3.5. ULTRASTRUCTURE DE LA SPERMIOGENESE ET DU SPERMATOZOÏDE DE *TAENIA PARVA* BAER, 1926 (CESTODA, CYCLOPHYLLIDEA, TAENIIDAE), PARASITE DE LA GENETTE *(GENETTA GENETTA)*

Résumé :

Nous avons étudié l'ultrastructure de la spermiogenèse et du spermatozoïde de *Taenia parva,* un cestode intestinal de la genette commune, *Genetta genetta.* Chez *T. parva,* la spermiogenèse est caractérisée par le développement de l'axonème hors de l'expansion cytoplasmique. Après une petite rotation, le flagelle libre fusionne avec l'expansion cytoplasmique médiane. Ce modèle de spermiogenèse correspond au type III défini par Bâ & Marchand (1995). La zone de différentiation ne présente ni racine striée ni corps intercentriolaire. Cependant une rotation flagellaire de près de 45º est observée chez cette espèce. D'autre part, le spermatozoïde mature de *T. parva,* comme chez les autres cestodes, est filiforme, éffilé à ses deux extrémités et dépourvu de mitochondrie. La présence d'un seul corps en crête, d'une gaine periaxonémale et de cloisons intracytoplasmiques transversales, sont aussi des particularités ultrastructurales du spermatozoïde de cette espèce. Nous avons comparé le type de spermiogenèse et l'organisation ultrastructurale du spermatozoïde de *T. parva* avec les données bibliographiques existantes chez les Cyclophyllidea et, en particulier, chez les espèces de la famille des Taeniidae.

Mots clés :

Ultrastructure, spermiogenèse, spermatozoïde, *Taenia parva*, Cestoda, Cyclophyllidea, Taeniidae.

ORIGINAL PAPER

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Ultrastructure of spermiogenseis and spermatozoa of *Taenia parva* Baer, 1926 (Cestoda, Cyclophyllidea, Taeniidae), a parasite of the common genet (*Genetta genetta*)

Abstract We studied the ultrastructure of spermiogenesis and of the mature spermatozoon of *Taenia parva*, an intestinal cestode of the common genet, *Genetta genetta*. Spermiogenesis in *T. parva* is characterized by the growth of the axoneme externally to a cytoplasmic extension. After a slight rotation, the free flagellum fuses with the cytoplasmic extension. This pattern corresponds to type III spermiogenesis according to the scheme proposed by Bâ and Marchand. The zone of differentiation lacks both striated roots associated with the centrioles and the intercentriolar body between them. Nevertheless, the flagellar rotation of about 45° is observed in this species. On the other hand, the mature spermatozoon of *T. parva*, as in other cestodes, is filiform, tapered at both extremities and lacks mitochondria. The presence of a single crest-like body, periaxonemal sheath, and transverse intracytoplasmic walls are also characteristic ultrastructural features. The pattern of spermiogenesis and the ultrastructural organization of the spermatozoon of *T. parva* are compared with the available data on cyclophyllideans and, in particular, species of the family Taeniidae.

Introduction

Over the past several years, many papers have provided evidence that the ultrastructural characteristics of the spermatozoon and the process of spermiogenesis are useful tools in the interpretation of the relationships between platyhelminths (Bâ and Marchand 1995; Hoberg et al. 1997; Justine 1998, 2001). For cestodes, most ultrastructural studies on spermatology have been carried out on the Cyclophyllidea (Justine 1998, 2001; Miquel et al. 1999, 2000; Bâ et al. 2000; Hidalgo et al. 2000; Swiderski et al. 2000; Brunanska et al. 2001; Conn 2001; Swiderski 2001). For the Taeniidae, and particularly for the genus *Taenia*, there are studies on the ultrastructure of sperm for only two species: *Taenia hydatigena* (Featherston 1971) and *Taenia mustelae* (Miquel et al. 2000). Other ultrastructural contributions in this genus involve *Taenia solium*, *Taenia saginata* and *Taenia pisiformis* (Tian et al. 1998).

This paper discusses the ultrastructural organization of spermiogenesis and of the spermatozoa of *Taenia parva* Baer, 1926 (Cyclophyllidea, Taeniidae), a cestode that usually infects genets both in Africa and in southwestern Europe.

Materials and methods

Adult specimens of *T. parva* were collected live from the small intestine of a naturally infected genet, *Genetta genetta* (Carnivora, Viverridae), that had been killed on the road in Santa Eulàlia de Ronçana (Barcelona, Spain). After dissection, the adult cestodes were kept in a 0.9% NaCl solution. Different portions of mature proglottids were dissected and fixed in cold (4°C) 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer at pH 7.2 for 1 h, rinsed in a 0.1 M sodium cacodylate buffer at pH 7.2, postfixed in cold (4°C) osmium tetroxide in the same buffer for 1 h, rinsed in a 0.1 M sodium cacodylate buffer at pH 7.2, dehydrated in ethanol and propylene oxide, embedded in Spurr, and polymerized at 60°C for 48 h. Ultrathin sections were cut on a Reichert-Jung Ultracut E ultramicrotome, placed in copper grids, and stained with uranyl acetate and lead citrate, according to the methodology of Reynolds (1963). The grids were examined with a Hitachi H-600 electron microscope at 75 kV.

Results

Figures 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 and 25 show the results of our ultrastructural examinations.

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Fig. 1–8 Spermiogenesis of *Taenia parva*

Fig. 1 Zone of differentiation showing the growth of the axoneme (*Ax*) externally to the cytoplasmic extension (*Ce*). B, cytoplasmic bud; *C*, centriole; *N*, nucleus. *Bar* = 1 μm

Fig. 2 Zone of differentiation after flagellar rotation. Ce, cytoplasmic extension. *Bar* = 1 μm

