

Germ cell Differentiation During Spermatogenesis, and Ultrastructural Characteristics of Mature Sperm in Male *Phacosoma japonicus* (Bivalvia: Veneridae)

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ABSTRACT

Some characteristics of germ cell differentiations during spermiogenesis and mature sperm ultrastructure in male *Phacosoma japonicus* were investigated by transmission electron microscope observations. The morphology of the spermatozoon of this species has a primitive type and is similar to those of other species in the subclass Heterodonta. Morphologies of the sperm nucleus and the acrosome of this species are the cylindrical type and cap shape, respectively. The spermatozoon is approximately 45-50 μm in length, including a long curved sperm nucleus (about 3.70 μm long with 45° of the angle of the nucleus, an acrosome (about 0.55 μm in length), and tail flagellum (about 42-47 μm). The axoneme of the sperm tail shows a 9+2 structure. As some characteristics of the acrosomal vesicle structures, the basal and lateral parts of basal rings show electron opaque part (region), while the anterior apex part of the acrosomal vesicle shows electron lucent part (region). These characteristics of the acrosomal vesicle were found in the family Veneridae and other several families in the subclass Heterodonta. These common characteristics of the acrosomal vesicle in the subclass Heterodonta can be used for phylogenetic and systematic analysis as a taxonomic key or a significant tool. The number of mitochondria in the sperm midpiece of this species are four, as one of common characteristics appear in most species in the family Veneridae and other families in the subclass Heterodonta. However, exceptionally, only three species in Veneridae of the subclass Heterodonta contain 5 mitochondria. The number of mitochondria in the sperm midpiece can be used for the taxonomic analysis of the family or superfamily levels as a systematic key or tools.

Key words: *Phacosoma japonicus*, spermiogenesis, mature sperm ultrastructural characteristics

INTRODUCTION

Germ cell differentiations during Spermatogenesis and mature sperm ultrastructural features have been documented in many species of bivalve molluscs using both light and transmission electron microscopy (Daniels *et al.*, 1971; Franzén, 1970, 1983; Dorange

and Le Pennec, 1989; Healy, 1989, 1995; Healy and Lester, 1991; Gaulejac *et al.*, 1995; Eckelbarger *et al.*, 1990, Eckelbarger and Davis, 1996; Chung and Ryou, 2000; Chung *et al.*, 2007, 2010). It is well-known that spermatogenesis occurs through spermatogenic germ cell differentiations in acini of the testis. Therefore, above all, it is important to investigate the processes of germ cell differentiations by developmental stages during spermatogenesis to clarify the reproductive mechanism.

To date, previously there have been only a few studies on reproduction and ecology in *Phacosoma japonicus*, including aspects of maturation and

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spawning (Chung *et al.*, 1997), and its distribution and ecology (Min *et al.*, 2004). Although reproduction and ecology of this species have been investigated by some authors, little information is available on germ cell differentiations during spermatogenesis, and ultrastructural characteristics of mature sperm of this species. Therefore, it is important to study some characteristics of germ cell differentiations during spermatogenesis to clarify the reproductive mechanism by transmission electron microscope observation, and to investigate the ultrastructural features of mature spermatozoa.

In bivalve molluscs, in particular, sperm ultrastructure is considered a valuable tool in assessing taxonomic and phylogenetic problems within the Bivalvia (Bacetti, 1970; Daniels *et al.*, 1971; Popham, 1979; Healy, 1989, 1995; Hodgson and Bernard, 1986; Eckelbarger *et al.*, 1990, Eckelbarger and Davis, 1996) and is especially useful when comparing closely related species (Popham, 1974; Popham, 1979). Thus sperm ultrastructures of bivalves are now widely used in taxonomic analysis (Healy, 1995; Chung *et al.*, 2010).

It is well-known that acrosomal morphologies of sperms have been used to organize bivalve subclasses (Popham, 1979; Healy, 1989), the number of mitochondria in the sperm midpiece tends to be stable within any given family or superfamily. For that reason, it need to study acrosomal morphology of the sperm and the number of mitochondria in the sperm midpiece for taxonomic analysis of this species. For the study of taxonomic analysis of *P. japonicus*, which belongs to Veneridae in the subclass Heterodonta, acrosomal morphorlogy of mature sperm needs to compare their similarities with many species in other families of Heterodonta, and the number of mitochondria need to compare with many species in the families or superfamilies in Heterodonta. Information on sperm ultrastructure is sorely needed for this important clade of bivalves. Therefore, the pupose of this study is to describe germ cell differentiation during spermatogenesis with some special features of mature sperm ultrastructure, and to clarify the types of sperm nucleus and acrosome as well as the number of mitochondria in the sperm midpiece by phylogenetic

and taxonomic analyses of *P. japonicum*.

