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Spermatophore Diversity Within and Among the Hermit Crab Families, Coenobitidae, Diogenidae, and Paguridae (Paguroidea, Anomura, Decapoda)

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Abstract. The spermatophore morphology of 13 species of hermit crab from the families Coenobitidae, Diogenidae and Paguridae is described and illustrated, and comparisons are made with existing descriptions to show that spermatophore form, at the light microscope level, can be used to separate three families of the Paguroidea. Spermatophores from members of the family Coenobitidae are robust in nature with large, ovoid-spherical ampullae mounted on short, thick stalks. Members of the family Diogenidae have more fragile spermatophores with small spherical ampullae mounted on long, slender stalks. The spermatophores of members of the family Paguridae are distinctive in possessing large, elongate, ampullae, an accessory ampulla at the base of the main ampulla and a pseudo-stalk analogous with the true stalk of the Coenobitidae and Diogenidae. The occurrence of double-headed spermatophores (two ampullae on a single stalk) is recorded for the first time, in a *Dardanus* species. The ultrastructure of the lateral ridge, which divides the ampulla of the paguroidean spermatophore into two halves, is described using both scanning and transmission electron microscopy. A simple, branching key for classifying the investigated hermit crabs (from the families Coenobitidae, Diogenidae and Paguridae only) into their respective family, based on the gross morphology of their spermatophore, is presented.

Introduction

The infraorder Anomura consists of 13 families of which only 6 have been investigated for spermatophore morphology. Representatives of three hermit crab families in the superfamily Paguroidea (*sensu* McLaughlin, 1983) have been investigated for spermatophore morphology. These are listed below. Coenobitidae: *Birgus latro* (Linnaeus), Matthews, 1956; Tudge and Jamieson, 1991. *Coenobita rugosus* H. Milne Edwards, Matthews, 1956. Diogenidae: *Clibanarius misanthropus* (Risso), Mouchet, 1930, 1931. *Dardanus arrosor* (Herbst), Mouchet, 1931 (as *Pagurus arrosor*). *Dardanus asper* (De Haan), Matthews, 1953. *Dardanus punctulatus* (Olivier), Matthews, 1956. *Diogenes pugilator* (Roux), Mouchet, 1930, 1931. *Paguristes oculatus* (Fabricius), Mouchet, 1931. *Aniculus maximus* Edmondson, *Trizopagurus maximus* (Herbst) and *T. strigatus* (Herbst), Matthews, 1957 (as *Aniculus strigatus*). Paguridae: *Anapagurus hyndmanni* (Thompson), Mouchet, 1930, 1931. *Anapagurus brevicarpus* A. Milne Edwards and Bouvier and *A. laevis* (Bell), Mouchet, 1931. *Cestopagurus timidus* (Roux), *Pagurus anachoretus* Risso, *P. cuanensis* (Thompson), *P. excavatus* (Herbst) and *P. sculptimanus* (Lucas), Mouchet, 1931 (all as *Eupagurus*). *Pagurus prideaux* Leach, Mouchet, 1931; Hamon, 1937 (both as *Eupagurus prideauxi*). *Pagurus bernhardus* (Linnaeus), Mouchet, 1931; Chevaillier, 1970 (both as *Eupagurus bernhardus*). *Pagurus novizealandiae* (Dana), Greenwood, 1972 (as *P. novae-zealandiae*). *Pagurus excavatus* (Herbst), Schaller, 1979 (as *P. meticulosus*). All of these previous studies of spermatophore structure have been at the light microscope level.

Except for *Trizopagurus maximus* and *T. strigatus* (Matthews 1957), the hermit crabs previously studied have a typically pedunculate spermatophore that can be divided into three major regions: a sperm-filled ampulla, a columnar stalk of variable length, and a foot or pedestal. The ampulla has a partition or line of division that runs around the lateral edge and separates the ampulla into two halves. This suture line is the point of weakness where the ampulla breaks to release the spermatozoa prior to fertilization.

