# Ultrastructure of sperm development in the free-living marine nematodes of the family Chromadoridae (Chromadorida: Chromadorina)

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**Summary** – Spermatogenesis in two species of free-living marine nematodes from the family Chromadoridae (*Panduripharynx pacifica* and *Euchromadora robusta*) was studied electron-microscopically. The spermatogonia of both species are undifferentiated polygonal cells with a large nucleus surrounded by a small amount of cytoplasm. In *P. pacifica* the cytoplasm of spermatocytes contains many Golgi bodies, cisternae of RER, ribosomes, mitochondria and dense spherical bodies. Filamentous material is accumulated in spermatids, which contain only mitochondria and a fragmented (or lobed) nucleus devoid of the nuclear envelope. The immature sperm resembles the late spermatid: its central filamentous area is surrounded by chromatine particles and occasional mitochondria. The immature sperm plasma membrane forms deep infoldings. Mature spermatozoa from the uterus consist of a small main cell body (MCB) bearing a prominent pseudopod filled with cytoskeleton filaments. The MCB contains a nucleus and mitochondria. Spermatogenesis in *E. robusta* (studied only in testes) resembles that described for *P. pacifica*, but spermatocytes of *E. robusta* show much lower metabolic activity and, as a result, a smaller mass of filamentous material is stored in the spermatids and immature sperm. The spermatozoa of *P. pacifica* and the immature sperm of *E. robusta* have the main ultrastructural features characteristic for nematodes (amoeboid nature, absence of axoneme, acrosome and nuclear envelope). No aberrant organelles special for many nematode sperm (membranous organelles, paracrystalline fibrous bodies and their complexes) were found during sperm development of the chromadorids studied. In this respect their spermatogenesis differs significantly from that in secernents and monhysterids.

Résumé – Ultrastructure du développement du sperme chez les nématodes libres de la famille des Chromadoridae (Chromadorida: Chromadorina) - La spermatogenèse a été étudiée en microscopie électronique à transmission chez deux espèces de nématodes libres marins (Panduripharynx pacifica et Euchromadora robusta) de la famille des Chromadoridae. Les spermatogonies, chez les deux espèces, sont des cellules indifférenciées avec un grand noyau entouré d'une petite quantité de cytoplasme. Chez P. pacifica, le cytoplasme des spermatocytes contient de nombreux corps de Golgi, des cisternae du RER, des ribosomes, des mitochondries et des corps sphériques denses. Le matériel filamenteux est accumulé dans les spermatides qui contiennent seulement des mitochondries et un noyau fragmenté (ou lobé) dépourvu d'enveloppe nucléaire. Le sperme immature resemble aux dernières spermatides: son aire centrale filamenteuse est entourée par des particules de chromatine et quelques mitochondries. La membrane plasmatique du sperme immature forme des invaginations profondes. Les spermatozoïdes matures, dans l'utérus, sont constitués par un petit corps cellulaire principal (MCB) portant un pseudopode proéminent rempli de filaments de cytosquelette. Le MCB contient un noyau et des mitochondries. La spermatogenèse chez E. robusta (étudiées seulement au niveau des testicules) ressemble à celle décrite chez P. pacifica, mais les spermatocytes d'E. robusta sont le siège d'une activité métabolique plus faible et, par conséquent, une masse plus faible de matériel filamenteux est stockée dans les spermatides et dans le sperme immature. Les spermatozoïdes de P. pacifica et le sperme immature d'E. robusta ont les mêmes caractéristiques ultrastructurales pour des nématodes (nature amiboïde, absence d'axonème, d'acrosome et d'enveloppe nucléaire) mais aucune des organelles aberrantes particuliéres à de nombreux spermes de nématodes (organelles membraneuses, corps fibreux paracrystallins et leurs complexes) n'ont été identifiées pendant le développement du sperme chez les Chromadorides étudiés. Par cet aspect, leur spermatogenèse diffère significativement de celle des Secernentes et des Monhysterides.

