Studies of male sexual tubes in hermit crabs (Crustacea, Decapoda, Anomura, Paguroidea). II. Morphology of the sexual tube in the land hermit crabs, *Coenobita perlatus* and *C. clypeatus* (Coenobitidae)

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Abstract.—Male gonopores on most hermit crabs (Paguroidea) occur as circular or oval apertures on the coxae of the fifth pereopods, are flush with the surrounding coxal segment, may be covered by a membrane or a calcified operculum, and are normally associated with numerous complex setae. In many male paguroids of the families Paguridae and Coenobitidae, elongate tube-like extensions called sexual tubes are found on the coxae. The sexual tubes of *C. perlatus* and *C. clypeatus* of the Coenobitidae are here investigated at the histological and SEM level for the first time. *Coenobita clypeatus* has two short, squarish, equal/subequal extensions of the coxae with the densely hirsute gonopore directed ventrally. In contrast, *C. perlatus* has large, unequal, tubular extensions of the coxae, directed posteriorly, and with the hirsute gonopores terminally on the tubes. The right sexual tube is the longest, and curves across the mid-line of the body. The left sexual tube is much shorter and extends posteriorly from the coxa. In both, the sexual tubes have various coarse setae of several different morphotypes. Histological sectioning reveals a *vas deferens* terminating at the gonopore, and that sexual tubes are as heavily calcified and muscular as the coxal segments they emanate from. This differs markedly from previously investigated sexual tubes in other paguroids.

INTRODUCTION

The semi-terrestrial hermit crab family Coenobitidae currently contains 16 species in the genus *Coenobita* Latreille, 1829, and the monotypic *Birgus* Leach, 1815, represented by the coconut crab *B. latro* (Linnaeus, 1767). Research investigating aspects of their reproduction span the last 50 years and for *Birgus* include papers on fertilization method and copulation behaviour (Matthews, 1956; Hallman, 1977), reproductive ecology and biology (Schiller et al., 1991), and morphology of spermatophores and spermatozoa (Tudge, 1991; Tudge & Jamieson, 1991). Similarly, for the genus *Coenobita* publications on reproduction cover a range of topics from probable method of fertilization (Matthews, 1956), through diverse aspects of breeding seasons, sites and behaviours, larval release, sexual maturity and egg size (Nakasone, 2001; Imafuku, 2001, 2002) to annual migrations and spawning behaviour (Nieves-Rivera & Williams, 2003). A more detailed analysis of the ultrastructure of spermiogenesis, spermatozoa and spermatophores for several species of *Coenobita* can be found in Hinsch (1980a, b), Tudge (1991) and the review by Jamieson & Tudge (2000).

The subject of the mechanics of spermatophore transfer in hermit crabs (Hess & Bauer, 2002; for *Clibanarius vittatus*), and in other anomurans (Kronenberger et al., 2004; for *Galathea intermedia*), has had very little attention until recently. The occurrence and form of male sexual tubes in hermit crabs has been a subject of interest for more then 100 years, but detailed morphological analyses have only recently been undertaken (e.g., Lemaitre & McLaughlin, 2003; Tudge & Lemaitre, 2004). Male sexual tubes are remarkably diverse among the family Paguridae where they are present in species of
about 56% of the genera (Tudge & Lemaitre, 2004). This paper, however, focuses on the morphology of male sexual tubes in the family Coenobitidae, using SEM and histology techniques to describe the external and internal structure of the calcified coxal extensions that form sexual tubes in two species of Coenobita, C. perlatus H. Milne Edwards, 1837, and C. clypeatus (Fabricius, 1787).

MATERIALS AND METHODS

Specimens used were from the collections of the National Museum of Natural History, Smithsonian Institution, Washington, D.C. (USNM). Three male individuals of each species were used for the procedures outlined below. The C. perlatus specimens (USNM 93775) were collected from Ujae Atoll, Marshall Islands, western Pacific Ocean and the C. clypeatus specimens (USNM 102641) from Albert Town, Long Cay, Bahamas, Atlantic Ocean. The specimens were originally fixed in 70% ETOH. The shield length measurement (sl) was taken from the tip of the rostrum to the mid-dorsal point of the posterior carapace.

