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Ultrastructural investigation of spermatogenesis in the nemertine worm *Procephalothrix* sp. (Palaeonemertini, Anopla)

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ABSTRACT: Spermatogenesis and sperm structure of the nemertine worm *Procephalothrix* sp. were studied by transmission electron microscopy. It is shown that a flagellum and proacrosomal vesicles are common in spermatogonia and spermatocytes as in spermatogenesis of a number of marine invertebrates with external fertilization. Originally, the animals were collected as *Procephalothrix spiralis* but they were found to have a type of spermatozoon different from that of *P. spiralis* as described by Turbeville & Ruppert (1985). The re-identification of the material collected in the Japan Sea has shown that the features are characteristic of *P. spiralis* (Coe, 1930). This finding suggests that *P. spiralis* shows variations in different parts of the world.

INTRODUCTION

The ultrastructure of the sperm cells of nemertines has been the subject of several investigations (Afzelius, 1971; Franzén, 1983; Turbeville & Ruppert, 1985; Stricker & Cavey, 1986; Franzén & Sensenbaugh, 1988; Reunov & Chernyshev, 1992; Jespersen, 1994). These studies have shown that some nemerteans have so-called primitive spermatozoa or aquasperm (Franzén, 1983; Turbeville & Ruppert, 1985). In other nemertines the head of the sperm may be modified, showing an elongated nucleus (Afzelius, 1971; Stricker & Cavey, 1986; Franzén & Sensenbaugh, 1988; Reunov & Chernyshev, 1992). Also aberrant spermatozoa without flagella have been found in nemertines by light microscopy (Gerner, 1969). Ultrastructural investigations of all cell stages of spermatogenesis were carried out on the hoplonemertines *Tetrastemma phyllospadicola* (Stricker & Cavey, 1986) and *T. nigrifrons* Reunov & Chernyshev, 1992) possessing modified spermatozoa. The data on the spermatogenesis of the most primitive nemertines (Palaeonemertea) are based on the description of spermiogenesis and sperm structure of *Cephalothrix rufifrons* (Jespersen, 1984). In our opinion it would be interesting to study the ultrastructure of all spermatogenic stages in palaeonemertines in more detail. It is known that in spermatogenesis of many marine invertebrates with external fertilization a flagellum and/or proacrosomal vesicles are common in spermatogonia and spermatocytes (Hodgson & Reunov, 1994; Reunov & Hodgson, 1994). This differentiation of flagellum and/or proacroso-

mal vesicles in early spermatogenesis appears to be an interesting problem of cell biology that has not yet been cleared up. In the nemertini with modified sperms described so far, *Tetrastemma phyllospadicola* (Stricker & Cavey, 1986) and *T. nigrifrons* (Reunov & Chernyshev, 1992), these characters were not detected. The production of the acrosomal material in both species is started in the spermatids. The tail formation in *T. nigrifrons* begins during spermiogenesis. On the other hand, the secondary spermatocytes of *T. phyllospadicola* can produce flagella which are considered atypical (Stricker & Cavey, 1986). The aim of this study is to describe all stages of spermatogenesis in *Procephalothrix* sp. and to check whether it is possible to observe the early arising of tails and proacrosomal vesicles during spermatogenesis of this palaeonemertine.

MATERIAL AND METHODS

The samples of *Procephalothrix* sp. (Anopla: Palaeonemertini) were collected from February to May 1992 in the intertidal zone of the Ussurian bay (Japan Sea, Russia), and identified by Dr. A. V. Chernyshev. The additional re-identification of this material by an analysis of histological sections of the worms from the gullet areas was made by Dr. W. Senz in the Zoological Institute at the University of Vienna. For the ultrastructural investigations the specimens were fixed in 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7,4 fixative osmolarity = 1090 mOsm) and in 2 % osmium tetroxide. Following dehydration in a graded series of ethanol and propylene oxide, the material was embedded in Epon. Sections were cut on an Ultracut-E (Reichert) ultramicrotome using glass and diamond knives, stained with uranyl acetate and lead citrate, and examined with a Zeiss EM 9S-2 transmission electron microscope. Five samples of mature males were studied. For scanning electron microscopical investigations, samples of *Procephalothrix* sp. were fixed as above. The material was passed through a graded ethanol series, critical-point dried, coated with gold and photographed with a JSM-35 CF scanning electron microscope. Most of the preparation for and the examination of the specimens with the electron microscopes was carried out in the Zoological Institute at the University of Vienna.

