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Ultrastructure of spermiogenesis and the spermatozoon of *Paramphistomum microbothrium* (Fischoeder 1901; Digenea, Paramphistomidae), a parasite of *Bos taurus* in Senegal

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Abstract The ultrastructural study of spermiogenesis and the spermatozoon of *Paramphistomum microbothrium* gave evidence of some characteristics of this digenean. The intercentriolar body located between two striated roots and two centrioles exhibits a symmetric plane. The two external bands of this intercentriolar body are made up of a line of granules. The striated roots are about 3.7-µm long. During spermiogenesis, a flagellar rotation of 90° is described. The spermatozoon is characterized in its anterior part by the existence of external ornamentations and a lateral expansion exhibiting a "spine-like body." Such a spine-like body has never been described before in a digenean. The region II of the spermatozoon is asymmetrical and also characterized by the absence of mitochondrion. The posterior end of this spermatozoon exhibits a nucleus and a few microtubules.

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Introduction

Ultrastructural studies of spermiogenesis and the spermatozoon of Paramphistomidae are still incomplete. In this family, which includes about ten genera and tens of species, only *Ceylonocotyle scoliocoelium* has been studied by electron microscopy (Li and Wang 1997). Spermiogenesis and the spermatozoon of this species were only partially described. According to Li and Wang (1997), the spermiogenesis of *C. scoliocoelium* is identical to that of the other trematodes. The only difference is that the external element of the intercentriolar body of *C. scoliocoelium* appeared in the form of a line of granules instead of a band as it is in the majority of the trematodes.

In the present study, we describe the ultrastructure of spermiogenesis and the spermatozoon of another Paramphistomidae, *Paramphistomum microbothrium*.

Material and methods

The specimens of *Paramphistomum microbothrium* [Fischoeder 1901] were gathered live 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, 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 LBK 8800A



Fig. 1 Spermiogenesis of *P. microbothrium*. Longitudinal section of a differentiation zone showing a flagellum (*F*) in a perpendicular position to the intercentriolar body (*Ib*). *C* Centriole, *Cm* corticol microtubules, *N* nucleus (bar= $0.4 \mu m$)

Fig. 2 Spermiogenesis of *P. microbothrium*. Longitudinal section of a differentiation zone showing arched membranes (*Am*). *F* Flagellum, N nucleus, *Sr* striated roots (bar= 0.4μ m)

Fig. 3 Spermiogenesis of *P. microbothrium*. Cross-section of a differentiation zone showing an intercentriolar body (*Ib*) between two centrioles (*C*). *F* Flagellum, *N* nucleus (bar= $0.4 \mu m$)

Fig. 4 Spermiogenesis of *P. microbothrium*. Longitudinal section of a differentiation zone at the stage of the flagellar rotation. The nucleus (*N*) has begun its migration in the median cytoplasmic process (*Mcp*). *Am* Arched membranes, *F* flagellum, Ib intercentriolar body, Sr striated roots (bar= $0.4 \mu m$)

Fig. 5 Spermiogenesis of *P. microbothrium*. Cross-section of a spermatid at the level of the two centrioles (*C*). *Cm* Cortical microtubules, *Ib* intercentriolar body, *N* nucleus (bar= 0.4μ m)

Fig. 6 Spermiogenesis of *P. microbothrium*. Cross-section of a median cytoplasmic process showing cortical microtubules (*Cm*) and attachment zones (*Az*). The latter are marked by a submembranous electron-dense material. *Ax* Axoneme (bar= 0.4μ m)

Fig. 7 Spermiogenesis of *P. microbothrium*. Cross-section of a spermatid with two axonemes (*Ax*) and cortical microtubules (*Cm*). *Az* Attachment zone (bar= $0.4 \mu m$)

Fig. 8 Spermiogenesis of *P. microbothrium*. Cross-section of a spermatid with two axonemes (Ax), a nucleus (N), a mitochondrion (Mt), and cortical microtubules (Cm; bar=0.4 µm)

Fig. 9 Spermiogenesis of *P. microbothrium*. Cross-section of an old spermatid at a level lacking the mitochondrion (*Mt*). Ax Axoneme, *Cm* cortical microtubules, *N* nucleus (bar= $0.4 \mu m$)

