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VERSITA

Spermiogenesis and spermatozoon of the tapeworm *Parabothriocephalus gracilis* (Bothriocephalidea): Ultrastructural and cytochemical studies

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Abstract

Spermiogenesis and spermatozoon ultrastructure of the tapeworm *Parabothriocephalus gracilis* were described using transmission electron microscopy (TEM). Spermiogenesis is characterized by the formation of a zone of differentiation with two centrioles associated with striated rootlets, and an intercentriolar body between them. The two flagella undergo a rotation of 90° until they become parallel to the median cytoplasmic extension with which they fuse. Electron-dense material is present in the apical region of the zone of differentiation in the early stages of spermiogenesis. This electron-dense material is characteristic for the orders Bothriocephalidea and Diphyllobothriidea. The mature spermatozoon contains two axonemes of the 9 + '1' trepaxonematan pattern, nucleus, parallel cortical microtubules and electron-dense granules of glycogen. The anterior extremity of the spermatozoon exhibits a single helical electron-dense crested body 130 nm thick. One of the most interesting features is the presence of a ring of cortical microtubules surrounding the axoneme. This character has been reported only for species of the order Bothriocephalidea and may be unique in this cestode group.

Keywords

Eucestoda, Bothriocephalidea, *Parabothriocephalus gracilis*, spermiogenesis, spermatozoon, ultrastructure

Introduction

Spermatozoon ultrastructure and spermiogenesis are significant characters for phylogenetic studies in Platyhelminthes (Świderski 1968, 1986; Euzet *et al*. 1981; Justine 1998, 2001). Sperm characters were included in the definition of several major groups of helminths by Ehlers (1984, 1985, 1986) and Brooks (1989). Synapomorphies based on spermatozoon ultrastructure for the Eucestoda is mainly based on the absence of a mitochodrion in mature sperm and the presence of a crested body (Justine 1998).

Recent taxonomic and molecular studies proved that the order Pseudophyllidea consists of two unrelated clades: Diphyllobothriidea and Bothriocephalidea (Brabec *et al*. 2006, Kuchta *et al*. 2008). To present, ten species of orders Bothriocephalidea and Diphyllobothriidea have been studied for spermiogenesis and/or sperm ultrastructure, nominally, *Bothriocephalus clavibothrium* (Świderski and Mokhtar-Maamouri 1980), *Bothriocephalus claviceps* (Bâ *et al*. 2007), *Bothriocephalus scorpii* (Levron *et al*. 2006a), *Paraechinophallus japonicus* (Levron *et al*. 2006b), *Eubothrium crassum* (Bruňanská *et al*. 2001, 2002), *Triaenophorus nodulosus* (Levron *et al*. 2005), *Diphyllobothrium latum* (Bonsdorff and Telkkä 1965, Levron *et al*. 2006c), *Duthiersia fimbriata* (Justine 1986) and *Ligula intestinalis* (Levron *et al*. 2009).

The objective of the present study is to describe spermiogenesis and sperm ultrastructure of *Parabothriocephalus gracilis* (Echinophallidae) using transmission electron microscopy. For the first time, spermiogenesis has been studied within the family Echinophallidae. Obtained data were compared with information about other species of Bothriocephalidea and Diphyllobothriidea, and also other cestodes.

Materials and methods

Adult tapeworms *P. gracilis* Yamaguti, 1934 (Bothriocephalidea, Echinophallidae) were obtained from the intestine of *Psenopsis anomala* (Temminck et Schlegel, 1844) (Perciformes, Centrolophidae), collected from an Inland Sea area of Japan in 2004 by coauthor M.F. Living worms were rinsed in

