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ORIGINAL ARTICLE

MALE REPRODUCTIVE BIOLOGY OF A BRACHYURAN CRAB, *SARMATIUM PUNCTATUM* (A. MILNE EDWARDS, 1875)

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Abstract:

This paper envisages the morphology and function of the male reproductive system in a brachyuran crab, Sarmatium punctatum using histological and histochemical studies. The male reproductive system of S. punctatum comprises paired symmetrical structures of testis, coiled vas deferens, sac-like tubular accessory glands and narrow ejaculatory duct which open out as intromittent organ. Seminiferous tubules of the testis display two zones: germinal zone (GZ) and transformation zone (TZ). Spermatogenesis and spermatozoan maturation occurrs within the testes and subsequently reaches the vas deferens via collecting duct. Structurally and functionally the vas deferens is categorized into anterior vas deferens (AVD), middle vas deferens (MVD), and posterior vas deferens (PVD). AVD secretes glycoproteinaceous and mucoproteinaceous products which assist in sperm grouping and spermatiophore formation. MVD and PVD secrete mucoproteinaceous secretion akin to AVD and act as storage centers. The exact role of accessory glands is enigmatic. The copious amount of acidic materials secreted by these ductal glands possibly supports easy transport of spermatophores during copulation.

KEYWORDS:

Sarmatium punctatum, testis, spermatophores, accessory glands, crustaceans.

INTRODUCTION

The male reproductive system of decapod crustacean comprises testis followed by vas deferens leading to the external gonopore. Spermiogenesis has been reported to occur in the testis; sperm cells become spermatozoa and pass to the vas deferens (Beninger et al., 1988; Hinsch, 1988; Suganthi, 1996; Ganapiriya, 2013). The spermatozoa are surrounded by ductal glands that consolidate the sperm mass into spermatophores (Hinsch and Walker, 1974; Benhalima and Moriyasu, 2000; Vogt, 2002).

Detailed knowledge on the formation of spermatophores and the associated bioactive substances and the deposition of these into the female is essential to an understanding of the mechanics of insemination, sperm storage and sperm release during fertilization (Bauer, 1991). Notwithstanding, previous studies on male reproduction exposed dissimilarity among the closely related species (Knudsen, 1964; Erdman and Blake, 1988; Erdman et al., 1991) or the members of the same species (Krouse, 1980; Anilkumar and Adiyodi, 1983; Conan, 1985; Suganthi, 1996). Such discrepancy associated with spermatophores and the insemination morphology of the male and female are important in analyzing the evolutionary relationships and interpretation of their mating system of brachyuran decapods (Bauer, 1991). The present study deals on the histological, histochemical aspects of the male reproductive system of a

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brachyuran crab, *Sarmatium punctatum*, which left unnoticed irrespective of its ecological and medicinal importance. *S. punctatum* inhabits the mangrove region and found along the Manakudy, Thengapattinam, Rajakamangalamthurai and Kadiyapattinam of Kanyakumari District, Tamil Nadu, India. *S. punctatum* appears deep violet (Carapace) except in the dactyl and the distal portion of propodus, which is white coloured. The animals are less active and more visible during twilight. The carapace width ranges between 2.2 and 3.2 cm in males and 2.2-3.4 cm in females.

MATERIALS AND METHODS:

The crab, *S. punctatum* inhabits the burrows of the mangrove region and were collected by hand picking or clam bait from Kadiyappattinam (Latitude 10º58´and Longitude 78º31´) Kanyakumari District, Tamil Nadu. In the laboratory, the crabs were categorized based on their sex and moult stages (after Suganthi and Anilkumar, 1999) and maintained in separate plastic cisterns in near natural conditions and fed with clam meat and rice flakes. Healthy adult male crabs (intermoult stage) with carapace width ranging between 2.2 and 3.2cm were selected for the present study. The male reproductive system was removed and fixed in formal alcohol for about 24 hours. Sections of 5-7µm thickness were double stained with Harry's haematoxylin and aqueous eosin. Thickness of 5-7µm were used for staining Mercuric bromophenol blue, Periodic acid – Schiff (PAS) reaction, Best`s carmine, Alcian blue and Sudan black B (Humason, 1967). Microphotographs were taken under trinocular microscope of Deep Vision company (Model no DV- 47T) using digital camera.

RESULTS

Testis: The male reproductive system of *Sarmatium punctatum* is bilateral and consists of tubular testis followed by vas deferens, accessory gland and ejaculatory duct. Each testis is formed by interwoven tubule spread over the dorsal portion of the hepatopancreas, extending antero-dorsally on the cephalothorax. Length of the single uncoiled testicular tubule is 7- 9cm and the width is 1.5mm. Distal ends of the testis are fused with the vas deferens anteriorly forming an H-shaped commissure. Based on the nature of coiling vas deferens is categorized into highly coiled Anterior Vas Deferens (AVD), moderately coiled Mid Vas Deferens (MVD) and a relatively straight, tubular Posterior Vas Deferens (PVD). The anterior PVD is lodged with a tuft of accessory glandular tubules followed by individual tubules fringed laterally throughout the mid and posterior PVD. A narrow ejaculatory duct originates from the basal part of the PVD and opens out through the intromittent organ.

