ACADEMICIA: An International Multidisciplinary Research Journal
ISSN: 2249-7137 Vol. 11, Issue 12, December 2021 SJIF 2021 = 7.492
A peer reviewed journal

# EMBRYOGENESIS OF GALLS COTTON NEMATODE MELOIDOGYNE INCOGNITA ACRITA (TYLENCHIDA, HETERODERIDAE)

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**DOI: 10.5958/2249-7137.2021.02691.4**

## ABSTRACT

*The structure of M.incognita acrita egg does not reveal its polarity. During the fusion of pronuclei, the egg cytoplasm forms a pseudomembrane and small protrusions at the egg surface. The cleavage is almost equal and asynchronous. The first four blastomeres are distributed along the longer egg axis what appears to be characteristic of all Tylenchida. Gastrulation proceeds by means of epiboly. Endo- and mesoderm originate from the common rudiment, blastomere EM at the stage of 4 blastomeres, and are represented by independent blastomeres E and M at the stage of 7 blastomeres. Oesophagus and neural rudiment arise from the ectoderm of the anterior embryonic end. The genital rudiment P4 is separated at the stage of 11 blastomeres.*

**KEYWORDS:** *Polarity, Pronuclei, Pseudomembrane, Cleavage, Blastomeres, Mesoderm*

## I. INTRODUCTION

The embryonic development of root gall nematodes has been little studied. The available descriptions of the crushing of eggs of representatives of the order Tylenchida are fragmentary and superficial. The initial stages of cleavage of Heterodera radicicol [1], Heterodera marioni [2], Ditylenchus dipsaci [2, 3], and several species of Aphelenchoides [4, 5, 6] were studied in vivo. The embryogenesis of Meloidogyne javanica has been studied in more detail [3].

## II. MATERIALS AND METHODS

The material for this study was the eggs of the nematode of the dangerous cotton pest Meloidogyne incognita acrita (Chitwood, 1949). Infected roots of fine-staple cotton varieties C 60-30 were taken from the farm of the Andijan region. To preserve a pure culture of this species, cotton (varieties C 60-30) was grown in pots with sterile soil at 20-24 °C. When the plants

reached a height of 10-15 cm, nematode egg sacs were introduced into the near-root soil. Thus, we were able to use live nematodes all year round.

Embryonic development was studied in vivo, in sections and on total preparations. Intravital observations were carried out under an MBR-3 microscope with a magnification of 10 x 20 and 10 x 40 in a humid chamber.

We used mixtures of "Susa" and Carnoy as fixing agents. The dyes used were Mayer, Karacchi, Ehrlich, Heidenhain hematoxylins and Schiff's reagent.

To obtain sections from sexually mature females and egg sacs, they were fixed in Susa liquid, passed through terpineol, and embedded in paraffin. Sections were made with a thickness of 5-7 μm. Drawings were made using a drawing apparatus, RA-4 with a 10x90 microscope magnification.

## III. RESULTS

### 3.1. Biological features

Meloidogyne incognita acrita is a dioecious, oviparous phytonematode. It infects cotton plants, tomatoes, cucumbers and other crops. The invasive larva penetrates into the plant root; in the places of accumulation, the female of these nematodes forms a thickening of the root-gall. The female here has a vulva facing outward. The female secretes a substance similar in chemical composition to the cuticle, which hardens and forms an egg sac (ootheca). The female lays eggs in this bag. Sexual dimorphism is pronounced. Males are small and worm-shaped, while females are pear-shaped. The female reproductive system consists of two parallel genital tubes, each of which is an ovary, oviduct, seminal receptacle and uterus [6]. Both tubes near the vagina are connected to each other and open the vulva outward. On histological sections of the ovary, a cytoplasmic strand is clearly visible - rachis. Oogonia at the end of the germinal zone are located around the rachis in several rows, and in the growth zone in one row. Oocytes in the growth zone increase in size, nucleoli are clearly visible in their nuclei. When oocytes pass singly through the narrow lumen of the seminal receptacle, egg membranes are formed.

