Ciliopharyngiella intermedia (Plathelminthes, Neoophora) – enigmatic as ever? Ultrastructural features and phylogenetic implications

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ABSTRACT. Ultrastructural features of the epidermis, gonads and photoreceptors are presented. The following features of *Ciliopharyngiella intermedia* are considered to represent autapomorphies of this species or of the taxon *Ciliopharyngiella*: the special design of egg shell-forming granules in vitellocytes, the pattern of marginal granules in germocytes, an intercentriolar body with strong striated supporting beams, cilia with a pair of rootlets in young spermatids, as well as intracerebral photoreceptors with both ciliary and rhabdomeric light-sensing organelles arising from the same cell. Based on present information it is hypothesized, that *Ciliopharyngiella* represents the sister taxon of the Eulecithophora (= Prolecithophora + Rhabdocoela).

KEY WORDS: Platyhelminthes, *Ciliopharyngiella intermedia*, ultrastructure, phylogenetics.

INTRODUCTION

The taxon *Ciliopharyngiella* comprises the species *C. intermedia* Ax, 1952 and *C. constricta* Martens & Schockaert, 1981. Based on anatomical characteristics, i.e. organisation of female gonads and the brain, *C. intermedia* was first ascribed to the Proseriata (see Ax, 1952, p. 303ff), albeit with some hesitancy. Later the species was assigned to the "Typhloplanoida" (Rhabdocoela) because of the organisation of the male system and the pharynx, but considered to represent the most basal taxon of all rhabdocoels (see discussion in EHLERS, 1972, p. 71ff).

Meanwhile there exists a wealth of ultrastructural data giving reason to question whether *Ciliopharyngiella intermedia* belongs to the Rhabdocoela (see EHLERS, 1984, 1985; BRÜGGEMANN, 1986; SOPOTT-EHLERS, 1997a, 1999). This study presents some submicroscopic features that lead to a better understanding of the phylogenetic relationships of this taxon.

MATERIAL AND METHODS

Material derives from sand samples gathered from the type locality (island of Sylt, North Sea). EM – preparation followed conventional steps (see SOPOTT-EHLERS, 1999). Serial sections of four specimens were examined using a Zeiss EM 10B and a Zeiss EM 900 electron microscope.

RESULTS AND DISCUSSION

Epidermis

The epidermis of *C. intermedia* consists of cuboidal, multiciliary cells with intraepithelial nuclei. Clearly marked microvilli project from the surface membrane. Cilia show a short rod-like main rootlet pointing rostrally and a long and slender vertical rootlet. Ultrarhabdites closely beneath the apical membrane as well as processes of true lamellate rhabdite glands are missing.

The apical portion of epidermal cells is studded with secretory vesicles (epitheliosomes) (Fig. 1 A). This layer is similar to those found in Prolecithophora, some dalyellioid and typhloplanoid species (see BEDINI & PAPI, 1974; TYLER, 1984; EHLERS, 1985; RIEGER et al., 1991). This is to say, in respect to the epidermis, *C. intermedia* shares a feature with representatives of some Eulecithophora (= Rhabdocoela + Prolecithophora), but there are no epider-

mal features typical for representatives of the taxon Proseriata.

Female and male gonads

Two strings of vitellarian follicles are differentiated in the prepharyngeal region. These yolk-producing segments of the female gonad are not enwrapped by a sheath of tunica cells (see SOPOTT-EHLERS, 1997a). Cytoplasmic differentiations such as lipid droplets and yolk deposits do not show any peculiarities. Egg-shell forming granules, however, are of special appearance. Electron-lucent material forming islets of different shape is embedded in the osmiophilic polyphenolic ground substance (Fig. 1 B). This is to say, the substructure of eggshell granules does not show a regular pattern, i.e. a meandering pattern, a design of concentric rings or a mosaic pattern as known for other neoophoran species.

