

**Spermiogenesis and ultrastructure of the spermatozoon of *Wardula capitellata* (Digenea, Mesometridae), an intestinal parasite of the sparid teleost *Sarpa salpa* in Senegal**

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**Abstract**

The spermiogenesis process in *Wardula capitellata* begins with the formation of a differentiation zone containing two centrioles associated with striated rootlets and an intercentriolar body. Each centriole develops into a free flagellum orthogonal to a median cytoplasmic process. Later these flagella rotate and become parallel to the

median cytoplasmic process, which already exhibits two electron-dense areas and spinelike bodies before its proximodistal fusion with the flagella. The final stage of the spermiogenesis is characterized by the constriction of the ring of arched membranes, giving rise to the young spermatozoon, which detaches from the residual cytoplasm. The mature spermatozoon of *W. capitellata* presents most of the classical characters reported in digenean spermatozoa such as two axonemes of different lengths of the 9+“1” trepaxonematan pattern, nucleus, mitochondrion, two bundles of parallel cortical microtubules and granules of glycogen. However, some peculiarities such as two lateral expansions accompanied by external ornamentation of the plasma membrane and spinelike bodies characterize the mature sperm. Moreover, a new spermatological character is described for the first time, the so-called cytoplasmic ornamented buttons.

**Keywords**

*Wardula capitellata*, Mesometridae, Digenea, spermiogenesis, spermatozoon, ultrastructure

## **Introduction**

Over the years, the historical systematic position and relationships of the family Mesometridae have been controversial. Several studies have related this family with the superfamily Paramphistomoidea (La Rue 1957, Holliman 1961). Jousson and Bartoli (1999) supported the inclusion of Mesometridae in Paramphistomiformes, as proposed by Brooks *et al.* (1985), and its close relationship to the Microscaphidiidae. Moreover, in their molecular study, Cribb *et al.* (2001) include the Mesometridae in the Paramphistomoidea, as also supported later by Olson *et al.* (2003) in their classification of the Digenea based on complete ssrDNA and partial (D1-D3) lsrDNA sequences. However, Jones and Blair (2005) treated the Mesometridae as a family in the superfamily Microscaphidioidea together with the type family Microscaphidiidae. Such controversial classifications are recurrent in the Platyhelminthes in general and within the trematodes in particular.

In order to clarify the phylogenetic relationships of Platyhelminthes, several workers have resorted to the ultrastructural characters of reproduction in the Platyhelminthes (see Justine 2001, 2003; Levron *et al.* 2010; Bakhoun *et al.* 2011a,b).

With respect to the Trematoda several characters seem to be interesting tools for phylogenetic purposes. However, the insufficient database on trematodes (about 62 descriptions, corresponding to 35 families) emphasise the need for more studies within this class. This is the case of the family Mesometridae, which was unexplored until now. Thus, the present work presents for the first time ultrastructural data concerning spermiogenesis and the mature spermatozoon of *Wardula capitellata*, one of the seven species that compose the family Mesometridae.

## **Material and Methods**

Adult specimens of *Wardula capitellata* (Rudolphi, 1819) were collected from the digestive tract of a naturally infected *Sarpa salpa* (Teleostei, Sparidae) captured off coast of Dakar (Senegal). Living digeneans were placed in a 0.9% NaCl solution. After dissection, specimens were routinely processed for TEM examination. They were fixed in cold (4°C) 2.5% glutaraldehyde in a 0.1M sodium cacodylate buffer at pH 7.4 for a minimum of 2 hr, rinsed in a 0.1M sodium cacodylate buffer at pH 7.4, postfixed in cold (4°C) 1% osmium tetroxide in the same buffer for 1 hr, rinsed in a 0.1 M sodium cacodylate buffer at pH 7.4, dehydrated in an ethanol series and propylene oxide, and finally embedded in Spurr resin. After the testes and seminal vesicle were located in semithin sections, ultrathin sections were made using a Reichert-Jung Ultracut E ultramicrotome, placed on copper grids and double-stained with uranyl acetate and lead citrate according to Reynolds (1963). To locate glycogen, gold grids were also prepared according to the Thiéry (1967) test. Ultrathin sections were examined using a JEOL 1010 transmission electron microscope operating at 80kv.

