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## Gonopod tegumental glands: a new accessory sex gland in the Brachyura

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**Abstract** It is not yet known whether gonopod tegumental glands (GTG) previously described in one species of brachyuran crab (*Chionoecetes opilio*) are a general feature in this large taxon. In order to determine the prevalence and role of GTG in the Brachyura, the first gonopods of six species of boreo-temperate and tropical brachyurans belonging to four families were examined morphologically and histologically, using the PAS–Alcian-blue staining protocol: *Carcinus maenas*, *Portunus sebae*, and *Ovalipes ocellatus* (Portunidae), *Cancer irroratus* (Cancridae), *Grapsus grapsus* (Grapsidae), and *Petrolisthes armatus* (Porcellanidae). Discrete rosette-type GTG were found in all species examined, although the longitudinal extent and location differed somewhat between taxa. The GTG were invariably grouped about the ejaculatory canal, and communicated with the lumen of the ejaculatory canal via ducts which traversed pores in the cuticle; staining properties of secretions at the duct openings to the ejaculatory canal matched those of the GTG. Neither GTG, ducts, nor pores were observed in regions distal to the ejaculatory canal. These data indicate that the prime, if not exclusive, role of the GTG is in reproduction, and that GTG may therefore be considered accessory sex glands. Together with previous and current investigations such GTG have been observed in all eight brachyuran species examined from five families, and are thus probably ubiquitous within the Brachyura. The organization and nature of the gland secretions differed between taxa: alternating acid (AMPS) and

neutral mucopolysaccharide (NMPS) layers in the three Portunidae, AMPS only in *Cancer irroratus*, and NMPS only in *Grapsus grapsus* and *Petrolisthes armatus*. When combined with data on gonopod morphology and occurrence of spermatophore-less sealant in the ejaculate of various brachyurans, two plausible functions of the AMPS GTG secretions emerge: protection of the male's genetic investment (stored spermatophores) from opportunistic microbes following copulation, and the reciprocal processes of sperm competition and paternity assurance. The NMPS secretions may function as a lubricant to reduce mechanical wear of the ejaculatory canal by the second gonopod during copulation, and to reduce the viscosity of the ejaculate from the vas deferens as it enters the narrow ejaculatory canal.

### Introduction

Although crabs currently represent the greatest proportion of crustacean captures in the world's fisheries, at 1 738 407 tons declared in 1993 (Anonymous 1995), fisheries management has been limited by the availability of biological data concerning the extremely diverse assemblage of crab species (Haefner 1985; Bailey and Elnor 1989). In particular, investigations of crab reproduction are thinly scattered both in level of approach and taxa studied. The information which has emerged over the past 20 years indicates that in the largest group, the infraorder Brachyura, reproduction is much more complex than was previously believed (see Adiyodi 1983; Sastry 1983; Bauer and Martin 1991; Wenner and Kuris 1991; Elnor and Beninger 1995 for reviews and references). Multi-level data exist for very few species (Sastry 1983), the foremost being the circumboreal majid *Chionoecetes opilio*. Its status as a major fishery species of Canada, the United States, Japan, and Russia has doubtless contributed to the impetus of the research effort.

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An understanding of the functions of the reproductive system components is fundamental to the elucidation of reproductive processes in these organisms. Recent studies of the male and female systems have shown that events surrounding copulation, sperm transfer, storage, and fertilization in the Brachyura now appear to be quite complex, with possibilities of sperm competition and paternity assurance (Christy 1987; Beninger et al. 1991, 1993; Diesel 1990, 1991; Seigny and Sainte-Marie 1996), self-fertilisation by females whose broods are compromised (Beninger et al. 1993), differential spermatophore dehiscence (Beninger et al. 1988, 1993), and enhancement of oocyte and stored sperm viability through protection from opportunistic microbes (Beninger et al. 1993; Elner and Beninger 1995; Lanteigne et al. 1996).

The copulatory appendages, or gonopods, are of particular interest in the brachyuran male reproductive system, because they are morphologically diverse yet taxonomically very conservative, and hence provide valuable taxonomic and phylogenetic information (Stephenson 1946; Garth 1958; Williams 1965; Nishimura 1967; Abele 1971; Hartnoll 1975; Chambers et al. 1980; Bauer 1986; Martin and Abele 1986). These structures may be of even greater importance in the Brachyura, as a glandular function has recently been attributed to the first gonopods of *Chionoecetes opilio* (Beninger et al. 1991, 1995). Although considerable progress has been made in the study of the glandular functions of the vas deferens and spermatheca (Spalding 1942; George 1963; Ryan 1967; Johnson 1980; Beninger et al. 1988, 1993; Lanteigne et al. 1996), the gonopod tegumental glands (GTG) have received scant attention. Emerging from the debate concerning the functions of such glands in the crustacean exoskeleton in general is the overarching need for more data (see Stevenson 1985; Felgenhauer 1992; Fingerman 1992; Subramoniam 1993; Talbot and Demers 1993 for reviews). That the GTG are related to reproductive activity in the male was first suggested by Beninger et al. (1991) for *C. opilio*; subsequent investigations confirmed their role as male accessory sex glands in this species (Beninger et al. 1995). No further studies have been published to date concerning this aspect in other brachyurans, or indeed in other decapods, so no evaluation of its occurrence or role within these taxa has been possible. The present study reports on investigations into the distribution, histology, and secretion types of GTG discovered in six additional brachyuran species from four additional families, encompassing both boreo-temperate and tropical habitats, as well as both aquatic and semi-terrestrial species. The results are related to gonopod morphology, possible secretion roles, and evolution of the functions of the ventralmost ejaculate within the brachyuran spermatheca.

## Materials and methods

### Sampling

#### *Boreo-temperate species*

Mature male specimens of *Cancer irroratus* (carapace width, CW, 91.5 to 101.1 mm) were collected via SCUBA in the Northumberland Strait (46°14'N; 64°12'W), 4 July 1995, 25 July 1995, and 15 August 1995. The gonopods of these individuals were removed in the field and fixed in aqueous Bouin's solution for later histological processing. In order to detect any differences in pre- and post-copulatory gland characteristics, three couples in pre-copulatory embrace were collected via SCUBA on 2 July 1996 at the same sampling site and returned to the laboratory, where they were placed in a recirculating seawater system at the same temperature, salinity and approximate ambient light conditions as the sampling site (20 °C, 24‰, natural shaded daylight). The aquarium bottom contained large rocks as refuges. One male and one female were placed together in three separated portions of the aquarium. All couples formed pre-copulatory embraces of variable duration; the left first gonopods were removed and fixed as above upon observing pair formation, and prior to female moulting which precedes copulation in this species (Hartnoll 1969). Although removal of the remaining first gonopod was intended following copulation, males were unable to successfully copulate after removal of the left first gonopod.

Mature male *Ovalipes ocellatus* and additional mature male *Cancer irroratus* were subsequently collected at the sampling site 11 July 1996. The gonopods were removed and placed in aqueous Bouin's fixative immediately upon surfacing.

Mature male specimens of *Carcinus maenas* and an embracing pair (hard-shelled male, soft-shelled female) were sampled from the intertidal in Passamaquoddy Bay (45°7'N; 67°4'W) on 25 July 1995 and placed in the recirculating seawater system. The embracing pair were observed to copulate upon being placed in the aquarium. The first gonopods were removed and fixed as above (after completion of copulation in the case of the copulating male).

