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Protistology

The cortical zone of skittle-like cells of *Urospora chiridotae*, a gregarine from an apode holothuria *Chiridota laevis*

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Summary

Electron microscopic study revealed a unique structure of the cortical zone of the skittle-like cells of *Urospora chiridotae* (Dogiel, 1906) Goodrich, 1925 (Eugregarinida: Urosporidae), a eugregarine from an apode holothuria *Chiridota laevis* Fabricius, 1720. The cell surface is covered with numerous hair-like projections, cytopilia. Their inner structure is formed by numerous longitudinal microtubules. These tubules are paired with fibrillar bands, making up a uniform carcass within each cytopilion. Another unique feature of these cells is the presence of pellicular invaginations, pellicular canals, at the bottom of which micropores are situated. The structure and function of both these structures are discussed.

Key words: *Urospora chiridotae,* skittle-like cells, cytopilia, cortical zone, pellicular canals, micropores

Introduction

Eugregarines possessing gliding motility usually have a typical structure of the cortical zone, composed of the epicyte and the ectocyte. The epicyte forms longitudinal pellicular folds. Its inner structure is described for a great number of eugregarines. The pellicular folds have the same structure in all of them. (Vivier, 1968; Vávra, 1969; Vavra and Small, 1969; Walsh and Callaway, 1969; Vivier et al., 1970; Heller and Weise, 1973; MacMillan, 1973; Sanders and Poinar, 1973; Sathananthan, 1977; Tronchin and Schrével, 1977; Hildebrand, 1980; Philippe et al., 1982; Schrével et al, 1983; Lucarotti, 2000).

In eugregarines the structure of the cortical zone changes with the changing of the movement manner. For example, in a monocystid *Nematocystis magna* (Schmidt, 1854) Hesse, 1909 essential modification of the epicyte occur during peristaltic movements (MacMillan, 1973).

Some gregarines from the families Monocystidae, Lecudinidae and Urosporidae bear hair-like projections on their surface. They are immotile or slow-moving, and the cortical zone structure of these gregarines differs

completely from that in gregarines with glidding or peristaltic motility (Vivier, 1968; Vivier and Petitprez, 1968; Warner, 1968; Porchet-Hennerè and Fischer, 1973; Sanders and Poinar, 1973; Sathanantan, 1977; Tronchin and Schrével, 1977; Hildebrand, 1980; Philippe et al., 1982; Schrével et al., 1983; Hoshide and Todd, 1996).

Being outwardly very similar in different gregarines, hair-like projections vary considerably in their fine structure (Vivier and Petitprez, 1968; Warner, 1968; Porchet-Hennerè and Fischer, 1973; Sathanantan, 1977; Hoshide and Todd, 1996). This research is aimed at studying the structure of the hair-like projections covering the surface of the skittle-like trophozoites of *Urospora chiridotae*.

Material and Methods

Sea cucumbers *Chiridota laevis* were collected at the sublitoral zone of the Sidorov Island near the White Sea Biological Station of the Saint-Petersburg State University (the Chupa Inlet, the Kandalaksha Bay, the White Sea) and in the vicinity of the White Sea Biological Station of the Moscow State University, in the Rugozerskaya Inlet near the Yepanchinicha Island, the Kandalaksha Bay, the White Sea, during the summers of 2000-2003. The individuals were dissected after complete anaesthesia with $MgCl_2 \times 6H_2O$. Different organs were fixed with several fixators for both light and electron microscopy. Light microscopic techniques were described in Dyakin and Paskerova (2004).

For **transmission electron microscopy** holothurian's blood vessels with parasites were fixed in PBS containing 2.5% (v/v) glutaraldehyde, with osmomolarity 750 mOsm. After several rinses in buffer solution blood vessels were postfixed for 1.5 h in 1% (w/v) osmium tetroxide in PBS, with osmomolarity 750 mOsm. The specimens were then dehydrated in an ethanol series and propylene oxide and embedded in Epon 812. Sections were obtained with a Leica Ultracut R with glass knives. Ultrathin sections were transferred on the Formvar coated oval whole grids and stained with lead citrate using Reynolds techniques (Reynolds, 1963). Micrographs were obtained with Leo-910 electron microscope and scanned with the Hewlett Packard Scan Jet 5370C.