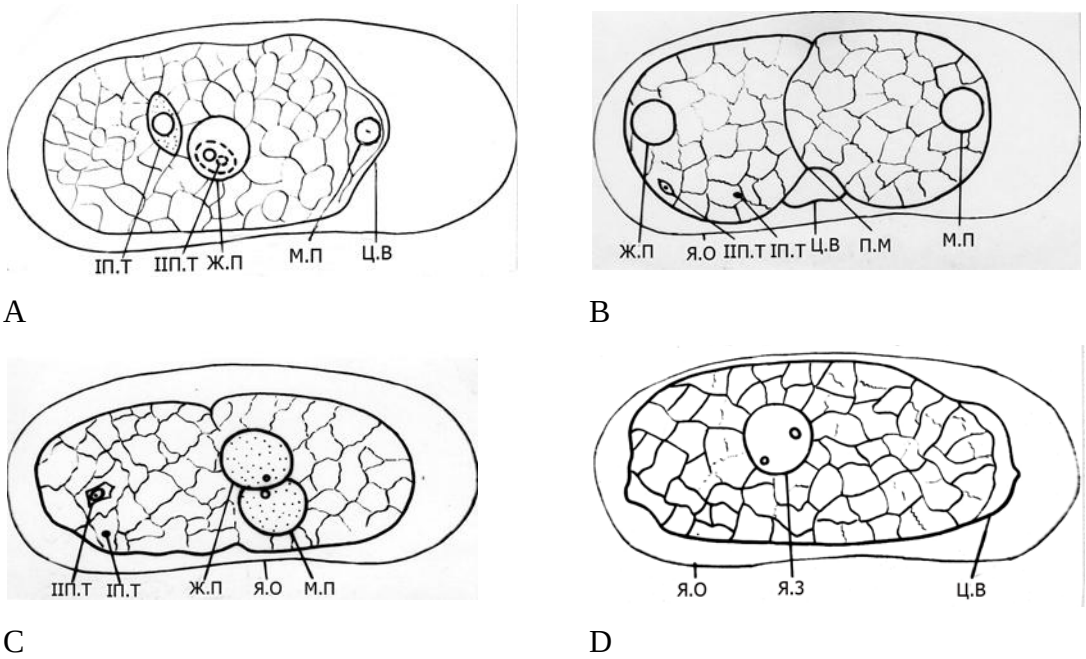
### 3.2. Features of the structure of the egg

The egg contains a lot of yolk, which is evenly distributed, in the middle of the egg is the core. The cytoplasm forms numerous bridges that make the egg structure cellular. The polarity of the egg is not pronounced. After penetration of the sperm, two polar bodies can be found in the egg, each of which contains a small amount of cytoplasm. The average egg size is 94 x 37 microns. The eggs are covered with two shells. The inner membrane is formed immediately after the sperm has entered the egg and is very thin, and the outer, thicker membrane is formed later in the genital tract.

### 3.3. Fertilization

Fertilization in M. incognita acrita occurs in the seminal receptacle. On total preparations, it was possible to study the stages of this process. At the point of contact with the sperm, the cytoplasm forms a protrusion into which the sperm is quickly immersed. At this point, a fertilization shell is formed on the surface of the egg. At this time, the nucleus of the egg is in the centre, at the same level are two polar bodies (Fig. 1, A). The cytoplasm is activated, on the surface of which protuberances (prominences) are formed. The nucleus of the ovum moves back, at the same time

a pseudomembrane forms in the middle of the cytoplasm (Fig. 1, B). In both pronuclei, the nucleoli are very clearly visible. Then the nuclei move towards each other. The pseudomembrane disappears. Pronuclei occur in the middle of the egg (Fig. 1, C) and merge to form the nucleus of the zygote (Fig. 1, D).




A B

C D

Fig 1. Sequential stages of egg fertilization

A-penetration of the sperm; B-formation of a pseudomembrane; C - meeting of pronuclei; D-zygote. I п.т, II п.т, respectively, the first and second polar bodies; я.я. - the nucleus of the egg; м.п., ж.п - male and female pronuclei, respectively; ц.в - cytoplasmic protrusions; п.м - pseudomembranes; я.з - the nucleus of the zygote; я.о - eggshell.

