

1 **Sperm ultrastructure of *Prodistomum polonii* (Digenea, Lepocreadioidea), an**
2 **intestinal parasite of horse mackerel, *Trachurus trachurus* (Teleostei, Carangidae),**
3 **from the Gulf of Gabes, Mediterranean Sea**
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9 **Hichem Kacem^{a, *}, Jordi Miquel^{b, c}**
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11 ^a Laboratoire de Biodiversité Marine et Environnement, Département des Sciences de la
12 Vie, Faculté des Sciences de Sfax, Université de Sfax, BP 1171, 3000 Sfax, Tunisia
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14

15 ^bSecció de Parasitologia, Departament de Biologia, Sanitat i Medi Ambient, Facultat de
16 Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, Av. Joan XXIII, sn,
17 08028 Barcelona, Spain
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22 ^cInstitut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona, Av. Diagonal,
23 645, 08028 Barcelona, Spain
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31 ***Corresponding author:**
32

33 Hichem Kacem
34

35 Laboratoire de Biodiversité Marine et Environnement, Département des Sciences de la
36 Vie, Faculté des Sciences de Sfax, Université de Sfax, BP 1171, 3000 Sfax, Tunisia
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Abstract

The present study describes the ultrastructural organization of the mature spermatozoon of the lepopocreadioid digenean *Prodistomum polonii* (Lepocreadiidae) by means of transmission electron microscopy (TEM). Live digeneans were collected from the digestive tract of the Atlantic horse mackerel *Trachurus trachurus* (Teleostei, Carangidae) captured in the coastal zone of the Mediterranean Sea, off La Chebba (Tunisia). The study reveals that the mature sperm cell of *P. polonii* is a filiform cell, which is tapered at both extremities. It exhibits the Bakhoum et al.'s type III of the digeneans spermatozoon characterized by the presence of: two 9+'1' axonemes, external ornamentation of the plasma membrane associated with cortical microtubules and located in the posterior part of the anterior region, two bundles of parallel cortical microtubules with their maximum number (15 microtubules) located in the middle part, and the presence of two mitochondria (one of them of moniliform type). According to the location of the external ornamentation, the male gamete of *P. polonii* presents a Quilichini et al.'s type 2 spermatozoon. Moreover, in the anterior spermatozoon extremity, there is a discontinuous and submembranous layer of electron-dense material. Whereas, the posterior spermatozoon extremity belongs to the Quilichini et al.'s cryptogonimid type. Our results are compared with the available data on digenean spermatology, in particular with species belonging to the superfamily Lepocreadioidea to highlight the potential criteria useful for phylogeny.

Keywords: *Prodistomum polonii*; Lepocreadiidae; Lepocreadioidea; Digenea; ultrastructure; sperm characters.

1. Introduction

The superfamily Lepocreadioidea Odhner (1905) is biologically of considerable interest as it comprises important groups of worms in a range of marine habitats (Abdel-Gaber et al. 2015). It comprises 10 families and 137 genera, inclusive lepecreadiids that parasite marine teleosts in both cold and warm waters (Bray 2005). The Lepocreadioidea is one of the complex and problematic digenean superfamilies (Toledo and Fried 2014). It is considered monophyletic and comprises six well-supported clades at the family rank namely: the Lepocreadiidae, Aephnidiogenidae, Lepidapedidae, Enenteridae, Gorgocephalidae, and Gyliuchenidae. Three of them had previously been considered as subfamilies of Lepocreadiidae (Bray 2005; Bray et al. 2009; Bray and Cribb 2012). Additionally, a new family, Gibsonivermidae (Bray et al. 2018), has been proposed. Moreover, according to Bray and Cribb (2012), the families Acanthocolpidae, Apocreadiidae and Brachycladiidae are now considered as less closely related to the Lepocreadiidae and should be placed outside the Lepocreadioidea.

Due to these uncertainties and the shortage of robustness in the existing classification of the Lepocreadioidea, ultrastructural studies of species belonging to this superfamily are of great importance for a better knowledge of relationships among these digeneans, since they provide additional information that could complement molecular and morphological data (Justine 1991, 1995, 2001; Quilichini et al. 2010a, 2011; Bakhoun et al. 2017a). To date, ultrastructural studies of the spermatozoon have been carried out for eleven lepecreadioidean species: two Aephnidiogenidae, *Aephnidiogenes senegalensis* and *Holorchis micracanthum* (Bâ et al. 2011, 2018); one Apocreadiidae, *Neoapocreadium chabaudi* (Kacem et al. 2010); one Deropristidae *Deropristis inflata* (Foata et al. 2007); two Gyliuchenidae, *Gyliuchen* sp. and *Robphildollfusium fractum* (Quilichini et al. 2011; Bakhoun et al. 2012); and five Lepocreadiidae: *Bianium*

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arabicum, *Bianium plicatum*, *Hypocreadium caputvadum*, *Neomultitestis aspidogastriformis* and *Opechona bacillaris* (Kacem et al. 2012; Bakhoum et al. 2015a; Ndiaye et al. 2015; Quilichini et al. 2015). The present study describes, for the first time, the mature spermatozoa of *Prodistomum polonii* (Molin, 1859) increasing the available data to the family Lepocreadiidae.

