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Ultrastructure of sperm of the Spotted scat (*Scatophagus argus*, Linnaeus, 1766) observed by scanning and transmission electron microscopy

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ABSTRACT

An investigation was conducted to understand the sperm cell morphology and ultrastructure of Spotted scat (*Scatophagus argus*) through scanning and transmission electron microscopy. The present study reveals that the sperm of *S. argus* can be differentiated into three major parts – an acrosome-less spherical head, a short mid-piece, and a cylindrical flagellum. The scat sperm cell had a mean total length of 21.32 ± 1.80 μm with the presence of ovoid electron-dense nucleus. The mean length and width of ovoid nucleus measured 1.44 ± 0.34 and 1.54 ± 0.33 μm, respectively. The structural characteristics of the nucleus were found to be a shallow axial nuclear fossa and centriolar complex. The two centrioles were positioned nearly perpendicular to each other with a conventional "9 + 0" pattern in the proximal centriole. The short mid-piece was located laterally to the nucleus and contains 5 or 6 spherical and unequal-sized mitochondria. The mitochondria were separated from the axoneme by a cytoplasmic canal. The flagellum was inserted at the base of the nucleus with the presence of an axoneme structure of 9 + 2 paired micro tubules. The sperm flagellum had short irregular lateral fins. The present study reveals that Spotted scat sperm can be categorized as being of a "primitive or ect-aquasperm type" and belongs to the teleostean "type I" sperm. This is the first report on the morphology and ultrastructure of sperm in Scatophagidae family.

1. Introduction

The structure of the sperm in teleost fish is influenced by both the mode of reproduction and systematic position of the species (Grier et al., 1978). Research has focused on both morphology and ultrastructural differences of sperm between species, which serve as a criterion for evolutionary studies and as a tool used for taxonomic studies, which help to establish phylogenetic relationships among fish species (Gwo et al., 1995; Hara and Okiyama, 1998; Jamieson, 1999). Spermiogenesis in teleosts shows a wide variety of patterns which is broadly categorized as two types (I and II) of spermiogenesis (Mattei, 1970). With Type I, rotation of the nucleus occurs and the diplosome enters the nuclear fossa and the flagellum is symmetrically located, while with Type II, there is no nuclear rotation, the diplosome remains outside the fossa and the flagellum is asymmetrically located. Mattei (1970) have reported that the teleost sperm exhibit a broad range of varying structural features that makes it difficult to depict a common sperm type. The structure of fish sperm vary within families from aflagellate to biflagellae, and depending on whether there is internal or external fertilization, and there are an enormous range of shapes, sizes, and structure (Baccetti

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1986; Jones and Butler, 1988). It is reported that, submicroscopical features of sperm cells may reflect the mode of reproduction, evolutionary significance, and can be used as additional characteristics for taxonomic classifications (Jamieson, 1991; Mattei, 1991). Moreover, knowledge of sperm structure is needed to understand sperm morphology which is valuable in the development of sperm cryopreservation methods and also useful for evaluating possible cell damage due to exposure of contaminants (Leveroni Calvi et al., 1994; Van Look and Kime, 2003).

The Spotted scat *Scatophagus argus* (family Scatophagidae) is brackishwater ornamental fish widely distributed in Indo-pacific regions (Nelson, 1976). It is highly preferred fish in the aquarium trade and also form as an important food fish in Southeast Asian countries (Barry and Fast 1992). Despite the importance of this species, very little is known about its reproductive biology. Reports on the breeding of this species under captivity are meagre (Cai et al., 2010; Kailasam et al., 2014 (submitted for publication)) and hatchery production of this species is yet to be developed for large scale farming. Considering the commercial importance of *S. argus* in aquaculture, it is essential to understand the reproductive biology of this species. Therefore, the present study was aimed to screen the ultrastructure of *S. argus* sperm using scanning and transmission microscopy.

