

Transmission electron microscopy of intra- tegumental sensory receptors in the forebody of *Crepidostomum metoecus* (Digenea: Allocreadiidae)

Zdeňka Žďárská and Jana Nebesářová

Institute of Parasitology, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic

Key words: Digenea, *Crepidostomum metoecus*, forebody tegument sensory receptors, transmission electron microscopy

Abstract. In the adult fish trematode *Crepidostomum metoecus* (Braun, 1900), four types of sensory receptors were observed inside the forebody tegument and one type beneath the tegument basal lamina. Two types of sensory receptors extend through the thickness of tegument and have a free cilium inside a pit (types I and II). Two types (III and IV) are nonciliate and entirely intra- tegumental in location. Type IV receptor with large horizontal and thin vertical rootlets was described earlier in aspidogastreans only. Below the basal lamina, nerve endings in close association with muscle fibres, comparable with those in the Aspidogastrea, were detected.

The present paper, describing the ultrastructure of sensory receptors of adult *Crepidostomum metoecus* (Braun, 1900), is part of the studies by the present authors on the ultrastructure of this trematode (Žďárská and Nebesářová 2002a, b, in press). As far as can be determined, no data on the ultrastructure of sensory receptors of this digenean have been published. This paper includes only descriptions of receptors localised in the basal part of the tegument. Sensory papillae of this species are dealt with in another paper (Žďárská and Nebesářová, in press).

Transmission electron microscope (TEM) studies of sensory receptors of adult digeneans of the genus *Crepidostomum* Braun, 1900 are not available. Only scanning electron microscope (SEM) studies of tegumental papillae distribution have been published of *Crepidostomum opeongoensis* Caira, 1985 (Caira 1989, Choudhury and Nelson 2000), *C. metoecus* and *C. farionis* (Müller, 1784) (Caira 1989, Moravec 2002), and *C. cooperi* Hopkins, 1931, *C. cornutum* (Osborn, 1903), *C. brevititellum* Hopkins, 1934, *C. ictaluri* (Suber, 1928) and *C. illinoiense* Faust, 1918 (Caira 1989). Sensory receptors were studied in detail by TEM only in the cercaria of *Crepidostomum* sp. (Bogea and Caira 2001).

In the genus *Crepidostomum*, in view of the special structure of the tegumental surface (small regularly arranged tubercles – Žďárská and Nebesářová 2002b) and sensory receptors (tegumental invagination into the bulb), it is necessary to compare the intra- tegumental sensory receptors of this genus with aspidogastreans (Halton and Lyness 1971, Ip and Desser 1984, Rohde 1989, 1990, Rohde and Watson 1989, 1990a, b, 1992) and paramphistome digeneans (Dunn et al. 1987a, b).

MATERIALS AND METHODS

Specimens of *Crepidostomum metoecus* were removed from the intestine of *Salmo trutta fario* L., collected in the Czech Republic. They were washed in saline, fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 2 h at 4°C, postfixed for 2 h at 4°C in 1% osmium tetroxide, dehydrated through an ethanol series and embedded in Durcupan via acetone. Series of ultrathin sections were cut using a Leica UCT ultramicrotome, double-stained with uranyl acetate and lead citrate and viewed in a JEOL 1010 transmission electron microscope operated at 80 kV. Semithin sections were stained in toluidine blue.

