

Ultrastructure of the forebody and foregut tegument and eccrine gland cells of *Crepidostomum metoecus* (Trematoda: Digenea: Allocreadiidae)

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Abstract. The forebody and foregut of *Crepidostomum metoecus* Braun, 1900 are invested with a tegument bearing regularly arranged surface tubercles comparable with the aspidogastrea surface structures. The tegument of the ventrolateral lobes and of the prepharynx is penetrated by ducts of eccrine gland cells. The frontal and prepharyngeal gland cells, localised in the parenchyma, discharge electron-dense granules. Their ducts are lined by peripheral microtubules and fixed to the tegument plasmalemma by a septate junction. The functional roles of these glands are discussed.

The ultrastructure of the tegument of adult *Crepidostomum metoecus* Braun, 1900 has been studied using scanning electron microscopy (SEM) by Caira (1989) and Moravec (2002). This species has not yet been examined by transmission electron microscopy (TEM). Of the genus *Crepidostomum* Braun, 1900 two other species, *C. opeongoensis* Caira, 1985 and *C. farionis* (O.F. Müller, 1784), were studied in detail by SEM only (Choudhury and Nelson 2000, Moravec 2002).

In TEM, the ultrastructure of the forebody and of the foregut tegument of *C. metoecus* differs from that in other digeneans examined (Bogitsh 1972, Smyth and Halton 1983, Dunn et al. 1987, Žďárská et al. 1990, Fried and Haseeb 1991, Fujino 1997, Halton 1997, Orido et al. 1998). The surface tegument displays small regularly arranged tubercles comparable with the surface tubercles in the Aspidogastrea (Rohde 1972, Fried and Haseeb 1991).

In the genus *Crepidostomum*, the eccrine gland cells present in the oral ventrolateral lobes and around the prepharynx of *C. metoecus* have not yet been studied. The existence of some gland cell ducts and openings only in the oral sucker lobes of *Crepidostomum cooperi* was reported by Caira (1989), and *C. opeongoensis* by Choudhury and Nelson (2000). The ultrastructure of these gland cells corresponds with the forebody gland cells described for other digeneans (Halton and Dermott 1967, Davies 1979, Žďárská and Soboleva 1984, Fujino 1997). Also the ultrastructure of prepharyngeal gland cells, known in a few digeneans (Žďárská et al. 1988, Orido et al. 1998), has not been studied in members of the genus *Crepidostomum*. In monogeneans, in contrast to digeneans, many types of eccrine gland cells penetrating the forebody and foregut tegument are known (Smyth and Halton 1983, Fried and Haseeb 1991).

