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Comparative sperm ultrastructure of *Neodasys ciritus* and *Musellifer delamarei*, two species considered to be basal among Chaetonotida (Gastrotricha)

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Abstract The spermatozoa of two species supposed to be basal to Gastrotricha Chaetonotida, *Neodasys ciritus* and *Musellifer delamarei*, were studied in order to supply further elements to the understanding of sperm evolution in Chaetonotida, a group in which a fully parthenogenetic reproduction is dominant. Two considerably different sperm patterns were found: the spermatozoon of *N. ciritus* has a simple, conical acrosome, a short, condensed nucleus, few conventional mitochondria randomly arranged along the sperm head, and a 9×2+2 flagellum perpendicular to the sperm major axis. The spermatozoon of *M. delamarei* is a filiform cell with a simple acrosome, a partially condensed nucleus, four mitochondria at the nuclear base, and a flagellum with a 9×2+2 axoneme and large accessory fibers. Some sperm features of *M. delamarei* are comparable to those of Xenotrichulidae, the only other Chaetonotida producing conventional spermatozoa, whereas the sperm of *N. ciritus* appears different from all the other patterns known among Gastrotricha, thus knowledge of it does not help in solving the problem of the discussed phylogenetic position of the genus.

Keywords Gastrotricha · Ultrastructure · Spermatozoa · Phylogeny

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Introduction

The vast majority of Gastrotricha Chaetonotida (over 450 species) are characterized by a loss of amphymixis and the acquisition of parthenogenetic reproduction, supposedly associated with the invasion of freshwater habitats (Balsamo 1992). In recent years, however, it has become progressively clear that parthenogenesis is often followed by a life phase characterized by a limited but regular production of few spermatozoa (reviewed in Weiss 2001). The discovery of sperm in such a large number of both freshwater and marine parthenogenetic Chaetonotida (Hummon 1966; Ruppert 1977; Balsamo and Todaro 1988) induced Weiss (2001), in his extensive review of the phenomenon, to consider them as functional (see also Kisielewska 1981; Hummon and Hummon 1983; Balsamo and Todaro 1988; Balsamo 1992), in spite of the peculiar ultrastructure of these cells. In both species of Chaetonotidae examined so far by electron microscopy the spermatozoa are, in fact, reduced to only a rod-like nucleus surrounded by a plasma membrane, and devoid of any recognizable organelle (Hummon 1984; Balsamo 1992). On the other hand, the few marine, functional hermaphroditic Chaetonotida, i.e., Neodasyidae, Xenotrichulidae, and *Musellifer* species (Chaetonotidae), produce more conventional spermatozoa, and apparently reproduce only through amphymixis (Weiss 2001). Current knowledge of these spermatozoa is limited to light microscopy observations: the only exceptions are the 'dart-shaped uniflagellated sperm' reported by Ruppert (1991) in a comment to a single TEM micrograph of *Neodasys* sp. from North Carolina, USA, and the TEM description of the spermatozoa of three species of Xenotrichulidae (*Heteroxenotrichula squamosa* Wilke, 1954, *Xenotrichula intermedia* Remane, 1934, and *X. punctata* Wilke, 1954, in Ferraguti et al. 1995). *Neodasys* sp. shows an apparently simple and primitive sperm *sensu* Franzén (1955), whereas the Xenotrichulidae (except *Draculiciteria*; see Ruppert 1979) show a filiform sperm with a simple acrosome, an uncondensed nucleus, a single mitochondrion at its base, and a flagellum containing

large accessory fibers each surrounded by an obliquely striated wall; in two species examined, two peculiar, long, para-acrosomal bodies lie at the sides of the acrosome. The sperm structure of Xenotrichulidae species appears completely different from that of the amphymictic species of the other gastrotrich taxon, Macrodasyida: the only possible synapomorphy may be the striated cortex of the accessory fibers and the striated cylinder surrounding the axoneme, respectively (Ferraguti et al. 1995).

We have shown elsewhere that the spermatozoon of the Macrodasyida species has a general plan based on peculiar features, and has proved to be a potentially useful tool for phylogenetic analyses (Ferraguti and Balsamo 1995; Fregni 1998). In contrast, current knowledge about the spermatozoa of Chaetonotida is still too scanty to allow the outlining of a model of the sperm in this taxon.

In the present paper we describe the ultrastructure of the spermatozoa of *Neodasys ciritus* Evans, 1992 and of *Musellifer delamarei* (Renaud-Mornant, 1968). The first is one of the three species known of Neodasyidae, the only representative taxon of the Chaetonotida Multitubulatina, which look similar to Macrodasyida rather than to all the other Chaetonotida, the Paucitubulatina, in having a worm-shaped body and lateral adhesive tubes. They are marine, quite rare, and hermaphrodite, and their reproductive apparatus includes two accessory organs, possible homologues of the 'frontal' and 'caudal' organs present in most Macrodasyida (cf. Ruppert 1991). *Musellifer delamarei* is a very rare, marine species belonging to Chaetonotidae, and like the two other congeneric species is a functional hermaphrodite, a sexual condition shared only with the Xenotrichulidae, among Chaetonotida Paucitubulatina.

