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The embryonic development of *Pontonema vulgare* (Enoplida: Oncholaimidae) with a discussion of nematode phylogeny

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Summary. The cell lineage in embryonic development of the marine nematode *Pontonema vulgare* (Enoplida, Oncholaimidae) was studied using cell labelling by fluorescent dye. After labelling at the two-cell stage, the border between labelled and unlabelled cells is different in different Lima bean and comma stage embryos crossing them in left-right, anterior-posterior, or intermediate direction. Therefore, in contrast to the majority of nematodes, *P. vulgare* has a variable type of development. However, as in other nematodes all the endoderm is derived from a single blastomere of the eight-cell stage, and the gastrulation process in *P. vulgare* is comparable to that in other nematodes. In general, the development of *P. vulgare* is similar to that in the previously studied *Enoplus brevis* (Enoplida, Enoplidae). Using the type of development, all nematodes can be placed in one of three major groups: 1, with variable cleavage (order Enoplida); 2, with invariant cleavage and anterior localization of endoderm material at the two-cell stage (remaining Enoplea); 3, with invariant cleavage and posterior localization of endoderm material at the two-cell stage (Chromadorea and Secernentea). This classification is independently supported by molecular phylogeny studies and by several anatomical characters.

Key words: nematodes, embryonic development, cell lineage, cleavage, gastrulation, phylogeny.

Study of nematode embryonic development is a well-developed field of science with fundamental results having been obtained with *Caenorhabditis elegans*. However, until now its significance in nematode phylogeny and taxonomy has been little recognized. This situation has arisen as a result of unequal study of development in different nematode taxa. However, some reports suggest that the cell lineage and other fundamental characteristics of nematode development may have utility for solving problems of nematode phylogeny (Drozdovsky, 1975, 1977, 1978; Skiba & Schierenberg, 1992; Goldstein *et al.*, 1998; Voronov *et al.*, 1998; Wiegner & Schierenberg, 1998). Thus, Drozdovsky (1969, 1975, 1977, 1978) demonstrated that the cell lineage of representatives of Enoplea is markedly different from that in Secernentea and in Chromadorea, and proposed an embryological criterion for distinguishing between these two branches of nematodes.

The cleavage pattern in the order Enoplida, in contrast to all previously studied nematodes, is highly variable (Cherdantsev *et al.*, 1972; Malakhov, 1994). This, together with untransparency of embryos due to high concentration of yolk granules, prevents study of the enoplidan cell lineage by direct observation. However, application of the technique of intracellular labelling by fluorescent dye can overcome these problems. Cell labelling enables observation to be made of the progeny of an arbitrarily chosen blastomere in a succession of developmental stages. Moreover, the bright luminescence of an appropriate label provides information of the redistribution of cells within the embryo, *e.g.* in studies of the gastrulation process.

The cell tracing technique revealed the absence of fixed cell lineage in the enoplidan species, *Enoplus brevis* (Enoplidae) (Voronov *et al.*, 1986, Voronov & Panchin, 1995b, 1998). However, *E. brevis* is the only enoplidan species for which cell tracing experiments have been made. In the present study the cell tracing technique was used to investigate the embryonic development of the enoplidan *Pontonema vulgare* (Oncholaimidae). The results obtained and their significance for nematode phylogeny are discussed.

MATERIALS AND METHODS

Specimens of the free-living marine littoral nematode *Pontonema vulgare* (Bastian, 1865) (Enoplida, Oncholaimidae) were collected from Kandalaksha Bay of the White Sea in the vicinity of the Kartesh Marine Biological Station of the Russian Academy of Sciences, and the White Sea Biological Station of Moscow State University. Uncleaved eggs were dissected from gravid females in filtered seawater. To observe both sides of the embryo, the eggs were placed on cover slips to which they stuck by their outer mucus sheath. During development eggs were kept at 15-16 °C.

