

Comparative ultrastructural study of the spermatozoa of *Cotugnia polycantha* (Cestoda, Cyclophyllidea, Davaineidae), the intestinal parasites of pigeons (*Columba livia domestica*) and doves (*Streptopelia senegalensis*) from Egypt

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Abstract: The present study compares ultrastructure of the spermatozoa of *Cotugnia polycantha* recovered from the intestine of the two different host, *Columba livia domestica* and *Streptopelia senegalensis* from Egypt. The spermatozoa of *C. polycantha* of the two different host are filiform, tapered at the anterior extremity and lack mitochondria. The anterior extremity has an apical cone of electron dense material and two helicoidal thick cord crested-like body. The axoneme possesses the 9+1" pattern of microtubules and contains the peri-axonemal sheath. The cortical microtubules are spiraled along the whole length of the spermatozoon. The spermatozoon of *C. polycantha* of *C. livia* consists of five regions (I-V), while the other consists of four regions (I-IV). The cytoplasm contains numerous and large electron dense granules only in the region V in case of *C. polycantha* of *C. livia* but, these granules are also found in the regions I, II and IV in the spermatozoon of *C. polycantha* of *S. senegalensis*. The nucleus is a fine compact cord and envelops the central axoneme once or twice, interposes itself between the cortical microtubules in case of *C. polycantha* of *C. livia* which is different in that of *C. polycantha* of *S. senegalensis* in which the nucleus is coiled in a helix around the axoneme. The cytoplasm of the both spermatozoa are of a very electron dense material at the posterior extremity of each spermatozoon.

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1. Introduction

The Cestoda constitutes the major group of the Platyhelminthes that has been studied for the spermatozoa ultrastructure (Justine, 2003).

The ultrastructural characters of spermiogenesis and the spermatozoon of parasitic Platyhelminthes have been shown to be useful in the interpretation of the phylogenetic relationships within this group of parasites (Bâ and Marchand 1995; Hoberg *et al.*, 1997; Justine, 1998, 2001). The sperm ultrastructure provides an important taxonomic feature, which can be considered as a modern tool to differentiate between Cestode taxa. The families of Cyclophyllidea which have been subjected to the ultrastructural studies of the spermatozoon are the Anoplocephalidae (Bâ and Marchand 1994a and Ahmed *et al.*, 2008), Catenotaeniidae (Justine, 1995 and Hidalgo *et al.*, 2000), Dilepididae (Swiderski and Tkach, 1996a; Swiderski *et al.*, 2000 and Ahmed, 2004), Mesocostoidae (Miquel *et al.*, 1999), Dipylidiidae (Miquel and Marchand, 1997; Miquel *et al.*, 1998 and Ndiaye *et al.*, 2003a), Nematotaeniidae (Mokhtar- Maamouri and Azzouz-Draoui, 1990) and Taeniidae (Miquel *et al.*, 2000; Ndiaye *et al.*, 2003b and Wilms *et al.*, 2004), Paruterinidae (Yoneva *et al.*, 2009, 2010), Hymenolepididae (Bâ and

Marchand, 1998; Sweiderski and Tkach, 1996b and Ahmed and Lasheen, 2011)

Concerning the family Davaineidae, there are few ultrastructural studies on spermatozoon (Ba and Marchand, 1994b and Ba *et al.*, 2005). Therefore the present work aimed to describe the ultrastructure of the spermatozoa of *Cotugnia polycantha* from two different hostes *Columba livia* and *Streptopelia senegalensis* in Egypt.

2. Materials and Methods

Specimens of adult *Cotugnia polycantha* were collected from the small intestine of naturally infected pigeons (*Columba livia*) and doves (*Streptopelia senegalensis*) from Egypt. Worms were kept in 0.9% NaCl solution. Different portions of mature proglottids were dissected and fixed in 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.2 for 3hr, rinsed in the same buffer at pH 7.2, four times at 4°C, then postfixed in cold (4°C) 1% osmium tetroxide in the same buffer for 1hr. The fixed specimens were washed in 0.1 m sodium cacodylate buffer at pH 7.2 dehydrated in graded concentrations of ethanol and propylene oxide at room temperature and embedded in Epon. Ultrathin sections were cut on LKB ultramicrotome placed on copper grids and stained with uranyl acetate and

lead citrate. The grids were examined in a JEOL transmission electron microscope.

3. Results

Ultrastructural examination of testes of *C. polycantha* from *C. livia domestica* and *S. senegalensis* showed the following main structure of the spermatozoa:

Spermatozoon structure of *C. polycantha* from *C. livia*

The mature spermatozoon of *C. polycantha* is filiform and lacks mitochondria. It is tapered at the anterior end and shows the 9+1 axoneme pattern (Ax) of the platyhelminths which does not reach the posterior extremity of the spermatozoon. It could be divided into different regions (I-V), showing no marked morphological discontinuity between them but exhibited distinctive ultrastructural features.

