Russian Journal of Nematology, 1998, 6 (1), 41 - 46

Embryological and histological peculiarities of the order Enoplida, a primitive group of nematodes

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Accepted for publication 10 January 1998

Summary. Unusual features of the Enoplida have been revealed in recent years. The type of enoplids embryogenesis differs sharply from all other nematodes. The cleavage of enoplids is characterized by the variability in the blastomeres arrangement. No strict determination of blastomeres has been revealed in the development of enoplids. Unlike in other nematodes, the bilateral symmetry appears late in the embryogenesis of enoplids. Enoplids do not have eutely which is considered to be a characteristic feature of nematodes. Enoplids are able to regenerate experimentally destroyed internal organs if the cuticle was not disrupted. These features suggest that enoplids are possibly more primitive than other nematode taxa.
Key words: Enoplida, embryogenesis, cell-lineage, eutely, regeneration.

Research of the various groups of nematodes has been highly variable, with those which contain the more economically important species having been the subject of most investigation. Most studies of the organization and biology of nematodes has been done with the parasitic and soil species. Data from investigations of the embryology and histology of soil and parasitic species have been used to formulate several fundamental tenets, *viz.,* i) nematodes have strictly determined cleavage with early formation of bilateral symmetry; ii) nematodes possess eutely, i.e., nematodes have a constant number of cells; and iii) nematodes are incapable of regeneration. These tenets are applied to all nematodes and are considered fundamental characteristics of the class.

Research data obtained mainly by researchers in Russia from investigations of the embryology and histology of marine enoplids, a primitive group of nematodes, is reviewed. These investigations of free-living marine nematodes reveal that these three tenets do not apply to all nematodes.

Regulative development in marine enoplid nematodes

Investigations of marine nematodes of the order Enoplida have revealed that regulative development determines late formation of bilateral symmetry in these nematodes (Malakhov & Cherdantsev, 1975; Malakhov & Akimushkina, 1976; Malakhov, 1986a, b, 1994; Voronov *et al.,* 1986; Voronov & Panchin, 1995a, b).

In enoplids, the furrow of the first cleavage occurs perpendicular to the longitudinal axis of the egg shell. The first furrow divides the egg into two equal blastomeres: the anterior and the posterior (Fig. 1). After division is completed, the interphase blastomeres display cytoplasmic activity which is apparent by the presence of blebs (Fig. 1). The blebbing is characteristic for interphase blastomeres in all successive stages of cleavage.

The second cleavage proceeds as one of several variations, *viz.*, tetrahedral, rhombic or exceptionally as a T-shaped configuration of 4 blastomeres. After division is complete the blastomeres migrate to form an asymmetrical tetrahedron or slightly swollen rhombic shape (Fig. 1). The arrangement of furrows of the third cleavage also varies. After each division the blastomeres migrate relative to each other.

During the transition to the fourth cleavage, one of the blastomeres of the embryo lags in the division and the other blastomeres grow around it. This "late" blastomere is the precursor of the endoderm (Fig. 3). The shrinking of this blastomere at the fifteen-cells stage can be regarded as the beginning of gastrulation, thus, the endoderm of the embryo originates from one of the blastomeres at the 8-cells stage. The subsequent cell divisions result in a more definite formation of a blastopore. The blastopore occupies the centre of the future ventral surface of the embryo.

[1] This paper is an expanded version of an invitational Plenary talk presented at the Second English Language International Nematology Symposium of the Russian Society of Nematology held in Moscow, Russia, 23rd to 30th August 1997.