RESULTS

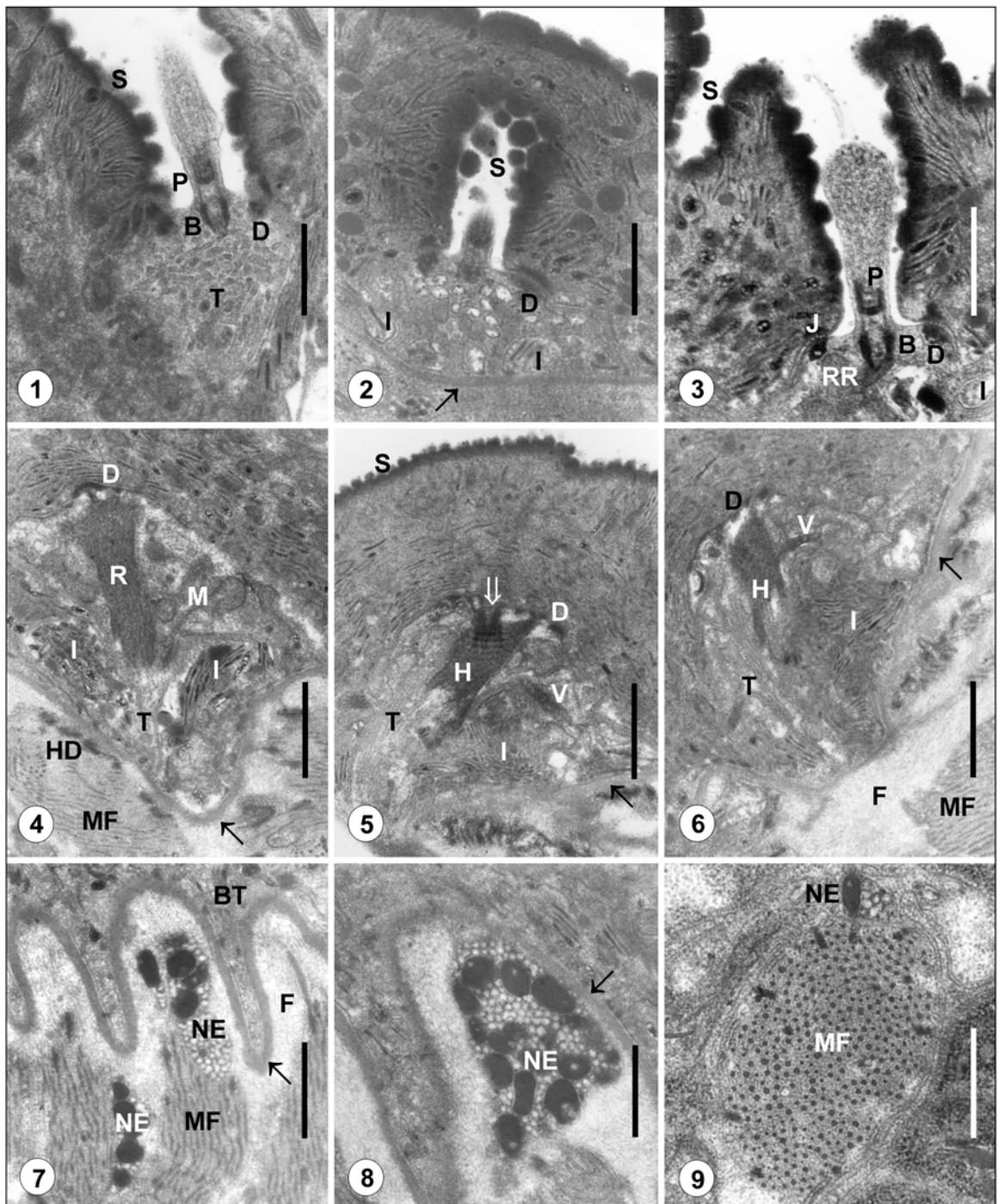
Four types of sensory receptors occur inside the forebody tegument of *C. metoecus*, namely, two types of uniciliate (types I, II) and two types of nonciliate (types III and IV) receptors. Below the tegument plasma membrane and basal lamina, structures which may be nerve endings in close association with muscle fibres were detected.

