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Spermatological characters of the digenean *Lecithostaphylus retroflexus* (Molin, 1859) (Microphalloidea: Zoogonidae), a parasite of the teleost fish *Belone belone gracilis*

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ABSTRACT

The ultrastructural organization of the spermatozoon of the digenean *Lecithostaphylus retroflexus* (Microphalloidea: Zoogonidae) was described. Alive digeneans were collected from *Belone belone gracilis* (Teleostei: Belonidae), caught from the Gulf of Gabès in Chebba (Tunisia). The mature spermatozoon of *L. retroflexus* exhibits two axonemes of different lengths with the 9+'1' Trepaxonematan pattern, a nucleus, two mitochondria, two bundles of parallel cortical microtubules and granules of glycogen. Additionally, the spermatozoon of *L. retroflexus* shows type 2 of the external ornamentation according to Quilichini et al. (2011), spine-like bodies and a continuous and submembranous layer of parallel cortical microtubules surrounding the axonemes at their anterior end. Moreover, the morphology of the posterior spermatozoon extremity in *L. retroflexus* corresponds to the fasciolidean type according to Quilichini et al. (2010).

1. Introduction

The family Zoogonidae includes digenean species mainly parasitizing marine fish. According to a recent review (Bray and Justine, 2014), this family contains 33 genera with a total of 159 species. Its placement as a microphalloidean has been demonstrated in several molecular analyses. The Microphalloidea includes two major clades. Three families are placed in the first clade (Pachypsolidae, Renicolidae and Eucotylidae). The Zoogonidae that was found closely related and probably paraphyletic to the Faustudidae is placed in the second clade. Thus, the second clade includes the Zoogonidae + Faustulidae as the most basal taxon with other taxa (Lecithodendriidae, Microphallidae, Pleurogenidae and Prosthogonimidae) as progressively more derived (see Olson et al., 2003; Bray and Justine, 2014).

In this context, the ultrastructural studies of species belonging to the Zoogonidae family are of great importance to bring additional information that complements the molecular results. The ultrastructural study of the mature spermatozoon provides numerous characters, which are useful for phylogenetic inference in parasitic Platyhelminthes (Justine, 1991, 1995; Levron et al., 2010; Quilichini et al., 2010, 2011).

Digenean trematodes have been the subject of numerous ultrastructural studies on spermatology. In fact, to our knowledge there are ultrastructural data of the spermatozoon for more than 75 species distributed among 45 families (Ndiaye et al., 2014). With respect to the Zoogonidae family and to the best of our knowledge, there is only one ultrastructural study on the sperm of *Diphterostomum brusinae* (Levron et al., 2004a).

The aim of the present study is to produce the first complete description of the ultrastructure of the spermatozoon of *Lecithostaphylus retroflexus* (Molin, 1859), contributing to the ultrastructural database concerning the Digenea. Our results were also compared with the available data on digenean spermatology, in particular, with those species belonging to the Microphalloidea

