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Fine structure of midgut cells of some White Sea free-living nematodes

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ABSTRACT: Midgut of nematodes is a straight tube consisting of a single layer of epithelial cells lined by layer of microvilli, which are covered by a glycocalyx. There are usual organelles in cells: nuclei, mitochondria, digestive vacuoles, endoplasmic reticulum, Golgi complexes. Gut cells of different nematode species could vary widely. We studied ten species of White Sea nematodes: *Bathylaimus arcticus* Kreis, 1963, *Oxystomina* sp., *Paracanthonchus caecus* Micoletzky, 1924, *Halichoanolaimus robustus* (Bastian, 1865), *Desmodora communis* (Bütschli, 1874), *Draconema ophicephalum* (Claparède, 1863), *Paramonhystera filamentosa* (Ditlevsen, 1928), *Sphaerolaimus balticus* Schneider, 1906, *Odontophora deconinki* Galtsova, 1976 and *Sabatieria ornata* (Ditlevsen, 1918). Almost all organelles have some their own features in the cells for each species. Some organelles seem to be dependent on the stage of food digestion, while others have been associated with a taxonomic position or with a food object. In cells of all species small digestive vacuoles are observed, but some species have big ones. They can be created by fusion of small digestive and enzyme vacuoles. Lipid droplets are indicated in cells of almost all species. Quantity of these droplets can be depending on digestive stage. Microvilli and glycocalyx do not have a clean connection either with the type of food, or with the taxonomic position of the nematodes. But if glycocalyx is complex we can see correlation with taxonomic position of nematodes: similar type of glycocalyx and microvilli are observed for nematodes from one order.

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KEY WORDS: Nematoda, White Sea, midgut, ultrastructure.

Ультраструктура клеток кишки некоторых видов свободноживущих нематод Белого моря

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РЕЗЮМЕ: Кишка нематод представляет собой простую прямую трубку, образованную однослойным эпителием. В апикальной части клеток имеются выросты — микровилли, покрытые гликокаликсом. В самих клетках имеется обычный набор органелл: ядро, митохондрии, пищеварительные вакуоли, ЭПР и аппарат Гольджи и связанные с ними везикулы. Тонкое строение клеток кишки у разных видов нематод может широко варьироваться. Нами было изучено десять видов беломорских нема-

тод: *Bathylaimus arcticus* Kreis, 1963, *Oxystomina* sp., *Paracanthonchus caecus* Micoletzky, 1924, *Halichoanolaimus robustus* (Bastian, 1865), *Desmodora communis* (Bütschli, 1874), *Draconema ophicephalum* (Claparède, 1863), *Paramonhystera filamentosa* (Ditlevsen, 1928), *Sphaerolaimus balticus* Schneider, 1906, *Odontophora deconinki* Galtsova, 1976 и *Sabatieria ornata* (Ditlevsen, 1918). В клетках у каждой нематоды почти все органеллы имеют какие-то особенности строения или распределения. Структура одних органелл может зависеть от стадии переваривания пищи, в то время как другие определяются систематическим положением нематоды или её объектом питания. Например, во всех клетках есть мелкие пищеварительные вакуоли, но некоторые виды имеют довольно крупные пищеварительные структуры, которые, по-видимому, образованы слиянием мелких вакуолей и ферментосодержащих везикул. Жировые капли есть почти у всех видов, но их количество зависит от стадии переваривания пищи. Структура микровиллей и гликокаликса не имеют четкой связи ни с систематикой, ни с типом питания нематод. Однако если гликокаликс многослойный, его строение сходно у нематод внутри одного отряда.
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КЛЮЧЕВЫЕ СЛОВА: Nematoda, Белое море, средняя кишка, ультраструктура.

Introduction

Midgut or intestine of nematodes is a straight tube consisting of a single layer of epithelial cells. There are usual organelles in cells: nuclei, mitochondria, digestive vacuoles, endoplasmic reticulum, Golgi complexes. All cells are bordered on the outside by a basal lamina while the internal lumen is lined by layer of microvilli, which are covered by a glycocalyx layer (Bird, Bird, 1991; Decraemer et al., 2013). Gut cells of different nematode species vary widely in the number of cells on a cross section as well as in their shape and size. In finer details, the structure of the glycocalyx and microvilli, the ratio of digestive vacuoles, and distribution of organelles may also differ between species.

The aim of the study is to outline structural diversity of the marine nematode intestine as well as attempt to find out conceivable relations between intestinal structures and diet or taxonomic position of species.

