Russian Journal of Nematology, 2002, 10 (1), 43 - 53

Ultrastructural study of the tadpole stage of the primitive marine nematode *Enoplus demani* (Enoplia: Enoplida)

Vladimir V. Yushin*, August Coomans, Gaetan Borgonie** and Vladimir V. Malakhov*****

*Institute of Marine Biology, Far East Branch of the Russian Academy of Sciences, Vladivostok 690041, Russia, e-mail: yushinvladimir@hotmail.com
**Rijksuniversiteit Gent, Instituut voor Dierkunde, K.L. Ledeganckstraat 35, 9000 Gent, Belgium,
***Faculty of Biology, Moscow State University, Moscow 119899, Russia.

Accepted for publication 25 November 2001

Summary. The ultrastructure of the tadpole (1½ fold) embryo of the free-living marine nematode *Enoplus demani* was studied. The cells of the tadpole embryo show no distinct cyto-differentiation. Cytoplasm contains mitochondria and is filled with yolk granules and lipid droplets; nuclei contain clumps of condensed chromatin. The cells at the different stages of the mitotic cycle occur frequently in all the primordial embryonic layers. The centrosomes of the mitotic spindles include centrioles. The cells of the epidermis and the intestine form distinct layers of polarized cells underlain by basal lamina and connected at the apical ends by adherent junctions. The apical membranes of epidermal cells are slightly undulated, occasional cisternae of RER and Golgi bodies occur in the apical cytoplasm. The epidermis of the head-end forms many small funnel-shaped invaginations, interpreted here as the result of morphogenetic cell movement associated with cephalic sensilla formation. There is no distinct lumen in the tadpole intestine, rare microvilli occur squeezed between the apical membranes of intestinal cells. Presumptive muscle cells have no myofilaments, but mitochondria are abundant at the future basal (contractile) part of the cells. The pair of primordial germ cells (PGC) are positioned in the blastocoel latero-ventrally to the mid-part of the intestine. These cells are about twice the diameter of other cells of the embryo. The cytoplasm of PGC does not differ from the cytoplasm of somatic cells of the embryo, and specific P-granules are absent. Data on cell structure of *E. demani* tadpole embryo are compared with data on cell differentiation in *Caenorhabditis elegans*.

Key words: *Caenorhabditis elegans*, centriole, development, *Enoplus demani*, germ cells, nematodes, P-granules, ultrastructure.

The free-living soil nematode *Caenorhabditis elegans*, belonging to the order Rhabditida of the subclass Rhabditia, has highly determinate development with invariant cell lineage, that is usual for this taxon of nematodes (Sulston, 1997). *C. elegans* has been studied extensively during the last two decades as a model organism for different fields of developmental biology. Cleavage and following morphogenesis were studied in detail, including ultrastructural observations of cell differentiation (Wood, 1988b; Riddle *et al.*, 1997).

To date, ultrastructural studies of the embryogenesis in the nematode species, except for *C. elegans*, have been focused mainly on the activity of the epidermis and the first cuticle formation (Bruce, 1970; Kozek, 1971; Bird & Stynes, 1981; Bird, 1977; Martinez-Palomo, 1978; Platzer & Platzer, 1988; Yushin & Malakhov, 1989, 1992). Little information is available on the structure of other embryonic tissues (Tongu *et al.*, 1978; MacKinnon, 1986).

Development of members of the order Enoplida, subclass Enoplia, differs from that of other nematodes. The pattern of cleavage in enoplid nematodes is indeterminate and cell lineage is not fixed (Malakhov, 1994; 1998; Voronov, 1999). The most detailed observations of enoplid embryogenesis were done on *Enoplus demani*

Table 1. Development of *Enoplus demani* at 18-20 °C.

Time from the initiation	Stage
1 day	2 blastomeres
3 days	start of gastrulation
3.5 days	slit-like blastopore
4 days	blastopore is closed
5 days	lima-bean
5.5 days	comma
6.5 days	tadpole
8 days	two-fold loop
10 days	three-fold loop
11 days	four-fold loop
13-18 days	pretzel
19-20 days	hatching, first stage juvenile

(Malakhov, 1994; Voronov & Panchin, 1998). These data were not accompanied by ultrastuctural observations of histogenesis and cyto-differentiation, which would assist understanding of the importance of different developmental patterns for embryonic morphogenesis.

Preliminary light microscope observations show the absence of distinct structural differences between cells of the early embryos of the free-living marine nematode *E. demani* to the tadpole stage (1 × fold embryo) (Malakhov & Akimushkina, 1976). Thus, the tadpole embryo may be considered as the starting point for embryonic cyto-differentiation. The purpose of the present study of *E. demani* was to describe in detail the ultrastructure of the cells in the primordial embryonic layers of the tadpole embryo.

MATERIALS AND METHODS

Gravid females of *Enoplus demani* Galtsova, 1976 were obtained from sand collected in the intertidal zone at the White Sea Biological Station of Moscow State University (Kandalaksha Bay, White Sea). Living specimens were cut into halves at the vulva; as a result the fertilized eggs were extruded from the body by internal pressure and began to develop. The eggs were maintained at room temperature (18-20 °C) in Petri dishes filled with filtered seawater. The egg shell was pierced by a glass capillary just before fixation for TEM. Embryos were fixed at 10 °C in 2.5% glutaraldehyde in 0.05 M cacodylate buffer containing 21 mg/ml NaCl, and then postfixed at room temperature in 2% osmium tetroxide in the same buffer containing 23 mg/ml NaCl. Post-fixation was followed by *en bloc* staining for 12 h in 1% uranyl acetate, and then specimens were dehydrated in an ethanol and acetone series and embedded in Spurr resin. Thin sections were cut with a Reichert Ultracut E ultratome, stained with lead citrate, and then examined with a JEOL JEM 100B or JEOL JEM 1010 electron microscope.

Development of free-living marine nematodes includes the same morphologically distinct stages as in *C. elegans* (Malakhov, 1994; Voronov & Panchin, 1998; Voronov, 1999). Table 1 shows original data on the approximate timing of development of *E. demani.*

RESULTS

The tadpole embryo of *E. demani* looks like a short worm with a massive dilated "head" and short, ventrally curved "tail" (Fig. 1). The anterior end comprises the ectodermal pharynx with an oral opening. Intestinal cells form a cylinder of relatively large cells; the short ectodermal proctodaeum opens ventrally to the exterior by the anus, close to the tip of the tail. The layer of mesodermal cells occurs between the epidermis and embryonic intestine.

