

Ultrastructure of the spermatozoon of the digenean *Lecithocladium excisum* (Rudolphi, 1819) (Hemiuroidea: Hemiuridae), a parasite of marine teleosts in Senegal

Papa Ibnou Ndiaye¹, Papa Mbagnick Diagne¹, Aminata Sène¹, Abdoulaye J.S. Bakhoun^{2,3} and Jordi Miquel^{2,3}

¹Laboratory of Evolutionary Biology, Ecology and Management of Ecosystems, Faculty of Sciences and Techniques, Cheikh Anta Diop University of Dakar, BP 5055, Dakar, Senegal;

²Laboratori de Parasitologia, Departament de Microbiologia i Parasitologia Sanitàries, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII, s/n, 08028 Barcelona, Spain;

³Institut de Recerca de la Biodiversitat, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal, 645, 08028 Barcelona, Spain

Abstract: The present study describes the ultrastructure of the mature spermatozoon of *Lecithocladium excisum* (Rudolphi, 1819) (Digenea: Hemiuroidea: Hemiuridae) from the stomach of the marine teleost *Scomber japonicus* Houttuyn (Scombridae) captured in the Atlantic Ocean, off Dakar (Senegal). The ultrastructural organization of the spermatozoon of *L. excisum* follows the general model described in most digeneans. It presents two axonemes of the 9+‘1’ pattern of the Trepaxonemata, nucleus, mitochondrion and parallel cortical microtubules, among other characters. However, some particularities of the spermatozoon of *L. excisum* are (i) the presence of a membranous ornamentation not associated with cortical microtubules in its anterior extremity, (ii) the presence of a very reduced number of cortical microtubules located only in the ventral side of the spermatozoon and (iii) the absence of several structures described in most digeneans such as spine-like bodies and cytoplasmic expansions.

Keywords: *Lecithocladium excisum*, Hemiuridae, Digenea, spermatozoon, ultrastructure, *Scomber japonicus*, Scombridae, fish, Senegal

The hemiuroid trematode *Lecithocladium excisum* (Rudolphi, 1819) is the type-species of the genus and it is characterized, between other aspects, by the plications of the body surface, well-developed ecsoma, long sinus-sac and the presence of seven long and tubular vitelline lobes. The genus *Lecithocladium* Lühe, 1901 includes parasites of the stomach of marine teleost fishes (see Gibson 2002a). It is one of the four genera belonging to the subfamily Elytrophallinae Skrjabin et Guschanskaja, 1954. Within the Hemiuroidea Looss, 1899, the family Hemiuridae Looss, 1899 was erected from the subfamily Hemiurinae Looss, 1899 and, now, this family includes the Elytrophallinae and other 11 subfamilies (see Gibson 2002a,b).

Amount of data on the ultrastructure of spermatozoa of flatworms (Platyhelminthes) has accumulated considerably during last decades (for a review see Justine 2001, Levron et al. 2010). In the case of tapeworms, particularly the eucestodes, several authors have postulated different patterns of spermiogenesis and recently different types of spermatozoa (Świdorski 1986, Bâ and Marchand 1995,

Levron et al. 2010). The ultrastructural characters of the spermatozoon are known to be useful when interpreting relationships among Platyhelminthes, especially those among cestodes (Hoberg et al. 1997, 2001, Justine 1998, 2001, Olson et al. 2001, Levron et al. 2010). In fact, despite the numerous studies on digenean spermatozoon ultrastructure, to date the application of these characters for phylogenetic studies is much scarcer in digeneans than in tapeworms. Nevertheless, during the last years, several authors worked in this direction and in the near future the ultrastructural characters of the spermatozoon may become very useful for the interpretation of relationships between the Digenea (see Miquel et al. 2006, Quilichini et al. 2010a,b, 2011, Bakhoun et al. 2011a,b, in press, Ndiaye et al. 2012).

To date, there are no data on the spermatozoon ultrastructure in the family Hemiuridae. The present study on *L. excisum* is the first approach to the ultrastructural organization of the mature spermatozoon in this family. Our results are particularly compared with the available spermatological data of hemiuroideans.

