




Reproductive system of the genus *Crasiella* (Gastrotricha, Macrodasysida)

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Abstract *Crasiella diplura* from Sweden and *Crasiella* sp. from Italy were studied alive and with transmission electron microscopy (TEM). The two species are simultaneous hermaphrodites and share the same reproductive system layout: paired ovaries extend along the posterior part of the intestine and join mid-dorsally, while club-shaped testes lie at the sides of the anterior gut extending as deferentia that fuse on the mid-ventral plane and open into a single pore; gametes mature in a caudo-cephalic and centripetal direction. The bulky, sac-like frontal organ is lined by a simple epithelium and lies dorsolaterally to the intestine, on the left side of the body. The spindle-shaped caudal organ is musculo-glandular and is located ventrolaterally to the gut on the right side. It is characterized by the presence of a roughly Y-shaped internal channel that opens into two pores close to each other, which function for the intake and outlet of the (auto)sperm, respectively. The spermatozoa, which are peculiar and similar in the two species, are characterized by a long and complex acrosome consisting of four structurally distinct regions, three of which

Keywords *Crasiella* Gastrotricha Macrodasysida Reproductive system Spermatozoon

Introduction Gastrotricha is a phylum comprising about 750 species of ctenophore and comb jelly-like animals, which are well represented in benthic communities and are grouped into two orders: Macrodasysida, consisting of 300 marine species, which are generally simultaneous or sequential hermaphrodites with cross-fertilization, and Chaetonotida, comprising 450 mostly freshwater, parthenogenic species, with few marine, Neodasys, Heteroxenotrichula, Diuronotus, Musellifer (Balsamo et al. 2009; Hummon and Todaro 2010).

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The reproductive system of Macrodasysida is anatomically quite complex and diverse, probably because of the different modalities of internal cross-fertilization. Usually, there is a pair of testes, two (or one) ovaries and two sexual accessory organs, which are respectively known as frontal and caudal organs from their relative positions in the trunk region (Ruppert 1978). The elongated testes lie at the sides of the anterior intestine and extend posteriorly in sperm ducts, opening ventrally into either a common or separated pores. The ovaries are lateral to the caudal intestine, and oocytes generally mature in a caudo-frontal direction, gradually shifting dorsally. In some taxa (e.g. Cephalodasys and Dolichodasys), oocytes mature in a fronto-caudal direction. The dorsal fusion of the two

ovaries in order to form an unpaired uterus has been also been collected in July 2002 at S. Isidoro, Lecce, Italy. reported in *Dactylopodola typhle* (Kieneke et al. 2008). Gastrotrichs were extracted from the sediment by the narrow connection made of either a solid cell band or an epi-cotization-decantation technique, using an isosmotic (7%) thelial duct between the ovary and an external, dorsal pore magnesium chloride solution (Gier 2009). The fauna- (mating pore?), has only been described in *Acanthodasys* containing supernatant was then poured directly into a thrinax and *Diplodasys ankei* (Thaumastodermatidae), 5-cm diameter Petri dish and scanned for specimens under and *Dactylopodola typhle* respectively (Ruppert 1978b) a Wild M3 dissecting microscope set at 50 magnification. Kieneke et al. 2008). For optical microscopy, the gastrotrichs were removed with

The sac-like frontal organ is lined by an epithelium and a micropipette from the Petri dish, fresh-mounted on slides lies in proximity to the most mature egg; it displays an and observed using a Nikon Eclipse 80i equipped with external pore through which it probably receives allosperm Differential Interference Contrasts (Nomarski). During during copulation, thus functioning as a seminal receptacle observation, the animals were photographed with a DSN An internal pore allows the stored sperm to leave the organ 5 M Nikon digital camera and measured with the Nikon and fertilize the eggs. The elongated caudal organ is wholly NIS software.

muscularized and lies ventrally; it opens close to or within For the transmission electron microscopy, 10 specimens the anus and almost certainly acts as a copulatory organ of *Crasiella diplura* and five of *Crasiella* sp. were fixed (Ruppert 1991). In the *Macrodasys* species, the caudal overnight in 2% glutaraldehyde in a 0.1 M sodium cacodylate buffer (pH 7.4) and stored in a 0.1 M sucrose/sperm are packed (Ruppert 1978a), while in some Uroda- sodium cacodylate buffer. In the laboratory, the specimens *Macrodasys* species, it has a cuticularized stylet (Schoepfer-Sterrer 1974; Fregni et al. 1998; Balsamo et al. 2007). Mating and 0.1 M sodium cacodylate, washed in a clean 0.1 M sodium cacodylate buffer, dehydrated in a graded ethanol series and embedded in Araldite. Semi-thin and ultra-thin sections were cut with a LKB Ultratome 2088 V and were contrasted with toluidine blue and lead citrate, respectively. The semi-thin sections were observed in transmission light under a VANOX AHBT3 Olympus optical microscope, whereas the ultra-thin sections were observed under a Philips CM10 transmission electron microscope. The

The studies carried out in the last 30 years have proved that the morphology of the reproductive organs and the ultrastructure of spermatozoa are very important diagnostic characters for taxonomic use, as well as being a valid tool

for phylogenetic purposes (e.g. Thane-Fendler 1970; Evans 1994; Marotta et al. 2005; Kieneke et al. 2009). Three-dimensional reconstruction of the spermatozoon of *C. diplura* was performed by CimatronE software

However, no morphological data on the reproductive organs and spermatozoa of the *Planodasyidae* family are yet available. Herein, we provide a description of the

reproductive system and the spermatozoon of two species of *Crasiella*, which is one of the two genera of the family.

