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VERSITA

Ultrastructure of the spermatozoon of *Calliobothrium verticillatum* (Cestoda, Tetraphyllidea, Oncobothriidae)

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Abstract

The ultrastructure of the spermatozoon of *Calliobothrium verticillatum* (Cestoda, Tetraphyllidea, Oncobothriidae), parasite of the smoothhound shark, *Mustelus mustelus* L. (Pisces, Carcharhiniformes), was studied by transmission electron microscopy. This spermatozoon presents five regions characterized by several ultrastructural elements: an apical cone, a crested body, two axonemes of 9 + "1" pattern, electron-dense granules, a nucleus and cortical microtubules. In the present study, three of these features were the subject of a detailed attention. The first is the presence of two axonemes, which confirms that the Tetraphyllidea, Oncobothriidae possess two axonemes whereas the Tetraphyllidea, Phyllobothriidae possess only one axoneme. The second is the presence of one crested body, a criterion homogeneous in the Tetraphyllidea but heterogeneous among the different orders of Cestoda. The third is the number and the disposition of cortical microtubules. These three criteria seem to be interesting for phylogeny.

Key words

Cestoda, Tetraphyllidea, Oncobothriidae, *Calliobothrium verticillatum*, spermatozoon, ultrastructure, fish, *Mustelus mustelus*

Introduction

For more than forty years, many studies on the ultrastructure of the spermiogenesis and spermatozoon of parasitic worms have been carried out. Many authors employed these criteria of ultrastructure in phylogeny like Ehlers (1986) for the Platyhelminthes or Brooks *et al*. (1985), Brooks (1989), Rohde (1990) and Justine (1991, 1997) for the only parasitic Platyhelminthes. Whilst the ultrastructure of spermiogenesis and the spermatozoon is relatively homogeneous in the digenetic trematodes, in the cestodes it is very diversified. Euzet *et al.* (1981) and Bâ and Marchand (1995) wrote on this complex subject. Among the fifteen orders comporting the Cestoda (Schmidt 1986), differences have been remarked in the spermatozoon like the number of crested bodies (Bâ and Marchand 1994a), the disposition of cortical microtubules (Bâ and Marchand 1992a, b; Eira *et al*. 2006) or the presence of an apical cone (Bâ *et al*. 1991, Bâ and Marchand 1994b, Bâ, *et al*. 2005). Moreover, if the axonemes are 9 + "1" pattern of Trepaxonemata like in most other parasitic Platyhelminthes, the number of axonemes changes according to the order or the family. Indeed, in the Tetraphyllidea, the spermatozoon of Phyllobothriidae species presents one axoneme, whereas the Oncobothriidae species present two axonemes in the sperm cell (Euzet *et al*. 1981). Present study concerns the ultrastructural analysis of the spermatozoon of a Tetraphyllidaea, Oncobothriidae: *Calliobothrium verticillatum*.

Only few Tetraphyllidea have been studied at this day. In the present work, we compare the results of our study with those previously described in other cestodes and especially in the Phyllobothriidae.

Materials and methods

The specimens of *Calliobothrium verticillatum* (Rudolphi, 1819) Beneden, 1850 were removed from the spiral valve of *Mustelus mustelus* collected near Senegal coasts (Atlantic Ocean). Then, the worms were kept active in physiological saline solution (0.9% NaCl). Portions of strobila, made up of mature proglottides, were quickly taken and then stretched out with a brush soaked in cold (4°C) 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate solution at pH 7.2. Portion containing male genitalia were removed under a binocular microscope, fixed for about 24 h in glutaraldehyde, rinsed several times in a sodium cacodylate buffer, then left overnight in the same buffer, postfixed with cold (4°C) 1% osmium tetroxide for 1 h, dehydrated in an ethanol series and propylene oxide, and then embedded in Epon. Ultrathin sections (60–90 nm) were cut on an LKB 8800A Ultrotome III.