2. Materials and methods

2.1. Semen collection

Mature semen releasing males (Total body length = 16.44 ± 3.46 cm, body weight = 132.75 ± 57.21 g, $n = 10$) were obtained from Kasimedu coastal waters, Chennai, Tamil Nadu, India. Immediately after collection, the fish were anesthetized in 150 ppm 2-Phenoxy ethanol. The genital pore of the fish was carefully cleaned to remove water, urine, and feces. After wiping the genital area, the semen were expelled by applying gentle pressure on the abdomen and collected into a 1 ml Eppendorf tube which was placed on crushed ice (4 ± 2 °C).

2.2. Ultrastructural study

The samples were fixed in 0.1 M cacodylate buffer (pH 7.5) containing 2.5% glutaraldehyde (Karnovsky, 1965) for 2 h at 4.0 °C. Afterwards, samples were washed in cacodylate buffer for 1 h, and then post fixed in 1% cacodylate-buffered osmium tetroxide for 1 h at 4.0 °C. After double fixation, the sperm pellets were processed for scanning (SEM) and transmission (TEM) electron microscopy. For SEM, the sperm samples were dehydrated through an ascending series of ethanol and the dehydrated samples were critical-point dried, mounted on specimen holders and sputter coated with 20 nm gold palladium. Preparations were examined using a SEM, Carl Zeiss MA 15/EV 018 SED at 20 kV (Greven and Schmahl, 2006). For TEM, the samples were dehydrated as described above, and embedded in epoxide resin. Then, ultra-thin sections of 60–100 nm thickness were collected using glass knives. Later, the sections were placed on copper grids, stained with uranyl acetate and lead citrate, and screened under a TEM (TECHNAI10-Philphs) (Ravaglia and Maggese, 2003) for screening the ultra-cellular structure of sperm.

All measurements were performed for both SEM and TEM were evaluated using the Olympics Image software (Version 4.0.1 for MS windows, 1998) to measure sperm morphological characteristics and expressed as mean ± SD and range.

3. Results

Morphologically, the sperm of *S. argus* consists of three major parts – head, mid piece, and flagellum (Fig. 1A and B). The acrosome is absent in the head portion and, therefore, the *S. argus* sperm cells can be categorized as a primitive type of cell with an acrosome-less aquasperm. Morphological measurements of scat sperm are shown in Table 1. The sperm of *S. argus* had a mean total length of 21.32 ± 1.80 μm (Fig. 1B). The head of the sperm is small, round shaped, and had a length and width of 1.57 ± 0.39 and 2.00 ± 0.37 μm, respectively (Fig. 1C). The nucleus contains an electron dense chromatin material with the condensed granular pattern. The nucleus had an average total length of 1.44 ± 0.34 μm and diameter of 1.54 ± 0.33 μm. The nucleus is covered with a nuclear envelope and at the base of the nucleus the nuclear envelope is invaginated and forms a depression which is termed the nuclear fossa. It accommodates a centriolar complex (proximal and distal centrioles) and the anterior portion of flagellum. This nuclear fossa facilitates connection of the nucleus posterior side of the sperm with the axial components of the nucleus and with the mid piece. The centriolar complex is formed by the proximal (anterior region) and the distal centriole (posterior region) by lying inside the nuclear fossa. This distal centriole positioned in such a way functions as a basal body for the flagellum. The sperm cell also had a classical arrangement of "9 + 0" pattern to the centrioles with the presence of electron-dense filaments. This electron dense material connects to the pair of centrioles. The centriolar complex is oriented perpendicularly to each other with a right angle position at the base of the head (Fig. 2A).

The mid-piece of the scat sperm is short and has an irregular shape. The mean length and mean diameter are 1.20 ± 0.53 and 1.86 ± 0.55 μm, respectively. The ultra section of the mid-piece indicated the presence of 5–6 distinct unequal sized spherical mitochondrial derivatives, which are positioned at the posterior region to the nucleus (Fig. 2B and C). These mitochondria contain irregular cristae with an electron lucent matrix the size of 0.43 ± 0.09 μm in a ring type arrangement. In the mid-piece, the axoneme is located posterior to the distal centriole and separated from the plasma membrane by a cytoplasmic canal. The cytoplasmic canal is located between the flagellum and plasma membrane. The mean width of the cytoplasmic canal is 63.30 ± 3.16 nm (Fig. 2B and C).

