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# Ultrastructure of Spermatozoa of the Light Bullhead *Leiocassis nitidus* (Teleostei, Siluriformes, Bagridae)

**Kgu-Hwan Kim*, Ae-Sook Kwon[1] and Byung-Chan Lee[2]**

*Department of Radiologic Technology, Daegu Health College, Daegu 702-722, Korea*
*[1]Department of Animal Pet manegement, Taegu Science College, Daegu 702-260, Korea*
*[2]Daegu Technopark, Nano Practical Application Center, Dalseo-Gu, Daegu 704-801, Korea*

The spermatozoa of *Leiocassis nitidus* are relatively simple cells composed of a spherical head, a short midpiece, and a tail, as in most Siluriformes. The ultrastructure is characterized by the following features: Acrosome absent, as in most teleosts; a round nucleus about 1.8 μm long, with a deep nuclear fossa containing the proximal and distal centrioles and mitochondria. Two centrioles approximately 180° from each other; 10 or more mitochondria surrounding the axoneme (with a 9+2 microtubular pattern), arranged in two layers in the postnuclear cytoplasm and separated from the axoneme by the cytoplasmic canal. Two lateral fins on the same plane as the two central microtubules; doublets 3 and 8, which are ultrastructural characteristics of the sperma tail unlike other siluroids laking the lateral fins.

Key words: Bagridae, Fish, Sperm, Spermatozoa, Ultrastructure

## Introduction

The ultrastructure of the spermatozoa of about 300 species of teleost fishes is known (Jamieson, 1991), and many studies have used spermatozoal ultrastructure for phylogenetic analyses of teleosts (Mattei, 1991). Teleost spermatozoa are relatively simple cells composed of a spherical head devoid of an acrosome, a short midpiece and an elongated tail. Siluriformes are a diverse group of marine and freshwater teleosts with about 2,400 species in 412 genera and 34 families. They have uniflagellate and biflagellate anacrosomal aquasperm (Poirier and Nicholson, 1982; Maggese et al., 1984) usually with deep nuclear fossa and short midpiece. However, accounts of siluroid sperm ultrastructure are scarce and restricted to *Ictalurus punctatus* (Poirier and Nicholson, 1982; Emelyanova and Makeyeva, 1991), *Clarias senegalensis* (Mattei, 1970), *Liocassius ussuriensis* (Emelyanova and Makeyeva, 1991; Kim and Lee, 2000), *Pseudobagrus fulvidraco* (Lee, 1998), *P. brevicorpus* (Kim and Lee, 2003), and *Rhamdia sapo* (SEM only; Maggese et al., 1984). Here we describe the ultrastructure of the spermatozoa of the light bullhead *Leiocassis nitidus* and compare it to those of other teleosts.

*Corresponding author: guhwan@hanmail.net

## Materials and Methods

Adult *L. nitidus* were collected during the breeding season from the Kumho River in the city of Kyungsan, Korea, and maintained in a controlled environment. Mature spermatozoa were obtained by pressing both sides of the abdomen, and maintained in physiological saline in a small petri dish. Part of the material was examined and photographed with a phase contrast microscope.

For transmission electron microscopy (TEM), semen and pieces of testis were dissected and fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer and postfixed in 1% osmium tetroxide in the same buffer. Then they were dehydrated in a graded ethanol series and embedded in Epon 812. The samples were sectioned with an ultramicrotome (RMC, MTXL, Germany), stained in 4% aqueous uranyl acetate, poststained with lead citrate, and examined using TEM (Hitachi, H-7500, Japan).

For scanning electron microscopy (SEM), testes were fixed and dehydrated using the same procedures as for TEM. They were then replacement in isoamyl acetate and critical point-dried. They were coated with gold using an ion sputter and examined with SEM (Hitachi, S-4100, Japan).

