

## Ultrastructure of sperm cells in the female gonoduct of free-living marine nematodes from genus *Enoplus* (Nematoda : Enoplida)

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**Summary** – The ultrastructure of mature spermatozoa in two marine enoplid nematodes *Enoplus demani* and *Enoplus anisospiculus* was studied with a transmission electron microscope. Both species have very similar bipolar sperm. The sperm cells show an amoeboid motility and are subdivided into anterior pseudopod and rigid cone-shaped posterior part filled with organelles. The cytoplasm of the pseudopod contains only a fibre network of cytoskeleton. In *E. anisospiculus* pseudopod numerous tube-like vesicles were found. The nucleus is built up of a strongly condensed mass of chromatin surrounded by the distinct nuclear envelope. Numerous mitochondria tend to concentrate at the base of the pseudopod before the nucleus. Membranous organelles of two types were found in the mature sperm of both species. Spherical organelles located more centrally are not connected with plasma membrane and contain an electron-dense material penetrated by internal microvilli. Electron-transparent membranous organelles are concentrated beneath the plasma membrane and open to the exterior via pores. These organelles have a more elaborate system of internal microvilli.

**Résumé** – *Ultrastructure des cellules spermatiques présentes dans le gonoducte femelle des nématodes libres marins du genre Enoplus (Nematoda : Enoplida)* – L'ultrastructure de spermatozoïdes matures de deux nématodes enoplides marins – *Enoplus demani* et *E. anisospiculus* – a été étudiée par microscopie électronique à transmission. Les deux espèces possèdent des spermatozoïdes bipolaires. Les cellules spermatiques sont douées d'une motilité amiboïde et sont divisées en un pseudopode antérieur et une partie postérieure conique, rigide, remplie d'organites. Le cytoplasme du pseudopode contient seulement le réseau fibreux du cytosquelette. Chez les pseudopodes de *E. anisospiculus* de nombreuses vésicules tubulaires ont été observées. Le noyau est constitué par une masse extrêmement concentrée de chromatine entourée par une enveloppe nucléaire distincte. De nombreuses mitochondries tendent à se concentrer à la base du pseudopode, près du noyau. Deux types d'organites membraneux ont été observés dans les spermatozoïdes matures des deux espèces. Les organites sphériques situés plus centralement ne sont pas connectés à la membrane plasmique et contiennent un matériel opaque aux électrons pénétré par des microvillosités internes. Les organites membraneux transparents aux électrons sont concentrés près de la membrane plasmique et s'ouvrent vers l'extérieur par des pores. Ces derniers organites montrent un système plus élaboré de microvillosités internes.

**Key-words** : *Enoplus*, ultrastructure, sperm, nematodes.

There are about fifty papers concerning nematode sperm cell ultrastructure, but the overwhelming majority of the species studied are animal or plant parasites. All reviews are based on these data (Bird, 1971; Anya, 1976; Bacetti *et al.*, 1983; Foor, 1983). Little information is available about structure of the sperm cells in relatively primitive free-living marine nematodes. Only two species have been studied up to date, both belonging to the order Enoplida – *Deontostoma californicum* (Wright *et al.*, 1973) and *Mesacanthion hirsutum* (Bacetti *et al.*, 1983). Their sperm cells have markedly different ultrastructural features. Moreover unusual sperm of *M. hirsutum* is believed to possess primitive characters according to Bacetti *et al.* (1983). Thus, the general features of enoplid sperm cells are not well defined. In the present paper we describe the ultrastructure of the sperm cells found in the female gonoduct of two free-

living marine species of the genus *Enoplus* belonging to the order Enoplida.

