

ULTRASTRUCTURE OF THE SPERMATOPHORE LATERAL RIDGE IN HERMIT CRABS (DECAPODA, ANOMURA, PAGUROIDEA)

BY

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ABSTRACT

The pedunculate spermatophore is characteristic of the Anomura, and the Paguroidea in particular. It has a sperm-filled ampulla which is composed of two valves joined at a conspicuous lateral ridge. This lateral ridge is a thickening of the spermatophore wall but also a discontinuity between the wall morphology of each valve. Within the hermit crabs (Paguroidea) the spermatophore wall morphology can be divided into three categories: fibrillar, heterogeneously granular and homogeneously granular. The fibrillar form is found in representatives from the families Coenobitidae and Diogenidae, with the exception of those of the genus *Clibanarius* which have the heterogeneously granular form, while the homogeneously granular form has been recorded from members of the family Paguridae. A single galatheid crab, *Allogalathea elegans* is shown to have a homogeneously granular spermatophore wall and ridge like this latter group of paguroids. These differences in spermatophore wall and ridge morphology between the various taxa in the Anomura appear to be phylogenetically significant.

RÉSUMÉ

Le spermatophore pédonculé est caractéristique des Anomoures, et des Paguroidea en particulier. Il présente une ampoule contenant les spermatozoïdes, formée de deux valves unies par une crête latérale bien visible. Cette crête latérale est un épaississement de la paroi du spermatophore mais constitue également une discontinuité entre la morphologie de la paroi de chaque valve. Chez les pagures (Paguroidea), la morphologie de la paroi du spermatophore peut être divisée en trois catégories: fibrillaire, granulaire hétérogène et granulaire homogène. On rencontre la forme fibrillaire chez les représentants des familles Coenobitidae et Diogenidae, avec pour exception le genre *Clibanarius* qui présente la forme granulaire hétérogène tandis que la forme granulaire homogène a été observée chez les membres de la famille des Paguridae. Seul, un galathéide, *Allogalathea elegans*, présente une paroi du spermatophore de type granulaire homogène et une crête identique à ce dernier groupe de paguroïdes. Ces différences observées dans la morphologie de la paroi du spermatophore et de la crête entre les différents taxons semblent être phylogénétiquement significatives.

INTRODUCTION

Anomuran crabs, including the paguroidean hermit crabs, all reproduce via the use of externally deposited spermatophores. The predominant spermatophore type encountered in these groups is the stalked or pedunculate spermatophore which is composed of three primary components (tripartite), the pedestal, the peduncle and the ampulla (Tudge, 1991, 1995; Subramoniam, 1993). These pedunculate spermatophores are constant in size and shape within a species, but above the species rank they constitute taxonomically specific structures (Tudge, 1991, 1995). The spermatozoa are contained within the distal ampulla which is composed of two halves or valves joined along their lateral edge. This thickened and raised joint is termed the lateral ridge (Tudge, 1991, 1995). The presence of a lateral ridge has now been recorded in 23 species of hermit crab and a further five species of other anomurans (Mouchet, 1931; Hamon, 1937, 1939a; Tudge, 1991, 1995; Tudge & Jamieson, 1996).

It has been known since the 1930's (Mouchet, 1931; Hamon, 1937, 1939a) that anomuran pedunculate spermatophores, and those in hermit crabs in particular, split along a designated fracture zone to release their contained spermatozoa. This line of dehiscence was termed the "ligne de suture" by Hamon (1937). Hamon also suggested two main mechanisms of spermatozoal release from the ampulla; changes in internal pressure due to mechanical or osmotic forces and chemical dissolution of the suture. She stated that these may act alone or in concert.

The present paper describes and illustrates the lateral ridge ultrastructure of 11 species of hermit crab and a single species of galatheid crab and suggests that the spermatophore ridge and wall morphology may be taxonomically and phylogenetically significant.

