Unusual spermatozoa and reproductive modalities of *Xenodasys eknomios* (Gastrotricha: Xenodasyidae)

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Abstract

The rare macrodasyidan *Xenodasys eknomios* is the first member of the genus to be found in the Mediterranean Sea and the fourth species known worldwide. As *Xenodasys* has proved to be at the base of the Macrodasyida clade, we provide new data on the reproductive system and spermatozoa to try and shed light on the ground pattern of gastrotrich reproduction. The hermaphroditic system of *X. eknomios* consists of two testes with ventrolateral pores and two caudal ovaries. A sac-like frontal organ, generally containing a spermatophore, is enveloped by a basal lamina and attached to the body wall by muscular fibres, appearing as a permanent structure. The spermatophore contains mature, filiform, spermatozoa, each composed of acrosome, spiralized nucleus, connecting piece and flagellum. The complex acrosome is the predominant element and forms the axis of the sperm. Most of the acrosome, is surrounded by two helixes, the external one is the nucleus and the internal one is a crystalline-like ribbon structure. The peculiar acrosome–nucleus complex, and the long connecting piece appear as autapomorphies. The structural plans of the reproductive system and the spermatozoa support the current systematization of Xenodasyidae and provide evidence for a possible sperm transfer modality in these species.

Keywords: *Xenodasys*, Gastrotricha, Macrodasyida, reproductive system, spermatozoa, ultrastructure, fertilization modalities

Introduction

The gastrotrich *Xenodasys eknomios* Todaro, Guidi, Leasi & Tongiorgi, 2006 is a rare macrodasyidan species recently described from a submarine cave along the Ionian coast of Apulia, Italy. It is the first species of *Xenodasys* to be found in the Mediterranean Sea and the third known species in the genus. The other two species in the genus include *X. sanctigoulveni* Swedmark, 1967 from the northern Europe, and *X. riedli* (Schoepfer-Sterrer 1969) recorded from the southern Atlantic coast of the USA (Todaro et al. 2006). An additional new species from Australia is yet to be described (Boesgard & Kristensen 2001). The taxonomic status and systematization of gastrotrichs affiliated to the sister genera *Xenodasys* and *Chordodasys* Schoepfer-Sterrer, 1969 have been the subject of much debate (d’Hondt 1970; Hummon 1974, 1982; Kiselewski 1987). However, new morphological information obtained from the Mediterranean specimens using different microscopical techniques (DIC, SEM, TEM and cLSM) have been clarifying most of the previous uncertainties. The new data include the discovery of a “chordoid” organ in species of *Xenodasys*, an organ only previously known from species of *Chordodasys* (Schoepfer-Sterrer 1969), prompted Todaro et al. (2006) to undertake a taxonomic revision of the genera *Xenodasys* and *Chordodasys* genera. Todaro et al. (2006) made the following amendments: (a) the transfer of the type species of the genus *Chordodasys* (i.e. *C. riedli*) to *Xenodasys*, (b) the establishment of a new genus, *Chordodasiopsis*, to allocate the single remaining *Chordodasys* species (*Chordodasys antennatus* Riegler, Ruppert, Rieger & Schoepfer-Sterrer, 1969) to the sister genus *Xenodasys* and (c) the establishment of the new genus, *Chordodasiopsis*, to allocate the single remaining *Chordodasys* species (*Chordodasys antennatus* Riegler, Ruppert, Rieger & Schoepfer-Sterrer, 1969).
1974), and (c) the institution of the new family Xenodasyidae to allocate both Xenodasyis and Chordodosasiops. The new family was separated from the remaining Dactylopodolidae (i.e. Dactylopoda Strand, 1929, Dendrodsayis Wilke, 1954 and Dendrodosayis Hummon, Todaro & Tongiorgi, 1992, which lack the “chordoid” organ (Todaro et al. 2006).

The aim of this study is to shed light on the morphology and ultrastructure of the male reproductive system and spermatooza of X. eknomios, and to hypothesize a possible sperm transfer modality in these animals. As the most inclusive phylogenetic analyses based on morphological characters (Hochberg & Litvaitis 2000, 2001a) have set Xenodasyis among the most basal taxa along the Macrodasyida evolutionary branch, it is likely that in a larger framework the new information might assume relevance for reconstructing the morphological ground pattern of the whole phylum.

