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Studies on the so-called plasmodium of *Ciliocincta sabellariae* (Phylum Orthonectida), with notes on an associated microsporan parasite.

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Abstract: The orthonectid *Ciliocincta sabellariae*, which parasitizes *Sabellaria cementarium* (Annelida: Polychaeta), creates extensive "galleries" in the epidermis of its host. The matrix ("plasmodium"), occupied by germinal cells, embryos, males, and females, consists of epidermal cytoplasm that has been greatly modified as a result of parasitism. Stages in the differentiation of cysts of a microsporan (microsporidian) are often found in the matrices and also in orthonectid embryos.

Résumé : *Étude du "plasmodium" de* Ciliocincta sabellariae *(Phylum des Orthonectides) et notes sur une microsporidie parasite associée.*
L'orthonectide *Ciliocincta sabellariae* infeste l'épiderme de *Sabellaria cementarium* (Annelida: Polychaeta), formant des "galeries" étendues. La matrice ("plasmodium"), qui renferme les cellules-germes, les embryons, les mâles, et les femelles, se compose de cytoplasme épidermique très modifié par les parasites. On trouve souvent, dans la matrice ainsi que dans les embryons, les stades de développement de kystes d'une microsporidie.

Keywords: Orthonectida, *Ciliocincta*, Polychaeta, *Sabellaria*, plasmodium, Microspora.

Introduction

A plasmodium is defined as an undivided mass of cytoplasm with several to many nuclei. The use of this term in connection with the life history of orthonectids began when Metschnikoff (1881) proposed Plasmodialschlauch (plasmodial tube, plasmodial sac) for the semifluid matrix in which embryos and adults develop. Caullery & Lavallée (1912), in a detailed study of the early stages of parasitism of the brittle star *Amphipholis squamata* by *Rhopalura ophiocomae* Giard, described young plasmodia of this species as consisting of cytoplasm in which germinal cells were embedded. The germinal cells multiplied, and their progeny developed into adult male or female orthonectids. Sooner or later, according to Caullery & Lavallée, numerous extremely small nuclei appeared. Unlike the nuclei in germinal cells, embryos, and adults, these were not within cells. Caullery & Lavallée believed that they originated by fragmentation of nuclei of some of the germinal cells, reproduced, and became scattered through the plasmodial matrix.

After studying plasmodia of *Rhopalura ophiocomae,* I came to the conclusion (Kozloff, 1994) that the matrices containing germinal cells, embryos, and adults consist of modified cytoplasm of contractile cells of the genital bursae or intestine of the host. Presumably, infective cells of a larva penetrate the epithelium of the gut or a bursa, then become lodged in contractile cells. As parasitized cells hypertrophy, they bulge into the perivisceral coelom. The peritoneum, pushed inward by each enlarging cell, persists and remains

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tightly applied to it. Contiguous matrices are thus separated by two peritoneal layers. I found no evidence for small plasmodial nuclei of the sort described by Caullery & Lavallée. Their idea that the first plasmodial nuclei were derived by fragmentation of a nucleus of a germinal cell may not have seemed far-fetched in 1912, and I do not doubt that Caullery & Lavallée saw stained bodies in the matrix. That these structures were plasmodial nuclei is not likely, however. Perhaps they were mitochondria.

In the present paper, I will describe the matrices occupied by *Ciliocincta sabellariae* Kozloff, 1965, an orthonectid whose development takes place in epidermal tissue of the body wall and dorsal cirri of *Sabellaria cementarium* (Polychaeta: Sabellariidae). In *C. sabellariae*, sexual dimorphism is obvious, but is not so striking as it is in *R. ophiocomae*. Both males and females may originate within the same matrix. Furthermore, matrices occupied by *C. sabellariae* often contain stages in the development of cysts of a microsporan (microsporidian) parasite.

Material and Methods

Specimens of *S. cementarium* were collected by dredging at a depth of about 12 meters off Dot Rock, close to the eastern shore of Decatur Island, in the San Juan Archipelago, Washington. For ultrathin and 1 µm sections, dorsal cirri of parasitized worms were fixed in osmium tetroxide buffered with *s*-collidine, then embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate; 1 µm sections were stained with methylene blue-azure II. For paraffin sections, pieces consisting of several segments were fixed in Bouin's fluid. The sections were cut at 5 µm and stained with iron hematoxylin.

Results

In sections of parasitized *S. cementarium,* the epidermis, including that of the dorsal cirri, is thoroughly pervaded by *C. sabellariae* (Fig. 1). It is rare, in fact, to find a substantial portion of the epidermis that is not parasitized. Many if not all of the numerous "galleries" of parasites seen in a single transverse section are probably connected at other levels. Because the frequency of parasitized worms is low (about 1.5% at the locality where the present material was collected), it is likely that the high level of parasitism in a single *Sabellaria* is due to proliferation and spread of germinal cells rather than to repeated infection.

Sections through a dorsal cirrus (Fig. 2A) confirm that the galleries containing *Ciliocincta* are confined to the epidermis which, in its unmodified state, consists mostly of large epithelial cells. These have a border of microvilli, and some of them are ciliated. There are also gland cells that open to the outside through pores in the cuticle and a few muscle bundles. Most of the musculature, however, is beneath the epidermis.

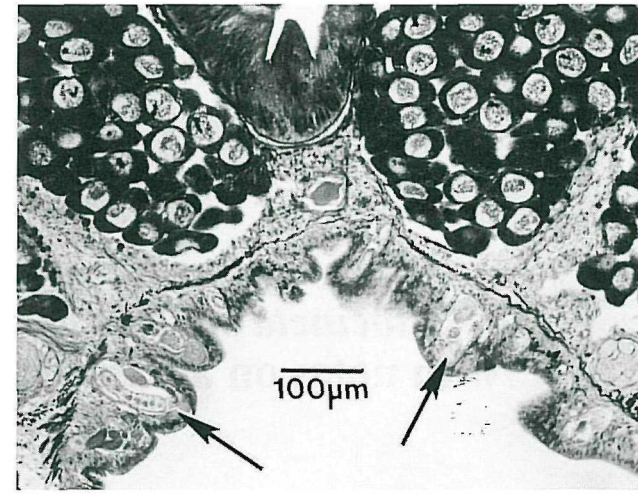


Figure 1. *Ciliocincta sabellariae* in the epidermis of the ventral body wall of a female *Sabellaria cementarium* (5 µm-thick section, iron hematoxylin). Although much of the epidermis is parasitized, the host's production of oocytes seems not to have been affected. At least many if not all of the galleries of the orthonectid (*arrows*) are probably part of a continuous matrix.