UNICILIATE SENSORY RECEPTORS

Type I receptor

Figs. 1, 10A

The bulb of the receptor, localised in the basal part of the tegument, contains neurotubules concentrated in the apical part, together with neurotubules and some mitochondria in the basal part. The neurilemma of the bulb is fixed to the tegumental plasma membrane by a septate junction at the level of a dense collar. The bulb is not lobed. The basal body of the short cilium is situated at the level of the dense collar. The basal plate is apical to the basal body. Doublets of the ciliary



Figs. 1–9. Sensory receptors of *Crepidostomum metoecus*, transmission electron micrographs. **Fig. 1.** Longitudinal section through uniciliate type I receptor with a cilium with basal plate (P) and basal body (B). The nerve bulb contains neurotubules (T) and one electron-dense collar (D); S – tegument surface tubercles. **Fig. 2.** Oblique section through type II receptor with electron-lucent vesicles, mitochondria, one electron-dense collar (D) and invaginations of tegument (I). Arrow – basal lamina; S – tegument surface tubercles. **Fig. 3.** Detail of the cilium of type II sensory receptor. P – basal plate; B – basal body; RR – rudimentary rootlet; J – septate junction; D – dense collar; I – invagination of tegument; S – tegument surface tubercles. **Fig. 4.** Nonciliate sensory receptor type III with large nonstriated rootlet (R), two dense collars (D), neurotubules (T) and invaginations of tegument (I). Arrow – basal lamina; HD – hemidesmosomes; M – mitochondria; MF – muscle fibres. **Fig. 5.** Nonciliate

microtubules are regularly arranged around two central microtubules. The tegument with surface tubercles is infolded to form a pit around the cilium.

Type II receptor

Figs. 2, 3, 10B

The bulb of this receptor is lobed. The plasma membrane of the bulb is tightly connected with the tegumental plasma membrane by a septate junction at the level of a dense collar. The bulb possesses many electron-lucent vesicles with small electron-dense inclusions and mitochondria. The cilium is short, broader at the apical part than at the level of the basal plate (Fig. 3). A basal body and a rudimentary rootlet are present. The microtubules of the cilium are irregularly arranged. The cilium is located within a pit-like depression and does not extend to the surface of the tegument.

NONCILIATE SENSORY RECEPTORS (THESE TYPES ARE ENTIRELY INTRA-TEGMENTAL IN LOCALISATION)

Type III receptor

Figs. 4, 10C

The bulb of this receptor is lobed with deep tegumental invaginations. This receptor has been detected on the ventrolateral oral lobe only. The bulb contains a large nonstriated rootlet with branched forepart, neurotubules, mitochondria and two narrow dense collars in front of the rootlet. The dendrite and the basal part of the bulb are surrounded by the basal lamina, fibrose layer and hemidesmosomes fixing the muscle fibres to the fibrose layer and basal lamina.

Type IV receptor

Figs. 5, 6, 10D

The dendrite of this receptor, containing a bundle of neurotubules, penetrates the basal lamina vertically and extends horizontally for a short distance below the basal tegument plasma membrane. This dendrite terminates in a bulb with deep tegumental invaginations. The bulb contains a basal body and a large flat horizontal (disc-shaped) and narrow vertical rootlet, neurotubules and mitochondria. The horizontal rootlet is partly striated and partly granular, whereas the vertical rootlet is striated. In the apical part of the bulb there are four dense collars. This receptor is located on the edge line of the ventrolateral oral lobe near a gland cell duct.

NERVE ENDINGS (? NEUROMUSCULAR JUNCTIONS)

Figs. 7–9, 10E

In the subtegumental part of the forebody wall, i.e. below the basal lamina, there are structures interpreted as nerve endings (? neuromuscular junctions) distributed

in the fibrous interstitial tissue and among the muscle fibres. These dendrites contain small electron-lucent (50–55 nm) and large electron-dense (200–280 nm) vesicles. The dendrite plasma membrane is in tight connection with the muscle fibre plasma membrane.