SEM

The abdomen was removed from the specimens prior to processing. 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 70, 95 and 100% for 10 min each, and finally a fresh change of 100% ethanol. The critical point-dried specimens were attached to glass cover slips on SEM stubs and sputter-coated with 25 nm of gold palladium. The specimens were viewed and photographed at 10–20 kV on a Leica Stereoscan 440 scanning electron microscope.

Histology

Each specimen had a complete transverse section of the body between the regions of the fourth and fifth pereopods dissected from the rest of the body. This dissected tissue was initially decalcified according to the Gooding and Stewart protocol (Carleton & Short, 1954). After dehydration and clearing, the tissue was embedded in Surgipath Formula R paraffin embedding compound. Thick sections (6 μm) were cut on a Spencer 820 rotary microtome and later stained with Crandall’s polychrome staining protocol (Crandall et al., 1998). Cover slips were applied using Fisher Permount mounting medium. Sections were viewed and photographed with a Leitz Orthoplan 2 optical microscope equipped with a Leica DFC 300 digital camera.

RESULTS

External morphology

Coenobita perlatus: The sexual tubes are asymmetrical, large and heavily calcified structures with an abundance of obvious setae occurring in patches (Fig. 1A–C). The left tube is roughly rectangular in shape, with a short posterior projection on which the gonopore is placed (Fig. IB, C). The entire structure appears to be an extension of the coxa, as there is no appreciable division from the coxa. The tube is approximately 1.5 mm long (on specimen sl 8 mm) from the last sternal plate posteriorly and 1.2 mm at its widest point. The gonopore is positioned on a small posterior projection (approximate 400 μm in width), and the slightly oval gonopore itself has a diameter just smaller than the width of the conical projection upon which it sits.

Obvious setae occur in three main areas: 1) on the mesial face of the coxal segment and sexual tube is a sparse field of short, simple setae spreading in a broken line posteriorly and ending in a ventrally directed patch of 15 adjacent setae (Fig. 1B, E). The largest (unbroken) seta is 300 μm in length; 2) laterally, and spreading in an isolated dense patch onto the ventral face of the sexual tube, is an irregular field of coarse setae of various lengths (Fig. 1B, C, E). The smallest setae are simple but develop into serrulate setae (see Garm, 2004) as they lengthen. The small, multi-cusped setules occur in longitudinal rows in the post-anunnular region (Fig. 1F). The longest setae in this field
Sexual tubes of *Coenobita perlatus* & *C. clypeatus*

Fig. 1. *Coenobita perlatus*, SEM of male sexual tubes. A. Ventral surface of crab (abdomen removed) showing the asymmetrical coxae of pereopod 5 (P5) with elongate sexual tubes. B. Ventral view of sexual tubes and coxae of P5 showing difference in size and shape of the left (L) and right (R) sexual tubes and the various fields of setae. C. Lateral view of left sexual tube and tip of right sexual tube showing extensive coarse setae on the surface and surrounding each gonopore. D. Detail of right sexual tube gonopore occluded by long, densely-packed, simple setae, with a few shorter setae present peripherally (arrowheads). E. Posterior view of the surface of the left coxae and base of sexual tube showing two distinct setal fields. F. Serrulate setae on the lateral surface of the left sexual tube with longitudinal rows of cusped setules. Note the obvious socketed base of these setae.

can reach 800 μm; 3) terminally, around the periphery of the oval gonopore, and virtually occluding it, is a long tuft of aligned simple setae (Fig. 1B, C). The largest of these can be over 1.5 mm in length.

The right sexual tube extends as a calcified tube posteriorly and mesially across the end of the left sexual tube (Fig. 1A, B). The coxa from
Fig. 2. *Coenobita clypeatus*, SEM of male sexual tubes. A. Ventral surface of crab (abdomen removed) showing the paired gonopores on the nearly symmetrical coxae of pereopod 5 (P5). B. Detail of paired sexual tubes showing distribution of coarse setae on the coxal surface and immediately surrounding the gonopores (arrowheads). C. Posterior view of paired sexual tubes. D. Detail of paired gonopores showing coarse setae surrounding each gonopore. E. Further detail of single gonopore and arrangement of smaller simple setae (arrow) and longer serrulate setae (arrowhead). F. Detail of largest serrulate setae surrounding the gonopore showing the longitudinal rows of post-annular cusped setules.

