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THE ANATOMY AND ULTRASTRUCTURE OF THE PROBOSCIS IN *ZONORHYNCHUS*-SPECIES AND IMPLICATIONS FOR PHYLOGENETIC RELATIONSHIPS WITHIN THE EUKALYPTORHYNCHIA MEIXNER, 1928 (PLATYHELMINTHES, RHABDOCOELA)

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Abstract. The ultrastructural morphology of the proboscis in species of the genus *Zonorhynchus* Karling, 1952 is investigated. The proboscis epithelia are organized in four belts. The apical cone epithelium has intra-epithelial nuclei, and the distal belt of the sheath epithelium is formed by numerous cells lacking a distinct pattern. Multiciliary receptors are lacking. The diversity in ultrastructural data on the proboscis is used to elucidate the phylogenetic relationships within the Cicerinidae and Eukalyptorhynchia. A bipartite cone epithelium as found in *Zonorhynchus*-species is a common feature for all eukalyptorhynch species investigated. A syncytial basal cone epithelium is a synapomorphic character for all species except *Toia calceformis* Brunet, 1973 and *Nannorhynchides herdlaensis* Karling, 1956. A syncytial proximal belt of the sheath epithelium, and intra-epithelial nucleus(i) in the apical cone epithelium constitute apomorphic features for species of the genus *Zonorhynchus*. The absence of multiciliary receptors is regarded as the plesiomorphic state and distinguishes *Zonorhynchus*-species, *T. calceformis* and *N. herdlaensis* from other Eukalyptorhynchia investigated. *Cicerina remanei* Meixner, 1928, *Psammorhynchus tubulipenis* Meixner, 1938 and *Cytocystis clitellatus* Karling, 1953 have two insunk sensory organs with muticiliary receptors. The presence of four epithelial belts in *Zonorhynchus*-species as in *C. remanei*, *P. tubulipenis* and *C. clitellatus* is considered a shared plesiomorphic state within the Eukalyptorhynchia The presence of a third belt in the sheath epithelium is a synapomorphic character of all Koinocystididae, Cystiplanidae and Polycystididae.

Key words: Platyhelminthes, Eukalyptorhynchia, proboscis, phylogeny, ultrastructure.

INTRODUCTION

The family Cicerinidae Meixner, 1928 comprises ten genera. In the diagnosis of the family the presence of germovitellaria and of a glandular ring at the junction of the proboscis in most species are the main diagnostic features. However, germovitellaria are present in *Cytocystis clitellatus* Karling, 1953 as well (KARLING, 1964). Although less prominent, a ring of glandular secretion in the sheath epithelium just above the junction is

present in species of other families such as Psammorhynchidae, Gnathorhynchidae and Placorhynchidae (DE VOCHT, 1990; DE VOCHT, 1992). Because of this a distinct apomorphic character to define a monophyletic taxon Cicerinidae is lacking. After the establishment of the taxon Cicerinidae by MEIXNER (1928), KARLING (1952) proposed the name Zonorhynchidae with two subfamilies: Zonorhynchinae (including the genera *Zonorhynchus* Karling, 1952, *Cicerina* Giard, 1904, *Paracicerina* Meixner, 1928, *Ptyalorhynchus* Ax, 1951 and *Blennorhynchus* n.n.), and Ethmorhynchinae (for *Ethmorhynchus* Meixner, 1938). With the description of several new species and genera, as for instance by MARCUS (1952), KARLING (1956) and AX (1959), the family was named Cicerinidae again by KARLING (1964). A few years later, BRUNET (1965, 1973) described eight new species and two new genera and made a revision of the family based on light microscopic observations on twelve species. BRUNET (1973) distinguished two groups of genera within the Cicerinidae. One group contains the genera *Toia* Marcus, 1952, *Nannorhynchides* Karling, 1956 and *Pocillorhynchus* Brunet, 1973. The other group includes the genera *Cicerina*, *Paracicerina*, *Ptyalorhynchus*, *Ethmorhynchus*, *Zonorhynchus*, *Xenocicerina* Karling, 1956 and *Didiadema* Brunet, 1965. In this non-cladistic approach the distinction between the two groups was based on several criteria, and the first group is characterized by their small dimensions (0.2-0.7 mm), their oval body-shape and excellent swimming capabilities. Histologically these species are characterized by two frontal glands, pigmented eyes with lenses and a ciliated proboscis sheath epithelium. The second group is characterized by a body length exceeding 1 mm, in general paired vesiculae seminales and opposite or different character states of the former mentioned characters. EVDONIN (1977) distributed the genera into three subfamilies: the Cicerininae Meixner, 1928, Nannorhynchinae Evdonin, 1977 and Xenocicerininae Evdonin, 1977. More recently new species of Cicerinidae have been described by SCHOCKAERT (1982), KARLING (1989) and ARMONIES & HELLWIG (1987).

Species of the genus *Zonorhynchus* have been collected from salt marshes, lagunes and muddy sediments (KARLING, 1952; AX, 1959; ARMONIES & HELLWIG, 1987). Up to now four species of *Zonorhynchus* have been described (ARMONIES & HELLWIG 1987, KARLING 1952, 1956). All four species are light microscopically characterized by the position of the copulatory organ and the genital pore in the middle of the body, non-sclerotized bursal mouthpieces and a proboscis with long proboscis- and integument retractors.

