

Ultrastructural aspects of vitellogenesis in *Archezogozetes longisetosus* Aoki, 1965 (Acari, Oribatida, Trhypochthoniidae)

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Abstract

We studied the process of vitellogenesis in the oribatid mite *Archezogozetes longisetosus* using light and electron microscopy. Both vitellogenesis and the formation of the first egg shell occur within the meroi of the ovary, starting after detachment from the medulla and completing with ovulation of the egg into the oviductal lumen at the ovarial bulb. Numerous microvilli appear on the surface, and abundant endocytotic pits and coated vesicles occur in the peripheral plasma of the oocyte. Accumulation of fatty yolk does not precede accumulation of proteineous yolk. Differentiation of ooplasm and formation of a perivitelline space beneath a continuous follicular epithelium were observed. Vitelline envelope material appears to be uniform. We compare details of vitellogenesis and propose a classification of the ovarian type as panoistic and the vitellogenesis as exogenic in *A. longisetosus*.

Keywords: mite, oogenesis, yolk, transmission electron microscopy, microvilli, ovary

1. Introduction

The vast diversity of the Acari is reflected by their diversity of reproductive modes and their associated processes such as oogenesis, vitellogenesis and hormonal regulation (Norton et al. 1993). In the last few years, great advances towards a unifying model for vitellogenesis and its hormonal regulation in mites have been achieved (James & Oliver 1999, Cabrera et al. 2009), though the body of basic knowledge still seems heavily biased with respect to the anactinotrichid Ixodidae (ticks), with their life histories greatly diverging from those exhibited by Actinotrichida (Cabrera et al. 2009). Thus, the general model of the evolutionary history of reproduction in mites yet remains unsure, partly due to unresolved phylogenetic issues in such an old clade (Dunlop & Alberti 2008). Within Actinotrichida, studies of oogenesis, albeit seldomly regarding the molecular background, have been undertaken in Trombidiformes (Alberti 1974, Witte 1975, Witaliński 1986, Shatrov 1997, Di Palma & Alberti 2001), Astigmata (Heinemann & Hughes 1970, Walzl et al. 2004), Brachypylina (Woodring & Cook 1962, Baker 1985, Witaliński 1986) and Desmonomata (Taberly 1987a,b, Smrž 1989).

One species from the group of Desmonomata, the thelytokous trhypochthoniid *Archeogozetes longisetosus* Aoki, 1965, however, received much attention during recent years as a potentially valuable model organism for a variety of issues, making it the most intensely studied oribatid mite so far (Alberti et al. 2003, Smrž & Norton 2004, Heethoff et al. 2007, and cited references).

We studied the structural aspects of vitellogenesis in *A. longisetosus* by means of light and electron microscopy. Previous studies on the genital system of this species (Bergmann et al. 2008) revealed its composition and yielded first results regarding the time course and location of oogenetical processes. Oocytes start development in a round, radially organised region termed rhodoid. These cells are connected to a central medulla via radially arranged protrusions. Cells detach from the medulla and in the course of oogenesis, including meiosis (Laumann et al. 2008), form paired extensions of the ovary, termed meroi (singular: meros). These are folded in a proximal portion, oriented rostrally and a distal portion dorsally of the proximal and oriented caudally (Fig. 1).

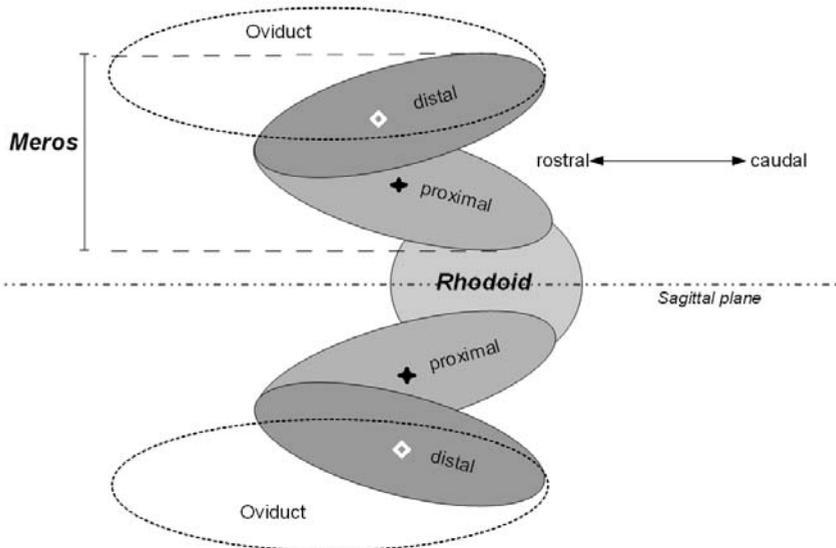


Fig. 1 Schematic representation of the major subdivisions of the ovary in *A. longisetosus* and their spatial arrangement in dorsal view. Black asterisks mark location of previtellogenesis. White diamonds mark location of vitellogenesis, i.e. the vitellarium.

The transition from previtellogenesis to vitellogenesis and the simultaneously ensuing deposition of egg shell material coincides with the passing of the oocytes into the distal part of the meros, as has been previously shown (Bergmann et al. 2008). The oocytes within the ovary are wrapped in and separated from each other by one to few layers of flattened follicular cells (Bergmann et al. 2008).

