

Ultrastructure of sperm morphology of *Trilobodrilus axi* and *T. heideri* (Dinophilidae, Polychaeta)

Petra Scharnofske*

II. Zoologisches Institut und Museum der Universität Göttingen; D-3400 Göttingen,
Federal Republic of Germany

ABSTRACT: The ultrastructure of the sperm of the dinophilids *Trilobodrilus axi* and *Trilobodrilus heideri* was investigated. The sperms are extremely modified and show an elongated head, middle piece, and tail region, which are overlapping. This condition is a synapomorphic feature for the dinophilids. The sperm structure has to be related to the specialized mode of sperm transfer by hypodermal injection in these species.

INTRODUCTION

The dinophilids *Trilobodrilus axi* and *Trilobodrilus heideri* display a specialized mechanism of copulation – the hypodermal injection of sperm (Ax, 1968; Westheide & Schmidt, 1974), as described in species in which the females do not have any organs for sperm uptake. Like other modified modes of internal fertilization, hypodermal injection is associated with the evolution of modified sperms, the morphology of which is related to the mode of sperm transfer (see Franzén, 1977b; 1982). Sperms with an elongated head and middle piece which show various modifications are typical of invertebrates with internal fertilization (Franzén, 1956). Furthermore, in sperms used in hypodermal injection the axoneme is extremely elongated and there are usually special supporting structures which can give stability to this very mobile sperm type (Franzén, 1975a, 1977a, b; Westheide, 1984a, b).

MATERIAL AND METHODS

Mature males of *Trilobodrilus axi* Westheide 1967 (from intertidal areas of the Island of Sylt, North Sea) and *Trilobodrilus heideri* Remane, 1925 (from subtidal sediments off the Island of Helgoland, North Sea; for data of the collecting site see von Nordheim, 1984) were investigated with TEM techniques. After relaxation in a MgCl₂-solution the animals were fixed at 4 °C in SPAFG (sucrose-picric-acid-formaldehyde-glutaraldehyde) in phosphate buffer (+10 % sucrose) for 1h (Ermak & Eakin, 1976). Then the specimens were rinsed in phosphate buffer (+10 % sucrose; pH 7.3) for 2h, postfixed at 4 °C in phosphate-buffered osmium tetroxide for 1h, dehydrated in an ethanol series,

*Present address: Fachbereich Biologie/Chemie der Universität Osnabrück, Spezielle Zoologie; Postfach 4469, D-4500 Osnabrück, Federal Republic of Germany

embedded in an Epon-Araldite mixture, and sectioned with a diamond knife on a Reichert Ultracut. The sections were stained with aqueous uranyl acetate and lead citrate; the photographs were taken with a Zeiss EM 10.

RESULTS

Trilobodrilus axi

Form and size

The mature sperm of *Trilobodrilus axi* is elongated and thread-like (ca 200 μm long). Acrosome, nucleus, mitochondria, and axoneme partly run parallel to each other (Fig. 1A). Therefore, no clear differentiation between head, middle piece, and tail region can be made. These parts cannot be distinctly differentiated with light microscope techniques (for a photograph of living sperms see Westheide, 1984a). The sperm diameter is 0.12 to 0.35 μm in the acrosomal region, up to 0.6 μm in the nuclear region, and 0.7 μm in the mitochondrial region. Due to the shape of acrosome and nucleus and the pattern of supporting structures in the mitochondrial region the sperm has a bilaterally symmetrical structure. (In the following text the term proximal refers to the acrosomal part, and distal to the tail region of the sperm.)

Acrosome

The elongated and tapering acrosomal vesicle has a bilaterally symmetrical structure and extends to the beginning of the mitochondrial region (Fig. 1A). It consists of an inner and an outer membrane (distance ca. 35 nm). Between the two membranes a homogeneous grey substance can be observed. The acrosomal vesicle surrounds a central rod-like structure apically like a ring and basally like a horseshoe. The rod-like structure has radiate spurs directed towards the acrosomal vesicle. The distal part of the acrosomal vesicle envelops the nucleus like a cap (Figs 1A, 2A, C).

Nucleus

The elongated rod-like nucleus originates in the acrosomal region and extends into the mitochondrial region. Proximally it is tube-like and encloses the axoneme; here the nuclear material is only 15–20 nm thick (Fig. 2A, C, 4C). More distally the nucleus surrounds the axoneme like a furrow which enlarges to form a massive rod with a triangular cross-section with rounded edges (Fig. 2A). The nucleus consists of electron-dense homogeneous chromatin material (length ca. 5.3 μm).

