DITRYPANOCYSTIS SP. (APICOMPLEXA, GREGARINIA, SELENIDIIDAE): THE MODE OF SURVIVAL IN THE GUT OF *ENCHYTRAEUS ALBIDUS* (ANNELIDA, OLIGOCHAETA, ENCHYTRAEIDAE) IS CLOSE TO THAT OF THE COCCIDIAN GENUS *CRYPTOSPORIDIUM*

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A selenid gregarine *Ditrypanocystis* sp. (Apicomplexa, Gregarinia, Selenidiidae), harboring the gut lumen of the oligochaete *Enchytraeus albidus*, was studied by light and electron microscopy. The trophozoite of *Ditrypanocystis* sp. is attached to the gut wall with its apical end to be anchored eventually between enterocytes in the crypts. Simultaneously, between the surfaces of the parasite and the host cell a peculiar contact is formed made of membranous channels and vesicles of unknown origin, the host cell surface in the contact area lacking cilia. The trophozoite becomes progressively enclosed within a parasitophorous vacuole made of layers of fused ciliar membranes of enterocytes. The fused cilia may be a source of membranes lining channels and vesicles of the contact area. Such a mode of parasitophorous arrangements has never been described before for gregarines, however, it bears a some likeness with that of the coccidian genus *Cryptosporidium* (similarity and differences being discussed). With regard to some molecular phylogeny constructions, claiming the «sister» relationship between gregarines and the coccidian genus *Cryptosporidium* (Carreno et al., 1999; Leander et al., 2003), this common feature in host-parasite relationships enabled us to put forward an idea of a possible evolutionary route from extracellularity of gregarines to intracellularity of coccidia, as exemplified by species of *Cryptosporidium*.

Key words: gregarine, *Ditrypanocystis*, Selenidiidae, ultrastructure, phylogeny, *Cryptosporidium*.

The obvious diversity of mutual adaptations of parasites and host cells is one of most challenging areas of comparative cytology. This diversity is rather well known for parasites of vertebrate hosts, but still remains poorly investigated for the majority of parasites of invertebrate hosts, primarily non-profit ones. The sea invertebrates serve a rich source of the relevant information because of their huge biodiversity and substantial evolutionary age. This study deals with protozoan gut parasites of the oligochaete *Enchytraeus albidus* inhabiting storm discharge of the White Sea.

Material and methods

Polychaetes *Enchytraeus albidus*, harboring in their guts selenid gregarines of the genus *Ditrypanocystis*, were collected at the White Sea littoral during a low tide.

Pieces of the gut were fixed with 2.5 % glutaraldehide and 2 % osmium tetroxide for 2 and 1 h, respectively. All fixatives were made in PBS, the osmolarity being brought with sucrose to that of sea water, 780 mOsm. Following ethanol and acetone treatment, the material was embedded in Araldit.

Semithin and ultrathin sections were done with the Reichert Ultracut R ultratome. Semithin (1000 nm) sections were stained with a mixture of 1 % azur and 1 % metylene blue and examined in a Axiolab ZEISS microscope. Images were received with the camera digital Nikon E990. Ultrathin sections were contrasted with 2 % uranyl acetate in 70 % ethanol and lead citrate solutions and examined in a ZEISS EM 912 Omega electron microscope.

Glutaraldehyde, osmium tetroxide, Araldit kit, PBS, acetone, uranyle acetate, lead citrate and nitrate were supplied by SERVA, USA.

Results

Trophozoites of *Ditrypanocystis* sp. are localized in the oligochaete gut lumen being attached to the gut wall in the crypt area (Fig. 1, *a*). The attachment is realized by the apical part of the trophozoite, which forms deep lobes towards the gut wall between enterocytes (Fig. 1, *a*, *b*).

The trophozoites under study are elongated bodies, measuring 30—40 μm by 20 μm. The nucleus has a big nucleolus (Fig. 1, *a*).

About half of the parasite surface is covered with 26 longitudinal ridges (Fig. 2, *a*, *b*) being up to 0.4 μm high and strengthened with 10 microtubules running through every ridge and aggregate of microfilaments in the basic part of the ridge (Fig. 2, *c*). The microfilaments go along the ridges enforcing them. Besides ridges, the parasite surface makes several longitudinal folds, which are considerably higher than ridges, differing from the latter in size and shape (Fig. 2, *d*, *e*). There are no microtubules but only microfilaments are

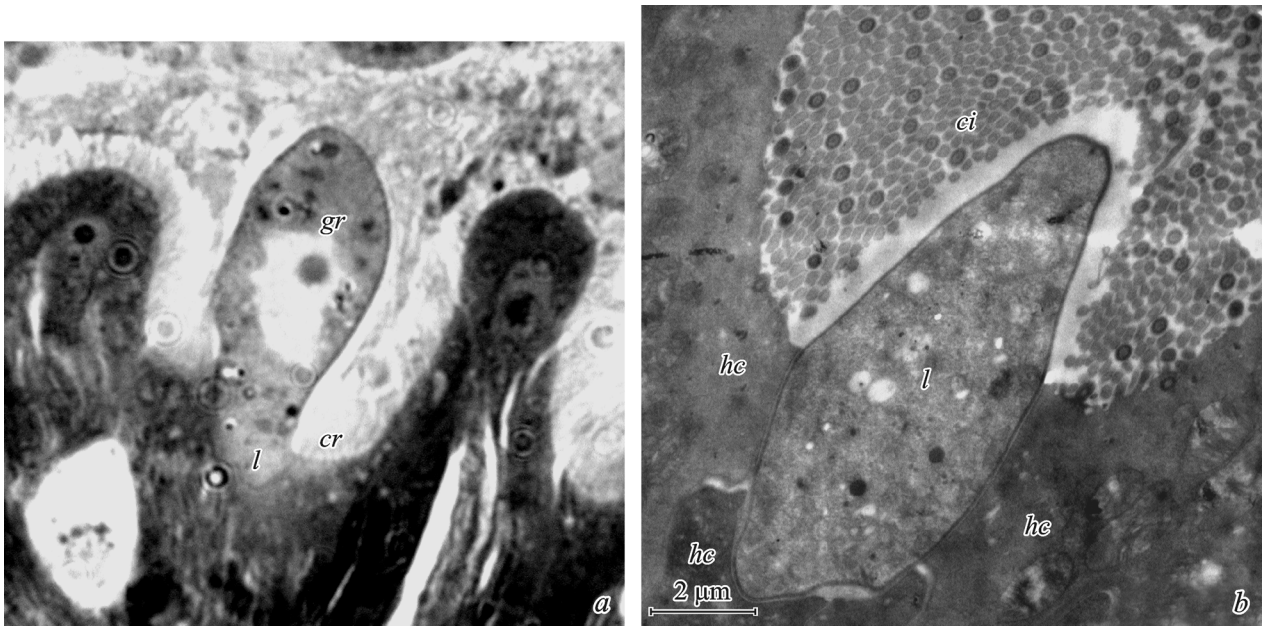


Fig. 1. Mode of attachment of gregarine *Ditrypanocystis* sp. to the gut of oligochaete *Enchytraeus albidus*.

a — mode of parasite attachment between enterocytes in crypts with lobed anterior part, light microscopy; ob. 100×, oc. 10×. *b* — ultrastructure of the contact area; parasite forms a close contact with host cell surfaces. *ci* — cilia, *cr* — crypt, *gr* — gregarine, *hc* — host cell, *l* — lobe.

