New spermatological characteristics in the Hemiuroidea inferred from the ultrastructure of the spermatozoon of *Gonocerca phycidis* a stomach parasite of *Gephyroberyx darwinii* (Teleostei: Trachichthyidae) off the Senegal coast

Aïssatou Bâ1 · Abdoulaye J. S. Bakhoum1 · Papa Ibnou Ndiaye1 · Rodney Alan Bray2 · Cheikh Tidiane Bâ1 · Bernard Marchand3 · Yann Quilichini3

Received: 14 April 2022 / Revised: 8 September 2022 / Accepted: 11 September 2022 / Published online: 30 September 2022

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Abstract

The present study provides the first ultrastructural data on the digenean *Gonocerca phycidis* belonging to the family Gonocercidae. Live adults digenean were collected from the stomach of the marine fish *Gephyroberyx darwinii* (Teleostei: Trachichthyidae) caught in the Atlantic Ocean, off Dakar (Senegal). This is also the first report of *G. phycidis* from Senegal and from this fish. The mature spermatozoon exhibits (i) two axonemes with the 9 + ’1’ pattern of the Trepaxonemata, (ii) a lateral expansion associated with external ornamentations and without cortical microtubules, (iii) two types of external ornamentation of the plasma membrane located in the anterior region of the spermatozoon, (iv) two bundles of cortical microtubules in the mitochondrial and nuclear areas of the spermatozoon, (v) one mitochondrion and (vi) a nucleus. The maximum number of cortical microtubules is located in the median part of the spermatozoon. Moreover, the mature spermatozoon of *G. phycidis* presents some peculiarities. The presence of a lateral expansion without cortical microtubules associated with external ornamentations is described here, for the first time, in digenean spermatozoa. The presence of two elongated bundles of cortical microtubules is also reported for the first time in the superfamily of Hemiuroidea. However, the ultrastructure of the posterior spermatozoon extremity confirms that already described in other hemiuroidean species.

Keywords *Gonocerca phycidis* · Hemiuroidea · Gonocercidae · Ultrastructure · Spermatozoon

Introduction

The systematic position of the genus *Gonocerca* has been the subject of many controversies. It was considered as a member of the subfamily Gonocercinae Skrjabin and Guschanskaja 1955, within the family Derogenidae in the Superfamily of Hemiuroidea (Gibson and Bray 1979; Gibson 2002). However, based on molecular studies, Olson et al. (2003) and Panklov et al. (2006) were the first to propose the reconsideration of the Gonocercinae as an independent family. According to these authors, the family Derogenidae is polyphyletic. Likewise, *Hemipera manteri*, a member of the Gonocercinae, and *Derogenes varicus*, the type genus of the Derogenidae, are phylogenetically distant from others. This fact was also supported posteriorly by Sokolov et al. (2016). More recently, based on molecular studies carried out on four gonocercids including the type species *G. phycidis*, Sokolov et al. (2018) have considered the Gonocercidae as a separate family from the Derogenidae. Molecular studies show also that this family is basal to the remaining families of the Hemiuroidea (Sokolov et al. 2019). To date, the Gonocercidae includes only two genera namely *Gonocerca* (the type genus) and *Hemipera*. However, according to Sokolov et al. (2018) the generic composition of this new family requires further
clarification as the molecular data do not support the inclusion of the genus *Hemipera* in the Gonocercidae.

In this context, the ultrastructural studies of species belonging to the family Gonocercidae are of great importance to bring additional information to complement the existing molecular data. *Gonocerca phycidis* is the type species of the genus *Gonocerca* and the first member of the Gonocercidae family to be studied by transmission electron microscopy.

