

Ultrastructure of spermatozoa of *Bullacta exarata* (philippi) and its significance on reproductive evolution and physio-ecological adaptation*

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Abstract: The morphology and ultrastructure of *Bullacta exarata* spermatozoa observed by light and transmission electron microscopy are presented in this paper. The spermatozoon is composed of head with a simple acrosomal complex and an elongated nucleus, and tail with a midpiece, principal piece and an end piece. The midpiece consists of a mitochondrial ring, and the principal piece is composed of axoneme and lateral fin. The structure of *B. exarata* spermatozoa differs significantly from that of other gastropods, especially in the lateral fin and the principal piece, which was described scarcely before. A comparison is made between *B. exarata* and other gastropods, and its significance on reproductive evolution and physio-ecological adaptation is preliminarily discussed.

Key words: *Bullacta exarata*, Spermatozoon, Ultrastructure, Evolution, Physio-ecological adaptation

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INTRODUCTION

The mud snail, *Bullacta exarata* Philippi is an Asia molluscan species noted for its delicious taste and commercial importance and is an important economic resource in eastern China. In our research on the reproductive biology of this species, we studied the anatomy of its reproductive system (Ying et al., 2002a), its spermatogenesis (Ying et al., 2002b), oogenesis (Ying, 2002), and organelles variation during its vitellogenesis (Ying and Yang,

2001). The ultrastructure of the spermatozoa remains unknown up to date.

The fine structures of spermatozoa of different groups of mollusks have been a popular topic in the past decades. The spermatozoa morphological features can be used to determine taxonomic characteristics, to delineate the evolutionary route (Justine, 1991), and to detect marine pollution (Ke and Li, 1992). The structure of gastropod spermatozoon had been extensively investigated (Buckland-Nicks and Howley, 1997; Healy, 1986; 1988; 1996; Healy and Willan, 1984; Jamieson et al., 1991; Bojat et al., 2001; Ponder and Lindberg, 1997). Healy and Willan (1984) expounded on the significance of the gastropod spermatozoa structure

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in phylogeny. Hodgson and Bernard (1988) compared the structures of the spermatozoon from 16 species of patellid limpets, and analyzed the relationships among genera of patellid limpets. However, very little work on gastropod spermatozoon was published in China. Bao *et al.* (1998), and Ke and Li (1992) investigated spermatozoon structures of *Haliolis discus* Hannai and *Babylonia formosae*. The spermatozoa of *Haliolis discus* is of primitive type, while *Babylonia formosae* is of modified type. Both of these species are gastropods, prosobranchias; *Haliolis discus* is in the order archaeogastropoda and *Babylonia formosae* is in the order neogastropoda.

Bullacta exarata belongs to opisthobranchia and is in the order cephalaspidea. The ultrastructural characteristics and its physio-ecological adaptation remain unknown. This paper presents the morphological features of *B. exarata* at the light microscope and electronic microscope level.

MATERIAL AND METHODES

Animals for this project were collected from a Huanghua aquatic breeding farm, Yueqing City, Zhejiang Province, monthly during the following periods: March to November, 2000; March to May, 2001; and April 2002. Testes were immediately pre-

fixed with 2.5% glutaraldehyde in 0.1 mol/L phosphate-buffered (PB, pH7.4) after dissection, fixed 1 hour at 4 °C and post-fixed 1 h in 1% osmium tetroxide. After post-fixation, testes were dehydrated in an ethanol series, infiltrated with mixtures of propylene oxide and Epon 812 (1:1, 1:3 for 1–2 h each) before being transferred to pure Epon overnight at room temperature. After one change to pure Epon for one hour, testes were embedded for 48 h at 60 °C.

For light microscopy observation, semi-thin sections were cut on an LKB2088 microtome, stained with Toluidine Blue and observed under Olympus BX40 microscopy.

For transmission electron microscopy observation, thin sections (600~900 Å, golden/gray interference color) were obtained with an LKB2088 microtome with a glass knife, counter-stained with alcoholic uranyl acetate and lead citrate and examined with a JEM-100CXII electron microscope (75 kV).