Material and methods

We studied ten species of White Sea nematodes belonging to different taxa: *Bathylaimus arcticus* Kreis, 1963 (Enoplida, Tripyloididae), *Oxystomina* sp. (Enoplida, Oxystominidae), *Paracanthonchus caecus* Micoletzky, 1924 (Chromadorida, Cyatholaimidae), *Halichoanolaimus robustus* (Bastian, 1865) (Chromadorida, Selachinematidae), *Desmodora communis* (Bütschli, 1874) (Desmodorida, Desmodoridae), *Draconema ophicephalum* (Claparède, 1863) (Desmodorida, Draconematidae), *Paramonhystera filamentosa* (Ditlevsen, 1928) (Monhysterida, Xyalidae), *Sphaerolaimus balticus* Schneider, 1906 (Monhysterida, Sphaerolaimidae), *Odontophora deconinki* Galtsova, 1976 (Araeolaimida, Axonolaimidae) and *Sabatieria ornata* (Ditlevsen, 1918) (Araeolaimida, Comesomatidae). Specimens of all the species were fixed with glutaraldehyde and then with osmium tetroxide using standard methods developed for transmission electron microscopy (TEM).

Results

Almost all intestinal organelles have some features in common for each species.

Mitochondria are distributed evenly throughout the cell (except for *Paramonhystera*). They are present in large numbers in all species ex-

