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**MORPHOLOGY OF CESTODE
WITH ATYPICAL MODE OF ATTACHMENT**

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Morphological features of *Rauschitaenia ancora* (Mamaev, 1959), a cestode with atypical mode of attachment («anchoring» in the wall of the host intestine), are considered. At the center of the overgrown scolex there is a rostellum, size of which is close to that of the developed metacestode. Large suckers are composed mainly of loose parenchyma and fine radial muscle fibers. Fragments of host tissue are noted in the suckers' cavity. Surface of the scolex is covered with large microtriches, which are in contact with the host tissue having signs of degradation. Distal cytoplasm is filled with vesicles coming from tegumental cytons. Rostellar glands that are common to cyclophyllids are not found. Excretory canals contain fluid (closer to the surface) and numerous lipid droplets (deeper located canals).

Key words: Cestoda, ultrastructure, attachment organs, host-parasite relationships, excretory system, rostellar glands.

**МОРФОЛОГИЯ ЦЕСТОДЫ
С НЕТИПИЧНЫМ СПОСОБОМ ПРИКРЕПЛЕНИЯ**

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Рассматриваются особенности морфологии *Rauschitaenia ancora* (Мамаяев, 1959), цестоды с нетипичным способом прикрепления («заякоривание» в стенке кишки хозяина). В центре разросшегося сколекса расположен хоботок, сохранивший размеры, близкие к таковым хоботка сформированной метацестоды. Крупные присоски состоят в основном из рыхлой паренхимы с включением радиальных мышечных волокон. В полости присосок отмечены фрагменты ткани хозяина. Поверхность сколекса покрыта крупными микротрихиями, которые контактируют с тканями хозяина, имеющими признаки деструкции. Дистальная цитоплазма заполнена везикулами, поступающими из цитонов тегумента. Хоботковых желез, обычных для циклофиллид, не

обнаружено. Экскреторные каналы содержат жидкость (поверхностные каналы) и многочисленные липидные капли (более глубокие каналы).

Ключевые слова: Cestoda, ультраструктура, органы прикрепления, паразито-хозяйинные взаимоотношения, экскреторная система, хоботковые железы.

The new genus of cestodes with a single species *Rauschitaenia ancora* (Mamaev, 1959) was described within the family Dilepididae on the material from three common snipes *Gallinago gallinago* L., obtained in the Chaun lowland, Magadan Province (Bondarenko, Tomilovskaya, 1979). Representatives of this species differed by atypical mode of attachment to the host intestine. If the majority of «higher» cestodes attach with the help of the rostellum and four suckers, besides, providing movement of the parasite, then the scolex and the anterior part of *R. ancora* strobila penetrate deeply into the intestinal wall almost perforating it. The scolex of the cestode increases almost by 10 times in comparison with the larva; a clearly visible capsule on the outer surface of the intestine is formed around the front part of the worm excluding any change of localization (Bondarenko, Tomilovskaya, 1979). Another structural peculiarity of *R. ancora* concerns the presence of multiple anastomosed excretory vessels, among which it is impossible to distinguish two pairs of main longitudinal canals common for cestodes.

The atypical attachment mode which defines tissue localization of the front part of the cestode with the rest of strobila situated in the intestinal lumen was determined as «anchoring» (Dogel, 1947). Penetration and overgrowth of the scolex considerably increase contact area of the parasite with host tissues, causing degradation of the mucosa and leading to the development of immune response of the host. Thus, the study of the morphology and ultrastructure of *R. ancora* is of great interest for understanding of both biology peculiarities of cestodes with atypical attachment mode and morphological aspects of relations between penetrated parasite and its host.

