Limburgs Universitair Centrum

Faculteit Wetenschappen

Anatomie en ultrastructuur van de proboscis van Eukalyptorhynchia (Platyhelminthes, Rhabdocoela).

Anatomy and ultrastructure of the proboscis in Eukalyptorhynchia (Platyhelminthes, Rhabdocoela).

Proefschrift voorgelegd tot het behalen van de graad van Doctor in de Wetenschappen aan het Limburgs Universitair Centrum te verdedigen door Alain DE VOCHT

Promotor : Prof. Dr. E. Schockaert

Academiejaar 1991-1992

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Aan Monique en Heleentje

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List of abbreviations

A	:	Apical cone epithelium
B	:	Basal cone epithelium
bl,bm	:	Basement membrane
Bu	:	Bulb
cg	:	Cytoplasmic girdle
ci	:	Cilium
Co	:	Proboscis cone
D	:	Dilator
Ep	:	Epidermis
F	:	Fixator
ga	:	Glandular ampulla
gp	:	Gland necks around the porboscis pore
gr	:	Glandular ring
g1 - g11	:	Different types of gland necks in the proboscis epithelia
iA	:	Insunk cell parts of the apical cone epithelium
iB	:	Insunk cell parts of the basal cone epithelium
ibp	:	Infoldings of the basal plasma membrane
icm	:	Inner circular muscle
iem	:	Intra-epithelial muscle
ilm	:	Inner longitudinal muscle
IR	:	Integument retractor
iS_1	:	Insunk cell parts of S_1
iS_3	:	Insunk cell parts of S_3
mcr	:	Multiciliary receptor
mv	:	Microvilli
nu	:	Nucleus
ng	:	Nucleoglandular girdle
ocm	:	Outer circular muscle
olm	:	Outer longitudinal muscle
P	:	Protractor
pr	:	Primary rootlet
PR	:	Proboscis retractor
PR_1	:	Anterior set of proboscis retractors
PR_2	:	Posterior set of proboscis retractors
s	:	Bulbar septum
sr	:	Secondary rootlet
S_1	:	Distal belt of the sheath epithelium
S_2	:	Median belt in tripartite sheath epithelia, proximal belt in bipartite sheath epithelium
S3	:	Proximal belt of the sheath epithelium
so	:	Sensory organ
ucr	:	Uniciliary receptor

Chapter 1.

General introduction

Ultrastructural characters have proved to be important and systematically significant. The last twentyfive years characters obtained from ultrastructural research have made an important contribution to phylogenetic systematics in freeliving Platyhelminthes (Ehlers 1985). Extensive comparative studies have been carried out on the ultrastructure of the pharynx (Doe 1981), the epidermis (Bedini & Papi 1974), rhabdites (Reisinger & Kelbletz 1964, Reisinger 1969, Smith *et al* 1982), sperm (Hendelberg 1974), epidermal receptors (Ehlers 1977, Sopott-Ehlers 1984) and adhesive systems (Tyler 1976, 1977). A wide variety of ultrastructural information on "Turbellaria" is presently available (for a bibliography till 1984 see Tyler *et al* 1986).

In the phylogenetic system of the Platyhelminthes proposed by Ehlers (1985), the Kalyptorhynchia are considered a monophyletic taxon with two autapomorphic characters; the frontal proboscis and full incorporation of both axonemata in the sperm cell during spermiogenesis. The taxon Kalyptorhynchia was placed in the taxon "Typhloplanoida", which probably does not represent a monophyletic but a paraphyletic taxon, characterized by plesiomorphic characters, within the Rhabdocoela. A pharynx of bulbosus-type with pharyngeal septum and transition of the muscle layer at the pharynx rim is considered an autapomorphy for the Rhabdocoela Ehrenberg, 1831. Rhabdocoela, Seriata and Prolecitophora possibly form a monophyletic taxon (Taxon N.N.1) with autapomorphy, the uniciliary collar receptors with a fixed number of eight microvilli (Ehlers 1985).

Within the free-living Platyhelminthes a proboscis is present in several taxa. In some species of Macrostomida, a differentiation of the anterior part of the body is called a proboscis (Meixner 1938), and an invaginated proboscis is present in some Rhabdocoela. Three families within the rhabdocoel taxon Typhloplanoida contain representatives with a proboscis: Typhloplanidae, Trigonostomidae and Kytorhynchidae (Meixner 1938, Rieger 1974). The proboscis, which is present in these families, differs from the proboscis in Kalyptorhynchia. In all these species a bulbar septum is lacking. Within Kytorhynchidae different proboscis types are present, ranging from a frontal invagination with sensory cells and gland necks, to a increased presence of muscles and glands and reduction of the number of receptors. In all species of Typhloplanidae and Trigonostomidae the proboscis pore is not situated at the terminal end but subterminally at the ventral side.

The taxon Kalyptorhynchia, comprising the taxa Eukalyptorhynchia Meixner, 1928 and Schizorhynchia Graff, 1905, is characterized by a frontal proboscis with a muscular part retracted in a sheath. The Eukalyptorhynchia possess a undivided muscular bulb, which is separated from the parenchyma by a circular muscle layer surrounded by a thin layer of

extracellular matrix (ECM) or septum (Meixner 1938). The anterior part of the bulb is differentiated in a cone and covered by an epithelium. The cone protrudes into the proboscis cavity, which is covered by a sheath epithelium. In Schizorhynchia two dorso-ventrally opposing muscular parts, enclosed by a layer of extracellular matrix, are present, which are to a large extend covered by epithelia. At the proximal end the two muscular parts are connected to each other. Information on the proboscis obtained from light microscopic investigations plays an important role in the current systematic classification of Kalyptorhynchia (Dean 1977, Evdonin 1977, Karling 1953, 1964, Meixner 1925, 1929, 1938, Rieger and Sterrer 1975, Schilke 1969). Hard structures as teeth and hooks, well developed glandular structures and muscular differentiations are distinct morphological characters gained from LM-observations. Now electron microscopic analysis of the eukalyptorhynch proboscis structure allows us to use other and more detailed information and gives us new insights in the relationships within the Eukalyptorhynchia.

Other invertebrate taxa, such as nemerteans, are also typified by an organ which is named proboscis. Because these organs definitly do not represent homologous characters, information on their structure has not been included in the discussion.

In the current systematic classification the taxon Eukalyptorhynchia Meixner, 1928 comprises twelve families. The Polycystididae Graff, 1905 was the first established family. A redefinition of the diagnosis of the family was given by Karling (1955). The family Gyratricidae Graff, 1905 and Polycystididae Meixner, 1924 are synonymous with Polycystididae Graff, 1905. Meixner erected the families Koinocystididae (1924), Cicerinidae (1928), Gnathorhynchidae (1929) and the family Placorhynchidae (1938). New diagnosis of the family Koinocystididae have been given by Karling (1954, 1980). The family Zonorhynchidae Karling, 1952 is synonymous with Cicerinidae Meixner, 1928. Karling (1947) presented a new diagnosis of the latter two families. Karling erected three monotypic families Psammorhynchidae (1956), Cytocystidae (1964) and Acrumenidae (1980) as well as the family Cystiplanidae (1964). The monotypic families Aculeorhynchidae and Crassicollidae have been erected by Schilke (1969) and Dean (1977) respectively. The family Bertiliellidae was established by Rieger and Sterrer (1975). A number of species with uncertain systematic position, such as *Gnorimorhynchus dividuus* Brunet, 1972, *Marirhynchus longasaeta* Schilke, 1970, *Mesorhynchus terminostylis* Karling, 1956 and *Elvertia krusei* Noldt, 1989 is not incorporated in these families. The most recent diagnosis of the different families within Eukalyptorhynchia have been presented by Karling (1947, 1964) and later by Schilke (1969), Brunet (1973), Rieger and Sterrer (1975) and Karling (1980) or by Evdonin (1977).

Upto now the ultrastructural organization of the proboscis epithelia has been described for *Polycystis naegelii* (Polycystididae) by Schockaert and Bedini (1977) and later for *Cicerina remanei* (Cicerinidae) by De Vocht and Schockaert (1988). The complete organization of the

proboscis was described by De Vocht (1989, 1990, 1991) in *Cystiplex axi*, *Cystiplana paradoxa* (Cystiplanidae), *Psammorhynchus tubulipenis* (Psammorhynchidae), *Cytocystis clitellatus* (Cytocystidae) and *Mesorhynchus terminostylis* (species incertae sedis). Fragmentary information is available from electron microscopic studies on *Gyratrix hermaphroditus* (Reuter 1975), *Florianella bipolaris* (Rieger & Sterrer 1975, Rieger 1981) and *Gnathorhynchus* (Doe 1976). Now information on 35 species is given.

Within a specific genus the differences in organization of the proboscis are not great. Only in some genera relatively small differences have been found. In a few species individual differences, which concern the number of cells or nuclei in a syncytium, have been encountered. The stability of ultrastructural characters, such as the component parts of the proboscis, at the species and genus level show significance of the variability encoutered at higher levels. Such ultrastructural characters can be applied and have proved to be significant in phylogenetic systematics, especially in metazoan phylogeny (Tyler 1977, Rieger 1986).

The presence of five families within the Eukalyptorhynchia, which actually represent one species, and several species incertae sedis form a indication for the problems which are encountered in classifying species and genera in this taxon. Often the genital structures are distinctly different and exclude incorporation in more extensive families or features of the proboscis, pharynx or epidermis do not coincide with characteristics of the genital system and have been used to justify the establishment of a new taxon of higher rank (Dean 1977, Karling 1953, 1964, Schilke 1969).

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Chapter 2.

Material, methods and terminology

Material

In this study 35 species of Eukalyptorhynchia, classified in nine families or species with uncertain sytematic postion, have been investigated ultrastructurally. Except for one specimen of *Zonorhynchus* from Lafranqui, all specimens have been identified up to species level. Specimens of the species, which form the monotypic families Crassicollidae Dean, 1977, Acrumenidae Karling, 1980 and Aculeorhynchidae Schilke, 1969, have not been found. The light microscopic type material of these species, however, has been investigated. Table 1. gives a summary of the investigated species, the location of the collecting sites and a brief description of the habitat. Table 2. gives a summary of the species of which the type material has been investigated by light microscopy as well as the origin of the material. No specimens of the monotypic families Crassicollidae, Acrumenidae and Aculeorhynchidae have been encountered or investigated. The type material of these species has been investigated light microscopically (see table 2).

Collection

Extraction of undamaged living sanimals for identification and fixation for electron microscopic investigations has been carried out. Interstitial species were qualitatively extracted from the sandy sediment using the $MgCl_2$-decantation method (Sterrer 1968, Hullings & Gray 1971). Animals inhabiting muddy sediments with high organic matter content were extracted by sieving the fine mud and washing the remaining sediment and animals into a petri-dish or by flodding the samples and covering them with a layer of clean sand of approximately 2 cm. These samples were kept at room temperature (25°C) and turned anaerobic quickly. The animals migrate upwards into the oxiginated sandy layer. Animals were extracted from the sand by washing and stirring the sediment with seawater or a $MgCl_2$-solution isotonic to seawater and subsequent decantation through a sieve (Armonies & Hellwig 1986). Specimen of *Zonorhynchus*, *Ethmorhynchus* and *Mesorhynchus* were collected in this way. Epiphytal species were obtained by stirring the algae vigorously in seawater or in a isotonic solution of $MgCl_2$. Samples were taken by hand, scuba diving or Ockelmann sledge.

Preparation for electron microscopy

Before primary fixation, animals were anaestetized in a solution of $MgCl_2$ isotonic to the seawater. Primary fixation was at first carried out with 0.1 M phosphate buffered 2% glutaraldehyde at 4°C for 2h, later a O.1 M cacodylate buffer was used and shorter times

Species	Locality	Habitat
Cicerinidae Meixner, 1928		
Toia calceformis Brunet, 1973	France (Banyuls-sur-Mer, Ile des Embiez, Calvi)	Algae (*Codium*), 8-11 m depth
Nannorhynchides hardlaensis Karling, 1956	Sweden (Kristineberg)	Algae, 10-12 m depth
Zonorhynchus seminascatus Karling, 1956	Sweden (Kristineberg)	Sand, 40 cm depth
Zonorhynchus salinus Karling, 1952		
Zonorhynchus spec.	France (La Franqui)	Etang de la Palme, sand
Ethmorhynchus anophthalmus Meixner, 1938	Sweden (Kristineberg)	Fine sand, 15 m depth
Cicerina remanei Meixner, 1928	Belgium (Mariakerke, Zeebrugge)	Litoral, sand
Cicerina brevicirrus Meixner, 1928	Belgium (Heist); Sweden (Kristineberg)	Litoral-40 cm depth, sand
Paracicerina deltoides Martens & Schockaert, 1981	Belgium (Oostende)	Litoral, sand
Ptyalorhynchus coecus Ax, 1951	Belgium (Oostende, Knokke)	Litoral, sand
Psammorhynchidae Karling, 1964		
Psammorhynchus tubulipenis Meixner, 1938	Germany, Sylt (List)	Litoral, sand
Cytocystidae Karling, 1964		
Cytocystis clitellatus Karling, 1953	Sweden (Kristineberg)	Algae, 10 m depth
Bertilellidae Rieger & Sterrer, 1975		
Florianella bipolaris Rieger & Sterrer, 1975	Florida, Eastward Cruise	25 m depth
Placorhynchidae Meixner, 1938		
Placorhynchus octaculeatus Karling, 1931	France (Ambleteuse)	Sand, estuary of La Slack
Gnathorhynchidae Meixner, 1929		
Gnathorhynchus conocaudatus Meixner, 1929	Belgium (Knokke)	Litoral, sand
Drepanorhynchides diodonthus L'Hardy 1966	France, Corse (Calvi)	Coarse sand, 12 m depth
Paragnathorhynchus subterraneus Meixner, 1938	Germany, Sylt (List)	Litoral, sand
Cystiplanidae Karling, 1964		
Cystiplex axi Karling, 1964	France, Corse (Calvi)	Sand, 9-12 m depth
Cystiplana paradoxa Karling, 1964	France (Ile des Embiez)	Sand, 2 m depth
Polycystidae Graff, 1905		
Progyrator mamertinus Graff, 1874	France, Corse (Calvi) and (Banyuls-sur-Mer)	Algae, 0-6 m depth
Phonorhynchus helgolandicus Metschnikoff, 1865	Sweden (Kristineberg)	Algae, 0-12 m depth
Gyratrix hermaphroditus Ehrenberg, 1831	Germany, Sylt (List)	Litoral, sand
	France, Corse (Calvi)	Coarse sand, 12 m depth
Neopolycystis ridentata Karling 1955	Belgium (Oostende, Knokke); Germany, Sylt (List)	Litoral, sand
Gallorhynchus mediterraneus Schockaert & Brunet, 1971	France, Corse (Calvi)	Coarse sand, 12 m depth
Rogneda pakula Brunet, 1969	France (Ile des Embiez)	Old salina, 10-20 cm depth
Polycystis riedli Karling, 1956	France (Ile des Embiez)	Algae, 20-30 cm depth
Alcha evelinae Marcus, 1949	Kenya	
Paraustrorhynchus nov. spec.	Kenya	
Typhlopolycystis rubra Noldt & Reise, 1987	Germany, Sylt (List)	Pocket sand of *Arenicola marina* burrows
Danorhynchus gosoensis Karling, 1955	Sweden (Kristineberg)	Mud with *Pennatula*, 40 m depth
Koinocystididae Meixner, 1925		
Tenarrhynchus magnus Brunet, 1972	France, Corse (Calvi)	Sand, 10 m depth
Itaipusa karlingi Mack-Fira, 1968	France(Ile des Embiez)	Old salina, 15 cm depth
Paraetalga biliai Karling, 1964	Sweden (Kristineberg)	Mud with *Pennatula*, 40 m depth
Species incertae sedis		
Marirhynchus longasaeta Schilke, 1970	Germany, Sylt (List)	Litoral, sand
Mesorhynchus terminostylis Karling, 1956	Sweden (Kristineberg)	Mud with *Pennatula*, 40 m depth

Table 1. Collected species which have been investigated ultrastructurally. The collecting sites and habitats are listed in the second and third column.

Species	Material	Reference
Cicerinidae Meixner, 1928		
Toia calceformis Brunet, 1973	1 whole mount	Brunet 1973
Toia ycia Marcus, 1952	1 whole mount S.M.N.H.	Marcus 1953
Nannorhynchides herdlaensis Karling, 1956	1 serially sectioned sp. Holotype 2720 S.M.N.H.	Karling 1956
Pocillorhynchus agilis Brunet, 1973	1 serially sectioned sp. Holotype 2807 S.M.N.H.	Brunet 1973
Ethmorhynchus anophthalmus Meixner, 1938	5 serially sectioned sp. S.M.N.H.	Meixner 1938
Blennorhynchus egregius Meixner, 1938	1 serially sectioned sp. 4.V.28 S.M.N.H.	Meixner 1938
Didiadema picardi Brunet, 1965	1 serially sectioned sp. S.M.N.H.	Brunet 1965, 1973
Xenocicerina gracilis Karling, 19565	1 serially sectioned sp. 2719 S.M.N.H.	Karling 1956, 1964
Psammorhynchidae Karling, 1956		
Psammorhynchus tubulipenis Meixner, 1938	1 serially sectioned sp. S.M.N.H.	Karling 1956, 1964
Aculeorhynchidae Schilke, 1969		
Aculeorhynchus glandulis Schilke, 1969	2 serially sectioned sp. 1 total mount Univ. Göttingen Holotype 2131, Paratype 2132	Schilke 1969, 1970 Hoxhold 1974
Cyctocystidae Karling, 1964		
Cytocystis clitellatus Karling, 1953	3 serially sectioned sp. Lectotype 2790 S.M.N.H.	Karling 1953
Placorhynchidae Meixner, 1938		
Harsa obnixa Marcus, 1951	Syntype 3130 S.M.N.H.	Marcus 1951
Cystiplanidae Karling, 1964		
Cystiplana rubra Dean, 1977	1 serially sectioned sp. Paratype 2952, 2953 S.M.N.H.	Dean 1977
Cystiplana paradoxa Karling, 1964	1 serially sectioned sp. Holotype 2756 S.M.N.H.	Karling 1964
Cystiplex axi Karling, 1964	1 serially sectioned sp. Holotype 2757 S.M.N.H.	Karling 1964
Cystirete graefei Brunet, 1965	1 serially sectioned sp. S.M.N.H.	Brunet 1965
Nigerrhynchus opisthoporus Schilke, 1970	2 serially sectioned sp. Dr. U. Noldt	Noldt 1989
Koinocystididae Meixner, 1924		
Brunetia camarguensis Brunet, 1965	1 serially sectioned sp. S.M.N.H.	Brunet 1965, Karling 1980
Groveia unicornis Karling, 1980	1 serially sectioned sp. Holotype 3128 S.M.N.H.	Karling 1980
Neoutelga inermis Karling, 1980	1 serially sectioned sp. Holotype 3123 S.M.N.H.	Karling 1980
Tenerrhynchus magnus Brunet, 1972	2 serially sectioned sp. Holotype 3038 Holotype 3038a S.M.N.H.	Brunet 1972
Parautelga bilioi Karling, 1964	1 serially sectioned sp. Holotype 2758 S.M.N.H.	Karling 1964
Utelga pseudoheinckei Karling, 1980	2 serially sectioned sp. S.M.N.H.	Karling 1980
Utelga heinckei Attems, 1897	2 serially sectioned sp. S.M.N.H.	Karling 1980
Rhinolasius dillonicus Karling, 1980	1 serially sectioned sp. Holotype 3122 S.M.N.H.	Karling, 1980
Rhinolasius sartus Marcus, 1951	Syntype 3130 S.M.N.H.	Marcus 1951
Crassicolilidae Dean, 1977		
Crassicolum musculare Dean, 1977	1 serially sectioned sp. Paratype 2954, S.M.N.H.	Dean, 1977
Acrumenidae Karling, 1980		
Acrumena marsiliensis Brunet, 1965	1 serially sectioned sp. Holotype 3035 S.M.N.H.	Brunet 1965
Incertae sedis		
Mesorhynchus terminostylis Karling, 1956	1 serially sectioned sp. Holotype 2759 2955a, 2955b S.M.N.H.	Karling 1956
Lekanorhynchus remanei Meixner, 1938	1 serially sectioned sp.	
Gnorimorhynchus dividuus Brunet, 1965	1 serially sectioned sp. Holotype 3036 S.M.N.H.	Brunet 1965, Karling 1980

Table 2. Species which have been investigated by light microscope, the kind of material and reference to the original description of the material.

were used. After double rinsing in the same buffer specimens were postfixed in 1%-cacodylate-buffered osmiumtetroxide at 4°C for 1h, prestained in 2% uranyl acetate solution (20 min) before dehydration in a graded acetone series and embedded in Araldite. Primary fixatives with a osmolality ranging between 480-900 mosm. and a vehicle osmolality of 240-680 mosm. have been used. After testing different total and vehicle osmolalities, a primary fixative with a total osmolatity of approximately 680 mosm. total osmolality and 450 mosm. vehicle osmolality has been adopted for most fixations. Serial sectioning was carried out with Reichert OMU 3 and Reichert Ultracut microtomes. Semi-thin sections (0.3-0.5 µm thick) were stained with a thionine blue solution, thin section were double stained with a 2% aqueous uranyl acetate (5 min) and 1.2 aqueous lead citrate (7 min), manualy or using a LKB-ultrostain. Thin sections were mounted on carbon-coated pioloform-covered grids and stained with 2 % aqueous uranyl acetate (5 min) and 1.2 % aq. lead citrate (7 min), using a LKB-ultrostain. Fine structural examination was performed with a Philips EM 400 electron microscope.

Terminology

As far as the terminology is considered, terms used in earlier literature have been taken over if possible. Some terms are now used in a more restricted sense than before en new terms have been added in order to give a more adequate description and to facilitate the comparison between different species. The most important terms are listed below and indicated in a schematic figure of a eukalyptorhynch proboscis (Fig. 1). Their correct sense as used in this study is given and will give the reader a better understanding of the comming descriptions and discussions.

Apex : Apicalmost part of the cone.

Belts : Limited, circumferential parts of the epithelium, either the sheath or the cone epithelium. They can be formed by one cell, several cells or a syncytium.

Bulb : Muscular part of the proboscis separated form 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.

Cytoplasmic girdle : Epithelial cell parts situated below the junction around the anterior part of the bulb, containing nuclei and cytoplasmic cell strands of proboscis epithelia.

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

Gland necks : The apical parts of gland cells of which the secretory parts are located in the parenchyma. They ususally store secretion granules.

Inner musculature : The muscles situated within the proboscis septum.

Insunk cell parts : Epithelial cell parts situated in the parenchym below the epithelial basement membrane.

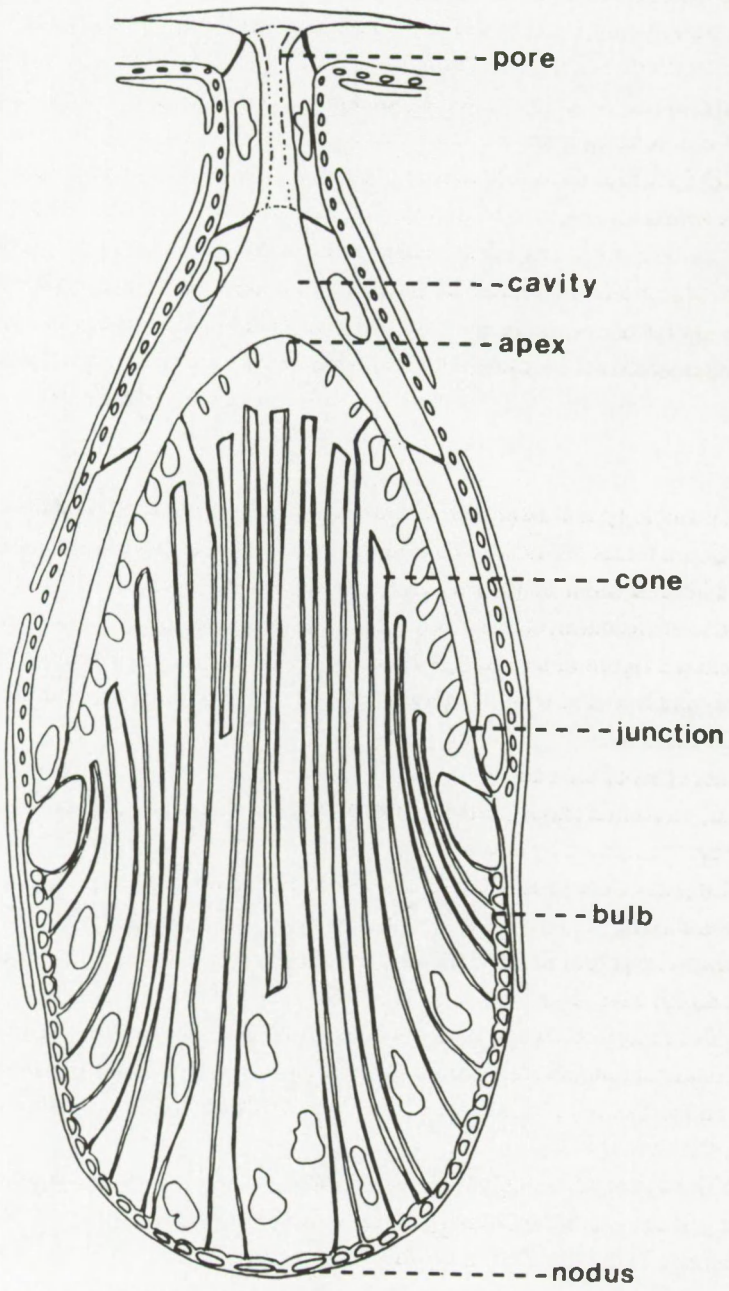


Fig. 1. Schematic representation of the proboscis in Eukalyptorhynchia. Explanation see text.

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

Intra-epithelial muscles : Muscles of which the anterior part passes through the proboscis epithelia at the juntion and cone and of which the posterior part is situated along the sides of the bulb.

Intrabulbar nuclei : Nuclei of the proboscis epithelia located inside 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 probiscis in the body. Bundles of muscle fibres seizing upon the proboscis and adhering upon the epidermal basement membrane. The motional muscles include, dilators, protractors, fixators, proboscis retractors and integument retractors. Although integument retractors do not seize upon the proboscis itself, they are also delt with as motional muscles because their muscle action is related to the functioning of the proboscis.

Nodus : Posterior end of the bulb.

Nucleiferous : Parts of the cell or syncytia which contain the nucleus or nuclei.

Nucleo-glandular girdle : Epithelial cell parts situated below the junction around the anterior part of the bulb, containing the nuclei of the epithelial cells or syncytia and conspicuous gland necks and fully surrounded by extracellular matrix.

Outer musculature : The muscles situated at the outside of the proboscis septum.

Proboscis : Frontal organ mainly composed of epithelia and muscles with associated glands and sensory cells. Used to capture preys.

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 cells : 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 cavity. Formed either by two or three epithelial belts.

Transition zone of outer in inner circular muscles : Place where the system of outer circular muscles , sheath epithelial basement membrane converts into the system of bulbar septum, inner circular muscles.

Distal(ly) and *proximal(ly)* are used as synonyms of *anterior(ly)* and *posterior(ly)*. In order to avoid confusion, the former is consistantly used to distinguish belts in the sheath epithelium.

In this study our attention has been focussed on the following characteristics of the proboscis: the organization of the proboscis epithelia, the presence of gland necks and sensory cells in the epithelia and the organization of the proboscis musculature. The full organization and localization of the glands associated with the proboscis as well as the overall nervous system of the proboscis have not been studied. Both aspects can not properly been addressed to in a pure ultrastructural studies, histological light or electron microscopic investigations are necessary to deal with these problems.

The prolonged division applied in the descriptive parts of the text is of course artificial and has been addopted for convenience of comparison and easy reference.

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Chapter 3.

The anatomy and ultrastructure of the proboscis in Cicerinidae Meixner, 1928.

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 form the major diagnostic features. But germovitellaria are present in *Cytocystis clitellatus* as well. Karling (1952) proposed the name Zonorhynchidae with two subfamilies; Zonorhynchinae (*Zonorhynchus*, *Cicerina*, *Paracicerina*, *Ptyalorhynchus* and *Blennorhynchus* n.n.) and Ethmorhynchinae (*Ethmorhynchus*). 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 microscipic investigations on twelve species. Recently new species of Cicerinidae have been described by Karling (1989) and Armonies and Hellwig (1987). Evdonin (1977) subdivided the genera in three subfamilies; the Cicerininae Brunet, 1973, Nannorhynchinae Evdonin, 1977 and Xenocicerininae Evdonin, 1977.

From light microscopic observations the genera of the family Cicerinidae show differences in organization of the proboscis (Meixner 1938, Karling 1952, Brunet 1973). Brunet (1973) distinguishes two groups of genera within the Cicerinidae. One group contains the genera *Toia*, *Nannorhynchides* and *Pocillorhynchus*, the other group includes the genera *Cicerina*, *Paracicerina*, *Ptyalorhynchus*, *Ethmorhynchus*, *Zonorhynchus*, *Xenocicerina* and *Didiadema*. The distinction between the two groups is based on several criteria and the former group is characterized by their small dimensions (0.2-0.7 mm), their ovale body-shape and excellent swimming capabilities. Histologically the species of the genera in this group are characterized by two frontal glands, pigmented eyes with lenses and a ciliated proboscis sheath epithelium.

This group of genera comprises two species in the genus *Toia* (Marcus 1952, Brunet 1973), five in the genus *Nannorhynchides* (Karling 1956, Brunet 1965, Evdonin 1971) and three species of *Pocillorhynchus* (Brunet 1973, Schockaert 1982).

Zonorhynchus species are bulky animals, they have been collected from a salt marsh, a lagune and muddy sediment. Up to now four species of *Zonorhynchus* have been described (Armonies & Hellwig 1987, Karling 1952, 1956). All four species are characterized by the position of the copulatory organ and the genital pore in the middle of the body, not sklerotized bursa mouthpieces and a proboscis with long proboscis and integument

retractors. The species considered here are *Z. seminascatus*, *Z. salinus* and a unidentified specimen of the genus.

Meixner (1938) described the proboscis of *Ethmorhynchus anophthalmus* and established the family Ethmorhynchidae, based on the morfological differences of the proboscis in comparison with known Eukalyptorhynchia at that time. Later the genus *Ethmorhynchus* was incorporated in the family Cicerinidae Meixner, 1928 or Zonorhynchidae Karling, 1952 in the subfamily Ethmorhynchinae Karling, 1952. Evdonin (1977) finally included *Ethmorhynchus* in the subfamily Cicerininae. Only two species of the genus *Ethmorhynchus* are known and found in muddy sediments. *E. anophthalmus* is found in marine sediments in northern Europe at 8 to 80 metres depth and *E. youngi* is recorded from a freshwater habitat in a upper stream in Italy (Meixner 1938, Karling 1956, Kolasa 1977). According to the author the taxonomic position of the latter species is not certain, because sectioned material was not available to make a detailed reconstruction of the reproductive system.

The genus *Cicerina* and *Paracicerina* include each six species (Ax 1959, Brunet 1973, Evdonin 1971, Giard 1904, Martens & Schockaert 1981, Meixner 1928, Karling 1952, 1989). As correctly noted by Brunet (1973) the genus *Blennorhynchus* Meixner, 1938 nom. nud. is identical to *Paracicerina* Meixner, 1928. The genus *Ptyalorhynchus* includes two species (Brunet 1973, Meixner 1938). The ultrastructural organization of the proboscis epithelia has been investigated in the latter group for *Cicerinaremanei* (De Vocht & Schockaert 1988).

The species *Xenocicerina gracilis* Karling, 1956 and *Didiadema picardi* Brunet, 1965 have not been found at or near the type localities. Type specimens present in the Swedish Museum Natural History have been investigated by light microscope.

The description of the proboscices in Cicerinidae is subdivided into four parts because of the considerable differences between the genear or groups of genera as *Toia* and *Nannorhynchides*, *Zonorhynchus*, *Ethmorhynchus* and finally *Cicerina*, *Paracicerina* and *Ptyalorhynchus*.

Material and methods

Specimens of *Toia calceformis* were collected from algae (in particular from *Codium vermilara*) in the Mediterranean at Banyuls-sur-Mer (France) at 3-11 meters depth and at Ile des Embiez (France) at 2-3 meters depth (October 1987). Samples were taken by scuba diving. *T. calceformis* was encountered on algae at 3 meters depth at Calvi (Corse) as well (April 1986). Specimens of *Nannorhynchides herdlaensis* were encountered on algae collected near Skulevik (Färlevfjorden) in the Gullmar-fjord (Swedish West coast, 58°25'31"N and 11°36'23"E) in June 1988. Samples were taken by Ockelmann-sledge at 10 to 15 meters depth. Extraction was carried out by decantation with a $MgCl_2$-solution

isotonic to seawater. Specimens of *Zonorhynchus seminascatus* 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 (France) in October 1987. *Zonorhynchus salinus* 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). Specimens of *Ethmorhynchus anophthalmus* Meixner 1938 were collected from muddy sediment harboring *Pennatula* at 40 meters depth near Gåsö, Gullmarfjord (Kristineberg) at the Swedish West Coast in June 1988. Animals were extracted either by sieving the fine mud and washing the remaining sediment and animals into a petri-dish or by the "Übersand" method. Specimens of *Ptyalorhynchus coecus*, *Paracicerina deltoides*, *Cicerina remanei* and *Cicerina brevicirrus* were collected from sandy beaches at the Belgian coast (Mariakerke, Heist and Zeebrugge). Type material of all genera of Cicerinidae was studied in the Swedish Museum of Natural History.

Results

Toia and *Nannorhynchides* (Figs 1-18)

Species of the genera *Toia* and *Nannorhynchides* are among the smallest Kalyptorhynchia and free-living Platyhelminthes in general. The total body length of fixed mature specimens of *T. calceformis* is 200-250 µm. *N. herdlaensis* is somewhat larger and measures 350 µm after fixation. Both species live epiphytic as species of the related genus *Pocillorhynchus*. The proboscis of *T. calceformis* is about 50 µm long and takes up 1/5 of the body length. Both the proboscis sheath and bulb are 25 µm long. The cone is dome-shaped and only 8 µm high. In *N. herdlaensis*, the 70 µm long proboscis takes up 2/9 of the body length. The sheath and the bulb are each about 35 µm long. The proboscis sheath is surrounded by two large mesenchymatic cells which possess an aqueous cytoplasm with only few mitochondria and which may function as hydrostatic compensation sacks when the proboscis is protruded.

Epithelia, glands and sensory cells

Epithelia. The proboscis epithelia in *T. calceformis* and *N. herdlaensis* are composed of four circumferential belts (Figs 1, 2). Two belts constitute the sheath epithelium and two

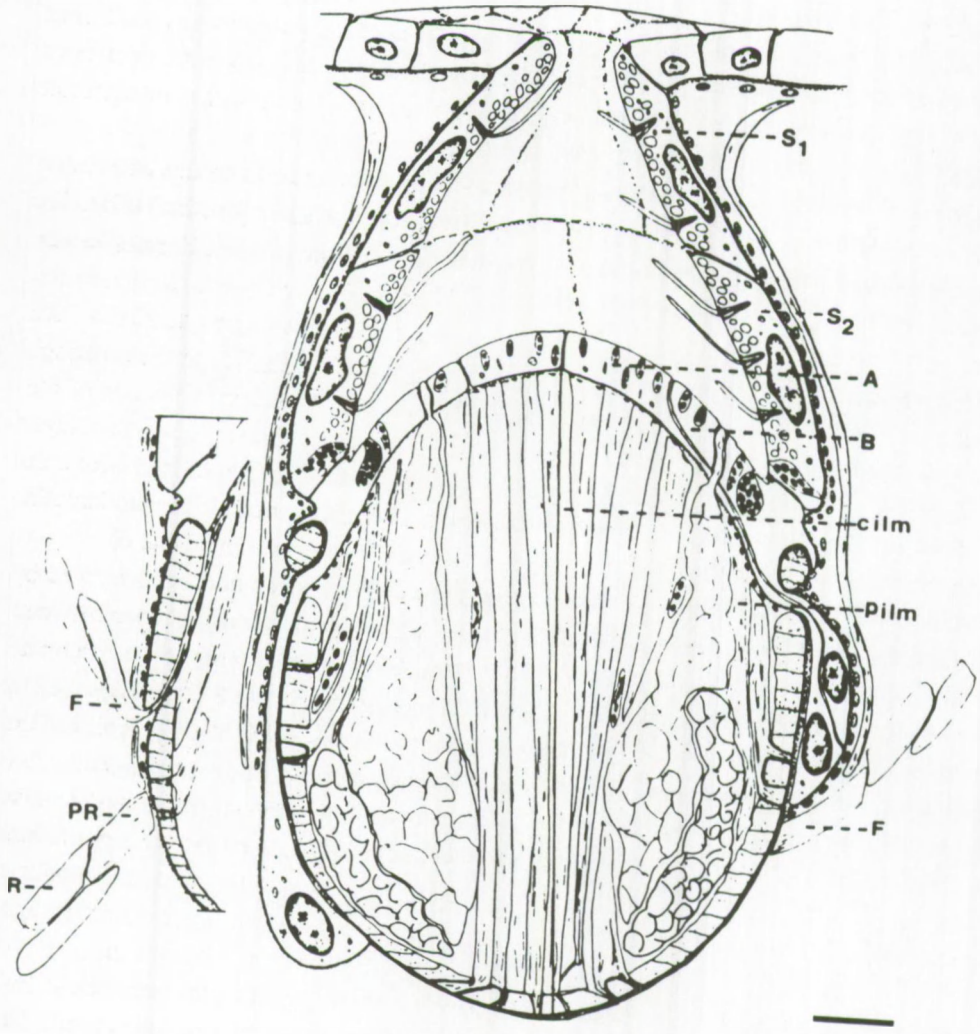


Fig.1. Reconstruction of the proboscis in *Toia calceformis* from electron micrographs. *Numbered arrows* indicate the level of cross sections in Fig 3. Scale bar: 5 µm.

belts cover the cone. All belts are cellular, the cells are devoided of cilia. Cell junctions are formed by zonulae adhaerentes and septate junctions.

In *T. calceformis* both the distal and proximal belt of the sheath epithelium consist of three cells (Fig. 3). The epithelium is 2.5 µm high and the cells of both belts are covered by long and slender microvilli, 850 nm long (7 per linear µm), with minute electron dense condensations in the tips Fig. 4). A terminal web could not be discerned. Underlying the epithelium a uniform 40 nm thick basement membrane is present. The basement membrane follows the inner margin of the surrounding circular muscles. The cytoplasm of the sheath epithelium cells is characterized by many empty vacuoles. Basally in the cells patches of endoplasmatic reticulum are found. Few mitochondria and Golgi complexes are present as well. All nuclei are intra-epithelial, almost globular and measure 3-4 µm in diameter (Fig. 5).

In *N. herdlaensis* the distal and proximal belt of the sheath epithelium consist of two and four cells respectively. The epithelium is 2 (at the porus) to 5 µm (at the junction) thick. The 850 nm long microvilli are more densely packed (12 per linear µm) than in *T. calceformis*. A dense terminal web is not found but a 500 nm thick fine fibrillar layer is present in the apical part of the cells (Figs 6, 7). The bipartite basement membrane is about 350 nm thick near the porus and changes in a uniform basement membrane medially. At the proboscis pore the basement membrane under the sheath epithelium is seen as a continuation of the median and proximal layer of the basement membrane of the epidermis. The median layer varies in thickness from 100 nm at the pore to 30 nm at the junction. The cells of both belts have intra-epithelial nuclei (6 µm long). Intra-nuclear crystalline inclusions with lamellar aspect are present in the nuclei of the proximal belt of the sheath epithelium (Fig. 6).

The cone epithelium in both species consists of two belts. The apical belt is formed by two cells, the basal one by five cells (Fig. 3). In *T. calceformis* the cell junctions between the proximal belt of the sheath epithelium and the basal belt of the cone epithelium are found exactly at the junction. The cone epithelium is 2.8 µm high and both belts are covered by stubby microvilli, 7 per linear µm and less than 300 nm long (Fig. 4). The microvilli of the basal belt are characterized by lateral and terminal condensations. A dense terminal web is not present but bundles of tonofilaments run from the basal plasma membrane to the apical part of the cells (Fig. 4). A uniform thin basement membrane supports the cone epithelium. The cytoplasm of the cell parts, which covers the cone, only contains some mitochondria. The nucleiferous cell parts of the cone epithelium are situated in four sagged packages around the bulb (Figs 13, 14). Two packages are located laterally around the anterior part of the bulb and the other two dorsally and ventrally around the posterior part of the bulb (Figs 8, 10). Surrounding the packages a thin layer of extracellular matrix is found. The two packages situated anteriorly and the distal, thin strands leading to the posterior

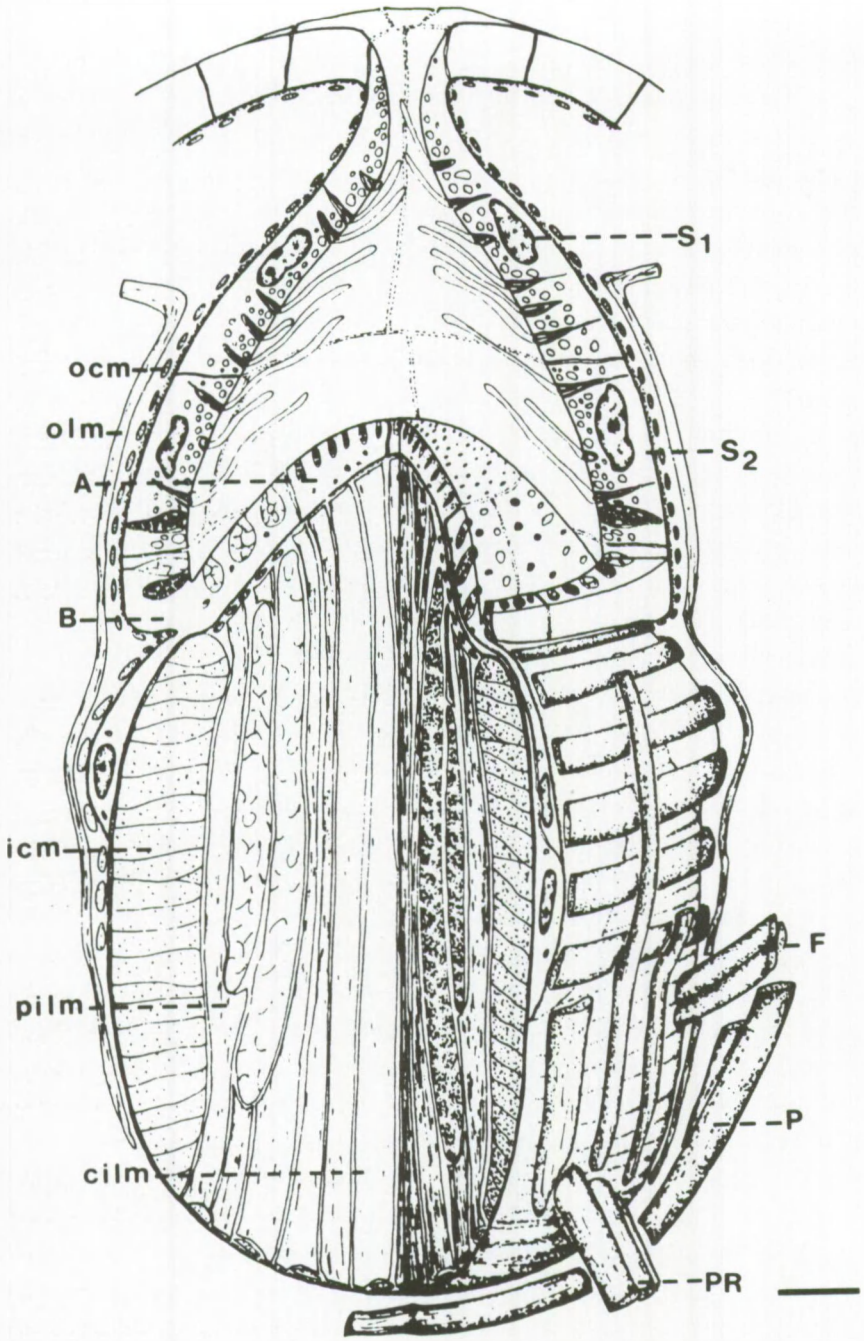


Fig. 2. Reconstruction of the proboscis of *Nannorhynchides herdlaensis*. Scale bar: 5 μm.

packages are surrounded by the circular and longitudinal muscles underlying the sheath epithelium (Figs 8, 9). Four nuclei are found in the anterior packages; two of which belong to the apical cone epithelium and two to the basal cone epithelium. The other three nuclei of the cells of the basal cone epithelium are situated in the posterior packages.

The cone epithelium in *N. herdlaensis* is 4 µm high basally to 2 µm apically. The basal belt of the cone epithelium lines the basal part of the sheath as well. The microvilli covering the cone epithelium are short and stubby about 200 nm long and 8 per linear µm (Fig. 7). The microvilli of the basal belt have electron dense condensations at their margins. A uniform thin basement membrane supports the cone epithelium. A distinct terminal web is not visible but the upper halfs (or even 2/3) of the cells are filled with gland necks and show a dense fibrous aspect (Fig. 7). In the cytoplasm mitochondria are present only in the basal part of the cells. The nucleiferous cell parts of the cone epithelium are grouped in four wide packages around the bulb (Figs 17, 18). The nuclei of the basal cone epithelium are distributed over the four cell packages around the anterior part of the bulb. The two nucleiferous cell parts of the apical cone epithelium are present at the inner side of and at the posterior end of two diametrally arranged packages, which are situated around the median part of the bulb. The nucleiferous cell parts are enveloped by a thin layer of extracellular matrix (Fig. 18).

Glands. All gland necks that penetrate the epithelium cells are apically connected to these cells by circumferential septate junctions. The sheath epithelium of both species is practically devoided of gland necks. In *T. calceformis* only few gland necks (g_1) with moderately electron dense secretion granules (300 nm) and flocculent appearance are present in the distal belt of the sheath epithelium (Fig 5). Just above the junction the proximal belt is pierced by numerous gland necks (g_2). These gland necks contain a moderatlye electron dense secretion packed in 400 nm ovoid granules (Figs 8, 12)).

In *N. herdlaensis* no gland necks were found in the distal belt of the sheath epithelium. In the proximal belt two types of gland necks are present, concentrated in the posterior part above the junction. One type (g_2) forms a circular glandular ring of 2.5 µm high gland necks. The secretion granules (350-450 nm) show varying degrees of electron density (Fig. 7). The other type (g_3) is less common and contain electron dense secrtion granules with light spots. The granules are irregular in shape and 300-700 nm large (Fig. 7).

The basal cone epithelium in *T. calceformis* is pierced by four types of gland necks. Wide open gland necks (g_6) of the most conspicuous type form eight to ten circular arranged groups of necks and are filled with secretion throughout the full length of the bulb (Figs 10, 14). These gland necks appear in two different forms, one with light granule margins and one with dense granule margins (Fig. 8). The apical parts of the necks piercing the

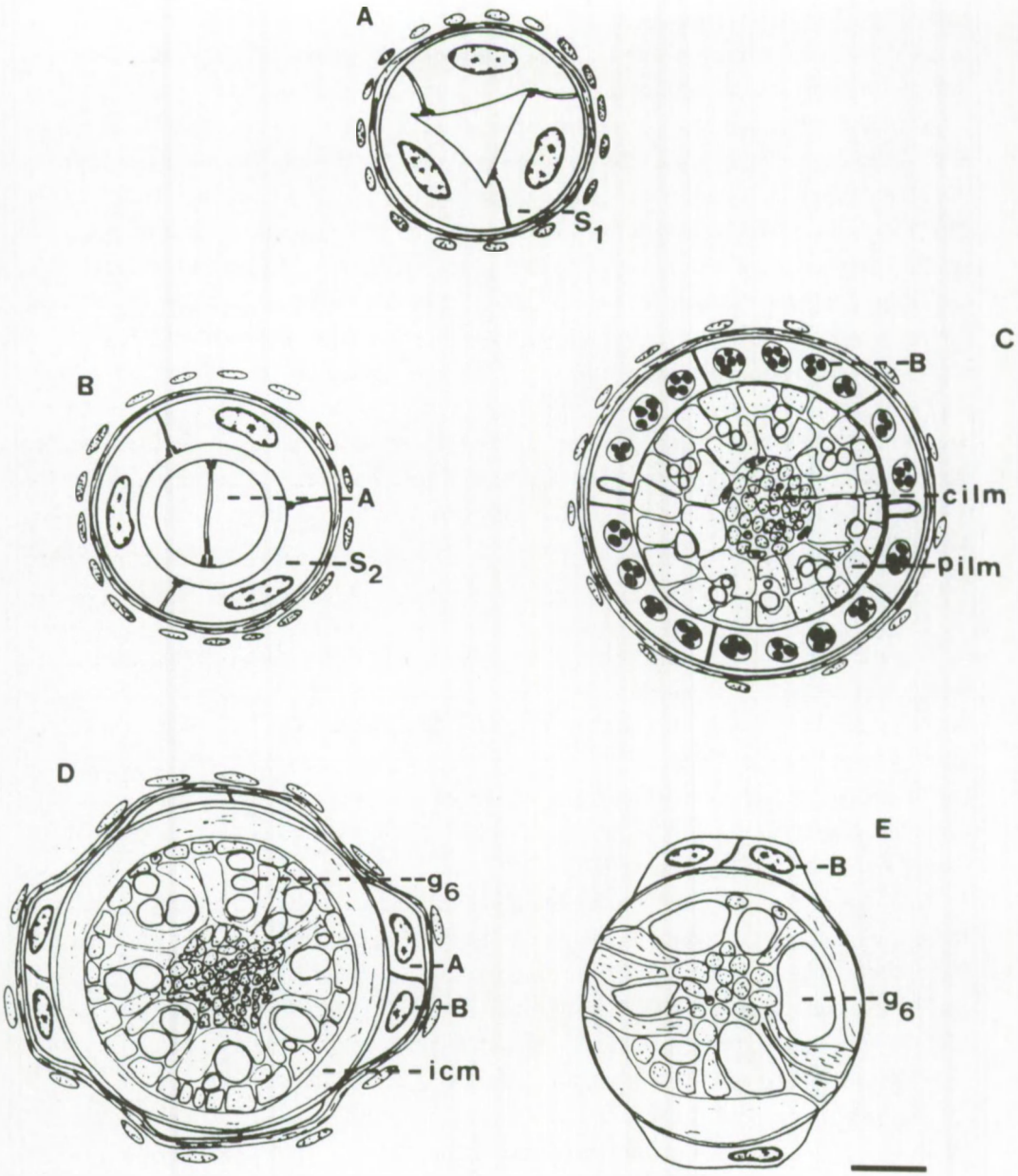


Fig. 3. Cross sections of the proboscis in *Toia calceformis* showing the distal belt of the sheath epithelium (A), the proximal belt of the sheath epithelium and apical cone epithelium (B), the basal cone epithelium just below the junction (C), the two distalmost nucleiferous cell parts (D), the two proximal nucleiferous cell parts (E). Scale bar: 5 μm.

epithelium are reinforced by peripheral microtubules and surface in the median part of the basal cone epithelium. Eighteen well filled gland necks (g_5 4.5 µm long) with electron dense secretion granules (800 nm) are located apically of the junction (Fig. 13). The secretion in the granules has shrunk during preparation. Twentysix of this type of gland necks are present in the whole mount of *Toia ycia*. Apically of the former type, gland necks (g_4) contain ovoid secretion granules (500 nm) with irregular condensed contents (Fig. 9). Throughout the basal cone epithelium, but mainly in the apical part of the belt just below the apical cone epithelium, numerous small gland necks (g_7) with electron dense secretion granules (320 nm) are present (Figs 5, 11). The apical cone epithelium is pierced by numerous collumnar and narrow gland necks (g_9), filled with spherical electron dense secretion granules (230 nm) (Fig. 11).

The cone epithelium in *N. herdlaensis* can be divided in an apical and basal part not only by the arrangement of the cells but also by the presence of specific gland necks. The basal cone epithelium in turn can be divided into two regions. The region at the junction contains the same gland necks at the side of the sheath epithelium as at the side of the cone (Fig. 16). Gland necks (g_4 2.3 µm long) with empty vesicles (700-900 nm) are found throughout this part of the epithelium together with gland necks (g_5) with moderately electron dense secretion granules (950 nm). Sometimes light granules with one pronounced and several smaller condensations can be seen in the latter. Gland necks (g_3) which contain electron dense secretion granules with light spots and of irregular shape (300-700 nm) are present as well. The region along the cone is pierced by two types of gland necks. One is characterized by empty vesicles about 900 nm large (g_6). Surfacing parts of the gland necks are up to 2 µm wide, in the bulb they are upto 3.5 µm wide and form eight groups (Figs 7, 16, 17). The other type has 2 µm wide surfacing gland necks (g_7) and contains a flocculent secretion (Figs 15, 16). Separate granules are hard to discern and measure about 900 nm. In the bulb these gland necks are seen as eight narrow strands of secretion. In *Nannorhynchides corneus* eight pairs of gland necks are present in the bulb (Brunet 1973, Fig. 6). The apical cone epithelium is penetrated by numerous gland necks (g_9) that contain electron dense, mostly spherical secretion granules (280 nm) (Fig. 6). The apical part of the gland necks piercing the epithelium is reinforced by peripheral microtubules.

Sensory cells. Both the distal and the proximal belt of the sheath epithelium in *T. calceformis* and *N. herdlaensis* are characterized by the presence of many uniciliary receptors. Marcus (1952) and Karling (1964) considered the sheath epithelium to be ciliated, which is not the case. The uniciliary receptors possess well developed primary rootlets as long as the height of the epithelium (Fig. 4). The rootlets are directed slantingly downwards in the epithelium. The cilia show the normal 9+2 configuration of microtubules and

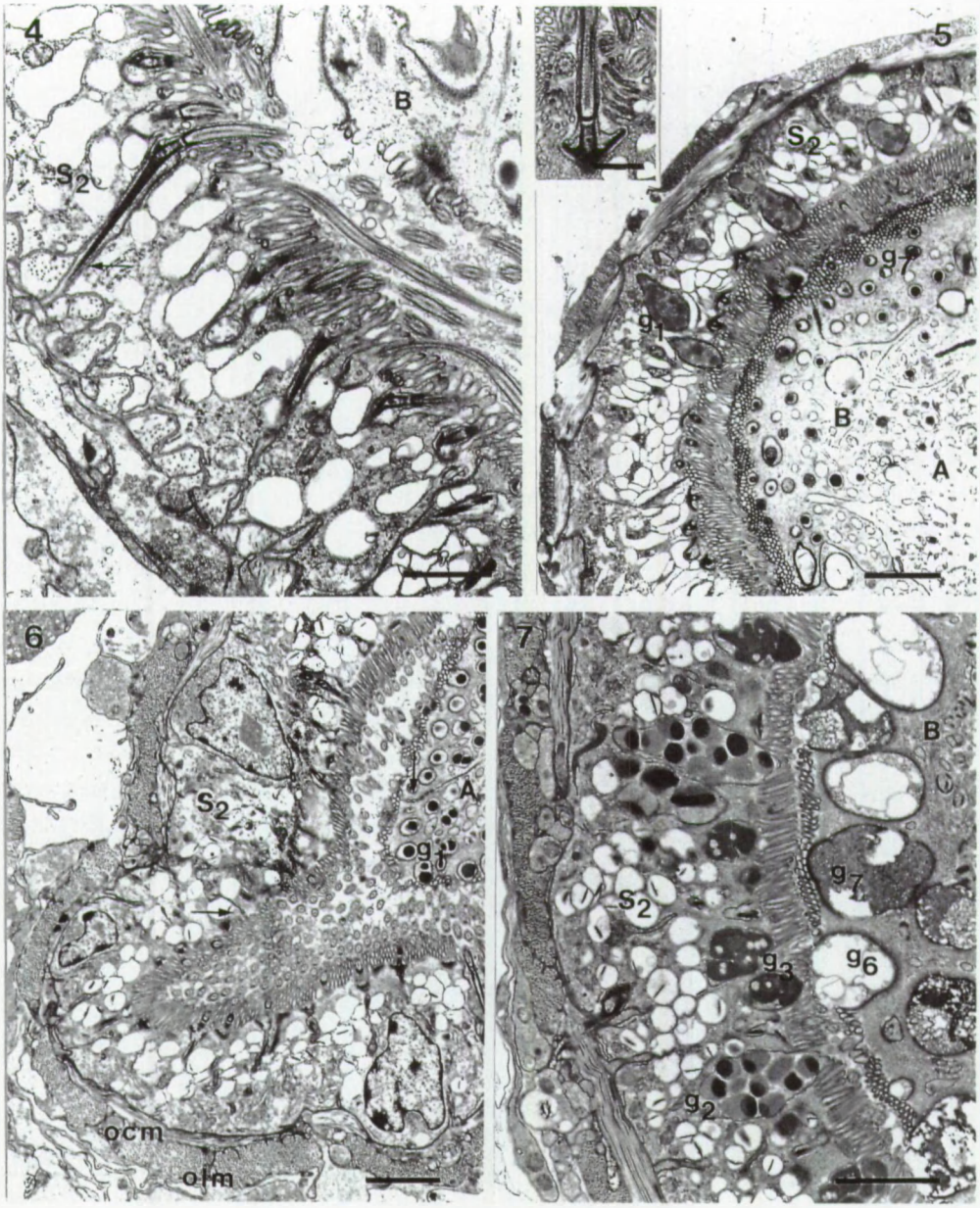


Fig. 4. Toia calceformis. Transverse section of the proximal belt of the sheath epithelium (S_2), with numerous vacuoles and uniciliary receptors, and the basal cone epithelium (*B*). Note the outer circular muscles and basement membrane, which uncloses the proximal part of the dendrite in the epithelium (*arrow*). Scale bar: 1 µm.

Fig. 5. Toia calceformis. Cross section of proximal belt of the sheath epithelium (S_2)with type g_1 gland necks, basal cone epithelium (*B*) pierced by type g_7 gland necks and cell strand of apical cone epithelium. Scale bar: 2 µm. *Inset*:: Uniciliary receptor with lateral extensions of the basal body. Scale bar: 1 µm.

extend into the cavity. The basal bodies are embedded in the epithelium and not lifted above the epithelium surface. The terminal ends of the dendrites are connected to the epithelium by zonulae adhaerentes and septate junctions. Two lateral condensations protrude from the underside of the basal bodies and form two lateral extensions of the dendrite endings which rest on the epithelium surface (Fig. 5 *inset*). The lateral condensations are connected to the zonulae adhaerentes. The ciliary shafts have electron dense condensations in their tips. The cone epithelium is pierced by uniciliary receptors as well. They consist of short ciliary shafts, basal bodies and rootlets. Intra-epithelial multiciliary receptors as described for *Cystiplex axi* and *Cystiplana paradoxa* (De Vocht 1989) or insunk multiciliary receptors as in *Psammorhynchus tubulipenis* and *Cytocystis clitellatus* (De Vocht 1990) are lacking.

Proboscis musculature

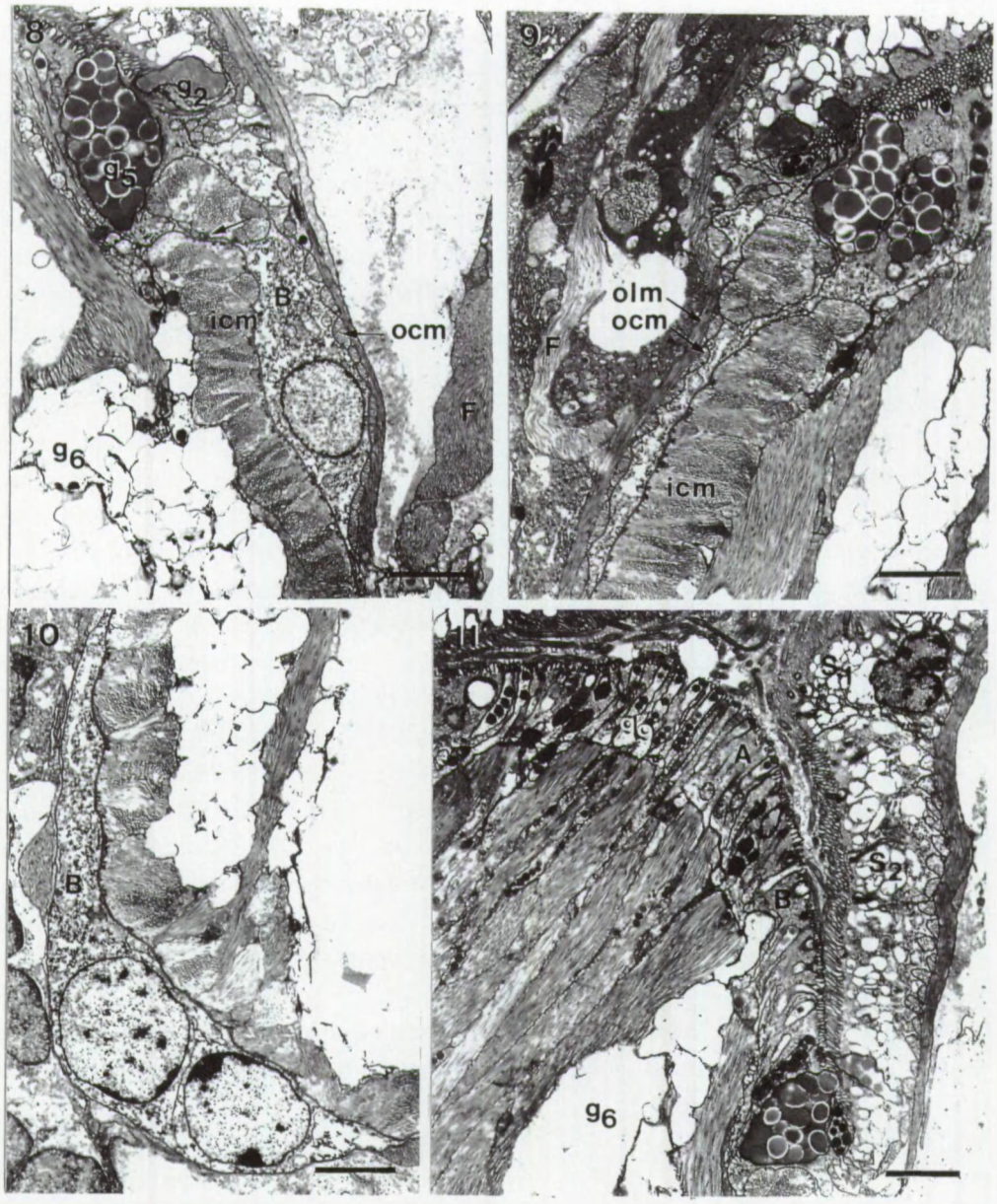
All muscle fibres contain mitochondria but a sarcoplasmatic reticulum is absent. Nuclei are not observed in the inner proboscis musculature.

Outer proboscis musculature. The sheath epithelium in *T. calceformis* is surrounded by a inner circular and outer longitudinal muscle layer from the porus on towards the junction and further down around the anterior part of the bulb and the anterior two nucleiferous cell packages (Figs 8, 9). The circular muscle layer forms a continuation of the circular musculature of the body wall. Their inner margin is coated by the thin uniform basement membrane (Fig. 4). The number of outer longitudinal muscles is 17 in the cross sectioned specimen. They are arranged in two lateral groups of 5 muscle fibres and one set of three and an other of four muscle fibres dorsally and ventrally. The two lateral groups have a more electron dense appearance than the dorsal and ventral groups. Beneath the junction the circular muscle layer forms a constriction and enclose the proboscis bulb and nucleiferous cell parts of the cone epithelium (Fig. 9). The longitudinal muscle layer does not follow the constriction of the circular muscles and runs straight down along the bulb and nucleiferous cell packages.

In *N. herdlaensis* a circular and longitudinal muscle layer surround the sheath epithelium as well. The outer circular muscles are found from the proboscis pore down to

Fig. 6. Nannorhynchides herdlaensis. Cross section of the proximal belt of the sheath epithelium (S_2) with vacuoles, pierced by uniciliary receptors and surrounded by circular and longitudinal muscles (*ocm*, *olm*) and apical cone epithelium (*A*)with type *g9* gland necks. *Arrow*: cell junctions. Scale bar: 2 µm.

Fig. 7. Nannorhynchides herdlaensis. Cross sections of the glandular ring in the sheath epithelium (S_2), with type g_2 and g_3 gland necks, and the basal cone epithelium (*B*) with type g_6 and g_7 gland necks. Scale bar: 2 µm.



Figs 8-11. Toia calceformis.
Fig. 8. Transverse section of the junction and nucleiferous cell part of the basal cone epithelium (*B*) around the distal part of the bulb. Note type *g2*, *g5* and *g6* gland necks, the outer and inner circular muscles (*ocm*, *icm*) with perforation (*arrow*). Scale bar: 2 µm.
Fig. 9. Transverse section of the junction and distal part of the bulb, showing type *g2*, *g4*, *g5*, *g7* inner circular muscles (*icm*), outer circular and longitudinal muscles along the bulb (*ocm*, *olm*)and fixator muscle (*F*). Scale bar: 2 µm.
Fig. 10. Proximal part of the bulb and insunk nucleiferous cell part of the basal cone epithelium (*B*). Scale bar: 2 µm.

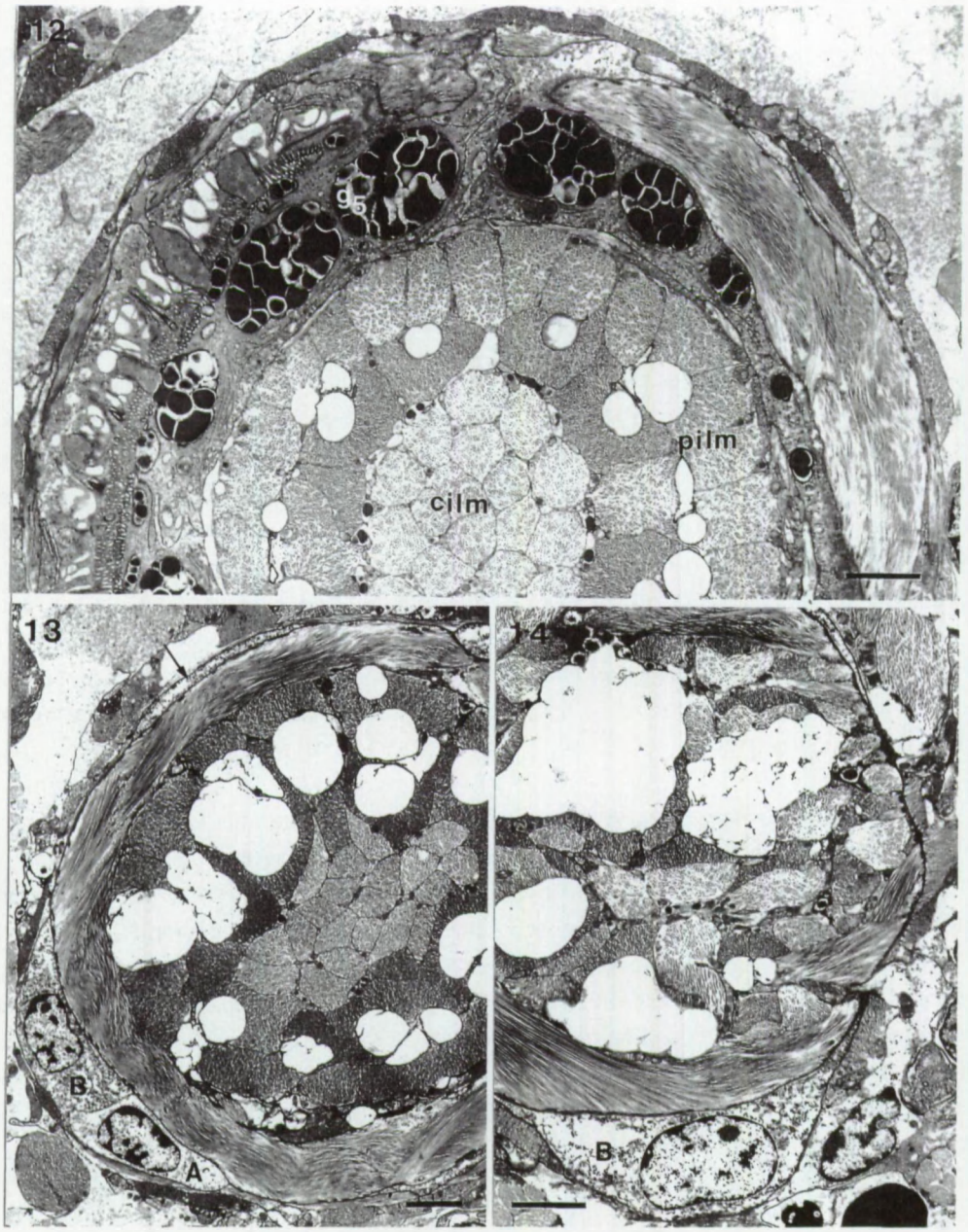
the posterior end of the nucleiferous cell packages around the bulb (Figd 17, 18). The circular muscles are surrounded by longitudinal muscles. The number of longitudinal muscle fibres varies from 14 below the distal belt of the sheath epithelium to about 20 at the junction. These muscles are continuous with the longitudinal muscles of the body wall. The connections form dilators of the sheath and are situated 8 to 10 µm below the proboscis pore. The other ends of the muscle fibres run down along the nucleiferous cell packages and the bulb. They adhere laterally on the the bulbar septum (Fig. 2).

The motional muscles of the proboscis in *T. calceformis* include protractors, fixators, proboscis retractors and one pair of ventral integument retractors (Fig. 1). Probably three pairs of protractors are present, one pair ventro-laterally and two pairs laterally. The protractors are formed by single muscle fibres with loosely arranged myofilaments. The fixators insert on the bulbar septum just below the anterior set of nucleiferous cell packages and adhere on the epidermal basement membrane at the level of the distal belt of the sheath epithelium. The proboscis retractors insert on the septum at the same level as the fixators and adhere on the epidermal basement membrane in the medial part of the body. Fixators and proboscis retractors are composed of at least three muscle fibres.

In *N. herdlaensis* the motional muscles are composed of protractors, fixators, proboscis retractors and integument retractors (Fig. 2). They have been described by Karling (1964), their organization is the identical as in *Toia* except that the muscles are formed by a higher number of muscle fibres.

Inner proboscis musculature. The inner musculature of the bulb consists of circular muscles surrounding the longitudinal muscles fibres or cone retractors. In *T. calceformis* a 1.2-2.5 µm thick inner circular muscle layer is found from the nodus up to 2.5 µm below the junction (Figs 9, 10). Few very thin muscle fibres (400 nm) surround the inner longitudinal muscles from here on as far up as the junction (Fig. 9). At the outside the inner circular muscle layer is covered by a 50-80 nm thick layer of extracellular matrix or septum. The inner circular muscle layer and bulbar septum show four perforations below the six or seven distal-most muscle fibres (Figs 8, 9). They leave passage to the nucleiferous cell parts of the cone epithelium. The bundle of isolated muscle fibres is totally surrounded by extracellular matrix. This ECM is continuous with the ECM surrounding the nucleiferous cell packages laterally of the bulb. Seven to eight micrometer from the top of the circular muscle layer in between the nucleiferous cell packages four separate flat 2.5 µm wide muscle fibres and gland necks perforate the septum and the inner circular muscle layer (Fig. 1).

Fig. 11. Transverse section showing the distal (S_1) and proximal (S_2) belt of the sheath epithelium, the basal (*B*) and apical (*A*) cone epithelium. Type *g9* gland necks in *A* possess spherical granules. Wide *g6* gland necks are present in the bulb. Scale bar: 2 µm.



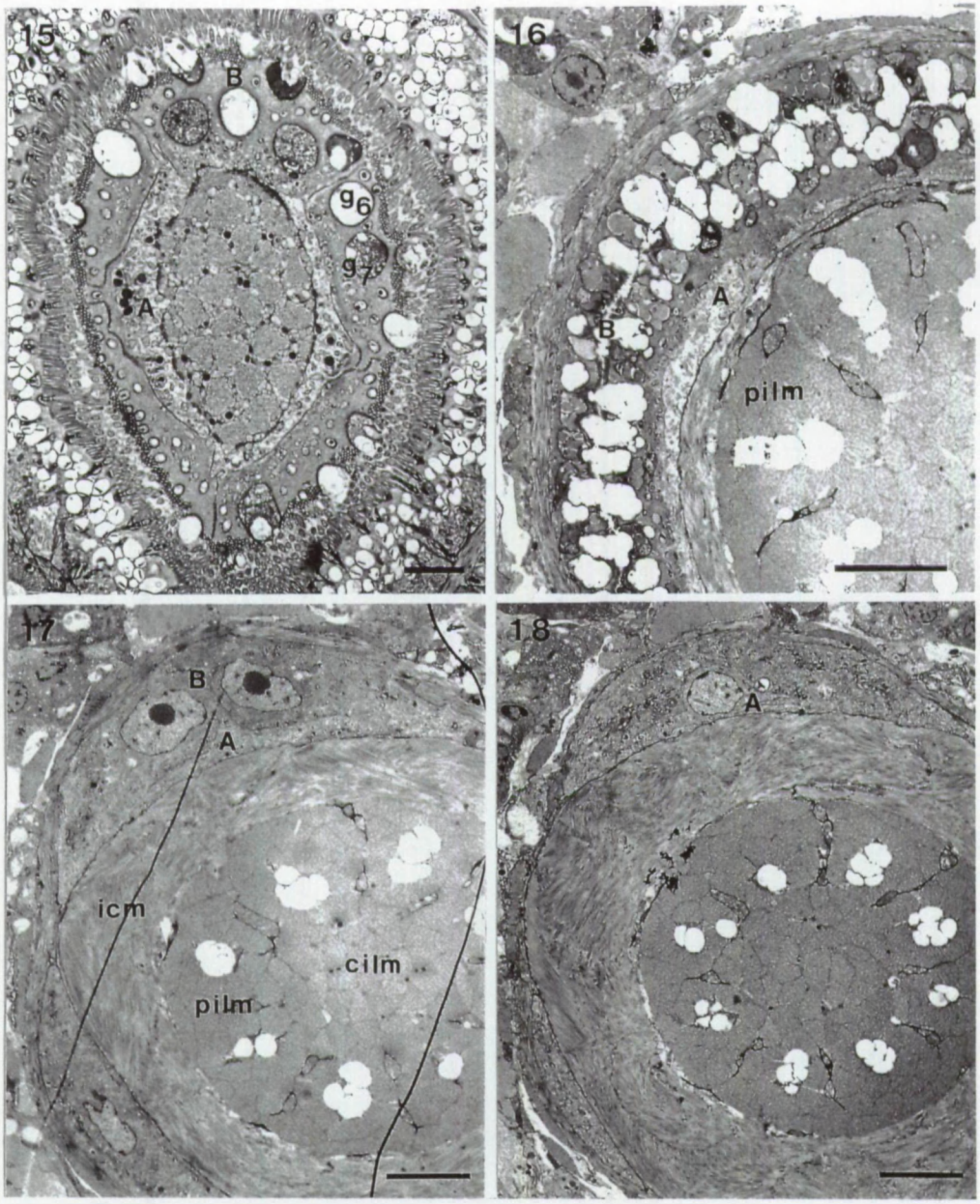
Figs 12-14. Toia calceformis.
Fig. 12. Cross section at the junction showing *g5* gland necks, the central (*cilm*) and peripheral (*pilm*) inner longitudinal muscles. Scale bar: 2 µm.
Fig. 13. Cross section of the bulb with *cilm* and *pilm*, *g6* gland necks and nucleiferous cell parts of the cone epithelium (*A* and *B*) and cytoplasmic strands (*arrow*) that lead to the posterior packages. Scale bar: 2 µm.
Fig. 14. Cross section of the posterior portion of the bulb with a posterior cell package (*B*). Scale bar: 2 µm.

These muscle fibres enter the bulb between the four cell packages and run peripherally of the inner longitudinal muscles in the bulb towards the basal part of the cone and join the fixators outside the bulb. At the nodus types g_6, g_7 and g_9 gland necks enter the bulb (Fig. 14).

The inner longitudinal muscles can be divided into a central cylinder of muscle fibres (*cilm*) under the apical cone epithelium and a double layer of muscle fibres at the periphery (*pilm*) under the basal cone epithelium (Figs 3, 12). The central cylinder is composed of 20 muscle fibres, the inner circle of peripheral muscles is composed of 14 fibres and the outer circle of 26 fibres. Type g_6 gland necks run between the inner and outer layer of peripheral muscles. Type g_7 gland necks run between the central cylinder and the inner layer of peripheral muscles. Type g_5 gland necks run in between the fibres of outer layer of peripheral muscles and between this layer and the inner circular muscles. The muscle fibres insert on the septum, the fibres of the central cylinder near the terminal end of the bulb, those of the peripheral layers more laterally. In the cone the muscle fibres adhere on the basement membrane of the cone epithelium. They are connected by desmosomes with 80 nm wide intercellular spaces with central condensations. In the epithelial cells, bundles of tonofilaments run up to the apical part of the cells. Nuclei of the muscle cells of the inner musculature are not found.

In *N. herdlaensis*, the inner circular muscle layer is very thick in the anterior part of the bulb as in all species of this genus (Figs 17, 18). Very thin circular muscle fibres are present beneath the basal cone epithelium, they thicken towards the junction (Fig. 16). Muscle fibres increase in thickness from 0.3 µm in the cone to 2 µm at the junction and decreases in thickness from 7 µm below the junction to 1.5 µm near the nodus. At the outer side the muscles are surrounded by thin layer of ECM forming the bulbar septum.

The inner longitudinal muscle layer is formed by distinct groups of fibres. Beneath the apical cone epithelium, 28 muscle fibres form a central cylinder (Fig. 16 *cilm*). These fibres insert on the septum in the terminal end of the bulb and adhere on the basement membrane under the apical cone epithelium. Below the basal cone epithelium eight blocks of peripheral longitudinal muscles are found (Fig. 16 *pilm*). These muscle fibres surround eight groups of radial oriented type g_6 gland necks. Eight groups of type g_7 gland necks are situated between the blocks. The muscle fibres insert on the septum at the postero-lateral side and adhere on the basement membrane under the basal cone epithelium.



Figs 15-18. Nannorhynchides herdlaensis.
Fig. 15. Cross section of the cone showing the cellular basal belt (*B*) with alternating g_6 and g_7 gland necks and two strands of the apical cone epithelium (*A*). Scale bar: 2 µm.
Fig. 16. Section just above the junction with g_3, g_4 and g_5 gland necks in the basal cone epithelium (*B*) and g_6 and g_7 gland necks in between the peripheral longitudinal muscles (*pilm*). Scale bar: 5 µm.
Fig. 17. Cross section of the bulb with thick layer of circular muscles (*icm*), peripheral (*pilm*) and central (*cilm*) longitudinal muscles and epithelial cell parts of the cone epithelium (*A, B*). Scale bar: 5 µm.
Fig. 18. Cross section of the bulb with eight blocks of *pilm* and equal number of g_6 and g_7 gland necks and nucleus of *A* in the epithelial cell packages. Scale bar: 5 µm.

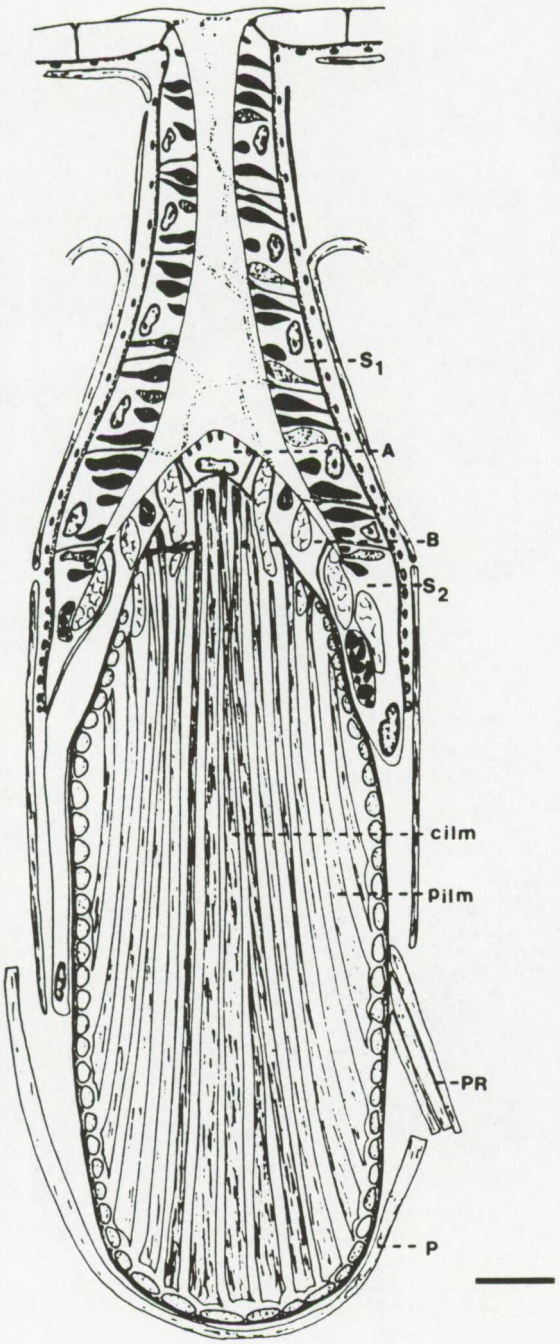
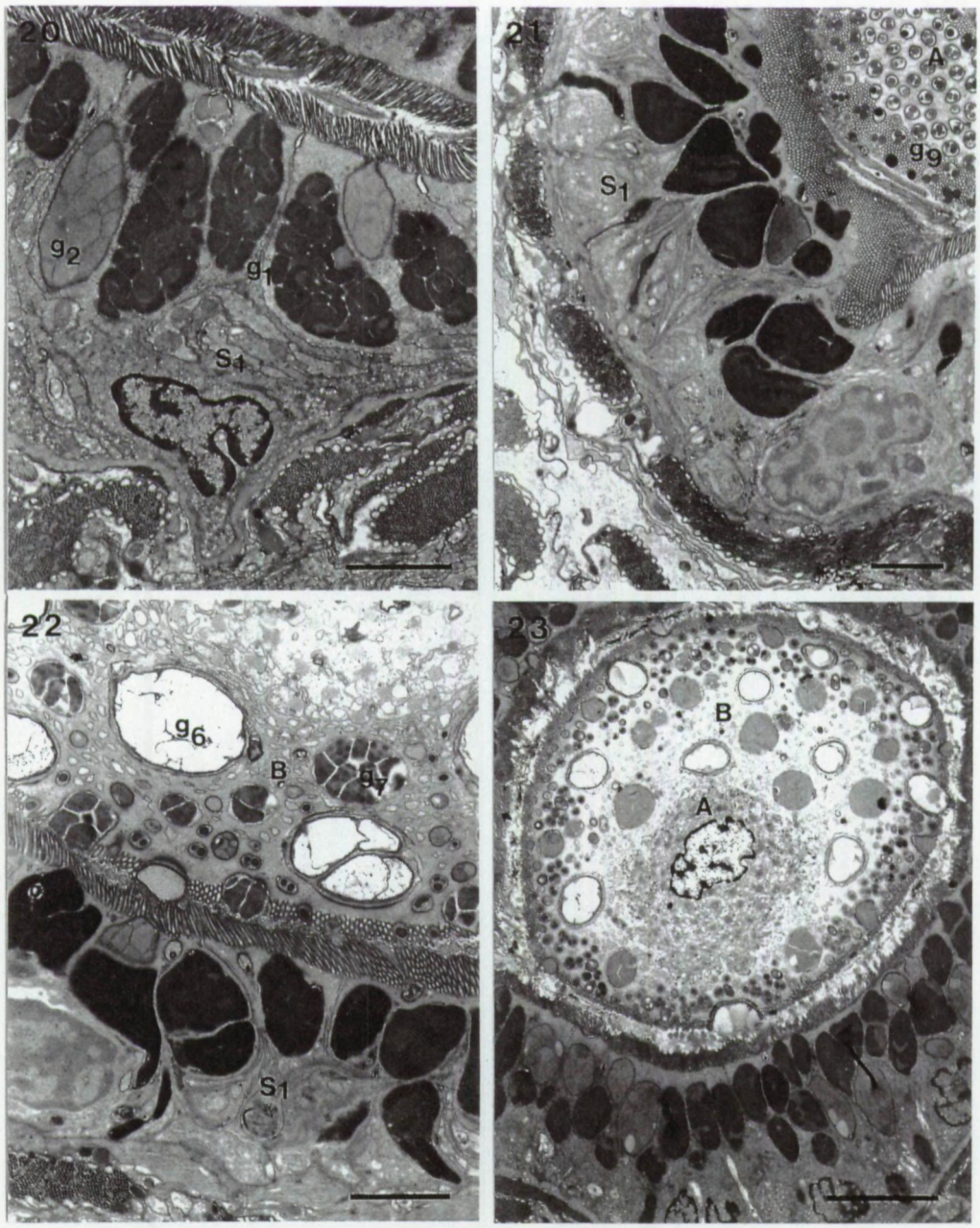


Fig. 19. Reconstruction of the proboscis of *Zonorhynchus seminascatus*. Scale bar: 10 µm.



Figs 20-21. Zonorynchus seminascatus.
Fig. 20. Cells of the distal belt (S_1) with nucleus and g_1 and g_2 gland necks. Scale bar: 2 µm.
Fig. 21. 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.
Fig. 22. 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.
Fig. 23. Zonorhynchus 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.