Fig. 3 Several cross-sections showing the axoneme and the cytoplasmic extension (*Ce*) after flagellar rotation but before proximodistal fusion. Note the parallel disposition of cortical microtubules in the cytoplasmic extension. *Bar* = 0.5 μm
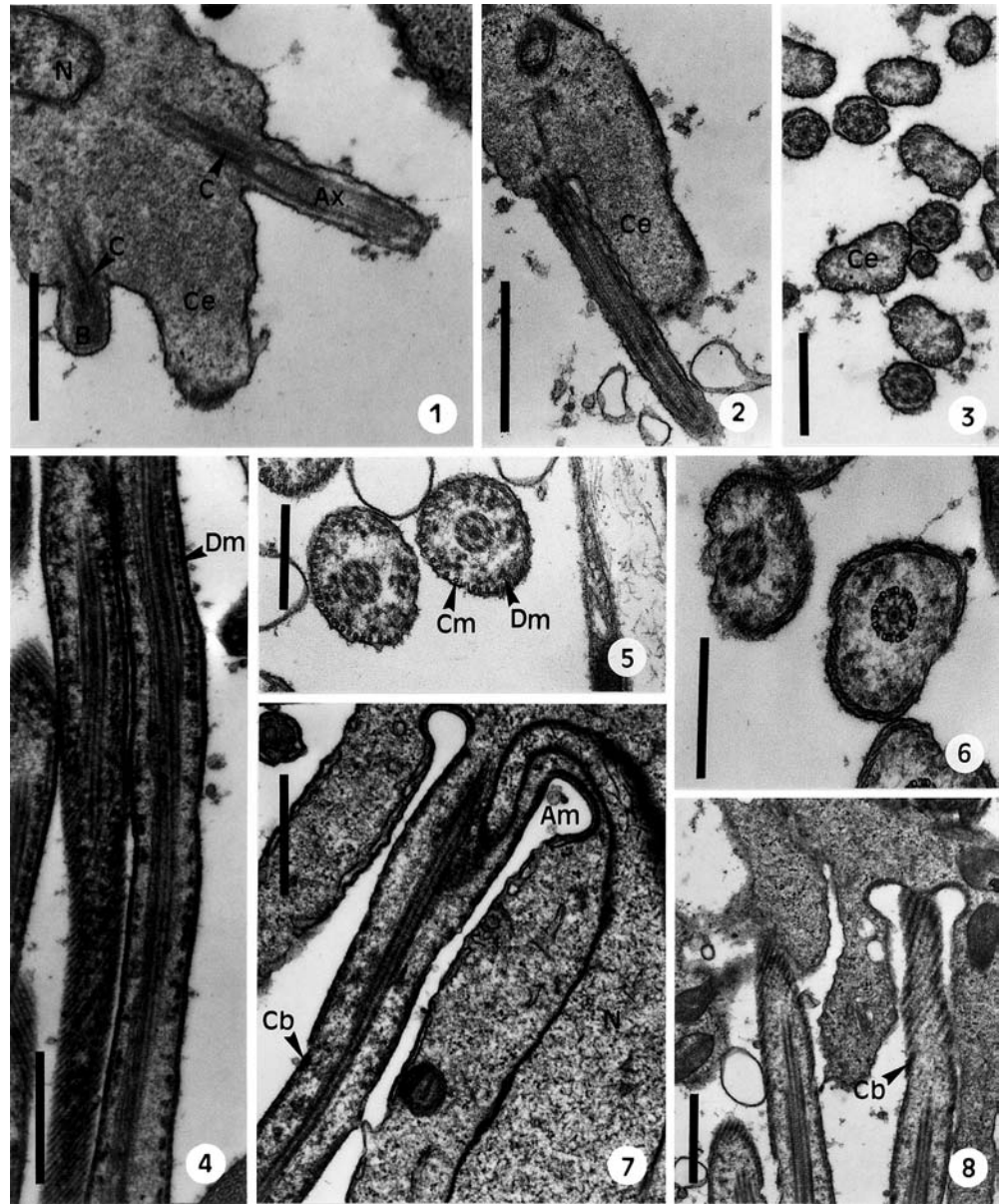
Fig. 4 Longitudinal sections of spermatids showing the presence of dense material (*Dm*). *Bar* = 1 μm

Fig. 5 Cross-sections of spermatids showing the presence of dense material (*Dm*). Cm Cortical microtubules. *Bar* = 0.5 μm

Fig. 6 Cross-sections in the area immediately posterior to the proximodistal fusion between the axoneme and the cytoplasmic extension. *Bar* = 0.5 μm

Fig. 7 Longitudinal section showing the migration of the nucleus (*N*) after the proximodistal fusion. Note the appearance of crest-like body (*Cb*). *Am* Arched membranes. *Bar* = 1 μm

Fig. 8 Longitudinal section at the level of the final stages of spermiogenesis, prior to the constriction of the ring of arched membranes. *Cb* Crest-like body. *Bar* = 1 μm

The spermiogenesis in *T. parva* (Figs. 1, 2, 3, 4, 5, 6, 7, 8, 24a–f) begins with the formation of a differentiation zone. This area is delimited at the proximal extremity by a ring of arched membranes and is bordered by cortical microtubules. The zone of differentiation contains two centrioles: one gives rise to a flagellum that grows externally to a median cytoplasmic extension with an angle of about 45°, and the other remains oriented in a cytoplasmic bud, and aborts posteriorly (Figs. 1, 24a, b). At this stage of spermiogenesis, the nucleus has already penetrated the ring of arched membranes (Fig. 24b). The flagellum moves parallel to the cytoplasmic extension and fuses with it (Figs. 2, 3). After this proximodistal fusion of the free flagellum with the cytoplasmic extension, the cortical microtubules become spiralled (Figs. 5, 6), then the nucleus migrates along the cytoplasmic extension (Fig. 7). At this stage, the cytoplasm becomes more dense (Fig. 6) and a crest-like body (Fig. 7) can be observed. Density is increased posteriorly in the periphery of the spermatid and is probably the origin of the transverse intracytoplasmic walls which are present in the mature spermatozoon (Figs. 4, 5). At the end of spermiogenesis the ring of arched membranes narrows (Fig. 8) until the spermatid detaches itself from the residual cytoplasm.

The mature spermatozoon of *T. parva* (Figs. 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25a–d) is filiform, tapered at both ends and lacks mitochondria. From the anterior to posterior extremities, we have distinguished four regions (I-IV) with differential ultrastructural features.

Region I (Figs. 9, 10, 11, 12, 13, 25a) is about 350 nm wide and constitutes the anterior extremity of the mature spermatozoon. Characteristically, this area contains an

Figs. 9–17 Mature spermatozoon of *Taenia parva*

Fig. 9 Longitudinal section of the anterior extremity of the spermatozoon. *Ac* Apical cone; *Cb* crest-like body. *Bar* = 1 μm

Fig. 10 Longitudinal section at the transition level of region *I* into region *II*. *Cb* Crest-like body; *Iw* intracytoplasmic walls. *Bar* = 1 μm

Fig. 11 Cross-section of region I at the apical cone level. *Cb* Crest-like body. *Bar* = 0.5 μm

