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Internal anatomy of the terminal ampoule of *Metapenaeus monoceros* (Fabricius, 1798) and its role in spermatophore formation

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ABSTRACT

Internal anatomy of terminal ampoule of *Metapenaeus monoceros* and the role played by this organ in spermatophore formation were elucidated employing histological and ultrastructural techniques. Terminal ampoule of mature male specimens consisted of an outer layer of connective tissue surrounding a layer of circular and longitudinal muscle fibres. The lumen of terminal ampoule was divided into four chambers lined by basophilic glandular epithelial cells, which showed intense secretary activity. One of the chambers had sperm mass surrounded by primary and secondary spermatophore layers. Formation of an eosinophilic matrix and adhesive globules resulting from secretions by glandular epithelial cells were observed in other chambers. Ultrastructural studies revealed that the cytoplasm of the epithelial cells had numerous free ribosomes, concentric arrangement of rough endoplasmic reticulum with vesicles often discharged into the lumen, and numerous electron dense bodies, all confirming the intense secretary activity taking place in the ampoule.

Keywords: Adhesive globules, Histology, *Metapenaeus monoceros*, Terminal ampoule, Ultrastructure

Introduction

Metapenaeus monoceros (Fabricius, 1798), the speckled shrimp is one of the commercially important penaeid shrimp species found along both the coasts of India. The species forms an important component of the shrimp catch from the Pokkali fields of Kerala in India and the rice-prawn filtration units in Bangladesh. The estimated landing of penaeid shrimps of India in 2010 was 2,17,900 t, forming 51% of the total crustacean landings (CMFRI, 2011) and *M. monoceros* is a commercially important species among large sized penaeids of the country. The species attains a maximum length of about 190 mm and has high export potential. There is very good scope for this species to be taken up for semi-intensive culture practice in India due to its larger size among the *Metapenaeus* spp. The present work elucidating the structural and functional details of the terminal ampoule of *M. monoceros* was carried out for the first time considering the importance of this species as a potential candidate for shrimp culture diversification.

Reproductive quality of males do play an important role in the productivity of broodstock of captive penaeids (Diaz *et al*., 2002), which is currently regarded as one of the major drawbacks in the fragile shrimp aquaculture industry (Parnes *et al*., 2004). However, studies on the reproductive system of male penaeids are fewer compared to their female counterparts. A thorough knowledge of the structure and functional aspects of reproductive system of the animal is a prerequisite in formulating and standardising methodologies for broodstock maintenance and large scale seed production.

The male reproductive system of penaeid shrimps consist of paired testes and vasa deferentia (King, 1948; Malek and Bawab, 1974a). In most crustaceans including penaeids, sperm transfer during copulation is accomplished by making use of a specialized sperm packet called spermatophore comprised of spermatozoa surrounded by layers of acellular secretions produced by the vas deferens (Aiken and Waddy, 1980). The envelopment of the sperm mass by layers of non-cellular materials is initiated in the median vas deferens (Malek and Bawab, 1974b), the process of spermatophore formation gets completed in the terminal ampoule (King, 1948). Parnes *et al*. (2004) classified the male reproductive system in *P. vannamei* based on external appearance of terminal ampoule. Even though there are several studies on the process of spermatophore formation in penaeids (Malek and Bawab, 1974a, b; Ro *et al*., 1990; Chow *et al*., 1991; Vasudevappa, 1992; Mohamed and Diwan, 1993; Joseph, 1996), the role played by the ejaculatory duct or terminal ampoule in the process is less understood. The large and complex structure of the terminal ampulla has hindered histological clarification of its role in spermatophore formation and the origin of certain spermatophoric materials (Chow *et al*., 1991).

Materials and methods

Specimens of *M. monoceros* were collected during November 2001 - June 2003 from trawlers operating from Kalamukku and Murikkumpadam fish landing centres of Vypeen Island (lat. 10° 08' N, long. 76° 21' E) in Kerala. Live adult shrimps of size ranging from 90 to 160 mm were used for the study. Twenty five litre plastic bins and aerators were used for live transport of shrimps from the fishing ground to the landing centres. Shrimps were then transported live to the laboratory of Central Marine Fisheries Research Institute at Cochin where they were segregated sexwise and kept in 1 t fibre glass tanks with aeration. Total length (TL) and carapace length (CL) of the shrimps were measured to the nearest mm and total weight (TW) to the nearest mg. The specimens were subjected to careful examination of the terminal ampoule, and the petasma and testes were carefully dissected out, proceeding from the terminal ampoule. The various parts of the male reproductive system, *viz*., the testicular lobes, proximal vas deferens, median vas deferens and distal vas deferens were observed under the light microscope.

