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COMPARATIVE MORPHOLOGY, ULTRASTRUCTURE AND FUNCTIONS OF THE EXCRETORY ORGAN (POSTVENTRICULAR MIDGUT) IN THE PARASITENGONA (ACARIFORMES)

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ABSTRACT — The excretory organ of terrestrial mites *Leptotrombidium orientale* (Schluger, 1948), *Euschoengastia rotundata* (Schluger, 1955), and *Hirsutiella zachvatkini* (Schluger, 1948) (Trombiculidae); *Platytrombidium fasciatum* (Koch, 1836) and *Camerotrombidium pexatum* (Koch, 1837) (Microtrombidiidae) as well as of water mites *Teutonia cometes* (Koch, 1837) (Teutoniidae) and *Piona carnea* (Koch, 1836) (Pionidae) in different developmental stages was studied using transmission electron microscopy and on semi-thin sections. Irrespectively of the species studied and developmental stages, the excretory organ is represented by a simple thin-walled blind sac that occupies an axial position. The excretory organ is not divided into obvious morphologically or functionally different parts. Posteriorly, the excretory organ transforms into the short cuticular-lined excretory duct, the proper hindgut, derivative of proctodeum. The excretory duct terminates by the axially orientated fissured opening (excretory pore, uropore) that anatomically corresponds to the anus, located on the ventral body wall. In larvae *P. carnea*, the excretory duct and the excretory pore are lacking. The lumen of the excretory organ is variously filled with yellowish double-refractive crystals of excretory wastes (probably guanine) and sometimes also contains single electron-dense globule. The walls of the excretory organ are formed of the single-layered endodermal epithelium, composed of the uniform cells greatly variable in their shape and size. The basal plasma membrane forms round digital indentations frequently containing corresponding projections of the underlying connective tissue cells penetrating through the basal lamina. Clear vacuoles resulting from these indentations immerse into the cells and migrate through the cytoplasm to the apical plasma membrane where they are discharged from the cells. The cells may also contain round or irregularly shaped electron-dense bodies resembling secondary lysosomes or residual bodies. These inclusions may come close to the apical plasma membrane and may be released from the cells. The apical plasma membrane forms irregular microvilli as well as larger irregular projections also bearing microvilli. Golgi bodies and profiles of granular endoplasmic reticulum occur only rarely. Passive transport of residual precursors, vacuolar transport as well as excretion of the electron-dense material provides the accumulation of wastes in the lumen of the organ.

KEYWORDS — ultrastructure; posterior midgut; guanine excretion; Parasitengona

INTRODUCTION

It is known that in the higher and most specialized Actinedida posterior portion of the midgut that most likely corresponds to the postcolon of more generalized groups has evolutionary transformed

into the dorsomedian excretory organ (Alberti and Coons, 1999).

The latter has lost its direct morphological connection with the ventriculus in most if not all of the Parasitengona (Henking, 1882; Schaub, 1888; Bader,

FIGURE 1: See next page

1954; Mitchell, 1964, 1970; Vistorin-Theis, 1977; Shatrov, 2000) maybe with the exception of the Erythraeidae (Witte, 1995). The excretory organ terminates by the short cuticular-lined ectodermal excretory duct (corresponded to the hindgut or rectum) opening by the excretory pore (uropore, anatomical anus) on the ventral body side. However, Thor (1904) stated that in prostigmatid mites posterior portion of the proctodeum (hindgut proper) has evolutionary been lost as a result of the liquid food, and a true anus is substituted afterwards by a newly developed excretory pore.

The excretory organ is shown to function in the excretion and removing of nitrogenous metabolic wastes, presumably guanine (Gasser, 1951; McEnroe, 1961; Wiesmann, 1968; Hazan *et al.*, 1974; van der Geest, 1985) from mite organism and to a great lesser extent in the regulation of ion and water balance (Alberti and Coons, 1999). Nevertheless, the process of the guanine production remains in many respects enigmatic because there are no direct evidences of transport, secretion and formation of the residuals by the epithelial cells of the excretory organ. As far as is known, only few works deal with the ultrastructural examination of the excretory organ in Actinedida, in particular in Tetranychidae (Mothes and Seitz, 1980, 1981, Alberti and Crooker, 1985, Mothes-Wagner, 1985) and in the Parasitengona (Alberti and Coons, 1999; Shatrov, 1989, 2000). No special works are available on the morphology and ultrastructure of the excretory organ of water mites.

The main purpose of this study is to provide detail ultrastructural observations of the excretory organ and to elucidate a possible mechanism of the guanine excretion in the representatives of terrestrial (Trombiculidae, Trombidiidae, Microtrombidiidae) and water mite (Teutoniidae and Pionidae) families of the cohort Parasitengona.

MATERIALS AND METHODS

The following species and developmental stages were used in this study: Trombiculidae - prelarvae and fed adult mites of *Leptotrombidium orientale* (Schluger, 1948), unfed larvae and fed adult mites of *Euschoengastia rotundata* (Schluger, 1955), unfed and feeding larvae, quiescent protonymphs, unfed and fed deutonymphs, quiescent tritonymphs, unfed and fed adults of *Hirsutiella zachvatkini* (Schluger, 1948); Microtrombidiidae - unfed larvae and fed adult mites of *Platytrombidium fasciatum* (Koch, 1836) and *Camerotrombidium pexatum* (Koch, 1837); Teutoniidae - fed adult mites of *Teutonia cometes* (Koch, 1837); Pionidae - unfed larvae and fed adult mites of *Piona carnea* (Koch, 1836).

