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Structure of vasa deferentia and spermatophores in *Parapenaeopsis stylifera* (H. Milne Edwards) (Decapoda: Penaeidae)

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The structure of vasa deferentia and spermatophores has been described in *Parapenaeopsis stylifera*. The male reproductive system consists of two symmetrical halves; each half bears testis, vas deferens and an ejaculatory duct. Each testis comprised of two to three short, broad and milky white lobes; vas deferens is divisible into proximal, medial and distal parts. The proximal vas deferens (PVD) is a convoluted mass made up of an extremely long and thin tube having elongated rod like spermatozoa. The median vas deferens (MVD) is broad, straight, somewhat flattened and bears many membranous folds internally and contained complete spermatophores; the distal vas deferens (DVD) is straight and cylindrical tube. The ejaculatory duct is a simple dilation with a tubular basal part tapered posteriorly for extrusion of spermatophores. The spermatophores are minute, spindle shaped bodies present in large numbers in each ejaculatory duct; the size varied from 0.148 to 0.161 mm; each spermatophore bears six to eight rows of regularly arranged spermatozoa. Histological studies reveal no internal partitioning of either vas deferens or ejaculatory duct.

Key words: *Parapenaeopsis stylifer*, vasa deferentia, spermatophore.

INTRODUCTION

Parapenaeopsis stylifera is purely a littoral species. Its distribution is in Indo-west Pacific (Holthuis, 1980). It supports a major fishery in Pakistan and India. From Pakistani waters, 25 species and seven genera of family Penaeidae have been recorded (Kazmi, 2003), among which only 12 species have commercial significance (Majid, 1988); namely, *Marsupenaeus japonicus*, *Penaeus monodon*, *P. semisulcatus*, *Fenneropenaeus indicus*, *F. merguiensis*, *F. penicillatus*, *Metapenaeus affinis*, *M. monocers*, *M. stebbingi*, *Parapenaeopsis hardwickii*, *P. sculptilis* and *P. stylifera*. The studies on the male reproductive organs and structure and formation of spermatophores have been undertaken in many penaeid genera of commercial importance like *Fenneropenaeus* (*Penaeus*) (King, 1948; Tirmizi, 1958; Subrahmanyam, 1965; Tuma, 1967; Tirmizi and Khan, 1970; Huq, 1981; Chen, 1986; Sultana, 1986; Champion, 1987); *Penaeus* (Motoh, 1981), *Melicertus* (*Penaeus*) (Malek and Bawab, 1974a, 1974b), *Litopenaeus* (*Penaeus*) and *Farfantepenaeus* (*Penaeus*) (Leung and Lawrence, 1987; Rao et al., 1990; Bauer and Cash, 1991; Chow et al., 1991), *Trachypenaeus similes* (Raymond et al., 1993), *Sicyonia disdorsalis* (Jeri, 1998) and *Aristeus antennatus* (Demestre and Fortuno, 1992), *Astacus leptodactylus* (Erkan et al., 2009; Mirheydari et al., 2012), red claw crayfish *Cherax quadricarinatus*

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(Lo'pez-Greco et al., 2007). Some studies on the male reproductive organs of *P. stylifera* were undertaken by Shaikhmahmud and Tembe (1958), Subrahmanyam (1963) and Sultana et al. (1994); whereas, Tirmizi (1968) presented the structure and developmental stages of genitalia. Besides this, certain studies were also done in crabs such as giant hermit crab *Petrochirus diogenes* (Raquel and Fernando, 2012).

