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Histology and histochemistry of the ventriculus of *Dolichoderus* (=*Monacis) bispinosus* (OLIVIER, 1792) (Hymenoptera: Formicidae)

M.L. Bution [a], F.H. Caetano [a,*], F.B. Britto [a], G.A. Tomaino Gomes [b], F.J. Zara [c]

[a] *Universidade Estadual Paulista (UNESP), Departamento de Biologia, Rio Claro, SP 13506-500, Brazil*
[b] *Universidade Estadual de Campinas (UNICAMP), Campinas, SP 13083-863, Brazil*
[c] *UNESP-Campus Litoral Paulista, São Vicente, SP 11330-250, Brazil*

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Abstract

In this study we histologically and histochemically describe the ventriculus of *Dolichoderus bispinosus.* The epithelium consists of two basic cell types, highly basophilic generative cells, and digestive cells. The latter present several cytoplasmic vesicles, rich in acidic and neutral polysaccharides, and basic proteins. Also, these cells exhibit an apocrine secretion pattern. A mass of fibrous material is observed on the surface of the epithelium. Finally, we discuss the results obtained.
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1. Introduction

Dolichoderus (=Monacis) bispinosus (Olivier, 1792) is one of the most common species of this cosmopolitan genus, living in very altered areas (such as coffee and cocoa plantations), as well as little disturbed tropical forests above 2100 m of altitude (Longino, 2003).

The diet of this species includes nectar, floral exudates of several plants, liquids extracted from homopteran insects. *D. bispinosus* is also an efficient termite predator (Swain, 1977; Fisher et al., 1990). As most adult hymenopterans, this ant feeds preferentially on liquid foods (Eisner, 1957), for which its digestive tract is specialized (Caetano, 1984).

The digestive tract in Formicidae, as well as in other insects, is divided into three regions with sphincters controlling the flux of food. The foregut and hindgut, of ectodermic origin, are lined by a thin cuticle layer (intima). The midgut derives from the endoderm and therefore is not lined by a cuticle. Instead, the peritrophic matrix lines the midgut and protects it from injuries (mechanical or chemical) that can occur as the food bolus passes (Chapman, 1975; Caetano, 1984, 1988; Zara & Caetano, 1998; Caetano et al., 2002). In addition to this role, the peritrophic matrix compartmentalizes the midgut, allowing an efficient circulation of enzymes in the endo and ectoperitrophic compartments (Terra, 2001; Terra & Ferreira, 1994; Terra et al., 1996).

Despite the homogeneous structure of the digestive tract, the ventriculus exhibits one of the most conspicuous structural plasticities. According to Caetano et al. (2002), in more primitive species, such as the predators *Myrmecia regularis* and *Dinoponera gigantea*, the ventriculus is elongated, while in more derived ants with an omnivorous diet, it is round. In Dolichoderinae, such as *D. bispinosus,* however, the structure of the ventriculus is pear shaped, indicating an intermediary characteristic between more primitive and more derived ants (Caetano et al., 1990).

Chapman (1975) correlates variations in the size of the digestive tract and the insect's diet. Insects with a diet rich in proteins exhibit a short digestive tract, while those with a diet rich in carbohydrates present a long digestive tract. For Caetano (1984, 1988, 1989), however, this dos not apply to Formicidae.

The ventriculus in ants consists of one single layer of epithelium resting on a continuous basal lamina (Lehane & Billingsley, 1996). Histologically, digestive cells are columnar cells and may exhibit two physiologically distinct types: the ones with several long microvilli, with concentric lamellar bodies (spherocrystals) and myelin bodies, usually in groups, (absorption and excretion), and a second type with few and short microvilli, secretion granules and single myelin bodies

* Corresponding author. Tel.: +55 19 3526 4141; fax: +55 19 3526 4136.
E-mail address: fcaetano@rc.unesp.br (F.H. Caetano).

