Spermatophore Transfer in the Hermit Crab *Clibanarius vittatus* (Crustacea, Anomura, Diogenidae)

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ABSTRACT Although mating has been described in several hermit crab species, the mechanics of spermatophore transfer have not previously been demonstrated. Evidence from pleopod and gonopore morphology, video observations, and inseminated females indicates that in *Clibanarius vittatus* the male applies a spermatophoric mass directly onto the female via the gonopores rather than with modified pleopods 1–2 (gonopods) and/or genital papillae as in many other decapods. The single second pleopod of males of *C. vittatus* has a simple endopod with no apparent modifications for sperm transfer. There are no genital papillae extending from the male gonopores. The globular spermatophores are aligned in rows surrounded by a seminal secretion in the male ducts (vasa deferentia that terminate in ejaculatory ducts opening to the exterior via the gonopores). During copulation, described from time-lapse video recordings, the ventral surface of the last thoracic segment of the male, bearing the gonopores, was apposed to the ventral cephalothorax of the female. A massive amount of seminal secretion containing spermatophore ribbons, termed here the spermatophoric mass and described for the first time in a hermit crab species, was observed covering the sternites and coxae of pereopods 1–5 of a recently copulated female. It is suggested that during copulation the male emits the contents of the ejaculatory ducts directly onto the female without the aid of gonopods or genital papillae. Although spermatophore transfer is simple in *C. vittatus*, the presence of modified anterior pleopods or elongate genital papillae (sexual tubes) in other paguroidean species suggests the possibility of a more complex insemination process in these other hermit crabs. J. Morphol. 253:166–175, 2002.

KEY WORDS: gonopods; gonopores; hermit crab; spermatophore; sperm transfer

In decapod crustaceans, the immotile spermatozoa are surrounded by protective and adhesive materials when transferred to the female (Subramoniam, 1993). These sperm-bearing structures (spermatophores), formed prior to or during ejaculation, vary in size, shape, and complexity. The placement of spermatophores on the exterior of the female or within spermathecae is often achieved with one or more pairs of male gonopods, which are modified anterior pleopods (abdominal appendages) utilized for sperm transfer in many decapods (Bauer, 1986), such as caridean shrimps (Bauer, 1976), penaeoidean shrimps (Bauer, 1991, 1996a,b), cambarid crayfishes (Andrews, 1911), nephropid lobsters (Farmer, 1974), and brachyuran crabs (Cronin, 1947; Ryan, 1967). In some decapods, such as stenopodid shrimps (Bauer, 1986) and palinurid lobsters (Phillips et al., 1980), the male anterior pleopods are not modified for sperm transfer.

Males of anomuran species, including the hermit crabs of the superfamily Paguroidea (McLaughlin, 1983), produce characteristic spermatophores that apparently are deposited on the external surface of the female (Tudge, 1999a,b). Tudge (1991, 1995, 1999a,b) and Subramoniam (1993) described the predominant spermatophore type encountered in paguroidean crabs as stalked or pedunculate with three distinct components, the distal ampulla, stalk, and proximal pedestal. The spermatozoa are stored within the ampulla, which is composed of two halves joined along their lateral ridges (Tudge, 1999a). The distal ampulla rests atop the stalk, which is underlain by the pedestal or base of the stalk. The pedestal appears to function as the point of attachment for the spermatophore to the external surface of the female during copulation (Tudge, 1999b). Mouchet (1930, 1931) and Hamon (1937, 1939) suggested that the ampulla splits along this ridge, termed the line of dehiscence (Tudge 1999a) or "*ligne de suture*" (Hamon, 1937), thereby releasing the spermatozoa (Tudge 1991, 1995, 1999a,b). Hamon (1937) suggested that mechanical or osmotic forces might cause the ampulla to fracture and release the spermatozoa.

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Bauer RT. 1998. Gill-cleaning mechanisms of the crayfish *Procambarus clarkii* (Astacidea: Cambaridae): experimental testing of setobranch function. Invert Biol 117:129–143.

Bauer RT. 1999. Gill-cleaning mechanisms of a dendrobranchiate shrimp, *Rimapenaeus similis* (Decapoda, Penaeidae): description and experimental testing of function. J Morphol 242:125–139.

Berg ABV, Sandifer PA. 1984. Mating behavior in the grass shrimp *Palaemonetes pugio* Holthuis (Decapoda, Caridea). J Crustacean Biol 4:417–424.

Cronin LE. 1947. Anatomy and histology of the male reproductive system of *Callinectes sapidus* (Rathbun). J Morphol 81:209–240.

Farmer ASD. 1974. Reproduction in *Nephrops norvegicus* (Decapoda: Nephropidae). J Zool 174:161–183.

Greenwood JG. 1972. The male reproductive system and spermatophore formation in *Pagurus novaezealandiae* (Dana) (Anomura: Paguridae). J Nat Hist 6:561–574.

Hamon M. 1937. Les mécanismes produisant la déhiscence des spermatophores d' *Eupagurus prideauxi* Leach. CR Acad Sci Paris 204:1504–1506.

Hamon M. 1939. La constitution chimique des spermatophores de Crustacés supérieurs du groupe des pagurides. CR Soc Biol Paris 130:1312–1315.

Hazlett BA. 1966. Social behavior of the Paguridae and Diogenidae of Curacao. Stud Fauna Curacao 23:1–143.

Hazlett BA. 1996. Reproductive behavior of the hermit crab *Clibanarius vittatus* (Bosc, 1802). Bull Mar Sci 58:668–674.

Hobbs HH Jr. 1974. Synopsis of the families and genera of crayfishes (Crustacea: Decapoda). Smith Contrib Zool 164:1–32.

Kamalaveni S. 1949. On the ovaries, copulation and egg-formation in the hermit-crab, *Clibanarius olivaceous* Henderson (Crustacea, Decapoda). J Zool Soc India 1:120–128.

Lemaitre R. 1994. Crustacea Decapoda: deep-water hermit crabs (Parapaguridae) from French Polynesia with descriptions of four new species. Mém Mus Natl Hist Nat 161:375–419.

Lemaitre R, McLaughlin PA. 1995. *Alainopagurus crosnieri*, gen. et sp. nov. (Decapoda, Anomura, Paguridae) from the Western Pacific. Bull Mus Natl Hist Nat, Paris, 4e sér 17:272–282.

Mathews DC. 1953. The development of the pedunculate spermatophores of a hermit crab, *Dardanus asper* (De Haan). Pac Sci 7:255–267.

