

SPERMATOGENESIS OF THE FRESHWATER CLAM *CORBICULA* AFF. *FLUMINEA* MÜLLER (BIVALVIA: CORBICULIDAE)

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ABSTRACT Spermatogenesis of the hermaphrodite freshwater clam, *Corbicula* aff. *fluminea* Müller was described from light and electron microscopy. During the spermatogenic process, difference between primary and secondary spermatocytes was hardly recognized. Mature spermatozoon consists of an elongated head ($13.9 \pm 0.32 \mu\text{m}$ in length) with a rod-like acrosome, indistinct midpiece, and two long flagella, 2.5 times as long as the head, each flagellum with a very thin undulating membrane. Comparison of the sperm morphology in *Corbicula* species presents two major groups, uniflagellated and biflagellated. Spermatozoa of *C. leana* and *C. aff. fluminea*, in spite of their close similarity, were distinguished by their size and number of mitochondria.

KEY WORDS: spermatogenesis, ultrastructure, flagella, hermaphrodite, *Corbicula*

INTRODUCTION

Studies on the reproductive biology of freshwater corbiculid clams, especially detailed studies of their spermatology, are few in spite of its commercial importance: of all the inland water fisheries in Japan, about 30% of the annual catch is accounted for by *Corbicula* clams. Ultrastructural studies of the spermatozoa of the Japanese corbiculid clams are published for only two species: *Corbicula sandai* Reinhardt and *C. leana* (Prime) (Hachiri and Higashi 1970, Komaru and Konishi 1996). These studies distinguished two types of spermatozoa, uniflagellate and biflagellate, were found within the same genus. Further, only a few previous works have described the ultrastructure of the complete spermatogenic process in freshwater bivalves (Higashi 1964, Rocha and Azevedo 1990), and no data are available for Corbiculidae. Recently, in a course of the genetic study on populations of *C. leana* in Kyushu, southern Japan, we sampled many specimens that were almost morphologically identical with *C. fluminea* Müller, and the gonads of these clams were histologically examined to allow a comparison of the spermatogenic process within *Corbicula*. The taxonomy of the corbiculid species, however, is complicated and confused at present. Four *Corbicula* species have been described from Japan (Habe 1977, Masuda and Habe 1988), although some authors listed *C. fluminea* in the bivalve fauna of Japan (Wang 1988, Hu and Tao 1995). Morton (1979), however, proposed only a two-species-complex, *C. fluminea* and *C. fluminaris*, from the Asian region. We herewith tentatively use the name *C. aff. fluminea* Müller (for details, see Komaru *et al.* 1998).

In this paper, we describe the ultrastructure of spermatogenesis of *C. aff. fluminea* from Japan and compare its morphological characters with those of other freshwater bivalves including *Corbicula* species.

MATERIAL AND METHODS

Clams were collected from the Tade River, Saga Prefecture, Japan. Intact sperm were obtained by dissecting fresh gonads, and the length of the head and flagella were measured with an ocular micrometer for specimens just after the termination of their movement by adding 1% formalin from the fringe of the cover slip. The soft part of the clams were fixed with Bouin's solution and pro-

cessed as for standard paraffin embedding methods. Serial sections in 5 μm thickness were stained with Mayer's hematoxylin and eosin. The sections were observed with an Olympus BH-2 microscope. Measurements of testicular cells were performed in 20-50 cells, ranging at least two clams, based on these sections using an ocular micrometer.

For electron microscope observations, small pieces of gonads were dissected under a binocular microscope, and prefixed with 2% glutaraldehyde and 2.5% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.5) at 4°C. The prefixed tissue was rinsed with the same buffer three times and then post-fixed with 1.0% osmium tetroxide in the same buffer. After fixation, the tissues were dehydrated through a graded acetone series and embedded in an epoxy resin Quetol 812 (Nisshin EM Co., Tokyo). Ultrathin sections were stained with aqueous uranyl acetate and lead nitrate, and then observed with a JEOL 1200 EX transmission electron microscope.

RESULTS

Gross morphology of testis

Testes are found adjacent to the ovaries, and occasionally both gonads are mingled. From outer wall to inner core of the testis, various stages of germ cells are observable. Mature spermatozoa form spherical or hemispherical clusters in the lumen of the testis (Fig. 1A, asterisk) attaching their head onto a large Sertoli-like cell (Fig. 2C, asterisk).

