SPERM STRUCTURE OF THE PANDALID SHRIMP *PANDALOPSIS JAPONICA* (DECAPODA, PANDALIDAE)

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ABSTRACT

The sperm structures of pandalid shrimps were previously undescribed. We determined the mature sperm morphology and ultrastructure of *Pandalopsis japonica* by means of light and electron microscopy and compared them with the known morphology and ultrastructure from other carideans. Spermatogenesis is initiated in the testicular tissue of the male-phased *Pandalopsis japonica* ranging from 14 to 23 mm in carapace length. The sperms are 52 to 55 μm in length and consist of a cup-shaped main body and a spike extending from the convex surface of the main body. The main body, of which the size is 24 to 26 μm in width and 5 to 7 μm in height, has a nucleus and a cup-shaped base. The spike, 40 to 44 μm long and approximately 3 μm thick, consists of the central core filled with electron-lucent materials and the electron-dense wall containing a number of tubular-like structures that are aligned along the longitudinal spike. The unique structure of *Pandalopsis japonica* sperm, particularly in the spike possessing tubular-like structures of approximately 24 nm in the outside diameter and 15 nm in the inside diameter, offers a good taxonomic tool among shrimp species in the carideans.

The sperm morphology of mature decapod crustaceans is interesting because they lack a flagellated tail and mitochondrial midpiece, unlike the sperm of most other animals. Most decapod sperm are nonmotile and have only a main body with stellate appendages (spikes). In general, decapod sperms are divided into two groups of characteristic natantian (shrimps) and reptantian (crayfish, lobster, and crab) morphological types on the basis of the number and structure of sperm spikes. Natantian sperm possess a nucleus and a single spike that may contain microfilaments (Brown *et al.*, 1976; Kleve *et al.*, 1980), whereas reptantian sperm possess a main body and multiple spikes that mostly consist of microtubules; however, these differ from the typical flagellated sperm of other animals in the lack of a true axoneme (Hinsch, 1969; Talbot and Summers, 1978; Dudenhausen and Talbot, 1982; Tudge and Jamieson, 1991; Lohrmann and Raineri, 1995).

In natantian decapods, many studies on sperm ultrastructure have been done in penaeidean shrimps of the suborder Dendrobranchiata (Clark *et al.*, 1973; Kleve *et al.*, 1980; Ogawa and Kakuda, 1987; Clark and Griffin, 1988;

Dougherty and Dougherty, 1989; Krol *et al.*, 1992; Medina, 1994; Medina *et al.*, 1994a, 1994b) and shrimps belonging to the suborder Pleocyemata. Within the suborder Pleocyemata, as reviewed by Jamieson and Tudge (2000), sperm ultrastructure has been studied in some caridean shrimps of the superfamilies Palaemonoidea (*Palaemon serratus* (see Papathanassiou and King, 1984); *Palaemonetes paludosus* (see Koehler, 1979); *Macrobrachium rosenbergii* (see Lynn and Clark, 1983); and *Macrobrachium australiense* (see Butcher and Fielder, 1994)), Rhynchocinetoidea (*Rhynchocinetes typus* (see Dupré and Barros, 1983)), and Crangonoidea (*Crangon septemspinosa* (see Arsenault *et al.*, 1979, 1980)). There are some morphological differences in the structure of sperm spikes among the caridean superfamilies. The spikes of palaemonoid shrimps are composed of cross-striated fibres, whereas the spikes of crangonoid and rhynchocinetoid shrimps are not. Moreover, the spike of the rhynchocinetoid shrimp is a hollow tube-like form and much longer than that of other caridean shrimps. Such differences reflect that spike components of sperm may vary according
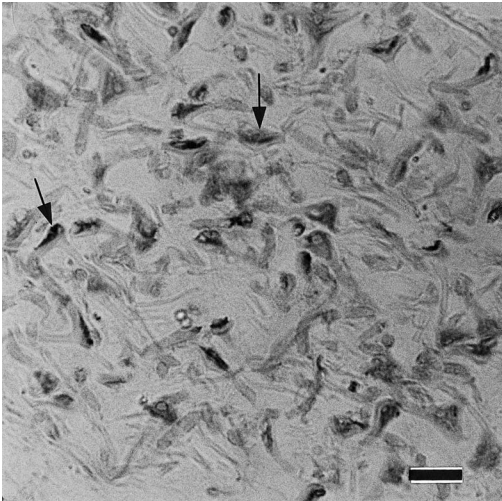
Fig. 1. Light micrographs showing sperm in the vas deferens of *Pandalopsis japonica*. Feulgen-positive reaction. The nucleus (arrowhead) is restricted to the posterior part of the main sperm body. Scale bars represent 20 μm.

to superfamilies. Recently, the ultrastructures of sperm have been extensively used for recognizing phylogenic linkages in decapod taxa (Jamieson, 1989; Tudge and Jamieson, 1991; Medina, 1994; Tudge, 1997; Medina *et al.*, 1998). Therefore, it appears that knowledge of sperm morphology and components might be very useful for estimating phylogeny in decapod crustaceans.