MATERIALS AND METHODS

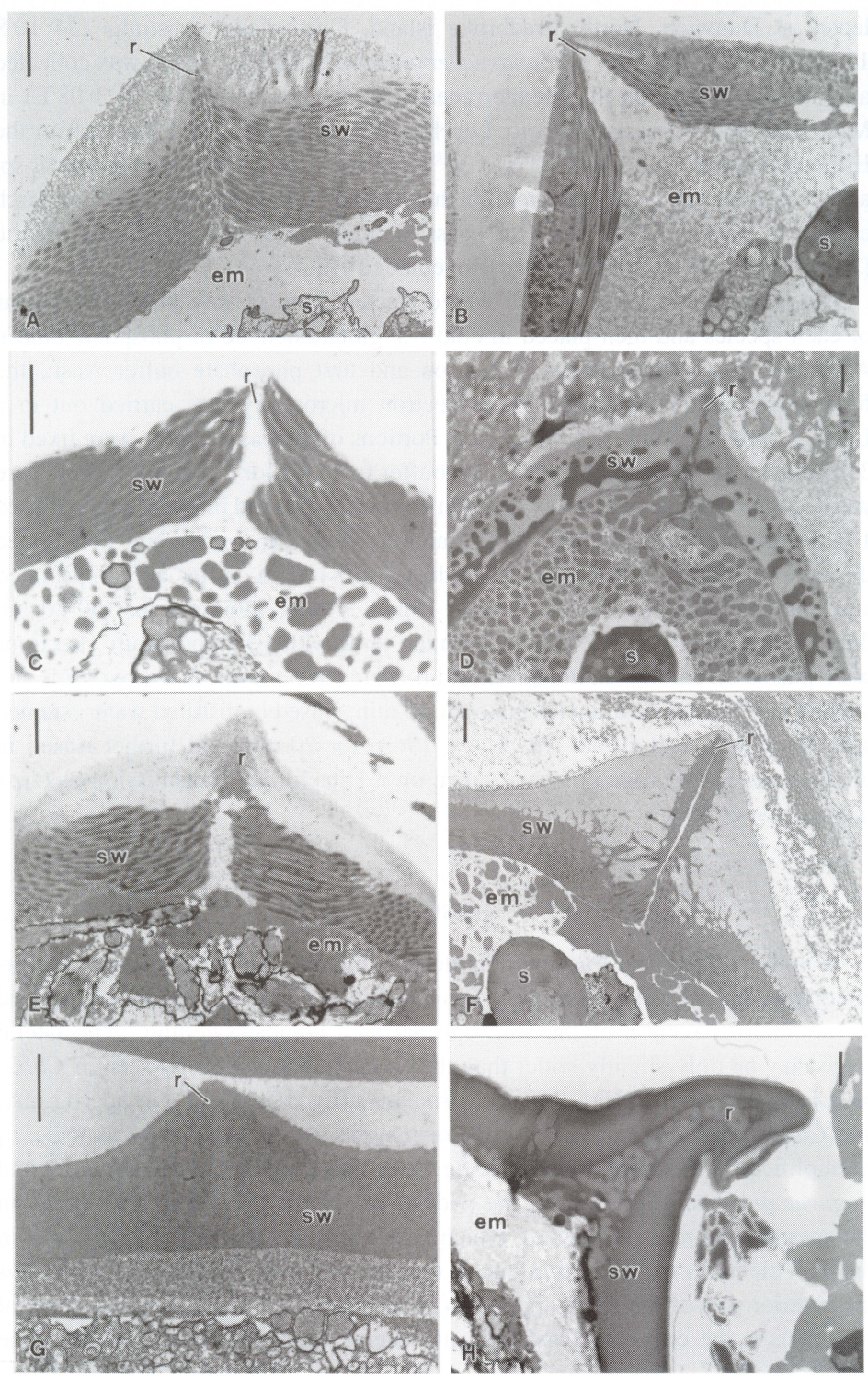
Mature male specimens of the following species were collected between 1988 and 1993 at the following locations. *Coenobita rugosus* H. Milne Edwards, 1837 was collected from the Cook Islands, southwest Pacific (20°S 160°W) in November 1990. *Calcinus gaimardii* (H. Milne Edwards, 1848), *Calcinus laevimanus* (Randall, 1840), *Dardanus scutellatus* (H. Milne Edwards, 1848), *Pagurus hirtimanus* Miers, 1880, and *Allogalathea elegans* (Adams & White, 1848) were collected on Heron Island, Great Barrier Reef, Queensland, Australia (23°27'S 151°55'E) in December 1988 (*Allogalathea* only) and 1990. *Diogenes gardineri* Alcock, 1905 was collected at Mooloolaba, southeast Queensland (26°41'S 153°07'E) in October 1990. *Clibanarius longitarsus* (De Haan, 1849) was col-