Рис. 1. Способ прикрепления грегарины *Ditrypanocystis* sp. в кишечнике малощетинкового червя *Enchytraeus albidus*.

a — прикрепление паразита между энтероцитами в криптах передним концом; световая микроскопия; об. 100×, ок. 10×. *b* — ультраструктура зоны контакта; паразит контактирует с поверхностью клетки хозяина. *ci* — реснички, *cr* — крипта, *gr* — грегарина, *hc* — клетка хозяина, *l*— лопасть.

present in the epicyte folds. The folds go from the apical part along the body of the trophozoite, but not to the very end (Fig. 2, *a*).

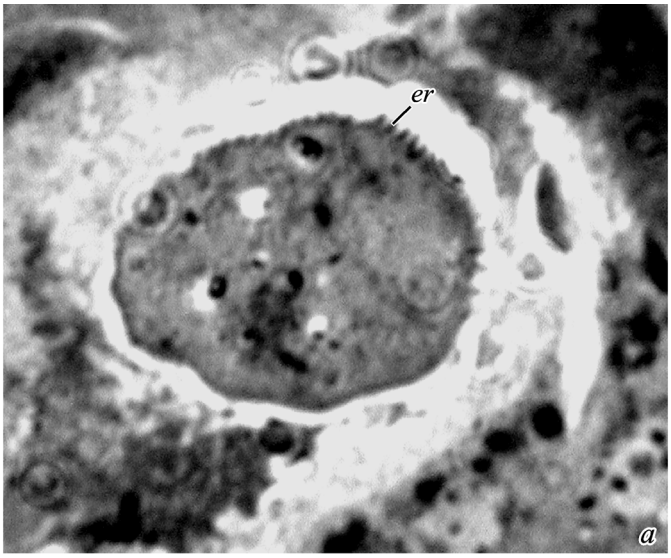
The trophozoite pellicle consists of a plasmalemma and inner membrane complex underlined with a layer of subpellicular microtubules (Fig. 3, *a*). Under the inner membrane complex, there is a layer of electron-dense substance denoted as a subpellicular dense layer (Fig. 2, *c*). The pellicle at the ridges is additionally enforced with electron-dense substance being deposited above the inner membrane complex (Fig. 2, *c*). The are few rhoptries and typical micronemes with electron-dense contents. In addition, in the apical part of the trophozoite in the cytoplasm numerous vesicular electron-lucent organelles are seen, resembling empty micronemes (Fig. 2, *f*). The cytoplasm is filled with dense granules, known to be shared by all sporozoan zoites (Fig. 3, *a*). Mitochondria with

Fig. 2. Morphology and ultrastructure of the trophozoite of *Ditrypanocystis* sp.

a — transversal section of the trophozoite posterior part supplied with epicyte ridges, light microscopy; ob. 100×, oc. 10×. *b* — ultrastructure of the trophozoite posterior part (transversal section) with 4 rhoptries and 26 epicyte ridges. *c* — ultrastructure of the epicyte ridges crossed with numerous microtubules and microfilaments. Inputs (shown by *arrows*): the pellicle at the epicyte ridges and between them, respectively. The pellicle of ridges differs in the presence of an additional electron-dense layer situated between the outer membrane and inner membrane complex, while the pellicle membranes and the subpellicular electron-dense layer, localized in the cytoplasm under the inner membrane complex, are equally developed on the whole parasite body. *d* — oblique section of the trophozoite anterior part with 3 epicyte folds; ob. 100×, oc. 10×. *e* — ultrastructure of the trophozoite anterior part (oblique section) differing from the posterior one in the availability of epicyte folds, in addition to epicyte ridges and 5 rather than 4 roptries. *f* — the apical part of trophozoite displaying typical apicomplexan features: rhoptries, subpellicular microtubules, electron-lucent vesicles, mitochondria with ampular cristae (*boxed* region). *g* — a magnified fragment of the *boxed* region in panel *f*, including mitochondria with ampular cristae. *cm* — cristae of mitochondria, *er* — epicyte ridge, *ef* — epicyte folds, *im* — inner pellicle membranes, *m* — mitochondria, *mt* — microtubules, *n* — nucleus, *om* — outer pellicle membrane, *r* — rhoptry, *sdl* — subpellicular dense layer, *smt* — subpellicular microtubules, *v* — electron-lucent vesicles. *Arrows* show imputs magnifying loci the *arrows* arise from; *arrowhead* — electron-dense substance in the pellicle of ridges.

Рис. 2. Морфология и ультраструктура трофозоита *Ditrypanocystis* sp.