In penaeid shrimps, the male reproductive system typically consists of paired testes, vasa deferentia and ejaculatory ducts. Each testis is comprised of several lobes of variable shapes (TL); The vas deferens is a long tube of variable diameter and length in different species, originating from the main axis of the testis and extends ventro laterally towards the base of fifth pereiopod; it is differentiated into the following parts: i) proximal vas deferens (PVD), ii) medial vas deferens (MVD), iii) and distal vas deferens (DVD), terminating into ejaculatory duct (ED); the ejaculatory duct is located on the basis of fifth pereiopod and opens to the exterior through the genital opening on the arthrodial membrane of fifth pereiopod. The penaeid shrimp vasa deferentia are more complex than most other decapods and unusual in having a large dilated ampoule termed as ejaculatory duct. The vas deferens and ejaculatory duct both play important roles in the formation of spermatophores (Malek and Bawab, 1974a, 1974b; Chow et al., 1991; Bauer and Min, 1993, Bauer and Cash, 1991). The morphological variations in different parts of vas deferens are associated mostly to the type of spermatphores. *P. stylifera* have large number of spermatophores suspended in spermatic fluid (Shaikhmahmud and Tembe, 1958; Tirmizi, 1958; Sultana et al., 1994), whereas, in other penaeid genera like *Farfantepenaeus*, *Fenneropenaeus*, *Marsupenaeus*, *Melicertus* and *Penaeus*, only one pair of complete spermatophores was found (King, 1948; Subrahmanyam, 1965; Tuma, 1967; Tirmizi and Khan, 1970; Huq, 1981; Motoh, 1981; Chen, 1986; Sultana, 1985; Champion, 1987, Malek and Bawab, 1974).

In all these species, spermatophores, consists of a single sperm sac attached to some non spermatic accessory structures; that is for this reason, vas deferens and ED are divided partially or completely into two equal or unequal halves for separate transportation of spermatic and non spermatic materials. The study has therefore been conducted mainly on morphological variation of vas deferens and ejaculatory duct in relation to numerous spermatophores of *P. stylifera*, suspended in fluid and devoid of any accessory structure. The histology was done to reveal the presence of any internal partitioning of vas deferens and ejaculatory duct, if exists.

MATERIALS AND METHODS

The fresh samples were collected from commercial fish landing sites along Sindh Coast. The shrimps were identified using key by Tirmizi (1970), the collected samples were transported to the laboratory in an insulated box containing ice. In the laboratory, shrimps were stored at -40°C in deep freezer. The dissection were followed on the next day and samples were proceeded for histological studies, samples were fixed in 10% formalin, dehydrated by Isopropanol, embedded in paraffin and 5 to 7 µm sections were obtained using rotary microtome. Standard H&E staining protocol was followed. The morphological and histological variations were studied under microscope fitted with a digital camera (Nikon SMZ800 and Nikkon trinocular Eclips 50i). Spermatophores were obtained by pressing the ejaculatory duct. A sum of 30 specimens was dissected for morphological studies; total size range was 6.0 to 7.5 cm while the size range for carapace was 0.5 to 1.0 cm.

RESULTS

Morphology

The male reproductive system (Figures 1A and 2A) consists of two halves; each half is comprised of testis, vas deferens and ejaculatory duct (Figures 1A, 2A and 2D). The testes are milky white and un-pigmented (Figure 2C) located dorsal to the hepatopancreas under the carapace and comprises of two to three (mostly three) short, broad and flattened lobes on each side; commonly three lobes were found. The opening of testicular lobes is not visible and has to be traced down after displacing the testicular lobes (Figure 2C).

The vas deferens originates from the main axis of the testis and differentiated into four distinct parts: (Figure 2A), i) proximal vas deferens (PVD); (Figure 2B), ii) medial vas deferens (MVD), iii) distal vas deferens (DVD) and, iv) an ejaculatory duct (ED) (Figures 1A and 2A). The PVD is a convoluted mass made up of an extremely long, thin and greatly convoluted tube it roughly resembles the testicular lobes in appearance but the tubules are much broader than testis; the convoluted mass of PVD can easily be differentiated from the testicular lobes. The PVD contains elongated rod like spermatozoa in large numbers. The posterior part of PVD is continued into MVD (Figure 2B).

