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Microanatomical and microbiological characteristics of the quiescent state of *Scutovertex minutus* (Acari: Oribatida)

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Abstract. Both adults and juveniles of the oribatid mite *Scutovertex minutus* (Scutoverticidae) may enter an immobile quiescent state under extreme dry conditions. The microanatomy of the alimentary tract, contents of parenchyma tissue and internal extraintestinal microbial communities were observed in these states. The quiescent state lasted at least 10 days and was generally characterized by an empty gut, guanine deposition and, in adults, by the resorption of spermatids or oocytes and eggs. The homogenate of mites was sterile, without microorganisms. The reverse processes were recorded two hours after remoistening: the mites started to move again and accompanying histological changes were shown.

Introduction

Dormancy in mites can be triggered by several factors. A regular dormant state occurs in the ontogenetical development of the cohort Uropodina (Acari: Gamasida) and in some Acaridida (Krantz 1978; Evans 1992). In the former group this state seems to apply to a regular developmental stage (Krantz 1978). In the latter, the progressive diminishing of food resources appears to be one of the triggers for its occurrence (Volgin 1973). These stages are non-feeding and frequently mouthparts are lacking. Internal changes during this state have been studied by Woodring and Carter (1974) in the species *Caloglyphus boharti* Cross. Premoulting resting stages occur in the ontogenetical development of mites including oribatids as described by Woodring and Cook (1962). They formulated the modern anatomical terminology of oribatids as well. Dormant states of adults have been recorded in antarctic invertebrates including oribatids (e.g. *Alaskozetes antarcticus* (Michael)) under extremely low temperatures (Block 1980). The influence of humidity has been studied in oribatids or soil animals in general by Madge (1961, 1964a, 1964b) and Vannier (1978).

Scutovertex minutus (C.L. Koch) is able to survive severe abiotic conditions for some time (Smrž 1994), especially very low moisture (about 5% of substrate – gravimetric estimation) in its habitat (moss covers on rocks or buildings). Smrž (1994) described the various responses of this species on several substrates, types

of potential food and under different moisture regimes from drying to flooding. In addition to behavioural responses (consumption of food, migration), one can imagine internal, microanatomical changes when conditions become arid. The internal anatomy has been described in the general textbooks (Krantz 1978; Evans 1992; Alberti and Coons 1999) and in studies of oribatid species (Smrž (1989, 1992a, 1995)).

This paper describes in detail the changes in microanatomy, contents of the alimentary tract and reproductive organs, internal microflora and enzyme activity in *S. minutus* during strong dessication as well as the reverse processes after re-moistening.

Materials and methods

Scutovertex minutus has been cultured in our laboratory for several years. For this experiment, mites were placed in glass jars (220 cm^3) with a plaster of Paris/charcoal substrate (group of 30 tritonymphs and 30 adults per jar) under two moisture regimes: 1. moistening every third day (10 drops of water), or 2. no moistening for three weeks. After three weeks, 10 adults and 10 nymphs of each group were sampled and fixed for histology and the others (again 10 + 10) were homogenized. After ten days under the second moisture regime ("drought"), 10 adults and 10 nymphs were re-moistened (50 drops of water per jar). The mites began to move after two hours. Then one half (5 + 5) were fixed and the others (5 + 5) were homogenized. All experiments were performed at laboratory temperature (20–22 °C) and repeated three times.

Two types of analysis were performed:

1. the sampled mites were fixed in Bouin-Dubosque-Brasil modified for oribatids (Smrž 1989), sectioned on an MSE rotary microtome (section thickness 5000 nm) and stained in Masson's triple stain. They were observed with a Provis AX 70 microscope (Olympus) and images edited with the Microimage 3.0 image analysis (Olympus). The application of Nomarski DIC in microscope and colour inversion of images was very useful.
2. the homogenized mites were tested for chitinase and cellulase activity and presence of internal bacteria by plating portions of homogenate on malt agar (for fungi) and MP agar (for bacteria); both agars with pH=7, temperature of plating 25 °C (for details of methods see Smrž et al. (1991) and Smrž (2000)). All phenomena reported occurred in all specimens in each group; mites were quite synchronized in their responses under moistening, drying, as well as during re-moistening after the drying.