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Earlier pioneers in hermit crab spermatophore morphology include Mouchet (1930, 1931) and Matthews (1953, 1956, 1957), whose important contributions to this field also covered other decapods. These references, along with many other papers on decapod spermatophores, are adequately tabulated in Dudenhausen and Talbot (1983). Some additional, more recent, references to anomuran and brachyuran spermatophore studies can be found in Mann's (1984) book on spermatophores. Studies on hermit crab spermatophores have been neglected for the past 10 years, except for a recent review by Hinsch (1991); although some important works on brachyuran (Jeyalectumie and Subramoniam, 1989; Subramoniam, 1991) and caridean shrimp (Chow *et al.*, 1989) spermatophores have been published. Comparisons of the functional morphology of genitalia and subsequent sperm transfer and storage mechanisms among taxa can provide useful information on phylogenetic relationships and evolutionary divergence; especially in the Decapoda (Bauer, 1986, 1991). The present paper describes and illustrates the spermatophore structure of 13 species of hermit crab, from 3 families in the Paguroidea, and introduces a simple key to classify these hermit crabs to family, based on light microscope observations of their spermatophores.

Materials and Methods

Collection sites and dates for the species in this paper are as follows:

Family Coenobitidae

Birgus latro (Linnaeus 1767), Malaita Island, Solomon Islands, SW Pacific, October, 1988; *Coenobita brevimanus* Dana 1852, *Coenobita perlatus* H. Milne Edwards 1837 and *Coenobita rugosus* H. Milne Edwards 1837, Suwarrow Atoll National Park, Cook Islands, SW Pacific, August, 1990; *Coenobita spinosus* H. Milne Edwards 1837, Darwin, Northern Territory, Australia, May, 1990.

Family Diogenidae

Calcinus latens (Randall 1839), *Calcinus minutus* Buitendijk 1937 and *Clibanarius corallinus* (H. Milne Edwards 1848), Heron Island, Queensland, Australia, December, 1990; *Clibanarius virescens* (Krauss 1843), Dunwich, North Stradbroke Island, Queensland, Australia, April, 1990; *Dardanus lagopodes* (Forskål 1775) and *Dardanus megistos* (Herbst 1804), Heron Island, Queensland, Australia, December, 1990; *Diogenes gardineri* Alcock 1905, Mooloolaba, Queensland, Australia, October, 1990.

Family Paguridae

Pagurus hirtimanus Miers 1880, Heron Island, Queensland, Australia, December, 1990.

The male reproductive system was dissected from fresh specimens, 10% buffered formalin-fixed or 3% glutaraldehyde-fixed specimens. The spermatophores were teased out of the distal part of the vas deferens onto microscope slides. Spermatophores were viewed and photographed with an Olympus BH2 microscope equipped with Nomarski interference contrast optics. After the 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 colloidin-coated 200 μm 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.