For scanning electron microscopy trophozoites of gregarines were fixed in 1.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) with NaCl up to 717 mOsm and postfixed in 2% OsO_4 in 0.1 M cacodylate buffer with the same osmomolarity. The trophozoites were dehydrated in a graded ethanol series, a mixture of 96% ethanol and acetone (1:1 v/v), and absolute acetone, and critical-point dried with CO_2. Dried specimens were mounted on stubs, sputter-coated with gold-palladium, and examined under a CamScan-S2 scanning electron microscope.

Results

Light microscopy

Both young and mature trophozoites of *Urospora chiridotae* were localized in the lumen of blood vessels. The former were sausage-like, up to 10 μm in length (Fig. 1 A). The latter were skittle-shaped, up to 500 μm in length and up to 100 μm in width in the widest region. Their narrow end was attached to the wall of the blood vessel (Fig. 1 B). Both young and mature gregarines were usually observed in lateral syzygy.

The surface of both young and mature trophozoites was covered with hair-like projections (Fig. 1). According to Warner (1968), these projections are cytopilia (see Discussion). During trophozoites' growth the length of cytopilia is not changed, remaining approximately 10-15 μm, but their number is increased. In mature trophozoites cytopilia are oriented perpendicularly to the cell surface. At the free end of the parasite they are longer and are parallel to the long axis of the cell (Fig. 1 B). On the attached end of the parasite cytopilia seem to be less long, but denser and more rigid than on the rest of the cell surface. They have a narrow conic shape and injure the epithelium of the vessel.

Electron microscopy

The pellicle, about 30 nm in thickness, of trophozoites is formed by three membranes. The distance between the membranes is usually constant. In the space between plasmalemma and alveoli fibrillar material can be observed. This material forms cross bands connecting the membranes. The innermost membrane of the internal membrane complex seems to be thicker than the others, because of the fibrillar material that can accumulate on its cytoplasm-oriented surface (Fig. 2 A).

No longitudinal or transverse pellicular folds were observed, though the surface of the gregarine was slightly rough (Fig. 3). As mentioned above, the surface is covered with numerous cytopilia (Figs 2 B; 4). The cytopilia are cylindrical, slightly narrowing to the distal end. These projections are highly variable in size. Their diameter at the basis varies from 200 to 700 nm, and the diameter of the tip on cross sections can reach 100 nm.

The inner structure of each cytopilion comprises numerous microtubules. They are oriented parallel to the long axis of cytopilion and to each other (Fig. 4 A). At a basis their number can exceed a hundred (Fig. 4 B), while in distal parts of cytopilion the number of microtubules is reduced to 8 or less (Fig. 4 C). Both at

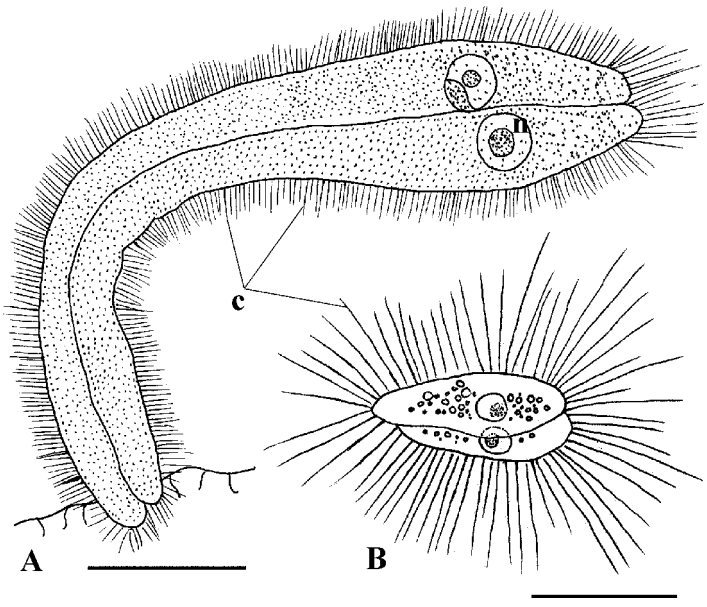

Fig. 1. Light microscopic drawing of a skittle-like cell of *Urospora chiridotae*. A - young trophozoites; B - mature trophozoites. *Abbreviations*: c - cytopilia, n - nucleus. Scale bars: A - 10 μm, B - 50 μm).

transversal and at longitudinal sections through various parts of cytopilia these microtubules were paired by fibrillar bands (Figs 4 A, B). Microtubules and fibrillar bands together are organized into a uniform carcass.

