Cah. Biol. Mar. (1996) 37 : 205-219

Structure of prostomial photoreceptor-like sense organs in *Protodriloides* species (Polychaeta, Protodrilida)

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Abstract: Two different types of ciliary sensory structures have been found in the prostomium and palps of *Protodriloides chaetifer* and *Protodriloides symbioticus*. There are three serially arranged pairs in the prostomium close to the brain and up to six have been found along the length of the palps. The sensory structures in the palps and the anterior pair in the prostomium belong to the first type. It is a so-called basal ciliated cell not associated with a supporting cell. This sensory cell bears a bundle of a few cilia forming a loop around the cell body. Most likely they have a function other than photoreception. The type-2 sense organs are multicellular, comprise one to two sensory units each consisting of a multiciliated sensory cell and a glial supporting cell, and probably have a photoreceptive function. The cilia are unbranched, form a regularly coiled bundle, and the 9x2+0 axoneme is transformed distally into a 3x1 pattern. There is a degree of intra- and interspecific variation in number of organs and cytological features. Comparison with the sense organs in other invertebrates with special emphasis on polychaetes and the remaining genera of the Protodrilida reveals that neither type has yet been observed in other polychaetes. This finding clearly corroborates the supposed phylogenetic relationship of *Protodriloides* within the Protodrilida.

Résumé : *Structure des organes sensoriels prostomiaux, comparables à des photorécepteurs, chez les espèces de Protodriloides (Polychaeta, Protodrilida).* Deux catégories différentes de structures sensorielles ciliées ont été mises en évidence dans le prostomium et les palpes de *Protodriloides chaetifer* et *P. symbioticus*. Trois paires se succèdent dans le prostomium le long du cerveau et six organes sont répartis le long des palpes. Les structures sensorielles des palpes et la paire la plus antérieure du prostomium appartiennent à une première catégorie : il s'agit d'une cellule dite cellule basale ciliée, non associée à une cellule de soutien. Cette cellule sensorielle porte un faisceau de quelques cils formant une boucle autour du corps cellulaire. Très vraisemblablement elle a une fonction autre que la photoréception. Les organes sensoriels de type 2 sont multicellulaires, formés d'une ou deux unités sensorielles comprenant chacune une cellule sensorielle multiciliée et une cellule gliale de soutien, et ont très probablement un rôle photorécepteur. Les cils ne sont pas ramifiés et forment un faisceau régulièrement enroulé où les axonèmes de type 9x2+0 sont transformés distalement en axonèmes de type 3x1. Il y a une variation intra- et interspécifique dans le nombre des organes sensoriels et dans leurs caractères cytologiques. Nos données sont comparées avec celles obtenues sur les organes sensoriels d'autres invertébrés, en particulier les polychètes et les autres genres de Protodrilida. Les deux types de structures sensorielles décrits sont nouveaux chez les polychètes et corroborent ainsi les relations supposées du genre *Protodriloides* au sein des Protodrilida.

Keywords : Photoreceptor, sense organs, *Protodriloides*, Polychaeta, phylogeny.

Introduction

Protodriloides species are interstitial polychaetes that are characteristic of sandy beaches and intertidal sand flats.

Reçu le 30 août 1996 ; accepté après révision le 18 octobre 1996.
Received 30 August 1996 ; accepted in revised form 18 October 1996.

Only two species have been described: *Protodriloides chaetifer* (Remane, 1926), that is one of the so-called cosmopolitan species, and *Protodriloides symbioticus* (Giard, 1904), that is only known from European coasts (Westheide, 1990). Like all other Protodrilida they have a long and slender body, reduced parapodia, a prostomium with a pair of palps and a pygidium with two adhesive lobes. The taxon comprises only four genera: *Saccocirrus* Bobretzky, 1872, *Protodrilus* Czerniavsky, 1881, *Protodriloides* Jouin, 1966 and *Parenterodrilus* Jouin, 1992 with about 60 known species. In a tentative phylogenetic analysis (Purschke & Jouin, 1988) the genus *Protodriloides* was assigned a somewhat isolated position and turned out to be the sister group of all other taxa. This unexpected position is on the one hand due to the lack of synapomorphies between *Protodriloides* and *Protodrilus* (the latter formerly included *P. chaetifer* and *P. symbioticus),* and on the other hand to the presence of several autapomorphies in *Protodriloides*, for instance the specific position and structure of the palps as well as the peculiar reproductive biology including aflagellate spermatozoa, large oocytes and direct development (Swedmark, 1954; Jouin, 1966, 1978/79). Electron microscopic investigations have also been conducted to test the proposed monophyly of the Protodrilida and their subtaxa, to evaluate their relationships and to find synapomorphies with their supposed sister group, the Spionida, as well as to clarify the functional morphology of selected organs (see Purschke & Jouin-Toulmond, 1994 for references). These investigations showed that the sense organs and the central nervous system are rather complex structures providing numerous characters which might be useful for phylogenetic considerations. Among the sense organs, photoreceptor-like organs are of special interest because a great variety of these organs, which have either rhabdomeric or ciliary sensory cells, have been found in the other Protodrilida.

The main intention of the present investigation was to examine the *Protodriloides* species for the presence of sense organs that might be homologous to unpigmented photoreceptor-like organs occurring in the other genera of the Protodrilida. Of particular interest are the so-called statocysts, sense organs only known in *Protodrilus* and until now regarded as an autapomorphy of this taxon (Purschke, 1990b). Remane (1926) mentioned the presence of statocysts in *P. chaetifer.* However, in subsequent light microscopic investigations statocysts similar to those of *Protodrilus* were not found, but two pairs of another type of sense organ were observed close to the complex cerebral ganglia (Jouin, 1966, 1970). Thus, the proof of the questionable occurrence of such sense organs in *Protodriloides* would seriously question its proposed phylogenetic relationship. The present study completes a series of investigations on the sense organs of the Protodrilida. Here two types of internal ciliary sensory structures in the anterior end of *P. chaetifer* and *P. symbioticus* are described on the basis of confocal laser scanning microscopy and transmission electron microscopy. The significance of these findings for functional morphology and phylogeny is discussed. They fit well into the proposed phylogenetic relationship of the subordinate taxa of the Protodrilida, that can now be based on a larger number of characters.

Materials and Methods

Specimens of *Protodriloides chaetifer* (Remane, 1926) and *Protodriloides symbioticus* (Giard, 1904) were collected in the eulittoral of sandy beaches at List (North Sea Island of Sylt, Germany).

For transmission electron microscopy specimens were anaesthetized in a solution of $MgCl_2$ isotonic to sea water prior to fixation. The fixative was a mixture of sucrose, picric acid, paraformaldehyde and glutaraldehyde (SPAFG; see Purschke, 1993) in 0.075 M phosphate buffer. After rinsing in buffer, specimens were postfixed in buffered 1% OsO_4 and embedded in an Epon-Araldite mixture. Series of ultrathin sections of 5-7 specimens of each species were cut with a diamond knife on Reichert Ultracut microtomes, and collected on single slot grids coated with pioloform support films (0.3% pioloform in trichlormethane) in order to obtain complete series of sections. The sections were stained with uranyl acetate and lead citrate in a Leica Ultrostainer and examined in Zeiss EM 109 and 902 A transmission electron microscopes. Reconstructions were done from micrographs taken at intervals of 0.5-0.7 µm (every 8th to 10th section).

For confocal laser scanning microscopy (CLSM) relaxed animals were fixed on ice overnight in 4% paraformaldehyde in 0.15 M phosphate buffer (PBS, pH 7.4) containing 8% sucrose. After several rinses with PBS the specimens were preincubated for 1 h in PBS with 0.1% Triton X-100. The primary antibody, mouse-anti-acetylated α-tubulin (Sigma, dilution 1:100), was incubated for 12 h at room temperature. After rinsing with PBS and incubation with the secondary antibody, either goat-anti-mouse TRITIC conjugated or goat-anti-mouse FITC conjugated (both Sigma, dilution 1:100), specimens were mounted in Citiflour. Preparations were viewed with a Zeiss LSM 410.

Results

Position of sense organs

There are three pairs of ciliary sensory structures belonging to two different types in the prostomium of *Protodriloides symbioticus* and *Protodriloides chaetifer.* They have an almost identical position in both species (Figs. 1, 2A, B). The pairs are serially arranged and lie below the epidermis.