Abbreviations:

a	acrosome	gp	glycogen particles
an	anulus region	md	mitochondrial derivatives
ax	axoneme	mi	mitochondrion
bb	basal body	mt	microtubule
cb	cytoplasmic bridge	n	nucleus
ec	electron dense cap	rs	rod-like structure
er	electron dense ring	ss	supporting structure
g	Golgi-apparatus		

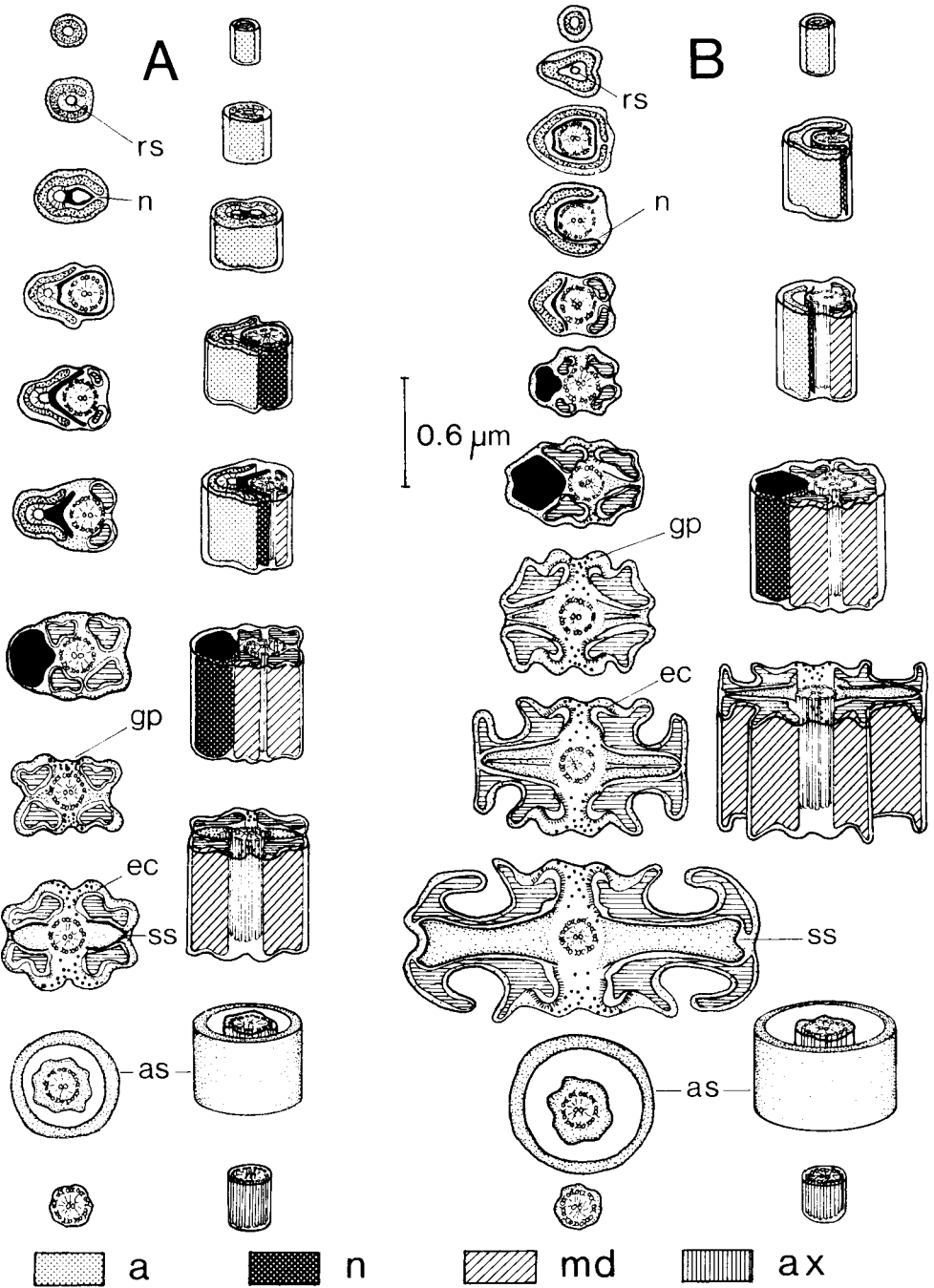


Fig. 1. Semidiagrammatic representation of the sperms of A: *Trilobodrilus axi* and B: *Trilobodrilus heideri*

