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# Spermiogenesis and sperm ultrastructure of *Asetocalamyzas laonicola* Tzetlin, 1985 (Polychaeta), an ectoparasite of the large spionid *Scolelepis* cf. *matsugae* Sikorsfi, 1994, from the White Sea

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SUMMARY: The sperm ultrastructure and spermatogenesis of the ectoparasitic polychaete *Asetocalamyzas laonicola* Tzetlin, 1985 (Calamyzidae) is investigated. The male cells are located freely in the coelom. The spermatocytes are large cells of irregular shape; their nuclei have condensed chromatin in the periphery. Spermatocyte cytoplasm is granular and electron-dense with several spherical mitochondria. During early developmental stages spermatids are aggregated into a rosette (four cells). The early spermatids have a tiny acrosomal vesicle at one side of the cell, a few round mitochondria at another, and electron dense nuclei. The late spermatids have elongated mitochondria, a well-developed acrosome and a flagellum. The mature sperm are threadlike with a round acrosomal vesicle, an electron-dense structure. The elongated nuclei have anterior and posterior depressions. The supporting root zone of the acrosome is located behind the acrosomal vesicle in the anterior invagination of the nuclei. Six elongated mitochondria surround the flagellum and form the midpiece of the sperm. A single centriole lies in the posterior depression of the nucleus. The middle part of the flagellum possesses a normal (9+2x2) pattern. Probably, the terminal part of flagellum is modified. The sperm structure suggests internal fertilization or another type of specialized sperm transfer in *A. laonicola*.

*Keywords*: spermiogenesis, sperm ultrastructure, *Asetocalamyzas laonicola*, *Scolelepis* cf. *matsugae*.

RESUMEN: ESPERMATOGÉNESIS Y ULTRAESTRUCTURA DEL ESPERMA DE *ASETOCALAMYZAS LAONICOLA* TZETLIN, 1985 (POLYCHAETA), ECTOPARASITO DEL ESPIÓNIDO *SCOLELEPIS* CF. *MATSUGAE* SIKORSFI, 1994, DEL MAR BLANCO. – La ultraestructura del esperma y la espermatogénesis del poliqueto ectoparásito *Asetocalamyzas laonicola* Tzetlin, 1985 (Polychaeta) ha sido investigada. Las células masculinas están localizadas libres en el celoma. Los espermatocitos son células grandes de forma irregular cuyos núcleos presentan cromatina condensada en su periferia. El citoplasma de los espermatocitos es granular y presenta alta densidad a los electrones, así como distintas mitocondrias esféricas. Durante el desarrollo inicial, las espermatidas se encuentran agregadas en rosetas de cuatro células. Las espermatidas iniciales presentan una minúscula vesícula acrosomal a un lado de la célula, unas pocas mitocondrias redondas y un núcleo denso a los electrones. En su fase más tardía, las espermátidas contienen mitocondrias alargadas, un buen desarrollado acrosoma y un flagelo. El esperma maduro parece estar enebrado con una vesícula acrosomal redonda y una estructura densa a los electrones. El núcleo alargado presenta depresiones posteriores y anteriores. La zona raiz de soporte del acrosoma se encuentra localizada detrás de la vesícula acrosomal, en una invaginación anterior del núcleo. Seis mitocondrias alargadas rodean el flagelo y forman la pieza central del esperma. Un centriolo único descansa en la depresión posterior del núcleo. La parte central del flagelo posee un patrón normal (9+2x2). Probablemnente la parte terminal de dicho flagaleo está modificada. La estructura del esperma sugiere una fertilización interna u otro tipo de transferencia del esperma muy especializada en *A. laonicola*.

*Palabras clave*: espermegiogénesis, ultraestructura del esperma, *Asetocalamyza laonicola*, *Scolelepis cf. matsugae*.

## INTRODUCTION

*Asetocalamyzas laonicola* Tzetlin 1985 is an aberrant polychaete and a parasite of a large spionid polychaete inhabiting the subtidal zone of the White Sea. In the original description of *A. laonicola*, *Laonice cirrata* (Spionidae) was named as its host (Tzetlin, 1985). Further investigations revealed that the host spionid was probably undescribed and unknown for the White Sea. These spionid polychaetes are morphologically close to *Scolelepis matsugae* described by Sikorski (1994) from the Barents Sea. However, the taxonomic position of the latter species is doubtful because species of the genus *Scolelepis* are characterized by a pointed prostomium, whereas in *Scolelepis matsugae* the prostomium is blunt with three semicircular anterior projections (Sikorski, 1994, our data).