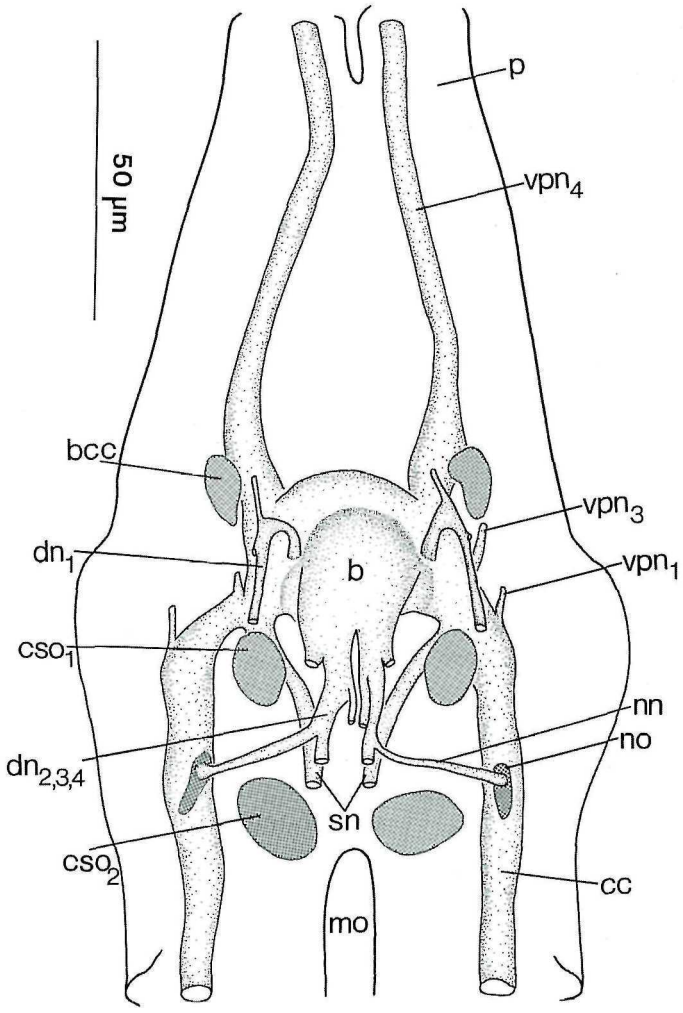

Figure 1. *Protodriloides symbioticus.* Position of prostomial sensory organs in relation to the central nervous system. *b* brain, *bcc* basal ciliated cell, *cc* circumoesophageal connective, $cso_{1,2}$ ciliary sense organ$_{1,2}$, $dn_{1,2,3,4}$ dorsal nerve$_{1,2,3,4}$, *mo* mouth, *no* (vestigal) nuchal organ, *nn* nuchal nerve, *p* palp, *sn* stomatogastric nerve, $vpn_{1,3,4}$ ventral palp nerve$_{1,3,4}$. Reconstruction from electron micrographs.

Figure 1. *Protodriloides symbioticus.* Position des organes sensoriels prostomiaux par rapport au système nerveux central. *b* cerveau, *bcc* cellule ciliée basale, *cc* connectif périœsophagien, $cso_{1,2}$ organe sensoriel cilié$_{1,2}$, $dn_{1,2,3,4}$ nerf dorsal$_{1,2,3,4}$, *mo* bouche, *no* organe nucal (vestigial), *nn* nerf nucal, *p* palpe, *sn* nerf stomatogastrique, $vpn_{1,3,4}$ nerfs ventraux$_{1,3,4}$ du palpe. Reconstruction d'après des observations faites en microscopie électronique.

The anterior pair represents the first type of ciliary sensory structures. Because they are unicellular, they are referred to as basal ciliated sensory cells. The middle and posterior pair belong to the second type, and in the following they are called ciliary sense organs. All sensory structures are closely associated with the brain. As previously described (see Purschke, 1993), structure and position of the brain are correlated with the arrangement of the muscle fibres connecting the palp musculature to that of the body wall. These muscle fibres together with coelenchyme cells form two dorsoventrally oriented ribbons of mesodermal tissue between which the brain (neuropile and perikarya) is situated (Fig. 6A). The basal ciliated cells lie at the inner side of these ribbons somewhat lateral to and above the main palp nerve (vpn_4, terminology of Purschke, 1993). In *P. symbioticus* the posterior part of the cell is lateral to the neuropile of the brain (Fig. 4A). These sensory cells are also present in the palps not only of *P. chaetifer* (see Purschke, 1993) but also in *P. symbioticus* (Fig. 2D). The organs are somewhat irregularly distributed in the palps, though, in a given specimen, they are similarly arranged in both palps (Fig. 2D, E). Usually the first sensory cell lies 85 µm (*P. symbioticus*) or 110 µm (*P. chaetifer*) above the palp base, the others follow at irregular intervals. Up to 6 sensory cells were counted in *P. chaetifer* and 5 in *P. symbioticus.*

The two pairs of ciliary sense organs are situated outside the ribbons of muscle fibres (Figs. 2A, B, 6A-C). The anterior organs lie directly behind the neuropile of the brain; the posterior ones are approximately on a level with the nuchal organs (Figs. 1, 6A). In certain specimens of both species one ciliary organ can either be lacking or be situated close to the remaining one on the same side of the body, which results in an asymmetrical appearance of the arrangement of these organs (Fig. 2B).

Basal ciliated sensory cell

The basal ciliated sensory cells (type 1 sensory structure) are primary sensory cells that are not associated with supporting cells. The sensory cells are oval to spindle-shaped, and each cell gives rise to a varying number of cilia (Figs. 3, 4A-E). There are three to nine cilia in *P. chaetifer* and seven to 15 cilia in *P. symbioticus*. The bundle of cilia emerges from a depression 2-2.5 µm deep. The diameter of the depression varies according to the number of cilia. From this depression the cilia run to the surface of the sensory cell and twist once around the cell body. Thus the bundle of cilia forms a loop (Figs. 2D, E). In the prostomium the ciliary loop is longitudinally oriented, whereas in the palps the orientation differs between the individual sensory cells (Figs. 2 A, B, D, E). The cilia lie close together (10-15 nm apart) in a flat furrow (Figs. 4B, C); only in *P. chaetifer* has splitting into two to three bundles been observed. The cilia border, on the interstitial space between the sensory cell and adjacent cells, usually glial cells. At the opening of the depression the sensory cell forms small pores around the cilia, so that each cilium is completely surrounded by a small part of the sensory cell (70-100 nm thick, 500 nm

