Spermatozoon Ultrastructure of the Davaineid Cestode

*Raillietina* (*R.*) *vinagoi* Ortlepp, 1938 from Egypt

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**Abstract:** The ultrastructure of the spermatozoon of the davaineid cestode *Raillietina* (*R.*) *vinagoi* Ortlepp, 1938 (Cestoda, Cyclophyllidea) is reported for the first time in Egypt. These tapeworms are collected from the small intestine of the naturally infected pigeons *Columba livia domestica* from Egypt. The mature spermatozoon is filiform, tapered at both ends and lacks mitochondria. Five regions can be distinguished along the whole length of the mature spermatozoon. The anterior extremity exhibits an apical cone and two crest-like bodies. The axoneme shows the 9+"1" pattern of the Platyhelminthes and becomes surrounded with a periaxonemal sheath. The cortical microtubules are spirally arranged and in close contact with the plasma membrane. Transverse intracytoplasmic walls joined the periaxonemal sheath to the cortical microtubules. Moreover, the nucleus is coiled in a helix around the axoneme. In the posterior extremity of the spermatozoon the axoneme is disorganized and the spiralled microtubules become parallel to the spermatozoon axis.

**Key words:** Spermatozoa %Cyclophyllidea %Davaineidae

**INTRODUCTION**

Ultrastructural studies of spermiogenesis and spermatozoa have been demonstrated as a valuable tool for the elucidation of phylogenetic relationships in the Platyhelminthes [1-6] and have been studied up till now in more than 80 species belonging to most orders of Cestoda [7].

Order Cyclophyllidea comprises 15 families [8] among which are the Davaineidae which include parasites of birds and mammals at their adult stages [9, 10]. Family Davaineidae comprises four genera *Fuhrmannetta*, *Paroniella*, *Raillietina* and *Skrjabinia* [10]. Up till now, only six species belonging to three genera of Davaineidae have been the subject of spermiogenesis and/or spermatozoon ultrastructural studies. These are *Raillietina carneostrobilata* [11], *Cotugnia polyacantha* [12], *Raillietina* (*R.*) *tunetensis* [13], *Paroniella reynoldsae* [14], *Raillietina* (*R.*) *baeri* [15] and *Raillietina* (*R.*) *micracantha* [7]. So the purpose of the present research was to describe the main ultrastructure characters and organization of the mature spermatozoon of *Raillietina* (*R.*) *vinagoi* and compare the current results with the available data obtained from other cyclophyllideans particularly those of davaineids.

**MATERIALS AND METHODS**

The specimens of *Raillietina* (*R.*) *vinagoi* were collected alive from the small intestine of naturally infected pigeons *Columba livia domestica* from El-Sharkia Governorate, Egypt. Worms were cooled in 0.9% NaCl solution and then fixed for 3 h in a 3% gluteraldehyde in 0.1 M cacodylate buffer, pH 7.2 at 4°C, then post-fixed in 1% cacodylate buffered OsO₄ at room temperature for 2 h. Fixed worms were dehydrated in a graded ethanol series and embedded in epoxy resin. A series of ultrathin sections were cut using a diamond knife of an LKB 4800 ultratome, mounted on uncoated copper grids and double stained with alcoholic uranyl acetate and aqueous lead citrate. Sections through the testes were viewed in a Joel 1200 EX2-transmission electron microscope operating at 80 kV.

**RESULTS**

About 25-30 testis lobules are present in each mature segment of the cyclophyllidean cestode *Raillietina* (*R.*) *vinagoi* Ortlepp, 1938 (Family Davaineidae). Each lobule contains various stages of the spermatozoon development (Fig. 1). Note that these stages may lay free
Fig. 1: A testis lobule of Raillietina (R.) vinagoi showing various stages of spermatozoon development. Fig. 2: Cross-section of the testis showing various stages of spermatozoon development that lay free or in groups. Fig. 3: Longitudinal section of the anterior area of Region I showing the apical cone (AC) and two crest-like bodies (CB) spiralled around the axoneme (Ax). Note the spirally arranged microtubules (Cm). Figs. 4, 5: Longitudinal sections of the posterior area of Region I showing the presence of only one crest-like body (CB). Ax, Axoneme; Cm, cortical microtubules. Fig 6: Cross-sections of the posterior area of Region I showing the Axoneme (Ax) and the cortical microtubules (Cm). Figs. 7, 8: Cross-sections of the posterior area of Region I showing one crest-like body (CB) and few granules (G) of electron dense material in the cytoplasm. Ax, Axoneme; Cm, cortical microtubules. Scale bar of Figs. (1, 2) =2mm and of Figs. (3-8) =500nm.

