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UNUSUAL TESTICULAR LOBE SYSTEM IN THE WHITE SHRIMPS, *PENAEUS SETIFERUS* (LINNAEUS, 1761) AND *P. VANNAMEI* BOONE, 1931 (DECAPODA, PENAEIDAE); A NEW CHARACTER FOR DENDROBRANCHIATA?

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RÉSUMÉ

Contrairement aux testicules de la plupart des Crustacés Décapodes, ceux de deux espèces de crevettes pénéides, *Penaeus setiferus* et *P. vannamei*, sont composés de seize lobes indépendants, chacun d'entre eux étant constitué par un tubule séminifère très replié sur lui-même. Les lobes testiculaires sont reliés un par un au canal déférent antérieur proximal grâce à un canal collecteur court et fin. Le canal déférent antérieur proximal a la forme d'un "U" renversé et passe à la base de chaque lobe testiculaire. Ces observations suggèrent que ce système de testicules composés de multiples lobes indépendants pourrait être une caractéristique nouvelle du sous-ordre des Dendrobranchiata.

Des observations histologiques indiquent que la population de spermatogonies (zone germinale) se trouve toujours à la périphérie du tubule séminifère. Ces tubules séminifères apparaissent soit dans une phase pleine, dite "en corde", composée de cellules germinales (spermatocytes ou spermatides jeunes ou vieux) et de cellules sustentatrices soit dans une phase tubulaire dite "à lumen" composée de spermatides vieux, soit encore comme une phase intermédiaire entre les deux précédentes. Ainsi les deux phases, "en corde" et "à lumen" semblent être réversibles, les cellules sustentatrices s'organisant en matrice pour former la lumière (épithéliation) et se désorganisant pour la supprimer (de-épithéliation).

INTRODUCTION

Testes of decapod crustaceans have generally been described as single lobed bilaterally paired structure: in Caridea (Chow et al., 1982), in Astacura (Matthews, 1954), in Palinura (Matthews, 1951), in Anomura (Fasten, 1917) and in Brachyura (Fasten, 1917). Similarly, King (1948) described the gross morphology of testes of *Penaeus setiferus* (Linnaeus, 1761) as a paired structure. His diagrams and descriptions indicate that each testis has several lobular projec-

tions which unite with one another at the main trunk, and that sperm are passed only from the last testicular lobe to the proximal vas deferens. Similar descriptions on testicular morphology of penaeid shrimps can be seen in Heldt (1938), Eldred (1958), Sybrahmanyam (1965), Malek & Bawab (1974), Motoh (1979), Huq (1981), Chen & Qui (1986) and Champion (1987). However, these studies did not pay much attention to the distinct testicular morphology of this animal group, which none of the other decapod crustaceans possess.

This paper presents anatomical and histological evidences on multiple independent testicular lobe system and observations on cellular dynamics in the seminiferous tubule in the American white shrimps, *Penaeus setiferus* (Linnaeus, 1761) and *P. vannamei* Boone, 1931.

MATERIALS AND METHODS

Mature males (25 to 30 g) of *Penaeus setiferus* (Linnaeus, 1761) were collected by trawl in Charleston Harbor from April to September, 1988 and 1989. Mature males (20 to 30 g) of *Penaeus vannamei* Boone, 1931 were obtained from rearing ponds at the Waddell Mariculture Center near Bluffton, South Carolina. Testes and vasa deferentia were dissected from live shrimps. Tissues were fixed in 5% glutaraldehyde-0.07M sodium cacodylate in artificial sea water (pH 7.4) for 4 to 12 hrs and rinsed in 0.1M sodium cacodylate buffer (pH 7.4) containing 7% sucrose for a minimum of one day. These tissues were dehydrated by alcohol or acetone and embedded in methacrylate or Spurr, or critical point dried for scanning electron microscope observation. Thick methacrylate sections (0.5-1.0 μm) were stained with chromotrope 2R/methylene blue (C2R/MB) (Dougherty & King, 1984), and thick Spurr sections were stained with 1% toluidine blue. Thin Spurr sections were stained with uranyl acetate and lead citrate for transmission electron microscope observation.

RESULTS

Anatomical Observation on the Male Reproductive Tract

The testes of *P. setiferus* and *P. vannamei* were located dorsal to the hepatopancreas anteriorly, and ventral to the heart posteriorly. Careful dissection suggested that the testes of both species consisted of 16 independent testicular lobes (figs. 1, 3 and 4). A thin tube lay along the bases of each testicular lobe forming an inverted "U", with the opening of the "U" directed posteriorly. We defined this U-shaped thin tube as anterior proximal vas deferens (APV) (figs. 2-6). The distal portion of the anterior proximal vas deferens, beyond the connection with the last testicular lobe, became moderately thick in diameter and was defined as posterior proximal vas deferens (PPV) (figs. 1, 2, 3 and 6). In both species, the last two pairs of the testicular lobes (7th and 8th) appeared to connect to the anterior proximal vas deferens through a thin short

