

The fat body in *Rhinocricus padbergi* (Diplopoda, Spirobolida)

Carmem S. Fontanetti, Maria I. Camargo-Mathias & Bianca M. S. Tiritan

Departamento de Biologia, Instituto de Biociências, Universidade Estadual Paulista (UNESP), Av 24A, 1515, 13506-900 Rio Claro, SP, Brasil. (fontanet@rc.unesp.br)

ABSTRACT. The distribution and morphology of fat body of Brazilian diplopod *Rhinocricus padbergi* Verhoeff, 1938 are analyzed by scanning electron microscopy and histology. A terminology is proposed for description of the diplopods fat body.

KEYWORDS. Fat body, diplopod, *Rhinocricus*, terminology.

INTRODUCTION

The fat body of millipedes is a diffuse tissue that almost fills the body cavity and has as a primary function the storage of lipids, glycogen, proteins and uric acid, being a permanent site for the storage of wastes (HOPKIN & READ, 1992). According to HUBERT (1974, 1975, 1979), fat body cells present a variety of granules containing metal ions or potassium urate.

The fat body of *Spirostreptus asthenes* Pocock, 1892 is distributed in two regions: a peripheral portion attached to the epidermis and a central anastomosed mass surrounding organs and filling the corporal cavity, although the two portions may be continuous in different body parts. The central mass, termed visceral fat body, forms a continuous layer around the gut, containing cells with well-defined limits, spherical nucleus, and a cytoplasm filled with globules of different sizes, which have lipids, glycogen and proteins, suggesting that the chemical composition and the physiology of the fat body of *Spirostreptus asthenes* are similar to the ones found in insects (SUBRAMONIAM, 1972).

HUBERT (1974), studying the fat body of *Cylindroiulus teutonicus* Pocock, 1900, observed the same structure found in *S. asthenes*, concluding that there is only one type of cell in that tissue, the adipocytes. CAMARGO-MATHIAS & FONTANETTI (2000) demonstrated that the fat body of diplopods has, besides the adipocytes, a second cellular type similar to the oenocytes found and described in the fat body of some insects.

The aims of this study were to describe the morphology of the fat body of *Rhinocricus padbergi* Verhoeff, 1938 and to propose an appropriate nomenclature to be used for the description of the fat body of diplopods.

MATERIAL AND METHODS

Males and females of *Rhinocricus padbergi* were collected around the campus of UNESP, IB, Rio Claro, São Paulo, Brazil, from October 1998 to March 2000 by B.S.Tiritan.

For the scanning observation the material was fixed in Karnovsky, dehydrated in criptical point and sputter

coated with gold and photographed in scanning electronic microscopy (SEM). For the histological studies, fragments of fat body were routinely fixed with paraformaldehyde 4% or Carnoy I (ethanol 3: acetic acid 1), dehydrated in a graded series of 70-100% alcohol, and embedded with JB-4 Resin at 4° C in a dark bottle for 24 hs. Embedding of the material occurred first at 4° C to retard premature polymerization and was then completed at room temperature. Sectioning was carried out (5 µm) with a dry glass knife. The sections were collected and transferred to a room-temperature water bath before being placed on precleaned glass slides. They were air-dried before staining with Haematoxylin and Eosin (HE).

RESULTS AND DISCUSSION

The parietal fat body (fig. 1), located dorsally in the animal body, is easily separated from the viscera, however the fat body located in the ventral region can sometimes be confused with the perivisceral fat body by being continuous with the latter at many points. The perivisceral fat body surrounding the digestive tract (fig. 2) is found as a layer of cells weakly adhered to the pylorus (beginning of the hind gut) at the level where the Malpighian tubules are inserted; this layer is easily separated from the rest of the perivisceral fat body by being restricted to the pyloric region. The perivisceral fat body that surrounds the gonads (figs. 3, 4) also fills the body cavity, forming an irregular and anastomotic mass; a similar organization was observed in both males and females.