An evolutionary scenario recently outlined on morphological bases (Hochberg and Litvaitis 2000) depicts *Neodasys* on an early, separate clade of the Chaetonotida branch, according to the current systematization, whereas *Musellifer* appears as the sister group of all the other Paucitubulatina, in partial contrast with the current view considering *Musellifer* as a basal taxon within the Chaetonotidae. Thus the study of *N. ciritus* and *M. delamarei* spermatozoa was intended to shed some light on the possible plesiomorphic design of chaetonotid spermatozoa and to address a comparison with the xenotrichulid sperm patterns known. Moreover, detailed observations on the reproductive structures might allow to obtain some information about the fertilization process.

Materials and methods

Specimens of *M. delamarei* were collected on 20 June 2001 at a water depth of 5 m in a submarine cave along the Ionian coast of Apulia (Grotta della Principessa, Lecce, Italy; Balsamo et al. unpublished data). Individuals of *N. ciritus* were collected on 22 July 2001 from the shallow sublittoral of Long Beach Island, New Jersey, USA. In both cases animals were extracted from the sediment by the narcotization–decantation technique using a 7% magnesium chloride aqueous solution (Pfannkuche and Thiel 1988). Some specimens were observed in vivo under Nomarski differential interference contrast optics under a Leitz Dialux 20 microscope. Living specimens were photographed with a Nikon 995 Coolpix digital camera, and measured with an ocular micrometer; location of the principal morphological characters along the body are reported in percentage units (U) from anterior to posterior.

Transmission electron microscope analysis

Some specimens were fixed for several days in a 0.1 M cacodylate buffered paraformaldehyde–glutaraldehyde mixture in a saturated solution of picric acid with sucrose added (SPAFG; Ermak and Eakin 1976) for TEM analysis. The animals were then washed in 0.1 M cacodylate buffer (pH 7.4), postfixed in 2% osmium tetroxide in the same buffer for 2 h, rinsed in the same buffer, dehydrated in a graded acetone series, prestained en bloc for 2 h in the dark in uranyl acetate in 70% acetone, and embedded in Araldite. Ultrathin sections were cut with a Reichert Ultracut E and an LKB Ultratome 2088 V, contrasted again with a saturated solution of 50% uranyl acetate in alcohol followed by lead citrate solution, and observed and photographed with either a Jeol 100SX, a Philips 300, or a Zeiss 902 transmission electron microscope. Measures of TEM-prepared material were derived directly from micrographs.

Results

Neodasys ciritus

Mature spermatozoa were observed in the testes as well as in the putative seminal receptacle ('frontal organ'; Ruppert 1991). The two testes extend at the sides of the intestine from U31 to U61 (Fig. 1A, B); a frontal organ was observed in the posterior portion of the body, at U75, where it occupied most of the body's diameter (Figs. 1A, 2J); a caudal organ was not seen.

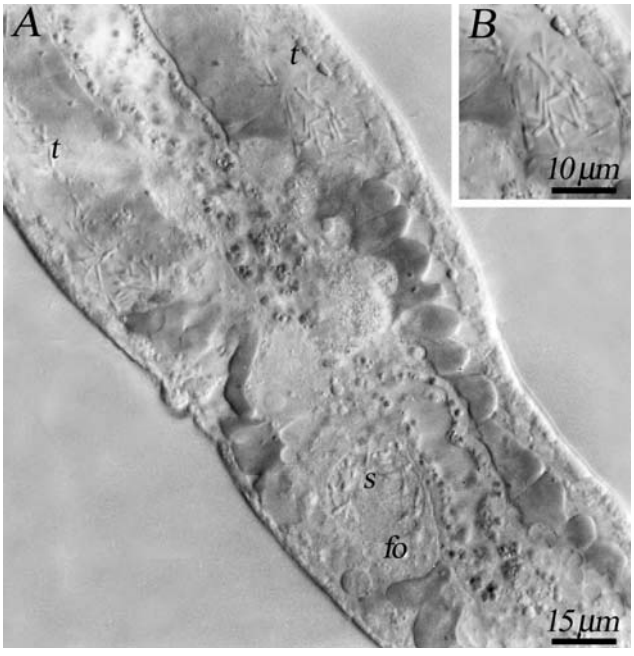


Fig. 1A, B *Neodasys ciritus* (Nomarski interference contrast). **A** Internal anatomy of the midtrunk region with location of the testes (*t*) and the frontal organ (*fo*) containing a spermatophore with spermatozoa (*s*). **B** Detail of the testicular spermatozoa