MATERIAL AND METHODS

Adult cestodes *R. ancora* from common snipes *G. gallinago*, taken with capsules and fragments of intestine wall, are served as the material. For convenience some capsules were dissected, and scolices of cestodes were fixed separately. Fixation and preparation of the material for the light and electron microscopy were conducted according to the standard method with some modifications (Pospekhova, Regel, 2015). Semi-thin sections obtained with the use of LKB Bromma 2088 and LKB Nova (Sweden) ultramicrotomes were stained with methylene blue according to Morgenstern (Morgenstern, 1969) and examined under an Olympus CX41 microscope (Olympus Corporation, Japan) with an Olympus E-420 digital camera. Ultra-thin sections were examined under JEM-1011 and JEM-1400Plus transmission electron microscopes (JEOL, Japan).

RESULTS AND DISCUSSION

Light microscopy. In sections, the overgrown scolex taken from the capsule possesses loose net-like structure (fig. 1, *a, b*, see ins.). Muscular wall of the rostellum, rostellar sac and suckers, covers of the scolex and the neck look denser. Loose parenchyma of the scolex is formed mainly of widened processes, some part of the latter containing dense round bodies (apparently, lipid droplets), others have no visible content. Large suckers also have loose structure; they are mainly formed of multiple processes without visible content divided by radial musculature.

The rostellum is located in the center of the scolex and is deeply submerged in the bottom of the retraction channel (fig. 1, *b*). The size of the rostellum in the developed metacestode (fig. 1, *c*) is 0.12×0.04 mm; penetrated cestode possesses slightly enlarged rostellum, $0.19\text{--}0.22 \times 0.07\text{--}0.08$ mm, that is disproportionately small comparing to the scolex of the penetrated cestode ($1.7\text{--}1.8 \times 1.2\text{--}1.4$ mm) (Bondarenko, Tomilovskaya, 1979). Rostellum length of the penetrated cestode in our work does not exceed 0.1 mm (fig. 1, *d*), diameter of the scolex, 0.9 mm, and diameter of suckers, 0.4 mm.

The front part of the rostellum with closely fitting hooks protrudes into the cavity, which, apparently, corresponds to that of the rostellar sac, and under normally developed rostellum forms its lateral surface when the rostellum is protruded. Walls of the cavity lack microtriches (we use here the term «microtrix» (pl. «microtriches») according to Chervy, 2009), which appear antieriad and cover the surface of the twisted retraction canal (fig. 1, *d*). Microtriches («spines», according to Bondarenko, Tomilovskaya, 1979) are large, varying from 3 to 5 μm . Their fine structure was described earlier (Pospekhov, Pospekhova, 1993).

Electron microscopy. Muscular walls of the rostellum, that is up to 10 μm thick in its basal part, are formed of inner circular and outer longitudinal fibers. The widened base of the rostellum is crossed by retractor muscles of the hooks. Processes of muscle cells containing lipid drops and separate cells resembling tegumental cytons in the shape and density of the cytoplasm, or glandular cytons without secretory inclusions are found in spaces between hooks. The wall of the rostellar sac has insignificant thickness, about 4 μm , and is formed of annular fibers. The outer layer of longitudinal musculature, typical of other species of the family Hymenolepididae, is not expressed here. No accumulations of glandular elements were noted in the rostellar sac. Tegumental cytons are large, with well-developed synthetic apparatus and numerous light vesicles, formed in the Golgi complex (fig. 2, *a*, see ins.). Cytons are located in the front part of the rostellar sac; their secretion (light vesicles with flaky content) runs into the tegument of apical part of the scolex (fig. 2, *b*). Morphology of the scolex tegumental cytons is identical to that described in cytons of the rostellar sac.

Sensory endings, both with and without cilia, were found in scolex and neck covers, at that, specific endings with central dense body (fig. 2, *c*) were noted in suckers' tegument, those were also found in suckers' covers of other representatives of Cyclophyllidea (Pospekhov, Krasnoshchekov, 1992; Pospekhova, Pospekhov, 1998; Pospekhova, Bondarenko, 2014). Authors suppose, those endings can register deformation degree of tegument in localization area of fixatory microtriches.

