

Spermatozoon ultrastructure in two monorchiid digeneans (#11566)

1

First submission

Please read the **Important notes** below, and the **Review guidance** on the next page.
When ready [submit online](#). The manuscript starts on page 3.

Important notes

Editor and deadline

Tokuko Haraguchi / 21 Jul 2016

Files

4 Figure file(s)

1 Table file(s)

Please visit the overview page to [download and review](#) the files not included in this review pdf.

Declarations

No notable declarations are present



Please in full read before you begin

How to review

When ready [submit your review online](#). The review form is divided into 5 sections. Please consider these when composing your review:

1. BASIC REPORTING

2. EXPERIMENTAL DESIGN

3. VALIDITY OF THE FINDINGS

4. General comments

5. Confidential notes to the editor

 You can also annotate this **pdf** and upload it as part of your review

To finish, enter your editorial recommendation (accept, revise or reject) and submit.

BASIC REPORTING

-  Clear, unambiguous, professional English language used throughout.
-  Intro & background to show context. Literature well referenced & relevant.
-  Structure conforms to [PeerJ standard](#), discipline norm, or improved for clarity.
-  Figures are relevant, high quality, well labelled & described.
-  Raw data supplied (See [PeerJ policy](#)).

EXPERIMENTAL DESIGN

-  Original primary research within [Scope of the journal](#).
-  Research question well defined, relevant & meaningful. It is stated how research fills an identified knowledge gap.
-  Rigorous investigation performed to a high technical & ethical standard.
-  Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

-  Impact and novelty not assessed. Negative/inconclusive results accepted. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
-  Data is robust, statistically sound, & controlled.
-  Conclusion well stated, linked to original research question & limited to supporting results.
-  Speculation is welcome, but should be identified as such.

The above is the editorial criteria summary. To view in full visit <https://peerj.com/about/editorial-criteria/>

Spermatozoon ultrastructure in two monorchiid digeneans

Yann Quilichini ^{Corresp., 1}, **Abdoulaye J S Bakhoun** ^{1,2}, **Jean-Lou Justine** ³, **Rodney A Bray** ⁴, **Cheikh T Bâ** ², **Bernard Marchand** ¹

¹ GEM - Service d'Etude et de Recherche en Microscopie Electronique (SERME), UMR 6134 - SPE, CNRS - Università di Corsica, Corte, Corsica, France

² Laboratory of Evolutionary Biology, Ecology and Management of Ecosystems, University Cheikh Anta Diop of Dakar, Dakar, Senegal

³ ISYEB, Institut de Systématique, Évolution, Biodiversité (UMR7205 CNRS, EPHE, MNHN, UPMC), Muséum National d'Histoire Naturelle de Paris, Sorbonne Universités, Paris, France

⁴ Department of Life Sciences, National History Museum of London, London, United Kingdom

Corresponding Author: Yann Quilichini

Email address: quilichini@univ-corse.fr

Spermatological characteristics of species from two monorchiid genera, *Opisthomonorchis* and *Paramonorchoides*, have been investigated, for the first time, by means of transmission electron microscopy. The ultrastructural study reveals that the mature spermatozoon of *Opisthomonorchis dinema* and *Paramonorchoides selaris* share several characters such as the presence of two axonemes of different lengths showing the 9+“1” pattern of the Trepaxonemata, a nucleus, two mitochondria, two bundles of parallel cortical microtubules, external ornamentation, spine-like bodies, granules of glycogen and similar morphologies of the anterior and posterior extremities. The slight differences between the male gamete of *O. dinema* and *P. selaris* are the length of the first axoneme and the position of the second mitochondrion. This study also elucidates the general morphology of the spermatozoon in all monorchiid species described so far, which corresponds to a unique spermatozoon type. Other interesting finds concern the spermatological similarities between monorchiid spermatozoa and the mature spermatozoon reported in the apocreadiid *Neoapocreadium chabaudi*. These similarities allow us to suggest a close phylogenetical relationship between the Monorchiidae and the Apocreadiidae, although more studies are needed, especially in the unexplored taxa.

Spermatozoon ultrastructure in two monorchiid digeneans

YANN QUILICHINI^{1,*}, ABDOULAYE J.S. BAKHOUM^{1,2}, JEAN-LOU JUSTINE³, RODNEY
A. BRAY⁴, CHEIKH T. BÂ², BERNARD MARCHAND¹

¹UMR 6134 – SPE, CNRS - Università di Corsica, SERME Service d'Étude et de Recherche en
Microscopie Électronique, 20250 Corte, Corsica, France

²Laboratory of Evolutionary Biology, Ecology and Management of Ecosystems, Cheikh Anta
Diop University of Dakar, B.P. 5055, Dakar, Senegal

³ISYEB, Institut de Systématique, Évolution, Biodiversité (UMR7205 CNRS, EPHE, MNHN,
UPMC), Muséum National d'Histoire Naturelle, Sorbonne Universités, CP 51, 55 rue Buffon,
75231 Paris cedex 05, France

⁴Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD,
United Kingdom

*Corresponding author: Yann Quilichini, UMR 6134 – SPE, CNRS - Università di Corsica,
SERME Service d'Étude et de Recherche en Microscopie Electronique, 20250 Corte, Corsica,
France; Email: quilichini@univ-corse.fr

19 ABSTRACT

20 Spermatological characteristics of species from two monorchiid genera, *Opisthomonorchis* and
 21 *Paramonorchoides*, have been investigated, for the first time, by means of transmission electron
 22 microscopy. The ultrastructural study reveals that the mature spermatozoon of *Opisthomonorchis*
 23 *dinema* and *Paramonorchoides selaris* share several characters such as the presence of two
 24 axonemes of different lengths showing the 9+“1” pattern of the Trepaxonemata, a nucleus, two
 25 mitochondria, two bundles of parallel cortical microtubules, external ornamentation, spine-like
 26 bodies, granules of glycogen and similar morphologies of the anterior and posterior extremities.
 27 The slight differences between the male gamete of *O. dinema* and *P. selaris* are the length of the
 28 first axoneme and the position of the second mitochondrion.
 29 This study also elucidates the general morphology of the spermatozoon in all monorchiid species
 30 described so far, which corresponds to a unique spermatozoon type.
 31 Other interesting finds concern the spermatological similarities between monorchiid spermatozoa
 32 and the mature spermatozoon reported in the apocreadiid *Neoapocreadium chabaudi*. These
 33 similarities allow us to suggest a close phylogenetical relationship between the Monorchiidae
 34 and the Apocreadiidae, although more studies are needed, especially in the unexplored taxa.

35

36

INTRODUCTION

The description of ultrastructural characteristics of the mature spermatozoon of the Platyhelminthes, and in particular the Digenea, has recently proved to be an interesting tool for phylogenetic purposes (Justine, 1995; Levron et al., 2010; Miquel et al., 2013; Bakhoun et al., 2012a, b; 2015a, b; Ndiaye et al., 2015a, b). The Monorchioidea comprise two families of trematodes namely, the Lissorchiidae and the Monorchiidae (Bray, 2008). Among the latter, which includes 40 valid genera (Madhavi, 2008), only two species, *Monorchis parvus* and *Helicometroides atlanticus* (Levron et al., 2004a; Diagne et al., 2015) have been explored for spermatological characteristics. Moreover, the mature spermatozoon of *M. parvus* and *H. atlanticus* exhibit some differences especially in their respective anterior extremity.

The aim of this work was to bring more spermatological descriptions from two additional genera, *Opisthomonorchis* and *Paramonorchoides*, in order to elucidate the general morphology of the spermatozoon in the Monorchiidae. In addition, the mature spermatozoon of the monorchiids was compared to those of the other digeneans with a brief comment on its phylogenetic relevance.

MATERIALS AND METHODS

Fish were bought dead, but very fresh, at the fishmarket in Nouméa, New Caledonia. Specimens of *Opisthomonorchis dinema* Bray & Justine, 2013 were collected from the digestive tract of *Carangoides dinema* Bleeker (Perciformes, Carangidae) caught off Nouméa, New Caledonia, from the same fish specimen (MNHN JNC3224) as the paratypes of the species (Bray & Justine, 2013). Specimens of *Paramonorchoides selaris* Lakshmi & Madhavi, 2009 were collected from the digestive tract of *Selar crumenophthalmus* (Perciformes, Carangidae) caught off Nouméa, New Caledonia, on 10 Sept. 2009 (fish specimen MNHN JNC 3043).