Results

The behavioural response of adults and nymphs to drying (second moisture regime) was the same – after five days without moistening they became immobile without any response to touch. They survived for at least ten days in this state. In this dormant state, the walls in the anterior part of the mesenteron thickened due to vacuolization (Figure 1). These vacuoles were filled with guanine crystals (Figures 2–4). In the posterior part, the mesenteral walls were thin (Figure 1). Food boli, if any, were very small, without any regular structure, rather of a mucoid nature (Figures 1–2). Activity in the walls of the mesenteral caeca was low, although some secretions occurred. The caecal cells contained guanine crystals as well (Figure 5). Only a few hemocytes were incorporated within the caecal walls, and these hemocytes were small. Faecal pellets were also very small, smooth and homogeneous, without any visible internal structure (Figures 6, 7). No chitinase or cellulase activity was found. The homogenates of mites were sterile, as no bacteria or micromycetes were plated from them.

The cavities in the parenchyma tissue contained guanine crystals (Figure 8), but only few. Opisthosomal glands in nymphs were small, dark and slightly visible in intact specimens. They possesed only a crevice-like lumen and thick walls.

In adult males, testes contained fewer than normal cysts with spermatogonia, spermatocytes and spermatids (cf. the moistened group). There were conspicuous gaps between cysts (Figures 9, 10). Spermatids and spermatozoa in the vesicula seminalis were very scarce (Figures 11–13) and caverns or gaps occurred in vesiculae as well. The female ovaria contained oogonia and oocytes attacked by hemocytes (Figure 14). Resorption of reproductive cells occurred in all females in this group. At first, the hemocytes formed some clusters near the ovaria (Figure 15), then a layer of hemocytes coated the chorion (Figure 16). These hemocytes were small and their shape was somewhat different from hemocytes in the mesenteral caeca (cf. Figure 5).

The active mites from the control, moistened group (i.e. the first moisture regime) exhibited many differences from dormant mites: their mesenteral walls were thick, vacuolized and they contained the dark granulation of enzymes. Food boli contained particles as well as some fungal propagula (spores, hyphae). Secretion in the mesenteral caeca was intensive, many hemocytes were incorporated in their walls. The faecal pellets were similar to food boli in structure, but they were more compressed. Cavities in parenchymal tissue were filled by glycogen deposits, especially near the alimentary tract. Some of these cavities contained groups of bacterial cells (mycetome-like bodies sensu Smrž (1995)). Opisthosomal glands in nymphs were bright red, they possessed a voluminous lumen and their walls were relatively thin. In adult males, testes were filled with cysts without gaps and the densities of spermatids and spermatozoa in the vesicula seminalis were much higher in comparison to the dormant specimens. In females, no resorption of eggs and no small types of hemocytes were observed. The plated homogenates of mites yielded a mixture of bacterial colonies and a few colonies of sterile mycelia. They were not