The fluorescent dye 5(6)-carboxyfluorescein (CF) (Sigma, Mw 376.3) was iontophoretically injected at the two- or eight-cell stage to visualize the progeny of labelled cells in the developing embryo. Microelectrodes used for injections were made of glass with inner filaments (Clark Electromedical Instruments, GC100F-15) and backfilled with injection solution. Single blastomeres were injected using a several seconds pulse of 1-5 nA negative current through 30-50 MegΩ glass microelectrodes filled with 3% solution of potassium carboxyfluorescein in distilled water. Injections were performed using a fluorescent microscope (ML-2, LOMO, Russia) with a filter set optimized for fluorescein. The development of the labelled embryos was traced from the beginning to the comma stage, when the pattern of label distribution gradually became unclear due to the loss of CF by differentiating cells.

Drawings were made with a camera lucida. Illustrations of labelled embryos were taken on Kodak Gold 400 film or a high sensitivity video-camera (DeltaTex, Russia).

RESULTS

Only a brief general description of *P. vulgare* development is presented as it has been reported previously (Cherdantsev *et al.*, 1972; Malakhov & Cherdantsev, 1975; Malakhov, 1994).

The zygote diameter in *P. vulgare* eggs is about 130 μm. Egg-shell shape is ellipsoidal, often visibly asymmetrical (Fig. 1), and the egg-shell is large providing a large free space for the embryo. Before cleavage, two polar bodies are formed. Their position, relative to the longitudinal egg-shell axis, varied in different eggs from polar to equatorial.

The interphase zygote has prominent cytoplasmic movement (Fig. 1A), which cease prior to mitosis (Fig. 1B). Similar movements are characteristic for interphase blastomeres, and these can interfere with direct observation of cell lineage.

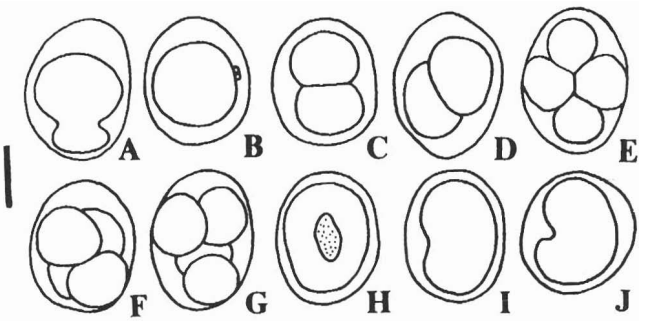

Fig. 1. Some stages of *P. vulgare* development. Camera lucida drawings of living embryos. A: Interphase cytoplasmic movements of zygote; similar movements are also peculiar for interphase blastomeres; B: The rounded zygote before the first division of cleavage; two polar bodies are seen near the equator of the egg-shell; C: Two-cell stage, the usual arrangement of blastomeres along the longitudinal egg-shell axis; D: The oblique disposition of blastomeres at the two-cell stage; E, F, and G: Examples of blastomere distribution at the four-cell stage (E, rhombus; F, tetrahedron; G, T-shaped figure); H: The beginning of ventral cleft formation in ventral view with the ventral cleft seen as an elliptical depression on the ventral side of the embryo (stipled); I: Lima bean stage in left lateral view with the anterior at the top; J: Comma stage in left lateral view with the anterior at the top. Scale bar - 100 μm.

The early cleavage divisions occur approximately every six hours. They are usually (up to the 16-cell stage) synchronous, but sometimes delays of divisions of some blastomeres were observed. These delays can last up to 2-3 hours, but do not perturb further development.

At the 2 to 16-cell stage blastomeres are indistinguishable. Early blastomeres are approximately equal, but sometimes slight irregular differences in their size are visible. The spatial-temporal pattern of early cleavage varies between different embryos, *e.g.* blastomeres at the two-cell stage are usually located along the longitudinal egg-shell axis, but sometimes they occur at a random angle to the axis (Fig. 1C, D). Blastomeres at the four-cell stage can be arranged as a tetrahedron, rhombus, quadrate, or T-shaped configuration (Fig. 1E, F, G). These configurations can transform from one to another due to interphase motility of the blastomeres.

