Spermiogenesis and spermatozoon ultrastructure of *Hunterella nodulosa* (Cestoda: Caryophyllidea), a monozoic parasite of suckers (Catostomidae) in North America

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Abstract: Spermiogenesis and ultrastructure of mature spermatozoon of the caryophyllidean cestode *Hunterella nodulosa*, a parasite of suckers (Catostomidae), have been studied by transmission electron microscopy. This monozoic tapeworm is unique in its mode of attachment and represents the second North American species studied. The process of spermiogenesis of *H. nodulosa* follows the general pattern already described in other caryophyllideans. The most characteristic feature is the presence of a slight rotation of the flagellar bud, which seems to be a typical character of spermiogenesis in this cestode group. The mature spermatozoon of *H. nodulosa* is characterized by the presence of one axoneme of $9+1$ type of the trepaxonematan flatworms surrounded by a semi-arc of cortical microtubules in its anterior extremity, parallel nucleus and cortical microtubules arranged in a parallel pattern, which corresponds to the Type III pattern of cestode spermatozoa according to Levron et al. (2010). Comparison of the present data with those available for other caryophyllideans did not reveal substantial differences, even though they belong to different families, infect different hosts (catostomid, cyprinid and siluriform fishes) and occur in distant zoogeographical regions. This indicates uniformity of the process of sperm formation and spermatozoon ultrastructure in one of the evolutionarily most ancient groups of tapeworms.

Keywords: sperm morphology, *Hunterella nodulosa*, Caryophyllacea, Caryophyllidea, common sucker, North America

Data on spermiogenesis and ultrastructure of mature spermatozoa have been increasingly used as an additional source of phylogenetic information on parasitic flatworms, including tapeworms (Cestoda) (Świderski 1986, Justine 1991, 1998, 2001, Bá and Marchand 1995, Hoberg et al. 1997, 1999, 2001, Olson et al. 2001, Levron et al. 2010, Bruňanská and Kostič 2012). Caryophyllidean cestodes, intestinal parasites of freshwater cypriniform and siluriform fishes with monopleurid body, have been the subject of several spermatological studies (see Levron et al. 2010 for a review, Yoneva et al. 2011, 2012, Bruńanská and Kostič 2012). Ultrastructural data available on eight species of three of the four currently recognized caryophyllidean families, namely Capingentidae Hunter, 1930, Caryophyllaeidae Leuckart, 1878 and Lytocestidae Hunter, 1927, enabled us to define the most important characteristics of spermiogenesis and the mature spermatozoon in this cestode order, the phylogenetic position of which still remains controversial (Olson et al. 2001, Waeschbach et al. 2007). The family Caryophyllaeidae currently comprises 20 genera and about 80 nominal species, but only three species were studied in detail, namely *Glaridacris catostomi* Cooper, 1920, a parasite of common sucker, *Catostomus commersoni* (Lacépède, 1803), in North America; *Wenyonia virilis* Woodland, 1923 from *Synodontis* Cuvier, 1816 catfish in Africa, and *Caryophyllaeus laticeps* (Pallas, 1781), parasitizing numerous cyprinids in the Palaearctic region (Świderski and Mackiewicz 2002, Gamil 2008, Miquel et al. 2008, Bruňanská and Kostič 2012).

The purpose of this study is to provide the first information on spermiogenesis and sperm ultrastructure of the caryophyllidean cestode *Hunterella nodulosa* Mackiewicz et McCrae, 1962, one of the two caryophyllidean species that is buried deeply in mucosal pockets of the host (Mackiewicz and McCrae 1962).

**MATERIALS AND METHODS**

Specimens of *Hunterella nodulosa* used for this study were found in the common sucker, *Catostomus commersoni* (Cypriniformes: Catostomidae), from Duck Creek River, Wisconsin, USA. Live worms were removed from the intestine, rinsed in 0.9% NaCl solution and fixed with 1.5% glutaraldehyde and 1.5% paraformaldehyde in 0.1 M Hepes at pH 7.4. After
washing with 0.1 M Hepes at pH 7.4, they were post-fixed in cold (4 °C) 1% OsO₄ in the same buffer for 1 h, dehydrated in a graded series of acetone and embedded in Spurr’s epoxy resin. Ultrathin sections (60–90 nm in thickness) were cut on a Leica UCT ultramicrotome, placed on copper grids and double stained with uranyl acetate and lead citrate according to Reynolds (1963). Sections were examined under a JEOL 1010 transmission electron microscope at 80 kV.