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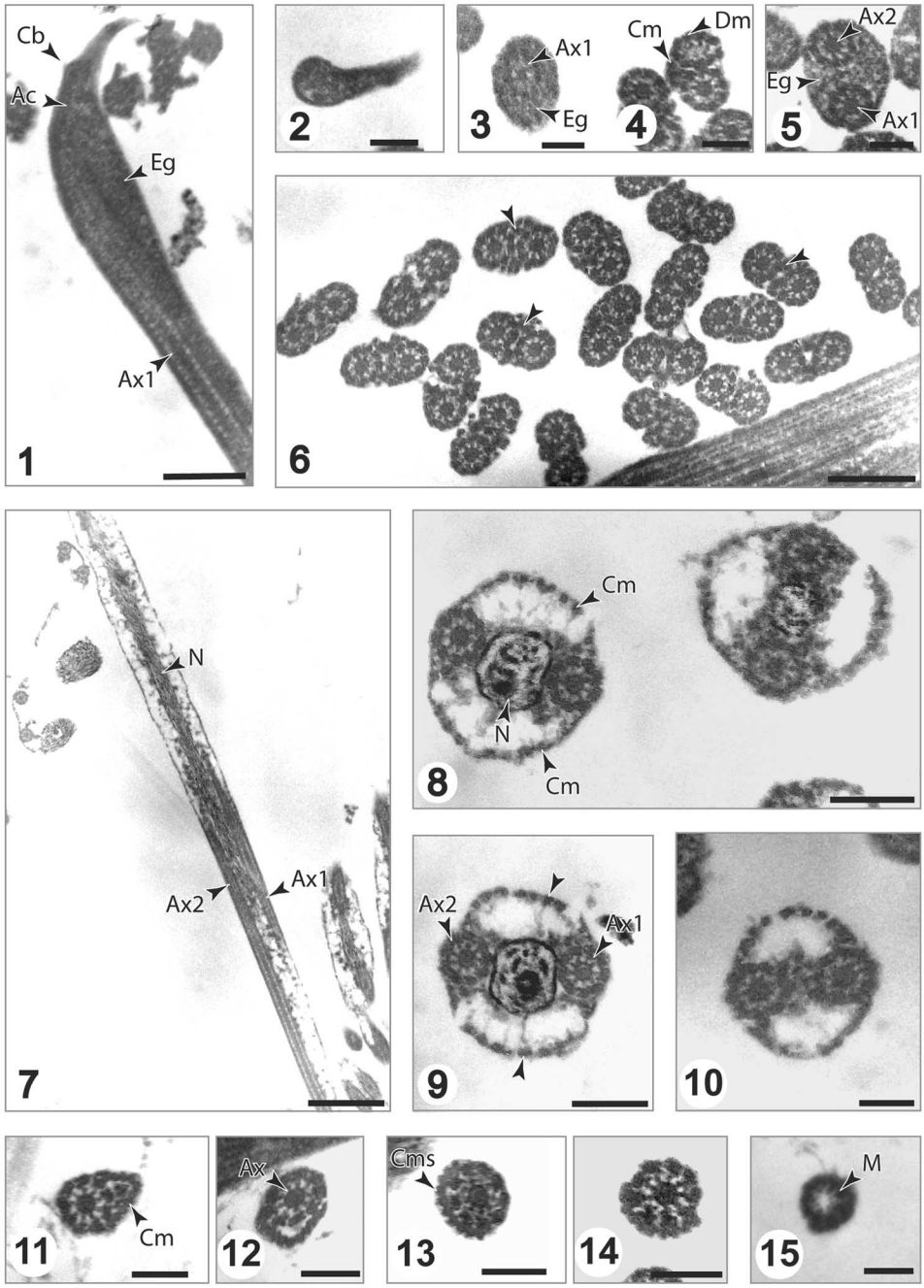

