JOURNAL OF MORPHOLOGY 259:106–118 (2004)

Studies of Male Sexual Tubes in Hermit Crabs (Crustacea, Decapoda, Anomura, Paguroidea). I. Morphology of the Sexual Tube in *Micropagurus acantholepis* (Stimpson, 1858), With Comments on Function and Evolution

Christopher Tudge[1,2]* and Rafael Lemaitre[2]

[1]*Biology Department, American University, Washington, DC 20016-8007*
[2]*Department of Systematic Biology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20013-7012*

ABSTRACT The external morphology and internal structure of the male sexual tube of the hermit crab *Micropagurus acantholepis*, a member of the family Paguridae from Australian waters, is described in detail using histological thick sectioning and scanning and transmission electron microscopy techniques. This is the first in-depth study of a sexual tube in the Paguroidea, a group where a remarkable number of genera (55.9% in the family Paguridae) with species having these intriguing sexual structures are known. In *M. acantholepis* a sexual tube is present on the left side, whereas only a gonopore is present on the right side. The tube is used for the delivery of spermatophores to the female and consists of a sheath of cuticular origin surrounding an internal, functional extension of the posterior vas deferens. Pedunculate spermatophores were observed within the lumen and partially extruding from the terminal opening of the tube in preserved specimens. The tube protrudes from the left coxa of the fifth pereopod as an elongate 3-mm-long, hollow, coiled structure with a terminal opening. Exteriorly the tube consists of a conspicuous thick chitinous cuticular ridge throughout its length, and a thin chitinous cuticle with sparse, regularly arranged simple setae. Interior to the cuticle, the tube contains loose connective tissue, secretory cells, oblique muscle, circular muscle, and epithelial cells. The latter cells line a central lumen that runs the length of the sexual tube. The morphology, cellular composition, and function of the tube are discussed. J. Morphol. 259: 106–118, 2004.

KEY WORDS: sexual tube; hermit crab; *Micropagurus*; Paguridae; histology; TEM; SEM

Sperm transfer in hermit crabs (superfamily Paguroidea, see Martin and Davis, 2001) is most commonly accomplished while the male/female pair are in a quasi-embrace, by simple direct release of spermatophores from the male gonopores located on the coxae of the fifth pereopods to the vicinity of the female gonopores located on the coxae of their third pereopods (e.g., Hess and Bauer, 2002). Fertilization is believed to be external in all hermit crabs. In some cases, such as in the Diogenidae genera *Paguristes* and *Pseudopaguristes*, Pylochelidae, and most Parapaguridae, the male first and second pairs of abdominal appendages, or pleopods, are modified as gonopods to act as transfer agents, as is seen in other decapods such as penaeoid shrimp, crayfish, lobsters, and many brachyuran crabs. However, some semiterrestrial hermit crabs of the family Coenobitidae, and at least 38 (or 55.9%) of the 68 known genera of the family Paguridae, have developed entirely different, specialized structures for sperm transfer called "sexual tubes" (e.g., Ingle, 1993; Sandberg and McLaughlin, 1998). These sexual tubes are believed to be functional prolongations of the vas deferens from one or both of the male gonopores, or may be formed by elongations of one or both coxae into often hardened, tubular protrusions (Lemaitre and McLaughlin, 2003).

The existence of sexual tubes in hermit crabs has been known for nearly two centuries (e.g., Milne Edwards, 1837; De Haan, 1849; Stimpson, 1858; Henderson, 1888). However, it is only recently that the astonishing variety and complex detail of these sexual tubes has been recognized (e.g., McLaughlin, 2003). Until the present investigation, no detailed information has been available on the external morphology and internal structure of a pagurid sexual tube. The shape and position of the sexual tubes have been used as distinguishing taxonomic characters at the generic and species levels (e.g., de Saint Laurent-Dechancé, 1966a,b; de Saint Laurent, 1970a,b; Nakasone, 1988; Ingle, 1993; McLaughlin,

*Correspondence to: C. Tudge, Biology Department, American University, 4400 Massachusetts Ave. NW, Washington, DC 20016-8007. E-mail: ctudge@american.edu or tudge.christopher@nmnh.si.edu

DOI: 10.1002/jmor.10174

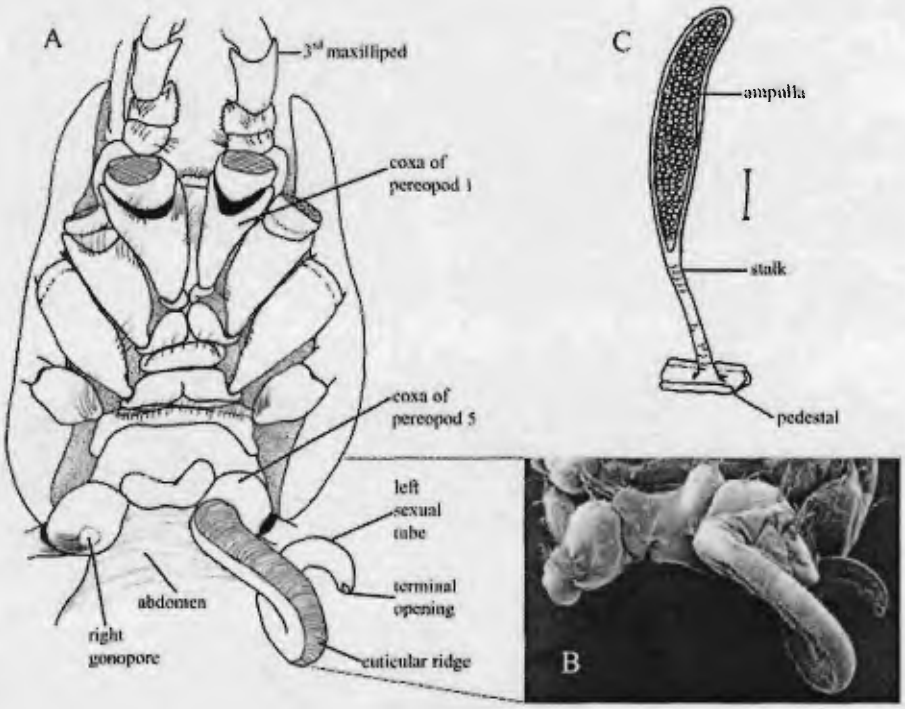

Fig. 1. *Micropagurus acantholepis*, male. **A:** Diagrammatic sternal region showing coxae of pereopods 1–5, right gonopore and left sexual tube. **B:** Coxae and sternite of pereopods 5 showing gonopore, and sexual tube. SEM. **C:** Diagrammatic morphology of single pedunculate spermatophore (scale bar = 100 μm). Drawings not to scale.

2003), but details of tube morphology, internal structure, function, and evolution have remained poorly studied.

This study of sexual tube morphology, microstructure, and ultrastructure in *Micropagurus acantholepis*, a species distributed along the southern coast of Australia (Gunn and McLaughlin, 1988), is the first in a series designed to explore the complexities of sexual tube morphology, development, functionality, and evolution, which are essential to future research into hermit crab reproductive behavior. However, due to the paucity of studies on sexual tubes, and hermit crab mating behavior, it is not yet possible to explain the striking variety of sexual tube shapes, differences in function, development, and evolution, or to make comparisons of phylogenetic significance with sperm transfer organs in other decapods.

In *Micropagurus acantholepis*, a sexual tube is present on the left side and is recurved or coiled upward to the level of the coxa of the fifth pereopod (Fig. 1). On the right side there is no sexual tube and only an open gonopore is present, although other species of *Micropagurus* lack the right gonopore (McLaughlin, 1986, 2003; Gunn and McLaughlin, 1988). Histological sectioning and staining for light microscopy (LM) and both scanning electron microscopy (SEM) and transmission electron microscopy (TEM) techniques are here used to identify the external and internal microstructure, as well as the cellular composition of the sexual tube.

MATERIALS AND METHODS

Four individuals of *Micropagurus acantholepis* (Stimpson, 1858) were used for the histological procedures outlined below. No decalcification was undertaken prior to the histology or electron microscopy.