METHODS AND MATERIALS

1. Sampling

A total of 65 male clams of *P. japonicus* were collected in the intertidal zone of Biin Bay, Chungcheongnam-do, Korea, from January to December, 2008. The clams were transported to the laboratory where they were maintained in seawater at 20°C. The specimens were used for ultrastructural study of germ cells and mature spermatozoa by transmission electron microscopy.

2. Ultrastructures of germ cells and mature sperm

For transmission electron microscope observations, excised pieces of the gonads were cut into small pieces and fixed immediately in 2.5% paraformaldehydegutaraldehyde in 0.1 M phosphate buffer solution (pH 7.4) for 2 hours at 4°C. After prefixation, the specimens were washed several times in the buffer solution and then postfixed in a 1% osmium tetroxide solution in 0.2 M phosphate buffer (pH 7.4) for 1hour at 4°C. Specimens then were dehydrated in increasing concentrations of ethanol, cleared in propylene oxide and embedded in an Epon-Araldite mixture. Ultrathin sections of Epon-embedded specimens were cut with glass knives on a Sorvall MT-2 microtome and LKB ultramicrotome at a thickness of about 80-100 nm. Tissue sections were mounted on collodion-coated copper grids, doubly stained with uranyl acetate followed by lead citrate, and observed with a JEM 100 CX-II (80-KV) electron microscope.

RESULTS

1. Developmental stages of spermatogenesis

In general, spermatogenesis occurs in acini in the testis and can be divided into four stages: (1) spermatogonia, (2) spermatocytes, (3) spermatids, and (4) spermatozoa. On the whole, the process of spermatogenesis of this species appears to be similar to those of other bivalve species.

1) Spermatogonia

Spermatogonia are present along the acinus wall

relatively large germ cells (approximately 6.3 to 7.2 μm in diameter), irregularly shaped cells having little cytoplasm and a large spherical nucleus (about 3.5-3.9 μm in diameter) containing scattered chromatin materials. At this time, a number of mitochondria and vacuoles appear in the cytoplasm, while the cytoplasm of this cell is largely devoid of organelles (Fig. 1).

2) Spermatocytes

Two stages of spermatocyte development, presumed to be primary and secondary, appear in the acinus walls of some specimens. The spermatogonia differentiate into primary spermatocytes which resemble the former except for the larger diameter of their nuclei. Primary spermatocytes (approximately 5.4-6.6 μm) are slightly smaller cells that are distinguished by nuclei (approximately 3.2-3.8 μm) with more abundant and more darkly staining chromatins. At this time, synaptonemal complexes appear in the nucleus during the pachtene phase in the prophase of maturation division. The primary spermatocytes differentiate into secondary spermatocytes by the secondary maturation division. At this time, the nucleolus is no longer prominent (Fig. 2).

3) Spermatids

The secondary spermatocyte is transformed into the spermatids by the secondary meiotic division (Fig. 2). For convenience, spermiogenesis has been classified arbitrarily into two stages: the early and late stages. In the early stage of spermiogenesis, spermatids (approximately 4.3-4.6 μm in diameter) are oval in shape, and the nucleus is spherical and occupies the center of the cell. The nucleus (about 3.2-3.4 μm in diameter) contains scattered electron-dense granular heterochromatins, and the cytoplasm contains several mitochondria (Fig. 3). The morphology of the spermatid nucleus changes gradually during the differentiation of the spermatid. At this time, small granules are formed by the Golgi complex in the cytoplasm move to a position just in front of the nucleus, while mitochondria move to a position just behind the nucleus. After all, the morphologies of the

spermatid nuclei were gradually elongated, and one or a few granules in the cytoplasm of the spermatid become a proacrosomal vesicle (Fig. 4). The proacrosomal vesicle migrates to the presumptive anterior end of the nucleus of spermatid, where they coalesce to form a single electron-dense vesicle. The mitochondria become reduced in number but increase in size by mitochondrial fusion. The larger mitochondria form a close association with the nucleus, however, the shape of the nucleus is modified and becomes greatly elongated. A proacrosomal vesicle becomes cone-like acrosomal vesicle on the nucleus (Fig. 5). Thereafter, a cone-like acrosomal vesicle becomes an acrosome. And then the acrosome lying on the sperm nucleus become cone in shape (Fig. 6). In the basal part of the nucleus, the mitochondria become reduced in number but increase in size by mitochondrial fusion. Posterior to the nucleus is the midpiece. This region consists of four spherical mitochondria surrounding a pair of triplet substructure centrioles. The cristae of each mitochondrion are randomly arranged (Fig. 7). Larger mitochondria form a close association with the nucleus and in many cases appear tightly apposed to the nuclear envelope. At this time, of the two centrioles lying in the midpiece of the spermatozoon, the two centrioles, at right angles, show the classic nine triplets of microtubules. The proximal centriole lies at 90° relative to the distal centriole and sperm longitudinal axis, and is connected to a shallow invagination of the nucleus by a thin layer of dense material. The distal centriole is attached to the plasma membrane. The distal centriole lies parallel to the sperm longitudinal axis and forms the point of origin for flagellar axoneme (Fig. 7). In the late stage of spermiogenesis, the spermatid nucleus is long elongated, and the cytoplasm is greatly reduced, and so the rate of nucleo-cytoplasm is high. After the sperm nucleus (average 3.70 μm long) is long elongated, an acrosomal vesicle is also gradually very elongated (average 0.52 μm long). The acrosome is composed of electron-opaque part. In particular, the axial filaments are not found in the acrosomal lumen, while subacrosomal granular materials are present in the subacrosomal space between the anterior