2. Materials and methods

Live adult specimens of *P. polonii* were gathered live in March 2019 from the digestive tract of the Atlantic horse mackerel *Trachurus trachurus* (Linnaeus, 1758) (Pisces, Teleostei, Carangidae) caught in the Mediterranean Sea, off La Chebba (34°14'N, 11°06'E) (Tunisia).

After their extraction, live 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 a 0.1 M sodium cacodylate buffer at pH 7.4. They were then postfixed in cold (4 °C) 1% osmium tetroxide with 0.9% potassium ferricyanide in the same buffer for 1 h, rinsed in MilliQ water (Millipore Gradient A10), dehydrated in an ethanol series and propylene oxide, embedded in Spurr resin, and finally polymerized at 60 °C for 72 h. Ultrathin sections were obtained using a Reichert-Jung Ultracut-E ultramicrotome, placed on copper grids, and double-stained with uranyl acetate and lead citrate according to Reynolds (1963). Finally, all stained grids were studied with a JEOL 1010 transmission electron microscope operated at 80 kV in the 'Centres Científics i Tecnològics' of the University of Barcelona (CCiTUB).

The Thiéry (1967) technique was used for cytochemical detection of glycogen at an ultrastructural level. Gold grids were treated 30 min in 10% periodic acid and rinsed in

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MilliQ water; 24 h in thiocarbohydrazide and rinsed in acetic solutions and MilliQ water; then 30 min in 1% silver proteinate in the dark, and rinsed in MilliQ water.

3. Results

The observation and interpretation of numerous cross- and longitudinal sections of the mature spermatozoon of *P. polonii* allow us to distinguish three different regions (I–III) from the anterior to the posterior spermatozoon extremities (see Figs. 1 to 3).

Region I (Figs. 1a-k and 3I) corresponds to the anterior spermatozoon extremity. The anterior tip forms a sharp point (Fig. 1a, b). Singlets and doublets of the first axoneme appear progressively to become the first axoneme (Fig. 1b-d). When the first axoneme of the 9+1' trepaxonematan pattern is already formed, an anterior electron-dense material that partly surrounds the central core of the second axoneme is observed (Fig. 1d). The first mitochondrion also appears in this part of the sperm cell (Fig. 1d). It is a moniliform mitochondrion, composed of a mitochondrial cord with joined mitochondrial bulges (Fig. 1d-j). In this area, the parallel cortical microtubules appear and progressively increase in number (Fig. 1e, f, i, j). In the middle part of region I, an external ornamentation of the plasma membrane appears in the mitochondrial side of the sperm cell associated with cortical microtubules (Fig. 1i, j). In the posterior part of region I, the external ornamentation disappears and, thus, the spermatozoon has the two axonemes, cortical microtubules, granules of glycogen and the first mitochondrion (Fig. 1k). The maximum number of cortical microtubules arranged into two fields is of 2+13 (Fig. 1i–j). Moreover, the presence of granules of glycogen progressively increases along region I (Fig. 1f-j). The transition toward region II is marked by the disappearance of the first mitochondrion (Fig. 1k).

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Region II (Figs. 11, m, 2a and 3II), which is the mid-region of the spermatozoon is mainly characterized by the above-mentioned absence of the first mitochondrion and external ornamentation of the plasma membrane (Figs. 11, m and 2a) and by the presence of the second mitochondrion in its posterior part (Figs. 1m and 2a). Thus, the anterior part of this region shows a simultaneous presence of both axonemes, granules of glycogen and two bundles of about 2+5 or 3+5 cortical microtubules (Fig. 11). In the posterior part of this region, the second mitochondrion appears and the two bundles of cortical microtubules are constituted by about 6+6 elements (Fig. 2a).