lected at Dunwich, North Stradbroke Island, Queensland, Australia (24°10'S 151°53'E) in April 1992. *Clibanarius erythropus* (Latreille, 1818) was collected at Banyuls-sur-Mer on the Mediterranean coast of France (42°29'N 3°08'E) in May 1992 and *Pagurus prideaux* Leach, 1815 was collected at Roscoff on the Brittany coast of France (48°43'N 3°59'W) in June 1992. *Porcellanopagurus* sp. and *Cancellus* sp. were collected on the BATHUS 1 and BATHUS 3 research cruises, respectively, off the east coast of New Caledonia, southwest Pacific (22°16'S 166°26'E) in March and November 1993.

The male reproductive system was dissected from freshly killed specimens of each species and then placed in cold 3% glutaraldehyde in phosphate buffer. After this initial glutaraldehyde fixation and first phosphate buffer wash, the fixation procedure for transmission electron microscopy was carried out in a Lynx-el. Microscopy Tissue Processor. Portions of the vas deferens were fixed in 3% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 1 h at 4°C. They were washed in phosphate buffer (3 washes in 15 min), postfixed in phosphate buffered 1% osmium tetroxide for 80 min; similarly washed in buffer and dehydrated through ascending concentrations of ethanol (40-100%). After being infiltrated and embedded 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 collodion-coated 200 μ m mesh copper grids and stained in 6% aqueous uranyl acetate for 40 min; rinsed in distilled water; stained with Reynold's lead citrate (Reynolds, 1963) for 20 min and further rinsed in distilled water. Micrographs were taken on a Hitachi 300 transmission electron microscope at 80 kV.

RESULTS

At both the light and transmission electron microscope (TEM) level the spermatophore lateral ridge is seen as a thickening of the spermatophore wall and in longitudinal section of the wall appears as a conical swelling. The lateral ridge may be only slightly wider than the spermatophore wall, as seen in *Clibanarius erythropus* (fig. 1D), *Pagurus hirtimanus* (fig. 1G), *Cancellus* sp. (fig. 2A) and *Clibanarius longitarsus* (fig. 2B), or may be two to three times as wide, as exemplified by *Coenobita rugosus* (fig. 1A), *Diogenes gardineri* (fig. 1F) and *Pagurus prideaux* (fig. 1H). At the widest part of the ridge there is a break in the spermatophore wall structure which may take the form of a physical gap in the wall elements or only a thin line of discontinuity. In *Clibanarius longitarsus* the exterior part of the ridge appears to be plugged with electron-dense material (fig. 2B). In the coenobitid and diogenid lateral ridges the inner portion of the