### Material and methods

Adult females of *Enoplus demani* Galtsova, 1976 were obtained from sand collected in the intertidal zone at White Sea Biological Station of Moscow State University (Kandalaksha bay, White Sea). Females of *E. anisospiculus* Nelson, Hopper & Webster, 1972 were collected from the druses of bivalve *Crenomytilus grayanus* at Vostok marine biological station of the Institute of Marine Biology, Vladivostok, Vostok Bay, Sea of Japan. Extracted female genitalia were fixed for transmission electron microscopy in 2.5 % glutaraldehyde in 0.05 M cacodylate buffer containing 13 mg/ml (for *E. demani*) and 21 mg/ml (for *E. anisospiculus*) NaCl and then post-fixed in 2 % osmium tetroxide in the same buffer con-

taining 14 mg/ml and 23 mg/ml NaCl accordingly. 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 Araldite. Ultrathin sections cut with a Reichert Ultracut E ultratome were stained with lead citrate and observed with a JEOL JEM-100B electron microscope. For light microscopy the suspension of live spermatozoa was extracted from the uterus of *E. anisospiculus* and was observed with a Reichert Polyvar microscope.

## Results

Sperm ultrastructure of both closely related species studied is very uniform and the following description is not subdivided into parts. Some existent differences are mentioned in special remarks.

Spermatozoa in the uteri of female worms as well as those activated *in vitro* represent distinctly bipolar cells (Fig. 1 A). Interference contrast microscope observations on spermatozoa taken from the body of the females of both species show an amoeboid activity and the broad anterior end is capable of this function. The posterior part of sperm cells is cone-shaped and has more constant form. Live spermatozoa attached to the glass slide measure 18-20  $\mu\text{m}$  (for *E. demani*) and 24-28  $\mu\text{m}$  (for *E. anisospiculus*) length and can crawl across the surface of glass.

The mature sperm found in the female displays the structure as seen in Figs 1 B and 4. Most sperm cells are oriented at right angles to the longitudinal axis of the uterus with their pseudopod part towards the uterus wall. A large lobate pseudopod has highly irregular contour and contains only a rich network of fibrillar elements. Pseudopods are interdigitated with the projections of the uterus wall, which may be important for the holdfast (Fig. 1 C). The pseudopod cytoplasm is filled with randomly distributed thick fibre complexes connected with the numerous radially running fibres 8-10 nm thick (Fig. 2 A). Fine fibres form an elaborate network attached to the plasma membrane. At the base of the pseudopod near the front line of the part of the sperm filled with organelles we note in *E. anisospiculus* the system of numerous membrane bound smooth vesicles having irregular or tube-like profiles (Fig. 2 B).

The centrally situated sperm nuclei often have irregular or slightly elongate shape (Figs 1 B; 2 C, D), but in *E. anisospiculus* they tend to be highly elongated (Fig. 3). Nuclei of both species are made up of strongly condensed masses of chromatin surrounded by a distinct nuclear envelope (Figs 2 B-D; 3). Such an envelope is evident in sperm cell nuclei as a continuous 25 nm wide electron-dense lamella separated from the chromatin core by electron-translucent space. In *E. anisospiculus* a bilayered feature of nuclear envelope is clearly visible as a double membrane system (Fig. 2 B, D). Prominent evaginations of nuclear envelope occur in sperms cells of

both species. These outfoldings cross over the broad electron-translucent space (halo) surrounding the nucleus and often terminate between closely packed outer cytoplasm organelles (Fig. 2 C, D). The very polymorphic nuclei of *E. anisospiculus* are often not surrounded by the halo and then come into close contact with peripheral organelles (Figs 2 D; 3).

Only two types of organelles constitute the rest of the cytoplasm of the sperm. Numerous round or polygonal mitochondria containing moderately dense matrix and lamellate cristae measure 0.5-0.7  $\mu\text{m}$  in diameter. They are dispersed throughout the cone part of the cell (Fig. 1 B). However, most of them are concentrated anteriorly to the nucleus at the base of the pseudopod (Figs 1 B; 2 B; 3). A thin dense fibre layer separates the anterior layer of mitochondria from the pseudopod cytoplasm (Figs 1 B; 2 B; 3). This evident fibrous border continues to the posterior half of the cell where it separates the closely packed membranous organelles from the plasma membrane (Fig. 1 B).

