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Anatomical Ultrastructure of Spermatozoa of *Paralichthys olivaceus* (Paralichthyidae, Perciformes) from Korea

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ABSTRACT Fine structure of spermatozoa of the bastard halibut (*Paralichthys olivaceus*) is described. Its structure is characterized by acrosomeless, having a spherical head region and a small midpiece containing five mitochondria disposed one row. The flagellum inserts centrally on the nucleus and has lateral ribbons. The present study supports the suggestion that spermatozoal ultrastructure is of taxonomic importance and may be a phylogenetic criterion in teleostei.

Key words : Bastard halibut, *Paralichthys olivaceus*, spermatozoa, ultrastructure

INTRODUCTION

The bastard halibut *Paralichtys olivaceus* is distributed along the coasts of Korea, Kuril island, Japan and south China (Kim *et al.*, 2005). In Korea, this species is found in the subtidal zone of the eastern, western and southern parts of Korea.

Ultrastructure of spermatozoa has most recently served as a criticism for taxonomic and phylogenetic classification of over 200 fish species (Jamieson, 1991; Mattei, 1991). The fine structure of many marine fishes as yet lacks detailed investigation. The spermatozoa of *Paralichthys olivaceus* has been examined by electron microscopy (Hara and Okiyama, 1998; Kim *et al.*, 2003). Although the study Hara and Okiyama (1998) and Kim *et al.* (2003) provided data on some spermatozoon structure, but the detail structure and the wide considerations with other species were not enough. Ultrastructural study of spermatozoa on teleost fish have revealed important morphological characteristics in different species (Afzelius, 1982; Gwo *et al.*, 1996). These data have helped to clarify several questions about phylogeny of these animals (Jamieson, 1991).

For that reason, the present report provides a description of the ultrastructure of the mature spermatozoon of the bastard halibut *Paralichtys olivaceus*.

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MATERIALS AND METHODS

During their spawning period lasting from the beginning to the end of April (2009), 5 male flatfish (total length 50 ~ 60 cm) were collected in the east sea of Uljin (Korea). The fish was identified and kept in the fish collection of laboratory. Gonad fragment were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2. the samples was postfixed for 2 hour in 1% O_sO_4 in the same buffer. Specimens were dehydrated in a graded ethanol series and embedded in Epon. Section were cut with diamond knife on an Leica ultramicrotome, sectionstained with uranyl acetate and lead citrate, and examined in a Hitachi H-7500 electron microscopy. For scanning electron microscopy, specimens were fixed in glutaraldehyde and postfixed in O_sO_4 as described above. Following dehydration in a graded ethyl alcohol series, sample were critical-point dried, coated with gold, and observed with Hitachi S-4800.

RESULTS

The spermatozoa of *Paralichtys olivaceus* have a head, a midpiece and a flagellum (Fig. 1a). The nucleus is spherical shaped, contains homogeneous granular, strongly electron-dense choromatin and is covered by a typical double nuclear envelope (Fig. 1b). The undulating nuclear envelope and plasma membrane are applied tightly to the anterior of nucleus (Fig. 1c), but no acrosome is pre-