Figure 1. *Ciliocincta sabellariae* dans l'épiderme de la paroi ventrale du corps d'une femelle de *Sabellaria cementarium* (coupe 5 µm, hématoxyline ferrique). Même lorsque l'épiderme est fortement parasité, la production d'ovocytes par l'hôte n'est pas réduite. La plupart des galeries d'orthonectide (*flèches*) sont probablement en continuité.

Nearly all galleries of *C. sabellariae* contain embryos or advanced stages of development, or both (Fig. 2B, C). Cells substantially larger than those of young embryos are interpreted as germinal cells (Fig. 2C). A distinct line of demarcation separates the matrix of a gallery from the surviving cells or portions of cells of the epidermis. In some cirri, the galleries contain cysts of a microsporan parasite (Fig. 2D).

An electron micrograph useful for orientation is reproduced in figure 3A. It shows the outer portion of a dorsal cirrus of the host, including what is left of the epidermis, a muscle bundle, and a substantial portion of a gallery. The matrix contains three embryos of *Ciliocincta* and a single nucleus that seems not to be enclosed within a cell. Such nuclei are neither common nor especially rare. Small lipid inclusions are found in the epidermal cells and also in the matrix, but not in the embryos. The line that separates the matrix from the epidermal cells is more pronounced than that which separates the matrix from the outer cells of embryos.

Figure 3B shows, at a higher magnification than that used for figure 3A, the dense line that separates a matrix from epidermal cells. Still higher magnification (Fig. 3C) reveals that this line consists of two membranes, one of which is

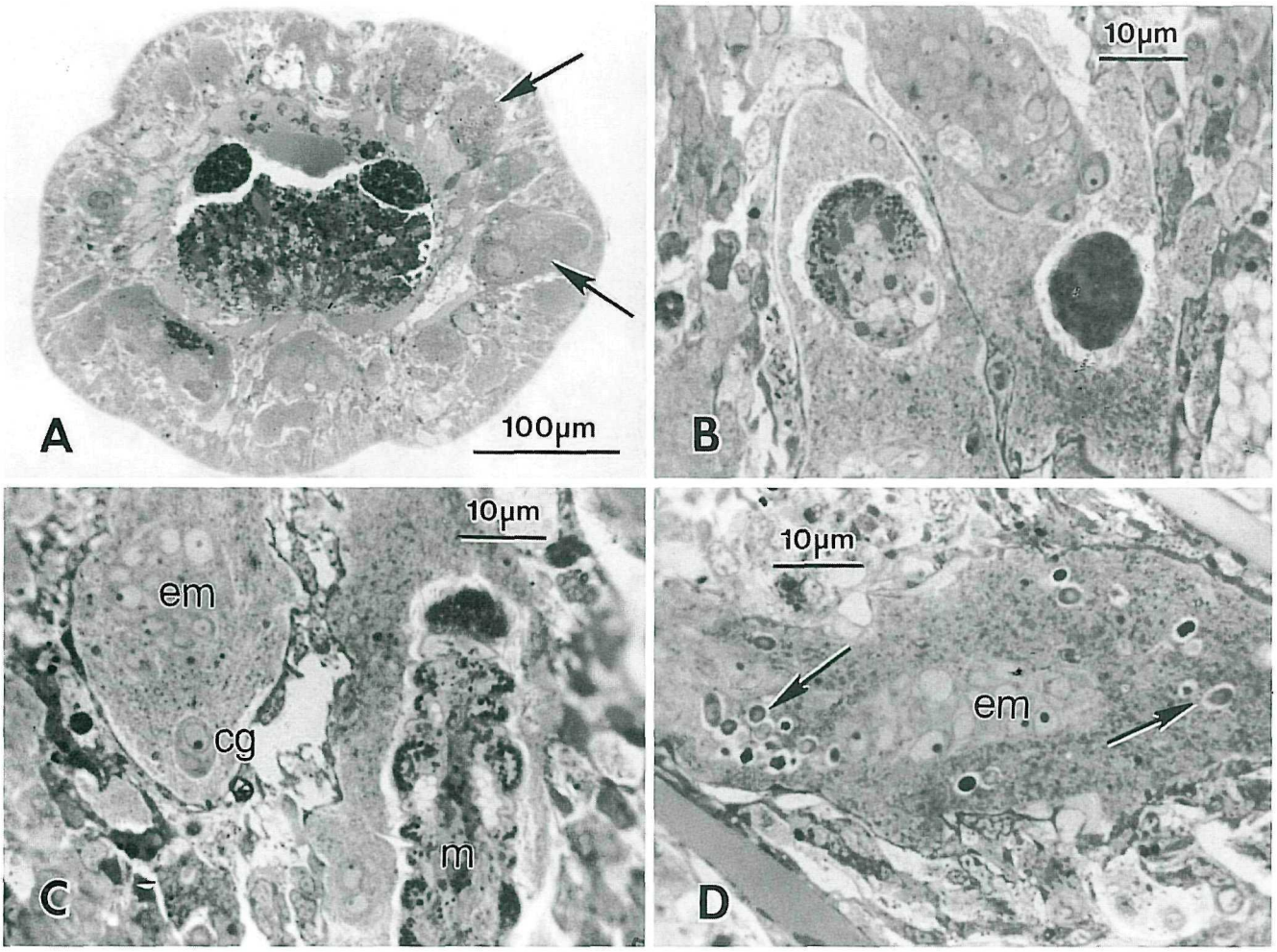
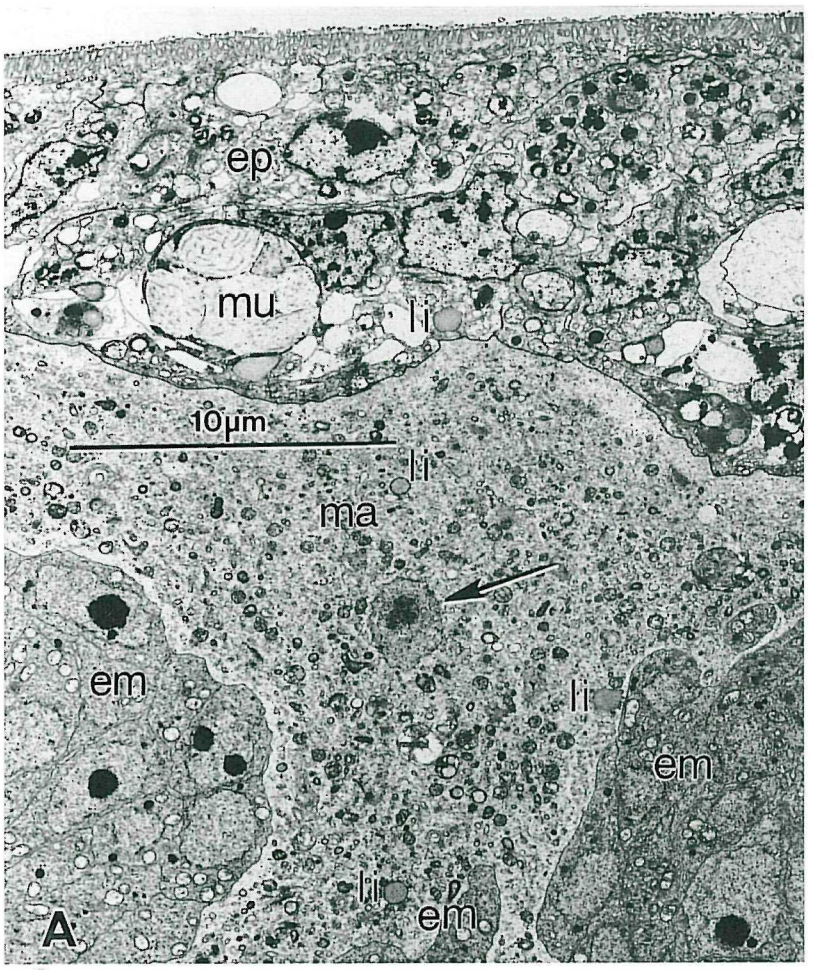


