Spermiogenesis and ultrastructure of the spermatozoon of the trypanorhynch cestode *Aporhynchus menezesi* (Aporhynchidae), a parasite of the velvet belly lanternshark *Etmopterus spinax* (Elasmobranchii: Etmopteridae)

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Abstract: Spermiogenesis and the ultrastructural organisation of the spermatozoon of the trypanorhynch cestode *Aporhynchus menezesi* Noever, Caira, Kuchta et Desjardins, 2010 are described by means of transmission electron microscopy. Type I spermiogenesis of *A. menezesi* starts with the formation of a differentiation zone containing two centrioles separated by an intercentriolar body constituted by five electron-dense plates. Each centriole gives rise to a free flagellum, which grows at an angle of 90° in relation to a median cytoplasmic process. The nucleus and cortical microtubules elongate along the spermatid body. Later, both flagella rotate and fuse with the median cytoplasmic process. At the final stage of spermiogenesis, the young spermatozoon is detached from the residual cytoplasm by a narrowing of the ring of arched membranes. The mature spermatozoon is a long and filiform cell, tapered at both ends, lacking mitochondria. It is characterized by the presence of two axonemes of the 9+‘1’ trepaxonematan pattern, the absence of crested bodies, the presence of parallel cortical microtubules and nucleus. This pattern corresponds to the type I spermatozoon of the eucestodes. The anterior extremity of the spermatozoon is characterized by the presence of an arc-like row of up to seven parallel cortical microtubules that partially surrounds the first axoneme. These anterior cortical microtubules are thicker than the posterior microtubules and, consequently, the sperm cell of *A. menezesi* exhibits two types of cortical microtubules. Another interesting aspect is the presence of α -glycogen rosettes. This spermatological pattern is similar to that observed in the spathebothriidean and diphyllbothriidean cestodes.

Keywords: *Aporhynchus menezesi*, Cestoda, Trypanorhyncha, Gymnorhynchoidea, Aporhynchidae, ultrastructure, spermiogenesis, spermatozoon

Trypanorhynch cestodes are amongst the most common metazoan parasites of marine fish. Whilst adults are typically found in the stomach and intestine of sharks and rays, larval forms infect a wide variety of marine invertebrates and teleosts. This order includes marine polyzoic cestodes, which are unique in the possession of a rhyncheal apparatus. The Trypanorhyncha Diesing, 1863 is characterized by a scolex bearing two or four bothria (Jones et al. 2004) and a tentacular apparatus consisting of four retractile tentacles adorned with hooks as extensions of tentacle sheaths that are attached to four bulbs (Dollfus 1942). This complex attachment appara-

tus is unique within the cestodes, and provides a strong synapomorphy that supports the monophyly of this order (see Palm et al. 2009).

Palm (2004) recognized 254 trypanorhynch species. However, in the last years, several authors have added 25 more species to the order (see Palm et al. 2009, Noever et al. 2010). Thus, a total of 279 trypanorhynch species can be considered valid, with additional species and genera being described every year.

All these species were previously inserted into four different superfamilies (see Campbell and Beveridge 1994), namely Homeacanthoidea Dollfus, 1942, Hetera-

canthoidea Dollfus, 1942, Obothrioidea Dollfus, 1942, and Poecilacanthoidea Dollfus, 1942. However, considering characters other than the patterns of tentacle hook organisation, Palm (2004) reorganized the order Trypanorhyncha into five superfamilies and created a new family (Progrillotiidae Palm, 2004). These superfamilies are the Tentacularioidea Poche, 1926, Gymnorhynchoidea Dollfus, 1935, Lacistorhynchoidea Guiart, 1927, Obothrioidea and Eutetrarhynchoidea Guiart, 1927. The cladistic analysis also presented by Palm (2004) and based on a matrix of morphological data shows the Trypanorhyncha as a polyphyletic order. In this sense, the molecular study of Palm et al. (2009) is congruent with this condition and demonstrates that the trypanorhynchs consist of two well-supported major clades. The first clade groups the superfamily Eutetrarhynchoidea and the Tentacularioidea while the second clade groups the Gymnorhynchoidea, the Lacistorhynchoidea and the Obothrioidea.