In general, the ultrastructure of the cells of the tadpole embryo is uniform and shows no distinct cyto-differentiation. The average sizes of the cells vary from 8 μm in the epidermis to 12 μm in the intestine. Their cytoplasm contains mitochondria, and is filled with yolk granules and lipid droplets (Fig. 2A, B). The nuclei of the cells are 5-6 μm in diameter and contain clumps of condensed chromatin. The cells at the different stages of the mitotic cycle occur frequently in all the primary embryonic layers (Fig. 3A). The centrosomes of the mitotic spindles include centrioles 180 nm long, and 150 nm in diameter (Fig. 3A, B).

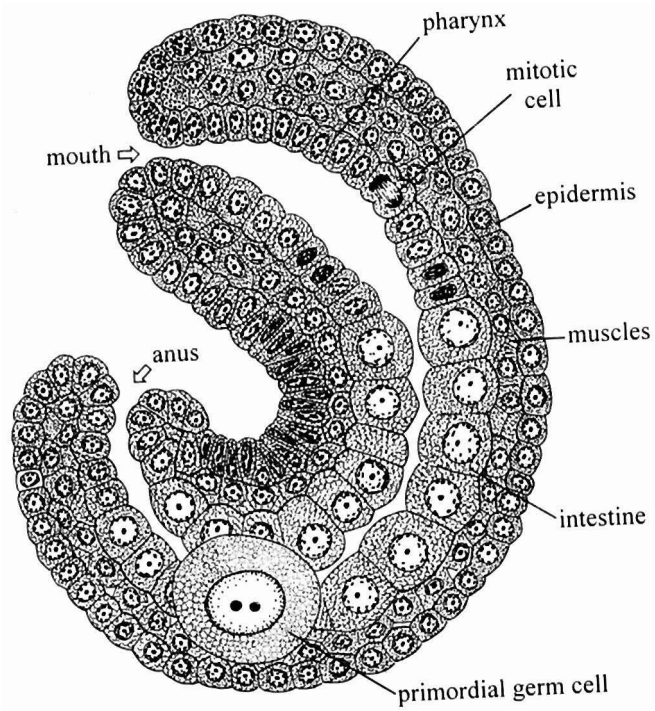

Fig. 1. *Enoplus demani*, schematic representation of the structure of the tadpole embryo as revealed by light microscopy.

The main feature of the tadpole histology is a clear epithelization of ectodermal derivatives (epidermis, stomodaeum, proctodaeum) and endoderm (intestine). The epithelial cells form distinct layers of polarized cells underlain by basal lamina and connected at the apical ends by adherent junctions (Figs 3C, D; 4A, B). The apical membrane of the epidermis is slightly undulated (Fig. 4C) and forms rare, short microvilli. No evidence of exocytosis or cuticle formation were found, but occasional cisternae of RER and Golgi bodies occur in the apical cytoplasm.

The epidermis of the head end forms many small funnel-shaped invaginations each consisting of several cells with extremely narrow apical and broad basal ends (Fig. 4D, 5). The apical ends of these cells form microvilli and long processes strengthened with longitudinally oriented microtubules (Fig. 4D). We interpret these invaginations of epidermis as the result of morphogenetic cell movement associated with head sensilla formation.

Intestinal cells are arranged radially and stretched along their apical-basal axes (Fig. 2B). There is no distinct lumen in the tadpole intestine and apical surfaces of the opposite cells are in close contact (Fig. 4A). Rare microvilli occur squeezed between the apical membranes of the intestinal cells. Microtubules and filamentous material are abundant in the apical cytoplasm. Broad basal parts of the cells are underlayen by basal lamina (Fig. 4B).

The undifferentiated cells positioned between the basal laminae of the epidermis and the intestine are interpreted here as presumptive muscle cells (Figs. 2A, 3D). The cells of this layer have no myofilaments, but mitochondria are abundant at the future basal (contractile) part of the cells (Fig. 3D).

A pair of very large cells, which we interpret as primordial germ cells (PGC), are positioned in the blastocoel latero-ventrally (one to the left and another to the right) to the midpart of the intestinal cylinder close to the flexion point of the embryo (Fig. 1). These cells are about twice as large in diameter (24 μm) than other cells of the embryo (Fig. 6A), and completely fill the blastocoel. The eccentric nuclei of these cells are large, about 10 μm in diameter. They differ distinctly from the nuclei of surrounding somatic cells by the uniform dispersion of chromatin. Small vesiculated nucleoli