We also suggest a possible modality of mating and fertilization in the two studied species of this genus and compare this to the information available on other species of *Macrodasysida*.

The overall arrangement of the reproductive system is the same in the two species of *Crasiella* examined. Mature individuals are simultaneous hermaphrodites. One pair of club-shaped testes lies at the sides of the anterior gut, extending into two sperm ducts that fuse on the mid-ventral plane and open into a single ventral pore (Fig. 1c, e).

Two bilateral ovaries run along the posterior part of the intestine and join mid-dorsally (Fig. 1a, d). In the posterior trunk region, there are two accessory organs, both of which are very close to each other. The voluminous, sac-like frontal organ is lined by an epithelium and lies

Sand samples of *Crasiella diplura* Clausen, 1968 were collected in September 2007 during a 2-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). Sand samples of *Crasiella* sp. had

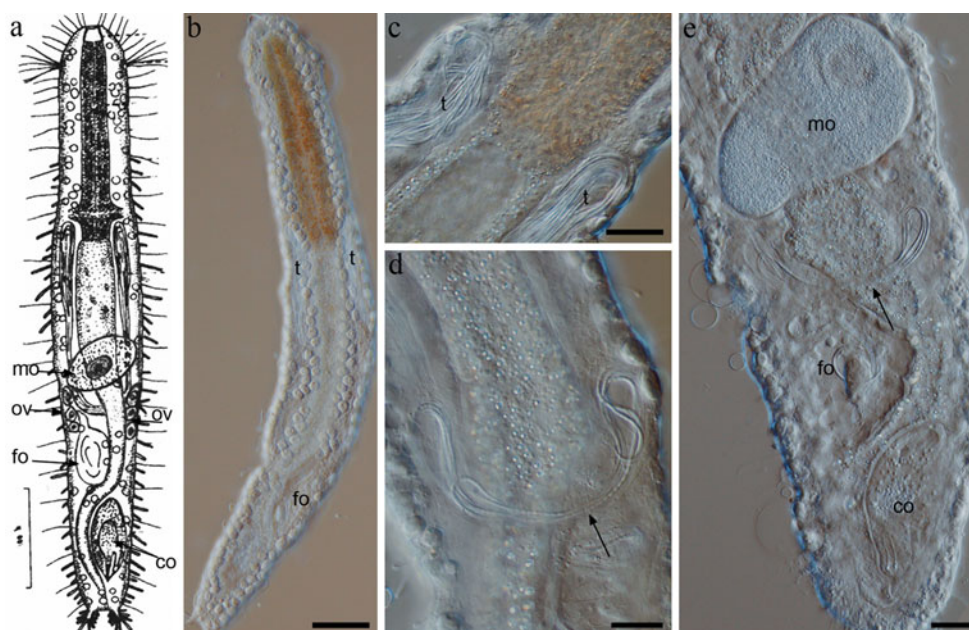


Fig. 1 Morphology of the reproductive system of *Crasiella diplura* (b, c, d, e DIC microscopy) a Drawing of a dorsal view of *Crasiella diplura* showing the reproductive system (modified from Clausen 1968). b Habitus of an adult specimen at the level of the pharyngo-intestinal junction. c Proximal portion of the testes. d Fusion of the sperm ducts on the mid-ventral plane (arrow). e Posterior region of the body: the mature oocyte, the fusion of the sperm ducts (arrow), and the frontal and caudal organs are clearly visible. co caudal organ, fo frontal organ, mo oocyte, ov ovary, t testes. Scale bars: b = 50 μ m; c–e = 15 μ m

dorsolaterally to the intestine on the left side of the body, a homogeneous, moderately electron-dense material, and between the largest oocyte and the caudal organ. The numerous small, strongly electron-dense vesicles. These spindle-shaped caudal organ is musculo-glandular and progressively pile up, forming the basal portion of the located ventrolaterally to the gut on the right side of the body (Fig. 1a, d).

Testes

The two testes extend from U34 to U50 and narrow into sperm ducts, which open into a single ventral pore at U66 (Fig. 2e). When the acrosome formation has been completed, spermatids enter the lumen of the anterior portion of each testis, and gametes mature in a caudo-cephalic and centripetal direction so that early germinal cells, spermatogonia and spermatocytes lie in the posterior part of each gonad, while spermatids gather in the internal lumen of the most anterior part (Fig. 2a).