Contrary to the vitellaria, the germaria are enclosed by tunica cells (see SOPOTT-EHLERS, 1997a). Mature germocytes do not basically differ in their inclusions from other neoophoran female generative cells. The most conspicuous inclusions, the marginal (cortical) granules, however, differ strongly from known data. Granules are about 1.5µm in diameter and show electron dense material interrupted by electron-lucent substances deposited in winding profiles appearing like a labyrinth (Fig. 1 C). Marginal granules of a substructure as found in *C. intermedia* are unknown for Seriata, Rhabdocoela and Prolecithophora (see GREMIGNI, 1988; LUCCHESI et al., 1995; GREMIGNI & FALLENI, 1998).

Since neither the design of eggshell-forming granules nor the pattern of marginal granules in *C. intermedia* corresponds to features typical of Seriata or of representatives of the Eulecithophora (= Prolecithophora + Rhabdocoela including the Temnocephalida and Neodermata), these characteristics are hypothesized as autapomorphic features of *C. intermedia* or of the taxon *Ciliopharyngiella* (see SOPOTT-EHLERS, 1997a,b; 1999).

The unpaired testis is not enclosed by a layer of tunica cells. Somatic tissue such as gut tissue lies directly adjacent to male generative cells, not even separated by ECM.

Cytogenesis of male gametes is fairly synchronized. Therefore, only preliminary data on the development could be obtained. Nuclei of the rosette stage show evenly distributed chromatin with a few dense islets. These nuclei develop two tip-like dilatations. Between these tips the intercentriolar body is located. It consists of several discs different in electron density. Well developed crossfilaments with split ends and delicate striation form supporting beams extending between the peripheral and central pair of dark plates (Fig. 1 D,E). Bundles of microtubules encircle the basal bodies inserting on the intercentriolar body (Fig. 1 E). The cytoplasm surrounding this organelle is scattered with dense particles, presumably chromatoid bodies. A pair of short rod-shaped rootlets

originates close to each basal body. One rootlet of each pair runs to the tip of the nucleus, the other to the cell membrane (Fig. 1 E,F).

The fine structure of mature male gametes corresponds to the pattern of sperm cells typical of free-living Trepaxonemata: filiform in shape, two free cilia of the 9+"1" axonemal pattern, dense bodies, numerous mitochondria, a sheath of cortical microtubules and a threadlike nucleus.

Near the functional frontal tip of the spermatozoa the cortical microtubules leave their peripheral course and turn to the interior in irregular lines (Fig. 2 A-C). At the rear end microtubules form two semicircles enclosing the hind end of the nucleus (Fig. 2 D). In the most caudal tip of the spermatozoa just a single microtubule is seen. Ciliary basal bodies are weakly expressed and surrounded by just a delicate layer of dark material. Special applications such as dense caps or spur-shaped dark appositions do not exist. The transition zone between basal bodies and axonemata has a small diameter (Fig. 2 A, B, G).

Roundish electron-dense as well as grayish dense bodies (Fig. 2 E, F), both with granular structure, extend over two-thirds of the length of the spermatozoa. The first dense body appears in front of the insertion of the cilia (Fig. 2 C). Neither dense bodies nor mitochondria are arranged in a special pattern. Big elongated mitochondria (Fig. 2 F, I) lying slightly staggered to each other, show almost the same extension as the dense bodies. Therefore, different numbers of mitochondrial profiles are seen in transverse sections. The nucleus passes nearly through the whole length of the spermatozoa except for the frontal tip and the very hind end.

While mature spermatozoa correspond to the basic pattern of Trepaxonemata, some features just present during spermiogenesis do not fit the basic pattern. There is first of all an intercentriolar body equipped with strongly developed supporting beams, a pair of striated rootlets inserting on each basal body, and microtubules surrounding the intercentriolar and basal bodies.