Region III (Figs. 2b-j and 3III) corresponds to the nuclear and posterior spermatozoon extremity. It begins with the simultaneous presence of the posterior part of the second mitochondrion, two axonemes, nucleus, granules of glycogen and cortical microtubules whose number progressively decreases till finally disappears (7+5; 3+3; 3+0; 0+0 –Fig. 2b-e). In the middle part, the mitochondrion disappears and the sperm cell contains two axonemes, a nucleus and granules of glycogen (Fig. 2f). The transition of characters towards the posterior tip of the spermatozoon is as follows: (i) disorganization of the first axoneme, (ii) disappearance of the nucleus, (iii) disappearance of the first axoneme, and (iv) disorganization of the second axoneme (see the transition in Fig. 2g-j). Therefore, the posterior spermatozoon tip is characterised by the presence of only granules of glycogen (Fig. 2j).

The glycogenic nature of the electron-dense granules observed along the mature spermatozoon (Figs. 1f-l and 2a-j) has been specifically evidenced by applying the Thiéry's test (Fig. 2k).

4. Discussion

1 The mature spermatozoa of *P. polonii* share the common ultrastructural features
2 described in the majority of digeneans. Some of these features are considered as
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4 potentially significant for phylogenetic inference. Because of this, Bakhoum et al.
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6 (2017a) analysed and regrouped characters into principal and secondary sets, and used
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8 the principal set to establish five different models of sperm cells in the Digenea.
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10 However, the usefulness of the secondary characters remains ambiguous because of
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12 they may be considered as homoplastic features. These characters have recently been
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14 described or vary within the types of spermatozoa but also within some digenean taxa
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16 (Bakhoum et al. 2017a). The mature spermatozoon of *P. polonii* presents an
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18 ultrastructural organization following the sperm cell's type III of Bakhoum et al.
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20 (2017a). Thus, the male gamete is characterized by the presence of two axonemes with
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22 the 9+'1' pattern of the Trepaxonemata (Ehlers 1984) as all digeneans with the exception
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24 of schistosomes with a special 9+'1' pattern (Justine et al. 1993; Jamieson and Justine
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26 2017) and *Didymozoon* with a 9+0 pattern (Justine and Mattei 1983). Other present
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28 features in the type III of digenean spermatozoon are two bundles of parallel cortical
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30 microtubules, external ornamentation associated with cortical microtubules which are
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32 located in the posterior part of the anterior region of the sperm cell, two mitochondria
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34 and the maximum number of cortical microtubules located in the middle part of the
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36 spermatozoon. The latter has been described in the mature spermatozoon of digeneans
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38 belonging to the superfamilies Haploporoidea, Opecoeloidea, Opisthorchioidea and
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40 Lepocreadioidea in which certain species such as *D. inflata* (Foata et al. 2007) or *H.*
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42 *caputvadum* (Kacem et al. 2012) present the type IV. Additionally, there are several
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44 specific features with great variability mainly located in both anterior and posterior
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46 extremities of the spermatozoon of *P. polonii* which might be suitable for taxonomic
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1 and/or phylogenetic considerations (Quilichini et al. 2010a, 2011; Bakhoum et al.
2 2017a).

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4 The anterior part of the mature spermatozoon exhibits an electron-dense material. It
5 appears as a submembranous layer located on the opposite side of the first axoneme and
6 disappears when the second axoneme is already present. This organization has been
7 described in all the Lepocreadioidea studied so far, except in apocreadiids and
8 deropristids (Foata et al. 2007; Kacem et al. 2010). Regarding other digeneans, the
9 anterior electron-dense material has been reported particularly in the Atractotrematidae
10 (Bakhoum et al. 2015b), the Opecoelidae (see Kacem et al. 2019a; Bâ et al. 2020a) or
11 the Cryptogonimidae (see Kacem and Miquel 2019). However, it is important to stress
12 that the morphology of the anterior dense material is variable depending on species.
13 Indeed, in *P. polonii* it appears as a discontinuous submembraneous layer of an
14 electron-dense material on the opposite side of the first axoneme beneath the plasma
15 membrane. However, in the mature spermatozoa of *B. plicatum* and *B. arabicum*
16 (Quilichini et al. 2015), it appears as a mass of electron-dense material placed in a
17 cytoplasmic expansion.