Mathews DC. 1956. The probable method of fertilization in the terrestrial hermit crabs based on a comparative study of spermatophores. Pac Sci 10:303–309.

McLaughlin PA. 1980. Comparative morphology of Recent Crustacea. San Francisco: W.H. Freeman.

McLaughlin PA. 1983. Hermit crabs—are they really polyphyletic? J Crustacean Biol 3:608–621.

McLaughlin PA, Provenzano AJ Jr. 1974. Hermit crabs of the genus *Paguristes* (Crustacea: Decapoda: Diogenidae) from the Western Atlantic. II. Descriptions of six new species. Bull Mar Sci 24:885–938.

Mouchet S. 1930. Morphologie comparée des canaux déférents de quelques Pagures. CR Acad Sci Paris 190:396–398.

Mouchet S. 1931. Spermatophores des Crustacés Décapodes Anomures et Brachyoures et castration parasitaire chez quelques Pagures. Ann Stat Océan Salammbô 6:1–203.

Phillips BF, Cobb JS, George RW. 1980. General biology. In: Cobb JS, Phillips BF, editors. The biology and management of lobsters. New York: Academic Press. p 1–82.

Ryan EP. 1967. Structure and function of the reproductive system in the crab *Portunus sanguinolentus* (Herbst) (Brachyura, Portunidae). I. The male system. Mar Biol Ass India, Symposium 2:506–521.

Subramoniam T. 1984. Spermatophore formation in two intertidal crabs *Albunea symnista* and *Emerita asiatica* (Decapoda: Anomura). Biol Bull 166:78–95.

Subramoniam T. 1993. Spermatophores and sperm transfer in marine crustaceans. Adv Mar Biol 29:129–214.

Tudge CC. 1991. Spermatophore diversity within and among the hermit crab families, Coenobitidae, Diogenidae, and Paguridae (Paguridae, Anomura, Decapoda). Biol Bull 181:238–247.

Tudge CC. 1992. Comparative ultrastructure of hermit crab spermatozoa (Decapoda: Anomura: Paguroidea). J Crustacean Biol 12:397–409.

Tudge CC. 1995. Ultrastructure and phylogeny of the spermatozoa of the infraorders Thalassinidea and Anomura (Decapoda, Crustacea). Mem Mus Natl Hist Nat 166:251–263.

Tudge CC. 1999a. Ultrastructure of the spermatophore lateral ridge in hermit crabs (Decapoda, Anomura, Paguroidea). Crustaceana 72:77–84.

Tudge CC. 1999b. Spermatophore morphology in the hermit crab families Paguridae and Parapaguridae (Paguroidea, Anomura, Decapoda). Invert Reprod Dev 35:203–214.

Uma K, Subramoniam T. 1984. A comparative study on the spermatophore I. *Scylla serrata* (Forskal) (Decapoda: Brachyura) and *Clibanarius longitarsus* (De Haan) (Decapoda: Anomura). J Mar Biol Assoc UK 26:103–108.

pleopods bear complex setules covered with multidigitate scales like those used in cleaning of gills and other structures in a variety of other decapods (Bauer, 1979, 1998, 1999). This bristling array of complex setae may serve to clean the embryos as the pleopods beat and circulate water among them. In male pleopod setae, the setules are sparse and morphologically simple.

The gonopores of male *Clibanarius vittatus* are unremarkable apertures guarded by flap-like opercula and are not borne on genital papillae (external extensions of the ejaculatory ducts), as in several other decapod groups. In cambarid crayfishes (Andrews, 1911) and brachyuran crabs (Cronin, 1947; Ryan, 1967), elongate genital papillae fit into pleopodal gonopods that are inserted into a median spermatheca (crayfishes) or female gonopores (brachyuran crabs). During copulation, ejaculated male products are conducted from the male ducts into the female via the genital papillae and gonopods. In the penaeoid shrimp *Sicyonia dorsalis*, short genital papillae may inject ejaculated material directly into the female spermathecae without involvement of the complex gonopods (petasma) (Bauer, 1996b). The lack of genital papillae in males of *C. vittatus* indicates that ejaculated material simply exits and flows directly onto the female.

During the brief copulation of *Clibanarius vittatus*, the gonopores of the male are near to or in contact with the ventral surface of the female cephalothorax. Our interpretation is that when ejaculation occurs, adhesive seminal secretion bearing spermatophores is emitted from both male gonopores directly onto the female as a spermatophoric mass. The rows of spermatophores, neatly arranged in the vasa deferentia of the male, are scattered about in the mass, which might be expected, as the seminal secretion is spewed out onto the female ventral surface. Spawning occurs within hours of copulation. Eggs spawned from the female gonopores must pass over the spermatophoric mass in passing to the abdomen, where they will be attached to the pleopods of the female. The jostling and contact of eggs with spermatophores during spawning may cause the splitting of their lateral ridges subsequent release of spermatozoa for fertilization. The spermatophoric mass quickly disappears after spawning, perhaps by cleaning with the fifth pereopods, the major cleaning and grooming appendage of hermit crabs.

Although we conclude that transfer of spermatophores in *Clibanarius vittatus* is a morphologically simple affair, without the involvement of gonopods or genital papillae, this may not be the case in other hermit crabs. In many paguroidean species, males have first and second pleopods, often referred to as gonopods because of their complex modifications (McLaughlin and Provenzano, 1974; Lemaitre, 1994) of the type seen in other decapod species in which the anterior pleopods do serve as gonopods. In addition, many other paguroidean species have pronounced genital papillae, termed "sexual tubes" (McLaughlin, 1980; Lemaitre and McLaughlin, 1995) that might be used to place spermatophoric masses or spermatophores directly on the female in a more complex fashion than that observed in *C. vittatus*. Spermatophore transfer in hermit crabs species with "gonopods" and sexual tubes needs to be investigated to determine if and how these structures are involved in insemination.

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LITERATURE CITED

Aiken DE, Waddy SL. 1980. Reproductive biology. In: Cobb JS, Phillips BF, editors. The biology and management of lobsters. New York: Academic Press. p 215–276.

Ameyaw-Akfumi C. 1975. The breeding biology of two sympatric species of tropical intertidal hermit crabs, *Clibanarius chapini* and *C. senegalensis*. Mar Biol 29:15–28.

Andrews EA. 1905. The sperm-receptacle of *Cambarus*. Johns Hopkins University Circular 178:1–8.

