Russian Journal of Nematology, 2001, 9 (2), 119 - 126

Ultrastructure of sperm development in *Heth mauriesi* Adamson, 1982 (Rhigonematida: Hethidae)

Vladimir V. Yushin* and Sergei E. Spiridonov**

***Institute of Marine Biology, Far East Branch of the Russian Academy of Sciences, Vladivostok 690041, Russia, e-mail: yushinvladimir@hotmail.com**

****Institute of Parasitology of Russian Academy of Sciences, Leninskii prospect 33, Moscow 117071, Russia.**

Accepted for publication 15 August 2001

Summary. Spermatogenesis in the nematode *Heth mauriesi* (Rhabditia, Rhigonematida, Hethidae), which inhabits diplopod hind gut, was studied by using transmission electron microscopy. During the late stages of development spermatocytes have voluminous cytoplasm filled with ribosomes, numerous cisternae of RER, Golgi bodies, and complexes of aberrant organelles comprised of membranous organelles and fibrous bodies (MO-FB complexes). Each complex includes paracrystalline FB associated, at one end, with MO reduced to a simple flattened cistern. Mature spermatids contain a condensed amorphous nucleus surrounded by mitochondria and MO-FB complexes and synthetic components of the cell are segregated into the residual body. The immature spermatozoon is a highly elongated and clearly polarized cell. The central longitudinal axis of the sperm cell is occupied by the long thread-like nucleus. The plasmalemma of the anterior part is smooth, but forms numerous deep infoldings that protrude into the transparent cytoplasm with rare mitochondria, lipid droplets and sparse filamentous material. The plasmalemma of the posterior part of the spermatozoon is arranged into short and wide microvilli of uniform size. Here, peripheral cytoplasm contains numerous spindle-shaped FB orientated along the longitudinal axis of the cell. The layer of small mitochondria positioned under the FB surrounds the central transparent cytoplasm that is filled with sparse filamentous material. MO, or their derivatives, were not observed in immature sperm. The pattern of sperm development in *Heth mauriesi* is similar to that described for other nematodes of the subclass Rhabditia, but differs significantly from the spermatogenesis in oxyurids.

Key words: nematode parasites of diplopods, sperm, spermatogenesis, ultrastructure.

Aberrant amoeboid sperm of nematodes are characterized by absence of an axoneme, acrosome and nuclear envelope (Foor, 1983; Bird & Bird, 1991; Justine & Jamieson, 1999). The basic type of nematode spermatozoon is 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 'membranous organelles' (MO), that are unique organelles characteristic of most nematode sperm cells (Foor, 1983; Justine & Jamieson, 1999).

Spermatogenesis and the ultrastructure of nematode sperm has been studied mainly in animal and plant-parasitic species belonging to the subclass Rhabditia (Bird & Bird, 1991; Justine & Jamieson, 1999). The development of the unique aberrant organelles [MO and fibrous bodies (FB)] has been described for many orders of this subclass: Rhabditida, Strongylida, Diplogasterida, Ascaridida, Spirurida and Aphelenchida (Justine & Jamieson, 1999). The MO in spermatocytes are derived from Golgi bodies, and appear to be a part of the bipartite MO-FB complexes, of which each includes a paracrystalline FB associated with a membranous cistern (= MO) (Foor, 1983; Justine & Jamieson, 1999). During spermatogenesis these MO-FB complexes dissociate into: 1) separate MO, that move to the periphery of immature sperm, and 2) free FB, that transform during sperm activation inside the uterus to become the cytoskeleton of the pseudopod.

Preliminary light and electron microscope observations of rhigonematid nematodes (order Rhigonematida) revealed several peculiarities in the morphology and internal structure of their sperm (Adamson, 1983; Van Waerebeke, 1984; Adamson & Van Waerebeke, 1985b; Van Waerebeke *et al.*, 1990; Justine & Jamieson, 1999). Sperm development in these rhabditians has been studied only on wholemount specimens (Van Waerebeke, 1984. Adamson & Van Waerebeke, 1985b). An ultrastructural examination of spermatogenesis would reveal if rhigonematid sperm have unique pattern development, therefore we examined spermatogenesis in the testes of the rhigonematid nematode *Heth mauriesi* Adamson, 1982 (family Hethidae) by using transmission electron microscopy. The results from our study are reported here.

