

### **3.6. ULTRASTRUCTURE DE LA SPERMIOGENESE ET DU SPERMATOZOÏDE DU GENRE *JOYEUXIELLA* FUHRMANN, 1935 (CESTODA, CYCLOPHYLLIDEA, DIPYLIDIIDAE) : ETUDE COMPAREE DE *J. ECHINORHYNCHOIDES* (SONSINO, 1889) ET *J. PASQUALEI* (DIAMARE, 1893)**

#### **Résumé :**

Dans ce travail, nous décrivons l'ultrastructure de la spermiogenèse et du spermatozoïde mature de deux cestodes Dipylidiidae : *Joyeuxiella echinorhynchoides* et *J. pasqualei*. Chez les deux espèces, la spermiogenèse suit le type III décrit par Bâ & Marchand (1995) pour les Cyclophyllididea. Cependant, il nous paraît important de signaler la présence de racines striées associées aux centrioles. Le spermatozoïde présente les mêmes caractères ultrastructuraux chez *J. echinorhynchoides* et *J. pasqualei*. Le cône apical, situé au niveau de la région antérieure du spermatozoïde mesure plus de 2 000 nm de long chez les deux espèces. Chacune des espèces présente un seul corps en crête qui a une épaisseur de 150 nm chez *J. echinorhynchoides* et 75 nm chez *J. pasqualei*. Les microtubules corticaux sont spiralés et forment un angle d'environ 40 - 45° avec l'axe du spermatozoïde chez les deux espèces. Une gaine périaxonémale et des granules de glycogène ont été également décrits dans le spermatozoïde mature. Nous décrivons pour la première fois chez les cestodes la disposition des granules de glycogène suivant deux cordons spiralés et parallèles entre eux ainsi que la formation de la gaine périaxonémale avant la fin de la spermiogenèse.

#### **Mots clés :**

Ultrastructure, spermiogenèse, spermatozoïde, *Joyeuxiella echinorhynchoides*, *J. pasqualei*  
Cestoda, Cyclophyllidea, Dipylidiidae.

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## Ultrastructure of spermiogenesis and the spermatozoon in the genus *Joyeuxiella* Fuhrmann, 1935 (Cestoda, Cyclophyllidea, Dipylidiidae): comparative analysis of *J. echinorhynchoides* (Sonsino, 1889) and *J. pasqualei* (Diamare, 1893)

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**Abstract** This paper describes the ultrastructure of spermiogenesis and the mature spermatozoon of two Dipylidiidae cestodes, *Joyeuxiella echinorhynchoides* and *J. pasqualei*. In both species, spermiogenesis follows the type III described by Bâ and Marchand for the cyclophyllideans. Nevertheless, it is interesting to note the presence of striated roots associated with the centrioles. The spermatozoon presents the same ultrastructural features in *J. echinorhynchoides* and *J. pasqualei*. The apical cone in the anterior extremity of the sperm is more than 2.0 µm long in both *J. echinorhynchoides* and *J. pasqualei*. Both species present a single crest-like body, 150 nm thick in *J. echinorhynchoides* and 75 nm thick in *J. pasqualei*. The cortical microtubules are spiralled at an angle of 40–45° to the spermatozoon axis in both *Joyeuxiella* species. A periaxonemal sheath and glycogen granules are also described in the mature sperm. We also describe, for the first time, the disposition of glycogen granules in two opposed and spiralled cords in cestodes and the formation of the periaxonemal sheath in the final stage of spermiogenesis.

### Introduction

The taxonomy of the dilepidid cestodes sensu lato at the family level and below has long been a subject of debate. Some authors have recognized one family, Dilepididae, with three subfamilies: Dilepidinae, Dipylidiinae and Paruterininae (e.g. Yamaguti 1959; Schmidt 1986).

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Others have given each of these subfamilies the rank of family (e.g. Matevosyan 1953, 1963; Wardle et al. 1974). According to Jones (in Khalil et al. 1994), the revision of the group has resulted in the recognition of the families Dilepididae Fuhrmann, 1907; Metadilepididae Spasskii, 1959; Paruterinidae Fuhrmann, 1907 and Dipylidiidae Stiles, 1896. The Dipylidiidae are represented by only three genera: *Dipylidium* Leuckart, 1863; *Diplopylidium* Beddard, 1913 and *Joyeuxiella* Fuhrmann, 1935, all parasites of carnivores.

It has now been clearly demonstrated that spermatozoon and spermiogenesis ultrastructure reveal significant characters for phylogenetic inference in parasitic Platyhelminthes (Euzet et al. 1981; Justine 1991, 1998; Bâ and Marchand 1995). In Cyclophyllidea, data are available for nine of the 15 families recognized by Khalil et al. (1994) (see Ndiaye et al. 2003). Ultrastructural studies of the male gamete in Dipylidiidae have only been carried out in *Dipylidium caninum* (Miquel and Marchand 1997; Miquel et al. 1998).

In the present work, we describe the ultrastructural features of spermiogenesis and the spermatozoon of other Dipylidiidae species belonging to the genus *Joyeuxiella*, a genus that comprises only three species *J. echinorhynchoides* (Sonsino, 1889), *J. fuhrmanni* (Baer, 1924) and *J. pasqualei* (Diamare, 1893) (see Jones 1983). The two species investigated in terms of their spermatozoa and spermiogenesis in the present work are *J. echinorhynchoides* and *J. pasqualei*. Following our study, the available ultrastructural data for three species belonging to two of the three genera of the Dipylidiidae allow us to establish several characteristics of spermiogenesis and the mature sperm in this family.

### Materials and methods

Live specimens of *J. echinorhynchoides* and *J. pasqualei* were collected from the small intestine of a road-killed red fox *Vulpes vulpes* in Corte, Corsica, France, and a wild cat *Felis lybica* in Thiès, Senegal, respectively.

Adult cestodes were first placed into a 0.9% NaCl solution. Subsequently, 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 2 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 an ethanol series and propylene oxide, embedded in Spurr's medium, and polymerized at 60°C for 48 h. Ultrathin sections were cut on a Reichert-Jung Ultracut E ultramicrotome, placed on 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 at the University of Barcelona and the University of Corsica.

## 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 show spermiogenesis and the spermatozoon of *J. echinorhynchoides* and *J. pasqualei*.

### Spermiogenesis

Spermiogenesis in *J. echinorhynchoides* and *J. pasqualei* starts with the formation of a differentiation zone. This is a conical area bordered by submembranous cortical microtubules and containing two centrioles. Each of these is associated with a striated root and exhibits a cytoplasmic process between the centrioles (Fig. 1). One of the centrioles rapidly develops a flagellum that grows parallel to this cytoplasmic expansion (Figs. 1, 3). The other centriole remains oriented in a cytoplasmic bud and aborts posteriorly (Fig. 1). Later, a proximodistal fusion occurs between the axoneme and the cytoplasmic expansion (Fig. 5). Cortical microtubules are elongated parallel to the sperm axis (Fig. 3) and become twisted posteriorly (Figs. 5, 6, 7). A ring of arched membranes is present at the base of the differentiation zone (Fig. 2). The nucleus becomes conical, elongates and migrates along the spermatid body before the proximodistal fusion of the flagellum (Fig. 2). Two attachment zones are visible in the cytoplasmic expansion in a stage previous to the proximodistal fusion of the free flagellum and the cytoplasmic extension (Fig. 3). After the proximodistal fusion, a condensation of cytoplasmic material occurs in the periphery of the spermatid (Fig. 6). At this stage, the cortical microtubules are still parallel to the sperm axis. Posteriorly, this striated structure becomes attached around the axoneme, and the cortical microtubules become twisted (Fig. 7). This process of cytoplasmic condensation is the origin of the periaxonemal sheath present in the mature sperm. Finally, an electron-dense material appears between the cortical microtubules in the anterior part of the differentiation zone. This electron-dense material progressively fuses and forms a crest-like body that appears at the anterior part of the old spermatid in the final stage of spermiogenesis (Fig. 4). At this stage, the nucleus has not yet finished its migration along the spermatid body (Fig. 4). The ring

**Fig. 1** Zone of differentiation of *Joyeuxiella pasqualei* showing the growth of the flagellum parallel to the cytoplasmic extension (Ce). B Cytoplasmic bud, C centrioles, N nucleus, Sr striated roots. Bar 0.5 µm

**Fig. 2** Zone of differentiation of *Joyeuxiella echinorhynchoides* showing the migration of the nucleus (N) along the spermatid body. Am Arched membranes, Sr striated roots. Bar 0.5 µm

**Fig. 3** Transverse sections of spermatids of *J. pasqualei* at a stage previous to the proximodistal fusion of the free flagellum and the cytoplasmic extension (Ce). Arrowheads indicate attachment zones. Bar 0.5 µm

**Fig. 4** Final stage of spermiogenesis in *J. echinorhynchoides* showing the appearance of a crest-like body (arrowheads). At this stage the nucleus (N) has not finished its migration. Ax Axoneme. Bar 1 µm

of arched membranes narrows until the spermatid detaches itself from the residual cytoplasm. The spermatozoa are liberated into the lumen of the vas deferens and pass to the seminal vesicle for storage.