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19 Another character found in *P. polonii* is the presence of parallel cortical
20 microtubules as occur in a majority of digeneans, with the exception of *Didymocystis*
21 and *Didymozoon* species (Justine and Mattei 1983; Pamplona-Basilio et al. 2001). Their
22 disposition, the location of their maximum number, and the number of bundles are
23 variable depending on the species and considered to be phylogenetically significant (see
24 Bakhoum et al. 2017a). In the mature sperm of parasitic flatworms such as digeneans
25 these submembranous ultrastructural elements are present as parallel tubular structures
26 underlying the plasma membrane. However, in more advanced cestodes, such as
27 tetrabothriideans and cyclophyllideans (excluding mesocestoidids), cortical
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1 microtubules have a spiralled disposition (Stoitsova et al. 1995; Miquel et al. 1999;
2 Levron et al. 2010). Among the majority of digeneans, the cortical microtubules are
3 usually arranged into two bundles in the sperm cell, as occurs in *P. polonii* and in the
4 remaining studied lepecreadioideans (see Table 1). However, they are arranged into a
5 single field for the species of the Hemiuridae, the lecithasterid *Aponurus laguncula*, the
6 sclerodistomid *Sclerodistomum italicum*, the sclerodistomoidid *Sclerodistomoides*
7 *pacificus* and the faustulid *Pronoprymna ventricosa* (Quilichini et al. 2007a, 2010b;
8 Ndiaye et al. 2017; Bâ et al. 2020b; Kacem et al. 2020). In the Sclerodistomidae there
9 are two studied species and only the above-mentioned *S. italicum* presents a single field
10 of cortical microtubules in their sperm cells, whereas *Prosorchis palinurichthi* has two
11 bundles (Ndiaye et al. 2013). Another aspect related to cortical microtubules that
12 presents great phylogenetic interest is the location of the maximum number of these
13 elements along the spermatozoon. In the light of this last character, Quilichini et al.
14 (2007b) and posteriorly Bakhom et al. (2017a) proposed that the spermatozoon of
15 digenean parasites could be divided into two groups: (i) first one in which the highest
16 number of cortical microtubules is located in the anterior part of the spermatozoon and
17 (ii) a second one with the maximum number located in the middle or more posterior part
18 of the spermatozoon. In *P. polonii*, as in all lepecreadiids studied so far, the maximum
19 number of cortical microtubules is located in the middle region of the sperm cell, with
20 the exception of *H. caputvadum* (Kacem et al. 2012), which exhibits its maximum
21 number in the anterior part of the spermatozoon (see Table 1). Therefore, *P. polonii* can
22 be classified in the Quilichini et al.'s type 2 of sperm cell.

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Spine-like bodies and external ornamentation are other structures in digenean
spermatozoa that can be useful to understand phylogenetic relationships and are
probably involved in the fertilization process (Justine and Mattei 1984; Miquel et al.

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2013). Spine-like bodies were first described by Miquel et al. (2000) from *Opecoeloides*
furcatus, and since then these submembranous electron-dense structures have frequently
been reported in mature spermatozoa of digenean species, inclusive species of
superfamily Lepocreadioidea (Table 1). However, *P. polonii* lacks these spine-like
bodies. This absence has also been reported for the aepnidiogenids *A. senegalensis* and
H. micracanthum, the deropristid *D. inflata* and the lepocreadiid *H. caputvadum* (Foata
et al. 2007; Bâ et al. 2011, 2018; Kacem et al. 2012 –see Table 1).

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The external ornamentation of the plasma membrane has been described in the
male gamete of numerous digeneans. The presence of external ornamentation as well as
its appearance, location and eventual association with the cortical microtubules are all
features of phylogenetic interest (see Quilichini et al. 2007a, 2011; Bakhoum et al.
2017a). Regarding the location of the external ornamentation along the spermatozoon,
Quilichini et al. (2011) suggested that digenean spermatozoa can be classified into three
types: (i) type 1 presents external ornamentation in the anterior extremity of the
spermatozoon; (ii) type 2 presents ornamentation at a more posterior level; and (iii) type
3 lacks external ornamentation. Based on all these criteria, *P. polonii* belongs to
Quilichini et al.'s type 2 spermatozoon. Among lepocreadioids, only the male gamete of
N. chabaudi (Kacem et al. 2010) differs from this type, belonging to Quilichini et al.'s
type 1. On the other hand, the association of the external ornamentation with cortical
microtubules is a commonly observed feature in the mature spermatozoon of all the
Lepocreadioidea studied so far (see Table 1).