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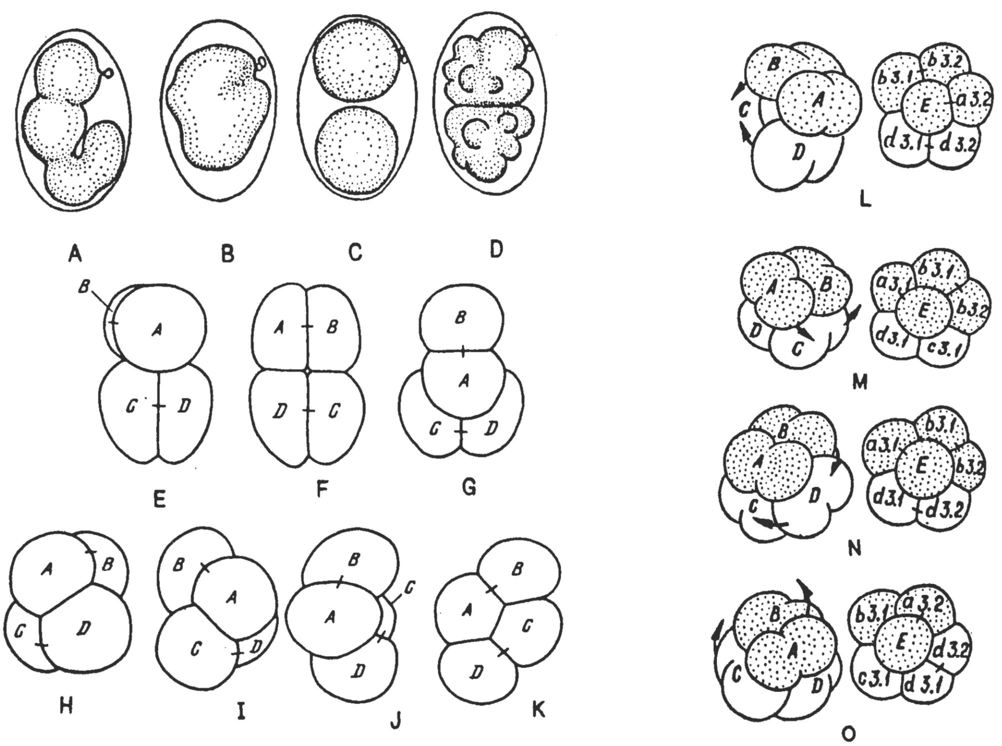

Fig. 1. Early stages of cleavage in enoplid nematodes: A-B: *Enoplus demani*; E-O: *Pontonema vulgare*. A: Extrusion of the 1st polar body; B, extrusion of the 2nd polar body; C: 2 blastomeres stage, immediately after the 1st cleavage; D: The blebbing of interphase blastomeres; E-F: Positions of blastomeres immediately after the 2nd cleavage; H-K: Positions of blastomeres prior to the 3rd cleavage; L-O: Variations of the 3rd cleavage with arrows indicating the direction of movement of the blastomeres; descendants of AB blastomere are dotted. (A-B, after Malakhov, 1991; E-K and L-O, after Malakhov, 1986).

The embryo preserves the organization of a gastrula up to the development stage of approximately 500 cells. The blastopore initially has a spherical shape, but then stretches to become an elongated oval-shape (Fig. 2). Subsequent development results in the lateral edges of the blastopore approaching and eventually connecting with the centre. Two openings, one at the anterior end the other at the posterior end of the embryo, are persistent remnants of the blastopore. The anterior opening provides the beginning of the definitive mouth, and the posterior one, the definitive anus. After the blastopore closes, the embryo starts to bend. From this development stage the embryo of enoplids appears similar to that present in other groups of nematodes. The "bean", "tadpole", and "loop" stages develop subsequently.

Histological sections have revealed that the mesoderm originates from the cells of the blastopore edges (Fig. 3) and the nerve system from the row of cells along the ventral cleft on the blastopore (Fig. 3). In the "bean" stage, two large cells appear situated to the right and left of the rudiment of the gut and are probably the precursors of the reproductive system. Juveniles of the larger species, i.e., *Enoplus, Pontonema*, hatch at 20-30 days and newly hatched 1st stage juveniles contain approximately 2000 cells. The number of cells in these specimens is more than twice the number of cells reported present in an adult *Caenorhabtitis* sp. (Ehrenstein & Schierenberg, 1980).