Wax Sectioning

The sexual tube from a male specimen collected in southern Port Phillip, Victoria, Australia, on 18 April 1987 (Museum Victoria catalogue number J17494) was dissected and placed in Bouin's fixative for 2 h, several changes of 50% ETOH, and then stored in 70% ETOH. An ascending ethanol series of 80, 90, and 2× 100% for 30 min each was applied, followed by two changes of toluene for 30 min each, wax for 30 min, and then wax in a vacuum for 1 h. Thick sections (6 μm) were cut on a Spencer 820 rotary microtome and later stained with Mayer's hematoxylin-eosin. Sections were viewed and photographed with an Olympus BH2 optical microscope equipped with Nomarski interference contrast optics, with an attached Olympus OM-2 camera.

Scanning Electron Microscopy

Two male specimens, one from the Northwest Bank in the mouth of the Tamar Estuary, Tasmania, Australia, and the other from Lime Bay National Park, Norfolk Bay, Tasmania, were collected on 25 July 1995 and 15 August 1995, respectively. Both were originally preserved in 70% ETOH. The protocol for SEM can be summarized as prior sonication for 20 sec in 70% ETOH (Felgenhauer, 1987), progressive dehydration in an ascending ethanol series of 80, 90, and 100% for 5 min each, and finally a fresh change of 100% ethanol. The critical point-dried specimens were attached to glass coverslips on SEM stubs and sputter-coated with gold. The specimens were viewed and photographed at 10–20 kV on a Leica Stereoscan 440 scanning electron microscope.

Transmission Electron Microscopy

A male specimen was collected under Queenscliff Pier, Queenscliff, Victoria, Australia, on 10 October 1998. Observations were made of the live animal prior to fixation in 3% glutaraldehyde in phosphate buffer (PB). The TEM protocol is that described in Tudge et al. (2001) and was carried out in a Lynx-el microscopy tissue processor.

Lynx-el Microscopy Tissue Processor

Portions of the vas deferens were washed in PB (three washes of 15 min), postfixed in PB 1% osmium tetroxide for 80 min, similarly washed in buffer, and dehydrated through ascending concentrations of ETOH (20–100%). After infiltrating and embedding in Spurr's epoxy resin (Spurr, 1969), thin sections (50–80 nm thick) were cut on a LKB 2128 UM IV microtome with a diamond knife. Sections were placed on carbon-stabilized colloidin-coated 200 μm mesh copper grids and stained in 6% aqueous uranyl acetate for 30 sec, rinsed in distilled water, stained with Reynold's lead citrate (Reynolds, 1963) for 4 min, and further stained in uranyl acetate for 2 min before a final rinse in distilled water (Daddow, 1986). Micrographs were taken on a JEOL 1200EX transmission electron microscope at 80 kV.

RESULTS

Tube Size and External Morphology

As previously mentioned, the male of *Micropagurus acantholepis* has a single, coiled sexual tube projecting from the left gonopore (Figs. 1A,B, 2A). The coiled nature of the tube makes determination of an exact length difficult, but of the four specimens used an average length of 3.0 mm was obtained. In comparison, the shield length (the standard measure of size in hermit crabs, measured from tip of rostrum to midpoint of posterior margin of shield) of the two individuals used for the SEM was 2.9 mm and 3.0 mm, respectively. This gives an indication of the substantial nature of the sexual tube in these relatively small hermit crabs.

The tube is slightly greater than 500 μm in diameter at its base on the coxal segment, but attenuates gradually along its length to ~60 μm at the tip. The tube completes one-and-a-half coils throughout its length (Figs. 1B, 2A), and on the exterior plane of the coiled tube a conspicuous, cuticular ridge runs from base to tip. This cuticular ridge appears as a raised, cross-furrowed, relatively smooth strip distinct from the irregularly wrinkled exterior of the rest of the tube surface (Figs. 2A,C, 3), and decreases in width from just over 200 μm at the base of the tube to ~20 μm near the tip. The ridge consists of a thickened region of the cuticle characterized by whorled microfibrils (Fig. 5B,C) similar to the chitinous or calcified cuticle of other crustaceans (Stevenson, 1985; Felgenhauer, 1991), and probably provides the structural integrity needed for the coiling as well as a strengthened area for any internal muscle attachment.

The tip of the sexual tube has a terminal opening or pore that ranges in diameter from slightly less than 20 μm (Fig. 4C) to 56 μm (Fig. 3C). The pore has a small, distended lip around it (Figs. 3A, 4C) when in a contracted state.

Many short, simple setae occur on the exterior surface of the sexual tube over its entire length, although they are conspicuously absent from the cuticular ridge (Figs. 2A,C, 3A,C). Observations using SEM and TEM indicate that the setae are cuticular in origin. Based on the presence at their base of cellular material (dendritic?) which penetrates into the socket of the setal stalk, the setae can be considered to be innervated (Fig. 6E).

Right Gonopore

The right gonopore (Figs. 1A, 2B) consists of a spherical pore ~190 μm in diameter and is surrounded posteriorly by dense, simple, and serrulate setae that can obscure the pore. Within and around the gonopore (Fig. 2B) are membranes that appear to be cuticular in structure, similar to those on the external surface of the sexual tube. The gonopore is flush with the surface of the coxal segment, and no distinct operculum sealing the opening is apparent.

Tube Internal Organization and Ultrastructure

Transverse sections (TS) of the sexual tube at both the LM and TEM level show three main regions: 1) a thin outer chitinous, cuticular layer and a thickened cuticular ridge; 2) an inner muscle-lined lumen; and 3) connective tissue intervening between the two previous regions (Fig. 4A). At the light microscope level the outermost cuticular layer, 10–16 μm thick, appears as multiple thin layers or laminae (Fig. 4B). The thickened cuticular ridge is on the outer side of the sexual tube, and differs from the thinner multilaminate layer in having the appearance of thick whorls (Fig. 4A,B). The ridge, being on average 100 μm thick, is 6–10 times thicker than the adjacent layer of thin cuticle. In longitudinal section (LS) the aligned, serial nature of the lamellar whorls is clearly visible (Fig. 4B). At the ultrastructural level, the multilaminate appearance of the thin cuticular layer, and the serial whorling pattern of the thickened ridge, are evident. Similarly, the entire cuticular layer has no obvious mineralized inclusions and parallel bundles of chitin microfibrils are often present (Figs. 5B,C, 6E).

A central lumen runs the full length of the sexual tube (Figs. 4A–C, 5E,F), ending at the terminal pore on the tip (Fig. 4C). The lumen is circular in TS (Fig. 4A), although it appears as a thin line (Fig. 4B) due to tangential sectioning, or partial collapsing (Fig. 4C). The latter case is probably a fixation and/or sectioning artifact.

The LM and TEM revealed four principle cell types between the cuticle and the central lumen. From exterior to interior these are: 1) loose connective tissue cells, 2) secretory or glandular cells, 3)