Zonorhynchus (Figs 19-31)

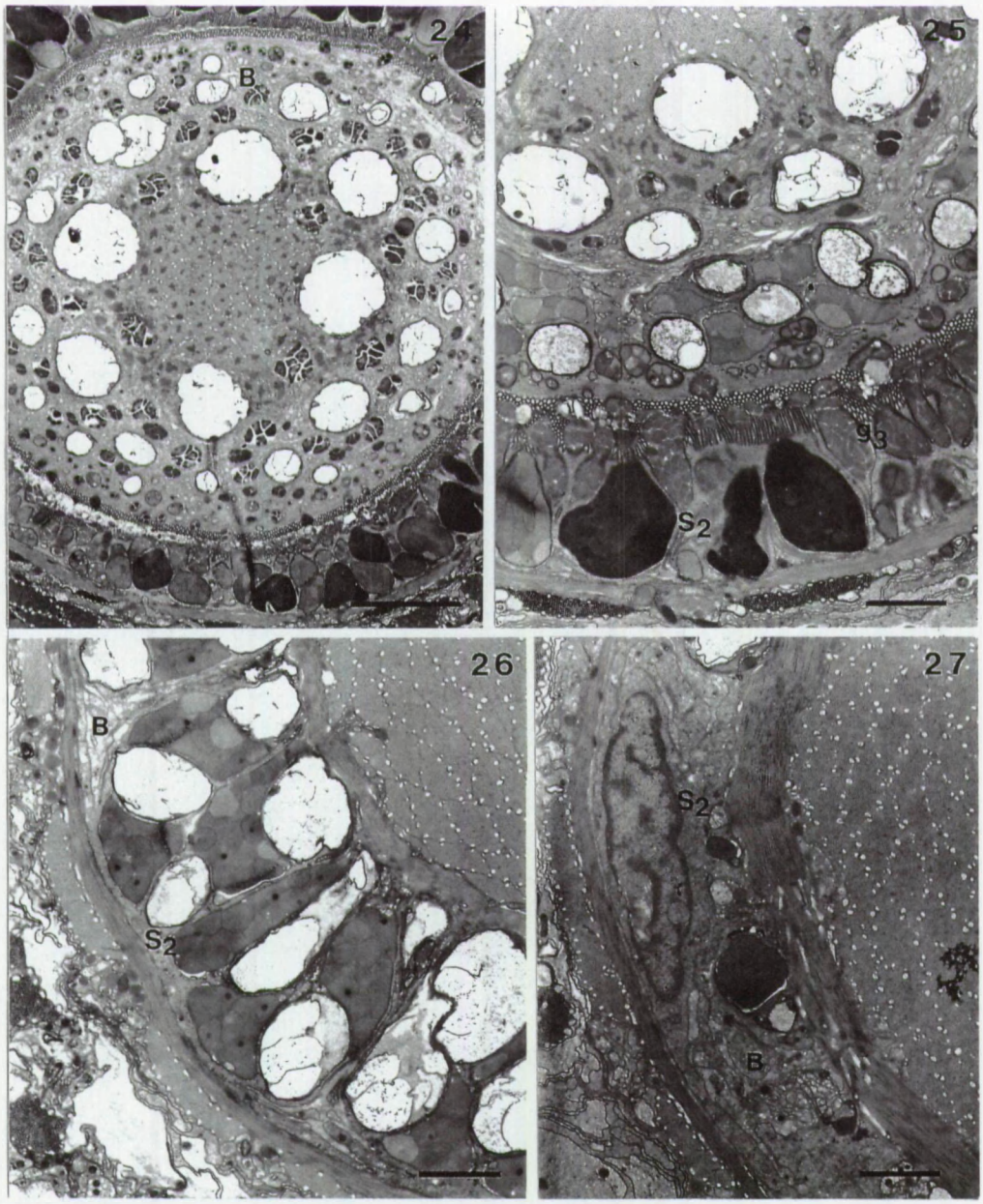
Zonorhynchus species are large bulky animals compared to most Eukalyptorhynchia. The brownish animals measure 0.8-1.5 µm and possess a relatively small proboscis. Characteristic for the proboscis in *Zonorhynchus* is the large amount of gland necks that pierce the epithelia of the sheath as well as the 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, glands and sensory cells

Epithelia. The proboscis epithelia in *Zonorhynchus* are composed of four belts (Fig. 19). One belt forms almost the whole sheath epithelium, one is found at the junction and two belts cover the cone. The epithelia are devoid of cilia and cells are interconnected by apical zonulae adhaerentes, subsequent septate junctions and dispersed desmosomes.

The distal belt constitutes the main part of the epithelium lining the proboscis cavity; it is formed by separate cells arranged without a distinct pattern (Figs 19, 20). The proximal part of the sheath epithelium is formed by a circumferential syncytium, which covers the basal part of the cone as well. The cells of the distal belt are 5 µm high and bear slender microvilli, 900 nm long and about 10 per linear µm (Fig. 20). 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 E.R. 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 a uniform 140 nm thick basement membrane is present. The proximal belt of the sheath epithelium is 5 to 0.5 µm high and is found at the junction (Fig. 25). The microvilli are distincly shorter (350 nm long) than in the distal belt. In the specimen from Banyuls-s-Mer, nine cytoplasmic strands are found in the nucleo-glandular girdle around the distal part of the bulb (Fig. 26). These strands fuse and four nuclei are present in the posterior end of this girdle. In *Zonorhynchus seminascatus*, eight cell strands stuffed with swollen gland necks are found in the nucleo-glandular girdle (Fig. 27). 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. 28). The basement membrane under the sheath epithelium proceeds around the nucleo-glandular girdle.

In *Z. seminascatus* the cone epithelium is formed by one apical cell and a basal syncytium that covers the flanks of the cone (Fig. 23). The epithelium is about 5 µm high. The stout microvilli are 350 nm long. The microvilli of the basal belt do not possess significantly

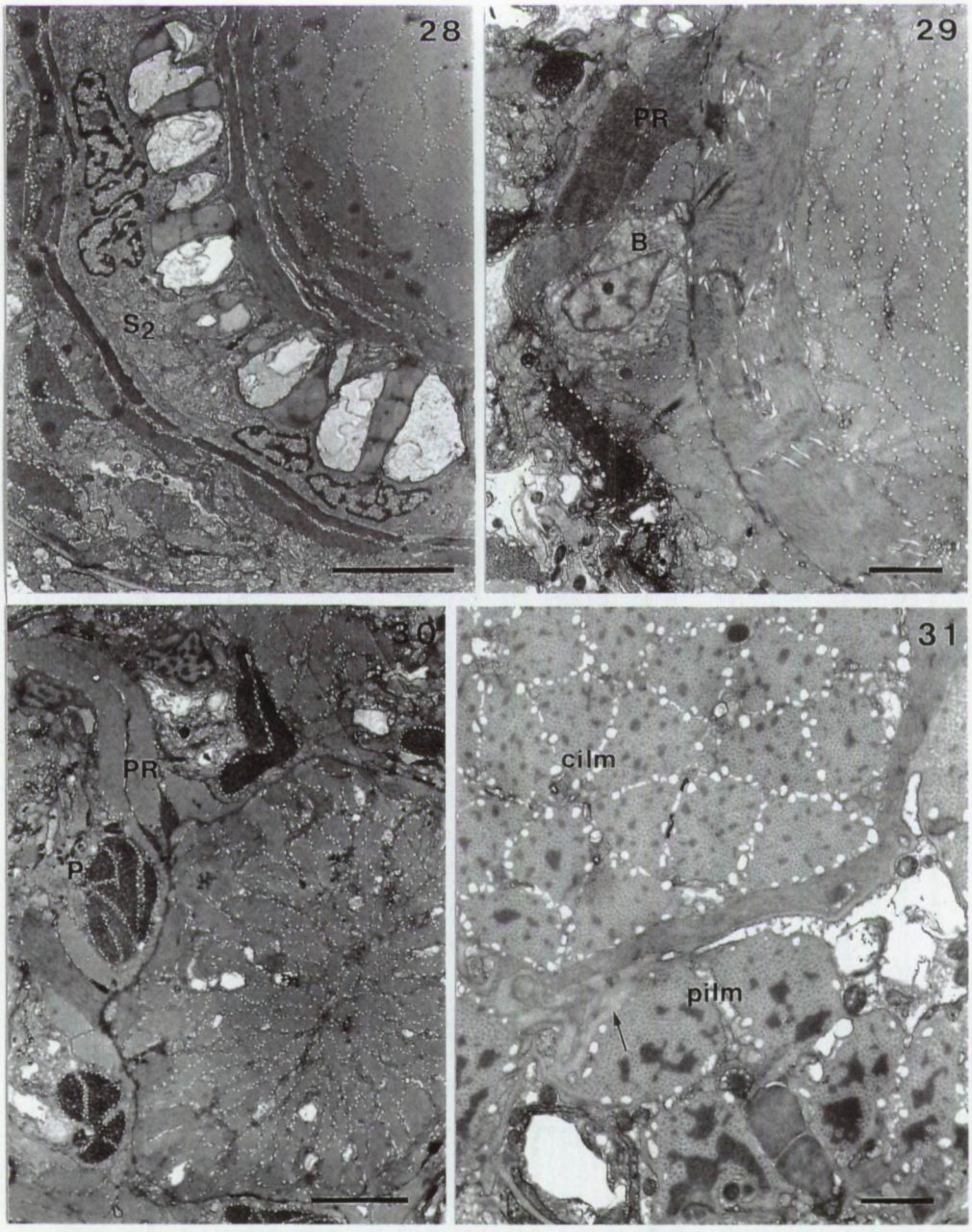


Figs 24-27. Zonorhynchus sp.
Fig. 24. Cross section of the cone clearly showing alternating $g6$ and $g7$ gland necks with in between $g8$ and $g10$ gland necks. Scale bar: 5 µm.
Fig. 25. Proximal belt of the sheath epithelium with $g1$, $g2$ and $g3$ gland necks, basal cone epithelum with goups of $g4$ and $g5$ gland necks and $g6$ necks in the bulb. Scale bar: 2 µm.
Fig. 26. Cross section of the nucleo-glandular girdle showing type $g4$ and $g5$ gland necks and cytoplasmic cell parts of $S2$ and *B*. Scale bar: 2 µm.
Fig. 27. Posterior portion of the nucleo-glandular girdle with nucleus of $S2$ and cell strands of *B*. Scale bar: 2 µm.

denser tips than those in the apical belt. This cell has no sunken cell parts. The apical cell measures 8 µm in diameter and has a 6 µm long intra-epithelial nucleus. Underlying the epithelium a basement membrane is present, irregular in thickness and frequently pierced by the gland necks. A terminal web is not present but the cytoplasm shows a fibrous appearance. The syncytial basal cone epithelium has five insunk nucleiferous cell parts. Narrow strands of the syncytium are situated in between the eight or nine cell strands of the proximal belt of the sheath epithelium (Figs 26, 27). The nuclei of the syncytium are situated in five cytoplasmic strands around the median part of the bulb (Fig. 29).

Glands. The sheath epithelium in *Zonorhynchus* 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 20, 22). Sometimes granules with light centre or lighter granules are present. A second type of gland necks (g_2) piercing the distal belt of the sheath epithelium is stowed with moderately electron dense secretion granules of about 900 nm in diameter (Figs 20, 22). 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. 25). These gland necks are filled with secretion in the apical half of the epithelium and stand wide open at the surface in contrary to the first mentioned types. 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, the other type (g_5) is piled with closely packed moderately electron dense granules of approximately 1000 nm diameter (Figs 26, 27). These granules sometimes possess a darker spot in the centre. Type 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, empty secretion granules and lined by a upto 200 nm thick peripheral margin of cytoplasm appear in a regular pattern (Figs 22, 23, 25). In a first circle, four gland necks alternate with four gland necks of type g_7 (Fig. 24). The latter type of gland necks contains 700 nm wide, electron dense secretion granules and pull through the muscles in the cone. However, they are not present in the bulb and enter the proboscis at the same position where the epithelial cell strands of the basal cone epithelium sink in (Fig. 19). Dispersed between type g_6 and g_7 gland necks two other types of gland necks are present. The narrow 700 nm wide necks contain a closely packed moderately electron dense secretion



Figs 28-31. Zonorhynchus seminascatus.
Fig. 28. Nucleo-glandular girdle with groups of g_4 and g_5 gland necks and nuclei of S_2. Scale bar: 5 µm.
Fig. 29. Cross section of the median part of the bulb with insunk nucleiferous cell part of *B* in between proboscis retractors (*PR*). Scale bar: 2 µm.
Fig. 30. Cross section of the posterior part of the bulb with proboscis retractors (*PR*) and protractors (*P*). Scale bar: 5 µm.
Fig. 31. 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.

350 nm in diameter (g_8) or spherical to ovoid more electron dense secretion granules 250 nm in diameter (g_{10}) (Fig. 24). The apical cell is pierced by gland necks (g_9) pulling through the central part of the bulb (Fig. 21). Moderately electron dense secretion granules (200 nm) are sparcely present near the terminal end of the gland necks.

Sensory cells. Uniciliary receptors are spread throughout the sheath epithelium (Fig. 22). 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 oriented 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 neither intra-epithelial nor insunk. Only very few sensory cells were encountered in the basal cone epithelium.

Proboscis musculature

Muscle fibres possess a sarcoplasmic reticulum, a cross striation is not observed.

Outer proboscis musculature. The sheath epithelium in *Zonorhynchus* is surrounded by circular and longitudinal muscles (Fig. 24). The outer circular muscles are present around the cavity from the dilators of the sheath upto the nucleo-glandular girdle, more specific up to the position of the nuclei of the junctional belt (S_2). Around de anterior part of the distal belt of the sheath epithelium, nine very thin longitudinal muscle fibres are found. Further down 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. The connective parts that pull through the parenchyma form the dilators of the sheath. From here on, the outer longitudinal muscle layer is composed of eighteen or twenty fibres. Towards the junction the fibres bifurcate and insert on the basement membrane. 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 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 and corresponds to the description of *Z. tvaerminensis* (Karling 1952). Proboscis retractors adhere on the postero-lateral sides of the bulb behind the nucleiferous cell parts of the basal cone epithelium in between the six protractor muscles (Fig. 30).

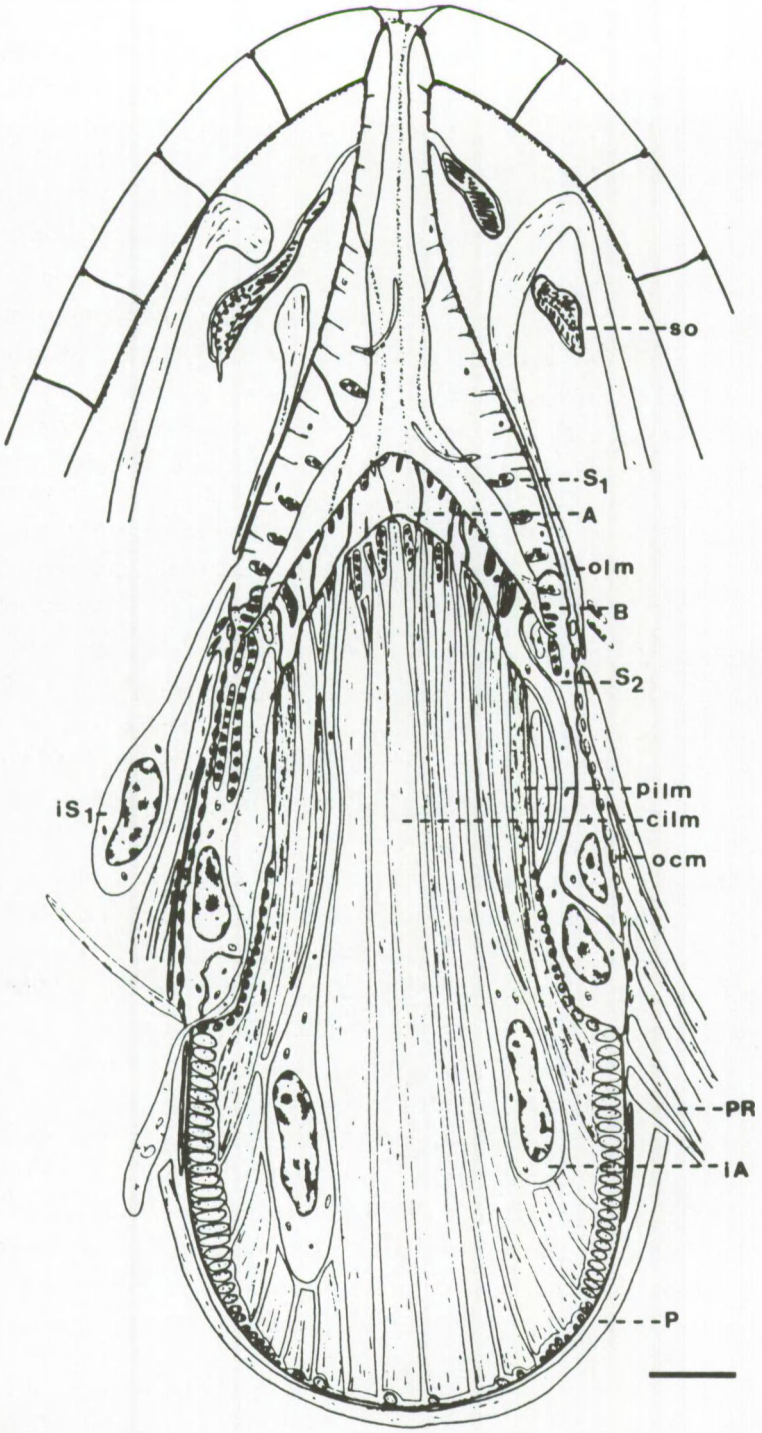


Fig. 32. Reconstruction of the proboscis in *Ethmorhynchus anophthalmus*. Scale bar: 10 µm.

Inner proboscis musculature. The inner circular muscles surround the internal cone retractors (inner longitudinal muscles) from the nodus nearly up to the junction (Figs 26, 27, 28, 29). Behind the nucleo-glandular girdle this layer is about 1.7 µm thick. In the cone the inner longitudinal muscles can be divided in a central cylinder (*cilm*) and eight peripheral blocks (*pilm*). The muscles which 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 between the peripheral blocks (Fig. 31). The radiating muscle fibres all bifurcate and include an other 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. 31). Sometimes they appear to be conical. 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 which 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 a distinct layer of extracellular matrix or septum is present. 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 basement membrane of the cone epithelium (Fig. 19).

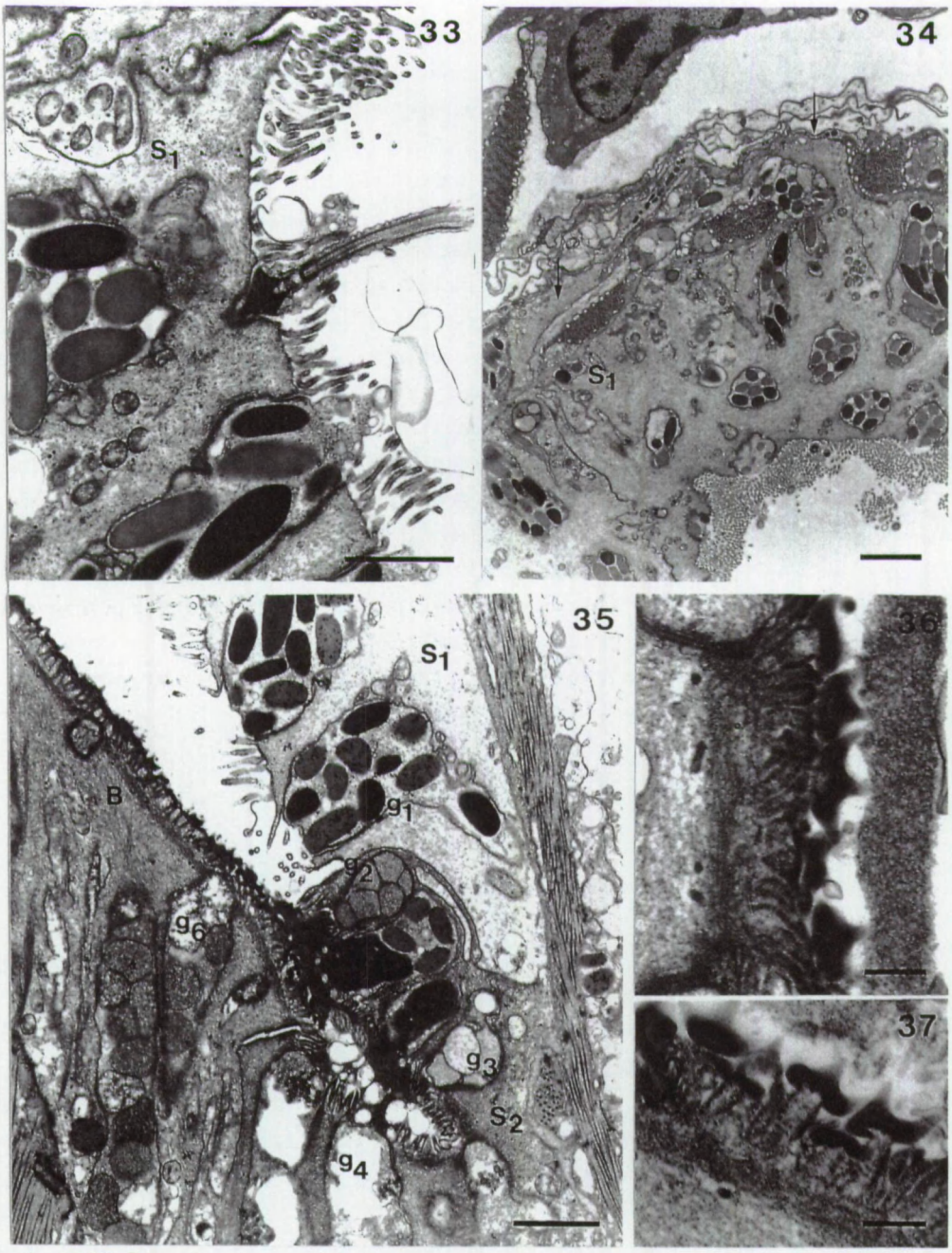
Ethmorhynchus (Figs 32-44)

The 105 µm long and 40 µm wide proboscis of *E. anophthalmus* takes up 1/5 of the body length.

Epithelia, glands and sensory cells

The proboscis epithelium is formed by cellular belts. Intra-epithelial nuclei are not found in the sheath epithelium, but the nucleo-glandular girdle below the junction contains twelve nuclei and many gland necks. Four nuclei are situated between the inner longitudinal muscles in the bulb. Swollen gland necks are present in the proximal half of the sheath epithelium, the nucleo-glandular girdle and the cone epithelium. Uni- and multiciliary receptors are found in the epithelia.

Epithelia. The proboscis epithelia are composed of four cellular belts (Fig. 32). One belt constitutes the major part of the sheath epithelium, another belt is found at the junction and two belt constitute the cone epithelium. All belts are devoided of cilia and cells are



Figs 33-37. Ethmorhynchus anophthalmus.
Fig. 33. Cell of the distal belt of the sheath epithelium (S_1) with uniciliary receptor and $g1$ gland necks. Scale bar: 1 µm.
Fig. 34. Cross section of the proximal part of the distal belt with cell strands sinking in the parenchyma. Scale bar: 2 µm.
Fig. 35. Transverse section of the junction with the distal (S_1) and proximal belt (S_2) of the

interconnected by apical zonulae adhaerentes, subsequent septate junctions and desmosomes (Fig. 33).

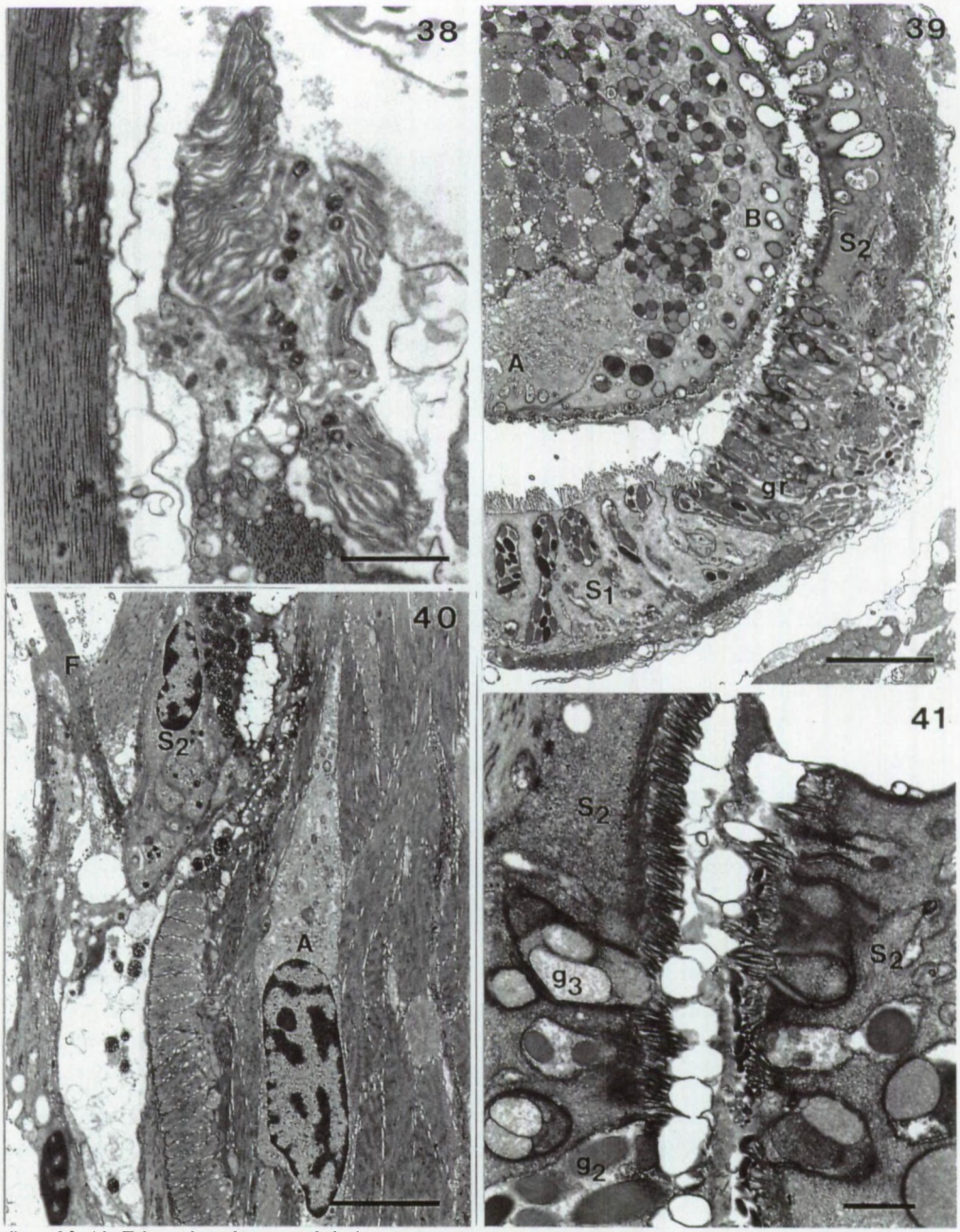
The sheath epithelium is formed by at least ten cells but the number can not be stated with certainty. The cells are oriented longitudinally; at the proboscis pore three or four cells are found, the other cells are only present in the median and proximal part of the epithelium. The epithelium is about 5 µm high and bears 900 nm long and slender microvilli (7 per linear µm) (Fig. 33). A dense terminal web is absent but the cytoplasm of the cells has a fibrous appearance. The basement membrane underlying the epithelium varies from 450 to 180 nm in thickness and is continuous with the proximal layer of the epidermal basement membrane at the pore. Small hemidesmosomes connect the basal plasmalemma to the basement membrane. In the cytoplasm few infoldings of the basal plasmalemma, mitochondria and Golgi apparatus are present. Some multilamellar bodies are found as well. The nuclei of these cells are situated in insunk cell parts in the mesenchyme around the bulb (Fig. 32). Narrow cell strands pierce the basement membrane and run down along the sheath and junction to the nucleiferous parts. The perforations in the basement membrane are situated in the posterior part of the cells, just above the proximal belt of the sheath epithelium (Fig. 34). The maculae adhaerentes between the cells have wide open intercellular spaces with inconspicuous intracellular condensations.

The proximal belt of the sheath epithelium or the "junctional" belt is composed of eight cells. This belt lines the proximal part of the sheath epithelium and covers the base of the cone (Fig. 35). The narrow part lining the cavity is 4 to 3 µm high. The major part of this belt is found in the nucleo-glandular girdle. The apical plasmalemma bears 400 nm long microvilli with condensations at their periphery and in the upper half, they narrow towards the tip (Fig; 38). Some 50 nm under the apical plasmalemma lies a 250 nm thick terminal web. The cytoplasm of the cells in this belt is always denser than this of cells in the previous belt. Cell organelles are only present in the lower parts of the cells in the nucleo-glandular girdle. Here the eight nuclei (8-12 µm) are found as well (Fig. 39). The zonulae adhaerentes between the cells are deep (400 nm) (Fig. 35).

The cone epithelium is about 4 µm high and formed by a basal and apical belt of four cells each. The basement membrane under the cone epithelium varies in thickness but does not exceed 150 nm. The basal belt of the cone epithelium (*B*) bears two different types of short microvilli. Normally shaped 180 nm long microvilli with dense tips appear among 270 nm

sheath epithelium and the basal cone epithelium (*B*). Apart of *g1* gland necks, *g2*, *g3*, *g4* and *g6* gland necks are visible. Bundles of tonofilaments connect desmosomes with the cell web in *B*. Scale bar: 1 µm.

Figs 36-37. Microvilli of the basal cone epithelium with wide electron dense caps. Scale bar: 0.2 µm.



Figs 38-41. Ethmorhynchus anophthalmus.

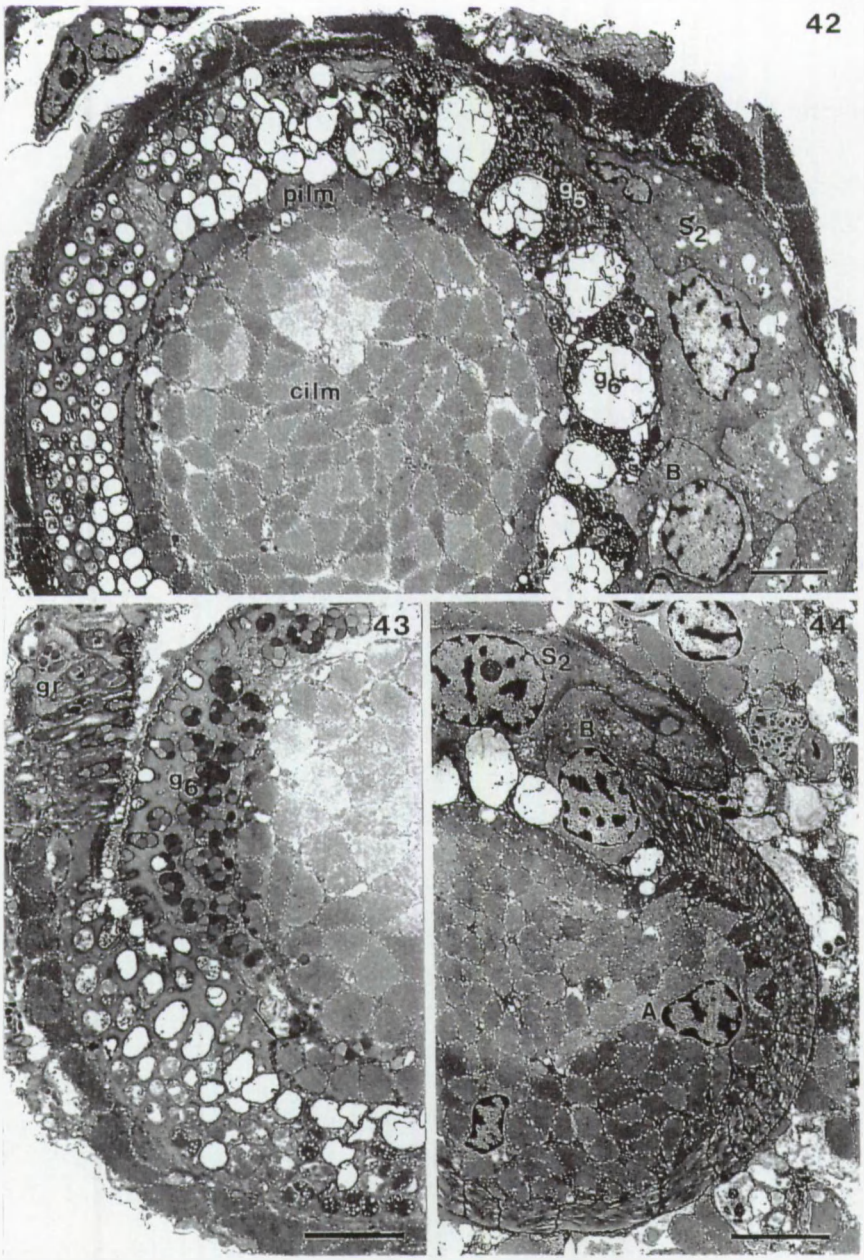
Fig. 38. Part of a sensory organ containing multiciliary receptors with flat sheetlike ciliary shaft. Scale bar: 1 µm.

Fig. 39. Oblique section showing distal (S_1) and proximal (S_2) belt of the sheath epithelium, apical (*A*) and the cellular basal (*B*) cone epithelium. *A* with g_9 gland necks, *B* predominantly with g_6 gland necks and *S2* with g_4 gland necks at the junction and g_2 and g_3 gland necks forming the glandular ring (*gr*). Scale bar: 5 µm.

long mushroom-shaped microvilli with wide dense caps (Figs 36, 37). Pointed ailes reach out laterally and dorsally from the caps. Some 50 nm under the apical plasmalemma a 120 nm thick terminal web is present. Mitochondria, few Golgi complexes and nuclei (10 µm) are situated in the cell parts in the nucleo-glandular girdle. The cells in the apical belt of the cone epithelium (*A*) bear 180 nm long stubby microvilli and lack a terminal web. Bundles of microfilaments, connected to the desmosomes with the inner longitudinal muscles, run to the apical layer of the cells. The nucleiferous cell parts of these cells are located between the inner longitudinal muscles in the bulb. Narrow cell strands pierce the basement membrane under the basal cone epithelium and run down between the internal cone retractors. The cell strands widen below the nucleo-glandular girdle to form the nucleiferous cell parts (Figs 39, 42). They contain up to 8 µm long nuclei and few small mitochondria and Golgi complexes.

Glands. The distal belt of the sheath epithelium contains gland necks only in its posterior half. The 1.5 µm wide terminal parts of the gland necks (g_1), which pierce the epithelium cells, are filled with closely packed ovoid (up to 1500 nm long) secretion granules with different degrees of electron density (Figs 33, 34, 35). In some specimens, the confluent contents of their secretion granules is found in the cavity at the junction and near the apex. The cell parts of the proximal belt of the sheath epithelium, situated anteriorly of the outer circular musculature, are except for type g_1 gland necks additionally pierced by two other types of gland necks. One type (g_2) contains ovoid, moderately electron dense secretion granules, 300 nm long. The other type (g_3) contains tightly packed globular and granular secretion granules with low electron density, 800 nm in diameter (Fig. 35). The entire epithelium is filled with these gland necks. Around the junction outside the proboscis, secretion packages of all three types of gland necks are found. The part of this belt at the junction is pierced by two different types of glands. Empty gland necks (g_4) probably contained 700 nm wide spherical secretion droplets (Figs 39, 40, 41). Type g_5 gland necks contain (moderately) electron dense secretion granules with several light spots (Fig. 40). The globular droplets measure up to 1000 nm in diameter. Apically the secretion granules dissolve and become less electron dense. Both types of gland necks are not wider than 1.5 µm in the superficial part of the epithelium (Meixner 1938 "Sieb aus Plasmapfeilern") but further down in the nucleo-glandular girdle they widen and form twenty pairs of 7 µm wide necks filled with secretion (Fig. 42). They enter the proboscis at the posterior end

Fig. 40. Transverse section of the lateral side of the bulb with posterior part of the nucleo-glandular girdle containing a nucleus of S_2 and g_4 and g_5 gland necks, fixator muscle (*F*) and intrabulbar nucleiferous cell part of *A*. Scale bar: 5 µm.
Fig. 41. Cross section above the junction with S_2 with g_2 and g_3 gland necks and *B*. Scale bar: 1 µm.



Figs 42-44. Ethmorhynchus anophthalmus.
Fig. 42. Oblique section of nucleo-glandular girdle showing surfacing g4 and g5 gland necks (*left*), which form wide alternating necks more proximally (*right*), and nucleiferous cell parts of S_2 and *B*. Note central (*cilm*) and peripheral (*pilm*) inner longitudinal muscles. Scale bar: 5 µm.
Fig. 43. Oblique section at the junction with glandular ring (*gr*) surfacing *g4* and *g5* gland necks and *g6* gland necks in the basal cone epithelium. Note the apical ends of the *pilm* (*arrow*). Scale bar: 5 µm.
Fig. 44. Oblique section showing the posterior part of the nucleo-glandular girdle with nuclei of S_2 and *B* and intrabulbar nuclei of *A*. Scale bar: 5 µm.

of the nucleo-glandular girdle apically of the thick layer of inner circular muscles. The basal belt of the cone epithelium is pierced by gland necks (g_6), tightly packed with secretion granules (800 nm in diameter) that vary in electron density, or in other specimens with granular contents, and small inconspicuous gland necks (g_7) with flocculent contents (Figs 35, 41, 43). The necks run between the internal retractors of the cone and the peripheral layer of longitudinal muscles. The apical cone epithelium contains small gland necks (g_9) with lightly stained spherical to ovoid secretion granules of 400 nm (Fig. 39).

Sensory cells. Uniciliary receptors are spread throughout the distal belt of the sheath epithelium and the part of the junctional belt that constitutes the posterior part of the sheath (Fig. 33). The receptors possess 2.5 µm long primary rootlets and very short secondary rootlets which form a connection with the zonulae adhaerentes. Multiciliary receptors are encountered in elongated insunk parts of cells belonging to the distal belt of the sheath epithelium (Fig. 41). The dendrites possess many cilia with axonemata only composed of single microtubules. Basal bodies and terminal plates are present but rootlets are lacking. The ciliary shafts form up to 1.7 µm broad sheets. The ciliary axonemata split in two sets of microtubules, which support two lateral stalks. In between these stalks a thin sheet is formed. The ciliary sheets form several long stacks.

Both cells of the apical and basal belt of cone epithelium contain uniciliary nerve endings. These receptors possess vertical rootlets but lack secondary rootlets and have short ciliary shafts (Fig. 38).

Proboscis musculature

All muscle fibres have a sarcoplasmic reticulum and the outer musculature is nucleated. Nuclei of muscle fibres inside the bulb have not been found. Muscle fibres can fuse or split and in this way they give rise to a variability in the number of fibres between different specimens and even within one particular specimen in cross sections at different levels.

Outer proboscis musculature. Circular muscles are not present around the distal belt of the sheath epithelium (Fig. 34). The distal most fibre is found at the level of the proximal belt just above the junction and is about twice as thick (1 µm) as the subsequent fibres (Fig. 35). This distal fibre is pressed in the epithelium and forms a kind of sphincter. It is strongly attached to the epithelium by numerous desmosomes and hemi-desmosomes (in the epithelium) (Fig. 38). The circular muscles enclose the nucleo-glandular girdle (Fig. 39). Below this girdle circular muscles are present inside the bulbar septum (transition zone). Sixteen longitudinal muscles enclose the sheath epithelium, the nucleo-glandular girdle and the anterior part of the bulb (Figs 34, 35). These muscle fibres are continuous with the well developed longitudinal muscles of the body wall musculature and are attached to the bulbar

septum at the postero-lateral sides of the bulb. The flattened fibres around the sheath epithelium narrow around the nucleo-glandular girdle and become very thin around the bulb.

The motional muscles include protractors, fixators, proboscis retractors and integument retractors. The fixators consist of fine muscle fibres that insert on the posterior end of the nucleo-glandular girdle and on the body wall at the level of the median part of this girdle (Fig. 39). Two sets of proboscis retractors attach on the basement membrane and outer circular muscles at the junction and on the bulbar septum at the postero-lateral side of the bulb (Fig.42). The first set of proboscis retractors forms sixteen groups of five muscle fibres closely surrounding the nucleo-glandular girdle and situated between the fibres of the outer longitudinal musculature (Fig. 44). At the posterior end and below the nucleo-glandular girdle these groups form eight bundles running posteriorly through the mesenchym, together with fibres attaching on the lateral side of the bulb below the nucleo-glandular girdle (Fig. 32).

Inner proboscis musculature The inner musculature comprises circular and longitudinal muscles. As mentioned above the inner circular muscles are found from below the nucleo-glandular girdle but they are not present at the posterior end of the bulb. The fibres are closely associated. They are surrounded by a thin layer of extracellular matrix, which forms the septum. About five to six fibres are found at the inner side of the nucleo-glandular girdle anteriorly of the thick layer of inner circular muscles (Fig. 39). They appear as a prolongation of this layer. The longitudinal muscles in the bulb, which function as retractors of the cone, form a central cylinder (*cilm*) and a single peripheral layer of longitudinal muscles (*pilm*) (Figs 42, 43, 44). A total of 135 muscle fibres form the central cylinder which is found below the apical and basal cone epithelium. The fibres are connected to the underlying basement membrane by desmosomes and hemi-desmosomes (with condensations in the muscles only). The peripheral muscles form a single layer of 54 fibres. The apical ends of these muscles insert on the basal parts of the cells of the basal cone epithelium (Fig. 43). In the bulb they insert on the layer of inner circular muscles immediatly beneath the nucleo-glandular girdle. More posteriorly the longitudinal muscles of the central cylinder insert mostly on the septum.

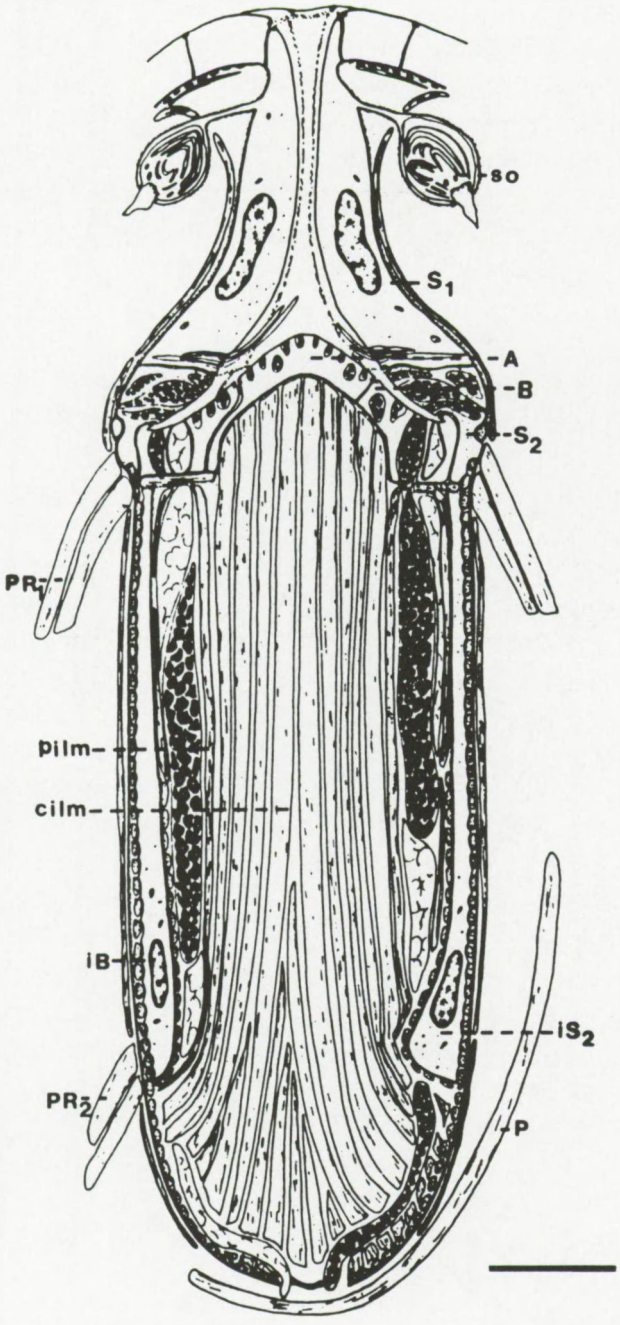


Fig. 45. Reconstruction of the proboscis of *Ptyalorhynchus coecus*. Scale bar: 10 μm.

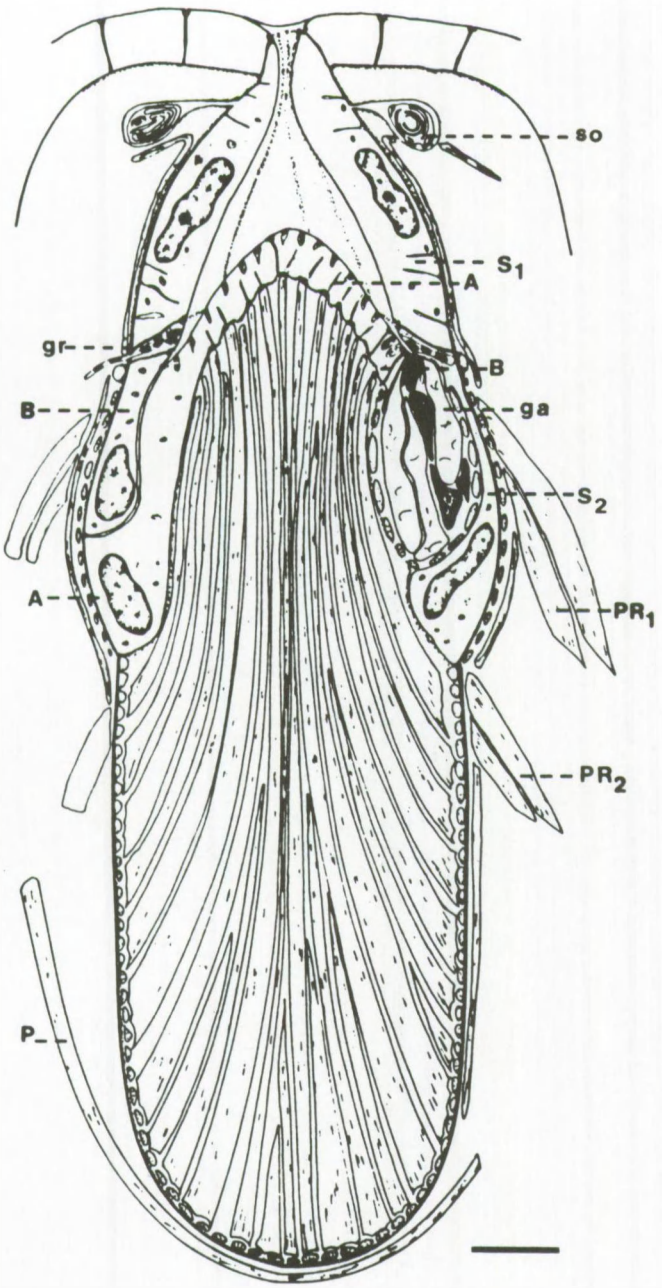


Fig. 46. Reconstruction of the proboscis of *Cicerina remanei*. Scale bar: 5 µm.

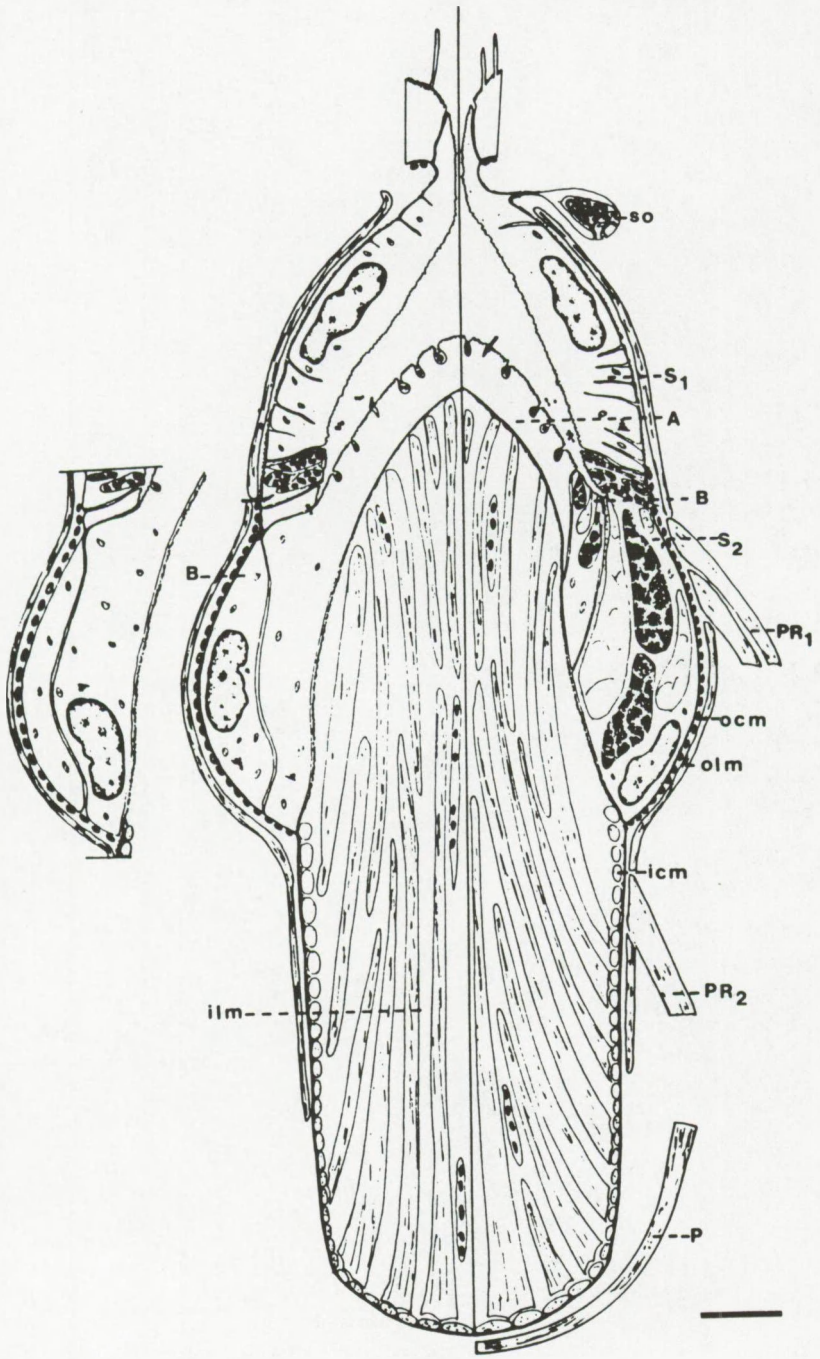


Fig. 47. Reconstruction of the proboscis in *Paracicerina deltoides*. Scale bar: 5 µm.

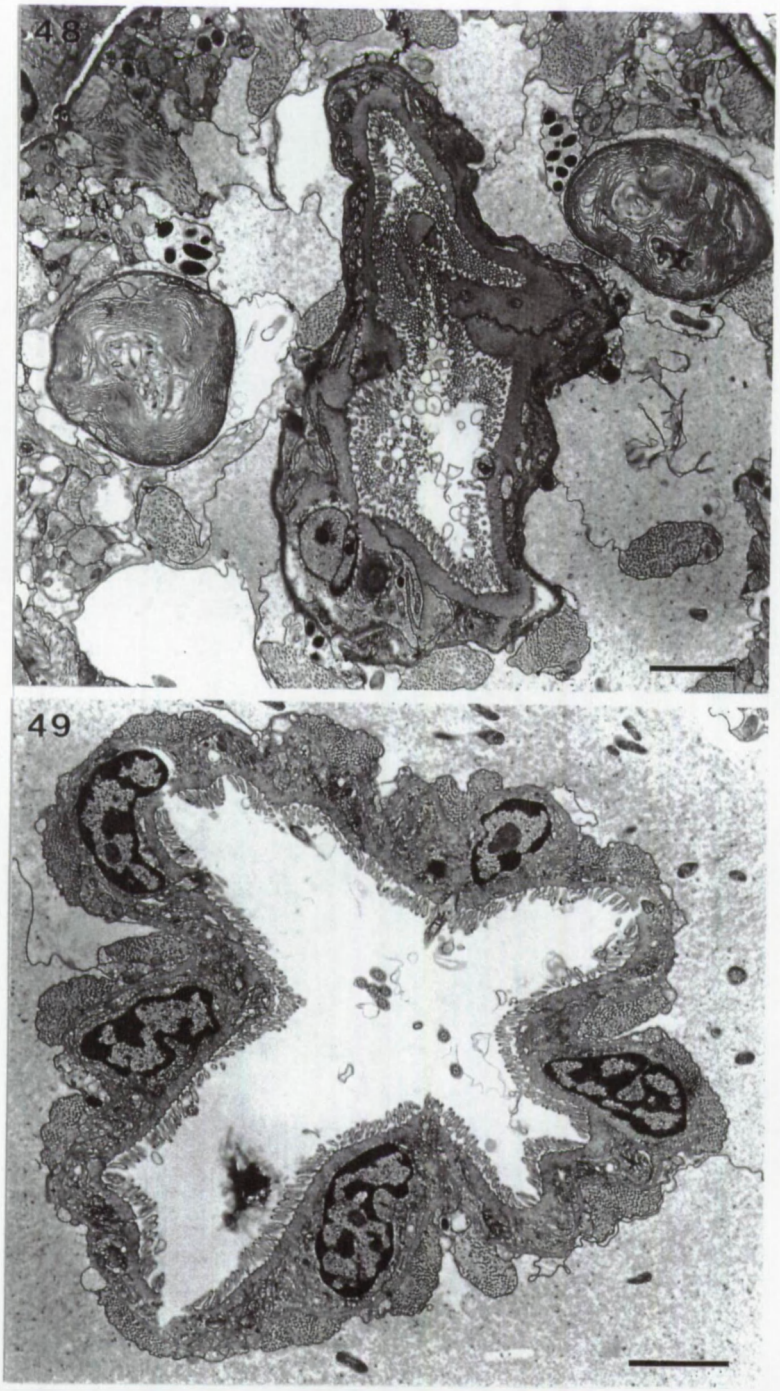


Fig. 48. Ptyalorhynchus coecus. Cross section of the cellular distal belt with two sensory organs, filled with concentric arranged memebranes of the ciliary shafts of the multiciliary receptors. Scale bar: 2 µm.
Fig. 49. Paracicerina deltoides. Cross section of the distal belt formed by five cells. Only longitudinal muscles surround the cavity. Scale bar: 2 µm.

Ptyalorhynchus*, *Paracicerina* and *Cicerina (Figs 45-78)

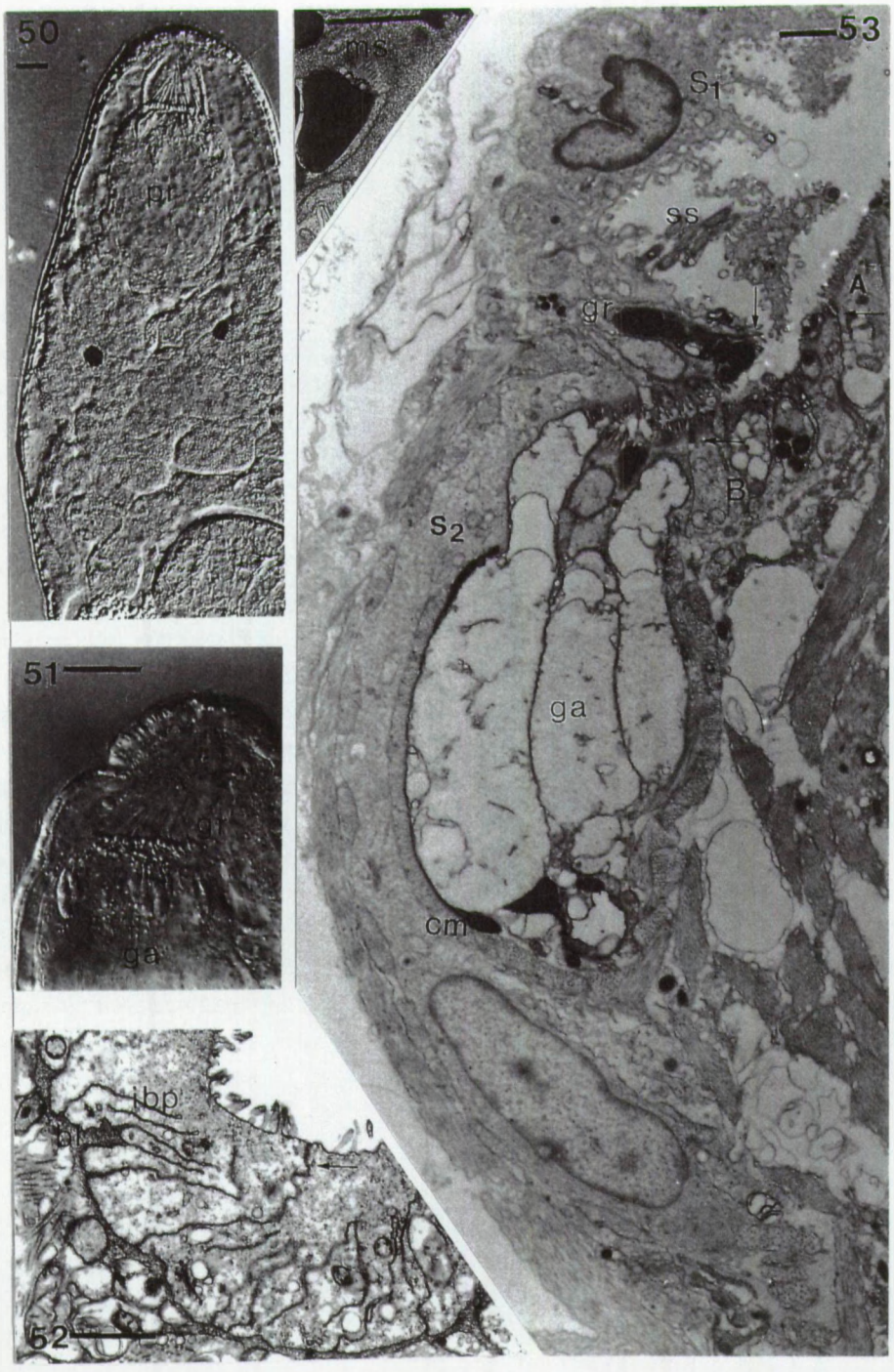
In the description common features for the three genera are delt with first, differences between the genera are stated explicitely afterwards. Often features in *Paracicerina* and *Cicerina* are delt with together but separately of *Ptyalorhynchus*.

Epithelia, gland necks and sensory cells

In the three genera a nucleo-glandular girdle is present below the junction (De Vocht & Schockaert 1988). The gland necks in this girdle pass through the proximal belt of the sheath epithelium and the basal belt of the cone epithelium. The nuclei in the girdle belong to the proximal belt of the sheath epithelium and the cone epithelium. In *Paracicerina* and *Cicerina*, four glandular ampullae and four intermediate nucleiferous cell parts are present. In *Cicerina* these glandular ampullae are enclosed by circular muscles. In *Ptyalorhynchus* these gland necks are incorporated in the bulb, while the nucleiferous cell parts are located peripherally. The gland necks and epithelial nucleiferous cell parts are, except for the anterior 10 µm, separated by a layer of extracellular matrix. The gland necks pass through the bulb, which is surrounded by a peripheral cytoplasmic girdle.

Epithelia. The proboscis epithelium in *P. coecus*, *P. deltoides*, *C. remanei* and *C. brevicirrus* is formed by four belts (Figs 45, 46, 47, 54). Three belts are cellular and the belt which covers the basal part of the cone is syncytial. All belts are devoided of cilia and cell junctions are formed by zonulae adhaerentes and septate junctions and sparse maculae adhaerentes. In *C. brevicirrus* wide intercellular spaces (2 µm) are present below the septate junctions (Fig. 55). Zonulae adhaerentes are 300 to 500 nm long in *Cicerina*, 100 to 150 nm in *Paracicerina* and *Ptyalorhynchus*.

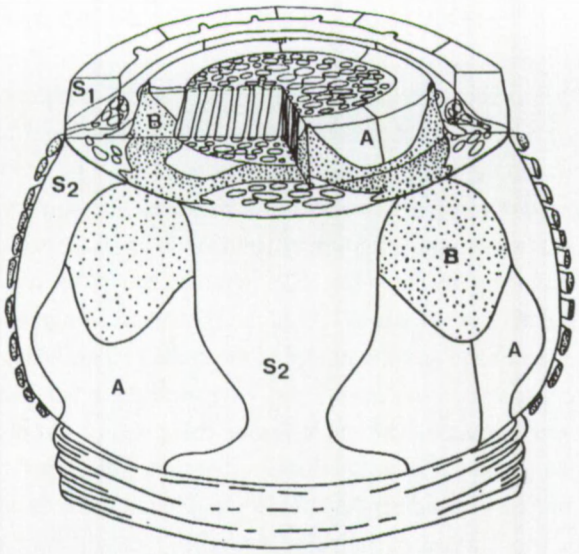
The sheath epithelium is formed by two cellular belts, of which the distal one constitutes the major part of this epithelium. In *Paracicerina* and *Ptyalorhynchus* this distal belt is formed by five cells (Figs 48, 49), in *C. brevicirrus* by seven and in *C. remanei* by eight. The proximal belt in all four species is formed by four cells. The epithelium is 1 to 1.5 µm thick near the proboscis pore and 1.5 to 3 µm further down. In *Cicerina* the distal belt of the epithelium forms several ridges upto 5 µm high (Figs 53, 55). The distal belt of the sheath epithelium is covered by long, slender microvilli, irregular in shape in *Cicerina* and about 350 nm long (Fig. 52). They measure 550 nm in *Paracicerina* and 400 to 500 nm in *Ptyalorhynchus* (Figs 48, 49). The long and slender microvilli, that cover the proximal belt of the sheath epithelium, are 350 to 450 nm long in *Cicerina*, in *Paracicerina* they measure 450 nm between the surfacing gland necks but just above the junction they increase to a length of approximately 600 nm. In *Ptyalorhynchus* the microvilli are 500 nm long. The microvilli in the proximal belt are more densely packed than those of the distal belt;


50
pr
51
ga
52
ibp
53
ms
S1
ss
gr
A
B
S2
ga
cm

in *C. brevicirrus* for instance there are 12 per µm in the proximal to 4-5 per µm in the distal belt. A terminal web is not present in the distal belt in *Cicerina*; in *Paracicerina* and *Ptyalorhynchus* a 300-400 nm thick peripheral fine fibrillar layer which is devoided of cell organelles is present in the apical part of the cells (Figs 48, 49). The basal parts of the cells in the distal belt contain a fine granular cytoplasm with patches of free ribosomes, rough endoplasmic reticulum, mitochondria, few Golgi apparatuses and multilamellar bodies. Infoldings of the basal plasma membrane are present and reach up to the apical fibrillar layer or upto 2/3 of the cell height in *Cicerina* (Fig. 52). Cells in the distal belt possess intra-epithelial nuclei but the nuclei of the proximal belt of the sheath epithelium are situated in the nucleo-glandular girdle. The lobate and flat nuclei of the distal belt are 6 µm long and 5 µm wide in *Cicerina* and *Paracicerina*, but upto 7 µm long with thick peripheral patches of heterochromatin in *Ptyalorhynchus*. The nucleiferous cell parts of the proximal belt are situated below the swollen gland necks in the nucleo-glandular girdle in *Paracicerina* and *Cicerina* (Figs 53, 64). In *Ptyalorhynchus* they are situated in the cytoplasmic girdle which surrounds the anterior and median part of the bulb (Fig. 60).

The cone epithelium in the three genera is bipartite. The belt covering the basal part of the cone is syncytial in all species. In *Ptyalorhynchus* this belt is found all around the base of the cone but in *Cicerina* and *Paracicerina* only four narrow strips of the syncytium situated at the glandular ampullae line the cone (Figs 54, 57). The epithelium on the cone is less than 1 to 2 µm thick. The basal belt of the cone epithelium bears uniform microvilli (450 nm long) in *Ptyalorhynchus*. In *Paracicerina* and *Cicerina* two types of microvilli are present; normal shaped microvilli measure 300 to 350 nm and have dense margins in *Paracicerina*. Broad microvilli with dense tips and sharp edges (450 nm) are present among the former in *Cicerina*. In *Paracicerina* the second type of microvilli are large with dense margins (300-700 nm) and near the junction they are often dichotomously branched. The apical belt of the cone epithelium bears stout microvilli about 450 nm high in *Ptyalorhynchus* and *Paracicerina* but in *Cicerina* they are shorter and about 250 nm long. A thin (100 nm) electron dense peripheral terminal web is present. Bundles of tonofilaments connect the terminal web to desmosomes with wide intercellular spaces at the insertion of the internal longitudinal

Figs 50-53. Cicerina remanei.
Fig. 50. Live specimen in I.C. showing the proboscis (*pr*), the brain with two pigmented eyes and part of the pharynx. Scale bar: 10 µm.
Fig. 51. Front half of the proboscis of the same specimen. The glandular ring (*gr*) glandular ampullae (*ga*) and some nuclei are visible. Scale bar: 10 µm.
Fig. 52. Distal belt of the sheath epithelium, showing cell boundary, infoldings of the basal plasma membrane (*ibp*) and underlying basement membrane (*bl*). Scale bar: 1 µm.
Fig. 53. Longitudinal section with distal (S_1) and proximal belt (S_2) of the sheath epithelium. S_2 with glandular ampulla (*ga*) and glandular ring (*gr*), basal (*B*) with g_6 and g_7 gland necks and apical cone epithelium (*A*). Note the circular muscles (*cm*) around *ga*. Scale bar: 1 µm.



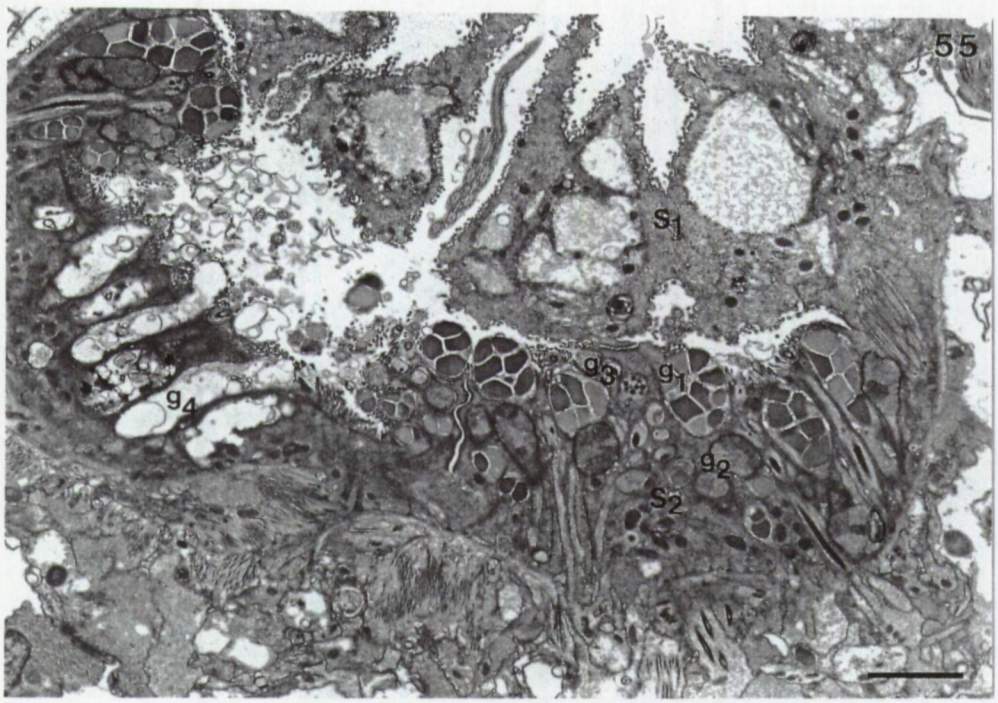
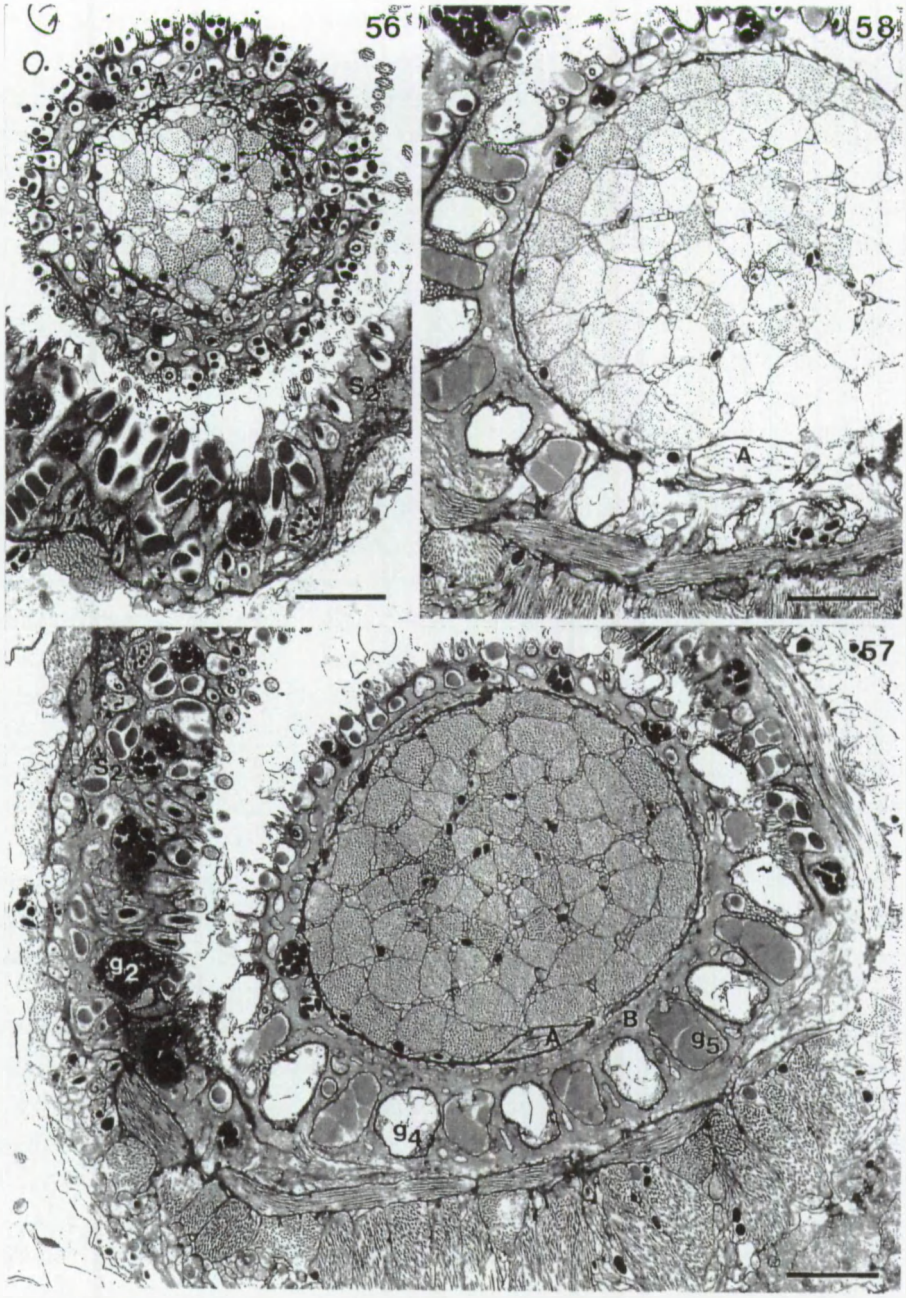


Fig. 54. Cicerina remanei. Three dimensional reconstruction of the junction and nucleo-glanudlar girdle. Have been removed: a) the upper part of the S_1 and *A*. b) the front of the remaining part of S_1 and the apical part of S_2. c) a section at the same level into the cone. Scale bar: 5 µm.
Fig. 55. Cicerina brevicirrus. Oblique section showing distal (S_1) and proximal belt of the sheath epithelium (S_2) with g_1, g_2, g_3 and g_4 gland necks in S_2. *Arrows*: groups of uni- and biciliary receptors. Scale bar: 2 µm.

muscles (Figs 71, 72, 73). The cone epithelium is connected to a 100 nm thick and uniform basement membrane by numerous small hemidesmosomes. The cytoplasm in the cell part lining the cone contains some mitochondria (with dense matrix in *C. brevicurrus*) but hardly any other cell organelles. Cytoplasmic cell parts which contain the nuclei, mitochondria and Golgi complexes (in *Cicerina* also some dense bodies) are situated in the nucleo-glandular girdle. In *Cicerina* and *Paracicerina*, the gland necks in the nucleo-glandular girdle form four glandular ampullae with in between four nucleiferous cell parts of both the basal and apical cone epithelium (Figs 61, 69). In *Paracicerina* the cytoplasmic cell strands between the gland necks are very narrow and widen at the posterior end and below the swollen gland necks (Fig. 64). In *Cicerina* the four groups of gland necks are enclosed by circular muscles and form four clearly limited glandular ampullae (Figs 53, 69). Two nuclei of the apical cone epithelium are found at the inner side of the cell packages diametral one of the other. Two nuclei of the basal cone epithelium are situated peripherally in the other nucleiferous cell packages at cross angles of the former. Below the glandular ampullae four nucleiferous cell parts of the proximal belt of the sheath epithelium are situated (Figs 53, 70). In *Ptyalorhynchus* the swollen gland necks pass through the bulb upto 5 µm of the epithelium surface and surface at the base of the cone (Figs 57, 58). They pull through the basal cone epithelium in the upper 5 µm. The gland necks form a closed circle at the inside close to or further down below in the bulb, while the nucleiferous cell parts of the proximal belt of the sheath epithelium and both belts of the cone epithelium form an outer cytoplasmic girdle around the anterior and median part of the bulb (Figs 59, 60). The apical cone epithelium is formed by a single cell, the part which covers the apex is connected to nucleiferous part by one cell strand (Fig. 58). This cell strand runs under the basal cone epithelium and runs under the single circular muscle fibre which encloses the central cylinder towards the cytoplasmic girdle. In *Paracicerina* and *C. brevicirrus* four cells constitute the apical belt of the cone epithelium. In *C. remanei*, two cells and two binucleated syncytia were found.

Glands. The distal belt of the sheath epithelium in all three species is practically devoided of gland necks. Occasionally in *Paracicerina* and *Ptyalorhynchus* type g3 gland necks pierce the cells immediately above the proximal belt of the sheath epithelium. The part of the proximal belt lining the posterior part of the cavity, however, is pierced by numerous gland necks. Three types of gland necks are found in this part of the epithelium and form a glandular ring above the junction. Most of the different types of gland necks can be homologized between the three genera. Type g1 gland necks are the most prominent and the apical parts transversing the epithelium are stuffed with secretion. The moderately to fairly electron dense secretion is closely packed in irregular shaped granules in *Cicerina*

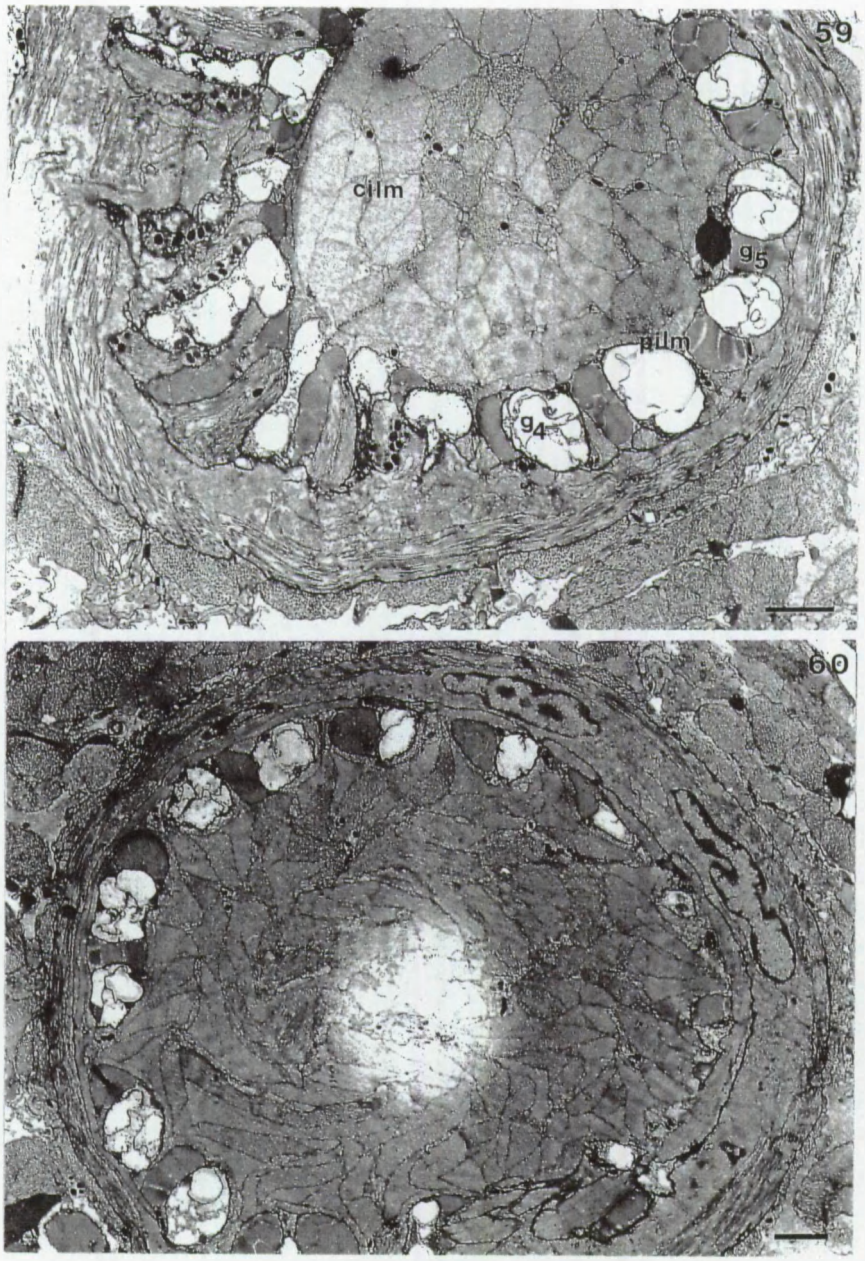


Figs 56-58. Ptyalorhynchus coecus.
Fig. 56. Cross section of the cone covered by the apical cone epithelium (*A*) with g9 gland necks and a part of the proximal belt of the sheath epithelium (S_2) with g_1, g_2 and g_3 gland necks. Scale bar: 2 µm.
Fig. 57. Oblique section at the junction, showing S_2 predominantly with g_2 gland necks and uniciliary receptors, basal cone epithelium (*B*) with alternating type g_4 and g_5 gland necks at the junction and g_6 and g_7 gland necks on the cone, insunk cell strands of the (*A*). Scale bar: 2 µm.

(600 nm) and *Paracicerina* (800 nm) or in large ovoid granules (1200 nm) in *Ptyalorhynchus* (Figs 53, 55, 56, 62). The second type of gland necks (g_2) has a flocculent content in *Cicerina* and *Paracicerina* packed in 350-500 nm large granules (Figs 55, 63). In *Ptyalorhunchus* this secretion appears as 450 nm large spherical, electron dense secretion granules (Fig. 56). These type g_2 gland necks in *Ptyalorhynchus* are situated further down towards the junction as type g_1 and g_3 gland necks. The type g_3 gland necks in *Cicerina* and *Ptyalorhynchus* contain small spherical to slightly ovoid secretion granules (120-200 nm) with variable electron density (Figs 55, 56). Type g_3 gland necks in *Paracicerina* are situated proximally of type g_1 and g_2 gland necks and contain 350 nm wide secretion granules (Fig. 63). Type g_3 gland necks in *Paracicerina* might not be homologous to type g_3 gland necks in *Cicerina* and *Ptyalorhynchus*.

The belt lining the proximal part of the sheath is found at the junction in four quadrants as well in *Cicerina* as *Paracicerina* (Figs 61, 67). Here, two types of gland necks (g_4 and g_5) surface. They pass through the the nucleo-glandular girdle and in *Cicerina* they are enclosed by circular muscles upto 2 µm of the surface and form four glandular ampullae (Figs 51, 61). Mostly the secretion of type g_4 gland necks is washed out during preparation. The remaining membranes indicate the presence of 850 to 1200 nm wide granules in the 2.5 to 3.5 µm wide gland necks in *Cicerina* and *Paracicerina* (Figs 53, 61, 68). In some specimens of *C. remanei* a moderately electron dense granular content could still be observed. The other type of gland necks (g_5) surfacing in this part of the epithelium contains electron dense and closely packed secretion granules (700-1000 nm) in the 4 µm wide necks (Figs 53, 61, 69). As the basal belt of the cone epithelium is found all around at the junction in *Ptyalorhynchus*, type g_4 and g_5 gland necks surface through this belt. The apical part of the gland necks are 2 µm wide and surrounded by an inner circle of microtubules. Exactly at the junction, fifteen gland necks of both types surface alternatingly (Fig. 57). Beneath the radiating muscles the gland necks pass through the peripheral inner longitudinal muscle layer in the bulb (Fig. 59). They first form a closed layer of gland necks but further down in the bulb twelve groups of gland necks are formed, each group enclosed by mostly two flat fibres of the peripheral inner longitudinal muscles and all of them also surrounded by an inner layer of circular muscles (Fig. 60). The gland necks enter the bulb at the nodus. The part of the basal cone epithelium which covers the base of the cone is pierced by two types of gland necks in *Ptyalorhynchus*. Type (g_6) gland necks with moderately electron dense, spherical to ovoid secretion granules (400 nm) surface near the junction through the proximal belt of the sheath epithelium as well (Fig. 57). Type g_7 gland necks contain very electron dense

Fig. 58. Cross section proximally of Fig. 57. with one cytoplasmic strand of *A* and a part of the circular muscle fibre (*arrow*) which surrounds the central longitudinal muscles in the bulb. Scale bar: 2 µm.



Figs. 59-60. Ptyalorhynchus coecus.
Fig. 59. Oblique section of the distal portion of the nucleo-glandular girdle with central (*cilm*) and peripheral (*pilm*) longitudinal muscles, the latter enclosing *g4* and *g5* gland necks and thin cytoplasmic strands. Scale bar: 2 µm.
Fig. 60. Proximal portion of the glandular girdle with wide cytoplasmic parts containing nuclei of S_2, *B* and *A*. Scale bar: 2 µm.

secretion granules as present in type g_2 gland necks (Fig. 57). In *Paracicerina* three types of gland necks organized in three rows surface through the basal cone epithelium belt at the junction (Fig. 61). From the outer side to the inner side a double row of five gland necks of type g_8 and g_7 is found close to type g_4 and g_5 gland necks. Between these two rows, four gland necks of type g_6 surface in each quadrant. Type g_8 gland necks contain moderatlye electron dense almost spherical secretion granules (700-800 nm), type g_6 gland necks contain empty vesicles (700 nm wide) and the 1.5 µm gland necks of type g_7 are filled up with 400 nm wide secretion granules with granular contents (Fig. 61). Type g_8 gland necks are peripherally enforced by microtubules. The four strips of the basal cone epithelium which run up the cone are devoided of gland necks. In *Cicerina* two types of gland necks pierce the basal cone epithelium. Type g_6 gland necks are filled with 400 nm wide empty secretion granules, type g_7 gland necks with a spherical electron dense secretion in *C. remanei* and closely packed secretion granules (350 nm) with flocculent contents in *C. brevicirrus* (Fig 67). Type g_6 and g_7 gland necks in *Cicerina* are much smaller than in *Paracicerina*.

The apical cone epithelium in *Ptyalorhynchus* is pierced by type g_9 gland necks with ovoid somewhat less electron dense secretion granules 180 nm wide and 300 nm long (Fig. 56). The apical cone epithelium in *Paracicerina* is pierced by two types of gland necks with ovoid electron dense secretion granules. Type g_9 gland necks are found more apically, secretion granules measure 220 nm in diameter and are about 350 nm long. Type g_{10} gland necks are situated more basally in the epithelium and the secretion granules measure 250 nm in diameter and are up to 420 nm long (Fig. 62). In *C. remanei* two types of gland necks are present. Type g_8 gland necks contain electron dense ovoid secretion granules 280 nm wide and upto 500 nm long, while type g_9 gland necks, mostly found towards the apex, contain smaller electron dense secretion granules 150 nm wide and 300 nm long (Fig. 71). In *C. brevicirrus* three types of gland necks are found in the apical cone epithelium. Type g_8 gland necks found basally in the epithelium contain secretion granules of variable electron density, which are irregular in shape and about 400 nm wide. Type g_9 gland necks contain moderate electron dense secretion granules (200 nm). Type g_{10} gland necks contain a 450 to 500 nm wide secretion granules with flocculent contents as in type g_7 gland necks.

Sensory cells. Only very few uniciliary receptors are spread throughout the anterior part of the distal belt of the sheath epithelium (Figs 48, 49). Through the posterior end of this belt in *Cicerina* and *Paracicerina* twelve groups of uni- and biciliary receptors protrude into the cavity (Figs 53, 62). Their rootlets transverse the epithelium obliquely. In *Cicerina* they are situated in twelve oblique funnels partially covered by the proximal belt of the sheath epithelium. The ciliary shafts with normal axonemata form rigid stalks which point

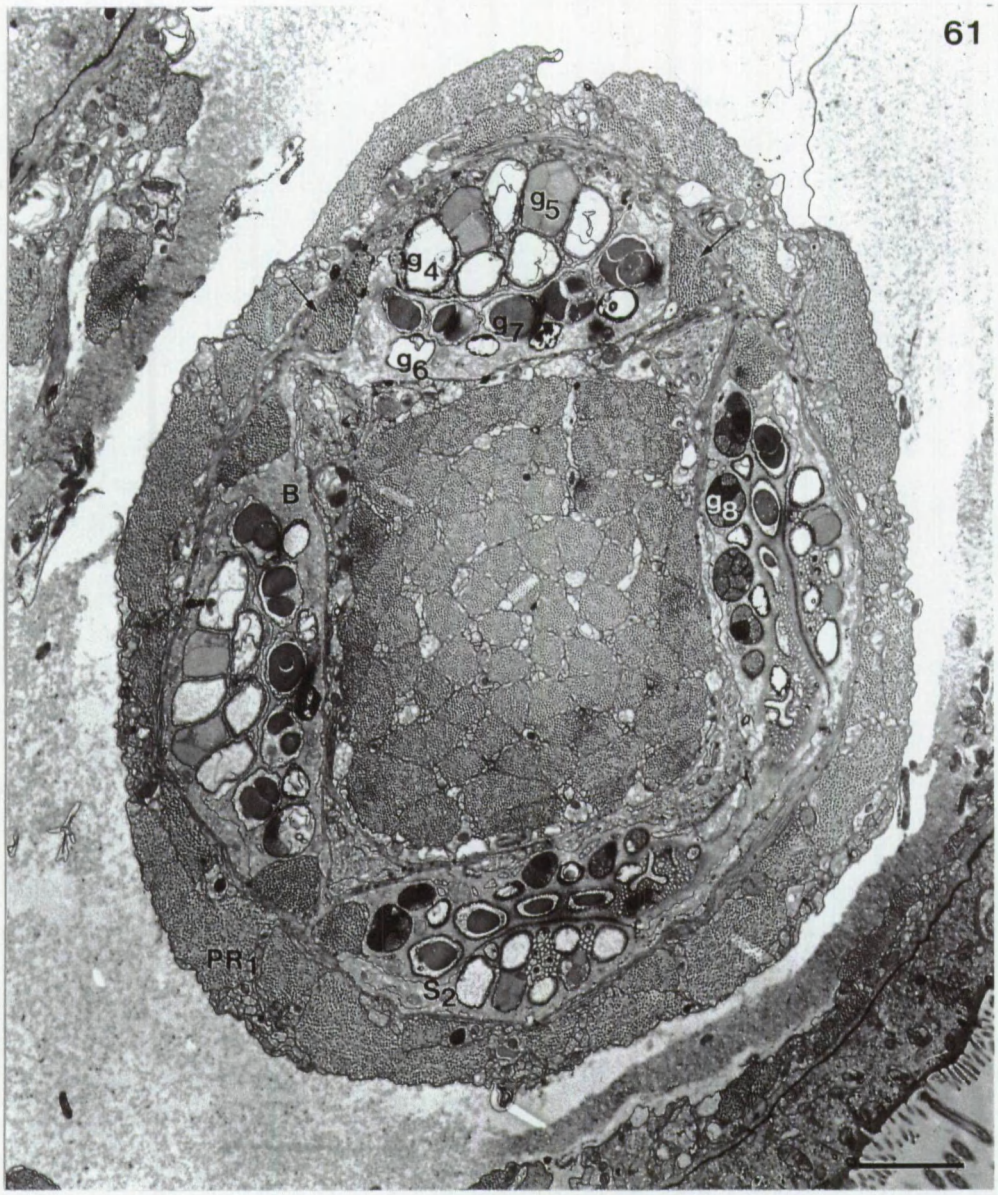


Fig. 61. Paracicerina deltoides. Cross section showing four glandular ampullae with g4 and g5 gland necks in the proximal belt of the sheath epithelium (S_2) and the basal cone epithelial belt (*B*) pierced by rows of *g6*, *g7* and *g8* gland necks. Note the longitudinal muscles (*arrows*) in the nucleo-glandular girdle and the the first set of proboscis retractors (PR_1). Scale bar: 2 µm.

