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ORIGINAL PAPER

Spermiogenesis and sperm ultrastructure of *Cotylophoron cotylophorum* (Trematoda, Digenea, Paramphistomidae), a parasite of *Bos taurus* in Senegal

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Abstract Ultrastructure of spermiogenesis and mature spermatozoon of *Cotylophoron cotylophorum* are described with transmission electron microscopy. The description gives evidence of some characteristics of this digenean. The intercentriolar body situated between two striated roots and two centrioles exhibits a symmetric plane. The external bands of this intercentriolar body are formed of a line of granules. During spermiogenesis, a flagellar rotation of 90° is described. The old spermatid does not present external ornamentations. The spermatozoon is characterized, in its anterior part, by the presence of a lateral expansion exhibiting a spinelike body. External ornamentations of the plasmic membrane are only present at the level of the ventral field of cortical microtubules. The posterior end of this spermatozoon exhibits a nucleus surrounded by a plasmic membrane without cortical microtubules, but exhibiting a small lateral expansion.

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Introduction

Ultrastructural studies of spermiogenesis and the spermatozoon of Paramphistomidae are still incomplete. In this family which counts about ten genera and tens of species, only four species have been studied in electronic microscopy. These are *Ceylonocotyle scoliocoelium* (Li and Wang 1997), *Basidiodiscus ectorchus* (Ashour et al. 2007), *Paramphistomum microbothrium* (Seck et al. 2007), and *Sardonia sudanensis* (Ashour et al. 2007). According to these authors, spermiogenesis of these species is similar to that of other trematodes.

The aim of the present study is to elucidate the characteristics of spermiogenesis and sperm ultrastructure of *Cotylophoron cotylophorum* (Paramphistomidae).

Materials and methods

The specimens of *C. cotylophorum* were gathered alive from the rumen of *Bos taurus* (Ndama cattle) at the slaughterhouse of Kolda (area located in southern Senegal). After extraction, the trematodes were kept in a 0.9% NaCl solution. The adult specimens were fixed for 24 h at 4°C with 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer at pH 7.2, postfixed for 1 h with 1% osmium tetroxide in the same buffer, then dehydrated with ethanol and propylene oxide before being embedded in Epon. Ultrathin sections (70–90 nm in thickness) were cut on a LBK 8800A Ultrome III ultramicrotome, and then stained with uranyl acetate and lead citrate according to Reynolds (1963). They were examined in a Hitachi H-600 electron microscope at 75 kV.

Results

Spermiogenesis

Spermiogenesis of *C. cotylophorum* starts with the formation of a differentiation zone. Arched membranes delimit this zone, which is bordered by cortical microtubules. The young spermatid contains a large nucleus, surrounded by cytoplasm containing the mitochondria, two centrioles and the intercentriolar body (Fig. 1).

In cross section, this zone shows an almost circular form, contains a nucleus, two centrioles situated in the same level, separated by the intercentriolar body and associated with two striated roots (Figs. 2, 3 and 12). The development of this differentiation zone is marked by the formation of a median cytoplasmic process (Figs. 4, 6 and 12).

The intercentriolar body presents a symmetry organization. On both sides of a fine central band, there are three thicker dark bands separated by clear spaces. The two external dark bands are made up of a line of granules (Figs. 3 and 12).

Each centriole develops a flagellum (Figs. 5 and 12). The flagella undergo a 90° rotation and become parallel to the median cytoplasmic process (Figs. 6 and 12).

The nucleus and the mitochondria migrate inside the median cytoplasmic process (Figs. 4, 6 and 12). Cross-sections of the cytoplasmic process show, before the fusion of the flagella, four attachment zones and two fields of cortical microtubules (Figs. 7 and 12). The flagella fuse, one after the other, with the median cytoplasmic process. This fusion is proximo-distal. After the fusion, the two flagella become parallel inside the median cytoplasmic process (Figs. 8 and 12).

At the end of spermiogenesis, the nucleus finishes its migration as it reaches the distal end of the spermatid, but the mitochondrion terminates its migration when it reaches the middle of the differentiation zone. So, depending on the level of the sections in mature spermatid, one can observe two axonemes, a plasmic membrane bordered by cortical microtubules, the presence or absence of a mitochondrion and a nucleus with electron-lucent chromatin (Figs. 9, 10, 11 and 12). The mature spermatid is detached from the residual cytoplasm by constriction of the arched membranes.

Spermatozoon

Ultrastructural study of the spermatozoon of *C. cotylophorum*, has allowed to distinguish six regions from anterior to posterior extremity.

Region I

It is the anterior extremity of the spermatozoon. It shows two axonemes of the 9+“1” pattern and a lateral expansion exhibiting a spinelike body. The most anterior part of the spermatozoon presents only one axoneme. The second axoneme then appears and is followed by the lateral expansion. Numerous cortical microtubules can be observed on the inner side of the plasmic membrane, starting at the same level of the second axoneme. The plasmic membrane presents external ornamentations only on its ventral field (Figs. 13, 14, 15, 16 and 25).

