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ULTRASTRUCTURE OF THE TAIL OF ECHINOSTOMA REVOLUTUM CERCARIA

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Abstract. The ultrastructure of the tail fins and muscles of *Echinostoma revolutum* cercaria is described. The longitudinal muscles consist of a primitive type of transversely striated muscle fibres with a well-developed sarcoplasmic reticulum in the Z-band. The fins are formed by a longitudinal tegument fold containing the same components as the remaining part of the tail tegument. The difference between the body and tail tegument is discussed.

The paper is a part of a complex study of *E. revolutum* cercaria from *Planorbarius corneus*. It was preceded by morphological (Našincová 1986), histochemical (Žďárská and Našincová 1985), and ultrastructural (Žďárská et al. 1987) studies of this cercaria. The aim of this paper is the elucidation of the tail ultrastructure in echinostome cercariae of the group "revolutum".

MATERIALS AND METHODS

Free-swimming cercariae *Echinostoma revolutum* (Froehlich, 1802) Dietz, 1909 were obtained from spontaneously infected snails *Planorbarius corneus*. For the study in a transmission electron microscope, the material was fixed at 4 °C in 3 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 2 h, postfixed in 1 % OsO_4 for 2 h, dehydrated through an alcohol series, and embedded through acetone into Durcupan and Vestopal. Ultrathin sections were cut with Reichert's OM-U2 ultramicrotome, contrasted with 20 % uranyl acetate and Reynold's solution of lead citrate, and examined with Philips 420, Philips 300 and JEM 100 B electon microscopes.

For the scanning electron microscopy, the cercariae were fixed in warm (70—80 °C) neutral formalin and dehydrated through alcohol series and acetone, dried at critical point, coated with gold, and examined in TESLA BS—300 scanning electron microscope at accelerating voltage of 15 kV.

RESULTS

The tail of cercaria is provided with two dorsal, three ventral, and two ventrolateral fins (Pl. I, Figs. 1, 2). Most conspicuous is the lower dorsal fin leading almost from the middle of the tail up to the beginning of its narrowed part. The smallest of the fins was identified only by SEM. It is situated on the ventral side of the tail between the two ventrolateral fins (Pl. I, Figs. 1, 2). Lateral rows of uniciliate sensory endings running almost along the whole length of the tail, except for its basal part, are visible in Pl. I, Fig. 1.

In the transmission electron microscope, both the ventral and dorsal fins are formed by a fold of the tail tegument (Pl. I, Fig. 3; Pl. II, Fig. 1) and contain the same components as the remaining parts of tegument. These are the mitochondria, secretory granules, lipid droplets, and small vesicles. The tegument is attached by hemidesmosomes to lamina basalis at some points. The longitudinal muscles of the tail are well developed, whereas the circular muscles, orientated perpendicularly to the tail axis, form only thin rings. The contractile fibres of the longitudinal muscle cells are concentrated on the ventral and dorsal sides of the tail (Pl. I, Fig. 3). Both the ventral and dorsal blocks of longitudinal muscle fibres are divided into two groups. The nerve fibres run between these groups. The sarcoplasmic parts of myocytes containing α — and β — glycogen and mitochondria with numerous cristae occupy the central part of the tail. There are also nuclei of myocytes with chromatin concentrated in clusters under nuclear membrane and with a compact nucleolus (Pl. I, Fig. 3). The longitudinal muscles of the tail consist of a primitive type of cross-striated muscle fibres. In the isotropic band (I), there are only thin myofilaments, in the anisotropic band (A), both thick and thin filaments, in H-band, only thick myofilaments, and in Z-band, a network of sarcoplasmic reticulum and electron-dense bodies (Pl. II, Fig. 2). Numerous flattened vesicles of sarcoplasmic reticulum are situated under the sarcolemma of longitudinal muscle fibres (Pl. II, Fig. 2). The nerve fibres running between the muscle cells contain small mitochondria, neurotubules and electron-lucid or electron-dense vesicles. These fibres form mutual synapses or synapses with muscle cells. Multipolar neurons are localized in the tail axis.

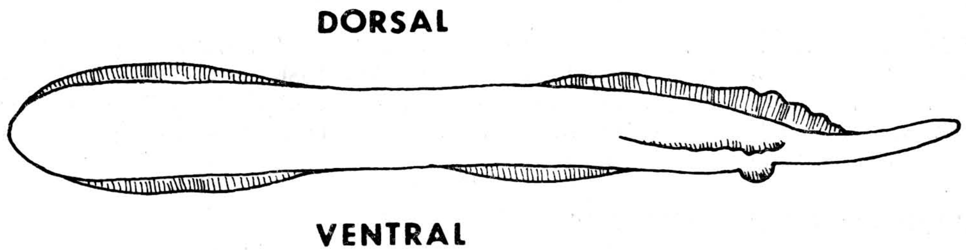

Fig. 1. Arrangement of membranes on the tail of *Echinostoma revolutum* cercaria.