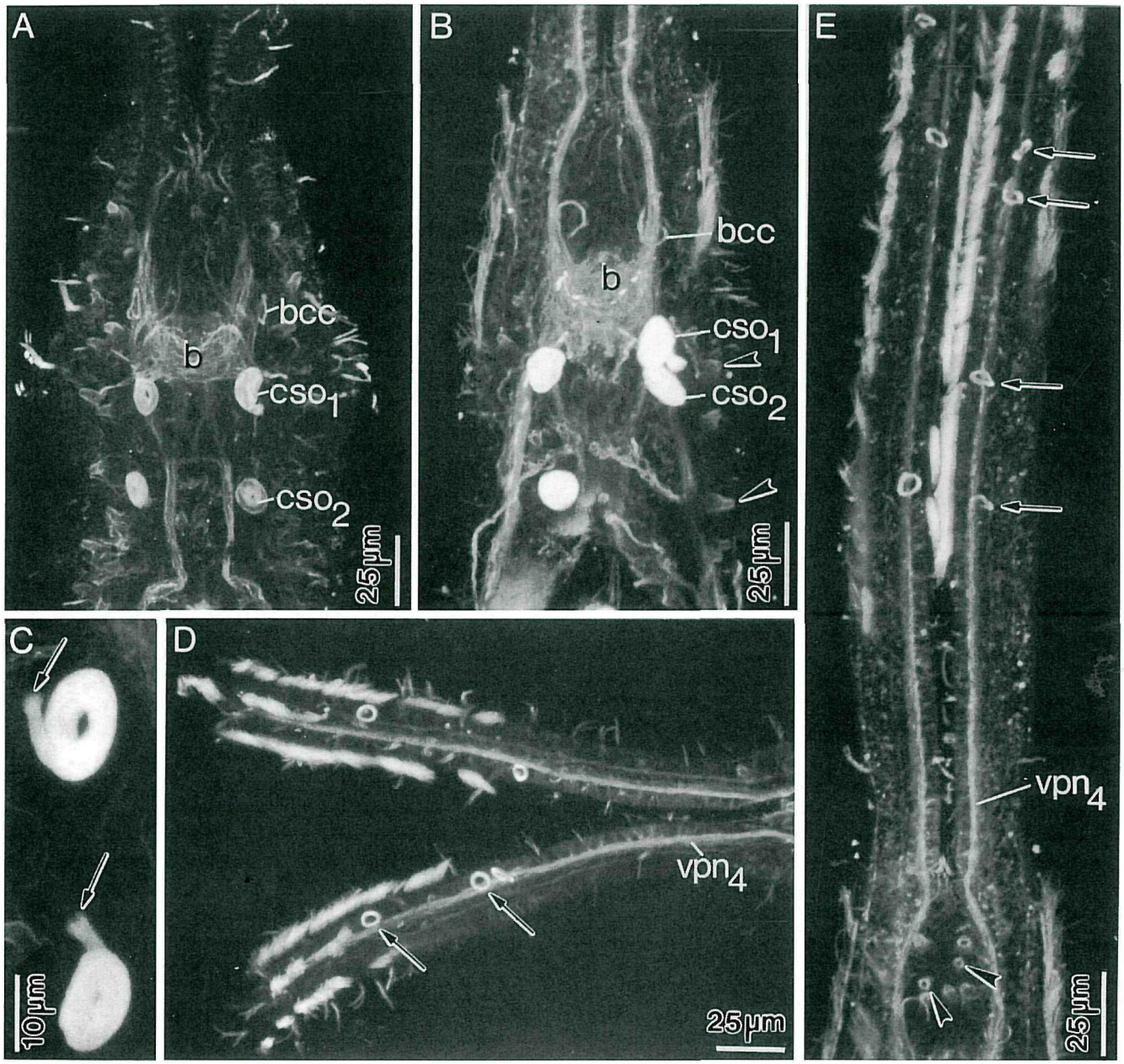

Figure 2. Anti-acetylated α-tubulin immunoreactivity. A, C, D *Protodriloides symbioticus*; B, E *Protodriloides chaetifer.*
A: Serially arranged prostomial sense organs. *b* brain, *bcc* basal ciliated cell, $cso_{1,2}$ ciliary sense $organ_{1,2}$.
B: Asymmetrically arranged sense organs. On the right side the ciliary sense $organ_2$ (cso_2) is located close to cso_1; *arrowheads* point to gland cell openings.
C: Emerging (*arrows*) and coiled sensory cilia of cso_1 and $_2$.
D, E: Basal ciliated sensory cells (*arrows*) in the palps; *arrowheads* point to gland cell openings. vpn_4 ventral palp $nerve_4$.

Figure 2. Immunoréactivité anti α-tubuline acétylée. A, C, D *Protodriloides symbioticus* ; B, E *Protodriloides chaetifer.*
A : organes sensoriels prostomiaux successifs. *b* cerveau, *bcc* cellule ciliée basale, $cso_{1,2}$ organe sensoriel $cilié_{1,2}$.
B : organes sensoriels disposés de manière asymétrique. Du côte droit l'organe sensoriel 2 cso_2 est situé près de cso_1 ; les *têtes de flèches* indiquent les pores des cellules glandulaires.
C : cils sensoriels émergeant (*flèche*) et formant une boucle autour du corps cellulaire, dans les organes sensoriels ciliés cso_1 et cso_2.
D, E : cellules sensorielles basales ciliées (*flèches*) dans les palpes ; les *têtes de flèches* indiquent les pores des cellules glandulaires. vpn_4 nerf $ventral_4$ d'un palpe.

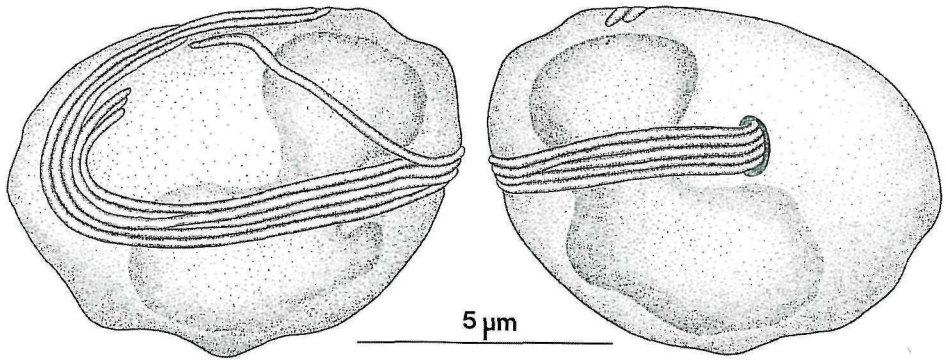

Figure 3. *Protodriloides chaetifer.* Basal ciliated cell (type-1 ciliary sensory structure). Reconstruction with computer-aided program showing both sides of the cell and course of the bundle of sensory cilia. Sensory cell shown as transparent to demonstrate position of nucleus; axon omitted. Program by courtesy of Dr. R. Windoffer, Osnabrück.

Figure 3. *Protodriloides chaetifer.* Cellule basale ciliée (structure sensorielle de type 1). Reconstruction à l'aide d'un programme informatique montrant les deux côtés de la cellule et le trajet du faisceau de cils sensoriels. La cellule sensorielle est représentée transparente pour montrer la position du noyau ; axone non représenté. Programme utilisé avec la permission du Dr. R. Windoffer, Osnabrück.

high) and leaves the depression separately (Fig. 4E). In this area rings of electron-dense material are present on the cytoplasmic side of the cell membrane of the sensory cell. A few short microvilli arise together with the cilia in the depression (Fig. 4D). The cilia have a typical 9x2+2 axoneme. However, dynein arms are lacking (Fig. 4E). The cilia rest on 0.5 µm high basal bodies that are connected to small rootlets in *P. symbioticus* only (Fig. 4A).

Ciliary sense organ

The ciliary sense organs (type 2 sensory structures) are multicellular and form internally projecting hemispheres (Fig. 6A). The microanatomy is basically similar in *P. chaetifer* and *P. symbioticus*. Usually each organ comprises two ciliary sensory units lying close together (Fig. 6B); in *P. chaetifer* occasionally only one unit was found. In *P. symbioticus* one ciliary sensory unit per ciliary sense organ was observed more often, especially in the anterior organs. In some specimens the anterior organs may be lacking and in this case the posterior organs always comprise two sensory units, or *vice versa*. Each unit consists of two cells: a glial supporting cell and a large multiciliary sensory cell. The cells form an extracellular cavity which is filled with the sensory cilia (Figs. 5A, B, 6B, C, 7A-C). Apically sensory and supporting cells are connected by a *zonula adhaerens*. A septate junction, however, could not be detected with certainty. These two cells interdigitate with the perikarya of a few other neurons and glial cells. However, these additional neurons do not show any apparent functional relationship with the ciliary sensory cells. In the immediate vicinity lie a gland cell and a vacuole-containing epidermal cell which are almost spherical in shape (Fig. 6A).

The cilia are unbranched and emerge in a spherical depression of the sensory cell (Figs. 2C, 5A, B, 7B, C). As a single bundle they enter the cavity formed by the supporting cell. This bundle of cilia is regularly coiled and runs three to four times to and fro. The length of the cilia has been estimated from evaluation of serial sections to be 45-50 µm in *P. symbioticus* and 90-110 µm in *P. chaetifer*. In *P. chaetifer* the supporting cell always forms a single cavity, and the different turns of the coil are more or less close together (Figs. 5A, 7B). In *P. symbioticus* the coils are separated by processes of the glial supporting cell, either for their entire length (Figs. 5B, 7A) or at least for the first turns. The sensory cell bears 220±40 (*P. chaetifer*) or 120±23 (*P. symbioticus*) cilia (mean values ± standard deviation, n = 7).

In *P. symbioticus* the cilia arise close together from the sensory cell, and in the bundle they are densely packed (15 nm apart) in all specimens investigated (Figs. 6C, 7A, 8F, G). In *P. chaetifer* the cilia are 120-350 nm apart in the cavity of the sensory cell; distally, in the cavity formed by the supporting cell, the distance is 20-100 nm (Figs. 7B, C, 8A, D). The space between the cilia is filled with granular material. It is not clear whether this material is secreted from the sensory or the supporting cell. Between the cilia there are a few short microvilli, 0.5-1 µm long (Fig. 7B).