which the long tube emanates is approximately the same size and shape as the left coxa but appears to be slightly flatter (dorso-ventrally) and has the base of the tubular extension roughly demarcated as a shallow crease on the cuticle. The coxal width is 1.1 mm at its widest point,
Sexual tubes of *Coenobita perlatus* & *C. clypeatus*

and the cylindrical tube extends 2.3 mm posteriorly, maintaining a diameter between 300 and 400 µm (Fig. 1A, B). Both the proximal section of the tube and the posterior tip are slightly wider (400 µm and 350µm respectively) than the middle portion (300 µm). The gonopore is positioned terminally on the sexual tube, is oval with a widest diameter of about 330 µm, and is similarly occluded by dense, elongate, simple setae (Fig. 1B–D). The longest setae are just less than 1 mm in length (930 µm). Other simple setae of various lengths are spread sparingly and randomly down the mesial and lateral faces of the tube, but a concentration of stout, serrulate setae (as on the left tube) occurs in a dense field on the lateral edge of the coxa (Fig. 1B).

**Coenobita clypeatus:** The sexual tubes are nearly symmetrical in size and appearance, and each is broadly conical in shape with the gonopore at the blunt apex (Fig. 2A–C). Their mesial surfaces are straight, smooth and parallel, while the lateral sides slope away from the gonopore to meet the ischium of pereopod 5. Both sexual tubes are directed ventrally, rather than posteriorly as in *C. perlatus*. The sexual tubes are approximately 1.6 mm long and 0.9–1 mm at their widest point (on a specimen of 6 mm sl) (Fig. 2A–C). The length of the sexual tube from the sternal segment to the terminal gonopore is 0.7 mm. The gonopores are oval in shape and are 290–300 µm in greatest diameter and 220 µm perpendicular to this (Fig. 2B–D).

The numerous setae are concentrated laterally on the sexual tubes, with an irregular pattern of various lengths. The short setae appear simple but longer ones can reach over 500 µm in length, and these are serrulate with longitudinal rows of cusped setules (Fig. 2B–E). The shorter setae (100 µm or less) are unarmed basally, and at least beyond 300 µm in length from their bases, the setae are serrulate with longitudinal rows of cusped setules (Fig. 2B–F). The setules are always post-annular.

**Internal morphology**

**Coenobita perlatus:** The asymmetrical sexual tubes and coxae show similar contents when sectioned, but present very different profiles due to their different external morphologies (Fig. 3A). Each tube is surrounded by thick, calcified cuticle (with red exocuticle and blue endocuticle in this staining regime) embedded with numerous socketed setae, and is penetrated for its entire length by a circular to oval *vas deferens* (Fig. 3B–F).Sparse blocks of striated muscle appear distally in the long tube (right hand side) along with connective tissue adjacent to the *vas deferens* (Fig. 3B–F); and both increase in prominence proximally. The shorter, stouter, left tube has more striated muscle present within it, especially proximally to the coxa, where it fills nearly the entire coxa (Fig. 3D, E).

Each *vas deferens* is internally lined by epithelial tissue and external to this several layers of circular muscle (Fig. 3B–H). The *vas deferens* lumen contents (in both sexual tubes) sometimes reveal sections of sperm-filled pedunculate spermatophores (Fig. 3H), as well as thickened epithelial tissue swelling in one region, comparable to a typhlosole (Fig. 3H) (see Subramoniam, 1984).

**Coenobita clypeatus:** Reflecting their external symmetry, the paired sexual tubes are virtually symmetrical internally as well (Fig. 4A, B). The tubes are a direct extension of the coxae, and each is surrounded by a thick, heavily calcified cuticle, armed with many socketed setae. The distal, conical part of the tubes is filled with large, aligned blocks of striated muscle penetrated mesially by the single *vas deferens* (Fig. 4B), that leads to the terminal gonopore. Each *vas deferens* is lined by several layers of circular muscle on the outside and has epithelial cells lining the inside (Fig. 4C–E). Pedunculate
spermatophores filled with spermatozoa are seen in many sections (Fig. 4C, D).