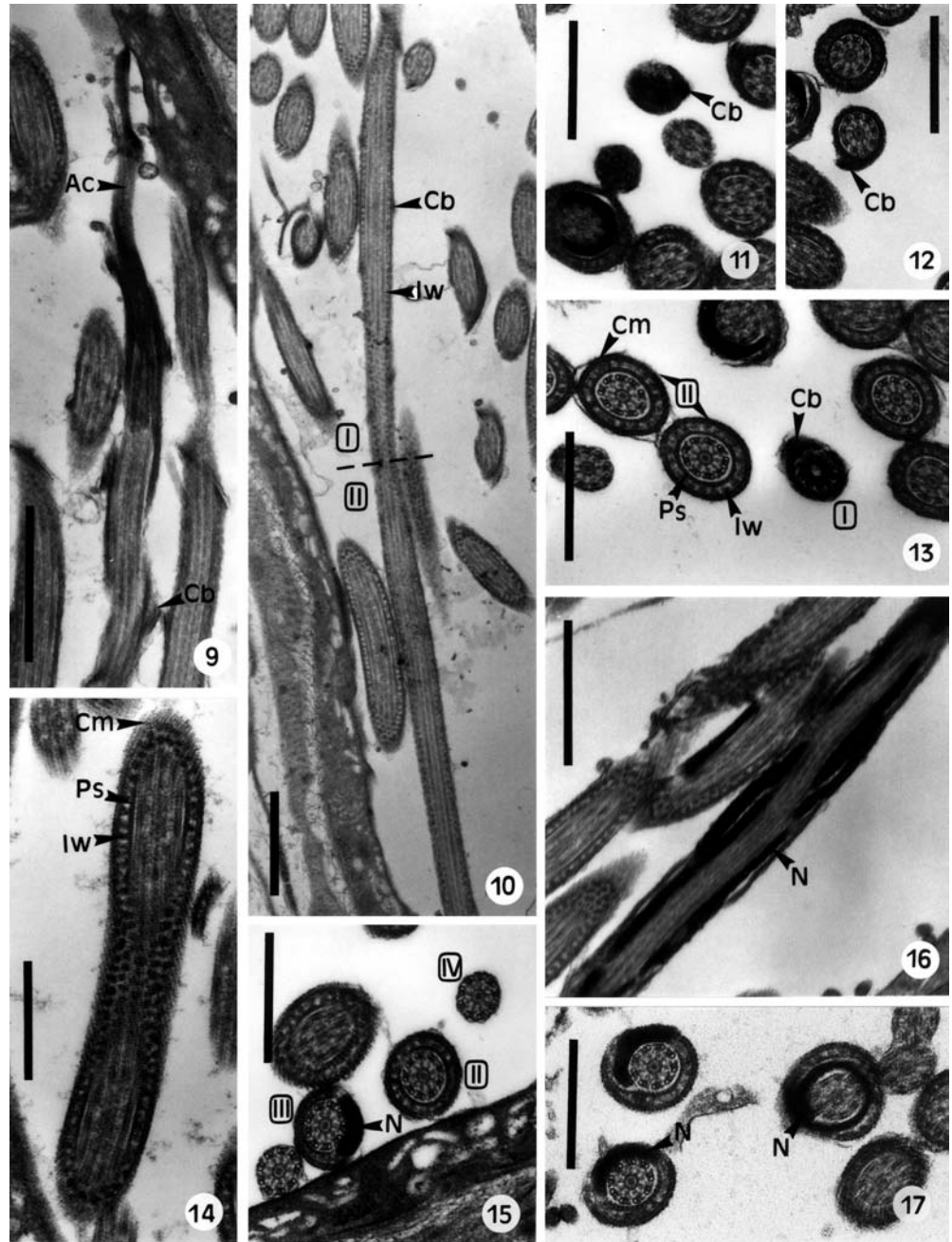**Fig. 12** Another cross-section of region I. *Cb* Crest-like body. *Bar* = 0.5 μm

Fig. 13 Cross-sections of region *I* at the level of centriole and region *II*. *Cb* Crest-like body; *Cm* cortical microtubules; *Iw* intracytoplasmic walls; *Ps* periaxonemal sheath.- *Bar* = 0.5 μm

Fig. 14 Tangential section of region II. *Cm* Cortical microtubules; *Iw* intracytoplasmic walls; *Ps* periaxonemal sheath. *Bar* = 0.5 μm

Fig. 15 Cross-sections of regions *II*, *III* and *IV*. *N* Nucleus. *Bar* = 0.5 μm

Fig. 16 Longitudinal section of region III showing areas in which the nucleus (*N*) surrounds the axoneme more than once. *Bar* = 1 μm

Fig. 17 Cross-sections of region III showing the horseshoe shape and the annular shape of the nucleus (*N*). *Bar* = 0.5 μm

apical cone (Figs. 9, 11) comprising electron dense material more than 1,900 nm long and 225 nm wide and a single helical crest-like body with a maximum thickness of 60 nm (Figs. 9, 10, 11, 12, 13). The axoneme, of the 9 + '1' pattern of Trepaxonemata, is situated in a central position. It is surrounded by a thin layer of electron lucent cytoplasm and an electron dense submembranous layer of cortical microtubules spiralled at an angle of about 45° to the hypothetical spermatozoon axis. In the posterior areas of region I, a periaxonemal sheath and a thin layer of transverse cytoplasmic walls appear between the axoneme and the cortical microtubules layer (Fig. 10).

Region II (Figs. 10, 13, 14, 15, 18, 21, 25b) is about 375 nm wide and is characterized by the absence of the crest-like body and by the gradual increase of the transverse intracytoplasmic wall layer with respect to region I (Figs. 10, 13, 14, 15, 21). The axoneme is additionally surrounded by the periaxonemal sheath (Figs. 13, 14, 15, 21). Externally to the transverse walls, the submembranous layer of spiralled cortical microtubules can be observed.

Region III (Figs. 16, 17, 18, 21, 25c) is about 485 nm wide and corresponds to the nuclear area of the mature spermatozoon. This nucleus is spiralled, surrounds the axoneme and the cross-section has a horse-shoe