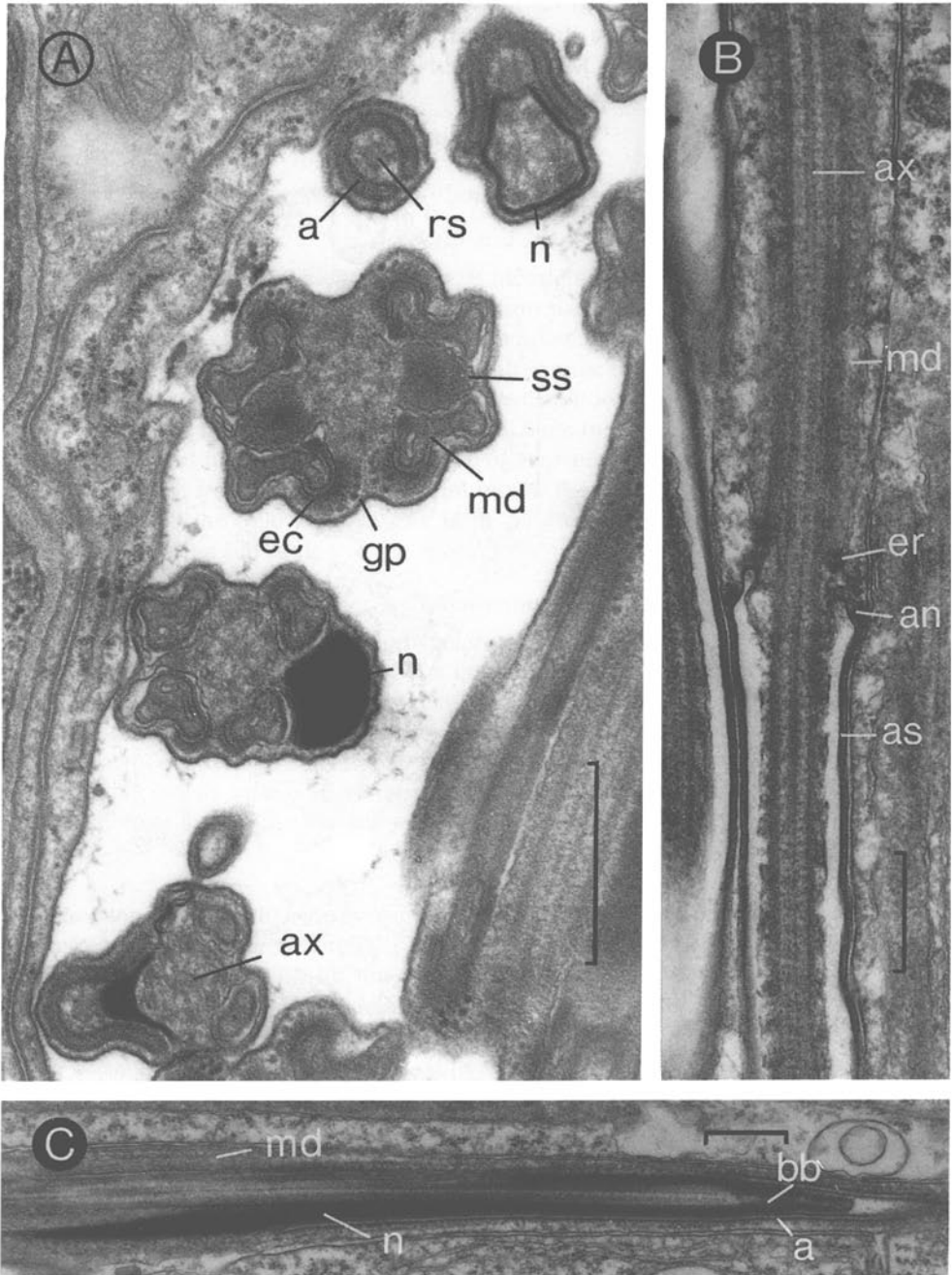


Fig. 2. *Trilobodrilus axi*, *A*: Cross-sections through different parts of the mature sperm. *B*: Longitudinal section through the annulus region and the tail region proper of a late spermatid. *C*: Longitudinal section through the proximal nuclear region of a late spermatid. Scale bar: 0.5 μ m

Mitochondrial derivatives

The four elongated mitochondrial derivatives, which are formed by fusion of several spherical mitochondria during spermiogenesis, begin in the distal part of the acrosomal region and run parallel to nucleus and axoneme; they do not show the typical cristae-structure (Fig. 1 A). Proximally the cross-section of the mitochondrial derivatives is oval or bean-shaped; distally they have the shape of dumb-bells (Fig. 2 A). Those protrusions of the mitochondrial derivatives which are situated near the axoneme show electron-dense caps close to the cell membrane. Between these dark caps and the cell membrane glycogen-particles arranged in rows can be found in the largest part of the mitochondrial region (Fig. 2 A).