Fig. 10 Spermiogenesis of P. microbothrium. Reconstruction of the main stages of spermiogenesis of P. microbothrium. a Formation of the first flagellum (F1). C1 Centriole 1, C2 centriole, Cm cortical microtubules, Ib intercentriolar body, N nucleus, Sr striated roots. b Formation of the second flagellum (F2). Am Arched membranes, F1 flagellum 1, Fr rotation flagellar, Mcp median cytoplasmic process, Mt mitochondrion, N nucleus. c Rotation of both flagella. Am Arched membranes, Az attachment zone, C1 centriole 1, C2 centriole 2, Mcp median cytoplasmic process, N nucleus, Pf proximo-distal fusion. d Fusion of both flagella with the median cytoplasmic process. F1 Flagellum 1, F2 flagellum 2, Mt mitochondrium, N nucleus



Ultrome III ultramicrotome, then stained with uranyl acetate and lead citrate following Reynolds' (1963) methodology. They were examined in Hitachi H-600 electron microscope at 75 kV.

Results

Spermiogenesis

The start of spermiogenesis in *P. microbothrium* is marked by the formation of a differentiation zone (Fig. 1). This is delimited at the front by arched membranes (Figs. 2 and 4), bordered by cortical microtubules and contains a nucleus and two centrioles. The latter are linked together by an intercentriolar body and are surmounted by striated rootlets (Figs. 1, 2, and 3). In cross-section, the cortical microtubules form a discontinuous layer and appear hollow centered (Figs. 5, 6, 7, 8, and 9). A median cytoplasmic expansion rapidly forms (Fig. 4), lined with hollow cortical microtubules (Fig. 6).

The intercentriolar body is located between the two centrioles (Figs. 3 and 5). Its surface is increasingly more important than that of the centrioles. It extends between the two striated roots (Fig. 4). It exhibits a perfectly symmetrical 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. 4 and 5).

In longitudinal section, the striated roots appear very long and triangular (Fig. 4). They can reach a length of $3.7 \mu m$.

Each centriole develops a flagellum. The two flagella increase (Figs. 1, 2, and 3) before undergoing a rotation of 90° and become parallel to the median cytoplasmic expansion (Fig. 4).

The nucleus and the mitochondrion migrate inside the median cytoplasmic expansion (Figs. 2 and 4). Thereafter, the flagella fuse proximo-distally with the median cytoplasmic expansion (Fig. 7).

Cross-sections on the median cytoplasmic expansion show, before and after the fusion of the flagella, four attachment zones (Figs. 6 and 7) and two distinct fields of cortical microtubules. The field that is near the nucleus corresponds to the ventral one (Figs. 6 and 10). The other, which encloses the mitochondrion, corresponds to the dorsal one (Figs. 8 and 10).

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. Therefore, depending on the level of the sections in mature spermatid, one can or cannot observe the presence of a mitochondrion, two Fig. 11 Cross-sections of regions I–VI of the mature *P. microbothrium* spermatozoon. Region I showing many axonemes surrounded by a thin layer of cytoplasm and a plasmic membrane. At this level of the cut, one can see the absence of cortical microtubules (bar= $0.4 \mu m$)

Fig. 12 Cross-sections of regions I–VI of the mature *P. micro-bothrium* spermatozoon. Region I showing two axonemes (*Ax*), numerous cortical microtubules (*Cm*), and external ornamentations (*Eo*) (bar= 0.4μ m)

Fig. 13 Cross-sections of regions I–VI of the mature *P. microbothrium* spermatozoon. Region I showing a lateral expansion (*Le*) of the cytoplasm and a spine-like body (*Sb*). *Ax* Axonemes, *Eo* external ornamentations (bar= $0.4 \mu m$)

Fig. 14 Cross-sections of regions I–VI of the mature *P. microbothrium* spermatozoon. Region II: One can see that there are neither external ornamentations nor a lateral expansion of the cytoplasm exhibiting a spine-like body. *Ax* Axonemes, *Cm* cortical microtubules (bar= $0.4 \mu m$)

Fig. 15 Cross-sections of regions I–VI of the mature *P. microbothrium* spermatozoon. Region III, showing a mitochondrion (*Mt*) and a few cortical microtubules (*Cm*; bar= 0.4μ m)

Fig. 16 Cross-sections of regions I–VI of the mature *P. micro*bothrium spermatozoon. Region IV, showing the nucleus (*N*) alongside the two axonemes (*Ax*). *Mt* mitochondrion (bar= 0.4μ m)