RESULTS

Spermatogonia

In February and March the testes of *Procephalothrix* sp. are full of spermatogonia (Fig. 1). Usually these cells are elongate (8–9 µm long and 3–4 µm wide) and they have a nucleus (about 4–5 µm long and 2 µm wide) with small patches of chromatin scattered throughout the nucleoplasm. In the nucleoplasm there are one or two nucleoli about 0.8 µm in diameter. The spermatogonia have flagella which emerge from the centriole just below the cell membrane (Fig. 3). The cytoplasm contains Golgi bodies, elongated mitochondria and numerous membrane-bound electron-dense vesicles which are assumed to be proacrosomal vesicles (Fig. 4). Intercellular bridges connect the tightly packed spermatogonia (Fig. 5). The wall of the testis is lined by elongated peritoneal cells with an ovoid nucleus (Fig. 2). In the cytoplasm of these cells there is a considerable number of vacuoles.

Spermatocytes

In April the gonads contain spermatocytes and spermatids (Fig. 6). The primary spermatocytes are round to ovoid (about 6 × 3 µm) and they have a flagellum. The diameter of the spheroidal nucleus of the primary spermatocytes is about 3 µm. The chromatin within the nucleus is now highly condensed. In the zygotene/pachytene stages there are typical synaptonemal complexes within the chromatin (Fig. 7). The intercellular bridges connect the spermatocytes (Fig. 8). The cytoplasm contains ovoid mitochondria, Golgi bodies and numerous electron-dense proacrosomal vesicles which are randomly distributed (Fig. 8). Spermatocytes also have one flagellum each (Fig. 9). It was not possible to investigate the second spermatocytes. Usually, the primary spermatocytes, spermatids and dividing cells (supposedly the meiotical maturation stages) may be seen (Fig. 6). It should be stressed that more than two nuclei can be observed in these dividing cells (Fig. 10).

Spermatids

The early spermatids are about 4 µm long and 1.5 µm wide. Each cell has one round or ovoid nucleus about 1.3 µm in diameter. Within the cytoplasm the proacrosomal vesicles are situated at the presumptive posterior pole of the cell (Fig. 11) and fuse to form one large acrosomal vesicle near the centrioles and the tail. This vesicle eventually has a diameter of 0.6 µm and is full of electron-dense material inside of which globules of even higher electron density may be seen (Fig. 12). During the course of development the acrosomal vesicle migrates to the presumptive anterior pole of the late spermatid (Fig. 13) and becomes concave (Figs 14, 16). Some periacrosomal material appears in the cytoplasm within the inner curvature of the concave vesicle (Figs 16, 17). In the late spermatid the acrosomal vesicle is located lateral to the apical pole of the nucleus (Fig. 17). During spermiogenesis the mitochondria congregate around the base of the nucleus (Fig. 13). The nucleus changes its shape from round to elongated. The condensation of chromatin continues and is almost complete in the late spermatids. Throughout spermiogenesis the developing spermatids are connected by intercellular bridges (Fig. 15).

Spermatozoon

In May the gonads contain spermatozoa only. The head of the spermatozoon is about 4 µm long (Figs 19–23). The spermatozoon has a cylindrical nucleus with an acrosome at its apical pole (Figs 18, 19). The cup-shaped acrosomal vesicle consists of some homogenous electron-dense substance with a light centre. The granular periacrosomal material contains central light substances and peripheral dark substances. The acrosome remains in a lateral position relative to the nucleus (Figs 18, 19). The nucleus is rounded at its apical pole and it has a centriolar fossa on its basal pole (Figs 19, 21). The mid-piece of the spermatozoon comprises one ring-shaped mitochondrium (Fig. 20) which surrounds the basal part of the nucleus and the proximal and distal centrioles. An electron-dense vesicle which is thought to represent a storage vesicle is present in connection with the centrioles (Fig. 21) The distal centriole has an anchoring fiber apparatus that consists of nine radially oriented forked elements (Fig. 22). It is the basal body of the tail (flagellum), which has the typical arrangement of the axonemal microtubules (9+2).


