

Ultrastructure and organisation of the proboscis epithelia in *Cicerina remanei* Meixner, 1928 (Turbellaria, Eukalyptorhynchia)

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Introduction

Ultrastructural data on the proboscis epithelia of Eukalyptorhynchia have been given by RIEGER and STERRER (1975) for Bertiliellidae, by DOE (1976) for Gnathorhynchidae, by REUTER (1975) and SCHOCKAERT and BEDINI (1977) for Polycystididae. Only the latter authors dealt with the overall organisation of the epithelia in the proboscis, and this for *Polycystis nägelii*. The anatomy of the proboscis is indeed an important element in the taxonomy of the Eukalyptorhynchia (see e. g. KARLING, 1963). It is to be expected that the ultrastructure will provide further clues to indicate phylogenetic relationships between the major taxa in Eukalyptorhynchia when more data become available (RIEGER, 1981). In view of further comparative studies on the ultrastructure of the proboscis of Eukalyptorhynchia, we here describe the organisation of the epithelia in *Cicerina remanei* Meixner, 1928.

Material and methods

Specimens of *C. remanei* were collected from sandy beaches at Mariakerke and Zeebrugge (Belgium). Preparation for electron microscopy was performed as in MARTENS (1986).

Observations

Within the Eukalyptorhynchia the proboscis of the Cicerinidae Meixner, 1928 is characterized by a girdle of glands at the junction of the sheath and cone (KARLING, 1964; BRUNET, 1973). In the genus *Cicerina* the girdle consists of four glandular ampullae (MEIXNER, 1928), surrounded by circular muscles (BRUNET, 1973).

The proboscis epithelia of *C. remanei*, with the exception of the cone, may be divided in circumferential belts, which can be either cellular or syncytial (Fig. 1; 2). One cellular belt lines the proboscis cavity, as reported for *C. tetradactyla* (KARLING, 1952) and two belts, an outer cellular one and an inner (i. e. at the conus side) syncytial one, form the so-called

junction (the transition zone between sheath and cone). Two cells and two binuclear syncytia cover the cone. All epithelia are devoid of cilia. Beneath the junction a girdle, surrounded by circular and longitudinal muscles, contains the nuclei of the junctional belts and the cone epithelium, as well as gland necks enclosed by circular muscles, forming four glandular ampullae (Fig. 6).

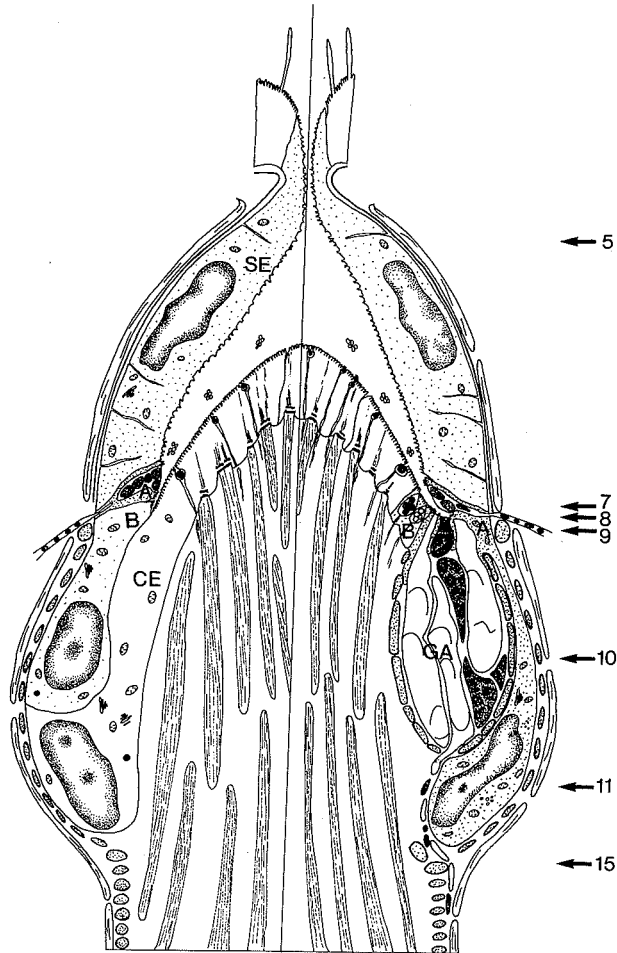


Fig. 1: Reconstruction of the proboscis epithelia in *C. remanei* from electron microscopic observations. Numbered arrows indicate where cross sections are taken. Scale bar = 5 μ m