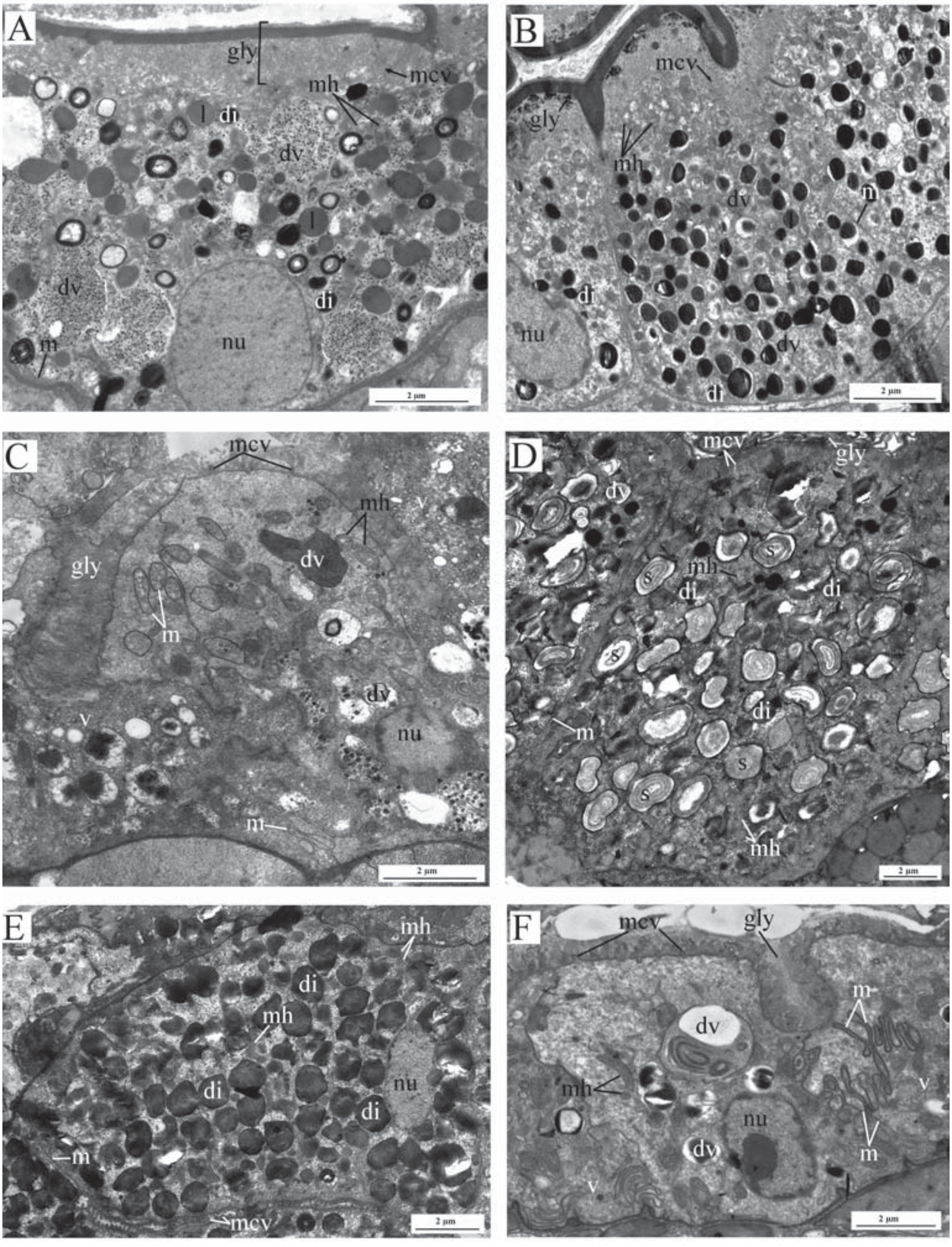

Fig. 1. Intestinal cells in cross sections, TEM: A — *Sphaerolaimus*, B — *Paramonhystera*, C — *Halichoanolaimus*, D — *Odontophora*, E — *Sabatieria*, F — *Paracanthonchus*.

Abbreviations: gly — glycocalyx, di — dense inclusions, dv — digestive vacuoles, l — lipid droplets, m — membrane, mcv — microvilli, mh — mitochondria, n — nucleus, s — spherocrystalls.

Рис. 1. Поперечный срез кишки, ТЕМ. A — *Sphaerolaimus*, B — *Paramonhystera*, C — *Halichoanolaimus*, D — *Odontophora*, E — *Sabatieria*, F — *Paracanthonchus*.

Обозначения: gly — гликокаликс, di — плотные включения, dv — пищеварительные вакуоли, l — жировые капли, m — мембрана, mcv — микровилли, mh — митохондрия, nu — ядро, s — сферокристаллы.

cept *Sabatieria*. In *Paramonhystera* mitochondria are concentrated in the apical and less in the middle part of cells (Fig. 1B).

Digestive vacuoles are distributed uniformly throughout the cell in almost all species (in *Paramonhystera* they are shifted to the basal part of the cell, although occur also in the apical part, Fig. 1B). They have a great variety in size and shape. All the species have small vacuoles with various content (vesicles, dense particles or lamellas). But in some species (*Draconema*, *Bathylaimus* (Fig. 2C), *Halichoanolaimus* (Fig. 1C), *Paracanthonchus* (Fig. 1F) and rarely *Sphaerolaimus*), there are large "phagosomes" that can contain lamellae and vesicles or large dense particles inside.

Endoplasmic reticulum, dictyosomes and associated vesicles are expressed as such in only two species, *Halichoanolaimus* and *Paracanthonchus*. In cells of other species, just rare vesicles can be seen (Fig. 1C, F).

Several types of inclusions are present: (termed according to Chitwood, Chitwood, 1950):

Dense inclusions are the most numerous in *Sabatieria* (Fig. 1E): they fill the whole cell. In a smaller number, they are present in *Odontophora* and *Sphaerolaimus,* and very few in *Halichoanolaimus*.

Main storage inclusions are lipid droplets. They can be large, occupying almost the entire cell (*Oxystomina*, *Desmodora*) (Fig. 2A, D), in other species they are rather small. The droplets can be present in almost all cells (*Sphaerolaimus*) (Fig. 1A), or in only some of cells (*Paracanthonchus*, *Paramonhystera*, *Sabatieria*). *Draconema*, *Halichoanolaimus* and *Bathylaimus* had not lipid droplets at all.

Spherocrystals are present in large numbers in *Odontophora*'s cells (Fig. 1D). They vary in number of concentric rings and in electron-density. Shape of the spherocrystals is not strictly round.

Microvilli are cylindrical in all species. They are long and sparse in *Bathylaimus* (Fig. 2C, H), very long and closely located in *Paramonhystera* (Fig. 2G), rather short and dense in *Sphaerolaimus*, moderate long and sparse in *Halichoanolaimus* (Fig. 1C), *Oxystomina* (Fig. 2A) and *Paracanthonchus* (Fig. 1F), short and sparse in *Sabatieria* (Fig. 1E), *Odontophora* (Fig. 2F) and *Desmodora* (Fig. 2D). In *Draconema*, the microvilli are short and arranged in groups of 4–8 (Fig. 2B, E).

Glycocalyx may be amorphous (*Sabatieria*, *Desmodora*, *Draconema*, *Oxystomina*) (Fig. 2E), amorphous with vesicles (*Bathylaimus*) (Fig. 2H), two-layered with amorphous basal layer and lamellar upper layer (*Odontophora*, *Halichoanolaimus*, *Paracantonchus*) (Fig. 2F) and three-layered with basal amorphous, intermediate dense and upper layer lamellar (*Sphaerolaimus* and *Paramonhystera*) (Fig. 2G).