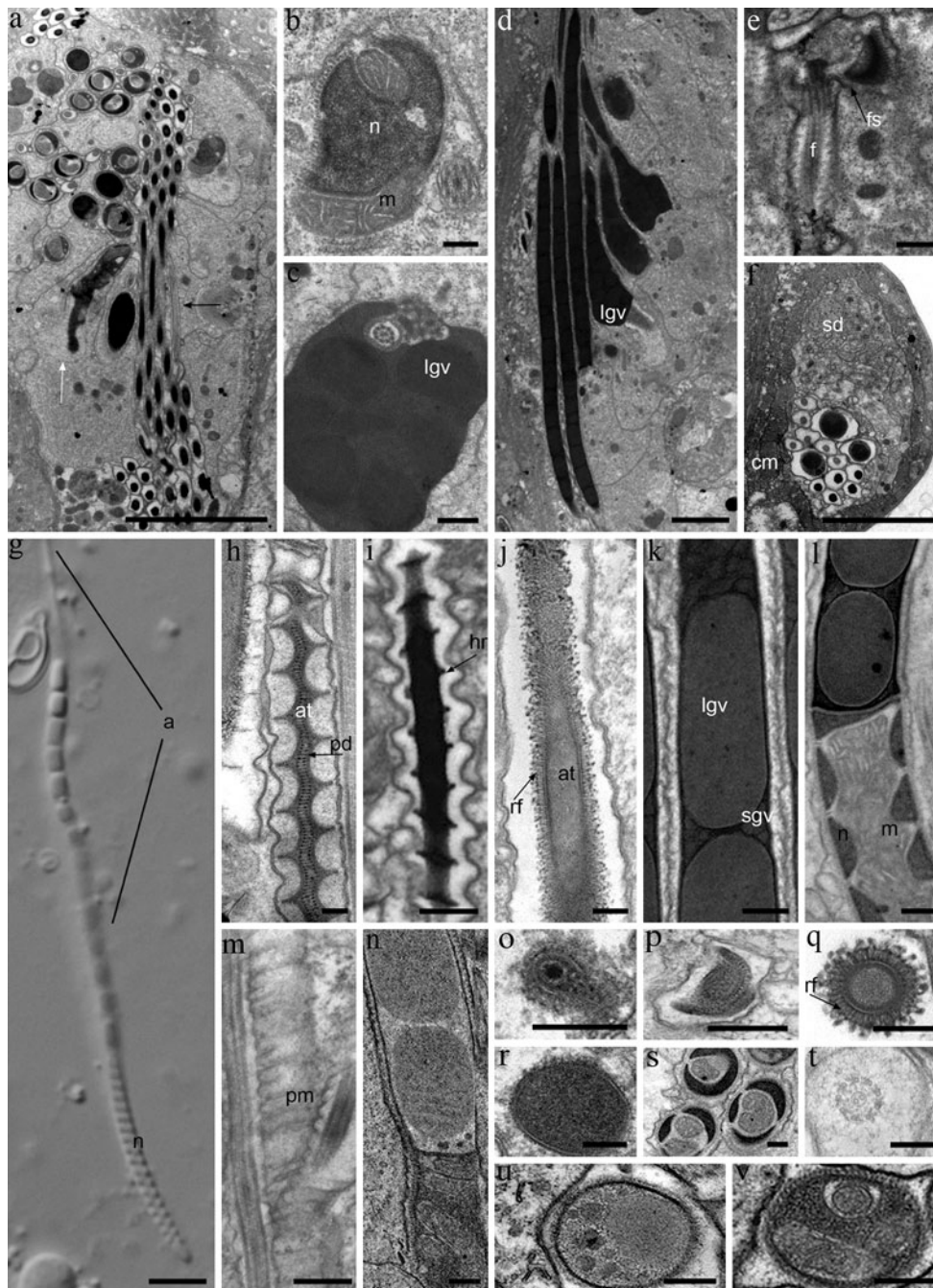
Spermatogenesis

In spermatids, the nuclear chromatin progressively condenses, while the nucleus starts elongating. At the same time, a long mitochondrion, arising from the fusion of many smaller mitochondria, sticks to the nuclear envelope and gradually surrounds it, before then sinking into the nucleus. A manchette of microtubules is visible around the nucleus-mitochondrion complex (Fig. 2a, b). At that point, the acrosome starts to develop: the cytoplasm of the spermatid is almost entirely filled with large vesicles containing

acrosomal complex is growing, the axoneme is already apparent and starts lengthening (Fig. 2d). Once the nucleus has reached its definitive size, it rotates medially and then posteriorly with respect to the axoneme. Due to this transformation, the spermatid takes on a clear U-shape (Fig. 2e). When the acrosome formation has been completed, spermatids enter the lumen of the anterior portion of each testis and rotate with respect to the nucleus, getting in line with the acrosomal complex. Mature spermatozoa migrate caudally along the testis lumen towards the sperm duct, which is completely surrounded by circular muscles belonging to the trunk musculature (Fig. 2f).

Spermatozoa

The spermatozoon of *Crasiella diplura* is long, pliform and morphological regions over its length (Fig. 3a, b). The apical region, which is 8 μ m long, is a helically twisted tube with an inner structure made of piled discs (Fig. 2h, o); its diameter increases in a basal direction



from 0.1 to 0.2 μm. The next region is a straight tube, which is 7 μm in length and surrounded by a helical ridge (Fig. 2i, p), with a diameter increasing from 0.2 to 0.3 μm. The third region, 4 μm long, is a straight tube surrounded by numerous short radial processes (Fig. 2j, q). The last region is much larger, being 2 μm in length and 0.8 μm in diameter, and has large, piled, globular vesicles (0.7 μm in diameter). Between these large vesicles, there are several smaller globular vesicles (0.1 μm in diameter), which are embedded within a very electron-dense plasma material (Fig. 2k, r).

The nucleo-mitochondrial portion is about 2 μm long and rounded by the nucleus, forming a characteristic 'spring' shape, which is a ribbon of compacted chromatin spirally coiled around the mitochondrion for a total of 20 turns (Figs. 2g, l, s, 3a, b). The flagellum has a 2 + 2 axoneme, characterized by a dense and prominent central sheath surrounding the central tubules; a wide space separates the axoneme from the tail membrane. Another peculiarity of the sperm tail in *Crasiaella* is the fact that, apparently, the plasma membrane is curled (Figs. 2m, t, 3a, b).