Figs. 18–23 Mature spermatozoon of *Taenia parva*

Fig. 18 Longitudinal sections of mature spermatozoa showing the transition from region *II* into region *III* and region *III* into region *IV*. *Bar* = 1 µm
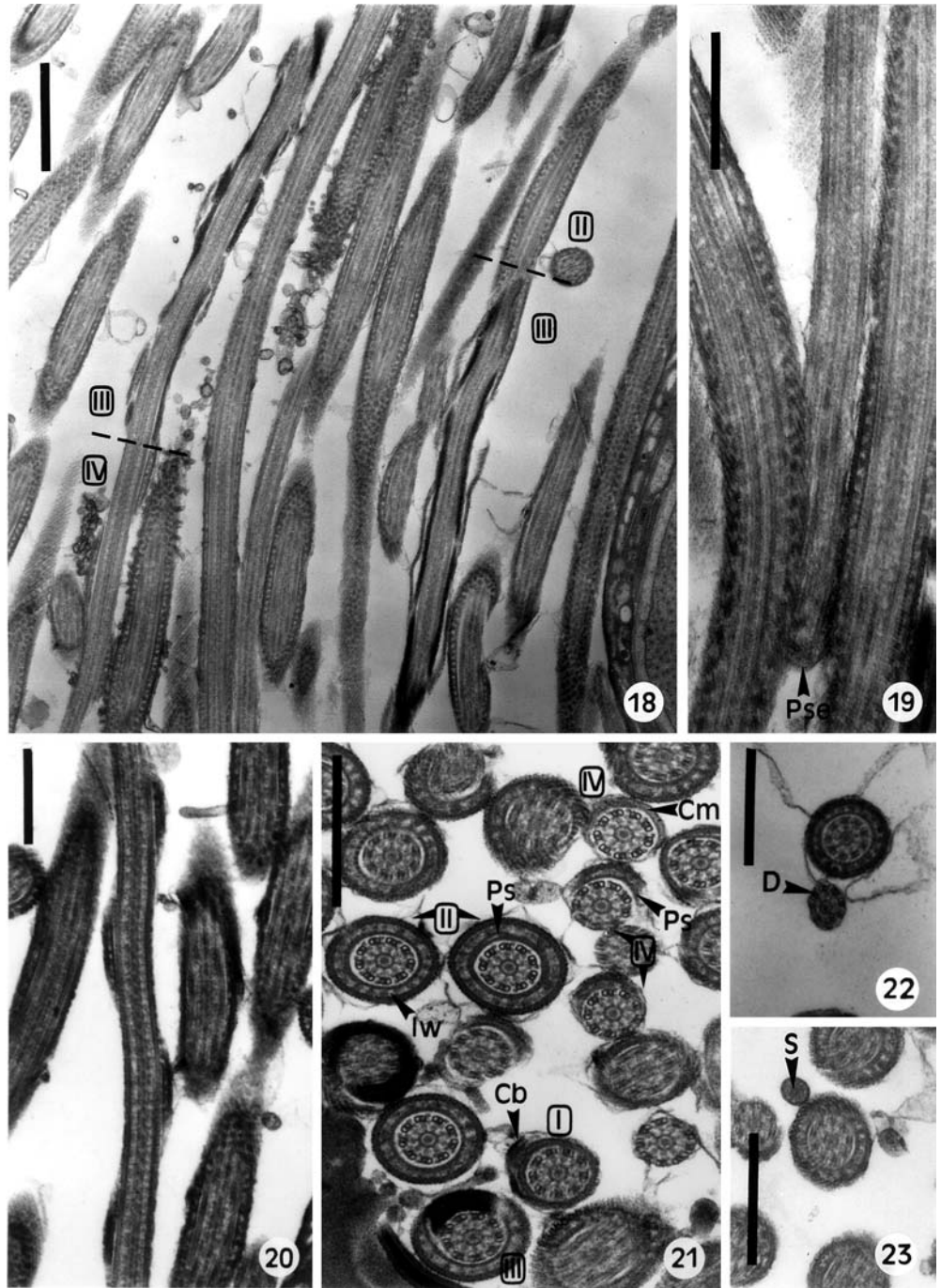
Fig. 19 Longitudinal section of the posterior extremity of the spermatozoon (*Pse*). *Bar* = 0.5 µm

Fig. 20 Longitudinal section of region IV before the disappearance of the periaxonemal sheath, intracytoplasmic walls and cortical microtubules. *Bar* = 0.5 µm

Fig. 21 Cross-sections of regions *I*, *II*, *III* and *IV*. *Cb* Crest-like body; *Cm* cortical microtubules; *Iw* intracytoplasmic walls; *Ps* periaxonemal sheath. *Bar* = 0.5 µm

Fig. 22 Cross-section of region IV showing the axonemal disorganization. *D* Doublet. *Bar* = 0.5 µm

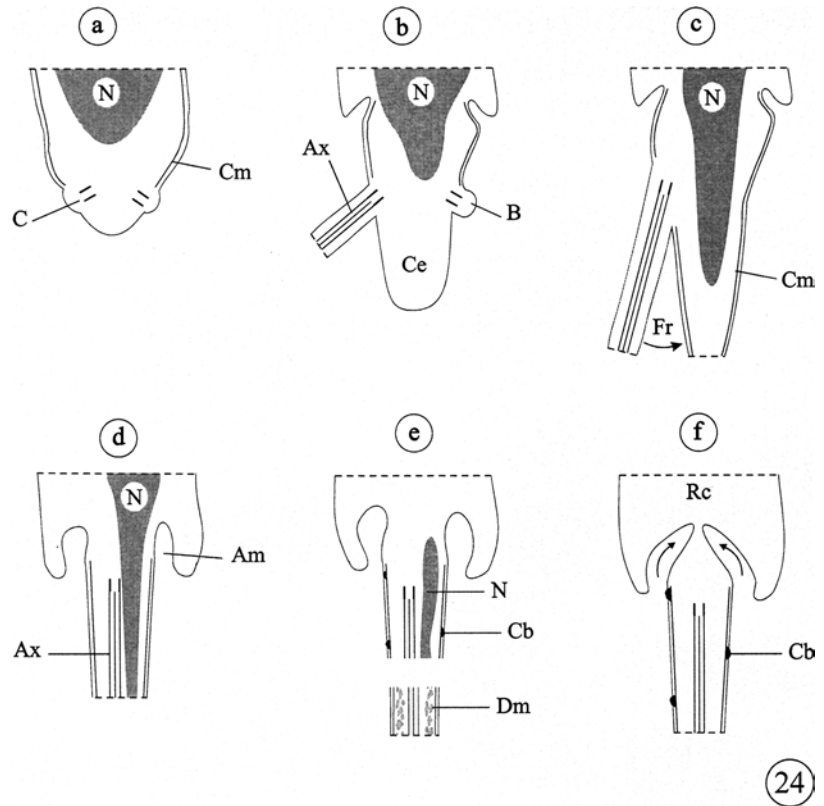**Fig. 23** Cross-sections of spermatozoon near to the posterior extremity. *S* Singlet. *Bar* = 0.5 µm

(Figs. 15, 17) or annular shape (Fig. 17). This is interposed between the rods of the periaxonemal sheath and the transverse intracytoplasmic walls (Fig. 15). Sometimes it envelopes the axoneme more than once (Fig. 16). As the nucleus increases its development, we also noted the progressive disappearance of a periaxonemal sheath, transverse intracytoplasmic walls and cortical microtubules (Fig. 18).

Region IV (Figs. 15, 18, 19, 20, 21, 22, 23, 25d) is about 280 nm wide. It is characterized by the absence of a nucleus. We noted that first the intracytoplasmic walls and periaxonemal sheath disappear (Fig. 18, 20, 21) followed by the posterior cortical microtubules (Figs. 15, 21). The cross-sections show the axoneme surrounded only by the layer of cortical microtubules (Fig. 21). Finally, the cortical microtubules disappear and the axoneme is surrounded by the plasma membrane (Figs. 15, 19, 21). Near to the posterior extremity the axoneme becomes disorganized (Fig. 22). Doublets transform into singlets before the disappearance of the central core. A few singlets reach the posterior extremity of the spermatozoon (Fig. 23).

