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Types of haemocytes in saprophagous soil mites (Acari: Oribatida, Acaridida), and the correlation between their presence and certain processes within mites

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Abstract. The microanatomy of several oribatid and one acaridid mite was studied to determine the role of free cells (haemocytes) in mites. Mites from the field as well as laboratory cultures were observed and analyzed histologically using Masson triple stain. The mites were offered various foods and kept in fluctuating moisture conditions. The presence of haemocytes was significantly correlated with the transport between internal organs of various substance. Three types of transport were recorded: (i) enzymes into the alimentary tract, including the incorporation of haemocytes into the gut walls. This process seemed to be correlated with the amount and type of food and frequently with the presence of internal extraintestinal bacteria associated with mesenchyma; (ii) metabolites, like guanine from mesenchyma into the alimentary tract followed by expulsion from the body via the gut. This process is correlated with food of high nitrogen content or dry conditions; (iii) resorption of nutrients from eggs during an induced quiescent state under unfavourable conditions by small haemocytes.

INTRODUCTION

For arachnids, including mites and ticks, several types of free cells have been reported in the body cavity (Opilionida: Romer & Gnatzy, 1981; Ixodida: Brinton & Burgdorfer, 1971; Balashov, 1979; Gamasida: Jakeman, 1961; Woodring & Galbraith, 1976; Acaridida: Kanungo, 1969; Woodring & Carter, 1974). The function of these free cells in Opilionida was discussed by Romer & Gnatzy (1981). These authors considered these cells to be oenocytes homologous with these of insects. Brinton & Burgdorfer (1971) recorded the transport of haemoglobin by haemocytes in Ixodida. Alberti & Coons (1999) and Alberti et al. (2003) studied the anatomy of the alimentary tract and fat body of oribatids, but did not record free cells.

The first description of the various morphological types of free cells in Oribatida was published by Smrž (1995). One type are the haemocytes. Observations on quiescent *Scutovertex minutus* (Smrž, 2002) revealed that haemocytes have a role during the drying and moistening of its environment.

This paper describes the correlations between the presence of haemocytes and digestion, excretion and reproductive processes in several non- related species of the soil saprophagous mites kept under various conditions.