Supporting structures

In the major part of the mitochondrial region two electron-dense cytoplasmic structures between axoneme and cell membrane can be observed; they show a regularly fine-striped substructure in longitudinal sections. These structures occupy the space between the parallel mitochondrial derivatives. They join the axoneme and the mitochondrial membrane and have a cone-shaped cross-section (Fig. 2 A). In longitudinal sections they present a rod-like shape; they probably serve as supporting structures (Fig. 1 A).

Axoneme

The basal body of the axoneme is situated in the proximal tip of the nuclear tube (Fig. 2 C). Centriolar structures could not be observed. The axoneme runs parallel to the nucleus and the mitochondrial derivatives (Fig. 1 A). Its microtubules show the typical 9 + 2 pattern. Immediately underneath the mitochondrial derivatives there is a double electron dense ring (Fig. 2 B). At the junction of the mitochondrial region and the tail region proper an anulus is found (Fig. 2 B) behind which an extensive folding of the cell membrane forms a cylindrical sheath (diameter ca 0.6 μm) around the axoneme. This sheath is filled with homogenous grey material (Figs. 2 B, 4 A). The distal part of the axoneme is only surrounded by a single membrane.

Trilobodrilus heideri

Form and size

The thread-like mature sperm of *Trilobodrilus heideri* is about 200 μm long and has a diameter of 0.12–0.4 μm in the acrosomal region, up to 0.75 μm in the nuclear region, up to 2 μm in the mitochondrial region and 1 μm in the tail region. A division into head, middle piece and tail cannot be made, because acrosome, nucleus, mitochondrial derivatives, and axoneme partly run parallel. Due to form and position of acrosome, nucleus and mitochondrial derivatives the sperm has a bilaterally symmetrical shape (Fig. 1 B).

Acrosome

The acrosomal vesicle extends into the beginning of the mitochondrial region. It begins with a tube-like part which then envelops nucleus and axoneme and more distally surrounds the nucleus like a furrow (Fig. 1 B, 5 D). The acrosome consists of an inner and an outer membrane between which is a layer of homogeneous grey material; it surrounds a central rod-like structure.

