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Fine structure of the ovary of *Schizomus palaciosi* (Arachnida: Schizomida)

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Abstract

The ovary of *Schizomus palaciosi* is an unpaired structure located in the medioventral opisthosoma. It consists of a flat tube with compressed lumen. The wall of the ovarian tube is composed of a monolayer of epithelial cells and muscle cells. Early oocytes are embedded in the dorsal wall of the ovarian tube. Here the first growing phase starts increasing the amount of cytoplasm and the size of the nucleus. This increase results in an outward movement (externalization) of the oocyte, i.e., the oocyte grows into a pouch made of the basal lamina of the epithelium and is finally almost completely exposed towards the hemolymphatic space or adjacent tissues. A funicle made of epithelial cells connects the oocyte with the ovarian tube. Now, solitary vitellogenesis starts and the oocyte becomes much larger (second growth phase) depositing a complex protein yolk and lipid yolk in the cell body. In the outward stages, the funicle cells facing the oocyte secrete material that is deliverd into the space between funicle and oocyte. Thus it is added to the simple basal lamina of the ovarian epithelium which first formed the pouch alone. The pouch thus consists finally of two more or less distinct layers. A vitelline envelope which is deposited between pouch and oocyte in most Chelicerata as a primary egg shell parallel to vitellogenesis was not observed. Fine structure of the cell types involved are dealt with for the first time for a schizomid species. Results are discussed under comparative and functional aspects.

Keywords externalization | short-tailed whip-scorpions | oogenesis | ultrastructure

1. Introduction

Schizomida or short-tailed whip-scorpions is a small group of Arachnida comprising 284 species world wide of which 35 occur in Mexico (Palacios Vargas et al. 2014). It is ranked as an arachnid order equal with, e.g., Scorpiones, Araneae, Amblypygi or Thelyphonida (giant whip-scorpions) (Levi 1982, Zhang 2011). Thelyphonida, Schizomida, Amblypygi and Araneae comprise the monophyletic taxon Tetrapulmonata. Thelyphonida and Schizomida represent sister-groups forming the taxon Uropygi (e.g., Moritz 1993, Sharma et al. 2014). Schizomida (mostly around 4–5 mm in body length) live as predators of small arthropods in humid leaf-litter of soils, under stones and in caves mostly in tropical to subtropical regions (Moritz 1993).

Sexes in Schizomida may easily be recognized due to the differently shaped so-called flagellum at the posterior end of the opisthosoma. It is, in contrast to Thelyphonida, which have a long annulated flagellum in both sexes, a short appendage in females with only 3–5 flagellomeres. In male schizomids it is a broad process with more or less distinct dorsal depressions and/or projections to which the female clings with its chelicerae during mating (Sturm 1958, 1973, Kraus & Beck 1967). After a tandem-walk, the male deposits a spermatophore onto the substratum and guides the female over it, which takes up the spermatophore with its genital opening. Spermatozoa are

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stored in small pouches of the genital atrium, the seminal receptacles (= spermathecae; Börner 1904, Rowland & Reddell 1979, Reddell & Cokendolpher 1986, 1995, 2002, Miyazaki et al. 2001). Only few observations have been reported on egg laying and parental care (Warren 1939, Rowland 1972, Brach 1976). Up to 30 eggs stick to the female's ventral opisthosoma due to secretion. It is not known where this secretion is produced. Soil dwelling schizomids burrow small chambers into the substratum, where they stay for oviposition and some further time carrying the hatched offsprings on the opisthosoma (Rowland 1972). Cave dwelling species are also carrying eggs active on the surface (Moritz 1993).

Schizomida are rarely studied and belong to the least investigated arachnids with regard to internal anatomy. In particular the genital system has rarely been investigated. There are only few observations included in the studies of, e.g., Börner (1904), Kästner (1931–1941) and Millot (1939, 1949) concerning the female genital tract and recent additional results by Miyazaki et al. (2001). According to these authors, the female genital tract consists of an unpaired tubular ovary located in the ventral opisthosoma. From its anterior end, a pair of oviducts runs anteriorly. These join to form a short unpaired uterus that opens into the cuticle-lined genital atrium. This leads towards the genital opening located in the second opisthosomal segment; the opening is covered by an unpaired genital plate. Up to three pairs of seminal receptacles open into the genital atrium. In *Schizomus palaciosi* there are only two pairs (Reddell & Cokendolpher 1986). Not earlier than 1960, the first study on the male genital system was published (Modder 1960). The only fine structural study on a schizomid genital system published until now, is that of Alberti & Palacios-Vargas (1987) on spermatogenesis and spermatozoa of *Schizomus palaciosi*. Here, we report on the fine structure of the ovary of this species.

2. Materials and methods

Three specimens of *Schizomus palaciosi* Reddell & Cokendolpher, 1986 including one female were collected by one of us (P.-V.) in March 1983 from the Grutas de Acuitlapán, Mexico. Later they were cut into pieces and immersed in ice-cold glutaraldehyde (3.5 % in phosphate buffer, pH 7.5, 0.1 M) for 2 hours. Subsequently, they were transferred into the fixative diluted with bufffer solution (1:4) and were then mailed to Germany for further treatment. After rinsing with the buffer solution (2h) and postfixation with OsO_4 (2 % aqueous solution, 2h), the tissues were rinsed again with buffer solution (10 min), dehydrated in graded ethanols (70 %, 80 %, 90 %, 95 %, absolute) and embedded in Araldite using propylenoxide as an intermedium. Ultrathin sections (70 nm) of the female specimen were obtained with a Leica Ultracut (Leica EM UC6) using a Diatome diamond knife. The sections were stained for 8 min with uranylacetate and for a further 8 min in lead citrate according to Reynolds (1963). Transmission electron microscopic (TEM) investigations were performed on a JEOL Jem-1011. For general orientation with the light microscope semithin sections (400 nm) were stained according to Richardson et al. (1960). For light microscope (LM) an Olympus BX60 provided with an Axio Cam MRC Zeiss digital camera was used.

3. Results

General

The ovary is an unpaired flat tube extending medioventrally through the opisthosoma (Fig. 1A, B). It consists of an ovarian wall composed of epithelial cells that surround a hardly detectable, narrow lumen. Small muscle cells are located between basal regions of epithelial cells. Germ cells, oogonia and early oocytes are embedded mainly in the dorsal ovarian wall and are thus surrounded by the somatic cells mentioned. During growth, previtellogenic oocytes start to bulge laterally from the ovarian tube pushing aside other tissues, e.g., the fat body (= intermediate tissue). The later, much enlarged stages of oogenesis full of yolk are only found at the ventral side of the tubular ovary, where these almost mature eggs bulge widely from the ovarian tube into the body cavity and may almost touch the ventral integument (Figs 1A; 6F, H).

Ovarian epithelium

The main component of the ovarian wall consists of a monolayer of rather small epithelial cells that contain, as their most conspicuous and distinctive structure nuclei of slightly irregular shape (Figs 1B, 2A, 9). The nuclei are densely staining because of their high amount of heterochromatin (Figs 2A, F; 3A; 4A–C). Small nucleoli may also be found (Fig. 3A). Some nuclei are almost completely electron-dense (Figs 2A, 4B). Free ribosomes are quite abundant (Fig. 2E). Other organelles are rare, e.g., small mitochondria, few rough cisternae of the endoplasmic reticulum and some microtubules (Fig. 2F–I). Occasionally some clusters of glycogen rosettes (α-particles) and lipid inclusions (Figs 2G,

3C) are observable. The strongly interdigitating cells are apically connected via short zonulae adhaerentes (Fig. 2D, E) followed by long cell junctions, probably representing septate junctions (Fig. 2B–F). But since the intercellular cleft is filled with dense material, septae were hardly visible. Also gap junctions seem to occur (Fig. 2D). As mentioned already, the lumen is hard to detect (Fig. 2A,B). In most areas the ovarian tube is compressed. In small regions where it is slightly widened, it contains a dense homogenous material (Fig. 2A, C, E). The ovarian wall is underlain by a thin basal lamina (Fig. 2G, 3C, about 50 nm) which frequently is intensively folded following the folds of the basal cell membranes of the epithelial cells (Fig. 2G, J; 7A).

Muscle layer

Small strands of cross-striated muscles are regularly found embedded in the basal parts of the ovarian epithelium thus forming an integral part of the ovarian wall (Figs 2A, F, G, J; 3A–C). The nuclei of the muscle cells (Fig. 3B) are rather similar to those of the epithelial cells. The nuclei containing somata are on the same level with the nuclei of the epithelial cells. A pair of coaxially arranged centrioles was occasionally observed close to the nucleus (Fig. 3B). Gap junctions appear to exist between muscle cells and epithelial cells (Fig. 3C).

Germ cells

Only four stages of oogenesis could be observed in the studied specimen. Indications of cell divisions were not seen.