Of the caridean superfamilies, spermatozoal ultrastructure of the pandaloid shrimps, including *Pandalopsis japonica*, has not previously been described. *Pandalopsis japonica* is a protandrous hermaphrodite, and starts reversing sex from male-phase to female-phase at the age of 3.75 years (Ito, 1978). An individual of this species that has a fully developed appendix masculina that is subequal in length to the appendix interna on the second pleopod and has a well-developed appendix interna on the first pleopod is in the male phase. The female-phase is recognized by the absence of the appendix interna from the first pleopod in an individual with a carapace length of 29 mm or more (Komai, 1994). *Pandalopsis japonica* inhabits the offshore reefs and bank areas in waters 180 to 530 m deep from the northern area of the East Sea of Korea (the Sea of Japan) to the Pacific coasts of Saghalin of Russia, providing one of the important fisheries resources in these areas (Ito, 1978; Komai, 1994).

However, the characteristics of reproductive biology, including sperm structure, in this species are little known.

This paper presents the morphology of mature sperm in the vas deferens of *Pandalopsis japonica* to exemplify the structural characteristics of sperm of superfamily Pandaloidea and to give a comparison among sperms in decapod crustaceans, including caridean shrimps.

MATERIALS AND METHODS

A total of 55 specimens of *Pandalopsis japonica* were collected by shrimp traps at depths of 200 m or less from the oceanic zone off the coast of eastern Korea in January 2001. After the selection of individuals, which had the external characteristics of males (Ito, 1978), their *vasa deferentia* were dissected, and fixed with Bouin's solution for 24 hours. Tissues were dehydrated in ethanol and embedded in paraffin. Sections (6–7 μm) were cut with a rotary microtome and affixed to albuminized slides. Alternate slides were stained with PAS (periodic acid-Schiff) and Feulgen stains for light microscopic observation. For examination with transmission electron microscopy, the *vasa deferentia* were dissected and fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.2) for 4 h in cold. The tissues were postfixed in 1% osmium tetroxide in the same buffer for 2 h, rinsed in distilled water, and embedded in Epon 812. The thin sections were picked up on copper grids, stained with uranyl acetate and lead citrate, and examined with a Jeol Jem 1200 EX-II transmission electron microscope at 80 kV.

RESULTS

Spermatogenesis occurs in the testicular tissue of the male-phased *Pandalopsis japonica* with carapace lengths ranging from 14 to 23 mm. Examination of the vas deferens of the shrimp at this size reveals spermatophores filled with mature sperm. Sperm in the vas deferens are nonmotile in sea water during microscopic observation and resemble a thumbtack when observed with a light microscope (Fig. 1). Sperm cells are approximately 52 to 55 μm long. At the ultrastructural level the sperm can be divided into two morphologically distinct regions: the cup-shaped main body containing a nucleus and the spike extending from the convex surface of the main body (Fig. 2).

