

## SPERMATOPHORE FORMATION IN THE WHITE SHRIMPS *PENAEUS SETIFERUS* AND *P. VANNAMEI*

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### ABSTRACT

The medial and distal vasa deferentia and terminal ampullae of *Penaeus setiferus* and *P. vannamei* were studied by light and electron microscopy to assess their roles in spermatophore formation. The ascending medial vas deferens of each species consisted of 2 parallel epithelium-lined ducts, referred to as the spermatophoric and accessory ducts, with the accessory duct fitting into a groove along the spermatophoric duct. In the spermatophoric duct, the sperm mass was surrounded by a thick primary spermatophore layer secreted by the epithelial lining. Two secretions forming accessory layers 1 and 2 were deposited by the epithelial cells of the accessory duct.

The lumina of the two ducts partially merged at the flexure between the ascending and descending portions of the medial vas deferens. Upon confluence, accessory layer 1 flowed into the spermatophoric duct and formed an additional layer around the primary spermatophore layer. Additional spermatophore components were deposited in the terminal ampulla, which consisted of 5 interconnecting chambers or lumina in *P. setiferus* and 4 in *P. vannamei*, respectively. The spermatophoric and accessory ducts terminated in chambers I and II, respectively. New secretions from chamber I were a thick dorsal plate and a thin adhesive layer. In chamber II, structural alteration of accessory layer 2 into "corky" reticulate and collapsed or "fibrous" reticulate portions occurred. Chamber III was a branching duct and contained glutinous material. Chamber IV seemed to be an extension of chamber II, but formed a distinct large lumen located in the proximal to medial regions of the terminal ampulla. This chamber contained a large amount of "fibrous" reticulate substance similar to that observed in chamber II. Chamber V of *P. setiferus* contained the wing portion of the spermatophore. The terminal ampulla of *P. vannamei* possessed neither this chamber nor wing material.

Upon ejaculation, each spermatophore joined mesially along the adhesive layer and formed a compound spermatophore. Accessory layer 1 and the "corky" reticulate layer were hard and functioned as a sheath for the sperm mass. These layers also supported the structure of the compound spermatophore. The dorsal plate, glutinous material, "fibrous" reticulate layer, and wing served to attach the compound spermatophore to the open thelycum.

The male reproductive tract of shrimps of the genus *Penaeus* consists of paired testes and vasa deferentia (King, 1948; Malek and Bawab, 1974a). Each vas deferens consists of four distinct regions: (1) a narrow proximal portion, (2) a thickened medial portion which tapers to form (3) a distal, relatively long narrow portion terminating in (4) a greatly dilated terminal ampulla surrounded by a thick layer of muscle. Malek and Bawab (1974a) further divided the medial vas deferens into ascending and descending portions at the flexure. They also noted that the medial vas deferens was subdivided into two independent ducts, referred to as "spermatophoric" and "wing" ducts, because of their proposed roles in the formation of spermatophore components. Whereas the envelopment of the sperm mass by layers of noncellular materials appears to be initiated in the medial vas deferens

(Malek and Bawab, 1974b), the terminal ampulla apparently functions to complete spermatophore formation (King, 1948). King (1948) described the compound spermatophore (extruded paired spermatophores) of *Penaeus setiferus* as roughly pod-like, with a pair of "wings" which anchor it to the open thelycum of the female. The posterodorsal region of the compound spermatophore is extended to form a flange or shelf which also functions in attachment. Several studies have dealt with the formation and/or structure of the spermatophore in this group (Heldt, 1938; Hudinaga, 1942; King, 1948; Eldred, 1958; Tirmizi, 1958; Subrahmanyam, 1965; Tirmizi and Khan, 1970; Malek and Bawab, 1974a, b; Farfante, 1975; Huq, 1981; Champion, 1987; Bell and Lightner, 1988; Ro *et al.*, 1988; Talbot *et al.*, 1989; Ro *et al.*, 1990). However, 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.

In this paper, we report on the structures of the vas deferens, terminal ampulla, and compound spermatophore; the process of spermatophore matrix depositions; and the role of each matrix in the compound spermatophore in the two American white shrimps *Penaeus setiferus* and *P. vannamei*.

### MATERIALS AND METHODS

Males (25–30 g) and mated females of *Penaeus setiferus* were collected by trawl in Charleston Harbor. Males (20–30 g) of *P. vannamei* were obtained from rearing ponds at the Waddell Mariculture Center near Bluffton, South Carolina. About 20 male specimens of each species were used in this investigation.

Vasa deferentia, including the terminal ampullae, were dissected from live males. The compound spermatophores were carefully stripped from the thelyca of the mated females. The procedures reported by Dougherty *et al.* (1986) were used to prepare samples for light and electron microscopy. Methacrylate and paraffin sections were stained with chromotrope 2R/methylene blue (C2R/MB) (see Dougherty and King, 1984), hematoxylin and eosin, or periodic acid Schiff reaction followed by Alcian Blue at pH 2.5 (PAS/AB).

### RESULTS

Since the gross morphology of the male reproductive tract of different species of the genus *Penaeus* is virtually the same, the terminology used in this study followed that of Malek and Bawab (1974a).