Figure 2. *Ciliocincta sabellariae* in the epidermis of dorsal cirri of *Sabellaria cementarium* (1 µm sections, methylene blue-azure II).
A: Transverse section through an entire cirrus, showing numerous galleries of parasites (*arrows*) in the epidermis.
B: Two adjacent galleries, both with advanced stages of development.
C. Gallery with an embryo (*em*) and a germinal cell (*cg*), and a gallery with a nearly mature male (*m*).
D: Gallery containing an embryo (*em*) of the orthonectid and stages in the development of cysts of a microsporan (*arrows*).
Figure 2. *Ciliocincta sabellariae* dans l'épiderme des cirres dorsaux de *Sabellaria cementarium* (coupes 1 µm, bleu de méthylène-azur II).
A : Coupe transversale d'un cirre entier, montrant plusieurs galeries de parasites (*flèches*) dans l'épiderme.
B : Deux galeries voisines, avec des stades avancés du développement.
C : Galerie avec embryon (*em*) et cellule-germe (*cg*), et une galerie avec un mâle (*m*) presque mûr.
D : Galerie qui contient un embryon (*em*) d'orthonectide et des stades de développement de kystes d'un microsporidien (*flèches*).

continuous with those separating adjacent cells of the epidermis. Both membranes, however, are much thicker and rougher than those surrounding epidermal cells, and the space between them is not clearly continuous. They are probably abnormal unit membranes, marking the border between portions of cells that are still more or less intact and cytoplasm that has become disorganized as a result of parasitism.

More than half of a two-celled embryo is shown in figure 4A. The matrix has a unit membrane that is tightly applied to the membranes covering the embryonic cells. Thus the matrix is as intimately bound to the embryo as it is to epidermal cells on its opposite side.