Spermatogonia (Fig. 1B)

In the peripheral region of the testis, large polygonal spermatogonia are visible; mean value \pm S.D. of the nuclear diameter is $5.84 \pm 0.47 \mu\text{m}$ ($n = 20$), and the nucleus contains a prominent electron-dense nucleolus in some sections (Fig. 1B, arrow). Chromatins are not condensed. A number of mitochondria are scattered around the nucleus.

Spermatocytes (Fig. 1C)

Round cells, $3.7 \pm 0.47 \mu\text{m}$ in mean nuclear diameter ($n = 50$). The nucleus is somewhat condensed and volume of cytoplasm is

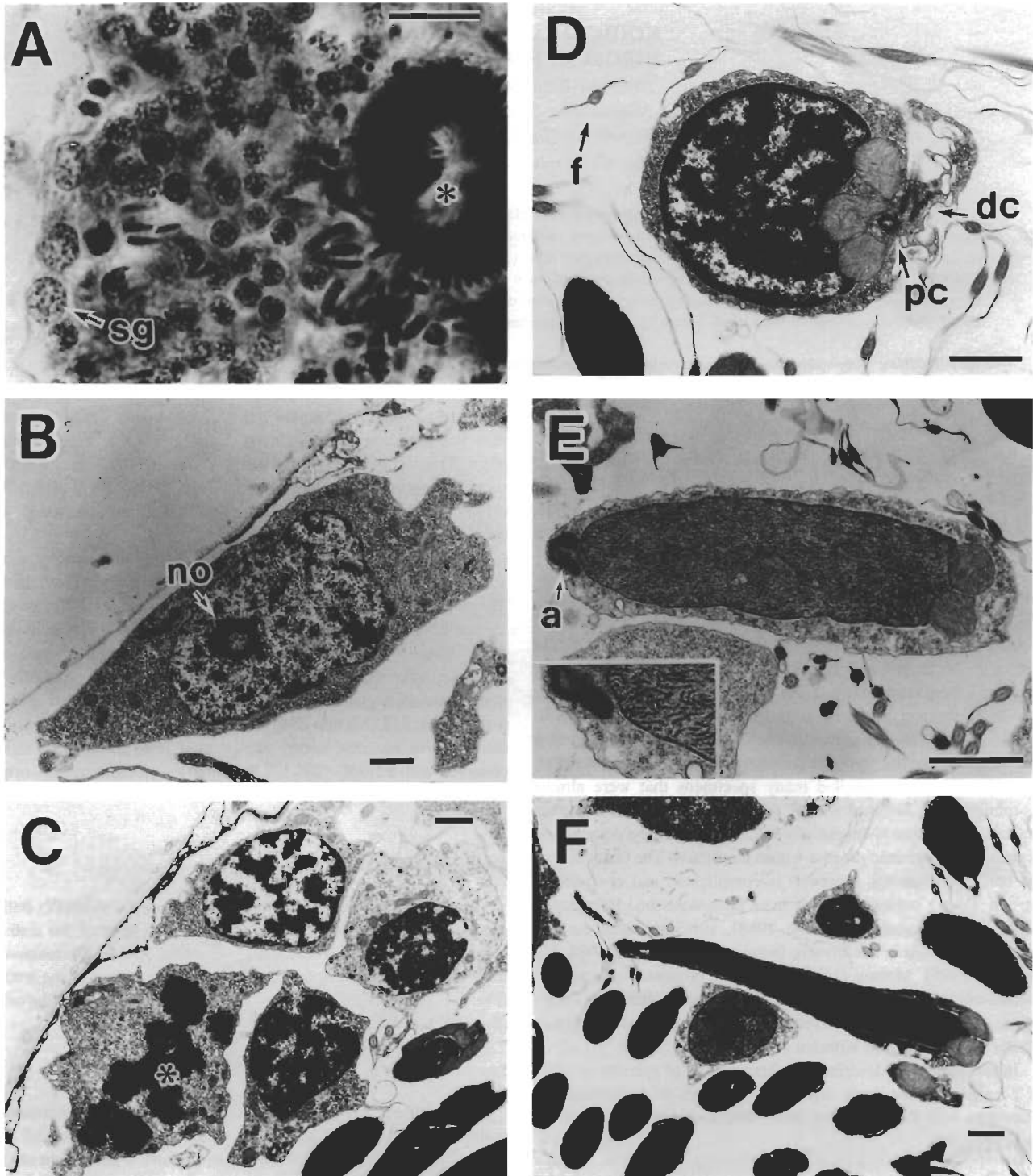


Figure 1. Spermatogenic process of *Corbicula* aff. *fluminea* Müller. (A) Gross morphology of the testis by light microscope. Spermatogonia (sg) are found at the peripheral region. Asterisk shows Sertoli-like cells onto which mature spermatozoa attach their head forming a cluster. (B) Spermatogonia. Nucleolus (no) is found. (C) Spermatocyte. (D) Early spermatid. Note proximal (pc) and distal (dc) centrioles. Transverse section of flagella (f) is also recognized. (E) Middle spermatid. Acrosomal structure (a) is not apparent. Note numerous fibrils of chromatin arranged with the longitudinal axis (in detail, see inlet). (F) Late spermatid. Scale bars = 10 μ m for A and 1 μ m for B-F.

more reduced than in the spermatogonia. Meiotic figures are frequently recognized (Fig. 1C, asterisk). The primary and secondary spermatocytes are hardly distinguishable in morphology.

Spermatids (Figs. 1D–F)

