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SCANNING ELECTRON MICROSCOPY OF THE CERCARIA OF DICROCOELIUM LANCEATUM

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Abstract. Completely developed cercariae of *Dicrocoelium lanceatum* have a continuous fringe of long, thin microvilli around the oral sucker, whereas the microvilli on the ventral sucker are arranged only in two lateral groups. Both suckers bear distinct tubercles. The stylet pocket is surrounded by a large number of sensory papillae with or without cilia. The same papillae are also situated around the oral sucker.

The scanning electron microscopy (SEM) has been increasingly used in the comparative studies of larval stages of trematodes, particularly of cercariae. Some small differences between the adults of individual trematode species leading to doubts about their species independence might be easily elucidated on the basis of surface structures of cercariae well visible in the SEM.

The present work represents a further contribution to the complex histochemical and ultrastructural investigations of larval stages of *D. lanceatum*. A previous paper on this subject has been published recently (Žďárská and Panin 1977).

MATERIAL AND METHODS

The material was obtained from spontaneously infected terrestrial snails, *Bradybaena lantzi*, collected in the pastures in the vicinity of Alma-Ata, in cooperation with the workers of the Zoological Institute of the Academy of Sciences of the Kazakh SSR. The cercariae and sporocysts from the hepatopancreas were repeatedly washed in saline and fixed in 3 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) at 4 °C for 2 h or in Baker's solution (Pearse 1968) at 4 °C for 14—21 days. The cercariae and sporocysts were then washed for 2—12 h in 0.1 M cacodylate buffer or distilled water and dehydrated through an ethanol series and amylacetate. Dehydrated cercariae and sporocysts were subjected to critical point drying (Anderson 1951), coated with carbon and gold or gold-palladium and viewed with a Jeol JSM-35 scanning electron microscope or the JXA-5 scanning device used with the JEM 100 B electron microscope at an accelerating voltage of 20 kV.

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RESULTS

The body surface of completely developed *D. lanceatum* cercaria has transverse ridges and at higher magnification folds of the tegument are well visible (Plate I, Figs. 1, 2, 4, 5). The body is provided with a long, smooth tail (Plate I, Fig. 1). The oral sucker is surrounded at its periphery by a large number of long and thin microvilli. Solitary tubercles appear on the surface of the oral sucker inside the fringe of microvilli (Plate I, Fig. 3). Outside this fringe there are some sensory papillae on the body of cercaria. The stylet pocket (Plate I, Figs. 2, 5) with a potruding part of stylet is located anterodorsally to the oral sucker. The openings of penetrating glands are not visible in this species, since they open at the base of the stylet pocket. The anterior

part of body surrounding the stylet pocket is provided with sensory papillae with or without a short cilium (Plate I, Figs. 2, 4, 5) and tubercles, in which the tegumentary folds are lacking only on their tips (Plate I, Figs. 4, 5). The ventral sucker, located in the middle of body, differs from the oral sucker in its structure. The ventral sucker has no continuous fringe of microvilli at its periphery. The microvilli are arranged in a small cluster only on the right and left lateral parts of the ventral sucker (Plate II, Figs. 1, 2) and only solitary tubercles are present on the remaining part (Plate II, Figs. 1, 2, 3). No sensory endings were observed on the posterior part of body and on the tail. At very high magnification, tegumentary folds and short, wide microvilli can be seen on the tail (Plate II, Fig. 4).

DISCUSSION

The SEM studies of cercariae reported by several authors (Hockley 1968, Robson and Erasmus 1970, Rees 1971, Køie 1971, 1973, Lo et al. 1975) concerned only the cercariae developing in water snails, i.e., those which actively leave the snail and swim in water. This is the first SEM study of cercariae developing in terrestrial snail which leave their host passively, in form of slimeballs. It was of interest to ascertain what was the difference between the surface structures of this cercaria and that of cercariae developing in water snails and living freely in water for some time. The surface structures of the oral sucker (microvilli and tubercles) and their localization in *D. lanceatum* cercariae are almost the same as in *Allopodocotyle lepomis* (Lo et al. 1975), *Zoogonoides viviparus* (Køie 1971) and *Parorchis acanthus* (Rees 1971) cercariae. However, the structure of the oral sucker differs from that of *Neophasis lageniformis* (Køie 1973) and *Schistosoma mansoni* cercariae (Hockley 1968, Robson and Erasmus 1970) which does not possess the fringe of microvilli. The ventral sucker of *D. lanceatum* cercaria differs from ventral suckers of all mentioned cercariae, with the exception of *Allopodocotyle lepomis*, in the presence of microvilli, but it is identical with them in the presence of tubercles.