DISCUSSION

This paper describes the ultrastructure of the intra- tegumental sensory receptors from the region of the forebody; it does not include observations on receptors localised in the surface papillae (Žďárská and Nebesářová, in press). In adult digeneans, little is known about the ultrastructure of sensory receptors of the basal part of the general body tegument, particularly about the types enclosed entirely by the tegument, i.e. not communicating with the exterior or communicating with it by a narrow pit only. Thus one type of receptor with no access to the exterior was observed in *Fasciola hepatica* by Bennett (1975) and in *Clonorchis sinensis* (Cobbolt, 1875) by Fujino et al. (1979) and four types in *Brachylaimus aequans* (Looss, 1899) by Žďárská et al. (1990) and Žďárská (1993).

With the exception of paramphistomes (Dunn et al. 1987a, b), there have been no previous descriptions in adult digeneans of lobed bulbous parts to ciliate and nonciliate sensory receptors, i.e. bulbs with deep tegument invaginations, as observed in *C. metoecus* (types II, III and IV). However, some of them are known to be present in aspidogastreans (Rohde 1989, 1990, Rohde and Watson 1989, 1990a, b, 1992). In *C. metoecus*, two types of sensory receptors are ciliate, two types nonciliate and one type is represented by nerve endings (? neuromuscular junctions). One of the two ciliate types of sensory receptors (types I and II) could be similar to the receptor with a submerged cilium as observed by scanning electron microscopy by Moravec (2002). The bulb content (concentration of neurotubules in the apical part) of the type I receptor of *C. metoecus* resembles the type V receptor of the adult aspidogastrean, *Lobatostoma manteri* Rohde, 1973 (Rohde and Watson 1989). The type II receptor corresponds with the anterior receptor with a short cilium of larval *L. manteri* (Rohde and Watson 1992). The type III receptor resembles in general (not in detail) the type VII A of adult *L. manteri* (Rohde 1989, Rohde and Watson 1989). The main difference is the nonstriated rootlet in *C. metoecus*. The type IV receptor with a large horizon-

sensory receptor type IV with a large flat horizontal (H) and a narrow vertical (V) rootlet and basal body (double arrow). D – dense collars; T – neurotubules; I – tegument invaginations; arrow – basal lamina; S – tegument surface tubercles. **Fig. 6.** Serial section to Fig. 5. Horizontal (H) and vertical (V) rootlets; D – dense collars; T – neurotubules; I – tegument invaginations; F – fibrose layer; MF – muscle fibres; arrow – basal lamina. **Fig. 7.** Nerve endings (NE) in fibrose layer (F) tightly connected with circular muscle fibres (MF). BT – basal part of tegument; F – fibrous interstitial tissue; arrow – basal lamina. **Fig. 8.** Nerve ending (NE) tightly connected with basal lamina (arrow). The nerve ending contains small electron-lucent and large electron-dense vesicles. **Fig. 9.** Cross-section of a nerve ending (NE) and muscle fibre (MF). Scale bars = 600 nm.

