Sperm characters of the bucephalid digenean *Prosorhynchoides arcuatus* and their phylogenetic significance

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Abstract

The present study describes the ultrastructural organisation of the mature spermatozoon of the bucephalid digenean *Prosorhynchoides arcuatus* (Bucephalinae) collected from the bluefish *Pomatomus saltatrix* (Teleostei: Pomatomidae) caught from the Gulf of Gabès in Chebba (Tunisia) by means of transmission electron microscopy. The ultrastructural study reveals that the mature spermatozoon of *P. arcuatus* is a filiform cell which is tapered at both extremities. It exhibits the Bakhoum et al.'s type V of spermatozoon of digeneans characterised by the presence of two axonemes with the 9+‘1’ pattern of the Trepaxonemata, lateral expansion, external ornamentation of the plasma membrane located in the anterior part of the proximal region of the spermatozoon and associated with cortical microtubules, two bundles of parallel cortical microtubules with maximum number located in the anterior part of the spermatozoon, and one mitochondrion. Other characteristics are the presence of spine-like bodies, a posterior extremity containing only the nucleus, and the presence of a large amount of glycogen granules. Results of the present study are compared with the existing data in other digeneans, particularly bucephalids.

Keywords: Bucephalinae, Bucephalidae, Digenea, Spermatozoon, Ultrastructure.

1. Introduction

The Bucephalidae is a major family of digeneans which has a wide host and geographic distribution. It comprises about 25 genera and 380 species. Parasites belonging to this family may invade various internal organs, such as the swim bladder, the body cavity, the stomach or the intestine of mainly marine, brackish, and freshwater fish (Nolan et al., 2015). The Bucephalidae includes five subfamilies, namely, the Bucephalinae, Dolichoenterinae, Heterobucephalopsinae, Paurorhynchinae, and Prosorhynchinae (Overstreet and Curran, 2002). A molecular and morphological study, performed by Nolan et al. (2015), showed that the subfamily Heterobucephalopsinae was basal to the remaining subfamilies. Moreover, both the Dolichoenterinae and the Prosorhynchinae represent monophyletic sister clades which are basal to the subfamilies Bucephalinae and Paurorhynchinae. Additionally, the Bucephalinae appear as a paraphyletic group.

In this context, ultrastructural studies of sperm characters in bucephalids are of great importance since they contribute with additional information that could complement molecular results. In fact, it has been proved that ultrastructural characters of sperm cells are useful when interpreting relationships among Platyhelminthes, not only among cestodes and monogeneans but also in digeneans (Bâ and Marchand, 1995; Bakhoum et al., 2017; Justine, 1991a, 1991b, 1998, 2001; Justine & Poddubnaya, 2018; Levron et al., 2010).

Therefore, the aim of the present study is to produce the first complete description of the sperm characters and their organisation along the mature spermatozoon in a representative of the subfamily Bucephalinae: *Prosorhynchoides arcuatus*. The present study will also contribute with data to the ultrastructural spermatological knowledge on the Bucephalidae family, especially considering that the currently available data concern only three species: the Prosorhynchinae *Prosorhynchus aculeatus* (Miquel et al., 2017), and the Bucephalinae *Prosorhynchoides gracilescens* published as *Bucephaloides gracilescens* (Erwin and Halton, 1983) and *Pseudorhipidocotyle elopichthys* (Tang et al., 1998). It is remarkable that the ultrastructural studies of the two latter species show numerous lacuna, containing several misinterpretations of TEM micrographs, and present a very scarce amount of data (for details, see Miquel et al., 2017, p. 303 and p. 304).

2. Materials and methods

2.1. Materials

Live adult specimens of *Prosorhynchoides arcuatus* were collected from the digestive tract of the Bluefish *Pomatomus saltatrix* (Pomatomidae) captured in the coastal zone of the Mediterranean Sea, off La Chebba (34°14′N, 11°06'E) (Tunisia) during November 2016. A voucher specimen, stained with Semichon’s acetic carmine and mounted in Canada balsam, was deposited in the parasitological collection of the Muséum National d’Histoire Naturelle

(Paris) (MNHN): one slide of *P. arcuatus* ex *P. saltatrix* (no. 2016112201) off La Chebba (Tunisia), 22 November 2016 – accession number MNHN HEL738.

2.2. Transmission electron microscopy

After their extraction, adult worms 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 for a minimum of 2 h, rinsed in 0.1 M sodium cacodylate buffer at pH 7.4, post-fixed in cold (4°C) 1 % osmium tetroxide with 0.9 % potassium ferricyanide in the same buffer for 1 h, rinsed in Milli-Q water (Millipore Gradient A10), dehydrated in an ethanol series and propylene oxide, embedded in Spurr's resin and polymerized at 60°C for 72 h. Ultrathin sections (60–90 nm thick) at the level of the seminal vesicle were obtained using a Reichert-Jung Ultracut E ultramicrotome. Sections were placed on 200-mesh copper and gold grids. Sections placed on copper grids were double-stained with uranyl acetate and lead citrate according to the Reynolds (1963) procedure. Copper grids were examined in a JEOL 1010 transmission electron microscope operated at an accelerating voltage of 80 kV, in the "Centres Científics i Tecnològics" of the University of Barcelona (CCiTUB).