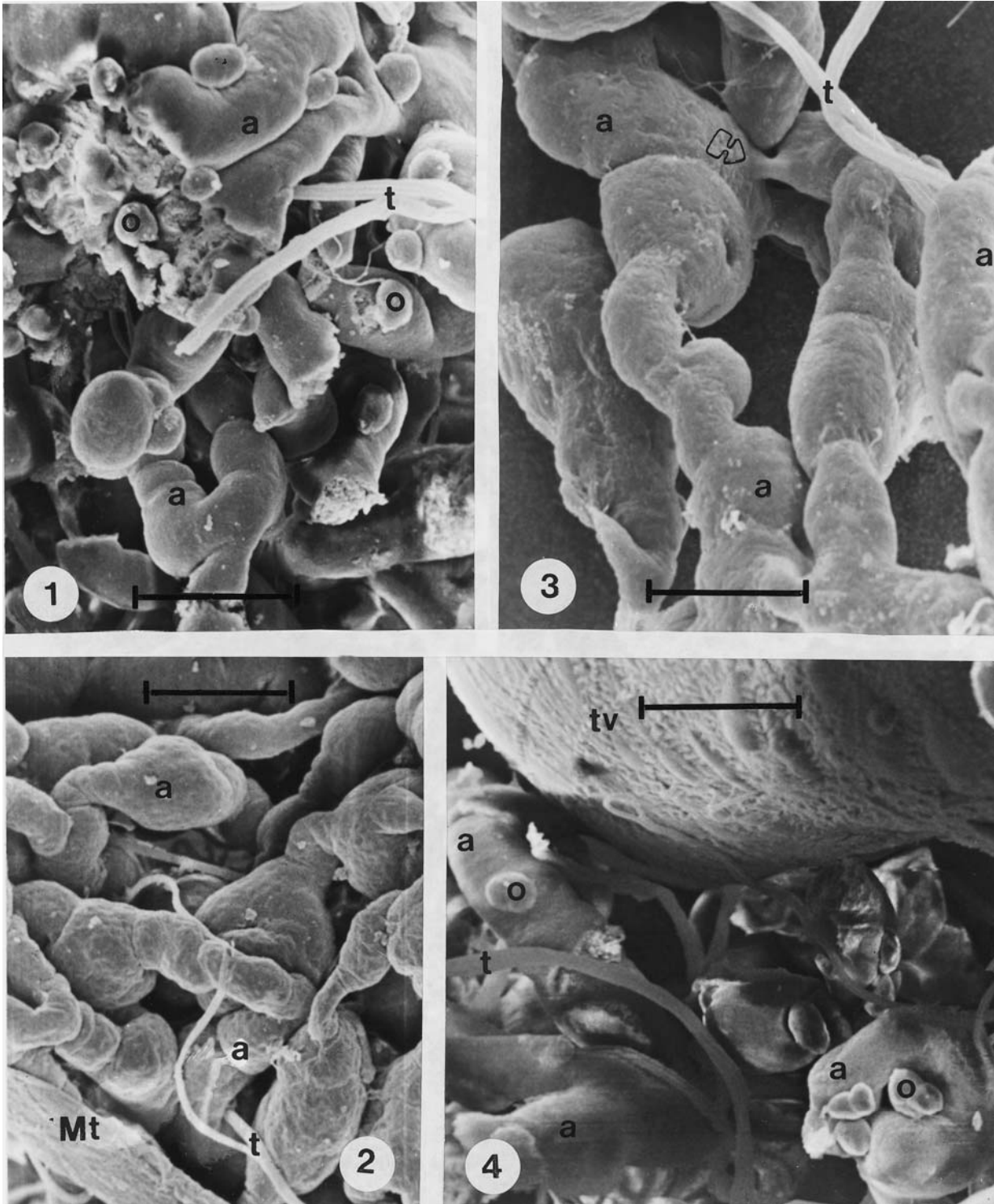
In general, the fat body has a whitish appearance, with cells disposed in rows, the adipocytes (figs. 1-4), where countless tracheas arrive, suggesting high metabolic activity. In association with adipocytes, smaller cells were observed with shape varying from rounded to elliptical, named oenocytes (figs. 1, 4); these cells are only absent from the fat body located around the digestive tract. The adipocytes in *R. padbergi* are large, with well-defined limits and a spherical nucleus located in different positions. In the majority of the cells the presence of nucleolus was observed. The cytoplasm shows great quantity of stored material, probably of variable composition, including a lot of vacuoles. This morphology

is observed in the perivisceral fat body in females, around ovaries (figs. 5-7), and also in males, around testes (figs. 8, 9). The perivisceral fat body observed at pylorus (figs. 10, 11) and the parietal fat body (fig. 12) show the same morphology.

