Ultrastructure of three types of scolex gland cells in adult *Bothriocephalus claviceps* (Cestoda: Pseudophyllidea)

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**Abstract.** The ultrastructure of three types of unicellular scolex gland cells in adult cestode *Bothriocephalus claviceps* (Goeze, 1782) is described. The first type – apocrine gland cells transport their secretion (small rounded electron dense granules) via thin ducts into the tegument where it accumulates as projections on the body surface. The second type – eccrine gland cells press out their secretion (large oval electron dense granules) through ducts which open to the exterior surface of the tegument. The third type – microapocrine gland cells transport their secretion (large rounded electron dense granules) through thin cytoplasmic processes into the distal cytoplasm of the tegument. The secretory discharge occurs by means of evaginations of the outer tegmental plasmalemma and their subsequent detachment. The possible functions of the scolex gland cells are discussed.

During the complex helminthological investigation of eels in the Czech Republic we had the opportunity to study the ultrastructure of cestodes parasitizing this host. The fine structure of the scolex (frontal, head) glands in *Bothriocephalus claviceps* (Goeze, 1782) Rudolph, 1810, has not yet been described. Two other *Bothriocephalus* species, *B. scorpit* and *B. gowkongensis* (= *B. achetolognathi*) were studied in transmission electron microscope (TEM) by Kuperman and Davydov (1982) and Kuperman (1988). In adult cestodes the presence of tegumental projections containing secretory granules has been studied by light microscopical and electronmicroscopical methods in Pseudophyllidea – *Bothriocephalus scorpit* (Jones 1975, Kuperman and Davydov 1982), *Eubothrium crassum* (Arme and Threadgold 1976, Kuperman and Davydov 1982), *E. salvelini* (Boyce 1976, Kuperman and Davydov 1982), *E. rugosum*, *E. acipenserinum*, *B. gowkongensis*, *B. claviceps* (Kuperman and Davydov 1982), and in Trypanorhynchia – *Grilletia erinaceus* (Davydov and Bisserova 1985). The ultrastructure of the apocrine gland cells, forming these projections, has been studied in adult *E. salvelini* by Tedesco and Coggins (1980), *B. scorpit*, *B. gowkongensis*, *E. crassum*, *E. salvelini*, *E. acipenserinum*, *Trienophorus nodulosus*, *T. crassus* and *T. meridionalis* by Kuperman and Davydov (1982). The ultrastructure of the apocrine scolex gland cells in *B. claviceps* was studied by us, and corresponds generally with the results of Kuperman and Davydov (1982) in species of the genus studied by them. In contrast to these authors, who found in adults of the genus *Bothriocephalus* only apocrine scolex gland cells, we detected two other types more – one with eccrine secretion and the second with microapocrine-like secretion.

**MATERIALS AND METHODS**

Adult specimens of *Bothriocephalus claviceps* were removed from the intestine of *Anguilla anguilla* (Linnaeus, 1758) collected from the Břehyně brook (Czech Republic). Then 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% OsO₄, dehydrated through an alcohol series and embedded in Durcupan via acetone. A series of ultrathin sections were cut using a Reichert-Jung Ultracut E ultramicrotome, double-stained with uranyl acetate and lead citrate and viewed in a Philips 420 electron microscope operated at 80 kV. Semi-thin sections were stained in toluidin blue.

**RESULTS**

In the scolex of *Bothriocephalus claviceps* three types of unicellular scolex glands have been detected.

1. **Apocrine gland cells.** The secretion of these gland cells forms secretory projections of the distal cytoplasm of the tegument. The matrix of the tegument (Figs. 1,4) represents the contents of the tegumental cells (Figs. 5, 6,13) transported via thin cytoplasmic processes (Fig. 13).
Figs. 1–5. Bothriocephalus claviceps. **Fig. 1.** Transversal section of the scolex with well visible dimensional differences of the secretory granules of apocrine (A) and microapocrine (B) gland cells. C – projection of the distal cytoplasm of the tegument (T) containing secretory granules of the apocrine gland cell. D – perikaryon of a microapocrine gland cell, × 9500. **Fig. 2.** Perikaryon of an apocrine gland cell with Golgi complexes (arrows), granular endoplasmic reticulum (R), secretory granules (S), small vacuoles (V) and nucleus (N), × 20000. **Fig. 3.** Detail of Fig. 1. Duct and tegumental projection containing secretory granules of
Figs. 6–10. *Bothriocephalus claviceps*. **Fig. 6.** Cross section of the scolex with well visible four types of cell processes. A – tegumental cell, B – apocrine, C – eccrine and D – microapocrine gland cells, E – projection of tegument with granules of the apocrine gland cell, arrow – basal lamina, CM – circular muscle fibres, LM – longitudinal muscle fibres, × 12000. **Fig. 7.** Tegument with the opening of an eccrine gland cell duct. Arrow – septate desmosome, × 33500. **Fig. 8.** Longitudinal section of an eccrine gland cell duct (A) localized in the vicinity of a tegumental projection (B) containing apocrine gland cell secretion, × 15400. **Fig. 9.** Cross section of an eccrine gland cell duct with well visible microtubules, × 33500. **Fig. 10.** Detail of the secretory granules and microtubules of an eccrine cell duct penetrating the basal lamina (arrows), × 33500.