## **Results**

### **Spermiogenesis**

Spermiogenesis process in *W. capitellata* is described in Figures 1-3.

The differentiation zone marks the beginning of spermiogenesis. Both longitudinal and cross-sections show the elongation of a cytoplasmic projection bordered by submembranous cortical microtubules and containing a nucleus, two centrioles, an intercentriolar body, and mitochondria (Figs. 1a, b). The intercentriolar body is made up by six electron-dense layers alternating with electron lucent ones (Fig. 1c). In an early stage of the spermiogenesis process, both centrioles originate free flagella that grow orthogonally to a median cytoplasmic process (Fig. 1a). Posteriorly,

the two flagella rotate and become parallel to the median cytoplasmic process (Fig. 1c, d). Additionally, in this stage the nucleus is observed in migration toward the median cytoplasmic process while the mitochondria are still in the differentiation zone (Fig. 1d, e, 3b). Before the fusion of flagella, the median cytoplasmic process exhibits electron-dense areas separating two parallel bundles of cortical microtubules (Fig. 1f, g). At this stage, it is also possible to observe a spinelike body in the median cytoplasmic process (Fig. 1g, h). Several observations of cross and longitudinal sections show that the nuclear migration occurs before the mitochondrial migration (Figs. 1d, e, 2a and 3a, b). The final stage of spermiogenesis in *W. capitellata* is characterized by the constriction of the ring of arched membranes (Figs. 2a, b, 3d). During the final stages of spermiogenesis, the mitochondrion is observed in migration and the striated rootlets disappear (Figs. 2a, b). Cross-sections of the young spermatozoon in the testicular tissue exhibit the presence of two cytoplasmic expansions (Fig. 2c) and two cytoplasmic buttons (Fig. 2d). The latter structure, described for the first time, contains an internal element and exhibits external ornamentation. We propose the term cytoplasmic ornamented button (COB) for this newly described structure (Fig. 2d).

## **Spermatozoon**

The observation of numerous ultrathin sections of the spermatozoon of *W. capitellata* allows us to distinguish three regions from the anterior to the posterior spermatozoon extremity (Figs. 4–7).

Region I (Figs. 4a–j, 6, 7I) corresponds to the anterior spermatozoon extremity and is characterized by the presence of external ornamentation of the plasma membrane, two lateral expansions and spinelike bodies. Moreover, two axonemes of the 9+‘1’ pattern of trepaxonematan Platyhelminthes and parallel cortical microtubules are also

present. Between the anterior tip of the spermatozoon and region II it is possible to distinguish several areas:

(a) area containing two lateral expansions, a continuous layer of parallel cortical microtubules, external ornamentation and spinelike bodies (Figs. 4a-c, i, 7I). It is interesting to remark the absence of attachment zones in this area.

(b) area containing one or two cytoplasmic ornamented buttons (Figs. 4d-f, 7I). The four attachment zones are visible in this area (Fig. 4d) and the cortical microtubules are clearly arranged into two fields (Fig. 4d, e). The cytoplasmic ornamented buttons contain an electron-dense element centrally located. They present an electron-dense material in the internal surface of plasma membrane and also exhibit external ornamentations (Fig. 4d, e, f).

(c) posterior area presenting external ornamentation associated to the cortical microtubules, with the progressive presence of granules of glycogen and disappearance of the external ornamentation (Figs. 4g, h, j, 6).