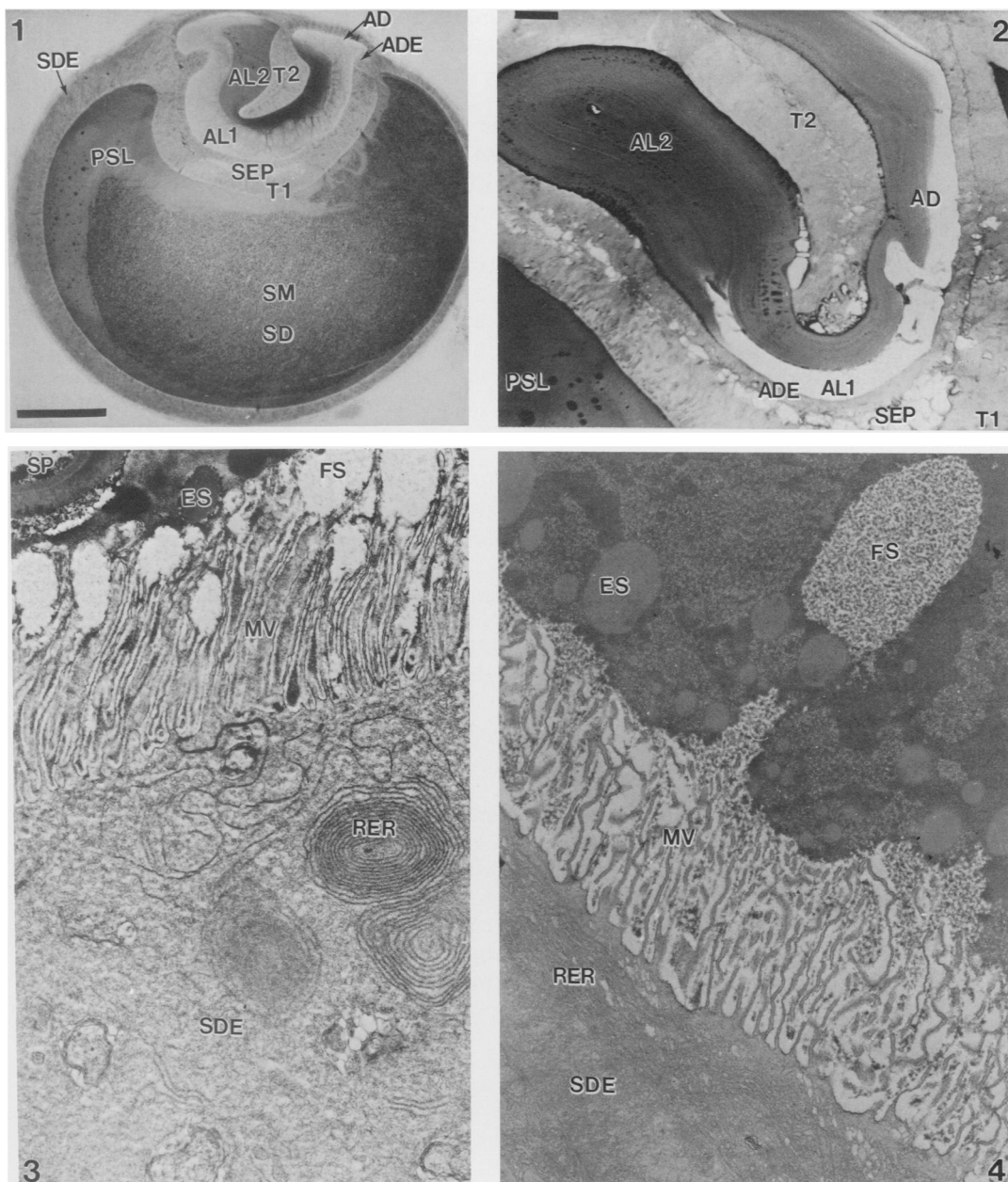
#### Medial Vas Deferens

*Ascending Portion.*—In both *P. setiferus* and *P. vannamei*, transverse sections revealed that this portion consisted of two independent ducts running parallel to one another, a larger spermatophoric duct (SD) and narrower accessory duct (AD) (Figs. 1, 2). The accessory duct was closely applied to the surface of the spermatophoric duct, fitting into a depression or groove in the wall of the spermatophoric duct. A layer of connective tissue separated the epithelia of the accessory and spermatophoric ducts where they adjoined, forming a joint septum (SEP) between the two ducts in this region. Since the depression in the wall of the spermatophoric duct is a continuation of the distinct typhlosolic invagination occurring in the proximal vas deferens and this invagination

preceded formation of the septum and accessory duct (unpublished observation), this depression will be referred to as typhlosole 1. The lumen of the spermatophoric duct was continuous with that of the proximal vas deferens, while the accessory duct originated at about the junction of the proximal and medial portions of the vas deferens. After the occurrence of the accessory duct, the epithelium also invaginated to form typhlosole 2 (unpublished observation), which was a bilayered projection of the epithelial lining subdividing the lumen of the accessory duct.

Deposition of three acellular layers was characteristic of this portion of the vas deferens. Epithelial cells of the spermatophoric duct (SD) were found to be highly active in synthesis and secretion, discharging an electron-dense substance (ES) and a flocculent substance (FS) (Figs. 3, 4). The primary spermatophore layer (PSL) was composed of these two substances which entirely surrounded the sperm mass (SM) (Figs. 1, 2). No differences were observed between the secretions of the epithelial cells of typhlosole 1 and the rest of the spermatophoric duct. No distinct boundaries were apparent between the two substances (ES and FS) composing the primary spermatophore layer (PSL).

In the accessory duct (AD), accessory layers 1 and 2 (AL1 and AL2) were deposited along the accessory duct epithelium (ADE) and around typhlosole 2 (T2), respectively (Figs. 1, 2). The primary spermatophore layer (PSL) and accessory layers (AL1 and AL2) were acidophilic. Accessory layer 1 gave a negative PAS/AB reaction, whereas PSL and AL2 gave strong PAS reactions (Fig. 2). Accessory layer 2 (AL2) appeared “frothy” and presented a laminate structure under the light microscope (Fig. 2). The fine structures of AL1 and AL2 were also different (Figs. 5, 6, 7). While AL1 appeared as a finely grained homogeneous matrix, AL2 consisted of granules or globules embedded in an electron-dense matrix (Figs. 5, 6, 7). Diameters of the granules of AL2 varied considerably (0.08–1.5  $\mu\text{m}$ ). Mixing of the two matrices was obvious at their interface (Fig. 5). Epithelial cells lining the accessory duct (AD) (Fig. 6) and typhlosole 2 (T2) (Fig. 7) appeared to be active in synthesis.



**Figs. 1-4.** Ascending medial vas deferens of penaeid shrimps. **Figs. 1, 2.** Transverse thick methacrylate sections of ascending medial vas deferens of *Penaeus setiferus* (Fig. 1, C2R/MB stain, bar = 0.1 mm) and *P. vannamei* (Fig. 2, PAS stain, bar = 0.1 mm). This portion consists of spermatophoric (SD) and accessory (AD) ducts separated by septum (SEP). Sperm mass (SM) is surrounded by thick primary spermatophore layer (PSL) secreted by spermatophoric duct epithelium (SDE) and epithelium on typhlosole 1 (T1). Depositions of two acellular matrices are observed in accessory duct. Accessory layers 1 and 2 (AL1 and AL2) are deposited along accessory duct epithelium (ADE) and around typhlosole 2 (T2), respectively. The primary spermatophore layer and accessory layer 2 are acidophilic and strongly PAS reactive. Accessory layer 1 is acidophilic but PAS negative (Fig. 2). **Figs. 3, 4.** Thin Spurr sections (Spurr, 1969). Cells of spermatophoric duct epithelium (SDE) exhibit abundant rough endoplasmic reticulum (RER) in *Penaeus setiferus* (Fig. 3,  $\times 6,450$ ) and *P. vannamei* (Fig. 4,  $\times 12,900$ ). Secretory product is observed at cell apices which possess numerous microvilli (MV). Primary spermatophore layer (PSL) consists of electron-dense substance (ES) and flocculent substance (FS).

*Descending Portion.*—The recurved portion between the ascending and descending medial vas deferens (see Malek and Bawab, 1974a), roughly coincided with detachment of one end of the septum (SEP), causing confluence of the lumina of spermatophoric (SD) and accessory (AD) ducts (Figs. 8, 9). The resultant detached septum, which now takes the form of a typhlosole partially separating the two lumina, is referred to as typhlosole 1. It appeared that the accessory layer 1 (AL1) began to flow into the spermatophoric duct (SD) through the confluence (Fig. 8) and formed a thin layer around the primary spermatophore layer (PSL) (Fig. 9). As accessory layer 1 penetrated between the primary spermatophore layer and the spermatophoric duct epithelium (SDE), the formation of a new, differently stained layer was observed at the interface between AL1 and PSL (Fig. 10, arrowheads). This new layer was weakly basophilic and AB reactive. Since this new layer always occurred where accessory layer 1 came into contact with the primary spermatophore layer, we assumed that it was a localized mixture of AL1 and PSL. In this portion, AL1 no longer presented a homogeneous fine structure (Fig. 11). It was not clear whether the epithelial cells lining the spermatophoric duct contributed to the changed consistency of AL1 and matrix formation. Although the cells appeared to be actively synthesizing, they were clearly not secreting the primary spermatophore layer.

#### Distal Vas Deferens

The distal vas deferens (DV) was very narrow compared with other portions of the vas deferens. Although the epithelium was compacted, serial transverse sections through the entire length of the distal vas deferens indicated that the two typhlosoles (T1 and T2) appeared to remain and there was little spermatophoric material (Fig. 12). Therefore, the passage of spermatophoric

materials from medial vas deferens to terminal ampulla is apparently discontinuous.

#### Terminal Ampulla

Dorsal views of the left terminal ampullae of the two species are shown in Figs. 13 and 14. The terminal ampulla of *P. setiferus* is cylinder-shaped, while that of *P. vannamei* is pear-shaped. Whitish ducts (WD) are observed through the translucent muscular layer. A wing (WI) is also observable at the distal region of the ampulla of *P. setiferus* but not in *P. vannamei*. A thick muscle layer was prominent in this portion.