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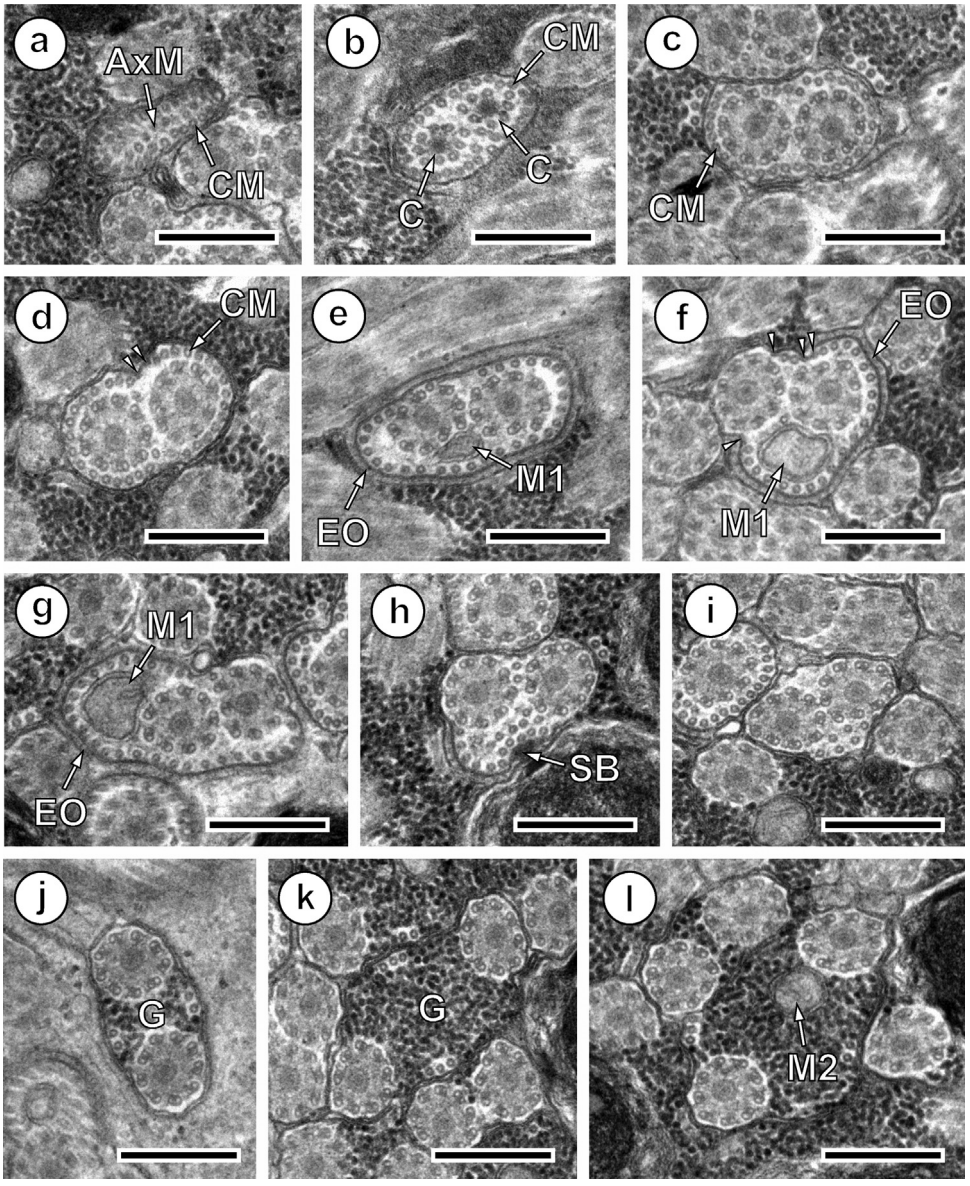

Fig. 1. Spermatozoon of *Lecithostaphylus retroflexus*: (a–d) cross-sections of region I showing the appearance of both axonemes; (e–h) cross-sections of the ornamented areas of region I; (i–k) cross-sections of region II showing the appearance of glycogen; (i) cross-section of posterior area of region II showing the second mitochondrion. Arrowheads: attachment zones; AxM, axonemal microtubules; C, centriole; CM, cortical microtubules; EO, external ornamentation of the plasma membrane; G, granules of glycogen; M1, first mitochondrion; M2, second mitochondrion; SB, spine-like bodies. Bars = 0.3 μm.

superfamily in order to select the possible criteria useful for phylogeny.

2. Materials and methods

Specimens of *L. retroflexus* (Molin, 1859) were collected from the digestive tract of *Belone belone gracilis* (Teleostei, Belonidae) caught from the Gulf of Gabès off Chebba (34°14′N, 11°06′E) (Tunisia).

The worms were isolated from their host. 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. They were then 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 obtained using a Reichert-Jung Ultracut-E ultramicrotome, placed on copper grids and double-stained with uranyl acetate and lead citrate according to Reynolds (1963) methodology. Finally, all stained grids were studied with a JEOL 1010 transmission electron microscope operated at 80 kV.

The Thiéry (1967) technique was used for glycogen localization. Gold grids with ultrathin sections 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 Milli-Q water.

3. Results

The observation of numerous ultrathin sections in the seminal vesicle of *L. retroflexus* allows the distinction of three different regions, I, II and III, from the anterior to the posterior spermatozoon extremities, considering the presence of different ultrastructural characteristics (Figs. 1–4). The mature spermatozoon of *L. retroflexus* exhibits the usual structures found in