At cross sections through the cytopilion's base microtubules are disposed without any order, although they are connected with fibrillar bands, forming the internal carcass (Fig. 4 B). In the cytoplasm all these microtubules extend more or less radially away from the base, and parallel to cell surface (Fig. 4 A, D). In contrast to the basal part of cytopilion, in proximal parts the complex of microtubules was strictly ordered. There was a unique microtubule in the centre surrounded by seven or less microtubules at the periphery (Fig. 4 C).

No fibrills or microtubules organized in systems, like in *Rhynchcystis pilosa* (Cuenot 1901) Hesse 1909, *Gregarina blaberae* Frenzel and other gregarines (see Warner, 1968; Schrével et al., 1983 and many others) are present in the cytoplasm under the pellicle of skittle-like cells of *Urospora chiridotae*. Neither longitudinal or circular microtubules nor microfilaments are present.

The pellicle of the skittle-like parasite also forms numerous cylindrical invaginations into the cytoplasm up to 1-1.5 μm in depth (Fig. 5 A). We suggest to call these structures pellicular canals. The number of pellicular canals per 1 square μm is sometimes rather large, up to 2-3, with an average diameter of the opening about 230 nm (Fig. 3 B). The internal membrane complex reaches down to the bottom of each canal. There it forms an electron-dense collar up to 120 nm in diameter (Fig. 5 B). The plasmalemma goes through this collar and forms an invagination extended into cytoplasm. The diameter of this plasmalemmal invagination can reach 170 nm (Fig. 5 B). On some sections a rather wide channel limited with a cell membrane can be observed (Fig. 5 C). However, small sac-like plasmalemmal invagination can also be found (Fig. 5 B). It probably depends on the plane of the sections that pass through the pellicular canals. Occasionally small ve-sicles budding from invaginations were obse-rved (Fig. 5 D).

A generalized scheme of the cortical zone structure in *Urospora chiridotae* is given in Fig. 6.

Discussion

This study has revealed two unique ultrastructural features of the aseptate gregarine *Urospora chiridotae*: the presence of pellicular canals and the fine structure of cytopilia.

Cytopilia are rare and unusual structures for the gregarines, they can be observed in some monocystids, lecudinids and urosporids.

In a monocistid *Zeylanocystis burti* Dissanaike, 1953 cytopilia are situated on the tip of the conical papillae, which form at the rim of the saucer-shaped cell. The inner structure of cytopilia is similar to that in *Urospora chiridotae*. It is composed of microtubules, but in contrast to the latter species, the number of microtubules is much less and they were not observed to form a uniform carcass. They continue into the papillae and form an internal cortex in them and in the pellicular folds, that cover the rest of the surface of the saucer-shaped cell (Sathanantan, 1977).

In another monocystid, *Rhynchocystis pilosa*, cytopilia are extensions of the primary pellicular ridges of the cortical zone and cover all the surface of the parasite, except the anterior part of the cell where the attachment organelle is situated. The carcass of these cytopilia consists of several microfilaments, oriented parallel to the long axis of cytopilion. Microfilaments are extended into primary ridges and pass longitudinally at the tip (Warner, 1968).