Here, we show that vitellogenesis is exogenic, with the simultaneously deposited vitelline envelope staying porous until its completion. The oocytes exhibit signs of intense communication with surrounding tissues throughout oogenesis, e.g. a dense microvilli fringe and abundant coated pits and coated vesicles. While microvilli are especially abundant during the early stages of vitellogenesis, coated pits dominate in later stages. Differentiations of the ooplasm are observed during the simultaneous accumulation of proteinaceous and fatty yolk. We address differences as well as similarities to existing studies of oogenesis, and describe the ovarian type as panoistic. We further discuss the possible role of follicle cells and surrounding tissues during vitellogenesis and prospects of future research.

2. Materials and methods

2.1. Rearing

Specimens were taken from our laboratory culture of *Archegozetes longisetosus* ran (Heethoff et al. 2007), a lineage established from a single female from Puerto Rico by Roy Norton in 1993, and presently hatched in various laboratories all over the world.

Molting aggregations of immobile individuals were removed from the culture and placed in the wells of tissue culture plates (Tissue Culture Cluster, Nunc, Roskilde, Denmark) for further development. Wells were filled with Plaster-of-Paris:charcoal (6:1) mixture. The plates were kept in constant dark; air humidity was kept at 90 % and temperature at 20 °C.

The wells were checked daily and freshly moulted individuals were removed with a fine brush and transferred to new wells. In these, bark of various trees covered with green algae (mainly *Protococcus* spec.) was supplied as a food source. Adults were collected for further processing 3 and 5 days after hatching.

2.2. Light and electron microscopy

Specimens were submerged in Karnovsky's fixative diluted in 0.05 M HEPES buffer to 1/3 strength (1.33 % formaldehyde; 1.66 % glutardialdehyde) with 4 % sucrose and 6.6 μ M $MgSO_4$ added at pH 7.8 and 0 °C, and punctured with a fine needle (Norton & Sanders 1985), then transferred into 12 ml glass vials with conical bottom filled with fixative prechilled to 0 °C. Fixation time was 80 min at 0 °C and ~ 200 mbar.

The fixative was prepared using freshly depolymerised paraformaldehyde (Fluka, Buchs, Switzerland) and EM -grade glutardialdehyde 25 % (Science Services, Munich, Germany).

After rinsing three times for 10 min in HEPES 0.05 M at 0 °C, postfixation and en-bloc staining was conducted with 1 % OsO_4 and 0.54 % uranyl acetate in 0.05 M HEPES at pH 7.8 and 0 °C for 1h.

After rinsing for 10 min twice in 0.05 M HEPES at 0 °C, dehydration was carried out in a graded acetone series at 30 % and 50 % for 10 min each at 0 °C and at 70 %, 80 %, 95 % and 100 % for three times 10 min each at room temperature. Samples were gradually infiltrated (acetone / resin mixture 3:1 for 90 min and 1:1 for 60 min on a vibratory plate at 100 rpm, 1:3 in open vials overnight) and embedded in epoxy resin (Araldite CY212 Premix Kit, Plano GmbH Wetzlar, Germany). Polymerisation was conducted at 60 °C for 48 h. Semithin (0.5 μ m) and ultrathin (70 nm) sections were cut using a Reichert Ultracut (Leica-Jung, Vienna, Austria) microtome and diamond knives (Diatome 45°, Biel, Switzerland).

Semithin sections were stained with ferric haematoxyline and light microscopy (LM) was conducted with a Zeiss Axioplan light microscope. Photographs were processed in AxioVision 4.0 (Carl Zeiss, Oberkochen, Germany). Ultrathin sections for transmission electron microscopy (TEM) were contrasted with ethanolic (50 %) uranyl acetate for 12 min and lead citrate for 10 min. TEM was conducted on a Siemens Elmiskop 1A transmission electron microscope at 80 kV.

3. Results

Two distinct phases were observed during oogenesis. After their detachment from the medulla of the rhodoid, the oocytes migrate to the proximal part of the meros, situated latero-rostrad. Here, the first phase of autogenous growth, multiplication of mitochondria and accumulation of numerous free ribosomes and polysomes ensues, which is termed previtellogenesis (Figs 1, 2). Vitellogenesis, indicated by the accumulation of yolk material from exogenous sources, ensues in the second part of the meros, situated dorsal of the proximal part and oriented caudally. Oocytes entering this part are cuboid, approximately 20–35 µm in diameter and, as a nucleus, contain a large central germinal vesicle with numerous nuclear pores and a prominent reticulate nucleolus. They stain basophilic in light microscopy, usually exhibiting a zone of even darker staining, the nuage, characteristic of previtellogenesis. In electron microscopy, they show a dense, finely grained cytoplasm containing numerous free ribosomes and polysomes, regions of well developed smooth endoplasmic reticulum (ER) containing an electron-lucent material and groups of mitochondria of the crista-type scattered throughout the cell volume.

An overview over a semithin sectioning of *A. longisetosus* is shown in Fig. 2. The follicular cells encompassing the individual oocytes at the onset of vitellogenesis possess a cytoplasm less electron dense than the oocytes and contain large, more or less flattened nuclei, with a single dense nucleolus and several heterochromatin bodies embedded in uniformly grained euchromatin. They generally contain several mitochondria and strands of rough and smooth ER as well as well-developed Golgi bodies. Follicular cells are delimited against the haemolymph space and surrounding tissues by a fine basal lamina (Fig. 3).

