

SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Morphological Study of the Hindgut in Larvae of *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae)

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Estudo Morfológico do Intestino Posterior em Larvas de *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae)

RESUMO - A lagarta da soja (*Anticarsia gemmatalis* Hübner) tem grande interesse econômico, pois afeta significativamente a cultura da soja em todo o mundo. Este trabalho descreve a morfologia do intestino posterior de larvas de *A. gemmatalis*, com ênfase nos seus aspectos histológicos. O intestino posterior é constituído por regiões morfológicamente distintas, identificadas como piloro, íleo, cólon e reto. Independente da região, a parede do intestino posterior é constituída por fina cutícula, epitélio simples e camada muscular. A íntima cuticular apresenta espículas no anel intersticial posterior, entre o intestino médio e o posterior, e na região posterior do piloro. A musculatura do reto é formada por camada única de largas fibras circulares, diferindo das demais regiões do intestino posterior que apresentam duas camadas de fibras musculares. As extremidades distais dos túbulos de Malpighi atravessam as paredes do reto, constituindo o sistema criptonefrídial característico de Lepidoptera.

PALAVRAS-CHAVE: Tubo digestivo, histologia, lagarta da soja, inseto

ABSTRACT - The velvetbean caterpillar (*Anticarsia gemmatalis* Hübner) has great economical interest as it affects the soybean crop worldwide. This work describes the morphology of the hindgut in *A. gemmatalis* larvae emphasizing their histological aspects. Distinct morphological regions, identified as pylorus, ileum, colon and rectum, constitute the hindgut of *A. gemmatalis*. A thin cuticular intima, a simple epithelium and muscular layer compose the hindgut wall, independent of the region. Microspines project from the cuticular intima in the posterior interstitial ring, between the midgut and the hindgut, and the posterior pyloric region. A single circular layer of large fibers, differing from the other hindgut regions that present two layers of muscular fibers, forms the rectal musculature. The distal ends of Malpighian tubules cross the rectum wall and constitute the cryptonephric excretory system typical in Lepidoptera larvae.

KEY WORDS: Digestive tube, histology, velvetbean caterpillar, insect

The digestive tract of insects is considered an effective physical and chemical barrier against the potentially invasive pathogens that are ingested with the feeding. Three main regions constitute the digestive tract: foregut, midgut and hindgut (Eaton 1988, Terra & Ferreira 1994, Chapman 1998).

It is well known that the insect midgut is responsible for food digestion and nutrient absorption (Santos *et al.* 1984, Billingsley & Lehane 1996, Cristofolletti *et al.* 2001). The undigested material goes straight to the hindgut where the water absorption and feces formation and elimination occur. The hindgut of most Lepidoptera larvae may be morphologically subdivided in pylorus, ileum, colon and rectum (Drecktrah *et al.* 1966, Judy & Gilbert 1970, Chi *et al.* 1975). The Malpighian tubules open into the pylorus; their distal ends are associated with the rectal wall and form the

Lepidopteran characteristic cryptonephric excretory system (Wigglesworth 1984) that conserves water by absorbing it actively from the feces either to the hemolymph or to the lumen of the Malpighian tubules (Maddrell & O'Donnell 1992).

The larvae of *Anticarsia gemmatalis* Hübner are considered one of the most serious pests of soybean crop, and they are known as velvetbean caterpillar. Although there is an effective program for the biological control of *A. gemmatalis* using virus that get into the insect through the digestive tract (Moscardi & Carvalho 1993, Flipsen *et al.* 1995), the internal morphology of this insect is not well known; besides, studies of the hindgut distinct regions, concerning their histological aspects have not been published. This work provides a description of the hindgut

in larvae of *A. gemmatilis*, under light and scanning electron microscopy.

Material and Methods

A. gemmatilis larvae were obtained from the Laboratório Entomológico of the Centro Nacional de Pesquisa da Soja (CNPSo)/Embrapa, Londrina - PR, Brazil. The larvae were maintained in the laboratory with artificial diet (Hoffmann-Campo *et al.* 1985), under controlled temperature (25-27°C), photoperiod (14h light/10h dark) and 80% relative humidity.

Larvae of the 4th and 5th instars (12-16 days old), after a rapid rinsing in 70% alcohol, were dissected in insect saline solution (ISS- 1.8 g de NaCl; 1.88 g de KCl; 0.16 g de CaCl₂; 0.004 g de NaHCO₃; distilled water - q.s.p. 100 ml) under stereomicroscope.

For studies with light microscope, the hindgut was isolated and fixed in glutaraldehyde (2.5%) and paraformaldehyde (4%) solution in phosphate buffer (0.1 M, pH 7.3). After dehydration in graded ethanol series, the material was embedded in JB4 hystoresin. The 3 µm sections were stained with hematoxylin-eosin (H.E.), analyzed and

photographed under an Axiophot (Zeiss) photomicroscope.