Stage 1: Small cells, which are completely embedded into the ovarian epithelium, are observed as the earliest stage of oogenesis representing early oocytes (Figs 1B, 2A, 4A, B; 9). The cells are slightly larger than those of the epithelial and muscle cells, quite electron-lucent and contain a larger, elongated nucleus with some heterochromatin patches. These patches appear less compact than those of the somatic cells. The nuclear envelope has some pores (Figs 4B inset). Small

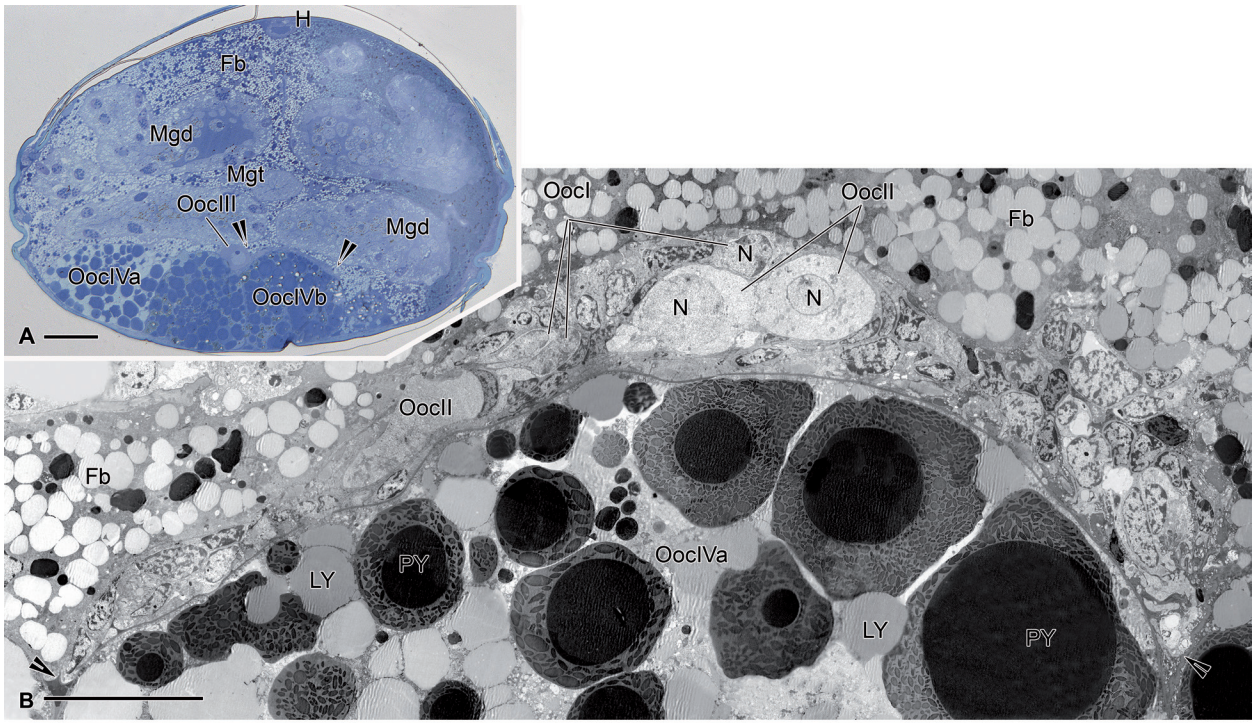

Figure 1. Overview of a transversely sectioned opisthosoma with the ovary of *Schizomus palaciosi*. (**A)** Location of the the ovary in the opisthosoma (LM). Note the relatively small, flat ovarian tube and the large, nearly mature oocytes (stage IV). In stage IVa the yolk and cytoplasm is not as condensed as in stage IVb. Arrowheads indicate lateral borders of the flat ovarian tube. A previtellogenic oocyte in stage III protrudes from the ovarian tube. Scale bar: 100 µm. (**B)** Overview of the ovary in higher magnification (TEM) sectioned in a position in the opisthosoma different from Fig. 1A. Arrowheads indicate lateral borders of the flat ovarian tube. A lumen is not visible in the ovarian tube in this magnification.Oocytes in stages I, II and IV are shown. Oocyte I and II are located in the epithelium of the ovarian tube. Note the increased size of the oocytes after vitellogenesis. In the stage IVa oocyte, the complex structure of the protein yolk is evident. Scale bar: 25 µm.

Abbreviations: **Fb** – fat body, **H** – heart, **LY** – lipid yolk, **Mgd** – midgut diverticle, **Mgt** – midgut tube, **N** – nucleus, **OocI, II, III, IVa,IVb** – oocyte in stages I, II, III, IVa, IVb; PY, protein yolk.

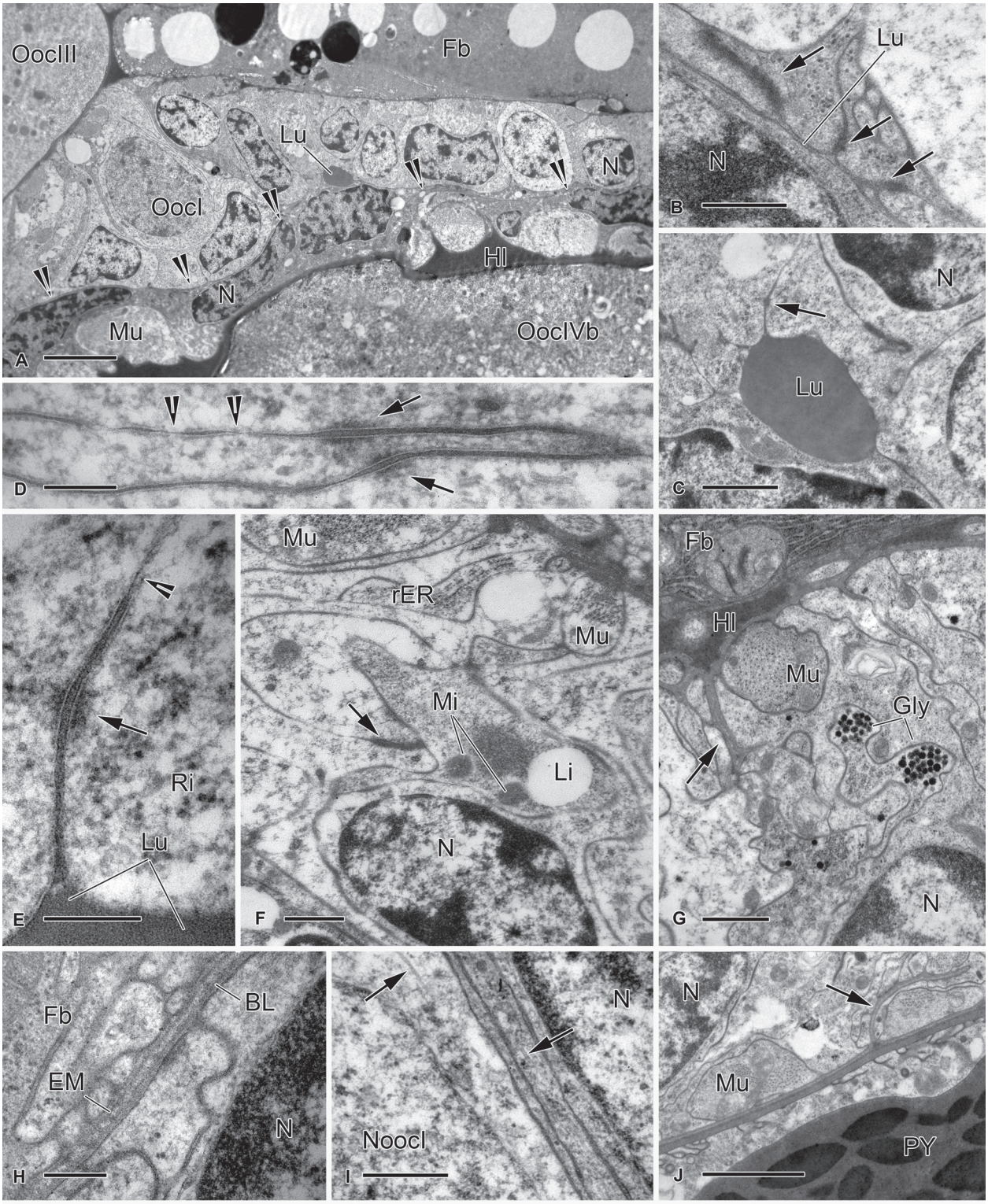

Figure 2. The ovarian tube (TEM). (**A)** Part of the ovarian tube showing a mostly inconspicuos lumen (arrowheads). The wall of the ovarian tube is made of epithelial cells and muscle cells. An oocyte I is embedded in the epithelium. Oocytes III and IV are externalized. Scale bar: 5 µm. (**B)** A detail showing the narrow lumen of the ovarian tube. Arrows point to zonulae adhaerentes. Scale bar: 0.5 µm. (**C)** Detail showing a region of the ovarian tube with widened lumen (compare Figs 2A, E). Arrow points to zonula adhaerens. Scale bar: 1 µm. (**D)** Here zonulae adhaerentes (arrows) connecting adjacent cells close to their apices and two putative gap junctions are shown. Scale bar: 0.2 µm. (**E)** Detail of Fig. 2C showing short zonula adhaerens (arrow). Note dense material in the intercellular cleft. Arrowhead points to putative gap junction. Scale bar: 0.2 µm. (**F)** Some organelles in the epithelial cells of the ovarian tube: heterochromatin-rich nucleus, few mitochondria, cisterns of rough ER and lipid inclusions. Arrow indicates zonula adhaerens. Note interdigitating cells. Scale bar: 1 µm. (**G)** Here some glycogen rosettes (α-particles) are visible in the epithelium. Muscle cells are embedded in the epithelium (compare Fig. 3). Arrow points to deep fold of the epithelium. Scale bar: 1 µm.

mitochondria are scarce. The peripheral cell membrane is smooth and there are no cell junctions between the germ cells and adjacent epithelial cells. This latter feature also applies for the following stages.