Trombiculid mites were originally collected as engorged larvae crawling off their natural hosts *Myodes glareolus* Schreber, 1780 (*H. zachvatkini*) and *Myodes rufocanus* Sundevall, 1846 (*L. orientale* and *E. ritundata*), which were trapped in North-Western (*M. glareolus*) and Far-Eastern (*M. rufocanus*) regions of Russia for the period from 1980 till 1994. Collected engorged larvae were taken for the laboratory cultures (see Shatrov, 2000) that have been maintained in the laboratory for several generations and were the source of mites at different developmental stages.

Adult trombidiid mites were collected from the soil surface in Leningrad province in spring-summer period from 1996 till 2003. Approximately two weeks after capture, females began to lay eggs, from which active unfed larvae hatched around two weeks later and were taken for fixation.

Water mites were collected in the fresh-water lakes in the region of the Chupa bay basin of the Kandalaksha gulf of the White Sea near the White Sea Biological Station of Zoological institute of the Russian Academy of Science "Kartesh" during the summer periods of 2000-2003. Unfed larvae of *P.*

FIGURE 1: Morphological organization of the excretory organ. Semi-thin toluidine blue stained sections. a - transverse section of adult mite *Hirsutiella zachvatkini* (Schluger) on the level of excretory pore. Scale bar - 50 μ m; b - transverse section of adult mite *Platytrombidium fasciatum* (Koch) on the level of excretory pore. Scale bar - 100 μ m; c - Higher magnification of the excretory organ of adult mite *Platytrombidium fasciatum* (Koch); transverse section through posterior body region. Scale bar - 50 μ m. ED - excretory duct; EO - excretory organ; EP - excretory pore; Mg - midgut; Ts - testes.

FIGURE 2: See next page

carnea were obtained from adult mites collected in the Volga river basin in Yaroslavl province in summer 2003.

For TEM examinations, mites at different stages were initially fixed in 2-2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2-7.4) for 2-6 h. After immersion into the fixative fluid, integument of active instars (larva, deutonymph and adult) was carefully ruptured from the dorsal side, and the body of quiescent instars (prelarva, protonymph and tritonymph) was pierced through with tiny insect pins for a better penetration of the fixative, or some mites were left intact. Mites were then washed in several changes of 0.2 M phosphate buffer, postfixed in 2% osmium tetroxide in 0.1 M phosphate buffer for 6 h to overnight, dehydrated in ethanol and acetone series, and finally embedded in an araldite mixture. Serial ultra-thin sections both in transverse and longitudinal planes were made on a LKB-III and Leica UC-6 ultramicrotomes and, after staining with uranyl acetate and lead citrate, were examined and photographed with Tesla BS-500 and LEO-900 transmission electron microscopes at 60-80 kV.

For preliminary and general observations, semi-thin sections were stained with toluidine blue and methylene blue and investigated under an Amplival and Leica DM LS-2 light optical microscopes.

RESULTS

Anatomy

The excretory organ in the species studied is represented by a simple thin-walled anteriorly blind sac that generally occupies a middle (axial) position in the body and is not divided into obvious morphologically or functionally different parts.

The simplest anatomical organization of the excretory organ is found in the most specialized and derived trombiculid mites living in soil (Figure 1, a).

No substantial differences in the form and shape of the organ are observed in these mites both throughout the life cycle from quiescent prelarva to adult mite of *H. zachvatkini* and in adults of the species studied. In trombiculids, the excretory organ is vertically orientated and mostly flattened from the lateral sides being squeezed between the midgut diverticula (Figure 1, a). It runs from the excretory pore in anterior direction and on going forward is gradually raised to the dorsal body wall and slightly dilated laterally especially in adult mites. The excretory organ terminates blindly approximately at the level of the brain (synganglion). Morphologically, it clearly differs from the surrounding midgut due to rather thin walls and the variously present yellowish double-refractive crystals, supposedly guanine, in the lumen.

Conversely, in terrestrial trombidids, the excretory organ resembles to some extent the midgut diverticula by the comparable width of the folded walls (Figures 1, b, c). Its anatomy is also more complicated than in trombiculids. In the adult mites studied, the anal atrium is divided anteriorly into two lateral branches. The left one is short and does not extend forward evidently. The right one, in contrast, gives rise to the excretory organ as such occupying the horizontal position in the body being oppressed dorso-ventrally between the midgut lobes and frequently has a nearly collapsed lumen. The excretory organ terminates blindly ('disappears' among the midgut diverticula) at the level of the brain and gives no conspicuous lateral or anterior projections. In contrast to adult mites, in unfed larvae, the excretory organ is represented by a simple small sac lying mostly adjacent to the dorsal body wall, flattened in dorsal-ventral direction (Figure 4, c) and is going down to the excretory pore.