Fig. 17 Cross-sections of regions I–VI of the mature *P. microbothrium* spermatozoon. Region V, showing the nucleus (*N*) between and alongside the two axonemes (*Ax*). *Cm* Cortical microtubules (bar= $0.4 \mu m$)

Fig. 18 Cross-sections of regions I–VI of the mature *P. microbothrium* spermatozoon. Region VI, showing the nucleus (*N*) and 1 axoneme (*Ax*). At this posterior end of the spermatozoon, the axoneme has disappeared. *Cm* Cortical microtubules (bar= 0.2μ m)

axonemes, a plasmic membrane bordered by cortical microtubules, and a nucleus with electron lucent chromatin (Figs. 8 and 9). The mature spermatid is detached from the residual cytoplasm by constriction of the arched membranes (Fig. 10).

Spermatozoon

The mature spermatozoon of *P. microbothrium* has a mitochondrion, is filiform, and tapered at both ends (Figs. 11, 12, 13, 14, 15, 16, 17, 18, and 19). Six regions from anterior to posterior could be distinguished.

Region I

It corresponds to the anterior extremity of the spermatozoon. It is characterized by the presence of two axonemes of the 9 + "1" pattern and a lateral expansion exhibiting a "spine-like body." The anterior extremity of the spermatozoon exhibits only one axoneme. The second axoneme appears then, followed by the lateral expansion. Numerous cortical microtubules can be observed on the inner side of the plasmic membrane that exhibits external ornamentations (Figs. 11, 12, 13, and 19).



Region II

It lacks external ornamentations and a mitochondrion. Cross sections of this region show two axonemes, 8 to 10 dorsal cortical microtubules and 13 to 16 ventral ones. A clear bilateral asymmetry can be observed (Figs. 14 and 19).

Region III

It is characterized by the presence of a mitochondrion that interposes itself between the two axonemes. At the posterior end of this region, the cortical microtubules terminate gradually (Figs. 15 and 19).



Fig. 19 Reconstruction of the *P. microbothrium* mature spermatozoon. Ase Anterior spermatozoon extremity, AxI axoneme 1, Ax2 axoneme 2, Az attachment zone, Cm cortical microtubules, Eo external ornamentations, Mt mitochondrion, N nucleus, Pse posterior spermatozoon extremity, Sb spine-like body

Region IV

It is characterized by the presence of a nucleus that is a fine compact cord of electron-dense material, located at the ventral field. The two axonemes are always separated by the mitochondrion that is located dorsally. The cortical microtubules are still present, but in reduced number (Figs. 16 and 19).

Region V

It lacks a mitochondrion. The nucleus is larger in crosssection than in the other regions. One can observe a few cortical microtubules underlying the plasmic membrane (Figs. 17 and 19).

Region VI

It corresponds to the posterior end of the gamete. It contains the nucleus and only one axoneme. The latter disorganizes gradually and then disappears. As a matter of fact, at the end of the spermatozoon, the cytoplasm exhibits only the nucleus and a few cortical microtubules (Figs. 18 and 19).

Discussion

In *P. microbothrium*, the general process of spermiogenesis is identical to that of other digenean species (Burton 1972; Rees 1979; Jamieson and Daddow 1983; Erwin and Halton 1983; Gracenea et al. 1997; Iomini and Justine 1997; Miquel et al. 2000; Baptista-Farias et al. 2001; Ndiaye et al. 2002, 2003a,b).

At the start of spermiogenesis, a differentiation zone forms. Flagella, elaborated from the centrioles, become parallel to the median cytoplasmic expansion and fuse with the latter after having undergone a rotation of 90° . This rotation was already observed in the majority of the digeneans. However, some trematodes show a flagella rotation of 120° (Table 1). The fusion of the flagella with the median cytoplasmic expansion is proximo-distal. This phenomenon was regularly observed in trematodes. The transverse sections of the spermatid show that fusion takes place at the level of the attachment zones that are four in number and are still seen after fusion.