MATERIAL AND METHODS

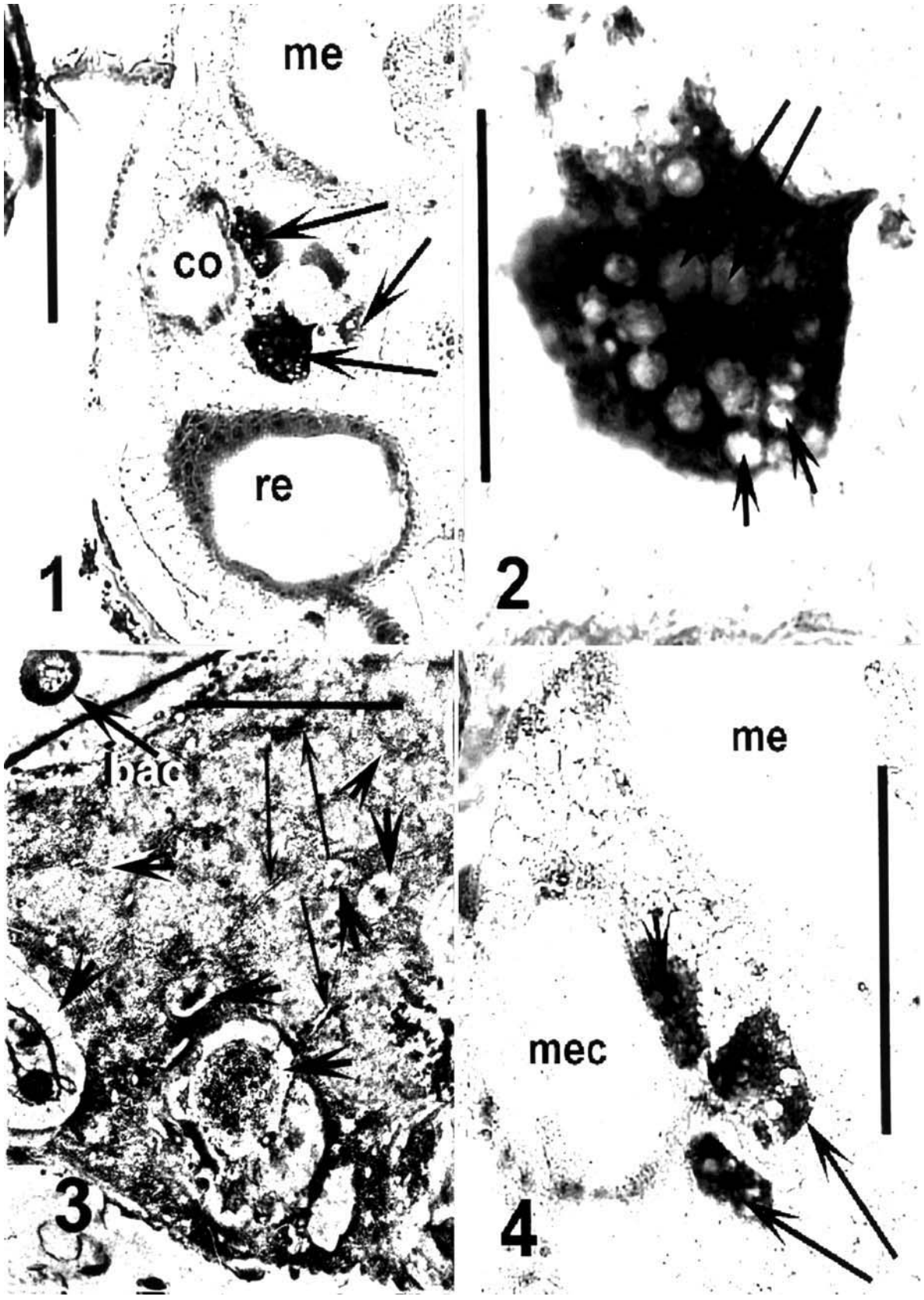
Several saprophagous mites [*Archegozetes longisetosus* Aoki, *Hermannia gibba* (C.L. Koch), *Scutovertex minutus* (C.L. Koch), *Tyrophagus putrescentiae* (Schrank)] were studied histologically and with TEM. *A. longisetosus* originated from a laboratory culture (see below). Samples containing the other mites were collected in the orchard in the village of Račice (near the city of Rakovník, Central Bohemia), extracted in Berlese-Tullgren funnels preserved in Bouin-Dubosque-Brasil modified for oribatids (Smrž, 1989), embedded in histoplast (Serva), sectioned on a MSE rotation microtome (section thickness 5000 nm), stained in Masson's triple stain and observed under a Provis AX 70 microscope (Olympus), and the microphotos edited by Microimage 3.0 image analysis (Olympus). The application of Nomarski DIC under the microscope and colour inversion of image in image analysis were very useful. For the histological studies, twenty specimens were of each species were sectioned.

For TEM, the mites were fixed in cacodylate-buffered glutaraldehyde (4%), postfixed in 1% osmium tetroxide, embedded in Spurr medium and sectioned using an Ultracut ultramicrotome (Reichert). Sections were stained in lead citrate and uranyl acetate and observed under a transmission electron microscope (TEM) Philips EM 300.

