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Living without mitochondria: spermatozoa and spermatogenesis in two species of *Urodasys* (Gastrotricha, Macrodasyida) from dysoxic sediments

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Abstract. The spermatozoa of two species of Macrodasyida (Gastrotricha), *Urodasys anorektoxys* and *U. acanthostylis,* show an ultrastructural organization diverging from one another and from other gastrotrichs: their main peculiarity is in the absence of mitochondria. In *U. anorektoxys,* the acrosome is a long, twisted column inserted into the nucleus, which is basally cylindrical, and the flagellum shows rows of peculiar, large globules parallel to the axonemal doublets. In *U. acanthostylis,* the acrosome is completely cork-screwed and surrounds the nucleus, and the tail shows columnar accessory fibers. At present, the absence of mitochondria in the mature sperm, and the peculiar fingerprint aspect of condensed chromatin are the only traits shared by the two species. The features of the spermatozoa of these two species of *Urodasys* widen the range of different models of gastrotrich spermatozoa, and place the genus in a peculiar position, from the spermatological point of view, within the Macrodasyida. The loss of mitochondria in mature spermatozoa is possibly related to either the dysoxic habitat of the two species or a peculiar fertilization mechanism.

Additional key words: Gastrotricha, spermatozoa, spermatogenesis, ultrastructure, mitochondria

Gastrotricha is a diverse taxon of microscopic worms characteristic of marine and freshwater sediments. Members of the marine order Macrodasyida are generally hermaphrodites and direct developers, and possess a bewildering array of reproductive organs, the real function of some of which is still unclear. This is especially true within the Macrodasyidae, where structural/functional studies have elucidated some bizarre methods of copulation and sperm transfer (Ruppert 1978). The cosmopolitan genus *Urodasys* is one of the most easily recognizable gastrotrich taxa because of its mobile tail, which is at least three times longer than the body. The genus currently includes 14 fully described species (Hummon 2001), and two described but unnamed species (Schoëpfer-Sterrer 1974; Valbonesi & Luporini 1984). Three species (*Urodasys anorektoxys* TODARO, BERNHARD, & HUMMON 2000, *U. apuliensis,* and *U. elongatus*) have two testes and no accessory organ, while one species (*U. mirabilis*) has two testes and a caudal organ without a stylet. Ten species (*U. acanthostylis* FREGNI, TONGIORGI, & FAIENZA 1998, *U. bucinostylis, U. calicostylis, U. cornustylis, U. nodostylis, U. remostylis, U. spirostylis, U. uncinostylis, Urodasys* sp.1 SCHOËPFER-STERRER 1974, and *Urodasys* sp.1 VALBONESI & LUPORINI 1984) possess only a single, left testis and a caudal organ containing a strongly cuticularized stylet. The parthenogenic species *Urodasys viviparus* lacks both testes and a caudal organ. Two distinct evolutionary lines have been hypothesized on the basis of the male system morphology; one line includes the species with two testes but no stylet, and the other line comprises the species with a single testis and a stylet and also *U. viviparus,* in which the male parts may have regressed (Todaro et al. 2000).

Schoëpfer-Sterrer (1974) made detailed drawings of the genital organs and spermatozoa of many different species of *Urodasys* using light microscopy. She concluded "The sperm, in all species, is characterized by a spiralized part, and, apart from *U. nodostylis* […] an arrow-shaped head." No ultrastructural data on spermatozoa or spermatogenesis are available for species of *Urodasys*. As several comparative studies of gastrotrich sperm ultrastructure have shown that the male cells bear both species-specific