Embryogenesis in enoplids appears to have several unusual features. Firstly, variability occurs in the blastomere arrangement in the stages of early cleavage. At the four-cells stage various configurations have been observed, *viz.*, tetrahedral, rhombic, T-shaped. These configurations have been variously encountered in the development of nematodes belonging to other groups. However, it is only in the Enoplida that each of these configurations have been observed occurring in a single species. In other groups of nematodes it appears that a species uses only one of these configuration of blastomeres in its development.

Secondly, variability is also exhibited in the blastomere arrangement in the late stages. The cell-lineage from the 2-blastomeres stage to the late cleavage stages has been studied (Malakhov, 1986a) (Fig. 4). The descendants of one of the blastomeres of the 2-cells stage, either the anterior or the posterior one, are arranged in different ways around the blastopore. Consequently, it is apparent that the organs of the embryos can originate from either of

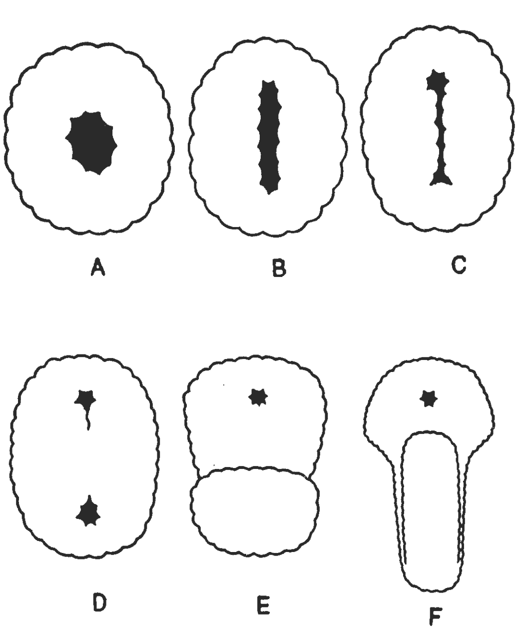

Fig. 2. Formation of the elongated oval-shape blastopore (A-B), the closing of the blastopore (C-D), and the bending of the embryo in *Pontonema vulgare*. (F). (After Malakhov, 1986).

the two blastomeres. Data from experiments have confirmed that the development of *Enoplus* is not strictly determined (Voronov *et al.*, 1986; Voronov & Panchin, 1995a, b). Experiments in which vital stain was injected into blastomeres of the 2-cells stage revealed that the stained cells from, for example, the anterior blastomere, could organise themselves in different arrangements (Fig. 4). These results support the observation of the occurrence of different development of cleavage as mentioned previously. Also, these experiments confirmed that the endoderm of the embryo originates from one blastomere of the 8-cells stage. Vital stain injected into an endodermal cell, at the 8-cells stage, subsequently was observed present only in the mid-gut of the juvenile. Generally, the endoderm cell is a descendant of the anterior blastomere of the 2-cells stage, although some exceptions have been observed (Voronov & Panchin, 1995a, b).

Thirdly, late establishment of bilateral symmetry is yet another primitive feature of enoplid development. The arrangement of blastomeres in enoplid cleavage has been observed not to be bilateral, and this absence of bilateral symmetry continues up to the stage of about 500 cells. Bilateral symmetry appears only when the blastopore begins to elongate (Fig. 2), which is quite different from all other nematode taxa where such symmetry is already present at the 4-cells stage of development (Strassen, 1896; Boveri, 1899; Martini, 1903; Pai, 1928; Ehrenstein & Schierenberg, 1980).