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The presence of mitochondria in the spermatozoa represents a plesiomorphic trait
for parasitic Platyhelminthes (Justine 1991). In the Digenea, all studied species contain
one, two or three mitochondria in their male gametes (see Bakhoum et al. 2017a). The
number, the morphology and the location of the mitochondrion/a vary depending on the

1 species, and have been considered as potentially informative for phylogenetic inference.
2 Concerning the number of mitochondria, in the Lepocreadioidea the majority of species
3 present two mitochondria in their spermatozoa (see Table 1). Only sperm cells of two
4 species namely *H. micracanthum* and *GyLIAUCHEN* sp. differ from this situation
5 presenting only one mitochondrion (Bâ et al. 2011; Quilichini et al. 2011). In general,
6 when the sperm cell contains two mitochondria, the first is placed in the anterior part
7 and the second in the posterior part. However, in *Dicrocoelium hospes*, both
8 mitochondria are located in a parallel disposition in the anterior part of the
9 spermatozoon (Agostini et al. 2005) and in *HaploSplanchnus caudatus* the first
10 mitochondrion appears in the anterior end of the spermatozoon before the complete
11 formation of both axonemes (Kacem et al. 2019b).

12 The first mitochondrion of *P. polonii* is moniliform and composed of a
13 mitochondrial cord with joined mitochondrial bulges. This shape of mitochondria was
14 first described from *H. micracanthum* by Bâ et al. (2011), and posteriorly reported in
15 the male gamete of some digeneans such as the Acanthocolpidae *Stephanostomoides*
16 *tenuis* (Bakhoun et al. 2015c), the Cryptogonimidae *Aphallus tubarium* and *Timoniella*
17 *imbutiforme* (Foata et al. 2012; Kacem et al. 2017a), the Opecoelidae *Allopodocotyle*
18 *pedicellata* and *Macvicaria obovata* (Bakhoun et al. 2017b; Kacem et al. 2017b), the
19 Plagiorchiidae *Enodiotrema reductum* (Ndiaye et al. 2012), and the Sclerodistomoididae
20 *S. pacificus* (Bâ et al. 2020b). Concerning the Lepocreadioidea, this type of
21 mitochondrion has been described in two species, the above-mentioned *H.*
22 *micracanthum* and *Opechona bacillaris* (Bâ et al. 2011; Ndiaye et al. 2015).
23 Additionally, Kacem et al. (2019a) have recently described, for the first time, another
24 morphological type of the first mitochondrion in *Allopodocotyle tunisiensis*

1 characterized by the presence of a circular fold in its posterior part making a
2 characteristic U-shaped posterior extremity.
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4 The posterior spermatozoon extremity is, just as the anterior tip, morphologically
5 diverse in digenean male gametes. This fact emphasizes its usefulness in the
6 establishment of spermatozoon models. Quilichini et al. (2010a) proposed three
7 different posterior spermatozoon extremities considering the sequence of the characters'
8 disappearance towards the posterior tip. Type 1 (also called opecoelidean type) is
9 characterized by the sequence 'axoneme, nucleus and cortical microtubules'. Type 2
10 (also called fasciolidean type) shows the sequence 'cortical microtubules, axoneme and
11 nucleus', whereas the sequence for type 3 (also called cryptogonimidean type) is
12 'corticalmicrotubules, nucleus and axoneme'. Nevertheless, some posterior
13 spermatozoon morphologies are not congruent with these three models, i.e. *D. inflata*
14 (Foata et al. 2007); *Scaphiostomum palaearticum* (Ndiaye et al. 2002); *A. laguncula*
15 (Quilichini et al. 2010b); and *H. caudatus* (Kacem et al. 2019b). This is the principal
16 reason argued by Bakhoum et al. (2017a) to scrutinize just the last spermatozoon
17 character instead of the sequence of characters. The present study shows that *P. polonii*
18 presents the second axoneme as the terminal character of the male gamete, as occurs in
19 all the studied leprocreadioideans, except *B. arabicum*, *B. plicatum*, *N. chabaudi*, *N.*
20 *aspidogastriformis* and *R. fractum*, which exhibit the nucleus as the posterior sperm
21 character (Kacem et al. 2010; Bakhoum et al. 2012, 2015a; Quilichini et al. 2015).
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51 **Compliance with ethical standards**

52 Conflict of interest: The authors declare that they have no conflict of interest.
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Fig. 1. Mature spermatozoon of *Prodistomum polonii*. (a, b) Longitudinal and cross-section of the anterior spermatozoon extremity. (c-f) Consecutive cross-sections of region I showing the progressive appearance of both axonemes. Note the location of the anterior electron-dense material as a submembranous layer in the opposite side of the first axoneme and the appearance of cortical microtubules. (g-j) Longitudinal and cross-sections of the ornamented area of region I showing the first mitochondrion of moliliform type, formed by a cord with several bulges. (k) Cross-section showing the absence of external ornamentation in the posterior part of region I (arrowheads). (l, m) Cross-sections of the anterior and posterior part of region II. Note the appearance of the second mitochondrion in the posterior part of this region. ADM, anterior electron-dense material; ASE, anterior spermatozoon extremity; Ax, axoneme; Ax1, Ax2, first and second axoneme; CC2, central core of the second axoneme; CM, cortical microtubules; D1, doublets of the first axoneme; EO, external ornamentation of the plasma membrane; G, granules of glycogen; M1, first mitochondrion; mB, mitochondrial bulge; mC, mitochondrial cord; S1, singlets of the first axoneme. Scale bars: 300 nm.