Scanning electronic microscope images showed thin prolongations between the cells (arrow, fig. 3), but no

such connections were observed in histological analysis; this suggests that these structures may be part of a continuous membrane or a material of connective nature.

The cytoplasm of adipocytes presents a lot of spherules with concentric layers (*, figs. 7, 11, 13, 14) which are easily removed during material microtomy, resulting in empty sites (arrows, figs. 7, 13); these were



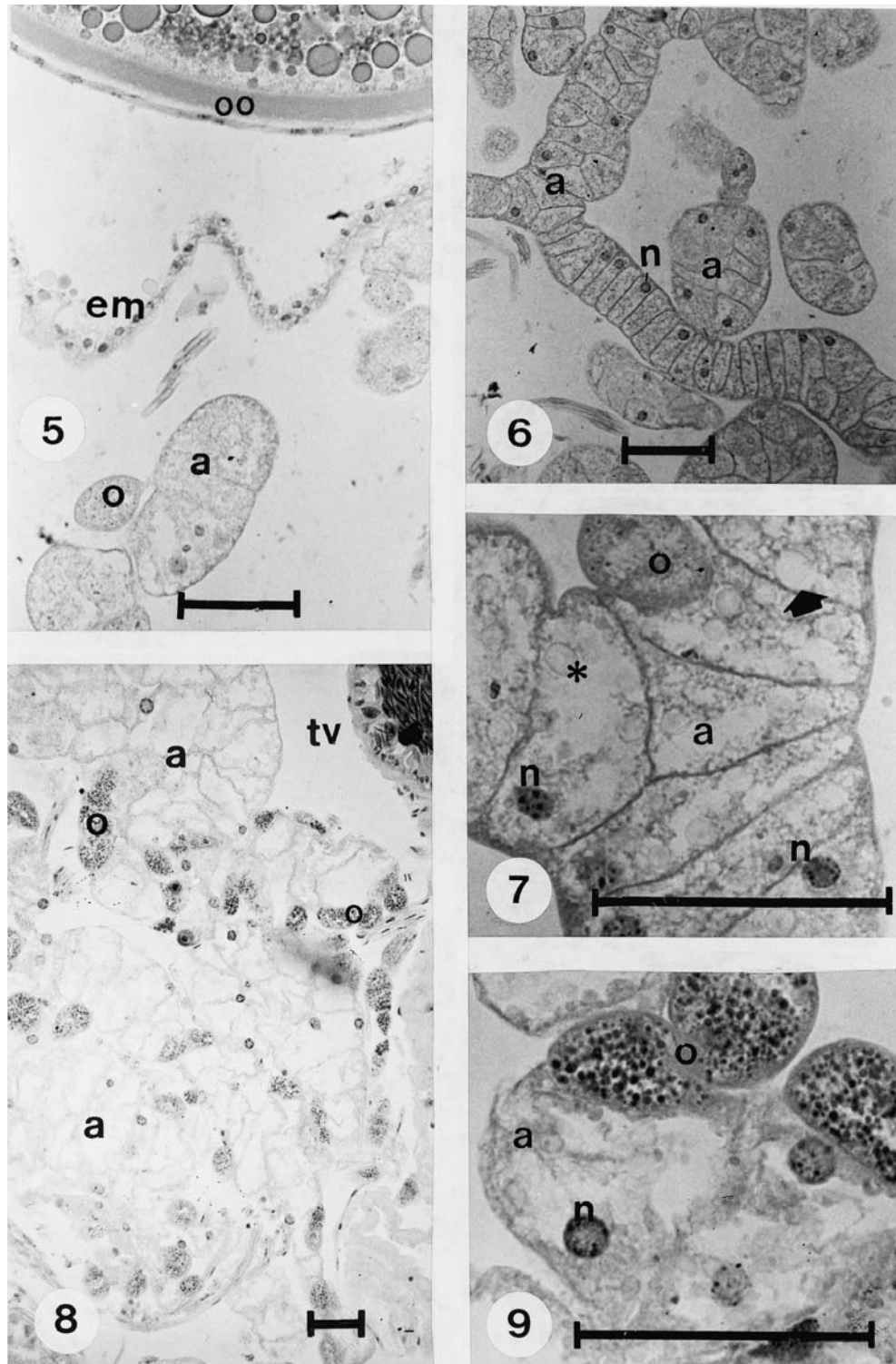
Figs. 1-4. *Rhinocricus padbergi* Verhoeff, 1938 fat body. 1, parietal; 2, perivisceral, around digestive tract; 3, perivisceral, around ovaries; 4, perivisceral, around testes (a, adipocytes; arrow, cellular expansion; Mt, Malpighian tube; o, oenocytes; t, tracheas; tv, testicular vesicle). Bars: figs. 1, 3, 4, 50 μ m; 2, 25 μ m.