The scat sperm flagellum has a mean length of 18.78 ± 1.77 μm with a side-fin (Fig. 2F). The mean width of the flagellum is 0.27 ± 0.10 μm (Fig. 2E). The average width of the flagellum fibre is 0.43 ± 0.07 μm. The flagellum membrane has irregular short fin like projections.

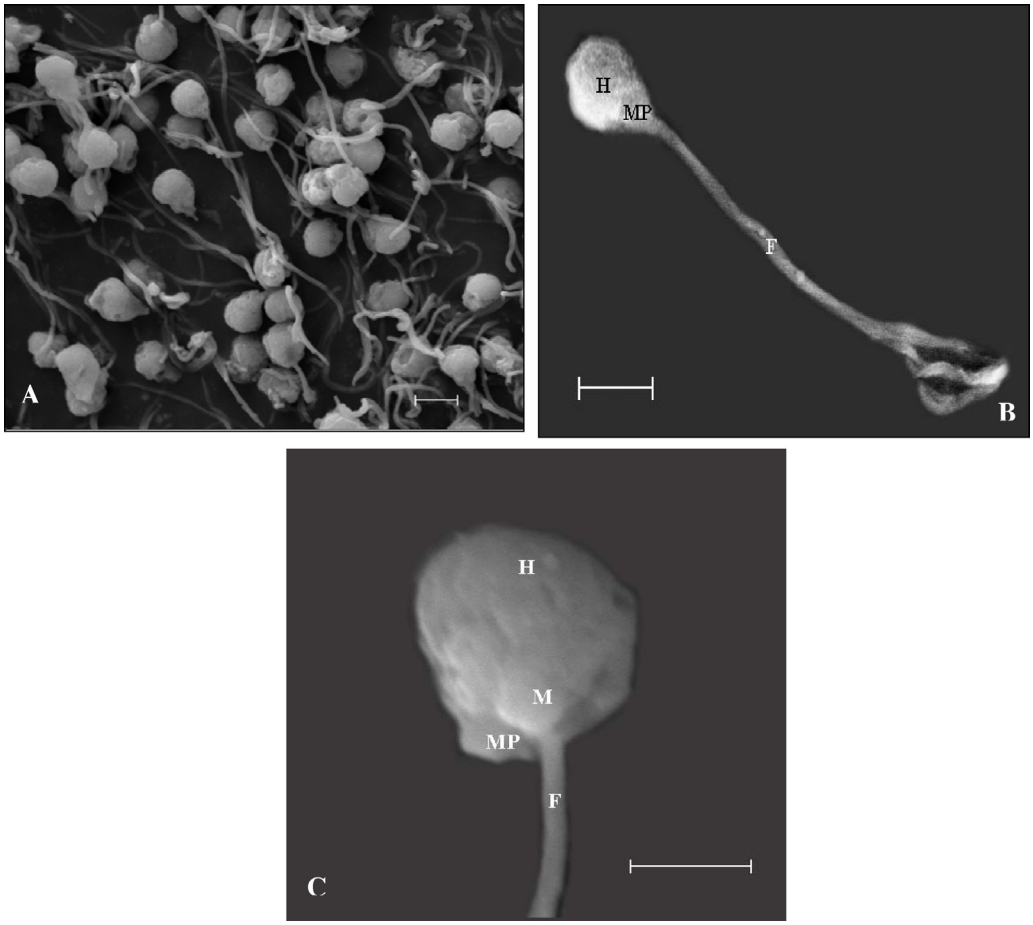

Fig. 1. Scanning electron microscopy (SEM) micrographs of *S. argus*. (A) The sperm bunch together in groups at low magnification. (B) A single sperm at high magnification. (C) General views of different rotations of the sperma with mitochondrial regions. H, head; MP, mid-piece; F, flagellum; M, mitochondria. Scale bars: 2 μm (A, B), 1 μm (C).