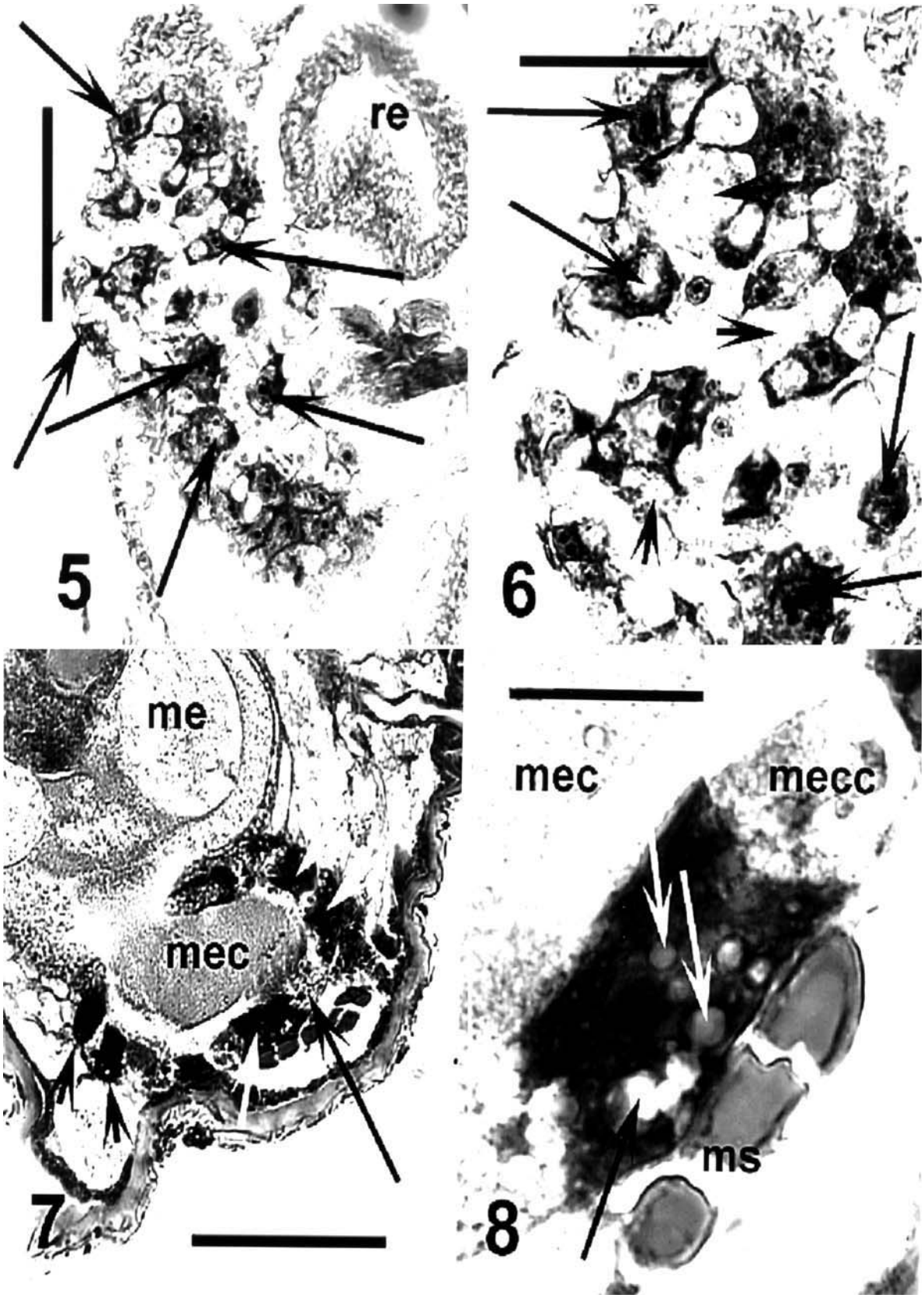
Hermannia gibba and *Scutovertex minutus* were collected from the field. The other mites (*Tyrophagus putrescentiae* and *Archegozetes longisetosus*) were placed in closed glass jars (220 cm^3) with a plaster of Paris/charcoal substrates to determine their food preferences. Several types of food were offered: the algae, *Protococcus* sp., from apple tree bark, fungi, *Stachybothrys* sp. and *Alternaria alternata*, from a fungal collection and filter paper. All these mites were kept at laboratory temperature (20–22°C) and humidity (approximately 50–60%, maintained by adding 10 ml of water per jar every third day for two weeks). The mites were removed after three or five days and placed in the histologicall fixative. as above mentioned. Furthermore, *Scutovertex minutus* was kept for three weeks in progressively dryer conditions until they became quiescent (see above, and Smrž, 1994), after which they were fixed in the same way.