**3.4. Splitting up.**

Egg crushing is complete, not quite uniform and asynchronous. The spindle of the first division is located along the long axis of the egg. 12-15 hours after fertilization, the egg divides into two blastomeres of almost the same size.

We will designate one of them as AC, the other as P1 (Fig. 2, A).

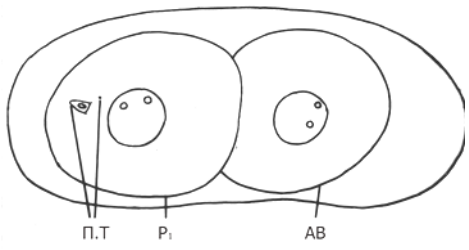
The blastomere AC 12-13 hours after the first crushing is divided transversely. Anterior blastomere A, posterior blastomere C appear. Each of them is almost two times smaller than P1. Blastomere P1 (Fig. 2, B) is also divided transversely. The division of this blastomere occurs 10-11 h after the division of the AC. Formed anterior cell EM and posterior P2, the latter differs from other blastomeres in smaller sizes. Thus, the first two divisions occur transversely, the emerging four blastomeres are arranged in one row linearly (Fig. 2, C). Then, after 5-6 hours, the blastomeres are rearranged. Blastomere B moves to the dorsal side, while blastomere A moves slightly back and comes into contact with the EM blastomere. Thus, blastomeres A, EM, and P2 are located in one row, and blastomere B takes place above A and EM; the resulting grouping of

blastomeres is somewhat rhombic. E.M. Drozdovsky (1978) calls this method of forming a rhombic figure linear (Fig. 2, D).

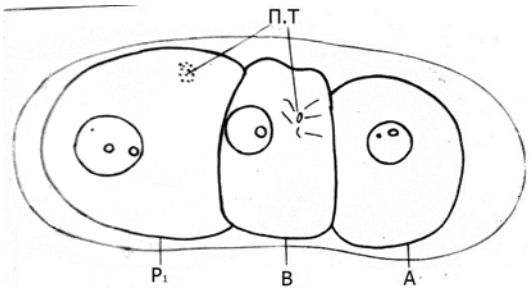
The next division occurs 3-4 hours after the formation of the rhombus. Blastomere A divides at an angle of approximately 25-30 ° with respect to the long axis of the egg into the right cell and the left α. Blastomere α is smaller than a (Fig. 2, E), then EM and B blastomeres divide almost simultaneously. From the first cell, the anterior M and posterior E blastomeres are formed. Blastomere B gives left anterior β and right b (Fig. 2, E). The P2 blastomere is divided into the dorsal –C and the ventral –P3 cells (Fig. 2, G). At the same time, granules appear in the cytoplasm of the P3 blastomere, around the nucleus, which is strongly stained with hematoxylin. Then the β blastomere is divided into anterior βI and posterior βII cells (Fig. 2, H). Blastomere P3 is divided into dorsal D and ventral P4, βII is divided into right βII.1 and left βII.2 cells (Fig. 2, I). Thus, the β blastomere is far ahead of other ectodermal blastomeres in divisions. After that, blastomere E is divided into anterior EI and posterior EII, b is divided into anterior bI and posterior bII (Fig. 2, K).

Then there is a division of blastomeres α and M. Blastomere α gives right α II and left posterior αI cells, blastomere a gives right aII and left aI, moreover, aI shifts slightly forward. In cell M, the division spindle is located approximately at an angle of 450 to the longitudinal axis, and a right-anterior m and a left-posterior blastomere μ are formed (Fig. 2, L).

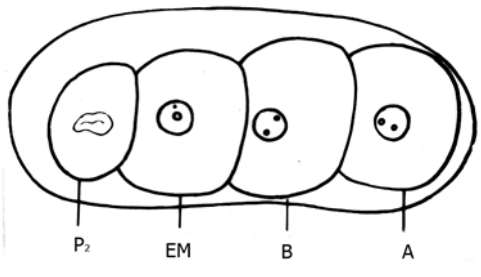
Further, the blastomere bI is divided into right bI.I, left bI.2, bII gives the posterior bII.1 anterior cell bII.2, from D a posterior right blastomere d is formed, left-posterior σ, C gives a right-posterior ɣ and left-anterior cell c. The embryo at this stage consists of 20 cells and is a sterroblastula (Fig. 2, M). It takes 6-7 days from the beginning of crushing to this stage.