◀ Fig. 2 Spermatogenesis of *Crasiella diplura* and the spermatozoa of *C. diplura* and *Crasiella* sp. (All figures refer to *C. diplura* exceptn, u, v; a–f, h–v are TEM images; g is a DIC image). a Testes: the gametes mature in a caudo-cephalward (white arrow) and centripetal direction (black arrow). b Nucleus/mitochondrion of a spermatid: the chromatin starts to condense and the mitochondrion is surrounding the nuclear envelope. c Spermatid showing the cytoplasm filled with large vesicles from which the basal region of the acrosome develops. d Basal regions of acrosomes: the large vesicles are piled up on top of each other. e The U-shaped connection between nucleus and flagellum in a late spermatid. f Sperm duct in longitudinal section. g A single spermatozoon as seen by differential interference contrast optics. h The more apical region of the acrosome of in longitudinal section: a twisted tube with an inner structure made up of piled discs. i The second region of the acrosome of in longitudinal section: a straight tube surrounded by a helical ridge. j The third region of the acrosome in longitudinal section: note the small radial fibres visible also in cross-section in q. k The more proximal region of the acrosome in longitudinal section formed by large, piled, globular vesicles surrounded by smaller globular vesicles. l Longitudinal section of the nuclear-mitochondrial region and the basal acrosome. m Tangential section of the curled plasma membrane of the flagellum. n Longitudinal section of connection between the acrosome and nuclear-mitochondrial region. *Crasiella* sp.: note the thin mitochondrion and the low and the high density of large and small globular vesicles, respectively. Note also the low density of the granular material surrounding both types of vesicles. o Cross-sections of the acrosome, ordered from distal to proximal. p Cross-section of nuclear-mitochondrial region. q Cross-section of the flagellum: note the prominent central sheath. r Cross-section of the proximal region of the acrosome and the nuclear-mitochondrial region of *Crasiella* sp. a acrosome at acrosomal tube; b circular muscles; c flagellum; fs basal spermatid; h helical ridge; l, g, v globular vesicles; m mitochondrion; n nucleus; pm plasma membrane; p, d piled discs; rf radial fibres; s sperm duct; g, v small globular vesicles. Scale bars a = 4 μm; b, d, e, h, i, k, u, v = 0.3 μm; c, f, g = 4 μm; t = 0.1 μm

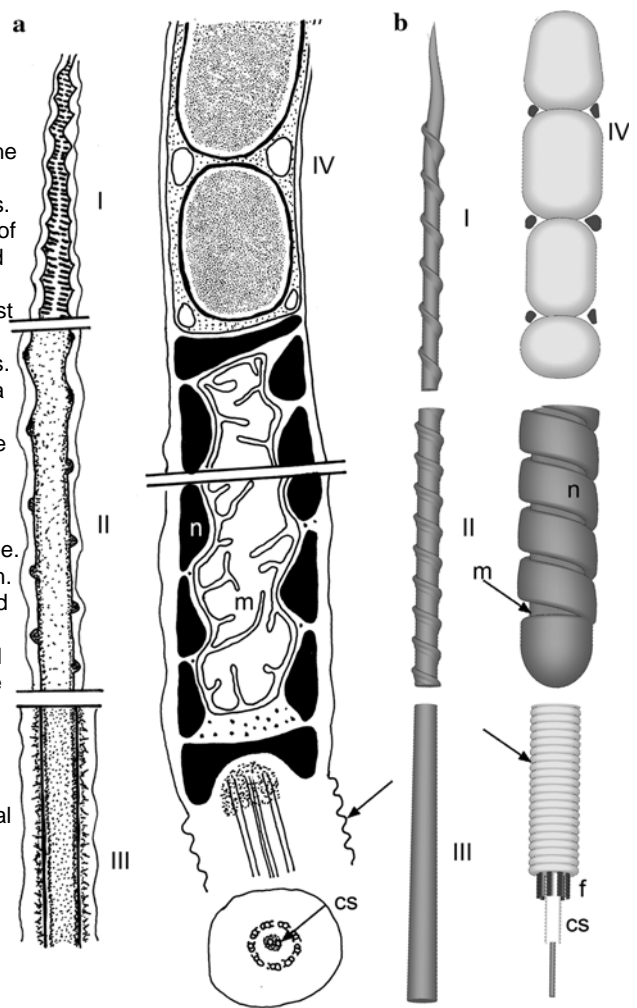


Fig. 3 Graphic reconstructions of the mature spermatozoon of *Crasiella diplura*. a Schematic drawing of the spermatozoon. I-IV: different regions of the acrosome, from the more distal (I) to the more proximal (IV) (see the description in the text). The nucleus is spiralized around the mitochondrion and the flagellum is surrounded by a curled plasma membrane (arrow). b 3D model of the spermatozoon showing the same regions of the spermatozoon represented in the 2D drawings. c central sheath; f flagellum (* in cross-section); m mitochondrion; n nucleus

The spermatozoon of *Crasiella* sp. differs from that of *Crasiella diplura* in the following ways: (1) the large globular vesicles of the acrosomal base are less electron-dense; (2) the small globular vesicles measure 0.07 versus 0.14 μm in diameter and are more electron-dense; (3) the granular material surrounding the vesicles is less electron-dense; (4) the mitochondrion inside the nucleus is much thinner, 0.18 versus 0.5 μm (Fig. 2n, u, v).