RESULTS

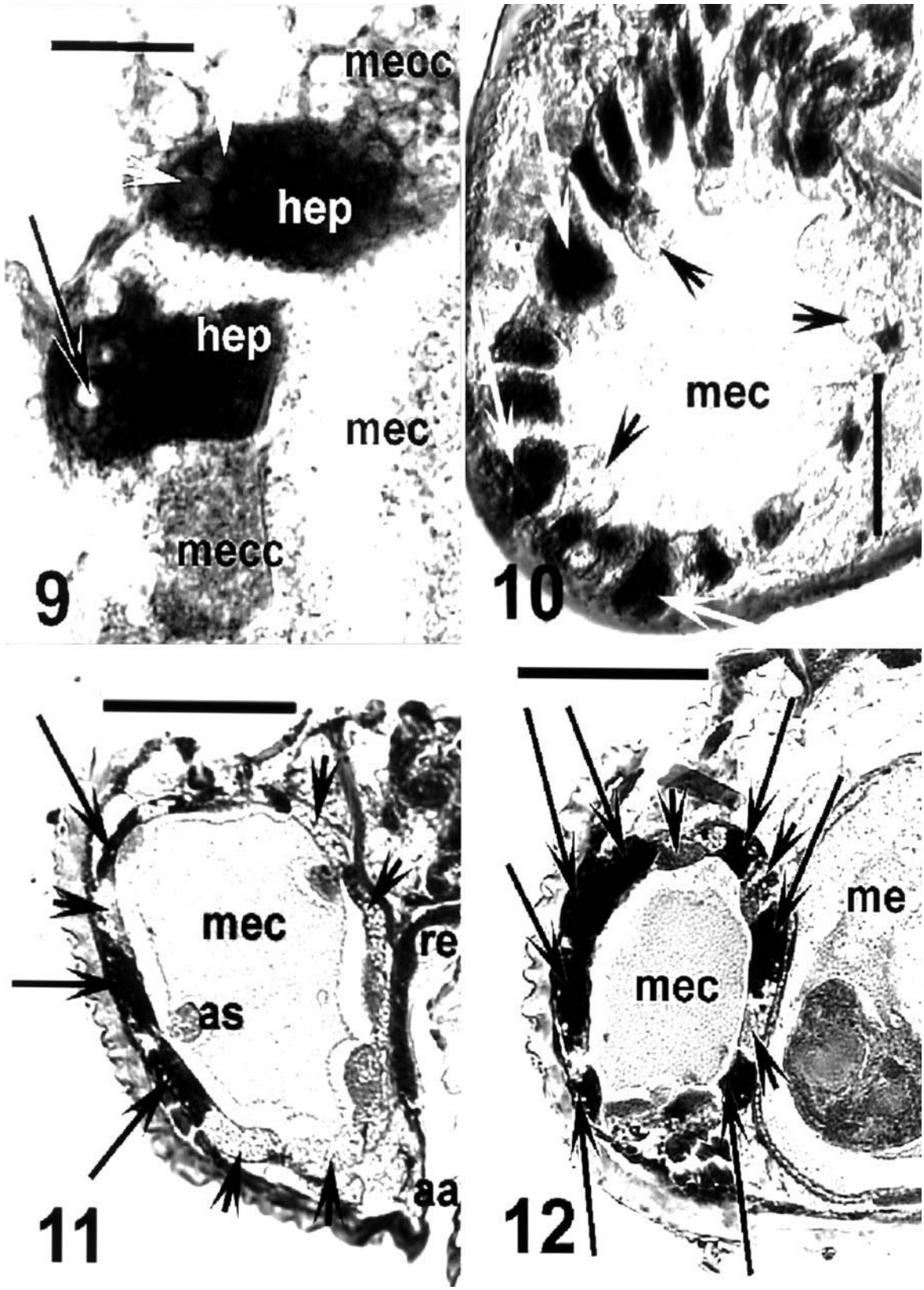
Generally, the haemocytes occurred primarily between the internal organs in the mesenchymal tissue in the opisthosoma. These cells were frequently large (up to 30 μm) and had conspicuous vacuoles (Figs 1, 2, 3). They contained substances of various kinds (of a bolus or fibrilar nature – Fig. 3). They were observed when the mites were fed on the cellulose rich food (filter paper) and algae, however, there were – only a few isolated cells. On the



