

Midgut of the diplopod *Urostreptus atrobrunneus*: structure, function, and redefinition of hepatic cells

C. Moreira-de-Sousa^a, M. Iamonte^a and C. S. Fontanetti^{a*}

^aDepartment of Biology, Biosciences Institute, Universidade Estadual Paulista – UNESP, Avenida 24-A, 1515, CEP 13506-900, Rio Claro, São Paulo, Brazil

*e-mail: fontanet@rc.unesp.br

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Abstract

Diplopods are considered important macroarthropods the soil as part of its maintenance and balance. These animals usually do not occur in high densities, but population explosions caused by environmental disturbances, climate changes, and use of pesticides that eliminate possible competitors, have been reported. The millipede *Urostreptus atrobrunneus* Pierozzi and Fontanetti, 2006 have become a nuisance to humans in infestation sites in urban centers of the state of Sao Paulo, Brazil. As a contribution to the understanding of this potential pest, this study describes the histology, histochemistry, and ultrastructure of the *U. atrobrunneus* midgut, and presents the redefinition of hepatic cells somewhat controversial in the literature. The region of the midgut is characterized by the absence of a cuticular intima, and composed of a pseudostratified epithelium on a thick basal membrane, followed by a muscle layer, a layer of hepatic cells, lined by an external membrane. The morphology observed in *U. atrobrunneus* is similar to that reported for other species of diplopods. The hepatic cells have been previously described as randomly without forming a layer, however, the present results clearly demonstrate that these cells form a continuous layer over the whole midgut.

Keywords: histology, histochemistry, ultrastructure, Diplopoda, digestive tract.

Intestino médio do diplópodo *Urostreptus atrobrunneus*: estrutura, função e redefinição das células hepáticas

Resumo

Diplópodos são considerados importantes macro-artrópodes do solo, uma vez que participam de sua manutenção e equilíbrio. Comumente estes animais não apresentam população numerosa, porém há relatos de explosões populacionais ocasionadas por desequilíbrios ambientais, mudanças climáticas e utilização de pesticidas que eliminam possíveis competidores. O milípede *Urostreptus atrobrunneus* Pierozzi e Fontanetti, 2006, têm apresentado pontos de infestação em centros urbanos, do estado de São Paulo, Brasil, causando muitos transtornos à população humana. Com objetivo de contribuir para o conhecimento desta potencial praga, este trabalho apresenta a descrição histológica, histoquímica e ultra-estrutural do intestino médio do milípede *U. atrobrunneus*, bem como apresenta a redefinição das células hepáticas, um tanto controversa na literatura pertinente. A região do intestino médio é caracterizada pela ausência da íntima cuticular, sendo formado por um epitélio pseudoestratificado, apoiado por uma membrana basal espessa, seguido de uma camada muscular, uma camada de células hepáticas, revestido por uma membrana externa. A morfologia observada neste trabalho assemelha-se bastante com as descrições de outras espécies de diplópodos estudadas até o momento. As células hepáticas foram previamente descritas como dispostas aleatoriamente sem a formação de uma camada, contudo, os presentes resultados demonstram claramente que estas células formam uma camada contínua ao longo de todo o intestino médio.

Palavras-chave: histologia, histoquímica, ultra-estrutura, Diplopoda, trato digestório.

1. Introduction

Diplopods usually do not occur in high densities, but population explosions have been reported, often caused by environmental disturbances (Cloudsley-Thompson, 1950; Nijjima and Shinohara, 1988; Boccardo et al., 1997, 2002; Kania and Tracz, 2005; Fontanetti et al., 2010a, b). The high proliferation rate of the millipede *Urostreptus atrobrunneus*, a species described by Pierozzi and Fontanetti

(2006), in some urban centers in the state of Sao Paulo, have drawn the attention of several researchers (Boccardo, 1998; Fontanetti et al., 2007, 2010a, b, 2012; Moreira-de-Sousa and Fontanetti, 2012).

The digestive tract of millipedes is a simple straight tube that starts in the mouth and ends in the anus, and is divided in foregut, midgut, and hindgut. Except for the midgut,

the entire digestive tube is internally lined by a cuticular intima. Salivary glands are found along the foregut and are responsible for the production of secretions that lubricate the ingested food and contain digestive enzymes that aid in the early stages of digestion (Nunez and Crawford, 1977; Fontanetti and Camargo-Mathias, 1997; Fantazzini et al., 1998; Moreira-de-Sousa and Fontanetti, 2012).

The midgut of millipedes located after the foregut and before the pyloric region, has a fundamental role in the digestive process of the animal. Some enzymes are secreted by epithelial cells on the food particles and others may be produced by microorganisms in the lumen (Hopkin and Read, 1992).

The structure and role of the midgut of diplopods have been described for few species (Bowen, 1968; Nunez and Crawford, 1977; Hubert, 1979b, 1988; Fontanetti and Camargo-Mathias, 1997; Fontanetti et al., 2001; Fantazzini et al., 2002; Camargo-Mathias et al., 2004; Sosinka et al., 2014). Studies on Brazilian species are rare and most of the literature is restricted to some orders. Fontanetti and Camargo-Mathias (1997) described the morphology and histology of the digestive tract of the *Plusioporus setiger* species (Spirostreptida). Fantazzini et al. (2002) described the anatomy of the midgut of *Rhinocricus padbergi* (Spirobolida) by means of histological and histochemical analysis, and Camargo-Mathias et al. (2004) later characterized the ultrastructure of the midgut of this species.