In three species from the family Lecudinidae (*Diplauxis hatti* Vivier, Ormières et Tuzet 1964, *Diplauxis schreveli* Porchet-Henneré et Fischer 1973 and *Filipodium ozakii* Hukui 1939) cytopilia have a similar structure. No microtubules or microfilaments supporting hair-like projections are observed. Instead there is an electron-dense tubular structure, originating probably from alveoli, serving as a carcass of cytopilia, that are

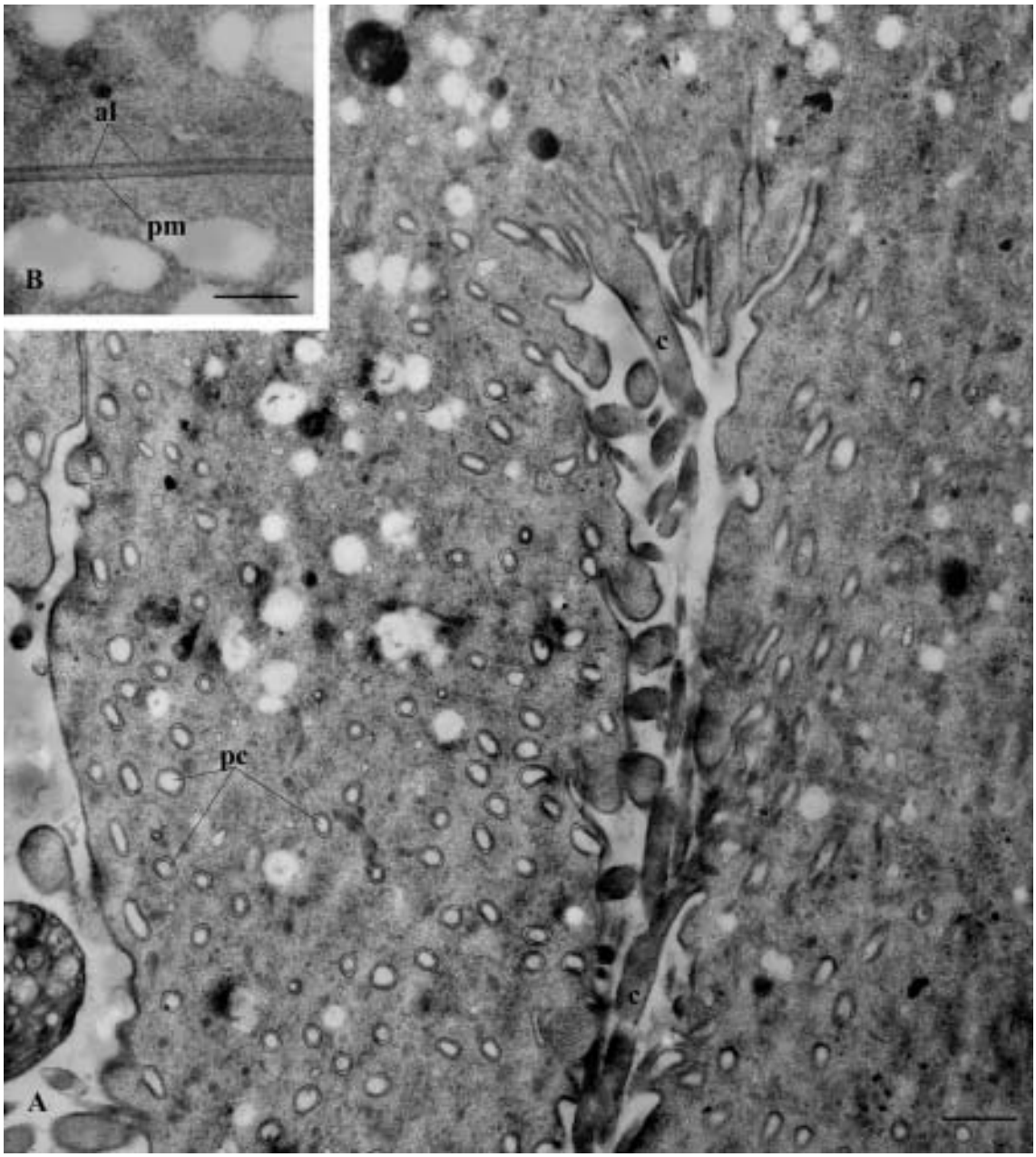

Fig. 2. Cortical zone of *Urospora chiridotae*. A - general view; B - pellicle of skittle-like cells (region of contact of two cells). *Abbreviations*: al - alveoli, pc - pellicular canals, pm - plasmalemma. Other abbreviations as in fig. 1. Scale bars: A - 1 μm, B - 0.5 μm.

present in these lecudinids (Vivier and Petitprez, 1968; Porchet-Hennerè and Fischer, 1973; Hoshide and Todd, 1996).