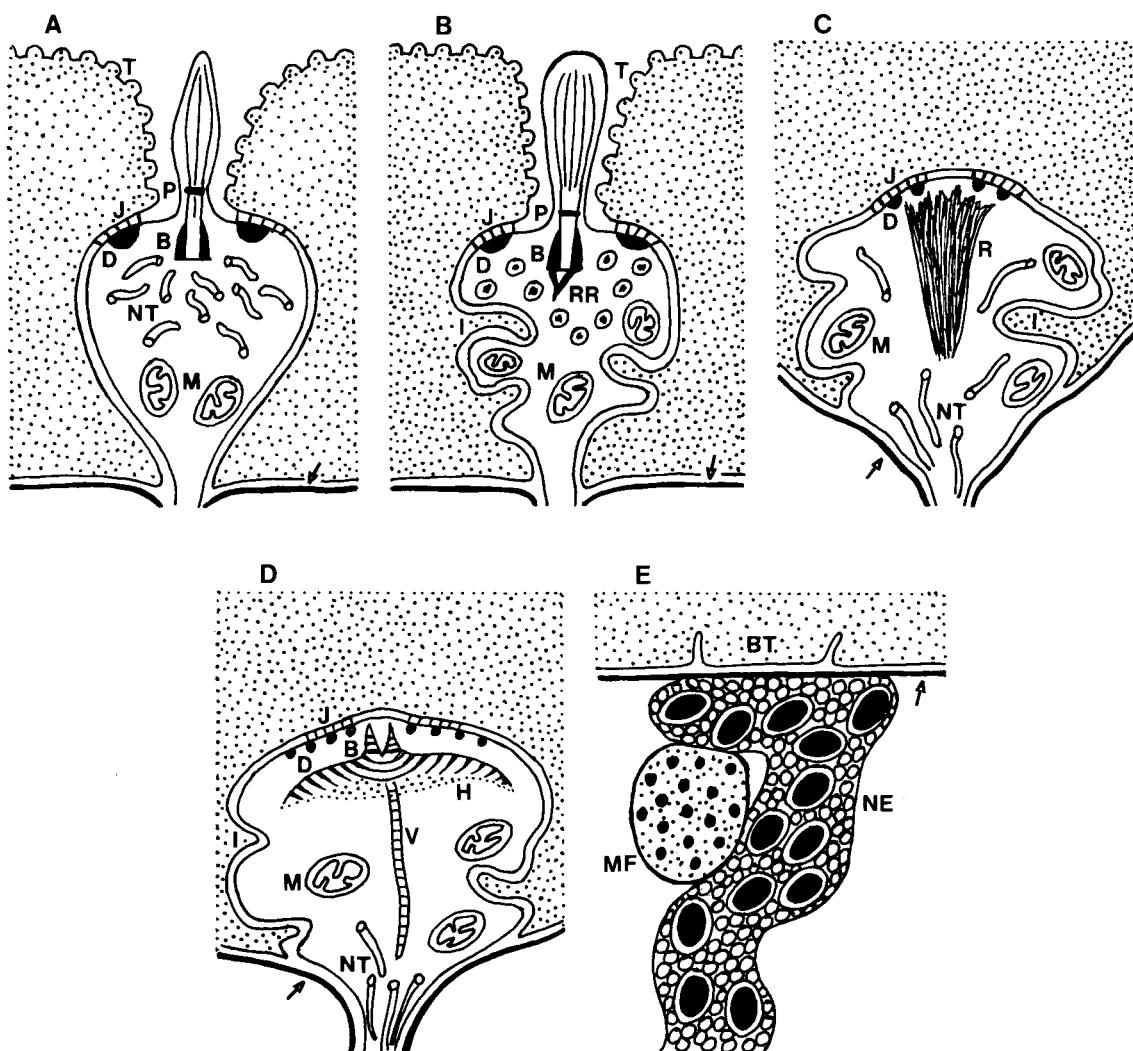


Fig. 10. Diagrams of sensory receptors of *Crepidostomum metoecus*. **A** – uniciliate type I receptor; **B** – uniciliate type II receptor; **C** – nonciliate type III receptor; **D** – nonciliate type IV receptor; **E** – nerve ending tightly connected with basal lamina and muscle fibre. *Explanation of abbreviations:* B – basal body; BT – basal part of tegument; D – dense collar; H – horizontal rootlet; I – invagination of tegument; J – septate junction; NE – nerve ending; NT – neurotubules; M – mitochondria; MF – muscle fibre; P – ciliary plate; R – rootlet; RR – rudimentary rootlet; T – surface tubercles of tegument; V – vertical rootlet; arrow – basal lamina.

tal and thin vertical rootlet corresponds with the type IV B receptor in adult *L. manteri* (Rohde 1989, Rohde and Watson 1989). Nerve endings (? neuromuscular junctions) of *C. metoecus* correspond to structures tentatively identified as free nerve endings in *L. manteri* by Rohde (1989) and Rohde and Watson (1989). The ultrastructure of these nerve endings in *C. metoecus* corresponds, too, to the ultrastructure of the neuromuscular junctions in cestodes (Gustafsson and Wikgren 1981, Biserova et al. 1996). These endings, closely associated with muscle fibres, may have a locomotor function.