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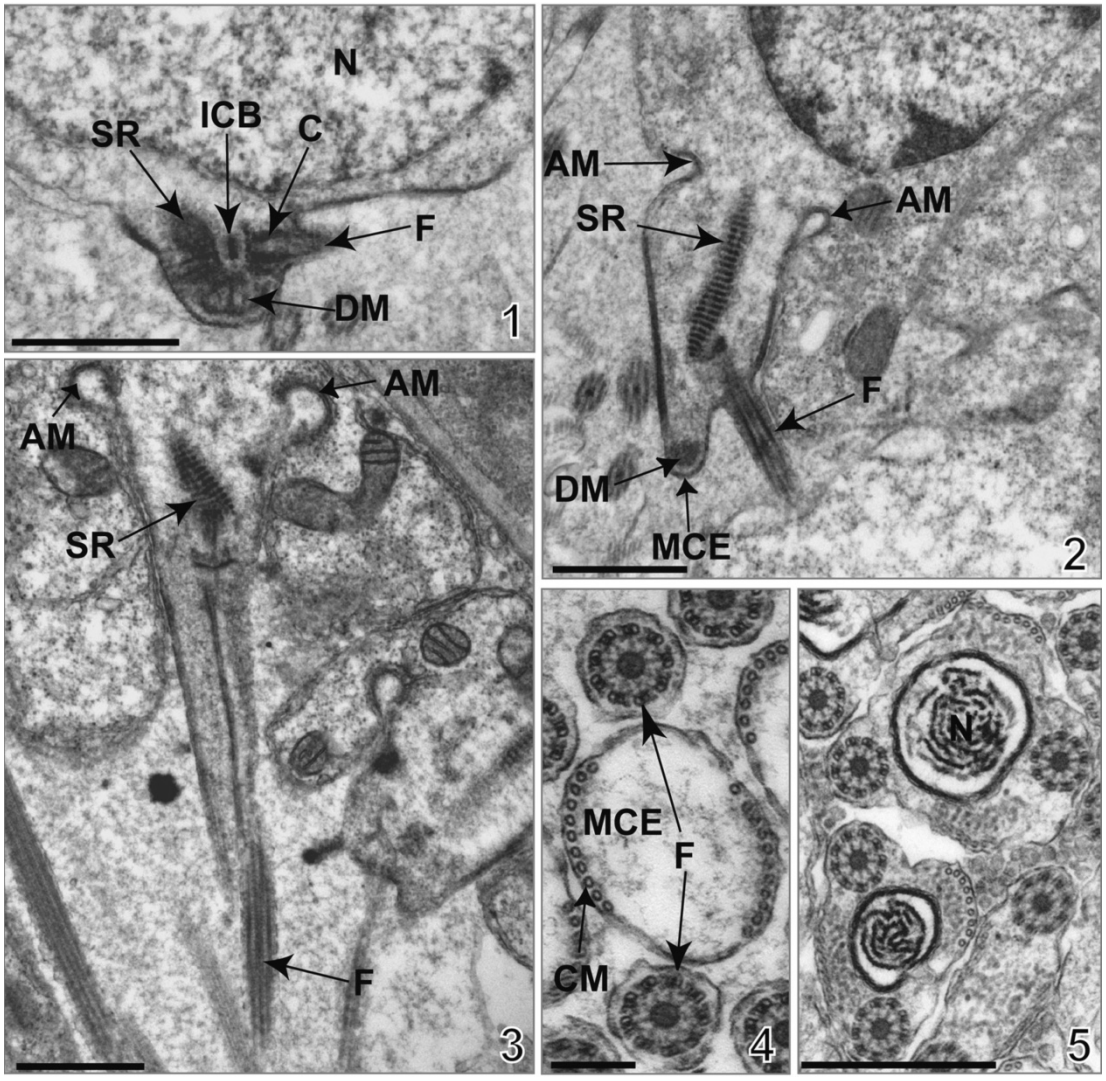
0.9% NaCl solution. Mature and gravid proglottids were separated and fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 1 day, washed overnight in 0.1 M cacodylate buffer at pH 7.4, postfixed with 1% OsO_4 in the same buffer for 1 h, washed again in 0.1 M cacodylate buffer, dehydrated in a graded series of acetone and embedded in Spurr resin within BEEM capsules. Ultrathin sections (60–90 nm in thickness) were cut on a Leica Ultracut UCT ultramicrotome, placed on copper grids, double stained with uranyl acetate and lead citrate. Sections were examined in a JEM 1010 (JEOL) transmission electron microscope operated at 80 kV.

The glycogen was detected using the periodic acid-thiosemicarbazide-silver proteinate (PA-TSC-SP), according to the method of Thiéry (1967).

Results

Spermiogenesis (Figs 1–6)

Spermiogenesis starts by the formation of a zone of differentiation situated at the periphery of each spermatid. This zone



Figs 1–5. Spermiogenesis of *Parabothriocephalus gracilis*: **1.** Longitudinal section of the zone of differentiation with two centrioles, intercentriolar body, striated rootlets, flagellum and electron-dense material. Scale bar = 1 µm. **2.** Longitudinal section showing the rotation of the flagella and the formation of the median cytoplasmic extension. Electron-dense material is visible in the median cytoplasmic extension. Scale bar = 1 µm. **3.** Longitudinal section of the zone of differentiation with the fusion of the flagella with the median cytoplasmic extension. Scale bar = 1 µm. **4.** Cross-section showing flagella parallel to the median cytoplasmic extension. Scale bar = 200 nm. **5.** Cross-section of the young spermatozoon with two axonemes, two parallel rows of cortical microtubules and nucleus. Scale bar = 500 nm. **Abbreviations used on all figures**: Ax – axoneme, AM – arched membranes, C – centriole, CB – crested body, CM – cortical microtubules, DM – electron-dense material, F – flagellum, G – glycogen, IB – intercentriolar body, MCE – median cytoplasmic extension, N – nucleus, PM – plasma membrane, S – singlets, SR – striated rootlets