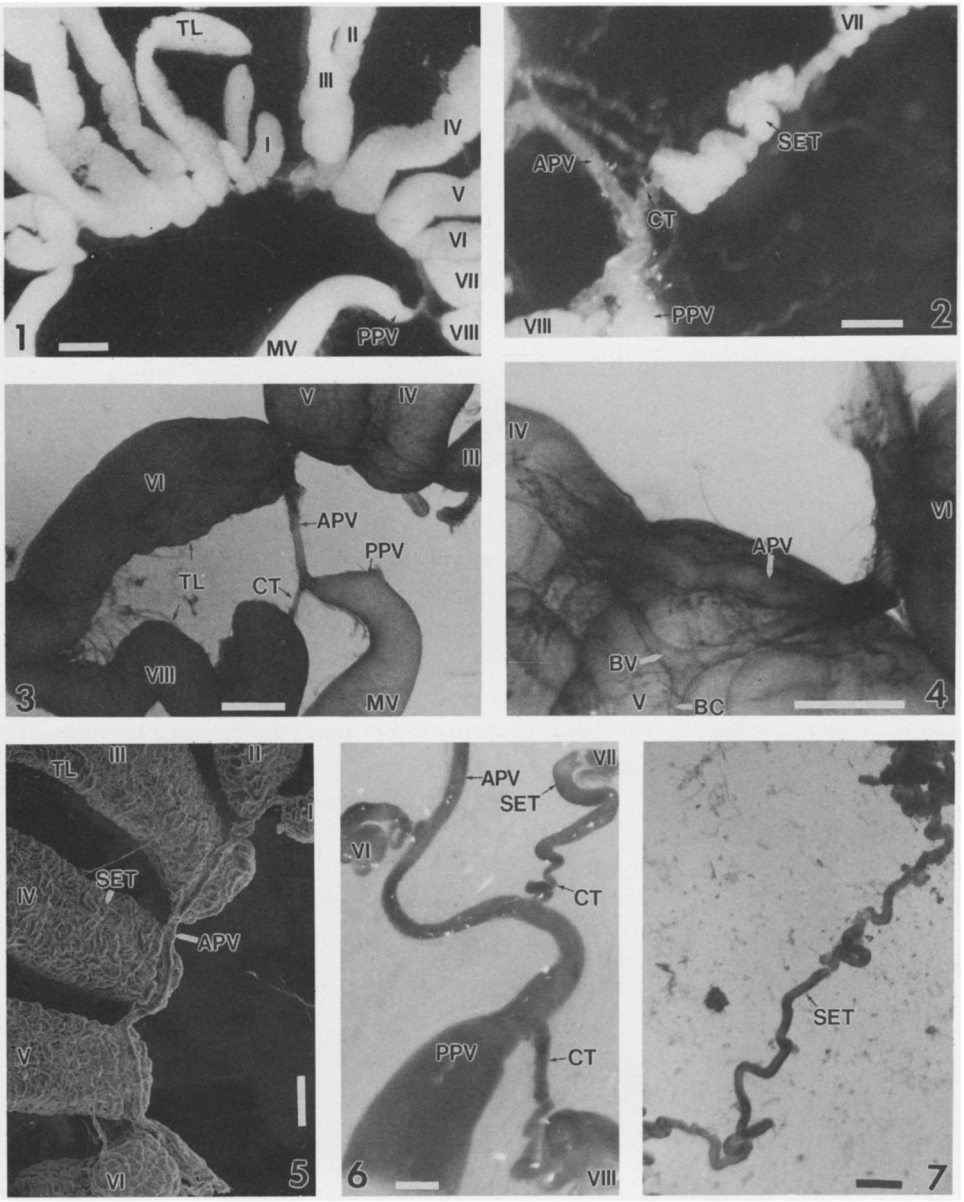

Fig. 1-7. Testicular structure and association with proximal vas deferens in *Penaeus setiferus* and *P. vannamei*. Fig. 1. Overview of entire testis and proximal vas deferens of *P. vannamei*. Note 16 independent testicular lobes (I-VIII on each side). MV, medial vas deferens; PPV, posterior proximal vas deferens; TL, testicular lobe. Bar = 1 mm. Fig. 2. Higher magnification of last two testicular lobes (VII and VIII) of *P. vannamei*. Distal portion of 7th testicular lobe (VII) stretched to show highly convoluted single seminiferous tubule (SET) connecting to anterior proximal vas deferens (APV) through thin, short collecting tube (CT). Bar = 0.5 mm. Fig. 3. Overview of 3rd to 8th testicular lobes (TL) (III-VIII) and association with proximal vas deferens of *P. setiferus*. 7th testicular lobe missing. 3rd to 6th lobes (III-VI) tightly attached on

tube (figs. 1, 2, 3 and 6). This tube was defined as a collecting tube (CT). The other testicular lobes (1st to 6th) seemed to attach directly to the anterior proximal vas deferens (figs. 1, 3, 4 and 5), but careful dissection indicated that all of the testicular lobes connected to the anterior proximal vas deferens in the same manner (figs. 2, 6). A blood vessel (BV) was observed running parallel to the anterior proximal vas deferens. A branch of the blood vessel projected at each testicular lobe and extended along and beneath or into each testicular lobe (fig. 4). Each testicular lobe appeared to consist of a highly convoluted seminiferous tubule (ST) (figs. 2, 4, 5, 6 and 7). It was nearly impossible to stretch the entire testicular lobe, but many portions which were unraveled suggested that the testicular lobe consisted of a single seminiferous tubule (figs. 2 and 7).

Fig. 8 illustrates whole male reproductive system. As in Malek & Bawab (1974), we defined the elbow shaped portion (''blind pouch'' by their description) of the proximal vas deferens as its end point (asterisk in fig. 8). The medial vas deferens was quite thick and it was distinguished into ascending (AMV) and descending (DMV) segments with a kink in between. A hyaline line (HL) can be observed along the entire length of the medial vas deferens. The distal vas deferens (DV) was a thin tube descending along the wall of the body cavity, and emptying into the bulky terminal ampulla (TA). The terminal ampullae of the two species were quite different in appearance. *P. vannamei* terminal ampulla was pear-shaped (fig. 8, on the left). Eight to twelve smooth, whitish ducts (WD) were characteristic. *P. setiferus* terminal ampulla was more bulky and cylindrical (fig. 8, on the right). The whitish ducts were not smooth, and they occupied a much larger area of the terminal ampulla than in *P. vannamei.*

Histological Observations

Association between seminiferous tubule and anterior proximal vas deferens

Thick sections through the long axis of the testicular lobes, including a transverse plane of the anterior proximal vas deferens, were made using

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anterior proximal vas deferens (APV), whereas last lobe (VIII) connects to APV through distinct collecting tube (CT). MV, medial vas deferens; PPV, posterior proximal vas deferens. Bar = 1 mm. Fig. 4. Higher magnification of base of 4th to 6th testicular lobes (IV-VI) of *P. setiferus.* Each lobe tightly attaches to anterior proximal vas deferens (APV). Blood vessel (BV) projects branch (BC) which extends beneath or into testicular lobe. Bar = 0.5 mm. Fig. 5. Scanning electron micrograph showing independent attachment of 1st to 6th testicular lobes (I-VI) to anterior proximal vas deferent (APV) of *P. setiferus.* Testicular lobes (TL) consist of highly convoluted seminiferous tubule (SET). Bar = 1 mm. Fig. 6. Higher magnification of last three testicular lobes (VI-VIII) of *P. setiferus.* Seminiferous tubules (SET) stretched to show identical connection to anterior proximal vas deferens (APV) through thin short collecting tube (CT). Bar = 0.5 mm. Fig. 7. Part of seminiferous tubule (SET) of *P. setiferus* stretched to show single tubular structure. Bar = 1 mm.