The MVD is broad, straight, somewhat flattened, curved upward and then bent down to form a curved portion (Figure 2B). It bears many folds internally; the folds can be seen clearly when empty (Figure 2B); whereas, it appears to be reflexed over itself when filled with the spermatophores.

A small notch is found at the junction where MVD transforms into the DVD. No partitioning or transportation of non-spermatic mass was seen through PVD, whereas, it was tightly packed with small spindle shape spermatophores of variable sizes in mature specimen. The DVD (Figures 1A and 2D) is almost equal to MVD in diameter, though rounded in shape and not flattened and bears no fold as found in MVD.

The ED is the dilation of DVD with a tubular enlargement, which opens to the exterior terminally (Figure 2D) with no septum or partition inside. Large numbers of spermatophores were extruded by pressing an ejaculatory duct. Figures 1D and 2E shows the ex-

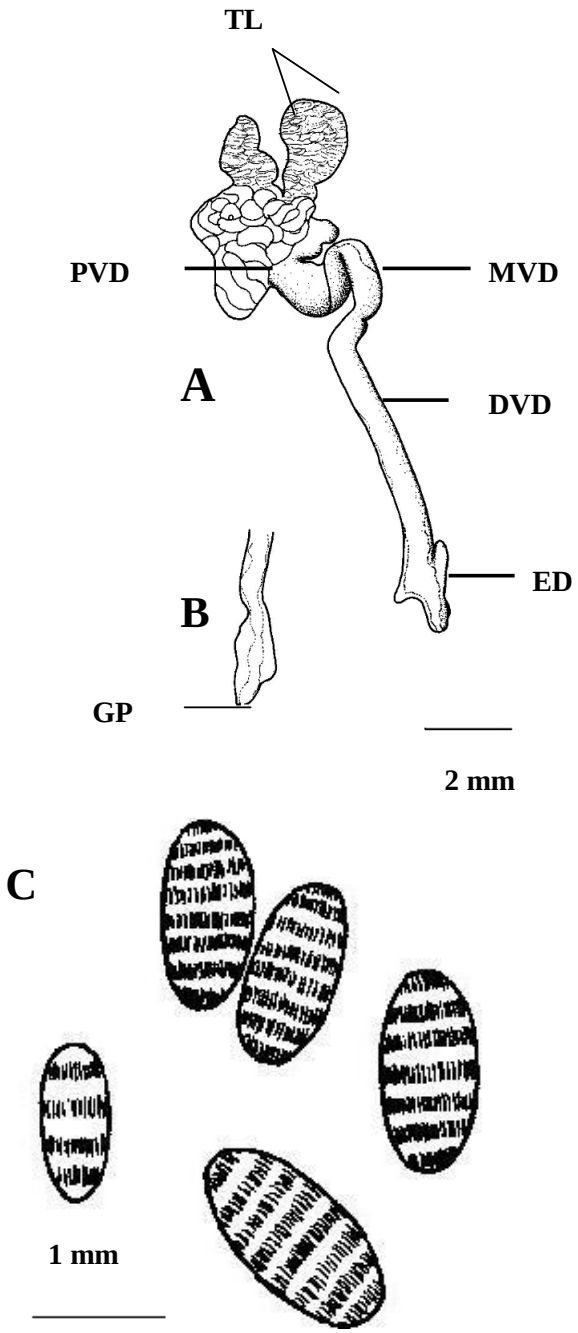

Figure 1. Line drawing of male reproductive system of *Parapenaeopsis stylifera*; A) Right half, dorsal view, proximal vasa deferens (PVD), medial vas deferens (MVD), distal vas deferens (DVD), ejaculatory duct (ED); B) ED, ventral view, gonopore (GP); C) spermatophores (Sultana, 1985, Sultana et al., 1994).

truded spermatophores which are minute and spindle shaped size varied from 0.148 to 0.161 mm; six to eight rows of fine striations were found on each spermatophore (Figure 2F), which are actually the sperms arranged in rows.