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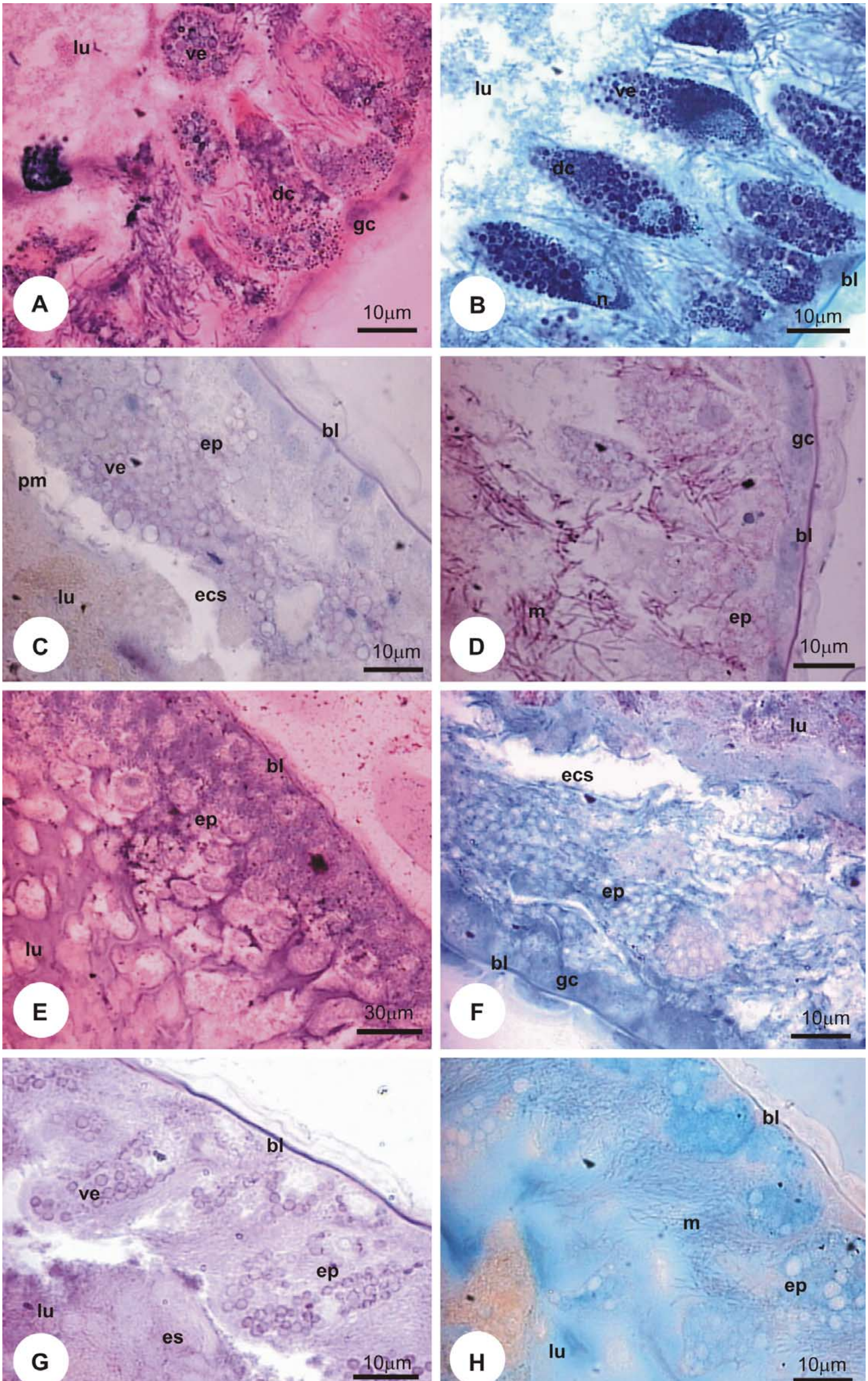

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(Caetano & Cruz-Landim, 1983). Another cell type found near digestive cells are generative cells. These cells are small, distributed in groups in the base digestive cells on the basal membrane (Caetano & Cruz-Landim, 1983; Caetano, 1984; 1988; Caetano et al., 2002). Circular and longitudinal muscle fibers are found externally to the basal membrane (Snodgrass, 1935; Caetano, 1984, 1988).

In between microvilli of globet cells of the ventriculus of ants of the genus *Atta*, fibrous elements and rod-shaped paracrystalline structures are observed (Caetano & Cruz-Landim, 1975).