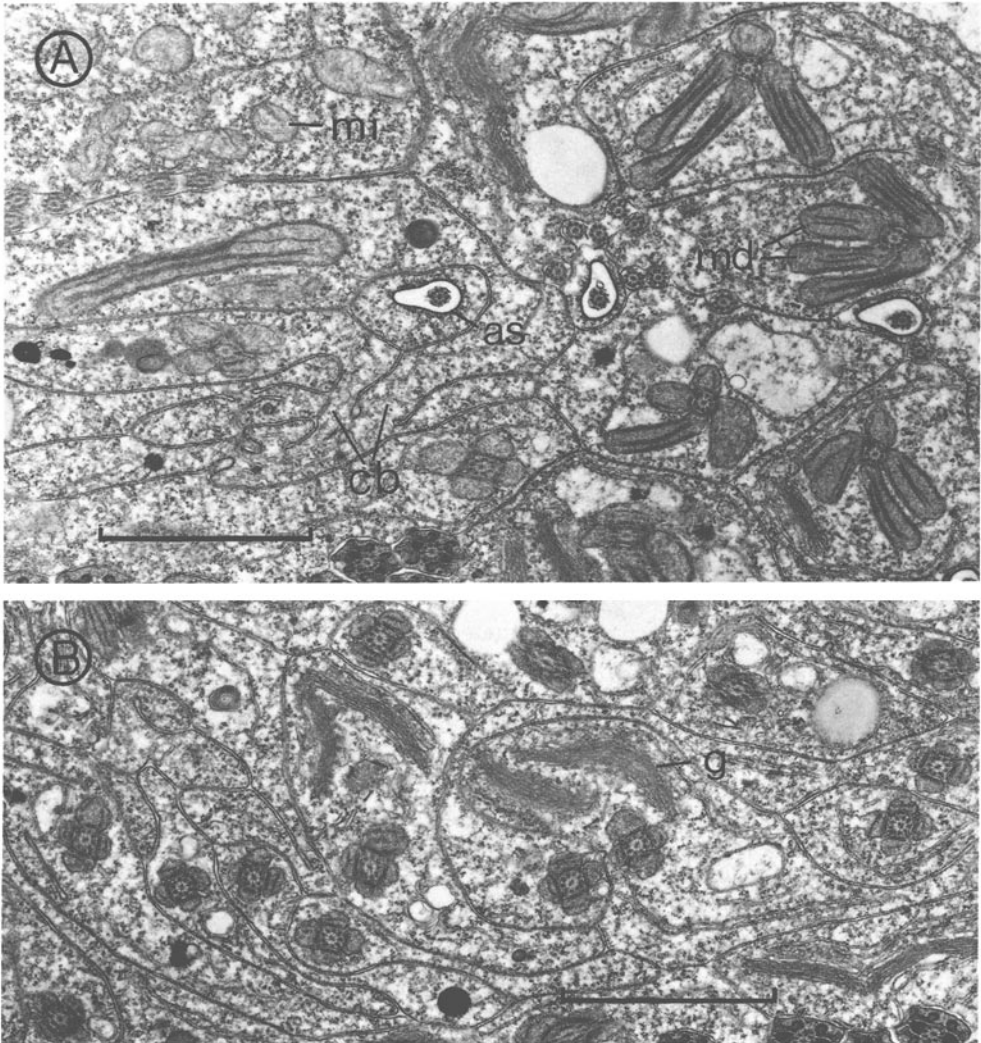


Fig. 3. *Trilobodrilus axi*. A and B: Testes with different stages of spermatids. Scale bar: 2 μm

Nucleus

The elongated rod-like nucleus originates in the middle of the acrosomal region and runs a considerable distance into the mitochondrial region. Proximally it encloses the axoneme completely, then it surrounds the axoneme like a furrow (Fig. 5 D); distally it forms a rod-like structure with a roughly triangular cross-section (diameter 0.3 μm ; Fig. 5 A).

Mitochondrial derivatives

Four rod-like mitochondrial derivatives begin directly behind the acrosome and run parallel to nucleus and axoneme (Fig. 1 B). More distally, the cross-sections increasingly show extreme protrusions. The cell membrane of the sperm follows these protrusions of

the mitochondrial derivatives; therefore, the sperm cross-sections have a bizarre shape (Fig. 5 A–C). In longitudinal sections these protrusions and the peripheral parts of the mitochondrial derivatives show a fine obliquely striped substructure (Fig. 5 C).