The main body, of which the size is 24 to 26 μm in width and 5 to 7 μm in height, is composed of a nucleus and a cup-shaped base. Nuclear material (indicated as a Feulgen-positive region) (Fig. 1) is restricted in the posterior part of the main body of the sperm. The nucleus, uncondensed and relatively electron-lucent, is delimited by a nuclear membrane, and is placed on the concave surface of the cup-shaped base which is thickened along the central basal margin lying near the spike (Figs. 2A and 3).
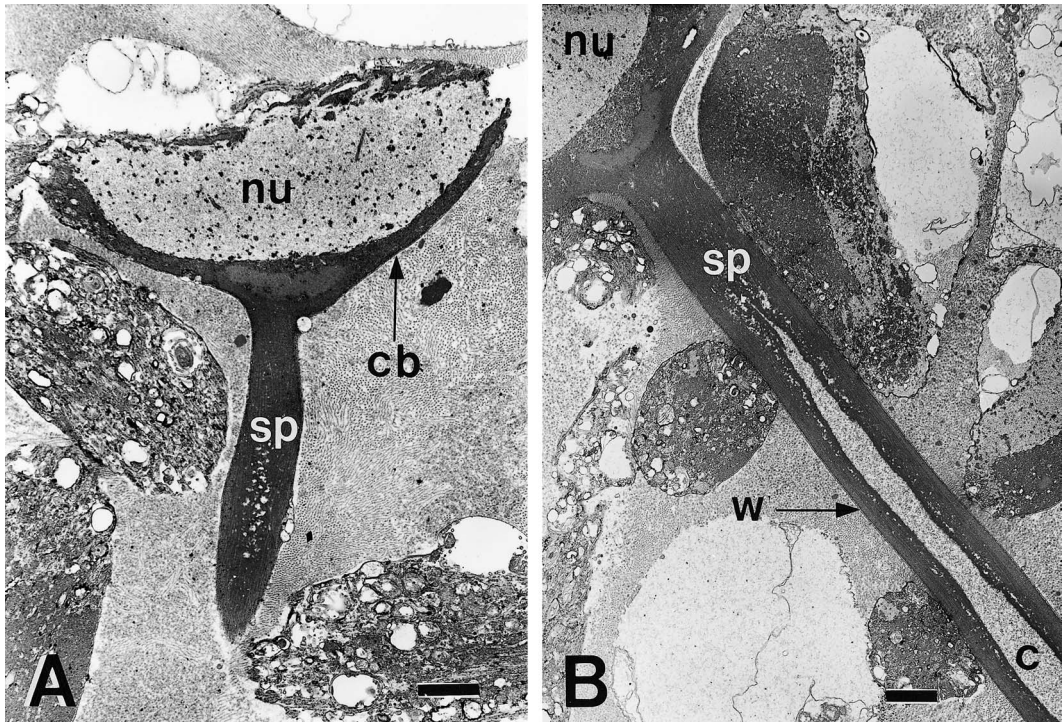
Fig. 2. Electron micrographs of longitudinal section (LS) revealing the location and general organization of components in the two ultrastructural regions of the *Pandalopsis japonica* sperm cell: the main body consisting a cup-shaped base (cb) and a nucleus (nu), and the spike (sp) consisting of electron dense wall (w) and electron lucent core (c). Scale bars represent 2 µm.

The cup-shaped base appears to be separated from the nucleus by a membrane (Fig. 3). The membrane limiting the cup-shaped base appears to be complete and to fuse with the single plasmalemma surrounding the sperm. In the cytoplasmic layer (as opposed to the nuclear region) of the cup-shaped base, a crescent-shaped structure that is housed centrally in relation to the base of the spike can be seen. A number of tubular-like structures extending into the spike are shown at the place adjacent to the convex surface of crescent-shaped structure (Fig. 3).

The spikes are approximately 40 to 44 µm in length and 3 µm in diameter, and consist of the central core filled with electron-lucent materials and the electron dense wall (Figs. 2B). The central core region of approximately 1.0 to 1.7 µm width is present along the length of the spike and gradually disappears near the base and the distal tip of the spike (Fig. 4). The wall of the spike contains a number of tubular-like structures aligned along the longitudinal spike, and exhibits two layers in terms of the density of tubular-like structures and the electron-density

of the matrix (Figs. 5 and 6). Although the boundary between the outer layer and the inner layer is not clear, the outer layer adjacent to the membrane is thinner and more lucent in the electron-density of the matrix than the inner layer. Tubular-like structures in the inner layer are more loosely located than those in the outer layer (Fig. 6). In the transverse and longitudinal sections near a halfway point along the spike, the mean thicknesses of the inner and outer layers are approximately 0.7 µm and 0.3 µm, respectively (Fig. 5). The tubular-like structure, of which the size is approximately 24 nm in the outer diameter and 15 nm in the inner diameter, is surrounded by an outer electron-lucent ring that is shown to be the region of loosening cytoplasm under high magnification (Fig. 6B).

