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VERSITA

Spermiogenesis and sperm ultrastructure of *Carmyerius endopapillatus* (Digenea, Gastrothylacidae), a parasite of *Bos taurus* in Senegal

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Abstract

Ultrastructural study of spermiogenesis and of the spermatozoon of *Carmyerius endopapillatus* has enabled to describe some characteristics of this digenea. The intercentriolar body situated between the two striated roots and the two centrioles, presents a symmetric organization. Both external bands of this intercentriolar body are made up of a row 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 region, by the presence of a lateral expansion exhibiting one spinelike body. In *C. endopapillatus*, external ornamentations are localized only at the level where the lateral expansion appears. The posterior extremity of spermatozoon exhibits a nucleus surrounded by a plasmic membrane lacking microtubules, but presenting a small lateral expansion. This is the first species of Gastrothylacidae family studied by transmission electron microscopy.

Keywords

Ultrastructure, spermiogenesis, spermatozoon, *Carmyerius endopapillatus*, Digenea, Gastrothylacidae

Introduction

Spermiogenesis characters and ultrastructure of spermatozoon of the platyhelminths appear to have a good phylogenetic interest (Justine 1991, 1995, 1998; Bâ and Marchand 1995). In Gastrothylacidae, the study of the spermatogenesis is still unexplored. From our knowledge, in this family which counts about ten genera and several tens of species, none of them has been studied by transmission electron microscopy.

In the present work, we describe the spermiogenesis and the ultrastructure of the spermatozoon of a first Gastrothylacidae: *Carmyerius endopapillatus*. The systematics of Gastrothylacidae, based essentially on the anatomical and morphological description of adults, is still a matter of controversy. This study of spermatogenetic characters could be a new contribution for a more coherent phylogeny of these digenean.

Materials and methods

The specimens of *C. endopapillatus* were gathered live from the rumen of *Bos taurus* (Ndama cattle) at the slaughterhouses of Kolda (area located in Southern Senegal). After extraction, the trematodes were kept in a 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, rinsed overnight 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 LKB 8800A Ultrome III ultramicrotome, and then stained with uranyl acetate and lead citrate according to Reynolds (1963). They were examined in Hitachi H-600 electron microscope at 75 kV.

Results

Spermiogenesis (Figs 1–9)

Spermiogenesis of *Carmyerius endopapillatus* starts with the formation of a differentiation zone. The young spermatid presents a large central nucleus, surrounded by cytoplasm containing mitochondria, two centrioles and an intercentriolar body (Fig. 1). The latter presents a symmetric organization. It