*A. laonicola* is an obligate ectoparasite that attaches to the host by inserting its pharynx into the body cavity through dorsal integument of the host. In the area where the pharynx of *A. laonicola* is embedded in the spionid body, the tissues of parasite and host fuse so that the border between them is undetectable even at the ultrastructure level. *A. laonicola* is oriented along the dorsal longitudinal axis of the host's body. One to three parasites may be found on an individual *S. matsugae* . *A. laonicola* is a small dorso-ventrally flattened polychaete-with a body width ( at the 1st segment without parapodia) of up to 0.5 mm and a body length of up to 2.5 mm. The number of segments is 9-14. The small eye patches are located at the perimeter of the prostomium in the smallest individuals only. Larger specimens lack eyes (our data).

In the original description (Tzetlin, 1985), *A. laonicola* was referred to the family Calamyzidae. *A. laonicola* differs from *Calamyzas amphictenicola*, also an obligatory polychaete ectoparasite, by the absence of prostomial appendages, podial tentacles, and any traces of chaetae and aciculae. The internal structure of *A. laonicola* slightly resembles that of *Calamyzas amphictenicola* (systems of organs are well developed in both species), though the structure of the intestine, nervous system, and body musculature are quite different. The position of *A. laonicola* in the Annelida is still unclear. Knowledge of the internal morphology of *A. laonicola* is very fragmentary (Tzetlin, 1985), and the absence of chaetae, aciculae, and any body appendages provides no information about its relationship with any other annelid. There is no information on the biology, reproductive mode and mode of sperm transfer of *A. laonicola*. Thus it is important to know the structure of a spermatozoon to comprehend the method of fertilization. The aim of the present study is to describe the sperm structure and spermiogenesis in *A. laonicola*.

## MATERIALS AND METHODS

In 1995-2003, eleven *A. laonicola* were found for the first time since the original discovery in 1985 in habitats typical for the species. Material was collected in August 1996, 1997, 2001 and 2002 at depths of 18-20 m near the Biological Station of the Moscow State University, Kandalaksha Bay, the White Sea, Russia.

Specimens for electron microscopy were fixed in 2.5% glutaraldehyde, buffered with 0.2 M sodium cacodylate buffer containing 0.3-0.36 M sucrose (pH 7.2-7.4). After rinsing in buffer, specimens were postfixed with 1% osmium tetroxide in the same buffer and dehydrated in a graded ethanol series followed by acetone. For transmission electron microscopy (TEM) specimens were embedded in Epon 812 resin. Semi-thin and ultra-thin sections were cut on a LKB microtome. Semi-thin sections were stained with 1% toluidine blue. The sections were examined in Jeol JEM 100-CX transmission electron microscopes.

Particular body fragments were critical-point dried, coated with platinum-palladium, and examined with a HITACHI 400A scanning electron microscope (SEM).

## RESULTS

Gametes lie free in the body cavity occupying almost the entire inner cavity of a segment in some sections and a large part of it in others. In one of the specimens studied, the ducts of segment nephridia were filled with mature spermatozoa, suggesting that the nephridia may serve as sperm ducts.

The following stages of spermiogenesis were found in the body cavity of mature *A. laonicola* males: early spermatids, late spermatids, and mature spermatozoa. The early spermatids (Figs. 1A, 2A) have an irregular oval or round shape 3.34-4.86 $\mu$m in diameter. Their cytoplasm is granular and electron-dense (Fig. 2A, B). The spherical nucleus,

