

## Replication of basal bodies during ciliogenesis in the epidermis of Prolecithophora and Lecithoepitheliata (Plathelminthes)

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**ABSTRACT.** Centriologenesi has been little studied in the Plathelminthes. Taking into account the importance of ultrastructural features for phylogenetic reconstructions, we studied the development of cilia in mature turbellarians *Friedmaniella* sp. (Prolecithophora), *Geocentrophora wagini*, and *Geocentrophora interstitialis* (Lecithoepitheliata) to compare the events of centriolo- and ciliogenesis in their epidermis. In all these species the formation of the multitude of centrioles follows the acentriolar pathway. In *Friedmaniella* sp., each centriole appears inside an individual filamentous accumulation. In *G. wagini*, the pro-centrioles arise in clusters of fibrous granules. Several pro-centrioles are usually produced in a separate cluster. Such a very big cluster of fibrous granules was also found in one cyton of *G. interstitialis*. The assemblage of cilia starts on the basal bodies with rootlets either in the cytoplasm of the epidermal layer or on the cell surface, after these basal bodies dock with the apical cell membrane. The last developmental mode is the only one found in epidermis of *G. interstitialis*. Both our results and the literature data suggest that the knowledge of ciliogenesis could assist in reconstructing phylogenetic trees of Plathelminthes.

**KEY WORDS:** centriole, ciliogenesis, fibrous granules, flatworms

### INTRODUCTION

Epidermal ciliation pattern is considered as a main autapomorphy of the phylum Plathelminthes (EHLERS, 1985, 1986; BAGUÑA & RIUTORT, 2004), however ciliogenesis has been little studied in the flatworms. The importance of ultrastructural features for internal phylogeny of the Plathelminthes is evident (RIEGER, 1981). It was shown that distinctive features of cilia in metazoans have significance for systematics (TYLER, 1979). Could the morphological aspects of ciliogenesis in the Plathelminthes be considered as phylogenetically significant? Here I tried to answer this question. In this paper evidence of the ciliogenesis in Prolecithophora and Lecithoepitheliata is presented and the available data of ciliogenesis in Acoela, Catenulida, Macrostomida, and Rhabdocoela are mentioned (TYLER, 1981, 1984; SOLTYSKA et al., 1976; EHLERS, 1992; CIFRIAN et al., 1992). Preliminary results of the study of centriologenesi in *Friedmaniella* sp. and *Geocentrophora wagini* have been published earlier (DROBYSHEVA, 1996, 2006).

### MATERIALS AND METHODS

Adult specimens of *Friedmaniella* sp., *Geocentrophora wagini* Timoshkin, 1984, and *Geocentrophora interstitialis* Timoshkin, 1984 were collected from Lake Baikal. For electron microscopy, samples were fixed in 2.5% glutaraldehyde, followed by 2% osmium tetroxide. All fixatives were buffered to pH 7.4 with 0.05 M cacodylate. Further

preparation was by standard methods for transmission electron microscopy. Ultrathin sections were examined using a LEO 100 and a JEM 1200-EX electron microscopes.

### RESULTS

The epidermis of *Friedmaniella* sp. was a monolayered, cellular, and ciliated epithelium with a well developed basement membrane, separating the epidermis from the inner tissues (DROBYSHEVA & MAMKAEV, 1995) (Fig. 1 A, B). The epidermis of *G. wagini*, and *G. interstitialis* was composed of insunk cellular epithelium (DROBYSHEVA & TIMOSHKIN, 2006; TIMOSHKIN, 1991). In these species each epidermal cell consisted of a thin epidermal plate located over the basement membrane, and the massive nucleated cell body (cyton) in the parenchyma (Figs 2, 3). The epidermal plate and the cyton were connected by a thin cytoplasmic bridge penetrating the basement membrane (Fig. 2 A). The whole body surface of *G. wagini* was covered with cilia, while only the ventral side of the body bore cilia in *G. interstitialis*. In all three species the microtubules of ciliary axonemes showed an ordinary 9 + 2 pattern and two striated rootlets (a long vertical and a short horizontal) extended from each basal body. The epidermal cells of the studied turbellarians often contained the various structures typical of differentiating ciliated cells, including free centrioles, rootlets, and axonemes.