Formation of spermatophores

From the testes, spermatozoa transferred to the anterior part of PVD which is the actual site where the sperms are arranged into rows and outer layer of the spermatophore is formed; the posterior part contained spindle shape spermatophores. The spermatophores inside the MVD are found into a more or less compact form but the spindle gets its perfect shape, when the spermatophores reached to the ED, the spermatozoa are arranged in perfectly regular rows in spermatophores present in ED.

Histology

The transverse sections of testicular lobes of *P. stylifera* reveal that it has a very thin transparent outer membrane and connective tissue septa dividing the testis into lobules. The larger cells are germ cells while smaller cells are glandular. In larger cells, the cell membrane is not very clear; these cells are termed as nutritive cells by King (1948). The nutritive cells were found at the peripheral portion of the tubules. No septum or partitioning of lumen or typhlosole was found. The most anterior part of PVD contains small irregular cells; whereas the posterior part bears the spermatozoa which are of variable sizes and shape. The MVD is lined with epithelial cells that may be glandular in nature and secrets some fluids to facilitate the transfer of spermatophores. No internal partition or septum is found in the lumen. The ejaculatory duct is lined with thick layer of muscle fibers. The lumen is oblong with many complete spermatophores floating in the seminal fluid (Figure 3A and 3B).

DISCUSSION

The basic division of vas deferens in *P. stylifera* has followed the same pattern found in other species of penaeid shrimps. On the basis of gross morphology, it is divisible into same four parts namely, PVD, DVD, MVD and ED though the shape and structure of different parts of vas deferens greatly varied (Sultana et al., 1994). In *P. stylifera*, the PVD is a convoluted and an extremely long and thin tube; the MVD is broad, straight, somewhat flattened with many internal membranous folds; the DVD is straight and cylindrical tube. The ED is a simple dilation with a tubular basal part tapered posteriorly for extrusion of spermatophores. Whereas, in species of genera *Fenneropenaeus, Litopenaeus*, *Penaeus* and *Melicertus*, PVD is small, straight and somewhat conical, MVD is broad and inverted u-shaped, DVD is thin and ED is a large muscular, 2-chambered, pear shaped or conical structure. In penaeid shrimps, the shape and the structure of vas deferens were found to be associated mainly with the shape of spermatophores. Among species of genera *Fenneropenaeus, Marsupenaeus,*