At the onset of vitellogenesis, a perivitelline space opens up between the oocyte and the surrounding follicular epithelium (Fig. 3). A coarsely grained, electron-lucent material is deposited in the perivitelline space, apparently constituting the first vitelline envelope. The oolemma develops densely arranged microvilli. These are of irregular shape and orientation, approximately 60–80 nm in diameter, and penetrate the vitelline envelope to come in close contact with the extensions of the follicular cells (Fig. 3).

With ongoing development, the cytoplasm of the oocyte shows an overall loss of basophily, as indicated by lighter staining with ferric haematoxyline (Fig. 2). Closer examination using electron microscopy reveals a complex development of cytoplasmic structures. First yolk vesicles appear in the cortical plasma (Fig. 4). The cytoplasm of the oocyte starts to differentiate into a dense portion, resembling that of the previtellogenetic oocytes, and a portion of reduced density and ribosome content (Fig. 5). The latter at first starts as a network of strands surrounding the germinal vesicle and, with growing total volume of the oocyte, develops into a voluminous zone of electron-lucent cytoplasm devoid of major organelles (Figs 5, 6). This lucent cytoplasm makes up for most of the oocyte volume, leaving areas of

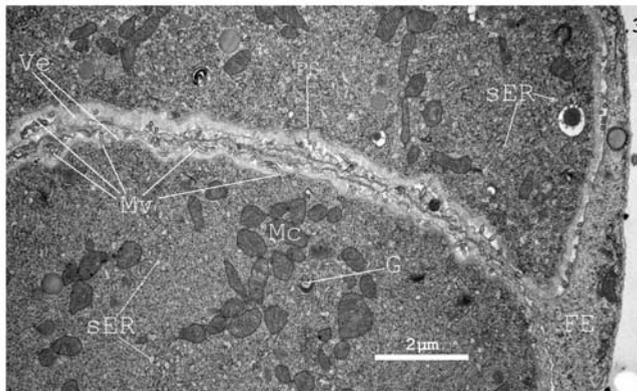
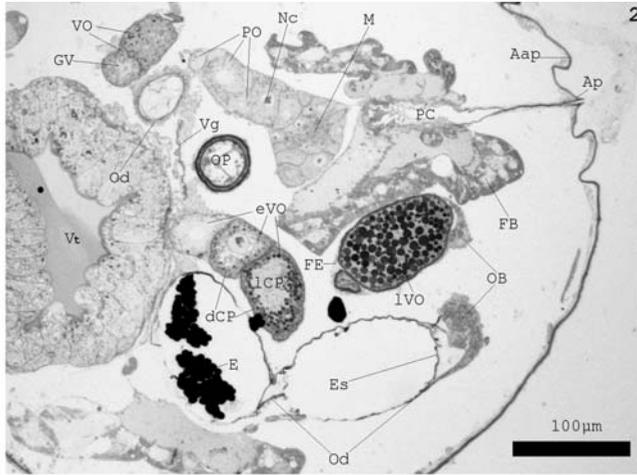
dense cytoplasm only in the vicinity of the germinal vesicle and along the cell periphery. Organelles important for the cell metabolism such as Golgi bodies, mitochondria and ER remain concentrated in this peripheral cytoplasm. Within this region, also new proteinaceous yolk vesicles and groups of electron lucent droplets, appearing pale greyish-yellow in light microscopy and likely representing lipids, start to emerge and develop independently of each other, generally showing a centripetal progression towards the centre of the oocyte (Fig. 2). Proteinaceous yolk vesicles appear simultaneously or slightly prior to lipid droplets. They invade the space occupied by the lucent cytoplasm, yet always stay embedded in and connected by strands of dense cytoplasm, leading to a voluminous central mass of yolk with a network of dense and lucent cytoplasm. Individual yolk vesicles start as medium-sized vesicles (1–3 μm), filled with a uniform material of medium electron density. With further development, they enlarge and a regular pattern of higher density starts to emerge from the uniform content. Fully developed yolk vesicles measure 7–10 μm in diameter and contain a central mass of dense material arranged in a crystalline pattern on a relatively clear background, likely to resemble tightly packed yolk protein. One to several (up to ~5) individual crystallites may be present within one yolk vesicle. Towards the vesicle membrane, this mass is surrounded by a finely grained zone of medium electron density (Figs 12–15). On one side, between vesicle membrane and central mass, yolk vesicles generally show a conspicuous disk-shaped cap of high electron density and uniformly grained structure (Fig. 9) that apparently emerges from an irregularly shaped precursor (Fig. 7). Lipid droplets develop either singularly or in groups between the proteinaceous yolk vesicles. They are apparently not surrounded by a phospholipid bilayer membrane. In late vitellogenesis, lipid droplets of about 0.5–1 μm in diameter occupy the space between yolk vesicles leaving only thin strands of dense and lucent cytoplasm in the centre of the oocyte.

The uptake of yolk precursors by the oocyte is indicated by the formation of a large number of coated pits at the base of the microvilli, being incorporated into the oocyte as coated vesicles measuring 120–140 nm in diameter (Fig. 7).

As the oocyte grows, the microvilli fringe gradually becomes less dense, yet more coated vesicles appear in the cell periphery (Figs 3, 7–9, 11).