Digenean specimens were fixed in cold (4 °C) 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.2, rinsed in 0.1 M sodium cacodylate buffer at pH 7.2, post-fixed in cold (4 °C) 1% osmium tetroxide in the same buffer for 1 h, dehydrated in ethanol and propylene oxide series, embedded in Spurr resin and polymerized at 60 °C for 24 h. Ultrathin sections (60–90 nm) of the seminal vesicle were obtained on an ultramicrotome (Power tome PC, RMC Boeckeler®). The sections were placed on 300 and 200 mesh copper grids and stained with uranyl acetate and lead citrate according to Reynolds (1963) methodology. To locate glycogen granules, the Thiéry technique (1967) was also used in several sections placed on gold grids. All grids were examined on a Hitachi H-7650 transmission electron microscope, operating at an accelerating voltage of 80 kV, in the “Service d’Étude et de Recherche en Microscopie Électronique” of the University of Corsica (Corte, France).

RESULTS

The organization of the mature spermatozoon of *Opisthomonorchis dinema* (Figs. 1, 4) and *Paramonorchoides selaris* (Figs. 2-4) is described after observations of cross- and longitudinal

sections. Thus, in both monorchiid species four regions, with distinctive ultrastructural characteristics, are evident, from the anterior to the posterior spermatozoon extremities.

Region I, corresponding to the anterior extremity of the mature spermatozoon, is characterized by the presence of the first axoneme accompanied by the anterior extremity of the second axoneme, cortical microtubules and external ornamentation of the plasma membrane. In *O. dinema* the anterior spermatozoon tip exhibits both anterior extremities of the axonemes, surrounded by a layer of cortical microtubules (about 26) and external ornamentation (Figs. 1A, 4I). In *P. selaris* longitudinal and cross-sections of the anterior spermatozoon tip show that it tapers to a point (Fig. 2A), and that it contains the anterior extremities of both axonemes and the cortical microtubules (about 26) (Fig. 2C) as well as exhibiting external ornamentation of the plasma membrane (Figs. 1A, B, 2B, C, 4I). In the middle part of the region I, when both axonemes are formed, spine-like bodies appear interrupting the external ornamentation of the spermatozoa of *Opisthorchis dinema* and *Paramonorchis selaris* (Figs. 1C, 2D, 4I). The posterior part of the region I is characterized, for each species, by the presence of external ornamentation of the plasma membrane associated with cortical microtubules and the first mitochondrion (Figs. 1D, E, 2E, 4I).

Region II corresponds to the transitional area before the nuclear region. For both *O. dinema* and *P. selaris* spermatozoa, it is characterized by the absence of external ornamentation and the presence of sections showing the axonemes and the first mitochondrion (Figs. 1F, 2F, 4II). In addition, for each monorchiid species, the posterior part of the region II contains a reduced number of cortical microtubules: about 5 in *O. dinema* (Fig. 1G) and 13 in *P. selaris* (Fig. 2G).

Region III exhibits several ultrastructural characteristics that distinguish the mature spermatozoa of *O. dinema* (Fig. 3a) from those of *P. selaris* (Fig. 3b):

- In the anterior part of the region III, the spermatozoon is characterized by the disorganisation of the first axoneme in *O. dinema* (Figs. 1H and 4II), whereas in *P. selaris* the

two axonemes and the second mitochondrion are observed (Figs. 2H, 4II).

- The middle part of the region III is characterized in *O. dinema* by the presence of only one axoneme, the nucleus, few cortical microtubules and the second mitochondrion (Figs. 1I and

4III). In contrast, in *P. selaris*, the second mitochondrion, the nucleus and few cortical microtubules were accompanied by the two axonemes (Figs. 2I, 4III). Note that this part

corresponds to the disorganization of the first axoneme in *P. selaris* (Figs. 2J, 4III).

- In the posterior part of the region III, the second mitochondrion, accompanied by one axoneme, the nucleus and cortical microtubules is observed in *O. dinema* (Figs. 1I, 4III) and in *P. selaris* (Figs. 3A, 4III).

Region IV represents the posterior spermatozoon extremity. When the second mitochondrion disappears in both *O. dinema* and *P. selaris* mature spermatozoa, cross-sections exhibit only one axoneme, the nucleus and very few cortical microtubules (Figs. 1J, 3B, 4IV). Moreover, for each species, the second axoneme disorganizes exhibiting doublets and singlets of microtubules (Figs. 1K, 3C). Consequently, the posterior spermatozoon tips in *O. dinema* and *P. selaris* show only the nucleus (Figs. 1L, 3D, 4IV).

The granules of glycogen present in *O. dinema* and *P. selaris* were detected by the cytochemical test of Thiéry in cross-sections (Fig. 3E).

DISCUSSION

The mature spermatozoon described in *Opisthomonorchis dinema* and *Paramonorchoides selaris* possesses two axonemes of the 9+“1” trepaxonematan pattern, two mitochondria, a nucleus, two

bundles of parallel cortical microtubules, an external ornamentation of the plasma membrane, spine-like bodies and granules of glycogen. In addition to these ultrastructural characters, the male gamete of *O. dinema* and *P. selaris* exhibits similar anterior and posterior spermatozoon morphologies.

Within the Monorchiidae most of these spermatological characters were described previously in *M. parvus* and *H. atlanticus* (Levron et al., 2004a; Diagne et al., 2015). Thus, the characters of the mature spermatozoon, its anterior and posterior morphologies were used here in comparative studies between the different species (Table 1).

General organisation of the mature spermatozoon in the Monorchiidae

From the anterior part to the posterior part of the gametes, six characteristics are presented here to describe the architecture of the mature spermatozoa in Monorchiidae. Moreover, monorchiid spermatozoa are compared to those reported from the other digenean species.

- Type of axoneme

The structure of the axoneme with 9+“1” pattern of Trepaxonemata (Ehlers, 1984) has been observed in *O. dinema* and *P. selaris* as described previously in *M. parvus* and *H. atlanticus* (Table 1). A similar morphology of the axoneme has been reported in most digenean species (Quilichini et al., 2011; Foata et al., 2012; Miquel et al., 2013; Ndiaye et al., 2012, 2015a) with the exception of schistosomes in which the 9+“1” special pattern was described and some didymozoid species showing the 9+0 pattern (Justine & Mattei, 1983; Justine, Jamieson & Southgate, 1993; Yang et al., 2003).

Their different length is another aspect related to axonemes. In both *O. dinema* and *P. selaris*, the posterior extremity of the axoneme 2 is longer than that of the first one. Moreover,

the spermatozoon of *O. dinema* is distinguished by the first axoneme that does not reach the nuclear region, while in *P. selaris* the first axoneme is observed in the nuclear area like in *M. parvus* and *H. atlanticus* (Levron et al., 2004a; Diagne et al., 2015).

- Anterior spermatozoon morphology

The anterior spermatozoon extremity of *O. dinema* and *P. selaris* contains two axonemes, cortical microtubules and external ornamentation of the plasma membrane. Within the Monorchidae, an anterior spermatozoon extremity showing two axonemes, cortical microtubules and external ornamentation has been reported in *Monorchis parvus* (Levron et al., 2004a). Whereas in the other monorchid species studied so far, namely *H. atlanticus* (Diagne et al., 2015), only electron-dense material and two axonemes have been observed in the anterior spermatozoon extremity.

Mature spermatozoa showing two axonemes and external ornamentation in their anterior extremities have also been reported in spermatozoa belonging to some digenean species such as the apocreadiid *Neoapocreadium chabaudi* (Kacem et al., 2010), the pronoccephalids *Pleurogonius truncatus* and *Cricoccephalus albus* (Ndiaye et al., 2011, 2012) and the mesometrids *Centroderma spinosissima* and *Wardula capitellata* (Bakhoun et al., 2012, 2013). In the two latter families the presence of a lateral expansion is described in the anterior spermatozoon extremity of the studied species.

Other types of spermatozoa showing two axonemes in their anterior extremities have been reported in several digenean species such as the deropristid *Deropristis inflata* (Foata, Quilichini & Marchand, 2007), the omphalometrid *Rubenstrema exasperatum* (Bakhoun et al., 2011) or the pleurogenids *Pleurogenes claviger*, *Pleurogenoides medians* and *Prosotocus confusus* (Miquel et al., 2013).

