ULTRASTRUCTURE OF THE SURFACE OF EXTERNAL GENITALS OF SIX SPECIES OF Digenetic Trematodes Studied By Scanning Electron Microscopy

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Abstract. The topography of the surface of external genitals of six species of digenetic trematodes has been studied by scanning electron microscopy. The cirrus surface in Haeomatothoeus corrigatus consists of tegumentary lamellae forming structures of irregular shape filled with a network of fibres. The cirrus surface in Echinopharynx recurvatus consists of flat, longitudinal tegumentary lamellae with irregularly indented margins. Anisodactyl papillae are in the middle part of the cirrus. Behind the invaginable apical part of cirrus in Hypoderum consideus, there are interrupted, longitudinally and obliquely orientated tegumentary folds with numerous papillae. The apical part of cirrus in Echinostoma revolutum is divided into two morphologically different parts. The surface of the part with the terminal cirrus opening is made up of rod-shaped processes. The other part consists of anastomosing tegumentary lamellae forming an irregular network with ciliated papillae. The cirrus surface in Notocotylus attenuatus is covered with spine-shaped protrusions, whereas that in Ophioligophloe locellus is formed from microvilli. The measurements of the evaginated part of cirrus are given for all of the studied trematode species.

A complete study of the topography of body surface of trematodes includes also the surface of their external genitals. The ultrastructure of these organs, however, has been studied only rarely by scanning electron microscopy (SEM). One of the reasons seems to be the commonly occurring curving of the fixed trematodes with concave ventral and convex dorsal side due to which the genital pore is covered by the tegument and the cirrus is invaginated. Another reason is the fact that the evagination of the cirrus before fixation can be hardly affected, particularly in small trematodes.

The ultrastructure of cirrus tegument has been studied by means of SEM in Leucochloridium sp., Urogonimus macrotomus and Leucochloridium variae by Bakke (1976, 1977, 1982), in Quinqueserialis quinqueserialis by Wittrock (1976), in Ribeiroia marini by Page et al. (1980), in Echinostoma revolutum and Ichthyophora melis by Smales and Blankespoor (1984), and in Concinnum epomopus by Otubanjo (1985). Partial data of the ultrastructure of cirrus surface may be obtained from the photomicrographs published by Ždárská et al. (1983) and Bušta and Našinová (1986).

Materials and Methods

The trematode species under study were obtained experimentally from cercariae released by naturally infected snails. The snails were collected in the localities near České Budějovice (150 km south of Prague, Czechoslovakia). The cercariae of Hypoderum consideus (Bloch, 1782) and Notocotylus attenuatus (Rudolph, 1809) were from the snail Lymnaea stagnalis, cercariae of Echinoparynx recurvatus (Lineow, 1873) from Galba corvus, cercariae of Echinostoma revolutum (Freilich, 1802) and Ophioligophloe locellus Kuseck, 1910 from Planorbius corneus. Snails L. stagnalis,
ECINOSTOMA REVOLUTUM (Frohlich, 1802) Plate I, Figs. 1-6

The genital pore is situated preacetabularly and medially on the ventral side of body. The evaginated cirrus (Pl. I, Fig. 1) is 225 μm long and 82 μm wide in front of corner spines. The characteristic division into two morphologically different parts is visible in the apical part of cirrus (Pl. I, Figs. 1 and 2). In the first part, around the terminal slit-like cirrus opening, there is an about 13 μm wide band of densely distributed rod-shaped protrusions of tegument with uneven surface. The other apical part of cirrus (Pl. I, Fig. 2) is situated about 25 μm distally to cirrus opening. It is a large protuberance with anastomosing tegumentary lamellae forming an irregular network. There is a large number of papillae with short cilia. As it is evident from the detail in Pl. I, Fig. 5, two types of cirrall papillae may be distinguished: large, dome-shaped (large white arrow) and small tubular ones (small white arrow).

The arrangement of the two above apical parts of cirrus is not constant, depending on the state of elongation of the cirrus and the related eversion of the part with cirrus opening. If it remains invaginated (Pl. I, Fig. 3), then the other part of cirrus bearing cirrall papillae takes the upper position.

The remaining part of the cirrus surface is verrucose, with flat tegumentary protuberances with rugged surface. A transition from the tegument found around the cirrus opening to the verruose surface covering the major part of cirrus is shown in Pl. I, Fig. 4. There are unfrequently papilla-like structures (Pl. I, Fig. 3, black arrow), which are clusters of concentrically arranged wart-like protuberances of tegument. A detail of such a structure from the middle part of cirrus measuring about 5 μm in diameter is visible in Pl. I, Fig. 6.