Although the genus *Aporhynchus* Nybelin, 1918 was first placed within the family Gilquiniidae Dollfus, 1942 by Campbell and Beveridge (1994), recently this genus has been attributed to the Aporhynchidae Poche, 1926 by Palm (2004). Thus, the family Aporhynchidae contains only the genus *Aporhynchus*. However, according to Beveridge et al. (1999) a cladistic analysis of the trypanorhynch genera revealed a close relationship between the genera *Aporhynchus*, *Gilquinia* Guiart, 1927 and *Deanicola* Beveridge, 1990. Therefore the precise phylogenetic position of gilquiniid and aporhynchid species is still unclear.

In the last years the ultrastructural data on spermatology have been demonstrated to be useful to interpret the relationships of the Platyhelminthes (Euzet et al. 1981, Justine 1991, 1998, 2001, Bâ and Marchand 1995, Levron et al. 2010) and they were incorporated with morphological characters and molecular data for a better interpretation of Platyhelminthes relationships (Hoberg et al. 1997, Olson et al. 2001). There is a large amount of ultrastructural studies on eucestode species, but most of them refer to species belonging to the order Cyclophyllidae van Beneden in Braun, 1900 (see Justine 1998, 2001, Levron et al. 2010). In contrast, considering the order Trypanorhyncha, data on the ultrastructural characters of spermatozoa or spermiogenesis refer only to two lacistorhynchiids, namely *Lacistorhynchus tenuis* (van Beneden, 1858) and *Grillotia erinaceus* (van Beneden, 1858), and two eutetrarhynchids, namely *Dollfusiella spinulifera* (Beveridge et Jones, 2000) and *Parachristianella trygonis* Dollfus, 1946 (see Świdorski 1976, 1994, McKerr 1985, Miquel and Świdorski 2006, Miquel et al. 2007a). Data concerning *L. tenuis* were presented in two conferences by Świdorski (1976, 1994) while data on *G. erinaceus* were included in the unpublished PhD thesis of McKerr (1985). With respect to the eutetrarhynchids, Miquel and Świdorski (2006) and Miquel et al. (2007a) only describe

the ultrastructural organisation of the mature spermatozoon.

The aim of the present study is to describe the ultrastructure of spermiogenesis and of the mature spermatozoon of a gymnorhynchoidean trypanorhynch, *Aporhynchus menezesi* Noever, Caira, Kuchta et Desjardins, 2010, thus providing the first ultrastructural study on this superfamily and increasing the available spermatological data concerning the Trypanorhyncha.

MATERIALS AND METHODS

Live adult specimens of *A. menezesi* were collected from the spiral valve of a naturally infected velvet belly lanternshark, *Etmopterus spinax* (L.) (Elasmobranchii, Etmopteridae) captured off the Faial Island (38°31'N, 28°37'W) (Azores Archipelago, Portugal).

Live cestodes were first placed in a 0.9% NaCl solution and the mature proglottids were fixed in cold (4 °C) 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer at pH 7.2 for a minimum of 2 h, rinsed in a 0.1 M sodium cacodylate buffer at pH 7.2, postfixed in cold (4 °C) 1% osmium tetroxide in the same buffer for 1 h, rinsed in a 0.1 M sodium cacodylate buffer at pH 7.2, dehydrated in an ethanol series and propylene oxide, and finally embedded in Spurr's resin. Ultrathin sections were obtained using a Reichert-Jung Ultracut E ultramicrotome, placed on copper grids and double-stained with uranyl acetate and lead citrate (Reynolds 1963). Ultrathin sections were examined using a JEOL 1010 TEM operated at an accelerating voltage of 80 kV.

The Thiéry (1967) technique was used for proving the presence of glycogen particles. Gold grids were treated in periodic acid, thiocarbonylhydrazide, and silver proteinate (PA-TCH-SP) as follows: 30 min in 10% PA, rinsed in distilled water, 24 h in TCH, rinsed in acetic solutions and distilled water, 30 min in 1% SP in the dark, and rinsed in distilled water.