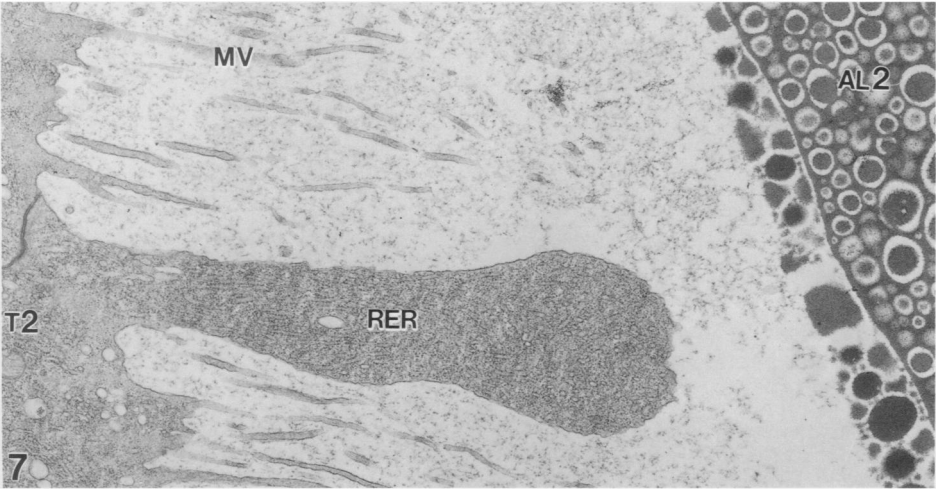
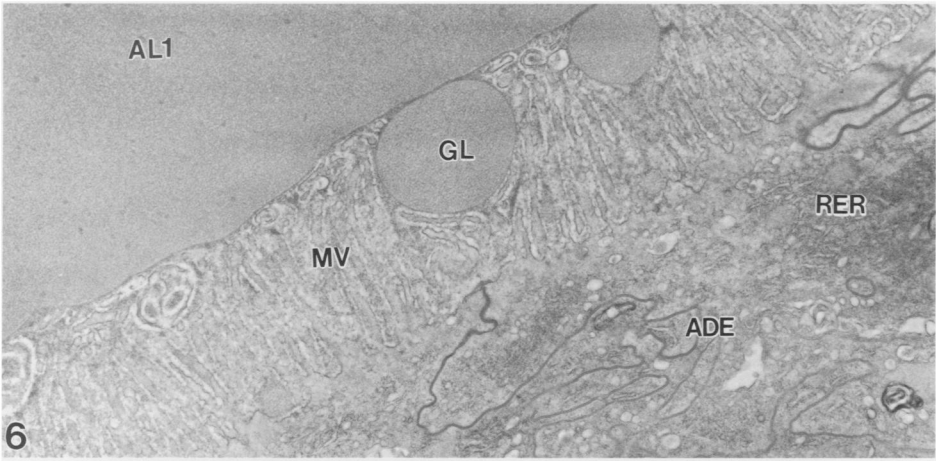
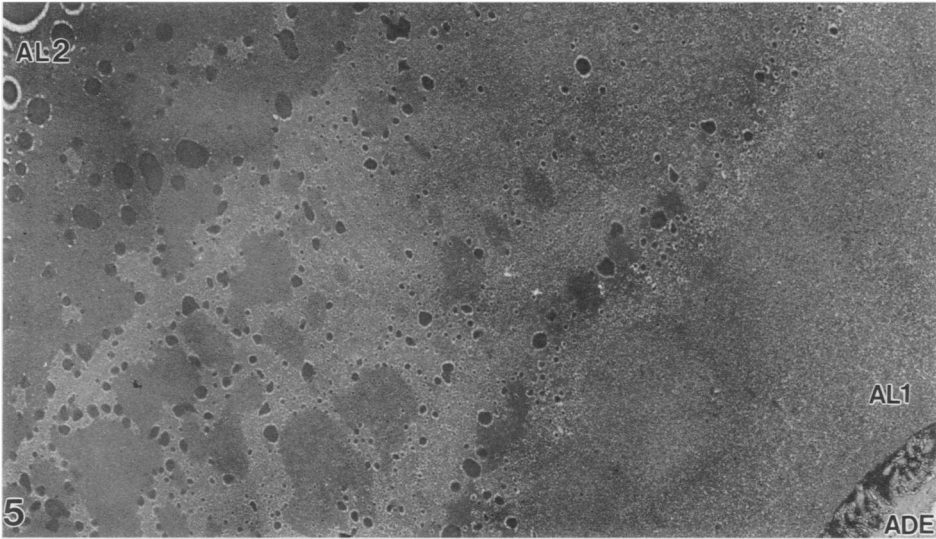
The terminal ampullae of *P. setiferus* and *P. vannamei* contained five and four interconnecting chambers or lumina, respectively. Chambers I, II, and III were evident in transverse sections of terminal ampullae of both species (Figs. 15, 16). Both typhlosoles (T1 and T2) could be tracked by observing serial transverse sections from the medial vas deferens to the terminal ampulla. Chamber I was partially separated from chamber II by typhlosole 1. Chamber II was subdivided by typhlosole 2. Chamber III was a glandular branching duct which opened into chamber II.

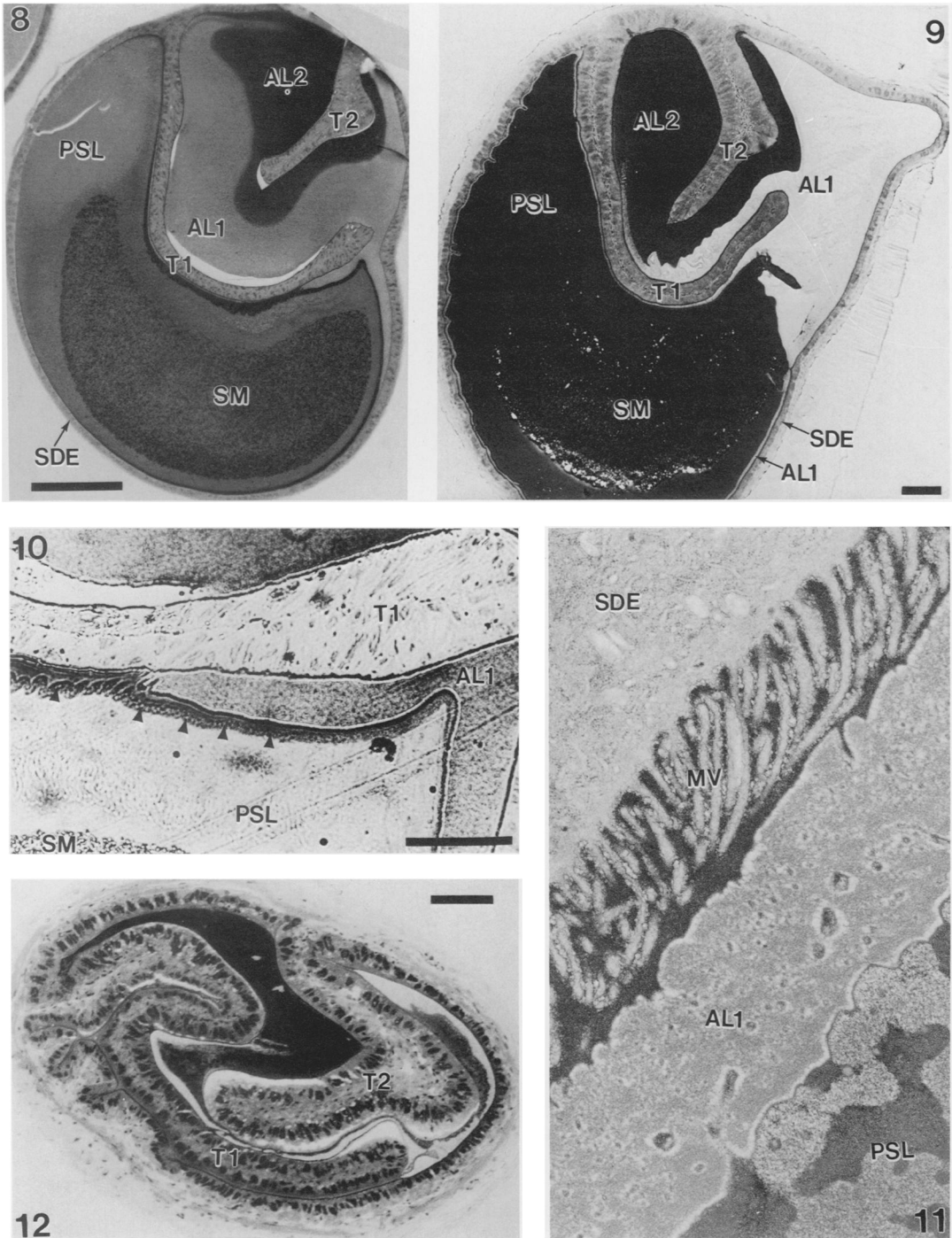
Based on the arrangements of the sperm mass (SM), typhlosoles (T1 and T2), and the other matrices (AL1, AL2, and PSL), chambers I and II appeared to correspond to the spermatophoric (SD) and accessory (AD) ducts, respectively. Chamber I contained the sperm mass surrounded by the primary spermatophore layer and accessory layer 1 (PSL and AL1), a newly deposited thick dorsal plate (DP), and a thin adhesive layer (ADL). A pouch (PC), or evagination of chamber I, was observed in both species, and the dorsal plate of the spermatophore was deposited along this pouch. The adhesive layer (ADL), which was continuous with the dorsal plate (DP), was deposited only along the ventral side of this chamber. These two new layers (ADL and DP) were both acidophilic and PAS reactive.