Figs 1–15. Mature spermatozoon of *Calliobothrium verticillatum*: **1**. Longitudinal section of the anterior part of the spermatozoon. Scale bar = 0.6 µm. **2**. Cross-section of the apical cone. Scale bar = 0.3 µm. **3**. Cross-section of the anterior part of the spermatozoon showing the first axoneme and electron-dense granules. Scale bar = 0.2 µm. **4**. Cross-section of the spermatozoon in region II showing cortical microtubules and the appearance of the second axoneme. Scale bar = 0.2 µm. **5**. Cross-section of the spermatozoon showing two axonemes and electron-dense granules. Scale bar = 0.2 µm. **6**. Cross-sections of several spermatozoa at the end of region II. Arrowheads indicate the electron-dense granules. Scale bar = 0.4 µm. **7**. Longitudinal section of a spermatozoon in region III. This region is characterized by the presence of the nucleus and the two axonemes. Scale bar = 1 µm. **8** and **9**. Cross-sections of the spermatozoon in region III showing the nucleus, the two axonemes and two rows of cortical microtubules (arrowheads). Scale bar = 0.3 µm. **10**. Cross-section of the spermatozoon in region IV characterized by the absence of the nucleus and the presence of the axonemes. Scale bar = 0.2 µm. **11**. Cross-section of the posterior part of the spermatozoon (region V). There is only one axoneme and a row of ten cortical microtubules. Scale bar = 0.2 µm. **12**. Cross-section of the spermatozoon showing the narrowing of the plasma membrane. Scale bar = 0.2 µm. **13** and **14**. Cross-sections of the spermatozoon in region V. The ten microtubules are disposed in a semi-circle around the axoneme. Scale bar = 0.2 µm. **15**. Cross-section of the posterior extremity of the spermatozoon showing some microtubules. Scale bar = 0.2 µm. **Abbreviations to all figures:** Ac – apical cone, Ase – anterior spermatozoon extremity, Ax – axoneme, Ax1 – axoneme 1, Ax2 – axoneme 2, Cb – crested-like body, Cc – central core, Cm – cortical microtubules, Cms – cortical microtubules in semi-circle, Dm – doublet of microtubules, Eg – electron-dense granules, M – microtubules, N – nucleus, Pm – plasma membrane, Pse – posterior spermatozoon extremity