By the time development of an embryo reaches an advanced stage, the membrane that had been closely applied to the young embryo separates from it and is no longer continuous. Figure 4B shows that the matrix surrounding a substantially mature orthonectid is in a very disorganized condition and that there is no longer a membrane separating it from the parasite. An orthonectid cell that may at first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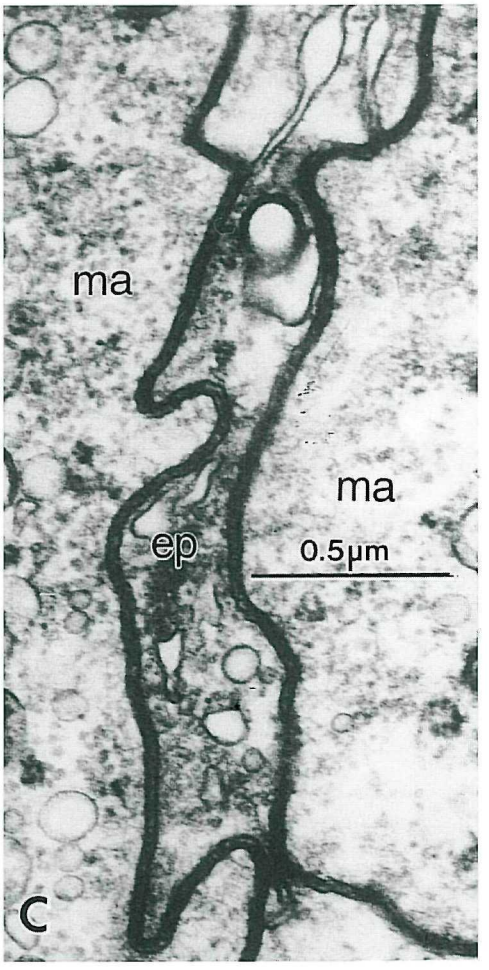
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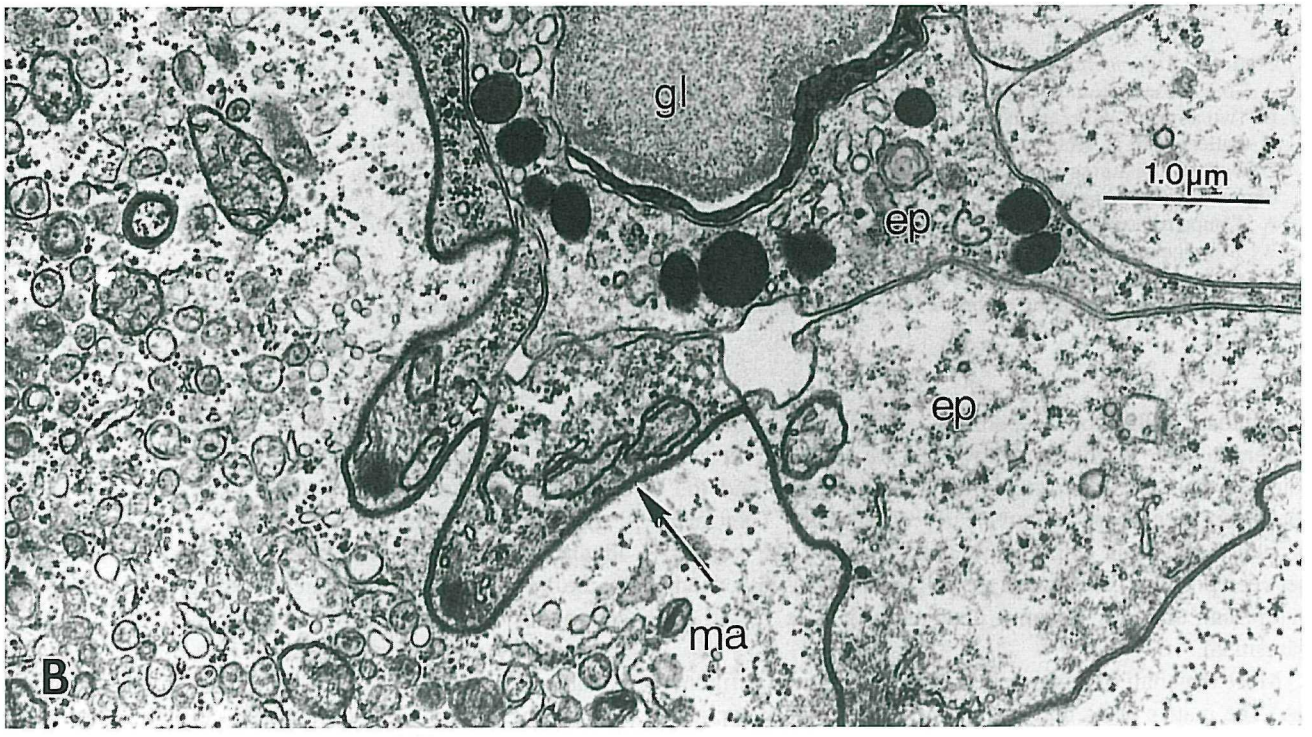
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B

glance appear to be a free nucleus is, however, surrounded by a thin layer of cytoplasm and a cytoplasmic membrane. It could be an old germinal cell or a cell that has become dissociated from an embryo. In many micrographs there are configurations that probably represent orthonectid cells that are losing or have lost their cytoplasm, or cells in which both nucleus and cytoplasm are being destroyed simultaneously (Fig. 6A). In most micrographs that show extensive expanses of a matrix, there are no bodies that can unequivocally be interpreted as plasmodial nuclei.

A diagram that shows the probable relationship of a matrix to persisting portions of adjacent epidermal cells is given in figure 5. This also illustrates events that conceivably could lead to the presence of isolated orthonectid nuclei within a matrix.

When developing cysts of the microsporan parasite (Figs. 6B, C) are present in the matrix, they are usually in groups. This suggests that they have originated close to one another. Developing cysts are also seen in embryos of the orthonectid, each cyst occupying most of a cell (Fig. 6B).

I have not been able to find, either in the matrices or embryos, concentrations of cells that are precursors of cysts. Perhaps binary or multiple fission takes place in a tissue of the host, and the products of multiplication enter the matrices and penetrate cells of the orthonectid, within which they differentiate into cysts. After an embryo has been destroyed, the cysts would be left free in the matrix. Sometimes cysts are arranged in such a way (Fig. 6B) that all of them appear to have begun their development within a single orthonectid embryo. But intact embryos in which more than a few cells were occupied by cysts have not been been observed.

Discussion

The matrices occupied by *C. sabellaria e* are very different from those of *R. ophiocomae* because they are within epidermal tissue, rather than within hypertrophied contractile cells that can bulge into a coelomic space. Furthermore, the persistence of the peritoneum around cells parasitized by *R. ophiocomae* clearly separates adjacent matrices. *Intoshia linei*, a parasite of the nemerteans *Lineus ruber* and *L. viridis*, is much like *C. sabellariae* in the way it forms galleries, but it is not restricted to epidermal tissue.

In his studies of *I. linei*, Haloti (1993) tested for various enzyme groups in tissues occupied by this orthonectid. He found acid phosphatases to be concentrated in the galleries, but not in the parasites. He proposed, therefore, that the orthonectid probably produces lytic substances that attack cells of the host, and that these cells then destroy themselves. This reasoning seems to be on the right track, and it may also apply to *Ciliocincta sabellariae*. The two comparatively thick and closely parallel unit membranes between a matrix and the epidermis presumably shift as destruction of the epidermis proceeds. Release of adult males and females from a matrix probably takes place when destruction of the epidermis reaches the point that it ruptures.

