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Ultrastructure studies of preadult *Proteocephalus longicollis* (Cestoda, Proteocephalidea): transmission electron microscopy of scolex sensory receptors

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Abstract The ultrastructure of five types of presumed sensory receptors in the scolex of preadults of *Proteocephalus longicollis* is described. Two types of nonciliate sensory receptors are situated on the inner surface of the lateral sucker. They differ from each other in the shape, presence, or absence of a large rootlet, electron-dense collars, desmosomes, microtubules, and/or vesicles. In addition, three types of ciliate sensory receptors are found along the edges of the lateral suckers. They can be differentiated by the length of the cilium, by the number of electron-dense collars (one or two), and by types of vesicles. Four types of vesicles were found inside the ciliate sensory receptors. One type of ciliate sensory receptor occurring in preadults differs markedly from any of the sensory receptors previously described in adult *P. longicollis*.

Introduction

Structurally the scolex of tapeworms represents a relatively well-defined organ, possessing in some taxa species-specific characters (Scholz et al. 1998). The scolex of *Proteocephalus* is formed early during the development of procercoids within the crustacean intermediate host and is morphologically similar to that of adult worms (Priemer 1980, 1987; Rusinek 1989; Scholz 1991, 1993; Scholz et al. 1997). Sysoev et al. (1994) have stated that the morphology of *Proteocephalus* larvae at the scanning electron microscopy (SEM) level appears to be basically similar to that of adults of the same species and is characterized rather by the absence of certain features than by the presence of any particular larval characters. However, a transmission electron microscopy (TEM) study revealing either similarities or differences in the fine structure of the scolex in various developmental stages of *Proteocephalus* is lacking. The sensory receptors in the scolex of adult *P. exiguus* (= *P. longicollis*, see Scholz and Hanzelová 1998), have been described by Bruňanská et al. (1998). The results of an immunocytochemistry study in *P. exiguus* show that the nervous system in the early larval stage is more or less at the same level of complexity as in the adult worm (Gustafsson et al. 1995). The present study was therefore done to provide information on the ultrastructure of sensory receptors occurring in the scolex of preadult *P. longicollis* and to compare it with that in adult worms.

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Materials and methods

Preadult worms of *Proteocephalus longicollis* were recovered from the intestines of whitefish (*Coregonus lavaretus*) from the Åland Islands, southwestern Finland. The worms measured < 1 cm and were void of proglottides. They were fixed overnight at 4 °C in 2.2% glutaraldehyde in 0.1 *M*-phosphate buffer (pH 7.4). The specimens were washed in the same buffer several times. They were postfixed in 1% phosphate-buffered OsO_4 at 4 °C for 2 h. The material was dehydrated through a graded series of acetone, saturated with Epon, and embedded in Epon. Ultrathin sections were cut on a Reichert ultramicrotome and were then stained with uranyl acetate and lead citrate in an LKB 2168 Ultrastainer. The sections were examined with a JEOL 100SX or a Philips 300 electron microscope operating at 60 kV.

Results

Sensory receptors

Five types of sensory receptor were observed in the tegument of the scolex of preadult *Proteocephalus longicollis*, including two nonciliate and three ciliate types.

Nonciliate sensory receptors

Nonciliate sensory receptors occurred on the surface facing the lumen of the lateral sucker (Figs. 1–3).

Type I sensory receptor

The type I sensory receptor was seen in the ventral tegument of the sucker (Fig. 1). In a series of longitudinal sections through the scolex of the preadult the single sensory receptor appeared as a club-like projection. It contained one electron-dense collar, a septate desmosome, some electron-dense vesicles (diameter about 50 nm), and microtubules.

Type II sensory receptor

This type of sensory receptor is situated deeply in the tegument (Fig. 2). It lacks a free cilium but has a basal body from which a large rootlet diverges (Fig. 3). An oblique section through this type of sensory ending confirms that the basal body is localized in the apical part. There are some desmosome-like dense patches in the apical area of the sensory ending. However, they do not appear to form complete collars. The rootlet shows cross-striation. Below the rootlet, some small vesicles (diameter about 20 nm) filled with a homogeneous matrix are present. The basal part of the sensory ending is occupied by numerous mitochondria measuring approximately 290 × 110 nm.