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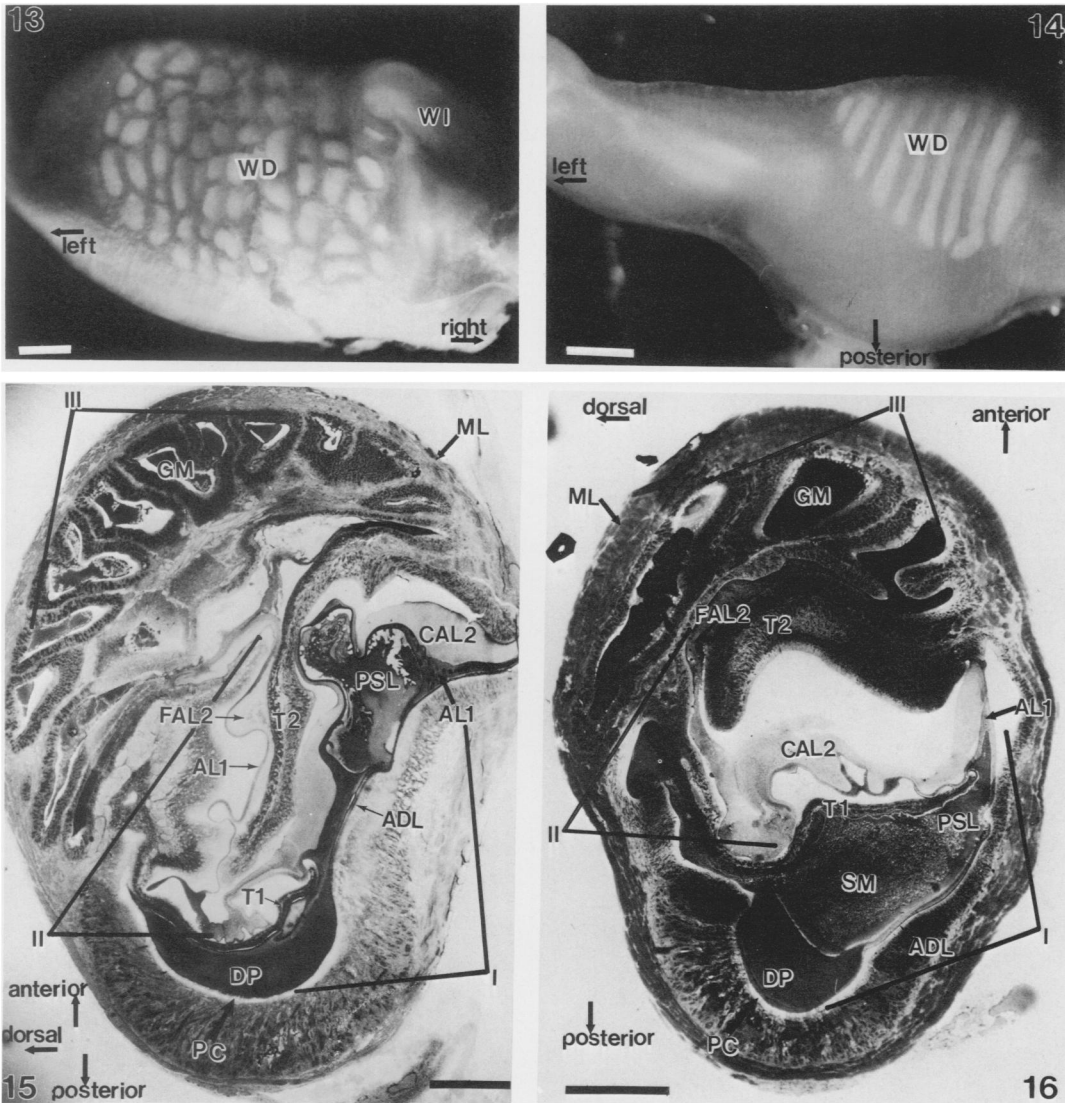
Figs. 5–7. Ascending medial vas deferens of penaeid shrimps. Fig. 5. Low magnification image of accessory layers (AL1 and AL2) of *Penaeus setiferus*, illustrating homogeneous matrix of accessory layer 1 and coarse granules in accessory layer 2. These two matrices mix at their interface.  $\times 6,450$ . Fig. 6. Higher magnification of accessory layer 1 (AL1) and accessory duct epithelium (ADE) of *P. setiferus*. Accessory duct epithelium possesses abundant rough endoplasmic reticulum (RER) and microvilli (MV). Secreted globules (GL) are observed on cell apex.  $\times 9,000$ . Fig. 7. Higher magnification of accessory layer 2 and typhlosole 2 (T2) of *P. vannamei*. Typhlosole 2 epithelial cells possess abundant rough endoplasmic reticulum and microvilli.  $\times 9,200$ .





Figs. 8–12. Vas deferens in penaeid shrimps. Figs. 8–11. Descending medial vas deferens. Figs. 8, 9. Transverse thick methacrylate sections in *Penaeus setiferus* (Fig. 8) and *P. vannamei* (Fig. 9). C2R/MB stain. Scale bars = 0.5 mm. One end of septum detached, which is newly referred to as typhlosole 1 (T1). At confluence of two ducts, accessory layer 1 (AL1) flows into spermatophoric duct (SD) (Fig. 8) and surrounds primary spermatophore layer (PSL) (Fig. 9). Fig. 10. Enlarged view of interface between accessory layer 1 and primary spermatophore layer in *P. setiferus*. Differently stained new layer (arrowheads) is observed. C2R/MB stain. Scale bar = 0.1 mm. Fig. 11. Thin Spurr section (Spurr, 1969) showing accessory layer 1 penetrating between primary spermatophore