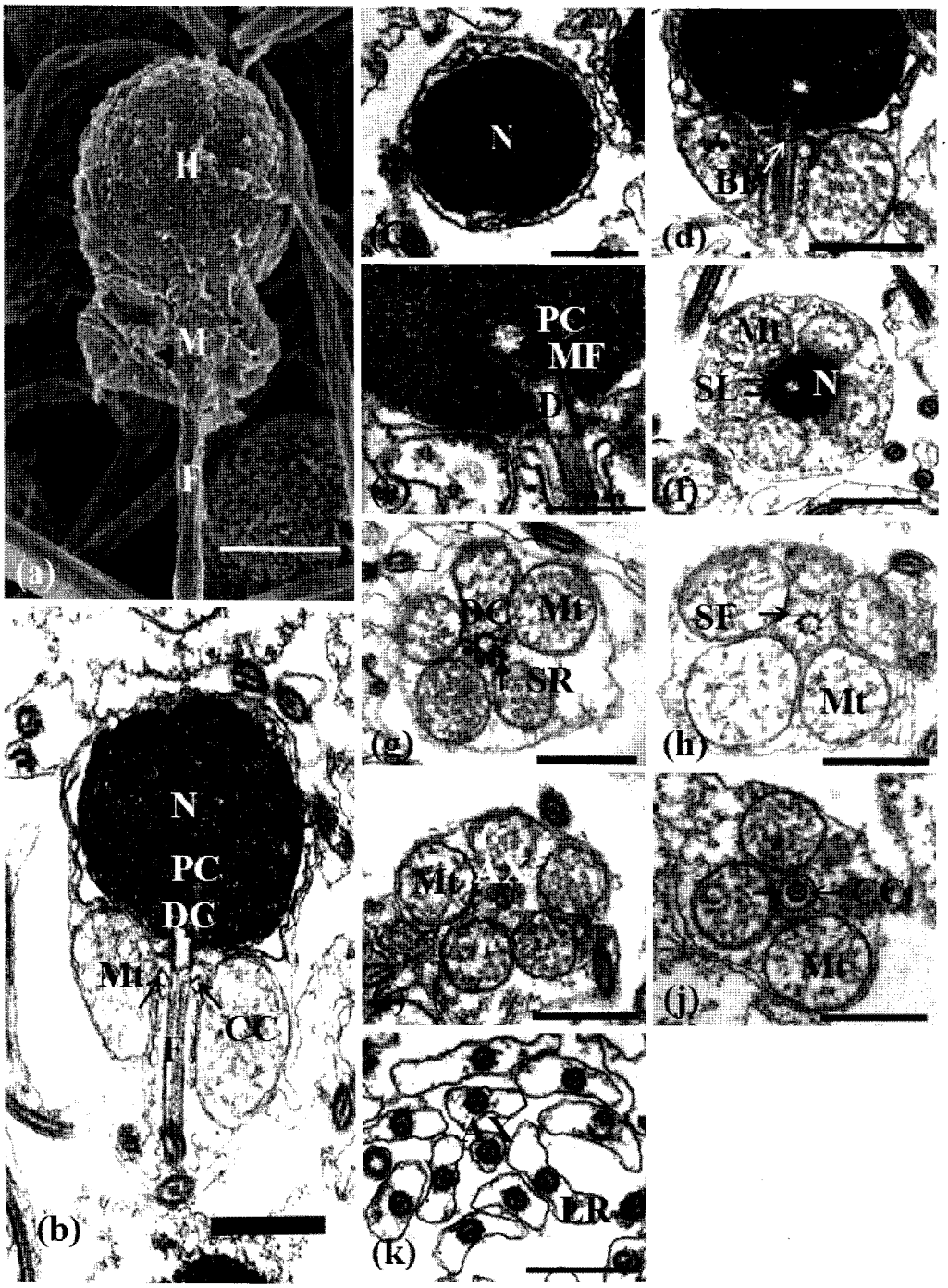

Fig. 1. The spermatozoa of *Paralichtys olivaceus* is peculiar in that the head is ball-shaped, the nuclear fossa is moderate and contains the proximal centriole, and 5 mitochondria are arranged in a single layer around the base of the flagellum. (a) SEM of spermatozoa showing a spherical head, short midpiece and a long tail possess lateral ribbon. (b) Longitudinal section through a head and midpiece region showing the base invaginated nucleus, two centriole and flagellum. (c) Cross-section of nucleus showing the plasma membrane and nuclear enveloper separated by each other. (d) Longitudinal section of nuclear fossa and midpiece portion showing moderatly invaginate the nuclear fossa contains proximal centriole and apical part of distal centriole. (e) Enlargement of nuclear fossa showing the proximal and distal centriole are fastened with each other by microfibril. (f)-(j) Cross-section of midpiece serial. (f) Midpiece cross-section showing the 5 mitochondria, and satellite lamellae surround the distal centriole. (g) Midpiece cross-section showing the satellite rays projected from distal centriole toward mitochondria. Note the satellite rays appear electron dense material stabilization of the distal centriole. (h) Midpiece cross-section showing the transitional area of base portion flagellum. (i) Midpiece cross-section showing axoneme and 5 mitochondria. (j) Midpiece cross-section showing axoneme separated by cytoplasmic canal (arrow). (k) Cross-section of flagellum, showing the 9+2 doublet structure and lateral ribbon. Abbreviations used in figure. AX: axoneme, BP: basal plate, CC: cytoplasmic canal, DC: distal centriole, F: flagellum, H: head, LR: Lateral ribbon, M: midpiece, MF: microfibril, Mt: mitochondria, N: nucleus, PC: proximal centriole, SL: satellite lamellae, SR: satellite rays.