Materials and methods

The specimens of Xenodasyis eknomios were found in sandy sediment collected on 21 June 2001 in an 80-m long cave near Santa Maria di Leuca, Lecce, Italy. Living gastrotrichs were extracted using the narcotization-decantation technique with a 7% MgCl$_2$ solution. Thirteen adult specimens were prepared for TEM analysis. They were fixed overnight in a 0.1 M phosphate-buffered solution (PBS) (pH 7.3) of paraformaldehyde, gluteraldehyde and picric acid (Ermak & Eakin 1976). Then, after washing in 0.1 M PBS, the gastrotrichs were postfixed in a 2% osmium tetroxide solution in the same buffer, then rinsed in PBS again, dehydrated in a graded acetone series, stained en bloc in uranyl acetate in 70% acetone, and embedded in Araldite. Ultrathin sections were cut with a LKB Ultrotome 2088V, contrasted with lead citrate, and observed under a Philips CM10 transmission electron microscope.

The locations of some morphological characteristics along the body are given in percentage units (U) of the total body length referring to the measuring made by Todaro et al. (2006).

Results

Reproductive system

The paired, tubular, elongate testes extend from U40 to U56, the approximate middle intestinal region. As it is usual for gastrotrichs, spermatogenesis occurs in a caudocephalic and centripetal direction (Figure 1A, B). Once mature, the spermatooza are turned and channelled towards the posterior extremity of the testes and enter in the sperm ducts containing a low number of mature spermatooza (less than 10). The sperm ducts end at U59; they probably lead to two ventro-lateral pores that were not detected with the electron microscope (Figure 1C). Two ovaries lie laterally to the terminal intestine (Figure 1D, E). Oocytes mature in a caudocephalic direction. A fully grown oocyte (approximately 60 μm in diameter) was seen in many specimens to fill most of the trunk central region (Figure 1F). A frontal organ, generally containing a single spermatophore, is located between the terminal tract of the right sperm duct and the most mature oocyte (Figures 1E, 2A). This organ is a pyriform sac up to 18 μm long, enveloped by a thick basal lamina and attached to the body wall by several muscular fibres (Figure 2B, E). It is full of electron-dense material produced by few subepidermal, glandular cells (Figure 2C). The rounded spermatophore, 4 μm in diameter, is located on the medial side of the frontal organ, just facing the intestine wall, and extends into a long, thin duct opening into a dorsolateral pore (Figure 2A-C). The spermatophore contains at least 14 mature spermatooza, apparently connected to each other by some sort of electron-dense material. No distinct internal connection of the frontal organ with the adjacent mature oocyte was seen. In three individuals lacking a fully grown oocyte, the frontal organ was reduced to a sac, up to 12 μm long, containing several degenerating sperm, but no spermatophore was present (Figure 2D, E). No caudal organ was present in any of the observed specimens.

The mature spermatoozon

The spermatoozon is a filiform cell composed of a peculiar acrosome, a spiralized nucleus, a long connecting piece and a long flagellum (Figure 3F). The acrosome is the dominant structure of the spermatoozon of the Xenodasyis eknomios: it forms the axis of the whole cell body from the anterior extremity to the connecting piece, linking the cell body to the axoneme. The acrosome is made up of three distinct regions: the apical region is tubular in shape, at least 6 μm long, 0.06 μm in diameter, and contains a moderately electron-dense material (Figure 3A). The middle region is longer and cylindrical, 0.25 μm in diameter, and is filled with material organized in a high number of disks stacked up in a pile. In longitudinal section the disks are generally rectangular in shape; only a few, randomly distributed, are triangular. Each disk is
electron-dense and is separated from the adjacent disks by electron-dense septa (Figure 3B). The basal region of the acrosome is a thin tubule similar in shape but longer than the apical one (Figure 3G). It widens abruptly near the connecting piece and slightly sinks into it (Figure 3H). The acrosome is almost completely surrounded by two helical structures (Figure 3F). The inner structure is a thin ribbon starting at the top of the cell and ending at the connecting piece; some sections reveal a crystalline appearance (Figure 3A–E, G, H). The outer helix is a rope-like spring beginning at a distance of 6 μm from the cell apex and running parallel to the inner ribbon (Figure 3B, D, E, G, H). Data from spermatogenesis indicate that the outer helix is the nucleus (Figure 3L). In mature spermatozoa the nucleus appears in cross-section like a right triangle with the short side oriented towards the acrosomal apex (Figure 3B, G). The nuclear helix gets progressively thinner and finally disappears (Figure 3F). The connecting piece is clearly divided into two parts. The anterior part is in contact with both the nucleus and the basal region of the acrosome; it is a thick, empty cylinder, 1.6 μm long and 0.36 μm in diameter (Figure 3H, J). The posterior part is in touch with the flagellum; it is thin, 1.2 μm in length and 0.18 μm in diameter. It is made of an electron-dense material organized into 2–4 coils and is surrounded by few, small mitochondria (Figure 3K). The flagellum shows a typical 9 × 2+2 axoneme, and lacks the striated cylinder and any accessory structure (Figure 3I).