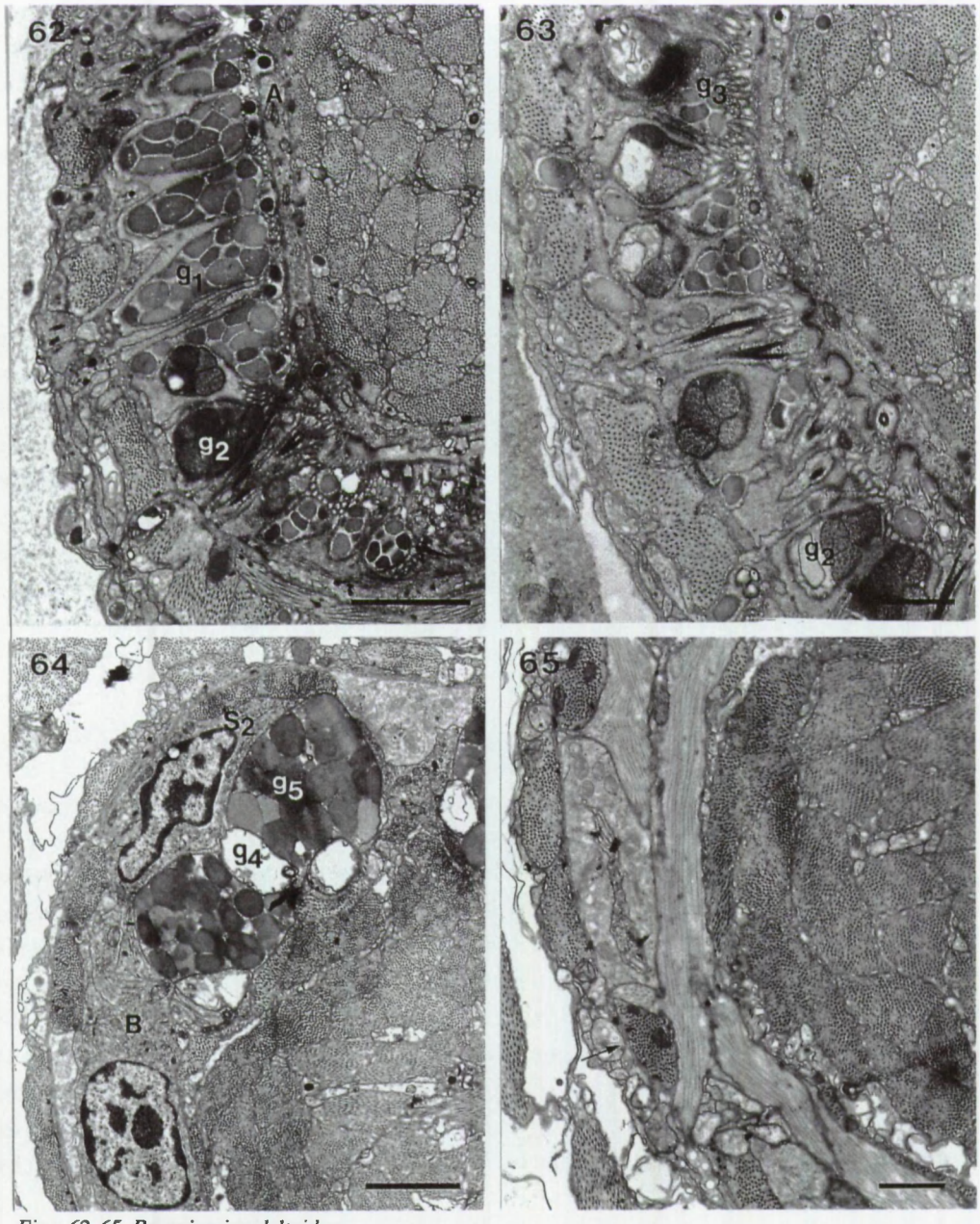
towards the proboscis pore (Figs 50, 51). In *Ptyalorhynchus* rather a circular groove with many scattered receptors is present in this part of the epithelium instead of twelve distinct groups of receptors (Fig. 56). The proximal belt of the sheath epithelium in the three genera is pierced by uniciliary receptors with long (3.5 µm) primary rootlets directed almost perpendicular to the epithelium (Figs 56, 63).

In the three genera multiciliary receptors are found in two insunk parts of cells of the distal belt of the sheath epithelium (Fig. 48). These spherical sensory organs are situated in the parenchym near the proboscis pore. A thin layer of extracellular matrix (30 nm) is found beneath the thin epithelium lining the two lumina and is continuous with the basement membrane (Figs 75, 78). Except for few mitochondria, cell organelles are absent in the epithelium and microvilli are not observed. The cilia of the multiciliary receptors form wide membranous sheets in the lumina of the insunk cell parts. The ciliary axonemata are composed of singlets only which split into two groups supporting the lateral stalks as in *Psammorhynchus tubulipenis* (De Vocht 1990). The ciliary sheets form stacks of membranes which are very closely packed, especially in *Paracicerina* and *Ptyalorhynchus*. The dendrites penetrate the insunk cell parts posteriorly and the highly modified ciliary shafts are directed forward. Immediately behind these two sensory organs in *Ptyalorhynchus* two rhabdomeric receptors are present (Fig. 76).

Proboscis musculature

Outer proboscis musculature. The musculature beneath the sheath epithlium and outside the bulbar septum is formed by circular and longitudinal muscles in *Cicerina* and *Paracicerina*. Around the distal belt of the sheath epithelium only a longitudinal muscle layer is found, composed of fourteen to sixteen fibres (Figs 49, 62). They are absent around the distal part of this belt in *Ptyalorhynchus*. The fibres are continuous with the longitudinal muscles of the body wall and enclose the proximal belt of the sheath epithelium and the nucleo-glandular girdle as well. They adhere on the lateral sides of the bulb. A layer of outer circular muscles is present from the junction on arround the nucleo-glandular girdle in *Cicerina* and *Paracicerina* (Figs 53, 61). The anterior most fibre is twice as thick as the subsequent fibres (fig. 67). In *Ptyalorhynchus* the anterior most circular muscle fibre is surrounded by a thin layer of ECM, but more posteriorly the circular muscles are covered by ECM at the outside (Figs 57, 59, 60).

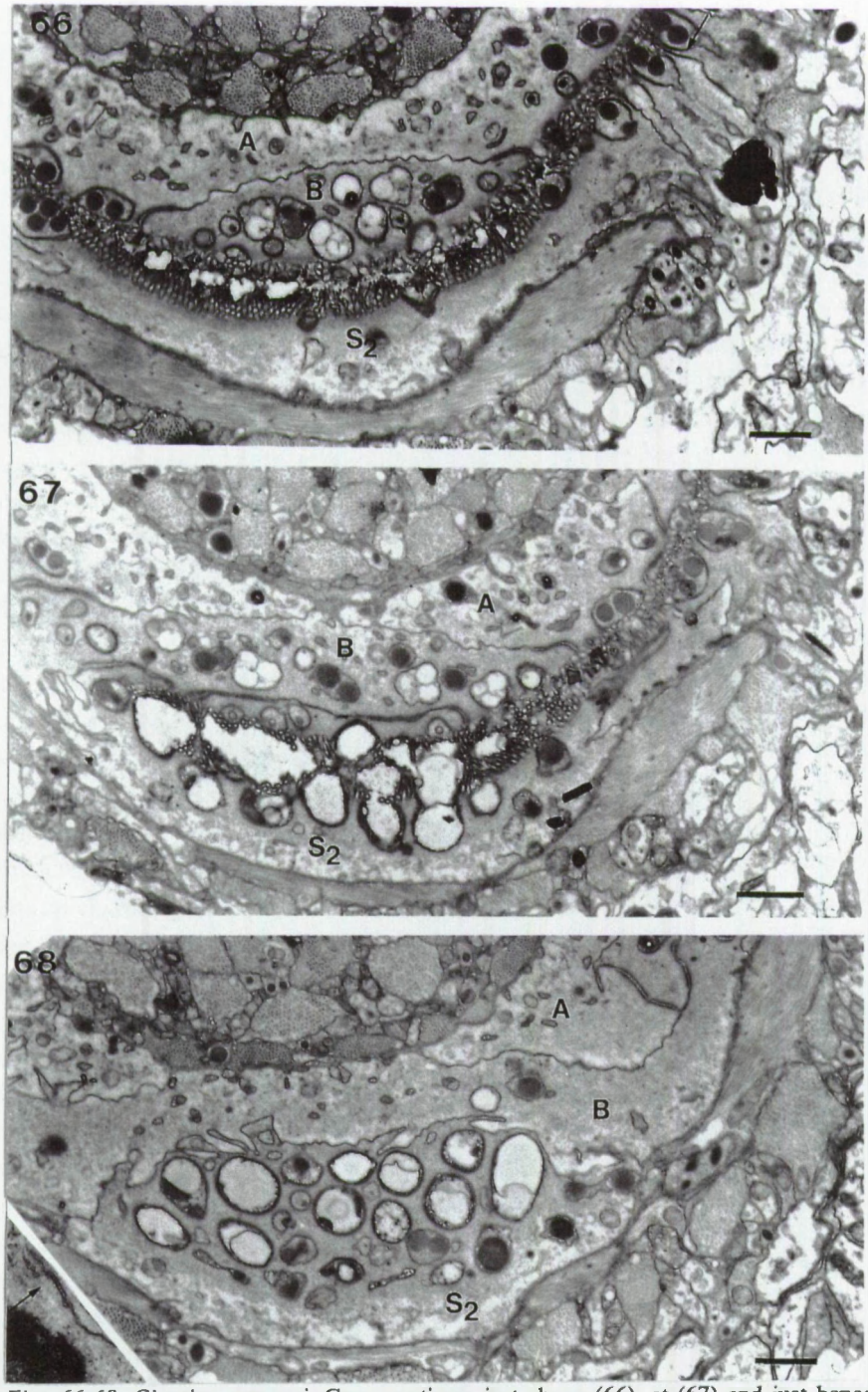
The motional muscles comprise thre pairs of protractors, two sets of proboscis retractors and one pair of ventral integument retractors. The protractors run from the nodus to the anterior body end. The anterior set of proboscis retractors inserts all around on the basement membrane at the junction and the anterior circular muscle fibres. Fourteen groups of four to five fibres are found between the fourteen outer longitudinal muscles in *Ptyalorhynchus*



Figs. 62-65. Paracicerina deltoides.
Fig. 62. Part of the glandular ring the proximal belt of the sheath epithelium (S_2) with g_1, g_2 and apical cone epithelium (*A*) with g_{10} gland necks. Scale bar: 2 µm.
Fig. 63. Proximal portion of the glandular ring with g_2 and g_3 gland necks and uniciliary receptors. Scale bar: 1 µm;
Fig. 64. Section of the posterior part of the nucleo-glandular girdle with g_4 and g_5 gland necks and nuclei of S_2 and *B*. Scale bar: 2 µm.
Fig. 65. Quarter of the bulb with posterior cytoplasmic parts of the nucleo-glandular girdle, split ends of the longitudinal muscle (*arrows*) and inner circular muscles. Scale bar: 1 µm.

(Fig. 57). The posterior set of proboscis retractors inserts on the postero-lateral sides of the bulb.

Inner proboscis musculature. Within the bulbar septum, which is formed by a thin layer of ECM, longitudinal muscles are surrounded by a single layer of circular muscles. The circular muscles are present from the nodus upto the posterior end of the nucleo-glandular girdle in *Cicerina* and *Paracicerina*. In *Ptyalorhynchus* this layer splits here in two layers; an inner layer encloses the longitudinal muscles and type g4 and g5 gland necks upto 30 µm of the junction (half the length of the bulb), an outer layer surrounds the nucleiferous cell parts of the proximal belt of the sheath epithelium and the cone epithelium upto the junction (Fig. 60). The anterior most fibre is twice as thick as the subsequent and isolated from these muscle fibres. Both layers are covered with a thin layer of ECM around the outside. Circular muscles are absent in the cone. In *Cicerina* circular muscles enclose the glandular ampullae, these muscle fibres run against the inner longitudinal muscles at the inside (Fig. 69). The upper fibres are attached to the outer circular muscles. At 5 µm below the junction in *Ptyalorhynchus*, a single circular muscle fibre surrounds the central cylinder (Fig. 58). This muscle fibre is connected to the outer layer of circular muscles by radiating extensions. Type g_4 and g_5 gland necks are strongly constricted at this level. In *Paracicerina* such a single circular muscle fibre is present below the junction with eight radiating extensions towards the outer circular muscles (Fig. 61). These muscle fibres with radiating extensions in *Paracicerina* and *Ptyalorhynchus* and the muscles of the glandular ampullae in *Cicerina*, which have connections to the outer circular muscle layer as well, probably have the same origin. The inner longitudinal muscles can be divided in a central cylinder (*cilm*) and a peripheral layer of longitudinal muscles (*pilm*) (Figs 59, 64, 68). The longitudinal muscles adhere on the bulbar septum between the inner circular muscles and insert on the basement membrane below the cone epithelium. The muscles are connected to the epithelium by desmosmones (Figs 71, 72). The former reach up into the cone, the latter do not reach the junction. The number of muscle fibres in the central cylinder varies for the three genera from 70 in *Ptyalorhynchus* over 75 in *Paracicerina* to 140 in *Cicerina*. The peripheral longitudinal muscles only reach up to the upper-most circular muscle fibre. In *Ptyalorhynchus* the peripheral muscles insert on the inside of the septum behind the nucleiferous cell parts of the epithelia (Fig. 45). The muscles run anteriorly at the inside of type g4 and g5 gland necks and curl outwards below the upper-most circular muscle fibre (Fig.59). They run halfway down the bulb till the full inner circular muscle layer appears . In *Paracicerina* and *Cicerina* only a thin layer of peripheral inner longitudinal muscles is present somewhat below the junction at the inner side of the glandular ampullae (Figs 64, 68). Nuclei of muscles within the septum have not been found. The muscle fibres contain two types of filaments, some



Figs 66-68. Cicerina remanei. Cross sections, just above (66), at (67) and just beneath (68) the junction. The cells of the proximal belt of the sheath epithelium (S_2) narrow towards the nucleiferous parts, leaving space for the interlaying cell strands of the basal (*B*) and the apical (*A*) cone epithelium. *Inset*: intercellular attachment of cell in S_2. Scale bar: 1 μm.

mitochondria and a sarcoplasmic reticulum. In *Ptyalorhynchus* a 10 µm wide area at the nodus is devoided of circular muscles. The gland necks which surface in the cone epithelium enter the bulb through a network of radiating muscle fibres.

In *Paracicerina* eight longitudinal muscle fibres are present in the nucleo-glandular girdle as well (Figs 61, 64). The muscles are found from the proximal end of the girdle, where the fibres are bifurcated and caught between the distalmost inserting inner cirucular muscles (Fig. 65) Apically the fibres are situated peripherally in the nucleo-glandular girdle, laterally of the four quadrants (Fig. 61). They adhere directly on the basal cone epithelium just below the junction. At this level the apicalmost fibre of the inner circular muscles radiates to the outer circular muscles (Fig. 61).

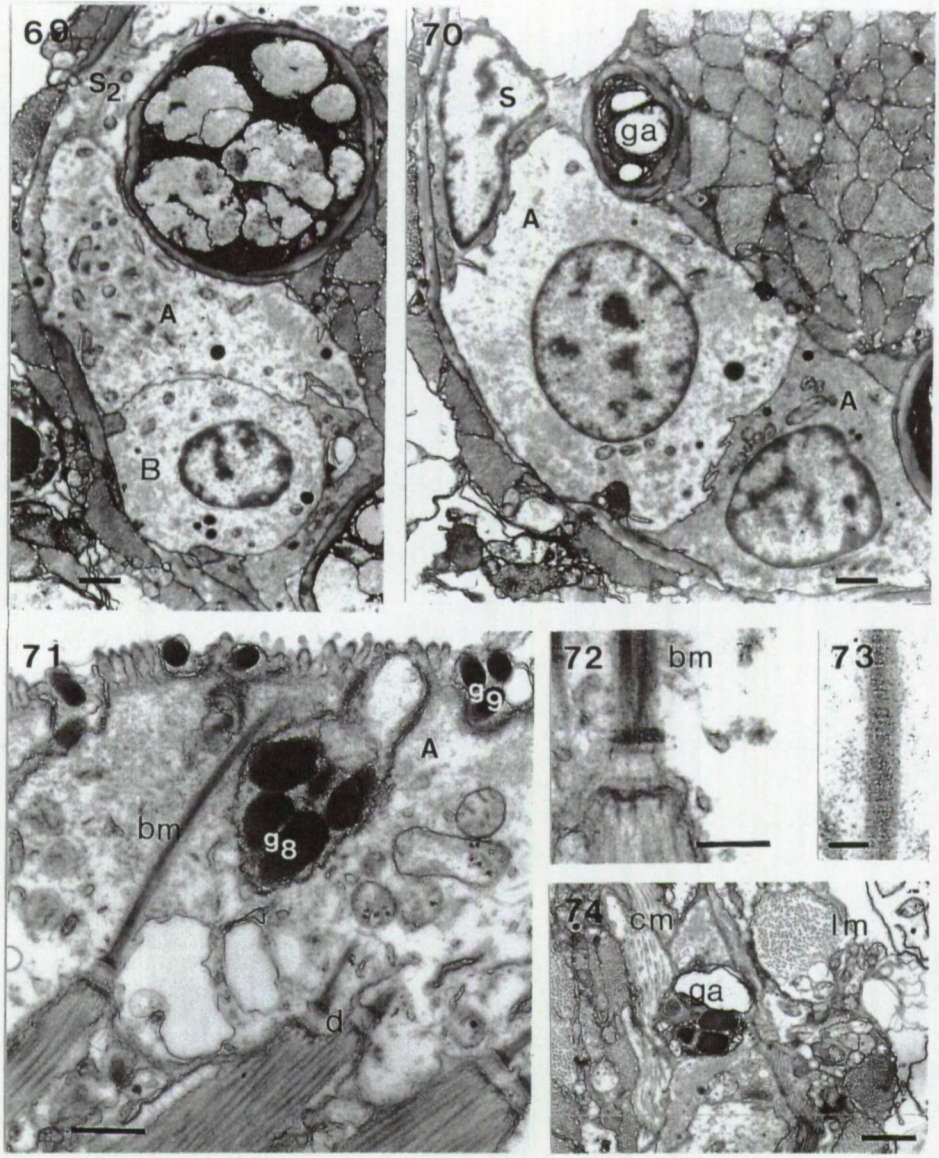
Discussion

In contrary to the findings of Brunet (1973) and Karling (1964) specimens of *T. calceformis* and *N. herdlaensis* were collected from algae and not from sediments and appeared to be fairly abundant in our samples. Samples of the previously mentioned authors were taken with a sledge probably mixing some algae with the surface sediment in the sample. This might explain the low number of individuals found in their samples as well. The first described species of this group, *Toia ycia* was collected from algae in the litoral zone by Marcus (1952) and appeared to be very numerous as well. The genera *Toia*, *Nannorhynchides* and *Pocillorhynchus* can be regarded as epiphytic as was indicated for *T. ycia* by Marcus (1952) and *Pocillorhynchus spiroductus* by Schockaert (1982).

A great difference in organization of the epithelia, sensory cells, glands and proboscis muscles is found within family Cicerinidae Meixner, 1928. In the descriptions the most important differences have been pointed out by separating the different genera. *Toia* and *Nannorhynchides* as well as *Cicerina* and *Paracicerina* have many features in common. The major differences will be discussed here and main characteristics for some genera will be pointed out as well.

The epithelium of the proboscis in all species of Cicerinidae Meixner, 1928 is divided in four belts as described for *Cicerina remanei*, *Psammorhynchus tubulipenis* and *Cytocystis clitellatus* (De Vocht & Schockaert 1988, De Vocht 1990). In *Polycystis naegelii* and Cystiplanidae, five circumferential belts constitute the proboscis epithelium (Schockaert & Bedini 1977, De Vocht 1989). The cone epithelium in all investigated species is formed by two belts.

In *Toia* and *Nannorhynchides* two belts each cover the cavity wall and the proboscis cone. In the other cicerinid species either the proximal belt of the sheath epithelium or both the proximal belt of the sheath epithleium and the basal belt of the cone epithelium tend to become incorporated in so-called nucleo-glandular girdle beneath the junction. In *Zonorhynchus*, *Ethmorhynchus* and *Ptyalorhynchus* the distal belt of the sheath epithelium



Figs 69-74. Cicerina remanei.
Fig. 69. Cross section through the middle region of the nucleo-glandular girdle with cell strands of the proximal belt of the sheath epithelium (S_2) and the cone epithelium (*A* and *B*). Scale bar: 1 µm.
Fig. 70. Cross section through the posterior portion of the nucleo-glandular girdle. Aside of the glandular ampulla (*ga*) the nucleus of a cell of S_2 and nucleiferous cell parts of *A* are present. Scale bar: 1 µm.
Fig. 71. Cone retractors adhering to *A* by means of desmosomes and bundles of microfibrils. Note g_8 and g_9 gland necks. Scale bar: 0.5 µm.
Fig. 72. Desmosome with wide open intercellular space, the parallel condensations are clearly visible. Scale bar: 0.1 µm.

practically lines the entire cavity wall. Additionally in *Cicerina* and *Paracicerina* the cone epithelium is practically exclusively formed by the apical belt and the basal belt is found only at the junction and in the nucleo-glandular girdle. In *Zonorhynchus* the nucleo-glandular girdle is proximally not enclosed by ECM.

Only *Toia* and *Nannorhynchides* have intra-epithelial nuclei in both belts of the sheath epithelium. In *Cicerina*, *Paracicerina*, *Ptyalorhynchus* and *Zonorhynchus* the cells of the distal belt have intra-epithelial nuclei but in *Ethmorhynchus* the cells of the distal belt have insunk nucleiferous cell parts. In the latter group of genera the nuclei of the cells in the proximal belt are located beneath the junction in the nucleo-glandular girdle. The significance and composition of the intra-nuclear crystalline inclusions present in *Nannorhynchides* are unclear.

A fully cellular cone epithelium is encountered in *Toia*, *Nannorhynchides* and *Ethmorhynchus*, in all other cicerinid genera investigated the basal belt of the cone epithelium is syncytial. The organization of the nucleiferous parts of the cone epithelium is highly divers and common tendencies are hard to discern. In *Toia* and *Nannorhynchides* the nucleiferous cell parts of the cone epithelium are situated along the sides of the proboscis bulb, with only minor differences. Four cell strands in *Toia* pierce the inner circular muscle layer, while in *Nannorhynchides* four wide cell strands slip in between the inner circular and outer circular muscle layer distally to the former. In *Toia* the insunk epithelial cell parts are situated around the median and posterior part of the bulb, while in *Nannorhynchides* they are found around the anterior and median part of the bulb. These cell parts represent elements of the cone epithelium and not of the sheath epithelium as postulated by Brunet (1973). In *Zonorhynchus* the apical cone epithelium has intracellular nuclei, one in *Z. tvaerminensis* and several in *Z. salinus* (Karling, 1952). *Zonorhynchus* is the only genus within the Eukalyptorhynchia, which has nuclei in the epithelium parts on the top of the cone. The basal cone epithelium in *Zonorhynchus* has insunk nuclei. These insunk epithelial cell parts have already been noted by Karling (1952). A distal belt formed by numerous cells, without distinct organization, is typical for all species of *Zonorhynchus*. In all other species the nuclei of the apical cone epithelium are situated subjunctionally in the nucleo-glanudlar girdle or inside the bulb. The nucleiferous parts of the basal cone epithelium are located in the nucleo-glandular girdle.

There is no difference in length between the microvilli of both belts in the sheath epithelium of *Toia* and *Nannorhynchides*, but the microvilli of the cone epithelium are distinctly shorter than those of the sheath epithelium. Both in *Zonorhynchus* and *Ethmorhynchus* the microvilli of the distal belt of the sheath epithelium are much longer than those of the

Fig. 73. Cross-striated aspect of a bundle of microfibrils. Scale bar: 0.1 µm.
Fig. 74. Type g4 and g5 gland necks (*ga*) are situated between the circular muscles in the bulb (*cm*) and longitudinal muscles outside the bulb (*lm*). Scale bar: 1 µm.

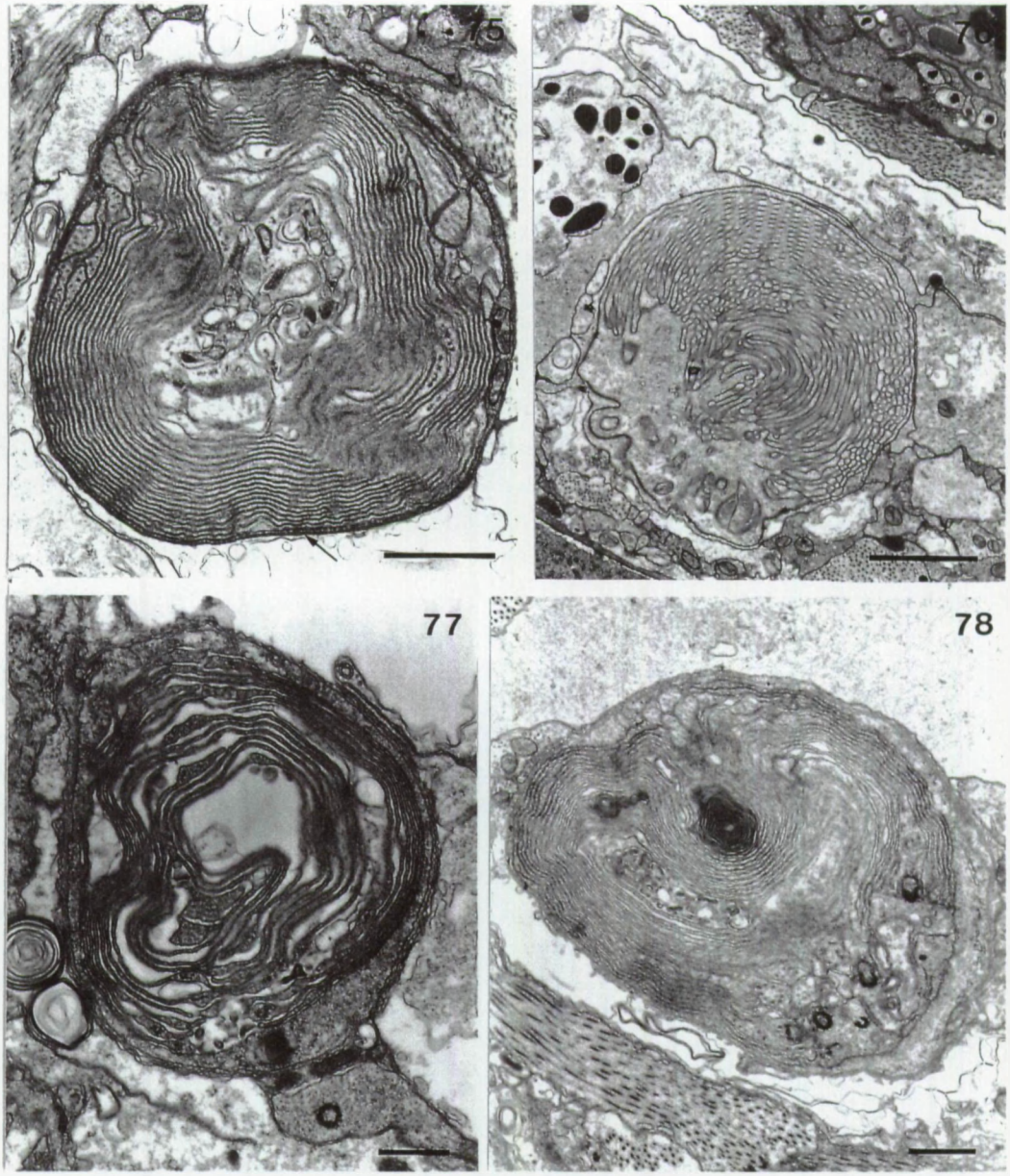


Fig. 75. Ptyalorhynchus coecus. Sensory organ formed by an insunk part of the distal belt of the sheath epithelium, surrounded by ECM (*arrow*) and multiciliary receptors with ciliary shafts that form wide flat membranous sheets. Scale bar: 1 µm.
Fig. 76. Ptyalorhynchus coecus. Rhabdomeric receptor situated posteriorly of the sensory organs. Scale bar: 1 µm.
Fig. 77. Cicerina brevicirrus. Sensory organ with *A* lining the lumen, which is filled with flat ciliary shafts. Scale bar: 0.5 µm.
Fig. 78. Paracicerina deltoides. Sensory organ with multiciliary receptors with flat ciliary shafts forming concentric rings. Scale bar: 1 µm.

proximal belt but about of the same length in both species. The microvilli of the cone epithelium in *Ethmorhynchus* are distinctly shorter than in *Zonorhynchus*. In *Cicerina* and *Paracicerina* the microvilli of the cone epithelium are slightly shorter than those of the sheath epithelium, while in *Ptyalorhynchus* the difference is almost negligible. Three tendencies can be discerned. One, if the proximal belt of the sheath epithelium is only found near the junction, the length of the microvilli seems to diminish in length, e.g. *Toia* and *Nannorhynchides* versus *Zonorhynchus* and *Ethmorhynchus*. Secondly, the length of the microvilli in the sheath epithelium namely the distal belt is about half the length in *Cicerina, Paracicerina* and *Ptyalorhynchus* as in *Toia, Nannorhynchides, Zonorhynchus* and *Ethmorhynchus*. Thirdly, the difference in length between the microvilli of the apical cone epithelium and the distal belt of the sheath epithelium (Chapter 11 table 4 *A/S1*) diminishes from *Ethmorhynchus* over *Nannorhynchides, Toia, Zonorhynchus* to *Cicerina, Paracicerina, Ptyalorhynchus.*

Stubby microvilli with dense tips or margins are typical for the basal cone epithelium and encountered in other eukalyptorhynch species as well. A basement membrane under the cone epithelium is present in all species. In *Ethmorhynchus*, however, small perforations in the basement membrane are present, which allow the passages to the nucleiferous cell parts. Although a clear terminal web is absent, microfibrillar reinforcements are visible in the cone epithelium, in all investigated species.

The sunken cell parts of the proboscis epithelia in Cicerinidae are fully enclosed by a layer of ECM. These cell parts are enclosed by the outer circular and longitudinal muscles of the sheath epithelium in all genera. The insunk cell parts in *Toia* and *Nannorhynchides* can not be regarded homologous to those in *Cicerina, Paracicerina, Ptyalorhynchus, Ethmorhynchus* and *Zonorhynchus* because they are solely formed by cell parts of the cone epithelium, while in the other genera mentioned both sheath and cone epithelial cell parts are present below the junction. Furthermore they do not form a circumferential girdle and are not pierced by type g4 and g5 gland necks. In the latter mentioned genera there is a tendency to integrate the nucleo-glandular girdle in the bulb from *Zonorhynchus* over *Ethmorhynchus* to *Paracicerina, Cicerina* and *Ptyalorhynchus*.

In *Toia, Nannorhynchides* and *Zonorhynchus* the circular muscles around the cavity wall enclose the anterior part of the bulb as well. In *Cicerina, Paracicerina, Ptyalorhynchus* and *Ethmorhynchus* outer ciruclar muscles are only present around the proximal belt of the sheath epithelium or in other words the nucleo-glandular girdle. In *Cicerina, Paracicerina* and *Ethmorhynchus* the subjunctional epithelial cell parts become more incorporated in the bulb. In *Ptyalorhynchus* these cell parts have become fully incorporated in the bulb and the ECM layer is found peripherally of the cirucular muscles. In this respect type g4 and g5 gland necks enter the proboscis at the nodus.

Clear light microscopic differences in the organization of the proboscis and the genital structures enables the separatation of the three genera, *Toia*, *Nannorhynchides* and *Pocillorhynchus*. According to Brunet (1973), *Toia* has twenty ot twentysix ampullae or wide gland necks, which surface in the basal part of the proboscis cone. In *T. ycia*, Marcus (1951) mentiond about twenty ampullae and in the toto preparation of the species from the Swedish Museum of Natural History, twentysix ampullae are present. For *T. calceformis* about twenty ampullae were mentioned as well (Brunet 1973). In our specimen, which was sectioned transversally, exactly eighteen ampullae were present. Apparently the two species of *Toia* can be idenditified by the number of glandular ampullae present in the proboscis base, where *T. calceformis* counts eighteen and *T. ycia* twentysix ampullae. From light microscopic observations ten to twelve glands are present in the proximal part of the epithelium lining the sheath of *Nannorhynchides vividus*. These glands are present as a ring of numerous small gland nekcs in *Nannorhynchides harparius*. Eight groups of two types of secretion, transversing the proboscis bulb and surfacing through the basal part of the cone, are present in *Nannorhynchides corneus* as well (Brunet 1973). In the voluminous proboscis of *Pocillorhynchus agilis*, *Pocillorhynchus minutus* and *Pocillorhynchus spiroductus* no glandular 'ampullae', in the sence of Brunet (1973) have been found. The type material of these species, however, contains no ideal sections of the proboscis to verify this.

The gland necks penetrating the proboscis epithelia in *T. calceformis* and *N. herdlaensis* show identical features. A delimited circumferential ring of gland necks above the junction is present in all species. In *Zonorhynchus* and *Ethmorhynchus* this glandular ring is less obvious but in the other genera a very limited glandular ring is present. The glanudalr ring can be formed by one, two or three gland types.

The basal cone epithelium shows two distinct types of gland necks; type g_5 gland necks in *T. calceformis* and *N. herdlaensis* contain empty granules while type g_3 in *T. calceformis* and type g_6 in *N. herdlaensis* contain an electron dense or flocculent secretion. Whether these two types of gland necks are homologous with the gland necks in the glandular ampullae of *C. remanei* or not, is not clear. The basal cone epithelium is pierced by two similar types of gland necks apically of the glandular ampullae as well (De Vocht & Schockaert 1988). These four types of gland necks all run through the bulb and enter it at the nodus. In *P. tubulipenis* and *C. clitellatus* homologous gland necks are found in the basal cone epithelium, types g_6 and g_9 contain empty secretion granules while types g_7 and g_{10} gland necks are filled with a electron dense secretion (De Vocht 1990). In *C. clitellatus* type g_9 glands form wide necks in the bulb as well but they do not form distinct groups. In Polycystididae and Cystiplanidae the basal cone epithelium is pierced by gland necks filled with electron dense granules; type g_7 in *P. naegelii* , sg_2 in *Gyratrix hermaphroditus* and type g_5 in Cystiplanidae (De Vocht 1989, Reuter 1975 and Schockaert & Bedini 1977).

Whether type g8 gland necks in *P. naegelii* and *G. hermaphroditus* and type g6 gland necks in Cystiplanidae are glandular elements is uncertain. They could also represent sensory elements, the electron dense core noted by Schockaert and Bedini (1977) in figure 6 of Reuter (1975) are probably cross sectioned ciliary rootlets. The secretion of gland necks piercing the apical cone epithelium is often present in the form of small electron dense granules. Two associated types of glands are described in the duo-gland adhesive system in many Turbellaria (Tyler 1976). The secretions show morphological differences with the glandular secretion in the basal cone epithelium. The stubby microvilli with dense tips of the basal cone epithelium could have the same function as the enforced microvilli of the anchor cells in the duo-gland adhesive system. The apical cone epithelium in *T. calceformis* and *N. herdlaensis* is pierced by one type of gland necks which contain a electron dense secretion packed in small granules (230-280 nm). This kind of secretion with relative small granules is found in *P. tubulipenis* (type g8) and *C. clitellatus* (type g11) as well (De Vocht 1990). In *C. remanei* and Cystiplanidae (De Vocht & Schockaert 1988, De Vocht 1989) two different types of secretions appear in this epithelium; small rod-like granules as in *P. naegelii* (Schockaert & Bedini 1977) and larger spherical granules. Gland necks with the small secretion granules are typical for the apical cone epithelium, a comparable secretion to the second type is found in the basal cone epithelium as well.

From light microscopic observations *T. ycia* and *N. herdlaensis*, were reported to have a ciliated sheath epithelium (Karling 1964, Marcus 1952). The epithelium is not ciliated but pierced by numerous uniciliary receptors. Uniciliary receptors spread throughout the sheath epithelium are present in *Zonorhynchus*-species and *Ethmorhynchus*. In *Cicerina*, *Paracicerina* and *Ptyalorhynchus* only very few receptors of this type are present. The rootlets are oriented perpendicular to the epithelium, the cilieary shafts point towards the proboscis pore. Secondary rootlets are present at the posterior part of the basal bodies. These receptors are homologous to uniciliary receptors found in the sheath epithelium of Cystiplanidae and Polycystididae (De Vocht 1989, Reuter 1975, Schockaert & Bedini 1977). In *Cicerina* and *Paracicerina* uni- and biciliary receptors form twelve groups or in *Ptyalorhynchus* a annulus in the proximal belt of the sheath epithelium just above the junction as reported for *Cicerina remanei* (De Vocht & Schockaert 1988). In *Cicerina* they appear to be rigid structures pointing in to the proboscis cavity. The uniciliary receptors present just above the junction in *Ethmorhynchus* resemble the first mentioned type and can not be regarded identical to the rigid receptors at the junction in *Cicerina*, *Paracicerina* and *Ptyalorhynchus*. Uniciliary receptors with blunt ciliary shafts, basal bodies and rootlets are present in the cone epithelium of all species investigated. They are identical to those found in *G. hermaphroditus* (type IV), *C. remanei*, *P. tubulipenis*, *C. clitellatus*, *P. naegelii* and Cystiplanidae (De Vocht 1989 &1990, De Vocht & Schockaert 1988, Reuter 1975, Schockaert & Bedini 1977).

The species investigated can be divided in two groups according to the presence or absence of multiciliary receptors in sensory organs associated with the distal belt of the sheath epithelium. Multiciliary receptors are found in *Ethmorhynchus*, *Ptyalorhynchus*, *Paracicerina* and *Cicerina*. They lack in *Toia*, *nannorhynchides* and *Zonorhynchus*. The receptors are situated in insunk cell parts of the epithelium. The multiciliary dendrites pierce the epithelium and the cilia merge in a closed lumen. The insunk cell parts in *Ethmorhynchus* have a elongated shape and the receptors short ciliary shafts as in *Cytocystis* (De Vocht 1990). In *Cicerina*, *Paracicerina* and *Ptyalorhynchus* two spherical sensory organs with flat sheet-like ciliary processes which are described for *Psammorhynchus* as well (De Vocht 1990). The multiciliary receptors have general characteristics as the presence of only single microtubules in the cilia, the presence of basal bodies and the absence of rootlets and are considered homologous to the intra-epithelial fingerlike multiciliary receptors in Cystiplanidae (De Vocht 1989).

A sarcoplasmatic reticulum as present in Cystiplanidae (De Vocht 1989 figs. 2 and 8), Gnathorhynchidae (Doe 1976 fig. 6, A and B), *P.tubulipenis* and *C. clitellatus* (De Vocht 1990 figs. 5, 6, 7 and 11) and *C. remanei* (De Vocht & Schockaert 1988 fig. 11) is absent *T. calceformis* and *N. herdlaensis*.

As in *P. naegelii* and Cystiplanidae (De Vocht 1989, Schockaert & Bedini 1977) both a circular and longitudinal muscle layer are present around the sheath epithelium in *T. calceformis*, *N. herdlaensis* and *Zonorhynchus*. The muscles continue around the distal part of the bulb. Circular muscles are not found around the epithelium lining the proboscis cavity in *Cicerina*, *Paracicerina*, *Ptyalorhynchus* and *Ethmorhynchus* but the nucleo-glandular girdle and consequently the proximal belt of the sheath epithelium, is surrounded by a layer of circular muscles. In *P. tubulipenis* and *C. clitellatus* circular muscles are only present around the proximal part of the sheath epithelium as well (De Vocht 1990). Longitudinal muscles in these species are found in all cicerinid species investigated from the pore or the median part of the sheath epithelium on uo to the lateral sides of the bulb. The variable number of longitudinal muscles around the sheath of *N. herdlaensis* probably originates from the fact that one muscle cell can form several fibres which can interdigitate. In this way the number of fibres is not found to be exactly the same in specific or different specimens. Both the circular and longitudinal muscles around the proboscis cavity are continuous with the body wall musculature.

Like in most Eukalyptorhynchia the motional muscles of the proboscis in *Toia*, *Nannorhynchides* and *Ethmorhynchus* include protractors, fixators, proboscis retractors and integument retractors. In *Ethmorhynchus* the fixators are reduced and only formed by a single muscle fibre. In *Zonorhynchus*,*Cicerina*, *Paracierina* and *Ptyalorhynchus* fixators are lacking. In *Ethmorhynchus* the fixator muscles are highly reduced and two sets of proboscis retractors or one set adhering on the full length of the nucleo-glandular girdle. Marcus

(1952) mentions the presence of protractors in *T. ycia*, but in his figures 68 and 69 the longitudinal muscles surrounding the sheath are marked as fixators. A double set of retractors is present in *Ethmorhynchus, Cicerina, Paracicierina* and *Ptyalorhynchus*. In *Ethmorhynchus* the distinction between the two sets is less pronounced.

All species of *Nannorhynchides* are characterized by a pronounced layer of circular muscles in the anterior part of the bulb. This layer decreases gradually in thickness towards the nodus. In *Zonorhynchus tvaerminensis* wide disk-shape fibres are present anteriorly in the inner circular muscle layer as well (Karling 1952). The organization of the inner longitudinal muscles in a central cylinder and peripheral layer is typical for *Toia* and *Nannorhynchides*. The appearance of the peripheral layer is somewhat different in both species. Such a clear distinction between groups of internal longitudinal muscles is encountered in *Zonorhynchus* as well. In light microscopic preparations, which often show contracted proboscis muscles, In *Ethmorhynchus, Cicerina, Paracicierna and Ptyalorhynchus* the peripheral longitudinal muscles are less pronounced and formed by a single layer of muscle fibres reaching up to the junction. In previously investigated species no distinction could be made in de inner longitudinal musculature.

In general the proboscises in *Toia, Nannorhynchides* and probably also *Pocillorhynchus* are characterized by fully cellular epithelia. The bipartite sheath epithelium with intra-epithelial nuclei is pierced by numerous uniciliary receptors and surrounded by both a circular and longitudinal muscle layer. The basal cone epithelium is composed of five cells, the nuclei of the cone epithelium are situated in four packages along the sides of the bulb. The internal musculature of the bulb is composed of circular and longitudinal muscles. The longitudinal muscle layer is constituted of a central cylinder and a peripheral layer. The inner circular muscle layer in *N. herdlaensis* is very pronounced in the anterior part of the bulb. In some aspects the genus*Zonorhynchus* approches *Toia* and *Nannorhynchides*, such as the absence of multiciliary receptors and the muscular organization of the bulb. The distal belt of the sheath epithelium is characterized by a random, multicellular organization. *Ethmorhynchus* differs strongly from the other genera by the presence of a cellular basal cone epithelium, insunk nuclei of the distal sheath epithelial belt, intrabulbar nuclei of the apical cone epithelium. Two insunk sensory organs are present as in *Cicerina, Paracicierina* and *Ptyalorhynchus* but they resemble more those in *C. clitellatus*. The presence of the a nucleoglandular girdle is a common feature with the three formerly mentioned genera. *Cicerina, Paracicerina* and *Ptylaorhynchus* are characterized by two spherical insunk sensory organs and receptors with rigid ciliary shafts in the sheath epithelium and two distinct sets of proboscis retractors. *Cicerina* and *Paracicerina* by the presence of four glandular ampullae, which are seperately enclosed by circular muscles in *Cicerina*.

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Chapter 4.

The ultrastructure of the proboscis in *Psammorhynchus tubulipenis* Meixner, 1938 and *Cytocystis clitellatus* Karling, 1953.[1]

Introduction

Psammorhynchus tubulipenis Meixner, 1938 has been studied extensively by Meixner (1938) and in particular by Karling (1956 and 1964) on the light microscopic level. An important feature to establish a new family for *P. tubulipenis* was the structure of the proboscis (Karling 1964). It somewhat resembles the proboscis of some genera in the family Cicerinidae by the presence of a well developed epithelium at the junction with associated glands, and the organization of the proboscis musculature with two girdles of short retractors. *Cytocystis clitellatus* Karling, 1953 is characterized by a small proboscis, which is enclosed in a sheath of four cells; behind the proboscis the epidermis forms a vacuolated girdle ('clitellum') (Karling 1953, 1964). The species has been found in muddy sediments at the Swedish West coast and is also recorded from the Mediterranian from muddy sand sediment at depth ranging from 27 up to 70 metres (Karling 1953, Brunet 1979). At first *C. clitellatus* was considered to be related to the family Zonorhynchidae Karling, 1952. The species was considered to represent a different evolutionary lineage in the family with many primitive features.

Materials and methods

Specimens of *P. tubulipenis* were collected from a sandy beach at the island of Sylt (Germany) in September 1985. Specimens of *C. clitellatus* were obtained from the Gullmarfjord at the Swedish west coast in June 1988. Samples were taken near Skulevik, Färlevfjorden at 10 meters depth with the Ockelmann-sledge. Altogether nine specimens of *P. tubulipenis* and six specimens of *C. clitellatus* have been investigated. Types of the Swedish Museum of Natural History, Section Invertebrate Zoology (S.M.N.H.I.) were studied as well.

Results

The organization of the proboscis in *P. tubulipenis* and *C. clitellatus* - and especially of its epithelia - is very much alike. The major differences in organization are related to the

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[1] The contents of this chapter has been published in Acta Zoologica (Stockholm) 71: 113-124, 1990.

musculature and the glands. The total length of the proboscis in *P. tubulipenis* is 70 µm and 100 µm in *C. clitellatus* .

Epithelia, gland necks and sensory cells

Epithelia. The proboscis epithelia in *P. tubulipenis* and *C. clitellatus* are organized in four circumferential belts (Figs 1, 2), which are divided in a sheath (S_1, S_2), and cone epithelium (*A*, *B*). In *P. tubulipenis* the sheath epithelium is composed of two cellular belts (Figs 3, 5). In *C. clitellatus* a single circumferential cell or syncytium distally and a cellular belt proximally constitute the sheath epithelium (Figs 4, 6). In the distal belt of the epithelium nuclei have never been observed. The basal part of the cone is covered by a syncytial belt and the apex by a cap formed by one or two cells. All belts are devoid of cilia. The belts are interconnected by zonulae adhaerentes, septate junctions and maculae adhaerentes (Figs 5, 7, 10).

In *P. tubulipenis* the total length of the sheath epithelium is about 30 µm, half of which is taken up by the distal belt and half by the proximal belt (Fig. 1). The basement membrane under the epithelium, lining the proboscis cavity is about 250 nm thick. It has the typical bipartite structure; adjacent to the epithelium a thin dense limiting layer is present, which becomes less pronounced towards the proximal belt of the epithelium, and an underlying thick microfibrillar layer. The cytoplasm contains mitochondria and small electron dense granules, which are less numerous in the distal belt (Fig. 5). The basal plasma membrane shows numerous infoldings into the cytoplasm (Figs 3, 5). The distal belt consists of 2 cells, 1.2 µm high. The apical plasma membrane bears microvilli, 400 nm long and 9 per linear µm. Cell organelles are found only in the cytoplasm among the infoldings. The apical layer of the cytoplasm above the infoldings is devoid of organelles and forms an electron dense terminal web, 250 nm thick (Fig. 17). Often a remarkable difference in electron density of the cytoplasm of the two cells could be seen. In none of the specimens investigated, nuclei of these cells could be discerned, either above the basement membrane or insunk. The proximal part of the distal belt is covered by the proximal belt. This belt has a less electron dense cytoplasm. The microvilli are comparable to those in the previous belt (8 per linear µm, 450-500 nm long). No clear terminal web can be discerned. In most specimens, including a paratype of the S.M.N.H., this belt is composed of 6 cells. However, one specimen with 7 and even one with 8 cells in this belt were observed, indicating that their number is not strictly fixed. The nucleiferous cell parts are situated peripherally in the bulb, among the retractors of the cone (Figs 9, 13, 15). The cell strands in the bulb contain only few cell organelles in their distal parts, but more proximally they contain a nucleus, mitochondria, rough endoplasmic reticulum and Golgi complexes (Fig. 9).

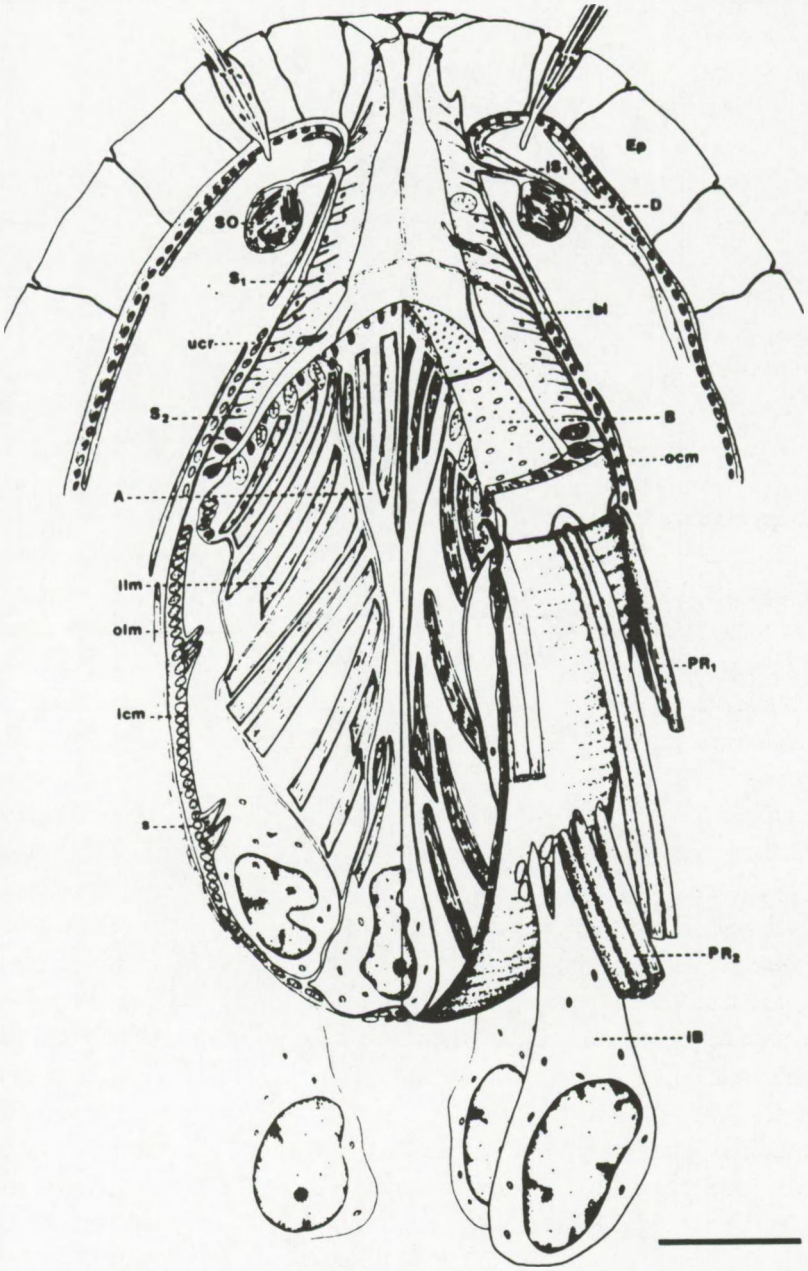


Fig. 1. Reconstruction of the proboscis in *Psammorhynchus tubulipenis* from electron micrographs. The left front quarter has been left out as well as the right part of the sheath epithelium above the junction. Scale bar: 10 μm.

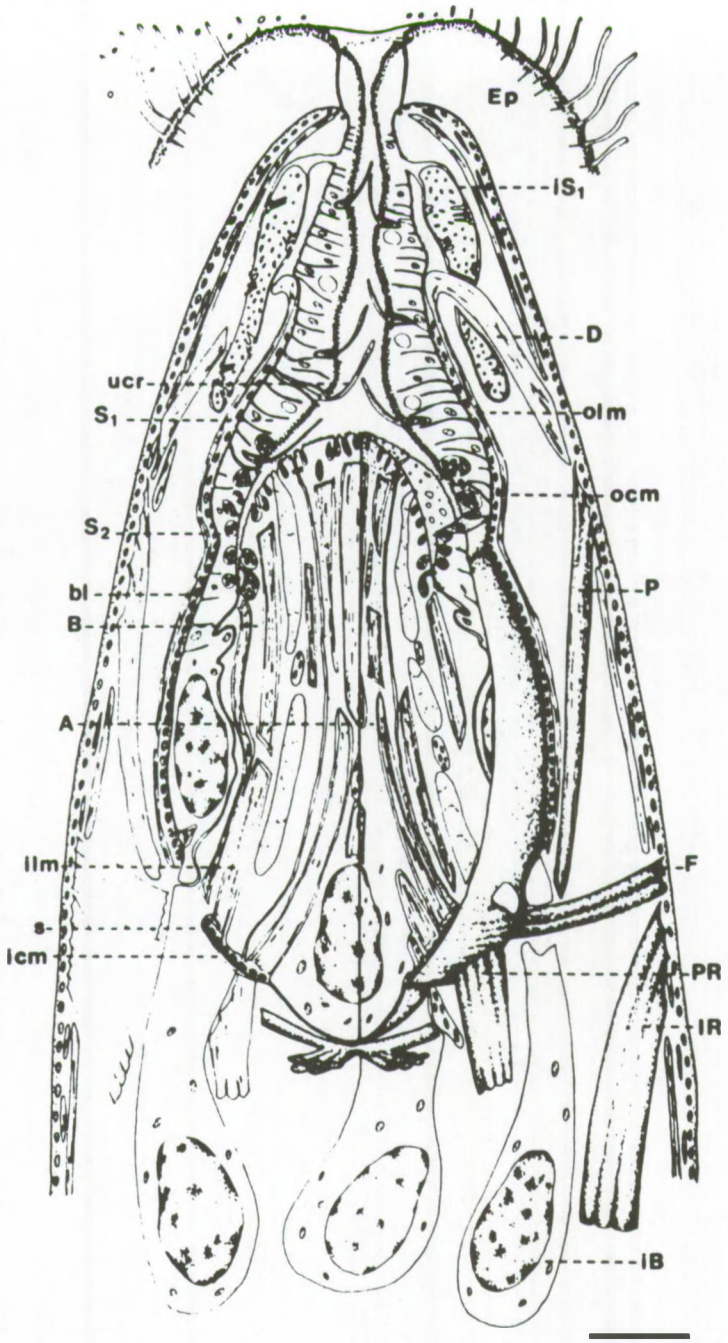


Fig. 2. Reconstruction of the proboscis of *Cytocystis clitellatus* from electron micrographs. Parts that have been left out are the same as in Fig. 1. Scale bar: 10 µm.

In *C. clitellatus* the sheath epithelium is about 60 µm long. The circumferential cell forming the distal part of the sheath epithelium is 2 - 6 µm high and 50 µm long. It is characterized by infoldings of the basal plasmalemma, which reach up to the 200 nm thick peripheral terminal web (Figs 2, 4). Fairly large mitochondria (up to 1 µm long) with dense matrices are located among the infoldings of the basal plasma membrane (Fig. 4). The slender and densely packed microvilli measure 1200 nm in length (7 per linear µm). The underlying basement membrane varies in thickness between 700 nm at the pore to 220 nm near the junction and is a continuation of the proximal layer of the epidermal basement membrane (Fig. 4). In none of the specimens a nucleus was observed. In all investigated specimens the proximal belt of the sheath epithelium is composed of four cells. The part of these cells lining the cavity is 2-3.5 µm high and about 10 µm long. Infoldings of the basal plasma membrane are present but less numerous and usually reach up only to the middle of the cell height (Fig. 2). The stubby microvilli are much shorter (320 nm) than in the distal belt. A weakly developed terminal web is present (Fig. 6). Mitochondria and electron dense condensations are found in the superficial part. The four cross-like arranged nucleiferous cell parts are situated peripherally in the bulb (Figs 11, 14). The basal plasma membrane shows numerous infoldings and apart of the nucleus, mitochondria are found in the cytoplasm. The nucleiferous cell parts belonging to this belt are not found further down as the insertion of the fixators.

A bipartite organization of the cone epithelium is found in *P. tubulipenis* and *C. clitellatus*. The belt covering the basal part of the cone is syncytial and of the insunk type. The superficial part of the cone epithelium is 1-1.4 µm thick in *P. tubulipenis* and 3.5-4 µm near the apex to 1.5-2 µm high at the basis in *C. clitellatus*. The microvilli of the basal belt are stout with dense tips and densely packed (300 nm, 10-12 per linear µm) (Figs 5, 6). The terminal web is weakly developed. There is no basement membrane under the cone epithelium and circular muscles surrounding the cone retractors are lacking. As in the sheath epithelium, mitochondria and small electron dense granules are present in the cytoplasm (Figs 5, 6). In *P. tubulipenis* the apex is covered by one or two cells, in *C. clitellatus* the apex is always covered by a single cell. The nucleus (nuclei) of the apical cone epithelium is located in the middle of the bulb behind the nuclei of the proximal belt of the sheath epithelium (Figs 1, 2). In *C. clitellatus*, only the bulbar septum is present below this cell part containing the nucleus; it forms a terminal sag at the proximal end of the bulb (Fig. 12). Apart from the nucleus also mitochondria, Golgi complexes and numerous electron dense inclusions are present in the insunk cell part (especially in *C. clitellatus*). The cytoplasmic cell strands of the basal cone epithelium pierce the septum in *P. tubulipenis* and form four insunk nucleiferous cell parts outside the bulb with one nucleus each (Figs 1, 15). The perforations are situated in between the insertions of the proximal set of proboscis retractors. In *C. clitellatus* the cell strands of the basal belt of



Fig. 3. Psammorhynchus tubulipenis. Cross section of the distal belt of the sheath epithelium (S_1), showing one insunk cell part (iS_1). Note the cell junctions (*arrow*), the infoldings of the basal plasma membrane (*ibp*) and the bipartite basement membrane (*bl*). The outer longitudinal muscles of the proboscis sheath (*olm*) are confluent with the dilators of the pore (*D*) and body wall musculature. Scale bar: 2 µm.

Fig. 4. Cytocystis clitellatus. Cross section of the distal belt of the sheath epithelium (S_1). There are no cell junctions but the basal plasma membrane shows numerous infoldings (*ibp*) which reach up from the basement membrane (*bl*) to the well developed terminal web (*tw*). Uniciliary receptors (*ucr*) pierce the epithelium. Scale bar: 2 µm.

the cone epithelium run between the retractors of the cone in the bulb. They leave the bulb at the transition zone of basement membrane of the sheath epithelium and septum of the bulb and form five insunk cell parts containing as many nuclei (Figs 2, 16).

Glands. Only few gland necks can be found in the distal belt of the sheath epithelium in *P. tubulipenis*. The same gland necks, however, are present in the proximal belt as well and most numerous in its posterior part (Fig. 5). They contain a moderately electron dense mucous secretion (g_1). Gland necks just above the junction contain electron dense secretion granules (g_2). Some of these gland necks, situated below the junction and in the bulb, contain a secretion with concentric lamellate granules (Fig. 7). They may be regarded as immature stages of the same secretion. Two types of gland necks pierce the distal belt of the sheath epithelium in *C. clitellatus* . The first type with wide open necks (up to 2 μm) contains moderately electron dense secretion granules (g_1) (Fig. 22), the second possesses electron dense secretion granules with a light centre (g_3). These gland necks are concentrated in the proximal part of the belt at the level of the apex. The proximal belt of the epithelium is pierced by two types of gland necks, one filled with moderately electron dense secretion granules (g_2), the other with electron dense secretion granules with light centre (g_3) (Figs 6, 10).

In *P. tubulipenis* two types of gland necks pierce the basal belt of the cone epithelium. The first type contains empty spherical vesicles or sometimes some electron dense secretion granules (g_6) (Fig. 7). Only the apical part of the gland necks is filled with secretion and the secretion packages are up to 3.5 μm long. The second type contains an electron dense secretion packed in small granules (g_7) (Figs 5, 7). Two types of gland necks surface in the apical cone epithelium, one kind of gland necks, however, appearing less numerous, is identical to the first mentioned type in the basal cone epithelium (g_6).

Fig. 5. Psammorhynchus tubulipenis. Cross section of the proximal belt of the sheath epithelium (S_2) and basal cone epithelium (*B*). Note the cell junctions (*arrow*) in S_2. The basal plasma membrane forms infoldings, gland necks (g_1) and uniciliary receptors (*ucr*) pierce the epithelium. The basal cone epithelium (*B*) bears microvilli with dense tips (*mv*). Gland necks (g_7) and uniciliary receptors are present as well. Inner longitudinal muscles (*ilm*) are connected directly to the basal plasma membrane, a basement membrane is absent. Cell strands of the apical cone epithelium (*A*) run among these muscles. Circular and longitudinal muscles surround the sheath epithelium. Scale bar: 2 μm.

Fig. 6. Cytocystis clitellatus. Slightly oblique cross section of sheath and cone epithelium. The distal belt of the sheath epithelium (S_1) with terminal web has numerous infoldings of the basal plasma membrane. Distinct cell junctions (arrows) and gland necks (g_2) are present in the proximal belt of the sheath epithelium (S_2). The sheath epithelium is surrounded by circular and longitudinal muscles (*ocm* and *olm*). A basement membrane is not present under the cone epithelium. Gland necks with small secretion granules and microtubular sheath (g_9) pierce the apical cone epithelium (*A*). The basal cone epithelium (*B*) bears microvilli with dense tips (*mv*). Scale bar: 2 μm.

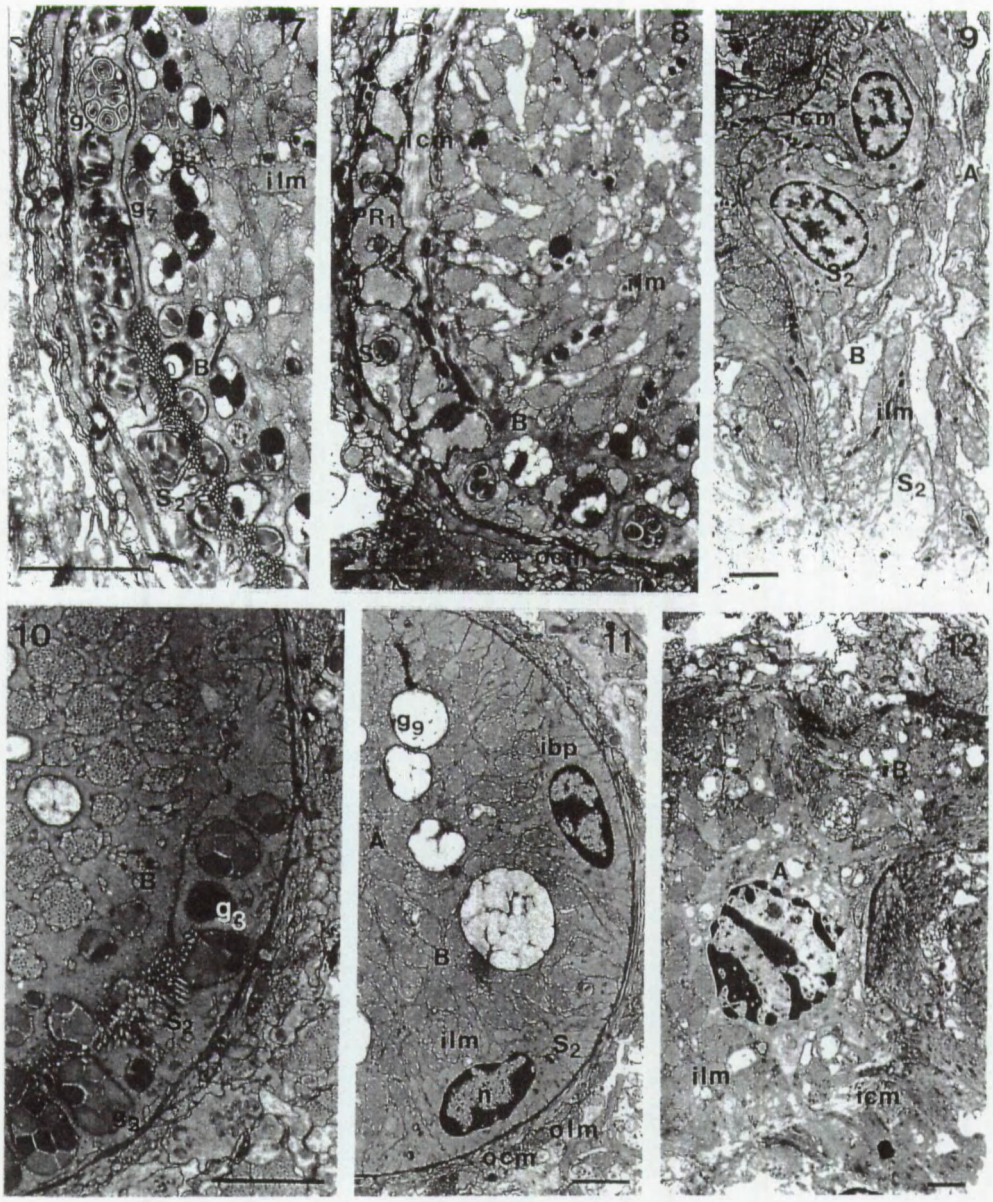


Fig. 7. Psammorhynchus tubulipenis. Slightly oblique cross section at the junction of sheath and cone epithelium, showing cell junctions (*arrow*) in the proximal belt of the sheath epithelium (S_2), the basal cone epithelium (*B*) and inner longitudinal muscles (*ilm*). A basement membrane, outer circular and longitudinal musculature are still present. Numerous gland necks penetrate the sheath epithelium above the junction (g_2). Two types of gland necks are found in the basal cone epithelium (g_6 and g_7). Scale bar: 2 µm.

Fig. 8. Psammorhynchus tubulipenis. Slightly oblique cross section of the anterior part of the bulb. Muscle fibres of the anterior set of retractors (PR_1) penetrate the proboscis and adhere to the basal cone epithelium (*B*) and the inner circular muscles (*icm*). Cell strands of the

The other kind, contains an electron dense secretion (250-350 nm) and is surrounded by a dense sheath in the epithelium (g_9) (Fig. 20). The inner distal margin of the gland necks is surrounded by a ring of semi-circular microtubules (Fig. 20). These gland necks enter the bulb at the nodus and run among the cone retractors (inner longitudinal muscles) to the apical cone epithelium.

In *C. clitellatus* three types of gland necks are found in the basal cone epithelium. The first and most conspicuous type forms long and wide open strands in the bulb (g_6) (Fig. 16). They run between the inner longitudinal muscles and are filled with secretion throughout the total length of the bulb. They enter the bulb at the proximal end of the belt formed by the inner circular muscles. Their secretion is washed out, sometimes leaving empty vesicles (1200 nm) in the necks (Fig. 11). A second type contains a secretion of electron dense, spherical granules, 400 nm in diameter (g_7) (Fig. 22). A third type has electron dense granules with a light centre (g_3), as present in the sheath epithelial belts. The apical cone epithelium contains one type of gland necks with small (200-250 nm) spherical moderately electron dense secretion granules (g_9) (Figs 6, 22). The terminal ends of the gland necks are consolidated by peripheral microtubules.

Sensory cells. In *P. tubulipenis* the basement membrane underlying the distal belt of the sheath epithelium leaves passage to two cell strands, one of each cell (Fig. 3). In the parenchyma, the insunk cell parts broaden and form lumina. The apical plasma membrane bears microvilli (Fig. 17). Numerous sensory cells pierce this part of the cells, their cilia

proximal belt of the sheath epithelium (S_2) are situated between the muscle fibres of the anterior set of retractors. Cell strands of the cone epithelium are found among the inner longitudinal muscles (*ilm*). Scale bar: 2 µm.

Fig. 9. Psammorhynchus tubulipenis. Cross section of the posterior part of the bulb with inner circular and longitudinal muscles (*icm* and *ilm*), nucleiferous cell parts of the proximal belt of the sheath epithelium (S_2), cell strands of basal (*B*) and apical (*A*) cone epithelium. Scale bar: 2 µm.

Fig. 10. Cytocystis clitellatus. Slightly oblique cross section at the junction. A basement membrane under the basal cone epithelium (*B*) is absent. Cell strands of the proximal belt of the sheath epithelium (S_2) are found at the periphery of the bulb. Outer circular and longitudinal muscles are present. Numerous gland necks are present at the junction (g_3 and g_2) Scale bar: 2 µm.

Fig. 11. Cytocystis clitellatus. Cross section of the posterior part of the bulb with two nuclei (*n*) of the proximal belt of the sheath epithelium (S_2). Note the infoldings of the basal plasma membrane (*ibp*) and the circular and longitudinal muscles (*ocm* and *olm*) surrounding the bulb. Cell strands of the apical (*A*) and basal (*B*) cone epithelium are situated between the inner longitudinal muscles (ilm). Gland necks (g_6) filled with empty secretion granules are present throughout the full length of the bulb. Scale bar: 2 µm.

Fig. 12. Slightly oblique cross section of the terminal end of the proboscis bulb with the nucleiferous part of the apical cone epithelium cell (*A*). Cell strands of the basal cone epithelium leave the bulb (*iB*). In this part of the bulb the inner circular muscles (*icm*) are found. Scale bar: 2 µm.

protruding into the spherical invaginations, formed by the epithelium cells. In each organ three groups of multiciliary receptors pierce through the lining epithelium. The cilia have highly modified axonemata and do not posses rootlets. The ciliary axonema is split in two sets of single microtubules, supporting two lateral stalks. In between those stalks, the ciliary membrane forms a thin sheet (Fig. 18). These ciliary sheets form numerous stacks of membranes, which indicates a possible photoreceptor function. The superficial cell parts

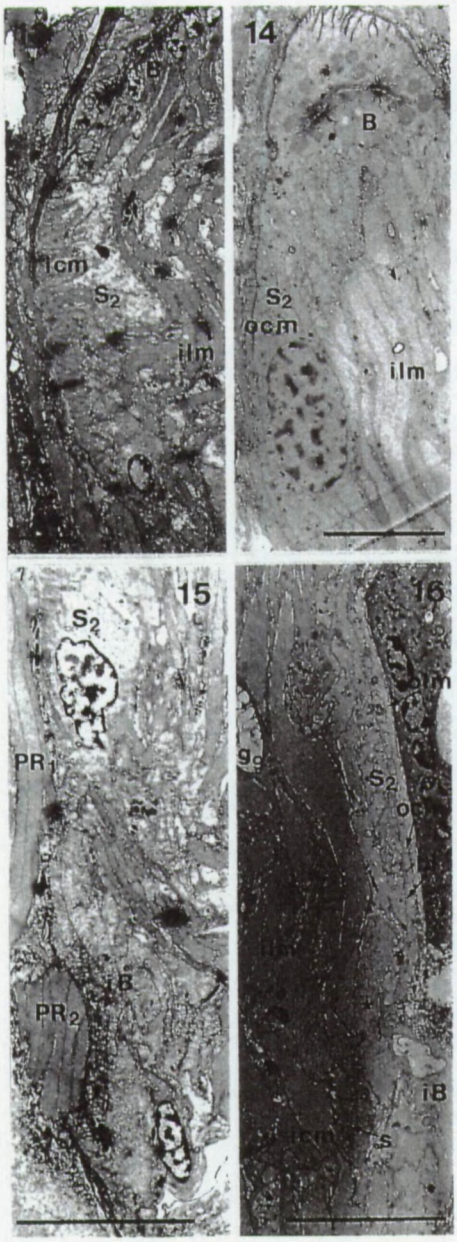


Fig. 13. Psammorhynchus tubulipenis. Sagittal section of the anterior part of the bulb. Cytoplasmic strands of cells of the proximal belt in the sheath epithelium(S_2) are situated in the bulb between inner circular muscles (*icm*) and longitudinal muscles (*ilm*). Scale bar: 10 µm.

Fig. 14. Cytocystis clitellatus. Sagittal section of the anterior part of the bulb. Cells of the proximal belt of the sheath epithelium have their nucleiferous parts in the bulb (S_2). They are surrounded by the basement membrane, outer circular (*ocm*) and longitudinal muscles. Scale bar: 10 µm.

Fig. 15. Psammorhynchus tubulipenis. Sagittal section of the posterior part of the bulb, with the nucleus of a cell belonging to the proximal belt of the sheath epithelium (S_2), cytoplasmic cell strands of the basal cone epithelium (*iB*) leave the bulb. A bundle of muscles from the anterior (PR_1) and posterior (PR_2) set of proboscis retractors run along the bulb. Scale bar: 10 µm.

Fig. 16. Cytocystis clitellatus. Sagittal section of the posterior part of the bulb, showing cytoplasmic cell strands of the proximal sheath epithelial belt (S_2) and basal cone epithelium (*B*), enfolded by basement membrane (*bl*), outer circular and longitudinal muscles (*ocm* and *olm*). Gland necks (g_6) surfacing in the cone epithelium run through the bulb. At the bottom right at the transition into inner circular muscles (*icm*) and bulbar septum (*s*), cytoplasmic cell strands of the basal cone epithelium (*iB*) leave the bulb. A inner longitudinal muscle fibre (*) leaves the bulb as well and joins the outer musculature. Inner and outer musculature, for instance proboscis retractors (PR) adhere on the bulbar septum. Scale bar: 10 µm.

of the sheath epithelium are pierced by uniciliary receptors with basal bodies and thin vertical rootlets. The basal body might have slanting secondary rootlets (Fig. 21) as in *Cystiplex axi*, but somewhat shorter (De Vocht 1989).

In *C. clitellatus* two sensory organs are found associated with the distal belt of the sheath epithelium. They reach from their invagination point, about 8 µm below the epidermal basement membrane, past the insertion of the dilators of the sheath over a distance of approximately 35 µm up to the level of the apex. The epithelium forms in this way two elongated sacs in the parenchyma (Fig. 2). Numerous multiciliary dendrites of sensory cells pierce these sacs. The axonemata of the cilia are composed of single microtubules but the cilia do not form stacks of flat sheets (Fig. 19). Rootlets are absent.

Numerous uniciliary receptors are spread throughout the distal belt of the sheath epithelium. These receptors possess rootlets up to 2.5 µm long, the basal body is situated in a small extension of the dendrite just above the epithelium surface. Very short secondary rootlets radiate slanting from the basal body to the narrow neck of the dendrite (Fig. 23). They are connected by the zonulae adhaerentes to the tonofilaments in the terminal web of the epithelium and form in this way a stable support for the ciliary shaft. Only few uniciliary receptors are found in the proximal belt of the sheath epithelium. They are mainly located just above the junction.

Uniciliary receptors, with rootlets, are spread throughout the cone epithelium in both species (Figs 5, 6).

Proboscis musculature

Outer proboscis musculature. In *P. tubulipenis* circular muscles surround only the proximal belt of the sheath epithelium. Seventeen or eighteen longitudinal muscles surround the cavity epithelium and are prolongations of the longitudinal muscles of the body wall musculature (Figs 3, 5). They are continuous with the retractors of the pore (or dilators) as well.

The outer musculature of the bulb consists of three pairs of protractors and two sets of short retractors. The anterior set of retractors forms six bundles of muscles fibres (Fig. 25). Distally they separate into twelve groups of two or three fibres, which penetrate the proboscis just beneath the junction (Fig. 24). Once penetrated in the bulb the two fibres curve away to opposite sides. They protrude into the basal cone epithelium and are attached directly to the plasmalemma and to the inner circular muscles (Fig. 8). Between the twelve groups of muscles and outside the loose sphincter, twelve cell strands of the proximal belt of the sheath epithelium sink into the bulb. The second set of proboscis retractors adheres more proximally on the septum and is composed of four pairs of bundles (Fig. 26).

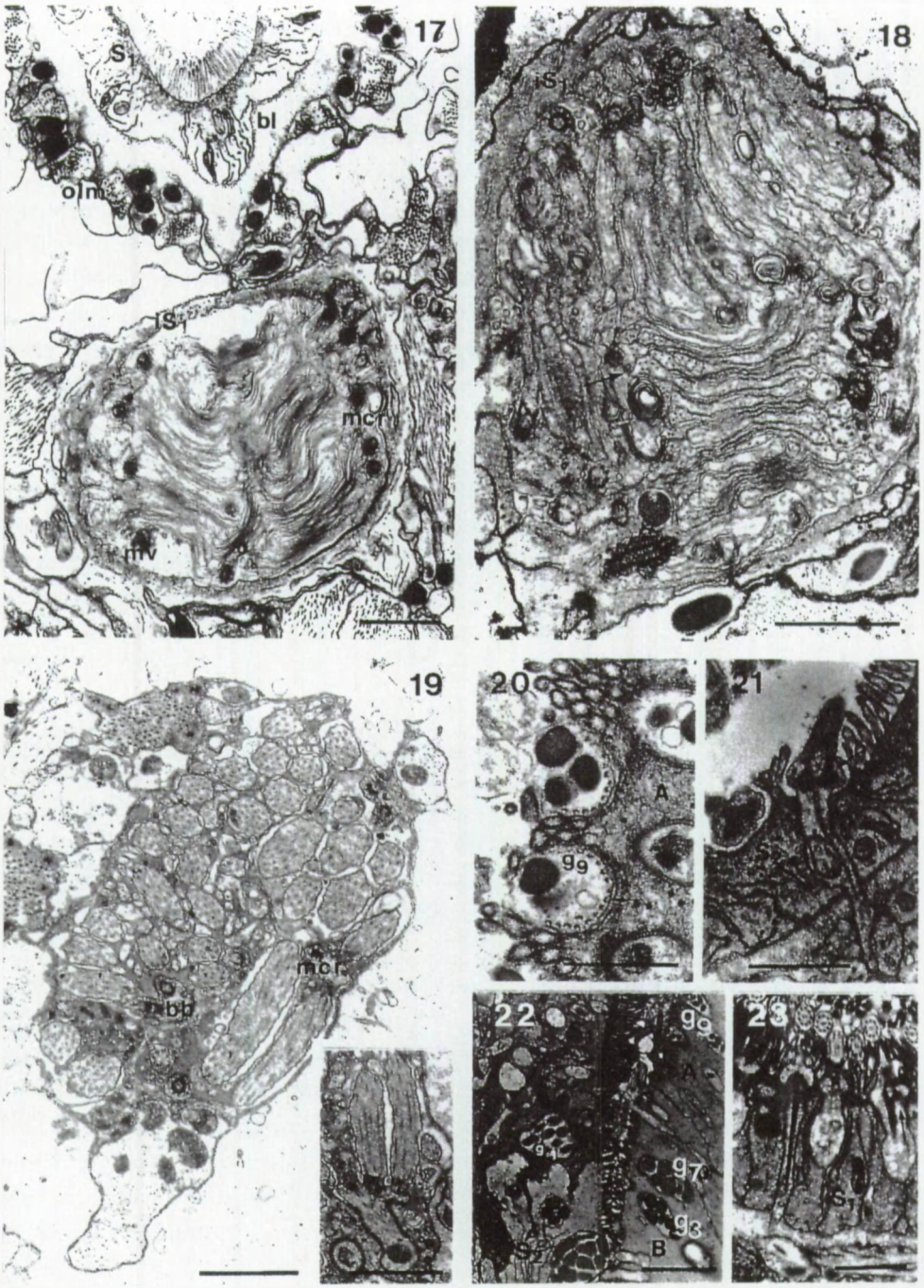


Fig. 17. Psammorhynchus tubulipenis. Cross section of distal belt of the sheath epithelium (S_1) enveloped by the bipartite basement membrane (*bl*) and longitudinal muscles (*olm*) and

In *C. clitellatus* a different organization of the outer musculature is found (Karling 1953). The circular and longitudinal muscles are present around the proximal half of the sheath epithelium up to the transition zone of basement membrane into septum (Figs 14, 16). About 50 circular muscle fibres surround this part of the sheath epithelium and the distal part of the bulb. The outer longitudinal muscles are distally continuous with the dilators of the sheath, which in turn are split from and are continuous with the longitudinal muscles of the body wall (Fig. 2). The outer longitudinal muscles are clearly visible up to the transition zone of basement membrane and septum, distally 21 in number to 46 around the bulb. The outer musculature of the bulb adheres on the septum behind the transition zone (Fig. 16). As noted by Karling (1953), three pairs of protractors, three pairs of proboscis retractors and two pairs of integument retractors are present. Inserting on the septum, just beneath the transition zone, some fibres of the fixators seem to enter the bulb through the perforations and act as cone retractors (Fig. 16, *). From our sections the number of fixators could not be determined and the diaphragm not verified.

Inner proboscis musculature. The proboscis bulb in *P. tubulipenis* is composed of a weakly developed circular muscle layer, enclosing longitudinal muscle fibres and the epithelial cell strands of the cone epithelium and of the proximal belt of the sheath epithelium (Fig. 9). The circular muscles are found from the nodus up to the junction;

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associated insunk sensory organ. Note the terminal web (*tw*) in the covering part of the epithelium. The central cavity of the spherical insunk cell part of the epithelium (iS_1) is filled with stacks of membranes. These are formed by the modified cilia of the multiciliary receptors (*mcr*), microvilli (*mv*) of the epithelium are present as well. Scale bar: 1 µm.

Fig. 18. Psammorhynchus tubulipenis. Cross section of a sensory organ. The axonemata of the flattened cilia are composed of single microtubules only. They form two lateral stalks (*arrows*) in between which the cilium membrane forms a thin sheet. Scale bar: 1 µm.

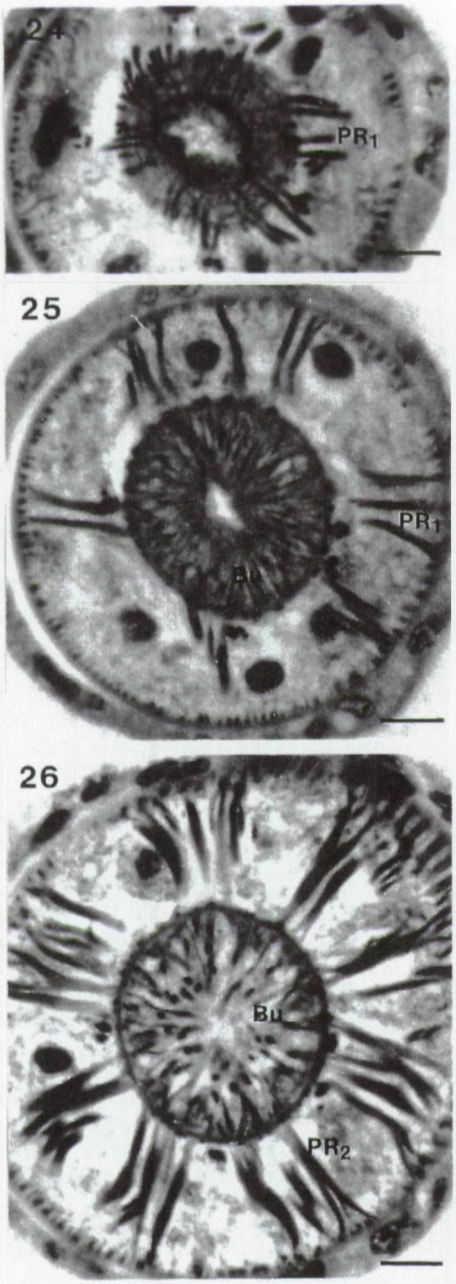
Fig. 19. Cytocystis clitellatus. Cross section of sensory organ, showing multiciliary receptors (*mcr*) with basal bodies (*bb*), aberrant axonemata composed of single microtubules only. Cilia do not form flat sheets as in *P. tubulipenis*. *Inset*: Multiciliary receptor showing cell junctions (macula adhaerens and septate junctions). Scale bar: 1 µm.

Fig. 20. Psammorhynchus tubulipenis. Surfacing gland necks ($g9$) in the apical cone epithelium (*A*), with secretion granules encircled by a ring of semi-circular microtubules (*arrow*). Scale bar: 0.5 µm.

Fig. 21. Psammorhynchus tubulipenis. Uniciliary receptors in the proximal belt of the sheath epithelium (S_2). Note the vertical rootlet and slanting secondary rootlets (*arrow*). Scale bar: 1 µm.

Fig. 22. Cytocystis clitellatus. Sagittal section of sheath and cone epithelium showing different gland necks. In the distal belt of the sheath epithelium (S_1) wide gland necks contain a moderate electron dense secretion (g_1). Gland necks contain small secretion granules ($g9$) in the apical cone epithelium (*A*), large spherical granules ($g7$) and granules with a light centre ($g4$) in the basal cone epithelium (*B*). The latter two types of gland necks are visible in the proximal belt of the sheath epithelium (S_2) as well. Scale bar: 1 µm.

Fig. 23. Cytocystis clitellatus. Uniciliary receptors in the distal belt of the sheath epithelium (S_1) with long vertical rootlets and short slanting rootlets radiating towards the cell junctions (arrow). Scale bar: 1 µm.



they are lacking under the cone epithelium. At the junction, the anterior fibres forming a loose sphincter are only connected to the septum on twelve spots. In between these spots, the fibres run close against the internal cone retractors, leaving space towards the septum for the insunk cell strands of the proximal belt of the sheath epithelium (Fig. 8). We consider the septum the thin layer of extracellular matrix at the outer side of the inner circular muscle layer.

In *C. clitellatus* a belt of inner circular muscles is only present in the proximal part of the bulb, behind the transition zone from basement membrane into septum up to the terminal sag of the nucleiferous cell part of the apical cone epithelium (Fig. 16). As mentioned by Karling nothing separates the inner longitudinal muscles from the nucleiferous cell parts of the sheath epithelium (*mantelzellen* Karling 1953). Together with the cell strands of the basal cone epithelium, some longitudinal muscle fibres leave the bulb as well and join the outer proboscis musculature (Fig. 16).

Discussion.

The proboscis of *P. tubulipenis* and *C. clitellatus* can generally be characterized by the following features: bipartite sheath epithelium and cone epithelium, distal belt of sheath epithelium with two sensory

Figs 24-26. Psammorhynchus tubulipenis. Light microscopic cross sections of the proboscis showing the insertion of the anterior set of retractors (PR_1)(*Fig. 24*), which form six bundles (*Fig. 25*) and the posterior set of retractors (PR_2) composed of eight bundles (*Fig. 26*). Scale bar: 2 µm.

organs containing multiciliary dendrites. Muscular bulb with loose inner longitudinal muscle fibres and epithelial nuclei of the proximal belt of the sheath epithelium and apical belt of the cone epithelium. Insunk nucleiferous cell parts of the basal cone epithelium pass through the proboscis bulb and are situated behind it. The basal belt of the cone epithelium is characterized by two types of glandular secretion.

As in the species in consideration, a division of the sheath epithelium into two belts is found in Cicerinidae, Gnathorhynchidae, Placorhynchidae and *Florianella* as well. In *Cicerina* the major part of the proximal belt, however, is enclosed in the nucleo-glandular girdle (De Vocht & Schockaert 1988 *belt A*). Both belts are cellular in all species with a bipartite sheath epithelium. The sheath epithelium is composed of three belts in *Polycystis naegelii* (Polycystididae) (Schockaert & Bedini 1977), *Cystiplana paradoxa* and *Cystiplex axi* (Cystiplanidae) (De Vocht 1989) and Koinocystididae. Nuclei of cells forming the distal belt are intra-epithelial all species, except *Ethmorhynchus* which has insunk nucleiferous cell parts. In *Psammorhynchus* and *Cytocystis*, however, they are not found. The nuclei of the proximal belt of the sheath epithelium are located among the cone retractors (inner longitudinal muscles) in *Psammorhynchus* and *Cytocystis*. This forms a typical feature for both species, which is not encountered in other species. These nuclei are found in the nucleo-glandular girdle which is separated from the cone retractors by a thin basement membrane or septum in species of the *Cicerina*-group or in a cytoplasmic girde in Cystiplandiae and some Polycystididae.