Abbreviations: A: Outer, cellular junctional belt; B: Inner, syncytial junctional belt; SE: Sheath epithelium; CE: Cone epithelium; \leftarrow : cell boundary; bl: basal lamina; bm: bundles of microfibrils; cm: circular muscle; d: desmosome; ga: glandular ampulla; gn: gland necks of glandular ampulla; gr: gland from or glandular ring; ibp: infoldings of the basal plasma membrane; lm: longitudinal muscle; ms: microtubular sheath; pr: proboscis.

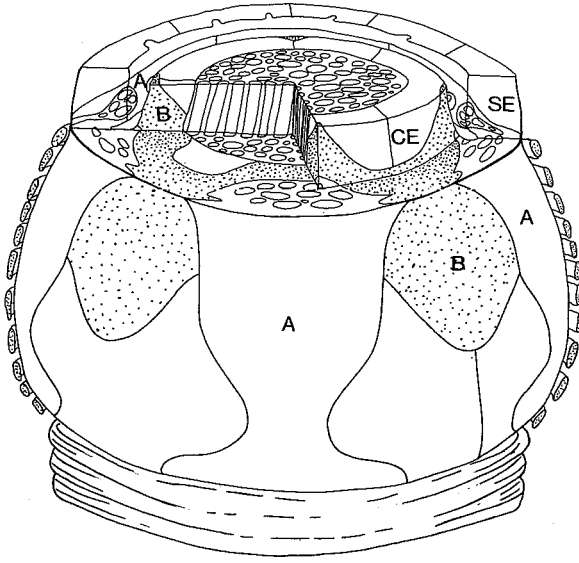


Fig. 2: Three dimensional reconstruction of the junction and nucleo-glandular girdle of the proboscis of *C. remanei*. Have been removed: a) the upper part of the sheath and cone epithelium. b) the front of the remaining part of the sheath epithelium and the apical part of the outer junctional belt (cellular). c) a section at the same level into the cone. Scale bar = 5 μ m