2.3. Cytochemistry

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

3. Results

The interpretation of numerous cross- and longitudinal sections of the mature spermatozoa of *P. arcuatus* allow us to distinguish three regions (I to III) from the anterior to the posterior extremities of the male gamete exhibiting different ultrastructural characteristics (Figs. 1-4). The mature spermatozoon of *P. arcuatus* is a filiform cell presenting the usual structures found in most digeneans. Indeed, it contains two axonemes of the 9+‘1’ pattern of trepaxonematan Platyhelminthes, external ornamentation of the plasma membrane, lateral expansion, spine-like bodies, nucleus, one mitochondrion, two bundles of parallel cortical microtubules, and granules of glycogen.

Region I (Figs. 1a-k, 2a-d and 4I) corresponds to the anterior region of the spermatozoon. It is mainly characterised by the presence of two axonemes which are displaced longitudinally (Figs. 1a-d and 4I). Other characteristics of region I are the presence of an external ornamentation of the plasma membrane (Figs. 1a-j and 4I), a lateral expansion (Figs. 1d-g and 4I), and spine-like bodies (Figs. 1f, i-k, 2b and 4I). Two different areas can be considered in this region: the anterior ornamented area (Figs. 1a-j and 4I) and the posterior area lacking ornamentation (Figs. 1j, k, 2a-d and 4I). At the level of the anterior spermatozoon extremity only the first axoneme is present (Figs. 1a-c) and the appearance of the external ornamentation can be observed as small prominent structures (Fig. 1a, c). When the second axoneme is present, the external ornamentation appears as well-developed elements and associated to cortical microtubules (Fig. 1d, e, g-i). Moreover, in this area a lateral expansion is present and located on the dorsal side of the second axoneme (Figs. 1d-g), and the maximum number of cortical microtubules, forming a submembraneous layer, consists of 43 microtubules (Fig. 1e). In addition, region I can be characterised by the presence of spine-like bodies both in the ornamented (Fig. 1f, i) and non-ornamented area (Figs. 1j, k and 2b). The transition between region I and region II is marked by the disorganisation of the first axoneme (Fig. 2c, d).

Region II (Figs. 2e-g, 3d and 4II) corresponds to the middle region of the spermatozoon which is mainly characterised by the sole presence of the second axoneme and the mitochondrion in its distal part (Figs. 2e-g and 4 II). Besides, a large amount of granular material, confirmed as glycogen by means of the Thiéry test, is observed along region II (Figs. 2e-g and 3d).

Region III (Figs. 2h-k, 3a-d and 4III) corresponds to the nuclear and posterior spermatozoon extremity. The presence of the nucleus is the main characteristic of this region (Figs. 2h-k, 3a-c). The transition of characters toward the posterior tip of the sperm cell is marked by the disorganisation of the second axoneme (Fig. 2j), followed by the disappearance of the mitochondrion (Fig. 3a, b), and finally the disappearance of cortical microtubules (Fig. 3c). Therefore, the posterior spermatozoon tip is characterized by the presence of only the nucleus (Fig. 3c).

4. Discussion

The mature spermatozoon of *Prosorhynchoides arcuatus* exhibits the usual ultrastructural characteristics found in most digeneans described so far: two axonemes of the 9+‘1’ trepaxonematan pattern, nucleus, mitochondrion, parallel cortical microtubules, and granules of glycogen. Moreover, lateral expansion, spine-like bodies, and external ornamentation of the plasma membrane are also present (see Table 1). Recently, Bakhoum et al. (2017) have analysed the ultrastructural characters of the mature spermatozoon of digeneans in order to evaluate their potential for phylogenetic purposes and to establish different models of spermatozoa in the Digenea. Therefore, two sets of characters (principal and secondary) have been distinguished. The principal characters were used for the definition of five types of sperm cells in digeneans. These characters included (i) the type of axonemes, (ii) the external ornamentation of the plasma membrane, its level of association with cortical microtubules and its location, (iii) the lateral expansion, (iv) the location of the maximum number of cortical microtubules and their number of bundles, and (v) the number of mitochondria. However, the usefulness of other secondary characters, such as spine-like bodies or terminal sperm characters was not clear according to the authors at the present state of knowledge (Bakhoum et al., 2017).

From the anterior to the posterior extremity of the spermatozoon of *P. arcuatus*, seven characters are discussed and compared with other digeneans, with particular emphasis on bucephalids.

4.1. Axonemes

The spermatozoon of *P. arcuatus* exhibits two trepaxonematan axonemes (Ehlers, 1984). This is the typical structure of axonemes observed in all digeneans except for the species of the genus *Schistosoma* with a special 9+‘1’ pattern (Jamieson and Justine, 2017; Justine et al., 1993) and species of *Didymozoon* with a 9+0 pattern (Justine and Mattei, 1983).

In the Bucephalidae studied up to now, namely, *P. aculeatus*, *P. gracilescens* and *P. elopichthys* (Erwin and Halton, 1983; Miquel et al., 2017; Tang et al., 1998), the spermatozoon presents two axonemes with different lengths which are slightly longitudinally displaced to one another, showing variability at the level of their disorganisation and disappearance. This aspect is particularly interesting in the sperm cell of *P. arcuatus* that shows a short first axoneme (only present in the anterior sperm region) and exhibits the disorganisation of the second axoneme before the mitochondrial end. To our knowledge, this organisation of axonemes and posterior area of sperm cell containing mitochondrion and nucleus and lacking axoneme has never been reported previously in digeneans.

