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A COMPARATIVE STUDY ON THE SPERMATOPHORE IN *SCYLLA SERRATA* (FORSKAL) (DECAPODA : BRACHYURA) AND *CLIBANARIUS LONGITARSUS* (DE HAAN) (DECAPODA : ANOMURA)

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ABSTRACT

Based on the anatomy and histology of the vas deferens of the brachyuran crab *Scylla serrata*, the vas deferens is classified into the proximal vas deferens (PVD), middle vas deferens (MVD), distal vas deferens (DVD) and the ejaculatory duct (ED), the latter opening into the intromittant organ. Proximal portion of PVD has cuboidal epithelial cells, secreting the substance A that encapsulises the sperm mass into spermatophores. The epithelial cells in the distal PVD region become columnar and secrete a granular material substance B to act as the medium for spermatophore transfer. Swollen MVD processes thin epithelial cells and stores the genital products pending ejaculation. Highly coiled DVD secretes a viscid, agranular substance C differing very much from substance secreted in the other regions. ED does not show any secretory activity, but appears to be filled with a substance D similar to that found in DVD. ED possesses a thick mesial muscular layer.

In the anomuran crab *Clibanarius longitarsus*, the proximal part of PVD possesses highly secretory cuboidal cells constituting the inner epithelial layer, the configuration of which significantly changes towards the distal part of PVD to give rise to a large typhlosole, the secretions which concentrate the loose spermatozoa into the sperm mass. In MVD, the epithelial cells are columnar in the lateral side of the lumen which is now elliptical. Here, the sperm mass is surrounded by a thick layer. The secretions accumulated in the ventral regions condense to form a cord which is attached to the pear-shaped ampule. In the DVD the lumen enlarges largely due to the thining of the lateral columnar epithelial cells. The spermatophore ampule is now kidney-shaped. The peduncle also get shortened and get connected to other spermatophores by a continuous gelatinous cord.

INTRODUCTION

CALMAN (1909) classified the spermatophores of Crustacea into pedunculate and non-pedunculate types. Pedunculate spermatophores are commonly met within the anomurans whereas the non-pedunculate, vesicular type is characteristic of Brachyura. In decapod Crustacea, the vas deferens plays a major role in the formation of spermatophores unlike the insects where special accessory glands are known to secrete the spermatophoric layers (Chapman, 1972). In general, the proximal vas deferens is secretory in nature and the distal part acts as a storage as well as ejaculatory organ of the seminal products. However, the stepwise formation of spermatophores and its associated seminal substances in different regions of the male genital tract is known only in a few forms (Cronin, 1947; Matthews, 1953, 1956 a, b; Ryan, 1967; Greenwood, 1972; Hinsch and Walker, 1974; Subramoniam, 1984).

In the present account, the comparative histo-morphology of the vas deferens in a brachyuran crab *Scylla serrata* and an anomuran crab *Clibanarius longitarsus* is presented with illustrations to elucidate the sequential synthesis of spermatophoric material and their final assembly.

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Materials and Methods

Scylla serrata were procured from the Pulicat Lake (Madras) and maintained in the laboratory. *Clibanarius longitarsus* were collected from the Ennore Backwaters. The male reproductive tracts were carefully removed and observed under microscope for the gross anatomy and morphology. To observe the final shape of spermatophores, squash preparations of vas deferens were made and stained with the vital stains such as 0 1% Congo red, 0 1% fast green and 0.1% meth lene blue). For histological studies, after fixing the reproductive tract for a few minutes in Bouin's fixative or 5% neutral buffered formalin, different regions of vas deferens were separated and fixed overnight. Paraffin sections were taken at 6 μm thickness and stained in Mallory's triple or haematoxylin-eosin. For the observation of the developing spermatozoa inside the spermatophores, smear preparations were stained with 0.1% Janus green, periodic acid - Schiff and Feulgen's stain (Pearse, 1968).

Results

Scylla serrata

i. *General morphology*

The male reproductive system consists of a pair of testes and vas deferens (VD). The paired testes are slender, white convoluted tubes interconnected medially by a commissure. The VD extends from the posterior end of testes and passes through the thoracic cavity and pereiopodal musculature of the 8th thoracic segmen where it ends in the penile papillae on the coxae of the 5th periopod.