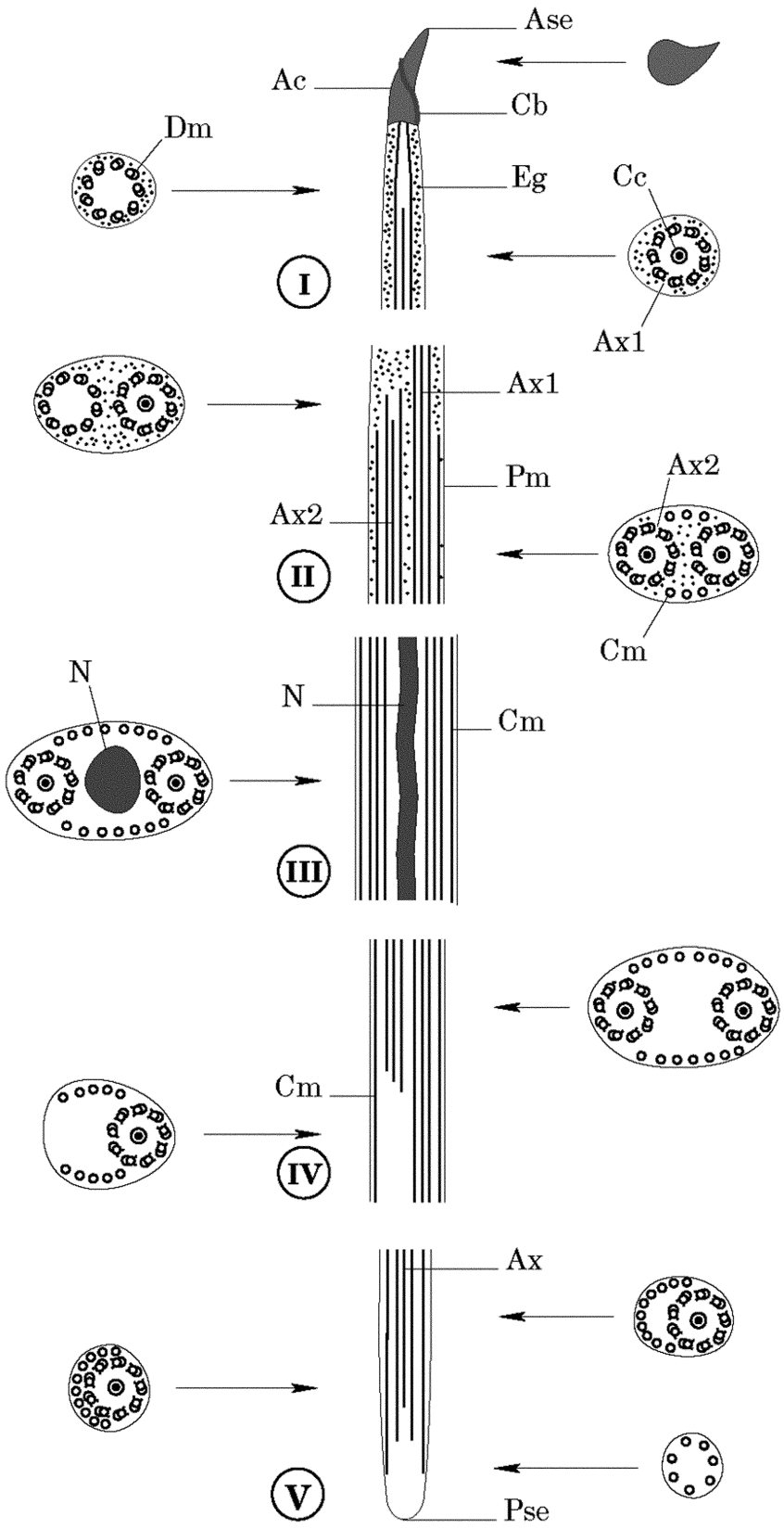

Fig. 16I–V. Diagram showing the ultrastructural organisation of the mature spermatozoon of *Calliobothrium verticillatum*

They were placed on 300-mesh copper grids and stained with uranyl acetate and lead citrate according to the method of Reynolds (1963).

The sections were then examined under a Hitachi H-600 transmission electron microscope, operating at an accelerating voltage of 75 kV, in the laboratory "Parasites et Ecosystèmes Méditerranéens" at the University of Corsica (Corte, France).

Results

Observations of numerous transverse and longitudinal sections of the mature spermatozoon of Calliobothrium verticillatum *enable us to establish five regions* (*I–V*) *with the following ultrastructural features*

Region I (Figs 1–3, 16I): This region constitutes the anterior part of the spermatozoon. It is characterized by an apical cone (Figs 1, 2 and 16I) and a short crested body (Figs 1 and 16I) in its anterior part. In transverse section, the apical cone is pear shaped (Fig. 2). The crested body has been observed only in this zone. Posteriorly, this region is characterized by the presence of the first axoneme (Figs 3 and 16I). The central core of the axoneme appears after the nine doublets of microtubules. In this region, a great number of electron-dense granules are observed (Figs 1, 3 and 16I).

Region II (Figs 4–6 and 16II): The anterior part of this region contains only the first axoneme. The second axoneme appears posteriorly (Figs 4 and 16II). Like in the first axoneme, in the second one, the nine doublets of microtubules appear before the central core. The region II is characterized by the presence of cortical microtubules in close contact with the plasma membrane (Figs 4 and 16II). They are disposed into two fields separated by the two axonemes. The maximum number of cortical microtubules is six, in the posterior part of this region. The electron-dense granules are less numerous than in region I (Figs 5, 6 and 16II).

Region III (Figs 7–9 and 16III): This region is characterized by the presence of the nucleus (Figs 7 and 8) and the disappearance of electron-dense granules. The two axonemes and cortical microtubules are still present (Figs 7 and 8). In this region, the number of cortical microtubules is about 15. They are always disposed into two fields (Fig. 9) separated by the axonemes, which are in close contact with the plasma membrane, and are never surrounded by cortical microtubules.

Region IV (Figs 10 and 16IV): The nucleus does not extend into this region. In the anterior part of this zone there are always about 15 cortical microtubules and the two axonemes (Fig. 10). In the posterior part, an axoneme disappears and the number of cortical microtubules decreases. At the end of this region there are only ten cortical microtubules.

Region V (Figs 11–15 and 16V): This region is the posterior zone of the spermatozoon. There are only one axoneme and ten cortical microtubules. In the anterior part of this zone the cortical microtubules are not in contact with the axoneme (Fig. 11). Posteriorly, with the reduction of the spermatozoon diameter, the cortical microtubules (always 10) (Fig. 12), are arranged in a semi-circle around the last axoneme (Figs 13, 14 and 16V).

Finally, the posterior end of the spermatozoon is characterized only by some microtubules (Fig. 15).

Discussion

The mature spermatozoon of *Calliobothrium verticillatum* possess a crested body, two axonemes, electron-dense granules, cortical microtubules and a nucleus.