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Fig. 1. A. Epidermis with epitheliosomes (ept), microvilli (mv) and cilia (ci). Scale = 2 µm. B. Segment of a vitellocyte. lp – lipid droplet; sg – egg shell-forming granule; y – yolk platelet. Scale = 2µm. C. Segment of a germocyte. gv – germinal vesicle; lp – lipid droplet; mg – marginal granule; tc – tunica cell; . Scale = 2 µm. D-F. Sections of young spermatids. D. Intercentriolar body with split, striated supporting beams (small arrow), basal bodies and microtubular bundles. bb – basal body; icb – intercentriolar body; mt – microtubules. Scale = 0.5 µm. E. Rootlet extending to a tip of the nucleus. bb – basal body; icb – intercentriolar body; n – nucleus; rt – rootlet. Scale = 0.5 µm. F. Rootlet running to the surface membrane of a spermatid. bb – basal body; icb – intercentriolar body; n – nucleus; rt – rootlet. Scale = 0.5 µm.
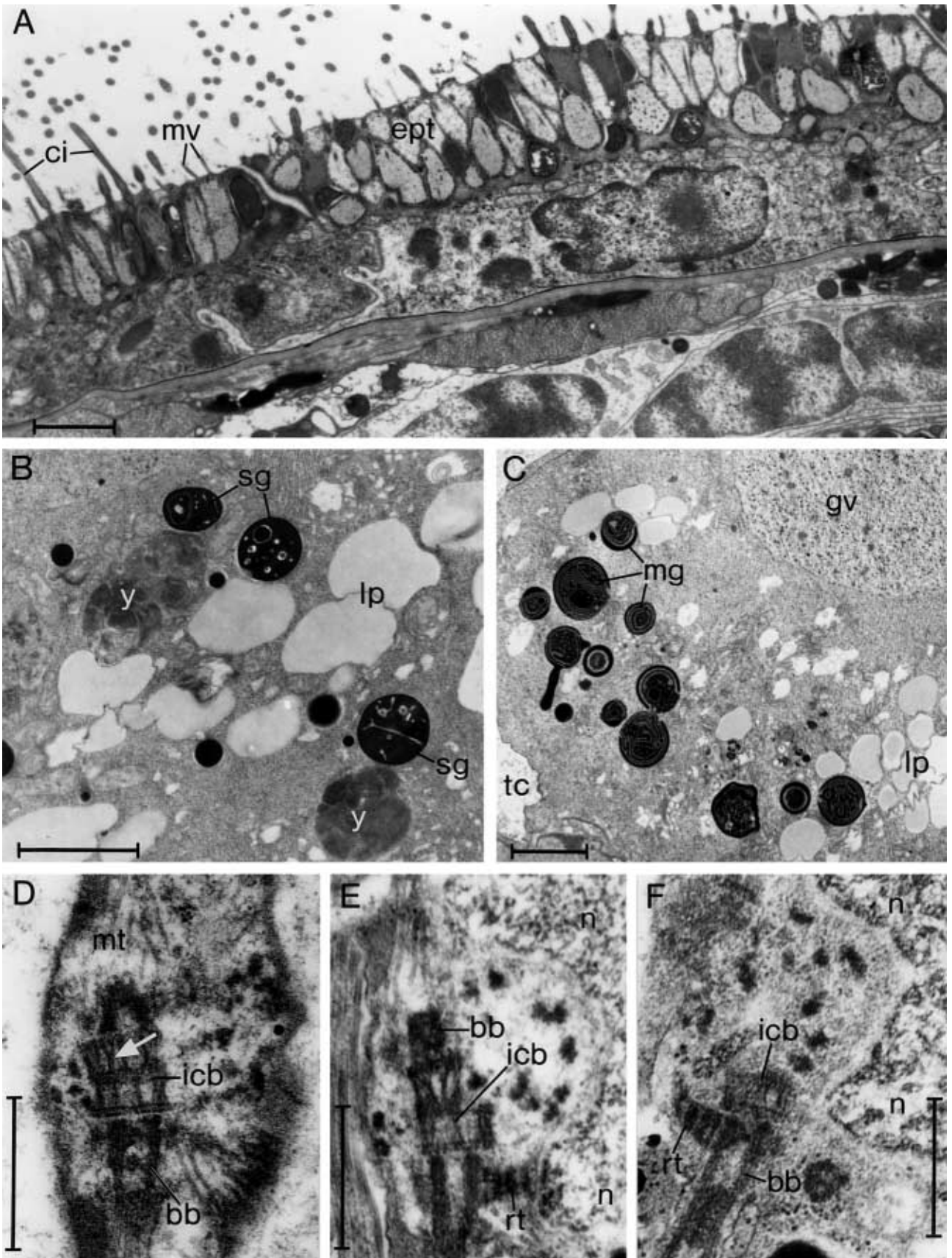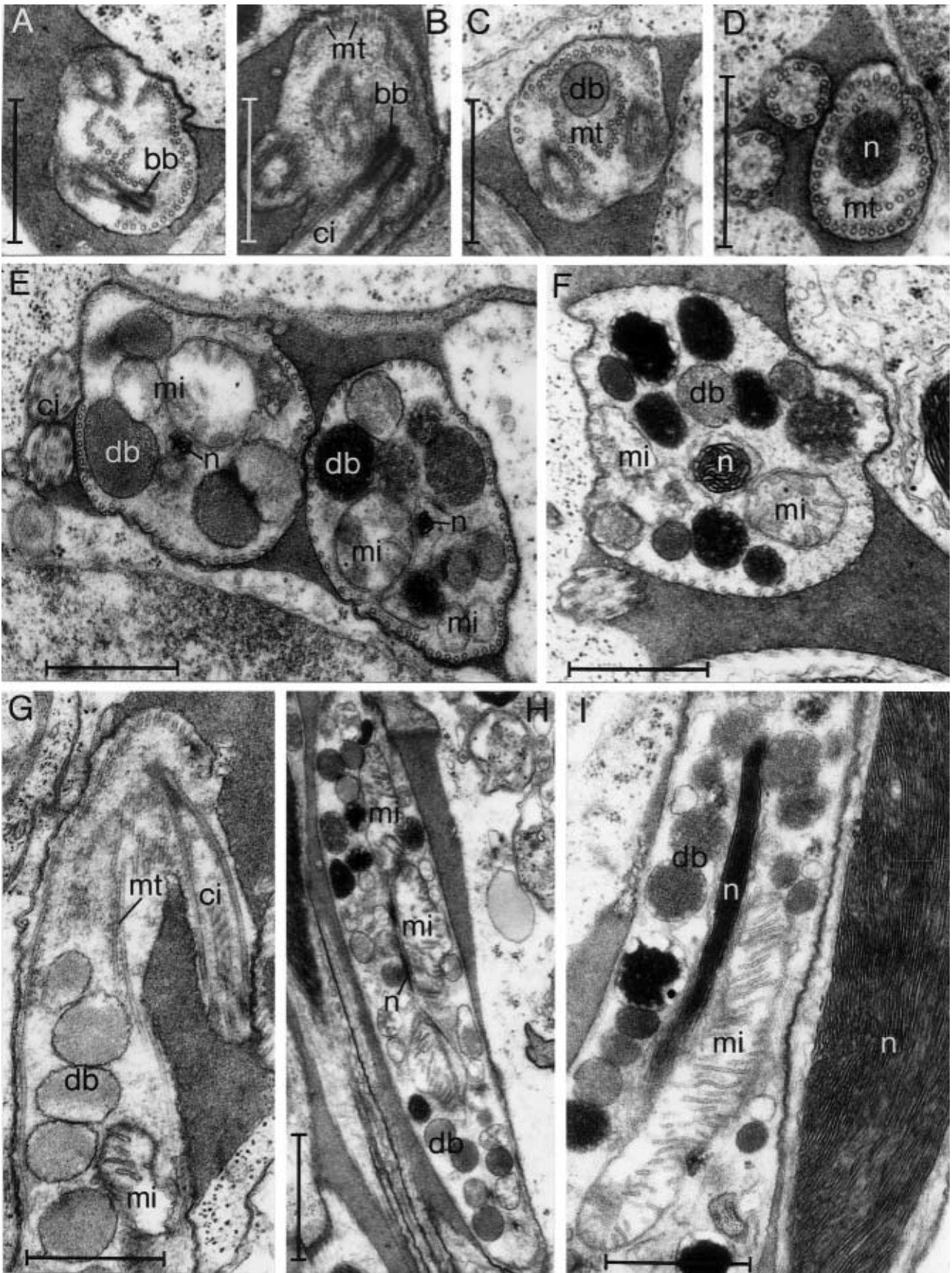
An intercentriolar body of the type as found in *C. intermedia* has hitherto not been reported for any other species of the Plathelminthes and is hypothesized as an autapomorphic feature of this species or the taxon *Ciliopharyngiella*. Residual rootlets extending towards the apical cell membrane are found in several taxa (see SOPOTT-EHLERS & EHLERS, 1986), and structures similar to an intercentriolar body as found in *Jensenia angulata* (see SOPOTT-EHLERS, 1997b) might also correspond to these residual rootlets. A pair of clearly differentiated rootlets originating from each basal body, however, is unknown for any other taxon of the Plathelminthes. This feature is also considered as an autapomorphy of *C. intermedia*. But, compared to other taxa a pair of rootlets might be a plesiomorphic condition, and residual rootlets a more derived characteristic.