MATERIALS AND METHODS

Adult *Heth mauriesi* males were extracted from the hindgut of the diplopod *Leptogoniulus naresi* (Pocock, 1893) collected near the seaside resort of Le Gosier, Guadeloupe (Grand-Terre), French West Indies. Live nematodes were cut and those pieces containing a whole testis were used in our study. Specimens were fixed for TEM during 3 hours in 2.5% glutharaldehyde in cacodylate buffer (pH = 7.4). The osmolarity of the fixative was adjusted up to 700 mOsm with NaCl. The specimens were then rinsed three times in cacodylate buffer, postfixated in 1% osmium tetroxide, dehydrated in an ethanol and acetone series, and embedded in Epon epoxy resin. Ultrathin sections were cut with a Reichert Ultracut E ultratome, stained with uranyl acetate and lead citrate and then examined with a JEOL JEM 100B and a JEOL JEM 1010 electron microscopes. Immature sperm from testes was the final stage studied.

RESULTS

The distal third of the testis in *H. mauriesi* is filled with relatively small cells, probably spermatogonia and early spermatocytes. Clearly visible growth of spermatocytes started at the point of the testis flexure. Three distinct zones in the proximal two thirds of the testis were observed: 1) zone of growing spermatocytes, 2) zone of spermatids, and 3) seminal vesicle that is a slightly dilated proximal part of the testis tube filled with immature sperm.

Spermatocytes at the testes flexure are polygonal cells, 10 μm in diameter, with their central nucleus surrounded by cytoplasm containing ribosomes, mitochondria, rare cisternae of rough endoplasmic reticulum (RER), Golgi bodies and transparent vesicles (Fig. 1A). Spermatocytes present in the growth zone attained a diameter of 14 μm and had voluminous cytoplasm filled with ribosomes, numerous cisternae of RER, and Golgi bodies (Figs. 1B & 3) indicative of substancial synthetic activity in these cells. The formation of cylindrical FB 0.6 μm long and 0.3 μm in diameter may be a consequence of this activity. Each FB has a paracrystalline structure and consists of densely packed parallel filaments associated at one end with a flattened cistern (Figs. 1C & 3). These cisternae probably represent the simplified MO, and the whole structure the MO-FB complex that is typical for spermatocytes of other rhabditian nematodes (Justine & Jamieson, 1999).

Meiotic stages of sperm development in the testes were not observed in any of specimens studied and the zone of spermatids was immediately adjacent to the area containing mature spermatocytes. In the early spermatids the central amorphous nucleus, without a nuclear envelope, was surrounded by voluminous cytoplasm with many organelles and MO-FB coplexes. Subsequently the bulk of cytoplasm with ribosomes, RER, Golgi bodies, and occasional lipid droplets, segregates into the residual body (Fig. 3). The nucleus of the late developed spermatid is surrounded only by numerous MO-FB complexes and mitochondria (Fig. 2D).

Spermiogenesis is completed rapidly after detachment of the residual body. Immature sperm of uniform structure is present in the testes immediately adjacent to the late developed spermatids. Immature sperm are highly elongated cells about 70 μm long and 2 μm thick and arranged in parallel rows along the testis. The spermatozoon is clearly polarized and its smooth (probably anterior) 20 μm long part is orientated toward the cloacal opening, with the posterior part appearing rough due to numerous short microvilli covering its surface.

The central axis of the sperm cell is occupied by a long thread-like nucleus, the diameter of which decreases gradually from 0.3 μm anteriorly to 0.1 μm posteriorly (Figs. 2 A-D & 3). The plasmalemma of the anterior part is smooth but forms numerous deep infoldings that protrude into the cytoplasm, up to the region of the nucleus (Figs. 2 A, B & 3). The cytoplasm of the anterior part is transparent and contains only occasional mitochondria, lipid droplets and sparse filamentous material.

