Tissue and Cell 44 (2012) 15–21

Contents lists available at SciVerse ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

Sperm ultrastructure of the digenean *Aphallus tubarium* (Rudolphi, 1819) Poche, 1926 (Platyhelminthes, Cryptogonimidae) intestinal parasite of *Dentex dentex* (Pisces, Teleostei)

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ARTICLE INFO

Article history:
Received 26 September 2011
Received in revised form 7 October 2011
Accepted 10 October 2011
Available online 14 November 2011

Keywords:
Ultrastructure
Spermatozoon
Dentex dentex
Digenea
Cryptogonimidae
TEM

ABSTRACT

The ultrastructural organization of the spermatozoon of a cryptogonimid digenean, *Aphallus tubarium*, a parasite of *Dentex dentex*, is described. The spermatozoon possesses the elements found in other digeneans: two axonemes with 9+"1" pattern, a mitochondrion, a nucleus, cortical microtubules, external ornamentation and spine-like bodies. However, the mitochondrion appears as a cord with a bulge; this characteristic has never been described in other studied cryptogonimid and in other digeneans except in one lepocreadiid, *Holorchis micracanthum*. Likewise, the presence of a thin cytoplasm termination in the anterior part of the spermatozoon has never been pointed out in the cryptogonimids.

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1. Introduction

Digenan trematodes have been the subject of many ultrastructural surveys of spermatozoa (e.g., Gracenea et al., 1997; Baptista-Farias et al., 2001; Kacem et al., 2010).

These results have been used in different phylogenetic studies (e.g., Ehlers, 1986; Brooks et al., 1989; Justine, 1997, 2001; Rohde et al., 1993).

The present work concerns the ultrastructural survey of the spermatozoon in a digenean species, *Aphallus turbarium* (Rudolphi, 1819) Poche, 1926 (Cryptogonimidae), a parasite collected in common dentex *Dentex dentex* Linnaeus 1758, belonging to Sparidae family from Valinco gulf (Mediterranean sea). The knowledge on the parasite of *D. dentex* is interesting because this fish is: a highly valued table fish in the Mediterranean region and elsewhere in the tropics; one of the favorite fishes of Mediterrannean fishermen, for his combativeness and its succulent taste; and of great potential for mariculture.

The superfamily Opisthorchioidea Loos, 1899 comprises three families: Cryptogonimidae, Heterophyidae and Opisthorchiidae (Bray et al., 2008).

Hitherto only three species of the family Cryptogonimidae have been the subject of this kind of study: *Stemmatostoma pearsoni* [also designated as *Neochasmus* sp. (Jamieson and Daddow, 1982; Cribb, 1986)], *Anisocoelium capitellatum* (Ternengo et al., 2009) and *Adlardia novaecaledoniae* [also designated as *Siphoderina elongata* (Quilichini et al., 2009; Miller et al., 2009)].

This investigation aims to compare first, sperm ultrastructural features of *Aphallus tubarium* with the three others Cryptogonimidae species, and then with others Platyhelminthes in order to try to highlight criteria that can contribute to improve the phylogeny of Platyhelminthes.

2. Materials and methods

Adults specimens of *A. tubarium* (Rudolphi, 1819) Poche, 1926 were collected live from the common dentex *D. dentex* (Linné, 1758), caught in the Valinco gulf (Mediterranean sea). The worms were removed alive from the digestive tract of their hosts. The parasites were carefully dissected, the entire genital apparatus of males were removed and the testes and seminal vesicle isolated fixed in cold (4 °C) 2.5% glutaraldehyde in 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 (1969) and polymerised at 60 °C for 24 h. Ultrathin sections (60–90 nm) of testis and seminal vesicle were cut on an

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0040-8166/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tice.2011.10.001

ultramicrotome (Power tome PC, RMC Boeckeler®). The sections were placed on 300 and 200-mesh copper grids and stained with uranyl acetate and lead citrate according to Reynolds (1963).

Sections were examined on a Hitachi H-7650 transmission electron microscope, operating at an accelerating voltage of 80 kV, in the "Service d'Étude et de Recherche en Microscopie Électronique" of the University of Corsica (Corte, France).

3. Results

The study was carried out on mature spermatozoon of *A. tubarium* of the testis and seminal vesicle. Observations mainly showed the presence of a thin cytoplasm termination, two axoneme of 9 + "1" model of the Trepaxonematan, four attachments zones, one mitochondrion, cortical microtubules, external ornamentations of a plasma membrane, spine-like bodies, and a nucleus.

Observations of a great number of cross and longitudinal sections arranged in an antero-posterior sequence according to morphological information, allowed us to distinguish four regions (I–IV) with specific ultrastructural features acquired by TEM.

