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Ultrastructure of the spermatozoon of the digenean *Tergestia acanthocephala* (Stossich, 1887) (Gymnophalloidea: Fellodistomidae): An intestinal parasite of *Belone belone gracilis* (Pisces: Teleostei)

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ABSTRACT

The ultrastructural organization of the spermatozoon of the digenean *Tergestia acanthocephala* (Gymnophalloidea: Fellodistomidae) is described. Live digeneans were collected from *Belone belone gracilis* (Teleostei: Belonidae), caught off the Gulf of Gabès in Chebba (Tunisia). The mature spermatozoon of *T. acanthocephala* exhibits the general pattern described in numerous digeneans, characterized by the presence of two axonemes of the different length of the 9 + '1' pattern of the Trepaxonemata, a nucleus, two mitochondria, two bundles of parallel cortical microtubules, external ornamentation, spine-like bodies and granules of glycogen. Moreover, the morphology of the posterior spermatozoon extremity in *T. acanthocephala* corresponds to the fasciolidean type of Quilichini et al. (2010a).

1. Introduction

Over the last decades, it has been clearly demonstrated that the ultrastructural study of male gametes reveals significant characters which are considered as useful tools for understanding the phylogenetic relationships within the Platyhelminthes at several taxonomic levels (Justine, 1991, 1995; Levron et al., 2010; Quilichini et al., 2010a, 2011a). About seventy-five species only have been studied (Ndiaye et al., 2014), whereas the Digenea comprises at present about 150 recognized families containing nearly 2700 nominal genera and about 18,000 nominal species (Littlewood et al., 1999).

The present work describes the spermatozoon of *Tergestia acanthocephala* (Stossich, 1887), a parasite of *Belone belone gracilis*. Moreover, among the five families and 42 genera which constitute the superfamily Gymnophalloidea Odhner, 1905, to our knowledge, there are only a scarce ultrastructural data on the spermatozoon of two species, namely *Proctoeces maculatus* Fellodistomidae (Justine, 1995), *Parvatrema minutus* Gymnophallidae (Davies, 1975). The aim of the present study is to provide the first complete description of the ultrastructure of the spermatozoon of a Gymnophalloidea with the analysis of *T. acanthocephala* and to compare to that of other digeneans in order to highlight the possible criteria useful for phylogeny.

2. Materials and methods

Adult specimens of *T. acanthocephala* were removed alive from the digestive tract of *Belone belone gracilis* caught off the Gulf of Gabès in Chebba (34°14′ N, 11°06′ E) (Tunisia).

After dissection, digeneans were routinely processed for transmission electron microscopy examination. Therefore, 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,

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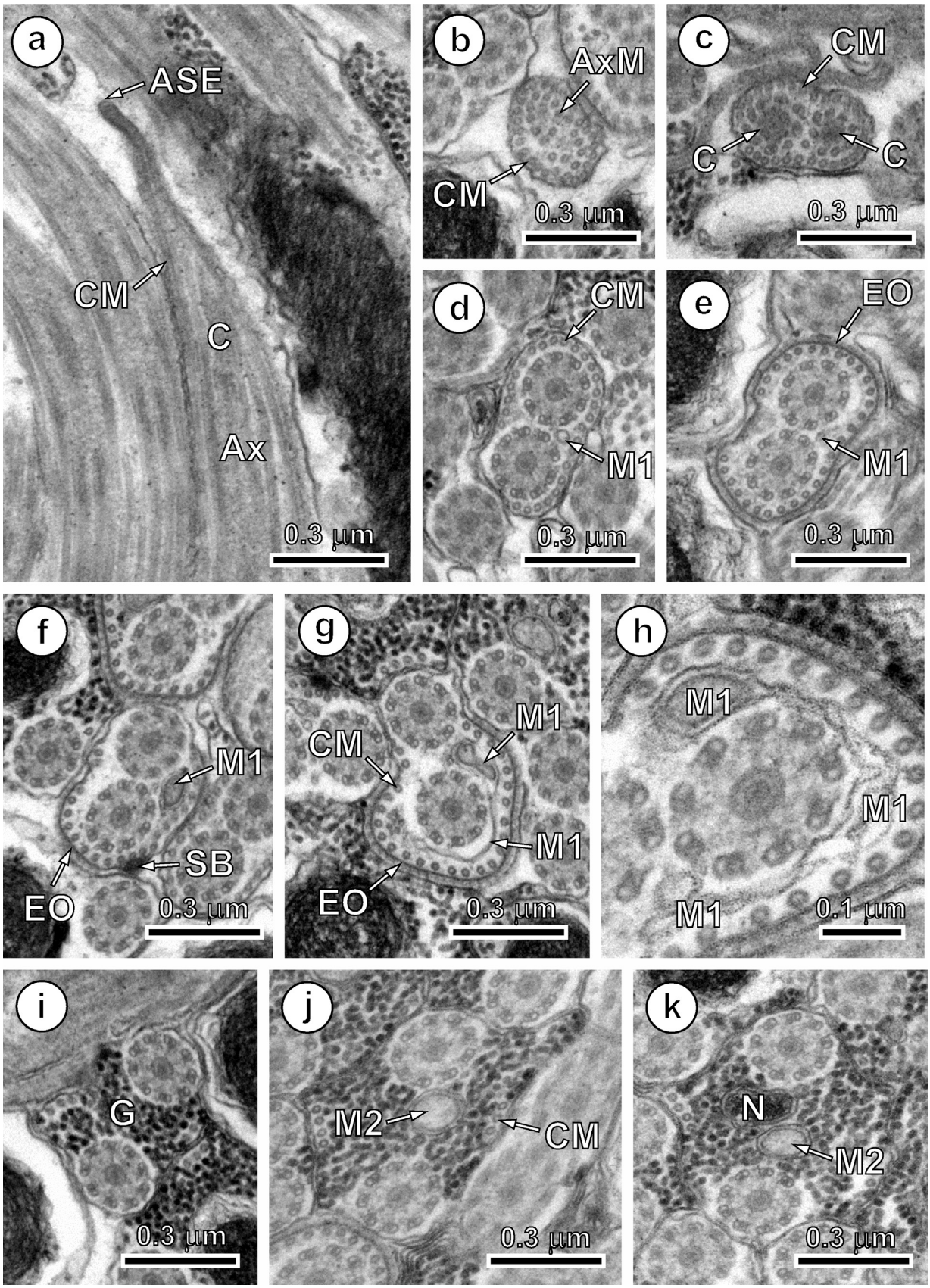

Fig. 1. Anterior (a–h), middle (i, j) and posterior (k) regions of the spermatozoon of *Tergestia acanthocephala*. (a) Longitudinal section of the anterior spermatozoon extremity. (b) Cross-section showing the axonemal and cortical microtubules. (c–g) Correlative cross-sections from centrioles to the mitochondrial area. (h) Detail showing the first mitochondrion encircling one of the axonemes. (i, j) Cross-sections showing the appearance of granules of glycogen and the second mitochondrion. (k) Cross-section of the anterior part of the nuclear region. ASE – anterior spermatozoon extremity, Ax – axoneme, AxM – axonemal microtubules, C – centriole, CM – cortical microtubules, EO – external ornamentation, G – granules of glycogen, M1 – first mitochondrion, M2 – second mitochondrion, N – nucleus, SB – spine-like bodies. Bars (a–g, i–k) = 0.3 μm, (h) = 0.1 μm.