a — поперечный срез задней части трофозоита с эпицитарными гребнями; об. 100×, ок. 10×. *b* — ультраструктура задней части трофозоита (поперечный срез) с 4 роптриями и 26 эпицитарными гребнями. *c* — ультраструктура эпицитарных гребней, внутри которых проходят микротрубочки и микрофиламенты. На *вставках*, указанных *стрелками*, — пелликула на эпицитарных гребнях и между ними соответственно. Пелликулу гребней отличает дополнительный электронно-плотный слой, лежащий между наружной мембраной и внутренним мембранным комплексом, тогда как мембраны пелликулы и субпелликулярный электронно-плотный слой, подстилающий внутренний мембранный комплекс, в равной мере развиты во всех участках тела паразита. *d* — косой срез через переднюю часть трофозоита, видны 3 эпицитарные складки; об. 100×, ок. 10×. *e* — ультраструктура передней части трофозоита (косой срез), отличающейся от задней наличием эпицитарных складок в дополнение к эпицитарным гребням и 5 роптрий вместо 4. *f* — апикальная часть трофозоита, имеющего типичные для зоитов особенности ультраструктуры: роптрии, субпелликулярные микротрубочки, электронно-прозрачные везикулы, митохондрии с ампулированными кристами (участок цитоплазмы с митохондриями выделен *рамкой*. *g* — увеличенный фрагмент на рис. 2, *f*, выделенный *рамкой*, содержащий митохондрии с ампулированными кристами. *cm* — кристы митохондрии, *er* — эпицитарный гребень, *ef* — эпицитарная складка, *im* — внутренняя мембрана пелликулы, *m* — митохондрия, *mt* — микротрубочки, *n* — ядро, *om* — наружная мембрана пелликулы, *r* — роптрия, *sdl* — субпелликулярный электронно-плотный слой, *smt* — субпелликулярные микротрубочки, *v* — электронно-прозрачные везикулы; *стрелки* отходят от участков, увеличенных на указанных ими вставках; *головка стрелки* — электронно-плотное вещество пелликулы эпицитарных гребней.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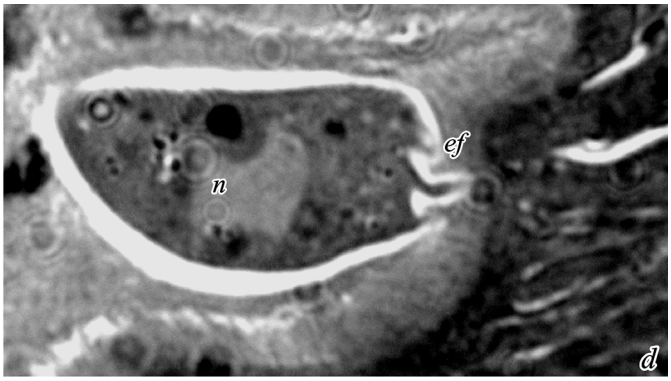
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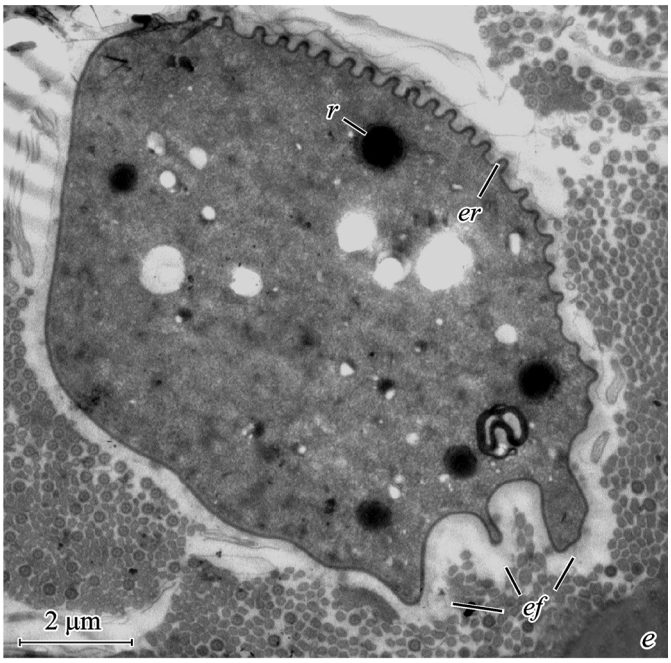
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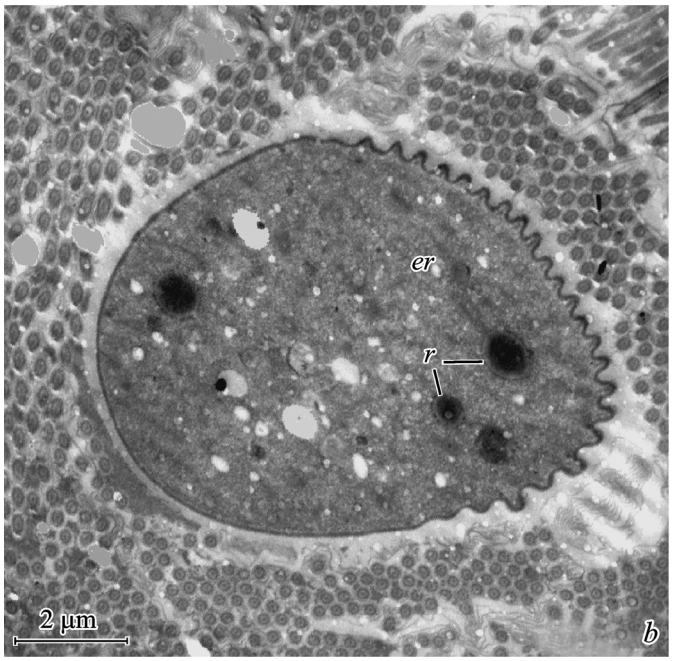
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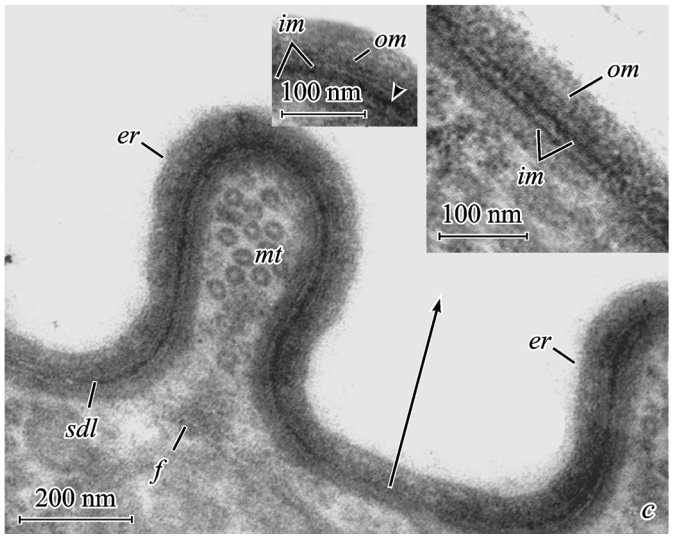
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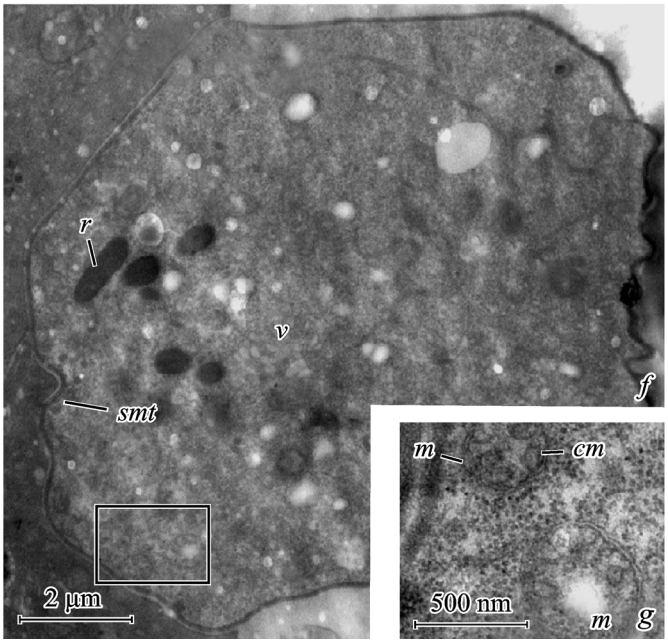
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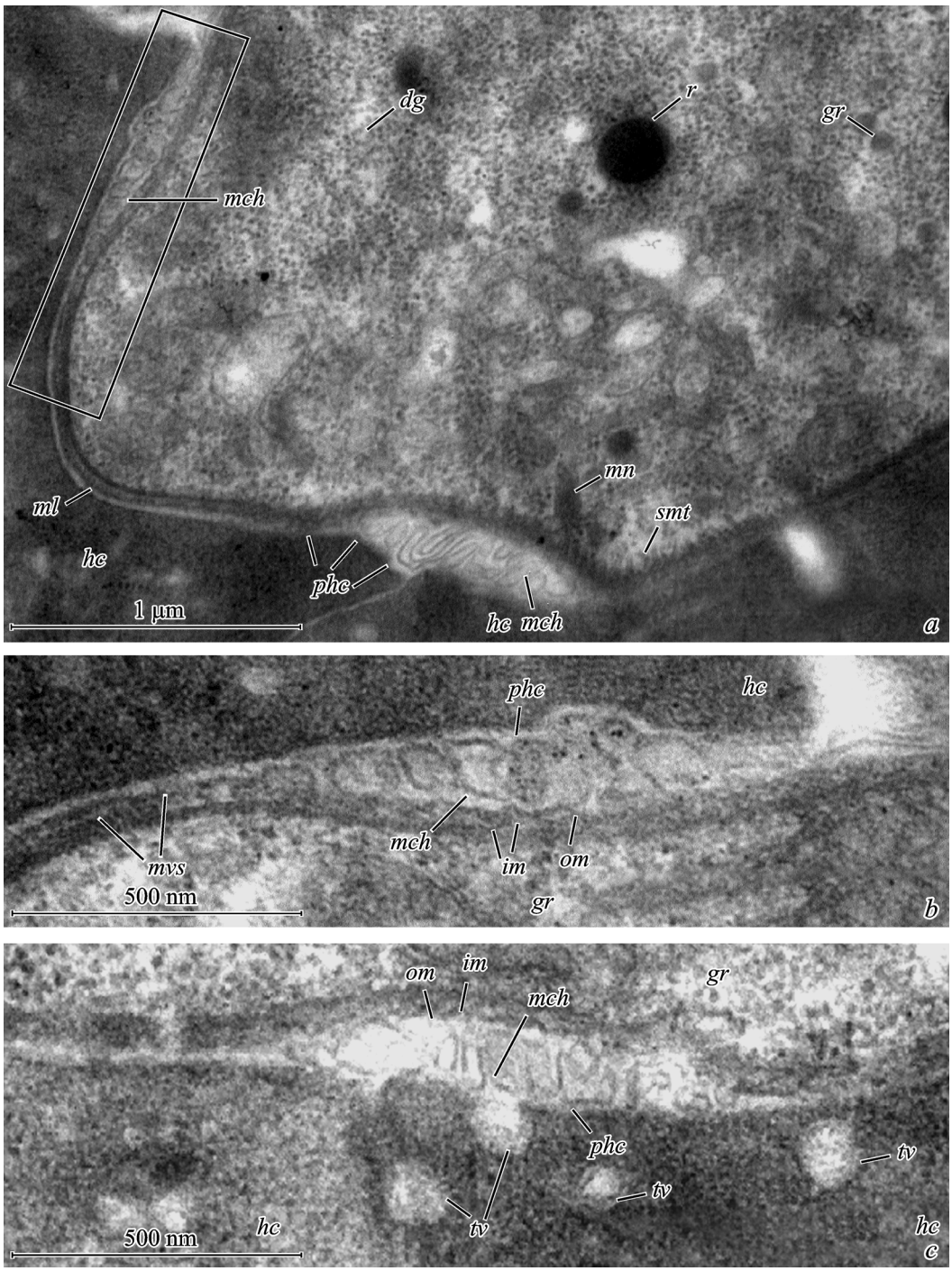