All crabs in the aquarium were fed daily with pollock and occasionally mussels, scallops, or oysters. Excess food was removed 3 h after presentation, and feces were removed daily. The aquarium filters were cleaned daily with boiling water in order to prevent microbial proliferation.

#### *Tropical species*

Mature male *Portunus sebae* were collected from Discovery Bay, Jamaica (18°24'N; 77°27'W) via SCUBA on 20 and 29 December 1995. Mature specimens of *Grapsus grapsus* were also collected in the intertidal in Discovery Bay on 26 December 1995. Mature specimens of *Petrolisthes armatus* were collected via SCUBA from Runaway Bay (18°24'N; 77°20'W) on 27 December 1995.

### Histological processing

In order to improve paraffin penetration, the gonopod cuticles were perforated using entomological needles in the region opposite the ejaculatory canal. The gonopods were stored in aqueous Bouin's fixative for at least 3 months, in order to achieve sufficient softening of the cuticle (Beninger et al. 1995). Scientific drawings were made under stereoscopic microscope of the first gonopods for all species, from both the anterior and posterior aspects. The gonopods were bisected transversally and processed for histology as described in Beninger et al. (1995). The two gonopod lengths were embedded side-by-side in order to facilitate serial sections. Transverse serial sections were cut at 10 µm (optimum thickness for intact sections) in order to determine gland distribution within the first gonopod of each species. Once this distribution was ascertained, subsequent sections were performed directly in the gland region of the gonopods.

Preliminary tests using the PAS–Alcian-blue staining protocol (Beninger et al. 1995) resulted in the use of staining times as indicated in Table 1. These protocols allowed visualization of both cuticle and gonopod tissues, although in some cases staining of the glands and hypodermis was unavoidably intense due to the weak staining affinities of the cuticle.

Diastase-digested negative controls were run to ensure that the stained substances were mucopolysaccharides and not glycogen deposits. Photomicrographs of gonopod internal structure, gland presence, position, and secretion type were obtained using a Leitz DMRB microscope. The secretion colours were standardized using the Pantone standard colour scale (Beninger et al. 1993).

## Results

### Gland type and cuticle pores

Glands were observed within the first gonopods of all specimens studied (Figs. 1 to 4). Their histology corresponded to the rosette-type tegumental glands (Talbot and Demers 1993). The longitudinal positions of the glands varied somewhat between species, as did the type of secretion (see below). However, in all species, glands were grouped about the ejaculatory canal, which invariably presented large pores (diameter 2.5 to 3.0  $\mu\text{m}$ ), lined with ducts often containing the same secretions of the glands (Figs. 3, 4). Continuity between the ducts of the gland system and the pores was often apparent in sections (e.g. Fig. 4.6, 4.7, 4.9). No pores or ducts were observed elsewhere than in the cuticle of the ejaculatory duct.

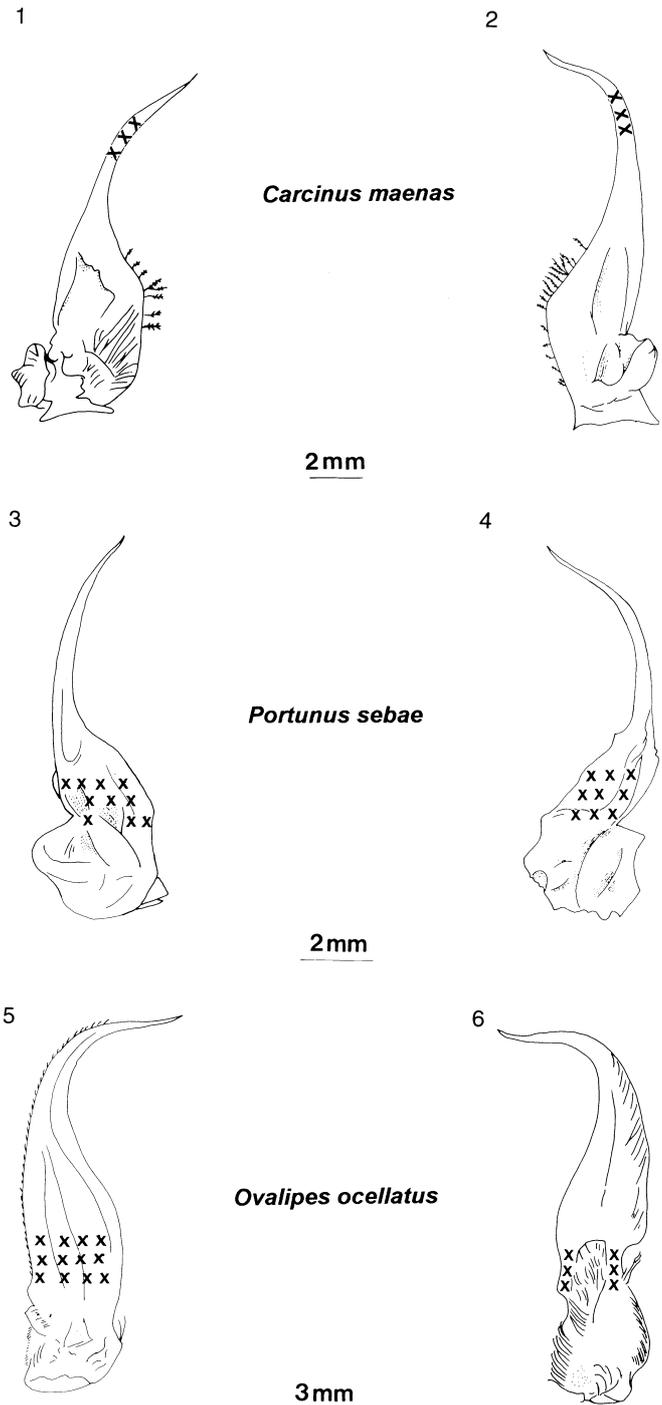
### Secretion types

Table 1 shows the visual colours of the gland secretions, their corresponding Pantone equivalents, and mucopolysaccharide (MPS) types. Both acid and neutral mucopolysaccharides were observed, depending on the species (see below).