Region I (Figs. 1, 2, 3 and 4)

Region I is capped by an apical cone (Ac) of electron-dense material had shown in (Fig. 1). The anterior axonemal extremity is situated immediately below the apical cone. The axoneme of the 9+1 pattern is in a central position and is surrounded by a thin layer of electron-lucent cytoplasm which contains a sheath of electron-dense material under the cortical microtubules (Fig. 2). These cortical microtubules (Cm) are spiralized forming a continuous layer of dense submembranous material around the cytoplasm.

Externally, the tubules are partially surrounded by two helicoidal thick cord of electron-dense material that forms a crested-like body (Cb) (Fig. 3 & 4).

Region II (Figs. 5&6)

Region II lacks the crested-like body and has the central axoneme with a periaxonemal sheath (Fig. 5). The axoneme is enveloped by a slightly electron-dense cytoplasmic layer and is surrounded by the twisted cortical microtubules that form a continuous layer of electron-dense and submembranous material (Fig. 6).

Region III (Figs. 7, 8, &9)

This region is characterized by the presence of a spiralized electron-dense nucleus (N) in close contact with the axoneme. The nucleus is a fine compact cord enveloping the central axoneme once or twice and interposes itself between the cortical microtubules (Fig. 7). These cortical microtubules are spiralized and are seen in cross sections (Fig. 8) and cross sections (Fig. 9) as a discontinuous layer of dense

submembranous material. The cytoplasm is electron-lucent.

Region IV (Figs. 10&11)

It is characterized by the presence of a central axoneme which is enveloped by a fine continuous sheath of electron-dense peri-axonemal material and a thick layer of electron-lucent cytoplasm. The cytoplasm is surrounded by the spiralized cortical microtubules forming a discontinuous layer of electron-dense and submembranous material. The cytoplasm is subdivided into several compartments by partitions of electron-dense material (intracytoplasmic proteinaceous walls) which join the fine peri-axonemal sheath to the spiralized cortical microtubules (Figs. 10&11).

Region V (Figs. 12,13,14.15&16)

This region is characterized by the presence of a large protein granules. The cytoplasm is also subdivided into several compartments by partitions of electron-dense material (Figs. 12&13). The spiralized cortical microtubules form a discontinuous layer of dense submembranous material (Figs. 14&15). At the posterior extremity of the spermatozoon, the axoneme, the peri-axonemal sheath, the intracytoplasmic walls and the cortical microtubules disappear, and the cytoplasm becomes very electron-dense (Fig. 16).

Spermatozoon structure of *C. polycantha* from *Streptoplia senegalensis*

Four different regions can be distinguished (I-IV) that showed no marked morphological discontinuity between them, but exhibited distinctive ultrastructural features.

Region I (Figs. 17,18,19&20)

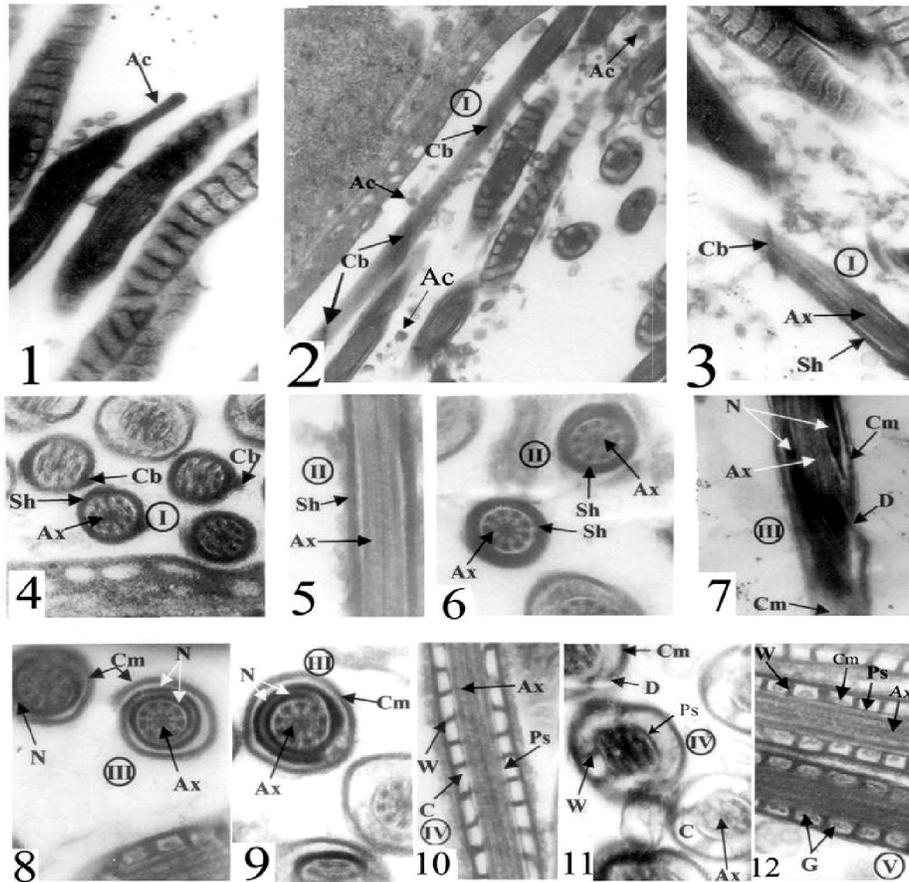
Region I is capped by an apical cone of electron-dense material. The axoneme of the 9+1 pattern is in a central position and is surrounded by a fine layer of electron-lucent cytoplasm (Figs. 17&18). The cortical microtubules are spiralized and form a continuous layer of dense submembranous material. They are accompanied on the outside by two helicoidal crested-like bodies. The cytoplasm exhibits small granules of electron-dense material forming a continuous layer under the cortical microtubules (Figs. 19&20).