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Fig. 2. Mature spermatozoon of *Prodistomum polonii*. (a) Cross-section of the posterior part of region II showing the second mitochondrion. (b-e) Correlative cross-sections of the anterior part of region III with the simultaneous presence of the second mitochondrion and the nucleus. Note the progressive reduction in the number of cortical microtubules. (f-j) Consecutive cross-sections showing the disappearance of characters toward the posterior spermatozoon tip. (k) Cytochemical test of Thiéry evidencing glycogen at ultrastructural level. Ax2, second axoneme; CM, cortical microtubules; D1, D2, doublets of the first and the second axoneme; EO, external ornamentation of the plasma membrane; G, granules of glycogen; M1, M2, first and second mitochondrion; N, nucleus; G, granules of glycogen; N, nucleus. Scale bars: 300 nm.

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Fig. 3. Schematic drawing showing the ultrastructural organization of the mature spermatozoon of *Prodistomum polonii*. The sperm cell is organized in three different regions: region I or anterior part, region II or middle part and region III or posterior part. In order to make the diagram clearer, granules of glycogen are not shown in longitudinal sections. ADM, anterior electron-dense material; ASE, anterior spermatozoon extremity; Ax1, Ax2, first and second axoneme; CC2, central core of the second axoneme; CM, cortical microtubules; D1, D2, doublets of the first and second axoneme; EO, external ornamentation of the plasma membrane; G, granules of glycogen; M1, M2, first and second mitochondrion; mB, mitochondrial bulge; mC, mitochondrial cord; N, nucleus; PM, plasma membrane; PSE, posterior spermatozoon extremity.

Table 1

Table 1. Spermatological characteristics in the superfamily Lepocreadioidea

Families and species (References)	Spermatozoon characteristics															
	TAx	ASC	LE	TAR	EO	EO+CM	LEO	BCM	MCM	LMCM	M	ADM	SB	TPR	PSC	TS
Aephnidiogenidae																
<i>Aephnidiogenes senegalensis</i> (Bâ et al. 2018)	9+'1'	1Ax-ADM	-	2	+	+	PostA	2	21	MedS	2	+	-	3	Ax	III
<i>Holorchis micracanthum</i> (Bâ et al. 2011)	9+'1'	1Ax-ADM	+?	2	+	+	PostA	2	22	MedS	1*	+	-	3	Ax	III
Apocreadiidae																
<i>Neoapocreadium chabaudi</i> (Kacem et al. 2010)	9+'1'	2Ax-EO-CM?	-	1	+	+	AntA	2	18	AntS	2	-	+	2?	N?	V?
Deropristidae																
<i>Deropristis inflata</i> (Foata et al. 2007)	9+'1'	2Ax-CM?	-	2	+	+	PostA	2	28	AntS	2	-	-	3?	Ax	IV
Gyliauchenidae																
<i>Gyliauchen</i> sp. (Quilichini et al. 2011)	9+'1'	1Ax?	-	2	+	+	PostA	2	26	MedS?	1	+	+	3	Ax	III-IV?
<i>Robphildollfusium fractum</i> (Bakhoum et al. 2012)	9+'1'	1Ax?	-	2	+	+	PostA	2	20	MedS?	2	+	+	2	N	III-IV?
Lepocreadiidae																
<i>Bianium arabicum</i> (Quilichini et al. 2015a)	9+'1'	1Ax-ADM	+?	2	+	+	PostA	2	22	MedS	2	+	+	2	N	III
<i>Bianium plicatum</i> (Quilichini et al. 2015a)	9+'1'	1Ax-ADM	+?	2	+	+	PostA	2	17	MedS	2	+	+	2?	N	III
<i>Hypocreadium caputvadum</i> (Kacem et al. 2012)	9+'1'	2Ax-ADM	-	2	+	+	PostA	2	26	AntS	2	+	-	3	Ax	IV
<i>Neomultitestis aspidogastriformis</i> (Bakhoum et al. 2015)	9+'1'	1Ax-ADM	-	2	+	+	PostA	2	22?	MedS	2	+	+	2?	N	III
<i>Opechona bacillaris</i> (Ndiaye et al. 2015)	9+'1'	1Ax-ADM-M1	-	2	+	+	PostA	2	15	MedS	2(1*)	+	+	3	Ax	III
<i>Prodistomum polonii</i> (present study)	9+'1'	1Ax-ADM	-	2	+	+	PostA	2	15	MedS	2(1*)	+	-	3	Ax	III