In *P. aculeatus*, one of the axonemes disorganises and disappears in the posterior extremity of the sperm cell before the disappearance of the mitochondrion. The other axoneme disorganises in the posterior spermatozoon tip. Thus, the axoneme constitutes the posterior spermatozoon character. In the remaining bucephalids, the published TEM micrographs of *P. gracilescens* and *P. elopichthys* show a posterior extremity with the presence of the second axoneme as well as some cortical microtubules (Erwin and Halton, 1983; Tang et al., 1998).

4.2. External ornamentation

The mature spermatozoon of *P. arcuatus* displays external ornamentation of the plasma membrane in a large area of the anterior region of the sperm cell as occurs in numerous digeneans. The possible presence of ornamentation, the variability in its location along the spermatozoon and its association or not with cortical microtubules are of particular interest for the establishment of spermatozoa models and for phylogenetic purposes in the Digenea (Bakhoum et al., 2017; Quilichini et al., 2011). So, according to Quilichini et al. (2011) three types of spermatozoa can be recognized based on the external ornamentation: spermatozoa with external ornamentation in the anterior region, with ornamentation in a more posterior part, and sperm cells lacking ornamentation. Recently, Bakhoum et al. (2017) have retained the Quilichini et al.'s criterion and considered the presence/absence as well as the location of the external ornamentation as an interesting character for phylogeny. But these authors added an additional aspect concerning the external ornamentation: its association or not with cortical microtubules. Based on all these criteria, *P. arcuatus* can be classified in the Quilichini et al.'s type 1 of sperm cell according to the external ornamentation, as *P. aculeatus* (Miquel et al., 2017). Moreover, the spermatozoa of both *P. arcuatus* and *P. aculeatus* also exhibit the ornamentation located in the anterior extremity and associated with cortical microtubules.

Concerning the bucephalines *P. gracilescens* (Erwin and Halton, 1983) and *P. elopichthys* (Tang et al., 1998), both species present external ornamentation associated with cortical microtubules and, probably, also located in the anterior area of the sperm cell (see discussion of lateral expansion).

4.3. Cortical microtubules

The location of the maximum number of cortical microtubules along the sperm cell has been considered as a useful character for phylogenetic inference between the Digenea (Bakhoum et al., 2017; Quilichini et al., 2007). Sperm cells of digeneans could be classified according to the presence of the maximum number of cortical microtubules in the anterior part or in a middle area of the gamete. In the spermatozoa of species belonging to the family Bucephalidae, the maximum number of cortical microtubules is located in the anterior region of the male gamete at the level of ornamented area (Erwin and Halton, 1983; Miquel et al., 2017; Tang et al., 1998; present study).

The number of bundles that cortical microtubules exhibited along the mature spermatozoon is another interesting characteristic for the establishment of sperm models in digeneans (Bakhoum et al., 2017). Most of digeneans show an arrangement of cortical microtubules into two fields, as occurs in *P. arcuatus* (present study) and also in the remaining bucephalids (Erwin and Halton, 1983; Miquel et al., 2017; Tang et al., 1998). Other digeneans, particularly of the superfamily Hemiuroidea, do not follow this pattern. In fact, in lecithasterids, hemiurids, and sclerodistomids cortical microtubules are arranged in a single field (Ndiaye et al., 2012, 2013a, 2013b, 2014; Quilichini et al., 2010a) and in didymozoids the sperm cells lack cortical microtubules (Justine and Mattei, 1983).

4.4. Lateral expansion

The lateral expansion is another structure present in the anterior area of the spermatozoon of certain digeneans. Bakhoum et al. (2017) considered the lateral expansion as an interesting characteristic for the establishment of sperm models in digeneans and distinguished two groups of spermatozoa based on the criterion presence/absence of lateral expansion.

In *P. arcuatus* a lateral expansion is observed in the anterior part of region I. Referring to the remaining studied bucephalids, the lateral expansion has never been described. However, this absence in *P. gracilescens*, *P. elopichthys* and *P. aculeatus* (Erwin and Halton, 1983; Miquel et al., 2017; Tang et al., 1998) should be considered with caution taking into account the importance of this character to establish the sperm models. In the prosorhynchine *P. aculeatus*, Miquel et al. (2017) do not mention the presence of a lateral expansion in the anterior extremity of the spermatozoon. But it can be observed before the presence of the second axoneme (see p. 301, Fig. 1a-d). In the bucephalines *P. gracilescens* and *P. elopichthys* the published TEM micrographs also indicate the possible presence of this type of lateral expansion (see p. 420, Fig. 15a, b of Erwin and Halton, 1983, and p. 169, Fig. 13 of Tang et al., 1998, respectively). As it is the case other characters, this is a good example to demonstrate the necessity of an accurate analysis of numerous sections in order to avoid gaps in the ultrastructural organisation of sperm cells.