MATERIAL AND METHODS

Specimens of *Zonorhynchus seminascatus* Karling, 1956 were collected from fine sediment of a shallow lagoon (0.4 m) near Blåbergsholmen in the Gullmar-fjord near Kristineberg (Sweden) in June 1988. An unidentified specimen of the genus was collected from fine sand from the bord of Etang de Lapalme at the Mediterranean coast near Banyuls-sur-Mer (France) in October 1987. *Zonorhynchus salinus* Karling, 1952 was collected from a salt marsh near Königshaven at the island of Sylt (Germany) in April 1988. Extraction was carried out by decantation with a $MgCl_2$-solution isotonic to seawater for the unknown species of *Zonorhynchus* and *Z. seminascatus*. *Z. salinus* was extracted by the «Übersand»-method described by ARMONIES & HELLWIG (1986).

Before primary fixation, animals were anaesthetized in a solution of $MgCl_2$ isotonic to the seawater. Primary fixation was carried out with 0.1 M cacodylate-buffered 2 % glutaraldehyde at 4°C for 2h. After double rinsing in the same buffer, specimens were postfixed in 1%-cacodylate-buffered osmium tetroxide at 4°C for 1h, pre-stained in 2% uranyl acetate solution (20 min) before dehydration in a graded acetone series and embedded in Araldite. Serial sectioning was carried out with Reichert OMU 3 and Reichert Ultracut microtomes. Thin sections were mounted on carbon-coated pioloform-covered grids and stained with 2 % aqueous uranyl acetate (5 min) and 1.2 % aqueous lead citrate (7 min), using an LKB-ultrostain. Fine structural examination was performed with a Philips EM 400 electron microscope.

Terminology and abbreviations used in the text and figures

Apex: Apical-most part of the cone.

Belts: Limited, circumferential parts of the epithelium, either the sheath (S_1 and S_2) or the cone epithelium (*A* and *B*). They can be formed by one cell, several cells or a syncytium.

Bulb: Muscular part of the proboscis separated from the surrounding parenchyma by a layer of extracellular matrix.

Cone epithelium: The part of the epithelium that covers the muscular part of the proboscis which protrudes into the cavity, constituted of an apical cone epithelium (*A*) and basal cone epithelium (*B*).

Dilators (D): Dilating muscles around the sheath.

Extracellular matrix (*ECM*): Intercellular components of connective tissue or the product of epithelia or muscles cells.

Gland necks (*g1-g2*): The apical parts of gland cells of which the secretory parts are located in the parenchyma. They usually store secretion granules.

Inner musculature: The circular (*icm*), central cylinder and peripheral blocks of longitudinal muscles (*cilm* and *pilm*) situated internally to the proboscis septum.

Insunk cell parts: Epithelial cell parts situated in the parenchym below the epithelial basal lamina.

Intra-epithelial: This notation is used in contrast with insunk or subepithelial in respect of the position of nuclei or receptors for instance.

Intrabulbar nuclei: Nuclei of the proboscis epithelia located internally to the bulbar septum in between the inner proboscis muscles.

Junction: The place where the apical part of the sheath epithelium makes contact with the apical part of the cone epithelium.

Motional muscles: The positioning muscles of the proboscis in the body. The motional muscles include dilators *(D)*, protractors (*P*), fixators, proboscis retractors (*PR*) and integument retractors.

Nodus (*n*): Posterior end of the bulb.

Nucleo-glandular girdle (ngg): Epithelial cell parts situated below the junction around the anterior part of the bulb, containing the nuclei of the epithelial cells or syncytia and gland necks.

Outer musculature: The muscles situated at the outside of the proboscis septum or underlying the sheath epithelium.

Proboscis: Frontal organ, mainly composed of epithelia and muscles with associated glands and sensory cells, used to capture prey.

Proboscis cavity: Cavity with terminal pore in which a part of the proboscis protrudes.

Proboscis cone: Terminal part of the muscular part of the proboscis, which protrudes into the proboscis cavity

Sensory receptors: Receptive terminal parts of nerve cells, which pierce the epithelia.

Septum: Layer of extracellular matrix surrounding the proboscis bulb.

Sheath epithelium: The part of the proboscis epithelium that lines the wall of the proboscis cavity, formed by a distal (S_1) and a proximal belt (S_2).

RESULTS

Zonorhynchus-species are large bulky animals compared to most Eukalyptorhynchia. The brownish animals measure 0.8-1.5 mm and possess a relatively small proboscis. Characteristic for the proboscis in species of the genus *Zonorhynchus* is the large amount of gland necks that pierce the epithelia of the sheath as well as the cone epithelium. The relatively long bulb shows a particular organization with internal circular and radial muscle fibres between the inner longitudinal muscles in the cone.

Epithelia. The proboscis epithelium in *Zonorhynchus*-species is formed by four belts (Fig. 1). The distal belt constitutes the main part of the epithelium lining the proboscis cavity and is formed by separate cells arranged without a distinct pattern (Figs 1, 2). A circumferential proximal belt of the sheath epithelium forms the junction between sheath and cone. The epithelia are devoid of cilia, and cells are interconnected by apical zonulae adhaerentes, septate junctions and dispersed desmosomes (Fig. 2). The cells of the distal belt are 5 µm high and bear slender microvilli, 900 nm long and about 10 per µm (Fig. 2). A fine fibrillar but not electron-dense layer, about 450 nm thick, is present in the apical parts of the cells just beneath the apical plasmalemma. All cells in the distal belt have intra-epithelial lobate nuclei (5-6 µm long). The cytoplasm contains only very few mitochondria, patches of endoplasmic reticulum and Golgi complexes. Anteriorly, differences in electron density of the cytoplasm of the cells can be seen. The lateral cell borders form many very narrow interdigitations with neighbouring cells. Underlying the epithelium is a uniform 140 nm-thick basal lamina. The proximal belt of the sheath epithelium is 5 to 0.5 µm high and is found at the junction (Fig. 7). The microvilli are distinctly shorter (350 nm long) than in the distal belt. In the specimen from Banyuls-sur-Mer, nine cytoplasmic strands are found in the nucleo-glandular girdle containing four nuclei (Fig. 8). In another species, *Z. seminascatus*, eight cell strands packed with swollen gland necks are found in the nucleo-glandular girdle (Fig. 9). Eight oblong and lobate nuclei (9-10 µm long) of the syncytium are found in a circle at the posterior end of this girdle (Fig. 10). The basal lamina underlying the sheath epithelium proceeds around the nucleo-glandular girdle but is not continuous with the bulbar septum.

