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# Ultrastructure of the spermatozoon of *Centroderma spinosissima* (Stossich, 1886) (Digenea: Mesometridae) and its phylogenetic potential

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ABSTRACT

The Mesometridae includes only five genera and eight species. The available data on the ultrastructure of sperm cells of mesometrid species referred to two species only, *Elstia stossichianum* and *Wardula capitellata*. The present study revealed the ultrastructure of the spermatozoon of a third genus and third species of Mesometridae, *Centroderma spinosissima*. The mature spermatozoon of *C. spinosissima* presents two axonemes with different lengths of the Ehlers' 9 + '1' trepaxonematan pattern, a nucleus, two mitochondria, two bundles of parallel cortical microtubules, external ornamentation of the plasma membrane, a lateral expansion, spine-like bodies, cytoplasmic ornamented buttons and granules of glycogen. The spermatozoon of *C. spinosissima* is similar to those of the previously studied mesometrids. However, some peculiarities such as the presence of two mitochondria, the disposition of the external ornamentation of the plasma membrane and the morphology of the posterior spermatozoon extremity, characterize the male gamete of *C. spinosissima*. Moreover, the presence of cytoplasmic ornamented buttons is a characteristic found only in the mature spermatozoon of mesometrids and it probably represents an autapomorphy for this family.



## 1. Introduction

The Mesometridae is a small family of Digenea comprising only eight recognized species belonging to five genera (Jones and Blair, 2005; Pérez-del Olmo et al., 2006). The eight species are *Centroderma spinosissima*, *Elstia stossichianum*, *Mesometra brachycoelia* and *M. orbicularis*, *Parawardula elongata*, and *Wardula bartolii*, *W. capitellata* and *W. sarguicola*.

Historically, the classification of the family Mesometridae has been controversial. These controversies are common to most digenean taxa, with recurrent problems when trying to establish phylogenetic relationships among taxa (Jones and Blair, 2005). In order to clarify such relationships, several authors have explored the ultrastructural characteristics of sperm cells in the Platyhelminthes, including the Digenea (Euzet et al., 1981; Ehlers, 1984; Brooks et al., 1985; Świderski, 1986; Bâ and Marchand, 1995; Justine, 1998, 2001, 2003; Levron et al., 2010; Quilichini et al., 2010, 2011; Ndiaye et al., 2011; Bakhoum et al., 2012a,b, 2013). Ultrastructural studies of spermatozoa in the Platyhelminthes in general revealed a very high diversity of characters, which is of great importance and usefulness for phylogenetic inferences. Also, in the Cestoda several patterns of spermiogenesis and different types of spermatozoa have been established (Świderski, 1986; Bâ and Marchand, 1995; Levron et al., 2010) and numerous spermatological synapomorphies have been postulated (Justine, 1991a, 1998, 2001). Moreover, several synapomorphies on the basis of spermatozoal ultrastructural characters have been used in the cladistic analysis of the Monogenea (Justine et al., 1985; Justine, 1991a,b, 1993). Contrarily, with respect to digeneans, there are numerous gaps concerning sperm characters of a great number of families (see Bakhoum, 2012). Nevertheless, the recent increase of ultrastructural studies in digeneans (for a review see Bakhoum, 2012) demonstrated a high diversity of sperm characters thus emphasizing their potential use in phylogenetic analysis.

Until now, there was ultrastructural data on two mesometrid species, *E. stossichianum* and *W. capitellata* (Bakhoum et al., 2012a,

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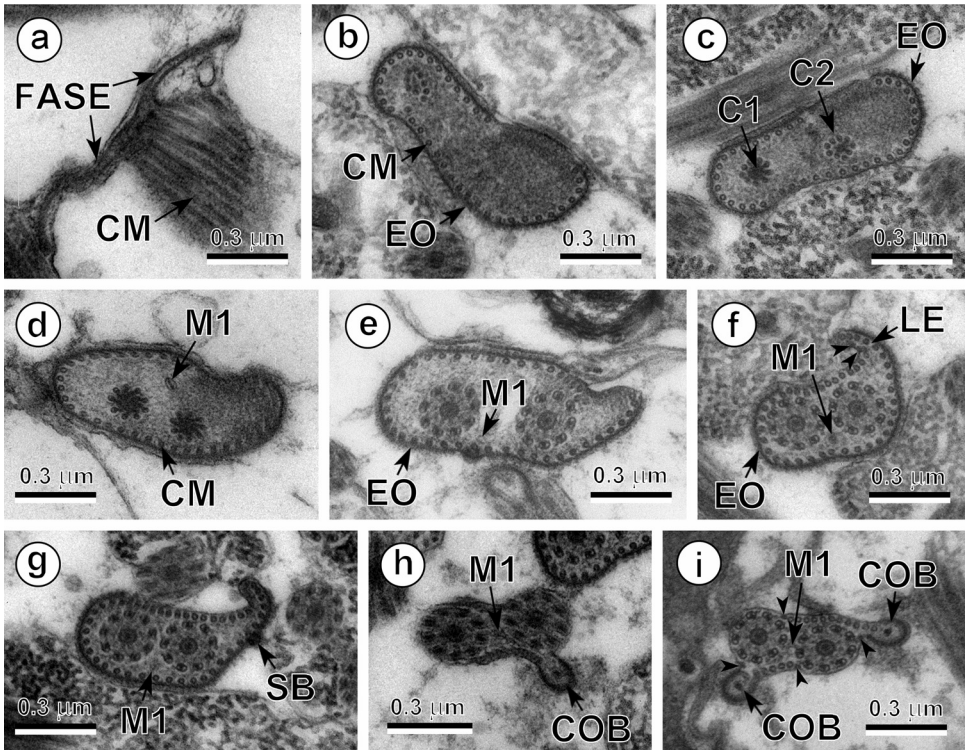