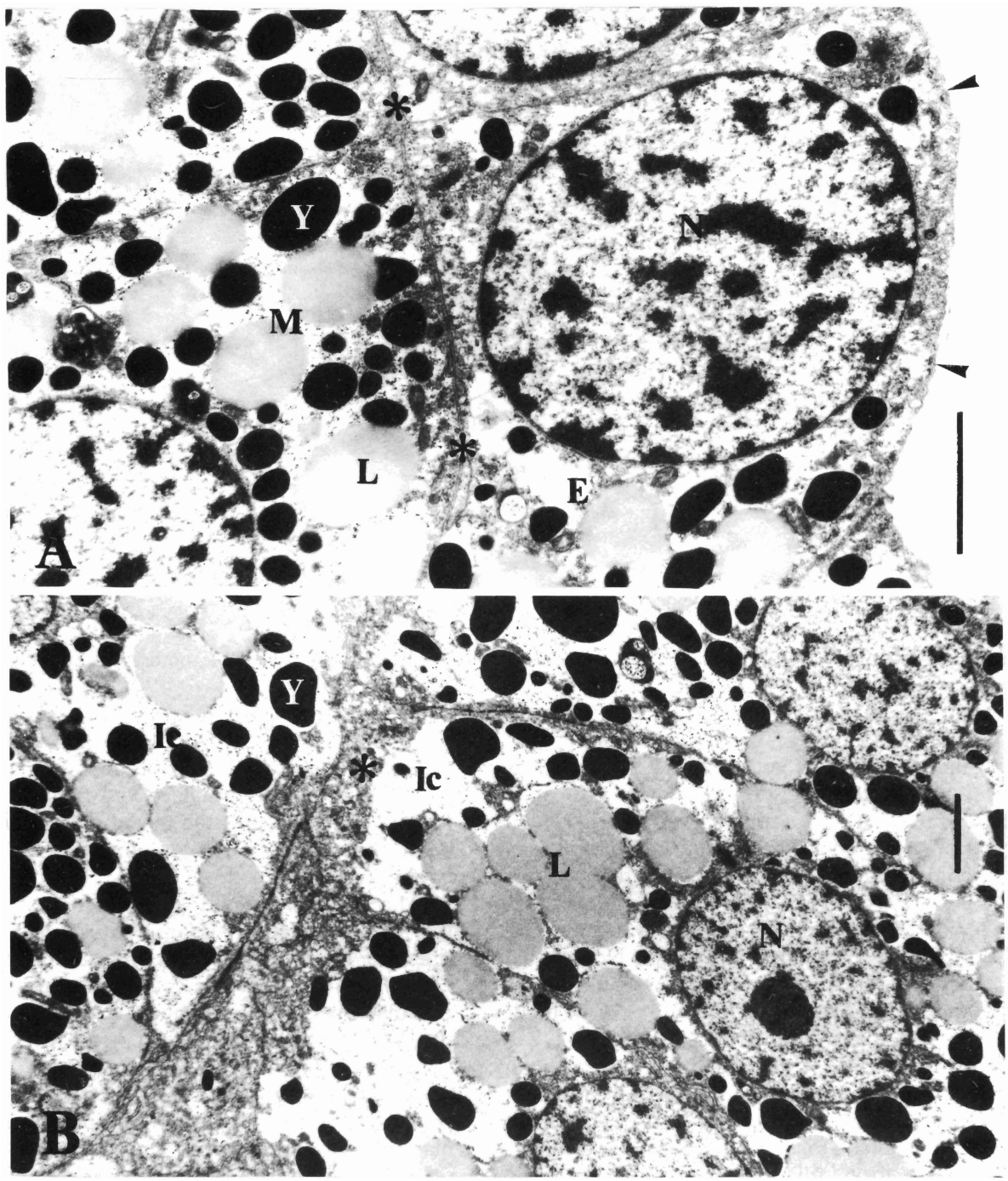

Fig. 2. *Enoplus demani*, sagittal sections through the tadpole embryo. A: Epidermal and muscle cells of the dorsal side, the asterisks indicate the basal lamina, the arrowheads show the apical membrane of the epidermal cell; B: Intestinal cells, the asterisk indicates the apical part of the intestinal cell. (Scale bar - 2 μm). Abbreviations, ch, chromosome; E, epidermal cell; gb, Golgi body; Ic, intestinal cell; L, lipid droplet; M, muscle cell; mi, mitochondria; mv, microvilli; pr, cell process; N, nucleus; ng, nucleus of the primordial germ cell; nu, nucleolus; Y, yolk granule.

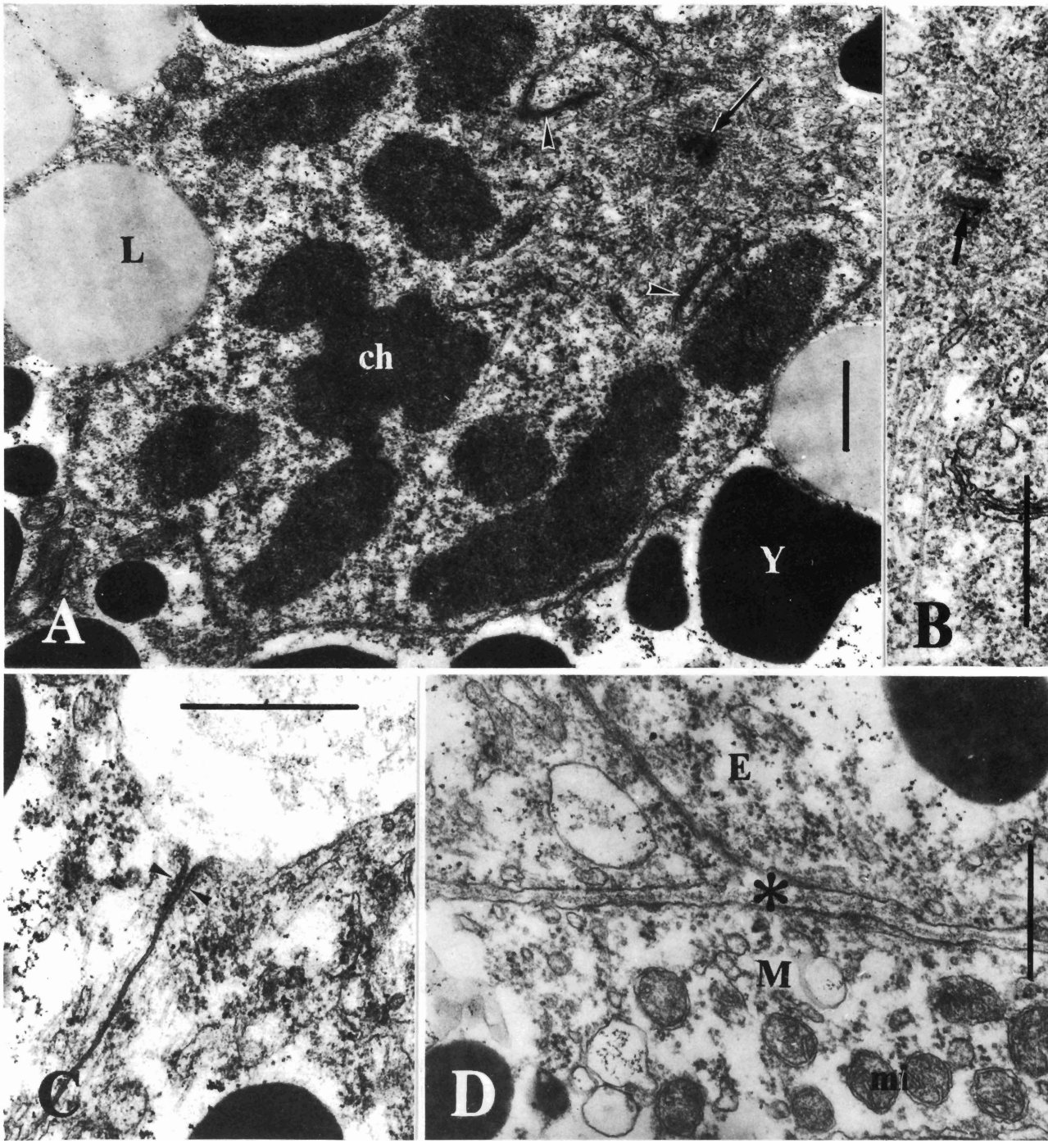

Fig. 3. *Enoplus demani*, sagittal (A-C) and transverse (D) sections through the tadpole embryo. A: Telophase stage of mitosis in the intestinal epithelium, chromosomes are surrounded by newly formed nuclear envelope (arrowheads), the arrow shows the centriole; B: Centriole (arrow) in the centrosome of a mitotic cell; C: Adherent junction (arrowheads) between epidermal cells; D: Basal lamina (asterisk) between epidermal and muscle cells. (Scale bar - 0.5 μm). For abbreviations see Figure 2.

