The spermatozoon of *Thaumastoderma moebjergi* with the description of the sperm model for the family Thaumastodermatidae (Gastrotricha, Macrodasyida)

Roberto Marotta*, Maria Balsamo**, Marco Ferraguti*, Cristina Fondello*, Loretta Guidi** and M. Antonio Todaro***

Abstract

The spermatozoon of *Thaumastoderma moebjergi* (Gastrotricha, Macrodasyida, Thaumastodermatidae) was described, and its structural features were compared to those of the other thaumastodermatid sperm models so far described. Although many of the sperm characters were present in different macrodasyidan species, the following complete set of characters was only found in all the thaumastodermatid spermatozoa at now described: a tubular structure within the acrosome formed by a pile of disks, the mitochondria arranged inside the nuclear spring, the striated cylinder in the flagellum, and a perinuclear helix. Thus the family Thaumastodermatidae appears uniform as far as sperm characters are considered.

Key words: Ultrastructure, systematics, spermatozoa, Gastrotricha

Introduction

We have few data on fertilization modalities in Gastrotricha, but, as far as we know, it is internal, through copulation, or transmission of spermatophores or dermal impregnation (Ruppert 1991). It is known that internal fertilization is correlated to changes in sperm morphology aimed to improve the movement inside the body's liquids of the partner (Franzén 1956), so we might expect a more slender and filiform cell, often accompanied by some kind of helical shape at least in some parts of the cell. If, however, in an evolutionary line, internal fertilization has been "invented" more than once, the modifications undergone by the sperm cells may be very different from one species to another, due to different evolutionary pressures. Among Gastrotricha it seems that this is the case (Schmidt-Rhaesa 2007).

Our knowledge of gastrotrich sperm models has considerably increased in the last years (Marotta et al. 2005). We now have data on sperm

* Dipartimento di Biologia, Università degli Studi di Milano, via Celoria 26, 20133, Milano, Italy; e-mail: marco.ferraguti@unimi.it
** Dipartimento di Scienze della terra, della Vita e dell'Ambiente (DiSTeVA), Università di Urbino 'Carlo Bo', Loc. Crocicchia, 61029, Urbino, Italy
*** Dipartimento di Biologia, Università di Modena e Reggio Emilia, via Campi 213/d, 41125, Modena, Italy

morphology of species belonging to 21 different genera from twelve families. At least one species for each of the eight macrodasyidan families has been investigated, and we have data on eight species from the only four chaetonotidan families with amphigonic reproduction. As the number of species investigated increased, it became more and more evident that it is not possible to define a general model for gastrotrich spermatozoa (compare Ferraguti & Balsamo 1995 with Balsamo et al. 1999 and Marotta et al. 2005). Among macrodasyidans, the spermatozoa, besides being all filiform and flagellated cells (with the exception of *Dactylopodola baltica*: Fischer 1996 and *Dolichodasys:* Ruppert & Shaw 1977) which however is a character common to many animal species with internal fertilization, do not show characters common to all the species investigated. For example, one of the most peculiar characters of macrodasyidan gastrotrich spermatozoa, i. e. the presence of one or more mitochondria surrounded by the nucleus is not present in the family Macrodasyidae and in *Xenodasys eknomios* (Guidi et al. 2009). Another macrodasyidan sperm character, the striated cylinder involving the flagellar axoneme is absent in the *Lepidodasys* species examined (Guidi et al. 2004), in one of the two *Urodasys* species (Guidi et al. 2007), in two *Crasiella* species (Guidi et al. 2010), in *Xenodasys eknomios* (Guidi et al. 2009) and in the three Turbanellidae studied (Balsamo et al. 2002), but is present in all the remaining twelve species examined.

Thaumastodermatidae is the most speciose family within Macrodasyida, with near 150 species (Hochberg 2001, Hummon & Todaro 2010, Hummon 2011) distributed in eight genera and two recognized subfamilies: *Acanthodasys* and *Diplodasys* (Diplodasyinae) vs. *Hemidasys*, *Oregodasys* (= *Platydasys*), *Pseudostomella*, *Ptychostomella*, *Tetranchyroderma* and *Thaumastoderma* (Thaumastodermatinae). It is worth to notice that recently *Hemidasys* has been considered extinct (Hummon & Todaro 2010). Phylogenetic relationships within the family are debated, with contrasting scenarios hypothesized on gross anatomy level data (e. g. Hochberg & Litvaitis

2000 vs. Hochberg & Litvaitis 2001 vs. Kieneke et al. 2008).