The water mites studied show a somewhat intermediate anatomical organization of the excretory organ. Like in trombiculids, it is a relatively thin-walled voluminous sac with a conspicuous lumen

FIGURE 2: Morphological organization of the excretory organ in adult water mite *Teutonia cometes* (Koch). Transverse semi-thin toluidine blue stained sections on the level of (a) posterior body region, (b) genital apparatus and (c) opening of the oesophagus into the ventriculus. Scale bars - 100 μ m (a) and 50 μ m (b-c). EO - excretory organ; GA - genital apparatus; HS - haemocoelic space; Mg - midgut; Ms - muscles; Oe - oesophagus.

FIGURE 3: See next page

but possessing more complicated outlines (Figures 2, a - 3, c). In general, in the posterior body region, the excretory organ is vertically orientated and occupies nearly the whole height of the body totally separating posterior gut diverticula (Figure 2, a). Anteriorly, at the level of the genital apparatus, it becomes significantly narrower (Figure 2, b), but more anterad it is again enlarged in lateral directions and occupies nearly the whole width of the mite body being located just beneath the dorsal body wall (Figure 2, c). In both water mites' species studied, the excretory organ gives two antero-lateral diverticula extending far ahead in the body cavity among the midgut lobes (Figures 3, a, c). In larvae *P. carnea*, the walls of the excretory organ, in contrast with trombiculid and trombidid larvae, may form folds or lacunas.

No muscle layer is present around the walls of the excretory organ in the species studied. However, single muscle fibers may be sometimes seen in the vicinity of the organ. The epithelial cells of the excretory organ rest on the basal lamina (see below).

The lumen of the excretory organ is variously filled with the yellowish double-refractive crystals of the excretory material (Figures 2, a - 3, b) and sometimes also contains single electron-dense globules, mostly in water mites (Figures 2, a, b and 3, b). The excretory material in the lumen of the organ is already present in prelarvae. In unfed larvae its quantity is rather decreased, and in feeding trombiculid larvae it remains low. Later on, in the life cycle, the residual materials in the excretory organ are increased again in their quantity in quiescent protonymph and tritonymph and become reduced in active instar. The latter obviously ejects the residuals after hatching from the old exuvia of the previous quiescent instars that is seen in the culture of trombiculid mites (Shatrov, 2000).

Posteriorly, the excretory organ transforms into the short cuticular-lined excretory duct, the proper

hindgut, derivative of proctodeum (Alberti and Coons, 1999) (Figures 1, a, b). The latter terminates by the axially orientated fissured opening (excretory pore) flanked by the two lateral cuticular plates extending above the surface of the surrounding body wall (Figures 1, a, b). The excretory pore is provided with small muscle fibers helping in its dilation during ejection of wastes. In adult trombidids, the particular anal atrium is present lined with a thick cuticle and dividing anterad into two branches (see above) (Figure 8, e). In the water mite *T. cometes*, in comparison with trombiculids and trombidids, the excretory pore occupies the most posterior (caudal) position on the ventral body wall. In larvae *P. carnea*, the excretory duct and the excretory pore are found totally absent so the excretory organ appears to be isolated from the external milieu. In adult mites of this species, the short excretory duct with mostly collapsed lumen is present provided with relatively thick walls resembling those of the organ with darker cytoplasm and lined with a thin cuticle. The duct terminates by a small elongated aperture on the ventral body wall bordered by the ring of a thickened cuticle. It is located not far from the caudal end of the body and devoid of the muscles armament.

Ultrastructure

Irrespective of species and developmental stages, the wall of the excretory organ is formed of the single-layered entodermal epithelium composed of the uniform cells greatly variable in their shape and size (Figures 4, a, e, 5, b and 6, f). The cell's height from the basal lamina to the apical plasma membrane varies from 0.2 up to 12-15 μm even in the adjacent parts of the organ's wall. Generally, the thickness of the walls is smaller in quiescent instars and un-engorged mites (Figures 4, a-g), and larger in fed animals (Figures 7, a, c-e). The places where nuclei situated are typically visible by bulges of the

FIGURE 3: Morphological organization of the excretory organ in adult water mite *Piona carnea* (Koch). a - transverse section on the level of posterior portion of the brain (synganglion), showing two antero-lateral diverticula; toluidine blue. Scale bar - 100 μm ; b - sagittal longitudinal section through the organ approximately at the middle line showing differences in the epithelial structure. Scale bar - 50 μm ; c - 3D reconstruction of the organ with voluminous axial portion and two antero-lateral diverticula. EO - excretory organ; Mg - midgut; Ms - muscles; Oe - oesophagus; Ov - ovary; Sn - synganglion.

FIGURE 4: See next page

wall of the organ (Figures 5, c - 6, a). The lateral cell margins are extremely folded, leave no conspicuous extracellular space and connected each other by the long septate junctions (desmosomes) with an electron-dense matrix (Figure 4, d).