The cilia rest on basal bodies (350-370 nm high) without rootlets. A basal foot associated with a few microtubules is present. The basal bodies give rise to 9x2+0 axonemes that are finally reduced to a 3x1 pattern 150 nm above the basal

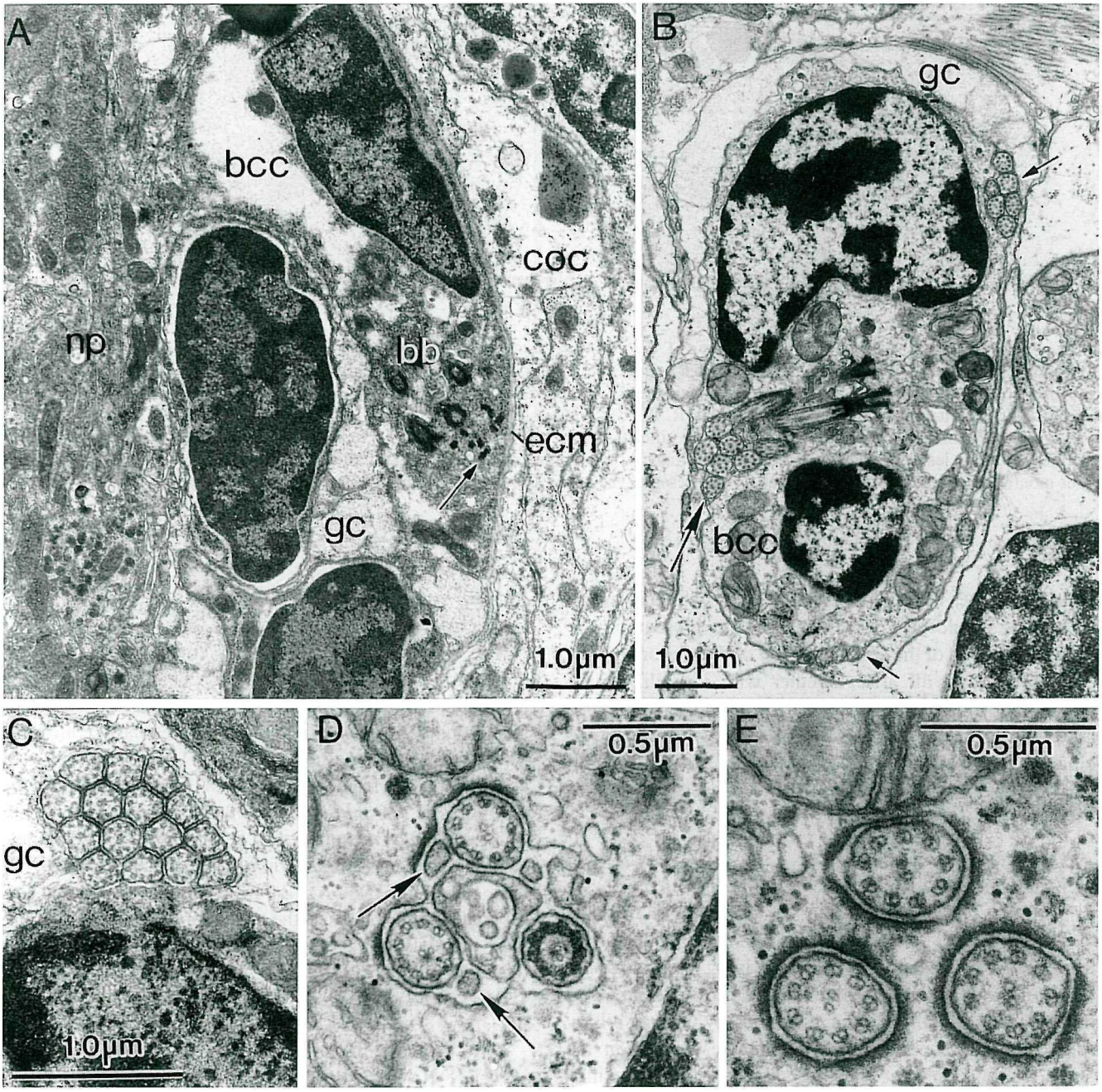

Figure 4. Basal ciliated sensory cell (*bcc*) (type-1 sensory structure) A, C *Protodriloides symbioticus*; B, D, E *Protodriloides chaetifer.*
A: Sensory cell with basal bodies (*bb*) and small rootlets (*arrow*) close to neuropile (*np*) of the brain. *coc* coelenchyme cell, *ecm* extracellular matrix, *gc* glial cell.
B: Bundles of sensory cilia cut proximally at the depression (*large arrow*) and distally (*small arrows*). Same cell as in Figure 3.
C: Cross section of bundle of sensory cilia surrounded by glial cells (*gc*).
D: Cross section of depression with cilia and microvilli (*arrows*).
E: Cross section through pores penetrated by cilia at opening of depression. Note electron-dense material within sensory cell, surrounding each pore.

Figure 4. Cellule basale ciliée sensorielle (*bcc*) (structure sensorielle de type 1). A, C *Protodriloides symbioticus* ; B, D, E *Protodriloides chaetifer.*
A : près du neuropile (*np*) du cerveau, cellule sensorielle avec corpuscules basaux (*bb*) et de petites racines ciliaires (*flèche*). *coc* cellule du coelenchyme, *ecm* matrice extracellulaire, *gc* cellule gliale.
B : faisceaux de cils sensoriels coupés dans la partie proximale de la dépression (*grande flèche*) et dans la partie distale (*petite flèche*). Même cellule que dans la Figure 3.
C : coupe transversale d'un faisceau de cils sensoriels entourés par des cellules gliales (*gc*).
D : coupe transversale de la dépression avec cils et microvillosités (*flèches*).
E : coupe transversale à travers les pores traversés par les cils à l'ouverture de la dépression. Noter le matériel de forte densité électronique autour de chaque pore de la cellule sensorielle.