DISCUSSION

E. revolutum cercaria is the third species of the family Echinostomatidae in which the tail ultrastructure has been studied. The other two species were *Himasthla quissetensis* studied by Cardell and Philpott (1960) and *H. secunda,* which was studied by Chapman (1973). The tail is the locomotory organ of the cercaria and it is adapted to rapid swimming motion.

Našincová (1986) demonstrated by SEM 2 dorsal. 2 ventrolateral and 3 ventral fins on the tail of *E. revolutum* cercaria.

The studies in transmission electron microscope revealed that the fins of the tail consist of a longitudinal tegument fold without any reinforcement and contain the same components as the remaining part of the tail tegument. Of a similar structure is also the caudal fin of *Cryptocotyle lingua* cercaria described by Rees (1975). In our studies of the tail of *E. revolutum* cercaria, like in those by Rees (1975) concerning *C. lingua,* no subtegumental cells and interruption of the tegument between the body and tail were observed. It is therefore supposed that the tail tegument contents is identical with the tegument contents of body, as it is reported by Galaktionov and Dobrovolskiy (1987). However, the tail tegument of *E. revolutum* differs from that of *C. lingua* (Rees 1975) only in that it contains, in contrast to the body tegument, only one type of secretory granules and that it is lower. Three types of granules were demonstrated in the body-tegument of *E. revolutum* cercaria (Žďárská, in press). The secretory granules in the tail tegument of *E. revolutum* cercaria are similar to the secretory granules of dorsal gland cells released into the body tegument, but they may be also a product released from the caudal gland cells during the development of the tail, the structure of which remains unknown. The elucidation of this question requires further studies of developing cercariae and their comparison with the free-swimming ones, which were the subject of the present paper.

In agreement with the findings in other cercariae (Vickers 1940, Pearson 1956, 1961, Kruidenier and Vatter 1960, Cardell and Philpot 1960, Lumsden and Foor 1968, Rees 1971, 1974, 1975, Nuttman 1974, Reger 1976, Sundararaman and Nakadel 1979), the tail of *E-revolutum* cercaria was found to contain both cross-striated and smooth muscles. The representative of the smooth muscles are the circular muscles. The longitudinal muscles, which are responsible for the rapid movements of the tail, are a primitive type of cross-striated muscles known in invertebrates (Kryvi 1973). These muscles differ, e.g., from the muscles of suckers in flatworms, which according to Kryvi (1973) have slower contractions than the longitudinal muscles of the tail. Like in other cercariae, the longitudinal muscles of the tail form two blocks — ventral and dorsal. Each block consists of two groups of contractile parts of myocytes, running longitudinally immediately below the circular muscles. The sarcoplasmic parts of myocytes, which contain a large number of mitochondria and α- and β-glycogen, and the nucleus are localized in the centre of the tail. Isotropic (I), anisotropic (A) and Z-bands are distinctly visible in longitudinal sections of the tail. In the transverse sections through myofibrils, the isotropic (I) bands contain only thin myofilaments of actin. In anisotropic (A) bands the thick myofilaments of myosin are surrounded by thin myofilaments of actin, in H-bands, there are only thick myofilaments, while sarcoplasmic reticulum and electron dense bodies with attached thin myofilaments prevail in the Z-bands.

Longitudinal muscle fibres of the tail in *E. revolutum* cercaria differ from its somatic muscle fibres in the orientation of electron-dense bodies into distinct transverse disks (= Z-bands), in the division of myofibrils into sarcomers with typical A-, I-, and H-bands, and in the presence of extensive sarcoplasmic reticulum, which penetrates between the myofibrils and forms a network between the electron-dense bodies in the Z-band and contains a larger number of mitochondria. According to Rees (1975), both the logitudinal muscles of tail and the radial musles of suckers of *Cryptocotyle lingua* cercaria are cross-striated. However, we have not managed to demonstrate the cross-striation in the radial muscles of *E. revolutum* cercaria. Consequently, the tail is the single organ of *E. revolutum* cercaria in which the cross-striated muscles occur. The circular muscles of tail are smooth and consist of thick myofilaments surrounded by a variable number of thin myofilaments. The tail is innervated with dorsal and ventral nerves localized between the two groups of muscle fibres. The intracellular content of axons consists of neurotubules, small mitochondria, as well as electron-lucid and electron-dense vesicles, which conform to the description of axons in *Fasciola hepatica* cercaria (Dixon and Mercer 1965) and *Cryptocotyle lingua* cercaria (Rees 1975).