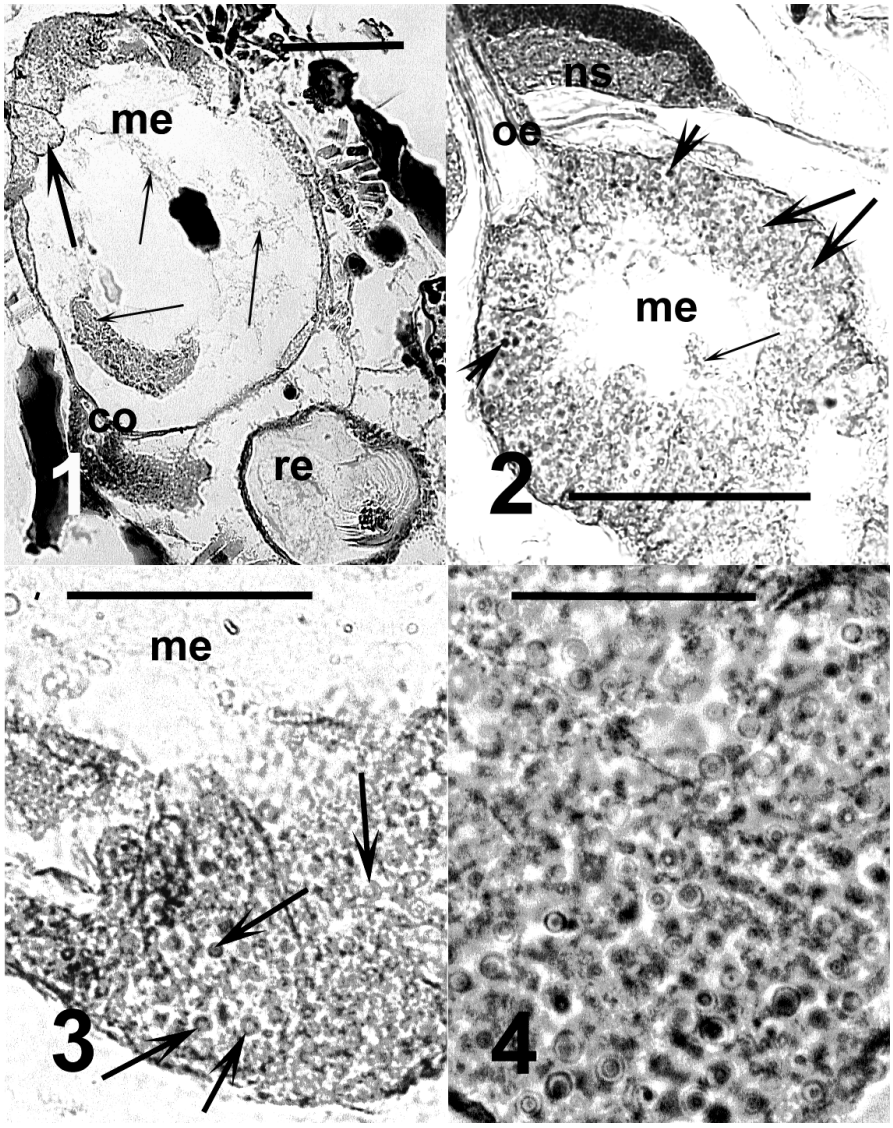

Figure 1. *Scutovertex minutus*, adults, dormant state, sagittal sections: 1 – alimentary tract, arrow points to the proliferation of the mesenteral cells, small arrows point to mucoid food boli of the various structurre, 2 – anterior part of mesenteron, arrows point to guanine crystals, arrowheads point to enzyme granules, small arrows point to mucoid substance, 3 – part of mesenteral wall with guanine crystals – arrows, 4 – detail of the same figure with conspicuous guanine crystals of concentric structure. Masson's triple stain. Scales: 20 μm (1,2), 10 μm (3), 5 μm (4). Abbreviations used: co – colon, me – mesenteron, ns – central nervous system – synganglion, oe – oesophagus, re – rectum.

identified, as the aim was only the comparison with the sterile plates from dormant states.