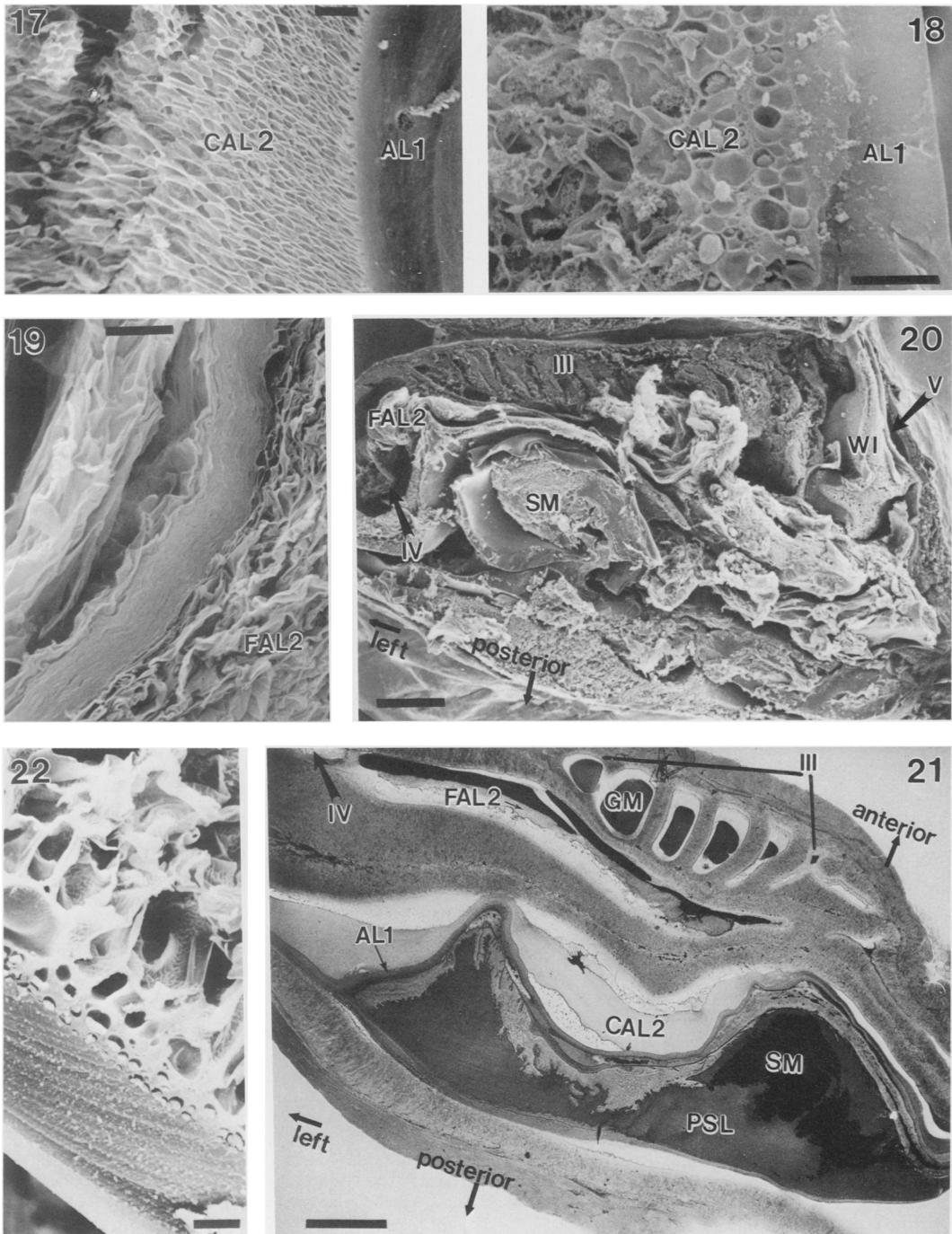




Figs. 13–16. Terminal ampullae in male reproductive system of penaeid shrimps. Figs. 13, 14. Dorsal views of left terminal ampullae of *Penaeus setiferus* (Fig. 13) and *P. vannamei* (Fig. 14). Whitish duct (WD) occupies larger area of terminal ampulla of *P. setiferus* than in *P. vannamei*. Wing (WI) portion of spermatophore can be observed in distal portion of terminal ampulla of *P. setiferus*, but not in *P. vannamei*. Scale bars = 1 mm. Figs. 15, 16. Transverse thick methacrylate sections of terminal ampullae of *P. setiferus* (Fig. 15) and *P. vannamei* (Fig. 16). Thick muscular layer (ML) is characteristic. Three chambers (I, II, and III) are shown. Chamber I contains sperm mass (SM), primary spermatophore layer (PSL), accessory layer 1 (AL1), newly deposited dorsal plate (DP), and adhesive layer (ADL). Dorsal plate is thick and deposited along concave pouch (PC), whereas adhesive layer is thin and deposited only along ventral epithelium. Chamber II contains accessory layer 1 on typhlosole 1 (T1) and accessory layer 2 (AL2) around typhlosole 2 (T2). Accessory layer 2 was altered into “corky” reticulate (CAL2) and collapsed or “fibrous” reticulate (FAL2) structures. Chamber III is glandular duct containing glutinous material (GM) and corresponding to whitish duct (WD). C2R/MB stain. Scale bars = 0.5 mm.

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layer and spermatophoric duct epithelium (SDE) of *P. vannamei*. Note that accessory layer 1 is no longer homogeneous.  $\times 17,200$ . Fig. 12. Transverse thick methacrylate section of distal vas deferens of *P. vannamei*. The epithelium is compacted and few spermatophoric materials are observed in lumen. Two typhlosoles (T1 and T2) remain intact. Scale bar = 0.1 mm.



Figs. 17–22. Male reproductive system in penaeid shrimps. Figs. 17, 18. Scanning electron micrographs of “corky” reticulate portion of accessory layer 2 (CAL2) closely associated with accessory layer 1 in *Penaeus setiferus* (Fig. 17) and *P. vannamei* (Fig. 18). Fig. 19. SEM of collapsed or “fibrous” reticulate portion of accessory layer 2 (FAL2) in *P. setiferus*. Scale bars = 5  $\mu$ m. Fig. 20. Scanning electron micrograph, horizontally sliced longitudinal section, revealing two more chambers (IV and V) in terminal ampulla of *Penaeus setiferus*. Chamber IV occurs at proximal to medial region and contains large amount of fibrous matrix tentatively assumed as homologous material to collapsed or “fibrous” reticulate portion of accessory layer 2 (FAL2) observed in chamber II. Chamber V is located in distal region and contains wing (WI). Scale bar = 1 mm. Fig. 21. Methacrylate thick



Accessory layers 1 and 2 (AL1 and AL2) filled chamber II, as they did in the accessory duct. AL1 was thin and adjacent to typhlosole 1 (T1), while AL2 was thick and surrounded typhlosole 2 (T2). In both species, structural and histochemical changes were found for AL2 in the terminal ampullae. In the medial vas deferens, this layer appeared "frothy" under the light microscope and acidophilic and highly PAS reactive, whereas it was granular at the electron microscope level. In the terminal ampulla, two different but closely related structures, which obviously originated from AL2, were found around T2. One was "corky" and reticulate in appearance (Figs. 17, 18), while the other was also reticulated but collapsed or "fibrous" (Fig. 19). These were termed "corky" accessory layer 2 (CAL2) and "fibrous" accessory layer 2 (FAL2), respectively, and stained neutral to weakly basophilic, with very weak PAS reactivity.

Chamber III appeared as a whitish duct (WD) which was observed through the translucent muscle layer (Figs. 13, 14). The external appearance (Figs. 13, 14) and transverse and longitudinal sections (Figs. 15, 16, 20, 21) indicated that chamber III of *P. setiferus* was convoluted, whereas that of *P. vannamei* was more regular in appearance. This chamber deposited glutinous material (GM) which was acidophilic and PAS reactive. The glutinous material interlaced with FAL2 at the opening to chamber II.

Longitudinal sections of the terminal ampullae revealed two other chambers (IV and V) in *P. setiferus* and one (IV) in *P. vannamei* (Figs. 20, 21). Chamber IV was a distinct large lumen located in the proximal to medial region of the terminal ampulla, and interconnecting to chamber II through a narrow opening. Chamber IV contained a large amount of the "fibrous" reticulate matrix similar to FAL2 (see also Figs. 23, 24). Although chamber IV might be an extension of chamber II, the distinct morphology allows us to define it as a separate structure.

We tentatively assume that the matrices in chambers II and IV are homologous and that chamber IV functions for storage of the matrix.

Chamber V was located in the distal region only of the terminal ampulla of *P. setiferus*, interconnecting to chamber II. This chamber contained the wing (WI) portion of the spermatophore (Figs. 20, 23). The wing (WI) consisted of two layers, one homogeneous and the other reticulate or "corky" in appearance (Fig. 22). The terminal ampulla of *P. vannamei* did not possess a wing (WI) or chamber V. Based on serial transverse and longitudinal sections, reconstructed illustrations of the entire ampullae of both species were prepared (Figs. 23, 24).