In electron micrographs, it is rare to find a deteriorating epidermal nucleus trapped within a matrix. This, and the presence of apparently normal nuclei in surviving portions of epidermal cells that border a matrix, suggest that as parts of these cells are slowly converted into matrix material, most nuclei are shifted away from the zone of destruction.

By grafting pieces from a *Lineus* parasitized by *I. linei* to healthy worms, Vernet & Fargette (1990), Haloti, Vernet, & Bierne (1992), Haloti (1993), and Haloti & Vernet (1994) have been able to observe the spread of the orthonectid from donors to recipients. An especially detailed summary of this work, involving both intra- and interspecific grafts, has been presented by Haloti (1993). He concluded that germinal cells are the "pioneers" that pervade the tissues of the recipient, perhaps by way of blood vessels. If, however, germinal cells have lytic capabilities, they could perhaps

Figure 3. *Ciliocincta sabellariae* in the epidermis of dorsal cirri of *Sabellaria cementarium*; transmission electron microscopy (TEM).
A: Portion of a gallery, the matrix (*ma*) showing parts of three embryos (*em*) and what appears to be a nucleus (*arrow*) not enclosed by cytoplasm and a cell membrane. Note, however, the absence of any other free nuclei. *ep* epidermis, *li* lipid, *mu* muscle.
B: Zone of contact between epidermal cells (*ep*) and a parasitized matrix (*ma*). Note that the two unit membranes at the zone of contact (*arrow*) are not so distinct as those that separate epidermal cells from one another. No free nuclei are evident in the matrix. *gl* gland cell.
C: Matrices of two galleries separated by a partition consisting of surviving portions of epidermal cells. Electron-lucent spaces can be seen between the two relatively thick unit membranes that separate the matrices (*ma)* from the epidermis (*ep*).

Figure 3. *Ciliocincta sabellariae* dans l'épiderme des cirres dorsaux de *Sabellaria cementarium* ; microscopie électronique à transmission (MET).
A : Une galerie avec des portions de trois embryons (*em*) et un noyau libre (*flèche*) non entouré de cytoplasme ni de membrane cellulaire. À noter l'absence d'autres noyaux libres dans la matrice. *ep* épiderme, *li* lipide, *mu* muscle.
B : Zone de contact entre cellules épidermiques (*ep*) et matrice parasitée (*ma*). À noter que les deux membranes de la zone de contact (*flèche*) sont moins distinctes que celles qui séparent les cellules épidermiques. On ne voit pas de noyaux libres dans la matrice. *gl* cellule glandulaire.
C : Matrices de deux galeries séparées par des portions de cellules épidermiques survivantes. On peut voir quelques espaces transparents aux électrons entre les deux membranes épaisses qui séparent les matrices (*ma*) de l'épiderme (*ep*).


ma
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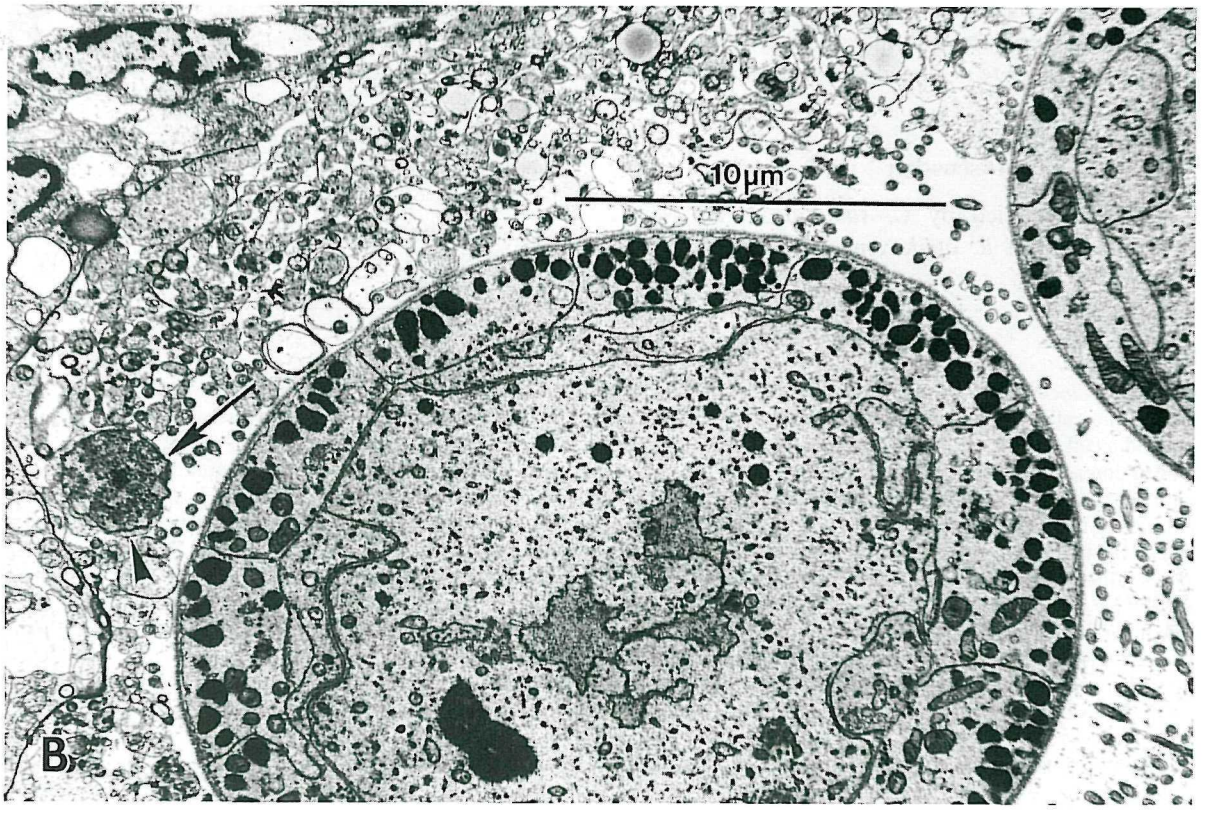
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B

Figure 4. *Ciliocincta sabellariae* in the epidermis of dorsal cirri of *Sabellaria cementarium* (TEM).