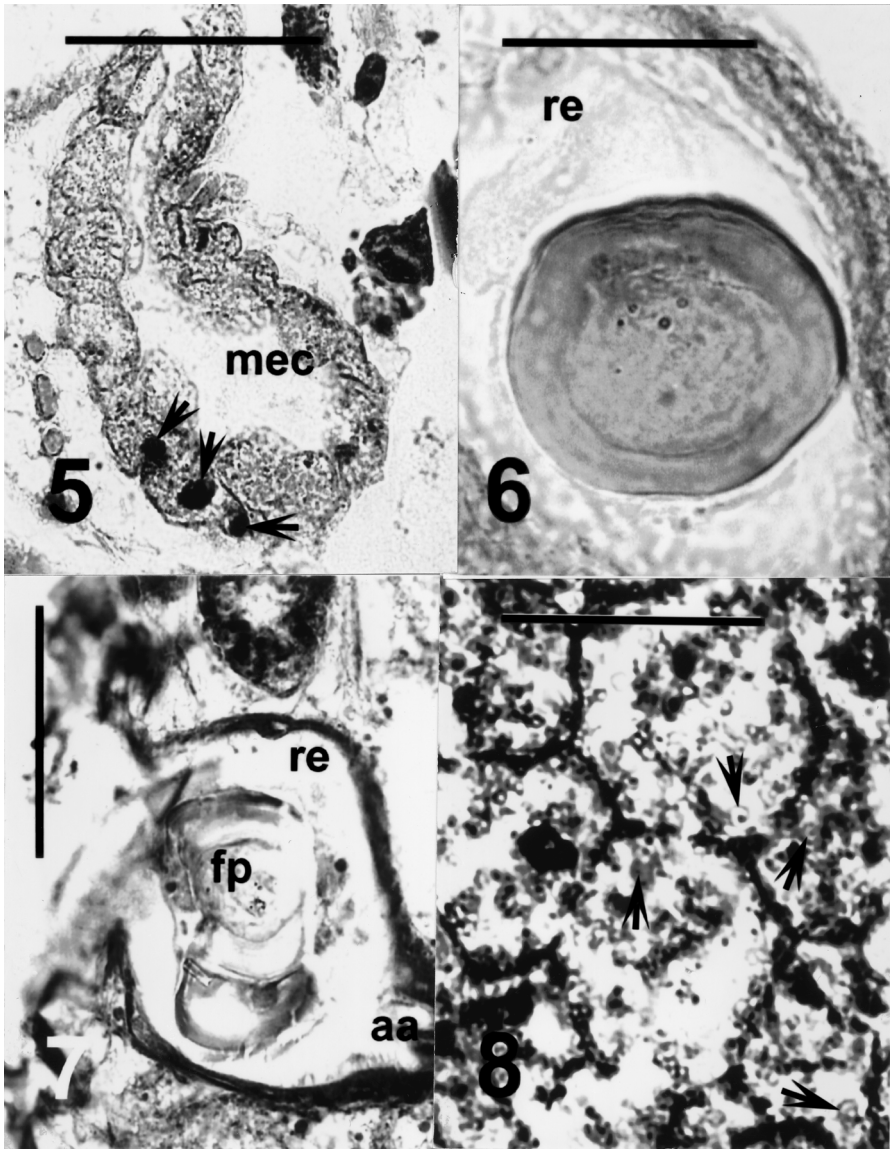

Figure 2. Scutovertex minutus, adults, dormant state, sagittal sections: 5 – mesenteral caecum, arrowheads point to hemocytes, 6 – faecal pellet in rectum, 7 – rectum of another specimen, 8 – parenchyma tissue, arrowheads point to guanine crystals.
Masson's triple stain. Scales: 20 μm (5), 10 μm (6,7), 5 μm (8). Abbreviations used: aa – anal atrium, fp – faecal pellet, mec – lumen of the mesenteral caecum, re – rectum.

Re-moistening resulted in the moving of mites after two hours. Microanatomically, the gradual return into the "active state" was compared to the microanatomical and microbiological characteristics in the "active state" of mites in the moist