Region II (Figs. 5a, b, 7II) is the mitochondrial region. It begins with the appearance of the mitochondrion, while exhibiting both axonemes, cortical microtubules and granules of glycogen (Figs. 5a, 7II). In posterior areas of region II the nucleus appears and thus, the sperm exhibits both nucleus and mitochondrion (Figs. 5b, 7II). The transition from region II towards region III is marked by the disappearance of the mitochondrion.

Region III (Figs. 5c-f, 7III) corresponds to the posterior spermatozoon extremity, characterized in its proximal area by the presence of nucleus, two axonemes, cortical microtubules and granules of glycogen (Figs. 5c and 7III). In the anterior part of this region the nucleus is located between the axonemes (Fig. 5c) and later, it becomes eccentric (Fig. 5d). Towards the posterior tip of the sperm cell the first axoneme (Fig.

5e), then the second axoneme and the cortical microtubules disappear (Fig. 5f). Areas near the posterior tip exhibit a reduced section of nucleus and some granules of glycogen (Fig. 5f).

## Discussion

### Spermiogenesis

The spermiogenesis process of *Wardula capitellata* does not differ significantly from those of other digenean species described until now, even though some peculiarities were detected. As described in the present study, most digeneans present a spermiogenesis process characterized by the formation of a differentiation zone containing two centrioles that give rise to two free flagella growing orthogonally to the median cytoplasmic process, becoming parallel after rotation and fusing proximodistally with the median cytoplasmic process. The flagellar rotation of 90° described in *W. capitellata*, has been reported in most digeneans: e.g. *Haematoloechus medioplexus*, *Fasciola gigantica*, *Neoapocreadium chabaudi* or *Diplodiscus subclavatus* (Justine and Mattei 1982; Ndiaye *et al.* 2004; Kacem *et al.* 2010; Bakhoun *et al.* 2011a). However, some recent studies have described flagellar rotations greater than 90°. It is the case of *Helicometra fasciata*, *Monorchis parvus*, *Fasciola hepatica*, *Dicrocoelium hospes*, *Nicolla wisniewskii* or *Crepidostomum metoecus* (Levron *et al.* 2003, 2004a; Ndiaye *et al.* 2003; Agostini *et al.* 2005; Quilichini *et al.* 2007a,b).

In *W. capitellata*, spinelike bodies were observed in an early stage of spermiogenesis before the proximodistal fusion. In fact, spinelike bodies are observed associated with the plasma membrane of the median cytoplasmic process before the fusion of the flagella and also after the fusion of both flagella with this median cytoplasmic process. Such description and location of spinelike bodies have been

reported recently in the spermiogenesis of *D. subclavatus* and *Rubenstrema exasperatum* (see Bakhoun *et al.* 2011a,b). Thus, in these species the formation of spinelike bodies occurs in an early phase of spermiogenesis.

During spermiogenesis and before proximodistal fusion, several mitochondria and the nucleus migrate toward the median cytoplasmic process. In most digeneans mitochondria migrate after the nucleus as postulated by Burton (1972). Such a migration is observed in *W. capitellata*. In fact, when the nucleus migrates toward the median cytoplasmic process, the mitochondria are still in the differentiation zone. Moreover, in several sections during the final stages of spermiogenesis, the mitochondrion is observed in migration. Contrarily, a mitochondrial migration before nuclear migration has been reported in the spermiogenesis of *Dicrocoelium dendriticum* (Cifrian *et al.* 1993), *Postorchigenes gymnesicus* (Gracenea *et al.* 1997) and *R. exasperatum* (Bakhoun *et al.* 2011b).