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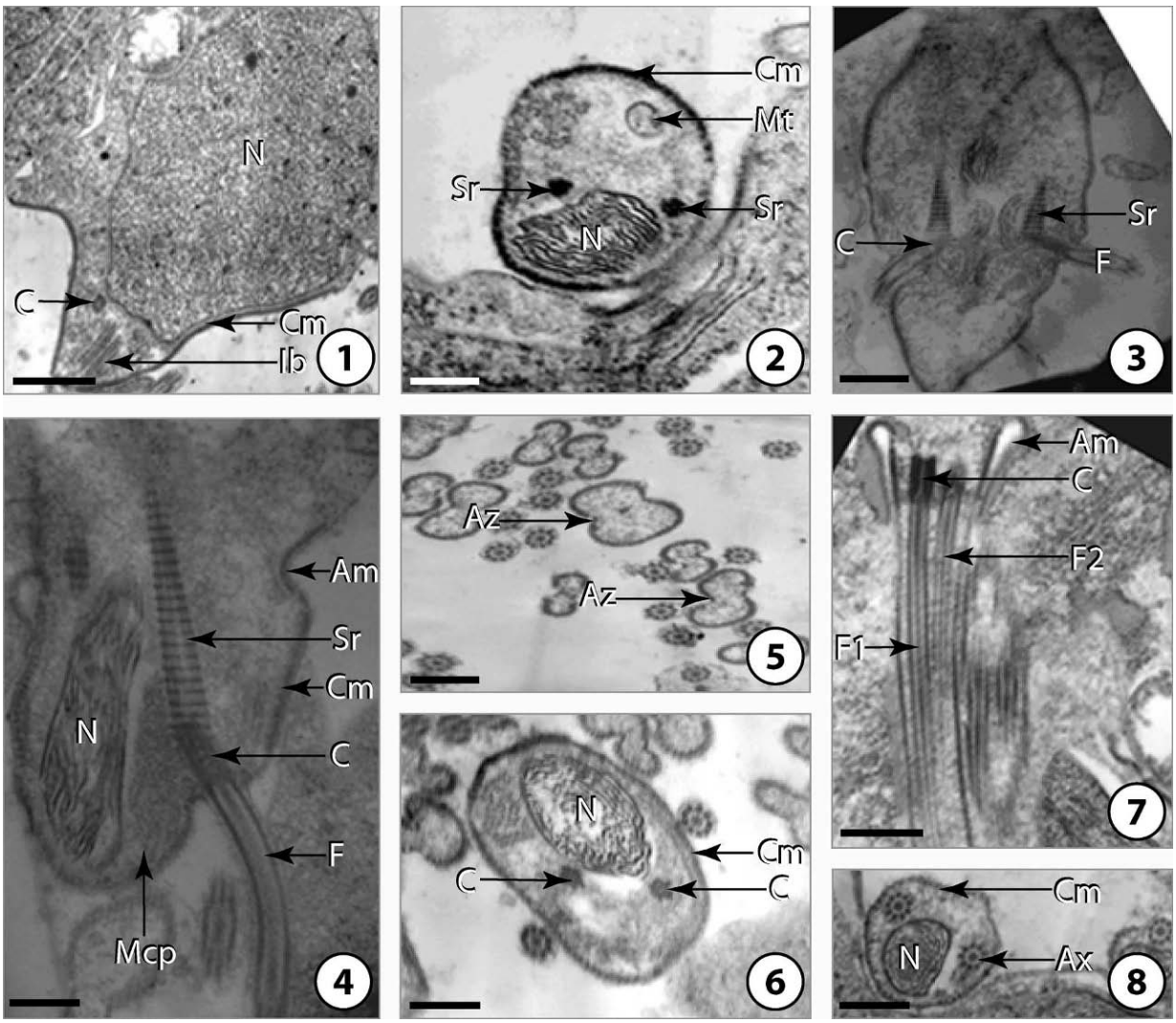
appears composed of a thin electron-dense central layer and 6 electron-dense lateral layers separated by electron-light spaces. Each of both external electron-dense layers is made of juxtaposed granules (Fig. 1). The striated roots appear lengthened and have a triangular form in longitudinal section (Figs 3 and 4). Both centrioles give rise, one after the other and in opposite directions, to a flagellum (Fig. 3). The development of this differentiation zone is marked by the formation of a median cytoplasmic expansion surrounded by cortical microtubules (Fig. 4). The nucleus and mitochondria migrate in this median cytoplasmic expansion (Fig. 4). Cross-sections of the spermatid, at different levels, show different structures. Some of them show the median cytoplasmic expansion and the two axonemes (Fig. 5); others show the median cytoplasmic expansion, the two centrioles and the nucleus (Fig. 6). The flagella undergo a 90° rotation, become parallel to the median cytoplasmic expansion and fuse with it. This fusion is proximo-distal (Figs 4 and 7). Cross-sections of the median cyto-



Figs 1–8. Spermiogenesis of *Carmyerius endopapillatus*: **1.** Longitudinal section of a differentiation zone showing a nucleus, centrioles, intercentriolar body and cortical microtubules. Scale bar = 0.8 µm. **2.** Cross-section of a differentiation zone showing both striated roots, a mitochondrion, a nucleus and cortical microtubules. Scale bar = 0.5 µm. **3.** Longitudinal section of a differentiation zone showing two centrioles and two striated roots. Each centriole develops a flagellum in opposite direction. Scale bar = 0.5 µm. **4.** Longitudinal section of a differentiation zone at the stage of flagellar rotation. The nucleus has begun its migration in the median cytoplasmic process. Scale bar = 0.6 µm. **5.** Cross-section of a median cytoplasmic process showing attachment zones. The latter are marked by a submembranous electron dense material. Scale bar = 0.5 µm. **6.** Cross-section of a median cytoplasmic process showing both centrioles, cortical microtubules and the nucleus. Scale bar = 0.6 µm. **7.** Longitudinal section showing the fusion of both flagella with the median cytoplasmic process. Scale bar = 0.8 µm. **8.** Cross-section of an old spermatid with two axonemes, a nucleus and cortical microtubules. Scale bar = 0.6 µm. **Abbreviations used on all figures:** Am – arched membrane, Ase – anterior spermatozoon extremity, Ax – axoneme, Az – attachment zone, C – centriole, C1 – centriole 1, C2 – centriole 2, Cm – cortical microtubules, Eo – external ornamentations, F1 – flagellum 1, F2 – flagellum 2, Ib – intercentriolar body, Le – lateral expansion, Mcp – median cytoplasmic process, Mt – mitochondrion, N – nucleus, Pf – proximodistal fusion, Pm – plasmic membrane, Pse – posterior spermatozoon extremity, Sb – spinelike body, Sr – striated roots