Stage 2: Previtellogenic oocytes have considerably grown but are still surrounded completely by the epithelial cells (Figs 1B, 4G, 9). The nucleus is much larger and more electron-lucent, almost devoid of heterochromatin. It is provided with a spherical nucleolus and the nuclear envelope has numerous pores (Fig. 4C–E). The cytoplasm is granular containing numerous ribosomes. The number of small mitochondria forming a cluster at one pole of the cell has increased (Fig. 4D). Small Golgi bodies were seen that produce electron-lucent vesicles (Fig. 4C, E).

Stage 3: The oocytes of this stage are mostly located close to the lateral edges of the ovary and bulge from the ovarian tube pushing aside the adjacent tissues being thus transferred outward (externalized) (Figs 1A, 5A, 7A, 9). They are connected with the ovarian tube via epithelial cells which form a more or less extended funicle (Figs 5A, 7A; see below). Stage-3-oocytes are, except for that pole which faces the ovarian tube or funicle, surrounded by a thin, dense layer of extracellular material of varying thickness according to the stage of its development (Figs 5A inset, 8A, E, F). The cells have a large spherical nucleus containing no heterochromatin. A spherical nucleolus is found close to the nuclear envelope (Fig. 5B). The nuclear envelope has numerous pores (Fig. 5C). Small dense granules are distributed around the nuclear envelope.These particles eventually fuse to form larger aggregates (Fig. 5B, C). Except for numerous ribosomes and small mitchondria there are only very few dense inclusions and some electron-lucent vesicles of different sizes visible (Figs 5A, D; 7A). The mitochondria are frequently connected to small dense spheres (Fig. 5D). The cell membrane of the oocyte is nearly smooth (Figs 5A, 7A).

Stage 4: These are huge vitellogenic cells (3–4 in a cross section), which are exclusively located ventrally of the ovarian tube (Fig. 1A, B) and may touch and compress the ventral integument (Fig. 1A, 6H). Compared to stage 1 oocyte, they have increased in size at least 20 times. Due to differences in density of the cytoplasm and yolk we could distinguish two substages IVa and IVb with the most condensed (advanced) stages located close to the medioventral axis of the ovary (Fig. 1A).

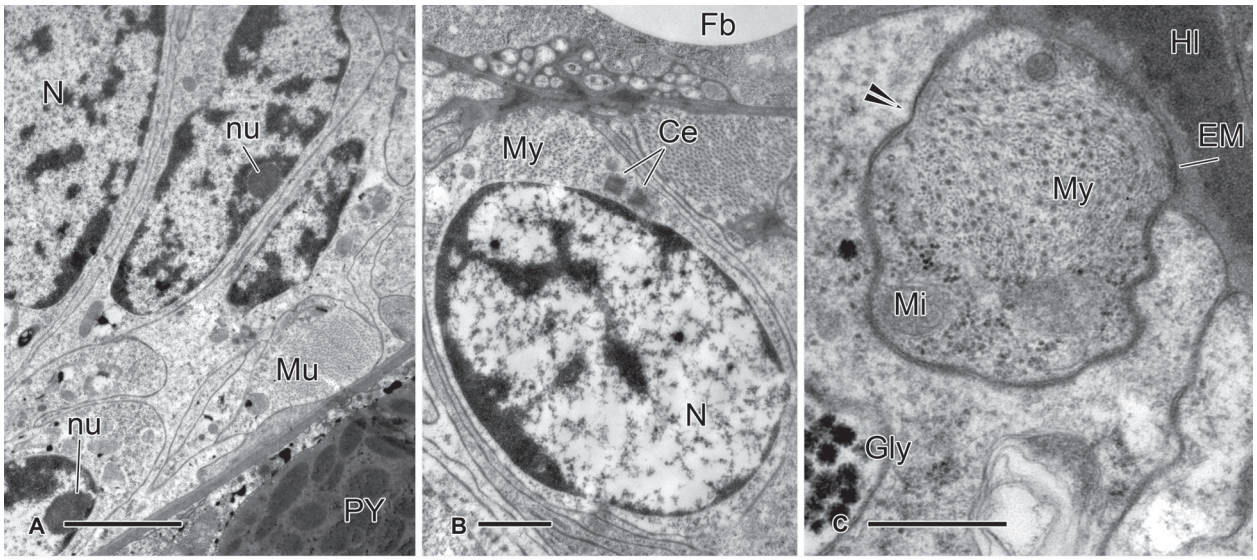

Figure 3. Muscle cells of the ovarian tube (TEM). (**A)** Epithelial and muscle cells form the wall of the ovarian tube. Nuclei of epithelial cells with nucleoli. Scale bar: 2 µm. **(B)** Nuclear region of muscle cell. Note pair of centrioles. Scale bar: 1 µm. (**C)** Cell process of muscle cell with myofilaments. Arrowhead points to putative gap junction. Glycogen rosettes in adjacent epithelial cell. Both cell types are underlain by a common extracellular matrix.Scale bar: 0.5 µm.

Abbreviations: **Ce** – centriole, **EM** – extracellular matrix, **Fb** – fat body, **Gly** – glycogen, **Hl** – hemolymphatic space, **Mi** – mitochondrium, **Mu** – muscle, **My** – myofilaments, **N** – nucleus, **nu** – nucleolus, **PY** – protein yolk.

◄ (**H)** A basal part of the ovarian wall adjacent to the fat body. Note the thin layers of extracellular matrices of both tissues. Scale bar: 0.2 µm. (**I)** The epithelial cells contain microtubules, which are also visible in an oocyte I (arrows). Scale bar: 0.5 µm. (**J)** A basal part of the ovarian wall adjacent to an almost mature oocyte (stage IVa). The base of the wall is deeply folded (arrow). Scale bar: 2 µm.

Abbreviations: **BL** – basal lamina of ovarian epithelium, **EM** – extracellular matrix of fat body, **Fb** – fat body, **Gly** – glycogen, **Hl** – hemolymphatic space, **Li** – lipid inclusion, **Lu** – lumen, **Mi** – mitochondrium, **Mu** – muscle, **N** – nucleus (of epithelial cells), **NoocI** – nucleus of oocyte I, **OocI, III, IVb** – oocytes in stage I, III, IVb, **PY** – protein yolk in oocyte IVa, **rER** – rough endoplasmic reticulum, **Ri** – ribosome.