In *Z. seminascatus* the cone epithelium is formed by a single apical cell and a basal syncytium that covers the flanks of the cone (Fig. 5). The epithelium is about 5 µm high. The stout microvilli are 350 nm long. The apical cell has no sunken cell parts, measures 8 µm in diameter and has a 6 µm long intra-epithelial nucleus. A basal lamina underlies the epithelium; it is irregular in thickness and frequently pierced by the gland necks. A terminal web is not present but the cytoplasm has a fibrous appearance. The syncytial basal cone epithelium has five insunk nucleated cell parts. The microvilli of the basal belt do not possess significantly more dense tips than do the microvilli in the apical belt. Narrow strands of the syncytium are situated in between the eight or nine cell strands of the proximal belt of the sheath epithelium (Figs 8, 9). The nuclei of the syncytium are situated in five cytoplasmic strands around the median part of the bulb (Fig. 11).

Glands. The sheath epithelium in *Zonorhynchus*-species is not only characterized by its particular cellular organization but also by the many gland necks that pierce the epithelium from pore to junction. The most numerous type of gland necks (g_1) contains electron-

dense secretion granules that measure 800 to 900 nm (Figs 2, 4). Sometimes granules with a light centre or lighter granules are present. A second type of gland neck (g_2) piercing the distal belt of the sheath epithelium stows moderately electron-dense secretion granules of about 900 nm in diameter (Figs 2, 4). The gland necks of both types are constricted by the terminal web of the epithelium. These two types of gland necks (g_1 and g_2) are also found in the proximal belt at the junction but a third type (g_3) is present as well (Fig. 7). Type g3 gland necks are filled with secretion in the apical half of the epithelium and stand wide open at the surface. The moderately electron-dense secretion granules measure about 500 nm in diameter. Two different types of gland necks surface through the part of this belt covering the basal part of the cone. One type (g_4) is empty because the secretion granules are washed out during preparation, while the other type (g_5) is piled with closely-packed, moderately electron-dense granules of approximately 1000 nm diameter (Figs 8, 9). These granules sometimes possess a darker spot in the centre. Types g_4 and g_5 gland necks are grouped in eight (*Z. seminascatus*) or nine (*Zonorhynchus* spec. B-s-M) cytoplasmic strands of the junctional belt (S_2) beneath the junction.

The basal belt of the cone epithelium is pierced by four types of glands. Large gland necks (g_6) with 1400 nm wide, transparent secretion granules and lined by an up to 200 nm thick peripheral margin of cytoplasm appear in a regular pattern (Figs 4, 5, 7). In a first circle, four g6-type gland necks alternate with four gland necks of type g_7 (Fig. 6). The latter type of gland necks, which contain 700 nm wide, electron-dense secretion granules, are also found between the muscles in the cone. However, they are not present in the proximal part of the bulb and enter the proboscis at the same position where the epithelial cell strands of the basal cone epithelium sink in (Fig. 1). Dispersed between types g_6 and g_7 gland necks are two other types of gland necks. Narrow (700 nm) necks contain a closely-packed, moderately electron-dense secretion, 350 nm in diameter (g8) or spherical to ovoid, more electron-dense secretion granules 250 nm in diameter (g10) (Fig. 6). The apical cell is pierced by gland necks (g9) passing through the central part of the bulb (Fig. 3). Moderately electron-dense secretion granules (200 nm) are sparsely present near the terminal end of the gland necks.

Sensory receptors. Uniciliary receptors are spread throughout the sheath epithelium (Fig. 4). The apical part of the sensory cells is connected to the epithelium by zonulae adhaerentes and septate junctions. The cilia possess normal 9+2 axonemata, basal bodies and rootlets. Over 2 µm long primary rootlets are orientated perpendicularly to the epithelium and short slanting secondary rootlets radiate from the basal bodies towards the plasmalemma forming the zonulae adhaerentes. Multiciliary receptors are not present, either intra-epithelially or insunk. Only very few sensory cells were encountered in the basal cone epithelium.

Outer proboscis musculature. The sheath epithelium in *Zonorhynchus*-species is surrounded by circular and longitudinal muscles (Fig. 6). The outer circular muscles are present around the cavity from the region of the dilators of the sheath up to the nucleo-glandular girdle, more specifically up to the position of the nuclei of the junctional belt (S_2). Around the anterior part of the distal belt of the sheath epithelium, nine very thin longitudinal muscle fibres are found. More posteriorly a distinct layer of longitudinal and circular muscles appears under the epithelium. Longitudinal muscle fibres of the body

wall musculature deflect and continue around the sheath epithelium (Fig. 1). The connective parts that run through the parenchyma form the dilators of the sheath. Posteriorly of the dilators of the sheath, the outer longitudinal muscle layer is composed of eighteen or twenty fibres. Towards the junction the fibres bifurcate and insert on the basal lamina. In between these muscle fibres and between their bifurcations a new set of outer longitudinal muscle fibres appears below the junction and around the nucleo-glandular girdle. Their bifurcated anterior ends form eighteen or twenty muscle fibres as well. They are present around the bulb and the 2 μm thick insunk cytoplasmic cell strands. They finally insert on the postero-lateral sides of the bulb.