RESULTS

Spermiogenesis

The sperm differentiation or spermiogenesis in *A. menezesi* starts with the formation of a rosette containing numerous peripheral spermatids attached to the central cytophore (Fig. 1). The cytoplasm of the spermatids contains a nucleus and a large, densely packed accumulation of mitochondria (Fig. 1). Later the plasma membrane of the spermatid cluster invaginates and forms the differentiation zone situated on the periphery of each spermatid (Figs. 2, 3, 9a). This is a conical protrusion bordered by cortical microtubules. It contains two centrioles interconnected by an intercentriolar body and associated with striated rootlets (Figs. 2–4, 9a). Each centriole gives rise to a flagellum, which grows externally (Figs. 3, 9b, c). The intercentriolar body is composed of five plates: a central thick electron-dense plate, one thin electron-dense plate on each side of the central plate and one thicker electron-dense plate externally on each side of the thin plates (Fig. 4). Cortical microtubules lengthen along a grow-

Figs. 1–8. Spermiogenesis of *Aporhynchus menezesi*. **Fig. 1.** Rosette showing numerous peripheral spermatids attached to the central cytophore (Cy). N – nucleus. **Fig. 2.** Cross-section of the zone of differentiation showing the two centrioles (C) and the intercentriolar body (IB). **Fig. 3.** Longitudinal section of a zone of differentiation showing the two flagella (F), the nucleus (N) and the intercentriolar body (IB). CM – cortical microtubules. **Fig. 4.** Detail of the intercentriolar body showing five electron-dense plates. **Fig. 5.** Longitudinal section of a spermatid during flagellar rotation. AM – arched membranes; CM – cortical microtubules; F – flagellum; MCP – median cytoplasmic process; N – nucleus; SR – striated roots. **Fig. 6.** Longitudinal section of a spermatid showing the two parallel flagella (F) before the proximodistal fusion. AM – arched membranes; CM – cortical microtubules; MCP – median cytoplasmic process. **Fig. 7.** Longitudinal section of a spermatid after the proximodistal fusion, showing one axoneme (Ax). AM – arched membrane; C – centriole. **Fig. 8.** Final stage of the spermiogenesis showing the strangulation of the ring of arched membranes (AM). Scale bars: Fig. 1 = 2.5 μm ; Fig. 2 = 0.5 μm ; Figs. 3, 5–8 = 1 μm ; Fig. 4 = 0.25 μm .

Fig. 9(a–f). Schematic drawing showing the main stages of spermiogenesis of *Aporhynchus menezesi*. AM – arched membranes; Ax1 – axoneme 1; Ax2 – axoneme 2; C1 – centriole 1; C2 – centriole 2; CM – cortical microtubules; F1 – flagellum 1; F2 – flagellum 2; IB – intercentriolar body; MCP – median cytoplasmic process; N – nucleus; SR – striated rootlets.

ing median cytoplasmic process. The nucleus initiates its elongation and the flagella undergo a 90° rotation (Figs. 3, 5, 6, 9c, d) becoming parallel to the median cytoplasmic process (Figs. 6, 9d). The flagella rotation is followed by their proximodistal fusion with the median cytoplasmic process (Figs. 7, 9e). Finally, the ring of arching membranes is strangled and the young spermatozoon is detached from the residual cytoplasm (Figs. 8, 9f).

Spermatozoon

The observation of numerous longitudinal and cross-sections has enabled us to establish four (I–IV) different regions in the mature spermatozoon of *A. menezesi*. It is a long filiform cell, tapered at both ends, which lacks mitochondria and crested body. The cytoplasm contains two axonemes of different lengths of the 9+1 pattern of trepaxonematan Platyhelminthes, a parallel nucleus,

α -glycogen rosettes and two types of cortical microtubules: the first type refers to microtubules that exhibit an opaque electron-dense centre and the second type refers to microtubules that exhibit a hollow centre.

Region I (Figs. 10–14, 25I) constitutes the anterior extremity of the spermatozoon. The anterior tip contains four to seven thick and electron-dense cortical microtubules (Figs. 10, 11) that later form a semi-arc beneath the plasma membrane partially surrounding the first centriole (Figs. 12, 13) and also the proximal areas of the first axoneme (Fig. 14).