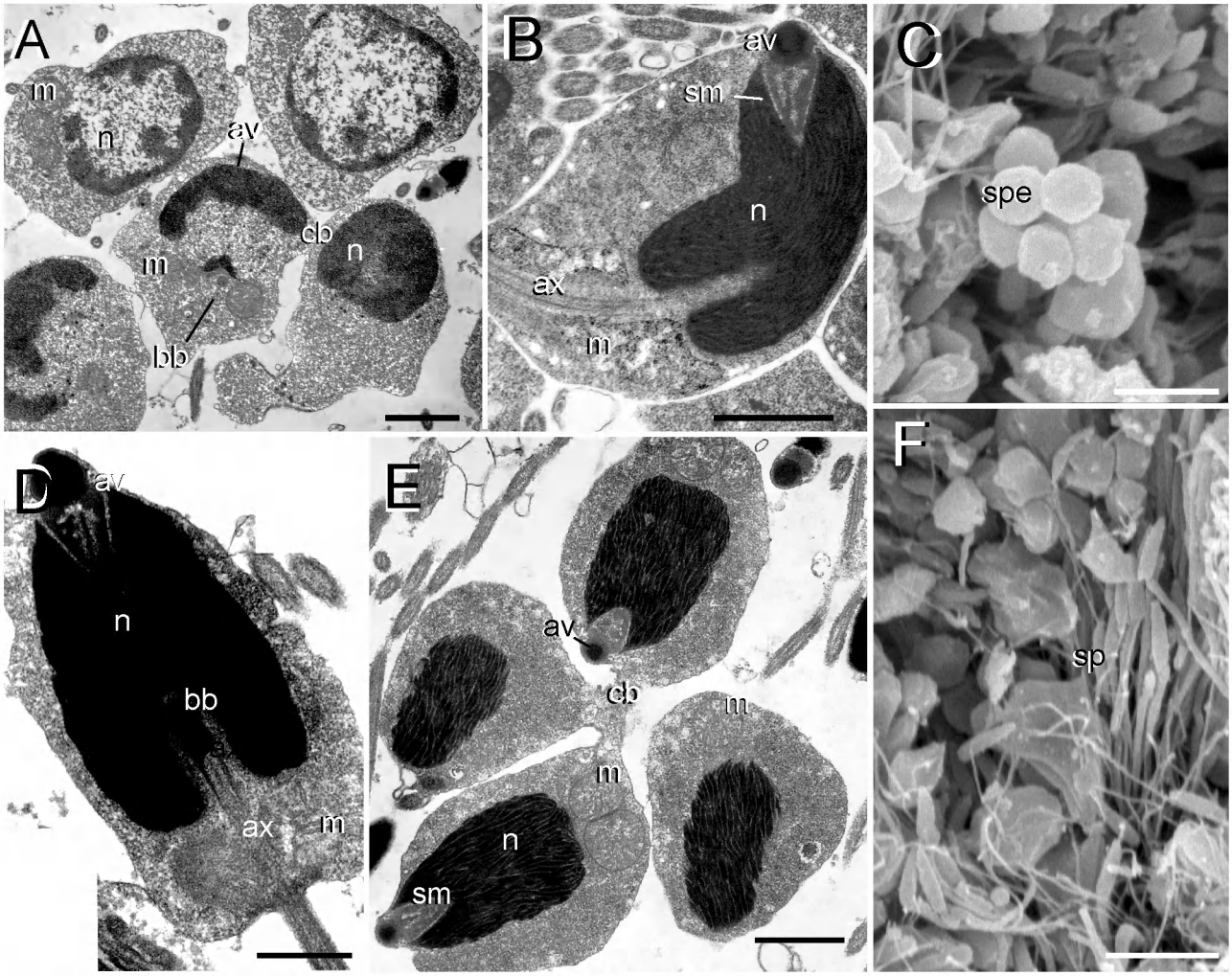


FIG. 1. – *Asetocalamyzas laonicola*: A, early spermatids connected with each other by the cytoplasmic bridges (TEM); B, spermatid with elongate mitochondria and two nuclear depressions (TEM); C, several spermatids connected by the bridge (SEM); D, late spermatid with round mitochondria and nucleus with two depressions (TEM); E, late spermatids connected by cytoplasmic bridge (TEM); F, mature spermatozoon and spermatids inside the body cavity (SEM). Scale: A, B, 1 μm; C, 1.5 μm; D, 0.5 μm; E, F, 1.5 μm.

2.45-3.02 $\mu$m in diameter, occupies almost the entire cell. Condensed chromatin at the periphery of the nucleus was up to 0.43 $\mu$m across and forms granules in the centre of the nucleus up to 0.64 $\mu$m in diameter. The central part of the nucleus is light-coloured. On one side, the cytoplasm contains a number of round mitochondria with a maximum diameter of 0.70 $\mu$m (Figs. 1A, 2A). Spermatids at this stage are assembled in tetrads.