The intercentriolar body is another structure of great phylogenetic interest present in the differentiation zone. In general the intercentriolar body in digeneans presents seven electron-dense layers morphologically formed by a fine central one and three external layers on each side. For example, seven electron-dense layers were described in the Paramphistomoids *Paramphistomum microbothrium*, *Cotylophoron cotylophorum*, *Carmyerius endopapillatus*, *Basidiodiscus ectorchus*, *Sandonia sudanensis* and *D. subclavatus* (Seck *et al.* 2007, 2008a,b; Ashour *et al.* 2007; Bakhoun *et al.* 2011a), the allocreadiid *C. metoecus* (Quilichini *et al.* 2007b), or the Opecoelids *Opecoeloides furcatus* (Miquel *et al.* 2000), and *Poracanthium furcatum* (Levron *et al.* 2004b). Intercentriolar bodies composed of nine electron-dense layers are described in *Cryptocotyle lingua* (Rees 1979) and *M. parvus* (Levron *et al.* 2004a) and others composed of six layers in *Deropristis inflata* by Foata *et al.* (2007). In *W. capitellata*



the intercentriolar body is composed of six electron-dense layers. However, the intercentriolar body may contain a seventh very thin central band, which is not clearly visible due to the orientation and level of sections.

According to Burton (1972) the intercentriolar body and also the striated rootlets participate in the stabilization of the differentiation zone, where the intercentriolar body serves as a reserve of material for microtubule polymerization. Phylogenetically, this structure is considered a plesiomorphic character present in Digenea and also in most Cestoda, except for some Tetrabothriidea and some Cyclophyllidea (Justine 1998, 2001). Moreover, there is a progressive reduction of this character in more evolved taxa of the Cestoda (see Bruňanská *et al.* 2005, Miquel *et al.* 1999). Variations in intercentriolar bodies should be further assessed considering its potential importance to the phylogenetic analysis of Digenea.

### **Spermatozoon**

Presently, the ultrastructural organization of digenean spermatozoa cannot be described as homogeneous. In fact, recent descriptions emphasise the variability of many characters such as spinelike bodies, number of mitochondria, lateral expansions, distribution of cortical microtubules or morphology of both spermatozoon extremities.

The anterior spermatozoon extremity of *W. capitellata* is characterized by the presence of two axonemes (slightly longitudinally shifted) of the 9+‘1’ trepaxonematan pattern. In this anterior extremity there are two lateral expansions with external ornamentation and the centrioles are surrounded by a continuous layer of cortical microtubules. Two slightly longitudinally shifted axonemes have been reported for example, in *H. medioplexus* (Justine and Mattei 1982), *Metorchis orientalis* (Liu and Pan 1990), *Echinostoma caproni* (Iomini and Justine 1997), *Nicolla testiobliquum*

(Quilichini *et al.* 2007c) or *R. exasperatum* (Bakhoun *et al.* 2011b). However, the particularity of *W. capitellata* sperm is the presence of two lateral expansions at centriolar level. Such an anterior tip exhibiting both axonemes is rare in digenean spermatozoa. In fact, most species described until now present only one axoneme as in *P. gymnesicus*, *Anisocoelium capitellatum* or *D. subclavatus* (Gracenea *et al.* 1997, Ternengo *et al.* 2009, Bakhoun *et al.* 2011a).

Considering the presently available data, there are several ultrastructural characters which are typical of anterior areas of the male gamete of digeneans, such as centrioles, the external ornamentation of the plasma membrane, lateral expansions, spinelike bodies, mitochondrion (in the case of species with more than one mitochondrion) as well as the lack of glycogen granules. In the present study, we describe a new character in this region of sperm cells, the so-called cytoplasmic ornamented buttons.

The centrioles mark the beginning of the axonemes and their observation in cross-section gives real evidence for the localization of electron micrographs. Thus, they would be good and unequivocal elements for determining the anterior spermatozoon tip. Moreover, in the case of species showing one axoneme in both spermatozoon extremities, such as *Troglorema acutum* (Miquel *et al.* 2006), the observation of centrioles is useful when differentiating anterior and posterior spermatozoon extremities.