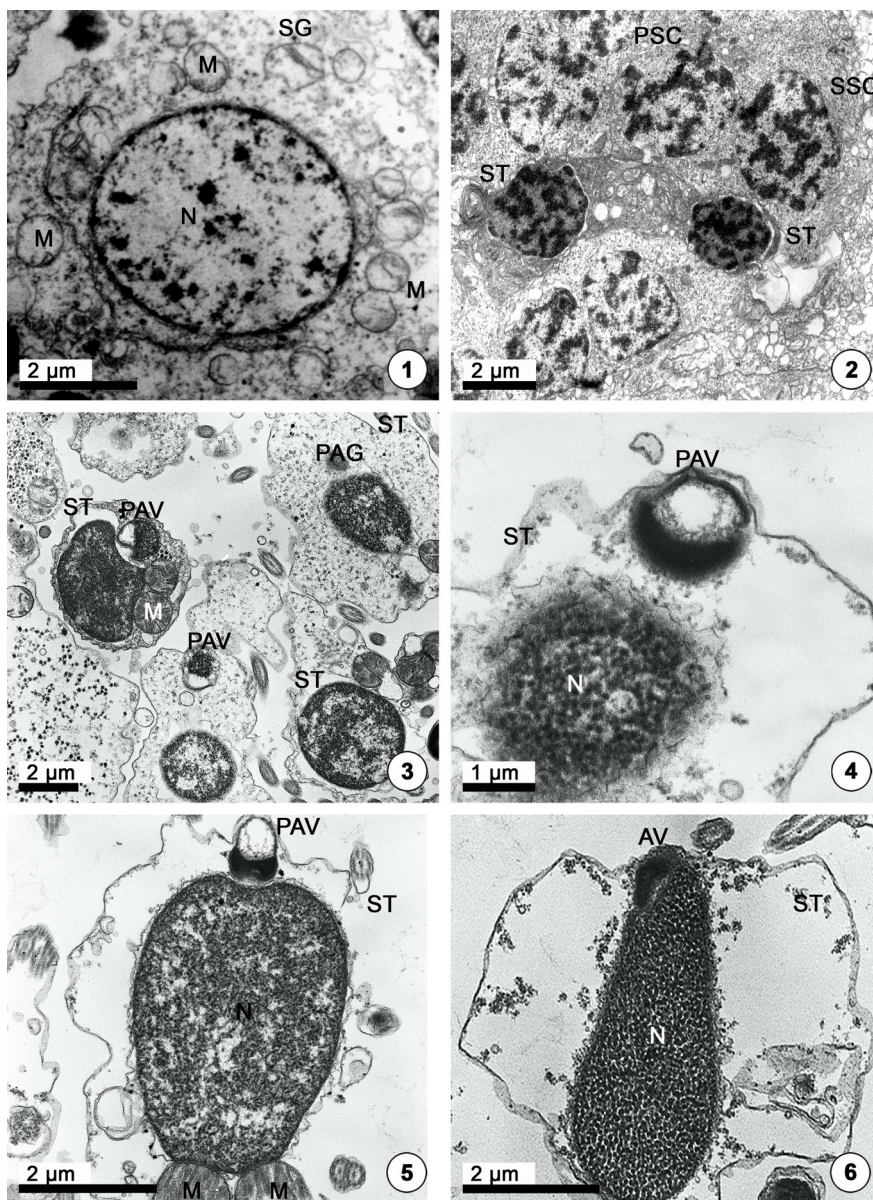


Fig. 1-6. Transmission electron micrographs of spermatogenesis and spermiogenesis in male *Phacosoma japonicus*. **Fig. 1.** A, spermatogonium (SG). Note a spermatogonium containing a nucleolus in the nucleus and several mitochondria (M) in the cytoplasm, **Fig. 2.** Primary spermatocytes (PSC), secondary spermatocytes (SSC) and spermatids (ST). Note several synaptonemal complexes in the nucleus of the primary spermatocytes (PSC) during the prophase of the primary maturation division, and heterochromatins in the nuclei of secondary spermatocytes (SSC) and spermatids (ST). **Fig. 3.** A spermatid (ST) in the early stage of differentiation during spermiogenesis. Note proacrosomal granules (PAG) and a proacrosomal vesicle (PAV) just before the nucleus (N) of the spermatid. **Fig. 4.** A spermatid (ST) in the same stage of differentiation during spermiogenesis. Note proacrosomal vesicle (PAV) just before the nucleus (N) of the spermatid. **Fig. 5.** A spermatid (ST) and a proacrosomal vesicle (PAV) on the nucleus (N). Note a proacrosomal vesicle (PAV) attached to the spermatid nucleus and mitochondria (M) beneath the nucleus (N). **Fig. 6.** A spermatid and elongated nucleus (N). Note an acrosomal vesicle (AV) on the elongated nucleus (N) of spermatid (ST).

