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7. Ultrastructure of spermiogenesis and the spermatozoon in cyclophyllidean cestodes

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Abstract. The usefulness of the ultrastructural characters of spermiogenesis and of the spermatozoon in the interpretation of relationships in the Platyhelminthes has been widely demonstrated. The present paper provides a review and an update on the ultrastructural knowledge on spermiogenesis and on the spermatozoon in cyclophyllidean cestodes. For each family of cyclophyllideans the pattern of spermiogenesis and the type of sperm cell is provided. Moreover, the most interesting characteristics of both spermiogenesis and the spermatozoon are compiled and illustrated for each family. Finally, new spermatological data on some species of the Anoplocephalidae and the Taeniidae are provided.

Introduction

The utility of ultrastructural characters of spermiogenesis and spermatozoa as a valuable tool for the elucidation of phylogenetic relationships in the

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Platyhelminthes has been widely demonstrated [1-13]. To date, the ultrastructure of spermiogenesis and/or the spermatozoon has been studied in more than 125 species of eucestodes belonging to the orders Bothriocephalidea, Caryophyllidea, Cyclophyllidea, Diphyllidea, Diphyllbothriidea, Haplobothriidea, Lecanicephalidea, Proteocephalidea, Spathebothriidea, Tetrabothriidea, Tetraphyllidea and Trypanorhyncha [14-27].

The Cyclophyllidea is undoubtedly the most extensively studied order, and data on the ultrastructure of spermiogenesis and/or spermatozoa is available for more than 60 species. According to the Global Cestode Database [28], the order Cyclophyllidea is constituted by 14 to 18 families, 380 to 400 genera and more than 3,000 species. To date, there are spermatological data on 12 families of cyclophyllideans, which will be analyzed in the present work. The studied families are Anoplocephalidae, Catenotaeniidae, Davaineidae, Dilepididae, Dipylidiidae, Gryporhynchidae, Hymenolepididae, Mesocestoididae, Metadilepididae, Nematotaeniidae, Paruterinidae and Taeniidae. Thus, the aim of the present chapter is to provide a review and an update concerning the ultrastructural knowledge on spermiogenesis and on the spermatozoon of cyclophyllidean cestodes.

1. Materials and methods

In the present chapter, new and preliminary spermatological results are showed concerning three species, namely *Thysanotaenia congolensis*, *Echinococcus granulosis* and *Echinococcus multilocularis*. Other illustrations refer to original TEM micrographs of previously published studies. These are *Gallegoides arfaai* [29], *Raillietina micracantha* [30], *Molluscotaenia crassiscolex* [31], *Mesocestoides lineatus* [32] and *Taenia taeniaeformis* [33].

Live specimens of *T. congolensis* were isolated from naturally infected black rats (*Rattus rattus*) from Sao Domingos and Orgaos (Cape Verde). Specimens of *E. multilocularis* were obtained from naturally infected red foxes (*Vulpes vulpes*) from La Roche sur Foron (France). Finally, *E. granulosis* were isolated from experimentally infected dogs from Sidi Thabet (Tunisia).

Adult studied tapeworms were immediately rinsed with a 0.9 % NaCl solution. Later, they were fixed in cold (4 °C) 2.5 % glutaraldehyde in a 0.1 M sodium cacodylate buffer at pH 7.4 for a minimum of 2 h, rinsed in 0.1 M sodium cacodylate buffer at pH 7.4, post-fixed in cold (4 °C) 1 % osmium tetroxide with 0.9 % potassium ferricyanide [K₃Fe(CN)₆] in the same buffer for 1 h, rinsed in milliQ water, dehydrated in an ethanol series and propylene

oxide, embedded in Spurr's resin and polymerised at 60 °C for 72 h. Ultrathin sections (60–90 nm thick) of mature segments at the level of the *vas efferens* and testes were obtained in a Reichert-Jung Ultracut E ultramicrotome. Sections were placed on copper grids and double-stained with uranyl acetate and lead citrate according to the Reynolds [34] methodology. The grids were examined in a JEOL 1010 transmission electron microscope operated at 80 kV, in the “Centres Científics i Tecnològics” of the University of Barcelona (CCiTUB).

2. Spermiogenesis and the spermatozoa in the Cyclophyllidea

Spermatogenesis in tapeworms is of the rosette type. The primary spermatogonium divides mitotically, forming two secondary spermatogonia. All further divisions occur simultaneously, resulting in a rosette of four tertiary, then eight quaternary spermatogonia and sixteen primary spermatocytes. The first meiotic division forms thirty-two secondary spermatocytes and after the second meiotic division, sixty-four spermatids are formed.