Region II (Figs. 21, 22&23)

Region II lacks crested-like bodies and has the central axoneme enveloped by a layer of electron-lucent cytoplasm (Figs. 21). The cytoplasm contains dense material forming a peripheral granular layer which is discontinuous and situated under the cortical

microtubules. The cytoplasm is surrounded by the twisted cortical microtubules that form a

discontinuous layer of electron-dense and submembranous material (Figs. 22&23).



Figs.(1-16): Transmission electron micrographs of the spermatozoa of *C. polycantha* infecting *C. livia*.

(Fig. 1): The anterior extremity, showing the apical cone (Ac). (X: 15000).

(Fig. 2): Longitudinal sections, showing the crested-like bodies (Cb). Apical cone (Ac). (X: 10000).

(Fig. 3): Longitudinal sections, showing the crested-like bodies (Cb), the central axoneme (Ax) and a sheath of electron-dense material (Sh). (X: 12000).

(Fig. 4): Cross sections, showing the crested-like bodies (Cb), the central axoneme (Ax) and a sheath of electron-dense material (Sh). (X: 20000).

(Fig. 5): Longitudinal section, showing the central axoneme (Ax), sheath (Sh). (X: 20000).

(Fig. 6): Cross sections, showing the axoneme (Ax) sheath (Sh). (X: 25000).

(Fig. 7): Longitudinal section, showing the discontinuity (D) in the electron-dense layer of cortical microtubules (Cm). The nucleus (N), the axoneme (Ax). (X: 20000).

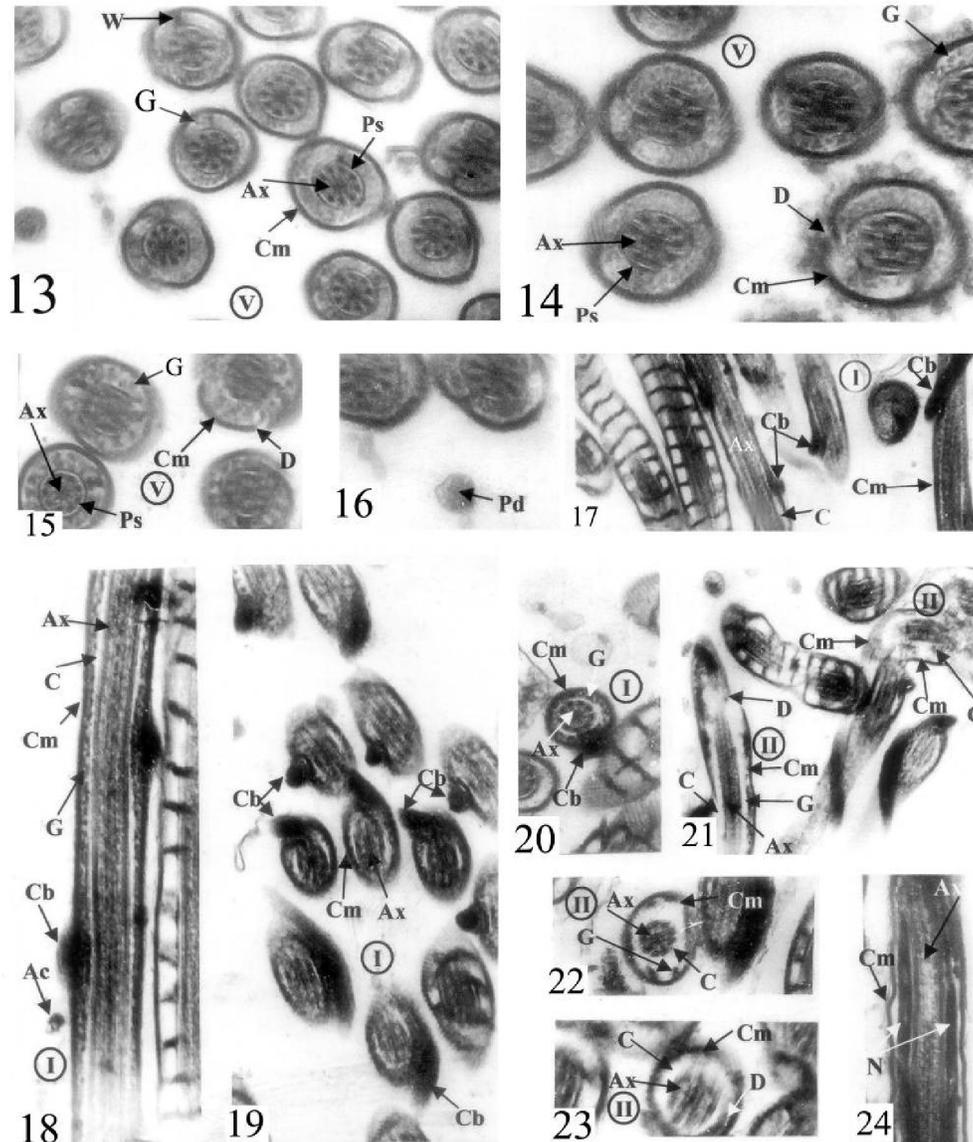
(Fig. 8): Cross sections, showing the nucleus (N) spiralled around the central axoneme (Ax). Cortical microtubules (Cm), discontinuity in the layer of cortical microtubules (D). (X: 25000).