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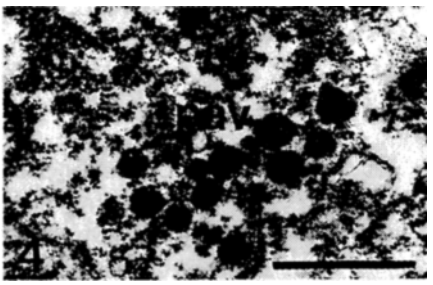
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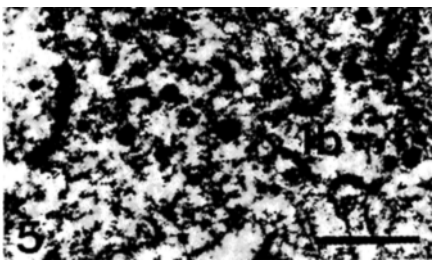
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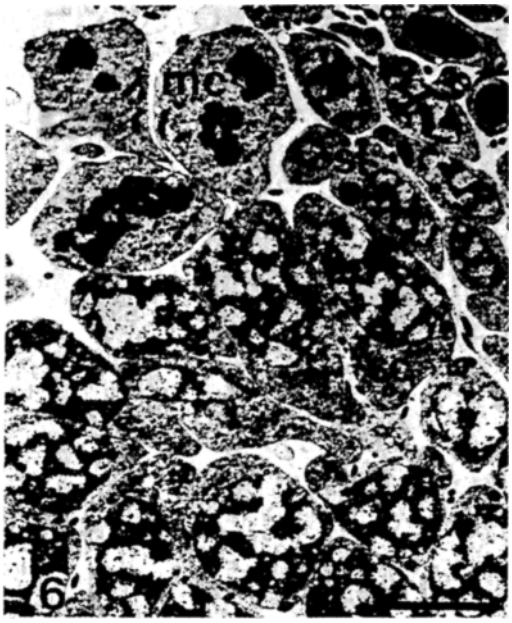
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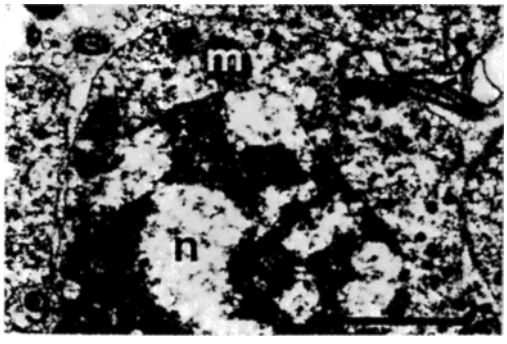
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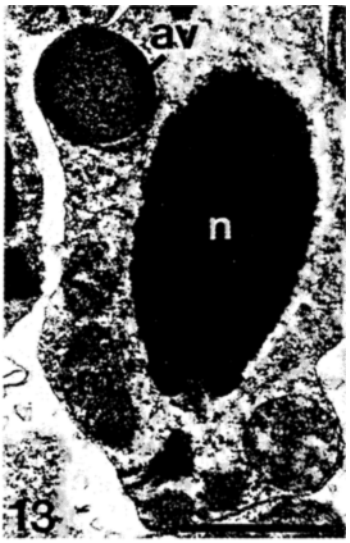
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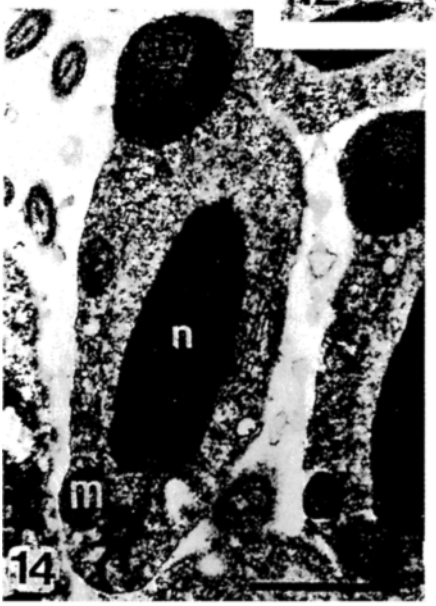
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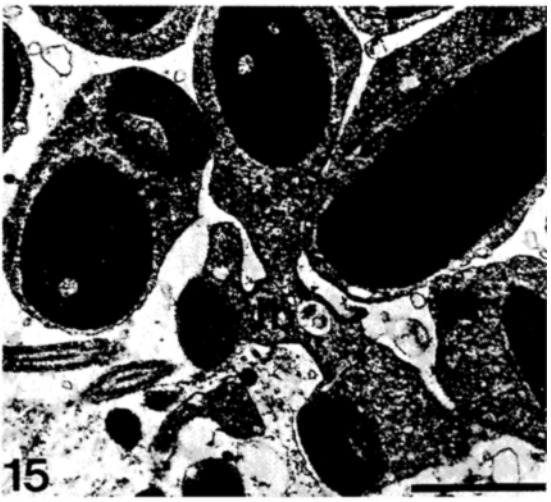
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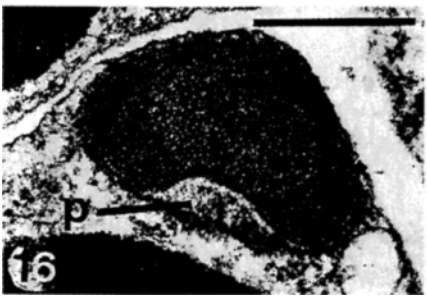
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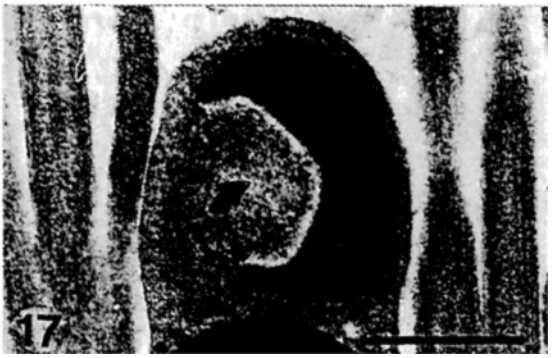
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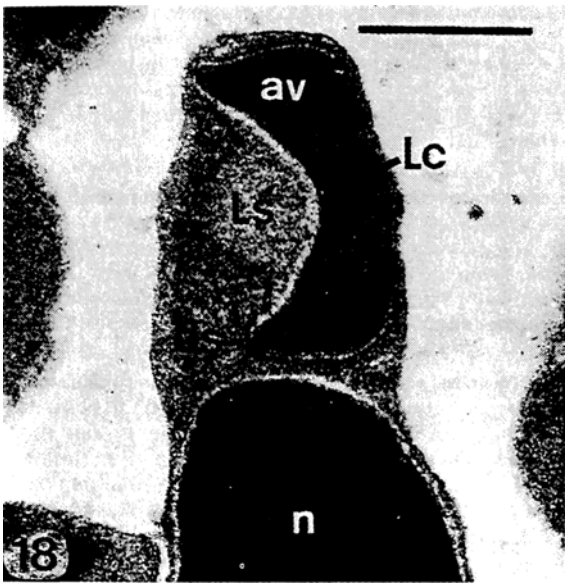