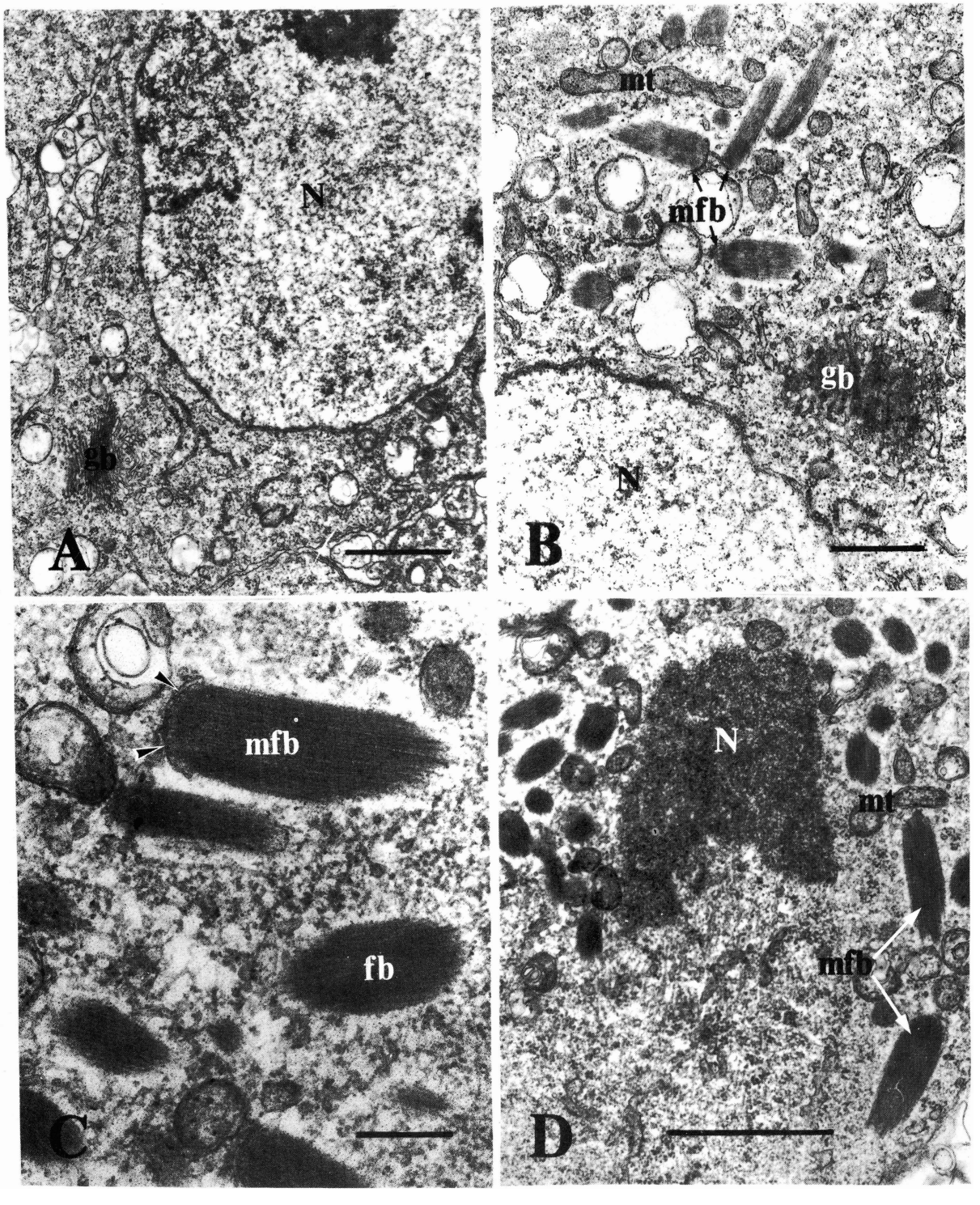

Fig. 1. *Heth mauriesi.* A: Early spermatocyte; B: Late spermatocyte with forming MO-FB complexes; C: MO-FB complexes in the early spermatid with arrows indicating flattened cistern (MO) associated with FB; D: Late spermatid with nucleus surrounded by mitochondria and MO-FB complexes. (Abbreviations: cy, cytoplasm; fb, FB; gb, Golgi body; I, plasmalemma infoldings; mfb, MO-FB complexes; mt, mitochondria; mv, microvilli; N, nucleus; rb, residual body. Scale bar: A, B & D - 1 μm; C - 0.25 μm).