We have spermatological data on species of four out of the eight genera: *Acanthodasys aculeatus* (Guidi et al. 2003), *Diplodasys ankeli*, *Pseudostomella etrusca*, *Tetranchyroderma* (three species) (Ferraguti & Balsamo 1995). It appears that the general structure of the spermatozoa in the family Thaumastodermatidae is pretty constant. In particular all sperm models have a more or less complex, helical acrosome containing a tubular structure made of piled hollow cylinders, a nuclear-mitochondrial region in which one or more mitochondria are surrounded by a helical nucleus, and a tail in which the flagellar axoneme is surrounded by a striated cylinder (sensu Ferraguti & Balsamo 1994). To assess to what extent this general model is valid for other members of the family, and in search for possible character ground pattern we have studied the spermatozoa of *Thaumastoderma moebjergi* Clausen, 2004. Within this framework the study bears supplementary relevance as a recent phylogenetic investigation based on molecular traits found *Thaumastoderma* to be one of the most basal taxon along the Thaumastodermatinae evolutionary line (Todaro et al. 2011).

Materials and methods

Marine sediment containing *Thaumastoderma moebjergi* Clausen, 2004 was collected in September 2007 during a two-week workshop held at the Sven Lovén Centre for Marine Sciences on Tjärnö, an island on the Swedish west coast (for details see Willems et al. 2009). Gastrotrichs were extracted from the sediment by the narcotization-decantation technique, using an isosmotic (7 %) magnesium chloride solution (Todaro & Hummon 2008). The fauna-containing supernatant was then poured directly into a 5-cm diameter Petri dish and scanned for specimens under a Wild M3 dissecting microscope set at 50 × magnification. For optical microscopy, the gastrotrichs were removed with a micropipette from the Petri dish, fresh-

Fig. 1. Spermatozoa of *Thaumastoderma moebjergi*. **A.** Main portion of the acrosome with the coiled tubular structure surrounded by the helical ridge (arrow). Inset: the apical portion of the spermatozoon. **B.** Basal portion of the acrosome to show the connection between the acrosome and the nucleus and the tubular vesicle (*) surrounding the distal portion of the nucleus. **C–D.** The nucleo-mitochondrial region of the spermatozoon in longitudinal (**C**) and cross (**D**) section. Note the two mitochondria in sequence (arrow). **E.** Basal portion of the nucleus with the beginning of the flagellum. **F.** Proximal region of the flagellum in longitudinal section. Note the two layers
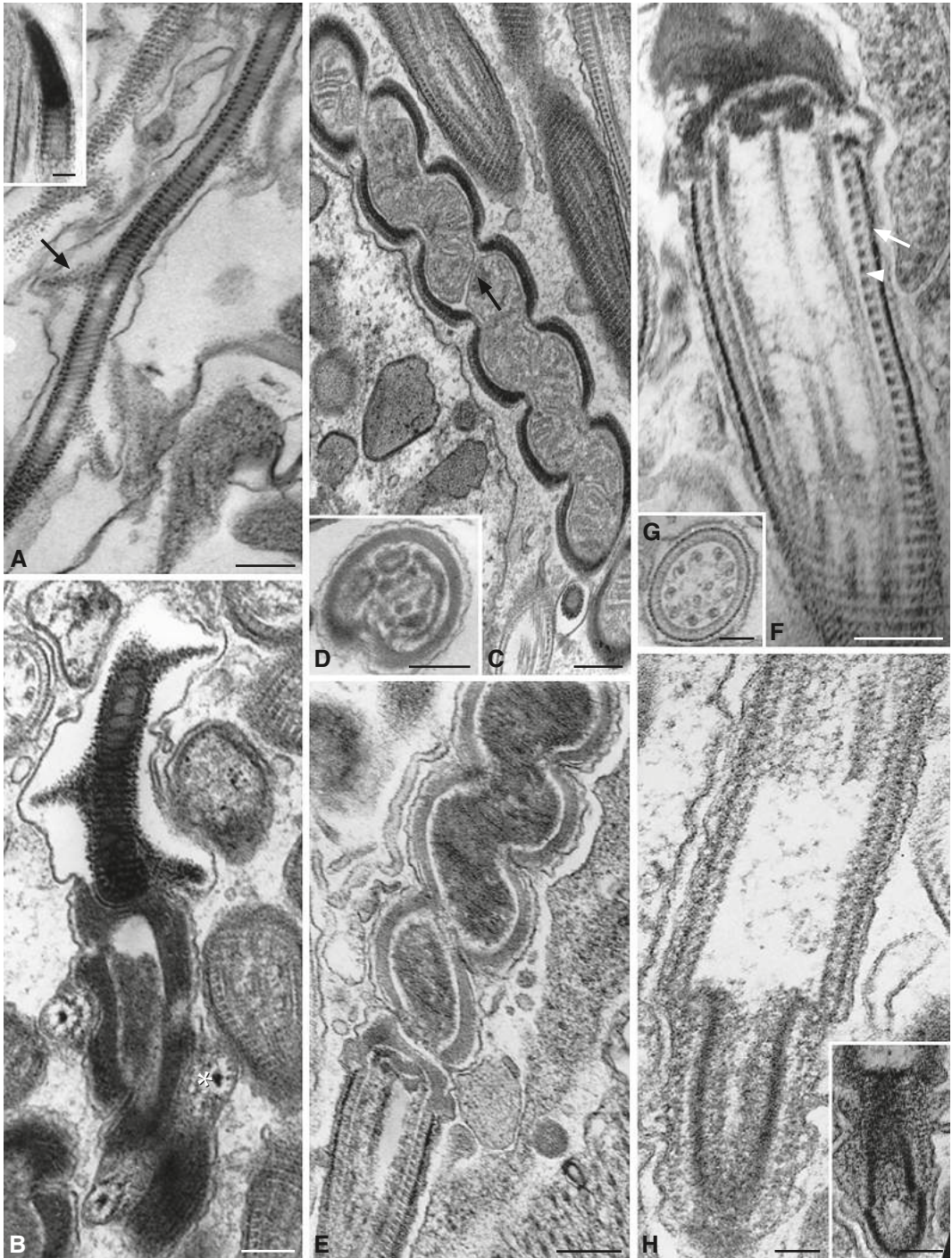
forming the striated cylinder: the external, denser, one (arrow) and the inner, larger and more transparent (arrowhead). **G.** Cross section of the flagellum at the distal region of the axoneme. Note the irregular aspect of the $9\times2+2$ structure. **H.** distal portion of the tail, where the striated cylinder appears "empty" and closed distally by a double "cork" (inset). Scale bars: A–F, 0.2 µm; G–H and insets, 0.1 µm.

