Sperm characters of the digenean *Nephrotrema truncatum* (Troglotrematidae): a kidney parasite of *Crocidura russula* (Soricidae) and their phylogenetic significance

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Abstract

Spermatological characteristics of the troglotrematid digenean *Nephrotrema truncatum*, a parasite of *Crocidura russula*, have been investigated by means of transmission electron microscopy. The ultrastructural study reveals that the mature spermatozoon of *N. truncatum* exhibits many ultrastructural characters previously described in most gorgoderoideans. These are two axonemes of the 9+'1' trepaxonematan pattern, four attachment zones, a lateral expansion, an external ornamentation of the plasma membrane associated with spine-like bodies and cortical microtubules, and in the posterior part of the anterior spermatozoon region, two bundles of parallel cortical microtubules with the maximum number located in the anterior part of the spermatozoon, a nucleus, two mitochondria, and granules of glycogen. The obtained results are compared with those of other digeneans, particularly the Gorgoderoidea. The sperm cells gorgoderoideans are of type IV characterised by a 9+'1' pattern of axonemes, the presence of an external ornamentation associated with cortical microtubules and located in posterior area of the anterior extremity, the presence of two bundles of cortical microtubules, the maximum number of cortical microtubules located in the anterior region of the spermatozoon, and the presence of generally two mitochondria. However, dicrocoeliids and troglotrematids have spermatozoa with ornamentation of the plasma membrane and lateral expansions.

Keywords: Digenea, Gorgoderoidea, Spermatozoon, Ultrastructure

Introduction

The Gorgoderoidea is one of the largest superfamilies in the Digenea, which includes 12 families and 123 genera (Littlewood et al. 2015). The systematics of gorgoderoideans is controversial and recently several families have been moved in or out (Curran et al. 2006; Bray and Blair 2008;

Pérez-Ponce de León et al. 2011; Henerek and Literák 2013; Littlewood et al. 2015). This is the case of the Brachycoelidae and Collyriclidae, considered by Bray and Blair (2008) in the Gorgoderoidea, but recently moved to Plagiorchiodea and Microphalloidea, respectively (Pérez-Ponce de León et al. 2011; Henerek and Literák 2013). Contrary to this, the Allocreadiidae family is included in the Gorgoderoidea (Curran et al. 2006). Among the 12 families of gorgoderoideans is the Troglotrematidae, which includes cosmopolitan species of parasites in different organs and tissues of mammals and seabirds. The systematic status of the members of this family is controversial (for a review, see Blair et al. 2008). According to these authors, the Troglotrematidae has 10 genera. Among them, is the genus *Nephrotrema* and its type species *N. truncatum* (Leuckart, 1842), a frequently recorded parasite of the kidney of small insectivorous mammals in Europe and objective of the ultrastructural study of the spermatozoon carried out in the present work.

The usefulness of ultrastructural characters of spermiogenesis and/or the spermatozoon in the interpretation of relationships among parasitic platyhelminthes has been demonstrated for many years (for a review, see Justine 1991 and Levron et al. 2010). In the last decade, the potential of this source of characters for phylogenetic purposes in the Digenea has been analysed by various authors (Quilichini, Foata et al. 2007; Quilichini et al. 2010a, 2011; Bakhoum et al. 2017). To date, there are more or less complete spermatological studies in a large number of digeneans belonging to 18 superfamilies and more than 50 families (Justine 1995; Bakhoum et al. 2017; Kacem and Miquel 2018; Ndiaye et al. 2018). In the Gorgoderoidea published works on the ultrastructure of the spermatozoon involve the Acanthocolpidae, Allocreadiidae, Dicrocoeliidae, Paragonimidae and Troglotrematidae families (Robinson and Halton 1982; Orido 1988; Cifrian et al. 1993; Agostini et al. 2005; Miquel et al. 2006; Quilichini, Foata, Orsini et al. 2007; Bakhoum, Quilichini, Justine et al. 2015; Bakhoum, Quilichini, Miquel et al. 2015). According to the above-

mentioned systematic reorganisations (Pérez-Ponce de León et al. 2011; Henerek and Literák 2013), the ultrastructural studies of spermatozoa of *Brachycoelium salamandrae* (Brachycoeliidae) and *Collyricloides massanae* (Collyriclidae) (see Bakhoum et al. 2013, 2014) have not been considered for comparative purposes in the Gorgoderoidea.

