

Spermatophore Structure and Anatomy of the Ejaculatory Duct in *Penaeus setiferus*, *P. duorarum*, and *P. aztecus* (Crustacea: Decapoda): Homologies and Functional Significance¹

RAYMOND T. BAUER AND CORA E. CASH

Center for Crustacean Research, University of Southwestern Louisiana,
Lafayette, Louisiana, 70504-2451, U.S.A

Abstract. Spermatophore structure inside the male ejaculatory duct, immediately following ejaculation, and after exposure to seawater is described and compared for three species of *Penaeus*, *P. setiferus* (external attachment of the spermatophore to an "open" thelycum of the female) and *P. duorarum* and *P. aztecus* (spermatophore storage behind a "closed" thelycum). Detailed investigation of the origin and disposition of spermatophore materials in the ejaculatory duct permits hypotheses of homologies among the species of *Penaeus* investigated. The sperm sac of the spermatophore of *P. setiferus* is homologous to the main body of the spermatophore of *P. duorarum* and *P. aztecus*. The adhesive glands and material have been lost entirely in the latter two species, and the appendages of their spermatophores are homologous to the glutinous material of *P. setiferus*. Structures for mechanical attachment found in the externally attached spermatophore of *P. setiferus* are lost or reduced in the phylogenetically advanced *P. duorarum* and *P. aztecus*. Reduction in complexity of the spermatophore in the species with closed thelycum is postulated to have evolved concomitantly with spermatophore enclosure and storage. In the species with closed thelycum, exposure of the spermatophore to seawater causes a striking reaction, consisting of an unfolding of its "appendage." Spreading of the appendage material throughout the aperture of the seminal receptacle to insure its complete seal after insemination is suggested as the function of the spermatophore reaction. In *P. setiferus*, the spermatophore reaction consists of changes in the adhesive and glutinous materials, as well as in extrusion of the sperm mass.

In penaeoid shrimps, there is considerable variation in external and internal morphology associated with insemination; i.e., the transfer of sperm to the female and its temporary or long-term storage until fertilization. In sicyoniid shrimps, spermatophores deposited in the seminal receptacles of the female are little more than spermatozoa in a seminal fluid (Bauer, 1991; Pérez Farfante, 1985). At the other morphological extreme in the Penaeoidea, sperm is transferred in much more complex spermatophores. In shrimps of the genus *Penaeus*, spermatozoa are enclosed in a variety of substances secreted by the male reproductive tract, forming a complex spermatophore. Twin spermatophores, one from each male ejaculatory duct, are deposited on or within the female's thelycum, a modification of the posterior sternites of the female cephalothorax. Species of *Penaeus* may be divided into two groups, one in which spermato-

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phores are attached externally to an "open" thelycum, and another in which spermatophores are deposited within a median cuticular pocket behind the "closed" thelycum (Bauer, 1991; Burkenroad, 1934, 1936; Champion, 1987; Chow et al., 1990; Eldred, 1958; King, 1948; Malek & Bawab, 1974a,b; Pérez Farfante, 1969, 1975; Tirmizi, 1958).

To understand the mechanics of insemination in penaeoid shrimps, the structure of spermatophores within the ejaculatory duct and after ejaculation must be compared. Pérez Farfante (1975) thoroughly described spermatophores in species of *Penaeus* with open thelyca (subgenus *Litopenaeus*). Studies on the origin of spermatophore materials were conducted by Malek & Bawab (1974a,b) for *Penaeus kerathurus* (Forskål, 1775), Champion (1987) for *P. indicus* H. Milne-Edwards, 1837, Chow et al. (1990) for *P. vannamei* Boone, 1931, with additional observations on *P. setiferus* (Linnaeus, 1767), *P. aztecus* Ives, 1891, and *P. duorarum* Burkenroad, 1939, and by Ro et al. (1990) for *P. setiferus*. Talbot et al. (1989) made observations on the spermatophores of *P. setiferus* in a study of degeneration of the male reproductive tract. However, considerable uncertainty still exists about the site of production, fate upon emission, function, and phylogenetic significance of spermatophore structures.

The major objectives of this report are: (1) to compare morphology and origin of certain spermatophore materials from the ejaculatory duct of *P. setiferus*, an open thelycum species, with that of *P. aztecus* and *P. duorarum*, two species with closed thelyca; (2) to describe structural changes in spermatophores after ejaculation with subsequent exposure to seawater ("spermatophore reaction"); and (3) to postulate the functional significance and homologies of spermatophore materials and ejaculatory duct structures in the species of *Penaeus* investigated.

MATERIALS AND METHODS

Adult specimens of *Penaeus setiferus*, *P. aztecus*, and *P. duorarum* were obtained by trawling during spring, summer, and autumn 1986–1989 from a variety of locations in the Gulf of Mexico off Texas, Louisiana, and Mississippi. Living specimens were transported to laboratory holding facilities in chilled, oxygenated water, but other specimens were preserved for later study. Various regions of male reproductive tracts were prepared for histological examination (serial sections for light microscopy) and/or scanning electron microscopy (SEM) from 22 adult males of *P. setiferus*, 13 adult males of *P. aztecus*, and 7 adult males of *P. duorarum*. Davidson's and 37–40% formaldehyde (=100% formalin) solutions were used as initial fixatives for specimens prepared for histology. A compound of paraffin and plastic polymers was the embedding medium, and Mallory's triple stain was used in staining sections. Ejaculatory ducts, vasa deferentia, and spermatophores of some specimens were partially sectioned and prepared for examination of cut surfaces using SEM. All structures and sections prepared for SEM were critical-point dried and sputter-coated to a thickness of 10–20 nm with gold or gold/palladium. In addition, dissections of numerous other preserved specimens were done to observe and illustrate reproductive structures.

Spermatophores were obtained from live males by external mechanical pres-

sure near the ejaculatory ducts. Ejaculated spermatophores were fixed immediately in 100% formalin or first placed in dishes with ambient seawater for 5–60 min in order to observe changes in spermatophore structure prior to preservation in 50% seawater formalin.