The motional muscles include three pairs of protractors, four pairs of proboscis retractors and one pair of integument retractors. This arrangement corresponds to the description of *Zonorhynchus tvaerminensis* (KARLING, 1952). Proboscis retractors adhere on the postero-lateral sides of the bulb behind the nucleated cell parts of the basal cone epithelium in between the six protractor muscles (Fig. 12).

Inner proboscis musculature. The inner circular muscles surround the internal cone retractors (inner longitudinal muscles) from the nodus nearly up to the junction (Figs 8-11). Behind the nucleo-glandular girdle this layer is about 1.7 μm thick. In the cone the inner longitudinal muscles can be divided into a central cylinder (*cilm*) and eight peripheral blocks (*pilm*). The muscles that form the central cylinder are found beneath the apical cell. About halfway down in the cone they are surrounded by thin, circular muscle fibres, which are continuous with eight radiating muscles fibres in between the peripheral blocks (Fig. 13). The radiating muscle fibres all bifurcate and include another eight blocks of longitudinal muscles at the periphery. These muscle fibres are probably continuous with the inner circular muscles that surround all longitudinal muscles in the bulb. The circular muscles are found when the radiating muscles disappear. Apically the longitudinal muscles show electron-dense condensations (Fig. 13). Sometimes they appear to be conical. All muscle fibres, including those in the outer proboscis musculature, possess a sarcoplasmic reticulum but cross striation is not observed. The circular muscles are present from the nodus up into the cone, below the basal belt of the cone epithelium. The apical-most fibres are connected to the radiating fibres in the cone that separate the eight blocks of peripheral longitudinal muscles and the one or few circular muscle fibres enclosing the central cylinder of longitudinal muscle fibres.

Surrounding the inner circular muscles is a distinct layer of extracellular matrix (ECM) or septum. Apically this ECM-layer continues with the inner circular muscles underneath the cytoplasmic epithelial cell parts forming the nucleo-glandular girdle, and forms the uniform basal lamina of the cone epithelium (Fig. 1).