We notice the absence of mitochondria. This absence is a characteristic of the Eucestoda and is considered as an ultrastructural synapomorphy (Brooks 1989; Justine 1997, 1998). Indeed one or more mitochondria are found in mature spermatozoon of the other Platyhelminthes (Justine 1997).

One crested body is present in the anterior part of the spermatozoon. This element is short and only observed at the level of the apical cone. This element has been described in many cestodes and is considered a synapomorphy for the Eucestoda (Bâ and Marchand 1995). It would play an important role during the fertilisation process (Mokhtar-Maamouri 1980). The number of crested bodies varies according to the species. Among Pseudophyllidea, some species, *Diphyllobothrium latum* (Levron *et al*. 2006a) and *Bothriocephalus clavibothrium* (Świderski and Mokhtar-Maamouri 1980), are devoid of this element. In the same order, other species, *Paraechinophallus japonicus* (Levron *et al*. 2006b), *Bothriocephalus scorpii* (Levron *et al*. 2006c), *Eubothrium crassum* (Bruňanská *et al*. 2002), *Duthiersia fimbriata* (Justine 1986) and *Triaenophorus nodulosus* (Levron *et al*. 2005), possess only one crested body. This variability is also observed in the Proteocephalidea where *Corallobothrium solidum* (Bruňanská *et al*. 2004) and *Sandonella sandoni* (Bâ and Marchand 1994c) possess only one crested body whereas *Nomimoscolex* sp. (Sène *et al*. 1997) possess 3 of these elements. But, the greatest variability of that criterion is observed among the Cyclophyllidea where the number of crested bodies varies from one, e.g. *Skrjabinoporus merops* (Yoneva *et al*. 2006, Dipylidiidae (Miquel *et al*. 2005), *Dipylidium caninum* (Miquel and Marchand 1997), to twelve in *Hymenolepis nana* (Bâ and Marchand 1992a). To our knowledge, in the Tetraphyllidea, *C. verticillatum* is the eighth studied species and all of them possess only one crested body (Table I). If this criterion appears homogeneous in this order, the number of axonemes shows more variations.

The spermatozoon of *C. verticillatum* presents two axonemes of the 9 + "1" pattern of the Trepaxonemata (Ehlers 1984). The order of Tetraphyllidea has the particularity to possess some species with one axoneme and other with two axonemes (Table I). The Oncobothriidae and the Triloculariidae present two axonemes and the Phyllobothriidae only one. Euzet *et al*. (1981) proposed a classification of cestode spermatozoa based on the number of axonemes. According to them, the presence of a single axoneme would be an evolved

Table I. Comparison of some characters present in the spermatozoon of Tetraphyllidea

Taxon	Cb	Ax	Cms	References
Oncobothriidae				
Acantobothrium filicolle var. *filicolle*	1	2	+	Mokhtar-Maamouri (1982)
Acantobothrium filicolle benedenii	1	2	+	Mokhtar-Maamouri and Świderski (1975)
Calliobothrium verticillatum	1	2	+	present study
Onchobothrium uncinatum	1	2	+	Mokhtar-Maamouri and Świderski (1975)
Phyllobothriidae				
Phyllobothrium gracile	1	1	+	Mokhtar-Maamouri (1979)
Pseudanthobothrium hanseni	1	1	+	MacKinnon and Burt (1984)
Echeneibothrium beauchampi		1	+	Mokhtar-Maamouri and Świderski (1976)
Triloculariidae				
Trilocularia acanthiaevulgaris	1	2	+	Mahendrasingam *et al*. (1989)

Ax – number of axonemes, Cb – number of crested bodies, Cms – 10 cortical microtubules disposed in semi-circle around the axoneme, + – presence.

character. In this case, the Cyclophyllidea, always presenting one axoneme, would be related to the Tetraphyllidea.

We notice the presence of electron-dense granules in the anterior part of the spermatozoon. These granules could be glycogen reserves. The position of these granules into the spermatozoon varies according to the species of Cestoda (Świderski and Mackiewicz 2002).