For scanning electron microscope studies, the hindgut was fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3), post-fixed in 1% osmium tetroxide solution in the same buffer, dehydrated in graded ethanol solutions, critical point dried and gold coated in a sputtering device. The materials were analyzed and photographed using SEM 515 (Phillips) scanning electron microscope.

Results and Discussion

The hindgut in *A. gemmatilis* larvae is the most complex portion of the digestive tract, being constituted by the pylorus, ileum, colon and rectum. It is internally lined by a continuous epithelium covered by a thin chitinous cuticular intima (Figs. 1A, 1C, 2B and 2D), as described for other Lepidoptera species (Drecktrah *et al.* 1966, Mathur 1966, Chi *et al.* 1975). According to Gillott (1995) the cuticle of the hindgut is thinner than the one of the foregut because this region is responsible for some water and nutrient absorption.

A posterior interstitial ring lined by small cuboidal epithelial cells with central spherical nuclei marks the junction

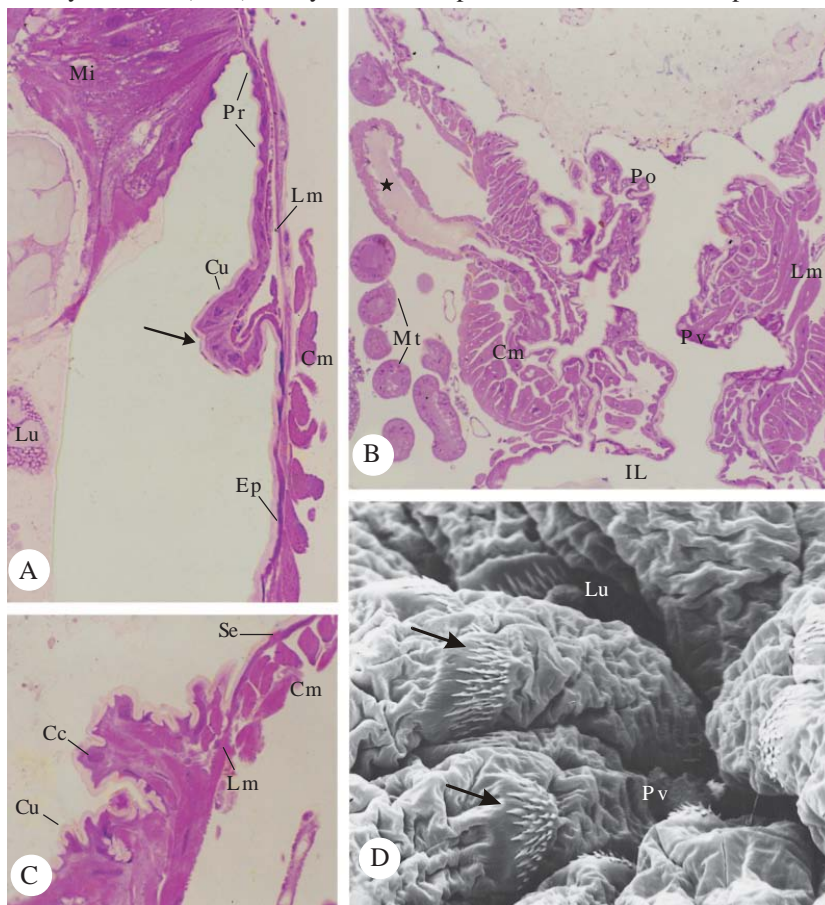


Figure 1. Morphology of the pylorus in *A. gemmatilis* hindgut. A - Junction between the midgut (Mi) and hindgut: the posterior interstitial ring (Pr) with small spines (arrow). H.E. 320x. B - General view of the anterior and posterior (Po) pyloric portions. H.E. 62x. C - Detail of the transition between anterior and posterior pyloric portions, showing the squamous (Se) and the cubic (Cc) epithelial cells. H.E. 250x. D - Scanning electron micrograph of the hindgut lumen surface showing groups of spines (arrow) near to pyloric valve (Pv). 720x. Epithelium (Ep), Lumen (Lu), longitudinal (Lm) and circular (Cm) muscular layers, cuticular intima (Cu), pyloric valve (Pv), Malpighian tubules (Mt), common ducts of the Malpighian tubules (C), and ileum (IL).

of mid-hindgut, and the cuticle intima at this region presents small spines (Fig. 1A); the longitudinal (external) and circular (internal) muscles cover the ring (Fig. 1A).