Fig. 24 Diagram showing the main stages of spermiogenesis. *Am* Arched membranes; *Ax* axoneme; *B* cytoplasmic bud; *C* centriole; *Cb* crest-like body; *Ce* cytoplasmic crest extension; *Cm* cortical microtubules; *Dm* dense material; *Fr* flagellar rotation; *N* nucleus; *Rc* residual cytoplasm

Discussion

There are no complete data on spermiogenesis for representatives of the Taeniidae family. The study by Featherston (1971) on *T. hydatigena* contains several electron micrographs of diverse stages of spermiogenesis, although it is difficult to establish a pattern. In the early stages of spermiogenesis, the micrographs seem to indicate a migration of the nucleus to a cytoplasmic mass surrounded by cortical microtubules (Featherston 1971). The absence of an axoneme in this image makes us think of a probable type III spermiogenesis, but the author does not describe this. In this sense, a similar situation is observed to that described in the study of Tian et al. (1998). In their paper, unidentified observations of three *Taenia* species (*T. solium*, *T. saginata* and *T. pisiformis*) also seem to indicate a probable type III spermiogenesis. In addition, recent observations on sperm formation in another *Taenia* species, *T. polyacantha* infecting the red fox *Vulpes vulpes*, (Ndiaye, unpublished data) also reflects a pattern of spermiogenesis that matches with the type III proposed by Bâ and Marchand (1995). In the other genus of Taeniidae, the ultrastructural organization of the spermatozoon of *Echinococcus granulosus* was described by Morseth (1969). In this paper, the author shows an electron micrograph with transverse sections of the free flagellum without cortical microtubules and the cytoplasmic extension with cortical microtubules before the proximo-distal fusion. In the same image, Morseth (1969) also shows transverse sections of the spermatid after the proximodistal fusion. This micrograph obviously corresponds with spermatids and with type III spermiogenesis.

Bâ and Marchand (1995) postulated four types of spermiogenesis in the Eucestoda. Type I has been found in the Tetraphyllidea–Onchobothriidae, Proteocephalidea, Trypanorhyncha and Pseudophyllidea. It is characterized by the flagellar rotation and proximodistal fusion of two free flagella with a median cytoplasmic extension. Type II has been found in the Tetraphyllidea-Phyllobothriidae, Caryophyllidea and Tetrabothriidae, and is characterized by the flagellar rotation and proximodistal fusion of a single flagellum with a cytoplasmic extension. Type II has recently been also described for the spermiogenesis of a Mesocestoidid cyclophyllidean (*Mesocestoides litteratus*; Miquel et al. 1999). Types III and IV are restricted to the cyclophyllideans. Thus, type III has been found in certain Cyclophyllidea and is characterized by the absence of flagellar rotation and the presence of proximodistal fusion. Finally, type IV, also found in certain Cyclophyllidea, is characterized by the absence of both flagellar rotation and proximodistal fusion.

Our work represents the first complete study of spermiogenesis in a taeniid cestode. It shows that the spermiogenesis of *T. parva* corresponds to type III as defined by Bâ and Marchand (1995) for the Eucestoda.
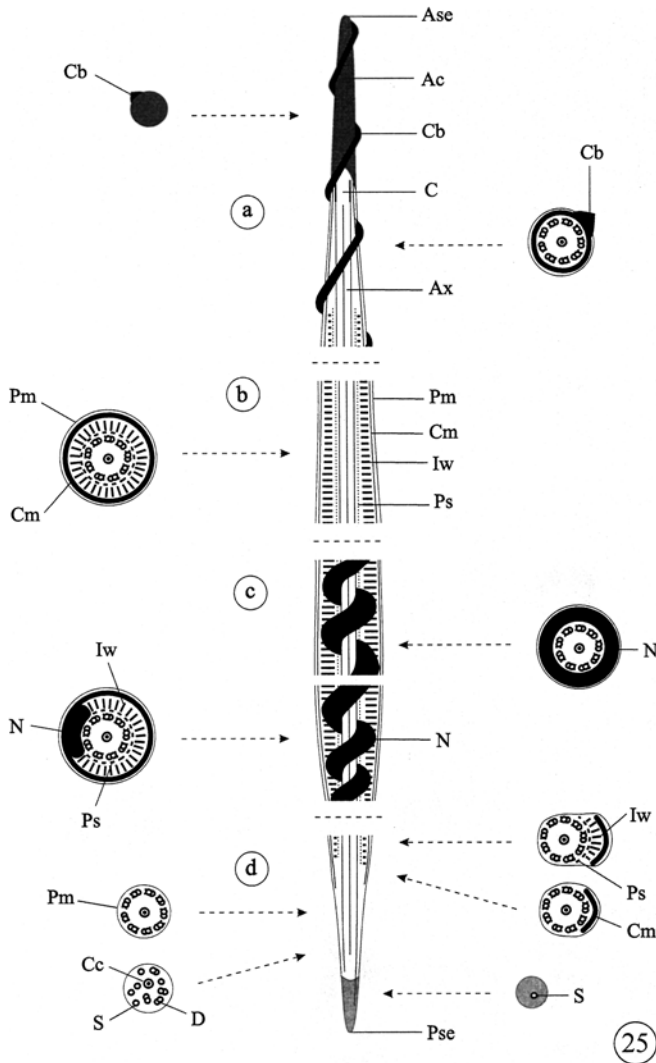
Fig. 25 Diagram showing the ultrastructural organization of the mature spermatozoon. *Ac* Apical cone; *Ase* anterior spermatozoon extremity; *Ax* axoneme; *C* centriole; *Cb* crest-like body; *Cc* central core; *Cm* cortical microtubules; *D* doublet; *Iw* intracytoplasmic walls; *N* nucleus; *Pm* plasma membrane; *Ps* periaxonemal sheath; *S* singlet

This pattern is characterized by the growth of a single axoneme outside of the cytoplasmic mass, by the absence of flagellar rotation prior to proximodistal fusion and by the absence of both striated rootlets and a intercentriolar body. In fact, the absence of flagellar rotation was described by Bâ and Marchand (1995) as an apomorphic character for cyclophyllideans. Nevertheless, in our study a slight rotation of the free flagellum of about 45° was observed. This has also recently been seen in other cyclophyllidean cestodes such as *Catenotaenia pusilla* (Catenotaeniidae) by Hidalgo et al. (2000). For the Hymenolepididae *Rodentolepis myoxi*, an oioxenous parasite of the garden dormouse *Eliomys quercinus*, a similar situation occurs (Ndiaye, unpublished data). Yet, in all of these species (*T. parva*, *C. pusilla* and *R. myoxi*) the pattern of spermiogenesis shows certain differences

in relation to type II described by Bâ and Marchand (1995) in which a single axoneme also grows externally but orthogonally to the cytoplasmic extension and posteriorly a 90° flagellar rotation occurs. In species that follow type II spermiogenesis, striated rootlets associated with the centrioles and intercentriolar body between centrioles are also present in the zone of differentiation. However, in *T. parva* this zone of differentiation lacks both striated roots associated with the centrioles and the intercentriolar body between them.