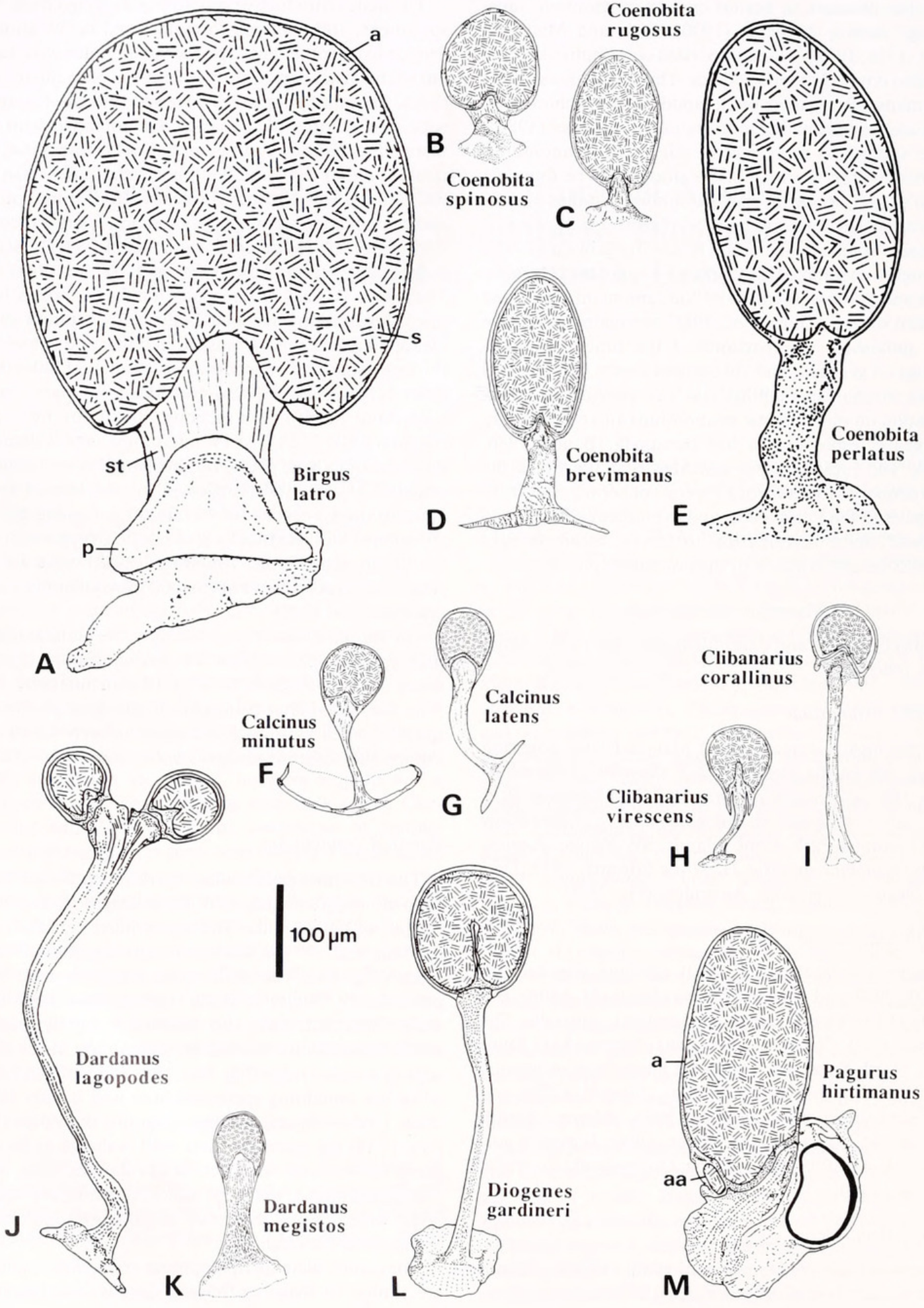
For scanning electron microscopy, 3% glutaraldehyde-fixed spermatophores were dehydrated through a graded series of ethanol (80–100%) at 10 min intervals. They were then dried after being placed in a drop of ether and sputter coated with gold; micrographs were taken on a Philips 505 scanning electron microscope at 20–30kV.

Results

Family Coenobitidae

The tripartite, pedunculate spermatophores of *Birgus latro* are approximately 650 μm in length from pedestal to apex of the ampulla. The sperm-filled ampulla is 440 μm long and 450 μm wide and forms an inverted heart-shape (Fig. 1A). The stalk of the spermatophore is approximately 100 μm in length. The spermatophore is laterally wider than deep, and therefore is slightly spatulate and is composed of two halves, which meet at the lateral edge as a raised ridge (Fig. 3A). This ridge is 20 μm thick while the remaining spermatophore wall is only 10 μm thick. Under a double Mallory's staining procedure (Mallory, 1936) the spermatophore wall is shown to be composed of two layers: an inner, darker staining layer, which is discontinuous at the ridge, and an outer, lighter staining layer, which covers the entire ampulla and increases in thickness at the ridge.