## Results

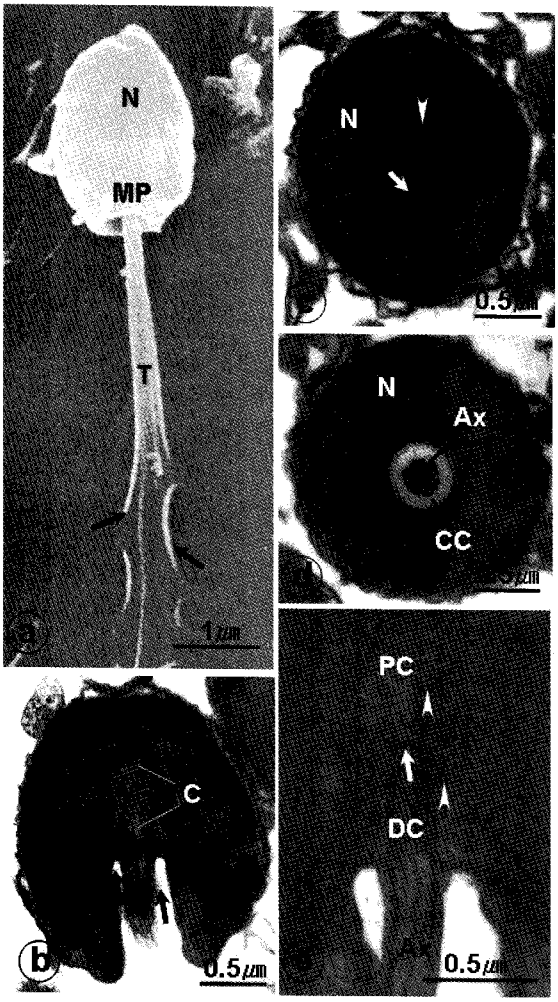


Fig. 1. Electron micrographs of spermatozoon from the *Leiocassis nitidus*. ⓐ, SEM of a spermatozoon showing a spherical head (N), a short midpiece (MP), and a tail (T). Note the two lateral fins (arrows). Bar=1 μm. ⓑ, longitudinal section through the head and midpiece region showing the nucleus containing two centrioles (C ) in a nuclear fossa, midpiece had 2 layer of mitochondria (M), and cytoplamic canal (arrows). There is no acrosome. Bar=0.5 μm. ⓒ-ⓓ, transverse section of the level of the basal nucleus (N). ⓒ, centriole connected to nuclear envelope (whitearrowhead) by fibrous materials (whitearrow). Bar=0.5 μm. ⓓ, posterior region of nucleus(N) showing the axoneme(Ax) separated to nucleus by cytoplasmic canal(CC). Bar=0.5 μm. ⓔ, longitudenal section through the posterior region of the nucleus (N) showing the two centriloe deposite in nuclear fossa, inter centriole plate (white arrow) and satellite fiber (white arrow head). Two centriolar axes are in the same plane. Bar=0.5 μm.

The spermatozoa of *L. nitidus* are relatively simple and elongated cells composed of a round head, short midpiece, and tail with two lateral fins (Fig. 1a). There is no acrosome (Fig. 1b). The nucleus is spherical, and about 1.8 μm in length and 2 μm in diameter. Its anterior end is covered by a nuclear envelope and plasma membrane. The posterior region of the nucleus is deeply penetrated by the nuclear fossa containing the centriolar complex and mitochondria (Fig. 1b). The transverse section of the nuclear fossa is circular (Fig. 1c, d). The chromatin is highly electron-dense and granular in appearance (Fig. 1b-d). The two centrioles are located in the anterior nuclear fossa, and are connected to each other by osmiophilic filament (Fig. 1e). They have a conventional 9+0 microtubular triplet construction. The proximal centriole is approximately 180° relative to the distal centriole, and their axes lie in the same plane, longitudinal to the axis of the cell. The distal centriole forms the basal body of the flagellum. There are electron-dense filamentous materials between the centrioles and the nuclear envelope lining the nuclear fossa (Fig. 1e). Ten or more mitochondria are arranged in two layers that surround the axoneme (Fig. 2b) at the anterior region of the postnuclear cytoplasm. They are completely separated from the axoneme by a cytoplasmic canal (Fig. 2a-c). The mitochondrial matrix is rather loose, and the internal membranes or cristae are sometimes difficult to distinguish (Fig. 2b, c). The flagellum is about 76.1 μm long, and composed of a typical 9+2 microtubular doublet structure. It is flanked by two lateral fins, which are in line with the two central microtubules. The cytoplasmic matrix of these lateral fins is more electron-dense than the rest of the flagellum (Fig. 2d).