Type III sensory receptor

The sensory receptor known as type III in adult *P. longicollis* is absent in preadults.

Ciliate sensory receptors

Type IV sensory receptors

A sensory receptor with a cilium of intermediate length (approx. 1.5 μm; Figs. 4–6) is situated close to the edge of the lateral suckers. An oval bulb, which is regularly present, is held in place by a distal circular septate desmosome. One or two electron-dense collars are present in the apical part of the sensory bulb. In cases in which two electron-dense rings are observed, the apical ring is larger than the distal one. A cilium is situated on a basal body that lacks a rootlet (Fig. 4). The cilium consists of microtubules (Figs. 4–6) and has an electron-dense cytoplasm. It is surrounded by a thick sheath. Some mitochondria measuring 265 × 130 nm are present in the bulb, the rest of which is filled with numerous vesicles of different morphology. Four types of vesicles were distinguished: (1) large electron-lucent vesicles (llv, size 65–100 nm; Figs. 4, 6), (2) large vesicles filled with a homogeneous matrix (lv, size 60–110 nm; Figs. 4, 6), (3) dense-core vesicles (dcv, size 50–120 nm) with a small or point-like center (size 15–30 nm; Fig. 5), and (4) electron-dense drop-like vesicles (dv; Fig. 4). In addition, some small vesicles of intermediate electron density (sv) occasionally occurred in the gap between the sensory bulb and the tegument (Fig. 6).

Fig. 1 Longitudinal section through a type I sensory receptor, showing many microtubules (*arrowheads*) (*c* Electron-dense collar, *d* septate desmosome, *s* small electron-dense vesicle). *Bar* 0.25 μm
Fig. 2 Localization of a type II nonciliate sensory receptor (*SO*) in the tegument (*t*) of the lateral sucker (*MC* Muscle cells). *Bar* 1 μm
Fig. 3 A type II nonciliate sensory receptor is characterized by a large rootlet (*r*) and by numerous mitochondria (*m*) situated in the basal part of the sensillum (*b* Basal body, *s* small vesicle). *Bar* 0.25 μm
Fig. 4 A type IV ciliate sensory receptor with a cilium (*cl*) of intermediate length (approx. 1.5 μm). It consists of microtubules (*mt*). The cilium is settled on a basal body (*b*) (*d* Desmosome, *c* electron-dense collar, *m* mitochondrion, *llv* large electron-lucent vesicle, *lv* large vesicle filled with homogeneous matrix, *dv* drop-like electron-dense vesicle, *GC* gland cell). *Bar* 0.3 μm