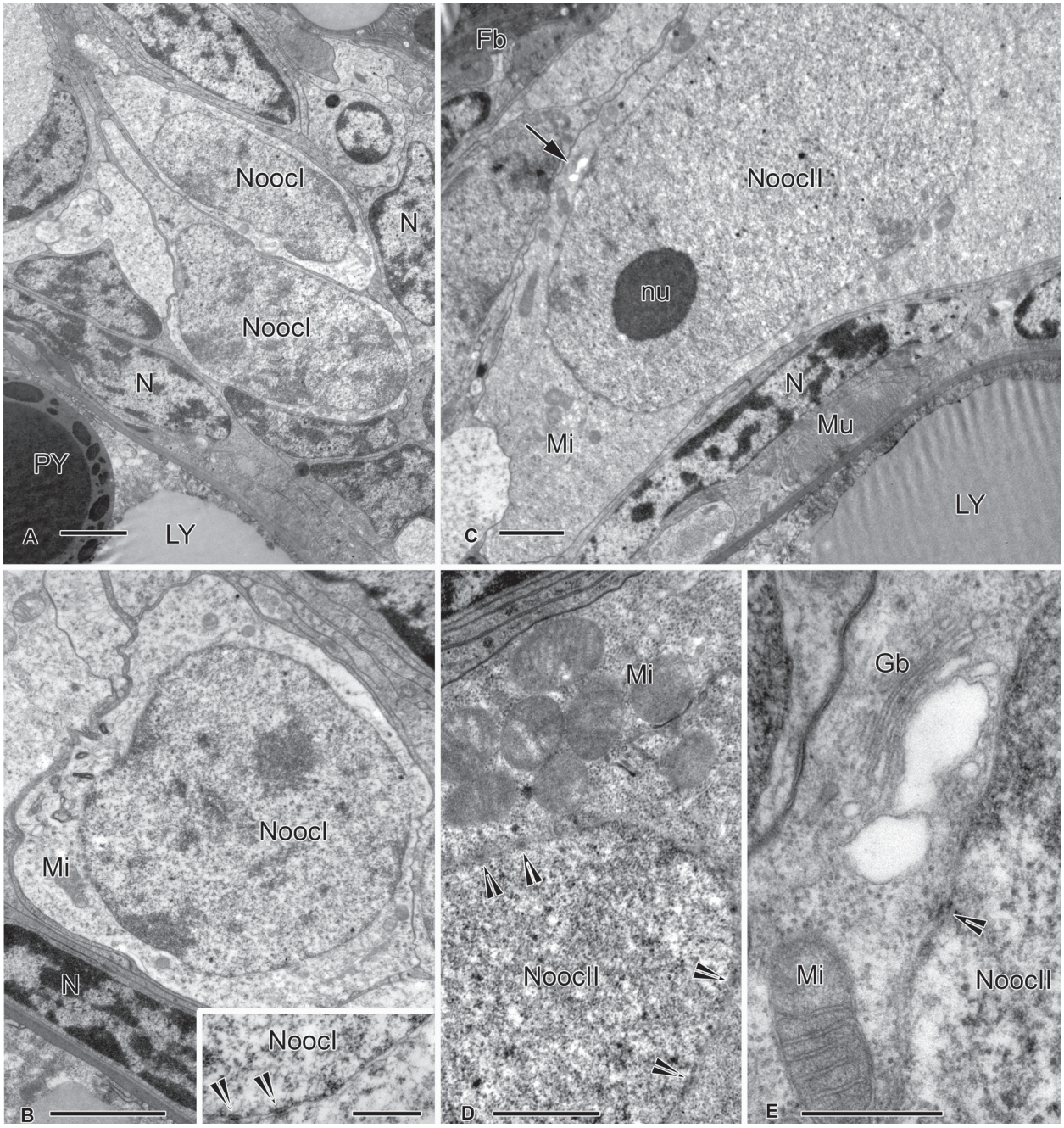

Figure 4. Oocytes in stage I and II embedded in the ovarian tube (TEM). (**A)** Two oocytes I. Note heterochromatin less condensed than in the nuclei of the epithelial cells. Scale bar: 2 µm. (**B)** Another oocyte I. The nucleus is surrounded by a relatively small amount of cytoplasm that contains only few organelles. Scale bar: 2 µm. Inset: Nuclear envelope of oocyte I shows rather few nuclear pores (arrowheads). Scale bar: 0.5 µm. **(C)** Oocyte II still embedded in the ovarian epithelium has grown considerably. Arrow points to Golgi body shown in Fig. 4E. Scale bar: 2 µm. (**D)** A cluster of mitochondria in an oocyte II. Arrowheads indicate nuclear pores. Scale bar: 1 µm. (**E)** Golgi body and mitochondrium in an oocyte II (compare Fig. 4C). Arrowhead points to nuclear pore. Scale bar: 0.5 µm.

Abbreviations: **Gb –** Golgi body, **LY –** lipid yolk, **Mi** – mitochondrium, **N –** nucleus (of epithelial cell), **NoocI, II –** nucleus of oocyte I, II, **PY –** protein yolk.

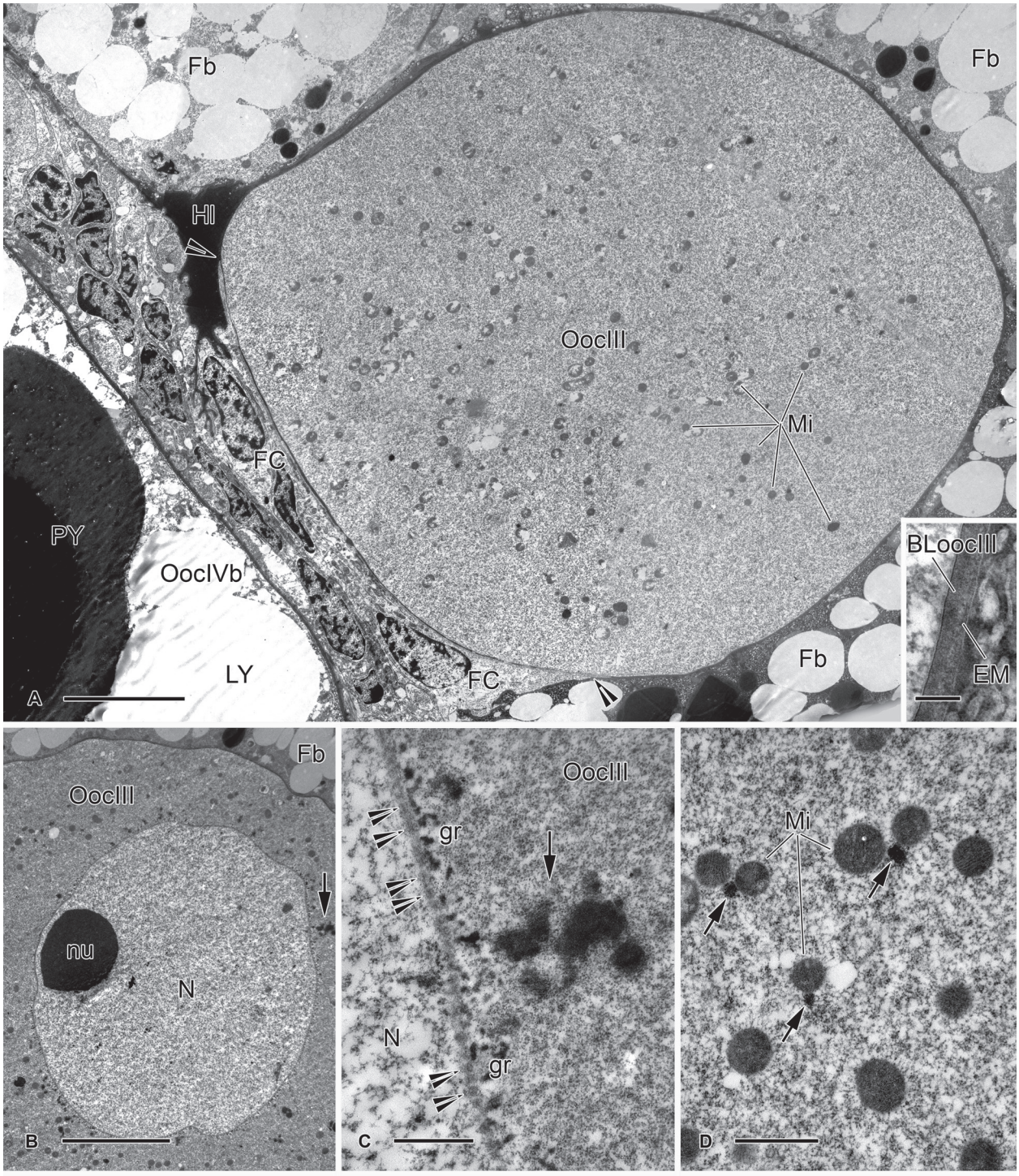

Figure 5. Oocyte in stage III (TEM). (**A)** The oocyte in stage III is externalized, i.e. protrudes from the ovarian tube into the surrounding hemolymphatic space touching adjacent tissues, e.g. the fat body.The oocyte is surrounded by the much extended basal lamina of the ovarian tube and is connected to the ovarian tube by funicle cells forming a rather short funicle in this case. Arrowheads indicate the border of the funicle. Scale bar: 10 µm. Inset: The basal lamina surrounding the oocyte III is thicker than the layer of extracellular matrix under the fat body cell. Scale bar: 0.2 µm. (**B)** The nucleus of an oocyte III is surrounded by numerous small dense granules. Arrow indicates an area where these granules cluster (compare Fig. 5C). Scale bar: 10 µm. (**C)** The nuclear envelope of oocyte III bears numerous nuclear pores. Small granules surround the nucleus and eventually form small clusters (arrow; compare Fig. 5B). Scale bar: 1 µm. (**D)** The number of mitochondria has increased in oocytes III. Frequently dense spheres (arrows) are closely associated with mitochondria. Scale bar: 1 µm.

Abbreviations: **BLoocIII –** basal lamina surrounding oocyte III, **EM** – extracellular matrix of fat body cell, **Fb –** fat body, **FC –** funicle cell, **gr –** granulum, **Hl –** hemolymphatic space, **LY –** lipid yolk, **Mi** – mitochondrium, **N –** nucleus, **nu** – nucleolus, **OocIII, OocIVb –** oocyte III, IVb, **PY –** protein yolk.