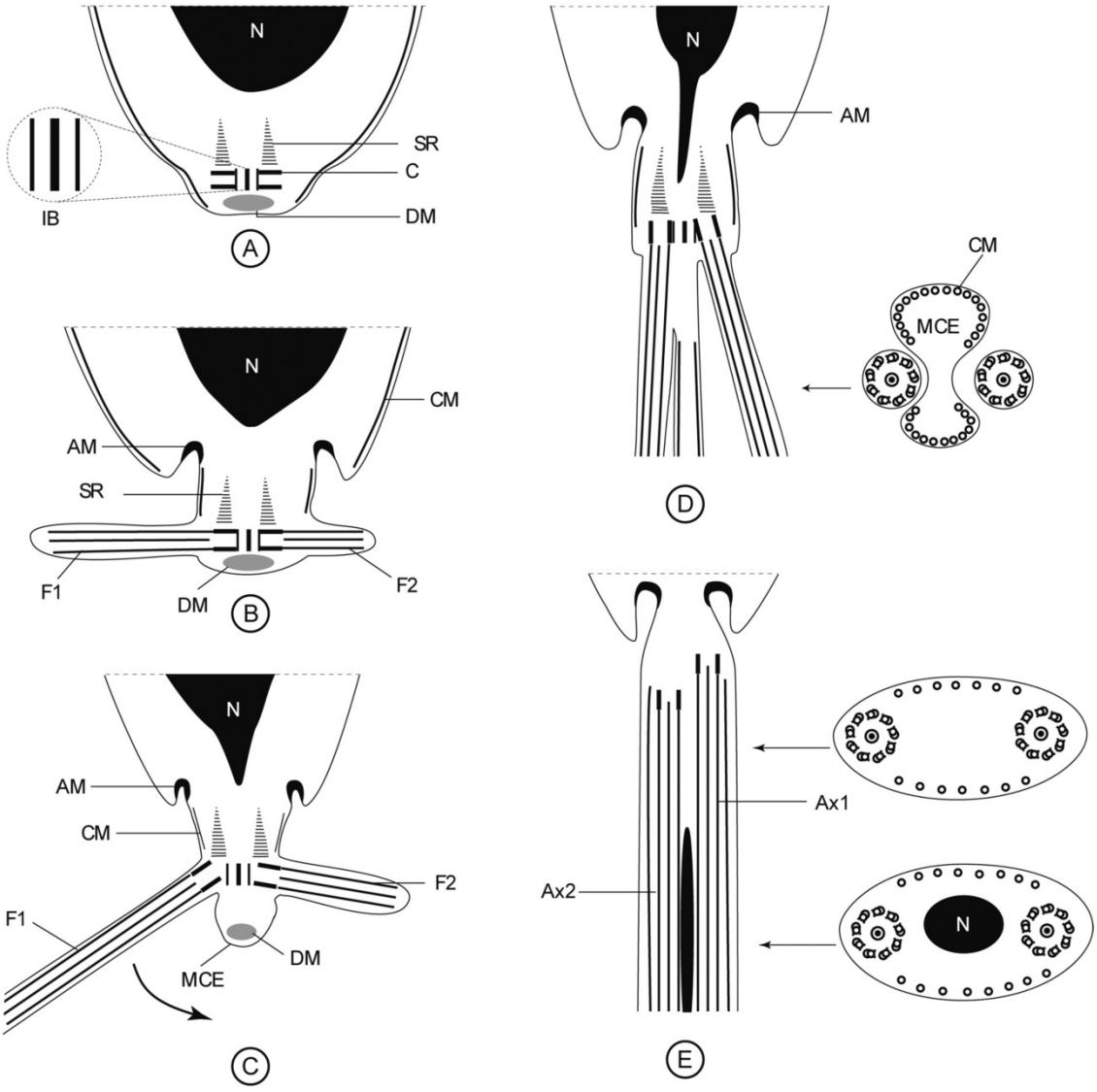


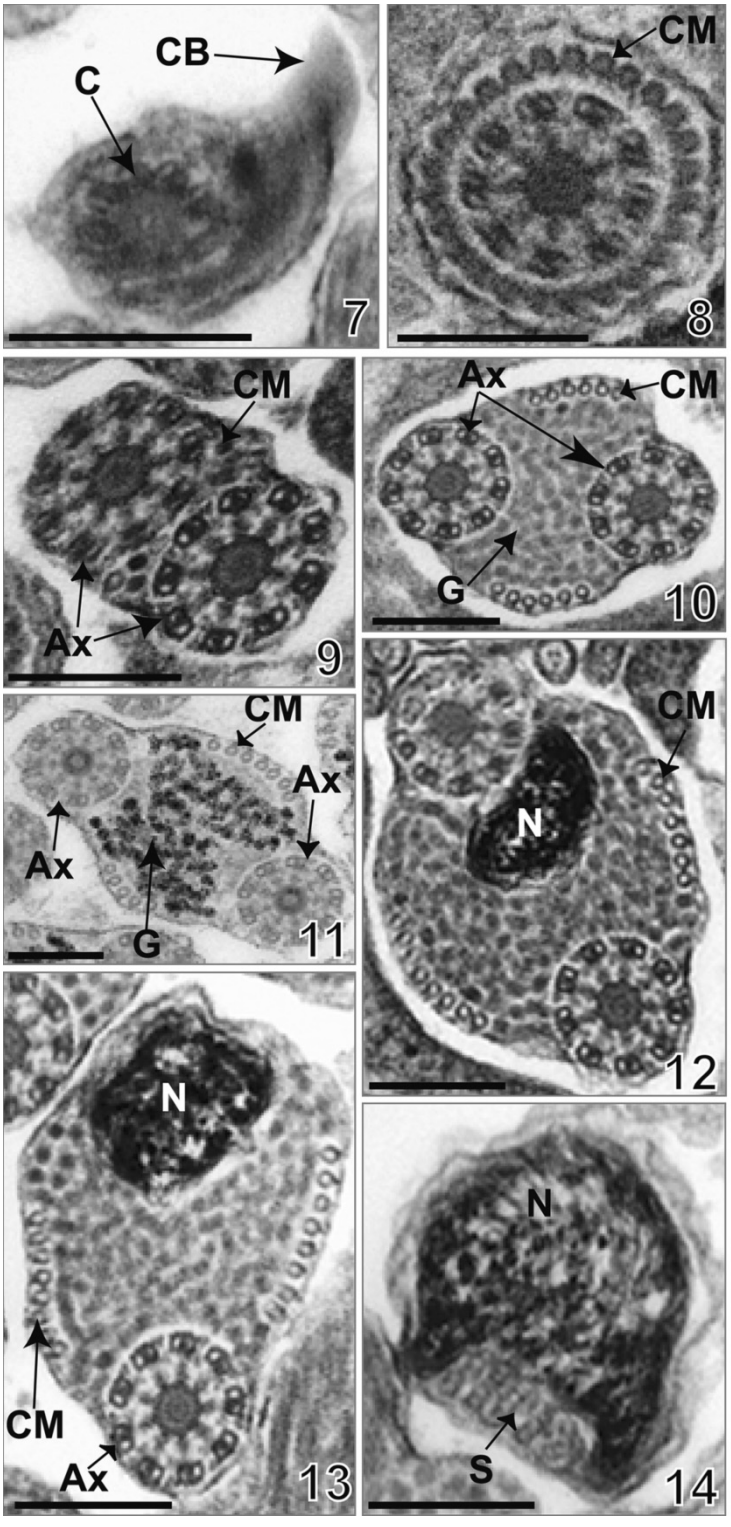
Fig. 6A-E. Diagram showing the main stages of spermiogenesis of *Parabothriocephalus gracilis*. For abbreviations see Figures 1–5