The aim of this study is to describe the sperm characters and their organisation in the spermatozoon of *N. truncatum*. It represents the second study of this family, the sperm of *Troglotrema acutum* being the other troglotrematid described to date (Miquel et al. 2006). Additionally, our results on the ultrastructure of the spermatozoon are compared with those of other digeneans, in particular troglotrematids and other gorgoderoideans, to highlight the potential of sperm characters in phylogeny.

Materials and methods

Materials

Live adult specimens of *Nephrotrema truncatum* were collected from the kidneys of a naturally infected *Crocidura russula* (Hermann, 1780) (Soricidae) accidentally trapped in the Natural Reserve of Py (Pyrenean Mountains, France) in October 2017.

Transmission electron microscopy (TEM)

For TEM, several live adult worms were immediately rinsed with a 0.9% NaCl solution and fixed in cold (4 °C) 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer at pH 7.4 for a minimum of 2 h, rinsed in 0.1 M sodium cacodylate buffer at pH 7.4, post-fixed in cold (4 °C) 1% osmium tetroxide with 0.9% potassium ferricyanide in the same buffer for 1 h, rinsed in Milli-Q water (Millipore Gradient A10), dehydrated in an ethanol series and propylene oxide, embedded in

Spurr's resin and polymerised at 60 °C for 72 h. Ultrathin sections (60–90 nm thick) at the level of the seminal vesicle were obtained using a Reichert-Jung Ultracut E ultramicrotome. Sections were placed on 200-mesh copper and gold grids. Sections placed on copper grids were double-stained with uranyl acetate and lead citrate according to the Reynolds (1963) procedure. Copper grids were examined in a JEOL 1010 transmission electron microscope operated at an accelerating voltage of 80 kV, in the 'Centres Científics i Tecnològics' of the University of Barcelona (CCiTUB).

Cytochemistry

Sections placed on gold grids were treated according to the Thiéry (1967) test to reveal the presence of glycogen. Thus, they were treated in periodic acid (PA), thiocarbohydrazide (TCH) and silver proteinate (SP) as follows: 30 min in 10% PA, rinsed in Milli-Q water, 24 h in TCH, rinsed in acetic solutions and Milli-Q water, 30 min in 1% SP in the dark and rinsed in Milli-Q water. Sections were examined in a JEOL 1010 transmission electron microscope in the CCiTUB.

Results

The observation of numerous ultrathin sections at the level of seminal vesicle allowed us to distinguish three regions in the mature spermatozoon of *N. truncatum* (Figures 1-4). These regions exhibit different ultrastructural characteristics and organisation.

Region I

Region I corresponds to the anterior extremity of the spermatozoon (Figures 1a-l, 2g and 4I). The anterior spermatozoon tip is sharp and lacks axonemes (Figure 1a). The two axonemes of the

9+'1' pattern appear simultaneously and they are surrounded by a continuous and submembranous layer of cortical microtubules (Figure 1b-e). This is the area of the sperm cell that has the maximum number of cortical microtubules (about 37) (Figure 1e). Two and four attachment zones appear progressively (Figure 1e-g). Different characteristics can be observed in areas with the four attachment zones: (i) cortical microtubules distributed in two bundles (Figures 1g, j-l and 4I), (ii) appearance of an external ornamentation of the plasma membrane (Figures 1g-l and 4I), (iii) presence of spine-like bodies (Figures 1i-k and 4I), (iv) presence of a reduced lateral expansion (Figures 1g, h and 4I), and (v) appearance of the first mitochondrion in the posterior part of region I (Figures 1i-l and 4I). Granules of glycogen irregularly distributed are also observed along this region (Figures 1f-l and 2g).

Region II

Region II is the middle region of the spermatozoon (Figures 2a-g and 4II). It is characterised by the disappearance of the external ornamentation of the plasma membrane and the spine-like bodies. The anterior part of this region presents the posterior extremity of the first mitochondrion (Figures 2a, b and 4II) while the posterior part exhibits the anterior extremity of the second mitochondrion (Figures 2e, f and 4II). The inter-mitochondrial area has only the two axonemes, cortical microtubules and granules of glycogen (Figure 2c, d, g).