Based on the anatomy and functional morphology, the VD is divided into four regions, the proximal vas deferens (PVD), mid vas deferens (MVD), distal vas deferens (DVD) and ejaculatory duct (ED) (Pl. I A.)

The proximal vas deferens is tightly coiled, 5-8 mm in length and dull white in colour, and lies anterior to the pericardial region. The milky white MVD is loosely coiled constituting the massive part of the system; this swe llen portion is extremely thin-walled and rup tures very easily. The DVD is a transparent, highly convoluted (50-130 mm) part extending straight into the muscular ED.

ii. *Histology of the vas deferens*

The VD is generally composed of three layers; an outer connective tissue covering (CT), middle muscular layer (ML) and an inner epithelial lining (EL). The CT stains red and ML, blue with Mallory's triple stain in all the regions of VD. The EL of the proximal portion of PVD and DVD stains blue, and MVD and distal portion of DVD stain red with Mallory's triple.

a. *Proximal vas deferens*

In the most proximal portion of PVD, the CT and ML are thin (0.5 - 0.7 μm) and EL is cuboidal. The lumen is filled with viscous fluid (Pl. I B). In the middle portion of PVD, EL is formed of low columnar cells with prominent nuclei. Sperm masses are seen in the midst of luminal substance A (Pl. I C). The substance A staining blue with Mallory slowly encapsulises the individual sperm masses by a process of condensation to become the spermatophoric layer (Pl. I D, II A). In the distal portion, EL consists of tall columnar cells showing foliation towards the lumen with vacuoles. The secretory products from this are in the form of tiny globules (Substance B) (Pl. I E). In the lumen they are intermingled with the spermatophores. The substance B stains red, whereas the spermatophores take blue with Mallory's triple. The granules vary in size from 1-4 μm in diameter (Pl. I F, II A).

b. *Mid vas deferens*

The most proximal portion of MVD and distal portion of the PVD are similar in their histological features. But the distal portion

