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Ultrastructure of distal vas deferens and terminal ampoule of the green tiger prawn *Penaeus semisulcatus* De Haan 1844

K. Sivachandrabose
Thiruvalluvar University, Serkkadu, Vellore-632 115, Tamil Nadu, India.
*Correspondence e-mail: drksivachandrabose@gmail.com

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Short Communication

Abstract

The present study was conducted on male prawn *Penaeus semisulcatus* De Haan 1844, to assess the spermatophore formation around the chambers containing sperm mass in distal vas deferens and terminal ampoule. For the study, the technique recommended by Felgenhauer (1987) for preparing crustacean tissues for scanning electron microscopic studies was used. The transverse sections of the terminal ampoule and vas deferens have been studied in detail using Scanning Electron Microscopy. The study showed that the vas deferens is formed of three chambers and three spermatophoric layers and the terminal ampoule is formed of three chambers and five spermatophoric layers. The sperm mass accumulated in the three chambers surrounded by five spermatophoric layers is discussed in detail.

Keywords: *Penaeus semisulcatus, vas deferens, terminal ampoule and spermatophoric layers*

Introduction

The reproductive physiology of crustaceans has received considerable attention all over the world in recent years on account of its importance in broodstock management associated with aquaculture. Detailed histological studies on spermatogenesis in prawns have been carried out on many cultivable penaeid species. Some of the notable contributions are those of King (1948) on *Penaeus setiferus* by Subrahmanyam (1965) and Mohamed (1989) on *Penaeus indicus* by Joshi *et al.* (1982) on *Parapenaeopsis stylifera* by Vasudevappa (1992) on *Metapenaeus dobsoni* and by Bauer and Min (1993) on *Trachypenaeus similis*. Very few light and ultrastructural studies have been made on spermatogenesis Ro *et al.* (1988) in *P. setiferus*, Mohamed (1989) in *P. indicus*, Chow *et al.* (1991) in *Aristeus antennatus*, Joice Abraham *et al.* (2012) in *Metapenaeus monoceros* and Sumate *et al.* (2015) in *Penaeus monodon.* Hence, the present work was attempted on the ultrastructural details of the distal vas deferens and the terminal ampoule of *P. semisulcatus* de Haan.

Material and methods

The green tiger prawn, *P. semisulcatus,* commonly known as green tiger prawn was collected from the Gulf of Mannar off Mandapam coast, Tamil Nadu located at Lat. 8°50'N to 9°10'N and Long.78°35'E to 79°40'E. A fully mature male prawn of (29.5 g) was selected for ultrastructural studies on distal vas deferens and terminal ampoule since these portions play a vital role in the formation of spermatophore layers and ejaculation of sperm mass. An electrocautery apparatus was used for the extrusion of spermatophore. The internal male reproductive organ was carefully dissected in a fresh condition as shown in Fig. 1. The technique recommended by Felgenhauer (1987) was used for preparing tissues of *P. semisulcatus* from the vas deferens and terminal ampoule for a Scanning Electron Microscopic (SEM) study. The tissues were prefixed in buffered glutaraldehyde,

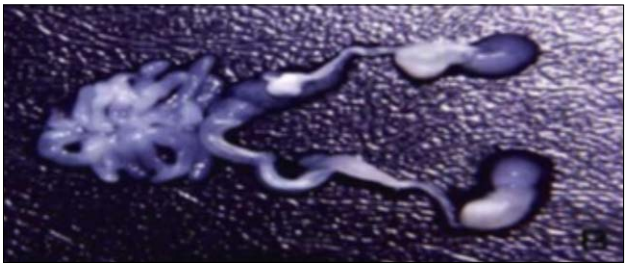

Fig. 1. A dissected out male reproductive organ in fresh condition

dehydrated in an ethanol series (35 to 100 %) critically point dried on CO_2 and sputter-coated with gold-palladium, mounted and observed.

Results

A detailed histological investigation was carried out to study the structure of the vas deferens and the role played by its various regions in the formation of the spermatophore. Three successive stages could be recognized during the formation of the spermatophore. In all three stages, the participation of certain secretions produced by the glandular epithelial cells, which line the vas deferens was involved. In the first stage, the sperm cells, which are concentrated at the centre of the seminiferous tubule, are drained into the proximal vas deferens where they became a compact sperm mass. In the second stage, the sperm mass further got compacted and the main layers of the spermatophore and the partly independent accessory wings were formed. In the third stage, the compact and convoluted spermatophore enters the terminal ampoule taking the final complete shape before extrusion.