- External ornamentation and its location

In all monorchiid species studied, the presence of external ornamentation associated with cortical microtubules has been observed in the anterior region of the spermatozoon as in most digenean species (Justine & Mattei, 1982; Miquel et al., 2006; Quilichini, Foata & Marchand, 2007; Bâ et al., 2011; Ndiaye et al., 2012, 2015a, b; Bakhoum et al., 2015a, b). However, the location of the external ornamentation distinguishes the spermatozoa of *O. dinema* and *P. selaris* from those of other digenean species. In fact, based on the diagram of the localization of the external ornamentation established by Quilichini et al. (2011a), the mature spermatozoon of *O. dinema* and *P. selaris* presents the type 1 of external ornamentation, i.e., located in proximal part of the anterior spermatozoon region. It is also interesting to note that in both *O. dinema* and *P. selaris*, the external ornamentation is extended from centriole level to the area containing the first mitochondrion. Such disposition of the external ornamentation is also reported in the monorchiid *M. parvus* but not in *H. atlanticus* (Table 1). This latter species seems to exhibit type 2 external ornamentation according to the diagram of Quilichini et al. (2011).

- Spine-like bodies

Since their first description by Miquel et al. (2000) the spine-like bodies have frequently been reported in the mature spermatozoon especially in its anterior part. In the male gamete of *O. dinema* and *P. selaris*, spine-like bodies are present in the ornamented area associated with cortical microtubules. Moreover, in both monorchiid species the morphology of the spine-like bodies follows that reported in most digenean species i.e., appearance of a small vesicle interrupting the external ornamentation of the plasma membrane (Miquel et al., 2006; Quilichini et al., 2011b; Bakhoum et al., 2015b; Ndiaye et al., 2015a, b).

Within the Monorchiidae, the absence of spine-like bodies was mentioned in the male gamete

191 of *M. parvus* and *H. atlanticus* (Levron et al., 2004a; Diagne et al., 2015) (Table 1). Hence the
 192 spine-like bodies are described here, for the first time, in the mature spermatozoon of monorchiid
 193 species.

194 Taking into account all these aspects, the presence or absences of spine-like bodies in the
 195 Monorchiidae needs more ultrastructural investigations.

196 - Number of mitochondria

197 The presence of mitochondria in the mature spermatozoon is considered as a plesiomorphic
 198 character in the Digenea (Bakhoun et al., 2014) whereas in other groups, such as the Eucestoda,
 199 the absence of mitochondria has been highlighted as a synapomorphy (Justine, 1995). Besides its
 200 interest in phylogenetic relationships, the other criterion related to mitochondria is their number.

201 Based on many cross and longitudinal sections, two mitochondria are evident in the mature
 202 spermatozoon of *O. dinema* and *P. selaris*. The presence of two mitochondria have also been
 203 reported in *M. parvus*, *H. atlanticus* (Table 1) and in several species belonging, for instance, to
 204 the families Acanthocolpidae (Bakhoun et al., 2015a), Apocreadiidae (Kacem et al., 2010),
 205 Deropristidae (Foata, Quilichini & Marchand, 2007) or Notocotylidae (Ndiaye et al., 2003,
 206 2015b). Other digenean species possess one mitochondrion [e.g. *Carmyerius endopapillus* (Seck
 207 et al., 2008), *Wardula capitellata*; (Bakhoun et al., 2012b)] or three mitochondria [(e.g.
 208 *Anisocoelium capitellatum* (Bakhoun et al., 2009), *Euryhormis squamula*; (Ternengo et al.,
 209 2009)].

210 In the present study, the position of the second mitochondrion is an ultrastructural
 211 characteristic that distinguishes the mature spermatozoa of *O. dinema* from those of *P. selaris*, *H.*
 212 *atlanticus* and *M. parvus*. The second mitochondrion appears after the disorganization of the first
 213 axoneme in *O. dinema*, whereas in the remaining monorchiid the appearance of the second

214 mitochondrion is noted before the posterior extremity of the first axoneme.

215 - Posterior spermatozoon characters

216 In Digenea the posterior spermatozoon extremity is morphologically variable and is, therefore,
217 valuable in the establishment of spermatozoon models (Quilichini et al., 2010; Ndiaye et al.,
218 2015b; Bakhoun et al., 2012a, 2015a). In all monorchiid species described so far the posterior
219 spermatozoon extremity contains a nucleus as in *O. dinema* and *P. selaris* (Table 1). This
220 morphology of the posterior spermatozoon extremity is frequently reported in digenean (Kacem
221 et al., 2010, 2015a, b; Zhukova et al., 2014; Quilichini et al., 2015) and corresponds to the
222 fasciolidean type or type 2 according to the diagram of Quilichini et al. (2010).

223 Other types of posterior spermatozoon extremities have been reported in digenean species. This
224 is the case of mature spermatozoa exhibiting one axoneme as described in most microphalloid
225 species (Bakhoun et al., 2012a; Miquel et al., 2013; Bruňanská et al., 2014) and opisthorchioid
226 species (Quilichini et al., 2009; Foata et al., 2012; Zhukova et al., 2014). In the other hand, in the
227 families Opecoelidae and Opistholebetidae (Miquel et al., 2000; Levron et al., 2003, 2004b;
228 Quilichini et al., 2010, 2011b), there are mature spermatozoa showing only cortical microtubules
229 in their posterior extremities.

230

231 Phylogenetic perspectives of ultrastructural characteristics

232 The ultrastructural differences observed in the spermatozoa are phylogenetically informative in
233 the Neodermata, particularly in the Cestoda and Monogenea (Justine 1991, 1998, Levron et al.,
234 2010). In the Digenea a large amount of spermatological data accumulated over the recent years
235 could serve as a complementary resource for understanding their phylogenetic relationships
236 (Quilichini et al., 2010; Miquel et al., 2013; Bakhoun et al., 2015a; Diagne et al., 2015).

The phylogenetic relationships in the Monorchiidae and its systematic placement within the Digenea have been controversial. The Monorchiidae has been considered polyphyletic because of the great diversity exhibited in the morphology of its members (Madhavi, 2008). The most recent classification based on DNA sequences includes the Monorchiidae and Lissorchiidae in the superfamily Monorchioidea (Bray, 2008), which was included in the new suborder Monorchiata (Olson et al., 2003).

Here, the understanding of the relationships and phylogenetic affinities in Monorchiidae are attempted with ultrastructural characteristics of the spermatozoa, although there still remain some unexplored groups. The morphology of the spermatozoa described in *O. dinema*, *P. selaris* and other monorchiid species is similar in several points to that reported in the apocreadiid *Neopocreadium chabaudi* (Kacem et al., 2010). The main similarities concern (1) the type of axoneme, (2) the morphology of both anterior and posterior spermatozoon extremities, (3) the presence of type 1 external ornamentation according to Quilichini et al. (2011a), (4) the two bundles of parallel cortical microtubules and (5) the presence of spine-like bodies.

Besides these similarities, it is interesting to note that the systematic position of the Apocreadiidae is unresolved. Some authors have grouped this family with the Haploporoidea and Monorchioidea (Cribb et al., 2001). Whereas other researchers suggested the removal of the Apocreadiidae from the Lepocreadioidea (see Bray & Cribb, 2012). Thus, to our knowledge no robust data are available to validate the phylogenetic position of the Apocreadiidae.

The present spermatological findings suggest, for the first time, a close relationship between the Monorchiidae and the Apocreadiidae based on ultrastructural characteristics of their mature spermatozoa. However, further studies are needed in order to support our hypothesis.

Moreover, ultrastructural studies in the Lissorchiidae (probably a sister group) are also needed to test their close relationships with the Monorchiidae.

Conclusion

The mature spermatozoa of *Opisthomonorchis dinema* and *Paramonorchoides selaris* share several ultrastructural features with those reported in *Monorchis parvus* and *Helicometroides atlanticus* (Table 1). These similarities allow us to define the type of spermatozoon in the Monorchiidae, which exhibits the following features:

- Presence of an anterior extremity showing two axonemes accompanied by cortical microtubules and external ornamentation,
- Presence of the association “external ornamentation+cortical microtubules”,
- Location of the external ornamentation corresponding to the type 1 according Quilichini et al. (2010),
- Presence of two bundles of parallel cortical microtubules and two mitochondria,
- Posterior spermatozoon extremity containing only the nucleus.

The model of mature spermatozoon described here for the monorchiid species is relatively similar to that reported to the apocreadiid *Neoapocreadium chabaudi*. These similarities allow us to suggest a close relationship between the Monorchiidae and Apocreadiidae, although additional spermatological evidences are needed especially, from the unexplored taxa belonging to the families mentioned above and the Lissorchiidae.