During the course of later development it is possible to distinguish the stages similar to that in other nematodes (Fig. 1H, I, J), *i.e.*, ventral cleft formation (beginning at about 90 hours at 15-16 °C), Lima bean stage (140 hours), comma stage (170 hours). The first stage juvenile hatches after approximately 30 days of development. The dorsal-ventral

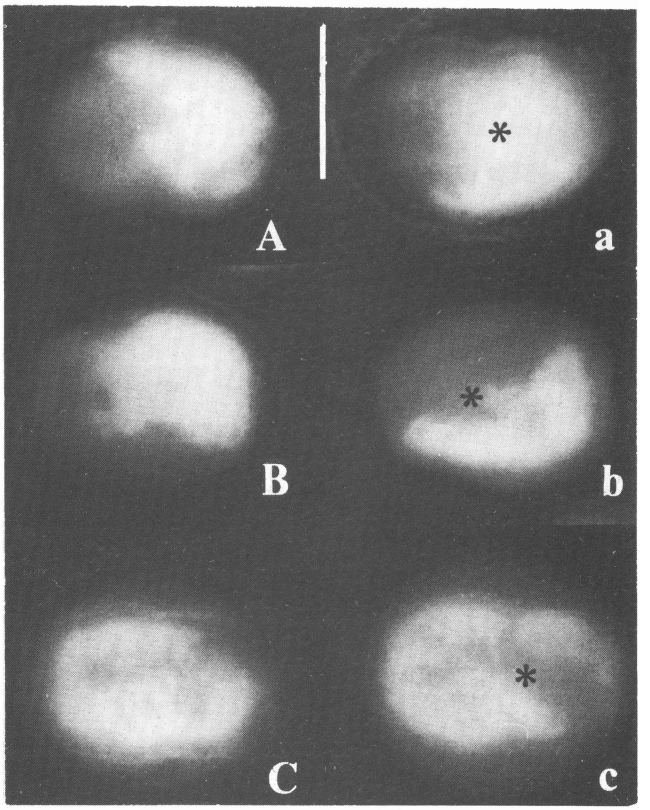

Fig. 2. The distribution of descendants of the first two blastomeres in the comma stage embryos of *P. vulgare*. These embryos were labelled by carboxyfluorescein (CF) at the two-cell stage in an arbitrarily chosen blastomere. Anterior at the left in ventral view (capital letters) and dorsal view (lower case letters). Initial position of injected blastomere at the two-cell stage was at the right in A, a, B, b and at the left in C, c: A, a, the transverse border between descendants of two-cell stage blastomeres; B, b, the oblique border between descendants of two-cell stage blastomeres; C, c, the label is approximately evenly distributed along the longitudinal embryo axis and therefore the first two blastomeres can not be designated as being "anterior" or "posterior". In this embryo the main part of the surface cells is labelled. In the embryo A, a the mid-gut was stained and their fluorescence is clearly seen through unstained epithelial cells at the dorsal side of the embryo (a, asterisk). In B, b and C, c the mid-gut was not stained and it was seen as a dark mass under the dorsal surface (b, c, asterisk). Scale bar - 100 μm.

polarity of the embryo is evident after the beginning of gastrulation, the direction of the anterior-posterior axis is visible after the ventral cleft stretching, and the polarity of the anterior-posterior axis is discernible only at the Lima bean stage.

Distribution of descendants of the two-cell stage blastomeres. Forty three embryos were studied in which one blastomere was labelled at the two-cell stage and the distribution of its progeny was followed up to the comma stage.

The distribution of progeny of the first two blastomeres was unique for each studied embryo. At the Lima bean and comma stages the border between the descendants of the first two blastomeres varied from transverse to longitudinal (Fig. 2). Among 43 studied embryos the transverse or oblique border was observed in 26, and predominantly the longitudinal border in the remaining 17 (for this classification the distribution of label on the surface and also within the embryo was used). Therefore, in different eggs of *P. vulgare* the first two blastomeres give rise to different parts of the embryo, ranging from anterior-posterior to left-right distribution with a variety of intermediate patterns.