contains two centrioles associated with an intercentriolar body (Figs 1, 6A). The intercentriolar body, cylindric structure, consists of one central thicker electron-dense plate and two thinner electron-dense plates on each side, each ones separating by electron-lucent layers (Figs 1, 6A). Striated rootlets, cone-shaped structure, are composed of electron-dense strips (Figs 2, 3 and 6A, B). Electron-dense material occurs in the first stages of spermiogenesis in peripheral region of the differentiation zone (Fig. 1). The zone of differentiation is delimited by arching membranes (Figs 2, 3 and 6B-D). Initially, both centrioles are oriented in the same plane (Figs 1 and 6A, B). Each centriole gives rise to a flagellum (Figs 1 and 6B, C). Subsequently, a median cytoplasmic extension is formed at the distal part of the zone of differentiation (Figs 2, 6C). The two flagellae of unequal length are formed and undergo a rotation of 90° (Figs 2 and 6C, D). Then, they become parallel to the median cytoplasmic extension with which they fuse in a proximo-distal way (Figs 3, 4 and 6E). The nucleus migrates into the median cytoplasmic extension (Figs 5, 6C-E). The region of arching membranes narrows at the end of spermiogenesis, and mature sperm separates from residual cytoplasm (Fig. 6E).

Spermatozoon (Figs 7–15)

The mature spermatozoon of *P. gracilis* is a long fibrillar cell, without mitochondria, which narrows at both ends. From the anterior to the posterior extremities of the spermatozoon, five regions with different ultrastructure characters can be distinguished.

Region I (Figs 7, 8, 15 I) corresponds to the anterior part of sperm. First, it is characterized by the presence of a centriole and a single helical electron-dense crested body 130 nm



Figs 7–14. Mature spermatozoon of *Parabothriocephalus gracilis*: **7.** Cross-section of the anterior extremity of the region I. Scale bar = 300 nm. **8.** Cross-section of the region I showing the ring of electron-dense cortical microtubules. Scale bar = 200 nm. **9.** Cross-section of the region II with two axonemes close to each other and separated by few cortical microtubules. Scale bar = 200 nm. **10.** Cross-section of the region II. Scale bar = 200 nm. **11.** Cross-section of the region II with granules of glycogen bolded with Thiéry method. Scale bar = 200 nm. **12.** Cross-section of the region III with the nucleus. Scale bar = 200 nm. **13.** Cross-section of the region IV with a single axoneme, nucleus, cortical microtubules and glycogen. Scale bar = 200 nm. **14.** Cross-section of the posterior extremity of spermatozoon (region V) with nucleus, and axonemal doublets and singlets. Scale bar = 200 nm. For abbreviations see Figures 1–5