The mature spermatozoon of *N. ciritus* (Fig. 5) was described as commaform in shape, since the head and the tail are orthogonally oriented with respect to each other (Fig. 2A). The head, about 5.2 μm in length, is formed by an elongated and conical acrosome (average length 2.9±0.4 μm, n=6), followed by a roughly cylindrical, short nucleus (average length 2.3±0.2 μm, n=14; average diameter 0.73±0.1 μm, n=8) (Fig. 2A, B). A varying number of mitochondria with a conventional aspect are variably arranged around the nucleus and, more frequently, around the acrosome (Fig. 2G). The conical acrosome is composed of a basal, electron-dense, small region containing a crystalline structure (Fig. 2A, E), and of an apical region including a thin tubule, which runs laterally (Fig. 2B–D) and may be formed by an invagination of the acrosomal membrane. The apex has a narrow tip ending in a small button (Fig. 2G). A 'wig' of many filaments of an uncertain nature is attached to the outer side of the acrosome membrane, and protrudes into the surrounding cytoplasm (Fig. 2B, C, G); the acrosome membrane seems to have some sort of specialization allowing the connection of these filaments (Fig. 2B, G). In the apical region of the acrosome, the filaments are shorter and arranged more densely than in the middle and the basal regions (Fig. 2G). The nucleus is a simple chromatin rod, composed of two concentric regions with different electron density, the inner of which constitutes the central nuclear axis, and is surrounded by an external, thinner one (Fig. 2A, F). The basal part of the latter forms five to eight regularly displaced longitudinal ridges, with a semicircular profile in cross-section (Fig. 2F). The contact area between the acrosome and the nucleus shows some sort of membrane specialization (Fig. 2B). The nuclear base show a depression hosting some electron-dense material derived from the proximal centriole, and connected to the nucleus by means of thin fibrils (Figs. 2H, 5). In a few favorable sections only 'ghosts' of the triplets inside this structure could be observed (Fig. 2H). The tail is almost perpendicular to the sperm head, and shows a simple 9×2+2 axoneme arising from the modified distal centriole (Fig. 2A, H). This one is nearly parallel to the proximal centriole, and is formed by a ring of dense material surrounding the base of the axoneme. Microtubules are barely visible in this unconventional basal body that is connected to the plasma membrane by an anchoring apparatus (Fig. 2I).

The spermatozoa contained in the frontal organ are hosted into a large mass of spongy material with numerous, large cavities (Fig. 2J). Single spermatozoa are present in several cavities: they look similar to the testicular sperm, but their cytoplasm is markedly reduced, mitochondria are smaller, with hardly recognizable cristae, and the filaments of the acrosomal 'wig' appear to be so pressed one against the other that they assume a crystalline aspect (Fig. 2J).

Musellifer delamarei

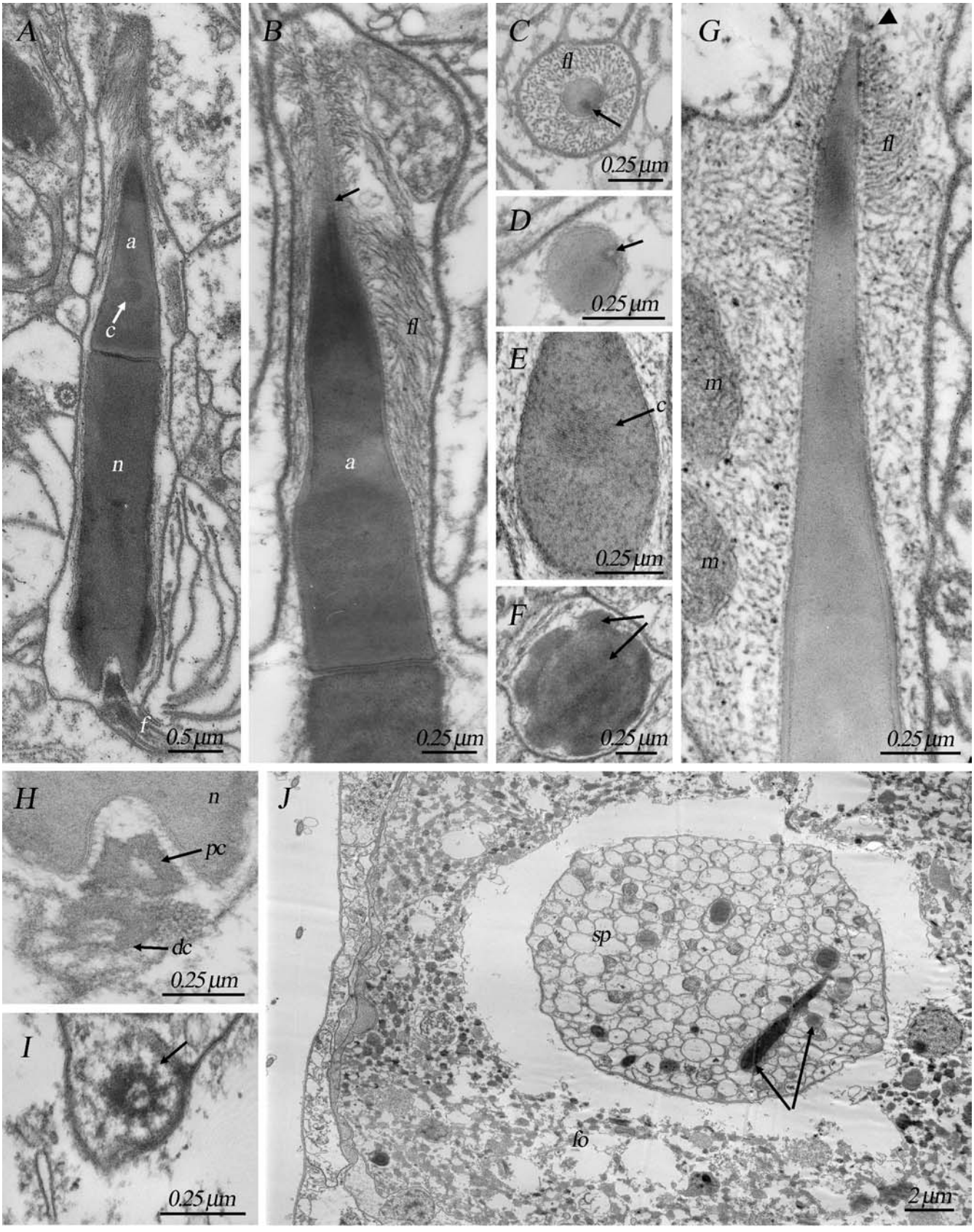
The two testes are small, simple bags surrounded only by two plasma membranes with some electron-dense material deposited between them. They are paired organs situated in the posterior-lateral region of the body (Fig. 3A, B) between the gut and the body wall at U51, in close proximity to a large, maturing oocyte (Figs. 3B, 4I). A few (about 20–30) spermatozoa are present in each testis (Fig. 3E), often 'coiled' around one or two large central, degenerating cells (Fig. 4I).