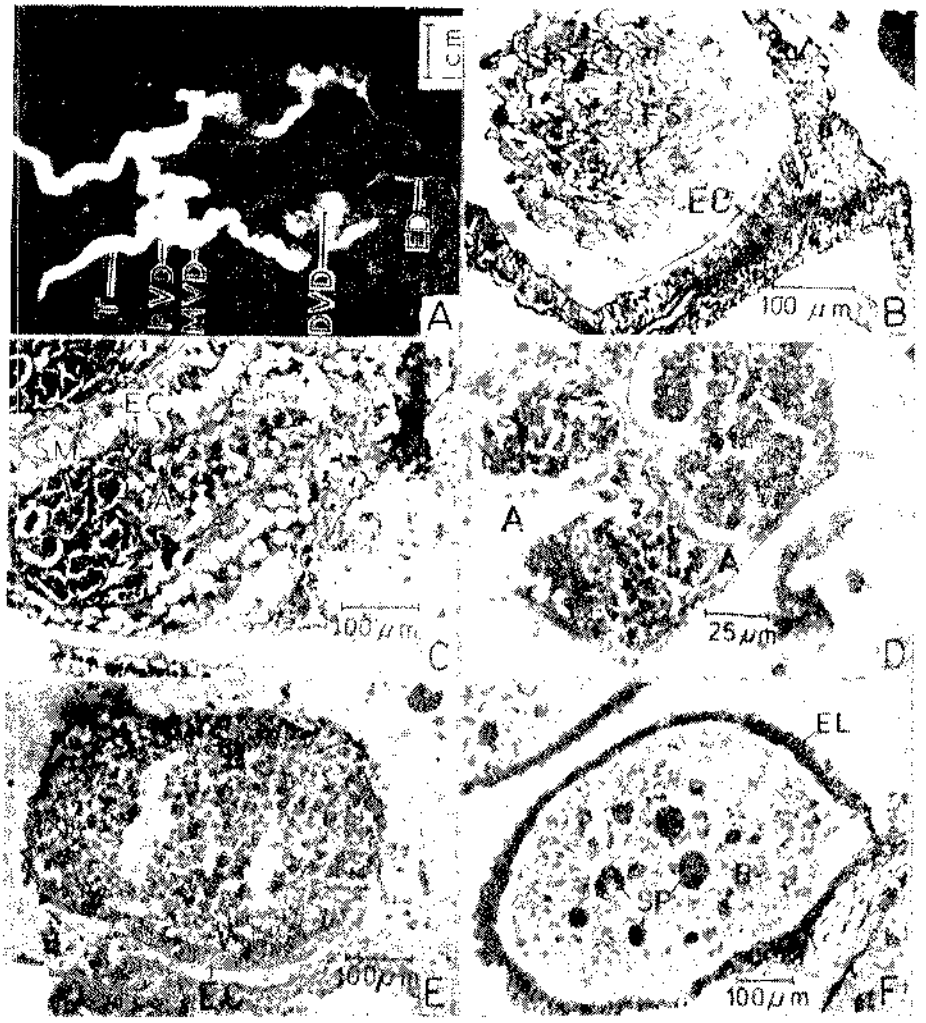

PLATE 1 A. Male reproductive system of *Scylla serrata* : Testis (T), Proximal vas deferens (PVD), Middle vas deferens (MVD), Distal vas deferens (DVD) and Ejaculatory duct (ED); B. Transverse section (T. S.) of proximal portion of PVD showing epithelial cells (EC) and fluid secretion (FS) in the lumen stained in haematoxylin-eosin. C. T. S. of middle portion of PVD showing substance A (A) (arrow) from the columnar epithelial cells (EC). Note the sperm masses (SM) in the lumen stained in haematoxylin-eosin, D. Showing substance A (A) surrounding the sperm masses (SM) stained in heamatoxylin-eosin. E. T. S. of distal portion of PVD showing tall columnar epithelial cell (EC) and numerous vacuoles (V) release the granular substance B (B) into the lumen stained haematoxylin-eosin and F. T.S. of the most distal region of PVD showing thinning of the epithelial layer (EL). Lumen with substance B (B) and few spermatophores (SP) stained in Mallory's triple.

of MVD is distinctly different from the preceding region in that the EL and ML are not only thin (0.6 0.7 μm), but the epithelial cells show no secretory activity. Such changes lead to the increase in the size of the lumen (600-780 μm) which is filled with only substance B along with spermatophores (Pl. II B).

c. *Distal vas deferens*

Epithelial layer is consisted of small cuboidal cells showing numerous infoldings into the lumen. They secrete viscid eosinophilic agranular substance C with no spermatophores in it (Pl. II C).

d. *Ejaculatory duct*

Muscular layer is thicker (2 μm) than EL, which is formed of cells with indefinite boundaries. Lumen is filled with an eosinophilic gelatinous substance D (Pl. II D).

Clibanarius longitarsus

i. *General morphology*

The male reproductive tract, confining to the abdominal region, consists of paired testes and vas deferens. PVD is highly coiled with its proximal portion, very difficult to differentiate from testes; whereas the MVD and DVD are larger in size and not much coiled (Pl. II E).

ii. *Histology of the vas deferens*

a. *Proximal vas deferens*

Proximal portion of PVD possesses highly secretory cuboidal epithelial cells that are ensheathed by ML and CT. The lumen has only free sperm (Pl. II F). In its distal region, the epithelial cells on the ventral side become highly glandular and from a typhlosole like structure (Pl. II G) staining blue with haematoxylin-eosin.

b. *Mid vas deferens*

The inner epithelium is in the form of long columnar cells spreading dorsolaterally to form an oval shaped lumen. Substances secreted from them are deposited around the sperm mass as sperm sheath (Pl. III A). Towards the distal region, secretion from the lateral epithelial cells are accumulated in the ventral groove found in the oval shaped lumen (Pl. III B). This secretion condenses into a gelatinous cord which establishes connection with ampule of each spermatophore (Pl. III C).

c. *Distal vas deferens*

The inner epithelial cells are reduced very much in length, but muscle layer is much thickened (2 μm). The lumen is greatly increased in size and assume a rectangular shape. A cross section of the DVD reveals the presence of several spermatophores with long peduncles (Pl. III D, IV A). Interestingly, the peduncles originate from all side of the lumen, although in the mid vas deferens the peduncle originates only from a ventral groove, which gets completely obliterated in the DVD region. Towards the more distal region of the DVD, the long peduncles of spermatophores tend to coalesce with each other to form a central cord to which are attached the spermatophore ampule, which is now kidney-shaped. Consequent to this change in shape, the peduncle shows a tendency for internalization into the ampule giving out radiating filaments (Pl. IV B). Inside the spermatophores, the spermatozoa are in different stages of maturation (Pl. IV B, C). Such a condition of the completion of spermiogenesis inside the spermatophores has also been reported in brachyuran crabs (Fasten, 1918; Moses, 1961). The spermatophoric ribbon is finally embedded in a gelatinous matrix (Pl. IV D, E).