MATERIALS AND METHODS

Live adult specimens of *Lecithocladium excisum* were collected during April 2011 from the stomach of two marine teleosts, the chub mackerel *Scomber japonicus* Houttuyn, 1782 (Scombridae) and the false scad *Caranx rhonchus* Geoffroy Saint-Hilaire, 1817 (Carangidae), both captured in the Atlantic Ocean, off Dakar (Senegal). Voucher specimens as whole mounts stained with Semichon's acetic carmine and mounted in Canada balsam are deposited in the parasitological collection of the Muséum National d'Histoire Naturelle (Paris) (MNHN): one slide of *L. excisum* ex *S. japonicus* (no. 11042606) off Ouakam (Dakar, Senegal), 26 April 2011 – accession number MNHN HEL253; one slide of *L. excisum* ex *C. rhonchus* (no. 11042608) off Soumbédioune (Dakar, Senegal), 26 April 2011 – accession number MNHN HEL254.

For the present TEM study, several worms collected from *S. japonicus* were 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, rinsed in 0.1 M sodium cacodylate buffer at pH 7.4, post-fixed in cold (4°C) 1% osmium tetroxide with K_4FeCn_6 in the same buffer for 1 h, rinsed in a 0.1 M sodium cacodylate buffer at pH 7.4, dehydrated in an ethanol series and propylene oxide, embedded in Spurr's resin and polymerized at 60°C for 72 h.

Ultrathin sections (60–90 nm thick) of seminal ducts and seminal vesicle were obtained using a Reichert-Jung Ultracut E ultramicrotome using a diamond knife. Sections were placed on copper and gold grids. Sections placed on copper grids were double-stained with uranyl acetate and lead citrate according to the Reynolds (1963) procedure. 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 milliQ water, 24 h in TCH, rinsed in acetic solutions and milliQ water, 30 min in 1% SP in the dark, and rinsed in milliQ water.

The grids were examined in a JEOL 1010 transmission electron microscope operated at 80 kV, in the "Centres Científics i Tecnològics de la Universitat de Barcelona (CCiTUB)".

RESULTS

The observation of a great number of sections of mature spermatozoa of *Lecithocladium excisum* enabled us to reconstitute its ultrastructural organization and to distinguish three different regions (I–III).

Region I (Figs. 1a–f, 2I) corresponds to the anterior part or premitochondrial area of the spermatozoon. The anterior spermatozoon extremity exhibits only an external ornamentation of the plasma membrane and a few singlets of the first axoneme (Fig. 1a). Soon, the first axoneme appears immediately followed by the second axoneme (Fig. 1b,c). At this level, the external ornamentation of the plasma membrane surrounds only one

of the axonemes (Fig. 1c). Areas of region I presenting both axonemes exhibit four attachment zones (Fig. 1c–e). The posterior areas of region I are characterized by an enlarged section (Fig. 1e), the appearance of a small number of cortical microtubules (four elements) located only in one side (Fig. 1e) and a scarce amount of granules of glycogen (Fig. 1f).

Region II (Figs. 1g–i, 2II) is the middle region or mitochondrial area of the spermatozoon. In addition to the structures observed in the posterior part of region I, this region presents a mitochondrion and a higher number of cortical microtubules, up to eight elements coinciding with the greatest size of the mitochondrion (Fig. 1h). Then, the number of cortical microtubules decreases progressively until reaching four elements in the transitional area to region III where the nucleus appears (Fig. 1i).