Table 1
Ultrastructural variables of spermatozoa.

Spermatozoa	Variables				
Sperm size (μm)	Total length				
	21.32 ± 1.80				
Head (μm)	Length	Width	Nucleus length	Nucleus width	
	1.57 ± 0.39	2.00 ± 0.37	1.44 ± 0.34	1.54 ± 0.33	
Mid-piece (μm)	Length	Width	Mitochondria diameter	Mitochondria number	
	1.20 ± 0.53	1.86 ± 0.55	0.43 ± 0.09	5–6	
Flagellum	Length (μm)	Width (μm)	Fibre width (μm)	Axoneme width (μm)	Cytoplasmic canal width (nm)
	18.78 ± 1.77	0.27 ± 0.10	0.43 ± 0.07	0.19 ± 0.03	63.30 ± 3.16
Axoneme (nm)	Peripheral doublets of microtubules width (PDM)	Central doublets of microtubules width (CDM)	Length of radial spoke	Microtubule diameter	Axoneme pattern
	34.84 ± 6.19	41.47 ± 4.21	35.90 ± 5.82	17.35 ± 1.28	9 + 2

Data are mean ± SD (n = 20).

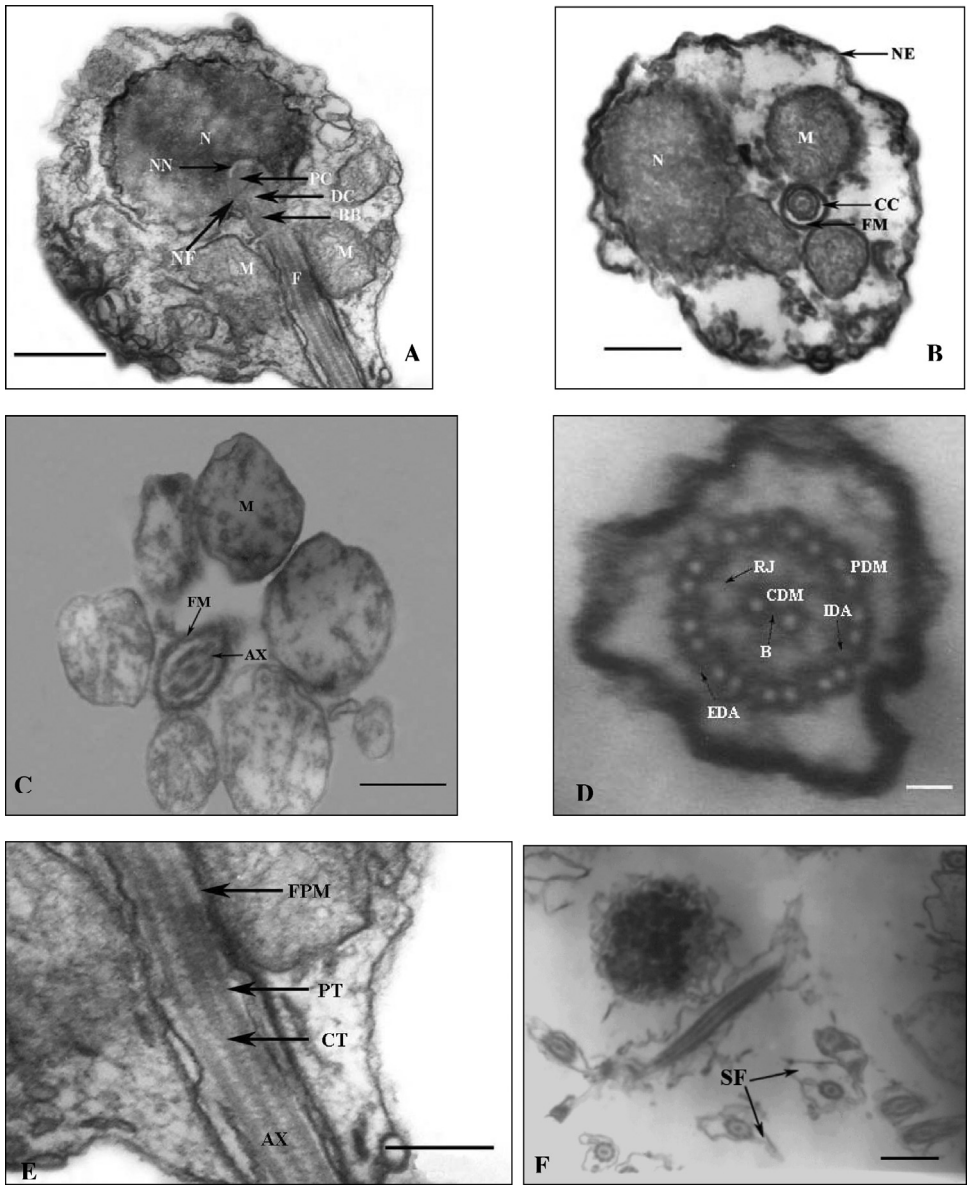

Fig. 2. TEM micrographs of *S. argus*. (A) Longitudinal section of a sperm showing the round nucleus with axial nuclearfossa which contains the centriolar complex. (B) Cross section of head and mid-piece. (C) The mitochondria ring with six mitochondrion. (D) Cross section of flagellum showing 9 + 2 axonemal doublet configuration. (E) Longitudinal section of flagellum. (F) Cross section of flagellum with side-fins. H, head; MP, mid-piece; F, flagellum; N, nucleus; NF, nuclear fossa; NN, nuclear notch; M, mitochondria; PC, proximal centriole; DC, distal centriole; BB, basal body; NE, nuclear envelope; CC, cytoplasmic canal; FM, flagellar membrane; AX, axoneme, PT, peripheral microtubules; CT, central microtubles; FPM, flagellar plasma membrane; PDM, peripheral doublets of microtubules; EDA, external dynein arms; CDM, central doublets of microtubules; IDA, internal dynein arms; RJ, radial joins; SF, side-fins. Scale bars: 500 nm (A, B, C, E, F), 50 nm (D).