Early spermatid, $3.0 \pm 0.11 \mu\text{m}$ in mean nuclear diameter ($n = 20$), has scant cytoplasm, and large mitochondria which are displaced to one side of the cell. A pair of centrioles is found near the basal end of the cell, consisting of proximal and distal centriole (Fig. 1D, arrows). A flagellum originates from the distal centriole at this stage in some specimens. In the middle stage, the nucleus becomes elongated, and numerous fibrils are arranged longitudinally in it (Fig. 1E and inlet). The acrosomal structure is recognizable at the anterior part of the head (Fig. 1E, arrow). The nucleus of the late spermatids becomes more elongated with condensed chromatin and the acrosome is now conspicuous. The

proximal centriole which was perpendicular to the distal one was not recognized. The shape of the mitochondria is elongated and flattened (Fig. 1F).

Spermatozoon (Figs. 2A–F)

The head, $13.9 \pm 0.32 \mu\text{m}$ in length ($n = 20$), consists of a rod-like acrosome and a long nucleus capped with four tightly packed mitochondria which form a calyx-like cluster (Fig. 2D). The length of the flagella is approximately 2.5 times as long as the head. In the head region, the acrosome is of a tapered form and the outer electron-lucent layer and inner moderate dense region are distinguished (Fig. 2B). The chromatin of the nucleus is more condensed and numerous small low-electron dense patches are found. Two centrioles are located parallel to longitudinal axis at basal part of the head (Fig. 2, E and F) and long flagella originate from each centriole. The basal part of one centriole (Fig. 2E,