The epithelial belts lining the proboscis cavity are characterized by numerous infoldings of the basal plasma membrane in all species investigated ultrastructurally (De Vocht 1989, De Vocht & Schockaert 1988 and Schockaert & Bedini 1977). Infoldings of the basal plasma membrane in epidermal cells or syncytia have been recorded in Kalyptorhynchia : *Carcharodorhynchus* and *Gnathorhynchus* (Doe 1976, Rieger & Doe 1975), *Gyratrix* (Bedini & Papi 1974, Reuter 1975), *Florianella* (Rieger & Sterrer 1975). The presence of a terminal web in the sheath epithelium is typical for *P. tubulipenis* and *C. clitellatus*, most pronounced in the latter where a terminal web is also found in the syncytial epidermis. A distinct terminal web in the sheath epithelium was not observed in *C. remanei* and Cystiplanidae (De Vocht 1989, De Vocht & Schockaert 1988). Electron dense granules with light centre were also encountered in the epithelia of *P. naegelii* (Schockaert & Bedini 1977).

A bipartite cone epithelium seems to be a common feature for the Eukalyptorhynchia (Karling 1964, De Vocht 1989). In addition to six families which possess a bipartite cone as known from light microscopic observations (see De Vocht 1989), Psammorhynchidae and Cytocystidae can be added . The basal belt of the cone epithelium in *Psammorhynchus* and *Cytocystis* bears microvilli with dense tips (Figs 5, 6) as in other eukalyptorhynch families studied by E.M. so far: Polycystididae, Koinocystididae, Cicerinidae, Bertiliellidae, Gnathorhynchidae, Cystiplanidae (De Vocht 1989, De Vocht & Schockaert 1988, Doe 1976,

Reuter 1975, Rieger 1981, Rieger & Sterrer 1975 and Schockaert & Bedini 1977). The presence of this type of microvilli of the basal belt of the cone epithelium in eight eukalyptorhynch families and a bipartite organization of the cone indicates that these features are ancestral for the Eukalyptorhynchia.

A circular muscle layer and basement membrane are lacking in the cone in *Psammorhynchus* and *Cytocystis*, as in Koinocystididae and *Mesorhynchus*. Without a basement membrane beneath the cone epithelium, the nuclei sink in between the internal cone retractors and are found inside the bulb or even behind it if perforations in the septum are present. Two species, *Ethmorhynchus* and *Paragnathorhynchus*, possess a basement mebrane in the cone with perforations, which leave passages to the nucleiferous cell parts of the apical cone epithelium (see Chapter 3 and 6).

In *Psammorhynchus* the perforations, leaving passage to the insunk nucleiferous cell parts of the basal cone epithelium, are located laterally at the proximal end of the bulb (Figs 1, 15). They do not indicate a transition of basement membrane of the sheath epithelium into septum of the bulb. This transition is found just beneath the junction. In *Cytocystis*, however, the basement membrane underlying the sheath epithelium and the outer circular and longitudinal muscle layer surround the distal part of the bulb. A bulbar septum is found at the posterior end of the bulb only (Figs 2, 16). Insunk epithelial cell parts leave the bulb at the transition zone as the insunk cell parts of the Cystiplanidae and Polycystididae. Generalizing the observations in *Cytocystis* a origine of the lateral bulbar septum from sheath epithelial basement membrane can be postulated. In *Psammorhynchus*, consequently, the circular muscles should have shifted from the outside to the inside of the extracellular matrix layer. This would imply that the origine of the lateral septum in species without intrabulbar epithelial cell parts must have been established in a different way. The situation in *Cytocystis*, however, can also have originated by a inversion of the circular muscles layer and septum in *Psammorhynchus*.

The gland necks surfacing above the junction in the sheath epithelium of *P. tubulipenis* and *C. clitellatus* are probably homologous (g_2). They are characterized by moderately electron dense secretion granules packed in wide gland necks. The part of the gland necks piercing the epithelium is totally filled with secretion. The gland necks penetrate the basement membrane of the epithelium and are orientated perpendicular to it. The gland necks with mostly empty vesicles which pierce the basal cone epithelium, are probably homologous as well (g_6). In both species the secretion is usually washed out. The gland necks run through the bulb and enter it at the nodus (the posterior end of the bulb). This kind of secretion is present in the proximal belt of the sheath epithelium in *Cystiplex axi* and *Cystiplana paradoxa* (De Vocht 1989, g_4). In *Cicerina remanei* the secretion found in gland necks in the glandular ampullae and in the inner junctional belt is washed out during preparation as well (De Vocht & Schockaert 1988). An electron dense secretion is present in

the basal cone epithelium of both species as well (g7). The glands surfacing in the apical cone epithelium in both species (g9) are characterized by small secretion granules and peripheral microtubules in the part of the gland necks piercing the epithelium.

In Cystiplanidae a wreath of multiciliary receptors is present in the distal belt of the sheath epithelium at the pore (De Vocht 1989). The cilia with basal bodies but without rootlets possess aberrant axonemata which are composed of single microtubules only. Rieger and Sterrer (1975) mention cilia with irregular pattern of microtubular arrangement in the sheath epithelium of *Florianella bipolaris*. In *P. tubulipenis* and *C. clitellatus* the dendrites pierced the distal belt of the sheath epithelium as well, although they are situated in two distinct insunk cell parts and form two sensory organs. The dendrites merge in invaginations which are not continuous with the proboscis cavity. This type of sensory cells is encountered by *Cicerina*, *Paracicerina*, *Ptyalorhynchus* and *Ethmorhynchus* as well. The presence of two insunk sensory organs with multiciliary receptors can be interpreted as an synapomorphic feature for the species mentioned above. The fine structure of the receptors and the position makes a homologation with intra-epithelial multiciliary receptors possible. In all species, the receptors have some features in common: the dendrites are multiciliary, the axonemata of the cilia are formed by single microtubules only and rootlets are absent. The receptors are in all cases associated with the distal belt of the sheath epithelium.

The uniciliary receptors in the sheath epithelium have the same characteristic organization of the rootlets as in Cystiplanidae. The radiating secondary rootlets in *P. tubulipenis* and *C. clitellatus* are shorter as in Cystiplanidae (De Vocht 1989).

The inner longitudinal musculature of the proboscis in *P. tubulipenis* and *C. clitellatus* is composed of loose muscle fibres, this in comparison to the organization of these muscles in for instance Cystiplanidae (De Vocht 1989). This is due to the presence of epithelial cell parts and gland necks among these muscle fibres. In Cystiplanidae, the internal cone retractors are connected to each other and only leave passage to a few gland necks. In *C. clitellatus* the gland necks are very conspicuous and form wide lumina in the bulb (Fig. 11).

Although the proboscis of *P. tubulipenis* resembles very much that of *C. clitellatus* for what the epithelial organization is concerned, the muscular system of the proboscis differs in both species. The organization of the outer proboscis musculature in *P. tubulipenis* resembles the organization in species of the *Cicerina*-group. A double set of proboscis retractors is present as in *Xenocicerina*, *Didiadema*, *Cicerina*, *Paracicerina*, *Ptyalorhynchus* and *Ethmorhynchus* (Brunet 1965, 1973, Karling 1952, 1964, see Chapter 3). In *Toia*, *Nannorhynchides*, *Pocillorhynchus* and *Zonorhynchus* only one set of proboscis retractors is present (Brunet 1973, Karling 1952, 1964, Marcus 1952, see Chapter 3). Genera of the Cicerinidae which possess a double set of proboscis retractors, only possess an outer circular muscle layer around the proximal sheath epithelium, which is almost fully incorporated in the

nucleoglandular girdle. In *P. tubulipenis* and *C. clitellatus* the proximal part of the sheath epithelium is surrounded by a weakly developed circular muscle layer (Figs 5, 6).

In *C. clitellatus* only one set of proboscis retractors and a set of fixators is present. The fixators in Cystiplanidae and Polycystididae insert on the bulbar septum immediately below the transition zone into epithelial basement membrane as well. In *C. clitellatus* this originates in a far posterior location of the fixators. We can homologize these muscles on the principle of identical insertion positions. Protractors are encountered in all known eukalyptorhynch families.

To conclude we can state that a bipartite sheath epithelium (*e.g. Cicerina, Psammorhynchus* and *Cytocystis*) is ancestral to a sheath epithelium composed of three belts (*Polycystis, Cystiplex* and *Cystiplana*). In species with a bipartite sheath epithelium, the epithelial belts of the proboscis epithelia are mostly cellular. The presence of two insunk sensory organs associated with the distal belt of the sheath epithelium is typical for *P. tubulipenis* and *C. clitellatus*. These receptors are homologous to those encountered in the distal belt of the sheath epithelium in for instance *C. axi* , *C. paradoxa* and *Florianella bipolaris*. The organization of the proboscis epithelia is a synapomorphic character for *P. tubulipenis* and *C. clitellatus*. These epithelia are characterized by the presence of intrabulbar nuclei of the apical cone epithelium and proximal belt of the sheath epithelium as well as insunk nucleiferous cell parts of the basal cone epithelium, which leave the bulb laterally at the posterior end

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Chapter 5.

The anatomy and ultrastructure of the proboscis in *Florianella bipolaris* Rieger and Sterrer, 1975.

Introduction

Rieger and Sterrer (1975) described a few remarkable freeliving Platyhelminthes with a common characteristic, namely the presence of spicules in the epidermis. An overall and thourough description has been made by the authors and now a detailed description of the proboscis is given. The authors included valuable information on the proboscis with some electron microscopic details.

Material and methods

A preserved specimen of *Florianella bipolaris* was kindly donated by Prof. Dr. R. Rieger. The specimen was collected from 25 m depth (N33° 25.8' - W78° 11.3') Eastward Cruise in march 1976 . For sampling, specimen extraction and fixation procedure see Rieger and Sterrer (1975) and Rieger & Ruppert (1978).

Results

The proboscis in *F. bipolaris* differs very much from the proboscis in other Eukalyptorhynchia. Especially the organization of the bulb and the insunk cell parts, which pierce the distal part of the bulb, make it difficult to draw close affinties with other species or genera. The proboscis is relatively small and measures 120 µm in length including the insunk cell parts, the bulb only measures 35 µm from the apex of the cone to the nodus. It possesses a low cone and the insunk cell parts, located behind the bulb are extensive. The number of nuclei in the insunk cell parts can not be stated with certainty but exceeds fifteen.

Epithelia, glands and sensory cells

Epithelia. The proboscis epithelium in *F. bipolaris* is formed by four circumferential belts (Fig. 1). Both sheath and cone epithelium are bipartite and devoided of cilia. Cell junctions are formed by zonulae adhaerentes, subsequent septate junctions and dispersed maculae adhaerentes. The basement membrane under the sheath epithelium has a bipartite structure near the pore and varies in thickness from 150 to 300 nm normally, with upto 1.2 µm thick protrusions into the cavity. Further downwards a uniform 50 to 75 nm thick basement membrane is present. The basement membrane is not only continuous with the

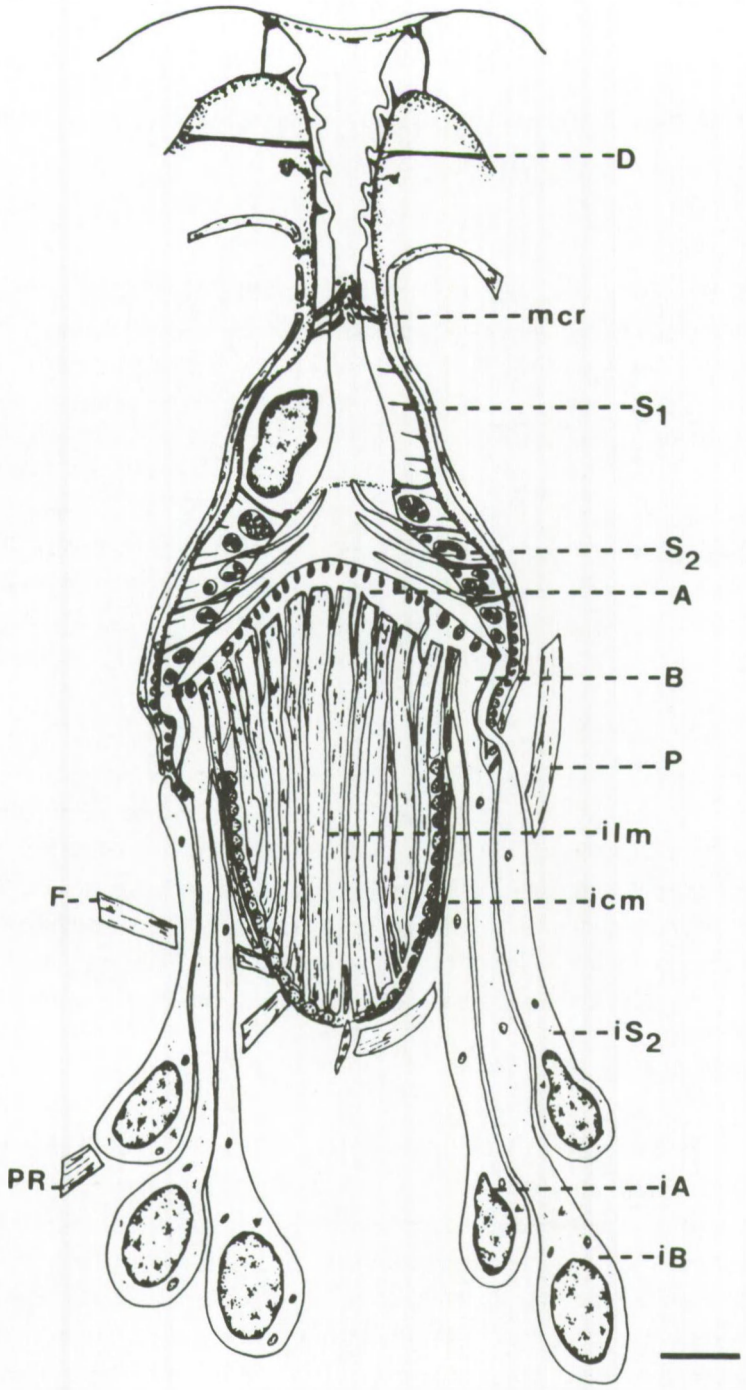


Fig. 1. Reconstruction of the proboscis in *Florianella bipolaris*. Scale bar: 5 µm.

epidermal basement membrane but also with extracellular matrix (ECM) in the parenchyma. This ECM appears as homogenous electron dense patches with peripheral vesicles (Fig. 2). These vesicles seem to originate from parenchymal cells (Fig.). The distal part of the sheath epithelium is formed by a single circumferential cell of which the large nucleus is situated in its posterior part half way down the sheath epithelium. A thin strand of cytoplasm (50-250 nm) covers the basement membrane and forms long and tender extensions into the cavity, practically filling it (Fig. 2). The apical plasmalemma is devoided of microvilli and any form of terminal web. In the cytoplasm, infoldings of the basal plasmalemma, mitochondria and patches of free ribosomes are present. In the parenchyma a large nucleus is situated close to the nucleus of the distal cell (Fig. 3). Whether this nucleus is to be considered an insunk nucleus of the distal cell (syncytium in that case) or not is unclear (Rieger & Sterrer 1975). Light microscopic observations of the embedded specimen previous to sectioning also gave the impression of the presence of two nuclei in the middle of the sheath epithelium (M. Gehlen, personal communication). At the transition of the distal cell to the proximal belt the proboscis cavity widens and the epithelium becomes up to 3 µm thick (Fig. 4). The belt is probably formed by four cells, although the cell junctions could not be discerned in all the sections due to the large amount of piercing gland necks. The cells of this belt bear stubby, 300 nm long microvilli. Directly above the junction the cells are only 100 nm tick bearing upto 200 nm long microvilli (Fig. 6). The most basal part of the cone is lined by this belt as well, the microvilli of this part are longer (500 nm) than the other. The cytoplasm shows infoldings of the basal plasmalemma, mitochondria and few ribosomes. Above the junction often intrusions of the basement membrane in the epithelium can be seen. The insunk cell strands of this belt, below the junction, are situated peripherally against the basement membrane and outer musculature (Fig.7). Just above the sudden narrowing of the bulb, four wide perforations leave passage to the nucleiferous cell parts of this belt which are situated behind the bulb in front of the secretory cell bodies of the proboscis glands.

The bipartite cone epithelium is formed by a circumferential syncytial basal belt and an apical syncytium (Fig. 1). A basement membrane is lacking under the 1 to 1.8 µm thick epithelium. The basal cone epithelium bears 500 nm long microvilli with electron dense condensation at their margins in the distal part. The apical cone epithelium bears stout, about 100 nm long microvilli (Fig. 4). A subterminal cell web (100 nm thick) is present in the fibrous cytoplasm of the basal syncytium. Epithelial cell strands are present in between the apical parts of the inner longitudinal muscles in the cone. The apical fibrillar layer, which is found on top of the internal cone retractors, is void of cell organelles; the cell strands among the muscles fibres, however, contain mitochondria with electron dense matrices. In cross section at the level of the junction, the apical cone epithelium is formed by twelve thin proliferations between the internal cone retractors and enclosed by the basal

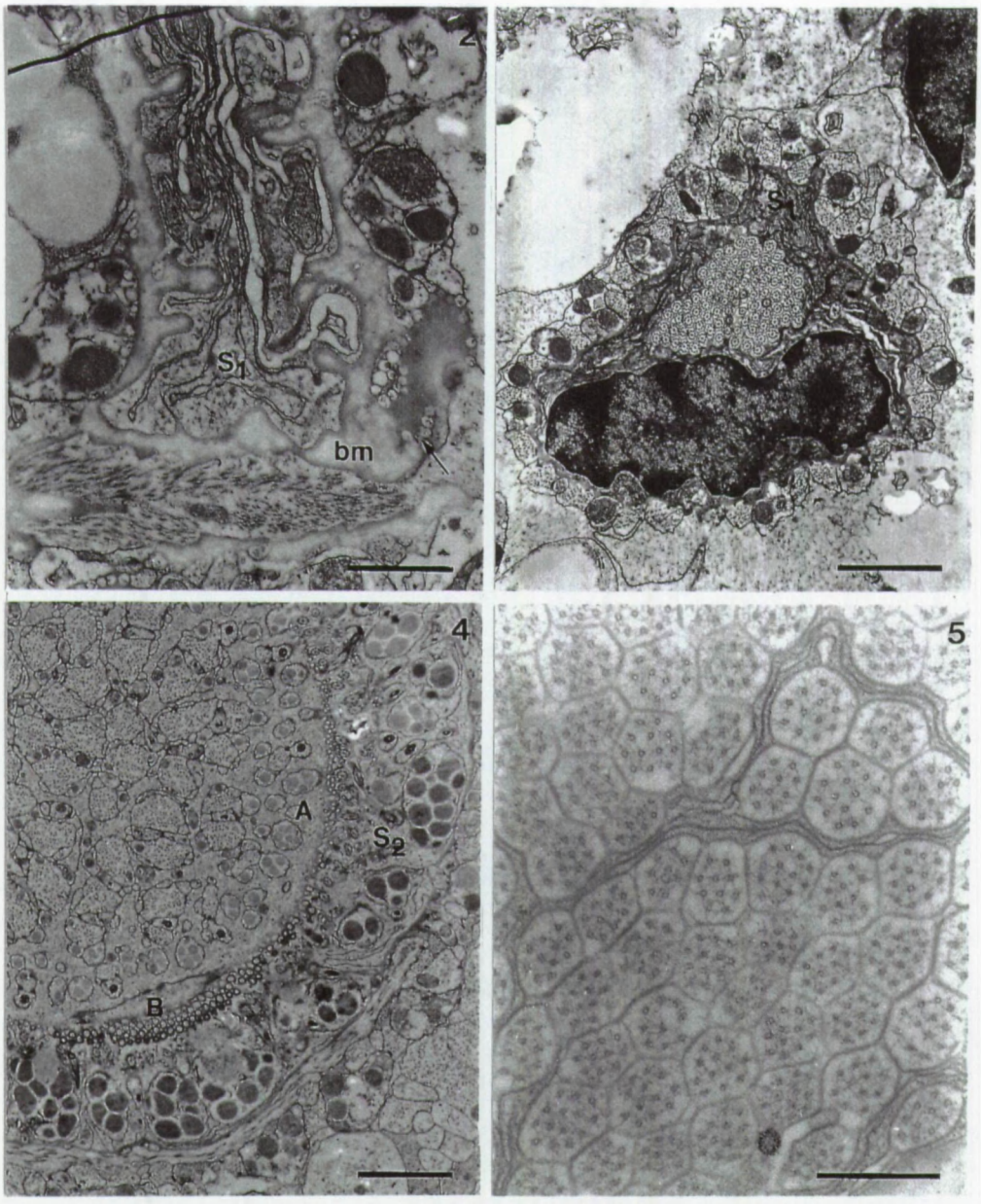


Fig. 2. Cross section of the folded distal belt of the sheath epithelium (S_1), with basement membrane (*bm*) connected to patches of ECM (*arrow*). Scale bar: 1 µm.
Fig. 3. Cross section showing the nucleus of S_1 with ciliary shafts of uniciliary receptors in the lumen. Only longitudinal muscles surround the sheath. Scale bar: 2 µm.
Fig. 4. Quarter of the proboscis showing proximal belt of the sheath epithelium (S_1), apical (*A*) and a part of the basal (*B*) cone epithelium. Scale bar: 2 µm.
Fig. 5. Ciliary shafts of multiciliary receptors with axonemata formed by single microtubules. Scale bar: 0.5 µm.

cone epithelium (Fig. 6). From the junction downwards to the transition of outer circular muscles layer into internal circular muscles, the epithelial cell strands migrate to the periphery, while the inner longitudinal muscles form a tight block in the centre (Fig. 7). The distal part of the bulb is surrounded by a thick girdle of epithelial cell parts of the proximal belt of the sheath and the cone epithelium. This girdle is surrounded by the outer ciruclar and longitudinal muscles. At the transition zone to inner probosics musculature, the cell strands sink in the parenchyma through wide gaps in the basement membrane. They are situated between the central muscle bulb and bundles of protractors and reach 40 µm behind the proboscis up to the secretory parts of the proboscis glands (Fig. 10). The insunk cell parts of the proximal belt of the sheath epithelium and cone epithelium have been regarded as secretory cells by Rieger and Sterrer (1975).

Glands. Seven different types of gland necks can be identified on morphological grounds in the proboscis epithelia of *F. bipolaris*. The distal cell of the sheath epithelium is void of gland necks but two types of gland necks fill the cells of the proximal belt of the sheath epithelium (Fig. 2). Most numerous are large type g_1 gland necks, about 2 µm high and closely packed with electron dense spherical secretion granules upto 700 nm in diameter. Type g_2 gland necks are somewhat smaller and contain 380-600 nm less electron dense, sperical to ovoid secretion granules. The narrowing part of the proximal belt of the sheath epithelium above the junction is void of gland necks. Three types of gland necks surface through the basal cone epithelium (Fig. 6). Type g_7 gland necks contain moderately electron dense spherical secretion granules, 350 nm in diameter with a 190 nm electron dense centre. Upto 1 µm wide type g_6 gland necks store 370 nm empty vesicles and type g_5 necks, basally in the belt near the junction, contain small ovoid 180 nm long electron dense secretion granules surrounded with a fluffy layer. Type g_7 and g_6 gland necks enter the proboscis between the insunk nucleiferous cell parts and through the wide peripheral cytoplasmic girdle in the distal part of the bulb. The apical cone epithelium is pierced by two types of gland necks; type g_9 necks contain moderately electron dense spherical secretion granules, 250 nm in diameter and type g_{10} necks 200 nm spherical electron dense secretion granules with weblike structure (Fig. 4). Both type g_9 and g_{10} gland necks are reinforced by cortical microtubules.

Sensory cells. The proboscis epithelia of *F. bipolaris* house many receptors. In the middle of the distal belt of the sheath epithelium is pierced by many multiciliary receptors. Dendrites with up to five, over 7 µm long cilia penetrate the epithelium. The cilia lack rootlets and have modified axonemata, composed of fourteen single microtubules, arranged in a regular pattern (Fig. 5). Five rows of 2-3-4-3-2 microtubules can be detected. The number of microtubules gradually decreased towards the tip of the cilia, first twelve and

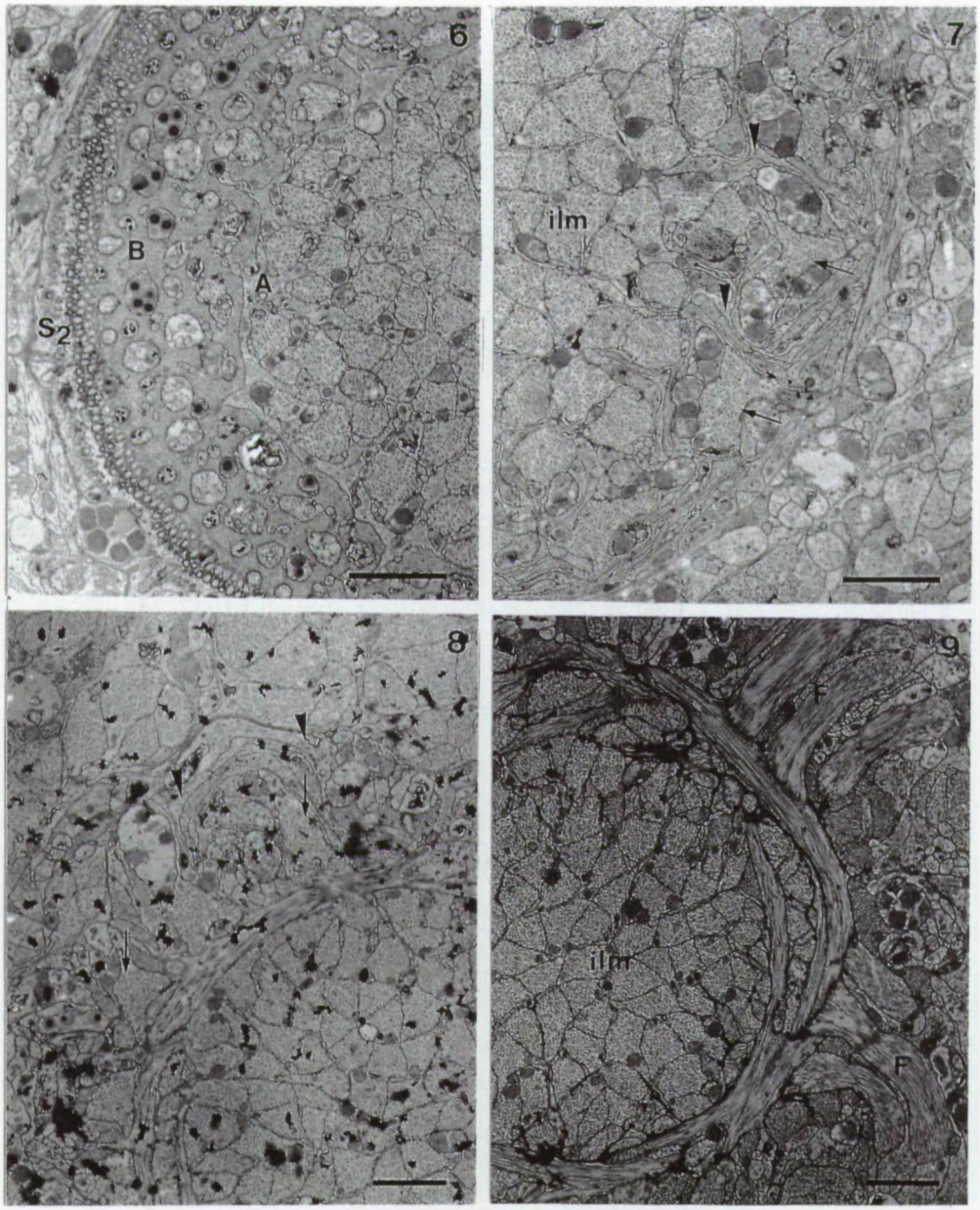


Fig. 6. Cross section just above the junction with thin S_2 epithelium and *B* in the cone with underlying *A* and inner longitudinal muscles. Scale bar: 2 µm.
Fig. 7. Quarter of the proboscis below the junction with inner longitudinal muscles (*ilm*) and cell strands of S_2, *B* and *A* (*arrowheads*). Note the intra-epithelial muscles (*arrow*). Scale bar: 2 µm.
Fig. 8. Quarter of the proboscis with insunk cell parts of the proboscis epithelia (*arrowheads*) and intra-epithelial muscles around the bulb (*arrow*). Scale bar: 2 µm.
Fig. 9. Posterior part of the bulb, inner longitudinal muscles (*ilm*) and fixators (*F*) adhere on the septum between circular muscles. Scale bar: 2 µm.

later only ten microtubules persist. The two outer rows with two microtubules each dissappear. Dense cross bridges connect the microtubules with those in the immediate surrounding or with the ciliary membrane (Fig.).

The proximal belt of the sheath epithelium is pierced by numerous uniciliary receptors which can give the impression of a ciliated epithelium in light microscopic sections (Fig. 3) (Rieger & Sterrer 1975). The cilia have normal 9+2 axonemata and possessed solid, over 3 µm long rootlets which reach past the basement membrane.

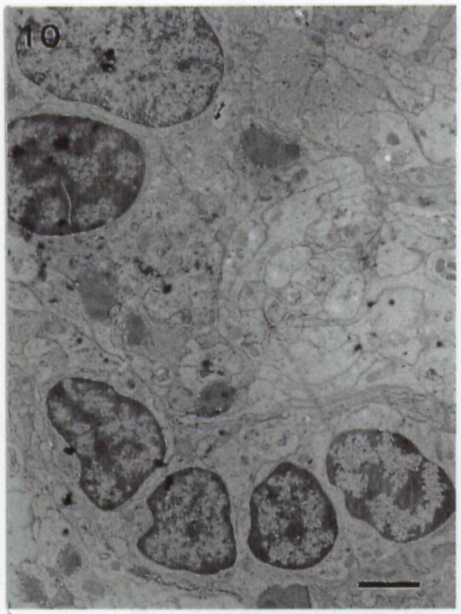
Proboscis musculature

The muscle fibres have a loose arrangement of myofilaments and a peripheral sarcoplasmic reticulum is lacking. Nuclei of the muscles have not been found.

Outer proboscis musculature. Outer circular muscles surround the proximal belt of the sheath epithelium down from the level of the basal cone epithelium on (Figs. 4, 6, 7). They are found over the junction around the wide anterior part of the bulb upto where circular muscles closely surround the inner longitudinal muscles. Below the junction the outer circular muscle layer forms a constriction of 1.5 µm. Below the junction around the distal part of the bulb, the outer cirucular and longitudinal muscle layer surround a intrabulbar cytoplasmic girdle. A total number of 31 outer longitudinal muscle fibres surround the sheath epithelium from the dilators of the sheath, around the distal belt of the epithelium on to the junction (Fig. 3). Below the junction 24 muscle fibres are present around the distal part of the bulb. They pull through the insunk epithelial cell strands and surround the proximal part of the bulb together with the intra-epithelial muscles (see below). The muscle fibres are connected to the uniform basement membrane and surrounding parenchymal cells by hemidesmosomes.

The motional muscles include strong protractors and fixators, which insert on the posterior part of the bulb and integument retractors. Three pairs of protractors interdigitate at the nodus. Three pairs of fixators insert on the posterior part of the bulb and adhere on the epidermal basement membrane in the same region (Fig. 9). One pair of ventral integument retractors is present. Proboscis retractors pull through the insunk epithelial cell parts behind the bulb. There exact organization could not be seized.

Inner proboscis musculature. The circular muscles in the bulb are actually not enclosed by the bulbar septum at the outside but are individually surounded by extracellular matrix (Fig. 9). In the cone only longitudinal muscles are present. The fibres contain fairly large mitochondria with electron dense matrices. Apically they intrude in the cone epithelium and are connected directly to the epithelial plasmalemma. Proximally they insert on the bulbar septum or ECM-layer between the inner circular muscles. As mentioned by Rieger





and Sterrer (1975) extensions of the ECM-layer around the circular muscles intrude between the inner longitudinal muscles in the proximal end of the bulb (Fig. 9).

Intra-epithelial muscles. About eighteen muscle fibres, with irregular shape in cross section, are situated around the bulb in between the 24 other longitudinal muscles (see above) (Fig. 8). In contrary to the latter, the intra-epithelial muscles fibres intrude into the epithelial cell strands, which form the intrabulbar cytoplasmic girdle (Fig. 7). Here, the muscle fibres are located peripherally of the inner longitudinal muscles and reach up to the junction. They adhere on the basal cone epithelium.

Discussion

Florianella bipolaris is a remarkable species within the Eukalyptorhynchia, not only because of the spiculae in the epidermal basement membrane but also because of the anatomy of the proboscis (Rieger &Sterrer 1975). The bipartite sheath epithelium and the low cone indicate a relationship with Cicerinidae, *Psammorhynchus*, *Cytocystis*, Placorhynchidae and Gnathorhynchidae. However, many special features, such as the form of the distal belt of the sheath epithelium, the complex organization of the insunk epithelial cell parts of the proximal

Fig. 10. Insunk nucleiferous cell parts of the proboscis epithelia behind the proboscis bulb. Scale bar: 2 µm.

Fig. 11. Trigonostomum setigerum. Invagination of the front end of the body, the epithelium is pierced by many gland necks, cilia are lacking and muscles without sarcoplasmic reticulum. Scale bar: 5 µm.

belt of the sheath epithelium and the cone epithelium, the structure and organization of the basement membrane and extracellular matrix illustrate the isolated position of the species. The proboscis sheath is not ciliated as mentioned by Rieger and Sterrer (1975) because the 'ciliation' is formed by the many uniciliary receptors in the proximal belt of the sheath epithelium. Such a high concentration of uniciliary receptors in the sheath epithelium is encountered in *Toia*, *Nannorhynchides* and *Cytocystis* as well. Multiciliary receptors in the distal belt of the sheath epithelium are present in many other species as well in Polycystididae, Cystiplanidae, Koinocystididae as in *Placorhynchus* and *Paragnathorhynchus*. The dense tips of the microvilli in the basal belt of the cone epithelium is present in other Eukalyptohynchia as well. A distinct basement membrane beneath the cone epithelium is not observed; however, intrabulbar epithelial nuclei are not present.

The structure and formation of the extracellular matrix in *Florianella* is remarkable. The presence of extracellular matrix in the parenchyma, continuous with epidermal and proboscis sheath epithelial basement membrane, represents a new character within the Eukalyptorhynchia. Several large patches of ECM are found especially around the distal belt of the sheath epithelium.

A sarcoplasmic reticulum is lacking in *Toia* and *Nannorhynchides* as well. This can be considered a reduction but most likely it still represents the plesiomorphic condition in Eukalyptorhynchia. In a species of *Trigonostomum* a sarcoplasmic reticulum in muscle fibres is lacking as well (Fig. 11). Rieger and Sterrer (1975) described the circular muscles fibres below the junction as a sphincter, without a special thickening of the fibres. This corresponds to the constriction as described above. The same type of intra-epithelial muscles are recorded for *Mesorhynchus* as well (De Vocht 1991). In Cystiplanidae and Polycystididae, the intra-epithelial muscles are found up to the apical cone epithelium (De Vocht 1989, Schockaert & Bedini 1977). In Cystiplanidae the bifurcated proximal end of the intra-epithelial muscle fibres adhere on the flanks of the bulb and join the fixators. The insertion site in the proboscis is different in the former two mentioned species and Polycystididae and Cystiplanidae, basal versus apical cone epithelium.

Rieger and Sterrer (1975) pointed out that the male copulatory organ shows closest relationship with the Polycystididae and especially with genera of the subfamily Gyratricinae. The phylogenetic position of *Florianella* remains still uncertain, the ultrastructure of the proboscis does not fully clarify the relationship within the Eukalyptorhynchia.

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Chapter 6.

The anatomy and ultrastructure of the proboscis in Placorhynchidae and Gnathorhynchidae.

Introduction

Placorhynchidae and Gnathorhynchidae are easily distinguished from other Eukalyptorhynchia by their proboscises. Two dorso-ventrally opposing muscular sheets enclose the bulb in Placorhynchidae, while two dorso-ventrally opposing hooks are present in Gnathorhynchidae. These characters can not be overlooked when looking at the animals through a dissecting microscope or light microscope. The two muscular sheets or muscle plates in Placorhynchidae have been regarded homologe to the split proboscis in Schizorhynchia (Karling 1961). If this is so, Schizorhynchia and Eukalyptorhynchia can not be considered sister taxa. The proboscis hooks in Gnathorhynchidae were found to be intra-epithelial differentiations related to electron dense condensations in microvilli of the epithelium at the junction (Doe 1976). In other species of Eukalyptorhynchia, electron dense condensations in the microvilli of the basal belt of the cone epithelium have been found as well (De Vocht 1989, 1990, 1991, Rieger & Sterrer 1975). Two hooks at the junction are also present in Aculeorhynchidae Schilke, 1969, a monogeneric family with one species *Aculeorhynchus glandulis* Schilke, 1969. As stated by Karling (1983), Gnathorhynchidae and Aculeorhynchidae can not be considered sister groups because there is no synapomorphy to unite the Gnathorhynchidae and separate it from *A. glandulis*. The family Placorhynchidae contains six genera and fifteen species, while the family Gnathorhynchidae now comprises eleven genera and thirty species. The species investigated are *Placorhynchus octaculeatus* Karling, 1931, *Paragnathorhynchus subterraneus* Meixner, 1938, synonimized with and a valid name for *Prognathorhynchus stylofer* Schilke, 1970 (Karling 1983), *Gnathorhynchus conocaudatus* Meixner, 1929 and *Drepanorhynchides diodonthus* L'Hardy, 1966. Within the genera the anatomy of the proboscis seems to be constant.

Material and methods

Specimens of *Placorhynchus octaculeatus* were collected from sediment in shallow water of the estuary of the river La Slack in Ambleteuse (Pas de Calais, France). Specimens of *Gnathorhynchus conocaudatus* were collected from a sandy beach at the Belgian coast (Knokke) in May 1986, the specimen of *Drepanorhynchides diodonthus* was obtained from coarse sand at 12 metres depth near Calvi (Corse, France) in April 1986. Specimens of *Paragnathorhynchus subterraneus* were collected from sand at the transition of 'Sandhang' to 'Sandwatt' in Königshafen near List (Sylt, Germany) in April 1988 (Schilke

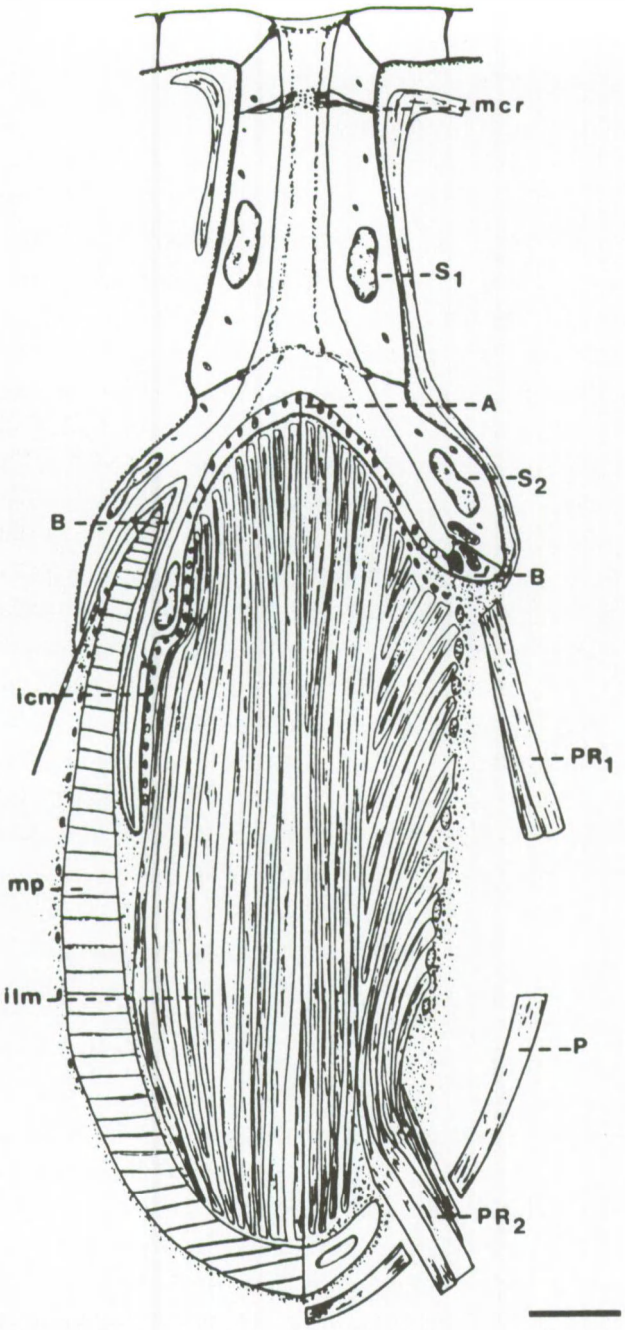


Fig. 1. Reconstruction of the proboscis in *Placorhynchus octaculeatus*. At the *right* a section through a muscle plate, at the left a section of the lateral side. Scale bar: 10 μm.

1970, Hoxholdt 1974). The type material of *Aculeorhynchus glandulis*, obtained from the University of Göttingen, has been investigated by light microscope.

Results

Due to the great differences in structure, the descriptions of the proboscises in Placorhynchidae and Gnathorhynchidae are given separately. The description of the Gnathorhynchidae is mainly based on *P. subterraneus*, differences in *G. conocaudatus* and *D. diodonthus* are stated explicitely.

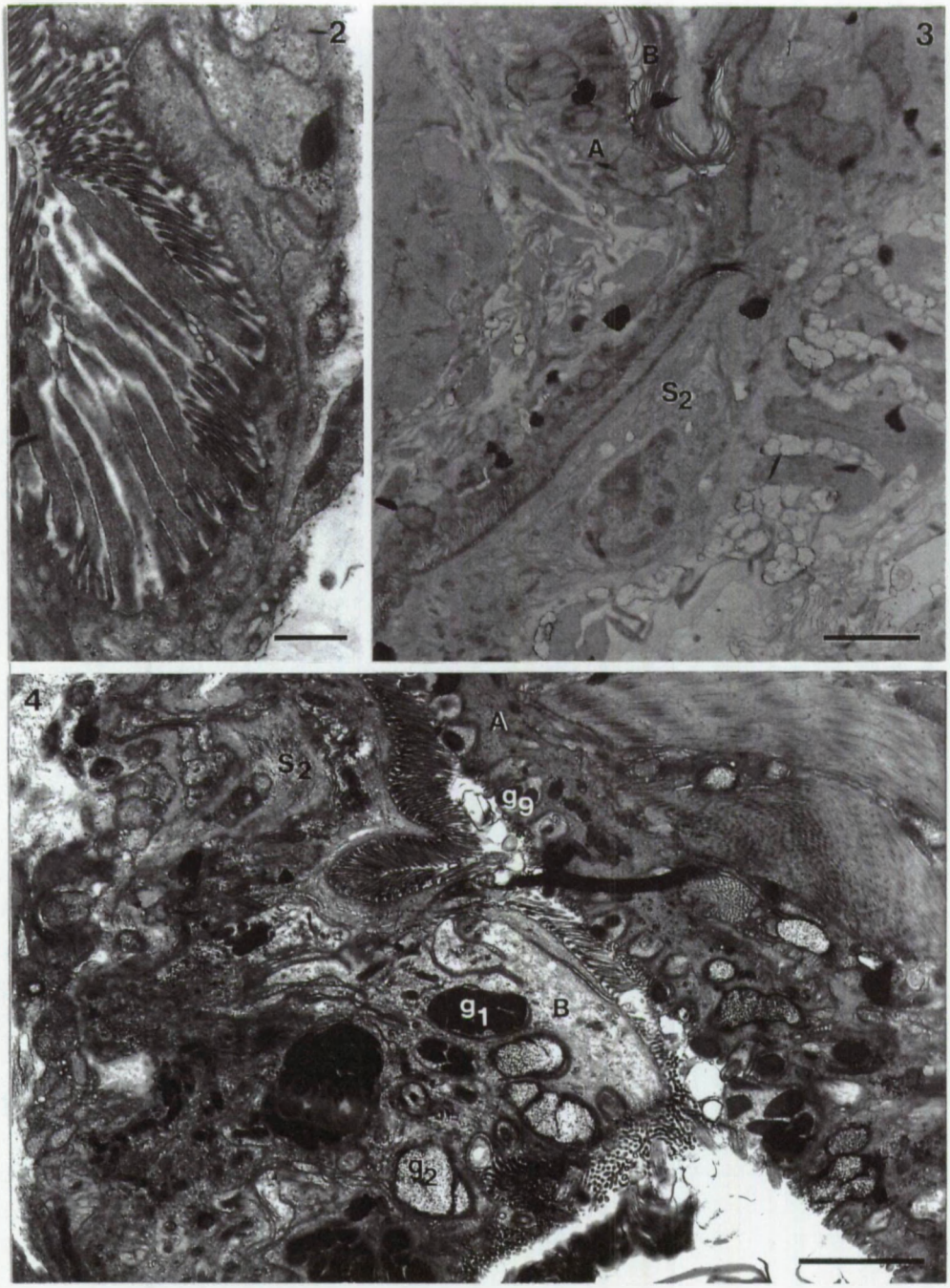
Placorhynchus octaculeatus

Placorhynchidae possess a proboscis with an elaborated system of extracellular matrix. The layer of extracellular matrix surrounding the two muscle plates is continuous with the extracellular matrix at the lateral sides of the bulb as with the basement membrane under the sheath epithelium and cone epithelium. The tips of the muscle plates extend into the proboscis cavity. The junction of sheath and cone epithelium, or the most posterial situated margin of the epithelia at the level of the two muscle plates, is located deep in between the proboscis bulb and the muscle plates. The inner longitudinal muscles are organized in a central and two lateral groups. The inner circular muscles only surround the longitudinal muscles fully in the anterior part of the bulb. Four extensive concentrations of gland necks are found at the junction and multiciliary receptors are present in the distal belt of the sheath epithelium. In *P. octaculeatus* two frontal glands with electron dense secretion granules are present dorsally. The gland necks are located dorsolaterally behind the proboscis. This type of gland necks is present in Schizorhynchia as well.

Epithelia, glands and sensory cells

Epithelia. The proboscis epithelia are formed by four belts; two cellular belts in the sheath epithelium and two syncytial belts in the cone epithelium (Fig. 1). The proboscis epithelium is void of cilia as in other investigated Eukalyptorhynchia. Cell junctions are formed by zonulae adhaerentes and septate junctions.

The distal belt of the sheath epithelium lining the cavity is highly folded and very variable in thickness (1-5 µm). The proximal belt decreases in height dorsally and ventrally near the terminal ends of the two muscle plates and is here only 200-500 nm high, at the lateral sides this belt continues as a 3 µm thick epithelium. The two transverse belts are formed by four cells each. At their transition cytoplasmic strands of cells from both belts interdigitate. The cells of both belts bear slender, 650-500 nm long microvilli, about 13 per linear µm in the distal belt and 16 per linear µm in the proximal

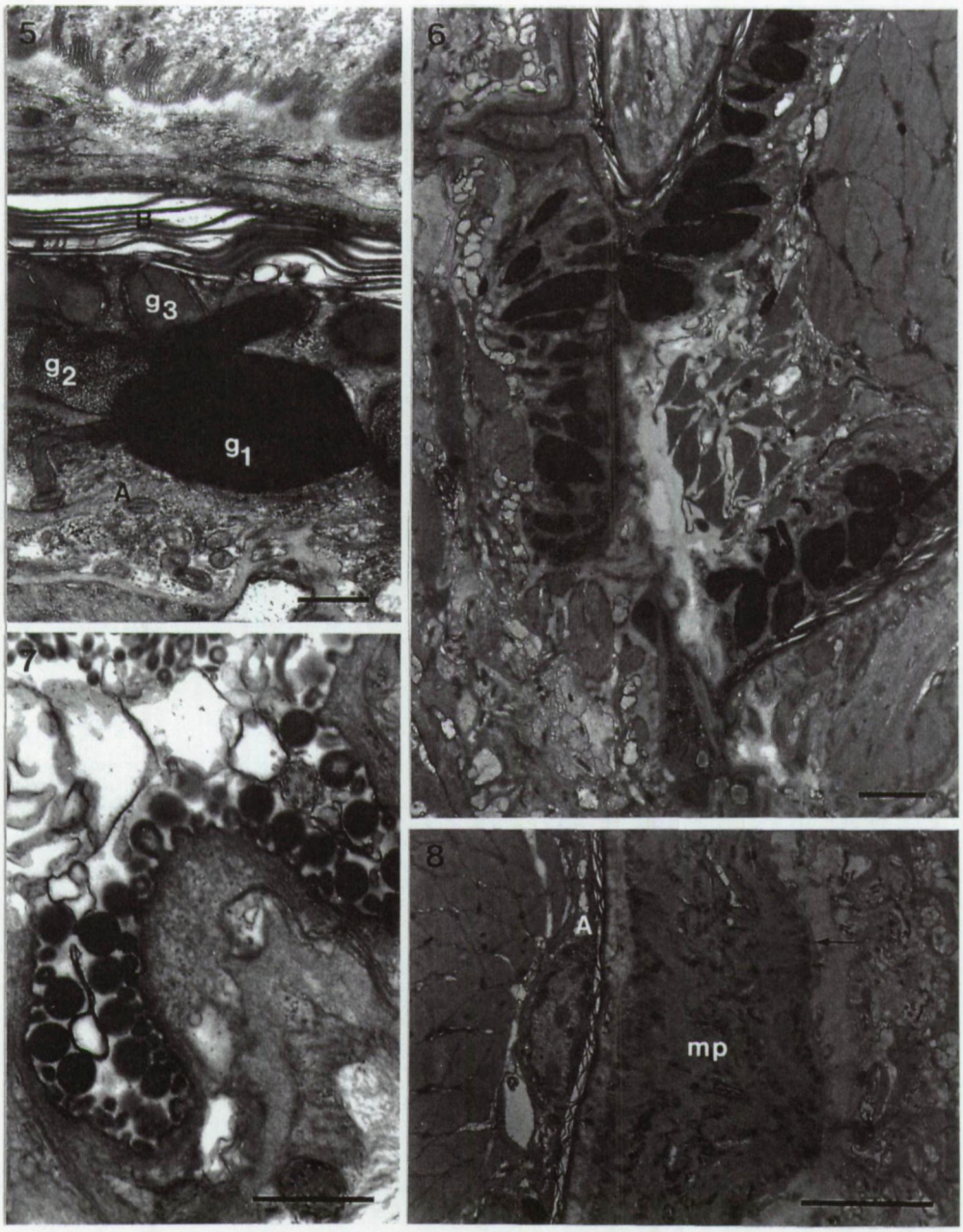


Figs. 2-4. Placorhynchus octaculeatus.
Fig. 2. Intra-epithelial multiciliary receptors in the distal belt of the sheath epithelium. Scale bar: 1 μm.

belt (Figs 2, 3). The basement membrane under the epithelium has a variable thickness, ranging from about 350 nm apically over 500 nm near the tips of the muscle plates to 2.5 µm when the outer longitudinal muscles are enclosed. The granular cytoplasm shows mitochondria with dense matrices, rough endoplasmic reticulum, free ribosomes and 3.5 µm large, globular nuclei (Fig. 3). The proximal belt of the sheath epithelium covers to a large extend the outer side of the muscle plates and is found upto the junction at the lateral sides of the proboscis. The parts of the cells of the proximal belt covering the outer side of the muscle plates are penetrated by single muscle fibres which run towards the tips of the two muscle plates.

The cone epithelium is formed by an apical and a basal syncytium. The basement membrane under the cone epithelium is about 30 nm thick as well apically in the cone as dorsally and ventrally where the two muscle plates are present (Fig. 5). Laterally especially under the basal cone epithelium it thickens to a 100-300 nm thick uniform layer (Fig. 6). The apical syncytium varies in height from 700 nm apically to 400 nm basally and has two intra-epithelial nuclei (6.5 µm) situated dorsally and ventrally (Fig. 8). The apical cell membrane shows undeep foldings or 150 nm high and thick microvilli (Fig. 5). The basal cone epithelium is very variable in thickness as well as appearance according to the position. The anterior parts of the epithelium which line the cavity laterally, are upto 3 µm and high pierced by numerous gland necks (Fig. 6). The part of the syncytium lining the muscle plates at the inner side is only 100-500 nm thick with a 400-1500 nm thick basement membrane and void of gland necks (Fig. 5). The epithelium, covering the coen laterally, is about 300 nm high and spherical, 200 nm wide vesicles are pinched of from the apical cell border (Fig. 7). During formation the vesicles show a primary condensation at the margin but later the vesicles are totally filled with a electron dense contents. The thin epithelial cell parts covering the muscle plates at the inside show numerous flat proliferations of the apical cell border (Fig. 5). They cover the epithelium and fill the space between the muscle plates and the dorsal and ventral side of the cone. The junction or transition from the epithelium lining the sheath in the epithelium, which covers the cone, is situated at different heights dorsally and ventrally in comparison to the lateral sides (Fig. 1). Laterally the posterior part of the sheath epithelium is formed by the basal cone epithelial belt and the junction is situated much more distally than at the dorsal and ventral sides were deep klefts are found between the cone and the muscle plates (Figs 1, 8). The position of the nuclei of the basal belt could not be determined with certainty. Nuclei of the basal cone epithelium have not been encountered.

Fig. 3. Cross section showing the proximal belt of the sheath epithelium (S_2) with nucleus, a tip of the muscle plate covered by the folded basal belt (*B*) and the cone lined by the apical cone epithelium (*A*). Scale bar: 2 µm.
Fig. 4. Section of S_2, *B* and *A*, with g_1, g_2, g_3 and g_9 gland necks. Scale bar: 1 µm.



Figs 5-8. Placorhynchus octaculeatus.
Fig. 5. Section of a muscle plate covered by the folded basal belt (*B*) and the apical cone epithelium(*A*), pierced by g_1, g_2 and g_3 gland necks. Scale bar: 0.5 µm.
Fig. 6. Section of the lateral side of the proboscis showing concentrations of gland necks (mainly g_1), central and lateral longitudinal muscles and a lateral margin of a muscle plate. Scale bar: 2 µm.

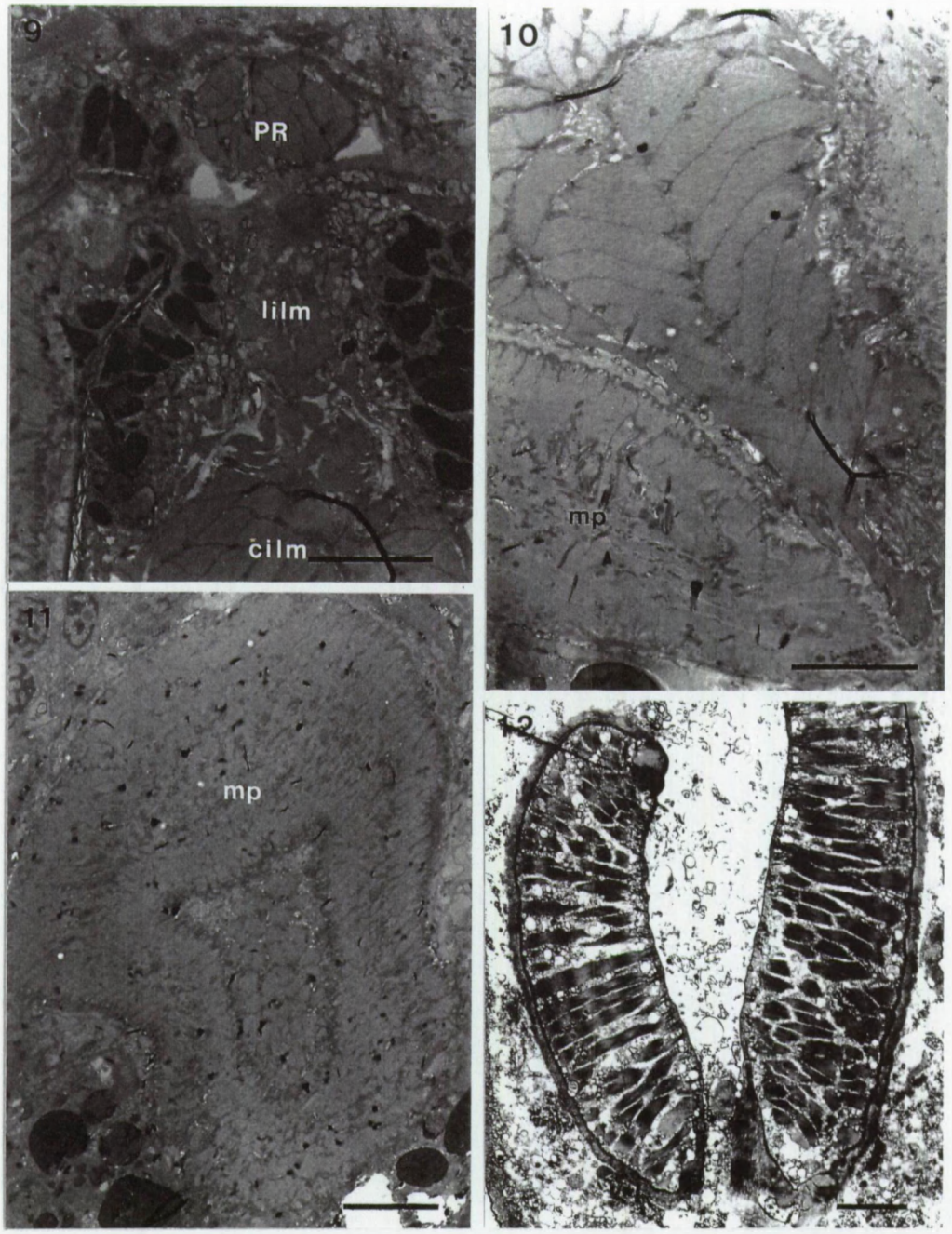
Glands. The distal belt of the sheath epithelium is void of gland necks as the major part of the proximal belt. Only the posterior part of the cells of the proximal belt are pierced by two types of gland necks at the lateral sides as present in the subsequent part of the basal cone epithelium which lines the posterior most part of the cavity laterally (Fig. 4). The epithelium, situated anteriorly of the muscle plates, on the muscle plates itself and dorsally and ventrally on the cone, below the nuclei of the apical cone epithelium, are void of gland necks. Most conspicuous are two types of gland necks at the lateral sides of the proboscis in the posterior part of the sheath epithelium (also around the edges of the muscle plates) and in the cone epithelium in the four corners at the lateral edges of the muscle plates (Fig. 6). They surface near the junction through the posterior part of cells of the proximal belt of the sheath epithelium as well as through the basal and apical cone epithelium. The most numerous are type g_1 gland necks which are upto 3 µm long and closely packed with elongated upto 1000 nm long, electron dense secretion granules (Figs 4, 5, 6). Type g_2 gland necks appear between the former and contain closely packed 700 nm wide, moderately electron dense secretion granules ovoid or slightly elongated of shape (Fig. 5). Type g_3 gland necks store closely packed 700 nm wide secretion granules with granular contents (Fig. 5). In the apical cone epithelium distally of the nuclei, two types of gland necks are found. Type g_9 gland necks are present in the basal region and contain irregular shaped secretion granules with flocculent contents about 300 nm in diameter. Near the apex many small gland necks (type g_{10}) with ovoid upto 400 nm long, moderately electron dense secretion granules pierce the epithelium (Fig. 4).

Sensory cells. Somewhat below the proboscis pore, the distal belt of the sheath epithelium is pierced by multiciliary receptors with short ciliary shafts (Figs 1, 2). The ciliary axonemata are composed of single microtubules only and ciliary rootlets are absent. Basally in the sheath epithelium and in the cone epithelium uniciliary receptors are present as well (Fig. 4). Those in the sheath epithelium have long rigid ciliar shaft, the ones in the cone possess blunt ciliary processesses. The proximal belt of the sheath epithelium contains four groups of uniciliary receptors with rigid ciliary shafts at the junction (Fig. 6). They are situated laterally of the muscle plates.

Proboscis musculature

Outer proboscis musculature. Only longitudinal muscles surround the sheath epithelium in *P. octaculeatus*, outer circular muscles lack. Below the proboscis pore

Fig. 7. Basal belt with spherical, electron dense extensions. Scale bar: 0.5 µm.
Fig. 8. Section through a nucleus of *A* and opposite muscle plate, surrounded by a thick ECM layer. *Arrow*: transverse muscle fibre connecting the lateral margins. Scale bar: 5 µm.



Figs 9-11. Placorhynchus octaculeatus.
Fig. 9. Section proximally of Fig. 6. Lateral proboscis retractors (*PR*), central (*cilm*) and lateral inner longitudinal muscles (*lilm*) and concentrations of glands opposite the lateral margins of the muscle plates. Scale bar: 5 µm.

twelve groups of longitudinally oriented muscle fibres are present; they can be divided into a ventral (3), ventrolateral (4), latero-ventrolateral (6), lateral (3), dorsolateral (10) and dorsal (5) group at either side (numbers indicate the number of separate muscle fibres). At the level of the cone, the muscle fibres form two lateral groups and seem to be embedded in the epithelial basement membrane or a layer of extracellular matrix (Fig. 3).

The motional muscles comprise protractors, proboscis retractors and integument retractors. The exact organization could not be reconstructed from our sections, a extensive description of these muscles is given by Karling (1931, 1947). Two proboscis retractors insert on the lateral side of the bulb below the junction (Figs 6, 9) and can be homologized with the anterior set of proboscis retractorsin the *Cicerina*-group. The muscle fibres form two delimited bundles which run posteriorly in the body and adhere on the epidermal basement membrane.

Inner proboscis musculature. The inner musculature comprises circular and longitudinal muscle fibres. Sparse cirucular muscles are present in the basal part of the cone from the level of the anterior tip of the muscle plates on. The appear as thin fibres which run directly under the basement membrane of the cone epithelium. Below the junction at the lateral side of the proboscis, transverse fibres connect the extracellular matrix at the two lateral edges of the two muscle plates.

The inner longitudinal muscles resemble the cross striated muscle type without the light H-zones (Fig. 4). The longitudinal muscle fibres can be divided into a central core of about 140 fibres, two groups of lateral longitudinal muscles and peripheral muscle fibres (Figs 6, 9, 10). The fibres in the central core adhere on the apical cone epithelium with desmosomes with wide extracellular matrix in between. Posteriorly the fibres insert on the layer of extracellular matrix at the inside of the fussion of both muscle plates. The thin peripheral muscle fibres are present from the basal part of the cone down into the bulb. In the cone they appear in a single layer between the inner circular muscles and the central core of longitudinal muscles. Each group of lateral muscles is composed of a ventral and a dorsal row of muscle fibres. Apically some muscle fibres adhere on the basement membrane of the cone epithelium but most of them insert on the thick layer of extracellular matrix (1.5-2.5 µm) at the lateral side of the proboscis bulb. The muscle fibers enter the bulb anteriorly of where the two muscle plates unite (Fig. 10). Outside the proboscis the dorsal and ventral bundle curve upwards and downwards respectively.

Fig. 10. Cross section of the dorsal and ventral row of lateral longitudinal muscles in the bulb and the muscle plates (*mp*). Scale bar: 5 µm.
Fig. 11. At the nodus the dorsal and ventral muscle plate are continuous. Scale bar: 5 µm.
Fig. 12. *Carcharodorhynchus flavidus*. Sagittal section of the proboscis showing the bipartite bulb with perpendicular muscle fibres, connecting the ECM layers. Scale bar: 5 µm.

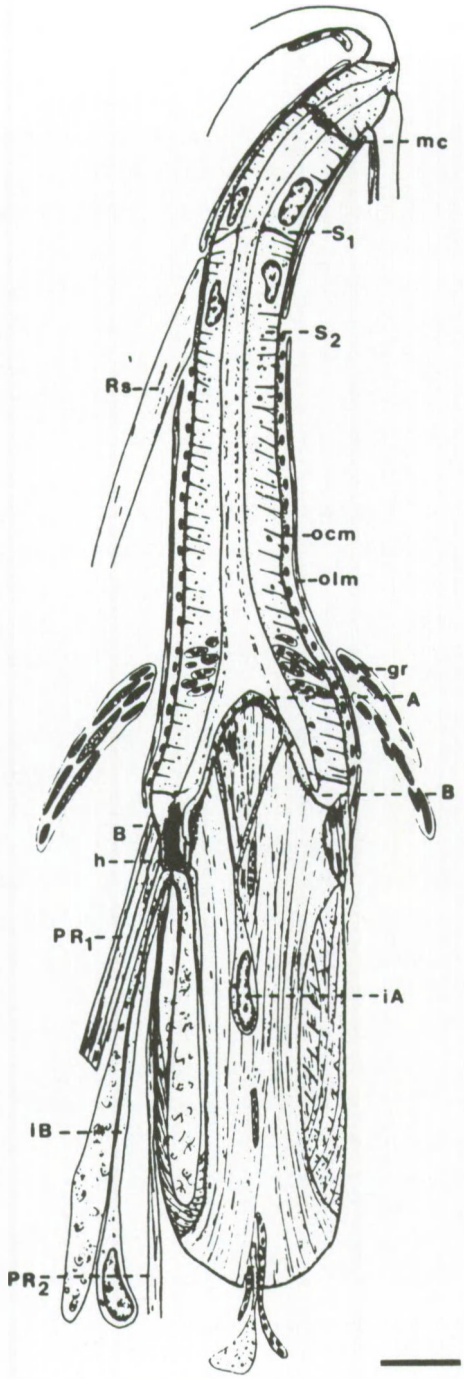


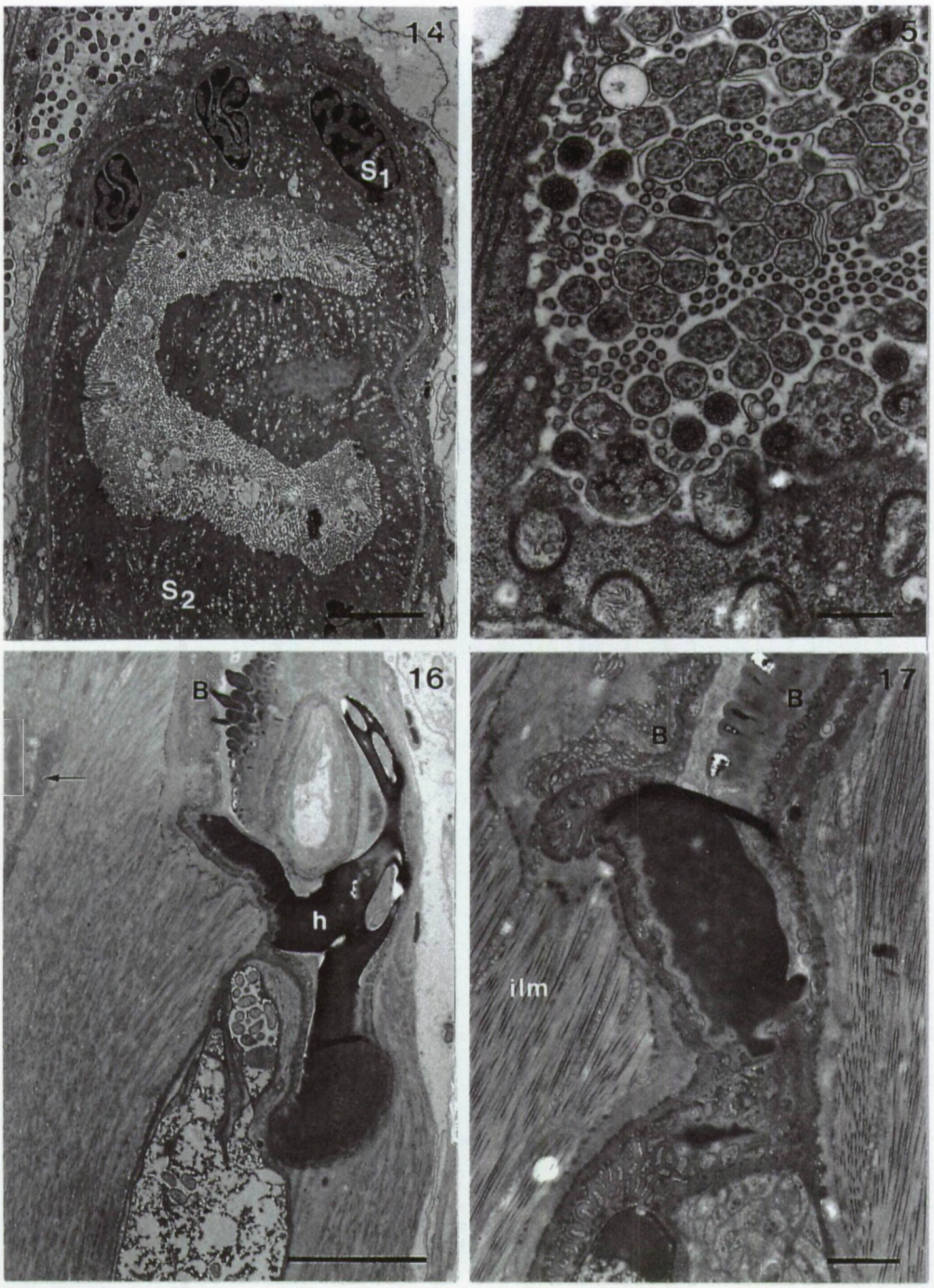
Fig. 13. Reconstruction of the proboscis in *Paragnathorhynchus subterraneus*. Scale bar: 10 µm.

Muscle plates. Most characteristic of the proboscis in Placorhynchidae are the two muscle plates ('Muskelplatten' Karling, 1931). They are formed by transverse lamellae which are oriented perpendicular on the proboscis bulb. The lamellae are caught in a pronounced layer of extracellular matrix (2-5 µm at the outside) (Fig. 8). The lamellae do not contain contractile elements and are separated one from another by transverse septa (ECM) (Fig. 5). The appear as cells with a granular cytoplasm but without nuclei. A extensive folding of the plasmalemma is present where the lamellae are connected to the surrounding ECM-layer (Fig. 5). Patches of ribosomes, mitochondria and few Golgi complexes are present in the cytoplasm along the margins. Individual muscle fibres, which are situated between the lamellae, connect the inner and outer margin of the muscle plates. At the outer side of the muscle plates thin transverse muscle fibres are present between the ECM-layer and the lamellae (Fig. 8). Longitudinal muscle fibres enter the proximal part of the sheath epithelium at the junction and pass through the epithelium where they adhere on the basement membrane of the epithelium which covers the outer free end of the muscle plates (Fig. 1). Below the junction the muscle fibres pass through the thick ECM-layer and run posteriorly to the body wall. In this respect the lamellae appear to be static and moved by thin muscle fibres, as well transverse fibres on the outer margin of the muscle plates which lift the lateral margins as retracting longitudinal fibres.

In the proboscis of *P. octaculeatus*, an extensive network of extracellular matrix is present. The basement membrane under the sheath epithelium is continuous with the lateral septum of the bulb as well as with the layer of extracellular matrix which surrounds the muscle plates (Fig. 6). The extracellular matrix extends into the bulb from the lateral septum and spreads among the inner longitudinal musculature (Fig. 9).

Gnathorhynchidae

The proboscis in Gnathorhynchidae possesses a deep cavity, about 110 µm long in *P. subterraneus*. The proboscis pore is situated subterminal at the ventral side. The proboscis bulb measures 85 µm from apex to nodus but the cone is not very pronounced, not more than 10 µm high. Beside the two hooks at the junction dorsally and ventrally, the proboscis is characterized by a wide glandular ring in the posterior part of the sheath epithelium (not in *G. conocaudatus*) and wide gland necks, which pull through the bulb and surface in the proboscis cavity through the hooks.



Figs 14-17. Paragnathorhynchus subterraneus.
Fig. 14. Transverse section of distal (S_1) and proximal belt (S_2) of the sheath epithelium. Scale

Epithelia, glands and sensory cells

Epithelia. The proboscis epithelia in Gnathorhynchidae are formed by four circumferential belts (Fig. 13). Two belts form the sheath epithelium; one covers the cone and one is found around the junction. The epithelium is void of cilia and cell junctions are formed by apical zonulae adhaerentes (180 nm deep) and subsequent septate junctions.

In *P. subterraneus* the two belts, which form the sheath epithelium, contain four cells each. The distal belt is only 30 µm long, while the major part of the cavity is lined by the proximal belt (80 µm). The uniform epithelium is about 1.5 to 1.7 µm thick and bears slender microvilli 1.7 µm long (Figs 14, 18). The uniform 200 nm thick basement membrane is continuous with the proximal layer of the epidermal basement membrane. The electron dense cytoplasm contains numerous infoldings of the basal plasma membrane, mitochondria, endoplasmic reticulum and empty vesicles (Figs 14, 15). The epithelium, which covers the cone apicallly and lines the sides of the cone as well, is homologous to the apical cone epithelium in other species of Eukalyptorhynchia (De Vocht 1989, 1990). The basal cone epithelial belt is found at the junction, higher up at the side of the sheath epithelium than on the lateral sides of the cone. The syncytial apical cone epithelium is only 700 nm thick and bears 150-180 nm long microvilli (Fig. 18). The basement membrane is apically 100 nm thick and shows some perforations for the nucleiferous cell parts. Basally at the lateral margins of the cone, the basement membrane thickens to a 1.8 µm thick band (Fig. 18). The cytoplasm above the basement membrane contains free ribosomes; the insunk cell parts in the bulb (intrabulbar) possess mitochondria, endoplasmic reticulum and two 14 µm long nuclei. The basal cone epithelial belt is syncytial as well and has a thin basal granular layer (130-200 nm) (Fig. 18). The apical plasmalemma forms folds with intra-epithelial condensations in the underlying apical layer. These condensations can be electron opaque in the lower folds or form thick electron dense proliferations which actually form the proboscis hooks (Figs 16, 17) (see Doe 1976 as well). The nucleiferous cell parts of the basal cone epithelial belt are found laterally behind the bulb and sink in beside the gland necks, which enter the proboscis bulb below the junction (Fig. 13). Four upto 10 µm long nuclei could be counted.

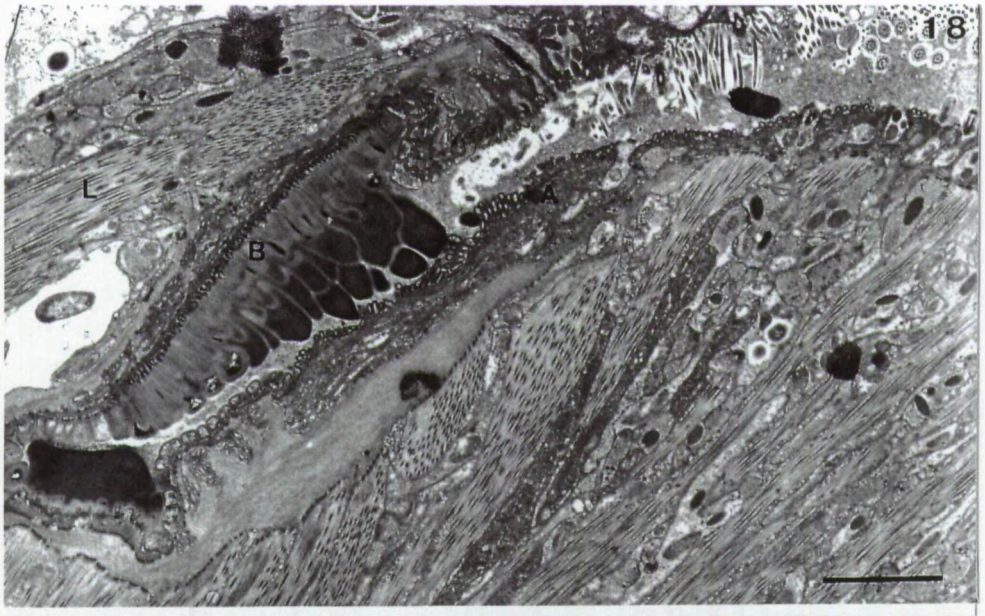
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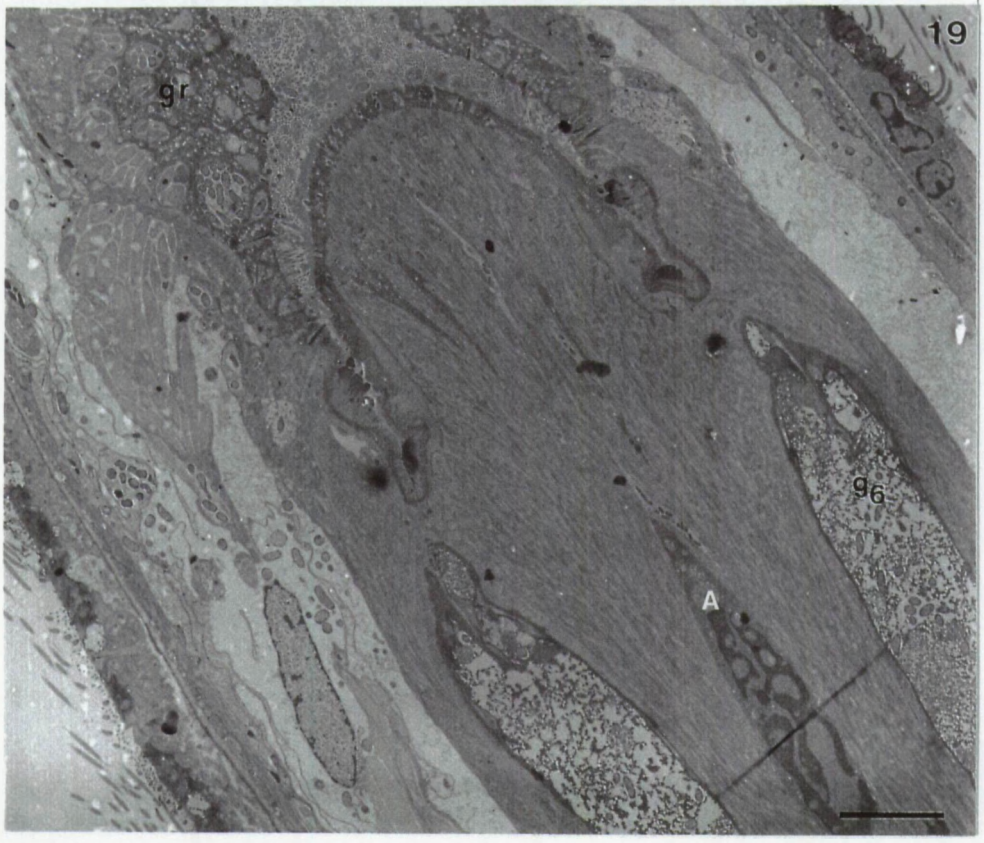
bar: 5 µm.

Fig. 15. Intra-epithelial multiciliary receptors in the distal belt of the sheath epithelium. Scale bar: 0.5 µm.

Fig. 16. Transverse section of a hook (*h*) with basal extension and g_6 gland necks in the basal cone epithelium (*B*). *Arrow*: cytoplasmic cell strands of the apical cone epithelium in the bulb. Scale bar: 5 µm.

Fig. 17. Magnifcation of the basal cone epithelium with intra-epithelial condensations in the apical part of the belt and thcik layer of ECM beneath. At the left inner longitudinal muscles. Scale bar: 1 µm.


18
L
B
A

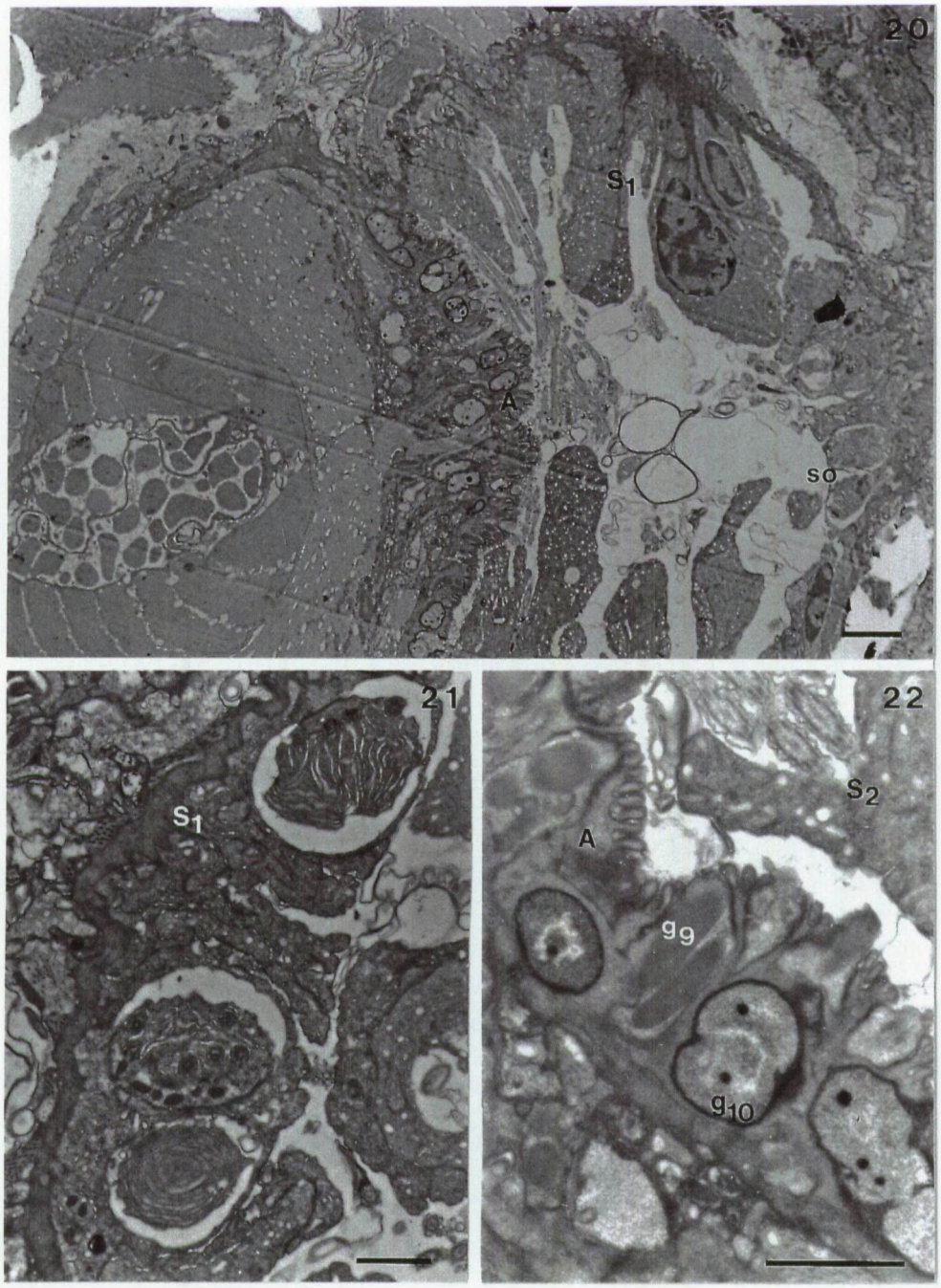

19
gr
g6
A

In *G. conocaudatus* ten cells are present in the sheath epithelium; six cells form the distal belt and four cells constitute the proximal belt. The apical plasmalemma bears no microvilli but contains 200-250 nm deep folds (Fig. 21). The uniform basement membrane is dense and 600 nm thick. The cytoplasm contains infoldings of the basal plasma membrane, endoplasmic reticulum, few mitochondria and empty vesicles. The intra-epithelial nuclei are 6 µm long (Fig. 20). The cone is covered by the apical cone epithelium and the basal belt is found at the junction only as a very narrow strand below the proximal belt of the sheath epithelium between the two hooks. Proliferations of the epithelium in a dorsal and ventral invagination of the basal belt of the cone epithelium give origine to the two proboscis hooks. The microvilli of the apical cone epithelium measure 230 nm in length. The insunk nucleiferous cell parts of the apical cone epithelium are situated around the distal part of the bulb (Fig. 20). The cell strands run under the narrow zone of the basal cone epithelium. The insunk nucleiferous cell parts of the basal belt of the cone epithelium pass together with the gland necks through the surrounding muscle layer at the proximal part of the bulb.

In *D. diodonthus* the organization of the sheath epithelium could not be seized. The median part of the epithelium is 500 nm high and bears 900 nm long microvilli about 10 per linear µm (Fig. 24). The uniform basement membrane is 1.2 µm thick and the electron dense cytoplasm contains infoldings of the basal plasma membrane.

Glands. In *P. subterraneus* two types of gland necks pierce the posterior part of the proximal belt of the the sheath epithelium. The 15-20 µm wide circumferential zone is contains mainly type g_1 gland necks with 1000 nm long ovoid secretion granules (Fig. 19). Below the epithelium basement membrane the necks are stuffed with upto 10 µm long granules. Type g_2 gland necks are less numerous and have a flocculent contents, packed in 600 nm wide spherical secretion granules. The secretory parts of the gland cells are situated behind the bulb. Type g_6 gland necks pierce the basal cone epithelial belt and surface in the cavity through the dorsal and ventral hook (Fig. 16). Each hook contains two wide gland necks next to each other, which pass through the intrabulbs (Karling 1983), and two gland necks, which enter the proboscis below the junction and which are situated laterally of the intrabulbar gland necks. The latter, extrabulbar gland necks join the

Figs. 18-19. Paragnathorhynchus subterraneus.
Fig. 18. Transverse section of the lateral extension of a hook formed by the basal cone epithelium (*B*), the apical cone epithelium (*A*) with g_9 gland necks covers the cone . Levator muscles (*L*) adhere on the basement membrane. Scale bar: 2 µm.
Fig. 19. Transverse section showing the proboscis cone and proximal belt of the sheath epithelium with concentration of gland necks (*gr*). In the bulb a nucleus of the apical cone epithelium (*A*) is situated between the wide g_6 gland necks which are surrounded by muscle fibres and an ECM layer. Scale bar: 5 µm.



Figs 20-22. Gnathorhynchus conocaudatus.
Fig. 20. Oblique section of the proboscis showing sensory organs (*so*) and a nucleus in the distal belt of the sheath epithelium (S_1), a part of the cone with the apical cone epithelium (*A*) and *g6* or *g7* gland necks in the muscular pad. Note the absence of microvilli in the sheath epithelium.

intrabulbar necks at the base of the hook. The gland necks in the bulb are surrounded by several layers of diagonal oriented muscle fibres (intrabulbs), which are lacking at the periphery of the bulb just below the hooks (Fig. 19). The gland necks, which run through the parenchyma have their secretory parts behind the brain. The electron dense nucleiferous cell parts, posterolateral of the bulb, belong to the basal cone epithelium and not to the gland cells (Karling 1983, Meixner 1938). The necks contain spherical, 1700 nm wide secretion granules with flocculent contents. The granules in the intrabulbs are mostly dissolved. The apical cone epithelium is pierced by numerous small gland necks (g8) filled with ovoid 500 nm long secretion granules (Fig. 18). The gland necks enter the bulb through the nodus and the secretory parts are located behind the proboscis.

In *G. conocaudatus* the sheath epithelium is void of gland necks, there is no glandular girdle above the junction. The basal cone epithelial belt is pierced by four wide gland necks (g_6 or g_7) with electron dense secretion granules upto 1300 nm wide (Fig. 20). The apical cone epithelium is pierced by two types of gland necks; type g_9 with 1000 nm long ovoid electron dense secretion granules and type g_{10} with spherical secretion granules 750 nm in diameter with flocculent contents with small electron dense condensations and dense margins (Fig. 22).

In *D. diodonthus* a wide glandular girdle is present in the posterior part of the sheath epithelium as well. The moderately electron dense, ovoid secretion granules in type g_1 gland necks are 1.5 μm long (Fig. 24). The basal cone epithelium is pierced by gland necks (type g_6 or g_7), which contain electron dense spherical but often deformed secretion granules upto 1700 nm in diameter.