Posterior from this ring, the epithelial cells become increasingly flatten at the pyloric region (Figs. 1A, 1B and 1C). The pylorus of *A. gemmatalis* is the most complex histological region comparing with the other hindgut regions. Two distinct pyloric portions were recognized in *A. gemmatalis* (Fig. 1B), as described for *Hyalophora cecropia* L. (Judy & Gilbert 1970). The anterior pyloric portion is lined with a simple basophilic squamous epithelium and the chitinous intima has no spines; the muscle layer presents inner circular and outer longitudinal fibers (Figs. 1A, 1B and 1C). The posterior pylorus has a smaller lumen; irregular shaped cuboidal epithelial cells (Fig. 1C) and microspines project from the chitinous intima (Fig. 1D). Similar spinner cuticle lining the pyloric posterior region was also observed in *H. cecropia* (Judy & Gilbert 1970), *Heliothis zea* Boddie, *Heliothis virescens* Fabricius and *Spodoptera frugiperda* Smith (Chi *et al.* 1975). According to Barth (1972), the presence of this chitinous structure can be correlated with the insects feeding habits: if they feed with fluid material, like the phytophagous and hematophagous insects, the internal wall is smooth; in solid feeding species, the cuticular layer showed chitinous microspines that helps in the undigested material transport toward the other portions of the hindgut. However, microspines were found in fluid-feeding insects, as described in different species of ants (Caetano *et al.* 2002). Elzinga (1998) suggested that the microspines might be correlated with the presence of well-developed hindgut subdivisions and valves between these sections. Such chambers permit regulation of food movement and retention of digested food remnants to be acted upon by symbionts, as observed in crickets, cockroaches and termites; the microspines appear to aid in retaining bacteria and protozoan (Elzinga & Hopkins 1995, Elzinga 1996).

The posterior pyloric region has a thick musculature constituted by an inner circular layer and an outer longitudinal layer, which characterized the pyloric valve (Figs. 1B and 1C). The outer longitudinal muscle fibers penetrate among the circular muscle fibers (Fig. 1B). The circular muscle layer helps in the pyloric valve contraction, while the longitudinal muscle layer is responsible for the pyloric valve relaxes after the food movements toward the ileum (Gillott 1995).

A pair of common Malpighian ducts, which are ampulla-shaped, inserted through the musculature on the ventrolateral sides of the pyloric valve (Fig. 1B), characterizing the end of pyloric region in *A. gemmatalis*. The same aspect was described for other Lepidoptera (Standlea & Yonke 1968, Mathur 1972, Eaton 1988), Coleoptera (Areekul 1957, Vasques 1988), Diptera (Patil & Govindan 1984) and Hymenoptera (Caetano & Overal 1984, Arab & Caetano 2002).

The ileum epithelium in *A. gemmatalis* has acidophilic flattened polygonal epithelial cells with elongated nuclei and apical cytoplasm exhibit brush border-like structure lined by cuticular intima (Figs. 2A and 2B). The brush border structure represents the apical plasma membrane invaginations associated with mitochondria described under transmission electron microscopy in the ileum cell of *Schistocerca gregaria*

Forsk. which allows the water absorption that occurs in this region (Irvine *et al.* 1988). This absorption is necessary to maintain the insect metabolism as well to promote the feces dehydration that starts into the ileum (Barth 1972). The outer circular muscles are more developed than the inner longitudinal fibers (Figs. 2A and 2B); similar aspects were observed in Lepidoptera species (Mathur 1966, Judy & Gilbert 1970, Chi *et al.* 1975) and in some Coleoptera (Areekul 1957). However, Vasques (1988) described a different muscular arrangement in *Pyrearinus termitilluminans* Costa, with inner circular and outer longitudinal muscular layer.

The histological characteristics of the colon in *A. gemmatalis* (Figs. 2A and 2C) are similar to those described in other insets (Judy & Gilbert 1970, Gonçalves 1980). The colon epithelium is composed by a simple squamous epithelial cells, and the thicker cuticular intima is thrown into longitudinal folds; because of this folding, the lumen is very restricted and has a compound tubular appearance (Fig. 2C), giving to the lumen a potential for expansion when large amount of food pass through (Chi *et al.* 1975). The outer circular muscular layer is more developed than the inner longitudinal layer (Fig. 2C).

A fold rectal wall penetrates into the distal colon regional and constitutes the rectal valve in *A. gemmatalis* (Fig. 2C). Well-developed bounds of dilator muscles detected in this region (Fig. 2C) allow the valve contraction and dilatation, so helping feces transport to the rectum; besides, the rectal valve does not allow the feces reflux to the colon when the rectum contracts to defecation. This structure was described in most of insects, but it is less developed in fluid feeding species (Barth 1972).