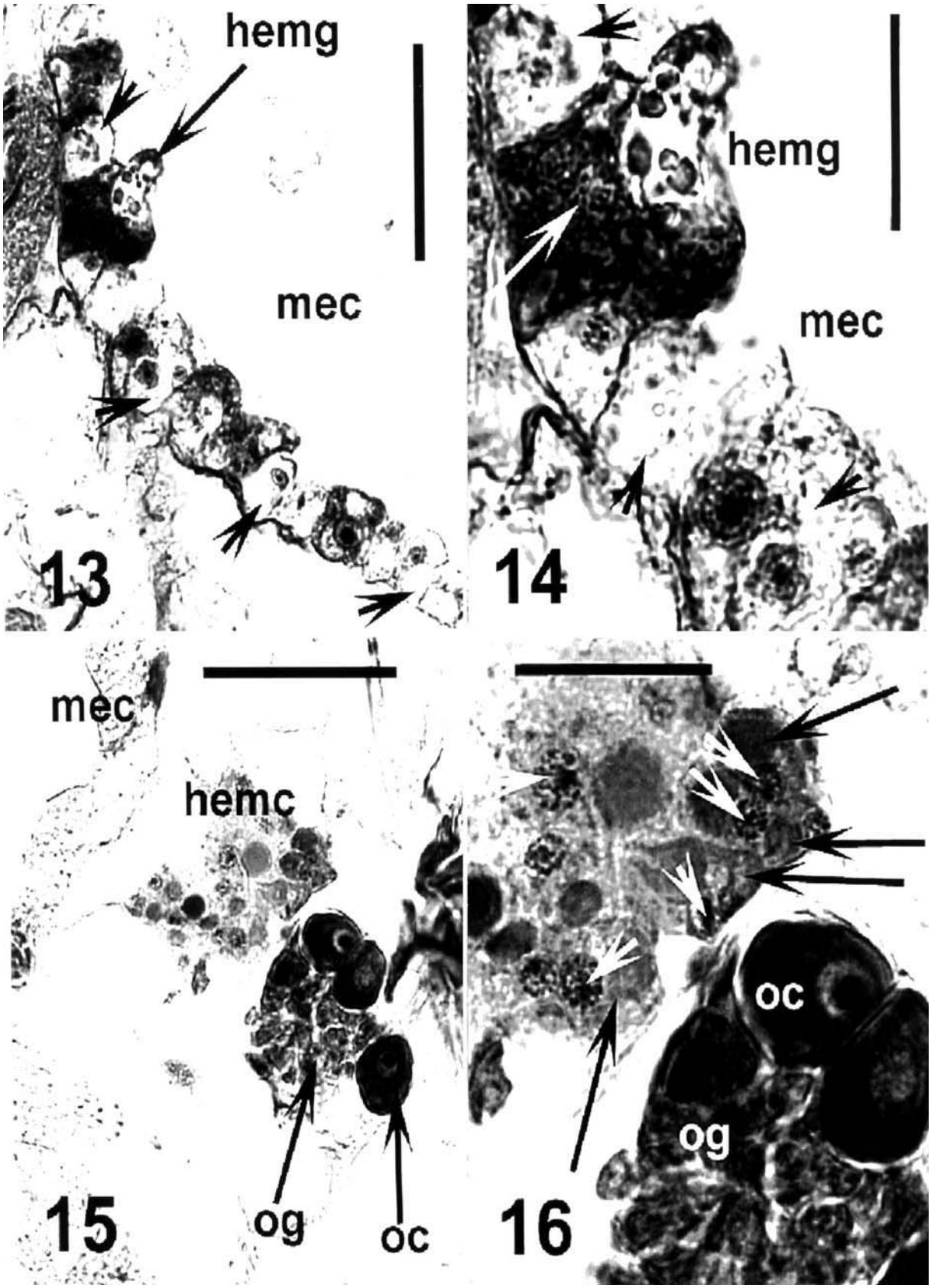
Figs 1–4: Haemocytes: *Hermannia gibba*: 1 – large haemocytes in the mesenchyma (arrows), sagittal section, 2 – details of one cell, arrows point to the hyaline content of the vacuoles, arrowheads to empty vacuoles. *Scutovertex minutus*: 3 – details of haemocyte with bacteria (TEM), black arrowheads point to vacuoles with various inclusions, black/white ones to cytoplasmic granulation, slim arrows point to endoplasmic reticulum. *Hermannia gibba*: 4 – haemocytes moving towards a mesenteral caecum, arrows point to the free cells, arrowhead to a haemocyte incorporating itself into the caecal wall, sagittal section. Masson's triple stain (1, 2, 4), TEM (3). Scales: 50 μm (1, 4), 20 μm (2), 5 μm (3). Abbreviations: bac – bacterial cell, co – colon, me – mesenteron, mec – mesenteral caeca, re – rectum.



