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ULTRASTRUCTURE OF THE CERCARIA OF BRACHYLAIMUS AEQUANS

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Abstract. It was demonstrated that the tegument structure in cercariae of *Brachylaimus aequans* is identical with the tegument structure in cercariae of other previously studied members of the superfamily Brachylaimoidea — *Leucochloridium paradoxum* and *Hasstilesia ovis*. The tegument of a developed *B. aequans* cercaria contains rod-shaped and oval, strongly electron-dense granules and is covered with a high glycocalyx. Multiciliated sensory receptors are localized in the tegument. The penetration gland cells are filled with large electron-dense granules and their ducts have a supporting ring of microtubules.

This paper is a continuation of previous studies (Žďárská and Soboleva 1980 a, b) dealing in detail with the larval stages of *B. aequans*. It is a part of complex investigations concerning the adaptation of trematode larvae of the superfamily Brachylaimoidea to the existence under terrestrial conditions (Žďárská 1981, 1983, Ždárská and Soboleva 1981 a, b, 1982 and Žďárská et al. 1982).

MATERIAL AND METHODS

The cercariae were recovered by Dr. T. N. Soboleva (Zoological Institute of the Kazakh Academy of Sciences, Alma-Ata) from spontaneously infected snails *Macrochlamys kazachstanica* Tzwetkov, 1940 collected in the vicinity of Alma-Ata. Parts of hepatopancreas with sporocysts and cercariae were 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 in Epon and Araldit. Ultrathin sections were cut with Reichert OM-U2 ultramicrotome, contrasted with 20 % uranyl acetate and Raynolds solution of lead acetate and examined in JEM 100B electron microscope.

RESULTS

The body of cercaria is covered with a tegument the structure of which changes with the age of the cercaria. In a very young cercaria (Plate II, Fig. 2) the tegument is formed by a thin cytoplasmic layer the outer part of which projects in single short microvilli (Plate II, Fig. 1) or by a narrow cytoplasmic layer with large vacuoles (Plate II, Fig. 3). The tegument of a fully developed cercaria (Plate III, Fig. 1, 2, 3) is higher and contains a large number of oval and rod-shaped electron-dense granules. The rod-shaped granules are situated mostly in the outer layer of tegument, whereas the oval ones are at its base. The rod-shaped granules are oriented mainly horizontally, only some of the granules near the surface vertically. These granules are produced by subtegumental cells. Under the tegument is a thin lamina basalis. The basal plasmalemma of the tegument forms numerous infoldings reaching up to the middle of tegument height. Under lamina basalis are bundles of circular and longitudinal muscle fibres (Plate I, Fig. 1). The surface of body tegument is covered with a high glycocalyx (Plate I, Fig. 1), the fibres of which are connected with the outer lamina of the trilaminar unit membrane (Plate III, Fig. 2). The glycocalyx in the cavity of oral sucker is much lower and the tegument forms larger infoldings (Plate, I. Fig. 3). In the tegument of the oral sucker

are sensory receptors with 4 short cilia, which are covered by a thick infolding of tegument (Plate IV, Fig. 3). In the anterior part of oral sucker open penetration gland cells, the ducts of which are situated immediately under the body muscle layer (Plate I, Fig. 1). The walls of the penetration gland cell ducts contain microtubules (Plate I, Fig. 2). The content of the penetration gland cells consists of large, irregular, strongly electron-dense granules lying close to one another (Plate I, Fig. 2). The walls of excretory canals form single microvilli (Plate IV, Fig. 2). The flame cells possess a large number of cilia, some of which exhibit deviations in the structure of central double microtubules (Plate IV, Fig. 1).