The distal centriole appears as a basal body of the flagellum axoneme, which extends from the anterior end of the cytoplasmic canal to the deep basal nuclear fossa. The axoneme is covered by a plasma membrane and the flagellum axoneme and is composed of microtubule configurations of a 9 + 2 microtubule pattern (Fig. 2D). The cross-section of the basal body resembles as cartwheel structure with the alar sheets extending from the microtubules to the plasma membrane. The mean width of axoneme is $0.19 \pm 0.04\,\mu m$ and the mean diameter of microtuble is 17.35 ± 1.28 nm. The mean width of the outer doublet and the central pair is 34.84 ± 6.19 and 41.47 ± 4.21 nm, respectively. The accessary microtubules are connected to each other by electron-dense microfilaments and radial spokes. The mean length of radial spoke which is situated between peripheral and central pairs is 35.90 ± 5.82 nm. The external and internal dynein arms act as a junction between the peripheral doublets. The pair of central microtubules is linked to the plasma membrane by Y-shaped bridges and is encased in the central sheath (Fig. 2D and E). The cylindrically shaped scat flagellum is well fixed perpendicularly at the basement of the nucleus

to its full length. Based on the ultrastructure description, the mature sperm of Spotted scat can be catergorized as "Type I or primitive type" as in the case of other Perciformes fishes (Fig. 2A–E).