The late spermatids are characterized by a nucleus with more condensed chromatin than early spermatids (Fig. 1A). The nucleus has an irregular round or oval shape; it is 2.67-3.84 $\mu$m in diameter and is filled with more or less condensed chromatin (Fig. 2B). Chromatin condensation starts from the zone adjacent to the zone of acrosome formation. A small posterior depression is noticeable on the nucleus at this stage. This depression is the second centre of chromatin condensation. A flattened acrosomal prominence 0.22 × 0.73 $\mu$m is formed on one side of the spermatid. The prominence has a distinct round electron-dense acrosomal vesicle (Figs. 1A, 2B). Round mitochondria up to 0.86 $\mu$m in diameter are located opposite the acrosome; a maximum of three mitochondria was found within a section. The cytoplasm and the central parts of the nuclei of these cells are coloured more intensely than in the early spermatids. During this stage, the spermatids are connected by cytoplasmic bridges that form links between spermatids at different stages of development (Fig. 1B, C). Cross-sections of late spermatids reveal a single centriole in the posterior depression of a nucleus (Figs. 1A, 2B). The flagellum developed in the next stage.

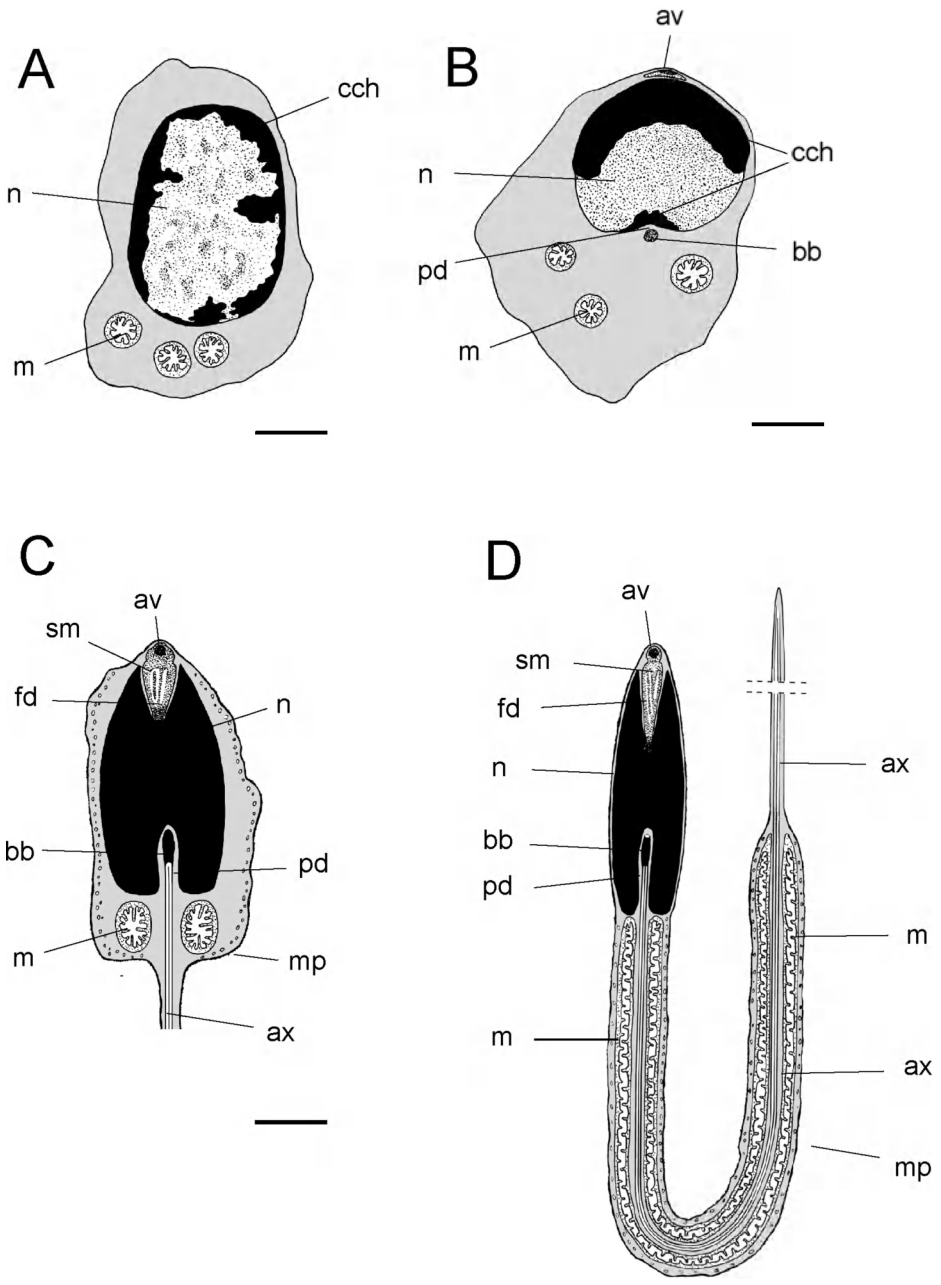