The distribution of sensory endings on the body surface of cercariae is of importance in their systematics. Many authors have used whole mounts stained with silver nitrate for the detection of these structures. The chaetotaxy of *D. lanceatum* cercaria has not been studied by us and therefore it cannot be assessed what is the ratio of sensory papillae with and without cilia to argentophilic structures on the surface of other cercariae (Ginetsinskaya 1968, Richard 1971, Balušek and Vojtek 1973, Short and Cartrett 1973). It is probable that even the tubercles in the oral and ventral suckers of *D. lanceatum* cercaria are identical with the type of sensory endings without cilium which are present on the anterior part of body of cercaria and were described in other cercariae (Matricon—Gondran 1971, Žďárská 1975). According to Matricon—Gondran (1971), these sensory endings ("terminaisons en massue, sans contact avec l'extérieur") have a tactile function. The tubercles in the oral and ventral suckers seem to be concerned with host finding, attachment of suckers or penetration (Lo et al. 1975).

The tail, which is an organ of motion, loses its significance in *D. lanceatum* cercaria, since there is no possibility of its active utilization. We failed to detect sensory endings on its surface, in contrast to free-swimming cercariae.

The microvilli in oral and ventral suckers seem to perform a resorbing function. It is generally assumed that the microvilli increase the adsorptive surface of cercariae and occasionally function during secretion of the material. It has been lately reported

(McLaren and Hockley 1976) that they are also important for the removal of periphery components (e.g., glycocalyx) during transition from one developmental stage to another. Their function in *D. lanceatum* cercariae will certainly be elucidated by further studies in the transmissive electron microscope.

ИЗУЧЕНИЕ ЦЕРКАРИЙ *DICROCOELIUM LANCEATUM* ПОД СКАНИРУЮЩИМ МИКРОСКОПОМ

З. Ждярска

Резюме. Ротовая присоска совершенно развитых церкарий *Dicrocoelium lanceatum* окружена непрерывной каймой состоящей из долгих микроворсинок, тогда как микроворсинки на брюшной присоске расположены только в двух латеральных группах. На обеих присосках отчетливые бугорки. Стилетный карман окружен большим числом сенсорных сосочков с волоском или без волоска. Эти же сосочки имеются также вокруг ротовой присоски.