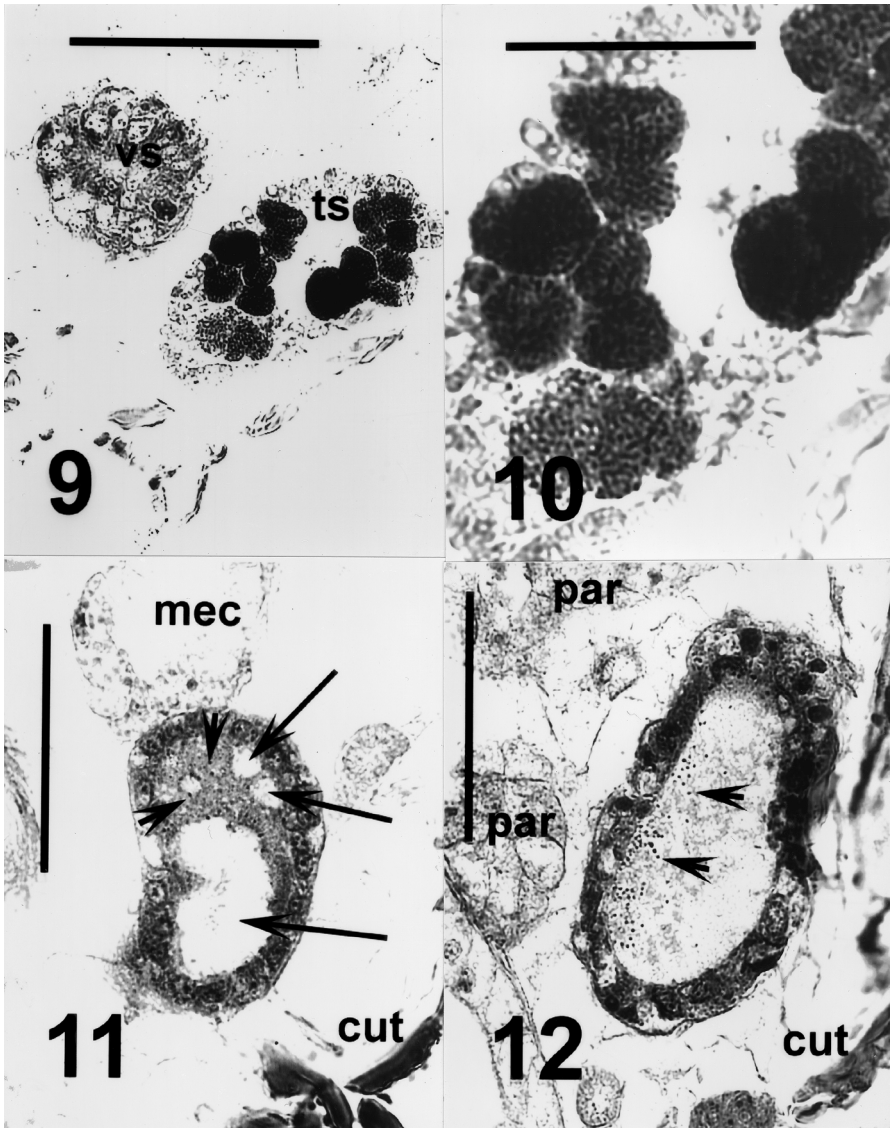

Figure 3. Scutovertex minutus, adults, dormant state, horizontal sections: 9 – male, reproductive system, 10 – detail of the same figure, testis with bundles (cysts) of male cells, 11 – the vesicula seminalis, arrows point to the gaps, arrowheads point to the rest of spermatids or spermatozoans, 12 – the vesicula seminalis of another male, arrowheads point to spermatids.
Masson's triple stain. Scales: 20 μm (9), 10 μm (11,12), 5 μm (10). Abbreviations used: cut – cuticle, mec – mesenteral caecum, par – parenchyma tissue, ts – testis.

conditions (first moisture regime). The guanine crystals were gradually expelled via apocrine secretion from the mesenteral walls (Figures 17–18). Their bundles were