Region III

Region III corresponds to the posterior region of the spermatozoon (Figures 2g, 3a-i and 4III). It is mainly characterised by the presence of the nucleus. In the anterior part of region III, the second mitochondrion and the nucleus are both present (Figures 3a-c and 4III). The nucleus increases progressively its size and the number of cortical microtubules are reduced (from 3 or 4

+ 6 to 2 + 2 or 3) (Figure 3a-c). Posteriorly, the second mitochondrion disappears and the first axoneme is disorganized (Figure 3 d, e). Transition toward the posterior spermatozoon tip shows the termination of cortical microtubules, the disorganisation and disappearance of the second axoneme and the posterior extremity of the nucleus (Figure 3f-i). Several singlets from the second axoneme reach the posterior tip of the spermatozoon (Figure 3i).

The granular material observed in all the regions has been determined as glycogen by means of the test of Thiéry (Figure 2g).

Discussion

The mature spermatozoon of *N. truncatum* exhibits many ultrastructural characters previously described in most gorgoderoideans and also in other digeneans. These are two axonemes of the 9+'1' trepaxonematan pattern (Ehlers 1984), a nucleus, two mitochondria, two bundles of parallel cortical microtubules, four attachment zones, a lateral expansion, an external ornamentation of the plasma membrane, spine-like bodies and granules of glycogen (see Table 1).

Anterior spermatozoon extremity

The anterior extremity of the spermatozoon of *N. truncatum* has two axonemes surrounded by a continuous and submembranous layer of cortical microtubules. This arrangement has been described previously in other gorgoderoideans, namely *Stephanostomoides tenuis*, *Brachylecithum eliomydis* and *Troglotrema acutum* (Miquel et al. 2006; Bakhoum, Quilichini, Justine et al. 2015; Bakhoum, Quilichini, Miquel et al. 2015). However, variability in the anterior extremity is evident in the Gorgoderoidea. Thus, other species have only one of the axonemes accompanied or not by cortical microtubules (see Table 1).

Another character frequently present in some digenean sperm is the anterior electron-dense

material. This dense material has been found in numerous species belonging to the Aephnidiogenidae, Atractotrematidae, Cryptogonimidae, Gyliauchenidae, Monorchiidae or Opecoelidae (see Bakhoum et al. 2017). However, all the studied gorgoderoideans lack this character.

Lateral expansion, external ornamentation and spine-like bodies

Bakhoum et al. (2017) have considered diverse morphological types of cytoplasmic expansions associated with cortical microtubules under the general term of lateral expansions. All these expansions are located in the anterior area of the spermatozoon and they are usually associated with other characters such as the external ornamentation of the plasma membrane and spine-like bodies. In the Gorgoderoidea only *T. acutum* exhibits a lateral expansion with a hook-shaped morphology (Miquel et al. 2006), whereas the remaining studied species lack a lateral expansion. Nevertheless, the mature spermatozoa of the dicrocoeliid *Dicrocoelium hospes* seem to have a reduced expansion (Agostini et al. 2005). In the present study we have also observed a reduced cytoplasmic expansion in *N. truncatum*.

An external ornamentation of the plasma membrane has been described in the mature spermatozoon of numerous digeneans and various authors analysed the potential of this character for phylogenetic inference (Quilichini, Foata et al. 2007; Quilichini et al. 2011; Bakhoum et al. 2017). Three types of anterior extremities according to the presence/absence of this character and, if present, its location have been considered by these authors: ornamentation (i) present in the anterior extremity, (ii) present in a more posterior area or (iii) absent. In the Gorgoderoidea, all the studied species have ornamentation of the plasma membrane located in posterior areas of the anterior region, except in the dicrocoeliids *Corrigia vitta* and *Dicrocoelium dendriticum*, which

lack ornamentation in their sperm cells (Robinson and Halton 1982; Cifrian et al. 1993).

Concerning the external ornamentation of the plasma membrane there is another important criterion useful for phylogenetic purposes and useful to establish sperm types in the Digenea: its association or not with cortical microtubules (Bakhoum et al. 2017). In the case of the Gorgoderoidea, all the studied species have external ornamentation associated with cortical microtubules with the exception of the above-mentioned dicrocoeliids that lack ornamentation (Robinson and Halton 1982; Cifrian et al. 1993).

Spine-like bodies were originally described as prominent submembranous electron-dense structures that contain a sort of vesicle (Miquel et al. 2000). Among the Gorgoderoidea, as occurs in *N. truncatum*, the spermatozoon of the majority of studied species have spine-like bodies (see Table 1). There are only two dicrocoeliids (*C. vitta* and *D. dendriticum*) that lack these structures (Robinson and Halton 1982; Cifrian et al. 1993). According to various authors, these structures could have been misinterpreted as artefacts of fixation in older papers (see Bakhoum et al. 2017). For example, in the paragonimid *Paragonimus ohirai* (Orido 1988) and in the haematoloechid *Haematoloechus* sp. (Justine and Mattei 1982) spine-like bodies are clearly present but authors omitted these in the respective descriptions of sperm cells. In fact, since its original description, spine-like bodies have been usually found in numerous digeneans. We think that the absence of these ultrastructural elements in certain species should be considered with caution, particularly in those belonging to groups containing a large number of records. This would be the case of gorgoderoideans with the two above-mentioned dicrocoeliids lacking spine-like bodies (Robinson and Halton 1982; Cifrian et al. 1993).