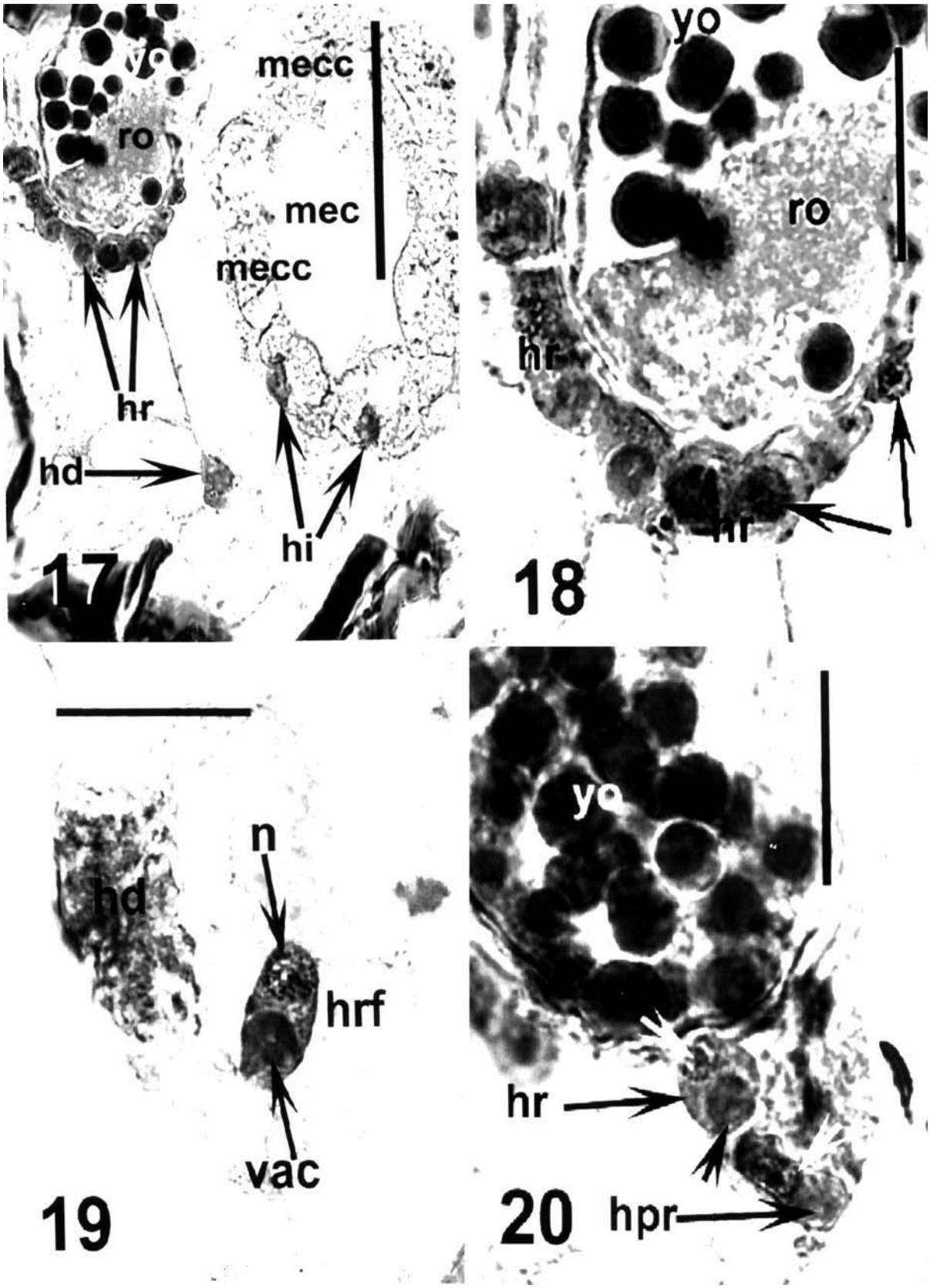
Figs 5–8: Haemocytes: *Archegozetes longisetosus*: 5 – haemocytes (arrows) adhering to the lateral mesenteral caeca, sagittal section, 6 – detail of the same figure, arrows point to the adhering haemocytes, arrowheads to the hyaline caecal cells, sagittal section. *Hermannia gibba*: 7 – mesenteral caecum with haemocytes, black arrow points to a caecal cell, shorter white arrow points to an incorporated haemocyte, black arrowheads point to the haemocytes just penetrating into the caecal wall, transversal section, 8 – haemocyte incorporated into the caecal wall, black arrow points to an empty vacuole, the white ones point to the hyaline content of vacuoles, sagittal section. Masson's triple stain. Scales: 60 μm (5, 7), 30 μm (6), 12 μm (8). Abbreviations: me – mesenteron, mec – mesenteral caeca, mecc – caecal cell, ms – muscle, re – rectum.