To our knowledge ultrastructural data of the mature spermatozoon exist for only fourteen species belonging to five of the fourteen families of the Hemiuroidea. These are three Didymozoidae: *Didymocystis wedli*, *Didymozoon* sp. and *Gonapodasmius* sp. (Justine and Mattei 1982, 1983, 1984; Pamplona-Basilio et al. 2001), seven Hemiuridae: *Aphanurus stossichii*, *Ectenurus lepidus*, *Hemius appendiculatus*, *Lecithochirium microstomum*, *L. musculus*, *Lecithocladium excisum* and *Parahemiurus merus* (Ndiaye et al. 2012a, 2013a, 2014; Dione et al. 2016; Kacem et al. 2020), one Lecithasteridae: *Aponurus laguncula* (Quilichini et al. 2010a), two Sclerodistomidae: *Prosorchis palinurichthi* and *Sclerodistomum italicum* (Ndiaye et al. 2013b, 2017) and one Sclerodistomoididae: *Sclerodistomoides pacificus* (Bà et al. 2020).

To increase the data on spermatological studies in the Hemiuroidea, the present study provides, for the first time, the spermatological characteristics of the genus *Gonocerca* and the family Gonocercidae. We compare our results with those obtained in other hemiuroideans previously described.

**Material and methods**

Adult specimens of *Gonocerca phycidis* Manter, 1925 were collected from the stomach of *Gephyroberyx darwini* (Trachichthyidae) caught off the coast of Dakar (Atlantic Ocean). The adult specimens were washed in 0.9% NaCl solution and fixed in cold (4 °C) 2.5% glutaraldehyde 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, embedded in Spurr’s resin and finally polymerized at 60 °C for 48 h.

Ultrathin Sects. (60–90 nm thick) were cut on an ultramicrotome (Power tome PC, RMC Boeckeler) with a diamond knife, placed on copper and gold grids. Sections on copper grids were double-stained with uranyl acetate and lead citrate. The ultrathin sections were examined using a Hitachi H-7650 electron microscope operated at 80 kV, in the “Service d’Étude et de Recherche en Microscopie Électronique” of the University of Corsica (Corte, France).

**Results**

The interpretation of several cross and longitudinal sections of the mature spermatozoon of *G. phycidis* allows us to establish three distinctive regions (I–III) from the anterior to the posterior spermatozoon extremity.

Region I (Figs. 1A–H and 3I) corresponds to the anterior extremity of the spermatozoon. Cross-sections of the anterior tip show axonemal microtubules surrounded by a continuous and submembranous layer of parallel cortical microtubules with a maximum number around 24 (Fig. 1A, B).

Then, the two centrioles appear almost simultaneously and they are covered entirely by filamentous ornamentation associated with the layer of cortical microtubules (Fig. 1C, D). A well-developed lateral expansion associated only with external ornamentation of the plasma membrane appears before the complete formation of the second axoneme (Fig. 1E). At this level the layer of cortical microtubules becomes discontinuous and their number decreases progressively (Fig. 2E, H). The transition toward region II is characterized by the disappearance of lateral expansion.

Region II (Figs. 2A–F and 3II) corresponds to the middle region of the spermatozoon. In the anterior part of this region, cross-sections show only two axonemes, their attachment zones, and four cortical microtubules (Fig. 2A). The number of cortical microtubules progressively increases in this region by 4 (Fig. 2A) to 8 (Fig. 2B), then 32–36 (Fig. 2C) which corresponds to the maximum number. Several cross-sections in the distal part of region II show the progressive decrease of cortical microtubules (Fig. 2D–F), the appearance of the mitochondrion (Fig. 2E), and the arrangement of cortical microtubules into two fields (Fig. 2F).