1. The proboscis sheath epithelium

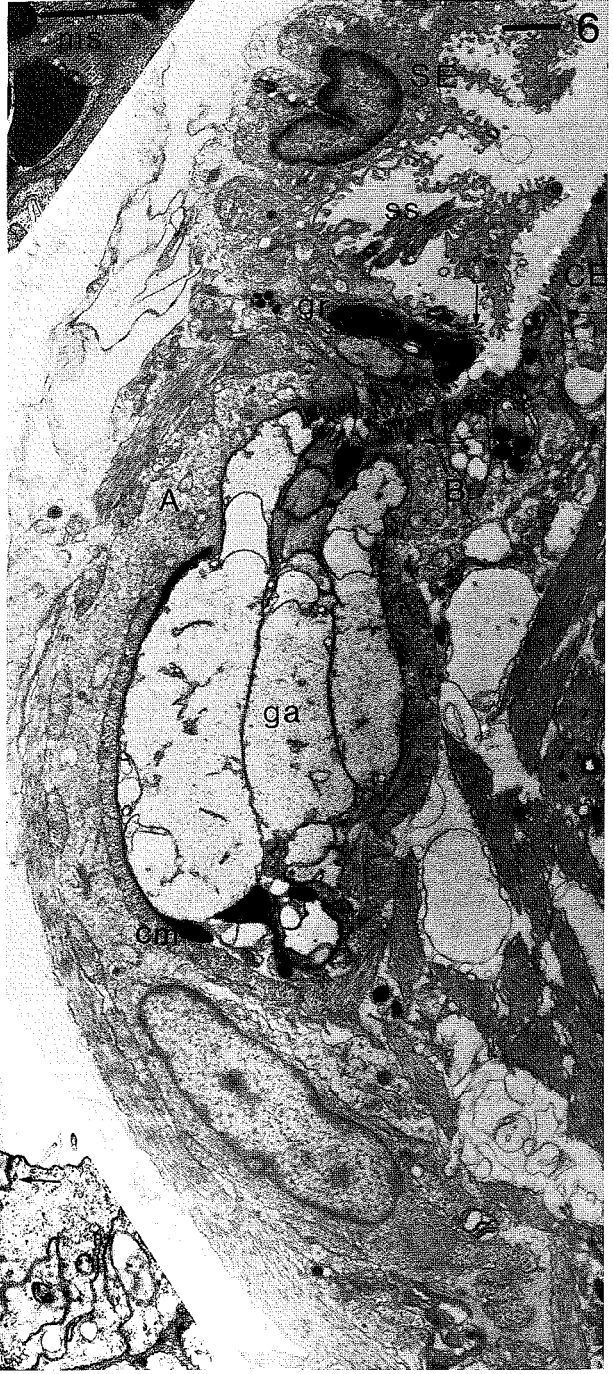
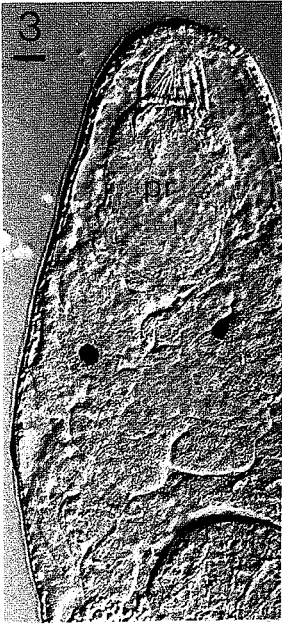
The eight cells lining the proboscis cavity are longitudinally arranged (Fig. 1; 2), about 20 μ m long and 2–5 μ m high, with longitudinal ridges. The lobate and flattened nuclei are to be found about halfway along the cavity (Fig. 1; 6). The basal plasma membrane shows some deep infoldings, extending up to 2/3 of the cell height (Fig. 5). Except for a few mitochondria and golgi complexes, no cellular organelles are present in the moderately granular cytoplasm. The cell surfaces bear irregular microvilli (length: 250–300 nm, 3–5 / μ m) with neither distinct microfibrillar support nor an underlying terminal web. The basal lamina (100 nm thick), which is bilayered in some specimens, is continuous with the epidermal basal lamina. No gland necks penetrate the epithelium. Intercellular contacts are made by zonulae adhaerentes and septate desmosomes.

Surrounding the sheath only a longitudinal muscle layer is to be found, and this contains 16 fibers or more. At the pore a sphincter closes the proboscis cavity.

Near the junction the epithelium cells form 12 oblique funnels, the deep parts being covered by the outer junctional belt. At the bottom of each, a group of mono- and biciliary sensory cells penetrates the basal lamina (Fig. 6; 7).

2. The junction and nucleo-glandular girdle

The outer junctional belt consists of four cells (Fig. 2) which are attached to the cavity epithelium cells and to each other by zonulae adherentes and septate desmosomes (Fig. 7; 9, inset). Each cell occupies the quadrant of the junction on top of a glandular ampulla (Fig. 7).