A peritrophic matrix consisted of chitin fibers, proteins, and carbohydrates is produced in the anterior region of the ventriculus. According to its composition, it can be classified as gel or matrix (Terra & Ferreira, 1994; Terra, 2001). The peritrophic matrix presents pores that allow the transit of small food particles and bacteria towards the cells located in this region of the intestine (Gullan & Cranston, 2000). Therefore, this matrix limits two internal spaces of the ventriculus, the ectoperitrophic space, between the epithelium and matrix, and the endoperitrophic space, inside the matrix, usually represented by the food bolus (Terra & Ferreira, 1990; Terra, 2001).

To Caetano et al. (1994), secretion vesicles produced in the ventriculus of *Pachycondyla striata* cross the peritrophic membrane. As soon as they reach the peritrophic space, these vesicles begin to exhibit signs of disintegration.

This study presents histological and histochemical characteristics of the ventriculus of *D. bispinosus* regarding distribution of basic proteins, acidic and neutral carbohydrates, DNA and RNA in the epithelium as well as in the food. The production and the chemical composition of the peritrophic matrix, and the presence of fibrous material are also described.

2. Material and methods

2.1. Collection of specimens

Workers of *D. bispinosus* were collected in the trunks of *Caesalpinia peltophoroide* (Leguminosae) trees, at the UNESP campus/Rio Claro, (22°24′36″S; 47°33′36″W, 612 meters of altitude).

2.2. Histology

For histological analysis and detection of chemical compounds, the dissected material was fixed with 4% paraformaldehyde for 2 h, and then buffered with sodium phosphate (pH 7.4) for 24 h.

Following fixation, the material was dehydrated in an ascending series of alcohol (70 to 95%) for 30 min each. The material was later embedded in resin (Leica).

Histological sections of 6–8 μm were mounted on slides.

2.3. Morphology

Some slides were stained with Hematoxylin-Eosin (Junqueira & Junqueira, 1983) for morphological analysis of the ventriculus.

2.4. Histochemistry

Part of the slides were stained with PAS (Junqueira and Junqueira, 1983) for neutral carbohydrates; Toluidine Blue (pH 3.5) for acidic polysaccharides and DNA; Picrosirius Red (Junqueira & Junqueira, 1983) for collagen; Bromophenol Blue (Pearse, 1960), to detect protein compounds; Mallory Trichromic (Junqueira & Junqueira, 1983), to detect basophilic and acidophilic structures, and Resorcin Fuchsin (Junqueira & Junqueira, 1983) for elastic fibers and nuclei. All slides were examined with a Leica photomicroscope. The images were captured and digitalized with the software Leica Qwin.

3. Results and discussion

The epithelium of the ventriculus basically consists of two types of cell: the digestive and generative cells, as observed by Caetano and Cruz-Landim (1983), Caetano (1984, 1988, 1989a).

To Zara and Caetano (1998); Caetano and Zara (2001), a third type of cell is also found in the transition region between the proventriculus and ventriculus of *Ectatomma edentatum* and *Pachycondyla* (=*Neoponera*) *villosa* larvae. This cell seems to be specialized in the production of peritrophic matrix, which is classified, based on its characteristics, as type I peritrophic matrix by Terra (2001).

This type of matrix may be produced by epithelial cells of the whole ventriculus or only by part of it. In *D. bispinosus*, it is produced at the transition region between the epithelium of the proventriculus and ventriculus. This has also been shown by Caetano (1988) for ants of the subfamily Ponerinae (*Ectatomma* and *Neoponera*). The peritrophic matrix produced by these cells enclosing the food in the ventriculus, compartmentalizing it in several layers in different stages of digestion (Wigglesworth, 1972; Chapman, 1975; Terra & Ferreira, 1994; Terra, 2001; Caetano & Rodriges, 2001; Caetano et al., 2002).

Fig. 1. Histological sections of the ventriculus of *Dolichoderus bispinosus*. (A) H–E Staining. Epithelium (ep) with digestive cells (dc) and generative cells (gc). Digestive cells with filamentous masses and vesicles (ve) in the cytoplasm. (B) Toluidine Blue. Digestive cells (dc) with vesicles (ve). The nucleus (n) occupies the median portion of the cell. Basal lamina (bl) strongly stained. (C and D) Picrosirius staining. (C) Epithelial cells (ep) with vesicles (ve) with positively stained periphery. Basal lamina (bl) and peritrophic membrane (pm) positively stained. (D) Generative cells (gc) in the base of epithelium (ep). Basal lamina (bl) strongly stained. Filamentous masses (m) present in epithelial cells. (E) Resorcin Fuchsin. Basal lamina (bl) positively stained. (F) Bromophenol Blue. Basal lamina (bl), generative cells (gc) and peritrophic membrane (pm) positively stained. (G) Periodic Acid Schiff (PAS). Basal lamina (bl) and vesicles (ve) inside epithelial cells positively stained. (H) Mallory Trichromic. Epithelial cells (ep) with fibrous masses (m). lu = lumen, ecs = ectoperitrophic space, ep = epithelium, es = endoperitrophic space, bl = basal lamina.