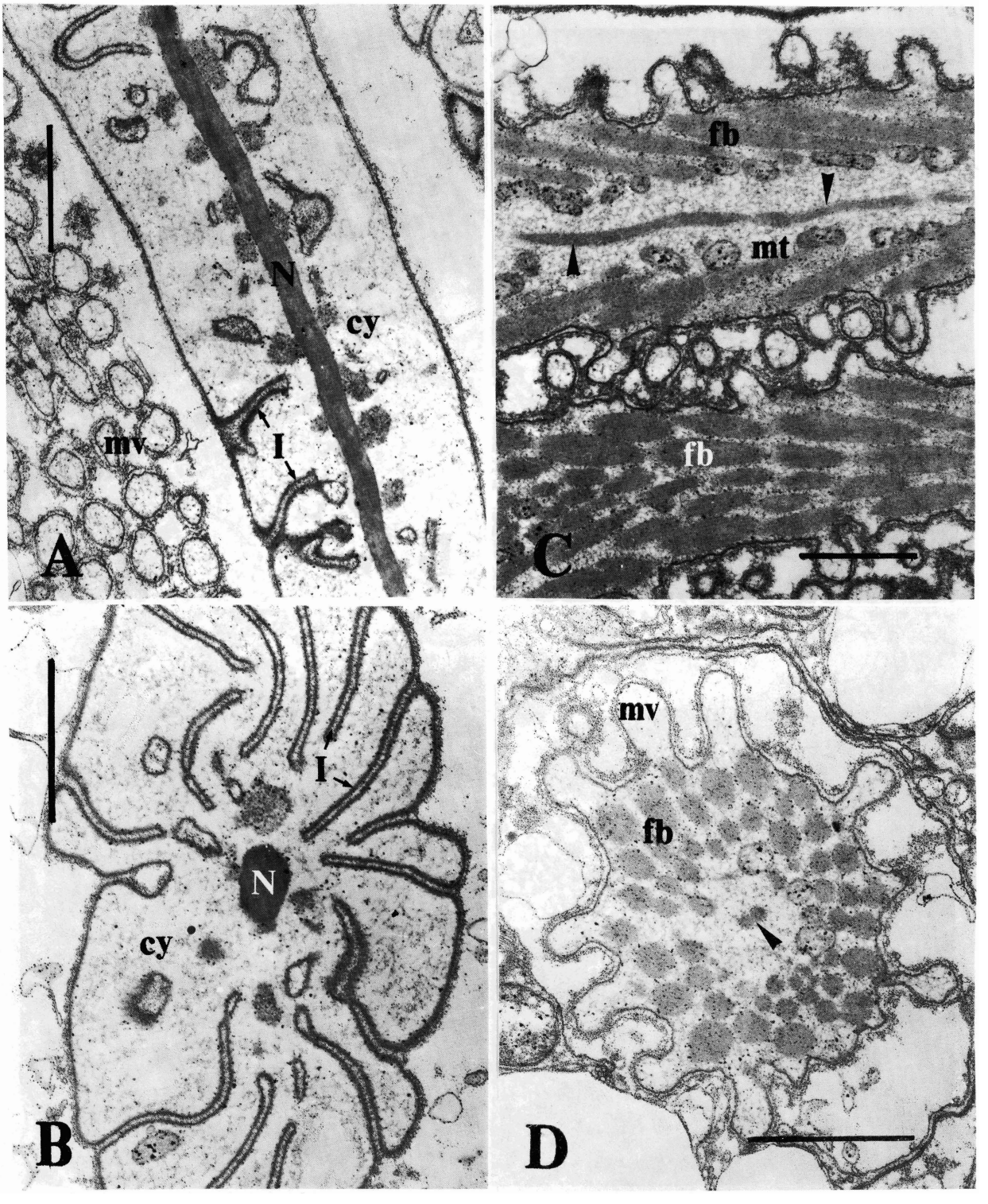

Fig. 2. *Heth mauriesi*, immature sperm. A & B: Longitudinal and transverse sections (respectively) through the anterior part; C & D: Longitudinal and transverse sections through the posterior part, arrowheads indicating nucleus. (For abbreviations see Fig. 1. Scale bar - 1 μm).