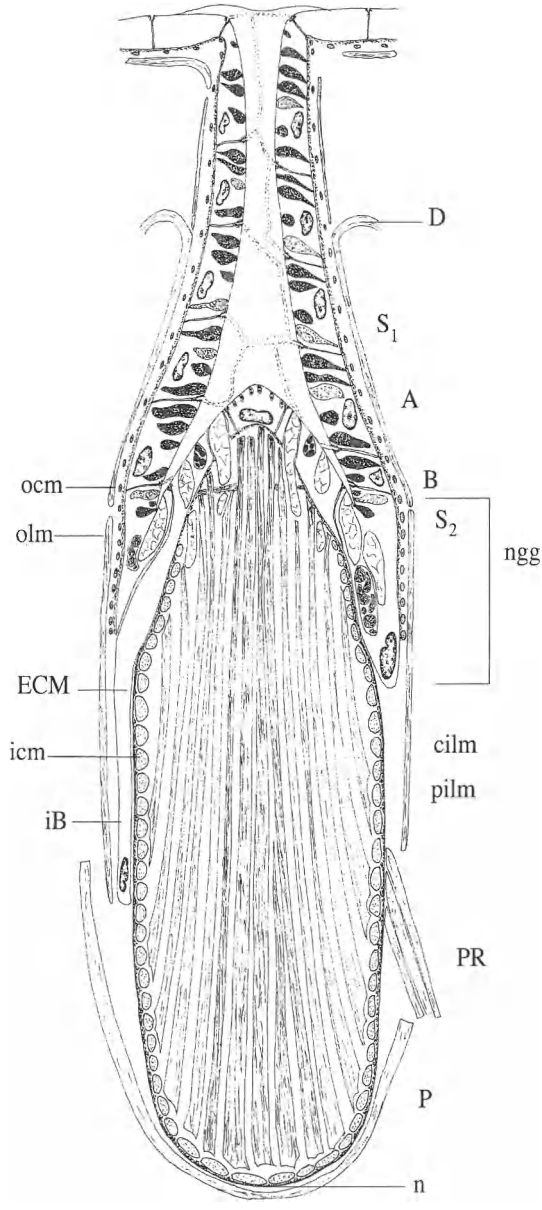
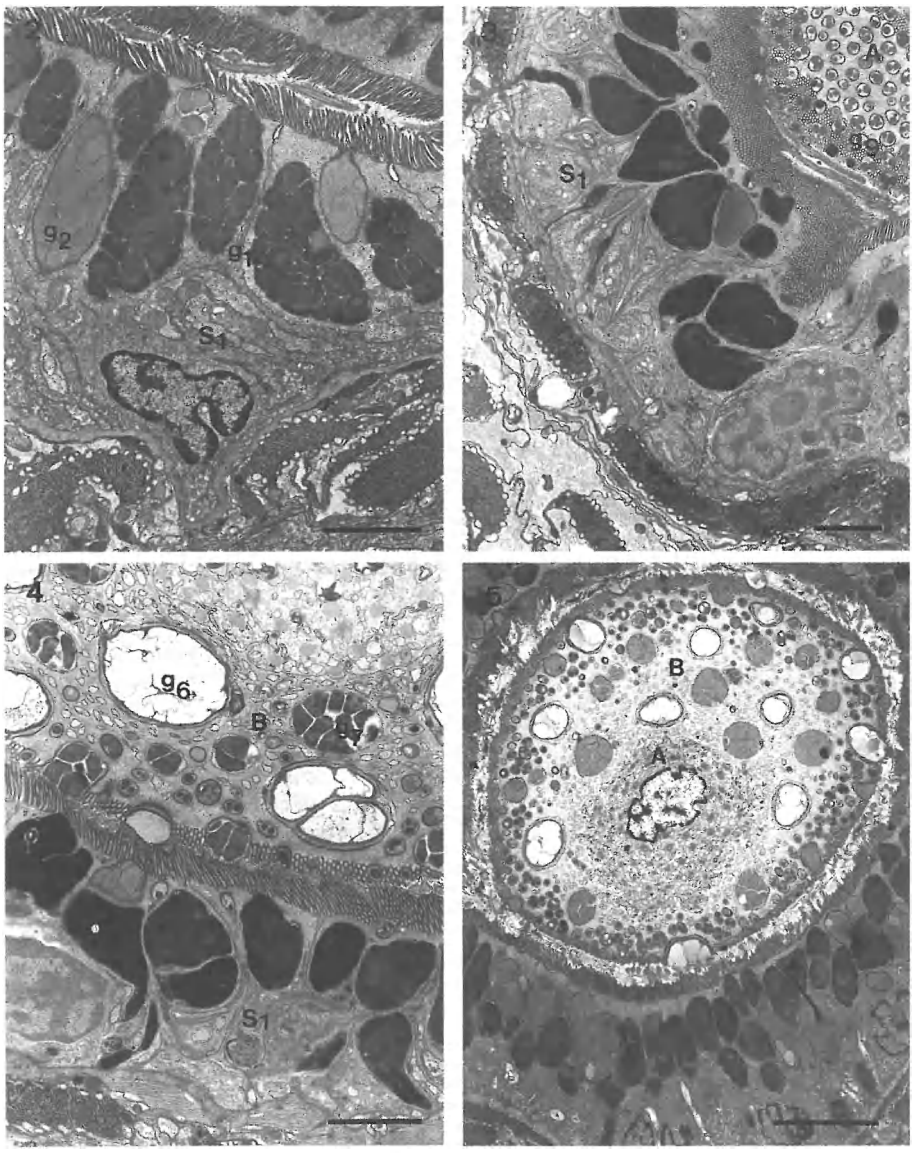
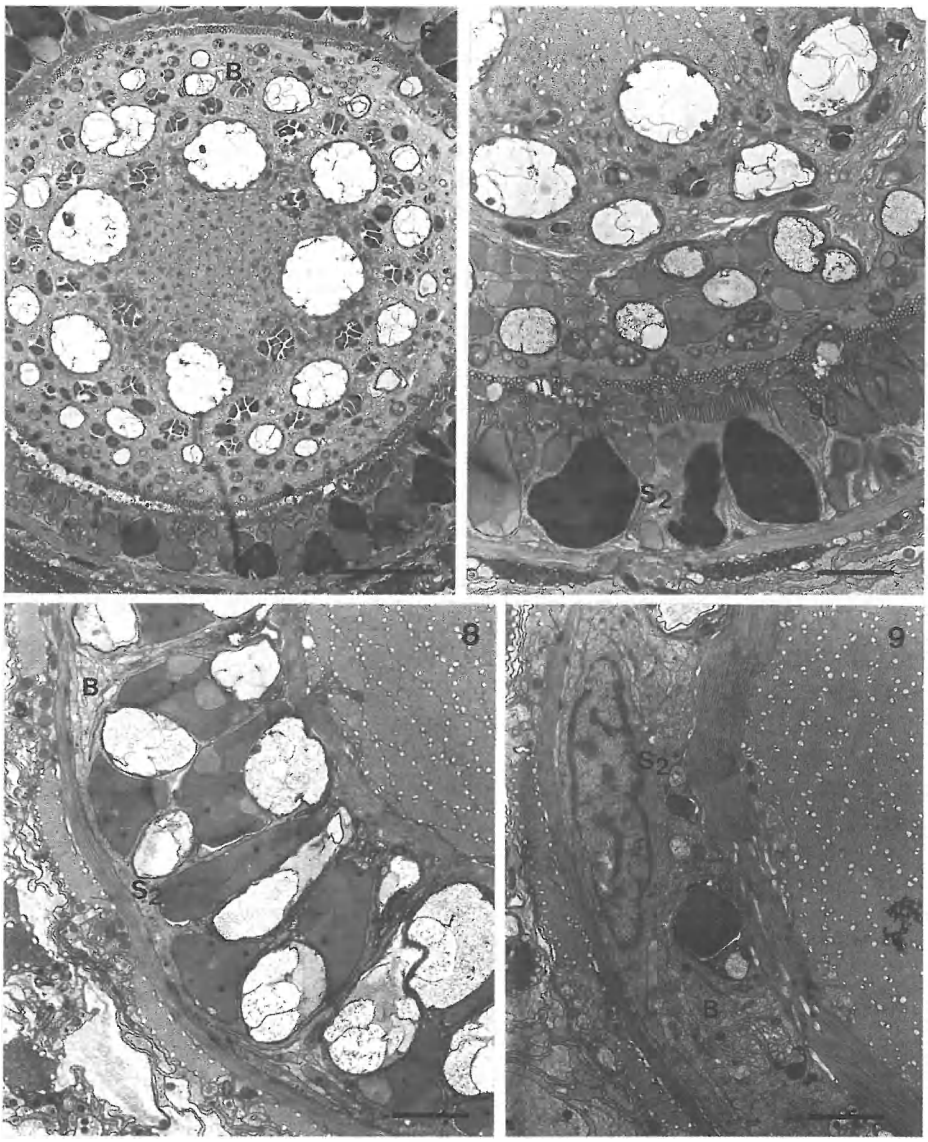