Region II

Cross-section of this region shows two axonemes and two distinct fields of cortical microtubules: one dorsal and the other ventral. At this level, the spinelike body has disappeared. This region is always characterized by the presence of external ornamentations on the plasmic membrane of the ventral field (Figs. 17 and 25).

Region III

It is characterized by the appearance of a mitochondrion between the two axonemes and the diminution of the number of cortical microtubules. External ornamentations of the ventral field have disappeared (Figs. 18 and 25).

Region IV

The appearance of the nucleus in the ventral field characterizes this region. The two axonemes are always separated by the mitochondrion. The cortical microtubules are still present, but in a reduced number (Figs. 19 and 25).

Region V

The absence of the mitochondrion characterizes this region. The nucleus is larger. A few cortical microtubules underlying the plasmic membrane can be observed (Figs. 20, 21 and 25).

Region VI

This region corresponds to the posterior end of the gamete. The disorganization and the disappearance of the first

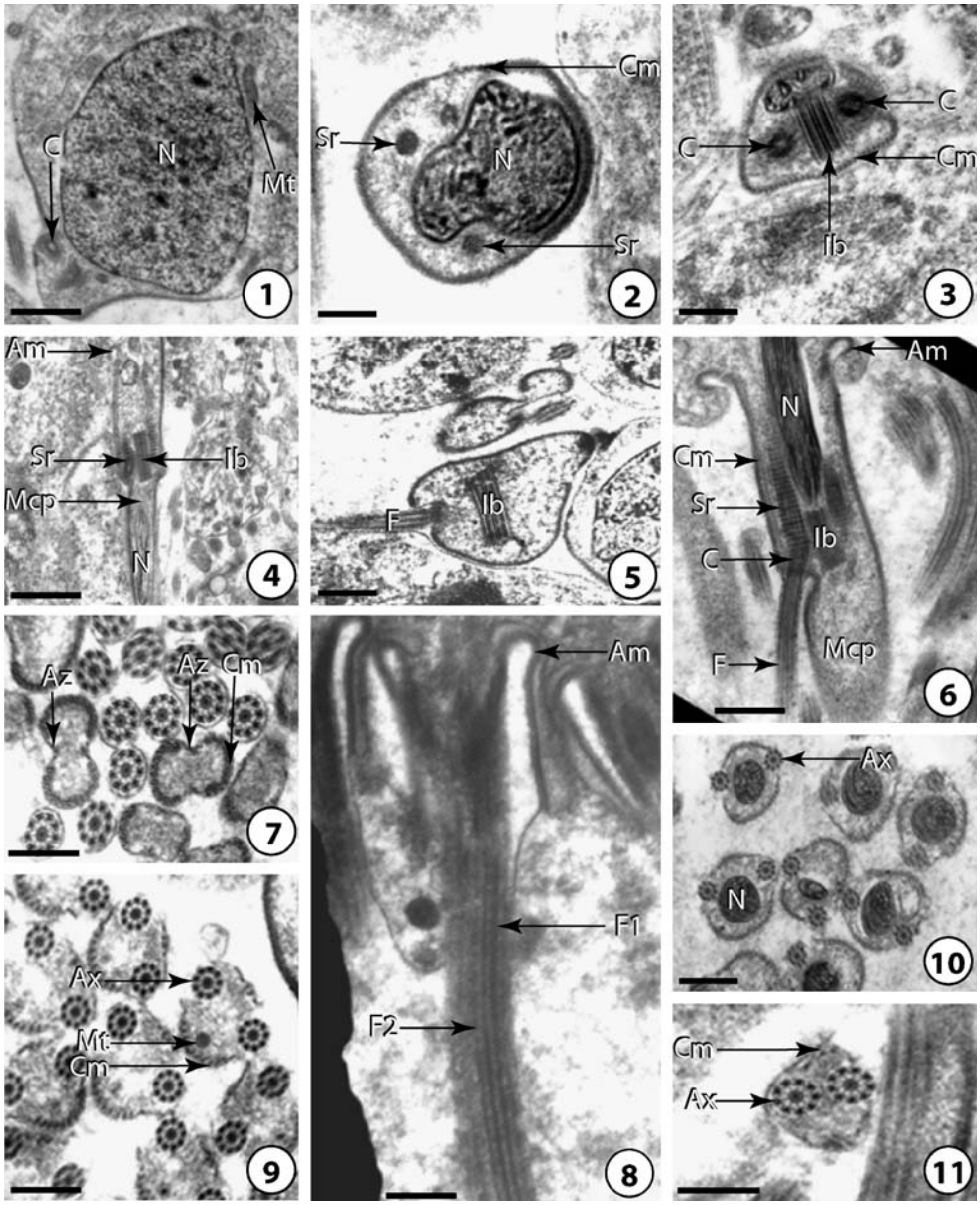

Fig. 1 Spermiogenesis of *Cotylophoron cotylophorum*. Longitudinal section of a differentiation zone showing a nucleus (*N*), mitochondrion (*Mt*) and centriole (*C*; *bar*=0.8 µm)