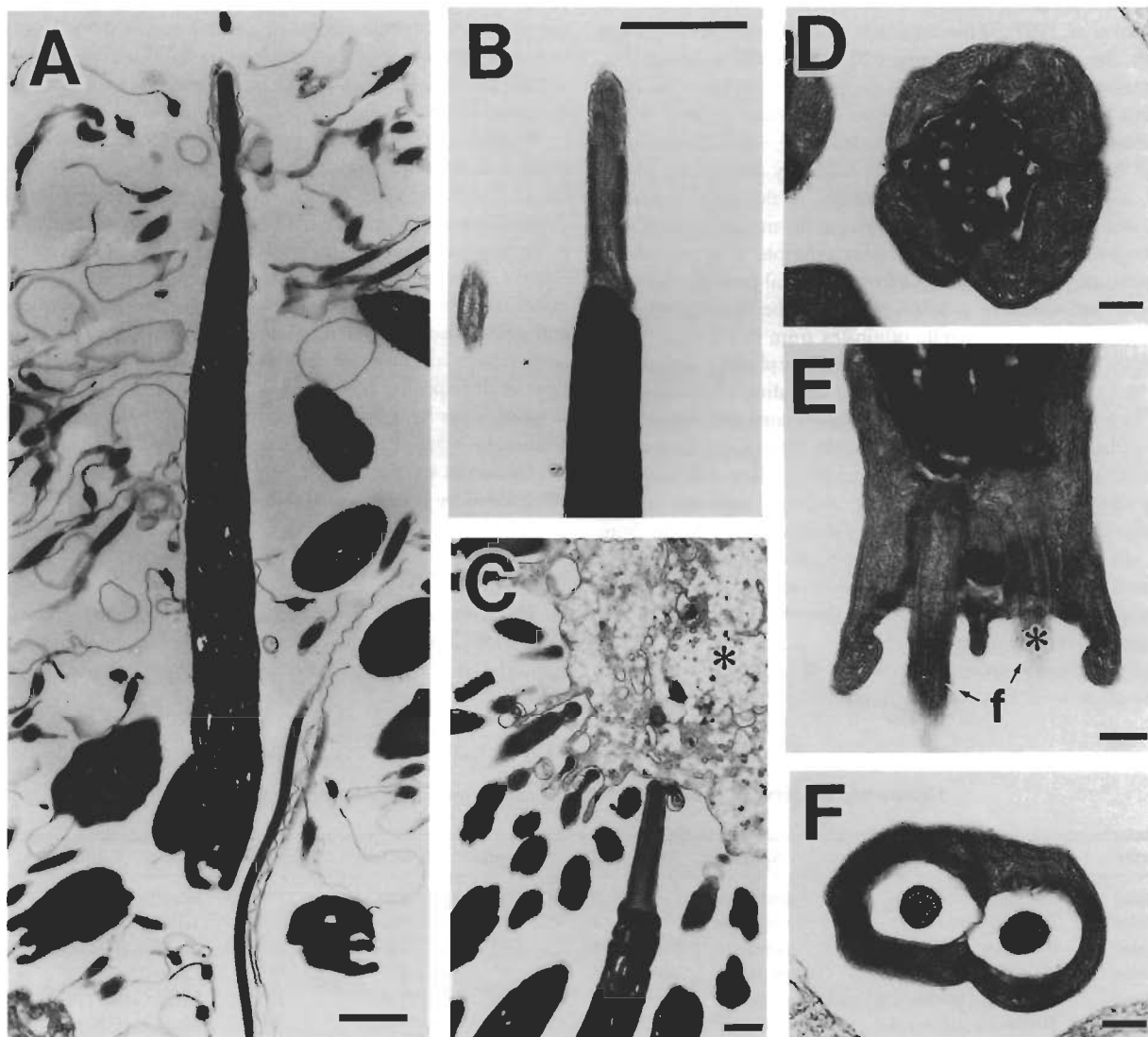


Figure 2. Spermatozoa of *Corbicula* aff. *fluminea* Müller. (A) Mature spermatozoa. (B) Acrosome on the head. (C) Spermatozoa which the head attaching to a Sertoli-like cell (asterisk). (D) Transverse section of posterior midpiece. Four mitochondria surround nucleus. (E) Longitudinal section of posterior midpiece. Two flagella (f) originate from each centriole, but not that the basal part of one flagellum is curved forward to the base of another flagellum (asterisk). (F) Transverse section of posterior end of midpiece. Scale bars = $1 \mu\text{m}$ for A–B, 500 nm for C, and 200 nm for D–F.

asterisk) is curved toward the base of the other centriole suggesting its proximal origin. Each flagella is fringed with a very thin undulating membrane (see arrow in Fig. 1D).

DISCUSSION

In spermatogenic studies of bivalves, it has been noted that secondary spermatocytes were rarely observed. For this reason, Sastry (1979) stated that the division rate was too rapid to recognize the second meiosis histologically. Two stages of spermatocyte, however, were recognized in *C. leana* (Ikematsu and Yamane 1977) and *C. japonica* (Maru 1981) by light microscopy. According to Takahashi and Takano (1970), the secondary spermatocytes are distinguishable from the primary spermatocytes by their size. In the present histological observations, no distinct groups were detected in the nuclear size of the spermatocytes. Electron microscopic figures also support this result. On the other hand, the chromosome number and DNA content of the three *Corbicula* species including *C. aff. fluminea* suggest the possibility of first or second meiosis being omitted in the reproduction of these clams (Komaru *et al.* 1997). If this omission actually exists in the present species, the absence of a definite mitotic step will be reasonable.

Kraemer (1983) distinguished two types of biflagellate spermatozoa in *C. fluminea*: wide-headed and slender-headed. In the wide-headed type, which was suggested to be a non-mature form, one of the pair of flagella was often relatively motionless. In this study, a pair of centrioles is recognized in the early spermatid stage, and one of the parallel centrioles in the mature spermatozoa is curved toward the base of the other centriole. It is most likely that after one flagellum emerged from the distal centriole, then the proximal one moves its position parallel to the longitudinal axis, and finally the second flagella originates from it.