The morphology of the lateral expansion is variable depending to the species. In fact, certain digeneans exhibit a simple lateral expansion as described in *Scaphiostomum palaearticum* (Ndiaye et al., 2002) or *Diplodiscus subclavatus* (Bakhoum et al., 2011). Other digeneans, as *P. arcuatus*, present a hook-shaped dorsolateral expansion as described in the echinostomatid *Echinostoma caproni* (Iomini and Justine, 1997), in the troglotrematid *Troglotrema acutum* (Miquel et al., 2006), in the mesometrids *Centroderma spinosissima* and *Elstia stossichianum* (Bakhoum et al., 2013a, 2013b), or in the fasciolids *Fasciola hepatica* and *Fasciola gigantica* (Ndiaye et al., 2003, 2004). Concerning *P. aculeatus* (Miquel et al., 2017), the morphology of lateral expansion is also hook-shaped, but located more anteriorly. Probably, this is also the case for *P. gracilescens* and *P. elopichthys* (Erwin and Halton, 1983; Tang et al., 1998).

It is interesting to remark that in all these species, the region of the hook-shaped dorsolateral expansion is the area of the sperm cell presenting external ornamentation associated to cortical microtubules and also the area containing the maximum number of cortical microtubules. This is a crucial aspect in order to establish the type of spermatozoon (see Bakhoum et al., 2017).

4.5. Mitochondria

The presence of mitochondria is always observed in digenean spermatozoa. It is considered as a plesiomorphic character present in all the parasitic Platyhelminthes with the exception of the Eucestoda (Justine, 1991a). The mitochondrion present in the mature spermatozoon is formed during spermiogenesis. Indeed, several mitochondria are present in the zone of differentiation. They penetrate into the spermatid body and fuse to form a long mitochondrion (Burton, 1972; Rees, 1979). However, the number of mitochondria in the spermatozoon of digeneans is still a matter of controversy (see Miquel et al., 2006). For many years, following Burton’s statement, the digenean sperm cells have been described containing only a single mitochondrion. However, sometimes there are evident problems for a logical interpretation of sections considering the presence of a single mitochondrion. At the current state of knowledge, there are digeneans with one, two, and even three mitochondria in the male gamete (see Bakhoum et al., 2017).

The mature spermatozoon of *P. arcuatus* contains one mitochondrion. As in most digeneans, when a single mitochondrion is present, it is located in the middle region of the sperm cell and it usually overlaps the anterior part of the nucleus. Nevertheless, the most interesting aspect concerning the mitochondrion in *P. arcuatus* is its placement. We describe for the first time a digenean sperm cell in which the mitochondrion is still present when the axonemes are no longer present. In the remaining studied bucephalids (*P. gracilescens, P. elopichthys* and *P. aculeatus*, Erwin and Halton, 1983; Miquel et al., 2017; Tang et al., 1998) there is also only one mitochondrion in their spermatozoa, but it always disappears before the second axoneme.

4.6. Spine-like bodies

Spine-like bodies were described for the first time in the opecoelid *Opecoeloides furcatus* by Miquel et al. (2000) as submembraneous and prominent electron-dense elements which contain a sort of vesicular structure. When present, spine-like bodies have been reported in the anterior part of the spermatozoon, normally associated with the external ornamentation. In fact, to our knowledge, only *Neoapocreadium chabaudi* (Kacem et al., 2010) exhibits spine-like bodies in the non-ornamented area of the spermatozoon. Since its original description, spine-like bodies have frequently been reported in digenean mature spermatozoa. Moreover, this character can be observed in TEM micrographs of older studies on male gametes, which were omitted and probably misinterpreted as artefacts of fixation (Justine and Mattei, 1982; Orido, 1988). In our opinion, spine-like bodies are important structures in digenean spermatozoa that are probably involved with external ornamentation in the fertilization process (see Justine and Mattei, 1984).

In the Bucephalidae, there is variability in the presence/absence of spine-like bodies in the spermatozoon. These structures are lacking in male gametes of *P. gracilescens* and *P. elopichthys* (Erwin and Halton, 1983; Tang et al., 1998) while they are present in *P. aculeatus* and *P. arcuatus* (Miquel et al., 2017; present study). Thus, as for most digeneans, the spermatozoon of *P. aculeatus* presents spine-like bodies in its ornamented area (Miquel et al., 2017). From *P. arcuatus*, we describe the presence of spine-like bodies both in the ornamented and non-ornamented region of the gamete. Moreover, in both bucephalids the number of spine-like bodies seems to be higher and smaller in size than in most digeneans.