3.1. Region I (Figs. 1–9 and 27(I))

This region corresponds to the anterior extremity of the spermatozoon. In longitudinal section, a thin cytoplasm termination is reported (Fig. 1). Firstly, appears the first axoneme with 9 doublets of microtubules associated with the central core (Fig. 2). External and internal dynein arms are present along each doublet of microtubules (Fig. 2). Gradually, the second axoneme forms and the anterior part of the mitochondrion appears (Fig. 3). Then four attachment zones are observed and the two axonemes are completely formed (Fig. 4). In the middle of region I, cortical microtubules emerge and number gradually increases from 1 to 7 (Figs. 5–9). One cortical microtubule first appears on each side (Fig. 5). Then, two cortical microtubules occur on one side and one is present on the other side (Fig. 6). Number of cortical microtubule, respectively reaches two and three on each side (Figs. 7 and 8) and finally three on one side and four on the second one (Fig. 9).

3.2. Region II (Figs. 10–12 and 27(II))

This region is characterized by the presence of external ornamentations of the plasma membrane located on one side (Figs. 10–12) and associated with 8 cortical microtubules (Figs. 10 and 12). On the opposed side of the external ornamentations, only one cortical microtubule is counted. Spine-like bodies are also observed in this region and are always associated with external ornamentations (Figs. 11 and 12). Cortical microtubules occur in a semi-circle between two attachment zones. They are related to the external ornamentations and around mitochondrial elements (Fig. 12). In cross sections, there is a mitochondrial bulge and a mitochondrial cord which can be distinguish by its diameter (Figs. 10 and 12).

3.3. Region III (Figs. 13–17 and 27(III))

This region is characterized by the lack of external ornamentations and spine-like bodies. The cortical microtubules are organized in two fields (Figs. 13–16). The first axoneme extremity occurs in the middle part of this region and is associated to the increase of the mitochondrion diameter and the maximal number of cortical microtubules (12) counted in the entire spermatozoon (Fig. 15). At this spermatozoon level, the anterior extremity of the nucleus is also observed. The posterior part of this region is characterized by the posterior extremity of the mitochondrion and the decrease of the cortical microtubules number (Figs. 16 and 17). Four attachments zone are present in this region.

3.4. Region IV (Figs. 18–26 and 27(IV))

This region is the posterior part of the spermatozoon. The anterior part of this region is characterized by a reduction in number of cortical microtubules: 8 (Fig. 18), 7 (Fig. 19), 6 (Fig. 20), 5 (Fig. 21), 3 (Fig. 22) and 0 (Figs. 23–26). Four attachment zones are observed (Figs. 18–22) excepted in the posterior part where only two are present (Fig. 23). Finally this part, ends with the disorganization of the posterior extremity of the second axoneme from doublets: nine (Fig. 24), three (Fig. 25) and into one singlet (Fig. 26).

4. Discussion

The spermatozoon of *A. tubarium* exhibits an antero-lateral expansion, two axonemes of 9 + "1" model of the Trepaxonematan, four attachment zones, one mitochondrion, cortical microtubules, external ornamentations of a plasma membrane, spine-like bodies, and a nucleus.

4.1. The axonemes

There are two axonemes with the typical 9 + "1" pattern reported in the Trepaxonematan Platyhelminthes. Nine rays allow the junction between the central core and each outer doublet of microtubules. This structure has already been reported in all the digenean spermatozoa studied e.g., *Haematoloechus* (Justine and Mattei, 1982), *Aponurus laguncula* (Quilichini et al., 2010a), *Helicometra epinepheli* (Quilichini et al., 2011). In contrast, a distinctive pattern axoneme has been found in the genus *Didymozoon* which exhibits two axonemes of 9 + 0 type (Justine and Mattei, 1983). The presence of two axoneme points out the homogeneity of this criterion in the digeneans.

The length of the two axonemes, a determination character in phylogeny of digeneans, differs (Iomini and Justine, 1997). In the mature spermatozoon of *A. tubarium*, the anterior extremity of the first axoneme shows a shift, compared to the second one. The posterior extremity of the first axoneme seems to be more longer than that of the second one. As in most of the digeneans, the two axonemes of the spermatozoon extend for different distance anteriorly and posteriorly (Ternengo et al., 2009).

4.2. The external ornamentations, spine-like bodies and cortical microtubules

In *A. tubarium*, external ornamentations have been only reported in the region II of the spermatozoon. Commonly found in Digenean species, e.g., *Scraphiostommum palearcticum* (Ndiaye et al., 2002), *Disphterostomum brusinae* (Levron et al., 2004), *Troglotrema acutum* (Miquel et al., 2006), *Deropristis inflata* (Foata et al., 2007) *Heterolebes maculosus* (Quilichini et al., 2010b), *Cricocephalus albus* (Ndiaye et al., 2011), their localization along the spermatozoon varies according to the species. Quilichini et al. (2007) proposed a location classification of these elements on the basis of two groups, one with the ornamentation in the anterior extremity of the spermatozoon (group 1) and another one at a more posterior level and not around the anterior part of the two axonemes (group 2). According to this classification, *A. tubarium* could be class in the second group.

Spine-like bodies always associated to ornamentations have been observed. They are randomly organized and their number varies among the Cryptogonimidae (Table 1).

In *A. tubarium*, we clearly see that ornamentations are only present on the mitochondrial side and are always related to

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