RESULTS

Light microscopy observation showed that the spermatozoon of *B. exarata* consists of two parts, the head and the tail (Fig. 1a). The head is composed of nucleus and acrosome, and the tail consists of pr-

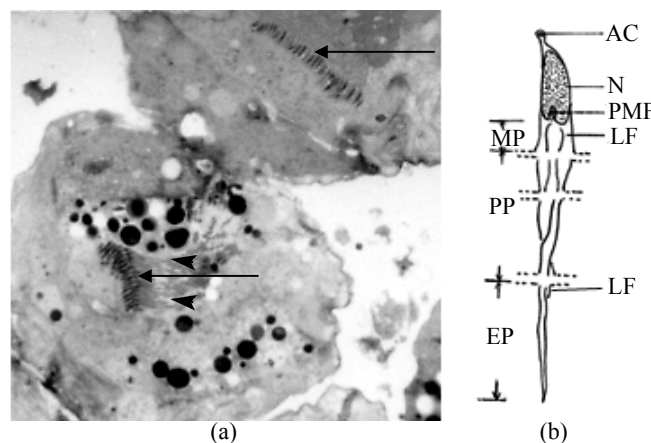


Fig.1 Mature testis of *B. exarata* was semi-sectioned and stained with Toluidine Blue. The nuclei are clear, and arranged in parallel (a, arrows); the tails are indicated by arrow heads. Two parts of the spermatozoon are fully labeled in the diagram (b)

AC, acrosome; EP, end piece; LF, lateral fin; MP, mid-piece; N, nucleus; PNF, posterior nuclear fossa; PP, principal piece

incipal piece, middle piece and end piece. All parts of the spermatozoa are labeled in the diagram (Fig.1b).

Transmission electron microscopy observations

The sperm head, about 4.4 μm in length, consisted of an acrosome and a nucleus (Figs.2a, 2b). The simple acrosome, cap-shaped and about 0.87 μm in length, is composed of electron-dense outward region and a less electron-dense inward region. There is also a subacrosomal space (Fig.2b). The

elongated cylindrical nucleus, about 3.6 μm in length \times 0.93 μm in width, tapers gradually. The contents of the whole nucleus have an even electron-density, and the nuclear membrane is clear. Both the nucleus and the midpiece become surrounded by a helical sheath of microtubules (Figs.2c, 2d). Posteriorly, there is a fossa, 0.6 μm in length, known as a posterior nucleus fossa, from which the axonemes originated (Figs.2b, 2e, 2f).

The midpiece, closely joined to the posterior portion of the head, consists of a mitochondrial ring,