(Fig. 9): Cross section, showing the nucleus (N), the axoneme (Ax), cortical microtubules (Cm), discontinuity in the layer of cortical microtubules (D). (X: 30000).

(Fig. 10): Longitudinal section, showing the axoneme (Ax), peri-axonemal sheath (Ps), lucent cytoplasm (C), intracytoplasmic walls (W), cortical microtubules (Cm). (X: 20000).

(Fig. 11): Cross section, showing the central axoneme (Ax), the peri-axonemal sheath (Ps), lucent cytoplasm (C), intracytoplasmic walls (W) and discontinuity (D) in the layer of cortical microtubules (Cm). (X: 20000).

(Fig. 12): Longitudinal sections, showing the central axoneme (Ax), the peri-axonemal sheath (Ps), intracytoplasmic walls (W), granules (G) and cortical microtubules (Cm). (X: 20000).



Figs. (13-16): Transmission electron micrographs of the spermatozoa of *C. polycantha* infecting *C. livia*.

(Fig. 13): Cross sections, showing the central axoneme (Ax), the peri-axonemal sheath (Ps), cortical microtubules (Cm) and intracytoplasmic walls (W). (X: 20000).

(Fig. 14): Cross sections, showing the discontinuity (D) in the layer of cortical microtubules (Cm). The axoneme (Ax), the peri-axonemal sheath (Ps), granules (G). (X: 25000).

(Fig. 15): Cross sections, showing the discontinuity (D) in the layer of cortical microtubules (Cm). The axoneme (Ax), the peri-axonemal sheath (Ps), granules (G). (X: 20000).

(Fig. 16): Cross section, showing the posterior electron-dense material (Pd). (X: 25000).

Figs. (17-24): Transmission electron micrographs of the spermatozoa of *C. polycantha* infecting *S. senegalensis*.

(Fig. 17): Longitudinal sections, showing the crested-like bodies (Cb), the central axoneme (Ax), the cortical microtubules (Cm) and lucent cytoplasm (C). (X: 12000).

(Fig. 18): Longitudinal section, showing apical cone (Ac), the crested-like bodies (Cb), the central axoneme (Ax), the cortical microtubules (Cm), lucent cytoplasm (C) and continuous layer of granules (G). (X: 25000).

(Fig. 19): Oblique sections, showing the central axoneme (Ax), the crested-like bodies (Cb) and the cortical microtubules (Cm). (X: 20000).