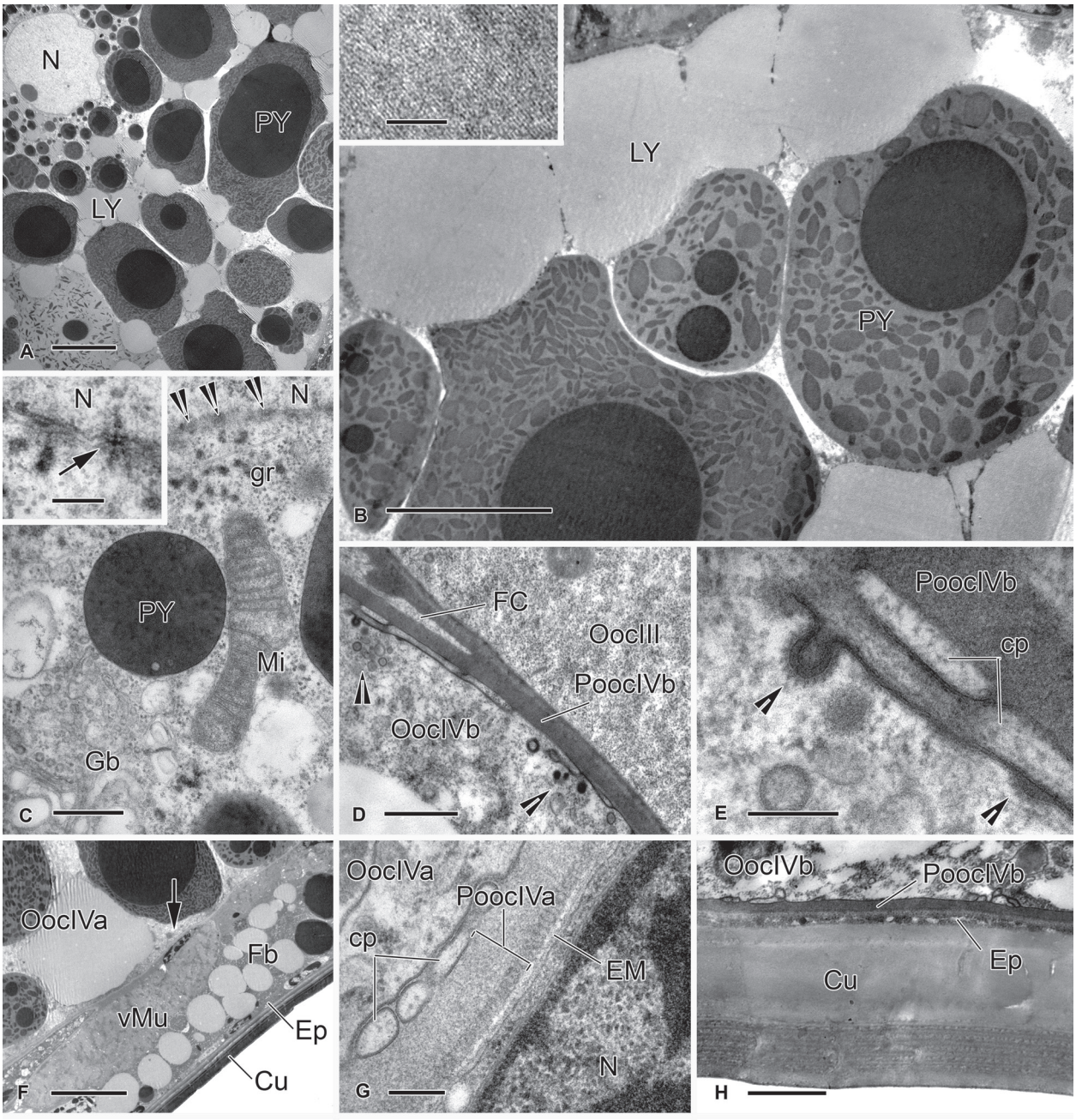

Figure 6. Oocyte in stage IV (TEM). (**A)** Detail of an oocyte IVa with nucleus and yolk granules. Note smaller granules close to nucleus. Scale bar: 20 µm. (**B)** Lipid and complex protein yolk granules. The latter contain a homogenous spherical inclusion and numerous small lens-like structures. Scale bar: 10 µm. Inset: Lens-like structure with paracrystalline substructure. Scale bar: 50 nm. (**C)** Structures close to the nucleus in an oocyte IVa. Note dense granules close to nuclear envelope provided with numerous pores (arrowheads). Scale bar: 0.5 µm. Inset: The dense granules are likely extruded through the nuclear pores (arrow). Scale bar: 0.2 µm. (**D)** Periphery of an oocyte IVb close to border of funicle showing small cytoplasmic processes and pinocytotic activity (arrowheads; compare Fig. 6E). Scale bar: 1 µm. (**E)** Coated vesicles indicate adsorptive pinocytosis (endocytosis). Scale bar: 0.2 µm. (**F)** An oocyte IVa close to the ventral integument. A ventral muscle and an extension of the fat body fill the space between oocyte and epidermis. Arrow indicates region figured in Fig. 6G in higher magnification. Scale bar: 10 µm. (**G)** The thick basal lamina surrounding the oocyte IVa reveals two layers with the external layer exhibiting a regular striation. Note different thickness of the basal lamina of the oocyte and the layer of extracellular matrix of the muscle cell. Scale bar: 0.2 µm. (**H)** The oocyte IVb touches the flat epidermis. Scale bar: 1 µm.

Abbreviations: **cp** – cytoplasmic processes, **Cu** – cuticle, **EM** – extracellular matrix of muscle cell, **Ep** – epidermis, **Fb** – fat body, **FC** – funicle cell, **Gb** – Golgi body, **gr** – dense granule, **LY** – lipid yolk, **Mi** – mitochondrium, **N** – nucleus, **OocIII, IVa, IVb** – oocyte III, IVa, IVb, **PoocIVa**, **IVb** – basal lamina pouch of oocyte IVa, IVb, **PY** – protein yolk, **vMu** – ventral muscle.