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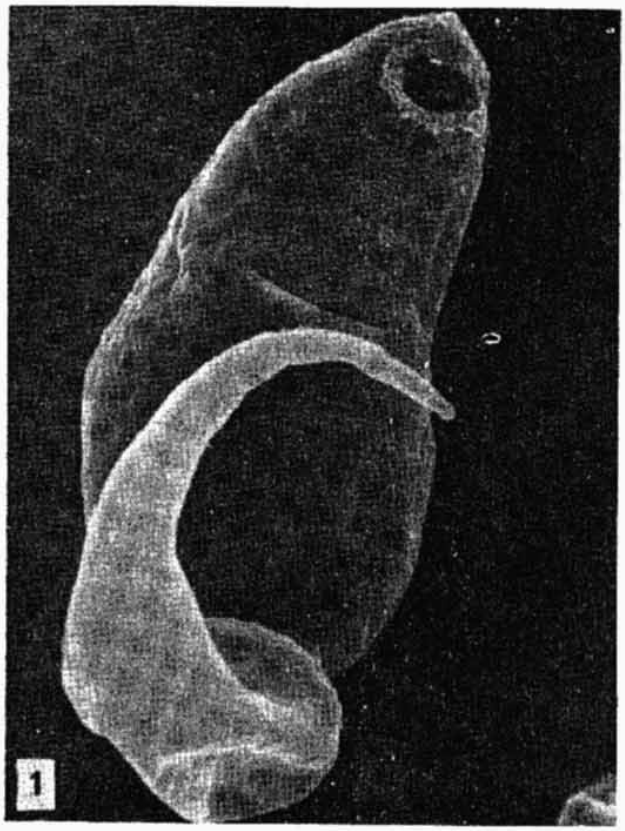
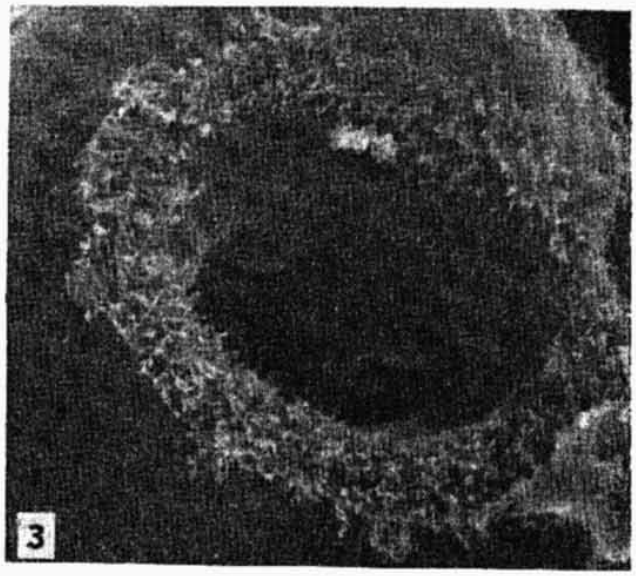
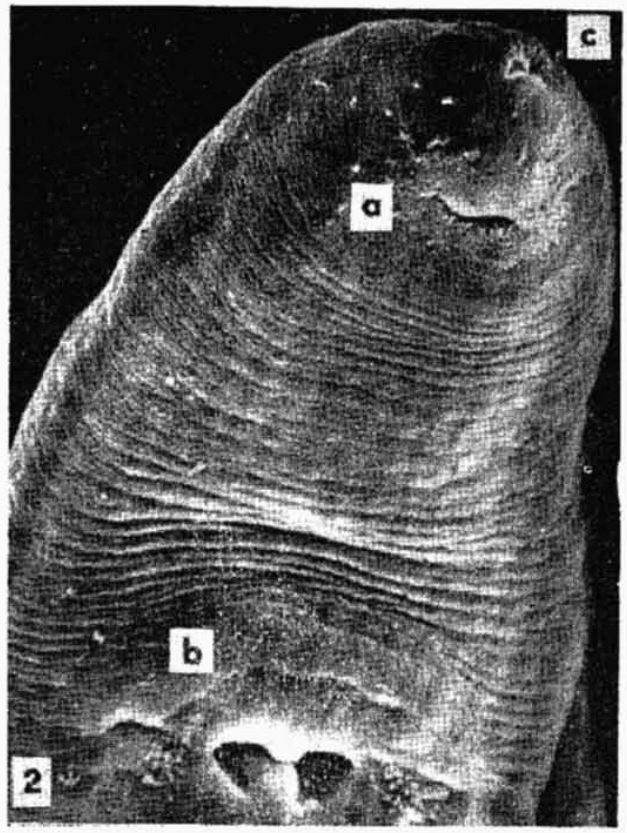
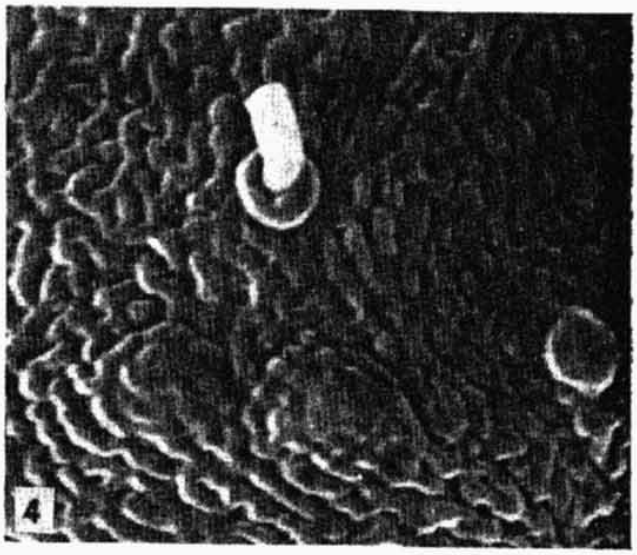
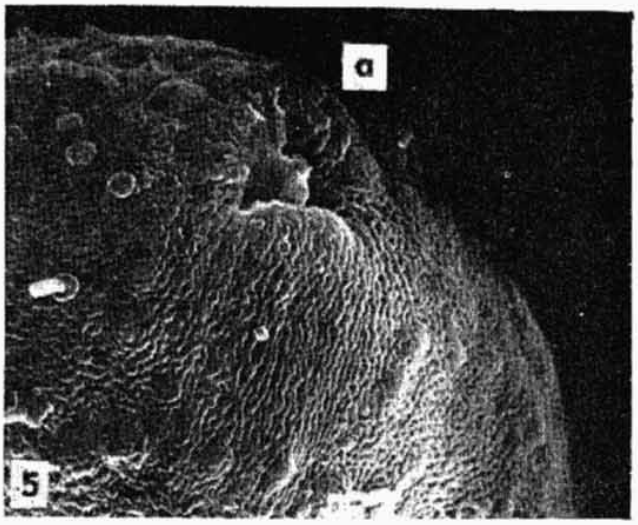