Region I corresponds to the anterior extremity of the spermatozoon. It exhibits a small apical cone of electron lucent material and two helicoidal crest-like bodies, which, based on the level of the section, differ in number between 1 and 2. Both crest-like bodies begin their helical course at level behind that of the apical cone and they

or enclosed and forming small groups within the lobule (Fig. 2). The mature spermatozoon of R. vinagoi is filiform, tapered at both ends and has no mitochondria. Observation of most transversal and longitudinal sections of the spermatozoon helped us to distinguish five regions (I–V) with different ultrastructural characters (Fig. 19).
Figs. 9, 10: Cross-sections of Region II showing the periaxonemal sheath (PS) surrounding the Axoneme (Ax) with the presence of scarce granules (G) of electron dense material granules in the cytoplasm round the axoneme. Cm, Cortical microtubules. Fig. 11: Longitudinal section of Region II showing the scarce granules (G) of electron dense material in the cytoplasm round the axoneme (Ax). Cm, Cortical microtubules; PS, periaxonemal sheath. Fig. 12: Longitudinal section of Region III showing the transverse intracytoplasmic walls (W) joining the periaxonemal sheath (PS) to the cortical microtubules (Cm). Ax, Axoneme. Fig. 13: Cross-section of region III showing the transverse intracytoplasmic walls (W). Ax, Axoneme; Cm, Cortical microtubules; PS, Periaxonemal sheath. Scale bar of Figs. (9, 11-13)= 500nm and of Fig. (10)= 100nm.

The axoneme, of the 9 + “1” pattern of the trepaxonematan Platyhelminthes, is situated centrally and surrounded by a thin layer of electron-lucent material and by a slightly electron-dense cytoplasm. The latter is provided with few granules of electron-dense material spiral at an angle of 45° in relation to the spermatozoon axis. Note that the crest-like bodies firstly run parallel to each other, while posteriorly, only one run spirally around the spermatozoon (Figs. 3-5).
at the posterior end of this region (Figs. 7, 8). The cortical microtubules appear as an electron-dense submembranous layer spirally arranged and in close contact with the plasma membrane (Fig. 6).

Region II lacks crest-like bodies. The central axoneme becomes surrounded by a periaxonemal sheath and the cytoplasm contains some scarce granules of electron-dense material (Figs. 9-11) with the extension of the spiralized cortical microtubules (Fig. 11).

Region III is provided with its central axoneme and periaxonemal sheath and surrounded by a lucent cytoplasm that is divided into compartments, taking the form of transverse intracytoplasmic walls, by electron-dense partitions which join the periaxonemal sheath to the cortical microtubules (Figs. 12, 13).

Region IV constitutes the principal nuclear region of the sperm, in which both the axoneme and nucleus coexist. The nucleus is a compact cord coiled in a helix round the axoneme (Fig. 14). The nucleus exhibits a different morphology in diverse cross-sections. It begins as an annular structure, initially round on cross-sections and progressively flattens towards the posterior end, becoming crescent-shaped, thus it envelopes partially or entirely the axoneme (Figs. 15, 16). Few transverse intracytoplasmic walls are still present (Fig. 15). The layer of electron-lucent material is more evident, especially between the nucleus and the cytoplasm (Fig. 16).