Fig. 3. The contact area of gregarine and host cells with membranous structures between their plasmalemmas.

a — general view showing different membranous structures: membranous channels and membranous vesicles (*boxed* region), membranous layer. *b* — magnified fragment of the *boxed* region in panel *a* including the contact area with membranous channels and vesicles, membranous vesicles are sequential to membranous channels. Surface membranes of channels and vesicles do not contact with plasmalemmas of either host, or parasite. *c* — view of the membranous channel opened to the contents of a transport vacuole crossing the host cell plasmalemma. Both the outer and inner membranes of the parasite pellicle retain in the contact area. *dg* — dense granules, *mch* — membranous channels, *ml* — membranous layer, *mn* — microneme, *mvs* — membranous vesicles, *phc* — host cell plasmalemma, *tv* — transport vacuole; other designations are the same as in Figs 1, 2.

Рис. 3. Зона контакта грегарины и клетки хозяина с мембранными структурами между их плазмалеммой.

a — общий вид зоны контакта, видны различные мембранные структуры: мембранные каналы и мембранные везикулы (на участке, выделенном *рамкой*), слой мембран. *b* — увеличенный фрагмент рис. 3, *a*, выделенный *рамкой*, включающей в себя зону контакта, в которой выявляются мембранные каналы и везикулы; видно, что мембранные везикулы являются продолжением мембранных каналов. Поверхностные мембраны каналов и везикул не сливаются с плазмалеммой ни хозяина, ни паразита. *c* — мембранный канал, открытый содержимому транспортной вакуоли, идущей через плазмалемму клетки хозяина. Наружная и внутренние мембраны пелликулы паразита сохраняются в зоне контакта. *dg* — плотные гранулы, *mch* — мембранные каналы, *ml* — мембранный слой, *mn* — микронемы, *mvs* — мембранные везикулы, *phc* — плазмалемма клетки хозяина, *tv* — транспортная вакуоль; остальные обозначения те же, что и на рис. 1, 2.