DISCUSSION

The sperm of crustaceans are very diverse in their morphology; therefore, it is difficult to designate sperm features that characterize the entire class. Nevertheless, sperm data are extremely useful in determining relationships
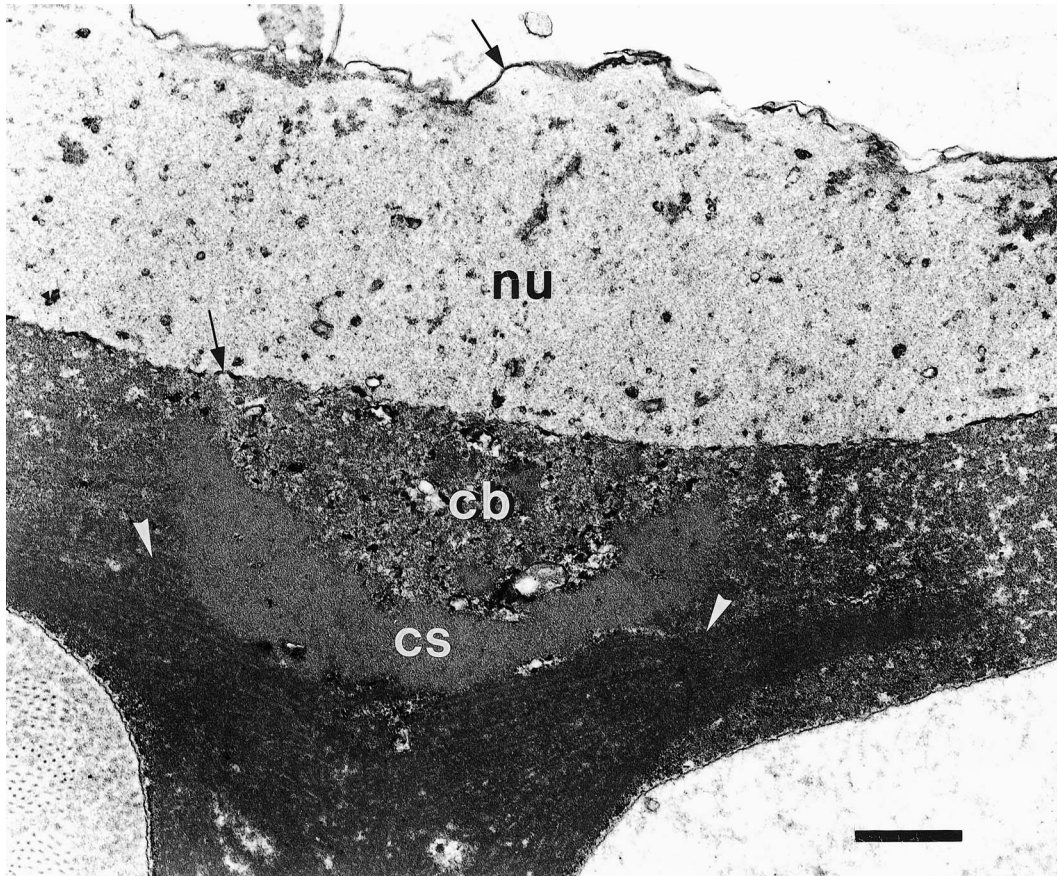
Fig. 3.   Electron micrograph of magnified LS of the main body of *Pandalopsis japonica* sperm cell. The nuclear materials (nu) are decondensed and delimited by nuclear membrane (arrow). The cup-shaped base (cb) is housing a crescent-shaped structure (cs) and has a number of tubular-like structures (white arrowheads) near the convex surface of crescent-shaped structure. Scale bars represent 2 μm.

between crustacean taxa (Jamieson, 1989; Krol *et al.*, 1992; Medina 1994; Medina *et al.*, 1994a, 1994b; Tudge, 1992, 1997). Jamieson and Tudge (2000) reviewed the sperm structure of decapod crustaceans, noting that some parts of the sperm structure, including spikes and the nucleus, are markedly different enough to be used in taxonomic keys.

Mature decapod crustacean sperm are aflagellate and have general characteristics of a decondensed nucleus and spikes. In general, decapod sperm are divided into two groups of characteristic natantian and reptantian morphological types on the basis of the number and structure of sperm spikes. Reptantian sperms are characterized by multiple spikes that are bound, or partially bound, by a nuclear envelope that lies against the plasma membrane and usually the spikes contain chromatin and/or micro-

tubules. Natantian sperm possess a single spike which is not continuous with the nucleus and may contain microfilaments (Brown *et al.*, 1976; Kleve *et al.*, 1980; Dupré and Barros, 1983).