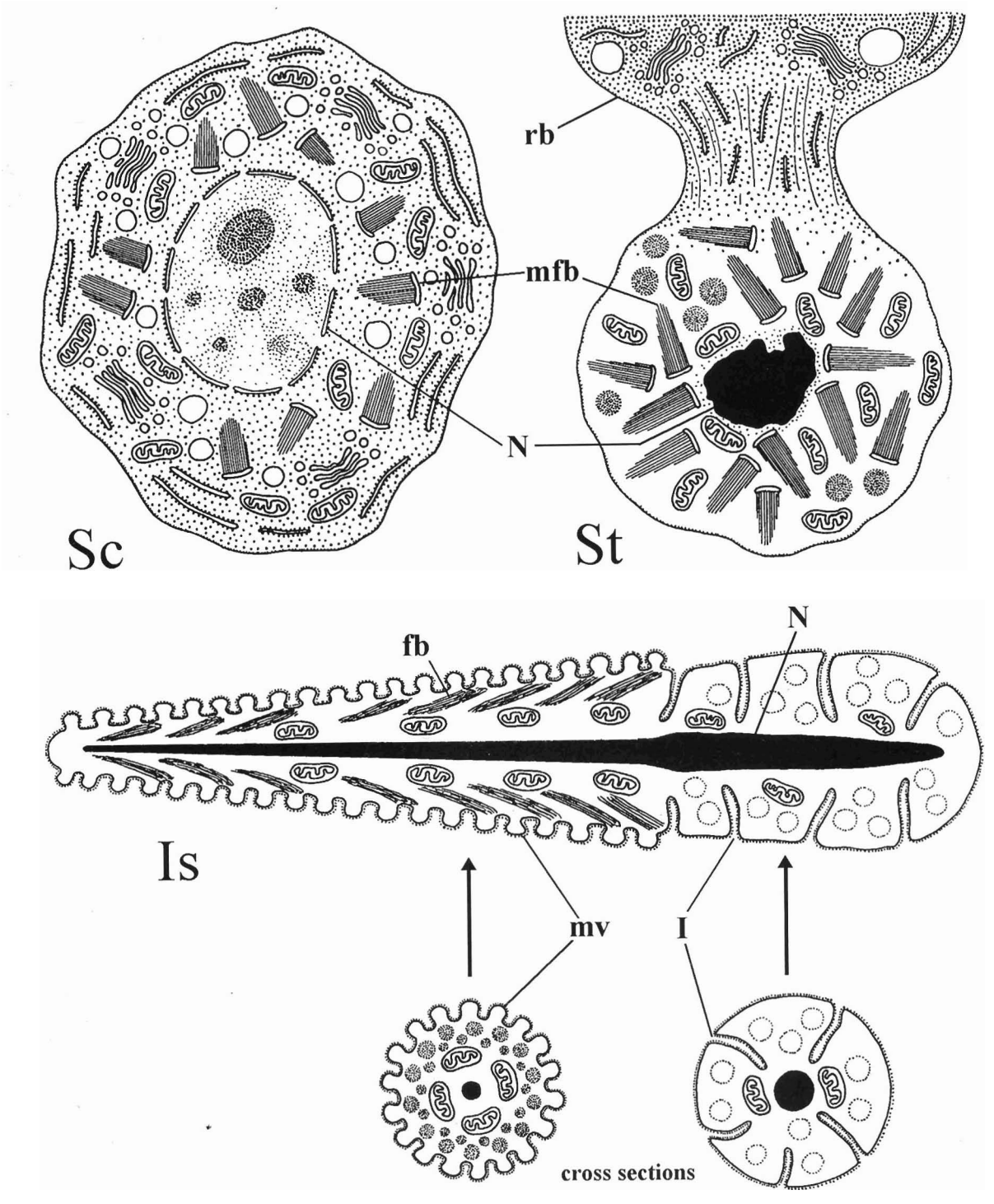

Fig. 3. Schematic representation of sperm develoment in *Heth mauriesi.* MO-FB complexes (where MO component is reduced to a simple flattened cistern) form in the cytoplasm of the spermatocyte (**Sc**). In the spermatid (**St**) the synthesized components of the cytoplasm aggregate to form residual body and the condensed amorphous nucleus is surrounded by MO-FB complexes and mitochondria. In immature sperm (**Is**) the nucleus is highly elongated, FB are retained in the posterior part of the cell as longitudinally arranged dense bands associated with microvillous plasmalemma and MO disappear. The anterior part (right) of immature sperm is smooth, whereas the posterior part (left) is microvillous.