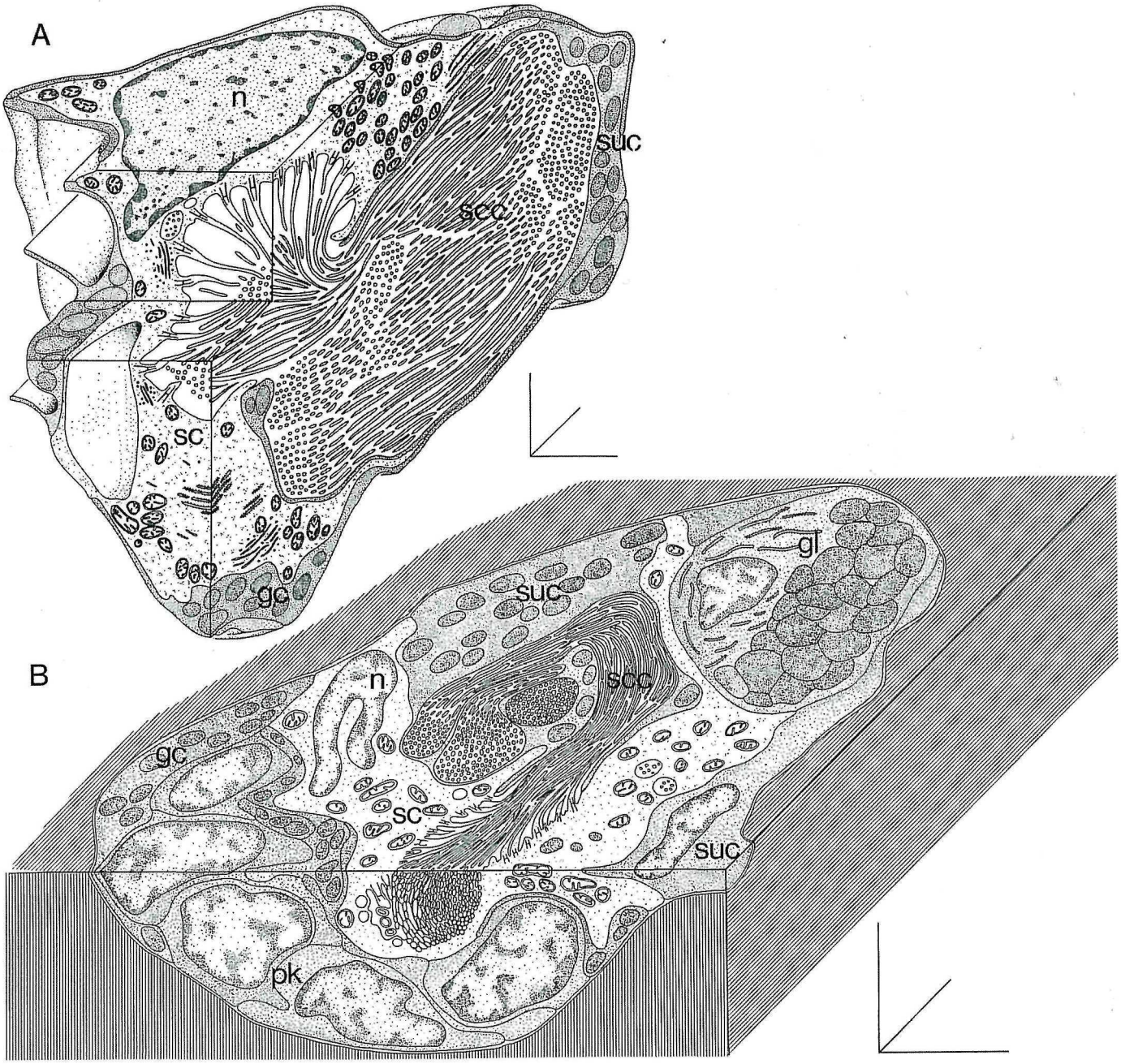

Figure 5. Reconstructions of ciliary sense organs (type-2 sensory structure) from serial sagittal sections, seen towards median plane. Only one unit consisting of one multiciliated sensory cell (*sc*) and one glial supporting cell (*suc*) shown in A and B. *gc* glial cell not serving as supporting cell, *gl* gland cell, *scc* cilia of the sensory cell, *n* nucleus of the sensory cell, *pk* perikarya of other neurons. Scale bars equal 2.5 µm in each direction.

A: *Protodriloides chaetifer*. Cells not belonging to single sensory unit have been left out.

B: *Protodriloides symbioticus*. Hatched areas represent muscle and coelenchyme cells.

Figure 5. Reconstruction tridimensionnelle des organes sensoriels ciliés (structure sensorielle de type 2), à partir de coupes sagittales sériées, vue du plan médian. Une seule unité sensorielle comprenant une cellule sensorielle multiciliée (*sc*) et une cellule gliale de soutien (*suc*) est représentée en A et B. *gc* cellule gliale qui n'est pas une cellule de soutien, *gl* cellule glandulaire, *scc* cils de la cellule sensorielle, *n* noyau de la cellule sensorielle, *pk* péricaryons d'autres neurones. Echelles : 2,5 µm dans chaque direction.

A : *Protodriloides chaetifer*. Les cellules n'appartenant pas à l'unité sensorielle n'ont pas été représentées.

B : *Protodriloides symbioticus*. Les zones hachurées représentent les cellules musculaires et les cellules du coelenchyme.

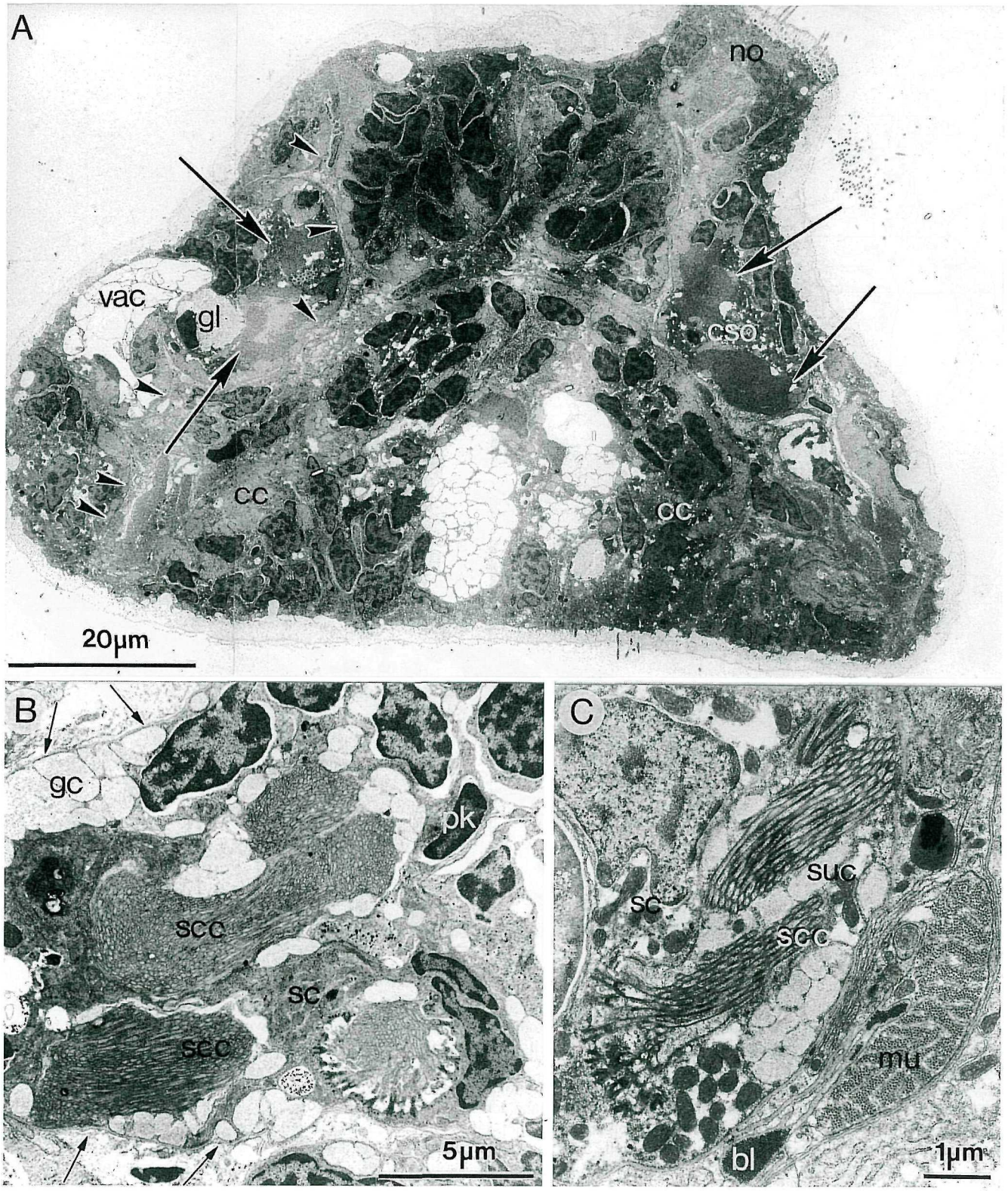
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plate (Fig. 8A-G); occasionally cilia with two microtubules have been observed in both species (Fig. 8G), cilia with four microtubules in *P. chaetifer* only (Fig. 8D). The transition zone is comparatively short; first the B-tubules are lost (Fig. 8B, C) and then six of the remaining A-tubules. The pattern of reduction does not appear to be uniform; consecutive cross sections through the transition zone showed that most likely different microtubules continue (e.g., tubules 1, 3, 7 or 1, 6, 9 in *P. chaetifer*). However, because the central singlets are lacking, the peripheral ones cannot be clearly distinguished. The conversion is correlated with a decrease in diameters of the cilia from 200-230 nm to 60-100 nm. The transition is rather sharp and the profiles of the cilia are flattened in *P. chaetifer* (Figs. 7B, C, 8A), whereas in *P. symbioticus* the diameter decreases continuously over a length of about 250 nm (Fig. 8F). In one specimen of *P. chaetifer* an aberrant cilium with a basal branch devoid of microtubules was found (Fig. 8E).

In the sensory cells a dendritic process cannot morphologically be distinguished from the cell body. In every organ of *P. chaetifer* the nuclei lie close to the surface of the sensory cell somewhat below the basal bodies of the cilia (Figs. 5A, 6B, 7C), whereas they are more distant in *P. symbioticus* (Figs. 5B, 6C, 7A). Mitochondria and glycogen particles are numerous and more or less uniformly distributed in the cell body. Rough ER, Golgi apparatus, multivesicular bodies and other organelles are well-developed. Characteristically, small ovoid vesicles (100-180 nm by 40-60 nm) with electron-dense contents (Figs. 7A-C, 8A) occur in low numbers all over the cell bodies. A system of cisternae of apical agranular ER is not present, nor were any signs of increased exo- or endocytic activity observed.