Discussion

Comparative morphology of decapod spermatophores suggests their relationship with fertilization. In the brachyuran crabs, with internal fertilization, the spermatophores are non-pedunculate and vesicular (Uma and

Subramoniam, 1979). They are, in general, transmitted through definite copulatory organs in seminal plasma to the spermatheca for storage and utilization during fertilization. On the other hand, in the anomurans with external fertilization the sperm transfer is through the pedunculate spermatophores embedded in a gelatinous matrix for the purpose of deposition into the female sternum. Whereas the spermatophores of brachyuran crabs have limited structural variation, the anomuran spermatophores exhibit a species - specific morphological variation (Subramoniam, 1977, 1984).

The results presented on the histology of the epithelial cells lining the vas deferens of the anomuran and the brachyuran together with the secretory products reflect such a difference in the structural organization of spermatophores of the two decapod groups. The proximal portion of PVD in *S. serrata* secretes substance A which agglutinates sperm and form the spermatophore layer. In their distal part, the secretory nature differs in that a granular substance B is released and that it forms the medium for storing the completed spermatophores. This morphological distinction in the final secretory products of these two regions is also reflected in the histological nature of the epithelial cells. In the proximal portion, they are cuboidal in nature; whereas in the distal portion, these cells are columnar and show foliation towards the lumen with large vacuoles. Granular secretory products from the vas deferens epithelium has been reported in a number of decapods such as *carcinus maenas* (Spalding, 1942), *Callinectes sapidus* (Cronin, 1947), *Portunus pelagicus* (Ryan, 1967) and *Libinia emarginata* (Hinsch and Walker, 1974). In a marine viviparous teleost fish *Cymatogaster aggregata,* the Sertoli cells have been implicated with the contribution of materials for spermatophore extra cellular matrix (Gardiner, 1978).

Unlike the PVD, the MVD shows very little secretory activity, but is membranous and acts as the storage region of spermatophores and other seminal products, derived from the PVD. In the DVD the epithelial cells are active again assuming a cuboidal shape. The fluid-like secretion C is, however, not mixed with the stored seminal products in the MVD region. It is possible that this secretion, in view of its mucoid nature, may be employed for the formation of sperm plug as described in several other brachyuran crabs (Spalding, 1942; Ryan, 1967). Following DVD there is a short straight tube, the ED, having a thin epithelial layer probably with very low secretory activity. Its lumen is filled with homogeneous secretion D which is almost identical to that found in the DVD. Ryan (1967) also reported in *Portunus* a similar condition of storage of seminal products in the MVD, however in *Libinia emarginata,* Hinsch and Walker (1974) reported that both the DVD and MVD are used for storing the seminal products including spermatophores. Yet another deviation is found in *Carcinus maenas* (Spalding, 1942) and *Uca lacteus* (Uma, 1978) where a distinct dilated portion in the form of vesicles in the DVD is serving a storage function for spermatophores.

Pedunculate spermatophores are characteristic of anomurans and certain macrurans (Matthews, 1953, 1956 a). Each spermatophore consists of ampule, peduncle and pedestal. The structure and size of the spermatophores may differ from one species to another. In *Diogenes pugilator* (Matthews, 1956 a), the ampule is round with a long and stretched peduncle; in *Pagurus bernherdus* (Mouchet, 1931) and *Pagurus novae-zealandia* (Greenwood, 1972), the ampule is very much elongated and connected to the gelatinous matrix by a shortened peduncle, whereas in *Dardanus asper* (Matthews, 1953) and *Dardanus punctulatus* (Matthews, 1956 a) ampules are small with a long peduncle. The spermatophores of *Coenobito rugosus* and *Birgus latro* have stumpy and short peduncle. Unlike other hermit crabs, in the sand crab *Hippa pacifica,* the spermatophore is in the form of a long convoluted tube