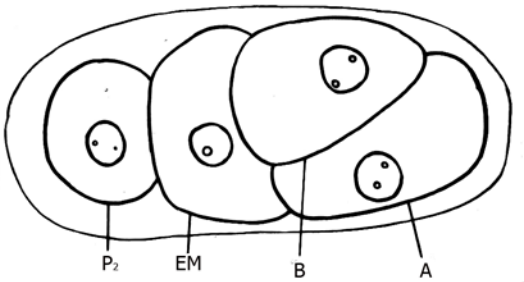

A




B




C




D

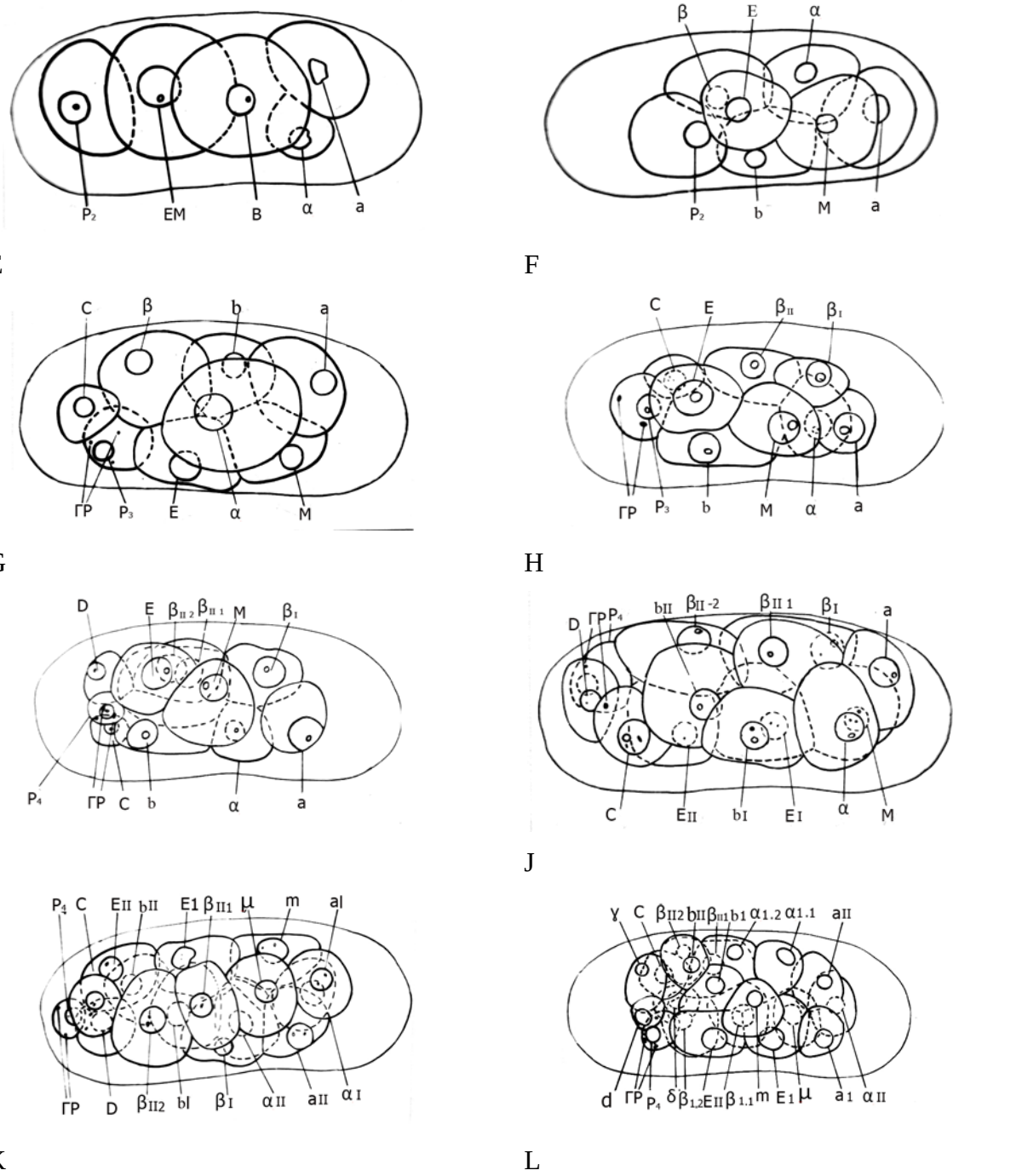
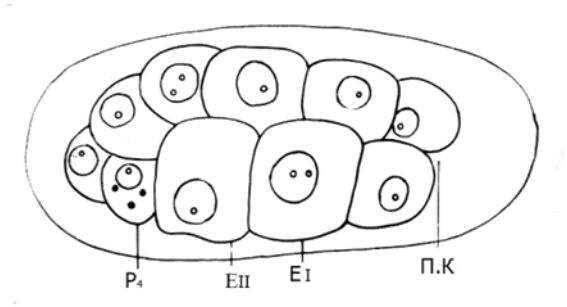