The supporting cells are similar to regular glial cells. They contain numerous ovoid granules, 1.1 by 0.6 µm, with fine fibrillar contents of medium electron-density (Figs. 5A-B, 7A-C). These cells send numerous interdigitating processes between the nerve cells of the ciliary sense organ (Fig. 7A-B). Usually the cell body with the nucleus lies apart from the process surrounding the cavity with the cilia. In regions without granules the process is rather thin and usually measures only 40-60 nm across (Fig. 7B).

The two other cell types lying close to the sense organ, the gland cell and the vacuole-containing cell, are abundant in the epidermis and represent a typical feature of the epidermis in *Protodriloides* (Fig. 2B, E; see Jouin, 1966). The gland cell is characterized by its large secretory granules (2.5-3 µm) containing fine granular material of low electron density (Fig. 5B). The gland cell opens to the exterior through a 1.5-µm-wide pore. The apex of the gland cell is surrounded by numerous microvilli. The other cell type contains large vacuoles of irregular outline that appear to be empty with the exception of a few membranous structures (Fig. 6A).

Discussion

The main intention of the present investigation was to examine the two species of the genus *Protodriloides* for the presence of photoreceptor-like sensory organs which might be homologous to such organs occurring in the other taxa, either to disprove or to corroborate the tentatively proposed phylogenetic relationship within the Protodrilida (Purschke & Jouin, 1988). This taxon, comprising the genera *Protodriloides*, *Parenterodrilus*, *Protodrilus* and *Saccocirrus*, is characterized by the occurrence of a variety of photoreceptor-like sense organs in its subtaxa (Purschke,

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Figure 6. Ciliary sense organ (type 2).

A: *Protodriloides chaetifer*. Entire cross section of the prostomium of a specimen at the level of nuchal organ (*no*) showing position of posterior pair of ciliary sense organs (*cso*); in each organ both ciliary units are marked with *arrows*. At left a gland cell (*gl*) and a vacuole-containing epidermal cell (*vac*) visible. *Arrowheads* point to dorsoventral ribbon of muscle and coelenchyme cells. *cc* circumoesophageal connective.

B: *P. chaetifer*. Cross section through hemispherical ciliary sense organ (exterior at right) with bundles of sensory cell cilia (*scc*) belonging to two sensory cells (*sc*), glial cells (*gc*) and perikarya (*pk*) of other neurons. *Arrows* point to extracellular matrix.

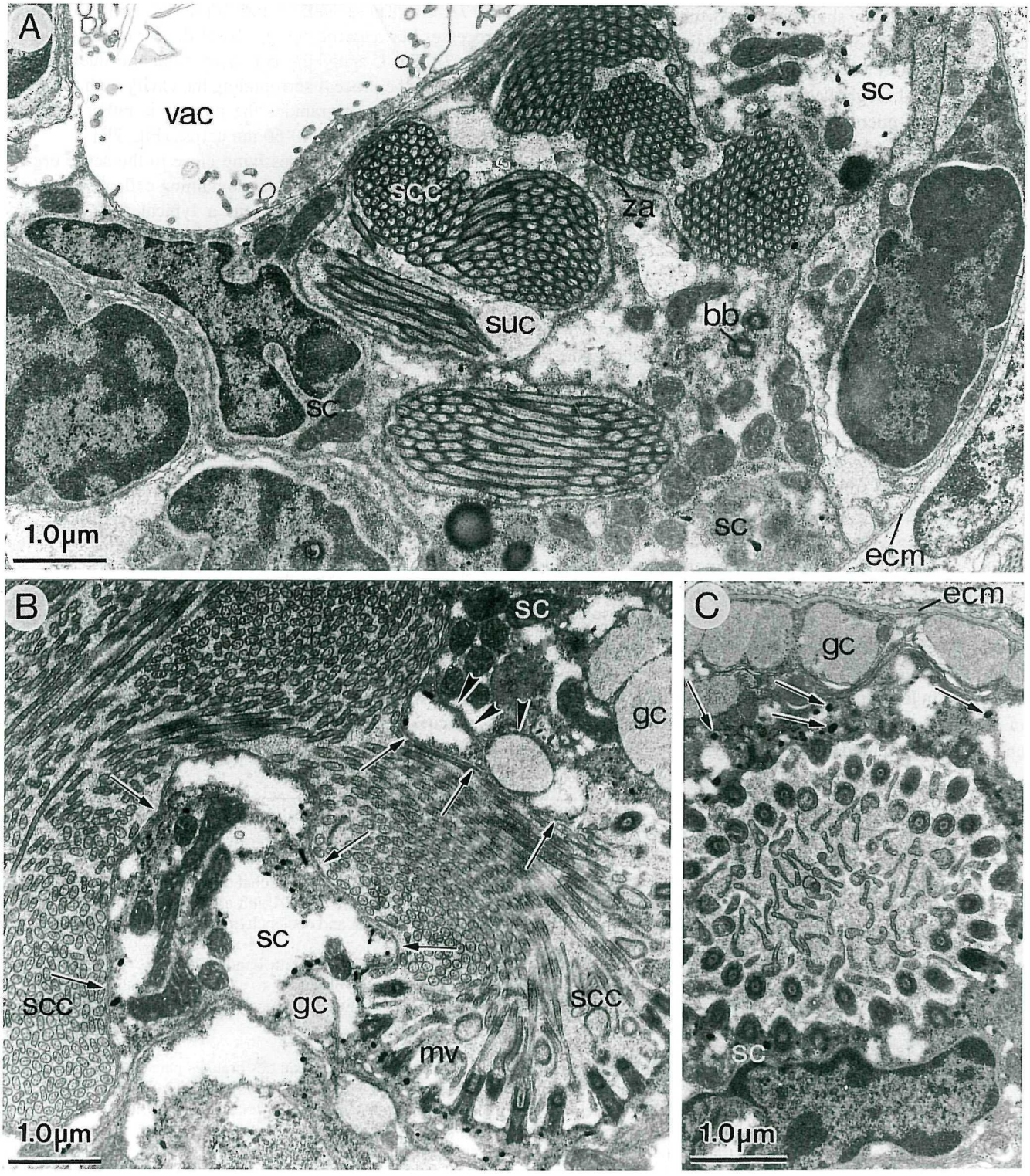
C: *Protodriloides symbioticus*. Sensory cell (*sc*) with bundle of cilia (*scc*) ensheathed by glial supporting cells (*suc*). *bl* blood vessel, *mu* muscle fibre.

Figure 6. Organe sensoriel cilié (type 2).

A : *Protodriloides chaetifer*. Coupe transversale entière du prostomium d'un spécimen au niveau des organes nucaux (*no*) montrant la position de la paire postérieure d'organes sensoriels ciliés (*cso*) ; dans chaque organe les 2 unités ciliées sont marquées par des *flèches*. A gauche une cellule glandulaire (*gl*) et une cellule épidermique vacuolaire (*vac*) sont visibles. Les *têtes de flèches* indiquent le ruban dorso-ventral de cellules musculaires et de cellules du coelenchyme. *cc* connectif périœsophagien.

B : *Protodriloides chaetifer*. Coupe transversale à travers les organes sensoriels ciliés hémisphériques (l'extérieur est à droite), avec les faisceaux de cils sensoriels (*scc*) appartenant à deux cellules sensorielles (*sc*), les cellules gliales (*gc*) et les péricaryons (*pk*) d'autres neurones. Les *flèches* indiquent la matrice extracellulaire.

C : *Protodriloides symbioticus*. Cellule sensorielle (*sc*) avec un faisceau de cils (*scc*) enveloppé par une cellule gliale de soutien (*suc*). *bl* vaisseau sanguin ; *mu* fibre musculaire.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1990a, b, 1993; Purschke & Jouin-Toulmond, 1993). Usually each supraspecific taxon possesses a specific type of these sense organs - often with species-specific characters. In this context the so-called statocysts of *Protodrilus* are of particular interest because homology of the sense organs of *Protodriloides* with these statocysts would seriously question the proposed phylogenetic relationship within the Protodrilida.

The ciliary sensory structures, situated at the same position in the palps and heads of the two species, *Protodriloides chaetifer* and *Protodriloides symbioticus*, belong to two types completely different from each other. The first type consists of a single cell; like similar sensory cells in oligochaetes it is called the basal ciliated sensory cell (Myhrberg, 1979). The second type, termed ciliary sense organ, consists of a multiciliated sensory cell and a glial supporting cell. The sensory structures of the same type in *P. chaetifer* and *P. symbioticus* show species-specific differences, especially in number, length and arrangement of cilia.