### Gonopod morphology, gland distribution and secretion types by species

#### *Carcinus maenas*

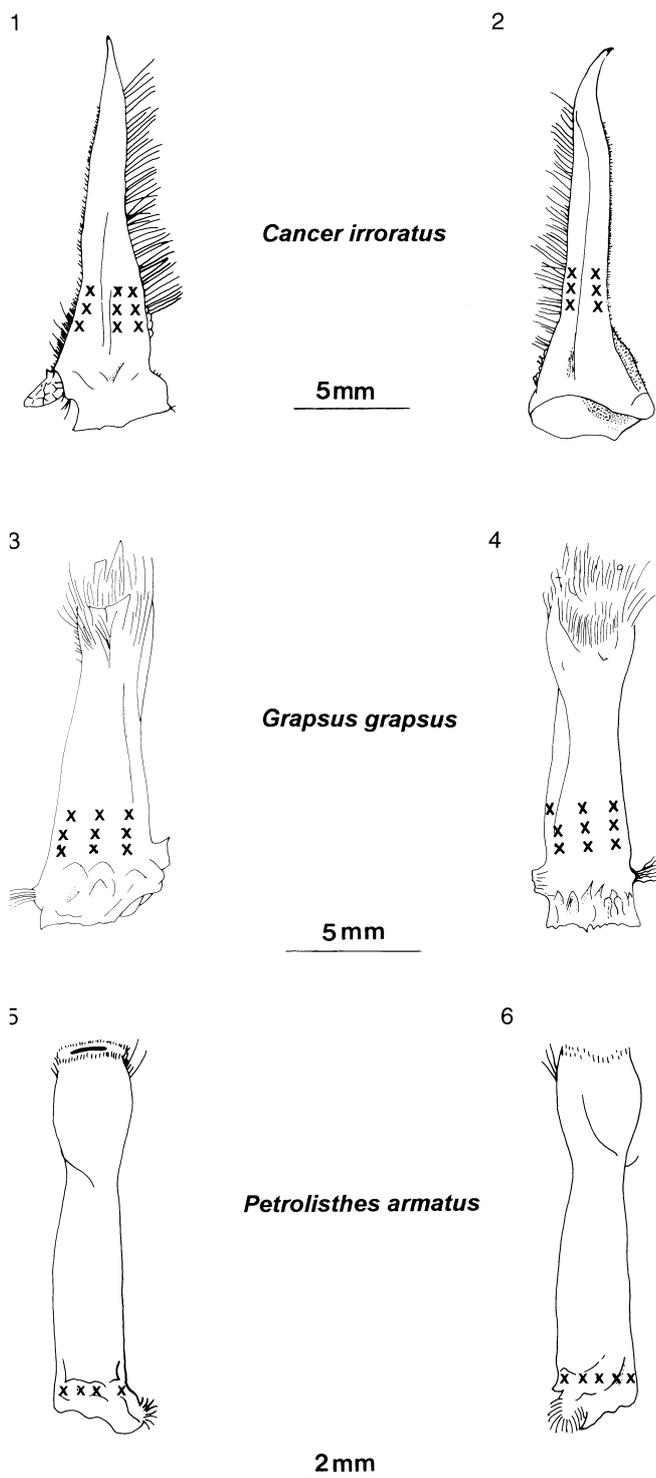
The first gonopod tapered quickly to a thin, curved process terminating in a point. Tegumental glands were situated much further distally than in the remaining species, between approximately 3/5 and 4/5 of the gonopod length (Fig. 1.1, 1.2). In the single males the glands appeared to be paired, with an intensely staining neutral mucopolysaccharide (NMPS) layer beneath an intensely staining acid mucopolysaccharide (AMPS) layer (Fig. 4.2, 4.3; Table 2). In the male which had copulated, only NMPS secretions were visible, indicating that all of the AMPS had been transferred during copulation (Fig. 4.4, 4.5). The remaining NMPS entirely filled the glands, suggesting that none had been transferred (Fig. 4.5).



**Fig. 1** Schematic representation of first gonopods of boreo-temperate brachyurans studied. 1.1, 1.3, 1.5, anterior aspect; 1.2, 1.4, 1.6, posterior aspect. Hatched areas indicate segments of gonopod in which tegumental glands are localized

#### *Portunus sebae*

The first gonopod tapered abruptly to a thin, curved process. The tegumental glands were located at approximately 2/5 of the gonopod length (Fig. 1.3, 1.4), and showed alternating layers of NMPS and AMPS glands tightly appressed to one another, as in *Carcinus maenas* (Table 2).



**Fig. 2** Schematic representation of first gonopods of tropical brachyurans studied. **2.1, 2.3, 2.5**, anterior aspect; **2.2, 2.4, 2.6**, posterior aspect. Hatched areas indicate segments of gonopod in which tegumental glands are localized

### *Ovalipes ocellatus*

Like the other portunids *Carcinus maenas* and *Portunus sebae*, the gonopods were tapered and curved, with tegumental glands located in the proximal 2/5 of the

gonopod (Fig. 1.5, 1.6). The glands were organized in bilayers of intensely staining NMPS-containing glands beneath intensely staining AMPS-containing glands (Fig. 4.6; Table 2). The contours of the two gland types were almost exactly identical.

### *Cancer irroratus*

The first gonopods were quite robust, very slightly curved at the distal extremity, tapering to a point. The tegumental glands were confined to the proximal 2/5 of the gonopod (Fig. 2.1, 2.2). Only AMPS was present in these glands (Fig. 4.1; Table 2), both in the single males and in the males which had formed precopulatory embraces.