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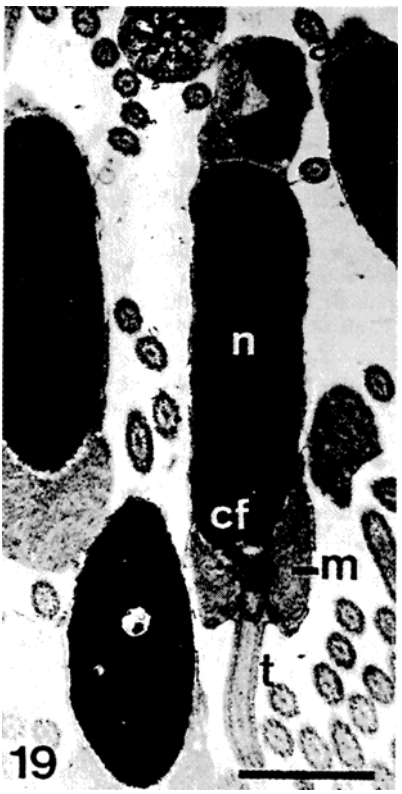
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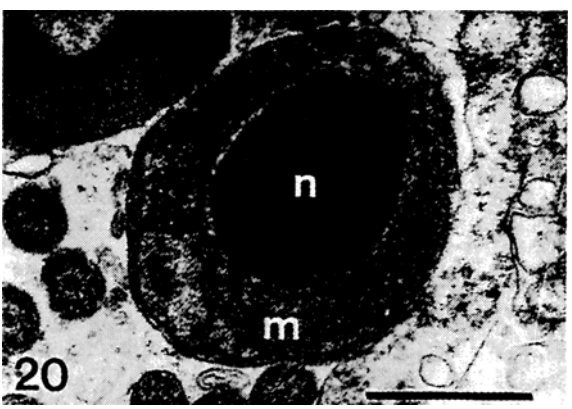
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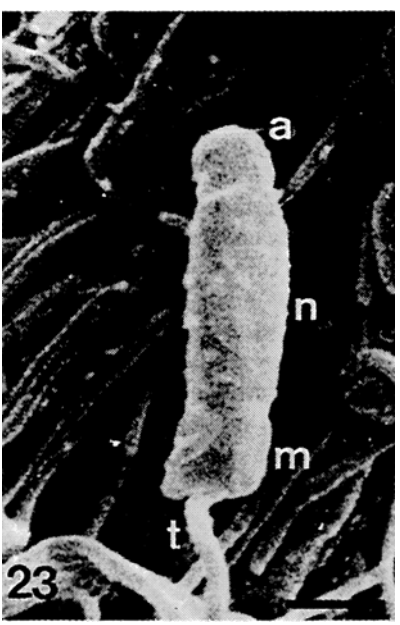
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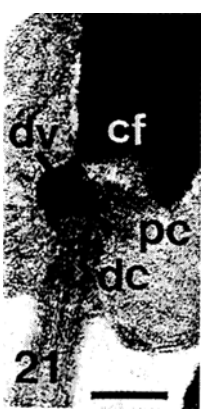
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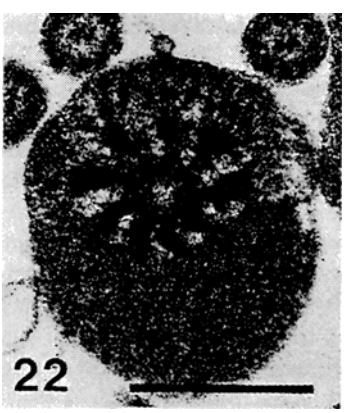
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Figs 1–5. *Procephalothrix* sp. Transmission electron microscopy (TEM). 1: Spermatogonia (sg) in contact with the gonad wall. n – nucleus, nu – nucleolus, bl – basal lamina. Scale bar – 2 µm. 2: Somatic peritoneal cell (sc) lining the wall of the testis. sg – spermatogonia, bl – basal lamina. Scale bar – 3 µm. 3: Spermatogonium with flagella (f). Scale bar – 1 µm. 4: Proacrosomal vesicles (pav) in spermatogonia. Scale bar – 0.5 µm. 5: Intercellular bridge (Ib) between spermatogonia. Scale bar – 1 µm