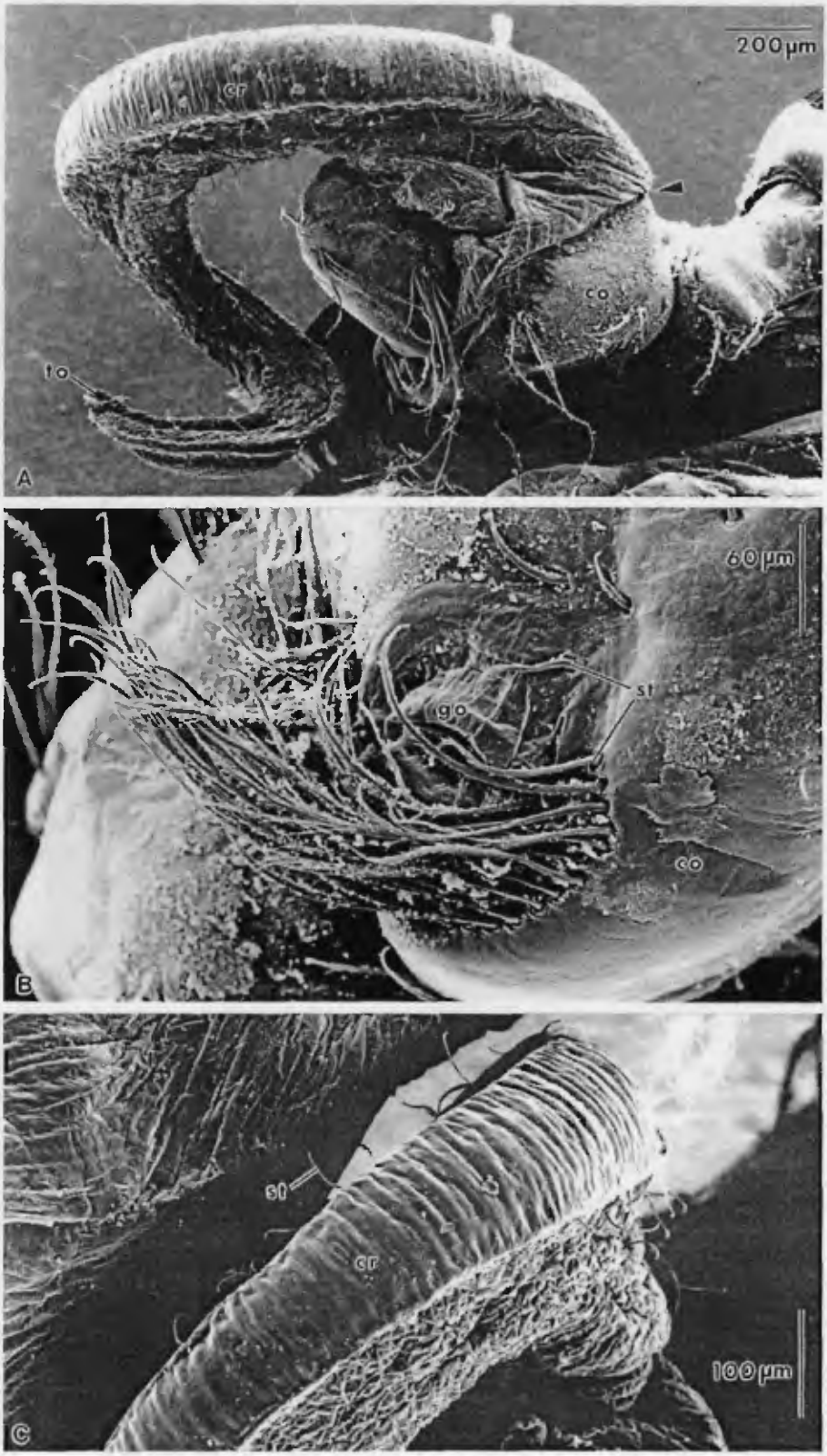

Fig. 2. *Micropagurus acantholepis*. Male sexual tube and gonopore. SEM. **A:** Lateral view of sexual tube showing spiral shape, junction (arrowhead) with coxa of left pereopod 5, and terminal opening. **B:** Detail of right gonopore and setae. **C:** Detail of thickened, cuticular ridge of the sexual tube. co, coxa; cr, cuticular ridge; go, gonopore; st, seta; to, terminal opening.

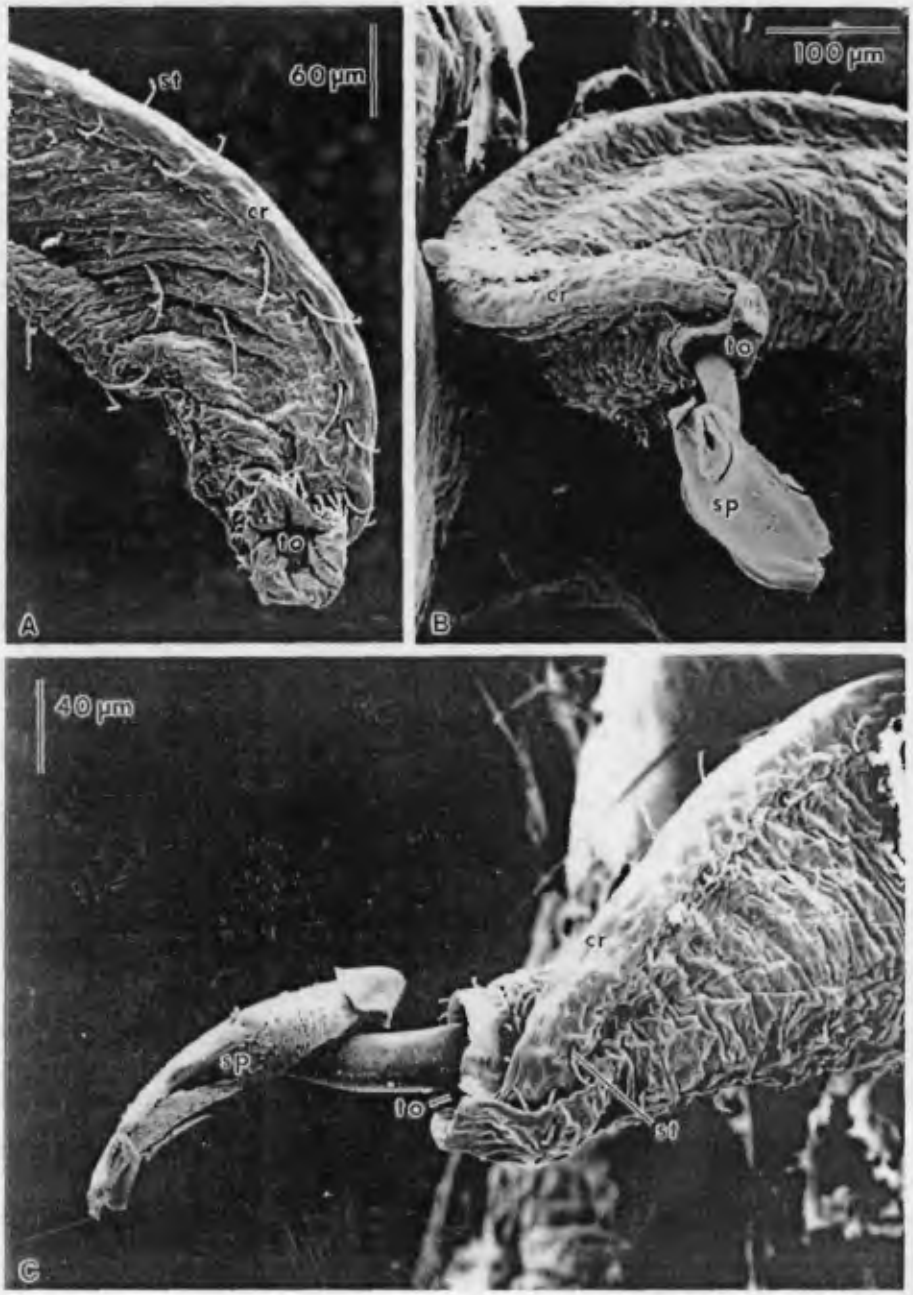

Fig. 3. *Micropagurus acantholepis*. Distal portion of male sexual tube. SEM. **A:** Terminal opening of sexual tube. **B:** Terminal opening with partially extruded spermatophore. **C:** Different view (lateral) of same terminal opening with partially extruded spermatophore. **cr**, cuticular ridge; **sp**, spermatophore pedestal; **st**, seta; **to**, terminal opening.

muscle or myocyte cells, and 4) epithelial cells. The description of these cell types follows.

1) The darkly staining cells of the connective tissue are widely scattered and are often only loosely connected to one another (Figs. 4A,B, 5D). In some sections (Fig. 4A,B) the large translucent spaces between the cells of the connective tissue may be artifactual, as more densely packed cells are seen in other light and TEM sections (Figs. 4C, 5D, 6B).

2) Several large secretory or glandular cells were identified within the connective tissue adjacent to the lumen (Fig. 4A,D,E), and are characterized by their large size of about 170 µm long by 70 µm wide and distinctive shape. These cells are much larger than the surrounding loose connective tissue cells and are immediately recognizable by their shape and contents. They are sometimes referred to as goblet cells because of this distinctive teardrop shape (Bloom and Fawcett, 1968). Both electron-dark (Figs. 4E) and electron-lucent (Figs. 4A,D, 6D) vesicles are present inside these secretory cells, with the darker vesicles concentrated at the tapered end.