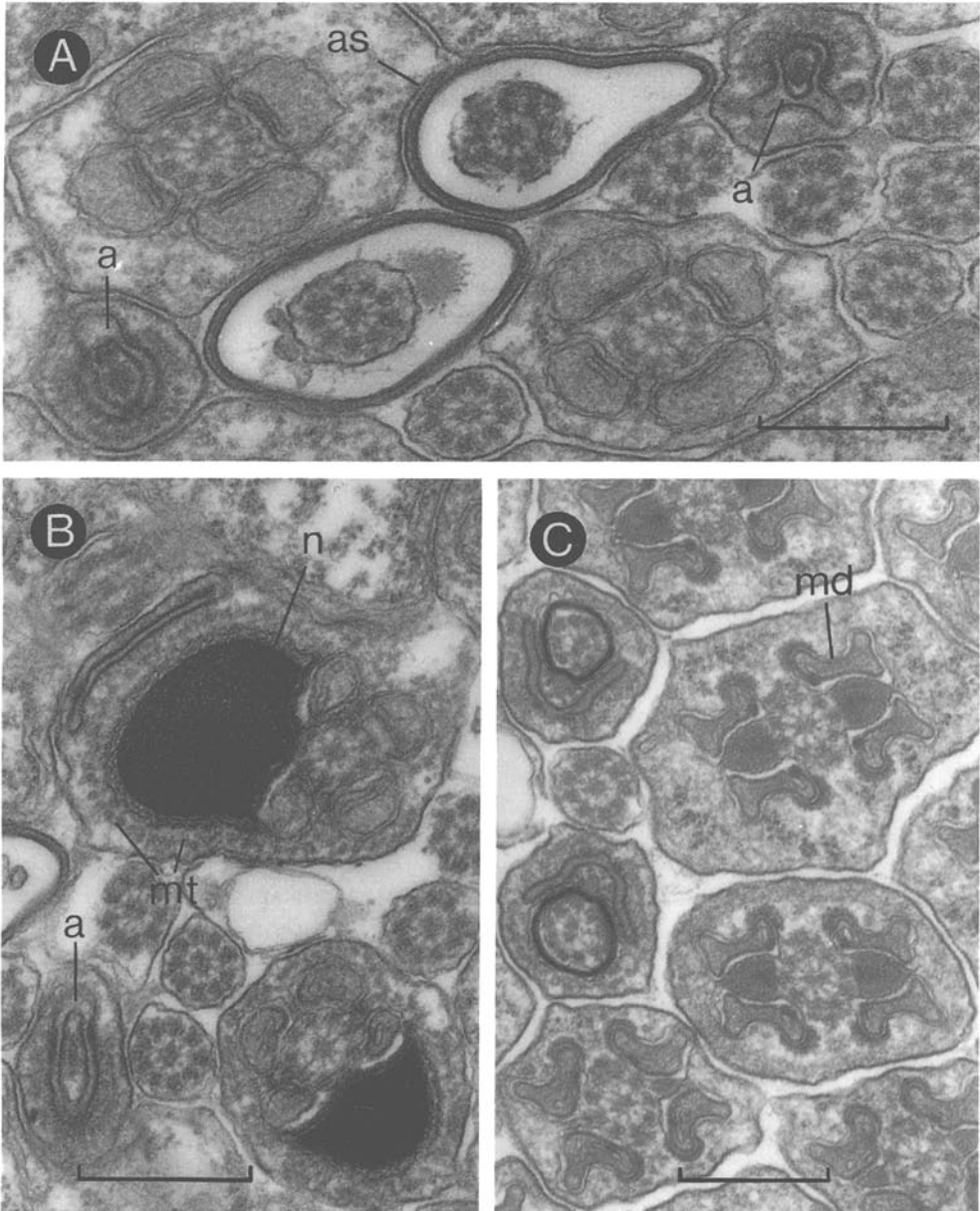


Fig. 4. *Trilobodrilus axi*. Cross-sections of spermatids. A: Acrosomal, mitochondrial, and tail region. B: Nuclear region surrounded by microtubules. C: Nuclear and mitochondrial region of late spermatids. Scale bar: 0.5 μm

Supporting structures

Between the two parallel mitochondrial derivatives, electron-dense, rod-like cytoplasmic structures extend through the mitochondrial region. They show a light margin and a darker matrix and have a rectangular cross-section with rounded edges (up to $0.25 \times 0.8 \mu\text{m}$). These structures can be interpreted as supporting structures. Between those parts of the mitochondrial derivatives which do not enclose the supporting structures, rows of electron-dense, dark glycogen particles can be observed (Fig. 5 A–C).

Axoneme

The axoneme begins in the proximal part of the acrosomal region and runs parallel to acrosome, nucleus and mitochondrial derivatives. No centriolar structures could be found. Behind the anulus region the proximal part of the tail region proper is surrounded by a cylindrical sheath (diameter $1 \mu\text{m}$), which is formed by a folding of the cell membrane (Fig. 5 E, F). Homogeneous grey material is situated between the inner and the outer membrane. The posterior part of the axoneme has only a single membrane.

Spermiogenesis

Considering the different stages of spermiogenesis the development of the modified sperms of *Trilobodrilus axi* and *T. heideri* becomes evident. Early spermatids have a spherical nucleus with scattered heterochromatin. Several spherical mitochondria with typical cristae-structure are spread in the cytoplasm among ribosomes, granular endoplasmic reticulum, and Golgi-apparatus (Fig. 3 A, B). Every four spermatids are connected by cytoplasmic bridges (Fig. 3 A).

Even at an early stage of development the mitochondria begin to fuse. This fusion leads to the formation of four large mitochondrial derivatives which form a ring around the developing axoneme (Fig. 3 A). During spermiogenesis the mitochondrial derivatives become more and more elongated and lose their typical cristae-structures.

During the elongation of the mitochondrial derivatives a large Golgi-apparatus begins to separate vesicles with granular contents (Fig. 3 B). Thus the primary acrosomal vesicle is formed, which also becomes more and more elongated. During the following differentiations the basal part of the acrosome envelops the apical part of the nucleus (Fig. 4 C). Chromatin condensation and elongation of the nucleus take place at the same time. At this stage, a ring of microtubules can be observed around the nucleus (Fig. 4 B) and the acrosome (Fig. 4 A). These microtubules disappear with progressing maturity of the sperm.