Region II (Figs. 15–18, 24, 25II) constitutes the prenuclear area of the spermatozoon containing two axonemes. At the end of region I, the semicircle of electron-dense cortical microtubules is interrupted by scattered tubular elements (Fig. 15), indicating the appearance of the second axoneme. Later the second centriole emerges, mate-

Figs. 10–23. Mature spermatozoon of *Aporhynchus menezesi*. **Fig. 10.** Longitudinal section of Region I near the anterior spermatozoon extremity. **Figs. 11, 12.** Cross-sections of the anterior part of Region I showing the electron-dense microtubules (CM). **Fig. 13.** Cross-section showing the appearance of the first centriole (C1). CM – cortical microtubules. **Fig. 14.** Cross-section of Region I at the level of the first axoneme showing the arc-like row of electron-dense cortical microtubules (CM). **Figs. 15, 16.** Cross-sections of anterior areas of Region II showing the first axoneme (Ax1) and the appearance of the second centriole (C2). CM – cortical microtubules. **Fig. 17.** Cross-section of Region II illustrating the presence of two pairs of attachment zones (AZ). **Fig. 18.** Cross-sections of Region II showing the appearance of granules of glycogen (G). **Fig. 19.** Cross-section of Region III showing the nucleus (N). CM – cortical microtubules; G – granules of glycogen. **Fig. 20.** Micrograph of the nuclear area showing a cross-section located before and another cross-section located after (*) the disorganisation of one of the axonemes. CM – cortical microtubules; N – nucleus. **Fig. 21.** Cross-section of Region III at the beginning of the disappearance of the central core of one of the axonemes. D – doublets; N – nucleus. **Fig. 22.** Cross-sections of Region IV showing the axoneme surrounded by the plasma membrane. **Fig. 23.** Cross-sections of Region IV near the posterior spermatozoon extremity. D – doublets; S – singlets. Scale bars: Fig. 10 = 0.5 μ m; Figs. 11–23 = 0.25 μ m.

Fig. 24. Cross- and longitudinal sections of the mature spermatozoon of *Aporhynchus menezesi* showing rosettes of α -glycogen evidenced by Thiéry staining. Scale bar = 0.5 μ m.

rialized by the appearance of nine centriolar doublets in the cytoplasm (Fig. 16). At this level the presence of the hollow-centre cortical microtubules is observed (Figs. 17, 18). They are distributed in two opposite fields and their quantity progressively increases (Figs. 17, 18). A narrow cytoplasmic bridge connects the axonemes in some spermatozoa (Fig. 17) and the attachment zones are also observed at this stage (Fig. 17). Later, two opposite rows of cortical microtubules are situated under the plasma membrane (Figs. 17, 18). The granules of glycogen, in the form of α -glycogen rosettes, appear progressively as the spermatozoon section enlarges (Figs. 18, 24).

Region III (Figs. 19–21, 25III) constitutes the nuclear area of the spermatozoon. In cross-sections, the nucleus is oval to circular, situated centrally between the axonemes (Figs. 19, 20). It contains strands of fine fibrillar material and enlarges gradually towards the posterior end. In the first part, this region also encloses two axonemes, cortical microtubules, and a large amount of glycogen (Fig. 19). Later the presence of glycogen gradually decreases (Fig. 20). The nuclear region is the area of the spermatozoon that contains the maximum number of cortical microtubules arranged in two parallel fields (Figs. 19–21). Subsequently, one of the axonemes loses its central element (Fig. 21); doublets disorganize and transform into singlets, which finally disappear. The posterior part of the nuclear region contains only one axoneme, the nucleus and some cortical microtubules (Fig. 20).

Region IV (Figs. 22, 23, 25IV) constitutes the postnuclear area of the spermatozoon. This is the posterior end and contains the axoneme surrounded by cytoplasmic membrane (Fig. 22). This single axoneme later disorganizes and disappears at the level of the posterior tip of the sperm cell (Fig. 23).

Fig. 25(I–IV). Schematic drawing showing the ultrastructural organisation of the mature spermatozoon of *Aporhynchus menezesi*. ASE – anterior spermatozoon extremity; Ax1 – axoneme 1; Ax2 – axoneme 2; AZ – attachment zone; C1 – centriole 1; C2 – centriole 2; CDM – electron-dense cortical microtubules; CLM – electron-lucent cortical microtubules; D – doublets; G – α -glycogen rosette; N – nucleus; PM – plasma membrane; PSE – posterior spermatozoon extremity; S – singlets.