RESULTS

General Structure of the Male Reproductive Tract

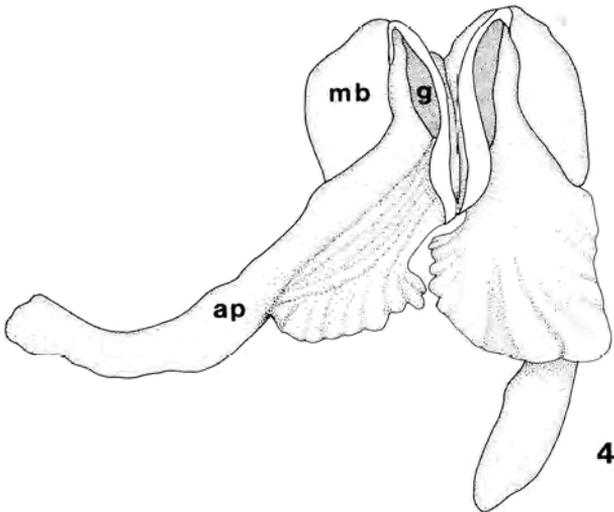
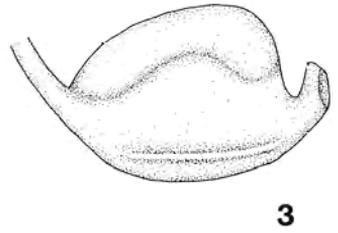
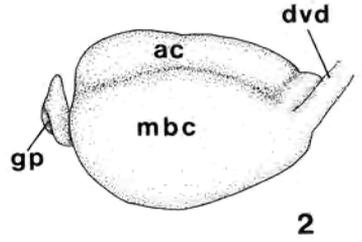
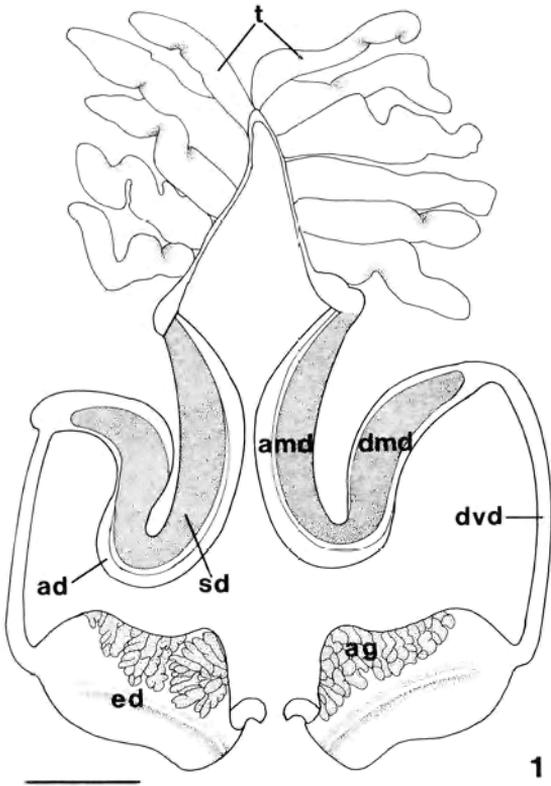
The reproductive tract dissected from a male of *P. setiferus* is illustrated to show the general features of the male system for all three species (Fig. 1). Each multilobed testis connects to a long tube, the proximal vas deferens, leading into the u-shaped medial vas deferens. The descending part of the medial vas deferens leads into the thin, apparently distensible distal vas deferens that is dilated at its distal end to form the ejaculatory duct, where a single spermatophore is stored prior to ejaculation. The ejaculatory duct connects by a fleshy, collapsed canal to the complexly folded, membranous area surrounding the gonopore externally (Figs. 1–3, 5).

The ejaculatory ducts are located in the ventral part of the last cephalothoracic segment of the male. In *P. aztecus* and *P. duorarum*, the proximal to distal axis of the duct is transverse within the body, but in *P. setiferus*, this axis is somewhat oblique to the sagittal plane, with the distal (gonopore) end somewhat anterior to the proximal end of the duct. In addition, the ejaculatory ducts of *P. aztecus* and *P. duorarum* lie flat inside the sternum, but the proximal ends of the ducts of *P. setiferus* are tilted dorsally toward the cephalothoracic wall. In describing the ejaculatory duct of *P. setiferus*, the terms "dorsal," "ventral," "anterior," and "posterior" will be used as if the duct had the same orientation in the body as the other species investigated.

Spermatophore Configuration and Formation in the Ejaculatory Duct

The ejaculatory duct is a highly modified extension of the vas deferens. The lumen of the descending vas deferens is divided into channels by typhlosoles, folds of connective tissue and inner epithelium of the wall of the vas deferens (Figs. 1, 6, 7). Typhlosole "1" incompletely separates the sperm duct from the accessory duct. The sperm duct contains a sperm mass (spermatozoa in a seminal matrix) surrounded by a homogeneous acellular secretion (Figs. 6–8). The accessory duct is filled with at least two different secretions surrounding typh-

FIG. 1. Male reproductive tract of *Penaeus setiferus*, ventral view. ad, accessory duct; ag, adhesive glands; amd, ascending medial vas deferens; dmd, descending medial vas deferens; dvd, distal vas deferens; ed, ejaculatory duct; sd, sperm duct; t, testicular lobe. FIG. 2. Right ejaculatory duct of *P. aztecus*, dorsal view. ac, appendage chamber; dvd, distal vas deferens; gp, gonopore; mbe, chamber of main body. FIG. 3. Right ejaculatory duct of *P. duorarum*, ventral view. FIG. 4. Compound spermatophore of *P. duorarum*, formed from spermatophores ejaculated from right and left male ejaculatory ducts. ap, appendage; g, groove; mb, main body. Scale bar represents 5.0 mm in Fig. 1 and Fig. 2, 3.5 mm in Fig. 3, and 3.0 mm in Fig. 4.



losole "2." The distal vas deferens, the conduit between the medial vas deferens and the ejaculatory duct, has both typhlosoles but usually is empty (Figs. 9, 10). We have traced the two typhlosoles in serial sections from the medial through the distal vas deferens into the ejaculatory duct of each species of *Penaeus*.

Transverse sections of the ejaculatory ducts of *P. aztecus* and *P. duorarum* show that the appendage and main body of the spermatophore (Figs. 4, 29, 31) occupy separate chambers that also may be recognized in external views of the ejaculatory duct (Figs. 2, 3, 11-14). The main body consists of a sperm mass surrounded by layers of acellular material, as in the medial vas deferens, but additional secretion appears to be added by the epithelium of the chamber of the main body (Fig. 15). The lumen of this chamber corresponds anteriorly to that of the sperm duct and posteriorly to part of the lumen of the accessory duct of the vas deferens. Typhlosole 2 enlarges as it enters the proximal end of the ejaculatory duct and is the principal structure forming and continuing to secrete the material of the groove between the folds of the main body (Figs. 11, 12, 14, 16). Typhlosole 1, the larger of the two typhlosoles in the distal vas deferens, becomes quite small, and the space between the bases of the typhlosoles, corresponding to part of the accessory duct of the distal vas deferens, closes in the ejaculatory duct. Both typhlosoles continue distally for most of the length of the chamber of the main body.

The appendage chamber has no counterpart in the vas deferens. A section of the septum that separates the appendage chamber from the chamber of the main body is open ventrally so that the two major parts of the spermatophore are confluent in this region (Figs. 12-14). The appendage material of the spermatophore appears to be derived from the lightly staining secretion in the accessory duct of the descending vas deferens (Figs. 6, 7). This secretion often appears as tightly packed layers that may separate into those observed in the appendage chamber (Figs. 13, 17, 18). There is no evidence of secretory activity by the epithelium of the appendage chamber.

The internal anatomy of the ejaculatory duct of *P. setiferus* is much more intricate than that of *P. aztecus* and *P. duorarum*, with various parts of the complex spermatophore located in several interconnecting chambers (Figs. 19, 20). Serial sections show that the posterior part of the ejaculatory duct is a modified extension of the vas deferens. At the point of juncture of the distal vas deferens and the ejaculatory duct, typhlosole 2 runs ventrally in the duct, continuing some distance distally, with its free edge projecting upward posterodorsally (Fig. 21). As in the other two *Penaeus* spp., typhlosole 2, the smaller of the two typhlosoles in the vas deferens (Figs. 6, 7, 10), is the largest and most prominent fold projecting into the ejaculatory duct. Typhlosole 1 is relatively small in the ejaculatory duct and anterodorsal in position (Figs. 19-21). The small space just posterodorsal to typhlosole 1 corresponds to the sperm duct lumen of the vas deferens. The secreted layers surrounding typhlosole 2 in the proximal part of the ejaculatory duct are the flap, glutinous material, and flange of the spermatophore (Fig. 21). The glutinous material inside the flap is composed of a web of delicate layers on the anterior side of typhlosole