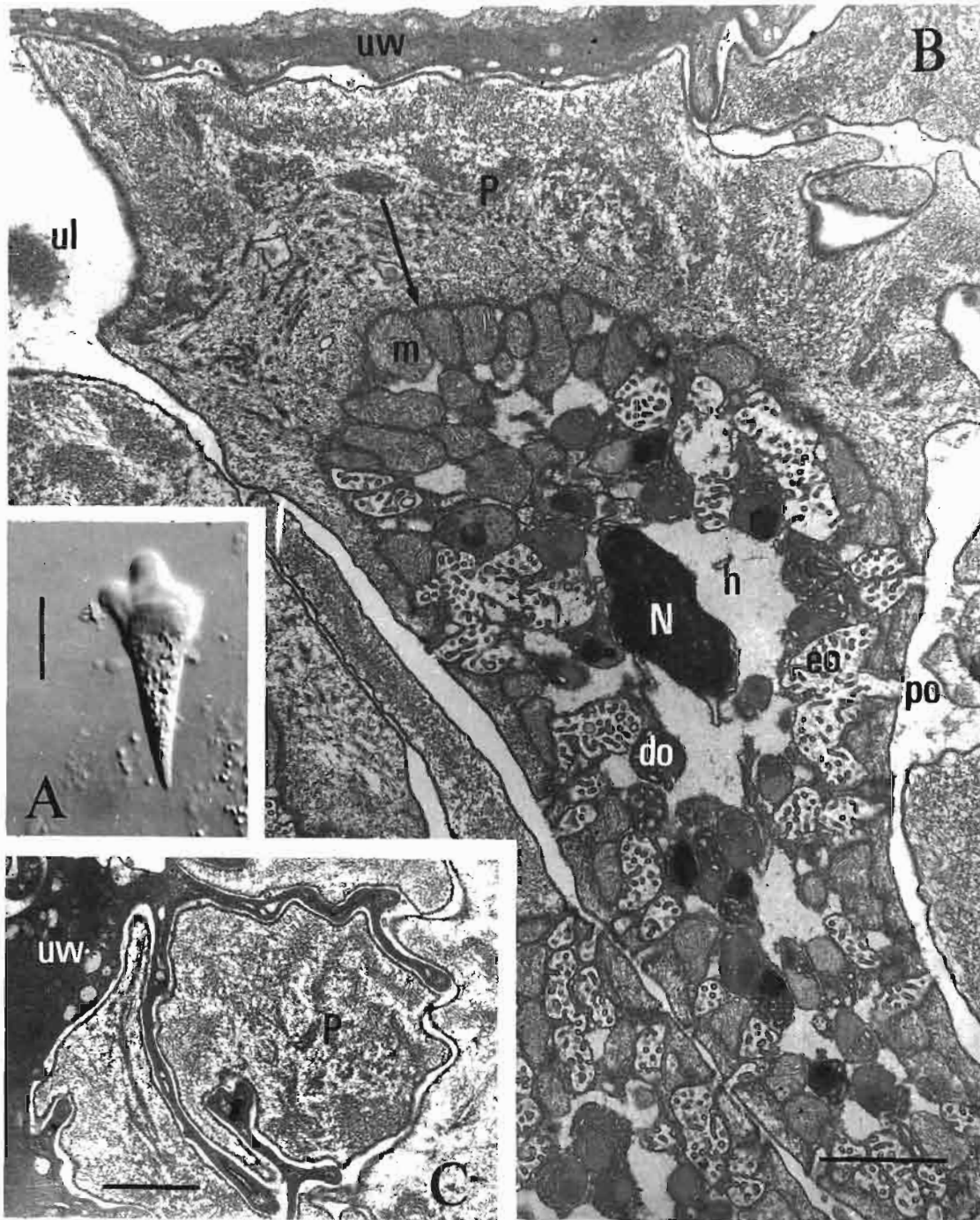
The so-called "membranous organelles" (for terminology see Foor, 1983) which are very characteristic of nematode sperm cells constitute the middle and posterior compact mass interspersed by mitochondria (Figs 1 B; 3). In uteri the sperm of both species contain two types of membranous organelles. Those located more centrally have spherical contours, measure 1.0-1.3  $\mu\text{m}$  (for *E. demani*) and 0.4-0.6  $\mu\text{m}$  (for *E. anisospiculus*) in diameter and are filled with homogeneous electron-dense material penetrated by many microvillus-like extensions of the outer membrane within the organelle (Figs 1 B; 2 D; 3). Distinct dense cores are often evident in this type of membranous organelle.