The ultrastructural characteristics described in digenean and in particular, the Monorchiidae could be used as phylogenetic tools and when establishing spermatozoon models, given that they may allow the distinguishing of genera, families or superfamilies within the Digenea.

REFERENCES

- Bâ CT, Ndiaye PI, Dione A, Quilichini Y, Marchand B. 2011.** Ultrastructure of the spermatozoon of *Holorchis micracanthum* (Digenea: Lepocreadiidae), an intestinal parasite of *Plectorhinchus mediterraneus* (Pisces, Teleostei) in Senegal. *Parasitology Research* **109**:1099-1106.
- Bakhoun AJS, Bâ CT, Fournier-Chambrillon C, Torres J, Fournier P, Miquel J. 2009.** Spermatozoon ultrastructure of *Euryhormis squamula* (Rudolphi, 1819) (Digenea, Opisthorchioidea, Heterophyidae), an intestinal parasite of *Mustela vison* (Carnivora, Mustelidae). *Revista Ibero-latinoana de Paramericasitologia* **1**:37-45.
- Bakhoun AJS, Bâ CT, Shimalov VV, Torres J, Miquel J. 2011.** Spatological characters of the digenean *Rubenstrema exasperatum* (Rudolphi, 1819) (Plagiorchioidea, Omphalometridae). *Parasitology Research* **108**:1283-1293.
- Bakhoun AJS, Feliu C, Bâ CT, Miquel J. 2012a.** Spermiogenesis and spermatozoon of the liver fluke *Mediogonimus jourdanei* (Microphalloidea: Prosthogonimidae), a parasite of *Myodes glareolus* (Rodentia: Cricetidae). *Folia Parasitologica* **59**:32-42.
- Bakhoun AJS, Kacem H, Neifar L, Miquel J. 2013.** Ultrastructure of the spermatozoon of *Centroderma spinosissima* (Stossich, 1886) (Digenea: Mesometridae) and its phylogenetic potential. *Tissue and Cell* **45**:428-33.
- Bakhoun AJS, Ndiaye PI, Sène A, Bâ CT, Miquel J. 2012b.** Spermiogenesis and ultrastructure of the spermatozoon of *Wardula capitellata* (Digenea, Mesometridae), an intestinal parasite of the sparid teleost *Sarpa salpa* in Senegal. *Acta Parasitologica* **57**:34-45.

- 305 **Bakhoun AJS, Quilichini Y, Justine J-L, Bray RA, Bâ CT, Marchand B. 2015a.**
 306 Ultrastructural study of sperm cells in Acanthocolpidae: the case of *Stephanostomum murelæ*
 307 and *Stephanostomoides tenuis* (Digenea). *PeerJ* 3, e744.
- 308 **Bakhoun AJS, Quilichini Y, Justine J-L, Bray RA, Bâ CT, Marchand B. 2015b.**
 309 *Neomultitestis aspidogastriformis* Bray and Cribb, 2003 (Digenea, Lepocreadiidae): mature
 310 spermatozoon and sperm morphologies in the Lepocreadiidae. *Cell Biology International*
 311 **39**:799-807.
- 312 **Bakhoun AJS, Quilichini Y, Miquel J, Feliu C, Bâ CT, Marchand B. 2014.** *Collyricloides*
 313 *massanae* (Digenea, Collyriclidae): spermatozoon ultrastructure and phylogenetic importance.
 314 *Parasite* **21**:59.
- 315 **Bray RA. 2008.** Superfamily Monorchioidea Odhner, 1911. In: Bray RA, Gibson DI, Jones A,
 316 eds. *Keys to the Trematoda, vol. 3*. London: CAB International and The Natural History
 317 Museum, 143-144.
- 318 **Bray RA, Cribb TH. 2012.** Reorganisation of the superfamily Lepocreadiidae Odhner, 1905
 319 based on an inferred molecular phylogeny. *Systematic Parasitology* **83**:169-177.
- 320 **Bray RA, Justine J-L. 2013.** Three species of opisthomonorchine monorchids (Digenea) in
 321 *Carangoides* spp. (Perciformes: Carangidae) from off New Caledonia, with a description of
 322 *Opisthomonorchis dinema* n. sp. *Systematic Parasitology* **85**:147-156.
- 323 **Bruňanská M, Brázová T, Zhokhov AE, Poddubnaya LG. 2014.** Ultrastructural features of
 324 the spermatozoon and its differentiation in *Brandesiaturgida* (Brandes, 1888) (Digenea,
 325 Microphalloidea, Pleurogenidae). *Parasitology Research* **113**:2483-2491.

- 326 **Cribb TH, Bray RA, Littlewood DTJ, Pichelin SP, Herniou EA. 2001.** The Digenea. In:
327 Littlewood DTJ, Bray RA, eds. *Interrelationships of the Platyhelminthes*. London: Taylor and
328 Francis, 168-185.
- 329 **Diagne PM, Quilichini Y, Bâ CT, Ndiaye PI, Dione A, Marchand B. 2015.** Ultrastructure of
330 the spermatozoon of **Helicometroides atlanticus** (Digenea, Monorchidae), an intestinal parasite
331 of **Parapristipoma octolineatum** (Pisces, Teleostei) in Senegal. *Tissue and Cell* **47**:198-204.
- 332 **Ehlers U. 1984.** Phylogenetisches System der Plathelminthes. *Verhandlungen des*
333 *Naturwissenschaftlichen Vereins Hamburg*, NF **27**:291-294.
- 334 **Foata J, Quilichini Y, Greani S, Marchand B. 2012.** Sperm ultrastructure of the digenean
335 *Aphallus tubarium* (Rudolphi, 1819) Poche, 1926 (Platyhelminthes, Cryptogonimidae) intestinal
336 parasite of *Dentex dentex* (Pisces, Teleostei). *Tissue and Cell* **44**:15-21.
- 337 **Foata J, Quilichini Y, Marchand B. 2007.** Spermogenesis and sperm ultrastructure of
338 *Deropristis inflata* Molin, 1859 (Digenea, Deropristidae), a parasite of *Anguilla anguilla*.
339 *Parasitology Research* **101**:843-52.
- 340 **Justine J-L. 1991.** Cladistic study in the Monogenea (Platyhelminthes), based upon a parsimony
341 analysis of spermiogenetic and spermatozoal ultrastructural characters. *International Journal for*
342 *Parasitology* **21**:821-838.
- 343 **Justine J-L. 1995.** Spermatozoal ultrastructure and phylogeny of the parasitic Platyhelminthes.
344 *Mémoires du Muséum National d'Histoire Naturelle* **166**:37-54.
- 345 **Justine J-L. 1998.** Spermatozoa as phylogenetic characters for the Eucestoda. *Journal of*
346 *Parasitology* **84**:385-408.
- 347 **Justine J-L, Jamieson BGM, Southgate VR. 1993.** Homogeneity of sperm structure in
348 **sixspecies** of Schistosomes (Digenea, Platyhelminthes). *Annales de Parasitologie Humaine et*

- 349 *Comparée* **68**:185-187.
- 350 **Justine J-L, Mattei X. 1982.** Réinvestigation de l'ultrastructure du spermatozoïde
351 d'**Haematoloechus** (Trematoda: Haematoloechidae). *Journal of Ultrastructure Research* **8**:322-
352 332.
- 353 **Justine J-L, Mattei X. 1983.** A spermatozoon with two 9+0 axonemes in a parasitic flatworm,
354 *Didymozoon* (Digenea: Didymozoidae). *Journal of Submicroscopic Cytology and Pathology*
355 **15**:1101-1105.
- 356 **Kacem H, Bakhounm AJS, Neifar L, Miquel J. 2010.** Spermiogenesis and spermatozoon
357 ultrastructure of the digenean *Neopocreadium chabaudi* (Apocreadiidae), a parasite of *Balistes*
358 *capricornus* (Pisces, Teleostei). *Parasitology International* **59**:358-366.
- 359 **Kacem H, Ndiaye PI, Neifar L, Torres J, Miquel J. 2015a.** Ultrastructure of the spermatozoon
360 of the digenean *Tergestia acanthocephala* (Stossich, 1887) (Gymnophalloidea: Fellodistomidae):
361 an intestinal parasite of *Belone belone gracilis* (Pisces: Teleostei). *Tissue and Cell* **47**:235-241.
- 362 **Kacem H, Ndiaye PI, Neifar L, Torres J, Miquel J. 2015b.** Spermatological characters of the
363 digenean *Lecithostaphylus retroflexus* (Molin, 1859) (Microphalloidea: Zoogonidae), a parasite
364 of the teleost fish *Belone belone gracilis*. *Tissue and Cell* **47**:431-437.
- 365 **Levron C, Miquel J, Oros M, Scholz T. 2010.** Spermatozoa of tapeworms (Platyhelminthes,
366 Eucestoda): advances in ultrastructural and phylogenetic studies. *Biological Reviews* **85**:523-543.
- 367 **Levron C, Ternengo S, Marchand B. 2003.** Ultrastructure of spermiogenesis and the
368 spermatozoon of *Helicometra fasciata* (Digenea, Opecoelidae), a parasite of *Labrus merula*
369 (Pisces, Teleostei). *Acta Parasitologica* **48**:255-264.
- 370 **Levron C, Ternengo S, Marchand B. 2004a.** Ultrastructure of spermiogenesis and the
371 spermatozoon of *Monorchis parvus* Looss, 1902 (Digenea, Monorchidae), a parasite of