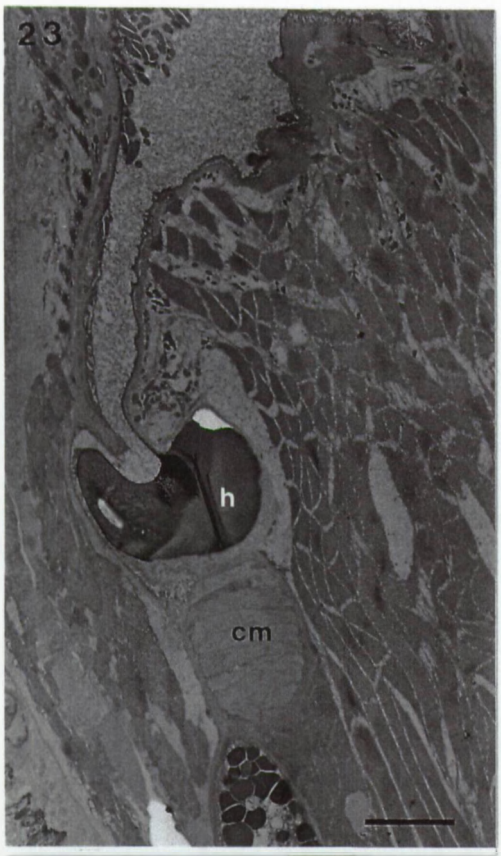
Sensory cells. Both uni- and multiciliary receptors are present in the proboscis epithelia of *P. subterraneus*. Uniciliary receptors with primary and short slanting secondary rootlets pierce the two belts of the sheath epithelium (Fig. 14). Uniciliary receptors with well developed primary rootlets but without secondary rootlets pierce the proximal belt of the sheath epithelium just before the basal belt of the cone epithelium lines the posterior part of the cavity. The distal belt of the sheath epithelium is close below the pore circumferentially pierced by numerous multiciliary receptors (Fig. 15). The short ciliary shafts protrude into the proboscis cavity. The ciliary 9+2 axonemata expanding from the basal bodies reduce to single microtubules apically. Ciliary rootlets lack. Uniciliary receptors with blunt ciliary processes and short rootlets are found in the apical cone

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Scale bar: 2 μm.

Fig. 21. Intra-epithelial sensory organs in S_1 with flat ciliary shafts forming concentric rings. Scale bar: 1 μm.

Fig. 22. The apical cone epithelium (*A*) with g_9 and g_{10} gland necks and short microvilli. A group of uniciliary receptors is present in the proximal belt of the sheath epithelium (S_2), of which the apical plasmalemma shows a few infoldings. Scale bar: 1 μm.





epithelium as well. Receptors are present in the base of the hooks in the basal cone epithelium.

In *G. conocaudatus* twelve groups of uniciliary receptors penetrate the sheath epithelium above the junction (Fig. 20). The closely associated long and rigid ciliar shafts have normal axonemata. Thick rootlets which pierce the basement membrane are present. The distal belt of the sheath epithelium contains ten to twelve spherical sensory organs in its epithelium (Fig. 21). These sensory organs are formed by multiciliary receptors with thin sheet-like ciliary processes. The axonemata are formed by single microtubules as well.

In *D. diodonthus* groups of conspicuous multiciliary receptors with normal axonemata and thick, 5.5 µm long rootlets are present in the proximal belt of the sheath epithelium apically of the extensive annulus of gland necks (Fig. 24).The holotype of *A. glandulis* shows a bipartite sheath epithelium; two nuclei are present in the distal and six in the proximal belt. The small proboscis bulb is dorsally and ventrally flanked by long muscular extrabulbs (Karling 1983), which surround gland necks. These gland necks surface in the cavity through the

Figs 23-24. Drepanorhynchides diodonthus.
Fig. 23. Transverse section showing a part of a hook (*h*), circular muscles (*cm*) of the muscular pad and *g6* or *g7* gland necks. Scale bar: 5 µm.
Fig. 24. A group of uniciliary receptors and *g1* gland necks in the proximal belt of the sheath epithelium. Scale bar: 1 µm.

two hooks. The hooks are less robust than in Gnathorhynchidae. The organization of the cone epithelium could not be clarified. The muscle fibres in the extrabulbs are oriented longitudinally.

Proboscis musculature

Outer proboscis musculature. Circular and longitudinal muscles surround the sheath epithelium in Gnathorhynchidae. The longitudinal muscles are continuous with the longitudinal muscles in the body wall and are present from the proboscis pore upto the junction (Figs 14, 18). Distally, the fibres are usually very thin but from the retractors of the sheath on they are more pronounced. In *D. diodonthus* their number exceeds 120. In the three species, the outer circular muscles lack below the distal part of the sheath epithelium, they appear proximally of the insertion of the retractors of the sheath. The retractors of the sheath are most pronounced at the dorsal side. In *D. diodonthus* the circular muscle fibres are embedded in the basement membrane under the proximal belt of the sheath epithelium.

The organization of the motional muscles could not be fully clarified from our sections and the information given here is only partial. Bundles of longitudinal muscles adhere on the base of the hooks and run along the sides of the bulb against the septum or extend caudad, peripherally of the insunk cell parts of the basal cone epithelium (Fig. 16). The muscles come of the bulb at the posterolateral side and proceed posteriorly in the body. They function as levators (extensors) of the hooks, antagonistic to some inner longitudinal muscle which adhere on the inner processes of the hooks and function as flexors. They are probably homologous to the first set of proboscis retractors in some Cicerinidae. Laterally of the hooks, in *P. subterraneus*, short longitudinal muscles connect the basement membrane below the proximal part of the sheath epithelium, which is formed by the basal belt of the cone epithelium, with the extracellular matrix surrounding the type g_3 gland necks below the junction (Figs 13, 17). In *G. conocaudatus* fixators which insert on the epidermal basement membrane at the level of the junction, adhere on the proboscis just below the junction. Laterally proboscis retractors adhere on the bulbar septum.

Inner proboscis musculature. In all three species of Gnathorhynchidae only longitudinal muscles are found within the septum. The fibres adhere on the cone epithelial basement membrane by numerous small desmosomes (Fig. 17). Dorsally and ventrally, some muscle fibres adhere on the basement membrane below the inner processes of the hooks and function as depressor (flexors). A circumferential inner circular muscle layer is lacking. In *P. subterraneus* two muscular pads or groups of flat and broad muscle fibres with diagonally arranged myofibrils are present around the wide type g_3 gland necks, dorsally and ventrally (Figs 13, 19). They are surrounded by and separated from the inner longitudinal muscles by

a layer of extracellular matrix. The muscles are situated laterally of the gland necks. The organization of the myofibrils resembles the cross-striated muscle type; A- and I-zones are present as well as Z-discs but H-zones are lacking. In *G. conocaudatus* the fibres of the muscular pads which surround the two dorsal and ventral gland necks are oriented transversally. They fully enclose the two gland necks except apically below the hooks (Fig. 20). In *D. diodonthus* the gland necks are laterally flanked by oblique, almost transverse muscles, enclosed by a layer of extracellular matrix as well (Fig. 23). The appearance of the "muscular pads" or intrabulbs differs for the different genera. In *Gnathorhynchus* these muscle fibres form a closed cylinder, while in *Paragnathorhynchus* and *Drepanorhynchides* spirally winded fibres partially enclose the gland necks.

Discussion

Karling (1947) mentioned the presence of numerous nuclei in the sheath epithelium for Placorhynchidae in his extensive and thorough description of *Placorhynchus octaculeatus*, *P. echinulatus* and *Clyporhynchus monolentis*. The absence of outer circular muscles is probably a common feature for all Placorhynchidae. As noted by Karling (1947), circular muscles are only present at the pore and form a distal sphincter. Insunk cell bodies below the junction as described for *P. octaculeatus* and *C. monolentis* are not present in our material and could be misinterpreted for the concentrations of gland necks at the junctions in between the muscle plates (Karling 1931, 1947, 1952). From the electron microscopic sections it is clear that the epithelial cell parts laterally between the muscle plates are not insunk but that a narrow slit of the cavity is present and that a very thin epithelium covers the cone or bulb. The intrabulbar nuclei as described for *P. octaculeatus* by Karling (1931) are not present. In the drawings of this species in his later paper of 1947, intrabulbar nuclei can not be discerned, although they are still mentioned in the text. However, intrabulbar nuclei are present in *C. monolentis* (Karling 1947). The nuclei of the apical cone epithelium have been noted by Karling as well (1931 *Fig. 36 n4*,1947 *Abb. 1*).

The gland cells with weakly stained cytoplasm along the sides of the bulb mentioned by Karling (1947) could be insunk epithelial cell parts of the basal cone epithelium and the extensive loose mass of ECM laterally of the bulb.

The posterior proboscis retractors in *P. octaculeatus* and *Clyporhynchus monolentis* mentioned by Karling (1947) must be identical to the extrabulbar parts of the peripheral inner longitudinal muscles. As noted by Karling (1947), the inner longitudinal muscles, which insert on the posterior part of the septum, are homologe to the central cylinder of longitudinal muscles in other species as for instance in the family Cicerinidae. Inner circular muscles are present as well in the cone as in the anterior part of the bulb. They are present at the same level as the muscle plates and do not show a connection to the muscle plates. Therefor the muscle plates and the inner circular muscles can not be considered to have the same origin.

From light microscopic sections the vertical lamellae of the muscle plates can be discerned as well (Karling 1931, 1947, 1963), but the lamellae can not be ascribed a contractile function because of the absence of contractile filaments in the lamellae itself. With a strong eosinophylic coloration of the cytoplasm of the lamellae in light microscopic sections, the muscle plates have a lobated appearance; with a predominantly cyanophylic coloration of the sections, the surrounding and intermediate layers of ECM show most (Karling 1947). They rather form a hydrostatic body enclosed by a thick layer of ECM and positioned by thin individual muscle fibres. The muscle plates can not be considered as derivatives of the inner circular muscles as postulated by Meixner (1929) and Karling (1947). The muscle plates are situated outside the bulb, peripheral of the inner longitudinal and circular muscle layer. They must be regarded as new structures without homologe in other eukalyptorhynch genera.

The structure of the two muscular parts of the proboscis in Schizorhynchia might resemble the muscle plates in Placorhynchidae in shape, ultrastructurally their organization is distinctly different. In Schizorhynchia the muscular parts of the bulb are formed by muscle fibres with contractile filaments connecting the dorsal and ventral margins (Fig. 12) (Rieger and Doe 1975). The epithelium, which covers the muscular parts has the same folded appearance as the epithelium lining the inner side of the muscle plates in *Placorhynchus*. The occurence of cytoplasmic sheets in these epithelia probably has a functional meaning in order to overcome the high friction by the scraping of the muscular parts or muscle plates against the sheath and cone epithelium.

The proboscis in species of the eukalyptorhynch families Gnathorhynchidae and Aculeorhynchidae is typified by the presence of proboscis hooks. However, the relationship of these families within the Eukalyptorhynchia is uncertain. Within the Gnathorhynchidae great differences in organization of the copulatory organ exist in respect to the position of the ejaculatory duct and the presence of a stylet or penis papilla, the position of the pharynx and structure of the proboscis, especially of the hooks and muscular structure (Ax 1952, 1953, Brunet 1966, 1973, Den Hartog 1968, Karling 1947, 1956, L'Hardy 1963, 1964, 1966, Meixner 1929). Most Gnathorhynchidae possess a deep proboscis cavity. The epithelium, however, is only formed by two belts, especially the proximal belt constitutes the major part of the sheath epithelium. A bipartite sheath epithelium is present in many families such as Cicerinidae, Psammorhynchidae, Cytocystidae, Bertiliellidae, Placorhynchidae and probably in Aculeorhynchidae as well. A bipartite sheath epithelium is considered as a plesiomophic charcacter state. The cellular organization of the both belts and the intraepithelial position of the nuclei are regarded plesiomorphic as well. Nuclei in the sheath epithelium are present in all Gnathorhynchidae except for *Neognathorhynchus suecicus*. The large insunk cells at the junction, of which the nucleiferous parts are not included in the drawing (Karling 1956). Intrabulbar nuclei of the apical cone epithelium as in *P. subterraneus* were also noted by

Karling (1983) and are present in *Odontorhynchus lonchiferus* and *Prognathorhynchus campylostylus* as well (Karling 1947). The position of these nuclei in *G. conocaudatus* is the same in *Uncinorhynchus flavidus* (Karling 1947). The notation of intrabulbar nuclei for the latter species in Karling (1952) must in my opinion be based on a mixing of the species *U. flavidus* and *O. lonchiferus*. Intrabulbar nuclei are present in different families such as Cicerinidae, Psammorhynchidae, Cytocystidae, Koinocystididae, and have to be regarded parallel tendencies in proboscis formation. In the three gnathorhynchid species, which have been investigated, the hooks are formed by intra-cellular differentiations in the basal belt of the cone epithelium as described for *Gnathorhynchus* sp. by Doe (1976). The shape of the proboscis hooks is typical for the different genera within the Gnathorhynchidae. The horseshoe-shaped base of the proboscis hooks is most pronounced in and typical for the genus *Uncinorhynchus*. A webfoot-like base of the hooks is encountered in *Gnathorhynchus* , round basal plates in *Prognathorhynchus* or ovale to square basal plates are encountered in *Odontorhynchus*, *Psittacorhynchus* and *Orbiculorhynchus* (Brunet 1973, Den Hartog 1968, Karling 1947, 1956, L'Hardy 1964, Noldt 1989).

Distinctive for the three investigated species of Gnathorhynchidae is the presence of a large ring of gland necks surfacing in the posterior part of the sheath epithelium. Although not always mentioned, these gland necks are usually present in all gnathorhynchid species. The gland necks are not only filled with secretion in the apical part piercing the epithelium but typically a large amount of secretion is stored in the necks under the epithelium.

Circumferential organized uni- or multiciliary receptors in the proximal belt of the sheath epithelium as in *Paragnathorhynchus* and *Gnathorhynchus* are present in some species of Cicerinidae, such as *Cicerina*, *Paracicerina* and *Ptyalorhynchus*, as well. The scattered groups of receptors in *Drepanorhynchides* are considered homologous to these receptors. The presence of this kind of receptors indicates a close relationship between Gnathorhynchidae and species of the *Cicerina*-group and Placorhynchidae. The presence of eight tot ten spherical sensory organs with multiciliary receptors in the distal belt of *Gnathorhynchus* must represent a parallel evolution in comparison to the two insunk spherical sensory cells in species of the *Cicerina*-group. Both types of sensory organs have probably evolved from intra-epithelial multiciliary receptors as present in representatives of most families of Eukalyptorhynchia.

"Muscular pads" (Den Hartog 1968), "Muskelwülsten" or "intrabulbs" (Karling 1947, 1983), "Muskellängswulst" (Meixner 1929), "Bourrelets musculaires" (L'Hardy 1963, 1966, Brunet, 1966) are present in all species of Gnathorhynchidae except for species of the genus *Uncinorhynchus* (Brunet 1973, Karling 1947, 1952, 1963). In this genus inner circular muscles are present; they lack in species and genera with muscular pads. A transformation lineage can be discerned from inner circular muscles, not separated from the longitudinal muscles by extracellular matrix in *Uncinorhynchus*, over the presence of dorsal

and ventral pads of muscle lamellae without inner layer of extracellular matrix in *Neognathorhynchus* and broad muscle pads enclosed by ECM in *Prognathorhynchus, Odontorhynchus, Psittacorhynchus* and *Orbiculorhynchus* to cylindrical closed muscle pads in *Gnathorhynchus, Drepanorhynchides, Ancistrorhynchus* (Brunet 1973, Den Hartog 1968, Karling 1947, 1956, L'Hardy 1963, Meixner 1929, Noldt 1989). The "muscular pads" may resemble the dorso-ventrally opposing muscles plates in Placorhynchidae but the fine structure is distinctly different and does not permit to homologize muscular pads in Gnathorhynchidae and muscle plates in Placorhynchidae. The muscular pads are formed by muscle fibres and not by lamellae as in the muscle plates of Placorhynchidae. Protractors inserting at the nodus are present in species of *Gnathorhynchus, Uncinorhynchus, Prognathorhynchus* and *Odontorhynchus* mostly with additional protractive musles, which insert on the anterior part of the bulb (Brunet 1973). The system of motional muscle is very variable within the family but seems to be constant within each genus. If no or incomplete sectioned material is available this character has to be treated with care. According to Karling protractors are lacking in *Neognathorhynchus* and *Gnathorhynchus* (1956). The retractors of the pore in Gnathorhynchidae (see also Karling 1956) are continuous with the longitudinal muscle of the body wall as in Cystiplanidae (De Vocht 1989). A double set of proboscis retractors seems to be present in Gnathorhynchidae. The first set functions as levators of the hooks. In *Uncinorhynchus*, a anterior pair of proboscis retractors inserts laterally on the proboscis at the junction. Three pairs of retractors adhere on the posterior part of the bulb (Karling 1947). The author mentions two pairs of anteriorly situated retractors inserting on the median part of the bulb and two pairs inserting on the posterior part of teh bulb for *Prognathorhynchus duhius*. In *Odontorhynchus*, only one pair of retractors adheres posteriorly on the bulb (Karling 1947). *G. conocaudatus* was reported to have a anterior (one pair) and posterior (five pairs) set of proboscis retractors (Meixner 1929). For *Orbiculorhynchus* only proboscis retractors inserting on the bulb have been recorded (Noldt 1989). Both sets are proboably presetn in *Neognathorrhynchus suecicus* as well (Karling 1956) and have been described for *Ancistrorhynchus ischnurus* by L'Hardy (1963).

The close affinity between *Aculeorhynchus glandulis* and Gnathorhynchidae, more particulary the genus *Paragnathorhynchus* has been pointed out by Karling (1983). The author concluded that *A. glandulis* and all the genera of the Gnathorhynchidae form a monophyletic taxon characterized by the presence of two dorsoventrally opposed hooks in the proboscis. A proboscis with a pair of extrabulbar glands (long gland necks), reduced proboscis bulb and large secretory granules in the proximal part of the sheath epithelium of the proboscis form autapomorphic characters for *A. glandulis*. A part of the family Gnathorhynchidae forms the sister taxon of *A. glandulis*.

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Chapter 7.

The anatomy and ultrastructure of the proboscis in Cystiplanidae. [1]

Introduction

The family Cystiplanidae comprises four genera. The family was established by Karling (1964) for the species *Cystiplex axi* and *Cystiplana paradoxa*.. Brunet (1965) included the species *Cystirete graefei*, Schilke (1970) the species *Nigerrhynchus opithoporus* and Dean (1977) *Cystiplana paradoxa*. Type material of all species has been studied and the proboscis of *Cystiplex axi* and *Cystiplana paradoxa* have been examined by electron microscope. From light microscopic observations a well developed cone, with clearly marked apex and a cytoplasmatic girdle surrounding the bulb at the junction of sheath and cone epithelium are known. Insunk nucleiferous cell parts have been recorded for *Cystiplex axi* (Karling 1964).

Materials and methods

Specimens were collected from sandy sediments in the Mediterranean; *Cystiplex axi* at Calvi (Corse) at 10 meters depth, *Cystiplana paradoxa* at Ile des Embiez (France) at 3 meters depth. The holotypes of *Cystiplex axi* and *Cystiplana paradoxa* as well as paratypes of *Cystiplana rubra* and *Cystirete graefei* from the Swedish Museum of Natural History (SMNH) have been studied by light microscope. Sectioned material of *Nigerrhynchus opisthoporus* was provided by Dr. U. Noldt from the university of Göttingen. Life observations of *Cystiplana karlingi* Hoxhold, n. nud. (1974) were made at List on the isle of Sylt (Germany).

Results

The description is valid for *Cystiplex axi* and *Cystiplana paradoxa*, differences are stated explicitly. The proboscis in both species has a length of approximately 160 to 210 um.

Epithelia, glands and sensory cells

Epithelia. The epithelium lining the proboscis cavity and covering the cone consists of five belts (Fig. 1). A first belt, surrounding the proboscis pore is formed by two cells, while two syncytial belts constitute the rest of the sheath epithelium. The next belt covers

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[1] The contents of this chapter has been published in Zoomorpholgy 109: 1-10.

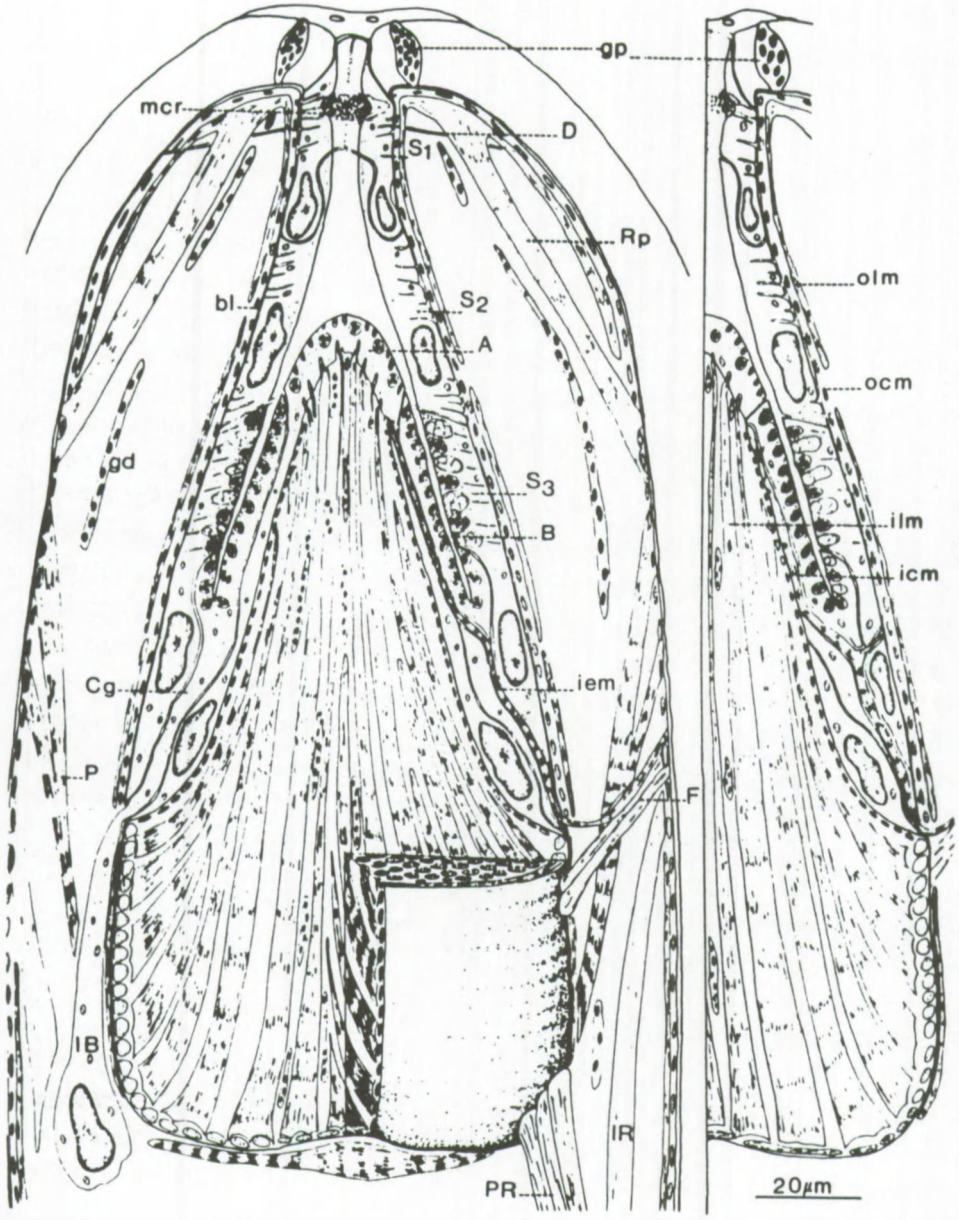


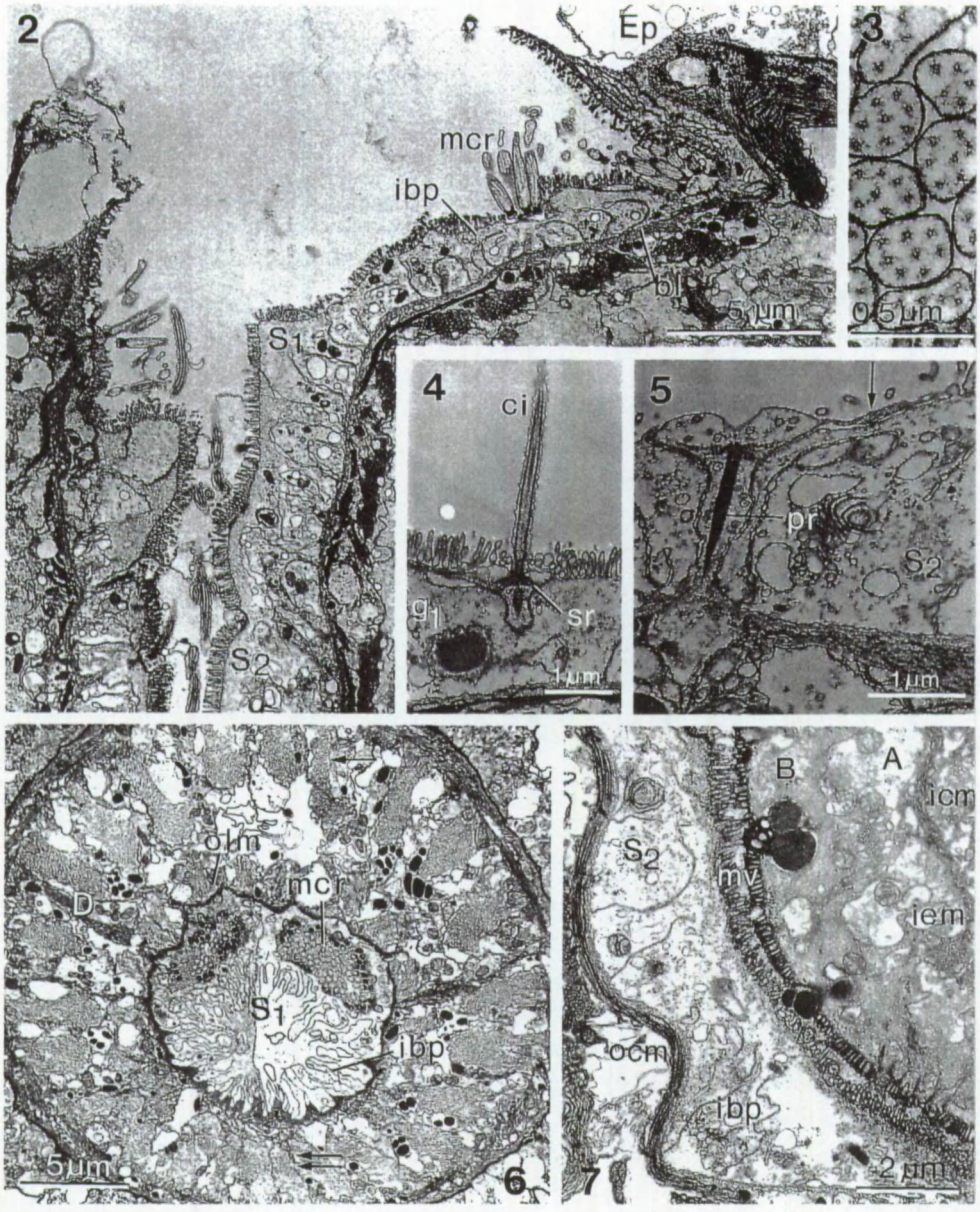
Fig. 1. Reconstruction of the proboscis of *Cystiplex axi* and *Cystiplana paradoxa* from electron microscopic observations. The left half of the proboscis of *Cystiplana paradoxa*, showing the insunk cell part of the basal cone epithelium, is left out.

the most proximal part of the cavity wall and the basal part of the cone. The apex is covered by a syncytial cap. Proximally of the junction of sheath and cone epithelium, cell parts of the proximal cavity belt, the basal and the apical cone epithelium form a cytoplasmic girdle around the anterior part of the proboscis bulb. This girdle is surrounded by the basement membrane and by the circular and longitudinal muscle layer underlying the sheath epithelium. The various belts are interconnected by inconspicuous zonulae adhaerentes, septate desmosomes and maculae adhaerentes. Contact between epithelial elements and gland necks or sensory cells are made by circumferential septate desmosomes. All belts are devoid of cilia.

At the pore two cells constitute the first short belt of the sheath epithelium, which bears a wreath of multiciliary receptors in a circular groove (Fig. 2). The two cells contain some mitochondria with electron dense matrices and bear irregularly arranged microvilli. The basal plasma membrane shows numerous infoldings (Fig. 2 and 6). Proximally, separate cell strands slip under the distal end of the next belt. Two of these saggings contain the lobate nuclei of both cells. The second belt is syncytial, containing up to five lobate nuclei. The basal plasma membrane forms the same infoldings as in the previous belt and the syncytium contains few mitochondria with electron dense matrices (Fig. 7). The numerous microvilli (12 per linear um) are shorter (400 nm) than those of the epidermis. A few gland necks pierce this belt, they are filled with spherical 750 nm wide, moderately electron dense secretion granules (g_1) (Fig. 4). The next circumferential belt lining the proboscis cavity is syncytial as well and has a dense cytoplasm. It is characterized by many penetrating gland necks which can be divided into three types: one with 700 nm wide, electron dense granules (g_2), one with 900 nm wide moderately electron dense secretion granules (g_3) and one with large, 1000 nm wide, empty vesicles (g_4) (Fig. 8). The microvilli are shorter (260 nm), more electron dense and more densely packed (14 per linear µm) than those in the previous belts. The nuclei (up to five) are located peripherally in the cytoplasmic girdle (Fig. 1). Underlying the cavity epithelium, a basement membrane is found continuous with the internal layer of the epidermal basement membrane (Fig. 2).

The cone epithelium is subdivided into two syncytia. At the junction a circumferential belt lines the proximal part of the cavity and covers the basal part of the cone. The apex is covered by a separate syncytium, which forms a cap (Fig. 1).

The superficial part of the basal cone epithelium covers cell strands of the most proximal belt of the sheath epithelium and the apical cone epithelium (Fig. 9). The apical plasmalemma bears densely packed microvilli (15 per linear µm) about 250 nm long. They have characteristic electron dense tips (Figs 8, 11, 13). The cytoplasm is divided in a superficial fibrillar and a basal granular part. The granular layer is characterized by free



Figs. 2-7. Cystiplex axi
Fig. 2. Electron micrograph of the proboscis pore in *Cystiplex axi*. Note the characteristic infoldings of the basal lamina (*ibp*) and the multiciliary receptors (*mcr*) with blunt cilia and without rootlets.
Fig. 3. Cross section of cilia (*ci*) belonging to the multiciliary sensory cells, showing eight singlets.

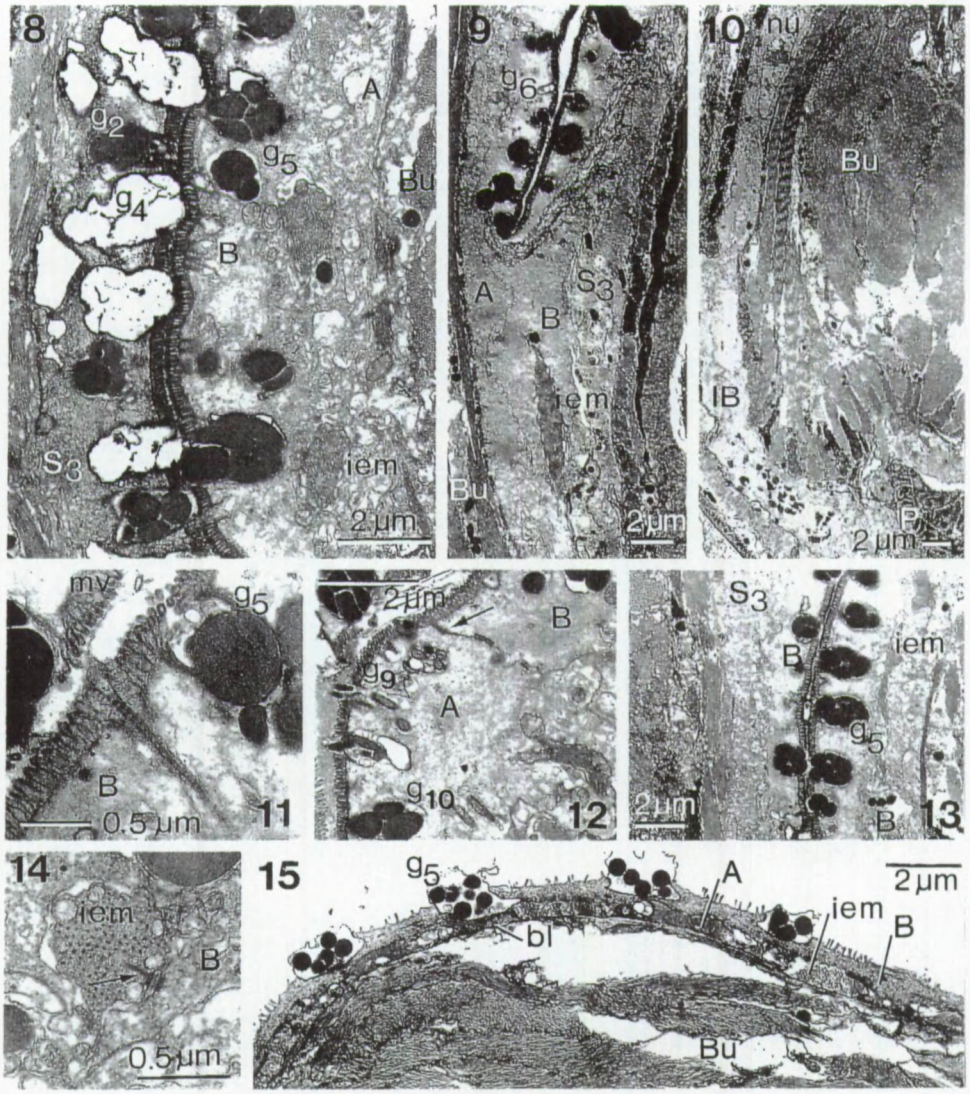
ribosomes and mitochondria. The fibrillar layer forms a kind of cell web (2.5 µm thick), through which numerous gland necks erupt. The most conspicuous gland necks contain a spherical, electron dense secretion (g_5), 700 nm in diameter in *Cystiplex axi* and 460 nm in *Cystiplana paradoxa*. The secretion of the second type of glands penetrating this belt is washed out, leaving the small and inconspicuous gland necks empty (g_6) (Fig. 9). The secretion granules have a diameter of 200-250 nm. In *Cystiplana paradoxa*, the part of this belt lining the cavity is adventitiously pierced by the same empty gland necks as the adjacent belt of the sheath epithelium. In the cytoplasmic girdle, a circumferential cell strand is situated between the nucleiferous cell parts belonging to the proximal belt of the cavity epithelium and the apical cone epithelium (Fig. 9). It connects the superficial with the insunk nucleiferous cell parts (Fig. 10). The basement membrane surrounding the cytoplasmic girdle is continuous with the septum surrounding the bulb. Twelve perforations leave passage to the nucleiferous cell parts of the basal cone epithelium (Fig. 10). The insunk cell parts form six groups around and just beneath the proximal part of the bulb. Up to nine nuclei could be counted in the insunk cell parts.

The syncytium, covering the apex, is 4.5 µm high. The underlying loose and irregular basement membrane is sometimes hard to discern. The superficial part of the syncytium is characterized by piercing gland necks, containing small, rodlike (700 nm long , 170 nm broad), electron dense granules with dense periphery and core (g_9) or spherical (700 nm in diameter), electron dense granules (g_{10}) (Fig. 12). Their ducts run among the cone retractors, entering the bulb at the nodus. The distal endings of the cone retractors intrude half way in the epithelium (Fig. 12). Like the microvilli of the basal cone epithelium, those of the apical syncytium are shorter (280 nm) than those of the sheath epithelium and supported by a dense peripheral layer. However, they lack the dense tips. In *Cystiplex axi*, a circumferential cell strand underneath the basal cone epithelium connects the superficial part of the apical cone epithelium with the nucleiferous part in the cytoplasmic girdle (Fig. 13). In *Cystiplana paradoxa*, 32 separate cell strands connect the superficial with the

Figs. 4-5. Sensory cells in the median and proximal belt of the sheath epithelium. Note the well developed primary rootlet (*pr*) and the slanting secondary rootlets (*sr*), as well as the dishlike protrusion (*arrow*) on the epithelium surface.

Fig. 6. Cross section of the anterior body end, showing in the centre the distal belt of the sheath epithelium (S_1) composed of two cells. The multiciliary receptors (*mcr*) and infoldings of the basal plasma membrane (*ibp*). Surrounding the sheath epithelium dilatators (*D*) can be seen, as well as longitudinal muscles underlying the sheath epithelium (*olm*), connected to the retractors of the proboscis pore and the longitudinal muscles of the body wall (*single/double arrow* : cross section of longitudinal muscle of the body wall and retractor of the pore, without/with outer longitudional muscles).

Fig. 7. Cross section of the sheath (S_2)and cone epithelium (*B*), which covers cell strands of the apical cone epithelium (*A*) and intra-epitlelial muscles (*iem*). Note the stout microvilli with dense tips of the *B*, the inner cirucular and longitudinal muscles.



Figs. 8-14. Cystiplex axi.
Fig. 8. Cross section of the proximal belt of the sheath epithelium (*S3*) and basal cone epithelium (*B*). Two types of gland necks (*g2* and *g4*) pierce the sheath epithelium and type *g5* glands the cone epithelium. Notice the intra-epithelial muscles (*iem*) and cell strands of the apical cone epithelium (*A*).
Fig. 9. Transverse section of the junction and part of the cytoplasmatic girdle. The bulb (Bu) and the cone are situated at the left. The basal cone epithelium (B), with type *g5* and *g6* gland necks forms the junction. From the periphery to the bulb, cell strands of the proximal belt of the sheath epithelium, the basal (*B*) and apical cone epithelium (*A*) can be seen. Note the intra-epithleial muscle (iem).

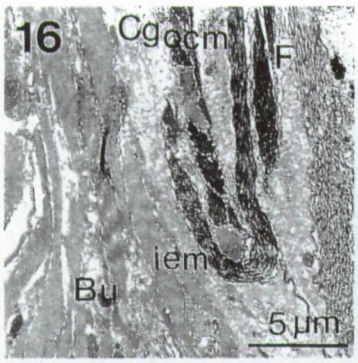
nucleiferous part (Fig. 15). As well as lobate, ovoid nuclei, the nucleiferous cell parts also contain mitochondria, free ribosomes and dense bodies (Fig. 10).

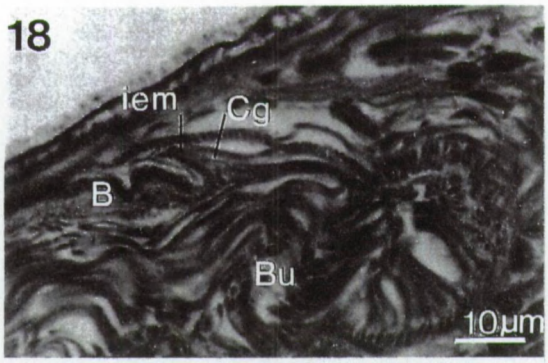
Sensory cells. Three distinct types of receptors are found in the proboscis epithelia. Multiciliary receptors, already mentioned above, are limited to the distal belt of the sheath epithelium and located in a circular groove. The sensory cells pierce the epithelium and form dishlike extensions on its surface, bearing several modified cilia. The cilia lack rootlets and their basal bodies are situated above the epithelium surface. The aberrant axonemata of the short cilia are composed of one central and seven peripheral singlets (Figs 2, 3). Some clear vesicles are found in the dishlike extension and the penetrating cell parts. The cell necks are attached to the epithelium by means of septate desmosomes. Monociliary sensory cells are mainly located in the proximal half of the median belt. The apical part of these receptors extends in a dishlike protrusion, resting on the epithelium surface. These sensory cells possess well developed rootlets directing downwards and short secondary rootlets, which radiate slanting from the basal body to the plasma membrane (Figs 4, 5). Receptors with short rootlets and blunt ciliary processes are spread throughout the cone epithelium (Fig.11). They barely exceed the microvilli and are easily overlooked.

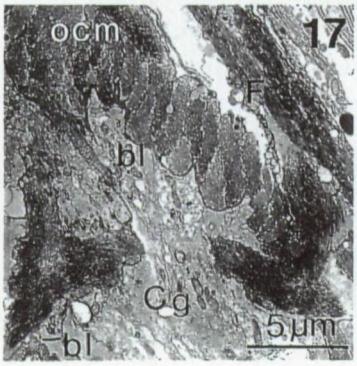
Proboscis musculature

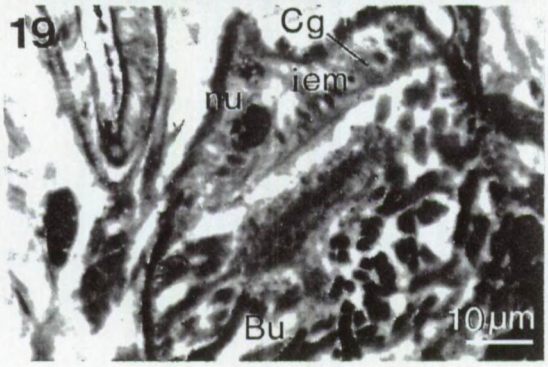
Outer proboscis musculature. The musculature of the body wall is continuous with the circular and longitudinal muscle layer surrounding the sheath epithelium (Fig. 2). In *Cystiplex axi*, 24 longitudinal muscles underlay the sheath epithelium, in *Cystiplana paradoxa* their number is 32. They are attached to the septum at the lateral side of the bulb. The longitudinal muscles are not only continuous with those in the body wall but also

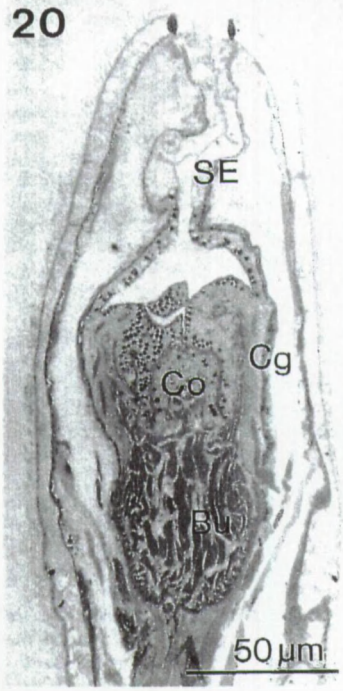
Fig. 10. Transverse section of the hind part of the bulb (*Bu*) and cytoplasmatic girdle with insunk cell parts of the basal cone epithelium (*IB*). A nucleus (nu) of the apical cone epithelium is located in the cytoplasmatic girdle. Protractor muscles (*P*) join each other at the nodus.
Fig. 11. Receptors with rootlet and blunt ciliary process in the basal cone epithelium (*B*). Notice the *g5* type of gland necks with circumferential septate desmosomes and the stout microvilli with dense tips (*mv*).
Fig. 12. Transverse section of contact zone (*arrow*) of the basal (*top*) and apical cone epithelium (*bottom*) with stout microvilli and type *g9*and *g10* gland necks.
Fig. 13. Cross section of the basal cone epithelium (*B*) and proximal belt of the sheath epithelium, clearly showing the fibrous superficial layer, microvilli with dense tips and intra-epithelial muscles (*iem*) in the former.
Fig. 14. Cross section of an intra-epithelial muscle (*iem*) making contact with the basal cone epithelium (*B*) by means of a macula adhaerens. Notice the dense body in the muscle and the central condensation in the intercellular space.
Fig. 15. Organization of the basal cone epithelium (*B*) in *Cystiplana paradoxa* with g5 type glands and alternating intra-epithelial muscles (iem) and cell strands of the apical cone epithelium (*A*) located against the basal lamina (*bl*).


16
Cg
ocm
F
iem
Bu
5 µm

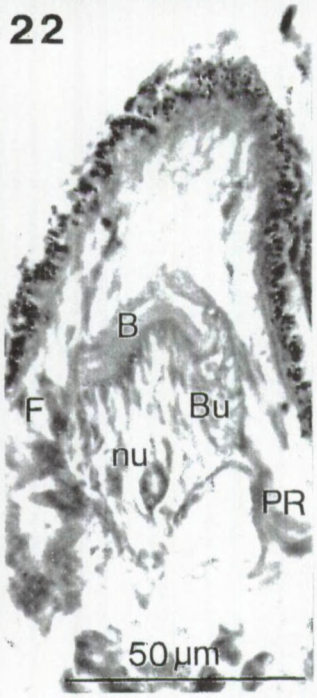

18
iem
Cg
B
Bu
10µm


ocm
17
F
bl
Cg
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19
Cg
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10µm


20
SE
Cg
Co
Bu
50µm


21
50µm
Cg
Bu
IB


22
B
F
Bu
nu
PR
50µm

with the 24 or 32 retractors of the proboscis pore (Figs 1, 6). Besides retractors of the pore, a set of 24 or 32 dilators of the pore is also found (Figs 1, 6). They consist of thin and single muscle fibres connecting the epidermal basement membrane with the basement membrane of the sheath epithelium. From and down from the transition zone between the second and third belt of the sheath epithelium, the longitudinal layer is composed of some 50 muscle fibres, because a second set of muscles appears in between the former. They are attached to the distal part of the bulb and form the principal component of the longitudinal muscle layer surrounding it. Only a longitudinal layer surrounds the bulb. The circular muscle layer, surrounding the cavity, is more pronounced towards the junction and encloses the cytoplasmic girdle as well (Figs 2, 7, 9, 10).

The motional muscles include fixators, protractors, retractors and integument retractors. Six protractors, forming the nodus of the proboscis, twelve fixators and three (*Cystiplana paradoxa*) or four pairs (*Cystiplex axi*) of integument retractors are attached to the epidermal basement membrane at the level of the cytoplasmic girdle (Fig. 20). The fixators seize upon the distal part of the bulb, beneath the cytoplasmic girdle. They are arranged in six groups, two by two, in this way that the insunk epithelial cell parts are situated in between the groups. Their attachment on the epidermal basement membrane is situated behind the insertion of the protractors and in front of the insertion of the integument retractors. The insertion of the protractors is bifurcated, one branch inserting just above the fixators, the other more anteriorly (Fig. 1). The protractors resemble the cross-striated muscle type, with clear A- and I-zones, sometimes with Z-discs but without H-zones. Four pair of retractors insert on the hind part of the bulb.

Fig. 16. Bulb (*Bu*) and cytoplasmatic girdle (*Cg*) in *Cystiplex axi*. An intra-epithelial muscle (iem) joins the fixators (*F*).

Fig. 17. Cystiplana paradoxa, oblique section showing bifurcations of the intra-epithelial muscles (*iem*). At the left the splitting results in one end connected to the basal lamina of the sheath epithelium (*bl*) and one end running into the cytoplasmatic girdles (*Cg*) to the right. The distal end of the muscle adheres to the basal lamina of the cone epithelium (*bl*) at the bottom of the picture. At the right, the splitting results in one branch joining the fixators (*F*) and one adhering on the outer longitudional muscles.

Fig. 18. Light microscopic transverse section (3µm) of the proboscis in *Cystiplana rubra* showing the bulb (*Bu*), basal cone epithelium (*B*), cytoplasmatic girdle (*Cg*) surrounded by circular muscles and intra-epithelial muscles (*iem*).

Fig. 19. Light microscopic oblique section of the proboscis of *Cystirete graefei*. A part of the cytoplasmatic girdle (*Cg*) with nuclei (*nu*) and intra-epithelial muscle (*iem*) can be seen.

Fig. 20. Semithin section of the proboscis of *Cystiplex axi* showing the overall organization of sheath epithelium (*SE*), retracted cone (*Co*), cytoplasmatic girdle (*Cg*) and proboscis bulb (*Bu*).

Fig. 21. Light microscopic semithin section of the proboscis of *Cystiplana paradoxa* with nuclei in the cytoplasmatic girdle (*Cg*) and insunk cell parts (*IB*) beside the bulb (*Bu*).

Fig. 22. Light microscopic sagittal section of the proboscis of *Nigerrhynchus opisthoporus* (leg. Noldt) with a nucleus (*nu*) inside the bulb (*Bu*), high uniformly stained basal cone epithelium (*B*), fixators (*F*) and proboscis retractors (*PR*) adhering on the side of the bulb.

Inner proboscis musculature. The inner musculature consists of longitudinal muscles surrounded by a single layer of circular muscles. The furcated insertion of the longitudinal muscles is caught onto the septum surrounding the bulb between the fibres of the inner circular muscles. Their distal ends insert on the cone epithelium. Like the protractors and the outer circular and longitudinal musculature, the inner musculature resemble the cross-striated muscle type. The inner circular muscle layer surrounds the cone retractors in the bulb and in the cone up to the superficial contact zone between the apical and basal cone epithelium. Muscles fibres from the cytoplasmic girdle on are thinner than those in the bulb. The inner musculature appears to be anucleated.

Intra-epithelial muscles. At the proximal end of the cytoplasmic girdle, muscle fibres appear in between the cell parts of the basal cone epithelium and the proximal belt of the sheath epithelium (Figs 16, 17). They resemble the cross-striated muscle type. Their number differs for the two genera, 24 for *Cystiplex axi* and 32 for *Cystiplana paradoxa*. In the cytoplasmic girdle, the intra-epithelial muscles pass through the basal cone epithelium and run under the basal cone epithelium in the cone (Fig. 9). In *Cystiplex axi* they are situated between the basal belt and the underlying cell strand of the apical belt. They finally sink into the latter and are connected directly to the epithelial plasma membrane by means of maculae adhaerentes. From the attachment site, dense bodies run to the centre of the muscle fibre, perpendicular to the myofilaments (Fig. 14). The distal tip of the muscles is connected to the basement membrane of the apical syncytium. In *Cystiplana paradoxa* the intra-epithelial muscles alternate with cell strands of the apical cone epithelium. They are connected to the basement membrane underlying the cone epithelium, from the junction up to the upper edge of the inner circular muscle layer (Fig. 15). At the junction the muscles are bifurcated, one end running into the cytoplasmic girdle, the other short end is fixed laterally to the basement membrane surrounding the sheath epithelium in the distal part of the cytoplasmic girdle. The proximal end of the fibres is bifurcated, one short branch adheres directly on the longitudinal muscles surrounding the sheath and the bulb. The other longer branch, twisting a little to the right or left, slips through the basement membrane surrounding the sheath epithelium and joins the fixators (Figs 16, 17). This end is fixed to the epidermal basement membrane together with the fixators, protractors and integument retractors.

Discussion

According to Karling (1964), *Cystiplana paradoxa* has no insunk nucleiferous cell parts, but only a cytoplasmic girdle with some nuclei, surrounding the distal part of the bulb. The holotype, however, clearly shows six groups of insunk cell parts containing about 9 nuclei (Fig. 21). Contrary to the observations of Dean (1977), a cytoplasmic girdle with nuclei and intra-epithelial muscles could be discerned in the paratypes of *Cystiplana rubra* deposited in

the S.M.N.H. (Fig. 18). The number of intra-epithelial muscles certainly exceeds 30 and might well be 32 as in *C. paradoxa*. Life observations on *C. karlingi* revealed the same organization as could be seen in *C. paradoxa* and *C. rubra*. *Cystirete graefei* possesses a small proboscis which is about 160 µm long. A nucleiferous girdle at the junction and insunk nucleiferous cell parts, as mentioned in the description could be verified on the paratypes. Intra-epithelial muscles, not mentioned by the author, are present as well; their number could not be determined (Fig. 19). Schilke (1970) mentions a small, 140 µm long proboscis in *Nigerrhynchus opisthoporus*, the sheath epithelium connected to and as long as the bulb. In the sectioned material of *N. opisthoporus* (leg. Noldt) a different type of proboscis is found. The sheath epithelium is fairly thin except for the thickened proliferations in the proximal part. The basal cone epithelium is thickened and differently stained, resembling a intracellular support which still remains flexible (Fig. 22). The small epithelial cap covering the apex is somewhat thinner than the basal cone epithelium. Several nuclei can be seen inside the bulb. Three or four of them are located near the junction and one is found in the posterior end of the bulb. The shape of the bulb is not spherical as in Schilkes description but has a elongation at its posterior end due to contraction of the proboscis retractors adhering terminally on the bulb near the nodus. The proximal part of the bulb is surrounded by a loose parenchymatous tissue. A cytoplasmic girdle and intra-epithelial muscles are lacking. The type of proboscis is identical to the one found in *Lekanorhynchus remanei* Meixner, 1938. At this moment, we can not decide if this sectioned material is identical to *N. opisthoporus* Schilke, 1970 or *L. remanei* Meixner, 1938. Or if both species are one and the same.

The proboscis epithelia in Cystiplanidae, Polycystididae and Koinocystididae are formed by five circumferential belts. Syncytial belts are encountered in species of all three families. In Cystiplanidae the distal belt lining the proboscis cavity is composed of two cells, whereas the more proximal belts are syncytial as in *Polycystis naegelii* (Schockaert & Bedini 1977). The main characteristics of the distal and median belt of the sheath epithelium are the infoldings of the basal plasma membrane and the microvilli, without obvious internal support. In *P. naegelii* the infoldings can be seen as ribbonlike arrangements of small vesicles (Schockaert and Bedini 1977 *Figs 5, 6*). Together with the swollen microvilli of the epidermis, we regard this as an artefact due to osmotic damage. The bipartite sheath epithelium is mostly fully cellular and shows the same characteristic as the currently investigated species (De Vocht and Schockaert 1988 *Fig. 5*). The proximal belt of the sheath epithelium is to its full extend pierced by numerous gland necks. In *P. naegelii* the gland necks are principally located in the proximal part of this belt, just above the junction (Schockaert & Bedini 1977).

The division of the cone epithelium in a apical part, characterized by gland necks containing a fine secretion and a basal part, characterized by gland necks containing a more

conspicuous contents, is known from light microscopical observations (Dean 1977, Karling 1964, Rieger and Sterrer 1975). From our observations a bipartite cone epithelium is found to be general in all Eukalyptorhynchia. The apical part is often referred to as the sensory part of the cone, because its secretion is rather inconspicuous in the light microscope. The belt, covering the basal part of the cone bears densely packed, stout microvilli with dense tips in *P. naegelii* (Schockaert and Bedini 1977 *Figs 7, 13*) and both cystiplanid species (Figs 8, 13). For *Gyratrix hermaphroditus* (Polycystididae), Reuter (1975) mentions short and coarse microvilli covering the cone epithelium. Reuter's Fig. 6 clearly shows the cone epithelium, divided in an apical and basal belt. The former is pierced by gland necks containing small ovoid, electron dense secretion granules; the latter by gland necks containing a larger spherical, electron dense secretion. Here the microvilli show a dense periphery as well. In *Psammorhynchus tubulipenis* (Psammorhynchidae) and *Cytocystis clitellatus* (Cytocystidae) this belt bears the same kind of microvilli (De Vocht 1990). Rieger and Sterrer (1975) described the same kind of microvilli for the basal secretory part in the cone epithelium of *Florianella bipolaris* (see Doe 1976 *Fig. 6 E*, Rieger 1981 *Fig. 11*, Rieger and Sterrer 1975 *Figs 7 B and C*). In *Gnathorhynchus*, the proboscis hook electron dense material is deposited in the microvilli of the epithelium lining the basal region of the cone (Doe 1976 *Fig. 6*). The appearance of the microvilli of this belt in all investigated eukalyptorhynch families gives an indication that all belts, covering the basal part of the cone epithelium, are homologous.

Except for *N. opisthoporus* (leg. Noldt), all listed species of Cystiplanidae possess a cytoplasmic girdle around the bulb, containing nuclei and intra-epithelial muscles. A cytoplasmic girdle surrounding the distal part of the bulb is not only found in Cystiplanidae but in many Polycystididae as well; e.g. *Phonorhynchus*, *Cincturorhynchus* and *Danorhynchus*, *Scanorhynchus*, *Annulorhynchus* and *Neopolycystis* (Evdonin 1970, Karling 1955, 1956). In *P. naegelii* (Schockaert and Bedini 1977) the proximal sheath epithelium belt and the syncytia covering the cone have insunk nucleiferous cell parts. The number of breakthroughs is not exactly known. In *Danorhynchus duplostylis*, the author describes 12 "Junkturtaschen" which represent bulges of the cytoplasmic girdle. They probably lead towards the insunk cell parts of the basal cone epithelium (Karling 1955 *Fig. 19*). Six groups of insunk cell parts are present in *P. naegelii* and *Typhlopolycystis rubra* (Noldt and Reise 1987).

A circular groove in the distal belt of the cavity epithelium, containing multiciliary receptors with highly modified axonemata and without rootlets, is at present recorded for two out of four genera of Cystiplanidae. The cilia do not exceed the cavity but they can be protruded with a short prolapse of the sheath epithelium. They can be interpreted as tactile- or chemoreceptors, as their position allows either interpretation. This type of intra-epithelial multiciliary receptor in the distal belt of the sheath epithelium is found in many

eukalyptorhynch families, such as Polycystididae, Koinocystididae, Placorhynchidae, Gnathorhynchidae and Bertiliellidae. The monociliary receptors in sheath and cone epithelium are similar to those present in other eukalyptorhynch species.

In Polycystididae three pairs of fixators are found; one pair subdorsal, lateral and subventral. In Cystiplanidae, in contrary, twelve fixators are present. The insunk cell parts of the basal cone epithelium, however, clearly divide them two by two in six groups. In *Opisthocystis goettei* Bresslau, 1906, the fixators also show a splitting at their insertion on the epidermal basement membrane (Meixner 1925). In most Polycystididae, eight protractors are found, the dorsal and ventral pair contain more fibres and often splitting more distally. From light microscopy six protractors (three pairs) are recorded for Cystiplanidae. All species of Cystiplanidae possess four pair of integument retractors, except *Cystiplex axi*, which according to Karling has three pairs. The integument retractors and the proboscis retractors in Cystiplanidae are more numerous as in Polycystididae, where usually one pair of integument retractors and four pairs of proboscis retractors or two pairs of integument retractors and three pairs of proboscis retractors occur. Clearly, we can homologize the proboscis musculature of Polycystididae and Cystiplanidae.

In Cystiplanidae, the inner circular muscle layer is only found in the basal part of the cone. In *P. naegelii* this musculature is found in the cone as well, presumably up to the apex (Schockaert and Bedini 1977 *Figs 13, 14*). In *C. remanei* the inner circular muscle layer is not found in the cone or nucleo-glandular girdle (De Vocht and Schockaert 1988).

In light microscopic descriptions of Cystiplanidae, intra-epithelial muscles were never mentioned (Brunet 1965, Dean 1977, Karling 1964, Schilke 1970). They have been recorded for many Polycystididae (see Schockaert and Bedini 1977). The authors regarded these structures as typical polycystidid features. However, they are present in all species of Cystiplanidae as well, except for *N. opisthoporus*. In *Cystiplana paradoxa*, the organization of the cone epithelium, with 32 intra-epithelial muscle fibres alternating with as many epithelial strands belonging to the apical cone epithelium, very much resembles the organization in *P. naegelii*, where 12 muscles alternate with 12 cell strands (Meixner 1925, Schockaert and Bedini 1977 *Fig. 15*). The latter considered the intra-epithelial muscles and alternating cell strands under the basal cone epithelium typical features for the Polycystididae. In Cystiplanidae they are present as well, stressing the relationship between the two families even more (Karling 1964). The organization in *Cystiplex axi*, where the apical cone epithelium forms a circumferential strand running against the cone retractors, could be considered as derived from an organization as in *Cystiplana paradoxa* or *P. naegelii*. When the separate epithelial strands unite, the intra-epithelial muscles are lifted up and run in between the two belts of the cone epithelium. The organization of the epithelia, where the distal tip of the intra-epithelial muscles are connected to the cone retractors, resembles the organization in *Cystiplana paradoxa* and *P. naegelii*.

Thus far, the Cystiplanidae have been characterized by a high and strongly vacuolated epidermis, a pharynx with neither grasping fold nor cuticular knobs and with dispersed gland pores in its lumen, and a male copulatory apparatus like in Koinocystididae but without penial structures (Karling 1964). The proboscis in Cystiplanidae and Polycystididae are very similar. The proboscis epithelia in both families are composed of five circumferential syncytial belts. Intra-epithelial muscles are also present in both families. A cytoplasmic girdle surrounding the bulb is also present in some Polycystididae and corresponds with the distal part of the insunk nucleiferous cell parts in *P. naegelii* (nuclei of proximal belt of the sheath epithelium and apical cone epithelium). As far as the proboscis musculature is concerned, Cystiplanidae possess six pairs of fixators, three pairs of protractors and four pairs of proboscis retractors and integument retractors. In Polycystididae, only three pairs of fixators and four or five pairs of protractors are found, while the total number of integument and proboscis retractors never exceeds five pairs.

N. opisthoporus was considered to be a member of the Cystiplanidae, mainly because of the organization of the genital system. The epidermis, however, is not vacuolated and flat, detailed information on the proboscis and pharynx is lacking. The organization of the proboscis and its musculature in the material in loan from Dr. Noldt is identical to *Lekanorhynchus remanei*, including the presence of a large intrabulbar nucleus and probably insunk nucleiferous epithelial cell parts behind the bulb (Meixner 1938) (Fig. 22). The systematic position of *N. opisthoporus* still remains obscure, till more data become available.

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Chapter 8.

The anatomy and ultrastructure of the proboscis in Polycystididae and *Marirhynchus longasaeta* Schilke, 1970.

Introduction

The family Polycystididae includes the highest species diversity in Eukalyptorhynchia. About 109 species and 37 genera have been described, divided into ten subfamilies (Evdonin 1977). Schockaert and Bedini (1977) described the ultrastructural organization of the proboscis epithelia as well as the glandular and the sensory elements in *Polycystis naegelii*. The authors found that the proboscis epithelia were formed by five syncytial, circumferential belts and postulated a reduction in the number of belts in the sheath epithelium in some other polycystidid species. From light microscopic observations differences in the organization of the proboscis can be discerned and have been mentioned in descriptions (e.g. Meixner 1925, Karling 1955). The presence of either a cytoplasmic girdle or mantle cells around the bulb have been noted by several authors as Meixner (1925) and Karling (1955). The inversion of the circular and longitudinal muscles surrounding the sheath is another striking feature, which has been recorded for several genera within the Gyratricinae (Karling 1955). Special features in the structure of the proboscis sometimes typify specific genera, such as the thick epithelium covering the base of the cone in *Fungorhynchus pistillatus* (Karling 1952). In this study eleven species of Polycystididae Graff, 1905 and *Marirhynchus longasaeta* Schilke, 1970, species incertae sedis (Karling 1980) have been investigated ultrastructurally.

Materials and methods

Specimens of *Gyratrix hermaphroditus* were obtained from sand in the littoral, specimens of *Typhlopolycystis rubra* were collected from pocket sand of *Arenicola marina* burrows in Königshafen all at List (Sylt, Germany) in September 1985 and April 1988 (Hoxholdt 1974, Noldt & Reise 1987, Schilke 1970). Specimens of *Rogneda palula* and *Polycystis riedli* were collected respectively from fine sediment in an old salina near the Mediterranean and from algae near the water surface at Ile des Embiez (France) in March and October 1987. Specimens of *Gallorhynchus mediterraneus* were obtained from sandy sediment collected by scuba diving at 12 metres depth near Calvi (Corse, France). Specimens of *Neopolycystis tridentata* were collected from sandy sediment at the Belgian coast (Oostende and Knokke) and at List (Sylt, Germany), specimens of *Phonorhynchus helgolandicus* and *Progyrator mamertinus* were collected form algae at 0-12 metres depth at Banyuls-sur-Mer and Calvi (France) and Kristineberg (Sweden) respectively. The specimens of *Danorhynchus*

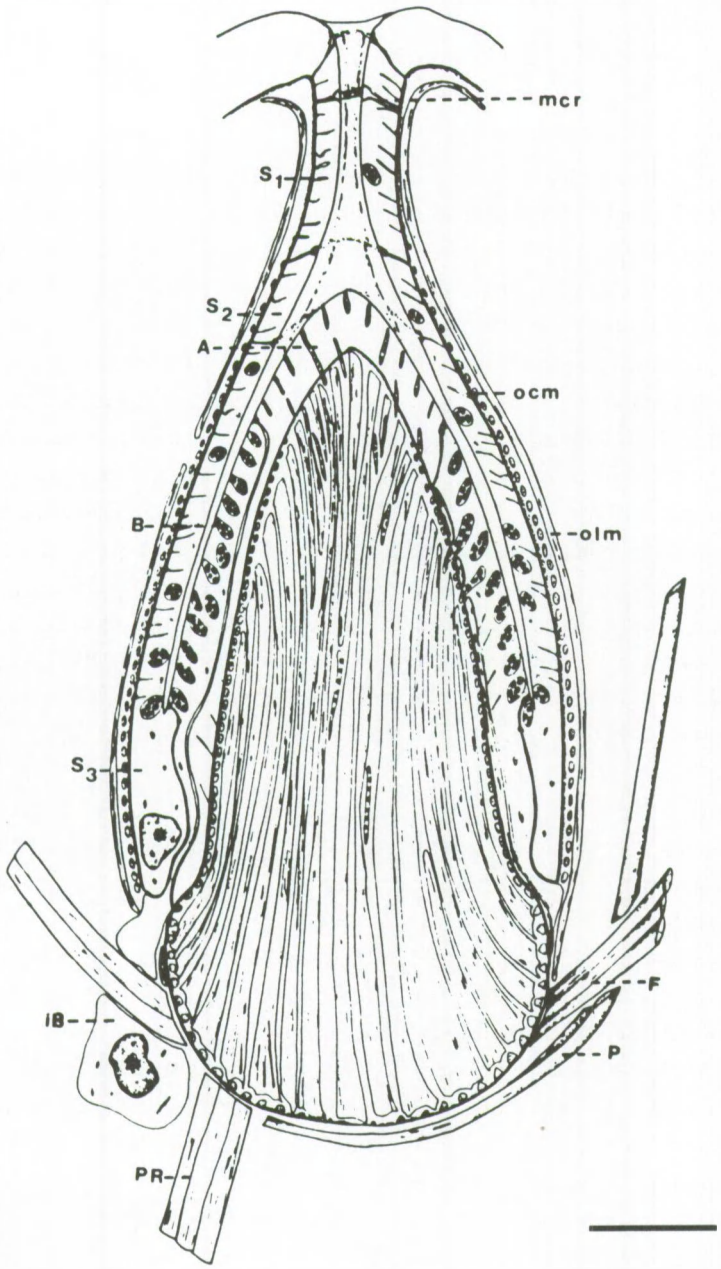


Fig. 1. Reconstruction of the proboscis in *Phonorhynchus helgolandicus*. Scale bar: 10 µm.

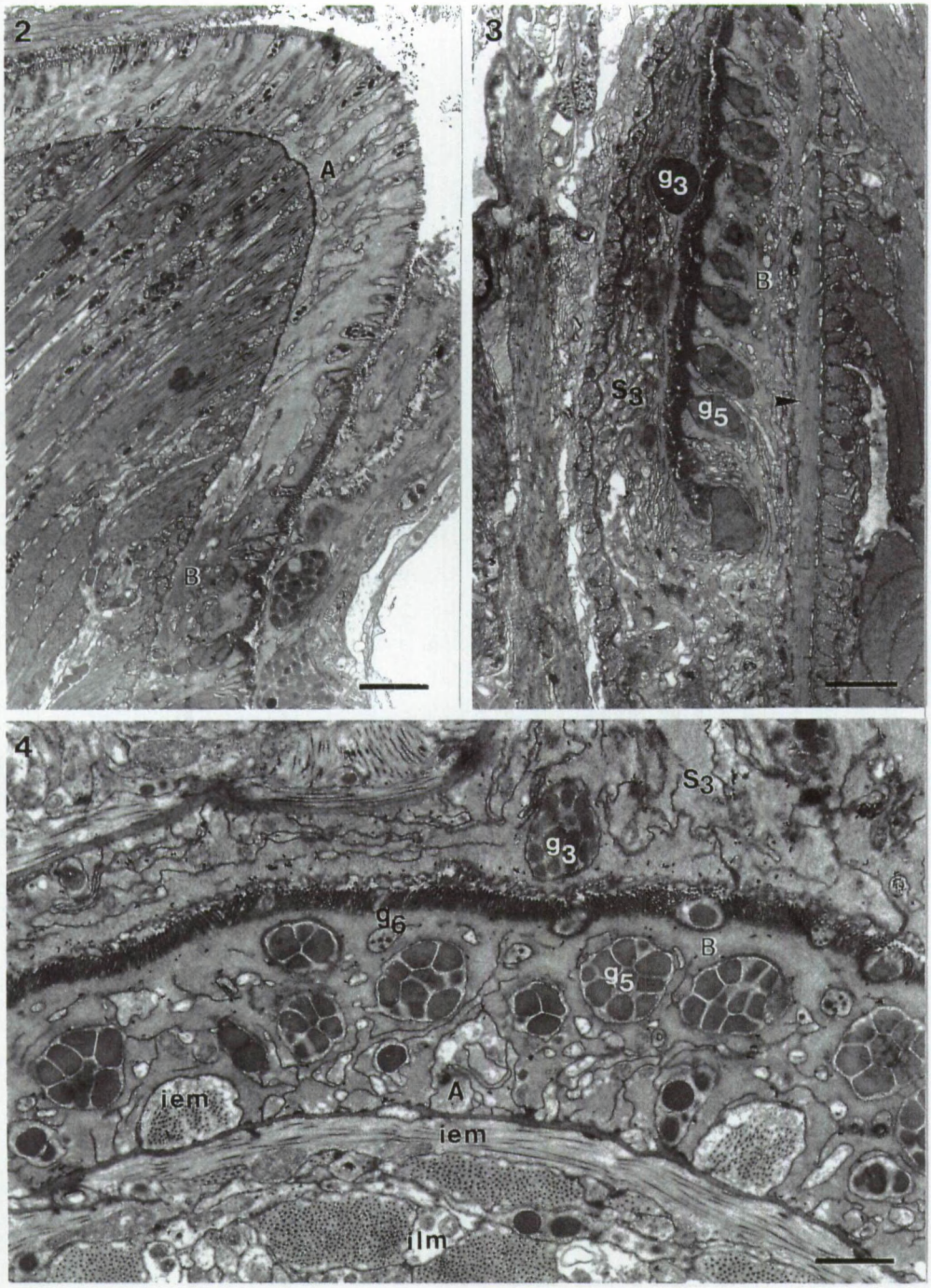
gosoeensis was collected from muddy sediment in the Gullmar-fjord (Swedish West coast, 58°25'31"N and 11°36'23"E) in June 1988. Samples were taken by Ockelmann-sledge at 40 metres depth. *Alcha evelinae* and the new species of *Paraustrorhynchus* were provided by P. Jouk and collected from algae near Mombasa (Kenya) (Jouk & De Vocht 1989). *Marirhynchus longasaeta* was collected from station 12 (see Schilke 1970) at List (Sylt, Germany) and one embedded specimen was provided by Dr. J. Brüggemann as well.

Results

For convenience and because of the stability of the proboscis structure within the genera, the genera names are used in the text to refer to the species investigated. If not indicated, the statements are general for all investigated species.

Epithelia, glands and sensory cells

Most Polycystididae possess a large proboscis which can take up 1/5 of the body length in some genera (*e.g. Typhlopolycystis* and *Danorhynchus*). The proboscis in living animals is characterized by a large cone. Sclerotic differentiations, specialized muscular structures or distinct gland necks are lacking. Only small differences are found in the proboscis organization. The proboscis epithelia in all Polycystididae and *Marirhynchus* are composed of five circumferential belts, three of which constitute the sheath epithelium and two covering the cone (Figs 1, 30). The distal belt of the sheath epithelium is usually short, the median and proximal belts constitute the major part of the cavity epithelium. In *Phonorhynchus*, and to a smaller extend in *Polycystis*, the median belt is reduced and forms a narrow belt above the apex of the cone (Fig. 1). In *Rogneda* the proximal belt is reduced and found as a narrow rim above the junction. The cone is usually covered by two belts but in *Progyrator* and especially in *Alcha*, *Paraustrorhynchus* and *Marirhynchus* a large part of the belt, which lines the proximal part of the sheath epithelium covers the base of the cone as well (Figs 8, 30). In these cases these belts can be called junctional belts because they are found at both sides of the junction. In *Polycystis*, *Rogneda*, *Progyrator*, *Alcha*, *Paraustrorhynchus* nov. spec. and *Marirhynchus*, the basement membrane of the sheath epithelium is continuous with the bulbar septum just below the junction (Figs 9, 10). Nucleiferous cell parts of the proximal belt of the sheath epithelium and the total cone epithelium sink in through perforations at the contact zone. In *Phonorhynchus*, *Typhlopolycystis*, *Gyratrix*, *Danorhynchus* and *Neopolycystis* a cytoplasmic girdle is present below the junction around the proboscis bulb (Figs 1, 5, 14). All belts are void of cilia and cell junctions are formed by apical zonulae adhaerentes, subsequent septate junctions and maculae adhaerentes (Fig. 35). Hardly any gland necks pierce the distal and median belt of the sheath epithelium, while the proximal belt can either

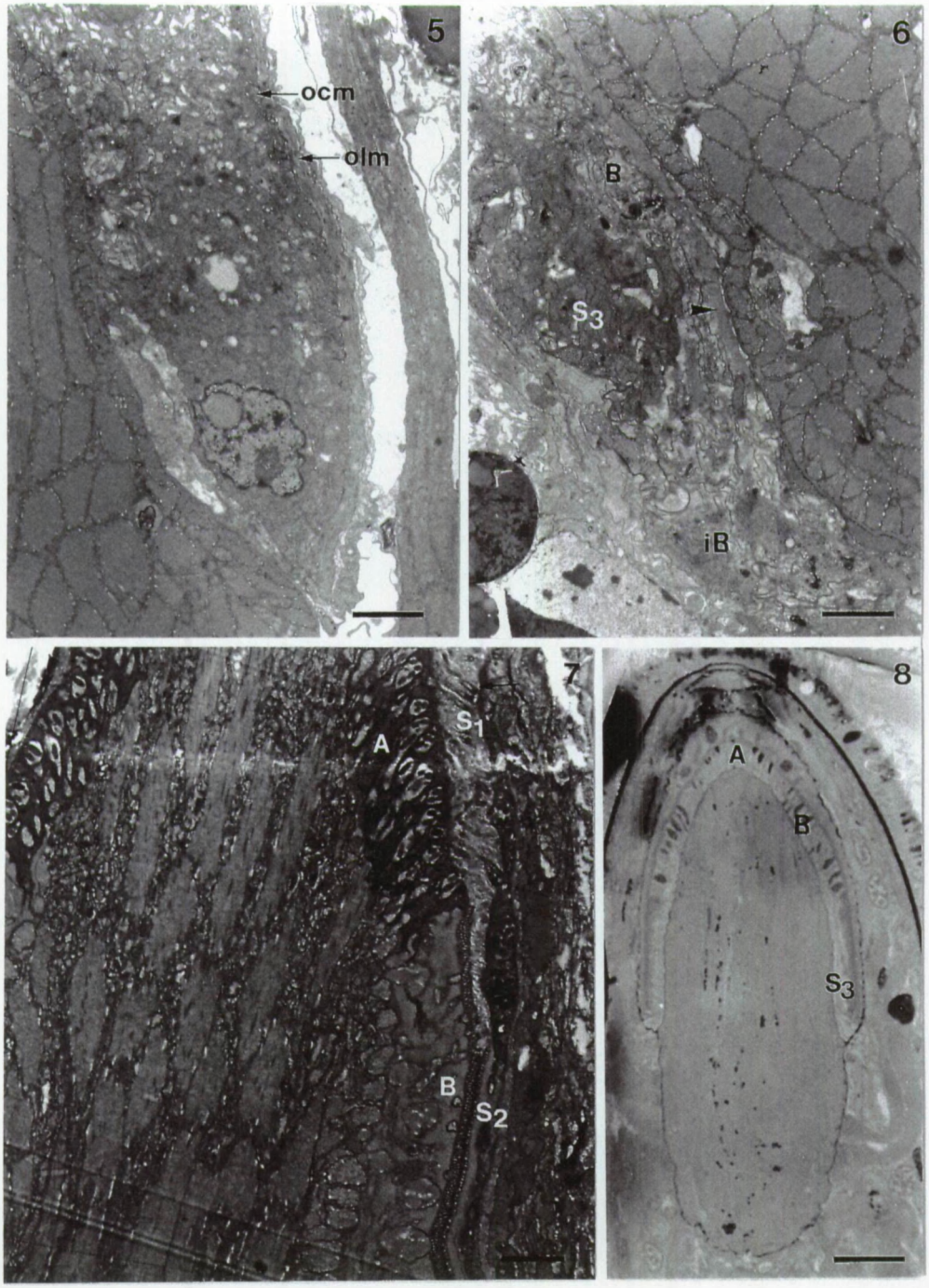

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be filled with surfacing gland necks or totally devoided of glands. The two belts in the cone epithelium can easily be distinguished by the erupting gland necks.

Epithelia. In *Phonorhynchus* the sheath epithelium is formed by three cellular belts. In *Scanorhynchus* and *Marirhynchus*, the proximal belt of the sheath epithelium is syncytial and the distal and median belt have a cellular organization, the proximal belt is syncytial[1]. In *Polycystis* and *Typhlopolycystis*, all three belts of the sheath epithelium are syncytial. For the other species, unfortunately, the exact organization of the sheath epithelium could not be reconstructed from the sections. The sheath epithelium varies in height from 0.5 µm in *Gallorhynchus* to 3 µm in most species and is covered by microvilli. If present, the microvilli are 900-600 nm long and slender near the proboscis pore about 12 per linear µm, they decrease in length towards the junction and measure approximately 300-350 nm in the median and proximal belt. Sometimes their number increases (16 per linear µm in *Pol. riedli*) or slightly decreases (8 per linear µm in *Rogneda*) towards the junction. The microvilli of the posterior part of the distal belt in *Typhlopolycystis* have long and thin tips (1.5 µm long) (Fig. 19), while the microvilli near the junction appear in two forms one with tips about 350 nm long and one without tips only 200 nm long. In *Danorhynchus* the distal belt bears sparse up to 1000 nm long and slender microvilli, while the microvilli of the median belt are irregular in shape and the proximal belt is totally void of microvilli (Figs 9, 10, 11). In *Marirhynchus* the microvilli measure 800-650 nm in the distal and median belt and about 300 nm in the proximal belt. In *Neopolycystis* the epithelium is devoid of microvilli (Fig. 13). Mostly a uniform basement membrane is present under the sheath epithelium, sometimes very thin as in *Gallorhynchus* (55 nm) or more distinct 100-300 nm thick in *Phonorhynchus*, *Rogneda*, *Polycystis* and *Marirhynchus* (Figs 3, 24, 36). In *Typhlopolycystis* a bipartite basement membrane is present near the pore and the basement membrane in *Neopolycystis* and *Danorhynchus* even shows a four-layered structure anteriorly and a three-layered structure more posteriorly (Figs 11, 13). The total thickness of the basement membrane in

Figs. 2-4. Phonorhynchus helgolandicus.
Fig. 2. Transverse section of the proboscis cone. The apical cone epithelium (*A*) is characterized by *g9* gland necks, the basal cone epithelium (*B*) by *g5* and *g6* gland necks. Inner circular muscles are present in the apical part of the cone. Scale bar: 2 µm.
Fig. 3. Section of the proximal belt of the sheath epithelium (S_3) with *g3* gland necks, *B* with *g5* gland necks and the upper part of the cytoplasmic girdle. An intra-epithelial muscle is present in *B* (*arrowheads*). Scale bar: 2 µm.
Fig. 4. Cross section of S_3 and *B* showing *g3*, *g5* and *g6* gland necks and alternating intra-epithelial muscles (*iem*) and cytoplasmic strands of *A* in *B*. Longitudinal (*ilm*) and circular (*icm*) muscles in the bulb. Scale bar: 1 µm.

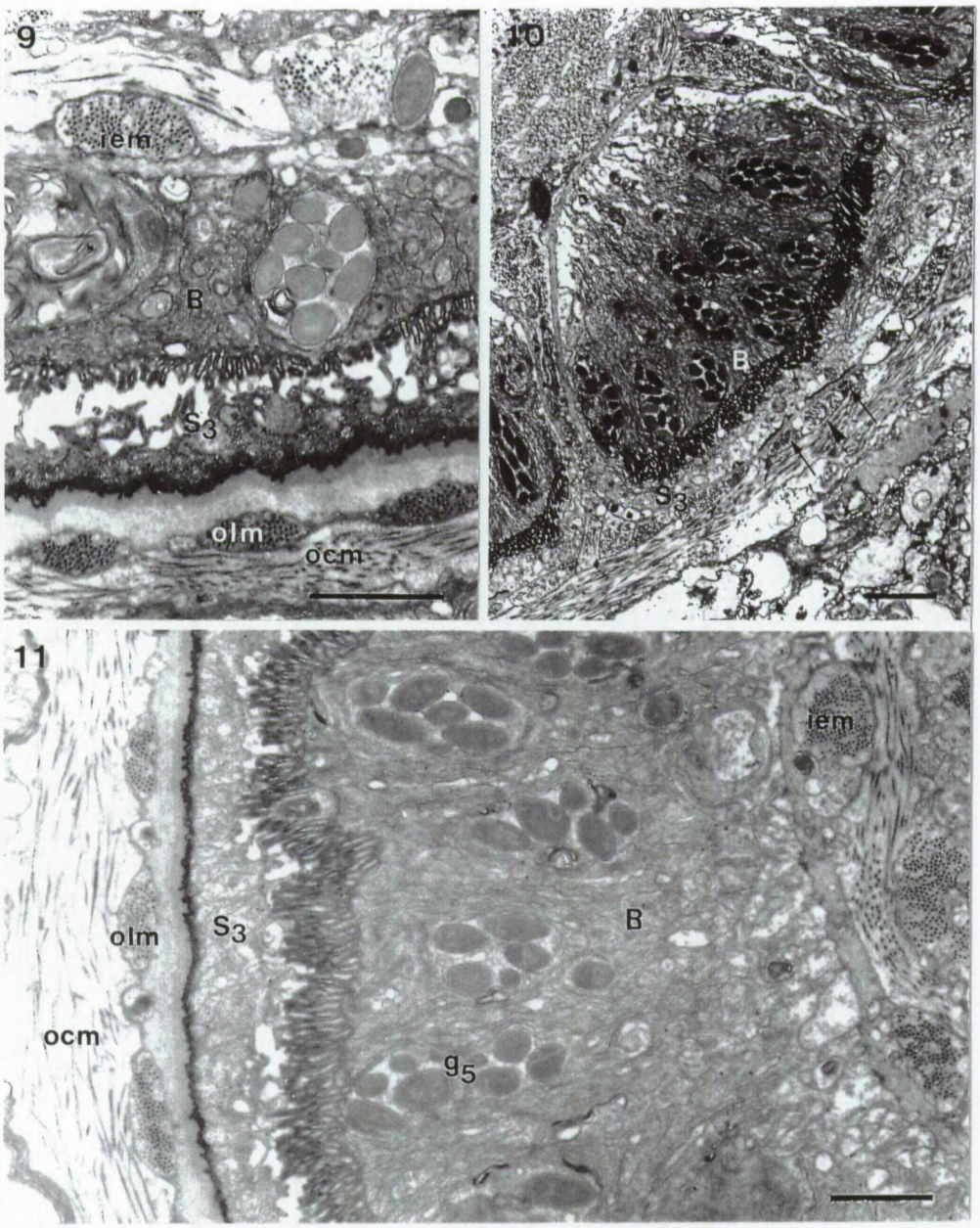
1 For an overview of the organization of the epithelia see *chapter 11 table 1*.


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B
S3

the anterior part varies between 400-500 nm in *Neopolycystis* to 500-600 nm in *Danorhynchus*. The basement membrane is composed of a very electron dense top layer with subsequent apical electron lucent layer, a median electron dense layer and basally a thick electron lucent layer. The second layer disappears towards the junction and the total thickness diminishes to 380-550 nm. In *Neopolycystis* apically dense patches (20 nm) are present above a thick moderately electron opaque layer (70 nm). Here below a 110 nm thick electron dense layer is found with a 200 nm thick electron opaque layer basally. Apically in the cytoplasm a fine fibrillar layer is present. The basal coarse granular cytoplasm contains mitochondria, patches of endoplasmic reticulum, few Golgi apparatuses, free ribosomes and numerous infoldings of the basal plasma membrane reaching up to the fibrillar layer. The mitochondria in *Neopolycystis* are spirally winded and the cytoplasm in *Rogneda* is filled with empty vacuoles. In many species aggregates of dense bodies are present which can be aggregates of glycogen. The number of nuclei in the syncytia is variable. In *Typhlopolycystis* two nuclei are present in the distal and median belt, while their number is four and six in *Polycystis*. In *Phonorhynchus* the three belts of the sheath epithelium are cellular and contain two, four and four nuclei respectively. The nuclei are flat or globular in shape and about 8 µm long or wide. Those of the distal belt are located in saggings posteriorly in the belt, apically covered by the median belt. The nucleiferous cell parts of the proximal belt are either situated at the periphery of a cytoplasmic girdle, which surrounds the anterior part of the bulb (*Neopolycystis*, *Danorhynchus*, and *Phonorhynchus*) or insunk in the parenchyma around the proboscis bulb (*Polycystis*, *Rogneda*, *Scanorhynchus*, *Progyrator* and *Marirhynchus*) (Figs 5, 31). In *Gallorhynchus* a short prolongation of the sheath epithelial basement membrane and the surrounding musculature forms a small cytoplasmic girdle without nuclei around the distal part of the bulb.

In all species investigated the cone epithelium has a bipartite organization formed by a basal and an apical syncytium. The cell junctions between the proximal belt of the sheath epithelium and the basal cone epithelium is mostly found at the junction but can be situated on the cone as in *Progyrator* or extremely high on the cone in *Marirhynchus*,

Fig. 5. Phonorhynchus helgolandicus. The cytoplasmic girdle, surrounded by circular and longitudinal muscles (*ocm*, *olm*), with nucleiferous cell part of the proximal belt of the sheath epithelum (S_3). Scale bar: 2 µm.
Fig. 6. Phonorhynchus helgolandicus. Posterior part of the cytoplasmic girdle and insunk part of the basal cone epithelium (*B*). Note the intra-epithelial muscle (*arrowhead*). Scale bar: 2 µm.
Fig. 7. Gyratrix hermaphroditus. Distal (S_1), with multiciliary receptors (*arrow*) and g_1 gland necks, median (S_2) belt of the sheath epithelium, apical (*A*) and basal (*B*) cone epithelium. Scale bar: 2 µm.
Fig. 8. Alcha evelinae. Light microscopic photograph of a semithin section of the proboscis, showing the apical (*A*), basal (*B*) cone epithelium and proximal belt of the sheath epithelium (S_3), which covers the base of the cone. Note the fixators (*F*). Scale bar: 15 µm.