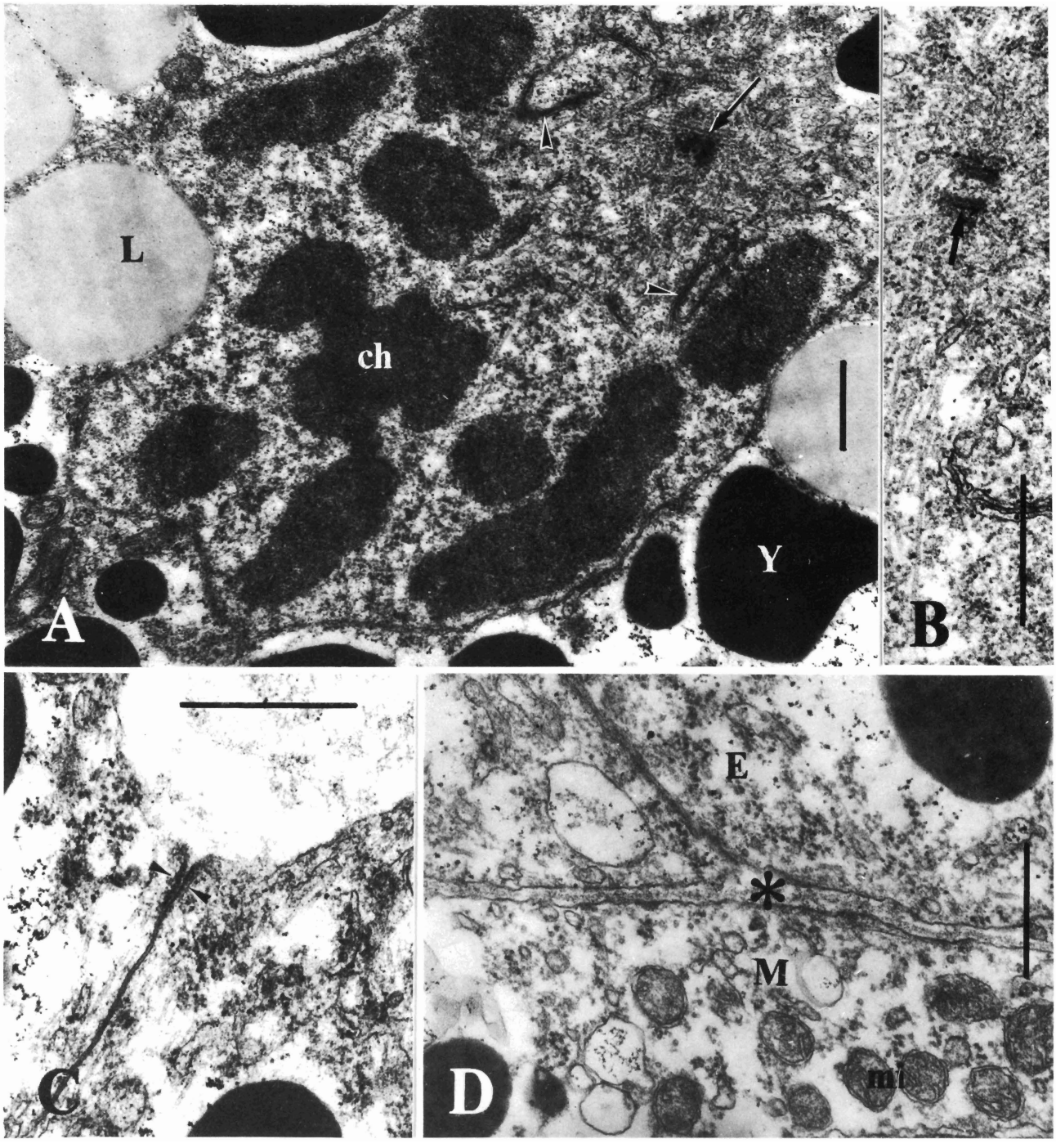

Fig. 3. *Enoplus demani*, sagittal (A-C) and transverse (D) sections through the tadpole embryo. A: Telophase stage of mitosis in the intestinal epithelium, chromosomes are surrounded by newly formed nuclear envelope (arrowheads), the arrow shows the centriole; B: Centriole (arrow) in the centrosome of a mitotic cell; C: Adherent junction (arrowheads) between epidermal cells; D: Basal lamina (asterisk) between epidermal and muscle cells. (Scale bar - 0.5 μm). For abbreviations see Figure 2.