ADM, anterior electron-dense material; AntA, anterior part of the anterior region; AntS, anterior part of the spermatozoon; ASC, anterior spermatozoon character; Ax, axoneme; BCM, number of bundles of cortical microtubules; EO, external ornamentation of the plasma membrane; EO+CM, association 'external ornamentation-cortical microtubules'; LE, lateral expansion; LEO, location of external ornamentation; LMCM, location of maximum number of cortical microtubules; M, number of mitochondria; MCM, maximum number of cortical microtubules; MedS, median part of the spermatozoon; PostS, posterior part of the spermatozoon; PSC, posterior spermatozoon character; SB, spine-like bodies; TAR, type of anterior region according to Quilichini et al. (2011); TAx, type of axoneme defined by Justine (2001); TPR, type of posterior region according to Quilichini et al. (2010a); TS, type of spermatozoon according to Bakhoum et al. (2017a); +/-, presence/absence of considered character; *, moniliform mitochondrion; ?, doubtful or unknown data.

Figure 1
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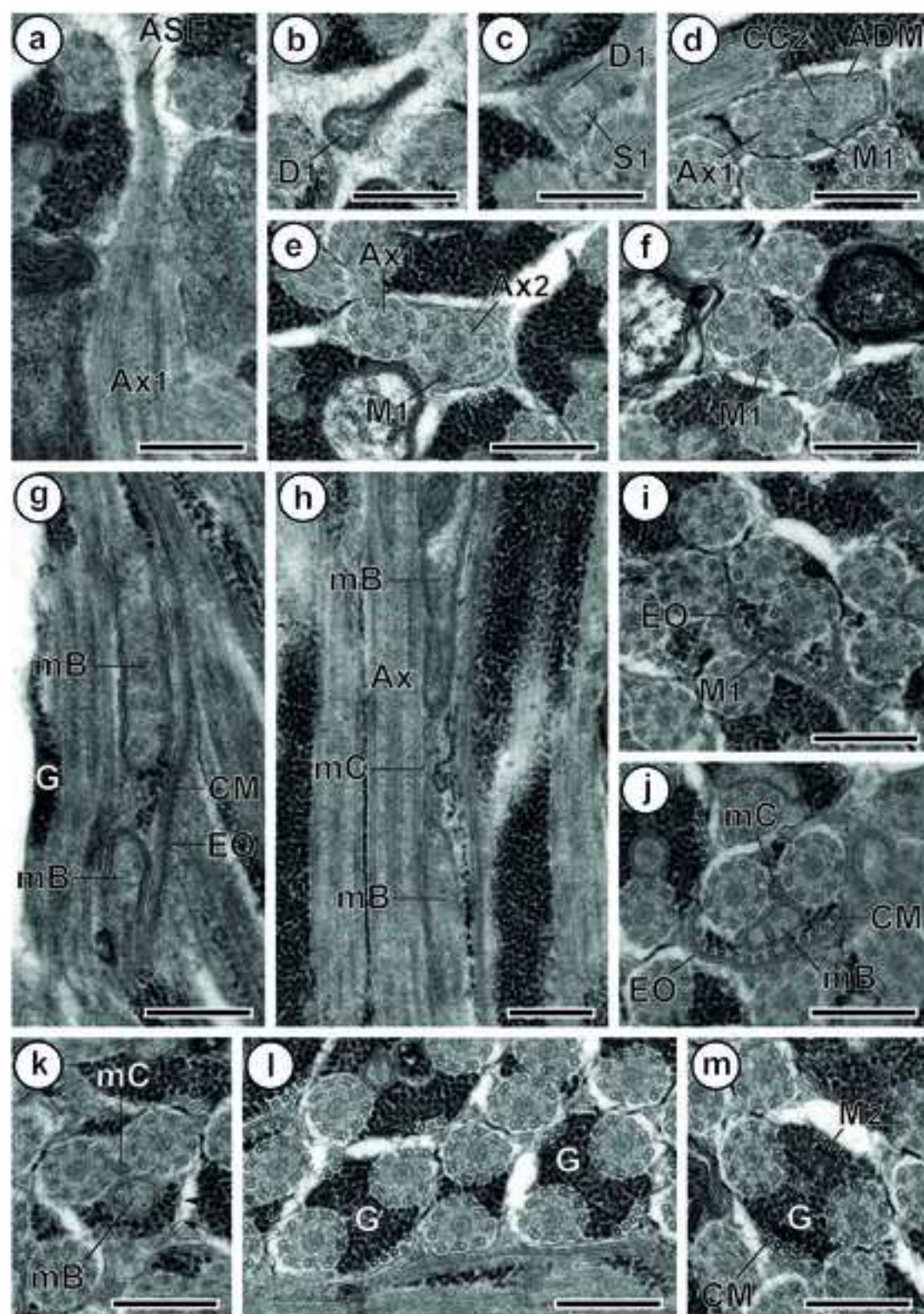