The pedunculate spermatophores of *Coenobita spinosus* are similar in shape to those of *Birgus latro* but much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s
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Birgus latro
A
B
Coenobita spinosus
Coenobita rugosus
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D
Coenobita brevimanus
E
Coenobita perlatus
Calcinus minutus
F
Calcinus latens
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Clibanarius corallinus
Clibanarius virescens
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Dardanus lagopodes
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Dardanus megistos
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Diogenes gardineri
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a
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Pagurus hirtimanus
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smaller (Fig. 1B). Each individual spermatophore is approximately 170 μm in length from base of the pedestal to the top of the ampulla. The ampulla, which is approximately 110 μm long, is composed of two halves that join at a conspicuous lateral ridge. The stalk of the spermatophore is only 20 μm long. The width of the ampulla from ridge to ridge is approximately 110 μm, while the depth of the ampulla is only 100 μm. This gives the ampulla a slightly spatulate shape, with the ridge running around the lateral edge. The ampulla is surrounded by a spermatophore wall 2 μm in thickness, which increases to 3 μm at the ridge, and is filled with many closely packed spermatozoa, separated by an extracellular matrix. Ultrastructural studies show the ridge to be a break in the fibrillar structure of this wall (Fig. 3C).

The spermatophores of *Coenobita rugosus* are larger than those of *Coenobita spinosus,* being 190 μm long, with an ampullar length and width of 150 and 100 μm, respectively. The ampulla, which is slightly more elongate than either *Birgus latro* or *Coenobita spinosus,* sits on a stalk 32 μm high (Fig. 1C).

Coenobita brevimanus has a spermatophore 290 μm in length which is composed of a stalk that is 90 μm long and an ampulla that is 180 μm long and 110 μm wide. Although the ampulla is larger than that of *Coenobita rugosus,* its shape is very similar (Figs. 1D, 2A).

The spermatophore of *Coenobita perlatus* is the largest of the four species of *Coenobita* studied. At 600 μm in length, it is only slightly smaller than the spermatophore of *Birgus latro* (Fig. 1A). The ampulla, 400 μm long and 230 μm wide, is similar in shape to those of *Coenobita rugosus* and *Coenobita brevimanus* and is situated on top of a stalk that is 180 μm long (Fig. 1E).

Family Diogenidae

The spermatophores of *Calcinus minutus* are 180 μm long, composed of a stalk that is 110 μm long and an ampulla 60 μm long and 70 μm wide. The stalk is very slender, only 10 μm wide, and gradually thickens distally to envelop the spherical ampulla (Figs. 1F, 3D). This shape is also found in *Calcinus latens,* but the entire spermatophore is smaller in the latter. The over-all length of the spermatophore of *Calcinus latens* is 150 μm, the thin stalk being 110 μm long and 15 μm wide, and the ampulla dimensions are 50 by 50 μm (Figs. 1G, 2C).

The spermatophore morphology of the two species of *Clibanarius* is similar to that of the *Calcinus* species. *Clibanarius virescens* has a small spermatophore, only 150 μm long with a short, thin stalk 70 μm long and 10 μm wide. The ampulla is 75 μm in length and width and has ventrally directed projections (25 μm long) which appear to be extensions of the lateral ridge (Fig. 1H). The distal end of the stalk penetrates into the ampulla as a dorsally directed projection.

The spermatophore of *Clibanarius corallinus* is twice the length (300 μm) of that of *Clibanarius virescens,* but otherwise it is morphologically similar (Fig. 1I). The 220 μm stalk is slightly wider at 15 μm. The ampulla is 60 μm long and 75 μm wide and possesses the same extensions of the lateral ridge, although the extensions in this species are only 12 μm in length. The ampulla is only slightly penetrated by a broad dorsal projection at the distal end of the stalk (Figs. 1I, 2E).

Dardanus lagopodes has a distinctive spermatophore, in comparison to the rest of the Paguroidea studied, in that some have two ampullae at the apex of a single stalk (Figs. 1J, 2D). Of the observed spermatophores, approximately 20% were double-headed. Both single and double-headed spermatophores were the same length, and each had similar ampullae. The spermatophores were 600 μm long with a 530 μm stalk, that had a mean width of 20 μm. Each ampulla was 70 μm long and 80 μm wide. In the spermatophores with two ampullae the stalk forked only at the most distal 50–70 μm (Fig. 2D).