Other ultrastructural elements present in this spermatozoon are the cortical microtubules. The reconstruction of the spermatozoon is principally based on these elements. Absent in the anterior part of the spermatozoon (region I), their number increases until the nuclear region (region III). The maximum number of cortical microtubules is 15. In this region they are arranged in two rows located between the two axonemes. Similar maximum number and disposition of cortical microtubules are found in other Tetraphyllidea, Oncobothriidae: *Acanthobothrium filicolle* var. *filicolle* (Mokhtar-Maamouri 1982), *Acanthobothrium filicolle benedenii* (Mokhtar-Maamouri and Świderski 1975). This maximum number is lower than that of the majority of Digenea and polyopisthocotylean Monogenea. For these groups, this number is about 40 longitudinal microtubules spaced regularly at the periphery (Justine 1991). Moreover, these microtubules are not spiralled as in Cyclophyllidea (Justine 1991; Bâ and Marchand 1992a, b; Miquel *et al*. 1999; Bâ *et al*. 2000, 2002, 2005). An other ultrastructural particularity is the presence of 10 cortical microtubules disposed in semi-circle around the axoneme in the region V. This observation was made in all the Tetraphyllidea (Table I). Świderski and Mokhtar-Maamouri (1980), notice that the spermatozoon of *Bothriocephalus clavibothrium* (Pseudophyllidea) differs of those of Tetraphyllidea, Oncobothriidae by the absence of this semi-circle of 10 microtubules around the axoneme. We notice that this structure is also found in *Nomimoscolex* sp. (Sène *et al*. 1997), a Proteocephalidea, who is a phylogenetically close group of Tetraphyllidea. This structure shows that cortical microtubules are interesting for phylogeny.

References

Bâ A., Bâ C.T., Marchand B. 2000. Ultrastructure of spermiogenesis and the spermatozoon of *Sudarikovina taterae* (Cestoda, Cyclophyllidea, Anoplocephalidae) intestinal parasite of *Tatera gambiana* (Rodentia, Gerbillidae). *Journal of Submicroscopic Cytology and Pathology*, 32, 137–144.

Bâ A., Bâ C.T., Marchand B. 2002. Ultrastructural study of the spermatozoon of *Echinocotyle dolosa* (Cestoda, Cyclophyllidea, Hymenolepididae). *Acta Parasitologica*, 47, 131–136.

Bâ C.T., Bâ A., Marchand B. 2005. Ultrastructure of the spermatozoon of *Paroniella reynoldsae* (Cyclophyllidea, Davaineidae) an intestinal parasite of *Corvus albus* (Aves, Corvidae). *Acta Parasitologica*, 50, 208–214.

Bâ C.T., Marchand B. 1992a. Reinvestigation of the ultrastructure and the spermatozoon of *Hymenolepis nana* (Cestoda, Cyclophyllidea), parasite of the small intestine of *Rattus rattus*. *Molecular Reproduction and Development*, 33, 39–45. DOI: 10.1002/mrd.1080330106.

Bâ C.T., Marchand B. 1992b. Ultrastructural particularities of the spermatozoon of *Stilesia globipunctata* (Cestoda) parasite of the small intestine of sheep and goats in Senegal. *Journal of Submicroscopic Cytology and Pathology*, 24, 29–34.

Bâ C.T., Marchand B. 1994a. Similitude ultrastructurale des spermatozoïdes de quelques Cyclophyllidea. *Parasite*, 1, 51–55.

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

Bâ C.T., Marchand B. 1994c. Ultrastructure of the spermatozoon of *Sandonella sandoni* (Cestoda, Proteocephalidea, Sandonellinae). *Invertebrate Reproduction and Development*, 25, 9–17.

Bâ C.T., Marchand B. 1995. Spermiogenesis, spermatozoa and phyletic affinities in the Cestoda. In: (Eds. B.G.M. Jamieson, J. Ausio and J.-L. Justine) *Advances in spermatozoal phylogeny and taxonomy*. *Mémoires du Muséum National d'Histoire Naturelle*, 166, 87–95.

Bâ C.T., 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. *Journal of Submicroscopic Cytology and Pathology*, 23, 605–612.

Brooks D.R. 1989. A summary of the database pertaining to the phylogeny of the major groups of the parasitic platyhelminths,

with a revised classification. *Canadian Journal of Zoology*, 67, 714–720.

Brooks D.R., O'Grady R.T., Glen D.R. 1985. The phylogeny of the Cercomeria Brooks, 1982 (Platyhelminthes). *Proceedings of the Helminthological Society of Washington*, 52, 1–20.