ECHINOGRAPHUM RECURRENTUM (Linstow, 1873) Plate II, Figs. 1-3

The cirrus projecting from the genital pore situated preacetabularly and medially on the ventral side of body is illustrated in Pl. II, Fig. 1. The evaginated part of cirrus measures approximately 110 μm in length and 30 μm in width in the middle. The cirrus surface consists of flat, longitudinal tegumentary lamellae, the margins of which are irregularly indented. The cirrus is covered in a manner so that they fit into one another. The cirrus surface is covered with large, dome-shaped, scillate papillae measuring 7.5—8.5 μm in width at base and approximately 3 μm in height (Pl. II, Figs. 2 and 3). The distance between the first papillae and cirrus tip depends on the rate of cirrus eversion. In this specimen (Pl. II, Fig. 2) it is 20 μm, in other specimens it may be even 60 μm. No papillae were found on the apical and proximal part of cirrus. The cirrus opening is subterminal (Pl. II, Fig. 1).

HYPODERAEOMA CONOIDEUM (Bloch, 1782) Plate I, Plate II, Figs. 4—6

Large evaginated cirrus protruding from the genital pore situated preacetabularly and medially on the ventral side of body is shown in Pl. II, Fig. 4. It is cylindrical and its evaginated part is 225 μm long and 130 μm wide in the middle. The apical part of the cirrus is conical, with cirrus opening on the tip. This part can be completely invaginated or partly or completely evaginated (Fig. 1). Pl. II, Figs. 4—6 show the partly evaginated apical part. Its maximum width is 38 μm (Pl. II, Fig. 6), the cirrus opening measures 6.8 μm in diameter. The surface of this part of cirrus is covered with a slightly folded tegument without papillae and is markedly different from the surface of the remaining parts of cirrus. The tegument on the wide cylindrical part of cirrus forms small folds, which are rounded in the proximal part of cirrus and form a verrucose structure on its surface. In the distal part of cirrus, these folds are longitudinally and obliquely orientated and directed radially towards the tip (Pl. II, Fig. 6). In the distal part, there are numerous dome-shaped acillate papillae measuring 3.0—5.7 μm in width at base. Most of them are concentrated in the tip part of the wide part of cirrus and their number and size decrease in proximal direction (Pl. II, Figs. 4—6).

HAEMATOLECHEUS VARIIGATUS (Rudolphi, 1819) Plate III, Figs. 1—5

The evaginated, club-shaped cirrus with rounded apical end is shown in Pl. III, Fig. 1. It projects from the genital pore situated medially on the ventral side of body, immediately behind the oral sucker. In the figure, the evaginated part of cirrus measures about 113 μm in length and about 40 μm in width in the middle. The surface tegument on the middle part of cirrus (Pl. III, Fig. 2) consists of transversal anastomosing lamellae which form irregular pictures. Shallow depressions in individual pictures bordered with lamellae are filled with a reticulate structure. The cirrus opening situated terminally at the apical end of cirrus and several short, radially directed lamellae distinctly project from it (Pl. III, Figs. 3 and 4). Marked transversal orienta-
tion of the pictures was observed also in the basal part of cirrus (Pl. III, Fig. 5). The whole surface of cirrus is without papillae and spines.

**Notocorylus attenuatus** (Rudolph, 1889) Plate IV, Figs. 1—2

The evaginated, cylindrical cirrus projecting from the genital pore situated praectacularly and medially on the ventral side of body is shown in Pl. IV, Fig. 1. The evaginated part of cirrus measures 157 µm in length and 25 µm in width in the middle part. The cirrus surface is covered with posteriorly directed, spinellike, triangular spines, measuring about 4—5 µm in width at base and 3—4 µm in length (Pl. IV, Fig. 2). The spines are arranged like a checkerboard. No papillae were found on the cirrus surface.

**Opisthioglyphe locellus** Kossack, 1910 Plate IV, Figs. 3—4

The evaginated cylindrical cirrus, coiled in a circle and projecting from the genital pore situated medially and praectacularly on the ventral side of body is shown in Pl. IV, Fig. 3. The cirrus measures approximately 90 µm in length and 15 µm in width. Its surface is covered with densely arranged microvilli. The cirrus opening from which protrudes a bundle of sperm is situated subterminally (Pl. IV, Fig. 4). No papillae or spines were found on the cirrus.