Histologically, the epithelium of the ventriculus presents several basophilic and globular generative cells forming small groups on the base of digestive cells (Fig. 1A,B). This characteristic indicates that the rough endoplasmic reticulum, ribosomes, and RNAs are highly active in the cytoplasm due to cell growth, and that the cell is prepared to synthesize compounds when necessary.

Caetano (1984) described these cells as more abundant in highly derived reproductive casts of *Atta sexdens rubropilosa* than in casts of other ant species compared in his studies. In *D. bispinosus*, exhibiting a morphology considered evolutionarily intermediary (Caetano et al., 2002), generative cells are numerous, however, less than the observed for *A. s. rubropilosa.*

Digestive cells are tall and apparently of one histological type. These, like generative cells, are basophilic structures that frequently present dilated apical end and sometimes ruptured. Therefore, these are clear evidences that these secretory cells are apocrine (Fig. 1B). This mechanism of secretion is very similar to the one observed in *P. striata* by Caetano et al. (1994). The authors, however, pointed out that the digestive cells of *P. striata* are slightly acidophilic, unlike the observed in *D. bispinosus*, indicating different compounds between the two species.

Histological analysis revealed that digestive cells exhibit nuclei in the medial portion of the cell, as well as several vesicles differently stained with H.E, characterizing them as secretory cells, as described by Chapman (1975) and Wigglesworth (1974). These vesicles detach from the apical region of the digestive cells, cross the peritrophic membrane, and then empty their contents into the lumen containing food. This is similar to the described for adults of *P. striata* (Caetano et al., 1994) and larvae of *P. villosa* (Caetano & Zara, 2001). To these authors these vesicles contain digestive enzymes, which are discharged and added to the food bolus (endoperitrophic space) after fusion with the peritrophic matrix. Therefore, *D. bispinosus* exhibits the same characteristics observed for the larvae and adults of Ponerinae, a subfamily considered less derived by Holldobler and Wilson (1990). This pattern of secretion seems to be the basic model for Formicidae ants.

For the Toluidine Blue technique, a cationic dye that forms electrostatic bonds with radicals of opposite charge present in the tissue (Junqueira & Junqueira, 1983), acidic polysaccharides in the vesicles were strongly stained as well as in the basal lamina and generative cells. The cytoplasm, which presents several vesicles, was moderately stained (Fig. 1B).

Histochemically, the peritrophic matrix was positively stained with Bromophenol Blue, PAS, and Toluidine Blue, techniques detecting proteins and polysaccharides, respectively (Fig. 1F,G,B).

Among the detected d protein compounds, however, collagen was positively stained with Picrosirius red and Resorcin Fuchsin in the basal lamina, as well as in the peritrophic matrix (Fig. 1C,D,E). The presence of collagen in the composition of the peritrophic matrix is reasonable, since its properties provide flexibility and resistance to this structure**.** Reid and Lehane (1984) describe peritrophin as another compound of the peritrophic matrix that exhibits similar properties to those of collagen.

The use of these techniques to detect collagen resulted in paler reactions in the periphery of digestive vesicles, indicating the similarities between the periphery of vesicles and the peritrophic matrix.

According to King and Akai (1982, 1984), only collagen IV was identified in the basal lamina of insects. We have strong indications of these fibrillated elements in the conjunctive tissue of *D. bispinosus*.

Regarding the Bromophenol Blue method used to detect protein compounds, the results revealed strong staining of generative cells and basal lamina. Moderate staining was detected in the cytoplasm of digestive cells and peritrophic matrix. However, vesicles in the cytoplasm of digestive cells were very weakly stained, nearly imperceptible (Fig. 1F). The staining observed in the vesicles indicates the presence of small concentrations of protein in the enzyme composition. Studies conducted by Jeantet (1971) on *Formica* and Caetano (1988) on several ants, such as *Ectatomma quadridens, Neoponera villosa, Camponotus rufipes* and *Azteca bicolor*, observed the same weak staining pattern.