Eutely is not present in marine enoplid nematodes

The concept of eutely in nematodes was developed from early studies and more recent investigations (Martini, 1907, 1908, 1916; Pai, 1928;

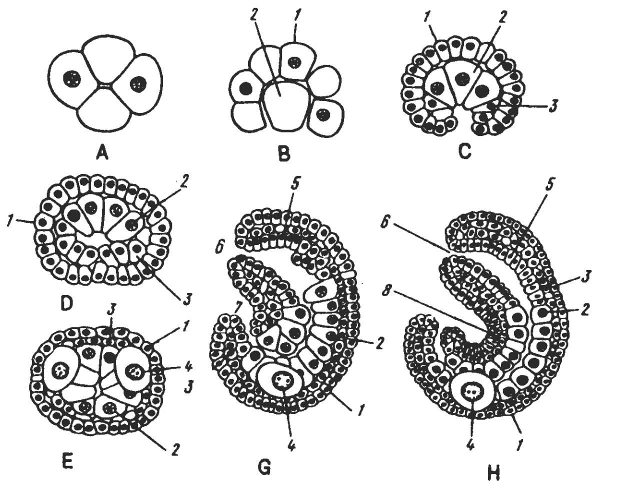

Fig. 3. Histological section of the embryos of *Enoplus* (A-E: Transverse sections; G-H: Longitudional sections). A: 8 blastomeres; B: 15 blastomeres, C: Elongated oval-shape blastopore stage; D: Closing of blastopore; E-G: "Bean" stage; H: "Tadpole" stage. 1, ectoderm; 2, endoderm; 3, mesoderm; 4, primary genital cells; 5, stomodeum; 6, mouth; 7, anus; 8, the precursor of the ventral nerve cord. (After Malakhov & Akimushkina, 1976).

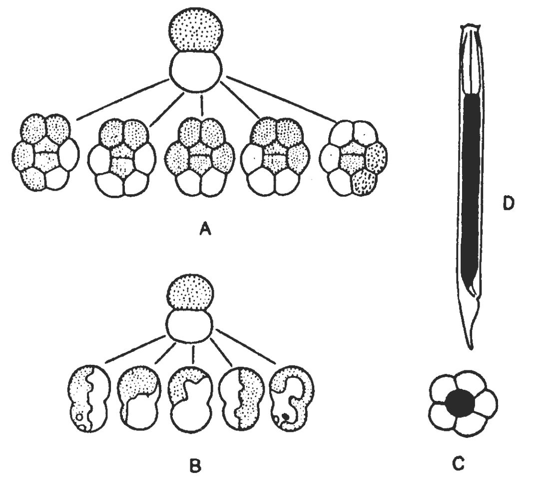

Fig. 4. Variations of the cell lineage in *Enoplus*: A: Variations of the descendants of the anterior blastomere of the 2-cells stage at the stage of 30 cells, ventral view; B: Distribution of the material of the anterior blastomere of the 2-cells stage at the "bean" stage; C & D: The occurence of stained cells in the mid-gut where one blastomere of 8 cells stage had been injected with vital stain. (A, after Malakhov, 1986; B-D, modified from Voronov *et al.*, 1986).