We suppose that the hair-like projections described previously are analogous but not homologous, because their fine structure varies. Obviously, cytopilia arose independently in different taxa of the eugregarines. Therefore, we propose to distinguish certain morphological types of cytopilia. In *Diplauxis* spp. the surface of trophozoites is covered only with cytopilia (Vivier, Petitprez, 1968; Porchet-Hennerè and Fischer, 1973). In contrast, in *Filipodium ozakii* rather wide pellicular ridges supported with longitudinal microtubules are formed on the cell surface. These microtubules pass directly under the pellicle parallel to the long axis of the cell (Hoshide and Todd, 1996).

Most monocystids, lecudinids and urosporids lack cytopilia and possess either typical epicyte with the

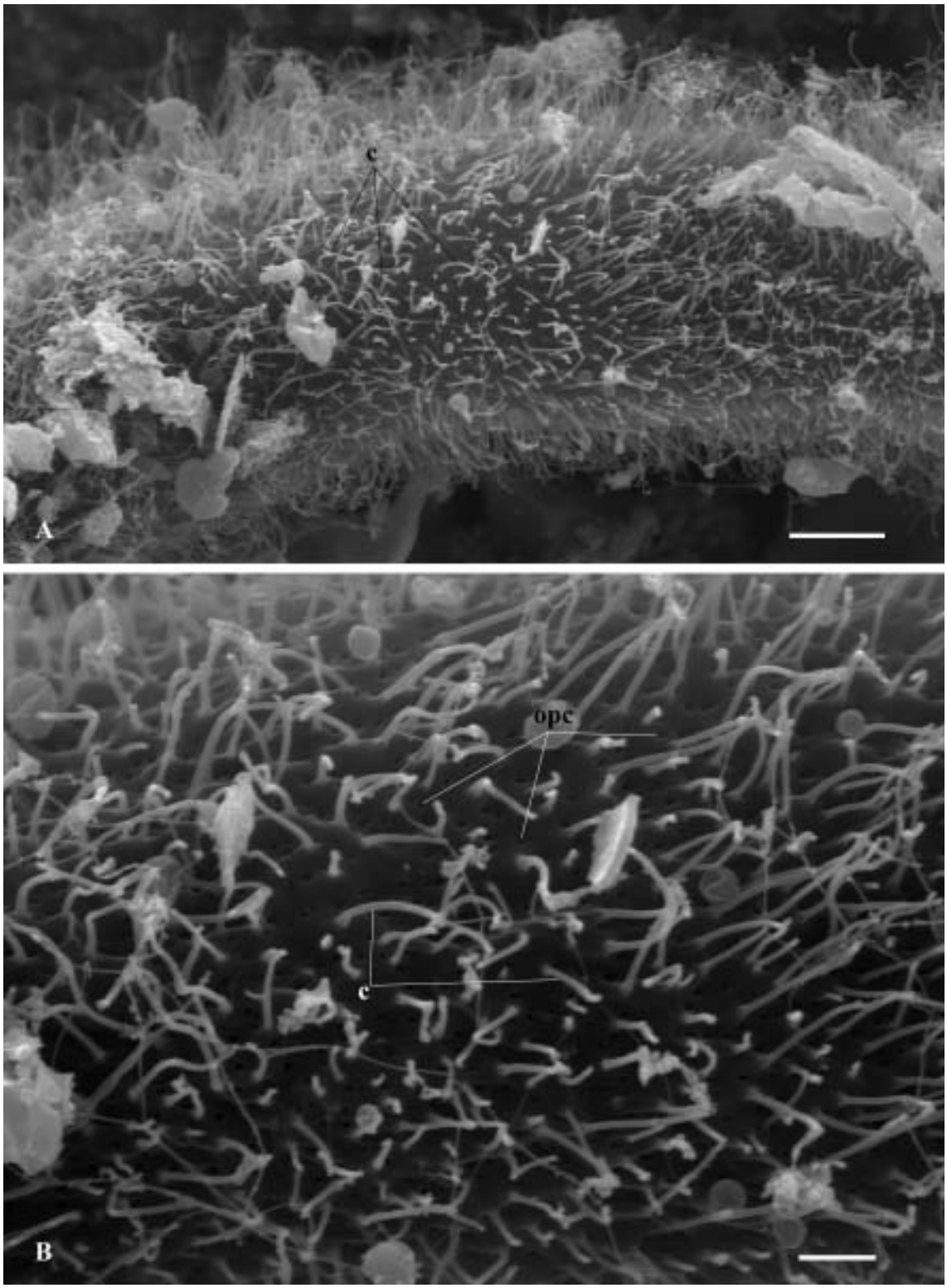

Fig. 3. Scanning electron microscopy of the surface of mature trophozoites. A - general view of the cell; B - part of the surface. *Abbreviations*: opc - openings of the pellicular canals. Other abbreviations as in figs 1, 2. Scale bars: A - 10 μm, B - 3 μm.