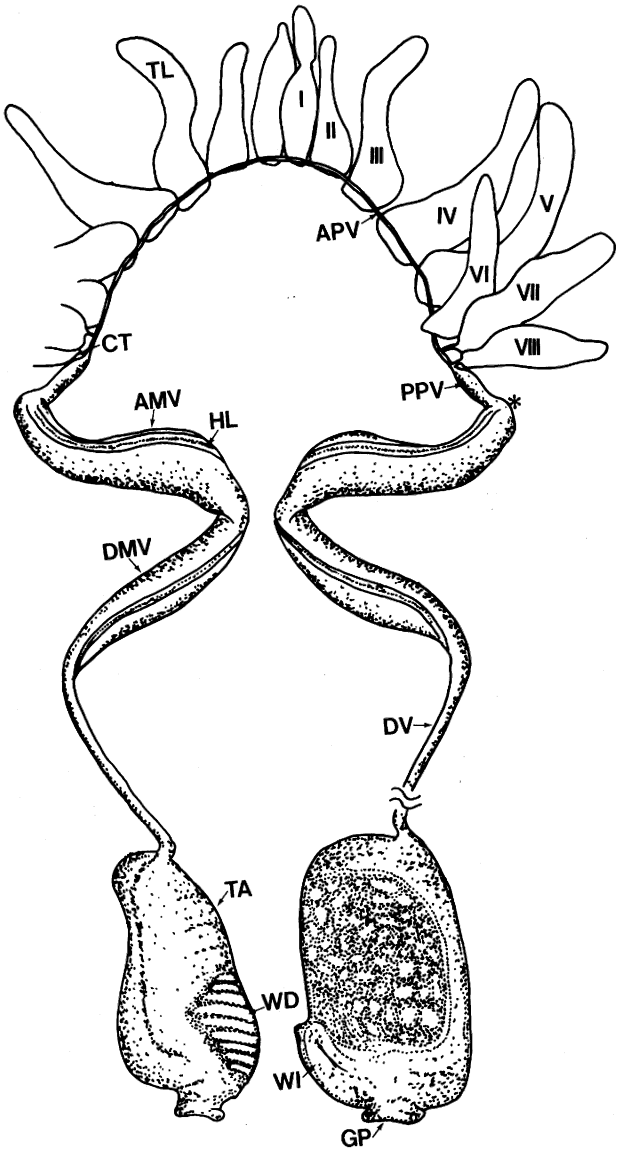


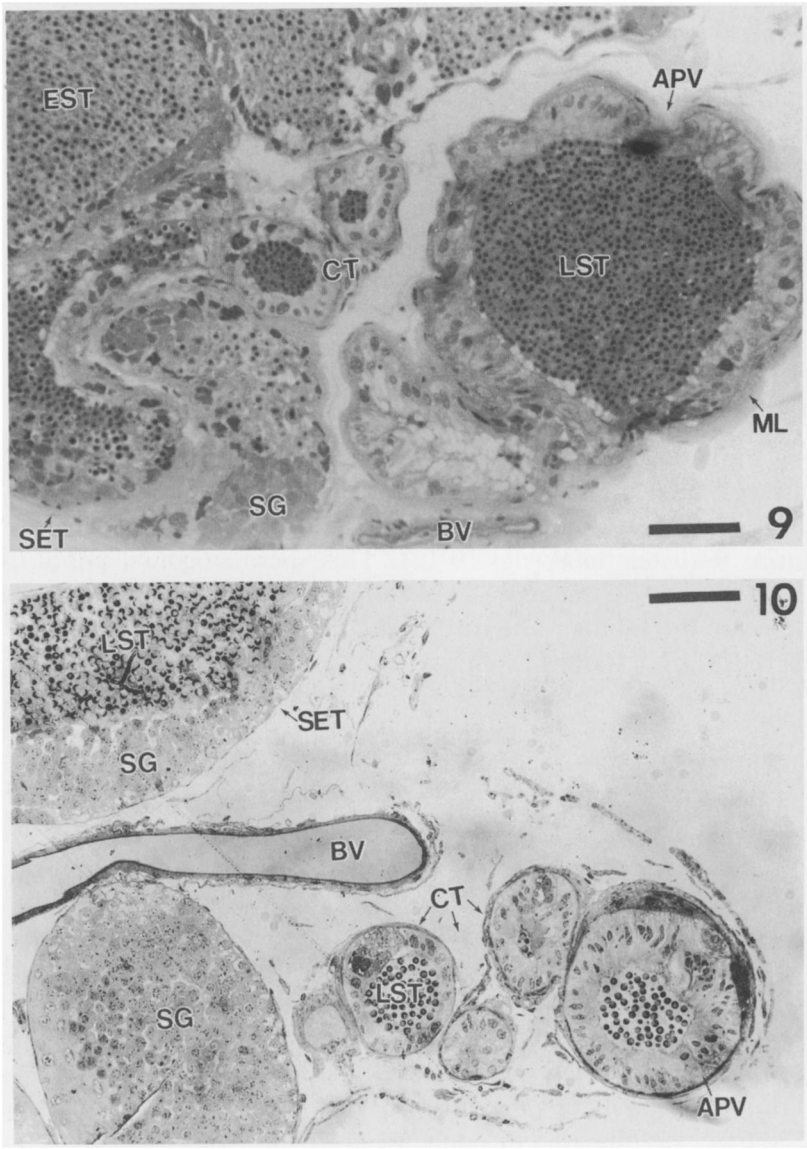
Fig. 8. Diagrammatic illustration of male reproductive system. Thin and long "U" shaped anterior proximal vas deferens (APV) lies along base of each testicular lobe (TL). Posterior proximal vas deferens (PPV) is short, but much thicker than APV. Medial vas deferens divided into ascending (AMV) and descending (DMV) segments, putting a kink between them. Thin distal vas deferens (DV) empties to large terminal ampulla (TA) which is pear-shaped in *P. vannamei* (left) and bulky and cylindrical in *P. setiferus* (right). CT, collecting tubule; GP, gonopore; HL, hyaline line; WD, whitish duct; WI, wing.