plasmic expansion show the attachment zones (Fig. 5) and two fields of cortical microtubules: one dorsal (small) and the other ventral (greater). The old spermatid is characterized by two axonemes, a nucleus with a partially condensed chromatin and a plasmic membrane associated with the cortical microtubules (Fig. 8).

Spermatozoon (Figs 10–21)

The ultrastructural study of the spermatozoon of *C. endopapillatus* allowed to distinguish six regions from anterior to posterior extremity.

Region I (Figs 10–12 and 21 I): The first region corresponds to the anterior part of the spermatozoon of *C. endopapillatus*. It shows two 9 + '1' axonemes typical of Trepaxonemata and a lateral expansion exhibiting at its extremity a spinelike body. The most anterior part of this region is characterized by the presence of only one axoneme. Cortical microtubules, in close contact with the internal side of the plasmic membrane, begin at the same level of the second axoneme. At the level of the lateral expansion, the plasmic membrane is surrounded by external ornamentations.

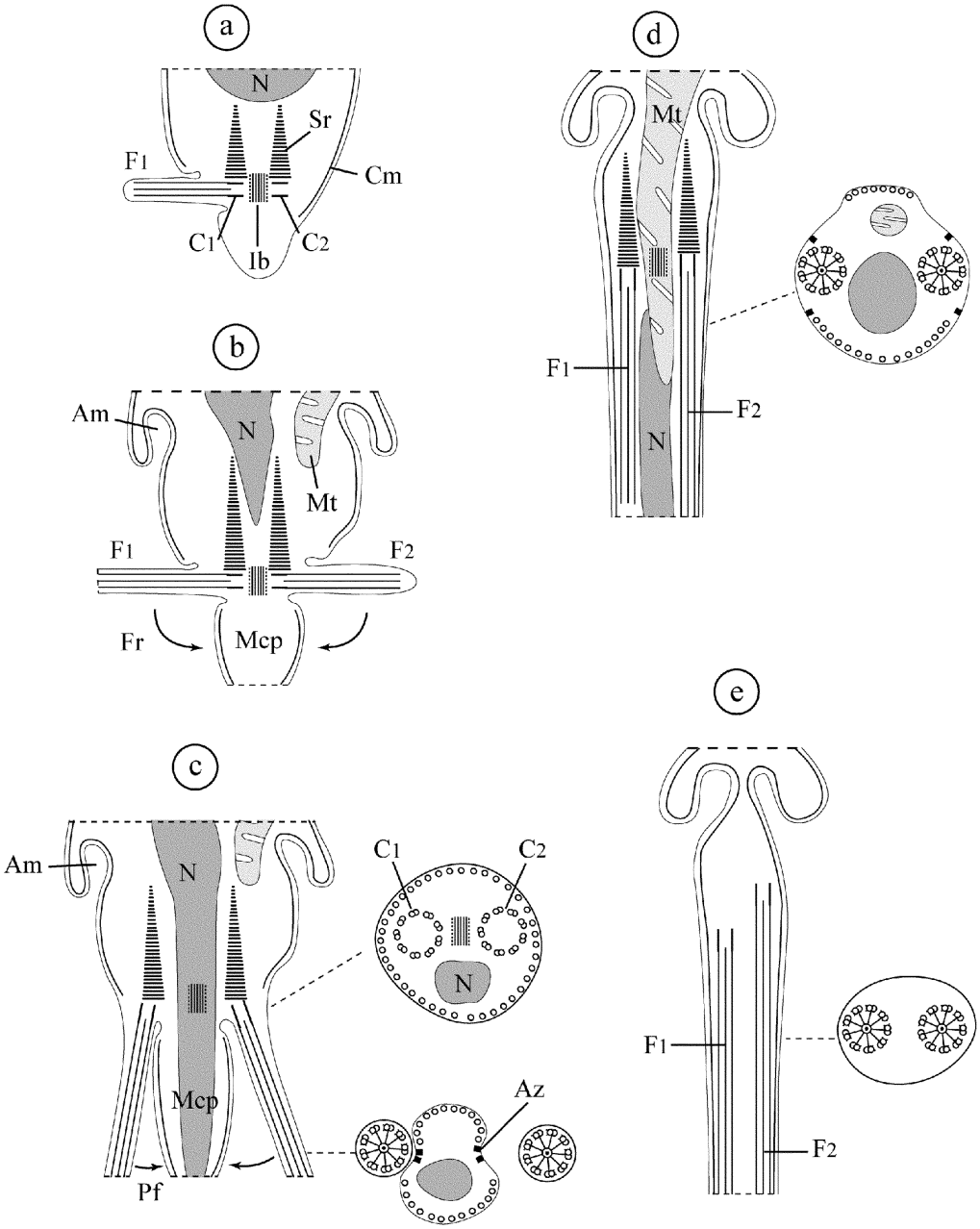
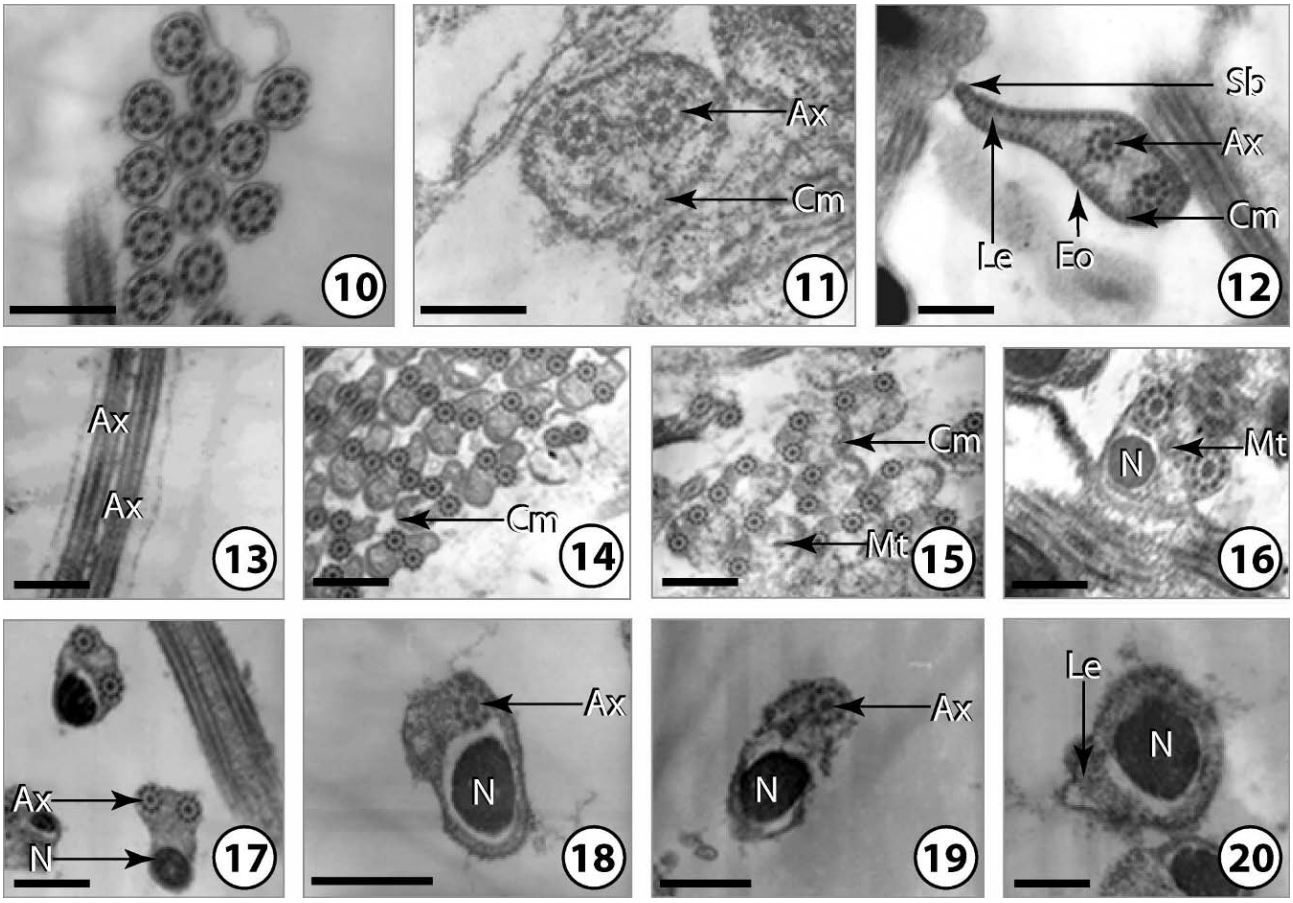