Keywords - Euchromadora robusta, Panduripharynx pacifica, spermatogenesis.

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The nematode sperm are characterised by the absence of an axoneme, acrosome and nuclear envelope (Foor, 1983; Bird & Bird, 1991). The basic type of nematode spermatozoon may be described as a bipolar cell with anterior pseudopod and posterior main cell body (MCB). The MCB has a condensed nucleus without nuclear envelope surrounded by mitochondria and so called 'membranous organelles' (MO), unique organelles, which are characteristic of most nematode sperm cells and which open to the surface in mature spermatozoa (Foor, 1983; Bird & Bird, 1991).

Spermatogenesis and ultrastructure of nematode sperm has been studied mainly in animal and plant-parasitic species (Foor, 1983; Bird & Bird, 1991). Publications concerning the sperm structure of free-living marine nematodes are limited to seven papers. Five of them deal with the sperm ultrastructure of the members of the order Enoplida (Enoplia) (Wright *et al.*, 1973; Baccetti *et al.*, 1983; Yushin & Malakhov, 1994, 1998; Turpeenniemi, 1998). Two papers are devoted to the sperm development in marine species belonging to the order Monhysterida (Chromadoria): Noury-Sraïri *et al.* (1993) for *Sphaerolaimus hirsutus* and Nicholas and Stewart (1997) for *Gonionchus australis*. There are no other data concerning the sperm ultrastructure in Chromadoria, which include mainly freeliving aquatic species of nematodes.

Noury-Sraïri *et al.* (1993) revealed that spermatogenesis in the monhysterid *S. hirsutus* is close to the pattern of sperm development described in many secernentean species (Foor, 1983). This is true when the development of the unique aberrant organelles of nematode sperm (MO and fibrous bodies [FB]) is considered. In much the same way as found in many secernenteans, MO in spermatocytes of *S. hirsutus* derive from the Golgi bodies and appear as a part of bipartite FB-MO complexes each including a crystalline FB associated with membranous cisternae. During spermatogenesis these FB-MO complexes dissociate into: *i*) separate MO, which move to the periphery of immature sperm and *ii*) free FB, which transform during sperm maturation into ectoplasmic filaments of the cytoskeleton (Noury-Sraïri *et al.*, 1993).

It is interesting to investigate whether or not the secernentean features of spermatogenesis are widespread amongst Chromadoria. In this paper we describe the development of the sperm of two species of free-living marine nematodes (*Panduripharynx pacifica* Belogurov, Dashchenko & Fadeeva, 1985 and *Euchromadora robusta* Kulikov, Dashchenko, Koloss & Yuhsin, 1998) both belonging to the family Chromadoridae of the order Chromadorida. These data are aimed to widen our knowledge of the spermatogenesis and sperm ultrastructure in Chromadoria.

## Material and methods

The adult males and females of *P. pacifica* were collected from silty sand at 1 m depth, the adult males of *E. robusta* were extracted from clusters of the bivalve *Crenomytilus grayanus* collected at 10 m depth. Both species were collected at Vostok Marine Biological Station of the Institute of Marine Biology (Vladivostok, Russia) located in Vostok Bay, Sea of Japan.

Live males were cut into pieces each containing a whole testis. Females of P. pacifica were cut at head and tail regions to give a piece containing uteri with fertilised eggs and mature sperm. These specimens were fixed for TEM in 2.5% glutaraldehyde in 0.05 M cacodylate buffer containing 21 mg/ml NaCl and then postfixed in 2% osmium tetroxide in the same buffer containing 23 mg/ml NaCl. Postfixation was followed by en bloc staining for 12 h in 1% uranyl acetate and then the specimens were dehydrated in ethanol and acetone series and embedded in Spurr resin. Thin sections were cut with a Reichert Ultracut E ultramicrotome, stained with lead citrate and then examined with a Philips EM 300, JEOL JEM 100B and JEOL JEM 1010 electron microscopes. The testes of four males were studied in each species, uteri of two females of P. pacifica were observed. Thus, the full cycle of spermatogenesis (up to the mature sperm from uterus) was studied only in P. pacifica. In E. robusta the immature sperm from the testes was the final stage of sperm development studied.