Region V corresponds to the posterior end of the spermatozoon with neither axoneme nor nucleus. The periaxonemal sheath also disappears at this level. The cortical microtubules are still spiralized, forming a discontinuous layer of electron-dense and submembranous material and ending their helicoidal course by becoming parallel to the spermatozoon axis (Fig. 17). There is a progressive disorganization of the axoneme towards the posterior end; the central element disappears first and then the doublets transform into singlets (Fig. 18).
**Fig. 19**: An attempted reconstruction of the mature spermatozoon. Ax, Axoneme; Aae, axonemal anterior extremity; Ac, Apical cone; Ape, axonemal posterior extremity; Ase, anterior spermatozoon extremity; C, centriole; CB, crest-like body; Cm, cortical microtubules; G, electron-dense granule; N, nucleus; Ps, periaxonemal sheath; Pse, posterior spermatozoon extremity; W, transverse intracytoplasmic walls. Scale bar= 1mm.

**DISCUSSION**

The mature spermatozoon of the davaineid *Raillietina (R.) vinagoi* lacks mitochondria and presents crest-like bodies sharing all the studied Eucestoda to date; furthermore these two features are synapomorphic characters for the Eucestoda [5]. Absence of a mitochondrion has been observed in more than 50 studied cestode genera to date [16], while the crest-like body (bodies) has been recorded to be formed after the migration of the nucleus in the differentiation zone of the old spermatids and usually marks the end of spermiogenesis and the beginning of the anterior extremity of the spermatozoon [17].

The number of crest-like body (bodies) varies from 1 to 12 and the Cyclophyllidea is the unique order within the Eucestoda which includes species with two crest-like bodies spiralled round the spermatozoon, as already recorded in some members of the families Anoplocephalidae [17-22], Catenotaeniinae [23, 24] and in all members of the Davaineidae studied to date [7, 12-15]. The presence of two crest-like bodies in the davaineid *Raillietina (R.) vinagoi* is in agreement with all the examples of davaineids studied to date. Note that the angle of spiralization in addition to the thickness of crest-like bodies in the spermatozoon vary according to the cestode species [14]. In the present study, it makes an angle of 45°, in agreement with the recorded results in other davaineids to be either 45° or 50° [7, 14, 15]. The maximal thickness of crest-like bodies has been shown in spermatozoa of the davaineid *Raillietina tunetensis* [13]. In most cases, the apical cone of the cestode spermatozoon is made up of material which may be electron-dense, slightly electron-
dense or electron lucent as demonstrated by Bâ et al. [14] this is also the case for all the species belonging to the Davaineidae [7].

The scarce granules of electron-dense material in the cytoplasm in posterior part of Region I and whole Region II of Raillietina (R.) vinagoi may represent glycogen granules that compensate for the absence of mitochondria in the spermatozoon. Similar small granules of electron-dense material are also recorded in the davaineids Paroniella reynoldsae and Raillietina (R.) baeri [14, 15] but with no interpretation, while granules of glycogen nature are recorded in the davaeid R. microcantha [7].

The disposition of glycogen in two opposed and spiralled cords round the studied axoneme has been described previously in the diplidiids Joyeuxiella echinorhynchoides and J. pasqualei [25]. According to Euzet et al. [1], glycogen seems to be the energy spring of the spermatozoon. On the other hand, the electron-dense granules located between the periaxonemal sheath and the submembranous layer of cortical microtubules of the dilepidid cestode Molluscotaenia crassiscolex are of non-glycogenic nature [26].

Except for Mesocestoides litteratus and M. lineatus [27, 28] all of the studied cyclophyllideans to date present twisted submembranous and electron-dense layer of cortical microtubules for most the lengths of the spermatozoa [22, 29], then, in most cyclophyllidean species, these microtubules become parallel to the spermatozoon axis at the posterior extremity [12, 13, 17-19, 30, 31]. The character of twisted cortical microtubules has been considered a synapomorphy for Cyclophyllidea [2-4] and the angle of the cortical microtubules to the hypothetical spermatozoon axis ranges from 15° to 60° in all members of order Cyclophyllidea as mentioned by Ndiaye et al. [16] and from 45° to 60° in the davaineids [7].