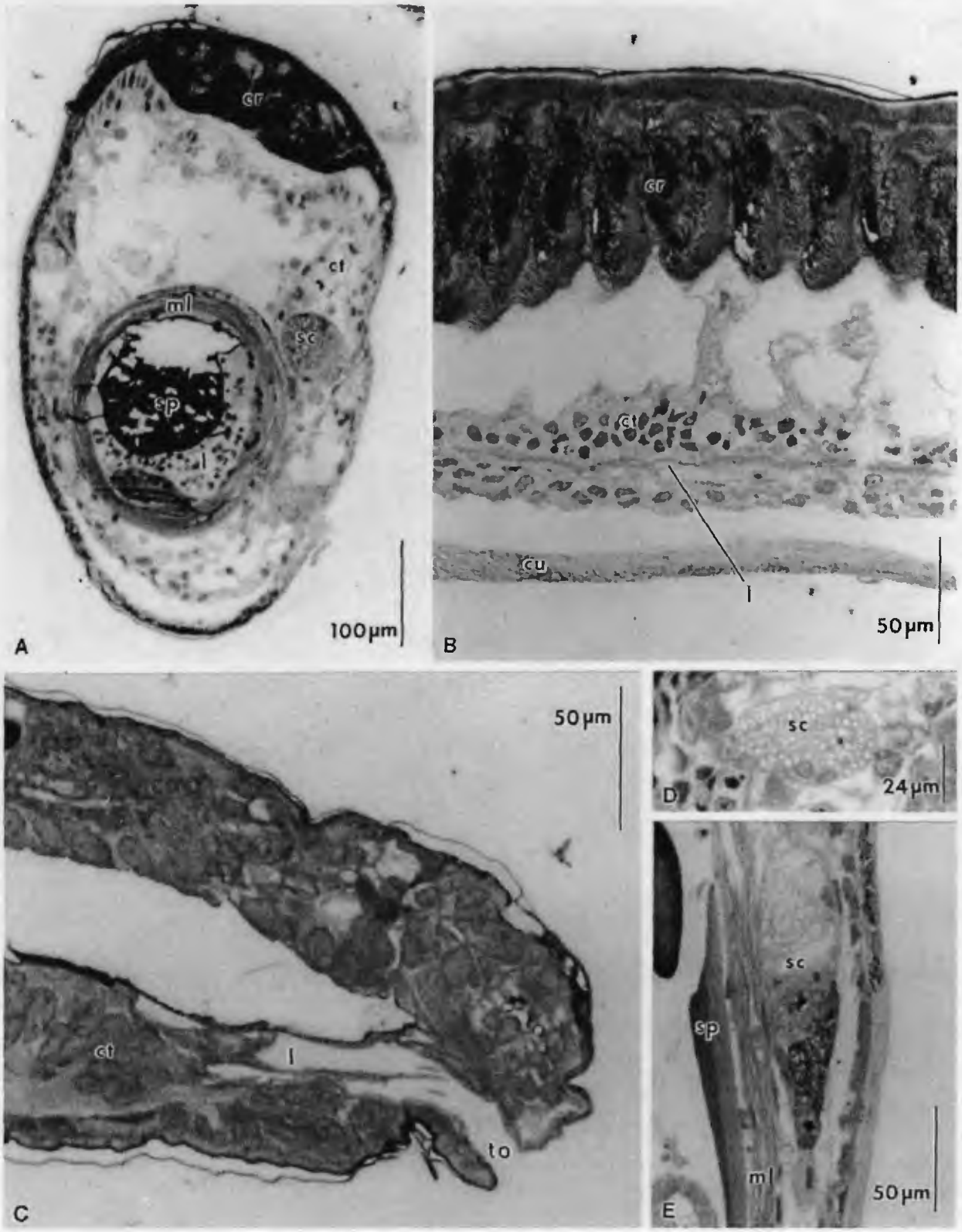

Fig. 4. *Micropagurus acantholepis*. Wax thick sections of male sexual tube. LM. **A:** Transverse section (TS) through sexual tube showing thickened cuticular ridge, loose connective tissue, secretory cell, and muscle-lined lumen enclosing a spermatophore. **B:** Oblique longitudinal section (LS) through sexual tube showing detail of cuticular ridge, loose connective tissue, and distinct cuticular nature of the thinner sexual tube wall. **C:** LS through the tip of sexual tube at the terminal opening. **D,E:** Goblet-shaped secretory cell in TS (**D**) and LS (**E**). cr, cuticular ridge; ct, connective tissue; cu, cuticle; l, lumen of sexual tube; ml, myocyte layers; sc, secretory cell; sp, spermatophore; to, terminal opening.

The change in density of the vesicles from electron lucent to dark may indicate the accumulation of secretory product within the vesicles (Fig. 6D), or two different types of vesicles.

3) The sexual tube lumen is lined with multiple layers of myocytes (Figs. 4A,E, 5E,F, 6A–C,F), about 20 μm thick in total, and the inner wall is composed of cells with membranes extended as many small microvillar projections (Fig. 5E,F). The microvillar projections ("microvilli") are separated from the muscle layers by a thin basal lamina (Fig. 5E,F). The interior of the lumen contains spermatozoa bound into pedunculate spermatophores (Figs. 4A,E, 6F), and even some loose spermatozoa (Figs. 5F, 6F). Two morphologically distinct myocyte arrangements are identifiable within the tube, and have been tentatively identified as oblique and circular muscle. The oblique muscle, consisting of myocytes 27 μm long by 10 μm wide, occurs as randomly arranged, infrequent bundles interspersed with connective tissue cells and extending across the sexual tube between the cuticle and the lumen. The associated myocyte nuclei and the distinctive myofibril bands or striations are clearly seen (Fig. 6B). The more obvious muscle type is the circular muscle that surrounds the lumen in several concentric layers. As with the oblique muscle, the occasional muscle cell nucleus and less distinct periodic banding can be seen in electron micrographs (Fig. 6A,C,F).

4) Interior to the band of concentric circular muscle, and directly adjacent to the lumen of the sexual tube, is a layer of epithelial cells. These cells are only visible at the TEM level and exhibit extremely diverse shapes with many intertwining cellular extensions and tiny "microvilli" (Fig. 5E,F). These "microvilli" are extremely numerous and project into the sexual tube lumen. The lumen itself appears reasonably clear of cellular or extracellular material, with the obvious exceptions being the large pedunculate spermatophores (Fig. 4A) and the occasional free sperm cell (Figs. 5F, 6F). In some sections the ampullae of the spermatophores occupy the majority of the lumen and parts of the stalk and pedestal are also visible (Figs. 4A, 6F).

Spermatophores

During dissection of the sexual tube of *Micropagurus acantholepis*, and mounting of specimens for SEM, spermatophores were observed inside the sexual tube and reproductive system, and also stuck to the external cuticle of one hermit crab specimen. The general spermatophore morphology (Fig. 1C) is typical for that of hermit crabs (Tudge, 1991) and the family Paguridae in particular (Tudge, 1999). The stalked or pedunculate spermatophore is composed of three main regions (tripartite) with a pedestal (or base), a stalk, and a sperm-filled ampulla. Each spermatophore is about 800 μm in height and the ampulla is about 100 μm at its widest point. One specimen was preserved with a spermatophore partially extruded from the end of the sexual tube, and the pedestal and stalk of the spermatophore can be clearly seen (Fig. 3B,C). It was not possible to determine whether the spermatophore was exiting when the hermit crab was fixed, or whether the partially extruded position of the spermatophore was an artifact of fixation.