Fig. 2 Spermiogenesis of *Cotylophoron cotylophorum*. Cross-section of a differentiation zone showing the nucleus (*N*), two striated rootlets (*Sr*) and cortical microtubules (*Cm*; *bar*=0.4 µm)

Fig. 3 Spermiogenesis of *Cotylophoron cotylophorum*. Cross-section of a differentiation zone showing a nucleus, cortical microtubules (*Cm*) and the intercentriolar body (*Ib*) between two centrioles (*C*; *bar*=0.5 µm)

Fig. 4 Spermiogenesis of *Cotylophoron cotylophorum*. Longitudinal section of a differentiation zone showing arched membranes (*Am*), striated rootlets (*Sr*) and intercentriolar body (*Ib*). The nucleus (*N*) has begun its migration in the median cytoplasmic process (*Mcp*; *bar*=1 µm)

Fig. 5 Spermiogenesis of *Cotylophoron cotylophorum*. Cross-section of a spermatid with an intercentriolar body (*Ib*) and a flagellum (*F*) forming an angle of 90° (*bar*=0.5 µm)

Fig. 6 Spermiogenesis of *Cotylophoron cotylophorum*. Longitudinal section of differentiation zone at the stage of the flagellar rotation (*bar*=0.8 µm). *Am* Arched membranes, *C* Centriole, *F* Flagellum, Median cytoplasmic process (*Mcp*), *N* Nucleus, *Sr* Striated rootlets (*bar*=0.4 µm)

Fig. 7 Spermiogenesis of *Cotylophoron cotylophorum*. Cross-section of a median cytoplasmic process showing cortical microtubules (*Cm*) and attachment zones (*Az*; *bar*=0.4 µm)

Fig. 8 Spermiogenesis of *Cotylophoron cotylophorum*. Longitudinal section showing both flagella (*F*) after fusion and which becomes parallel. *Am* Arched membranes (*bar*=1 µm)

Fig. 9 Spermiogenesis of *Cotylophoron cotylophorum*. Cross-section of a spermatid showing a mitochondrion (*Mt*), both axonemes (*Ax*) and cortical microtubules (*Cm*). (*bar*=0.4 µm)

Fig. 10 Spermiogenesis of *Cotylophoron cotylophorum*. Cross-section of a spermatid with two axonemes (*Ax*), a nucleus (*N*) and cortical microtubules. (*bar*=0.5 µm)

Fig. 11 Spermiogenesis of *Cotylophoron cotylophorum*. Cross-section of an old spermatid with two axonemes (*Ax*) and cortical microtubules (*Cm*). (*bar*=0.4 µm)