Based on external morphology, the male specimens were grouped into three maturity stages according to Abraham *et al*. (2007). The terminal ampoules of shrimps belonging to three maturity stages were fixed for both light microscopy and transmission electron microscopy. For light microscopy, tissues were fixed in Bouin's fluid for 24 - 48 h. Tissue preparation for light microscopy was performed according to Bell and Lightner (1988). The tissues were washed overnight to remove excess picric acid, dehydrated in propanol series (30-100%), cold impregnated in a mixture of wax and chloroform (1:1) and tissue blocks were prepared. Serial sections of 5-6 µm were taken, affixed on glass slides and subjected to routine haematoxylin-eosin staining. Stained sections were repeatedly washed in an ascending series of propanol grades to remove excess eosin and cleared in xylene. Sections were then mounted with DPX, examined and photomicrographs were taken using a LEICA MPS 60 binocular microscope.

For electron microscopy, the terminal ampoules dissected out from the three maturity stages were fixed in 3% buffered glutaraldehyde solution for 2 h at 4 °C, washed with buffer (0.1 M sodium cacodylate), post-fixed in 1% osmium tetroxide, dehydrated in acetone series, infiltrated in Spurr's resin (Spurr, 1969) and blocks were prepared. From the polymerized blocks, ultra-thin sections (60-90 nm) were taken, double-stained with uranyl actetate and lead citrate, mounted on grids and the images observed and photographed in a Hitachi H 600 Transmission Electron Microscope.

Results and discussion

Male reproductive system of *Metapenaeus monoceros* consisted of paired testes, vas deferens and terminal ampoules (Fig. 1). The testis was an unpigmented, translucent organ which composed of seven or eight lobes located in the cardiac region, dorsal to the hepatopancreas. The testicular lobes were connected to each other at their inner ends and led to the vas deferens. The paired vas deferens in the species originated from the posterior margin of the main axis of the testicular lobes and consisted of four portions. The short and narrow proximal vas deferens (PVD) was located nearer to the testis, the thickened and long median vas deferens (MVD) at the middle portion and the long and narrow distal vas deferens (DVD) at the farther end of the testicular duct just prior to the terminal ampoule (Fig. 1), which is a greatly dilated muscular organ embedded in the coxal muscles of the fifth pereopod. Hence the general plan of organization of male reproductive system in the species conforms to that of other penaeids as reported by King (1948); Malek and Bawab (1974a, b); Vasudevappa (1992); Mohamed and Diwan (1993) and Joseph (1996).

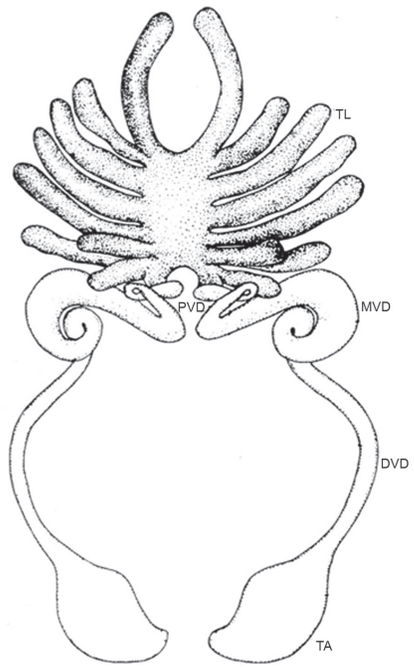

Fig. 1. Diagram of male reproductive system of *Metapenaeus monoceros* showing testicular lobes (TL), proximal vas deferens (PVD), median vas deferens (MVD), distal vas deferens (DVD) and terminal ampoule (TA)

Based on external morphology, male specimens of *M. monoceros* were classified into three stages of maturity, *viz*., immature, maturing and mature. In the present study, it was observed that in immature males, the terminal ampoule was a thin, delicate, membranous bag but in

maturing ones, the organ became more muscular and thick. In mature males, terminal ampoule was a thick, muscular, membranous bag and appeared white due to the presence of fully developed spermatophores inside. Unlike females, male shrimps provide no visible clues as to the physiological state of the gonad. Hence, very few workers have described well defined maturity stages in males. Using the small variations in the opacity and size of the testes with the size of the animal, Subrahmanyam (1965) described five maturity stages in male *Penaeus monodon*. Parnes *et al*. (2004) classified the male reproductive system in *Penaeus vannamei* into four categories based on external appearance of spematophores as observed in the terminal ampoule.