The spermatozoon of *M. delamarei* is a filiform cell, approximately 28 μm long, and is composed by a sequence of acrosome, nucleus, and flagellum (Figs. 3F, 5). Four mitochondria are gathered eccentrically at the nuclear base (Fig. 4B, G, H) and a supernumerary external membrane is present all around the cell (Fig. 4C–G). Due to the small number and the slenderness of the spermatozoa, we have few informative longitudinal sections, thus our reconstruction is mainly based on the comparison of the cross-sections.

The acrosome, 8 μm long, is a sort of an elongated cone gradually reducing its diameter from the base, 0.3 μm, to the apex, 0.07 μm (Fig. 4A, C, E). Two regions are clearly recognizable due to the different texture of the contents (Fig. 4A), which appears basally loose (Fig. 4D) but becomes more compact apically (Fig. 4C). The contact area with the nucleus is obliquely oriented with respect to the longitudinal axis of the sperm (Fig. 4E), and some electron-dense material is always present between the plasma and the acrosomal membranes (Fig. 4C *arrow*).

The nucleus is an elongated cylinder, approximately 0.4 μm in diameter, with chromatin scarcely condensed in the form of thin threads (Fig. 4F, H). The nuclear base is irregular in shape since it is flanked by four mitochondria, two of them parallel to each other (Fig. 4B, G, H).

The flagellum, about 0.3 μm in diameter in its main portion, contains a 9×2+2 axoneme surrounded by accessory fibers (Fig. 4L) and ends in a very peculiar way (Fig. 4J, K, M–O). There is no trace of a basal body: the axoneme starts directly from the nuclear/mitochondrial region (Fig. 4B). Nine accessory fibers are arranged externally to and in correspondence with each axonemal doublet. Their shape in cross-section is peculiar and each accessory fiber is connected to the subfibers A and B of the corresponding doublet as well as to the subfiber B of the following doublet (Fig. 4L). The terminal portion of the flagellum shows the axoneme progressively becoming less visible and giving rise to a region slightly narrower, 0.25 μm in diameter, which in cross-section shows only nine peripheral and two central electron-dense masses (Fig. 4M). More distally the flagellum gradually reduces in diameter up to about 0.18 μm, and is entirely occupied by electron-dense material somewhat marked by nine denser peripheral septa; in the center of these cross-sections 11 aggregates, each composed of eight small whitish spots, are present (Fig. 4N). Furthermore distally, the aggregates disappear and only the peripheral septa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remain, while the tail diameter continues its progressive reduction (Fig. 4O).

Light microscopy revealed a few other spermatozoa (presumably allosperm) located medially and ventrally to the caudal intestine, at U54.5, within a well-defined cavity (possibly a seminal receptacle) lying posteriorly to a vertebra-shaped structure (Fig. 3C). Another small, rounded structure (caudal organ?) was visible just caudally to the previous one: a clear connection between them was not evident (Fig. 3D).

Discussion

The spermatozoa of *N. ciritus* and of *M. delamarei* are very different from one another: the first looks like a 'primitive' sperm (*sensu* Franzén 1955), due to the simple, short head, the low number of normal mitochondria, the two centrioles, and the 9×2+2 flagellum, whereas the sperm of *M. delamarei* belongs to the category of the 'modified' spermatozoa (*sensu* Franzén 1955), being a filiform cell with a structurally complex flagellum devoid of a conventional basal body. However, both sperm patterns depart from the generally accepted schemes of 'primitive' and 'modified' spermatozoa showing an assemblage of plesiomorphic and apomorphic character states.

The putatively 'primitive' sperm of *N. ciritus* has a complex specialization: the 'wig' of filaments is attached to the outer face of the acrosomal membrane, its nucleus appears completely condensed, the few mitochondria are of conventional type but they are randomly disposed around the acrosome and the nucleus, and a large amount of cytoplasm persists even in the mature spermatozoon, in which the two centrioles are still present, even if deeply modified.

On the other hand, the 'modified-pattern' spermatozoon of *M. delamarei* shows both characters typical of the 'primitive' spermatozoon (a very simple acrosome, few mitochondria, a scarcely condensed nucleus) and possible autapomorphies (presence of a supernumerary membrane, shape of the flagellar endpiece).