The vitelline envelope is at the same time increasing both in thickness and apparent electron density, yet retaining its granular appearance. In the latest vitellogenic oocytes, the layer of vitellar envelope material is roughly 1 μm in thickness. In pores and channels not occupied by microvilli as well as in the remaining perivitelline space, a finely grained substance appears. This substance resembles the content of coated pits and coated vesicles found in these stages (Fig. 7). The follicular epithelium in middle vitellogenesis shows well developed Golgi bodies as well as rough and smooth ER and mitochondria with circular cross sections of their cristae in a very lucent ground cytoplasm (Figs 7, 8).

Towards the end of vitellogenesis, the follicular cells tend to lose contact to each other, leaving only the basal lamina as delimitation between the haemolymph space and perivitelline space (Figs 9, 10).

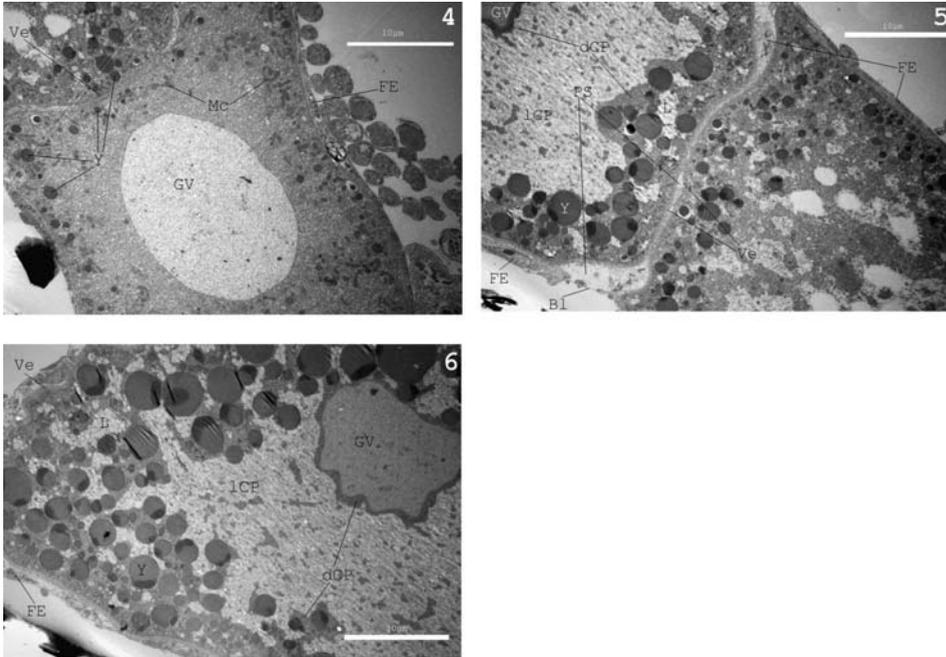


Figs 2–3 2: Light microscopy image of parafrontal semithin sectioning through adult *A. longisetosus*, stained with ferric haematoxyline. The plane of sectioning is slightly tilted to the right and close to the ventral body wall. All major parts of the genital system and oocytes in all major phases. Eggs break out of section due to insufficient fixation and infiltration through egg shell. Apparently empty spaces are artefacts of very large and delicate fat body cells being lost during the sample preparation.

Abbreviations: Aap: Adanal plate; Ap: anal plate; dCP: dense cytoplasm of vitellogenic oocyte; E: remains of egg within oviduct (partly broken out of section due to improper infiltration via eggshell); Es: egg shell; eVO: early vitellogenic oocytes; FB: fat body; FE: follicular epithelium; GV: germinal vesicle; ICP: lucent cytoplasm of vitellogenic oocyte; IVO: late vitellogenic oocyte; M: medulla; Nc: nucleolus of oocyte; OB: ovarian bulb; Od: oviduct; OP: ovipositor; PC: postcolon; PO: previtellogenic oocyte; Vg: vagina; VO: vitellogenic oocyte; Vt: ventriculus.

3: Transmission electron microscopy (TEM) image of ultrathin section from *A. longisetosus*. Proximal meros of an adult ovary. Two oocytes at the onset of vitellogenesis. Numerous microvilli and early vitellar membrane.