DISCUSSION

Sperms are highly complicated cells that have gone through an evolution which has no parallel in other cell types. Most features can be traced back to the demands of fertilization (reaching the egg, penetrating the egg membrane). Two species, no matter how closely related they may be, probably never have structurally completely identical sperms (Baccetti & Afzelius, 1976). This notion can be confirmed using the example of *Trilobodrilus axi* and *T. heideri* which both have the same mode of sperm transfer, but show pronounced differences in the structure of the acrosome, nucleus and mitochond-

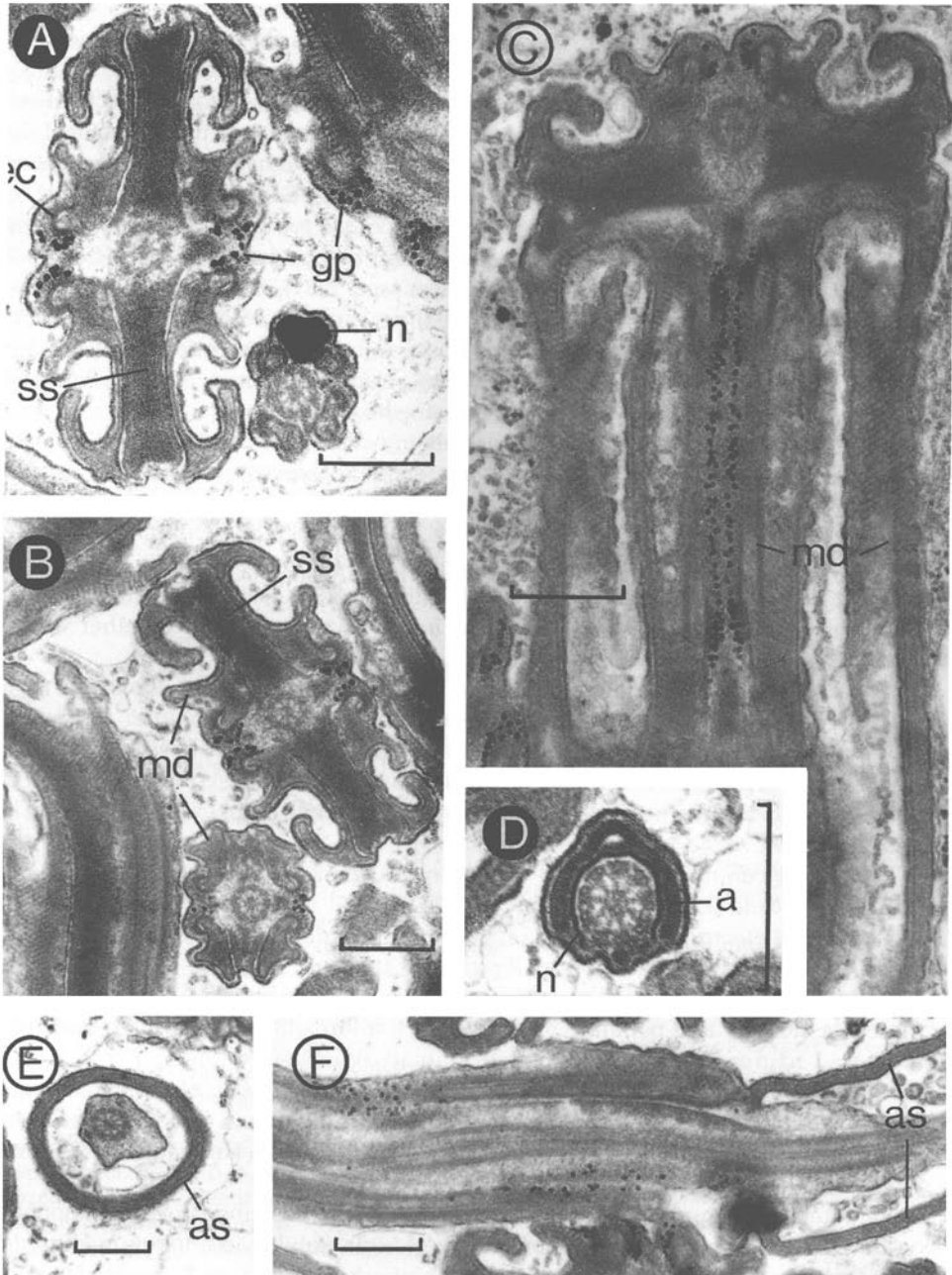


Fig. 5. *Trilobodrilus heideri*. A and B: Cross-sections through nuclear and mitochondrial region. C: Longitudinal section of the mitochondrial region. D: Cross-section through the proximal nuclear region. E: Cross-section of axoneme with surrounding sheath. F: Longitudinal section of the annulus region. Scale bar: 0.5 μ m.

rial region. The same is true for several morphologically similar *Protodrilus*-species, in which sperms are transferred with spermatophores (von Nordheim, unpublished).