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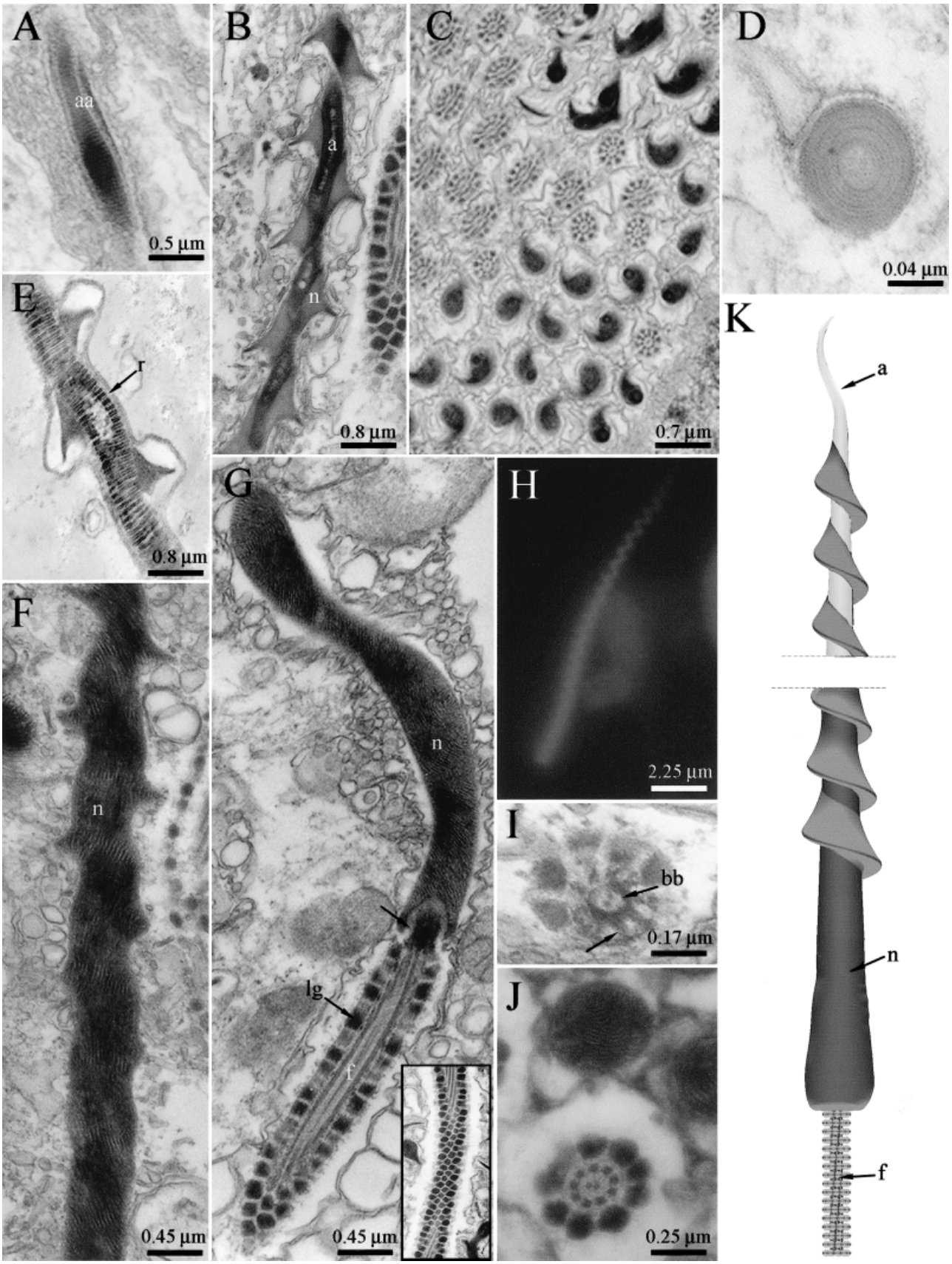
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traits and strongly informative phylogenetic characters (Balsamo et al. 1999, 2002; Guidi et al. 2003a, b, 2004), we have undertaken a study on the fine structure of the spermatozoa of two species of *Urodasys,* namely *U. anorektoxys,* belonging to the first putative evolutionary line, and *U. acanthostylis,* belonging to the second evolutionary line.

The present study compares the sperm structure of these two species, with the dual purpose of broadening the knowledge of reproductive characters in the genus *Urodasys* and of providing new morphological information. The latter information should be included in a comprehensive morphological data matrix from which inferences about phylogenetic relationships within Gastrotricha may be drawn, and in particular among members of the Macrodasyidae, a family almost unknown spermatologically. The scarce data available point to a sperm model unusual for Macrodasyida, which have spermatozoa generally characterized by the position of mitochondria inside a spring-shaped nucleus (Marotta et al. 2005). In contrast, the two Macrodasyidae studied so far, *Macrodasys* sp. and *M. caudatus,* possess spermatozoa in which a single mitochondrion coils around the nucleus (Ruppert 1978; Marotta et al. 2005).

Methods

Specimens of *Urodasys anorektoxys* were collected from bathyal (588–592 m depth) muddy sediment of severely dysoxic to anoxic waters in the Santa Barbara Basin, California, U.S.A. (34°13′–34°17′ N, 119°58′–120°02′ W; see also Todaro et al. 2000). Specimens of *U. acanthostylis* were obtained from fine to medium volcanic sand, collected at 3.5 m depth at Cala la Nave, Island of Ventotene, Tyrrhenian Sea, Italy (40°47′ N, 13°26′ E; see also Todaro et al. 2003). Animals were extracted after narcotization with 7% $MgCl_2$, aqueous solution, following decantation (Higgins & Thiel 1988). They were then rinsed in filtered artificial seawater and fixed in a mixture of glutaraldehyde and paraformaldehyde in cacodylate-buffered picric acid (SPAFG: Ermak & Eakin 1976). The fixation was extended for several months. After an overnight washing in cacodylate buffer, the specimens were postfixed in 2% osmium tetroxide in the same buffer. Next they were rinsed in the same buffer, dehydrated in a graded acetone series, prestained *en bloc* in uranyl acetate in 70% acetone, and embedded in araldite. Ultrathin sections were observed under a Philips EM300 and a Philips CM10 transmission electron microscope (FEI-Philips, Hillsboro, OR, USA) at the University of Urbino, or under a JEOL 100 SX (JEOL Ltd., Tokyo, Japan) at the University of Milan. Three-dimensional reconstructions of the spermatozoa were performed by CimatronE software (Cimatron Ltm, Tel Aviv, Israel; www.cimatron.com).