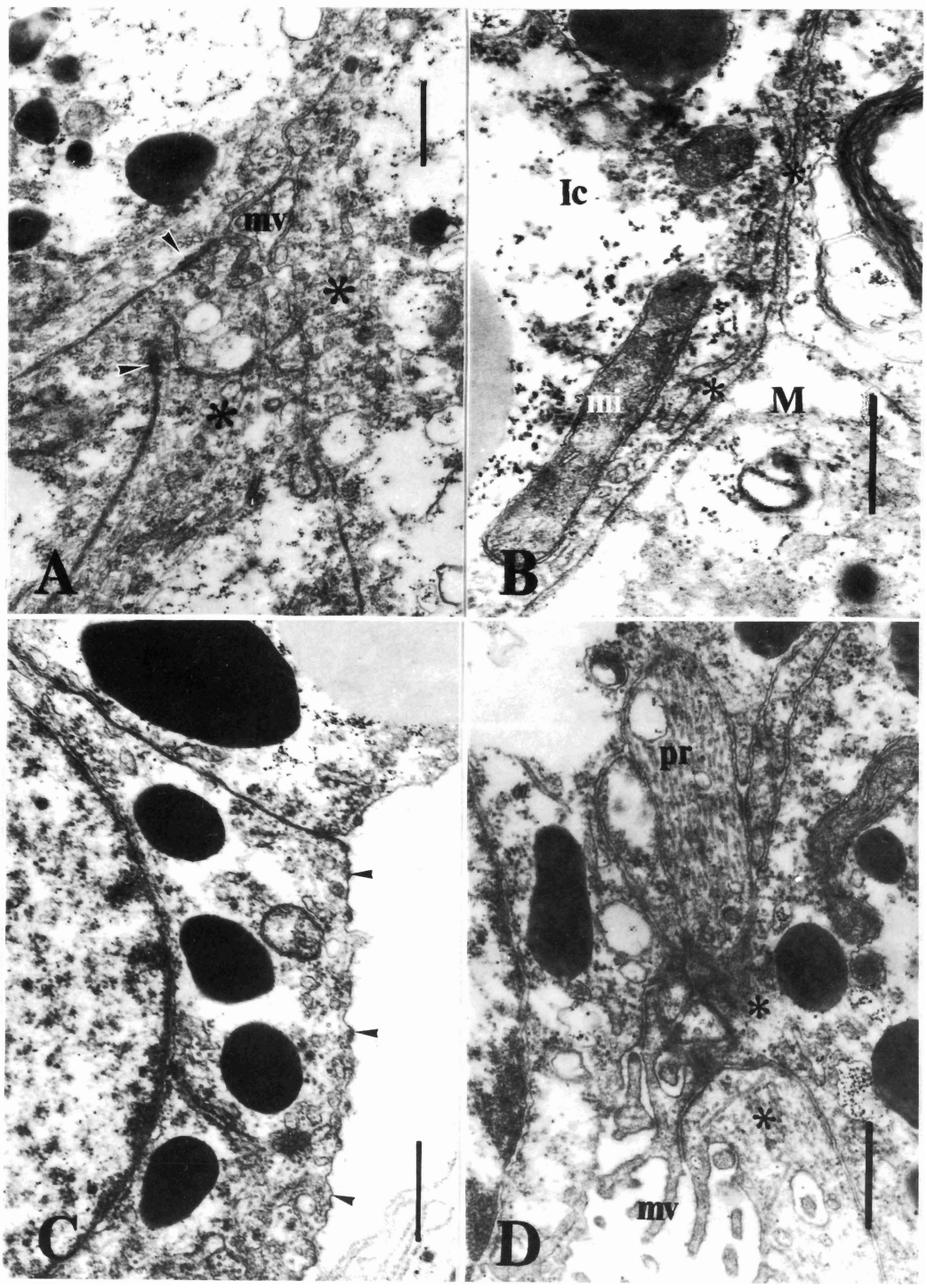

Fig. 4. *Enoplus demani*, transverse (A, B, D) and sagittal (C) sections through the tadpole embryo. A: Apexes (asterisks) of the intestinal cells, the arrowheads show adherent junctions; B: Basal lamina (asterisks) between intestinal and muscle cells; C: Apical surface (arrowheads) of the epidermal cell; D: High magnification of epidermal invagination at the anterior end of the embryo, the asterisks indicate the apical parts of epidermal cells. (Scale bar - 0.5 μm). For abbreviations see Figure 2.

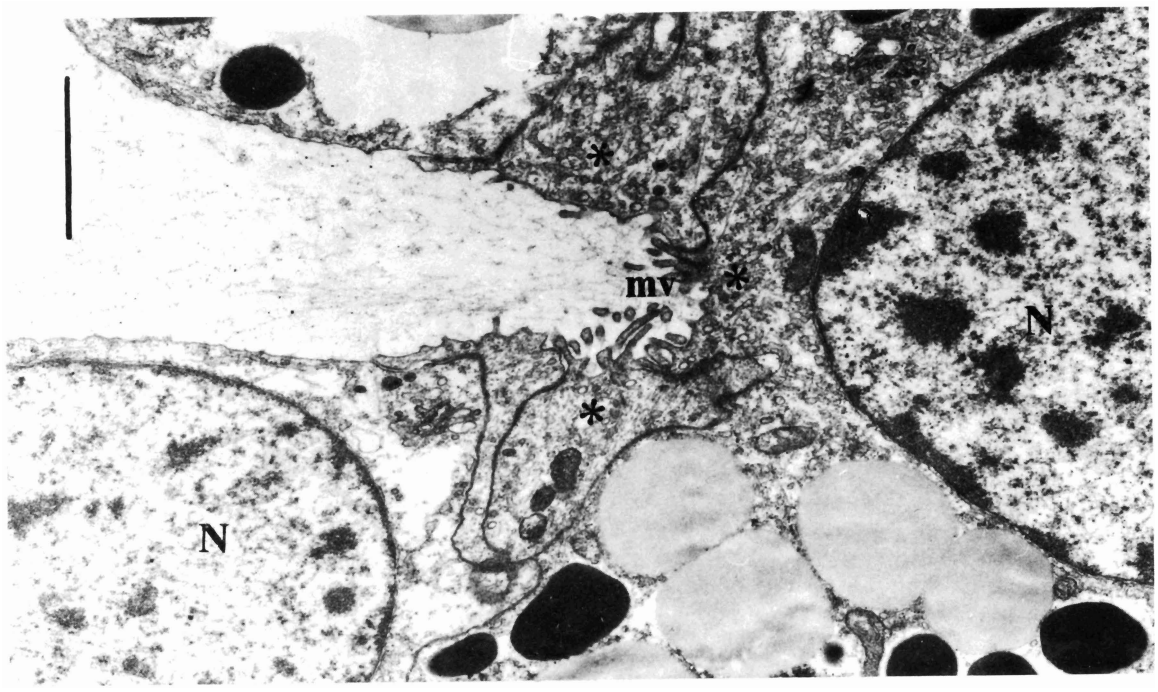

Fig. 5. *Enoplus demani*, sagittal section through the tadpole embryo. Epidermal invagination at the anterior end of the embryo, the asterisks indicate the apical parts of epidermal cells. (Scale bar- 2 μm). For abbreviations see Figure 2.

occur rarely in the nucleoplasm, and the nuclear envelope has numerous pores (Fig. 6B).

The voluminous cytoplasm of the PGC does not differ from the cytoplasm of somatic cells of the embryo (Fig. 6A). It contains mitochondria and is filled with yolk granules and lipid droplets. The thin cytoplasm region near the nuclear envelope contains Golgi bodies, cysternae of RER, ribosomes and vesicles. We were unable to identify distinct cytoplasmic features or inclusions that may be used as morphological markers of the tadpole PGC.

DISCUSSION

The tadpole embryo of *E. demani* consists of almost uniform cells, but finishing organogenesis results in epithelia formation, start of early morphogenesis, and cyto-differentiation.

The epidermis of *E. demani* forms in the comma embryo as five cell rows: two lateral, two subventral, and one dorsal (Voronov *et al.*, 1989). This five row arrangement of embryonic epidermal cells occurs with *C. elegans* (Priess & Hirsch, 1986). The tadpole stages of both species do not show cuticle formation. In *C. elegans* this process starts only after strong elongation in the three-fold loop embryo (Costa *et al.*, 1997). The same situation might be expected in the development of *E. demani*, where the tadpole embryo shows poor metabolic activity of epidermal cells.

Myoblasts in the tadpole embryo of *E. demani* are positioned between the epidermis and the intestine as two lateral layers of uniform thickness, without division into quadrants. Future muscle cells of the embryo show no evidence of cyto-differentiation except mitochondria grouping at the basal (future contractile) part of the cell. No myofilaments were detected there. In *C. elegans* the first muscle proteins appear in 290 min old embryos, long before the comma (390 min) and tadpole (420 min) embryos show twitching movement followed by a slow movement of the two-fold loop embryo (450 min) inside the egg shell (Wood, 1988a; Moerman & Fire, 1997). Muscle cells of the *C. elegans* tadpole (1× fold) embryo are divided into quadrants and polarized due to a concentration of myofilaments in the basal part of the cells (Moerman & Fire, 1997). It is evident, that muscle formation in *E. demani* is shifted to a later stage of morphogenesis than in *C. elegans*.