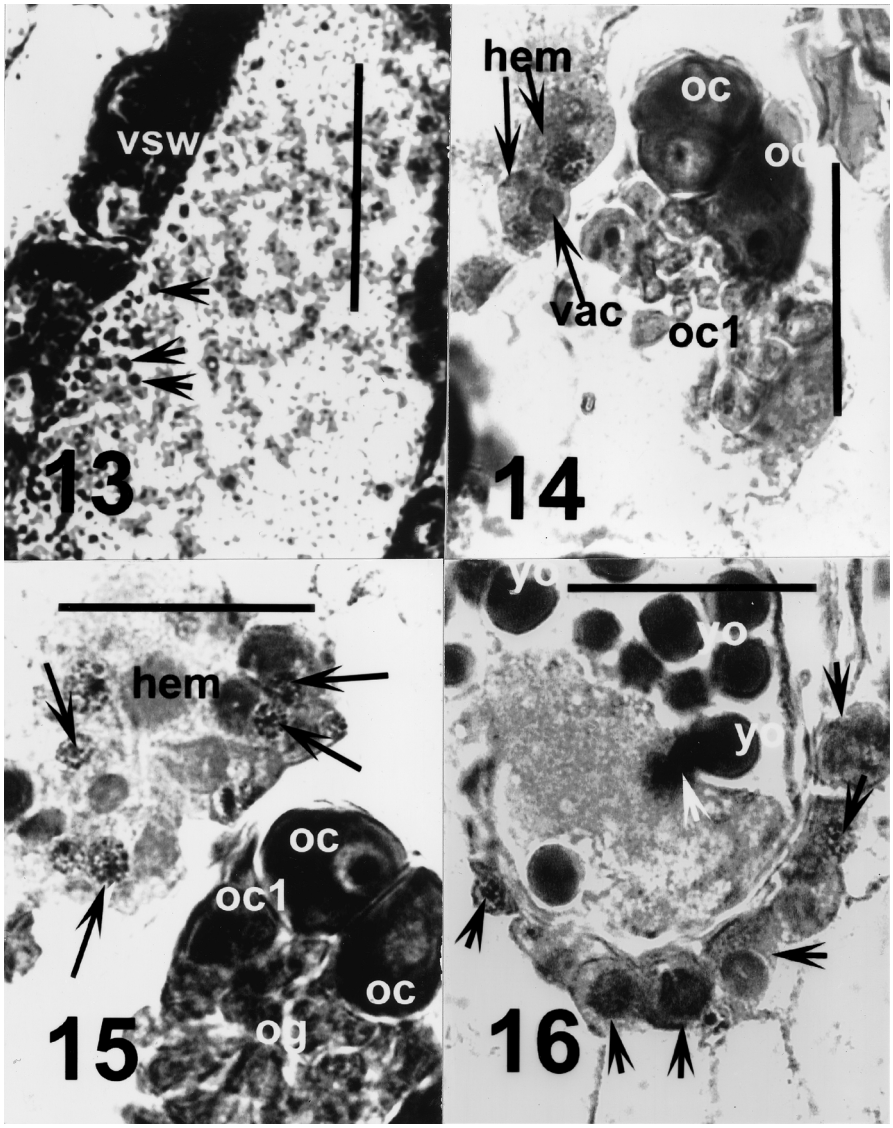

Figure 4. Scutovertex minutus, adults, dormant state, horizontal sections: 13 – male, vesicula seminalis, a detail of Figure 12, arrowheads point to spermatids and spermatozoa, 14 – female, ovarium with two hemocytes, 15 – another female, ovarium with bundle of hemocytes, arrows point to the hemocyte nuclei, 16 – another female, ovarium, egg resorbed by the hemocyte cover (black arrowheads), white arrowhead points to the disturbed yolk granule.
Masson's triple stain. Scales: 10 μm (14, 15) 5 μm (13, 16). Abbreviations used: hem – hemocyte, oc – highly developed oocyte (2.oocyte), oc1 – 1.oocyte, og – oogonia, vac – vacuole in hemocyte, vsw – wall of vesicula seminalis, yo – yolk granule.

found in food boli in the mesenteron (Figure 19) and in faecal pellets in the rectum (Figure 20) during this process.

Discussion

Scutovertex minutus is very resistant to the drying of its environment (Smrž (1992a, 1994)). In our experiments, immobile dormant states were formed only in this oribatid. Other tested species have died under the long-term dry conditions over several days (Smrž (1992a, 1992b, 1994, 1996)). *S. minutus* exhibited drought resistance in juvenile as well as adult stages. Although its juveniles (nymphae plisée sensu Grandjean (1953)) are poorly sclerotized in comparison to adults, they survive nearly the same extent of drought as adults. This has been explained by their physiological adaptations (guanine deposition, opisthosomal gland function; Smrž (1994)). The immobile dormant state appears dead, but is readily reversible up to ten days after onset. Survival was variable; all mites survived 10 days of the experiment, but some survived as much as 20 days. Since these data relate to conditions on the base floor of the experimental jars, survival may be longer when mites are within their natural moss substrate.

The empty gut and reduced enzyme secretion in mesenteron and caeca appear to be logical phenomena in dormant states. With respect to the role of associated internal bacteria, the absence of the mycetome-like bodies in the parenchyma tissue corresponds with the general diminishing of metabolism (Smrž 1995). The interesting sterility of mite homogenate may be associated with this decrease of metabolism as well (cf. Smrž (1996)). The gut was empty, hence the number of colony forming units was nearly zero in the gut. Other bacteria usually occurring in parenchyma tissue have been associated with digestion as well (Smrž et al. 1991; Smrž and Trelová 1995; Smrž (1996, 1998)). Therefore, they were lacking in this type of dormant state. Guanine deposition resulted from moisture decrease as noted in previous papers (Smrž (1992a, 1994, 1996, 1998)).

The gradually decreasing water content of the environment and consequently of the mite body probably induced resorption of energy-rich substances like glycogen, but mainly resorbed were reproductive cells in males and females. The substantial role of hemocytes and their morphological and functional variability were confirmed (cf. Smrž (1995)). Physiological processes were readily reversed after remoistening, which results in the return of mobility and activity.