Andrews EA. 1906. The annulus ventralis. Proc Bost Soc Nat Hist 32:427–497.

Andrews EA. 1908. The sperm-receptacle in the crayfishes, *Cambarus cubensis* and *C. paradoxus*. Proc Wash Acad Sci 10:167–185.

Andrews EA. 1911. Male organs for sperm-transfer in the crayfish, *Cambarus affinis*: their structure and use. J Morphol 39: 419–434.

Bauer RT. 1976. Mating behavior and spermatophore transfer in the shrimp *Heptacarpus pictus* (Stimpson) (Decapoda: Caridea: Hippolytidae). J Nat Hist 10:415–440.

Bauer, RT. 1979. Antifouling adaptations of marine shrimp (Decapoda: Caridea): gill cleaning mechanisms and cleaning of brooded embryos. Zool J Linn Soc 65:281–303.

Bauer RT. 1986. Phylogenetic trends in sperm transfer and storage complexity in decapod crustaceans. J Crustacean Biol 6:313–325.

Bauer RT. 1991. Sperm transfer and storage structures in penaeoid shrimps: a functional and phylogenetic perspective. In: Bauer RT and Martin JW, editors. Crustacean Sexual Biology. New York and Oxford: Columbia University. p.183–207.

Bauer RT. 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 185:174–185.

Bauer RT. 1996a. A test of hypotheses on male mating systems and female molting in decapod shrimp, using *Sicyonia dorsalis* (Decapoda: Penaeoidea). J Crustacean Biol 16:429–436.

Bauer RT. 1996b. Role of the petasma and appendices masculinae during copulation and insemination in the penaeoid shrimp, *Sicyonia dorsalis* (Crustacea: Decapoda: Dendrobranchiata). Invertebr Reprod Dev 29:173–184.

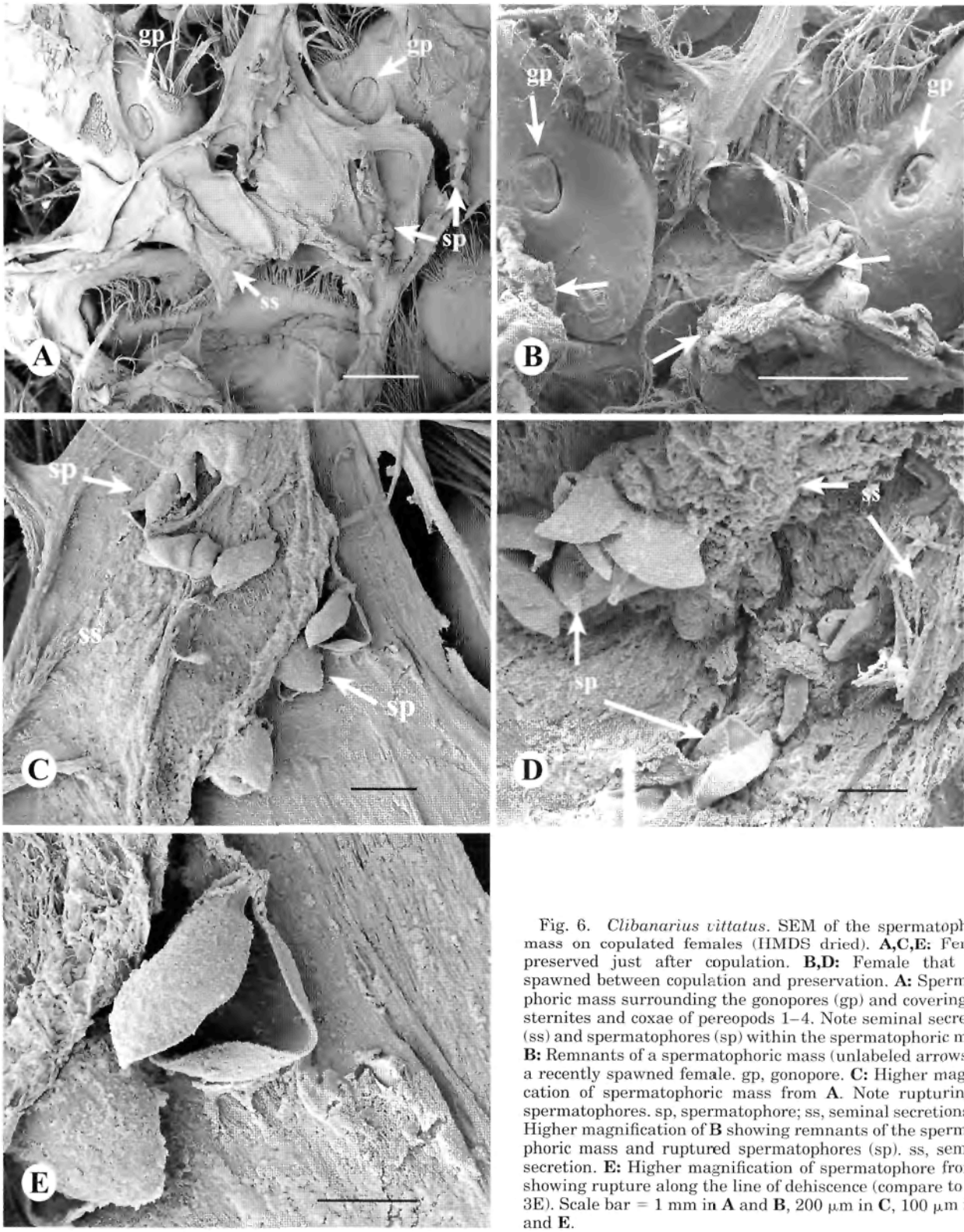

Fig. 6. *Clibanarius vittatus*. SEM of the spermatophoric mass on copulated females (HMDS dried). **A,C,E:** Female preserved just after copulation. **B,D:** Female that had spawned between copulation and preservation. **A:** Spermatophoric mass surrounding the gonopores (gp) and covering the sternites and coxae of pereopods 1–4. Note seminal secretion (ss) and spermatophores (sp) within the spermatophoric mass. **B:** Remnants of a spermatophoric mass (unlabeled arrows) on a recently spawned female. gp, gonopore. **C:** Higher magnification of spermatophoric mass from **A**. Note rupturing of spermatophores. sp, spermatophore; ss, seminal secretions. **D:** Higher magnification of **B** showing remnants of the spermatophoric mass and ruptured spermatophores (sp). ss, seminal secretion. **E:** Higher magnification of spermatophore from **C** showing rupture along the line of dehiscence (compare to Fig. 3E). Scale bar = 1 mm in **A** and **B**, 200 μm in **C**, 100 μm in **D** and **E**.