Figs 6–10. *Procephalothrix* sp. TEM. 6: Spermatocytes (sc) and spermatids (st) in gonad of *Procephalothrix* sp. mc – meiotically dividing cells. Scale bar – 3 µm. 7: Primary spermatocyte. s – synaptonemal complex. Scale bar – 1 µm. 8: Primary spermatocytes connected by intercellular bridges (Ib). m – mitochondria, pav – proacrosomal vesicles. Scale bar – 2 µm. 9: Primary spermatocyte with flagella (f). m – mitochondrium, n – nucleus. Scale bar – 1 µm. 10: Meiotically dividing spermatocytes. n – nucleus. Scale bar – 1 µm

Figs 11–17. *Procephalothrix* sp. TEM. 11: Proacrosomal vesicles (pav) situated at the presumptive posterior pole of the spermatid. t – tail. Scale bar – 0.5 µm. 12: Acrosomal vesicle (av) in basal part of the spermatid. m – mitochondrion, dc – distal centriole, pc – proximal centriole, t – tail. Scale bar – 0.5 µm. 13: Acrosomal vesicle (av) migrating to lie at the presumptive anterior pole of the spermatid. n – nucleus, m – mitochondrion. Scale bar – 1 µm. 14: Acrosomal vesicle (av) changing form. n – nucleus, m – mitochondrion. Scale bar – 1 µm. 15: Spermatids connected by intercellular bridges (Ib). Scale bar – 1 µm. 16: Concave acrosomal vesicle (av) in spermatid. p – periacrosomal material. Scale bar – 0.5 µm. 17: Acrosomal vesicle situated lateral to the nucleus of spermatid. Arrow shows the periacrosomal material. Scale bar – 0.5 µm