Figs 9–12: Haemocytes: *Hermannia gibba*: 9 – detail of Fig. 7, haemocytes penetrating into the caecal wall, black arrow points to an empty vacuole, white arrowhead to the hyaline content of haemocyte vacuole, transverse section. *Archegozetes longisetosus*: 10 – caecum showing intense apocrine secretion, white arrows point to the haemocytes, black arrowheads to the apocrine secretion, sagittal section. *Hermannia gibba*: 11 – the caudal part of caecum, transverse section, arrows point to incorporated haemocytes, arrowheads to caecal cells, sagittal section. 12 – the more anterior part of caecum, arrows point to incorporated haemocytes, arrowheads to caecal cells, sagittal section. Masson's triple stain. Scales: 60 μm (11, 12), 30 μm (10), 12 μm (9). Abbreviations: aa – anal atrium, as – apocrine secretion, hep – penetrating haemocyte, me – mesenteron, mec – mesenteral caecum, mecc – cell of caecal wall, re – rectum.



Figs 13–16: Haemocytes: *Archegozetes longisetosus*: 13 – incorporated haemocytes with guanine crystals (arrows), arrowheads point to caecal cells, 14 – details of the same figure, white arrow pointing to haemocyte, black arrowheads point caecal cells. *Scutovertex minutus*: 15 – resorption of eggs, 16 – details of the same figure, black arrows point to haemocytes in the cluster, white arrowheads point to their nuclei. All sagittal sections. Masson's triple stain. Scales: 30 μm (13, 15), 10 μm (14, 16). Abbreviations: hemc – cluster of haemocytes, hemg – haemocytes with guanine crystals, mec – mesenteral caeca, oc – oocytes, og – oogonia.



Figs 17–20: Resorption of eggs, *Scutovertex minutus*: 17 – haemocytes on surface of egg, 18 – detail of the same figure, arrows point to nuclei of haemocytes, bursting yolk granule in the center of figure, 19 – mesenchyma with haemocytes, 20 – show the close contact of the haemocytes with egg chorion, white arrowhead points to the nucleus, black arrowhead to the intracellular inclusions. All sagittal sections. Masson's triple stain. Scales: 30 μm (17), 12 μm (18 – 20). Abbreviations: hd – destroyed free haemocyte, hi – the small haemocytes leaving the ovary and being incorporated into the caecal wall, hrf – free haemocyte with intracellular inclusion passing through the mesenchyma tissue , hr – haemocyte closely adhering to egg chorion, hpr – haemocyte before close contact with egg chorion, n – nucleus, ro – remains of yolk, vac – intracellular inclusions, yo – yolk.

other hand, the presence of fungal fragments in the alimentary tract seemed to be correlated with an increase of in the number of haemocytes. Moreover, this type of food also induced an increase in the bacterial populations within the mesenchyma. In some cases, contact between haemocytes and bacteria were observed (Fig. 3).