Figure 2
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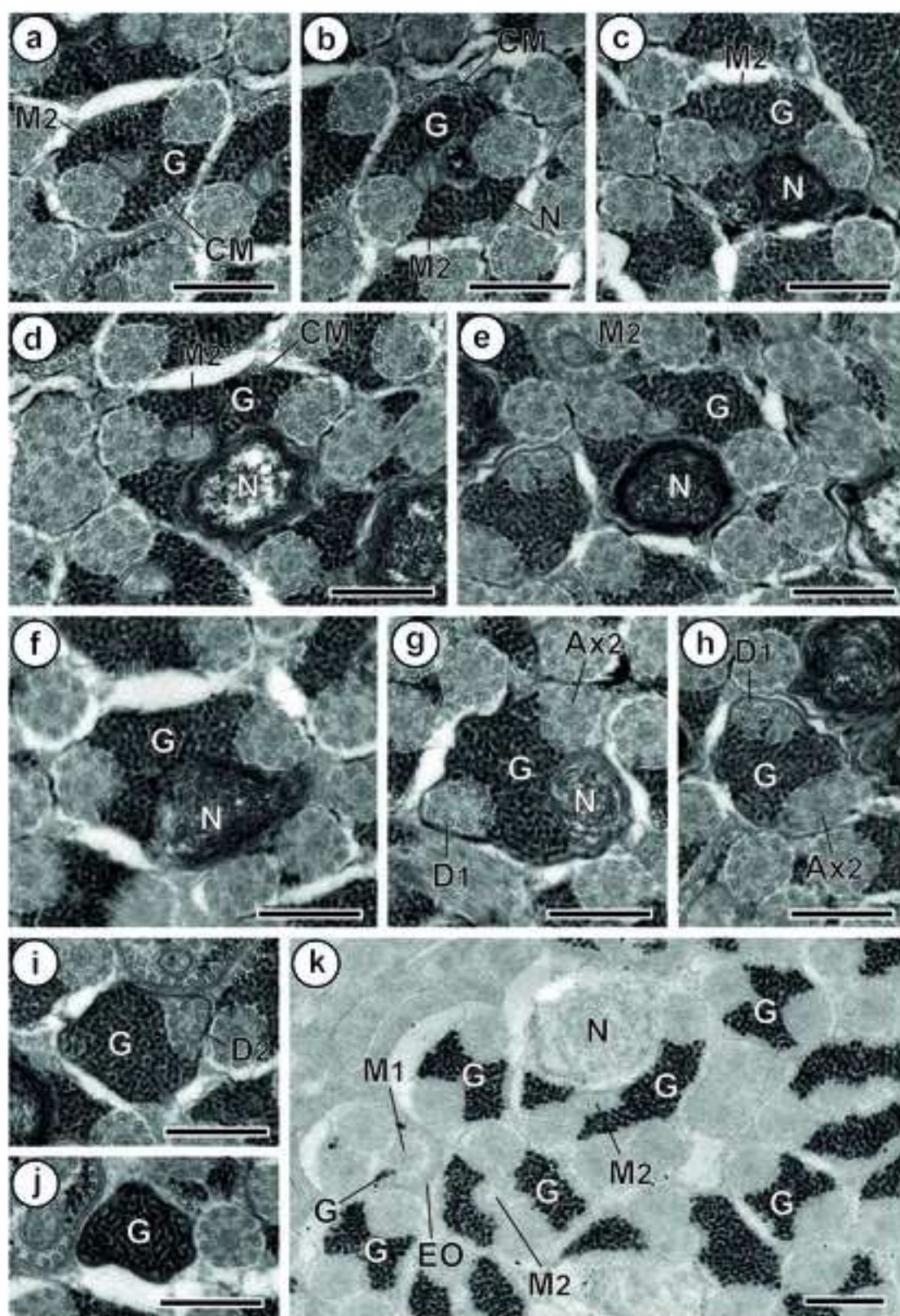


Figure 3