The ultrastructural features present in the mature spermatozoon of *T. parva* are similar to those observed in the spermatozoon of the related Taenidae species *T. hydatigena* and *T. mustelae* described respectively by Featherston (1971) and Miquel et al. (2000). One of the most distinctive particularities observed in the spermatozoon of *T. parva* is the length of the apical cone (around 1,900 nm). In fact, it is one of the longest of all the apical cones described in the cyclophyllideans (Table 1). Only *Skrjabinotaenia lobata* (Catenotaeniidae) (Miquel et al. 1997) has a longer one (2,500 nm). It is interesting to note the close resemblance between the morphometry of the anterior extremity of the sperm in *T. mustelae* observed by Miquel et al. (2000) and *T. parva* (present paper). According to the study of Bâ et al. (1991), a crest-like body or bodies always mark the anterior extremity of the cestode spermatozoon. It is also necessary to orient the spermatozoon of *T. parva* in relation to the four regions, from the anterior to the posterior, based on many cross-sections and longitudinal sections of different levels of the mature spermatozoon. In the Cyclophyllidea, the number of crest-like bodies varies from 1 to 12, but apart from the work of Bâ and Marchand (1994a) on *Aporina delafondi* (Anoplocephalidae), Bâ et al. (2000) on *Sudarikovina taterae* (Anoplocephalidae) and Bâ and Marchand (1992a, 1993, 1996, 1998) on Hymenolepididae species, all of the other cyclophyllideans exhibit 1 or 2 crest-like bodies (Table 1). In the Taeniidae (Table 1), only a single crest-like body has been previously described; in *T. mustelae* by Miquel et al. (2000). Moreover, in *T. parva* as in *T. mustelae* (Miquel et al. 2000) the crest-like body initiates its helical course along the spermatozoon at the level of the apical cone.

A periaxonemal sheath surrounding the axoneme has been described by many authors in the spermatozoon of 12 cyclophyllidean cestodes (Table 1). This structure is seen in regions I–III of the mature *T. parva* spermatozoon. In all of these regions, we have also observed transverse intracytoplasmic walls that progressively disappear at the beginning of region IV. The presence of these two features (periaxonemal sheath and transverse intracytoplasmic walls) in the mature spermatozoon and the absence of electron dense granular material are in agreement with the relationship established by Justine (1998) that shows a mutual exclusion between the presence/absence of both periaxonemal sheath-transverse intracytoplasmic walls and dense granules. Furthermore, Justine (1998) indicates a possible presence of dense granules only in sperm originating from a type IV

Table 1 Available data on the ultrastructural features of spermiogenesis and spermatozoon of some cyclophyllideans. All measurements are given in nm. *G* Granules; *Iw* transverse intracytoplasmic walls; *n* number of crest-like bodies; *Ps* periaxonemal sheath; +/−, presence/absence of considered characters

Families and species of Cyclophyllidea	Type of spermiogenesis	Crest-like bodies		Apical cone	Cortical microtubules	Ps	G	Iw	Reference
		n	Thickness						
Anoplocephalidae									
Anoplocephaloides dentata	IV[b]	2	140	1,400×350	30°	−	+	−	Miquel and Marchand (1998a)
Aporina delafondi	IV	5	15–40	300×150	15°	−	+	−	Bâ and Marchand (1994a)
Moniezia benedeni		2	30–40	1,000×250	Twisted	−	+	−	Bâ and Marchand (1992b)
Moniezia expansa		2	30–60	1,000×250	40°	−	+	−	Swiderski (1968), Bâ and Marchand (1992b)
Monoecocestus americanus		2			30–35°	−	+	−	MacKinnon and Burt (1984)
Paranoplocephala omphalodes		2	180	900×200	25–35°	−	+	−	Miquel and Marchand (1998b)
Sudarikovina taterae	IV	7	50–100	500×−	Twisted	+	+	−	Bâ et al. (2000)
Inermicapsifer guineensis		2	40		30–35°	+	−	+	Bâ and Marchand (1994c)
Inermicapsifer madagascariensis		2	40		30–35°	+	−	+	Swiderski (1984), Bâ and Marchand (1994c)
Mathevotaenia herpestis	III	1	70	700×300	40°	+	−	−	Bâ and Marchand (1994b)
Avitellina centripunctata		1	150–200	1,250×500	35°	−	−	+	Bâ and Marchand (1994d)
Stilesia globipunctata		1	150		50°	+	−	−	Bâ and Marchand (1992c)
Thysaniezia ovilla	IV	2	80	600×200	40–50°	−	+	−	Bâ et al. (1991)
Catenotaeniidae									
Catenotaenia pusilla	III[c]	2	75	1,750×225	40°	+	−	−	Swiderski (1970), Hidalgo et al. (2000)
Skrjabinotaenia lobata		2	60–80	2,500×200	40°	+	−	−	Miquel et al. (1997)
Davaineidae									
Cotugnia polyacantha	III	2	50–100		Twisted	+	−	+	Bâ and Marchand (1994e)
Raillietina tunetensis		2	100–200	−×300	60°	+	−	+	Bâ and Marchand (1994f)
Dilepididae									
Dilepis undula		1			35–45°	+	+	+	Swiderski et al. (2000)
Dipylidiidae									
Dipylidium caninum	III[b]	1	150	600×400	40°	−	−	−	Miquel and Marchand (1997), Miquel et al. (1998)
Hymenolepididae									
Dicranotaenia coronula	IV								Chomicz and Swiderski (1992)
Retinometra serrata		6	30	500×350	35°	−	+	−	Bâ and Marchand (1993)
Hymenolepis nana	IV	12	30–40		15°	−	+	−	Bâ and Marchand (1992a)
Hymenolepis straminea		8	50–100	750×100	30°	−	+	−	Bâ and Marchand (1996)
Vampirolepis microstoma	IV	6	100–200		20–30°	−	+	−	Bâ and Marchand (1998)
Mesocestoididae									
Mesocestoides litteratus	II	1	100–150		0°	−	+	−	Miquel et al. (1999)
Nematotaeniidae									
Nematotaenia chantalae	III	1	80		Twisted	−	+	−	Mokhtar-Maamouri and Azzouz-Draoui (1990)

	Type								Reference
Taeniidae									
Echinococcus granulosus	III				Twisted	–	–	–	Morseth (1969)
Echinococcus multilocularis					Twisted	+	+	–	Barrett and Smyth (1983), Shi et al. (1994)
Taenia spp.[a]	III				Twisted	+	+	+	Tian et al. (1998)
Taenia hydatigena					40–50°	+	–	+	Featherston (1971)
Taenia mustelae	III[c]	1	75	1,900×250	45°	–	–	+	Miquel et al. (2000)
Taenia parva	III[c]	1	60	>1,900×225	45°	+	–	+	Present paper