The spermatophores of *Dardanus megistos* are very different from that of *Dardanus lagopodes* in both size and morphology (Figs. 1K, 2B). They appear more similar to the spermatophores of the two *Calcinus* species. *Dardanus megistos* has a spermatophore that is only 160 μm long, a stalk that is 90 μm long and 20 μm wide and an ampulla that is 60 μm long and 45 μm wide. As in *Calcinus minutus* and *Calcinus latens* (Figs. 1F, G), the stalk gradually widens distally so as to appear to envelop the ampulla, but unlike these species, the broad end of the stalk projects considerably into the ventral aspect of the ampulla (Fig. 1K).

The spermatophore of *Diogenes gardineri* is different from those of any of the other diogenid genera in this paper. The entire spermatophore is 430 μm in length, with 300 μm of that being the thin (15 μm) stalk. The ampulla is approximately square in outline with length and width dimensions both being 130 μm. The ampulla

Figure 1. Semidiagrammatic representations of spermatophores of hermit crabs from three families of the Paguroidea. (A–E) Coenobitidae. A. *Birgus latro,* B. *Coenobita spinosus,* C. *Coenobita rugosus,* D. *Coenobita brevimanus,* E. *Coenobita perlatus.* (F–L) Diogenidae. F. *Calcinus minutus,* G. *Calcinus latens,* H. *Clibanarius virescens,* I. *Clibanarius corallinus,* J. *Dardanus lagopodes,* K. *Dardanus megistos,* L. *Diogenes gardineri.* (M) Paguridae. M. *Pagurus hirtimanus.* Drawn from light micrographs. All to the same scale. a; ampulla, aa; accessory ampulla, p; pedestal, s; spermatozoa, st; stalk.

is penetrated ventrally by a thin projection, which originates at the distal end of the stalk, to a depth of 60 μm (Figs. 1L, 2G–I).

Family Paguridae

The spermatophores of the single representative collected from this family, *Pagurus hirtimanus,* are morphologically very different from any other Paguroidea in this paper. The spermatophores, although considered to be stalked (pedunculate), do not appear to have a definite stalk lifting the ampulla above the pedestal. Another important difference is the presence of a secondary or accessory ampulla, at the base of the main ampulla but much smaller than the latter (Figs. 1M, 2F). This accessory ampulla has only been described in representatives from this family. The entire spermatophore of *Pagurus hirtimanus* is 400 μm in length and the attached stalk is 100 μm long by 50 μm wide. The main ampulla is 280 μm long and 150 μm wide while the accessory ampulla is only 36 μm long and 12 μm wide (Fig. 1M). The accessory ampulla is often devoid of spermatozoa but may contain one or two sperm cells (Fig. 2F).

Discussion

Coenobitidae

The spermatophores of the five representatives in the Coenobitidae (Fig. 1A–E) all show a similar over-all morphology and only size differences distinguish them. The spermatophores in this family are characterized by large spherical to ovoid ampullae with short thick stalks, giving them a generally robust appearance. I consider the genera in the Coenobitidae to be either semi-terrestrial, *Coenobita* spp., or terrestrial, *Birgus latro,* based on their differing abilities to withstand desiccation and their subsequent dependence on the marine environment.

In the Paguroidea, spermatophores are attached to the exoskeleton of the female hermit crab or on the surface of the temporarily inhabited gastropod shell, by the sticky base or pedestal (Matthews, 1956; Greenwood, 1972), and generally are embedded in a gelatinous mass. In an aquatic habitat, the spermatophoric mass does not dry out and thus can be carried around for long periods; but for terrestrial species or species that venture from the water for periods of time, desiccation of the spermatophoric mass is a problem. Matthews (1956) assumed that copulation, spermatophore deposition, and fertilization in the semi-terrestrial species, *Coenobita rugosus,* and the terrestrial coconut crab, *Birgus latro,* occurred in water, mainly because the remainder of the reproductive cycle is aquatic and also because no one had observed a terrestrial mating in this family.