Just above the junction numerous gland necks and monociliary receptors penetrate this part of the belt, forming a glandular ring (Fig. 4; 6). The gland necks, penetrating the basal lamina, have an internal microtubular sheath (Fig. 6, inset) and contain spherical, electron dense granules (400–700 nm). In the middle of each quadrant the gland necks from the ampullae pierce the surface. The glandular ampullae are pocket-like muscular sheaths enclosing swollen parts of gland necks, and containing two types of secretion, a granular, electron lucid, and an electron dense secretion (Fig. 10). Contrary to the opinion of MEIXNER (1928) these ampullae do not form part of the sheath epithelium. Further down, the necks run in between the circular and longitudinal muscles of the bulb to the nodus (Fig. 15), their secretory parts being situated behind the proboscis, as mentioned by KARLING (1964). Some monociliary receptors are visible between the surfacing gland necks. The cell surface bears densely packed microvilli, $\pm 10/\mu\text{m}$, 300–400 nm long, with a fibrous core and a dense terminal web underneath. The whole superficial cytoplasm looks fibrous and rather homogeneous. Whereas at the surface each cell occupies a quadrant of the junction, it narrows towards its nucleiferous part, leaving space for the nucleiferous parts of the inner junctional belt and the cone epithelium in between the glandular ampullae (Fig. 2; 9; 10; 11). The flattened nuclei, about $6 \mu\text{m}$ long, $6 \mu\text{m}$ broad and only $1.5 \mu\text{m}$ thick, are located under the glandular ampullae. The cytoplasm contains some mitochondria, golgi-complexes, fractions of rough endoplasmatic reticulum and patches of smooth endoplasmatic reticulum.

The inner junctional belt is syncytial and visible at the surface as a narrow strip between the outer belt and the cone epithelium (Fig. 2; 6). The nucleiferous parts are situated in between the glandular ampullae, at the peripheric side of the girdle, leaving space for the nucleiferous parts of the cone epithelium against the bulb (Fig. 1; 9). The four nuclei lie at the level of the ampullae. The cellular contents and matrix are similar to those of the outer belt, except for some electron dense vesicles. Where the syncytium surfaces, monociliary receptors and two types of gland necks – one being empty and the other with electron dense granules – pierce through (Fig. 6; 8).

Fig. 3: Live specimen in I.C. showing the proboscis, the brain with two pigmented eyes and part of the pharynx. Scale bar = $10 \mu\text{m}$

Fig. 4: Front half of the proboscis of the same specimen. The glandular ring, glandular ampullae and some nuclei are visible. Scale bar = $10 \mu\text{m}$

Fig. 5: Electron micrograph of cross sectioned sheath epithelium cells, showing a cell boundary, infoldings of the basal plasma membrane and underlying basal lamina. Scale bar = $1 \mu\text{m}$

Fig. 6: Longitudinal section through a glandular ampulla. At the top a sheath epithelium cell with nucleus is attached to a cell of the outer junctional belt with its nucleus being situated under the ampulla. The apical part is pierced by a gland neck (glandular ring) and sensory cells. The swollen gland necks, surrounded by a sheath of circular muscles, surface at the junction. The syncytial junctional belt, with two types of glands, is seen against the cone in between the former cell and the cone epithelium. At the bottom the circular muscles surrounding the girdle are continuing around the bulb. Inset: Detail of gland neck, penetrating the basal lamina, with internal microtubular sheath. Scale bar = $1 \mu\text{m}$

