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 superfamly 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 Microsaphidiidae. 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 Microsaphidioidea together with the type family Microsaphidiidae. Such controversial classifications are recurrent in the Platyhelminthes in general and within the trematodes in particular.

In order to clarify the phylogenetic relationships of Platynhelminthes, several workers have resorted to the ultrastructural characters of reproduction in the Platynhelminthes (see Justine 2001, 2003; Levron *et al.* 2010; Bakhoum *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

Fig. 1. Spermiogenesis of *Wardula 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 µm (a, b, d, f), 0.3 µm (e, g, h), 0.2 µm (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