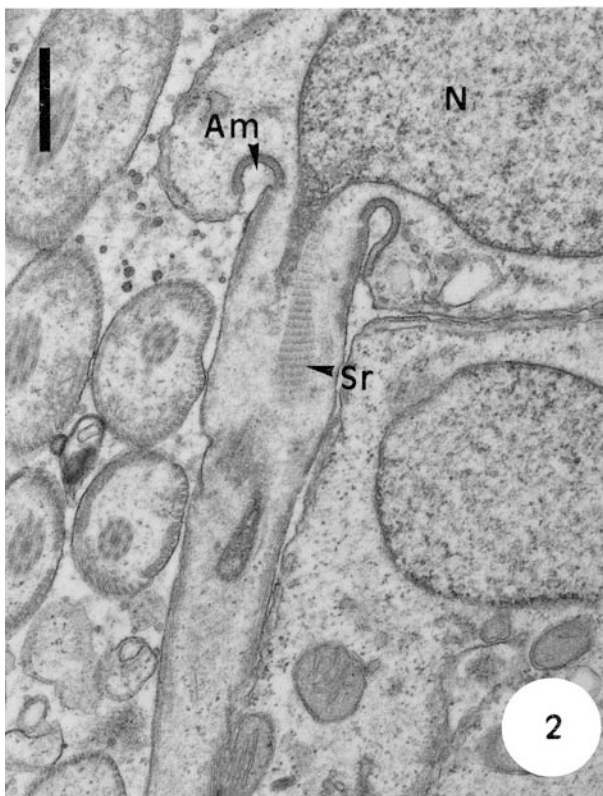
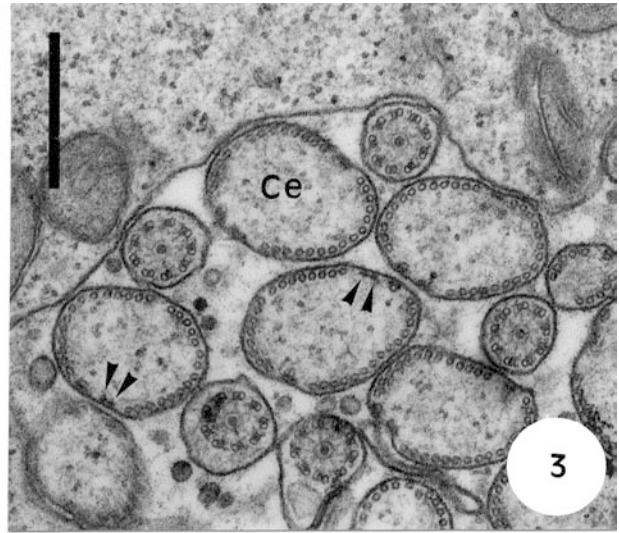
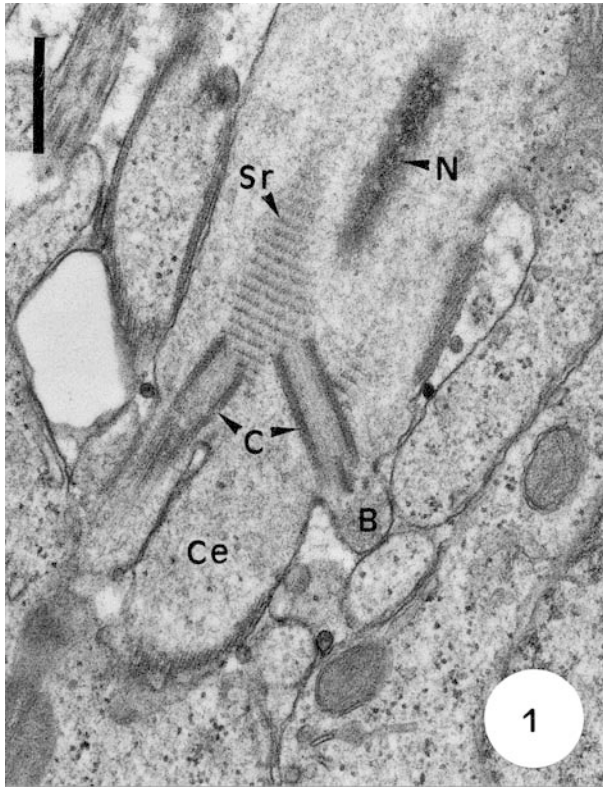
Figure 22 depicts the main stages of spermiogenesis in the genus *Joyeuxiella*.

### Spermatozoon

The mature spermatozoon of *J. echinorhynchoides* and *J. pasqualei* is a long filiform cell. It is tapered at both ends, shows the 9+1' axoneme pattern of Platyhelminthes and lacks mitochondria. Observation of most transversal and longitudinal sections of the spermatozoon permitted us to distinguish five regions (I–V) with different ultrastructural characters.

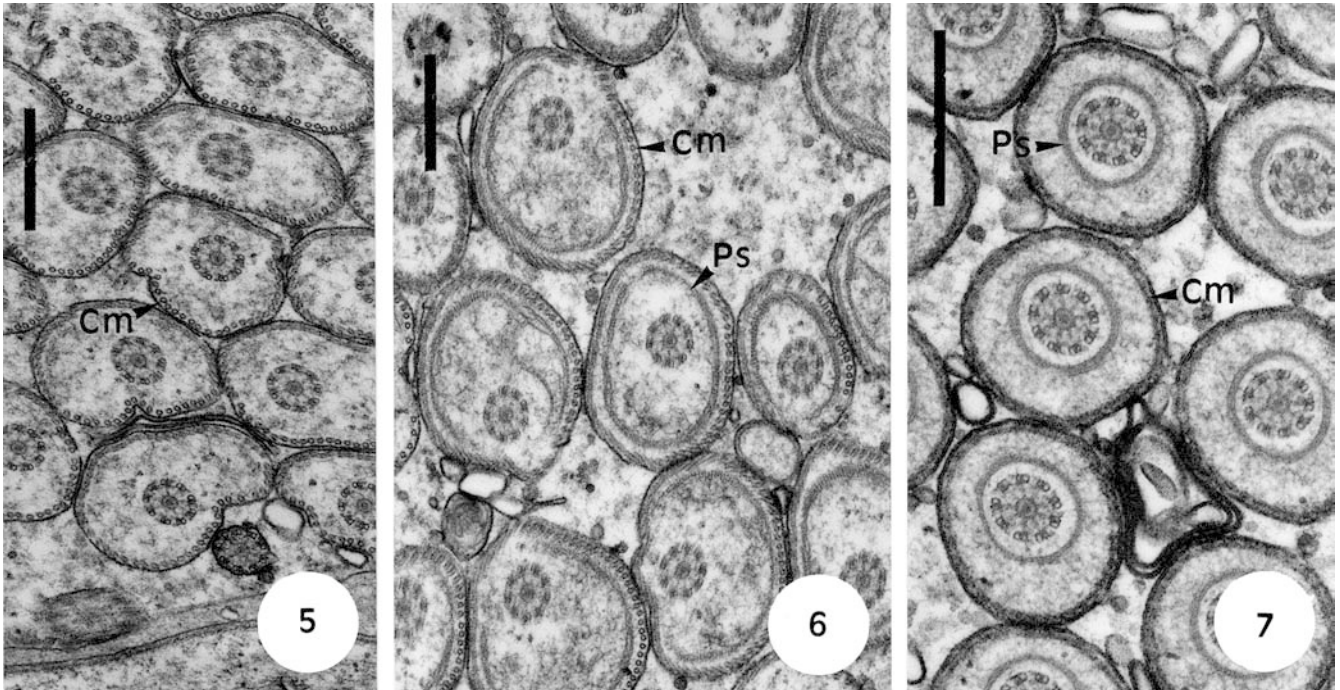
Region I (Figs. 8, 9, 16, 18, 21, 23I) corresponds to the anterior extremity of the spermatozoon. Its mean maximal width is about 375 nm in both *J. echinorhynchoides* and *J. pasqualei*. This region is capped by an apical cone of irregular electron-dense material more than 2.0 µm long and around 285 nm wide (Figs. 8, 9). The anterior axonemal extremity is situated immediately below the apical cone. In this extremity the centriole remains clearly visible (Fig. 8). The 9+1' axoneme pattern lacks a periaxonemal sheath and is surrounded by a thin layer of electron lucent material and by slightly electron-dense cytoplasm (Fig. 1). Cortical microtubules are twisted and present an angle of about 40° to 45° to the spermatozoon axis (Fig. 13). Externally, these spiralled cortical microtubules are partially surrounded by a thick helicoidal cord of electron-dense material that forms a single crest-like body (Figs. 8, 16, 21). This crest-like body is spiralled at the same angle to the sperm axis and has a maximal width of up to 150 nm for *J. echinorhynchoides* and 75 nm for *J. pasqualei*. The thickness of the crest-like body gradually decreases toward the proximal end of region II.

Region II (Figs. 10, 11, 20, 23II) has a maximal width of around 375–400 nm in both *J. echinorhynchoides* and



*J. pasqualei*. In both species, this region is characterized by the disappearance of the crest-like body and the presence of a periaxonemal sheath around the axoneme (Figs. 10, 11, 20).

Region III (Figs. 11, 12, 13, 17, 23III). The mean width of this region of the spermatozoon is around 450 nm in both species. Region III is characterized by the presence of granules of glycogen disposed in two



**Fig. 5** Transverse sections of spermatids of *J. pasqualei* after proximodistal fusion. Note the parallel disposition of the cortical microtubules (*Cm*). Bar 0.5  $\mu$ m

**Fig. 6** Cross-sections of spermatids of *J. echinorhynchoides* showing the formation of the periaxonemal sheath (*Ps*) in the periphery of the spermatid. *Cm* Cortical microtubules. Bar 0.5  $\mu$ m

**Fig. 7** Transverse sections of spermatids of *J. echinorhynchoides* in a posterior stage showing the displacement of the periaxonemal sheath (*Ps*) and its location around the axoneme. *Cm* Cortical microtubules. Bar 0.5  $\mu$ m

opposed and spiralled cords (Figs. 12, 17). These granules are located between the periaxonemal sheath and the submembranous layer of cortical microtubules (Figs. 11, 12, 17).