RESULTS

Spermiogenesis

The spermiogenesis of Hunterella nodulosa is illustrated in Figures 1 and 2. It commences with the appearance of a zone of differentiation at the periphery of each spermatid. The zone of differentiation is a conical area that contains electron-dense material in its apical region (Figs. 1A, 2A). This zone is lined by cortical microtubules and also includes a large nucleus surrounded by cytoplasm and two centrioles composed of nine sets of triplet tubules (Fig. 1A,C,E). The centrioles are associated with striated roots and connected to each other by an intercentriolar body (Fig. 1B,D,E). The latter is a symmetric structure composed of three layers differing in their thickness. Cross-section of the intercentriolar body reveals one central thick and one thinner electron-dense layer on each side of the central layer. These three electron-dense layers are separated by two electron-lucent layers (Figs. 1B, 2A). Initially, the centrioles are arranged in the same plane of symmetry and a small cytoplasmic protrusion is formed from the differentiation zone (Figs. 1B, 2B). Later, one of the centrioles develops into a free flagellum, which grows externally to the cytoplasmic protrusion. The second centriole remains oriented in a short flagellar bud that also undergoes a slight rotation but never forms a free flagellum (Figs. 1D, 2C). As development proceeds, the nucleus elongates and initiates its migration toward the spermatid body. This stage takes place before the fusion of the free flagellum with the cytoplasmic protrusion (Figs. 1E–G, 2D). When the nucleus is incorporated, these two structures fuse proximodistally (Figs. 1H,J, 2E). Spermiogenesis finishes with the detachment of the spermatid from the residual cytoplasm at the level of the arching membranes (Fig. 1J).

Spermatozoon

The spermatozoon of H. nodulosa is a filliform cell tapered at both ends and lacks mitochondrion and a ciliated body. The cytoplasm contains a single axoneme, parallel cortical microtubules and parallel nucleus. Five regions can be distinguished along the length of the gamete from the anterior to the posterior extremity (Figs. 3, 4).

Region I (Figs. 3A, 4I) corresponds to the anterior part of the spermatozoon. It is characterized by the presence of one axoneme of 9 + “1” type of the trepaxonematan platyhelminths. The axoneme is bordered by a semi-arc of parallel cortical microtubules situated beneath the plasma membrane. The width of this region of the spermatozoon is c. 250 nm.

Region II (Figs. 3B,C, 4II) differs from the region I in that cortical microtubules progressively increase in number. At the posterior part of this region the volume of the cytoplasm also increases and the diameter of the gamete is c. 390 nm. This region is also characterized by the presence of dense granules.

Region III (Figs. 3D–G, 4III) is a nucleated part of the spermatozoon with a diameter of c. 540 nm. The anterior part of this region contains a nucleus and an axoneme. The nucleus is parallel and located in the centre of the gamete (Fig. 3D), its diameter increases gradually. It is electron-dense, composed of fibrillar material and with a diameter of c. 315 nm. Towards the posterior end of the region, the diameter of the spermatozoon (c. 340 nm) and its nucleus are reduced. Dense granules are also present.

Region IV (Figs. 3H, 4IV) is a short zone containing the axoneme. The cortical microtubules disappeared and the axoneme is surrounded only by plasma membrane. The diameter of the gamete at this region is c. 260 nm.

Region V (Figs. 3I, 4V) is the posterior part of the spermatozoon. It is marked by disorganization of the axoneme. In the posterior end of the gamete, the central core of the axoneme disappears and the spermatozoon contains only doublets of microtubules.

DISCUSSION

Spermiogenesis

In this contribution, detailed ultrastructural study of spermiogenesis of the caryophyllidean cestode Hunterella nodulosa is presented. The general pattern of spermiogenesis that ends in the formation of mature spermatozoon possessing one axoneme is very similar to that described in all other caryophyllidean species studied to date (Świderski and Mackiewicz 2002, Arafa and Hamada 2004, Bruñanská and Podubnaya 2006, Gamill 2008, Miquel et al. 2008, Bruñanská 2009, Yoneva et al. 2011, 2012, Bruñanská and Kostič 2012). During this process one of the centrioles develops a single flagellum which rotates and fuses proximodistally with the cytoplasmic protrusion. It is also characterized by the presence of an intercentriolar body and typical striated roots associated with the centrioles. This pattern of spermiogenesis thus corresponds to the Type II pattern of spermiogenesis described by Bâ and Marchand (1995).