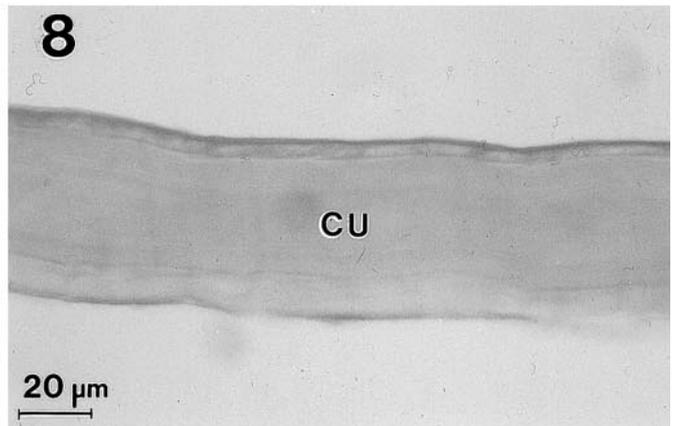
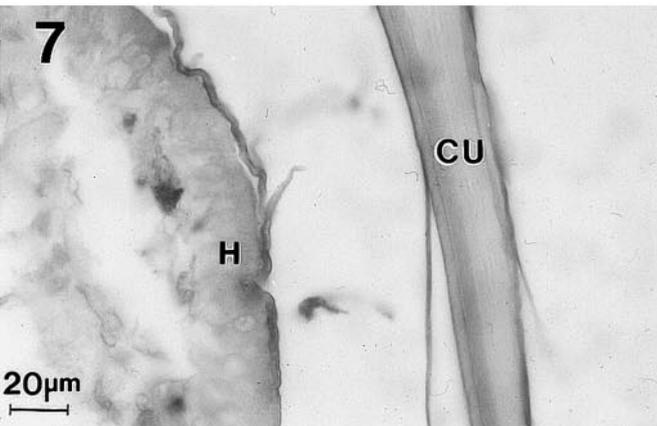
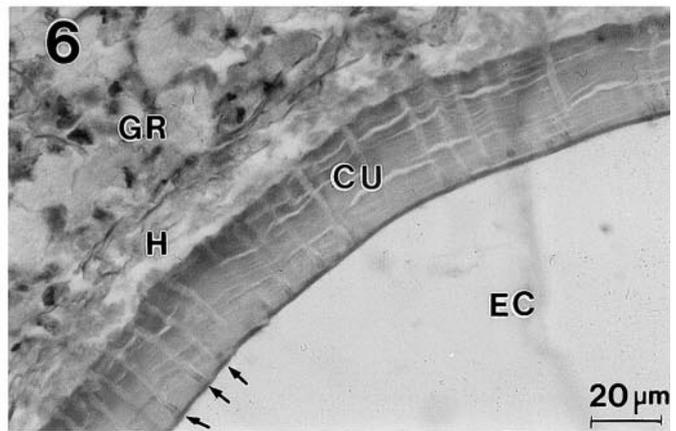
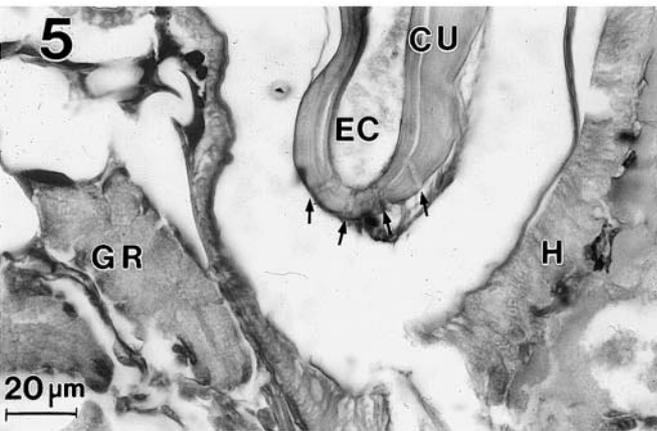
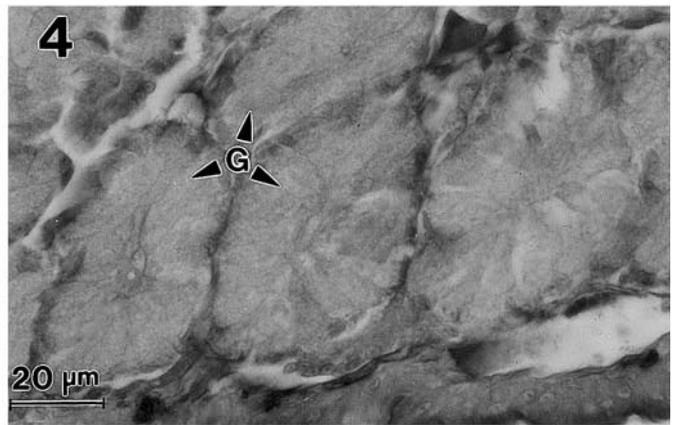
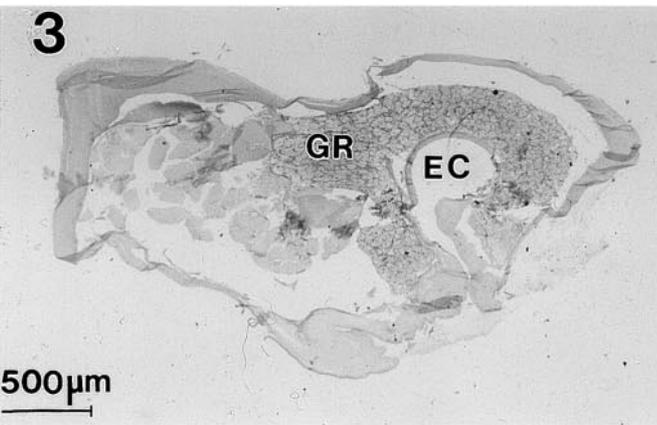
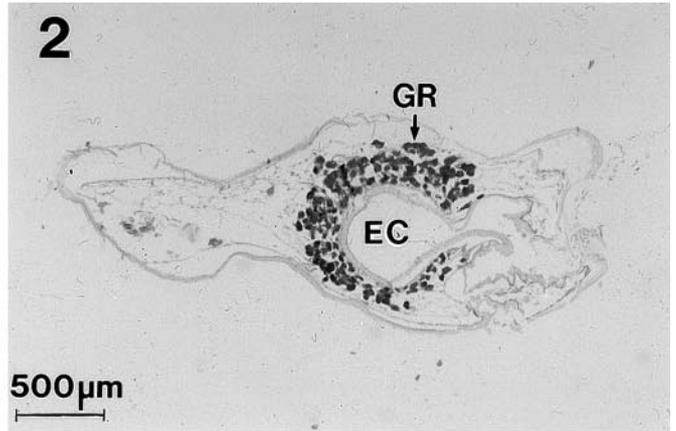
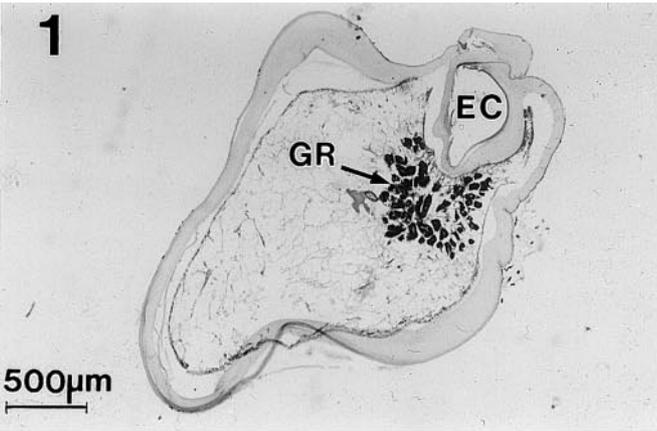
### *Grapsus grapsus*

The first gonopod was squat, straight, and presented a flat distal extremity. The tegumental glands were situated in the proximal 2/5 of the gonopod length (Fig. 2.3, 2.4). Only NMPS secretions were observed in these glands, and the intense fuschia colour (Table 1) indicated that the secretions were fully constituted. The mating status was unknown.

### *Petrolisthes armatus*

The first gonopod morphology was similar to that of *Grapsus grapsus*: straight, somewhat narrower, terminating in a flat tip. The tegumental glands were confined to a very restricted region (50  $\mu$ m) at the base of the gonopod (Fig. 2.5, 2.6). Although in all other species examined, the cuticle pores were grouped around the extent of the ejaculatory canal, in *P. armatus* the pores were clustered around the deepest part of the ejaculatory canal (Fig. 3.5). The shrunken duct walls lining the pores made their identification somewhat more difficult. The gland secretions consisted of NMPS only, and their

**Fig. 3** Transverse histological sections of the first gonopod in the brachyurans studied (periodic acid-Schiff-Alcian-blue protocol). Transverse section of whole gonopod in: **3.1** *Grapsus grapsus*, **3.2** *Carcinus maenas*, **3.3** *Cancer irroratus*. Note the position of the glandular region (GR) proximal to the ejaculatory canal (EC), typical of all species studied. **3.4** Detail of glands (G) in *Petrolisthes armatus*, showing rosette structure typical of all species studied. **3.5** Detail of ejaculatory canal (EC) region in *Petrolisthes armatus*. Note pores (arrows) in cuticle (CU) at base of ejaculatory canal (GR gland region; H hypodermis, mechanically detached from cuticle during histological processing). **3.6** Detail of ejaculatory canal (EC) in *Cancer irroratus*. Note pores (arrows) traversing cuticle (CU). Subjacent hypodermis (H) and gland region (GR) intact. **3.7** *Petrolisthes armatus*, detail of cuticle (CU) distal to ejaculatory canal. Note absence of pores in cuticle (H hypodermis). **3.8** *Cancer irroratus*, detail of cuticle (CU) in region distal to ejaculatory canal. Note absence of pores in cuticle



**Table 1** Visual and Pantone standard colours of gonopod tegumental glands (GTG) following staining with PAS–Alcian-blue protocol and corresponding mucopolysaccharide type (AMPS acid mucopolysaccharide; NMPS neutral mucopolysaccharide)

Species	Staining times (min)		Visual colours	Pantone standard colours	Secretion type
	Alcian-blue	PAS			
<i>Carcinus maenas</i> Before copulation	1	1	Alternating layers of blue and pink	2927 c	AMPS + NMPS
After copulation			Light pink	251 c	NMPS
<i>Portunus sebae</i>	6	6	Alternating layers of dark blue and pink	2745 c, 2727 c 239 c	AMPS + NMPS
<i>Ovalipes ocellatus</i>	3	4	Alternating layers of blue and pink	293 c 251 c	AMPS + NMPS
<i>Cancer irroratus</i>	6	6	Dark blue	293 c	AMPS
<i>Grapsus grapsus</i>	7	5	Fuschia pink	225 c	NMPS
<i>Petrolisthes armatus</i>	7	3	Very light purple-pink	677 c	NMPS

pale staining indicated that the secretions were not fully constituted (Table 1).