Table 1. Spermatological characters in trypanorhynch cestodes.

Superfamily, family and species (reference)	Spermiogenesis					Spermatozoon							
	Type	FR	PF	IB	SR	Type	ASE	AC	CB	ArcCM	AZ	G	PSE
Eutetrarhynchoidea, Eutetrarhynchidae													
<i>Dollfusiella spinulifera</i> (Miquel and Świderski 2006)						I	CM	–	–	10	+	α, β	1Ax
<i>Parachristianella trygonis</i> (Miquel et al. 2007a)						I	CM	–	–	10	+	α, β	1Ax
Lacistorhynchoidea, Lacistorhynchidae													
<i>Lacistorhynchus tenuis</i> (Świderski 1976, 1994)	I	+	+	5	+			–				β	
<i>Grillotia erinaceus</i> (McKerr 1985)	I	+	+	7	+			–				α, β	
Gymnorhynchoidea, Aporhynchidae													
<i>Aporhynchus menezesi</i> (present study)	I	+	+	5	+	I	CM	–	–	7	+	α	1Ax

AC – apical cone; ArcCm – number of cortical microtubules in the arc-like row; ASE – anterior spermatozoon extremity; Ax – axoneme; AZ – attachment zone; CB – crested body; CM – cortical microtubules; FR – flagellar rotation; G – type of glycogen; IB – number of plates of the intercentriolar body; PF – proximodistal fusion; PSE – posterior spermatozoon extremity; SR – striated rootlets; +/- presence/absence of character; no marked characters correspond to unknown data or requiring confirmation. Spermiogenesis and spermatozoa types are considered according to Bâ and Marchand (1995) and Levron et al. (2010), respectively.

DISCUSSION

Spermiogenesis

The process of spermiogenesis in *Aporhynchus menezesi* includes the presence of two centrioles, an intercentriolar body and two striated rootlets in the differentiation zone, flagellar rotation and proximodistal fusion. Therefore it corresponds to the basic pattern of the pseudophyllidean type of spermiogenesis described by Świderski (1986) or the type I of Bâ and Marchand (1995). Type I spermiogenesis has been reported in species belonging to the orders Spathebothriidea Wardle et McLeod, 1952, Diphyllbothriidea Kuchta, Scholz, Brabec et Bray, 2008, Bothriocephalidea Kuchta, Scholz, Brabec et Bray, 2008, Diphyllidea van Beneden in Carus, 1863, Trypanorhyncha, Tetraphyllidea Carus, 1863, and Proteocephalidea Mola, 1928 (see the reviews of Justine 1998, 2001, Bruňanská 2010, Levron et al. 2010) and was considered to be a plesiomorphic process in the eucestodes by Justine (1998). All the studied trypanorhynch cestodes exhibit this type of spermiogenesis. However, the number of plates (five or seven) forming the intercentriolar body is variable in these species (see Table 1). In fact, the number of intercentriolar body plates is seven in *G. erinaceus* (Lacistorhynchoidea) (see McKerr 1985) and five in the remaining studied species, namely *L. tenuis* (Lacistorhynchoidea) (see Świderski 1994) and *A. menezesi* (Gymnorhynchoidea) (present study).

The number of intercentriolar plates has been used in a recent phylogenetic analysis of the Eucestoda inferred both from ultrastructural data alone and from a combination of ultrastructural and morphological data (Levron et al. 2010). Within the Eucestoda, although the intercentriolar body is absent from the Tetrabothriidea and Cyclophyllidea, it is present as a single-plate body in the Lecanicephalidea Wardle et McLeod, 1952 and Proteocephalidea and as a multiplate body in the remaining orders. However, it is interesting to remark the dual condition of mesocestoidids, presenting both single and multiplate intercentriolar bodies (see Miquel et al. 1999, 2007b).