Among cestodes, a similar pattern of sperm development is known in species that belong to the Tetraphyllideidae (Phyllobothridiae), Tetrabothridiae and Mesocestoididae (Mokhtar-Maamouri 1979, Stoitsova et al. 1995, Miquel et al. 1999, 2007). This pattern has been found also in one species of the Diphylidiidae, contrary to the other diphyllideans, in which the spermiogenesis resembles the type I pattern (Azzouz-Draoui and Mokhtar-Maamouri 2010).
Fig. 1A–G. Spermiogenesis of *Hunterella nodulosa*. A – Differentiation zone showing a centriole (C) with an intercentriolar body (ib) and electron-dense material (Dm) in the apical region. B – Detail of an intercentriolar body (ib). C – Longitudinal section of a differentiation zone showing a centriole (C). D – Longitudinal section of a spermatid showing the free flagellum (F) and abortive centriole oriented in a cytoplasmic bud (B). E – Longitudinal section of a differentiation zone showing the presence of two centrioles (C). F – Several cross-sections of spermatids in stages before the proximodistal fusion between a free flagellum (F) and cytoplasmic protrusion (Cp). G – Cross-sections showing the migration of the nucleus (N) into the cytoplasmic protrusion before the proximodistal fusion. H – Cross-sections of spermatids showing the presence of the nucleus (N). I – Longitudinal section demonstrating the migration of the nucleus (N). J – Longitudinal section illustrating the final stage of spermiogenesis with detachment of the spermatid.

 Abbreviations: Am – arching membranes; Ax – axoneme; B – cytoplasmic bud; C – centriole; Cm – cortical microtubules; Cp – cytoplasmic protrusion; Dm – electron-dense material; F – free flagellum; Ib – intercentriolar body; N – nucleus; Rc – residual cytoplasm; Sr – striated roots. Scale bars: A = 0.8 μm; B = 0.2 μm; C, F, G = 0.3 μm; D = 1 μm; E = 0.5 μm; H, I, J = 0.4 μm.
1986/1988, Marigo et al. 2011). However, recent studies have shown that the process of spermiogenesis in most caryophyllideans presents some specific features, in which it differs from the typical Type II pattern of spermiogenesis present in acetabulate, i.e. much more derived cestode groups listed above. An interesting feature in the caryophyllaeid *Wenyonia virilis* Woodland, 1923 is the presence of an unusual rotation of the free flagellum more than 90° with the cytoplasmic protrusion (Miquel et al. 2008). This feature has never been observed in the other caryophyllideans. Our results on *H. nodulosa* support the previous observations, i.e., the presence of a slight rotation of the flagellar bud as one of the most particular characteristics. It was described for the first time by Bruňanská and Poddubnaya (2006) in *Khawia armeniaca* (Cholodkovski, 1915) and then observed also in other members of Caryophyllidea, namely, *Wenyonia virilis*, *Khawia sinensis* Hsü, 1935, *Breviscole* *orientalis* Kulakovskaya, 1962, *Lytocestus indicus* (Moghe, 1925) and *Caryophyllaeus laticeps* (Pallas, 1781) (Gamil 2008, Miquel et al. 2008, Bruňanská 2009, 2010, Yoneva et al. 2011, 2012, Bruňanská and Kostič 2012) and thus seems to be the most common feature of these monozoic cestodes. One of the more recent ultrastructural investigations, i.e. that on the spermiogenesis of *C. laticeps* by Bruňanská and Kostič (2012), recognized rotation of free flagellum + flagellar bud as a derived stage of spermiogenesis in the caryophyllideans.

The comparative analyses show that caryophyllidean species share a common spermiogenesis character, i.e., typical striated roots associated with the centrioles. The main differences observed between species belonging to three of the four families studied lie in the arrangement of striated roots. In most caryophyllideans, each of the cen-

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**Fig. 2.** Schematic representation of the main stages of spermiogenesis in *Hunterella nodulosa*. Abbreviations: Am – arching membrane; Ax – axoneme; B – cytoplasmic bud; C – centriole; Cm – cortical microtubules; Cp – cytoplasmic protrusion; Dm – electron-dense material; F – free flagellum; Ib – intercentriolar body; N – nucleus; Sr – striated roots.

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Am

C

Ib

Cm

Dm

F

Sr

Ax

Am

Cm

F

Cp

Ax

Am
trioles is associated with a typical striated root during spermiogenesis and the same pattern was observed in H. nodulosa. Nevertheless, in the caryophyllaeid C. laticeps and the lytocestids K. armeniaca and K. sinensis an atypical arrangement of the striated roots was observed: an additional striated root situated in the opposite direction to the typical roots, the presence of two thin striated roots joint with one centriole or the presence of striated root orient-
ed tangential to the long axis of the nucleus (Bruňanská and Poddubnaya 2006, Bruňanská 2010, Bruňanská and Kostič 2012). In addition, a similar arrangement of striated roots has been reported in some members of the Diphyllobothriidea [Diphyllobothrium latum (Linnaeus, 1758)] and Proteocephalidea (Barsonella lafoni de Chambrier, Scholz, Beletew et Mariaux, 2009) (Levron et al. 2006, Marigo et al. 2012), even though the latter species belongs to a more derived group of tapeworms, unrelated to caryophyllideans (Olson et al. 2001, Waeschenbach et al. 2007).