The initial stages of spermiogenesis in all cestodes are similar. They are characterized by the formation of a zone of differentiation delimited by a ring of arched membranes and supported by cortical microtubules, and by a change in the density of the nucleus. The zone of differentiation contains two centrioles and sometimes an intercentriolar body and striated rootlets. A cytoplasmic extension elongates from this zone of differentiation and the two centrioles give rise to one or two flagella. Bâ & Marchand [7] described four types of spermiogenesis in the Cestoda mainly characterized by the formation of one or two flagella and by the presence/absence of flagellar rotation and proximodistal fusion between the flagellum/a and the cytoplasmic extension. All studied cyclophyllideans present spermiogenesis of pattern 3 or 4, except for mesocestoidids [32, 35] (see Table 1).

The spermatozoa of tapeworms are filiform cells, tapered at both extremities and lacking mitochondria. The absence of mitochondria in eucestode spermatozoa is a clear spermatological synapomorphy. Considering diverse characters, Levron *et al.* [13] described seven types of spermatozoa in the eucestodes. These characters include the presence of one or two axonemes, the parallel or spiralled pattern of cortical microtubules and nucleus, and the presence/absence of crested bodies, periaxonemal sheath and intracytoplasmic walls. All cyclophyllideans exhibit spermatozoa of types V, VI or VII, containing only one axoneme, with crested body/ies, twisted cortical microtubules and a nucleus spiralled around the axoneme

(see Table 2). Some characters such as the presence/absence of a periaxonemal sheath surrounding the axoneme and the presence/absence of transverse intracytoplasmic walls connecting the layer of cortical microtubules and the periaxonemal sheath allow differentiating these three types of sperm cells in cyclophyllideans. Mesocestoidids are the unique exception in the model of sperm cells within cyclophyllideans, showing a parallel disposition of cortical microtubules [32, 35] and thus a type IV spermatozoon.

2.1. Family Anoplocephalidae

In the family Anoplocephalidae there are ultrastructural studies on the spermiogenesis and/or the spermatozoon in 16 species belonging to the different subfamilies of anoplocephalids. These are *Anoplocephaloides dentata*, *Aporina delafondi*, *Gallegoides arfaai*, *Moniezia benedenii*, *Moniezia expansa*, *Monoecocestus americanus*, *Mosgovoyia ctenoides*, *Paranoplocephala omphalodes* and *Sudarikovina taterae* (Anoplocephalinae) [29, 36-44], *Inermicapsifer guineensis* and *Inermicapsifer madagascariensis* (Inermicapsiferinae) [45, 46], *Mathevotaenia herpestis* and *Oochoristica agamae* (Linstowiinae) [47, 48], and *Avitellina centripunctata*, *Avitellina lahorea*, *Stilesia globipunctata* and *Thysaniezia ovilla* (Thysanosomatinae) [49-52]. The type of sperm cells varies in this family. Thus, the studied anoplocephalines and also *Thysaniezia* [29, 36, 37, 39, 42-44, 52] exhibit the type V sperm of Levron *et al.* [13], which is mainly characterized by the absence of periaxonemal sheath and intracytoplasmic walls (Fig. 1a). Both *Inermicapsifer* studied species exhibit type VII sperm, characterized by the presence of periaxonemal sheath and intracytoplasmic walls. Moreover, preliminary observations on another inermicapsiferine (*Thysanotaenia congolensis*) show the same type VII sperm cells (Fig. 1d). In the Linstowiinae the spermatozoon is of type VI with periaxonemal sheath but without intracytoplasmic walls. In the subfamily Thysanosomatinae the three studied genera exhibit three different types of male gametes: the above mentioned *Thysaniezia* presents a type V spermatozoon [52], whereas *Stilesia* presents a type VI spermatozoon [51] and *Avitellina* presents a type VII spermatozoon [49].

Crested body or bodies always mark the anterior extremity of the spermatozoon, constituting a constant character in all cyclophyllideans. In this order the number of crested bodies is usually one or two. Most anoplocephalids exhibit one or two crested bodies in the anterior spermatozoon extremity (see Table 2). However, there are two species belonging to the subfamily Anoplocephalinae that exhibit more than two

crested bodies, namely *A. delafondi* [37] with five and *S. taterae* [44] with seven crested bodies.