The transverse section of the distal vas deferens has been studied using SEM (Fig. 2A). The ultrastructure of DVD showed that it contains three chambers. Chamber I was found filled with sperm mass covered by the primary spermatophore layer (PSL). An accessory layer could be seen along with PSL. A thick secondary spermatophore layer (SSL) was found to be overlapped with PSL and forms chamber II which contains the sperm mass. Chamber III was covered with thick muscle spermatophore layer III. The transverse section of the terminal ampoule was studied in detail by SEM (Fig. 2B and Fig. 3A and B). SEM showed that the terminal ampoule is formed of three chambers (1, II, III) and five spermatophore layers. The sperm mass accumulated in chambers I and II. Chamber I contains sperm mass, which is

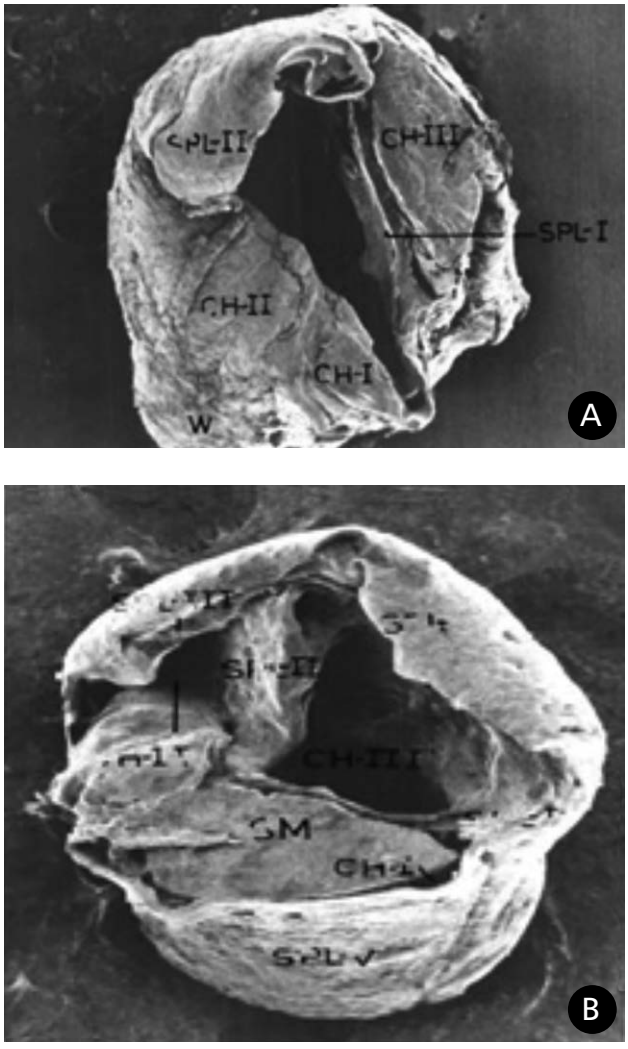

Fig. 2. A). Scanning electron micrograph of a section of the distal vas deferens (DVD) showing the presence of three chambers (CH-I, II, III) containing sperm mass (SM) and spermatophore layers. x 58.5 and B) a cross-section of the terminal ampoule, showing five spermatophore layers (SPL-I, II, III, IV, V) and three chambers containing sperm mass. x 92

Fig. 3. A). Scanning electron micrograph of a section of the terminal ampoule (TA) showing the sperm mass surrounded by spermatophore layers. x 44 and B) section of TA showing five spermatophore layers (SPL-I, II, III< IV, V). x 39

surrounded by PSL on which the adhesive layer is deposited. With the continuing invagination of the primary spermatophore layer, chamber II was found full of sperm mass. The spermatophore layer III is deposited on the SSL and made up of glutinous material. Chamber III is an empty concave pouch without sperm mass but surrounded by thick plate-like spermatophore layer IV and located in the distal region of the terminal ampullae. This chamber contains the wing portion of the spermatophore and is made up of spermatophore layer V which is reticulate or corky in appearance. The respective accessory layer I or II located on chambers I and II are known to function as a supportive sheath for the sperm mass and the spermatophore layers I and II. The function of the dorsal plate made of spermatophore layer IV is to attach the spermatophore onto the thelycum, while the anterior portion of the spermatophore is anchored by the wing or the spermatophore layer V.