Discussion

The intestine of nematodes is believed to be an organ of more or less similar structure in gross morphology for all nematodes. However, the fine cell structures may vary considerably. Some organelles seem to be dependent on the stage of food digestion, while others are associated with a taxonomic position or with food items. According to conventional classification of Wieser (1953), the species are distributed in four feeding types: 1A, no real buccal cavity, selective deposit-feeders or bacterivores, here *Oxystomina*; 1B, buccal cavity unarmed, non-selective deposit-feeders, here *Paramonhystera* and *Sabatieria*; 2A, buccal cavity with small armature, epistrate-feeders, here *Paracanthonchus*, *Desmodora*, *Draconema* and *Odontophora*; 2B, large and heavy armed buccal cavity, carnivores, here *Halichoanolaimus* and *Sphaerolaimus* ingesting prey nematodes; special case is presented by *Bathylaimus* feeding on ciliates. But manner of food consumption and its consistency in the gut may not depend on the feeding type, different feeding types there may be the same mechanism of digestion.

Presence of a large number of mitochondria indicates active synthetic processes in the cell. It means that in *Sabatieria* these processes are not very active, and the main function of the cells (perhaps at this stage of digestion) is the storage (there are many dense particles). Dense particles can be metabolic products.

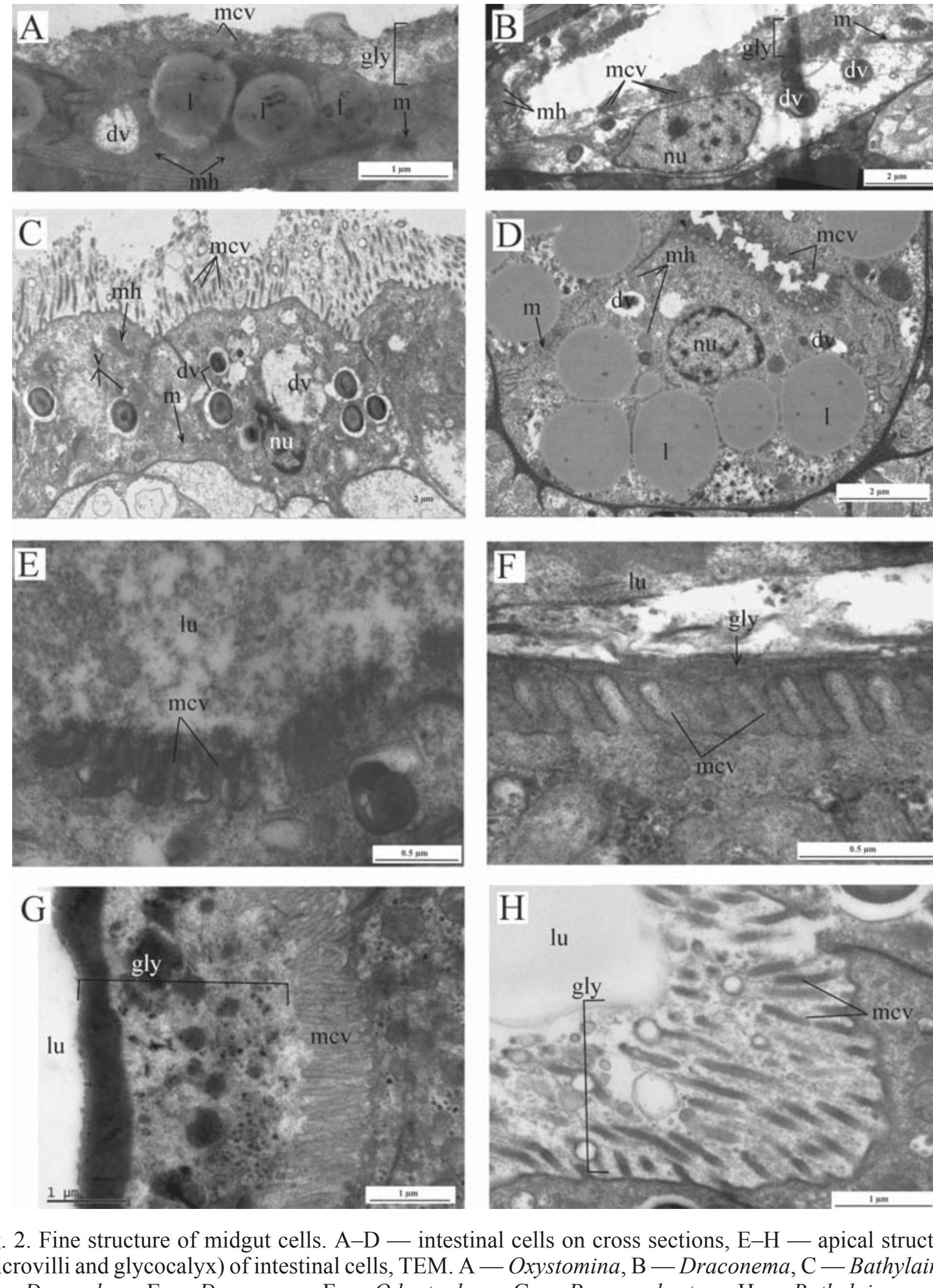

Fig. 2. Fine structure of midgut cells. A–D — intestinal cells on cross sections, E–H — apical structures (microvilli and glycocalyx) of intestinal cells, TEM. A — *Oxystomina*, B — *Draconema*, C — *Bathylaimus*, D — *Desmodora*, E — *Draconema*, F — *Odontophora*, G — *Paramonhystera*, H — *Bathylaimus*.

Abbreviations: gly — glycocalyx, dv — digestive vacuoles, l — lipid droplets, lu — lumen, m — membrane, mcv — microvilli, mh — mitochondria, n — nucleus.

Рис. 2. Ультраструктура клеток кишки. A–D — поперечный срез клеток кишки, E–H — апикальные структуры (микровилли и гликокаликс) клеток кишки, TEM: A — *Oxystomina*, B — *Draconema*, C — *Bathylaimus*, D — *Desmodora*, E — *Draconema*, F — *Odontophora*, G — *Paramonhystera*, H — *Bathylaimus*.

Обозначения: gly — гликокаликс, dv — пищеварительные вакуоли, l — жировые капли, lu — просвет кишки, m — мембрана, mcv — микровилли, mh — митохондрия, nu — ядро.

Digestion in small vesicles takes place in intestine cells of all species. But the digestion of larger amounts of food in vacuoles is observed in only certain species. Since phagocytosis has not been seen as such anywhere, it is likely that such large "phagosomes" are already formed in the cells due to the fusion of small digestive vacuoles and vesicles with enzymes which are numerous in cells of some species (*Halichoanolaimus* and *Paracantonchus*).