Type V sensory receptors

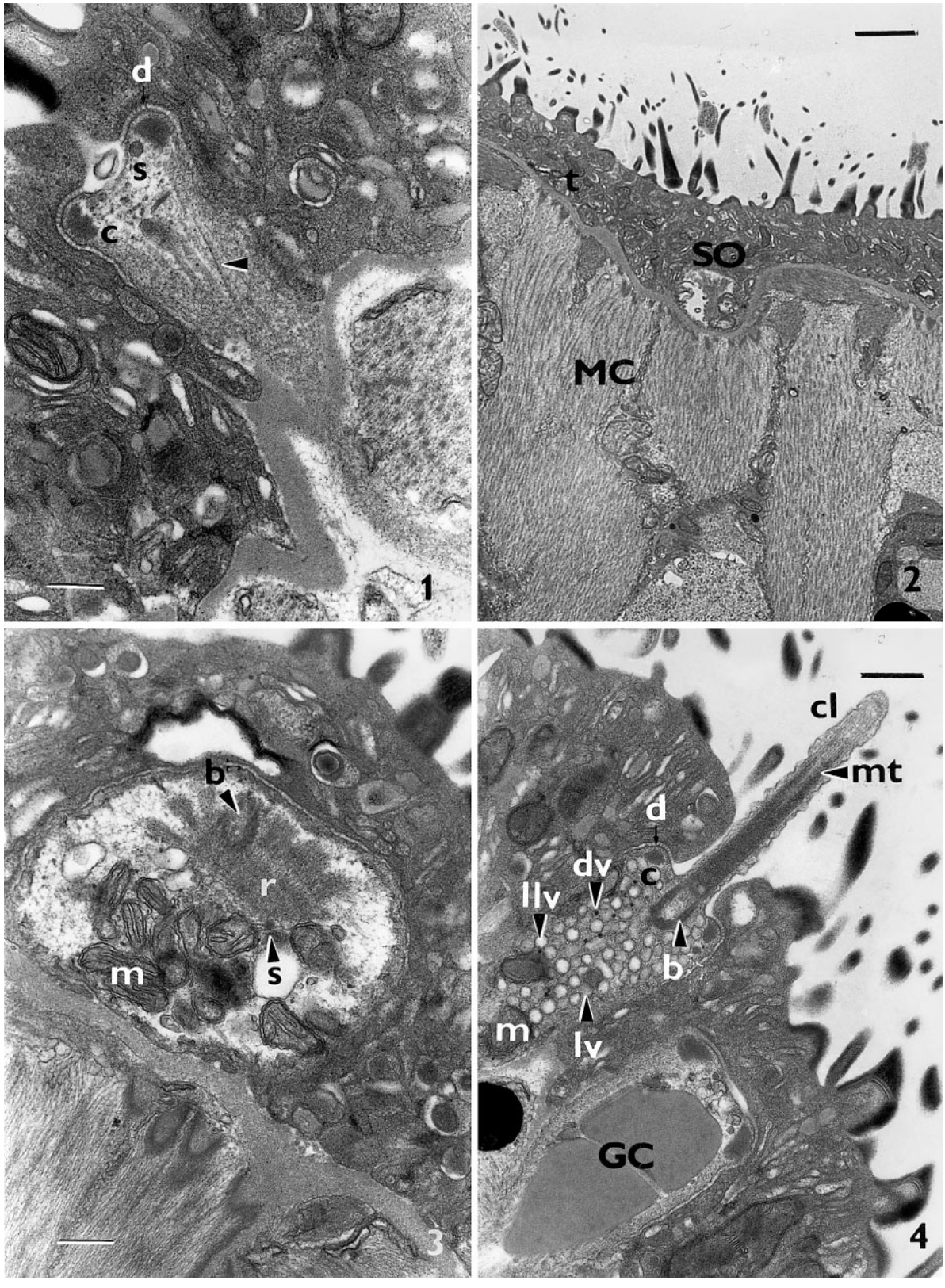
A sensory receptor with a short cilium (approx. 0.5 μm; Fig. 7) occurs on the lateral rim of the lateral suckers. The cilium contains microtubules. In addition, the bulb contains three types of vesicles: large electron-lucent vesicles (llv), large vesicles filled with homogeneous matrix (lv), both types measuring 75–100 nm, and electron-dense drop-like vesicles (dv). Two to five large mitochondria (340 × 160 nm) and some microtubules are present in the basal part of the sensory bulb.

