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The ultrastructure of oocyte and vitellocyte inclusions in a scutariellid (Platyhelminthes, Rhabdocoela, Temnocephalida) with phylogenetic implications

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Ultrastructural investigations of the female gonad have provided useful data on platyhelminth phylogeny (1). In particular, the structure and composition of eggshell-forming globules in vitellocytes, as well as those of peripheral egg granules, and the presence or lack of nutritive materials in the oocytes, have been considered suitable features for understanding the phylogenetic relationships (2, 3, 4).

Members of Rhabdocoela (Dalyellioida, Kalyptorhynchia, Typhloplanoida) studied so far, display peripheral egg granules with a granular content in the oocytes, and eggshell-forming globules with a multigranular/mosaic-like pattern in the vitellocytes. These characteristics of the female gonad are considered apomorphic features shared with the taxon Prolecithophora (synapomorphies of the two taxa) and have contributed to the assignation of the name Eulecithophora to the monophylum Prolecithophora+Rhabdocoela (5).

To date, ultrastructural data on the female gonad of Temnocephalida are still scarce. The only detailed ultrastructural investigation examined *Temnocephala dendyi* and *T. minor* belonging to the family Temnocephalidae (6). In the present study we have examined the germarium and the vitellarium of a temnocephalid belonging to the family Scutariellidae, *Troglocaridicola* sp., in order to obtain additional information on the structure and composition of oocyte and vitellocyte inclusions. These ultrastructural findings are compared with those from other platyhelminths.

Specimens of *Troglocaridicola* sp. were removed from the gill cavity of the shrimps *Troglocaris* sp. collected in the cave of Comarie, locality Doberdò del Lago, near Gorizia (Italy). Specimens were processed for transmission electron microscopy, and cytochemical tests were performed according to the procedure described in Raikova et al. (7)

The female gonad of *Troglocaridicola* sp. consists of a single germarium located posterior to the pharynx, and of several paired, dorsal vitelline follicles. The germarium is delimited from the surrounding somatic tissues by a sheath of flattened accessory cells and a thin extracellular lamina. Packed free-ribosomes, mitochondria, chromatoid bodies, annulate lamellae, RER profiles and Golgi complexes are the main organelles of the developing oocytes (Fig. 1). RER and Golgi complexes appear to be involved in the production of two types of vesicle, some smaller containing an electron-dense material, others larger with a translucent material (Fig. 2). Repeated fusions of the electron-dense vesicles give rise to a few acorn-shaped granules (1.3-1.5 μm in diameter), which remain scattered throughout the cytoplasm during oocyte maturation (Fig. 1). They have a protein content that is partially extracted by protease (Fig. 3), and do not contain polyphenols. These egg granules differ from those observed in most rhabdocoels where they have a cortical localization and a granular content with polyphenols. The larger, translucent Golgi-derived vesicles undergo only a minimal coalescence process and, as soon as they are formed, migrate to the cortical ooplasm (Fig. 4). Some of them are seen in the process of fusing with the plasma membrane (Fig. 5). They have never been observed in other rhabdocoels.

Vitellocytes are similar to secretory cells with well-developed RER and Golgi complexes involved in the production of two types of membrane-bound inclusions. The first type to appear has an electron-dense content, which, in early stages of maturation, may show a mixed pattern consisting of both multigranular and concentric material, and in later stages a pattern with alternating dark and clear rings (Fig. 6). These inclusions measure 1.8-2 μm in diameter, contain polyphenols (Fig. 7) and have been interpreted as eggshell globules. The peculiar design of their content does not correspond either to the multigranular pattern prevailing in representatives of the Rhabdocoela and Prolecithophora or to the homogeneous/convoluted pattern of the Lecithoepitheliata; in fact, it somehow resembles that of eggshell globules in Proseriata and Tricladida. The second type of membrane-bound inclusion in the vitellocytes has a homogeneous, glycoprotein content of medium electron-density, is devoid of polyphenols, and represents yolk (Fig. 8). In addition, mature vitellocytes contain glycogen and lipid droplets.

In conclusion the female gonad of *Troglocaridicola* sp. exhibits some autapomorphic features (the presence of ooplasmic peripheral translucent vesicles, the substruc-