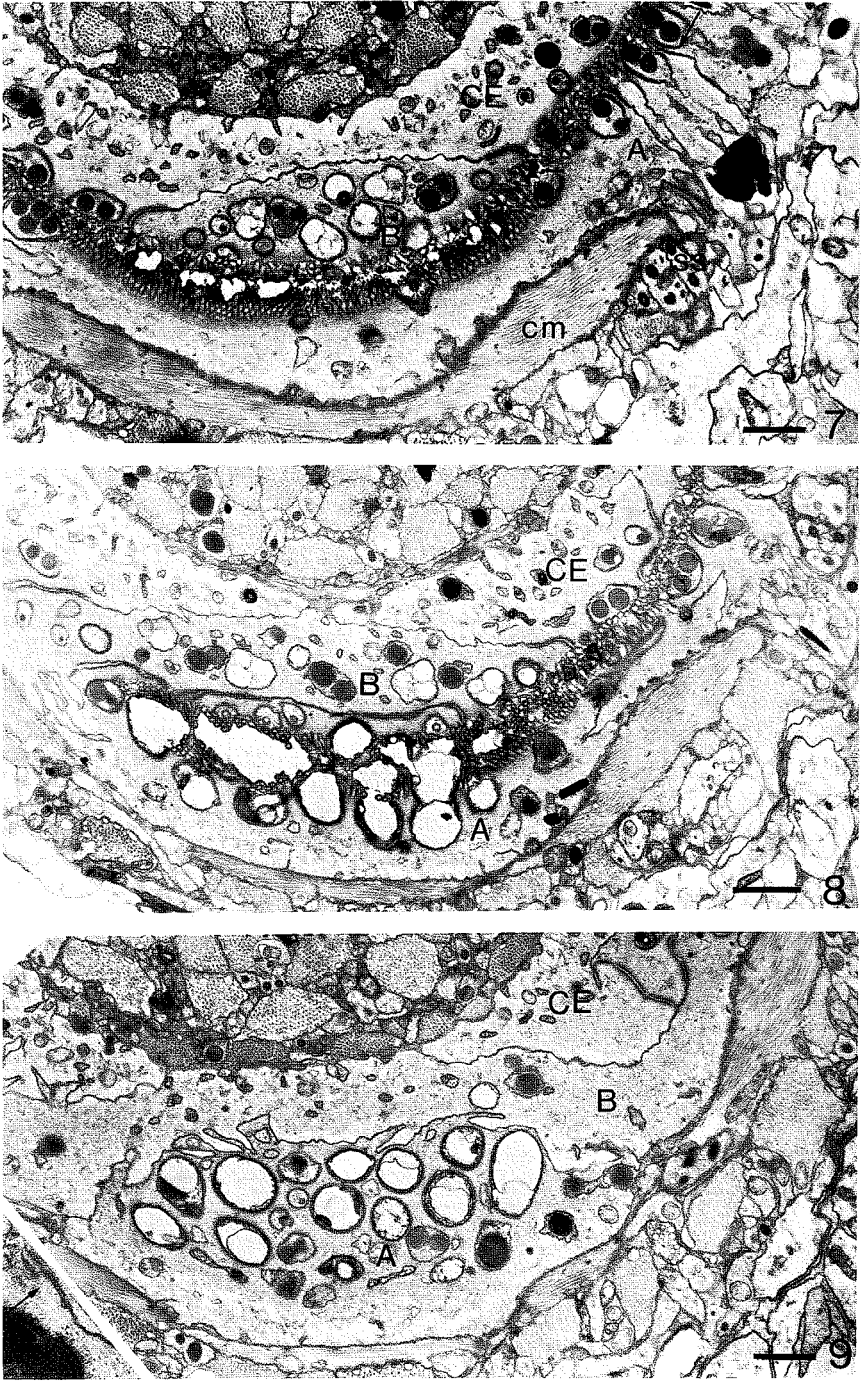


Fig. 7, 8, 9: Cross sections, just above (7), at (8) and just beneath (9) the junction. The cells of the outer junctional belt narrow towards the nucleiferous parts, leaving space for the interlaying cell strands of the inner junctional belt and cone epithelium cells. Inset: Intercellular attachment of cells in outer junctional belt. Scale bar = 1 μ m