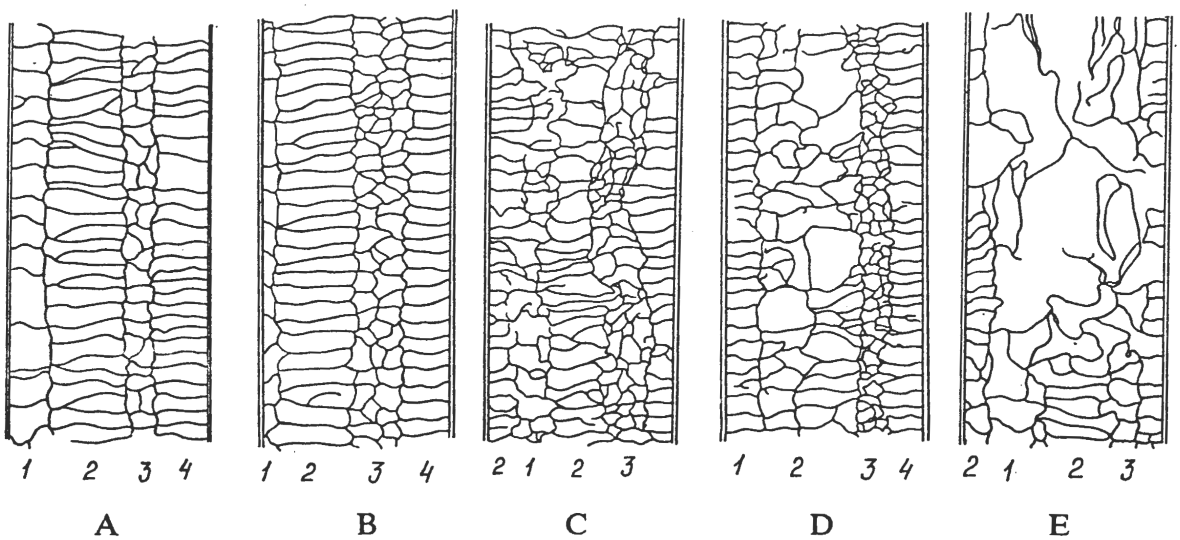

Fig. 5. Variation of the hypoderm cells boundaries in the middle of the body of *Pontonema vulgare.* A: Normal arrangement; B & C: Disrupted arrangement; E: Irregular arrangement (in this example it is not possible to count the number of cells). Rows of hypoderm cells: 1, ventral; 2, subventral; 3, lateral; 4, subdorsal. (After Rusin & Malakhov, 1998).

Chitwood & Chitwood, 1950; Sulston *et al.*, 1983; Wood, 1988). Counting of cells present in small soil nematodes, e.g., *Caenorhabditis,* is a relatively simple procedure as the organization of these nematodes consists of about 1000 cells. However, counting the cells present in the much larger, free-living marine nematodes is a more difficult procedure as the adults are comprised of several thousand, or even tens of thousands, cells. In these larger nematodes it is more practical to count cells which comprise the separate tissues. The hypoderm of free-living nematodes consists of cells rather than syncytial hypoderm present in parasitic nematodes. The simple method of impregnation enables the cell boundaries in the hypoderm to be revealed (Malakhov & Spiridonov, 1982). The total cell number in the hypoderm ranges from about 300, in the small enoplid *Anoplostoma viviparum,* to 5000 - 6000 cells in large species, e.g., *Pontonema vulgare* (Rusin & Malakhov, 1998). However, individual specimens from a population differ in their number of cells (Table 1). A silver impregnation technique revealed large variation in the mosaic of hypoderm cell boundaries (Fig. 5) and, thus, the concept of eutely is not reconcilable with data of the hypoderm structure of enoplids.

The ability of marine enoplid nematodes to regenerate internal tissues

Experiments with marine enoplids provided data of the ability of these nematodes to regenerate tissues. Korotkova & Agafonova (1976) investigated the ability of the marine enoplid *Pontonema vulgare* to regenerate tissue. In common with other nematodes, this species dies after being cut open. However, if the nematode is compressed the intestine can be ruptured (Fig. 6) and the nematode survives if the cuticle remains intact. In compressed nematodes, in which