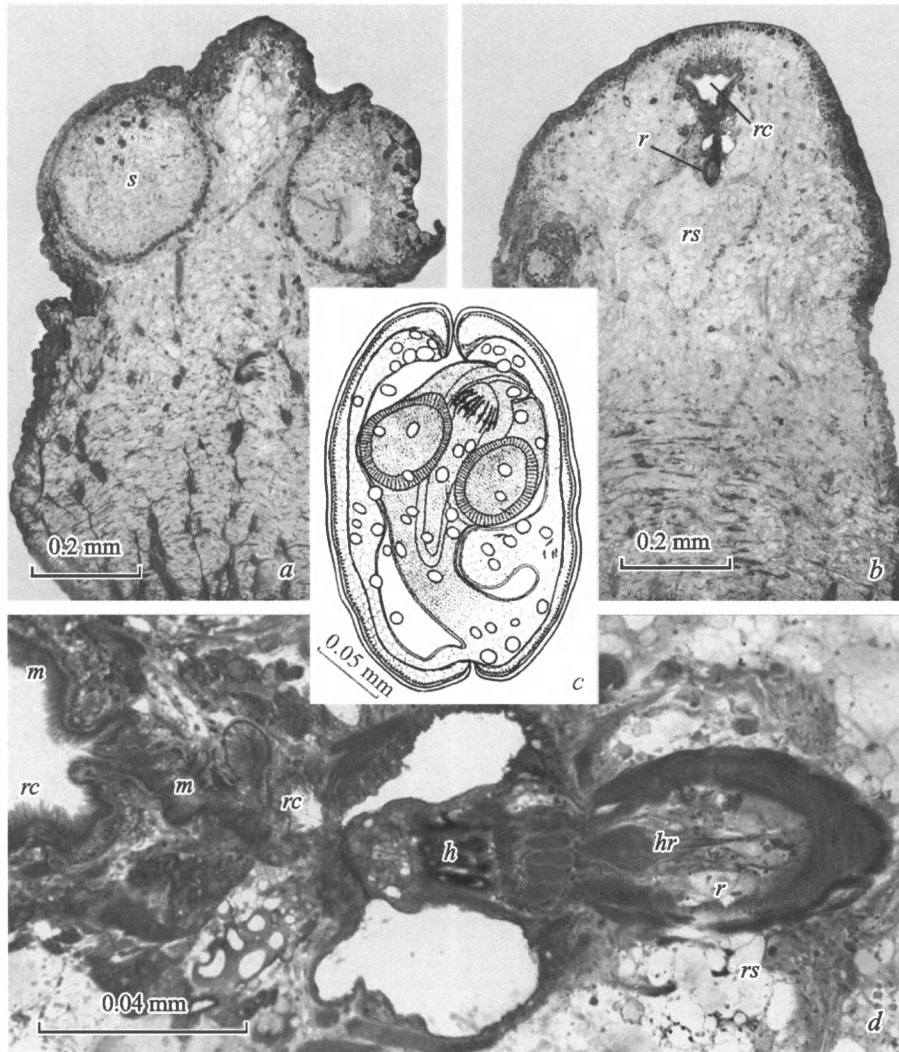


Fig. 1. Semi-thin sections of the scolex of *Rauschitaenia ancora* without capsule (*a*, *b*, *d*) and scheme of developed metacystode (after Bondarenko, Tomilovskaya, 1979, with courteous permission of authors) (*c*).

a — tangential section, *b* — longitudinal section through the middle of the scolex, *d* — region of the rostellum. *h* — hooks, *hr* — hook retractor, *m* — microtrix, *r* — rostellum, *rc* — retraction channel, *rs* — rostellar sac, *s* — sucker.