The basal plasma membrane is either flat or slightly folded (Figures 4, a, b, e, g, 5, a and 6, e) and forms round or elongate digital indentations, which may contain corresponding projections of the underlying connective tissue cells (Figures 5, b-d, 7, b, e and 8, c). The latter penetrate the delimiting basal lamina and form the type of intimate contact with the basal plasma membrane of the epithelial cells of the organ (Figures 5, c, d and 8, c). In some cases, however, these indentations do not contain projections of the connective tissue cells and look empty (Figure 6, b). These structures appear to be constantly present in the wall of the excretory organ and sometimes, in a water mite *P. carnea*, reach a very high number (Figure 8, c). A somewhat different situation is observed in a water mite *T. cometes*, where the basal lamina follows the indentations of the basal plasma membrane of the epithelial cells (Figures 8, a, b). The basal lamina is relatively thin having moderate electron density and follows folds of the basal plasma membrane (Figure 6, b). In contrast, in adult water mite *P. carnea* and sometimes in adult trombiculid mite *H. zachvatkini*, the basal lamina is extremely thick with a low electron density (Figure 8, c). Round vacuole-like inclusions losing contact with the indentations immerse into the cells and obviously migrate through the cytoplasm to the apical plasma membrane where they are apparently released from the cells into the lumen of

the excretory organ (Figures 7, b, c and 8, c). This transportation process is most prominent in a water mite *P. carnea* (Figures 8, c, d).

The apical plasma membrane forms irregular microvilli (Figures 4, b, f, 6, b, f) as well as larger irregular projections also bearing microvilli (Figure 6, f) that sometimes, in adult mites *H. zachvatkini*, look like 'trees' with the lateral 'branches' (Figures 7, a, b). Microvilli may be sparse and short like in trombiculid prelarvae *L. orientale* and unfed instars of *H. zachvatkini* (Figures 4, a and 6, c), or, contrary, long, branched and intermingled like in other instars and especially in adult trombiculid mite *Pl. fasciatum* (Figures 7, d, e). In a water mite *P. carnea* they even form a kind of microvillar labyrinth, within which the releasing of the clear vacuoles is proceeded (Figure 8, d). Such microvilli and larger projections contain the same cytoplasmic matrix as the remaining cell volume with ribosomes and occasionally glycogen particles (Figures 4, e, 5, a, b and 6, e). No apical mitochondrial pool is expressed beneath the microvilli in any specimens studied. In feeding trombiculid larvae the apical cell surface, besides microvilli, may form figures resembling pinocytotic or even phagocytotic pits, as in the midgut cells, which however, do not immerse into the cells (Figure 5, a). In prelarvae *L. orientale* and adult water mites *T. cometes*, aggregations of an electron-dense material of unknown origin may be closely applied to the apical plasma membrane from the side of the lumen (Figures 4, a, 8, a, b). In these cases, electron-dense globules of different sizes are also seen in the lumen.

Nuclei are rather irregularly shaped with folded or indented outlines and may sometimes bulge the

FIGURE 4: Ultrastructure of the excretory organ in prelarval and larval stages. TEM. a - portion of the wall of the excretory organ adjacent to the midgut in prelarva *Leptotrombidium orientale* (Schluger). Scale bar - 0.5 μm ; b - lateral wall of the excretory organ in close contact with the midgut in unfed larva *Euschoengastia rotundata* (Schluger). Scale bar - 1 μm ; c - transverse section through the organ closely applied to the dorsal body wall in the middle body region in unfed larva *Camerotrombidium pexatum* (Koch). Scale bar - 1 μm ; d - septate junction (desmosome) between the cells of the organ in unfed larvae *Camerotrombidium pexatum* (Koch). Scale bar - 0.5 μm ; e - portion of the wall of the organ in unfed larva *Camerotrombidium pexatum* (Koch). Note dense ground cytoplasm and branched microvilli. Scale bar - 1 μm ; f - longitudinal section through the organ's wall in unfed larva *Camerotrombidium pexatum* (Koch) showing small Golgi body and electron-dense bodies coming close to the apical plasma membrane. Scale bar - 0.5 μm ; g - longitudinal section through the organ's wall in unfed larva *Platytrombidium fasciatum* (Koch) showing small Golgi body and electron-dense body in the vicinity of the apical plasma membrane. Scale bar - 0.5 μm . BL - basal lamina; CTC - connective tissue cell; DB - dense bodies; EO - excretory organ; G - Golgi body; Gl - glycogen; Hm - haemocyte; Li - lipid inclusions; Lu - lumen of the organ; M - mitochondria; Mg - midgut; Ms - muscles; Mv - microvilli; N - nucleus; Nu - nucleolus.

FIGURE 5: See next page

cell portion into the lumen (Figures 5, c, 6, a, f) or may be flattened along the wall of the organ (Figures 4, a, c, 6, e). Nuclei contain small chromatin particles scattered throughout the nucleoplasm and applied to the nuclear envelope as well as large round nucleolus. The ground cytoplasm is electron-dense containing ribosomes, lipid inclusions and glycogen particles in various proportions (Figures 4, e and 7, e). In adult water mite *T. cometes* and adult trombidid mite *Pl. fasciatum* glycogen is observed in relatively large associations (Figures 7, e, 8, a, b).