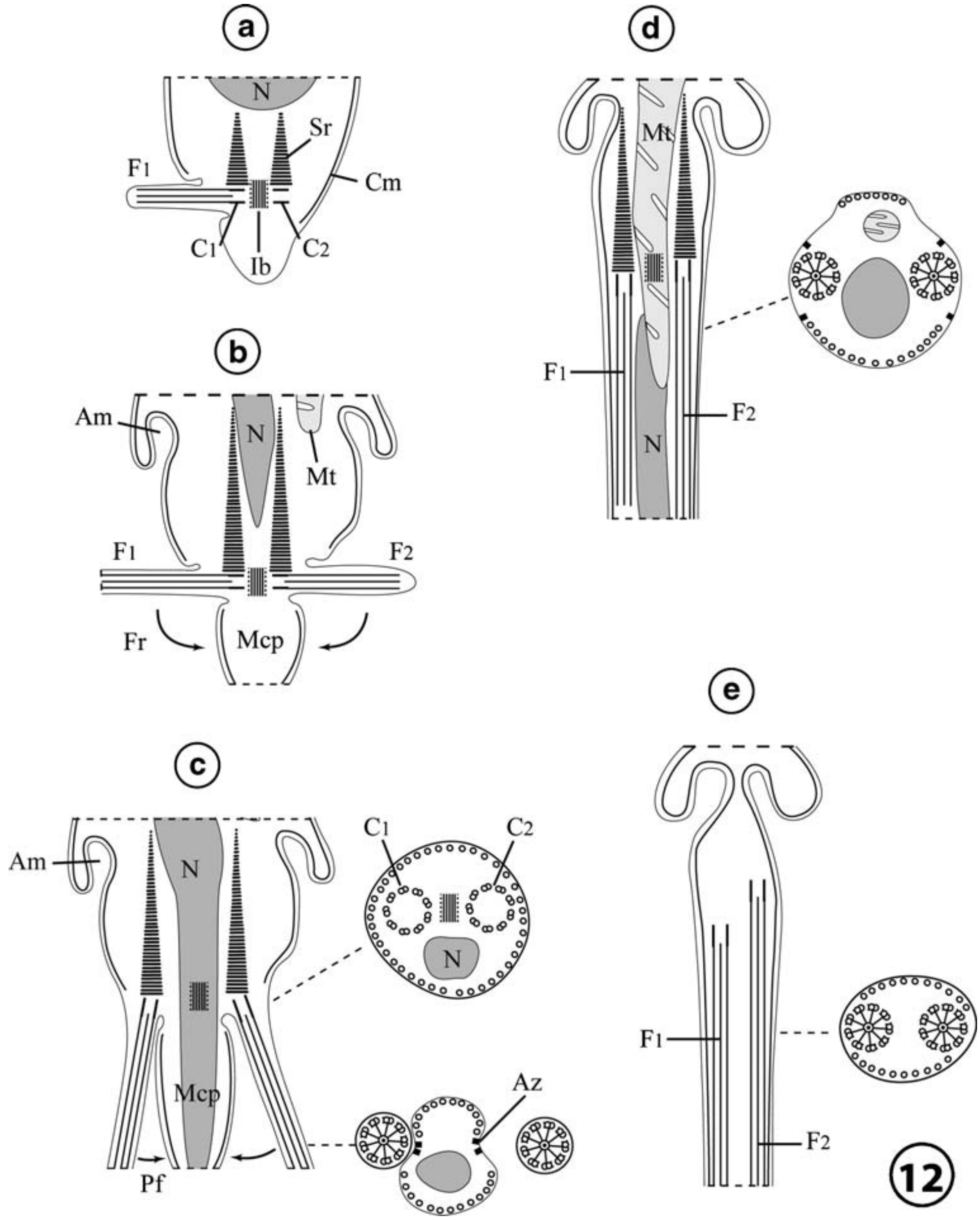

Fig. 12 Diagram showing the main stages of spermiogenesis of *Cotylophoron cotylophorum*. *Am* Arched membrane, *Az* Attachment zone, *C1* Centriole 1, *C2* Centriole 2, *Cm* Cortical microtubules, *F1* Flagellum 1, *F2* Flagellum 2, *Fr* Rotation flagellar, *Ib* Intercentriolar body, *Mcp* Median cytoplasmic process, *Mt* Mitochondrion, *N* Nucleus, *Pf* Proximo-distal fusion, *Sr* Striated roots

axoneme mark the beginning of this region. Then, the second axoneme disappears. At the end of the spermatozoon, the cytoplasm exhibits only the nucleus, a few cortical microtubules and a small lateral expansion (Figs. 22, 23, 24 and 25).

Discussion

The general process of spermiogenesis of *Cotylophoron cotylophorum* is similar to that of other digenean species. A differentiation zone appears on the side of the spermatid, and then a median cytoplasmic expansion and two flagella develop. These flagella elaborated from the centrioles, undergo a 90° rotation and become parallel to the median cytoplasmic expansion. This rotation has been already observed in the majority of the digeneans (Table 1). However, some trematodes show flagella rotation greater than 90° (Table 1). Simultaneous to these rotations, the nucleus and the mitochondria migrate to the median cytoplasmic expansion. In *C. cotylophorum*, the fusion of the flagella with the median cytoplasmic expansion is proximo-distal. This phenomenon is regularly observed in trematodes. The fusion zone is marked by the presence of four electron-dense lines, which appear during the spermiogenesis.

Other elements such as the intercentriolar body and the striated roots are characteristic of the differentiation zone. According to Burton (1972), each of these two elements plays a role during spermiogenesis. The purpose of the