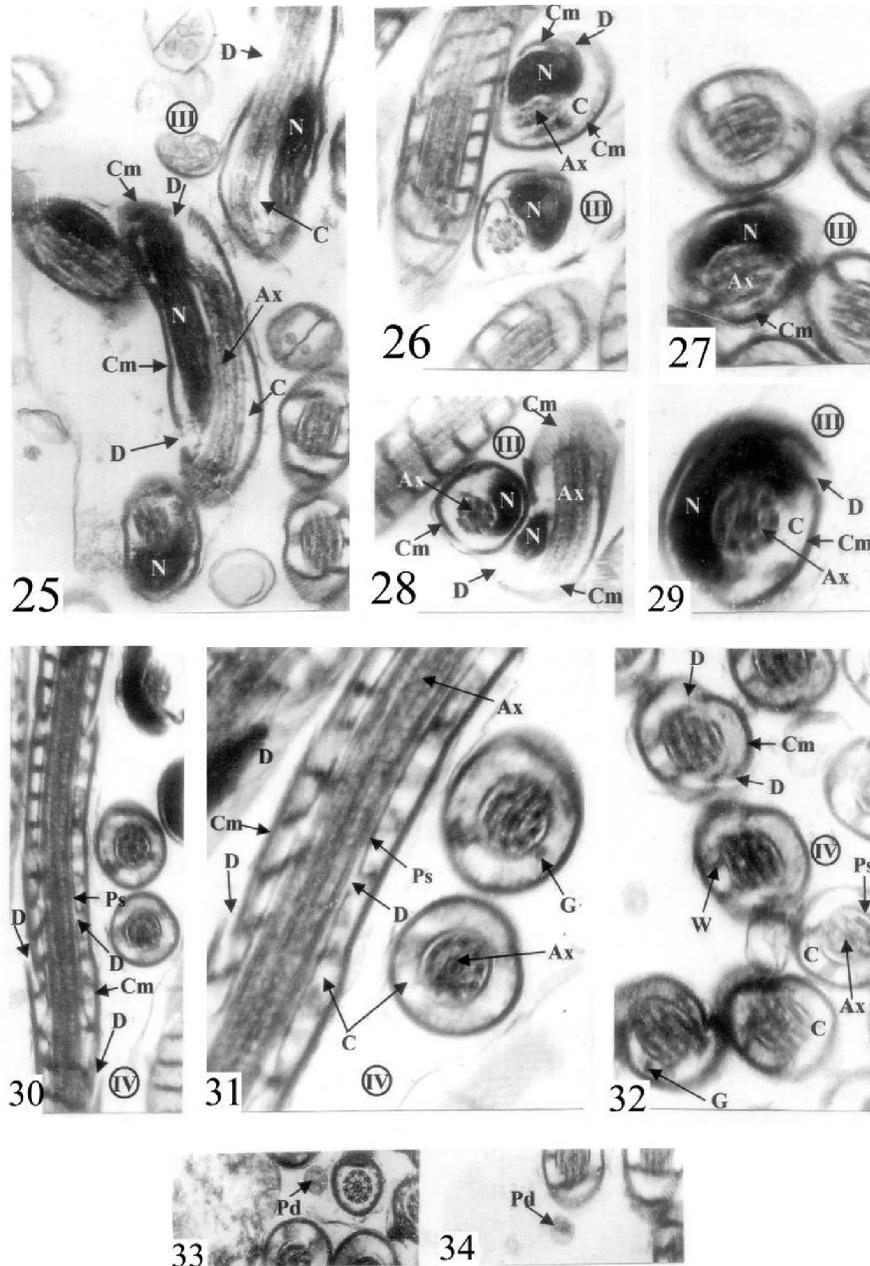
(Fig. 20): Cross section, showing the axoneme (Ax), the cortical microtubules (Cm), lucent cytoplasm (C), the crested-like bodies (Cb) and layer of granules (G). (X: 40000).

(Fig. 21): Longitudinal sections, showing the central axoneme (Ax), the lucent cytoplasm (C), the discontinuity (D) in layer of cortical microtubules (Cm) and a discontinuous layer of granules (G). (X: 12000).

(Fig. 22): Cross section, showing the central axoneme (Ax), the cortical microtubules (Cm), the lucent cytoplasm (C) and granules (G). (X: 20000)

(Fig. 23): Cross section, showing the discontinuity (D) in the layer of cortical microtubules (Cm). The axoneme (Ax), lucent cytoplasm (C). (X: 30000).

(Fig. 24): Longitudinal section, showing the nucleus (N) around the axoneme (Ax). Cortical microtubules (Cm). (X: 20000).



Figs. (25-34): Transmission electron micrographs of the spermatozoa of *C. polycantha* infecting *S. senegalensis*.

(Fig. 25): Oblique section, showing the elongate portions of nucleus (N) alternating on both sides of the axoneme (Ax) and the discontinuity (D) in the layer of cortical microtubules (Cm). Lucent cytoplasm (C). (X: 20000).

(Fig. 26): Cross section, showing the nucleus (N), the axoneme (Ax), lucent cytoplasm (C) and the discontinuity (D) in the layer of cortical microtubules (Cm). (X: 20000).

(Fig. 27): Cross section, showing the nucleus (N), the axoneme (Ax) and cortical microtubules (Cm). (X: 30000).

(Fig. 28): Cross and oblique sections, showing the nucleus (N), the axoneme (Ax) and the discontinuity (D) in the layer of cortical microtubules (Cm). (X: 20000).