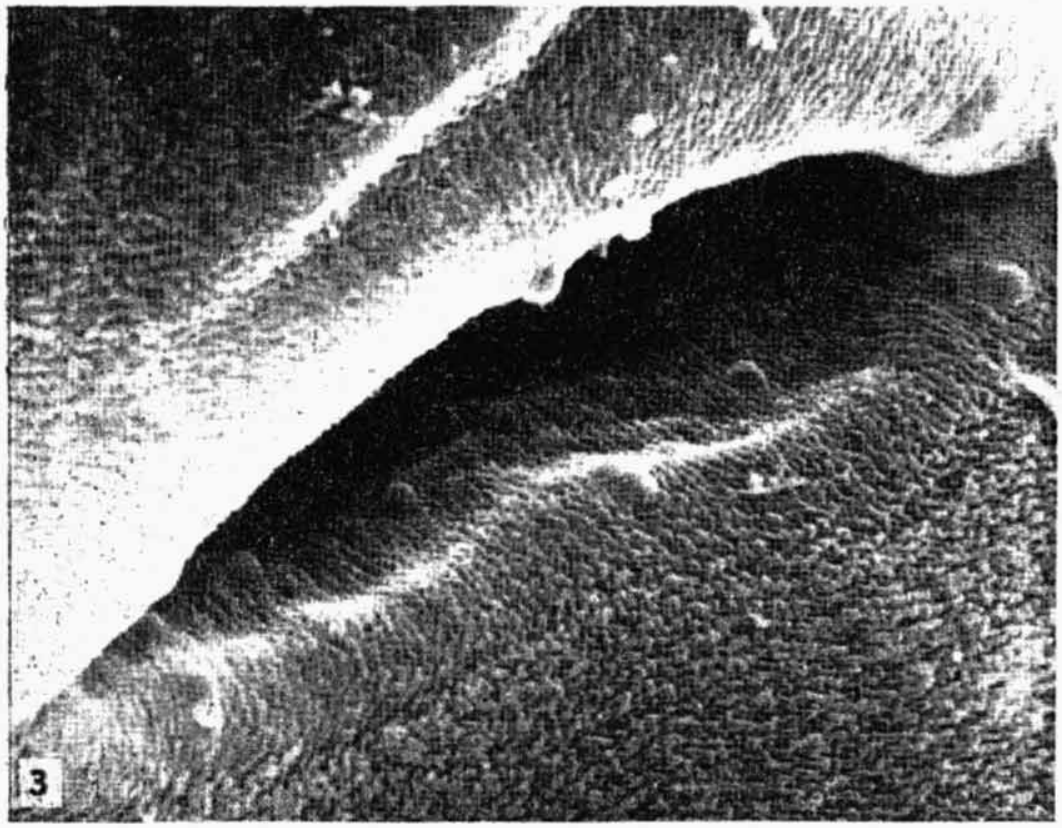








Figs. 1—5. Scanning electron micrographs of *Dicrocoelium lanceatum* cercariae. **Fig. 1.** Cercaria with non-contracted oral sucker and contracted ventral sucker. (×310). **Fig. 2.** Anterior part of body of cercaria. a — oral sucker, b — non-contracted ventral sucker, c — stylet pocket. (×1,000). **Fig. 3.** Detail of oral sucker from Fig. 1 with well visible fringe of thin microvilli. (×1,800). **Fig. 4.** Detail of sensory endings from Fig. 5 distributed around the stylet pocket. Sensory papilla with cilium (at the top), sensory papilla without cilium (right at the bottom) and two tubercles (left at the bottom). (×10,200). **Fig. 5.** Distribution of sensory endings ventrolateral to stylet pocket (a) from which protrudes the tip of stylet. (×3,400).



Figs. 1—4. Scanning electron micrographs of *Dicrocoelium lanceatum* cercariae. Fig. 1. Ventral sucker (lateral view). Note the group of long thin microvilli and tubercles. (×3,600). **Fig. 2.** Ventral sucker (ventral view) with well visible two groups of microvilli located laterally and some tubercles situated in anterior half of the sucker. (×3,000). **Fig. 3.** Contracted ventral sucker (ventrolateral view). Note location of tubercles and arrangement of tegumentary folds in posterior half of ventral sucker. (×3,000). **Fig. 4.** Detail of tail tegument. (×15,000).