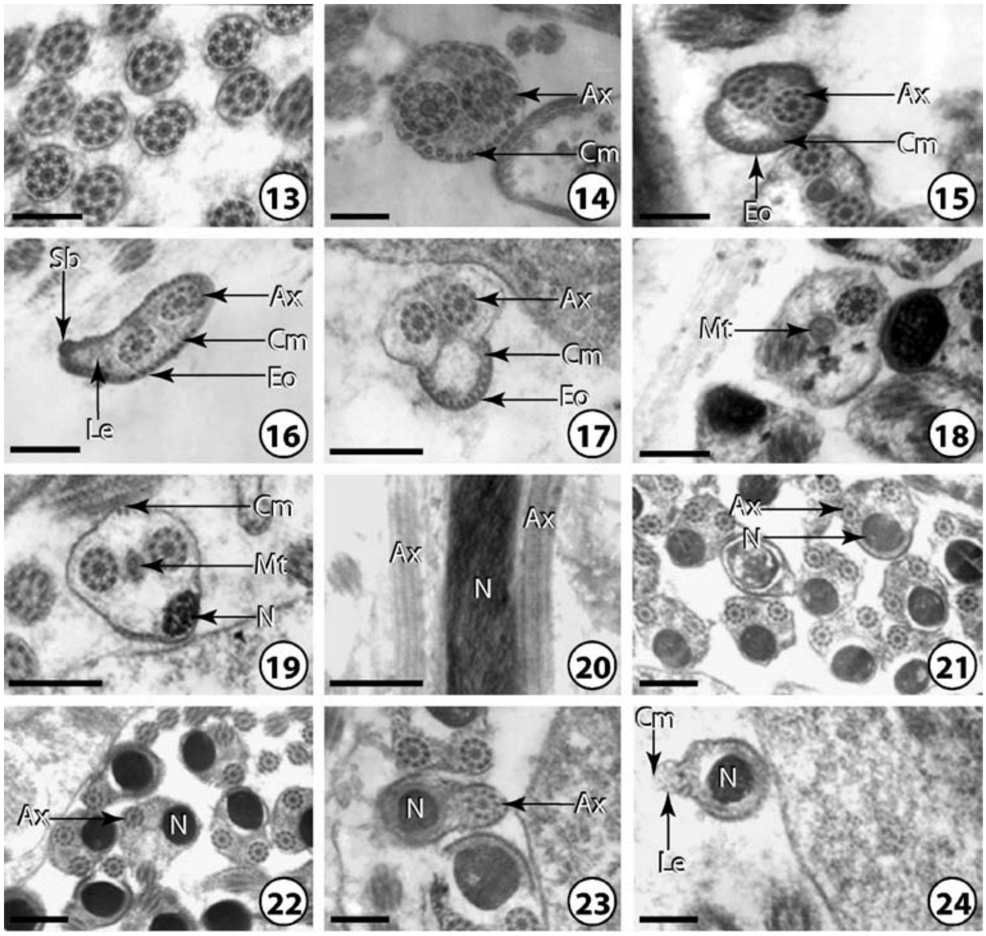

Fig. 13 Spermatozoon of *Cotylophoron cotylophorum*. Cross-section of region I showing many axonemes surrounded by a thin layer of cytoplasm and a plasmic membrane. A this level of section, one can see the absence of cortical microtubules (*bar*=0.4 µm)
Fig. 14 Spermatozoon of *Cotylophoron cotylophorum*. Cross-section of region I showing two axonemes (*Ax*) and numerous cortical microtubules (*Cm*; *bar*=0.2 µm)
Fig. 15 Spermatozoon of *Cotylophoron cotylophorum*. Cross-section of region I showing two axonemes (*Ax*), numerous cortical microtubules (*Cm*). The ventral field presents external ornamentations (*Eo*; *bar*=0.4 µm)
Fig. 16 Spermatozoon of *Cotylophoron cotylophorum*. Cross-section of region I showing a lateral expansion (*Le*) of the cytoplasm and a spinelike body (*Sb*). Only the ventral field exhibits external ornamentations (*Eo*). *Ax* Axonemes, *Cm* Cortical microtubules (*bar*=0.4 µm)
Fig. 17 Spermatozoon of *Cotylophoron cotylophorum*. Cross-section of region II showing both axonemes (*Ax*) and cortical microtubules (*Cm*). The ventral field presents external ornamentations (*Eo*; *bar*= 0.4 µm)
Fig. 18 Spermatozoon of *Cotylophoron cotylophorum*. Cross-section of region III showing both axonemes, cortical microtubules and anterior extremity of mitochondrion (*Mt*). The plasmic membrane is devoid of external ornamentations (*bar*=0.4 µm)
Fig. 19 Spermatozoon of *Cotylophoron cotylophorum*. Cross-section of region IV showing cortical microtubules (*Cm*), two axonemes, mitochondrion (*Mt*) and nucleus (*N*) (*bar*=0.4 µm)
Fig. 20 Spermatozoon of *Cotylophoron cotylophorum*. Longitudinal section of region V showing a nucleus (*N*) and two axonemes (*Ax*) (*bar*=0.4 µm)
Fig. 21 Spermatozoon of *Cotylophoron cotylophorum*. Cross-section of region V showing a nucleus (*N*), two axonemes (*Ax*) and the disappearance of mitochondrion (*bar*=0.5 µm)
Fig. 22 Spermatozoon of *Cotylophoron cotylophorum*. Cross-section of region VI showing one axoneme (Ax) which starts to disorganize. *N* Nucleus (*bar*=0.5 µm)
Fig. 23 Spermatozoon of *Cotylophoron cotylophorum*. Cross-section of region VI showing one axoneme (*Ax*) and a nucleus (*N*; *bar*= 0.5 µm)
Fig. 24 Spermatozoon of *Cotylophoron cotylophorum*. Cross-section of region VI showing the nucleus (*N*). The posterior end of the spermatozoon is devoid of axonemes. *Le* Lateral expansion, *Cm* Cortical microtubules (*bar*=0.5 µm)

striated roots could be to stabilize the flagella during fusion with the median cytoplasmic expansion; the intercentriolar body could be used to store material for the polymerization of the microtubules. In *C. cotylophorum*, the intercentriolar body appears composed of a thin electron-dense central layer and six electron-dense lateral layers separated by electron-light spaces. The external bands are made up of a line of granules, and not of a continuous band as it is the case in the majority of the trematodes. Today, such an intercentriolar body has only been observed in *Ceylonocotyle scoliocoelium* (Li and Wang 1997), *Troglotrema acutum* (Miquel et al. 2006), *Paramphistomum microbothrium* (Seck et al. 2007), and *Carmyerius endopapillatus* (Seck et al. 2008). In other trematodes, the intercentriolar body is