probably inclusions or crystals of different composition, mainly calcium. The presence of minerals stored in cells of different tissues is common in diplopods, mainly in fat body (HUBERT, 1975; 1979).

The oenocytes show cytoplasm replete of granules of different staining (figs. 7, 9).

The oenocytes, commonly described in insects,

have ectodermal origin, according to LOCKE (1969), and possess their own ultrastructure, a natural cycle of specialization and function, according to their location. Morphological aspects indicate that the oenocytes have a high capacity of absorbing substances from the haemolymph, through invaginations of their cell surface membrane. The development of their smooth endoplasmic

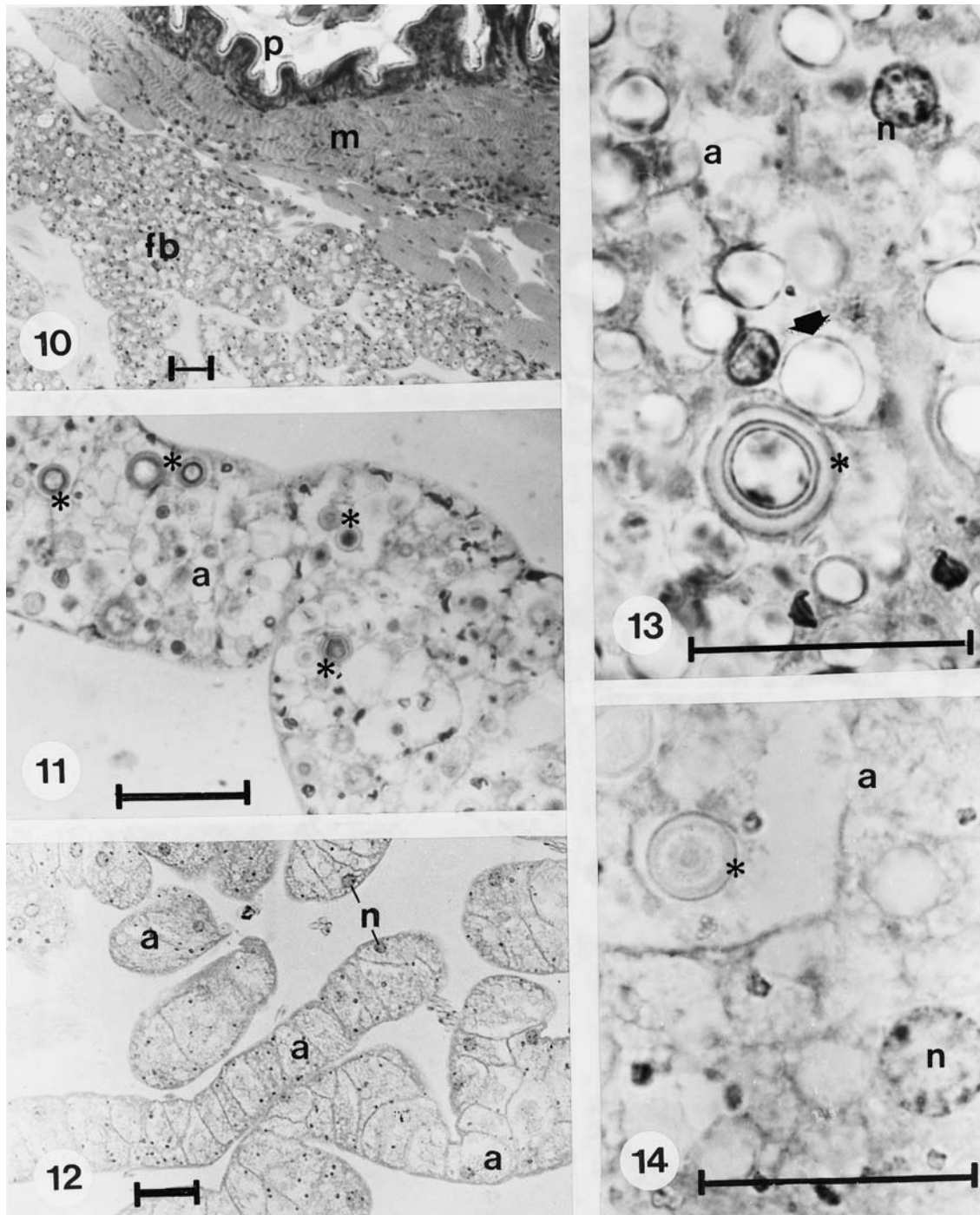


Figs. 5-9. Perivisceral fat body of *Rhinocricus padbergi* Verhoeff, 1938 located around the gonads (hematoxylin and eosin). 5-7, around the ovary; 8, 9, around testes (a, adipocytes; em, external membrane of ovary; n, nuclei; o, oenocytes; oo, oocyte; tv, testicular vesicle; arrow in 7, place where the circular structure was removed; *, circular structure). Bars: 25 μ m.

reticulum suggests that they are mainly involved with the metabolism of lipids. GEE (1911) cited a secretory function for these cells and POISSON (1924) reported a storage function, having observed fat spheres and glycogen granules in their cytoplasm. In *R. padbergi* the ultrastructural analysis suggested that the oenocytes are involved in the synthesis and storage of proteins, rather than lipids (CAMARGO-MATHIAS & FONTANETTI, 2000).

As in insects, the fat body of male and female *R. padbergi* is well-developed, especially when located