Region IV (Figs. 13, 14, 19, 20, 23IV) corresponds to the nuclear area of the spermatozoon. The mean maximal width of this region is around 425 nm for both species. The nucleus coils around the axoneme in a helicoidal form and occupies the sheath position (Figs. 13, 14, 19, 20). In cross-sections, it appears horseshoe-shaped or almost annular in form (Figs. 19, 20).

Region V (Figs. 15, 17, 19, 23V) corresponds to the posterior part of the spermatozoon. Its mean maximal width is 370 nm in both species. This region lacks a nucleus and periaxonemal sheath. The cortical microtubules form a spiralled submembranous layer in the anterior part of this region (Fig. 19). Posteriorly, the cortical microtubules run parallel to the spermatozoon axis and disappear progressively (Fig. 17). In more distal areas of this region, only the axoneme is observed (Figs. 15, 17), and its doublets are progressively disorganized into singlets (Fig. 17). A few axonemal microtubules extend to the posterior extremity of the spermatozoon (Fig. 17).

## Discussion

### Spermiogenesis

In the Eucestoda, striated roots have been described in the orders Caryophyllidea, Trypanorhyncha, Tetrphyllidea, Pseudophyllidea, Proteocephalidea, Tetrabothriidea and in some species of Cyclophyllidea (Table 1). In the order Diphyllidea, Mokhtar-Maamouri and Azzouz-Draoui (1984), and Azzouz-Draoui and Mokhtar-Maamouri (1986/88) have described "granular masses" associated with the two centrioles. These were interpreted as homologous to striated roots. It is our view, in line with Justine (1998), that the great differences observed in the spermiogenesis of Diphyllidea species belonging to a

**Fig. 8** Longitudinal section of region I of the spermatozoon of *J. pasqualei*. *Ac* Apical cone, *C* centriole, *Cb* crest-like body. Bar 0.5  $\mu$ m

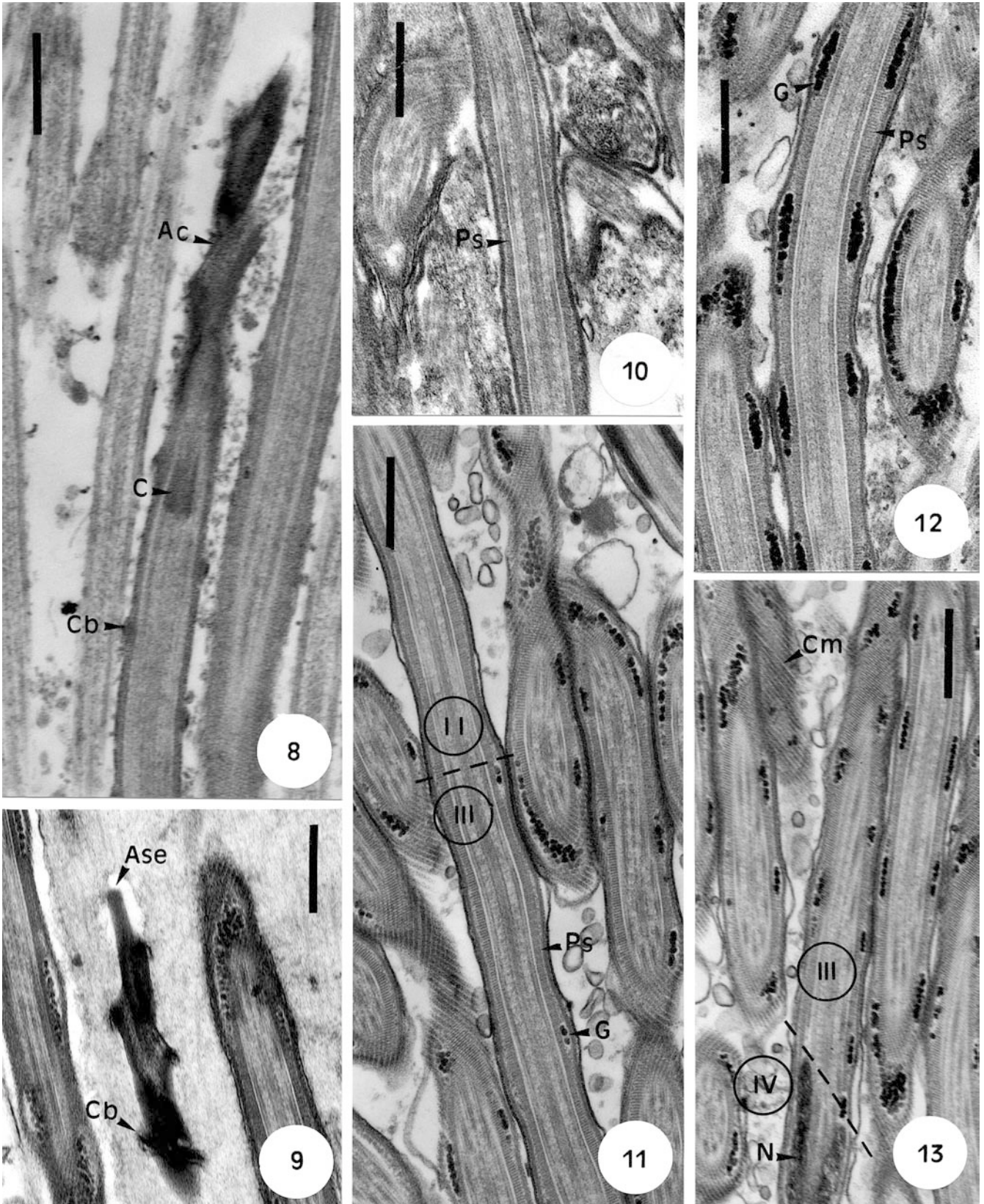
**Fig. 9** Detail of the anterior spermatozoon extremity (*Ase*) of *J. echinorhynchoides*. *Cb* Crest-like body. Bar 0.5  $\mu$ m

**Fig. 10** Longitudinal section of region II of the spermatozoon of *J. pasqualei*. *Ps* Periaxonemal sheath. Bar 0.5  $\mu$ m

**Fig. 11** Longitudinal section of the region II-region III transition of the spermatozoon of *J. echinorhynchoides*. *G* Granules of glycogen, *Ps* periaxonemal sheath. Bar 0.5  $\mu$ m

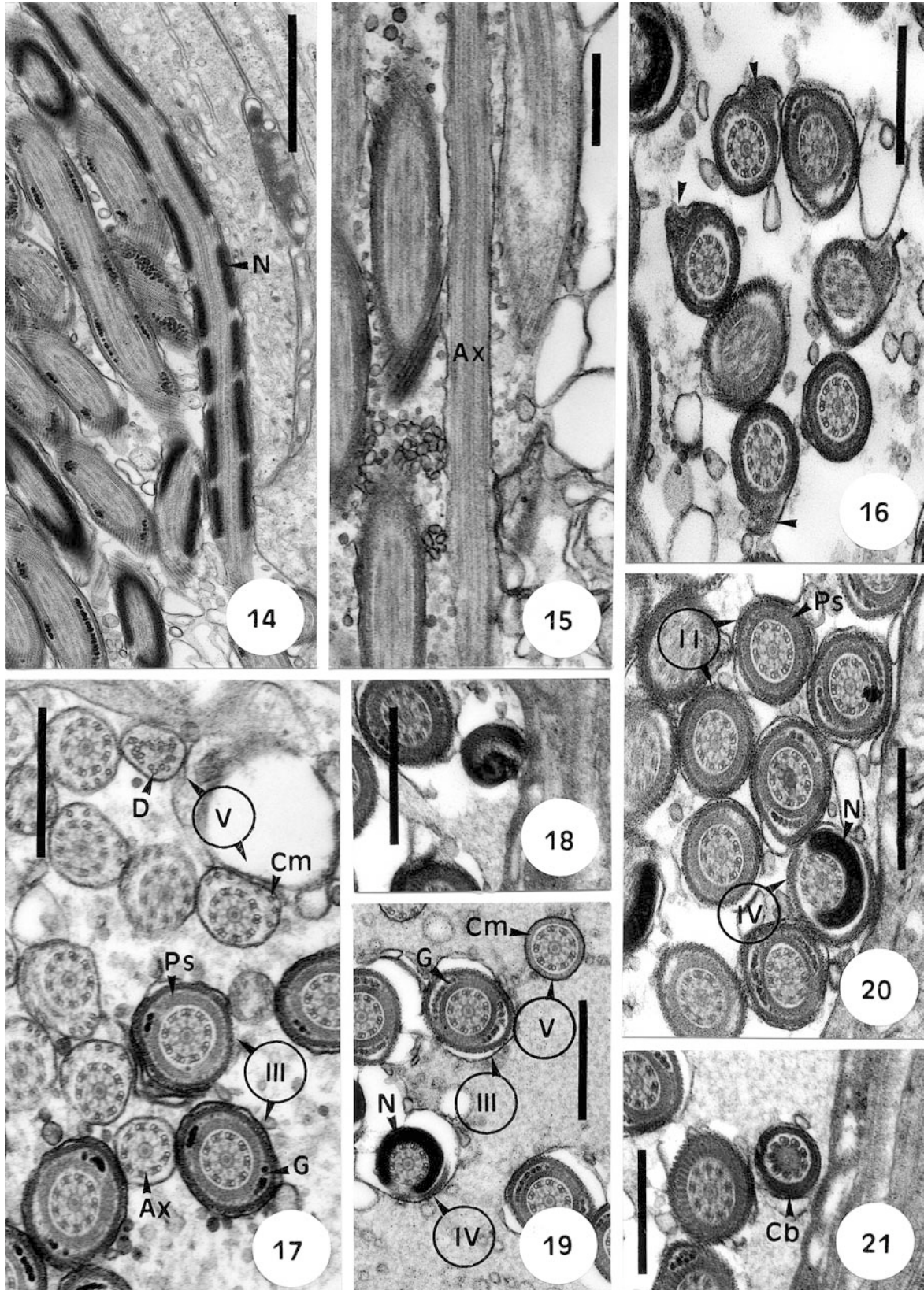
**Fig. 12** Longitudinal section of region III of the spermatozoon of *J. pasqualei*. *G* Granules of glycogen, *Ps* periaxonemal sheath. Bar 0.5  $\mu$ m