Many single centrioles and small centriole groups were scattered throughout the epidermal cell cytoplasm of *Friedma-*



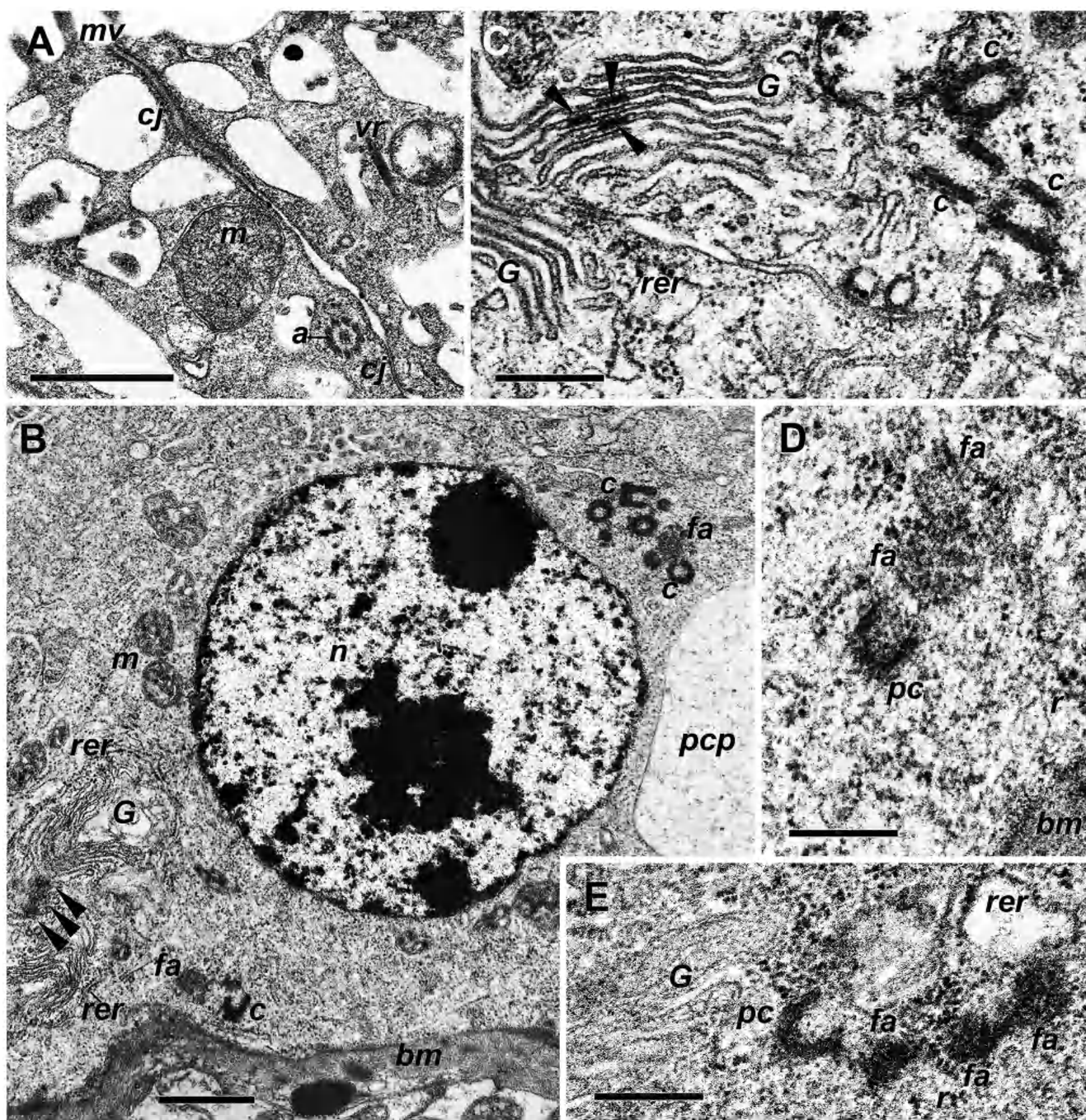


Fig. 1. – *Friedmaniella* sp. Fragments of epidermal cells. (A) Intracellular axoneme and cell junctions between two epidermal cells. (B) Basal part of epidermal cell with nucleus, Golgi apparatus, centrioles, and filamentous accumulations. (C) Golgi apparatus and centrioles. (D, E) Procentrioles and filamentous accumulations. a, axoneme; bm, basement membrane; c, centriole; cj, cell junction; fa, filamentous accumulations; G, Golgi apparatus; m, mitochondria; mv, microvilli; n, nucleus of epidermal cell; pc, procentriole; pcp, parenchymal cell process; r, free ribosomes; rer, profiles of rough endoplasmic reticulum; vr, vertical rootlet; arrowheads mark parts of Golgi apparatus cisternae with electron-dense contents (C). Scale bars = 1 µm (A, B), 0.5 µm (C–E).

*niella* sp., while intracellular axonemes and rootlets occurred more rarely (DROBYSHEVA, 1996) (Fig. 1 A–E). Moreover, some relatively amorphous structures resembling local cytoplasmic condensations were found in the epidermal cells of *Friedmaniella* sp. (Fig. 1 A, D, E). Such structures appeared to consist of interlacing filaments. These filamentous accumulations

were not membrane-enclosed and had a more -or -less spherical shape with diameter of about 200–300 nm. Centrioles and filamentous accumulations formed mixed groups (Fig. 1 D, E). Subsequent stages of centriole maturation suggested that each centriole arose inside an individual filamentous accumulation.