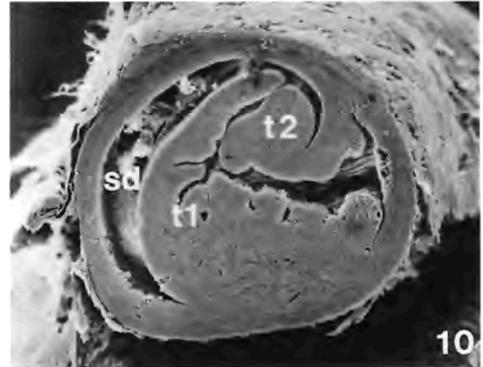
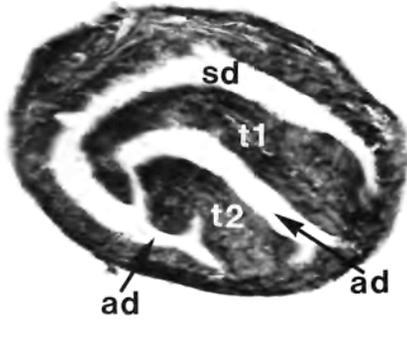
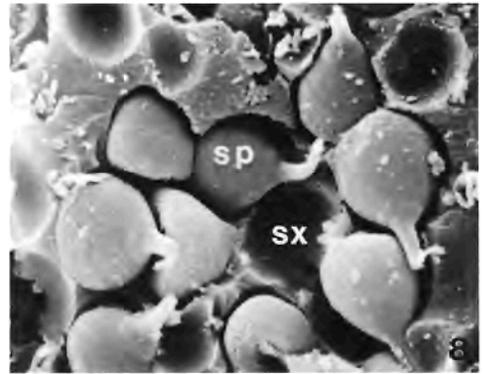
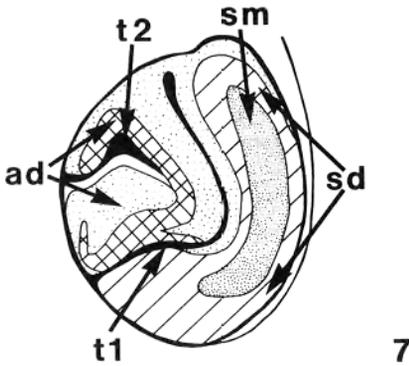
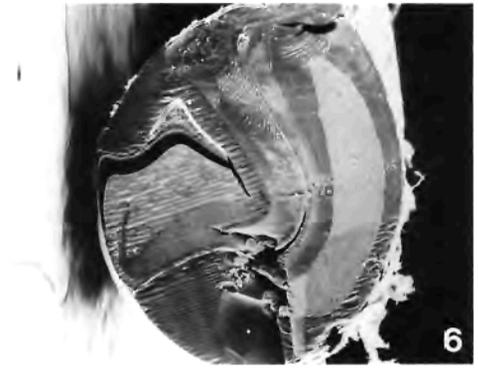


FIG. 5. Gonopore area on medial side of coxa, right pereiopods, *Penaeus setiferus*. gp, gonopore lips surrounding slit-like gonopore. FIGS. 6, 7. Scanning electron micrograph and labelling diagram for transverse section of descending vas deferens (right side, proximal view) of *P. setiferus*, showing sperm mass (heavy stipple, sm) in sperm duct (sd), sperm duct secretion (hatching), and lightly staining (light stipple) and densely staining (cross-hatching) secretions of accessory duct (ad). t1, t2, typhlosoles 1, 2, respectively. FIG. 8. Spermatozoa (sp) of *P. setiferus* surrounded by seminal matrix (sx) from sperm mass of descending medial vas deferens. FIGS. 9, 10. Transverse section of empty, collapsed left distal vas deferens near the ejaculatory duct in *P. aztecus* (Fig. 9, distal face of section) and *P. setiferus* (Fig. 10, proximal face of section). ad, accessory duct; sd, sperm duct; t1, t2, typhlosoles 1, 2, respectively. Scale bar represents 1.0 mm in Fig. 5, 560 μ m in Fig. 6, 4 μ m in Fig. 8, 150 μ m in Fig. 9, and 260 μ m in Fig. 10.

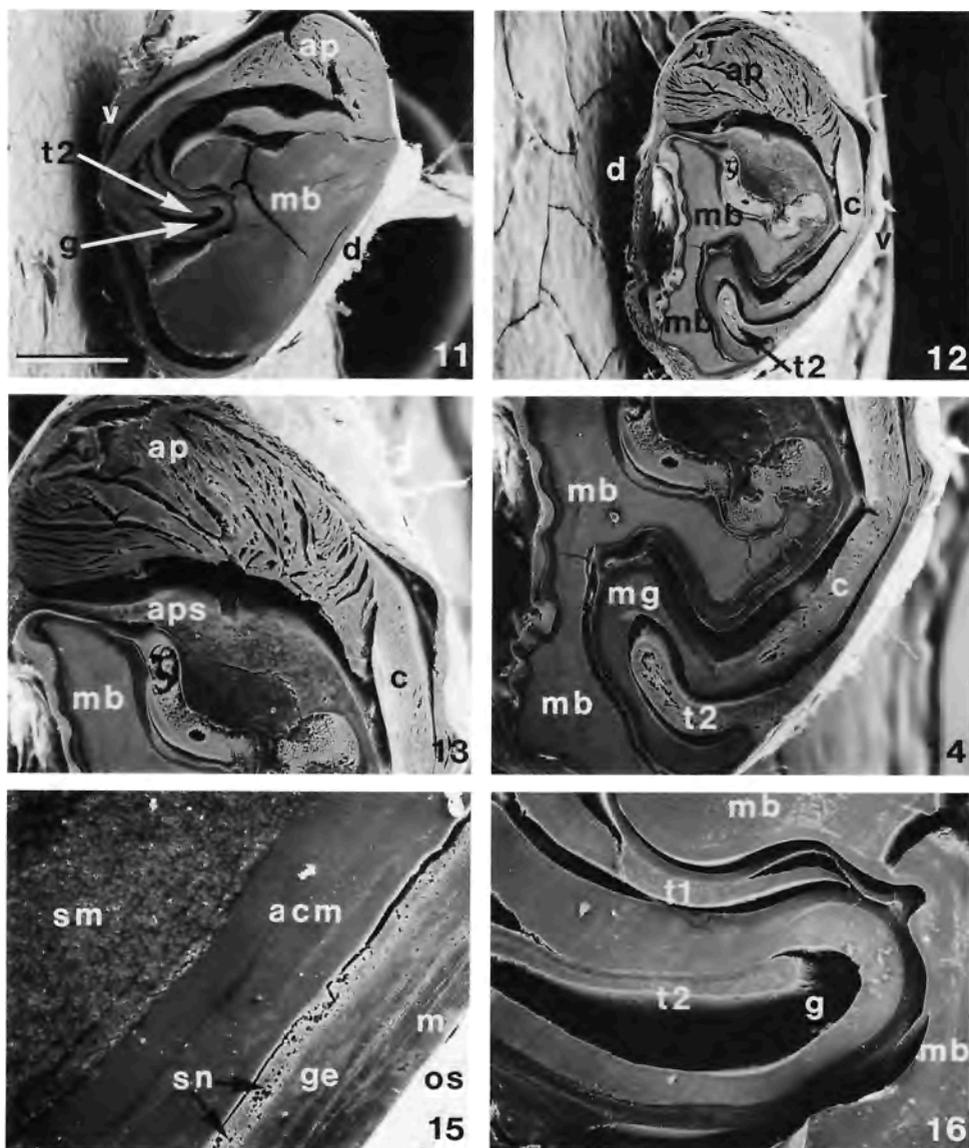


FIG. 11. Distal view of transverse section of right ejaculatory duct, *Penaeus aztecus*. ap, appendage (anterior in duct); d, dorsal side of duct; g, groove between folds of main body; mb, main body; t2, typhlosole 2; v, ventral side of duct. FIG. 12. Distal view of transverse section of left ejaculatory duct, *P. duorarum*, showing complete connection (c) between appendage and main body groove. Other labels same as for Fig. 11. FIGS. 13, 14. Anterior (Fig. 13) and posterior (Fig. 14) parts of ejaculatory duct of *P. duorarum* from section illustrated in Fig. 12. aps, septum separating appendage and main body chambers; mg, secreted material forming groove between main body folds; other labels same as for Figs. 11, 12. FIG. 15. Structure of outer wall of chamber containing main body in *P. aztecus*, showing, from outer surface (os), successive layers of muscle (m), glandular epithelium (ge), and secretion (sn) being added onto layers of acellular material (acm) surrounding inner sperm mass (sm) of main body of the spermatophore. FIG. 16. Typhlosole 1 (t1) and 2 (t2) area of ejaculatory duct of *P. aztecus*. Note layers of secreted material surrounding typhlosole 2 and between typhlosoles 1 and 2, forming external groove (g) of main body (mb) of spermatophore. Scale bar in Fig. 11 represents 1.1 mm in Fig. 11 and Fig. 12, 590 μ m in Fig. 13 and Fig. 14, 67 μ m in Fig. 15, and 260 μ m in Fig. 16.