Cortical microtubules

The presence of parallel cortical microtubules has been described in digenean spermatozoa

except in the genus *Didymozoon* (Justine and Mattei 1983). In all the digeneans in which these structures were detected, the existing variability in the male gamete refers to the number, disposition and location of the maximum number. As occur in *N. truncatum* and other gorgoderoideans, in digeneans cortical microtubules are arranged into two bundles, at least in principal regions of the sperm cell containing the nucleus and/or mitochondria. Only in certain hemiuroideans (Hemiuridae, Lecithasteridae and Sclerodistomidae) these ultrastructural elements are grouped in a single bundle (for a review, see Bakhoum et al. 2017).

The location of the maximum number of cortical microtubules is another interesting character. According to Bakhoum et al. (2017), all the species of the Gorgoderoidea have the maximum number of cortical microtubules in the anterior region of the spermatozoon (see Table 1). The maximum number of microtubules is quite homogeneous among species of this superfamily, being between 30 and 44 microtubules. This is a range usually found between digenean spermatozoa. However, as above-mentioned, it is interesting to note the absence of cortical microtubules in *Didymozoon* sp. and also their reduced number (5 to 10 microtubules) in certain hemiuroideans. Other species, e.g. *Pronoprymna ventricosa*, *Adlardia novaecaledoniae* or *Aphallus tubarium* (Quilichini, Foata et al. 2007; Quilichini et al. 2009; Foata et al. 2012) also exhibit a reduced number (around 10 microtubules). By contrast, *Scaphiostomum palaearcticum* (Ndiaye et al. 2002) and other species with a large lateral expansion, such as *Diplodiscus subclavatus* (Bakhoum et al. 2011) or *D. amphicurus* (Diagne unpublished data) have a higher number (65, 75 and 63 microtubules, respectively).

Mitochondria

In the Digenea, the number and morphology of the mitochondrion/a vary according to the species. The number of mitochondria varies between 1 and 3 (for a review, see Bakhoum et al.

2017). In the Gorgoderoidea, all the species analysed until now have two mitochondria except the dicrocoeliids *C. vitta* and *D. dendriticum* (Robinson and Halton 1982; Cifrian et al. 1993).

Concerning their morphology, various authors have described the presence of moniliform mitochondria constituted by several mitochondrial bulges joined by a mitochondrial cord. This is the case of *Holorchis micracanthum* (Aephnidiogenidae), *Timoniella imbutiforme* (Cryptogonimidae), *Opechona bacillaris* (Lepocreadiidae), *Macvicaria obovata* (Opecoelidae) and *Enodiotrema reductum* (Plagiorchiidae) (Bâ et al. 2011; Ndiaye et al. 2012, 2015; Kacem et al. 2017; Kacem, Quilichini et al. 2017). In the Gorgoderoidea only *Stephanostomoides tenuis* (Acanthocolpidae) has this type of mitochondrion (Bakhoum, Quilichini, Justine et al., 2015).

Posterior spermatozoon extremity

The posterior spermatozoon extremity is another region with a great variability of characteristics. Considering the sequence of disappearance of principal characters (nucleus, axoneme and cortical microtubules) in the posterior extremity of the male gamete, and after comparison of 19 species of Digenea belonging to 12 families, Quilichini et al. (2010a) distinguish three types of posterior spermatozoon extremities. These are: type 1 (opecoelidean type) characterised by the sequence 'axoneme, nucleus and cortical microtubules'; type 2 (fasciolidean type) with the sequence 'cortical microtubules, axoneme and nucleus' and type 3 (cryptogonimidean type) with the sequence 'cortical microtubules, nucleus and axoneme'. Based on the posterior mitochondrion and axonemes of the spermatozoon of *Aponurus laguncula* described by (Quilichini et al., 2010b), Bakhoum et al. (2017), recommend that only the terminal character of the spermatozoon should be used. Thus, three types of posterior spermatozoon extremities have been established: (i) a posterior spermatozoon extremity containing only cortical microtubules, (ii) a posterior spermatozoon extremity containing only the nucleus, and (iii) a posterior spermatozoon extremity