**Fig. 13** Longitudinal section of the region III-region IV transition of the spermatozoon of *J. pasqualei*. The cortical microtubules (*Cm*) are spiralled at an angle of 40–45° to the sperm axis. *N* Nucleus. Bar 0.5  $\mu$ m



single genus (*Echinobothrium affine*, *E. brachysoma*, *E. harfordi* and *E. typus*, see Table 1) indicate that further observations are needed for this family. In the

Cyclophyllidea, striated roots have only been described in *Dipylidium caninum* (Miquel et al. 1998), *Anoplocephaloides dentata* (Miquel and Marchand 1998) and



*Mesocestoides litteratus* (Miquel et al. 1999). The latter species showed striated roots similar to those observed in the primitive orders of Eucestoda (Table 1) and in the

digenean trematodes, while in *D. caninum* and *A. dentata*, striated roots were reduced. As a result of these observations, the term “striated roots” has been recoded as

**Fig. 14** Longitudinal section of region IV of the spermatozoon of *J. echinorhynchoides*. N Nucleus. Bar 1  $\mu$ m

**Fig. 15** Longitudinal section of region V of the spermatozoon of *J. pasqualei*. Ax Axoneme. Bar 0.5  $\mu$ m

**Fig. 16** Several cross-sections of region I of the spermatozoon of *J. echinorhynchoides*. Arrowheads indicate crest-like bodies. Bar 0.5  $\mu$ m

**Fig. 17** Several cross-sections of regions III and V of the spermatozoon of *J. echinorhynchoides*. Note the parallel disposition of terminal cortical microtubules (Cm). Ax Axoneme, D doublets, G granules of glycogen, Ps periaxonemal sheath. Bar 0.5  $\mu$ m

**Fig. 18** Transverse section of region I of the spermatozoon of *J. pasqualei* at the level of the apical cone. Bar 0.5  $\mu$ m

**Fig. 19** Three cross-sections of regions III, IV and V of the spermatozoon of *J. pasqualei*. Cm Cortical microtubules, G granules of glycogen, N nucleus. Bar 0.5  $\mu$ m

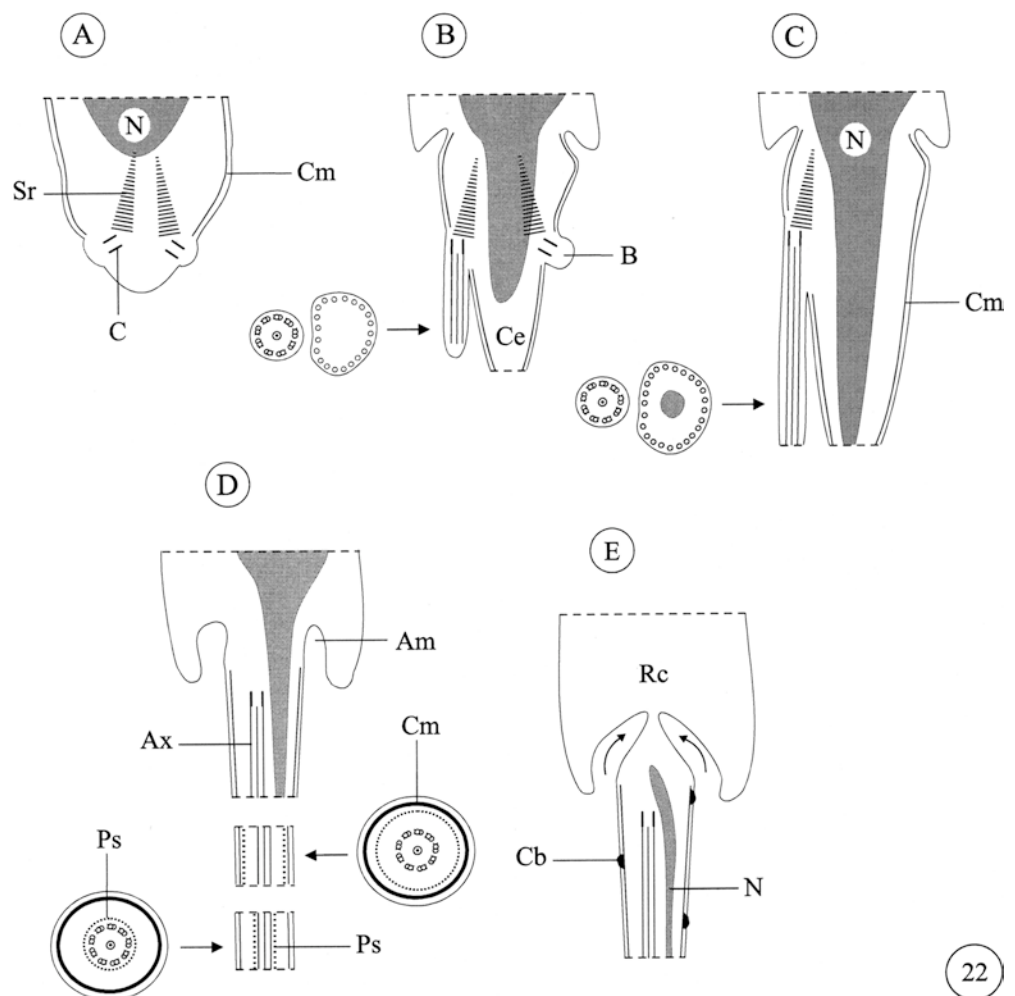
**Fig. 20** Cross-sections of regions II and IV of the spermatozoon of *J. echinorhynchoides*. N Nucleus, Ps periaxonemal sheath. Bar 0.5  $\mu$ m

**Fig. 21** Transverse section of region I of the spermatozoon of *J. echinorhynchoides* at the level of the centriole. Cb Crest-like body. Bar 0.5  $\mu$ m

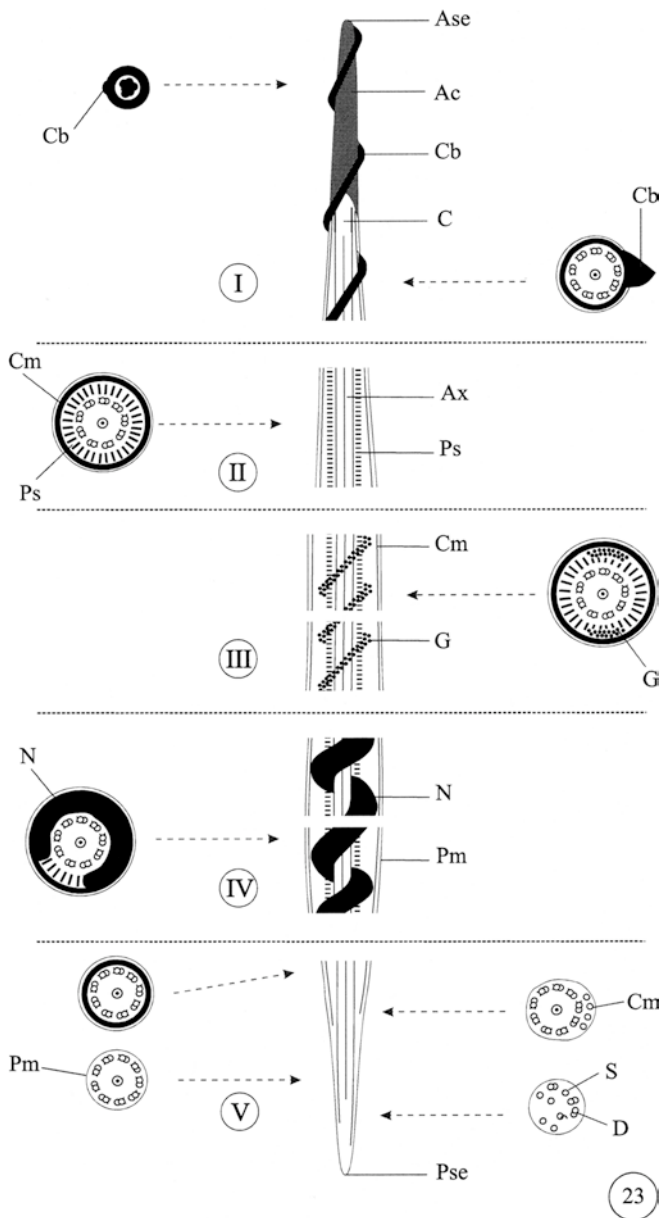
“typical striated roots” by Justine (2001). In the case of *Mesocestoides*, the morphological, biological and molecular data, along with the ultrastructural observations of sperm (Rausch in Khalil et al. 1994; Justine 1998; Mariaux 1998; Miquel et al. 1999), demonstrate the unclear position of this group within the cyclophyllideans. Several authors have suggested that the family Mesocestoididae should be excluded from the Cyclophyllidea order, to maintain the monophyly of the cyclophyllideans (Mariaux 1998). On the other hand, Bâ et al. (1991) described electron-dense masses associated with centrioles in the cyclophyllidean Anoplocephalidae *Mathevo-taenia herpestis* similar to those observed in the diphyllidean Echinobothriidae. Our work also describes well-developed striated roots in two species belonging to another genus of cyclophyllideans of the Dipylidiidae family: *J. echinorhynchoides* and *J. pasqualei*.