A: Portion of a two-celled embryo. The unit membranes of the cells (*arrows*) are separated from the matrix (*ma*) by a second membrane (*arrowheads*).

B: Nearly mature female. There is no distinct membrane on the side of the matrix facing the orthonectid. What may seem to be a free nucleus *(arrow)* is enclosed by a thin layer of cytoplasm and by a cell membrane (*arrowhead*).

Figure 4. *Ciliocincta sabellariae* dans l'épiderme des cirres dorsaux de *Sabellaria cementarium* (MET).

A : Portion d'un embryon de deux cellules. Les membranes plasmiques (*flèches*) sont séparées de la matrice (*ma*) par une autre membrane (*têtes de flèches).*

B : Femelle presque mûre. La membrane du côté de la matrice a disparu. Le noyau qui semble être libre (*flèche*) est entouré par une mince couche de cytoplasme et par une membrane cellulaire (*tête de flèche*).

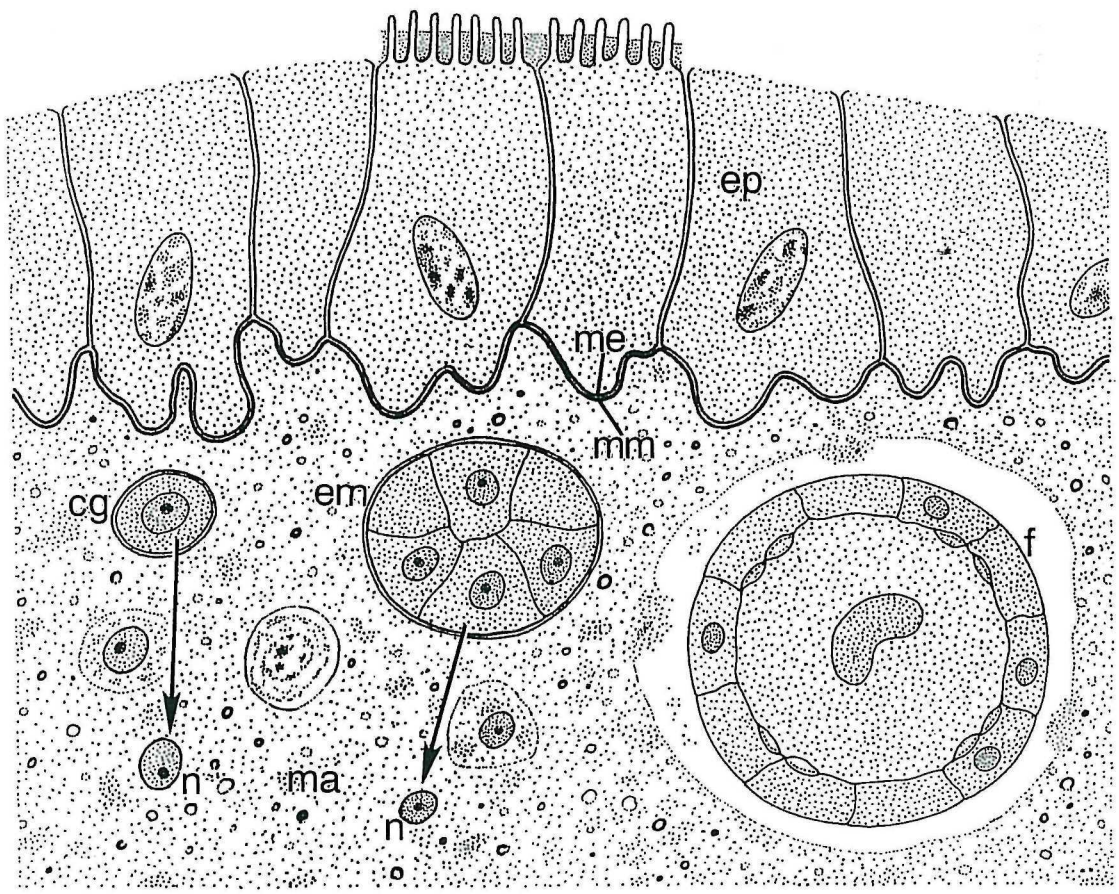
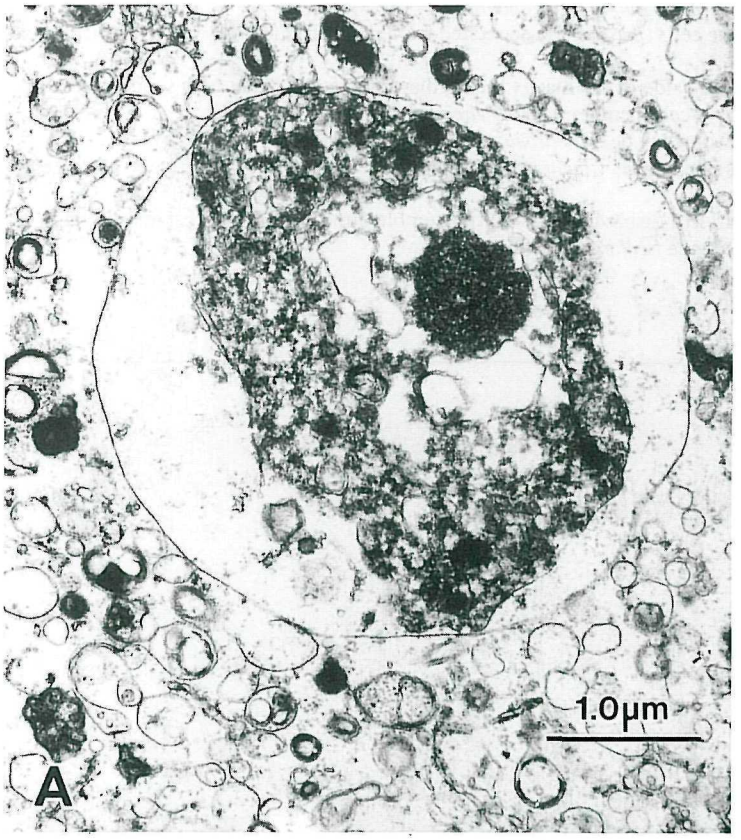