We observed in *P. microbothrium* a differentiation zone, two striated roots, and an intercentriolar body. According to Burton (1972), each of these two elements plays a role during spermiogenesis. The purpose of the 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 our study, the intercentriolar body is large and located between the two centrioles and the striated roots. On both sides of a fine central band, there are three thicker dark bands separated by clear spaces. Such a structure was also observed with Corrigia vitta (Robinson and Halton 1982), Postorchigenes gymnesicus (Gracenea et al. 1997), Brachylaima (Gracenea et al. 2000), Helicometra fasciata (Levron et al. 2003), and Poracanthium furcatum (Levron et al. 2004b). However, in P. microbothrium, the external bands of the intercentriolar body are different; they 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. An intercentriolar body of the same type has, for the moment, been observed only in C. scoliocoelium (Li and Wang 1997).

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

Families and digenean species	Rf	Az	Le	Eo	Sb	Mt	Pse	References
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, 1983)
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	20					1	11	
Didymozoon sp			_	_	_	1		Justine and Mattei (1983–1984a)
Gonanodasmius sp		2	_	+	_	1		Justine and Mattei (1982a, 1984b)
Didymocystis wedli		2	_	_	_	1		Pamplona-Basilio et al. (2001)
Dinlostomatidae						1		r ampiona Basino et al. (2001)
Pharmaostomoides procuonis	00°		_	_	_			Grant et al. (1976)
Echinostomatidae	<i>J</i> 0							
Echinostoma agreni		4	-	_	_	1	N+1E	Jamini and Justina (1007) Justina (1005)
Econosioma caproni		4	Ŧ	_	_	1	IN+II	Tommi and Justine (1997), Justine (1995)
Essential housting	1200					2	117	Stitt and Esimulation (1000), Miliana et al. (2002h)
Fasciola nepalica	120	4	+	_	+	ے 1		Nultives et al. (2004)
Fasciola gigantica	90°	4	+	+	+	1	N	Ndiaye et al. (2004)
Fellodistomidae								I: (1005)
Proctoeces maculatus		4	_	+	-	1		Justine (1995)
Haematoloechidae								
Haematoloechus medioplexus	90°	4	+	+	-	1		Burton (1972) ; Justine (1995) , Justine and Matter $(1982b)$
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	Ν	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)
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		•				-		
Cevlonocotyle scoliocoelium			_	_	_			Li and Wang (1997)
Paramphistomum microbothrium	90°	4	+	+	+	1	N + Cm	Present work
	20			•			1, · Ciii	- research work

Az Attachment zones, Cm cortical microtubules, Eo extramembranous ornamentations, F flagellum, G glycogen granules, Le lateral expansion, Mt mitochondrion, N nucleus, Pse posterior spermatozoon extremity, Rf rotation of flagella, Sb spine-like body; "+" and "-" indicate the presence or the absence of the considered character, respectively

In other trematodes, the intercentriolar body is composed of nine dense bands. This is the case in *Cryptocotyle lingua* (Rees 1979), *Microphallus primas* (Castilho and Barandela 1990), and *Monorchis parvus* (Levron et al. 2004a).

The striated roots of *P. microbothrium* are very long, even exceeding the arched membranes. Their average lengths are 3.7 μ m. This phenomenon is scarce. To our knowledge, it has already been described in *Fasciola gigantica* (Ndiaye et al. 2004). Generally, the length of the striated roots does not exceed the arched membranes. This is the case, in particular, with *C. vitta* (Robinson and Halton 1982) and *Fasciola hepatica* (Ndiaye et al. 2003b). The absence of striated roots and of 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).

In *P. microbothrium*, the mature spermatid does not exhibit external ornamentations, as it is the case in the majority of studied digenean, nor "spine-like bodies," as it is in *Echinostoma caproni* (Iomini and Justine 1997), *F. hepatica* (Ndiaye et al. 2003b), and *F. gigantica* (Ndiaye et al. 2004).

The P. microbothrium, spermatozoon shows ultrastructural features similar to those described in the majority of the digeneans. It comprises two axonemes of the 9 + "1"pattern that is 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 et al. 1997; Miquel et al. 2000; Levron et al. 2003; Ndiaye et al. 2003a, 2003b). Besides this 9 + "1" pattern, there exist some other examples such as the 9 + "0" pattern. The latter is found, in particular, in the schistosomes: Schistosoma mansoni (Kitajima et al. 1976), S. bovis (Justine and Mattei 1981), S. curassoni, S. rodhaini, S. intercalatum, S. bovis, S. margrebowiei, and S. mansoni (Justine et al. 1993), and in the didymozoïdes Didymozoon sp. (Justine and Mattei 1983, 1984a). In spite of this common scheme of the spermatozoon of the Digenea, P. microbothrium spermatozoon exhibits some specific characters.