4. Discussion

In the Scatophagidae family, the Spotted scat *S. argus*, is one of the most economically important candidate species, useful as food fish and for ornamental purposes. The present study reveals that *S. argus* sperm have three distinct parts – head, mid-piece, and flagellum. Spotted scat sperm, possess an acrosome-less aquasperm, which usually participate in external fertilization once released into the water column (Fürböck et al., 2010). The absence of an acrosome in the head, as in *S. argus*, is a common feature in many teleost species such as Tilapia (Don and Avtalion, 1993) and Turbot (Suquet et al., 1993). According to the Kim et al., 2011; the sperm with an acrosome-less head is closely associated with with the egg micropyle diameter of the species and it is the likely result of co-evolution with possessing a micropyle, an opening in the *zona pellucid* through which sperm enter to fertilize the egg (Medina et al., 2000). The present investigation confirmed that Spotted scat sperm possess the configuration of the Uniflagellate acrosome-less aquasperm which is consistent with this species having external fertilization.

It is reported that the shape of the head is highly variable among teleosts sperm. The Spotted scat sperm head has spherical in shape and contains an ovoid nucleus. The small spherical head found in *S. argus* is the result of a simple spermiogenesis process. However, Psenicka et al. (2007) concluded that an elongated head present in Siberian sturgeon (*Acipenser baerii*) indicated a more complex spermiogenic process that is considered as an advanced morphological sperm feature. The hydrodynamic shape of the head can moderate swimming ability and velocity (Malo et al., 2006) and is considered to be a primitive or ect-aquasperm. The spherical shaped head in sperm have been reported in other Perciformes species such as Golden grey mullet (Brusle, 1981), Gilthead Sea bream (Maricchiolo et al., 2007), Zebra Sea bream (Shabana, 2012), and Pikeperch (Kristan et al., 2014). The sperm head shape has, however, been reported to be different in other perciform species as indicated by the ovoid shape in Cardinal fish, *Apogon limberbis* (Lahnsteiner, 2003), flattened shape in Goat fish, *Upeneichthys lineatus* (Gwo et al., 2004a) and elongated shape in Golden mandarin, *Siniperca scherzeri* (Luo et al., 2011). The shape of the nucleus in fish sperm is species specific and it varies from species to species (Maricchiolo et al., 2010). In *S. argus*, the nucleus is almost fully located in the head portion and the depression aspect of the nuclear fossa is found to be shallow. The relative position of the centriolar complex varies among species from being parallel to perpendicular (Morisawa, 1999). In *S. argus*, the centriolar complex is located inside the nuclear fossa and is perpendicular to each other.

According to Baccetti et al. (1984), swimming speed and duration of motility of sperm are influenced by the size of the mid-piece. In *S. argus*, the mid-piece is short which is a common feature in teleost where fertilization is external (Vergílio et al., 2013). Species with internal fertilization have sperm with a more complicated mid-piece (Lahnsteiner et al., 1997). The small mid-piece length and a long flagellum in *S. argus* sperm clearly suggests the sperm cell can move quickly through the water column to fertilize a free floating egg which is released by the female brooder. It is believed that swimming speed of sperm help to reduce the distance between the dispersed gametes within the water column. A cross-section of the mid-piece in *S. argus* shows the presence of 5–6 mitochondria as a ring like structure positioned between the opening of the nuclear fossa and the proximal region of the flagellum. The role of mitochondria in the mid-piece of the sperm is an energy source for generating adenosine triphosphate (ATP) for sperm motility as previously documented (Billard et al., 2000). The ATP is also used by the dynein arms, which helps in a self-oscillatory bending behavior of the flagellar axoneme (Maricchiolo et al., 2004). The number of mitochondria present in the mid-piece of sperm of various species have been reported for the Common two-banded seabream *Diplodus cervinus cervinus* which have 1 mitochondrium (Mahmoud, 2010); and there are 4–6 in Common barbel *Barbus barbus* (Alavi et al., 2008); 5–6 in Leopard coralgrouper *Plectropomus leopardus* (Gwo et al., 1994); 6 in Atlantic bluefin tuna *Thynnus thynnus* (Abascal et al., 2002) and 6–9 in Longtooth grouper *Epinephelus bruneus* (Kim et al., 2013). The presence of 5 or 6 mitochondrion in the Scatophagidae family of *S. argus* indicate a greater energy delivering capacity for the sperm compared with those species possessing a fewer number of mitochondria. Thus, the number of mitochondria is an important factor for sperm motility as a source of energy and thereby has a significant role in fertilizing eggs (Lahnsteiner and Patzner, 1995). Movement of the flagellum depends on how it is attached to the mid-piece and the structural support provided by the associated membranes. The mid-piece, has several foldings of the cytoplasmic membrane and a shallow collar-like structure providing structural support for the movement of flagellum during swimming (Markovina, 2008).