MATERIALS AND METHODS

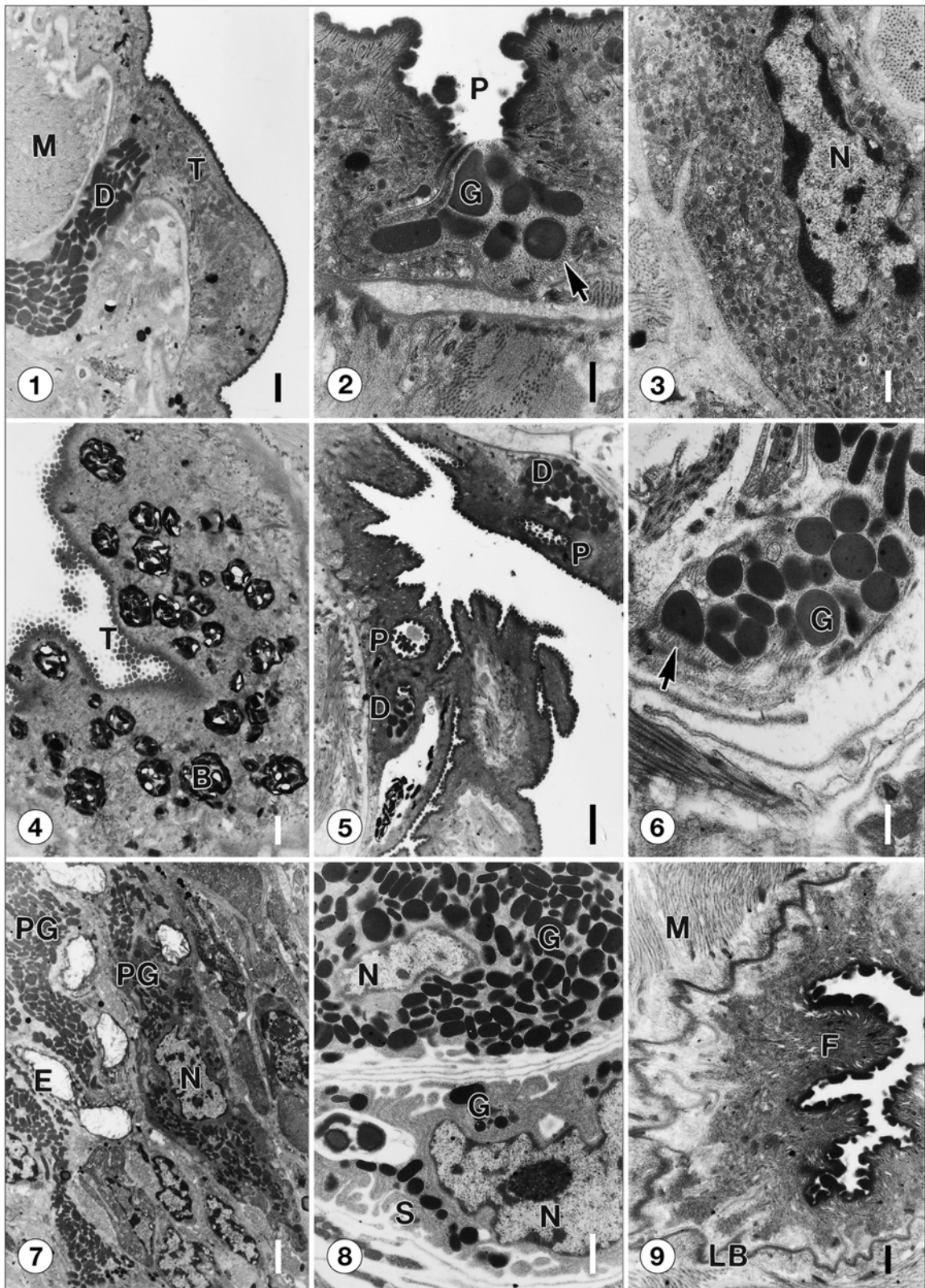
Specimens of *Crepidostomum metoecus* Braun, 1900 were removed from the intestine of *Salmo trutta fario* L., collected in the Czech Republic. They were washed in saline, fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 2 h at 4°C, postfixed for 2 h at 4°C in 1% osmium tetroxide, dehydrated 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 with toluidine blue.

RESULTS

Tegument

The lining of the oral sucker, lobes, mouth, prepharynx, pharynx and oesophagus is composed of tegument which is continuous with and structurally similar to that of the general body surface. The surface of the forebody and foregut tegument is elevated into a series of regularly arranged tubercles (Figs. 1, 2, 4, 5, 9). The tegument distal cytoplasm contains mitochondria, ribosomes and electron-dense secretory bodies (Fig. 2). Other components occur regionally in the distal cytoplasm. The forebody is lined with a tegument with sensory endings, and the tegument of the ventrolateral lobe region is perforated by gland cell ducts (Figs. 1, 2).

The tegument of the foregut is without sensory receptors and its distal cytoplasm is regionally modified. In the mouth tegument there are concentrated large membraneous multilamellar bodies (Fig. 4). In the pharynx (Fig. 9), and mainly in the oesophagus, the distal cytoplasm forms long extensions. The basal plasmalemma of the tegument is closely connected with



the basal lamina. At the sites where the distal cytoplasm connects with the processes of the tegumental cell bodies (Fig. 3), the basal lamina and fibrous layer is perforated. The ducts of the prepharyngeal gland cells also perforate the basal lamina and fibrous layer.

Eccrine gland cells of ventrolateral lobes (frontal glands)

These unicellular glands open to the exterior at the edge line along the anterior part of the ventrolateral lobes (Figs. 1, 2). The perinuclear parts of the gland cells are situated in the parenchyma below the thick muscle layer. The glands contain a large nucleus, granular endoplasmic reticulum with cisternae, Golgi complexes, mitochondria and large oval electron-dense secretory granules (900×500 nm). The secretory product is concentrated in the considerably broadened ducts (Figs. 1, 2). The ducts are supported by longitudinal microtubules and attached to the basal tegument plasmalemma by a ring-like septate junction (Fig. 2). An electron-dense collar is juxtapositional to the junction.