Region III (Figs. 2G–M and 3III) corresponds to the nuclear region and posterior spermatozoon extremity. In its proximal part, we notice the appearance of the nucleus with the simultaneous presence of the posterior part of the mitochondrion, two axonemes, and cortical microtubules disposed in two fields (Fig. 2G–H). Consecutive cross-sections show the disappearance of the mitochondrion (Fig. 2H–I), the gradual increase of the nucleus size (Fig. 2H–J) and the progressive disorganization of the first and the second axoneme (Fig. 2J–K). When cortical microtubules disappear completely, the cross-section show only the posterior extremity of the second axoneme and the nucleus with a reduced diameter (Fig. 2L). The posterior spermatozoon tip is characterized by the disappearance of the nucleus and the presence of only the doublets of the second axoneme (Figs. 2M and 3III).
Discussion

The mature spermatozoon of *Gonocerca phycidis* exhibits the general pattern described in most hemiuroideans: two axonomes of the 9 + ‘1’ pattern of Trepaxonemata (Ehlers 1984), nucleus, mitochondria, external ornamentation of the plasma membrane, and parallel cortical microtubules (Kacem et al. 2020). However, compared with hemiuroideans studied to date, the mature spermatozoon of *G. phycidis* presents some unusual characteristics (Table 1).

Anterior spermatozoon morphology

The mature spermatozoon of *G. phycidis* exhibits in its anterior tip two centrioles corresponding to the two axonomes of 9 + ‘1’ trepaxonematan pattern (Ehlers 1984).
In the Hemiuroidea, mature spermatozoa exhibiting two axonemes in their anterior extremity are reported only in two species namely the Didymozoidae *Gonapodasmius* sp. (Justine and Mattei 1982) and the Sclerodistomoididae *Sclerodistomoides pacificus* (Bâ et al. 2020), whereas in the remaining studied species there is only one axoneme in their anterior tip (see Table 1).
In this anterior tip, there is also the presence of a continuous and submembranous layer of cortical microtubules. Within the Hemiuroidea, this feature was previously observed only in the didymozoid *Gonapodasmius* sp. by Justine and Mattei (1982). However, some cortical microtubules were also observed in the anterior spermatozoon extremity of other hemiuroideans namely the Hemiuridae *Hemiurus appendiculatus* and *Lecithochirium microstomum* (Ndiaye et al. 2014; Dione et al. 2016) and the Sclerodistomidae *Prosorchis palinurichthi* and *Sclerodistomum italicum* (Ndiaye et al. 2013b, 2017) whereas in the remaining hemiuroideans, cortical microtubules are absent in the anterior tip.

**Fig. 3** Schematic reconstruction of the mature spermatozoon of *Gonocerca phycidis*. *Ase* anterior spermatozoon extremity, *Ax1* first axoneme, *Ax2* second axoneme, *Az* attachment zones, *C1* centriole of the first axoneme, *C2* centriole of the second axoneme, *Cm* cortical microtubules, *Eo* external ornamentation of the plasma membrane, *Feo* filamentous external ornamentation, *Le* lateral expansion, *M* mitochon- drion, *N* nucleus, *Pm* plasma membrane, *Pae1* posterior extremity of the first axoneme, *Pae2* posterior extremity of the second axoneme, *Pse* posterior spermatozoon extremity.
of the spermatozoon (Table 1). Nevertheless, their appearance is noted only when both axonemes are already formed. However, it is important to remark that in the Didymozoids *Didymocystis wedli* and *Didymozoon sp.* (Justine and Mattei 1983; Pamplona-Basilio et al. 2001), the sperm cell lacks cortical microtubules.

Another peculiarity observed in the anterior spermatozoon region is the presence of a well-developed lateral expansion that distinguishes the mature spermatozoon of *G. phycidis* from those of the remaining hemiuroids studied until now. A lateral expansion has been described at the anterior extremity of the spermatozoon of digeneans belonging to five superfamilies: the Echinostomatoidea, the Microscaphidioidea, the Paramphistomoidea, the Pronocephaloidea and the Bucephaloidea (Bakhoum et al. 2017a; Kacem and Miquel 2018; Ndiaye et al. 2019). In the present work, we describe for the first time its presence in a Hemiuroidea. Its morphology is variable according to the species and it is generally associated with external ornamentation, cortical microtubules, and sometimes with spine-like bodies. In *G. phycidis* the lateral expansion is associated with only external ornamentation. This type of lateral expansion is described here for the first time in the anterior extremity of the digenean spermatozoa.