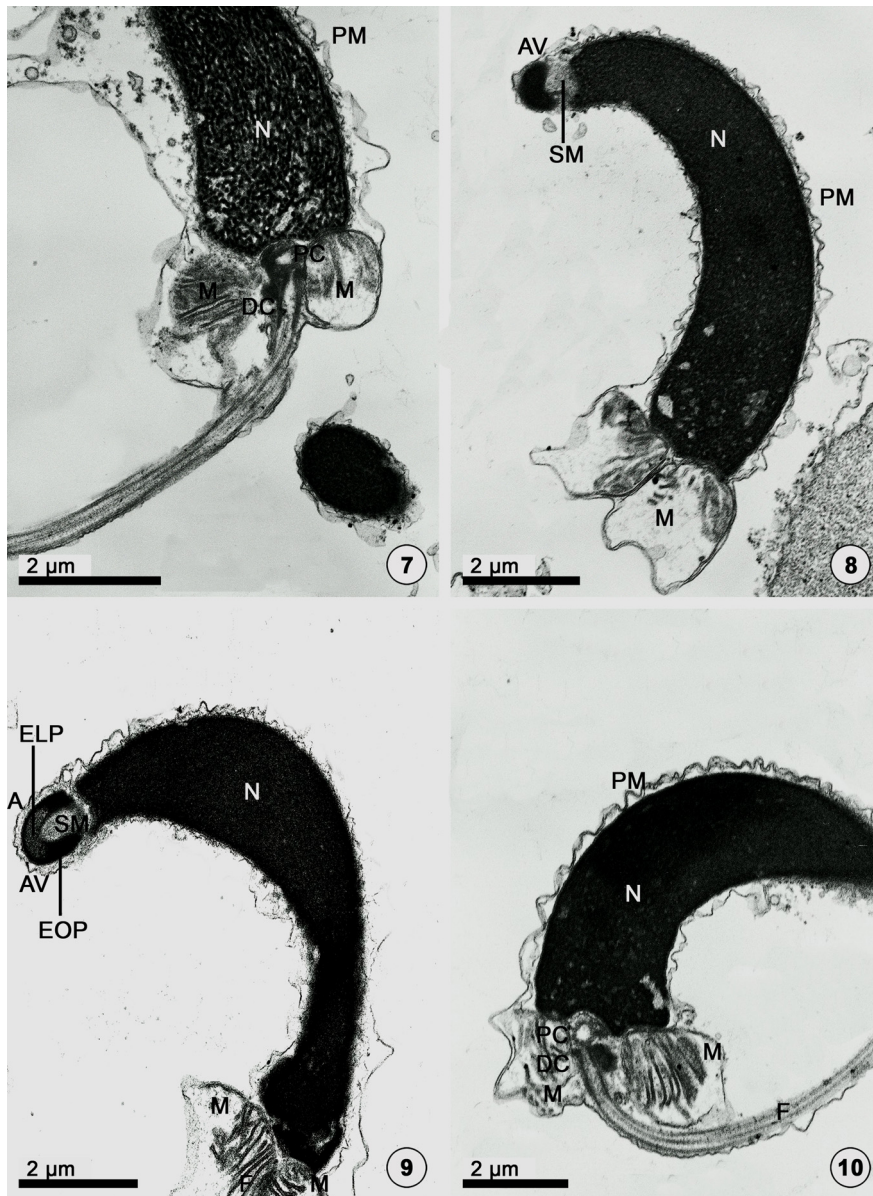


Fig. 7-10. Transmission electron micrographs of the spermiogenesis in male *Phacosoma japonicus*. **Fig. 7.** Sperm midpiece beneath the curved nucleus (N) and a flagellum. Note the proximal centriole and distal centriole with spherical mitochondria beneath the posterior nuclear fossa and a tail flagellum. **Fig. 8.** Subacrosomal materials between the acrosomal vesicle and the nucleus. Note subacrosomal materials (SM) between the acrosomal vesicle (AV) and the anterior nuclear fossa of curved nucleus with the plasma membrane (PM), and large mitochondria (M) beneath posterior nuclear fossa. **Fig. 9.** A complete spermatozoon with the acrosomal vesicle, nucleus and large mitochondria (M). Note subacrosomal materials (SM) between basal and lateral parts of basal rings showing electron opaque parts (EOP), and the apex part showing electron lucent part (ELP) on the nucleus (N). **Fig. 10.** A complete spermatozoon with the head part, sperm midpiece, and tail part. Note the head part containing an acrosome and the curved nucleus (N) with plasma membrane (PM), the proximal (PC) and distal centrioles (DC) surrounding large mitochondria (M) in the sperm midpiece part, and a flagellum (F) in the tail part.

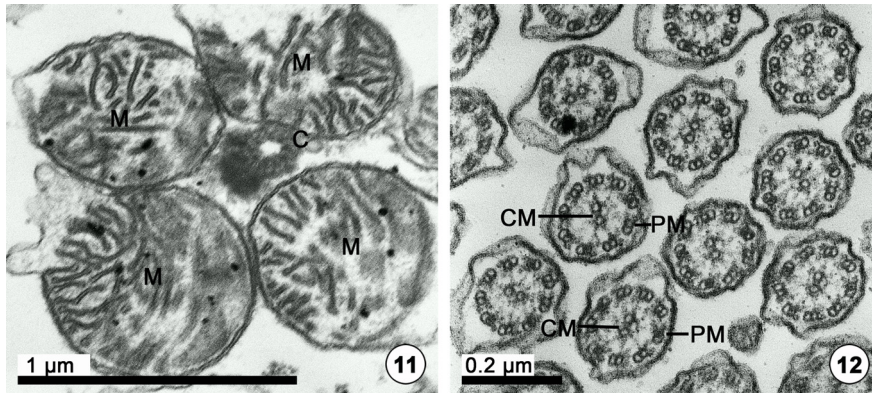


Fig. 11-12. Transmission electron micrographs of spermatozoa in male *Phacosoma japonicus*. **Fig. 11.** A cross sectioned sperm midpiece. Note a pair of centrioles surrounding with 4 mitochondria. **Fig. 12.** Cross sectioned sperm tail flagellum. Note the axoneme of the sperm tail flagellum showing a 9+2 structure (nine peripheral microtubules (PM) and a pair of central microtubules (CM)).