Basal ciliated sensory cells are unknown in other polychaete species. They are not restricted to the vicinity of the brain but were found in the palps of *P. chaetifer* as well (Purschke, 1993). With confocal laser scanning microscopy the number of these cells could easily be determined and they could also be detected in *P. symbioticus*. In annelids basal ciliated sensory cells have previously been described only in the epidermis of *Lumbricus terrestris* (Myhrberg, 1979). These cells may be compared with ciliated sensory cells present in the brain of several oligochaete species (Golding & Whittle, 1975; Basmer & Greven, 1993). All of these sensory cells are apparently not associated with a supporting cell and their cilia project into the intercellular space. However, in no species has an aperture been observed in the sensory cell and the cilia do not coil around the sensory cell. Such cells may be more widely distributed in polychaetes than is known today. Owing to the small number of cilia these structures are rather delicate and may easily have been overlooked. Advanced immunological methods might help to detect such cells. They are absent in all probability in other members of the Protodrilida: series of ultrathin sections of species of every genus have been thoroughly examined by us to look for such sensory cells. During these studies other small sensory cells were found in the palps of *Parenterodrilus taenioides* and *Protodrilus* species; these proved to be true phaosomes (Purschke, 1993, Purschke & Jouin-Toulmond, 1994).

Nothing is known about the function of these sensory cells; photoreceptive and osmoreceptive functions have been discussed (Myhrberg, 1979). The sensory cells of *Protodriloides* clearly differ from presumed photoreceptors in having only a few cilia, resulting in a comparatively small increase of the membrane surface, and in not being associated with a supporting cell (see e.g., Eakin & Hermans, 1988; Verger-Bocquet, 1992). In ocelli the presumed photoreceptive microvillar or ciliary processes lie in extracellular cavities which are generally isolated from the interstitium by septate junctions. However, projection of the sensory cilia into the interstitium is a characteristic feature of these sensory cells in *Protodriloides*. For these reasons photoreception appears to be unlikely and sensitivity to other modalities, e.g. chemoreception or hydrostatic pressure, should be taken into consideration.

Remane (1926) mentioned the presence of a pair of so-called statocysts in *Protodriloides chaetifer*. This could not be confirmed by Jouin (1966), who first discovered the ciliary sense organs and just termed these structures

Figure 7. Ciliary sense organ (type 2).

A: *Protodriloides symbioticus*. Sensory organ with coiled bundle of sensory cilia (*scc*), turns separated by glial supporting cell (*suc*); note irregular outline of sensory cell (*sc*) interdigitating with adjacent cells. *bb* basal body, *ecm* extracellular matrix, *vac* vacuole-containing cell, *za zonula adhaerens*.

B: *Protodriloides chaetifer*. Spherical cavity of sensory cell (*sc*) with emerging cilia (*scc*) which enter the single cavity surrounded by a thin glial supporting cell (*arrows*); *arrowheads* point to glial process interdigitating with the sensory cell. *gc* glial cell, *mv* sensory cell microvilli.

C: *P. chaetifer*. Base of sensory cell (*sc*) with sensory cilia cross-sectioned at different levels and microvilli, *arrows* point to electron-dense vesicles. *ecm* extracellular matrix, *gc* glial cell.

Figure 7. Organe sensoriel cilié (type 2).

A : *Protodriloides symbioticus*. Organe sensoriel avec faisceau de cils sensoriels enroulés (*scc*) séparés par des cellules gliales de soutien (*suc*). Noter le contour irrégulier de la cellule sensorielle (*sc*) au contact des cellules adjacentes. *bb* corpuscule basal, *ecm* matrice extracellulaire, *vac* cellule vacuolaire, *za zonula adhaerens*.

B : *Protodriloides chaetifer*. Cavité sphérique d'une cellule sensorielle (*sc*), dans laquelle émergent les cils (*scc*), entourée par une mince cellule gliale de soutien (*flèches*). Les *têtes de flèches* indiquent des prolongements cellulaires imbriqués avec la cellule sensorielle. *gc* cellule gliale, *mv* microvillosités de la cellule sensorielle.

C : *P. chaetifer*. Base de cellule sensorielle (*sc*) avec des cils sensoriels coupés transversalement, à différents niveaux et des microvillosités ; les *flèches* indiquent des vésicules à forte densité électronique. *ecm* matrice extracellulaire, *gc* cellule gliale.

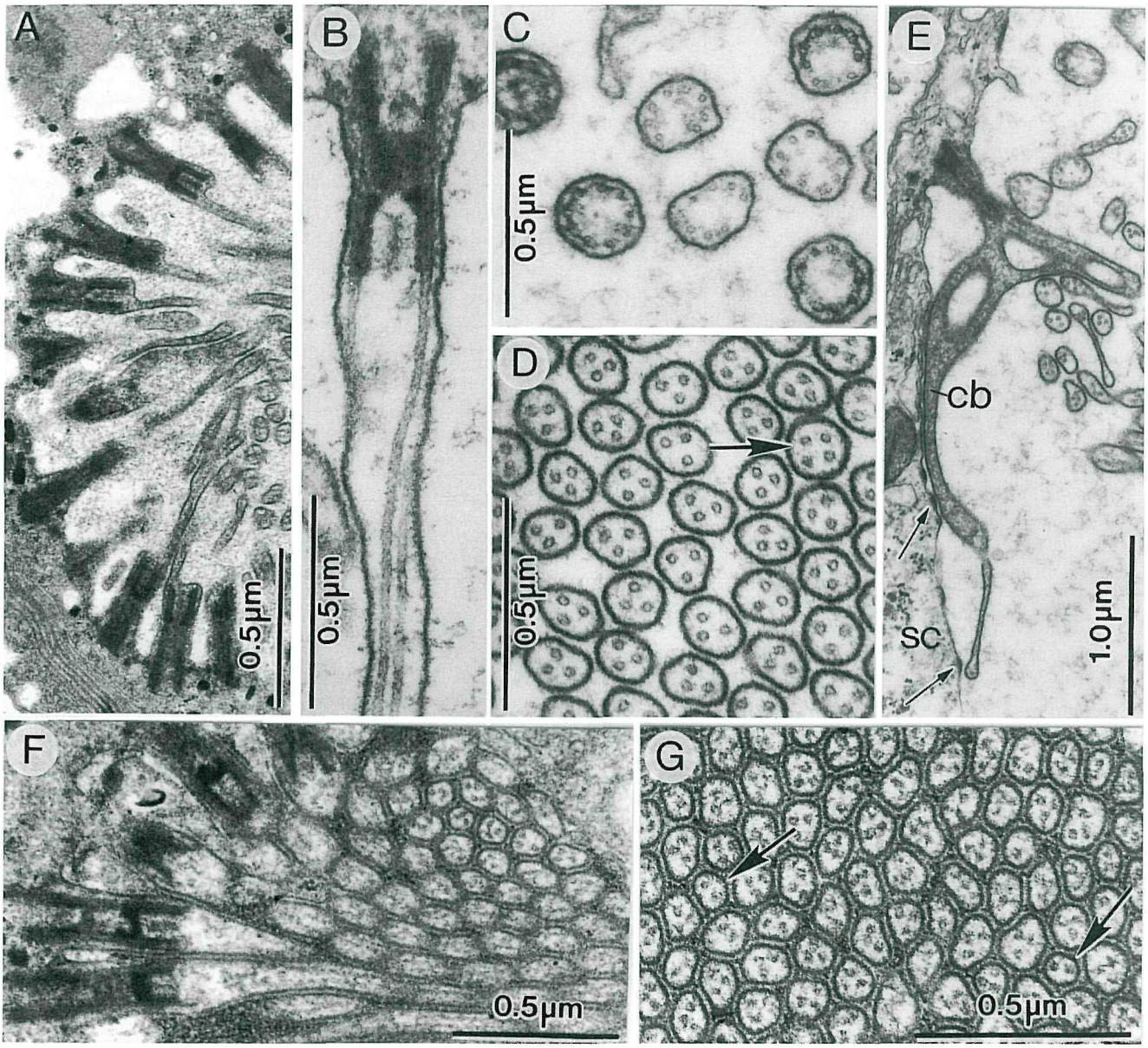

Figure 8. Ciliary sense organ (type 2). Structure of sensory cilia.
A-E: *Protodriloides chaetifer.*
A: Ciliary bases and arrangement of cilia.
B: Longitudinal section of sensory cilium.
C: Cross sections through ciliary bases at different levels.
D: Distal cross sections of cilia, *arrow* points to cilium with 4 microtubule singlets.
E: Cilium with branch (*cb*) lying on sensory cell (*sc*) surface; *arrows* point to electron-dense plaques.
F, G: *Protodriloides symbioticus.*
F: Ciliary bases and arrangement of cilia.
G: Distal cross sections of cilia; *arrows* point to cilia with 2 microtubule singlets.