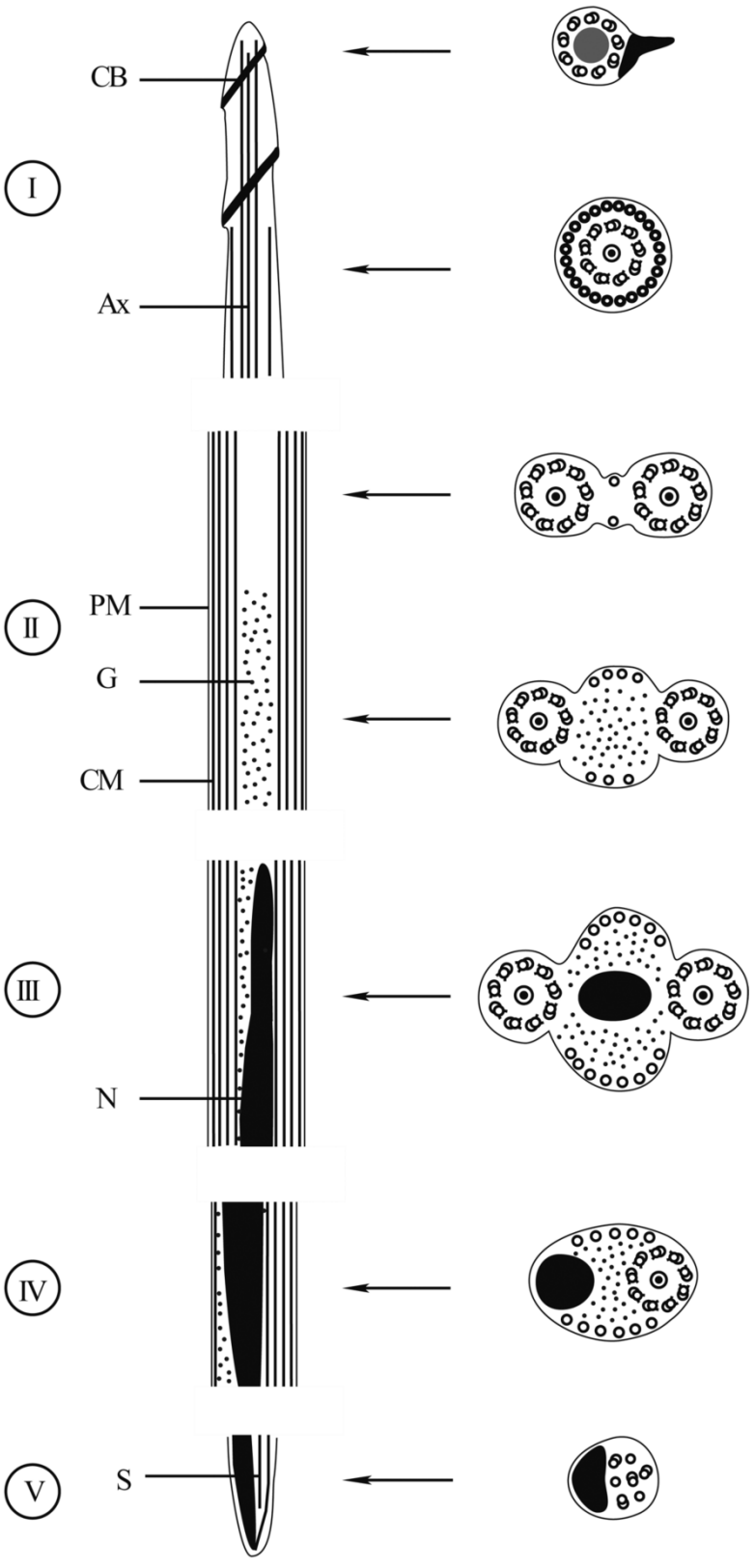


Fig. 15 (I–V). Diagram showing the ultrastructural organization of the mature spermatozoon of *Parabothriocephalus gracilis*

thick (Fig. 7). The centriole gives rise to an axoneme of the 9 + '1' trepaxonematan pattern. Then, the crested body disappears and cortical microtubules (25 in number) form a ring surrounding the axoneme (Fig. 8). The microtubules are electron-dense with a small electron-lucent centre. At the end of this region, the spermatozoon is approximately 260 nm wide.

Region II (Figs 9–11, 15 II) is characterized by the presence of two axonemes, cortical microtubules and electron-dense granules of glycogen. First, both axonemes are very close to each other (Fig. 9). The diameter of the spermatozoon is approximately 420 nm. Both axonemes are separated from each other by only few cortical microtubules (Fig. 9). Subsequently, width of the spermatozoon increases and reaches 700 nm. At this stage, 4 to 7 cortical microtubules are organized in two fields. Cortical microtubules are characterized by a bigger electron-lucent centre than those of the region I. Granules of glycogen, revealed by the method of Thiéry, are present in the cytoplasm of the cell (Fig. 11).

Region III (Figs 12, 15 III) contains two axonemes, cortical microtubules, glycogen and nucleus. Maximal diameter of the sperm is approximately 800 nm. The nucleus is electron-dense with fibrillar patches of chromatin (Fig. 12). Each field of cortical microtubules is composed of 6 to 9 subunits. At the end of the region III, one axoneme disappears.

Region IV (Figs 13, 15 IV) consists of one axoneme, nucleus, glycogen and cortical microtubules. The diameter of the gamete is approximately 600–700 nm. Two fields of cortical microtubules are still present and disappear in the posterior part of this region.

Region V (Figs 14, 15 V) corresponds to the posterior extremity of the spermatozoon. It is characterized by a disorganization of the axoneme. Central core of the axoneme disappears and doublets of microtubules loose their arms. At this stage, the nucleus occupies all the cytoplasm of the cell. The posterior extremity of the sperm is characterized by the presence of a nucleus and few axonemal singlets. The diameter of the spermatozoon is approximately 150 nm.