several testicular lobes from three individuals of each species (figs. 9 and 10). Essentially the same connecting system between the lobe and anterior proximal vas deferens was found among testicular lobes and between species. The highly convoluted seminiferous tubule (ST) continued to the collecting tube (CT) which, in turn, joined the anterior proximal vas deferens (APV). While the collecting tubes of the 1st to 6th testicular lobes were convoluted and folded between the lobe and vas, those of the 7th and 8th lobes were somehow stretched and unfolded. The blood vessel (BV) appeared to be closely associated with the seminiferous tubule and the anterior proximal vas deferens. These results support the anatomical observations and confirm the independent nature between the testicular lobes.

Seminiferous tubule

In six individuals of *P. vannamei* and five of *P. setiferus* examined, transverse sections indicated that spermatogonia (SG) were always observed to form a nested population in a peripheral region of the seminiferous tubule throughout the entire testicular lobe (figs. 9-12). This spermatogonial population was assumed to be a germinal zone. The seminiferous tubule exhibited two distinct assemblies of the tubular structure containing the spermatogenic and sustentacular cells (figs. 11 and 12). In one arrangement or phase, the seminiferous tubule appeared as a solid "cord" in which spermatogenic cells (spermatocytes, or early or late spermatids) were supported by the sustentacular cells (STC) (fig. 11A and B). In the other phase, the late spermatids (LST) were suspended in a lumen of the seminiferous tubule (fig. 12A, B and C). An incomplete lumen (fig. 12A) seemed to be a transition phase between the "cord" and "lumen" phases.

In both species, variations of the tubular phase and the spermatogenic cell stage were observed between regions within a testicular lobe and between lobes in an individual. Three individuals of *P. vannamei* exhibited both "cord" and "lumen" phases in one testicular lobe. In these three individuals, observations through the entire lobe indicated that two individuals possessed several spermatogenic cell stages from the spermatocytes to late spermatids and one possessed only late spermatids. Of the other three individuals in which only the "cord" phase was observed through the entire lobe, two individuals possessed spermatocytes and early spermatids and the other possessed only spermatocytes. Of the two individuals of *P. setiferus* in which the "cord" phase was observed, one possessed only late spermatids, while the other possessed spermatocytes and early and late spermatids. The other two individuals of *P. setiferus* had the "lumen" phase through the entire testicular lobe possessing only late spermatids. In the other individual of this species, the proximal region of one lobe showed the "cord" phase and exhibited only spermatocytes. The distal region of this lobe possessed a very small lumen (fig. 12C, asterisk) with a small number of late spermatids, although a large portion of the



Figs. 9, 10. Thick sections of distal portion of testicular lobes, including transverse plane of anterior proximal vas deferens (APV). Both *P. vannamei* (Fig. 9; 6th lobe) and *P. setiferus* (Fig. 10; 3rd lobe) show spermatogonia (SG) at periphery of seminiferous tubule (SET). Highly convoluted and folded collecting tube (CT) shown in close association with testicular lobe (TL) and blood vessels (BV). EST, early spermatids; LST, late spermatids. Bars = 50 μm.

seminiferous tubule was occupied by spermatocytes (SC) (fig. 12C). In both species, only the late spermatids appeared in the "lumen".

Cross sections of testicular lobes showed that the spermatogenic cell stage in one region was usually synchronized, but a different region may contain another spermatogenic cell stage. The variation of the spermatogenic cell stage between regions within one testicular lobe was more commonly observed in *P. vannamei* than in *P. setiferus*.

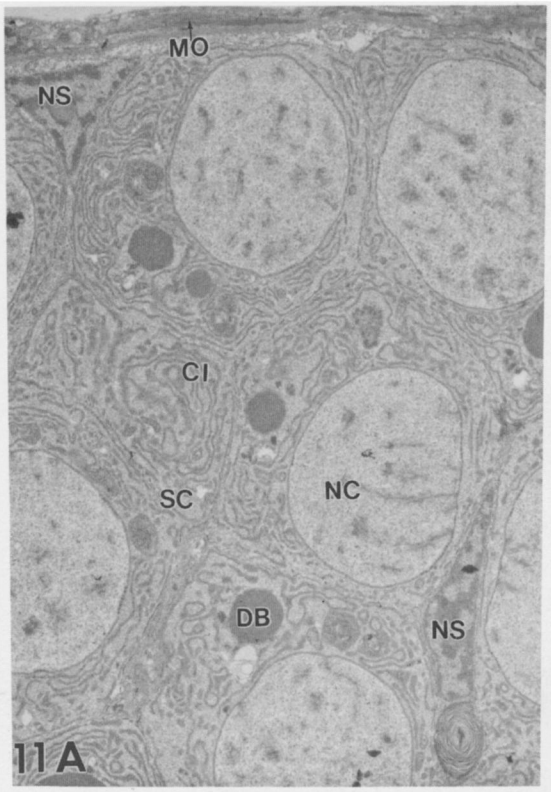
TEM observations indicated that the sustentacular cells supporting the late spermatids in the "cord" phase contained numerous mitochondria (MT), smooth (SER) and rough (RER) endoplasmic reticulum, somewhat irregularly shaped large nuclei (N), and well developed Golgi Body (GB) (fig. 13). A flocculent matrix (FM) was sometimes observed to fill the narrow space between the late spermatids, and the presence of a number of pits in the plasma membranes suggested secretion of the matrix material (fig. 13, arrow). In the "lumen" phase, late spermatids were suspended in the flocculent matrix (FM). The epithelial cells lining the lumen were very similar to the sustentacular cells but appeared to be more active in synthesis. A number of pits on the cell apices suggested exocytotic activity for secretion of the flocculent matrix (fig. 14, arrows). These observations indicated that the sustentacular cells which previously intermingled with and supported the late spermatids retreated to peripheral areas of the seminiferous tubule, thus creating an epithelial lined lumen.