## Discussion

### Gland distribution

Gonopod tegumental glands were found in all six species of the present study; when combined with published reports on the circumboreal majid *Chionoecetes opilio* (Beninger et al. 1991, 1995) and current investigations on the tropical majid *Mithrax spinosissimus* (author's personal observations), such glands have been revealed in all eight of the species studied, comprising five brachyuran families. Their presence in the Brachyura thus appears to be general, and several salient features are common. (1) The glands are invariably grouped around and in proximity to the ejaculatory canal; no glands are observed distal to the canal. (2) The glands communicate with the lumen of the ejaculatory canal via ducts which pass through large (2.5 to 3.0  $\mu\text{m}$ ) cuticular pores. No such ducts or pores are observed elsewhere in the gonopod. (3) Staining affinities of secretions at the pore openings to the ejaculatory canal lumen match those of the glands.

These three common features confirm that the gonopod tegumental glands of brachyurans are involved chiefly, if not exclusively, in reproduction. Experimental confirmation of this function can be found in the difference between pre- and post-copulatory secretion types of the *Carcinus maenas* glands, where it is clear that AMPS were transferred to the female during copulation.

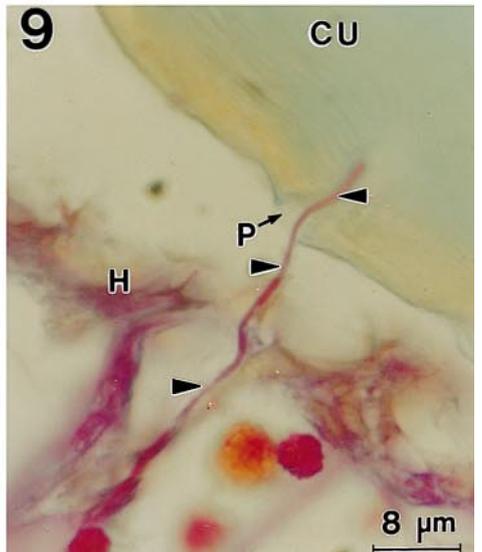
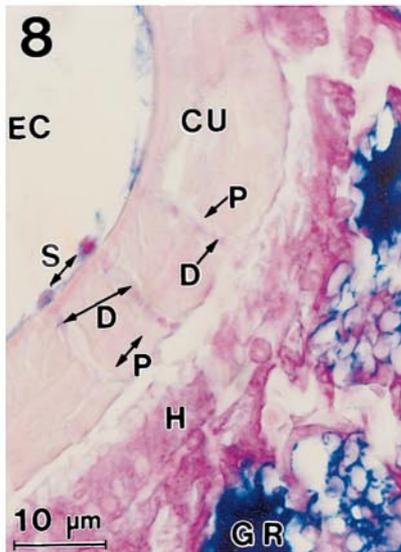
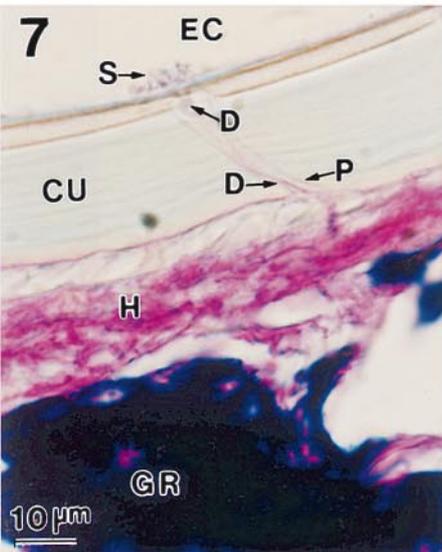
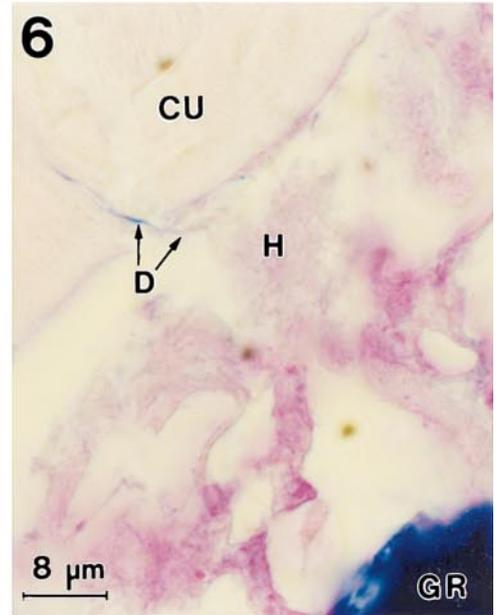
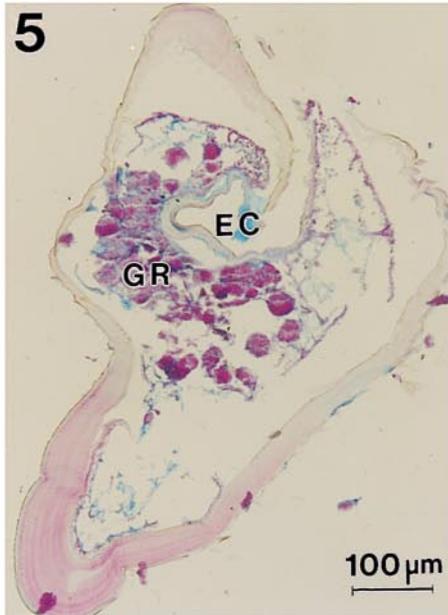
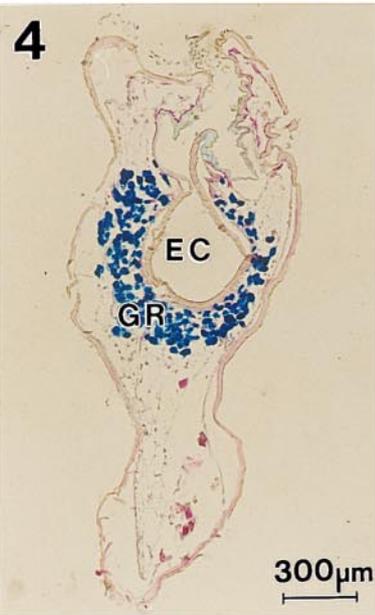
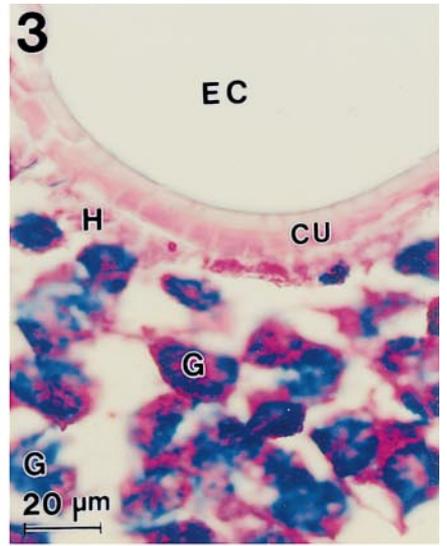
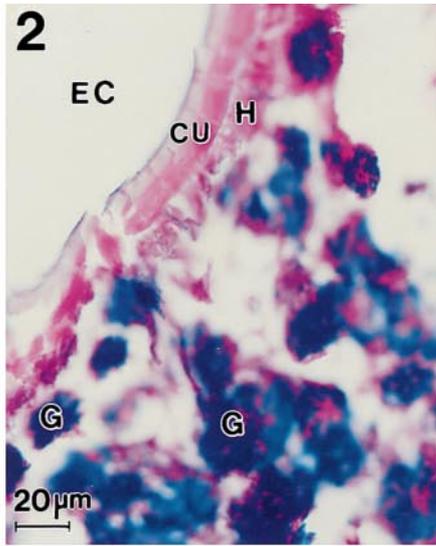
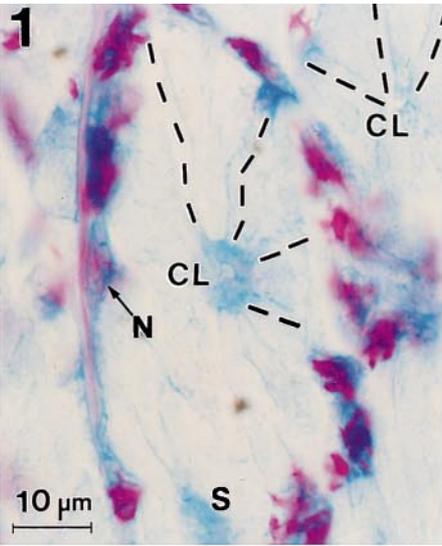
The major differences between taxa were in the longitudinal position of the glands within the gonopods and in the gland secretion types, as highlighted below.