mounted on slides and observed using a Nikon Eclipse 80i equipped with Differential Interference Contrast (Nomarski). During observation, the animals were photographed with a DS-5M Nikon digital camera and measured with the Nikon NIS software. For the transmission electron microscopy, 10 specimens were fixed overnight in 2 % glutaraldehyde in a 0.1 M sodium cacodylate buffer (pH 7.4), and stored in a 0.1 M sucrose/sodium cacodylate buffer. Subsequently, the animals were washed for two hours in 0.1 M cacodylate buffer, postfixed for two hours in aqueous 0.1 % Osmium Tetroxide in 0.1 M cacodylate buffer, washed in distilled water, pre-stained in a saturated solution of uranyl acetate, dehydrated in a graded ethanol series, and embedded in Spurr's resin.

Thin sections were obtained with a Reichert Ultracut E microtome, stained with 2 % lead citrate, carbon-coated and observed with a JEOL 100SX electron microscope.

The three dimensional reconstructions of the sperm model have been performed using Rhinoceros® NURBS (Non-Uniform Rational B-splines) modelling for Windows starting from the TEM micrographs.

Results

The spermatozoon of *Thaumastoderma moebjergi* is a long (more than 50 μm), filiform cell formed, in sequence, by a complex acrosome, a nucleo-mitochondrial region (together 16 μm long) and a tail (Fig. 2A–F).

The acrosome is a long organule with a diameter decreasing from about 0.2 μm at the proximal end, close to the nucleus (Fig. 1B) to about 0.1 μm apically, where it terminates with a short (0.5 μm), dense, conical end (Fig. 1A, inset; 2C). The acrosome contains a coiled tubular structure formed by a pile of electrondense rings (Fig. 1A), containing, basally, a series of tightly packed vesicles (Fig. 1B). The coiled tubular structure is surrounded by a lateral expansion in the form of a ridge following the coils of the tube, with a pitch increasing from 0.6 μm basally to 1 μm apically (Fig. 1A, 2A). The ridge is not surrounded by an acrosomal membrane, but is formed by a dense granular material. The plasma membrane surrounding the acrosome is somewhat loose. The acrosome is basally hosted in a short concavity formed by the nucleus (Fig. 1B).