DISCUSSION

The ultrastructure of tegument in *B. aequans* cercaria is the same as in other species of the superfamily Brachylaimoidea studied previously. In *B. aequans* cercaria, like in the cercariae and metacercariae of *Hasstilesia ovis* (Žďárská and Soboleva 1982) and *Leucochloridium paradoxum* (Žďárská 1981, Žďárská et al. 1982), a high layer of glycocalyx connected with the outer lamina of the trilaminar unit membrane was demonstrated on the surface of the tegument. Like in the cercaria and metacercaria of *H. ovis*, the glycocalyx in the cercaria of *B. aequans* does not contain calcium salts, which were detected in *L. paradoxum* metacercariae (Žďárská 1981). The tegument proper contains a large number of rod-shaped and oval electron-dense granules in all of the three species. The ultrastructure of the tegument in a very young cercaria differs from that in a fully developed cercaria in the presence of microvilli and absence of glycocalyx. The tegument of a fully developed cercaria is covered by a high layer of glycocalyx. Also the histochemical studies revealed some differences. The tegument of a very young cercaria exhibits a high activity of alkaline phosphatase, which is lacking in the tegument of a fully developed cercaria (Žďárská and Soboleva 1982 b — Plate II, Fig. 2). The presence of microvilli and activity of alkaline phosphatase in a developing cercaria indicate an intense transport of substances in the young developmental stages. The presence of microvilli in young cercariae and of glycocalyx in mature cercariae was observed also by Køie (1971) in *Zoogonoides viviparus*. It is interesting that the ultrastructure of larval tegument in *B. aequans* is the same as in *L. paradoxum* and *H. ovis*, though the life cycle of *B. aequans* is triheteroxenous and that of *L. paradoxum* and *H. ovis* secondarily diheteroxenous (Žďárská and Soboleva 1982, Žďárská et al. 1982). Although the cercaria of *B. aequans* actively leaves the first intermediate host and in the second intermediate host slowly changes into a non-encysted metacercaria, its tegument does not differ from that of the cercaria and metacercaria of *L. paradoxum* and *H. ovis* which never leave the first intermediate host. It remains unsolved whether the terms used for these types of metacercariae are correct with regard to their envelopes. Are they encysted or non-encysted? In our opinion, having regard to the division of metacercariae published by Dönges (1969), non-encysted metacercariae are involved in case of the three above-mentioned species of the superfamily Brachylaimoidea. The high glycocalyx on the tegument surface of *L. paradoxum* and *H. ovis* metacercariae appears like a cyst, but it is no cyst in the proper sense. The metacercaria does not lie freely in the cavity, but its tegument (outer lamina of trilaminar unit membrane) is connected with the high glycocalyx on the body surface, in the cavities of both suckers and even in pharynx (Žďárská 1983).

The contents and structure of penetration gland cells in *B. aequans* cercaria are the same as in *Schistosoma mansoni* (Erasmus 1972), *Zoogonoides viviparus* (Køie 1971) and *Microphallus similis* cercaria (Davies 1980). A similar structure have also the gland cells of the lappet region of the adult trematode *Apatemon gracilis minor* (Erasmus 1969). The presence of peripheral microtubules in the penetration gland ducts has been reported also by other authors (Krupa et al. 1968, Kemp 1970, Robson and Erasmus 1970, Køie 1971, Morris 1971).

Only one type of sensory receptors (multiciliated) was found in the oral sucker, though the scanning electron microscopy revealed more types (Žďárská and Soboleva 1970a). This is the first finding of multiciliated receptor in a member of the superfamily Brachylaimoidea. This type of receptor is identical with multiciliated receptors in the cercariae of *Paryphostomum segregatum* and *Echinostoma paraense* described by Matricon-Gondran (1971). The ultrastructure of sensory receptors in members of Brachylaimoidea was described only in case of *H. ovis* metacercaria, where only uniciliated receptors were present (Žďárská and Soboleva 1982).

УЛЬТРАСТРУКТУРА ЦЕРКАРИИ *BRACHYLAIMUS AEQUANS*

З. Ждярска

Резюме. Ультраструктура тегумента церкарий *Brachylaimus aequans* соответствует ультраструктуре тегумента раньше изучаемых видов надсемейства Brachylaimoidea — *Leucochloridium paradoxum* и *Hasstilesia ovis*. Тегумент развитой церкарии *B. aequans* содержит палочковидные и овальные, сильно электронноплотные гранулы и покрыт высоким гликокаликсом. В тегументе находятся многосничные сенсорные рецепторы. Клетки желез проникновения выполнены большими электронноплотными гранулами и в их протоках большое количество микротрубочек.