The anterior extremity of the *P. microbothrium* spermatozoon is characterized by the presence of external ornamentations outside on the plasmic membrane. The presence of such structures has also been described in the spermatozoon of other digeneans (Jamieson and Daddow 1982; Justine and Mattei 1982a,b; Erwin and Halton 1983; Orido 1988; Justine 1995; Gracenea et al. 1997; Iomini and Justine 1997; Tang et al. 1998; Miquel et al. 2000; Ndiaye et al. 2002, 2003a, 2004). On the other hand, these external ornamentations are lacking in the spermatozoa of *H. fasciata* (Levron et al. 2003) and *P. furcatum* (Levron et al. 2004b).

The external ornamentations are variably located along the spermatozoon, but they always appear at the same time as the cortical microtubules. According to Justine and Mattei (1982b), the role of these structures is unknown, but they suppose that they would occur in the fusion between the spermatic and ovular membranes during fertilization.

The anterior part of the spermatozoon of *P. microbothrium* exhibits a lateral expansion. Such an expansion has already been described in other digeneans (Table 1).

"Spine-like bodies" were described for the first time by Miquel et al. (2000) in the spermatozoon of *Opecoeloides furcatus* (Opecoelidae). More recent studies have also shown the existence of "spine-like bodies" in *Notocotylus neyrai* (Ndiaye et al. 2003a), *F. gigantica* (Ndiaye et al. 2004), and *P. furcatum* (Levron et al. 2004b). But, in these species, the "spine-like bodies" are not located on the spermatozoon lateral expansion. *P. microbothrium* spermatozoon is distinguished from that of the other digeneans by the presence of a "spine-like body" on its lateral expansion.

According to Burton (1972), the attachment zones in mature spermatozoon mark the site of the surface of fusion of flagella with the median cytoplasmic expansion. In our study, in cross-sections, four attachment zones are seen in the spermatozoon of *P. microbothrium*, as in the majority of the other species of Digenea (Table 1).

In P. microbothrium, we observed only one mitochondrion in the spermatozoon. This mitochondrion results from the fusion of several mitochondria, having migrated into the differentiation zone. The same phenomenon was described in Cryptocotyle lingua (Rees 1979), Neochasmus sp. (Jamieson and Daddow 1982), C. vitta (Robinson and Halton 1982), Bucephaloides gracilescens (Erwin and Halton 1983), Microphallus primas (Castilho and Barandela 1990), Mesocoelium monas (Iomini et al. 1997), Echinostoma caproni (Iomini and Justine 1997), O. furcatus (Miquel et al. 2000), Scaphiostomum palaearcticum (Ndiaye et al. 2002), H. fasciata (Levron et al. 2003), and F. gigantica (Ndiave et al. 2004). In other digeneans, the presence of two mitochondria was described, one located at the same level as the ornamentations; the other in the posterior part at the level of the nucleus (Orido 1988; Stitt and Fairweather 1990; Ndiaye et al. 2003a; Levron et al. 2004a,b).

The posterior part of the *P. microbothrium* mature spermatozoon contains an eccentric axoneme, a nucleus, and cortical microtubules. In cross-section, the distal extremity of this area contains only the nucleus with a broad diameter and few cortical. A posterior end of the same type, without cortical microtubules, was found only in *F. gigantica* (Ndiaye et al. 2004). In all other studied digeneans, the structure of the distal extremity exhibits a different structure (Table 1).

Cross-sections of the spermatozoon show a progressive reduction of the number of cortical microtubules from anterior to posterior extremity. In other species such as *C. vitta* (Robinson and Halton 1982), *F. hepatica* (Stitt and Fairweather 1990), and *Dicrocoelium dendriticum* (Cifrian et al. 1993), such a gradual reduction continues until complete disappearance before reaching the posterior extremity of the spermatid.

In spite of their importance in veterinary pathology, the system in Paramphistomidae, based primarily on the morphological and anatomical description of the adults, remains difficult and controversial. The ultrastructural study of spermiogenesis and the spermatozoon of these species appears to us as a tool that would be very interesting in the future to understand the taxonomy and phylogeny of the Paramphistomidae.

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