The cells may also contain round or irregularly shaped electron-dense bodies resembling secondary lysosomes or residual bodies of digestive cells (Figures 6, e-g, 5, b, 7, a, c, f). The midgut digestive cells are frequently closely applied to the organ from the outside being divided by the long and narrow extensions of connective tissue cells (Figures 4, a, 5, a-c, 6, a, 7, b and 8, b). Typically, in adult trombidid mite *Pl. fasciatum* and, to a lesser extent, in adult trombiculid mites *H. zachvatkini* and *L. orientale* these dense inclusions are numerous (Figures 7, a, c, d). This type of inclusions may also show a fine-granular matrix of moderate electron density, and may come close to the apical plasma membrane that is obviously seen in unfed larvae and adult mites *Pl. fasciatum* and unfed deutonymphs *H. zachvatkini* (Figures 4, f, g, 6, c, d, 7, e, f). However, an evident process of detachment of these granules into the lumen is observed with certainty only in unfed deutonymphs *H. zachvatkini* (Figures 6, c, d) and maybe in variation in adult water mite *T. cometes* (Figures 8, a, b). In a latter case, single vacuoles and deep exocytotic pits with a dense rim and an electron-lucent core discharge their contents into the lumen

where these dense globules and smaller particles associate into large round or irregularly shaped boluses (Figure 8, a). In feeding larvae *H. zachvatkini*, these dense inclusions, highly resembling residual bodies of the midgut cells, are relatively sparse but may achieve large sizes (Figure 5, b). In quiescent and unfed instars these dense inclusions are poorly or moderately represented.

Granular endoplasmic reticulum (GER) is rather poorly represented in any specimens studied; although in feeding larvae *H. zachvatkini* long curved single profiles may be seen in different cell portions (Figures 5, a, b) and may sometimes be extraordinary dilated revealing a fine-granular matrix of moderate electron density. Small Golgi bodies may be mostly observed in unfed larvae of the species studied (Figures 4, f, g) and, rarely, in fed adults of *H. zachvatkini*. At the same time, this organelle is especially expressed in feeding larvae of *H. zachvatkini*, where it has a form of large and loosely organized association of the electron-lucent to electron-dense vesicles and short curved cisterns in the middle cell portion accompanied with GER elements (Figure 5, a). Oval mitochondria with a matrix of moderate electron density and loosely arranged cristae are scattered freely throughout the cell volume. The ground cytoplasm typically shows high electron density but in some cases, for instance, in feeding larvae and unfed adults of *H. zachvatkini*, some cells may have electron-lucent cytoplasm, so adjacent cells demonstrate contrast density.

Connective tissue cells, typically delimiting the excretory organ and surrounding tissues, form long thin processes with mostly an electron-lucent cytoplasm containing single ribosomes, mitochondria

FIGURE 5: Ultrastructure of the excretory organ in feeding larvae *Hirsutiella zachvatkini* (Schluger). TEM. a - portion of the wall of the excretory organ separated from the midgut by a narrow strip of connective tissue cells. Note large Golgi bodies and long profiles of granular endoplasmic reticulum. Scale bar - 0.5 μm ; b - part of the wall of the excretory organ containing large electron-dense bodies and profiles of granular endoplasmic reticulum. Note round indentations in the basal cell portion. Scale bar - 0.5 μm ; c - elongated nucleus in the wall of the excretory organ. The basal plasma membrane both of the cells of the excretory organ and of the midgut cell forms numerous round indentations, which may or may not contain corresponding projections of connective tissue cells. Scale bar - 0.5 μm ; d - part of the wall of the excretory organ with the large elongated nucleus and digital indentation in the basal cell portion containing corresponding projection of connective tissue cell. Scale bar - 0.5 μm . BL - basal lamina; CTC - connective tissue cells; DB - dense bodies; G - Golgi bodies; GER - granular endoplasmic reticulum; Lu - lumen of the excretory organ; M - mitochondria; Mg - midgut; Mv - microvilli; N - nucleus; Nu - nucleolus; SJ - septate junction; *arrows* indicate digital indentations in the basal cell zone.

FIGURE 6: See next page

and occasionally electron-dense bodies and clear vacuoles (Figures 4, a, 4, g, 5, a, b, 6, a, e, 7, b, c and 8, b). Nuclei are elongated and occur rarely. Sometimes, these cells may form short processes extending into the basal portion of the midgut cells (Figure 5, b). Neither precursor of the guanine formation nor transportation processes is seen with certainty in the connective tissue cells.

Excretory duct

The excretory organ terminates by an ectodermal excretory duct lined with a relatively thin cuticle of moderate to low electron density (Figure 8, f). This excretory duct corresponds to the hindgut of the more generalized groups and originates from the proctodeum. In trombiculid mites, the cells forming the duct are irregularly shaped and rich in ribosomes, mitochondria and lipid inclusions. Nuclei are predominantly round to oval and are provided with a round centrally located nucleolus and large chromatin globules. These cells, in particular in unfed adults *H. zachvatkini*, and to a lesser extent in other instars of this species may form short irregular microvilli on the apical cell surface reaching the cuticle. Besides this, in deutonymphs and adult mites *H. zachvatkini* and in adult mites *Pl. fasciatus* the apical plasma membrane of the duct epithelial cells may form a type of plications that suggests the possibility of transportation processes. Cell debris of unknown origin may be sometimes seen in the duct lumen at its base. The terminal opening of the excretory duct is armed with two cuticular plates extending above the ventral surface of the body and joining with the duct cuticle at the top of the pore (Figure 8, g). No accessory glands were found to

empty through the excretory pore in trombiculids as have been shown in males *Blankaartia acuscutellaris* (Walch, 1922) (Mitchell, 1964).