FIG. 2. – *Asetocalamyzas laonicola*, reconstruction of spermatogenesis: A, early spermatid at the first stage, nucleus without depressions; B, early spermatid at the second stage (posterior depression in the nucleus, acrosomal vesicle and basal body in the cytoplasm); C, late spermatid, nucleus with two depressions. Frontal depression contains acrosome, posterior depression contains basal body. midpiece and two well developed depressions. Scale: A, B, C, D, 1.0 μm.

During the next stage, the spermatids become elongated with the maximum size of 4.08 $\mu$m (Fig. 1B, D, E). The chromatin of the nucleus is completely condensed (Fig. 2C). The acrosome is located in the frontal depression of the nucleus and the depth of the depression reaches 0.97 $\mu$m (Fig. 1B). The round electron-dense acrosomal vesicle is up to 0.33 $\mu$m in diameter and the subacrosomal space which changes its structure depending on the rate of spermatozoon maturity is distinguishable in the acrosome (Figs. 1B, 2C). Granular cytoplasm still occupies a relatively large volume. During this stage, late spermatids are still connected by cytoplasmic bridges (Fig. 1E).

The mature spermatozoon is elongated and torpedo-shaped and has a long flagellum (Figs. 1F, 2D, 3A, K). The distal centriole is located in the posterior depression of the nucleus whilst the proximal one