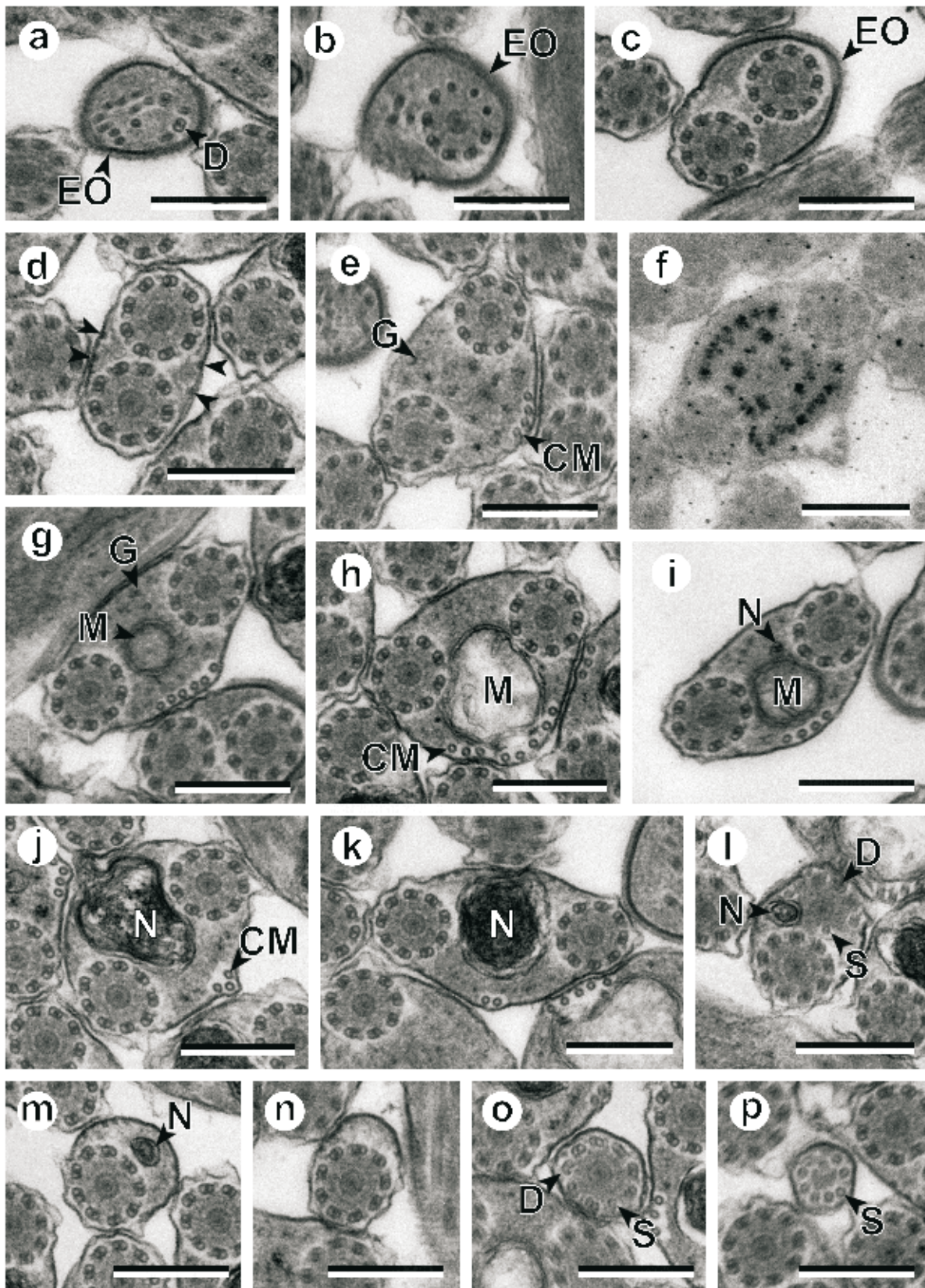
Region III (Figs. 1j–p, 2III) corresponds to the posterior part or nuclear area of the mature spermatozoon. It is characterized by the disappearance of the mitochondrion, the presence of the nucleus, and the progressive decrease of the number of cortical microtubules (Fig. 1j–m). In this region, the disorganization of the first axoneme occurs (Fig. 1l) as well as the disappearance of the nucleus (Fig. 1m,n). The posterior extremity of this region exhibits only the second axoneme (Fig. 1n) that disorganizes into doublets and singlets (Fig. 1o,p).

DISCUSSION

The mature spermatozoon of *Lecithocladium excisum* shows the basic ultrastructural features described in most digeneans so far: two axonemes of the 9+1' pattern of trepaxonematans (Ehlers 1984), nucleus, mitochondrion and parallel cortical microtubules (Burton 1972, Jamieson and Daddow 1982, Daddow and Jamieson 1983, Iomini and Justine 1997, Tang et al. 1998, Miquel et al. 2000, Levron et al. 2004a, Seck et al. 2008, Bakhoun et al. 2012, Ndiaye et al. 2012). However, the mature spermatozoon of *L. excisum* differs from those of other digeneans studied until today in several ultrastructural features, such as (i) the presence of a single field of parallel cortical microtubules, (ii) the lack of cortical microtubules in much of the anterior spermatozoon region, (iii) the presence of external ornamentations of the plasma membrane located in the anterior spermatozoon extremity, and (iv) the absence of both spine-like bodies and cytoplasmic expansions.