Until recently, many studies have focused on the sperm structure in dioecious natantian species (Pochon-Masson, 1969; Lu, 1976; Koehler, 1979; Kleve *et al.*, 1980; Lynn and Clark, 1983; Papathanassiou and King, 1984; Shigekawa and Clark, 1986; Felgenhauer *et al.*, 1988; Griffin *et al.*, 1988). However, in hermaphroditic natantian shrimps, the sperm structure is still poorly understood as compared to dioecious natantians. As far as the general feature of sperm is concerned, sperm of the hermaphroditic pandalid shrimp *Pandalopsis japonica* is not different from that of other dioecious natan-
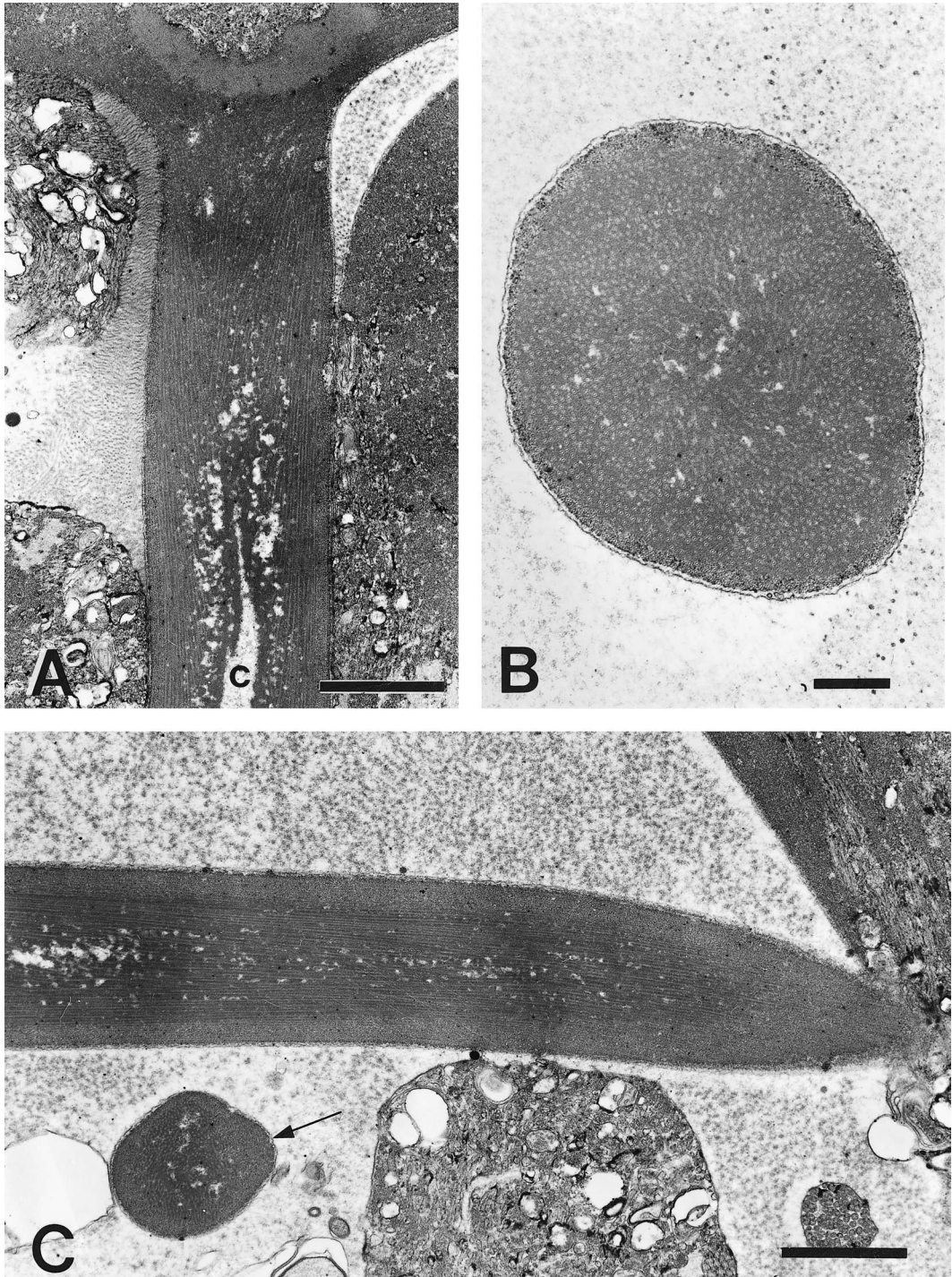
Fig. 4.   Electron micrographs of LS (A) and transverse section (TS) (B) of the area near the base and LS of the distal tip (C) of the spike in the sperm cell of *Pandalopsis japonica*. The core region (c) present along the length of the spike gradually disappears near the base and the distal tip of the spike. The arrow indicates the TS feature near the distal tip of the other spike. Scale bars represent 2 μm.