Ovaries

The two lateral ovaries extend along the intestine from U60 to U76. Each ovary is formed by a few oocytes (4–5) that migrate anteriorly and dorsally during maturation. The fully grown oocyte lies anterior to the frontal organ and dorsal to the intestine (Fig. 4a, d). No epithelial sheath enveloping the germ cells was observed. Circular and longitudinal trunk muscles surround the ovaries. The early oocytes have many free ribosomes and mitochondria; the nuclear chromatin is granulated and moderately electron-dense, and the nucleolus is large and well organized (Fig. 4b). Later oocytes contain yolk granules, which extending from U65 to U80. An external, well-developed

progressively increase both in number and size during oogenesis, until they finally fill the entire cytoplasm. The nucleolus is absent (Fig. 4c). Mature oocytes are very large (100 μm in diameter), which are packed with yolk granules, free ribosomes and fragments of rER. The egg envelope consists of a thin, inner layer of electron-dense granular material and an outer layer of densely packed microfilaments (Fig. 4d, e). The frontal organ is a large sac (80 μm long, 26 μm wide),

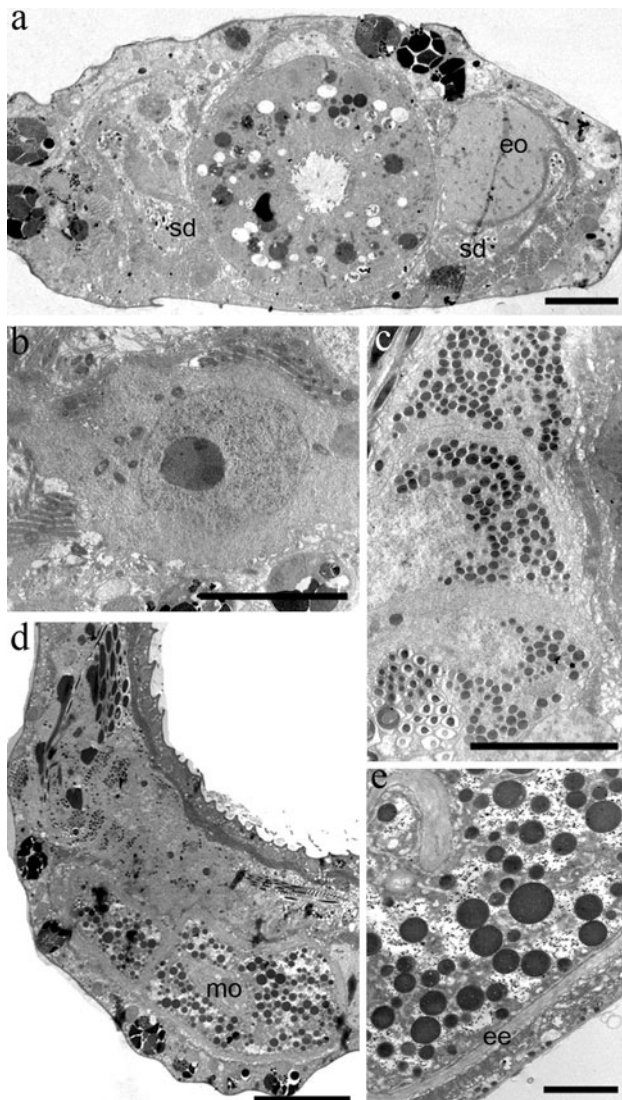


Fig. 4 Ovaries of *Crasiella diplura* (TEM microscopy). **a** Cross-section of the body of a whole animal at the level of the early oocytes. The sperm ducts are sectioned close to their fusion with an early oocyte. **b** Three later oocytes. **c** Mature dorsal oocyte packed with yolk granules (longitudinal section). **d** Egg envelope: the inner thin layer made of electron-dense granular material and the outer one made of densely packed microfilaments are visible. **e** Egg envelope of an early oocyte, **mo** mature oocyte, **sd** sperm duct. Scale bars: **a–d** = 8 μm ; **e** = 1 μm

dorsolateral pore is present, and another small pore is directed anteriorly towards the most mature oocyte. The frontal organ is lined by a monolayered columnar epithelium, delimiting a cavity in which usually numerous active spermatozoa are visible in living specimens (Fig. 5g). The epithelial cells have many microvilli extending into the inner cavity, and a cytoplasm with secretory vesicles of different sizes, varying from 0.2 to 1 μm . The spermatozoa inside the lumen are surrounded by a uniformly numerous interdigitations. They contain many mitochondria, a well-developed Golgi apparatus, a lot of ribosomes

belonging to the trunk musculature, surround the frontal organ (Fig. 5c).

Caudal organ

The caudal organ is spindle-shaped (70 long, 30 μm large) and extends from U73 to U84. It is formed by an anterior, glandulo-muscular region (50 μm long) and by a posterior muscular region (20 μm in length). This organ is completely surrounded by a thick basal lamina and well-developed circular muscles and also contains an internal duct (Figs. 5b, f, 6).

This internal duct is Y-shaped, with one branch lying inside the anterior region and forming a blind end (Figs. 5b, e, 6), while the other two branches extend along the posterior region and open into the exterior through two distinct ventral pores (Figs. 5b, e, f, g, 6).

The anterior, blind duct is surrounded by cells with the cytoplasm full of secretory granules. Numerous microvilli extend into the lumen. Inside it, in some cases, spermatozoa were observed (Fig. 5d, e).