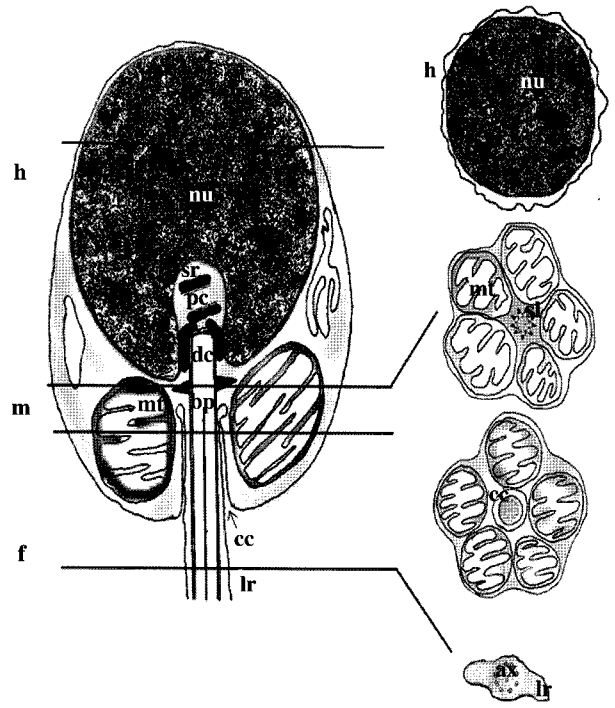

Fig. 2. Schematic organization of the spermatozoon of the flatfish, *Paralichthys olivaceus*. ax, axomene; cc, cytoplasmic canal; dc, distal centriole; f, flagellum; h, head; lr, lateral ribbon; m, midpiece; mt, mitochondria; nu, nucleus; pc, proximal centriole; sl, satellate lamellae; sr, satellate rays.

sent anterior portion of nucleus (Fig. 1d). The base region of the nucleus is indented by a nuclear fossa, the length of which is about one-fourth of the length of the nucleus (Fig. 1e).

The two centrioles are present in the nuclear fossa which comprise it are located at a right angle to each other (Fig. 1e). The centrioles shows a characteristic nine-triplet pattern. The distal centriole is the basal body of the flagellum (Fig. 1d, e). Proximal and distal centrioles are fastened with each other by microfibril (Fig. 1e), these interconnect the electron-dense ring of the distal centriole with the lateral side of the proximal centriole (Fig. 1f). The proximal centriole itself is connected to the nuclear envelope by microfibrils (Fig. 1e). Nine satellite fibers project from the centriole to the mitochondria (Fig. 1g). The satellite rays projected from distal centriole, appear nine electron dense lamella materials (Fig. 1g). These satellites inference is to anchor the distal centriole to the midpiece (Fig. 1g, h).

The midpiece contains five spherical mitochondria arranged in the distal centriole and anterior portion of axoneme (Fig. 1h-j). The mitochondria are closely related to the nucleus and often the nuclear envelope contacts the exterior mitochondrial membrane (Fig. 1f). The cytoplasmic canal is formed by an invagination of the plasmalemma and runs longitudinally from the caudal to the cranial end of the midpiece (Fig. 1d, j).

The flagellum emerges from the distal centriole (Fig. 1e). Between the base of the axoneme and the distal centriole is the transitional area, where the axoneme have of nine double outer tubules and no central tubules (Fig. 1g, h). The flagellum has paired lateral ribbon. In each lateral ribbon, there is electron lucent contents (Fig. 1k). Fig. 2 is a diagram of flatfish spermatozoa. Fine structure of the head region, midpiece region and flagella is illustrated.

DISCUSSION

The organization of spermatozoa of *Paralichtys olivaceus* is similar to typical external fertilizing teleost (Jamieson, 1991; Jun *et al.*, 2006). They have no acrosome, and small midpiece include several mitochondria and a long flagellum separated by cytoplasmic canal.

According to Jamieson (1991), the spermatozoa head of *P. olivaceus* is spherical shaped which is regarded as a lower developed feature than ovoid sperm head. These differences in the shape of spermatozoa may have consequences in flow resistance and in the swimming behaviour as swimming types or swimming velocities (Lahnsteiner and Patzner, 1995).

The invagination of nucleus, basal fossa, which is also found in other species (Cyprinidae, Siluridae, Salmonidae) with diversity (Baccetti *et al.*, 1984; Gwo *et al.*, 1996; Kim and Lee, 2003). Previous investigation also reported that structure (Hara and Okiyama, 1998; Kim *et al.*, 2003). But we can provide the types of nuclear fossa in Perciformes. In Siganidae, the nuclear fossa appeared very shallow (Gwo *et al.*, 2004). In Mullidae, the nuclear fossa deeply invaginated inversely U shape structure (Gwo *et al.*, 2004). But Cichlidae, nuclear fossa shows moderately type similar to *P. olivaceus* (Kim *et al.*, 2003).