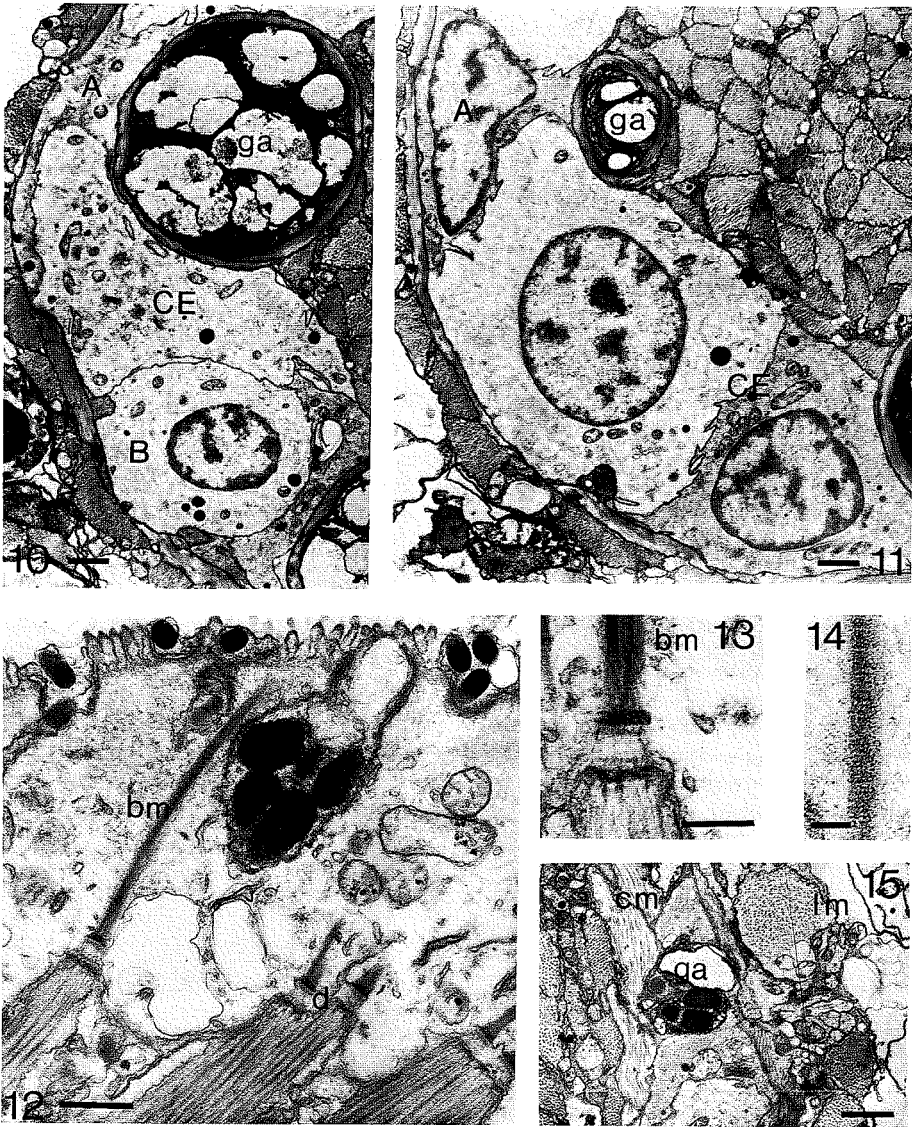


Fig. 10: Cross section through the middle region of the nucleo-glandular girdle. Cell strands of the outer junctional belt and cone epithelium run down. In between two cone epithelium cells, a nucleiferous part of the syncytial belt is found. Scale bar = 1 μ m

Fig. 11: Cross section through the lower region of the nucleo-glandular girdle. Aside of the glandular ampulla the nucleus of a cell belonging to the cellular junctional belt is seen. The nucleiferous cell parts of the cone epithelium are situated between two ampullae. Scale bar = 1 μ m

Fig. 12: Cone retractors adhering to the cone epithelium by means of desmosomes and bundles of microfibrils. Note the two types of glands and the stout microvilli with underlying terminal web. Scale bar = 0,5 μ m

Fig. 13: Desmosome with wide open intercellular space. The parallel condensations are clearly visible. Scale bar = 0,1 μ m

Fig. 14: Cross-striated aspect of a bundle of microfibrils. Scale bar = 0,1 μ m

Fig. 15: Gland necks are situated between the circular and longitudinal muscles layer of the bulb. Scale bar = 1 μ m