*H. nodulosa* presents an intercentriolar body composed of three electron-dense layers delimiting two electron-lucent ones and possesses electron-dense material in the apical region of the differentiation zone in the initial stages of spermiogenesis. This is consistent with what has been described in all but three other caryophyllidean species examined so far (Bruňanská and Poddubnaya 2006, Miquel et al. 2008, Bruňanská 2009, Yoneva et al. 2011, 2012, Bruňanská and Kostič 2012). In the caryophyllaeid *Glaridacris catostomi*, a parasite of suckers from North America and the lytocestid *Monobothrioides chalmersius* (Woodland, 1924), a parasite of catfish of the genus *Clarias* Cuvier, 1816 in Africa, no electron-dense material was observed in the apical region of the zone of differentiation. In addition, in *M. chalmersius* an intercentriolar body was also absent (Świderski and Mackiewicz 2002, Arafà and Hamada 2004). In *K. armeniaca*, another lytocestid parasitic in barbels (Cyprinidae: Barbinæ) in the Palaearctic region (Oros et al. 2010, Scholz et al. 2011), intercentriolar body is composed of a single electron-dense layer, similarly as in members of some more derived cestode groups, namely the Proteocephalidea and Mesocestoidea (Miquel et al. 1999, Świderski and Mackiewicz 2002, Bruňanská et al. 2003, 2004, 2005, Arafà and Hamada 2004, Bruňanská and Poddubnaya 2006, Marigo et al. 2012). Progressive reduction of intercentriolar layers occurs in the “higher” cestodes and thus the presence of a reduced intercentriolar body in *K. armeniaca* may represent a homoplasic character due to secondary reduction of layers.

In *H. nodulosa*, the nucleus migrates along the spermatid body before the proximodistal fusion of the free flagellum with the cytoplasmic protrusion takes place. Interestingly, the same pattern was observed in unrelated species *W. virilis* from African catfish (Gamil 2008, Miquel et al. 2008), and this is in contrast with the pattern documented in the other caryophyllideans in which the nucleus migrates after the proximodistal fusion.

**Spermatozoon**

The existing ultrastructural data on the Caryophyllidea indicate that their spermatozoa are rather uniform and do not differ considerably between species infecting unrelated hosts (cyprinids, suckers and siluriforms) from distant zoogeographical regions. The present study has also revealed that the mature spermatozoon of *H. nodulosa* corresponds to the Type III pattern according to the classification of Levrón et al. (2010), which is typical for caryophyllideans.

The anterior extremity of *H. nodulosa* spermatozoa contains a single axoneme surrounded by a semi-arc of
a few cortical microtubules. It is interesting to note the presence of only one axoneme in the gamete of all caryophyllideans studied to date (Bruňanská 2010, Levron et al. 2010). A single axoneme has also been reported in the mature sperm of Tetraphyllidea (Phyllobothriidae), Proteocephalidea, Mesocestoididae, Tetrabothriidea and Cyclophyllidea, which are all derived groups of cestodes unrelated to caryophyllideans. The fact that only one axoneme is also present in the mature spermatozoon of the Cyclophyllidea, one of the most basal orders of the Eucestoda, is thus in contradiction with the phylogenetic position of this order (Olson et al. 2001, Waeschenbach et al. 2007).

The posterior region of the male gamete of H. nodulosa is characterized by the lack of cortical microtubules and by the gradual disorganization of the axoneme. Similar posterior extremity was observed in the spermatozoa of all studied caryophyllideans (Arafà and Hamada 2004, Gamîl 2008, Bruňanská 2009, Yoneva et al. 2011, 2012, Bruňanská and Kostič 2012), as well as in species of other cestode groups (Bruňanská 2010, Levron et al. 2010).

Conclusions

The type of spermiogenesis found in H. nodulosa is identical to that found previously in the Cepingintidae (B. orientalis) and in some caryophyllaeids (W. virilis, C. laticeps) and lytocestids (K. sinensis, L. indicus). It is characterized by (1) the presence of electron-dense material in the apical region of the differentiation zone, (2) the presence of typical striated roots, (3) an intercentriolar body composed of three electron-dense layers, and (4) rotation of both the free flagellum and the flagellar bud. This process of spermiogenesis corresponds to the Type II spermiogenesis as characterized by Bâ and Marchand (1995), but there are some slight differences from the same pattern observed in species of much more evolved orders (Levron et al. 2010). Therefore, future studies should provide more robust evidence that spermiogenesis in caryophyllideans and that in unrelated, much more derived acetabulate cestode orders actually represents the same process, i.e. it is homologous. New data on the second North American species studied also indicate that spermatozoon ultrastructure is uniform among all caryophyllideans.

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