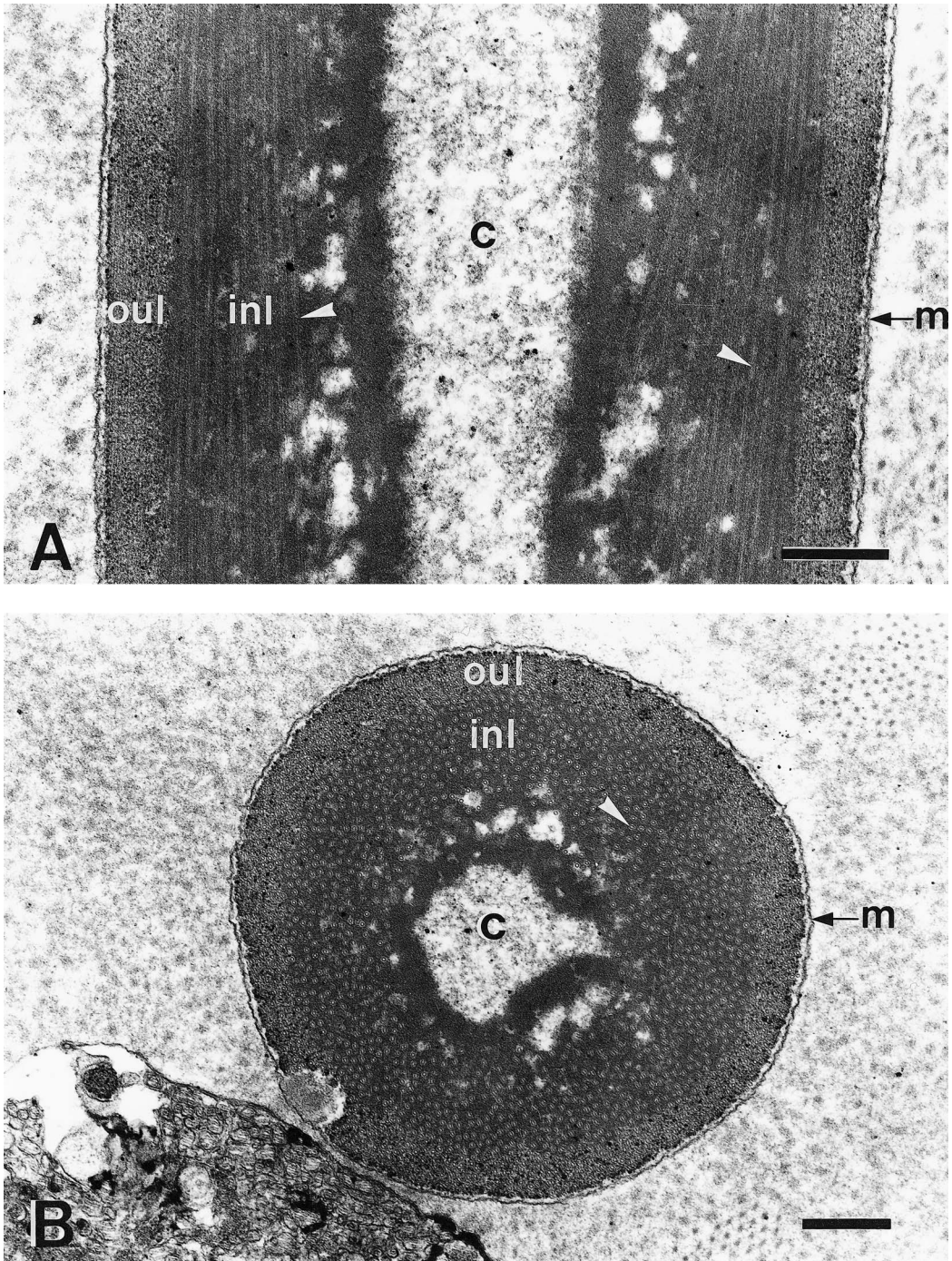
Fig. 5.   Electron micrographs of an LS (A) and TS (B) near the halfway point along the spike in sperm cell of *Pandalopsis japonica*. The wall of spike contains a number of tubular-like structures (white arrowheads) aligned along the longitudinal spike, and exhibits two layers: inner layer (inl) and outer layer (oul). c, core region; m, membrane. Scale bars represent 500 nm.
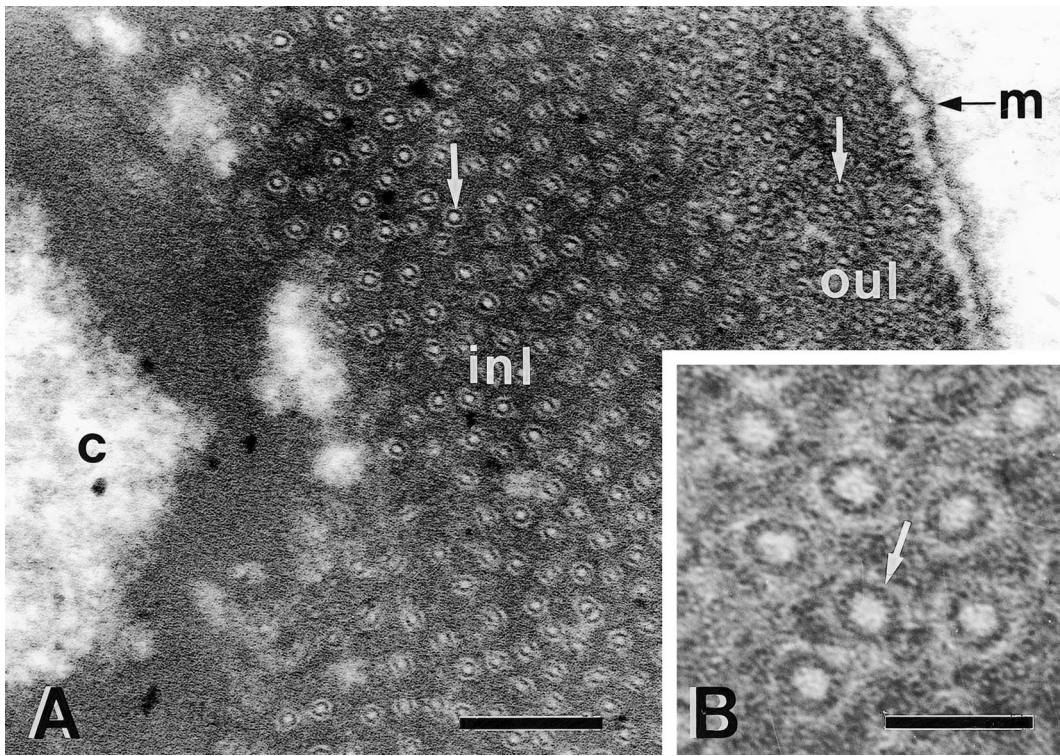
Fig. 6. Electron micrographs of magnified TS of the tubular layers (A) and detail showing tubular-like structure (B) in the sperm spike of *Pandalopsis japonica*. The tubular-like structures (white arrows) in the outer layer (oul) are dense compared to those in the inner layer (inl). c, core region; m, membrane. Scale bars in A and B represent 200 nm and 50 nm, respectively.