How do these sperm patterns compare to the other known ones from Chaetonotida, i.e., those of Xenotrichulidae? The spermatozoon of *N. ciritus* appears quite different, except perhaps in the peculiar shape of the acrosomal tubular structure: this may well be a subacrosomal space, if its lining by the acrosomal membrane is proved. The peculiar 'wig' surrounding the acrosome is visible also in the single micrograph of *Neodasys* sp. published by Ruppert (1991) and is possibly a synapomorphy for the genus *Neodasys.* The chromatin clearly

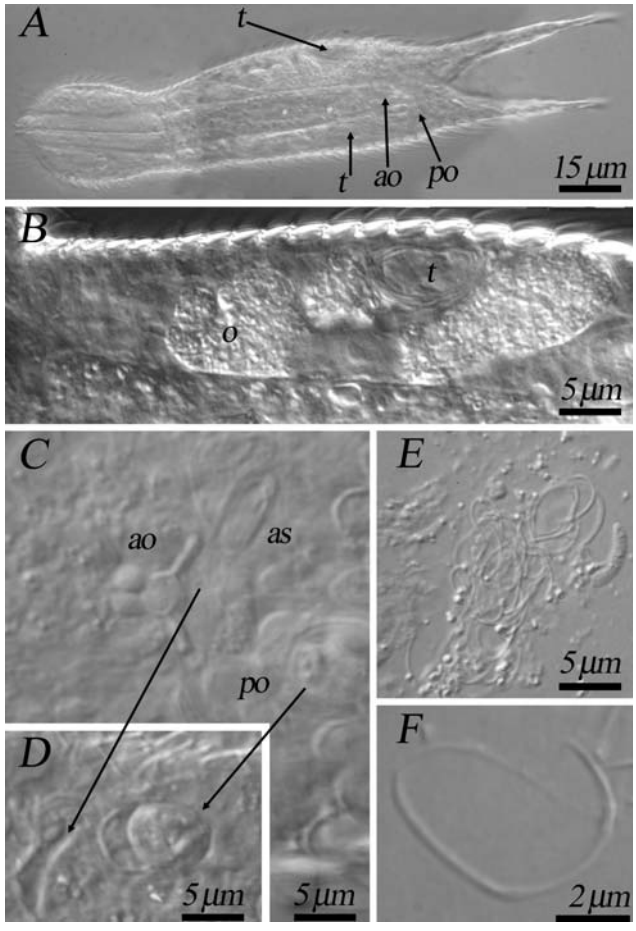
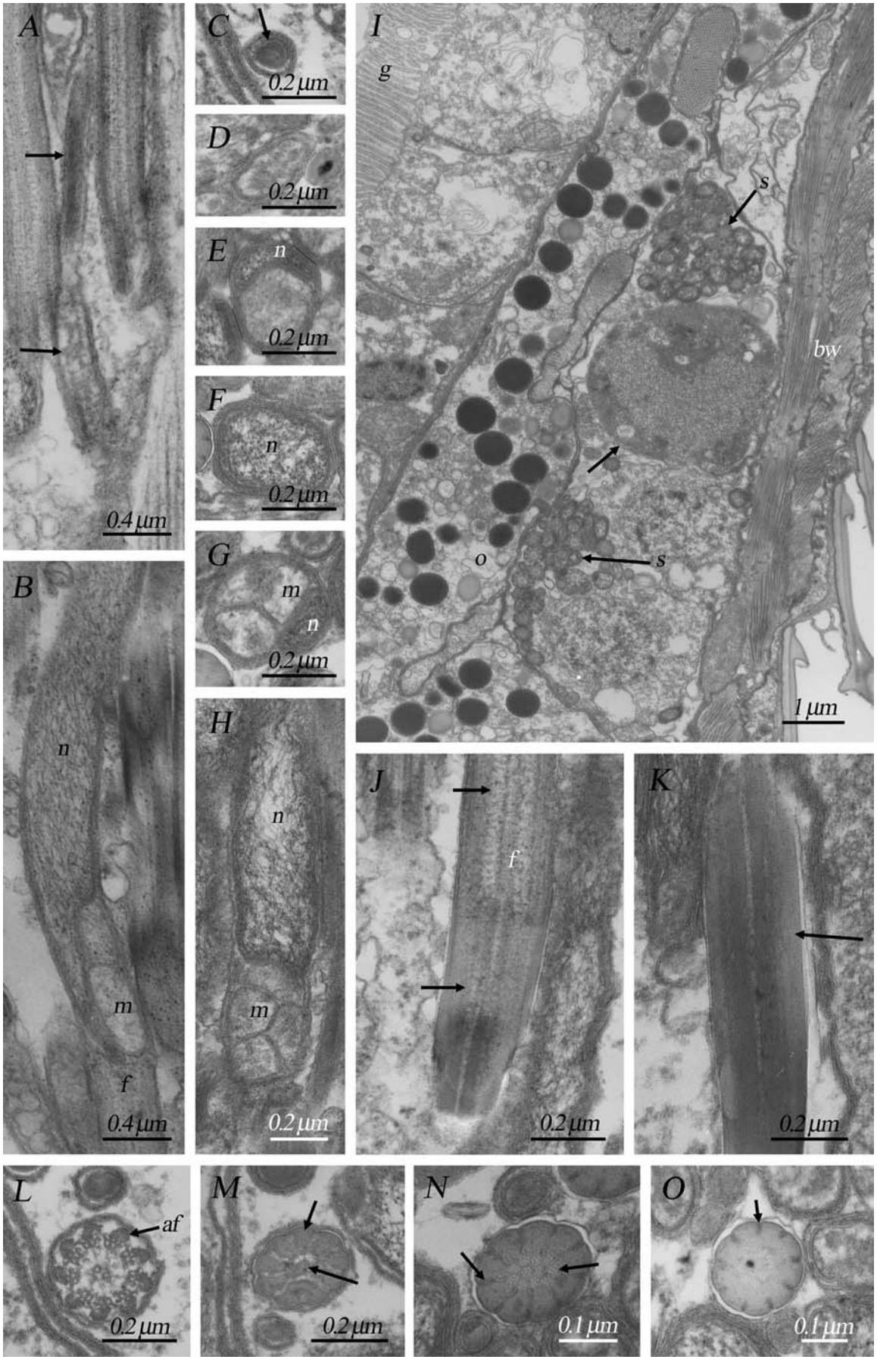