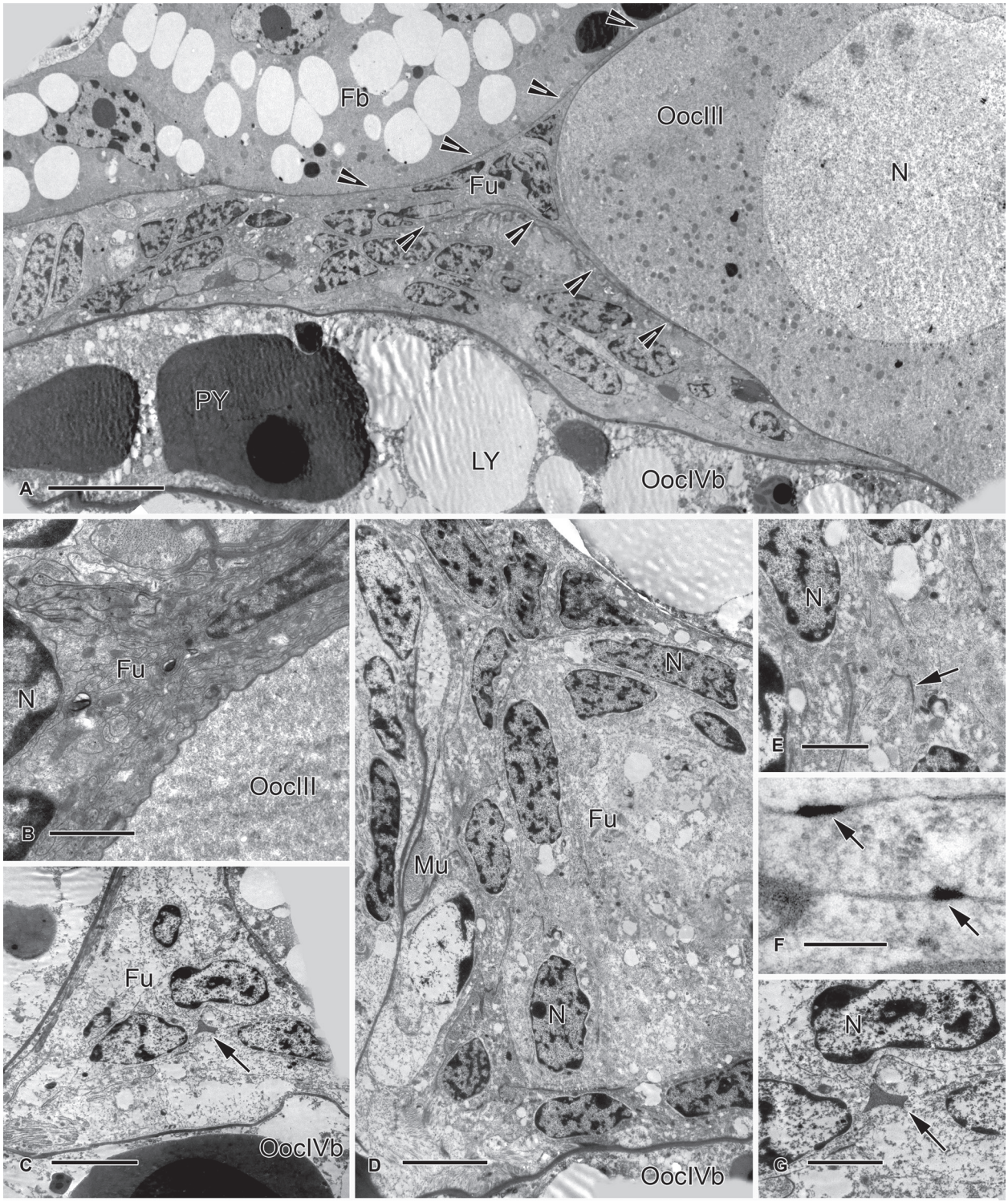

Figure 7. Funicle (TEM). (**A)** An externalized oocte III connected to the ovarian tube by an extended funicle (borders indicated by arrowheads). Scale bar: 10 µm. (**B)** Detail showing border between funicle and an oocyte III. Funicle cells and oocyte touch each other almost directly. Not dense and structured cytoplasm of funicle cells. Scale bar: 2 µm. (**C)** Another funicle connected with an oocte IVb. Arrow points to slightly widened funicle lumen. Cytoplasm of funicle cells appears less structured and light than that in Fig. 7A, B. Scale bar: 5 µm. (**D)** A funicle similar to Fig. 7C. The central part of the funicle appears light and less structured than funicle shown in Figs 7A, B. Scale bar: 5 µm. (**E)** Detail of a funicle connected to an oocyte IVb. Arrow points to zonula adhaerens. Scale bar: 2 µm. (**F)** Two zonulae adhaerentes (arrows) from a follicle of an oocyte IVb. Scale bar: 0.2 µm. (**G)** Detail of Fig. 7C showing slightly widened lumen (arrow). Scale bar: 2 µm.

Abbreviations: **Fb –** fat body, **Fu –** funicle, **LY –** lipid yolk, **Mu –** muscle, **N –** nucleus, **OocIII, IVb –** oocyte III, IVb, **PY –** protein yolk.

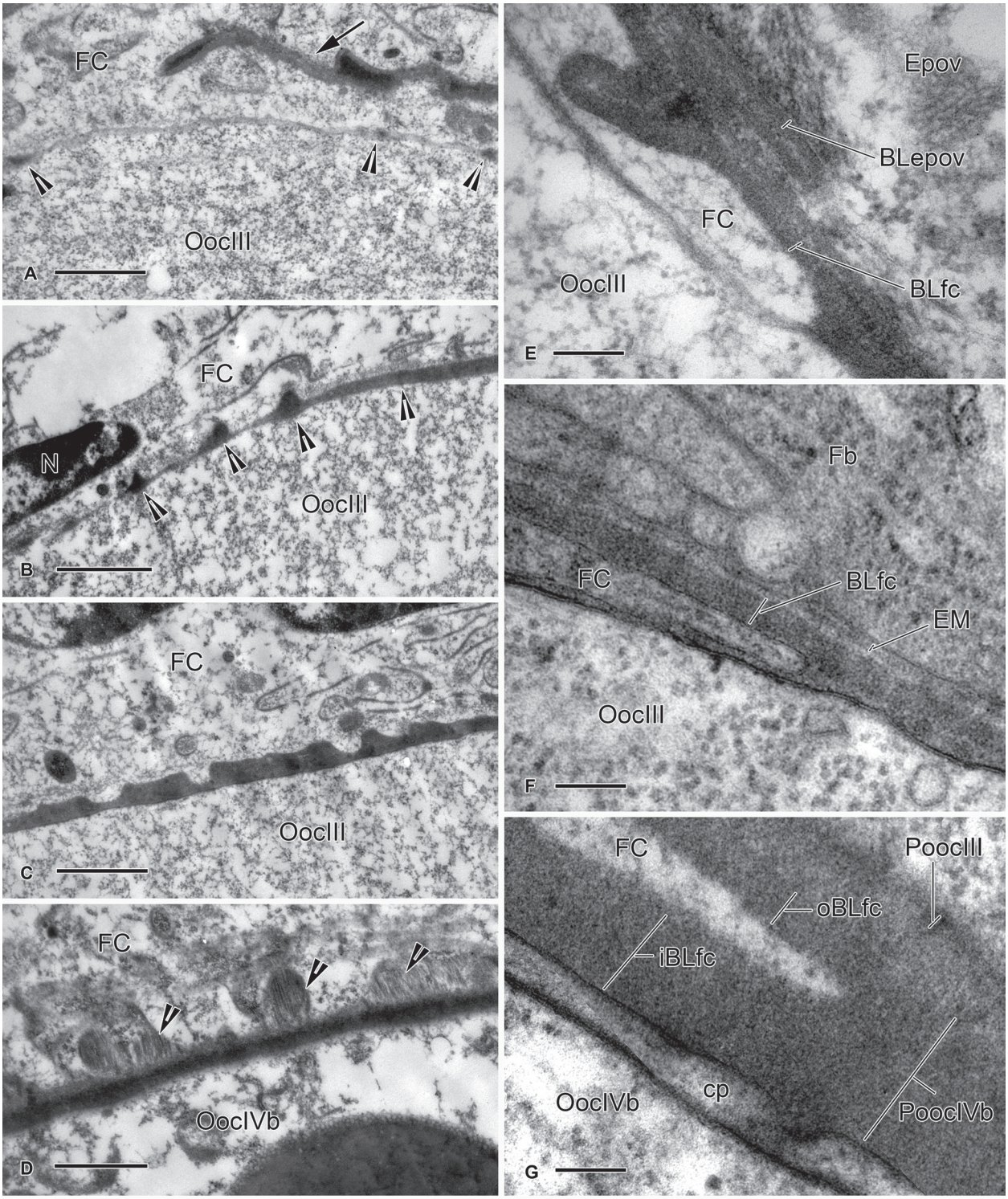

Figure 8. Formation of basal lamina pouch surrounding externalized oocytes (TEM). **(A–D)** Aspects of border between funicle and oocyte close to center of funnel-like funicle. Scale bars: 1 µm. **(A)** An only recently externalized oocyte III. The intercellular cleft between funicle cell and oocyte contains only few, small patches of dense material (arrowheads). **(B)** Slightly more advanced stage. Note from left to right increasing deposition of material into the intercellular cleft between funicle cell and oocyte. At right a continuous layer begins. **(C)** Here the layer of material deposited between funicle cell and oocyte shows still an irregular outline. Note small vesicles in the funicle cell. **(D)** Here the funicle cell apparently adds a fibrous component to the material deposited between funicle and oocyte IVb. **(E–G)** Aspects of border between the funicle and oocyte at the distal rim of the funicle. Scale bars: 0.5 µm. **(E)** Recently externalized oocyte III. Only few or no material is deposited between funicle cell and oocyte III. Note rather thin basal laminae of funicle and adjacent ovarian epitelium. **(F)** Somewhat advanced stage. The basal lamina of the funicle has increased in thickness. A distinct space is visible between funicle cell and oocyte III. **(G)** Here it becomes evident that the definite basal lamina pouch surrounding the oocytes IVb is established from two sources:

The nucleus is spherical, rather electron-lucent and with numerous nuclear pores (Fig. 6A, C). A spherical nucleolus is present. Numerous small dense particles are located around the nucleus close to its envelope. This dense material apparently is released through the very numerous nuclear pores (Fig. 6C inset). More peripherally small, dense and homogenous yolk granules are located (Fig. 6A). These granules fuse and most of the remaining cytoplasm is filled with large, dense and complex granules. Each granule contains a large homogenous inclusion and many smaller dense, lens-shaped particles (Figs 1A, B; 6A, B). These smaller particles show a paracrystalline substructure (Fig. 6B inset). In more advanced stages IVb, the yolk granules are much condensed and this substructure is often hard to detect. In the spaces between the large, dense complex granules, lipid inclusions are frequent (Figs 1B, 6A, B, F). The remaining space is occupied by the cytoplasm with mitochondria, microtubules and small Golgi bodies with electron-lucent vesicles (Figs 1B, 6C). The periphery of the oocyte is almost smooth (Fig. 1B). Only in a few restricted areas small cytoplasmic processes sharply bent towards the surface of the oocyte are observable (Fig. 6D, E, G; 8G). Pinocytotic vesicles are quite frequent (Fig. 6D, E). The cell is surrounded by a thin, dense layer of extracellular material (Figs 1B, 6D–E, G, H; 7A, 8D, G). In certain sections, this layer appears to be composed of two sublayers: an inner homogenous layer (about 200 nm) and an outer layer (about 70 nm) that in some sections shows a striated substructure. The striae are orientated perpendicular to the surface of the oocyte (Fig. 6G).