In the Hemiuroidea, ultrastructural studies on the spermatozoon of members of only three families have been carried out, namely the Didymozoidae Monticelli, 1888 (see Justine and Mattei 1982a, 1983, 1984, Pamplona-

Fig. 1. Consecutive cross-sections of the mature spermatozoon of *Lecithocladium excisum* from anterior to posterior extremities. **a–c** – cross-sections of the anterior extremity of the spermatozoon showing the ornamented area; **d–f** – cross-sections of the posterior part of the region I showing a progressive increase in the number of cortical microtubules placed in only one side; note the scarce amount of granules of glycogen stained by the method of Thiéry (in f); **g–i** – sections in the region II or mitochondrial area of the



spermatozoon; j–p – cross-sections of the region III or posterior part of the spermatozoon from an area containing two axonemes, nucleus and cortical microtubules to the posterior tip exhibiting only singlets of the second axoneme. *Abbreviations:* CM – cortical microtubules; D – doublets; EO – external ornamentation of the plasma membrane; G – granules of glycogen; M – mitochondrion; N – nucleus; S – singlets. Arrowheads indicate attachment zones. Scale bars: a–p = 0.3 μ m.

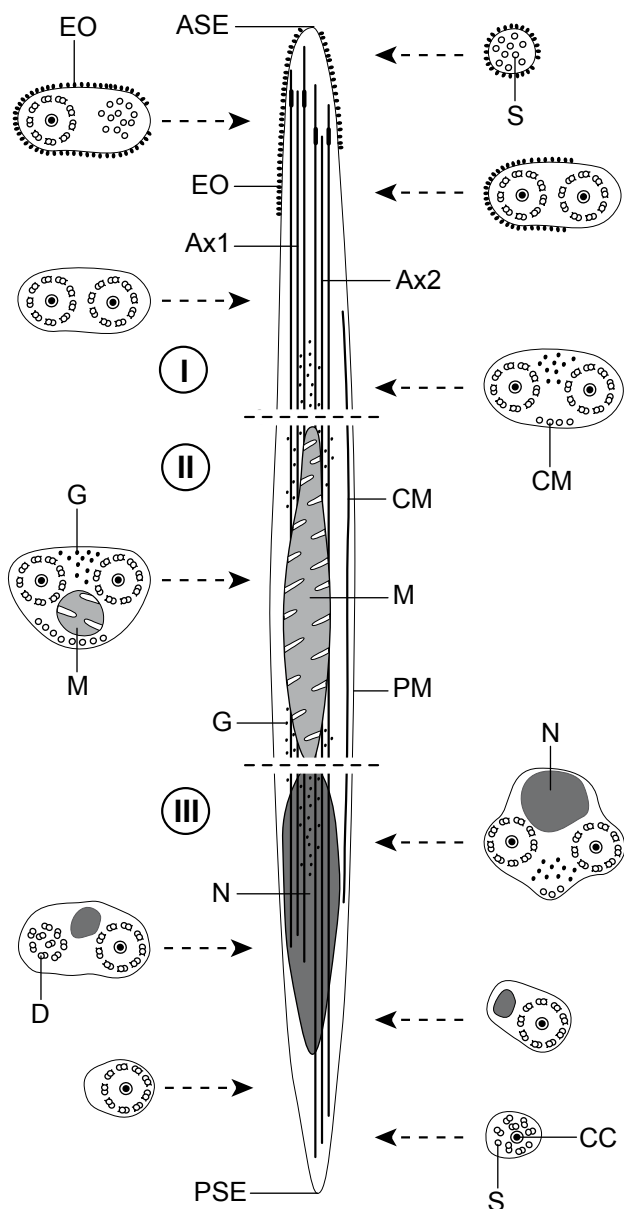


Fig. 2 (I–III). Schematic reconstruction of the mature spermatozoon of *Lecithocladium excisum*. Abbreviations: ASE – anterior spermatozoon extremity; Ax1 – first axoneme; Ax2 – second axoneme; CC – central core; CM – cortical microtubules; D – doublets; EO – external ornamentation of the plasma membrane; G – granules of glycogen; M – mitochondrion; N – nucleus; PM – plasma membrane; PSE – posterior spermatozoon extremity; S – singlets.

Basilio et al. 2001), Sclerodistomidae Odhner, 1927 (see Justine 1995) and Lecithasteridae Odhner, 1905 (see Quilichini et al. 2010a). With the present study we add data on a fourth family (Hemiuridae). Furthermore, extensive data on the ultrastructure of the spermatozoon are available only for the didymozoid *Gonapodasmius* sp., the lecithasterid *Aponurus laguncula* Looss, 1907 and the hemiurid *L. excisum* (Justine and Mattei 1982a, Quilichini et al. 2010a, present study).