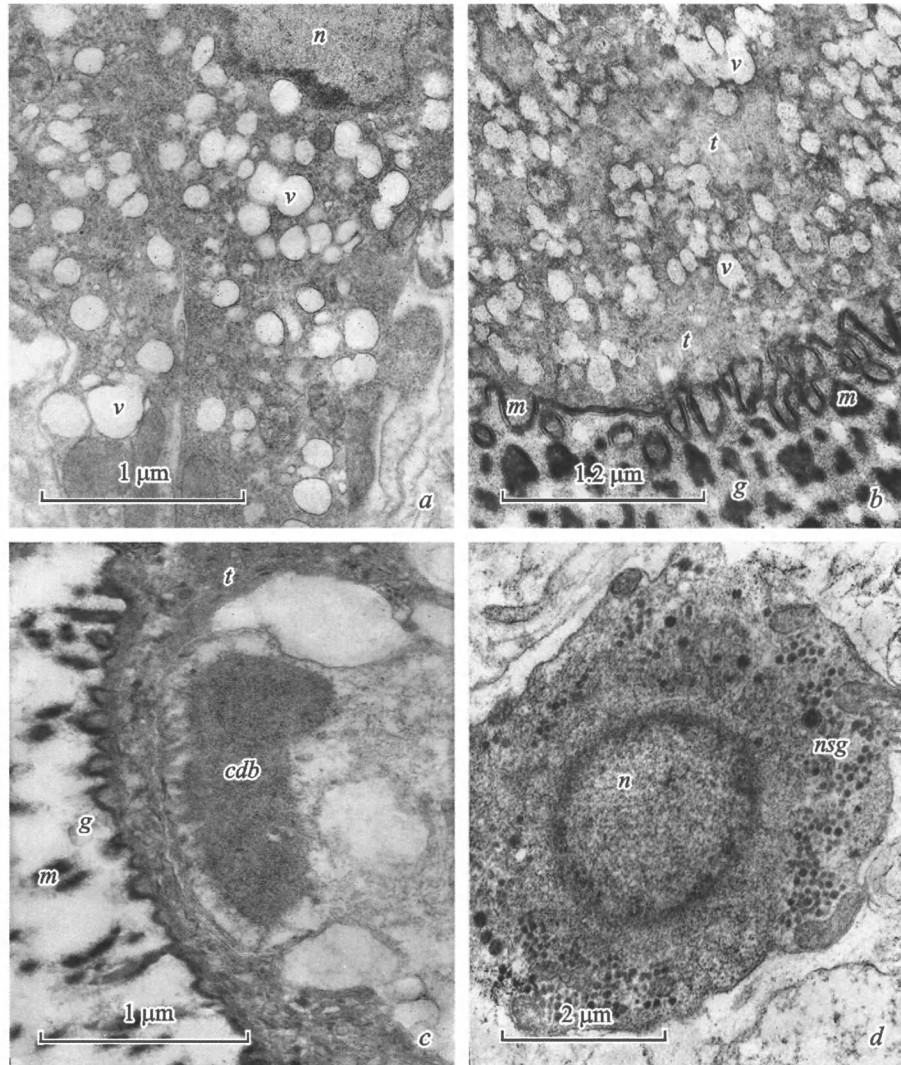


Fig. 2. Ultrastructure of the scolex of *Rauschitaenia ancora*.

a — tegumental cyton of rostellar sac, *b* — distal cytoplasm of scolex tegument, *c* — specialized sensory endings in the suckers' tegument, *d* — neuron. *cdb* — central dense body, *g* — glycocalyx, *m* — microtrix, *n* — nucleus, *nsg* — neurosecretory granules, *t* — distal cytoplasm of tegument, *v* — vesicles with flocculent material.

Within the scolex at least two types of large (about 15 μm) neurons (fig. 2, *d*) were found, however, no large nerve formation were noted with the exception of two nerve cords going from neck. Probably, during overgrowth of scolex, some compact formations (i. e., nerve ganglia) could transform into diffuse accumulations of tissue hardly distinguishable in semi-thin sections.