2 in a large space corresponding to that which closes between typhlosoles 1 and 2 in the ejaculatory ducts of *P. duorarum* and *P. aztecus*. The flange is located posterior to typhlosole 2, but is connected with the flap dorsally (Fig. 21). The flap and flange are composed of low and high density layers that stain purple with Mallory's triple stain, similar to the layers surrounding typhlosole 2 in the ejaculatory ducts of *P. duorarum* and *P. aztecus*. The secretions forming the flap and flange are morphologically similar to the densely staining secretion surrounding typhlosole 2 in the descending vas deferens. The flap and flange continue distally as the wall of the sperm sac of the spermatophore. Additional secretion onto the wall of the sperm sac and its extensions occurs in the ejaculatory duct because droplets or blebs of secreted material may be seen emerging from the surrounding epithelium in histological sections. Figure 21 also illustrates the proximal end of the spermatophore's dorsal plate in the postero-dorsal part of the ejaculatory duct.

More distally, the sperm sac, enclosing the sperm mass and surrounding acellular secretion from the sperm duct of the descending vas deferens, is located behind typhlosole 2 in a space corresponding to part of the accessory duct lumen in the vas deferens (Figs. 19, 20). Glutinous material, continuous with that in the proximal part of the ejaculatory duct, is abundant in a chamber beneath the adhesive glands (Figs. 22, 23). This chamber is broadly confluent with the space anterior to typhlosole 2, but is demarcated from that space by short anterior and posterior projections of the wall of the ejaculatory duct.

The adhesive glands are one of the most distinctive features of the ejaculatory duct of *P. setiferus*, both externally and internally (Figs. 1, 19, 24, 25). Located anterodorsally throughout most of the length of the duct, the glands consist of a series of tubular ducts emptying into the chamber containing the glutinous material (Fig. 26). Sections through the glands reveal a secretion composed of irregularly shaped particles or droplets (Figs. 27, 28). In general, the particles decrease in size from the outer to the inner areas of the secreted mass. Although the secretion may be seen entering the chamber behind the adhesive glands, we have never observed abundant quantities.

Other chambers in the ejaculatory duct of *P. setiferus* have no counterpart in the ejaculatory ducts of *P. aztecus* and *P. duorarum*. The "wing" of the spermatophore is contained in a chamber connecting to the distal part of the sperm sac and apparently is secreted there (Fig. 25). Because we have not observed the dorsal plate of the spermatophore anywhere in the vas deferens, perhaps the dorsal plate also is secreted in the ejaculatory duct.

Spermatophore Morphology Immediately Following Ejaculation

When mechanical pressure is applied externally to the area around an ejaculatory duct of a living male, a single spermatophore is emitted. However, in one observation, spermatophores from the right and left ejaculatory ducts of *P. duorarum* were ejaculated simultaneously, forming a compound spermatophore (Fig. 4). In *P. aztecus* and *P. duorarum*, a single ejaculated spermatophore is composed of the two basic divisions found in the ejaculatory duct, the appendage and main body (Figs. 29, 31A). On one side, the main body has two

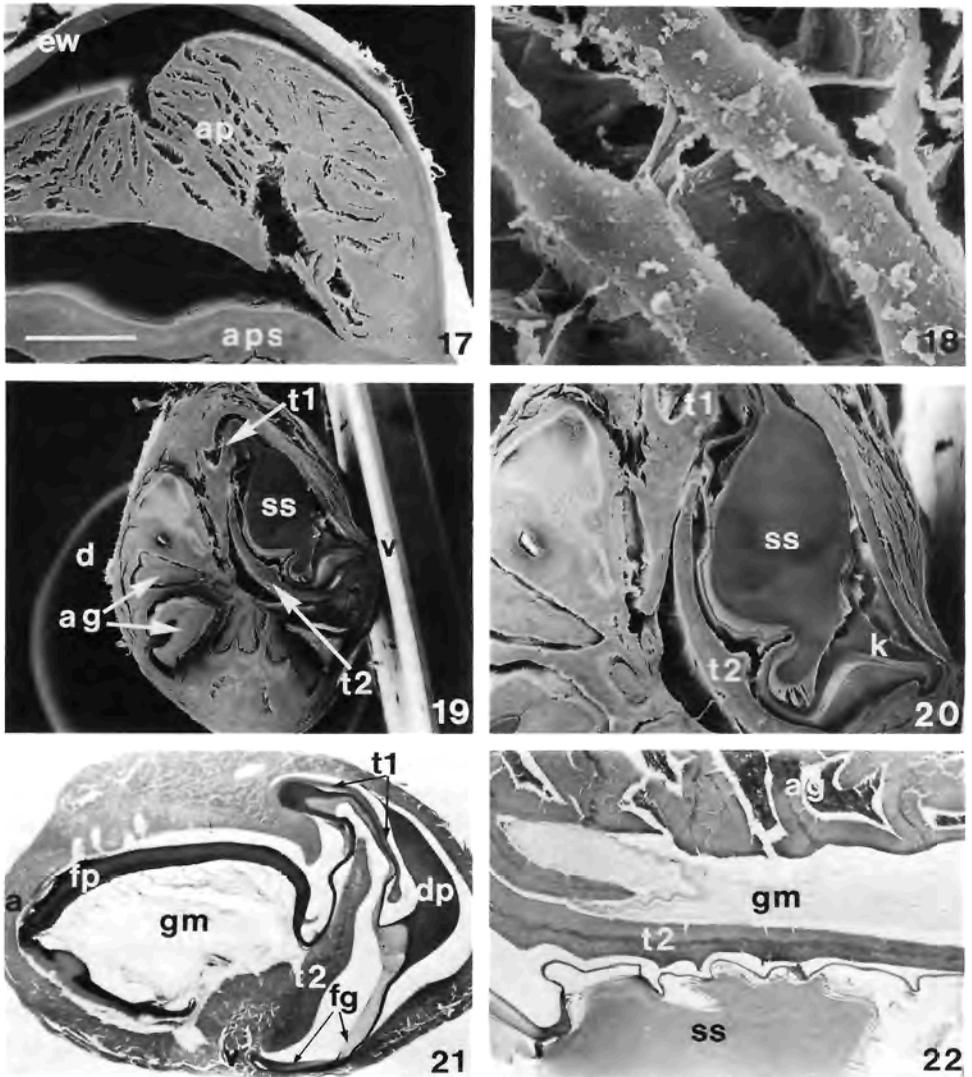
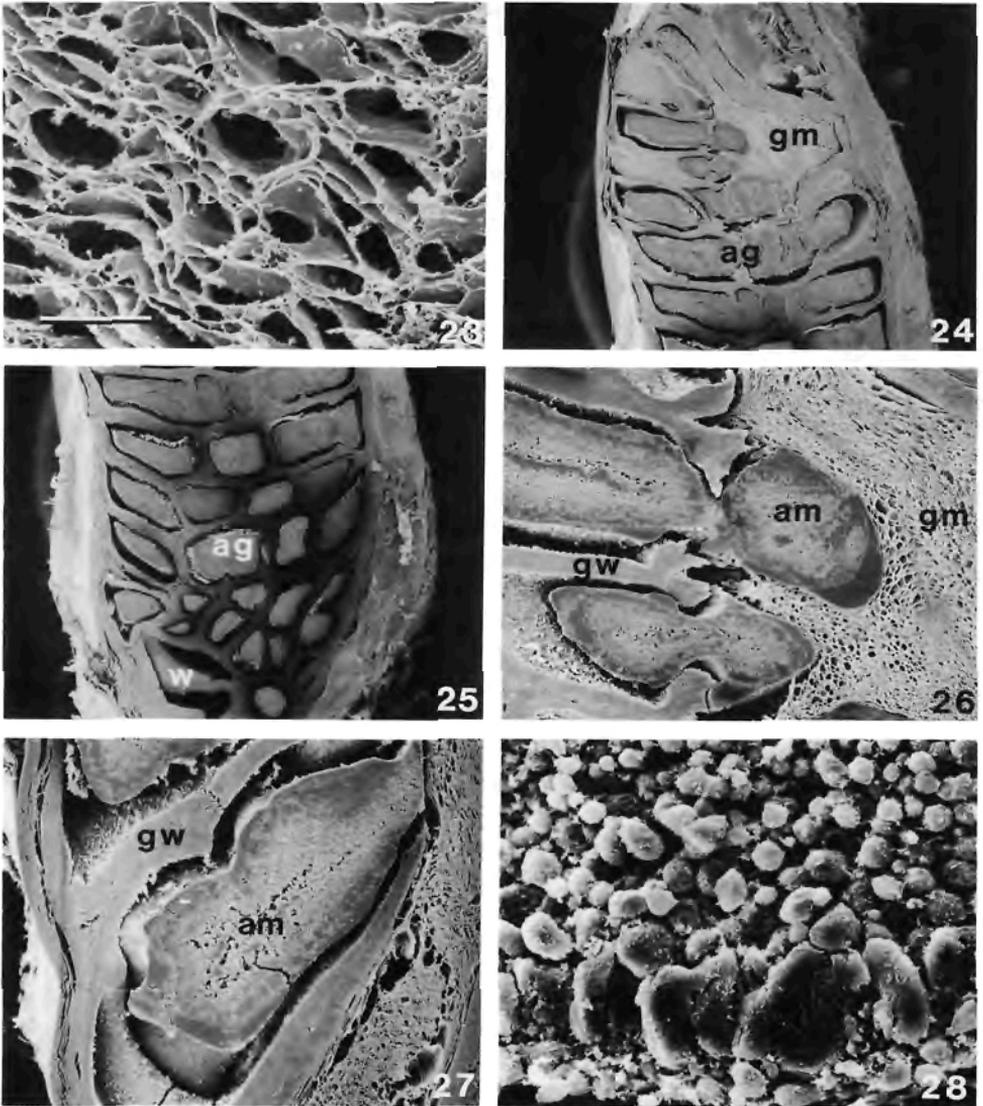


