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Ultrastructure of the spermatozoon of *Helicometroides atlanticus* (Digenea, Monorchiidae), an intestinal parasite of *Parapristipoma octolineatum* (Pisces, Teleostei) in Senegal



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ABSTRACT

The mature *Helicometroides atlanticus* spermatozoon exhibits an anterior electron-dense material, two axonemes of the 9+"1" pattern, a nucleus, two mitochondria, extramembranous ornamentations located at the level of the first mitochondrion and cortical microtubules arranged into two fields: a ventral field corresponding to the mitochondrial side and a dorsal field corresponding to the nuclear side. It lacks of spine-like body and a cytoplasmic expansion as in *Monorchis parvus* the only species of Monorchioidea which spermatozoon has been described until now. Nevertheless, it is distinguished from *M. parvus* by the presence of an anterior electron-dense material and two axonemes which appear one after the other.

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1. Introduction

The classification of the Platyhelminthes in general and of the Digenea in particular has been subject of profound changes ought to the use of molecular and ultrastructural data on spermiogenesis and/or spermatozoon (Euzet et al., 1981; Ehlers, 1984; Justine, 1991, 1993, 1995, 1997, 1998; Bâ and Marchand, 1994, 1995; Cribb et al., 2001; Olson et al., 2003).

In the Digenea, some ultrastructural characters of the mitochondria, the axonemes, the nucleus, the arrangement of cortical microtubules, the presence or not of spine-like bodies, the extramembranous ornamentations and the cytoplasmic expansions have been purposed as tools for the reconstitution of the phylogeny and the systematic (Justine and Mattei, 1982; Miquel et al., 2000; Ndiaye et al., 2003a; Ashour et al., 2007; Kacem et al., 2010; Quilichini et al., 2010a,b, 2011a,b; Bakhoum et al., 2012a).

The superfamily Monorchioidea Odhner, 1911 comprises two families namely the Monorchiidae Odhner, 1911 and the Lissorchiidae Magath, 1917 which are found respectively in the intestine of marine and freshwater fishes. To our knowledge, only *Monorchis*

parvus spermatozoon has been described in the Monorchiidae (Levron et al., 2004). In this work, we describe the ultrastructure of the mature *Helicometroides atlanticus* spermatozoon.

2. Materials and methods

Adult specimens of *H. atlanticus* (Gayevskaya and Aljoshkina, 1983) were gathered alive from the intestine of *Parapristipoma octolineatum* (Valenciennes, 1833) captured in the Atlantic coast of Dakar. Then they were rinsed in a 0.9% NaCl solution and fixed in cold (4° C) 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer at pH 7.2, rinsed in 0.1 M sodium cacodylate buffer at pH 7.2, post-fixed in cold (4° C) 1% osmium tetroxide in the same buffer for 1 h, dehydrated in ethanol and propylene oxide, embedded in Spurr's resin and polymerized at 60° C for 24 h.

Ultrathin sections (60–90 nm thick) were obtained using the ultramicrotome (Power tome PC, RMC Boeckeler®) with a diamond knife. Sections were placed on copper and gold grids.

Sections on copper grids were stained with uranyl acetate and lead citrate (Reynolds, 1963). Sections on gold grids were stained with periodic acid, thiocarbohydrazide and silver proteinate (Thiéry, 1967). This latter technique allows to evidence the presence of glycogen in the mature spermatozoon.

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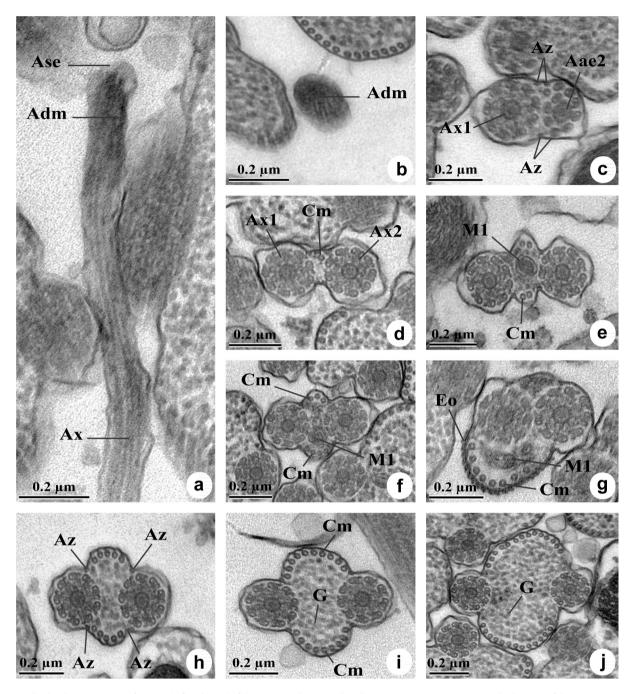


Fig. 1. Longitudinal and cross-sections of region I (a-f) and II (g-j) of the mature *Helicometroides atlanticus* spermatozoon. a: Longitudinal section of the anterior spermatozoon extremity (Ase) showing an anterior electron-dense material (Adm) and one axoneme (Ax); b: Cross-section of the anterior electron-dense material (Adm); c: presence of the first axoneme (Ax1), the anterior extremity of the second axoneme (Aae2) and attachment zones (Az); d-f: presence of two axonemes (Ax1, Ax2), gradual increase in the number of cortical microtubule (Cm), which are organized into two fields and the first mitochondrion (M1); g: presence of extramembranous ornamentations (Eo) and the first mitochondrion (M1); h-j: presence of attachment zones (Az) and gradual increase of the number of cortical microtubules (Cm) and of the granules of glycogen (G).

All grids were examined in a Hitachi H-7650 electron microscope operated at 80 kV, in the "Service d'Étude et de Recherche en Microscopie Électronique" of the Pascal Paoli University of Corsica.

3. Results

The observation of several cross and longitudinal sections of the mature spermatozoon of *H. atlanticus* allowed us to distinguish from front to back five different regions (I–V).

Region I (Figs. 1a–f and 5I) corresponds to the anterior extremity of the spermatozoon. It is characterized by the presence of an

anterior electron-dense material (Fig. 1a and b), two axonemes of the 9+"1" pattern of the Trepaxonemata which appear one after the other (Fig. 1c and d) and two fields of cortical microtubules which number increases progressively from 1 to 8 (Fig. 1d-f). Cross-sections in the posterior part of this region show the presence of the first mitochondrion (Fig. 1e and f).

Region II (Figs. 1g–j and 5II). The anterior part of this region is marked by the presence of extramembranous ornamentations on the ventral side (mitochondrial) of the spermatozoon (Fig. 1g) and by the posterior part of the first mitochondrion. The posterior part of this region, devoid of mitochondria and extramembranous

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