raised by a continuous ribbon like stalk, attached to a broad foot (Matthews, 1956 b). In another hippid mole crab *Emerita asiatica*, two types of spermatophores with their peduncles connected a common base have been described by Subramonian (1977). A similar type of continuous spermatophore ribbon has also been found in *E. talpoida* and *E. analoga* (Wharton, 1942; Macginite and Macginite, 1949 respectively). In *Albunea symnista*, the spermatophore is non-pedunculate, and comprised of a highly convoluted tube which is embedded in a gelatinous matrix (Subramoniam, 1984). Such a diversity in the morphology of spermatophores among different anomuran crabs is further magnified in the present study on the spermatophores of the hermit crab *Clibanarius longitarsus*. Here, the spermatophores are kidney-shaped and arranged eitherwise of a gelatinous connecting cord which is further embedded in a gelatinous matrix. A similar condition also exists in *C. olivaceous* (Rathnavathy, 1941). The spermatophores specifically lack a peduncle with the ampules directly attached to the common gelatinous cord. The free inner end of this process gives radiating filamental structures inside the ampule. Possibly, they may aid in the transport of materials from outside into the spermatophores where the maturational changes of sperm occur. The anomurans are apparently a heterogeneous group which has both pedunculate and non-pedunculate spermatophores. To bring out the final shape of spermatophore, the basic structure of the different regions of vas deferens undergo stepwise histological changes, as revealed in the present study.

In almost all anomurans, the cuboidal cells of the proximal portion of PVD become columnar in its distal portion. In *Clibanarius longitarsus*, the formation of oval shaped lumen occurs as in *Dardanus asper* (Matthews, 1953) and *Pagurus novae-zealandia* (Greenwood, 1972). The substance secreted by the columnar cells of the ventral side condenses into a cord which connects the ampule, whereas in *Dardanus asper*, there is specific stalk forming material which is seen in the closing arches of the epithelial cells (Matthews, 1953) and in *P. novae-zealandia*, the tongue of the sperm sheath projects into the lower groove to assume an inverted T shape. However, in *C. longitarsus* the gelatinous cord initially originates from the epithelial cells in the ventral groove; later several peduncles originate from all regions of the vas deferens in the DVD region. The peduncles are eventually detached from the epithelial wall and are interconnected to give a continuous cord. Then there is a shortening of the cord, possibly due to its internalisation into the ampule.

Structural specificity of spermatophores in different groups of decapod crustaceans may tempt one to interpret these features on phylogenetic basis. However, it is more instructive to relate the structure of spermatophores with reference to the mode of fertilization in the species concerned. For instance, almost all the brachyuran forms in which the spermatophores exist as vesicles without any peduncles or gelatinous matrix, have well defined intromittant organ to transfer them to the female gonopore where they would be stored in the spermatheca for internal fertilization. On the contrary, in the majority of anomurans the spermatophoric deposition as well as the fertilization occur externally. Here, an intromittant organ is normally lacking; and therefore, a direct spermatophore transfer may entail wastage of the male gametes if they are transported as individual vesicular type of spermatophores, as in the case of brachyurans. The development of peduncle, a connecting cord and an embedding sticky gelatinous matrix favours quick fastening of these structures to the ventral surface of the female, from where they will either fertilize directly (*Emerita asiatica*, Subramoniam, 1977) or may slide into the oviduct, to effect internal fertilization as in *Clibanarius olivaceous* (Kamalaveni, 1947).

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PLATE II. Male reproductive system of *Scylla serrata*: A. Showing the spermatophoric layer (SL). Note the accumulation of granular substance (B) in between the spermatophores stained in Mallory's triple. B. T. S. of MVD, showing thin epithelial layer (EL); lumen is filled with a viscid agranular substance (C) stained in heamatoxylin-eosin. C. T. S. of DVD, showing the epithelial lining (EL). Lumen is filled with a viscid agranular substance (C) stained in haematoxylin-eosin. D. T. S. of ED showing thick muscular layer (ML). The section (D) is similar to that of DVD stained in haematoxylin-eosin. E. Male reproductive system of *Clibanarius longitarsus*: Testes (T). Proximal vas deferens (PVD), Middle vas deferens (MVD) and Distal vas deferens (DVD). F. T. S. of the proximal portion of PVD showing cuboidal epithelial cells (EC) and free sperm (S) in the lumen stained in haematoxylin-eosin and G. T. S. of the distal portion of PVD showing typhlosole (TS) on the ventral side stained in haematoxylin-eosin.