FIG. 17. Appendage chamber and contents in *Penaeus aztecus*. ap, appendage; aps, appendage chamber septum; ew, outer (anterior) wall of ejaculatory duct. FIG. 18. Anastomosing folds from appendage illustrated in Fig. 17. FIG. 19. Distal view of ejaculatory duct through middle part of ejaculatory duct of *P. setiferus*. ag, adhesive glands; d, dorsal; ss, sperm sac; t1, t2, typhlosoles 1, 2, respectively; v, ventral. FIG. 20. Posterior part of ejaculatory duct of *P. setiferus* (from Fig. 19) showing sperm sac under typhlosole 2. k, keel of wall of sperm sac; other labels same as for Fig. 19. FIG. 21. Distal view of transverse section through proximal part of right ejaculatory duct of *P. setiferus*. a, anterior; dp, dorsal plate; fg, flange; fp, flap; gm, glutinous material; t1, t2, typhlosoles 1, 2, respectively; v, ventral. FIG. 22. Posterior view of oblique section through right ejaculatory duct of *P. setiferus* showing glutinous material (gm) in chamber below adhesive glands (ag). ss, sperm sac. t2, typhlosole 2. Scale bar represents 430 μ m in Fig. 17, 19 μ m in Fig. 18, 1.5 mm in Fig. 19, 800 μ m in Fig. 20, and 570 μ m in Figs. 21, 22.



FIGS. 23-28. *Penaeus setiferus*. Fig. 23. Transverse section through glutinous material in ejaculatory duct. Figs. 24, 25. Anterior views of sections through the proximal (Fig. 24) and distal (Fig. 25) halves of anterior part of ejaculatory duct, showing numerous adhesive glands, glutinous material (Fig. 24), and wing (Fig. 25). Fig. 26. Secretion of adhesive gland discharged into chamber with glutinous material in ejaculatory duct. Fig. 27. Section through adhesive gland, showing undischarged adhesive material inside duct of gland. Fig. 28. Particles of secretion in adhesive gland, showing typical decrease in particle size from outer (below) to inner (above) part of gland lumen. ag, adhesive gland; am, adhesive material; gm, glutinous material; gw, wall of gland duct; w, wing. Scale bar represents 16 μm in Fig. 23, 1.5 mm in Fig. 24 and Fig. 25, 320 μm in Fig. 26, 260 μm in Fig. 27, and 19 μm in Fig. 28.

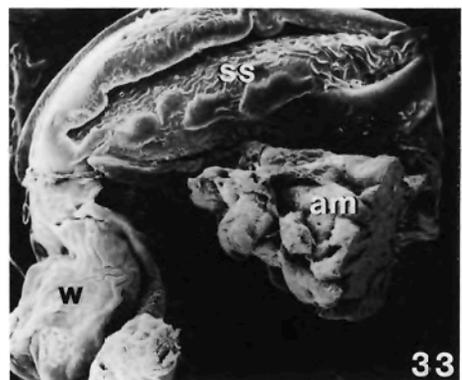
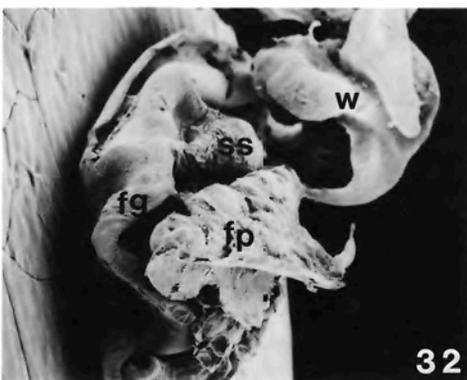
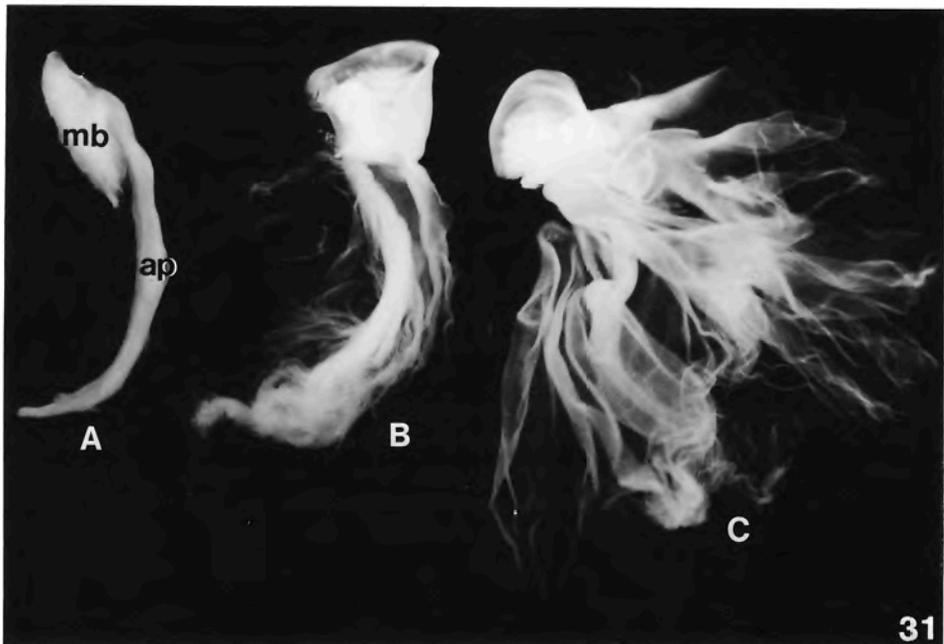
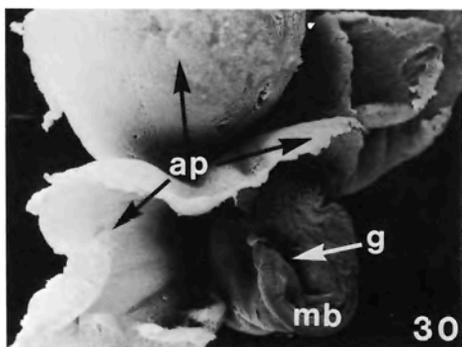
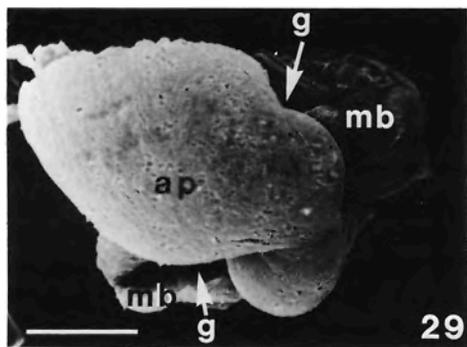
folds separated by a groove (Figs. 4, 29, 30). The appendage of *P. aztecus* is relatively much shorter than that of *P. duorarum* (Figs. 29, 31). The spermatophore of *P. setiferus* is more complex than that of *P. aztecus* and *P. duorarum*, consisting of the flap and flange, in the proximal part of the ejaculatory duct, the sperm sac proper, surrounding the sperm mass and its coat of acellular material, the dorsal plate, and the wing, from the distal part of the ejaculatory duct (Fig. 32). In addition, a lobate mass of material discharged from the adhesive glands of the ejaculatory duct is attached to the ejaculated spermatophore (Fig. 33). Glutinous material is located in the space between the flap and flange.