Only one of the first two blastomeres forms the mid-gut (see below). After injection of CF at the two-cell stage the mid-gut could be easily recognized in the Lima bean and comma stage embryos as a bright (labelled) or dark (unlabelled) axial mass beneath the dorsal surface in the posterior half of the embryo (Figs. 2a-c & 3F). In the 26 embryos with anterior-posterior distribution of descendants of the first two blastomeres the mid-gut precursor was derived in 16 embryos from the anterior blastomere and in 10 from the posterior blastomere (Table 1).

Distribution of descendants of the eight-cell stage blastomeres. The labelling at the eight-cell stage was

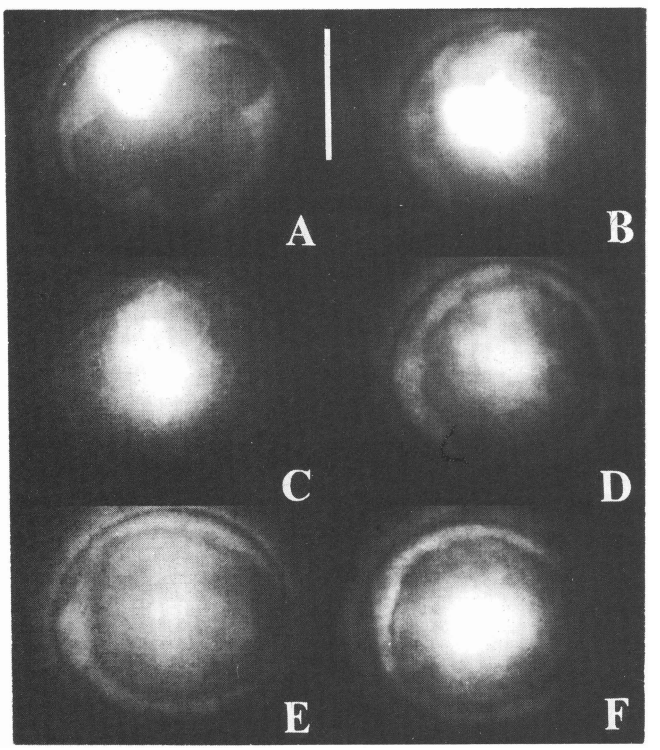

Fig. 3. An example of CF labelling of a *P. vulgare* embryo at the eight-cell stage. The label was injected in an arbitrarily chosen blastomere at the eight-cell stage. In this embryo the labelled blastomere was the endoderm precursor and that enabled the gastrulation process to be followed. A-E, ventral view with anterior at the left; F, the same stage as E but in dorsal view. In A, the eight-cell stage, the label fluorescence is seen at the one-celled endoderm precursor. In B, the beginning of gastrulation at the 30-cell stage, the two-celled endoderm precursor is still visible at the surface of the embryo. Note the embryo rotation between the eight- and the 30-cell stages. In C, the 60-cell stage, the endoderm precursor is almost completely submerged. In D, the endoderm precursor is completely submerged and is the onset of ventral cleft formation (90 hours after the beginning of development). In E, the comma stage, the luminescence of labelled mid-gut is just visible through the unlabelled ventral surface cells. In F, the same embryo as E but in dorsal view, the considerably brighter luminescence of labelled mid-gut can be seen through the unlabelled dorsal surface cells. Scale bar - 100 μm.

used to study the process of cell redistribution in 37 embryos up to the comma stage.

In 6 embryos the label was found only in the endoderm precursor, and in the remaining 31 was found exclusively in unendoderm blastomeres. Therefore, the pure endoderm precursor is formed in the embryo of *P. vulgare* at the eight-cell stage.