containing only one axoneme. The posterior extremity of the mature spermatozoon of *N. truncatum* as in the other troglotrematid (*T. acutum* -Miquel et al. 2006) follow the cryptogonimidean type. The remaining studied families of gorgoderoideans, the allocreadiids, paragonimids and dicrocoeliids (except *D. hospes* -Agostini et al. 2005) also seem to follow the cryptogonimidean pattern, while acanthocolpids differ from them and exhibit the fasciolidean type of posterior extremity. This variability within gorgoderoideans is clearly reflected in Table 1 at the level of the posterior spermatozoon character: axoneme or nucleus.

Sperm model for the Gorgoderoidea

Bakhoum et al. (2017) have defined five spermatological models in the Digenea based on the principal spermatological characters described in the Digenea. These principal characters concern axonemes, the external ornamentation of the plasma membrane, the lateral expansion, cortical microtubules and the mitochondrion. The mature spermatozoon of species of the Gorgoderoidea is of type IV characterised by a 9+'1' pattern of axonemes, the presence of an external ornamentation associated with cortical microtubules and located in posterior area of the anterior extremity, the presence of two bundles of cortical microtubules, the maximum number of cortical microtubules located in the anterior region of the spermatozoon, and the presence of generally two mitochondria. There are, however, certain discrepancies concerning the Dicrocoeliidae and Troglotrematidae concerning ornamentation of the plasma membrane and lateral expansions.

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Disclosure statements

No potential conflict of interest was reported by the authors.

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Captions of figures

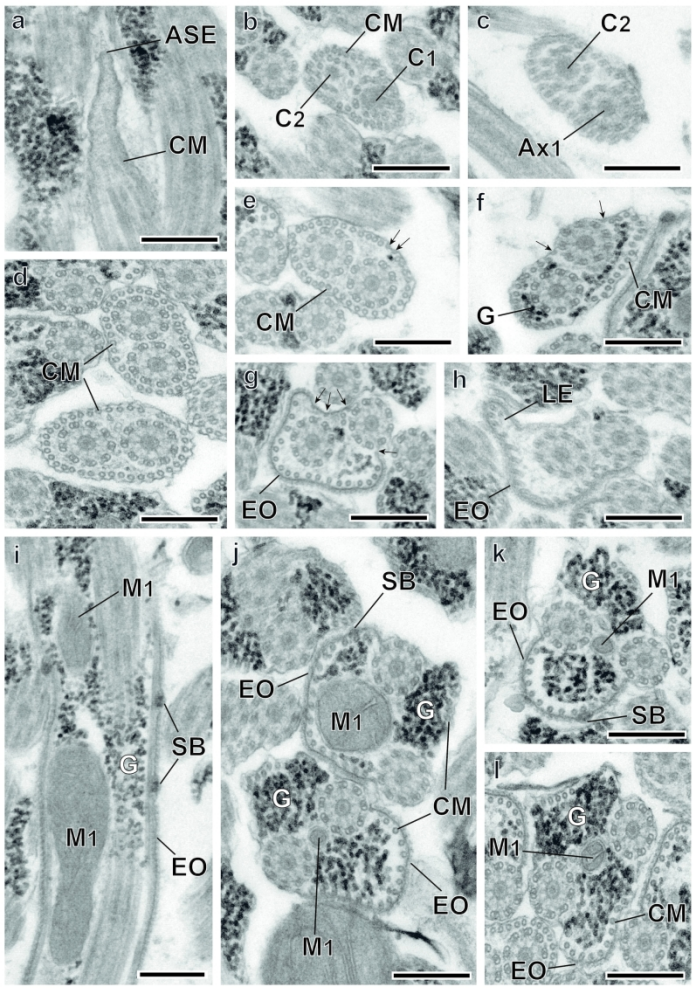
Figure 1. Region I of the spermatozoon of *Nephrotrema truncatum*. (a) longitudinal section of the anterior extremity of the spermatozoon, (b-d) cross-sections showing the progressive appearance of axonemes. Note the presence of a continuous and submembranous layer of cortical microtubules, (e, f) cross-sections showing the presence of two attachment zones (arrows), (g, h) cross-sections of anterior area of the ornamented region. Arrows indicate the presence of four attachment zones, (i) longitudinal section of the posterior area of the ornamented region showing the simultaneous presence of external ornamentation, spine-like bodies and the first mitochondrion, (j-l) cross-sections of posterior area of the ornamented region. ASE: anterior spermatozoon extremity, Ax1: first axoneme, C1 and C2: centrioles of the first and second axonemes, CM: cortical microtubules, EO: external ornamentation of the plasma membrane, G: granules of glycogen, LE: lateral expansion, M1: first mitochondrion, SB: spine-like bodies, scale bars: 0.3 µm.