### Longitudinal position of glands

The precise longitudinal position of the glands within the first gonopods showed considerable variation

between families. While the glands were usually located somewhere in the proximal region of the endopod, they were found in the distal region in *Carcinus maenas*. Similarly, the longitudinal extent of the glandular region differed between taxa, ranging from 50  $\mu\text{m}$  in *Petrolisthes armatus* to 2–2.5 mm in the remaining species, for similar gonopod sectional diameters. While it is impossible at this time to conclude whether the differences in longitudinal position correspond to functional attributes, it is clear that the volume of secretion will be proportional to the gland volume, and hence much reduced in *P. armatus*. The significance of these differences, in turn, must await further studies on the exact roles of the GTG in reproduction in the various brachyuran families. In the meantime, it must be emphasized that the site of the GTG must be determined by serial section for each new species studied.

**Fig. 4** Color photomicrographs of histological sections of first gonopods in the brachyurans studied, illustrating gland secretion types (periodic acid–Schiff–Alcian-blue protocol). **4.1** Detail of two rosette glands in *Cancer irroratus* (CL common locus; N nucleus; S secretions; dashed lines indicate cell contours). **4.2, 4.3** *Carcinus maenas* rosette glands (G) proximal to the ejaculatory canal (EC). The acid mucopolysaccharide secretions (AMPS, Alcian-blue positive, Fig. 4.2) are situated in a plane of focus above the neutral mucopolysaccharides (NMPS, periodic acid–Schiff positive, Fig. 4.3), indicating that the two secretion types are typically stratified in this manner (CU cuticle; H hypodermis). **4.4, 4.5** AMPS of *Carcinus maenas* present prior to copulation (Fig. 4.4) are absent in a male known to have copulated recently, which shows only NMPS (Fig. 4.5) (GR gland region). **4.6–4.9** Details of pores and gland ducts in ejaculatory canal cuticle. **4.6** *Ovalipes ocellatus*, gland region (GR) containing Alcian-blue positive secretions, and a cuticular duct (D) containing Alcian-blue positive secretions (H hypodermis). **4.7** *Portunus sebae*, detail of cuticular pore (P), and duct (D), with secretions (S) on the pore opening to the ejaculatory canal (EC) (CU cuticle; GR gland region; H hypodermis). **4.8** *Ovalipes ocellatus*, detail of cuticular pores (P) and ducts (D), with secretions (S) on the pore opening to the ejaculatory canal (EC) (GR gland region; H hypodermis). **4.9** *Grapsus grapsus*, detail of duct (arrowheads) from gland region penetrating pore (P) in cuticle (CU) of ejaculatory canal (H hypodermis)



**Table 2** Characteristics of gonopods and GTG secretions for Brachyura investigated to date

Family	Species	Gonopod morphology		GTG secretions		
		Tapered	Curved	AMPS	AMPS + NMPS alternating layers	NMPS
Portunidae	<i>Carcinus maenas</i>	+	+			
	Before copulation				+	
	After copulation					+
	<i>Portunus sebae</i>	+	+		+	
	<i>Ovalipes ocellatus</i>	+	+		+	
Canceridae	<i>Cancer irroratus</i>	+	-	+		
Majidae	<i>Chionoecetes opilio</i>	+	+	+		+
	(Beninger et al. 1991, 1995)					
	<i>Mithrax spinosissimus</i>	+	+	+		+
	(Beninger unpublished)					
Grapsidae	<i>Grapsus grapsus</i>	-	-			+
Porcellanidae	<i>Petrolisthes armatus</i>	-	-			+

### Cuticular pores

The cuticular pores associated with the GTG system appear to be specific to this context. The normal ubiquitous cuticular pores of the crustacean exoskeleton are much smaller than those observed in the present study and in Beninger et al. 1995 [0.06 to 0.2  $\mu\text{m}$ , Halcrow (1993) vs 2.5 to 3  $\mu\text{m}$ ]. The size of the GTG cuticular pores probably constitutes an adaptation for the secretion of relatively large quantities of MPS, as would be necessary during copulation.