invaginated part of the nucleus and the acrosomal vesicle of the acrosome (Fig. 8). However, the axial filament is not found in the subacrosomal materials in the acrosomal vesicle. At this time, the acrosomal vesicle is composed of well-developing basal rings, and the curved nucleus (angle of the nucleus: 45°) is covered with the plasma membrane. The anterior nuclear fossa is exist in front of an acrosomal vesicle and the posterior nuclear fossa is present near the proximal centriole and distal centriole with some spherical mitochondria in the sperm midpiece (Fig. 8).

4) Spermatozoa

After spermiogenesis, differentiation of spermatozoon is completed. Sperm morphology is primitive, as found in most bivalve species that undergo external fertilization. As some characteristics of the acrosomal vesicle structures in an acrosome of this species, the basal and lateral parts of basal rings show electron opaque part (region), while the anterior apex part of the acrosomal vesicle shows electron lucent part (region, Figs. 9, 13). These characteristics of the acrosomal vesicle were found in the family Veneridae and other several families in the subclass Heterodonta.

Mature spermatozoa of this species measure approximately $45\text{-}50\ \mu\text{m}$ long, and consist of conical acrosome positioned at the top of an elongated nucleus, a pair of centrioles surrounded by a ring of

four spherical mitochondria, and a flagellum. The sperm head part is about $5.20\ \mu\text{m}$ long and comprises a long, electron-dense nucleus (about $3.70\ \mu\text{m}$ long), with the anterior nuclear fossa, and an acrosome. The acrosomal vesicle is about $0.55\ \mu\text{m}$ long, membrane-bound, and deeply invaginated. The acrosomal vesicle is a cone shape. The morphology of the sperm nucleus and the acrosomes of this species