DNA cytochemistry

4′,6-Diamidino-2-henylindol (DAPI). An adult specimen of *U. anorektoxys,* fixed in SPAFG, was washed in phosphate buffer and carefully dissected on a microscope slide with micro-needles. One drop (25 μL) of Vectashield Mounting Medium (Vectro Laboratories, Inc., Burlingame, CA, USA) with DAPI was added to the specimen. The slide was immediately observed under a Vanox Olympus fluorescence photomicroscope equipped with a DM400 dichroic mirror and a UG1 excitation filter (Olympus Optical Co., Hamburg, Germany). The excitation light wavelength was 334–365 nm.

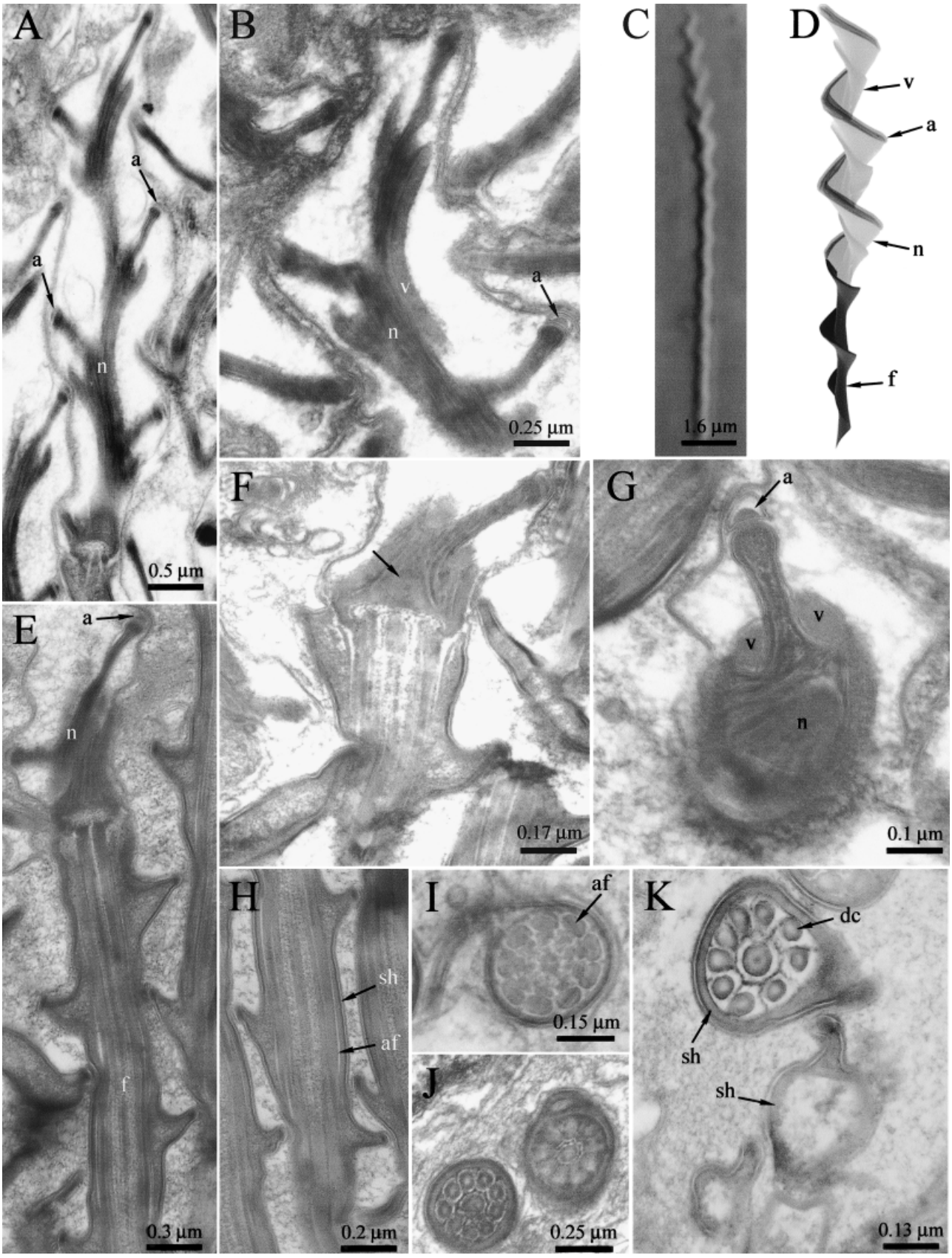
Osmium amine. For DNA-specific staining, sections on gold grids were first floated on 5 N HCl for 20 min at room temperature, rinsed in water, and processed with a 0.1% osmium amine solution (treated with SO_2) for 60 min at room temperature (Cogliati & Gautier 1973; Olins et al. 1989). Sections were then rinsed in distilled water and dried.