Spermatophore Reactions in Seawater

The appendage of the spermatophore of *P. aztecus* and *P. duorarum* quickly undergoes a drastic change in morphology when exposed to seawater (Figs. 29–31). When preserved immediately in 100% formalin, the appendage retains its original shape and condition (Figs. 29, 31A). When placed in seawater, the appendage rapidly (within 5 min) unravels to form an expanded, ruffled membrane (Figs. 30, 31C). Spermatophores placed immediately in 50% seawater formalin show a partial appendage reaction (Fig. 31B). Transverse sections of the appendage of an unreacted spermatophore (Fig. 34) show the anastomosing layers of material found in the ejaculatory ducts of both *P. duorarum* and *P. aztecus* (Figs. 13, 17). Sections through a reacted appendage of *P. duorarum* show that these layers unravel and stretch when exposed to seawater (Fig. 35).

Parts of the spermatophore of *P. setiferus* also undergo changes when exposed to seawater. Mechanically ejaculated spermatophores of this species were left longer in seawater (10–60 min) in an attempt to observe the same kind of obvious morphological change that occurs within minutes in the appendage of the spermatophores of *P. duorarum* and *P. aztecus*. In seawater, the adhesive material seems to change very little (Fig. 33) within the first hour following exposure to seawater, unless it touches some solid object to which it will adhere. Sections through unreacted adhesive material show that it is composed of particles or droplets identical in structure (Figs. 36, 37) and staining properties using Mallory's triple stain to the secretions found inside the adhesive glands

FIG. 29. Newly ejaculated spermatophore of *Penaeus aztecus*, with unreacted appendage (ap) above main body (mb). g, groove of main body. FIG. 30. Spermatophore of *P. aztecus*, showing folds (ap) of reacted appendage. g, groove of main body (mb). FIG. 31. Spermatophore reaction in *P. duorarum*. A, spermatophore placed in 100% formalin immediately following ejaculation; B, spermatophore placed in 50% seawater formalin (partial reaction); C, spermatophore exposed to seawater for 10 min before preservation (completed reaction). ap, appendage; mb, main body. FIG. 32. Newly ejaculated (unreacted) spermatophore of *P. setiferus*, viewed from proximal end of spermatophore. fg, flange; fp, flap; ss, sperm sac; w, wing. FIG. 33. Distal part of ejaculated spermatophore of *P. setiferus* showing unreacted adhesive material (am) (proximal part of spermatophore sectioned off). ss, sperm sac; w, wing. Scale bar represents 1.2 mm in Fig. 29, 1.3 mm in Fig. 30, Fig. 32, and Fig. 33, and 4.2 mm in Fig. 31.