One instance of terrestrial copulation between two individuals of *Birgus latro* was later observed and reported by Helfman (1977). He also observed several terrestrial matings in the semi-terrestrial hermit crab, *Coenobita perlatus.* These observations indicate that, in the Coenobitidae, spermatophore transfer and fertilization can occur on land. The robust nature of the spermatophores in this family may be an adaptation to facilitate spermatophore survival on the external surface of the female while she is on land. Large spermatophores with short, thick stalks would be harder to damage or dislodge from the gelatinous spermatophoric mass attached to the female than slender stalked spermatophores.

Diogenidae

The Diogenidae is a large hermit crab family encompassing many genera including *Calcinus, Clibanarius, Dardanus,* and *Diogenes.* The representatives in this study, from the above genera, show a great diversity of spermatophore form (Figs. 1F–L), but within the limits of a characteristic familial morphology. The spermatophores generally have long, thin stalks (less than 20 μm in diameter) and small spherical ampullae approximately 50–70 μm in size. The investigated *Diogenes gardineri* is an exception to the latter case, as it has larger ampullar dimensions (Figs. 1L, 2G–I).

Each of the genera can be distinguished by its possession of specific structural features. These distinctive generic forms can be complicated by unusual morphologies, such as the double-headed spermatophores of *Dardanus lagopodes* (Figs. 1J, 2D). The 20% occurrence rate of this form among the majority of single-headed spermatophores suggests that they might not be aberrant. This is the first record of double-headed spermatophores in the Diogenidae (also for the Paguroidea), and further study may reveal whether this is more common among other genera.

All the representatives in the Diogenidae are aquatic; mating and fertilization occur in water. Spermatophores are placed externally on either the carapace of the female or the gastropod shell she inhabits. The fact that desiccation of the spermatophoric mass is not a problem in an aquatic habitat may be reflected in the fragile structure of the slender-stalked spermatophores of these species. The long slender stalks in *Clibanarius corallinus, Dardanus lagopodes,* and *Diogenes gardineri* (Figs. 1I, J, L) may reflect the need to use the water for the extra support of the sperm-filled ampullae.

Paguridae

The 12 species within the family Paguridae have been studied for spermatophore morphology (see Introduction), making it the most studied family of the Paguroidea. The single species for which the spermatophore is illustrated