second pleopod shows no obvious modification for spermatophore transfer. Although the second pleopod of *C. vittatus* is sexually dimorphic, the differences between the sexes can be attributed to the role of female pleopods in incubation of embryos. The pleopods of females, relatively larger and more setose than those of males, serve as attachment sites for embryos. The setae of female

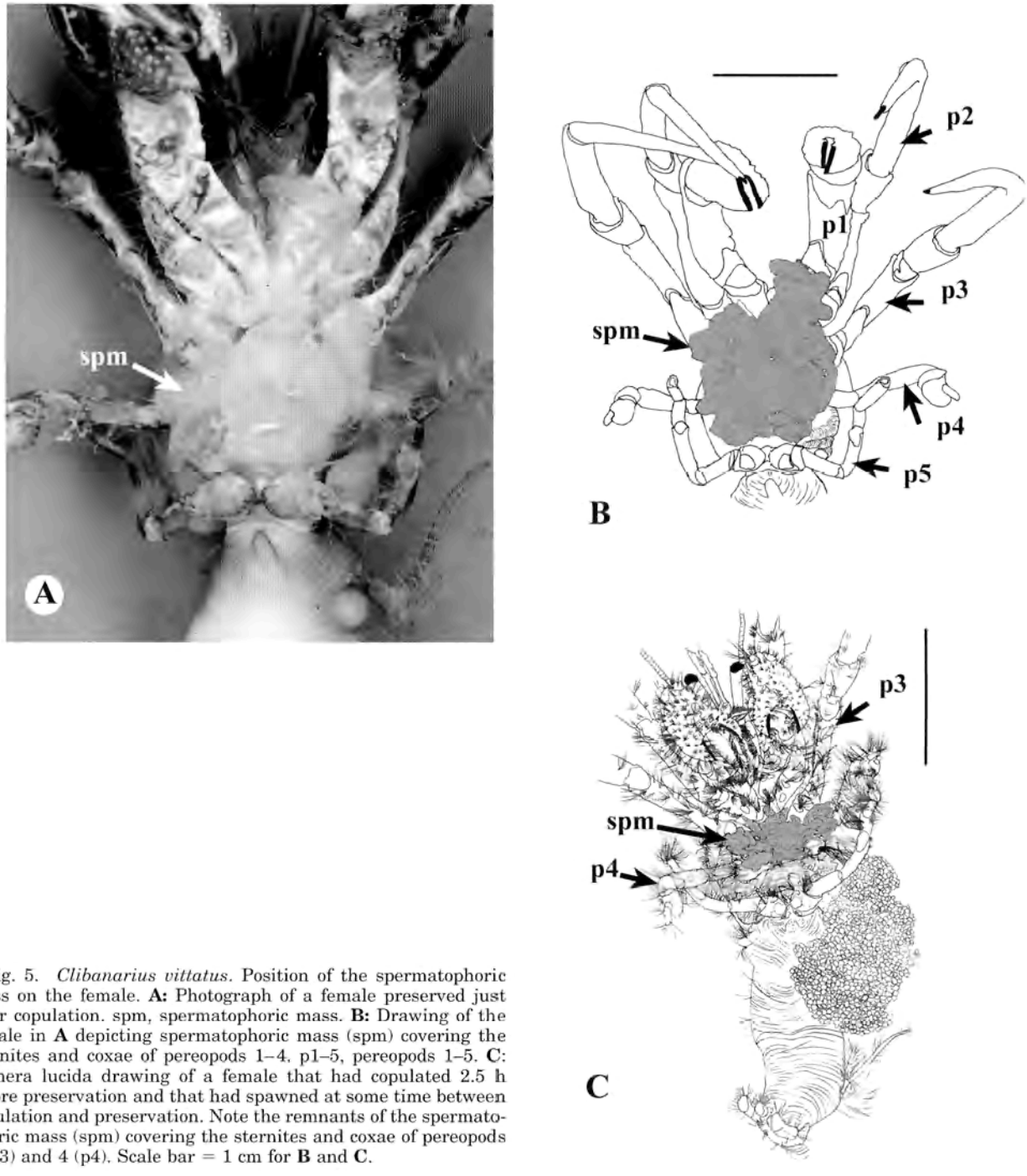

Fig. 5. *Clibanarius vittatus.* Position of the spermatophoric mass on the female. **A:** Photograph of a female preserved just after copulation. spm, spermatophoric mass. **B:** Drawing of the female in **A** depicting spermatophoric mass (spm) covering the sternites and coxae of pereopods 1–4. p1–5, pereopods 1–5. **C:** Camera lucida drawing of a female that had copulated 2.5 h before preservation and that had spawned at some time between copulation and preservation. Note the remnants of the spermatophoric mass (spm) covering the sternites and coxae of pereopods 3 (p3) and 4 (p4). Scale bar = 1 cm for **B** and **C**.

the viscous, adhesive seminal secretion and spermatophores observed in the vasa deferentia and ejaculatory ducts of the male. Our conclusion about transfer of the spermatophoric mass from the male to the female is based on the morphology of the anterior pleopods and gonopores of the male, the position of male and female during copulation, and the position of the spermatophoric mass on the female after copulation. The evidence presented here indicates that the spermatophoric mass is applied onto the external surface the female directly from the male gonopores rather than with the aid of gonopods or other intromittent structures.