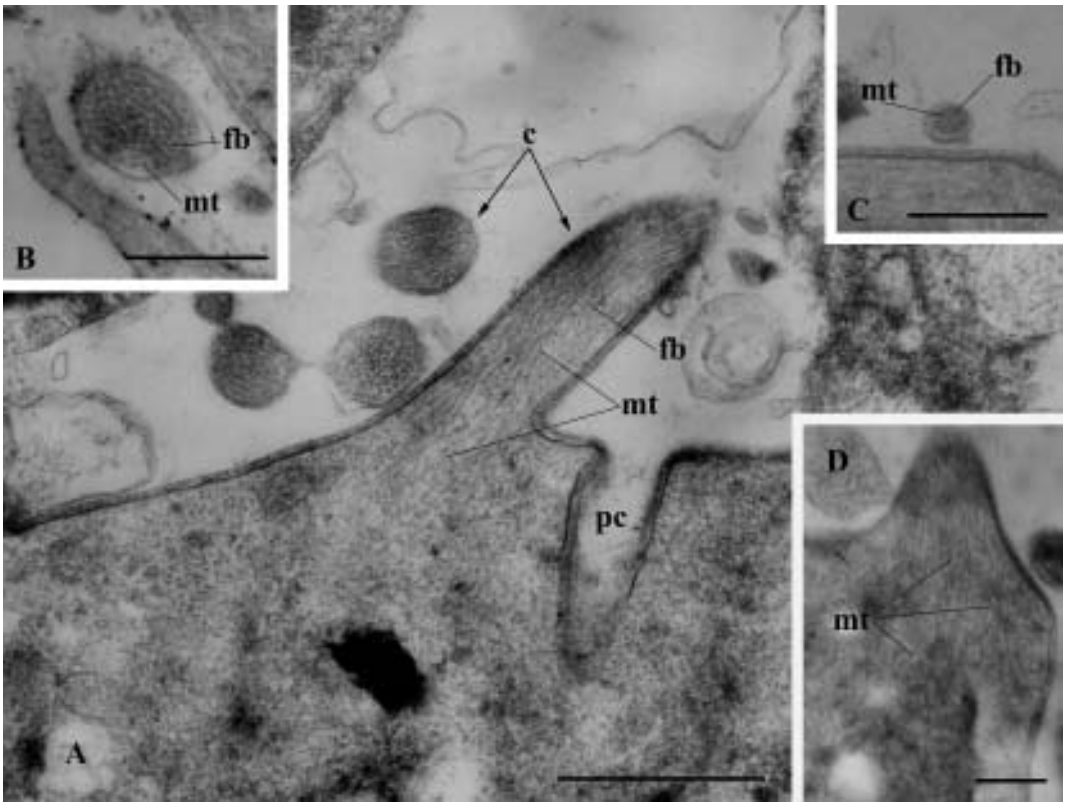

Fig. 4. Ultrastructure of the cytopilia. A - longitudinal section of a cytopilion at the basis; B - transverse section of a cytopilion at the basis; C - transverse section of a cytopilion at the tip, D - longitudinal section of a cytopilion at the basis. *Abbreviations*: fb - fibrillar band, mt -microtubules, pc - pellicular canals. Other abbreviations as in figs 1-3. Scale bars: A - 1 μm, B, C - 0.25 μm, D - 0.5 μm.

longitudinal folds or somewhat modified epicyte and cortical zone. For example, in the pellicle of the *Gonospora holoflora* Pomory et Lares 1998 (Urosporidae), *Lithocystis schneideri* Giard, 1876 (Urosporidae) and *Lecudina pellucida* (Kölliker, 1849) Mingazzini, 1891 (Lecudinidae) longitudinal folds are formed that are club-shaped in cross sections (Vivier, 1968; Coulon and Jangoux, 1988; Pomory and Lares 1998). In *Pterospora floridensis* Landers, 2001 (Urosporidae) the cell surface varies from smooth to crenulate, with occasional ridges, numerous pockets and folds (Landers, 2002).