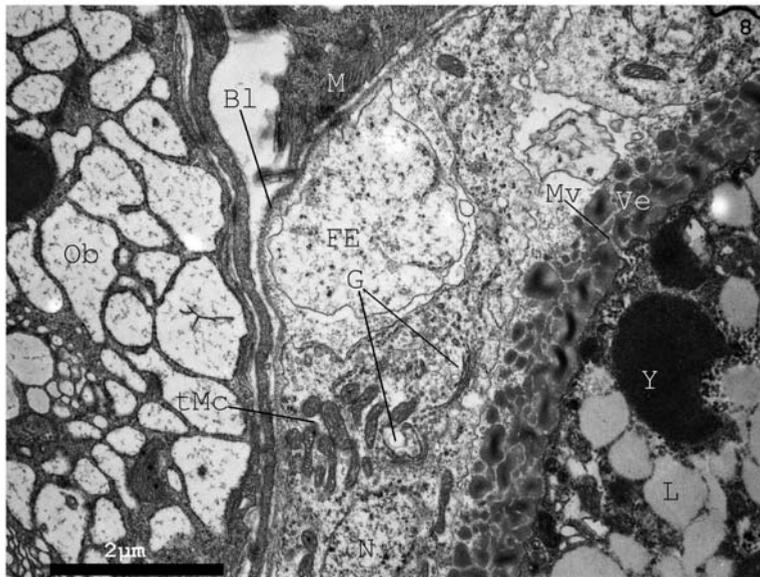
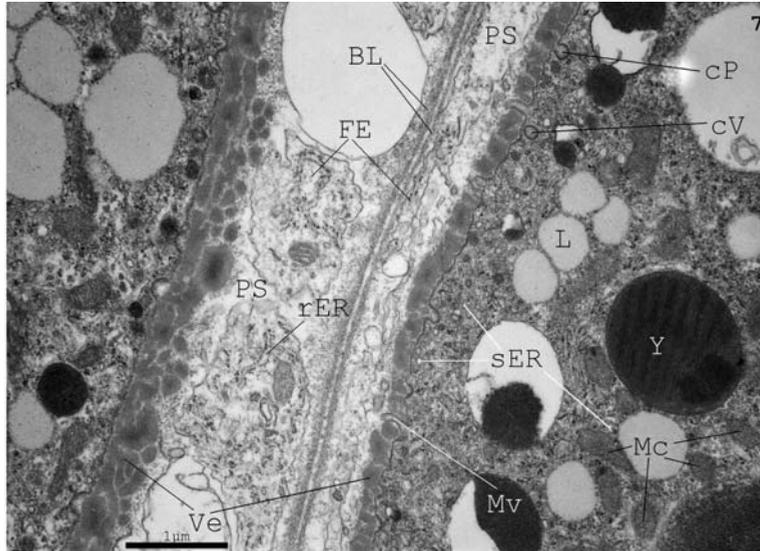
Abbreviations: FE: follicular epithelium; G: Golgi body; Mc: mitochondria; Mv: microvilli; sER: smooth endoplasmic reticulum; PS: perivitelline space; Ve: vitelline envelope.



Figs 4–6 4: TEM image of ultrathin section from *A. longisetosus*. Early vitellogenetic oocyte. Germinal vesicle in central position. Abbreviations: FE: follicular epithelium; GV: germinal vesicle; Mc: mitochondria; NP: nuclear pores; Ve: vitelline envelope; Y: yolk vesicles containing proteinaceous yolk.

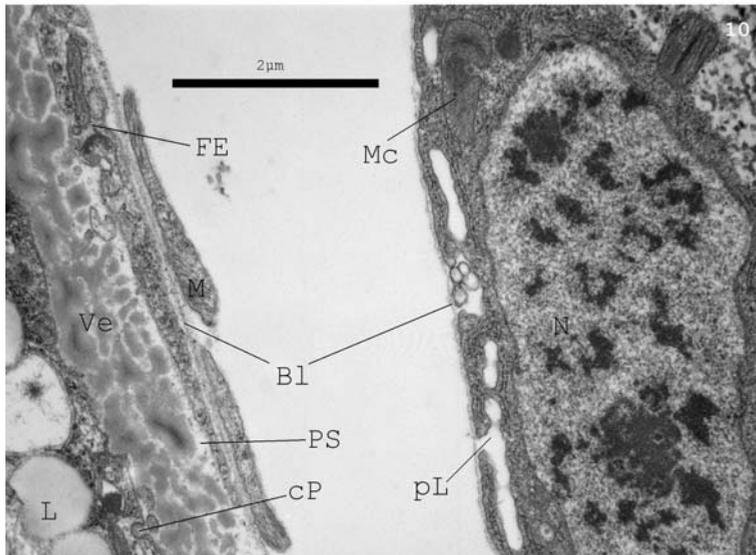
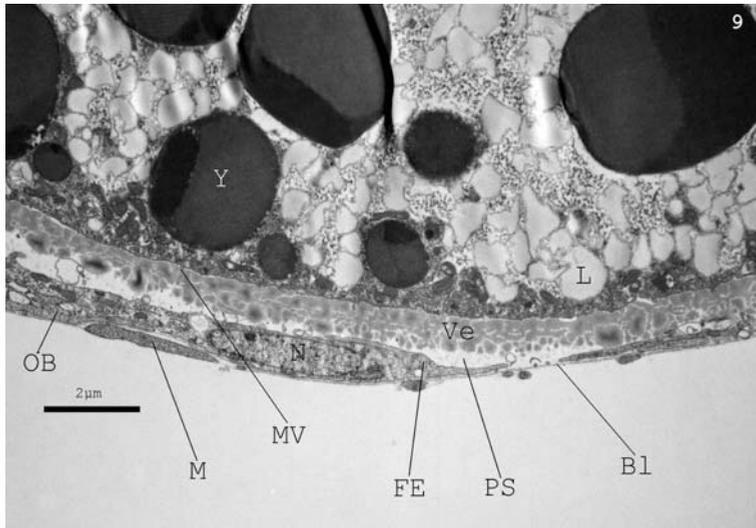
5: TEM image of ultrathin section from *A. longisetosus*. Oocytes later in vitellogenesis, adjacent to cell in Fig. 4. Abbreviations: BL: basal lamina; dCP: dense cytoplasm; FE: follicular epithelium; GV: germinal vesicle of oocyte in central position; L: lipid droplets; ICP: lucent cytoplasm; PS: perivitelline space; Ve: vitelline envelope; Y: yolk vesicle containing proteinaceous yolk.