Fig. 3A–F *Musellifer delamarei* (Nomarski interference contrast). **A** Habitus showing the internal anatomy with the location of the testes (*t*) and of the anterior and posterior reproductive accessory organs (*ao, po*). **B** Close-up of the left body side with a mature testis (*t*) and a large, fully grown oocyte (*o*). **C**, **D** Details of the reproductive accessory organs (*ao*, *po*) and possible allosperm (*as*) at different focal planes. **E** Bundle of spermatozoa from the left side testis. **F** Close-up of a single spermatozoon

Fig. 2A–J *Neodasys ciritus* (TEM). **A** Longitudinal section of a spermatozoon. The acrosome (*a*) is conical and its basal portion contains a crystalline structure (*c*). The nucleus (*n*) is a rod of chromatin composed of two concentric regions with different electron densities. The flagellum (*f*) is perpendicular to the sperm head. **B** Detail of the acrosome (*a*). In the apical portion a thin tubule is visible (*arrow*). A 'wig' made up of numerous filaments (*fl*) is attached to the outer side of the acrosomal membrane and protrudes into the surrounding cytoplasm. **C** Cross-section of the apical region of the acrosome containing a thin tubule (*arrow*) and entirely surrounded by densely arranged short filaments (*fl*). **D** Cross-section of the acrosomal apex showing the eccentric position of the thin tubule (*arrow*). **E** Longitudinal section of the basal part of the acrosome including a crystalline structure (*c*). **F** Cross-section of the nuclear base showing that chromatin is organized in two concentric regions (*arrows*) with different electron densities. **G** Longitudinal section of the acrosome. Note the button (*arrowhead*); the filaments (*fl*) of the wig are arranged more densely in the apical region than in the middle and basal ones. Two mitochondria (*m*) lying along the acrosome are visible. **H** Longitudinal section of the nuclear base (*n*), indented to form a depression hosting some electron-dense material derived from the proximal centriole (*pc*). The modified distal centriole (*dc*) is parallel to the proximal one and gives rise to the flagellum. **I** The modified distal centriole is connected with an anchoring apparatus (*arrow*) to the plasma membrane. **J** Section of the frontal organ (*fo*) containing a large mass of a spongy material, the spermatophore (*sp*). Several spermatozoa, two of which are indicated by *arrows*, are visible in the large cavities of the spermatophore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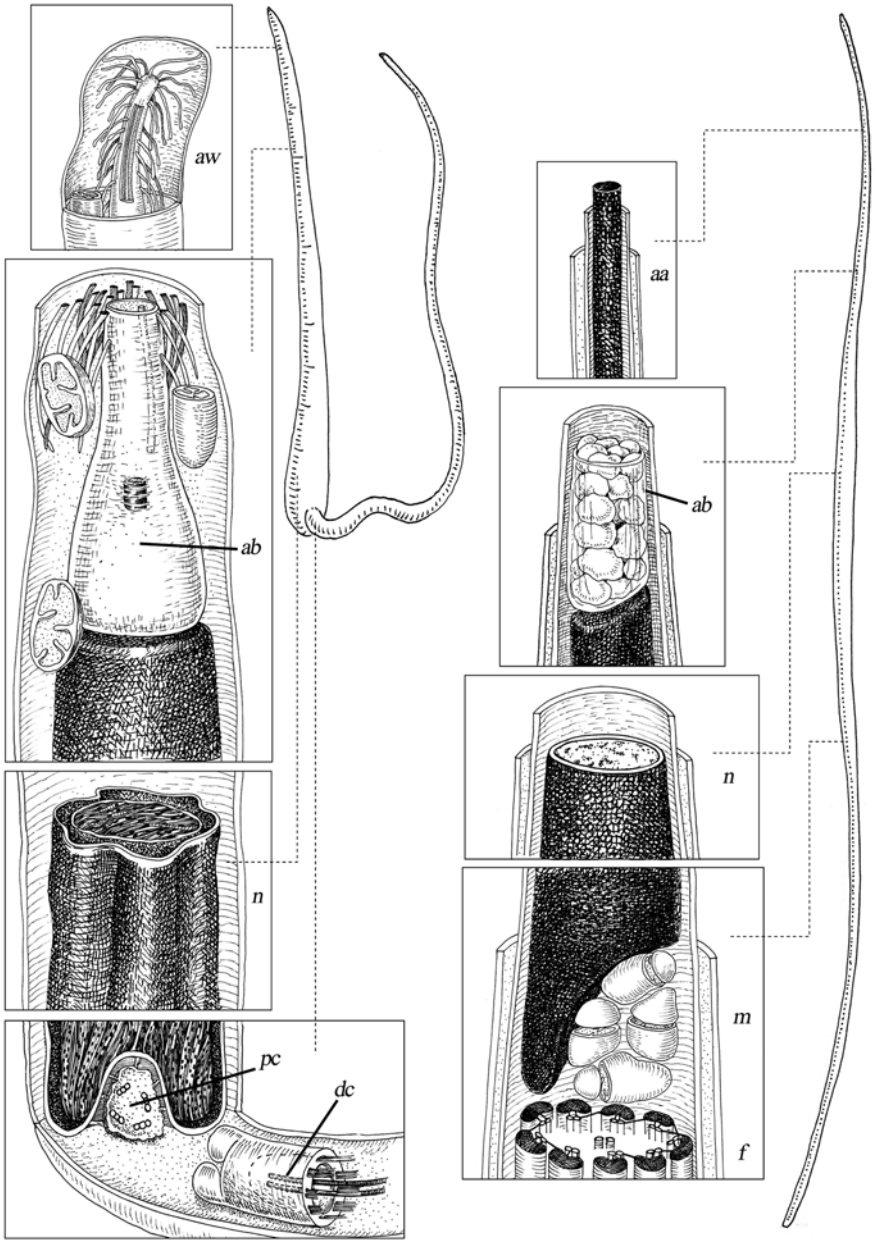