- 372 **Diplodus annularis** (Pisces, Teleostei). *Parasitology Research* **93**:102-110.
- 373 **Levron C, Ternengo S, Marchand B. 2004b.** Spermiogenesis and sperm ultrastructure of
 374 *Poracanthium furcatum* (Digenea, Opecoelidae), a parasite of *Mullus surmuletus* (Pisces,
 375 Teleostei). *Acta Parasitologica* **49**:190-200.
- 376 **Madhavi R. 2008.** Family Monorchidae Odhner, 1911. In: Bray RA, Gibson DI, Jones A, eds.
 377 *Keys to the Trematoda, vol. 3.* London: CAB International and The Natural History Museum,
 378 145-175.
- 379 **Miquel J, Fournier-Chambrillon C, Fournier P, Torres J. 2006.** Spermiogenesis and
 380 spermatozoon ultrastructure of the cranial digenean *Troglorema acutum* (Leuckart, 1842).
 381 *Journal of Parasitology* **92**:441-453.
- 382 **Miquel J, Nourrisson C, Marchand B. 2000.** Ultrastructure of spermiogenesis and the
 383 spermatozoon of *Opecoeloides furcatus* (Trematoda, Digenea, Opecoelidae), a parasite of *Mullus*
 384 *barbatus* (Pisces, Teleostei). *Parasitology Research* **86**:301-310.
- 385 **Miquel J, Vilavella D, Świdorski Z, Shimalov VV, Torres J. 2013.** Spermatological
 386 characteristics of Pleurogenidae (Digenea) inferred from the ultrastructural study of *Pleurogenes*
 387 *claviger*, *Pleurogenoides medians* and *Prosotocus confusus*. *Parasite* **20**:1-28.
- 388 **Ndiaye PI, Bakhoun AJS, Sène A, Diagne PM, Miquel J. 2015a.** The ultrastructural
 389 characters of the mature spermatozoon of *Opechona bacillaris* (Molin, 1859) (Digenea,
 390 Lepocreadiidae) a parasite of *Scomber colias* Gmelin, 1789 (Scombridae) off the coast of Dakar
 391 (Senegal). *Acta Zoologica (Stockholm)* **96**:91-98.
- 392 **Ndiaye PI, Miquel J, Feliu C, Marchand B. 2003.** Ultrastructure of spermiogenesis and
 393 spermatozoa of *Notocotylus neyrai* González Castro, 1945 (Digenea, Notocotylidae), intestinal
 394 parasite of *Microtus agrestis* (Rodentia: Arvicolidae) in Spain. *Invertebrate Reproduction and*

395 *Development* **43**:105-115.

396 **Ndiaye PI, Quilichini Y, Sène A, Bâ CT, Marchand B. 2011.** Ultrastructure of the
397 spermatozoon of the digenean *Cricocephalus albus* (Kuhl & van Hasselt, 1822) Looss, 1899
398 (Platyhelminthes, Pronocephaloidea, Pronocephalidae), parasite of “the hawksbill sea turtle”
399 *Eretmochelys imbricata* (Linnaeus, 1766) in Senegal. *Zoologischer Anzeiger* **250**:215-222.

400 **Ndiaye PI, Quilichini Y, Sène A, Tkach VV, Bâ CT, Marchand B. 2012.** Ultrastructural study
401 of the male gamete of *Pleurogonius truncatus* Prudhoe, 1944 (Platyhelminthes, Digenea,
402 Pronocephalidae) parasite of *Eretmochelys imbricata* (Linnaeus, 1766). *Comptes Rendus*
403 *Biologies* **335**:239-246.

404 **Ndiaye PI, Torres J, Eira C, Shimalov VV, Miquel J. 2015b.** Ultrastructure of the
405 spermatozoon of the trematode *Notocotylus noyeri* (Digenea: Notocotylidae), a parasite of
406 *Microtus arvalis* (Rodentia: Cricetidae). *Folia Parasitologica*, **62**:001.

407 **Olson PD, Cribb TH, Tkach VV, Bray RA, Littlewood DTJ. 2003.** Phylogeny and
408 classification of the Digenea (Platyhelminthes: Trematoda). *International Journal for*
409 *Parasitology* **33**:733-755.

410 **Quilichini Y, Foata J, Justine J-L, Bray RA, Marchand B. 2009.** Sperm ultrastructure of the
411 digenean *Siphoderina elongata* (Platyhelminthes, Cryptogonimidae) intestinal parasite of
412 *Nemipterus furcosus* (Pisces, Teleostei). *Parasitology Research* **105**:87-95.

413 **Quilichini Y, Foata J, Justine J-L, Bray RA, Marchand B. 2010.** Ultrastructural study of the
414 spermatozoon of *Heterolebes maculosus* (Digenea, Opistholebetidae), a parasite of the
415 porcupinefish *Diodon hystrix* (Pisces, Teleostei). *Parasitology International* **59**:427-434.

416 **Quilichini Y, Foata J, Justine J-L, Bray RA, Marchand. 2011a.** Spermatozoon ultrastructure
417 of *Gyliauchen* sp. (Digenea: Gyliauchenidae), an intestinal parasite of *Siganus fuscescens*

- 418 (Pisces: Teleostei). *Biological Bulletin* **221**:197-205.
- 419 **Quilichini Y, Foata J, Justine J-L, Bray RA, Marchand B. 2011b.** Sperm ultrastructure of
 420 *Helicometra epinepheli* (Platyhelminthes, Digenea, Opecoelidae), parasite of *Epinephelus*
 421 *fasciatus* (Pisces, Teleostei). *Histology and Histopathology* **26**:1019-1028.
- 422 **Quilichini Y, Foata J, Marchand B. 2007.** Ultrastructural study of the spermatozoon of *Nicolla*
 423 *testiobliquum* (Digenea, Opecoelidae) parasite of brown trout *Salmo trutta* (Pisces, Teleostei).
 424 *Parasitology Research* **101**:1295-1301.
- 425 **Quilichini Y, Foata J, Orsini A, Marchand B. 2007.** Spermiogenesis and spermatozoon
 426 ultrastructure of *Nicolla wisniewskii* (Digenea: Opecoelidae), an intestinal parasite of brown trout
 427 *Salmo trutta* (Pisces: Teleostei). *Journal of Parasitology* **93**:469-478.
- 428 **Quilichini Y, Ndiaye PI, Sène A, Justine JL, Bray RA, Tkach VV, Bâ CT, Marchand B.**
 429 **2015.** Ultrastructural characters of the spermatozoa in Digeneans of the genus *Bianium* Stunkard,
 430 1930 (Digenea, Lepocreadiidae) parasites of fishes: a comparative study of *Bianium plicatum* and
 431 *Bianium arabicum*. *Parasitology Research* **114**:3747-3757.
- 432 **Reynolds ES. 1963.** The use of lead citrate at high pH as an electron-opaque stain in electron
 433 microscopy. *Journal of Cell Biology* **17**:208-212.
- 434 **Seck MT, Marchand B, Bâ CT. 2008.** Spermiogenesis and sperm ultrastructure of *Carmyerius*
 435 *endopapillatus* (Digenea, Gastrothylacidae), a parasite of *Bos taurus* in Senegal. *Acta*
 436 *Parasitologica* **53**:9-18.
- 437 **Ternengo S, Quilichini Y, Katharios P, Marchand B. 2009.** Sperm ultrastructure of the gall
 438 bladder fluke *Anisocoelium capitellatum* (Digenea: Cryptogonimidae), a parasite of *Uranoscopus*
 439 *scaber* (Pisces: Uranoscopidae). *Parasitology Research* **104**:801-807.
- 440 **Thiéry JP. 1967.** Mise en évidence des polysaccharides sur coupes fines en microscopie

441 électronique. *Journal of Microscopy* **6**:987-1018.