around the gonads. Due to this location and its high development, some authors have demonstrated the importance of this tissue in the production of vitellogenin that is incorporated in the oocytes and also produced in the males, although at a smaller scale (HAGEDORN & JUDSON, 1972; HAGEDORN *et al.*, 1973; NADARAJALINGAM & THANUMALAYA, 1984). The association of adipocytes with oenocytes suggests that the latter are involved in the production of elements that may possibly take part in the final processes of vitellogenesis, or even in the



Figs. 10-14. *Rhinocricus padbergi* Verhoeff, 1938 fat body. 10, 11, 13, perivisceral, around pylorus; 12, 14, parietal (a, adipocytes; arrow, place where the circular structure was removed; fb, fat body; p, pylorus; m, musculature; n, nuclei; *, circular structures). Bars: 25 μ m.

production of the chorion which involves the eggs of insects (THIELE & CAMARGO-MATHIAS, 1999).

Upon revising several studies on this tissue, many authors emphasized the similarity of the body of diplopods with that observed in insects (SUBRAMONIAM, 1972; HUBERT, 1974; CAMARGO-MATHIAS & FONTANETTI, 2000). TREGS & MANTON (1958) considered that the presence of the fat body in Insecta and in Myriapoda could be a character suggesting a phylogenetic proximity between the two groups.

The results obtained by us showed that the diplopod fat body is distributed in two areas: a peripheral layer that covers internally the whole body of the animal, adhered firmly to the tegument, for which we suggest the term parietal fat body, and a tissue that fills the cavities of the body and surrounds different organs, including the digestive tract, for which we suggest the term perivisceral fat body.

Due to the morphological and functional similarity of this tissue between representatives of Diplopoda and Insecta, it is suggested that the same terminology used for insects (CRUZ-LANDIM, 1975; DEAN *et al.*, 1985; THIELE & CAMARGO-MATHIAS, 2003) is adequate to describe the morphology and the location of the fat body in diplopods.

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