The trophozoites of *Urospora chiridotae*, similarly to the trophozoites of *Diplauxis* spp., are immotile. This phenomenon can be easily explained. Motile stages of gregarines (Septata, Selenidiidae) are characterized by gliding (in most Septata and Aseptata), peristaltic (Aseptata: Monocystidae) movement or active undulation (Selenidiidae). Such movements become possible due to presence of a well-developed typical pellicular fold as in Septata and Aseptata, a modifying cortical zone as in Monocystidae and longitudinal wide groves as in Selenidiidae, with well-developed contractile systems (Grassé, 1953; Miles, 1966; Warner, 1968; Vivier, 1968; Walsh and Callaway, 1969; Heller and Weise, 1973; MacMillan, 1973; Frolov, 1991). So, the trophozoites of the *U. chridotae* and *Diplauxis* spp. are immotile in consequence of absence of contractile systems (Vivier and Petitprez, 1968; Porchet-Hennerè and Fischer, 1973; present study).

In most eugregarines specialized structures, micropores, are situated between pellicular folds or cytopilia. They are invaginations of the plasma membrane into the cytoplasm of the cell. These invaginations are reinforced by an electron-dense collar formed from internal membrane complex. Micropores in eugregarines lie on the cell surface (Schrével, 1968; Vivier, 1968; Scholtyseck and Mehlhorn, 1970; etc.). This study has revealed that on the surface of trophozoites of *Urospora chiridotae* pellicular canals are formed. At the bottom of these canals plasmalemmal invaginations are situated. These invaginations correspond precisely to the description of zoites' micropores of others sporozoans in general and gregarines in particular.

In other gregarines pellicular canals with the micropores at the bottom are not found. However, a previous investigation revealed deep plasmalemmal

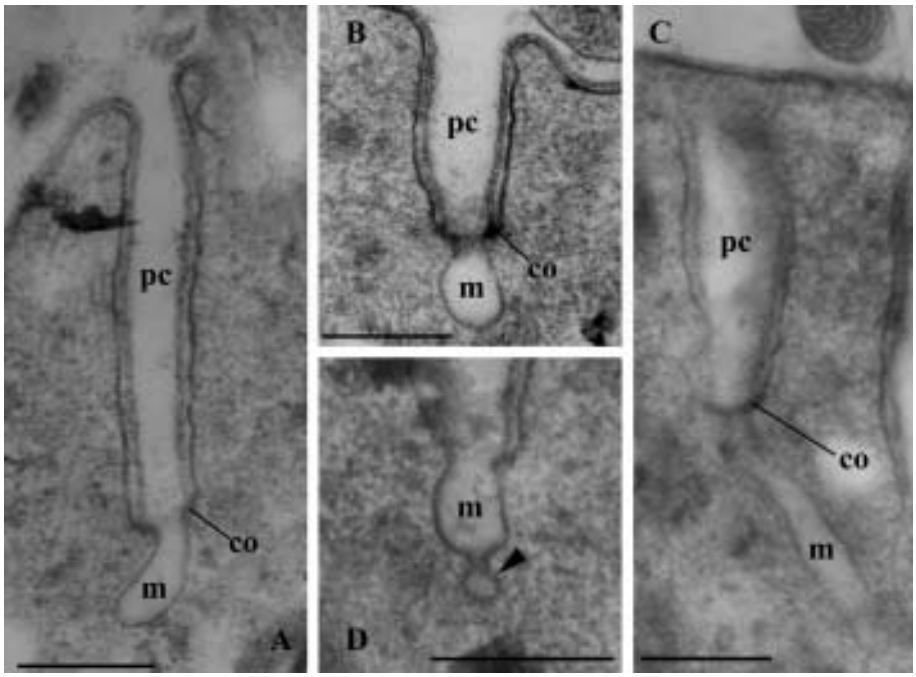

Fig. 5. Ultrastructure of the pellicular canals and micropores. A, B - general structure of the pellicular canals and micropores, C - micropore represented by a wide channel, D - a small vesicle budding from micropore (arrowhead). *Abbreviations*: co - electron dense collar, m - micropore. Other abbreviations as in fig. 2. Scale bars: 0.5 μm.