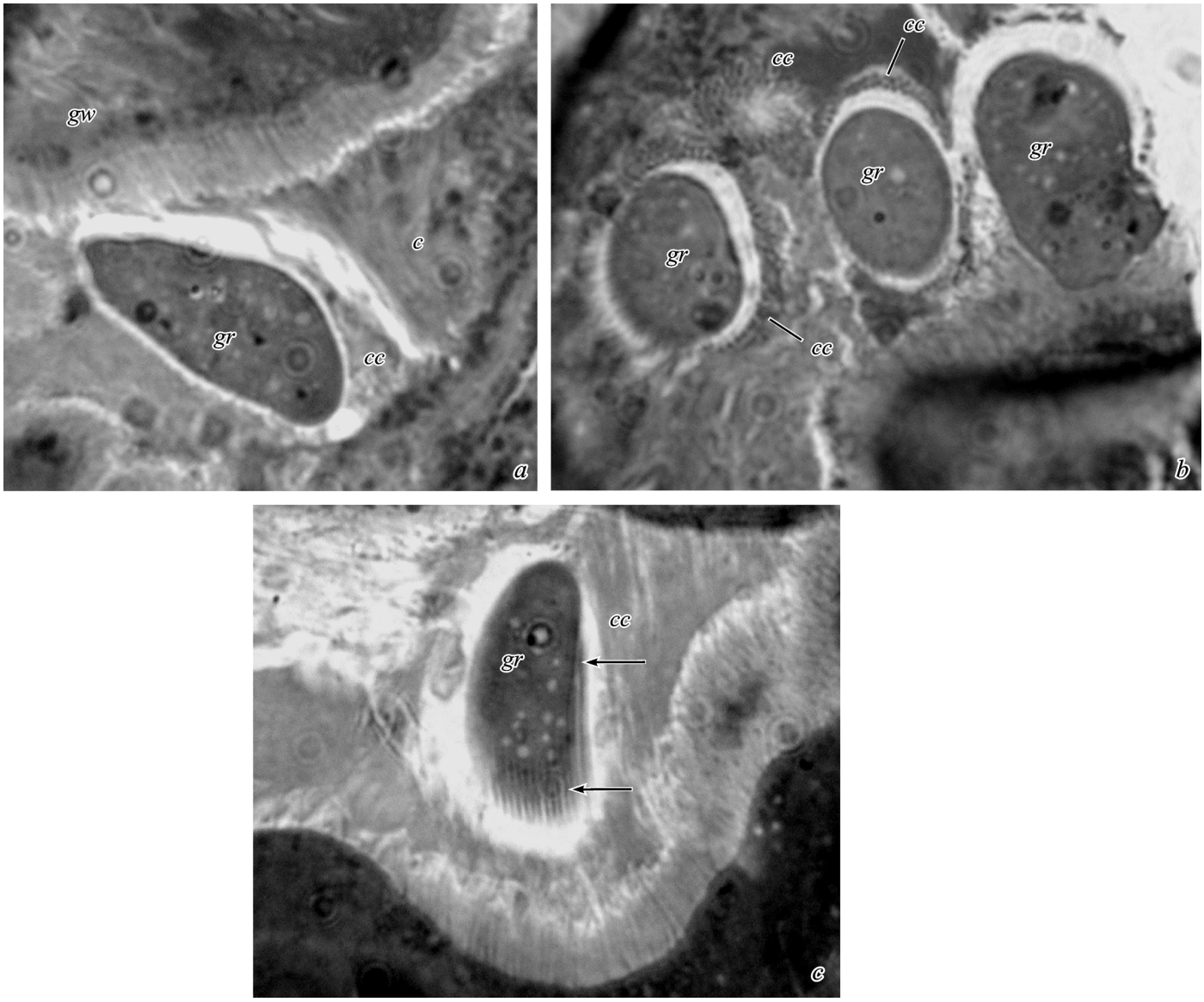

Fig. 4. Morphology of *Ditrypanocystis* sp. trophozoites inside parasitophorous vacuoles.

a — longitudinal section of cilia clusterized around the parasite, normal cilia are seen nearby. *b* — cross section of parasitized brush border, clusterization occurs around each trophozoite. *c* — oblique section through the periphery of parasitophorous vacuole; *arrows* — clusterized cilia adjacent to the parasite surface. *cc* — clusterized cilia, *gw* — gut wall; other designations are the same as in Fig. 1. Ob. 100×, oc. 10×.

Рис. 4. Морфология трофозоитов *Ditrypanocystis* sp. внутри паразитофорной вакуоли.

a — продольный срез через слой ресничек, кластеризовавшихся вокруг паразита; нормальные реснички рядом. *b* — поперечный срез щеточной каемки зараженного червя; кластеризация ресничек происходит вокруг каждого трофозоита. *c* — косой срез через периферическую часть паразитофорной вакуоли; *стрелки* — кластеризованные реснички вплотную прилежат к поверхности паразита. *cc* — кластеризованные реснички, *gw* — кишечная стенка; остальные обозначения те же, что и на рис. 1. Об. 100×, ок. 10×.

ampular crysts are not numerous and localized at the periphery of the trophozoite. We detected them only in the apical part of the trophozoite (Fig. 2, *g*).

The surface of the trophozoite front part makes a close contact with the enterocyte surface. In the attachment zone, there is a prominent space filled with numerous membranous structures (Fig. 3, *a*), making channels of different configuration, and being limited by host enterocyte and parasite surfaces, respectively (Fig. 3, *b*). But through the most part of the contact area they look as either associations of tiny vesicles, or a layer of electron dense substance (Fig. 3, *a*). Gut epithelial cells demonstrate high transport activity, being seen by the presence of numerous vacuoles in the cytoplasm. The channels of contact area, opened in the immediate proximity to enterocyte plasmalemma, are shown to be in contact with the contents of the transport vacuoles crossing the host cell plasmalemma (Fig. 3, *c*).

The source of channels in the contact area is not clear. The channels are not homologous to enterocyte outgrowths, referred to as cilia. Nevertheless, both cilia and the substrate of micronemes, found immediately under the trophozoite pellicle, may be, presumably, involved in the formation of this specialized host-parasite interface under the influence that the parasite exerts on the host enterocyte.

The rest part of the trophozoite is surrounded by cilia-free space (Fig. 4, *a*—*c*). Cilia of enterocytes, normally deflecting

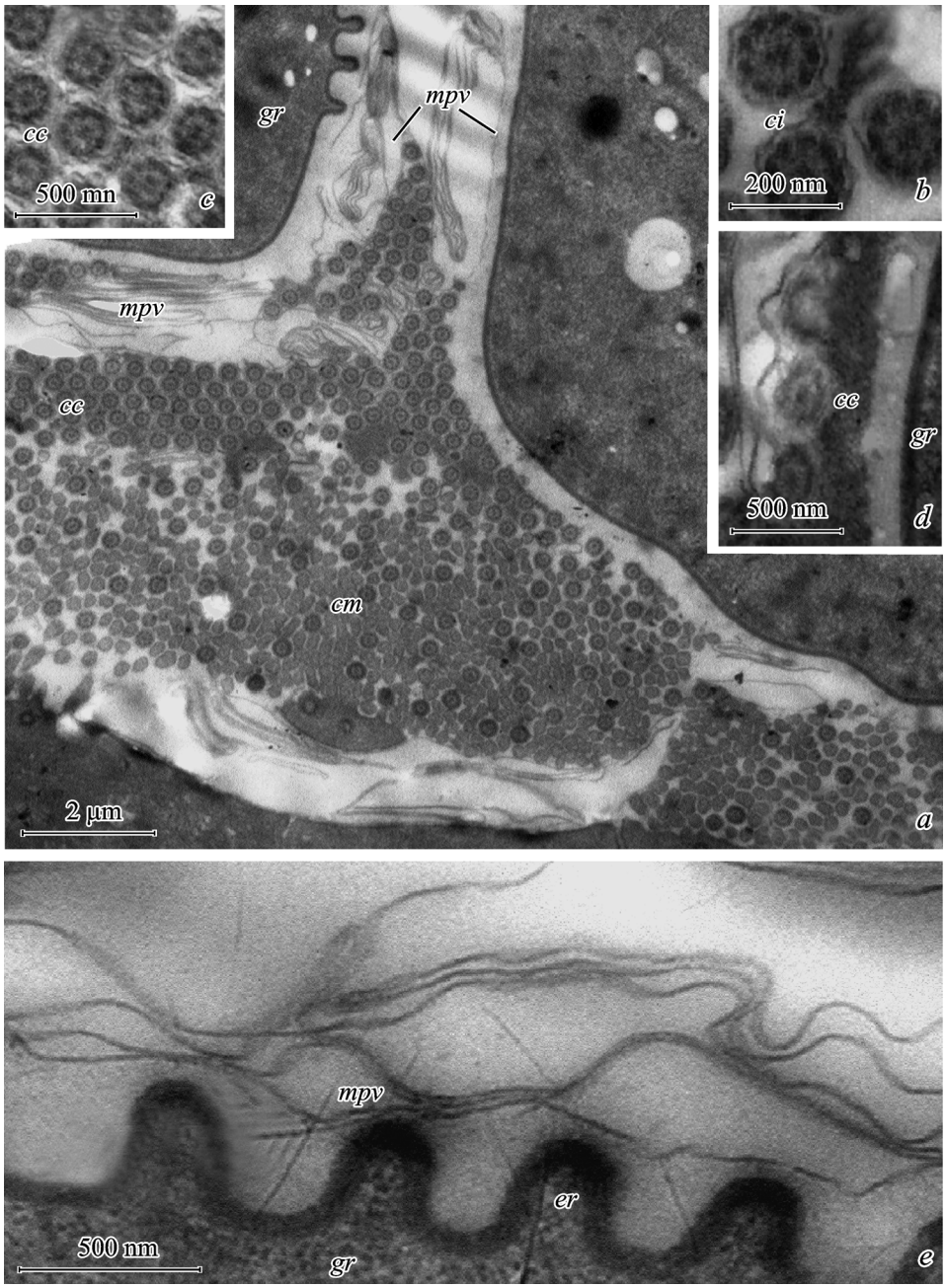

Fig. 5. Fine structure of the trophozoite in parasitophorous vacuole, and clusterized cilia around it.