In inermicapsiferines only scarce data on spermatogenesis of *I. madagascariensis* in known [46], and details on spermiogenesis are not given. For the Anoplocephalinae in which spermiogenesis has been studied [36-38, 40, 42, 44], this process follows the type 4 of Bâ & Marchand [7] characterized by the absence of proximodistal fusion. In the Linstowiinae, pattern 3 spermiogenesis has been described in *M. herpestis* [47]. For the Thysanosomatinae only data on *T. ovilla* are known and spermiogenesis follows the pattern 4 [52].

Table 1. Ultrastructural data on spermiogenesis in cyclophyllideans.

Family and subfamily	Character				
	Type*	SR	IB	FR	PF
Anoplocephalidae					
Anoplocephalinae	4	-/vsr	-	-	-
Inermicapsiferinae	?	?	?	?	?
Linstowiinae	3	-	-	-	+
Thysanosomatinae	4	-	-	-	-
Catenotaeniidae					
Catenotaeniinae	3	-	-	+ (<90°)	+
Skrjabinotaeniinae	?	?	?	?	?
Davaineidae					
Davaineidae	3	-	-	-	+
Dilepididae					
Dilepididae	4	-	-	-	-
Dipylidiidae					
Dipylidiidae	3	+/vsr	-	-	+
Gryporhynchidae					
Gryporhynchidae	4	-	-	-	-
Hymenolepididae					
Hymenolepididae	4	-	-	-	-
Mesocestoididae					
Mesocestoididae	2	+	+	+ (90°)	+
Metadilepididae					
Metadilepididae	3	vsr	-	-	+
Nematotaeniidae					
Nematotaeniidae	3	-	-	-	+
Paruterinidae					
Paruterinidae	3	vsr	-	+ (<90°)	+
Taeniidae					
Echinococcinae	3	vsr	?	?	+
Taeniinae	3	-/vsr	-	+ (<90°)/-	+

FR – flagellar rotation, IB – intercentriolar body, PF – proximodistal fusion, SR – striated rootlets, vsr – vestigial striated rootlets, ? – unknown data, +/- – presence/absence of character, * – type according to Bâ & Marchand [7].

During spermiogenesis, some anoplocephalids such as *A. dentata*, *G. arfaai*, *M. expansa* and *M. ctenoides* exhibit thin striated rootlets or spiral rootlets associated to centrioles [36, 38, 40, 42]. In order to increase homogeneity in the designation of the previously described non-typical striated rootlets, the common designation of vestigial striated rootlets was proposed [see 38]. Similar root-like structures, named as thin, filamentous or vestigial striated rootlets, also occur in species from other families (Dipylidiidae, Metadilepididae, Paruterinidae and Taeniidae) [21, 53-58].

2.2. Family Catenotaeniidae

In catenotaeniids only two species were studied, *Catenotaenia pusilla* (Catenotaeniinae) and *Skrjabinotaenia lobata* (Skrjabinotaeniinae) [59, 60]. The spermatozoon has been characterized in both subfamilies and corresponds to the type VI [13]. With respect to spermiogenesis, there is data concerning only *C. pusilla* [59] and it follows the pattern 3 [7]. However, a flagellar rotation of about 45° was observed in this species [59].

Table 2. Ultrastructural data on the spermatozoon in cyclophyllideans.

Family and subfamily	Character						
	Type*	CB	CM	N	PS	IW	G
Anoplocephalidae							
Anoplocephalinae	V	2, 5, 7	15-45°	Spi	-	-	+
Inermicapsiferinae	VII	2	45°	Spi	+	+	-
Linstowiinae	VI	1	40°	Spi	+	-	-
Thysanosomatinae	V/VI/VII	1, 2	35-50°	Spi	+/-	+/-	+/-
Catenotaeniidae	VI	2	40°	Spi	+	-	-
Davaineidae	VII	2	45-60°	Spi	+	+	+
Dilepididae	VI	1, 2	30-45°	Spi	+	-	+
Dipylidiidae	VI	1	40°	Spi	+	-	-
Gryporhynchidae	VI	1	45°	Spi	+	-	+
Hymenolepididae	V	6, 8, 9, 10, 12	15-30°	Spi	-	-	+
Mesocestoididae	IV	1	0°	Spi	-	-	+
Metadilepididae	VII	1	30-40°	Spi	+	+	-
Nematotaeniidae	V	1	Spi	Spi	-	-	+
Paruterinidae	VII	1	35-40°	Spi	+	+	+
Taeniidae							
Echinococcinae	VII	1, 2?	Spi	Spi	+	+	-
Taeniinae	VII	1	40-50°	Spi	+	+	-

CB – number of crested bodies, CM – angle of cortical microtubules, G – electron-dense granules, IW – intracytoplasmic walls, N – nucleus, PS – periaxonemal sheath, Spi – spiralled, ? – unknown data, +/- – presence/absence of character, * – type according to Levron *et al.* [13].