The embryos with the labelled endoderm precursor were used to observe the gastrulation process (Fig. 3). After the 16-cell stage the divisions of endoderm blastomeres are always delayed. At the 30-cell stage the embryo is organized as a compact morula with the two-celled endoderm precursor lying on the surface and indicating the future ventral side (Fig. 3B). After this stage gastrulation commences, and the endoderm precursor sinks into the embryo (Fig. 3C). Such behavior is not peculiar for the progeny of unendoderm blastomeres which never becomes completely immersed in the embryo up to the comma stage.

The endoderm primordium rapidly becomes covered by other cells (Fig. 3D), which subsequently also migrate into the embryo. At the position of their immersion the ventral cleft is formed as a depression, initially rounded, then elongated. As a result of this cell redistribution process the endoderm precursor moves inside the embryo in the dorsal direction and therefore its luminescence is more clearly visible on the dorsal side (Fig. 3E, F).

After labelling at the eight-cell stage, all 31 embryos with label in the unendoderm blastomeres were different from one another. In these embryos the gastrulation cell movement was clearly visible (Fig. 4). During gastrulation the unendoderm cells situated near the endoderm precursor sank into the embryo next to the endoderm. The cells situated at the dorsal side moved along the surface of the embryo in a ventral direction. The ventral cleft was formed in the region of the cell entrance, and no significant cell migrations were observed in other parts of the

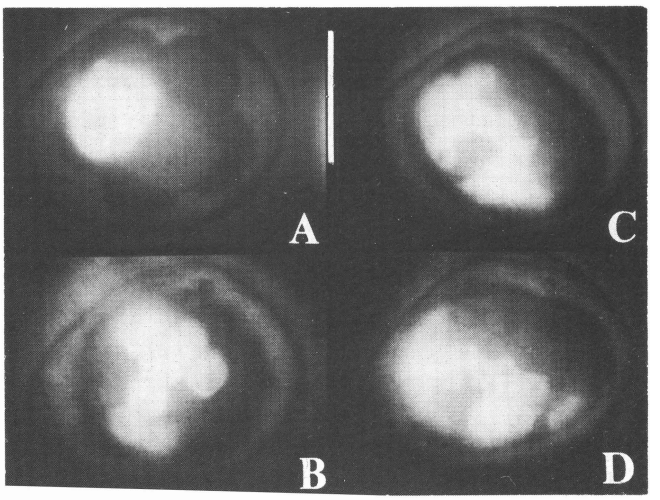

Fig. 4. An example of CF labelling of a *P. vulgare* embryo at the eight-cell stage. The label was injected in an arbitrarily chosen blastomere at the eight-cell stage, and its progeny gave rise presumably to the dorsal structures of the comma stage embryo. Pictures in dorsal view with the anterior to the left. In A, the eight-cell stage, the label fluorescence is visible only in one blastomere. In B-D, the label redistribution is shown in the 60-cell stage (B), at the beginning of ventral cleft formation (C), and at the comma stage (D). The labelled cells were progressively spread across the surface of the embryo to its ventral side where part of them submerged through the ventral cleft. Note the significant embryo rotation between stages presented at B and C. Scale bar - 100 μm.

embryo. The ventral cleft progressively closed before the Lima bean stage.

DISCUSSION

In embryos of different nematode species low molecular weight dyes such as fluorescein (Mw 332.3) and Lucifer yellow (Mw 445.4) can leak into uninjected blastomeres through gap junctions (Voronov *et al.*, 1986; Bossinger & Schierenberg, 1992a, 1996). However, in embryos of *P. vulgare* up to the comma stage the small molecules of CF do not visibly diffuse through gap junctions. In *C. elegans* several types of tracer molecules can be transferred into uninjected mid-gut from labelled cells (Bossinger & Schierenberg, 1992b), but this phenomenon did not occur in *P. vulgare* labelled by CF. Therefore, CF can be successfully used as a cell tracer. The embryos of *P. vulgare* and other Enoplida are characterized by the very high yolk concentration but the use of fluorescent labels allows study *in vivo* of the general features of cell movement inside the untransparent embryo (Voronov & Panchin, 1995b, 1998).