Ultrastructural study of suckers showed their frame to be formed of radial muscles, while multiple processes of muscle cells form parenchyma of the sucker. The diameter of radial muscles (about 10 μm) looks small in comparison with suckers' size of the overgrown scolex. Suckers are separated from the scolex parenchyma by a thin basal lamina (basal matrix) adjacent to fibers of circular muscles. In longitudinal sections suckers are cup-shaped, their cavities are constantly filled with fragments of the host intestinal epithelium, recognized by the presence of microvilli (fig. 3, *a*, see ins.). This, apparently, indicates rather strong attachment to intestinal lining with the help of suckers at first stages of attachment. Later, probably, penetration into host tissues occurs with the help of hooks and rostellar musculature, while fragments of the intestinal epithelium stay in suckers' cavities after penetration of the scolex into intestinal wall. Isolated fusiform cells resembling fibroblasts were noted between fragments of the intestinal epithelium and microtriches of suckers. Besides, host leukocytes, mainly neutrophilic granulocytes, are found in the suckers' cavity (fig. 3, *b*). We should note rather frequent occurrence of separate erythrocytes close to the scolex tegument.

A layer of the glycocalix (fig. 3, *c*), integrating separate microtriches into united structure — microtrichial border, is constantly registered on the surface of the scolex and neck microtriches. It is in close contact with host tissues and evidently represents rather strong (both mechanical and biochemical) barrier, protecting parasite from immune reactions of the host. Generally, intact tissues and cells of the host are separated from the microtrichial border by layer of detritus and cells with signs of destruction (fig. 3, *d*).

Elements of the excretory system (cyrtocytes and canals of different diameter) are scattered in scolex parenchyma, however, we did not find penetration of canals into cavity of the rostellar sac, as is usually observed in other representatives of the suborder Hymenolepidata (Pospekhova, 2009b, c). Sections of large excretory canals surround suckers along the perimeter; they are located in cortical parenchyma of scolex base and neck. Part of them is filled with fluid (fig. 4, *a*, see ins.), while another part has no visible content. Beside fluid, which is stained with methylene blue into light blue, lipid drops, both isolated drops and accumulations of drops of different sizes, are regularly registered in canals' lumen, where large lipid accumulations are found around these canals (fig. 4, *b*). Ducts with fluid and lipids, however, differ in the depth of location: canals with fluid are closer to the surface, whereas lipid accumulations are registered in deeper canals. Those differences can be caused by flow direction and functional meaning of the canals.

Walls of canals have dense cytoplasm, as well as rounded processes of the inner surface — microvilli. Numerous processes of canal and muscle cells are fastened to duct walls by dense contacts (fig. 4, *c*). Large number of dense contacts around canal walls and in the parenchyma of the scolex lets us suppose that they help to provide structural and functional integrity of the loose tissue of the overgrown scolex.