Membranous organelles of another type are concentrated beneath the plasma membrane and have more irregular outlines. They measure 0.3-1.6  $\mu\text{m}$  in *E. demani* and 0.3-1.0  $\mu\text{m}$  in *E. anisospiculus* (Figs 1 B; 3). These organelles open to the exterior by short neck-like pores of about 0.1-0.3  $\mu\text{m}$  in diameter and their membranes are continuous with plasma membrane. Membranous organelles and corresponding pores show relatively regular longitudinal arrangement. Pore-bearing membranous organelles have a more elaborate system of internal microvilli and are devoid of dense material. Their matrix is electron-translucent and contains only scarce flocculent material.

Axonemes, centrioles and microtubules were not found in mature sperm cells of either species.

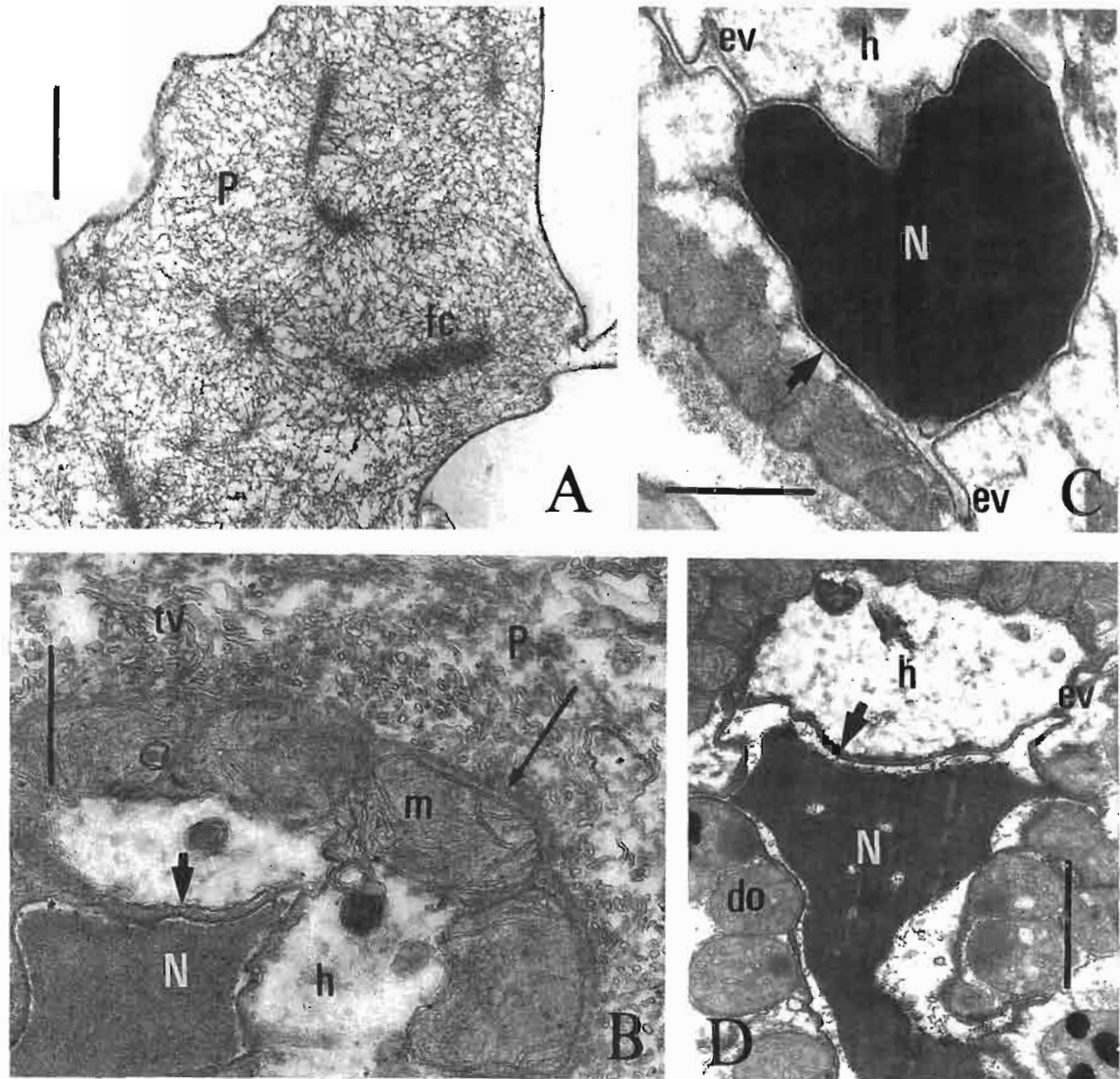
## Discussion

The aflagellate amoeboid bipolar spermatozoa of *Enoplus* spp. show all the basic characters of nematode sperm. They have a highly condensed nucleus free of any organelles, an anterior pseudopod filled with fibres, numerous mitochondria and membranous organelles.



**Fig. 1.** *A*: *Enoplus anisospiculus* spermatozoon freed from the uterus and viewed with Nomarski interference contrast; *B*: *Enoplus demani* spermatozoon attached to the uterus wall, transverse section through the uterus; *C*: *E. demani*, detail of the interdigitation of the sperm pseudopod with the uterus wall. (Bar: *A* = 10  $\mu\text{m}$ ; *B* = 2  $\mu\text{m}$ ; *C* = 1  $\mu\text{m}$ .)

Abbreviations in the figures: do = membranous organelle containing electron-dense material; eo = emptied membranous organelle; ev = evagination of nuclear envelope; fc = fiber complex in pseudopod cytoplasm; h = halo; m = mitochondrion; N = nucleus; P = pseudopod; po = pore in the emptied membranous organelle; tv = tube-like vesicles; ul = uterus lumen; uw = uterus wall. Arrow indications: thin long arrow = fiber layer at the base of pseudopod; thick short arrow: nuclear envelope.