In the epidermal cells of *G. wagini*, centrioles were found



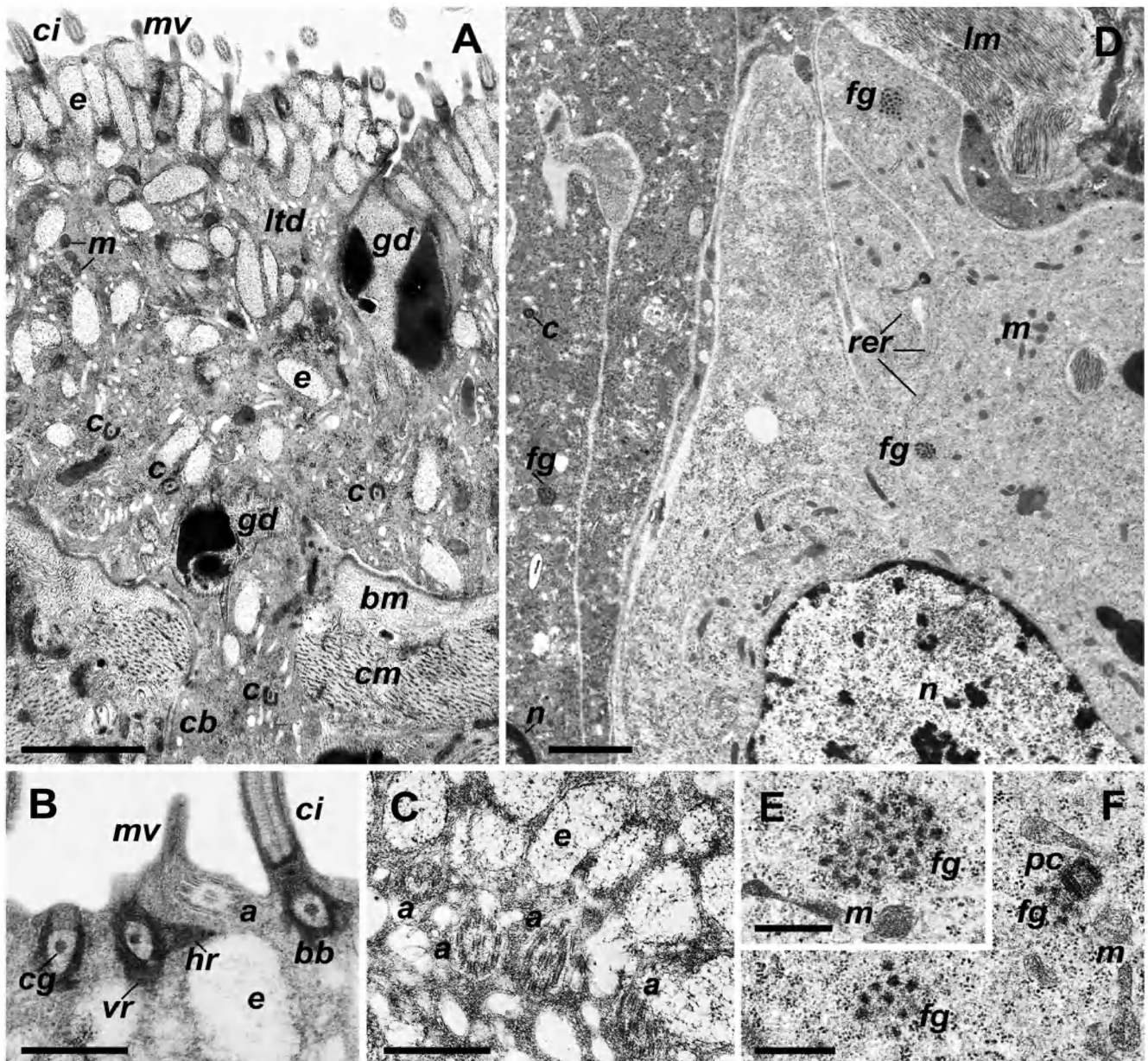


Fig. 2. – *Geocentrophora wagini*. Fragments of epithelial layer and cytons. (A) Epidermal plate connected to insunk part of epidermal cell (cyton) by a cytoplasmic bridge, as seen on the oblique section of ventral body side. (B, C) Apical zone of epidermal plates with cilium, basal bodies, and intracellular axonemes. (D) Parts of dark and light cytons with clusters of fibrous granules. (E, F) Clusters of fibrous granules and one procentriole at higher magnification. a, axoneme; bb, basal body; bm, basement membrane; c, centriole; cb, cytoplasmic bridge; cg, central granule in centriolar cylinder; ci, cilia; cm, circular muscles; e, epitheliosome; fg, fibrous granules; gd, parenchymal gland duct; hr, horizontal rootlet; lm, longitudinal muscles; ltd, light tubules and vesicles; m, mitochondria; mv, microvilli; n, nucleus of epidermal cell; pc, procentriole; rer, rough endoplasmic reticulum; vr, vertical rootlet. Scale bars = 2  $\mu$ m (A, D), 0.5  $\mu$ m (B, C, E, F).

in the cytoplasm of epidermal plates, cytons, and connecting bridges (DROBYSHEVA, 2006). Single free centrioles occurred more frequently than the mature basal bodies with rootlets (Fig. 2 A). The basal bodies with rootlets and intracellular axonemes were positioned only in the cytoplasm of epidermal plates (Fig. 2 B, C). The intracellular axonemes were observed mainly in their apical parts (Fig. 2 C). In *G. wagini*, cilia were assembled on the basal bodies both in the cytoplasm of the epidermal layer and on the cell surface, following the docking

of basal bodies to apical cell membrane (Fig. 2 B).

In the ventral epidermis of *G. interstitialis*, the overwhelming majority of centrioles were present as units consisting of basal body and rootlets, located in the epidermal plates (Fig. 3 A-C). In the cytons, centrioles were extremely rare. No intracytoplasmic axonemes could be seen in any of the epidermal cell compartments. Cilia assemblage started on the basal bodies, as soon as these basal bodies docked with the outer cell membrane (Fig. 3 A-C).