The features, intercentriolar body with strongly developed striated supporting beams and a pair of rod-shaped rootlets originating from each basal body, are hypothesized as autapomorphic features of *Ciliopharyngiella intermedia*.

Bundles of microtubules attached to the basal bodies of young spermatids are not widespread among the Plathelminthes. This feature has been found in the dalyellioid *Jensenia angulata* (see SOPOTT-EHLERS, 1997b) and in a triclad and in a fecampiid species (see i.al. WATSON & ROHDE, 1993; ROHDE & WATSON, 1995). Since data on microtubules inserting on the basal bodies are very sparse, it appears premature to discuss their phylogenetic implication. Furthermore, it cannot be excluded, that these organelles appearing during spermiogenesis, are very short-term transient structures and are therefore rarely reported.

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Fig. 2. A – C. Transverse sections through functional fore ends of spermatozoa . A, B. Insertion of cilia with basal bodies accompanied by dense material. bb – basal body; ci – cilium; mt – microtubules. Scales = 0.5 μm. C. Anterior dense body on the level of the insertion of cilia. db – dense body; mt – microtubules; Scale = 0.5 μm. D. Rear end of a male gamete with posterior segment of the nucleus surrounded spirally by microtubules. mt – microtubules; n – nucleus. Scale = 0.5 μm. E-F. Transverse sections through median segments of spermatozoa. ci – cilia; db – dense bodies; mi – mitochondria; n – nucleus. Scales = 0.5 μm. G-I. Longitudinal sections of spermatozoa. G. Frontal end with a cilium leaving the sperm body, dense bodies and anterior mitochondrion. ci – cilium; db – dense bodies; mi – mitochondrion; mt – microtubules. Scale = 0.5 μm. H. Anterior-median segment with mitochondria arranged staggered to each other, dense bodies and nucleus. db – dense bodies; mi – mitochondria; n – nucleus. Scale = 1 μm. I. Posterior-median segment with long vermiform mitochondrion, dense bodies and nucleus, at the right a rear end containing the nucleus only. db – dense bodies; mi – mitochondrion; n – nucleus. Scale = 0.5 μm.

Photoreceptors

C. intermedia possesses two different kinds of submicroscopic light-sensing organs, five pairs of circumcerebrally located ciliary aggregations and one pair of intracerebral photoreceptors. Ciliary aggregations are presumed light-perceiving organs consisting of a single cell having an intracellular lumen into which axonemata of modified cilia project (Fig. 3 A). Photoreceptors of this type are widespread within representatives of Rhabditophora and do also exist in a representative of the Prolecithophora (see PISKUREK et al., 1998). These differentiations are in all instances combined with fibrous capsules of the brain. Ciliary aggregates are considered either as a plesiomorphic feature or as having evolved more than ones (for ref. see SOPOTT-EHLERS, 1999, 2000).