a — cross section of two neighboring trophozoites with adjacent parasitophorous vacuole membranes. *b*—*e* — steps of parasitophorous vacuole formation: *b* — the beginning of cilia clusterization (neighboring cilia approximate to each another); *c* — clusterization of cilia (cilia become tightly adjacent to each other); *d* — a further step of cilia disorganizing (fusion of membranes of clusterized cilia and disorganizing of microtubules); *e* — material of microtubules of clusterized cilia is completely resorbed, membranes of these cilia are fused to produce a multimembraned layer limiting the parasitophorous vacuole. *cm* — membranes of cilia, *mpv* — membranes of parasitophorous vacuole; other designations are the same as in Figs 1, 2, 4.

Рис. 5. Ультраструктура трофозоита в паразитофорной вакуоли и ресничек, кластеризованных вокруг него.

a — два соседних трофозоита с плотно прилегающими к ним мембранами паразитофорных вакуолей, поперечный срез. *b*—*e* — стадии образования паразитофорной вакуоли: *b* — начало кластеризации ресничек (рядом лежащие реснички тесно соприкасаются); *c* — кластеризация ресничек (реснички плотно прилежат друг к другу); *d* — следующая стадия изменения ресничек (слияние мембран кластеризованных ресничек и разрушение микротрубочек); *e* — материал микротрубочек кластеризованных ресничек полностью резорбируется, мембраны бывших ресничек сливаются, образуя мультимембранный слой вокруг паразитофорной вакуоли. *cm* — мембраны ресничек, *mpv* — мембраны паразитофорной вакуоли; остальные обозначения те же, что и на рис. 1, 2, 4.

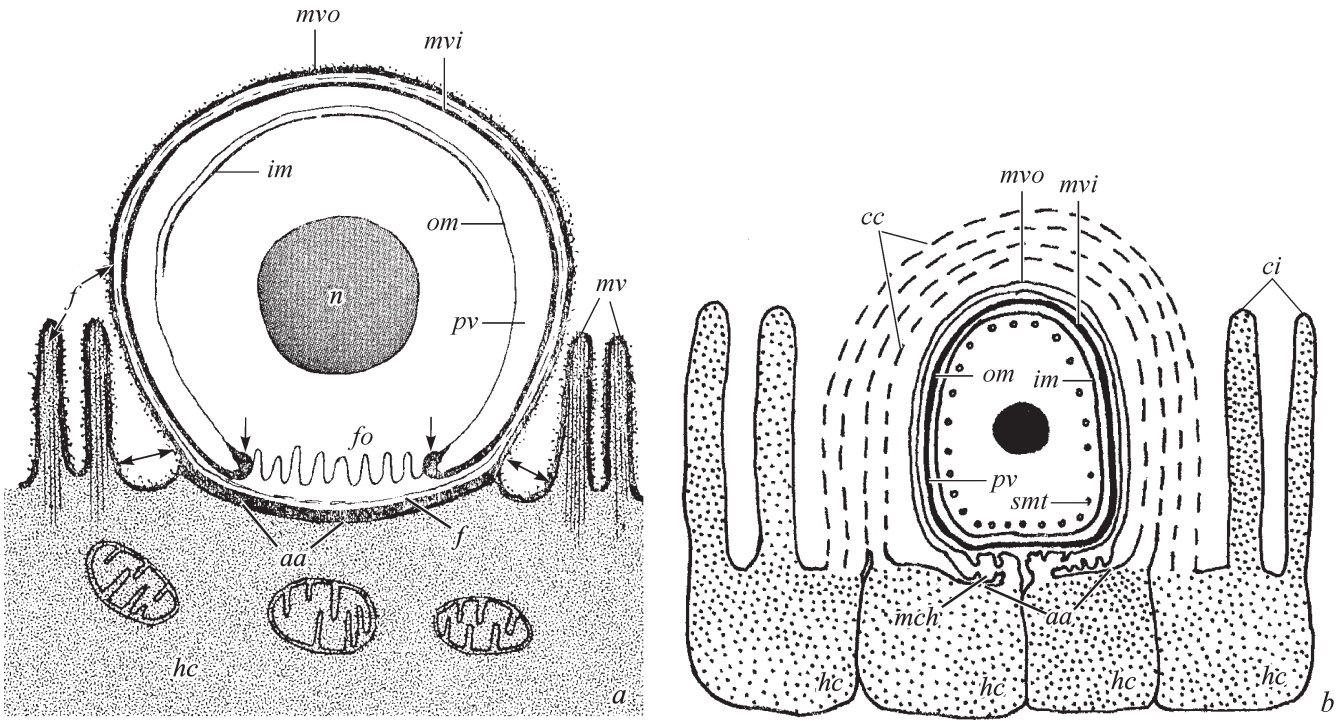

Fig. 6. Comparative schematic representation of model of extracytoplasmic intracellular localization in *Cryptosporidium* and *Ditrypanocystis* sp.

a — *Cryptosporidium* in the gut of suckling mouse (from: Goebel, Braendler, 1982) possessing a double-membrane parasitophorous vacuole of fused microvilli preserving filaments; feeder organelle is seen in the region of contact. *b* — *Ditrypanocystis* sp. in the gut of oligochaete worm (hypothetical scheme) possessing a multimembrane parasitophorous vacuole of fused and next to fuse clusterized cilia lacking microtubules, see membranous structures at the region of contact. *aa* — attachment area, *f* — microfilaments of villi, *fo* — feeder organelle, *mv* — microvilli, *mvi* — inner membrane of parasitophorous vacuole, *mvo* — outer membrane of parasitophorous vacuole, *pv* — parasitophorous vacuole; other designations are the same as in Figs 1—4.