## Results

Males of both species studied (as well as in other Chromadoridae) have a single (anterior) testis, longitudinal sections through which allows observation of all the stages of sperm development — from spermatogonia through immature sperm.

## PANDURIPHARYNX PACIFICA

Spermatogonia occur at the distal tip of the testis as slightly flattened (8  $\mu$ m long, 4  $\mu$ m wide) cells with a large irregular nucleus filled with large clumps of chromatin (Fig. 1A). The cytoplasm contains mitochondria, ribosomes, small vesicles and endoplasmic reticulum.



**Fig. 1.** Panduripharynx pacifica. A: Spermatogonium; B: Late spermatocyte; C: Late spermatocyte, filamentous material (F) condenses between outfoldings (ou) of the nuclear envelope (arrowheads). (Abbreviations: Ch = nuclear chromatin; cy = cytoplasm; F = filamentous material; fp = filopodia; G = Golgi bodies; i = infoldings of outer membrane; mt = mitochondria; N = nucleus; n = nucleolus; ou = outfoldings of nuclear envelope; P = pseudopod; RB = residual body; rer = rough endoplasmic reticulum; Scale bar = 1  $\mu m$ .)

The nuclei of spermatocytes are strongly lobed, their nuclear envelope forms numerous outfoldings deeply protruded into the cytoplasm (Figs 1B, C; 7). The nucleoplasm contains patches of chromatin and large nucleoli, the cytoplasm comprises many mitochondria, Golgi bodies, cisternae of rough endoplasmic reticulum (RER), granular or filamentous spherical bodies and ribosomes. (Fig. 1C). The late spermatocytes attain a diameter of about 12  $\mu$ m.

The stage of the spermatid is limited to the period from the last meiotic division to the detachment of the residual body (Shepherd, 1981). The spermatids consist of a small spherical chromatin-containing part connected to the large residual body by a narrow neck-like cytoplasmic bridge (Figs 2A, B; 7). The nuclear chromatin in the early spermatids is arranged in randomly distributed and interconnected pieces, some of which show filamentous structure (Fig. 2A). In the late spermatids the nucleus consists of strongly condensed chromatin particles without any traces of the nuclear envelope (Fig. 2B).

Several mitochondria, concentrated mostly at the cytoplasmic bridge, are the only organelles surrounding the nuclear chromatin. The cytoplasm of the residual body is filled with ribosomes, large Golgi bodies, cisternae of RER, vesicles and clumps of granular material (Fig. 2A, B).

The region of spermatids occupies up to the one third of the whole length of the testis; this suggests a long period of spermatid development. This process includes the growth of the nucleus containing part (from 3.5 to 4-5  $\mu$ m diam.) coinciding with the accumulation of the dense filamentous material. The latter occupies the centre of the late spermatid shifting the chromatin particles and mitochondria to the periphery (Fig. 2B).

After detaching from the residual body the central region of the immature sperm resembles that of the spermatid: the central filamentous area is surrounded by chromatine particles which seem to be immersed into the filamentous matrix (Figs 3A; 7). The peripheral cytoplasm of the immature sperm contains only occasional mitochondria. The sperm plasma membrane is arranged into deep infoldings which look in some sections like cisternae, but all these cisternae are open to the exterior by 'pores' (Fig. 3A). Spherical unpolarised immature sperm 4-5  $\mu$ m in diameter devoid of both aberrant (MO and FB) and normal organelles (with exception of mitochondria) are the final stage of sperm development in the testes. Activation of sperm after insemination changes their structure significantly. The mature spermatozoa (found inside the uteri of the adult females) are clearly polarised cells (Figs 3B; 7). They consist of small (only 1-2  $\mu$ m in diam.) MCB bearing a prominent (1-2  $\mu$ m long) pseudopod filled with cytoskeleton filaments. The MCB contains the nucleus and mitochondria, with the latter concentrated mainly at the base of the pseudopod. Nucleus in the mature sperm is condensed into a single slightly lobed mass. Plasma membrane of the mature sperm is without infoldings.