Table 1 summarizes the gross sperm morphology and developmental mode in freshwater bivalves including *Corbicula* species. Two distinct groups are recognized among the corbiculids: uniflagellate and biflagellate spermatozoa. The groups correspond with other aspects of their reproduction. Biflagellate and hermaphrodite species, includes *C. leana*, *C. fluminea*, and the present species. The spermatozoa of *C. aff. fluminea* are different from those of *C. leana* in two aspects: 1) the number of mitochondria is four in *C. aff. fluminea* and five in *C. leana*, and 2) the head length of the present species is smaller than that of *C. leana* (16.9 and 13.9 μm in average, respectively).

Early spermatological studies suggested that spermatozoa of the Bivalvia were classified as a primitive type (e.g., Franzén

1956). Successive works, however, have revealed the presence of modified spermatozoa in bivalves in different families: *Tellina* and *Codakia* (Mouëza and Frenkiel 1995). Popham (1979) was the first to review the class from sperm morphology, and suggested that the most useful taxonomic application of comparative sperm morphology in the Bivalvia seems to lie at the species and genus levels.

Most spermatozoa of freshwater bivalves are of a primitive type which have a rudimentary acrosome, a short head, five spherical mitochondria, and a long simple flagellum (e.g., Higashi 1964, Trimble and Gaudin 1975, Peredo *et al.* 1990, Rocha and Azevedo 1990, Lynn 1994). The gross sperm morphology of freshwater *Corbicula* is a modified type among bivalve spermatozoa. This form may be related with their specialized mode of reproduction: hermaphroditic and internal fertilization (Komaru and Konishi 1996). Franzén (1983) also noted that there was a correlation between the evolution of elongated sperm nuclei and large, yolk-rich eggs. According to Healy's (1996) definition, the sperm of corbiculid clams belongs to an ent-aquasperm of which sperm are released into the ambient water and fertilization occurs within the confines of the mantle cavity. He also commented on the resemblance of sperm morphology between freshwater Corbiculidae and marine Tellinidae in both having an elongated nucleus and similar midpiece structures.

Popham (1979) pointed out that the presence or absence of acrosomal structures may be indirectly correlated with the brooding of developing young. Peredo *et al.* (1990) compared the sperm morphology and development in freshwater bivalves, and came to a similar conclusion. In fact this is true for the upper six species in Table 1. Based on recent data, however, their category seems to be inadequate for the freshwater bivalves such as found in Japanese corbiculid species. As shown in Table 1, the spermatozoa of *C. sandai* have a prominent acrosome although the developmental type of this species is non-brooding: e.g., this clam releases developing eggs to ambient water (Miyazaki 1936, Furukawa and Mizumoto 1953). This suggests that the reproductive strategies in the *Corbicula* species and their sperm morphology are not simply categorized by previous models and classifications.

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TABLE 1.
Comparison of sperm morphology and developmental mode in freshwater bivalves.

Family	Species	Sperm	As.	Mit.	Flagella	Brooding	Sexuality	Author(s)
Unionidae	<i>Hyriopsis schlegelii</i>	primitive	-	4	1	Yes	D	Higashi (1984)
Unionidae	<i>Ligumia subrostrata</i>	primitive	-	4	1	Yes	D	Trimble and Gaudin (1975)
Unionidae	<i>Anodonta cygnea</i>	primitive	-	5	1	Yes	D	Rocha and Azevedo (1990)
Unionidae	<i>Anodonta grandis</i>	primitive	-	5	1	Yes	D	Lynn (1994)
Hyriidae	<i>Diplodon chilensis chilensis</i>	primitive	-	5	1	Yes	D	Peredo <i>et al.</i> (1990)
Dreissenidae	<i>Dreissena polymorpha</i>	primitive	+	4-5	1	No	D	Franzén (1983)
Corbiculidae	<i>Corbicula sandai</i>	modified	+	4	1	No	D	Hachiri and Higashi (1970)
Corbiculidae	<i>Corbicula leana</i>	modified	+	5	2	Yes	H	Komaru and Konishi (1996)
Corbiculidae	<i>Corbicula fluminea</i>	modified	+	?	2	Yes	H	Kraemer (1983)
Corbiculidae	<i>Corbicula aff. fluminea</i>	modified	+	4	2	Yes	H	this study

As, acrosome; D, dioecious; H, hermaphrodite; Mit., mitochondria; +, present; -, absent.

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