Fig. 9a-e. Reconstruction of the main stages of spermiogenesis of *Carmyerius endopapillatus*: Formation of the first flagellum (**a**). Formation of the second flagellum (**b**). Rotation of both flagella (**c**). Fusion of both flagella with the median cytoplasmic process (**d**). End of the spermiogenesis (**e**)



Figs 10–20. Sections of regions I–VI of the *Carmyerius endopapillatus* mature spermatozoon: **10.** Region I showing many axonemes surrounded by a thin layer of cytoplasm and a plasmic membrane. At this level of section, one can notice the absence of cortical microtubules. Scale bar = 0.4 µm. **11.** Region I showing two axonemes, numerous cortical microtubules. Scale bar = 0.4 µm. **12.** Region I showing external ornamentations, a lateral expansion of the cytoplasm and a spinelike body. Scale bar = 0.4 µm. **13.** Longitudinal section of region I showing both axonemes. Scale bar = 0.4 µm. **14.** Region II. One can see that there are neither external ornamentations nor a lateral expansion of the cytoplasm exhibiting a spinelike body. Scale bar = 0.5 µm. **15.** Region III showing a mitochondrion and a few cortical microtubules. Scale bar = 0.5 µm. **16.** Region IV showing the nucleus alongside the two axonemes. Scale bar = 0.4 µm. **17.** Region V showing the nucleus and both axonemes. Scale bar = 0.5 µm. **18.** Region VI near the end of the first axoneme. Scale bar = 0.4 µm. **19.** Region VI showing the nucleus and an axoneme. Scale bar = 0.4 µm. **20.** Region VI showing the nucleus. At this posterior end of the spermatozoon, the axonemes have disappeared. The plasmic membrane exhibiting a small lateral expansion. Scale bar = 0.2 µm

Region II (Figs 13, 14 and 21 II): Sections of this region show two axonemes and four zones of attachment that delimit two fields of different sizes. One observes a small dorsal field with 10 cortical microtubules and a ventral one with 17 cortical microtubules.

Region III (Figs 15 and 21 III): It is characterized by the presence of a mitochondrion in the dorsal field. It always shows the two axonemes, attachment zones and cortical microtubules.

Region IV (Figs 16 and 21 IV): The appearance of the nucleus in the ventral field characterises this region. The cortical microtubules are always present but in a reduced number.

Region V (Figs 17 and 21 V): This region is devoided of mitochondrion, attachment zones and cortical microtubules. Only the two axonemes and the nucleus are still present.

Region VI (Figs 18–20 and 21 VI): This zone corresponds to the posterior extremity of spermatozoon. The disorganization and the disappearance of the first axoneme mark the beginning of this region. Then the second axoneme disappears. The end of spermatozoon is marked by the nucleus surrounded by a plasmic membrane without cortical microtubules, but presenting a small lateral expansion.

Discussion

The general process of spermiogenesis of *Carmyerius endopapillatus* is similar to that described in many other digenean species (Burton 1972; Rees 1979; Daddow and Jamieson 1983; Erwin and Halton 1983; Gracenea *et al*. 1997; Iomini and Justine 1997; Iomini *et al*. 1997; Li and Wang 1997; Miquel *et al*. 2000; Baptista-Farias *et al*. 2001; Ndiaye *et al*. 2002, 2003a, b, 2004; Levron *et al*. 2003, 2004a, b, c; Seck *et al*. 2007).