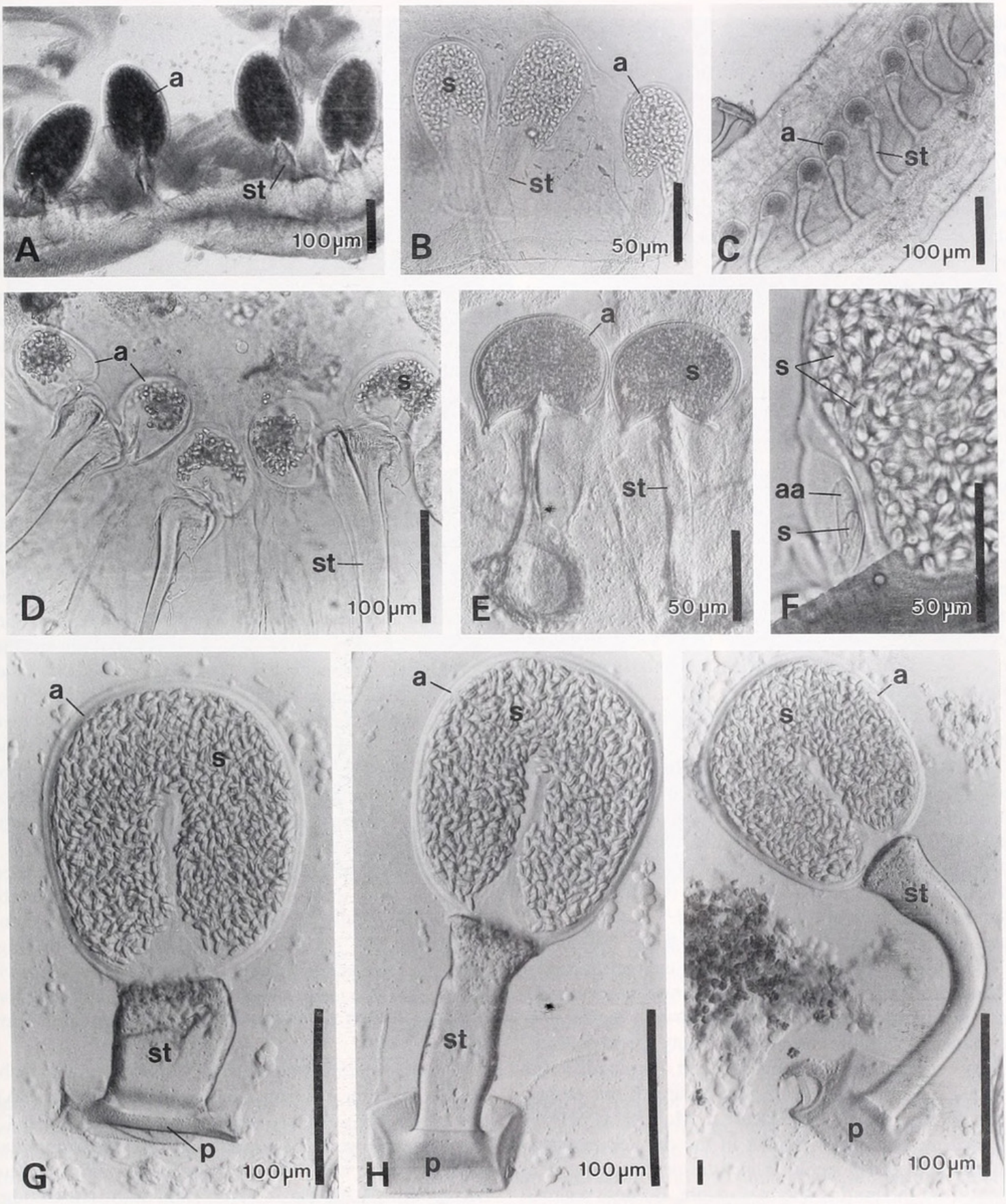

Figure 2. Light micrographs of spermatophores of hermit crabs. (A) *Coenobita brevimanus* (Coenobitidae). (B) *Dardanus megistos* (Diogenidae). (C) *Calcinus latens* (Diogenidae). (D) *Dardanus lagopodes* (Diogenidae). Note the double-headed spermatophores. (E) *Clibanarius corallinus* (Diogenidae). (F) *Pagurus hirtimanus* (Paguridae). Detail of the accessory ampulla containing a single spermatozoa. (G–I) *Diogenes gardineri* (Diogenidae). Series of three micrographs from the proximal to the distal end of the vas deferens showing the progressive lengthening of the spermatophore stalk. a; ampulla, aa; accessory ampulla, p; pedestal, s; spermatozoa, st; stalk.

and described in this paper conforms with the general familial morphology (Fig. 1M). Pagurid spermatophores differ from those of the Coenobitidae and Diogenidae in three important ways:

1. Although considered pedunculate (Greenwood, 1972), the spermatophores of the Paguridae tend to have only a short stalk, which is often incorporated into the pedestal and gives the appearance of being non-pedunculate (Matthews, 1957). Greenwood (1972), following the terminology of Mouchet (1931), divided the vas deferens of the hermit crab, *Pagurus novizealandiae,* into nine discrete regions. The different musculature in each of the nine regions is responsible for fragmentation of the continuous sperm ribbon into spermatophores. The presence or absence of each of the nine regions dictates the form of the spermatophore in the Paguroidea. In the Paguridae, regions four and six (areas of stalk secretion and elongation respectively) are absent. This led Matthews (1957) to state that the spermatophores of members of the Paguridae were non-pedunculate. However, Greenwood (1972) found that there was a stalk in *Pagurus novizealandiae* but that is is moulded from the capsule sheath. He suggested that, in accordance with Mouchet's (1931) description of the spermatophores of other members of the Paguridae, a "pseudo-pedicule" forms the stalk in this family, which is analogous to the true stalk in the Coenobitidae and Diogenidae.