#### EUCHROMADORA ROBUSTA

Spermatogonia fill the very distal region of the testis where mitosis was rarely observed. Spermatogonia are closely packed cells about 6  $\mu$ m in diam., with 4-5  $\mu$ m wide spherical nucleus surrounded by a thin layer of cytoplasm with ribosomes, mitochondria and transparent vesicles (Fig. 4A).

Spermatocytes are about 7  $\mu$ m diam. The cytoplasm of late spermatocytes contains mitochondria, occasional Golgi bodies, cisternae of RER and transparent vesicles (Figs 4B; 7). No other organelles and inclusions appear in spermatocytes.

The meiotic telophases during the spermatogenesis of E. robusta are not fully completed and spermatids develop in tetrads where four spermatids are shared by a common residual body. At the final stages of meiosis, four centres of the nuclear chromatin condensation with associated mitochondria are formed (Fig. 5A). Later each spermatid segregates into its own spherical bud connected to the large residual body (cytophore) by a narrow cytoplasmic bridge (Figs 5B; 7). Occasional filopodia occur on the surface of the spermatids (Fig. 5B). The process of chromatin condensation continues and finally small patches are integrated into the solid concave or fragmented nucleus without nuclear envelope (Fig. 6A). Mitochondria immersed into the filamentous matrix are the only organelles of the spermatids. The ectoplasm of the late spermatid contains microtubules, the plasma membrane is arranged into small outfoldings or filopodia (Fig. 6A). The large residual body accumulates a mass of ribosomes and numerous cisternae of RER belonging to the maternal spermatocyte (Fig. 6A).

Immature sperm from the proximal region of the testes are small (about 2.5  $\mu$ m in diam.) rounded cells with slightly irregular contours (Figs 6B, C; 7). The surface of immature sperm may be arranged into several small pseudopods with sparse filamentous content, but these pseudopods do not polarise the cell. It seems that nuclei of the immature sperm are not uniform in shape. Some sections demonstrate a cup-shaped form, but on other sec-



**Fig. 2.** Panduripharynx pacifica. A: Early and B: late spermatids are connected to residual body by cytoplasmic bridge (arrowheads), arrows indicate the cisternae of rough endoplasmic reticulum (For abbreviations see Fig. 1; Scale bar =  $1 \mu m$ ).



**Fig. 3.** Panduripharynx pacifica. A: Immature sperm (arrowheads: pores connecting outer membrane infoldings with the lumen of testis); B: Mature (in utero) sperm (For abbreviations see Fig. 1; Scale bar =  $1 \mu m$ ).

tions the nucleus appears to consist of separate chromatin particles (Fig. 6B, C). It is conceivable that some of these sperm nuclei are multilobed. The dense filamentous cytoplasm of the immature sperm contains only mitochondria, which are concentrated mainly at the cell periphery (Fig. 6C). No other organelles (including MO and FB) were observed in the immature sperm of *E. robusta*.

#### Discussion

The spermatozoa of *P. pacifica* and *E. robusta* have the main ultrastructural features characteristics of many nematodes studied so far: they are amoeboid cells without axoneme, acrosome and nuclear envelope around the strongly condensed nuclear chromatin (Foor, 1983; Bird & Bird, 1991; Scott, 1996). Sperm of the chromadorids studied contain only a nucleus and several mitochondria around it. Mature sperm of *P. pacifica* stored inside the female reproductive system are bipolar cells with prominent pseudopod and compact MCB containing the nucleus and mitochondria. It is possible, that polarisation and pseudopod formation may occur also in sperm of *E. robusta* after their activation in uterus.