The measurements of cirri of the six trematode species obtained by the studies of permanent mounts in the light microscope and in scanning electron microscope are presented in Table 1.

<table>
<thead>
<tr>
<th>Species</th>
<th>Values obtained from permanent mounts</th>
<th>Values obtained from SEM</th>
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<tbody>
<tr>
<td></td>
<td>length</td>
<td>width</td>
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<tr>
<td><strong>Notocorylus attenuatus</strong></td>
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<tr>
<td><strong>Haematoloechus variegatus</strong></td>
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+ The cirrus was invaginated in all studied specimens.

**DISCUSSION**

Our results concerning the topography of tegument of external genital organs of the studied trematode species and the relating metrical characteristics could be compared with few literary data.

The metrical values obtained by SEM are of an orientation character.

As it is evident from Table 1, the mean cirrus length in the permanent mounts is twice to three times greater than the values obtained by SEM. According to the data in the literature, the cirrus length in the species under study is also much greater. For example, Rašin (1933) reported 380—430 µm, Moravec et al. (1974) 270—600 µm, and Fain and Galal (1977) 400—750 µm in E. recurvatum. Kowalewski (1897) reported the length of cirrus in *Hypodermis conoides* to be more than 1 mm and according to Bashkirova (1941) it was up to 12 mm.

The differences in the metrical values obtained by the study of permanent mounts and by SEM are caused by different preparation of the material, as well as by the fact that in the specimens studied by SEM, the whole evaginated part of cirrus is usually not visible.

Of the cuticular structures of cirri of digenetic trematodes, probably only the large papillae or spines can be differentiated in the light microscope. Moreover, the sclerotized spines can be easily differentiated from the surrounding tissue by their markedly different refractivity. For example, Groschaft and Sitko (1970) found in *Leucocordium papillosum* a large number of spherical papillae of markedly different size on the proximal (80 µm) and distal (75 µm) parts of cirrus. Alm (1985) observed spines measuring 15—20 µm on the anterior part of terminal organs in two species of trematodes from sea fish. On the other hand, some of the extensive systematical studies often do not give any data of the length and surface of cirri, or the descriptions are inexact. Kowalewski (1987) described incorrectly cylindrical spines measuring 60 × 80 µm instead of papillae in the distal half of cirrus of *H. conoides*. Rašin (1933) did not find any papillae on the cirrus of *E. recurvatum* and considered it to be smooth. Odening (1985) in his comprehensive study (1985) in his comprehensive study dealing with the revision of the genus *Haematoloechus* did not mention the cirrus surface at all.

A similar cirrus surface as that observed by us in *H. variegatus* and in one tip part of *E. revolutum* cirrus, i.e., anastomosed lamellae or ridges filled with an irregular, net-like structure, was observed also in SEM by Bakke (1976, 1982) in *Leucocordium sp.* and *Leucocordium variis*, by Page et al. (1980) in *Benevidesia marci*, and by Otubanj (1985) in *Concinum epomisopis*. In contrast to our studies, however, Bakke (1976, 1982) and Otubanj (1985) noted the presence of cirrus papillae situated in shallow depressions in the net-like structure. These papillae possessed sensory endings in *L. variis* and *I. sp.*, which were lacking in *C. epomitisopis*.

We have found cirrus papillae in *E. recurvatum*, *H. conoides* and *E. revolutum*. In *E. recurvatum* and *H. conoides*, there were acellic cirrus, large or of medium size, situated on the distal end of cirrus, but not directly on its tip part. The papillae found in *E. recurvatum* were situated on one of the two apical parts of cirrus, they possessed a short central cilium and were similar to the papillae found on the tegument of adult trematodes (Nadaravskaja and Nollen 1975, Font and Witterg 1980). Oliver et al. (1984), Bañiti (1983 and others). There were also small tubular papillae with cilium, also observed in adult trematodes (Koie 1987).

In all of the three species (*E. recurvatum*, *H. conoides* and *E. revolutum*) the papillae gradually became less numerous in the direction from the apical part of cirrus and the major part of cirrus was without papillae. This is in agreement with Smales' and Blankenpoel's (1984) finding in *E. recurvatum* and Otubanj's finding in *C. epomisopis* who observed cirrus papillae only on the anterior tip of cirrus.

From the functional viewpoint is of importance the presence of the papillae performing the sensory function on that part of cirrus which penetrates into female genitalia during the copulation.