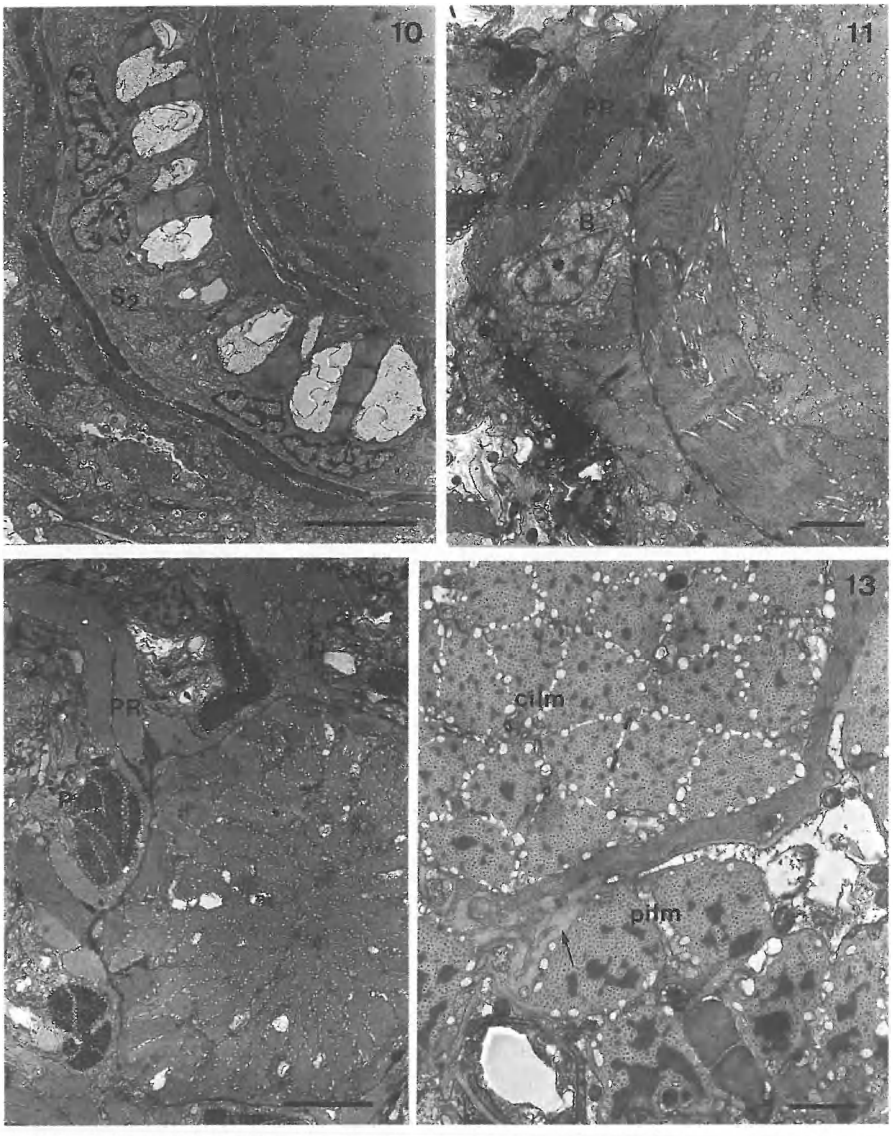
Fig. 1. – Reconstruction of the proboscis of *Zonorhynchus seminascatus*. – S_1 and S_2: resp. the distal and proximal belt of the sheath epithelium; cone epithelium formed by an apical cell (*A*) and a basal syncytial belt (*B*) with insunk nucleated cell parts (*iB*). Proboscis musculature with central (*cilm*) and peripheral (*pilm*) internal longitudinal muscle fibres, inner circular muscles (*icm*), outer circular (*ocm*) and longitudinal muscles (*olm*), proboscis retractors (*PR*) and proboscis protractors (*P*). The position of the nucleo-glandular girdle (*ngg*) is indicated.



Figs 2-5. — 2. *Z. seminascatus*. Cells of the distal belt (S_1) with nucleus and g_1 and g_2 gland necks. Scale bar: 2 µm. – 3. *Z. seminascatus*. Distal belt of the sheath epithelium (S_1) surrounded by circular and longitudinal muscles, and apical cone epithelium (A) with g_9 gland necks. Scale bar: 2 µm. – 4. *Zonorhynchus* sp. Distal belt of the sheath epithelium (S_1), and basal cone epithelium (B) with g_6 and g_7 gland necks. Scale bar: 2 µm. – 5. *Z. seminascatus*. Cross section of the cone with nucleus of the apical cone epithelium (A), and basal cone epithelium (B) with alternating g_6 and g_7 gland necks. Scale bar: 5 µm.



Figs 6-9. – *Zonorhynchus* sp. – 6. Cross section of the cone clearly showing alternating g_6 and g_7 gland necks with in between g_8 and g_{10} gland necks. Scale bar: 5 µm. – 7. Proximal belt of the sheath epithelium with g_1, g_2 and g_3 gland necks, basal cone epithelium with groups of g_4 and g_5 gland necks, and g_6 necks in the bulb. Scale bar: 2 µm. – 8. Cross section of the nucleo-glandular girdle showing type g_4 and g_5 gland necks and cytoplasmic cell parts of S_2 and *B*. Scale bar: 2 µm. – 9. Posterior portion of the nucleo-glandular girdle with nucleus of S_2 and cell strands of *B*. Scale bar: 2 µm.