The use of modified first and second pleopods as gonopods for spermatophore transfer has been demonstrated in decapod groups such as caridean shrimps (Bauer, 1976; Berg and Sandifer, 1984), penaeoidean shrimps (Bauer, 1991, 1996b), cambarid crayfishes (Andrews, 1911), nephropid lobsters (Farmer, 1974), and brachyuran crabs (Cronin, 1947; Ryan, 1967). Males of *Clibanarius vittatus* do not have first pleopods and the single

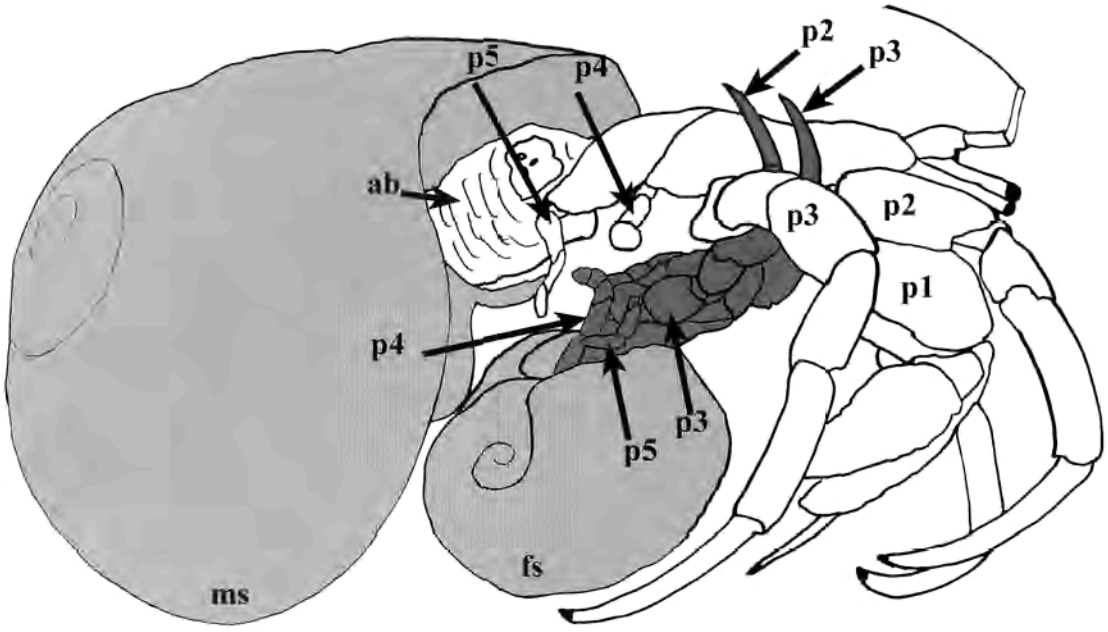

Fig. 4. *Clibanarius vittatus*. Copulatory position modeled from video recordings of copulation and from preserved specimens placed in mating posture. The male (unshaded, above female) has the gonopore area near the ventral surface of the posterior cephalothorax of the female (dark shading). ab, abdomen; p1–p5, pereopods 1–5.

ing of the female in its gastropod shell, as described by Hazlett (1966, 1996). In the copulations observed, the female partially emerged from the shell after several hours and assumed a copulatory position, in which the ventral surfaces of the cephalothorax of the mating pair are apposed. Examination of the copulatory position, based on a model based on video observations and arranged preserved specimens, reveals that the male gonopores are situated opposite the posterior ventral surface of the pereopod 1–4 segments (Fig. 4). The male and female walking legs (pereopods 2–3) are intertwined while pereopods 4–5 are held outward from the cephalothorax at approximately a 90° angle. The portion of the male abdomen bearing the pleopods does not extend out beyond the aperture of the shell and does not come into contact with the female. In the three mating pairs in which copulation was recorded on video, the copulatory position was held for 20 sec in two matings, but only for 5 sec in the third. One of the three females from these pairs was preserved 2.5 h after copulation; spawning had occurred and remnants of a spermatophoric mass were observed. In the other two females, preserved at least 6 h after copulation, spawning had taken place but no spermatophoric mass remained. In another female whose copulation was observed directly on a laboratory water table, preservation took place immediately after copulation. Spawning had not taken place and a large spermatophoric mass was observed on the female (Fig. 5A,B).

Spermatophoric Mass

The single female preserved just after copulation had a large opaque gelatinous mass covering the ventral surface of the cephalothorax from pereopods 1–4, including the gonopores (Fig. 5A,B). The adhesive seminal secretions attached the spermatophores to the sternites and coxae of the female. Microscopic examination of the mass prior to SEM revealed long ribbons of unruptured spermatophores embedded within a mass of seminal secretion. The dehydration process for SEM caused these spermatophores to rupture (Fig. 6A,C,E). Dehydration also caused some shrinkage in both the seminal secretion and spermatophores.

In another copulated female, preserved 2.5 h after copulation, spawning had occurred but remnants of a spermatophoric mass remained, covering the coxae of pereopods 4–5 as well as the sternites of pereopods 3–5 (Figs. 5C, 6B,D). Observations made first with a stereomicroscope and then SEM showed that ruptured spermatophores were still present on the coxae of pereopods 3 as well as the coxae and sternites of pereopods 4 (Fig. 6B,D).

DISCUSSION

Hazlett (1996) first noted the presence of a spermatophoric mass on recently copulated females of *Clibanarius vittatus*. In the present study, we describe the spermatophoric mass of a hermit crab species in detail for the first time. It is composed of