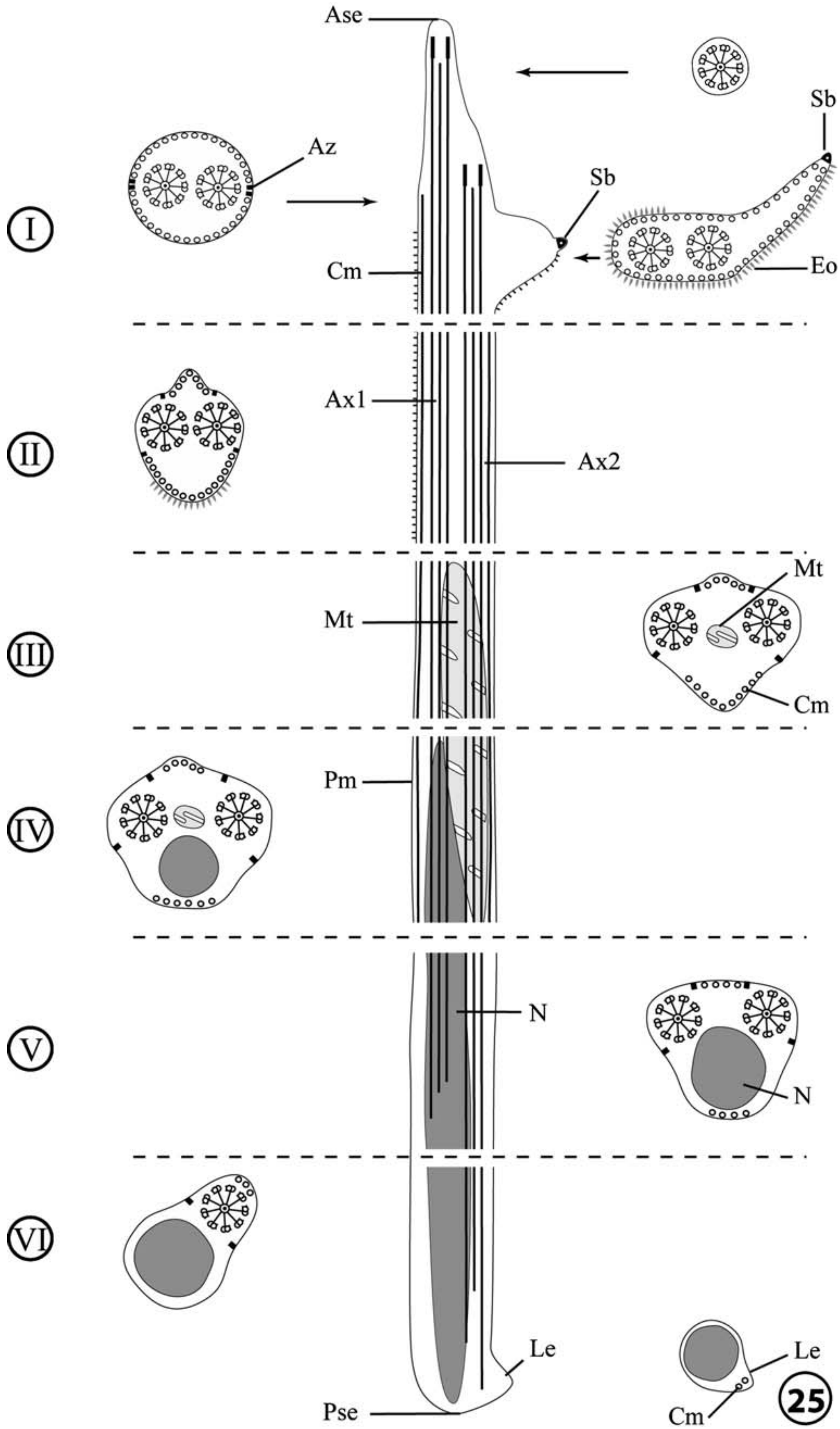

Fig. 25 Diagram showing regions I–VI and the ultrastructural organization of a mature spermatozoon of *Cotylophoron cotylophorum*. *Ase* Anterior spermatozoon extremity, *Ax1* Axoneme 1, *Ax2* Axoneme 2, *Az* Attachment zone, *Cm* Cortical microtubules, *Eo* External ornamentations, *Le* Lateral expansion, *Mt* Mitochondrion, *N* Nucleus, *Pm* Plasmic membrane, *Pse* Posterior spermatozoon extremity, *Sb* Spinelike body