Figs 10-13. – *Zonorhynchus seminascatus*.– 10. Nucleo-glandular girdle with groups of g_4 and g_5 gland necks and nuclei of S_2. Scale bar: 5 µm. – 11. Cross section of the median part of the bulb with insunk nucleated cell part of *B* in between proboscis retractors (*PR*). Scale bar: 2 µm. – 12. Cross section of the posterior part of the bulb with proboscis retractors (*PR*) and protractors (*P*). Scale bar: 5 µm. – 13. Cross section in the cone with central cylinder (*cilm*) and peripheral longitudinal muscles (*pilm*), separated by a circular muscle fibre. Note the ECM (*arrow*). Scale bar: 1 µm.

DISCUSSION

The epithelium of the proboscis in *Zonorhynchus*-species is divided into four belts as described for *Cicerina remanei* Meixner, 1928, *Psammorhynchus tubulipenis* Meixner, 1938 and *Cytocystis clitellatus* Karling, 1953 (De Vocht & Schockaert, 1988, De Vocht, 1990). In *Polycystis naegelii* K^lliker, 1845, *Cystiplana paradoxa* Karling, 1964 and *Cystiplex axi* Karling, 1964 and *Mesorhynchus terminostylis* Karling, 1956 five circumferential belts constitute the proboscis epithelium (Schockaert & Bedini, 1977; De Vocht, 1989; De Vocht, 1991). The cone epithelium in all investigated species is always formed by two belts while the number of belts in the sheath epithelium is two or three.

As in *C. remanei*, the distal belt of the sheath epithelium in *Zonorhynchus*-species lines the major part of the proboscis cavity. A distal belt formed by numerous cells, without distinct organization, is typical for all species of *Zonorhynchus,* and has not been recorded for any other species up till now.

A basal lamina under the cone epithelium, as in *C. remanei*, *P. naegelii* and Cystiplanidae is present in *Zonorhynchus*-species as well (Schockaert & Bedini, 1977; De Vocht & Schockaert, 1988; De Vocht, 1989). In *C. axi*, *P. tubulipenis* and *M. terminostylis* and Koinocystididae in general such a basal lamina is lacking (De Vocht, 1989; De Vocht, 1990; De Vocht, 1991; Karling, 1980). In all species of Eukalyptorhynchia investigated, with the exceptions of *Toia calceformis* Brunet, 1973 and *Nannorhynchides herdlaensis* Karling, 1956, the basal belt of the cone epithelium is syncytial (De Vocht, 1992). The organization of the nucleated parts of the cone epithelium is diverse and common tendencies are hard to discern. In all species of the genus *Zonorhynchus* the apical cone epithelium has intra-epithelial nuclei, one in *Z. seminascatus* and *Z. tvaerminensis,* and several in *Z. salinus* (Karling, 1952). Within the Eukalyptorhynchia, only species of the genus *Zonorhynchus* have intra-epithelial nuclei in the cone. In all other species the nuclei of the apical cone epithelium are situated beneath the junction in the nucleo-glandular girdle or inside the bulb. The basal cone epithelium in *Zonorhynchus*-species has insunk nucleated cell parts, not enclosed by a layer of ECM. These epithelial cell perikarya have already been noted but not characterized as such by Karling (1952).

The perikarya of the proboscis epithelia in *C. remanei* are fully enclosed by a layer of ECM and outer circular and longitudinal muscles (De Vocht & Schockaert, 1988). They are not considered insunk. In *Zonorhynchus*-species the nucleo-glandular girdle is not as such incorporated in the bulb as in *C. remanei*, and proximally not enclosed by a layer of ECM. As in *C. remanei*, but less pronounced, a grouping of g_4 and g_5 gland necks is present in this girdle in *Zonorhynchus*-species as well.

A delimited circumferential ring of gland necks above the junction is present in all species classified in the family Cicerinidae. However, such a ring is also present in Gnathorhynchidae and Placorhynchidae (De Vocht, 1992). In *Zonorhynchus*-species this glandular ring is less obvious because the sheath epithelium as a whole is pierced by numerous gland necks.

The conspicuous gland necks of type g_4 and g_5 are also found in *C. remanei*, but are not present in either *P. tubulipenis* or *C. clitellatus* (De Vocht & Schockaert, 1988; De

VOCHT, 1990). Type g_6 and g_7 gland necks in the basal cone epithelium show common features with gland necks found in *C. remanei, P. tubulipenis* and *C. clitellatus*. In *C. remanei* the basal cone epithelium is pierced by two similar types of gland necks ($g_6$and g_7) apically of the glandular ampullae as well (DE VOCHT & SCHOCKAERT, 1988). These gland necks pass through the bulb and enter it at the nodus. In *P. tubulipenis* and *C. clitellatus* homologous gland necks are also found in the basal cone epithelium (DE VOCHT, 1990). In *C. clitellatus*, type g_9 glands also form wide necks in the bulb but they do not form distinct groups.

The apical cone epithelium in *Zonorhynchus*-species is pierced by one type of gland necks that contain an electron-dense secretion packed in small granules (200 nm). This kind of secretion with relatively small granules is also found in *P. tubulipenis* (type g_8) and *C. clitellatus* (type g_{11}) (DE VOCHT, 1990). In *C. remanei* and Cystiplanidae two different types of secretions appear in this epithelium: small rod-like granules as in *P. naegelii* and larger spherical granules (SCHOCKAERT & BEDINI, 1977; DE VOCHT & SCHOCKAERT, 1988; DE VOCHT, 1989). Apparently gland necks with the small secretion granules in the apical cone epithelium are typical and a common feature for all species investigated.