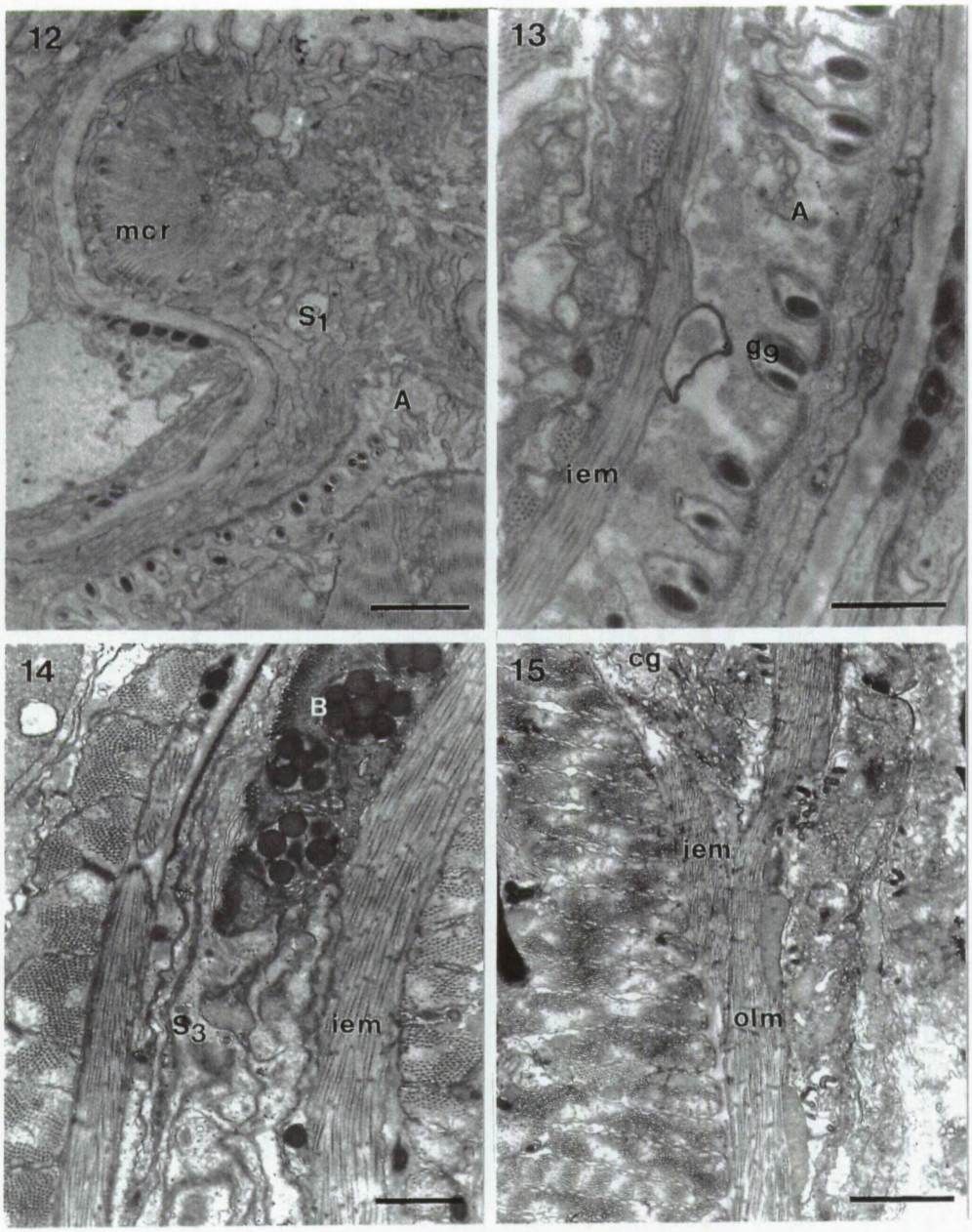


Figs 9-11. Danorhynchus gosoensis.
Fig. 9. Section of the proximal belt of the sheath epithelium (*S3*) with three-layered basement membrane and the basal belt of the cone epithelium (*B*), containing type g_5 gland necks. Intra-epithelial muscles (*iem*) are situated between the inner circular muscles and the basement membrane. Outer longitudinal (*olm*) and circular muscles (*ocm*) are inversed. Scale bar: 1 μm.

Alcha and *Paraustrorhynchus* (Figs 8, 25, 32) The epithelium is 1.5-3 µm thick and covered by short microvilli. Those in the basal belt have electron dense tips or dense margins and vary in length from 200-250 nm in *Neopolycystis*, *Typhlopolycystis*, *Gallorhynchus* and *Marirhynchus*, over about 350 nm in *Rogneda* and *Phonorhynchus* to 700 nm in *Danorhynchus*. The microvilli of the apical cone epithelium are as long or shorter as those of the basal belt, they measure 120-200 nm about 10-12 per linear µm in *Danorhynchus* up to 20 per linear µm in *Rogneda*. A uniform 50-60 nm thick basement membrane is always present under the epithelium (*e.g.* Figs 2, 9, 18, 23). The cytoplasm of the part of the epithelium lining the cone only contains few mitochondria. Other cell organelles and the nuclei are situated in the insunk cell parts, in the cytoplasmic girdle if present or in the surrounding parenchyma (spirally winded mitochondria in *Neopolycystis*). The cytoplasm of the basal belt of the cone epithelium in *Neopolycystis* is distinctly more electron dense than the cytoplasm in the apical belt, while the opposite is encountered in *Gyratrix* (Fig. 7). In genera, which have a cytoplasmic girdle around the anterior part of the bulb as *Phonorhynchus*, *Gallorhynchus*, *Danorhynchus* and *Neopolycystis*, the nucleiferous cell parts of the apical cone epithelium are situated at the inner side of this girdle, while the nucleiferous cell parts of the basal cone epithelium sink in the parenchyma below (Figs 6, 15). A thin cell strand of this belt is found between the proximal belt of the sheath epithelium and the apical cone epithelium in the cytoplasmic girdle (Fig. 1). In genera without cytoplasmic girdle as *Polycystis* species, *Paraustrorhynchus*, *Rogneda*, *Progyrator* and *Marirhynchus* , the nucleiferous cell parts of the apical and basal belt are found insunk close against the lateral sides of the bulb ('Myoblasten' Meixner (1925), 'Zellenmantel' Karling (1953) or 'mantle cells' Noldt & Reise (1987)) (Figs 21, 31). In most species six insunk cell parts are found around the posterior part of the bulb (*Neopolycystis*, *Scanorhynchus*, *Danorhynchus*, *Typhlopolycystis*). Twelve perforations in the basement membrane are present, but the cell strands fuse to form six more or less distinct groups. The part of the apical cone epithelium, which lines the cone, is connected to nucleiferous cell parts by narrow cell strands under the basal cone epithelium just above the basement membrane. The number of cell strands equals the number of intra-epithelial muscles (Figs 4, 18).

Glands. The proboscis in Polycystididae and *Marirhynchus* is not considered a glandular type of proboscis as for instance in some Cicerinidae (De Vocht & Schockaert 1988). A

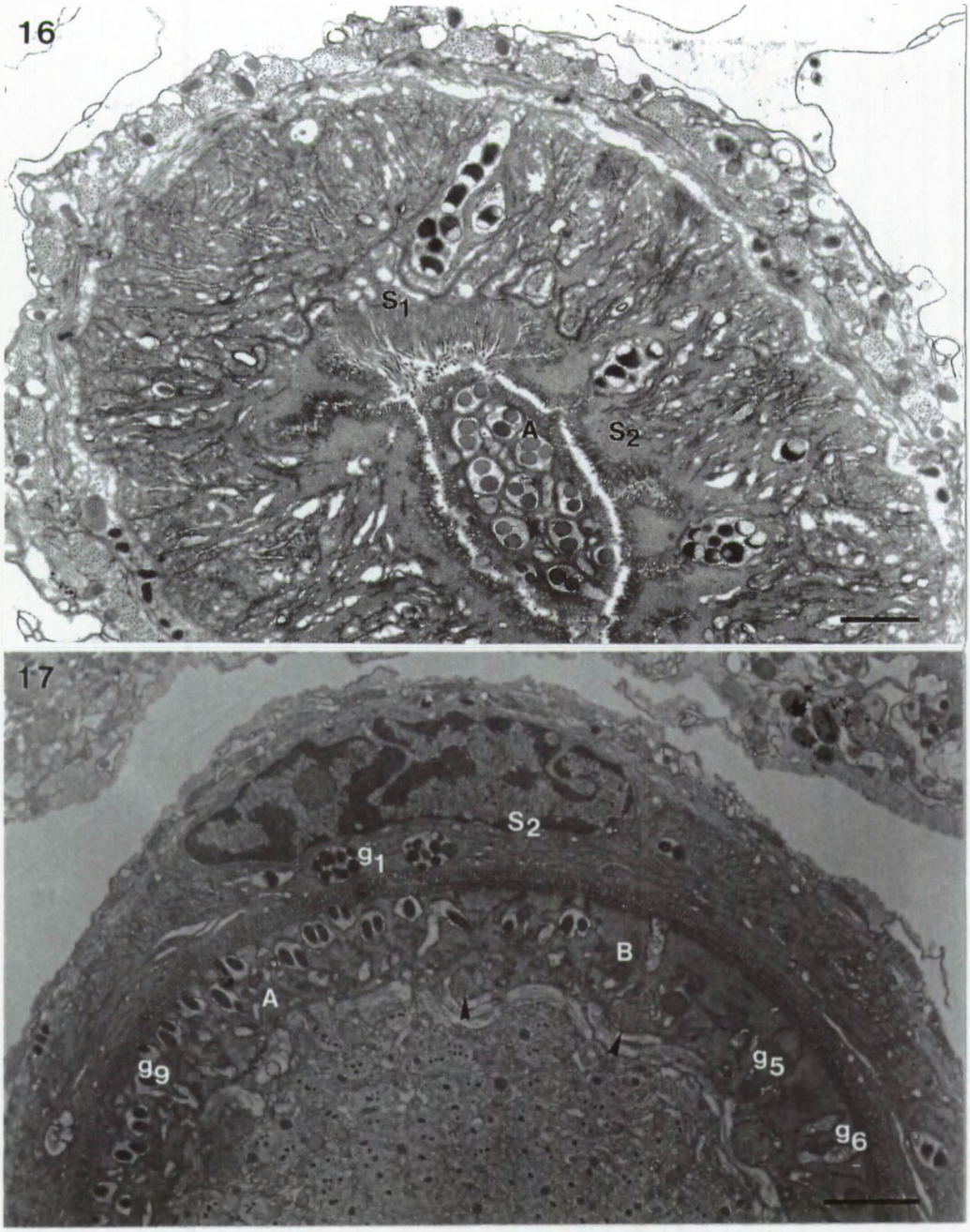
Fig. 10. Section of S_3 and *B* at the junction, showing connection of the *iem* to the outer muscles around S_2. Two sets of outer longitdinal muscles are present (*arrow* and *arrowhead*). Scale bar: 2 µm.
Fig. 11. Cross section of S_3 and *B* with inversed *olm* and *ocm*, g_5 gland necks, typical microvilli of *B* and *iem*. Scale bar: 1 µm.



Figs 12-15. Neopolycystis tridentata.
Fig. 12. Transverse section of the distal belt of the sheath epithelium (S_1) with multiciliary receptors (*mcr*) and apical cone epithelium (*A*) with g9 gland necks. Scale bar: 2 µm.
*Fig. 13.*Transverse section of A with intra-epithelial muscle (*iem*) piercing the basement membrane and *g9* gland necks. A four-layered basement membrane is present under the sheath epithelium. Scale bar: 1 µm.

maximum of six but usually four (g_1, g_5, g_6 and g_9) different types of gland necks and secretions is present in each species. Six types of gland necks are present in *Phonorhynchus*. Differences in the position of the gland necks do occur between the different species. Schockaert and Bedini (1977) identified four different gland necks in the proboscis epithelia of *Polycystis naegelii* (g_6 up to g_9 in Schockaert and Bedini 1977). The same types of gland necks are present in *Pol. riedli*, *Gyratrix*, *Rogneda* and *Typhlopolycystis* as well. In the latter species type g_1 gland necks appear in the three belts of the sheath epithelium and contain spherical electron dense secretion granules, 350 nm in diameter (Fig. 16). Sometimes the contents is partially dissolved. The basal cone epithelium is pierced by two types of gland necks. Type g_5 necks contain spherical moderately electron dense secretion granules, 630 nm in diameter packed in 3 µm long gland necks (Fig. 18). The narrow and inconspicuous type g_6 gland necks are filled with small (200 nm) empty secretion granules (Fig. 17). The apical cone epithelium is pierced by narrow gland necks (type g_9) with ovoid 500 nm long electron dense secretion granules (Figs 16, 17). In species with a thin sheath epithelium as *Neopolycystis*, *Danorhynchus*, *Gallorhynchus* and *Progyrator*, no gland necks are found in the sheath epithelium (Figs 9, 13). Only three different types of gland necks are present; two types in the basal cone epithelium and one type in the apical belt. Type g_5 gland necks are the most conspicuous and contain spherical to ovoid electron dense secretion granules respectively 380 nm and 280–450 nm in diameter in *Neopolycystis* and *Gallorhynchus*, elongated 720-1300 nm long electron dense granules in *Danorhynchus* and spherical 900 nm wide electron dense granules in *Progyrator* with accumulations below the basement membrane (Figs 11, 14, 27). The small, type g_6 gland necks contain empty, spherical (120-200 nm) secretion granules. Type g_9 gland necks in the apical cone epithelium contain ovoid electron dense secretion granules, 450-500 nm long in *Neopolycystis* or electron dense, 580 nm long rodlike granules in *Danorhynchus* in both cases with a light periphery (Fig. 13). In *Scanorhynchus* two types of gland necks appear in the sheath epithelium, sparse type g_1 gland necks are found in the median belt, filled with spherical, electron dense secretion granules 450 nm in diameter, while very numerous type g_2 gland necks are situated in the proximal belt and filled with moderately electron dense secretion granules, 700 nm in diameter. One type of gland necks (type g_5) also with 700 nm wide granules could be determined in the basal cone epithelium. Type g_9 gland necks in the apical cone epithelium contain electron dense 500 nm long ovoid secretion granules. In *Phonorhynchus* three different types of glands pierce the sheath epithelium. Type g_1 and

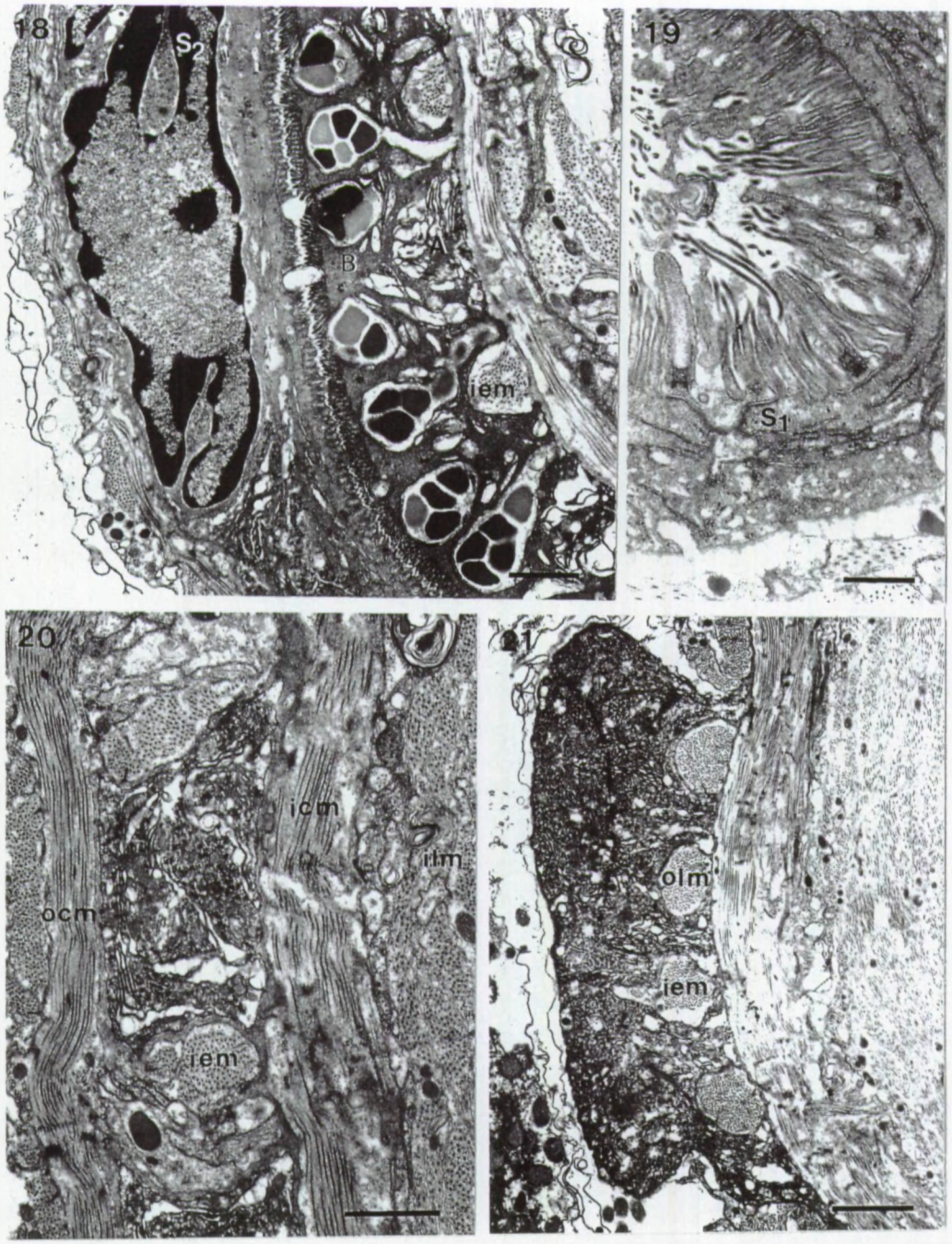
Fig. 14. Transverse section at the junction showing the proximal belt of the sheath epithelium (S_3), *B* with g_5 gland necks and an *iem*. Note the inversed longitudinal and circular muscles around the sheath epithelium and cytoplasmic girdle. Scale bar: 1 µm.
Fig. 15. Section showing the proximal end of the cytoplasmic girdle (*cg*) with *iem*, *olm* and flat fibres of the inner circular muscles (*icm*). Scale bar:2 µm.



Figs 16-17. Typhlopolycystis rubra.
Fig. 16. Cross section of apical tip (*A*) of the cone with g9 gland necks and transition of the distal (S_1) and median (S_2) belt of the sheath epithelium with g_1 gland necks. Scale bar: 1 μm.
Fig. 17. Cross section of S_2 and the transition of apical (*A*) and basal (*B*) cone epithelium. Type g_1, g_5, g_6, g_9 gland necks and intra-epithelial muscles are present (*arrowheads*). Scale bar: 2 μm.

few type g_2 gland necks are found in the median belt, the former type has wide gland necks filled with ovoid electron dense secretion granules up to 500 nm long, the latter have narrow gland necks and contains spherical, 300-400 nm wide secretion granules with moderately electron dense contents. The proximal belt contains wide type g_3 gland necks closely packed with electron dense secretion granules 700 nm in diameter (Figs 3, 4). The gland necks in the cone epithelium are homologous to those described above. Type g_5 gland necks contain 500 nm wide electron dense secretion granules with flocculent contents, they pierce the bulbar septum laterally below the cytoplasmic girdle and run peripherally through the distal part of the bulb (Figs 2, 4). The inconspicuous type g_6 gland necks contain empty granules and type g_9 necks a electron dense ovoid secretion, 300 nm long and 100 nm wide (Figs 2, 4). Type g_9 gland necks pull through the bulb medially. In *Marirhynchus* three types of gland necks are present in the sheath epithelium, type g_1 necks contain electron dense spherical granules, 500 nm in diameter, while type g_2 gland necks are filled with moderately electron dense, spherical granules, 450 nm in diameter. The somewhat wider type g_3 necks are stuffed with slightly more electron dense granules, 500-750 nm diameter, often with light central spot (Fig. 32). The basal and apical cone epithelium is pierced by gland necks with ovoid electron dense secretion granules, 700 nm long (g_9) (Fig. 32).

Sensory cells. Multiciliary receptors associated with the distal belt of the sheath epithelium have been found in *Polycystis, Typhlopolycystis, Gallorhynchus, Neopolycystis* and *Phonorhynchus* (Figs 7, 19, 26). Their presence or absence can not be stated with certainty for *Rogneda* and *Paraustrorhynchus* nov. spec. But they are probably lacking in *Danorhynchus, Scanorhynchus, Progyrator* and *Marirhynchus*. In the first mentioned group of species, the receptors are always situated in the proboscis cavity below the pore as in Cystiplanidae (De Vocht 1989). The aberrant axonemata are probably composed of single microtubules only and rootlets are always lacking. In *Marirhynchus* multiciliary receptors are present in the epidermis at the level of teh distal belt of the sheath epithelium. Uniciliary receptors with long ciliary shafts are sparsely present in the sheath epithelium. They have primary rootlets and short slanting secondary rootlets. The median belt of the sheath epithelium of *Progyrator* is pierced by very numerous uniciliary receptors. The terminal ends of the dendrites form small flat extensions on the epithelium surface (Fig.37) . These extensions carry the long and thick ciliary rootlets which point out into the proboscis cavity (Fig. 38). The 1 µm wide bases of the rootlets are connected to the zonulae adhaerentes and have a central aperture. The rootlets are about 1.6 µm long, as thick as the basal bodies and show a cross striation. The ciliary shaft contain axonemata with two central singlets and nine doublets, which are interconnected by electron dense condensations. The cone epithelium contains uniciliary receptors with blunt ciliary shafts and primary rootlets in all species.



Figs 18-21. Typhlopolycystis rubra.
Fig. 18. Section of the median belt of the sheath epithelium (S_2) with nucleus and *B* with g_5 gland necks, intra-epithelial muscles (*iem*) and cytoplasmic cell strands of the apical cone epithelium (*A*). Scale bar: 1 µm.

Proboscis musculature

The muscles associated with the proboscis resemble the cross-striated muscle type. The contractile parts possess thick and thin filaments organized in A- and I-zones, Z-discs are present as well but H-zones are lacking. The fibres have a sarcoplasmic reticulum and peripheral mitochondria are present.

Outer proboscis musculature. The outer musculature is considered the circular and longitudinal muscles around the sheath and the motional muscles as well as the muscles which connect the bulb with the body wall. The muscles surrounding the proboscis sheath are continuous with the musculature of the body wall at the pore. Both circular and longitudinal muscles are present around the sheath epithelium. In *Neopolycystis*, *Scanorhynchus* and *Danorhynchus* the circular muscles are found peripherally of the longitudinal muscles (Figs 11, 14), while in all other species the longitudinal muscles are located peripheral of the circular muscles. In the first mentioned species, both muscle layers are present around the sheath from the proboscis pore on, over the junction and around the cytoplasmic girdle. At the posterior end of the girdle, the outer circular muscles are replaced by circular muscles inside the septum. In *Danorhynchus* the longitudinal muscles around the sheath bifurcate above the junction and a second set continues from here on around the cytoplasmic girdle and the bulb (Fig. 10). A total number of 34 fibres (14 of which are actually intra-epithelial muscles and 20 fibres of the second set of outer longitudinal muscles) is present around the bulb and the muscles adhere on the bulbar septum laterally. The outer longitudinal muscles in *Neopolycystis* are 1.8 µm thick around the cytoplasmic girdle and adhere on the flanks of the bulb (Figs 14, 15). In *Phonorhynchus*, also a species with a cytoplasmic girdle, the outer longitudinal muscles surround the sheath and the circular muscles from the pore on. The circular muscles only appear from the median belt of the sheath epithelium on (Fig.1). In *Typhlopolycystis*, both the circular and longitudinal muscles are present from the pore on. At the pore the first circular muscles form a sphincter, the fibres are embedded in an electron dense layer of extracellular matrix (or basement membrane) together with nerves and glands. A total number of 42 longitudinal fibres (46 in *Pol. riedli*) surround the sheath and adhere on the bulbar septum laterally together with the 12 intra-epithelial muscles. In *Gallorhynchus* circular muscles are only present around the proximal belt of the sheath epithelium, while the longitudinal muscles enclose the median and proximal belt.

Fig. 19. Intra-epithelial multiciliary receptors (*mcr*) in the distal belt of the sheath epithelium (S_1). Scale bar: 0.5 µm.

Fig. 20. Cross section of the cytoplasmic girdle showing outer longitudinal and circular muscles (*olm*, *ocm*) , intra-epithelial muscles (*iem*), inner circular and longitudinal muscles(*icm*, *ilm*). Scale bar: 1 µm.

Fig. 21. Insunk epithelial cell part, *iem*, *olm*, *icm* and *ilm*. Scale bar: 2 µm.

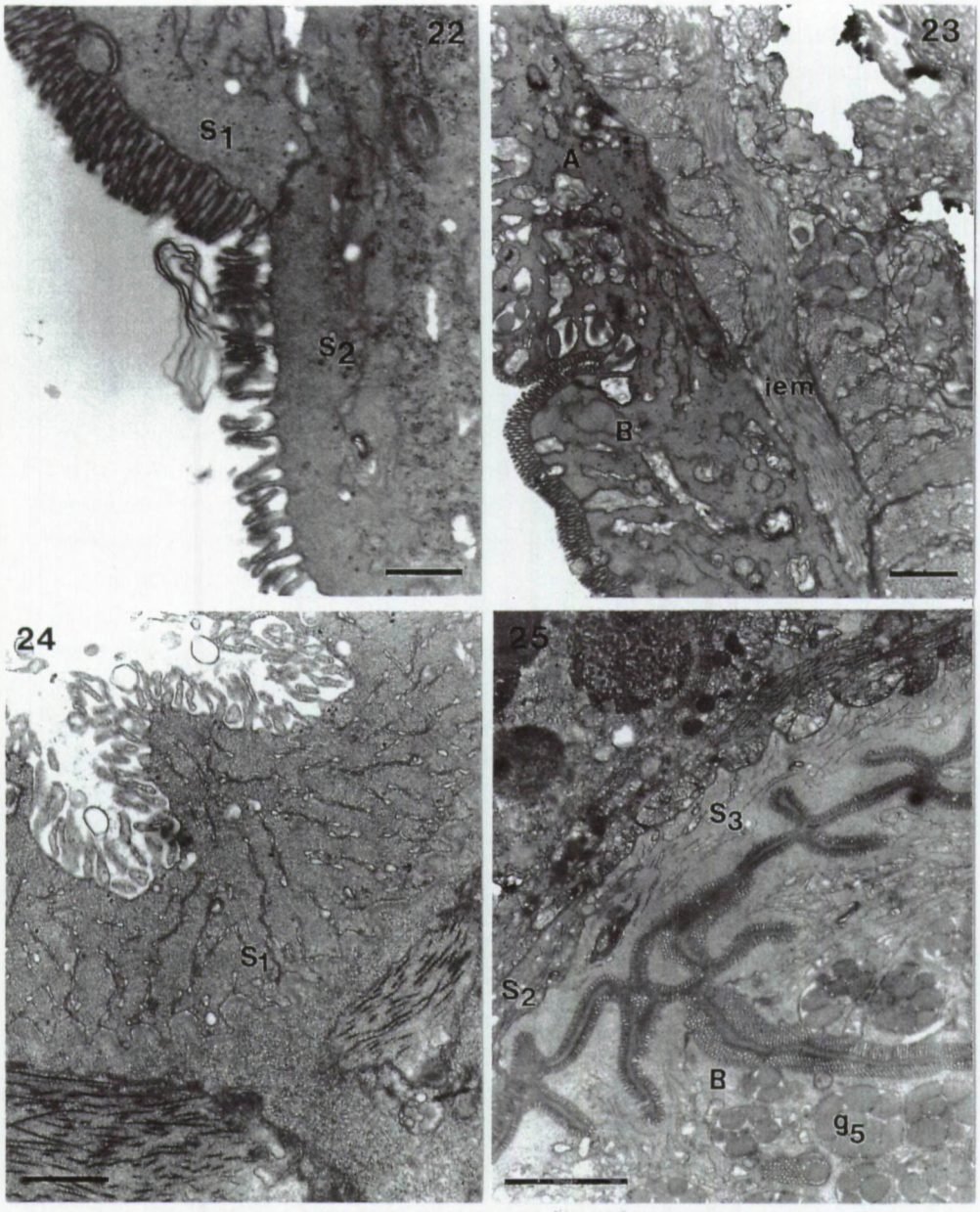


Fig. 22. Rogneda palula. Transition of the distal (S_1) and median belt (S_2) of the sheath epithelium showing the difference in length and abundancy of the microvilli, the apical fibrous and basal granular cytoplasm. Scale bar: 0.5 µm.
Fig. 23. *Rogneda palula.* Transition of the apical (*A*) and basal (*B*) cone epithelium showing *g5* and *g9* gland necks, an intra-epithelial muscle (*iem*), which slips under the basement membrane and inner circular muscles. Scale bar: 1 µm.

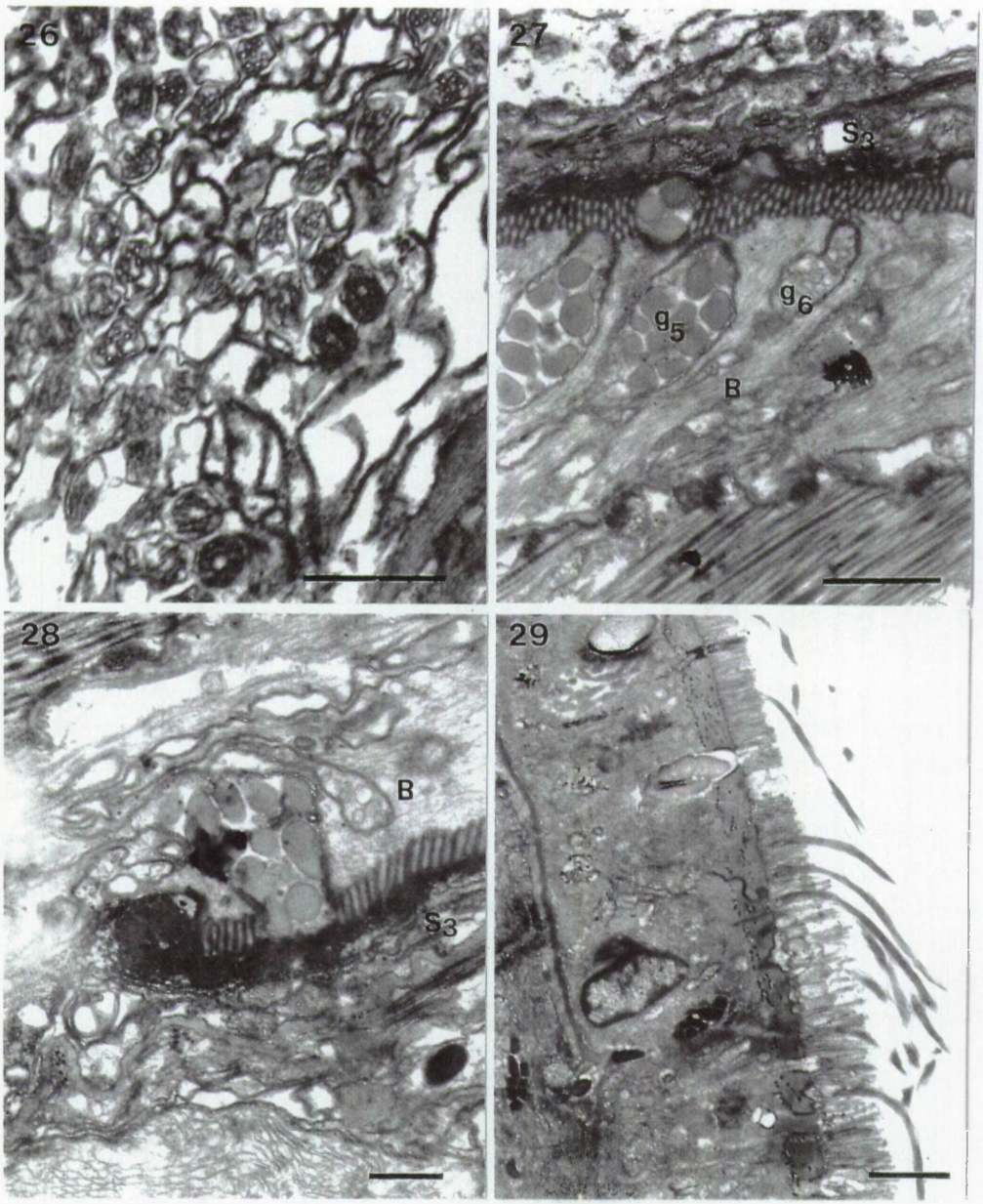
Dilators of the sheath, protractors, fixators, proboscis and integument retractors are regarded as the motional muscles. Four pairs of protractors are present in Polycystididae. Three pairs of fixators insert on the lateral sides of the bulb, below the junction or below the cytoplasmic girdle and run rostrad towards the body wall (Fig. 8). They are situated between the insunk cell parts of the epithelia. Proboscis retractors insert on the posterolateral side of the bulb and pull caudad through the body. One or two pairs of integument retractors are present. The protractors form a kind of knot behind the bulb and run forward towards the anterior body wall. For what the organization of the motional muscles is concerned, the description of Meixner (1925) is still valid for species such as *Progyrator*, *Phonorhynchus*, *Gyratrix* and species of the genus *Polycystis*. In *Pol. riedli* six dilators of the sheath divide the outer longitudinal muscles in six groups; two of 10-11 fibres and four of 6 fibres each.

Inner proboscis musculature The inner musculature is composed of longitudinal muscles surrounded by a single layer of circular muscles. The latter is found from the nodus up to the junction and in the cone although as much thinner fibres. They proceed up to the apex in species as *Typhlopolycystis*, or half way the apical cone epithelium as in *Rogneda* and *Neopolycystis* or only up to the transition of basal in apical cone epithelium as in *Gyratrix* (Figs 7, 13, 17). In *Progyrator*, *Scanorhynchus* and *Marirhynchus*, the circular muscle layer in the bulb is thick and formed by flattened fibres (10-12 µm, 8-9 µm and 3 µm respectively). Just below the junction this layer changes into a thin layer (0.6 µm, 1 µm and 0.7 µm respectively). In species with a cytoplasmic girdle, a more gradual diminishing in thickness is found but in *Neopolycystis* there is still a difference in thickness of the circular muscles above or below the proximal end of the cytoplasmic girdle (Figs 13, 14, 15). The longitudinal muscles in the bulb insert with furcated proximal ends between the circular muscles on the inside of the bulbar septum. The distal ends adhere on the basement membrane of the cone epithelium by small desmosomes (Fig. 2). The central fibres adhere on the apex, the peripheral muscles on the basal cone epithelium.

The bulbar septum is a uniform 100-250 nm thick layer of extracellular matrix, which is continuous with the basement membranes of the sheath and cone epithelium. A perforation at the nodus leaves passages to the gland necks that surface in the cone epithelium (g_5, g_6, g_9).

Fig. 24. Polycystis riedli. Distal belt of the sheath epithelium (S_1) with infoldings of the basal plasma membrane and thick basement membrane and outer circular muscle. Scale bar: 0.5 µm.

Fig. 25. Paraustrorhynchus sp. Oblique section showing outer longitudinal and circular muscles, median (S_2) and proximal belt (S_3) of the sheath epithelium and basal cone epithelium (*B*) with g_5 gland necks. Scale bar: 2 µm.



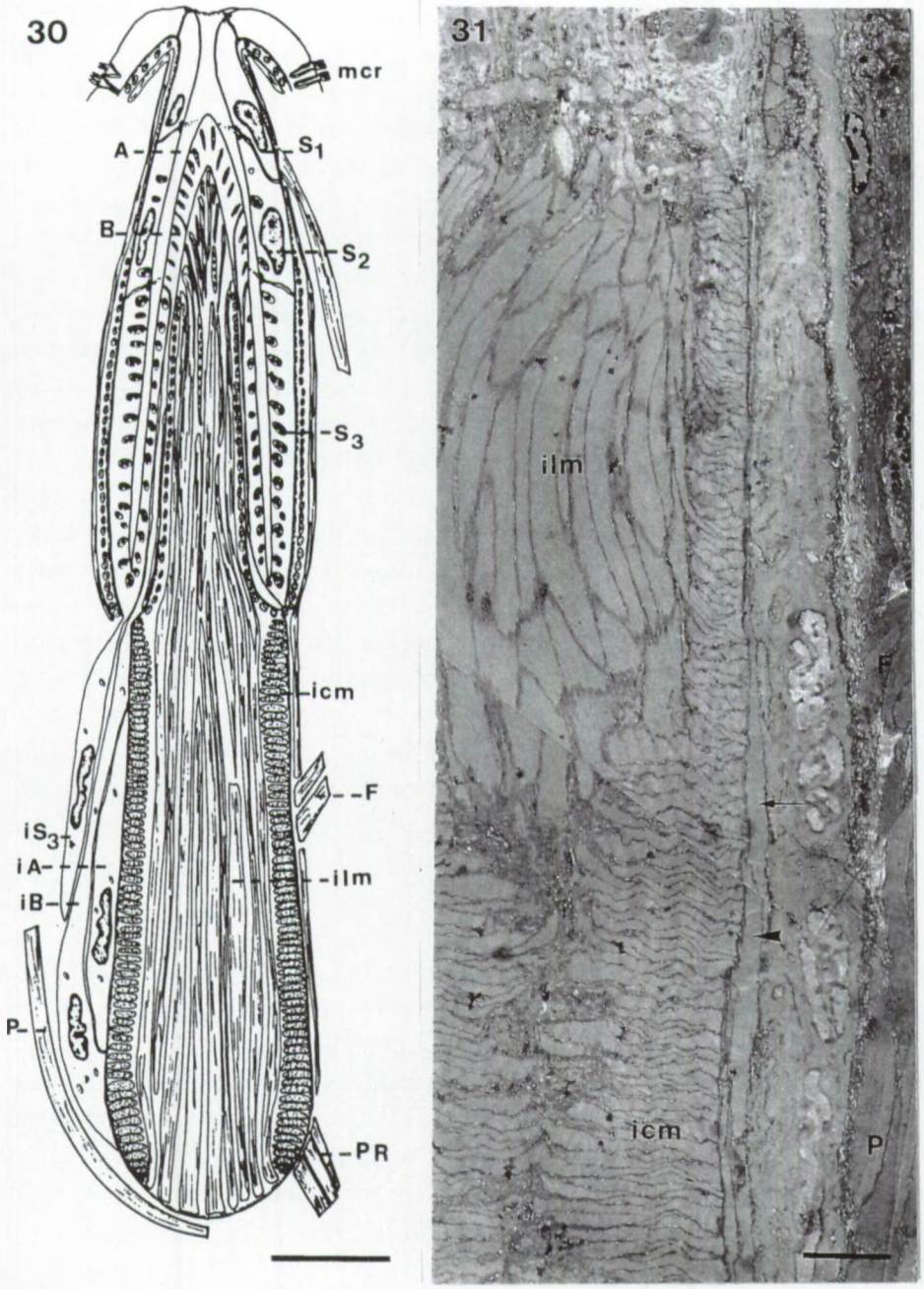
Figs 26-28. Gallorhynchus mediterraneus.
Fig. 26. Intra-epithelial multiciliary receptors (*mcr*) in the distal belt of the sheath epithelium. Scale bar: 0.5 μm.
Fig. 27. Basal cone epithelium (*B*) with fibrous cytoplasm and g_5 and g_6 gland necks, thin proximal belt of the sheath epithelium (*S3*). Scale bar:
Fig. 28. Junction of *S3* and *B* with g_5 and g_6 gland necks. Scale bar: 1 μm.
Fig. 29.

Intra-epithelial muscles. Intra-epithelial muscles are present in all species investigated. They are found between the outer longitudinal muscles around the bulb and enter the proboscis epithelia at the junction or at the proximal end of the cytoplasmic girdle (Figs 9, 15, 20, 31). In most species they run under the basal cone epithelium but above the basement membrane in this girdle and in the cone (Fig. 4). At the transition into apical cone epithelium or below the proximal part of this epithelium, the muscles fibres pierce the basement membrane and proceed between the basement membrane and the inner circular muscle layer. Their number is usually twelve as for instance in *Phonorhynchus* and *Typhlopolycystis* but in *Pol. riedli* fourteen muscle fibres are present. In *Danorhynchus* fourteen intra-epithelial muscles are present as well but they are always situated between the basement membrane and the inner circular muscle layer in the cone (Figs 9, 11). At the junction the fibres bifurcate; one end pulls through the cytoplasmic girdle, enveloped by a thin layer of extracellular matrix, the other end runs along the basement membrane of the bulb in the cytoplasmic girdle (Fig. 10). The first mentioned branch, which has a terminal bifurcation, inserts on the basement membrane and outer circular muscles. In *Neopolycystis* the number of intra-epithelial muscles could not be determined from our sections. Below the basal belt of the cone epithelium, the fibres are situated above the basement membrane but under the posterior part of the apical cone epithelium the fibres slip under the basement membrane and continue between the basement membrane and the inner circular muscles (Figs 13, 23). Below the junction the fibres bifurcate; one end runs to the outer longitudinal muscles, the other pierces the basement membrane at the junction and continues around the bulb. Twelve intra-epithelial muscles are present in *Phonorhynchus*, they run against the basement membrane in the cone and are connected to the bulbar septum by hemidesmosomes.

Discussion

Within the family Polycystididae there are ten subfamilies according to Evdonin (1977). The organization of the genital organs and the structure of the male copulatory organ form the main characters in order to establish the different subfamilies and genera. Only minor differences in the proboscis structure are present within this family, which is typified by the presence of four sclerotic knobs on the pharynx. Sometimes distinctive features in the proboscis form useful characters for diagnosing the subfamilies. Intra-epithelial muscles were considered typical features for Polycystididae by Schockaert and Bedini (1977) but have been recorded for Cystiplanidae as well (De Vocht 1989).

The organization of the proboscis epithelia in *Pol. naegelii* has been described by Schockaert and Bedini (1977). The proboscis epithelia of all species of Polycystididae investigated are formed by five circumferential belts, three belts line the cavity and two cover



Figs 30-31. Marirhynchus longasaeta.
Fig. 30. Reconstruction of the proboscis. Scale bar: 20 µm.
Fig. 31. Transverse section of the bulb and insunk nucleiferous cell parts of the proximal belt of the sheath epithelium, the apical and basal cone epithelium. Fixator (*F*) and protractor mucles (*P*) are present and intra-epithelial (*arrow*) and outer longitudinal muscles (*arrowhead*) surround the bulb. Scale bar: 5 µm.

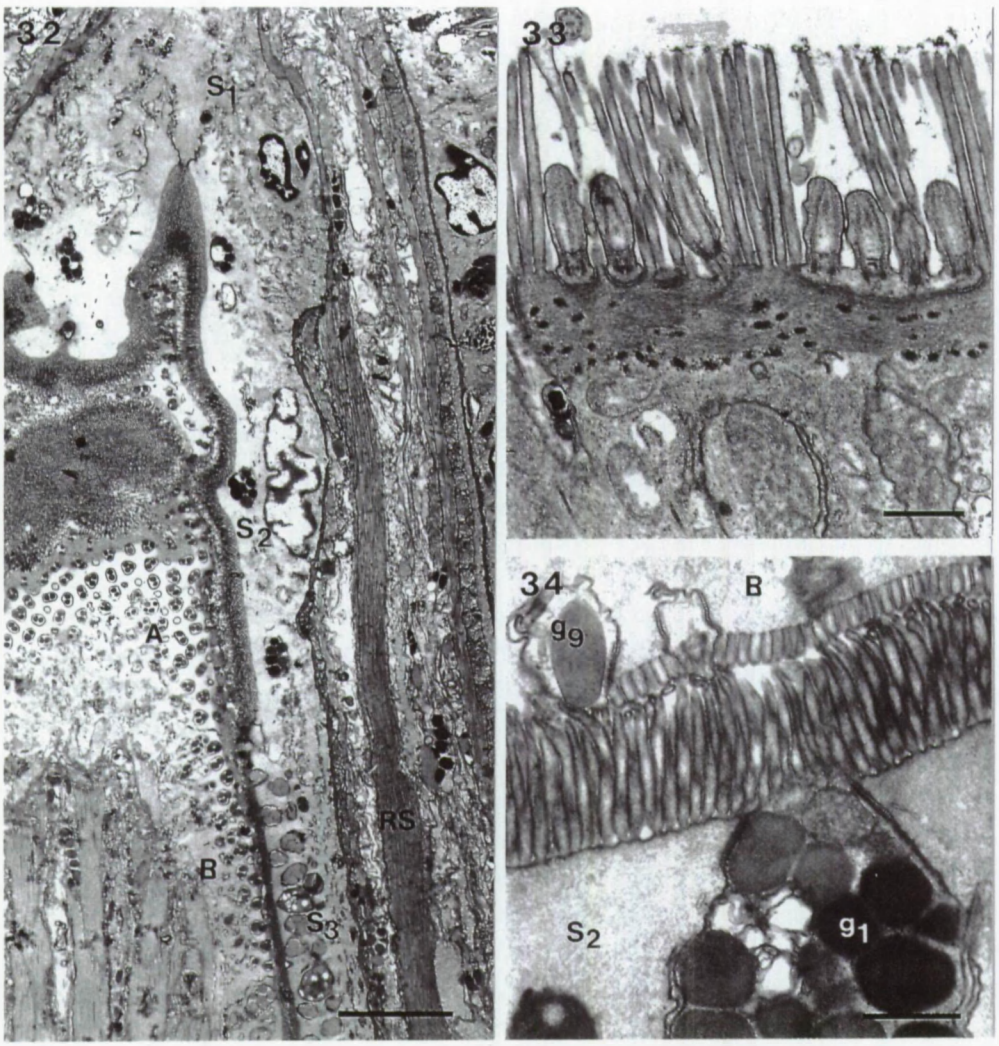
the cone as in Cystiplanidae, Koinocystididae and *Mesorhynchus terminostylis* (De Vocht 1989, 1991). The organization of the sheath epithelium differs from three fully cellular belts in *Phonorhynchus* to three syncytial belts in *Polycystis* and *Typhlopolycystis* (see chapter 11 table 1). Syncytialization in the sheath epithelium seems to occur first in the proximal belt, in a later stage in the median belt and finally in the distal belt. Such a progression is seen from *Phonorhynchus* over *Scanorhynchus* to *Polycystis* and *Typhlopolycystis*. From light microscopic observations a thin sheath epithelium without nuclei is described for *Gyratricella attemsi* (Karling 1955). An extensive "Zellenmantel" or cytoplasmic girdle, surrounded by the outer circular and longitudinal muscles of the sheath, is present around the bulb. Other ultrastructural differences are the lengths of the different belts in the sheath epithelium. In *Marirhynchus* for instance, the distal and median belt as well as the apical and basal cone epithelial belt are short, while the proximal belt of the sheath epithelium covers more than half of both the cavity wall and the cone.

Generally, the microvilli of the distal belt are longer than those of the median and proximal belt (see chapter 11 table 2). Characteristic for *Neopolycystis* is the absence of microvilli in the sheath epithelium (extensions of the cell survace are seen), in *Danorhynchus* only the proximal belt is void of microvilli. The functional aspect is not clear.

The presence of a cytoplasmic girdle, containing the nucleiferous cell parts of the proximal belt of the sheath epithelium and the apical cone epithelium, is typical for the genera *Phonorhynchus* (and probably *Cincturorhynchus*), some genera within the Gyratricinae and is found in Cystiplanidae as well (De Vocht 1989, Karling 1955). The insunk cell parts have been regarded as myoblasts (Meixner 1925) or epithelial cell parts of the cone epithelium (Karling 1931, 1953). In species with a cytoplasmic girdle only the basal cone epithelium has insunk cell parts. In the other species the proximal belt of the sheath epithelium and both belts of the cone epithelium sink in the parenchyma.

The bipartite cone epithelium is always formed by two syncytial belts. In *Marirhynchus*, *Alcha* and *Paraustrorhynchus* these two belts actually cover the apical part of the cone and a part of the proximal belt of the sheath epithelium covers the base of the cone. The length of the microvilli is more or less the same in all species except for *Danorhynchus gosoeensis*, where the basal cone epithelium has much longer microvilli (see chapter 11 table 2). The separate cell strands, which connect the apical parts of the cone epithelium with their nucleiferous cell parts are present in Cystiplanidae as well (De Vocht 1989).

Gland necks are relatively sparse in the sheath epithelium of Polycystididae and in species with very thin epithelium they are totally absent. The gland necks are concentrated in the proximal belt just above the junction. The basal cone epithelium is pierced by two types of



Figs 32-34. Marirhynchus longasaeta.
Fig. 32. Transverse section of distal (S_1), median (S_2), proximal belt (S_3) of the sheath epithelium, basal (*B*) and apical (*A*) cone epithelium. Type g_1, g_2, g_3 and g_9 gland necks are present in the epithelia and retractors of the sheath (*RS*) adhere on the basement membrane at the transition of S_1 and S_2. Scale bar: 5 µm.
Fig. 33. Muliciliary receptors in the epidermis. Scale bar: 0.5 µm.
Fig. 34. S_2 with long microvilli and g_1 gland necks oppposite of *B* with short microvilli, g_9 gland necks and uniciliary receptors. Scale bar: 0.5 µm.

glands (type g4 en g5), except for *Scanorhynchus forcipatus* where only one type is found. The glandular secretion emerging from the basal cone epithelium is, not produced by the insunk parts of the epithelium (Schockaert 1972). As the gland necks, which surface in the apical cone epithelium, the secretory cell parts of these glands is situated behind the proboscis. In general in Polycystididae and Eukalyptorhynchia the proboscis epithelia do not produce glandular secretions. Only the vacuoles in the sheath epithelium of *Toia* and *Nannorhynchides* could be interpreted as empty secretion granules of the epithelium.

Intra-epithelial multiciliary receptors associated with the distal belt of the sheath epithelium are known from Cystiplanidae, Koinocystididae and *Mesorhynchus* as well (De Vocht 1989, 1991). The receptors are reduced in number in some species as *Polycystis*, and are totally lacking in *Marirhynchus* and probably also in *Rogneda*. The uniciliary receptors have an identical structure as in Cystiplanidae (De Vocht 1989). This intra-epithelial type of multiciliary receptors is encounterd in some Eukalyptorhynchia genera with a bipartite sheath epithelium as well (*Paragnathorhynchus, Placorhynchus* and *Florianella*). The intra-epithelial form of multiciliary receptors is to be regarded the plesiomorphic form of this receptor in Eukalyptorhynchia.

The cross-striated organization of the proboscis muscle fibres is a typical feature for the investigated species of the family Polycystididae and the family Cystiplanidae (De Vocht 1989). A typical feature for most genera of the subfamily Gyratricinae (with the exception of *Gyratrix*) is the inversion of the outer circular and longitudinal muscles. Distally of the sphincter at the proboscis pore the circular muscles are found peripheral of the longitudinal muscles up to the junction and the cytoplasmic girdle. The functional meaning of this inversion is not clear. A second set of outer longitudinal muscles around the cytoplasmic girdle, replacing or joining the longitudinal muscles surrounding the sheath as in *Danorhynchus* is present in Cystiplanidae as well (De Vocht 1989).

Intra-epithelial muscle fibres with bifurcated proximal end, passing through the cone epithelium up to the apical cone epithelium is a homologous and synapomorphic character for Cystiplanidae and Polycystididae. Several features present in Cystiplanidae are found in *Phonorhynchus, Danorhynchus, Neopolycystis, Scanorhynchus* as well. This indicates a close relationship between the genera. The presence of a cytoplasmic girdle in polycystid species (Gyratricinae and *Phonorhynchus*) and in Cystiplanidae, indicates that a cytoplasmic girdle containing the nucleiferous cell parts of the proximal belt of the sheath epithelium and the apical cone epithelium forms the plesiomorphic state in Polycystididae in comparison to three belts with insunk cell parts. The inversion of outer circular and longitudinal muscles can be considered a synapomorphic character for the genera *Scanorhynchus, Danorhynchus, Neopolycystis* and *Gyratricella*.

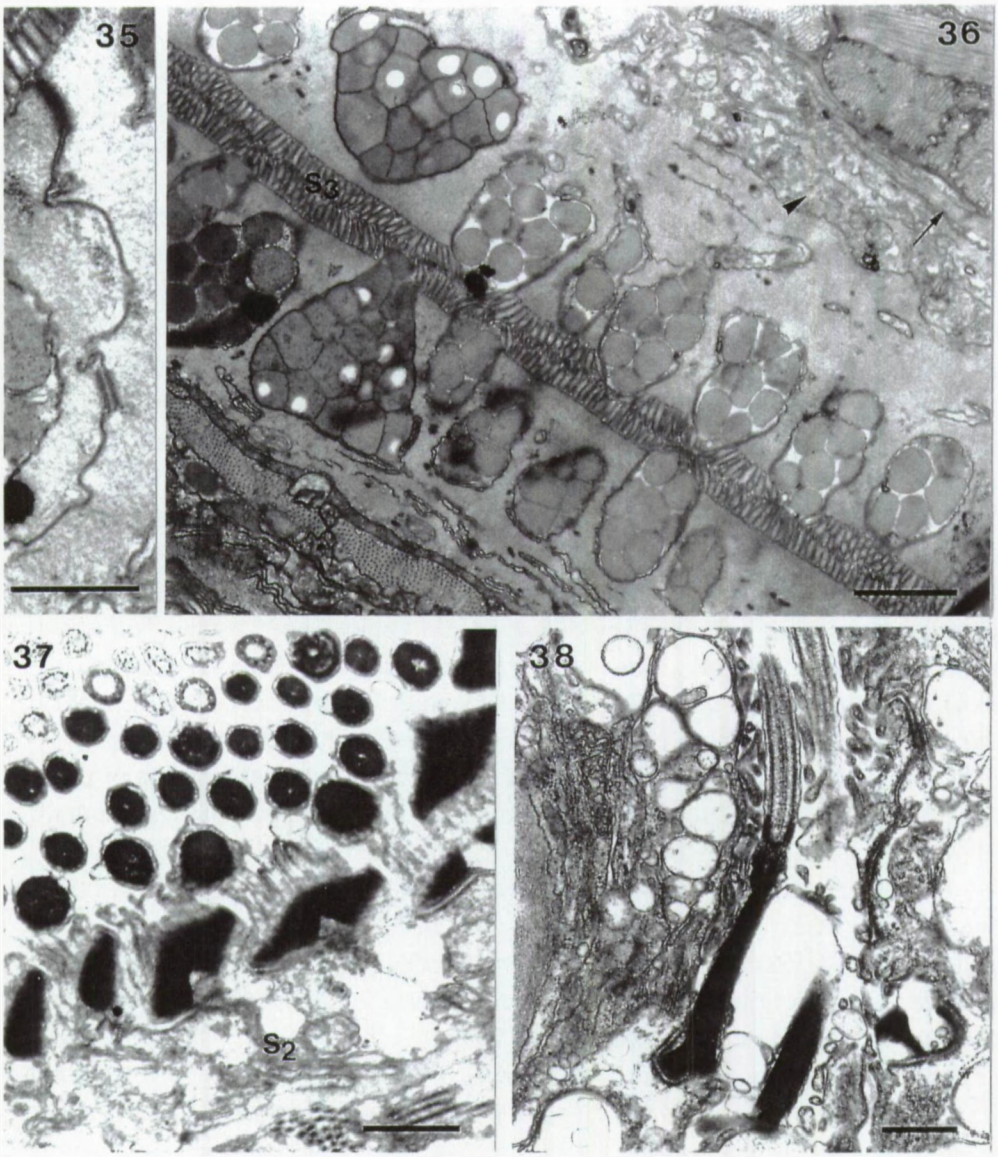


Fig. 35. Marirhynchus longasaeta. Cell junction between apical and basal cone epithelium with zonula adhaerens, septate junction and macula adhaerens. Scale bar: 0.5 µm.
Fig. 36. Marirhynchus longasaeta. Transverse section of the proximal belt of the sheath epithelium (S_3), which covers the cavity wall (bottom) and cone (top). Cytoplasmic strands of the cone epithelium (*arrowhead*) and intra-epithelial muscle (*arrow*) are present under S_3 on the cone. Scale bar: 1 µm.
Fig. 37. Progyrator mamertinus. Numerous uniciliary receptors lifted above the apical plasmalemma of the median belt of the sheath epithelium (S_2), with microvilli on the extensions covering the epithelium. Scale bar:0.5 µm.
Fig. 38. Progyrator mamertinus. Transverse section of the uniciliary receptors with long and thick rootlets lifted above the epithelium. Scale bar: 0.5 µm.

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Chapter 9.

The anatomy and ultrastructure of the proboscis in Koinocystididae Meixner, 1924.

Introduction

A thorough revision of the family Koinocystididae, published by Karling (1980), included eighteen genera of which seven, *Axiutelga*, *Brunetia*, *Getula*, *Groveia*, *Itaipusina*, *Leguta* and *Neoutelga*, have been introduced in the mentioned paper. Thirteen genera are monotypic, the genera *Itaipusa* including thirteen species and *Utelga* with five species are the two largest genera in the family. The formerly established system by Evdonin (1977) includes fourteen genera, of which three have been excluded by Karling (1980). For *Acrumena massiliensis* the latter author created a new family the Acrumenidae. In total 50 species of Koinocystididae have been described up to now. The systematic position of the genera *Marirhynchus* and *Gnorimorhynchus* is not certain as well as the position of *Utelga possjetica* and *Utelga spinosa* , two species of which Karling could not study the material. They are regarded as species incertae sedis. The organization of the proboscis in *Marirhynchus* has been described and discussed in the former chapter. From the proboscis structure follows a close affinity of *Marirhynchus*, Polycystididae and Cystiplanidae. Karling (1980) named three synapomorphies for the family Koinocystididae, although the characters are sometimes transformed or in some species uncertain. The first synapomorphy is a copulatory organ of duplex type with a eversible cirrus. The second synapomorphy is a copulatory bursa opening posteriorly in the common atrium, with proximal resorptive part and the third concerns the presence of paired oviducts and a common vitelloduct, opening into an unpaired seminal receptacle with strong distal sphincter. The author described four transformations of structures within the Koinocystididae among which the proboscis. He denoted some plesiomorphic characters of the proboscis such as a weakly muscular conorhynch with cell nuclei in the bulb, the absence of a cellular mantle, muscle plates, cuticular hooks and secretory girdles or ampullae and a proboscis cone with apex or in other words the presence of a bipartite cone epithelium. The author distinguishes two types of proboscises within the family: a *Koinocystis*-type, present in the *Koinocystis* species and a *Itaipusa*-type, wide spread in the family and present in *Itaipusa*, *Groveia*, *Brunetia*, *Falkla*, *Tenerrhynchus*, *Paratenerrhynchus*. The *Koinocystis*-type of proboscis has a strong sphincter at the junction built up of a single layer of ribbon-like muscle fibres, while the sphincter in the *Itaipusa*-type of proboscis is constructed of a bundle of filamentous muscle fibres enclosed in a layer of extracellular matrix (Karling 1980). Weakly developed sphincters of both types are found in for instance *Parautelga* and some species of *Utelga* (*Koinocystis*-type)

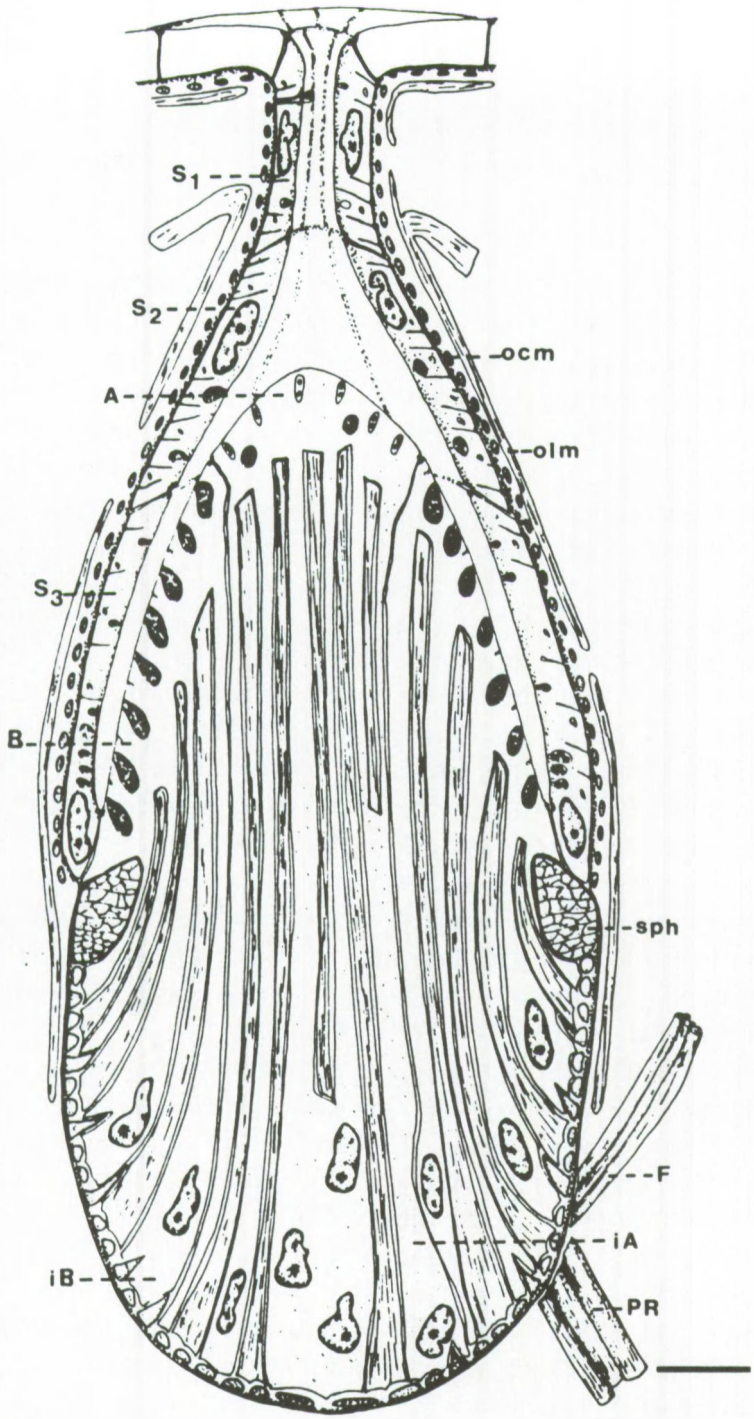


Fig. 1. Reconstruction of the proboscis in *Itaipusa karlingi*. Scale bar: 10 µm.

and *Groveia* (*Itaipusa*-type). In his phylogenetic reconstruction Karling principally takes into account the differences in the organization of the male copulatory organ and the female atrial organs. The differentiation of a proboscis juncture sphincter, enclosed by extracellular matrix, is sometimes additionally used but does not form a synapomorphy within the Koinocystididae (Karling 1980). Brunet (1972) paid more attention to the fact that the nuclei in the sheath epithelium are more or less organized in several rings and that one of them is often situated at the junction. Together with the absence of insunk nucleiferous cell parts along the sides of the bulb as in Polycystididae, this indicates an intra-epithelial localization of the nuclei of the sheath epithelium. The proboscis of three koinocystidid species and genera has now been studied ultrastructurally,the results clarify former light microscopic observations.

Materials and methods

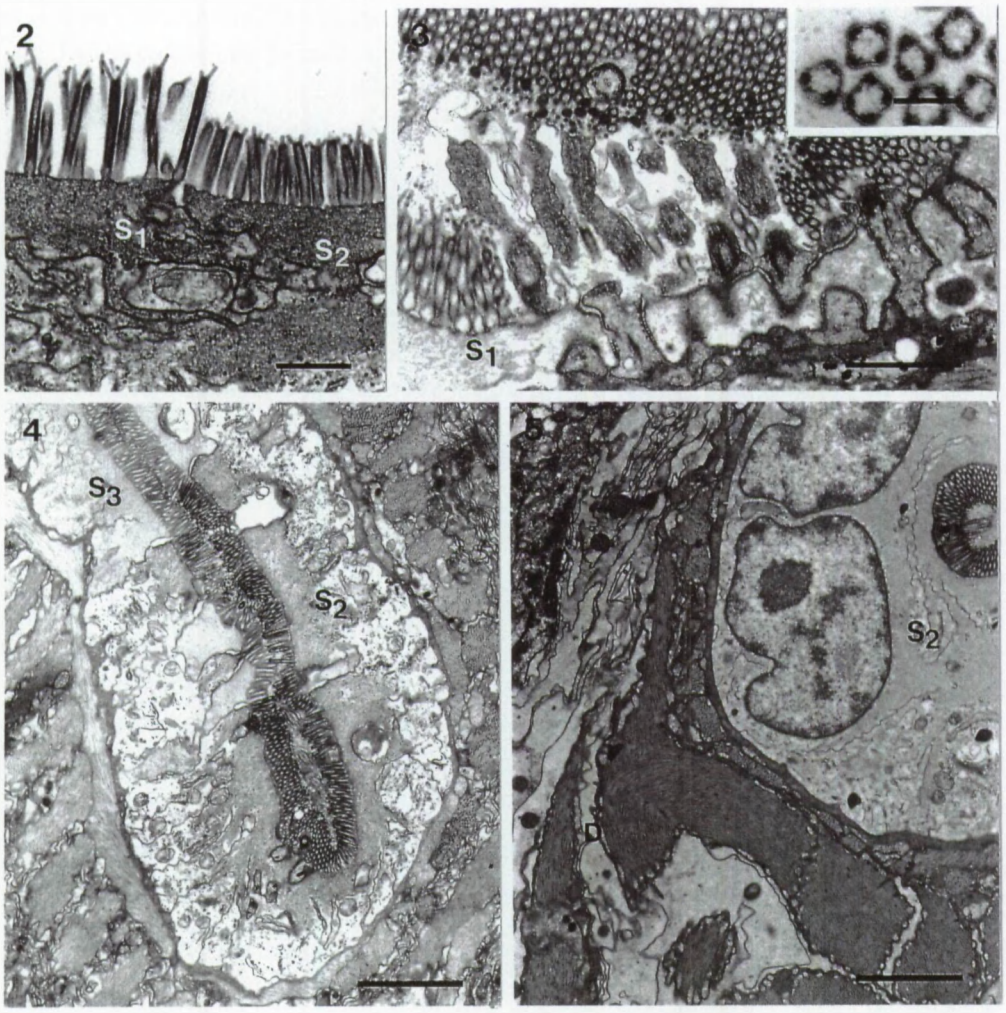
Specimens of *Itaipusa karlingi* were collected from muddy sediments in an old salina (40 cm depth) at the Mediterranean at Ile des Embiez (France, march 1987). The specimen of *Tenerrhynchus magnus* was collected at Calvi (Corse, April 1985) at 10 meters dept by scuba-diving. The specimen of *Parawelga bilioi* was obtained from the Gullmar-fjord at the Swedish west coast, June 1988 near Gåsö at 40 meters depth by means of an Ockelmann-sledge. Specimens of *I. karlingi* were extracted by sieving the fine mud and washing the remaining sediment and animals into a petri-dish. Extraction of specimens of *T. magnus* was carried out by decantation with a $MgCl_2$-solution isotonic to seawater. *P. bilioi* was extracted from the muddy sediment by the extraction method of Armonies & Hellwig (1986).

Results

The description of the proboscis is mainly based on *I. karlingi* but valid for *T. magnus* and *P. bilioi* as well. If differences do occur, they are stated explicitly in the text. The proboscis in *I. karlingi* and *T. magnus* is about 240 µm long, in *P. bilioi* only 85 µm (measured from the proboscis pore to the nodus).

Epithelia, glands and sensory cells

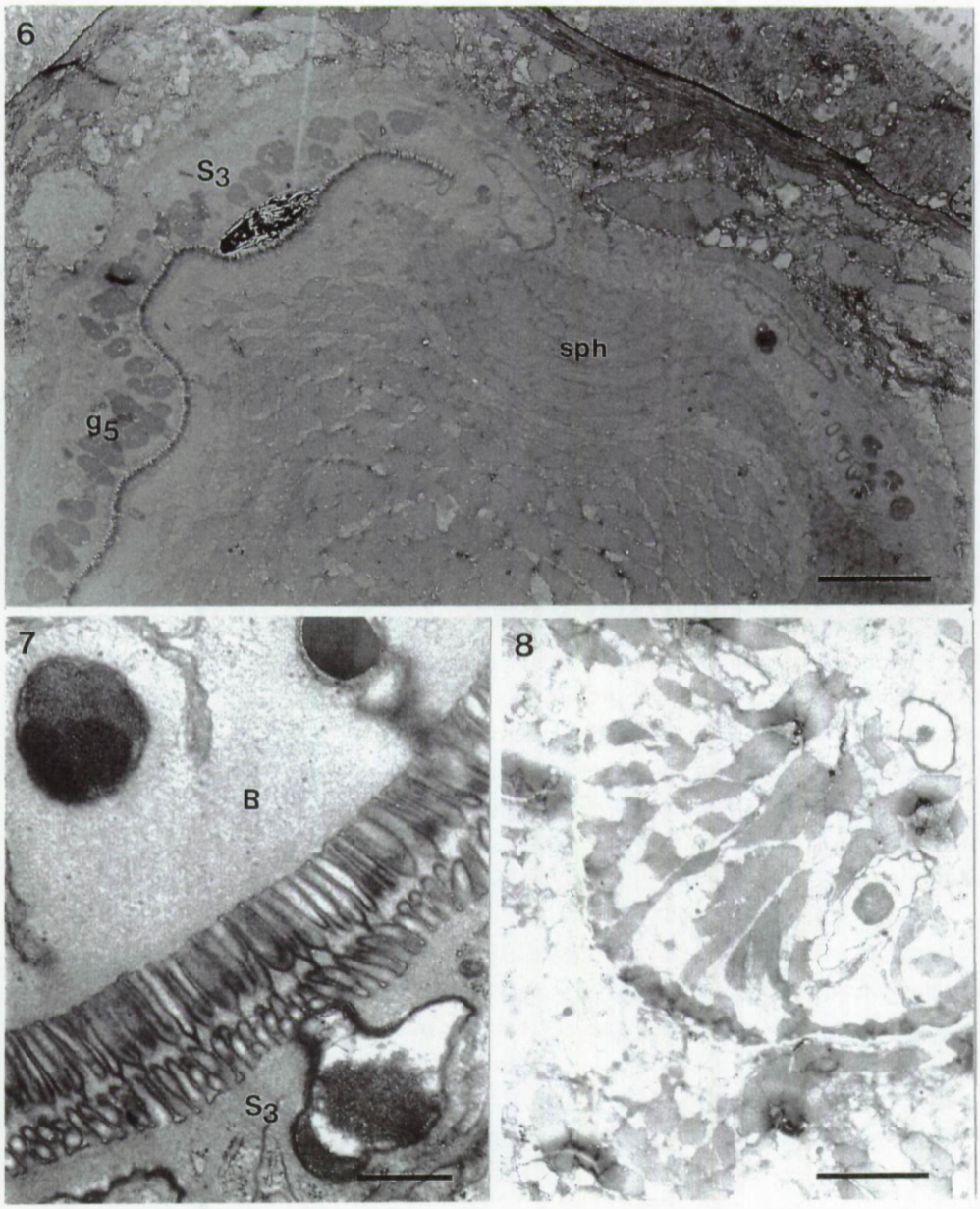
Epithelia. The proboscis epithelia of Koinocystididae are formed by five circumferential belt, three of which constitute the sheath epithelium and two of which cover the cone (Fig. 1). The distal and median belt of the sheath epithelium are cellular, the proximal belt of the sheath epithelium and the cone epithelium are syncytial. The total proboscis epithelium is devoid of cilia. Cell junctions are formed by up to 300 nm deep zonulae adhaerentes, subsequent septate junctions and maculae adhaerentes (Fig. 2).



Figs. 2-5. Itaipusa karlingi.
Fig. 2. Microvilli of the distal (S_1) and median (S_2) belt of the sheath epithelium with characteristic extensions. Scale bar: 0.5 µm.
Fig. 3. Intra-epithelial multiciliary receptors in S_1. Scale bar: 1 µm.*Inset*: microvilli of S_1 in cross section. Scale bar: 0.1 µm.
*Fig. 4.*Transition of S_2 and the proximal belt of the sheath epithelium (S_3). Scale bar: 2 µm.
Fig. 5. Nucleus of a cell of S_2, outer circular and longitudinal muscles, which are continuous with the dilators of the sheath (*D*). Scale bar: 2 µm.

In *I. karlingi*, the sheath epithelium varies in height from 3.5 µm at the pore to 1.5 µm near the junction if the proboscis muscles are not contracted. The distal belt of the sheath epithelium is formed by four cells with intra-epithelial nuclei. The cells bear characteristic microvilli, about 950 nm in length with two tentacles on the tip (Fig. 2). Four electron dense stalks are present in the microvilli, in cross-section they are seen as four condensations at the periphery of the microvilli (Fig. 2 *inset*). The median belt of the sheath epithelium is formed by four cells as well and bears microvilli of the same type as in the distal belt, however, much shorter in length (400-500 nm) (Fig. 2). The proximal belt of the sheath epithelium is a syncytium with five intra-epithelial nuclei (10 µm) and bears normal shaped, 400 nm long microvilli (Fig. 6). The microvilli are less closely packed in the distal belt (7 per linear µm) than in the median and proximal belt (11 per linear µm). A peripheral fibrillar layer is present in the three belts, although less prominent in the proximal belt, and varies in thickness from 1200 to 500 nm. Under the sheath epithelium a thin (150-200 nm) and uniform basement membrane is present (Figs 3, 4). A fibrous layer serves as cytoskeletal support and fills the upper part of the cytoplasm of the cells. Mitochondria, Golgi complexes, infoldings of the basal plasma membrane, which reach up to the apical fibrous layer, and the 8 µm long nuclei are restricted to the basal part of the cells (Figs 4, 5). The sheath epithelium of the proboscis in *P. bilioi* and *T. magnus* is formed by three circumferential belts as well, the number of cells in the belts could not be determined with certainty from our sections. In *T. magnus* a total number of nineteen up to 14 µm long nuclei is present in the distal and median part of the sheath epithelium. No intra-epithelial nuclei were observed in the proximal part of the epithelium near the junction. The microvilli of the distal and median belt measure about 900 nm, those of the proximal belt are only 600 nm in length (Figs 9, 10). In *P. bilioi* four 9.5 µm long nuclei are found in the proximal belt of the sheath epithelium at the junction. The distal belt of the sheath epithelium is covered by 1200 nm long microvilli (Figs 13, 14).

The bipartite cone epithelium in *I. karlingi* and *T. magnus* is 1.5 µm thick at the apex to 3 µm near the junction and formed by two syncytial belts. Cell junctions are formed by up to 1.7 µm deep zonulae adhaerentes, septate junctions and maculae adhaerentes. In *I. karlingi* the cone epithelium bears 450 nm long microvilli, near the junction those in the basal belt have electron dense, pointed tips (Figs 6, 7). These elongated electron dense tips disappear gradually towards the apex. In *T. magnus* the apical cone epithelium bears 700 nm long densely packed (10 per linear µm) microvilli. The basal cone epithelium bears two types of microvilli, 700 nm long normal-shaped microvilli with dense margins in the distal parts and larger arrow-shaped microvilli, 900 nm long with dense condensations at their periphery in the distal part (Fig. 10). In *P. bilioi* the microvilli of the apical cone

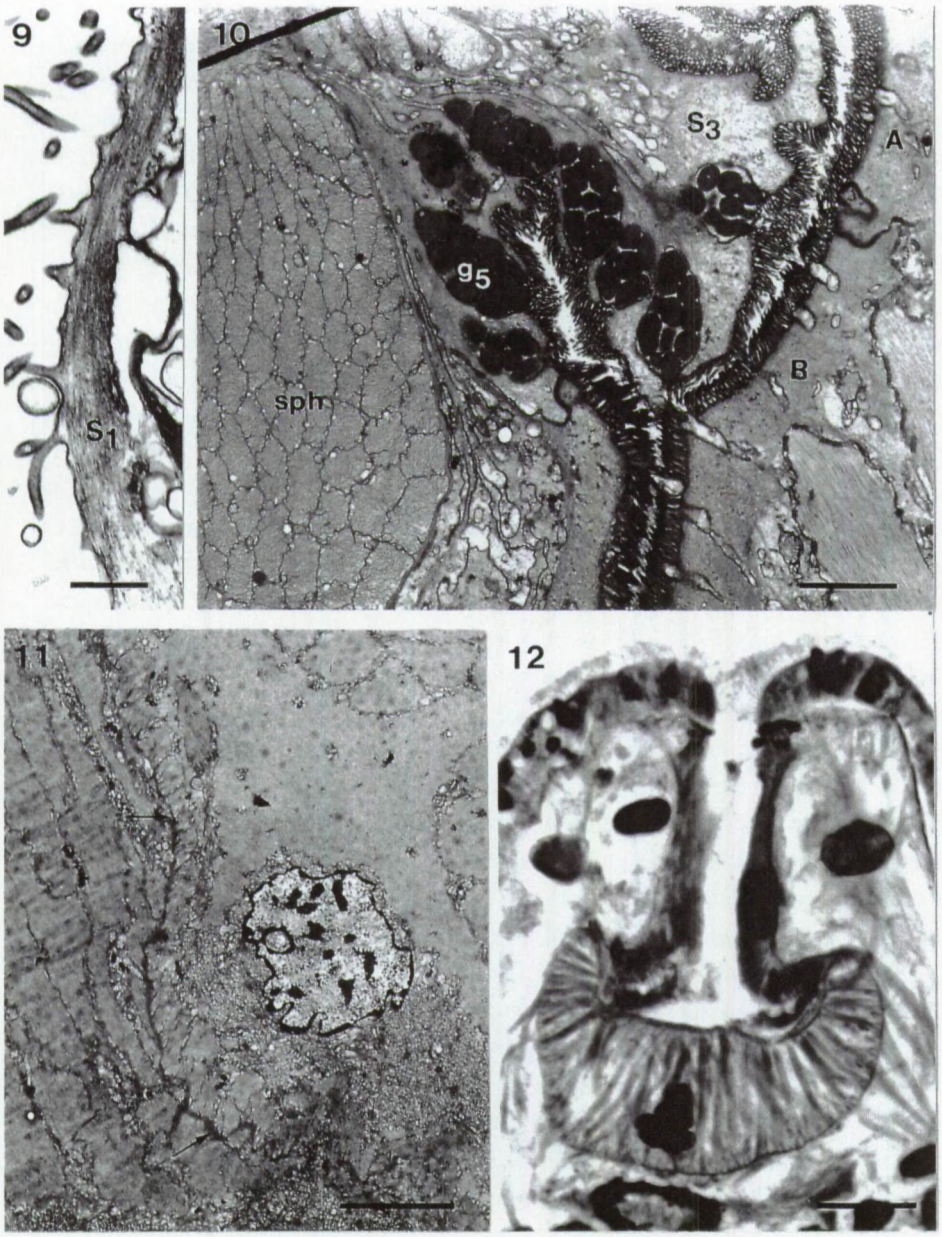


Figs 6-8. Itaipusa karlingi.
Fig. 6. Cross section at the junction with nuclei of the proximal belt of the sheath epithelium (S_3), $g5$ gland necks and a part of the proboscis sphincter (*sph*). Scale bar:5 µm.
Fig. 7. Basal cone epithelium (*B*) with g_7 gland necks and microvilli with apical condensations and sharp tips opposite of S_3. Scale bar: 0.5 µm.
Fig. 8. Bulb in cross section showing intrabulbar epithelial nuclei, circular and longitudinal muscles. Scale bar: 5 µm.

long; those of the basal cone epithelium are 550 nm long (11 per linear µm) with dense margins (Fig. 16). The apical layer of the epithelium, which covers the internal cone retractors, is void of cell organelles and shows a fibrous aspect. Sunken parts of both belts are found between the fibres of the inner longitudinal musculature. In *T. magnus* a 60 nm thick terminal web is present under the apical plasmalemma (Fig. 10). A basement membrane is not found under the cone epithelium. In *T. magnus* the epithelial cell parts are very large in the posterior part of the bulb and show patches of homogeneous fine and coarse granular cytoplasm (Fig. 11). The up to 10 µm long nuclei with large nucleoli (1.2 µm) and peripheral heterochromatin are situated in the posterolateral part of the bulb close against the inner circular muscles. In *I. karlingi* and *T. magnus* many nuclei are present in the bulb. In *P. bilioi* only one nucleus of the apical cone epithelium is found in the posterior part of the bulb. The nucleiferous cell parts of the basal cone epithelium are found behind the bulb. Minute perfoarations in the septum posterolaterally of the bulb, as in *Mesorhynchus terminostylis*, leave passages to the insunk cell parts of the basal belt (Fig. 17).

Glands. In *I. karlingi* the distal and median belt of the sheath epithelium are almost devoid of gland necks. However, few gland necks of two types are present; type g_1 gland necks are empty in our sections and type g_2 gland necks contain spherical electron dense secretion granules (700 nm) (Figs 5, 7). In the proximal belt of the sheath epithelium three types of gland necks are present. In the anterior part mostly type g_3 gland necks are found, containing granular, moderately electron dense secretion granules (700-800 nm). Above the junction most of the gland necks (type g_5) are filled with spherical electron dense secretion granules up to 900 nm in size (Fig. 6). Few empty gland necks (type g_4) are found between type g_3 and g_5 gland necks. In *T. magnus* only few gland necks (type g_2) pierce the median belt of the sheath epithelium, they contain 500 nm broad, electron dense secretion granules. Two types of gland necks pierce the proximal belt of the sheath epithelium. Type g_5 gland necks form a glandular ring above the junction as in *I. karlingi* and are stuffed with large secretion granules, up to 1000 nm in diameter (Fig. 10). Microtubules are present in the cortical layer. Type g_3 gland necks are less abundant and filled with small, electron dense secretion granules of 150 nm diameter. The proboscis of *P. bilioi* is characterized by the presence of only few gland necks. No gland necks pierce the distal and median belt of the sheath epithelium, type g_5 gland necks with moderately electron dense secretion granules are present above the junction in the proximal belt (Fig. 15).

In *I. karlingi* the basal cone epithelium is pierced only by very few 1 µm wide gland necks (type g_7) with densely packed electron dense secretion granules (420 nm) (Fig. 7). The apical cone epithelium is pierced by two types of glands. Type g_9 gland necks contain



Figs.9-11. Tenerrhynchus magnus.
Fig. 9. Distal belt at the proboscis pore (S_1), sparse and long microvilli and fibrous cytoplasm. Scale bar: 0.5 µm.
Fig.10. Transverse section showing the proximal belt of the sheath epithelium (S_3) with g_5 gland necks, basal (*B*) and apical (*A*) cone epithelium. Note the typical microvilli of *B* and the proboscis sphincter (*sph*) enclosed by ECM. Scale bar: 2 µm.

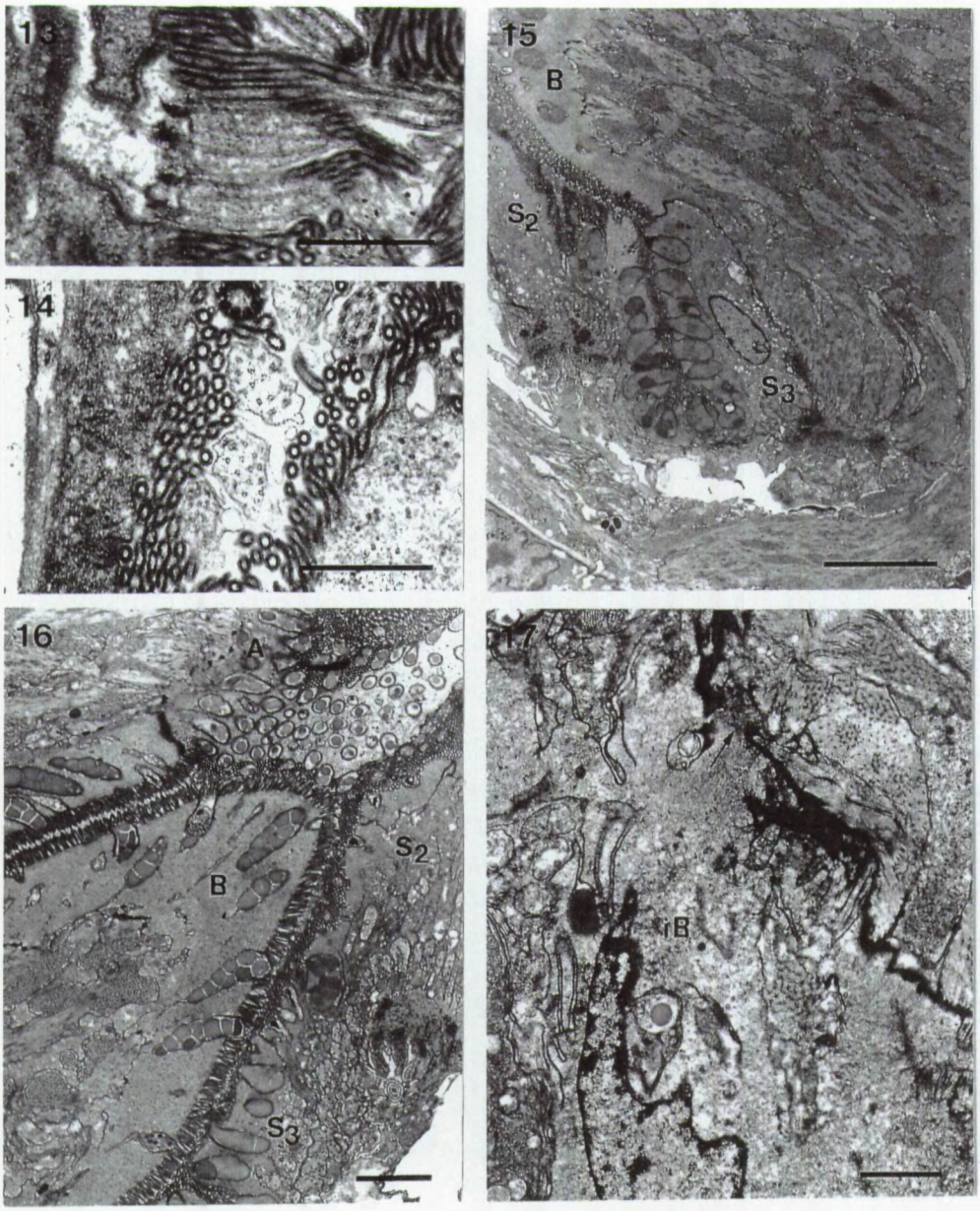
small, electron dense secretion granules (250 nm). The necks are reinforced by peripheral microtubules. Type g_{10} gland necks contain large, moderately electron dense secretion granules up to 1000 nm in diameter. In *T. magnus* two types of gland necks are present in the basal cone epithelium; small gland necks (type g_7) contain electron dense, spherical secretion granules, 400 nm in size, and type g_6 gland necks are filled with 220 nm wide, empty granules (Fig. 10). Type g_9 gland necks surface in the apical cone epithelium an contain ovoid 300 nm long electron dense secretion granules. In *P. bilioi* two types of gland necks are present in the basal cone epithelium, type g_7 gland necks are 3.5 μm long and contain closely packed 400-500 nm large secretion granules (Fig. 16). Type g_6 gland necks contain 180 nm great spherical secretion granules with fine content. Type g_9 gland necks with a cortical layer of microtubules and moderately electron dense secretion granules (300-350 nm in size) surface in the apical cone epithelium (Fig. 16).

Sensory cells. The distal and median belt of the sheath epithelium in *I. karlingi* are pierced by groups of uniciliary receptors. The receptors have long primary rootlets, the secondary rootlets radiate slanting from the basal bodies to the cell junctions (Figs 4, 5). The cilia have normal 9+2 axonemata. The same type of receptors is present in the proximal belt as well but they do not form groups. Numerous uniciliary receptors are found in the cone epithelium as well. The cilia have rootlets and blunt ciliary shafts. In *T. magnus* uniciliary receptors are present in the median and proximal belt of the sheath epithelium. Their primary rootlets point obliquely forward, as the ciliary shafts do. The secondary rootlets are found at the bottom of the basal bodies and form a half circle. In *P. bilioi* numerous uniciliary receptors are spread throughout the median belt of the sheath epithelium. They are less abundant in the proximal belt. The short secondary rootlets form a solid base posteriorly of the basal bodies. The cone epithelium contains uniciliary receptors with blunt ciliary shaft, they are especially abundant in *I. karlingi*. In the three species, the distal belt is additionally pierced by few multiciliary receptors (Fig. 3). The cilia have aberrant axonemata composed of single microtubules only and lack rootlets.