3. The proboscis cone epithelium

The cone is covered by two mononucleated cells and two binuclear syncytia, without obvious regular pattern. The superficial cell parts, 1.3 to 3.5 μm high, are joined to their deeper parts by cytoplasmic cell strands slipping under the junctional belts between two glandular ampullae. Their nucleiferous parts fill the spaces between these of the cellular junctional belt. The apical plasma membrane bears numerous stout microvilli, these being 200–250 nm long, at a density of 6/ μm , and with somewhat denser tips (Fig. 12). The terminal web is less dense (50–100 nm thick) and the superficial cytoplasm less fibrous than in the junctional belts. Two types of gland necks penetrate the cone epithelium (Fig. 12). The first contains small, electron dense granules which are elliptical to rod-like in shape (300 nm long; 150 nm broad). The second type contains larger, spherical, electron dense granules (500 nm). Monociliary receptors (type IV: REUTER, 1975) are spread throughout the epithelium as well.

Underlying the epithelium a uniform basal lamina is found, in the apical part becoming very diffuse and pushed upwards by the adhering retractors of the cone. The muscles are connected to the epithelium by means of desmosomes with wide open intercellular spaces (150–175 nm), with two parallel condensations and a less electron dense median portion (Fig. 13). Bundles of microfibrils connect them with the terminal web (Fig. 12; 14). The cells are connected to each other by well developed zonulae abhaerentes and septate desmosomes.

Considerations

In *P. năgeli* the proboscis epithelia are organised in five circumferential belts, which are syncytial, as in the epidermis. Three belts have their insunk nucleiferous cell parts outside the bulb. In *C. remanei*, however, we found a different organisation with four belts: two of them being cellular, as in the epidermis. At this stage no final conclusions on possible homologies can be made.

Acknowledgements

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References

- Brunet, M. (1973): La famille des Cicerinidae (Turbellaria, Kalyptorhynchia). *Zool. Scr.* 2, 17–31.
- Doe, D. A. (1976): The Proboscis Hooks in Karkinorhynchidae and Gnathorhynchidae (Turbellaria, Kalyptorhynchia) as Basement Membrane or Intracellular specializations. *Zool. Scr.* 5, 105–115.
- Karling, T. G. (1952): Studien über Kalyptorhynchien (Turbellaria). IV. Einige Eukalyptorhynchia. *Acta Zool. Fenn.* 69, 1–49.
- Karling, T. G. (1963): Die Turbellarien Ostfennoskandiens. V. Neorhabdocoela 3. Kalyptorhynchia. *Fauna Fenn.* 17, 1–59.
- Karling, T. G. (1964): Über einige neue und ungenügend bekannte Turbellaria Eukalyptorhynchia. *Zool. Anz.* 172, 159–183.

- Martens, E. E. (1986): Comparative ultrastructure of copulatory organs having a stylet in the Proseriata (Turbellaria). *Hydrobiologia* 132, 165–173.
- Meixner, J. (1928): Aberrante Kalyptorhynchia (Turbellaria, Rhabdocoela) aus dem Sande der Kieler Bucht. *Zool. Anz.* 77, 229–253.
- Meixner, J. (1938): Turbellaria (Strudelwürmer) I. Tierw. d. Nord. u. Ostsee. 46, 1–146.
- Reuter, M. (1975): Ultrastructure of the epithelium and sensory receptors in the body wall, the proboscis and the pharynx of *Gyatrix hermaphroditus* (Turbellaria, Rhabdocoela). *Zool. Scr.* 4, 191–204.
- Rieger, R. M. (1981): Morphology of the Turbellaria at the ultrastructural level. *Hydrobiologia* 84, 213–229.
- Rieger, R. M., Sterrer, W. (1975): New spicular skeletons in Turbellaria, and the occurrence of spicules in marine meiofauna. Part I. *Zeitschr. zool. Syst. Evolut.forsch.* 13, 207–248.
- Schockaert, E. R., Bedini, C. (1977): Observations on the ultrastructure of the proboscis epithelia in *Polycystis naegeli* Kölliker (Turbellaria Eukalyptorhynchia) and some associated structures. *Acta Zool. Fenn.* 154, 154–191.