In adult mites *Pl. fasciatus*, a particular anal atrium is expressed (Figure 8, e), which walls is formed of the ectoderm cells also showing plications. These cells are covered with a rather thick electron-light cuticle. The anal atrium is divided anterad into two branches (see above) lined with a thin cuticle and formed of the ectoderm cells with plications (Figure 8, e).

DISCUSSION

This study confirms the previous finding for some of the higher Actinedida (Thor, 1904; Alberti and Coons, 1999) that in the Parasitengona the excretory organ is likely of endodermal origin being devoid of a cuticle and has no morphological connection with the midgut. It is not divided into functionally different parts and is opened to the external milieu by the ectodermal cuticle-lined excretory duct terminated by the excretory pore, which is probably homologous to the anus. In spite of the fact that the excretory organ goes far ahead in the body cavity and has the anterior termination at the level of the brain, it is doubtfully derived from Malpighian tubules, as has been supposed previously (Thor, 1904; Reuter, 1909; Mitchell, 1970), at least in trombiculids and water mites. Conversely, in its present constitution, the excretory organ combines postventricular portions of the midgut of more generalized groups (Henking, 1882; Michael, 1895; Hughes, 1959; Alberti and Coons, 1999), namely colon, which is supposedly mostly reduced (Ehrnsberger, 1984; Alberti

FIGURE 6: Ultrastructure of the excretory organ in the nymphal stages of *Hirsutiella zachvatkini* (Schluger). TEM. a - wall of the excretory organ in the 4 day quiescent nymphochrysalis (protonymph). Note the large nucleus with indented outline forming a bulge in the wall of the organ. Scale bar - 1 μ m; b - wall of the excretory organ in the 8 day quiescent nymphochrysalis (protonymph). Note clear vesicles in the basal cell region and the intensive formation of the excretory material in the lumen of the organ. Scale bar - 1 μ m; c - excretion of a dense body from the wall of the organ in unfed deutonymph. Scale bar - 0.5 μ m; d - a type of excretion granule in the wall and lumen of the organ in unfed deutonymph in the form of the electron dense ring with a lucent core. Scale bar - 0.5 μ m; e - part of the wall of the excretory organ in the 2 day quiescent tritonymph without evidence of transportation processes. Scale bar - 1 μ m; f - portion of the wall of the excretory organ in the 4 day quiescent tritonymph showing large protrusions into the lumen filled with the forming excretory granules. Note muscle cells running close to the organ's wall. Scale bar - 2 μ m. BL - basal lamina; CTC - connective tissue cells; DB - dense body; Hm - haemocyte; Hs - haemocoelic space; Lu - lumen of the organ; Mg - midgut; Ms - muscles; Mv - microvilli; N - nucleus; arrows indicate basal vesicles.

FIGURE 7: See next page

and Coons, 1999) and postcolon. Division of the excretory organ into various branches in most water mite groups (Croneberg, 1878; Bader, 1938, 1954) may be considered as a secondary acquisition corresponding to relatively large sizes of these mites. The possible absence of the excretory duct and the excretory pore in some water mites (Hydrachnidia) (Mitchell, 1955) and representatives of Halacaridae (Thomae, 1925) has been explained by the evolutionary reduction of the proctodeum in this stock of mites (Thor, 1904; Bader, 1938, 1954; Stout, 1953). This reduction is accompanied with the subsequent development of these parts anew as the true excretory pore at the place of the former anus in all groups where the excretory organ is present. Most likely, however, that it is a secondary loss and atrophy of the posterior terminal portion of the excretory organ in more derived groups of water mites. *P. carnea* shows a possible way to this situation where the excretory duct and pore are developed in the course of postlarval ontogenesis.

Somewhat another situation is found in trombidiid mites where the excretory organ is divided from the anal atrium into two asymmetrical branches partly resembling midgut lobes with predominant development of one of these. Such an arrangement may suggest: (1) that the only small 'dam' between the proper midgut and the anal atrium is reduced, (2) that the branches of the present excretory organ are not derived from the 'principal line of the gut' (Henking, 1882; Alberti and Coons, 1999). It could be developed anew as the secondary lateral extensions of the most posterior portion of the gut endoderm. Bader (1954) also mentioned that in the terrestrial trombidiid mites

the excretory organ consists of the two tubes opening into the rectum. According to his point of view, this organ in water mites is also initially paired that may be seen from its anterior division into two lobes that is also found in *P. carnea* and to a lesser extent in *T. cometes*. In adult trombiculid mite *B. acuscutellaris*, the excretory organ is 'T' shaped anteriorly in cross sections having two lateral extensions (Mitchell, 1964). In accordance with the latest assumption, however, the excretory organ in the higher Actinedida, being paired or especially single, is not homologous to the Malpighian tubules (Alberti and Coons, 1999) but has developed independently and directly from the posterior midgut portions.