DISCUSSION

Morphological Features

Previous articles, almost exclusively taxonomic in nature, have documented only limited aspects of the external morphology of sexual tubes at a gross level (e.g., Alcock, 1905; Fenizia, 1937; de Saint Laurent, 1968a,b,c, 1969, 1970a,b; García-Gómez, 1994; McLaughlin, 1997; Asakura, 2001). In the present study, a number of features have been confirmed and elucidated in much finer detail. The most detailed description of the tip of a sexual tube, where the lumen opens to the exterior, was provided by de Saint Laurent (1968b: 1103, fig. 39) for *Decaphyllus junquai*. The tip of the tube in *Micropagurus acantholepis*, like in *D. junquai*, has a lip and short setae around it (Figs. 3A, 4C). Some setae have been found during our study to be innervated (Fig. 6E) and might serve a sensory function during the mating process. A thick, cross-furrowed cuticular ridge (Figs. 1A, 2A,C, 3A–C, 4A,B), similar to that present in *M. acantholepis*, has previously been depicted only for *Forestopagurus drachi* (see Forest, 1966: fig. 24, as *Anapagurus drachi*), and *Anapagurus* species (see García-Gómez, 1994: fig. 2M). We have also observed (unpubl. obs.) a similar ridge on the sexual tube of *Spiropagurus* species. Quite possibly this ridge serves as structural support for the tube, which in *M. acantholepis* appears to have very limited mobility.

Spermatophore Delivery

Sexual tubes containing spermatophores within the tube have been previously illustrated in *Decaphyllus junquai* (see de Saint Laurent, 1968b: fig. 39), and *Enneobranchus flavioculatus* (see García-Gómez, 1988: fig. 1h). We have also observed (unpubl. obs.) spermatophores in the tube of preserved specimens of *Cestopagurus timidus* and in live and preserved specimens of *Iridopagurus caribbensis*. These observations, as well as those described herein for *Micropagurus acantholepis* showing spermatophores within the tube and partially extruded at the tip (Figs. 3B,C, 4A, 6F), indicate that at least in these species the tube must have a spermatophore delivery capability. The terminal opening of the tube of *M. acantholepis* (Figs. 2A, 3A–C) appears to be capable of considerable expansion to allow exit of the tripartite spermatophore. The width of the terminal opening (~50 μm) of the tube is less than

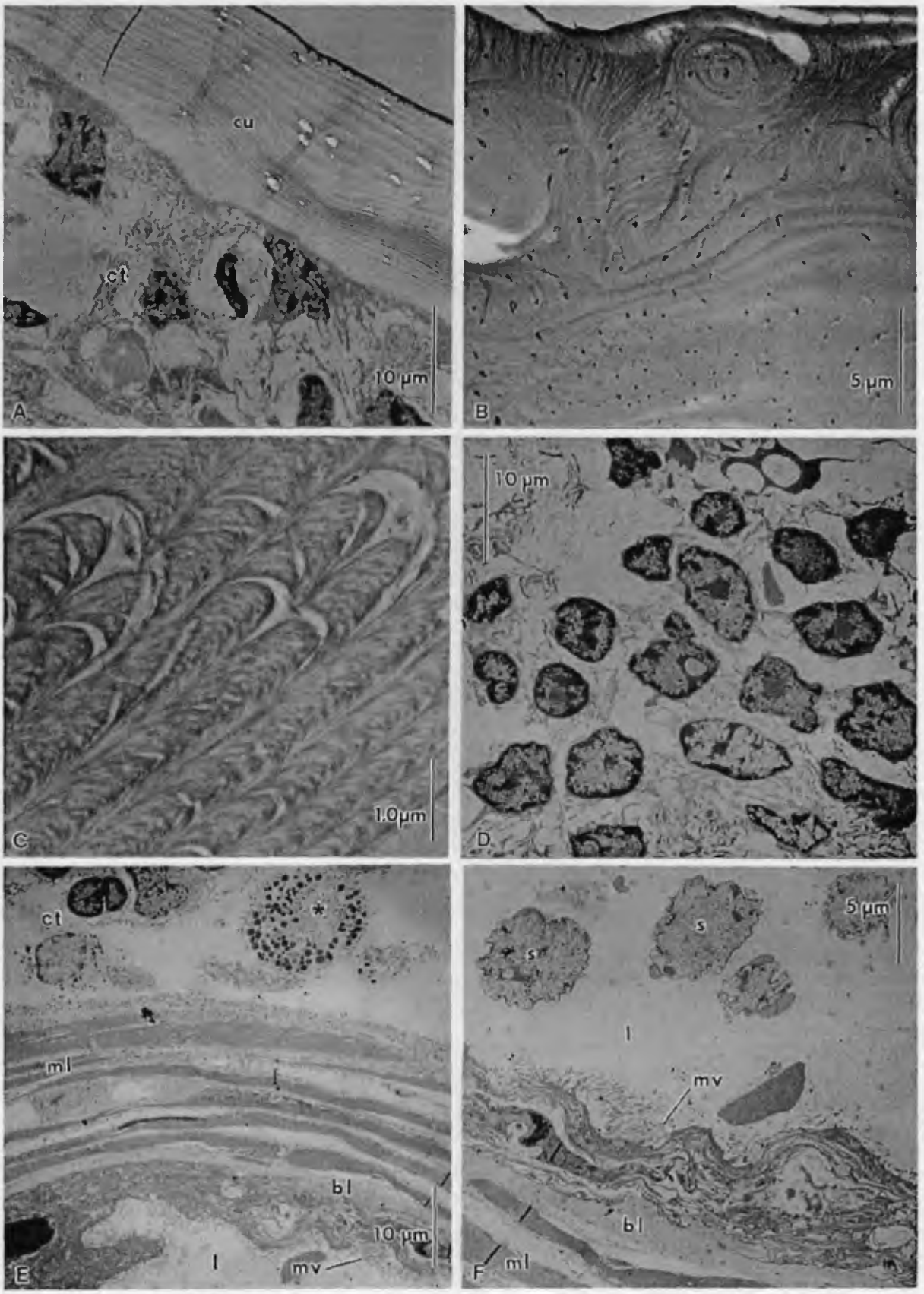
cu
ct
10 µm
A
5 µm
B
1.0µm
C
10 µm
D
ct
*
ml
bl
10 µm
l
mv
E
5 µm
s
s
l
mv
bl
F
ml

Figure 5

the width of the spermatophore pedestal (Fig. 3B,C). Also, the sperm-filled ampulla is of greater diameter than the preceding stalk (Fig. 1C), so the terminal opening of the tube would have to expand to expel it. Again, direct observations of mating behavior using live animals are needed to understand how this process functions.

Cellular Composition and Function

Both light and ultrastructural microscopy revealed important details of the microstructure of the sexual tube of *Micropagurus acantholepis*. The outermost membrane surrounding the sexual tube was found unequivocally to be made of typical, chitinous crustacean cuticle (Figs. 4A,B, 5A–C, 6B,E). The particular morphology of crustacean cuticle, with multilaminar appearance in some sections and a swirling pattern of chitin fibrils in others, is unmistakable (Johnson, 1980; Stevenson, 1985; Felgenhauer, 1991; Marlowe and Dillaman, 1995). The crustacean cuticle is primarily composed of the multilaminate, chitinous procuticle (exo- and endocuticle), but may also be calcified by the deposition of mineral crystals (usually calcium salts). When calcification occurs, the calcium salts fill spaces between the chitin microfibrils and generally appear as vertical canals of stacked crystals (Stevenson, 1985). When calcified cuticle is decalcified, these canals lose their salts and appear as aligned translucent spaces. Since the sexual tube tissue was not decalcified during preparations of our specimens, and no mineral crystal canals are present in light or TEM sections, we conclude that the cuticle consists only of chitin and its associated proteins. In fact, bundles of chitin microfibrils are clearly visible in some ultrastructural micrographs of the cuticle (Figs. 5B,C, 6E). The majority of the cuticular membrane surrounding the sexual tube in *M. acantholepis* is composed of thin cuticle (~13 μm thick), and under SEM appears as a highly wrinkled surface (Figs. 2A,C, 3A–C).

Glandular cells similar in morphology and shape to those found in the tube of *Micropagurus acantholepis*, have been recorded from other crustaceans (Felgenhauer, 1991; Fingerman, 1991), as well as from mucous-secreting tissues of oligochaetes (Jamieson, 1981) and bivalve molluscs (Norenburg and Ferraris, 1990). The presence of secretory cells adjacent to the tube lumen of *M. acantholepis* suggests that a secretion is being produced into the lumen, perhaps as lubrication to assist the passage of spermatophores, or as part of the complex formation of the spermatophores (Mouchet, 1931; Greenwood, 1972; Tudge, 1999).