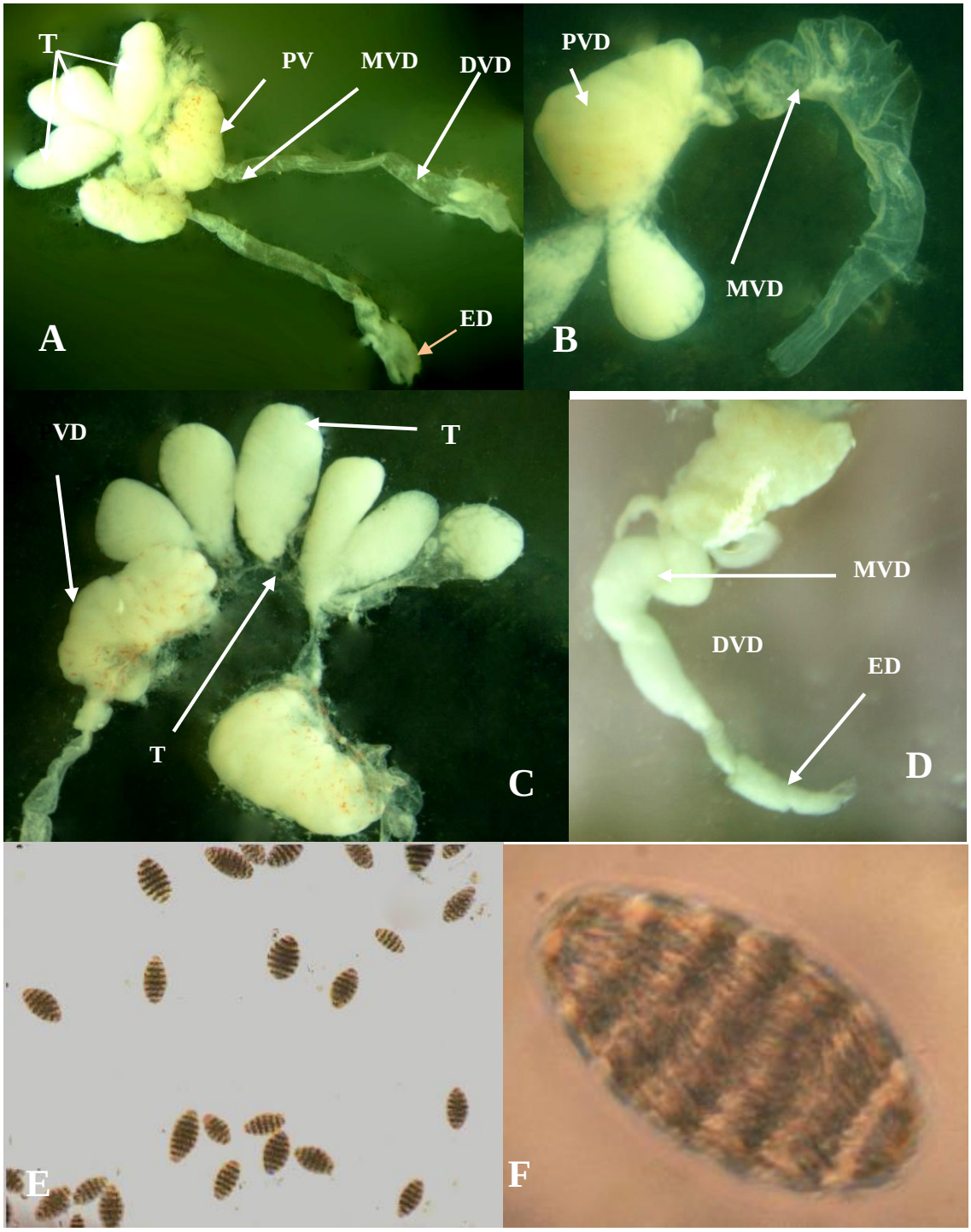

Figure 2. A) Male reproductive system of *Parapenaeopsis stylifera*; **B)** Median vas deferens, folds visible; **C)** corresponding magnification of testicular lobes, proximal, median and distal vas deferens; **D)** MVD filled with spermatic material; ED, ejaculatory duct; **E)** Spermatophores; F) corresponding magnification of spermatophore showing striations. DVD, distal vas deferens; ED, Ejaculatory duct; MVD, Median vas deferens; PVD, proximal vasa deferens; SP, spermatophore; T, testicular lobes; TB, tubules.

Litopenaeus, *Penaeus* and *Melicertus*, one large complete spermatophore is found from each ED, which is associated with a non spermatic accessory structure called as wing. In open thelycum penaeids (for example, *Liopenaeus* spp.), the spermatophores are more complex and bears many accessory structures to cling the sper-