The nucleo-mitochondrial region is formed by the compact nuclear chromatin forming a sort of "spring", with a diameter increasing from about 0.3 μm apically to 0.5−0.6 μm basally (Fig. 1C–E, 2B). The inner space delimited by the nuclear spring is often apically empty, in the first one-two coils (Fig. 1B, 2D), but is basally filled by the mitochondria (Fig. 1E). Since this area is quite long, it is difficult to establish the exact number of mitochondria present: in one instance we have seen two mitochondria disposed in line, one after the other (Fig. 1C). The nuclear chromatin forming the spring is uniform apically (Fig. 1C), but clearly divided in two parts basally, one more, the other less, electrondense (Fig. 2E). The distal portion of the nucleus, the one devoid of inner mitochondria, is surrounded by a tubular "vesicle" (0.1 μm in diameter) containing a dense central core surrounded by an electron transparent space, following externally the coils of the nucleus for 2.5 gyres (perinuclear helix in Guidi et al. 2003) (Fig. 1B, 2A,D).

The nucleo-mitochondrial region is followed by the flagellum, containing a conventional $9 \times 2 + 2$ axoneme, surrounded by a striated cylinder (Fig. 1G, 2E–F). This last is formed by two layers close one to the other: an external one, thinner and more electrondense, and an internal one, thicker (25 nm) which is formed by a U-shaped structure coiled around the axoneme and forming with its major axis an angle of 80−90 degrees (Fig. 1F). The tight coiling of the U-shaped structure gives to the organule the appearance of a striated cylinder (Fig. 1F, bottom). The axoneme originates from a complex connecting piece, without any conventional basal body, and terminates distally in an irregular way, so as many sections with odd number of microtubules have been found (Fig. 1G). The axoneme ends distally well before the end of the striated cylinder, thus leaving part of it with an "empty" appearance (Fig. 1H, 2F). The striated cylinder terminates distally in a peculiar, double end piece, the extremity of the spermatozoon (Fig. 1H and inset; 2F).

Discussion

The spermatozoa of Gastrotricha belong to the modified type sensu Franzén (1956) and are extremely complex cells, with a considerable variation of their characters among the taxa, so as we

were able to attempt a phylogenetic analysis of the group based on sperm morphology only (Marotta et al. 2005). The spermatozoon of *Thaumastoderma moebjergi*, when compared to the other sperm models known in the phylum, appears to share some of the characters with species of different families: the mitochondria surrounded by the nuclear spring are present also in Lepidodasyidae (Guidi et al. 2004), Cephalodasyidae (Ferraguti & Balsamo 1994, Fischer 1994), Planodasyidae (Guidi et al. 2010) and Turbanellidae (Balsamo et al. 2002); the striated cylinder around the axoneme is present in Cephalodasyidae (Ferraguti & Balsamo 1994, Fischer 1994, Balsamo et al. 1999) and Macrodasyidae (Marotta et al. 2005). However, all the members of the family Thaumastodermatidae, thus also *T. moebjergi* share the following complete set of characters:

– a tubular structure within the acrosome, formed by a pile of disks
– the mitochondria arranged inside the nuclear spring
– the striated cylinder in the flagellum
– the perinuclear helix. This last character has been overlooked in our former investigation of *Pseudostomella etrusca* (Ferraguti & Balsamo 1995), but a more careful observation has allowed us to recognize this character also in this species.

Thus the family Thaumastodermatidae appears uniform as far as sperm characters are considered. Each of the characters reported above do varies in the different species of the family:

– The tubular acrosomal structure in *Acanthodasys aculeatus* is formed by electron-dense rings surrounding an electron-lucent axis (Guidi et al. 2003), whereas in other species of the family the tubular structure is formed by thinner disks each with the shape of a thin circular crown. In *Diplodasys ankeli* (Balsamo et al. 1999) the tubular acrosomal structure is formed basally by electron-dense rings that apically become electron-dense disks. In the same species the tubular acrosomal structure ends laterally against the acrosomal membrane, whereas in *Pseudostomella etrusca* and in *Tetranchyroderma* sp. it ends basally.
– There is only one mitochondrion in *Diplodasys ankeli* (Balsamo et al. 1999), in *Acanthodasys aculeatus* (Guidi et al. 2003), and in *Tetranchyroderma* sp. 1 (Ferraguti & Balsamo 1995), at least two in *Thaumastoderma moebjergi*, but

many in *Pseudostomella etrusca* (Ferraguti & Balsamo 1995).
– The striated cylinder can be monolayered and very thick as in *Tetranchyroderma* sp. (Ferraguti & Balsamo 1995), or thinner as in *Acanthodasys aculeatus*: Guidi et al. 2003), but is thinner and formed by two layers in *Thaumastoderma moebjergi*. The distal extremity of the striated cylinder may be empty (i.e. it continues behind the axoneme) for a long tract, as in *Acanthodasys aculeatus* (Guidi et al. 2003), *T. moebjergi* and one *Tetranchyroderma* species.
– The perinuclear helix can can run for one gyre only around the nucleus, as in *Pseudostomella etrusca*, or for two and a half, as in *Thaumastoderma moebjergi*, or involve the whole nucleus, as in *Acanthodasys aculeatus* (Guidi et al. 2003) and *Diplodasys ankeli* (Ferraguti & Balsamo 1995, Balsamo et al. 1999). Our unpublished material on various *Tetranchyroderma* species allowed us to identify the perinuclear helix, but not to determine its length. We were not able to ascertain the origin of the perinuclear helix. Balsamo et al. (1999) proposed that the helix is a basal extension of the acrosome, and we had some confirmations from scattered images of the complex gastrotrich spermiogenesis (as for *Acanthodasys aculeatus*, Guidi et al. 2003), but its thinness and its coiled arrangement in the Thaumastodermatidae did not allow us to understand its real nature.

To summarize, the sperm characters of the family Thaumastodermatidae are fairly constant: a tubular structure inside the acrosome, the position of mitochondria inside the nuclear spring, the striated cylinder surrounding the axoneme, and the perinuclear helix.