The circular muscle cells found in the tube of *Micropagurus acantholepis* lack repeated banding or any particular pattern of myofibrils (Figs. 5E, 6A,C,F) and are similar to those described for pseudostriated muscle in nematodes (Hope, 1969) and nemerteans (Norenburg and Roe, 1998). The circular muscle development around the sexual tube lumen may produce voluntary, peristalsis-like movement of the lumen contents, and thus possibly aid in spermatophore expulsion or in holding the spermatophores in place within the sexual tube. The less frequent oblique myocytes may only provide limited movement of the entire sexual tube. Extensive mobility of the sexual tube seems doubtful considering the small amount of muscle tissue relative to the size and volume of the tube. Once again, direct observations of the sexual tube in live specimens are needed.

Tube Function and Evolutionary Comments

As previously indicated, sexual tube development in species of a substantial number of paguroid genera is diverse and highly variable. Heretofore, even studies on the external morphology of male hermit crab gonopores have been few (e.g., Lancaster, 1988; Manjón-Cabeza and García Raso, 2000; Hess and Bauer, 2002). Thus, it is not surprising that this first in-depth study of a sexual tube has generated more questions than it has provided answers. However, the present study has shown that at least in *Micropagurus acantholepis* the tube is much more complex than previously thought. The tube has elements of the associated coxal segment (chitinous cuticle, oblique muscle, and connective tissue), as well as tissues in common with the vas deferens (circular muscle, secretory cells, and epithelium) immediately surrounding the central lumen. It is clear that the tube is a functional extension of the posterior vas deferens, and exteriorly consists of a structural elongation or chitinous sheath, cuticular in structure, that originates from the coxal segment. But why in *M. acantholepis* do males have both a spermatophore-delivering left sexual tube and apparently also a functional right gonopore that lacks a similar tube? Other species of the genus reportedly lack a right gonopore altogether (McLaughlin, 1986; Haig and Ball, 1988). The right gonopore in *M. acantholepis* lacks an operculum, whereas it is present in other hermit crab species with paired functional gonopores, such as *Diogenes pugilator* and *Clibanarius vittatus* (see Manjón-Cabeza and García Raso, 2000; Hess and Bauer, 2002). An explanation cannot

Fig. 5. *Micropagurus acantholepis*. Male sexual tube. TEM. **A:** LS of cuticle of thin wall of sexual tube. **B,C:** Detail of thickened cuticular ridge showing characteristic patterns of chitinous crustacean cuticle. **D:** Multiple cells in loose connective tissue. **E:** TS through muscular lumen wall from connective tissue at top, to the lumen itself at bottom. Note: possible granular hemocyte (*). **F:** TS through muscular lumen wall showing microvilli and scattered sperm cells within the lumen. bl, basal lamina; ct, connective tissue; cu, cuticle; l, lumen of sexual tube; ml, myocyte layers; mv, microvilli; s, sperm cell.

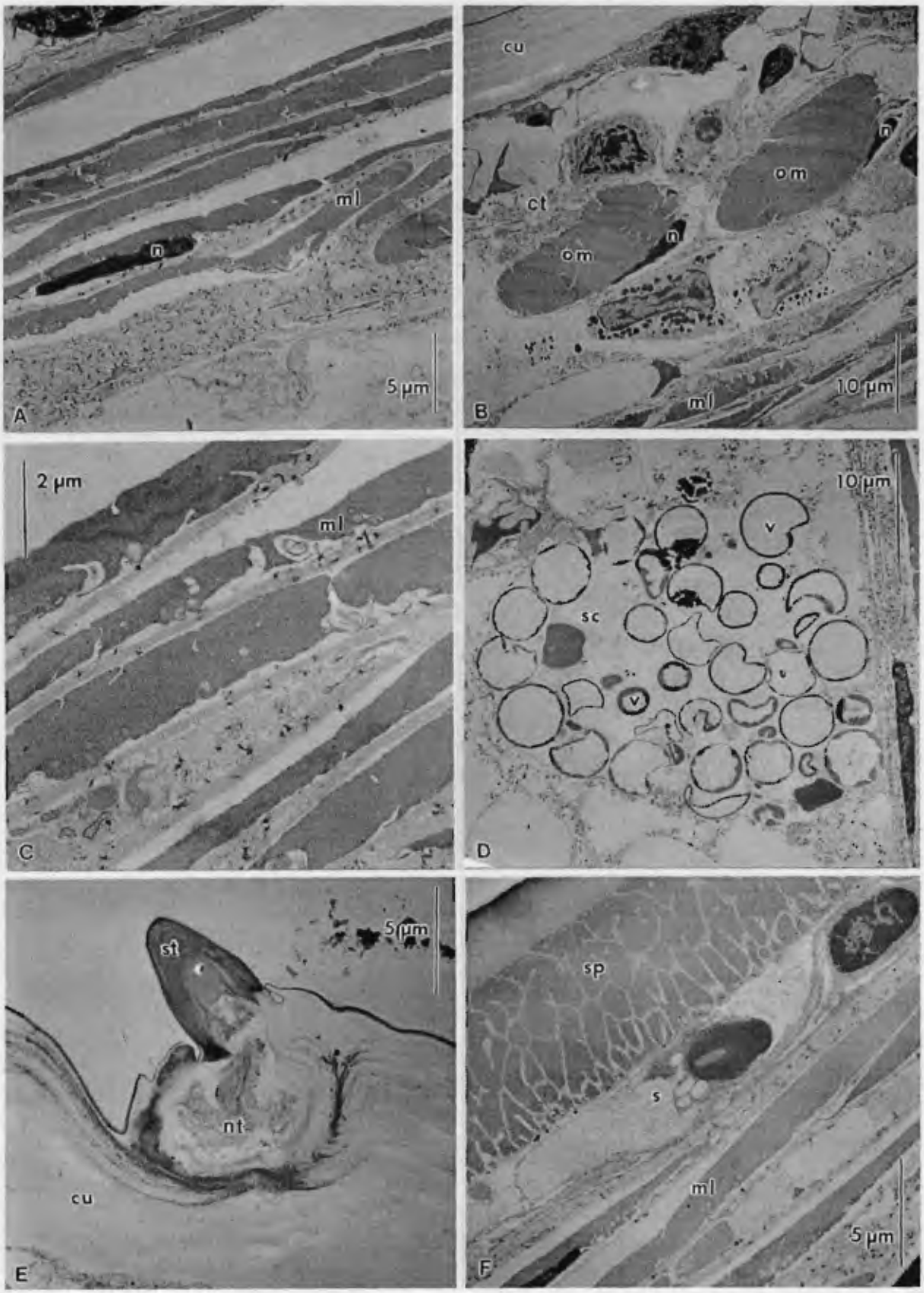
A
ml
n
5 µm
B
cu
n
om
ct
n
om
ml
10 µm
C
2 µm
ml
D
10 µm
v
sc
v
E
5 µm
st
nt
cu
F
sp
s
ml
5 µm

Figure 6

be provided at this time, as the focus of the present study was to establish preliminary insight into the structure of the pagurid sexual tube. However, the answer to this question, as well as those involving the evolution of sexual tube development within the entire family Paguridae, will provide a wealth of avenues for future research, some of which are already ongoing. In fact, until the phylogenetic relationships of paguroid families and genera are known, one can only speculate on the evolution of sexual tubes.