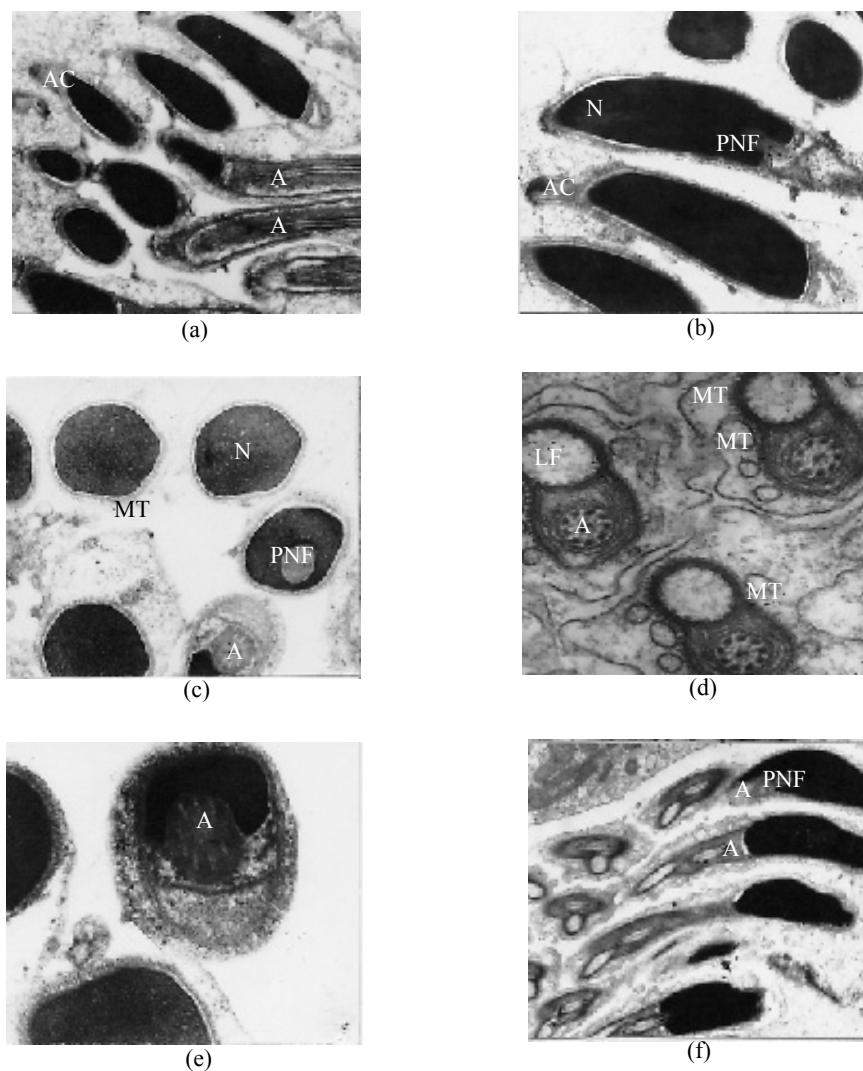


Fig.2 Ultrastructure of *B. exarata* (Philippi) spermatozoa (a) Mature spermatozoa of *Bullacta exarata* ($\times 8000$); (b) Mature acrosomal complex, nuclear and posterior nuclear fossa ($\times 15000$); (c) Transverse section of nucleus surrounded by microtubules ($\times 20000$); (d) Transverse section of an immature sperm, showing microtubules, lateral fins and the "9+2" structure ($\times 35000$); (e) Transverse section of nucleus, also showing the axoneme ($\times 35000$); (f) Longitudinal section of spermatozoa, showing axoneme and posterior nuclear fossa ($\times 8000$)

A: axoneme; AC: acrosome cap; LF: lateral fin; MT: microtubules; N: nucleus; PNF: posterior nuclear fossa

lateral fin and centrioles (Figs.3a, 3b). In the middle piece, the cell membrane process formed a bilaterally symmetrical structure named the lateral fin (Fig.3b). A pair of centrioles are also found in the middle piece. The proximal one lays in the posterior nucleus fossa and the distal one links the mid-piece and the head. The longitudinal axis of the distal centriole runs parallels to the spermatozoa, but is arranged orthogonally to that of the proximal centriole. The axoneme of the tail comes from the

distal centriole, and has the typical structure "9+2" (Fig.2d). The remarkable feature of the middle piece is that there is a mitochondrial ring surrounding the axoneme, with spherical mitochondria that are tightly apposed to the nuclear envelope.

The principal piece (Figs.3c, 3d), which is closely joined with the middle piece, is the longest part of the flagellum. The principal piece consists of axoneme and lateral fin, and the axoneme and lateral fins twist constantly. The lateral fin even ex-