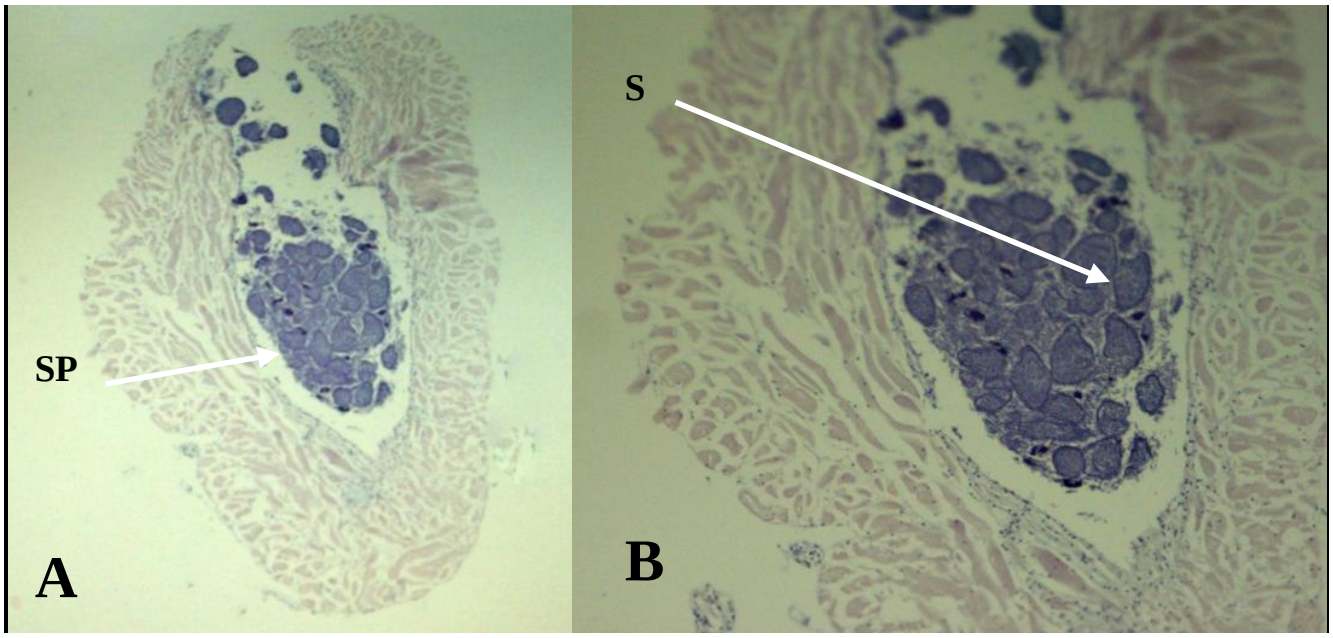

Figure 3. A) Horizontal section of ejaculatory duct of *Parapenaeopsis stylifea* showing the bulk of spermatophore; **B)** corresponding magnification of spermatophore in ejaculatory duct; S, spermatophore.

matophore on thelycum (Perez Farfante, 1969, 1975; Malek and Bawab, 1974); whereas, in closed thelycum penaeids genera, *Fenneropenaeus* and *Penaeus*, a large membranous wing is found.

In either type, the entire vas deferens was divided by a complete or partial internal septum into two ducts to separately process and transport the spermatic and non spermatic materials. The partitioning was complete in PVD; whereas in DVD and ejaculatory duct, septum was partial (Malek and Bawab, 1974a, 1974b, Champion, 1987; Sultana et al., 1994). In such species, the cellular structure revealed through histological sections was also complex and contained glandular cells which usually formed a thick lining and or typhlosole in both of the ducts. In contrary to this, in *P. stylifera*, several thousand tiny spindle shaped spermatophores can be extruded form a single ED; further no accessory structure is associated with the spermatophore; hence, no internal longitudinal partitioning of vas deferens and ejaculatory duct was found. The long convoluted PVD and MVD with extensive folds may contribute to the compaction and arrangement of many small spermatozoa in regular rows into the spermatophores, which are extruded through an elongated tubular opening at the end of ejaculatory duct. The tubular enlargement of ejaculatory duct may also be considered as a functional adaptation to extrude a fluid containing spermatophores.

The structures of vas deferens and spermatophore both have a close homology of structures found in *Trachypenaeus* (*Rimapenaeus*) *similis* (Bauer and Min, 1993) than species of other penaeid genera for having convoluted PVD, an undivided vas deferens and numerous spermatphores suspended in spermatic fluid, though no spermatic plug was found.

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