Similarly, within the superfamily Paguroidea there are questions about the need for a specialized spermatophore delivery system in species of some genera but not in others. As Hess and Bauer (2002) reported, males of the diogenid *Clibanarius vittatus*, which lacks sexual tubes, simply emit material containing spermatophores onto the sterna of the females without having any special delivery system, and during copulation the male/female pair position themselves so that the ventral surfaces of the cephalothorax are apposed. Is copulatory behavior in sexual tube-bearing species different from those without tubes? What is so different about the Paguridae, where species in 55.9% of the 68 known genera have a specialized sexual tube sperm transfer mechanism, and the Diogenidae, where only species of three genera have any form of sperm transfer specializations at all? Again, we do not as yet have any substantive information to answer these questions. Nor do we have sufficient data to begin to formulate hypotheses regarding the possible homologies between paguroid sexual tubes, brachyuran penes, and male sperm transfer organs in other decapods such as penaeoid shrimps, crayfishes, and lobsters. Based on anatomical position, however, the male sexual tubes of paguroids are entirely different structures than the sperm transfer organs of other decapods. In paguroids the sexual tubes originate from the coxae of the last pair of thoracic pereopods, and there are no pleopods to aid in spermatophore transfer. In contrast, in other decapods the spermatophore transfer is accomplished by the modified first two pairs of abdominal appendages (gonopods). In some brachyurans, such as the Dromiacea, tubular structures do develop from the gonopores (see Guinot and Tavares, 2003), although here again, the sperm transfer is aided by the gonopods. Evidently, much data must yet be accumulated if our understanding of comparative morphology and behavioral ecology is to be applicable to all decapods.

Our initial research has, nevertheless, revealed or confirmed some important information. For example, we have found that the sexual tube in *Micropagurus acantholepis*, and presumably all other pagurid species in which sexual tubes develop, is indeed a sperm transfer mechanism. How or why it has evolved in such elaborate diversity, particularly in the Paguridae, is still a matter of speculation. Species of several genera such as *Anapagrides* (see McLaughlin and Sandberg, 1995), *Paguritta* (see McLaughlin and Lemaitre, 1993), *Parapagurodes* (see McLaughlin and Haig, 1973; Komai 1999), and *Pylopagurus* (see McLaughlin and Lemaitre, 2001) are known to have very short tubes or simply papillary protrusions of the vas deferens. Do these cases represent early stages in tube evolution, or are they stages of the vas deferens that reflect potential for extension during the mating process? No studies of mating have been conducted, although such data are critical to our understanding of sexual tube function and evolution.

Fig. 6. *Micropagurus acantholepis*. Sexual tube. TEM. **A:** Myocyte layers in lumen wall. **B:** Two oblique myocytes between the outer cuticle and the inner lumen. **C:** Detail of myocyte layers around lumen. **D:** Detail of ring-like vesicles inside goblet-shaped secretory cells. **E:** LS through the base of a cuticular seta. **F:** LS through single sperm cell wedged between base of spermatophore in the lumen and the myocyte layers in the lumen wall. ct, connective tissue; cu, cuticle; ml, myocyte layers; n, nucleus; nt, nerve tissue; om, oblique myocyte; s, sperm cell; sc, secretory cell; sp, spermatophore; st, seta; v, cellular vesicles.

ACKNOWLEDGMENTS

The authors thank Gary Poore, John Moverley (Museum Victoria, Melbourne, Australia), Matt McArthur (Marine and Freshwater Resources Institute, Queenscliff, Victoria, Australia), and Karen Mudar for assistance with acquisition or collection of specimens. We thank Lina Daddow (Department of Zoology and Entomology, The University of Queensland, Brisbane, Australia) for patient training in histological staining techniques (CT). We thank Walter Brown, Susann Braden, Rose Gulledge, Duane Hope and Abbie Yorkoff (National Museum of Natural History, Smithsonian Institution, Washington DC, USA) for assistance with SEM and TEM and Patsy A. McLaughlin for insightful comments on an earlier version of the manuscript. This project was initially supported by an Australian ARC Postdoctoral Fellowship (1996-1999) to CT and has been completed while the senior author is a Smithsonian Institution Research Associate. The support of Kristian Fauchald and David Pawson (National Museum of Natural History, Smithsonian Institution, Washington DC, USA) to CT during this latter period is gratefully acknowledged. Our studies on hermit crab sexual tubes were partly supported by the Smithsonian Marine Station at Fort Pierce, Florida, and this is contribution no. 566 from that Station.

LITERATURE CITED

Alcock A. 1905. Anomura. Fasciculus. 1. Pagurides. Catalogue of the Indian decapod Crustacea in the collections of the Indian Museum. Part 2. Indian Museum, Calcutta: India.

Asakura A. 2001. A revision of the hermit crabs of the genera *Catapogurus* A. Milne-Edwards and *Hemipagurus* Smith from