Lipids are the main store material, since these free-living nematodes live under aerobic conditions, and such a reserve substance is more energy-efficient (Frantová, Moravec, 2004). Probably, the amount of lipid droplets in the cell varies depending on the satiety of the nematode: the droplets are bigger in size and more numerous in full specimens than in hungry ones.

Spherocrystals are rather unusual inclusion. In parasitic nematodes, they are considered either as calcium structures (Chitwood, Chitwood, 1938) containing metabolic products, or as digestive organelles containing hemoglobin-like substance (Riley, 1973). Spherocrystals of *Odontophora* are featured with a dense outer membrane and a varying light content, they are more likely digestion structures than stores. In addition, dense particles are always located outside the spherocrystals. But, the exact function of these structures can only be clarified by histochemical methods.

Microvilli do not show a clear connection either with the type of food, or with the taxonomic position of the species. Nevertheless, short and sparsely located microvilli are mostly associated with the amorphous glycocalyx while longer and denser microvilli with structurally complicated glycocalyx. With an increase in the length (in relation to cell) and density of microvilli, the apical surface of the cell also increases. Assuming that the glycocalyx is released from the apical part of the cells (Sheffield, 1964), the larger its surface the thicker glycocalyx layer can be formed.

Glycocalyx structure of some nematodes seems to be linked with systematic position: *Sphaerolaimus* and *Paramonhystera* have the similar glycocalyx, although they are predator and deposit-feeder respectively. Similarly, *Halichoanolaimus* and *Paracanthonchus* have a glycocalyx consisting of amorphous and lamellar layer, although they are predator and epistrate-feeder respectively. *Sabatieria*, *Draconema, Desmodora* and *Oxystomina* have the similar amorphous glycocalyx, although they belong to different orders and different types of feeding. Sometimes glycocalyx varies depending on presence of food in the gut lumen. Thus, glycocalyx of *Odontophora* is mostly amorphous, but when a content in the gut presents, lamellar structures appear and the glycocalyx thickens significantly. In *Bathylaimus*, the glycocalyx may be amorphous or amorphous with vesicles in different states.

As a result, we can say that such structures as mitochondria, vesicular apparatus, microvilli structure and partially structure of glycocalyx are more conditioned by the systematic position of nematodes, while the quantity and features of lysosomes, lipid droplets and inclusions (dense particles and spherocrystals) depend on the presence or absence of food and its character.

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