[a] Unidentified micrographs from *T. solium*, *T. saginata* and *T. pisiformis*
[b] Presence of thin striated roots associated with the centrioles in the zone of differentiation
[c] Presence of a flagellar rotation of about 45°

spermiogenesis. In this type of spermiogenesis, both periaxonemal sheath and transverse walls are absent. On the other hand, in all of the cyclophyllideans that have a periaxonemal sheath in the mature spermatozoon, the pattern of spermiogenesis is type III as it occurs in *T. parva*. This is the case for *Mathevotaenia herpestis* (Anoplocephalidae) studied by Bâ and Marchand (1994b), *Catenotaenia pusilla* (Catenotaeniidae) by Hidalgo et al. (2000) and *T. hydatigena* (Taeniidae) by Featherston (1971). The only exception is *Nematotaenia chantalae* (Nematotaeniidae) which follows a type III spermiogenesis and apparently lacks a periaxonemal sheath (Mokhtar-Maamouri and Azzouz-Draoui 1990).

Transverse intracytoplasmic walls are a characteristic found in the sperm of several groups of Eucestoda, such as Inermicapsiferinae and Linstowinae anoplocephalids (Bâ and Marchand 1994c, d), davaineids (Bâ and Marchand 1994e, f), dilepidids (Swiderski et al. 2000) and also in several taeniids, in particular, the *Taenia* genus (Featherston 1971; Tian et al. 1998; Miquel et al. 2000 and present paper).

The spiralled nucleus present in the mature spermatozoon of *T. parva* shows a horse-shoe to annular shape in cross-sections. The same characteristic is observed in other Taeniidae species studied in this way, such as *T. mustelae* (Miquel et al. 2000) and unidentified *Taenia* spp. *T. solium*, *T. saginata* and *T. pisiformis* (electron micrographs see Tian et al. 1998). Tian et al (1998) describe the presence of a mitochondrion in the mature spermatozoon of *Taenia* spp. We think that this represents a misinterpreted feature that probably corresponds with the annular morphology of the nucleus around the axoneme. It is important to consider that the absence of a mitochondrion is a synapomorphic character for the Eucestoda and has been observed in the more than 50 genera studied to date.

The presence of twisted cortical microtubules has been considered as a synapomorphic character for the Tetrabothriidea and Cyclophyllidea (Justine 1998). All of the cyclophyllideans studied so far present a sub-membranous layer of spiralled cortical microtubules (Table 1), with the unique exception of *Mesocestoides litteratus*, a mesocestoidid studied by Miquel et al. (1999). This cestode belongs to a group the systematic position of which has been the subject of controversy. *T. parva* also has this characteristic and the angle of the cortical microtubules to the hypothetical spermatozoon axis is about 45° as with the other *Taenia* species 40–50° in *T. hydatigena* (Featherston 1971) and 45° in *T. mustelae* (Miquel et al. 2000).

In conclusion, it appears that type III spermiogenesis, the length of the apical cone, the presence of a single crest-like body, periaxonemal sheath and also transverse intracytoplasmic walls are the most distinguishing features for *T. parva* and also for the other Taeniidae species. Nevertheless, more ultrastructural studies are needed and several characters, such as the absence of a periaxonemal sheath in the case of *T. mustelae*, require re-evaluation.

References

Bâ CT, Marchand B (1992a) Reinvestigation of the ultrastructure of spermiogenesis and the spermatozoon of *Hymenolepis nana* (Cestoda, Cyclophyllidea) parasite of the small intestine of *Rattus rattus*. Mol Reprod Dev 33:39–45

Bâ CT, Marchand B (1992b) Ultrastructural study of the spermatozoon of *Moniezia expansa* Rudolphi, 1810 and *M. benedeni* Moniez, 1879 (Cestoda, Cyclophyllidea, Anoplocephalidae). Ann Parasitol Hum Comp 67:111–115

Bâ CT, Marchand B (1992c) Ultrastructural particularities of the spermatozoon of *Stilesia globipunctata* (Cestoda) parasite of the small intestine of sheep and goats in Senegal. J Submicrosc Cytol Pathol 24:29–34

Bâ CT, Marchand B (1993) Ultrastructure of the *Retinometra serrata* spermatozoon (Cestoda), intestinal parasite of turtle-doves in Senegal. J Submicrosc Cytol Pathol 25:233–238

Bâ CT, Marchand B (1994a) Ultrastructure of spermiogenesis and the spermatozoon of *Aporina delafondi* (Cyclophyllidea, Anoplocephalidae) intestinal parasite of turtle doves in Senegal. Int J Parasitol 24:225–235

Bâ CT, Marchand B (1994b) Ultrastructure of spermiogenesis and the spermatozoon of *Mathevotaenia herpestis* (Cestoda) intestinal parasite of *Atelerix albiventris* in Senegal. Acta Zool (Stockh) 75:167–175

Bâ CT, Marchand B (1994c) Comparative ultrastructure of the spermatozoa of *Inermicapsifer guineensis* and *I. madagascariensis* (Cestoda, Anoplocephalidae, Inermicapsiferinae), intestinal parasites of rodents in Senegal. Can J Zool 72:1633–1638

Bâ CT, Marchand B (1994d) Ultrastructure of the spermatozoon of *Avitellina centripunctata* (Cestoda, Cyclophyllidea), a parasite of the small intestine of cattle in Senegal. Acta Zool (Stockh) 75:161–166

Bâ CT, Marchand B (1994e) Similitude ultrastructurale des spermatozoïdes de quelques Cyclophyllidea. Parasite 1:51–55

Bâ CT, Marchand B (1994f) Ultrastructure of spermiogenesis and the spermatozoon of *Raillietina (R.) tunetensis* (Cyclophyllidea, Davaineidae) intestinal parasite of turtle doves in Senegal. Int J Parasitol 24:237–248

Bâ CT, Marchand B (1995) Spermiogenesis, spermatozoa and phyletic affinities in the Cestoda. Mem Mus Natl Hist Nat 166:87–95

Bâ CT, Marchand B (1996) Ultrastructure of the spermatozoon of *Hymenolepis straminea* (Cyclophyllidea, Hymenolepididae), intestinal parasite of *Arvicanthis niloticus* in Senegal. Invertebr Reprod Dev 29:243–247

Bâ CT, Marchand B (1998) Ultrastructure of spermiogenesis and the spermatozoon of *Vampirolepis microstoma* (Cestoda, Hymenolepididae), intestinal parasite of *Rattus rattus*. Microsc Res Tech 42:218–225

Bâ CT, Marchand B, Mattei X (1991) Demonstration of the orientation of the Cestoda spermatozoon illustrated by the ultrastructural study of spermiogenesis and the spermatozoon of a Cyclophyllidea: *Thysaniezia ovilla* Rivolta, 1874. J Submicrosc Cytol Pathol 23:605–612