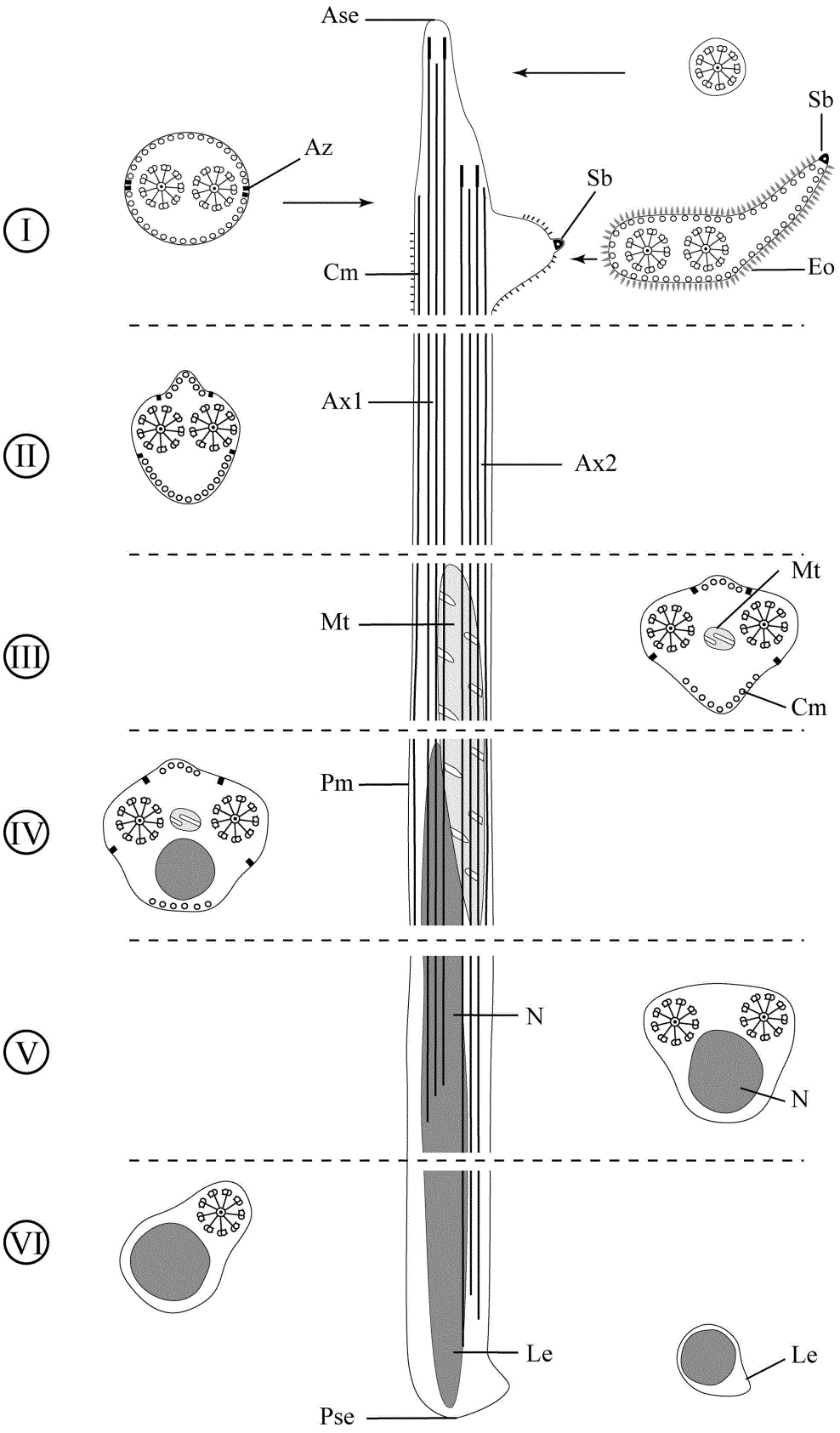


Fig. 21. Reconstruction of the *Carmyerius endopapillatus* mature spermatozoon

Differentiation zone appears on the side of the spermatid, then a median cytoplasmic expansion and two flagella appear and develop. These flagella undergo a 90° rotation and become parallel to median cytoplasmic expansion. This rotation has been already observed in most of the studied digenean species (Table I). However, flagella in six others trematodes: *Helicometra fasciata* (Levron *et al*. 2003), *Fasciola hepatica* (Ndiaye *et al*. 2003a), *Monorchis parvus* (Levron *et al*. 2004a), *Dicrocoelium hospes* (Agostini *et al*. 2005), *Nicolla wisniewskii* (Quilichini *et al*. 2007a) and *Crepidostomum metoecus* (Quilichini *et al*. 2007b) undergo a rotation greater than 90°. The flagella of the first four species have the particularity of a rotation of 120° before fusion with the median cytoplasmic expansion. This fusion in *C. endopapillatus* is proximo-distal as it is in all the studied trematodes (Cifrian *et al*. 1993; Miquel *et al*. 2000; Ndiaye *et al*. 2002; Levron *et al*. 2003, 2004a, b, c; Seck *et al*. 2007). The fusion of plasmic membranes begins near the cytoplasmic mass and ends at the distal extremity of the old spermatid. This fusion zone is marked by the presence of four electron dense lines, which appear during the spermiogenesis and persist in the mature spermatozoon. This phenomenon has also been described in many other digenean species: one Haematoloechidae, *Haematoloechus medioplexus* (Burton 1972), one Didymozoidae, *Gonapodasmius* (Justine and Mattei 1982a), one Dicrocoeliidae, *Corrigia vitta* (Robinson and Halton 1982), two Microphallidae, *Maritrema linguilla* (Hendow and James 1988) and *Microphallus primas* (Castilho and Barandela 1990), one Haploporidae, *Saccocoeliodes godoyi* (Baptista-Farias *et al*. 2001), three Opecoelidae, *Opecoeloides furcatus* (Miquel *et al*. 2000), *Helicometra fasciata* (Levron *et al*. 2003), *Monorchis parvus* (Levron *et al*. 2004a), one Brachylaimidae, *Scaphiostomum palaearcticum* (Ndiaye *et al*. 2002), one Notocotylidae, *Notocotylus neyrai* (Ndiaye *et al*. 2003b), two Fasciolidae, *Fasciola hepatica* (Ndiaye *et al*. 2003a) and *Fasciola gigantica* (Ndiaye *et al*. 2004), one Paramphistomidae, *Paramphistomum microbothrium* (Seck *et al*. 2007).