Figure 5. *Ciliocincta sabellariae* in the epidermis of *Sabellaria cementarium.* Diagram illustrating the probable relationships between persisting portions of epidermal cells (*ep*), a matrix (*ma*) consisting of disorganized epidermal cytoplasm, and a germinal cell (*cg*), embryo (*em*), and adult female orthonectid (*f*). Also suggested are possible ways *(arrows)* by which isolated nuclei (*n*) may be derived from those of germinal cells or embryonic cells. The two unit membranes that separate adjacent cells of the embryo and those of the female are indicated by single lines. *me* unit membrane of epidermal cells, *mm* unit membrane of matrix.

Figure 5. *Ciliocincta sabellariae* dans l'épiderme de *Sabellaria cementarium.* Dessin schématique montrant les rapports vraisemblables entre les portions persistantes des cellules épidermiques (*ep*), une matrice (*ma*), formée par le cytoplasme désorganisé des cellules épidermiques, et une cellule germe (*cg*), un embryon (*em*), et une femelle adulte (*f*). L'origine possible des noyaux isolés *(n),* à partir des cellules germes ou des cellules embryonnaires est aussi évoquée *(flèches).* Les deux membranes qui séparent les cellules contiguës de l'embryon et de la femelle sont indiquées par un seul trait. *me* membrane plasmique des cellules épidermiques, *mm* membrane unitaire de la matrice.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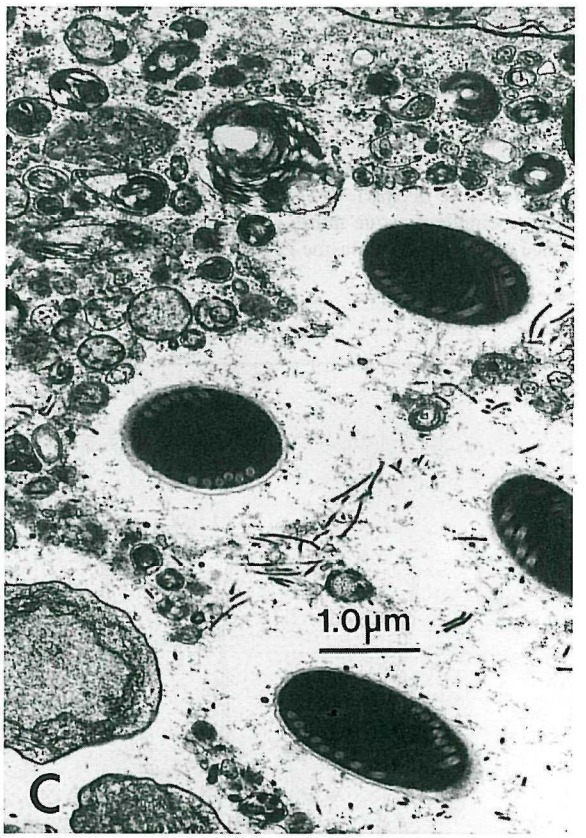
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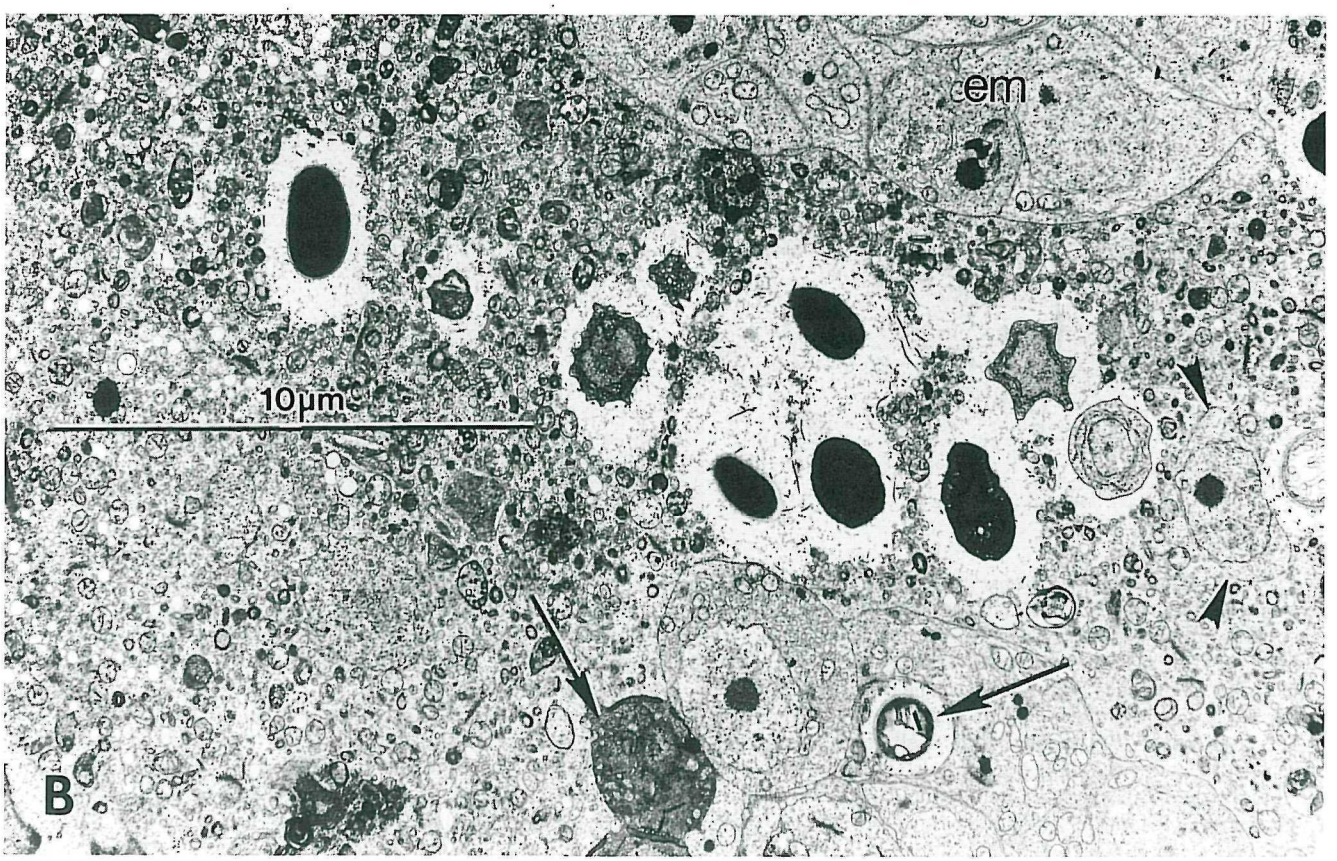
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Figure 6. *Ciliocincta sabellariae* in the epidermis of dorsal cirri of *Sabellaria cementarium* (TEM).
A: Orthonectid cell, perhaps an old germinal cell, undergoing destruction within a matrix.
B: Portions of a normal embryo (*em*) and an embryo containing cysts of a microsporan (*arrows*). The developing cysts between the two embryos perhaps mark the position of an embryo that was destroyed by the microsporan. A cell of the orthonectid type is also present, but its cell membrane (*arrowheads*) is difficult to distinguish.
C: Stages in the development of microsporan cysts.
Figure 6. *Ciliocincta sabellariae* dans l'épiderme des cirres dorsaux de *Sabellaria cementarium* (MET).
A : Cellule du type orthonectide, peut-être une vieille cellule-germe, en cours de destruction dans une matrice.
B : Portions d'un embryon normal (*em*) et d'un embryon qui contient des kystes d'une microsporidie (*flèches*). Les kystes entre les deux embryons indiquent peut-être l'emplacement d'un embryon qui a été détruit par une microsporidie. Une cellule du type orthonectide est aussi présente, mais sa membrane cellulaire (*têtes de flèches*) est difficile à voir.
C : Stades du développement des kystes de la microsporidie.