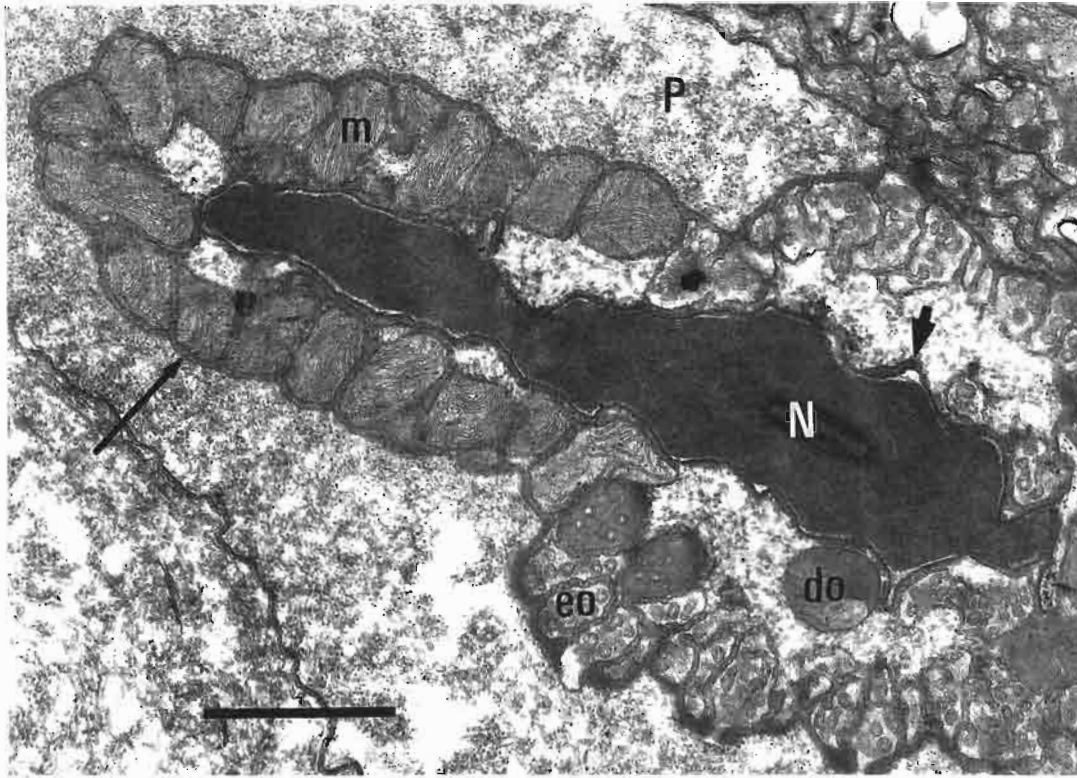


**Fig. 2.** A: *Enoplus demani*, longitudinal section through the pseudopod of the spermatozoon; B: *E. anisospiculus*, border between pseudopod and part of the sperm containing organelles, longitudinal section; C: *E. demani*, transverse section through the sperm nucleus with the nuclear envelope; D: *E. anisospiculus*, longitudinal section through the sperm nucleus with the nuclear envelope. (Bar: A, C, D = 1  $\mu\text{m}$ ; B = 0.5  $\mu\text{m}$ .) For abbreviations see Fig. 1.

But it is the nuclear envelope that draws attention before other ultrastructural features. Such envelopes were found in the sperm nuclei of both species of *Enoplus* studied. Moreover, the nuclear envelope of *E. anisospiculus* is made up of clear double membranes.

To date the nuclear envelope of the sperm nucleus was described in only one nematode species, *Mesacanthion hirsutum*, belonging to the same order Enoplida (Bacetti *et al.*, 1983). However, in earlier study some distinct structure resembling the *E. demani* nuclear envelope was observed in another enoplid, *Deontostoma*

*californicum* (Wright *et al.*, 1973). The thin layer of irregular granular material surrounding the sperm nucleus and connected with peripherious cytoplasm was not interpreted by Wright *et al.* (1973) as a nuclear envelope. Nevertheless, electron microscope images of the nuclear envelopes in *Enoplus* spermatozoa are vary similar to those observed for the perinuclear layer of *D. californicum*. The nuclear envelope of *E. demani* is represented by continuous electron-dense layer. This layer protrudes into prominent outfoldings crossing over the electron-translucent halo. Thus, four enoplid



**Fig. 3.** *Enoplus anisospiculus*, longitudinal section through the central part of the sperm cell. (Bar : 1  $\mu$ m.) For abbreviations see Fig. 1.

**Table 1.** List of nematode species for which bipolar amoeboid sperm possessing a nucleus, mitochondria and membranous organelles have been described.