Gastrulation in nematodes results in the formation of a central cylinder consisting of ectodermal pharynx and endodermal intestine (Wood, 1988a; Malakhov, 1994). In the tadpole embryo of *E.*

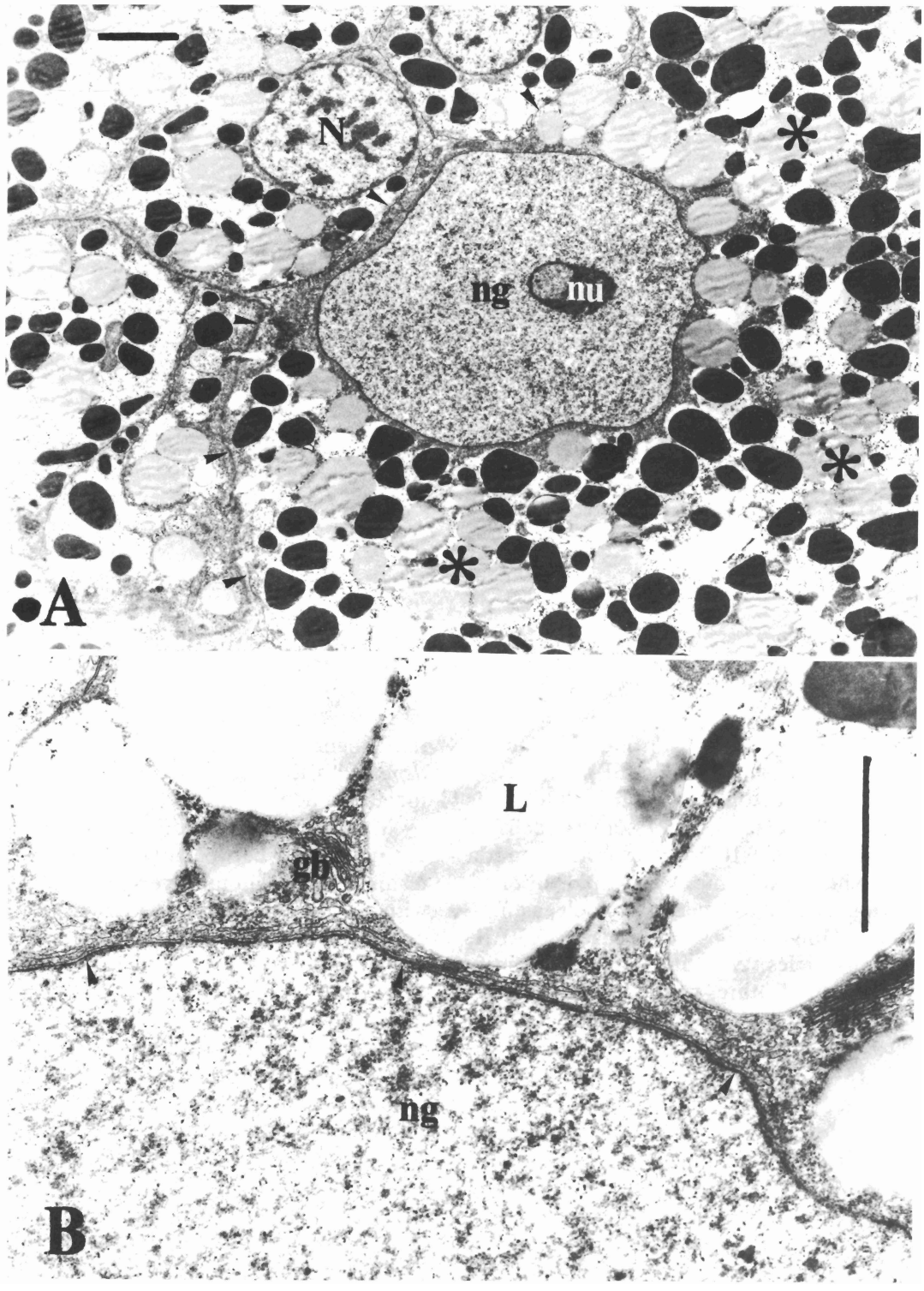

Fig. 6. *Enoplus demani*, primary germ cell (PGC) in a parasagittal section through the tadpole embryo. A: General view, the arrowheads show the border, the asterisks indicate the cytoplasm of the PGC; B: High magnification of the nuclear envelope (arrowheads) and surrounding cytoplasm. (Scale bar, A - 2 μm; B - 1 μm). For abbreviations see Figure 2.

demani the intestine consists of relatively large polarized epithelial cells with microvilli on the apical surface, but the lumen is indistinct and cells show no metabolic activity. At this stage the cells of the pharynx and the intestine have only size differences. It was shown for *C. elegans* that cell communication between intestinal and pharyngeal cells is completed at the tadpole stage (Bossinger & Schirenberg, 1992). This may be considered as the first evidence of intestinal cell differentiation.

Future nerve cells of early embryos of nematodes are undifferentiated and indistinguishable from surrounding cells up to the stage of morphogenesis (Krieg *et al.*, 1978; Malakhov, 1994). We were unable to identify neurons and axons inside tissues of the tadpole embryo of *E. demani*. The only evidence of nerve system (sense organs) formation was deep epidermal invaginations at the anterior end of the embryo. Specific microtubule filled processes of apical ends confirm the future neuronal nature of the invaginated cells. Concerted morphogenetic migration of the cells of future cephalic sensilla occurs in *C. elegans* and proceeds at the comma stage (Sulston, 1988), just before tadpole stage formation.

As stated by Voronov *et al.* (1989) for *E. demani* embryogenesis, active proliferation is finished at the comma stage. Our ultrastructural data show occasional mitoses in all the primordial tissues of the tadpole embryo. In *C. elegans* mitoses are completed at the lima-bean stage, and this coincides with the start of morphogenesis and cyto-differentiation (Ehrenstein & Schierenberg, 1980). The end of proliferation and start of cyto-differentiation in *E. demani* are shifted to a later stage of embryogenesis than occurs in *C. elegans*. This phenomenon may be derived from the multicellularity of *E. demani* juveniles and adults as compared to *C. elegans*. For example, the first stage juveniles (J1) of *E. brevis* consist of approximately 1500 cells (Voronov & Panchin, 1998), whereas the J1 of *C. elegans* are comprised of only 538 cells (Wood, 1988a).

Centrioles have only occasionally been described in somatic cells of nematodes (Wright, 1991). It was shown for *C. elegans* that sperm derived centrioles are incorporated into centrosomes of the first mitotic spindle (Albertson, 1984). Our data on the tadpole embryo of *E. demani* is the first ultrastructural evidence of the presence of centrioles in mitotic spindles of a primitive marine nematode. The structure of these centrioles is clear only in cross section, and the diameter (150 nm) coincides with the diameter of centrioles with triplets (Loewy & Siekewitz, 1974). In sperm of *C. elegans*, as well as of many other nematodes, centrioles have a reduced structure and consist of only 9 singlets of microtubules (Justine & Jamieson, 1999). Centrioles with nine doublets were found in somatic cells of *Capillaria hepatica* (Wright, 1976). It is possible that centrioles of *E. demani* also have a reduced number of microtubules.