The plasmalemma of the posterior part of the spermatozoon is arranged into short, wide microvilli of uniform size (Figs. 2 D, C & 3). The peripheral cytoplasm of the sperm contains numerous spindle-shaped FB that are orientated along the longitudinal axis of the cell and appear to be associated with plasmalemma. The layer of small mitochondria below FB surrounds the central transparent cytoplasm filled with sparse filamentous material. The FB in immature sperm are elongated (up to 2 μm) and no longer associated with MO. The latter disappear and their fate is unknown. The entire surface of immature spermatozoa is covered with a thin layer of an osmiophilic substance.

DISCUSSION

Spermatozoa of rhigonematid nematodes are usually elongated with slightly dilated anterior and tapering posterior parts (Adamson, 1983; Van Waerebeke, 1984; Adamson & Van Waerebeke, 1985b; Van Waerebeke *et al.*, 1990). Numerous rod-like longitudinally orientated particles in the cytoplasm of immature sperm are clearly visible under the light microscope (Van Waerebeke, 1984; Adamson & Van Waerebeke, 1985b; Van Waerebeke *et al.*, 1990). Electron microscope observations of *Rhigonema madecassum* (Rhigonematidae) revealed that immature spermatozoa of this species are divided into a smooth anterior part and a posterior region surface, which bears numerous protuberances with internal transparent vesicles (Van Waerebeke *et al.*, 1990). The latter were interpreted by Justine & Jamieson (1999) as MO. The posterior region of the sperm contains the nucleus, rare mitochondria, and numerous dense rod-like structures interpreted by these authors as FB.

Immature sperm in testes of rhigonematids from the genus *Heth* (Hethidae) are extremely elongated (up to 85 μm) cells with thread-like nucleus (Van Waerebeke *et al.*, 1990). Our observations on *H. mauriesi* revealed that this morphological peculiarity has little effect on the internal structure of the cell. Similar to *R. madecassum*, the sperm of *H. mauriesi* are bipartite and have a smooth anterior and rough (due to microvilli) posterior part (Van Waerebeke *et al.*, 1990). The FB of *H. mauriesi*, which start to form in spermatocytes, persists into immature sperm as dense longitudinally orientated bundles of the same structure as FB described from the spermatozoa of *R. madecassum*. We were unable to recognize MO in sperm of *H. mauriesi*, but these structures are present in spermatocytes and persist in spermatids as a component of MO-FB complexes. The fate of MO during spermiogenesis may differ between *H. mauriesi* and *R. madecassum*.

H. mauriesi and *R. madecassum* represent two different superfamilies of the order Rhigonematida: the Ransomnematoidea and Rhigonematoidea, respectively. Similarities in the internal structure of the sperm of these two species may be indicative of this type of spermatozoa being characteristic of the whole order. Alternatively, the present observation on *H. mauriesi* reveal that its sperm development resembles the pattern of spermatogenesis in many rhabditians. In most rhabditians studied (Rhabditida, Strongylida, Diplogasterida, Ascaridida, Spirurida, Aphelenchida) MO-FB complexes frequently present as aberrant organelles and occurring initially in spermatocytes (Pasternak & Samoiloff, 1972; McLaren, 1973; Shepherd & Clark, 1976; Wolf *et al.*, 1978; Ugwunna & Foor, 1982; Foor, 1983; Justine & Jamieson, 1999). The MO in these orders, unlike those in Rhigonematida, are spherical vesicles up to 1 μm in diameter with an internal system of microvilli. If the flattened cisternae associated with FB in spermatocytes and spermatids of *H. mauriesi* represent the real MO it can be concluded, that these structures are much simplified, with probably reduced function.

Spermatogenesis of the oxyurid nematode *Aspiculuris tetraptera* (Oxyurida, Oxyuridae) proceeds without MO and FB formation, but the sperm of this nematode appears to be complex in structure and to have several unique features not observed in other nematodes (Lee & Anya, 1967). Van Waerebeke *et al.* (1990) noted that sperm morphology, and internal structure in oxyurids is different from that of rhigonematids, thus rhigonematid nematodes be removed from the order Oxyurida, and considered as a separate order in the Rhabditia (Adamson & Van Waerebeke, 1985a; Justine & Jamieson, 1999). The observations on sperm structure and development in the rhigonematid *H. mauriesi* confirm this conclusion.