Discussion

Reproductive system

The hermaphroditic reproductive system of Xenodasys eknomios, X. riedli and Chordodasiopsis antennatus (Rieger et al. 1974) is characterized by the possession of two club-like testes that extend into sperm ducts, paired caudal ovaries, and a frontal organ.

This is likely also true for X. sanctigoulveni (Swedmark 1967), although there are no data on number clearly increase in the caudo-cephalic and centripetal direction. B, cross-section of a testis: in the lumen flagella of several mature spermatozoa are visible (arrows). C, oblique section of a sperm duct. D, habitus. E, central part of the body showing the testes, the frontal organ and a vitellogenetic oocyte. F, fully grown oocyte filling most of the trunk. fo, frontal organ; mo, mature oocyte; s, spermatozoa; sc, spermatocytes; st, spermatids; t, testis; vo, vitellogenetic oocyte.
the reproductive system of this species (see Kisielewski 1987).

This arrangement differs from the basic plan of the genital system of Gastrotricha Macrodasyida in lacking a caudal organ (see Ruppert 1991). A caudal organ is, on the contrary, present in species from at least two genera of Dactylopodolidae, a character further supporting the allocation of Xenodasys and Chordodasiopsis to a separate family (i.e. Xenodasyidae; Todaro et al. 2006). The fact that male pores were not detected on the body surface in X. riedli (Schoepfer-Sterrer 1969), as in X. eknomios and C. antennatus (Rieger et al. 1974), could be related to their extremely small size or their possible

Figure 2. Frontal organ of Xenodasys eknomios observed with transmission electron microscopy (TEM). A, longitudinal section of the frontal organ with the spermatophore in a hermaphrodite individual. The proximal part of the frontal organ duct is visible (arrow). B, longitudinal section of the frontal organ showing the proximal part of the duct opening dorso-laterally (arrow), and muscular fibres connecting the frontal organ to the body wall (arrowheads). C, detail of the subcuticular portion of the frontal organ duct (arrow). D, longitudinal section of an individual lacking mature oocyte: the frontal organ is reduced to a sac with degenerating sperm in which no spermatophore is visible. E, cross-section of the frontal organ in an individual lacking a mature oocyte: many degenerating spermatozoa are visible inside it. Note the muscular fibres connecting the frontal organ to the body wall (arrowheads). ds, degenerating sperm; fo, frontal organ; vo, vitellogenetic oocyte; sp, spermatophore.
Figure 3. Mature spermatozoon of *Xenodasys eknomios* observed with transmission electron microscopy. **A**, longitudinal section of the apical acrosome (arrow). **B**, longitudinal section of the middle acrosome formed by a high number of disks (asterisk) stacked on top of each other separated by electron-dense septa (arrows). Both ribbon and nuclear helixes are visible. **C–E**, cross-sections of the apical, middle and basal acrosome. **F**, three-dimensional reconstruction of a mature male gamete. **G**, longitudinal section of the basal acrosome surrounded by the two helixes. **H**, detail of the acrosomal base widening and sinking into the connecting piece. **I**, cross-section of several flagella. **J**, longitudinal section of the connecting piece formed by two parts. **K**, detail of the posterior part of the connecting piece surrounded by
appearance only during the male reproductive phase, as it is known for other macrodasyidans (Balsamo et al. 2002).

An elongate frontal organ, lateral to the intestine and opening into a ventrolateral pore, has been described in X. riedli (Schoepfer-Sterrer 1969) and also in C. antennatus (Rieger et al. 1974). However, in both species it appears larger and more complex than the simple, rounded frontal organ of X. eknomios, since two distinct parts of it can be clearly recognized (receptaculum seminis and bursa). The presence of a musculature firmly connecting the frontal organ of X. eknomios to the body wall suggests that it is a permanent structure, as it seems also in the other two species. A permanent frontal organ is known in a number of Macrodasyida (e.g. Macrodasyidae, Thaumastodermatidae), whereas in others it is apparently only temporary (e.g. Turbanella, Paraturbanella), or even absent (e.g. Mesodasys, Lepidodasys). Sperm have been observed inside the frontal organ of all the studied species of Xenodasyidae: they appeared free in C. antennatus and X. riedli and packed into a spermatophore in X. eknomios.

Species of Dendrodasys and Dactylopoda (fam. Dactylopodolidae) share with species of Xenodasyidae two elongate, lateral testes and two caudal ovaries, but clearly differ in possessing a caudal organ. A “receptaculum seminis” (i.e. a frontal organ) containing sperm and resembling in location and morphology the frontal organ of X. eknomios has been described in Dendrodasys species (Wilke 1954; Schmidt 1974), and a similar structure was reported for a single, unnamed species in the genus Dactylopoda (Ruppert 1991); for a different interpretation of the reproductive system in Dactylopoda see Kienke et al. (2008). No data are available for Dendropodola as the species was described on a single immature specimen (Hummon et al. 1993).