The late spermatids, which have almost the same morphology as spermatozoa, but lack the spike, were the most advanced spermatogenic cell stage observed in the testis.

Collecting tube to proximal vas deferens

Spermatogenic cells observed in the collecting tube were late spermatids (LST) which possessed no spike. Transmission electron microscope observations indicated that the epithelial cells lining the collecting tubes had few microvilli and little rough endoplasmic reticulum. This suggested that this tube was mere a pathway to deliver the late spermatids from the seminiferous tubule to the anterior proximal vas deferens (fig. 15). This supports the light microscopic observations by Malek & Bawab (1974) in *P. kerathurus* (Forskål, 1775).

In contrast to the collecting tube, cell apices of the anterior proximal vas deferens demonstrated abundant microvilli (MV) (fig. 16A). The lining cells containing numerous mitochondria (MT), rough endoplasmic reticulum (RER) and many free ribosomes (FRB) (fig. 16B) appeared highly active in synthesis. In this segment, late spermatids (LST) were embedded in an electron dense sperm embedding matrix (SEM), and secretion granules (SCG) were observed. The active secretion may facilitate aggregation into a sperm mass. The fine structure of the flocculent matrix observed in the collecting tube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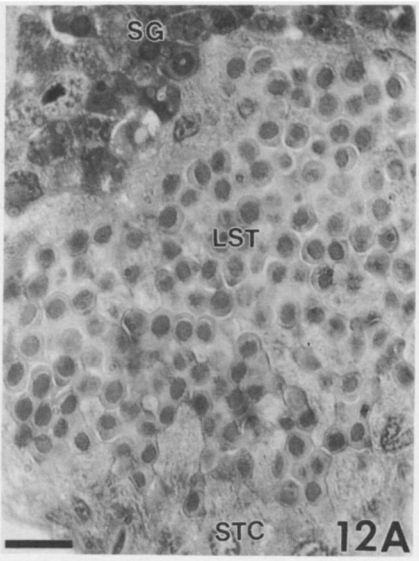
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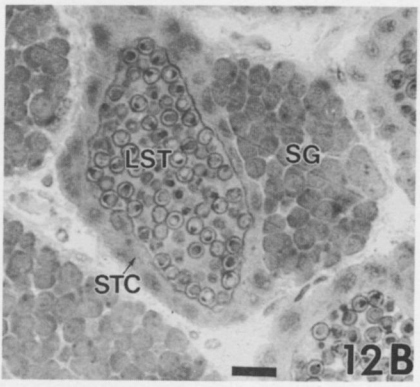
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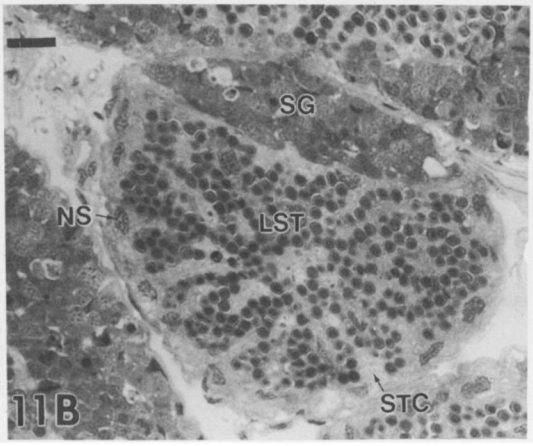
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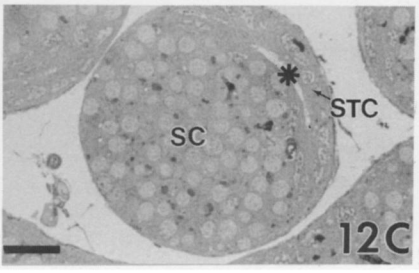
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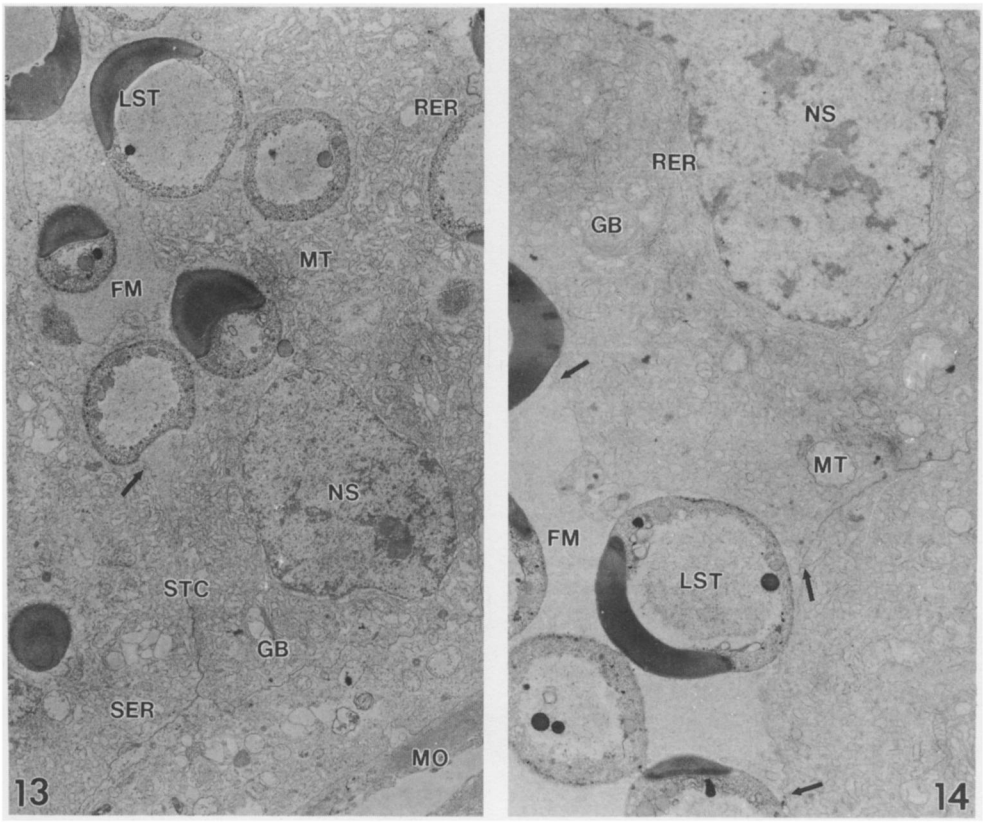
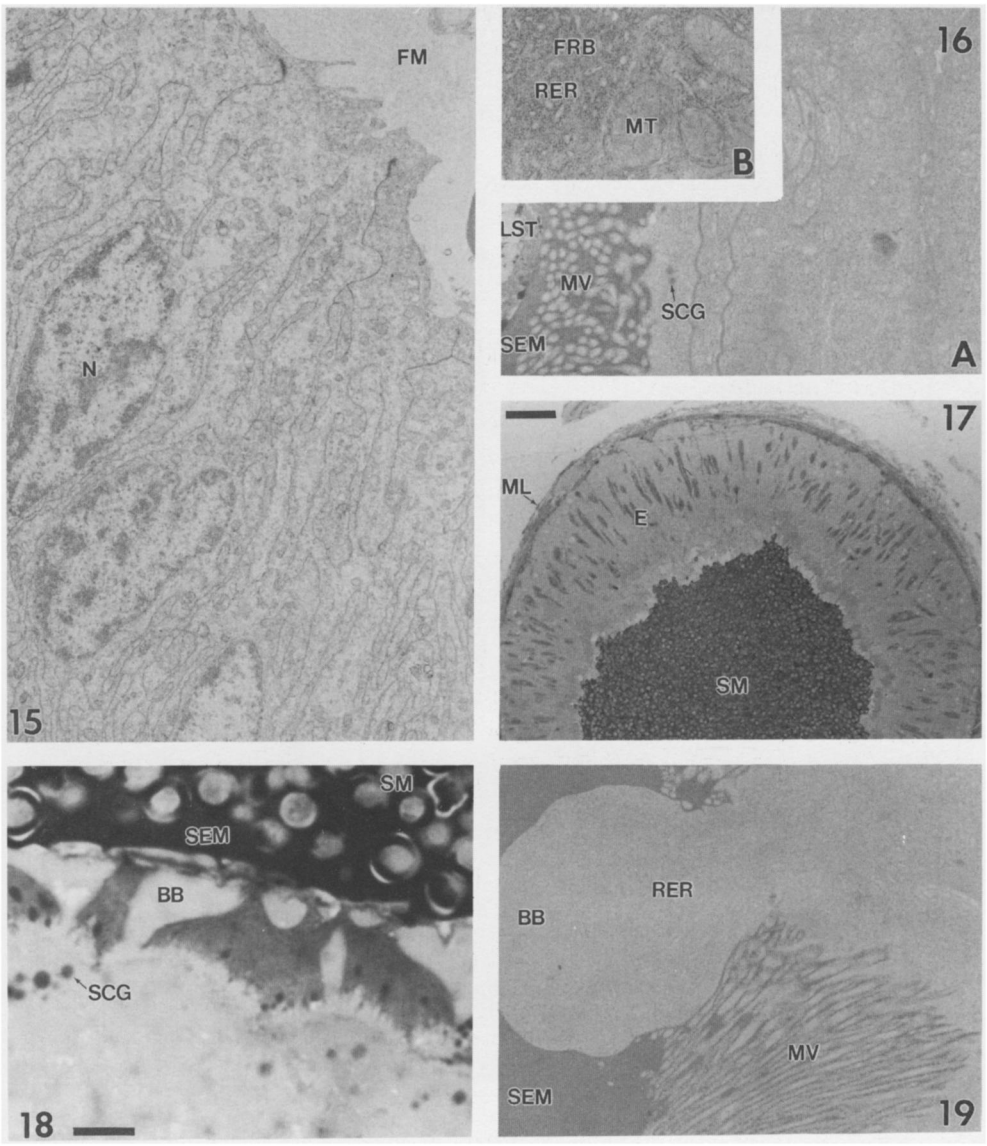