Discussion

The process of spermiogenesis in *Parabothriocephalus gracilis* is characterized by a flagellar rotation and proximo-distal fusion. Spermiogenesis of the type I of Bâ and Marchand (1995) is characteristic for *P. gracilis* and also for other cestodes of Bothriocephalidea, Diphyllobothriidea, Trypanorhyncha, Tetraphyllidea and Proteocephalidea (Justine 1998). This type of spermiogenesis is considered to be plesiomorphic character in Eucestoda (Justine 1998).

The intercentriolar body in *P. gracilis* consists of three electron-dense plates and two electron-lucent layers. This character was also reported in other members of Bothriocephalidea: *Bothriocephalus scorpii*, *Eubothrium crassum* and *Triaenophorus nodulosus* (Table I; Bruňanská *et al*. 2001; Levron *et al*. 2005, 2006a). In Diphyllobothriidae, four electron-lucent layers have been observed (Table I; Bonsdorff and Telkkä 1965, Levron *et al*. 2006c, 2009). Progressive reduction of the layers of the intercentriolar body occurs in the higher cestodes (Jus-

Table I. Comparison of spermiogenesis and spermatozoon characters in the Bothriocephalidea and Diphyllobothriidea

Species	Spermiogenesis			Spermatozoon				References
	Type	IB	DM	NAx	CB	R	PSE	
Bothriocephalidea								
Bothriocephalidae								
Bothriocephalus clavibothrium	I	+	–	2	–	+	Ax	Świderski and Mokhtar-Maamouri (1980)
Bothriocephalus claviceps				2	1	partial		Bâ *et al*. (2007)
Bothriocephalus scorpii	I	2	+	2	1	+	N	Levron *et al*. (2006a)
Echinophallidae								
Paraechinophallus japonicus				2	1	+	N+CM	Levron *et al*. (2006b)
Parabothriocephalus gracilis	I	2	+	2	1	+	N+CM	present study
Triaenophoridae								
Eubothrium crassum	I	2	+	2	1	+	Ax	Bruňanská *et al*. (2001, 2002)
Triaenophorus nodulosus	I	2	+	2	1	+	Ax	Levron *et al.* (2005)
Diphyllobothriidea								
Diphyllobothriidae								
Diphyllobothrium latum	I	4	+	2	–	–	N	Bonsdorff and Telkkä (1965), Levron *et al*. (2006c)
Ligula intestinalis	I	4	+	2	–	–	Ax	Levron *et al*. (2009)
Scyphocephalidae								
Duthiersia fimbriata				2	1	partial		Justine 1986

Ax – axoneme, CB – crested body, CM – cortical microtubules, DM – electron-dense material, IB – intercentriolar body (number of electron-lucent layers), N – nucleus, NAx – number of axonemes in the mature spermatozoon, PSE – posterior spermatozoon extremity, R – ring of cortical microtubules, +/– presence/absence.

tine 2001). Therefore, the reduction of the layers of the intercentriolar body in Bothriocephalidea with respect to those of Diphyllobothriidea could suggest a position higher for this order.

Spermatids of *P. gracilis* contain an apical electron-dense material in the first stages of spermiogenesis. For the first time, this material was observed in *E. crassum* (Bothriocephalidea) (Bruňanská *et al*. 2001). Later, electron-dense material has been reported in the bothriocephalideans *B. scorpii* and *T. nodulosus* (Levron *et al*. 2005, 2006a), in the diphyllobothrideans *Diphyllobothrium latum* and *Ligula intestinalis* (Levron *et al*. 2006c, 2009; see Table I), and also in the caryophyllideans *Khawia armeniaca* and *Wenyonia virilis* (Bruňanská and Poddubnaya 2006, Miquel *et al*. 2008). The presence of electron-dense material in the early stages of spermiogenesis appears to be characteristic for presumably most basal cestode orders (Caryophyllidea, Diphyllobothriidea and Bothriocephalidea).

Spermatozoon of *P. gracilis* contains two axonemes of the 9 + '1' trepaxonematan pattern (Ehlers 1984). Two axonemes were reported in Bothriocephalidea, Diphyllobothriidea, Spathebothriidea, Haplobothriidea, Trypanorhyncha, Tetraphyllidea and Proteocephalidea (Table I) (Justine 1998). Two axonemes are considered to be the plesiomorphic condition (Justine 1998). Only one axoneme is present in secondary cestodes, comprising the order Cyclophyllidea (Justine 1998, Miquel *et al*. 2007a).

A crested body, important phylogenetic character, has been reported in *P. gracilis*. The crested body is present in all species of Bothriocephalidea, except *Bothriocephalus clavibothrium* (Table I; Świderski and Mokhtar-Maamouri 1980). This character is absent in two diphyllobothriideans but is present in *Duthiersia fimbriata* of the same order (Table I; Justine 1986). Bâ and Marchand (1995) proposed this feature to be common for the Eucestoda. However, the crested body is missing in the most, evolutionary older, cestodes, Caryophyllidea, Spathebothriidea, Haplobothriidea and Trypanorhyncha (MacKinnon and Burt 1985; Świderski and Mackiewicz 2002; Bruňanská *et al*. 2006; Miquel and Świderski 2006; Miquel *et al*. 2007a, 2008; Gamil 2008).