Of the two ducts lying in the posterior region, the main duct is 10 μm long and 8 μm wide and ends with a feeder-bib extremity (12 μm long), which opens into a very small ventral pore (1.5 μm in diameter, Figs. 5b, 6). The second duct, the accessory one, is straight and much longer (25 μm long, 3 μm wide). It lies ventrally to the *Prst* and opens into a large ventral pore (6 μm in diameter) (Figs. 5f, 6). The lumen of the main duct is covered with a large number of packed secretion granules, which are small, round or ovoid in shape, and contains a homogeneous, moderately electron-dense material. The cytoplasm of the cells surrounding the lumen is filled with secretion granules (80–200 nm), globular mitochondria, numerous stacked membranes (endoplasmic reticulum and dictyosomes), ribosomes that are both free and attached to the reticulum membranes, and many groups of secretion granules. The nuclei often have annulated membranes (Fig. 5j). These cells also form many, very long and extremely thin cytoplasmic protrusions, which overlap each other, thus forming a multi-membranous sheath all over the lumen. The secretion granules are regularly stuck into these cytoplasmic protrusions. The content of the granules is progressively released into the lumen and appears as a granular material (Fig. 5e, k). The lumen of the feeder-bib-shaped extremity of the main duct is covered with typical secretion granules, which are ovoid in section, slightly electron-dense, and are regularly arranged in rows. Only the terminal part of the duct lacks these granules and is wholly surrounded by large cells that are peculiar for their irregular shape and numerous interdigitations. They contain many mitochondria, a well-developed Golgi apparatus, a lot of ribosomes

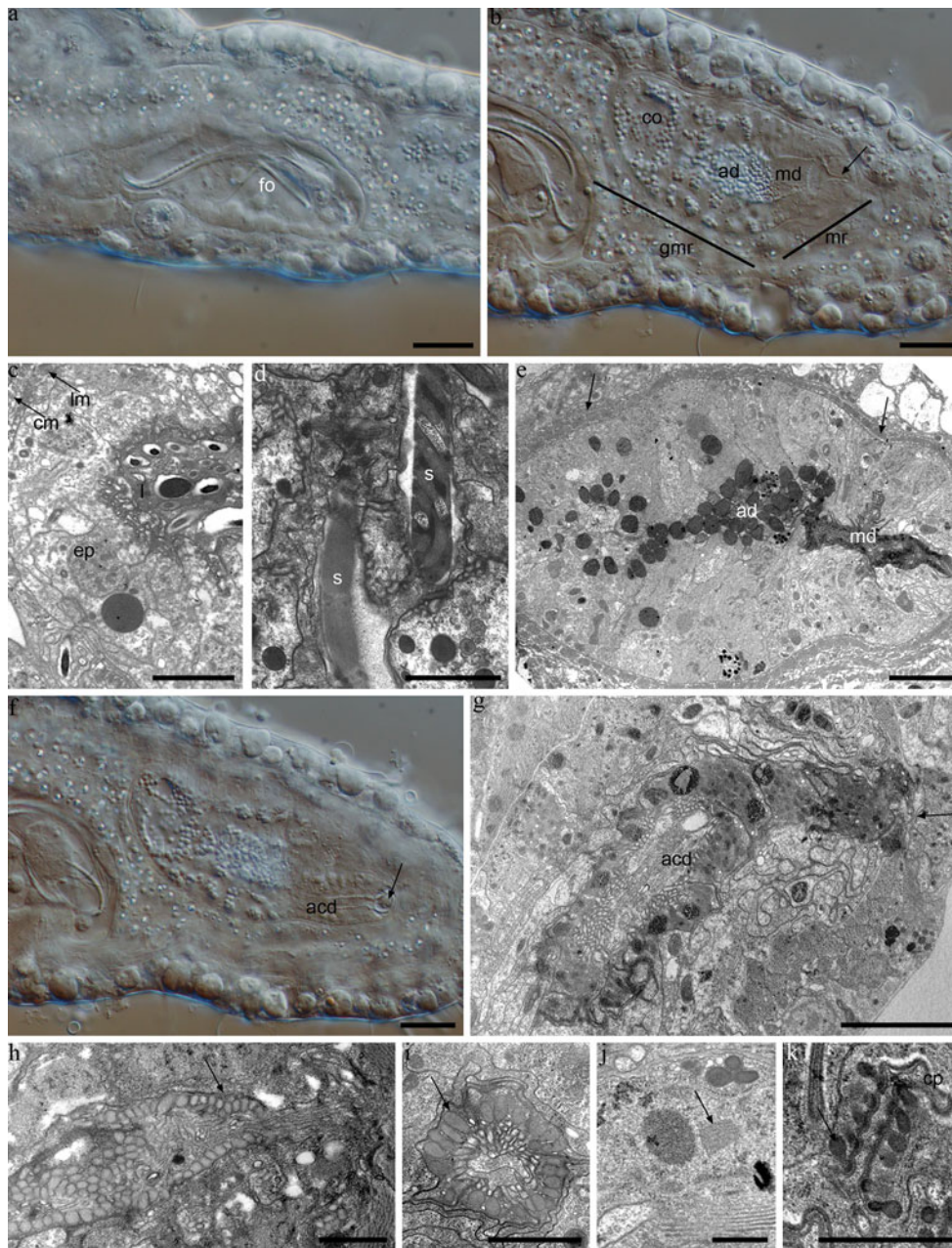


Fig. 5 Accessory organs of *Crasiella diplura* (All figures refer to *C. diplura* except d; c, e, g, h are TEM images, a, b, f are DIC images). a The frontal organ containing several spermatozoa to the caudal organ. The anterior, blind duct surrounded by secretory cells and the posterior, main duct ending in the “feeder bib” extremity (arrow) are clearly visible. c Epithelium of the frontal organ, which surrounds the lumen containing spermatozoa inside the anterior blind duct of the caudal organ. *Crasiella* sp. e Caudal organ showing the well-developed circular muscles (arrows), the anterior blind duct and the posterior main duct. Caudal organ in which the accessory duct opening into a large pore (arrow) is visible. g Accessory posterior duct of the caudal organ. The pore (arrow) is visible. h, i Longitudinal (h) and cross-sections (i) of the “feeder bib” extremity of the principal, posterior duct. Note the arrangement of its typical secretion granules (arrows). j Annulated membranes (arrow) in the nucleus of the cells surrounding the principal, posterior duct. k The cytoplasmic protrusions forming a multi-membranous sheath over the lumen of the main duct. Note the regular arrangement of the secretion granules (arrow) inside these cytoplasmic protrusions. acd accessory duct, ad anterior blind duct, cm circular muscles, co caudal organ, cp cytoplasmic protrusion, ep epithelium of frontal organ, fo frontal organ, gmr glandulo-muscular region, l lumen, lm longitudinal muscles, md main duct, mr muscular region, spermatozoon. Scale bars a, b, f = 15 μm; c, d = 2 μm; e, g = 8 μm; h, i = 1 μm

that are both free and attached to the rER membranes at secretion granules (Fig. 5h, i).