Intense staining of the peritrophic membrane, basal lamina and vesicles with PAS, used to detect polysaccharides, was observed. This technique allowed the detection of large concentrations of glycoproteins with 1-2 glycol inside vesicles, which were strongly stained (Fig. 1G). Similar PAS reactions were also observed by Caetano (1988) for *E. quadridens*, *N. villosa*, *C. rufipes*, and *A. bicolor*, and by Jeantet (1971) from larval stages to imago workers, males, and queens of *Formica*; and by Cruz-Landim (1981) for larvae and adults of *Trigona*.

The cytoplasm of digestive cells was moderately stained by PAS, indicating the presence of glycogen. According to Junqueira and Junqueira (1983), this technique precisely stains glycogen with 1-2 glycol groups. Therefore, considerable amounts of glycogen are present in the cytoplasm of these cells, although in smaller concentrations than those found in the basal lamina (Fig. 1G). It is very thick and intensively stained by dyes detecting protein compounds (collagen) as well as polysaccharides.

The results obtained with PAS were similar to those obtained with Toluidine Blue, confirming the accuracy of both techniques.

We also observed masses of filamentous structures in the ventriculus (Fig. 1A,D,H). These masses are similar to those described by Caetano (1984, 1988, 1989) for *Cephalotes atratus*, *Cephalotes (=Zacryptocerus) clypeatus*, and *Dolichoderus decollatus.* This author observed that this structure consisted of approximately 10 different bacteria and one fungus, which might be acting as symbionts. Caetano et al. (1989, 1990) describe the presence of fungus and bacteria between microvilli of digestive cells of the ventriculus of *Dolichoderus attalaboides*. Caetano et al. (1989) found similar results in the ventriculus of *C. atratus*, where bacteria were observed. In general, ants preferentially feed on fluid foods composed of mainly polysaccharides. Therefore, the presence of these microorganisms would be necessary to complement their diet (Caetano, 1989; Roche & Wheeler, 1997). Therefore, strong evidences indicate that microorganisms are present in *D. bispinosus*, since these masses were found in all dissected and analyzed individuals from different colonies.

The pH of the content of the ventriculus is an important characteristic directly affecting the activity of digestive enzymes (Terra, 1994). Inside the ventriculus of *D. bispinosus*, as well as other hymenoptera, the pH is acidic. This characteristic was observed using the Mallory trichromic method (Fig. 1H). Terra (2001), Schumaker et al. (1993) and Wigglesworth (1972) obtained pH values between 5 and 6 for hymenopteran insects, and also a decrease in pH towards the posterior region of the ventriculus.

Vesicles present in the cytoplasm of digestive cells are free in the ventriculus lumen. In this region vesicles also were strongly stained by dyes detecting polysaccharides, indicating the predominance of these compounds in the composition of digestive enzymes. The intensity of staining of proteins was again weakly positive.

Schumaker et al. (1993) describe the protein content of these vesicles in Hymenoptera as proteases consisting only of serine (trypsin and chymotrypsin). Among polysaccharides, however, several carbohydrases are present (α-Amylase, Cellulases, Hemicellulases, among others).

This study showed that the secretion pattern present in this ant is similar to the apocrine pattern described for adults of *P. striata* (Caetano et al., 1994) and larvae of *P. villosa* (Caetano & Zara, 2001). All ants previously studied by these authors exhibited the same pattern, suggesting that this might be the basic model among Formicidae.

The content of vesicles present in the cytoplasm of digestive cells, as well as in the lumen of ventriculus consisted of glycoproteins. Polysaccharides, however, exhibited more intense staining than protein compounds.

The mass of fibrous material is similar to the microorganisms described by Caetano (1984, 1988, 1989, 1990) in the interior of the digestive tract of *C. atratus, C. clypeatus* and *D. decollatus.*

The compounds observed in the periphery of digestive vesicles were also observed in the peritrophic membrane, indicating a similarity between the periphery of vesicles and the peritrophic matrix, explaining the permeability between them.

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