Another structure observed in the anterior region of the sperm cell is the external ornamentation of the plasma membrane. It is associated with cortical microtubules and also with spinelike bodies in some cases. It represents a character of great phylogenetic importance in the spermatozoon of digeneans. The external ornamentation is located in the anterior area of the spermatozoon, generally in the mitochondrial side (ventral side)

of the sperm cell. However, certain authors have described an external ornamentation associated with one of the axonemes in the anterior extremity of the spermatozoon. This is the case of *H. medioplexus*, *M. parvus*, *Pronoprymna ventricosa* and *N. chabaudi* (Justine and Mattei 1982, Levron *et al.* 2004a, Quilichini *et al.* 2007d, Kacem *et al.* 2010).

In some digeneans with several mitochondria, the external ornamentation is observed in the area containing the first mitochondrion (See Agostini *et al.* 2005, Miquel *et al.* 2006 or Bakhoun *et al.* 2011b).

In *W. capitellata*, the external ornamentation appears in two distinct areas: (a) in the region of the lateral expansions and (b) in another area containing only the axonemes and granules of glycogen. The external ornamentation associated with lateral expansions was also observed in final stages of spermiogenesis. According to Justine and Mattei (1982), it corresponds to external ornamentation belonging to the differentiation zone and, thus it is accompanied by a continuous layer of cortical microtubules and by the lack of attachment zones. The other type of external ornamentation, although morphologically similar to the first type, is probably formed in more advanced stages of spermiogenesis at more distal areas, where the proximodistal fusion has occurred. Consequently, this second type of ornamentation is associated with other structures such as attachment zones.

Concerning the lateral expansions, in *W. capitellata* these are observed in the anterior spermatozoon extremity associated with other characters such as cortical microtubules, external ornamentation and spinelike bodies. Lateral expansions are described in numerous digeneans. Between these species it is remarkable its description in all the paramphistomoid species studied until now including *C. cotylophorum*, *P. microbothrium*, *C. endopapillatus*, *B. ectorchus*, *S. sudanensis* and *D. suclavatus* (Seck

et al. 2007, 2008a, b; Ashour et al. 2007; Bakhoum et al. 2011a). It is interesting to notice that several authors (see Cribb et al. 2001; Olson et al. 2003) have nested the Mesometridae (which includes *W. capitellata*) within the Paramphistomoidea. Thus, these lateral expansions would be a good character for justifying the proximity of these taxa. However, there are different morphologies of lateral expansions in the digeneans such as in *H. fasciata*, *Scaphiostomum palaearticum* or *T. acutum* (Levron et al. 2003; Ndiaye et al. 2002; Miquel et al. 2006). The presence and variability of lateral expansions in mature spermatozoa of digeneans should be analysed carefully due to their potential phylogenetic interest.

In the present study we describe cytoplasmic ornamented buttons for the first time. Their appearance seems to be related with the reduction and disappearance of the lateral expansions. The cytoplasmic ornamented buttons appear to be formed by an internal electron-dense element surrounded by ornamented cytoplasmic membrane. However, these ornamentations seem to be different from those observed on lateral expansions. Considering that this is the first report of this structure, more studies are needed to verify its importance to phylogenetic studies.

Generally, digeneans presenting external ornamentation also present spinelike bodies, as observed in *W. capitellata*. However, some species exhibit external ornamentation without spinelike bodies, e.g. *M. parvus* (Levron et al. 2004a), *P. ventricosa* (Quilichini et al. 2007d), or *Euryhormis squamula* (Bakhoum et al. 2009). With respect to spinelike bodies, it is interesting to notice their formation during spermiogenesis. In *W. capitellata*, spinelike bodies appear before the fusion of the flagella with the median cytoplasmic process as observed in *D. subclavatus* and *R. exasperatum* (Bakhoum et al. 2011a, b). In the first report of spinelike bodies during

spermiogenesis (Miquel *et al.* 2000), these structures were observed only in old spermatids when both flagella were already fused with the median cytoplasmic process.