6: TEM image of ultrathin section from *A. longisetosus*. Oocyte still further in vitellogenesis. Figure adjacent to Fig. 5. Abbreviations: dCP: dense cytoplasm; FE: follicular epithelium; GV: germinal vesicle; L: lipid droplets; ICP: lucent cytoplasm; PS: perivitelline space; Ve: vitelline envelope; Y: yolk vesicle containing proteinaceous yolk. Note ‘caps’ of denser material.



Figs 7–8 7: TEM image of ultrathin section from *A. longisetosus*. Meros of ovary. Two oocytes in vitellogenesis and follicular epithelium. Younger oocyte to the right, older one to the left. Abbreviations: BL: basal lamina; cP: coated pit; cV: coated vesicle; FE: follicular epithelium; L: lipid droplet; Mc: mitochondria; Mv: microvilli; rER: rough endoplasmic reticulum; sER: smooth endoplasmic reticulum; PS: perivitelline space; Ve: vitelline envelope; Y: yolk vesicle containing proteinous yolk.

8: TEM image of ultrathin section from *A. longisetosus*. Follicular epithelium and oocyte in late vitellogenesis at tip of ovarian meros. Abbreviations: BL: basal lamina; FE: follicular epithelium; G: Golgi body; L: lipid droplet; M: muscle strand; Mv: microvilli; N: nucleus of follicular cell; Ob: epithelium of ovarian bulb (oviduct); tMc: tubular mitochondria; Ve: vitelline envelope; Y: proteinous yolk vesicle.



Figs 9–10 9: TEM image of ultrathin section from *A. longisetosus*. Latest vitellogenesis at tip of ovarian meros, adjacent to the ovarian bulb of the oviduct. Abbreviations: Bl: basal lamina; FE: follicular epithelium; L: lipid droplet; M: muscle; Mv: microvillus; N: nucleus of follicular cell; OB: tissue of ovarian bulb; PS: perivitelline space; Ve: vitelline envelope; Y: proteinaceous yolk vesicle with characteristic dark ‘cap’.

10: TEM image of ultrathin section from *A. longisetosus*. Oocyte in late vitellogenesis adjacent to extraovarian nutritive cell. Abbreviations: Bl: basal lamina; cP: coated pit; FE: follicular epithelium; L: lipid droplet; M: muscle strand; Mc: mitochondrion; N: nucleus; pL: peripheral labyrinth; PS: perivitelline space; Ve: vitelline envelope.

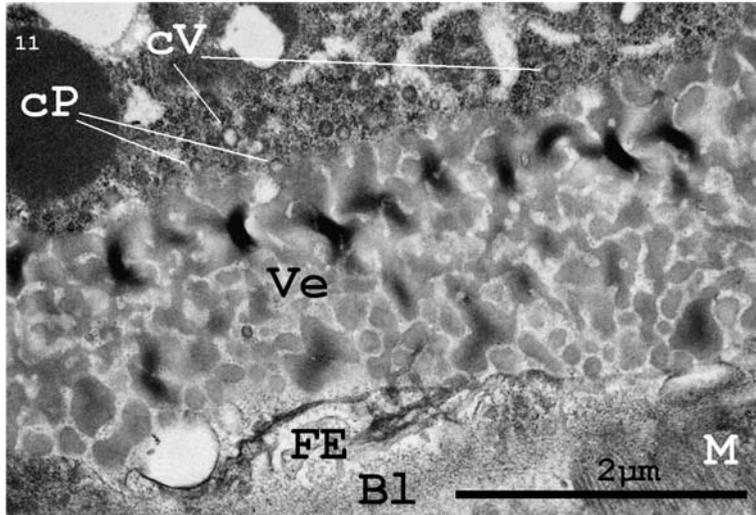
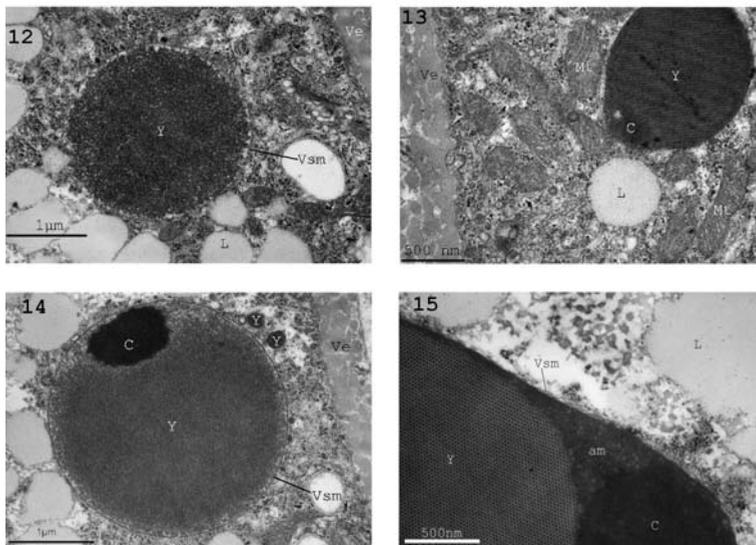


Fig. 11 TEM image of ultrathin section from *A. longisetosus*. Oocyte in late vitellogenesis. Vitellar envelope deposited, yet still porose. Microvilli mainly retracted, numerous coated pits and vesicles. Contact between adjacent follicular cells loosened and perivitelline space only delimited against haemolymph by basal lamina. Abbreviations: BL: basal lamina; cP: coated pits; cV: coated vesicles; FE: follicular epithelium; M: muscle; Ve: vitelline envelope.