One of the most interesting features in the spermatozoon of *P. gracilis* is the presence of a ring of electron-dense cortical microtubules, structure also reported in other Bothriocephalidea (Table I). This character was not observed in the diphyllobothriideans *Diphyllobothrium latum* and *L. intestinalis* (Bonsdorff and Telkkä 1965; Levron *et al*. 2006c, 2009). Partial ring of cortical microtubules was found in *Bothriocephalus claviceps* and *D. fimbriata* (Justine 1986, Bâ *et al*. 2007). The complete ring of cortical microtubules seems to be an autapomorphy for Bothriocephalidea. It is interesting to note that a partial ring of cortical microtubules has been also observed in the anterior part of the spermatozoon of numerous eucestodes, e.g., Spathebothriidea, Trypanorhyncha, Tetraphyllidea, Proteocephalidea, Mesocestoididae (see Miquel *et al*. 2007b).

The posterior part of the spermatozoon in *P. gracilis* and *Paraechinophallus japonicus* is characterized by the presence of the nucleus and microtubules (remains of the disorganisation of the axoneme) (Levron *et al*. 2006b). In *B. scorpii* (Bothriocephalidea) and *D. latum* (Diphyllobothriidea), only the nucleus was observed in the posterior extremity of the spermatozoon (Bonsdorff and Telkkä 1965; Levron *et al*. 2006a, c). In other bothriocephalideans (*B. clavibothrium*, *E. crassum* and *T. nodulosus*), the distal end of the gamete lacks the nucleus and only a single axoneme is present (Świderski and Mokhtar-Maamouri 1980, Bruňanská *et al*. 2002, Levron *et al*. 2005). Nucleus is absent also in *L. intestinalis* (Levron *et al*. 2009). The ultrastructure of the posterior extremity of spermatozoon does not appear to be suitable character for the differentiation of the orders Bothriocephalidea and Diphyllobothriidea, because its presence is variable in both orders and between families in a same order (see Table I).

On the basis of the present and previous studies of sperm characters in Bothriocephalidea and Diphyllobothriidea, it appears that the order Bothriocephalidea, with a reduced intercentriolar body, the presence of a crested body and a ring of electron-dense cortical microtubules, seems to be more evolved than the Diphyllobothriidea. These assumptions are in agreement with molecular studies (Brabec *et al*. 2006, Waeschenbach *et al*. 2007, Olson *et al*. 2008).

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References

Bâ C.T., Bâ A., Marchand B. 2007. Ultrastructure of the spermatozoon of *Bothriocephalus claviceps* (Cestoda, Pseudophyllidea): a parasite of *Anguilla anguilla* (Fish, Teleostei). *Parasitology Research*, 101, 77–83. DOI: 10.1007/s00436-006-0445-z.

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

Bonsdorff C.H. von, Telkkä A. 1965. The spermatozoon flagella in *Diphyllobothrium latum* (fish tapeworm). *Zeitschrift für Zellforschung und Mikroskopische Anatomie*, 66, 643–648. DOI: 10.1007/BF00339248.

Brabec J., Kuchta R., Scholz T. 2006. Paraphyly of the Pseudophyllidea (Platyhelminthes: Cestoda): Circumscription of monophyletic clades based on phylogenetic analysis of ribosomal RNA. *International Journal for Parasitology*, 36, 1535–1541. DOI: 10.1016/j.ijpara.2006.08.003.

Brooks D.R. 1989. The phylogeny of the Cercomeria (Platyhelminthes: Rhabdocoela) and general evolutionary principles. *Journal of Parasitology*, 75, 606–616.

Bruňanská M., Nebesářová J., Scholz T., Fagerholm H.-P. 2001. Spermiogenesis in the pseudophyllidean cestode *Eubothrium crassum* Bloch, 1779. *Parasitology Research*, 87, 579–588. DOI: 10.1007/s004360100392.

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., Poddubnaya L.G. 2006. Spermiogenesis in the caryophyllidean cestode *Khawia armeniaca* Cholodkovski, 1915. *Parasitology Research*, 99, 449–454. DOI: 10.1007/s00436-006-0155-6.

Bruňanská M., Scholz T., Dezfuli B.S., Poddubnaya L.G. 2006. Spermiogenesis and sperm ultrastructure of *Cyathocephalus truncatus* (Pallas, 1781) Kessler, 1868 (Cestoda: Spathebothriidea). *Journal of Parasitology*, 92, 884–892. DOI: 10.1645/GE-718R1.1.

Ehlers U. 1984. Phylogenetisches System der Platyhelminthes. *Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg* (*NF*), 27, 291–294.

Ehlers U. 1985. Das Phylogenetische System der Platyhelminthes. G. Fischer, Stuttgart, Germany, 317 pp.

Ehlers U. 1986. Comments on a phylogenetic system of the Platyhelminthes. *Hydrobiologia*, 132, 1–12. DOI: 10.1007/BF00046222.

Euzet L., Świderski Z., Mokhtar-Maamouri F. 1981. Ultrastructure comparée du spermatozoide des cestodes. Relation avec la phylogénèse. *Annales de Parasitologie* (*Paris*), 56, 247–259.