### Gland structure, secretion types, and possible roles

The GTG in all of the species examined to date present the same histological features, belonging to the class of crustacean tegumental glands termed rosette glands (Talbot and Demers 1993). The homogeneous distribution of secretions within the secretory cells corresponds to the profile of the common Type A subclass (Talbot and Demers 1993). In contrast to their histological homogeneity, the GTG present inter-familial differences in the types and distributions of secretions. The Portunidae are characterized by glands which possess distinct superimposed layers of AMPS and NMPS, and the result of the *Carcinus maenas* copulation indicate that, whereas NMPS remains following ejaculation, all of the AMPS is transferred. These observations suggest that independent control of the two secretion types is possible in the Portunidae, rendering previous hypotheses on their function plausible (Beninger et al. 1995). The low-viscosity NMPS could thus be used to facilitate the passage of the more viscous vas deferens ejaculate in the narrow ejaculatory canal, whereas the more viscous AMPS could constitute the layers of sealant (often unsuitably referred to as "sperm plugs") observed in the spermatheca and vagina following copulation in many species, including *Cancer irroratus*, *Carcinus maenas*, and *Ovalipes ocellatus* of the present study (Chidester 1911;

Spalding 1942; Ryan 1967; Hartnoll 1969; Bawab and El-Sherief 1989). The hypothesis that the sealant is derived at least in part from the gonopod tegumental glands was proposed by Spalding (1942) for *Carcinus maenas*, although he did not perform the most appropriate histochemical tests (PAS and Alcian blue) and therefore was unable to determine the dominant composition of the gland secretions and the sealant itself. Nevertheless, his hypothesis has been virtually ignored ever since. Beninger et al. (1995) reasoned that the origin of this substance must be found outside the vas deferens, which contains spermatophores, whereas the sealant does not; hence the most likely origin must be the GTG. The sealant in the vagina of *Chionoecetes opilio* is known to consist entirely of AMPS (Beninger et al. 1993). Phenolic tanning of the sealant is also generally supposed (Bawab and El-Sherief 1989), although hardening could also be accomplished by specific AMPS components such as chondroitin sulphate (Radha and Subramoniam 1985).

The sealant may participate in several complex functions due to its mechanical and chemical properties. AMPS are highly sulphated, and components such as hyaluronic acid and chondroitin sulphate are anti-microbial (Sasikala and Subramoniam 1987; Subramoniam 1991); these characteristics may therefore function to protect the female gonoduct and thus both directly and indirectly the male's genetic investment following transfer of spermatophores to the spermatheca (Beninger et al. 1993). The most complex and controversial role may, however, be in the interplay between sperm competition and paternity assurance.

First developed in the field of ecological entomology (Parker 1970), the concept of sperm competition was evoked in brachyurans independently by Diesel (1990, 1991) and Beninger et al. (1991). Briefly, since females can store large quantities of viable sperm for protracted periods, the sperm of several males could thus compete for fertilization of oocytes at ovulation. Since decapod sperm are non-motile, such competition essentially cen-

tres around the strategic positioning of sperm by the male within the spermatheca, i.e. closest to the oviduct. Males may increase the probability of representation of their genome either by offensively displacing and sealing off rival sperm and substituting their own, or by defensively “locking” their sperm into place (see Diesel 1991 for review). Ventral sealing of the vagina (“sperm plug”) has thus been ascribed a defensive role (e.g. Bauer and Min 1993), whereas displacement and sealing of a previous male’s spermatophoric mass accomplished by the gonopod and its ejaculate would fulfill an offensive role (e.g. Diesel 1990; Beninger et al. 1991). If indeed the origin of the sealant lies with the GTG, it is of interest to note that both offensive and defensive roles would therefore have evolved in the same structure (the first gonopod), as is the case for the insects in which sperm competition has been observed (Waage 1979).

The evolution of the reciprocal phenomena of sperm competition and paternity assurance in the Brachyura thus centres around the first gonopod. As any improvement in the defensive mechanisms automatically selects for improvements in offensive mechanisms, this evolutionary interplay would be synergistic. For example, Diesel (1991) has proposed that in the time required for hardening of the ventral sealant in *Portunus sanguinolentus*, the pointed extremity of a rival male’s first gonopod could pierce the sealant and deposit its sperm somewhat more dorsally in the spermatheca, displacing the previous male’s sperm ventrally and placing its own in a more favorable position with respect to the oviduct in this species. This is an intriguing area of biology, both from the practical and theoretical points of view. Unfortunately, although much indirect evidence points to the existence of sperm competition and paternity assurance in the Brachyura, very little experimental confirmation has been provided – in fact the hypotheses and concepts have far outstripped the available data base of two majid species, *Inachus phalangium* (see Diesel 1990, 1991) and *Chionoecetes opilio* (Séigny and Sainte-Marie 1996). Adequate understanding of these processes requires nearly simultaneous studies of multiple facets of the reproductive biology of each species, and a panorama of such research to provide the taxonomic comparisons necessary for evolutionary insight. In particular, such studies must carefully relate (1) gonopod morphology, (2) spermatheca morphology and oviduct position, (3) composition of GTG secretion transferred, (4) composition of sealant, and (5) behavioral elements such as mate guarding.

An interesting perspective opened by the studies to date on the GTG is that the primitive function of their secretions may have been simply to seal the vagina from entry of either seawater (which could cause premature spermatophore dehiscence and spermatozoan acrosome reaction; Beninger et al. 1988, 1993) or opportunistic microbes – both accomplished by the viscous AMPS observed in the ventral region of the vagina in the primitive majid *Chionoecetes opilio* (Beninger et al. 1993). The additional advantage of paternity assurance

may have appeared with the hardening of this AMPS mass in the more advanced families such as the Portunidae, within which the processes of paternity assurance have putatively been evolving synergistically ever since.

Although the potential roles of the mucopolysaccharide secretions in the GTG are considerable, it should be stressed that a complete chemical characterization of the GTG secretions will be necessary for a full understanding of their roles in reproduction. Research on insects, which is incomparably more advanced than that on Crustacea, has demonstrated biological activity in the secretions of male accessory sex glands, such as elevation of oviposition and repression of subsequent sexual receptivity (Chen 1984) – again increasing the probability of success for the copulating male’s genome. Having demonstrated the ubiquity and reproductive nature of GTG in the Branchyura, these multiple perspectives are open for exploration.

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## References

- Abele LG (1971) Scanning electron micrographs of brachyuran gonopods. *Crustaceana* 21: 218–219
- Adiyodi RG (1983) Reproduction and its control. In: Bliss DE, Mantel LH (eds) *The biology of Crustacea*. Vol. 9. Integument, pigments, and hormonal processes. Academic Press, New York, pp 147–215
- Anonymous (1995) *Fishery statistics – captures and landings*. FAO, Rome
- Bailey RF, Elnor RW (1989) Northwest Atlantic snow crab fisheries: lessons in research and management. In: Caddy JF (ed) *Marine invertebrate fisheries: their assessment and management*. John Wiley and Sons, New York, pp 261–280
- Bauer RT (1986) Phylogenetic trends in sperm transfer and storage complexity in decapod crustaceans. *J Crustacean Biol* 6: 313–325
- Bauer RT, Martin JW (eds) (1991) *Crustacean sexual biology*. Columbia University Press, New York
- Bauer RT, Min LJ (1993) Spermatophores and plug substance of the marine shrimp *Trachypenaeus similis* (Crustacea: Decapoda: Penaeidae): formation in the male reproductive tract and disposition in the inseminated female. *Biol Bull mar biol Lab, Woods Hole* 185: 174–185
- Bawab FM, El-Sherief SS (1989) Contributions to the study of the origin, nature, and formation of the plug in the spermatheca of the female crab *Portunus pelagicus* (Linnaeus, 1766) (Decapoda, Brachyura). *Crustaceana* 57: 9–24
- Beninger PG, Elnor RW, Foyle T, Odense P (1988) Functional anatomy of the male reproductive system and the female spermatheca in the snow crab *Chionoecetes opilio* (O. Fabricius) (Decapoda: Majidae) and a hypothesis for fertilization. *J Crustacean Biol* 8: 322–332
- Beninger PG, Elnor RW, Poussart Y (1991) Gonopods of the majid crab *Chionoecetes opilio* (O. Fabricius). *J Crustacean Biol* 11: 217–228
- Beninger PG, Ferguson A, Lanteigne C (1995) The gonopod tegumental glands of snow crab (*Chionoecetes opilio*) are accessory reproductive glands. *J Shellfish Res* 14: 365–370