Figs. 11-14. Seminiferous tubules in "cord" and "lumen" phases. Fig. 11(A). Thin section of "cord" phase seminiferous tubule of *P. setiferus*. Primary spermatocytes (SC) tightly packed; nuclei of sustentacular cells (NS) to be observed in narrow space between primary spermatocytes. CI, cisternae; DB, dense body; MO, myoid cells; NC, nucleus of primary spermatocyte. × 10,000. Fig. 11 (B). Transverse thick section of "cord" phase seminiferous tubule of *P. vannamei*. Spermatogonia (SG) populations exist at periphery of tubule. Late spermatids (LST) supported by sustentacular cells (STC). Bar = 15 μm. Fig. 12. Transverse thick sections of "lumen" phase seminiferous tubule. Fig. 12 (A). Late spermatids (LST) suspended in incomplete lumen (*P. setiferus*). Fig. 12 (B). Late spermatids suspended in entire lumen (*P. vannamei*). Bars = 10 μm. Fig. 12 (C). Large part of seminiferous tubule occupied by primary spermatocytes (SC) and lumen (asterisk) constricted. Fig. 13. Thin section of "cord" phase seminiferous tubule of *P. setiferus*. Sustentacular cells (STC) possess irregular shaped nuclei (NS), numerous mitochondria (MT), rough (RER) and smooth (SER) endoplasmic reticulum, and Golgi body (GB). Small spaces between sustentacular cells or late spermatids (LST) filled by flocculent matrix (FM). Presence of pit (arrow) suggest exocytotic activity. MO, myoid cell. × 7,900. Fig. 14. Thin section of "lumen" phase seminiferous tubule of *P. setiferus*. Late spermatids (LST) suspended in flocculent matrix (FM). Epithelial cells quite similar to sustentacular cells of "cord" phase. Presence of pits indicates exocytotic activity (arrows). GB, Golgi body; MT, mitochondria; NS, nucleus of sustentacular cell; RER, rough endoplasmic reticulum. × 10,000.