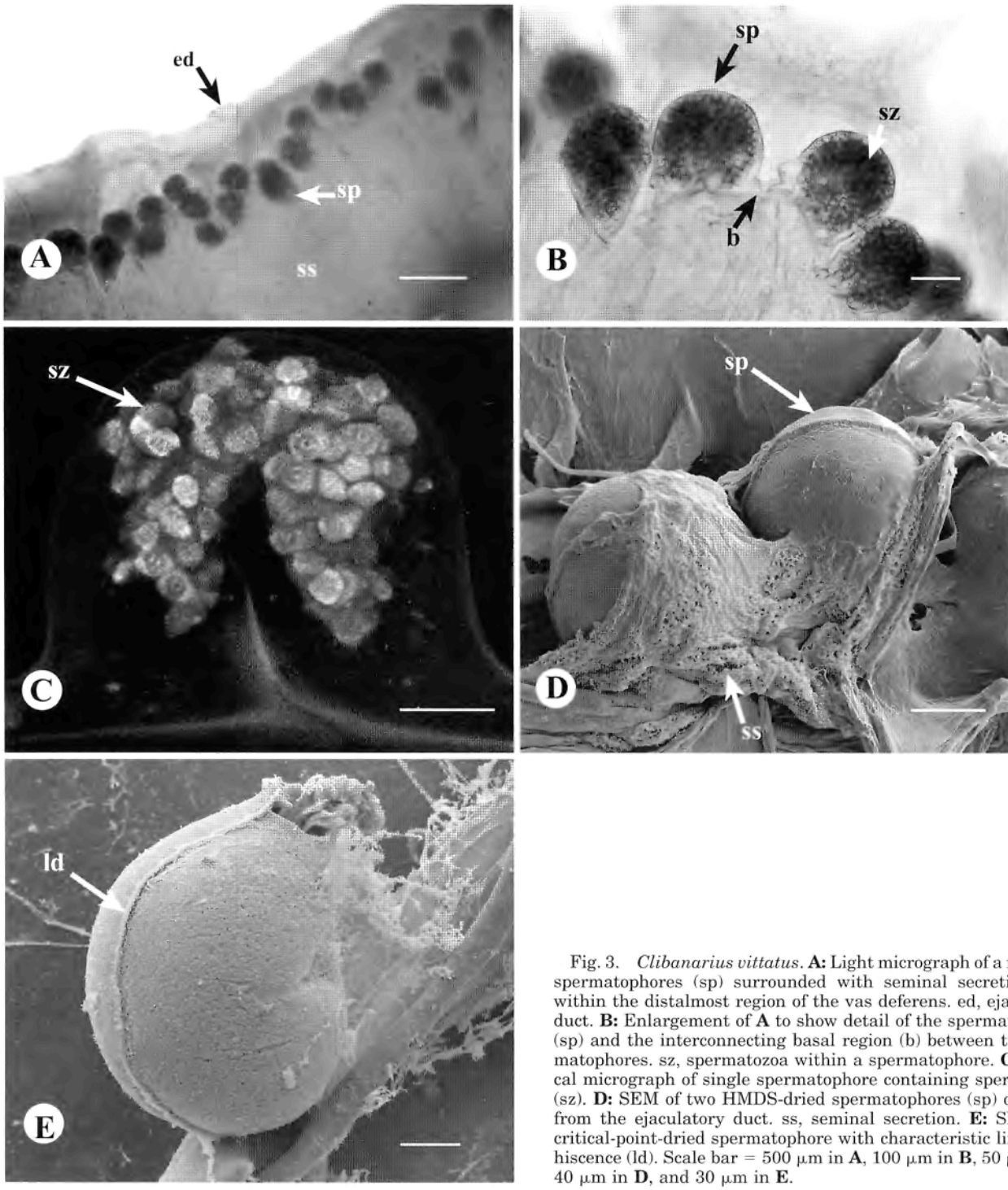

Fig. 3. *Clibanarius vittatus*. **A:** Light micrograph of a ribbon of spermatophores (sp) surrounded with seminal secretions (ss) within the distalmost region of the vas deferens. ed, ejaculatory duct. **B:** Enlargement of **A** to show detail of the spermatophores (sp) and the interconnecting basal region (b) between two spermatophores. sz, spermatozoa within a spermatophore. **C:** Confocal micrograph of single spermatophore containing spermatozoa (sz). **D:** SEM of two HMDS-dried spermatophores (sp) dissected from the ejaculatory duct. ss, seminal secretion. **E:** SEM of a critical-point-dried spermatophore with characteristic line of dehiscence (ld). Scale bar = 500 μm in **A**, 100 μm in **B**, 50 μm in **C**, 40 μm in **D**, and 30 μm in **E**.

lumens of the vasa deferentia and ejaculatory ducts (Fig. 1B). Surrounding the spermatophores (Fig. 3A,B,D) is an opaque, adhesive, mucoid material, termed here the seminal secretion, which clings to objects upon contact (Figs. 3D, 6A–D). Mechanical disturbance (e.g., touching with a probe) of freshly extruded seminal secretion or the spermatophores themselves caused the latter to rupture along their lateral ridges.

Description of Copulation

Upon contact with a receptive female, the male began precopulatory behavior, a tapping and rotat-