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УЛЬТРАСТРУКТУРА ХВОСТА ЦЕРКАРИИ *ECHINOSTOMA REVOLUTUM*

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Резюме. Описана структура плавательных мембран и мышц хвоста церкарии *Echinostoma revolutum.* Продольные мышцы состоят из примитивного типа поперечнополосатых волокон с могучей саркоплазматической сетью в Z-полосе. Плавательные мембраны образованы складкой тегумента, содержащей те же компоненты, как остальные части тегумента хвоста. Обсуждается разница между тегументом тела и хвоста.

CARDELL R. R., PHILPOTT D. E., 1960: The ultrastructure of the tail of the cercaria of *Himasthla quissetensis* (Miller and Northup, 1926). Trans. Am. Microsc. Soc. 79: 442—450.

CHAPMAN H. D., 1973: The functional organisation of fine structure of the tail musculature of the cercariae of *Cryptocotyle lingua* and *Himasthla secunda*. Parasitology 66: 487—497.

DIXON E. E., MERCER E. H., 1965: The fine structure of the nervous system of the cercaria of the liver fluke, *Fasciola hepatica* L. J. Parasitol. 51: 967—976.

GALAKTIONOV K. V., DOBROVOLSKIY A. A., 1987: The hermafroditic generation of trematodes. Nauka Publ. House, Leningrad, 192 pp. (In Russian.)

KRUIDENIER F. J., VATTER A. E., 1960: Microstructure of muscles in cercariae of the digenetic trematodes *Schistosoma mansoni* and *Tetrapapillotrema concavocorpa*. Proc. 4th Int. conf. Electron Microscopy 2: 332—335.

KRYVI H., 1973: Ultrastructural studies of the sucker muscle cells in *Hemiurus communis* (Trematoda). Norw. J. Zool. 21: 273—280.

LUMSDEN R. D., FOOR W. E., 1968: Electron miscroscopy of schistosome cercarial muscle. J. Parasitol. 54: 780—794.

NAŠINCOVÁ V., 1986: Contribution on the distribution and life history of *Echinostoma revolutum* (Trematoda) in Central Europe. Věst. čs. Společ. zool. 50: 70—80.

NUTTMAN E. J., 1974: The fine structure and organization of the tail musculature of the cercaria of *Schistosoma mansoni*. Parasitology 68: 147—159.

PEARSON J. C., 1956: Studies on the life cycles and morphology of the larval stages of *Alaria arisaemoides* Augustine et Uribe, 1927 and *Alaria canis* La Rue et Fallis, 1936 (Trematoda: Diplostomatidae). Can. J. Zool. 34: 295—387.

—, 1961: Observations on the morphology and life cycle of *Neodiplostomum intermedium* (Trematoda: Diplostomatidae). Parasitology 51: 133—172.

REES F. G., 1971: Locomotion of the cercaria of *Parorchis acanthus*, Nicoll and the ultrastructure of the tail. Parasitology 62: 489—503.

—, 1974: The ultrastructure of the body wall and associated structures of the cercaria of *Cryptocotyle lingua* (Creplin) (Digenea: Heterophyidae) from *Littorina littorea* (L). Z. Parasitenkd. 44: 239—265.

—, 1975: The arrangement and ultrastructure of the musculature, nerves and epidermis, in the tail of the cercaria of *Cryptocotyle lingua* (Creplin) from *Littorina littorea* (L). Proc. R. Soc. Lond. B. 190: 165—186.

REGER J. F., 1976: Studies on the fine structure of cercarial tail muscle of *Schistosoma* sp. (Trematoda). J. Ultrastruct. Res. 57: 77—86.

SUNDARARAMAN V., NADAKAL A. M., 1979: The ultrastructure of the striated muscle of the tail of *Cercaria chackai* Nadakal et al., 1969. Cell Tissue Res. 201: 479—486.

VICKERS G. G., 1940: On the anatomy of *Cercaria macrocerca* from *Sphaerium corneum*. Qu. J. Micr. Sci. 82: 311—326.

ŽĎÁRSKÁ Z., NAŠINCOVÁ V., 1985: Histological and histochemical studies of the cercaria of *Echinostoma revolutum*. Folia parasitol. 32: 341—347.