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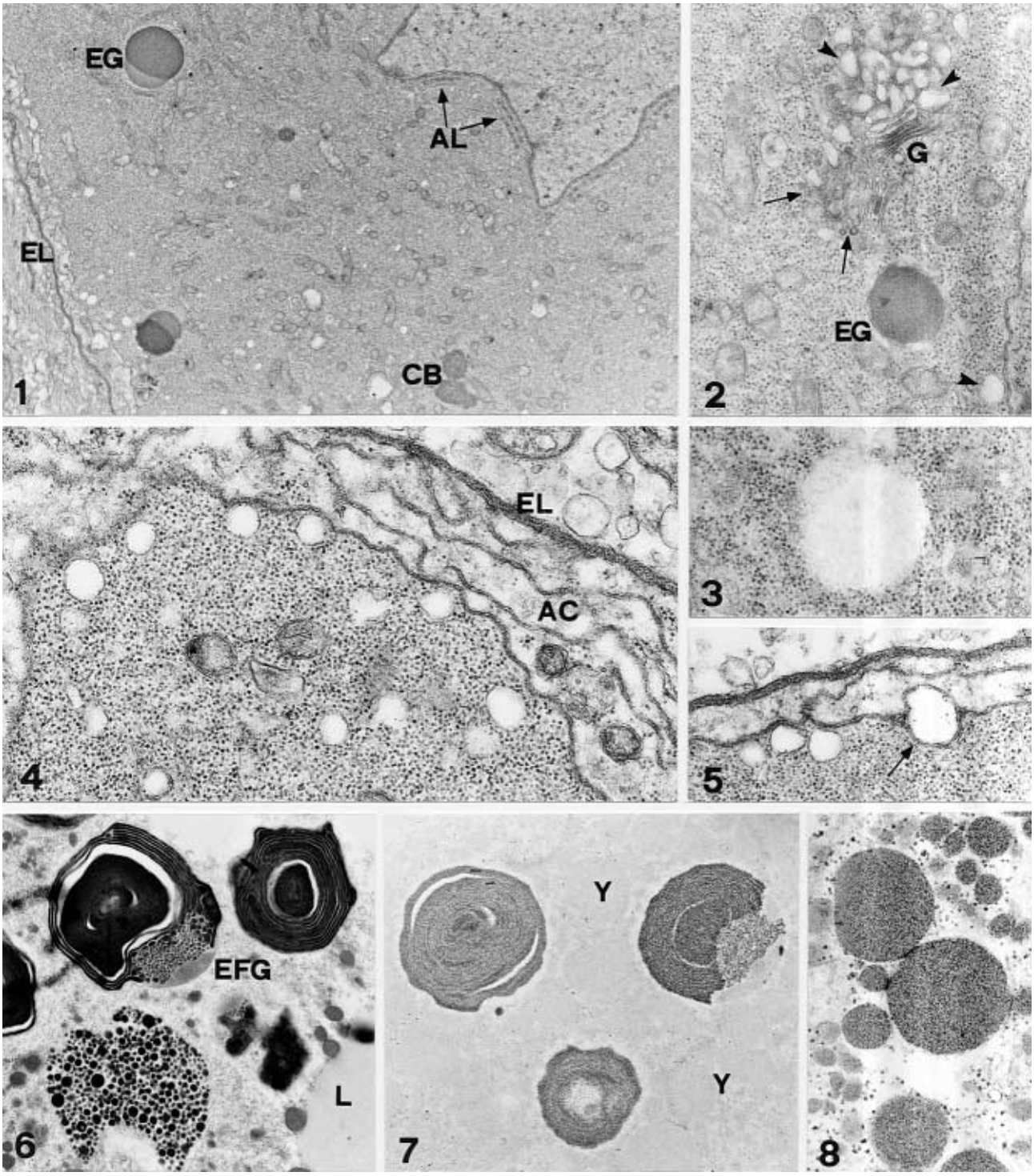

Fig. 1. – Growing oocyte. AL, annulate lamellae; CB, chromatoid body; EG, egg granule, EL, extracellular lamina. x17 500

Fig. 2. – Growing oocyte. A Golgi area (G) and a nascent egg granule (EG). Arrows point to small vesicles with a content of medium electron-density, arrowheads indicate larger vesicles with a translucent material . x24 000

Fig. 3. – Growing oocyte. Enzymatic extraction, Pronase incubation. The egg granule content is partially digested. x28 500

Fig. 4. – Nearly mature oocyte. Some translucent vesicles under the oolemma. EL, extracellular lamina, AC, accessory cells. x30 000

Fig. 5. – A translucent vesicle (arrow) in the process of fusing with the plasma membrane and releasing the content into the extracellular space. x25 000

Fig. 6. – Developing vitellocyte. Some eggshell-forming globules (EFG) at different stages of maturation. L, lipid. x12 800

Fig. 7. – Mature vitellocyte. Locke and Krishnan test for polyphenols, unstained section. A silver precipitate is exclusively on the eggshell-forming globules. Y, yolk. x12 000

Fig. 8. – Mature vitellocyte. Thiéry test for polysaccharides and glycoproteins, unstained section. A fine silver precipitate is visible on the yolk globules and on glycogen particles. x23 500

ture of eggshell-forming globules) rather than characteristics typical of the taxon Rhabdocoela + Prolecithophora. These data support the observation that the spermiogenesis of *Troglocaridicola* sp. differs from that of other temnocephalids and shows features that resemble those of Proseriata (8). Taken together, these ultrastructural findings on the male and female gonads give rise to some doubts about the place of scutariellids in the monophylum Eulecithophora and strongly suggest reconsideration of the supposed close phylogenetic relationships between Scutariellida and Temnocephalida.

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