**Fig. 1.** Mature spermatozoon of *Centroderma spinosissima*. (a) Longitudinal section of the forked anterior spermatozoon extremity (FASE) showing cortical microtubules (CM). (b–d) Consecutive cross-sections of the anterior part of Region I showing the appearance of the two centrioles (C1 and C2). This area is mainly characterized by the presence of external ornamentation of the plasma membrane (EO) associated to cortical microtubules (CM). First mitochondrion. (M1) (e–g) Cross-sections of the middle part of Region I exhibiting a lateral expansion (LE) and spine-like bodies (SB). Attachment zones (arrowheads), cortical microtubules (CM); external ornamentation (EO); first mitochondrion (M1). (h–i) Cross-sections of the posterior part of Region I showing the cytoplasmic ornamented buttons (COB). Note the two fields of cortical microtubules. Attachment zones (arrowheads), first mitochondrion (M1).

2013). The present study revealed the ultrastructure of the spermatozoon of a third genus and third species of Mesometridae, *C. spinosissima*, an intestinal parasite of Sparidae fishes, mainly *Sarpa salpa*, in the Mediterranean (Jones and Blair, 2005). Furthermore, the present study aims at comparing the ultrastructural organization of the spermatozoon in mesometrids, with emphasis to its phylogenetic potential in the Digenea.

## 2. Materials and methods

Live specimens of *C. spinosissima* were collected from the digestive tract of *S. sarpa* off Chebba (Tunisia). After dissection, live digeneans were routinely processed for transmission electron microscopy examination. They were fixed in cold (4 °C) 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer at pH 7.4 for a minimum of 2 h, rinsed in a 0.1 M sodium cacodylate buffer at pH 7.4, postfixed in cold (4 °C) 1% osmium tetroxide ($OsO_4$) with 0.9% potassium ferricyanide [$K_3Fe(CN)_6$] in the same buffer for 1 h, rinsed in milli Q water (Millipore Gradient A10), dehydrated in an ethanol series and propylene oxide, embedded in Spurr resin and finally polymerized at 60 °C for 72 h. 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). Finally, all stained grids were studied with a JEOL 1010 transmission electron microscope operated at 80 kV.

The Thiéry (1967) technique was used to locate glycogen. Gold grids were treated in periodic acid, thiocarbohydrazide and silver proteinate (PA-TCH-SP) as follows: 30 min in 10% PA, rinsed in milliQ water, 24 h in TCH, rinsed in acetic solutions and milliQ water, 30 min in 1% SP in the dark, and rinsed in milliQ water.

## 3. Results

The mature spermatozoon of *C. spinosissima* contains two axonemes of the 9 + '1' trepaxonematan pattern, two bundles of parallel cortical microtubules, a nucleus, two mitochondria, a lateral expansion, external ornamentation of the plasma membrane, cytoplasmic ornamented buttons and granules of glycogen. The observation of these structures in cross- and longitudinal sections enabled us to establish three regions (I–III).

Region I is the anterior spermatozoon extremity presenting a forked anterior tip (Figs. 1a and 3I). This anterior spermatozoon extremity exhibits the centrioles, a submembranous layer of parallel cortical microtubules and external ornamentation of the plasma membrane (Fig. 1b and c). The first mitochondrion appears at the centriolar level (Fig. 1d) and reaches the middle part of Region I, where it is possible to observe a lateral expansion associated with external ornamentation of the plasma membrane and spine-like bodies (Figs. 1g and 3I). In the lateral expansion it is also possible to observe a small segment of the plasma membrane without external ornamentation (Fig. 1e–g). The distal part of region I is characterized by the appearance of one or two cytoplasmic ornamented buttons (Figs. 1h and i and 3I). Moreover, the parallel cortical microtubules become arranged into two fields: one with about 11–12 microtubules and the other with about 5–9 microtubules (Fig. 1h and i).