Figs 18–23. *Procephalothrix* sp. TEM. 18: Acrosome of spermatozoon. av – acrosomal vesicle, lc – light centre of the acrosomal vesicle, ls – light substance of periacrosomal material, ds – dark substance of the periacrosomal material, n – nucleus. Scale bar – 0.5 µm. 19: Spermatozoon of *Procephalothrix* sp. a – acrosome, n – nucleus, cf – centriolar fossa, m – mitochondria, t – tail. Scale bar – 1 µm. 20: Ringshaped mitochondrium (m) in the mid – piece of spermatozoon. n – nucleus. Scale bar – 0.5 µm. 21: Mid-piece of spermatozoon. cf – centriolar fossa, dv – dark vesicle, pc – proximal centriole, dc – distal centriole. Scale bar – 0.3 µm. 22: Pericentriolar complex of distal centriole. Scale bar – 0.5 µm. 23: Scanning electron microscopy. Spermatozoon of *Procephalothrix* sp. a – acrosome, n – nucleus, m – mitochondria, t – tail. Scale bar – 1 µm

DISCUSSION

The gonad wall in *Procephalothrix* sp. consists of peritoneal cells with their basal lamina. Peritoneal cells forming the gonad wall are typical for nemertines (Stricker & Cavey, 1986; Turbeville & Ruppert, 1985; Reunov & Chernyshev, 1992; Jespersen, 1994) and many other invertebrates. The exception was described for the nemertine *Tubulanus rhabdotus* when these cells were absent in the male gonads (Turbeville & Ruppert, 1985).

The developing sperm cells in the gonads of *Procephalothrix* sp. are connected by intercellular bridges but they are not aggregated in clusters as is characteristic for *Tetrastemma* sp. (Stricker & Cavey, 1986; Reunov & Chernyshev, 1992). It is likely that the organization in clusters is a more advanced character among the nemertines.

It was shown that the flagellum and/or proacrosomal vesicles are common in prespermiogenic cells of *Procephalothrix* sp. as in other marine invertebrates such as sponges (Paulus, 1989), cnidarians (Dewel & Clark, 1972; Schmidt & Holtken, 1980; Larkman, 1984), polychaetes (Eckelbarger, 1984), echinoderms (Longo & Anderson, 1969; Kato & Ishikawa, 1982; Yamashita, 1983), chitons (Hodgson et al., 1988), bivalves (Reunov &

Hodgson, 1994), brachiopods (Hodgson & Reunov, 1994), sipunculids (Klepal, 1993; Reunov & Rice, 1993), priapulids (Adrianov et al., 1991).Therefore, it may be assumed that this early development of a flagellum and proacrosomal vesicles is characteristic of the development of a primitive sperm. The possible reasons for early arising flagella and proacrosomal vesicles were recently discussed (Hodgson & Reunov, 1994; Reunov & Hodgson, 1994). Yamashita (1983) suggested that the early development of the acrosome in the Ophiuroidea is correlated with its relatively large size and the short duration of spermatogenesis. Pre-spermiogenic development of the proacrosomal vesicles was also observed in holothurians the sperm of which has a large acrosome (Atwood, 1974). The duration of spermatogenesis of the Holothurioidea is not particularly short (Reunov et al., 1984). It is also doubtful that the early formation of the acrosomal material in the spermatogenesis of priapulids, sipunculids, polychaetes, brachiopods, nemertines and bivalves could be explained by a short spermatogenesis. However, it might be that there is some correlation between the early arising of proacrosomal vesicles and the size of the acrosome. For example, in the brachiopod *Discinisca tenuis* the spermatozoon has a large acrosome and the proacrosomal vesicles are formed in the spermatogonium whilst in the brachiopod *Kraussina rubra* the sperm has a very small acrosome, formed during spermiogenesis (Hodgson & Reunov, 1994). Unfortunately, this correlation does not appear to exist in the spermatogenesis of nemertines. The average diameter of the acrosome of the modified sperm (development of the acrosome during spermiogenesis) of *Tetrastemma phyllospadicola* is about 1.1 µm and that of *Tetrastemma nigrifrons* is about 0.5 µm (see Stricker & Cavey, 1986; Reunov & Chernyshev, 1992). The average diameter of the acrosome of the *Procephalothrix* sp. sperm (pre-spermiogenic development of the acrosome) is about 0.8 µm, so that the correlation between the sizes of the acrosomes and the beginning of the formation of the proacrosomal vesicles is not conclusive in this case. As an alternative it may be assumed that the pre-spermiogenic development of acrosomal elements is an ancestral feature characteristic for the development of the primitive spermatozoa.