The results from our study suggest that in the embryo of *P. vulgare* at the two- or eight-cell stages blastomeres have no regular cell lineage pattern. After labelling of one blastomere at the two-cell stage the border between labelled and unlabelled cells varied between the different Lima bean and comma stage embryos from transversal to longitudinal, with a variety of intermediate patterns. Therefore, the first two blastomeres produce different parts in different embryos, and frequently can not be classified as being a "posterior" or "anterior" blastomere, unlike the situation that occurs for all nematodes with a constant cell lineage (Fig. 2, C, c; Table 1). In these cases where such a classification is possible the mid-gut is produced by the "anterior" or the "posterior" blastomere (Table 1). With the eight-cell stage the cleavage patterns of all blastomeres are not determined. The only exception being the endoderm precursor, which segregates from the other blastomeres at the eight-cell stage. All these features of *P. vulgare* development are similar to that in *E. brevis* (Voronov & Panchin, 1995a, b, 1998).

The process of gastrulation in *P. vulgare* is similar to that in *E. brevis* (Voronov & Panchin, 1995b, 1998) and in other nematodes, including Secernentea (Sulston *et al.*, 1983; Skiba & Schierenberg, 1992). In this discussion the designations for the main branches of Nematoda, classes or subclasses, are: Enoplea, Chromadorea, and Secernentea (= Rhabditea). In all nematodes: 1, the entire endoderm is derived from a single blastomere at the eight-cell stage; 2, gastrulation starts after the 24 to 30-cell stage when the two-celled endoderm precursor begins to sink into the embryo; 3, other blastomeres move to the position of the endoderm immersion, sink into the embryo, and these cellular movements form a pronounced depression (= ventral cleft) on the ventral side of the embryo (Sulston *et al.*, 1983). In *P. vulgare* the non-endoderm cells that submerge during the ventral cleft formation

Table 1. The general pattern of label distribution in comma stage embryos after labelling of one blastomere at the two-cell stage, and its relation to the labelling of mid-gut.

The mid-gut	The border between labelled and unlabelled parts of the embryo:		
	longitudinal	transverse or oblique	
		The head end labelled	The tail end labelled
Labelled	8	6	6
Unlabelled	9	4	10

apparently are the precursors of the pharynx and body muscles, as reported to occur with *C. elegans* (Sulston *et al.*, 1983) and *E. brevis* (Voronov & Panchin, 1995b, 1998).

The main characteristics of morphogenetic processes appear to be similar in Secernentea and Enoplida. However, they differ in that: secernentean species have a regular spatial-temporal pattern of early cleavage and an invariant cell lineage (Sulston *et al.*, 1983; Skiba & Schierenberg, 1992) whereas enoplidan species have a very labile early cleavage (Cherdantsev *et al.*, 1972; Malakhov & Cherdantsev, 1975; Voronov & Panchin, 1995a, 1998) and a variable cell lineage (Voronov *et al.*, 1986; Voronov & Panchin, 1995b, 1998).

Cell lineages in other Enoplea (excluding the order Enoplida) and Chromadorea have not been described in detail. However, several important characteristics of their cleavage have been reported. These nematodes have a reproducible invariant cleavage pattern, therefore, it appears that they have a constant cell lineage. In all the studied species of these nematodes the first two blastomeres correspond to the anterior and posterior halves of the embryo, respectively. The cell lineage of all the studied chromadorean species (orders Araeolaimida, Chromadorida, Desmodorida, and Monhysterida) appears to be similar to that in the Secernentea (Drozdovsky, 1977, 1978; Malakhov, 1981; Milyutina, 1981). In particular, in chromadorean embryos at the two cell stage the endoderm material is localized in the prospective posterior blastomere. The cell lineage in Enoplea species in the orders Dioctophymida, Dorylaimida, Mermithida, Mononchida, and Trichurida differs from that in the Secernentea, as at the two-cell stage the endoderm material is localized in the prospective anterior blastomere (Drozdovsky, 1969, 1975, 1981; Malakhov & Spiridonov, 1981, 1983; Malakhov *et al.*, 1984).