With respect to the distribution of spinelike bodies along the spermatozoon, *O. furcatus* (Miquel *et al.* 2000) and *F. gigantea* (Ndiaye *et al.* 2004) show a periodicity of spinelike bodies of 1  $\mu\text{m}$ , *P. furcatum* (Levron *et al.* 2004b) a periodicity of 0.7  $\mu\text{m}$ , and *N. wisniewskii* (Quilichini *et al.* 2007a) a periodicity of 0.6  $\mu\text{m}$ . However, due to their irregular distribution no periodicity is observed in *W. capitellata* as in many other digeneans (see Bakhoun *et al.* 2011a). This character will probably be a good tool in elucidating the relationships between digeneans at the family, superfamily or order level.

One mitochondrion is observed in the spermatozoon of most digeneans as occurs in *W. capitellata*. This is the case of *Brachylaima aequans*, *O. furcatus*, *F. gigantea* or *D. subclavatus* (Žďárská *et al.* 1991; Miquel *et al.* 2000; Ndiaye *et al.* 2004; Bakhoun *et al.* 2011a). Other digeneans exhibit two mitochondria, one being located at the level of the ornamented region and the other in the area containing the nucleus. This is the case of the opecoelids *P. furcatum*, *N. wisniewskii*, *N. testiobliquum* (Levron *et al.* 2004b; Quilichini *et al.* 2007a, c), the dicrocoeliid *D. hospes* (Agostini *et al.* 2005), the troglotrematid *T. acutum* (Miquel *et al.* 2006), the apocreadiid *N. chabaudi* (Kacem *et al.* 2010) or the omphalometrid *R. exasperatum* (Bakhoun *et al.* 2011b). Additionally, a spermatozoon with three mitochondria has recently been described in *B. ectorchus*, *S. sudanensis*, *A. capitellatum* and *E. squamula*, (Ashour *et al.* 2007, Bakhoun *et al.* 2009, Ternengo *et al.* 2009). Thus, the variation in the number of mitochondria in the mature sperm could contribute to the interpretation of relationships at family level. It must be emphasized that the absence of mitochondria is considered a synapomorphy in some cestodes (Eucestodes) (Justine 1995) and its presence in basal cestodes (Gyrocotylidea

and Amphilinidea) and in other neodermatans (monogeneans, digeneans) is considered an ancestral or plesiomorphic character.

The distribution of glycogen granules along the spermatozoon of digenean species is not well documented. Nonetheless, in most digeneans studied until now glycogen granules are absent from the anterior tip of the spermatozoon. Also, in *W. capitellata* no glycogen granules were observed in the anterior extremity of the mature spermatozoon. The presence/absence of this character allows identifying those cross-sections which belong to the anterior extremity of the mature spermatozoon.

The morphology of the posterior extremity of the spermatozoon is variable. According to Quilichini *et al.* (2010a) three principal types of posterior spermatozoon extremities could be observed in digenean spermatozoa considering the succession of several characters. Those are (a) the type 1 (or opecoelidean type), characterized by a posterior extremity containing cortical microtubules, (b) the type 2 (or fasciolidean type), exhibiting only the nucleus and (c) the type 3 (or cryptogonimidean type) presenting a posterior tip containing the second axoneme. In *W. capitellata* the posterior spermatozoon extremity corresponds to the type 2, containing only nucleus and granules of glycogen. However, several species present posterior extremities that do not concur with any of the postulated types. This is the case of the spermatozoon of *D. subclavatus* (Bakhoun *et al.* 2011a) and other paramphistomoids that present the nucleus and some cortical microtubules in the posterior tip (see Seck *et al.* 2007, 2008a).

Another unique morphological pattern has recently been observed in the spermatozoon of *Aponurus laguncula* (Quilichini *et al.* 2010b), which presents a mitochondrion that reaches the posterior spermatozoon extremity along with both axonemes. Subsequently, it would be more interesting to consider the terminal character only rather than the succession of characters observed in the posterior spermatozoon tip

in order to overcome the minimal variations described in the posterior spermatozoon extremities of some species. Finally, the variability of the posterior spermatozoon extremity would be very useful to separate digeneans at family level.