An external ornamentation of the plasma membrane entirely covering the anterior extremity of the spermatozoon as described in *L. excisum* was previously reported only in two species, namely *Gonapodasmius* sp. and *A. laguncula* (Justine and Mattei 1982a, Quilichini et al. 2010a). However, a particularity that exists in the case of both *L. excisum* and *A. laguncula* is the absence of cortical microtubules associated with this external ornamentation. This organization has only been observed in *Pronoprymna ventricosa* (Rudolphi, 1819) (see Quilichini et al. 2007).

Two fields of parallel cortical microtubules are usually described in the spermatozoon of digeneans (Jamieson and Daddow 1982, Justine and Mattei 1982b, Robinson and Halton 1982, Daddow and Jamieson 1983, Cifrian et al. 1993, Iomini and Justine 1997, Miquel et al. 2000, Baptista-Farias et al. 2001, Ndiaye et al. 2002, 2011, Foata et al. 2007, Seck et al. 2008, Bakhoum et al. 2012). Nevertheless, in the case of the hemiuroideans *Gonapodasmius* sp., *A. laguncula* and *L. excisum*, a few cortical microtubules are located only in one side (ventral side) of the spermatozoon (Justine and Mattei 1982a, Quilichini et al. 2010a, present study).

Moreover, there is a particularity in the case of *L. excisum* and *A. laguncula*; both species lack cortical microtubules in much of the anterior region of the spermatozoon (Quilichini et al. 2010a, present study). In these species the maximum number of cortical microtubules is present in the mitochondrial region of the spermatozoon: there are ten and eight cortical microtubules in *A. laguncula* and *L. excisum* (Quilichini et al. 2010a, present study), respectively.

With respect to *Gonapodasmius* sp., Justine and Mattei (1982a) described a layer of cortical microtubules associated with the external ornamentation of the plasma membrane in the anterior part of the spermatozoon and a bundle of 20 to 25 cortical microtubules in the middle region. However, in *Didymozoon* sp. and *Didymocystis wedli* Ariola, 1902 spermatozoa lacking cortical microtubules were observed (Justine and Mattei 1983, Pamplona-Basilio et al. 2001).

Finally, the presence of a single field of cortical microtubules in the microphalloidean *P. ventricosa* should be mentioned (Quilichini et al. 2007). Considering the reported variability of this character, more studies on the superfamily Hemiuroidea and particularly on the unexplored families are necessary in order to elucidate the importance of this character.

Other structures described in most digeneans such as spine-like bodies and cytoplasmic expansions are not present in *L. excisum* (Miquel et al. 2000, Ndiaye et al. 2002, 2003, 2012, Levron et al. 2004b, Agostini et al. 2005, Seck et al. 2008, Kacem et al. 2010, Bakhoum et al. 2012). It is remarkable that they are also absent from the remaining species of the Hemiuroidea studied to date (Justine and Mattei 1982a, Pamplona-Basilio et al. 2001, Quilichini et al. 2010a).

The fundamental difference between the spermatozoon of *A. laguncula* and that of *L. excisum* is the type of the posterior extremity. In *A. laguncula*, Quilichini et al. (2010a) described a well-developed mitochondrion spread through a large part of the posterior end of the spermatozoon and the persistence of both axonemes. In addition, some microtubules from axoneme reach the posterior region of the spermatozoon and disappear just before the posterior tip. Thus, this type of the posterior extremity of the spermatozoon is different than that described in our study of *L. excisum* (without a mitochondrion and presenting only the second axoneme).