- Beninger PG, Lanteigne C, Elner RW (1993) Reproductive processes revealed by spermatophore dehiscence experiments and by histology, ultrastructure, and histochemistry of the reproductive system in the snow crab *Chionoecetes opilio* (O. Fabricius). *J Crustacean Biol* 13: 1–16
- Chambers CL, Payne JF, Kennedy ML (1980) Geographic variation in the first pleopod of the form I male dwarf crayfish, *Cambarellus puer* Hobbs (Decapoda, Cambaridae). *Crustaceana* 38: 169–177
- Chen PS (1984) The functional morphology and biochemistry of insect male accessory glands and their secretions. *A Rev Ent* 29: 233–255
- Chidester FE (1911) The mating habits of four species of the Brachyura. *Biol Bull mar biol Lab, Woods Hole* 21: 235–248
- Christy JH (1987) Competitive mating, mate choice, and mating associations of brachyuran crabs. *Bull mar Sci* 41: 177–191
- Diesel R (1990) Sperm competition and reproductive success in a decapod *Inachus phalangium* (Majidae): a male ghost spider crab that seals off rivals' sperm. *J Zool, Lond* 220: 213–224
- Diesel R (1991) Sperm competition and mating behavior in Brachyura, with special reference to spider crabs (Majidae). In: Bauer RT, Martin JW (eds) *Crustacean sexual biology*. Columbia University Press, New York, pp 145–163
- Elner RW, Beninger PG (1995) Multiple reproductive strategies in snow crab, *Chionoecetes opilio*: physiological pathways and behavioral plasticity. *J exp mar Biol Ecol* 193: 93–112
- Felgenhauer BE (1992) External anatomy and integumentary structures. In: Harrison FW, Humes AG (eds) *Microscopic anatomy of invertebrates*. Vol. 10. Decapod Crustacea. Wiley-Liss, New York, pp 7–43
- Fingerman M (1992) Glands and secretion. In: Harrison FW, Humes AG (eds) *Microscopic anatomy of invertebrates*. Vol. 10. Decapod Crustacea. Wiley-Liss, New York, pp 345–394
- Garth JS (1958) Brachyura of the Pacific coast of America. Oxyrhyncha. *Allan Hancock Pacif Exped* 21: 1–854
- George MJ (1963) The anatomy of the crab *Neptunus sanguinolentus* Herbst. Part IV. Reproductive system and embryological studies. *J Madras Univ (Sect B)* 33: 289–304
- Haefner PA (1985) The biology and exploitation of crabs. In: Provenzano AJ (ed) *The biology of Crustacea*. Vol. 10. Economic aspects, fisheries, and culture. Academic Press, New York, pp 111–166
- Halcrow K (1993) Pore canal systems and associated cuticular microstructures in amphipod crustaceans. In: Horst MN, Freeman JA (eds) *The crustacean integument: morphology and biochemistry*. CRC Press, Boca Raton, Florida, pp 39–77
- Hartnoll RG (1969) Mating in the Brachyura. *Crustaceana* 16: 161–181
- Hartnoll RG (1975) Copulatory structure and function in the Dromiacea, and their bearing on the evolution of the Brachyura. *Pubbl Stazi zool Napoli* 39(Suppl): 657–676
- Johnson PT (1980) Histology of the blue crab, *Callinectes sapidus*: a model for the Decapoda. Praeger Publishers, New York, pp 327–367
- Lanteigne C, Beninger PG, Gionet C (1996) Ontogeny of the female primary sexual characters in the majid crabs *Chionoecetes opilio* and *Hyas coarctatus*. *J Crustacean Biol* 16: 501–514
- Martin JW, Abele LG (1986) Notes on male pleopod morphology in the brachyuran crab family Panopeidae Ortmann, 1893, sensu Guinot (1978) (Decapoda). *Crustaceana* 50: 182–198
- Nishimura S (1967) Male first pleopods of the majid brachyurans *Chionoecetes opilio* (O. Fabricius) and *C. japonicus* Rathbun from the Japan Sea. *Publ Seto mar biol Lab* 15: 165–171
- Parker GA (1970) Sperm competition and its evolutionary consequences in the insects. *Biol Rev* 45: 525–568
- Radha T, Subramoniam T (1985) Origin and nature of the spermatophoric mass of the spiny lobster *Panulirus homarus*. *Mar Biol* 86: 13–19
- Ryan EP (1967) Structure and function of the reproductive system of the crab *Portunus sanguinolentus* (Herbst) (Brachyura: Portunidae). The female system. In: Proceedings of the symposium on Crustacea, Ernakulam. Part II. Marine Biological Association of India, Bangalore Press, Bangalore, India, pp 522–544
- Sasikala SL, Subramoniam T (1987) On the occurrence of acid mucopolysaccharides in the spermatophores of two marine prawns, *Penaeus indicus* (Milne-Edwards) and *Metapenaeus monoceros* (Fabricius) (Crustacea: Macrura). *J exp mar Biol Ecol* 113: 145–153
- Sastry AN (1983) Ecological aspects of reproduction. In: Vernberg FJ, Vernberg WB (eds) *The biology of Crustacea*. Vol. 8. Environmental adaptations. Academic Press, New York, pp 179–270
- Sévigny J-M, Sainte-Marie B (1996) Electrophoretic data support the last-male precedence hypothesis in the snow crab, *Chionoecetes opilio* (Brachyura: Majidae). *J Shellfish Res* 15: 437–440
- Spalding JF (1942) The nature and formation of the spermatophore and sperm plug in *Carcinus maenas*. *Q Jl microsc Sci* 83:399–422
- Stephensen K (1946) The Brachyura of the Iranian Gulf. Appendix: the male pleopoda of the Brachyura. In: Danish scientific investigations in Iran, Part IV. Ejnar Munksgaard, Copenhagen, pp 57–237
- Stevenson JR (1985) Dynamics of the integument. In: Bliss DE, Mantel LH (eds) *The biology of Crustacea*. Vol. 9. Integument, pigments, and hormonal processes. Academic Press, New York, pp 1–42
- Subramoniam T (1991) Chemical composition of spermatophores in decapod crustaceans. In: Bauer T, Martin JW (eds) *Crustacean sexual biology*. Columbia University Press, New York, pp 308–321
- Subramoniam T (1993) Spermatophores and sperm transfer in marine crustaceans. *Adv mar Biol* 29: 129–214
- Talbot P, Demers D (1993) Tegumental glands of Crustacea. In: Horst MN, Freeman JA (eds) *The crustacean integument: morphology and biochemistry*. CRC Press, Boca Raton, Florida, pp 152–191
- Waage JK (1979) Dual function of the damselfly penis: sperm removal and transfer. *Science* 203: 916–918
- Wenner A, Kuris A (eds) (1991) *Crustacean egg production*. A A Balkema Inc, Rotterdam
- Williams A (1965) Marine decapod crustaceans of the Carolinas. *Fish Bull US* 65: 1–271