Results

Spermatozoa of *Urodasys anorektoxys*

The spermatozoon of *U. anorektoxys* consists of a head ~49 μm in length, followed by a long tail. The

Fig. 1. Spermatozoon of *Urodasys anorektoxys*. **A.** Longitudinal section of the acrosome apex (aa). **B.** Longitudinal section of the acrosome region (a) surrounded by the nucleus (n). **C.** Cross-section of nuclei, acrosomes, and tails at different levels. **D.** Cross-section of the acrosome apex. **E.** Longitudinal section of the acrosome showing thin and thick rings (r). **F.** Longitudinal section of the spiralized, distal region of the nucleus (n) showing the peculiar fingerprint aspect of the chromatin. **G.** Longitudinal section of the basal nuclear region (n) and of the flagellar base (f). The conical piece (arrow) and the large flagellar globules (lg) are visible; inset, longitudinal tangential section of the flagellum showing the twisting of the external microtubules and of the globule rows. **H.** Spiralized nucleus stained with DAPI. **I.** Cross-section of the axonemal base, in which the modified basal body (bb) and the pericentriolar satellite processes (arrow) are visible. **J.** Cross-sections of the nucleus and flagellum. The latter shows the nine large globules corresponding to the axonemal doublets. **K.** Three-dimensional reconstruction of the spermatozoon; a, acrosome; f, flagellum; n, nucleus.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head is composed of a peculiar acrosome, which is a long, twisted, tubular structure almost completely surrounded by the spiralized nucleus, except for a short apical segment (Fig. 1A,K). The acrosome is formed by alternating thick (0.016 μm) and thin (0.005 μm) black rings (Fig. 1E) stacked to form a tube, which measures 0.1 μm in diameter at the two ends and 0.24 μm in its central part (Fig. 1A,B). The tube is filled with granular material except in the apex, where only five to six concentric electron-dense rings are visible (Fig. 1D).

Two nuclear regions can be recognized: a cylindrical, basal one, 12 μm long (Fig. 1G), and a spiralized, apical one, ~35 μm in length, which coils around the acrosome with a pitch increasing toward the apex 1–1.8 μm (Fig. 1B,C,F,K); the chromatin has a peculiar fingerprint aspect (Fig. 1F,G). The nuclear nature of the distal spring-shaped portion surrounding the acrosome has been confirmed by both DAPI and osmium amine staining (Fig. 1H). No mitochondria are present.

The tail shows a conventional $9 \times 2+2$ axoneme, in which the doublets are highly twisted (Fig. 1G inset, J). Nine regular, longitudinal rows, made of large globules with the shape of irregular polyhedra, surround the axoneme; each globule is external to, and in correspondence with, an axonemal doublet and is linked to it by some filamentous material (Fig. 1J). As the rows of globules run along the twisted doublets, in longitudinal tangential sections they also appear twisted (Fig. 1G, inset). At the axonemal base, a modified basal body is surrounded by nine radial processes, each extending up to the first large globule of the axoneme (Fig. 1I). A conical piece connecting head and tail is located in a nuclear fossa, and is joined to the nuclear membrane through some scattered filaments (Fig. 1G).

Spermatozoa of *U. acanthostylis*

The spermatozoon of *U. acanthostylis* is a short, spiralized cell, ~18 μm in length, composed of a head and tail. The head, 5 μm long, has a helical shape like a meat mincer, with 4–5 gyres, and contains a complex structure composed of both the acrosome and the nucleus (Fig. 2A–D).

The nucleus is a twisted column extending laterally in two parallel helices, one smaller than the other, and with a pitch ranging 1.2–1.5 μm. The chromatin shows a peculiar fingerprint appearance (Fig. 2A,B).