Figure 2. Region II and test of Thiéry of the spermatozoon of *Nephrotrema truncatum*. (a-f) consecutive cross-sections from the anterior area of region II with posterior extremity of the first

mitochondrion to the posterior area containing the anterior extremity of the second mitochondrion, (g) positive test of Thiéry for glycogen in regions I, II and III (R-I, R-II and R-III). CM: cortical microtubules, G: granules of glycogen, M1 and M2: first and second mitochondrion, N: nucleus, scale bars: 0.3 μm.

Figure 3. Region III of the spermatozoon of *Nephrotrema truncatum*. (a-c) cross-sections of areas with simultaneous presence of nucleus and second mitochondrion, (d, e) cross-sections showing the interruption of the second mitochondrion, the disorganisation of the first axoneme and the progressive reduction of cortical microtubules, (f) longitudinal section showing the posterior spermatozoon extremity, (g, h) cross-sections showing the disorganisation of the second axoneme, (i) cross-section at the level of posterior tip with some singlets. Ax2: second axoneme, CC1 and CC2: central core of the first and second axoneme, CM: cortical microtubules, G: granules of glycogen, M2: second mitochondrion, N: nucleus, PSE: posterior spermatozoon extremity, S2: singlets of the second axoneme, scale bars: 0.3 μm.

Figure 4. Schematic reconstruction of the spermatozoon of *Nephrotrema truncatum*. ASE: anterior spermatozoon extremity, Ax1 and Ax2: first and second axoneme, AZ: attachment zones, C1 and C2: centrioles of the first and second axoneme, CC1 and CC2: central core of the first and second axoneme, CM: cortical microtubules, EO: external ornamentation of the plasma membrane, G: granules of glycogen, LE: lateral expansion, M1 and M2: first and second mitochondrion, N: nucleus, PM: plasma membrane, PSE: posterior spermatozoon extremity, S2: singlets of the second axoneme, SB: spine-like bodies.



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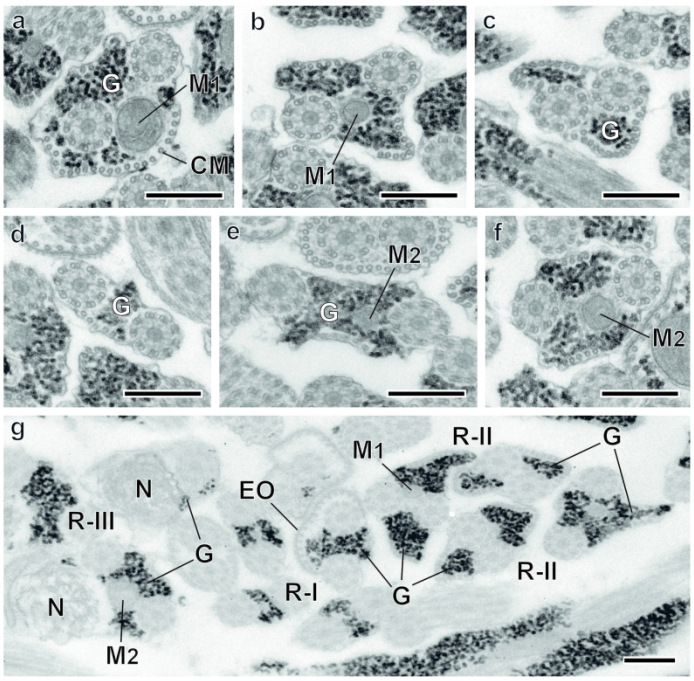

Figure 2. Region II and test of Thiéry of the spermatozoon of Nephrotrema truncatum. (a-f) consecutive cross-sections from the anterior area of region II with posterior extremity of the first mitochondrion to the posterior area containing the anterior extremity of the second mitochondrion, (g) positive test of Thiéry for glycogen in regions I, II and III (R-I, R-II and R-III). CM: cortical microtubules, G: granules of glycogen, M1 and M2: first and second mitochondrion, N: nucleus, scale bars: 0.3 μm.