The periaxonemal sheath surrounding the central axoneme of the mature spermatozoon has been revealed in several cyclophyllideans as the anoplocephalids [18, 19], the catenotaeniids [23, 24], the davaineids [7, 12-15], the dilepidids [26, 32, 33], the diplidiids [25] and the taeniids [16, 29, 34, 35]. In the Cyclophyllidea, the periaxonemal sheath has been suggested as a character for phylogenetic inference at the family level [3].

Transverse intracytoplasmic walls are a characteristic feature found in the sperm of several groups of Eucestoda, such as the anoplocephalids Inermicapsiferinae and Linstowinae [19, 36], the davaineids [7, 12-15] and in several taeniids [16, 29, 35] and according to Justine [3], the presence of both periaxonemal sheath and transverse intracytoplasmic walls are related characters. On the other hand, the cyclophyllideans Stilesia globipunctata and Catenotaenia pusilla are recorded to lack these transverse intracytoplasmic walls and exhibit only the periaxonemal sheath [24, 30].

Justine [3] has explained that there is mutual exclusion between the character dense granules and the paired characters periaxonemal sheath and transverse intracytoplasmic walls. Thus, species with dense granules in the spermatozoon lack a periaxonemal sheath. This feature is previously demonstrated in many cyclophyllideans [16, 21, 22, 24, 26]. Note that only the diplidiid Dipylidium caninum possesses neither granules nor periaxonemal sheath in its spermatozoon [32]. The present study is in agreement with the interpretation of Justine [3], as the mature spermatozoa of Raillietina (R.) vinagoi possesses both periaxonemal sheath and transverse intracytoplasmic walls with no dense granules appearing. Such granules are other than the dense granules of the Region II which may represent granules of glycogen nature.

According to Levron et al. [37], transverse intracytoplasmic walls and periaxonemal sheath are not necessarily associated in all cyclophyllideans spermatozoa and they added that among the cyclophyllidean types of spermatozoon, Type VI (found in the Catenotaeniidae, Dilepididae, Diplidiidae, Gryporhynchidae and in anoplocephalids of genera Mathevotaenia and Stilesia) presents only periaxonemal sheath, while Type VII (found in davaineids, metadilepidids, paruterinids, taeniids, tetrabothriids and certain anoplocephalids) exhibits both periaxonemal sheath and transverse intracytoplasmic walls.

Generally, the nucleus of the spermatozoon of most of the Cyclophyllidea is in close contact with the axoneme and coiled around it [5]. In Raillietina (R.) vinagoi, the spiralled nucleus shows an annular to a crescent shape in cross-sections. These morphological configurations are already described in most of the Cyclophyllidea [5, 7, 13-16, 19, 26, 29, 34, 35], however, irregular morphology of the nucleus has also recorded previously [17, 21, 22], in addition, the nucleus has taken a straight position and laid free in the spermatozoon cytoplasm of Retinometra serrata [38] and moreover it becomes closer to the cortical microtubules than to the axoneme.

In general, the posterior end of the spermatozoa in most of Platyhelminthes shows the disappearance of the central core of the axoneme before disorganization of the peripheral doublets and their transformation into singlets [22].
The ultrastructural characters of the mature spermatozoon of the davaineid Raillietina (R.) vinagoi show a great similarity to those reported in other davaineids studied previously [7, 12-15]. The presence of two crest-like bodies of different lengths, a periaxonemal sheath and intracytoplasmic walls are all constant features found in the spermatozoa of all these species. The most evident differences in the spermatozoon of Raillietina (R.) vinagoi are the presence of the spermatozoon developmental stages either free or enclosed forming small groups within the lobules of the testes, in addition to the presence of scarce granules of electron-dense material in the cytoplasm in the posterior part of Region I and the whole of Region II that may represent glycogen granules compensating for the absence of mitochondria.

More ultrastructural studies on the spermatozoon and the whole pattern of spermatogenesis are needed to establish possible differences in the ultrastructural organization of sperm between the different families of Cyclophyllidea in general and to subfamilies of the Davaineidae.

REFERENCES