—, —, ŠTĚRBA J., VALKOUNOVÁ J., 1987: Ultrastructure of a new type of sensory ending in *Echinostoma revolutum* cercaria (Trematoda: Echinostomatidae). Folia parasitol. 34: 311—315.

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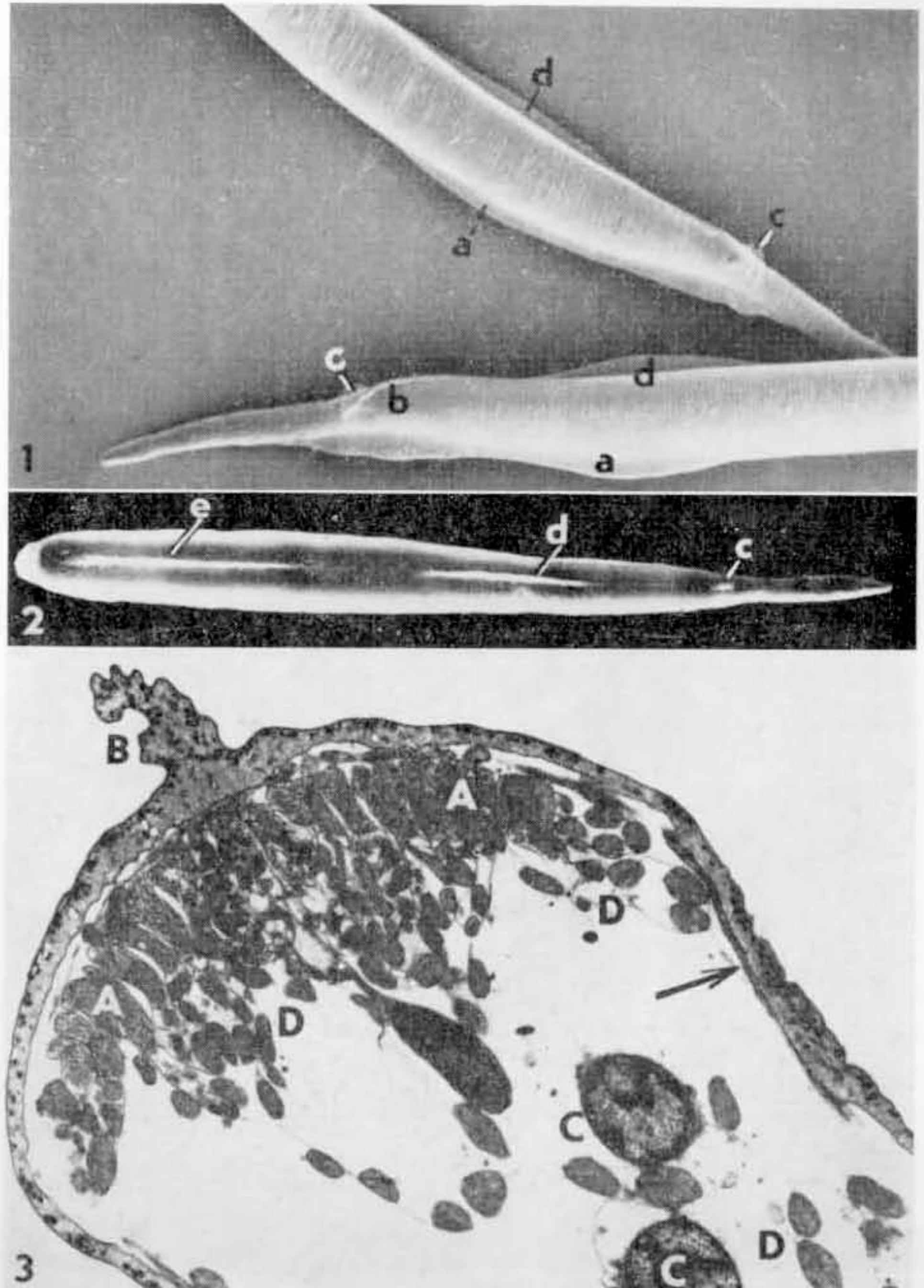

Fig. 1. Tail of *E. revolutum* cercaria — lateral view. a — lower dorsal, b — ventrolateral, c — lower ventral, d — middle ventral fins (×600). **Fig. 2.** Tail of *E. revolutum* cercaria -ventral view. c — lower, d — middle, e — upper ventral fins (×400). **Fig. 3.** Transverse section through the tail with a block of longitudinal muscle fibres (A) and tegument protruding into a fold (B) forming the fin.