Type VI sensory receptor

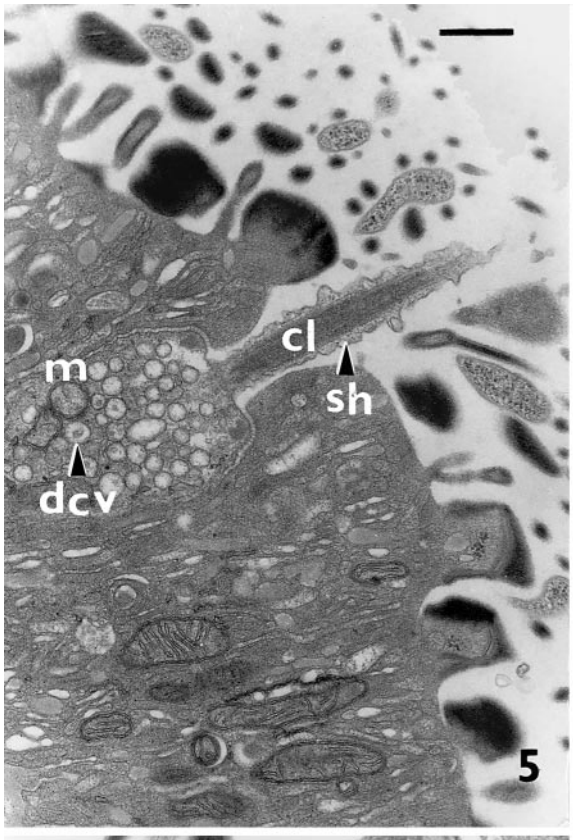
The sensory receptor known as type VI in adult *P. longicollis* is absent in preadults.

Type VII sensory receptors

A ciliated sensory receptor containing a dense formation under the basal body (Fig. 8) is localized near the edge of the lateral suckers in the vicinity of the sensory receptor with a cilium of intermediate length (type IV). The general ultrastructure largely resembles that of the type IV sensory receptor; it consists of a bulb supplied with desmosomes, two electron-dense collars, and vesicles. Three types of vesicle are present in this type of sensory receptor: large vesicles filled with a homogeneous matrix (lv), dense-core vesicles (dcv), and electron-dense drop-like vesicles (dv). One or two mitochondria (200 × 120 nm) occur in the basal part of the sensory bulb. A cilium (1.4 μm long) is associated with a basal body. In contrast to the type IV sensory receptor, the basal body is