This study was aimed at describing the morphology of the midgut of *U. atrobrunneus*, through histological, histochemical, and ultrastructural analysis, and its relationship with the roles played by this organ. It was also aimed to review the terminology and definition of hepatic cells, which are controversial in the literature.

2. Materials and Methods

The specimens (n=8) were collected by Edilberto Gianotti and by Raphael Baston de Souza in the surroundings of the city of Rio Claro, São Paulo, Brazil (22°23'59"S; 47°34'18"W). The animals were maintained in a terrarium with soil and leaf litter moistened regularly.

The midgut was obtained by dissecting specimens anesthetized with sulfuric ether on a dissection plate with saline solution. The digestive tube was fixed with Bouin solution, 4% paraformaldehyde or formol calcium for 24 hours. The material was embedded in historesin and stained with hematoxylin and eosin for histological analysis. The histochemical stains used were: Xylidine Ponceau (Melo and Vidal, 1980) and Bromophenol Blue (Pearse, 1985) for the detection of proteins; Periodic acid-Schiff (PAS) and simultaneous PAS and Alcian Blue (Junqueira and Junqueira, 1983) for polysaccharides; von Kossa method (Junqueira and Junqueira, 1983) for calcium detection; and Nile Blue (Lison, 1960) and Sudan Black B (Junqueira and Junqueira, 1983) for lipids detection.

The material processed for transmission electron microscopy was fixed with 2.5% glutaraldehyde in 0.1M cacodylate buffer at 4 °C, rinsed with cacodylate buffer and post-fixed with 1% osmium tetroxide for 2 hours. After this period, the material was then rinsed with the same buffer, immersed in 10% ethanol for 15 minutes and contrasted with 2% uranyl acetate in 10% ethanol for 4 hours. Following this procedure, the material was dehydrated in a crescent series of acetone and immersed in resin: acetone (1:1) for 12 hours, embedded in Epon-araldite resin with catalyzer for 24 hours and placed in the oven at 70 °C for 24 hours for resin polymerization. Sections were obtained with ultramicrotome and observed with a Phillips CM 100 transmission electron microscope.

3. Results

3.1. Histology

The midgut of *U. atrobrunneus* consists of a pseudostratified epithelium resting on a basal lamina, followed by a muscle layer, a layer of hepatic cells, and irregularly distributed muscles, lined externally by a membrane termed external membrane. Adjacent to the midgut there is the perivisceral fat body (Figure 1A).

The epithelium is composed of three cell types: principal or absorptive cells, generative or regenerative cells and secretory cells. The absorptive cells are the most numerous cells, which exhibit a well-defined brush border.

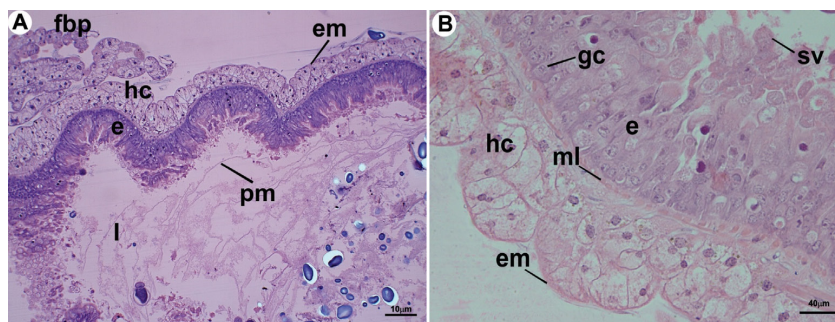


Figure 1. (A, B) Histological sections of the midgut of *U. atrobrunneus*, stained with haematoxylin/eosin. Abbreviations: e – epithelium; em – external membrane; gc – generative cell; hc – hepatic cell; l – lumen; ml – muscular layer; fbp – fat body perivisceral; pm – peritrophic membrane; sv – secretory vesicle.

The generative cells are smaller and located at the base of the epithelium (Figure 1B). The secretory cells are among the absorptive cells and release a secretion of the apocrine type, in which part of the cell is lost along with the secretion (Figure 1B).

Just beneath the muscle layer are the hepatic cells forming a continuous layer. These cells are irregular and have a spherical nuclei and heterogeneous cytoplasm (Figure 1A, B).

Along the entire midgut, a peritrophic membrane consisted of acellular layers is observed in the lumen (Figure 1A, B).

3.2. Histochemistry

The histochemical analysis revealed that the epithelium was strongly positive to proteins, while hepatic cells were moderately to weakly stained (Figure 2A, B).

The secretion released by epithelial cells contains glycoproteins. Some vesicles were strongly stained for proteins (Figure 2A, B) while others were more intensely stained for neutral polysaccharides.

Neutral polysaccharides were observed in the brush border, in the basal membrane, and some hepatic cells (Figure 2C).

Calcium was observed diffusely in the absorptive cells (Figure 2D), and small quantities of lipids were present

in the brush border of epithelial cells and some hepatic cells (data not shown).

3.3. Ultrastructure

Absorptive (principal) cells exhibit many microvilli in their free face (Figure 3A) and rest on a thick basal lamina, where cytoplasmic projections are observed (Figure 3B). The cytoplasm of absorptive cells exhibits well-developed rough endoplasmic reticulum (Figure 3C, D) and spherocrystals (Figures 3A, D, 4A).