The tubular testes of *S. punctatum* consist of several seminiferous tubule open individually into a central collecting duct (Fig. 1). Each testicular lobule is enveloped by an outer thin connective tissue and an inner epithelial layer. The epithelium is simple and squamous with sub-spherical nucleus (diameter approximately 0.8 µm). Two different zones are discernible; proliferation or germinal zone (GZ) and transformation zone (TZ) (Fig. 1). The peripheral region of the lobule constitutes the GZ, lodged with spermatogonial cells and few accessory cells. The primary spermatogonia are large, spherical shaped (8.1 ± 0.7 µm) with indistinct cytoplasm and prominent nucleus with one or two nucleoli (Fig. 2). Secondary spermatogonia are smaller (7.8 ± 0.6 µm) with basophilic chromatin clumps. Small spherical accessory cells with distinct nucleus and nucleolus is seen intermingled with the germinal cells (Fig. 2).

The TZ encompasses the germinal cells in different stages such as spermatocytes, spermatids. It is interesting to note that each germinal cell is coated by thin muco-proteinaceous secretion. Spermatocytes are relatively small (approximately 6.50 µm) with indistinct cytoplasm and chromatin clumps. The spermatid (4.8 ± 0.67 µm) undergoing spermiogenesis is often visible. Mature spermatozoan (3.15 ± 0.33 µm diameter) reaches the anterior vas deferens through the collecting duct.

Vas deferens: The wall of AVD comprises of outer connective tissue, middle muscle layer and inner epithelium (Fig. 3). In proximal AVD, the epithelium is columnar (42 ± 0.58 µm) and multinucleated (2.17 ± 0.29 µm) secretes glycoproteinaceous homogenous secretion (Fig. 3) into the lumen, where sperm groupings are perceptible. On the other hand the epithelium of distal AVD is cuboidal (22.2 ± 1.46 µm), secretes globular mucoproteinaceous substances. The spermatophores are simple, oval with single layered wall noticeable in the distal AVD onwards. The thick striated muscle layer of MVD (approximately 1.5 - 3 µm) enclosing cuboidal (8.1 ± 0.49 µm) epithelium with elongated nucleus (1.1 ± 0.22 µm) (Fig. 4). MVD secretes huge globular mucoprotenaceous substances amassed in the lumen. The accessory glands fringed throughout the PVD secretes entirely dissimilar secretion which differs in texture and chemistry (Fig.5). The wall of ED comprises thick longitudinal and circular musculature enclosing cubic epithelium with oval shaped nucleus. The narrow lumen encloses ductal secretions and spermatophores (Fig. 6).

DISCUSSION:

The macroscopic morphology of the male reproductive system of S. punctatum corroborates with the findings of other brachyuran crabs (Krol *et al*., 1992; Suganthi and Anilkumar, 1998; Garcia and Silva, 2006). The testis of crabs is either tubular (Binford, 1913, Hoestlandt, 1948, Kon and Honma, 1970; Sapelkin and Fedoseev, 1981, Chiba and Honma, 1972, Suganthi and Anilkumar, 1998; Ganapiriya, 2013) or lobular (Cronin, 1947; George, 1963; Gupta and Chatterjee, 1976; Hinsch, 1988a; Minagawa *et al.*, 1994; Cuartus and Petriella, 2007; Castilho *et al*., 2008). In *S. punctatum*, the testis is tubular and consists of several Seminiferous tubules also referred as lobules (Suganthi and Anilkumar, 1998) or testicular acini (Ganapiriya, 2013). Studies hitherto reported the presence of three zones within the testis; germinalzone, transformation zone and evacuation zone (Simeo *et al.,* 2009; Ganapiriya, 2012). However, the evacuation zone is not apparent in *S. punctatum*. Presumably, the presence of mature spermatozoa in the collecting duct corroborate with the evacuation zone, as described in *M. brachydactyla* (Simeo et al., 2009) and *P. plicatum* (Ganapiriya, 2012).

The accessory cells in *S. punctatum* are variously named as Sertoli cells (Hinsch, 1993; Simeo et al., 2009; Sal-Mayano *et al*., 2010), nurse cells, sustentacular cells and interstitial cells (Muthuraman, 1986) in other brachyurans. Electron microscopic studies on the accessory cells of *P. plicatum* revealed that they showed an intimate association between the germinal cells thus playing an important role in spermiogenesis, synchronization and maintenance of the germ cells (Ganapiriya, 2012). The presence of mucoproteinaceous secretion in the testis of *S. punctatum* is presumably the product of the accessory cells that may have nutritive role or form cytoplasmic bridge between the germinal cell (Ganapiriya, 2012) and any other functions reported by other researchers (Hinsch and Walker, 1974).