Using the terminology of Davidson (1990), the cleavage pattern in Nematoda can be classified as being variable or invariant. When the cleavage pattern is invariant the endoderm at the two-cell stage can be localized either in the anterior or posterior blastomere (Drozdovsky, 1975). Therefore, three main groups of nematodes can be distinguished: 1, Enoplida with a variable cleavage pattern and changeable localization of the endoderm material at the two-cell stage; 2, Enoplea, excluding Enoplida, with an invariant cleavage pattern, and at the two-cell stage the endoderm material is localized in the prospective anterior blastomere; 3, Chromadorea and Secernentea which have an invariant cleavage pattern, and at the two-cell stage the endoderm material is localized in the prospective posterior blastomere.

According to maximal parsimony analysis the variable (regulative) development is plesiomorphic, and this analysis is supported by recent molecular phylogenies (Valentine, 1997). Thus, it appears that the plesiomorphic condition of cleavage pattern is retained in the order Enoplida.

Nematode phylogeny and taxonomy have been discussed in several publications (Chitwood, 1933; Chitwood & Chitwood, 1933, 1950; Filipjev, 1934; Goodey, 1963; Maggenti, 1963, 1970; Gadea, 1973; Drozdovsky, 1975, 1981; Andrássy, 1976; Coomans, 1977; Lorenzen, 1981, 1994; Inglis, 1983; Adamson, 1987; Malakhov, 1994). Differences between the proposed variants of phylogeny and taxonomy are not exclusive as almost all recent authors discuss interrelations between three main evolutionary groups: 1, Enoplea; 2, Chromadorea; and 3, Secernentea, and there is a tendency to consider only two main phylogenetic groups: 1, Enoplea; and 2, Chromadorea and Secernentea. For example, Gadea (1973), Drozdovsky (1975, 1981), and Adamson (1987) supported the division of nematodes into these two groups; dendrograms in Maggenti (1963, 1970), Andrássy (1976), and Malakhov (1994) have these two main branches; and Inglis (1983, p. 246) wrote "... if it is felt that only two Classes or Subclasses should be recognized, then these should be (1) Enoplea; (2) Chromadorea+ Rhabditea; and *not* the historically older, and more usual: (1) Enoplea+Chromadorea; (2) Rhabditea."

Recently, comparison of 18S ribosomal RNA gene sequences was used to construct a phylogeny for Nematoda (Aleshin *et al.*, 1998a, b; Blaxter *et al.*, 1998; Kampfer *et al.*, 1998). The main branches

of the phylogenetic trees presented by Aleshin *et al.* (1998a, b) and Blaxter *et al.* (1998) concur with the three groups detected using the embryological criteria, and Enoplida and other Enoplea are more primitive then representatives of the chromadorean-secernentean clade (Aguinaldo *et al.*, 1997; Aleshin *et al.*, 1998a, b; Blaxter *et al.*, 1998).

Embryological and molecular data are in agreement when applied to the phylogeny of higher taxonomic groups of Nematoda (Voronov *et al.*, 1998). Embryological and molecular criteria also gave identical phylogenetic trees for the order Rhabditida (Blaxter *et al.*, 1998; Goldstein *et al.*, 1998; Wiegner & Schierenberg, 1998). Embryological criteria applied to the construction of nematode phylogeny appear to be pertinent as they do not reflect a simple feature, but rather are characteristic of a whole programme of development which is regulated by a hierarchy of numerous interrelated genes, as shown for *C. elegans* (Hengartner, 1997; Kemphues & Strome, 1997; Schnabel & Priess, 1997).