Figs 12–15 TEM image of ultrathin section from *A. longisetosus*. Progression (12–15) of individual yolk vesicles and accumulation of crystalline yolk protein. Abbreviations: am: amorphous material in vesicles periphery; C: cap of amorphous material; L: lipid droplet; Mc: mitochondria; Ve: vitelline envelope; Vsm: vesicle membrane; Y: yolk protein.

4. Discussion

4.1. Classification of the ovary type

As was already noted before (Heethoff et al. 2007, Bergmann et al. 2008), the overall structure of the ovary in *A. longisetosus* is in accordance with the basic pattern for oribatids (Michael 1884, Alberti & Coons 1999). However, ultrastructural examinations reveal further anatomical and functional features to be discussed.

In contrast to the situation found in many Anactinotrichida, namely in cattle ticks (de Oliveira et al. 2005, Saito et al. 2005), the ovary seems to be structured in readily distinguishable functional regions in correspondance with the time course of oogenesis, a feature present in most arthropods (Saito et al. 2005). In *A. longisetosus*, previtellogenesis takes place in the proximal, ventro-latero-rostrad part of the ovarian meros (marked with black asterisks in Fig. 1). The accumulation of visible yolk vesicles and lipid droplets is restricted to the distal part of the meros, situated latero-caudad and dorsally of the proximal part (Bergmann et al. 2008). This section of the ovary may therefore be termed the vitellarium (marked with white diamonds in Fig. 1).

The oocytes develop outward from the germarium towards the body cavity, a general feature in chelicerates and some maxillopod crustaceans (Ikuta & Makioka 2004), but clearly in contrast to the situation found in insects and most crustaceans, where oocytes develop inside tubular ovarioles towards a lumen (Anderson 1973, Ando & Makioka 1999).

Unfortunately, the typology of ovaries as panoistic, meroistic-polytrophic or meroistic-telotrophic was developed mainly on insect ovaries, and there is ongoing debate which type of ovaries is realised in different groups of mites (Cabrera et al. 2009, and cited references). Consequently, the transfer of this typology to mites, for want of a better alternative so far, has to rely on the convergently evolved conformance of functional and anatomical definitions and cannot bear implications of homology.

The oocyte in *A. longisetosus* loses contact with the medulla of the germarium early in previtellogenesis. From this event on, the oocyte is completely covered in follicular cells and never ruptures the basal lamina or even bulges individually into surrounding tissues throughout vitellogenesis (Bergmann et al. 2008), in contrast to many arachnid groups studied so far (Alberti 1974, Shatrov 1997, and cited references, Talarico et al. 2009). A situation resembling a meroistic ovary seems unlikely, as neither an individual association of ovarian nutritive cells with one oocyte (polytrophic), nor a connection of vitellogenic oocytes to the germarium via stalks (telotrophic) could be demonstrated. A nutritive central cell was described for Astigmata (Witaliński et al. 1990, Walzl 2004), which are undergoing nutritive oogenesis. This situation is very similar to meroistic-telotrophic ovaries in insects (Schwaha et al. 2008). Although *A. longisetosus* shares many similarities in vitellogenesis (see paragraph 4.2) to the solitary type of oogenesis described in the trombiculid mite *Hirsutiella zachvatkini* Schluger, 1948 (Shatrov 1997), trophic cells within the ovary and associated to oocytes would suggest a meroistic situation in the latter case. In view of the functional arrangement of tissues in the ovary of *A. longisetosus*, it most likely resembles a panoistic ovary. A panoistic ovary has also been described for the brachyphylax oribatid mite *Ceratozetes cisalpinus* Berlese, 1908 (Woodring & Cook 1962).

4.2. Vitellogenesis

The process of vitellogenesis during the accumulation of visible yolk vesicles, with a high rate of endocytosis indicated by the dense microvilli fringe in younger and numerous coated pits in later oocytes, resembles the exogenous type in *H. zachvatkini* (Shatrov 1997). This is in contrast to the endogenic vitellogenesis in the brachypiline mite *Hafenrefferia gilvipes* C. L. Koch, 1840 (Witaliński 1986). Accumulation of storage material is putatively accomplished by the uptake of yolk precursors as lipoproteins by the oocyte via the follicular cells. A likely source for these substances is the well-developed nutritive tissue (possibly fat body derivatives) in the region of the genital system of adult females with its abundant rough ER and peripheral labyrinth (Figs 2, 10). These nutritive cells, lying in the same body compartment as the fat body cells, i.e. the haemocoel, are likely to play an intermediate/synthetic role in the process, as the fat body cells in turn are connected to the digestive system via fingerlike protrusions of midgut wall cells (Alberti et al. 2003). The fat body structurally separates the digestive from the genital system, i.e. the calory source from the calory sink, in all specimens examined. Adults of *A. longisetosus* are rather slow-moving animals, yet produce an average of 1.3 eggs per day and up to over 300 eggs during their adult life span (Heethoff et al. 2007). Therefore, this pathway could well account for a major portion of the netto calory flux within the individual mite. An active role of the follicular epithelium in lipoprotein turnover may also be inferred by the presence of mitochondria with circular cross-sections of their cristae in late vitellogenesis (see paragraph 4.4).