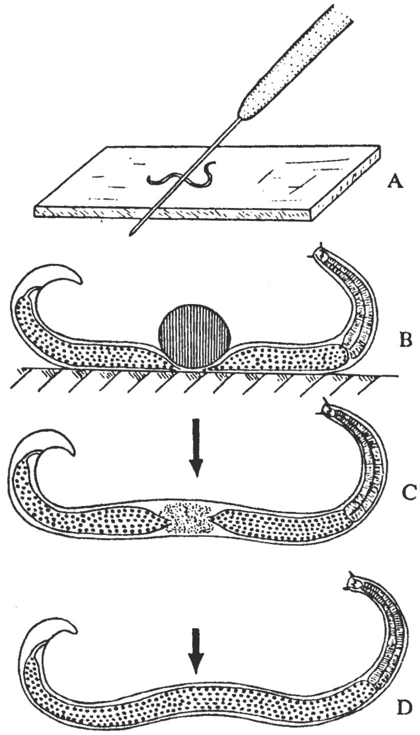

Fig. 6. Regeneration of the mid-gut in *Pontonema vulgare.* A & B: Nematode compressed by a needle; C: Ruptured mid-gut immediately after compressing; D: "Repaired" mid-gut 6 days after compression. (Based on the experiments of Korotkova & Agafonova, 1976).

Table 1. Total number of hypoderm cells in individual *Pontonema vulgare.*

Males		Females	
Length (mm)	Number of cells	Length (mm)	Number of cells
14.65	5529	17.03	5311
17.50	5052	17.15	4959
17.20	5313	17.40	5062
17.70	5664	18.20	5555
17.75	5310	18.40	5270
18.20	5453	18.40	5503
18.30	5216	19.50	6137
18.70	6470	20.04	5491
19.20	5141	20.40	5710
19.70	5072	20.95	6283

the gut is ruptured, mitosis start in the intestine epithelium. The edges of the intestine grow towards one another and the gut tube regenerates to its normal structure about 6 days after being severed (Korotkova & Agafonova, 1976).

DISCUSSION AND CONCLUSIONS

Marine enoplid nematodes have been the subject of fundamental research, especially concerning their embryology, at several research centres in Russia. For several reasons data from these studies, until recently, have had a restricted international availability. However, results from these studies have fundamental significance for understanding embryological development in Nematoda. The data obtained with marine enoplids used in these studies provide a basis for revising several fundamental tenets of Nematology. These investigations have revealed that free-living marine Enoplida have a regulative development which results in a late formation of bilateral symmetry. Also, eutely is not a characteristic of marine enoplids and, furthermore, this group of nematodes apparently can regenerate internal tissue to repair damaged organs. These characteristics represent plesiomorphic features of nematode organization and thus enoplids may be considered the most primitive group of the class. From these results it may be concluded that enoplids represent an early evolutionary branch, which seperated from the ancestral nematode stem prior to all other groups of nematodes.

REFERENCES

Boveri, T. 1899. Die Entwicklung von *Ascaris megalocephala* mit besonderer Rucksicht auf die Kernverhaltnisse. *Festschrift für R.Kupffer. Jena*: 383-430.

Chitwood, B. & Chitwood, M. 1950. *Introduction to Nematology.* Baltimore, Monumental Print Co. 213 pp.

Ehrenshtein, G. & Schierenberg, E. 1980. Cell lineage and development of *Caenorhabditis elegans* and other nematodes. In: *Nematodes as Biological Models* (M. Zuckerman. Ed.). pp. 1-74. Academic Press, New York.

Korotkova, G.P. & Agafonova, L.A. 1976. [Experimental morphological study of reparative abilities of the nematoda *Pontonema vulgare* (Bastian, 1865)]. *Arkhiv Anatomii, Gistologii i Embriologii* 70: 90-98.

Malakhov, V.V. 1986a. [New data on the embryonic development of free living marine nematode *Enoplus demani* (Enoplida, Enoplidae)]. *Zoologichesky Zhurnal* 65: 175-182.

Malakhov, V.V. 1986b. [*Nematodes: Structure, Development, Classification and Phylogeny*]. Moscow, Nauka. 215 pp.

Malakhov, V.V. 1991. [The analysis of embryonic development of Nematodes]. *Trudy Leningradskogo Obschestva Estestvoispytateley* 89: 101-123.