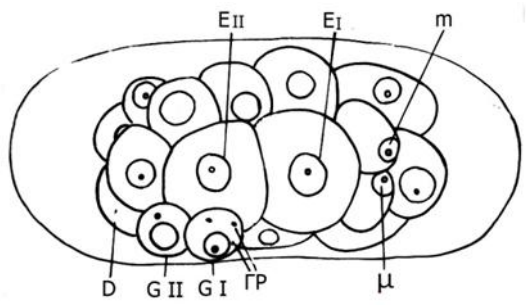

Fig. 2. Stages of egg crushing (for total preparations): A-two; B-three; C, G - four; D-five (left); E-seven (from the ventral side); F-eight (right); 3-nine (left); I-eleven (right); K-thirteen (right); L-sixteen; M-twenty (left) blastomeres (sterroblastula); г.р - granules, п.т - polar bodies. The rest of the notation is in the text.

### 3.5. Gastrulation and organogenesis

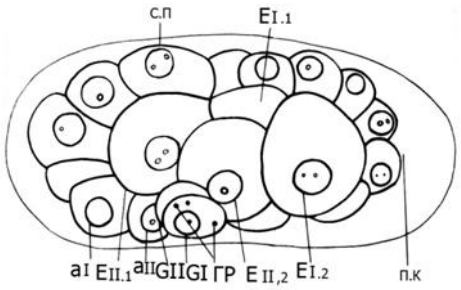
Gastrulation begins from the moment when the number of cells that make up the embryo reaches 20-22. At this stage, the anterior end and lateral sides of the embryo consist of the descendants of blastomeres A and B. At the posterior end are the descendants of blastomeres C and D. P4 takes place on the ventral side at the posterior end of the embryo, and the rudiments of the endoderm E I and E II are located almost in the centre of the ventral side (Fig. 3, A). In front of the endodermal cells, there are 2 cells (m and μ) of the future mesoderm. On large endodermal cells, ectodermal cells move from the posterior end and from the sides of the embryo, following the endodermal blastomeres, mesodermal cells also go inward (Fig. 3, B). At the stage when the embryo consists of 42-45 cells, 4 endodermal cells are already visible under the ectodermal layer, and in front of them, there are 2 mesodermal cells. By this time, the genital anlage - GI and GII are located at the posterior edge of the blastopore (Figure 3, C). Soon, due to the growth of ectodermal cells in front, behind and on the sides, the blastopores are concentrically closed. Thus, gastrulation in M. incognita acrita occurs with epibolism [8,9].