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## Legends of Figures

**Fig. 1.** Spermiogenesis of *W. capitellata*. **a.** Differentiation zone showing one of the flagella growing orthogonally and an intercentriolar body. **b.** Differentiation zone exhibiting two centrioles, nucleus, mitochondrion and intercentriolar body. **c.** Detail of the intercentriolar body. **d.** Differentiation zone with two flagella nearly parallel to the median cytoplasmic process. **e.** Longitudinal section showing the nucleus in migration and mitochondria staying in the differentiation zone. **f.** Section with the nucleus before proximodistal fusion. **g, h.** Several cross-sections before the fusion showing the electron-dense area (arrowheads) and spinelike body. Scale bars = 0.5  $\mu\text{m}$  (a, b, d, f), 0.3  $\mu\text{m}$  (e, g, h), 0.2  $\mu\text{m}$  (c). **Abbreviations to all figures:** AM—arched membranes, ASE—anterior spermatozoon extremity, Ax—axoneme, AZ—attachment zones, C—centriole, CE—cytoplasmic expansion, CM—cortical microtubules, COB—cytoplasmic ornamented button, EO—external ornamentation, F—flagellum, G—granules of glycogen,

IB–intercentriolar body, LE–lateral expansion, M–mitochondrion, MCP–median cytoplasmic process, N–nucleus, NM–nuclear membrane, PM–plasma membrane, PSE–posterior spermatozoon extremity, SB–spinelike body, SR–striated rootlets.

**Fig. 2.** Spermiogenesis of *Wardula capitellata*. **a.** Early stage of the constriction of the ring of arched membranes in which mitochondrion is observed in migration. **b.** Advanced stage of the arched membranes constriction characterized by absence of striated rootlets. **c.** Anterior region of the old spermatid showing cytoplasmic expansions and external ornamentation. **d.** Old spermatid with cytoplasmic ornamented buttons. Scale bars = 0.5  $\mu\text{m}$  (a, b), 0.3  $\mu\text{m}$  (c, d)

**Fig. 3.** Schematic reconstruction of the main stages of spermiogenesis in *Wardula capitellata*.

**Fig. 4.** Spermatozoon of *Wardula capitellata*. **a,b.** Anterior spermatozoon extremity with the two centrioles of the axonemes, external ornamentation, lateral expansions and spinelike body in a more posterior area when both axonemes are already formed. **c.** Cross-section showing reduced lateral expansions. **d,e.** Sections with two and one cytoplasmic ornamented buttons showing also attachment zones (arrowheads). **f.** Detail of a cytoplasmic ornamented button. **g,h.** Sections exhibiting external ornamentation of the plasma membrane associated with axonemes and cortical microtubules. **i.** Ornamented area showing several spinelike bodies. **j.** Cross-section with only two axonemes, cortical microtubules and granules of glycogen. Scale bars = 0.1  $\mu\text{m}$  (f), 0.3  $\mu\text{m}$  (a–e, g, h, j), 0.5  $\mu\text{m}$  (i)

**Fig. 5.** Spermatozoon of *Wardula capitellata*. **a.** Mitochondrial region. **b.** Cross-section at the mitochondrial level characterized by the appearance of the nucleus. **c,d.** Consecutive cross-sections in region III showing the central and eccentric position of the nucleus. **e.** Section exhibiting one axoneme, nucleus, few cortical microtubules and

granules of glycogen. **f.** Posterior spermatozoon tip containing only the nucleus and granules of glycogen. Scale bars = 0.3  $\mu\text{m}$  (a-f)

**Fig. 6.** Visualization of the granules of glycogen by the Thiéry methodology (1967).

**Fig. 7.** Attempted reconstruction of the spermatozoon of *Wardula capitellata*.



