An external ornamentation of the plasma membrane is described in the anterior region of the spermatozoa from most species of Digenea in general and in particular from all Hemiuroidea studied to date except in the Didymozoids *Didymocystis wedli* and *Didymozoon sp.* (Pamplona-Basilio et al. 2001; Justine and Mattei 1983) which lack external ornamentation (see Table 1). In *G. phycidis* the lateral expansion is associated with external ornamentation. This type of lateral expansion is described here for the first time in the anterior extremity of the digenean spermatozoa.

### Table 1 Spermatological characteristics in the superfamily Hemiuroidea

| Family and species | Ase | Le | Eo | FEo | MaxCm | BCm | LMaxCm | M | Pse | TypeSpz | References |
|-------------------|-----|----|----|-----|-------|-----|--------|---|-----|----------|------------|
| Didymozoidae      |     |    |    |     |       |     |        |   |     |          |            |
| *Didymocystis wedli* | ?  | −  | −  | −  | 0     | 0   | NA     | 1?| ?  | ?        | Pamplona-Basilio et al. (2001) |
| *Didymozoon sp.*  | 2Ax| −  | −  | −  | 0     | 0   | NA     | 1?| ?  | ?        | Justine and Mattei (1983) |
| *Gonapodasmias sp.* | 2Ax-Eo-Cm | −  | +  | 36  | 2     | AntS| 1? AX | IV?|     |          | Justine and Mattei (1982) |
| Gonocercidae      |     |    |    |     |       |     |        |   |     |          |            |
| *Gonocerca phycidis* | 2Ax-Cm | +  | +  | +  | 32    | 2   | MedS   | 1 | Ax | III?     | Present study |
| Hemiuridae        |     |    |    |     |       |     |        |   |     |          |            |
| *Aphanurus stossichii* | 1Ax-Eo | −  | +  | 11  | 1     | MedS| 1 Ax   | II|     |          | Kacem et al. (2020) |
| *Ectenurus lepidus* | 1Ax-Eo | −  | +  | 8   | 1     | PostS| 1 Ax   | II|     |          | Kacem et al. (2020) |
| *Hemiaris appendiculatus* | 1Ax-Eo | −  | +  | +  | 7     | 1   | MedS   | 1 | Ax | II       | Dione et al. (2016) |
| *Lecithochirium microstomum* | 1Ax-Eo | −  | +  | +  | 8     | 1   | MedS   | 1 | Ax | II       | Ndiaye et al. (2014) |
| *Lecithochirium masculus* | 1Ax-Eo | −  | + | ?  | 6     | 1   | MedS   | 1 | Ax | II       | Ndiaye et al. (2014) |
| *Lecithochloriadium excisum* | 1Ax+Eo | −  | +  | +  | 8     | 1   | MedS   | 1 | Ax | II       | Ndiaye et al. (2012a) |
| *Parahemiurus merus* | 1Ax+Eo | −  | +  | −  | 5     | 1   | MedS   | 1 | Ax | II       | Ndiaye et al. (2013a) |
| Lecithasteridae    |     |    |    |     |       |     |        |   |     |          |            |
| *Aponurus laguncula* | 1Ax-Eo | −  | +  | −  | 10    | 1   | MedS   | 1 | Ax | II       | Quilichini et al. (2010a) |
| Sclerodistomidae   |     |    |    |     |       |     |        |   |     |          |            |
| *Prosochis palinurichthi* | 1Ax-Cm | −  | +  | +  | 30    | 1   | MedS   | 1 | Ax | II       | Ndiaye et al. (2013b) |
| *Sclerodistomum italicum* | 1Ax-Cm | −  | +  | +  | 28    | 1   | MedS   | 1 | Ax | II       | Ndiaye et al. (2017) |
| Sclerodistomoididae|     |    |    |     |       |     |        |   |     |          |            |
| *Sclerodistomoides pacificus* | 2Ax-Eo | −  | +  | −  | 24    | 1   | PostS  | 1*| Ax | II       | Bâ et al. (2020) |