Рис. 6. Сопоставление способов экстрацитоплазматической внутриклеточной локализации *Cryptosporidium* и *Ditrypanocystis* sp. (схема).

a — *Cryptosporidium* в кишке новорожденного мышонка (из: Goebel, Braendler, 1982), имеющий двумембранную паразитофорную вакуоль из слившихся микроворсинок, сохраняющих микрофиламенты; питающая органелла выявляется в месте контакта. *b* — *Ditrypanocystis* sp. в кишке олигохеты (гипотетическая схема), имеющий мультимембранную паразитофорную вакуоль из слившихся и сливающихся ресничек, теряющих микротрубочки; видны мембранные структуры в зоне контакта. *aa* — зона контакта, *f* — микрофиламенты микроворсинок, *fo* — питающая органелла, *mv* — микроворсинки, *mvi* — внутренняя мембрана паразитофорной вакуоли, *mvo* — наружная мембрана паразитофорной вакуоли, *pv* — паразитофорная вакуоль; остальные обозначения те же, что и на рис. 1—4.

irregularly in the gut lumen, in the infected oligochaetes become oriented parallel to one another, being in some places adjacent to the parasite surface (Fig. 4, *c*). After being oriented parallel to one another the cilia become fused (Fig. 5, *b*), thus making clusters (Fig. 5, *c*). Afterwards plasmatic membranes of clusterized cilia fuse, and ciliar microtubules are resorbed (Fig. 5, *d*). In such a way, a network of multimembranous layers is formed in the space around the parasite (Fig. 2, *a*, *d*; 4, *a*; 5, *a*, *e*). Thus, the trophozoite becomes enclosed within a parasitophorous vacuole composed of membrane layers surrounded by cilia, which fuse eventually.

On Fig. 6, *a* comparative diagrammic representation is given summarizing patterns of similarity/dissimilarity between patterns of parasitiphorous vacuole formation around *Ditrypanocystis* sp. and *Cryptosporidium*.

On Fig. 7, *a* comparative diagrammic representation is given for illustrating successive stages of parasitophorous vacuole formation in *Ditrypanocystis* sp.

Discussion

The availability in trophozoites of *Ditrypanocystis* sp. of the epicyte with numerous regular longitudinal ridges and irregular folds enables us to qualify the gregarine as belonging to the monotypic genus *Ditrypanocystis* Burt et al. (1963) in the family Selenidiidae, so far comprizing only one species — *D. cirratuli* parasitic in polychaetes, rather than in oligochaetes.

The ultrastructure of *D. cirratuli* from the polychaete *Cirriformia tentaculata* was studied in detail by Mac Gregor and Thomasson (1965). Pellicle ridges of *D. cirratuli* are doubled at their ends, by this differing from non-doubled ridges of *Ditrypanocystis* sp., the subject of the present research. Both the species have almost similar numbers of epicyte ridges: 25 and 26 in *D. cirratuli* and *Ditrypanocystis* sp., respectively. But the compared species differ in the number and size of folds, making profiles of so called «undulating membranes» involved in trophozoite movement in the gut lumen before the attachment (Mac Gregor, Thomasson, 1965). All this makes it possible to consider *Ditrypanocystis* sp. from the oligochaete *Enchytraeus albidus* as a new species.

Ditrypanocystis sp. makes the first case of parasitism of selenid gregarines in the host, which is not primarily a marine animal. Te majority of selenids are parasitic in polychaetes, some of selenids are known from sipunculids, and only single species were reported from Echinodermata, Hemic-

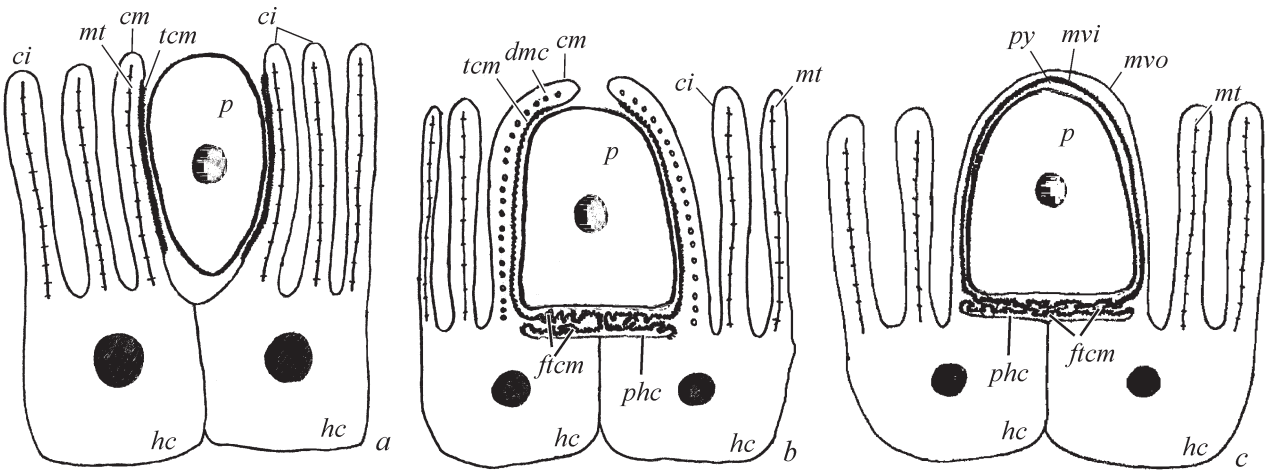

Fig. 7. A hypothetic scheme of the steps of host-parasite interface formation by *Ditrypanocystis* sp.

a — parasite contacting cilia of the neighboring enterocytes eventually transforming them. *b* — transformed cilia clusterize around the parasite, ciliary plasmalemmas form branched folds between host and parasite surfaces at the contact region. *c* — plasmalemmas of clusterized cilia become completely fused to form a closed parasitophorous vacuole with heavily folded contact area. *ftcm* — folds of transformed ciliary membrane, *p* — parasite, *tcm* — transformed cilia membrane; other designations are the same as in Figs 1—6.

Рис. 7. Гипотетическая схема стадий образования паразито-хозяинного взаимодействия у *Ditrypanocystis* sp.

a — паразит входит в контакт с ресничками окружающих его энтероцитов и трансформирует их. *b* — трансформированные реснички кластеризуются вокруг паразита, их плазмалемма формирует в зоне контакта разветвленные складки. *c* — плазмалемма кластеризованных ресничек сливается полностью с образованием замкнутой паразитофорной вакуоли, сильно складчатой в зоне контакта. *ftcm* — складки трансформированной плазмалеммы ресничек, *p* — паразит, *tcm* — трансформированная плазмалемма ресничек; остальные обозначения те же, что и на рис. 1—6.

hordata, and Chordata (Ascidia). The oligochaetes are known to originate in fresh water basins; subsequently, some of them must have been secondarily changed to adapt to marine survival. The occurrence of *Ditrypanocystis* sp. in oligochaete hosts, inhabiting storm discharge, suggests that selenid gregarines may have had a wider range of host specificity than it was thought before.

The present study has shown that *Ditrypanocystis* sp. lives in the host-parasite interface, unique for gregarines, which involves a peculiar pattern of membranous structures and a parasitophorous vacuole composed of membranes that are in fact membranes of fused cilia.