In terms of the four types of spermiogenesis defined by Bâ and Marchand (1995), the genus *Joyeuxiella* seems to present a modified type III spermiogenesis. Indeed, we observed: (1) the presence of two centrioles without an intercentriolar body between them, (2) the absence of flagellar rotation given that the axoneme grows parallel to the cytoplasmic process, and (3) a

**Fig. 22A–E** Reconstruction of the main stages of spermiogenesis in the genus *Joyeuxiella*. Am Arched membranes, Ax axoneme, B cytoplasmic bud, C centriole, Cb crest-like body, Ce cytoplasmic extension, Cm cortical microtubules, N nucleus, Ps periaxonemal sheath, Rc residual cytoplasm, Sr striated roots







**Fig. 23** Diagram showing the ultrastructural organization of regions I to V of the mature sperm of the genus *Joyeuxiella*. *Ac* Apical cone, *Ase* anterior spermatozoon extremity, *Ax* axoneme, *C* centriole, *Cb* crest-like body, *Cm* cortical microtubules, *D* doublets, *G* granules of glycogen, *N* nucleus, *Pm* plasma membrane, *Ps* periaxonemal sheath, *Pse* posterior spermatozoon extremity, *S* singlets

proximodistal fusion of the free flagellum with this cytoplasmic expansion. However, a difference was noted with respect to type III: the presence of typical and well-developed striated rootlets. Our results are comparable to the observations made by Miquel et al. (1998) in *D. caninum*, but this last species presents thin striated roots (Table 2). These observations in two of the three genera of Dipylidiidae indicate that type III spermiogenesis is probably the characteristic pattern for this family.

Another interesting and novel aspect of spermiogenesis is the formation of the periaxonemal sheath, which

is present in the mature spermatozoon. This occurs in the final stage of spermiogenesis and, to our knowledge, the process is described for the first time in the present work. The presence of a periaxonemal sheath has been reported in mature spermatozoa of numerous species belonging to different families of Cyclophyllidea (Anoplocephalidae, Catenotaeniidae, Davaineidae, Dilepididae and Taeniidae) by several authors (Swiderski 1984; Bâ and Marchand 1992a, 1994a, 1994b, 1994c, 1994d; Miquel et al. 1997; Hidalgo et al. 2000; Swiderski et al. 2000; Ndiaye et al. 2003), but has never been observed during spermiogenesis. In all of these studies, an electron-dense granular material has only been described in *Taenia parva*, but this densification has been interpreted as the origin of the transverse intracytoplasmic walls and not the periaxonemal sheath (Ndiaye et al. 2003). In the present paper, we describe for the first time a condensation of electron-dense material forming a striated structure that encircles the axoneme in the periphery of the mature spermatid. This striated structure progressively approaches the axoneme and forms the periaxonemal sheath.

### Spermatozoon

Analysis of numerous sections of *J. echinorhynchoides* and *J. pasqualei* revealed the same ultrastructural organization of the spermatozoon in both species. Differences are noted only at the level of the maximal width of the crest-like body. The crest-like body in *J. echinorhynchoides* is twice as large as that in *J. pasqualei* (150 nm vs 75 nm).

The ultrastructural features present in the mature spermatozoa of *J. echinorhynchoides* and *J. pasqualei* are quite different from those observed in the spermatozoon of *D. caninum* (Miquel and Marchand 1997). The most remarkable differences are noted at the level of the apical cone (shorter in *Dipylidium*: 600 nm; longer in *Joyeuxiella*: 2.0  $\mu$ m), in the presence of glycogen in *Joyeuxiella* spp., and in the morphology of the posterior extremity of the sperm (Table 2). In our study, we describe for the first time the presence of a periaxonemal sheath and glycogen granules in the Dipylidiidae.

In the 33 previous ultrastructural studies of spermatozoa in representatives of the order Cyclophyllidea (Bâ et al. 2002; Ndiaye et al. 2003), a periaxonemal sheath is described in only 13 species: *Inermicapsifer guineensis* and *I. madagascariensis* (Bâ and Marchand 1994a), *Mathevotaenia herpestis* (Bâ and Marchand 1994b), and *Stilesia globipunctata* (Bâ and Marchand 1992a) (Anoplocephalidae), *Catenotaenia pusilla* (Hidalgo et al. 2000), and *Skrjabinotaenia lobata* (Miquel et al. 1997) (Catenotaeniidae), *Cotugnia polyacantha* (Bâ and Marchand 1994c), and *Raillietina tunetensis* (Bâ and Marchand 1994d) (Davaineidae), *Dilepis undula* (Swiderski et al. 2000) (Dilepididae), *Echinococcus multilocularis* (Barret and Smyth 1983; Shi et al. 1994), *Taenia* spp. (Tian et al. 1998), *T. hydatigena* (Featherston 1971),

**Table 1** The current state of our knowledge based on spermiogenesis studies in Eucestoda. “Type” of spermiogenesis refers to the four patterns defined by Bâ and Marchand (1995). *Pf* Proximodistal fusion, *Fr* flagellar rotation, *Sr* striated root, *Ib* intercentriolar body, + presence of the character, – absence of the character

| Order, family and species                 | Type                   | Pf | Fr | Sr | Ib | Reference of spermatological work  |
|---|------------------------|----|----|----|----|--|
| Caryophyllidea                            |                        |    |    |    |    |  |
| Caryophyllaeidae                          |                        |    |    |    |    |  |
| <i>Glaridacris catostomi</i>              | II                     | +  | +  | +  | +  | Swiderski and Mackiewicz (2002)  |
| Diphyllidea                               |                        |    |    |    |    |  |
| Echinobothriidae                          |                        |    |    |    |    |  |
| <i>Echinobothrium affine</i>              | I or II <sup>a,b</sup> | +  | +  | –  | +  | Mokhtar-Maamouri and Azzouz-Draoui (1984),<br>Azzouz-Draoui and Mokhtar-Maamouri (1986/88) |
| <i>E. brachysoma</i>                      | I <sup>a,b</sup>       | +  | +  | –  | +  | Azzouz-Draoui (1985)   |
| <i>E. harfordi</i>                        | II <sup>a,b</sup>      | +  | +  | –  | +  | Mokhtar-Maamouri and Azzouz-Draoui (1984),<br>Azzouz-Draoui and Mokhtar-Maamouri (1986/88) |
| <i>E. typus</i>                           | I <sup>a,b</sup>       | +  | +  | –  | +  | Azzouz-Draoui (1985)   |
| Trypanorhyncha                            |                        |    |    |    |    |  |
| Lacistorhynchidae                         |                        |    |    |    |    |  |
| <i>Lacistorhynchus tenuis</i>             | I                      | +  | +  | +  | +  | Euzet et al. (1981), Swiderski (1994)  |
| Tetraphyllidea                            |                        |    |    |    |    |  |
| Onchobothriidae                           |                        |    |    |    |    |  |
| <i>Acanthobothrium filicolle benedeni</i> | I                      | +  | +  | +  | +  | Mokhtar-Maamouri and Swiderski (1975)  |
| <i>A. filicolle filicolle</i>             | I                      | +  | +  | +  | +  | Mokhtar-Maamouri (1982)  |
| <i>Onchobothrium uncinatum</i>            | I                      | +  | +  | +  | +  | Mokhtar-Maamouri and Swiderski (1975)  |
| Phyllobothriidae                          |                        |    |    |    |    |  |
| <i>Phyllobothrium gracile</i>             | II                     | +  | +  | +  | +  | Mokhtar-Maamouri (1979)  |
| <i>P. lactuca</i>                         | I                      | +  | +  | +  | +  | Sène et al. (1999)   |
| <i>Trilocularia acanthiaevulgaris</i>     | I                      | +  | +  | +  | +  | Mahendrasingam et al. (1989)   |
| Pseudophyllidea                           |                        |    |    |    |    |  |
| Bothriocephalidae                         |                        |    |    |    |    |  |
| <i>Bothriocephalus clavibothrium</i>      | I                      | +  | +  | +  | +  | Swiderski and Mokhtar-Maamouri (1980)  |
| Trienophoridae                            |                        |    |    |    |    |  |
| <i>Eubothrium crassum</i>                 | I                      | +  | +  | +  | +  | Brunanska et al. (2001)  |
| Proteocephalidea                          |                        |    |    |    |    |  |
| Proteocephalidae                          |                        |    |    |    |    |  |
| <i>Proteocephalus longicolis</i>          | I                      | +  | +  | +  | +  | Swiderski (1985)   |
| Monticelliidae                            |                        |    |    |    |    |  |
| <i>Nomimoscolex sp.</i>                   | I <sup>b</sup>         | +  | +  | +  | +  | Sène et al. (1997)   |
| Tetrabothriidea                           |                        |    |    |    |    |  |
| Tetrabothriidae                           |                        |    |    |    |    |  |
| <i>Tetrabothrius erostris</i>             | I                      | +  | +  | +  | –  | Stoitsova et al. (1995)  |
| Cyclophyllidea                            |                        |    |    |    |    |  |
| Anoplocephalidae                          |                        |    |    |    |    |  |
| <i>Anoplocephaloides dentata</i>          | IV <sup>c</sup>        | –  | –  | +  | –  | Miquel and Marchand (1998)   |
| <i>Aporina delafondi</i>                  | IV                     | –  | –  | –  | –  | Bâ and Marchand (1994e)  |
| <i>Mathevotaenia herpestis</i>            | III                    | +  | –  | –  | –  | Bâ and Marchand (1994b)  |
| <i>Sudarikovina taterae</i>               | IV                     | –  | –  | –  | –  | Bâ et al. (2000)   |
| <i>Thysaniezia ovilla</i>                 | IV                     | –  | –  | –  | –  | Bâ et al. (1991)   |
| Catenotaeniidae                           |                        |    |    |    |    |  |
| <i>Catenotaenia pusilla</i>               | III <sup>d</sup>       | +  | +  | –  | –  | Hidalgo et al. (2000)  |
| Davaineidae                               |                        |    |    |    |    |  |
| <i>Railletina tumetensis</i>              | III                    | +  | –  | –  | –  | Bâ and Marchand (1994d)  |
| Dipylidiidae                              |                        |    |    |    |    |  |
| <i>Dipylidium caninum</i>                 | III <sup>c</sup>       | +  | –  | +  | –  | Miquel et al. (1998)   |
| <i>Joyeuxiella echinorhynchoides</i>      | III                    | +  | –  | +  | –  | Present study  |
| <i>J. pasqualei</i>                       | III                    | +  | –  | +  | –  | Present study  |
| Hymenolepididae                           |                        |    |    |    |    |  |
| <i>Dicranotaenia coronula</i>             | IV                     | –  | –  | –  | –  | Chomicz and Swiderski (1992)   |
| <i>Hymenolepis diminuta</i>               | IV                     | –  | –  | –  | –  | Kelsoe et al. (1977)   |
| <i>Monorcholepis dujardini</i>            | IV                     | –  | –  | –  | –  | Swiderski and Tkach (1996)   |
| <i>Rodentolepis microstoma</i>            | IV                     | –  | –  | –  | –  | Bâ and Marchand (1998)   |
| <i>R. nana</i>                            | IV                     | –  | –  | –  | –  | Bâ and Marchand (1992b)  |
| Mesocestoides                             |                        |    |    |    |    |  |
| <i>Mesocestoides litteratus</i>           | II <sup>b</sup>        | +  | +  | +  | +  | Miquel et al. (1999)   |
| Nematotaeniidae                           |                        |    |    |    |    |  |
| <i>Nematotaenia chantalae</i>             | III                    | +  | –  | –  | –  | Mokhtar-Maamouri and Azzouz-Draoui (1990)  |
| Taeniidae                                 |                        |    |    |    |    |  |
| <i>Taenia parva</i>                       | III <sup>d</sup>       | +  | +  | –  | –  | Ndiaye et al. (2003)   |