*Ase* anterior spermatozoon extremity, *Ax* Axoneme, *BCm* number of fields of cortical microtubules, *Cm* cortical microtubules, *Eo* external ornamentation of the plasma membrane, *Feo* filamentous external ornamentation, *Le* lateral expansion, *LMaxCm* location of the maximum number of cortical microtubules, *M* number of mitochondria, *MaxCm* maximum number of cortical microtubules, *MedS* median region of the spermatozoon, *NA* not applicable, *PostS* posterior region of the spermatozoon, *Pse* posterior spermatozoon extremity, *TypeSpz* type of spermatozoon

*Moniliform mitochondrion

± Presence/absence of considered character

? Doubtful or unknown data
Gonapodasmium sp. (Justine and Mattei 1982; Ndiaye et al. 2013b, 2014, 2017; Dione et al. 2016; Kacem et al. 2020).

Cortical microtubules

In the Digenea, the number of cortical microtubules in the mature spermatozoon varies according to the species from zero in the Didymozoididae Didymozoon sp. and Didymocystis weddi (Justine and Mattei 1983; Pamplona-Basilio et al. 2001) to 73 in Diploidiscus subclavatus (Bakhoum et al. 2011). In most hemiuroids this number is between 5 to 11 as described in the Hemiuriidae and the Lecithasteridae (Quilichini et al. 2010a; Kacem et al. 2020). However, about 24 to 36 cortical microtubules have been reported in Sclerodistomidae (Ndiaye et al. 2013b, 2017), Sclerodistomoididae (Bâ et al. 2020), Didymozoididae (Justine and Mattei, 1982), and Gonocercidae (present study).

The location of the maximum number of cortical microtubules is also variable according to the species. Quilichini et al. (2007) proposed for the first time two groups of digeneans according to the location of the maximum number of cortical microtubules in the spermatozoa: type 1 with the maximum number of cortical microtubules in the anterior part and type 2 in the median part of the spermatozoon. In G. phycidis, the maximum number of cortical microtubules is located in the median part of the spermatozoon and corresponds to the type 2 sensu Quilichini et al. (2007). This type 2 is also present in all hemiuroideans studied until now, except Gonapodasmium sp. which exhibits the maximum number in the anterior region and S. pacificus in the posterior one (see Table 1).

Most digeneans show an arrangement of cortical microtubules into two fields in the mitochondrial and nuclear regions of the spermatozoon as occurs in G. phycidis (present study) and in the didymozooid Gonapodasmium sp. (see Table 1). In contrast, in the remaining hemiuroideans studied to date, only one field of cortical microtubules has been described (see Table 1).

Mitochondria

In the digenean spermatozoa, the number of mitochondria varies from one to three, depending on the species (Bakhoum et al. 2017a). The mature spermatozoon of G. phycidis exhibits one mitochondrion as described in the remaining hemiuroideans studied up to now (see Table 1). On the other hand, the morphology of the mitochondrion is variable according to the species. Recently, a mitochondrial matrix granule has been observed in the hemiurid Aphanurus stossichii (Kacem et al. 2020). Moreover, a moniliform mitochondrion has been described in the sclerodistomoidid S. pacificus (Bâ et al. 2020). The morphological particularity of this type of mitochondrion has been described by the authors as a succession of bulges and cords. This type was also described in the male gamete of some digeneans such as the acanthocolpid Stephanostomoides tenuis (Bakhoum et al. 2015), the aephnidiogenids Holorchis micracanthus and H. pycnoporus (Bâ et al. 2011; Kacem and Miquel 2020a), the cryptogonimids Aphallus tubarium and Timoniella imbutiformis (Foata et al. 2012; Kacem et al. 2017a), the lepocreadids Opechona bacilliaris and Prodistomum polonii (Ndiaye et al. 2015, Kacem and Miquel 2020b), the opecoelids Allopodocotyle pedicellata and Macvicaria obovata (Bakhoum et al. 2017b; Kacem et al. 2017b) and the plagiorchiid Enodiotrema reductum (Ndiaye et al. 2012b). Another morphology of mitochondrion with a U-shaped posterior extremity has been described in the opecoelid Allopodocotyle tunisiensis by Kacem et al. (2019).