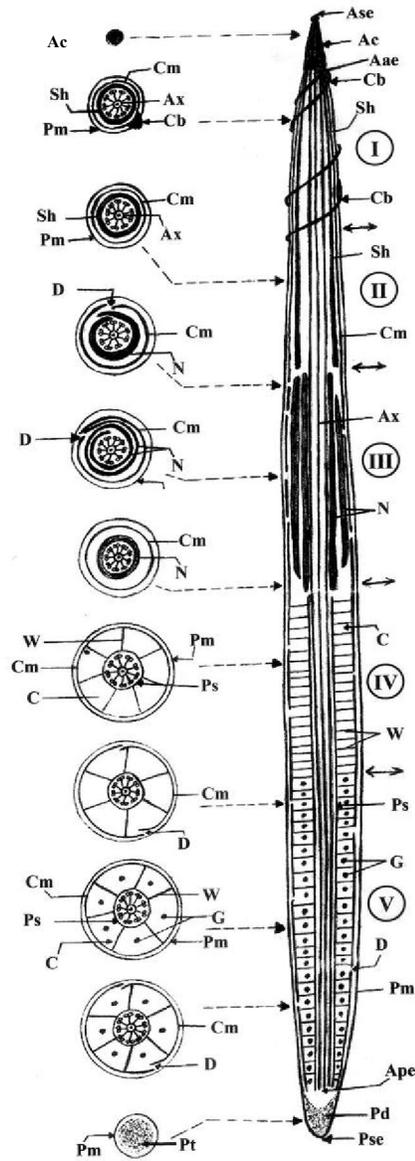
(Fig. 29): Cross section, showing the nucleus (N), the axoneme (Ax), lucent cytoplasm (C) and the discontinuity (D) in the layer of cortical microtubules (Cm). (X: 40000).

(Fig. 30): Longitudinal section, showing the discontinuity (D) in the layer of cortical microtubules (Cm) and the peri-axonemal sheath (Ps). (X: 20000).

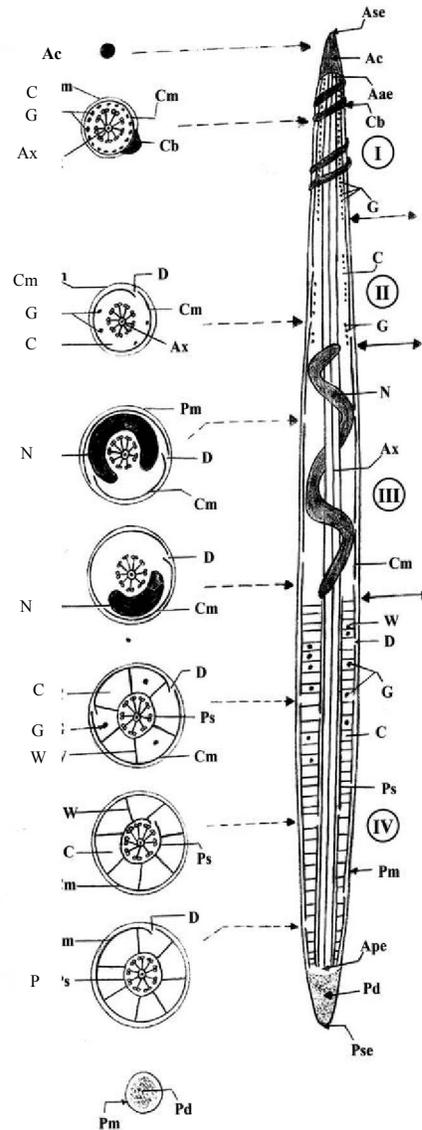
(Fig. 31): Longitudinal and cross sections, showing the granules (G) and the discontinuity (D) in the layer of cortical microtubules (Cm) and the peri-axonemal sheath (Ps). The axoneme (Ax), lucent cytoplasm (C). (X: 40000).

(Fig. 32): Cross sections, showing the discontinuity (D) in the layer of cortical microtubules (Cm), the axoneme (Ax), the peri-axonemal sheath (Ps), lucent cytoplasm (C), granules (G) and intracytoplasmic walls (W). (X: 30000).

(Figs. 33 and 34): Cross sections, showing the posterior electron-dense material (Pd). (X: 20000).



(Fig.35)



(Fig.36)

(Fig. 35): Attempted reconstruction of the mature spermatozoon of *C. polycantha* of *C. livia* in which distinguish five regions (I-V) from anterior to posterior. Aae = axonemal anterior extremity; Ac = apical cone; Ape = axonemal posterior extremity; Ase = anterior spermatozoon extremity; Ax = axoneme; C = lucent cytoplasm; Cb = crested-like bodies; Cm = cortical microtubules; D = discontinuity in the layer of cortical microtubules; G = granules; N = nucleus; Pd = posterior electron-dense material; Pm = plasma membrane; Ps = peri-axonemal sheath; Pse = posterior spermatozoon extremity; Sh = sheath of electron-dense material; W = intracytoplasmic walls.