<sup>a</sup> Presence of annexed centriolar bodies and not striated roots

<sup>b</sup> Presence of thin or reduced intercentriolar body

<sup>c</sup> Presence of thin striated roots

<sup>d</sup> Presence of a slight rotation of about 45 ° of the free flagellum

**Table 2** Available data on the ultrastructure of spermiogenesis and the spermatozoon in Dipylidiidae species. All measurements are given in nm. "Type" of spermiogenesis refers to the four patterns defined by Bâ and Marchand (1995). *Pf* Proximodistal fusion, *Fr* flagellar rotation, *Sr* striated root, *Ib* intercentriolar body,

*Cb* crest-like body, *n* number, *Ac* apical cone, *Cm* cortical microtubules, *Ps* periaxonemal sheath, *G* granular material, *Iw* transverse intracytoplasmic walls, + presence of the character, - absence of the character

| Dipylidiidae species   | Spermiogenesis |    |    |                |    | Spermatozoon |                |             |            |        |    |   |
|--|----------------|----|----|----------------|----|--------------|----------------|-------------|------------|--------|----|---|
|  | Type           | Pf | Fr | Sr             | Ib | Cb           | Ac             | Cm          | Ps         | G      | Iw |   |
| Reference  |                |    |    |                |    | <i>n</i>     | Thickness (nm) | Length (nm) | Width (nm) | Angle  |    |   |
| <i>Dipylidium caninum</i><br>Miquel and Marchand (1997),<br>Miquel et al. (1998) | III            | +  | -  | + <sup>a</sup> | -  | 1            | 150            | 600         | 400        | 40°    | +  | - |
| <i>Joyeuxiella echinorhynchoides</i><br>Present paper                            | III            | +  | -  | +              | -  | 1            | 150            | >2,000      | 385        | 40-50° | +  | - |
| <i>Joyeuxiella pasqualei</i><br>Present paper                                    | III            | +  | -  | +              | -  | 1            | 75             | >2,000      | 385        | 40-50° | +  | - |

<sup>a</sup>Presence of thin striated roots

and *T. parva* (Ndiaye et al. 2003) (Taeniidae). In our view, several images of *D. caninum* (Dipylidiidae) show structures that resemble periaxonemal sheaths in both nuclear and non-nuclear areas of the sperm, but the authors did not describe this aspect of the images. Probably, the presence of a periaxonemal sheath is another feature that characterizes the ultrastructure of spermatozoa in members of the family Dipylidiidae.

The disposition of glycogen in two opposed and spiralled cords described in the present study has never been reported previously in cestodes. According to Euzet et al. (1981), glycogen seems to be the energy spring of the spermatozoon, but the mechanisms by which it is utilized are not yet known. In the Cyclophyllidea, the distribution of glycogen varies according to the species.

The ultrastructure of the posterior end of the spermatozoon in the genus *Joyeuxiella* is different from that observed in *D. caninum*. In *Joyeuxiella* spp., cross-sections of the posterior end present only singlets resulting from the disorganization of the doublets of the axoneme. However, in *D. caninum* this posterior end is characterized by the presence of spiralled microtubules and a slightly electron-dense granular material (Miquel and Marchand 1997).

Thus, we believe that our study, together with those carried out in the genus *Dipylidium* (Miquel and Marchand 1997; Miquel et al. 1998), contributes to establishing the general pattern of spermiogenesis and the ultrastructural features of the spermatozoon in the family Dipylidiidae. However, further ultrastructural studies will be necessary for phylogenetic purposes at the family rank. Such studies should examine the gamete in Dipylidiidae (particularly the genus *Diplopylidium*) and in the Dilepididae, Metadilepididae and Paruterinidae, for which ultrastructural data on sperm are not available. Future studies in the Dilepididae may contribute to elucidating the status of this paraphyletic group.

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## References

- Azzouz-Draoui N (1985) Étude ultrastructurale comparée de la spermiogenèse et du spermatozoïde de quatre Cestodes Dipylidiidae. Thesis, University of Tunis, Tunis
- Azzouz-Draoui N, Mokhtar-Maamouri F (1986/88) Ultrastructure comparée de la spermiogenèse et du spermatozoïde de *Echinobothrium affine* Diesing, 1863 et *E. harfordi* MacVicar, 1976 (Cestoda, Dipylidiidae). Bull Soc Sci Nat Tunisie 18:9-20
- Bâ A, Bâ CT, Marchand B (2000) Ultrastructure of spermiogenesis and the spermatozoon of *Sudarikovina taterae* (Cestoda, Cyclophyllidea, Anoplocephalidae) intestinal parasite of *Tatera gambiana* (Rodentia, Gerbillidae). J Submicrosc Cytol Pathol 32:137-144
- Bâ A, Bâ CT, Marchand B (2002) Ultrastructural study of the spermatozoon of *Echinocotyle dolosa* (Cestoda, Cyclophyllidea, Hymenolepididae). Acta Parasitol 47:131-136
- Bâ CT, Marchand B (1992a) 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 (1992b) Reinvestigation of the ultrastructure 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 (1994a) 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 (1994b) Ultrastructure of spermiogenesis and the spermatozoon of *Mathevotaenia herpestis* (Cestoda), intestinal parasite of *Atelerix albiventris* in Sénégal. Acta Zool (Stockh) 75:167-175
- Bâ CT, Marchand B (1994c) Similitude ultrastructurale des spermatozoïdes de quelques Cyclophyllidea. Parasite 1:51-55
- Bâ CT, Marchand B (1994d) Ultrastructure of spermiogenesis and the spermatozoon of *Raillietina (Raillietina) tunetensis* (Cyclophyllidea, Davaineidae) intestinal parasite of turtle doves in Senegal. Int J Parasitol 24:237-248