Bâ A, Bâ CT, Marchand B (2000) Ultrastructure of the spermiogenesis and the spermatozoon of *Sudarikovina taterae* (Cestoda, Cyclophyllidea, Anoplocephalidae) intestinal parasite of *Tatera gambiana* (Rodentia, Gerbillidae). J Submicrosc Cytol Pathol 32:137–144

Barret NJ, Smyth JD (1983) Obsevations on the structure and ultrastructure of sperm development in *Echinococcus multilocularis*, both in vitro and in vivo. Parasitology 87: li

Brunanska M, Nebesarova J, Scholz T, Fagerholm HP (2001) Spermiogenesis in the pseudophyllid cestode *Eubothrium crassum* (Bloch, 1779). Parasitol Res 87:579–588

Chomicz L, Swiderski Z (1992) Spermiogenesis and ultrastructure of the spermatozoon of the Cestode *Dicranotaenia coronula* (Dujardin, 1845) (Cyclophyllidea, Hymenolepididae). Proceedings of the 5th Asia-Pacific Electron Microscopy Conference, Beijing, China, pp 324–325

Conn DB (2001) Early spermatogenesis, sperm ultrastructure and spermatoferic duct cytoarchitecture in *Mesocestoides lineatus* (Platyhelminthes: Cestoda). Proceedings of the 9th International Congress on Invertebrate Reproduction and Development, Grahamstown, South Africa, p 71

Featherston DW (1971) *Taenia hydatigena*. III. Light and electron microscope study of spermatogenesis. Z Parasitenkd 37:148–168

Hidalgo C, Miquel J, Torres J, Marchand B (2000) Ultrastructural study of spermiogenesis and the spermatozoon in *Catenotaenia pusilla*, an intestinal parasite of *Mus musculus*. J Helminthol 74:73–81

Hoberg EP, Mariaux J, Justine J-L, Brooks DR, Weekes PJ (1997) Phylogeny of the orders of the Eucestoda (Cercomeromorphae) based on comparative morphology: historical perspectives and a new working hypothesis. J Parasitol 83:1128–1147

Justine J-L (1998) Spermatozoa as phylogenetic characters for the Eucestoda. J Parasitol 84:385–408

Justine J-L (2001) Spermatozoa as phylogenetic characters for the Platyhelminthes. In: Littlewood DTJ, Bray RA (eds) Interrelationships of the Platyhelminthes. Taylor and Francis, New York, pp 231–238

MacKinnon BM, Burt MDB (1984) The comparative ultrastructure of spermatozoa from *Bothrimonus sturionis* Duv. 1842 (Pseudophyllidea), *Pseudanthobothrium hanseni* Baer, 1956 (Tetraphyllidea) and *Monoecocestus americanus* Stiles, 1895 (Cyclophyllidea). Can J Zool 62:1059–1066

Miquel J, Marchand B (1997) Ultrastructure of the spermatozoon of *Dipylidium caninum* (Cestoda, Cyclophyllidea, Dilepididae), an intestinal parasite of *Canis familiaris*. Parasitol Res 83:349–355

Miquel J, Marchand B (1998a) Ultrastructure of spermiogenesis and the spermatozoon of *Anoplocephaloides dentata* (Cestoda, Cyclophyllidea, Anoplocephalidae), intestinal parasite of Arvicolidae rodents. J Parasitol 84:1128–1136

Miquel J, Marchand B (1998b) Ultrastructure of the spermatozoon of the bank vole tapeworm, *Paranoplocephala omphalodes* (Cestoda, Cyclophyllidea, Anoplocephalidae). Parasitol Res 84:239–245

Miquel J, Bâ CT, Marchand B (1997) Ultrastructure of the spermatozoon of *Skrjabinotaenia lobata* (Cyclophyllidea, Catenotaeniidae), intestinal parasite of *Apodemus sylvaticus* (Rodentia, Muridae). J Submicrosc Cytol Pathol 29:521–526

Miquel J, Bâ CT, Marchand B (1998) Ultrastructure of spermiogenesis of *Dipylidium caninum* (Cestoda, Cyclophyllidea, Dipylidiidae), an intestinal parasite of *Canis familiaris*. Int J Parasitol 28:1453–1458

Miquel J, Feliu C, Marchand B (1999) Ultrastructure of spermiogenesis and the spermatozoon of *Mesocestoides litteratus* (Cestoda, Mesocestoididae). Int J Parasitol 29:499–510

Miquel J, Hidalgo C, Feliu C, Marchand B (2000) Sperm ultrastructure of *Taenia mustelae* (Cestoda, Taeniidae), an intestinal parasite of the weasel, *Mustela nivalis* (Carnivora). Invert Reprod Dev 38:43–51

Mokhtar-Maamouri F, Azzouz-Draoui N (1990) Étude de la spermiogénèse et de l'ultrastructure du spermatozoïde de *Nematotaenia chantalae* Dollfus, 1957 (Cestoda, Cyclophyllidea, Nematotaeniidae). Ann Parasitol Hum Comp 65:221–228

Morseth DJ (1969) Spermtail finestructure of *Echinococcus granulosus* and *Dicrocoelium dendriticum*. Exp Parasitol 24:47–53

Reynolds ES (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 17:208–212

Shi DZ, Liu DS, Wang SK, Craig PS (1994) The ultrastructure of *Echinococcus multilocularis*. Chin J Parasite Dis Control 7:40–41

Swiderski Z (1968) The fine structure of the spermatozoon of sheep tapeworm, *Moniezia expansa* (Rud., 1810) (Cyclophyllidea, Anoplocephalidae). Zool Pol 18:475–486

Swiderski Z (1970) An electron microscope study of spermatogenesis in cyclophyllidean cestodes with emphasis on the comparison of the fine structure of mature spermatozoa. J Parasitol 56:337–338

Swiderski Z (1984) Ultrastructure of the spermatozoon of davaineid cestode *Inermicapsifer madagascariensis*. Proc Electron Microsc Soc South Africa:131–132

Swiderski Z (2001) Ultrastructure of spermatogenesis and the mature spermatozoon of *Glaridacris catostomi* Cooper, 1920 (Cestoidea, Caryophyllidea). Proceedings of the 9th International Congress of Invertebrate Reproduction and Development, Grahamstown, South Africa, p 91

Swiderski Z, Salamatin RV, Tkach VV (2000) Electron microscopical study of spermatozoa of the cestode *Dilepis undula* (Cyclophyllidea, Dilepididae). Vest Zool 34:93–97

Tian X, Yuan L, Huo X, Han X, Li Y, Xu M, Lu M, Dai J, Dong L (1998) Ultrastructural observation on the transformation of the spermatozoon in spermatogenesis of taeniid cestodes. Chin J Parasitol Parasite Dis 16:269–273