Although the type of spermiogenesis in trypanorhynch cestodes is similar to that described in the caryophyllideans, in the spathebothriideans and in certain species of the former Pseudophyllidea Carus, 1863 (see Bruňanská et al. 2001, 2006, Levron et al. 2005, 2006a, b, 2009, Bruňanská and Poddubnaya 2006, 2010, Miquel et al. 2008, Bruňanská 2009, Šípková et al. 2010), the electron-dense material appearing in initial stages of spermiogenesis in the apical region of the differentiation zone is not observed in *A. menezesi* as in the other trypanorhynch species studied to date (McKerr 1985, Świderski 1994).

Spermatozoon

No evident differences were found in the ultrastructural organisation of the spermatozoon of *A. menezesi* in comparison with the other trypanorhynch species studied to date (Świderski 1976, McKerr 1985, Miquel and Świderski 2006, Miquel et al. 2007a). The spermatozoon possesses two axonemes of the 9+‘1’ trepaxonematan pattern (Ehlers 1984) of different lengths, a parallel nucleus, parallel cortical microtubules and lacks crested body, periaxonemal sheath and transverse intracytoplasmic walls. This type of sperm's schema corresponds to the type I of Levron et al. (2010) and is found in species of the orders Spathebothriidea, Diphyllbothriidea, Haplobothriidea Joyeux et Baer, 1961 and Trypanorhyncha.

As in *Dollfusiella spinulifera* and *Parachristianella trygonis*, the anterior extremity of the spermatozoon of *A. menezesi* contains a single axoneme partially surrounded by an arc-like layer of submembranous cortical microtubules parallel to the hypothetical spermatozoon axis. Moreover, the cortical microtubules that constitute this arc-like layer are thick and their centre is electron-dense, while posterior cortical microtubules present a hollow centre. Consequently, two types of cortical microtubules can be considered in the spermatozoon of these trypanorhynchs (Miquel and Świderski 2006, Miquel et al. 2007a, Levron et al. 2010).

This arc-like row of cortical microtubules has been observed in the anterior part of the spermatozoon of numer-

ous eucestodes, but there is variability in their aspect and number. In trypanorhynch species the number of cortical microtubules forming this arc-like layer varies from seven to ten microtubules. Thus, *D. spinulifera* and *P. trygonis* present an arc-like row constituted by ten microtubules (Miquel and Świdorski 2006, Miquel et al. 2007a) whereas in *A. menezesi* the maximal number is seven (see Table 1).

Anterior spermatozoon extremities showing a similar arrangement of cortical microtubules describing an arc-like layer were found in the Caryophyllidea (Gamil 2008, Bruňanská 2009), Spathelbothriidea (Bruňanská et al. 2006, Bruňanská and Poddubnaya 2010), Diphyllbothriidea (Justine 1986, Levron et al. 2006a, 2009), Bothriocephalidea (Bruňanská et al. 2002, Levron et al. 2006c, Bâ et al. 2007), Diphyllidea (Azzouz-Draoui and Mokhtar-Maamouri 1986/88), Tetraphyllidea (Mokhtar-Maamouri and Świdorski 1975, 1976, Mokhtar-Maamouri 1979, 1982, Mahendrasingam et al. 1989), Proteocephalidea (Bruňanská et al. 2003a, b, 2004a, b), and in the mesocestoid cyclophyllideans (Miquel et al. 1999, 2007b). However, the arc-like layer of thicker cortical microtubules described in the trypanorhynchs also occurs only in the former pseudophyllideans, in the tetraphyllideans, in the proteocephalideans and in the mesocestoidids.

However, particular aspects of this character are described in the anterior spermatozoon extremity of four bothriocephalidean cestodes. These are *Bothriocephalus scorpii* (Müller, 1781), in which Levron et al. (2006b) describe up to three arc-like layers of cortical microtubules, *Triaenophorus nodulosus* Pallas, 1791 (see Levron et al. 2005), which possesses a layer of microtubules in a spiral pattern forming one and a half open circles around the axoneme, and *Bothriocephalus clavibothrium* Ariola, 1899 and *Parabothriocephalus gracilis* Yamaguti, 1934 (see Świdorski and Mokhtar-Maamouri 1980, Šípková et al. 2010), which present a complete ring of cortical microtubules. Moreover, in the Proteocephalidea, two additional species, namely *Nomimoscolex* sp. and *Sandonella sandoni* (Lynsdale, 1960), possess two arc-like layers of cortical microtubules (see Bâ and Marchand 1994, Sène et al. 1997).