2.3. Family Davaineidae

Seven species of davaineids were analysed: *Cotugnia polyacantha*, *Paroniella reynoldsae*, *Raillietina baeri*, *Raillietina carneostrobilata*, *Raillietina micracantha*, *Raillietina tunetensis* and *Raillietina vinagoi* [30, 61-66]. The spermatozoon in this family corresponds to type VII according to Levron *et al* [13] (Fig. 1e). In davaineids, spermiogenesis has been studied only in *R. micracantha* and *R. tunetensis* [30, 65]. In both cases spermiogenesis follows the pattern 3 of Bâ & Marchand [7].

2.4. Family Dilepididae

There are spermatological studies concerning five species of Dilepididae. These are *Anomotaenia quelea*, *Angularella beema*, *Dilepis undula*, *Kowalewskiella glareola* and *Molluscotaenia crassiscolex* [18, 31, 67-69]. The ultrastructural organization of the mature spermatozoon of dilepidids corresponds to the type VI of Levron *et al.* [13] (Fig. 1c). Available data on spermiogenesis concerns only *A. beema* and *M. crassiscolex* [31, 67], corresponding to the pattern 4 of Bâ & Marchand [7].

2.5. Family Dipylidiidae

There are ultrastructural studies on the spermiogenesis and the spermatozoon of *Dipylidium caninum*, *Joyeuxiella echinorhyncoides* and *Joyeuxiella pasqualei* [53, 54, 70, 71]. The ultrastructural organization of their spermatozoa corresponds to the type VI [13]. Spermiogenesis in these species is also well described and follows the pattern 3 [7]. However, striated rootlets are described in these species. In the case of *Joyeuxiella* spp. two well-developed striated rootlets associated to both centrioles were observed [71]. In *D. caninum* there are several thin striated rootlets [53, 54].

2.6. Family Gryporhynchidae

In this family, only *Valipora mutabilis* [72] has been studied. Spermiogenesis follows the pattern 4 of Bâ & Marchand [7] and the mature spermatozoon is of type VI of Levron *et al.* [13].

2.7. Family Hymenolepididae

Numerous species of this family have been the subject of spermatological studies. To our knowledge these are *Cladogynia*

guberiana, *Cladogynia serrata*, *Dicranotaenia coronula*, *Diorchis parvogenitalis*, *Echinocotyle dolosa*, *Hymenolepis diminuta*, *Hymenolepis sulcata*, *Monorcholepis dujardini*, *Rodentolepis fraterna*, *Rodentolepis microstoma*, *Rodentolepis myoxi*, *Rodentolepis nana* and *Rodentolepis straminea* [73-85].

However, most of them consist in incomplete and partial analyses of the spermatozoon. There are consistent studies of the spermatozoon in the case of *C. serrata*, *E. dolosa*, *R. microstoma*, *R. nana* and *R. straminea* [74, 77, 83-85] and the ultrastructural organization of sperm cells for all these species corresponds to the type V [13].

Spermiogenesis is well known for *R. microstoma* and *R. nana* [83, 84], corresponding to the pattern 4 of Bâ & Marchand [7].

In hymenolepidids the high number of crested bodies present in the spermatozoon is particularly interesting (see Table 2). There are six crested bodies in *C. serrata* [74], up to eight in *E. dolosa* and *R. straminea* [77, 85], up to nine in *H. sulcata* and *R. myoxi* [81], up to 10 in *R. fraterna* [81] and 12 in *H. nana* [84].

2.8. Family Mesocestoididae

The spermiogenesis and the ultrastructural organization of the spermatozoon of two mesocestoidids (*Mesocestoides lineatus* and *Mesocestoides litteratus*) have been studied [32, 35].

In contrast with the remaining cyclophyllideans, these species show a parallel arrangement of cortical microtubules in the spermatozoon and thus, the sperm cell is of type IV of Levron *et al.* [13] (Fig. 1b).

With respect to spermiogenesis, the pattern is also particular in comparison with the remaining cyclophyllideans. Mesocestoidids follow the pattern 2 of Bâ & Marchand [7], which is characterized by the formation of a single flagellum that rotates and fuses proximodistally with a cytoplasmic extension. Both species also exhibit a reduced intercentriolar body and well-developed striated rootlets associated to centrioles in the zone of differentiation [32, 35].