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Parasitological problems at the XVIII International Ornithological Congress in Moscow

Among the numerous scientific meetings organized in 1982, the XVIII International Ornithological Congress held in Moscow on 16—24 August was of a special importance. The program was very rich: 7 plenary sessions, 47 specialized symposia, 43 round-table discussions and several meetings of international commissions and scientific societies. More than 900 scientists from about 40 countries participated in the Congress.

Among the versatile ornithological problems discussed at the Congress a great attention was paid to Symposium No. 23, Avian parasites. It was held on 22nd August and included the problems of the relationship between parasites and host birds. The significance of birds as hosts of various arthropods was dealt with in the report by Dr. V. Černý (Czechoslovakia). He deduced theoretical aspects particularly in the field of phylogenetic relations between birds and arthropods and stressed the role of arthropods as vectors of arboviruses. Prof. W. Eichler (G.D.R.), using Mallophaga as an example, analyzed in detail their parasitophyletic relations with birds of the order Anseriformes. The next three reports were devoted to wider problems of bird helminths. Prof. K. M. Ryzhikov and Prof. P. G. Oshmarin (U.S.S.R.) presented the results of a detailed analysis of helminth fauna of ducks with a special regard to *Anas platyrhynchos* L. The influence and significance of migration ways on the formation of helminth fauna in Charadriiformes was evaluated by Prof. M. D. Sonin and Dr. A. N. Pelgunov (U.S.S.R.) using a matrix analysis. The theoretical and practical consequences of the exchange of helminth fauna between free-living birds and water fowl were dealt with in the report by Prof. B. Ryšavý (Czechoslovakia). Summaries of these and other reports were published in XVIII CIO, Moscow 1982, Abstracts, pp. 77—82.

The interest in the problems discussed at this symposium continued at the round-table discussion held at the Helminthological Laboratory of the U.S.S.R. Academy of Sciences on 23rd August 1982. Many other topical questions were dealt with, as the effect of the process of synanthropization and synurbanization on the formation of helminth fauna in birds, the limits of application of parasitophyletic rules, practical importance of the phenomenon of reservoir parasitism and hostality, as well as some epidemiological and epizootological concepts. Parasitological problems, especially results of ecological-taxonomical analysis of helminths parasitizing birds, were the topic of posters presented by Prof. B. E. Kurashvili, Dr. A. P. Maksimova, Dr. N. M. Pronin and Prof. A. A. Spassky.

XVIII International Ornithological Congress was a very successful scientific meeting and we want to thank the Soviet zoologists for its organization. The synthesis of parasitological and ornithological problems and their evaluation at this wide international scientific forum was very useful and significant for further development of the two scientific fields.