Species with a particular mode of sperm transfer usually have specific sperms as well. The different types of modified sperms can each be related to the mechanism of sperm transfer and, therefore, must be regarded as analogous structures. This fact does not, however, exclude the existence of synapomorphous homologies in some sperms, e. g. in the dinophilids *Dinophilus* and *Trilobodrilus* (Westheide, 1984a).

Sperms with elongated head and middle piece are typical of invertebrates with internal fertilization (Franzén, 1956). In the annelids, this sperm type is characteristic for the Clitellata (see Wissocq & Malecha, 1975; Jamieson 1981), however, it has also been described in some polychaetes (see Olive, 1983). In comparatively primitive sperms of this type the nucleus is more or less distinctly separated from the middle piece, e. g. in *Protodrilus rubropharyngeus* (Franzén, 1975a), *Microphthalmus similis*, *Ikosipodus carolensis*, and *Apodotrocha prognerans* (Westheide, 1984a).

Species with sperm transfer by hypodermic injection show further modifications of their sperm structure. Here the sperm diameter decreases even more, the axoneme becomes more elongated and – as a result – no distinct differentiation between head, middle piece and tail can be made (see Franzén, 1977a). In *Microphthalmus listensis* (Westheide, 1984a) and *Dinophilus spec.* (Franzén, 1977a) the axoneme extends far into the nuclear region, in *Hesionides arenaria* (Westheide, 1984b) it begins directly behind the acrosome, and in *Trilobodrilus axi* and *T. heideri* it even extends together with the nucleus into the acrosomal region itself.

In the dinophilids the rod-shaped nucleus reaches apically into the acrosomal region and basally far into the mitochondrial region. In *T. axi* and *T. heideri* the nucleus apically forms a cylindrical tube-like sheath enclosing the axoneme, basally it covers the axoneme like a cap. An extremely modified nucleus has been described for *Hesionides arenaria* (Westheide, 1984b). Here the nucleus has the shape of an extended thin-walled tube-like cap that covers the entire head piece.

The lack of centriolar structures is not an uncommon feature in highly modified mature spermatozoa (Franzén, 1975b; Phillips, 1970). At this stage it can not be proved that the double electron dense ring in the anulus region of *Trilobodrilus* is a "ring centriole" similar to that described by Rice (1981) for some *Polydora* species.

An axoneme extending almost through the entire length of the sperm surely increases its mobility and penetration ability. Such a threadlike sperm, therefore, needs additional structures to guarantee a certain stability (Westheide, 1984b). For that reason the modification of the middle piece, i. e. the form and distribution of the mitochondrial derivatives and possibly the formation of additional structures in this part is of special importance for aberrant sperms (see Franzén, 1970; Schroeder & Hermans, 1975; Baccetti & Afzelius, 1976).

Elongated mitochondrial derivatives as have been described for the dinophilids have the function of energy supply for sperms of high mobility in a tough medium (see Favard & André, 1970; Franzén, 1973). In *Trilobodrilus axi* and *T. heideri* glycogen particles which can be observed in the mitochondrial region can serve as an additional energy source. Comparable glycogen particles in the middle piece and tail region have been described for several species (e. g. Anderson & Personne, 1970; Afzelius, 1970).

In the two species of *Trilobodrilus* investigated here, the electron-dense cytoplasmic

regions of the middle piece could provide the sperm with additional stability. Far more complicated differentiations of additional supporting structures have up to now been described for *Hesionides arenaria* (Westheide, 1984b) and *Protodrilus rubropharyngeus* (Franzén, 1975a) and seem to be generally common in *Protodrilus* (von Nordheim, unpublished).

In the dinophilids, the modified sperm structure is a synapomorphous homology which, in this family, has been developed in connection with the special mode of sperm transfer by hypodermal injection (Scharnofske, 1984). The general features of the aberrant thread-like sperm of the dinophilids (elongated head, middle piece and tail are overlapping) already show that their mode of reproduction is not of the primitive type. Furthermore, ultrastructural features (extremely elongated axoneme, special supporting structures) suggest that hypodermal injection is a derived mode of reproduction which has evolved as a secondary transformation from a 'normal' copulatory mechanism with sperm transfer directly into female genital openings (comp. Jägersten, 1943; Ax, 1968).

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