It is possible to observe that the materials in spherocrystals accumulate in concentric layers; spherocrystals are enclosed by a membrane and are in close contact with the rough endoplasmic reticulum (Figure 3D). Spherocrystals found in the cytoplasm of absorptive cells are easily removed during the preparation for microtomy, resulting in empty sites (Figure 4A, B).

Among the absorptive cells observed, secretory cells were seen releasing part of their cytoplasm with secretion (Figure 3A).

The generative cells, located at the base of the epithelium, exhibit large nucleus, an evident nucleolus, cytoplasm less electron-dense than that observed in absorptive cells with several mitochondria more electron-dense than that observed in absorptive cells (Figure 4C).

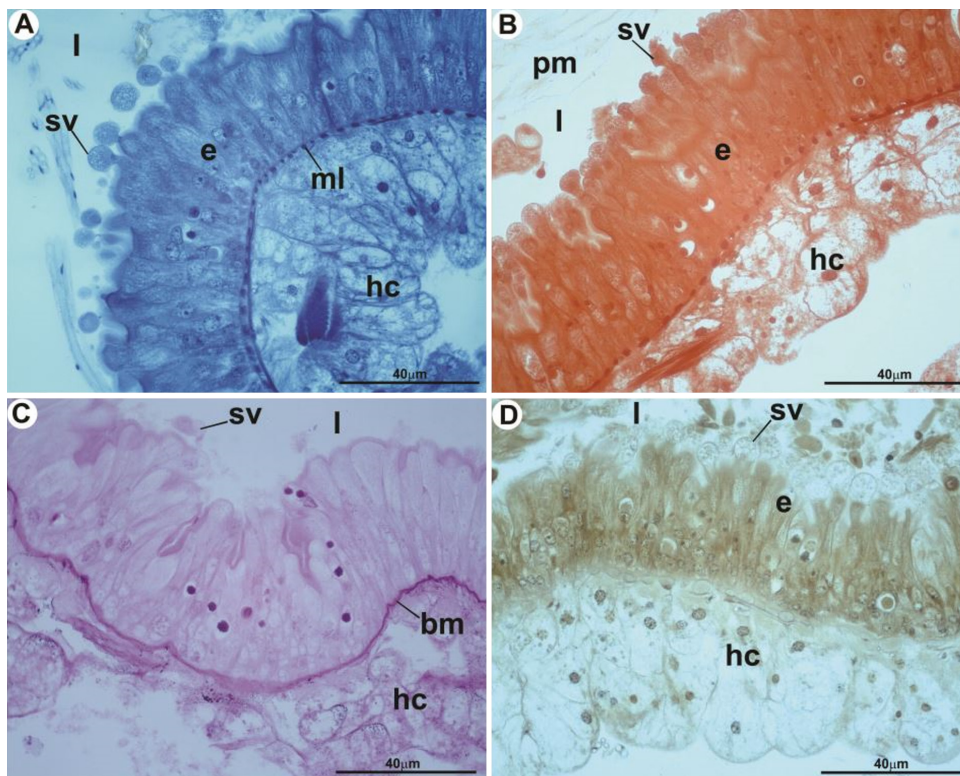


Figure 2. Histological sections of the midgut of *U. atrobrunneus*. (A) Submitted to the bromophenol blue technique; (B) Staining with xylydine ponceau; (C) Submitted to the PAS the technique; (D) Submitted to the von Kossa method (Calcium in brown color). Abbreviations: bm – basal membrane; e – epithelium; hc – layer hepatic cells; l – lumen; ml – muscular layer; pm – peritrophic membrane; sv – secretory vesicle.