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СТРУКТУРА ПОВЕРХНОСТИ ВНЕШНИХ ПОЛОВЫХ ОРГАНОВ НЕКОТОРЫХ СЕКСАЛЬНО РЕГУЛИРУЕМЫХ ЗООТЮРГИД, ИЗУЧАЕМЫХ НА ПОМОЩЬ ПРИ САНИРУЮЩЕМ ЭЛЕКТРОИЗОЛЯЦИОННОМ МИКРОСКОПА

Я. Бушта и В. Нашивко

Разом. Топографию поверхностей внешних половых органов строения видов дигенетических троматода с помощью санитарного электронного микроскопа. На поверхности

пиропара Haematoloechus variatus находятся тегументальные пластинки, образующие фигуры перегородок, выполненные сетью табличек узорчатыми пластинками. Поверхность шероховатая с нерегулярно зубчатыми краями. В средней части пиропара бывают видны сосочки без ресничек. У Hyperaurea pumila наблюдалась интактивная анимальная часть пиропара, а на амбулаторных, шероховатые, вытянутые, направленные вперед направление тегументальных выростов. С большим количеством сосочков. Амбулаторная часть пиропара Echinocotylus resedum разделена в два морфологические разные части. Поверхность первой части, в которой расположена отверстия пиропара, состоит из вытянутых выступов. Другая часть образована микроворсиками тегументными пластинками, образующими нерегулярную сетку. Между которыми видны сосочки с ресничками. У Notocotylus attenuatus на поверхности пиропара видны лапковидные выступы. Поверхность пиропара Opisthiaephile pusillus состоит из микроворсиками. Длина размеры микроворсиками части пиропара всех изученных видов троматод.

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FAIN A., GALAL A., 1975: Presence du trematode Echinocotylus resedum (von Linne, 1873) chez l’homme et chez un ram-
Figs. 1—5. Scanning electron microscopy of the cirrus of *Echinostoma revolutum* (Frolich, 1802). Fig. 1. Total view of evaginated cirrus (ventral side of the trematode) (×140). Fig. 2. Segmentation of the apical part of cirrus (×1800). Fig. 3. Detail of the apical part of cirrus with cirrus papillae and cirrus opening with projecting spines (×1200). Fig. 4. Detail of the segmentation on the apical part of cirrus (×3700). Fig. 5. Detail of cirrus papillae on the apical part of cirrus (×4800). Fig. 6. Detail of the tegument structure in the middle part of evaginated cirrus (×3500).

Figs. 1—3. Scanning electron microscopy of the cirrus of *Echinocystis revolutum* (Linstow 1872). Figs. 4—6. Scanning electron microscopy of the cirrus of *Hypoderaeus convolvens* (Bloch 1782). Fig. 1. Total view of evaginated cirrus (ventral side of the trematode) (×200). Fig. 2. Segmentation of the distal part of cirrus and cirrus papillae (×730). Fig. 3. Detail of papillae in the middle part of cirrus (×2250). Fig. 4. Total view of the cirrus (ventral side of the trematode) (×115). Fig. 5. Distal part of the cirrus with cirrus papillae and evaginated apical part (×330).
Figs. 1–5. Scanning electron microscopy of the cirrus of *Haematobdella coriogaster* (Rudolphi, 1819).

**Fig. 1.** Total view of the cirrus (ventral side of the trematode) (*× 720*).
**Fig. 2.** Detail of the tegument of the middle part of cirrus (*× 5 000*).
**Fig. 3.** Apical part of cirrus with cirrus opening (*× 1 400*).
**Fig. 4.** Detail of the apical part of cirrus with cirrus opening and radially directed lamellae (*× 4 000*).
**Fig. 5.** Tegument of the proximal part of cirrus with transversely directed lamellae (*× 1 300*).

Figs. 1–2. Scanning electron microscopy of the cirrus of *Notocotyphus attenuatus* (Rudolphi, 1809).

**Fig. 3–4.** Scanning electron microscopy of the cirrus of *Opisthobdella koznicki* Koznick, 1916.

**Fig. 1.** Total view of the evaginated cirrus (ventral side of the trematode) (*× 700*).
**Fig. 2.** Detail of cuticular spines of cirrus (*× 1 300*).
**Fig. 3.** Total view of evaginated cirrus (ventral side of body of the trematode) (*× 1 250*).
**Fig. 4.** Detail of the cirrus opening with sperms (*× 3 400*).