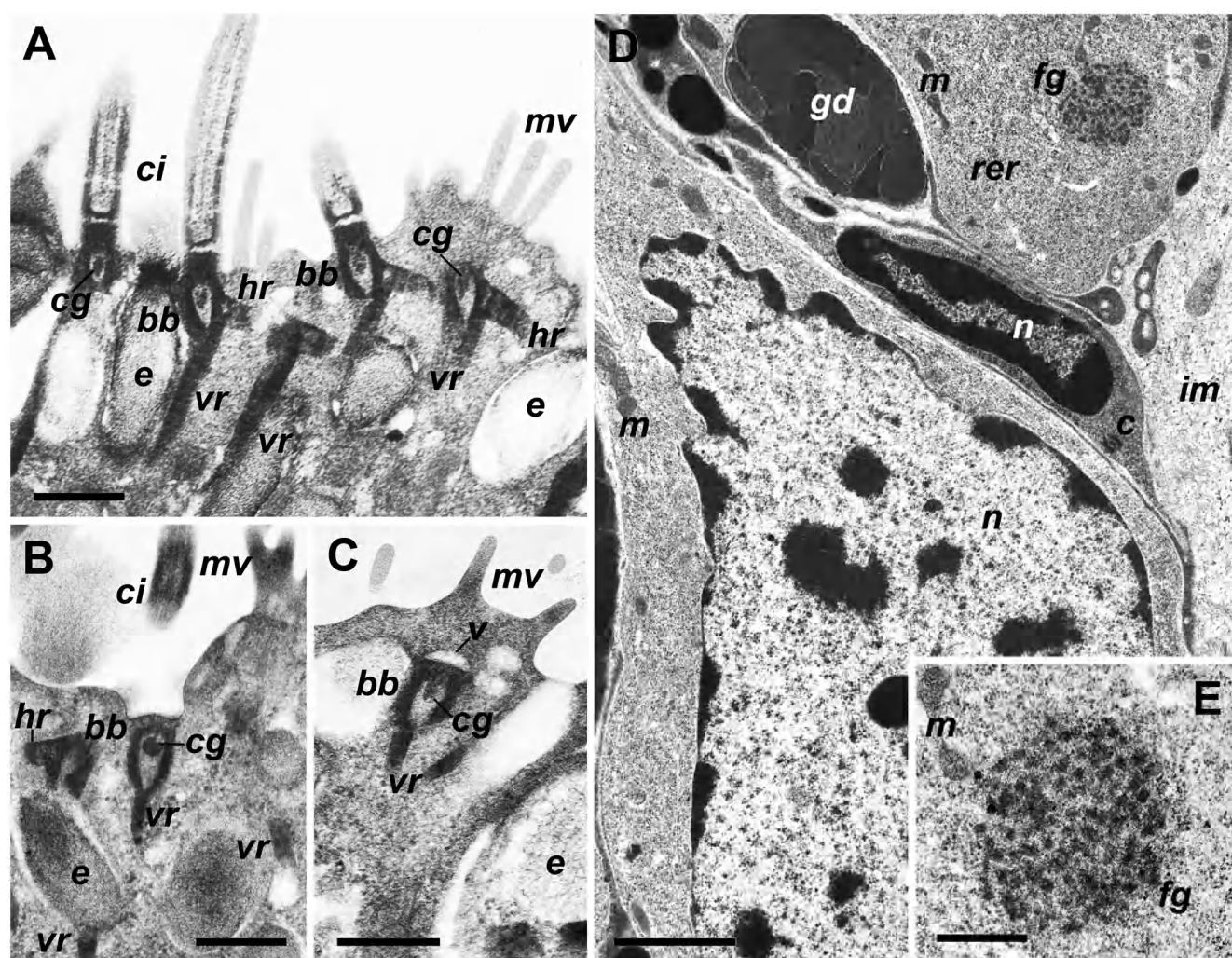


Fig. 3. – *Geocentrophora interstitialis*. Fragments of epithelial layer and cytons. (A, B, C) Parts of epidermal plates representing migration of basal body to cell surface. (A) Cilia on cell surface; free basal bodies with rootlets in cytoplasm. (B) Basal body anchored to cell membrane. (C) Basal body connected to a cytoplasmic vesicle. (D) Parts of two light and one dark cytons. (E) Cluster of fibrous granules in upper cyton (as on D) visible at higher magnification. bb, basal body; c, centriole; cg, central granule in centriolar cylinder; ci, cilia; e, epitheliosome; fg, fibrous granules; gd, parenchymal gland duct; hr, horizontal rootlet; im, intercellular matrix; m, mitochondria; mv, microvilli; n, nucleus of epidermal cell; rer, rough endoplasmic reticulum; v, cytoplasmic vesicle; vr, vertical rootlet. Scale bars = 0.5  $\mu$ m (A–C, E), 2  $\mu$ m (D).