Terminal ampoule of mature animals had an outer connective tissue layer (Fig. 2). A layer of circular and longitudinal muscle fibres was seen inner to the outermost layer. The lumen of the terminal ampoule was not continuous but was divided into four chambers (Fig. 1 and 2); each lined by basophilic glandular epithelial cells, which showed intense secretory activity. Muscle fibres were often encountered between the layers of glandular epithelial cells (Fig. 3). Terminal ampoule in immature animals was not well developed – the outer layer of connective tissue and muscle layers were present, but the glandular epithelial cells lining the lumen of mature specimens were absent. In maturing animals, the size of terminal ampoule increased considerably due to increased size of the muscle layers but the epithelial lining was not well developed and the lumen was found empty.

The chamber 1 of the ejaculatory duct of *M. monoceros* was occupied by sperm mass surrounded by primary spermatophore layer and secondary spermatophore layer (Fig. 3). Chamber 2 was filled by an eosinophilic matrix secreted by the highly secretory glandular epithelial cells lining the lumen of the duct (Fig. 2 and 3), while chamber 3 contained adhesive globules (Fig. 2). In chamber 4, which

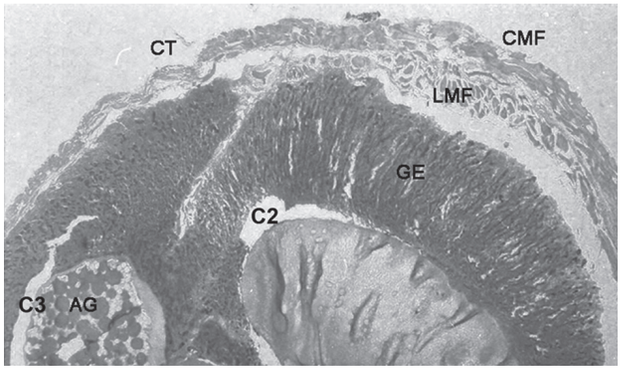

Fig. 2. Light micrograph of terminal ampoule showing connective tissue (CT), longitudinal muscle fibres (LMF), circular muscle fibres (CMF), glandular epithelial cells (GE), adhesive globules (AG) in chamber 3 (C3) and eosinophilic matrix in chamber 2 (C2); X50

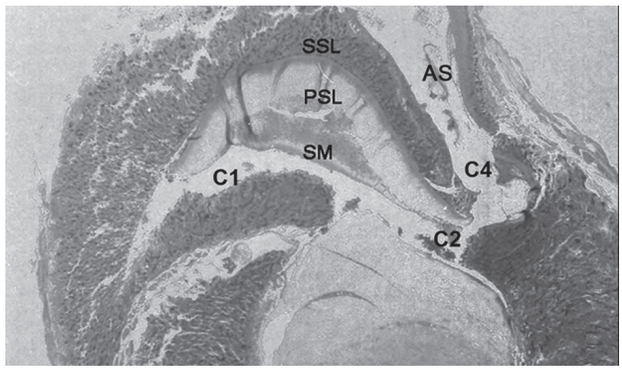

Fig. 3. Light micrograph of terminal ampoule showing sperm mass (SM) surrounded by primary spermatophore layer (PSL) and secondary spermatophore layer (SSL) in chamber 1 (C1). Chamber 4 (C4) with rudiments of accessory substance (AS) is also seen; X50

was found almost empty, remnants of accessory substance or wing was seen (Fig. 3). Bauer and Cash (1991) reported that in *Penaeus aztecus* and *Penaeus duorarum*, appendage and main body of the spermatophore occupy separate chambers which could be recognized in the external view of the ejaculatory duct.

Ultrastructural sections revealed that epithelial cells of the terminal ampoule were in a state of high secretory activity. Cytoplasm of the epithelial cells had numerous free ribosomes which serve as sites for the synthesis of proteins indicating a high protein turnover (Fig. 4). The cells also showed an extensive network of rough endoplasmic reticulum (Fig. 5). Concentrically arranged

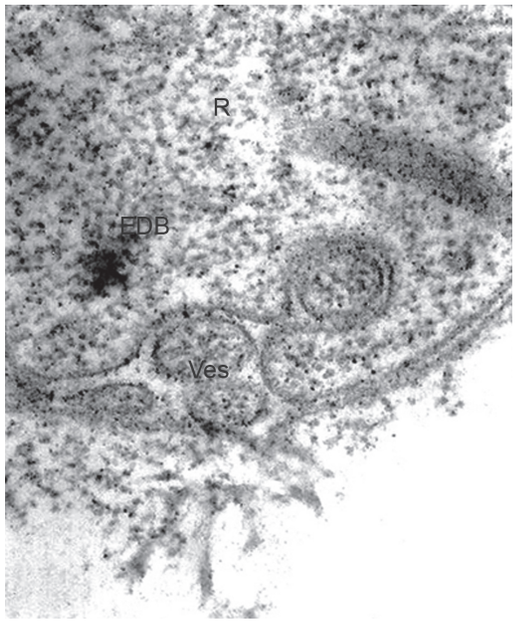

Fig. 4. Electronmicrograph of epithelial cells of terminal ampoule with free ribosomes (R), vesicles (Ves) and electron dense bodies (EDB); X35000

flattened parallel cisternae like structures studded with ribosomes were observed in the cytoplasm of some cells, indicating secretary nature of the cells (Fig. 6). The lumen of the vesicle of endoplasmic reticulum serves to store the recently synthesised proteins (Ham, 1969). Larger vesicles were found to discharge their contents into the lumen (Fig. 4). Besides, many electron dense bodies were also observed in the cytoplasm (Fig. 4 and 6).