A




B




C

Fig 3. Gastrulation (sagittal optical sections):

A - the beginning of gastrulation;

B — further stages of cell immersion into the blastocoel:

B-immersion of the reproductive primordium (40-45 cells):

п.к - front end; с.п - dorsal side;

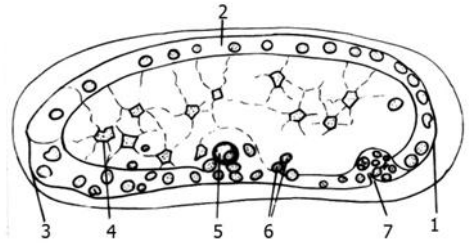
эн -endoderm.

### 3.6. Other designations in the text

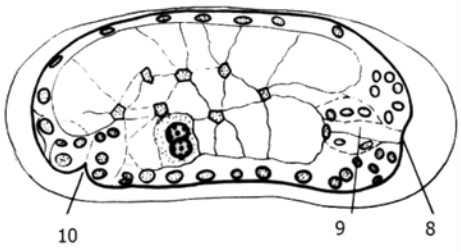
Organogenesis begins. When the embryo consists of about 200-220 cells, ectoderm cells divide intensively on the ventral side of the anterior end. Gradually, the newly formed cells are immersed inward. They are at first arranged randomly (Fig. 4, A), but then they are arranged in the form of a cylinder, and depression is noticeable at the place of immersion of the cells. This is how the anterior intestine is formed. At the end of organogenesis, it differentiates into the corresponding departments. At this time, endodermal cells occupy the central part of the embryo. They differ from other cells in their large size. Several free mesoderm cells are observed in front and behind them.

The ectoderm consists of a single layer of cells. At the posterior end, ectodermal cells are somewhat larger. At the next stages of development, the cells of the ectoderm of the abdominal side divide intensively and, as it were, pushes the oral cavity forward. On the ventral side, from the ectoderm (from the descendants of C and D), the hindgut is formed (Fig. 4, B).

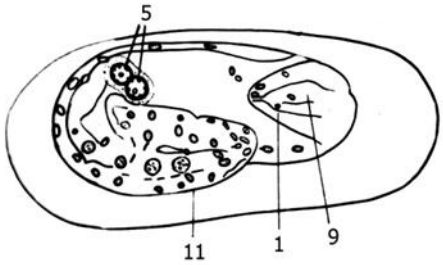
The growth of the embryo continues. The back and front ends are folded. Inside the embryo, at the bend site, there are 2 large cells of the reproductive rudiment. Meanwhile, the number of mesodermal cells increases, they form two stripes, which are very closely adjacent to the ectoderm. This stage in other nematodes is called the tadpole stage, but in this species the anterior end of the larva is thinner than the caudal end (Figure 4, C). In the following stages, the embryo lengthens and turns into a larva. On total preparations from larvae of the first instar, in the anterior part, under the ectoderm, a layer of dark-colored neuroblast cells is distinguished. In the area of the esophagus, their clusters form a nerve ring. Dorsal and ventral nerve trunks are well distinguished. Before hatching, the larva twists inside the shell in two turns. The first molt of the larva takes place in the egg. The second instar larva hatches from the egg, which is invasive. The duration of embryonic development at a temperature of 25-260 is 30-31 days.




A




B




C

Fig. 4 Organogenesis (sagittal optical sections):

A - the beginning of the laying of the stomodeum;

B — further stages of stomodeum formation;

B-stage "tadpoles";

1 — anterior end of the embryo; 2 – ectoderm;

3 – posterior end of the embryo; 4 – endoderm; 5 - sexual

primordium; 6 – mesoderm; 7 –stomodeum; 8-mouth cavity;

9 – foregut; 10 - the beginning of the posterior colon laying; 11-tail of the larva.