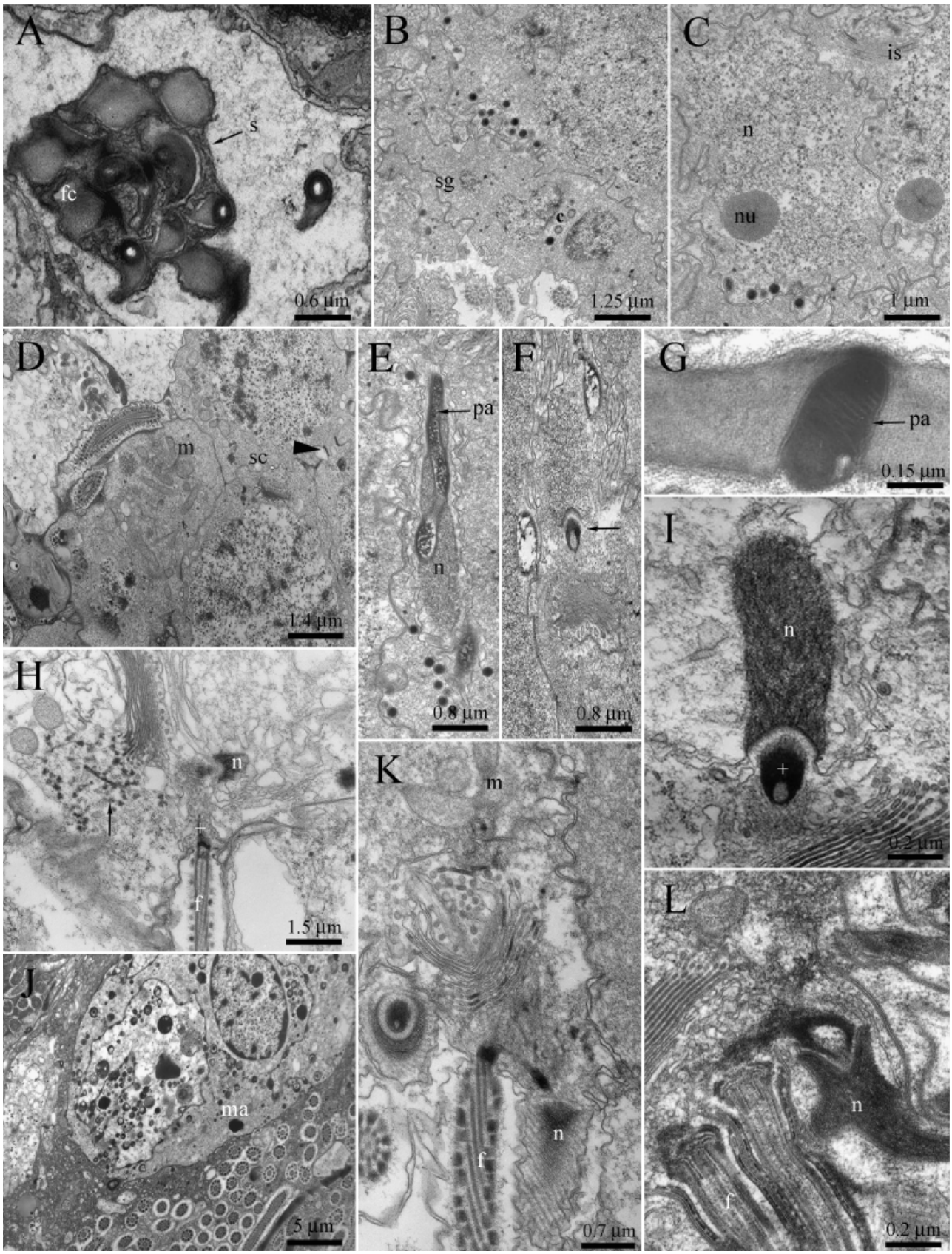
The acrosome is a simple, tubular vesicle, coiled around the edge of the larger helix. Two other peculiar, long and flattened vesicles, filled with moderately electron-dense material, run along the twisted axis of the nucleus (Fig. 2B,G). No mitochondria are present.

A distinct, conical piece lies at the nuclear base (Fig. 2F). An unresolved dense area takes the place of the basal body of the axoneme, and the central singlets arise from some central, dense material (Fig. 2E,F). The tail, ~13 μm long, has a conventional $9 \times 2+2$ axoneme surrounded by nine longitudinal accessory fibers, partially made of cross-striated material (Fig. 2H). An electron-dense striated sheath, probably homologous to the striated cylinder of many macrodasyidans (see Balsamo et al. 1999), encloses the whole tail. It lies between the plasma membrane and the accessory fibers, and coils into ~10 gyres, whose pitch is continuous with that of the head (Fig. 2H,I). In the distal part of the tail, 10 columns of dense material replace all the axonemal structures present in the proximal part; at the tail end, only a ring of electron-dense material surrounds a central, empty space (Fig. 2J,K).

Spermatogenesis

Spermatogenetic steps were followed in *U. anorektoxys,* in which the two testes of adults are lateral to the posterior extremity of the gut, which is a blind sac without an apparent anus as in other species of the genus *Urodasys*. Two sperm ducts extend posteriorly and join medially into a common duct opening on the ventral side (Fig. 3A). Spermatogenesis occurs in a cephalo-caudal direction, so that spermatogonia and spermatocytes lie in the anterior part of each testis, and are followed by spermatids. Spermatogonia are elongated cells, 27×6 μm, with two long