Table 1 Some ultrastructural characteristics of spermiogenesis and the spermatozoon in the Digenea

Families and digenean species	Fr	Az	Le	Eo	Sb	Mt	Pse	References
Allocreadiidae								
Crepidostomum metoecus	>90°	4	−	+	+	2	1F	Quilichini et al. (2007a)
Brachylaimidae								
Brachylaimus aequans	+		−	−	−	1		Ždărskă et al. (1991)
Scaphiostomum palaearcticum	90°	4	+	+	−	1	1F	Ndiaye et al. (2002)
Bucephalidae								
Bucephaloides gracilescens	90°		−	+	−	1	1F	Erwin and Halton (1983)
Pseudorhipidocotyle elpichthys			−	+	−	1		Tang et al. (1998)
Cryptogonimidae								
Neochasmus sp.	90°	4	−	+	−	2	1F	Jamieson and Daddow (1982), Daddow and Jamieson (1983)
Deropristidae								
Deropristis inflata	90°	4	−	+	−	2	1F	Foata et al. (2007)
Dicrocoeliidae								
Dicrocoelium dendriticum	90°	2	−	−	−	1	1F	Cifrian et al. (1993), Morseth (1969)
Dicrocoelium chinensis			−	−	−	1		Tang (1996), Tang and Li (1996)
Dicrocoelium hospes	120°	4	−	+	+	2	N	Agostini et al. (2005)
Corrigia vitta	90°	4	−	−	−	1	1F	Robinson and Halton (1982)
Didymozoidae								
Didymozoon sp.			−	−	−	1		Justine and Mattei (1983, 1984a)
Gonapodasmius sp.		2	−	+	−	1		Justine and Mattei (1982a, 1984b)
Didymocystis wedli			−	−	−	1		Pamplona-Basilio et al. (2001)
Diplostomatidae								
Pharyngostomoides procyonis	90°		−	−	−			Grant et al. (1976)
Echinostomatidae								
Echinostoma caproni		4	+	−	−	1	N+1F	Iomini and Justine (1997), Justine (1995)
Fasciolidae								
Fasciola hepatica	120°		+	−	+	2	1F	Stitt and Fairweather (1990), Ndiaye et al. (2003b)
Fasciola gigantica	90°	4	+	+	+	1	N	Ndiaye et al. (2004)
Fellodistomidae								
Proctoeces maculatus		4	−	+	−	1		Justine (1995)
Pronoprymna ventricosa		4	−	+	−	1	1F	Quilichini et al. (2007d)
Gastrothylacidae								
Carmyerius endopapillatus	90°	4	+	+	+	1	N+Le	Seck et al. (in press)
Haematoloechidae								
Haematoloechus medioplexus	90°	4	+	+	−	1		Burton (1972), Justine (1995), Justine and Mattei (1982b)
Haploporidae								
Saccocoelioides godoyi	90°	2	−	−	−	1		Baptista-Farias et al. (2001)
Heterophyidae								
Cryptocotyle lingua	90°	4	−	−	−	1	1F	Rees (1979)
Lecithodendriidae								
Postorchigenes gymnesicus	90°	4	−	+	−	2	1F+G	Gracenea et al. (1997)
Ganeo tigrum			−	−	−	1	1F	Sharma and Rai (1995)
Monorchiidae								
Monorchis parvus	120°		−	+	−	2	N+D	Levron et al. (2004a)
Mesocoelidae								
Mesocoelium monas			−	−	−	1	N	Iomini et al. (1997)
Microphallidae								
Maritrema linguilla	90°	4	−	−	−	1	1F	Hendow and James (1988)
Notocotylidae								
Notocotylus neyrai	90°	4	−	+	−	2	1F	Ndiaye et al. (2003a)
Opecoelidae								
Helicometra fasciata	120°	4	+	−	−	1	G	Levron et al. (2003)
Opecoeloides furcatus	90°	4	−	+	+	1	G	Miquel et al. (2000)
Poracanthium furcatum	90°		+	+	+	2	G	Levron et al. (2004b)

Table 1 (continued)