Prepharyngeal gland cells

The prepharyngeal gland cells release their contents into the lumen of the prepharynx (Fig. 5). The perinuclear part of these glands is localised deep in the parenchyma between the oral sucker and pharynx (Fig. 7). The cytoplasm contains a large nucleus with nucleolus, granular endoplasmic reticulum with cisternae, Golgi complexes, mitochondria and large elliptical electron-dense secretory granules (750×400 nm) (Fig. 8). The surface of the cell bodies is irregular, forming small lobes (Fig. 8). Long narrow ducts transport the secretory granules to the prepharyngeal tegument. The ducts open at the base of the prepharyngeal tegument and release their contents into the lumen of the prepharynx (Fig. 5). The ducts are at the periphery reinforced by longitudinally arranged microtubules (Fig. 6). The apical part of the ducts penetrates the muscle and fibrous layers and basal lamina and is fixed to the tegument basal plasmalemma by a septate junction.

DISCUSSION

The ultrastructure of the tegument of *Crepidostomum metoecus* differs from that in other digeneans. The forebody and foregut (mouth, prepharynx, pharynx and

oesophagus) are lined with a tegument bearing regularly arranged surface tubercles comparable with the aspidogastrea surface structures (Rohde 1972, Fried and Haseeb 1991). The pits at the gland cell openings are also surrounded by these tubercles. As in other digeneans, the forebody tegument bears sensory receptors. In the foregut tegument of *C. metoecus*, as in the foregut of *Paragonimus miyazakii* (Orido et al. 1998), no receptors were observed. Sensory receptors in the foregut of a digenean trematode have been detected in *Megalodiscus temperatus* only (Bogitsh 1972).

In adult digenean trematodes, in comparison with monogeneans (Smyth and Halton 1983), there is little information on the ultrastructure of forebody (Halton and Dermott 1967, Davies 1979, Žďárská and Soboleva 1984, Fujino 1997), foregut (Davies 1979, Žďárská et al. 1988, Orido et al. 1998), and their associated glands. The glands are designated according to the localisation at which the ducts of gland cells penetrate the tegument. In digeneans the forebody gland cells that open around the oral sucker are termed frontal gland cells (Halton and Dermott 1967, Davies 1979). In *C. metoecus* such glands could be designated the glands of the ventrolateral lobes.

In digeneans there are two types of glands opening into the foregut lumen, the prepharyngeal (Žďárská et al. 1988, Orido et al. 1998) and oesophageal (Davies 1979) gland cells. In our opinion, in *C. metoecus* both types, the frontal and prepharyngeal gland cells, are involved in digestion. The frontal glands function in extracorporeal digestion (and adhesion) as described in other digeneans (Halton and Dermott 1967, Davies 1979, Žďárská and Soboleva 1984, Fujino 1997). In *C. metoecus* the secretory product of the frontal and foregut glands consists of electron-dense homogenous granules as in most other digeneans (Halton and Dermott 1967, Davies 1979, Žďárská and Soboleva 1984, Žďárská et al. 1988) except *Paragonimus miyazakii* (Orido et al. 1998).

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Figs. 1-9. *Crepidostomum metoecus*, transmission electron micrographs. **Fig. 1.** Tegument (T) with regularly arranged surface tubercles of the oral sucker ventrolateral lobe, and duct (D) of frontal gland cell penetrating between the muscle fibres (M). **Fig. 2.** Detail of the frontal gland cell duct opening at the basal part of the tegument. Note the surface part of the pit tegument (P) with tubercles. G – secretory granules; arrow – microtubules of the duct. **Fig. 3.** Perinuclear part of a tegumental cell with electron-dense secretory granules. N – nucleus. **Fig. 4.** Tangential section of the mouth tegument containing electron-dense membranous bodies (B). T – surface tubercles. **Fig. 5.** Prepharyngeal tegument penetrated by ducts (D) of prepharyngeal gland cells. P – pit at the duct opening. **Fig. 6.** Detail of the prepharyngeal gland cell duct. Arrow – peripheral microtubules; G – secretory granules. **Fig. 7.** Perinuclear parts of prepharyngeal gland cells (PG) containing electron-dense secretory granules. N – nucleus; E – excretory duct. **Fig. 8.** Detail of perinuclear parts of two prepharyngeal gland cells. S – fine lobes of the gland cell surface; N – nucleus; G – secretory granules. **Fig. 9.** Transverse section of the pharynx. F – folds of tegument; LB – basal lamina; M – muscle fibres. Scale bars: Figs. 1, 4, 8 = 1 μ m; Figs. 5, 7 = 2 μ m; Figs. 2, 3, 6, 9 = 500 nm.

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