The single flagellum, which is found in scat sperm, is also observed in other families such as Siluridae, Clariidae, Bagridae, Schibeidae, Pimelodidae, Ariidae, and Malapteruridateae (Yao et al., 1999). The flagellum length of the scat is approximately 20-fold that of its head. According to Mattei (1991), the sperm with a flagellum perpendicular to the nucleus can be categorized as Type I sperm. In the present study, the scat sperm had a similar pattern of perpendicularly placed flagellum to the nucleus and can be categorized as Type I sperm as a consequence. The axoneme forms the cytoskeletal structure within the flagellum, providing support and structure to the flagellum primarily during movement (Gwo, 1995). The flagellum of Type I aquasperm contains an axoneme 9 + 2 configuration, typically common in teleostean sperm (Mattei, 1991). The present finding revealed the presence of 9 + 2 axoneme configurations in the sperm of Spotted scat which confirm that it has a Type I aquasperm. The flagellum of *S. argus* sperm has a fin-like projection of variable length. Even though the functions of side-fins are still

unknown (Maricchiolo et al., 2004), the presence could be accelerate the flagellar forward motion and also increase the friction with the surrounding medium which results in an increased probability of fertilization (Zhang et al., 1993; Cosson et al., 2000; Psenicka et al., 2007). The presence of side-fins is not order or family-specific, as shown by Maricchiolo et al. (2004) in Sparidae; Kristan et al. (2014) Percidae; and Hatef et al. (2011) Acipenseridae. Within species of the same family there is also variation in occurrence of lateral fins, which for the sparidae family *P. erythrinus* and *D. sargus* is one unpaired side-fin (Lahnsteiner and Patzner, 1995), whereas no side-fins were reported in *B. boops* and *D. puntazzo* (Mattei, 1970; Taddei et al., 1999).

Specifically, from the evolutionary viewpoint, the sperm of *S. argus* could be regarded as an evolved form of the "primitive Type I" of sperm which is characterized with a flagellum, positioned perpendicular to the base of the nucleus. The centriolar complex lies inside the nuclear fossa as a result the rotation of the flagellum axis relative to the nucleus during the process of spermiogenesis (Mattei, 1970). In the order Perciformes, many species are categorized as having Type II sperm but some families such as the Centracanthidae (Mattei, 1991), Sparidae (Taddei et al., 1999; Maricchiolo et al., 2007), Mullidae (Saperas et al., 1993; Gwo et al., 2004b), and Serranidae (García-Díaz et al., 1999; Kim et al., 2013) were found to have "Type I" sperm, where the centriolar complex lies inside the nuclear fossa with the nuclear rotation occurring during spermiogenesis.

5. Conclusion

The present study provides important evidence that the ultrastructure facets of sperm in *S. argus* provide a basis for comparing this species with other species of the Scatophagidae family. The present investigation confirmed that the Spotted scat possesses the configuration of the uniflagellate acrosomal-less aquasperm with external fertilization occurring in this species and sperm cells being categorized as Type I. Features such as a spherical shaped head, collar like mid piece which provide structural support to the flagellum, and long uniflagellum attribute to the potential swimming ability for *S. argus* sperm. The present information on ultrastructure of Spotted scat sperm is very important for an enhanced understanding on the reproductive biology and will facilitate devising improved protocols for cryopreservation, and artificial propagation for enhanced production with this species.

Conflict of interest

The authors declare no conflict of interest to any of the internal or external funding sources.

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