It is important to find morphological features corresponding to the proposed phylogeny. Morphological differences between Enoplea *sensu lato* and other nematodes have been extensively reviewed (Maggenti, 1963, 1970, 1981; Gadea, 1973; Andrássy, 1976; Drozdovsky, 1981; Lorenzen, 1981, 1994; Inglis, 1983; Adamson, 1987; Malakhov, 1994), therefore we consider exclusively the diagnostic morphological characteristics of the order Enoplida.

Specific mechanosensory organ metanemes have been described only for the Enoplida (Lorenzen, 1978, 1981, 1994) and are absent in non-enoplidan Enoplea (Dorylaimida, Mononchida and Mermithi-da), Chromadorea, and Secernentea (Lorenzen, 1994). Electron microscopy studies have revealed that the structure of metanemes (Hope & Gardiner, 1982) differs from the structure of mechanosensory sensillae in the secernentean *C. elegans* (White *et al.*, 1986).

Other potential diagnostic features of the Enoplida include the structure of the pharyngeal glands (Chitwood & Chitwood, 1950; Maggenti, 1963, 1970; Drozdovsky, 1981), somatic muscle cells (Hope, 1969; Malakhov, 1994), and spermatozoa (Yushin & Malakhov, 1998). For example, the presence of the nuclear envelope in the mature spermatozoa is the reliable characteristic of the order Enoplida.

Presence or absence of eutely (the constancy of body cell number) may be a useful characteristic for distinguishing between nematodes with variable and invariant cleavage patterns, *i.e.*, between the Enoplida and all other nematodes. Eutely is the result of strictly invariant development and, conversely, variable development can give rise to variable cell numbers. Secernentean nematodes have invariant cleavage patterns (Sulston *et al.*, 1983; Skiba& Schierenberg, 1992) and consequently also have eutelic first stage juveniles (Martini, 1907, 1916; Chitwood & Chitwood, 1950; Sulston *et al.*, 1983).Conversely, the enoplidans *P. vulgare* and *E. brevis* each have variable cleavage pattern (Voronov *et al.*, 1986, Voronov & Panchin, 1995b, 1998) and first stage juveniles have variable cell numbers (Voronov *et al.*, 1989; Voronov & Nezlin, 1994). However, until now this feature has not been studied for first stage juveniles of other Enoplea (outside the order Enoplida), or for Chromadorea.

Therefore, three independent approaches (embryological, molecular, and morphological) suggest that three main evolutionary branches can be recognized within Nematoda: 1, Enoplida; 2, the remaining Enoplea; and 3, Chromadorea and Secernentea, with the most primitive nematodes belonging to the Enoplida.

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Воронов Д.А. Эмбриональное развитие *Pontonema vulgare* (Enoplida: Oncholaimidae) с обсуждением филогении нематод.

Резюме. Генеалогию клеток в эмбриональном развитии морской нематоды *Pontonema vulgare* (Enoplida: Oncholaimidae) исследовали маркировкой клеток флуоресцентными красителями. После мечения на двухклеточной стадии граница между помеченными и немеченными клетками различалась у отдельных зародышей на стадии "боба" и "запятой", проходя в них слева направо, спереди назад и в промежуточных направлениях. Таким образом, в отличие от большинства нематод, *P. vulgare* характеризуется вариабельным развитием. Однако, как и у других нематод, вся эндодерма этой нематоды происходит из единственного бластомера на восьмиклеточной стадии и сам процесс гаструляции *P. vulgare* сравним с таковым у других нематод. В общем, развитие *Pontonema vulgare* сходно с развитием ранее изученной нематоды *Enoplus brevis* (Enoplidae, Enoplida). В соответствии с типом развития, все нематоды могут быть разделены на три главных группы: 1 - нематоды с вариабельным развитием (отряд Enoplida); 2 - с инвариантным дроблением и передней локализацией материала эндодермы на двуклеточной стадии (остальные Enoplea); 3 - с инвариантным развитием с задней локализацией материала эндодермы на двуклеточной стадии (Chromadorea и Secernentea). Такая классификация подтверждается исследованиями по молекулярной филогении нематод и некоторыми анатомическими особенностями.