Small marine nematodes lack a real body cavity (Malakhov, 1994; Van de Velde & Coomans, 1989, Ehlers, 1994). Narrow interstitial spaces between cellular layers are occupied by a thin basal lamina. This situation is considered primitive for nematodes (Ehlers, 1994). The tadpole embryo of *E. demani* has no distinct body cavity, but a thin basal lamina separates layers of epidermis, presumptive muscle cells and intestinal epithelium. However adult *E. demani,* a relatively large marine species, possesses a body cavity between the epithelial-muscle sac and the intestine (Malakhov, 1994).

Nematodes have one of the earliest determinations of primary germ cells (PGC) (Strome & Wood, 1982, 1983). For example, in *C. elegans* two PGC, termed in cell lineage as Z_1 and Z_2, are isolated in the 28 cell embryo and continue to proliferate only during the postembryonic period (Ehrenstein & Schierenberg, 1980). The cells of the germ line in the embryogenesis of all nematodes studied are clearly marked by P-granules, *viz.* specific amorphous cytoplasm inclusions, associated with nucleus (Kimble & Ward, 1988).

E. demani has less determined development than other nematodes (Malakhov, 1994; Voronov & Panchin, 1998). Although the cell cleavage pattern of this primitive nematode was studied intensively, the germ cell line remains undetermined. Putative PGC were found in late stages of development (comma, tadpole and two-fold loop), after examination of serial thick sections (Malakhov & Akimushkina, 1976). These were two giant cells positioned laterally to the primordial intestine.

The present study of serial thin sections confirms the existence of two cells of a special type in the tadpole embryo of *E. demani*. The number (2), position (blastocoel, midbody, latero-ventrally to the intestine), and comparatively large size of these cells suggest they are PGC. However they have no P-granules or other special cytoplasmic markers. In this respect the PGC of *E. demani* differ distinctly from PGC of other nematodes studied (Wisse & Daems, 1968; Krieg *et al.*, 1978; Wolf *et al.*, 1983; Sulston *et al.*, 1983; Endo, 1998). The absence of P-granules prevents tracing determination of PGC during the early cleavage of *E. de-*

mani. Also, a late determination of PGC in *E. demani* cannot be assumed, because it is possible that the PGC become isolated from somatic cells at an early stage, but are stored during development without easily detectable cytological events (Jeffery, 1988; Ikenishi, 1998).

ACKNOWLEDGEMENTS

This research was supported in part by grant G 005196 of FWO, Flanders to A. Coomans and RFBI grants 00-04-48896 and 00-15-97938. The authors are grateful to M. Claeys and D.V. Fomin for technical assistance.

REFERENCES

Albertson, D.G. 1984. Formation of the first cleavage spindle in nematode embryos. *Developmental Biology* 101: 61-72.

Bird, A.F. 1977. Cuticle formation and moulting in the egg of *Meloidogyne javanica* (Nematoda). *Parasitology* 74: 149-152.

Bird, A.F. & Stynes, B.A. 1981. The life cycle of *Anguina agrostis*: embryogenesis. *International Journal for Parasitology* 11: 23-33.

Bruce, R.G., 1970. *Trichinella spiralis*: fine structure of body wall with special reference to formation and moulting of cuticle. *Experimental Parasitology* 28: 499-511.

Bossinger, O. & Schierenberg, E. 1992. Cell-cell communication in the embryo of *Caenorhabditis elegans*. *Developmental Biology* 151: 401-409.

Costa, M., Draper, B.W. & Priess, J.R. 1997. The role of actin filaments in patterning of the *Caenorhabditis elegans* cuticle. *Developmental Biology* 184: 373-384.

Ehlers, U. 1994. Absence of a pseudocoel or pseudocoelom in *Anoplostoma viviparum* (Nematodes). *Microfauna Marina* 9: 345-350.

Ehrenstein, G. & Schierenberg, E. 1980. Cell lineages and development of *Caenorhabditis elegans* and other nematodes. In: *Nematodes as Biological Models*, Vol. 1 (B.M. Zuckerman. Ed.). pp. 1-71. London, Academic Press.

Endo, B.Y. 1998. *Atlas on ultrastructure of infective juveniles of the soybean cyst nematode, Heterodera glycines*. US Department of Agriculture, Agriculture Handbook No 711. 224 p.

Ikenishi, K. 1998. Germ plasm in *Caenorhabditis elegans, Drosophila and Xenopus*. *Development Growth and Differentiation* 40: 1-10.

Jeffery, W.R. 1988. The role of cytoplasmic determinants in embryonic development. In: *Developmental Biology. Vol. 5. The Molecular Biology of Cell Determination and Cell Differentiation* (L.W. Browder. Ed.). pp. 3-56. New York, Plenum.

Justine, J.-L. & Jamieson, B.G.M. 1999. Nematoda. In: *Reproductive Biology of Invertebrates*. Volume IX, part B (B.G.M. Jamieson. Ed.). pp. 183-266. New Delhi, Oxford & IBH.

Kimble, J. & Ward, S. 1988. Germ line development and fertilization. In: *The Nematode Caenorhabditis elegans* (W.B. Wood. Ed.). pp. 191-213. New York, Cold Spring Harbor Laboratory.

Kozek, W.J. 1971. The moulting pattern in *Trichinella spiralis*. II. An electron microscope study. *Journal of Parasitology*, 57: 1029-1038.

Krieg, C., Cole, T., Deppe, U., Schierenberg, E., Schmitt, D., Yodler, B. & Ehrenstein, G. 1978. The cellular anatomy of embryos of the nematode *Caenorhabditis elegans*. *Developmental Biology* 65: 193-215.

Loewy, A.C. & Siekewitz, P. 1974. *Cell Structure and Function*. London, Holt, Rinehart and Winston. 516 pp.

MacKinnon, B.M. 1986. *Nematospiroides dubius* (Nematoda): ultrastructure of the developing larva within the egg shell. *Proceedings of XI International Congress on Electron Microscopy*, Kyoto, p. 3319-3320.

Malakhov, V.V.& Akimushkina, M.I. 1976. [Embryogenesis of a free-living nematode *Enoplus brevis*]. *Zoologichesky Zhurnal* 55: 1788-1799.

Malakhov, V.V. 1994. *Nematodes: Structure, Development, Classification, and Phylogeny*. Washington & London, Smithsonian Institution Press. 286 pp.