Figs. 15-19. Fig. 15. Overview of epithelium of collecting tube of *P. setiferus*. Epithelial cells columnar and inactive in synthesis. FM, flocculent matrix; N, nucleus. × 7,200. Fig. 16. Thin sections of anterior proximal vas deferens of *P. vannamei*; (A) cell apices displaying abundant microvilli (MV), and late spermatids (LST) embedded in electron-dense sperm embedding matrix (SEM). Secretion granules (SCG) can be observed. × 17,000. (B) Higher magnification of epithelial cell showing abundant mitochondria (MT), rough endoplasmic reticulum (RER) and free ribosomes (FRB). × 27,000. Fig. 17. Thick section through initial position of posterior proximal vas deferens of *P. setiferus*. Muscle layer (ML), columnar epithelium (E) and sperm mass (SM) are shown. Bar = 50 μm. Fig. 18. Higher magnification of thick section of posterior proximal vas deferens of *P. setiferus*. Cell apices project blebs (BB). Secretion granules (SCG), sperm mass (SM) and sperm embedding matrix (SEM) shown. Bar = 5 μm. Fig. 19. Thin section through cell apex of posterior proximal vas deferens of *P. vannamei*. Abundant microvilli (MV), large bleb (BB), and numerous rough endoplasmic reticulum (RER) shown. SEM, sperm embedding matrix. × 12,000.

was quite different from the sperm embedding matrix. Coexistence of these two matrices was not observed. It is possible that the two matrices have similar properties but are of different densities, and that the minor amount of the flocculent matrix delivered through the collecting tube is entirely mixed with the sperm embedding matrix.

Most of the spermatogenic cells in the anterior proximal vas deferens were late spermatids. A few spermatozoa which possessed spikes could be observed through the anterior to posterior proximal vas deferens in both species. Therefore, spermiogenesis appears to be completed in the vas deferens. This confirms King's (1948) observations in *P. setiferus*.

As the diameter of the vas deferens increased, late spermatids and spermatozoa accumulated, forming a larger sperm mass in the lumen of the posterior proximal vas deferens (fig. 17). Secretion granules were observed in the apical cytoplasm of the epithelial cells lining this portion of the proximal vas deferens, with a number exhibiting blebs of the plasma membrane (figs. 18 and 19). Rough endoplasmic reticulum was abundant in these cells as were the blebs (fig. 19). The secreted matrix was similar to that of the anterior proximal vas deferens. Through the anterior and posterior proximal vas deferens, we did no observe irregularly distributed multicellular glands as described by Malek & Bawab (1974) in *P. kerathurus*. We observed that light micrographs of these epithelial cells showed abundant microvilli and blebs (fig. 18), sometimes giving the impression of glandular tissue like that described in Malek & Bawab (1974). Since neither King (1948) in *P. setiferus* nor Champion (1987) in *P. indicus* Milne-Edwards, 1837 observed the multicellular glands in the proximal vas deferens, we conclude that the cellular linings of the proximal vas deferens are simple secretory epithelia.

DISCUSSION

The anatomical and histological data presented in this study evidenced that the testes of *P. setiferus* and *P. vannamei* consisted of multiple, independent testicular lobes, each of which made connection with anterior proximal vas deferens by means of collecting tubes. Since we have preliminary observed that the testicular lobe systems of *Penaeus aztecus* Ives, 1981, *P. duorarum* Burkenroad, 1939, *P. japonicus* Bate, 1888 and *P. monodon* Fabricius, 1798 were essentially the same as those of *P. setiferus* and *P. vannamei*, we conclude that the independent multiple testicular lobe system is characteristic of the genus *Penaeus* Fabricius, 1798. Heldt (1938) observed multiple lobular projections of testes in some penaeid species of another genus (*Parapenaeus* Smith, 1885) and of other subfamily and family (Sicyoninae Ortmann, 1901 and Aristeidae Wood-Mason, 1891), suggesting that the multiple testicular lobe (possibly independent) is a widespread character in the suborder Dendrobranchiata Bate, 1888, but not in the other, Pleocyemata Burkenroad, 1963.

Huq (1981) reported the number of testicular lobes in six *Penaeus* species. He found that smaller individuals tended to possess fewer lobes than larger individuals, and that numbers of lobes were different between left and right sides, and between species. Intraspecific variation in the number of testicular lobes of *P. setiferus* and *P. vannamei* should not be ruled out. A few observations for small males of *P. setiferus* indicated a variation in the number of lobes (unpublished).

Histological observations on the testes in the present study coincide with those of King (1948) and Talbot et al. (1989), who reported that one testicular lobe of *P. setiferus* may contain several stages of spermatogenic cells. Lu et al. (1973) reported that spermatogenic cell stages of all testicular lobes in one individual of *P. setiferus* were synchronized. Talbot et al. (1989) suggested that the captive condition may interfere with spermatogenic synchronism in one testicular lobe of *P. setiferus*. This might be true to some extent, because pond-reared *P. vannamei* showed much more variation in spermatogenic cell stages between regions within one testicular lobe than wild *P. setiferus*.