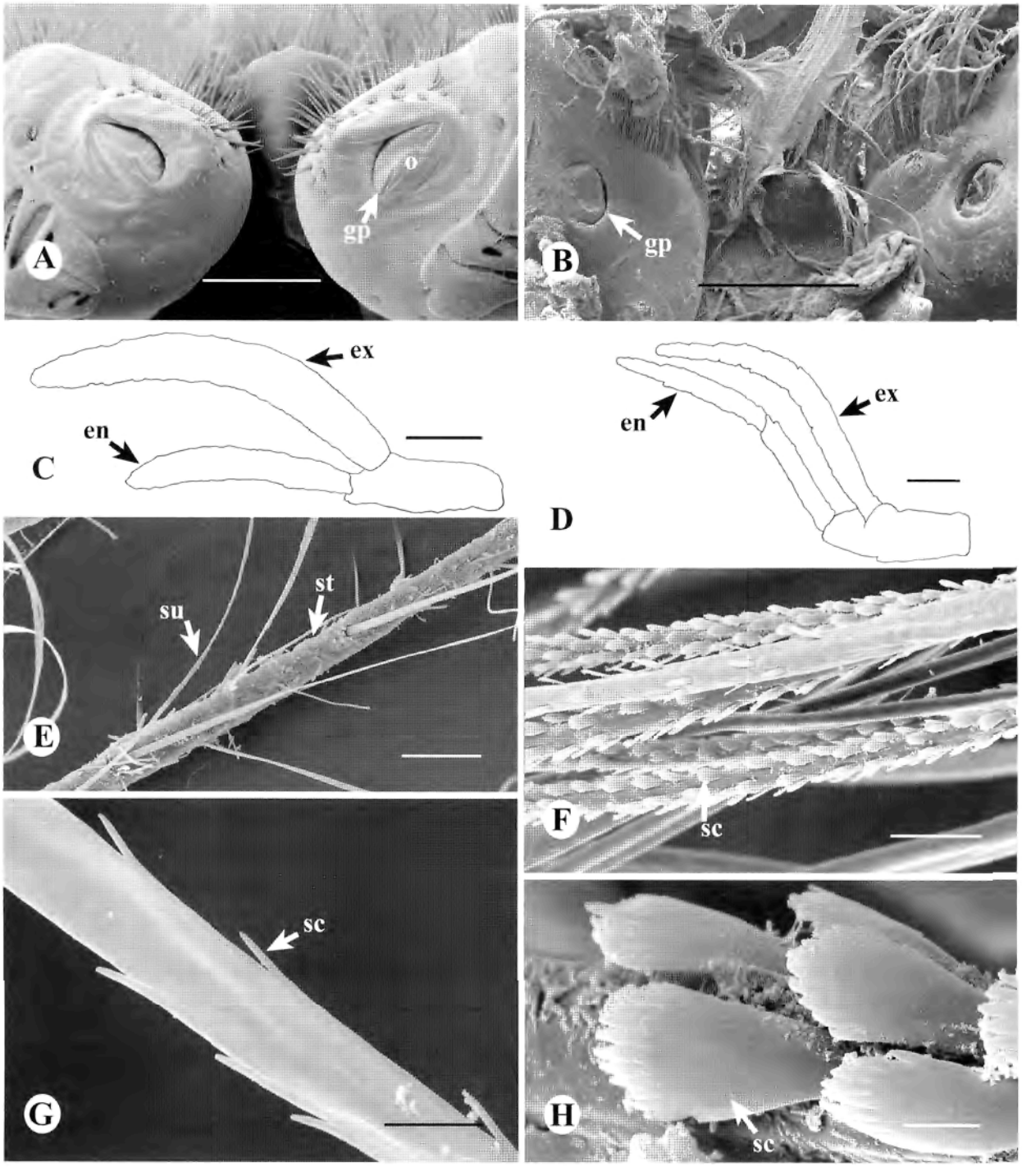

Fig. 2. *Clibanarius vittatus.* Sexual dimorphism in reproductive morphology of gonopores and second pleopods. **A:** SEM of male gonopores (gp) and operculum (o) located on the coxae of the fifth pereopods (HMDS dried). **B:** SEM of female gonopores (gp) on coxae of third pereopods (HMDS dried). **C:** Anterior view of second pleopod of male (shield length = 10.5 mm) with setae removed. en, endopod; ex, exopod. **D:** Anterior view of second pleopod of female (shield length = 6.3 mm) with setae removed. en, endopod; ex, exopod. **E:** SEM of male pleopod seta (st) and setules (su) (critical-point-dried). **F:** SEM of dense array of female pleopodal 2 setules; arrow indicates multidigitate scale subsetule (sc) on setule (HMDS dried). **G:** SEM of a single male pleopod 2 setule with simple scale subsetules (sc) (critical-point-dried). **H:** SEM of female setule with multidigitate scale subsetules (sc) (HMDS dried). Scale bar = 1 mm in **A** and **B**, 2 mm in **C** and **D**, 20 μm in **E**, 40 μm in **F**, 2 μm in **G**, and 4 μm in **H**.

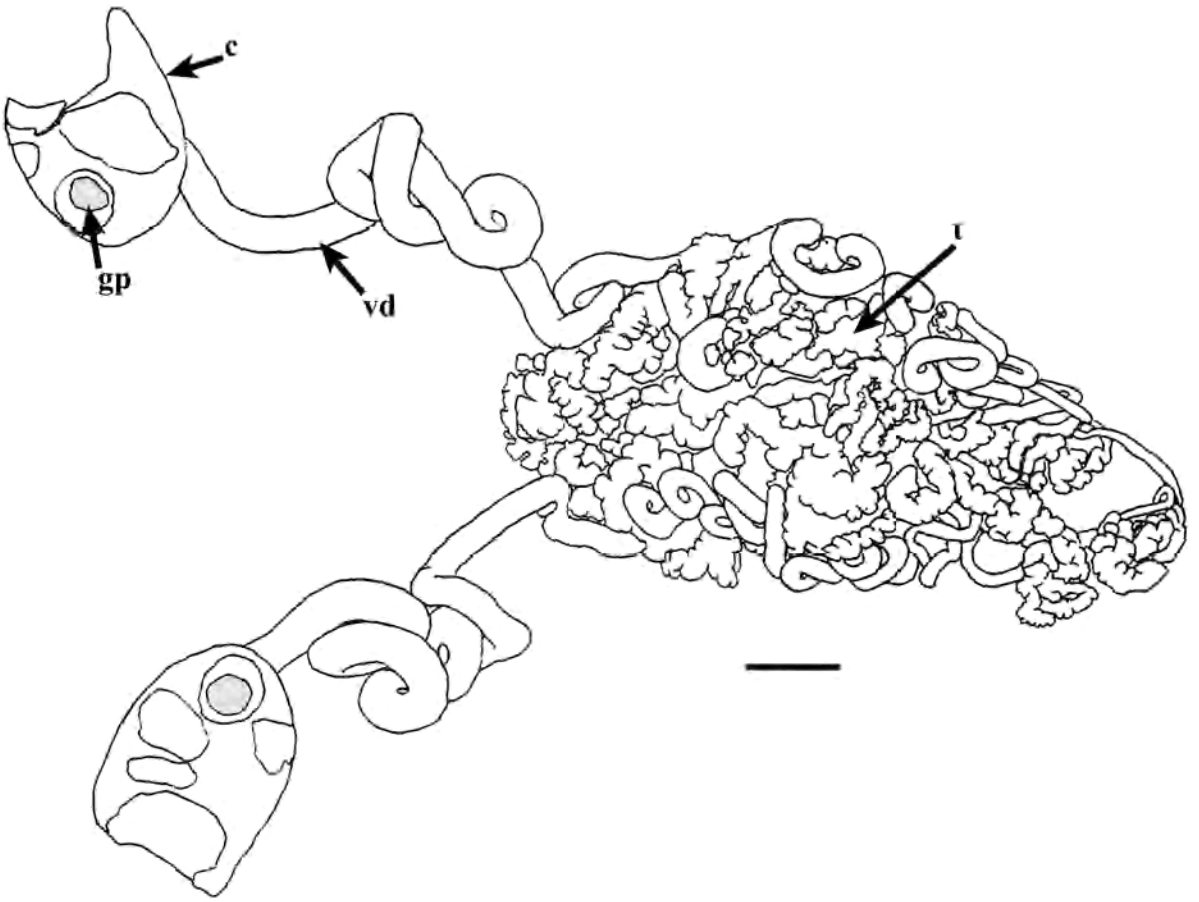

Fig. 1. *Clibanarius vittatus*. Testes and vasa deferentia terminating within the pereopod 5 coxae as ejaculatory ducts that open to the exterior through the gonopores. c, coxa; gp, gonopore; t, testis; vd, vas deferens. Scale bar = 1 mm.

similar in relative size and shape to those of the male (Fig. 2B). The sternum between the third pereopods (Fig. 2B) of the female is smooth and lacks any invaginations of the exoskeleton or apertures suggestive of the female sperm-storage structures found in many other decapods, such as the thelyca and spermathecae of penaeoidean shrimps (Bauer, 1991, 1993, 1996a,b), cambarid crayfishes (Andrews, 1905, 1906, 1908; Hobbs, 1974), and nephropid lobsters (Farmer, 1974; Aiken and Waddy, 1980).