(Fig. 36): Attempted reconstruction of the mature spermatozoon of *S. senegalensis* in which distinguish four regions (I-IV) from anterior to posterior. Aae = axonemal anterior extremity; Ac = apical cone; Ape = axonemal posterior extremity; Ase = anterior spermatozoon extremity; Ax = axoneme; C = lucent cytoplasm; Cb = crested-like bodies; Cm = cortical microtubules; D = discontinuity in the layer of cortical microtubules; G = granules; N = nucleus; Pd = posterior electron-dense material; Pm = plasma membrane; Ps = peri-axonemal sheath; Pse = posterior spermatozoon extremity; W = intracytoplasmic walls.

Region III (Figs. 24, 25, 26, 27, 28 & 29)

This region is characterized by the presence of a spiralized electron-dense nucleus in close contact with the axoneme. It appears as electron-dense elongate portions alternating on both sides of the axoneme, which suggests its helical course around the axoneme (Figs. 24 & 25). The nucleus is kidney to horseshoe-shaped in cross section (Figs. 26 & 27). The axoneme is located centrally and is moderately displaced by the presence of the nucleus (Figs. 28 & 29). The axoneme is surrounded by a layer of electron-lucent cytoplasm. The latter is enveloped by the spiralled cortical microtubules. These microtubules form a discontinuous layer of dense submembranous material (Figs. 28 & 29).

Region IV (Figs. 30, 31, 32, 33 & 34)

This region has the central axoneme which is surrounded by a fine discontinuous sheath of electron-dense material (a peri-axonemal sheath) and a thick layer of electron-lucent cytoplasm with a few granules (Figs. 30, 31 & 32). Cytoplasm is subdivided into several compartments by partitions of electron-dense material (intracytoplasmic proteinaceous walls). These cortical microtubules are spiralized and form a discontinuous layer of electron-dense and submembranous material (Fig. 32). At the posterior extremity of the spermatozoon, the axoneme, the peri-axonemal sheath, the intracytoplasmic partitions and the cortical microtubules disappear, and the cytoplasm which is electron-lucent, becomes very electron-dense (Figs. 33 & 34).

4. Discussion

The mature spermatozoon of *C. polycantha* in the present work does not differ significantly from the general pattern known for the one-axoneme type spermatozoa of cestodes (Justine, 1991). It is elongated, with no distinct separation between its basic parts and has a single axoneme of the 9+1 pattern and does not reach the posterior extremity of the spermatozoon. A single axoneme structure is considered apomorphic (Justine, 1998). The spermatozoon does not contain mitochondria. The absence of mitochondria in the mature spermatozoon is an obvious synapomorphy for the Eucestoda (Brooks and McLennan, 1993).

The apical electron-dense cone is found at the anterior extremity of the spermatozoon, the other posterior extremity, also sometimes exhibits an electron-dense structure in all Cyclophyllidea (Justine, 1998). As shown in the present study, the anterior extremity of the spermatozoon of *C. polycantha* from the both hosts has an apical cone of

electron-dense material, the other posterior extremity also exhibits an electron-dense structure.

The presence of the crested-like body (or bodies) in Eucestoda spermatozoa always marks the anterior extremity of the spermatozoon. This structure was considered a derived character as it is not known in other parasitic Platyhelminthes (Justine, 1998). It is also considered a synapomorphy for the Eucestoda (Justine, 1998). The crested-like bodies in the spermatozoon of *Killigrewia delafondi* of *Streptoplia senegalensis* are absent (Ahmed, et al., 2008). While, in the present study, the spermatozoon of *C. polycantha* of the both hosts exhibits two crested like bodies, as in *Paranoplocephala omphalodes* (Miquel and Marchand, 1998a), *Anoplocephaloides dentata* (Miquel and Marchand, 1998b), *Gallegooides arfaai* (Miquel et al., 2004) and *Mosgovoyia ctenoides* (Eira et al., 2006). While, a single crested-like body has been described in *Anomotaenia dehiscens* (Ahmed, 2004), *Anonchotaenia globate* (Yoneva, et al., 2010) and *Rodentolepis nana* (Ahmed and Lasheen, 2011).

In the present study, the spermatozoon of *C. polycantha* of *C. livia* and *S. senegalensis* has a central axoneme surrounded by a layer of electron-lucent cytoplasm and enveloped by a peri-axonemal sheath in regions IV and V in case of *C. polycantha* of *C. livia*, while in case of *C. polycantha* of *S. senegalensis* the peri-axonemal sheath enveloped the axoneme in region IV. Also, the regions IV and V of *C. polycantha* of *C. livia* and region IV of *C. polycantha* of *S. senegalensis* are subdivided into several compartments by intracytoplasmic walls. Similar results were observed in *Raillietina (Raillietina) tunetensis* (Ba and Marchand, 1994b); *Taenia parva* (Ndiaye et al., 2003a); *T. crassiceps strobilae* (Willms et al., 2004) and *Rodentolepis nana* (Ahmed and Lasheen, 2011).