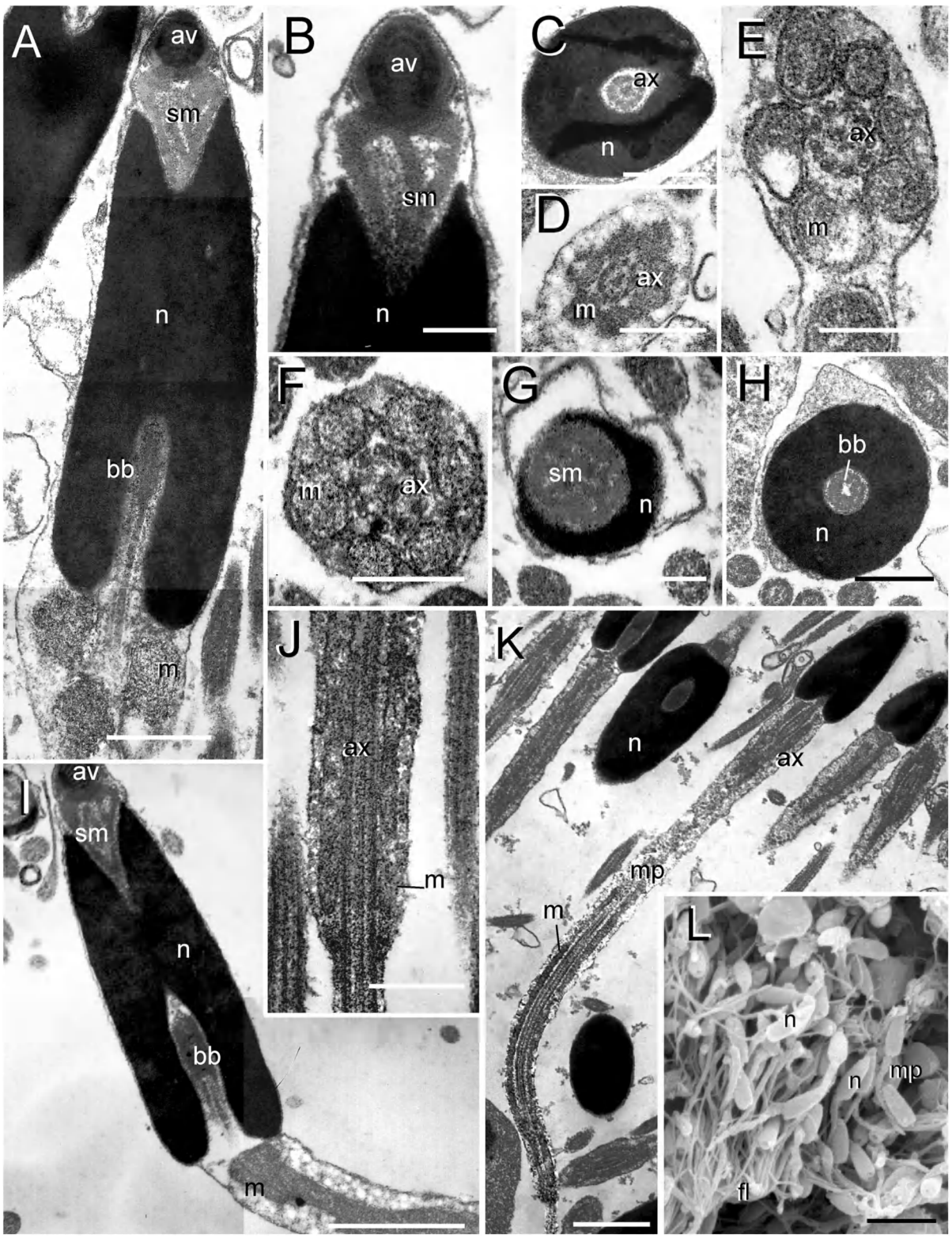


FIG. 3. – *Asetocalamyzas laonicola*, mature spermatozoon: A, longitudinal section through nucleus (TEM); B, acrosome of late spermatid, subacrosomal space well developed (TEM); C, transverse section through the posterior depression of mature spermatozoon (TEM); D, transverse section through transitional zone between midpiece and flagellum of mature spermatozoon, six mitochondria are seen (TEM); E, transverse section through midpiece, six mitochondria around the flagellum (TEM); F, transverse section through the midpiece, axonema has typical structure (9x2 + 2) (TEM); G, transverse section through nucleus, frontal depression (TEM); H - transverse section through nucleus, posterior depression (TEM); I, longidudinal section through nucleus and midpiece. Mitochondria have bright white vesicles along their whole length (TEM); J, transitional zone between midpiece and flagellum (TEM); K, midpiece, longitudinal section (TEM); L, spermatozoa in the body cavity (TEM). Scale: A, B, C, E, G, H, J, 0.5 μm; D, I, K, 1.0 μm; F, 0.3 mm; L, 3.0 μm.