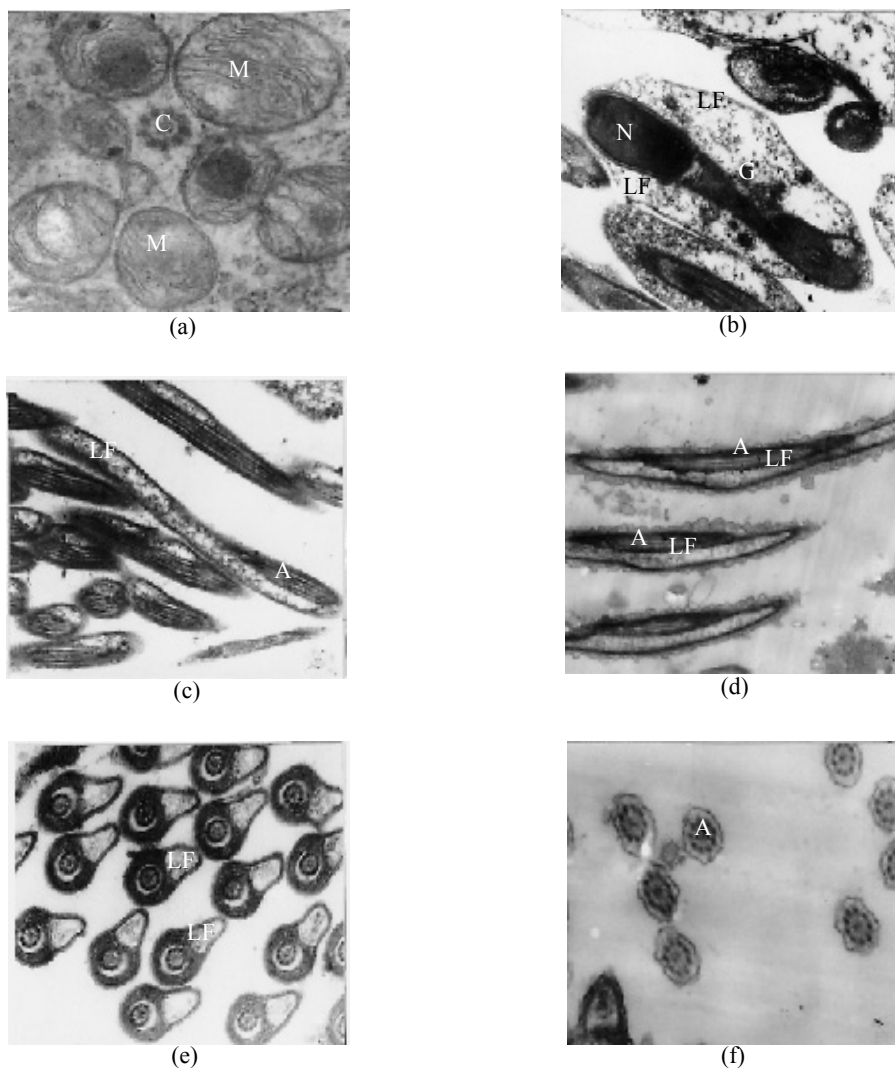


Fig.3 Ultrastructure of midpiece, principal and end piece of *B. exarata* (Philippi) spermatozoa (a) showing the mitochondria around the centriole ($\times 40000$); (b) Showing the lateral fins in the two sides of midpiece ($\times 17000$); (c) Longitudinal section of principal piece, showing the axoneme and lateral fins twisting together ($\times 15000$); (d) Longitudinal section of the sperm tail, showing the axoneme and lateral fins ($\times 12000$); (e) Transverse section of the sperm tail, showing the structure of the lateral fins ($\times 25000$); (f) Transverse section of the end piece of the sperm ($\times 25000$)

A: axoneme; C: centriole; G: glycogen; LF: lateral fin; M: mitochondria; N: nucleus

tends to the posterior of the nucleus (Fig.3b). Only one side of the distal flagellum has a lateral fin, which surrounds the axis filament with twisting (Figs.3c, 3d). The lateral fin becomes narrower as it extends caudally (Fig.3e).

The end piece is short, and its structure is relatively simple. The axoneme of the end piece is surrounded only by plasma membrane and gradually attenuates (Fig.3f).

DISCUSSION

Phylogenetic relations between *B. exarata* and other gastropods at different taxonomic level

The spermatozoon of mollusks are of two types, i.e., primitive type and modified type. Generally, the mollusk spermatozoa has a head with variable length, a simple mitochondrial mid-piece, and a tail with a "9+2" arrangement of microtubules. The proximal and distal centrioles separate the head from the mid-piece and the end piece (Hodgson and Bernard, 1988; Franzen, 1983; Bao et al., 1998). *B. exarata* spermatozoa is of the modified type and is composed of a head and a tail. The tail can be divided into three parts: the midpiece, the principal piece, and the end piece based on the lateral fin morphology.

The morphology of the acrosome and nucleus are the most important features for taxonomy. Hodgson and Bernard (1988) found that the acrosomes and nuclei of 16 species of patellid limpets were highly variable in size and morphology, had few variations in the structure of the midpiece and endpiece. At the genera level, the diversity of sperm morphology exists mainly in the acrosome, anterior nuclear fossa and posterior nuclear fossa or the morphology and structure of nucleus and subacrosomal space.