Notable in *A. longisetosus* is the well developed microvilli fringe of the oocyte, typical for many chelicerates, but not described in the brachypiline oribatid mite *H. gilvipes* (Witaliński 1986). For example, the combination of a microvilli fringe during vitellogenesis and endocytotic pits in late vitellogenesis with simultaneous deposition of a layered egg shell was also shown for a member of Ricinulei, *Pseudocellus boneti* Bolivar y Pieltain, 1941 (Talarico et al. 2009). The absence of microvilli on the oocyte during vitellogenesis therefore may not be a common oribatid feature, but represent a derived state.

However, the oribatids *A. longisetosus* and *H. gilvipes* share the feature of simultaneous vitellogenesis and deposition of the vitelline envelope, as is characteristic for mites (Witaliński 1986, Shatrov 1997, and cited references). Furthermore, they both exhibit the opening of the perivitelline space, first described in *H. gilvipes* (Witaliński 1986). The former two processes are completed within the ovary, as has been described also for the middle-derivative oribatid mites, *Plathynothrus peltifer* C. L. Koch, 1839 and *Trhypochthonius tectorum* Berlese, 1896 (Taberly 1987b), but neither for the brachypiline *C. cisalpinus* (Woodring & Cook, 1962) nor the astigmatic mite *Sancassania berleseii* Michael, 1903 (Walzl et al. 2004).

In *A. longisetosus*, the differentiation of the cytoplasm of the oocyte, the centripetal accumulation of yolk vesicles between the dense peripheral and perinuclear cytoplasm and the development of individual yolk platelets resemble the situation in *S. berleseii* (Walzl et al. 2004).

4.3. Vitelline envelope structure and microvilli fringe

In *A. longisetosus*, the vitelline envelope is neither lamellated as in *S. berlesei* (Walzl, et al. 2004), nor shows a layered structure nor distinct phases of deposition as in *H. zachvatkini* (Shatrov 1997) or *H. gilvipes* (Witaliński 1986). It also does not solidify prior to the end of vitellogenesis as in *H. gilvipes* (Witaliński 1986). By contrast, it stays porose until the completion of vitellogenesis, with oocytal microvilli protruding in the pores, as described for various members of Prostigmata and Mesostigmata (Witaliński 1986). The protruding microvilli of the oocyte in *A. longisetosus* do not exhibit the apical flattening noted in the two latter groups, and, although gradually becoming less electron lucent, the vitelline envelope never reaches the electron density as demonstrated for Mesostigmata and Prostigmata (Witaliński 1986). A porose vitelline envelope during vitellogenesis, but without the protruding oocytal microvilli, has also been shown to exist in *S. berlesei* (Walzl et al. 2004). The decreasing number of microvilli with a simultaneously increasing number of coated pits and vesicles could probably be due to different uptake mechanisms for different compounds in the course of yolk formation in *A. longisetosus*.

4.4. Considerations on molecular processes

As both follicular epithelium and oocytes contain organelles of synthetic activity, such as rough and smooth ER, or the Golgi bodies, it seems quite difficult to infer the origin of vitelline envelope material from ultrastructural analysis only. Vesicles and ER canals with contents matching the electron density of the vitellar envelope could be found in both follicular cells and early vitellogenic oocytes, yet seem to be missing in late vitellogenic oocytes. Without specific histochemical markers for the compounds involved in the formation of the vitelline envelope, we can not classify the observed uniform material as a primary or secondary vitelline envelope (*sensu* Raven 1961, Witaliński 1986) with certainty.

A peculiar feature of follicle cells in *A. longisetosus* is the presence of mitochondria that show circular cross-sections of their inner membrane. The internal organisation of mitochondrial membranes may vary significantly between and even within tissues of the same individual due to changing cytoplasmal conditions brought about either by physiological processes or artefacts of the fixation process (Frey & Manella 2000). Although differential reactions of the tissues to the fixation protocol cannot be ruled out in this study, the presence of tubular cristae rather than lamellar ones was found in both vertebrates and arthropods to coincide with a higher activity of oxygenases as opposed to ATP-synthesis. These are involved in lipid metabolism, e.g. the breakdown of cholesterol and steroid production (Sumegi et al. 1988, Blass & Ruthmann 1989, Scheffler 2001).

4.5. Perspectives

Vitellogenesis is a multi-stage process including nutrient uptake, synthesis of vitellogenin, its transformation into vitellin, transport of yolk precursors as lipoproteins and their incorporation into the oocyte as well as the production of regulating hormones. It seems plausible that different stages of the process are distributed among different tissues in *A. longisetosus*. As several of these metabolic events most likely include cytochrome-related enzyme complexes, it seems feasible for future studies to clarify the contribution of candidate tissues such as midgut wall, fat body, nutritive cells and, by view of their mitochondria, follicle cells, using specific markers and labelling techniques.

Histochemical and molecular studies of the process of vitellogenesis and its hormonal regulation by juvenile hormone and/or the steroid ecdysone have already been conducted in a number of acarine taxa, mostly in the Ixodidae (e.g. James & Oliver 1999, Cabrera et al. 2009). Yet, such studies are still lacking in actinotrichid mites. Corresponding examinations could confirm the model inferred from morphology involving the fat body and the nutritive cells surrounding the genital organs, locate synthesis and postranslational processing of the proteins, clarify the role of the follicular epithelium and adjacent tissues, and generally deepen our understanding of vitellogenesis in the Acari and its evolutionary history.

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