Subclass	Order	Species	References
Enoplia	Enoplida	<i>Enoplus</i> spp.	Present paper
	Trichinellida	<i>Trichinella</i> spp.	Baccetti <i>et al.</i> (1983)
Rhabditia	Trichurida	<i>Capillaria hepatica</i>	Neill & Wright (1973)
	Rhabditida	<i>Rhabditis pellio</i>	Beams & Sekhon (1972)
		<i>Caenorhabditis elegans</i>	Wolf <i>et al.</i> (1978)
	Aphelenchida	<i>Aphelenchoides blastophthorus</i>	Shepherd & Clark (1976)
	Strongylida	<i>Nippostrongylus brasiliensis</i>	Jamuar (1966)
		<i>Ancylostoma caninum</i>	Foor (1970)
		<i>Angyostrongylus cantonensis</i>	Foor (1970)
		<i>Nematospiroides dubius</i>	Wright & Sommerville (1985)
	Ascaridida	<i>Ascaris</i> spp.	Clark <i>et al.</i> (1967) Burghard & Foor (1978)
	Spirurida	<i>Dipetalonema viteae</i>	Foor <i>et al.</i> (1971)
<i>Dirofilaria immitis</i>		Lee (1975)	
<i>Brugia pahangi</i>		Burghard & Foor (1975)	

species studied appear to have a nuclear envelope in their mature sperm cells, even though it is the lack of nuclear envelope that is believed to be the fundamental feature of aberrant nematode spermatozoon (Foor, 1983). It seems that the enoplid nucleus retains the relatively primitive and unique nematode character. This

coincides well with the primitive position of the order Enoplida in the system of the class Nematoda supported by several authors (Maggenti, 1964; Malakhov, 1986).

A simple lenticular spermatozoa with the centrally located nucleus and nuclear envelope were described in *Mesacanthion hirsutum* (Baccetti *et al.*, 1973). Sperm



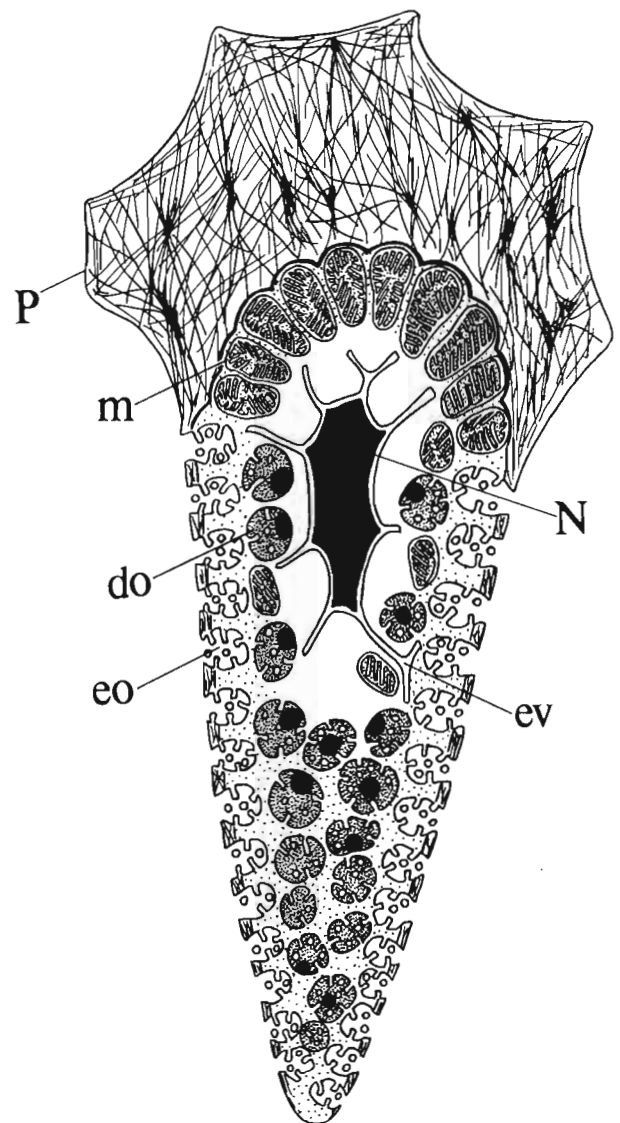
cytoplasm contains only compact group of membranous organelles interspersed with small mitochondria. Unlike *Enoplus* sperm that of *D. californicum* does not possess an anterior pseudopod and its end facing the uterus wall is indicated only by granulous filamentous ectoplasm (Wright *et al.*, 1973). The nuclear material in *D. californicum* sperm is represented by several longitudinally oriented electron-dense fibres. Such a mode of chromatin condensation is unusual for enoplids as well as for other nematodes studied. Thus, three genera of the order Enoplida show considerable divergences in their ultrastructural features.