Based on our findings, and earlier observations by several authors (Schoepfer-Sterrer 1969; Rieger et al. 1974; Todaro et al. 2006), we conclude that X. eknomios is a protandric hermaphrodite that develops into a simultaneous hermaphrodite after the first mating event (see below). This condition – protandry followed by simultaneous hermaphroditism – is characteristic of most species of Macrodasyida and is likely the “primitive” sexual condition in Gastrotricha (Balsamo et al. 1999).

Among Dactylopodolidae, species of Dendrodasys are reported as simultaneous hermaphrodites, whereas species of Dactylopoda are sequential hermaphrodites and, together with Xenodasys eknomios, are perhaps the only taxa of Macrodasyida in which the production of spermatophores is observed as part of the normal reproductive cycle (Teuchert 1968). A spermatophore has been recorded from a sac-like organ (putative frontal organ) in Turbanella varians (Maguire 1976), but this has not been confirmed. In fact, the original illustration (Maguire 1976, p. 5) shows free spermatozoa, and not a spermatophore, inside the frontal organ.

Our observations on X. eknomios using both brightfield and electron microscopy suggest a possible sperm transfer modality in this species. (1) In the first sexual male phase, individual gastrotrichs exchange spermatophores via cross-fertilization. Spermatophores are expelled through male pores and pressed against the pore of the partner’s frontal organ. The musculature of the frontal organ may function in the uptake of the spermatophore. This mating event may be the stimulus for entering into the second sexual phase. (2) As the individuals become simultaneous hermaphrodites, the most mature oocyte develops in the direction of the frontal organ. (3) Active, flagellate spermatozoa are released from the spermatophore and set free into the frontal organ. (4) Then the spermatozoa leave the frontal organ and fertilize the most mature oocyte, likely through a connection between the frontal organ and the most mature oocyte, as it is known for other Macrodasyida (i.e. Macrodasyidae). (5) Eventually, unused spermatozoa are resorbed by the frontal organ, and the organ itself shrinks in size.

A small-sized frontal organ, with a few sperm in degeneration, has been observed in the male specimens lacking a fully grown oocyte: it could be in a late stage, following the egg fertilization and laying. There is no evidence for a cyclic presence/absence of the frontal organ, as it has been described for Turbanellidae (Balsamo et al. 2002): on the contrary, the well-defined structure and the location of this organ in X. eknomios supports its permanent presence, and its possible re-utilization in following phases.
Phylogenetic implications

A growing body of evidence suggest Dactylopodola as one of the most basal taxa within Macrodasyida (e.g. Hochberg & Litvaitis 2001b); since genuine spermatophores are known to also occur in Neodasys (see Guidi et al. 2003), the supposedly most basal taxon in Chaetonotida, the sister group of Macrodasyida, a possible sperm transfer modality by means of spermatophores might be hypothesized in the common ancestor of both taxa. The presence of spermatophores also in another basal taxon such as Xenodasys may support this hypothesis.

The spermatoozon of X. eknomios presents a spiral head, which is the major synapomorphy supporting the Macrodasyida. Nevertheless, it differs from the basic sperm model of the order (Ferraguti & Balsamo 1995) in showing the nucleus surrounding most of the acrosome, and a peculiar, very long connecting piece between the head and the flagellum: these two characters are probably autapomorphic. The absence of a striated cylinder and any periaxonemal accessory structure appear to be synapomorphies, shared only, within Macrodasyidae, with Turbanellidae and species of Lepidodasys (Guidi et al. 2004). These features seem to confirm the basal position of Xenodasys within the Macrodasyida suggested by the morphological, cladistic analyses of Hochberg and Litvaitis (2000, 2001a).

The spermatoozon of X. eknomios is the first male gamete to be described at the ultrastructural level in the Xenodasyidae, since only optical observations are available so far on the spermatoozoa of X. riedli and C. antennatus (Schoepfer-Sterrer 1969; Rieger et al. 1974). All observations on species of Xenodasyidae agree on the structure of the filiform spermatoozon, formed by a distinct spiraled head and a long tail. This differs substantially from the unspiraled and flagellate sperm of the species of Dactylopodola, the only genus of Dactylopodolidae, which has been investigated from a spermatical point of view (Fischer 1996). The presence of two possible spermatozoal autoapomorphies in Xenodasys eknomios (the nucleus surrounding most of the acrosome and the very long connecting piece) suggests that future ultrastructural studies of the spermatooza of species of Xenodasyidae and Dactylopodolidae will shed light on the phylogenetic relationships among members of these two putative basal taxa.

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References


typhle (Gastrotricha: Macrodasyida) and their possible functions in sperm transfer. Invertebrate Biology 127:12–32.