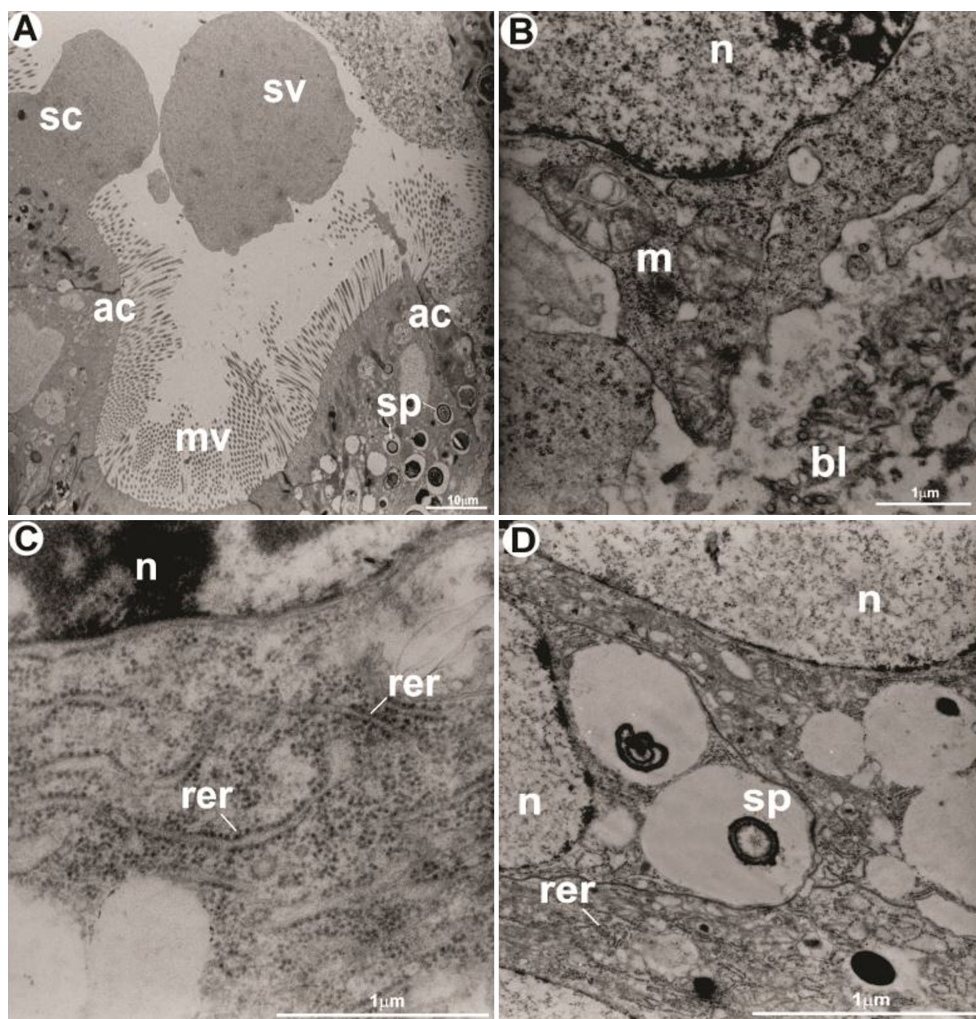


Figure 3. Electron micrographs of *U. atrobrunneus* midgut. (A) Detail of the apical region of the midgut; (B) Detail of the basal lamina; (C) Detail of the cytoplasm, showing the abundance of rough endoplasmic reticulum; (D) Details of the spherocrystals. Abbreviations: bl – basal lamina; m – mitochondria; mv – microvilli; n – nuclei; ac – absorptive cell; rer – rough endoplasmic reticulum; sc – secretory cells; sp – spherocrystal; sv – secretory vesicle.

The contact of epithelial cells occurs through interdigitations and occlusive junctions. However, in many regions, dilated intercellular spaces are generally observed (Figure 4D).

Hepatic cells exhibit heterogeneous cytoplasm (Figure 4A), large quantities of mitochondria, vacuoles of various aspects and large nucleus (Figure 4A, B). Many empty spaces are observed in the cytoplasm of these cells, due to the preparation for microtomy that removed granules present in the cells (arrows in Figure 4A, B). Between hepatic cells, hemocytes in different maturation stages are observed (Figure 4B).

4. Discussion

The midgut of *U. atrobrunneus* exhibited a pseudostratified epithelium. Bowen (1968) has reported the presence of a pseudostratified columnar epithelium in the midgut

of *Floridobolus penneri* and *Narceus gordanus*, both Spirobolida. For all other species of diplopods examined, a simple prismatic epithelium has been reported. This has been confirmed by Hubert (1979b) on the ultrastructural analysis of *Cylindroiulus londinensis*. On the contrary, in *R. padbergi*, the ultrastructural analysis has revealed a pseudostratified epithelium (Camargo-Mathias et al., 2004). Sosinka et al. (2014) reported that the midgut epithelium of the species *P. lagurus*, *A. gigas* and *J. scandinavius* is columnar pseudostratified. In this study, was also observed that the epithelium of the midgut of *U. atrobrunneus* is pseudostratified.

Along the midgut of the millipede *U. atrobrunneus*, a peritrophic membrane, which is closely associated to the intestinal cells, has also been observed. Nunez and Crawford (1977) have reported that this well-known structure in Diplopoda.