## IV. DISCUSSION

### Differences in early cleavage of nematode eggs

The arrangement of blastomeres at the initial stages of cleavage, in species such as Ascaridia galli [19], Sphaerularia bombi [20], Neoaplectana carpocapsae [10], is subject to significant individual variations, which suggests that cleavage is not yet deterministic in them. In some cases, the first four blastomeres are in the form of a tetrahedron; sometimes their arrangement then changes to rhombic, which is characteristic of the overwhelming majority of nematodes. This arrangement is achieved mainly in four ways: T-shaped, intermediate, parallel and linear [2,4]. We consider it possible to somewhat expand this scheme by including 2 more options: the formation of a rhombus through the tetrahedron stage and crushing without the rhombus stage. Thus, the following types of early crushing can be distinguished:

I. In marine nematodes from the order Enoplida, after the second division, a tetrahedral arrangement of blastomeres was found. The egg divides along the equator, and then both blastomeres divide meridionally in two mutually perpendicular planes. Immediately after the second division, the blastomeres are grouped in the form of a tetrahedron (Pontonema vulgare) [10-15]. The tetrahedral arrangement of blastomeres was later found in some other nematodes Anoplastoma vivipara, Enoplus bravis, Enoplus demani, Hupodontolaimus inaequalis [13,14,15,17]. V.V. Malakhov [9] classifies nematode crushing as spiral single-beam crushing and calls it malt crushing.

II. In Neoaplectana carpocapsae (up to 10% of cases), after the 2nd division, the blastomeres are also arranged in the form of a tetrahedron. Then, as a result of the rearrangement of the blastomeres, a rhombic figure appears. A similar transformation of a tetrahedral figure into a rhombic figure is described by Malakhov et al. In a part of the embryos of Eustrongyloides excisus. This type of cleavage can be called a rhombic tetrahedron.

III. The ascaris type is characterized by the formation of a T-shape at the stage of four blastomeres, followed by rearrangement of the blastomeres into a rhombus.

IV. In Neoaplectana carpocapsae 60-65% of cases, an intermediate type of cleavage is observed, in which a T-shaped figure arises at the stage of three blastomeres. But unlike roundworm, before division of the posterior blastomere (P1), cell B moves back. Under the pressure of blastomere B, the division spindle in the P1 cell lies obliquely. After the division of this

blastomere, a rhombic figure is immediately formed [10] This type of cleavage was also observed in Panagrellus silusioides [1].

V. Parallel type of cleavage, in which, during the transition from the two blastomeric stage to the four-blastomeric stage, the spindle of division in both blastomeres is parallel to one another and obliquely relative to the long axis of the egg; the resulting four blastomeres are immediately arranged in a diamond shape. Such cleavage was noted in Rhabitis elegans [1].

According to our observations, early cleavage of the saprophytic nematode Pelodera Strongyloides belongs to the parallel type [4]. Vi. The linear type of cleavage is characterized by the fact that the first two divisions occur in the transverse direction and the resulting four blastomeres are located in one row. The linear arrangement of blastomeres was observed in several species of Aphelenchoides [5, 6] and in Meloidogyne javanica [3]. We observed a linear arrangement of blastomeres (A, B, EM, P2) at the four-cell stage in Meloidogyne incognita acrita. Then the second blastomere (B) is displaced and located above the blastomeres A and EM. Consequently, this species does not form a typical rhombic figure.

Thus, the following types of four cell stages are observed in nematodes: tetrahedral, tetrahedral-rhombic, ascaris, intermediate, parallel and linear. However, the listed types do not exhaust the whole variety of nematode egg cleavage.

## V. CONCLUSION

1. The eggs of Meloidogyne incognita acrita are formed in the paired ovary. The ovary has a cytoplasmic core - rachis, which performs the function of feeding the oocytes.
2. Fertilization takes place in the seminal receptacle.
3. Egg crushing proceeds in a linear manner. Blastomere division occurs asynchronously. The first four blastomeres are linear from the very beginning.
4. (4) At the stage of 20 cells, the embryo is a sterroblastula with a not expressed blastocoel.
5. Gastrulation occurs by immersion inside a dense complex of cells and is combined with epiboly. The ectoderm arises from the blastomere AB, C, and D.
6. The endoderm is formed from the E blastomere and gives rise only to the midgut. The mesoderm comes from the primordium common with the endoderm - the EM blastomere. It goes inward at the end of gastrulation and forms two mesodermal stripes.
7. The stomodeum is formed by invagination from the ectoderm at the anterior end of the embryo, and not at the site of the blastopore. 7. The genital anlage (P4) is isolated at the stage of 11 blastomeres.

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