Based on histological observations, we suggest the following cycle of structural changes in the semiferous tubule accompanying development of the spermatogenic cells: (1) production of primary spermatocytes from the spermatogonial population (germinal zone), (2) spermatogenesis in the "cord" phase up to late spermatids, (3) retreat and lining of the sustentacular cells to peripheral areas to form a lumen ("epitheliation"), (4) transferring and emptying late spermatids (or sperm) to proximal vas deferens, and (5) contraction and disappearance of the lumen ("de-epitheliation") with resumption of the initial phase (1). If this cycle is correct, the sustentacular cells must change in morphology and in function by repeated epitheliation and de-epitheliation. Similar epitheliation of sustentacular, accessory, or nutritive cells to form a lumen has been observed in some brachyuran crabs (Ryan, 1967; Kon & Honma, 1970; Chiba & Honma, 1971). Ryan (1967) also noticed that Cronin's (1947) cross sections of the blue crab (*Callinectes sapidus* Rathbun, 1896) testis showed incomplete epitheliation, although Cronin did not mention this. Matthews (1954), Haley (1984) and Hinsch (1988) reported testes composed of a convoluted collecting tube which was arrayed with multiple follicles, in the red lobster *Enoplometopus occidentalis* (Randall, 1840) and golden crab *Geryon fenneri* Manning & Holthuis, 1984, respectively. But Haley (1984) mentioned that the follicles were not independent of each other, and he also observed the formation of a lumen by accessory cells which came to line the lumen. These follicles may be homologous to the lobules described in the blue crab by Cronin (1947) and Johnson (1980), and ultimately to the nested population of spermatogonia (germinal zone) observed in the present study. Nutritive, accessory, supportive or sustentacullar cells reported for decapod testes might be different phases of a kind of acinar cell having flexibility for morphological and functional change during spermatogenesis. Thus, the gross morphology of testis may

essentially differ between the suborders of Decapoda, whereas the functional structure of the seminiferous tubule seems to be common.

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REFERENCES

CHAMPION, H. F. B., 1987. The functional anatomy of the male reproductive system in Penaeus indicus. South Afr. J. Zool., **22**: 297-307.

CHEN, O. & W. QUI, 1986. On structure and development of the male reproductive system of the Chinese prawn Penaeus orientalis. Acta Zool. Sinica, **32**: 255-259.

CHIBA, A. & Y. HONMA, 1971. Studies on gonad maturity in some marine invertebrates-II. Structure of the reproductive organs of the lined shore crab. Bull. Japanese Soc. Sci. Fish., **37**: 699-706.

CHOW, S., Y. OGASAWARA & Y. TAKI, 1982. Male reproductive system and fertilization of the palaemonid shrimp, Macrobrachium rosenbergii. Bull. Japanese Soc. Sci. Fish., **48**: 177-183.

CRONIN, L. E., 1947. Anatomy and histology of the male reproductive system of Callinectes sapidus Rathbun. Journ. Morph., **81**: 209-239.

DOUGHERTY, M. M. & J. S. KING, 1984. A simple rapid staining procedure for methacrylate-embedded tissue sections using chromotrope2R and methylene blue. Stain Tech., **59**: 149-153.

ELDRED, B., 1958. Observations on the structural development of the genitalia and the impregnation of the pink shrimp, Penaeus duorarum Burkenroad. Flr. St. Board Conserv., Technical Series, **23**: 26 pp.

FASTEN, N., 1971. Male reproductive organs of Decapoda, with special reference to Puget Sound forms. Puget Sound Mar. St. Pub., **1**: 285-307.

HALEY, S. R., 1984. Spermatogenesis and spermatophore production in the Hawaiian red lobster Enoplometopus occidentalis (Randall) (Crustacea, Nephropidae). Journ. Morph., **180**: 181-193.

HELDT, J. H., 1938. La reproduction chez les crustacés décapodes de la famille des Pénéides. Ann. Inst. Océanogr., **18**: 31-206.

HINSCH, G. W., 1988. Morphology of the reproductive tract and seasonality of reproduction in the golden crab Geryon fenneri from the eastern Gulf of Mexico. Journ. Crust. Biol., **8**: 254-261.

HUQ, A., 1981. Reproductive system of a species of Penaeus (Decapoda). Bangladesh J. Zool., **8**: 81-88.

JOHNSON, P. T., 1980. Histology of the blue crab, Callinectes sapidus: a model for the Decapoda: i-xv, 1-440, text-figs. (Praeger Publishers, New York).

KING, J. L., 1948. A study of the reproductive organs of the common marine shrimp, Penaeus setiferus (Linnaeus). Biol. Bull., mar. biol. Lab. Woods Hole, **94**: 244-262.

KON, T. & Y. HONMA, 1970. Studies on the maturity of the gonad in some marine invertebrates-IV. Seasonal changes in the testes of the tanner crab. Bull. Japanese Soc. Fish., **36**: 1028-1033.

LU, C. C., W. H. CLARK & L. E. FRANKLIN, 1973. Spermatogenesis of the decapod Penaeus setiferus. J. Cell Biol., **59**: 202a.

MALEK, S. R. A. & F. M. BAWAB, 1974. The formation of the spermatophore in Penaeus kerathurus (Forskål, 1775) (Decapoda, Penaeidae). I. The initial formation of the sperm mass. Crustaceana, **26**: 273-285.

MATTHEWS, D. C., 1951. The origin, development and nature of the spermatophoric mass of the spiny lobster Panulirus penicillatus (Oliver). Pacif. Sci., **5**: 359-371.

——, 1954. The origin and development of the spermatophoric mass of a nephropsid lobster, Enoplometopus occidentalis (Randall). Pacif. Sci., **8**: 115-120.

MOTOH, H., 1979. Preliminary study on the internal genital organ of the giant tiger prawn, Penaeus monodon. Quart. Res. Rep. Aquacult. Department, SEAFDEC, **3**: 15-18.

RYAN, E. P., 1967. Structure and function of the reproductive system of the crab Portunus sanguinolentus (Herbst) (Brachyura: Portunidae). I. The male system. Proc. Symp. Crustacea, Ernakulam, **3**: 506-521.

SUBRAHMANYAM, C. B., 1965. On the reproductive cycle of Penaeus indicus (Milne Edwards). Journ. Mar. Biol. Ass. India, **7**: 284-290.

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