tian sperm. However, there are some differences in the structure and size of sperm.

The nucleus of *Pandalopsis japonica* is decondensed, does not have a nuclear envelope and does not extend into, or become continuous with, the spike, which is typical of natantians (Pochon-Masson, 1969; Clark *et al.*, 1973; Lynn and Clark, 1983). A nuclear membrane has been reported in the mature sperms of *Crangon septemspinosa* (see Arsenault *et al.*, 1979), and Lu *et al.* (1973) observed that the nuclear membrane broke down during spermiogenesis in *Penaeus setiferus*. The other unique structure found in the main body of *Pandalopsis japonica* sperm is the crescent-shaped body. To date, the function of this organelle is unknown. Centrioles lie at the base of the spike in *Palaemon elegans* (see Pochon-Masson, 1969), *Macrobrachium rosenbergii* (see Lynn and Clark, 1983), and *Macrobrachium australiense* (see Butcher and Fielder, 1994). *Palaemonetes kadiakensis*, *Palaemonetes paludosus*, and *Penaeus aztecus* have a centriole in developing spermatids, but the centriole is absent in the mature sperm (Lu, 1976; Koehler, 1979; Felgenhauer *et al.*, 1988). Centrioles have not been confirmed in developing spermatids of *Pandalopsis japonica*, and no centrioles are observed at least in the mature sperms.

The structure and size of the spike vary among the studies on natantian shrimps. The size of spike in *Pandalopsis japonica* is almost three times longer than that of palaemonid shrimps, in which the lengths of the spike are 12 to 15 μm in *Macrobrachium rosenbergii* (see Lynn and Clark, 1983), 13 to 15 μm in *Macrobrachium australiense* (see Butcher and Fielder, 1994), and 12 to 14 μm in *Palaemonetes paludosus* (see Koehler, 1979). The length of the spike in *Pandalopsis japonica* is smaller than that in *Rhynchocinetes typus* (see Dupré and Barros, 1983), whose spike is a hollow tube-like form and 53 μm in length. The spikes of *Macrobrachium rosenbergii* (see Lynn and Clark, 1983), *Palaemon elegans* (see Pochon-Masson, 1969), *Palaemon serratus*

(see Papathanassiou and King, 1984), and *Palaemone paludosus* (see Koehler, 1979) have cross-striations, whereas the spikes of *Rhyncho-cinetes typus* (see Dupré and Barros, 1983) and *Procaris ascensionis* (see Felgenhauer *et al.*, 1988) are striated. However, in *Pandalopsis japonica*, these striations are not observed in the spike; rather, it has a number of tubular-like structures.