Academician V. Baruš and
Prof. Dr. K. M. Ryzhikov

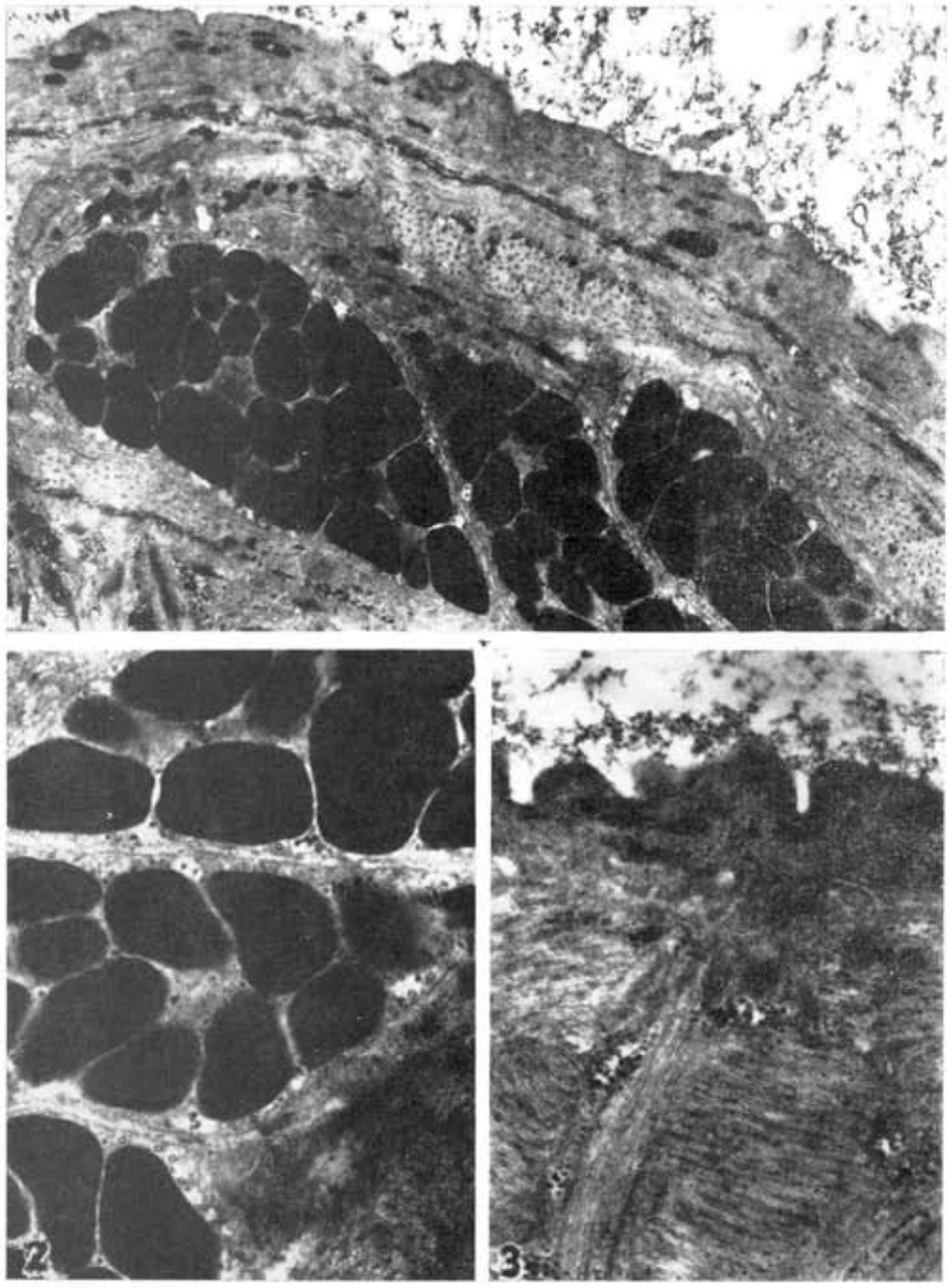

Fig. 1. Section through anterior part of body of *B. aequans* cercaria in the region of penetration gland cell ducts filled with irregular large electron-dense granules. The body tegument of cercaria is covered with high glycocalyx (at the top). (G, Os, UAc, Pb). (× 17 000). **Fig. 2.** Detail of penetration gland cell ducts with well visible microtubules under plasmalemma (G, Os, UAc, Pb) (× 41 500). **Fig. 3.** Detail of tegument and muscles of oral sucker (G, Os, UAc, Pb) (× 37 800).