REFERENCES

- AGOSTINI S., MIQUEL J., NDIAYE P.I., MARCHAND B. 2005: *Dicrocoelium hospes* Looss, 1907 (Digenea, Dicrocoeliidae): spermiogenesis, mature spermatozoon and ultrastructural comparative study. *Parasitol. Res.* 96: 38–48.
- BÂ C.T., MARCHAND B. 1995: Spermiogenesis, spermatozoa and phyletic affinities in the Cestoda. *Mém. Mus. Natl. Hist. Nat.* 166: 87–95.
- BAKHOUM A.J.S., BÂ C.T., SHIMALOV V.V., TORRES J., MIQUEL J. 2011a: Spermatological characters of the digenean *Rubensstrema exasperatum* (Rudolphi, 1819) (Plagiorchioidea, Omphalometridae). *Parasitol. Res.* 108: 1283–1293.
- BAKHOUM A.J.S., NDIAYE P.I., BÂ C.T., MIQUEL J.: Spermatological characteristics of *Elstia stossichianum* (Digenea, Mesometridae) from the intestine of the cow bream (*Sarpa salpa*) off Dakar, Senegal. *J. Helminthol.* (In press.)
- BAKHOUM A.J.S., NDIAYE P.I., SÈNE A., BÂ C.T., MIQUEL J. 2012: Spermiogenesis and ultrastructure of the spermatozoon of *Wardula capitellata* (Digenea, Mesometridae), an intestinal parasite of the sparid teleost *Sarpa salpa* in Senegal. *Acta Parasitol.* 57: 34–45.
- BAKHOUM A.J.S., TORRES J., SHIMALOV V.V., BÂ C.T., MIQUEL J. 2011b: Spermiogenesis and spermatozoon ultrastructure of *Diplodiscus subclavatus* (Pallas, 1760) (Paramphistomoidea, Diplodiscidae), an intestinal fluke of the pool frog *Rana lessonae* (Amphibia, Anura). *Parasitol. Int.* 60: 64–74.
- BAPTISTA-FARIAS M.F.D., KOHN A., COHEN S.C. 2001: Ultrastructure of spermatogenesis and sperm development in *Saccocoeleliodes godoyi* Kohn & Froes, 1986 (Digenea, Haploporidae). *Mem. Inst. Oswaldo Cruz* 96: 61–70.
- BURTON P.R. 1972: Fine structure of the reproductive system of a frog lung-fluke. III. The spermatozoon and its differentiation. *J. Parasitol.* 58: 68–83.
- CIFRIAN B., GARCIA-CORRALES P., MARTINEZ-ALOS S. 1993: Ultrastructural study of the spermatogenesis and mature spermatozoa of *Dicrocoelium dendriticum* (Plathelminthes, Digenea). *Parasitol. Res.* 79: 204–212.
- DADDOW L.Y.M., JAMIESON B.G.M. 1983: An ultrastructural study of spermiogenesis in *Neochasmus* sp. (Cryptogonimidae: Digenea: Trematoda). *Aust. J. Zool.* 31: 1–14.
- EHLERS U. 1984: Phylogenetisches System der Plathelminthes. *Verh. Naturwiss. Ver. Hamburg (NF)* 27: 291–294.
- FOATA J., QUILICHINI Y., MARCHAND B. 2007: Spermiogenesis and sperm ultrastructure of *Deropristis inflata* Molin, 1859 (Digenea, Deropristidae), a parasite of *Anguilla anguilla*. *Parasitol. Res.* 101: 843–852.
- GIBSON D.I. 2002a: Family Hemiuridae Looss, 1899. In: D.I. Gibson, A. Jones and R.A. Bray (Eds.), *Keys to the Trematoda*. Vol. 1. CABI Publishing, Wallingford and The Natural History Museum, London, UK, pp. 305–340.
- GIBSON D.I. 2002b: Superfamily Hemiuroidea Looss, 1899. In: D.I. Gibson, A. Jones and R.A. Bray (Eds.), *Keys to the Trematoda*. Vol. 1. CABI Publishing, Wallingford and The Natural History Museum, London, UK, pp. 299–304.
- HOBERG E.P., MARIAUX J., BROOKS D.R. 2001: Phylogeny among orders of the Eucestoda (Cercaromorphae): integrating morphology, molecules and total evidence. In: D.T.J. Littlewood and R.A. Bray (Eds.), *Interrelationships of the Platyhelminthes*. Taylor and Francis, London, UK, pp. 112–126.
- HOBERG E.P., MARIAUX J., JUSTINE J.-L., BROOKS D.R., WEEKES P.J. 1997: Phylogeny of the orders of the Eucestoda (Cercaromorphae) based on comparative morphology: historical perspectives and a new working hypothesis. *J. Parasitol.* 83: 1128–1147.
- IOMINI C., JUSTINE J.-L. 1997: Spermiogenesis and spermatozoon of *Echinostoma caproni* (Platyhelminthes, Digenea): transmission and scanning electron microscopy, and tubulin immunocytochemistry. *Tissue Cell* 29: 107–118.
- JAMIESON B.G.M., DADDOW L.M. 1982: The ultrastructure of the spermatozoon of *Neochasmus* sp. (Cryptogonimidae, Digenea, Trematoda) and its phylogenetic significance. *Int. J. Parasitol.* 12: 547–559.
- JUSTINE J.-L. 1995: Spermatozoal ultrastructure and phylogeny of the parasitic Platyhelminthes. *Mém. Mus. Natl. Hist. Nat.* 166: 55–86.
- JUSTINE J.-L. 1998: Spermatozoa as phylogenetic characters for the Eucestoda. *J. Parasitol.* 84: 385–408.
- JUSTINE J.-L. 2001: Spermatozoa as phylogenetic characters for the Platyhelminthes. In: D.T.J. Littlewood and R.A. Bray (Eds.), *Interrelationships of the Platyhelminthes*. Taylor and Francis, London, UK, pp. 231–238.
- JUSTINE J.-L., MATTEI X. 1982a: Étude ultrastructurale de la spermiogenèse et du spermatozoïde d'un Plathelminthe: *Gonapodasmius* (Trematoda: Didymozoidae). *J. Ultrastruct. Res.* 79: 350–365.
- JUSTINE J.-L., MATTEI X. 1982b: Réinvestigation de l'ultrastructure du spermatozoïde d'*Haematoloechus* (Trematoda: Haematoloechidae). *J. Ultrastruct. Res.* 81: 322–332.
- JUSTINE J.-L., MATTEI X. 1983: A spermatozoon with two 9 + 0 axonemes in a parasitic flatworm, *Didymozoon* (Digenea: Didymozoidae). *J. Submicrosc. Cytol.* 15: 1101–1105.