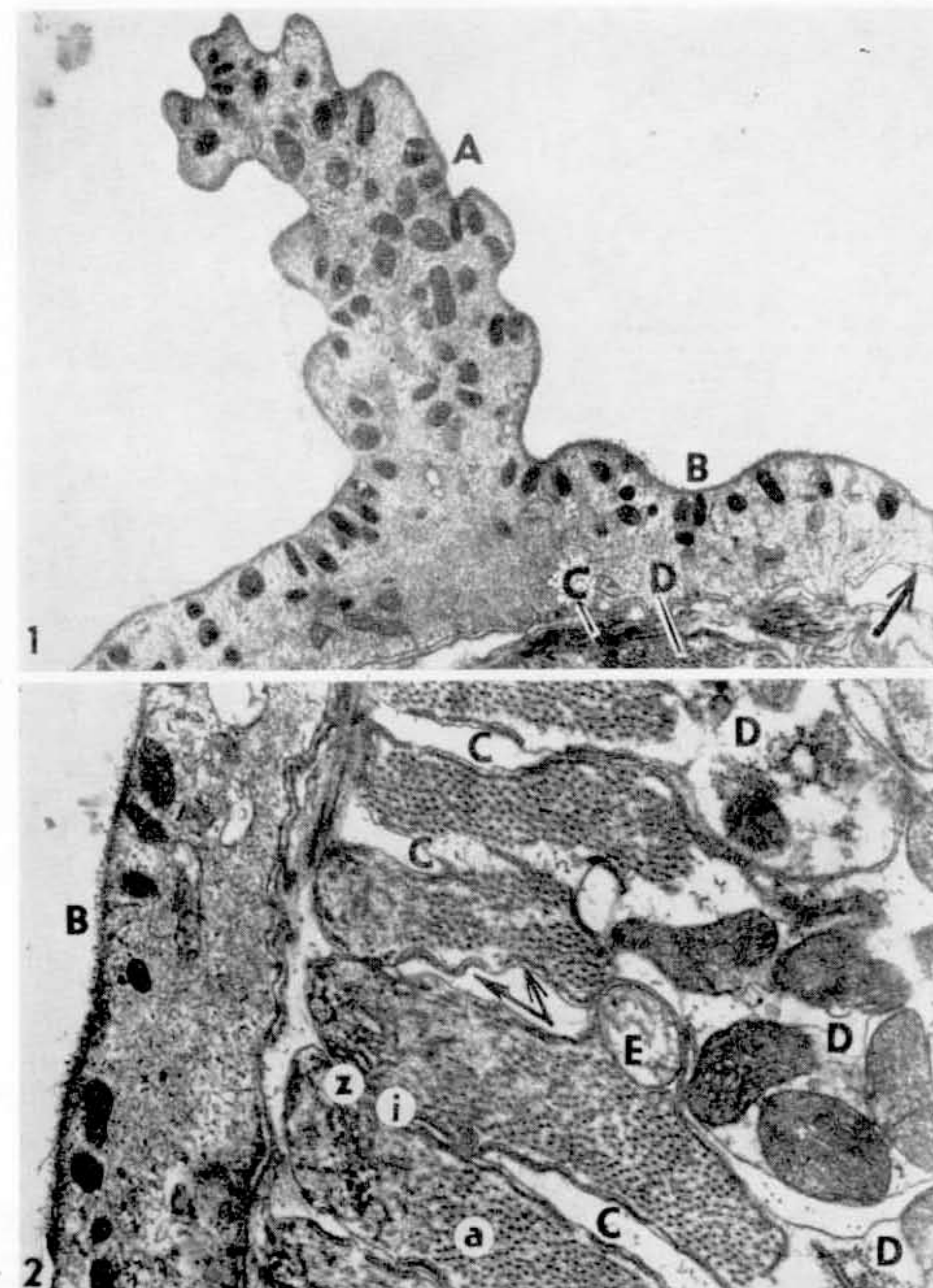

Fig. 1. Detail of fin fold (A) on the tail of *E. revolutum* cercaria. B — tail tegument, C — circular muscles, D — longitudinal muscles, arrow — lamina basalis. G, Os, UAC, Pb (×17,500). **Fig. 2.** Detail of tail tegument (B), contractil (C) and sarcoplasmic (D) parts of myocytes, nerve fibre (E), and vesicles of sarcoplasmic reticulum (arrows) below the sarcolemma. A-bands (a), I-bands (i),