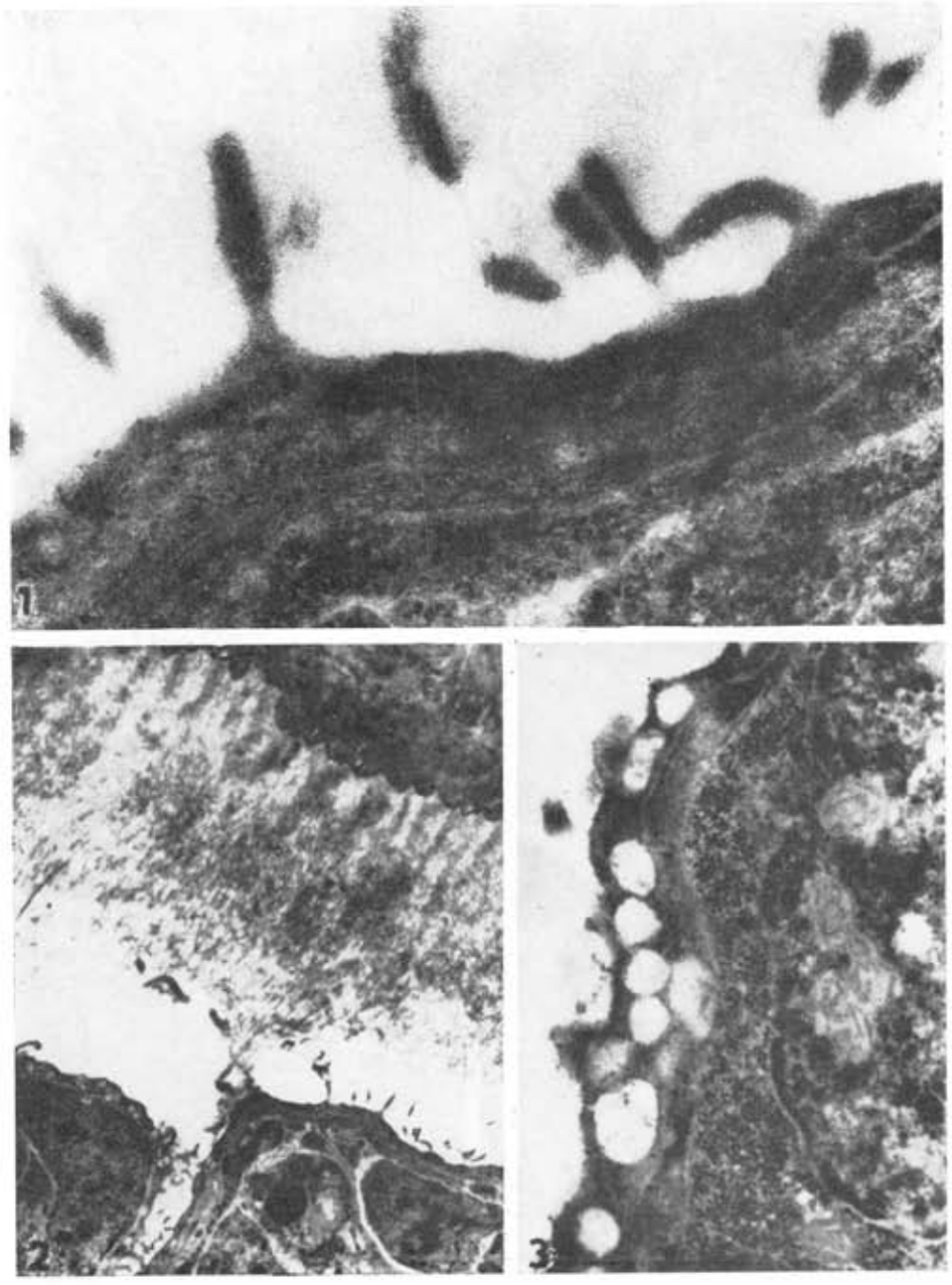

Fig. 1. Detail of tegument of a very young cercaria of *B. aequans* from Fig. 2. (right at the bottom) covered with microvilli (G, Os, UAc, Pb) (× 51 900). **Fig. 2.** Tegument of fully developed cercaria with a high glycocalyx (at the top) and two very young cercariae (left and right at the bottom). (G, Os, UAc, Pb) (× 7 700). **Fig. 3.** Detail of tegument of a very young cercaria from Fig. 2 (left at the bottom) the tegument of which contains large vacuoles (G, OS, UAc, Pb) (× 27 000).