Supposedly the flagella might be a character not only of early spermatogenical cells but also of early ovogenical cells. As was shown in the actinia *Actinia fragacea* (Larkman, 1984), the early germinative cells of the female may have flagella and centriolar complexes identical to those found in the germinative cells of the male. It could be suggested that the flagella in early germinative cells may be interpreted as plesiomorphic or rudimental. This may provide evidence for the possibility that the origin of Metazoan gametes is to be found in monociliated ancestral cells (Reunov & Hodgson, 1994). The possibility that flagella may be rudimental was discussed for muscle cells of brachiopods, phoronids, echinodermates, polychaetes and vertebrates (see Gardiner & Rieger, 1980). These authors support Sorokin's idea (Sorokin, 1962) that rudimentary cilia in muscle cells represent vestiges inherited from a primitive ancestor.

It was not possible to observe the second spermatocytes. The dividing cells between the spermatocytes I and the spermatids present the meiotical maturation stages. So it may be assumed that during the spermatogenesis of *Procephalothrix* sp. the first and second divisions of maturation occur within the spermatocyte I.

It is surprising that *Procephalothrix spiralis* (Coe, 1930) as identified by Dr. A. V. Chernyshev, collected from the intertidal zone of the Ussurian Bay (Russia), has a different type of sperm compared with that of *P. spiralis* described by Turbeville & Ruppert (1985). Unfortunately, these authors did not mention where they got their animals. The

sperm of *P. spiralis* described by Turbeville & Ruppert (1985) has a typical primitive structure with the exception of a mitochondrial collar around the centrioles which is usually more characteristic of modified sperms. The spermatozoon of *Procephalothrix* sp. described in this paper has some primitive features like forked pericentriolar projections (Ferraguti, 1984) and the organization of the tail (9+2). There are also modified elements like an elongated nucleus and a mitochondrial collar around the centriolar complex. The sideway position of the acrosome relative to the axis of the spermatozoon is most likely an aberrant feature. The composition of the middle piece of the *Procephalothrix* sp. sperm could be compared with that in spermatozoa of *Amphiporus cruentatus* (Turbeville & Ruppert, 1985) and *Gononemertes parasita* (Franzén & Sensenbaugh, 1988). In these cases the centriolar apparatus is also surrounded by one circular mitochondrion. In *Malacobdella grossa* (Afzelius, 1971), *Tetrastemma nigrifrons* (Reunov & Chernyshev, 1992) and *Cerebratulus lacteus* (Longo et al., 1988) the neck region includes some separate mitochondria. The sperms of *Tetrastemma phyllospadicola* (Stricker & Cavey, 1986) and *Cephalothrix rufifrons* (Jespersen, 1994) have one elongated mitochondrium which is situated laterally in the middle part of the spermatozoon. It should be stressed that the storage vesicle being in contact with the centrioles of the *Procephalothrix* sp. sperm was not described in spermatozoa of other nemertines. In relation to the asymmetrical position of the acrosome the sperm of this nemertine is similar to the spermatozoa of *Cephalothrix rufifrons* (Jespersen, 1994) and *Tetrastemma phyllospadicola* (Stricker & Cavey, 1986). A comparison of the ultrastructure of the sperm of *Procephalothrix spiralis* from the Japan Sea with that described originally by Turbeville & Ruppert (1985) leads to the conclusion that the differences may be due to different geographical distribution of the species. Nevertheless, this finding raised the interesting question concerning the necessity for comparative analysis of these two *P. spiralis* species from different parts of the world.

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