Gamil I.S. 2008. Ultrastructural studies of the spermatogenesis and spermiogenesis of the caryophyllidean cestode *Wenyonia virilis* (Woodland, 1923). *Parasitology Research*, 103, 777–785. DOI: 10.1007/s00436-008-1040-2.

Justine J.-L. 1986. Ultrastructure of the spermatozoon of the cestode *Duthiersia fimbriata* Diesing, 1854 (Pseudophyllidea, Diphyllobothriidae). *Canadian Journal of Zoology*, 64, 1545–1548. DOI: 10.1139/z86-230.

Justine J.-L. 1998. Spermatozoa as phylogenetic characters for the Eucestoda. *Journal of Parasitology*, 84, 385–408. DOI: 10.2307/3284502.

Justine J.-L. 2001. Spermatozoa as phylogenetic characters for the Platyhelminthes. In: (Eds. D.T.J. Littlewood and R.A. Bray) *Interrelationships of the Platyhelminthes*. Taylor and Francis, London, New York, 231–238.

Kuchta R., Scholz T., Brabec J., Bray R.A. 2008. Suppression of the tapeworm order Pseudophyllidea (Platyhelminthes: Eucestoda) and proposal of two new orders, Bothriocephalidea and Diphyllobothriidea. *International Journal for Parasitology*, 38, 49–55. DOI: 10.1016/j.ijpara.2007.08.005.

Levron C., Bruňanská M., Kuchta R., Freeman M., Scholz T. 2006b. Spermatozoon ultrastructure of the pseudophyllidean cestode *Paraechinophallus japonicus*, 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., 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., Bruňanská M., Poddubnaya L.G. 2006c. Spermatological characters in *Diphyllobothrium latum* (Cestoda, Pseudophyllidea). *Journal of Morphology*, 267, 1110–1119. DOI: 10.1002/jmor.10460.

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

Levron C., Sitko J., Scholz T. 2009. Spermiogenesis and spermatozoon of the tapeworm *Ligula intestinalis* (Diphyllobothriidea): Phylogenetic implications. *Journal of Parasitology*, 95, 1–9. DOI: 10.1645/GE-1646.1.

MacKinnon B.M., Burt M.D.B. 1985. Ultrastructure of spermatogenesis and the mature spermatozoon of *Haplobothrium globuliforme* Cooper, 1914 (Cestoda: Haplobothrioidea). *Canadian Journal of Zoology*, 63, 1478–1487. DOI: 10.1139/z85-221.

Miquel J., Eira C., Świderski Z., Conn D.B. 2007b. *Mesocestoides lineatus* (Goeze, 1782) (Mesocestoididae): new data on sperm ultrastructure. *Journal of Parasitology*, 93, 545–552. DOI: 10.1645/GE-1008R.1.

Miquel J., Świderski Z. 2006. Ultrastructure of the spermatozoon of *Dollfusiella spinulifera* (Beveridge and Jones, 2000) Beveridge, Neifar and Euzet, 2004 (Trypanorhyncha, Eutetrarhynchidae). *Parasitology Research*, 99, 37–44. DOI: 10.1007/s00436-005-0094-7.

Miquel J., Świderski Z., Mackiewicz J.S., Ibraheem M.H. 2008. Ultrastructure of spermiogenesis in the caryophyllidean cestode *Wenyonia virilis* Woodland, 1923, with re-assessment of flagellar rotation in *Glaridacris catostomi* Cooper, 1920. *Acta Parasitologica*, 53, 19–29. DOI: 10.2478/s11686-008-0013-z.

Miquel J., Świderski Z., Neifar L., Eira C. 2007a. Ultrastructure of the spermatozoon of *Parachristianella trygonis* Dollfus, 1946 (Trypanorhyncha: Eutetrarhynchidae). *Journal of Parasitology*, 93, 1296–1302. DOI: 10.1645/GE-1193.1.

Olson P.D. 2008. Hox genes and the parasitic flatworms: New opportunities, challenges and lessons from the free-living. *Parasitology International*, 57, 8–17. DOI: 10.1016/j.parint.2007.09.007.

Świderski Z. 1968. The fine structure of the spermatozoon of sheep tapeworm, *Moniezia expansa* (Rud., 1810) (Cyclophyllidea, Anoplocephalidae). *Zoologica Poloniae*, 18, 475–486.

Świderski Z. 1986. Three types of spermiogenesis in cestodes. In: Electron Microscopy – 1986. *Proceedings of the XI International Congress of Electron Microscopy*, *Kyoto*, *Japan*, 2959–2960.

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

Świderski Z., Mokhtar–Maamouri F. 1980. Etude de la spermatogénèse de *Bothriocephalus clavibothrium* Ariola, 1899 (Cestoda: Pseudophyllidea). *Archives de l'Institut Pasteur de Tunis*, 57, 323–357.

Thiéry J.-P. 1967. Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. *Journal of Microscopy*, 6, 987–1018.

Waeschenbach A., Webster B.L., Bray R.A., Littlewood D.T.J. 2007. Added resolution among ordinal level relationships of tapeworms (Platyhelminthes: Cestoda) with complete small and large subunit nuclear ribosomal RNA genes. *Molecular Phylogenetics and Evolution*, 45, 311–325. DOI: 10.1016/j.ympev.2007.03.019.

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