Some of the above mentioned characters are present also in other gastrotrich taxa, whereas the perinuclear helix, at the actual state of knowledge, is present only in the species of the family Thaumastodermatidae. Unfortunately we could not detect ultrastructural features to support the evolutionary scenario proposed recently on molecular ground, in which *Thaumastoderma* is hypothesized as basal to most Thaumastodermatinae. The homology of the perinuclear helix to the acrosome of the extremely aberrant spermatozoon of *Urodasys acanthostylis* (Macrodasyidae) (Balsamo et al. 2007) remains to be established.
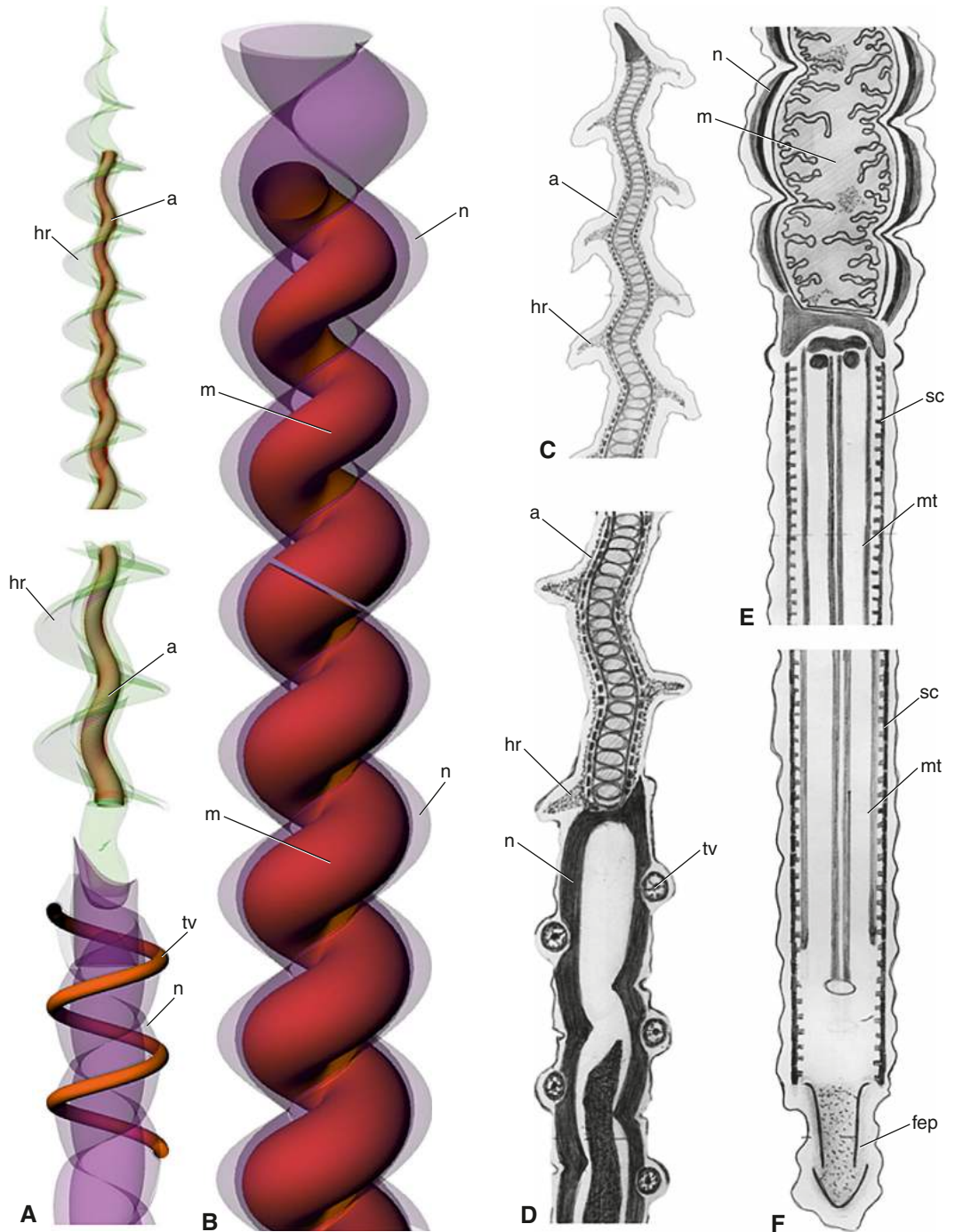
Fig. 2. Spermatozoon of *Thaumastoderma moebjergi*. **A-B.** 3D reconstructions of the acrosome (**A**) and nucleus (**B**). The acrosome is shown in its more distal (top) and proximal (bottom) regions. Basally, the acrosome is connected to the nucleo-mitochondrial region. Note the tubular vesicle coiled around the nucleus. **C–F.** Schematic drawing showing the apical regions of the acrosome (**C**), the acrosome-nuclear connection (**D**), the base of the flagellum (E) and the extremity of the tail (F). Abbreviations: **a**, acrosome; **hr**, helical ridge; **tv**, tubular vesicle; **n**, nucleus; **m**, mitochondria; **sc**, striated cylinder; **mt**, microtubules; **fep**, terminal structure of the flagellum.

Acknowledgements

References

Balsamo, M., E. Fregni & M. Ferraguti (1999). Gastrotricha. In: Reproductive Biology of the Invertebrates, vol. IX, part A, Adiyodi, K. G. & R. G. Adiyodi (eds.), pp. 171–192. J. Wiley, Chichester, UK.

Balsamo, M., M. Ferraguti, L. Guidi, M. A. Todaro & P. Tongiorgi (2002). Reproductive system and spermatozoa of *Paraturbanella teissieri* (Gastrotricha, Macrodasyida): implications for the sperm transfer modality in Turbanellidae. Zoomorphology 121: 235–241.

Balsamo, M., L. Guidi, L. Pierboni, R. Marotta, M. A. Todaro & M. Ferraguti (2007). Living without mitochondria : spermatozoa and spermatogenesis in two species of *Urodasys* (Gastrotricha, Macrodasyida) from dysoxic sediments. Invertebr. Biol. 126: 1–9.

Ferraguti, M. & M. Balsamo (1994). Sperm morphology and anatomy of the genital organs in *Mesodasys laticaudatus* Remane, 1951 (Gastrotricha, Macrodasyida). J. Submicrosc. Cytol. Pathol. 26: 21–28.

— (1995). Comparative spermatology of Gastrotricha. In: Advances in Spermatozoal Phylogeny and Taxonomy, vol. 166, Jamieson B. G. M., J. Ausio & J. L. Justine (eds.). Mém. Mus. Natn. Hist. Nat. Paris 166: 105–117.