The rectum wall in *A. gemmatalis* has large squamous epithelial cells with polymorphic nuclei and the chitinous cuticular intima is smooth (Figs. 2C and 2D), as described for other Lepidoptera (Judy & Gilbert 1970, Chi *et al.* 1975). Malpighian tubules are visualized between the epithelium and the thin rectal cellular membranes (Figs. 2C and 2D) and constitute the rectal complex that characterizes the cryptonephric excretory system typically found in Lepidoptera larvae (Drecktrah *et al.* 1966, Eaton 1988). The ability of the Malpighian tubules to excrete uric acid with very little water loss is an important factor for the success of insects in terrestrial environments as uric acid is the principal nitrogenous end product of protein metabolism in insects (Hegner & Engeman 1968, Maddrell & O'Donnell 1992). A thin sheet-like layer of muscle is found in the rectum wall, formed by large flattened muscle cells (Figs. 2C and 2D) that make this hindgut region different from the others concerning the musculature.

The rectal pads are absent in *A. gemmatalis*, as well as in other Lepidoptera larvae as *H. cecropia* (Judy & Gilbert 1970), *H. zea*, *H. virescens* and *S. frugiperda* (Chi *et al.* 1975). The histological analysis of the anus in *A. gemmatalis* was not possible to be a accomplished, as its strong junction to the exoskeleton did not allow the dissection.

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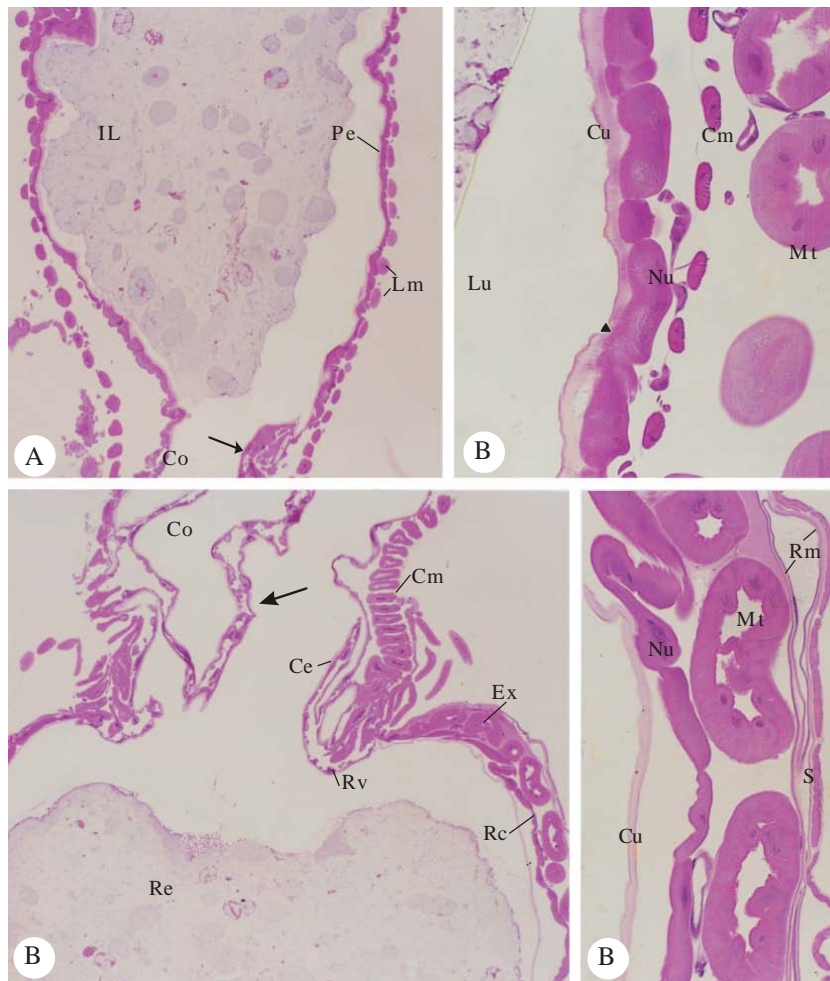


Figure 2. Morphology of the *A. gemmatilis* hindgut. A - Ileum (IL) with flattened polygonal epithelial cells (Pe). H.E. 62x. B - Detail of ileum wall: epithelial cells with elongated nuclei (Nu), and apical brush border-like structure (arrow head). H.E. 250x. C - Transition between the colon (Co) and rectum (Re): longitudinal folds (arrow) and rectal valve (Rv). H.E. 62x. D - Detail of the cryptonephric excretory system: rectum epithelial cells with polymorphic nuclei (Nu), rectal cellular membranes (Rm), and sheet muscle (S). H.E. 250x. Cuticular intima (Cu), colon epithelial cells (Ce), rectum epithelial cells (Rc), circular (Cm) muscular layer, Malpighian tubules (Mt), lumen (Lu) and cryptonephric excretory system (Ex).

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