The accessory duct is connected to the anterior end of the main duct and extends posteriorly along the medial side of the caudal organ up to a ventral pore that is clearly visible in both light and electron microscopy (Fig. 5f, g). The lumen of the accessory duct is covered with at least three types of secretion granules: the first kind are large and ring-shaped and made up of coarse granular material; the second kind are ovoid or round and full of coarse granular material; the third kind are small, cylindrical granules and consist of homogeneous material. The cells surrounding the lumen have many organelles and groups of all of the types of secretion granules. Numerous elongated microvilli extend from the cell surface into the luminal space (Fig. 5g).

Discussion

Members of the *Cyasiella* genus are hermaphroditic, with separate female and male gonads, as is the case in most species of *Dolichodasys carolinensis*, *Acanthodasys thrinax*, *Diplo-dasys ankei*, *Tetranchyroderma bunti*, *Thaumastoderma heideri*, *Macrodasys* spp.; Ruppert and Shaw (1977, 1978a, b). A thin epithelial lining the oocytes has trochrichs, as is the caudo-cephalic direction of the matured spermatozoa and oocytes (Ruppert and Fischer 1991; Fischer 1994; Balsamo et al. 2002).

The presence of *Cyasiella* of two club-shaped and hollow testes, which extend into sperm ducts confirms the basic plan of the male system of *Macrodasys* (Ruppert 1991; Fischer 1994; Guidi et al. 2003). However, it has been stated that *Lepidodasys* sp. and *Urodasys anorektoxya* have strip-shaped testes, with no inner lumen or defined sperm ducts (Guidi et al. 2004; Balsamo et al. 2007). The paired testes of *Dactylopodola typhle* are also strip-shaped, without distinct sperm ducts, but they do have a testicular lumen (Kieneke et al. 2008). The germinal epithelium of the *Cyasiella* species forms the testes wall, like in many species of *Macrodasys* (Ruppert 1991). The fusion of the two sperm ducts in a single median, ventral pore is a feature that *Cyasiella* shares with *Turbanellidae*, *Cephalodasys* and *Megadasys* (Remane 1926; Schmidt 1974), whereas most *Macrodasys* species have two separate, ventral male pores (Ruppert and Shaw 1977; Ruppert 1978a, b; Guidi et al. 2009; Hummon and Todar 2010). Only a few species have a direct connection between the sperm duct/s and the caudal organ (*Mesodasys* *Thaumastodermatidae*: Ruppert 1991; Ferraguti and Balsamo 1994; Fregni et al. 1999).

The lack of a sheath enveloping the oocytes is a feature shared by the *Cyasiella* species with other species of *Macrodasys* (*Mesodasys laticaudatus*, *Mesodasys adnotubulatus*, *Paraturbanella teissieri* Ferraguti and Balsamo 1994; Fregni et al. 1999; Balsamo et al. 2002). An incomplete ovarian covering has been reported in some species of *Macrodasys* which is the other genus

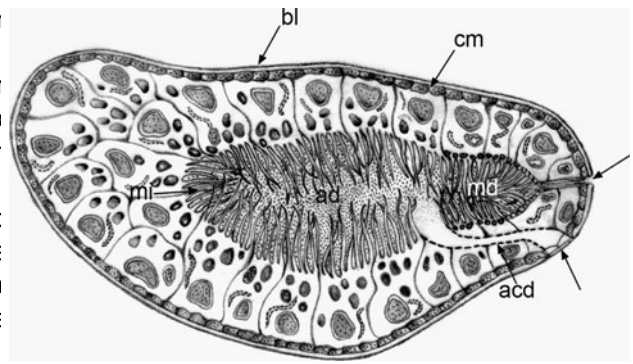


Fig. 6 Schematic drawing of the caudal organ of *Cyasiella diplura*. The basal lamina, the circular musculature and the three ducts of the caudal organ are shown. The accessory duct is on a different focal plane, so it is hatched. The position of the two pores of the main and of the accessory duct is clearly visible. acd accessory duct, ad anterior blind duct, bl basal lamina, cm circular musculature, md main duct, mi microvilli

of the family, the mitochondrion wraps itself externally although they are clearly separate: their morphology and arrangement correspond most closely to those of species of sperm and remains in this position. Hence Macrodasys, Macrodasys (Macrodasidae). The shape and size of the the formation of the nuclear-mitochondrial complex likely frontal organ, as well as the relative position of its external imply a single step, i.e. the fusion of the mitochondria in and internal pores, conform the observations on most single element (Marotta et al. 2005). This shortened process in species of Macrodasidae seems to be related to a secondary evolutionary process towards the loss of mitochondria, rather than to a primitive modality of spermatogenesis (Guidi et al. unpublished data).

Sperm

The spermatozoa of the two *Crasiella* species resemble those of other macrodasidan gastrotrichs in several ways. They have a long and complex acrosome with four stylets. The two regions are connected by a thin anterior straight tube with helical ridges; (3) a straight tube with radial pores; and (4) a long and wide region formed by piled vesicles.