The midpiece of *P. olivaceus* is small and transversed by the cytoplasmic canal containing 5 mitochondria separating the flagellum from the mitochondria by the cytoplasmic canal. This is a significant difference from spermatozoa of other vertebrates (Guraya, 1987) and invertebrates (Koch and Lambert, 1990). The physiological meaning of this structural separation of mitochondria and flagella is unknown. According to Baccetti *et al.* (1984) the number of mitochondria is closely linked with phylogeny. In Perciformes, there are 5~10 mitochondria placed in midpiece, around the initial flagellum (Mattei, 1991). In Labridae, the mitochondria, three-five in number, disposed closely to the nucleus and surround the initial region of flagellum (Mattei, 1991). In *P. olivaceus* spermatozoa, the mitochondria (five in number) are round and their distribution is very similar to Labridae (Mattei, 1991). In Cichlidae, the midpiece has 10 round of slightly elongated mitochondria disposed in two layers around the initial segment of the flagellum

(Quagio-Grassiotto *et al.*, 2003). In Siganidae, the midpiece has six spherical mitochondria arranged only one row like in *P. olivaceus* and in Siganidae, the mitochondria are arranged in two rows, five on the cranial and caudal below (Gwo *et al.*, 2004). In Embiotocidae, the midpiece shows 6 elongated mitochondria, placed in pairs in three layers (Gardiner, 1978). In these families, mitochondria have different forms and distribution patterns within the midpiece.

The interconnection between proximal centriole and distal centriole by microfibril is common in teleost (Lahnsteiner and Patzner, 1996). But the distal centriole's nine satellite lamellas structure is rarely in fish spermatozoa. The nine satellite lamellas are very common in lower metazoan sperm, but is reduced in size in higher phyla (Afzelius, 1979). According to Gwo (1995), the nine satellite lamellas were well developed in Perciformes and Atheriniformes. The basal body in various, since minute morphological differences might have functional and evolutionary significations (Gwo, 1995).

The flagellum of *P. olivaceus* is centrally inserted to the nucleus, and the sperm reveals an symmetrical organization. Such symmetrical spermatozoa have been observed in most Perciformes (Quagio-Grassiotto *et al.*, 2003), excepts Percidae (Lansteiner *et al.*, 1995). But other teleost fishes Cyprinidae, Trachinidae (Lahnsteiner and Patzner, 1996) and Esocidae (Billard, 1970) have asymmetrical spermatozoon. External fertilization of teleostei spermatozoa, the position of the flagellar axis, that may be perpendicular or parallel to the nucleus, has been took to characterize spermatozoa as type I or II, respectively (Mattei, 1970).

The presence of lateral ribbon of flagellum are not clear in flounder (Hara and Okiyama, 1998; Jun *et al.*, 2006). But spematozoon of *P. olivaceus* has it clearly. The sperm flagellar ultrastructure has great diversity according to species which has many way in the motility patterns (Afzelius, 1982).

The result of the present study compared Hara and Okiyma (1998) and Kim *et al.* (2003), reveal new structure and previously undescribed feature. To our knowledge, the satellate lamellae, satellate rays in midpiece, interconnection of two centriole and lateral fibbon in flagellum observed in bastard halibut *P. ovlivaceus*, spermatozoa has not been reported elsewhere. And we described nucler fossa type and mitochondria distribution pattern in Perciformes.

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한국산 넙치(*Paralichthys olivaceus*) 정자의 미세해부학적 구조

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요 약 : 한국산 넙치(*Paralichthys olivaceus*) 정자의 미세해부학적구조를 관찰한 결과 첨체가 없고, 두부는 둥글고, 중부는 작으며 단편모로 이루어져 있었다. 넙치정자의 구조는 경골어류의 일반적인 특징이나 형태 및 세포소기관의 미세구조에서 종간에 차이가 나타난다. 본 종의 정자는 5개의 미토콘드리아가 한 층으로 중편에서 중심립을 둘러싸고 있고, 말단부중심립과 관련된 9개의 구조물이 나타났다. 편모는 기부가 핵에 삽입되어 있고, 양측면으로 막이 확장되어 있었다. 이러한 특징은 경골어류의 종 분류와 계통학적 연구에 중요한 요소가 된다.

찾아보기 낱말 : 넙치, *Paralichthys olivaceus*, 정자, 미세구조