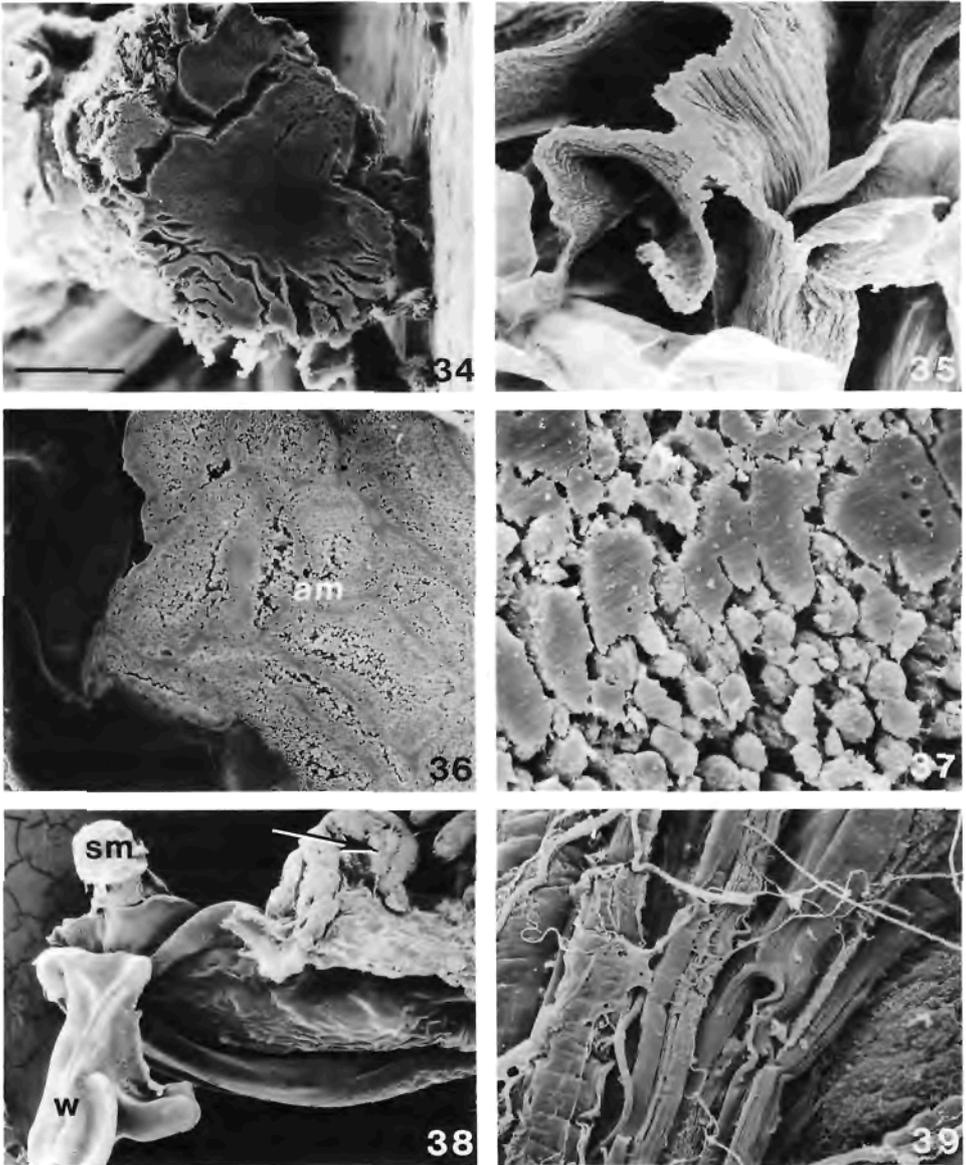


FIG. 34. Transverse section of appendage of unreacted spermatophore of *Penaeus duorarum*, showing tightly packed folds. FIG. 35. Transverse section of appendage of reacted spermatophore of *P. duorarum*, showing spreading of appendage folds. FIG. 36. Transverse section of unreacted adhesive material (am) from an ejaculated spermatophore of *P. setiferus*. FIG. 37. Higher magnification of sectioned particles of unreacted adhesive material shown in Fig. 36. FIG. 38. Distal part of spermatophore of *P. setiferus* showing "reacted" adhesive material (arrow) and extruded sperm mass (sm). w, wing. FIG. 39. Strings of reacted adhesive material from area at tip of arrow in Fig. 38. Scale bar represents 320 μm in Fig. 34, 500 μm in Fig. 35, 220 μm in Fig. 36, 14 μm in Fig. 37, 1.5 mm in Fig. 38, and 51 μm in Fig. 39.

of ejaculatory duct (Figs. 27, 28). The adhesive material attaches tenaciously and stretches out into elastic strands and strings if the spermatophore is removed from the object to which it is affixed (Figs. 38, 39). After several hours in seawater, the lobate mass of adhesive material changes into a flattened sheet-like structure.

The proximal end of the spermatophore of *P. setiferus* swells upon exposure to seawater (Figs. 32, 40). The source of this reaction is the glutinous material, located primarily between the flap and flange but extending distally along the spermatophore below the adhesive material (Figs. 41, 42). In some areas between the flap and flange, the glutinous material is densely packed just after ejaculation, but these sheets of material apparently separate upon exposure to seawater, causing inflation (Figs. 43, 44).

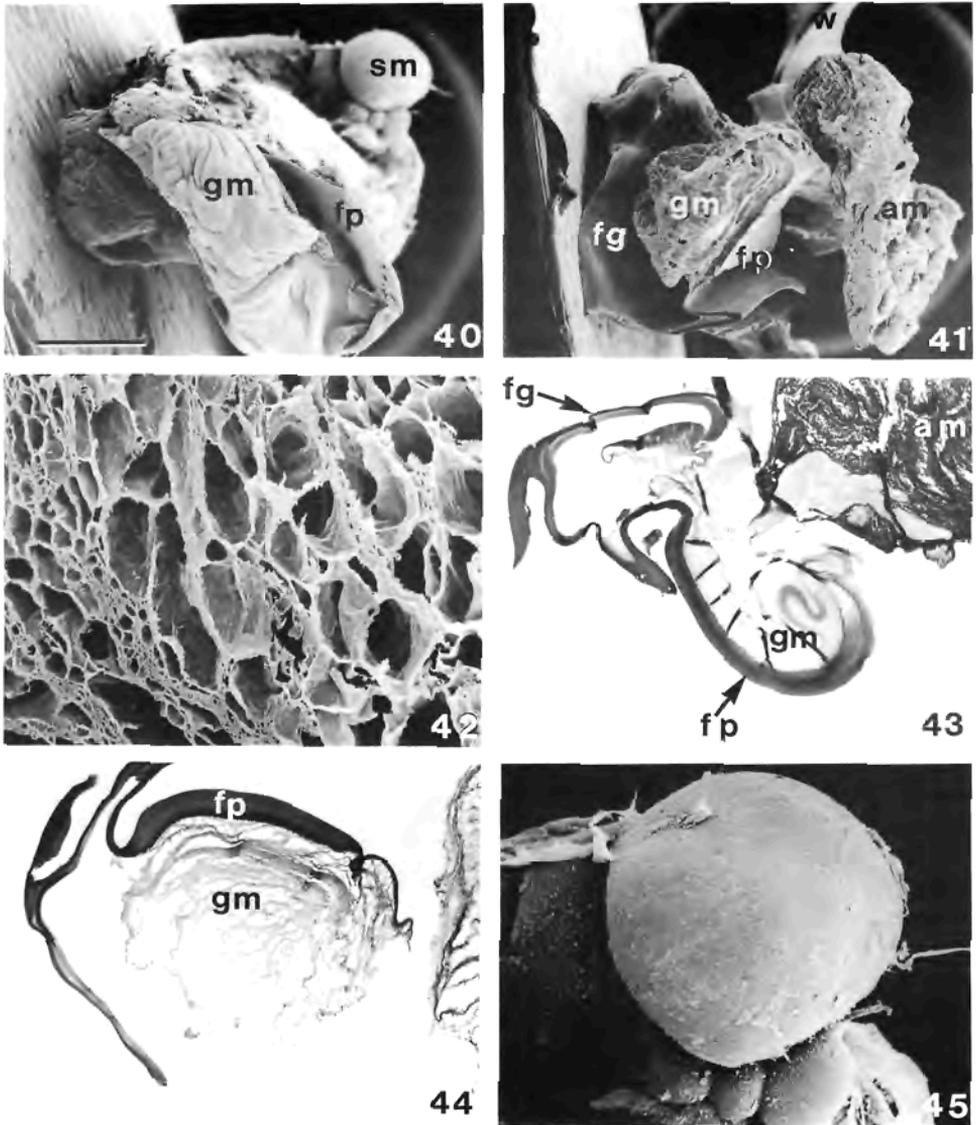
In addition to these changes, the other "reaction" of the spermatophore upon exposure to seawater is the production of a bulge or bulges of material at the distal end of the spermatophore (Figs. 38, 40, 45). These bulges are extrusions of the sperm mass from the sperm sac; spermatozoa and surrounding seminal fluid have been observed within them. The extruded sperm masses are highly adhesive and, unlike the adhesive material described earlier, retain this quality in seawater (up to 20 h during one observation).

DISCUSSION

The ejaculatory duct is, in part, an extension of the vas deferens in *Penaeus duorarum*, *P. aztecus*, and *P. setiferus*. Two typhlosoles have been traced from the medial and distal vas deferens into the posterior part of the ejaculatory duct. Typhlosole 1 of the vas deferens of *Penaeus* was termed the "septum" by Malek & Bawab (1974b) and Ro et al. (1990). This typhlosole originally is a septum that divides the vas deferens into two channels, but the septum detaches to become a typhlosole in the descending and distal vas deferens. We have followed Malek & Bawab (1974b) in calling the other typhlosole of the vas deferens "typhlosole 2."

Results of this study permit identification of homologies in the structure of the spermatophore and the anatomy of the ejaculatory duct when *P. setiferus*, *P. aztecus*, and *P. duorarum* are compared. The main body of the spermatophores of *P. aztecus* and *P. duorarum* clearly is homologous to the sperm sac of *P. setiferus*. Both the main body and the sperm sac originate in the channel of the vas deferens delimited by typhlosole 1, the sperm duct (Chow et al., 1990; spermatophoric duct of Malek & Bawab, 1974b; lumen 1 of Ro et al., 1990), and are composed of a sperm mass surrounded by a thick layer of acellular secretion originating in the sperm duct. In *P. duorarum* and *P. aztecus*, additional layers of material are secreted around the main body in the ejaculatory duct. The flap, flange, and wall of the sperm sac surrounding the sperm mass and its coat of acellular secretion in *P. setiferus* are formed from the densely staining substances secreted around typhlosole 2 in the vas deferens and in the ejaculatory duct.