Bruňanská M., Nebesářová J., Scholz T., Fagerholm H.P. 2002. Ultrastructure of the spermatozoon of the pseudophyllidean cestode *Eubothrium crassum* (Bloch, 1779). *Parasitology Research*, 88, 285–291. DOI: 10.1007/s00436-001-0532-0.

Bruňanská M., Scholz T., Ibraheem M.H. 2004. Ultrastructural characters of the spermatozoon of the cestode *Corallobothrium solidum* Fritsch, 1886 (Cestoda: Proteocephalidea), a parasite of the electric catfish *Malapterurus electricus*. *Parasitology Research*, 94, 421–426. DOI: 10.1007/s00436-004-1236-z.

Ehlers U. 1984. Phylogenetisches system der Plathelminthes. *Verhandlungen des Naturwissenschaftlichen Verein in Hamburg* (*NF*), 27, 291–294.

Ehlers U. 1986. Comments on a phylogenetic system of the Platyhelminthes. *Hydrobiologia*, 132, 1–12. DOI: 10.1007/BF000 46222.

Eira C., Miquel J., Vingada J., Torres J. 2006. Spermiogenesis and spermatozoon ultrastructure of the cestode *Mosgovoyia ctenoides* (Cyclophyllidea: Anoplocephalidae), an intestinal parasite of *Oryctolagus cuniculus* (Lagomorpha: Leporidae). *Journal of Parasitology*, 92, 708–718. DOI: 10.1645/GE-818R.1.

Euzet L., Świderski Z., Mokhtar-Maamouri F. 1981. Ultrastructure comparée du spermatozoïde des cestodes. Relations avec la phylogénèse. *Annales de Parasitologie Humaine et Comparée*, 56, 247–259.

Justine J.-L. 1986. Ultrastructure of the spermatozoon of the cestode *Duthiersia fimbriata* (Pseudophyllidea, Diphyllobothriidae). *Canadian Journal of Zoology*, 64, 1545–1548.

Justine J.-L. 1991. Phylogeny of parasitic Platyhelminthes: a critical study of synapomorphies proposed on the basis of the ultrastructure of spermiogenesis and spermatozoa. *Canadian Journal of Zoology*, 69, 1421–1440.

Justine J.-L. 1997. La classification générale des Plathelminthes parasites: changements récents et utilisation des caractères ultrastructuraux, en particulier des spermatozoïdes. *Bulletin de la Société Zoologique de France*, 122, 269–277.

Justine J.-L. 1998. Systématique des grands groupes de Plathelminthes parasites : quoi de neuf ? *Bulletin de la Société Française de Parasitologie*, 16, 34–52.

Levron C., Bruňanská M., Marchand B. 2005. Spermiogenesis and sperm ultrastructure of the pseudophyllidean cestode *Triaenophorus nodulosus* (Pallas, 1781). *Parasitology Research*, 98, 26–33. DOI: 10.1007/s00436-005-0009-7.

Levron C., Bruňaská M., Poddubnaya L.G. 2006a. Spermatological characters in *Diphyllobothrium latum* (Cestoda, Pseudophyllidea). *Journal of Morphology*, 267, 1110–1119. DOI:10.1002/jmor.10460.

Levron C., Bruňanská M., Kuchta R., Freeman M., Scholz T. 2006b. Spermatozoon ultrastructure of the pseudophyllidean cestode *Paraechinophallus japonicusi*, a parasite of deep-sea fish *Psenopsis anomala* (Perciformes, Centrolophidae). *Parasitology Research*, 100, 115–121. DOI: 10.1007/s00436-006-0224-x.

Levron C., Bruňanská M., Poddubnaya L.G. 2006c. Spermatological characters of the pseudophyllidean cestode *Bothriocephalus scorpii* (Müller, 1776). *Parasitology International*, 55, 113–120. DOI: 10.1016/j.parint.2005.11.055.

MacKinnon B.M., Burt M.D.B. 1984. The comparative ultrastructure of spermatozoa from *Bothrimonus sturionis* Duv. 1842 (Pseudophyllidea), *Pseudanthobothrium hanseni* Baer, 1956 (Tetraphyllidea), and *Monoecocestus americanus* Stiles, 1895 (Cyclophyllidea). *Canadian Journal of Zoology*, 62, 1059–1066.