Additional evidence for the origin of the excretory organ from the 'principal' midgut is the organization of the apical cell portions of the organ in larvae *H. zachvatkini* in the early feeding stages. The apical cell surface forms figures resembling phagocytotic pits and vacuoles found at the same stages in the midgut epithelium. These vacuoles, however, do not immerse into the cells but remain only reflecting the possibility of this process, which has lost evolutionary. Generally, the cells of the organ contain the same inclusions as the midgut cells, in particular large Golgi bodies, but subsequent accumulation of the nutrition vacuoles does not take place. Schmidt (1935) has also noted that the wall of the excretory organ is composed of the large irregularly shaped cells obviously having not the ectodermal origin.

The most important question concerning the functioning of the excretory organ is the formation

FIGURE 7: Ultrastructure of the excretory organ in fed adult mites *Hirsutiella zachvatkini* (Schluger) (a-b), *Leptotrombidium orientale* (Schluger) (c) and *Platytrombidium fasciatum* (Koch) (d-f). TEM. a - part of the wall of the excretory organ with protuberances bearing microvilli and projecting into the lumen. Note folded basal cell surface without vesiculation. Scale bar - 1 μ m; b - portion of the wall of the excretory organ revealing numerous basal digital indentation with corresponding projections of connective tissue cells as well as release of clear vacuole into the lumen. Scale bar - 1 μ m; c - wall of the excretory organ with nucleus and apical microvillar plexus with the applying excretory material in the form of irregular profiles. Note detachment of such membranous profile into the lumen and haemocytes adjacent to the organ. Scale bar - 2 μ m; d - portion of the excretory organ resembling midgut lobe, bearing microvilli and provided with the electron-dense bodies and basal indentations. Scale bar - 2 μ m; e - wall of the excretory organ containing glycogen particles and provided with numerous basal indentations. Scale bar - 2 μ m; f - realizing of the dense bodies into the lumen of the organ in its posterior portion. Scale bar - 0.5 μ m. CTC - connective tissue cells; DB - dense bodies; Gl - glycogen; HS - haemocoelic space; Lu - lumen of the organ; Mv - microvilli; Ms - muscles; N - nucleus; arrows indicate basal indentations; arrowhead points realizing of clear vacuole into the lumen.

FIGURE 8: See next page

of the excretory material in the lumen of the organ. As far as is known, there are no direct evidences either for transport of the excretory precursors through the organ's wall or for assemblage of the excretory granules in the lumen (Bader, 1938, 1954; Alberti and Coons, 1999). Basal labyrinth and basal infoldings as well as tight microvillar border that may suggest active water and ion transports through the organ's wall (Wharton and Brody, 1972) are totally absent from the cell composition of the organ in all stages of the life cycle. A narrow layer of the connective tissue cells is nearly always present between the excretory organ and other parts of digestive system preventing formation the close apposition of these organs that is just observed in tetranychids and eriophiids (Alberti and Crooker, 1985; Alberti and Coons, 1999). In the latter, a basal lamina may be lacking between both epithelia (Nuzzaci and Alberti, 1996; Alberti and Coons, 1999) that is never found in the Parasitengona. Conversely, in the parasitengonid mites the basal lamina underlying the epithelium of the excretory organ is always present even in prelarvae, and the delimiting connective tissue layer is thought to play a certain role in the transport of the excretory precursors.

In contrast to the opinion that mites, who have lost direct communication between the ventriculus and the postventricular midgut produce only white excrements, (Vistorin-Theis, 1977; Vistorin, 1980; Alberti and Coons, 1999), the examined species may produce besides large masses of 'white' excretions, also some amount of the type of black feces that is especially seen in water mites. These processes oc-

cur via the same cells of the excretory organ. Differentiation into two different portions with particular cell types that is found in tetranychids (Mothes and Seitz, 1980; Alberti and Crooker, 1985; Mothes-Wagner, 1985; Alberti and Coons, 1999) is not observed with certainty in the examined parasitengonids. However, in *P. carnea* some differences in the cell organization through the length of the organ are observed where the dorsal and posterior walls possess large cells giving projections into the lumen and may be broken down whereas the anterior wall has a squamous epithelium (Figure 3, b).

The formation of the guanine residuals in the lumen of the excretory organ remains enigmatic. It is highly doubtful that the apical cell processes projecting into the lumen and detaching via apocrine secretion are directly involved in the formation of the excretory granules as has been proposed by Alberti and Coons (1999) on the example of *Allothrombium fuliginosum* (Hermann, 1804). This point of view does not explain and show how the cytoplasmic cell portions transform into the birefringent granules, especially if microvillar projections are poorly developed. It is more likely that the combination of the following processes is involved in the formation of the excretory wastes and the excretory materials in the lumen of the organ.