Other elements such as the intercentriolar body and the striated roots are characteristic of the differentiation zone. According to Burton (1972), each of them plays a role in spermiogenesis. The role of striated roots would be to stabilize flagella during their fusion with the median cytoplasmic expansion; while intercentriolar bodies would serve as reserve of material for the polymerization of the microtubules. In *C. endopapillatus*, the intercentriolar body is formed of seven electron dense bands. Such a structure was previously described in *Corrigia vitta* (Robinson and Halton 1982), *Postorchigenes gymnesicus* (Gracenea *et al*. 1997), *Brachylaima* sp. (Gracenea *et al*. 2000), *Scaphiostomum palaearcticum* (Ndiaye *et al*. 2002), *Helicometra fasciata* (Levron *et al*. 2003), *Poracanthium furcatum* (Levron *et al*. 2004b), *Fasciola gigantica* (Ndiaye *et al*. 2004). In *C. endopapillatus*, we have noted a difference: both external bands of intercentriolar body are made up of a line of electron dense granules and not a continuous band as is the case in most 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) and *Paramphistomum microbothrium* (Seck *et al*. 2007). In other trematodes, the intercentriolar body is 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 signalled only in a Didymozoidae, *Gonapodasmius* sp. by Justine and Mattei (1982a) who mentioned that this absence does not affect the phenomena described by Burton (1972). As in the other trematodes, striated roots and intercentriolar body disappear at the end of spermiogenesis. They are no longer observed in the mature spermatozoon (Burton 1972, Rees 1979, Erwin and Halton 1983, Justine 1995, Ndiaye *et al*. 2002).

In *C. endopapillatus*, the old spermatid does not exhibit external ornamentations, as it is the case in other studied digeneans. On the other hand, the spermatid of *C. endopapillatus* does not present a spinelike body in most of the digenean except *Fasciola hepatica* (Ndiaye *et al*. 2003a) and *F. gigantica* (Ndiaye *et al*. 2004).

The spermatozoon of *C. endopapillatus* has ultrastructural characteristics which are similar to those of the majority of the digenean: two axonemes of the type 9 + '1' pattern characteristic of the Trepaxonemata (Ehlers 1984), a nucleus, one or two mitochondria and cortical microtubules (Burton 1972; Jamieson and Daddow 1982; Orido 1988; Stitt and Fairweather 1990; Iomini and Justine 1997; Levron *et al*. 2003, 2004a, b, c; Ndiaye *et al*. 2003a, b; Seck *et al*. 2007). In Trepaxonemata, only Schistosomatidae and Didymozoidae have flagella with 9 + 0 axonemes considered as a variant of the classic 9 + '1' pattern. This is the case of *Schistosoma mansoni*, *S. bovis*, *S. curassoni*, *S. rodhaini*, *S. intercalatum*, *S. margrebowiei*, and *Didymozoon* sp. (Kitajima *et al*. 1976; Justine and Mattei 1981, 1983, 1984a; Justine *et al*. 1993).