Besides the above-described ciliary elements, small electron-dense fibrous granules, 50–80 nm in diameter, were also found in the ciliated epidermal cells of both *G. wagini* and *G. interstitialis*. In *G. wagini*, these fibrous granules generally formed clusters, sometimes containing up to several tens of fibrous granules (Fig. 2 D–F). The granules were not membrane-enclosed and their clusters were usually embedded in a fine granular matrix whose density was greater than that of the surrounding cytoplasm (Fig. 2 E, F). These fibrous granule clusters were noticeably more frequent in the cytons than in the epidermal plates of *G. wagini*. A very big cluster of fibrous granules was also found in one cyton of *G. interstitialis* (Fig. 3 D, E). Separate fibrous granules were observed near single centrioles or centriole groups in epidermal cells of both *G. wagini* and *G. interstitialis*. Some clusters of fibrous granules in *G. wagini* contained

one to three centrioles or procentrioles (Fig. 2 F). This implied that fibrous granules either were built into centriolar cylinders as structural blocks or served only as a source of microtubule proteins, functioning as a microtubule organizing center (MTOC).

## DISCUSSION

Two multiplication pathways for centrioles are distinguished during ciliogenesis: centriolar and acentriolar pathways (ANDERSON & BRENNER, 1971). In the centriolar pathway, centrioles are generated in the contact with the parental centrioles. In the acentriolar pathway, centrioles are formed from centriole precursor structures without contact with preexisting centrioles. In the examined species centriologensis clearly follows the acentriolar pathway. However,

the ultrastructure of centriolar precursors is different between the species studied. In Lecithoepitheliata, centriolar precursors are fibrous granules usually gathered in clusters, while in Prolecithophora the precursor for each centriole is an individual filamentous accumulation. It is unclear if this difference is paralleled by variation in precursor molecular composition between two species.

EM-observations on ciliogenesis in other turbellarian taxa are rare and the available data suggest large variation in the pattern of centriologenesi among taxa. In the embryos of *Archaphanostoma* sp. (Acoela, Acoelomorpha), centriologenesi follows both classical and aberrant (in Bilateria) centriolar pathways (TYLER, 1984). In this acoel turbellarian, procentrioles arise not only on the centrioles and basal bodies, but also on the rootlets of established cilia. No centriolar precursors have been found during cilia development in the embryos of *Macrostomum hystricinum* (Macrostomida), where the procentrioles are generated “de novo” and by the centriolar pathway (TYLER, 1981). Clusters of centrioles are a prominent feature of the differentiating epidermal cells and epidermal stem cells in *Catenula lemnae*, *Stenostomum* sp., *Retronectes cf. sterreri* (Catenulida) (SOLTYSKA et al., 1976; EHLERS, 1986). Early genesis of centrioles for cilia has been shown in the catenulid *Rhynchoscolex simplex* (EHLERS, 1992). In this species centriole multiplication starts in the M-phase during mitoses of intraepidermal stem cells. Both diplosomal centrioles are surrounded by many procentrioles concentrated in the cloud of centrosomal material from which astral and polar microtubules spread out (EHLERS, 1992).

All presently available data suggest that the mode of centriole formation and the ultrastructure of the centriolar precursors could be useful in reconstructing phylogenetic relationships within the Plathelminthes. Fibrous granules of *G. wagini* and *G. interstitialis* (Lecithoepitheliata) are morphologically identical to dense granules in differentiating epidermal cells of *Syndesmis echinorum* and *Paravortex cardii* (Rhabdocoela) (CIFRIAN et al., 1992). This common trait of ciliogenesis (a possible synapomorphy for Lecithoepitheliata and Rhabdocoela) could support joining taxa Lecithoepitheliata and Rhabdocoela in a single monophyletic group on some phylogenetic trees (BAGUÑA & RIUTORT, 2004). Another argument to consider characteristics of ciliogenesis as phylogenetically significant is the acentriolar mode of centriologenesi observed in Lecithoepitheliata, Rhabdocoela, and Prolecithophora and presumably lacking in Acoela, Catenulida, and Macrostomida.

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