Very proximal sections through the sternal plate (Fig. 4D, E) reveal paired *vasa deferentia*, one in each coxa, surrounded by striated muscle blocks, some connective tissue and occasionally glandular tissue. In particular, a large gland composed of several roseate gland cells (or tegumental glands, see Fingerman, 1992 and Dworschak, 1998) is seen between the paired *vasa deferentia* (Fig. 4E).

**DISCUSSION**

Direct comparisons with the microscopic morphology of other coenobitid sexual tubes is not possible since this is the first study of this kind in species of this family, despite the fact that sexual tubes are key structures that have been used prominently in the taxonomy of *Coenobita* species (Nakasone, 1988). A comparison to sexual tubes in other hermit crab families is similarly limited, since the only study is that by Tudge & Lemaitre (2004) for a species of Paguridae, *Micropagurus acantholepis* (Stimpson, 1858).

The microstructure of the *vas deferens* and associated tissues in the two *Coenobita* species studied here is generally similar to that seen in *Micropagurus* (see Tudge & Lemaitre, 2004). In all three species, the *vas deferens* is surrounded by a thin layer of epithelial cells and several layers of smooth, circular muscle. Some connective and glandular tissue is also present, although the sexual tube of *M. acantholepis* has much less striated muscle and calcified cuticle than either of the *Coenobita* species in this study (Figs. 3B–F, 4B–E).

It is externally that sexual tubes of the pagurid *M. acantholepis* and the coenobitids used in this study differ most. The tubes of *C. clypeatus* and *C. perlatus* are more heavily calcified extensions of the coxae, and are more setose than in *M. acantholepis*. In all three species, sperm-filled spermatophores were observed in the lumen of the *vas deferens*, inside the sexual tube. As with *M. acantholepis*, this indicates a functional spermatophore delivery role in *C. perlatus* and *C. clypeatus*.

The heavily calcified sexual tubes in *Coenobita* species elicit some interesting functional and evolutionary questions. How does spermatophore transfer differ in aquatic hermit crabs with simple gonopores (surrounded by setae and sometimes operculate) from those with one (as in *M. acantholepis*) or two sexual tubes (as in *Coenobita* species)? Copulatory behavior in members of the semi-terrestrial Coenobitidae has previously been recorded for *Birgus latro* (Helfman, 1977), but it was not documented until recently for *Coenobita* species (Imafuku, 2002). Helfman (1977) illustrated a male and female *B. latro* adpressed ventrally, engaged in a copulatory embrace, and upon their separation observed a spermatophoric mass on the ventral surface of the female on the coxae of the third and fourth walking legs. Male *B. latro* have small papillae emanating from the coxae of the fifth pereopods (Fig. 4F), from which spermatophores presumably are delivered.

More definitive evidence of the male sexual tube in *Coenobita* species being part of a func-