The acrosome of *B. exarata* spermatozoa is simple. It has a cap-like structure covering the top of the nucleus. The same structure is also found in other Neogastropods. But in *Babylonia formosae* (Neogastropoda) (Ke and Li, 1992), the acrosome is more complicated. It consists of an acrosomal cap, acrosomal rod and subacrosomal space. The acro-

somal cap is in the shape of the letter "U", capping the anterior end of the nucleus. In the subacrosomal space, there is a small amount of floccules. An electron-dense acrosomal rod extends through the center of the subacrosomal space. The acrosome of *B. exarata* is also similar to that of *Haliotis discus hannai* (Archaeogastropoda) (Bao et al., 1998). The latter is bullet-shaped, consisting of two parts of differing electron density. The acrosome tip membrane alveolated. In the subacrosomal space lie microfilaments. The acrosome of *H. discus hannai* is particularly large, of the same volume as the nuclei (Bao et al., 1998). The acrosome of *Arianta arbustorum* (Pulmonata) is also very simple and consists of an apical vesicle and a less electron-dense acrosomal pedestal. The acrosome of *Arianta arbustorum* spermatozoa forms an angle of about 90° with the longitudinal axis of the nucleus (Bojat et al., 2001).

It is obvious that, at the genera and subclass level, *B. exarata* has close relationship with *A. arbustorum* and *H. discus*, but has distant relationship with *Babylonia Formosa*, based on the acrosome morphology. The difference between acrosomes of different species of Cephalaspidea is currently being further studied in our lab.

The morphology of the spermatozoal nucleus varies greatly between genera. In this study, the nucleus of *B. exarata* was elongated and cylindrical and different from that of the archaeogastropoda *Haliotis discus hannai*, which has a long columnar shape (Bao et al., 1998). Hodgson and Bernard (1988) compared the structure of spermatozoon and spermatids of 16 species of patellid limpets and found that the spermatozoa nucleus of the species of family patella is also cylindrically elongated, with length-to-breadth ratio of 2.1:1 to 23:1. However, they did not notice the significance of the nuclear morphology in pursuing its evolutionary route. The spermatozoa nucleus of *B. exarata*, described in this paper is cylindrically elongated, had a distinct posterior nuclear fossa, but no anterior nuclear fossa. The relationship between sperm length-width ratios, egg size and the fertilization efficiency is now under investigating in our lab, and will be reported at a later date.

Physio-ecological adaptation of gastropods

The complexity of the midpiece in gastropods spermatozoa has attracted the attention of scientists for a long time. The spermatozoa of *B. exarata* has lateral fin which twists around the axoneme of the middle piece and principal piece. In other phylum such as Osteichthyes (Pisces), some species have the same lateral fin as in *B. exarata* spermatozoa, which originated from the plasma membrane and were asymmetrical (Lin and You, 1998; Zhang et al., 1993; Stoss, 1983). Stoss (1983) and Zhang et al. (1993) suggested that the lateral fins could accelerate the velocity of the flagella, thus increasing the rate of fertilization. Among mollusks, the lateral fins of the spermatozoa tail could only be found in some species of pulmonates. Bojat et al. (2001) found the lateral fins in the spermatozoa of *Arianta arbustorum* (Pulmonata), but they did not discuss its function and origin. Healy (1988; 1996) found glycogen helices in the lateral fin (secondary helices) of stylommatophoran (Pulmonata) spermatozoa, and he thought that the large quantity of glycogen stored within the midpiece of the spermatozoa played a significant role in maintaining long periods of viability. Healy (2001) also suggested that the microtubules which surrounded the midpiece play a role in helping the formation of the secondary helices within the midpiece sheath. Thompson (1973) suggested that the helical shape (lateral fin) of the nucleus and midpiece may provide a means of converting uniplanar flagellar activity into helical forward movement. The function(s) of the lateral fin of the *B. exarata* spermatozoa remain unknown. It may share the same function(s) as other species, such as stylommatophoran, and it may also function in increasing the fertilizing efficiency. Further investigation on the relationship between sperm morphology and fertilization are undergoing in this lab.

We hypothesize that the spermatozoa of gastropods that occupy higher taxonomic status have simpler structure. Hodgson and Bernard (1988) suggested that in the same genera, spermatozoa with simpler acrosomes are of modified type. Our data supports our hypothesis. The acrosome of *B. exarata* spermatozoa is similar to that of some pa-

tellid limpets and *A. arbustorum* (Pulmonata). The middle piece and the principal piece of *B. exarata* spermatozoa is similar to those of pulmonata, but different from those of the prosobranchia. We conclude that *B. exarata* occupies higher evolutionary status.

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