#### Compound Spermatophore

Upon mating, each of the paired terminal ampullae expels a spermatophore. These two join together and form a compound spermatophore which is deposited on the open thelycum of the female. A ventral view of an artificially assembled compound spermatophore of *P. setiferus* is shown in Fig. 25. The compound spermatophore consisted of a main trunk which was termed the geminate body (GEB) by Farfante (1975), a pair of wings (WI) at the anterior portion, and glutinous material (GM) clinging to both sides of the trunk. A montaged transverse section of the compound spermatophore of *P. setiferus* at the midposterior portion is shown in Fig. 26. The symmetrical structure consisted of paired spermatophores joined along the mesial adhesive layer (ADL). As observed in the descending medial vas deferens and in chamber I of the terminal ampulla, accessory layer 1 entirely surrounded the primary spermatophore layer (PSL) (Fig. 26). Accessory layer 1 (AL1) and the "corky" reticulate accessory layer 2 (CAL2) were very thick at the ventral apex of the geminate body (Fig. 26, arrow). The dorsal apex of the compound spermatophore consisted of a dorsal plate (DP). The accessory layers

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section of *P. vannamei* terminal ampulla revealing only one additional chamber (IV). C2R/MB stain. Scale bar = 0.5 mm. AL1, accessory layer 1; CAL2, "corky" accessory layer 2; FAL2, "fibrous" accessory layer 2; GM, glutinous material; PSL, primary spermatophore layer; SM, sperm mass; III, chamber III. Fig. 22. Scanning electron micrograph of cut edge of wing (WI). Wing consists of two layers similar to accessory layers. Scale bar = 5  $\mu$ m.

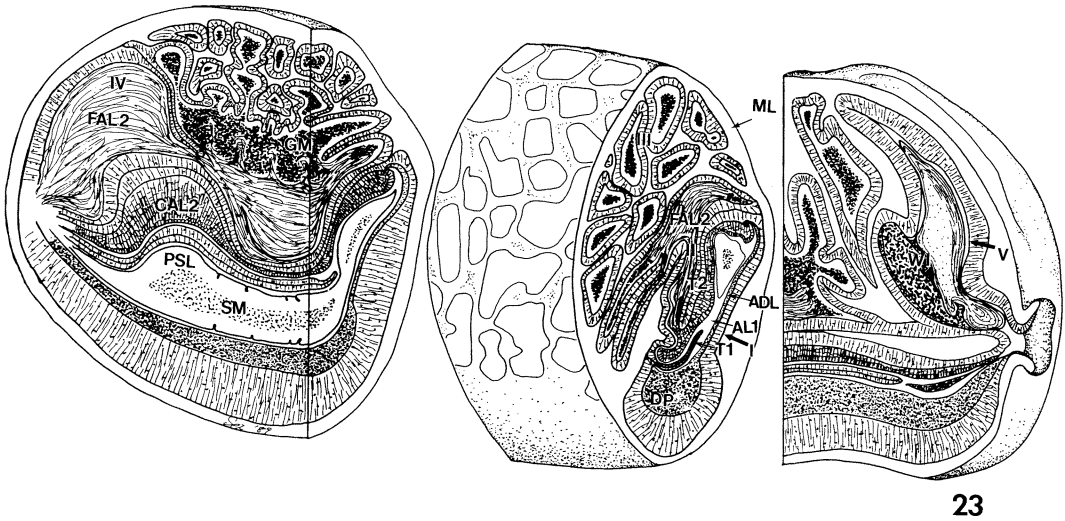


Fig. 23. Reconstructed anatomical illustrations of terminal ampulla of *Penaeus setiferus*. See Figs. 15–21 for abbreviations.

(AL1 and CAL2) were laid along the dorsal plate (DP). At the lateral edge of the DP, the accessory layers were bent toward the ventral side. After the bend, CAL2 became thin and was gradually replaced by the collapsed or “fibrous” reticulate accessory layer 2 (FAL2). AL1 and CAL2 are hard and function as a supportive sheath for the sperm mass and the primary spermatophore layer. FAL2 and the glutinous material (GM) are soft and interlacing. Upon ejaculation, the peripheral edge of the glutinous material attaches to the lateral side of the geminate body. Thus, the compound spermatophore

of *P. setiferus* may be attached to the thelycum of the female, by the dorsal plate, “fibrous” accessory layer, and glutinous material, whereas the anterior portion of the compound spermatophore is anchored by the wing (see Farfante, 1975).

The structural resemblance between the wing and the accessory layers observed in the terminal ampulla might suggest that the wing is molded in chamber V. However, the surface of the “corky” portion of the wing was observed to have an orderly structure (Fig. 27), whereas the geminate body surface exhibited a disordered structure (Fig. 28).

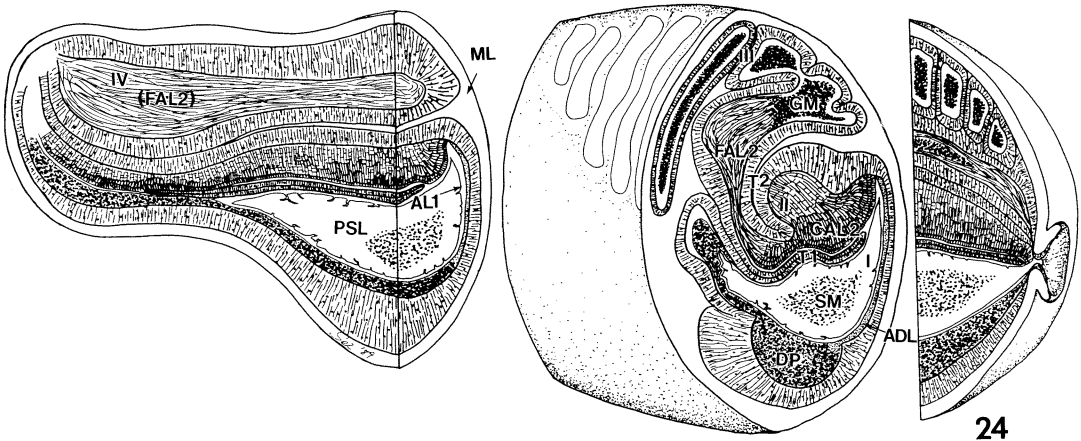


Fig. 24. Reconstructed anatomical illustrations of terminal ampulla of *Penaeus vannamei*. See Figs. 15–21 for abbreviations.

The reticulate "corky" spaces of the wing were usually larger than those of CAL2 (see Figs. 17, 18, 22). While most of the reticulate "corky" spaces of CAL2 were empty (Figs. 17, 18), those of the wing were often filled with a basophilic, PAS-reactive matrix (M) (Fig. 29). The wall of the wing "corky" spaces consisted of finer chambers (Fig. 29). The wing and the geminate body are separate structures and the wing is movable at its junction with the geminate body. The differences in structure and its separate formation indicate that the wing is formed *de novo* in chamber V, regardless of whether or not the matrices of the wing and accessory layers are homologous.

Since no mated females of *P. vannamei* were available during this study, the structure of the naturally inseminated compound spermatophore was not examined in this species. Nevertheless, based on the homologous arrangement of the spermatophoric materials observed in the terminal ampullae of these two species, it appears that the structure of the compound spermatophore of *P. vannamei*, as well as the roles of each of the spermatophore components, must be quite similar to those of *P. setiferus*, except for the absence of the wing.