On the other hand, the axoneme of the mature spermatozoon lacks a peri-axonemal sheath and is surrounded by a layer of slightly electron-dense cytoplasm, as in *Dipylidium caninum* (Miquel and Marchand, 1997), *Vampirolepis microstoma* (Ba and Marchand, 1998), *Sudarifeovina taterae* (Ba et al., 2000) and *K. delafondi* (Ahmed et al 2008).

The nucleus of the spermatozoon of most of the Cyclophyllidea is in close contact with the axoneme and is coiled around it (Ba and Marchand, 1995). However, in *Nematotaenia chantalae* (Mokhtar-Maamouri and Azzouz-Draoui, 1990), and *A. centripunctata* (Ba and Marchand, 1994c) the nucleus interposed between the cortical microtubules, while, in *V. microstoma* (Ba and Marchand, 1998), *Catenotania pusilla* (Hidalgo et al., 2000), *P. reynoldsae*, *R. (Raillietina) baeri* (Ba et al., 2005),

Mosgovoyia ctenoides (Eira *et al.*, 2006), it shows the typical spiralled pattern. The nucleus is a compact cord of electron-dense material coiled in a spiral around the axoneme and exhibits a crescent shape or horseshoe-shaped in cross sections.

In the present work, the nucleus of *C. polycantha* of *C. livia* is a fine compact cord enveloping the axoneme once or twice, thus in cross sections, the nucleus forms one or two coils around the axoneme (spiral), as in *R. (Raailletina) tunetensis* (Ba and Marchand, 1994b) and *K. delafondi* (Ahmed *et al.*, 2008) On the other hand, the nucleus of *C. polycantha* of *S. senegalensis* shows the typical spiralled pattern as in the most of the cyclophyllidean cestodes.

The cortical microtubules of the spermatozoa of all the Cyclophyllidea are spiralized (Miquel and Marchand, 1998a,b; Hidalgo *et al.*, 2000; Ndiaye *et al.*, 2003a,b; Willms *et al.*, 2004; Eira *et al.*, 2006; Ahmed, 2004; Ahmed *et al.*, 2008 ; Ahmed and Lasheen 2011). In the present study, the cortical microtubules of the spermatozoon of *C. polycantha* of the both hosts *C. livia* and *S. senegalensis* are spiralized to the spermatozoon axis throughout their length and end before the posterior extremity of the spermatozoon. Also, the cortical microtubules of the spermatozoon of *C. polycantha* of the both hosts form a discontinuous layer of electron-dense and submembranous material. On the other hand, the cortical microtubules of the spermatozoon of *K. delafondi* (Ahmed *et al.*, 2008) and (Ahmed and Lasheen 2011) remain spiralized along its whole length and reach the posterior end of the spermatozoon and form a continuous layer of electron-dense and submembranous material.

Granular electron-dense material in the cytoplasm of the spermatozoa of the cyclophyllidean cestodes has been described in the several species as in *P. omphalodes* (Miquel and Marchand, 1998a), *A. dentata* (Miquel and Marchand, 1998b), *S. taterae* (Ba *et al.*, 2000), *Anomotaenia dehiscense* (Ahmed, 2004), *M. ctenoides* (Eira *et al.*, 2006), *K. delafondi* (Ahmed *et al.*, 2008) and (Ahmed and Lasheen 2011) In the present study, the spermatozoon of *C. polycantha* of *C. livia* contains large granules in region V only, while in *C. polycantha* spermatozoon of *S. senegalensis* the cytoplasm exhibits small granules of electron-dense material forming a continuous layer in region I and a discontinuous layer in region II situated under the cortical microtubules. Also, region IV contains a few granules. On the other hand, the spermatozoon of *K. delafondi* (Ahmed *et al.*, 2008) contains numerous and large granules of electron-dense material in regions II, III, IV and V.

The present investigation shows the differences between the spermatozoon of *C. polycantha* from the

two different hostes, *C. livia* and *S. senegalensis* as follows:

1- The spermatozoon of *C. polycantha* of *C. livia* consists of five regions (I-V) while the other consists of four regions (I-IV).

2- The cytoplasm contains numerous and large electron dense granules only in the region (V) in case of *C. polycantha* of *C. livia* but, these granules are found in the region(I, II and IV) in the spermatozoon of *C. polycantha* of *S. senegalensis* .

3- The nucleus is a fine compact cord and envelops the central axoneme once or twice, interposes itself between the cortical microtubules in case of *C. polycantha* of *C. livia* which differs from that of *C. polycantha* of *S. senegalensis* in which, the nucleus is coiled in a helix around the axoneme.

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