Fig. 5 Three-dimensional reconstruction of the spermatozoa of *N. ciritus* (*left*) and *M. delamarei* (*right*) with some details of different parts. *N. ciritus*: anterior portion of the spermatozoon, with the plasma membrane cut off to show the acrosome surrounded by the 'wig' (*aw*); basal portion of the acrosome surrounded by three mitochondria (*ab*); nucleus (*n*); remnants of the proximal centriole (*pc*); distal centriole (*dc*). *M. delamarei*: anterior portion of the acrosome (*aa*) with compact contents; basal portion of the acrosome (*ab*) with a somewhat foamy content; nucleus (*n*); four mitochondria (*m*) flanking the nucleus base; flagellum (*f*)

Fig. 4A–O *Musellifer delamarei* (TEM). **A** Longitudinal section of the acrosome. The apical region is thinner and much more electron dense than the basal one (*arrows*). **B** Longitudinal section of the nucleus (*n*) basally flanked by the mitochondria (*m*), and touching the insertion of the flagellum (*f*), which does not show a true basal body. **C–G** Cross-sections of the acrosome and nucleus at different levels. **C** Acrosomal apex. **D** Acrosomal base. **E** Acrosomal base and anterior end of the nucleus (*n*). **F** Nucleus (*n*). **G** Nuclear base (*n*) flanked by the mitochondria (*m*). **H** Longitudinal section of the nucleus (*n*) and mitochondria (*m*) region. **I** Longitudinal section of a testis located between the gut (*g*) and the body wall (*bw*), and close to a large, maturing oocyte (*o*). Several spermatozoa (*s*) adjacent to a large, degenerating cell (*arrow*) are visible. **J** Longitudinal section of the flagellum (*f*) showing the different structure of the axoneme in the basal and in the terminal regions (*arrows*). **K** Detail of the terminal region of the flagellum: some cross-striations are visible on the accessory fibers (*arrow*). **L–O** Cross-sections of the flagellum at progressively distal levels. **L** Base of the flagellum: the 9×2+2 axoneme is surrounded by nine accessory fibers (*af*). **M** Postaxonemal region characterized by nine peripheral and two central electron-dense masses (*arrows*). **N** A more distal region, reduced in diameter, and showing an electron-dense homogeneous content marked by nine denser peripheral septa (*arrow*); 11 groups each composed of eight whitish spots are arranged in the center of the section (*arrow*). **O** Terminal region, further reduced in diameter, where the nine peripheral denser septa appear shorter (*arrow*)

organized in two concentric regions appear to be autapomorphic characters.

Some features of the *M. delamarei* sperm seem more informative: the aspect of the nucleus and the loose condensation of chromatin are nearly identical in the Xenotrichulidae. These ones show the mitochondria interposed between nucleus and flagellum. Such a condition is present elsewhere only in Clitellata and Onychophora (Ferraguti et al. 1995), and among Gastrotricha this condition is to be considered an autapomorphy for Xenotrichulidae. In *M. delamarei*, in fact, one of the mitochondria is in contact with the flagellum, but also a thin, basal part of the nucleus extends to touch it. Most similarities between *M. delamarei* and Xenotrichulidae sperm reside in the main structure of the flagellum, and particularly in the presence of accessory fibers with a very similar cross-striation, and a similar geometry of the connections to the doublets. The peculiar shape of the endpiece of the *M. delamarei* sperm as well as the intriguing presence of a supernumerary membrane surrounding the whole cell are autapomorphic features for the whole Animal Kingdom. The supernumerary membrane might be the remnants of the spermiogenetic process. The latter might occur in a cyst, as described for *Lepidodermella squamata*, where the cyst cells forms a complete cover around the spermatids (Hummon 1984).