## Discussion

The ultrastructure of the spermatozoa of *L. nitidus* is similar to that of other siluroids in having a spherical head with a deep nuclear fossa, a short midpiece, and an elongated tail. However, there are some differences in the orientation of the two centrioles, the distribution of the mitochondria, and the lateral fins. A deep nuclear fossa housing the centriolar complex is also found in other teleost species (Emelyanova and Makeyeva, 1991; Gwo et al., 1996), but is shallow in Cypriniformes and Characiformes. According to Jamieson (1991), the deep nuclear fossa is apomorphic compared to the shallow nuclear fossa. The relative positions of the two centrioles of fish spermatozoa vary considerably among species. In Siluriformes, they are mutually perpendicular in *Silurus glanis* (Gwo et al., 1995) and

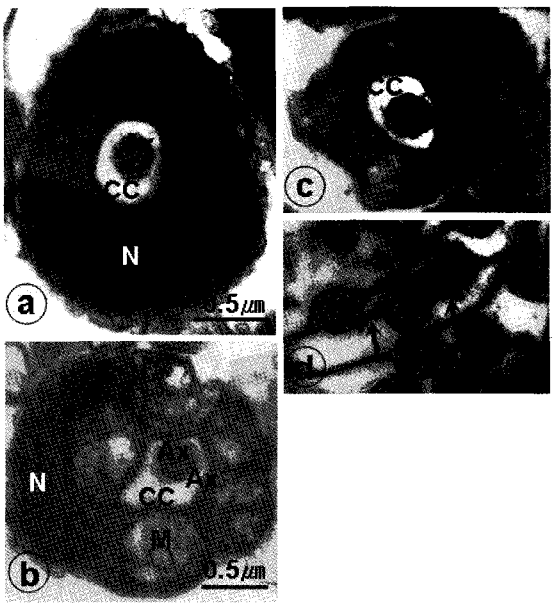


Fig. 2. Midpiece and flagellum transverse section of *L. nitidus*. ⓐ-ⓒ, transverse section through various levels of the cytoplamic canal (CC) showing the distribution of the mitochondria (M), which is separated from the axoneme by the cytoplasmic canal. ⓐ, anterior part of the cytoplasmic canal (CC) containing nucleus (N), mitochondria (M), and axoneme (Ax). Bar=0.5 μm. ⓑ, transverse section at the middle level of the cytoplasmic canal (CC) containing nucleus (N), and mitochondria (M), and axoneme (Ax). Bar=0.5 μm. ⓒ, transverse section at the posterior level of the cytoplasmic canal (CC) containing many mitochondria (M). Bar=0.5 μm. ⓓ, transverse section of endpiece. The two lateral fins (LF) are in line with the two central microtubules. Bar=0.5 μm.