← an apocrine gland cell. Arrow – basal lamina, F – fibrous layer, M – muscle fibres, × 20000. **Fig. 4.** Cytoplasmic process (A) of a tegumental cell at the site of connection with the distal cytoplasm of the tegument (T). Arrow – basal lamina, F – fibrous layer, M – muscle fibres, MS – microtriches, × 15400. **Fig. 5.** Tegumentary perikaryon with well visible flat vesicles (V) and nucleus (N). The processes of this cell expanding among the muscle fibres (M) are connected by intercellular junctions (arrows) with other parenchymal cells, × 15400.
Figs. 11–14. *Bothrioccephalus claviceps*. **Fig. 11.** Perikaryon (A) and duct (B) of an eccrine gland cell. N – nucleus, × 9200. **Fig. 12.** Detail of Fig. 11. N – nucleus, R – granular endoplasmic reticulum, G – Golgi complexes, S – secretory granules, M – mitochondria, × 15400. **Fig. 13.** Perikaryon of a microapocrine gland cell (A) and tegumental cell (B). C – process of an apocrine gland cell, N – nucleus, × 12000. **Fig. 14.** Processes of microapocrine (A) and apocrine (B) glands among the muscle fibres (C), × 15700.
4). The apocrine gland cells are of irregular shape (Fig. 2) with ducts extended towards the apical disc, edges of bothria and lateral parts of the scolex and neck. The glands contain a large nucleus, well developed granular endoplasmic reticulum, Golgi complexes, mitochondria, small rounded electron dense granules (143–175 nm) bound by a unit membrane and small electron lucid vacuoles (Fig. 2). The secretory ducts (Figs. 1, 3) of these gland cells fuse with the basal plasmalemma of the tegument. The secretion granules and parts of the cytoplasm containing small electron lucid vacuoles run through these ducts and accumulate under the apical plasmalemma of the tegument to form projections on the body surface (Figs. 1, 3, 6, 8). The projections bear no microtriches. The ducts are enforced by longitudinally oriented microtubules. Generally one duct leads to one projection, but in few cases we have detected projections with two ducts. The discharge of the secretion occurs by an apocrine-like mechanism with the destruction of the projections.

2. Eccrine gland cells. These unicellular glands (type 2) open outside at the apical disc and edges of the bothriae. The cells are situated deep in the parenchyma and a relatively long duct connects them with the body surface. The glands are irregularly shaped and contain a large nucleus, granular endoplasmic reticulum with cisternae, Golgi complexes, mitochondria and large oval electron dense secretory granules (405–475 nm × 218 to 276 nm) (Figs. 11, 12). This secretory product is concentrated in the ducts, which are strengthened by longitudinal microtubules (Figs. 7–10). The ducts, going to the tegument, are considerably broadened (Fig. 6) and form large reservoirs of secretory granules. The ducts are attached to the tegument by a ring-like septate desmosome. An electron dense collar is juxtapositional to the desmosome (Fig. 7). The ducts open outside between microtriches at the surface of the tegument (Fig. 7). The secretion is voided by an eccrine mechanism.

3. Microapocrine gland cells. These gland cells (type 3) transport their secretory product to the distal cytoplasm of the tegument along the cytoplasmic processes and thin ducts in the same manner as the apocrine gland cells (type 1), i.e. their secretion is intrategmental. The secretion discharge from the distal cytoplasm occurs by means of small evaginations of the apical plasmalemma of the tegument including the merged secretory granules, and by their subsequent detachment (microapocrine-like mechanism). These gland cells (Figs. 1, 13) contain a large nucleus, granular endoplasmic reticulum, Golgi complexes, mitochondria and fewer large round electron dense granules (317–375 nm). The cytoplasmic processes and ducts contain electron dense granules too (Figs. 1, 14). The ducts are enforced by microtubules (Fig. 18). It seems that there is a periodicity in the secretion activity. Some processes of these cells are filled with electron lucid cytoplasm only, and at the place where the ducts open into the distal cytoplasm a large electron lucid cavity-like formation is present. At the secretory phase the electron dense granules are transported into the electron lucid cavity (Figs. 15–18), where they merge and are later transported to the external plasmalemma of the tegument (Fig. 19) and extruded via detachment of small evaginations containing the merged electron dense material (Figs. 20, 21).