Posterior spermatozoon morphology

The posterior spermatozoon extremity is morphologically variable within digeneans. Quilichini et al. (2010b) were the first authors to propose three types of posterior spermatozoon extremities according to the disappearance of characters toward the posterior tip. Type 1 or opecoelidean type with the sequence “second axoneme, nucleus and cortical microtubules”, type 2 or fasciolidean type with the sequence “cortical microtubules, second axoneme and nucleus” and type 3 or cryptogonimidean type with the sequence “cortical microtubules, nucleus and second axoneme”. Following this criterion, the mature spermatozoon of G. phycidis exhibits type 3 of posterior spermatozoon extremity. This type has been observed in nearly all digenean species belonging to the Hemiuroidea (see Table 1).

Spermatozoa models in the Hemiuroidea

The ultrastructural study of species belonging to the Hemiuroidea reveals similarities in their mature spermatozoa. According to Bakhoum et al. (2017a), the hemiuroideans described until now show the type II sperm model. The latter is characterized by the presence of two 9 + ‘1’ axonemes, external ornamentation of the plasma membrane not associated with cortical microtubules and located in the anterior part of the spermatozoon, one bundle of cortical microtubules of which the maximum number is located in a middle part of the spermatozoon and one mitochondrion. However, the mature spermatozoon described in G. phycidis seems not to follow this type II sperm model. Several characters present in the male gamete of G. phycidis are absent in the mature spermatozoa of other hemiuroideans. Those are:

- The lateral expansion associated with external ornamentation of the plasma membrane and without cortical microtubules,
The two types of external ornamentation of the plasma membrane located in the anterior extremity of the spermatozoon,

- The two bundles of cortical microtubules in the mitochondrial and nuclear areas of the spermatozoon.

The position of the Gonocercidae within the Hemiuroidea seems to be confirmed. Our study confirms the previous molecular studies regarding the position of Gonocercidae compared with the rest of the hemiuroid trematodes, including Derogenes varicus, representative of the type genus of the family Derogenidae. Unfortunately, the family Derogenidae remains unexplored from the spermatological point of view. In the future, it would be important to perform ultrastructural studies of spermatozoon not only in representatives of the family Derogenidae but also in the remaining genera of Gonocercidae.

**Conclusion**

The present study enlarges the data on ultrastructural studies in the Digenea providing, for the first time, spermatological characteristics in the Gonocercidae. The most interesting findings are the presence of a lateral expansion associated only with external ornamentation and the presence of two fields of cortical microtubules in the mitochondrial and nuclear regions of the male gamete of *G. phycidis*. These features distinguish the mature spermatozoon of *G. phycidis* from those reported in other hemiurooids. However, additional spermatological studies are needed on this family, but especially on the Derogenidae from which the Gonocercidae has been separated to confirm and complete the results of the molecular studies.

**Acknowledgements** The authors are grateful of the “Collectivité Territoriale de Corse”. This study was also partly supported by the financial assistance of “Projet d’appui pour la Promotion des Enseignantes-chercheures du Sénégal (PAPES) du Ministère de l’Enseignement supérieur et de la Recherche du Sénégal”.

**Author contributions** All authors contributed to and reviewed the manuscript.

**Declarations**

**Conflict of interest** The authors declare no competing interests.

**Ethical approval** Collection of specimens was governed by national guidelines and permits.

**Informed consent** This article does not contain any studies with human participants performed by any of the authors.

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