- JUSTINE J.-L., MATTEI X. 1984: Ultrastructural observations on the spermatozoon, ovocyte and fertilization process in *Gonapodasmius*, a gonochoristic trematode (Trematoda: Digenea: Didymozoidae). *Acta Zool. (Stockh.)* 65: 171–177.
- KACEM H., BAKHOUM A.J.S., NEIFAR L., MIQUEL J. 2010: Spermiogenesis and spermatozoon ultrastructure of the digenean *Neopocreadium chabaudi* (Apocreadiidae), a parasite of *Balistes capriscus* (Pisces, Teleostei). *Parasitol. Int.* 59: 358–366.
- LEVRON C., MIQUEL J., OROS M., SCHOLZ T. 2010: Spermatozoa of tapeworms (Platyhelminthes, Eucestoda): advances in ultrastructural and phylogenetic studies. *Biol. Rev.* 85: 523–543.
- LEVRON C., TERNENGO S., MARCHAND B. 2004a: Ultrastructure of spermiogenesis and the spermatozoon of *Monorchis parvus* Looss, 1902 (Digenea, Monorchidae), a parasite of *Diplodus annularis* (Pisces, Teleostei). *Parasitol. Res.* 93: 102–110.
- LEVRON C., TERNENGO S., MARCHAND B. 2004b: Spermiogenesis and sperm ultrastructure of *Poracanthium furcatum* (Digenea, Opecoelidae), a parasite of *Mullus surmuletus* (Pisces, Teleostei). *Acta Parasitol.* 49: 190–200.
- MIQUEL J., FOURNIER-CHAMBRILLON C., FOURNIER P., TORRES J. 2006: Spermiogenesis and spermatozoon ultrastructure of the cranial digenean *Trogloitrema acutum* (Leuckart, 1842). *J. Parasitol.* 92: 441–453.
- MIQUEL J., NOURRISSON C., MARCHAND B. 2000: Ultrastructure of spermiogenesis and the spermatozoon of *Opecoeloides furcatus* (Trematoda, Digenea, Opecoelidae), a parasite of *Mullus barbatus* (Pisces, Teleostei). *Parasitol. Res.* 86: 301–310.
- NDIAYE P.I., MIQUEL J., BÂ C.T., FELIU C., MARCHAND B. 2002: Spermiogenesis and sperm ultrastructure of *Scaphiostomum palaearticum* Mas-Coma, Esteban et Valero, 1986 (Trematoda, Digenea, Brachylaimidae). *Acta Parasitol.* 47: 259–271.
- NDIAYE P.I., MIQUEL J., FELIU C., MARCHAND B. 2003: Ultrastructure of spermiogenesis and spermatozoa of *Notocotylus neyrai* González Castro, 1945 (Digenea, Notocotylidae), intestinal parasite of *Microtus agrestis* (Rodentia: Arvicolidae) in Spain. *Invert. Reprod. Dev.* 43: 105–115.
- NDIAYE P.I., QUILICHINI Y., SÈNE A., BÂ C.T., MARCHAND B. 2011: Ultrastructure of the spermatozoon of the digenean *Cricocephalus albus* (Kuhl & van Hasselt 1822) Looss, 1899 (Platyhelminthes, Pronocephaloidea, Pronocephalidae) parasite of the “hawkbill sea turtle” *Eretmochelis imbricata* (Linnaeus, 1766) in Senegal. *Zool. Anz.* 250: 215–222.