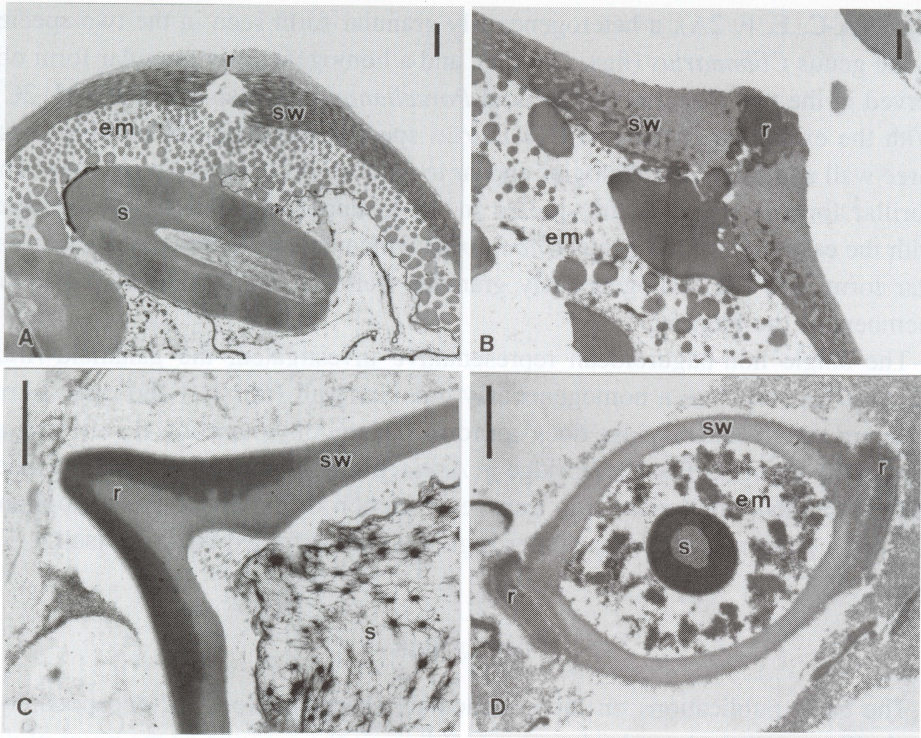


Fig. 2. Transmission electron micrographs of the lateral ridges of the spermatophores of hermit crabs (A-C) and a galatheid crab (D). A-B, Diogenidae. A, *Cancellus* sp.; B, *Clibanarius longitarsus* (De Haan, 1849). C, *Porcellanopagurus* sp. (Paguridae); D, *Allogathea elegans* (Adams & White, 1848) (Galatheidae). Abbreviations: em, extracellular matrix; r, lateral ridge; s, spermatozoa; sw, spermatophore wall. Scale bars = 1 μ m.

spermatophore wall is the most electron-dense, whether granular (figs. 1D, 2B) or fibrillar (figs. 1A-C, E, F, 2A), but in the three pagurid species the opposite arrangement occurs (figs. 1G, H, 2C).

The spermatophore wall and lateral ridge of the investigated hermit crabs have three distinctive and differing ultrastructural morphologies. These three morphologies are a predominantly fibrillar structure recorded in the representatives from the genera, *Coenobita*, *Calcinus*, *Dardanus*, *Diogenes*, and *Cancellus*

Fig. 1. Transmission electron micrographs of the lateral ridges of the spermatophores of selected hermit crabs. A, *Coenobita rugosus* H. Milne Edwards, 1837 (Coenobitidae); B-F, Diogenidae. B, *Calcinus laevimanus* (Randall, 1840); C, *Calcinus gaimardii* (H. Milne Edwards, 1848); D, *Clibanarius erythropus* (Latreille, 1818); E, *Dardanus scutellatus* (H. Milne Edwards, 1848); F, *Diogenes gardineri* Alcock, 1905. G-H, Paguridae. G, *Pagurus hirtimanus* Miers, 1880; H, *Pagurus prideaux* Leach, 1815. Abbreviations: em, extracellular matrix; r, lateral ridge; s, spermatozoa; sw, spermatophore wall. Scale bars = 1 μ m.