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Fig. 3. *Coenobita perlatus*, SEM (A) and stained wax thick sections (B–H) of male sexual tubes. A. Ventral view of asymmetrical sexual tubes showing approximate position of the sections B–H. B. Near transverse section (TS) through distal part of the elongate right sexual tube showing thick cuticle, central *vas deferens* and setae from gonopore of the left sexual tube. C. TS just proximal to B showing gonopore of left sexual tube as well (*). D. TS through both left (L) and right (R) sexual tubes reflecting the different shape of each. The *vas deferens* and blocks of striated muscle are visible in each. E. TS just proximal to D showing more striated muscle closer to the base of the sexual tubes. F. Detail of TS of right sexual tube from E. The thick cuticle is clearly distinguished into exocuticle (red) with setae and endocuticle (blue), the striated muscle blocks are seen in TS and the circular *vas deferens* has internal epithelial cells surrounded by a band of circular muscle. G. Composite of two TS’s through the base of the coxae and sternite. The paired *vasa deferentia* and extensive striated muscle are obvious. H. Detail of the *vas deferens* from one sexual tube showing peripheral circular muscle layers (arrow) and epithelial cells lining the lumen (arrowhead) which has a sperm-filled spermatophore in its center (*).
Fig. 4. Coenobita clypeatus, SEM (A) and stained wax thick sections (B–E) of male sexual tubes. A. Posterior view of paired sexual tubes showing approximate position of the sections B, D & E. B. TS through both left and right sexual tubes (coxae?) showing small, paired, oval vasa deferentia embedded in extensive striated muscle. C. Detail of a single vas deferens showing peripheral circular muscle layers, epithelial cells lining the lumen and sections of three sperm-filled spermatophores (*) in the lumen. D and E. TS’s through the base of the coxae and sternite showing paired vasa deferentia surrounded by connective tissue and striated muscle and, centrally a large tegumental gland (E only) under the sternite. F. Birgus latro (USNM 267615). Photograph of ventral view of the coxae of pereopod 5 (P5) with the gonopores terminally positioned on paired conical projections or papillae (arrowheads).
tional spermatophore delivery system is found in Imafuku (2002). Imafuku observed instances of pairs of *Coenobita purpureus* Stimpson, 1858, in copula on the beach at night, and for one adpressed pair observed the male, elongate, right sexual tube inserted into the space between the gastropod shell and the smaller female withdrawn into the shell. It is assumed that being so heavily calcified the sexual tube has no independent mobility from the coxa and is only as mobile as the entire segment. Several hours later, the same female was shown to have her ventral surface (and the aperture of the shell) covered with white sticky spermatophore material. This is the first recorded instance of a sexual tube being used during mating in any hermit crab, terrestrial or aquatic. This observation also suggests that the sexual tube may be used to mate (transfer spermatophores onto) with “uncooperative” females, although the female may not use the sperm for fertilization as there is a need for either mechanical or chemical dehiscence of the spermatophore ampullae for sperm release (see Hess & Bauer, 2002 for references). The fact that fertilization occurs under aquatic conditions for most hermit crabs raises the interesting question of how it is achieved by the semi-terrestrial coenobitids in an aerial environment. There are no aquatic coenobitid representatives but the sperm and spermatophores of species of this family have no major morphological differences to those in their aquatic relatives, in particular the Diogenidae, considered their sister taxon (e.g., Tudge, 1997; McLaughlin & Lemaitre, in press). This suggests that there might be physiological and microhabitat (both within and external to the gastropod shelter) differences required for terrestrial external fertilization.

Our present, limited knowledge suggests that despite their phallic appearance male hermit crab sexual tubes are not intromittent organs similar to penises, and are instead used to externally deposit spermatophores on the female for external fertilization. However, many questions remain to be answered. If the majority of hermit crabs complete spermatophore transfer and fertilization without the aid of sexual tubes, what advantage is gained by those species that have them? Are the tubes an adaptation for external fertilization in terrestrial environments for the Coenobitidae (Imafuku, 2002)? Is presence of sexual tubes in the Coenobitidae an evolutionary derived condition as it appears to be in the Paguridae, and are the sexual tubes homologous or not in these two families? Are sexual tubes an adaptation for minimizing the risky exposure of partial or complete emergence from their housing during mating? If this is the case then how does one explain the intrageneric variability that exists where members of the same genus may have one (left or right), two, or no sexual tubes at all (McLaughlin, 2003)? Why do many species have only one sexual tube on either the right or left side irrespective of the predominant asymmetry favoring the left side, or housing type? Why are sexual tubes not more commonly present on both sides considering that the usual male reproductive system is paired and bilaterally functional and the female usually has paired, presumably functional gonopores? Are hermit crab sexual tubes secondary sexual characteristics needed in complex mating behaviours? This may be so, but many aquatic hermit crabs that have complex mating behaviours (e.g., Hazlett, 1996, and references therein), do not need male sexual tubes to successfully breed. Some species characterized by small individuals (~1.5 mm sl) have sexual tubes that are equal to or larger than the size of the fifth pereopod, and thus appear to represent a significant evolutionary investment; could these be examples of structures under “runaway” (Darwin, 1871; Fisher, 1930) sexual selection?

Answers are elusive at this point. However, this study does provide baseline information on size, morphology and possible function of male sexual tubes in two *Coenobita* species. Only through a series of detailed studies of internal and external sexual tube structure, male and female reproductive morphology, phylogenetic relationships among paguroid families, and observations of mating behaviours, both in the wild and in the laboratory, will some of these intriguing questions be answered.
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