Fig. 2. Spermatozoon of *Urodasys acanthostylis*. **A.** Longitudinal section of the spiralized head comprising the acrosome (a) and nucleus (n). **B.** Oblique section of the head: the nucleus (n), the acrosome (a), and the flattened vesicle (v) are shown. **C.** Closeup of a single spermatozoon under Nomarski optics. **D.** Three-dimensional reconstruction of the spermatozoon (a, acrosome; f, flagellum; n, nucleus; v, flattened vesicles). **E.** Longitudinal section of the proximal region of the flagellum (f) and base of the head (a, acrosome; n, nucleus). **F.** Longitudinal section of the base of the nucleus with the conical piece connecting head and tail (arrow). **G.** Cross-section of the head showing the nucleus (n), acrosome (a), and the flattened vesicles (v). **H.** Longitudinal section of the flagellum, with accessory fibers (af) and striated sheath (sh). **I–K.** Cross-sections of flagellum at progressively more distal levels (af, accessory fibers; dc, dense column; sh, striated sheath).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cytoplasmic projections and a central, lobated nucleus; a few roundish electron-dense (0.005 μm) vesicles and the centrioles are easily visible in the abundant cytoplasm (Fig. 3B). The interphasic spermatocytes are irregular cells ($\sim 5.4 \times 10$ μm), with a large nucleus, a well-defined nucleolus, scarce cytoplasm, and also several vesicles (Fig. 3C). At first prophase, spermatocytes, $\sim 14 \times 8$ μm, have an eccentric, ovoidal nucleus and numerous, rod-shaped mitochondria mainly localized at one pole of the cell. Spermatocytes are interconnected by intercellular bridges (Fig. 3D); other meiotic stages were not observed.

Three spermatid stages may be recognized. In the first stage, the nucleus lengthens and chromatin gradually condenses. The nucleus is entirely wrapped by a long tubular, coiled structure, the "pro-acrosome" (Fig. 3E,F), which later shows thick black rings separated by thin electron-transparent spaces (Fig. 3G). The conical piece connecting head and tail is already located in the nuclear fossa, and is separated from the flagellum by a great amount of cytoplasm with a well-evident Golgi complex and large mitochondria. The flagellum lengthens but the accessory structures are still absent (Fig. 3F).

The middle spermatids are characterized by the gradual lengthening and shifting of the nucleus, which finally becomes perpendicular to the flagellum while its chromatin condenses more distally. Some mitochondria and a large Golgi complex are visible in the cytoplasm; Golgi vesicles migrate toward the cell surface and release their products into the interspace between the cell membrane and the flagellum. A very elongated conical piece links the nucleus to the axoneme (Fig. 3H,I).

The final spermatids, which were observed in both *U. anorektoxys* and *U. acanthostylis,* appear U-shaped, with the elongated nucleus and the flagellum parallel to one another. The nucleus lengthening and chromatin condensation are almost complete, and the chromatin in both species already has the peculiar fingerprint aspect of the mature sperm. Mitochondria, Golgi complexes, many polyribosomes, and free ribosomes are still visible between the nucleus and the flagellum (Fig. 3K,L). The residual cytoplasm is shed out of the cell and removed by several macrophages (Fig. 3J).

Discussion

Spermatozoa

Our observations point to considerable differences between the sperm of the two species of *Urodasys* that we have examined. Both are spiralized, for the whole cell length in *Urodasys acanthostylis* but only in the anterior region of the head in *U. anorektoxys*. The acrosome is a twisted column in *U. anorektoxys,* whereas in *U. acanthostylis* it is a simple vesicle lying along the nuclear helix. The tail contains peculiar globular accessory structures in *U. anorektoxys,* whereas in *U. acanthostylis* accessory fibers are present. The unusual fingerprint pattern of condensed chromatin and the absence of mitochondria are the only two spermatological synapomorphies of the two species that we could observe. To our knowledge, a fingerprint pattern of the chromatin has never been observed, whereas the absence of mitochondria from mature spermatozoa, although a rare feature, is known in scattered animal species. Baccetti & Afzelius (1976) gave a list of those species, among which are the platyhelminth *Echinococcus granulosus,* a few arthropods, a single vertebrate, the urodele amphibian *Cryptobranchus,* and all Acanthocephala. The fact that the only two gastrotrich species are devoid of mitochondria and belong to the same genus suggests an interesting autapomorphy. The study of other species of *Urodasys* could shed some light on this unusual feature.

In the last few years, many different models of macrodasyid spermatozoa have been described (for review, see Balsamo et al. 1999), and it has become more difficult to outline a basic sperm pattern valid for the whole group. Among the most widespread characters are the position of mitochondria enclosed by the spring-shaped nucleus, and the helical shape of

Fig. 3. Spermatogenesis of *Urodasys* (A–K, J: *Urodasys anorektoxys*; L: *U. acanthostylis*). **A.** Mature spermatozoa (s) into a sperm duct. The fingerprint chromatin (fc) is clearly visible. **B.** Spermatogonia (sg). **C.** Interphasic spermatocytes (is) (n, nucleus; nu, nucleolus). **D.** Prophasic spermatocytes (sc): cytoplasmic bridge connecting cells (arrowhead) are visible; m, mitochondria. **E, F.** First spermatids: the nucleus (n) is lengthening and the "pro-acrosome" (pa) is wrapping around it. The conical piece is located in the nuclear fossa (arrow). **G.** Pro-acrosome (pa): note the thick black rings separated by thin electron-transparent spaces. **H, I.** Middle spermatids: the nucleus (n) and flagellum (f) are perpendicular to each other. Golgi vesicles (arrow) are migrating toward the flagellum. An elongated conical piece (+) links the nucleus to the axoneme. **K, L.** Final spermatids: the nucleus (n) and flagellum (f) are parallel to each other. **J.** Macrophages (ma) removing the residual cytoplasm.