Depending on the nature of coiling and structural dissimilarity, VD is divided into three zones, in *S. punctatum* as in other crabs (Lopez Greco *et al*., 2007; Castilho *et al*., 2008) four (Hinsch and McKnight, 1988) and eight (Manjon- Cabeza and Raso, 2000) zones reported. The epithelium of AVD secretes glycoproteinaceous proximally and mucoproteinaceous distally and probably both these secretions aggregates the sperm and eventually transform into single walled spermatophores. In brachyurans, the spermatophores are either single layered as reported in brachyurans such as *Libinia emarginata, L. dubia, Ocypode ocellatus* (Hinsch, 1986), *S. quadratum* (Suganthi, 1996) and *M. brachydactyla* (Simeo *et al.*, 2009) or double layered in Scylla serrata (Uma and Subramonium, 1979), Portunus pelagicus (El-Serief, 1991), C. opilio (Chiba *et al*., 1992), *S. hydrodroma* (Kulasekharan, 1994) and *M. messor* (Suganthi and Anilkumar, 1992). It is supposed that the main role of MVD is to secrete copious amount of seminal fluid (evidenced by the presence of irregular shaped nuclei) and long term storage of semen within the male tract pending copulation (Costilho *et al*., 2007; Simeo et al., 2009; Sal Moyano *et al*., 2010). The structure of PVD is similar to MVD, except in its musculature which could perhaps facilitate the ejaculation of ductal fluids during coitus (Minagawa *et al*., 1994, Subramoniam, 1995, Suganthi, 1996; Simeo *et al*., 2009). The AG of *S. punctatum* appended to the lower part of the PVD warrants more research, however, the dissimilarity in the texture and chemical composition of the AG luminal contents claims its insignificant role in male reproduction. The thick musculature in the ED may play an important role in the extrusion and ejaculation of semen through the gonopore (Cronin, 1947; Suganthi, 1996; Simeo *et al*., 2009) during copulation.

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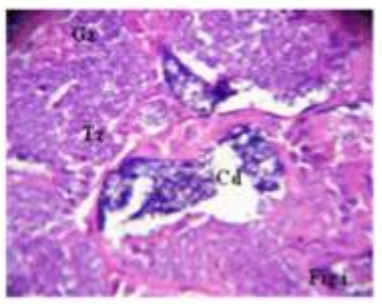

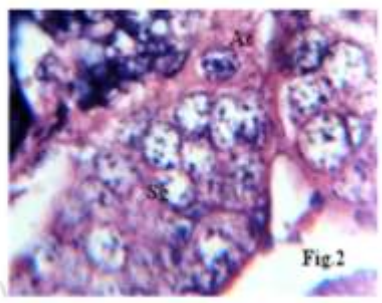

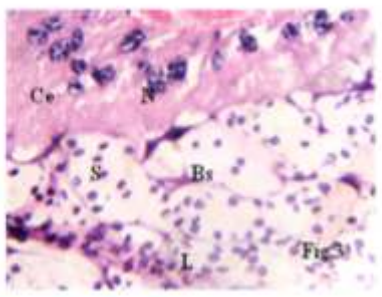

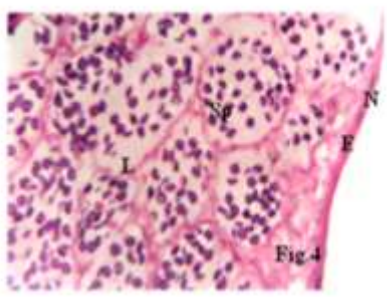

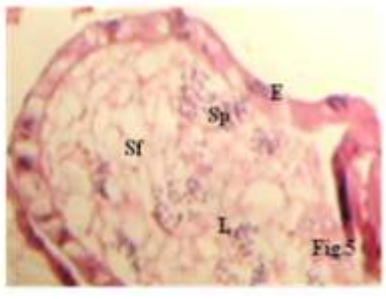

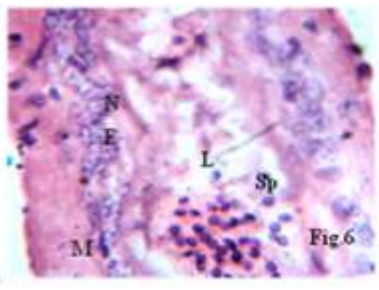

Fig. 1) Seminiferous tables with germinal zone (Gz), transformation zone (Tz) and collecting duct (Cd) x 100
Fig. 2) Germinal zone with spermatogonial cells (Sg) and accessory cells (Ac) under higher magnification x 1000
Fig. 3) Cross section of AVD showing multinucleated (N) columnar epithelium (Ce). The lumen (L) encloses spermatozoa (S) intermingled with basophilic secretion x 1500
Fig. 4) Cross section of MVD showing epithelium (E) with elongated nucleus (N). Lumen (L) encloses mucoproteinaceous secretion with the spermatophores (Sp) x 1200
Fig. 5) PVD: lumen (L) enclosing seminal fluid (Sf) and spermatophres (Sp) x 1200
Fig. 6) ED with thick musculature (M), epithelium (E) and nucleus (N) and Narrow lumen (L) x 1000