(1). Vacuolar transcellular transport may play a certain role in this process that may be, in particular, seen on the example of *P. carnea*. The clear vacuoles detaching from the basal digital indentations and frequently containing portions of the connective tissue cells migrate across the cell from the basal

FIGURE 8: Ultrastructure of the excretory organ, excretory ducts and excretory pore. TEM. a - wall of the excretory organ in an adult water mite *Teutonia cometes* (Koch) filled with lipid inclusions and glycogen particles and forming basal folds of the thin basal lamina. Note dense boluses in the lumen of the organ. Scale bar - 2 μ m; b - portion of the wall of the excretory organ in an adult water mite *Teutonia cometes* (Koch) at higher magnification. Note quite a narrow strip of the connective tissue cells separating the midgut and the excretory organ. Scale bar - 0.5 μ m; c - portion of the excretory organ in an adult water mite *Piona carnea* (Koch) showing numerous basal digital indentations penetrating the extremely thick basal lamina and forming vacuoles releasing into the lumen. Scale bar - 2 μ m; d - an apical portion of the epithelium of the excretory organ in an adult water mite *Piona carnea* (Koch) showing the apical microvillar labyrinth and numerous vacuoles realizing into the lumen. Scale bar - 2 μ m; e - anal atrium and collecting duct in an adult mite *Platytrombidium fasciatum* (Koch). Scale bar - 5 μ m; f - excretory duct in feeding larva *Hirsutiella zachvatkini* (Schluger). Scale bar - 2 μ m; g - terminal portion of the excretory pore in fed adult mite *Hirsutiella zachvatkini* (Schluger). Scale bar - 2 μ m. AA - anal atrium; BL - basal lamina; CD - collecting duct; CTC - connective tissue cell; DBI - dense bolus; Gl - glycogen; Li - lipid inclusions; Lu - lumen of the organ; M - mitochondrion; Mg - midgut; Ms - muscles; N - nucleus; V - vacuoles; arrows indicate basal digital indentation of the basal plasma membrane; arrowheads point to the indentation of the basal lamina into the basal cell zone.

to the apical plasma membrane and are delivered into the lumen as empty vacuoles with a slightly folded membranous envelope. In the lumen, the excess fluids are extracted from the excretions and reabsorbed by the epithelial cells owing to function of microvilli.

(2). Similar process may be expected, generally, in the excretion of black feces that is most characteristic in a freshwater mite *T. cometes*. In this case, concentrated excretions penetrated by unknown means through the apical plasma membrane and applied to it in the excretory pits and vacuoles, finally are associated and aggregated within the lumen of the organ in the form of the electron-dense boluses. On the other hand, some amount of black excrements may be immediately detached from the cells as residual bodies through the action of merocrine secretion as may be seen in the case of adults *L. orientale* and *Pl. fasciatum*.

(3). Along the direct excretion, the main mode of the guanine formation is thought to be a passive transport of precursors with a low molecular weight (molecule of guanine proper) through the organ's wall from the body cavity into the lumen. An important role in this process may apparently play the connective tissue cells with their processes penetrating through the basal lamina and extending into the basal portion of both the epithelial cells of the organ and the midgut cells. These connective tissue cells are thought to mediate the passive exchange and transport of ions into the organ and water back to the organism. The formation of projections and vesicles in the organ's cells just greatly increases the cell surface for the exchanging processes and transportation of precursors into the organ. In the lumen, these precursors associate into granules that gradually become crystallized. The excess of water is reabsorbed through the system of microvilli back to the organism.

However, proportions and sources of these processes are not known with certainty, and very often the walls of the organ do not show any direct transportation evidences. Because neither body muscles immediately attach to the excretory organ, nor muscle layer is expressed around the wall of the organ, the evacuation the organ is realized through

the body pressure via the action of both large dorso-ventral muscles (Mitchell, 1964) and smaller muscle fibers. These motions may be seen in living adult trombiculid mites in culture a short time later of their hatching from the exuvia of the previous quiescent tritonymphal instar, when waves of sporadic contractions runs throughout the mite body.

The unusual organization of the excretory organ provokes several different explanations of its origin and evolution in the Parasitengona.

(1). Trombidiids *sensu lato* had initially two large lateral extensions of the posterior midgut portion transforming afterwards into the specialized excretory organ, which is not significantly different from the midgut lobes. A small communication between the excretory organ and the midgut found in *Trombidium* (McLeod, 1884) may be considered as a secondary adaptation. In the course of evolution, the reduction of one of these branches and substitution and acquisition of functions of the lost branch by the remaining one has occurred. The two other large groups - Trombiculids and water mites have the excretory organ originated directly from the principal line of the midgut with the subsequent complication of its shape in water mites and reduction of the excretory pore in the earliest derived groups.

(2). The ancestral group of the Parasitengona had a single axially orientated simple excretory organ originated directly from the posterior midgut portion as may be apparently seen in recent larvae. Subsequently, the anatomy of the excretory organ has progressively changed mostly in trombidiids and water mites with retaining, however, its histology close to the midgut in trombidiids.

(3). Ancestral parasitengonids had the initially paired excretory organ formed of the lateral extensions of the posterior midgut with its subsequent significant simplification especially in trombiculids. Larvae as the possible result of desembryonization (i.e., hatching on the earlier stages of embryonic development) reproduce a simplified morphology of the excretory organ in combination with additional functional adaptations in other organ systems.

(4). The excretory organ has evolved totally independently of the digestive system from the part of the embryonic endoderm supposedly divided

from the main endoderm very early in the embryogenesis. The excretory duct and the excretory pore have developed from the ectoderm apart of the true hindgut and the true anus that subsequently have nearly totally reduced in parasitengonid groups.

Unfortunately, the present knowledge does not allow us to incline surely to one of these explanations. To resolve this problem adequately, it needs further comparative and embryological investigations and evidences.

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