Discussion

A considerable amount of work has been done on the formation of spermatophore and wing in proximal vas deferens, middle vas deferens and distal vas deferens as reported by Perez-Farfante (1975) in the Subgenus *Litopenaeus*; Chow (1982) in *Macrobrachium rosenbergii*: Radha and Subramoniam (1985) in *P. homarus*. Martin *et al.* (1987) in *Panulirus interruptus* and Berry and Heydorn (1970) in *P. homarus,* opined that the middle region of vas deferens is for storage of fully developed spermatophores. Chow *et al.* (1991) studied the middle and distal vas deferentia and terminal ampullae of *P. setiferus* and *P. vannamei* by light and electron microscopy to assess their roles in spermatophore formation and reported that the passage of spermatophoric materials from the middle vas deferens to the terminal ampullae is discontinuous. Leung-Trujillo and Lawrence (1991) studied the spermatophore developments in *P. setiferus, P. vannamei* and *P. stylirostris* and reported that the PSL and SSL are secreted in the vas deferens and the third and fourth spermatophore layers are completed in the terminal ampoule. In the present study, the PSL and SSL are formed in the middle vas deferens and the remaining spermatophore layers (III to V) are completed in the terminal ampoule as shown by scanning electron microscopy in the terminal ampoule. This is almost in agreement with the observation of Mohamed (1989) in *P. indicus*. Malek and Bawab (1974 b) have described the five successive stages involved in the formation of the complete spermatophore layer in *P. kerathurus*.

Agreeing with the histological observations of Malek and Bawab (1974 a, b) in *P. kerathurus* and Bizot-Espiard (1980) in *P. japonicus*, Heitzmann *et al.* (1993) suggested that the sperm mass in *P. vannamei* joined the spermatophore wings at the end of the middle vas deferens before reaching the terminal ampullae. In *M. dobsoni*, Vasudevappa (1992) observed the formation of four spermatophore layers and the presence of three conspicuous typhlosoles in the sperm duct and one small typhlosole in the wing duct in the middle vas deferens. Malek and Bawab (1974 b) in *P. kerathurus*, Mohamed (1989) in *P. indicus* reported the presence of a typhlosole each in the sperm duct and wing duct as encountered in the present study. The presence of blood supply to the typhlosole and the active secretion of the latter indicated the high metabolic rate. Penaeid spermatophores exhibit considerable variation. At one extreme, they are structurally complex external spermatophores, characterized by various wings, flanges, plates and adhesive materials (families *Aristeidae, Solenoceridae* and the subgenus *Litopenaeus*, genus *Penaeus* in the *penaeidae*). At the other extreme, they are highly internalised simple spermatophoric mass as found in *Sicyoniidae* (Bauer, 1990). Most workers have investigated spermatophores of crustaceans by using material teased out from the distal vas deferens (Chow, 1982; Subramoniam, 1984; Radha and Subramoniam, 1985). The fully formed spermatophore of *P. semisulcatus*, extruded using the electro-ejaculation technique (Kooda-Cisco and Talbot, 1982), during the present study is parachute or umbrella-like and the pattern of the attachment of the wing-like structures on the main body of spermatophore using short stalk is characteristically different from that of other penaeid prawns like *P. indicus* (Mohamed, 1989) and *M. dobsoni* (Vasudevappa, 1992). The wing-like structure in *P. semisulcatus* is attached in the middle region of the main body of spermatophore, whereas in *P. indicus* (Mohamed, 1989; Mohamed and Diwan, 1992 and *M. dobsoni* (Vasudevappa, 1992) the same is attached to one end of the body. Subramoniam (1993) reviewed spermatophore and sperm transfer in marine crustaceans and described the spermatophore morphology and origin of spermatophore in penaeid shrimp. In the present study, the transverse section of ejaculated spermatophore showed that it consists of five spermatophoric layers and three chambers containing sperm mass.

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