The appendage material of the spermatophore of *P. aztecus* and *P. duorarum* appears to be homologous to the glutinous material of the spermatophore of *P.*



Figs. 40–45. *Penaeus setiferus*. Fig. 40. Reacted spermatophore, showing swelling of glutinous material at proximal end of spermatophore. Fig. 41. Reacted spermatophore sectioned in proximal part, showing glutinous material between flap and flange. Fig. 42. Transverse section of glutinous material from reacted spermatophore. Fig. 43. Transverse section through flap/flange area of unreacted spermatophore, showing closely packed layers of glutinous material inside flap. Fig. 44. Transverse section through flap of reacted spermatophore, showing separation of layers of glutinous material. Fig. 45. Extruded sperm masses at distal end of reacted spermatophore. am, adhesive material; fg, flange; fp, flap; gm, glutinous material; sm, extruded sperm mass; w, wing. Scale bar represents 1.3 mm in Fig. 40, 1.2 mm in Fig. 41, 47 μ m in Fig. 42, 610 μ m in Fig. 43 and Fig. 44, and 440 μ m in Fig. 45.

setiferus. The appendage material is formed in the channel of the vas deferens into which typhlosole 2 projects (accessory duct of Chow et al., 1990; "wing duct" of Malek & Bawab, 1974b; lumen 2 of Ro et al., 1990). The secretion that is the source material of the appendage is a lightly staining secretion that surrounds the densely staining material around typhlosole 2 in the accessory duct. A secretion similar in position and appearance in the accessory duct of *P. setiferus* is the apparent source of its glutinous material. In the ejaculatory duct, both the glutinous material of *P. setiferus* and the appendage material of *P. aztecus* and *P. duorarum* are composed of delicate anastomosing sheets that delaminate or unfold upon exposure to seawater. The appendage of the spermatophores of *P. aztecus* and *P. duorarum* is homologous to the "wing" of the spermatophores of *P. kerathurus* (Malek & Bawab, 1974b) and *P. indicus* (Champion, 1987). The term "wing" has been used by King (1948) and Pérez Farfante (1975) for a structure of the spermatophore of *P. setiferus* that is formed completely in a chamber in the ejaculatory duct. Therefore, the wing of the spermatophore of *P. setiferus* is neither homologous to the appendage of *P. aztecus* and *P. duorarum* (Bauer, 1991) nor to the "wing" of the spermatophores of *P. kerathurus* and *P. indicus*.

The contents of the descending vas deferens of the three species investigated shift in relative position after entry into the ejaculatory duct. In *P. duorarum* and *P. aztecus*, the sperm mass and surrounding acellular secretion from the sperm duct of the vas deferens extend posteriorly behind typhlosole 2, the latter being larger and more prominent than typhlosole 1 in the ejaculatory duct. This movement of material into a space that was part of the accessory duct of the descending vas deferens apparently forces the appendage material anteriorly into the appendage chamber. In *P. setiferus*, typhlosole 1 becomes quite reduced in size, and the space corresponding to the lumen of the sperm duct in the vas deferens is nearly closed. The contents of the sperm duct from the descending vas deferens seem to be moved posteriorly behind the large typhlosole 2 upon entry into the ejaculatory duct. We postulate that the glutinous material entering the ejaculatory duct from the accessory duct of the vas deferens is pushed anteriorly into the chamber behind the adhesive glands by the movement of the sperm mass and surrounding acellular secretion.

The adhesive glands of the ejaculatory duct of *P. setiferus* have no counterpart in *P. aztecus* and *P. duorarum*. Talbot et al. (1989) termed the secretion of these glands the "adhesive," and we agree with these investigators that the "glutinous material" of Pérez Farfante (1975) is composed of two substances with different origins, the adhesive and glutinous materials. The adhesive secretion appears to be discharged onto the spermatophore when it is ejaculated, and the droplets or particles of the secretion form adherent strings and sheets upon contact with a surface. The adhesive material serves to attach the spermatophore to the open thelycum of the female, as Talbot et al. (1989) have noted. The swelling of the glutinous material may aid in lodging the spermatophore in spaces and crevices on the underside of the female. Talbot et al. (1989) reported that the glutinous material was adherent, although less so than the adhesive material.

The function of the appendage reaction in transfer and storage of the spermatophores of *P. duorarum* and *P. aztecus* is unclear. The appendages of the compound spermatophore transferred to the female may project initially from between the lateral plates of the female thelycum just after insemination. The reaction that changes the tightly packed appendage into a dispersed sheet may function in spreading the appendage material throughout the aperture of the seminal receptacle prior to the hardening of the material into a thelycum seal. The appendage has not been found inside the seminal receptacle but remnants of the appendage are lodged between the thelycum plates of inseminated females (Bauer, 1991). Excess appendage material projecting from the thelycum may be scraped off by substrate particles during burrowing of the female, as suggested by Heldt (1938) for *P. kerathurus*.

We observed the extrusion of sperm masses from the spermatophore of *P. setiferus* after its exposure to seawater. Pérez Farfante (1975) illustrated similar sperm masses for *P. stylirostris* Stimpson, 1874 and discussed the importance of expulsion of sperm masses in the release of sperm for fertilization in species of *Litopenaeus*.

There is considerable variation in reproductive morphology within the genus *Penaeus*. As Pérez Farfante (1969) pointed out, the greatest differences in reproductive biology are between species with an "open thelycum" (subgenus *Litopenaeus*) and those with a "closed thelycum" (subgenera *Penaeus* s.s., *Fenneropenaeus*, *Farfantepenaeus*, *Melicertus*, *Marsupenaeus*). Spermatophores are attached externally to females in the first group, but are inserted into a cuticular pocket behind the thelycum, the seminal receptacle, in the latter group (Bauer, 1991; Pérez Farfante, 1969). Mating occurs among females that have mature ovaries, but that have not recently molted, in *Litopenaeus* (see Bray & Lawrence, 1984; Pérez Farfante, 1969, Yano et al., 1988). In species of *Penaeus* with a closed thelycum, mating occurs when the female has molted recently, as in other penaeids in which spermatophores are stored, because the contents of the cuticularized seminal receptacle are cast off with the molt (Bauer, 1991; Pérez Farfante, 1969).

Pérez Farfante (1969) emphasized the importance of insemination morphology and spermatophore structure in the systematics of *Penaeus*. Based on these features, she suggested that the subgenus *Litopenaeus* is the most primitive of the subgenera of *Penaeus* (as did Burkenroad, 1934). Bauer (1986, 1991) proposed that the overall evolutionary trend in insemination morphology of penaeoid genera was from ancestral taxa in which females have open thelyca and complex external spermatophores to advanced taxa in which spermatophore storage is internalized and the complexity of the spermatophore is reduced. In this investigation, detailed comparisons of ejaculatory ducts and spermatophores between a *Litopenaeus* species, *P. setiferus*, and two *Farfantepenaeus* species, *P. aztecus* and *P. duorarum*, shows how reduction of a complex external spermatophore to an internally deposited one with simpler structure may have taken place. The adhesive glands of *P. setiferus*, which secrete a material that helps the external spermatophore adhere to the open thelycum of the female, have been lost in the *Farfantepenaeus* species. Extensions of the spermatophore,

such as the flap, flange, and wing, that mechanically aid in lodging the spermatophore of *P. setiferus* in the open thelycum and among the bases of the posterior pereopods of the female, are absent from the enclosed spermatophores of the *Farfantepenaeus* species.

Although there are major differences in the morphology of the ejaculatory duct and spermatophores between *Litopenaeus* and *Farfantepenaeus* species, the genealogical relationship of the taxa is confirmed by the structural homologies determined during this investigation. However, given the disparity in structure and function of insemination morphology, in reproductive behavior, and in other aspects of morphology (Pérez Farfante, 1969) among the subgenera of *Penaeus*, serious consideration should be given to raising the taxonomic rank of these taxa from subgeneric to generic status. Such a change would more closely reflect the true evolutionary relationships of these taxa, and, in turn, permit realistic descriptions of character distributions in phylogenetic studies of penaeoid genera.

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