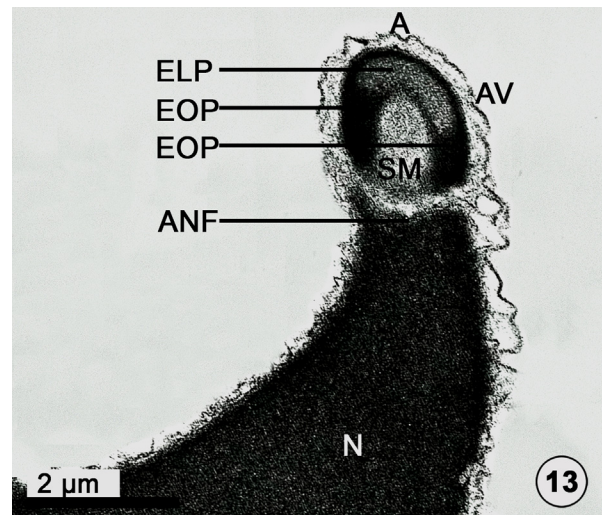


Fig. 13. A diagram of an acrosomal vesicle being composed of electron opaque part (EOP) and electron lucent part (ELP) in an acrosome on the anterior nuclear fossa of the curved nucleus in male *Phacosoma japonicus*. EOP: basal and lateral parts of the basal rings; ELP: the apex part of the basal ring.

are of a curved cylindrical type (the angle of the nucleus: 45°) and a cone shape, respectively (Fig. 9). Irregular electron-lucent lacunae are present in the nucleus (Fig. 10). Four spherical mitochondria with well-defined cristae occur near the posterior nuclear fossa of the nucleus (Fig. 11). However, the axial filaments or an axial rod and satellite fibres are not found in the ultrastructures of mature spermatozoa of this species. A cross-sectioned tail flagellum shows that the axoneme is composed of a classic 9+2 microtubular substructure (nine peripheral doublets surrounding a central pair of singlet microtubules) enclosed by a plasma membrane (Fig. 12).

DISCUSSION

1. Spermatogenesis

The testis of *P. japonicus* (Veneridae) is composed of a number of acini, spermatogenesis occurs in acini of the testis. The processes of germ cell differentiations and mature sperm ultrastructure of this species were similar to those in the species in bivalves (Chung *et al.*, 2010). Therefore, the processes of spermatogenesis of bivalves was similar to those in the species in bivalves (Sakker, 1984; Bernard & Hodgson, 1985; Chung, 2006; Chung *et al.*, 2007, Kim *et al.*, 2010a,b).

Spermatogonia were multiplied by the mitotic division and developed into the primary spermatocytes containing electron dense scattered chromatins in the nucleus. In this study, synaptonemal complexes appeared among scattered chromatins in the pachtene stage of the prophase during the first maturation division in the nucleus of the primary spermatocyte. Therefore, it shows a characteristics of the primary spermatocytes. Although it is hard to distinguish the species according to some characteristics of early developmental stages of germ cells such as spermatogonia and spermatocytes, the concentration degree and morphology of chromatins will be an important factor to determine the type of the nucleus in the course of spermiogenesis from spermatids to spermatozoa. During spermiogenesis, according to concentration degree of chromatins in the spermatid nucleus, the morphology of the nucleus remarkably changed into the anterior and posterior elongations or

right and left extentions.

Yasuzumi (1974) reported that the nuclei of spermatids can be classified into three morphological types according to the concentration morphology and degree of chromatins in the nuclei of spermatids: (1) granular type, (2) fibrous type, and (3) layer type. However, in case of bivalves, it was a granular type in the early stage of chromatin concentrations, however, as the concentration of chromatins progresses, it was changed to be the fibrous type. The nuclear types are determined according to the concentration and elongation degree of chromatins. Kim (2001) reported that the nuclear morphology of the species having wide nucleus from side to side (Ostreidae, Mytilidae, etc.) and that of the elongated species back and fourth (Veneridae, Pectinidae, Corbiculidae, *Mya arenaria*, *Panopea japonica*, etc.) is elongated with the concentration of fibrous chromatins. Thus, it is assumed that nuclear morphology is determined according to concentration degree and morphology of chromatins.

The acrosome is formed by various granular secretions secreted by the Golgi complex (Longo and Dornfeld, 1967; Sastry, 1979). In *Mytilus coruscus*, several small proacrosomal vesicle are formed by the Golgi complexes, and these vesicles were mixed with each other in the acrosomal vesicle during spermiogenesis (Kim *et al.*, 2010a).

In *P. yedoensis*, after several granules were formed by the Golgi complexes in the early stage of spermiogenesis, a proacrosomal vesicle (high electron dense vesicular granules) was formed from a mixture of these granules.

In general, morphologies of proacrosomal vesicles showed some different speciality with the species, therefore, it is assumed that proacrosomal vesicles are formed in different course of most bivalves.