In sensory receptors, the lack of connection to the surface indicates that the nonciliate receptors (type III and IV) are possibly mechanoreceptors, serving to respond to compression. Receptor IV, localised near an

opening of a gland cell duct on the oral ventral lobe, probably could serve for regulating the outflow of secretion. The ciliate receptors (type I and II), communicating with the exterior through a pit (inside which a short cilium is located) could be chemoreceptors.

Rohde (1990, 2001) suggests a sister-group relationship of the Aspidogastrea and the Digenea. Our TEM results support this relationship. In *C. metoecus* the type IV receptor with horizontal and vertical rootlet and the nerve endings (? neuromuscular junctions) corresponds partly with the aspidogastreans (Ip and Dresser 1984, Rohde 1989, Rohde and Watson 1989). The tegumental surface part with tubercles of *C. metoecus* (Žďárská and Nebesářová 2002b) is comparable with the tegument structure in aspidogastreans.

From the general point of view (not in details), the types of intra- tegumental sensory receptors of *C. metoecus* are close to the receptors of adult aspidogastreans. **Acknowledgements.** We wish to thank Dr. F. Moravec for collecting the parasites. We also appreciate the technical assistance of Mrs. B. Škoríková, Mr. A. Polák, Mrs. L. Nováková and Mrs. P. Masařová. This study was supported by the Grant Agency of the Czech Republic, project no. 524/00/0267 and by the Institute of Parasitology, Academy of Sciences of the Czech Republic, research project Z6 022 909.