442 **Yang M-Y, Dong H-F, Jiang M-S. 2003.** Ultrastructural observation of spermatozoa and
443 fertilization in *Schistoma japonicum*. *Acta Tropica* **85**:63-70.

444 **Zhukova MV, Mordvinov VA, Kiseleva E. 2014.** Ultrastructure of spermatozoa in the seminal
445 receptacle of the liver fluke *Opisthorchis felinus* (Rivolta, 1884). *Parasitology Research*
446 **113**:1093-1101.

Figure 1(on next page)

Cross-sections of the mature spermatozoon of *Opisthomonorchis dinema* observed in TEM.



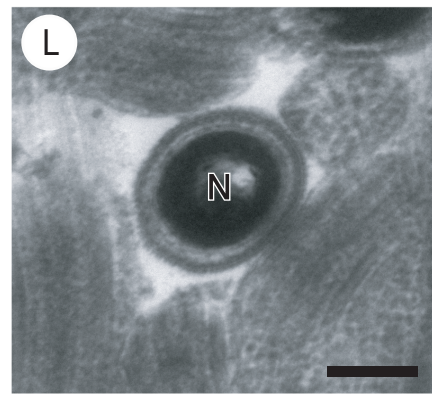
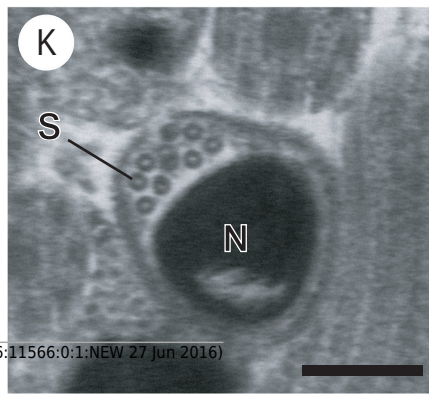
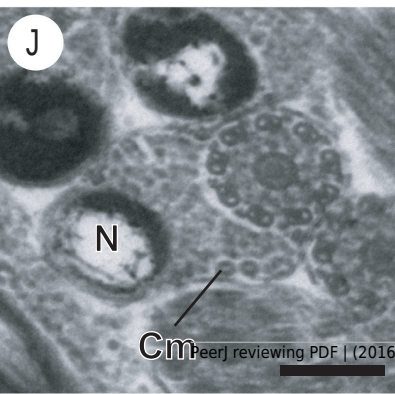
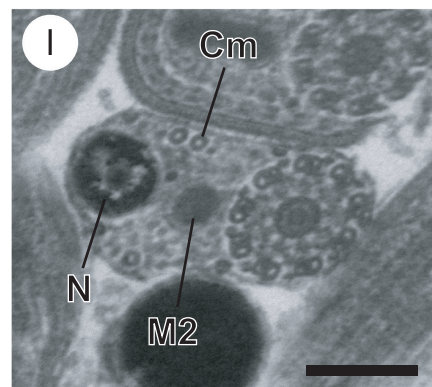
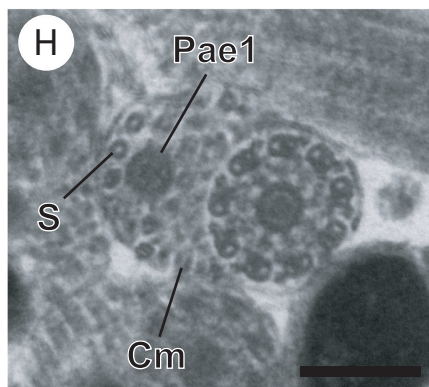
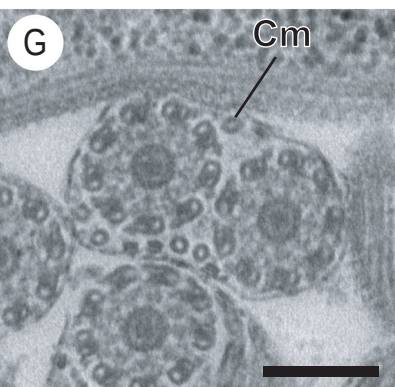
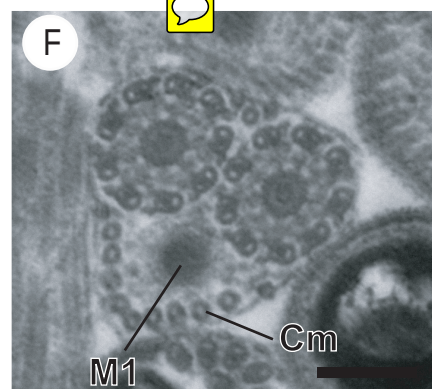
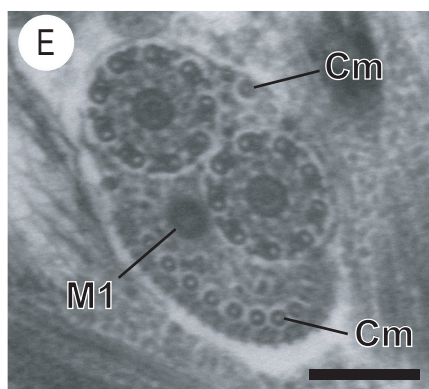
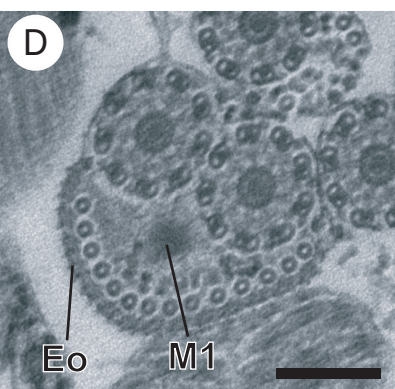
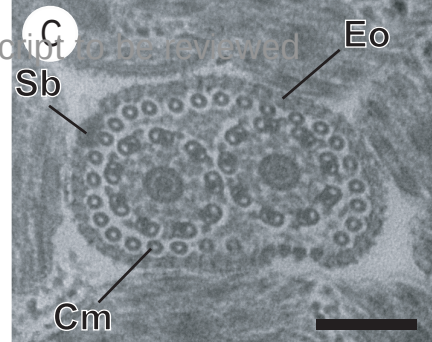
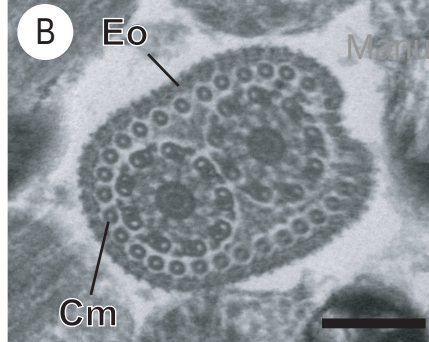
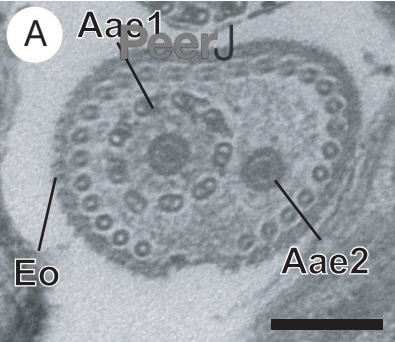


Figure 2 (on next page)

Regions I–III of mature spermatozoon of *Paramonorcheides selaris* observed in TEM.

(A–C) Longitudinal and cross-sections of region I corresponding to the anterior spermatozoon extremity; **(D)** longitudinal section in the ornamented area showing the spine-like body interrupting the external ornamentation of the plasma membrane; **(E)** cross-section in the posterior part of the region I characterized by the appearance of the first mitochondrion; **(F and G)** consecutive cross-sections in region II or transitional area showing only the axonemes, the first mitochondrion and a decrease of the cortical microtubules number; **(H)** anterior part of region III exhibiting the second mitochondrion; **(I and J)** cross-sections of middle part of region III showing the appearance of the nucleus. Note the disorganisation of the first axoneme (J). Scale bars: 0.2 μm . Aae1: anterior extremity of first axoneme; Aae2: anterior extremity of second axoneme; Ase: anterior spermatozoon extremity; Ax1: first axoneme; Ax2: second axoneme; Cm: cortical microtubules; Eo: external ornamentation of the plasma membrane; M1: first mitochondrion; M2: second mitochondrion; N: nucleus; Pae1: posterior extremity of axoneme 1;  spine-like body.