one or more regions of the cell, which is, however, a feature shared with many other animal taxa (Jamieson 1999). Only two macrodasyid species have mitochondria coiled around the nucleus—*Macrodasys* sp. (Ruppert 1978) and *M. caudatus* (Marotta et al. 2005); it may be relevant to note that *Macrodasys* is the only other genus forming, with *Urodasys,* the family Macrodasyidae, which is recognized as a monophyletic taxon on a morphological basis (Hochberg & Litvaitis 2000, 2001; Marotta et al. 2005). Other spermatological characters of one or the other of these species of *Urodasys* are shared with other gastrotrichs: the accessory fibers of *U. acanthostylis* have a shape and position similar to those of *Cephalodasys maximus* (Macrodasyida, Lepidodasyidae) (Fisher 1994), and are cross-striated like those of Xenotrichulidae (Chaetonotida); the acrosome coiled around the nuclear helix of *U. acanthostylis* recalls the perinuclear helix, which is a basal extension of the acrosome, of some Thaumastodermatidae (Macrodasyida) (Guidi et al. 2003a). The peculiar pattern of the axonemal distal portion of the flagellum of *U. acanthostylis* is similar to that of *Musellifer delamarei* (Chaetonotidae), which, however, belongs to the order Chaetonotida (Guidi et al. 2003b).

Spermatogenesis

Spermatogenesis in Macrodasyida is well documented in four species: *Turbanella cornuta* (Turbanellidae), *Cephalodasys maximus* and *Lepidodasys* sp. (Lepidodasyidae), and *Acanthodasys aculeatus* (Thaumastodermatidae) (Teuchert 1976; Fisher 1994; Guidi et al. 2003a, 2004).

The synchronous development of the spermatocytes of *U. anorektoxys,* connected by cytoplasmic bridges, agrees with the previous studies on *T. cornuta*, *Lepidodasys* sp., and *A. aculeatus*. In all the species studied so far, the mitochondria, or the unique giant mitochondrion, derived by the fusion of multiple individual mitochondria, wind around the elongating nucleus and then sink into it. In *U. anorektoxys,* mitochondria are absent in the mature sperm, but are present in the cytoplasm of all the spermatogenetic stages, even if never in association with the nucleus. It is likely that the mitochondria are expelled together with the cytoplasmic debris at the end of the spermatogenetic process, when the head and the flagellum become arranged in parallel. In the first and middle spermatids of *U. anorektoxys,* a tubular structure coils around the elongating nucleus: we hypothesize that this structure corresponds to a "pro-acrosome" because of its strong morphological similarity to the acrosome of the mature sperm. In the terminal spermatids of the same species, the pro-acrosome is located in the nucleus for most of its length and occupies the position usually taken by mitochondria in many other gastrotrichs. In *A. aculeatus, C. maximus,* and *T. cornuta,* the lengthening of the flagellum occurs parallel to the nuclear-acrosome complex, so that all spermatids become U-shaped. On the contrary, in *U. anorektoxys,* and in *U. acanthostylis,* as in *Lepidodasys* sp. (Guidi et al. 2004), the parallel growth of the flagellum and nucleus is achieved only at the end of spermatogenesis, again resulting in a U-shaped spermatid. Thus, the characteristic U-shaped late spermatid may be considered an autapomorphy of the Macrodasyida.

The absence of mitochondria in the mature spermatozoa in the two species of *Urodasys* might be related to the dysoxic habitat in which they were found. The very low oxygen tension and anoxia of sediments of the Santa Barbara Basin, the habitat of *U. anorektoxys,* is well known (Kuwabara et al. 1999; Bernhard et al. 2000), while the dysoxia of the sediment of the Ventotene Island, habitat of *U. acanthostylis,* is shown by the characteristic associated fauna, including *Kentrophoros* sp. (Ciliata), *Gnathostomula* sp. (Gnathostomulida), and at least two species of Stilbonematinae (Nematoda), all typical dwellers of poorly oxygenated environments (cf. Giere 1993).

On the other hand, the loss of mitochondria during the spermatogenetic process might also be related to some kind of peculiar internal modality of fertilization, as mitochondria are present in germinal and somatic cells of both species of *Urodasys*. Despite lacking mitochondria, mature spermatozoa in *U. acanthostylis* have been seen actively moving both isolated from mature animals and in their body after copulation, and therefore show normal behavior. Baccetti & Afzelius (1976:p. 68) comment that whereas in some cases, like some isopods, mitochondria-free spermatozoa appear non-motile, in other cases, like that of Acanthocephala, they are "… capable of rapid movements which may be of the same duration as those of sperm types with mitochondria." It is generally agreed that ATP needed for sperm movement is formed in these cases through glycolysis under anaerobic conditions, whereas in the species of *Urodasys* the persistence of mitochondria up to the final steps of spermatogenesis may easily explain a normal sperm activity. Moreover, the caudal organ, with a likely copulatory function, may favor the transferring of sperm to the partner during copulation in *U. acanthostylis* as in most Macrodasyida.