The intracerebral photoreceptors are built up by an unpigmented mantle cell enveloping a single sensory cell each. Outfoldings of membranes of three modified cilia (see SOPOTT-EHLERS 1999, p.490 fig. 11) and evaginations of the surface membrane of the sensory cell constitute the light-sensing elements (Fig. 3 B). Photoreceptors of this mixed type with both, ciliary and rhabdomeric photoreceptive organelles arising from the apical surface of the same cell, have hitherto not been reported for any other representative of the Plathelminthes. On the contrary, visual cells showing a striking similarity to the ones observed in *C. intermedia* are known for photoreceptors occurring on esthetes of Polyplacophora (for ref. see EERNISSE & REYNOLDS, 1994). This, however, is surely a convergence. The intracerebral photoreceptors of *C. intermedia* are hypothesized as an autapomorphorphic feature of this species or of the taxon *Ciliopharyngiella* (see discussion in SOPOTT-EHLERS, 1999).

CONCLUDING REMARKS

Most of the ultrastructural data presented in this contribution – i.e. egg shell-forming granules of vitellaria, marginal granules in germocytes, submicroscopic anatomy of the intercentriolar body and a pair of rootlets per spermatid cilium, and special intracerebral photoreceptors – are considered as autapomorphic features of the species. These characteristics as well as unpublished findings on some other organs such as protonephridia and adhesive structures indicate that *C. intermedia* is neither a representative of the Seriata nor of the Rhabdocoela.