Gregarines are known to display a broad scale of adaptations for survival in the brush border of host gut cells (e. g. Tuzet, Galangau, 1968; Hildebrand, 1976; Ormières, Marquès, 1976). But in all these cases enterocyte microvilli are commonly applied to the parasite's epicyte only at some particular regions, as, for example, in gregarine *Actinocephalus carrilynnae* from dragonfly *Enallagma civile* (Arthropoda, Odonata) (Cook et al., 2001). So far, no cases have been reported of a complete enclosing of gregarines within a parasitophorous envelope made of clustered or fused microvilli or cilia. Such a type of host-parasite interaction is characteristic of the only coccidian genus *Cryptosporidium* (Apicomplexa, Eimeriida), which is illustrated in Fig. 6. On being attached to the top of enterocyte, zoites of *Cryptosporidium* stimulate an, additional growth of microvilli and their eventual fusion around the parasitized cell (for reference see: Beyer et al., 2000). In the case of *Cryptosporidium* (Fig. 6, *a*) as well as of *Ditrypanocystis* sp. (Fig. 6, *b*) a specialized host-parasite interface is formed. But with *Cryptosporidium*, the inner membrane complex and subpellicular microtubules progressively disappear in the contact area. At the periphery of this area the outer membrane (plasmalemma) covering the parasite body fuses with the inner membrane of parasitophorous vacuole. Within the host cell, in the contact area a thick electron-dense band is formed. The filamentous content of joined microvilli is retained. The central part of the parasite plasmalemma forms a feeder organelle made of numerous folds directed to the host cell surface. The membranous folds of the surface organelle much enlarge the contact area between the host and parasite. Through this highly specialized host-parasite interface *Cryptosporidium* is presumably supplied with nutrients from the host cell, rather than from the gut lumen.

The pattern of parasitism of *Ditrypanocystis* sp. obviously differs from that in *Cryptosporidium* in spite of the extracytoplasmic localization common for both. The gregarine preserves pellicular and subpellicular microtubules in the contact area. In this area neither direct fusion of plasmalemma with host cell membrane, nor feeder organelle occur. The fused cilia of oligochaete enterocytes totally lack microtubular contents and, as a consequence, they may lose their motility. Unlike, microvillar filaments in the parasitophorous vacuole of *Cryptosporidium* are preserved.

The pellicle of *Ditrypanocystis* sp. trophozoite remains completely preserved in the contact area (Fig. 6, *b*). Likely as in *Cryptosporidium*, the contact area of *Ditrypanocystis* sp. is considerably enlarged. But in the latter, numerous additional membranous structures are seen to tightly fill the whole space between the host and parasite plasmalemmas. Neither folds of parasite plasmalemma (feeder organelle) nor its fusion with the host cell are observed in the gregarine under discussion.

In *Ditrypanocystis* sp., membranous structures, channels and vesicles, may originate from just the same enterocyte cilia to form the parasitophorous vacuole. Ciliary fusion of is triggered presumably by the parasite's contact with plasmalemma of cilia of neighboring enterocytes (Fig. 7, *a*). Under the influence of the parasite, cilia loose their microtubular

contents and become clusterized around the parasite. Then they fused to form membranes of the parasitophorous vacuole. The ciliary plasmalemma becomes heavily folded beneath the parasite at the attachment place (Fig. 7, *b*) and after the fusion it forms a network of membranous structures between the host cell and parasite surfaces in the contact area (Fig. 7, *c*).

As a result, a hypothesis has been put forward that in *Ditrypanocystis* sp. the surface of the host-parasite contact area is extremely enlarged, and that numerous branched channels are facing one another with joined plasmalemmas of cilia. The fact that these channels are opened to the gut cell surface and thus contact directly with the contents of enterocyte transport vacuoles supports the idea that the source of nutrients taken by gregarines is intracellular, similarly as is *Cryptosporidium*, rather than extracellular, from the gut lumen. Perhaps, *Ditrypanocystis* sp. may also use for some degraded microtubule material of fused cilia for nutrition.

Cryptosporidium sp. is the only coccidian parasite, whose zoites do not penetrate the host cell by means of its membrane invagination and form an extracytoplasmic parasitophorous vacuole. After Grassè (1953), in has been commonly considered that the Coccidia have evaluated in general as intracellular parasites, whereas the Gregarinia mostly remained extracellular. If this view is correct, the extracytopasmatic mode of parasitism seen in *Cryptosporidium* well compares with relevant data on molecular phylogeny, suggesting that *Cryptosporidium* may have the «sister» relationship with gregarines, being closer to the latter than to the Coccidia (Carreno et al., 1999; Leander et al., 2003).

Double-membrane parasitophorous vacuoles of *Cryptosporidium* and multimembrane vacuoles of *Ditrypanocystis* seem to be similarly formed with the involvement of analogous enterocyte outputs — microvilli and cilia. This may reflect similar modes of adaptation to parasitic survival in similar sites of location in the enterocyte brush border. Though there are substantial differences between these two parasites, which involve structural peculiarities of the contact area and formation of parasitophorous vacuole, the very fact of remote development of functionally similar features by different ways in phylogenetically different organisms must be considered as a typical case of evolutionary parallelism. So, the curious similarity between *Ditrypanocystis* sp., belonging to the archaic family Selenidiidae (Schrèvel et al., 1971), on the one hand, and *Cryptosporidium*, on the other one, in no case is a manifestation of any direct phylogenetic heritage, but may suggest a possible way followed by gregarine-like ancestors to produce the pattern of parasitophorous vacuole typical for *Cryptosporidium* species.

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DITRYPANOCYSTIS SP. (APICOMPLEXA, GREGARINIA, SELENIDIIDAE): СПОСОБ ПАРАЗИТИРОВАНИЯ В КИШЕЧНИКЕ *ENCHYTRAEUS ALBIDUS* (ANNELIDA, OLIGOCHAETA, ENCHYTRAEIDAE), ПОДОБНЫЙ ТАКОВОМУ КОКЦИДИЙ РОДА *CRYPTOSPORIDIUM*

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Приведены результаты светооптического и электронно-микроскопического исследования грегарины *Ditrypanocystis* sp. (Apicomplexa, Gregarinia, Selenidiidae) из кишечника олигохеты *Enchytraeus albidus.* Трофозоит *Ditrypanocystis* sp. прикрепляется передним концом между клетками ресничного эпителия (энтероцитами) в области крипт. В результате между трофозоитом и поверхностью энтероцитов формируется слой мембранных канальцев и везикул неизвестного происхождения. В зоне контакта на поверхности клеток хозяина отмечается отсутствие ресничек. Трофозоит оказывается внутри замкнутой паразитофорной вакуоли, ограниченной мембранами ресничек энтероцитов, слившихся вокруг паразита. При этом такие реснички утрачивают свои микротрубочки. Предполагается, что мембранные структуры возникают в зоне контакта паразита и хозяина из мембраны ресничек клетки хозяина, а не из плазмалеммы паразита. Этот способ формирования паразитофорной вакуоли, не описанный у грегарин, во многом сходен с выявленным у кокцидий рода Cryptosporidium, хотя имеет и некоторые отличия. В свете данных молекулярной филогении (Carreno et al., 1999; Leander et al., 2003), выявивших сестринское родство между грегаринами и кокцидиями рода Cryptosporidium, эта общая черта позволяет предположить возможный путь эволюции внеклеточного паразитизма грегарин в направлении внутриклеточого паразитизма кокцидий, как это видно у криптоспоридий.

Ключевые слова: грегарина, *Ditrypanocystis*, Selenidiidae, ультраструктура, филогения, *Cryptosporidium*.