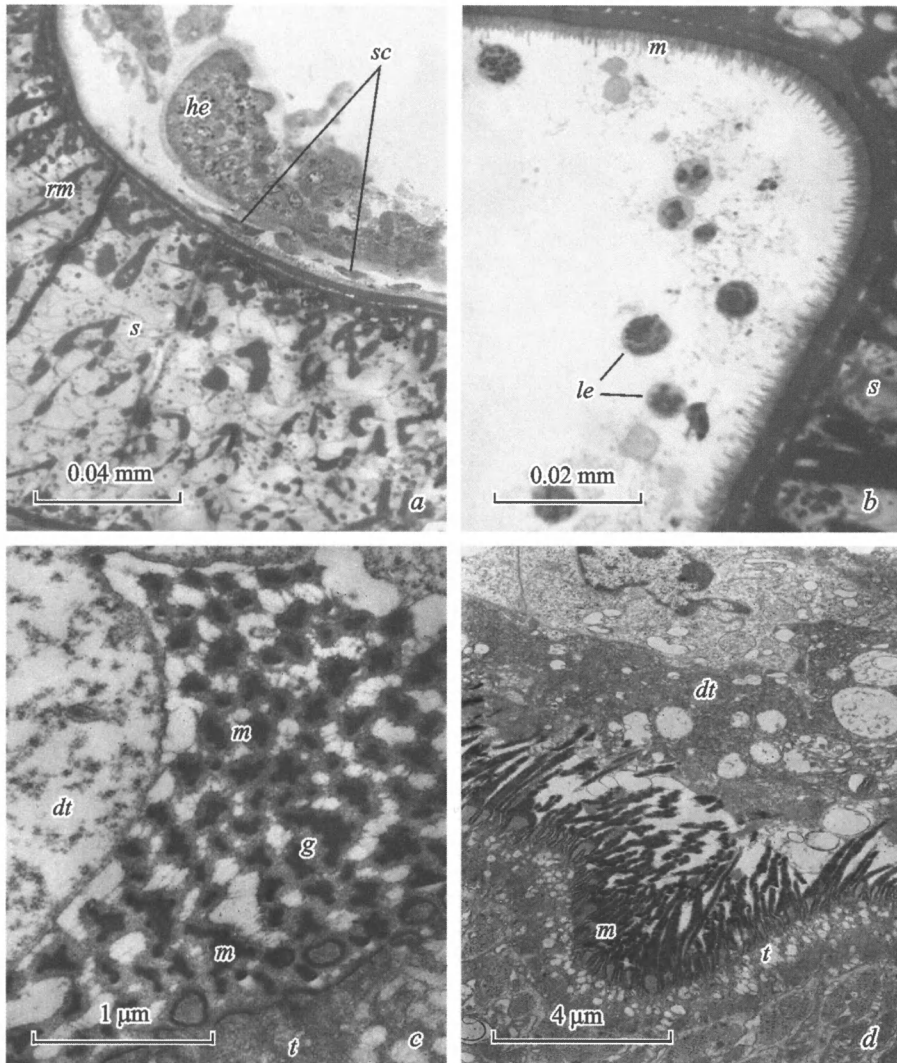


Fig. 3. Morphology of host-parasite relationships of *Rauschitaenia ancora*.

a, b — semi-thin section of suckers (*a* — the host intestinal epithelium in the suckers' cavity, *b* — white blood cells in the suckers' cavity); *c, d* — electron microscopy (*c* — a cross-section of microtrichia in contact with host tissue, *d* — microtrichial border of the scolex base in contact with host tissue). *dt* — degenerated host tissue, *g* — glycocalyx, *he* — host intestinal epithelium, *le* — leucocyte, *m* — microtrichia, *rm* — radial muscle, *s* — sucker, *sc* — spindle-shaped cells, *t* — distal cytoplasm of tegument.