the Indo-West Pacific (Crustacea: Decapoda: Anomura: Paguridae). Invert Tax 15:823–891.
Bloom W, Fawcett DW. 1968. A textbook of histology. Philadelphia: W.B. Saunders.
Daddow LYM. 1986. An abbreviated method of the double lead stain technique. J Submicrosc Cytol 18:221–224.
Felgenhauer BE. 1987. Techniques for preparing crustaceans for scanning electron microscopy. J Crust Biol 7:71–76.
Felgenhauer BE. 1991. External anatomy and integumentary structures. In: Harrison FW, Humes AG, editors. Microscopic anatomy of invertebrates, vol. 10. Decapod Crustacea. New York: Wiley-Liss. p 7–43.
Fenizia G. 1937. I Paguridi del Golfo di Napoli. 2° Contributo. Specie e varietà dei generi *Catapaguroides* M. Edwards e Bouvier, *Anapagurus* Henderson e *Nematopagurus* M. Edwards e Bouvier. Ann Mus Zool Univ Napoli (Nuova Serie) 7:1–40.
Fingerman M. 1991. Glands and secretion. In: Harrison FW, Humes AG, editors. Microscopic anatomy of invertebrates, vol. 10. Decapod Crustacea. New York: Wiley-Liss. p 345–394.
Forest J. 1966. Résultats scientifiques des campagnes de la "Calypso", 7 – Campagne dans le golfe de Guinée et aux Iles Principe, São Tomé et Annobon (1956). 17. Crustacés Décapodes; Pagurides. Ann Instit Océanogr nouvelle sér 44:125–172.
García-Gómez J. 1988. A new genus and three new species of hermit crabs (Crustacea: Decapoda: Paguridae) from the western Atlantic Ocean. Bull Mar Sci 42:44–64.
García-Gómez J. 1994. The systematics of the genus *Anapagurus* Henderson, 1886, and a new genus for *Anapagurus drachi* Forest, 1966 (Crustacea: Decapoda: Paguridae). Zool Verhandel 295:1–131.
Greenwood JG. 1972. The male reproductive system and spermatophore formation in *Pagurus novae-zealandiae* (Dana) (Anomura: Paguridea). J Nat Hist 6:561–574.
Guinot D, Tavares M. 2003. A new subfamilial arrangement for the Dromiidae de Haan, 1833, with diagnoses and descriptions of new genera and species (Crustacea, Decapoda, Brachyura). Zoosystema 25:43–129.
Gunn SW, McLaughlin PA. 1988. The rediscovery of *Pagurus acantholepis* (Stimpson) (Decapoda: Anomura: Paguridae). Mem Mus Vic 49:67–71.
Haan W De. 1849. 1833-1850. Crustacea. In: Siebold PF, editor. Fauna Japonica sive descriptio animalium, quae in itinere per Japoniam, jussu et auspiciis superiorum, qui summum in India Batava Imperium tenent, suscepto, annis 1823-1830 collegit, notis, observationibus et adumbrationibus illustravit. Leiden: Lugduni-Batavorum. p 243.
Haig J, Ball EE. 1988. Hermit crabs from North Australian and Eastern Indonesian waters (Crustacea Decapoda: Anomura: Paguroidea) collected during the 1975 *Alpha Helix* Expedition. Rec Aust Mus 40:151–196
Henderson JR. 1888. Report of the Anomura collected by H.M.S. "Challenger" during the years 1873–1876. Report on the scientific results of the voyage of HMS Challenger during the Years 1873–1876. Zoology 27:1–221.
Hess GS, Bauer RT. 2002. Spermatophore transfer in the hermit crab *Clibanarius vittatus* (Crustacea, Anomura, Diogenidae). J Morphol 253:166–175.
Hope WD. 1969. Fine structure of the somatic muscles of the free-living marine nematode *Deontostoma californicum* Steiner and Albin, 1933 (Leptosomatidae). Proc Helminth Soc Wash 36:10–29.
Ingle R. 1993. Hermit crabs of the northeastern Atlantic Ocean and Mediterranean Sea. London: Chapman and Hall.
Jamieson BGM. 1981. The ultrastructure of the Oligochaeta. London: Academic Press.
Johnson PT. 1980. Histology of the blue crab, *Callinectes sapidus*. A model for the Decapoda. New York: Praeger Scientific.
Komai T. 1999. Reexamination of the type material of *Pagurus sagamiensis* Miyake (Decapoda: Anomura: Paguridae). Nat Hist Res 5:79–92.
Lancaster I. 1988. *Pagurus bernhardus* (L.) — an introduction to the natural history of hermit crabs. Field Stud 7:189–238.
Lemaitre R, McLaughlin PA. 2003. A new species of *Goreopagurus* (Decapoda: Anomura: Paguridae) from Tasmania and reevaluation of sexual tubes in hermit crab systematics. Mem Mus Vic 60:85–90.
Manjón-Cabeza ME, García Raso E. 2000. Morphological reproductive aspects of males of *Diogenes pugilator* (Roux, 1829) (Crustacea, Decapoda, Anomura) from southern Spain. Sarsia 85:195–202.
Marlowe RL, Dillaman RM. 1995. Acridine orange staining of decapod crustacean cuticle. Invert Biol 114:79–82.
Martin JW, Davis GE. 2001. An updated classification of the Recent Crustacea. Nat Hist Mus of Los Angeles County Sci Series 39:1–124.
McLaughlin PA. 1986. Three new genera and species of hermit crabs (Crustacea, Anomura, Paguridae) from Hawaii. J Crust Biol 6:789–803.
McLaughlin PA. 1997. Crustacea Decapoda: hermit crabs of the family Paguridae from the KARUBAR cruise in Indonesia. In: Crosnier A, Bouchet P, editors. Résultats des Campagnes MUSORSTOM 16. Mém Mus nat Hist nat 172:433–572.
McLaughlin PA. 2003. Illustrated keys to families and genera of the superfamily Paguroidea (Crustacea: Decapoda: Anomura), with supplemental diagnoses of the genera of the Paguridae. Mem Mus Vic 60:111–144.
McLaughlin PA, Haig J. 1973. On the status of *Pagurus mertensii* Brandt, with descriptions of a new genus and two new species from California (Crustacea: Decapoda: Paguridae). Bull S Calif Acad Sci 72:113–136.
McLaughlin PA, Lemaitre R. 1993. A review of the hermit crab genus *Paguritta* (Decapoda: Anomura: Paguridae) with descriptions of three new species. Raffles Bull Zool 41:1–29.
McLaughlin PA, Lemaitre R. 2001. Revision of *Pylopagurus* and *Tomopagurus* (Crustacea: Decapoda: Paguridae), with descriptions of new genera and species. Part VI. *Pylopagurus* A. Milne-Edwards & Bouvier, 1891, *Haigia* McLaughlin, 1981, and *Pylopaguridium*, a new genus. Proc Biol Soc Wash 114:444–483.
McLaughlin PA, Sandberg L. 1995. Redescription of Gustaf Melin's *"Eupagurus (Pagurillus)" exiguus, "Eupagurus (Catapagurus)" vallatus, "Eupagurus (Spiropagurus)" facetus* (Decapoda: Anomura: Paguridae) based on the type material. J Crust Biol 15:569–587.
Milne Edwards H. 1837. Histoire naturelle des Crustacés, comprenant l'anatomie, la physiologie et la classification de ces animaux, vol. 2. Paris: Librairie Encyclopédique de Roret.
Mouchet S. 1931. Spermatophores des Crustacés Décapodes Anomoures et Brachyoures et castration parasitaire chez quelques Pagures. Annal Stat Océanogr Salammbô 6:1–203.
Nakasone Y. 1988. Land hermit crabs from the Ryukyus, Japan, with a description of a new species from the Philippines (Crustacea, Decapoda, Coenobitidae). Zool Sci 5:165–178.
Norenburg JL, Ferraris JD. 1990. Cytomorphology of the pedal aperture glands of *Mya arenaria* L. (Mollusca, Bivalvia). Can J Zool 68:1137–1144.
Norenburg JL, Roe P. 1998. Observations on musculature in pelagic nemerteans and on pseudostriated muscle in nemerteans. Hydrobiologia 365:109–120.
Reynolds ES. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J Cell Biol 17: 208–212.
Saint Laurent M de. 1968a. Révision des genres *Catapaguroides* et *Cestopagurus* et description de quatre genres nouveaux. I. *Catapaguroides* A. Milne Edwards et Bouvier et *Decaphyllus* nov. gen. (Crustacés Décapodes Paguridae). Bull Mus Hist nat Paris 2e sér no 5 and 6, 39:923–954.
Saint Laurent M de. 1968b. Révision des genres *Catapaguroides* et *Cestopagurus* et description de quatre genres nouveaux. I. *Catapaguroides* A. Milne Edwards et Bouvier et *Decaphyllus* nov. gen. (Crustacés Décapodes Paguridae) (suite). Bull Mus Hist nat Paris 2e sér no 6, 39:1100–1119.
Saint Laurent M de. 1968c. Révision des genres *Catapaguroides* et *Cestopagurus* et description de quatre genres nouveaux. II. *Cestopagurus* Bouvier (Crustacés Décapodes Paguridae). Bull Mus Hist nat Paris 2e sér no 3, 40:539–552.

Saint Laurent M de. 1969. Révision des genres *Catapaguroides* et *Cestopagurus* et description de quatre genres nouveaux. III. *Acanthopagurus* de Saint Laurent (Crustacés Décapodes Paguridae). Bull Mus Hist nat Paris 2e sér no 3, 41:731–742.

Saint Laurent M de. 1970a. Révision des genres *Catapaguroides* et *Cestopagurus* et description de quatre genres nouveaux. IV. *Solenopagurus* de Saint Laurent (Crustacés Décapodes Paguridae). Bull Mus Hist nat Paris 2e sér no 6, 41:1448–1458.

Saint Laurent M. de 1970b. Révision des genres *Catapaguroides* et *Cestopagurus* et description de quatre genres nouveaux. V. *Trichopagurus* de Saint Laurent (Crustacés Décapodes Paguridae). VI. Conclusion. Bull Mus Hist nat Paris 2e sér no 1, 42:210–222.

Saint Laurent-Dechancé M de. 1966a. *Iridopagurus*, genre nouveau de Paguridae (Crustacés Décapodes) des mers tropicales Américaines. Bull Mus Hist nat Paris 2e sér no 2, 38:151–173.

Saint Laurent-Dechancé M de. 1966b. Remarques sur la classification de la famille des Paguridae et sur la position systématique d' *Iridopagurus* de Saint Laurent. Diagnose d' *Anapagrides* gen. nov. Bull Mus Hist nat Paris 2e sér no 3, 38:257–265.

Sandberg L, McLaughlin PA. 1998. Crustacea, Decapoda, Paguridea. Mar Invert Scand 10:1–120.

Spurr AR. 1969. A low viscosity epoxy-resin embedding medium for electron microscopy. J Ultrastruct Res 26:31–43.

Stevenson JR. 1985. Dynamics of the integument. In: Bliss DE, Mantel LH, editors. The biology of the Crustacea, vol. 9. Integument, pigments, and hormonal processes. Orlando, FL: Academic Press. p 1–42.

Stimpson W. 1858. Prodromus descriptionis animalium evertebratorum, quae in Expeditione ad Oceanum Pacificum Septentrionalem, a Republica Federata missa, Cadwaladaro Ringgold et Johanne Rodgers Ducibus, observavit et descripsit. W. Stimpson. Pars VII. Crustacea Anomoura. Preprint (December 1858) from Proc Acad Nat Sci Philadelphia 10:225–252.

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

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

Tudge CC, Scheltinga DM, Jamieson BGM. 2001. Spermatozoal morphology in the "symmetrical" hermit crab, *Pylocheles (Bathycheles)* sp. (Crustacea, Decapoda, Anomura, Paguroidea, Pylochelidae). Zoosystema 23:117–130.