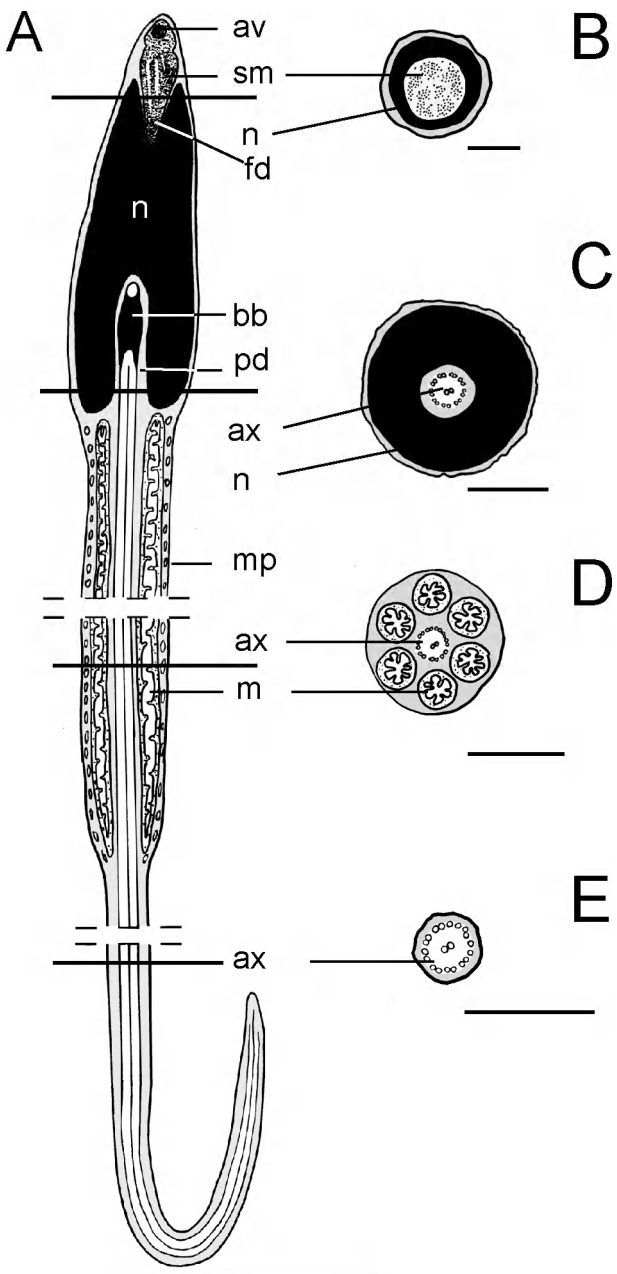


FIG. 4. – Asetocalamyzas laonicola, reconstruction of the mature spermatozoon: A, longitudinal section; B, transverse section through the subacrosomal space; C, transverse section through the nucleus, posterior depression; D, transverse section through the midpiece; E, transverse section through the flagellum. Scale: 1 μm. Abbreviations: m, mitochondria; av, acrosomal vesicle; n, nucleus; cb, cytoplasmic bridge; bb, basal body; sm, subacrosomal space; ax, axoneme; spe, spermatid; sp, spermatozoon; cch, condensed chromatin; pd, posterior depression of the nucleus; fd, frontal depression of the nucleus; mp, midpiece; fl, flagellum.

has disappeared. The following four parts can be distinguished: acrosomal zone, nuclear zone, midpiece, and flagellum (Fig. 4A). The acrosome is located in the frontal nuclear depression up to 1.35 $\mu$m deep and 0.45 $\mu$m wide (Fig. 3A, B, G). The round acrosomal vesicle is up to 0.48 $\mu$m in diameter and intensively coloured. The acrosomal vesicle overlies the subacrosomal space for 0.9 $\mu$m. The light colour and longitudinal striation of the subacrosomal space is characteristic of the late stages of spermiogenesis (Figs. 2D, 3B).

The nucleus is elongated with deep anterior and posterior depressions. It is 0.9 $\mu$m wide, 2.7 $\mu$m long, and completely electron-dense (Figs. 3 A, 4). The posterior depression is narrower and longer than the anterior one (1.2-1.5 $\mu$m long, 0.15-0.21 $\mu$m wide) and contains a single centriole 0.4 × 0.15 $\mu$m (Figs. 3A, C, H, 4A). In the anterior part, six long, round, or oval mitochondria coloured less intensively than the nucleus are arranged symmetrically around the flagellum (Fig. 3E, F). The length of mitochondria is 16.4 $\mu$m, the cross-section size is 0.15 × 0.21 $\mu$m (Fig. 3K). Mitochondria have bright white vesicles at the periphery up to 0.05 $\mu$m in diameter along their entire lengths (Fig. 3I). Mitochondria get smaller in diameter near the transitional zone between the midpiece and the flagellum (Figs. 3D, G, 4A).

The length of the flagellum could not be measured because no sections passed along the entire length; the maximal measured length reached 9.72 $\mu$m. The flagellum has a normal (9 + 2 × 2 microtubes) structure (Fig. 4E) and begins immediately behind the centriole (basal body (9+0 microtubes)) (Figs. 2D, 4C). In the midpiece, the structure is also normal (Figs. 3F, 4D).