The relationship between the absence of the mitochondria in the spermatozoa of *Urodasys* species and their anomalous position in the sperm of *Macrodasys*

species (see Marotta et al. 2005) should be examined in the phylogenetic context of the family Macrodasyidae.

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References

Baccetti B & Afzelius BA 1976. The Biology of the Sperm Cell. Karger, Basel, Switzerland, p. 254.

Balsamo M, Fregni E, & Ferraguti M 1999. Gastrotricha. In: Reproductive Biology of Invertebrate, Vol. 9. Adiyodi KG & Adiyodi RG, eds., Part A. Progress in Male Gamete Ultrastructure and Phylogeny. Jamieson BGM, ed., pp. 171–191. Oxford & IBH Publishing Co., New Delhi.

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

Bernhard JM, Buck KR, Farmer MA, & Bowser SS 2000. The Santa Barbara Basin is a symbiosis oasis. Nature 403: 77–80.

Cogliati R & Gautier A 1973. Mise en évidence de l'ADN et des polysaccharides à l'aide d'un nouveau réactif 'de type Schiff'. C. R. Acad. Sci. 276: 3041–3044.

Ermak TH & Eakin RM 1976. Fine structure of the cerebral pygidial ocelli in *Chone ecaudata* (Polychaeta: Sabellidae). J. Ultrastruct. Res. 54: 243–260.

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

Giere O 1993. Meiobenthology. The Microscopic Fauna in Aquatic Sediments. Springer-Verlag, Berlin.

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

Guidi L, Marotta R, Pierboni L, Ferraguti M, Todaro MA, & Balsamo M 2003b. Comparative sperm ultrastructure of *Neodasys ciritus* and *Musellifer delamarei,* two species considered to be basal among Chaetonotidae (Gastrotricha). Zoomorphology 122: 135–143.

Guidi L, Pierboni L, Ferraguti M, Todaro MA, & Balsamo M 2004. Spermatology of the genus *Lepidodasys* Remane, 1926 (Gastrotricha, Macrodasyida: towards a revision of the family Lepidodasyidae Remane 1927. Acta Zool. (Stockholm) 85: 211–221.

Higgins RP & Thiel H 1988. Introduction to the Study of Meiofauna. Smithsonian Institute Press, Washington, DC. 488pp.

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

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

Hummon WD 2001. Global database for marine Gastrotricha on CD. Ohio University Zoological Collections, Athens (hummon@ohio.edu).

Kuwabara JS, van Geen A, McCorkle DC, & Bernhard JM 1999. Dissolve sulphide distributions in the water column and sediment pore waters of the Santa Barbara Basin. Geochim. Cosmochim. Acta. 63: 2199–2209.

Jamieson BGM 1999. Reproductive Biology of Invertebrates. Vol. 9. Progress in Male Gamete Ultrastructure and Phylogeny, Parts A, B, C. Adiyodi KG & Adiyodi RG, eds., 271, 450, 342pp. John Wiley & Sons, Chichester, UK.

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

Olins AL, Moyer BA, Kim SH, & Allison DP 1989. Synthesis of a more stable osmium ammine electron-dense DNA stain. J. Histochem. Cytochem. 37: 395–398.

Ruppert EE 1978. The reproductive system of Gastrotrichs II. Insemination in *Macrodasys*: a unique mode of sperm transfer in Metazoa. Zoomorphologie 89: 207–228.

Schoëpfer-Sterrer C 1974. Five new species of *Urodasys* and remarks on the terminology of the genital organs in Macrodasyidae (Gastrotricha). Cah. Biol. Mar. 15: 229–254.

Teuchert G 1976. Elektronenmikroskopische Untersuchung über die Spermatogenese und Spermatohistogenese von *Turbanella cornuta* Remane (Gastrotricha). J. Ultrastruct. Res. 56: 1–14.

Todaro MA, Bernhard JM, & Hummon WD 2000. A new species of *Urodasys* (Gastrotricha, Macrodasyida) from dysoxic sediments of the Santa Barbara Basin (California, U.S.A.). Bull. Mar. Sci. 66: 467–476.

Todaro MA, Matinato L, Balsamo M, & Tongiorgi P 2003. Faunistics and zoogeographical overview of the Mediterranean and Black Sea marine Gastrotricha. Biogeographia 24: 131–160.

Valbonesi A & Luporini P 1984. Researches on the coast of Somalia. Gastrotricha Macrodasyoidea. Monit. Zool. Ital., N.S. XIX (Suppl. 1): 1–34.