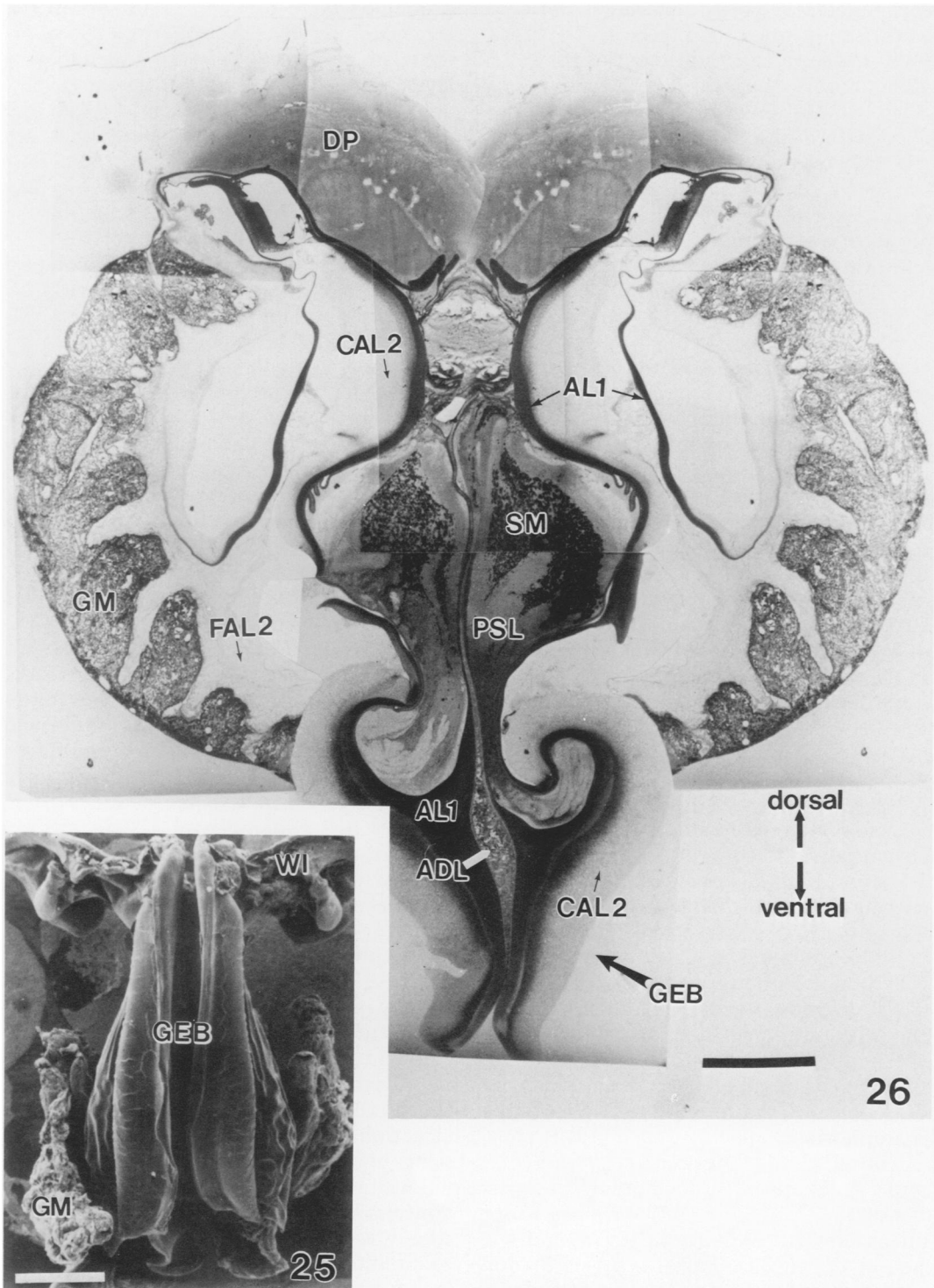
#### DISCUSSION

*Review on the Compound Spermatophore.*—The present study has clarified the structure of the compound spermatophore and the origin of each component of the spermatophore in *Penaeus setiferus* and *P. vannamei*. Our observations and terminology are compared with those of Farfante (1975) and Talbot *et al.* (1989) in Table 1. These previous studies dealt mainly with the external morphology of the compound spermatophore of *P. setiferus*, while our terminology is based on light and electron microscopic observations of the origin of spermatophoric materials.

The complex of the accessory layers (AL1 and CAL2) appears to be homologous with the structures described as blade, flap, flange, and lateral wall by Farfante (1975) and Talbot *et al.* (1989). Farfante's (1975) dorsomesial wall corresponds to the mesial portion of accessory layer 1 (AL1) of the present study. Talbot *et al.* (1989) reported that the glutinous material described by Farfante (1975) was composed of two matrices that

originated from different chambers in the terminal ampulla. These two matrices, referred to as glutinous mass and adhesive layer, respectively, by Talbot *et al.* (1989), coincide with glutinous material (GM) and collapsed or "fibrous" reticulate accessory layer 2 (FAL2) of the present study. Farfante's (1975) gelatinous substance is unclear in origin. This substance may be the glutinous material (GM), the collapsed or "fibrous" accessory layer 2 (FAL2), or a part of the primary spermatophore layer (PSL). The ventral wall of Farfante (1975) and Talbot *et al.* (1989) is apparently the thick portion of the "corky" reticulate accessory layer 2 (CAL2) of the present study. Farfante (1975) apparently overlooked the adhesive layer (ADL) which functioned to join together a pair of spermatophores.

*"Wing Duct" and Wing.*—Since Heldt (1938) first described the double duct structure of the vas deferens in *Penaeus trisulcatus* (= *P. kerathurus*), this character has been shown to be common in the genus *Penaeus* (King, 1948; Malek and Bawab, 1974b; Champion, 1987; Ro *et al.*, 1988). These two ducts were termed "spermatophoric" and "wing" ducts by Malek and Bawab (1974b) in *P. kerathurus*. Similarly, Champion (1987) described deposition of wing substance in the vas deferens of *P. indicus*. Bell and Lightner (1988) mistakenly presumed that the accessory duct (secondary lumen in their study) deposited the primary spermatophore layer in *P. stylirostris*. However, our observation that *P. vannamei* possesses a "wing" duct, but no wing (see also Farfante, 1975), clearly indicates that "wing" duct is an incorrect term. Further evidence of this incorrect terminology is the presence of a terminal ampulla chamber V which contains the wing portion of the spermatophore of *P. setiferus*, and the structural differences observed between the wing material and accessory layers. This confusion may have been caused by a misconception, since the gelatinous membrane-like matrix of the spermatophore was referred to as "wing" in various closed-thelycum species (Eldred, 1958; Tuma, 1967; Tirmizi and Khan, 1970; Huq, 1981; Champion, 1987). However, "wing" was first used by King (1948) to describe the unique paired wing-shaped projections



Figs. 25, 26. Male reproductive system of *Penaeus setiferus*. Fig. 25. Scanning electron micrograph illustrating ventral view of artificially assembled compound spermatophore. Compound spermatophore consists of main trunk called geminate body (GEB), pair of wings (WI) at anterior portion, and glutinous material (GM) at mid-to posterior portion. Scale bar = 2 mm. Fig. 26. Photographic montage of transverse thick section (methacrylate) of compound spermatophore. Pair of spermatophores expelled from paired terminal ampullae join together

which served to anchor the compound spermatophores of open-thelycum species (King, 1948; Farfante, 1975). Since Malek and Bawab (1974b) referred to the "wing" duct and "wing" materials as accessory structures, we retermed this duct an accessory duct.

The structure of the terminal ampulla of *P. indicus* examined by Champion (1987), and our preliminary observations on the terminal ampullae of three closed-thelycum species (*P. aztecus*, *P. duorarum*, and *P. monodon*; unpublished), indicate that these species possess a single, large chamber containing fibrous matrix. This large chamber and the fibrous matrix might coincide with chamber IV and the collapsed or "fibrous" reticulate accessory layer described here, respectively. Bell and Lightner (1988) also observed a similar large chamber in the terminal ampulla of an open-thelycum species, *P. stylirostris*. The fibrous matrix emerged from the terminal ampulla of chamber IV as a sticky matrix, but subsequently spread out into an extensive flattened sheet in sea water. Malek and Bawab (1971) described a similar matrix in *P. trisulcatus* (= *P. kerathurus*). Apparently, this membrane-like matrix serves to plug the slit of the closed thelycum (Tuma, 1967; Champion, 1987), although most of it would likely be lost within a short time after mating. Therefore, we conclude that the wing of the open-thelycum species is formed in the terminal ampulla and thus differs from the so-called "wing" in the closed-thelycum species. We also suggest that the "wing" described in the closed-thelycum species should be referred to as part of a stopper or plug.