- NDIAYE P.I., QUILICHINI Y., SÈNE A., TKACH V.V., BÂ C.T., MARCHAND B. 2012: Ultrastructural study of the spermatozoon of the digenean *Enodiotrema reductum* Looss, 1901 (Platyhelminthes, Plagiorchioidea, Plagiorchiidae) parasite of the green turtle *Chelonia mydas* (Linnaeus, 1758) in Senegal. *Parasitol. Res.* 111: 859–864.
- OLSON P.D., LITTLEWOOD D.T.J., BRAY R.A., MARIAUX J. 2001: Interrelationships and evolution of the tapeworms (Platyhelminthes: Cestoda). *Mol. Phylogenet. Evol.* 19: 443–467.
- PAMPLONA-BASILIO M.C., BAPTISTA-FARIAS M.F.D., KOHN A. 2001: Spermatogenesis and spermiogenesis in *Didymocystis wedli* Ariola, 1902 (Didymozoidae, Digenea). *Mem. Inst. Oswaldo Cruz* 96: 1153–1159.
- QUILICHINI Y., FOATA J., JUSTINE J.-L., BRAY R.A., MARCHAND B. 2010a: Spermatozoon ultrastructure of *Aponurus laguncula* (Digenea: Lecithasteridae), a parasite of *Aluterus monoceros* (Pisces, Teleostei). *Parasitol. Int.* 59: 22–28.
- QUILICHINI Y., FOATA J., JUSTINE J.-L., BRAY R.A., MARCHAND B. 2010b: Ultrastructural study of the spermatozoon of *Heterolebes maculosus* (Digenea, Opistholebetidae), a parasite of the porcupinefish *Diodon hystrix* (Pisces, Teleostei). *Parasitol. Int.* 59: 427–434.
- QUILICHINI Y., FOATA J., JUSTINE J.-L., BRAY R.A., MARCHAND B. 2011: Spermatozoon ultrastructure of *Gyliauchen* sp. (Digenea: Gyliauchenidae), an intestinal parasite of *Siganus fuscescens* (Pisces: Teleostei). *Biol. Bull.* 221: 197–205.
- QUILICHINI Y., FOATA J., MARCHAND B. 2007: Ultrastructural study of the spermatozoon of *Pronoprymna ventricosa* (Digenea, Baccigerinae), parasite of the twaite shad *Alosa fallax* Lacépède (Pisces, Teleostei). *Parasitol. Res.* 101: 1125–1130.
- REYNOLDS E.S. 1963: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17: 208–212.
- ROBINSON R.D., HALTON D.W. 1982: Fine structural observations on spermatogenesis in *Corrigia vitta* (Trematoda: Dicrocoeliidae). *Z. Parasitenkd.* 68: 53–72.
- SECK M.T., MARCHAND B., BÂ C.T. 2008: Spermiogenesis and sperm ultrastructure of *Cotylophoron cotylophorum* (Trematoda, Digenea, Paramphistomidae), a parasite of *Bos taurus* in Senegal. *Parasitol. Res.* 103: 157–166.
- ŚWIDERSKI Z. 1986: Three types of spermiogenesis in cestodes. *Proc. 11th Int. Cong. Electron Microsc.*, Kyoto, pp. 2959–2960.
- TANG J., WANG W., WANG G. 1998: Studies on ultrastructure of spermatogenesis and sperm in *Pseudorhipidocotyle elpichthys*. *Acta Hydrobiol. Sin.* 22: 168–173.
- THIÉRY J.P. 1967: Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. *J. Microsc.* 6: 987–1018.

Received 14 June 2012

Accepted 13 July 2012