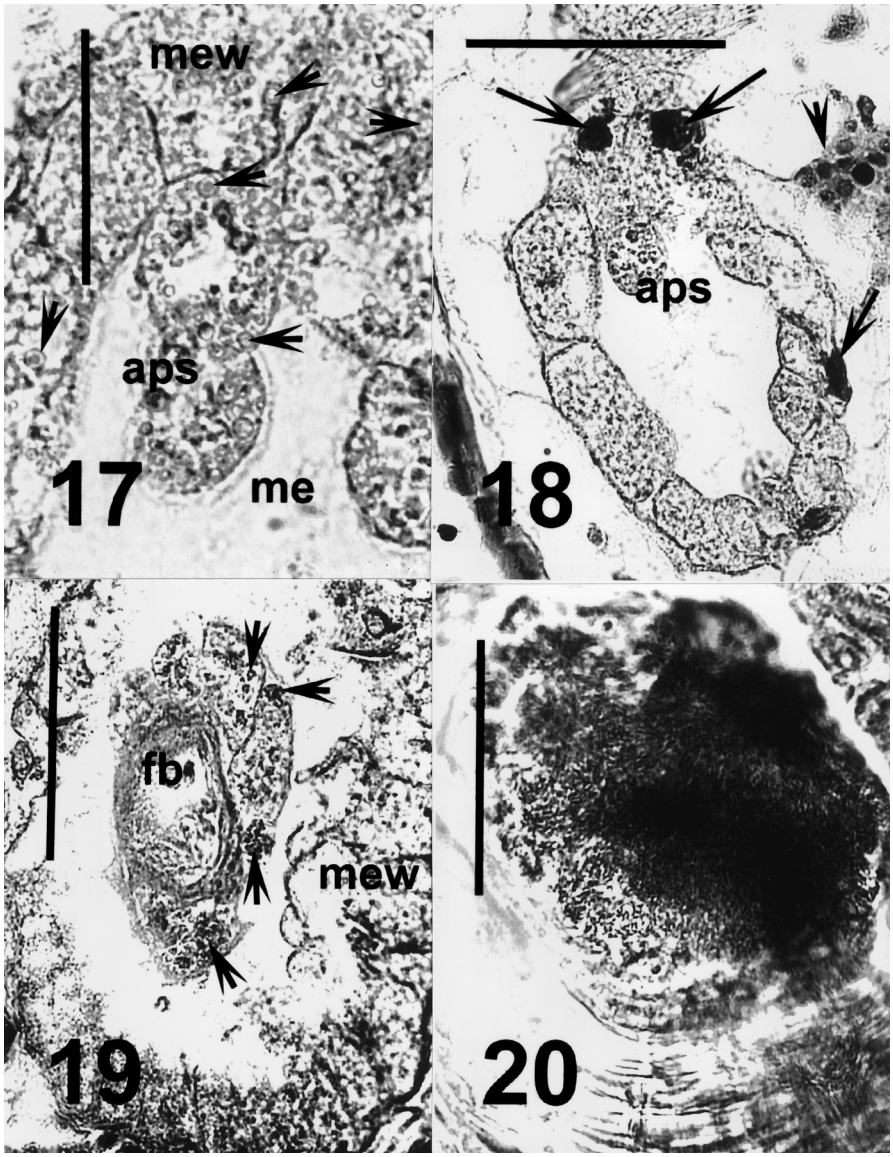

Figure 5. Scutovertex minutus, adults under re-moistening, sagittal sections: 17 – mesenteron, arrowheads point to guanine crystals expelled from the wall by apocrine secretion, 18 – mesenteral caecum, arrowhead points to the remaining hemocyte bundle, arrows point to hemocytes incorporated into the caecal wall, 19 – mesenteron, food bolus with guanine crystals (arrowheads), 20 – rectum, faecal pellet formed by guanine crystals.
Masson's triple stain. Scales: 20 μm (18), 10 μm (17,19), 5 μm (20). Abbreviations used: aps – apocrine secretion, fb – food bolus, me – mesenteral lumen, mew – mesenteral wall.

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