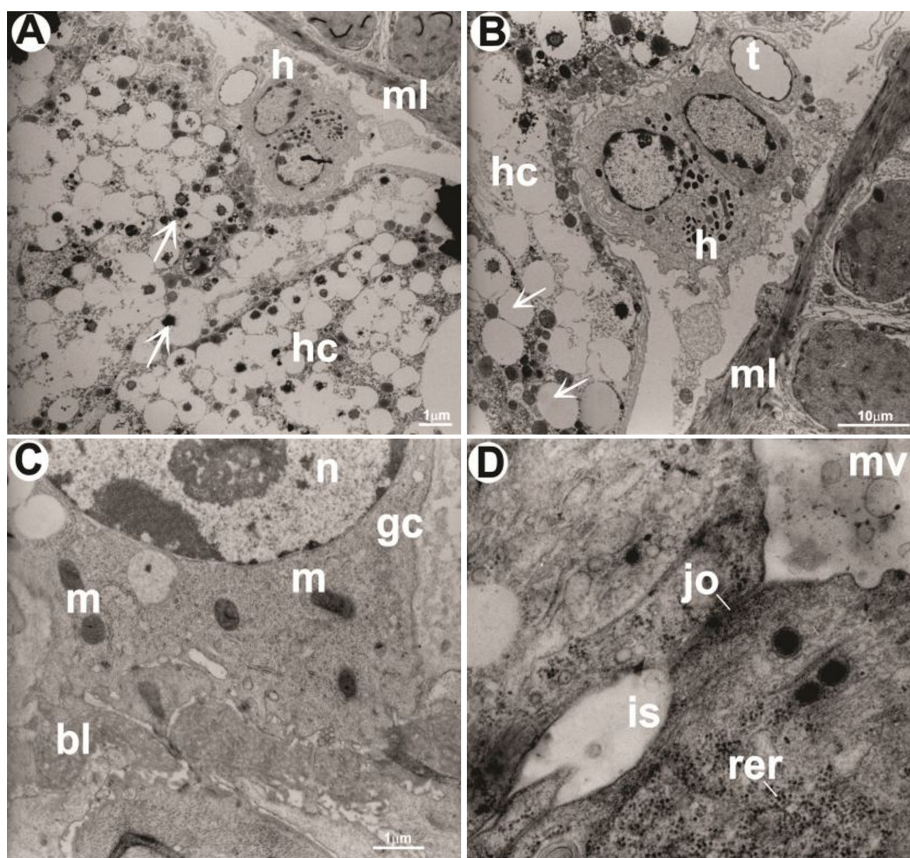


Figure 4. Electron micrographs *U. atrobrunneus* midgut. (A) Muscular layer + hepatic cells; (B) Detail of hemocytes; (C) Generative cell; (D) Detail of cellular junctions. Abbreviations: bl – basal lamina; gc – generative cell; h – hemocyte; hc – hepatic cell; is – intercellular space; jo – junction occlusion; m – mitochondria; mv – microvilli; n – nuclei; rer – rough endoplasmic reticulum; arrow in (A): spherocrystal; arrow in (B): spaces observed in the cytoplasm resulting from the removal of spherocrystals during microtomy.

The epithelium consists of absorptive cells (principal cell or digestive cell), generative cells (regenerative cell) and secretory cells, similar to the ones described for other species of diplopods, such as *Oxidus gracilis* (Neumann, 1985 apud Hopkin and Read, 1992), *Glomeris marginata* (Martin and Kirkham, 1989), *Plusiopurus setiger* (Fontanetti and Camargo-Mathias, 1997), *Rhinocricus padbergi* (Fantazzini et al., 2002) and *Polynexus lagurus*, *Archispirostreptus gigas* and *Julus scandinavius* (Sosinka et al., 2014).

The absorptive cells, also called by some authors as principal cells or digestive cells, are involved in both the absorption of food and in the transport elements to the hemolymph. There are descriptions that these cells accumulate heavy metals such as lead, cadmium, zinc and copper (Köhler et al., 1995) and calcium (Hubert, 1988; Fantazzini et al., 2002).

The observation of absorptive cells in diplopods, including *U. atrobrunneus* demonstrated the presence of numerous secretory vesicles. These vesicles are in the lumen near the microvilli, attesting that the absorptive cell can play the role of secretory cell according to the physiological demand. The content of these vesicles varied in coloration,

suggesting a maturation level or a dehydration process of the secretion during the transport from the cytoplasm to the intestinal lumen, as also proposed by Fantazzini et al. (2002) and Fontanetti et al. (2001).

Similar characteristics have been described for the digestive cells mite. According to Filimonova (2008), these cells are polyfunctional, once that digestion processes, absorption and excretion are carried out by cells in the different phases of the cell cycle. The author observed the presence of various different cell types representing a gradual change from the absorptive cells for secretory cells (Filimonova, 2008). The same should happen in the midgut of millipedes.

In diplopods, the mode of secretion can be apocrine, as observed in the present study and described by Fontanetti et al. (2001) for the species *P. setiger*, by Fantazzini et al. (2002) for *R. padbergi*, or merocrine as observed by Hefner (1929) for *Parajulus impressus*. The latter author also suggested that in other millipedes the type of secretion may be holocrine. Sosinka et al. (2014) reported the release of microapocrine secretion in the millipedes *Julus scandinavius* and *Archispirostreptus gigas*. The vesicle are

formed at the end of microvilli and then discharged into the lumen as double-membrane vesicles.

The generative or regenerative cells are located in the basal area of the epithelium and are distributed along the entire midgut. These cells are responsible for epithelial renewal (Hopkin and Read, 1992) and are considered unipotent stem cells of millipedes midgut (Sosinka et al., 2014).

Hubert (1988) reported that hepatic cells are not contiguous, and that the cohesion between them is ensured by a connective tissue. Regarding its embryonic origin there are no studies, however Hubert (1988) states that the hepatic cells are lined by a coelomic leaflet, which may indicate that they are of mesodermal origin.

On the similarity between these cells with those that form the fat body of these animals, many authors have confused the hepatic cells with the fat body, a totally separate body (Subramoniam, 1972; Fontanetti and Camargo-Mathias, 1997; Fantazzini et al., 1998; 2002). Further, according Seifert and Rosenberg (1977), the hepatic cells are distributed randomly, not forming a layer, while the results shown here indicate that these cells in fact form a continuous layer throughout the entire midgut. The hepatic cells of millipedes probably perform functions similar to the hepatopancreas of other arthropods or chloragogenous tissue surrounding the gut of the earthworms (Hopkin and Read, 1992). According to Hubert (1978b) these are cells rich in glycogen and probably interfere in intermediary metabolism.

According Hubert (1988) the hepatic cells branch out towards the basal portion of the midgut cells. According to the author the fusiform junctions suggest that there is a transport between the two cell types. In the present study, observed that hepatic cells form a continuous layer along the entire midgut, but without connections with epithelial cells.

Part of the products of digestion assimilated by the absorptive cells will pass to the hepatic cells (Hopkin and Read, 1992). Hubert (1978a, b, 1988) reports that the intestinal cells and hepatic cells perform complementary functions. The intestinal cells are involved in the absorption of the digestion products, while hepatic cells are involved in mineral retention. The hepatic cells can also accumulate toxic substances which are transported by hemolymph and then excreted. The hepatic cells actively participates in detoxification process of the organism. (Kohler, 2002; Nogarol and Fontanetti, 2010, 2011; Perez and Fontanetti, 2011; Merlini et al., 2012). These observations explain the fact that large quantities of spherocrystals are found in the cytoplasm of these cells in millipede *U. atrobrunneus*.