Observations made with light microscopy of sperm inside the uterus of the rhigonematid nematodes *Rhigonema madecassum* and *Obainia petteri* revealed substantial changes in sperm morphology and structure after insemination (Van Waerebeke, 1984; Adamson & Van Waerebeke, 1985b). Activated spermatozoa elongate, their cytoplasm becomes more transparent, rod-like particles (probable FB) are dissolved, and a prominent pseudopod forms at the anterior end of the cell. Ultrastructural observations were not made of

rhigonematid sperm activation, but it may be assumed that their FB are precursors of the pseudopod cytoskeleton, as has been shown for many other nematodes (Scott, 1996; Justine & Jamieson, 1999). In the activated spermatozoa of *Heth mauriesi* pseudopod formation probably also occurs. The deep infoldings at the smooth anterior end of the immature spermatozoon increases the surface and volume of the cell, thus facilitating the process of the pseudopod formation.

ACKNOWLEDGEMENTS

This research was supported by the Russian Foundation for Basic Research Grants 00-04-48896 and 00-15-97938, and by the University of Gent. The authors are grateful to M. Claeys and D.V. Fomin for technical assistance.

REFERENCES

Adamson, M.L. 1983. The genus *Ransomnema* Artigas, 1926 (Rhigonematidae) in South American diplopods. *Systematic Parasitology* 5: 119-129.

Adamson, M.L. & Van Waerebeke, D. 1985a. The Rhigonematida (Nematoda) of diplopods: reclassification and its cladistic representation. *Annales de Parasitologie Humaine et Comparée* 60: 685-702.

Adamson, M.L. & Van Waerebeke, D. 1985b. *Obainia petteri* n. sp. (Rhigonematidae, Nematoda) from *Pachibolus laminatus* (Diplopoda) in Ivory Coast: description and study of the spermatogenesis. *Revue de Nématologie* 8: 241-247.

Bird, A.F. & Bird, J. 1991. *The Structure of Nematodes.* San Diego, Academic Press. 316 pp.

Foor, W.E. 1983. Nematoda. In: *Reproductive Biology of Invertebrates*, Vol. 2. (K.G. Adiyodi & R.G. Adiyodi. Eds). pp. 223-256. Chichester, John Wiley & Sons.

Justine, M. & Jamieson, B.G.M. 1999. Nematoda. In: *Reproductive Biology of Invertebrates.* Vol. IX, Part B. (K.G. Adiyodi & R.G. Adiyodi. Eds). pp. 183-166. New Dehli, Calcutta, Oxford & IBH Publishing.

Lee, D.L. & Anya, A.O. 1967. The structure and development of the spermatozoon of *Aspiculuris tetraptera* (Nematoda). *Journal of Cell Sciences* 2: 537-544.

McLaren, D.J. 1973. The structure and the development of the spermatozoon of *Dipetalonema vitiae* (Nematoda: Filarioidea). *Parasitology* 66: 447-463.

Pasternak, J. & Samoiloff, M.R. 1972. Cytoplasmic organelles present during spermatogenesis in the free-living nematode *Panagrellus silusiae. Canadian Journal of Zoology,* 50: 147-151.

Scott, A.L. 1996. Nematode sperm. *Parasitology Today* 12: 425-430.

Shepherd, A.M. & Clark, S.A. 1976. Spermatogenesis and the ultrastructure of the sperm and of the male reproductive tract of *Aphelenchoides blastophthorus* (Nematoda: Tylenchida, Aphelenchina). *Nematologica* 22: 1-9.

Ugwunna, S.C. & Foor, W.E. 1982. Development and fate of the membranous organelles in spermatozoa of *Ancylostoma caninum. Journal of Parasitology* 68: 834-844.

Van Waerebeke, D. 1984. *Rhigonema madecassum* n. sp. (Rhigonematidae; Nematoda), parasite de diplopode a Madagascar: description et étude de la spermatogenèse. *Revue de Nématologie* 7: 271-276.

Van Waerebeke, D., Noury-Sraïri, N. & Justine, J.-L. 1990. Spermatozoa of rhigonematid nematodes: morphology of 25 species and ultrastructure of *Rhigonema madecassum. International Journal for Parasitology* 20: 779-784.

Wolf, N., Hirsch, D. & McIntosh, J.R. 1978. Spermatogenesis in males of the free-living nematode, *Caenorhabditis elegans. Journal of Ultrastructure Research* 63: 155-169.