These free cells were associated with the alimentary tract. They occurred near the anterior part of the mesenteron. More frequently, they adhered to the the walls of the mesenteral caeca (Figs 1, 4, 5, 6). Moreover, they were able to penetrate into the walls of the caeca and become incorporated between the walls of the caecal cells (Figs 7–9). Some vacuoles contained of the inclusions of a hyaline or even crystalline nature (Figs 2, 3, 4, 13, 14). Apocrine secretion by caecal cells usualy indicates digestion. The presence of haemocytes in caecal walls, however, was correlated with an increase in apocrine secretion in the caeca (Fig. 10). The most intensive secretion occurred in the cells closely adjoining those that incorporated haemocytes. In the grazing and digesting mites, these incorporated haemocytes also exhibited apocrine secretion (Fig. 10). The above mentioned adherence and incorporation of haemocytes occured only on the external lateral side of the mesenteral caeca (Fig. 11). The axial part of the caeca (adjacent to the alimentary canal – colon and rectum) consisted only of caecal cells without haemocytes. This pattern occured especially in the caudal part of the caeca (Fig. 11), whereas haemocytes were incorporated anteriorly into the whole caecal wall (Fig. 12).

The other correlation was recorded for *Scutovertex minutus* kept in dry conditons. The haemocyte vacuoles contained crystalline particles, which exhibited the usual structure of guanine. These haemocytes were smaller than those recorded previously (up to 15 μm), but had relatively voluminous vacuoles compared to the whole cell. These cells also occured in the mesenteral caeca (Figs 13, 14). The crystals accumulated in the mesenteral and caecal walls and were finally expelled into the alimentary canal. However, this expulsion proceeded only under more moist conditions.

The third, very different type of haemocyte occurred when conditions became extremely unfavourable – dry or progressive decrease in the amount of food. The females characteristically resorbed their eggs under these conditions. This type of haemocyte, however, was very small compared to the above mentioned cells (5 μm) (Figs 17–20). They had a high nucleus/cytoplasm ratio and contained unstructured spherical inclusions (Fig. 19). These haemocytes accumulated in the voluminous bodies in the mesenchyma near the ovaries (Figs 15, 16) and subsequently a single layer adhered to the egg chorion (Figs 17, 18). They penetrated the chorion (Fig. 20) and resorbed the yolk, which was accompanied by the bursting of the yolk granules (Fig. 18). This was followed by transport of the material through the body cavity from the ovaries into the alimentary tract (Fig. 19).

DISCUSSION

The morphology of the haemocytes appeared to be more diverse in their general characteristics (size, vacuoles) than indicated when first descried by Smrž (1995). This diversity is correlated with the various processes occurring in mites. The first type of haemocyte (large, with a number of voluminous vacuoles) is associated with the alimentary tract, with the number correlated with the amount and type of food. The changes in the microanatomy of the alimentary tract associated with different types of food are reported in several papers (Smrž, 1992a, 1996; Smrž & Čatská, 1989; Smrž & Trelová, 1995). Finally, their adherence and penetration into the mesenteral caeca and induction of apocrine secretion into caecal lumen seem to support a messenger role for haemocytes (Brinton & Burgdorfer, 1971). In oribatid species, they probably transport substances. The simultaneous presence of high populations of associated bacteria with the chininase activity (Smrž, 1996, 2000, 2001, 2002; Smrž & Trelová, 1995) in mesenchyma (similar to mycetome-like bodies – cf. Wigglesworth, 1974) and their mutual contacts suggest such an interpretation.

The presence of guanine crystals in the other types of haemocytes and their transport of this material into the gut, support the excretory function of this type of haemocytes (excretion see Smrž, 1994, 1995, 1996, 2001, 2002, 2003, Smrž & Norton, 2004).

The role of haemocytes in egg resorption is confirmed by the observation of this process, including the destruction of eggs (burst yolk granules, degenerative changes in egg). This process was described by Smrž (2003) in *Scutovertex minutus*, a very hardy oribatid mite that occurs in the moss on roofs and rocks. The specific morphology of this type of haemocyte confirms the different nature of this process compared to their role in digestion and excretion.

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