Similar tubular-like structures were also observed in *Macrobrachium rosenbergii* sperm (Lynn and Clark, 1983) and *Sicyonia ingentis* sperm (Clark and Griffin, 1988). In *Macro-brachium rosenbergii*, tubular-like structures of 26 nm in diameter were observed only in the cross section at or near the tip of the spike. *Sicyonia ingentis* sperm has the tubular structures in the acrosomal filament, which are formed during elongation of the filament proper between the nucleus and the extended saucer just after the occurrence of sperm-egg interaction. In an electron micrograph of low magnification, the tubular-like structures of the spike of *Pandalopsis japonica* is similar in feature to the cross section of that of the acrosomal filament in *Sicyonia ingentis*, of which a tubular-like structure contains an electron dense ring surrounded by an outer electron translucent ring. Under high magnification of the spike in *Pandalopsis japonica* sperm, however, the outer electron-lucent rings are shown to be regions of loosening cytoplasm, and neither organelles nor structures of cytoplasm. In *Rhynchocinetes typus*, microfilaments and tubulin-like proteins were found in the sperm rays and spine by immunocytochemical studies using antitubulin antibodies (Perez *et al.*, 1991) but not in the spike that has no tubular-like structure (Dupré and Barros, 1983). As biochemical and immu-nocytochemial studies on these tubular-like structures were not performed, the tubular-like structures of the spike in *Pandalopsis japonicus* sperm could not be construed as a microtubule composed of tubulin. However, the outer and inner diameters of the tubular-like structure is approximately the same as those of the typical microtubule whose size is 25 nm in outer diameter and 15 nm in inner diameter. Although tubular-like structures may be present in cross sections of the tip of *Macrobrachium* sperm spikes (Lynn and Clark, 1983), they are not a prominent feature in natantian sperm (Talbot and Summers, 1978; Krol *et al.*, 1992). Therefore, the tubular-like structure of the spike is thought to be one characteristic of pandalid shrimp sperm, including *Pandalopsis japonica*. This is supported in the spike structure of *Pandalus gracilis*, in which a tubular-like structure was also evident (unpublished data).

Pochon-Masson (1969) reported that the spike in the sperm of *Palaemon elegans* corresponds to the acrosome. In several studies, as well as in this study, regarding the sperm of natantians, however, neither the sperm nor spermatid have a PAS positive spike (Arsenault *et al.*, 1979; Koehler, 1979; Dupré and Barros, 1983; Lynn and Clark, 1983; Papathanassiou and King, 1984). Recently, Rios and Barros (1997) have shown the presence of a trypsin-like enzyme in *Rhynchocinetes typus* spermato-zoa that appears to be involved in sperm penetration through the egg-coats during fertil-ization.

The nucleoplasm of reptantian sperm has been observed to contain a number of micro-tubules that extend along the length of the radiating spikes (Pochon-Masson, 1968; Lan-greth, 1969; Talbot and Summers, 1978). Microtubules in various configurations are common in the acrosomal tubule of reptantian sperm (Talbot and Summers, 1978; Dudenhau-sen and Talbod, 1982; Lohrmann and Raineri, 1995; Tudge and Jamieson, 1996). The role of these microtubules may be to maintain the shape of the spike in reptantian sperm (Hinsch, 1969), although the precise role and biochem-ical composition of the spike, other than its proteinaceous nature, is uncertain. In *Panda-lopsis japonica*, the function of the tubular-like structures and their material composition are also uncertain. They are predominantly different from that of other carideans studied to date in that the spike contains multiple electron densi-ties (Arsenault *et al.*, 1979; Koehler, 1979; Lynn and Clark, 1983; Papathanassiou and King, 1984; Butcher and Fielder; 1994).

Pandalid sperm fit the general scheme for natantian sperm structure but differ in several important respects from other caridean sperm, particularly in the tubular-like structure of the spike, and more studies need to be conducted to determine the biochemical properties and role of the tubular-like structure.

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