The histochemical analysis has revealed that the epithelium and hepatic cells were positive for proteins, indicating a high concentration of proteins in the region. Except for secretory cells, the epithelium is weakly stained for neutral polysaccharides, suggesting that this epithelium participates in the production of neutral polysaccharides, but do not store them. Lipid staining techniques indicated the presence of this compound in small quantities in the

brush border in hepatic cells, indicating that the midgut of *U. atrobrunneus* has little participation in the metabolism of these elements.

The presence of calcium in the midgut of diplopods is well known, since these animals dwell in the soil. The presence of this element represents a form of detoxification, a well-described process for invertebrates, including diplopods (Fontanetti et al., 2006).

According to Fantazzini et al. (2002), the presence of calcium in absorptive cells suggests that the epithelium might be involved in the transportation of this element. In this case, the metal precipitation occurs as intracellular granules of different types which, after being stored, are directly discharged by secretory vesicles into the lumen or by substitution of the intestinal epithelium (Hubert, 1979a; Nogarol and Fontanetti, 2010; Godoy and Fontanetti, 2010).

Hopkin and Read (1992) also observed several granules of calcium phosphate arranged concentrically in intestinal cells of diplopods. According to Hubert (1979b), since the midgut is a site of mineral storage, it plays an important role in the ionic regulation of the organism, and consequently, the cyclic discharge of granules may be a form of excretion. The excretion of minerals in *U. atrobrunneus* may occur in a similar way, since several spherocrystals has also been observed in the midgut of this species and the accumulation of material inside them is also concentric.

According to Fontanetti et al. (2006), spherocrystals may be named mineralized bodies, and their structural organization can vary inside different cells, from different parts of the body, with distinct aspects. These authors have also observed that spherical bodies were enclosed by a membrane, although not associated to organelles such as the endoplasmic reticulum or the Golgi apparatus. In *U. atrobrunneus*, spherocrystals were also enclosed by a membrane, but in close contact with the rough endoplasmic reticulum.

According Camargo-Mathias et al. (2004), the structural organization of the basal portion of the absorptive cells in the midgut of *R. padbergi* supports the hypothesis that in addition to absorbing compounds from food, these cells may also be involved in the transport of elements from the hemolymph. For these authors, the morphology is similar to that of renal tubular cells of vertebrates and play a role in the transport of ions, the large number of mitochondria in this region suggests a similar role for absorptive cells (Camargo-Mathias et al., 2004). Analyzing the results of the present study, the same may occurs with *U. atrobrunneus*, since a significant quantity of mitochondria was also observed.

It concludes that the midgut structure millipede *U. atrobrunneus* is very similar to that described for other species. In the species studied in this work the epithelium is pseudostratified. The observation of hepatic cells in this species contributed to clarify a point of controversy in the literature sometimes these cells were referred to as “fat body layer midgut” and sometimes described as randomly arranged cells without forming a layer. It is clear from this analysis that these cells form a continuous layer around the entire midgut.

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References

BOCCARDO, L., 1998. Surto de diplópodos em área urbana impactada localizada no município de Paulínia-SP. In: *Anais do 22º XXII Congresso Brasileiro de Zoologia*, 1998, Recife, PE. Recife: Universidade Federal de Pernambuco. p. 36.

BOCCARDO, L., PENTEADO, C.H.S. and JUCÁ-CHAGAS, R., 1997. Swarming of millipedes, a new case noticed in the district of Patrocínio – MG - Brazil. *Journal of Advanced Zoology*, vol. 18, pp. 62-63.

BOCCARDO, L., PENTEADO, C.H.S. and JUCÁ-CHAGAS, R., 2002. Migration and population outbreaks of millipedes in the coffee plantations, region of Alto Paranaíba, MG, Brazil. *Holos Environment*, vol. 2, no. 2, pp. 220-223.

BOWEN, R.C., 1968. Histochemical studies on two millipedes species. *The Ohio Journal of Science*, vol. 68, pp. 85-91.

CAMARGO-MATHIAS, M.I., FANTAZZINI, E.R. and FONTANETTI, C.S., 2004. Ultrastructural features of the midgut of *Rhinocricus padbergi* (Diplopoda: Spirobolida). *Journal of Morphological Science*, vol. 21, no. 2, pp. 65-71.

CLOUDSLEY-THOMPSON, J.L., 1950. Economics of the 'spotted snake millipede' *Blaniulus guttulatus*. *Annals & Magazine of Natural History*, vol. 12, no. 3, pp. 947-962.

FANTAZZINI, E.R., FONTANETTI, C.S. and CAMARGO-MATHIAS, M.I., 1998. Anatomy of the digestive tube, histology and histochemistry of the foregut and salivary glands of *Rhinocricus padbergi* Verhoeff (1938) (Diplopoda: Spirobolida: Rhinocricidae). *Arthropoda Selecta*, vol. 7, pp. 257-264.

FANTAZZINI, E.R., FONTANETTI, C.S. and CAMARGO-MATHIAS, M.I., 2002. Midgut of the millipede, *Rhinocricus padbergi* (Verhoeff, 1938) (Diplopoda: Spirobolida): histology and histochemistry. *Arthropoda Selecta*, vol. 11, pp. 135-142.