Mahendrasingam S., Fairweather I., Halton D.W. 1989. Spermatogenesis and the fine structure of the mature spermatozoon in the free proglottis of *Trilocularia acanthiaevulgaris* (Cestoda, Tetraphyllidea). *Parasitology Research*, 75, 287–298. DOI: 10.1007/BF00931813.

Miquel J., Feliu C., Marchand B. 1999. Ultrastructure of spermiogenesis and the spermatozoon of *Mesocestoides litteratus* (Cestoda, Mesocestoididae). *International Journal for Parasitology*, 29, 499–510. DOI: 10.1016/ S0020-7519(98)00202-1.

Miquel J., Marchand B. 1997. Ultrastructure of the spermatozoon of *Dipylidium caninum* (Cestoda, Cyclophyllidea, Dilepididae), intestinal parasite of *Canis familiaris*. *Parasitology Research*, 83, 349–355. DOI: 10.1007/s004360050260.

Miquel J., Świderski Z., Marchand B. 2005. Spermatological characters in the Dipylidiidae Stiles, 1896 (Cestoda, Cyclophyllidea). *Acta Parasitologica*, 50, 65–73.

Mokhtar-Maamouri F. 1979. Étude en microscopie électronique de la spermiogenèse et du spermatozoïde de *Phyllobothrium gracile* Wedl, 1855 (Cestoda, Tetraphyllidea, Phyllobothriidae). *Zeitschrift für Parasitenkunde*, 59, 245–258.

Mokhtar-Maamouri F. 1980. Particularités des processus de la fécondation chez *Acanthobothrium filicolle* Zschokke, 1888 (Cestoda: Tetraphyllidea, Onchobothriidae). *Archives de l'Institut Pasteur de Tunis*, 57, 191–205.

Mokhtar-Maamouri F. 1982. Étude ultrastructurale de la spermiogenèse de *Acanthobothrium filicolle* var. *filicolle* Zschokke, 1888 (Cestoda, Tetraphyllidea, Onchobothriidae). *Annales de Parasitologie*, 57, 429–442.

Mokhtar-Maamouri F., Świderski Z. 1975. Étude en microscopie électronique de la spermatogénèse de deux cestodes *Acanthobothrium filicolle benedenii* Loennberg, 1889 et *Onchobothrium uncinatum* (Rud., 1819) (Tetraphyllidea, Onchobothriidae). *Zeitschrift für Parasitenkunde*, 47, 269–281.

Mokhtar-Maamouri F., Świderski Z. 1976. Ultrastructure du spermatozoïde d'un cestode Tetraphyllidea Phyllobothriidae, *Echeneibothrium beauchampi* Euzet, 1959. *Annales de Parasitologie*, 51, 673–674.

Reynolds E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology*, 17, 208–212.

Rohde K. 1990. Phylogeny of Platyhelminthes, with special reference to parasitic groups. *International Journal for Parasitology*, 20, 979–1007.

Schmidt G.D. 1986. CRC handbook of tapeworm identification. CRC Press, Inc., Boca Raton, Florida, 675 pp.

Sène A., Bâ C.T., Marchand B. 1997. Ultrastructure of spermiogenesis and the spermatozoon of *Nomimoscolex* sp. (Cestoda, Proteocephalidea) intestinal parasite of *Clarotes laticeps* (fish, teleost) in Senegal. *Journal of Submicroscopic Cytology and Pathology*, 29, 115–124.

Świderski Z., Mokhtar-Maamouri F. 1980. Étude de la spermatogénèse de *Bothriocephalus clavibothrium* Ariola, 1899 (Cestoda: Pseudophyllidea). *Archives de l'Institut Pasteur de Tunis*, 57, 323–347.

Świderski Z., Mackiewicz J.S. 2002. Ultrastructure of spermatogenesis and spermatozoa of the caryophyllidean cestode *Glaridacris catostomi* Cooper, 1920. *Acta Parasitologica*, 47, 83–104.

Yoneva A., Georgieva K., Mizinska Y., Georgiev B.B., Stoitsova S.R. 2006. Ultrastructure of spermiogenesis and mature spermatozoon of *Skrjabinoporus merops* (Cyclophyllidea, Metadilepididae). *Acta Parasitologica*, 51, 200–208. DOI: 10.2478/s11686-006-0031-7.

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