2. Morphology and ultrastructure of mature spermatozoon

In general, as a matter of convenience, the morphology of the spermatozoon of bivalve species can be divided into five types: (1) primitive type, (2) modified type, (3) biflagellate type, (4) biflagellate type, and (5) aflagellate type (Verdonk *et al.*, 1983). Most bivalves belong to the primitive type, sperm

ultrastructures are composed of three parts: (1) head, (2) midpiece, and (3) tail flagellum parts.

In the present study, the morphology of the spermatozoon of *P. japonicum* has a primitive type and is similar to those of other species in the subclass Heterodonta. The morphologies of the sperm nucleus and the acrosomal vesicle of this species are the cylindrical type and cap shape, respectively. Beside them, mature spermatozoa of this species contain subacrosomal material (embedded in a granular matrix), an elongated curved nucleus showing invaginated anteriorly, two triplet substructure centrioles surrounded by four spherical mitochondria.

The spermatozoon is approximately 45-50 μm in length including a long sperm nucleus (about 3.70 μm in length), an acrosome (about 0.45 μm in length), and tail flagellum (about 42-47 μm). 1983).

Ultrastructural characteristics of mature spermatozoa of this species showed somewhat different characteristics in many families in the subclass Heterodonta (Kim, 2001; Chung *et al.*, 2010).

3. Phylogenetic and taxonomic implications of mature spermatozoon ultrastructure

Sperm ultrastructure has long been viewed as a tool in assessing taxonomic problems and phylogenetic relationship in the Metazoa through the use of spermiocladistic analysis (Jamieson, 1991; Healy, 1989, 1995). In this study, *P. japonicus* belongs to external fertilization species and the type of spermatozoa is the primitive type. Many species of Veneridae in the subclass Heterodonta have three kinds of sperm nuclear types (cylindrical type) according to the angle of curved sperm nucleus: (1) no curved nuclear type (angle of the nucleus: 0°), (2) slightly curved nuclear type (angle of the curved sperm nucleus: 5°-10°), and (3) largely curved sperm nuclear type (angle of the curved nucleus: 15-80°).

Of them, *Protothaca jodoensis* (0°) belongs to no curved nuclear type, and three kinds of species (e.g. *Gomphina veneriformis* (5°), *Meretrix lusoria* (10°), *Cyclina sinensis* (10°)) to the slightly curved nuclear type, and four kinds of species (e.g. *Saxidomus purpuratus* (15°), *Ruditapes philippinarum* (25°),

Mercenaria stimpsoni (80°)) to the largely curved nuclear type. In particular, the nuclear type of *P. japonicus* belongs to largely curved nuclear type because the angle of the curved sperm nucleus is 45°. Thus, the angle of the sperm nuclear types vary with the species in Veneridae of Subclass Heterodonta.

Regarding the sizes of sperm nuclei (lengths) in the species of Veneridae in Subclass Heterodonta, Kim (2001) described that lengths of nuclei varied with the species of Veneridae: *Meretrix lusoria* is 1.49 μm , *Cyclina sinensis* 2.13 μm , and *G. veneriformis* 7.80 μm . Therefore, it is assumed that sperm nuclei could not be used for the taxonomical key because the sizes of sperm nuclei vary with the species (Healy, 1995).

However, Healy (1995) stated that acrosomal morphologies of sperm ultrastructures of bivalves can be used for systematical analysis. Recently, Popham (1979) stated that morphological characteristics of the acrosomes of sperms can be used for the composition of the subclass. Healy (1989) stated acrosomal morphology of each species in the subclass shows a unique characteristics and reported some differences between Subclasses.

Popham (1979) reported some differences according to the morphologies and positions of sperm acrosomes of the species in 5 subclasses of bivalves. Hodgson and Bernard (1986) reported that sperm acrosomes can be classified into 5 subclasses according to morphologies of acrosomal vesicles and described some characteristics of the families of the subclass. Systematically, the family Veneridae belongs to the subclass Heterodonta. In this study, as shown in Fig. 13, acrosomal morphologies of species in all families of Subclass Heterodonta were mostly cylindrical type or cap-like in shape. This point is one of common structural characteristics of the acrosomal vesicle.