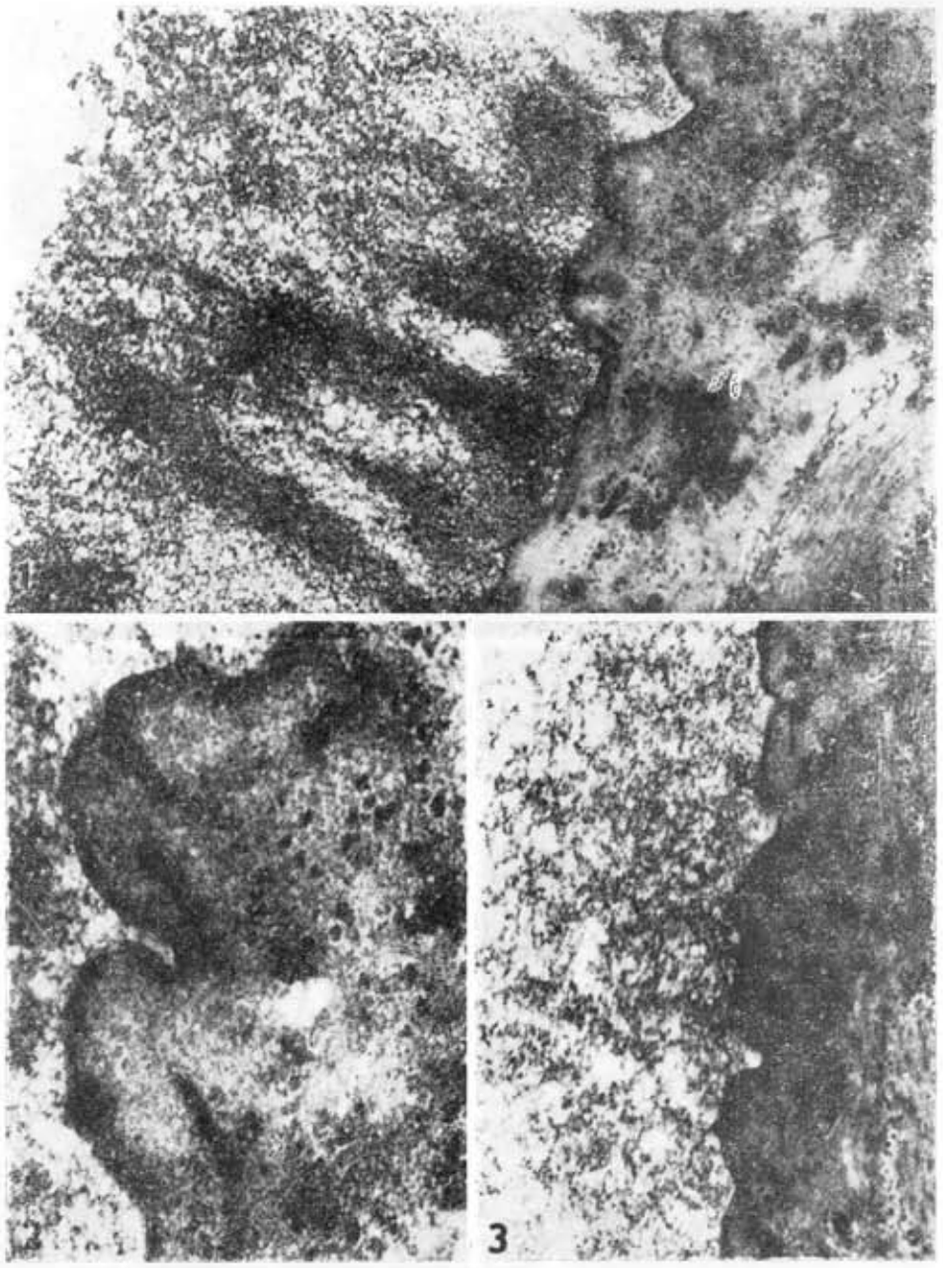

Fig. 1. Tegument of *B. aequans* cercaria the plasmalemma of which is united with a high glycocalyx (G, Os, UAc, Pb) (× 32 000). **Fig. 2.** Detail of unite membrane, the outer lamina of which is connected with glycocalyx filaments (G, Os, UAc, Pb) (× 70 000). **Fig. 3.** Distribution of dense granules in the tegument (G, Os, UAc, Pb) (× 26 950).