(figs. 1A-C, E, F, 2A), a heterogeneously granular form seen in the two species in the genus *Clibanarius* (figs. 1D, 2B), and a homogeneously granular form observed in the two *Pagurus* species and *Porcellanopagurus* sp. (figs. 1G, H, 2C). With the exception of the two *Clibanarius* species, the divisions between these three wall and ridge morphologies appear to conform to familial boundaries. The fibrillar form is found in the species in the Coenobitidae and the Diogenidae, with the exception of the genus *Clibanarius* which has the heterogeneously granular form, and the homogeneously granular wall and ridge is restricted to the members of the Paguridae.

The single non-paguroidean representative investigated, the galatheid *Allogalathea elegans*, has a homogeneously granular wall (fig. 2D) and ridge ultrastructure and the lateral ridge does not form such an obvious conical structure, as seen in the hermits. The lateral ridge in *A. elegans* is seen to be on opposite sides of the ampulla, when viewed in transverse section of the entire spermatophore, and has a more electron-dense outer region which is folded back on itself.

DISCUSSION

The early publications on the pedunculate spermatophores of decapods correctly illustrated the lateral ridge or suture line on the ampulla as the point of dehiscence of the two ampulla halves in a variety of hermit crabs and other anomurans, including *Pagurus prideaux* (see Mouchet, 1931; Hamon, 1937; as *Eupagurus prideauxi*), *Diogenes pugilator* (Roux, 1829) (in Mouchet, 1931), *Dardanus arrosor* (Herbst, 1796) (in Hamon, 1939a; as *Pagurus*) and *Pisidia longicornis* (Linnaeus, 1767) (in Mouchet, 1931; as *Porcellana*). Hamon (1937), in particular, illustrated that the ampulla of the spermatophore of *Pagurus prideaux* could open in a variety of ways to release the contained spermatozoa. This "unzipping" of the spermatophore lateral ridge, to release sperm, was later illustrated by Tudge (1991) for the diogenid hermit crab *Calcinus minutus* Buitendijk, 1937.

The lateral ridge was not investigated ultrastructurally until 1991 when a series of papers appeared showing the detail of the spermatophore wall and associated lateral ridge in a selection of anomurans (Hinsch, 1991a, b; Tudge, 1991, 1995; Tudge & Jamieson, 1996). Often the spermatophore wall and ridge structure were revealed incidentally during ultrastructural studies of the spermatozoa. These TEM studies confirm that there are three primary wall and ridge morphologies in the Anomura and that, in most cases, the changes occur between families and sometimes genera. The fibrillar wall and lateral ridge morphology seen in the representatives from the Coenobitidae and Diogenidae (except *Clibanarius*) (figs. 1A-C, E, F, 2A) is also shown in *Coenobita clypeatus* (Herbst,

1791) (in Hinsch, 1991b) and *Coenobita variabilis* McCulloch, 1909 (in Tudge, 1991, 1995). The heterogeneously granular morphology exhibited by the two *Clibanarius* spp. (figs. 1D, 2B) is further shown in a third species, *Clibanarius virescens* (Krauss, 1843) (in Tudge, 1991, 1995) and interestingly, may also be present in the spermatophore of the galatheid *Munida* sp. (Tudge, 1995). The third wall and ridge morphology encountered is the homogeneously granular form which is present in the three pagurid hermit crabs (figs. 1G, H, 2C), but a similar morphology has been illustrated in the galatheid, *Pleuroncodes planipes* Stimpson, 1860 (in Hinsch, 1991a, b) and the porcellanid, *Aliaporcellana suluensis* (Dana, 1852) (in Tudge, 1995; Tudge & Jamieson, 1996).

These principal differences in spermatophore wall ultrastructure, between taxa in the Anomura, have been used in combination with other spermatophore morphological characters in phylogenetic analyses of relationship within this important, and often enigmatic, decapod group (Tudge, 1995, 1997). The homogeneous spermatophore wall structure has been found in representatives from the anomuran families Paguridae, Parapaguridae, Porcellanidae, Lomidae, Hippidae, and some Galatheidae. It has also been observed in the decapod infraorders Brachyura and Palinura, but its homology is uncertain in these non-anomuran representatives. This pattern of occurrence of the homogeneous wall structure indicates that it is a plesiomorphic state for these decapods and particularly in the Anomura. The further two wall states (heterogeneously granular and fibrillar) occur in only one genus of diogenid hermit crab (*Clibanarius*), and the remainder of the Diogenidae and the Coenobitidae, respectively. These states are considered apomorphic and support a close relationship between these two hermit crab families (Tudge, 1997).