The presence of crest-like body or bodies in the anterior spermatozoon extremity has been postulated as a synapomorphy for the eucestodes (Bâ and Marchand 1995). In contrast to bothriocephalideans, tetraphyllideans, proteocephalideans, tetrabothriideans and cyclophyllideans, no crested body is present in the spermatozoon of caryophyllideans, spathelbothriideans, diphyllbothriideans and haplobothriideans (see Levron et al. 2010) as occurs in all the trypanorhynchs studied to date (see Table 1). Thus, the present work confirms the absence of this structure in the sperm cell of trypanorhynch cestodes and supports the idea that crest-like bodies are a synapomorphic character only for a part of the Eucestoda and not for the whole group (Justine 1998).

The two pairs of electron-dense attachment zones, visible in the median cytoplasmic process during spermiogenesis in numerous Platyhelminthes, mark the lines of proximodistal fusion between the median cytoplasmic process and the axonemes. These attachment zones persist in mature digenean spermatozoa (e.g. Miquel et al. 2006, Quilichini et al. 2010), but they are generally absent in the mature spermatozoa of the Eucestoda. To our knowledge, in the mature sperm cell of cestodes these submembranous structures have been observed only in some diphyllbothriidean, bothriocephalidean and trypanorhynch species (Bruňanská et al. 2002, Levron et al. 2006a, b, c, 2009, Miquel and Świdorski 2006, Miquel et al. 2007a). In fact, the attachment zones are also still present in the *A. menezesi* spermatozoon and their presence is probably a consequence of the proximodistal fusion.

According to the Thiéry's test, glycogen is present in the mature spermatozoon of *A. menezesi*. In the trypanorhynch species studied to date, the presence of two types of glycogen granules (α , β) has been demonstrated only in *D. spinulifera*, *P. trygonis* and *G. erinaceus* (McKerr 1985, Miquel and Świdorski 2006, Miquel et al. 2007a), while in *L. tenuis* (Świdorski 1994) and *A. menezesi* (present study) only one type of glycogen granules was found: the former species presents β -glycogen particles and the latter α -glycogen rosettes.

As in *D. spinulifera* and *P. trygonis* (see Miquel and Świdorski 2006, Miquel et al. 2007a), the posterior part of the spermatozoon of *A. menezesi* possesses one axoneme, which becomes disorganized towards the extremity of the cell. This pattern of posterior extremity is very common in the spermatozoon of the Eucestoda (e.g. Bruňanská et al. 2003b, Levron et al. 2005, 2009, Bruňanská 2009). However, in the eucestodes the posterior spermatozoon extremity may present different ultrastructural organisations: those formed by only a nucleus (e.g. Bruňanská et al. 2006, Levron et al. 2006a, b) or by a posterior electron-dense material usually containing cortical microtubules (e.g. Miquel and Marchand 1998, Miquel et al. 2009, 2010). The latter is particularly frequent in the sperm cell of cyclophyllidean cestodes.

Conclusion

The analysis of spermatological characters of all studied trypanorhynch species allowed discussing the phyletic relationships of trypanorhynchs based on molecular data. According to Palm et al. (2009), molecular evidence demonstrates that trypanorhynchs consist of two well-supported major clades (see introduction). However, considering two lacistorhynchoideans (*L. tenuis* and *G. erinaceus*) and one gymnorhynchoidean (*A. menezesi*) belonging to the same clade, their spermatological characters emphasize only a few similarities (see Table 1). On the other hand, the comparison of spermatozoa of both eutetrarhynchoideans *D. spinulifera* and *P. trygonis* with those of the gymnorhynchoidean *A. menezesi* reveals a high degree of

similarity between them, although they are placed in different clades. In conclusion, on the basis of the current spermatological knowledge, we are not able to explain the polyphyletic condition of the Trypanorhyncha. Subsequently, other spermatological studies focussing on species of different families are required to further elucidate this question.

Finally, it is interesting to reveal that spermatological features, specially the basic pattern of the spermatozoon, show resemblances between trypanorhynch, spathebothri-

riidean and diphyllbothriidean cestodes, as recently emphasized by Levron et al. (2010) according to both spermatological and morphological characters.

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