The feature "secretory vesicles closely beneath the epidermal surface membrane" is shared with some dalyellioid, typhloplanoid and prolecithophoran species and the absence of true lamellated rhabdites with representatives of the Prolecithophora. The fine structure and location of the secretory vesicles, however, might be a convergence. The lack of tunica cells enveloping gonads is also found in some Lecithoepitheliata and Prolecithophora. However, this characteristic is only partly present in *C. intermedia*, since germaria have a tunica sheath.
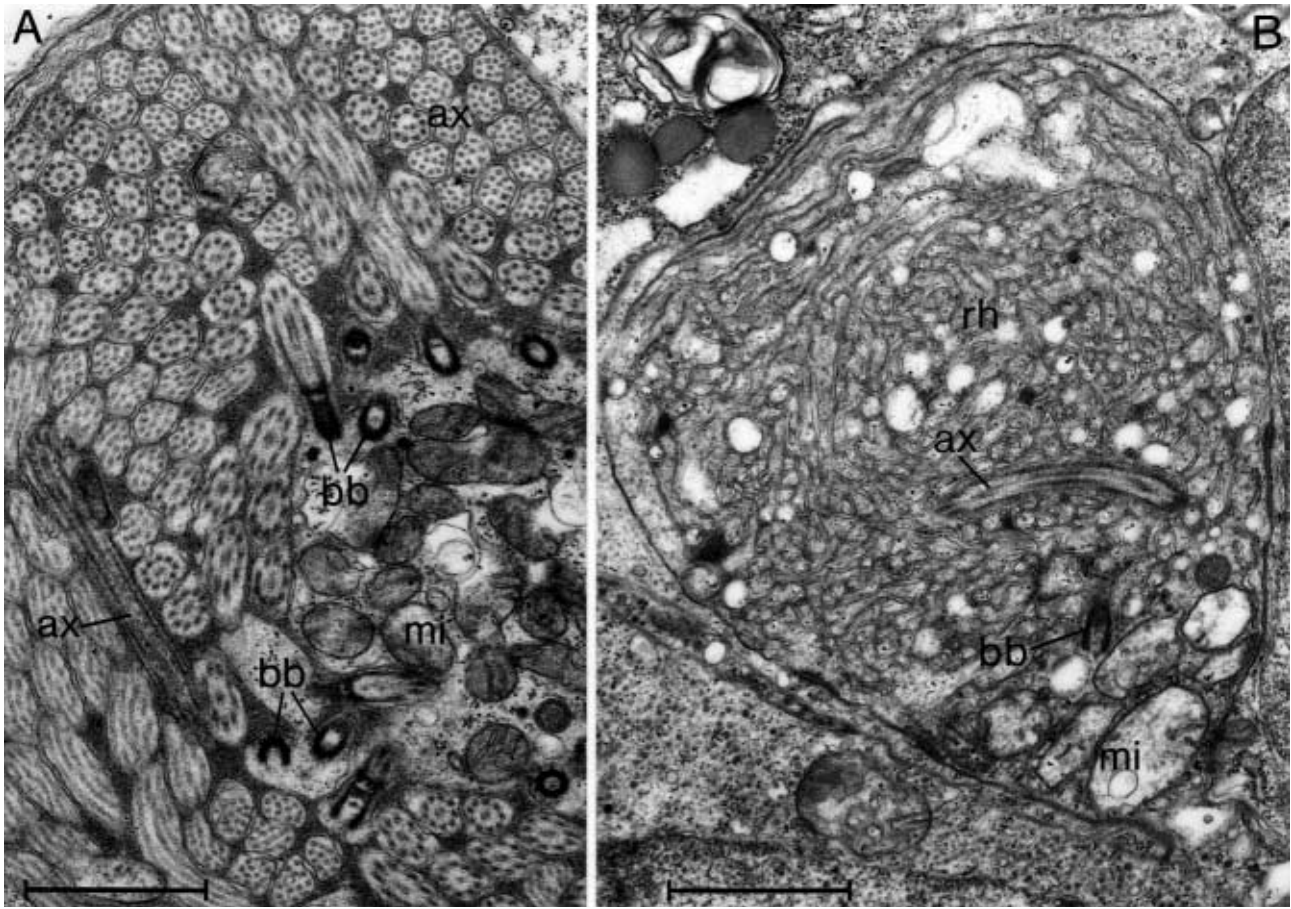
Fig. 3. A. Segment of a ciliary aggregation. ax – axonemata; bb – basal bodies; mi – mitochondria. Scale = 1 µm. B. Dendritic section of a sensory cell of an intracerebral photoreceptor. ax – axoneme; bb – basal body; mi – mitochondrion; rh – rhabdomeres. Scale = 0.1 µm.

Circumcerebral ciliary aggregations are widespread within the Rhabditophora and are either a plesiomorphic feature or have evolved more than once.

So, the data available clearly show that *Ciliopharyngiella intermedia* (or the taxon *Ciliopharyngiella* with both known species) does not belong to any of the taxa Proseriata, Prolecithophora or Rhabdocoela. As hypothesized by SOPOTT-EHLERS (1997b), Prolecithophora + Rhabdocoela constitute the monophylum Eulecithophora. This hypothesis is based on the special design of female gametes (ultrastructure of eggshell-forming granules in vitellocytes and marginal granules in germocytes). The muscular pharynx in most species of the Eulecithophora is of the 'bulbosus-type' (see RIEGER et al., 1991), a more or less barrel-shaped pharynx with an internal isolating muscular septum. Such a pharynx bulbosus is also present in *C. intermedia* and *C. constricta*, but not in any species of the Proseriata (the bulbosus-like pharynx in *Bulbotoplana acephala* lacks a septum). One hypothesis is that the pharynx bulbosus present in quite a number of prolecithophorans (here often named a pharynx variabilis) has evolved from a pharynx plicatus (as known from distinct prolecithophorans), independently of the pharynx bulbosus of all rhabdocoels (here called pharynx rosulatus and pharynx doliiformis). But it is also possible that the

different subtypes of the pharynx bulbosus are homologous and that a pharynx bulbosus can be hypothesized for the basic pattern of the Eulecithophora. This would support the view of a sister-group relationship of *Ciliopharyngiella* and Eulecithophora.

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