connected to an irregular, dense formation, showing different shapes according to the level of the section. This dense formation has two parallel membranes at the periphery, suggesting that it could be a giant mitochondrion. Small electron-dense drop-like vesicles (dv) occur on the surface of this dense formation. A schematic diagram of the type VII sensory receptor, which has not yet been described in cestodes, is shown in Fig. 9.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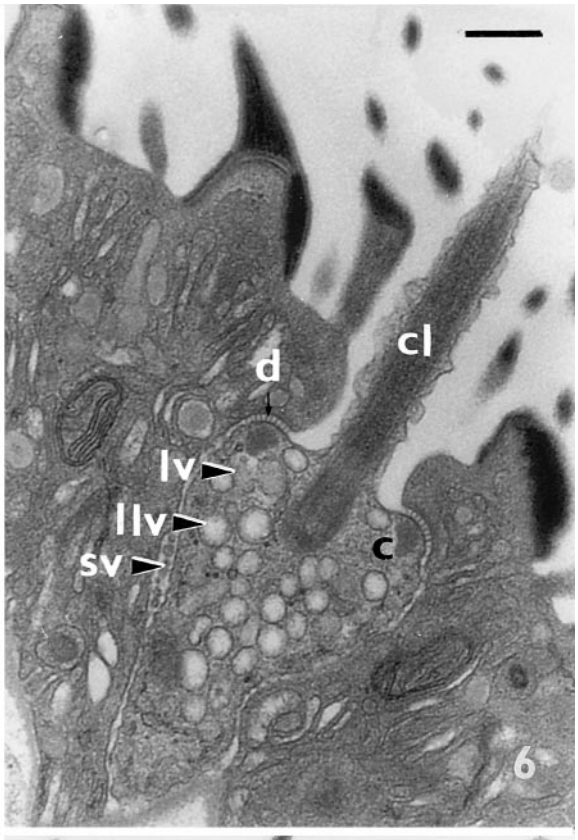
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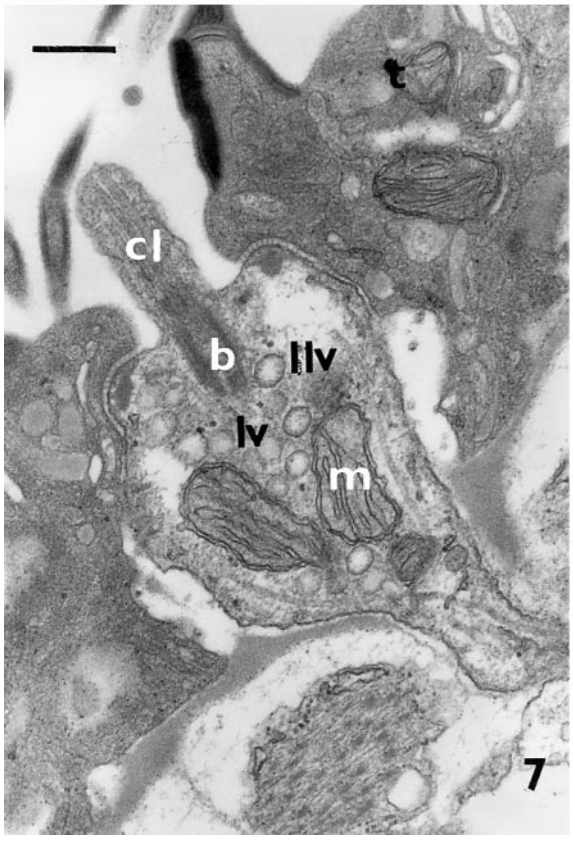
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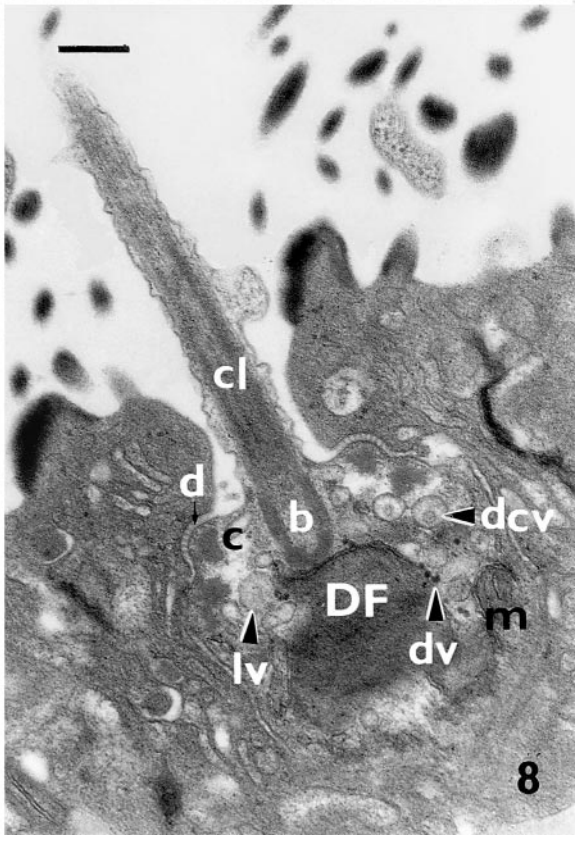
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Fig. 5 Electron micrograph showing dense-core vesicles (*dcv*), which are present in a type IV sensory receptor. They are characterized by a small, round, dense center situated inside an electron-lucent vesicle (*m* Mitochondrion, *cl* cilium, *sh* sheath of the cilium). *Bar* 0.3 μm

Fig. 6 In the gap between the tegument and a type IV sensory receptor, small membrane-bound vesicles (*sv*) occur (*cl* Cilium, *d* desmosome, *c* electron-dense collar, *llv* large electron-lucent vesicle, *lv* large vesicle filled with homogeneous matrix). *Bar* 0.2 μm

Fig. 7 Type V ciliate sensory receptor with a short cilium (*cl*) (*b* Basal body, *llv* electron-lucent vesicle, *lv* large vesicle filled with homogeneous matrix, *m* mitochondrion, *t* tegument). *Bar* 0.2 μm