- Bâ CT, Marchand B (1994e) 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 (1995) Spermiogenesis, spermatozoa and phyletic affinities in the Cestoda. In: Jamieson BGM, Ausió J, Justine JL (eds) Advances in spermatozoal phylogeny and taxonomy. *Mem Mus Natl Hist Nat* 166:87–95
- 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 Cestodes spermatozoon illustrated by the ultrastructural study of spermiogenesis of a Cyclophyllidea: *Thysaniezia ovilla*, Rivolta, 1874. *J Submicrosc Cytol Pathol* 23:606–612
- Barrett NJ, Smyth JD (1983) Observations 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 H-P (2001) Spermiogenesis in the pseudophyllid cestode *Eubothrium crasum* (Bloch, 1779). *Parasitol Res* 87:579–588
- Chomicz L, Swiderski Z (1992) Spermiogenesis and ultrastructure of the ultrastructure of the cestode *Dicranotaenia coronula* (Dujardin, 1845) (Cyclophyllidea, Hymenolepididae). Proceedings of the 5th Asia-Pacific Electron Microscopy Conference, Beijing, 324–325
- Euzet L, Swiderski Z, Mokhtar-Maamouri F (1981) Ultrastructure comparée du spermatozoïde des Cestodes. Relations avec la phylogénèse. *Ann Parasitol Hum Comp* 56:247–259
- 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
- Jones A (1983) A revision of the cestode genus *Joyeuxiella* Fuhrmann, 1935 (Dilepididae: Dipylidiinae). *Syst Parasitol* 5:203–213
- Justine J-L (1991) Phylogeny of parasitic Platyhelminthes: a critical study of synapomorphies proposed on the basis of the ultrastructure of spermiogenesis and spermatozoa. *Can J Zool* 69:1421–1440
- 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, London, pp 231–238
- Kelsoe GH, Ubelaker JE, Allison VF (1977) The fine structure of spermiogenesis in *Hymenolepis diminuta* (Cestoda) with a description of the mature spermatozoon. *Z Parasitenkd* 54:175–187
- Khalil LF, Jones A, Bray RA (1994) Keys to the cestode parasites of vertebrates. CAB International, Cambridge
- Mahendrasingam S, Fairweather I, Halton DW (1989) Spermatogenesis and the fine structure of the mature spermatozoon in the free proglottis of *Trilocularia acanthiaevulgaris* (Cestoda, Tetracyphylidae). *Parasitol Res* 75:287–298
- Mariaux J (1998) A molecular phylogeny of the Eucestoda. *J Parasitol* 84:114–124
- Matevosyan EM (1953) Re-organisation of the classification of dilepidid cestodes. Papers on helminthology presented to Academician K.I. Skryabin on his 75th birthday. Izdatel'stvo Akademii Nauk SSSR, Moscow, pp 392–397
- Matevosyan EM (1963) Principles of cestodology, vol. III. Dilepidoidea, tapeworms of domestic and wild animals. Akademiya Nauk SSSR, Moscow
- 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 (1998) Ultrastructure of spermiogenesis and the spermatozoon of *Anoplocephaloides dentata* (Cestoda, Cyclophyllidea, Anoplocephalidae), an intestinal parasite of Arvicolidae rodents. *J Parasitol* 84:1128–1136
- 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
- Mokhtar-Maamouri F (1979) Étude en microscopie électronique de la spermiogénèse et du spermatozoïde de *Phyllobothrium gracile* Weld, 1855 (Cestoda, Tetracyphylidae, Phyllobothriidae). *Z Parasitenkd* 59:245–258
- Mokhtar-Maamouri F (1982) Étude ultrastructurale de la spermiogénèse de *Acanthobothrium filicolle* var. *filicolle* Zschokke, 1888 (Cestoda: Tetracyphylidae, Onchobothriidae). *Ann Parasitol Hum Comp* 57:429–442
- Mokhtar-Maamouri F, Azzouz-Draoui N (1984) Spermiogénèse et ultrastructure du spermatozoïde de deux Cestodes Dipylidiidae. 4ème Ecole Franco-Africaine Biol Mol, Djerba, Tunisie, pp 467–469
- Mokhtar-Maamouri F, Azzouz-Draoui N (1990) Spermiogénèse et ultrastructure du spermatozoïde de *Nematotaenia chantalae* Dollfus, 1957 (Cestoda, Cyclophyllidea, Nematotaeniidae). *Ann Parasitol Hum Comp* 65:221–228
- Mokhtar-Maamouri F, Swiderski Z (1975) Étude en microscopie électronique de la spermatogénèse de deux Cestodes *Acanthobothrium filicolle benedenii* Loennberg, 1889 et *Onchobothrium uncinatum* (Rud., 1819) (Tetracyphylidae, Onchobothriidae). *Z Parasitenkd* 47:269–281
- Ndiaye PI, Miquel J, Marchand B (2003) Ultrastructure of spermiogenesis and spermatozoa of *Taenia parva* Baer, 1926 (Cestoda, Cyclophyllidea, Taeniidae), a parasite of the common genet (*Genetta genetta*). *Parasitol Res* 89:34–43
- 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
- Schmidt GD (1986) Handbook of tapeworm identification. CRC Press, Boca Raton, Florida
- Sène A, Bâ CT, Marchand B (1997) Ultrastructure of spermiogenesis and the spermatozoon of *Nomimoscolex* sp. (Cestoda, Proteocephalidae) intestinal parasite of *Claroetes laticeps* (Fish, Teleost) in Senegal. *J Submicrosc Cytol Pathol* 29:1–6
- Sène A, Bâ CT, Marchand B (1999) Ultrastructure of spermiogenesis of *Phyllobothrium lactuca* (Cestoda, Tetracyphylidae, Phyllobothriidae). *Folia Parasitol* 6:191–198
- Shi DZ, Liu DS, Wang SK, Craig PS (1994) The ultrastructure of *Echinococcus multilocularis*. *Chin J Parasit Dis Control* 7:40–41
- Stoitsova SR, Georgiev BB, Dacheva RB (1995) Ultrastructure of spermiogenesis and the mature spermatozoon of *Tetrabothrius erostris* Loennberg, 1896 (Cestoda, Tetrabothriidae). *Int J Parasitol* 25:1427–1436
- Swiderski Z (1984) Spermatogenesis in the davaineid cestode *Inermicapsifer madagascariensis*, a parasite of man and rodents: SEM observations. Proceedings of the 23th Annual Conference of the Electron Microscopy Society of South Africa, Stellenbosch, pp 29–130
- Swiderski Z (1985) Spermiogenesis in the proteocephalid cestode *Proteocephalus longicollis*. Proceedings of the 24th Annual Conference of the Electron Microscopy Society of South Africa, Pietermaritzburg, pp 181–182
- Swiderski Z (1994) Spermiogenesis in the Trypanorhynchid Cestode *Lacistorhynchus tenuis*. Proceedings of the 13th International Congress of Electron Microscopy, Paris, pp 691–692
- Swiderski Z, Mackiewicz JS (2002) Ultrastructure of spermatogenesis and spermatozoa of the caryophyllidean cestode *Glaridaeris catostomi* Cooper, 1920. *Acta Parasitol* 47:83–104

- Swiderski Z, Mokhtar-Maamouri F (1980) Étude de la spermatogénèse de *Bothriocephalus clavibothrium* Ariola, 1899 (Cestoda: Pseudophyllidea). Arch Inst Pasteur Tunis 57:323–347
- Swiderski ZP, Tkach VV (1996) Ultrastructure of the spermatozoon of the cestode *Monorcholepis dujardini* (Cyclophyllidea, Hymenolepididae). Proceedings of the 6th Asia-Pacific Electron Microscopy Conference, Hong Kong, pp 507–508
- Swiderski Z, Salamatin RV, Tkach VV (2000) Electron microscopical study of spermatozoa of the cestode *Dilepis undula* (Cyclophyllidea, Dilepididae). Vest Zool 34:3–7
- Tian X, Yuan L, Li Y, Huo X, Han X, Xu M, Lu M, Dai J, Dong L (1998) Ultrastructural observation on spermatocytogenesis in taeniid cestode. Chin J Parasitol Parasit Dis 16:209–212
- Wardle RA, McLeod JA, Radinovsky S (1974) Advances in the zoology of tapeworms, 1950–1970. University of Minnesota Press, Minneapolis
- Yamaguti S (1959) Systema Helminthum, vol II. The cestodes of vertebrates. Interscience, New York

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| Dipylidiidae species   | Spermiogenesis |    |    |                |    | Spermatozoon |                |             |            |        |    |   |   |
|--|----------------|----|----|----------------|----|--------------|----------------|-------------|------------|--------|----|---|---|
|  | Type           | Pf | Fr | Sr             | Ib | Cb           | Ac             | Cm          | Ps         | G      | Iw |   |   |
| Reference  |                |    |    |                |    | <i>n</i>     | Thickness (nm) | Length (nm) | Width (nm) | Angle  |    |   |   |
| <i>Dipylidium caninum</i><br>Miquel and Marchand (1997),<br>Miquel et al. (1998) | III            | +  | -  | + <sup>a</sup> | -  | 1            | 150            | 600         | 400        | 40°    | +  | - | - |
| <i>Joyeuxiella echinorhynchoides</i><br>Present paper                            | III            | +  | -  | +              | -  | 1            | 150            | >2,000      | 385        | 40–50° | +  | - | - |
| <i>Joyeuxiella pasqualei</i><br>Present paper                                    | III            | +  | -  | +              | -  | 1            | 75             | >2,000      | 385        | 40–50° | +  | - | - |

<sup>a</sup>Presence of thin striated roots

and *T. parva* (Ndiaye et al. 2003) (Taeniidae). In our view, several images of *D. caninum* (Dipylidiidae) show structures that resemble periaxonemal sheaths in both nuclear and non-nuclear areas of the sperm, but the authors did not describe this aspect of the images. Probably, the presence of a periaxonemal sheath is another feature that characterizes the ultrastructure of spermatozoa in members of the family Dipylidiidae.