Figure 8. Organe sensoriel cilié (type 2). Structure des cils sensoriels.
A-E : *Protodriloides chaetifer.*
A : Base et disposition des cils.
B : Section longitudinale d'un cil sensoriel.
C : Coupe transversale des bases ciliaires à différents niveaux.
D : Coupe transversale distale des cils, la *flèche* indique un cil avec 4 microtubules isolés.
E : Cil avec une ramification (*cb*) reposant sur la cellule sensorielle. Les *flèches* indiquent les zones denses aux électrons à la surface de la cellule.
F, G : *Protodriloides symbioticus.*
F : Base et disposition des cils.
G : Coupe transversale distale des cils, chaque *flèche* indique un cil avec 2 microtubules isolés.

"organes sensoriels" (Jouin, 1970). In fact, the ciliary sense organs of *Protodriloides* and the "statocysts" of *Protodrilus* show several differences: in *Protodrilus* there is only one pair of sense organs, always lying in front of the brain (Pierantoni, 1908; Jägersten, 1952). Each "statocyst" always consists of four cells; three different sensory cells and one supporting cell forming the cavity which encloses the receptor structures (Purschke, 1990a, b). The latter are densely packed ciliary branches arising from only a few cilia. These ciliary branches are often flattened and arranged in paracrystalline patterns or they are microvillus-like. In addition, the sensory cells bear true microvilli as well. Only a few ciliary branches of the two smaller sensory cells contain one to several microtubules and hence appear similar to the cilia in the sense organ of *Protodriloides*. Owing to these differences the sense organs of the two genera can hardly be considered homologous.

Among the other photoreceptor-like sense organs present in the Protodrilida - the type-1 ocelli of *Saccocirrus* and the type-3 ocelli of *Parenterodrilus taenioides* are of interest as well, owing to their ciliary sensory cells (Purschke, 1992; Purschke & Jouin-Toulmond, 1993). In both taxa the sense organs are composed of a sensory and a supporting cell. But the sensory cells always bear two cilia that give rise to numerous microvillus-like branches. In *Saccocirrus* these branches are parallel to each other only in the periphery of the cavity, in *P. taenioides* they form a single bundle running straight and parallel to the longitudinal axis of the cavity. These presumed ocelli are incorporated among the perikarya of the brain and, therefore, they are more closely associated with the brain than the sensory structures in *Protodriloides*. All other photoreceptor-like organs found in the Protodrilida show even larger differences. This likewise applies for the pigmented ocelli present in *Saccocirrus* and a few *Protodrilus* species (Eakin *et al.*, 1977; Purschke, 1992). Sense organs with multiciliated sensory cells bearing a regularly coiled bundle of unbranched cilia with a highly modified axoneme are unknown in *Parenterodrilus*, *Protodrilus* and *Saccocirrus*. This clearly corroborates the position of *Protodriloides* as the sister group of all of these three genera in the presumed phylogenetic system (Purschke & Jouin, 1988).

In other polychaete species unpigmented ciliary sensory cells have been found in *Lepidonotus helotypus, Eteone longa, Phyllodoce (Anaitides) mucosa, Eulalia viridis, Microphthalmus* species, *Nereis pelagica, Psammodrilus aedificator* and trochophores of *Harmothoe imbricata* (Dhainaut-Courtois, 1965; Holborow & Laverack, 1972; Whittle & Golding, 1974; Gotow, 1976; Kristensen & Nørrevang, 1982; Pietsch & Westheide, 1985; Rhode, 1991). The sensory cells in all of these organs differ from those of *Protodriloides* in that they only possess a few but always branched cilia. If the microvillus-like branches contain microtubules there is only one singlet or doublet. In none of these sense organs are the cilia as regularly arranged as in *Protodriloides*. Therefore this specific type of sense organ is here described in polychaetes for the first time and most likely represents an autapomorphy of the genus *Protodriloides*.

Outside the Annelida similar sensory cells have been described in a gastrotrich (Teuchert, 1976) and a few platyhelminthes (see Sopott-Ehlers, 1991, for references). There is also a certain similarity to sensory cells in the organ of Bellonci of the Crustacea: the outer dendritic segments represent ciliary branches of only two cilia per sensory cell (Chaigneau *et al.*, 1991; Juberthie & Pitzalis, 1993, 1994). Only in copepods are the dendrites multiciliated (Juberthie & Pitzalis, 1994; Hosfeld, 1995/96). The cilia taper and are transformed into microvillus-like structures containing only one microtubule. In the so-called third unit of the copepod *Canuella perplexa* (see Hosfeld, 1995/96) the cilia form a compact bundle comparable to that in the *Protodriloides* species. Under the assumption that these structures are homologous in the different Crustacea, this indicates that receptors bearing a few branched cilia and multiciliary receptors with unbranched cilia may develop from each other. For instance, such a development may occur in connection with a change of function (see Juberthie & Pitzalis, 1994). Even if this were conclusively established for polychaetes and the Protodrilida, it would not affect the interpretation of the ciliary sense organs of *Protodriloides* as an autapomorphy of this taxon because of their specific structure.

The ciliary sense organs of *P. chaetifer* and *P. symbioticus* resemble photoreceptors in that their sensory cells possess a great expanse of apical plasmalemma, the most characteristic feature of photoreceptors (Eakin & Hermans, 1988). Like such receptors they are situated beneath the epidermis, lie in close proximity to the brain and are associated with a supporting cell. Because the supporting cell is devoid of shading pigment, a photoreceptive function cannot definitely be inferred. Other functions suggested are chemoreception, including control of neurosecretory activity, of pH and osmoregulation, as well as detection of changes of hydrostatic pressure (Zahid & Golding, 1974; Golding & Whittle, 1975; Rhode 1991). The main feature of the ciliary sense organs of *Protodriloides* that might distinguish them from presumed ocelli is that the cilia are enclosed in an extracellular cavity which very likely is not sealed off from the interstitial space with septate junctions. This might allow access of all substances to the putative receptive processes without control by either the supporting or the sensory cell. Thus, another function besides photoreception cannot be excluded. The gland cell and the vacuole-containing cell lying close to the ciliary organs have no apparent functional

connection to the sensory units; such epidermal cells are present in other regions of the body as well.

In conclusion, the present study of a new type of probable unpigmented photoreceptor organ and the first record of the occurrence of basal ciliated cells in polychaetes confirms the diversity of these sense organs in annelids. No sense organ comparable in structure and position is known in the other Protodrilida; this new feature clearly supports the presumed position of *Protodriloides* as the sister group of the remaining three genera *Protodrilus, Parenterodrilus* and *Saccocirrus* in the phylogenetic system. The great structural diversity of unpigmented photoreceptor-like sense organs has led to the assumption that such organs evolved convergently several times, so that they are thought to have phylogenetic implications for taxa at a low level only (e.g., Pietsch & Westheide, 1985). As a second hypothesis, the widespread occurrence of photoreceptor-like organs in annelids with either rhabdomeric or ciliary sensory cells (see Purschke, 1992; Verger-Bocquet, 1992, for references) may also suggest that at least one type of these organs belongs to the ground pattern in annelids. However, at present it appears to be impossible to identify the basic type of this organ and to assess if the ciliary sense organ of *Protodriloides* represents an isolated branch in the evolution of these organs.

Acknowledgements

We are indebted to Professor W. Westheide, Osnabrück, for continuous support and helpful discussion. We express our thanks to Dr. Claude Jouin-Toulmond, Roscoff, for using her unpublished micrographs, for valuable suggestions and comments on the manuscript and for translating the abstract and figure legends into French. We thank Anna Stein, Osnabrück, who skilfully executed most of the drawings and figures and Andrea Noël, Osnabrück, who carefully typed the manuscript.

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