The sperm structure of *Enoplus* spp. follows the general pattern observed in many other nematodes. It should be emphasized that the spermatozoa described in this paper show the absence of any unique characters (only the nuclear envelope appears to be an unusual feature of *Enoplus* spp. sperm). Anya (1976) and Baccetti *et al.* (1983) consider the non-polarized spheroid sperm containing all usual organelles (*i.e.* nucleus, mitochondria, membranous organelles) as the least specialized type of nematode sperm. In both reviews the authors propose the sperm of certain species as the model of such relatively non-specialized sperm type. It is significant that these two model species belong to the very divergent nematode orders Rhabditida (for *Rhabditis pellio* proposed by Anya (1976)) and Enoplida (for *Mesacanthion hirsutum* proposed by Baccetti *et al.* (1983)). These authors postulate that the absence of pseudopod is a primary character for nematode spermatozoon; it should be noted here that Beams and Sekhon (1972) cited by Anya (1976) have described in *Rhabditis pellio* a clearly bipolar spermatozoon with a large anterior pseudopod.

However, our observations of the relatively non-specialized free-living marine nematodes *Enoplus* spp., as well as the results of other numerous studies of nematode sperm, allow the assumption that it is the distinctly polarized amoeboid spermatozoon containing typical organelles that appears to be the basic and primitive type of sperm. This point of view is supported by widespread distribution of such spermatozoa in the very different and undoubtedly divergent nematode taxa (Table 1).

Several species mentioned in Table 1 have sperm with additional features. But it is very likely that such sperm characters as refringent bodies in ascarid sperm and shifting of the nucleus to the tail-like process in strongylid sperm are more accessory features than the others. As shows Table 1, the polarized amoeboid sperm of uniform structure occurs in most of the orders studied.

It must be emphasized that the most aberrant sperm have the very individual characters which do not occur outside of certain orders or even families (Lee & Anya, 1967; Foor, 1970; Shepherd & Clark, 1983; Van Waebeke *et al.*, 1990; Van de Velde *et al.*, 1991; Kruger, 1991). All these unique sperm types may be derived from the common but relatively less aberrant ones (Bac-



**Fig. 4.** Schematic representation of spermatozoon ultrastructure in *Enoplus demani*. (For abbreviations see Fig. 1.)

cetti *et al.*, 1983). We agree with Baccetti *et al.* (1983) that one of the main directions of the nematode sperm evolution was the simplification and the loss of some basic characters. It should be mentioned, however, that the polarized amoeboid sperm does not appear to be a derived type of sperm. It is difficult to imagine that such a complicated and uniform sperm type was derived many times independently in such divergent orders as Enoplida, Trichinellida, Trichurida, Rhabditida, Aphelenchida, Strongylida, Ascaridida and Spirurida.

It seems that in *Enoplus* spp. the spermatozoa should be considered as the most primitive ones described for nematodes (Fig. 4). They have a nuclear envelope as a

distinct ancestor feature and are devoid of any unique specializations. Thus the primitive nematode spermatozoon may be described as a bipolar amoeboid cell consisting of an anterior pseudopod part and a posterior one containing organelles. The pseudopod is movable after activation and devoid of any organelle. Pseudopod cytoplasm is filled with a cytoskeleton network. The posterior part of the cell includes a highly condensed nucleus surrounded by a nuclear envelope, numerous mitochondria and membranous organelles.

As indicated in Table 1, very similar sperms but devoid of a nuclear envelope are retained in many orders of the class Nematoda. It is likely that the numerous types of significantly more aberrant sperm were derived independently in all orders including the order Enoplida.

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