also migrate directly through tissue as they promote its destruction.

Haloti avoided using the term plasmodium for matrices populated by germinal cells, embryos, and adults of *I. linei.* Furthermore, he did not mention the presence of free nuclei in the matrices. He reported, however, finding what he thought was a true plasmodial stage in a natural infection, but his photomicrograph of a 1 µm section is not convincing. Furthermore, Haloti's rather startling assertion that germinal cells originate when free nuclei detach from the plasmodium and become enclosed by cytoplasm is not documented. It is important to remember that if what he saw is indeed a genuine plasmodium, it is not the same thing as the matrix in which germinal cells, embryos, and adult orthonectids are distributed.

Because some of my electron micrographs of parasitized matrices show orthonectid nuclei in stages of disintegration, I think that the relatively few still-intact free nuclei observed in matrices probably belonged to germinal cells or to dissociated embryonic cells whose cytoplasm has disappeared. It is likely that after the cytoplasm of cells of the host's epidermis has been modified beyond a certain point, some germinal cells or cells of embryos simply deteriorate. It is also possible that a few nuclei that seem to lack cytoplasm are those of germinal cells or peripheral cells of embryos which have been sectioned in such a plane that the nucleus is distinct but the cell membrane is not.

The presence of microsporan cysts in the matrix and in embryos of *Ciliocincta* is perplexing, partly because the source of the cells that differentiate into cysts has not been identified. Perhaps, as I have already suggested, multiplication of the microsporan takes place in the epidermis or other tissue of *Sabellaria*, and that the products of this multiplication somehow get into the matrix as the epidermis is being modified. Differentiation of cysts could conceivably take place in the matrix, but the fact that the cysts of a group are usually rather evenly spaced suggests that they were left free in the matrix after the orthonectid embryo in which they began development was destroyed.

Acknowledgements

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References

Caullery M. & Lavallée A. 1912. Recherches sur le cycle évolutif des orthonectides. Les phases initiales dans l'infection expérimentale de l'ophiure, *Amphiura squamata, par Rhopalura ophiocomae* Giard. *Bulletin Scientifique de la France et de la Belgique,* **46**: 139-171.

Haloti S. 1993. Étude du parasite et du parasitisme : l'Orthonectide *Intoshia linei* endoparasite de l'Hétéronémerte *Lineus ruber*. Thèse de Doctorat en Sciences, Université de Reims Champagne-Ardenne. 133 pp.

Haloti S. & Vernet G. 1994. Avance dans la connaissance des mésozoaires. Étude de la spécificité d'*Intoshia linei* (Orthonectide) par greffe interspécifique de tronçons de *Lineus* (Hétéronémertes) parasités sur des sujets sains. *Bulletin de la Société Zoologique de France,* **119**: 355-363.

Haloti, S., Vernet G., & Bierne J. 1992. Reproduction asexuée de l'endoparasite *Rhopalura linei* (Orthonectide) au cours de la contamination expérimentale de l'hôte, l'hétéronémerte *Lineus ruber. Bulletin de la Société Zoologique de France,* **117**: 174-175.

Kozloff E. N. 1965. *Ciliocincta sabellariae* gen. and sp. n., an orthonectid mesozoan from the polychaete *Sabellaria cementarium* Moore. *Journal of Parasitology,* **51**: 37-44.

Kozloff E. N. 1994. The structure and origin of the plasmodium of *Rhopalura ophiocomae* (Phylum Orthonectida). *Acta Zoologica,* **75**: 191-199.

Metschnikoff, E. 1881. Untersuchungen über Orthonectiden. *Zeitschrift für wissenschaftliche Zoologie,* **35:** 282-303.

Vernet G. & Fargette C. 1990. Procédé expérimental de contamination de *Lineus ruber* (Hétéronémertes) par *Rhopalura linei* (Orthonectides). *Bulletin de la Société Zoologique de France,* **115**: 218-219.