In the present study no biochemical analyses were carried out to determine the composition of the spermatophore wall and ridge, but it has been previously indicated that the wall of anomuran pedunculate spermatophores, and therefore the lateral ridge, are predominantly composed of mucopolysaccharides. This has been shown in representatives from the anomuran superfamilies Paguroidea (Hamon, 1939a, b; Hinsch, 1991b), Hippoidea (Subramoniam, 1984) and Galatheaidea (Hinsch, 1991b). Further analysis of the ultrastructure and biochemistry of the spermatophore wall in the Anomura will prove useful to comparisons of the spermatophore wall structure in the Brachyura (capsular spermatophores) and the 'macrurous' infraorders Astacidea and Palinura (tubular spermatophores).

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REFERENCES

- HAMON, M., 1937. Les mécanismes produisant la déhiscence des spermatophores d'*Eupagurus prideauxi* Leach. C. R. Acad. Sci., Paris, **204**: 1504-1506.
- , 1939a. La constitution chimique des spermatophores de Crustacés supérieurs du groupe des pagurides. C. R. Soc. Biol., Paris, **130**: 1312-1315.
- , 1939b. Détermination de la présence de certains acides α aminés dans les spermatophores des pagurides. C. R. Soc. Biol., Paris, **131**: 109-110.
- HINSCH, G. W., 1991a. Ultrastructure of the sperm and spermatophores of the anomuran crab *Pleuroncodes planipes*. Journ. Crust. Biol., **11**: 17-22.
- , 1991b. Structure and chemical content of the spermatophores and seminal fluid of reptantian decapods. In: R. T. BAUER & J. W. MARTIN (eds.), Crustacean sexual biology: 290-307. (Columbia University Press, New York).
- MOUCHET, S., 1931. Spermatophores des Crustacés Décapodes Anomoures et Brachyours et castration parasitaire chez quelques Pagures. Annal. Stat. océanogr. Salammbô, **6**: 1-203.
- REYNOLDS, E. S., 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. Journ. Cell Biol., **17**: 208-212.
- SPURR, A. R., 1969. A low viscosity epoxy-resin embedding medium for electron microscopy. Journ. Ultrastruct. Res., **26**: 31-43.
- SUBRAMONIAM, T., 1984. Spermatophore formation in two intertidal crabs *Albunea symnista* and *Emerita asiatica* (Decapoda: Anomura). Biol. Bull., Woods Hole, **166**: 78-95.
- , 1993. Spermatophores and sperm transfer in marine crustaceans. Adv. mar. Biol., **29**: 129-214.
- TUDGE, C. C., 1991. Spermatophore diversity within and among the hermit crab families, Coenobitidae, Diogenidae, and Paguridae (Paguroidea, Anomura, Decapoda). Biol. Bull., Woods Hole, **181**: 238-247.
- , 1995. The ultrastructure and phylogeny of anomuran crab spermatozoa: 1-346. (Ph.D. Thesis, Zoology Department, The University of Queensland, Brisbane, Australia).
- , 1997. Phylogeny of the Anomura (Decapoda, Crustacea): spermatozoa and spermatophore morphological evidence. Contrib. Zool., Amsterdam, **67**: 125-141.
- TUDGE, C. C. & B. G. M. JAMIESON, 1996. Spermatophore and spermatozoal morphology in the Porcellanidae. I. *Aliaporcellana suluensis* and *Pisidia longicornis* (Decapoda: Anomura: Porcellanidae). Crust. Res., Tokyo, **25**: 73-85.

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