Both the parallel arrangement of cortical microtubules in the mature spermatozoon and the pattern 2 of spermiogenesis with their associated characteristics are unique within cyclophyllideans and constitute the plesiomorphic condition of these characters. These ultrastructural results are in agreement with other morphologic and molecular data, confirming the problematic systematics of mesocestoidids [see 32, 35].

2.9. Family Metadilepididae

Only *Skrijabinoporus merops* has been analysed in this family [55]. The spermatozoon is of type VII of Levron *et al.* [13], containing both periaxonemal sheath and intracytoplasmic walls. Spermiogenesis has also been described and follows the pattern 3 of Bâ & Marchand [7]. Additionally, vestigial striated rootlets associated to centrioles were observed [55].

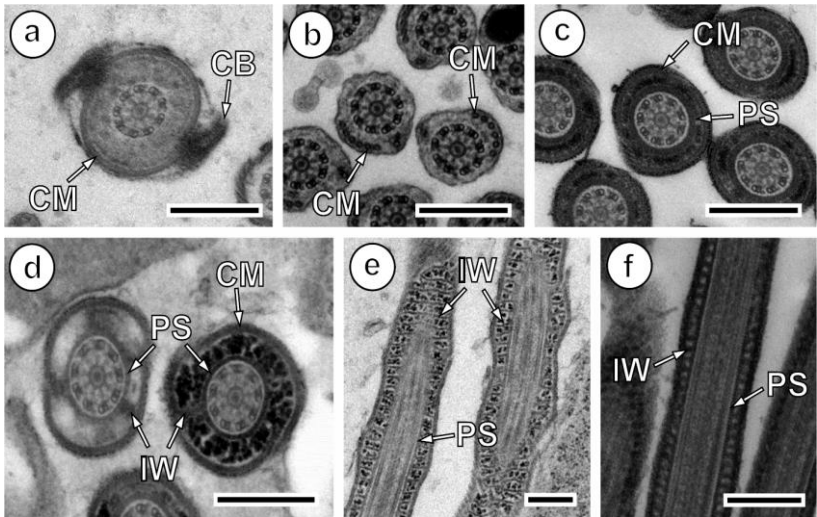


Figure 1. Longitudinal and cross-sections of spermatozoa of a) *Gallegoides arfaai*, b) *Mesocestoides lineatus*, c) *Molluscotaenia crassiscolex*, d) *Thysanotaenia congolensis*, e) *Raillietina micracantha*, and f) *Taenia taeniaeformis*. CB – crested bodies, CM – cortical microtubules, IW – intracytoplasmic walls, PS – periaxonemal sheath. Scale bars = 0.3 μm .

2.10. Family Nematotaeniidae

To date, spermatological data from nematotaeniids are available concerning only *Cylindrotaenia hickmani* and *Nematotaenia chantalae* [86–88]. In *C. hickmani* there are two ultrastructural studies on the cirrus pouch and sperm ducts [86, 87] in which some sections of spermatozoa are shown. As in *N. chantalae* [88], the ultrastructural organization of the spermatozoon seems to be of type V [13]. Concerning spermiogenesis, only data on

N. chantalae exists [88] and this process corresponds to pattern 3 of Bâ & Marchand [7].

2.11. Family Paruterinidae

Three paruterinids were the subject of ultrastructural studies on spermiogenesis and on the mature spermatozoon: *Anonchotaenia globata*, *Notopentorchis* sp. and *Triaenorhina rectangula* [21, 56, 57]. All three paruterinids exhibit a type VII spermatozoon according to the Levron *et al.* models [13] and the pattern 3 spermiogenesis of Bâ & Marchand [7]. As mentioned above, paruterinids present vestigial striated rootlets associated to centrioles. Moreover, a non-parallel development of the free flagellum was described and thus, a flagellar rotation occurs before the proximodistal fusion between flagellum and cytoplasmic extension.