invaginations, microcanaliculae, in *Lithocystis schneideri*. At the bottom of microcanaliculae micropores are situated. The wall of microcanaliculae is inlayed with a single cytoplasmal membrane, instead of three-membrane pellicle as in *Urospora chiridotae* (Coulon and Jangoux, 1988). From our point of view, these structures, microcanaliculae and micropores, as indicated in the original paper (Coulon and Jangoux, 1988), are micropore parts of highly metabolized parasite's cell. In *Pterospora floridensis*, instead of typical micropores present in other sporozoans, there are membrane-associated vesicles observed between irregular folds (Landers, 2002).

As mentioned above, some gregarines possess cytopilia, whose function is absolutely uninvestigated. Cytopilia are always immotile projections, different in length and diameter.

In *Zeylanocystis burti* cytopilia may serve as stilts during rotational movements of the saucer-like cell. It is a possible cytopilia function in *Z. burti* because of their shortness (only 4-8 μm in length) as compared to the total cell dimensions (150-200 μm in diameter) (Sathanantan, 1977). In *Rhynchocystis pilosa* the function of cytopilia is completely obscure.

We propose that in skittle-like cells of *Urospora chiridotae* cytopilia may serve as defense organelles. In the previous paper we described the differences in relationships between different morphotypes of parasite and host cell (Dyakin and Paskerova, 2004). The skittle-like cells of *Urospora chiridotae* are not covered with coelomocytes, while the globe-like cells, situated on the surface of the intestine, are covered with cells of coelomic epithelium. In contrast to skittle-like cells, globe-like cells are naked, i.e. do not possess cytopilia (Dyakin and Paskerova, 2004). So we assume that cytopilia of skittle-like forms prevent coelomocytes' settlement on the surface of the parasite cells. This is also the case for *Diplauxis* spp. early trophozoites inhabiting a similar biotope, i.e. blood vessels of polychaetes.

The function of the pellicular canals is completely unclear. Further investigations might shed light on this problem.

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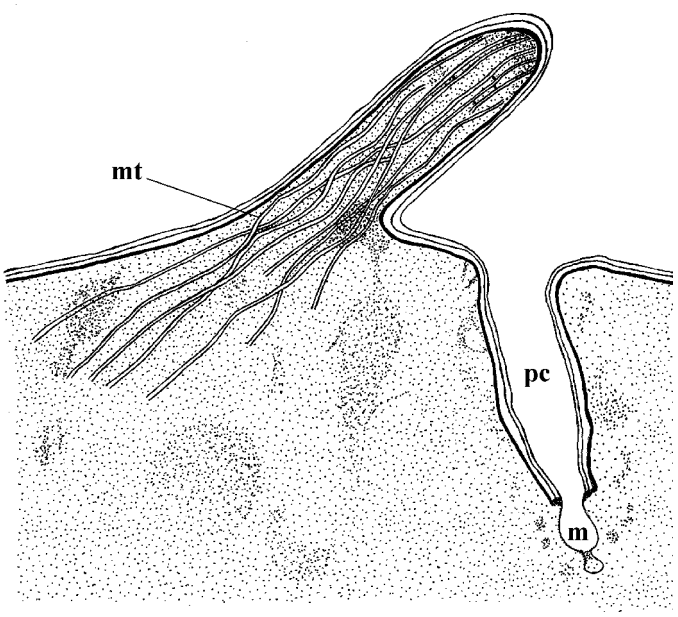

Fig. 6. Scheme of the cortical zone of the skittle-like cells of *Urospora chiridotae*. For abbreviations see figs 4, 5.

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