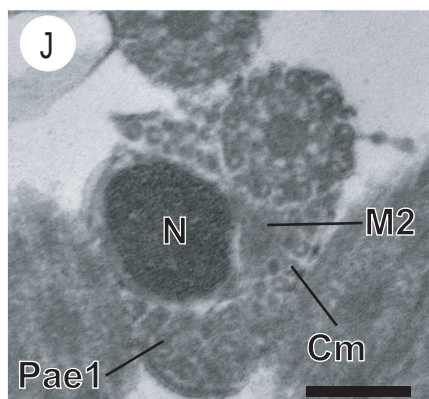
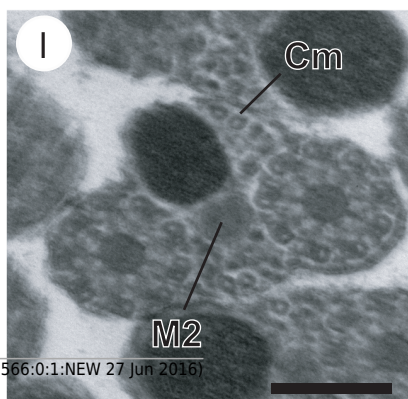
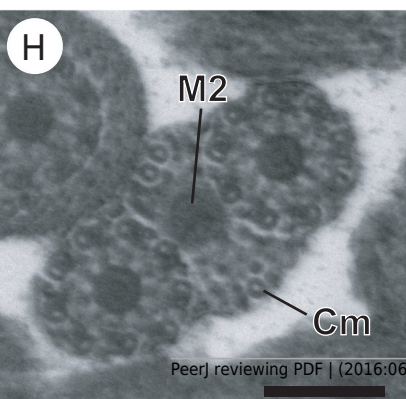
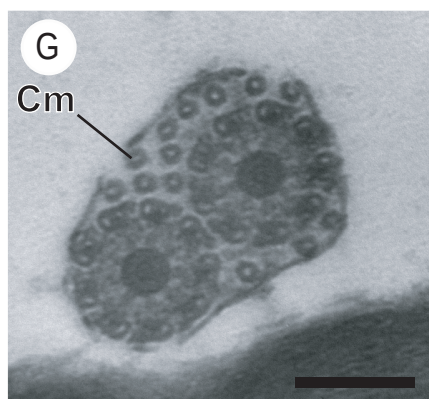
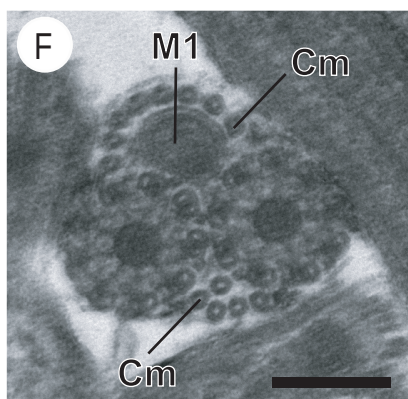
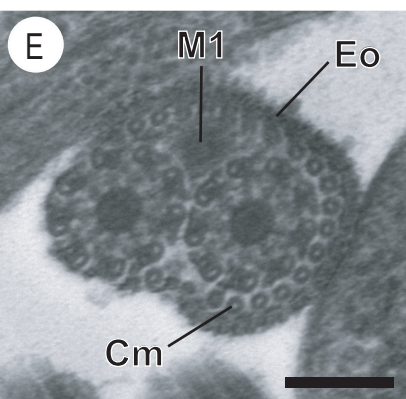
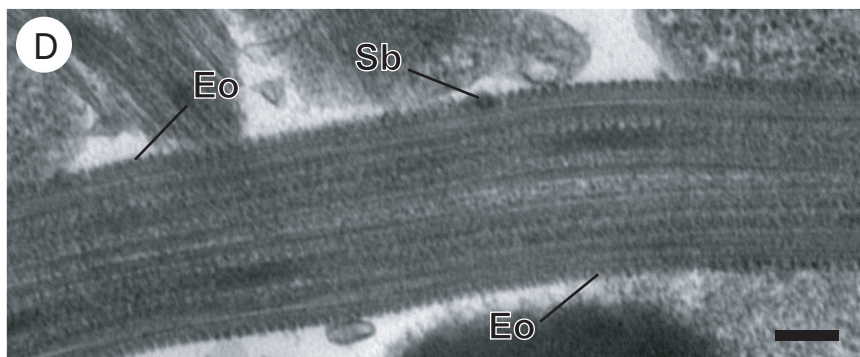
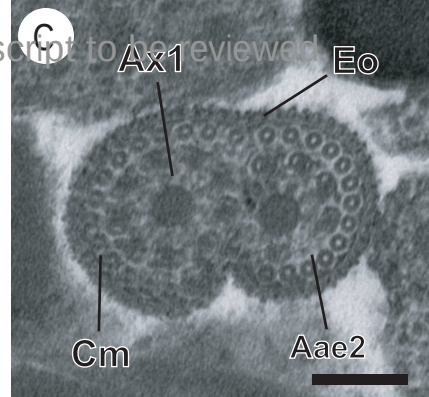
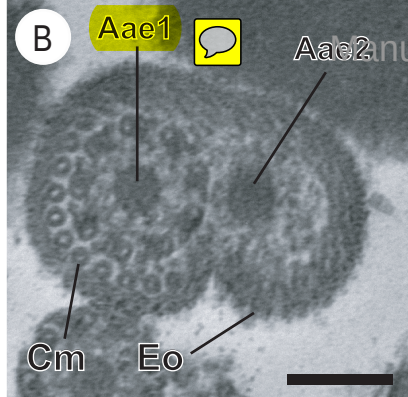
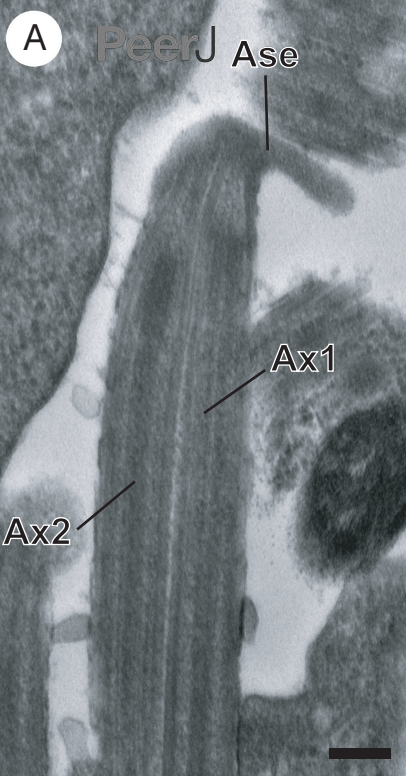


Figure 3(on next page)

Cross-section of regions III and IV of the mature spermatozoon of *Paramonorcheides selaris* observed in TEM.

(A) Posterior part of the region III characterized by the nucleus, the second axoneme, the second mitochondrion and cortical microtubules; **(B and C)** region IV characterized by the nucleus, the second axoneme which disorganizes progressively, and the posterior extremity of the last cortical microtubules; **(D)** posterior part of region IV containing only the nucleus; **(E)** positive test of Thiéry for labelling of glycogen. Scale bars: 0.2 μm (A–D), 0.5 μm (E). Cm: cortical microtubules; D: doublets of microtubule; Eo: external ornamentation of the plasma membrane; G: granules of glycogen; M2: second mitochondrion; N: nucleus.