REFERENCES

- BENNETT C.E. 1975: Surface features, sensory structures and movement of the newly excysted juvenile *Fasciola hepatica* L. *J. Parasitol.* 61: 886–991.
- BISEROVA N.M., GUSTAFSSON K.S., REUTER M., TERENINA N.B. 1996: The nervous system of the pike-tapeworm *Triaenophorus nodulosus* (Cestoda: Pseudophyllidea) – ultrastructure and immunocytochemical mapping of aminergic and peptidergic elements. *Invertebr. Biol.* 115: 273–285.
- BOGÉA T., CAIRA J.N. 2001: Ultrastructure and chaetotaxy of sensory receptors in the cercariae of a species of *Crepidostomum* Braun, 1900 (Digenea: Allocreadiidae). *J. Parasitol.* 87: 273–286.
- CAIRA J.N. 1989: A revision of the North American papillose Allocreadiidae with independent cladistic analyses of larval and adult forms. *Bull. Univ. Nebr. State Mus.* 11: 1–58 + 195 figs.
- CHOUDHURY A., NELSON P.A. 2000: Redescription of *Crepidostomum opeongoensis* Caira, 1985 (Trematoda: Allocreadiidae) from fish hosts *Hiodon alosoides* and *Hiodon tergisus* (Osteichthyes: Hiodontidae). *J. Parasitol.* 86: 1305–1312.
- DUNN T.S., HANNA R.E.B., NIZAMI W.A. 1987a: Ultrastructural and histochemical observations on the foregut and gut caeca of *Gigantocotyle explanatum*, *Gastrothylax crumenifer* and *Srivastavaia indica* (Trematoda: Paraphystomidae). *Int. J. Parasitol.* 17: 1141–1152.
- DUNN T.S., HANNA R.E.B., NIZAMI W.A. 1987b: Ultrastructural and cytochemical observations on the tegument of three species of paramphistomes (Platyhelminthes: Digenea) from the Indian water buffalo, *Bubalus bubalis*. *Int. J. Parasitol.* 17: 1153–1161.
- FUJINO T., ISHII Y., CHOI D.W. 1979: Surface ultrastructure of the tegument of *Clonorchis sinensis* newly excysted juveniles and adult worms. *J. Parasitol.* 65: 579–590.
- GUSTAFSSON M.K.S., WIKGREN M.C. 1981: Peptidergic and aminergic neurons in adult *Diphyllobothrium dendriticum* Nitzsch, 1824 (Cestoda, Pseudophyllidea). *Z. Parasitenkd.* 64: 121–134.
- HALTON D.W., LYNES R.A.W. 1971: Ultrastructure of the tegument and associated structures of *Aspidogaster conchicola*. *J. Parasitol.* 57: 1198–1210.
- IP S.H., DESSER S.S. 1984: Transmission electron microscopy of the tegumentary sense organs of *Cotylogaster occidentalis* (Trematoda: Aspidogastrea). *J. Parasitol.* 70: 563–575.
- MORAVEC F. 2002: External morphological differences between *Crepidostomum farionis* and *Crepidostomum metoecus* (Trematoda: Allocreadiidae), parasites of salmonids, as revealed by SEM. *Folia Parasitol.* 49: 211–217.
- ROHDE K. 1989: At least eight types of sense receptors in an endoparasitic flatworm: a counter trend to sacculization. *Naturwissenschaften* 76: 383–385.
- ROHDE K. 1990: Ultrastructure of the sense receptors of adult *Multicotyle purvisi* (Trematoda, Aspidogastrea). *Zool. Scrip.* 19: 233–241.
- ROHDE K. 2001: The Aspidogastrea: an archaic group of Platyhelminthes. In: D.T.J. Littlewood and R.A. Bray (Eds.), *Interrelationships of the Platyhelminthes*. Taylor and Francis, London, pp. 159–167.
- ROHDE K., WATSON N.A. 1989: Sense receptors in *Lobatostoma manteri* (Trematoda, Aspidogastrea). *Int. J. Parasitol.* 19: 847–858.
- ROHDE K., WATSON N.A. 1990a: Non-ciliate sensory receptors of larval *Multicotyle purvisi* (Trematoda, Aspidogastrea). *Parasitol. Res.* 76: 585–590.
- ROHDE K., WATSON N.A. 1990b: Uniciliate sensory receptors of larval *Multicotyle purvisi* (Trematoda, Aspidogastrea). *Parasitol. Res.* 76: 591–596.
- ROHDE K., WATSON N.A. 1992: Sense receptors of larval *Lobatostoma manteri* (Trematoda: Aspidogastrea). *Int. J. Parasitol.* 22: 35–42.
- ŽĎÁRSKÁ Z. 1993: Transmission electron microscopy of ventral sucker papillae of *Brachylaimus aequans* (Digenea: Brachylaimidae). *Folia Parasitol.* 40: 203–207.
- ŽĎÁRSKÁ Z., NEBESÁŘOVÁ J. 2002a: Ultrastructure of pigmented photoreceptor of adult *Crepidostomum metoecus* (Trematoda: Digenea: Bunoderidae). *Folia Parasitol.* 49: 165–166.
- ŽĎÁRSKÁ Z., NEBESÁŘOVÁ J. 2002b: Ultrastructure of the forebody and foregut tegument and eccrine gland cells of *Crepidostomum metoecus* (Trematoda: Digenea: Allocreadiidae). *Folia Parasitol.* 49: 291–294.
- ŽĎÁRSKÁ Z., NEBESÁŘOVÁ J.: Transmission electron microscopy of the forebody papillae of *Crepidostomum metoecus* (Trematoda: Allocreadiidae). *Folia Parasitol.* (in press).
- ŽĎÁRSKÁ Z., SOBOLEVA T.N., VALKOUNOVÁ J., ŠTĚRBA J. 1990: Ultrastructure of the general body tegument of the trematode *Brachylaimus aequans*. *Helminthologia* 27: 3–9.

Received 24 January 2003

Accepted 21 March 2003