FILIMONOVA, S.A., 2008. The fine structure of the midgut in the mite *Anystis baccharum* (L.) (Acari, Actinedida: Anystidae). *Arthropod Structure & Development*, vol. 37, no. 4, pp. 299-309. <http://dx.doi.org/10.1016/j.asd.2007.11.005>. PMID:18396461.

FONTANETTI, C.S. and CAMARGO-MATHIAS, M.I., 1997. Histoanatomy of the digestive tract in *Plusioporus setiger* diplopod (Brolemann, 1901) (Spirostreptida, Spirostreptidae), Brazil. *Journal of Morphology*, vol. 14, pp. 205-211.

FONTANETTI, C.S., CAMARGO-MATHIAS, M.I. and CAETANO, F.H., 2001. Apocrine secretion in the midgut of *Plusioporus setiger* (Brolemann, 1901) (Diplopoda, Spirostreptidae). *Naturalia (São José do Rio Preto)*, vol. 26, pp. 35-42.

FONTANETTI, C.S., TIRITAN, B. and CAMARGO-MATHIAS, M.I., 2006. Mineralized bodies in the fat body of *Rhinocricus padbergi* (Diplopoda). *Brazilian Journal of Morphological Sciences*, vol. 23, pp. 487-493.

FONTANETTI, C.S., CALLIGARIS, I.B. and SOUZA, T.S., 2007 [viewed 4 August 2015]. *Laudo sobre o surto de piolho-de-cobra no Parque das Universidades: infestação de piolhos-de-cobra em Campinas* [online]. Campinas. Available from: <http://www.barao/barao/pq-universidades/laudo.htm>.

FONTANETTI, C.S., CALLIGARIS, I.B. and SOUZA, T.S., 2010a. A millipede infestation of an urban area of the city of Campinas, Brazil and preliminary toxicity studies of insecticide Bendiocarb to the *Urostreptus atrobrunneus* Pierozzi & Fontanetti, 2006. *Arquivos do Instituto Biológico*, vol. 77, pp. 165-166.

FONTANETTI, C.S., SOUZA, T.S., CALLIGARIS, I.B. and BOZZATTO, V., 2010b. Ovarian morphology and oogenesis dynamic of the diplopod *Urostreptus atrobrunneus* Pierozzi & Fontanetti, 2006 (Spirostreptidae), a potential plague in urban centers. *Animals Biology*, vol. 60, no. 4, pp. 467-478. <http://dx.doi.org/10.1163/157075610X523323>.

FONTANETTI, C.S., CALLIGARIS, I.B., SOUZA, T.S. and IAMONTE, M., 2012. Ultrastructure of oocytes of the *Urostreptus atrobrunneus* (Diplopoda, Spirostreptida, Spirostreptidae) a potential urban centers plague. *Microscopy Research and Technique*, vol. 75, no. 11, pp. 1486-1491. <http://dx.doi.org/10.1002/jemt.22092>. PMID:22791626.

GODOY, J.A. and FONTANETTI, C.S., 2010. Diplopods as Bioindicators of Soils: Analysis of Midgut of Individuals Maintained in Substrate Containing Sewage Sludge. *Water, Air, and Soil Pollution*, vol. 210, no. 1-4, pp. 389-398. <http://dx.doi.org/10.1007/s11270-009-0261-z>.

HEFNER, R.A., 1929. Studies of parajulid diplopods. The micro-anatomy of the alimentary canal of *Parajulus impressus* Say. *Transactions of the American Microscopical Society*, vol. 48, no. 4, pp. 321-339. <http://dx.doi.org/10.2307/3222054>.

HOPKIN, S.P. and READ, H.J., 1992. *The biology of millipedes*. New York: Oxford University Press.

HUBERT, M., 1978a. Données histophysiologiques complémentaires sur les bio-accumulations minérales et puriques chez *Cylindroiulus londinensis* (Leach, 1914), (Diplopode, Iuloidea). *Archives de Zoologie Expérimentale et Générale*, vol. 119, pp. 669-683.

HUBERT, M., 1978b. Les cellules hépatiques de *Cylindroiulus londinensis* (Leach, 1814), (Diplopode, Iuloidea). *Comptes Rendus de l'Académie des Sciences, Série D*, vol. 286, pp. 627-630.

HUBERT, M., 1979a. Localization and identification of mineral elements and nitrogenous waste in Diplopoda. In: M. CAMATINI, ed. *Myriapod biology*. London: Academic Press, pp. 127-134.

HUBERT, M., 1979b. L'intestin moyen de *Cylindroiulus londinensis* Leach (psyllopygus Latzel) (Diplopoda, Iuloidea): observations ultrastructurales en relation avec la fonction d'accumulation. *Comptes Rendus de l'Académie des Sciences Série D*, vol. 289, pp. 749-752.

HUBERT, M., 1988. Le complexe anatomique et fonctionnel des cellules hépatiques-mesenteron de *Cylindroiulus londinensis* Leach (psyllopygus Latzel): étude ultrastructurale et spectrographique. *Bulletin de la Société Zoologique de France*, vol. 2, no. 113, pp. 191-198.

JUNQUEIRA, L.C.U. and JUNQUEIRA, M.M.S., 1983. *Técnicas básicas de citologia e histologia*. São Paulo: Editora Santos.