The aspects of these regions differ considerably, but three of them have an equivalent in other species. Region one is, in fact, also found in *Urodasys anorektoxy* (Balsamo et al. 2007), *Acanthodasys aculeatus* (Guidi et al. 2003), and in the Thaumastodermatinae examined so far; region two is present in *Acanthodasys aculeatus* while region four is similar to that observed in *Xenodasys eknomis* (Guidi et al. 2009) and *Thaumastoderma moebjergii* (Ferraguti et al., unpublished data). A nucleo-mitochondrial region, formed by a spring-shaped nucleus surrounding the mitochondrion, is also present in all of the Thaumastodermatidae, Lepidodasidae and Turbanellidae studied so far, as well as in *Mesodasys* (Ferraguti and Balsamo 1994). It is, however, absent in *Xenodasys* where, surprisingly, the nuclear surrounds part of the acrosome (Guidi et al. 2009). In *Crasiella*, the flagellum is devoid of a striated cylinder which is a characteristic present in Thaumastodermatidae, Cephalodasidae (Hummon and Todar 2010) and Macrodasidae, with the exception of *Urodasys anorektoxy* (Balsamo et al. 2007). The axoneme has a curious modification of the central axonemal apparatus, which is the prominent central sheath that is known only from Clitellatamuscular region and was regarded as an autapomorphy of that group (Ferraguti 1984). Accordingly, the prominent central sheath of the axoneme is the only characteristic, which clearly separates the spermatozoa of *Crasiella* from those of the other gastrotrichs.

Accessory sex organs and possible fertilization modality

The two accessory sexual organs of *Crasiella* species are always visible, well developed, and close to each other. The caudal organ is apparently very similar in the species of *Crasiella* and *Macrodasys* because of its shape, and well-developed, anterior glandular region. However, only in *Crasiella*, it shows two distinct ducts opening into two distinct pores. The presence of a short, blind duct in the glandular region and of the only circular musculature are two additional features that clearly distinguish the caudal organ of *Crasiella* from that of the other Macrodasidae. A possible mechanism for cross-fertilization in *Crasiella* is hypothesized here on the basis of morphological grounds and by making a comparison with the few observations available on the fertilization modalities in other Macrodasidae. A mature individual would first transfer spermatozoa from the testes to the caudal organ. It accomplishes this transfer through ventral body flexions, bringing the male pore close to the pore of the accessory duct of the caudal organ. Spermatozoa enter the accessory duct, and travel to the blind duct of the glandulo-muscular region. They are then stored in the secretion material produced by the surrounding glandular cells (i.e. the abundant electron-dense material that has been observed with TEM) until to be transferred to the mating partner. At mating, the glandulo-muscular region pushes spermatozoa from the blind duct through the main duct up to its feed-bib-shaped extremity, which might be protruded into the pore of the partner's frontal organ. Active sperm can then reach the mature oocyte via the internal pore of the frontal organ. The supposed fertilization mechanism in *Crasiella* has some correspondence with that described by Ruppel (1978a) for Macrodasys sp. Here, spermatozoa are transferred during copulation from the male pores of an individual to its own caudal organ through its pore. Inside the caudal organ, they are packed into the thin, copulatory tube at the same time that the latter is placed into the partner's frontal organ.

Then, the copulatory tube breaks off and releases the spermatozoa, which now head to the mature oocyte. Excess of spermatozoa are reabsorbed.

Some functional similarities and possible homologies between different parts of the reproductive system in the two genera can be suggested on the basis of ultrastructural observations. The lack of a direct connection between the male gonads and the caudal organ in both implies the need to charge the latter with autosperm before fertilization. In fact, the hypothesized mechanisms of fertilization in *Macrodasys* and *Crasiella* appear to be similar in that they comprise two subsequent phases, the first for transferring autospermatozoa from the male pore to the caudal organ and the second for transferring sperm to the frontal organ of the partner by means of a specialized anatomical structure belonging to the caudal organ.

According to Ruppert (1978) description, the terminal part of the caudal organ of *Macrodasys* called 'antrum' by Remane (1936), is a glandular region specialized in receiving autosperm, and thus it may be regarded as being at least functionally correspondent to the accessory lumen of the caudal organ of *Crasiella*. Similarly, the copulatory tube of *Macrodasys* (Ruppert 1978) would have a functional correspondence with the feeder bib-shaped extremity of the caudal organ of *Crasiella*, since both structures likely transfer autosperm to the partner's frontal organ. However, the timing of the two phases appears to be different for species of *Macrodasys* and *Crasiella*, respectively. In the former, the packaging of autosperm into the copulatory tube occurs during copulation, when the tube is already inside the partner's frontal organ; in *Crasiella*, on the contrary, the autosperm are packaged into the main duct of the caudal organ prior to copulation. All other species of *Macrodasys* for which information on reproductive anatomy and biology is available appear to be too different from the *Crasiella* species to be usefully compared.

Conclusions

This study has allowed us to trace a consistent plan of the reproductive system in two species of the genus *Crasiella*. The features of the accessory organs and, in particular, the highly complex structure of the caudal organ suggest that the *Crasiella* species are phylogenetically close to the *Macrodasys* species. If the functional similarities that have been proposed for the two genera can be proved to be real homologies, these would strongly support the proximity of the genus *Crasiella* to the *Macrodasys*idae, which has been suggested by the phylogenetic analysis of Hochberg and Litvaitis (2001). A morphological study of species of

Planodasyidae, the only other genus of *Planodasyidae*, would be the best way to test this hypothesis.

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