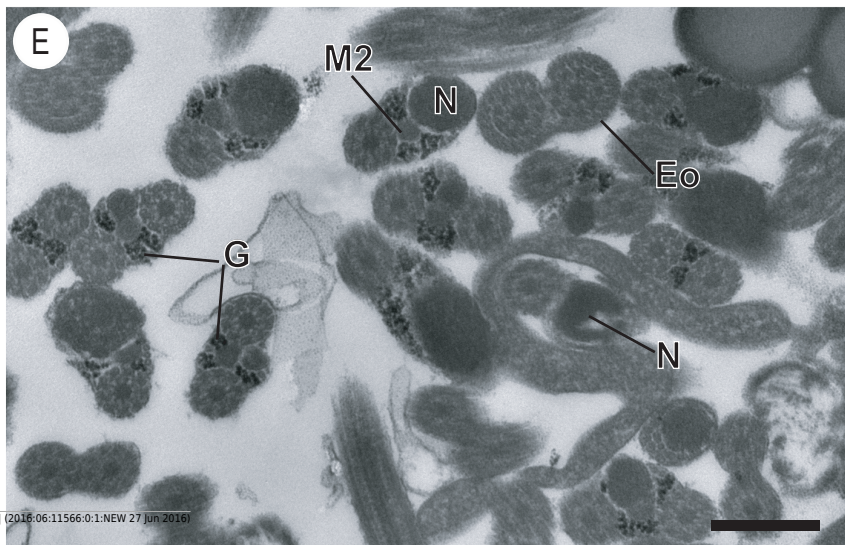
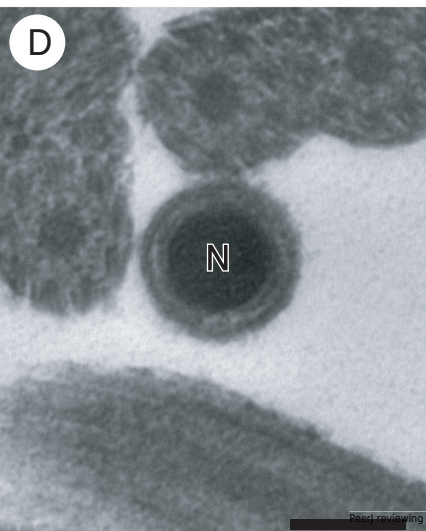
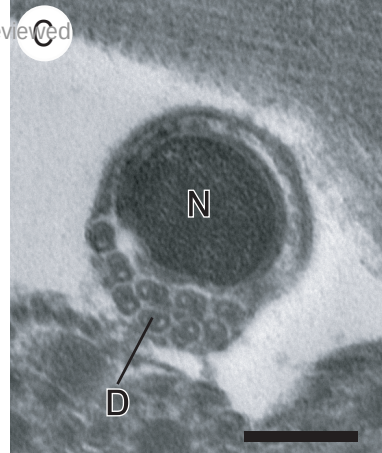
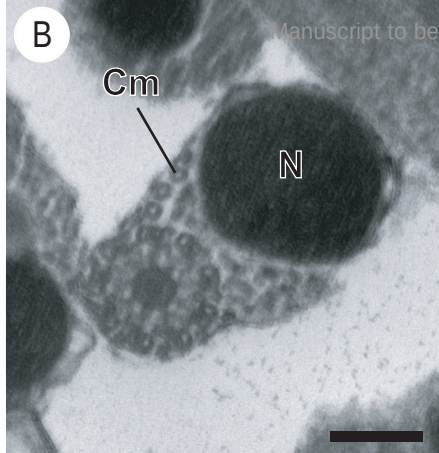
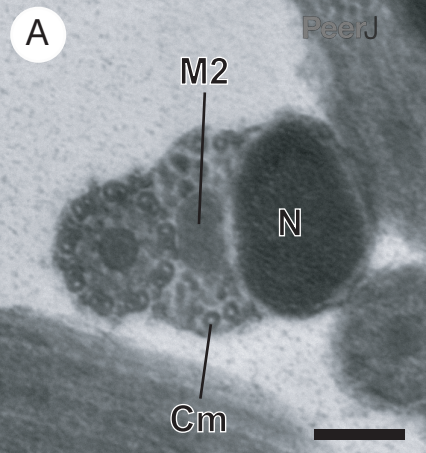


Figure 4 (on next page)

Comparative schematic reconstruction of the mature spermatozoa of *Opisthomonorchis dinema* (a) and *Paramonorchoides selaris* (b).

Aae1: anterior extremity of first axoneme; Aae2: anterior extremity of second axoneme; Ase: anterior spermatozoon extremity; Ax1: first axoneme; Ax2: second axoneme; Az: attachment zone; Cm: cortical microtubules; Eo: external ornamentation of the plasma membrane; M1: first mitochondrion; M2: second mitochondrion; N: nucleus; Pae1: posterior extremity of axoneme 1; Pae2: posterior extremity of axoneme 2; Pm: plasma membrane; Pse: posterior spermatozoon extremity; Sb: spine-like body.



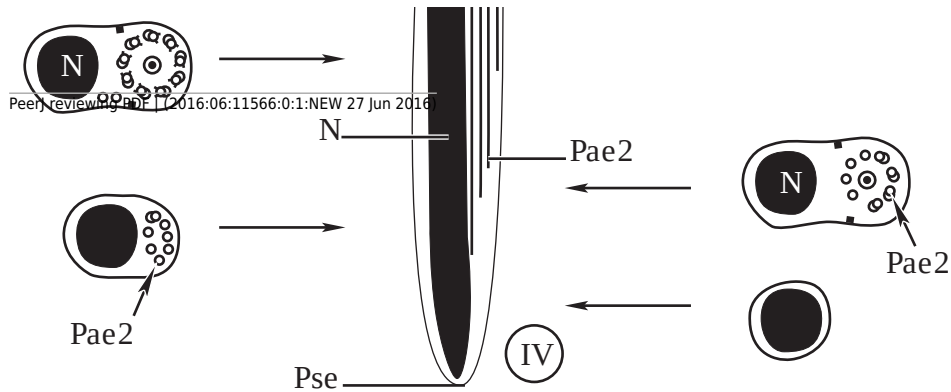
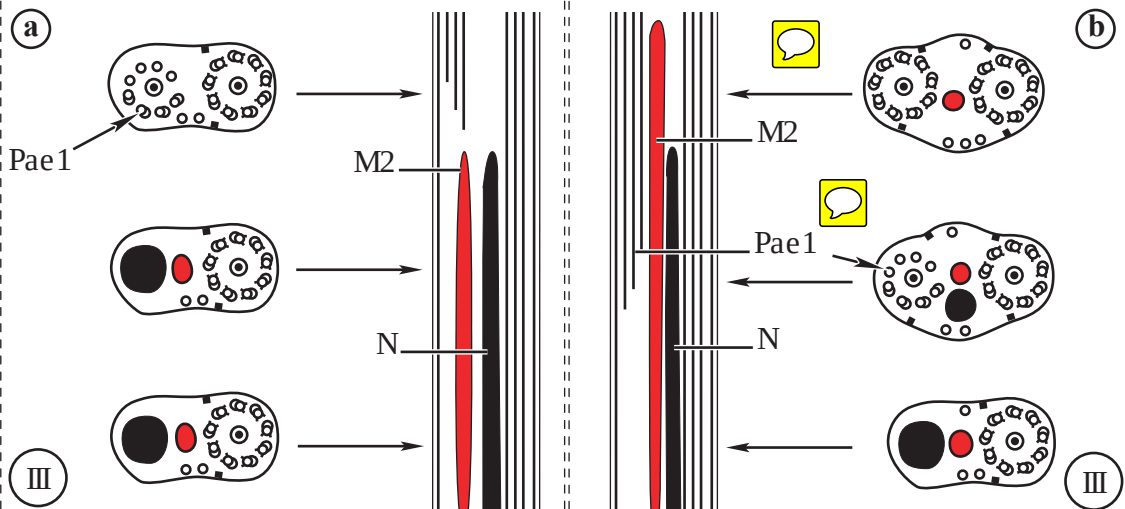


Table 1 (on next page)

Table 1: Comparative ultrastructural characteristics of the spermatozoon in the Monorchidae.

Ase, anterior spermatozoon extremity; Ax, axoneme; Cm, cortical microtubules; Edm, electron-dense material; Eo, external ornamentation of the plasma membrane; M, number of mitochondria; MCm, maximum number of cortical microtubules; N, nucleus; Tax, type of axoneme; Teo, type of external ornamentation according to Quilichini *et al.* (2011); Psc, posterior spermatozoon character; Sb, spine-like bodies; +/-, presence/absence of considered character.

1

Parasites species	Spermatological characteristics								References
	Tax	Ase	Eo	Teo	Sb	MCm	M	Psc	
<i>Helicometroides atlanticus</i>	9+“1”	2Ax+Edm	+	2	–	35	2	N	Diagne <i>et al.</i> (2015)
<i>Monorchis parvus</i>	9+“1”	2Ax+Eo	+	1	–	25	2	N	Levron <i>et al.</i> (2004a)
<i>Opisthomonorchis dinema</i>	9+“1”	2Ax+Eo	+	1	+	28	2	N	Present study
<i>Paramonorchoides selaris</i>	9+“1”	2Ax+Eo	+	1	+	26	2	N	Present study

2