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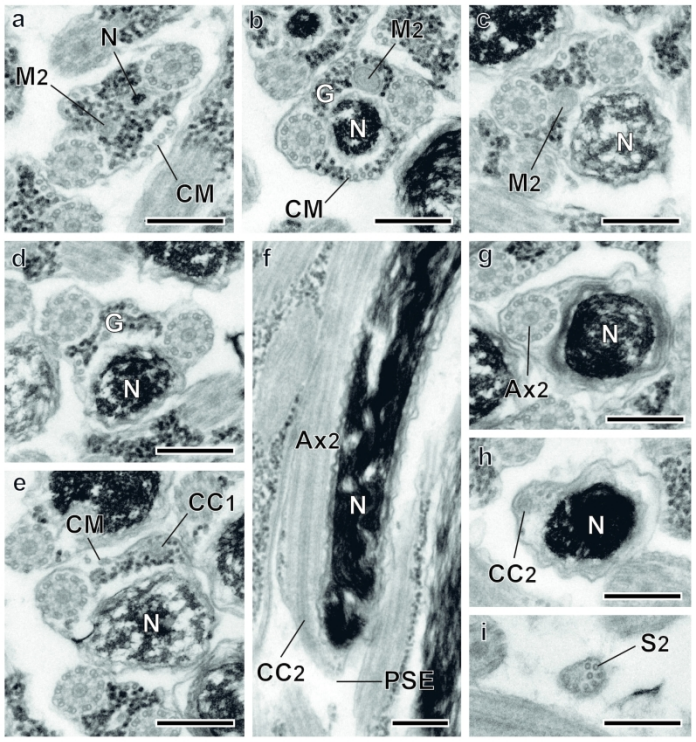

Figure 3. Region III of the spermatozoon of Nephrotrema truncatum. (a-c) cross-sections of areas with simultaneous presence of nucleus and second mitochondrion, (d, e) cross-sections showing the interruption of the second mitochondrion, the disorganisation of the first axoneme and the progressive reduction of cortical microtubules, (f) longitudinal section showing the posterior spermatozoon extremity, (g, h) cross-sections showing the disorganisation of the second axoneme, (i) cross-section at the level of posterior tip with some singlets. Ax2: second axoneme, CC1 and CC2: central core of the first and second axoneme, CM: cortical microtubules, G: granules of glycogen, M2: second mitochondrion, N: nucleus, PSE: posterior spermatozoon extremity, S2, singlets of the second axoneme, scale bars: 0.3 µm.