Funicle

The funicle is a more or less extended protrusion of the ovarian wall which connects the growing oocyte with the ovarian tube (Figs 5A, 7A–D, 9). It consists of epithelial and muscle cells similar to those of the ovarian tube. As in the ovarian tube, a lumen within the funicle is rarely visible (Fig. 7C, G). Towards the oocyte, the funicle is shaped like a wide funnel. Here the epithelial cells and their nuclei may be flattened and the cell membranes are intensively folded (Figs 5A, 7B). They are connected via cell junctions (Fig. 7F). The cell membranes of the funicle cells and the oolemma of the growing oocyte are closely adjacent to each other only separated by a thin intercellular cleft (Figs 5A, 7A, B; 8A, E). This cleft is later filled with dense material (Fig. 8).Towards the rim of the funnel made by the funicle cells, these cells are very flat (Figs 5A, 6D, 7A, 8E–G). More distally of this rim, the oocyte is only ensheathed by the thin, dense layer of extracellular material mentioned above (Figs 5A, 6D–H, 7A) which is continuous with the basal lamina of the ovarian epithelium and funicle (Fig. 8E, F). This layer forming the pouch containing the externalized oocyte becomes thicker during development of the oocyte (Figs 8E–G) and is also established between oocyte and funicle cells (Fig. 8A–D). In this region, the funicle cells show small apical indentations containing dense material that in some sections appeared striated (Fig. 8D). Straight microvilli neither of the funicle cells nor of the oocyte were not observed. In those funicles connecting the ovarian tube with a large, vitellogenic oocyte the central parts of the funicle show a less ordered, electron-lucent cytoplasm, and membranes between these cells appear disrupted (Fig. 7C–E). Muscle cells were never seen around the bulging oocytes.

4. Discussion

The gros morphology of the ovary of *Schizomus palaciosi* is similar to those described from other Schizomida (e.g., Börner 1904, Kästner 1931–1941, Millot 1939, 1949 and Miyazaki et al. 2001). It is composed of an unpaired tube located medioventrally. The Schizomida thus differ apomorphically from the closely related giant-whip scorpions (Thelyphonida), but also from whip spiders (Amblypygi) and (true) spiders (Araneae), which all have paired ovaries as most of the other chelicerate groups (Moritz 1993). A further difference which we can confirm is the presence of stages of oogenesis in the dorsal wall of the ovarian tube in contrast to Thelyphonida, Amblypygi and at least certain Araneae where oogenesis is said to be confined to the ventral walls of the ovarian tubes (*op. cit*., Weygoldt et al. 1972, Weygoldt 2000, Morishita et al. 2003). According to our results oogenesis may start probably in the mediodorsal wall of the ovarian tube and proceeds laterally around the edges of the ovarian tube with the most developed oocytes being located along the medioventral line (Fig. 9). This interpretation includes the assumption that the early stages of oogenesis including oogonia and mitotic and meiotic divisions, which we could not observe,

◄ the thickened outer basal lamina (= basal lamina of the funicle of previous stages and continuous with the basal lamina of the ovarian tube) and a thick inner basal lamina produced by the inner side of the funnel-like funicle.

Abbreviations: **BLepov –** basal lamina of epithelial cell of ovarian tube, **BLfc –** basal lamina of funicle cell, **EM** – extracellular matrix of fat body cell, **Epov –** ovarian epithelial cell, **FC**, funicle cell, **iBLfc –** inner basal lamina of funicle cell, **N –** nucleus, **oBLfc –** outer basal lamina of funicle cell, **OocIII, IVb –** oocyte III, IVb, **PoocIII, IVb –** pouch of oocyte III, IVb.

occur along a dorsomedian axis but had already ceased to exist in our specimen. Thus we support the similar interpretation by Miyazaki et al. (2001). Remarkably, Warren (1939) in a thelyphonid and Weygoldt et al. (1972) and Weygoldt (2000) in an amblypygid species did not observe cell division. In contrary, in spiders cell divisions were observed to continue over a long period (Seitz 1971, Morishita et al., 2003). Miyazaki et al. (2001) concluded that egg production is limited to a few 'cohorts' of eggs since a 'germarium', i.e. a layer with oogonia, was not present in the schizomid they observed and thus germ cell depletion would finally occur.

In the early, previtellogenic stages, we observed simply an increase in size of the oocyte, i.e. of both cytoplasm and nucleus (1st phase of growth). The nucleus seems to be more active, shows little heterochromatin and an increase of the number of nuclear pores. Ribosomes are the most frequent structures. There are some more mitochondria forming a cluster. A pronounced nebenkern, e.g. an accumulation of organelles such as cisterns of endoplasmic reticulum, mitochondria, Golgi bodies and/or annulated lamellae that may occur in spiders (Foelix 2011) was not observed.

A general feature of oogenesis in chelicerates is the 'externalization' of the oocytes, i.e. the growing oocyte, prior to vitellogenesis, is more and more bulging from the ovarian epithelium towards the hemolymphatic cavity. Finally, oocytes are connected to the ovarian tube only via more or less pronounced funicles made of epithelial cells. The ovaries are thus described as being grape-like or similar to those of birds (e.g., Börner 1904; Kästner 1931–1941; Dumont & Anderson 1967, Seitz 1971, Alberti 1974, Witte 1975, Diehl & Aeschlimann 1982, Witalinski 1987a,b, Moritz,1993, Fahrenbach 1999, Alberti & Coons 1999, Coons & Alberti 1999, Weygoldt 2000, Morishita et al. 2003, Alberti & Michalik 2004, Michalik et al. 2005, Miyazaki & Bilinski 2006, Talarico et al. 2009, Foelix 2011). This type of ovary must be regarded as plesiomorphic within Chelicerata or even Arthropoda (Mayr & Tait 2009). The oocyte is, except for the region where it attaches to the funicle, surrounded only by the extended basal lamina of the ovarian wall

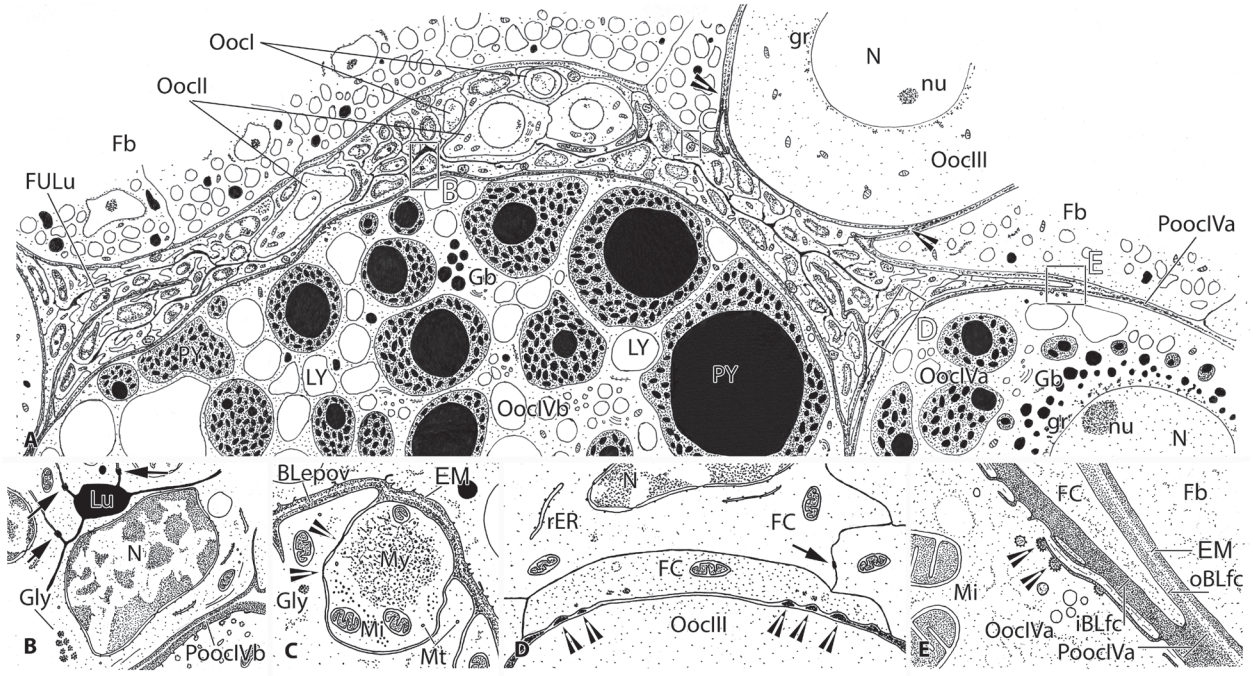

Figure 9. Drawings showing some characteristics of a cross-sectioned ovary of *Schizomus palaciosi* (acc. to different magnifications). **(A)** Overview showing flat ovarian tube with oocytes I–IVa, b (compare Figs 1, 2A). Ocyte IVb may be much denser than drawn in this figure. Squared areas labelled with white B–E represent areas comparable to those figured in Fig. 9B–E. **(B)** Detail showing region with slightly widened lumen of ovarian tube. Arrows point to zonulae adhaerentes. **(C)** Detail with muscle embedded in the ovarian wall. Arrowheads point to putative gap junctions (compare Fig. 3C). **(D)** Detail of border between funicle cell and recently externalized oocyte III. Arrowheads point to dense patches in intercellular cleft between funicle cell and oocyte indicating beginning of formation of inner basal lamina of funicle (compare Fig. 8A). Arrow marks zonula adhaerens (compare Fig. 7F). **(E)** Detail depicting rim zone of funicle of an oocyte IVb. Note pinocytosis with coated vesicles (arrowheads) and components establishing definite oocyte IV-pouch consisting of basal lamina material (compare Figs 6D,E, 8G).