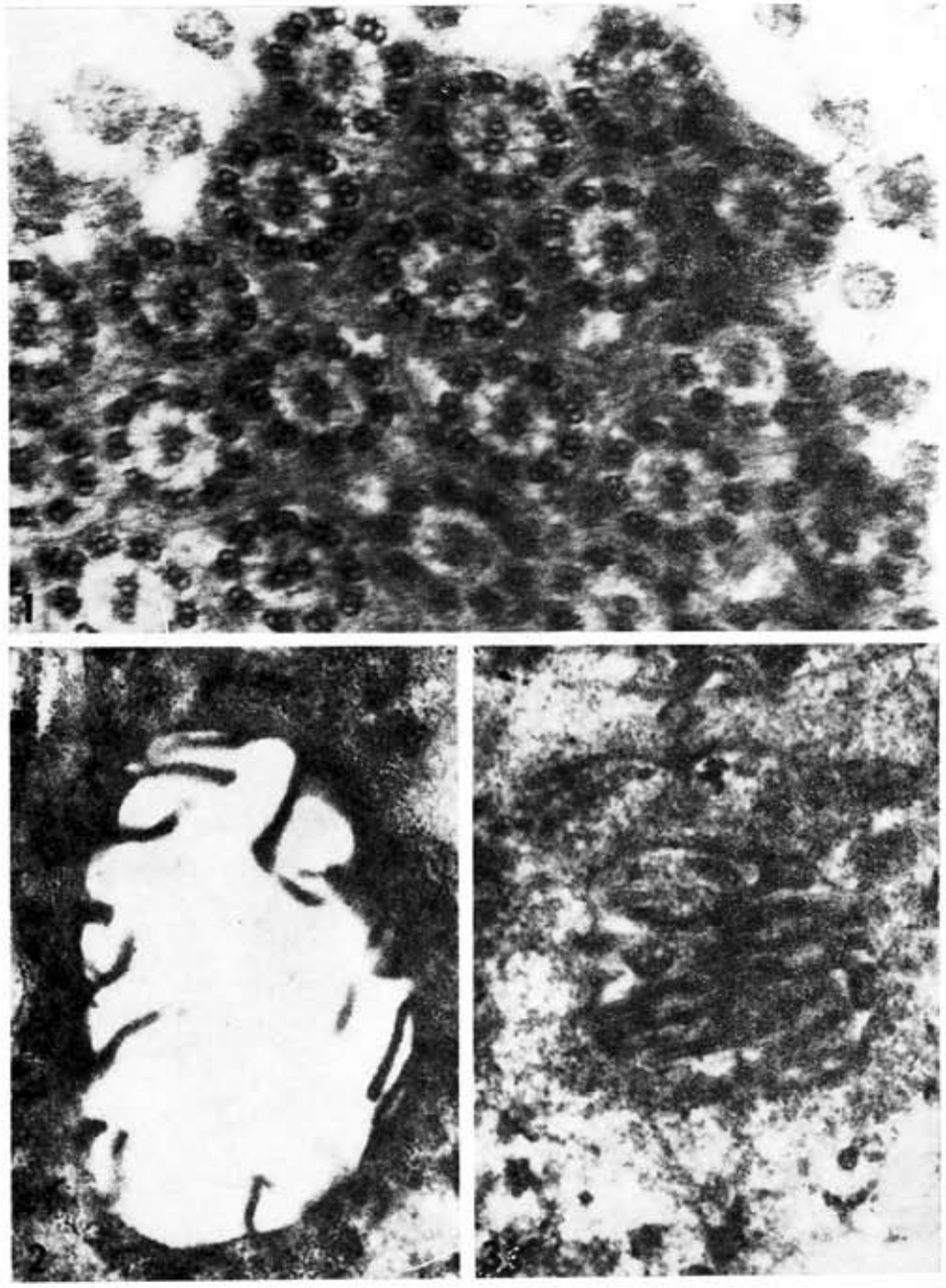

Fig. 1. Transverse section through cilia of flame cell in *B. aequans* cercaria. One of the cilia (in the middle) has only one central microtubule (G, Os, UAc, Pb) (× 80 250).
Fig. 2. Transverse section through wall of excretory canal (G, Os, UAc, Pb) (× 30 800). **Fig. 3.** Oblique section through multiciliate sensory ending in tegument of oral sucker of cercaria (G, Os, UAc, Pb) (× 53 900).