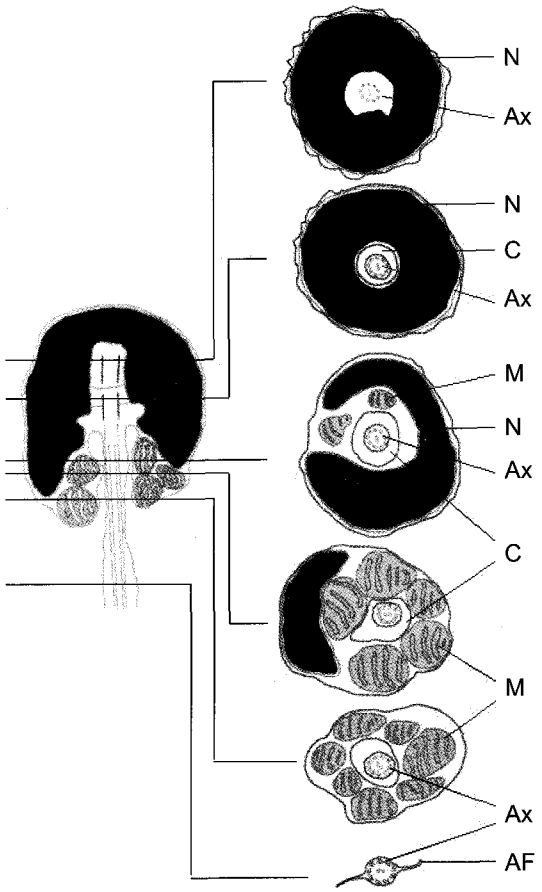


Fig. 3. Graphic illustration of longitudinal and transverse sections of the mature spermatozoon *of Leiocassis nitidus*. AF, axonemal fins. Ax, axonemae. C, cytoplamic canal. DC, distal centriole. M, mitochondria. N, nucleus. NF, nuclear fossa.

*S. microdorsalis* (Lee and Kim, 2001); at an obtuse angle in *Clarias senegalensis* (Mattei, 1970), *Liocassius ussuriensis* (Gwo et al., 1995; Kim and Lee, 2000), *Pseudobagrus fulvidraco* (Lee, 1998), *P. brevicorpus* (Kim and Lee, 2003), and *Silurus asotus* (Kwon et al., 1998); and parallel in *Amiurus nebelosus* (Emelyanova and Makeyeva, 1991). In *L. nitidus,* the proximal centriole is approximately 180° to the distal centriole. Baccetti et al. (1984) proposed that centriolar geometry is correlated with both nuclear and flagellar position. Jamieson (1991) reported that a proximal centriole perpendicular to a distal centriole is a plesiomorphic feature in fish spermatozoa. The midpieces of teleost fish spermatozoa are less developed than those in other vertebrate groups (Billard, 1970) and generally contain only mitochondria around the axoneme. The midpiece of *L. nitidus* is short and contains several mitochondria, as in most siluroids. Midpiece length may be related to fertilization strategy, as internal fertilization is associated with a long midpiece and external fertilization is associated with a short midpiece (Idelman, 1967). The number and distribution of mitochondria vary in teleosts (Afzelius and Mims, 1995; Gwo, 1995), and are useful taxonomic characters in Cyprinidae (Baccetti et al., 1984). The number of mitochondria varies from one in *Rhodeus ocellatus ocellatus* (Ohta, 1991) to ten in *Carassius auratus* (Baccetti et al., 1984), but is frequently three or four. Bagridae have 2-21 mitochondria located in the midpiece (Gwo et al., 1995; Kim and Lee, 2000, 2003). The mitochondria of *L. nitidus* are similar to those of other siluroids in number and layering, but differ in distribution. In *L. nitidus*, they are found in both the nuclear fossa and the peripheral postnuclear cytoplasm, whereas in other siluroids, they are found only in the latter. The structure of the spermatozoal

flagellar apparatus is diverse among animal species (Afzelius, 1982). Axonemal fins are very common in many fish species (Afzelius, 1978; Mattei, 1991), including those in the Siluriforme families Bagridae (Lee, 1998; Kim and Lee, 2003) and Amblycipidae (Lee and Kim, 1999). However, Siluridae (Kwon et al., 1998) and Ictaluridae (Emelyanova and Makeyeva, 1991) do not have axonemal fins. In view of the widespread occurrence of lateral fins throughout the Osteichthyes, the absence of lateral fins in Siluriformes appears to be an apomorphic loss and an ostariophysian synapomorphy (Jamieson, 1991). Lateral fins vary in size and number, and their presence is independent of reproductive mode (internal or external fertilization), type of spermatozoa (primitive or evolved), and number of flagella (Mattei, 1991). Axonemal fins might increase the efficiency of flagellar movement.

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