In males of many decapod species the endopods of the first two pairs of pleopods are modified as gonopods (Bauer, 1986). In *Clibanarius vittatus*, as in many other hermit crabs, pleopods are present only on the left side of the abdomen. The first abdominal somite of *C. vittatus* lacks a pleopod in both males and females while a single pleopod is present on abdominal somites 2–5. The pleopods are biramous, with both an endopod and an exopod, each bordered by long setae bearing setules. The second pleopods of males and females (Fig. 2C,D) were compared to detect possible modifications in the male related to spermatophore transfer. The female exopod is approximately equal in length and width to the endopod, while in males the exopod is longer and broader than the endopod (Fig. 2C,D). Both the endopod and exopod of female pleopod 2 possess a greater number and proportionally longer setae and setules than those of the male (Fig. 2E,F). The setules of pleopod 2 setae of the male are sparsely equipped with simple scale subsetules (Fig. 2G), while those of the female are densely covered with multidigitate scale subsetules (Fig. 2F,H).

Spermatophores and Seminal Secretion

Spermatophores of this species are formed prior to ejaculation and can be observed within the lumens of the vasa deferentia and ejaculatory ducts (Figs. 1, 3A,B). Each nonpedunculate spermatophore is composed of two halves meeting at a raised line termed the "lateral ridge" or "line of dehiscence" (Tudge, 1991, 1999a), forming the ampulla of the spermatophore (Fig. 3D,E). Spermatozoa can be observed within the ampulla (Fig. 3B,C). Below the ampulla is a basal region (Fig. 3B) that acts as a connecting cord attaching adjacent spermatophores within the

Mouchet (1931), Hamon (1937, 1939), Mathews (1953), Greenwood (1972), and Uma and Subramoniam (1984) have described the formation of paguroidean spermatophores within the testes and vasa deferentia. According to Mathews (1953), the spermatozoa of *Dardanus asper* (Diogenidae) form a continuous stream as they leave the testicular region of the male gonopods and are mixed with epithelial secretions. During this mixing process the spermatozoa are grouped and separated into distinct and visible spermatophores within the coiled vasa deferentia via contractions of the surrounding muscular layer. The contractions mold the seminal secretions surrounding the grouped spermatozoa into the characteristic shape of the spermatophore.

Although aspects of spermatophore formation have been investigated in several paguroidean genera (Mouchet, 1931; Mathews, 1953), the mechanism by which spermatophores are transmitted from the male gonopore to the body of a receptive female has only been hypothesized (Mouchet, 1931; Kamalaveni, 1949; Mathews, 1956; Hazlett, 1966, 1996; Ameyaw-Akfumi, 1975). The purpose of this study was to determine the mode of spermatophore transfer in the hermit crab, *Clibanarius vittatus*. Morphology of the anterior pleopods of the male, gonopores, spermatophores, and spermatophoric masses deposited on the female are described and observations on copulation recorded with time-lapse video are reported.

MATERIALS AND METHODS

Live specimens of *Clibanarius vittatus* (Bosc, 1802) were collected in July and August 1999 from Grand Terre Island and Isles Dernieres, Louisiana, for mating observations. The hermit crabs were placed in large plastic bags containing ambient seawater saturated with oxygen for transport back to the laboratory. They were maintained on a recirculating water table system with water temperature from 25–30°C, salinity 15–25 ppt, and a light:dark cycle of 14:10 h.

Testes and vasa deferentia containing spermatophores were dissected from freshly preserved male crabs. The crabs were first anesthetized by chilling, secured with insect pins to a wax-coated dissecting dish to straighten the abdomens for ease of dissection, and then fixed in 10% formalin. Ejaculatory ducts, the distalmost portions of the vasa deferentia contained within the coxae of the fifth pereopods, were stuck to glass coverslips precoated with poly-L-lysine and prepared as described below for scanning electron microscopy (SEM). The freshly dissected vasa deferentia of one male were placed in oxygenated seawater for 1 h and then placed in 10% seawater formalin for 20 min, during which time they spontaneously ejaculated their spermatophores and surrounding seminal secretions. These spermatophores and seminal secretions were prepared by washing with seawater for 30 min, stained with FM 464® (Red lipophilic marker) and Syto 13® (green nuclear marker) for 30 min and examined with a confocal microscope (Biorad® MRC 1024 ES).

Drawings of testes and pleopods were made using a camera lucida mounted on a stereomicroscope. Material photographed using light microscopy was first fixed in 10% seawater formalin for 1 h, washed twice with seawater for 20 min, stained with acid fuchsin, and cleared with CMCP-10 high viscosity mountant.

For observation of morphology with SEM, formalin-preserved vasa deferentia, spermatophores, appendage, and portions of the cephalothorax from various individual crabs were washed twice for 15 min with a 0.2 M PBS (pH 7.2) (Tudge, 1992) to remove residual formalin and then dehydrated with a graded ethanol series (20–100%). Specimens were either critical-point-dried with CO_2 or air-dried after treatment with a 1:1 solution of 100% hexamethyldisalizane (HMDS) and 100% ethanol for 20 min followed by immersion in 100% HMDS. All specimens were sputter-coated with gold for 1–6 min before viewing them in a JEOL 6300-FV SEM equipped with a digital image-capture system.

To assess ovarian maturity for mating observations, female hermit crabs were removed from shells by gently heating the apex of their gastropod shells with a butane cigarette lighter. Nineteen females with mature ovaries (full of vitellogenic oocytes), but without embryos or remnants of egg membranes on the pleopods, were individually paired with a male in aquaria. Activities were recorded for 1 day at a 24-h recording speed (five frames/sec) with a time-lapse video recorder system attached to an infrared-sensitive surveillance camera equipped with a 30–180 mm zoom lens. Illumination consisted of two 880 nm infrared lamps during the night (dark) period and two 40-watt fluorescent lamps for day (light) observations. Videotapes were reviewed daily to determine if copulation had occurred. When copulation was observed on a tape (3 of 19 pairings), the pairs were preserved in 10% seawater formalin and the females were later examined for the presence or absence of spermatophores and seminal secretions. One additional copulation was observed on a water table and the mating pair was immediately preserved and later examined as in the other three pairs.

RESULTS

Gonopore and Pleopod Morphology

The distal ends of the vasa deferentia, the ejaculatory ducts, are located within the coxae of the fifth pereopods of the male and open to the exterior via the gonopores (Figs. 1, 2A). These openings are guarded by flap-like opercula (Fig. 2A). The female gonopores, the external openings of the oviducts, are located on the coxae of the third pereopods and are