Fig. 8 Type VII ciliate sensory receptor containing a dense formation (*DF*) under the basal body (*b*) (*d* Desmosome, *c* electron-dense collar, *cl* cilium, *dcv* dense-core vesicle, *lv* large vesicle filled with homogeneous matrix, *dv* drop-like electron-dense vesicle, *m* mitochondrion). *Bar* 0.2 μm

Discussion

Bruňanská et al. (1998) have described six morphologically distinct sensory receptors with an apparent receptory function in adults of *Proteocephalus exiguus* (= *P. longicollis*). These putative sensory receptors include three nonciliate types, terminating within the tegument, and three ciliated types that reach the surface of the tegument of the scolex. Our present results indicate that in the scolex tegument of preadults of *P. longicollis*, five types of sensory receptor (two nonciliated and three ciliated types) occur. The localization of sensory receptors in adults and preadults are principally the same (see schematic diagram, Bruňanská et al. 1998).

Table 1 shows a comparison of sensory receptors observed in adult and preadult *P. longicollis*. The two types of nonciliate sensory receptor localized in the preadult closely resemble (with the exception of vesicles) types I and II of the presumed sensory receptors previously described in adult worms. The mitochondria situated below the large rootlet of the type II sensory receptor are smaller in preadult worms than in adult worms. The type III presumed sensory receptor, localized in the neck region of the adult worm, was not seen in preadults, as the neck region is not distinctly pronounced in the preadult stages of development (Priemer 1980).

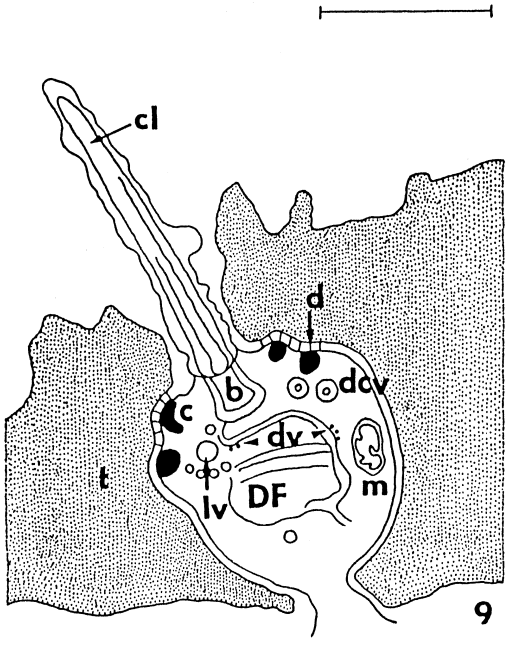

Fig. 9 Schematic diagram of a type VII ciliate sensory receptor of preadult *Proteocephalus longicollis* (*c* Electron-dense collar, *d* septate desmosome, *cl* cilium, *lv* large vesicle filled with homogeneous matrix, *dcv* dense-core vesicle, *dv* electron-dense drop-like vesicle, *DF* dense formation, *b* basal body, *m* mitochondria, *t* tegument). *Bar* 1 μm

Ciliate sensory receptors include one cilium, a basal body, vesicles, and mitochondria. The ciliate sensory receptors known as type VI in adults of *Proteocephalus* are not present in the preadult stage. This type of sensory receptor occurs occasionally in the tegument of scolices of adult cestodes, as has been shown in Table 1 of our previous paper (Bruňanská et al. 1998). The ciliate sensory receptor with a dense formation connected to a basal body, described in this study of preadults as the type VII sensory receptor, is not known to occur in adult *P. longicollis*. It resembles the type III sensory ending described from *Diplostomum pseudospathaceum* cercariae (Digenea; Czubaj and Niewiadomska 1996), which, however, have only a short cilium. It cannot be excluded that these sensory receptors gradually trans-