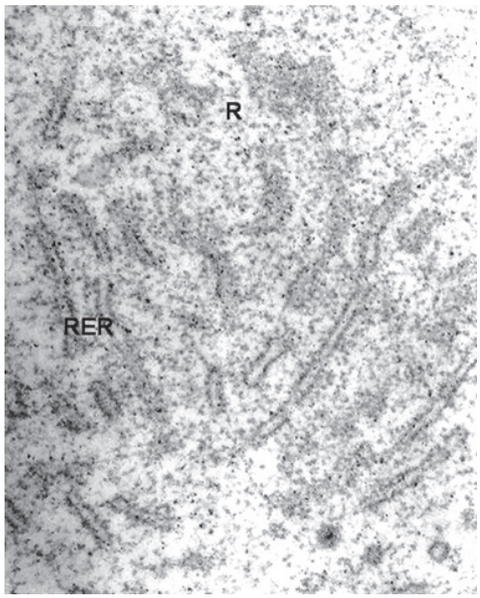

Fig. 5. Electronmicrograph of epithelial cells of terminal ampoule with rough endoplasmic reticulum (RER) and free ribosomes (R); X35000

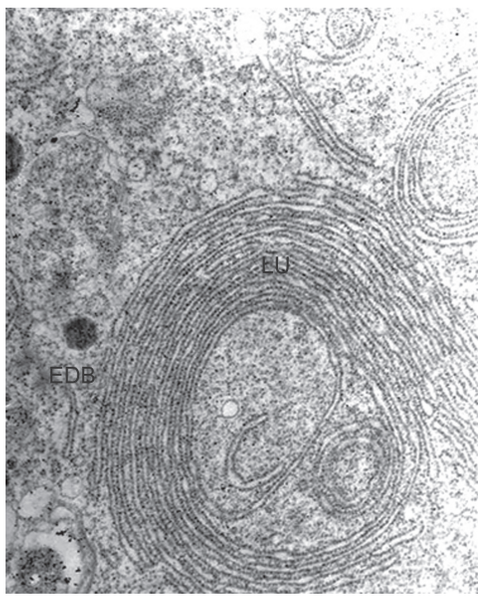

Fig. 6. Electronmicrograph of epithelial cells of terminal ampoule showing concentric arrangement of rough endoplasmic reticulum (RER). Lumen of vesicle (LU) of RER is shown; X17000

The partition of the ejaculatory duct into a number of chambers lined by glandular epithelial cells have been reported by some workers with the number of chambers differing from four in *P. vannamei* to five in *P. setiferus* (Chow *et al*., 1991a). In shrimp *Pleoticus muelleri*, the terminal ampoule has four interconnecting chambers, lined with infolded glandular epithelium and the final spermatophore maturation is attained in the organ (Diaz *et al*., 2002). Comparing the ejaculatory ducts of closed thelycum penaeids (*P. aztecus* and *P. duorarum*) with that of shrimps with open thelycum (*P. setiferus*), Bauer and Cash (1991) opined that the internal anatomy of the latter is much more intricate with various parts of the complex spertmatophore located in several interconnecting chambers.

In the present study in *M. monoceros*, no spermatophoric layers were found secreted by the terminal ampoule. Abraham (2005) has demonstrated the secretion of primary and secondary spermatophore layers and accessory substances in the testicular duct proximal to the terminal ampoule in *M. monoceros*. Bauer and Cash (1991) reported that in *P. aztecus* and *P. duorarum*, additional layers of materials are secreted around the main body of spermatophore in the terminal ampoule. In *M. monoceros*, the adhesive globules secreted by the glandular cells lining the chambers of the ejaculatory duct help to cement and join parts of the spermatophores ejaculated from the terminal ampoule on either side to form compound spermatophore as reported by Mohamed and Diwan (1993) in *Fenneropenaeus indicus.* Even though no spermatophoric layers are found secreted in the ejaculatory duct in *M. monoceros*, the eosinophilic acellular matrix secreted here helps in the final moulding of spermatophore. Terminal ampoule functions to complete spermatophore maturation (Chow *et al*., 1991b; Diaz *et al*., 2002).

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