## DISCUSSION

The general pattern of spermiogenesis in *Asetocalamyzas laonicola* is similar to that in other polychaetes. Stages of development representing gradual differentiation of gametes are the same from the initial stage, when the cells are connected with the cytoplasmic bridges (as shown on TEM photos in tetrads), to the final stage of mature threadlike spermatozoon. Certain traits of spermiogenesis are similar in sabellids and some syllids. In syllids *Typosyllis pulchra* (Heacox and Schroder, 1981) and *Sphaerosyllis hystrix* (Franzen, 1956), in Sabellinae (*Notaulax nudicollis*, *Jasmineira* sp.) (Rouse, 1999), *Oriopsis* sp. (Rice, 1992) spermatids are also assembled in tetrads. The spermatozoa are at different stages of maturity in one tetrad, as in *Petita amphophthalma* (Buhrmann *et al.*, 1996).

Spermiogenesis of *Asetocalamyzas laonicola* differs considerably from that of oligochaetes (Jamieson, 1992). In the latter, morulae are formed in the course of spermiogenesis. A morulae consists of spermatogonia connected by cytoplasmic bridges. Further differentiation may occur in the coelom or, more often, in the seminal vesicles with the diverticulae of the septa protruding into the adjacent segment. At the stage of 8-32 cells a morula contain a small cytophore, and at this stage morulae travel into the coelom or seminal vesicle. Up to the final stage of spermiogenesis, gametes are connected with the cytophore (Jamieson, 1992). Thus *A. laonicola* obviously does not belong to the Clitellata.

A nucleus of the mature spermatozoon of *A. laonicola* has two depressions. Anterior depressions are found in some species with spherical spermatozoa as in the magelonids *Magelona* sp., sabellids *Bispira melanostigmata* (Rouse, 1999) and nereids *Nereis diversicolor*, *Platinereis massiliensis*, *P. dumerilii* (Rice, 1992). The posterior depression with a single centriole (basal body) is present in a number of polychaetes, such as in the syllid *Petita amphophthalma*, in the interstitial hesionid *Hesionides arenaria* (Westheide, 1984), in the spionids *Polydora ligni* (Rice, 1981) and *Tripolydora* sp. (Rouse, 1988), as well as in some sabellids (such as two *Oriopsis* species (Rouse, 1992) and *Fabricia sabella* (Franzén, 1975)). Westheide (1984) suggested that such a structure enhanced sperm mobility and penetrative capacity. In *Pseudopolydora paucibranchiata* (Rice, 1992) and in the genus *Polydora* (Rice, 1981), the acrosome is located in the frontal depression of the nucleus during spermatogenesis, but not in mature spermatozoa. The midpiece of *A. laonicola* also differs greatly from that in other polychaetes. As a rule, a mature spermatozoon of *A. laonicola* has six elongated mitochondria. Such long mitochondria are known only for *Petita amphophthalma*; such a combination of the number and length of mitochondria is not observed in any other syllid species with spermatozoa of the introsperm type.

Mature spermatozoa in *Asetocalamyzas laonicola* and the second Calamyzidae species, *Calamyzas amphictenicola* are greatly modified. According to Franzén (1982), mature spermatozoa of *Calamyzas amphictenicola* lack a flagellum and have mitochondria and two centrioles in the cytoplasm. Rouse (1999) refers to spermatozoa such as this as being of the "introsperm" type.

Spermatozoa of *A. laonicola* differ considerably from oligochaete spermatozoa where the presence of acrosomal tubes and a particular midpiece structure are the characteristic traits (Jamieson, 1992).

*Asetocalamyzas laonicola* has modified thread-like spermatozoa containing the nucleus with two depressions, long midpiece, and a long flagellum. Based on these characteristics, the sperm of *A. laonicola* may be referred to as of introsperm type (Rouse, 1999) as are mature spermatozoa of *Calamyzas amphictenicola*. Spherical aflagellate spermatozoa of *C. amphictenicola* greatly differ from spermatozoa of *A. laonicola* (Franzén, Å. and S. A. Rice, 1988). Such sperm morphology suggests that *A. laonicola* is characterized by copulation, pseudocopulation or some other type of sperm transfer and inner fertilization. However, since no copulatory organs were found in *A. laonicola*, pseudocopulation appears to be likely and it probably occurs inside the host's (*Scolelepis*) tube.

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