Other nematode sperm from very different orders, however, may be more complicated. One of the usual aberrant structures appearing in the mature sperm or during the spermatogenesis of nematodes are MO, FB and their



**Fig. 4.** Euchromadora robusta. A: Spermatogonium (arrows: thin layer of cytoplasm); B: Late spermatocyte (For abbreviations see Fig. 1; Scale bar =  $1 \mu m$ ).



**Fig. 5.** Euchromadora robusta. A: Quartet of spermatids is forming (1, 2 and 3 indicate three of four centres of condensation of nuclear chromatin [arrowheads] and grouping of mitochondria [mt]); B: Early spermatid connected to residual body (RB) by cytoplasmic bridge (arrowheads). Note occasional filopodia (fp) on the surface of the spermatid. (For abbreviations see Fig. 1; Scale bar:  $A = 1 \ \mu m$ ;  $B = 0.5 \ \mu m$ .)

complexes. These organelle complexes were described in many nematodes as a necessary component of the differentiating cytoplasm during spermatogenesis (Foor, 1983). As a rule, the provisory FB-MO complexes appear in the spermatocytes in the form of membranous vesicles and cisternae connected with growing paracrystalline FB. The FB-MO complexes dissociate in the spermatids and give rise to the free FB (later disintegrated and transformed into the pseudopod cytoskeleton) and MO, which attach to the plasma membrane and release their content via pores (Foor, 1983). This pattern of sperm development is the usual one for most studied secernentean species (McLaren, 1973; Shepherd & Clark, 1976; Wolf et al., 1978; Ugwunna & Foor, 1982; Foor, 1983) and for the only representative studied from Chromadoria (Noury-Sraïri et al., 1993).

The chromadorids studied here demonstrate significant deviations from the above mentioned type of sperm organelle development. Both species have no MO and FB in all the stages of spermatogenesis. The deep infoldings of the immature sperm membrane in *P. pacifica* resemble to some extent the MO of many other nematodes, but we cannot interpret them as homologous with true MO. The membrane infoldings in *P. pacifica* have a distinctly divergent structure and their origin and fate is quite different. We found them only at the stage of immature sperm, but usually MO appear as derivatives of Golgi bodies in spermatocytes and persist through all the stages of spermiogenesis including mature sperm from uteri (Foor, 1983; Bird & Bird, 1991).

Alterations of the basic type of spermatogenesis are known both for secernentean and adenophorean nematodes. Two unusual variations of spermatogenesis in Rhabditia have been described for tylenchids and oxyurids. In *Meloidogyne incognita* (Tylenchida) the FB (also the precursors of pseudopod cytoskeleton) are formed freely in the cytoplasm of the spermatocytes, no MO were observed (Shepherd & Clark, 1983). The same situation was described recently in the tylenchid *Pratylenchus penetrans* (Endo *et al.*, 1998). The final (*in utero*) sperm of this nematode is a bipolar cell with pseudopod and MCB with the nucleus, mitochondria and remnants of FB.



**Fig. 6.** Euchromadora robusta. A: Late spermatid with strongly condensed nuclear chromatin (arrowheads: cytoplasmic bridge); B: Immature sperm, section showing a cup-shaped projection of the nucleus; C: Immature sperm, the nucleus on this section looks like separate chromatin particles (small arrows). (For abbreviations see Fig. 1; Scale bar =  $0.5 \mu m$ .)

These spermatozoa resemble the mature sperm of *P. paci-fica*. Mention may also be made of filopodia, which were described in spermatids and sperm of many tylenchids (Shepherd & Clark, 1983; Cares & Baldwin, 1995; Endo *et al.*, 1998) and are present in spermatids of the chromadorid *E. robusta* as described above.

The spermatogenesis of the oxyurid nematode *Aspiculuris tetraptera* proceeds without MO and FB, but its sperm is not simple in structure and is unique for nematodes (Lee & Anya, 1967).

The orders of Enoplia show great variability in cytological events during spermatogenesis. Relatively minor alterations were described in *Enoplus anisospiculus* (Enoplida), where MO and FB occur during spermatogenesis and their structure and fate is more or less typical (Yushin & Malakhov, 1998).