4.7. Posterior spermatozoon extremity

In digenean species, the posterior spermatozoon extremity exhibits a diversity of morphologies involving characters such as axonemes, cortical microtubules, mitochondrion and nucleus. The transition of three characters (axoneme, cortical microtubules and nucleus) toward the posterior spermatozoon tip was proposed as a character for phylogenetic purposes by Quilichini et al. (2010b) who postulated three different posterior spermatozoon extremities: Opecoelidean type characterized by the sequence axoneme, nucleus and cortical microtubules, the Fasciolidean type with the sequence cortical microtubules, axoneme and nucleus and the Cryptogonimidean type with the sequence cortical microtubules, nucleus and axoneme. Nevertheless, other posterior spermatozoon morphologies are not congruent with these three models (i.e. *Deropristis inflata* -Foata et al., 2007-, *S. palaearcticum* -Ndiaye et al., 2002- or *Aponurus laguncula* -Quilichini et al., 2010a). The posterior sequence of characters is also different in the case of *P. arcuatus* considering the posterior end of the mitochondrion after the stopping of the second axoneme. In the recent revision of Bakhoum et al. (2017), authors have recommended to use only the last spermatozoon character instead of the sequence of characters. There are differences in the posterior spermatozoon character among the bucephalids studied until now. Concerning bucephalines, sperm cells of *P. arcuatus* exhibit the nucleus as posterior character whereas spermatozoa of *P. gracilescens* and *P. elopichthys* present the axoneme or cortical microtubules as posterior character (Erwin and Halton, 1983; Tang et al., 1998). Finally, the spermatozoon of *P. aculeatus* shows the axoneme as posterior character (Miquel et al., 2017).

Concluding remarks

The comparative analysis of the ultrastructural organisation of the male gamete in the bucephalids studied until now shows that, probably, the Bakhoum et al.'s type V is the typical model for this family of digeneans. As we have argued in the discussion section (see 4.4) the presence/absence of lateral expansion is the most important character to establish a general pattern of the sperm cells for the Bucephalidae. In fact, there are two types of spermatozoa presenting the maximum number of cortical microtubules and external ornamentation of the plasma membrane simultaneously at the same level (types III and V) but located in different regions. However, from these two models, only the type V presents lateral expansion. In fact, these three characteristics are located in the anterior region of the spermatozoon. Consequently, in our opinion, the bucephalids sperm cell type is the type V, characterised by the presence of: (i) two 9+'1' axonemes, (ii) lateral expansion, (iii) external ornamentation associated with cortical microtubules and located in the anterior part of the spermatozoon, (iv) cortical microtubules organised in two bundles and with their maximum number in the anterior part of the sperm cell, and (v) one mitochondrion. Nevertheless, future studies involving species belonging to the three remaining subfamilies of the Bucephalidae (the Dolichoenterinae, Heterobucephalopsinae and Paurorhynchinae) are needed in order to establish a more consistent pattern of organisation of the spermatozoon and assess the phyletic relationships within this family from an ultrastructural approach.

Competing interests

The authors declare that they have no competing interests.

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Legends

Fig. 1. Spermatozoon of *Prosorhynchoides arcuatus* (region I). (a–c) Longitudinal and cross-sections of the anterior spermatozoon extremity (ASE). (d–g) Longitudinal and cross-sections of the ornamented area showing the external ornamentation (EO) associated with the lateral expansion (LE). (h, i) Two cross-sections of the ornamented area with and without spine-like bodies (SB). (j) Longitudinal section showing the interruption of the external ornamentation (interrupted line). (k) Longitudinal section showing the presence of spine-like bodies in the post-ornamented area of region I. Ax1 and Ax2, first and second axoneme; C1, centriole of the first axoneme; CC2, central core of the second axoneme; CM, cortical microtubules; Scale bars: 300 nm.

Fig. 2. Spermatozoon of *Prosorhynchoides arcuatus* (regions I, II and III). (a, b) Cross-sections of the post-ornamented area of region I with and without spine-like bodies (SB). (c, d) Posterior area of region I showing the disorganisation of the first axoneme. (e) Cross-section of region II showing the appearance of granules of glycogen (G). (f) Cross-section showing the presence of mitochondrion (M). (g) Longitudinal section of region II. (h–k) Correlative cross-sections of region III from the appearance of nucleus (N) to the disappearance of the second axoneme. Ax2, second axoneme; CM, cortical microtubules; D, doublets; S, singlets. Scale bars: 300 nm.

Fig. 3. Spermatozoon of *Prosorhynchoides arcuatus* (regions II and III). (a–c) Longitudinal and cross-sections showing the posterior spermatozoon extremity (PSE) (region III). (d) Positive test of Thiéry for glycogen (G). Ax2, second axoneme; CM, cortical microtubules; M, mitochondrion; N, nucleus. Scale bars: 300 nm.

Fig. 4. Schematic reconstruction of the mature spermatozoon of *Prosorhynchoides arcuatus*. The sperm cell is organised 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. ASE, anterior spermatozoon extremity; Ax1 and Ax2, first and second axoneme; C1 and C2, centriole of the first and second axoneme; CM, cortical microtubules; D, doublets; EO, external ornamentation of the plasma membrane; G, granules of glycogen; LE, lateral expansion; M, mitochondrion; N, nucleus; PM, plasma membrane; PSE, posterior spermatozoon extremity; SB, spine-like bodies.

Table

Table 1. Available data on the ultrastructure of the spermatozoon in the Bucephalidae.