In the acrosomal vesicle of spermatozoa of *P. japonicus*, the basal and lateral parts (regions) of the basal rings showed electron opaque part (region), while the anterior parts of the apex part of the acrosomal vesicle showed electron lucent part (region). Accordingly, these phenomena of this species showed remarkably similar patterns to various kinds of species of Veneridae, Mactridae, Tellinidae,

Solenidae, Myidae, Hiatellidae in the subclass Heterodonta. Therefore, this species showed a typical characteristics of the subclass Heterodonta. In particular, in the acrosomes of species of the family of Veneridae, Mactridae, Solenidae in the subclass Heterodonta, the anterior apex parts of acrosomal vesicles were very thin, and the boundaries of the inner and outer membranes were closely attached and acrosomal vesicles in acrosomes showed the cone types or cap-like shapes, as shown in common characteristics of the subclass Heterodonta. Hodgson and Bernard (1986) reported that in case of the subclass Pteriormorphia, the morphology of sperm acrosomal vesicles shows the same cone shape, as shown in the subclass Heterodonta. However, it is a common characteristics of the species in the families of the subclass Pteriormorphia that all parts of acrosomal vesicles are composed of electron opaque parts (regions), unlikely the subclass Heterodonta showing electron opaque parts at the basal and lateral parts of basal rings and electron lucent part (region) at the anterior apex part. Therefore, *P. japonicus* showed a common characteristics of the subclass Heterodonta.

Of sperm ultrastructures, the axial filament or axial rod are present in the subacrosomal granular materials of the acrosomal vesicles in the acrosomes of several species of the subclass Heterodonta and Pteriormorphia. However, in spermatozoa of some species, it is hard to find their ultrastructures the subacrosomal granular materials in the space of acrosomal vesicles or they did not find in mature sperm ultrastructures because their spermatozoa have not their structure in many species of families in the subclass Heterodonta and Pteriormorphia. Thus, appearances of the axial filament and axial rod varied with the species of families in subclasses (Kim *et al.*, 2010a; Chung *et al.*, 2010).

To date, the axial filament in the subacrosomal granular materials have been only found in a few species of Veneridae (Kim, 2001; Chung *et al.*, 2010) and Corbiculidae (Kim, 2001) in subclass Heterodonta, while in general, it was not found in the subacrosomal granular materials of sperm ultrastructure in subclass

Pteriormorphia, In addition, regarding the axial rod in the subacrosomal granular materials of the acrosomal vesicle, it was only found in a few species of Osteidae and Mytilidae in the subclass Pteriormorphia (Kim, 2001; Kim *et al.*, 2010a). In this study, the axial filament was not found in the subacrosomal granular materials. *P. japonicus* belongs to Veneridae such as *Saxidomus purpuratus*, *Meretrix lusoria* and *P. jedoensia* in subclass Heterodonta. However, the acrosomal vesicles of *R. philippinarum* (Kim, 2001) and *G. veneriformis* in Veneridae contained the axial filament in the subacrosomal granular materials (Chung *et al.*, 2010).

Regarding the satellite fibres of sperm ultrastructure, it was reported that they were not found in all species in Veneridae, Mactridae, Corbiculidae, Myidae, Solenidae, Tellinidae and Hiatellidae of the subclass Heterodonta, while they were found in many species of Ostreidae, Arcidae, Mytilidae and Pectinidae of the subclass Pteriormorphia except for Pinnidae and Pteridae. In this study, the satellite fibres of sperm ultrastructure of this species was not found because Veneridae species has not this structure, unlikely spermatozoa of Ostreidae species of the subclass Pteriormorphia.

Hodgson and Bernard (1986) and Healy (1989) stated that the number of mitochondria in the sperm midpiece tends to be stale within any family or superfamily varying from a maximum of 14 in the mytilord *Modiolus difficilis* (Drozdov and Reunov, 1986) to a minimum of 4 (Healy, 1989, 1995). Based on the results reported by some authors (Chung & Ryou, 2000; Kim, 2001; Chung *et al.*, 2006), the number of mitochondria at the midpiece of sperm of bivalves were four in Veneridae, Solenidae, Corbiculidae in subclass Heterodonta and Ostreidae in subclass Pteriormorphia, while those of mitochondria were five in Arcidae, Mytilidae in subclass Pteriormorphia and some species of Veneridae (*Saxidomus purpuratus*, *Meretrix lusoria*, *Cyclina sinensis*) and *Atrina pinnata japonica*.

Healy (1989) reported that the number of mitochondria at the midpiece of sperm showed unconstant and irregular characteristics in the level

of subclass, however showed stable a constant characteristics under the level of family or in superfamily. Accordingly, the results of the number of the mitochondria are coincided with that reported by Healy (1989). In this study, the number of mitochondria at the midpiece of the spermatozoon are four (common to many bivalve families), and satellite fibres are not found in the family Veneridae. Judging from the results on the ultrastructure of mature spermatozoon, it is supposed that this species belongs to family Veneridae and subclass Heterodonta because the axial rod in the acrosomal vesicle or satellite fibres in the sperm midpiece were not found in the sperm ultrastructure, unlikely the structures of the acrosomal vesicle of Ostreidae sperm in the subclass Pteriomorpha.

On the whole, appearances of the axial filament or axial rod, and satellite fibres varied with the species of families in Subclasses (Kim *et al.*, 2010b; Chung *et al.*, 2010).

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