Fischer, U. (1994). Ultrastructure of spermiogenesis and spermatozoa of *Cephalodasys maximus* (Gastrotricha, Macrodasyida). Zoomorphology 114: 213–225.

Fischer, U. (1996). Ultrastructure of penetrated spermatozoa, ovary, and oogenesis of *Dactylopodola baltica* (Gastrotricha, Macrodasyida). Invertebr. Reprod. Dev. 29: 71–78.

Franzén, Å. (1956). On spermiogenesis, morphology of the spermatozoon, and biology of fertilization among invertebrates. Zool. Bijdr. Uppsala 31: 355–482.

Guidi, L., M. Ferraguti, L. Pierboni & M. Balsamo (2003). Spermiogenesis and spermatozoa in *Acanthodasys aculeatus* (Gastrotricha, Macrodasyida): an ultrastructural study. Acta Zool. 84: 77–85.

Guidi L., L. Pierboni, M. Ferraguti, M. A. Todaro & M. Balsamo (2004). Spermatology of the genus *Lepidodasys*: towards a revision of the family Lepidodasyidae (Gastrotricha, Macrodasyida). Acta Zool. 85: 211–221.

Guidi, L., M. Ferraguti, M. A. Todaro, L. Pierboni & M. Balsamo (2009). Unusual spermatozoa and reproductive modalities of *Xenodasys eknomios* (Gastrotricha: Xenodasyidae). Ital. J. Zool. 76: 165–172.

Guidi, L., M. A. Todaro, M. Ferraguti & M. Balsamo (2010). Reproductive system of the genus *Crasiella* (Gastrotricha, Macrodasyida). Helgol. Mar. Res. 65: 175–185.

Hochberg, R. & M. K. Litvaitis (2000). Phylogeny of Gastrotricha: a morphology-based framework of gastrotrich relationships. Biol. Bull. 198: 299–305.

— (2001). Macrodasyida (Gastrotricha): a cladistic analysis of morphology. Invert. Biol. 120: 124–135.

Hummon, W. D. (2011). Marine Gastrotricha of the Near East: 1. Fourteen new species of Macrodasyida, along with a species described from Australia. Zookeys 94: 1–59.

Hummon, W. D. & M. A. Todaro (2010). Analytic taxonomy and notes on marine, brackish-water and estuarine Gastrotricha. Zootaxa 2392: 1-32.

Kieneke, A., O. Riemann & W. H. Ahlrichs (2008). Novel implications for the basal internal relationships of Gastrotricha revealed by an analysis of morphological characters. Zool. Scr. 37: 429–460.

Marotta, R., L. Guidi, L. Pierboni, M. Ferraguti, M. A. Todaro & M. Balsamo (2005). Sperm ultrastructure of *Macrodasys caudatus* (Gastrotricha: Macrodasyida) and a sperm-based phylogenetic analysis of Gastrotricha. Meiofauna Marina 14: 9–21.

Ruppert, E. E. (1991). Gastrotricha. In: Microscopic Anatomy of Invertebrates, Vol. 4, Aschelminthes. Harrison, F. W. & E. E. Ruppert (eds.). Wiley-Liss, New York: 41–109.

Ruppert, E. E. & K. Shaw (1977). The reproductive system of gastrotrichs. I. Introduction with morphological data for two new *Dolichodasys* species. Zool. Scr. 6: 185–195.

Schmidt-Rhaesa, A. (2007). The Evolution of Organ Systems. Oxford University Press.

Todaro, M. A. & W. D. Hummon (2008). An overview and a dichotomous key to genera of the phylum Gastrotricha. Meiofauna Marina 16: 3–20.

Todaro, M. A., T. Kånneby, M. Dal Zotto & U. Jondelius (2011). Phylogeny of Thaumastodermatidae (Gastrotricha: Macrodasyida) Inferred from Nuclear and Mitochondrial Sequence Data. PLoS ONE 6 (3): e17892. doi:10.1371/journal.pone.0017892

Willems, W. R., M. Curini-Galletti, T. J. Ferrero, D. Fontaneto, I. Heiner, R. Huys, V. N. Ivanenko, R. M. Kristensen, T. Kånneby, M. O. MacNaughton, P. Martínez Arbizu, M. A. Todaro, W. Sterrer & U. Jondelius (2009). Meiofauna of the Koster-area, results from a workshop at the Sven Lovén Centre for Marine Sciences (Tjärnö, Sweden). Meiofauna Marina 17: 1–34.