Subfamilies and species	Spermatozoon characters											References
	TS	TAx	LE	EO	EO+CM	LEO	BCM	LMCM	M	SB	PSC	
BUCEPHALINAE												
Prosorhynchoides arcuatus	V	9+‘1’	+	+	+	AntA	2	AntS	1	+	N	Present study
Prosorhynchoides gracilescens	V?	9+‘1’	+/-?	+	+	AntA	2	AntS	1	-	Ax/CM?	Erwin and Halton (1983)
Pseudorhipidocotyle elopichthys	V?	9+‘1’	+/-?	+	+	AntA	2	AntS	1	-	Ax/CM?	Tang et al. (1998)
PROSORHYNCHINAE												
Prosorhynchus aculeatus	V	9+‘1’	+	+	+	AntA	2	AntS	1	+	Ax	Miquel et al. (2017)

AntA, anterior part of the anterior region; AntS, anterior region of the spermatozoon; Ax, axoneme; BCM, number of bundles of cortical microtubules; CM, cortical microtubules; EO, external ornamentation of plasma membrane; EO+CM, association of external ornamentation with cortical microtubules; LE, lateral expansion; LEO, location of external ornamentation; LMCM, location of maximum number of cortical microtubules; M, number of mitochondria; N, nucleus; PSC, posterior spermatozoon character; SB, spine-like bodies; TAx, type of axoneme; TS, type of spermatozoon; +/-, presence/absence of considered character; ?, doubtful data.

Figure 1

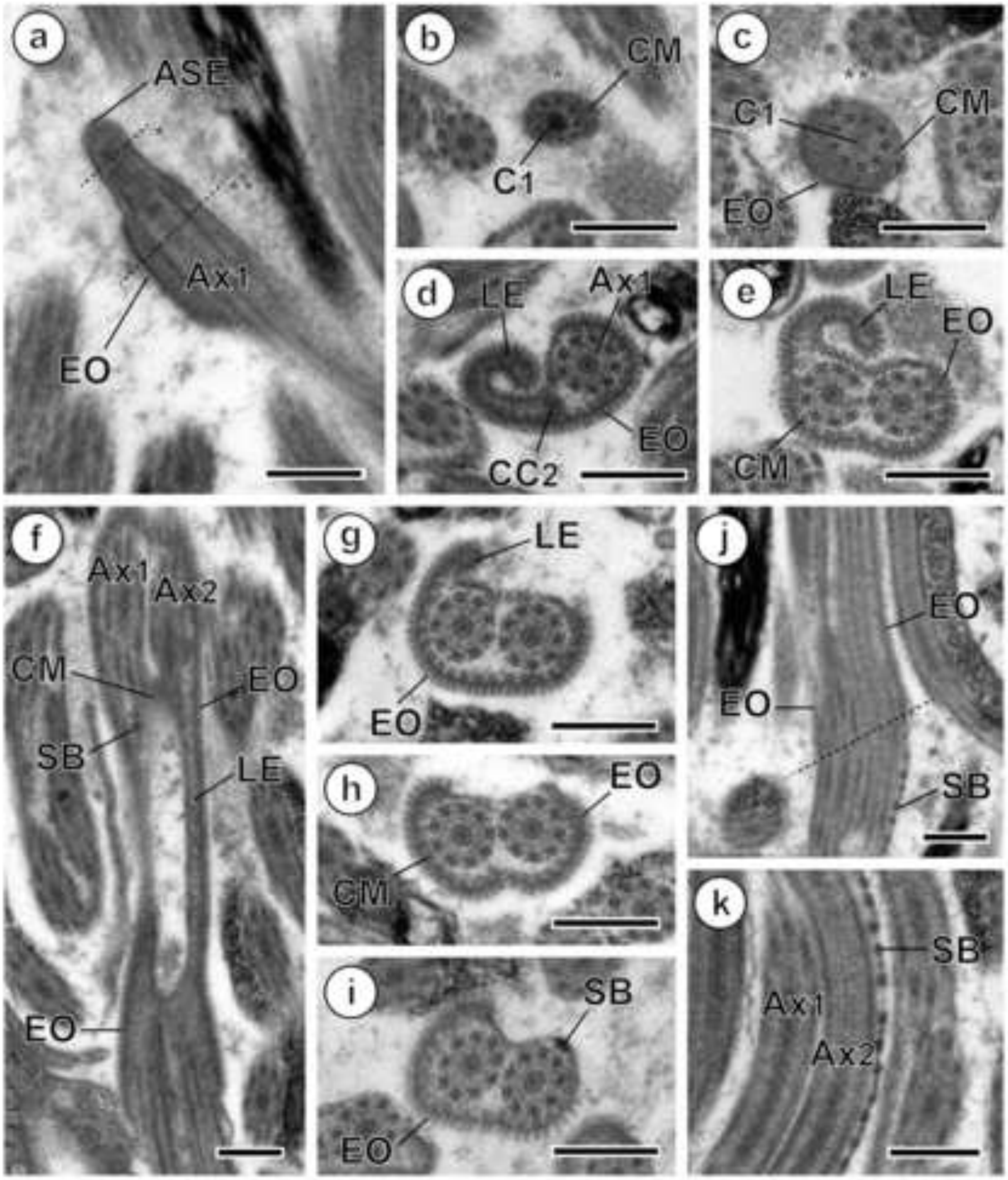
a
ASE
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Figure 2
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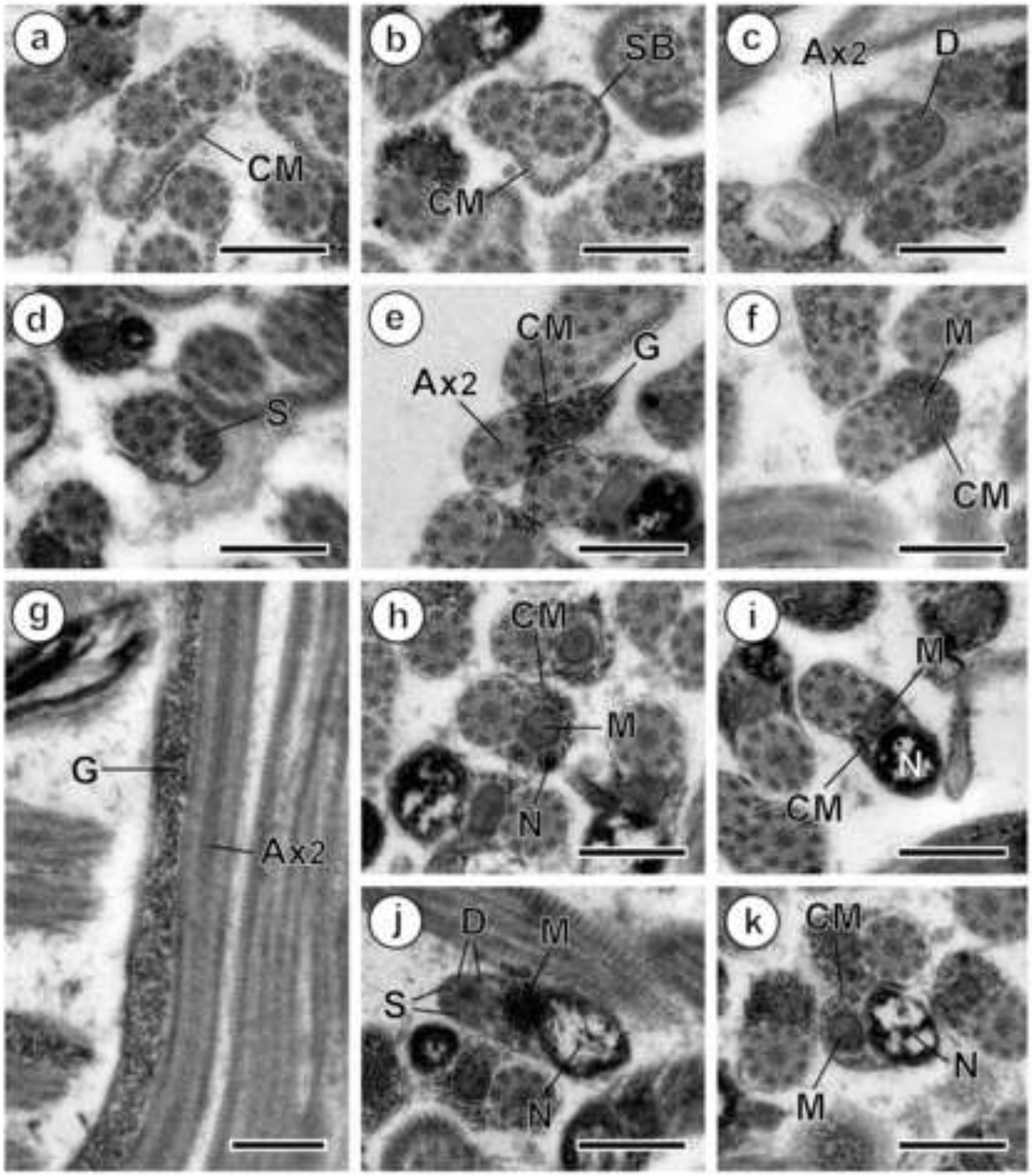