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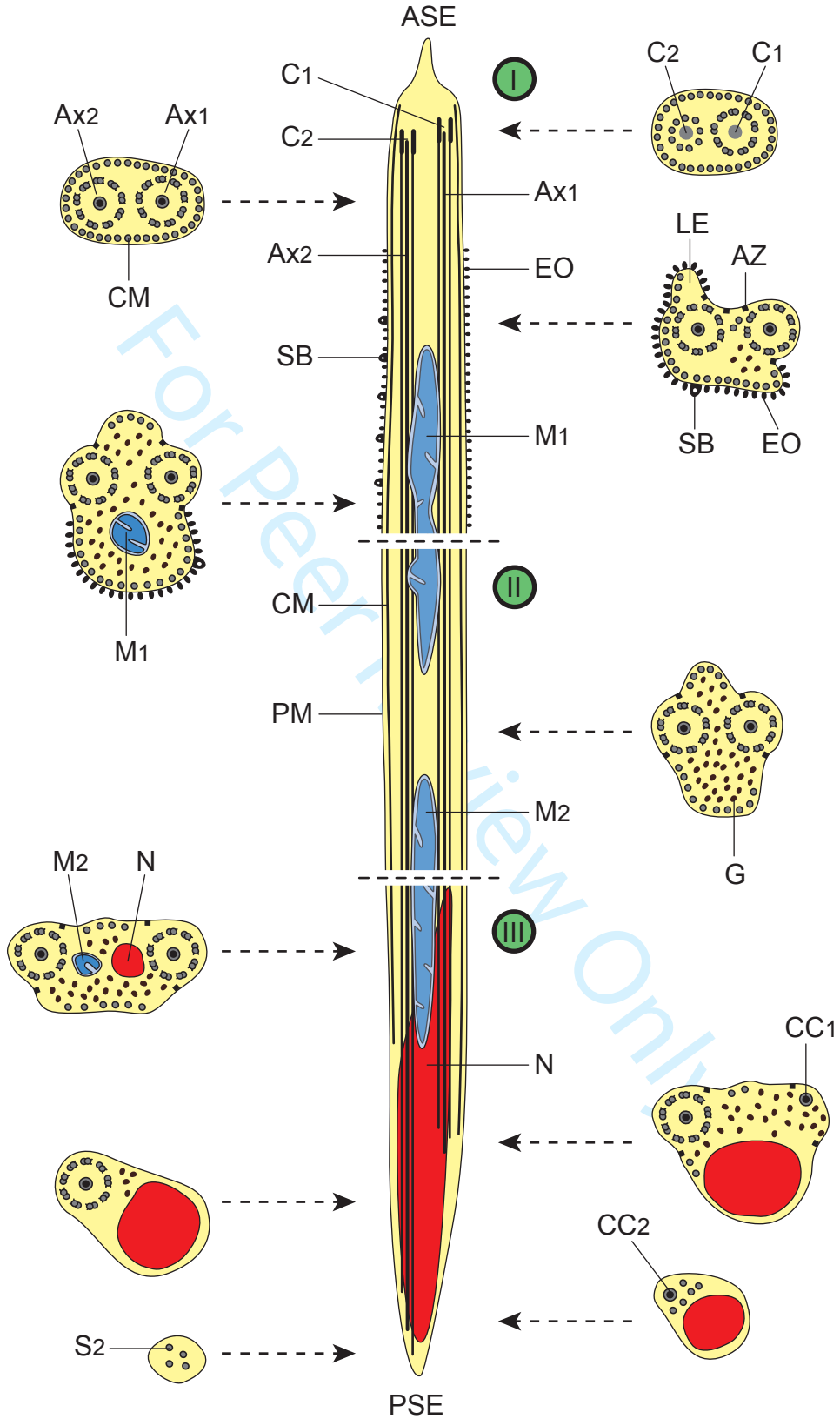

Table 1. Ultrastructural characteristics of the spermatozoon in the Gorgoderoidea

Families/Species	ASE	LE	EO	EO+CM	LEO	MCM	LMCM	M	SB	PSC	References
Acanthocolpidae											
Stephanostomoides tenuis	2Ax-CM	–	+	+	PostA	31	AntS	2*	+	N	Bakhoum, Quilichini, Justine et al. (2015)
Stephanostomum murielae	1Ax-CM	–	+	+	PostA	30	AntS	2	+	N	Bakhoum, Quilichini, Justine et al. (2015)
Allocreadiidae											
Crepidostomum metoecus	1Ax	–	+	+	PostA	32	AntS	2	+	Ax	Quilichini, Foata, Orsini et al. (2007)
Dicrocoeliidae											
Brachylecithum eliomydis	2Ax-CM?	–	+	+	PostA	44	AntS	2	+	Ax	Bakhoum, Quilichini, Miquel et al. (2015)
Corrigia vitta	?	–	–	NA	NA		AntS	1?	–	Ax?	Robinson and Halton (1982)
Dicrocoelium dendriticum	?	–	–	NA	NA	44	AntS	1	–	?	Cifrian et al. (1993)
Dicrocoelium hospes	1Ax	+ (red.)	+	+	PostA	36	AntS	2	+	N	Agostini et al. (2005)
Paragonimidae											
Paragonimus ohirai	1Ax-CM?	–	+	+	PostA	32	AntS	2?	+	Ax?	Orido (1988)
Troglotrematidae											
Nephrotrema truncatum	2Ax-CM	+ (red.)	+	+	PostA	37	AntS	2	+	Ax	Present study
Troglotrema acutum	2Ax-CM?	+	+	+	PostA	34	AntS	2	+	Ax	Miquel et al. (2006)

AntS: anterior part of the spermatozoon, ASE: anterior spermatozoon extremity, Ax: axoneme, BCM: number of bundles of cortical microtubules, CM: cortical microtubules, EO: external ornamentation of the plasma membrane, EO+CM: association external ornamentation and cortical microtubules, LE: lateral expansion, LEO: location of the external ornamentation, LMCM: location of maximum number of cortical microtubules, M: number of mitochondria, MCM: maximum number of cortical microtubules, N: nucleus, NA: not applicable, PostA: posterior part of the anterior region, PSC: posterior spermatozoon character, red.: reduced, SB: spine-like bodies, +/-: presence/absence of the considered character, ?: doubtful or unknown data, *one mitochondrion is moniliform.