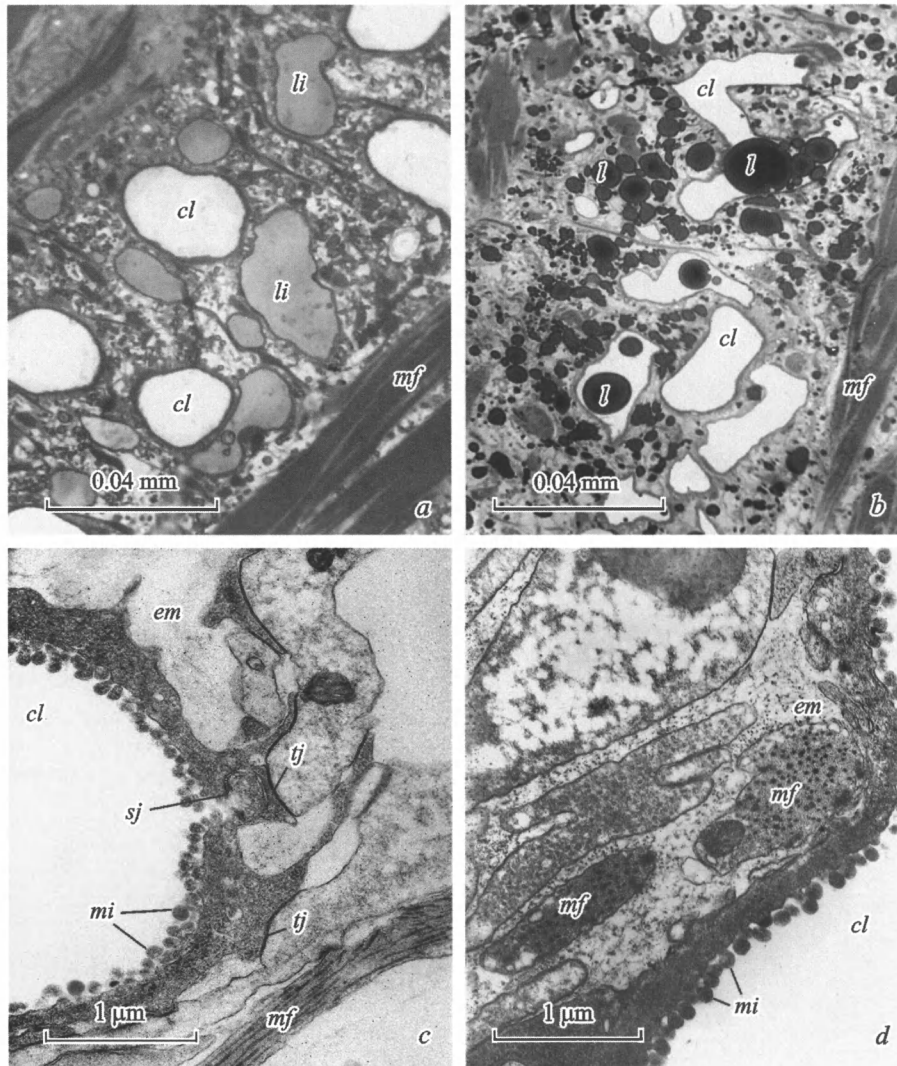


Fig. 4. Excretory system of *Rauschitaenia ancora*.

a, b — tangential semi-thin sections of neck (*a* — surface canals with a liquid, *b* — deeper canals with lipid droplets); *c, d* — electron microscopy (*c* — canal wall with attached processes, *d* — muscle fibers close to canal wall). *cl* — canal lumen, *em* — extracellular matrix, *l* — lipid droplet, *li* — liquid, *mf* — muscle fibers, *mi* — microvilli, *sj* — septate junction, *tj* — tight junction.

More or less expressed fibrous extracellular matrix with the immersed muscle fibers is located directly underneath the distal cytoplasm of the canal wall (fig. 4, *d*). Probably, muscle fibers provide indispensable tone of canal walls, or participate in their contraction transferring fluid and lipid drops. Areas of confluence of small collecting ducts with septate junctions are noted (fig. 4, *c*).

Among Cyclophyllidea, especially among Taeniidae, tissue parasitism is widely distributed at larval stages, whereas adult cestodes rarely use host tissues as a habitat, e. g., for instance, *Gastrotaenia dogieli* (Gynezinskaja, 1944) from the gizzard of Anseriformes. During study of covers' morphology of *G. dogieli* (Davydov et al., 1990; Pospekhova, 2009c), the presence of developed glands in the rostellar apparatus and a thick layer of glycocalyx were noted; according to Davydov and Mikryakov (1998) the glands are similar to that performing barrier and protecting functions in the cestode larvae in host tissues.