KANIA, G. and TRACZ, H., 2005. Mass occurrence and migration of *Ommatoiulus sabulosus* (Linnaeus, 1758) (Diplopoda, Julida: Julidae) in Poland. *Peckiana*, vol. 4, pp. 57-66.

KÖHLER, H.R., 2002. Localization of metals in cells of saprophagous soil arthropods (Isopoda, Diplopoda, Callembola).

- Microscopy Research and Technique*, vol. 56, no. 5, pp. 393-401. <http://dx.doi.org/10.1002/jemt.10039>. PMID:11877814.
- KÖHLER, H.R., KÖRTJE, K.H. and ALBERTI, G., 1995. Content absorption quantities and intracellular storage sites of heavy metals in Diplopoda (Arthropoda). *Biometals*, vol. 8, no. 1, pp. 37-46. <http://dx.doi.org/10.1007/BF00156156>.
- LISON, L., 1960. *Histochimie et cytochimie animales*. Paris: Gauthier Villans.
- MARTIN, J.S. and KIRKHAM, J.B., 1989. Dynamic role of microvilli in peritrophic membrane formation. *Tissue & Cell*, vol. 21, no. 4, pp. 627-638. [http://dx.doi.org/10.1016/0040-8166\(89\)90013-X](http://dx.doi.org/10.1016/0040-8166(89)90013-X). PMID:18620281.
- MELO, M.L.S. and VIDAL, B.C., 1980. *Práticas de biologia celular*. São Paulo: Edgard Blucher.
- MERLINI, V.V., NOGAROL, L.R., MARIN-MORALES, M.A. and FONTANETTI, C.S., 2012. Toxicity of trifluralin herbicide in a representative of the edaphic fauna: histopathology of the midgut of *Rhinocricus padbergi* (Diplopoda). *Microscopy Research and Technique*, vol. 75, no. 10, pp. 1361-1369. <http://dx.doi.org/10.1002/jemt.22075>. PMID:22623053.
- MOREIRA-DE-SOUSA, C. and FONTANETTI, C.S., 2012. Structure and function of the foregut and salivary glands of the synanthropic diplopod *Urostreptus atrobrunneus* (Spirostreptidae). *Animal Biology*, vol. 62, no. 4, pp. 493-504. <http://dx.doi.org/10.1163/157075612X650168>.
- NIJIMA, K. and SHINOHARA, K., 1988. Outbreaks of the *Parafontaria laminate* group (Diplopoda, Xystodesmidae). Japan. *Journal of Ecology*, vol. 38, pp. 257-268.
- NOGAROL, L.R. and FONTANETTI, C.S., 2010. Acute and subchronic exposure of diplopods to substrate containing sewage mud: Tissue responses of the midgut. *Micron (Oxford, England)*, vol. 41, no. 3, pp. 239-246. <http://dx.doi.org/10.1016/j.micron.2009.10.009>. PMID:20005118.
- NOGAROL, L.R. and FONTANETTI, C.S., 2011. Ultrastructural alterations in the midgut of diplopods after subchronic exposure to substrate containing sewage mud. *Water, Air, and Soil Pollution*, vol. 218, no. 1-4, pp. 539-547. <http://dx.doi.org/10.1007/s11270-010-0666-8>.
- NUNEZ, F.S. and CRAWFORD, C.S., 1977. Anatomy and histology of the alimentary tract of the desert millipede *Orthoporus ornatus* (Diplopoda: Spirostreptidae). *Journal of Morphology*, vol. 151, no. 1, pp. 121-130. <http://dx.doi.org/10.1002/jmor.1051510107>.
- PEARSE, A.T.E., 1985. *Histochemistry: theoretical and applied*. London: J&A Churchill.
- PEREZ, D.G. and FONTANETTI, C.S., 2011. Assessment of the toxic potential of sewage sludge in the midgut of the Diplopod *Rhinocricus pagbergi*. *Water, Air, and Soil Pollution*, vol. 128, no. 1-4, pp. 437-444. <http://dx.doi.org/10.1007/s11270-010-0657-9>.
- PIEROZZI, P.H.B. and FONTANETTI, C.S., 2006. A new species of *Urostreptus* (Diplopoda, Spirostreptidae): description and chromosome number. *Iheringia, Zoologia*, vol. 96, no. 2, pp. 209-212. <http://dx.doi.org/10.1590/S0073-47212006000200011>.
- SEIFERT, G. and ROSENBERG, J., 1977. Feinstruktur der leberzellen von *Oxidus gracilis* (C. L. Koch, 1847), (Diplopoda, Paradoxosomatidae). *Zoomorphology*, vol. 88, no. 2, pp. 145-162. <http://dx.doi.org/10.1007/BF01880651>.
- SOSINKA, A., ROST-ROSZKOWSKA, M.M., VILIMOVA, J., TAJOVSKÝ, K., KSZUK-JENDRYSIK, M., CHAJEC, L., SONAKOWSKA, L., KAMIŃSKA, K., HYRA, M. and POPRAWA, I., 2014. The ultrastructure of the midgut epithelium in millipedes (Myriapoda, Diplopoda). *Arthropod Structure & Development*, vol. 43, no. 5, pp. 477-492. <http://dx.doi.org/10.1016/j.asd.2014.06.005>. PMID:25038427.
- SUBRAMONIAM, T., 1972. Studies on the fat body of millipedes. I. Histological and histochemical features millipede *Spirostreptus asthenes* Pocock. *Zoologischer Anzeiger*, vol. 189, pp. 200-208.