In spite of this classic scheme of the spermatozoon of the Digenea, *C. endopapillatus* has some particularities. The anterior extremity of this spermatozoon is characterized by the presence of a lateral expansion, a spinelike body and external ornamentations after the beginning of the second axoneme. This lateral expansion and external ornamentations were already described in other digeneans (Table I). The location of these ornamentations is variable on the spermatozoon according to the species. In *C. endopapillatus*, they are localized only at the level of the lateral expansion. According to Justine and Mattei (1982b), the role of these ornamentations remains unknown, but they hypothesized that they participate in the fusion of spermatic and ovular membranes during the fertilization.

The main characteristic of *C. endopapillatus* consists of the presence of the spinelike body at the end of the lateral

Table I. 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		Zdarska *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)
Depropristidae								
Depropristis 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)
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	present study
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)
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)

Continuation: Table I

Families and digenean species	Fr	Az	Le	Eo	Sb	Mt	Pse	References
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			–	–	–			Li and Wang (1997)
Paramphistomum microbothrium	90°	4	+	+	+	1	N+Cm	Seck *et al.* (2007)
Sandonia sudanensis	90°	4	+	+		3	N	Ashour *et al.* (2007)
Basidiodiscus ectorchus	90°	4	+	+		1		Ashour *et al.* (2007)
Troglotrematidae								
Troglotrema acutum	90°	4	–	+	+	2	1F	Miquel *et al.* (2006)
Zoogonidae								
Diphterostomum brusinae	90°	4	–	+	–	1	N+G	Levron *et al.* (2004c)

Az – attachment 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, "+"

expansion. Such spinelike bodies were described for the first time by Miquel *et al*. (2000) in *Opecoeloides furcatus* (Opecoelidae). Other recent studies have shown the existence of spinelike bodies in *Notocotylus neyrai* (Ndiaye *et al*. 2003b), *Poracanthium furcatum* (Levron *et al*. 2004b), *Fasciola gigantica* (Ndiaye *et al*. 2004), *Dicrocoelium hospes* (Agostini *et al*. 2005), *Troglotrema acutum* (Miquel *et al*. 2006), *Crepidostomum metoecus* (Quilichini *et al*. 2007a), *Nicolla wisniewskii* (Quilichini *et al*. 2007b), *N. testiobliquum* (Quilichini *et al*. 2007c) and *Paramphistomum microbothrium* (Seck *et al*. 2007). But in these species, except for the last one, the spinelike bodies are not located on the lateral expansion. 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* (Paramphistomidae).

The spermatozoon of *C. endopapillatus* contains only one mitochondrion. It is also the case in many digeneans (Table I), while in some others, two mitochondria have been found in the spermatozoon. It is the case in *Neochasmus* sp. (Daddow and Jamieson 1983), *Maritrema linguilla* (Hendow and James 1988), *Paragonimus ohirai* (Orido 1988), *Fasciola hepatica* (Stitt and Fairweather 1990), *Postorchigenes gymnesicus* (Gracenea *et al*. 1997), *Notocotylus neyrai* (Ndiaye *et al*. 2003b), *Monorchis parvus* (Levron *et al*. 2004a), *Poracanthium furcatum* (Levron *et al*. 2004b), *Dicrocoelium hospes* (Agostini *et al*. 2005), *Troglotrema acutum* (Miquel *et al*. 2006), *Deropristis inflata* (Foata *et al*. 2007), and *Crepidostomum metoecus* (Quilichini *et al*. 2007a). The spermatozoon of *Sandonia sudanensis* contains three mitochondria (Ashour *et al*. 2007).

The posterior extremity of the spermatozoon of *C. endopapillatus* contains only the nucleus surrounded by a plasmic membrane devoided of cortical microtubules and presents a small lateral expansion. A posterior extremity with such a lateral expansion has been previously described only in *Bucephaloides gracilescens* (Erwin and Halton 1983). But in this species, the posterior extremity contains in addition one axoneme and cortical microtubules. In other digeneans, the structure of the posterior extremity is different (Table I).

In spite of their importance in veterinary pathology, the systematic in Gastrothylacidae, essentially based on the anatomical and morphological description of adults, is controversial. Therefore, the ultrastructural study of spermiogenesis and the spermatozoon of these helminths appear of a good interest and would allow us, in the end, to specify the taxonomy of Gastrothylacidae and to propose a more coherent phylogeny of these digeneans.

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