*Matrix Alterations.*—Structural alteration of the granular accessory layer 2 into reticulate "corky" AL2 (CAL2) and collapsed or "fibrous" AL2 (FAL2) was accompanied by hardening of CAL2. Similar structural alterations of spermatophoric materials have

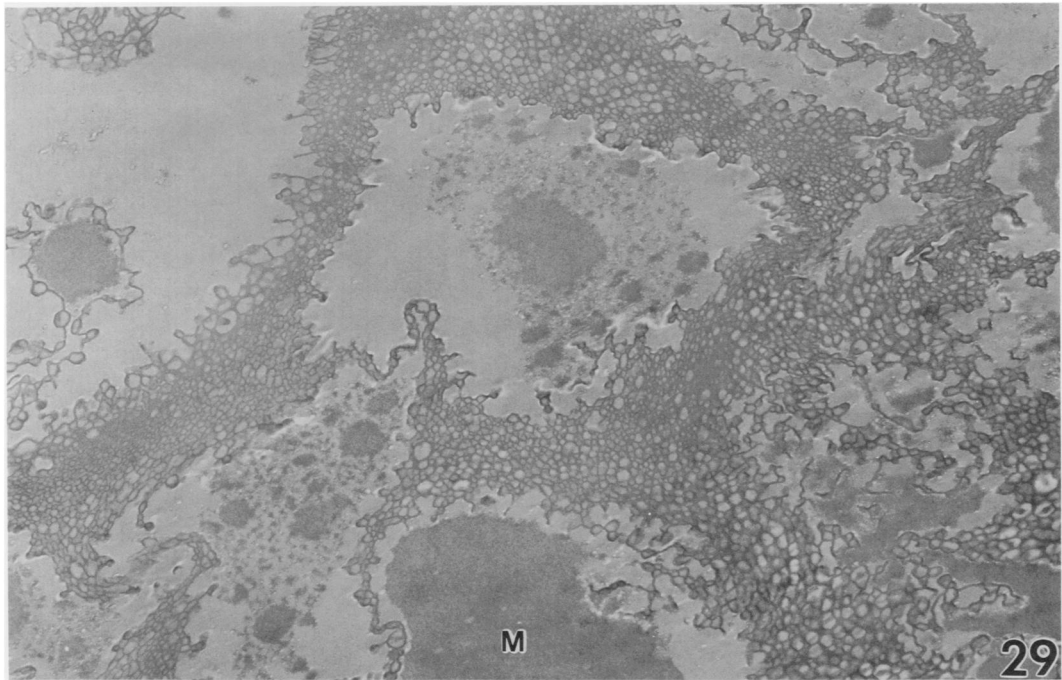
been observed in the fresh-water crayfish *Pacifastacus leniusculus* (see Dudenhausen and Talbot, 1983), and the spiny lobster *Panulirus interruptus* (see Martin *et al.*, 1987) by comparing soft and hardened spermatophores. In the crayfish, the middle granular layer in the soft spermatophore changed into an inner compact region and an outer reticulate region of the hardened spermatophore. In the spiny lobster, the granular matrix of the soft spermatophore became the brittle cap and matrices comprised of empty spheres and a filamentous network. Dudenhausen and Talbot (1983) described this matrix alteration as "reticulation"; this may be what occurred in the alteration of accessory layer 2 (AL2) observed here. It appears that reticulation per se is not the cause of hardening of AL2, since the CAL2 and FAL2 portions both became similarly reticulated, but the latter was not hardened. Chemical transformation of the matrix catalyzed by certain enzymes, such as those involved in quinone or phenolic tanning, might occur in the terminal ampulla, as suggested by Malek and Bawab (1971). Interestingly, this hardening and reticulation of the spermatophore matrices occurs within the terminal ampulla of the two penaeid shrimps studied here, whereas in the crayfish and lobster, matrix alteration occurs after ejaculation upon spermatophore contact with water.

Similar hardening also occurred in accessory layer 1, which was fluid in the vas deferens but hard in the compound spermatophore. No structural alteration was observed in the primary spermatophore layer (PSL). However, the PSL dissected from the medial vas deferens was quite sticky and maintained this glutinous nature in sea water, whereas, when dissected from the terminal ampulla, it was first sticky and glutinous, but became hard and lost its glutinous nature upon exposure to sea water. These

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along mesial adhesive layer (ADL) and form symmetrical compound spermatophore. Sperm mass (SM) and primary spermatophore layer (PSL) are surrounded by accessory layer 1 (AL1) and reticulate "corky" accessory layer 2 (CAL2). Dorsal apex of compound spermatophore consists of dorsal plate (DP). Accessory layer 1 is laid along dorsal wall layer, bends toward ventral side at edge of dorsal plate. "Corky" accessory layer 2 covering accessory layer 1 is gradually replaced by collapsed or "fibrous" reticulate accessory layer 2 (FAL2). "Fibrous" accessory layer 2 and glutinous material (GM) interlace facilitating close adhesion between them. Glutinous material attaches to geminate body (GEB), and forms lateral side of compound spermatophore. Accessory layer 1 and "corky" accessory layer 2 function as sheath for sperm mass. Dorsal plate, "fibrous" accessory layer 2, and glutinous material function as attachment instruments for mid- to posterior portions of compound spermatophore, whereas anterior portion is anchored primarily by wing (WI). C2R/MB stain. Scale bar = 0.5 mm.





Figs. 27–29. Male reproductive system in *Penaeus*. Fig. 27. Scanning electron micrograph of surface of “corky” portion of wing (WI). Scale bar = 5  $\mu$ m. Fig. 28. Scanning electron micrograph of geminate body surface of *P. setiferus*. Scale bar = 5  $\mu$ m. Fig. 29. Thin section of “corky” portion of wing (WI).  $\times 7,000$ .

Table 1. Comparison of terms used to describe the compound spermatophore structure of *Penaeus setiferus*. Abbreviations used in each study are shown in parentheses.

Farfante (1975)	Talbot et al. (1989)	Present study
Blade (b)	—	accessory layer 1 (AL1) "corky" reticulate accessory layer 2 (CAL2)
Dorsal plate (dp)	dorsal plate (DP)	dorsal plate (DP)
Dorsomesial wall (dw)	—	accessory layer 1 (AL1)
Flap (fp)	—	accessory layer 1 (AL1) "corky" reticulate accessory layer 2 (CAL2)
Flange (fg)	flange (F)	accessory layer 1 (AL1) "corky" reticulate accessory layer 2 (CAL2)
Glutinous material (gm)	glutinous mass (GM) adhesive (A)	glutinous material (GM) collapsed of "fibrous" reticulate accessory layer 2 (FAL2)
Gelatinous substance (gs)	—	glutinous material (GM)? collapsed or "fibrous" reticulate accessory layer 2 (FAL2)? primary spermatophore layer (PSL)?
Lateral wall (lw)	—	accessory layer 1 (AL1) "corky" reticulate accessory layer 2 (CAL2)
Ventral wall (vw)	ventral wall (VW)	"corky" reticulate accessory layer 2 (CAL2)
Wing (w)	wing (W)	wing (WI)
—	—	adhesive layer (ADL)

changes might be attributable to mixing of the matrices (e.g., AL1 and PSL) in the descending segment of the vas deferens, accompanied by biochemical reaction.

Thus, it appears that the terminal ampulla is not merely a site for organizing the components of the spermatophore, but that maturation of the spermatophore matrices and deposition of new matrices also occur there.

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The Crustacean Society presents a Best Student Paper Award (as announced in the *JCB*, vol. 2, no. 1, p. 53) for papers given at its annual meeting. The award consists of a free one-year subscription to the *Journal of Crustacean Biology*.

For 1990 the award is made to Suzanne C. Wache, University of Connecticut, Storrs, Connecticut, for her paper (with H. Laufer) entitled "Purification and characterization of PUFA in *Artemia salina* (Great Salt Lake) for the study of fatty acid synthesis patterns during development," presented during the San Antonio, Texas, meetings of the American Society of Zoologists and The Crustacean Society in December 1990.