As in all eukalyptorhynch species investigated, uniciliary receptors with blunt ciliary shafts, basal bodies and rootlets are also present in the cone epithelium of *Zonorhynchus*-species (REUTER, 1975; SCHOCKAERT & BEDINI, 1977; DE VOCHT & SCHOCKAERT, 1988; DE VOCHT, 1989; DE VOCHT 1990; DE VOCHT, 1991). Multiciliary receptors associated with the distal belt of the sheath epithelium, situated either intra-epithelially as in *C. paradoxa* and *C. axi,* or insunk as in *P. tubulipenis*, *C. clitellatus* and *C. remanei*, are not present in *Zonorhynchus*-species (DE VOCHT, 1989; DE VOCHT 1990; DE VOCHT 1992).

The motional muscles of the proboscis in *Zonorhynchus*-species include protractors, proboscis retractors and integument retractors. As in *C. remanei* fixators are lacking. In *Z. tvaerminensis* wide disk-shaped fibres are also present anteriorly in the inner circular muscle layer (KARLING, 1952). A clear distinction between groups of internal longitudinal muscles as encountered in *Zonorhynchus*-species is only found in *T. calceformis* and *N. herdlaensis* but in no other eukalyptorhynch species investigated thus far (DE VOCHT 1992).

Based on the ultrastructural data derived from investigations of the proboscis in different species of Eukalyptorhynchia, a tentative cladogram is proposed (Fig. 14). Within the Kalyptorhynchia the Eukalyptorhynchia forms a monophyletic taxon based on the presence of a conorhynch (proboscis with muscular bulb enclosed by a layer of ECM with a cone protruding in the proboscis cavity) (apomorphy 1). *T. calceformis* and *N. herdlaensis* are characterized by fully cellular proboscis epithelia. This character state is regarded as the plesiomorphic condition. Both *Toia*- and *Nannorhynchides*-species have pigmented eyes with lenses and a reduction of one or two sperm axoneme(s), which form synapomorphic features for both taxa (apomorphies 2 and 3) (BRUNET, 1973; DE VOCHT, 1992; WATSON, 1998). The presence of a syncytial basal belt on the cone epithelium forms a synapomorphy for *Zonorhynchus*-species and the other taxa investigated (apomorphy 4). A distal belt of the sheath epithelium with random, multicellular organization and an apical cone epithelium with intra-epithelial nuclei are apomorphic character states for *Zonorhynchus*-species (apomorphies 5 and 6). The presence of multiciliary receptors asso-

ciated with the distal belt of the sheath epithelium constitutes an apomorphic feature for the other taxa investigated (apomorphy 7). Two insunk sensory organs with multiciliary receptors are present in *C. remanei, P. tubulipenis* and *C. clitellatus* (apomorphy 8). The presence of a third belt in the sheath epithelium is a synapomorphic character for species of the families Koinocystididae, Cystiplanidae and Polycystididae (apomorphy 9) (De Vocht, 1989; De Vocht, 1991; De Vocht, 1992). *P. tubulipenis* and *C. clitellatus* are characterized by intrabulbar nucleated cell parts of the proximal belt of the sheath epithelium and the apical cone epithelium, as well as insunk nucleated cell parts of the basal belt of the cone epithelium (apomorphy 10) (De Vocht, 1990). *C. remanei* is characterized by the presence of four glandular ampullae, surrounded by circular muscles (apomorphy 11) (De Vocht & Schockaert, 1988). The presence of respectively one or three lens(es) in the pigmented eyes of species of the genus *Toia* and *Nannorhynchides* are autapomorphic characters for both taxa (apomorphies 12 and 13) (Brunet, 1973). The monospecific genera *Psammorhynchus* and *Cytocystis* are respectively typified by the appearance of multiciliary receptors which form stacks of flat sheet-like ciliary shafts in the sensory organs, and elongated insunk cell parts of the distal belt with 'normal'-shaped ciliary shaft (apomorphy 14 and 15) (De Vocht, 1990).

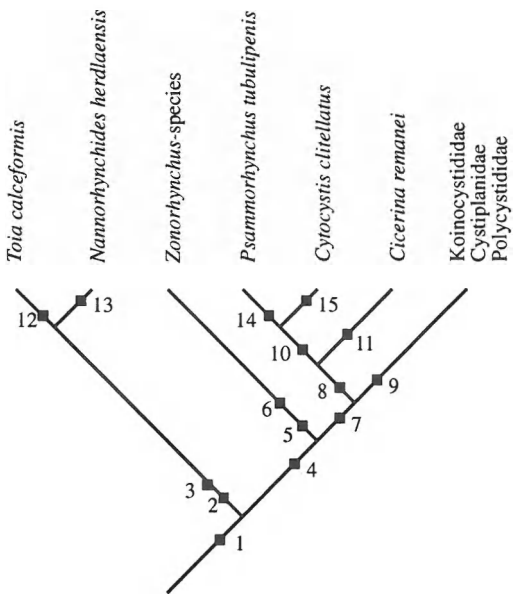


Fig. 14. – Tentative cladogram mainly based on characters derived from ultrastructural investigations on the proboscis of eukalyptorhynch species investigated. See text for the description of the apomorphic characters 1 to 15.

We conclude that the species or genera grouped in the family Cicerinidae lack a synapomorphic character. *Zonorhynchus*-species have an intermediate position in the group of species investigated thus far and have symplesiomorphic features in common with *T. calceformis* and *N. herdlaensis*. *Zonorhynchus* species are characterized by synapomorphic fea-

tures common with *P. tubulipenis*, *C. clitellatus* and *C. remanei*. *C. remanei* and the monospecific genera *Psammorhynchus* and *Cytocystis* can be regarded as monophyletic based on the presence of the sensory organs associated with the distal belt of the sheath epithelium.

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