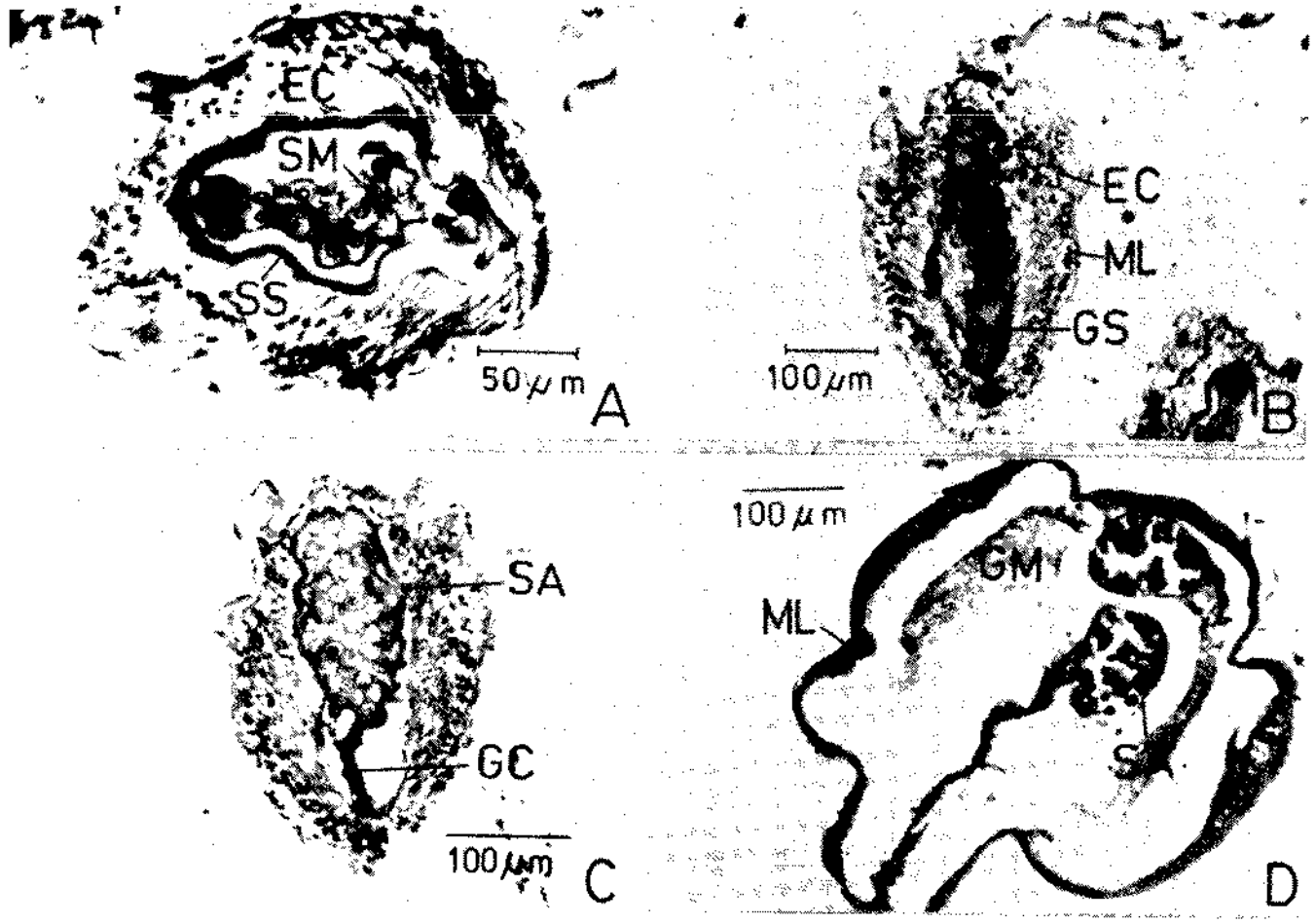

PLATE III Male reproductive system of *C. longitarsus*: A. T. S. of proximal portion of MVD showing the epithelial cells (EC) on the lateral sides with the secretion forming sperm sheath (SS) around the sperm mass (SM) stained in haematoxylin-eosin. B. T. S. of distal portion of MVD showings deposition of gelatinous substance (GS) on the ventral groove of the lumen. Note the thin muscular layer (ML), the tall columnar epithelial cell (EC) and spermatozoa (S) in the lumen; stained in haematoxylin-eosin. C. T. S. of MVD showing the formation of gelatinous cord (GC) from the deposited substance and it is connecting the spermatophoric ampule (SA) stained in haematoxylin-eosin and D. T. S. of DVD showing the gelatinous matrix (GM) in the lumen and the changing shape of the spermatophoric ampule (SA). Note thick muscular layer (ML) stained in haematoxylin-eosin.