Families and digenean species	Fr	Az	Le	Eo	Sb	Mt	Pse	References
Nicolla wisniewskii	> 90°	4	−	+	+	2	Cm	Quilichini et al. (2007b)
Nicolla testiobliquum		4	−	+	+	2	Cm	Quilichini et al. (2007c)
Opisthorchiidae								
Aphalloides coelomicola			−	+	−	1		Justine (1995)
Paragonimidae								
Paragonimus miyazakii			−	−	−	1		Sato et al. (1967)
Paragonimus pulmonalis			−	−	−			Fujino and Ishii (1982)
Paragonimus westermani			−	−	−			Fujino and Ishii (1982)
Paragonimus ohirai		4	−	+	+	2	1F	Fujino et al. (1977), Hirai and Tada (1991), Orido (1988)
Paramphistomidae								
Ceylonocotyle scoliocoelium			−	−	−	1	N+Cm	Li and Wang (1997)
Paramphistomum microbothrium	90°	4	+	+	+	3	N	Seck et al. (2007)
Sandonia sudanensis	90°	4	+	+		1		Ashour et al. (2007)
Basidiodiscus ectorchus	90°	4	+	+		1	N + Cm + Le	Ashour et al. (2007)
Cotylophoron cotylophorum	90°	4	+	+	+			Present work
Troglotrematidae								
Troglotrema acutum	90°	4	−	+	+	2	1F	Miquel et al. (2006)
Zoogonidae								
Diphterostomum brusinae	90°	4	−	+	−	1	N+G	Levron et al. (2004c)

Az Attachement zones, *Cm* cortical microtubules, *Eo* extramembranous ornamentations, *F* flagellum, *Fr* flagellar rotation, *G* glycogen granules, *Le* lateral expansion, *Mt* mitochondrion, *N* nucleus, *Pse* posterior spermatozoon extremity, *Sb* spinelike body "+ and −" indicate the presence or the absence of the considered character.

composed of nine electron-dense bands. It is the case in: *Cryptocotyle lingua* (Rees 1979), *Microphallus primas* (Castilho and Barandela 1990), and *Monorchis parvus* (Levron et al. 2004a). In some trematodes, the intercentriolar body exhibits a symmetrical organization and is composed by six electron-dense layers separated by five electron-lucent ones. The two most external layers are discontinuous. It is the case in *Deropristis inflata* (Foata et al. 2007).

The absence of striated roots and intercentriolar body was mentioned only in Didymozoide *Gonapodasmius* by Justine and Mattei (1982a, b) who reported that this absence does not affect the phenomena described by Burton (1972). The striated roots and the intercentriolar body degenerate at the end of the spermiogenesis. In the mature spermatid, external ornamentation and a spinelike body are absent.

The *C. cotylophorum* spermatozoon shows ultrastructural features similar to those described in the majority of the digeneans: two axonemes of 9+"1" pattern which is characteristic of the Trepaxonemata (Ehlers 1984), a nucleus, one or two mitochondria and cortical microtubules. In Trepaxonemata, only Schistosomatidae and Didimozoidae have flagella with 9+"0" axonemes considered as a variant of the classic 9+"1". This is the case of *Schistosoma mansoni* (Kitajima et al. 1976); *S. bovis* (Justine and Mattei 1981); *S. curassoni*, *S. rodhaini*, *S. intercalatum*, *S. bovis*, *S. margrebowiei*, *S. mansoni* (Justine et al. 1993), and *Didymozoon* sp. (Justine and Mattei 1983, 1984).

In spite of these classic schemes of spermatozoon of the Digenea, *C. cotylophorum* has some particularities. The anterior extremity of this spermatozoon is characterized by the presence of a lateral expansion after the appearance of the second axoneme. This lateral expansion was already signaled in other digenean species (Table 1). In *C. cotylophorum*, the spinelike body is situated at the tip of the lateral expansion. Spinelike bodies were described for the first time by Miquel et al. (2000) in the spermatozoon of *Opecoeloides furcatus*. Other recent studies have shown the existence of spinelike bodies (Table 1). But in these species, except *Paramphistomum microbothrium* and *Carmyerius endopapillatus* (spinelike body located on the lateral expansion), the spinelike bodies are present in the anterior areas of spermatozoon and associated with the external ornamentation of the plasmic membrane. The presence of spinelike body on the lateral expansion of the spermatozoon has been described for the first time by Seck et al. (2007) in *P. microbothrium*.

The anterior extremity of the *C. cotylophorum* spermatozoon presents external ornamentations outside on the plasmic membrane, only on its ventral field.

In *C. cotylophorum*, only one mitochondrion in the spermatozoon was observed. It is also the case in many digenean, while in others, the presence of two mitochondria was described (Table 1).

The posterior extremity of the *C. cotylophorum* mature spermatozoon contains a nucleus surrounded by a plasmic membrane, without cortical microtubules, but presenting a small lateral expansion. A posterior extremity with such a lateral process has been previously described in *Carmyerius endopapillatus* (Seck et al. 2008) and *Bucephaloïdes gracilenscens* (Erwin and Halton 1983). But in this last species, the posterior extremity contains in addition, an axoneme and cortical microtubules. In other digeneans, the structure of the posterior extremity is different (Table 1).

This work enables to describe characteristics useful for future phylogenetic studies: the two external dark bands of intercentriolar body are formed of a line of granules, lateral expansion exhibiting a spinelike body, and external ornamentations only at the level of the ventral field of cortical microtubules. Nevertheless, further studies are required for consideration of relationships within the Paramphistomidae.

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