Table 1 A comparison of sensory receptors in preadult and adult *Proteocephalus longicollis* (*s* Small vesicles filled with homogeneous matrix [size 20–50 nm], *sl* small electron-lucent vesicles [size 50 nm], *llv* large electron-lucent vesicles [size 65–100 nm], *lv* large vesicles filled with homogeneous matrix [size 50–110 nm], *dcv* dense-core vesicles [size 50–120 nm] with a point-like center [size 15–30 nm], *dv* drop-like electron-dense vesicles)

Types of sensory receptor	Preadult		Adult	
	Types of vesicle	Mitochondria (size in nm)	Types of vesicle	Mitochondria (size in nm)
I.	s	–	sl	–
II.	s	290 × 110	sl	320 × 210
III.	Absent		sl	200 × 160
IV.	llv, lv, dcv, dv	265 × 130	llv, lv	320 × 150
V.	llv, lv, dv	340 × 160	llv, lv	525 × 80
VI.	Absent		llv	380 × 150
VII.	lv, dcv, dv	200 × 120	Absent	

form into another type during ontogenesis. They may also play a role in development and might have a dual function as sense receptors and developmental agents (see discussion in Rohde and Garlick 1985).

The classification of types of sensory receptor on the basis of the presence or absence of a cilium does not involve ultrastructural differences in cytoplasmic vesicles. The presence of vesicles within the bulbs of sensory receptors is typical of cestodes, including preadults of *P. longicollis*. The type I sensory receptor in preadult worms contains small electron-dense vesicles, in contrast to the electron-lucent vesicles previously described in this type of sensory receptor of adult *P. exiguus* (= *P. longicollis*; Bruňanská et al. 1998). The morphology of the small vesicles present in the basal part of the rootlet of the type II nonciliate sensory receptor differs in preadult versus adult stages of *Proteocephalus* development; in preadults they are smaller (about 20 nm) and are filled with homogeneous matrix, whereas in adults, small electron-lucent vesicles (diameter about 50 nm) have been described inside this type of sensory receptor (Bruňanská et al. 1998). The small vesicles described in nonciliate sensory endings of *P. longicollis* are regarded as cholinergic (Reuter and Gustafsson 1995).

Four types of vesicle were described in the ciliate sensory receptors of *Proteocephalus* preadults: large lucent vesicles (llv), large vesicles filled with homogeneous matrix (lv), dense-core vesicles (dcv), and electron-dense drop-like vesicles (dv). Llv, dcv, and ldv (large, dense vesicles) occur in the nerve fibers of plerocercoid larvae of *Diphyllobothrium dendriticum* (Gustafsson 1984). Reuter and Gustafsson (1995) reviewed the role of various vesicle types. According to their study, ldv have been regarded as peptidergic, whereas dcv have been considered as aminergic. The large lucent vesicles were observed in nerve fibers regarded to be sensory. In a comparison of the vesicle types occurring in the same types of sensory receptor of preadults and adults of *Proteocephalus* it was found that sensory endings in preadults contain dcv, which were not present in the sensory endings of adults. The most common type of vesicles occurring in the sensory receptors of *P. longicollis* is the large lucent vesicle (llv), which usually occurs in the sensory neurons of flatworms (Reuter and Gustafsson 1995). The large vesicles (lv) filled with homogeneous matrix are observed together with llv, and the ultrastructural picture may suggest a relationship between the two types of vesicles. A transformation from lv to llv is plausible. Reuter and Gustafsson (1995) suggest that the vesicle ultrastructure depends on the developmental stage. The electron-dense drop-like vesicles present in all types of ciliate sensory receptors in preadults (but not in adults) of *P. longicollis* resemble the neurosecretory vesicles previously reported in the ciliated receptors of *Caryophyllaeus laticeps* (Richards and Arme 1982) and in the sensory processes of trematodes (Chapman and Wilson 1970; Bibby and Rees 1971).

Finally, it should be stressed that since there is little experimental evidence, if any, that any of the supposed sensory receptors mediate sensory modalities, any conclusion based solely on their ultrastructural characters can at best only be speculative (Halton et al. 1997).

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