In *Xiphinema theresiae* (Dorylaimida) the MO and FB appear in spermatocytes as separate cytoplasmic components (Kruger, 1991). Then the MO disappear, but FB



Fig. 7. Schematic representation of the sperm development in chromadorid nematodes. A: Panduripharynx pacifica (Spermatocyte [Sc], note filamentous spherical bodies and outfoldings of the nuclear envelope. Early spermatid is connected to the residual body by the cytoplasmic bridge [St]. Immature sperm [Is] from testis, nuclear chromatin is immersed into the filamentous matrix, outer membrane is organised into deep infoldings. Mature bipolar sperm [Ms] from the uterus, the main cell body contains only nucleus and several mitochondria, pseudopod is filled with filaments of cytoskeleton); B: Euchromadora robusta. (Sc: with poor synthetic activity; St: with condensing nucleus is connected to the residual body by cytoplasmic bridge; Is: the set of organelles is reduced to the nucleus and several mitochondria).

form the peripheral cytoskeleton of the unpolarised sperm cell.

In *Gastromermis* sp. (Mermithida) the MO-like structures begin developing from membrane-bound pockets only in immature spermatozoa (Poinar & Hess-Poinar, 1993). No structures resembling FB were indicated during the spermatogenesis of *Gastromermis* sp., even though the mature sperm possesses a distinct pseudopod (Foor, 1983). No FB were observed during the spermatogenesis of *Capillaria hepatica* (Trichurida) but the distinctly bipolar spermatozoa of this nematode are amoeboid and possess an anterior pseudopod (Neill & Wright, 1973). In the immature sperm of *C. hepatica* very simple MO appear as double membrane-bound vesicles formed by fusion of small cisternae.

An extremely simplified sperm for Enoplia was described in *Dioctophyme renale* (Dioctophymida) (Foor, 1970, 1983). This polarised sperm has a small pseudopod but no MO while FB were described in spermatocytes and spermatids. Moreover, the mature sperm is devoid of mitochondria, which are removed during sperm development with the cytophore.

Thus, there are several independent lines of nematode sperm simplification which is manifested in reduction of typical aberrant organelles — MO and FB. The result of this trend is illustrated by dioctophymid *D. renale* in Enoplia (Foor, 1970, 1983), tylenchid *P. penetrans* in Rhabditia (Endo *et al.*, 1998), and two chromadorid species in Chromadoria (present paper). In this respect the spermatogenesis of monhysterids which retains all the phenomena of sperm development in secernents (or Rhabditia) (Noury-Sraïri *et al.*, 1993; Nicholas & Stewart, 1997) may be referred to as a primitive for Chromadoria.

Despite total absence of FB during spermatogenesis of *P. pacifica* the distinct pseudopod filled with filaments is formed in the mature sperm. It means that cytoskeleton proteins are synthesised in the cell at the stages of spermatocytes and spermatids. This is confirmed by significant metabolic activity of the cells at these stages which is evidenced by proliferation of the RER and Golgi bodies together with accumulation of dense filamentous material. This material fills the central region of the late spermatid and transforms into the pseudopod cytoskeleton in the mature sperm. Pseudopod formation without the intermediary condensed stages resembling the FB is not an unique feature for the sperm studied here since it has been described in several other nematode species (Lee & Anya, 1967; Neill & Wright, 1973; Foor, 1983; Poinar & Hess-Poinar, 1993).

The two species of chromadorids studied represent two different subfamilies of the family Chromadoridae — Euchromadorinae (*E. robusta*) and Hypodontholaiminae (*P. pacifica*) (Lorenzen, 1994), but we cannot extend their pattern of sperm and sperm development throughout all the order Chromadorida. Light microscopical observations show great variability of sperm form, size and internal structure in different groups of the order (Cobb, 1928; Chitwood & Chitwood, 1977; Riemann, 1983). The ultrastructural study of this variability can provide the systematics of nematodes with new data of phylogenetic value.

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