Figure3

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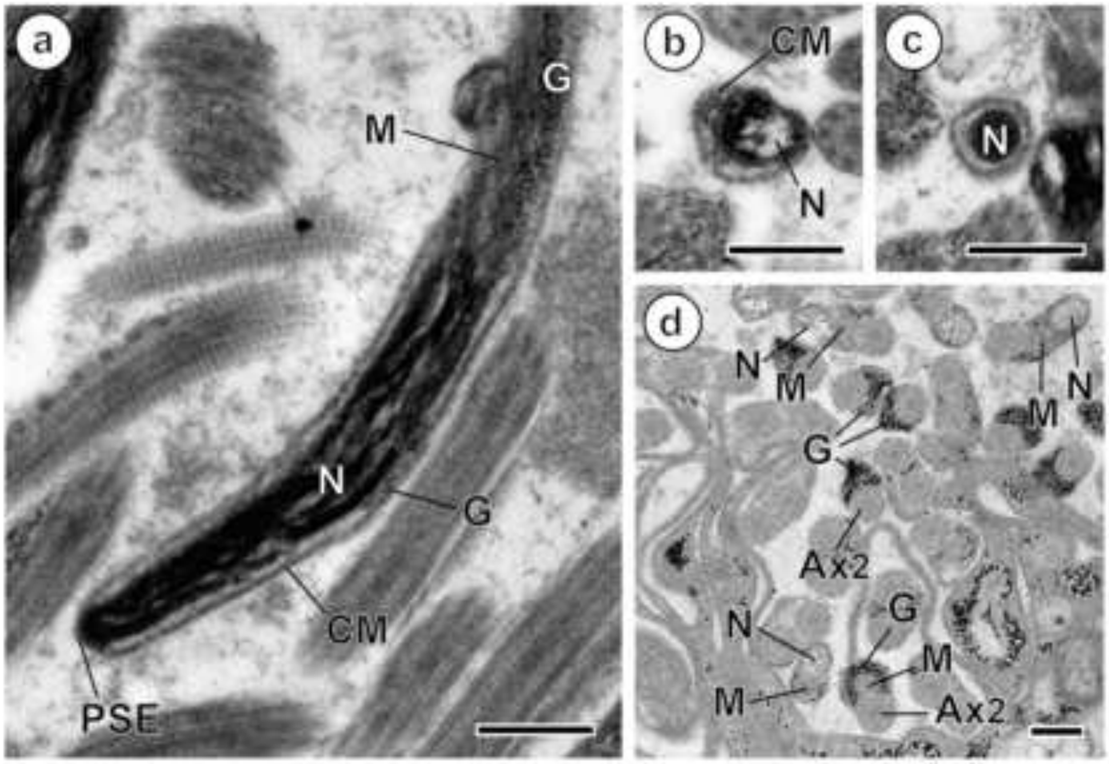
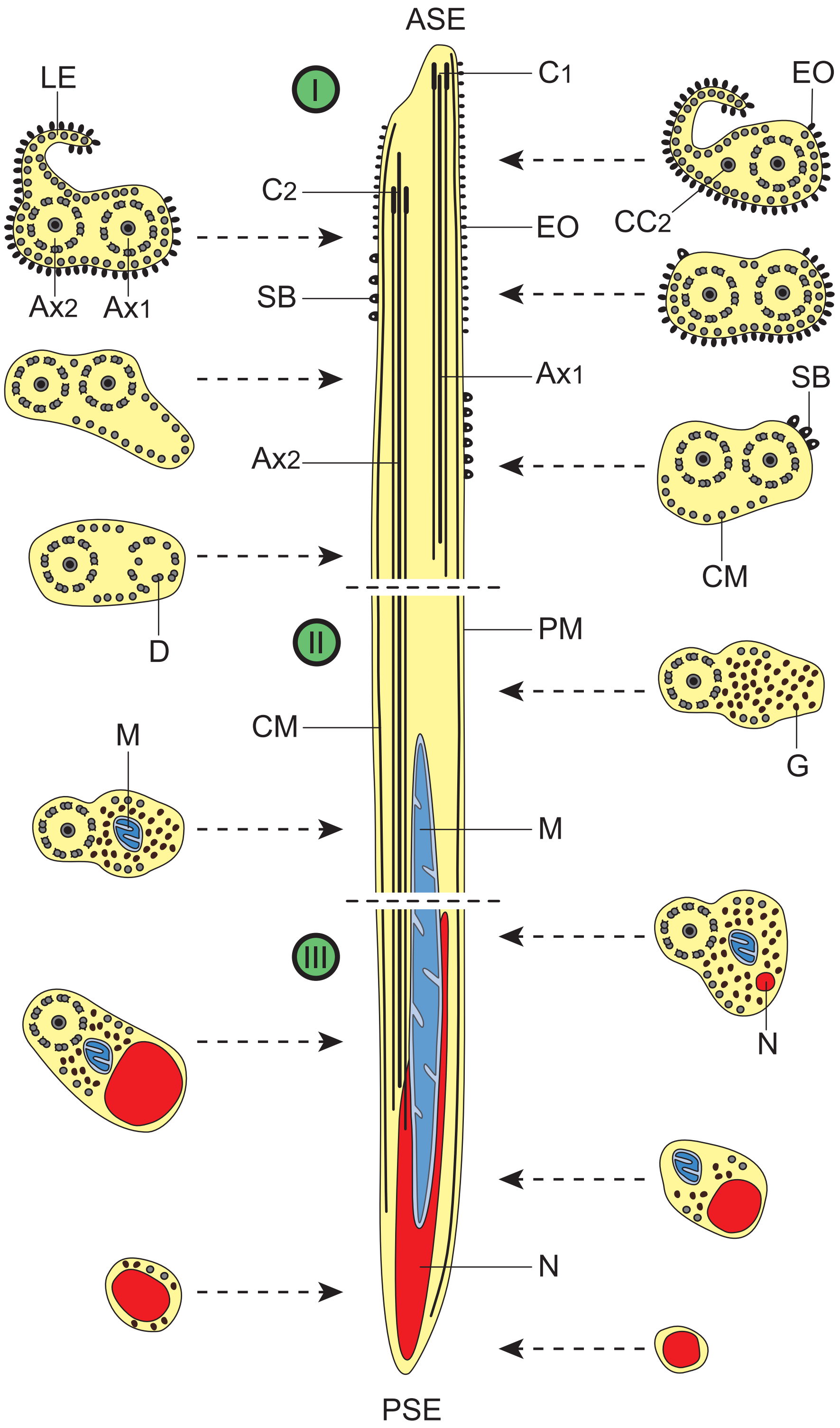


Figure 4


ASE
I
II
III
LE
Ax2
Ax1
C1
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M
M
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N
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