Malakhov, V.V. 1994. *Nematodes. Structure, Development, Classification, Phylogeny.* (W.D. Hope. Ed.). Washington & London. Smithsonian Institution Press. 286 pp.

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

Malakhov, V.V. & Cherdantsev, V.G. 1975. [Embryonic development of free living marine nematode *Pontonema vulgare*]. *Zoologichesky Zhurnal* 54: 165-174.

Malakhov, V.V. & Spiridonov, S.E. 1982. [Simple method of the impregnation of the hypoderm in nematodes]. *Zoologichesky Zhurnal* 61: 1419-1421.

Martini, E. 1903. Über Furchung und Gastrulation bei Cucullanus. *Zetschrift für wissenschaftliche Zoologie* 74: 501-557.

Martini, E. 1907. Über Konstanz histologisher Elementen bei erwachsenen Nematoden. *Sitzungsberichte der Naturforschungen Gesellschaft, Rostock* 61 (8): 1-14.

Martini, E. 1908. Über Subcuticula und Seitenfelder einiger Nematoden (mit Bemerkungen über determinierte Entwicklung. III. *Zeitschrift für wissenschaft-*

liche Zoologie 91: 191-235.

Martini, E. 1909. Über Subcuticula und Seitenfelder einiger Nematoden. IV. *Zeitschrift für wissenschaftliche Zoologie* 93: 535-622.

Martini, E. 1916. Die Anatomie der *Oxyuris curvila. Zeitschrifr für wissenschaftliche Zoologie* 116: 339-534.

Pai, S. 1928. Die Phasen des Lebenscyclus der *Anguillula aceti* Ehrbg. *Zeitschrift für wissenschaftliche Zoologie* 131: 293-344.

Rusin, L.Yu. & Malakhov, V.V. 1998. [Free living marine nematodes have no eutely]. *Doklady Rossiiskoy Akademii Nauk* (in press).

Strassen, O., Zur. 1896. Embryonalentwicklung der *Ascaris megalocephala. Archiv für Entwicklungsmechanik* 3: 27-105.

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

Voronov, D.A., Makarenkova, E.P., Nezlin, L.P., Spiridonov, S.E. & Panchun, Yu.V. 1986. [The study of the embryonic development of free living marine nematode *Enoplus brevis* using the method of the marking of blastomeres]. *Doklady Akademii Nauk SSSR* 286: 210-204.

Voronov, D.A. & Panchin, Yu.V. 1995a. [The early stages of the cleavage in free-living marine nematode *Enoplus brevis* (Enoplida, Enoplidae) in the normal and experimental conditions]. *Zoologichesky Zhurnal* 74 (6): 31-38.

Voronov, D.A. & Panchin, Yu.V. 1995b. [Gastrulation in free living marine nematode *Enoplus brevis* (Enoplida, Enoplidae) and the localization of enthodermal material at the stage of two blastomeres in the nematodes of the order Enoplida]. *Zoologichesky Zhurnal* 74 (7): 10-18.

Wood, W.B. 1988. *The Nematode Caenorhabditis elegans.* New York. Cold Spring Harbor. 450 pp.

Малахов В. В. Эмбриологические и гистологические особенности отряда Enoplida как примитивной группы нематод.

Резюме. В последние годы у представителей отряда Enoplida выявлен ряд необычных черт. Тип эмбриогенеза эноплид резко отличается от такового всех других нематод. Дробление эноплид характеризуется вариабельностью в расположении бластомеров. В дроблении не было выявлено жесткой детерминации. В отличие от других нематод, билатеральная симметрия в эмбриогенезе эноплид появляется поздно, обычно на стадиях гаструляции. Эноплиды не имеют эвтелии, которая считается характерной для нематод. Эноплиды способны регенерировать экспериментально разрушенные внутренние органы при условии сохранения целостности кутикулы. Предполагается, что эти черты свидетельствуют о том, что эноплиды - наиболее примитивная группа нематод.