Abbreviations: **BLepov** – basal lamina of ovarian epithelium, **EM** – extracellular matrix of fat body cell, **Fb** – fat body, **FC** – funicle cell, **FUL** – lumen of funicle, **Gb** – Golgi body, **Gly –** glycogen, **gr** – dense granule, **iBLfc** – inner basal lamina of funicle cell, **Lu** – lumen, **LY** – lipid yolk, **Mi** – mitochondrium, **My** – myofilaments, **Mt** – microtubules, **N** – nucleus, **nu** – nucleolus, **oBLfc** – outer basal lamina of funicle cell, **OocI, II, III, IVa, IVb** – oocyte I, II, III, IVa, IVb, **PoocIVa** – pouch of oocyte IVa, **PY** – protein yolk, **rER** – rough endoplasmic reticulum.

forming a pouch (or sac) surrounding the growing oocyte. This externalization, exposing the oocyte directly to the hemolymph, is considered to facilitate the uptake of vitellogenins (precursors of vitellin produced elsewhere in the animal) from the hemolymph by the oocyte (e.g., Fahrenbach 1999, Alberti & Michalik 2004). The pouches in which the oocyte is located and which primarily are formed by the basal lamina of the ovarian epithelium have frequently been called 'follicle' and their connection with the ovarian tube 'follicle stalk' (also 'stalk', 'pedicle' or 'peduncle' have been used; lat. *pedunculus* = stalk). Here we prefer the term funicle (= funiculus; lat. *funiculus* = strand, cord) (e.g., Seitz 1971, Alberti & Coons 1999, Coons & Alberti 1999, Michalik et al. 2005, Talarico et al. 2009). In some taxa follicle cells surrounding the externalized oocyte in addition to the pouch have also been described, e.g. in Scorpiones (Pawlowsky 1925) and Pseudoscorpiones (Weygoldt 1969).

Vitellogenesis, i.e. yolk production, is mainly done by the oocyte itself. During this phase, the oocytes grow enormously (2nd phase of growth). Thus chelicerates mostly represent the solitary type, which is likely plesiomorphic for this taxon.

Apomorphic exceptions occur in Scorpiones which evolved a very peculiar oogenesis and development (e.g., Moritz 1993, Farley 1999) and some 'Acari' with nutrimentary vitellogenesis (Witte 1975, Feiertag-Koppen & Pijnacker 1985, Alberti & Zeck-Kapp 1986, Alberti & Coons 1999, Di Palma & Alberti 2002) with accessory cells or tissues involved.

It is evident that schizomids represent the plesiomorphic mode of vitellogenesis as was already stated by Miyazaki et al. (2001). We could show that the nucleus is highly active shortly before and during vitellogenesis delivering dense materials through the nuclear pores, perhaps representing ribosomal material that clusters together to produce the peculiarly structured protein yolk (complex dense granules). Coated vesicles at the periphery of the cell indicate the uptake of materials, perhaps vitellogenins, from the hemolymph (compare spiders, e.g., Ōsaki 1972). Such yolk precursors may be produced in the well developed fat body nearby and may pass the pouch.

In most chelicerates exhibiting the plesiomorphic externalization of oocytes and solitary vitellogenesis parallel with yolk formation, a vitelline envelope (also termed vitelline membrane) is secreted by the oocyte which underlies the basal lamina surrounding the oocyte. Contact with the hemolymph is maintained by numerous microvilli extending more or less straightly from the oocyte surface and penetrating the still incomplete vitellin envelope (e.g., Dumont & Anderson 1967, Seitz 1971, Ōsaki 1972, Diehl et al. 1982, Witalinksi 1987a,b, Alberti & Coons 1999, Coons & Alberti 1999, Fahrenbach 1999, Michalik et al. 2005, Talarico et al. 2009, Foelix 2011). At the end of vitellogenesis, the microvilli are retracted and the vitelline envelope is continuous. Then the oocyte is ready for oviposition. Additional egg shell layers, chorions, may be added during the passage of ovarian tube and/or oviduct (e.g., Alberti & Coons 1999).

Since the relevant terms are used differently in the literature, it seems necessary to clarify explicitly our usage.

We use the term 'vitelline envelope' for an extracellular layer that is produced by the growing oocyte parallel to vitellogenesis and which is deposited between numerous microvilli extending more or less straightly from the oocyte. Thus, during the phase of vitellogenesis the microvilli maintain a passage way that facilitates uptake of vitellogenins from the outside.These microvilli are later retracted and the vitelline envelope becomes continuous and thus completed forming a primary egg shell. From this it is evident that the vitelline envelope develops underneath the peripheral pouch made of the basal lamina of the ovarian tube in most chelicerates.

A 'chorion' in our usage is a secondary egg shell produced in the oviduct following ovulation. Further layers may be added by accessory glands.

The term 'follicle' should be used for a cellular layer surrounding a developing oocyte. This is not the case in the species under concern. Hence we prefer the term 'pouch' for the envelope built by basal lamina-material.

It seems remarkable that no development of such a vitelline envelope was observed in *Schizomus palaciosi*. Neither we could see numerous (straight) microvilli at the surface of the oocyte nor a deposition of materials additional to the enveloping pouch provided by the basal lamina in a way as described above. Instead the basal lamina of the ovarian and funicle wall becomes thicker towards the oocyte. Furthermore it appears that material is added from the funicle cells that are adjacent to the oocyte to fill the intercellular space between oocyte and funicle cells and this material spreads farther between the surface of the oocyte and the original basal lamina envelope made from the ovarian epithelium. Thus, in certain stages of (external) oogenesis, a split of the basal lamina into two layers, an inner and an outer, is found at the rim of the funicle. The outer layer is continuous with the basal lamina of the funicle and ovarian tube and the inner layer runs into the space between oocyte and funicle. A similar observation was reported by Weygoldt et al. (1972) from an amblypygid species.These authors speculated that the two layers might represent an inner vitelline membrane/envelope and an outer chorion. On the contrary, we think that formation of a typical vitelline envelope has not yet started in our specimen and thus seems much retarded or reduced in schizomids and probably also amblypygids. Our observations clearly

show that the material that is added to the basal lamina of the ovarian wall to form the thicker pouch is produced by the funicle cells only. Interestingly, Morishita et al. (2003) described from a spider a 'proteic, PAS-positive thick band' that appears between the vitelline membrane/ envelope and the basement membrane. This layer very likely corresponds to the inner layer described herein.

Miyazaki et al. (2001) recently discussed the further fate of the oocytes. Regarding the questions whether the mature oocytes are delivered via the funicle into the ovarian tube or the so-called follicle (pouch) is finally disrupting and the egg passes through the hemolymphatic cavity somehow towards the genital opening, we prefer the first hypothesis which is supported by observations of mature oocytes passing through the funicle in a mite (Alberti 1974, Alberti & Coons 1999) and a spider (Morishita et al. 2003). In our specimen, the funicle cells of most developed oocytes seem to degenerate and may thus indicate the future passage of the oocyte/ egg. Shrunken 'follicles' (pouches, egg sacs) containing cells or cell debris indicating previous ovulations have frequently been observed in mites, ticks and spiders (e.g., Alberti & Coons 1999, Coons & Alberti 1999, Morishita et al. 2003, Michalik et al. 2005). Morishita et al. (2003) described that basal lamina (outer layer) and the PAS-positive band (inner layer) are left behind in a shrunken condition.The forces which push the large oocyte through the funicle into the ovarian lumen is still enigmatic since muscles surrounding these pouches are in most cases not present. Thus, the 'ovulation' of the oocytes can only be performed by indirect forces, e.g., creation of internal pressures through contraction of opisthosomal dorso-ventral muscles. Di Palma & Alberti (2002) suggested also the possibility of an active role of the pouch by a rearrangement of molecules in the basal lamina-material. A fibrillar component seems to be present in the pouch-material in the investigated *Schizomus palaciosi* and perhaps also in a spider (Michalik et al. 2005). During this process of ovulation, the funicle and ovarian tube evidently need to expand considerably.

Since we could not observe spermatozoa (Alberti & Palacios-Vargas 1987) in the female, the fertilization site remains speculative as in most arachnids except of scorpions, ticks and mites where it occurs in the ovaries (e.g., Alberti & Michalik 2004, Michalik et al. 2005). There is only one report, which claims that fertilization in the ovary occurs also in spiders (Suzuki, 1995). It may be worth mentioning in this respect that the area of the oocyte close to the center of the funicle remains the part in which formation of the vitelline envelope between oocyte and funicle is most retarded in the arachnid species studied (e.g. Diehl et al. 1982, Alberti & Coons 1999, Alberti & Michalik 2004, Michalik et al. 2005). Interestingly, this applies also for the depositon of basal lamina material (inner layer) in *Schizomus palaciosi*. This region has been suspected to represent a temporary micropyle which could be penetrated by a spermatozoon (Brinton & Oliver 1971). Permanent micropyles like in insects do not occur in arachnid eggs (Margaritis & Mazzini 1998).

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