Malakhov, V.V. 1998. Embryological and histological peculiarities of the order Enoplida, primitive group of nematodes. *Russian Journal of Nematology* 6: 41-46.

Martinez-Palomo, A. 1978. Ultrastructural characterization of the cuticle of *Onchocerca volvulus* microfilaria. *Journal of Parasitology* 64: 127-136.

Moerman, D.G. & Fire, A. 1997. Muscle: structure, function, and development. In: *C. elegans II* (D.L. Riddle, T. Blumental, B.J. Meyer & J.R. Priess. Eds.). pp. 417-470. Cold Spring Harbor, Cold Spring Harbor Laboratory Press.

Platzer, A. & Platzer, E.G. 1988. Early cuticle formation in an adenophorean nematode. *International Journal for Parasitology* 18: 793-801.

Priess, J.R. & Hirsch, D.I. 1986. *Caenorhabditis* morphogenesis: the role of the cytoskeleton in elongation of the embryo. *Developmental Biology* 117: 156-173.

Riddle, D.L., Blumental, T., Meyer, B.J. & Priess, J.R. (Eds.). 1997. *C. elegans II*. Cold Spring Harbor, Cold Spring Harbor Laboratory Press, 1222 pp.

Strome, S. & Wood, W.B. 1982. Immunofluorescence visualization of germ-line specific cytoplasmic granules in embryos, larvae, and adults of *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences of the USA* 79: 1558-1562.

Strome, S. & Wood, W.B. 1983. Generation of asymmetry and segregation of germ-line granules in early *Caenorhabditis elegans*. *Cell* 35: 15-25.

Sulston, J.E. 1988. Cell lineage. In: *The Nematode Caenorhabditis elegans* (W.B. Wood. Ed.). pp. 123-155. New York, Cold Spring Harbor Laboratory.

Sulston, J.E. 1997. Cell lineage. In: *C. elegans II* (D.L. Riddle, T. Blumental, B.J. Meyer & J.R. Priess. Eds.). pp. 123-155. Cold Spring Harbor, Cold Spring Harbor Laboratory Press.

Sulston, J.E., Schierenberg, E., White, E. & Thomson, J.N. 1983. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Developmental Biology* 100: 64-119.

Tongu, Y., Vincent, A.L. & Ash, L.R. 1978. The ultrastructure of early larval morphogenesis in *Brugia pahangi* (Nematoda: Filarioidea). *Japan Journal of Parasitology* 27: 245-260.

Van de Velde, M.-C. & Coomans, A. 1989. A putative new hydrostatic skeletal function for epidermis in monhysterids (Nematoda). *Tissue and Cell* 21: 525-533.

Voronov, D.A. 1999. The embryonic development of *Pontonema vulgare* (Enoplida: Oncholaimidae) with a discussion of nematode phylogeny. *Russian Journal of Nematology* 7: 105-114.

Voronov, D.A., Nezlin, L.P., Panchin, Y.V. & Spiridonov, S.E. 1989. [The first stage larva of the free-living marine nematode *Enoplus brevis*. Hypoderm and sensory organs]. *Ontogenez* 20: 416-422.

Voronov, D.A. & Panchin, Y.V. 1998. Cell lineage in marine nematode *Enoplus brevis*. *Development* 125: 143-150.

Wisse, E. & Daems, W.T. 1968. Electron microscopic observation on the second stage larvae of the potato root eelworm *Heterodera rostochiensis*. *Journal of Ultrastructure Research* 24: 210-231.

Wolf, N., Priess, J. & Hirsch, D. 1983. Segregation of germline granules in early embryos of *Caenorhabditis elegans*: an electron microscopic analysis. *Journal of Embryology and Experimental Morphology* 73: 297-306.

Wood, W.B. 1988a. Embryology. In: *The Nematode Caenorhabditis elegans* (W.B. Wood. Ed.). pp. 215-241. New York, Cold Spring Harbor Laboratory.

Wood, W.B. (Ed.) 1988b. *The Nematode Caenorhabditis elegans*. New York, Cold Spring Harbor Laboratory. 667 pp.

Wright, K.A. 1976. Somatic centrioles in the parasitic nematode, *Capillaria hepatica* Bancroft, 1893. *Journal of Nematology* 8: 92-93.

Wright, K.A. 1991. Nematoda. In: *Microscopic Anatomy of Invertebrates,* Vol. 4 (F.W. Harrison & E.E. Ruppert. Eds.). pp. 111-195. New York, Wiley-Liss.

Yushin, V.V. & Malakhov, V.V. 1989. [Cuticle formation in embryogenesis of the free-living marine nematode *Enoplus demani*]. *Doklady Akademii Nauk SSSR* 308: 497-499.

Yushin, V.V. & Malakhov, V.V. 1992. [Body cuticle formation in embryogenesis of free-living marine nematode *Halichoanolaimus sonorus* (Chromadorida, Selachinematidae)]. *Zoologichesky Zhurnal* 71: 23-30.

Юшин В. В., Команс А., Боргони Г., Малахов В. В. Ультраструктурное исследование стадии головастика у примитивной морской нематоды *Enoplus demani* (Enoplia: Enoplida).

Резюме. Изучена ультраструктура головастика – ранней стадии эмбрионального развития свободноживущей морской нематоды *Enoplus demani*. Клетки всех тканей эмбриона мало дифференцированы. Цитоплазма содержит митохондрии, желточные гранулы и липидные капли; ядра заполнены частицами конденсированного хроматина. Во всех зародышевых листках обнаружены клетки на различных стадиях митоза. Центросомы митотического веретена содержат центриоли. Клетки эпидермиса и кишечника поляризованы и формируют эпителии, подстилаемые базальной пластинкой. Апикальная мембрана эпидермальных клеток волнистая, в цитоплазме клеток встречаются тельца Гольджи и цистерны ШЭР. На головном конце эпидермис формирует множество воронковидных впячиваний, свидетельствующих о начале формирования головных чувствительных органов. В кишечной трубке просвет отсутствует, однако между апикальными мембранами клеток кишечника обнаружены микроворсинки. Презумптивные мышечные клетки не содержат миофиламентов, однако в их будущей базальной (сократительной) части наблюдается скопление митохондрий. Пара первичных половых клеток (ППК) располагается в бластоцеле эмбриона латеро-вентрально на уровне середины кишечной трубки. Диаметром эти клетки в два раза превосходят соматические клетки эмбриона. Цитоплазма ППК не отличается от цитоплазмы соматических клеток и не содержит специфических Р-гранул. Данные по ультраструктуре головастика *E. demani* сравниваются с имеющимися в литературе сведениями по клеточной дифференцировке в раннем эмбриогенезе почвенной нематоды *Caenorhabditis elegans*.