Summarizing, spermatological characters support the assumption that *M. delamarei* could be a sister group to the Xenotrichulidae, so confirming spermatologically its belonging to Chaetonotida, even if on a separate clade; however, they do not help to solve the controversial phylogenetic relationships of *Neodasys.* Perhaps some elements useful in answering to this question may come from a comparative study of the small spermatozoa of a few Macrodasyida species which share with *Neodasys* sperm a similar light microscopy morphology, i.e., the 'commaform' sperm of *Dolichodasys elongatus* Gagne, 1977, *D. carolinensis* Ruppert and Shaw, 1977, and *Macrodasys* sp. (Ruppert 1978).

The finding in both species examined of spermatozoa in anatomical districts far from and/or not connected to the testes suggest their external origin. If they were allosperm they would have derived from a partner through internal fertilization, likely mediated by some accessory reproductive structures, as is the general rule for Macrodasyida. Organs potentially involved in sperm transfer have been found in individuals of both species during our survey, but their actual role is still far from being understood. For instance, the rounded, spongy mass including sperm which has been observed in *N. ciritus* could be interpreted as a spermatophore, according to the finding by Ruppert (1991) in *Neodasys* sp. The frontal organ containing the spermatophore was clearly seen, but the caudal organ acting as a copulatory organ was not.

Even more difficult appears the interpretation of the accessory structures observed in *M. delamarei*. The presence of spermatozoa in a hollow organ suggests for this a function of seminal receptacle, like the frontal organ of Macrodasyida; however, the role played by the peculiar, stiff, vertebra-shaped structure associated with it remains obscure. Also the homology of the rounded, posterior organ of *M. delamarei* with the caudal organ of Macrodasyida is difficult to demonstrate on the basis of the few data available so far.

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References

Balsamo M (1992) Hermaphroditism and parthenogenesis in lower bilateria: Gnatosthostomulida and Gastrotricha. In: Dallai R (ed) Sex origin and evolution. Selected symposia and monographs. UZI Mucchi, Modena, pp 309–327

Balsamo M, Todaro MA (1988) Life history traits of two chaetonotids (Gastrotricha) under different experimental conditions. Invert Reprod Dev 14:161–176

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

Ferraguti M, Balsamo M (1995) Comparative spermatology of Gastrotricha. In: Jamieson BGM, Ausio J, Justine JL (eds) Advances in spermatozoal phylogeny and taxonomy. Mem Mus Natl Hist Nat Paris 166:105–117

Ferraguti M, Balsamo M, Fregni E (1995) The spermatozoa of three species of Xenotrichulidae (Gastrotricha, Chaetonotida): the two "dünne Nebengeisseln" of spermatozoa in *Heteroxenotrichula squamosa* are peculiar para-acrosomal bodies. Zoomorphology 115:151–159

Franzén Å (1955) On spermiogenesis, morphology of the spermatozoon, and biology of fertilization among invertebrates. Zool Bidr Uppsala 31:355–482

Fregni E (1998) The spermatozoa of macrodasyids gastrotrichs: observation by scanning electron microscopy. Invertebr Reprod Dev 34:1–11

Hochberg R, Litvaitis MK (2000) A morphology-based framework of gastrotrich relationships. Biol Bull 198:299–135

Hummon WD (1966) Morphology, life history, and significance of the marine gastrotrich *Chaetonotus testiculophorus* n. sp. Trans Am Microsc Soc 85:450–457

Hummon MR (1983) The peculiar sperm of the freshwater gastrotrich *Lepidodermella squammata*: fine structure and speculations on function. Ohio J Sci 83:1

Hummon MR (1984) Reproduction and sexual development in a freshwater gastrotrich. 2. Kinetics and fine structure and postparthenogenic sperm formation. Cell Tissue Res 236:619–628

Hummon MR, Hummon WD (1983) Gastrotricha. In: Adiyodi KG, Adiyodi AG (eds) Reproductive biology of invertebrates, vol II. Spermatogenesis and sperm function. Wiley, London, pp 195–205

Kisielewska G (1981) Hermaphroditism of freshwater gastrotrichs in natural conditions. Bull Acad Pol Sci 29B:167–171

Pfannkuche O, Thiel H (1988) Sampling processing. In: Higgins RP, Thiel H (eds) Introduction to the study of meiofauna. Smithsonian Inst Press, Washington, pp 134–145

Ruppert EE (1977) *Ichthydium hummoni* n.sp., a new marine chaetonotid gastrotrich with a male reproductive system. Cah Biol Mar 18:1–5

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

Ruppert EE (1979) Morphology and systematics of the Xenotrichulidae (Gastrotricha, Chaetonotida). Microfauna Meeresbodens 76:1–56

Ruppert EE (1991) Gastrotricha. In: Harrison FW, Ruppert EE (eds) Microscopic anatomy of invertebrates, vol 4. Aschelminthes. Wiley-Liss, New York, pp 41–109

Weiss MJ (2001) Widespread hermaphroditism in freshwater gastrotrichs. Invert Biol 120:308–341