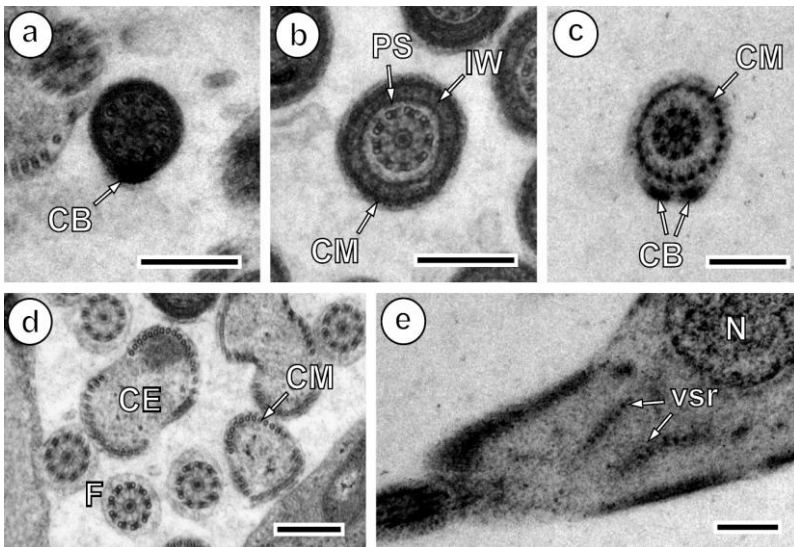


Figure 2. Spermiogenesis and the spermatozoon of *Echinococcus* spp. a) Spermatozoon of *E. granulosus*, b) Spermatozoon of *E. multilocularis*, c and d) Spermatids of *E. granulosus*, and e) Zone of differentiation of *E. multilocularis*. CB – crested bodies, CE – cytoplasmic extension, CM – cortical microtubules, F – flagellum, IW – intracytoplasmic walls, N – nucleus, PS – periaxonemal sheath, vsr – vestigial striated rootlets. Scale bars = 0.3 μ m.

2.12. Family Taeniidae

In this family there are spermatological studies on eight species of the genus *Taenia*, namely *T. crassiceps*, *T. hydatigena*, *T. mustelae*, *T. parva*, *T. pisiformis*, *T. saginata*, *T. solium* and *T. taeniaeformis* [27, 33, 58, 89-96], and in two species of the genus *Echinococcus*, namely *E. granulosus* and *E. multilocularis* [97-99]. However, for some of these species, the spermatological data are only partial [89, 90, 93, 94, 96, 97-99].

In the genus *Taenia*, a type VII spermatozoon of Levron *et al.* [13] was described (Fig. 1f). Spermiogenesis follows the pattern 3 of Bâ & Marchand [7], although good descriptions are published concerning only two species, *T. parva* and *T. taeniaeformis* [58, 93]. In both species a flagellar rotation was described and in the case of *T. taeniaeformis*, additionally, the presence of vestigial striated rootlets was illustrated [58].

In the genus *Echinococcus* there are some published papers that show scarce results on *E. granulosus* and *E. multilocularis* [97-99]. Nevertheless, new preliminary observations on these two species indicate a pattern 3 spermiogenesis of Bâ & Marchand [7] with the presence of vestigial striated rootlets associated to centrioles in both species (Fig. 2d,e). Concerning the spermatozoon, the presence of both periaxonemal sheath and transverse intracytoplasmic walls characterize the sperm cell as belonging to the type VII of Levron *et al.* [13] (Fig. 2a,b). In the case of *E. multilocularis*, two crested bodies have been observed during spermiogenesis, but this fact is still to be confirmed in the mature spermatozoon (Fig. 2c).

3. Conclusion

The present review highlights the usefulness of spermatological data for the systematics and phylogenetics of cestodes. Indeed, the last decade of ultrastructural research on spermiogenesis and sperm structure provided extensive information. This fact leads to a considerable increase in the existing dataset of characters suitable for the assessment of phylogenetic relationships of cestodes. In the case of Cyclophyllidea, future research should be focused on species belonging to those families lacking spermatological studies (Acoleidae, Amabiliidae, Dioecocestidae and Progynotaeniidae), but also on other families that have been poorly studied.

In cyclophyllideans, both spermiogenesis patterns and sperm models are quite homogeneous, with the exception of anoplocephalids, for which an important variability is observed. In this Order, the suitability of sperm characters is clearly shown in mesocestoidids or in inermicapsiferines. The plesiomorphic characters present during spermiogenesis and in the mature

spermatozoon of *Mesocestoides* are unique within cyclophyllideans and they are also observed in other primitive groups of tapeworms. Thus, these spermatological data support the separation of the Mesocestoididae from the Cyclophyllidea [see 32, 35]. On the other hand, the available data on the spermatozoa of *Inermicapsifer* and *Thysanotaenia* indicate their proximity to davaineids. However, as it is generally accepted, all these spermatological data should be combined with molecular studies to obtain a more robust phylogeny of cestodes [10-12].

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