**DISCUSSION**

In the scolex of *Bothriocephalus claviceps* three types of unicellular glands have been detected with the aid of transmission electron microscopy. The most common cells are apocrine gland cells (type 1) which secret small rounded electron dense granules which are transported via ducts into the tegument where they accumulate as projections on the body surface. These projections are distributed on the apical disc, lateral parts of the scolex and on the edges of bothriae. The mechanism of the release is an apocrine one, in which the discharge of the secretion is brought about by the partial or complete destruction of the projections.

The eccrine gland cells (type 2) in *B. claviceps* are less frequent. Their ducts are distributed in the apical disc and the edges of the bothriae. The secretory granules are released by an eccrine process, as the secretory ducts pass through the distal cytoplasm, and then open at the surface of the tegument via small pores. This means of releasing secretory material has been described earlier for the scolex glands of the genus *Diphyllobothrium* (Ohman-James 1973, Andersen 1975, Gustafsson and Vaihela 1981, Kuperman and Davydov 1982), *Eubothrium crassum* (Arme and Threadgold 1976), *Proteocephalus percae* (Andersen 1979, Kuperman and Davydov 1982), *P. torulosus*, *P. exigus* and *Khawia sinensis* (Kuperman and Davydov 1982), *Caryophyllaesa laticeps* (Richards and Arme 1981, Kuperman and Davydov 1982), *Grillotia erinacea* (Davydov and Biserova 1985), *Tentacularia coryphaenae* (Farooqi 1986) and *Trilocularia acanthiae vulgaris* (McCullough and Fairweather 1989).

The third type of gland cells occurs very rarely. The secretion products are large rounded electron dense granules which enter the distal cytoplasm of the tegument and merge. Their mode of secretion is microapocrine-like. The secretory discharge occurs by means of evaginations of the outer tegumental plasmalemma and their subsequent detachment. This type of secretion has been observed before in *Caryophyllidea* by Hayunga (1979) in *Hunterella nodulosa*, Kuperman and Davydov (1982) in *C. laticeps* and *K. sinensis*, and Davydov and Podubnaya (1988) in *K. sinensis* and *K. armeniaca*. 

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Figs. 15-18. *Bothriocephalus claviceps*. **Fig. 15.** Duct (A) of a microapocrine gland cell at the site of connection with the distal cytoplasm of the tegument (T). At the base of the tegument the expressed cytoplasm forms an electron lucid cavity-like formation (B) in which a partly merged secretion granule (S) is visible. In this section two other (C, D) cavity-like formations with secretions are present. Arrow—basal lamina, CM—circular muscle fibres, LM—longitudinal muscle fibres, MS—microtriches, × 20200. **Fig. 16.** Cavity-like formation (A) with secretory granules (S) and duct (B) of a microapocrine gland cell localized between two tegumental projections (C) containing the secretion granules of the apocrine gland cells (AS), × 27700. **Fig. 17.** Cavity-like formation (A) with merged secretion granules (S) and duct (B) of a microapocrine gland cell. Arrow—lamina basalis, F—fibrous layer, M—circular and longitudinal muscle fibres, × 27700. **Fig. 18.** Serial section to Fig. 17 with oblique sectioned two ducts strengthened by microtubules (arrows). A—cavity-like formation, S—merged secretion, F—fibrous layer, M—muscle layer, T—tegument, arrowhead—basal lamina, × 27700.
In *B. claviceps* scolex three types of cells have cytoplasmic continuity with the tegument. They transport their cytoplasm and secretory products along the cytoplasmic processes into the syncytial layer of the tegument. These three types of cells are localized in the parenchyma near the scolex surface. The contents of the sub tegumental cells are transported into the distal cytoplasm and represents the matrix of the tegument. The organelles and flat electron-lucid vesicles are regularly distributed throughout the syncytial layer of the scolex tegument. The secretory product of the two other types of cells (the apocrine and microapocrine gland cells) is localized only at the site where their ducts open into the syncytium. The apocrine gland cells accumulate at these foci their secretions thus forming the tegumental projections. The microapocrine gland cells periodically press out into the syncytium the electron lucid cytoplasm with few secretory granules which fuse and are extruded from the tegumental syncytium via detachment of small evaginations. The secretion is pushed out directly to the exterior only from the eccrine gland cells, the ducts of which penetrate through the syncytial tegument.

In adult cestodes the explanation of the functions of the gland cell secretions is at present purely speculative. From the results of different authors it is evident that the secretions play a role in parasite-host interface (Williams 1966, McVicar 1972, Hayunga 1979, Davydov and Mikryakov 1988, Smyth and McManus 1989, Brockerhoff and Jones 1995). To the scolex gland secretions in adult cestodes some functions (proteolysis, adhesion and protection) have been attributed. The proteolytic function may aid in the adult cestodes nutrition (Thompson et al. 1979). However, in adult cestodes enzyme activity has not yet been detected in the secretions of the scolex glands of any species.


It is possible that the scolex glands protect the worm against the host’s digestive enzymes or immune response. It would be of great interest to determine the function of each type of gland separately. In our opinion there will be special functions for each type of gland.

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