The study of the morphology of scolex tegument in *R. ancora* revealed four areas, characterized by different sets of cytoplasm inclusions and different morphology of microtriches: the area of suckers, the scolex behind suckers, neck, and strobila in intestinal lumen (Pospekhov, Pospekhova, 1993). It was noted, that even in cases when microtriches do not form a solid border, they bear a visible layer of glycocalyx. Contact of microtriches with host cells was observed everywhere, frequently it was accompanied with integrity violation of the cytoplasmic membrane of the latter. Erythrocytes, lymphocytes and fibroblasts, both intact and with signs of destruction, were noted in contact area. Also, evidences of the damage of the parasite tegument, from breaking of limiting membrane to complete absence of the surface syncytium over some areas, were seen.

Close contact between host tissues (capsule) and microtrichial border of *R. ancora* tegument was most frequently registered along the suckers' edge. Occasionally it was so strong, that dislocation of the parasite led to tearing of the layer of the tegument surface syncytium of the suckers from the basal plate lying beneath. This phenomenon was observed in cestodes fixed together with the capsule, and apparently it is explained by the suckers muscle contraction at the time of fixation. The presence of erythrocytes close to parasite surface (both with and without capsule) indicates damages of intestinal tissue, which, probably, are performed by microtriches during cestode movements or during peristaltic motions of host intestine. The host, in its turn, actively attacks parasite; it is evidenced by partial destruction of the surface syncytium of the tegument and adhesion of microtrichia to host tissues, resulting in separation of the surface syncytium from subjacent basal lamina.

According to literary data (Baer, 1940; Boertje, 1974) and our observations (Pospekhov, Pospekhova, 1993; Pospekhova, 2009a, б, c; Pospekhova, Bondarenko, 2014), atypical localization and attachment mode of cestode can lead to morphological changes of attachment organs, and, in some cases, of the entire scolex. Apparently, processes of morphological transformations of attachment organs have adaptive character, and in the long ago formed parasite systems they lead to hypertrophy or, on the contrary, to the loss of some fixation organs. In this case the latter does not form anlage at the stage of scolexogenesis and is absent in the developed metacestode. Such situation is observed in *Cloacotaeenia megalops* (Nitzsch in Creplin, 1829), a parasite of Anseriformes, that attaches to cloacal wall of the host with the help of strong suckers. Completely developed metacestode of this species has no rostellum, but possesses an accumu-

lation of glandular cells in its place (Gvozdev, Maksimova, 1978); later it develops into the gland of the rostellar sac, releasing secret into contact area between the parasite and the host (Pospekhova, 2009b).

As for visible absence of rostellar glands at *R. ancora*, which were noted practically for all representatives of higher cestodes, it can be connected with periodicity of secretory activity and fixation of material during decrease of secretion, when absence of specific secretion prevent distinguishing the regular cyton from the glandular one. There is, however, another probability: the rostellar glands of *R. ancora* stop their activity after penetration of the cestode into the intestinal wall, the overgrow of the scolex and the formation of the capsule, and their function is taken by regular tegumental cytons of the scolex, producing numerous vesicles coming into the tegument. Morphologically similar vesicles in contact area with host tissues were noted in two other representatives of the family Dilepididae: in the rostellar tegument of *Dichoanotaenia clavigera* (Krabbe, 1896) and in the front part of cestode scolex of *Platyscolex ciliata* (Furmann, 1913) (Pospekhova, 2009a). It should be mentioned, that *P. ciliata* has rudimentary rostellum with lack of armament even at the metacestode stage; in adults cestodes it does not function and is located at the bottom of the retraction channel (Krasnoshchekov, Pluzhnikov, 1981; Tomilovskaya, 1982). Thus, the rostellar apparatus of *P. ciliata* represents an intermediate variant between the rostellar apparatus of *C. megalops* (the rostellum is absent even at the metacestode stage) and *R. ancora* (the rostellum of proportional size is formed at the metacestode stage, and later it stops growing). Probably, these variants reflect different degree of parasite adaptation and different age of their parasite-host systems.

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