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## References

- Azzouz-Draoui N (1985) Étude ultrastructurale comparée de la spermiogenèse et du spermatozoïde de quatre Cestodes Dipylidiidae. Thesis, University of Tunis, Tunis
- Azzouz-Draoui N, Mokhtar-Maamouri F (1986/88) Ultrastructure comparée de la spermiogenèse et du spermatozoïde de *Echinobothrium affine* Diesing, 1863 et *E. harfordi* MacVicar, 1976 (Cestoda, Dipylidiidae). Bull Soc Sci Nat Tunisie 18:9–20
- Bâ A, Bâ CT, Marchand B (2000) Ultrastructure of spermiogenesis and the spermatozoon of *Sudarikovina taterae* (Cestoda, Cyclophyllidea, Anoplocephalidae) intestinal parasite of *Tatera gambiana* (Rodentia, Gerbillidae). J Submicrosc Cytol Pathol 32:137–144
- Bâ A, Bâ CT, Marchand B (2002) Ultrastructural study of the spermatozoon of *Echinocotyle dolosa* (Cestoda, Cyclophyllidea, Hymenolepididae). Acta Parasitol 47:131–136
- Bâ CT, Marchand B (1992a) 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 (1992b) Reinvestigation of the ultrastructure 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 (1994a) 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 (1994b) Ultrastructure of spermiogenesis and the spermatozoon of *Mathevotaenia herpestis* (Cestoda), intestinal parasite of *Atelexis albiventrix* in Sénégal. Acta Zool (Stockh) 75:167–175
- Bâ CT, Marchand B (1994c) Similitude ultrastructurale des spermatozoïdes de quelques Cyclophyllidea. Parasite 1:51–55
- Bâ CT, Marchand B (1994d) Ultrastructure of spermiogenesis and the spermatozoon of *Raillietina (Raillietina) tunetensis* (Cyclophyllidea, Davaineidae) intestinal parasite of turtle doves in Senegal. Int J Parasitol 24:237–248

- Bâ CT, Marchand B (1994e) 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 (1995) Spermiogenesis, spermatozoa and phyletic affinities in the Cestoda. In: Jamieson BGM, Ausió J, Justine JL (eds) *Advances in spermatozoal phylogeny and taxonomy*. *Mem Mus Natl Hist Nat* 166:87–95
- 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 Cestodes spermatozoon illustrated by the ultrastructural study of spermiogenesis of a Cyclophyllidea: *Thysaniezia ovilla*, Rivolta, 1874. *J Submicrosc Cytol Pathol* 23:606–612
- Barrett NJ, Smyth JD (1983) Observations 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 H-P (2001) Spermiogenesis in the pseudophyllid cestode *Eubothrium crasum* (Bloch, 1779). *Parasitol Res* 87:579–588
- Chomicz L, Swiderski Z (1992) Spermiogenesis and ultrastructure of the ultrastructure of the cestode *Dicranotaenia coronula* (Dujardin, 1845) (Cyclophyllidea, Hymenolepididae). *Proceedings of the 5th Asia-Pacific Electron Microscopy Conference, Beijing*, 324–325
- Euzet L, Swiderski Z, Mokhtar-Maamouri F (1981) Ultrastructure comparée du spermatozoïde des Cestodes. Relations avec la phylogénèse. *Ann Parasitol Hum Comp* 56:247–259
- 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
- Jones A (1983) A revision of the cestode genus *Joyeuxiella* Fuhrmann, 1935 (Dilepididae: Dipylidiinae). *Syst Parasitol* 5:203–213
- Justine J-L (1991) Phylogeny of parasitic Platyhelminthes: a critical study of synapomorphies proposed on the basis of the ultrastructure of spermiogenesis and spermatozoa. *Can J Zool* 69:1421–1440
- 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, London, pp 231–238
- Kelsoe GH, Ubelaker JE, Allison VF (1977) The fine structure of spermiogenesis in *Hymenolepis diminuta* (Cestoda) with a description of the mature spermatozoon. *Z Parasitenkd* 54:175–187
- Khalil LF, Jones A, Bray RA (1994) *Keys to the cestode parasites of vertebrates*. CAB International, Cambridge
- Mahendrasingam S, Fairweather I, Halton DW (1989) Spermatogenesis and the fine structure of the mature spermatozoon in the free proglottis of *Trilocularia acanthiaevulgaris* (Cestoda, Tetraphyllidea). *Parasitol Res* 75:287–298
- Mariaux J (1998) A molecular phylogeny of the Eucestoda. *J Parasitol* 84:114–124
- Matevosyan EM (1953) Re-organisation of the classification of dilepidid cestodes. *Papers on helminthology presented to Academician K.I. Skryabin on his 75th birthday*. Izdatel'stvo Akademii Nauk SSSR, Moscow, pp 392–397
- Matevosyan EM (1963) *Principles of cestodology*, vol. III. Dilepidoidea, tapeworms of domestic and wild animals. *Academiya Nauk SSSR*, Moscow
- 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 (1998) Ultrastructure of spermiogenesis and the spermatozoon of *Anoplocephaloides dentata* (Cestoda, Cyclophyllidea, Anoplocephalidae), an intestinal parasite of Arvicolidae rodents. *J Parasitol* 84:1128–1136
- 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
- Mokhtar-Maamouri F (1979) Étude en microscopie électronique de la spermiogénèse et du spermatozoïde de *Phyllobothrium gracile* Weld, 1855 (Cestoda, Tetraphyllidea, Phyllobothriidae). *Z Parasitenkd* 59:245–258
- Mokhtar-Maamouri F (1982) Étude ultrastructurale de la spermiogénèse de *Acanthobothrium filicollae* var. *filicollae* Zschokke, 1888 (Cestoda: Tetraphyllidea, Onchobothriidae). *Ann Parasitol Hum Comp* 57:429–442
- Mokhtar-Maamouri F, Azzouz-Draoui N (1984) Spermiogénèse et ultrastructure du spermatozoïde de deux Cestodes Diphyllidea. 4ème Ecole Franco-Africaine Biol Mol, Djerba, Tunisie, pp 467–469
- Mokhtar-Maamouri F, Azzouz-Draoui N (1990) Spermiogénèse et ultrastructure du spermatozoïde de *Nematotaenia chantalae* Dollfus, 1957 (Cestoda, Cyclophyllidea, Nematotaeniidae). *Ann Parasitol Hum Comp* 65:221–228
- Mokhtar-Maamouri F, Swiderski Z (1975) Étude en microscopie électronique de la spermatogénèse de deux Cestodes *Acanthobothrium filicollae benedenii* Loennberg, 1889 et *Onchobothrium uncinatum* (Rud., 1819) (Tetraphyllidea, Onchobothriidae). *Z Parasitenkd* 47:269–281
- Ndiaye PI, Miquel J, Marchand B (2003) Ultrastructure of spermiogenesis and spermatozoa of *Taenia parva* Baer, 1926 (Cestoda, Cyclophyllidea, Taeniidae), a parasite of the common genet (*Genetta genetta*). *Parasitol Res* 89:34–43
- 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
- Schmidt GD (1986) *Handbook of tapeworm identification*. CRC Press, Boca Raton, Florida
- Sène A, Bâ CT, Marchand B (1997) Ultrastructure of spermiogenesis and the spermatozoon of *Nomimosclex* sp. (Cestoda, Proteocephalidae) intestinal parasite of *Claroetes laticeps* (Fish, Teleost) in Senegal. *J Submicrosc Cytol Pathol* 29:1–6
- Sène A, Bâ CT, Marchand B (1999) Ultrastructure of spermiogenesis of *Phyllobothrium lactuca* (Cestoda, Tetraphyllidea, Phyllobothriidae). *Folia Parasitol* 6:191–198
- Shi DZ, Liu DS, Wang SK, Craig PS (1994) The ultrastructure of *Echinococcus multilocularis*. *Chin J Parasit Dis Control* 7:40–41
- Stoitsova SR, Georgiev BB, Dacheva RB (1995) Ultrastructure of spermiogenesis and the mature spermatozoon of *Tetrabothrius erosiris* Loennberg, 1896 (Cestoda, Tetrabothriidae). *Int J Parasitol* 25:1427–1436
- Swiderski Z (1984) Spermatogenesis in the davaineid cestode *Inermicapsifer madagascariensis*, a parasite of man and rodents: SEM observations. *Proceedings of the 23th Annual Conference of the Electron Microscopy Society of South Africa, Stellenbosch*, pp 29–130
- Swiderski Z (1985) Spermiogenesis in the proteocephalid cestode *Proteocephalus longicollis*. *Proceedings of the 24th Annual Conference of the Electron Microscopy Society of South Africa, Pietermaritzburg*, pp 181–182
- Swiderski Z (1994) Spermiogenesis in the Trypanorhynchid Cestode *Lacistorhynchus tenuis*. *Proceedings of the 13th International Congress of Electron Microscopy*, Paris, pp 691–692
- Swiderski Z, Mackiewicz JS (2002) Ultrastructure of spermatogenesis and spermatozoa of the caryophyllidean cestode *Glaridacris catostomi* Cooper, 1920. *Acta Parasitol* 47:83–104

- Swiderski Z, Mokhtar-Maamouri F (1980) Étude de la spermatogénèse de *Bothriocephalus clavibothrium* Ariola, 1899 (Cestoda: Pseudophyllidea). Arch Inst Pasteur Tunis 57:323–347
- Swiderski ZP, Tkach VV (1996) Ultrastructure of the spermatozoon of the cestode *Monorcholepis dujardini* (Cyclophyllidea, Hymenolepididae). Proceedings of the 6th Asia-Pacific Electron Microscopy Conference, Hong Kong, pp 507–508
- Swiderski Z, Salamatin RV, Tkach VV (2000) Electron microscopical study of spermatozoa of the cestode *Dilepis undula* (Cyclophyllidea, Dilepididae). Vest Zool 34:3–7
- Tian X, Yuan L, Li Y, Huo X, Han X, Xu M, Lu M, Dai J, Dong L (1998) Ultrastructural observation on spermatocytogenesis in taeniid cestode. Chin J Parasitol Parasit Dis 16:209–212
- Wardle RA, McLeod JA, Radinovsky S (1974) Advances in the zoology of tapeworms, 1950–1970. University of Minnesota Press, Minneapolis
- Yamaguti S (1959) Systema Helminthum, vol II. The cestodes of vertebrates. Interscience, New York