Proboscis musculature

Outer proboscis musculature. The outer musculature is composed of a circular and longitudinal muscle layer around the sheath epithelium and the motional muscles around

Fig. 11. Transverse section of the bulb with intrabulbar nucleiferous cell part of the basal cone epithelium with fine fibrillar and granular part containing a nucleus. *Arrows*: septum. Scale bar: 5 μm.
Fig. 12. Acrumena massiliensis. Sagittal section of the proboscis with nuclei in the median part of the sheath and in the bulb. Short proboscis retractors adhere laterally on the bulb. Scale bar: 10 μm.

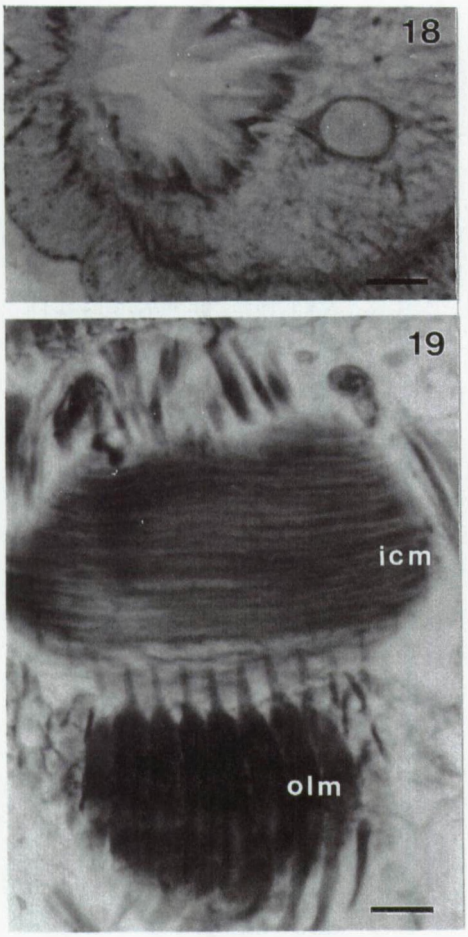


Figs 13-17. Parautelga bilioi.
Figs 13-14. Intra-epithelial multiciliary receptors in the distal belt in the sheath epithelium. Scale bar: 0.5 µm.
Fig. 15. Transverse section showing the jucntion, proximal belt of the sheath epithelium (S_3) with nucleus and g_5 gland necks, basal cone epithelium (B)with g_7 gland necks. Scale bar: 5 µm.
Fig. 16. Oblique section showing S_2, S_3, B, with g_5 and g_6 gland necks, and A, with g_9 gland necks. Scale bar: 2 µm.
Fig. 17. Insunk nucleiferous part of the B piercing the bulbar septum (*arrow*). Scale bar: 1 µm.

the bulb. In *I. karlingi* circular muscle fibres are found from the proboscis pore down to the junction (Figs 3, 4, 5). In *T. magnus* and *P. bilioi* the circular muscles are present from the median belt of the sheath epithelium on (this is from the insertion of the dilators of the sheath on) up to the junction or just past it. The circular muscles in all three species are surrounded by longitudinal muscles (about seventy in *I. karlingi*). Thin longitudinal muscle fibres, continuous with the longitudinal muscles of the body wall, surround the distal belt of the sheath epithelium (400 nm in *I. karlingi*). From the insertion of the dilators of the sheath on, the longitudinal fibres thicken (1300 nm in *I. karlingi*) and run along the epithelium over the junction along the bulb. Dilators of the sheath are continuous with the outer longitudinal muscles fibres (Fig. 4). The fibres adhere on the basement membrane between the circular fibres. In *P. bilioi* also a second set of longitudinal muscles surrounds the bulb. The fibres enter the epithelium below the bulging part of the proximal belt of the sheath epithelium below the junction, run first against the ECM layer and later freely in the basal cone epithelium (Fig. 15). Here the fibres are attached to the fibrillar layer of the cytoplasm by small desmosomes.

The motional muscles include protractors, fixators, proboscis and integument retractors. The fixators insert on the lateral sides of the bulb, far to the posterior end just in front of the insertion of the proboscis retractors in all three species. In *P. bilioi* they form 5 µm thick bundles and adhere about 15 µm below the junction on the epidermal basement membrane (Fig. 15).

Inner proboscis musculature. Circular and longitudinal muscles are present within the bulbar septum. The circular muscles are found from the nodus almost up to the junction. In *I. karlingi* the anterior most fibres form a well developed sphincter. It is composed of many circular muscles and is surrounded by a layer of extracellular matrix (Fig. 6). The other circular muscle fibres are thin and do not form as continuous layer, they reduce in thickness towards the nodus. The inner longitudinal muscles or internal cone retractors are loosely arranged with epithelial cell strand between them (Fig. 8). They adhere directly on the plasma membrane below the apical fibrillar layer of the cone epithelium by desmosomes. The muscle fibres insert on the inside of the bulbar septum between the circular muscles. In *T. magnus* a single layer of circular muscles is found from the nodus up to the proboscis sphincter and is surrounded by a thin layer of ECM (Fig. 11). The sphincter includes over 170 fibres and is surrounded by a layer of ECM (100 nm), which is thinner at the outside against the outer longitudinal muscles in comparison to the inner lining. The basement membrane of the sheath epithelium continues in the ECM layer at the inside of the sphincter. In *P. bilioi* flat circular muscle fibres are found in the anterior part of the bulb, decreasing in thickness towards the nodus. As in the two other species, circular muscles are lacking under the cone epithelium. The inner longitudinal muscles have irregularly arranged myofilaments;



Figs. 18-19. *Crassicollum musculare.*
Fig. 18. Light microscopic, oblique section showing one spherical insunk part of the distal belt. Scale bar: 10 µm.
Fig. 19. Tangential section showing thickening of outer longitudinal muscles (*olm*) and thick layer of inner circular muscles (*icm*). Scale bar: 10 µm.

a central core of longitudinal filaments seems to be surrounded by spirally arranged filaments.

Discussion

From light microscopic observations, the presence of epithelial belts in the sheath epithelium was already indicated by Brunet (1972), the author noted the presence of a ring of nuclei at the junction as a general feature for Koinocystididae. For *Utelga bocki* nuclei in the sheath epithelium have been figured at three levels; near the proboscis pore, at the insertion of the dilators and at proximal end of the epithelium (Karling 1954). The nuclei of the distal belt are often overlooked in light microcopic investigations and the presence of sheath epithelial nuclei are only indicated from the dilators on, up to the junction (Brunet 1972). From electron microscopic observations, a tripartite sheath epithelium is recorded for the families Cystiplanidae, Polycystididae, Koinocystididae and for *Marirhynchus longasaeta* and *Mesorhynchus terminostylis*, two species with uncertain systematic position. Insunk nucleiferous cell parts of the sheath epithelium are never present. Only the proximal belt of the sheath epithelium is syncytialized, the median and distal maintain their cellular organization. A decrease in length of the microvilli from the distal towards the proximal belt is found in Koinocystididae. In *Itaipusa* the reduction in length is found between the distal and the median belt, in *Tenerrhynchus* between the

median and the proximal belt. In *Acrumena massiliensis*, the large nuclei of the sheath epithelium are found on two levels only; in the median part of the cavity and proximally at the junction, as in Koinocystididae (Brunet 1965) (Fig. 12). The presence of a tripartite sheath epithelium remains possible but can not be stated with certainty. Possibly a shorth distal belt is present, of which the nuclei are not observed in the sections (Fig. 12). If a bipartite sheath epithelium is indeed present, *Acrumena* must be closely related to *Psammorhynchus*, *Cytocystis* and species of the *Cicerina*-group. The proboscis of *Acrumena* is furthermore characterized by short proboscis and integument retractors as in *Psammorhynchus tubulipenis* and Cicerinidae (Brunet 1979). The same author mentions the presence of a parenchymous tissue behind the proboscis bulb as in *Lekanorhynchus remanei* and *Nigerrhynchus opisthoporus* (Chapter 7 *Fig. 22*, Brunet 1979, Meixner 1938 *p.27*). A for Eukalyptorhynchia general bipartite cone epithelium is also present in Koinocystididae. The microvilli of the cone epithelium are fairly long in comparison to other Eukalyptorhynchia (see chapter 11 table 2). Especially in *Tenerrhynchus* the microvilli are long and some in the basal belt have characteristic sharp tips and give the basal part cone epithelium a sclerotic appearance in light microscopic observations (Brunet 1972). A basement membrane lacks beneath the entire cone epithelium as in *Psammorhynchus*, *Cytocystis* and *Mesorhynchus* (De Vocht 1990, 1991). The origin of the intrabulbar nuclei is different, however. In *Itaipusa* and *Tenerrhynchus* both the nuclei of the basal and the apical belt of the cone epithelium are located inside the bulb. In *Psammorhynchus* and *Cytocystis*, in contrary, only the nucleus of the apical cell is situated in the bulb, while the nucleiferous parts of the basal belt are found insunk behind the bulb (De Vocht 1990). The other peripheral intrabulbar nuclei belong to the proximal belt of the sheath epithelium. The organization of the cone epithelium in *Parautelga* is identical to the organization in *Mesorhynchus terminostylis*, the apical cone epithelium has a intra-epithelial nucleus, the nucleiferous cell parts of the basal cone epithelium are located insunk behind the bulb. Contrary to the original description of *Parautelga*, only one nucleus is present in the bulb in our specimen and not several as mentioned by Karling (1964). *Gnorimorhynchus dividuus*, a species which was provisionally included in the Koinocystididae, is the only related species with only a few, large nuclei in the posterior part of the bulb (Brunet 1972). The species was excluded from the Koinocystididae by Karling (1980) and the author doubted the absence of a glandular girdle at the junction as well as the presence of germovitellaria, to his opninion indicating a close relationship with the Cicerinidae. Brunet (1972) pointed out that the organization of the genital organs corresponds to the organization in *Parautelga bilioi*. *Rhinolasius sartus*, also a species which has been either incorporated in the Koinocystididae or formerly the Cicerinidae, contains a few large nuclei in the posterior part of the bulb as well (Marcus 1951).

The concentration of gland necks in the posterior part of the sheath epithelium is a general feature in Eukalyptorhynchia. The number of different types of glands and the appearance of the secretion granules is different for the three species. Type g_5 gland necks are present in all three species and form the principal component of the glandular ring near the junction. They might be homologous to type g_1 gland necks in *Paragnathorhynchus*, *Drepanorhynchides* or in *Ptyalorhynchus*, *Paracicerina* and *Cicerina*. *Itaipusa* is the only investigated species in the Eukalyptorhynchia with just one type of gland necks in the basal cone epithelium, but two types of glands pierce the apical syncytium. The gland necks in the basal cone epithelium of *T. magnus* are found in Cystiplanidae and Polycystididae as well (De Vocht 1989). Type g_{10} gland necks present in the apical cone epithelium of the three species under investigation are generally encountered in other eukalyptorhynch species as well.

Intra-epithelial multiciliary receptors associated with the distal belt of the sheath epithelium are present in many eukalyptorhynch species as Cystiplanidae, some species of Polycystididae, *Placorhynchus*, *Paragnathorhynchus*, *Drepanorhynchides* and *Florianella bipolaris*. Because multiciliary receptors are present in most eukalyptothynch species and because the intra-epithelial localization is encountered in six families, this condition is regarded as the plesiomorphic situation within the Eukalyptorhynchia. In *Crassicollum*, behind the proboscis pore, four spherical invaginations of the distal belt of the sheath epithelium are present (Fig. 18). The invaginations probably represent insunk sensory organs. Uniciliary receptors are present in the three investigated speices and most numerous in *Parautelga*. Theyt are recorded for the related species *Crassicollum musculare* as well (Dean 1977). Furthermore, numerous uniciliary receptors in the sheath epithelium are present in *Toia*, *Nannorhynchides*, *Cytocystis* and *Florianella*. The uniciliary receptors in the cone epithelium are a general characteristic for Eukalyptorhynchia.

Itaipusa and *Tenerrhynchus* both possess a *Itaipusa*-type of sphincter at the base of the cone. A layer of extracellular matrix surrounds the inner ciruclar muscles and the sphincter. *Parautelga* has a *Koinocystis*-type of sphincter, which is formed by flattened muscle fibres of the inner circular musculature. Flat and broad circular muscle fibres are presetn in other genera as well. *Nannorhynchides* and *Zonorhynchus* for instance possess a distinct circular muscle layer in the distal and median part of the bulb (Karling 1952, 1964). A sphincter composed of numerous thin muscle fibres and enclosed by a layer of ECM is only present in some genera of Koinocystididae as *Itaipusa*, *Brunetia*, *Falkla*, *Groveia*, *Tenerrhynchus*, *Paratenerrhynchus* and *Crassicollum* (Crassicollidae). The presence of such type of sphincter can be regarded as an apomorphy for the genera mentioned above. In the phylogenetic system proposed by Karling (1980), however, only characters and character states derived from the male copulatory organ and the female atrial organs are used as synapomorphies within the Koinocystididae. A *Itaipusa*-type of sphincter should have

arisen five times within this family and additionally in *Crassicollum*, placed in a separate monotypic family. To my opinion such a parallel formation of a identical structure is rather unlikely.

The terminal cone retractors mentioned by Dean (1977) in *Crassicollum* are thickening of the outer longitinal muscles at the junction and do not function as retractors of the cone (Fig. 19). A second set of muscle fibres around the bulb, which penetrate the cone epithelium as in *Parautelga* is present in *M. terminostylis* as well. They can be regarded as intra-epithelial muscles but their homology to the intra-epithelial muscles in Polycystididae is not certain as pointed out in the chapter on *M. terminostylis*. The proboscis of *P. bilioi* is has a similar organization as the proboscis in *M. terminostylis*.

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Chapter 10.

Anatomy and ultrastructure of the proboscis in *Mesorhynchus terminostylis* .[1]

Introduction

The monotypic genus *Mesorhynchus* was created by Karling (1956) and placed within the family Polycystididae. He based his conclusions on the organization of the genital structures, which are typical for Polycystididae (divisa-type). However, the four pharyngeal knobs (sclerotizations), a typical character for Polycystididae, were missing. Light microscopic observations also revealed a non-Polycystididae type of proboscis with a large intrabulbar nucleus. Due to these conflicting data, the systematic position of *Mesorhynchus* remained uncertain.

Materials and methods

Specimens of *Mesorhynchus terminostylis* Karling, 1956 were collected from muddy sediment with *Pennatula* at 40 meters depth near Gåsö in front of and in the mouth (58°15'68"N and 11°27'30"E) of the Gullmarfjord (Kristineberg) at the Swedish West Coast in June 1988 . Animals were extracted either by sieving the fine mud and washing the remaining sediment and animals into a petri-dish or using the method proposed by Armonies & Hellwig (1986). Type material of *M. terminostylis* from the Swedish Museum of Natural History was studied as well.

Results

Epithelia, gland necks and sensory cells

Epithelia. The proboscis epithelia of *M. terminostylis* comprise of five circumferential belts in the specimens studied (Fig. 1). As in all previously investigated species of Eukalyptorhynchia two belts form the cone epithelium (bipartite). The sheath epithelium is formed by three belts as in Polycystididae, Cystiplanidae and Koinocystididae. These three belts are cellular. The basal belt of the cone epithelium is syncytial, while the apex is covered by a single cell. The proboscis epithelia are devoid of cilia and junctions between cells include an apical-most zonula adhaerens (300 nm deep) and a more basal septate junction and maculae adhaerentes.

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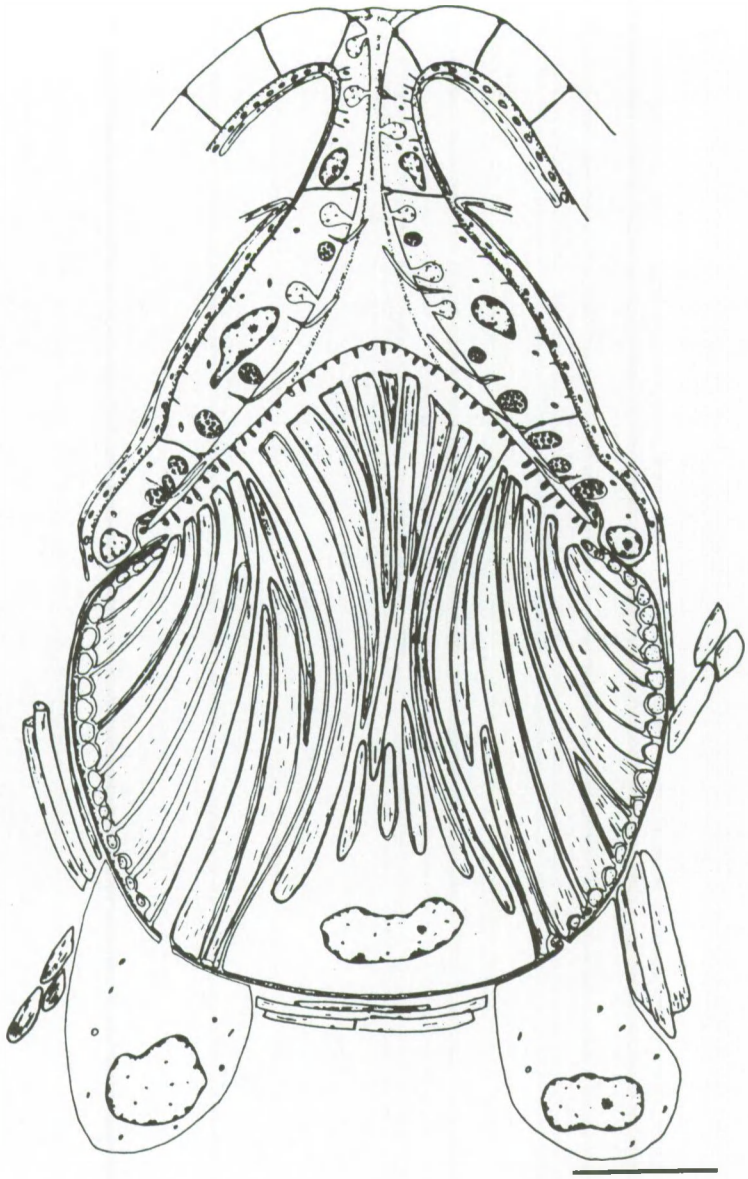


Fig. 1. **Sagittal reconstruction of the proboscis of *Mesorhynchus terminostylis* from electron microscopic observations. Three cellular epithelial belts (S_1, S_2, S_3) with intra-epithelial nuclei form the sheath epithelium, while two belts (*B*, *A*) cover the cone. The syncytial basal belt has insunk nucleiferous cell parts (*iB*), the nucleus of the apical cell is situated in the bulb. Glands and uniciliary receptors pierce the epithelia. Outer circular (*ocm*) and longitudinal muscles (*olm*) surround the sheath and protractors (*P*), fixators (*F*) and proboscis retractors (*PR*) adhere on the bulbar septum (*s*), which encloses the inner longitudinal (*ilm*) and circular muscles (*icm*). Intra-epithelial muscles (iem) are only present up to the junction. Scale bar: 10 μm.**

The distal belt of the sheath epithelium (S_1) is formed by two cells, the median (S_2) and proximal (S_3) belts by four cells (Figs. 2, 3). At the proboscis pore the epithelium is only 2 µm thick, but the major part of the distal belt, the median and proximal belts are 5 µm thick. The epithelium surface is covered by long and slender microvilli (10 per linear µm), which are of progressively shorter length from the distal (820 nm), through the median (700 nm), to the proximal belt (500 nm). A distinct terminal web is not present, but a fine fibrillar layer is found in the apical part (1/3) of the cells. The basal plasma membrane forms 300 to 400 nm high infoldings in the cytoplasm (Fig. 2). Mitochondria, few Golgi apparatuses and dense bodies are present in the granular cytoplasm. The distal and median belts have intra-epithelial bean-shaped nuclei, the ends pointing proximally (Fig. 2). The nuclei of the cells in the proximal belt are intra-epithelial as well but situated just below the junction of sheath and cone epithelium. The nuclei increase in length from the distal, over the median, to the proximal belt (7 µm, 8.5 µm and 15 µm respectively). The underlying basement membrane is bipartite, 500 to 350 nm thick at the pore and around the distal belt and 350 to 170 nm thick from the median belt on, and is continuous with the bipartite epidermal basement membrane at the pore. Below the junction the 170 nm thick basement membrane is continuous with the bipartite bulbar septum. The posterior part of the proximal belt of the sheath epithelium is separated from the inner musculature by a thin and electron dense layer of extracellular matrix (90 nm) (Fig. 4 *arrow*), which is found around the intra-epithelial muscle fibres (10-20 nm) as well. This layer is continuous with the 45 nm thick apical layer of the septum. Where the motional muscles insert on the septum only a 90 nm electron dense layer is present, which is continuous with the basal lamina of the septum (Fig. 6).

The bipartite cone epithelium is formed by a basal syncytium (*B*) and one apical cell (*A*) (Fig.1). The part of the epithelium covering the cone is 1 to 1.5 µm thick and forms a fibrillar apical layer with dense bodies but devoid of cell organelles. A part of the basal syncytium lines the posterior 3 µm of the cavity. The cone epithelium is covered by slender, 700 nm long microvilli; these of the basal belt have dense tips (Fig. 3). There is no basement membrane separating the epithelium from the inner proboscis musculature. Granular cytoplasmic cell strands with mitochondria are found among the inner longitudinal muscles in the bulb. The nucleus-bearing part of the apical cell lays in the centre of the bulb and is widest in the posterior part where the nucleus (6.5 µm) is situated. A fibrillar layer of cytoplasm is found around the nucleus (Fig. 5). The interdigitating cytoplasmic cell strands of the basal cone epithelium are situated all around the apical cell in the bulb. Light and dense patches are present in the cytoplasm, the dense parts are mainly found near the plasma membrane surrounding the muscle fibres. Bundles of microfibrils (100-150 nm thick) run from hemidesmosomes with the bulbar septum into the cytoplasm (Fig. 6 *arrow*). Strands of the basal cone epithelium leave the bulb at the

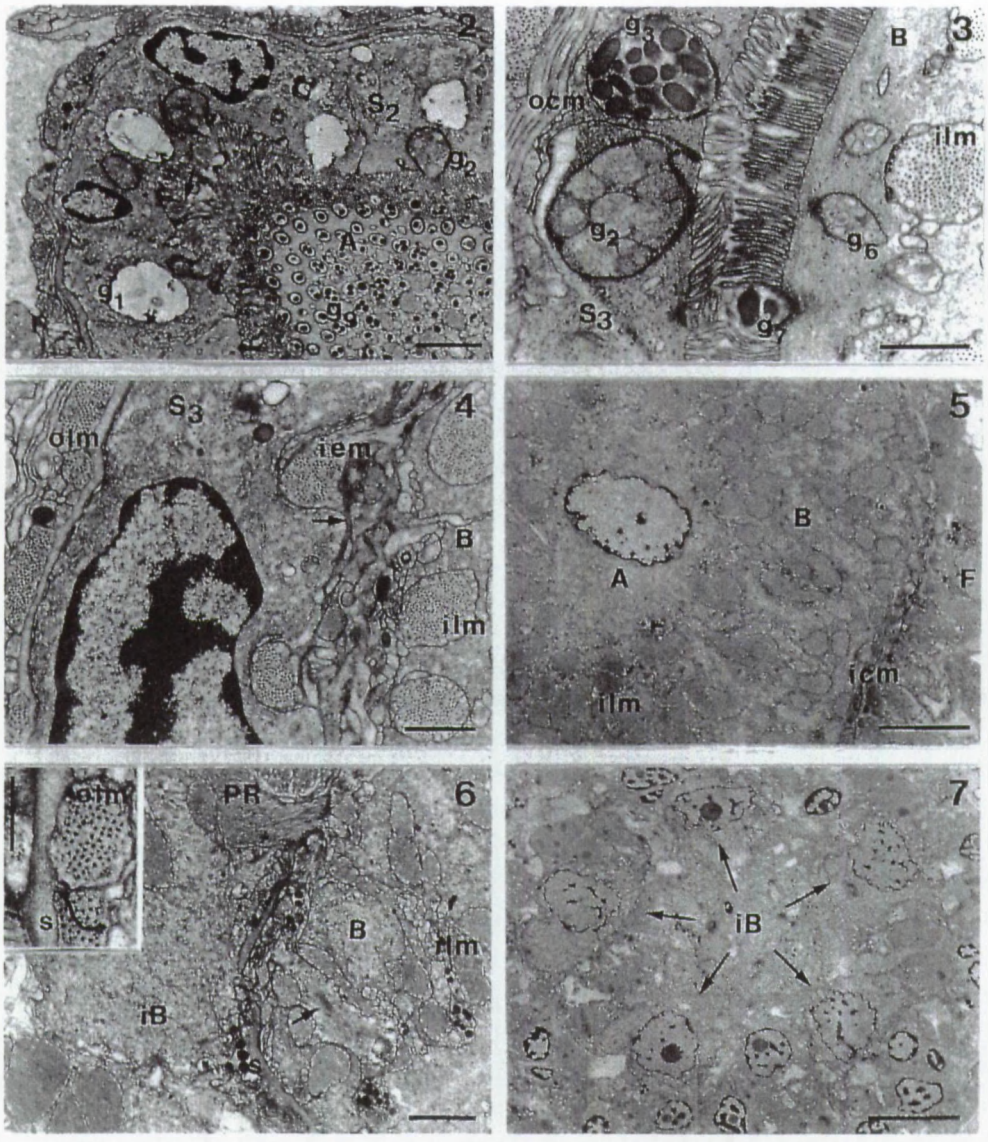


Fig. 2. Cross section through the median belt of the sheath epithelium (S_2) and the apical cell on the cone (*A*). Type g_1 and g_2 gland necks and uniciliary receptors (arrow) penetrate S_2, type g_9 gland necks and uniciliary receptors *A*. Scale bar: 2 µm.

Fig. 3. Cross section through the proximal belt of the sheath epithelium (S_3) and the basal cone epithelium (*B*). S_3 is surrounded by outer circular (*ocm*) and outer longitudinal muscles and pierced by type g_2 and g_3 gland necks. Type g_6 and g_7 gland necks surface through *B* which bears long microvilli with condensations at the tips. Scale bar: 1 µm.

Fig. 4. Cross section of the nucleiferous cell parts of the proximal belt of the sheath epithelium (S_3), showing the outer longitudinal muscles (*olm*), lipid droplets (*arrow*), intra-epithelial muscles, intrabulbar part of *B* and inner longitudinal muscles (*ilm*). Scale bar: 1 µm.

posterior end through narrow perforations in the bulbar septum (Fig. 6). Behind the bulb five insunk nucleiferous cell parts are formed; two dorsolaterally, two ventrolaterally and one ventrally (Fig. 7). The 6.5 µm large nuclei possess a narrow margin and scattered patches of heterochromatin and have a large nucleolus (2.5 µm) with less electron dense appearance.

Glands. Type g_1 gland necks in the distal and median belt of the sheath epithelium lost their contents during preparation and contain only patches of membranes (Fig. 2). Type g_2 gland necks with moderately electron dense granular secretion granules (570 nm) are situated in the median and proximal belt of the sheath epithelium (Figs 2,3). Often the central part of the granules appears less electron dense. Type g_3 gland necks with electron dense secretion granules up to 500 nm long and 230 nm wide (Fig. 3), are present in the posterior part of the proximal belt of the sheath epithelium together with type g_2 gland necks . The basal belt of the cone epithelium is pierced by two types of gland necks. Type g_6 gland necks are rather inconspicuous because the 200 nm secretion granules with low electron density (or empty) are only stored in the apical parts of the gland necks, which are less than 1 µm long. The granules have a centrally condensed contents (Fig. 3). Type g_7 gland necks are more numerous and contain ovoid, electron dense secretion granules (up to 700 nm long) stored in narrow necks only 500 nm wide (Fig. 3). Often less electron dense granules are present. The cell covering the apical part of the cone is pierced by type g_9 gland necks with terminal peripheral reinforcements of microtubules (Fig. 2). The 200 to 400 nm, spherical to ovoid secretion granules are usually very electron dense but less electron dense secretion granules are often present as in type g_7 gland necks.

Sensory cells. Two types of uniciliary receptors are present in the proboscis epithelia of *M. terminostylis*. One type of receptors is present in the sheath epithelium, only few in the distal belt but more numerous in the median and proximal belt (Figs 1, 2). The terminal ends of the dendrites are connected to the epithelium by apical zonulae adhaerentes

Fig. 5. Cross section through the posterior part of the bulb with the nucleiferous cell part of the apical belt (*A*) and peripheral cytoplasmic cell strands of the basal belt (*B*) between the inner longitudinal muscles (*ilm*). Inner circular muscles (*icm*) line the septum at inside while fixators (*F*) insert on the outside. Scale bar: 5 µm.
Fig. 6. Cross section in the posterior part of the bulb with a perforation in the septum and insunk cell part of the basal cone epithelium (*iB*) and insertion of a proboscis retractor (*PR*). Scale bar: 2 µm. *Inset* : septum at higher magnification showing an apical electron dense layer (*left*) and a basal less electron dense layer (*right*). A dense ribbon is present in the outer longitudinal muscle (*olm*). Scale bar: 0.5 µm.
Fig. 7. Cross section posteriorly of the bulb, showing the five insunk nucleiferous cell parts of the basal cone epithelium (*iB*). Scale bar: 10 µm.

and more proximal septate desmosomes. The basal body is situated in a small extension that protrudes slightly above the epithelium surface; it is connected to a long, thin primary rootlet (up to 3.5 µm long). From the basal body, cross-striated secondary rootlets radiate

obliquely to the zonulae adhaerentes at the base of the extension with the epithelium. Uniciliary receptors with short ciliary shafts, primary rootlets but without secondary rootlets are present in the cone epithelium.

Proboscis musculature

All muscle fibres have a peripheral sarcoplasmic reticulum; non are cross-striated.

Outer proboscis musculature. The outer musculature of the proboscis comprises circular and longitudinal muscles around the sheath and the motional muscles (*i.e.*, protractors, fixators and retractors) mostly inserting on the bulb. A circular muscle layer is present around the sheath epithelium from the median belt and the dilators of the sheath on up to the posterior end of the proximal belt were the transition into inner circular muscles and bulbar septum is situated. While no circular muscles are present around the distal belt of the sheath epithelium, more than twenty very thin longitudinal muscle fibres are present here. They are situated immediately below the basement membrane and are almost totally embedded in the basement membrane at the pore. From the median belt on about 32 thick muscle fibres enclose the sheath. Apically the fibres are continuous with the dilators of the sheath (*D*) and attached to the basement membrane; posteriorly, they are attached to the bulbar septum. They surround the bulb laterally together with the 34 or 35 intra-epithelial muscles and about 30 thin fibres situated between the outer longitudinal and intra-epithelial fibres just below the epithelium at the junction. The latter form a intermediate set of longitudinal muscles. All these muscle fibres are found from here to the posterior part of the bulb.

The motional muscles comprise protractors, fixators, proboscis retractors and integument retractors. Three pairs of protractors inserting in a knot at the nodus, originate on the epidermal basement membrane in the anterior body end. The ventrolateral pair is composed of three fibres each, the lateral pair of seven and the dorsolateral pair of nine. More anteriorly some fibres split and the final number of fibres in the bundles is somewhat higher. Fixators insert on the lateral side of the bulb and adhere on the epidermal basement membrane. Four pairs of proboscis retractors insert on the posterolateral part of the bulb and run towards the posterior part of the body. Additional thin muscle fibres run from the nodus posteriorly.

Inner proboscis musculature. The inner musculature includes circular and longitudinal muscles. The inner musculature has a loose appearance in that they do not form associated bundles but rather extended individually through the cone epithelium , separated by cytoplasmic cell strands of the cone epithelium (Fig. 5). No nuclei of the inner musculature could be found. The inner circular muscle layer is composed of very thin (700 nm) fibres that surround the inner longitudinal muscles from the nodus almost up to the junction. In the

anterior part of the bulb, the circular muscles are surrounded at the outer side by a bipartite layer of extracellular matrix (bulbar septum) (Fig. 6 *inset*). Anteriorly, the longitudinal muscles are attached to the apical fibrillar layer of the cone epithelium by desmosomes, posteriorly they connect to the bulbar septum by hemidesmosomes. Thick filaments measure 2-3 nm in diameter and show condensations all around which form connections to the 7 nm thick thin filaments.

Intra-epithelial muscles. As already mentioned 34 or 35 intra-epithelial muscles surround the bulb laterally together with the outer longitudinal muscle fibres and a intermediate set of muscles. The intra-epithelial muscles pierce the transition zone of sheath epithelial basement membrane and bulbar septum and enter the posterior part of the proximal belt of the sheath epithelium (Fig. 4). They adhere to the apical fibrillar layer of the basal belt of the cone epithelium just above the junction. Cell junctions are formed by narrow desmosomes. Laterally of the bulb the fibres are connected with dense ribbons (Czubaj & Malec 1988) to the bulbar septum (Fig. 6 *inset*).

Discussion

Salient features of the proboscis of *M. terminostylis* are these: sheath epithelium comprising three belts, bipartite cone epithelium, loose musculature in bulb and intrabulbar nucleus of apical cone epithelium. Five insunk nucleiferous cell parts of the basal cone epithelium pass through the proboscis bulb and are situated behind it. The presence of three epithelial belts in the epithelium lining the proboscis cavity and a bipartite cone epithelium is conform to the description of the proboscis in Polycystididae, Cystiplanidae and Koinocystididae (Schockaert & Bedini 1977, De Vocht 1989). However, the belts are cellular, contrary to the three or two syncytial belts in *Polycystis naegelii* and Cystiplanidae. The proximal belt has intra-epithelial nuclei as in Koinocystididae (see Brunet 1972, Karling *et al* 1972). The bipartite cone epithelium is a general characteristic for all Eukalyptorhynchia (De Vocht 1989,1990). The epithelial and muscular organization of the cone and the bulb resembles the organization in *P. tubulipenis*.

In the sheath epithelium, the length of the microvilli decreases from the distal to the proximal belt. The microvilli in the cone epithelium are extremely long compared to those in other species of Eukalyptorhynchia. Moreover, *M. terminostylis* is the only known species to have longer microvilli in the cone exceed than in the sheath epithelium (Fig. 3) (see chapter 11 table 2). The sheath epithelium is characterized by infoldings of the basal plasma membrane as known from other species.

The basement membrane of the sheath epithelium of *M. terminostylis* is continuous with the bulbar septum which, by its bipartite structure, can be homologized with the basement membrane of the cone epithelium. By this interpretation, only the basal epithelium would

have insunk nuclei and the inner longitudinal muscles can be regarded as intra-epithelial, perhaps originating from motional muscles which insert on the bulbar septum. The apical parts of the muscle fibres are squeezed off by the basement membrane during proboscis formation and so the inner longitudinal muscle fibres are void of nuclei. Muscle fibres intruding the bulb as in *Toia calceformis* and *Placorhynchus octaculeatus* confirm this hypothesis. Bundles of microfibrils, present in the intrabulbar cytoplasmic cell strands of the cone epithelium in *M. terminostylis*, were encountered in the cone epithelium on top of the cone in *Cicerina remanei* as well (De Vocht & Schockaert 1988). A syncytial basal cone epithelium is wide spread in Eukalyptorhynchia. The organization of the proboscis in *M. terminostylis* and *Parautelga bilioi* is almost fully identical. both species have a tripartite sheath epithelium with intra-epithelial nuclei, insunk nucleiferous cell parts of the basal cone epithelium, which pass through the bulb, and a intrabulbar nucleus of the apical cone epithelium. Considering the organization of the proboscis, *M. terminostylis and Parautelga bilioi* as well as *Nigerrhynchus opisthoporus* and *Lekanorhynchus remanei* are probably closely related (see also chapter 7. Discussion). The proboscis of *M. terminostylis* and *P. bilioi* can be derived from a Koinocystididae type of proboscis, by evolving from a intrabulbar nucleiferous cell parts of the basal cone epithelium to sunken nucleiferous cell parts or the other way around. Intrabulbar nuclei are known for Koinocystididae in general (Brunet 1972, Karling 1980), *P. tubulipenis* (Karling 1964), *C. clitellatus* (Karling 1953), some species of Cicerinidae and Gnathorhynchidae (Karling 1964, 1983, Meixner 1938) and *Lekanorhynchus remanei*, a species with uncertain taxonomic position (Meixner 1938). Formerly, these nuclei were regarded as myoblast nuclei but they are beyond any doubt epithelial nuclei. Not only nuclei of the cone epithelium are found inside the bulb, also nuclei of the proximal belt of the sheath epithelium can be situated within the bulbar septum (De Vocht 1990). In this case a ring of nuclei at the junction is lacking. In Koinocystididae this ring is present and only the cone epithelium has intrabulbar nuclei.

Relatively few types of glands pierce the proboscis epithelia. Type g_6, g_7, g_8 and g_9 gland necks in *P. naegelii* (Schockaert & Bedini 1977) are probably homologous to type g_2, g_7, g_6 and g_9 gland necks respectively in *M. terminostylis*. Type g_9 necks are typical for the apical cone epithelium in all Eukalyptorhynchia, type g_6 and g_7 necks are present in the basal cone epithelium of *P. naegelii*, Cystiplanidae and *C. clitellatus* as well (De Vocht 1989, 1990, Schockaert & Bedini 1977)

All sensory cells found in the epithelia are uniciliary receptors. The receptors in the sheath epithelium are homologous to those found in the sheath epithelium of Cystiplanidae, *P. tubulipenis* and *C. clitellatus* (De Vocht 1989, 1990). The receptors in the cone epithelium are present in other species as well (De Vocht 1989, 1990, type IV Reuter 1975, Schockaert & Bedini, 1977).

The myofilaments of the muscle fibres are loosely arranged contrary to the dense myofibrils which resemble the cross-striated muscle type in Cystiplanidae (De Vocht 1989). Intra-epithelial muscles are present in Polycystididae and Cystiplanidae as well. In the latter two families the muscle fibres are found up to the apical cone epithelium were they perforate the basement membrane. The same type of intra-epithelial muscles as in *M. terminostylis* is also encountered in *P. bilioi* and *Florianella bipolaris*.

In *M. terminostylis*, three belts constitute the sheath epithelium in Polycystididae, Cystiplanidae and Koinocystididae (De Vocht 1989, Schockaert & Bedini 1977). A bipartite sheath epithelium is known from Cicerinidae, Psammorhynchidae and Cytocystidae, placorhynchidae and Gnathorhynchidae (De Vocht 1990, De Vocht & Schockaert 1988). Concerning the organization of the cone epithelium, however, *M. terminostylis* might be closely related to *P. tubulipenis* and *C. clitellatus*. The absence of multiciliary receptors associated with the distal belt of the sheath epithelium can either originate from a loss or can be the ancient character state. The organization of the proboscis permit us to exclude *M. terminostylis* from the family Polycystididae. On grounds of the proboscis structure *M. terminostylis* can not be separated from *P. bilioi* and Koinocystididae. Creating a new taxon with higher rank for *M. terminostylis* and *P. bilioi* could be a taxonomic solution but still leaves the kinship problems open. Only after ultrastructural investigation of other koinocystid species without a distinct proboscis sphincter (probably with intrabulbar nucleiferous parts basal and apical cone epithelium but possibly with insunk basal cone epithelium), the exact sister taxon of the the formerly mentioned species can be defined. The presence of three belts in the sheath epithelium, the presence of intra-epithelial muscles and fixators indicate a close relationship with the families, Polycystididae, Cystiplanidae and Koinocystididae. The presence of three belts in the sheath epithelium can be used as synapomorphic character. The organization of the cone epithelium might, which occurs in a similar form in the out-group as well, might be considered the result of parallel evolution.

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Chapter 11.

General discussion

Introduction

Although the aim of this study was not to make a monographic revision of the Eukalyptorhynchia, there are some implications for the phylogenetic system of the group. The different anatomical organizations and ultrastructural details in the proboscis are analyzed and evaluated in order to detect useful characters to establish a phylogenetic system which reflects a relationship hypothesis in Eukalyptorhynchia. The proposed system is mainly based on morphological characters obtained from our ultrastructural study on the proboscis. Morphological data on epidermis, pharynx, spermatozoa and eyes are also incorporated in this system.

Not intending to give a complete summary or detailed analysis on phylogenetic systemactics, a few fundamental notitions on phylogenetic systematics are briefly discussed. The theoretical base, methods and principles for phylogenetic systematics have been formulated by Hennig (1966) and recently by Farris (1974), Wiley (1981), Maddison *et al* (1984) and Ax (1984) and applied for Platyhelminthes by Ax (1984) and Ehlers (1984, 1985, 1986). The methodology used to formulate phylogenetic hypotheses is a two-step process (Smith *et al* 1986, Westheide & Rieger 1987). At first the likelyhood that a particular similarity represents a homologous feature must be tested. Secondly the likelihood that one particular configuration of a homologous feature, present in two or more species, is a derived (synapomorphic) condition must be tested. Or in other words, how probable is the conclusion about the reading direction in morphological sequences? If two synapomorphies seem to overlap, the coincidence principle can be used to decide between the two. The principle says that the probability for each single synapomorphy is raised proportionally to the number and the probability of each of the coinciding synapomorphies (Rieger & Tyler 1979, Westheide & Rieger 1987).

A premise in phylogenetic analysis is the monophyletic origin of the taxon under consideration. Comparing organisms and structures in organisms in order to determine relationships among them, systematists have to rely on the use of the homology theorem (Bock 1973, Remane 1952, Riedl 1975). Hence, one needs criteria for identifying homologies or in other words, similarities of pattern that are most easily explained as being caused by inheritance from a common ancestor. Criteria used for identifying homology and analogy in structural and ultrastructural analysis have been given by Rieger and Tyler (1979) and are based on criteria proposed by Remane (1952).

A second principle in phylogenetic systematics is the character state analysis in order to determine monophyletic groups and methods for determining whether a given character state

of a homologous character is derived (apomorphic) or ancestral (plesiomorphic). In this study only ingroup and outgroup analysis have been used to clarify relationships within Eukalyptorhynchia (see Watrous & Wheeler 1981). Ontogenetic data are not available and fossil records to reconstruct a direct evolutionary lineage are lacking as well.

In some cases for the reconstruction of a phylogenetic tree the principle of parsimony has been used (Kluge & Farris 1969, Farris 1970). The principle of parsimony is an important selection criterium between competing phylogenetic hypotheses (see Wiley 1981, Farris 1983). The principle works under the assumption that the most probable estimate of the phylogenetic realtionships within a monophyletic group is the estimate that requires the smallest number of characters transformations. In other words a minimization of parallellisms. But what if parallel evolution does occur? Sluys (1989) pointed out that phylogenetic reconstruction can be made also under the assumption of abundant parallelism and that true parallelism concern homologous characters (Seather 1983). But one must distinguish between repeatedly evolved characters that presumably evolved in parallel or arose convergently. A detailed discussion on parallelism and convergence has been presented by Wiley (1981) and Ax (1984). The distinction is in practise based on the proximity of common ancestry.

The Wagner and Camin-Sokal methods are widely used in numerical cladistics and supported by computer programmes as PAUP (Phylogenetic Analysis Using Parsimony) (Swofford 1984) or PHYLIP (Phylogeny Interference Package version 3.2) (Felsenstein 1989). Probability decisions between competing relationships hypotheses have been made, and certain hypotheses are favorised above others using the either parsimony algorithm. The algorithm works by applying the principle of simplicity or parsimony. Additionally in the computer program PHYLIP, freely destributed by Joe Felsenstein, the probable ancestral state and weighing of the characters as well as putting forward a limited number of reversibilities of characters, have been used. The obtained relationship hypothesis can be transformed into a consequent phylogenetic system using the subordination method. The advantage of the subordination method in phylogenetic classification is that the relationships can be expressed clearly and unambiguously. But in practice also several disadvantages of this method are revealed, such as the creation of many new names and the loss of communicative significant system categories (Hasprunar 1986).

Homology of the eukalyptorhynch proboscis

The proboscis can be regarded as a homolog structure in Eukalyptorhynchia following Rieger and Tylers criteria for identifying homology and analogy (1979). Sufficient information is available to apply the criteria for homology and we can state briefly that the proboscis has the same position with respect to other structures in the organism in all

representatives and that a transformation sequence in probable phylogeny can be discerned (criteria 1 and 2). Furthermore, the component parts of the proboscis have similar positions. Concerning the coincidence with other homolog characters, we payed special attention to the collar receptors in the epidermis and spermatozoa in Kalyptorhynchia (see this chapter, Characters and character states in the eukalyptorhynch proboscis *5* and *6*). The hard structures in the male genital system, stylets or cirrus hooks, are formed by intracellular differentiations in all eukalyptorhynch species investigated.

Applying the criteria for identifying analogs, we conclude that the eukalyptorhynch proboscises are unlikely to be analogs because this structure is certainly not the only possible mean by which its function, namely prey capture, can be accomplished. Preys can also be captured by epidermal glandular function, associated epidermal structures or toxins instead of a specilized protrusile organ with a primary glandular system sometimes with additional structures as hooks or 'muscular' processes in order to catch preys. Our ecological knowledge of these animals does not allow us to make a judgement on the presence or absence of a common selection pressure. Hardly any information is available on food preference of the different species and strategies in prey capture for instance. The presence of an undivided proboscis bulb with septum and central cone protruding into the probscis cavity with terminal pore, forms a synapomorphic feature for the taxon Eukalyptorhynchia. This feature is easily distinguished in living specimens under light microscope.

Proboscises are present in other taxa of freeliving Platyhelminthes or 'Turbellaria'. Especially within the related taxon Typhloplanoida, several families include representatives with a frontal proboscis. In Trigonostomidae the proboscis pore is situated not terminal but subterminal at the ventral side. In *Trigonostomum* species the proboscis epithelium is abundantly pierced by gland necks and a ciliation is lacking (Chapter 5 *Fig. 11*). In Kytorhynchidae a terminal invagination is present but a muscular bulb is lacking in all species (Rieger 1974). Rieger described distinctly different types of proboscises, that are typified by differences in the number of receptors present, the number of nuclei in the proboscis epithelium (mostly four but three and six are also encountered), the abundancy of secretion granules and the number of retractor muscles. Within the Kytorhynchidae a tendency occurs with an increase of the number of retractor muscles together with a increase in glandular secretion and a reduction of the amount of receptors. The proboscis can be regarded as the result of a convergent evolution in comparison to the proboscis in Eukalyptorhynchia. A shift in function from sensory pit to glandular muscular proboscis for prey capture can be assumed in Kytorhynchidae. In Eukalyptorhynchia, however, the pimary function is prey capture, and a shift in function is not present. But alterations of the way of prey capture and changes in the abundancies of glands and organizations of the musculature can be detected within the Eukalyptorhynchia.

Characters and character states in the eukalyptorhynch proboscis

In order to establish on phylogenetic system, phylogenetically useful characters are identified in Eukalyptorhynchia. The following characters from our investigations are taken into account to reconstruct the phylogenetic relationships within the Eukalyptorhynchia.

Proboscis epithelia: overall organization, number of belts, cellular versus syncytial, position of the nucleiferous cell parts and intracellular aspects (hooks).

Gland necks: types, position.

Sensory cells: presence, structure.

Proboscis musculature: organization, muscular plates, intra-epithelial muscles.

Spermatozoa: aciliary, biciliary.

Eyes: lenses

By examining the proboscis ultrastructurally, several misinterpretations of light microscopic observations can be corrected and new structures are revealed. A ciliation of the proboscis epithelia, as described by several authors, is not present in the proboscis of Eukalyptorhynchia (Marcus 1951 for *Toia*, Karling 1953, 1964 for *Cytocystis* and *Nannorhynchides*, Brunet 1973 for *Nannorhynchides* and *Pocillorhynchus*). Species formerly described with such a ciliated sheath epithelium can there for not be regarded as being plesiomorphic (Karling 1953, 1964). On the other hand, a high number of uniciliary, unmodified receptors could form a plesiomorphic feature as well. Nuclei found inside the bulb have often been regarded myoblasts or myocytes of the inner musculature of the proboscis. In all species investigated, which were known to have nuclei in the bulb, these nuclei could clearly be identified as epithelial nuclei. Myocytes have never been found within the bulbar septum.

1. Epithelia

Several tendencies known to occur in the epidermis can be extrapollated to the proboscis epithelium as well. Among these characteristic tendencies, the trend towards syncytialization is known (Ax 1963, Ehlers 1985, Rieger 1981). A syncytial epidermis is present in different taxa and is known from Prolecitophora e.g. *Pseudostomum* (Ehlers 1985) and *Urostoma* (Burt & Fleming 1978), from Typhloplanoida and also in several Kalyptorhynchia e.g. *Gyratrix* (Bedini & Papi 1974), *Polycystis* (Schockaert & Bedini 1977), *Cytocystis* (Karling 1953), Placorhynchidae (Karling 1931, 1947) and *Florianella* (Rieger & Sterrer 1975). However, a syncytial epidermis is not general for Polycystididae or Eukalyptorhynchia (Bedini & Papi 1974). Both from light and electron microscopic investigations several eukalyptorhynch species, such as *Cicerina, Psammorhynchus, Utelga, Psammopolycystis, Papia, Mesorhynchus* and Gnathorhynchidae, are now known to have a cellular epidermis (De Vocht 1990, Karling 1947, 1954, Tyler 1984). Occasionally circumferential parts of the

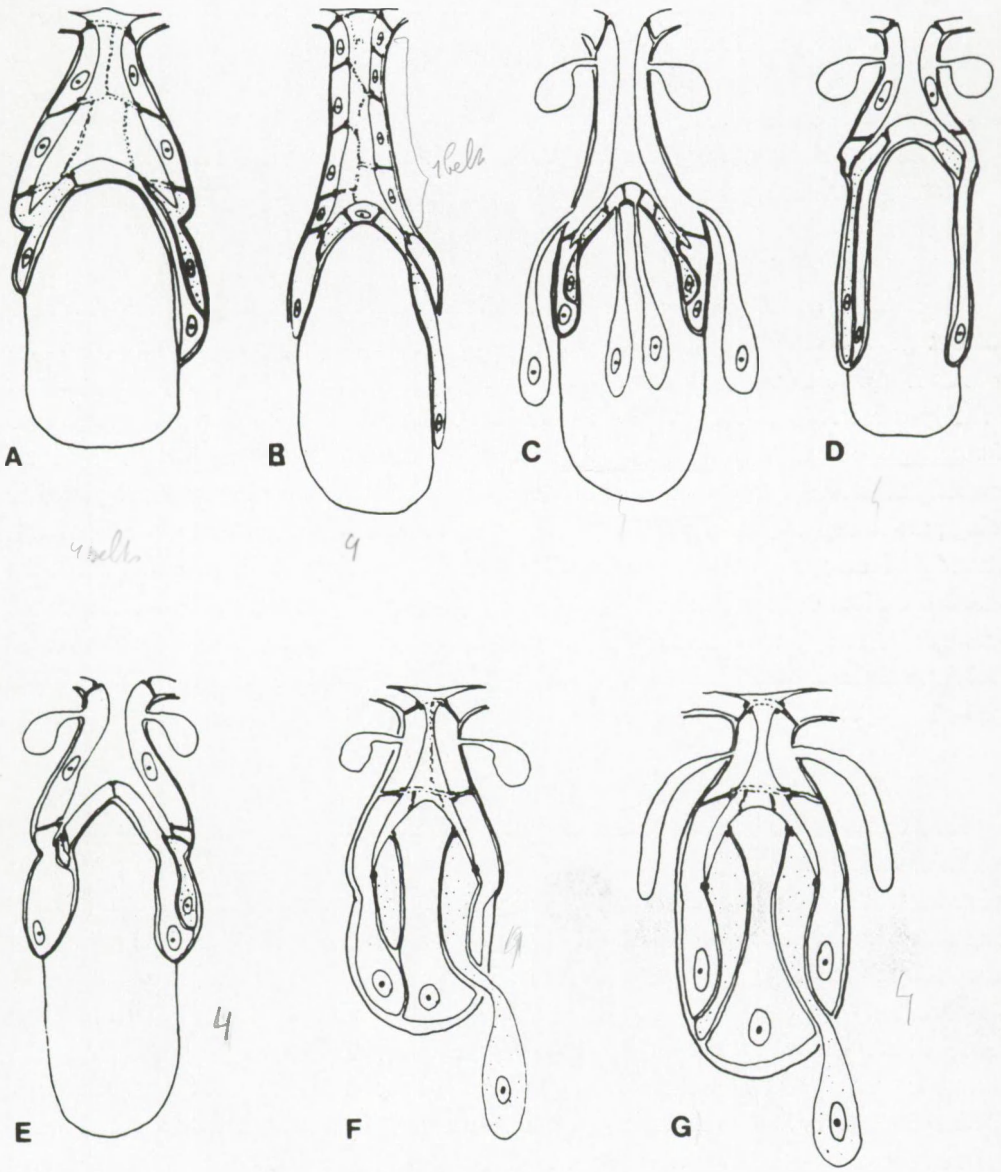


Fig. 1. Schematic presentation of the organization of the proboscis epithelia. A. *Toia*, *Nannorhynchides*, B. *Zonorhynchus*, C. *Ethmorhynchus*, D. *Ptyalorhynchus*, E. *Cicerina*, *Paracicerina*, F. *Psammorhynchus*, G. *Cytocystis*.

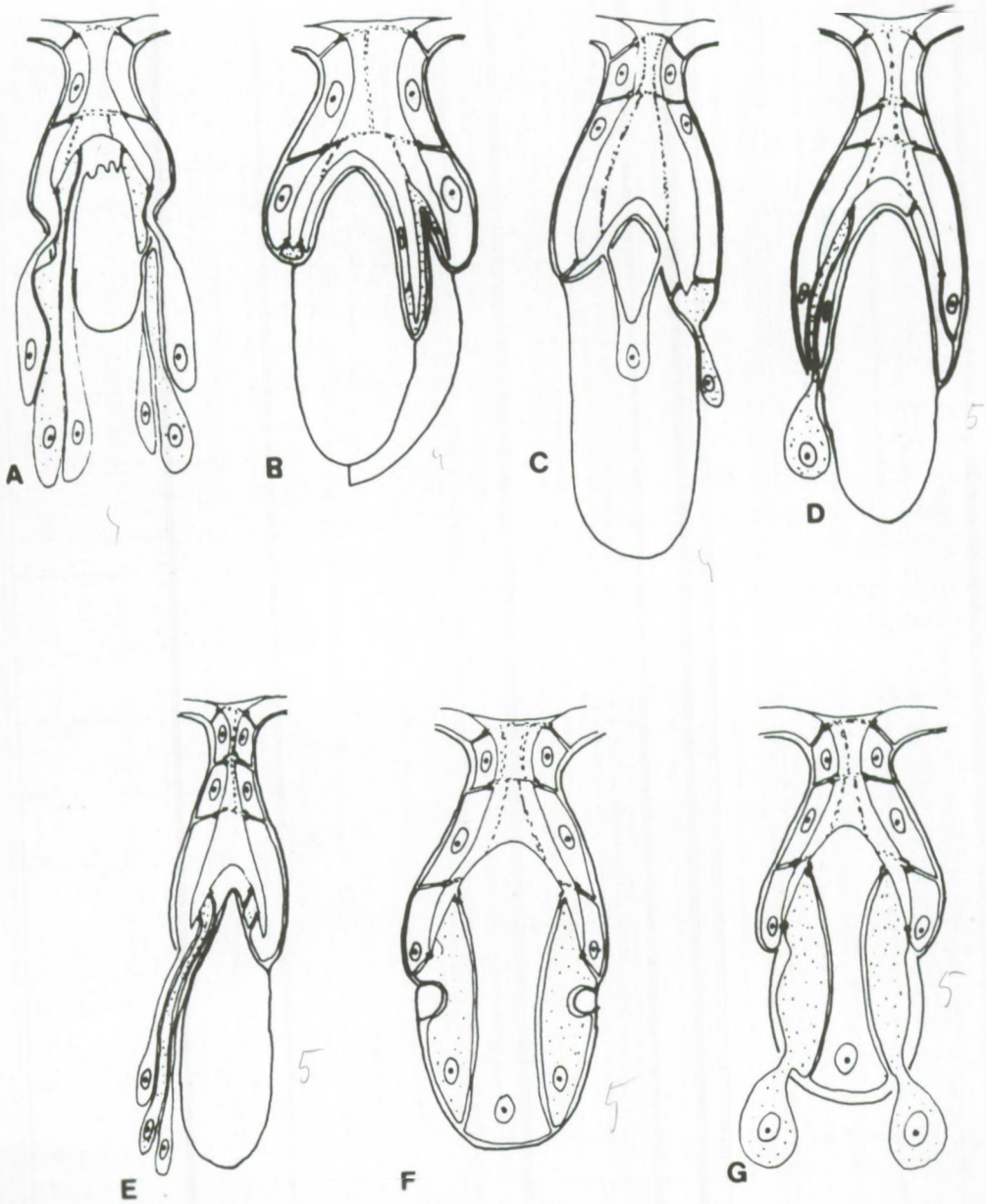


Fig. 2. Schematic presentation of the organization of the proboscis epithelia. A. *Florianella*, B. *Placorhynchus*, C. *Paragnathorhynchus*, D. *Phonorhynchus*, E. *Marirhynchus*, F. *Itaipusa*, G. *Mesorhynchus*, *Parautelga*.

epidermis form epithelial belts. In *Polycystis naegelii* the anterior body end is covered by a syncytial belt and a circumferential "clitellum" is present in *Cytocystis clitellatus* (Schockaert & Bedini 1977, Karling 1953). Consequently a syncytial epidermis is found in species of various monophyletic taxa and has arisen independently within several taxa within the Eukalyptorhynchia.

The proboscis epithelia of Eukalyptorhynchia are always composed of four or five circumferential belts (Table 1.).

- In *Toia*, *Nannorhynchides* and *Ethmorhynchus* all belts are cellular, including the belt which covers the basal part of the cone (*B*). A fully cellular organization of the proboscis epithelium can be considered plesiomorphic.
- Syncytialization occurs first in the basal belt of the cone epithelium and a single syncytial belt is present in *Psammorhynchus*, *Cytocystis* and *Ptyalorhynchus*.
- In *Paracicerina*, *(Cicerina)*, *Florianella*, *Placorhynchus*, *Paragnathorhynchus* and *Gnathorhynchus* the apical cone epithelium is syncytial as well. In *Cicerina* this belt is formed by two cells and two binuclear syncytia.
- *Zonorhynchus* is the only genus with bipartite sheath epithelium where apart of the basal cone epithelium also the proximal belt of the sheath epithelium is syncytial.
- In genera with five belts in the proboscis epithelium (Koinocystididae, Cystiplanidae and Polycystididae), the cone epithelium is syncytial (in *Parautelga* and *Mesorhynchus* the apical belt is formed by a single cell).
    - The genera *Mesorhynchus* and *Phonorhynchus* possess a fully cellular sheath epithelium.
    - In Koinocystididae, *Scanorhynchus*, *Neopolycystis* and *Marirhynchus* the proximal belt of the sheath epithelium is syncytial, the median and distal belts are cellular.
    - In Cystiplanidae both the median and the proximal belt are syncytial, while the distal belt retained a cellular organization.
    - In *Polycystis* and *Typhlopolycystis* the proboscis epithelium is formed by five syncytial belts.

The proboscis epithelia in *Toia* and *Nannorhynchides* are condisered to have maintained their original cellular organization. In *Ethmorhynchus*, a genus which has other characters in common with genera of the *Cicerina*-group, *Psammorhynchus* and *Cytocystis*, the cellular basal cone epithelium is regarded a re-establishment of the plesiomorphic state.

The nuclei of the epithelium cells are originally situated centrally in the cell parts forming the covering part of the epithelium (intra-epithelial). They can also be found apart from the covering epithelial cell parts but still above or enclosed by the basement membrane in a cytoplasmic or nucleo-glandular girdle. An other possibility is a insunk position of the nuclei, this is a position below the basement membrane. As will be demonstrated below, the

position of epithelial nucleiferous cell parts of the proboscis is very variable within the Eukalyptorhynchia.

Sheath epithelium

The sheath epithelium can be divided in two or three belts and is characterized by numerous infoldings of the basal plasma membrane. The position of the nuclei in the sheath epithelium is very variable.

- Two belts are present in Cicerinidae, *Psammorhynchus*, *Cytocystis*, Gnathorhynchidae, Placorhynchidae and *Florianella*.
  - In *Toia* and *Nannorhynchides* the two belts of the sheath epithelium contain intra-epithelial nuclei.
  - In *Zonorhynchus* the distal belt is cellular but without distinct pattern (in all other investigated species a belt is formed by a single circumferential row of cells or a syncytium). The proximal belt in *Zonorhynchus* is syncytial (apomorphy 4a). The nuclei of this syncytium are found below the junction, caught between the muscles surrounding the cavity and the bulb.
  - In *Ethmorhynchus* the cells, which form the distal belt, have insunk nuclei. This forms a unique character state for *Ethmorhynchus* (apomorphy 17b) The proximal belt forms the junction, the nuclei of the cells are situated in a nucleo-glandular girdle around the anterior part of the bulb.
  - In *Cicerina*, *Paracicerina* and *Ptyalorhynchus* the nuclei of the cells of the proximal belt are found posteriorly in the nucleo-glandular girdle around the bulb as in *Ethmorhynchus* (see synapomorphy 16). A concentration of gland necks is usually found just above the junction (glandular ring).
  - In *Psammorhynchus* and *Cytocystis*, the nucleiferous cell parts of the cells of the proximal belt are situated between the internal cone retractors in the bulb (synapomorphy 13a). Nuclei of the cells forming the distal belt have not been observed.
  - In *Florianella* the cell forming the distal belt has an intra-epithelial nucleus and the nucleiferous parts of the cells in the proximal belt are insunk and situated behind the proboscis.
  - In *Placorhynchus* and *Gnathorhynchus* both belts in the sheath epithelium are cellular and have intra-epithelial nuclei.
- Three belts forming the sheath epithelium are found in Polycystididae, Cystiplanidae, Koinocystididae and in *Marirhynchus* and *Mesorhynchus* (synapomorphy 8).
  - In Polycystididae the nuclei of the cells or syncytia forming the distal and median belts of the sheath epithelium are intra-epithelial. The nuclei of the syncytium forming the proximal belt can be situated in a cytoplasmic girdle around the anterior part of the bulb as

in *Phonorhynchus* or can be found insunk in the parenchyma around the posterior part of the bulb as in *Polycystis*.

- In Cystiplanidae the distal and median belt of the sheath epithelium have intra-epithelial nuclei, while the nuclei of the proximal belt are situated along the sides of the bulb in a cytoplasmic girdle as in *Phonorhynchus* (De Vocht 1989).
- The organization of the sheath epithelium in *Marirhynchus* is identical to the organization in some Polycystididae as *Alcha*.
- In Koinocystididae and *Mesorhynchus* all three belts have intra-epithelial nuclei.

To conclude I can state that the position of sheath epithelial nuclei is variable within the Eukalyptorhynchia and that insunk nuclei have arisen in different ways. The distal belt of the sheath epithelium in species with three sheath epithelial belts, is regarded as the most recently added belt to the proboscis epithelium. This belt preserves its cellular character longer than the other belts and is closest located to the epidermis of which the proboscis epithelium originates. The microvilli of this belt are longer than in the two subsequent belts and approach the microvilli of the epidermis in length. Therefor the distal belt is presumed to be latest belt added and is the appearance of the fifth belt, namely the distal belt of the sheath epithelium, in those species considered a new character and a synapomorph character in species with three belts in the sheath epithelium. Two belts in the sheath epithelium could be the result of the fusing of two belts of a tripartite sheath epithelium. But the number of cells or nuclei in either the distal or the proximal belt in the bipartite sheath epithelium does not contain a sum of cells or nuclei of two subsequent belts in the tripartite sheath epithelium (see Table 1). Genera with a bipartite sheath epithelium predominantly have a cellular organization, which indicates that this is the plesiomorphic state. Therefor the latter hypothesis is rejected. Additionally the presence of a fairly flat cone can be noted in all species with a bipartite sheath epithelium, while in Polycystididae, Koinocystididae and Cystiplanidae a more pronounced and much higher cone is found.

Cone epithelium

A bipartite cone epithelium is encountered in all species investigated (apomorphy 1) and is considered ancestral in Eukalyptorhynchia. Karling (1980) listed a proboscis with apex as an apomorphic feature common for some eukaplyptorhynch families. The author mentioned the possibility that a bipartite cone epithelium could be ancestral in all Eukalyptorhynchia, which is confirmed by our observations.

- A complete cellular organization of the cone epithelium is only present in *Toia*, *Nannorhynchides* and *Ethmorhynchus*. A fully cellular proboscis epithelium, including the cone epithelium, is probably also present in *Pocillorhynchus*, a genus related to *Toia* and *Nannorhynchides*. In all other investigated species a syncytial belt was found to cover the base of the cone (see also De Vocht 1989, 1990, 1991, De Vocht & Schockaert 1988 *belt*

B, Schockaert & Bedini 1977). The presence of a cellular basal belt can be considered as a secondary change from the more common syncytial basal belt or as a retention of the primitive character state. The cellular basal belt in *Ethmorhynchus* is regarded as a secondary cellularity or as a reduction from a syncytium to several cells, because of the coincidence with other homolog characters with *Cicerina*, *Paracicerina* and *Ptyalorhynchus*. In the *Toia-Nannorhynchides* group a fully cellular epithelium is present and this is considered the ancient character state for all Eukalyptorhynchia. A syncytial basal belt of the cone epithelium could have arisen several times independently. Because a syncytial belt of the cone epithelum is wide spread in Eukalyptorhynchia and because of the general appearance of the microvilli, a single syncytialization has occured in this homolog belt (synapomorphy 3).

- In *Zonorhynchus* intra-epithelial nuclei are found in the apical cone epithelium (apomorphy 4b) and the basal cone epithelium has insunk nucleiferous parts.
- In *Ethmorhynchus* the apical cone epithelium has intrabulbar nuclei although a basement membrane is present under the cone epithelium (apomorphy 17c). The basal cone epithelium is cellular (apomorphy 17a)
- In *Cicerina* and *Paracicerina* the nucleiferous cell parts of the cone epithelium are located in a nucleo-glandular girdle around the bulb. In *Ptyalorhynchus* the gland necks are over a large extend separated from the cytoplasmic epithelal parts.
- In *Psammorhynchus* and *Cytocystis* a basement membrane beneath the cone epithelium is lacking and both the proximal belt of the sheath epithelium and the apical cone epithelium have intrabulbar nuclei. The cytoplasmic cell strands of the basal cone epithelium pass through the bulb, pierce the septum at the posterolateral side of the bulb and form insunk nucleiferous parts behind the bulb.
- In *Florianella* the nucleiferous cell parts of the proximal belt of the sheath epithelium and both belts of the cone epithelium are insunk and located behind the proboscis. They leave the proboscis through a wide open ring around the distal part of the proboscis bulb.
- In *Placorhynchus* two intra-epithelial nuclei are present in the apical cone epithelium, the position of the nuclei of the basal belt could not be determined with certainty.
- In Gnathorhynchidae intrabulbar nucleiferous cell parts of the apical cone epithelium are present in *Paragnathorhynchus* and laterally insunk cell parts in *Gnathorhynchus*.
- In Cystiplanidae, *Phonorhynchus*, *Gallorhynchus*, *Danorhynchus* and *Neopolycystis* the nucleiferous cell parts of the apical cone epithelium are located in the cytoplasmic girdle around the bulb, while those of the basal belt are of the insunk type and situated behind the cytoplasmic girdle.
- In other Polycystididae such as *Polycystis*, *Paraustrorhynchus*, *Rogneda*, *Progyrator* and *Marirhynchus* both the nucleiferous cell parts of the apical and basal cone epithelium are situated insunk along the sides of the bulb.

- In the koinocystid genera *Itaipusa* and *Tenerrhynchus* the nucleiferous cell parts of the apical and basal cone epithelium are situated in the bulb (synapomorphy 19).
- In *Parautelga* and *Mesorhynchus* only the nucleiferous part of the apical cone epithelium is located in the bulb, the nucleiferous parts of the basal belt sink in through the bulb, pierce the septum posteriorly and are situated behind the proboscis bulb (synapomorphy 22).

Form these observations, parallel tendencies in the formation of insunk cell parts can be noticed. The location of the insunk parts or the perforations in the basement membrane or septum, however, differs in each case.

In most genera, if insunk cell parts are present, the basal cone epithelium has always insunk nuclei (*Psammorhynchus*, *Cytocystis*, *Polycystis*, *Cystiplex* and *Cystiplana*). The insunk cell parts in *Toia* represent elements of the cone epithelium and not of the sheath epithelium as postulated by Brunet (1973). *Ethmorhynchus* is the only species with insunk nucleiferous cell parts of the distal belt of the sheath epithelium.

In some species the expanse of a certain belt can diminish or increase. In *Zonorhynchus*, *Ethmorhynchus*, *Cicerina*, *Paracicerina* and *Ptylorhynchus* the proximal belt of the sheath epithelium is reduced, the nucleiferous cell parts are situated below the junction. In *Cicerina* and *Paracicerina* the proximal belt of the sheath epithelium and the basal belt of the cone epithelium have hardly any covering function and mainly form the nucleo-glandular girdle below the junction. In *Marirhynchus* for instance, the proximal belt of the sheath epithelium is extensive and lines the base of the cone as well. The actual basal cone epithelial belt is displaced upwards on the cone and reduced in expanse. In *Phonorhynchus* the median belt of the sheath epithelium forms a narrow annular belt in the sheath at the level of the apex.

An overview of the organization of the proboscis epithelia in 26 genera is given in table 3. Whether the different belts are formed by a specific number of cells or one syncytium is noted and sometimes the number of nuclei in the syncytium forming the basal belt of the cone epithelium is mentioned as well. The genera are groups in families. Genera with two belts in the sheath epithelium are listed first, genera which hold three belts in the sheath epithelium are listed in accordance to progressing syncytialization of the belts. The genera incertae sedis *Marirhynchus* and *Mesorhynchus* are listed below.

Genus	S_1	mcr	S_2	S_3	B	A
Toia	3C	-	3C	-	5C	2C
Nannorhynchides	3C	-	3C	-	5C	2C
Zonorhynchus	?C	-	1S	-	1S(5n)	1C
Ethmorhynchus	±10C	°	8C	-	4C	4C
Ptyalorhynchus	5C	°	4C	-	1S(2n)	1C
Paracicerina	5C	°	4C	-	1S(4n)	1S(2n)
Cicerina	8C	°	4C	-	1S(4n)	2C + 2S (*)
Psammorhynchus	2C	°	6C(*)	-	1S(5n)	1/2C
Cytocystis	1C/S	°	4C	-	1S(5n)	1C
Florianella	1C	°	4C**	-	1S	1S
Placorhynchus	4C	°	4C	-	1S	1S
Paragnathorhynchus	4C	°	4C	-	1S(4n)	1S
Gnathorhynchus	6C	°	4C	-	1S	1S
Itaipusa	4C	°	4C	1S(5n)	1S	1S
Tenerrhynchus	4C	°(?)	?C	1S(?)	1S	1S
Paravatelga	?C	°	?C	1S	1S	1C/S
Cystiplex	2C	°	1S	1S	1S(6iB	1S
Cystiplana	2C	°	1S	1S	1S 9n)	1S
Phonorhynchus	2C	°	4C	4C	1S	1S
Scanorhynchus	?C	?	?C	1S	1S	1S
Neopolycystis	?C	°	?C	1S	1S	1S
Polycystis	1S (4n)	°	1S(6n)	1S	1S	1S
Typhlopolycystis	1S (2n)	°	1S(2n)	1S	1S	1S
Rogneda	?	?	?	1S	1S	1S
Marirhynchus	2C	-	4C	?	1S	1S
Mesorhynchus	2C	-	4C	4C	1S	1C

Table 1. Organization of the proboscis epithelia in 26 species of Eukalyptorhynchia. The cellular (*C*) or syncytial (*S*) organization of the distal (S_1), median (S_2) and proximal (S_3) belt of the sheath epithelium, the basal (*B*) and apical (*A*) cone epithelium as well as the number of cells or nuclei in the syncytia (*n*) are indicated. The presence of multiciliary receptors (*mcr*) in S_1 is noted in the second column. * indicates a limited variation. ** not absolutely certain.

S_1	S_2	S_3	B	A	genus	A/S_1
850	850	-	<300	<300	*Toia*	0.35
850	850	-	200	200	*Nannorhynchus*	0.24
900	350	-	350	350	*Zonorhynchus*	0.39
900	400	-	180-(270)	180	*Ethmorhynchus*	0.20
550	450-600	-	300-350	450	*Paracicerina*	0.82
350	350-450	-	300-350	250	*Cicerina*	0.64
400-500	500	-	450	450	*Ptyalorhynchus*	±1
400	450-500	-	300		*Psammorhynchus*	0.67
1200	320	-	300		*Cytocystis*	0.25
0	300	-	500	100	*Florianella* A/S_2	0.33
650-500		-	0	150	*Placorhynchus*	0.23
0	?	-	?	150-180	*Gnathorhynhus*	-
1700	1700	-	?	150-180	*Paragnathorhynchus*	0.10
400	400	260	250	280	*Cystiplex, Cystiplana*	0.66
1000	?	0	700	120-200	*Danorhynchus*	0.15
0	0	0	200-250	120-200	*Neopolycystis*	-
900-600	300-350	300-350	200-250	120-200	*Typhlopolycystis*	±0.25
"	"	"	200-250	"	*Gallorhynchus*	±0.25
"	"	"	350	"	*Rogneda*	±0.25
"	"	"	350	"	*Phonorhynchus*	±0.25
"	"	"	200-250	"	*Marirhynchus*	±0.25
950	500-400	400	450	450	*Itaipusa*	0.47
900	900	600	700(900)	700	*Tenerrhynchus*	0.78
1200	?	?	550	300	*Parautelga*	0.25
820	700	500	700	700	*Mesorhynchus*	0.85

Table 2. Length (in nm) of the microvilli in the distal (S_1), median (S_2) and proximal (S_3) belt of the sheath epithelium, the basal (B) and apical (A) cone epithelium in 26 genera of Eukalyptorhynchia. The last column gives the ratio of microvilli length in the apical cone epithelium over the length of the microvilli in the distal belt of the sheath epithelium (A/S_1).

In the following paragraphs characters of the epithelia such as the microvilli, the cell junctions, the basement membrane and the terminal web are discussed. Some of these characters have no direct implications on the phylorgeny but are nevertheless worthwhile to mention.

Microvilli

The microvilli of the distal belt of the sheath epithelium are practically of equal length as those of the epidermis. The microvilli of the cone epithelium are much shorter than those of the sheath epithelium in all investigated species and represent 2/3 to 1/4 of the length of the microvilli of the distal belt of the sheath epithelium (Table 2). *Itaipusa*, *Parautelga*, *Tenerrhynchus* and *Mesorhynchus* have long microvilli. In *Tenerrhynchus* and *Mesorhynchus* especially, the differences in length between microvilli of the sheath and cone are very small. The ratio of the length of the microvilli in the apical belt over the length of the microvilli in the distal belt, gives an impression of the decline. Although a difference in length is indicated between the proximal and distal belt of the sheath epithelium in *Cicerina* (De Vocht & Schockaert 1988), they both bear microvilli of about the same length (350 nm). In most species with a bipartite sheath epithelium, the microvilli of both belts of the sheath epithelium are uniform in length. *Zonorhynchus*, *Ethmorhynchus* and *Cytocystis* form exceptions on this tendency. The microvilli of the proximal belt measure not yet half the length of those of the distal belt. *Gnathorhynchus* and *Florianella* are typified by the absence of microvilli in the distal belt. In species with a tripartite sheath epithelium, the microvilli in the sheath epithelium decline in length from the pore to the junction. Often the decrease in length is very abrupt between two belts. This can be at the transition from the distal to the median belt, as for instance in *Typhlopolycystis* and *Itaipusa*, or from the median to the proximal belt as in *Cystiplex* or *Tenerrhynchus*. In Cystiplanidae the microvilli of the proximal belt of the sheath epithelium bear shorter microvilli in comparison to those of the median and distal belt (De Vocht 1989). In *Polycystis* the microvilli of the median and proximal belt are shorter than those of the distal belt (Schockaert & Bedini 1977 *Figs 6 and 7*). In general the microvilli reduce in length down from the pore over the junction towards the apex. Stubby microvilli with dense tips are typical for the basal cone epithelium and are encountered in all investigated species (Fig. 1). They have been described or noted for Cystiplanidae, *Psammorhynchus*, *Florianella*, *Gnathorhynchus*, *Gyratrix* and *Polycystis* (De Vocht 1989, 1990, Reuter 1975, Rieger & Sterrer 1975, Schockaert & Bedini 1977). In *Toia* and *Nannorhynchides* dense margins are present instead of dense tips. In Gnathorynchidae the proboscis hooks are formed by electron dense condensations in microvilli or extensions of the apical plasmalemma (chapter 6 *Fig. 18*, Doe 1976 *Fig. 6*). Gland necks erupt through the hollow hooks. The presence of two dorsoventrally opposing intra-epithelial hooks in the basal cone epithelium forms a synapomorphic character

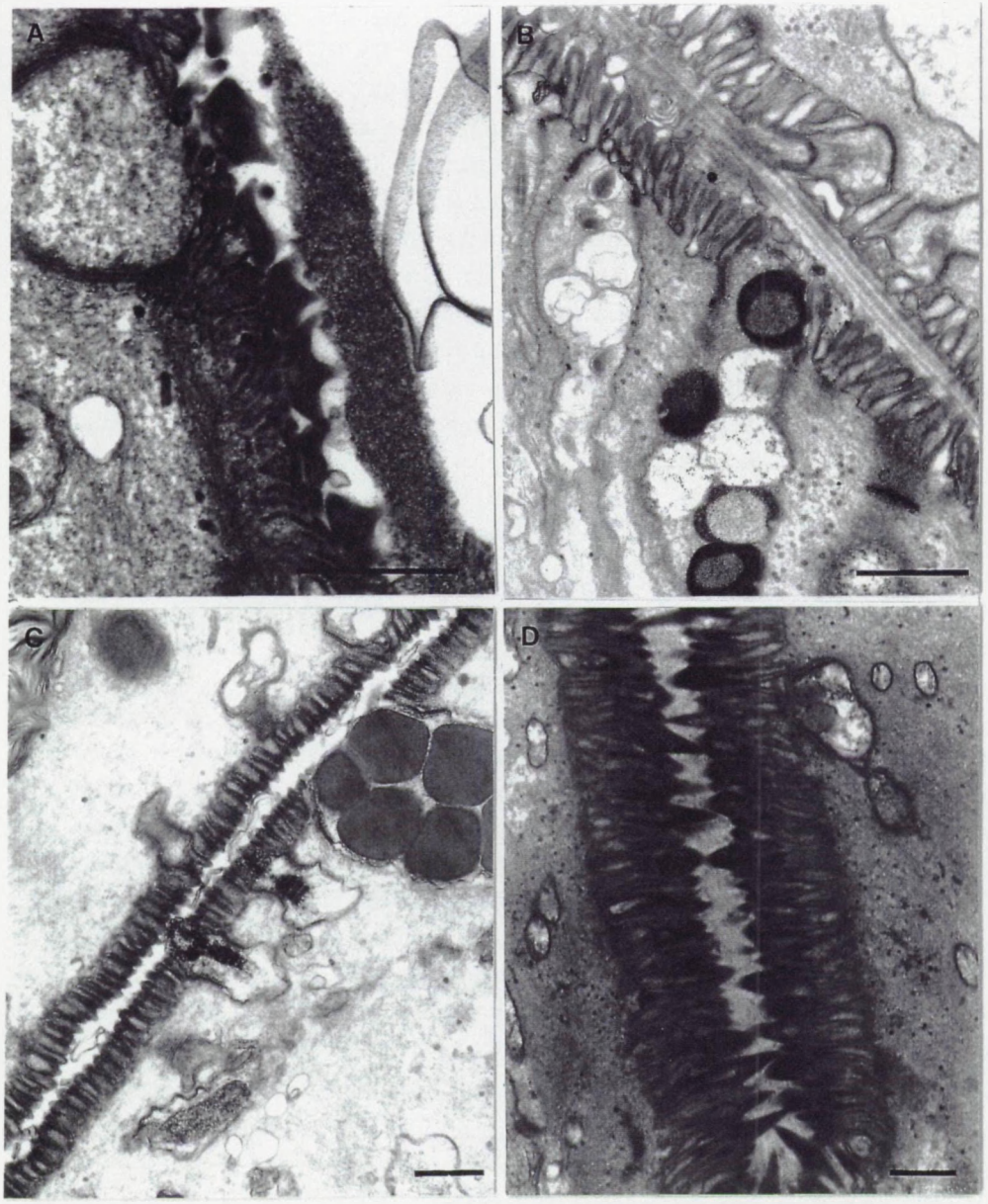


Fig. 3. Microvilli of the basal belt of the cone epithelium (*B*) in *Ethmorhynchus* (A), *Psammorhynchus* (B), *Cystiplex* (C) and *Tenerrhynchus* (D). Scale bar: 0.5 μm.

state for the families Gnathorhynchidae and Aculeorhynchidae (synapomorphy 10). In *Placorhynchus* the basal belt of the cone epithelium, which covers the inside of the muscle plates, bears no microvilli but shows numerous thin folds. This general characteristic for the basal belt of the cone epithelium indicates that these belts in all genera are homologous and stresses the hypothesis of a plesiomorphic bipartite cone epithelium.

Cell junctions

Bedini and Papi (1974) described the cell junctions in the epidermis of Rhabdocoela as a junctional complex formed by filamentous dismembranal junctions, apically and septate junctions or septate desmosomes, basally. The filamentous dismembranal junctions form the connection with the cell web. If filamentous dismembranal junctions are absent the distance between the septate desmosomes and the free cell surface is less than 100 nm and shows an thickening on the side of the plasma membrane. Usually these species lack a cell web or have a poorly developed cell web. To my opinion the filamentous dismembranal junctions in species with a well developed cell web and the less than 100 nm long tickening at the inner side of the plasmalemma in species with inconspicuous cell web or without cell web can be regarded as zonulae adhaerentes. In the descriptions the term zonula adhaerens has been used instead of filamentous dismembranal junction (Bedini & Papi 1974, Pedersen 1983), because it is generally used in morphology and in turbellarian morphology as well (Tyler 1984). Cell junctions in the proboscis epithelia are formed by zonulae adhaerentes, septate junctions and desmosomes.

Extracellular matrix, basement membrane and bulbar septum

Several important questions concerning the basement membranes of the epithelia and the origin of the bulbar septum should be answered first in order to establish a sound evolutionary system and to answer the question: how is the proboscis evolved, according to dynamic folding theories or by a parallel way of establishing a septum? Can the ECM layer on the proboscis beneath the cone epithelium be regarded as a basement membrane? If a basement membrane is considered to be at least a bipartite ECM layer, than should the ECM layer beneath the sheath epithelium in most species not be named basement membrane and the ECM layer beneath the cone epithelium should never be called so. However, because of the total organization of the sheath with apart of the epithelium, the presence of a circular and longitudinal muscle layer, this layer of ECM is to be regarded a basement membrane. The presence of both circular muscles and an epithelium indicates that this ECM layer is the product of both cell types (Hori 1979, 1980).

In species which lack an ECM layer in the cone, the nucleiferous parts in the bulb can be regarded as insunk in the bulb with a totally reduced basement membrane in the cone. Or the muscle fibres can be considered 'intra-epithelial' and intruded in the thick epithelium. In this

case the septum is to be considered the basement membrane of the cone epithelium. This bring us to the next question; what is the origin of the septum ? The septum plays a crucial role in theories on the origin and evolution of the proboscis and other structures of which the origin can be explained by an infolding theory. However, the origin of the epithelia from epidermis by infolding and the origin of the probosccis musculature from the body wall musculature does not exclude neoformation of extracellular matrix. An increase in muscles in these organs followed by the formation of well developed muscles individualized by muscular ECM sheaths allows a more autonomic way of functioning in the organism.

A basement membrane under the cone epithelium is present in Cicerinidae, Placorhynchidae, Gnathorhynchidae, Cystiplanidae and Polycystididae and has been described for *Cicerina remanei* (De Vocht & Schockaert 1988), *Polycystis naegelii* (Schockaert & Bedini 1977) and Cystiplanidae (De Vocht 1989). In some genera as *Ethmorhynchus* and *Paragnathorhynchus* small perforations in the basement membrane leave passage to epithelial cell stands containing the nuclei of the apical cone epithelium. In *Psammorhynchus, Cytocystis, Florianella, Mesorhynchus* and Koinocystididae a basement membrane is lacking in the cone (De Vocht 1989, 1990, 1991). The longitudinal muscles in the bulb adhere directly on the basal plasmalemma of the epithelium.

From our electron microscopic data, different formations of the septum and proboscis epithelium basement membrane can be illustrated.

- In *Mesorhynchus*, the bulbar septum has a bipartite basement membrane structure, supporting the viewpoint that the septum is derived from the proboscis epithelial basement membrane (chapter 10 *Fig. 6 Inset*). Because the sheath epithelium has intra-epithelial nuclei and does not extend further down than the junction, only cytoplasmic cell parts of the cone epithelium make contact with septum (De Vocht 1991). In this respect the septum represents the basement membrane of the cone epithelium. The inner musculature can be regarded as intra-epithelial, the longitudinal muscles are squeezed off from outer motional muscles, the inner muscles transplaced from the outer to the inner margin of the septum. If a common origin of the bulbar septum is assumed, the organization in *Mesorhynchus* is ancestral to a bulbar organization with, apart of the septum, the presence of a layer of ECM beneath the cone epithelium. This must have arisen by a delamination of the septum - original basement membrane - or by a new formation of a secondary basement membrane in the cone.

- In contrary, in *Florianella* a uniform ECM layer is present below the sheath epithelium and surrounds the individual inner circular muscles fibres. This ECM layer is continuous with parenchymal patches of ECM, which are present throughout the animal. A parenchyma containing ECM is not recorded in other species of Eukalyptorhynchia, but is present in other platyhelminth taxa such as Macrostomida for instance (Pedersen 1983, Rieger 1981). The septum does not seem to be evolved from the basement membrane of the cone epithelium but is rather a product of parenchymatic and muscle cells.

- In *Cytocystis*, the distal part of the bulb is enclosed by outer circular muscles and a prolongation of the basement membrane along with the sunken nucleiferous cell parts of the proximal belt of the sheath epithelium. Only the posterior part of the bulb contains inner circular muscles surrounded by ECM. So the bulb in *Cytocystis* is laterally formed by the basement membrane of the sheath epithelium. In *Psammorhynchus* a similar organization of the bulb is found but the transition of outer circular muscles and sheath epithelial basement membrane into septum and inner circular muscles is located just beneath the junction instead of in the posterior part of the bulb as in *Cytocystis*. (De Vocht 1990). If the organization in *Cytocystis* is ancestral, the septum on the lateral sides of the bulb originates from sheath epithelial bssement membrane. But a reduction of the septum and inner circular muscles with a replacement by outer circular muscles and basement membrane in *Cytocystis* is possible as well. These hypotheses are only valid for species which lack a basement membrane in the cone and which have intrabulbar nuclei.

- For species with insunk nucleiferous cell parts outside the bulb an other formation of the septum must be postulated, taking into account the orientation of the basement membrane structure in *Mesorhynchus*. On the other hand, if the addition of a third belt in the sheath epithelium only occured ones, an almost parallel evolution must have taken place in *Psammorhynchus* and *Cytocystis* on one hand and Koinocystididae and *Mesorhynchus* on the other hand. With this difference that in the latter group only the cone epithelium can be considered the precursor of the bulbar septum and that a participation of the sheath epithelium occurs in the septum formation in the former mentioned species.

If a common origin of the septum from epithelial basement membrane is postulated, the addition of the third belt must have occured independently within the Koinocystididae-*Mesorhynchus* and the Polycystididae-Cystiplanidae. For further discussion see also the discussion in 'Evolution of the proboscis' (this chapter).