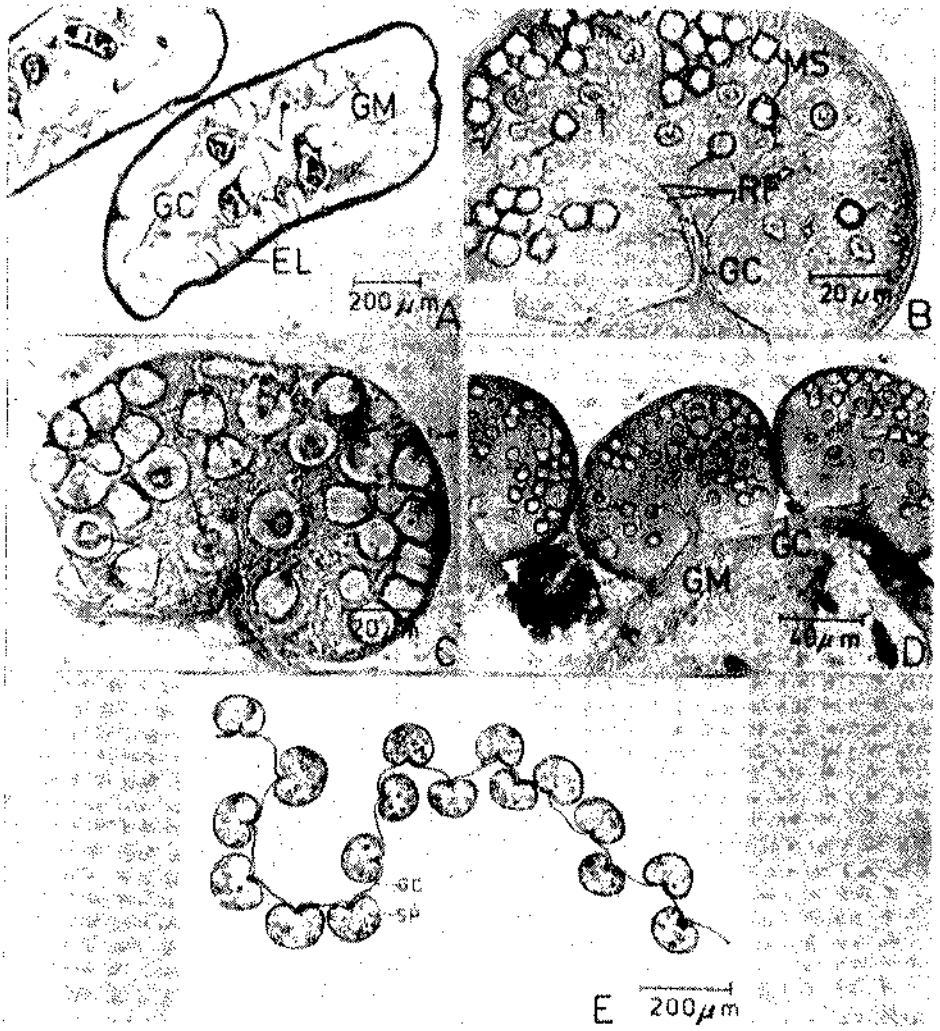

PLATE IV. Male reproductive system of *Clibanarius longitarsus*. A. Sagittal section of the coiled portion of DVD showing the detachment of gelatinous cord (GE) from the base and note thin epithelial layer (EL) and gelatinous matrix (GM) stained in Mallory's triple. B. Spermatophoric ampule showing the various stages in the final maturation of the spermatozoa (arrow) and mature sperm (MS). Note the radiating filaments (RF) from the internalised gelatinous cord (GC) at the base of the spermatophoric ampule. C. Spermatophoric ampule showing the various stages in the final maturation of the spermatozoa (arrow). D. Higher magnification of the spermatophores to show the common connecting gelatinous cord (GC), embedded on the gelatinous matrix (GM) and E. Diagrammatic representation of the arrangement of spermatophores (SP) on either side of the gelatinous cord (GC).