Terminal web

Although a clear terminal web is mostly lacking, microfibrillar reinforcements are present in the cone epithelium as in *Cicerina*, *Cystiplex* or *Mesorhynchus* (De Vocht 1989, 1991, De Vocht & Schockaert 1988). A terminal web is present in the distal belt of the sheath epithelium in *Psammorhynchus* and *Cytocystis* (De Vocht 1990).

2. Gland necks

Several types of glands merge in the proboscis epithelia of Eukalyptorhynchia. From our investigations only morphological information is obtained merely on the shape of the secretion granules and electron density of the secretion. Other characteristics of the gland necks in the epithelium as the presence of peripheral microtubules, the lenght of the necks

filled with secretion, the place of entering the proboscis are noted as well. Due to restrictions encountered in using electron microscopic information, the exact location of the secretory cell bodies of the glands could not be obtained in most cases. Light microscopic sections stained in various ways or cytochemical methods in light and electron microscopy might solve this problem more easily. In the descriptions the numbering of the different gland necks has been carried out in a more or less uniform way throughout the descriptions. However, only for some types of gland necks and specific species or genera, homology of gland necks and glands can be stated. Undoubtly glandular function is of overall importance for the functioning of the proboscis in all Eukalyptorhynchia. In Placorhynchidae, Gnathorhynchidae and *Aculeorhynchus* additional structures are present in order to catch the preys. In these animals the number of glands is diminished consequently. In some other species a reduction of the number of glands was found as well, for instance in a number of Polycystididae. In table 3 a summary of the different types of gland necks, more specific their secretion granules and contents, in 32 genera or species is given. In many species the glandular secretion is very prominent and visible under the dissecting microscope. The distinction, which has been made by many authors, between a basal glandular and an apical sensory part in the cone is based on the presence of larger and light microscopic visible glandular secretion in the basal cone epithelium and only small secretion granules in the apical cone epithelium. Considering the distribution of receptors in the cone, no specialized sensory part can be delimited although receptors appear in a higher number towards the apex of the cone.

Gland necks are not associated with a particular belt in the epithelium but seem to be restricted to a certain position or level in the proboscis epithelium. The glands emerging in the proboscis cavity through the posterior part of the sheath epithelium just above the junction and often referred to as the glandular ring (*gr*) as in Cicerinidae, can either pierce the anterior part of the proximal belt of the sheath epithelium (S_2) as in species of the *Cicerina*-group, through the posterior part of this belt in the *Toia* or *Nannorhynchides*, or through the proximal belt (S_3) in Koinocystididae, Cystiplanidae and Polycystididae. If the position of the epithelial belts has changed, the gland necks have maintained at their original position. For instance the swollen gland necks (g_4 and g_5) in the nucleo-glandular girdle pierce the basal belt of the cone epithelium (*B*) in *Ptyalorhynchus*. They are regarded homologous to gland necks forming the glandular ampullae (*ga*) in *Paracicerina* and *Cicerina* and those in *Ethmorhynchus*, which surface through the proximal belt of the sheath epithelium (S_2) at the junction. The presence of large g_4 and g_5 gland necks forms a synapomorphic feature for the five genera mentioned above. In *Marirhynchus* the basal part of the cone is formed by the proximal belt of the sheath epithelium (S_2) and the basal belt of the cone epithelium (*B*) is shifted upwards to the apex. Type g_9 gland necks, which in all other species are restricted to the apical cone epithelium, pierce the basal cone epithelial belt as well.

The proboscis in Kalyptorhynchia is used to capture preys such as nematodes and harpacticoids. This function can be achieved in different ways as glandular action or grabbing by means of hooks or musculare structures (Meixner 1938). The proboscis of Eukalyptorhynchia functions by thrusting the proboscis cone to the prey and hold on to it by glandular adhesives of the cone epithelium. Additional structures can be present as well. The proboscis is protruded and the prey sticks to the apical part of the cone by the viscid secretion of the glands (Graff 1874 *Taf. XIX*, Meixner 1925). The prey is paralyzed (own observations) and is brought to the pharynx by a ventral flexion (ventral or ventrolateral integument retractors). The prey is probably glued to the proboscis by the secretion of gland necks erupting in the apical part of the proboscis.

Type g9 gland necks are found in the apical cone epithelium and can be homologized in all species. The secretion is always electron dense to moderately electron dense and usually stored in ovoid granules. Only in *Toia* and *Nannorhynchides* these granules are spherical but peripheral microtubules are present in the distal part of the gland necks passing the epithelium in all species. Type g9 gland necks enter the proboscis at the nodus in all species. In the viscid gland of the duo-gland system, electron dense ovoid secretion granules are present as well and the necks are reinforced by peripheral microtubules as well (Tyler 1976). The granules of the viscid gland in *Cicerina* measure 250-350 nm in length and are 150 nm wide. In comparison type g9 secretion granules in *Cicerina* are 200-300 nm long and 150 nm wide. In the proboscis cone the microvilli do not form a collar around the gland necks as in the duogland system, but the apical cell surface with short microvilli does form the anchoring surface. The cone epithelium has a well developed fibrous apical layer and sometimes bundles of microfibrilles connect the apical layer to the inner longitudinal muscles in order to transduce the forces of the muscles on the apical cell surface. The typical dense tips or margins of the microvilli in the basal cone epithelium could be regarded as reinforcements of the attachment site and considered analoge structures of the anchor cells microvilli. How the prey is detached from the proboscis is less clear. Releasing gland types are not present between type g9 glands in the apical cone epithelium. Only the glands that erupt through the basal part of the cone are in the immediate vicinity of the sticking site if the proboscis is protruded. We presume that the releasing glands are situated in the proboscis and not for instance in the pharynx because the prey can be released immediately without bending the proboscis towards the pharynx (own obserations). The releasing glands of the duo-gland adhesive system contain small 100 nm, spherical secretion granules, moderately electron dense in *Cicerina*. The exact chemical nature of the glandular secretions of the duogland system or the proboscis is not known. The viscid glands in *Macrostomum* and

genus/species	g1	g2	g3	g4	g5	g6	g7	g8	g9	g10	g11
Toia	300	400	-	500	800	900-1400	320	-	230	-	-
	fl med	med		var	ed	empty	ed		sph mt		
	S_1	S_2/GR		B j	B j	B n	B n		A		
Nanno-	-	250-450	300-700	700-900	950	900	900	-	280	-	-
rhynchides		var	ed+ls	empty	med	empty	fl		sph mt		
		S_2/GR	S_2/B	B j	B j	B n	B n		A		
Zonorhynchus	800-900	900	500	1300	1000	1400	700	350	200	250	-
	ed	med	med	empty	med	empty	ed	med	med	ed	
	S_1/S_2	S_1/S_2	S_2	S_2 c	S_2 c	B	B	B	A	B	
Ethmorhynchus	1500	300	900	700	1000	800	500	-	400	-	-
	fl ed	med	led	empty	med ls	var	fl		med		
	S_1/S_2	S_2	S_2	S_2 c	S_2 c	B	B		A		
Ptyalorhynchus	1200	450	200	4500	1000	400	450	-	300/180	-	-
	ed	ed	var	empty	med	med	ed		ed		
	S_2 GR	S_2 GR	S_1/S_2	B	B	B	B		A		
Paracicerina	800	550	350	1200	1000	700	400	700-800	350/220	420/250	-
	ed	fl med	med	empty	ed	empty	med g	med mt	ed	ed	
	S_2 GR	S_2 GR	S_1/S_2	S_2 GA	S_2 GA	B	B	B	A	A	
Cicerina	600	500	120	850-1200	700	400	350	-	300/150	500/280	-
remanei	ed	fl med		empty	ed	empty	sph ed				
	S_2 GR	S_2 GR	S_2	S_2 GA	S_2 GA	B	B		A	A	
Cicerina	600	500	120	1200	700	400	350	-	200/150	400	450-500
brevicirrus	ed	fl med		empty	ed	empty	sph fl		med	var	fl
	S_2 GR	S_2 GR	S_2	S_2 GA	S_2 GA	B	B		A	A	A
Psammo-	300-500	430	-	-	-	450	300	-	250-350	-	-
rhynchus		led	ed lam				empty	ed		mt	
						or ed					
	S_1/S_2	S_2				B	B		A		
Cytocysts	220	300-700	250	-	-	1200	400	-	200-250	-	-
	ed	med	ed ls			empty	ed		mt		
	S_1/S_2	S_1/S_2	S_2/B			B	B		A		
Florianella	700	380-600	-	-	180	370	350	-	250	200	-
	sph med				ed	empty	edc		med	sph w	
						gn=1µ					
	S_2	S_2			B	B	B		A	A	

genus/species	g1	g2	g3	g4	g5	g6	g7	g8	g9	g10	g11
Placorhynchus	1000	700	700	-	-	-	-	-	400	300	-
	ed	med	gr						med	ir fl	
	S_2+B	S_2+B	S_2+B+A						A	A	
Paragnatho-	1000	600	-	-	-	1700	-	-	500	-	-
rhynchus		fl				empty/fl					
	S_2	S_2				B			A		
Gnathorhynchus	-	-	-	-	-	1300	-	1000	750	-	
						ed		ov	sph		
						B		A	A		
Drepano-	1500	-	-	-	-	1700	-	?	?	-	
rhynchides	med					ed					
	S_2					B		A			
Cystiplex	750	700	900	1000	700	250	-	-	700/170	700	-
	med	ed	med	empty	sph ed	empty			rod	sph ed	
	S_2	S_3	S_3	S_3	B	B			A	A	
Cystiplana	750	700	900	1000	460	200	-	-	700/170	700	-
	med	ed	med	empty	sph ed	empty			rod	sph ed	
	S_2	S_3	S_3	S_3/B	B	B			A	A	
Gyratrix	350	-	-	-	630	200	-	-	500	-	-
Typhlopolycystis	sph ed				sph med	empty			ov		
Polycystis	$S_1/S_2/S_3$				B	B			A		
Rogneda											
Neopolycystis	-	-	-	-	380	120-200	-	-	450-500	-	-
Danorhynchus	-	-	-	-	280-450	120-200	-	-	580	-	-
Gallorhynchus	-	-	-	-	720-1300	120-200	-	-		-	-
Progyrator	-	-	-	-	900	120-200	-	-		-	-
					ed	empty			ov ed mt		
					B	B			A		
Scanorhynchus	450	700	-	-	700	-	-	-	500	-	-
	sph ed	med			ed				ov mt		
	S_2	S_3			B				A		
Phonorhynchus	500	300-400	700	-	500	100	-	-	300/100	-	-
	ov ed	med	ed		med	ed			ov		
	S_2	S_2	S_3		B	B			A		
Marirhynchus	500	450	500-750	-	-	-	-	-	700	-	-
	sph ed	sph med	ed						ov		
	S_2/S_3	S_3 a+c	S_3 a+c						B/A		

genus/species	g1	g2	g3	g4	g5	g6	g7	g8	g9	g10	g11
Mesorhynchus	750	570	500/230	-	-	200	700	-	200-400	-	-
	empty	med	ed			empty	ed		mt		
	S_1	S_2/S_3	S_3			B	B		A		
Itaipusa	600	700	700-800	400	900	-	420	-	250	1000	-
	empty	sph ed	med	empty	sph ed	fl	ed		mt	med	
	S_1/S_2	S_1/S_2	S_3	S_3	S_3	B	B		A	A	
Tenerrhynchus	-	500	150	-	1000	220	400	-	300	-	-
		ed	sph ed		mt GR	empty	ed		mt		
		S_2	S_3		S_3	B	B		A		
Parautelga	-	-	-	-	400-600	180	400-500	-	300-350	-	-
					med	sph	ed		med mt		
					S_3	B	B		A		

Table 3. **Glandular secretions in the Eukalyptorhynch proboscis.**
***First line*: in nm, *-*: varying between, */*: length/width, *l*: length, -: not present, *?*: unknown.**
Second line: *ed*: electron dense, *edc*: electron dense centre, *empty*: secretion washed out during preparation, *fl*: flocculent contents, *g*: granular contents, *gn*: length of gland neck containing secretion, *lam*: lamellated secretion granules, *led*: low electron dense, *ls*: light spots, *med*: moderately electron dense, *mt*: peripheral microtubules in gland necks, *ov*: ovoid, *rod*: rodlike, *sph*: spherical, *var*: variable electron density, *w*: weblike contents. (*led*, *med* and *ed* are relative, in comparison with other secretions present in the specimen.)
Third line: *gr*: form glandular ring, *ga*: in glandular ampullae, $S_1/S_2/S_3/B/A$: belt in which the gland necks surface, *c*: part of the belt covering the cone, *j*: entering the proboscis laterally below the junction, *n*: entering the proboscis through the nodus, *s*: part of the belt covering the cavity wall.

Polystyliphora react with stains for basic proteins but releasing glands do not react on tests for endo- or ectopeptidases (Tyler & Melanson 1979).

A reduction of the number of glands is noted in several species. But the viscid gland type in the apical cone epithelium (g9) is still present in all species. Sometimes the presence of specializations in the proboscis forms an indication for the reduction of the glandular action. In *Placorhynchus* the presence of muscle plates in order to catch preys deminishes the importance of the glandular function (if the number of different types of glands is used as a measure for the importance of glands for prey capture). In Gnathorhynchidae the hooks are used in prey capture and a reduction of the number of glands is noticed as well. A reduction of the total number of gland types is found in some Polycystididae as well. In *Neopolycystis*, *Danorhynchus*, *Gallorhynchus* and *Progyrator* the sheath epithelium is void of gland necks. *Gyratrix*, *Typhlopolycystis*, *Polycystis* and *Rogneda* have only one type of

gland necks in the sheath epithelium. Two types are present in the sheath epithelium of *Scanorhynchus* but only one type of gland necks is present in the basal cone epithelium. In all other species of Polycystididae constantly two types of glands (g_5 and g_6) are present in the basal cone epithelium and type g_9 gland is the only gland type in the apical cone epithelium. Of all investigated genera of Polycystididae, the number of glands in *Phonorhynchus* is distinctly higher. Type g_5 gland necks in Polycystididae and Cystiplanidae are most probably homologous to type g_7 gland necks in other Eukalyptorhynchia.

In two species of Gnathorhynchidae, *Paragnathorhynchus* and *Drepanorhynchides*, a extensive glandular ring is present in the proximal part of the sheath epithelium. A limited but very distinct glandular ring is present in all species of Cicerinidae and only less obvious in *Zonorhynchus* due to the high concentration of gland necks in the entire epithelium. In *Ethmorhynchus* and *Zonorhynchus*, secretion is stored in the parts of the gland necks in the parenchyma under the basement membrane. This accumulation of secretion granules is distinctly present in the two gnathorhynchid species as well.

Two types of gland necks (g_4 and g_6) contain empty secretion granules after preparation for electron microscopy. Both types are present in Cicerinidae, surfacing through the proximal belt of the sheath epithelium or the basal belt of the cone epithelium. In *Cicerina* type g_4 and g_5 gland necks enter the proboscis at the posterior end of the nucleo-glandular girdle. The necks are situated between the septum and the longitudinal muscles along the sides of the bulb, converging to the nodus. In *Ptyalorhynchus* these gland necks are incorporated in the bulb and are found close to the inside of the bulbar septum. The different appearance (light and dense margins of secretion granules) of the wide gland necks with empty secretion granules (g_6) in *Toia* might find its explanation that these necks in fact represent both g_4 and g_6 gland necks present in the other cicerinid species. The as type g_4 gland necks indicated gland than must represent a different gland type. Type g_4, g_5, g_6, g_7 gland necks in the proximal belt of the sheath epithelium and the basal cone epithelium in Cicerinidae are organized in a strict pattern.

In *Paracicerina* and *Cicerina* g_4 and g_5 gland necks form four glandular ampullae (synapomorphy 26). In *Cicerina* the glandular ampullae are enclosed by circular muscles (apomorphy 27). In *Ptyalorhynchus* fifteen alternating g_4 and g_5 gland necks are present at the junction (apomorphy 25). Type g4 and g5 gland necks lack in *Psammorhynchus* and *Cytocystis* (synapomorphy 13b).

3. Sensory cells

Different types of terminal endings of sensory cells can be distinguished in the proboscis epithelia. Three types of uniciliary receptors have been identified.

- The first type are uniciliary receptors which pierce the sheath epithelium. These receptors have been described in the sheath epithelium of Cystiplanidae, *Gyratrix hermaphroditus* and *Polycystis naegelii* (De Vocht 1989, Reuter 1975, Schockaert & Bedini 1977). They are characterized by long ciliary shafts, basal bodies, primary and secondary rootlets, which radiate slanting from the basal body to the plasma membrane. These secondary rootlets can be short as in *Psammorhynchus* or prominent as in Cystiplanidae (De Vocht 1989, 1990). They can be present only at the side of the basal body oriented posteriorly or they can be found all around it. The secondary rootlets radiate from the basal bodies to the zonulae adhaerentes. If the basal bodies are situated below the epithelium surface, the secondary rootlets are directed towards the epithelium surface as in *Toia* and *Nannorhynchides*. In *Cicerina*, *Paracicerina* and *Ptyalorhynchus*, Polycystididae and Cystiplanidae, only few receptors are present. In Gnathorhynchidae, Placorhynchidae, *Mesorhynchus*, *Ethmorhynchus*, *Psammorhynchus* and *Cytocystis* they are more numerous and sometimes fairly abundant. They are extremely abundant in *Toia*, *Nannorynchides* and in the proximal belt of the sheath epithelium in *Florianella*. In a previous study on the proboscis of *Cytocystis* it was clear that the epithelium was not ciliated but pierced by numerous uniciliary receptors (De Vocht 1990). High densities of uniciliary receptors of this type have previously been interpreted as ciliated epithelia (Brunet 1973, Karling 1953, 1964, Marcus 1951, Rieger and Sterrer 1975). It is clear now that the proboscis epithelia are never ciliated and that a ciliated sheath epithelium is not the plesiomorphic state in Eukalyptorhynchia. The extremely high density of this kind of receptors could, however, be considered a derived state in Eukalyptorhynchia, if the presence of few to abundant receptors is regarded as the ancestral state.

- Peculiar uniciliary receptors are present in the median belt of the sheath epithelium in *Progyrator*. The base of the strong rootlets is connected to the zonulae adhaerentes but secondary rootltets are lacking (reduced?) because the receptors are lifted above the epithelium. These receptors are very characteristic because of their well-developed rootlets which reach out far above the epithelium surface. They form an autapomorphic character for this species and might be derived from the first type of uniciliary receptors (reduced secondary rootlets) or represent a totally new type of receptors (lacking secondary rootlets).

- In *Cicerina*, *Paracicerina*, *Ptyalorhynchus*, *Placorhynchus*, *Paragnathorhynchus* and *Gnathorhynchus* uni- and biciliary receptors form groups or an annulus in the proximal belt of the sheath epithelium just above the junction. In *Drepanorhynchides*, groups of multiciliary receptors are situated apically of the extensive ring of glands in the proximal belt. With interference contrast in light microscopic observations the long ciliary shafts in *Cicerina* appear to be rigid structures pointing into the proboscis cavity. The long ciliary shafts have normal 9+2 axonemata. In the former three genera they pierce the posterior part of the distal belt and the deeper parts are covered by the proximal belt. In *Cicerina* and *Paracicerina* they

form twelve groups, in *Cicerina* they merge through twelve funnels. The receptors are characterized by long, thick cross-striated rootlets but secondary rootlets are lacking. In *Cicerina* the terminal ends of the dendrites unite and two rootlets per dendrite are visible on cross section. In the other species the receptors are always uniciliary. These receptors represent a new character in the formerly mentioned species. This type of rigid receptors are considered chemoreceptors by Ferrero and Bedini (1989) but in my opinion they are even likely tactile mechanoreceptors. Long ciliary shafts with 9+2 axonemata pointing into the proboscis cavity far above the proboscis cone and stabilized by thick and long primary rootlets seem to be perfectly able to function as mechanoreceptors. In *Ethmorhynchus* uniciliary receptors are present in the proximal belt of the sheath epithelium just above the junction as well but they resemble the first mentioned type of receptors.

- Uniciliary receptors with blunt ciliary shafts, basal bodies and short primary rootlets in the cone epithelium are present in all investigated species as well. They are identical to those found in *Gyratrix* (Reuter 1975, *type IV*), *Cicerina*, *Psammorhynchus*, *Cytcystis*, *Polycystis* and Cystiplanidae (De Vocht 1989, 1990, De Vocht & Schockaert 1988, Reuter 1975, Schockaert & Bedini 1977). For *Polycystis naegelii* this type of receptor is reported for both the sheath and the cone epithelium but in our sections they only appear in the cone epithelium and not in the sheath epithelium. The dendrites pass through the proboscis bulb and are predominant in the apical part of the cone. According to Ferrero and Bedini (1989) these receptors should be assigned a chemoreceptive function in analogy with the receptors described by Gelei (1930) and identified as chemoreceptors by Mueller (1936) in other rabdocoelans.

- Multiciliary receptors associated with the distal belt of the sheath epithelium are found in most species investigated (Fig. 2). However, their position and appearance can differ.

  • The receptors can be situated intra-epithelial (e.g. in the part of epithelium lining the cavity) with fingerlike ciliary shafts. Such an organization is encountered in Cystiplanidae, some Polycystididae, Koinocystididae, Placorhynchidae, *Paragnathorhynchus* and *Florianella*. The receptors are situated in the anteriormost part of this belt just below the proboscis pore as in Cystiplanidae or in the posterior part as in *Florianella* (synapomorphy 5).

  • The multiciliary receptors can also form about ten spherical sensory organs situated in the epithelium lining the cavity. This situation is encountered in the genus *Gnathorhynchus* (apomorphy 12).

  • The receptors can be situated in two insunk cell parts of the epithelium, located in the surrounding parenchyma. The multiciliary dendrites pierce the epithelium and the cilia merge in a closed lumen. The insunk epithelial cell parts can either have an elongated or a spherical shape. The first is encountered in *Cytocystis* and *Ethmorhynchus*, the latter in *Cicerina*, *Paracicerina*, *Ptyalorhynchus* and *Psammorhynchus* (synapomorphy 11).

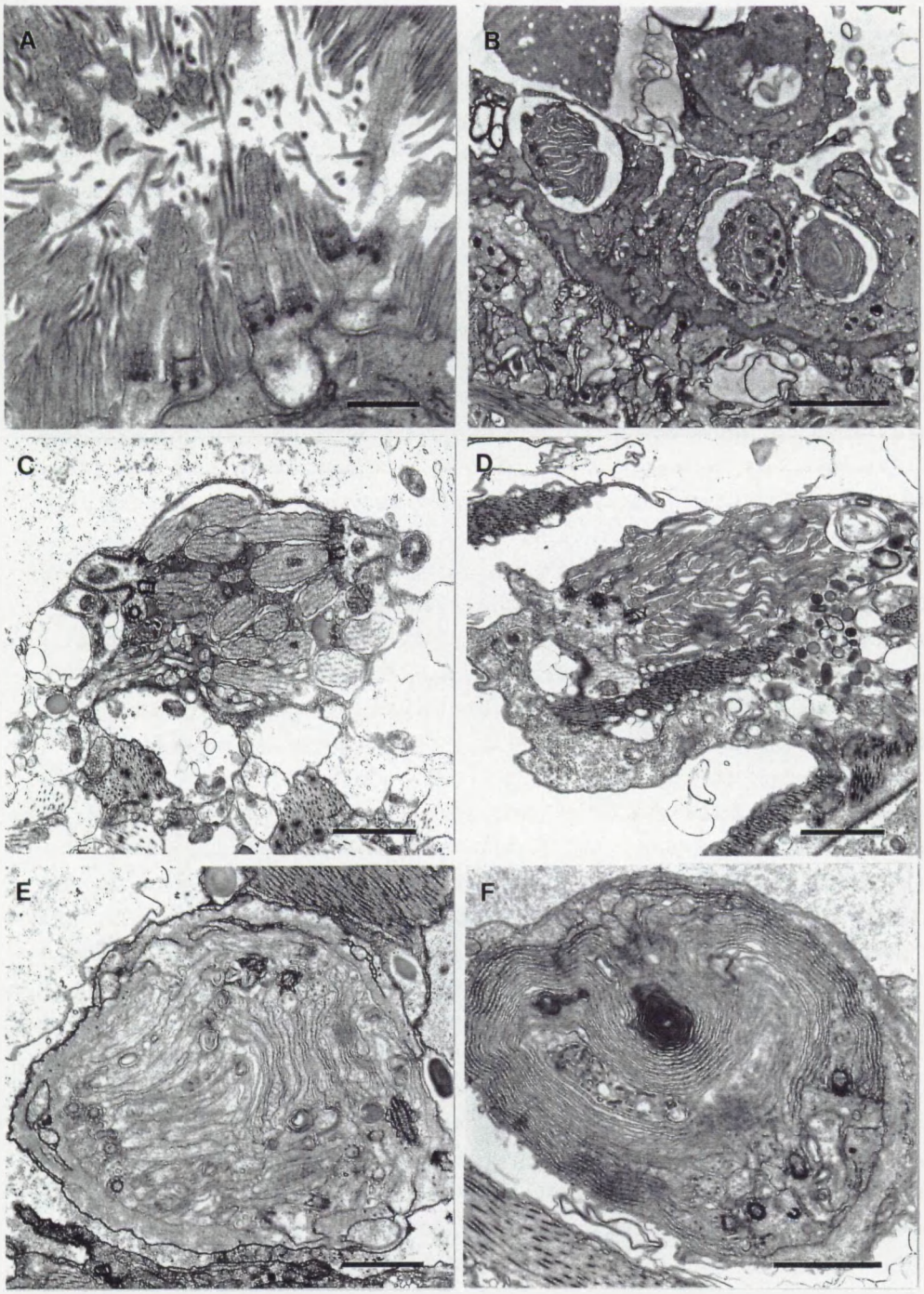


Fig. 4. Multiciliary receptors associated with the distal belt of the sheath epithelium. *Typhlopolycystis* (A), *Gnathorhynchus* (B), *Cytocystis* (C), *Ethmorhynchus* (D), *Psammorhynchus* (E), *Paracicerina* (F). Scale bar A: 0.5 µm. Scale bar B: 2 µm. Scale bar C-F: 1 µm.

* In *Marirhynchus* multiciliary receptors with short ciliary shafts and without rootlets are present in the epidermis around the anterior body at the level of the distal belt of the sheath epithelium. They might be homologous to the multiciliary receptors in the distal belt of the sheath epithelium. Their position can be derived from the ancestral position if the terminal ends of the dendrites switch to the epidermis near the proboscis. As for the gland necks, these receptors are bound to a particular position, below the pore, and not to a particular belt. They are always present in the distal belt of the sheath epithelium but as well in species with two belts as three belts in the sheath epithelium. The distal and proximal belt in species with only two belts are considered homologous to the median and proximal belt in species with three belts in the sheath epithelium.

Multiciliary receptors associated with the distal belt of the sheath epithelium are not present, neither intra-epithelial nor insunk in *Toia, Nannorhynchides, Zonorhynchus* and *Mesorhynchus*. In some species of Polycystididae it is not clear whether multiciliary receptors are totally absent or not. The material often does not exclude the possibility that sparce receptors could be present.

The function of the intra-epithelial multiciliary receptors can be explained by a prolapse of the distal part of the sheath when the proboscis is protruded, as present in several of our preserved specimens and noted by Meixner (1925 *p. 283 "zum Teil sicherlich unnatürlicher Vorgang"*). The intra-epithelial multiciliary receptors could function as chemoreceptors. Ferrero and Bedini (1989) designed chemoreceptive function to various types of receptors in Turbellaria, such as uniciliary receptors with long, rigid ciliary shafts and well-developed rootlets, branched receptor processes with short cilia and inconspicuous rootlets and the 'ciliated pits'. The authors regard the uniciliary receptors, present in the proboscis sheath and cone epithelium, as chemoreceptors. The multiciliary receptors could be interpreted as chemoreceptors because of the short length of the ciliary shafts, the aberant axonemata, the reduced rootlet and the association of the receptors with the proboscis and the position close to the proboscis pore. However, the fact that an open connection to the insunk receptors is lacking, does not favor a chemoreceptive function of these sensory organs and receptors. A function as tactile mechanoreceptor is unlikely because a rigid ciliary shaft and primary rootlet are lacking. The position of the insunk sensory organs favors a presumed mechanoreceptive function, more specific a pressurereceptive function, in order to protect the animal from extreem high pressure when the proboscis is protruded. The large parenchymal cells around the proboscis sheath, which only contain an aqueous cytoplasm with sparce mitochondria, function as hydrostatic compensation sacks by the use of the proboscis. The localization of multiciliary receptors in sunken spherical or elongated epithelial cell parts in the parenchyma supports this point of view. An alteration of the function is implied in this filosophy. We must notice that only on pure morphological grounds the function of these receptors or sensory organs will remain highly speculative.

The presence of intra-epithelial spherical sensory organs containing multiciliary receptors with modified ciliary shafts in *Gnathorhynchus* seems to form an intermediate stage in the evolution from intra-epithelial receptors with blunt ciliary shafts, to insunk sensory organs with receptors with flat ciliary shaft. However, in the other two gnathorhynchid species investigated intra-epithelial multiciliary receptors with fingerlike ciliary shafts are recorded. Furthermore, in *Cytocystis* the receptors in the elongated insunk sensory organs have blunt ciliary shafts, which do not form flat membranous sheets (apomorphy 15). So fingerlike ciliary shafts might form the plesiomorphic state for multiciliary receptors in spherical sensory organs. In *Ethmorhynchus* and *Psammorhynchus* the ciliary shafts form flat sheets. In *Ptyalorhynchus*, *Paracicerina* and *Cicerina*, the ciliary sheets form very wide flat sheets, which form concentric rings (synapomorphy 18). In *Gnathorhynchus* moreover, ten to twelve spherical sensory organs are present while in *Psammorhynchus*, *Cytocystis*, *Ethmorhynchus*, *Ptyalorhynchus*, *Paracicerina* and *Cicerina* only two insunk sensory organs are found. Spherical sensory organs, containing receptors with flat membranous ciliary shafts, have been formed at least two times in evolution: once intra-epithelial in *Gnathorhynchus* and once as two insunk organs with fingerlike receptors which gave origin to receptors with flat membranous ciliary sheets. Gnathorhynchidae are characterised by the presence of two hooks in the basal belt of the cone epithelium, excluding *Gnathorhynchus* from this taxon is impossible. If indeed four sensory organs are present in *Acrumena*, a third independent establishement of spherical sensory organs must have taken place. Intra-epithelial spherical sensory organs and insunk spherical sensory organs represent a parallel development and form homoplasies.

A summary of the multiciliary receptors associated with the distal belt of the sheath epithelium is given in table 4.

- Intra-epithelial receptors with normal fingerlike ciliary shafts	Cystiplanidae, most Polycystididae, Koinocystididae, Placorhynchidae, *Paragnathorhynchus* and *Florianella*.
- Intra-epithelial spherical sensory organs	*Gnathorhynchus*
- Insunk spherical sensory organs	*Psammorhynchus*, *Cicerina*, *Paracicerina* and *Ptyalorhynchus*
elongated sensory organs	*Ethmorhynchus* and *Cytocystis*
- Absent	*Toia*, *Nannorhynchides* and *Zonorhynchus* *Mesorhynchus*

Table 4. Multiciliary receptors and their appearance in the eukalyptorhynch proboscis.

Multiciliary receptors in sensory organs are present in many Platyhelminthes, freeliving as well as parasitic, and in many other invertebrate taxa such as Gnathostomulida (Lammert 1984). The sensory organs in the eukalyptorhynch proboscis, however, differ from all previously described ciliary receptors in free-living and parasitic Platyhelminthes. Within free-living Platyhelminthes, ciliary lamellate bodies or ciliary aggregations with presumptive photoreceptive function are found in addition to the rhabdomeric pigment-cup eyes. Multiciliary receptors with ciliary shafts that form lamellae are present in the Proseriata (Parotoplaninae) *Parotoplanina geminoducta* and *Parotoplana capitata* (Ehlers & Ehlers 1977a, Sopott-Ehlers 1986). Both species possess two pairs of lamellate bodies laterally of the brain and short ciliary rootlets are present in the latter species. In the former species, microvilli are present between the lamellae. Pericerebral ciliary aggregations are found in *Dicoelandropora atriopapillata* and *Notoplanella glandulosa*, two proseriate species of the taxon Otoplaninae, *Cirrifera aculeata* (Coelogynoporidae) and *Nematoplana coelogynoporoides* (Ehlers & Ehlers 1977b, Sopott-Ehlers 1982, unpublished in Sopott-Ehlers 1986). In *Microstomum lineare* (Macrostomida), ciliary epidermal pigmented eyespots are present below the epidermis (Palmberg *et al* 1980). In Müller's and Götte's larvae of Polycladida, ciliary epidermal eyes are present as well, while the cerebral eyes possess both cilia and microvilli (Eakin & Brandenburger 1981, Lacalli 1983, Lanfranchi & Bedini 1986, Lanfranchi *et al* 1981). Except for the lamellate bodies in Polycladida larvae, the receptors in the mentioned free-living and parasitic species are all formed by a single nerve cell (Brooker 1972, Lyons 1972, 1973, Short & Gagné 1975, Wilson 1970, Xylander 1984). The cilia merge in an intracellular lumen not open to the outside. This central cavity is lined by a thin rim of nerve cytoplasm and the basal bodies have aberrant axonemata. The cilia usually have a 9+0 or 9+2 organization of microtubuli near the basal body but distally mostly single microtubules are present. Except for *P. capitata*, the cilia lack rootlets. These ciliary lamellate bodies have always been regarded as potential photoreceptors (Kearn 1984, Short & Gagné 1975, Sopott-Ehlers 1982, Xylander 1984). They have been ascribed this photoreceptive function because they resemble proven ciliary photoreceptors in other invertebrates (Kearn 1973). Wilson (1970) gives an elaborated discussion on other possible functions for multiciliary lamellate bodies in the miracidium of *Fasciola hepatica* and does not exclude chemo- or tangoreception. The receptors in the sensory organs in Eukalyptorhynchia merge through an insunk part of an epithelial cell. In this case the central cavity, which has no connection to the outside either, is lined by the epithelial cell.

Multiciliary receptors are present in the anterior part of the epidermis as well. The cilia have long ciliary shafts with normal 9+2 axonemata and long rootlets. They have been described for *P. naegelii* by Schockaert and Bedini (1977) type VI. They are present in *Toia*, *Nannorhynchides* and *Mesorhynchus*.

4. Proboscis musculature

With respect to the ultrastructure of the muscle cells, some remarkable differences are revealed. A sarcoplasmic reticulum is present in almost all species investigated and have been recorded for Cystiplanidae (De Vocht 1989 *Figs 2 and 8*), Gnathorhynchidae (Doe 1976 *Fig. 6 A and B*), *Psammorhynchus tubulipenis* and *Cytocystis clitellatus* (De Vocht 1990 *Figs 5, 6, 7 and 11*) and *Cicerina remanei* (De Vocht & Schockaert 1988 *Fig. 11*). A sarcoplasmic reticulum is absent *Toia, Nannorhynchides* and *Florianella.* The myofilaments are loosely arranged or sparce in muscle fibres of *Florianella.* In Cystiplanidae and Polycystididae an organization of the myofilaments resembling the cross-striated muscle type is encountered. Often transverse condensations are present in both circular and longitudinal muscle fibres. The outer longitudinal muscles cling to the septum by means of hemidesmosomes and transverse condensations in the fibres (Czubaj & Malec 1988 'dense ribbons').

Concerning the organization of the proboscis musculature, the musculature has been subdivided in inner and outer musculature according to the location of muscles in or outside the bulbar septum. The musculature inside the bulbar septum comprises longitudinal and circular muscles. By outer musculature is meant the circular and longitudinal muscles below the sheath epithelium and around the bulb as well as the muscle system which acertains the protraction, retraction and fixation of the proboscis in the animal and which is often referred to as the motional muscles.

Practically all species possess outer circular muscles, only in *Placorhynchus* no circular muscles are present around the sheath (apomorphy 9b). Within the Cicerinidae Meixner, 1928, a circular muscle layer surrounding the sheath is only present in *Toia, Nannorhynchides* and *Zonorhynchus.* In other cicerinid species circular muscles surround the proximal belt of the sheath epithelium, which only lines the proximalmost part of the cavity, and the nucleo-glandular girdle. In *Psammorhynchus, Cytocystis, Florianella,* Gnathorhynchidae, some species of Polycystididae and *Mesorhynchus.* In Cystiplanidae, some Polycystididae and Koinocystididae the circular muscles are present beneath the distal belt of the sheath epithelium as well and form a continuation of the circular muscles of the body wall.

The longitudinal muscles surrounding the sheath epithelium are continuous with the longitudinal muscles of the body wall at the pore as for instance in Placorhynchidae, Gnathorhynchidae, Koinocystididae, Polycystididae and Cystiplanidae or with the dilators of the sheath as in *Mesorhynchus, Florianella, Cytocystis, Zonorhynchus* and *Nannorhynchides.* In Cystiplanidae, *Itaipusa* and *Psammorhynchus* it is clear that the retractors of the pore or the dialtors of the sheath are continuous both with the longitudinal muscles of the body wall and the sheath epithelium. In all species the longitudinal muscles adhere laterally on the flanks of the bulb. In some species of Polycystididae a second set of

longitudinal muscles appears at the junction and joins or replaces the former muscles fibres as in *Danorhynchus*.

Apart of the retractors of the pore and dilators of the sheath, the motional muscles include protractors, fixators, proboscis and integument retractors.

- Protractors are present in almost all species investigated, they have not been encountered in *Paragnathorhynchus* and *Gnathorhynchus*. In most species the muscles are interconnected behind the nodus but in Gnathorhynchidae they adhere on the bulbar septum (Karling 1947).
- Fixators are present in many species; in Cystiplanidae six pairs of fixators are found, in Polycystididae always three pairs. The fixators adhere on the lateral sides of the bulb, whether on the distal part of the bulb posteriorly of the junction or on the posterior part near the insertion of the proboscis retractors. In some species, such as *Ethmorhynchus*, *Cicerina*, *Paracicerina*, *Ptyalorhynchus*, *Psammorhynchus* and *Placorhynchus* the fixators muscles are reduced or absent.
- Proboscis retractors are present in all species, they can be short not reaching further than the pharynx as in species of the *Cicerina*-group or long reaching up to the posterior part of the body as in most species. The adhere on the posterolateral side of the bulb. Two sets of proboscis retractors are present in *Cicerina*, *Paracicerina*, *Ptyalorhynchus*, *Ethmorhynchus*, *Psammorhynchus* and probably in Placorhynchidae and Gnathorhynchidae (levators of the hooks) as well (synapomorphy 7). If a second set of short retractors is present, these muscles insert on the distal part of the bulb just below the junction.
- The number of integument retractors varies from one ventral pair as in most species up to four pairs in *Cystiplex*.

Inner circular muscles of the proboscis are only lacking in the three investigated species of Gnathorhynchidae, which only have longitudinal muscles in the bulb. Possibly the muscles in the "muscular pads" originate from the inner circular muscles, which are still present in the genus *Uncinorhynchus*. The other genera of Gnathorhynchidae have distinct muscular pads and lack inner circular muscles.

In many species a distinction within inner longitudinal muscles can be made. Peripheral longitudinal muscles sometimes form an extensive part of the inner longitudinal muscles as in *Toia* and *Nannorhycnhides*. But in most species a thin layer of peripheral longitudinal muscles is only present. In Koinocystididae, Polycystididae and Cystiplanidae no distinction can be made between central or peripheral longitudinal muscles on morphological grounds. A distinct difference between peripheral and central longitudinal muscles is most pronounced in species with many plesiomorphic character states.

Intra-epithelial muscles are present in Polycystididae and Cystiplanidae and reach up to the apical cone epithelium. The fibres pass through the basal cone epithelium up to the apical

cone epithelium. At the junction (or at the transition zone of outer into inner circular muscles, the fibres bifurcate, one end joining the fixators, the other end running along the sides of the bulb with the outer longitudinal muscles. In *Florianella*, *Mesorhynchus* and *Parautelga* similar muscle fibres are present but they never reach up into the cone and do not have a bifurcated proximal end. There homology to the intra-epithelial muscles in Cystiplanidae and Polycystididae is therefore doubtful.

Ontogenetically the inner longitudinal muscles probably originate from the outer motional muscles. They are cut of from these muscles and their nucleiferous parts by formation of the septum. In *Cytocystis* and *Toia* muscle fibres of respectively the proboscis retractors and fixators are still found to be continuous with the inner longitudinal muscles and adhere on the epithelium in the cone. The fixator fibres in *Toia*, which enter the proboscis, are situated peripheral in the bulb and adhere on the basal cone epithelium near the junction. The fibres of the proboscis retractors in *Cytocystis* enter the bulb posterolaterally and insert on the basal cone epithelium as well. This might indicate an origin of the peripheral inner longitudinal muscles from fixators and proboscis retractors.

Muscle plates in Placorhynchidae can not be homologized with any other structures in the proboscis in other species. They are formed by transverse lamellae enclosed by ECM. A thick ECM layer surrounds the muscle plates. Muscle fibres, which adhere on the ECM layer, can move the muscle plates.

5. Spermatozoa

The spermatozoa of practically all investigated species of Eukalyptorhynchia were examined and two types of spermatozoa have been encountered. Two axonemata were encountered in all species investigated except for *Toia* and *Nannorhynchides* which do not possess flagella or sperm axonemata in mature spermatozoa (Figs 3C&D, 4A&B) (synapomorphy 2a). The latter two genera have so-called thread-shaped spermatozoa or filiform spermatozoa with neither free or incorporated flagella nor undulating membranes (Fig. 3A&B). This type of spermatozoa has been reported for some Dalyellioida and Typhloplanoida (Hendelberg 1969, 1977) and is now for the first time recorded for Kalyptorhynchia. In one cross section of a spermatozoon of *Nannorhynchides* a axonema is visible. This indicates a possible reduction of the incorporated flagella. In comparison to Eukalyptorhynchia, only one axonema is present in Schizorhynchia (Fig. 4C&D). The genera of Schizorhynchia investigated include *Cheliplana*, *Carcharodorhynchus*, *Proschizorhynchus* and *Diascorhynchus*. This coincides with other records as for *Baltoplana* (Schizorhynchia), which also has monociliary spermatozoa (Hendelberg 1975). In contrary to our findings the schizorhynch species *Proschizorhynchus typhlus* was listed to have two axonemata as well (Hendelberg 1969). Other eukalyptorhynch genera, as *Acrorhynhcides*,

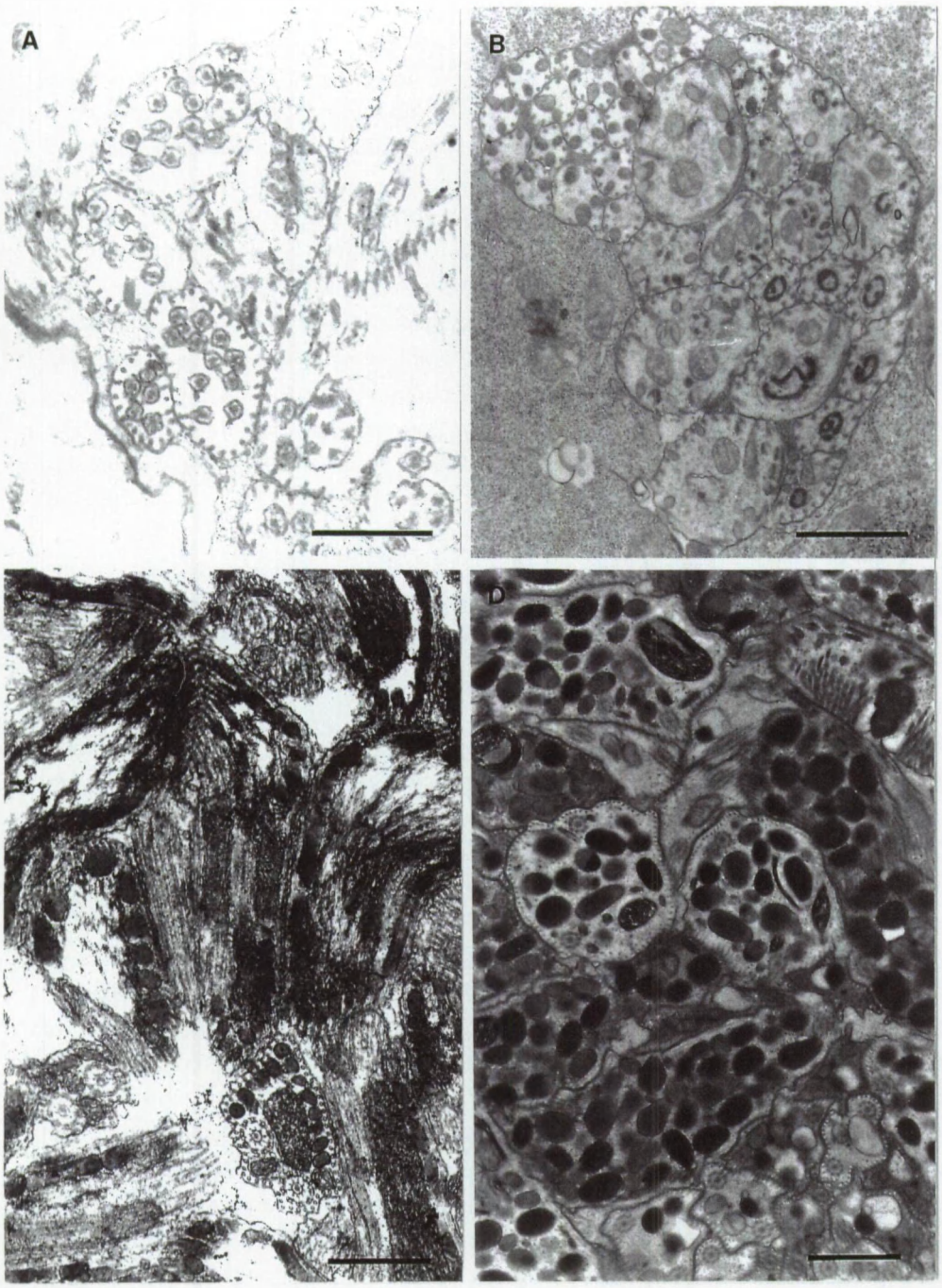


Fig. 5. Spermatozoa in *Toia* (A), *Nannorhynchides* (B), *Cicerina* (C) and *Ptyalorhynchus* (D). Scale bar A, C: 0.5 µm. Scale bar B,D: 1 µm.

Prognathorhynchus, *Odontorhynchus* and *Gyratrix*, are known to have two axonemata (Hendelberg 1969, 1986, Rhode *et al* 1987). In the phylogeny of Platyhelminthes the paired flagella have arisen early in flatworm evolution and might even be regarded as the plesiomorphic condition within Platyhelminthes (Tyler & Rieger 1975, Hendelberg 1977). In this respect parallel evolution of the thread-like spermatozoa has occurred in Dalyellioida, Typhloplanoida and Kalyptorhynchia. Monoflagellated spermatozoa have been reported for Schizorhynchia, Digenea, Monogenea and Cestoda (Hendelberg 1977).

A full incorporation of the two axonemata in the spermatozoon, named as one of the two synapomorphic characters for the taxon Kalyptorhynchia by Ehlers (1985) must be specified into a full incorporation of the axonemata (one or two). However, it does form an additional synapomorphic character for the Eukalyptorhynchia. Within the taxon Typhloplanoida (*s.s.*) spermatozoa with two free flagella (e.g. *Bothromesostama* and *Promesostoma*) or without axonemata (*Proxenetes*) are known (Hendelberg 1969). Within "Typhloplanoida" and "Dalyellioida" both spermatozoa with two or without flagella occur. Kalyptorhynchia have incorporated flagella. Schizorhynchia are apparantly typified by spermatozoa with one incorporated flagellum (synapomorphy).

6. Kinocilia

The kinocilia in both Eukalyptorhynchia and Schizorhynchia have been investigated ultrastructurally as well. These observations revealed small but distinct differences in the kinocilia of both taxa. In Lithophora and Rhabdocoela a fixed number of eight stereocilia is encountered and a electron dense differentiation as in Unguiphora and Archimonocilididae is lacking (Ehlers 1985, Sopott-Ehlers 1984). In Schizorhynchia the stereocilia are detached from the dendritic process and do not extend above the epidermal microvilli (Ehlers & Ehlers 1977c), while in Eukalyptorhynchia the stereocilia do not detach from the dendritic plasma where they form eight ridges and stretch out upto or above the epidermal microvilli (Figs 13-20). This forms an additional synapomorphic character for all Eukalyptorhynchia.

Evolution of the proboscis

Rieger (1974) summarizes Graff's, Meixner's and Karling's hypotheses on the evolution of the eukalyptorhynchid proboscis. The proboscis has either evolved from an invagination of the anterior body end (Graff 1908) or from a retraction of the anterior body end (Meixner 1925). The inner longitudinal muscles have always been assumed to orginate from the longitudinal muscles of the body wall (Meixner 1925, Rieger 1974). Irrespectively the two different hypotheses of the first stages, the author as well as Meixner (1938) postulate that the bulbar septum originates from the basement membrane. Rieger's hypothetical folding theory (the septum originates from an epithelial fold which encloses the proboscis bulb) implicates that the septum is derived from the cone epithelial basement membrane (except in

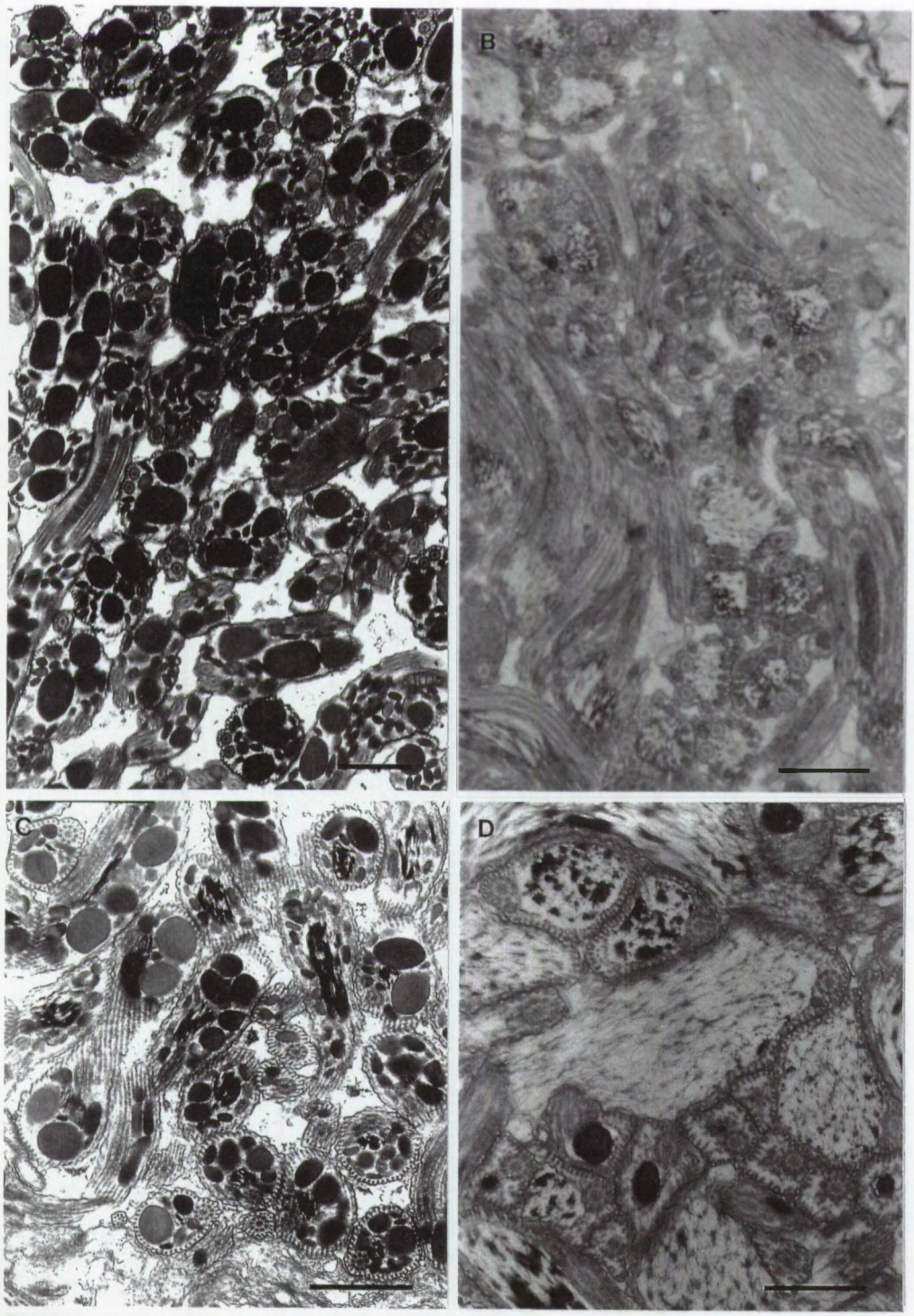


Fig. 6. Spermatozoa in *Progyrator* (A), *Scanorhynchus* (B), *Diascorhynchus* (C) and *Cheliplana* (D). Scale bar: 1 μm.

Cytocystis where the lateral septum is formed by the basement membrane below the proximal belt of the sheath epithelium). From the ultrastructural investigation of 35 species, a bilayered or structured bulbar septum is only present in two species with epithelial cell parts in the bulb, *Mesorhynchus* and *Psammorhynchus* . But if the septum should originate from the internal part of the basement membrane fold, the septum would have an opposite polarity as in the latter two species. In *Psammorhynchus* the septum should originate from the basement membrane of the sheath epithelium as in *Cytocystis*, with the only difference that the transition from outer circular muscles into inner circular muscles is situated near the junction instead of in the posterior part of the bulb. In *Mesorhynchus*, however, only cell parts of the cone epithelium are found in the bulb and the septum seems to be formed by the sunken cone epithelial basement membrane. The septum can originate from the cone epithelial basement membrane by a process of delamination or by sinking in. If sinking in is postulated, intrabulbar epithelial cell parts and nuclei would represent an ancestral state in comparison to extrabulbar cell parts and nuclei, which would by pushed up or aside by a delamination of the septum. If the bulbar septum originates from a direct delamination of cone epithelial basement membrane, the disappearance of this basement membrane is a secondary process and intrabulbar epithelial cell parts and nuclei are regarded as a new character state.

In Rieger's folding theory, epithelial cell parts with nuclei of the cone epithelium and sometimes of the proximal belt of the sheath epithelium should have an ancestral position laterally of the bulb, enfolded by basement membrane. A intrabulbar position of epithelial cell parts is hard to explain from this situation. The folding theory is generally considered the evolutionary way in the development of a pharynx plicatus outof a pharynx simplex and a shortening of the fold with formation of a closed septum the way of forming a pharynx bulbosus outof a pharynx plicatus (Ax 1963). Karling (1963) pointed out evolutionary lines in regard to the development of the male copulatory organ, the pharynx and the adhesive organ-proboscis. He postulated parallel evolutionary processes based on the folding theory. The parallelism is explained by a common selective factor, forcing the three organse to evolve to a functionally greater independence of the body. In all three organs a high degree of mobility is necessary either for copulation, feeding or prey capture. This high degree of mobility is achieved by separating the structure or organ from the parenchyma by a layer of ECM (or septum) and the presence of a independent musculature. Especially in the evolutionary stages of the copulatory organ and the adhesive organ-proboscis, the folding theory is very hypothetical. The adaptive response to overcome problems in construction is believed to be limited but only ultrastructural studies on the ontogeny of the proboscis in several species might solve the problem and show if other evolutionary trends occur in the establishment of the proboscis.

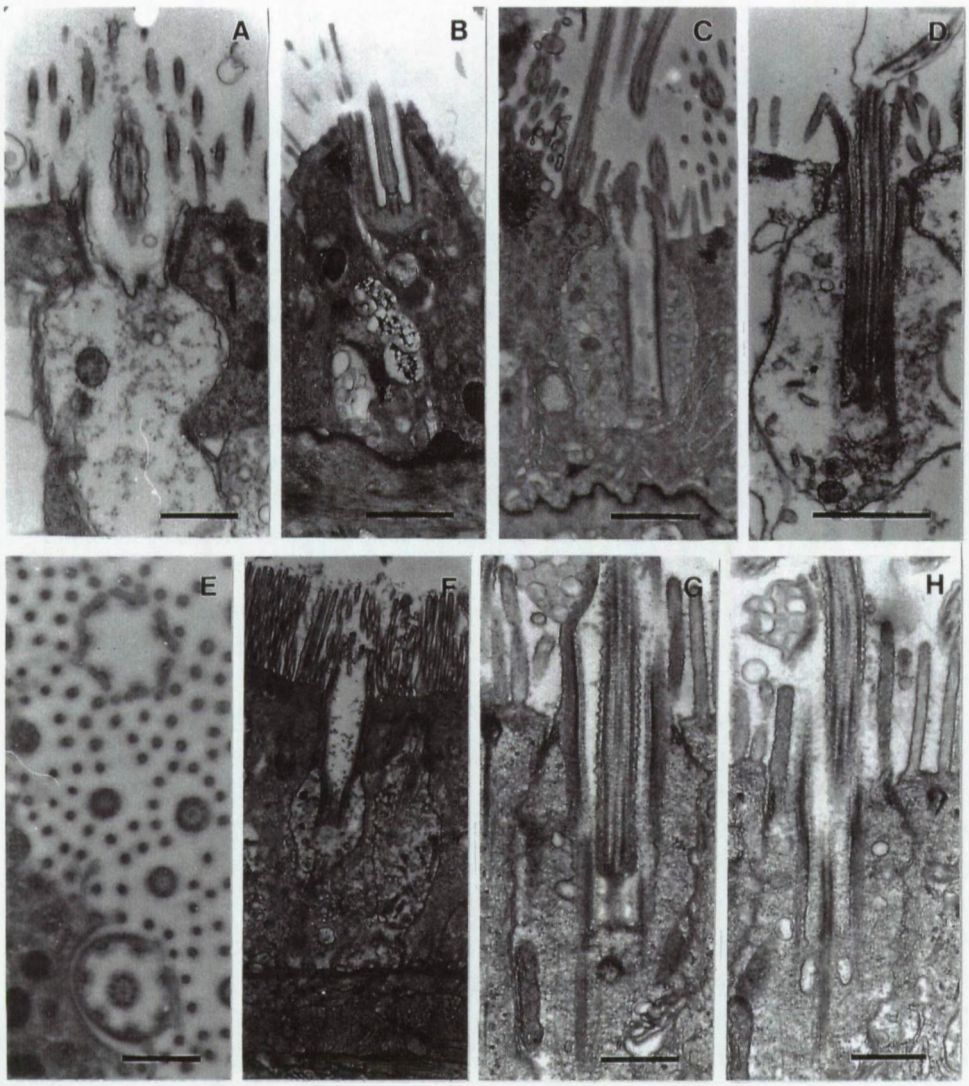


Fig. 7. Kinocilia in the epidermis of *Cytocystis* (A), *Placorhynchus* (B), *Gnathorhynchus* (C), *Cystiplex* (D), *Typhlopolycystis* (E), *Marirhynchus* (F) and *Itaipusa* (G, H). Scale bar A, E, G, H: 0.5 µm. Scale bar: B, C, D, F: 1 µm.

The way of functioning of the proboscis alters with the structure. The presence of two hooks or two muscle plates are two distinct ways of altering the method of catching preys in Eukalyptorhynchia. But the cone persists in all Eukalyptorhynchia and still has a glandular and probably adhesive function. Two muscular plates functioning as pincers are general in Schizorhynchia and the development of hooks can be regarded as a parallel evolution in both taxa of Kalyptorhynchia (Karling 1961). The conorhynch or the eukalyptorhynch proboscis does not function as a sucker and the retracted cone which results in a cup-shaped proboscis is not the resting position of the proboscis (Karling 1961) but a cone extending in the cavity is the normal situation (Meixner 1938 *Fig. 24*). The fact that so many proboscises in Eukalyptorhynchia are described or drawn with a retracted cone is due to the contraction of the inner longitudinal muscles by fixation. This can be prevented by anaestethizing and relaxing the animals in a isotonic $MgCl_2$ solution before fixation.

Records on feeding and food preferences in Eukalyptorhynchia are not abundant. Armonies (1986) mentions the preys for eight species in salt marches *Acrorhynchides robustus* (Polycystididae) fed on Copepoda, *Parautelga bilioi* (Koinocystididae) on Oligoschaeta, *Zonorhynchus salinus* and *Z. pipettiferus* on Nematoda, *Placorhynchus octaculeatus*, *P. dimorphis* and *Psittocorhynchus verweyi* (Placorhynchidae) were feeding on Nematoda and *Prognathorhynchus canaliculatus* (Gnathorhynchidae) on Nematoda and Oligochaeta.

Implications of the proboscis ultrastructure for phylogenetic systematics

A common origin of the eukalyptorhynch proboscis is accepted by an invagination of the epidermis at the anterior terminal body end. The sunken cell parts of the epithelium and the septum are probably established and formed in different parallel ways. A bipartite cone epithelium is encountered in all species investigated (character 1) and is considered ancestral in Eukalyptorhynchia. A glandular function of the proboscis is general for the eukalyptorhynch proboscis. A reduction of the number of gland types, the abundancy of the glands necks or the amount of secretion is noticed in some genera, sometimes associated with the appearance of additional structures, such as hooks or muscle plates. Type g_9 gland necks can be homologized in all species investigated. Type g_4, g_5, g_6 and g_7 gland necks can also be homologized in most species on morphological grounds. The gland necks, which predominantly form the glandular ring in the proximal part of the sheath epithelium, are mostly refered to as type g_2 gland necks. Uniciliary receptors in the sheath and the cone epithelium have the same characteristics in all species investigated and are regarded homologous. Intra-epithelial nuclei represent the ancestral state, while insunk nuclei in the surrounding parenchyma or the proboscis bulb form a derived state. Insunk nucleiferous cell parts outside and inside bulb represent a parallel development. Intrabulbar nuclei even have arisen different times independently (S_2, A or B). Especially the nucleiferous cell parts of

the basal cone epithelium have many different positions. A cellular organization is ancestral to a syncytial organization. The basal belt of the sheath epithelium is syncystialized in most species except for *Toia, Nannorhynchides* and *Ethmorhynchus*. The cellular organization in the former two genera (and probably in *Pocillorhynchus*) is regarded ancestral, in *Ethmorhynchus* this probably represent a secondary cellular state (reduction) of the syncytium (character 17a). Syncytialization of the belts in the sheath epithelium progresses from the proximal over the median to the distal belt. Cellular belts in the sheath epithelium are mostly encountered in bipartite sheath epithelia. Syncytializations of the sheath epithelium occurs mostly in the proximal belt of genera with a tripartite sheath epithelium. The microvilli decrease in length from the distal belt of the sheath epithelium to the apical cone epithelium. A basement membrane is present or absent as well in the group of genera with two as three belts in the sheath epithelium. The position of the nucleiferous parts differs, indicating a independent reduction of this basement membrane during evolution.

The genera *Toia* and *Nannorhynchides* are characterized by many plesiomorphic characters in the probsoscis as the presence of a fully cellular proboscis epithelium. But the presence of pigmented eyes with lenses forms a synapomorphic character for *Toia, Nannorhynchides* and *Pocillorhynchus* (character 2a). The presence of numerous uniciliary receptors with long rootlets in both belts of the sheath epithelium probably represents a synapomorphic feature in the proboscis for the three genera mentioned. The absence of incorporated flagellae and axonemata in the mature spermatozoa of both species investigated is an additional synapomorphic feature (character 2b). *Toia, Nannorhynchides* and *Pocillorhynchus* form a monophyletic taxon, the genera can be distinguished by the appearance of the inner circular muscles of the bulb and the number of g_6 and g_7 and the number of component parts in the lenses. The genus *Toia* is characterized by a thin inner circular muscle layer, and one-part lenses. *Pocillorhynchus* does not show an inner circular muscle layer in light microcopic preparations and this muscle layer might be very thin or lacking. Distinctive g_6 and g_7 gland necks are not present in the bulb and the lenses are bipartite. In *Nannorhynchides* the inner circular muscle layer is distinctly thicker and tripartite lenses are present. For further distinction between the three genera see chapter 3 and Brunet (1973). The other genera, except the formerly mentioned, are characterized by a syncytial basal cone epithelium (character 3). The numerous and irregular arranged cells of the distal belt of the sheath epithelium, the presence of nuclei in the apical cone epithelium on top of the cone and the syncytial proximal belt of the sheath epithelium form synapomorphic characters for the genus *Zonorhynchus* (characters 4a and 4b). Multiciliary receptors are present in the distal belt of the sheath epithelium of most species, they lack in *Toia, Nannorhynchides, Zonorhynchus* and *Mesorhynchus*. Multiciliary dendrites with short fingerlike ciliary shafts, piercing the cavity epithelium, are present in most species and are encountered in the family Polycystididae, Cystiplanidae, Koinocystididae as well as in

Paragnathorhynchus, Florianella and *Placorhynchus*. If multiciliary receptors associated with the distal belt of the sheath epithelium are considered to be a synapomorphic feature for all Eukalyptorhynchia, the absence of these receptors could be regarded as a synapomorphy for the genera *Toia, Nannorhynchides* and *Zonorhynchus*. The sister taxon, however, can not be characterized by a synapomorphy. The absence of multiciliary receptors can also form the plesiomorphic state, the presence of these receptors in the distal belt forms a synapomorphy for all genera except *Toia, Nannorhynchides* and *Zonorhynchus* (character 5). Within the sister group of *Toia, Nannorhynchides* and *Zonorhynchus*, the exact relationships remain uncertain to a certain extend. Three monophyletic taxa can be distinguished in this group (characters 6, 7, 8). Bertiliellidae form a monophyletic taxon based on the presence of spiculae in the epidermis (character 6). Two sets of proboscis retractors are present in *Cicerina, Paracicerina, Ptyalorhynchus, Ethmorhynchus, Psammorhynchus*, Placorhynchidae and Gnathorhynchidae (character 7). Placorhynchidae and Gnathorhynchidae are characterized by receptors with rigid ciliary shafts situated at the junction (character 28). Placorhynchidae are characterized by the presence of muscle plates (character 9) and Gnathohynchidae have a synapomorphy with *Aculeorhynchus glandulis*, namely two proboscis hooks in the basal cone epithelium (character 10). Within the Gnathorhynchidae, *Gnathorhynchus* is characterized by about ten intra-epithelial spherical sensory organs, containing receptors with flat sheetlike ciliary shafts (character 12). A parallel development must have taken place within the formation of these sensory organs as well (*Gnathorhynchus* versus *Cicerina*, etc). Both a circular and longitudinal muscles layer surround the cavity; only in *Placorhynchus* the circular muscles are lacking (character 9b). The genera *Cytocystis, Psammorhynchus, Ethmorhynchus, Ptyalorhynchus, Paracicerina* and *Cicerina* are characterized by the presence of two insunk sensory organs with multiciliary receptors (in plesiomorphic state with fingerlike ciliary shafts) (character 11). Within this group *Cytocystis* and *Psammorhynchus* are characterized by the presence of insunk nucleiferous cell parts of the basal cone epithelium, which sink in through the bulb, while the proximal belt of the sheath epithelium and the apical cone epithelium have intrabulbar nuclei (character 13a). Type g4 and g5 gland necks are lacking (character 13b). *Cytocystis* is typified by one set of posterior proboscis retactors and the presence of fixators (character 14) and the continuation of the basement membrane of the proximal belt of the sheath epithelium around the sides of the bulb. *Psammorhynchus* is characterized by multiciliary receptors in the insunk sensory organs, which are organized in three stacks of flat membranous sheets (character 15). *Ethmorhynchus, Ptyalorhynchus, Paracicerina* and *Cicerina* are characterized by a nucleo-glandular girdle. This girdle is formed by long gland necks of type g4 and g5 filled with secretion which are situated in subjunctional nucleiferous cell parts of the proximal belt of the sheath epithelium and the cone epithelium (character 16). *Ethmorhynchus* is characterized by insunk nucleiferous cell parts of the cells of the distal belt

of the sheath epithelium and intrabulbar nuclei of the apical cone epithelium and a cellular basal belt (characters 17a, 17b and 17c). *Ptyalorhynchus, Paracicerina* and *Cicerina* are characterized by multiciliary receptors in the two insunk sensory organs, which form concentric lamellae (character 18). *Ptyalorhynchus* possesses fifteen g4 and g5 gland necks which surface alternatingly (character 25). The gland necks are to a large extend incorporated in the bulb. *Paracicerina* and *Cicerina* are characterized by four glandular ampullae formed by g4 and g5 gland necks (character 26), which are enclosed by a muscular sheath in *Cicerina* (character 27).

The presence of two belts in the sheath epithelium is regarded the plesiomorphic state, while a tripartite sheath epithelium is considered to be apomorphic. Koinocystididae, *Mesorhynchus*, Cystiplanidae and Polycystididae are characterized by three belts in the sheath epithelium (character 8). *Itaipusa, Tenerrhynchus, Parautelga* and *Mesorhynchus* are characterized by intra-epithelial nuclei in the three belts of the sheath epithelium but this is considered the plesiomorphic condition. The intrabulbar nuclei of the apical and basal cone epithelium form a synapomorphic character for this group (character 19). Within this group *Itaipusa* and *Tenerrhynchus* are typified by the presence of a strong closed (by ECM) sphincter of inner circular muscles below the junction (character 21), while in *Parautelga* and *Mesorhynchus* the nucleiferous parts of the basal cone epithelium are evolved to insunk cell parts behind the bulb (character 22). Polycystididae and Cystiplanidae are characterized by the presence of intra-epithelial muscles in the cone epithelium (character 20). The two families can not be separated on basis of the proboscis structure. Polycystididae are characterized by four pharyngeal knobs on the proximal margin of the pharynx (characer 24). Cystiplanidae are typified by a highly 'vacuolated' epidermis, which in fact represent numerous empty gland necks (character 23). *Phonorhynchus, Neopolycystis, Danorhynchus* and *Scanorhynchus* have a cytoplasmic girdle surrounding the distal part of the bulb as in Cystiplanidae. This might represent the plesiomorphic condition in Cystiplanidae and Polycystididae. In *Neopolycystis, Danorhynchus* and *Scanorhynchus* the outer circular muscles are situated peripheral of the outer longitudinal muscles. The other genera within the Polycystididae of insunk nucleiferous cell parts of the proximal belt of the sheath epithelium and both belts of the cone epithelium. A special type of uniciliary receptors is present in *Progyrator*. If a male copulatory organ of the conjuncta type is considered plsiomorphic (Graff 1908, Karling 1956), the divisa type must have arisen several times independently, in Polycystididae and *Mesorhynchus* for instance.

List of autapomorphies

1. conorhynch with bipartite cone epithelium
2a. aflagellate spermatozoa
2b. lenses
3. *B* syncytial
4a. S_2 syncytial
4b. *A* with intra-epithelial nucle(i)us
5 intra-epithelial multiciliary receptors in S_1
6. spiculae in epidermal basement membrane
7. two sets of proboscis retractors, no fixators
8. three belts in sheath epithelium
9a. muscle plates
9b. no outer circular muscles
10. intra-epithelial hooks in *B*
11. two spherical sensory organs with multiciliary receptors with fingerlike ciliary shafts
12. ten spherical intra-epithelial sensory organs with multiciliary receptors
13a. insunk nucleiferous cell parts of *B* through bulb, those of S_2 and *A* intrabulbar
13b. absence of g_4 and g_5 gland necks
14. one set of proboscis retractors , fixators and basement membrane of S_2 forming the lateral septum
15. stacks of flat sheetlike ciliary shafts of multiciliary receptors in the sensory organs
16. g_4 and g_5 long gland necks filled with secretion, nucleo-glandular girdle fully enclosed by ECM
17a. *B* cellular
17b. S_1 insunk nuclei
17c. *A* intrabulbar nuclei
18. multiciliary receptors in sensory organs form concentric lamellae
19. *A* and *B* intrabulbar nuclei
20. intra-epithelial muscles
21. proboscis sphincter
22. insunk cell parts of *B* through bulb
23. vacuolated epidermis
24. four sklerotizations on the proximal magin of the pharynx
25. fifteen alternating g_4 and g_5 gland necks
26. four glandular ampullae formed by g_4 and g_5 gland necks
27. glandular ampullae enclosed by circular muscles
28. receptors with rigid ciliary shafts at the juntion

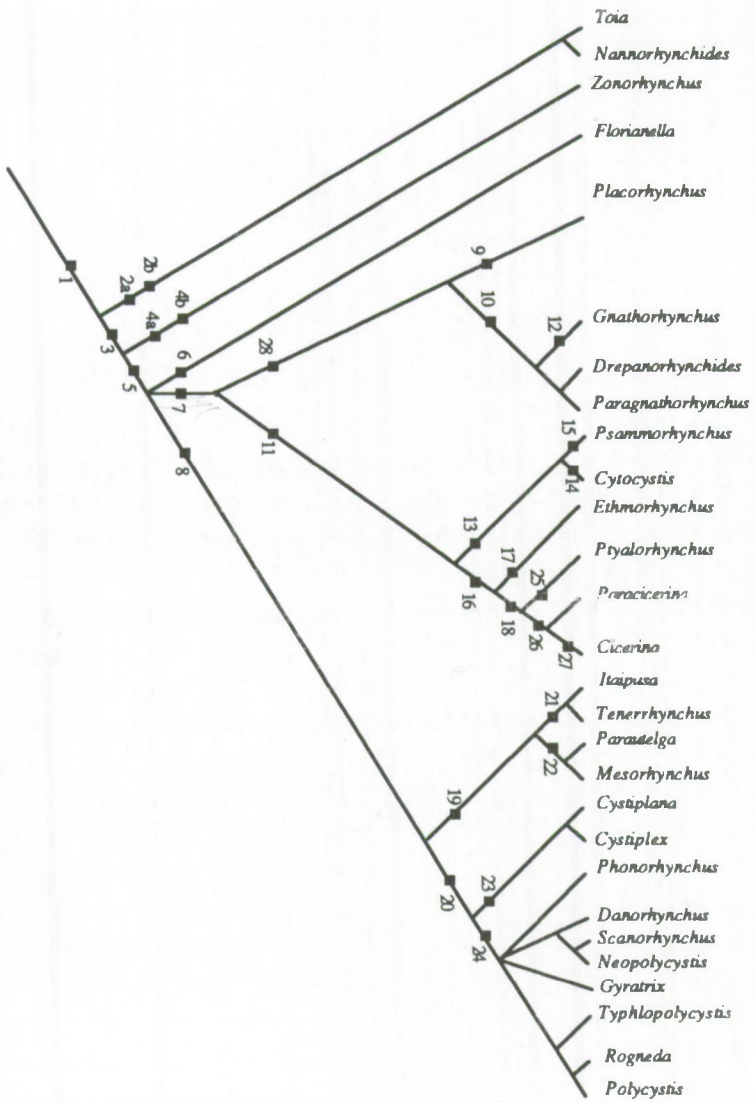


Fig. 8. Phylogram of the investigated species of Eukalyptorhynchia. The numbered black squares refer to the apomorphies that have been mentioned in the text.

In a phylogenetic classification modus this results in the subsequent classification:

Eukalyptorhynchia
- Nannorhynchidae
- N.N.1
  - Zonorhynchidae
  - N.N.2
    - Bertiliellidae (*s.m.*)
    - N.N.3 (*s.m.*)
      - N.N.4
        - Placorhynchidae
        - Gnathorhynchidae (+*Aculeorhynchus*)
      - N.N.5
        - Psammorhynchidae
        - Cicerinidae (*s.s.*)
    - N.N.6 (*s.m.*)
      - Koinocystididae (+*Mesorhynchus*)
      - N.N.7
        - Cystiplanidae
        - Polycystididae (+*Marirhynchus*)

Seven new taxa are formed, N.N.1 includes all species with a syncytial basal cone epithelium, N.N.2 includes all species with multiciliary receptors associated with the distal belt of the sheath epithelium. The exact relationship of the major taxa in N.N.2 is unclear. N.N.3 includes N.N.4 and N.N.6. N.N.4 is formed by Placorhynchidae and Gnathorhynchidae, including *Aculeorhynchus*. N.N.5 includes the genera *Psammorhynchus, Cytocystis, Ethmorhynchus, Ptyalorhynchus, Paracicerina* and *Cicerina*. The taxon N.N.6 comprises Koinocystididae and N.N.7 formed by Cystiplanidae and Polycystididae. *Mesorhynchus* is included in the Koinocystididae and *Marirhynchus* in the Polycystididae. The subfamilies Nannorhynchinae Evdonin, 1977 and Zonorhynchinae Karling, 1952 are given a higher taxonomic rank. The other genera, which were grouped in the family Cicerinidae Meixner, 1928, represent a monophyletic taxon that comprises the Cicerininae Meixner, 1938 and Ethmorhynchinae Meixner, 1938 and can now be referred to as the Cicerinidae (*s.s.*). The genera *Psammorhynchus* and *Cytocystis* are grouped in a monophyletic taxon, which could be alloted the name Psammorhynchidae Meixner, 1938. The family Nannorhynchidae comprises the genera *Toia, Nannorhynchides* and *Pocillorhynchus*. The family Zonorhynchidae only contains the genus *Zonorhynchus*. The families Bertiliellidae, Gnathorhynchidae and Placorhynchidae have been established by Rieger and Sterrer (1975) and Meixner (1938). This leads us to a classifaction of Eukalyptorhynchia with ten families; Nannorhynchidae, Zonorhynchidae, Bertiliellidae, Placorhynchidae, Gnathorhynchidae, Cicerinidae, Psammorhynchidae, Koinocystididae, Cystiplanidae, Polycystididae.

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Summary

All genera and species of the taxon Eukalyptorhynchia are characterized by a proboscis with terminal pore. The proboscis is an organ situated in front end of the animals and is used to capture preys. The morphology of this structure has been investigated ultrastructurally to gain better insight in the anatomy of this characteristic organ. In spite of the extensive amount of information on the genital system, the relationships witin the Eukalyptorhynchia have been unclear up to now. From our study useful ultrastructural characters have been obtained to establish a sound phylogenetic system.

The proboscis epithelia are formed by four or five circumferential belts. Two or three belts constitute the sheath epithelium. Three sheath epithelial belts are present in Polycystididae, Cystiplanidae and Koinocystididae. The presence of two belts in cone epithelium is considered the plesiomorphic state in Eukalyptorhynchia. The proboscis epithelia are generally void of cilia and covered by microvilli. The belts in the sheath epithelium in species with a bipartite sheath epithelium are mostly cellular. Syncytial belts are present in many species with tripartite sheath epithelia. The position of the nuclei varies from intra-epithelial to insunk in the parenchyma or intrabulbar. The sheath epithelium is characterized by infoldings of the basal plasma membrane. The cone epithelium in all species is bipartite. In some species a basement membrane is lacking in the cone. The basal belt is generally a syncytium. Only three species possess a cellular basal cone epithelium. The apical cone epithelium is in most species syncytial or formed by a single cell.

Different types of glands are present in the epithelia of the proboscis. Some types can be homologized in all investigated species. Type g_9 gland necks are homologous in all Eukalyptorhynchia and contain small (120-200 nm) ovoid, electron dense secretion granules. The terminal parts of the gland necks are reinforced by peripheral microtubules. Two types of gland necks (type g_6 and g_7) in basal cone epithelium can be homologized in all species as well.

The sensory cells associated with the proboscis include uni- and multiciliary receptors. Uniciliary receptors in the sheath epithelium are characterized by primary and secondary rootlets. Those in the cone epithelium have blunt ciliary shafts and only primary rootlets. Multiciliary receptors associated with the distal belt of the sheath epithelium appear in different forms; intra-epithelial with fingerlike ciliary processes or with ciliary processes forming concentric lamellae in spherical sensory organs. In several species two insunk spherical invaginations of the distal belt of the sheath epithelium contain the multiciliary receptors. In these insunk sensory organs the ciliary shafts can either be fingerlike or flat sheetlike.

The muscles of the proboscis show cytological differences. A peripheric sarcoplasmic reticulum is not present in *Toia*, *Nannorhynchides* and *Florianella*. In Polycystididae and

Cystiplanidae the muscles show a cross-striation without H-zones. The outer musculature of the proboscis is formed by outer circular and longitudinal muscles of the sheath and motional muscles (protractors, fixators, proboscis and integument retractors), which enable the positioning, pro- and retraction of the proboscis. Only in Placorhynchidae outer circular muscles are lacking. In many species these muscles only appear beneath the proximal belt of the sheath epithelium. Gnathorhynchidae, Placorhynchidae, *Psammorhynchus* and a number of genera of the Cicerinidae are characterized by the presence of two sets of proboscis retractors, while fixator muscles are lacking. The inner musculature within the bulbar septum comprises inner circular and longitudinal muscles. The inner circular muscles are found from the nodus upto the junction or even in the cone. The inner longitudinal muscles can either form a thigh muscular block or are loosely arranged. In many species a division in central and peripheral longitudinal muscles can be made. Intra-epithelial muscles in the cone epithelium are present in Cystiplanidae and Polycystididae.

The family Cicerinidae Meixner, 1928 appears to be a grouping of a number of genera based on plesiomorphic characters (germovitellaria, glandular proboscis). This family falls apart in three monophyletic families Nannorhynchidae, Zonorhynchidae and Cicerinidae (s.s.). The Cicerinidae (s.s.), *Psammorhynchus*, *Cytocystis*, Placorhynchidae and Gnathorhynchidae form a monophyletic taxon based on the presence of two sets of proboscis retractors. The position of *Florianella* (Bertiliellidae), of which the proboscis is characterized by a number of plesiomorphic characters and autapomorphic features, is doubtful. The families Koinocystididae, including *Mesorhynchus*, Cystiplanidae and Polycystididae form a monophytletic taxon based on the presence of a third belt in the sheath epithelium. Cystiplanidae and Polycystididae are characterized by intra-epithelial muscles in the cone.

Samenvatting

Ultrastructurele kenmerken leveren een belangrijke bijdrage tot de fylogenetische systematiek van de Platyhelminthes. Ondanks de variatie aan ultrastructurele gegevens over "Turbellaria", zijn dergelijke gegevens over de proboscis van Eukalyptorhynchia slechts beperkt. In deze studie werden 35 soorten van het taxon Eukalyptorhynchia elektronenmicroscopisch onderzocht.

Alle genera en soorten van het taxon Eukalyptorhynchia worden gekarakteriseerd door een proboscis met terminale porus. De proboscis is een orgaan dat in het vooreinde van het lichaam van deze dieren is gesitueerd, het wordt gebruikt om prooien te vangen. De morfologie van de proboscis werd ultrastructureel onderzocht om een beter inzicht te krijgen in de anatomie van dit karakteristieke orgaan. Ondanks de enorme hoeveelheid informatie betreffende het voortplantingsstelsel waren de verwantschappen in het taxon Eukalyptorhynchia onduidelijk. Uit deze studie werden bruikbare ultrastructurele kenmerken bekomen die toelaten een fylogenetisch systeem voor te stellen.

Het epitheel van de proboscis wordt gevormd door vier of vijf gordels. Twee of drie gordels vormen het schedeepitheel. Drie gordels in het schedeepitheel komen voor bij Polycystididae, Cystiplanidae en Koinocystididae. De aanwezigheid van twee gordels in het conusepitheel wordt als een plesiomorf karakter beschouwd bij Eukalyptorhynchia. De epithelen van de proboscis bezitten geen ciliën en zijn bedekt met mikrovilli. In soorten met een tweedelig schedeepitheel zijn de gordels, die het schedeepitheel vormen meestal cellulair. Syncytiale gordels worden in vele soorten met een driedelig schedeepitheel gevonden. De positie van de kernen varieert van intra-epitheliaal tot uitgezakt in het parenchym of in de bulbus. Het schedeepitheel wordt gekarakteriseerd door inplooïngen van de basala plasmamembraan. In sommige soorten ontbreekt echter een basale membraan in de conus. Het basale conusepitheel is meestal een syncytium. Enkel in drie soorten werd een cellulair basaal conusepitheel gevonden. Het apicale conusepitheel wordt ofwel gevormd door een syncytium ofwel door één enkele cel.

Verschillende klieren bevinden zich in de epithelen van de proboscis. Sommige types zijn homoloog in alle onderzochte soorten. Type g_9 klieren zijn homoloog in alle Eukalyptorhynchia en zijn gevuld met kleine (120 tot 200 nm), ovale, elektronendense secretiekorrels. De uiteinden van deze klierafvoergangen zijn versterkt door perifere mikrotubuli. Twee types van klierafvoergangen (g_6 en g_7) in het basale conusepitheel kunnen homoloog gesteld worden in alle onderzochte soorten.

De zintuigcellen, die geassocieerd met de proboscis voorkomen, omvatten uni- en multiciliaire receptoren. Uniciliaire receptoren in het schedeepitheel worden gekenmerkt door primaire en secundaire ciliënwortels. De uniciliaire receptoren van het conusepitheel hebben stompe cilia en enkel primaire wortels. Multiciliaire receptoren, geassocieerd met de distale gordel van het schedeepitheel, komen in verschillende vormen voor; intra-epitheliaal met

vingervormige ciliën of met ciliën die concentrische lamellen vormen in ronde zintuigorgaantjes. In een aantal soorten komen twee uitgezakte sferische invaginaties van de distale gordel van het schedeepitheel voor, die de multiciliaire receptoren bevatten. De ciliën in deze uitgezakte zintuigorganen kunnen vingervormig of plat en bladvormige zijn.

De spieren van de proboscis vertonen cytologische verschillen. Een perifeer sarcoplasmatisch reticulum komt niet voor in *Toia, Nannorhynchides* and *Florianella.* In Polycystididae en Cystiplanidae de spieren vertonen een dwarsstreping zonder H-zones. De buitenste musculatuur van de proboscis wordt gevormd door de buitenste circulaire en longitudinale spieren rond de schede en de bewegingsspieren (protractoren, fixatoren, proboscis- en integumentretractoren), die het positioneren, uitsteken en terugtrekken van de proboscis toelaten. Buitenste circulaire spieren ontbreken enkel in Placorhynchidae. In veel soorten komen laatstgenoemde spieren alleen voor onder de proximale gordel van het schedeepitheel. Gnathorhynchidae, Placorhynchidae, *Psammorhynchus* en een aantal genera uit de Cicerinidae worden gekenmerkt door de aanwezigheid van twee sets proboscisretractoren, terwijl fixatoren ontbreken. De musculatuur binnen van het septum omvat binnenste circulaire en longitudinale spieren. De binnenste circulaire spieren komen voor van de nodus tot aan de junctie of tot in de conus. De binnenste longitudinale spieren vormen of een hechte spiermassa of zijn los georganiseerd. In veel soorten kunnen deze spieren in een centrale en perifere longitudinale spieren onderverdeeld worden. Intra-epitheliale spieren in het conusepitheel komen voor in Cystiplanidae and Polycystididae.

De familie Cicerinidae Meixner, 1928 blijkt een groepering van een aantal genera te zijn op basis van plesiomorfe karakters (germovitellaria, kliërrijke proboscis). Deze familie valt uiteen in drie monofyletische families Nannorhynchidae, Zonorhynchidae en Cicerinidae (s.s.). De Cicerinidae (s.s.), *Psammorhynchus, Cytocystis,* Placorhynchidae en Gnathorhynchidae vormen een monofyletisch taxon gebaseerd op de aanwezigheid van twee sets proboscisretractoren. De positie van *Florianella* (Bertiliellidae), waarvan de proboscis gekenmerkt wordt door een aantal plesiomorfe alsook autapomorfe karakters, blijft onzeker. De families Koinocystididae, inclusief *Mesorhynchus*, Cystiplanidae en Polycystididae, inclusief *Marirhynhcus,* vormen een monofyletisch taxon gebaseerd op de aanwezigheid van een derde gordel in het schedeepitheel. Cystiplanidae en Polycystididae worden gekarakteriseerd door intra-epitheliale spieren in de conus.

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