2. The ampullae are often elongate or cylindrical in nature and fairly large.

3. Most importantly, the spermatophores have a secondary, smaller ampulla, which is termed the accessory ampulla. This accessory ampulla is found at the base of the main ampulla and generally contains no spermatozoa, although one or two individual spermatozoa may be present (Fig. 3D). This accessory ampulla is formed by the vesiculation of the empty sperm column sheath that separates each of the developing main ampullae (Mouchet, 1931; Matthews, 1957). The rhythmic muscle contractions of the reproductive tract fold the continuous sperm column into partially closed arches filled with developing spermatozoa, with the compressed empty sperm column in between. In the Coenobitidae and Diogenidae the elongation of the stalk is sufficient to stretch this empty sperm column sheath and obscure it. Mouchet (1931) illustrated an accessory ampulla in the developing spermatophore of the diogenid *Paguristes oculatus,* but it is absent from the mature spermatophore. Because regions four and six are absent in the Paguridae, stalk elongation is not significant. The sperm column sheath is, therefore, not stretched, leaving an empty vesicle at the base of each main ampulla. Some spermatozoa may be sealed in this vesicle but their release, upon dehiscence of the main ampulla, is doubtful.

This process of stalk lengthening, whether there is a pseudo-stalk or a true stalk, occurs during maturation of all spermatophores of the Paguroidea, with the exception of *Trizopagurus maximus* and *Trizopagurus strigatus* (Matthews, 1957). If spermatophore morphology is to be compared between species, care must be taken to obtain only mature spermatophores from the most distal portion of the vas deferens or to use extruded spermatophores. Immature spermatophore morphology of one family may be consistent with the mature spermatophore form of another family. An example of this can be seen in Figure 2G–I, where the short stalked, immature spermatophore of *Diogenes gardineri* is reminiscent of a coenobitid type spermatophore, while the mature spermatophore from the distal vas deferens shows clearly its diogenid origins (Fig. 1L).

Spermatophore lateral ridge

The conspicuous lateral partition seen by Mouchet (1930) on the ampulla of the spermatophore of *Diogenes pugilator* and later by Hamon (1937) in a similar position for *Pagurus prideaux* (as *Eupagurus prideauxi*) was correctly identified by both workers as the joining line of the two halves of the ampulla. Hamon (1937) called this partition the "*ligne de suture*" and showed that it ran around the elongate spermatophore of *Pagurus prideaux* and was where the two halves of the ampulla separated to release the contained spermatozoa. Hamon also suggested two main mechanisms of spermatophore dehiscence: one being changes in internal pressure due to mechanical or osmotic forces and the other being the chemical dissolution of the suture that unites the two halves of the ampulla.

This suture line, or lateral ridge, has now been recorded in species from three paguroidean families, the Coenobitidae [*Birgus latro,* Tudge and Jamieson, 1991; present study (Fig. 3A); *Coenobita spinosus,* present study (Fig. 3C)], the Diogenidae [*Calcinus minutus, Clibanarius virescens,* present study (Figs. 3B, D); *Diogenes pugilator,* Mouchet, 1930], and the Paguridae (*Pagurus prideaux,* Hamon, 1937).

Light microscope studies using Mallory's staining techniques, scanning electron microscope studies (Fig. 3A), and transmission electron microscope studies (Fig. 3B, C) show that the lateral ridge is a thickening of the ampullar spermatophore wall. The spermatophore wall of *Coenobita spinosus* is fibrillar in nature, and at the lateral ridge there is a break in this structure (Fig. 3C). Although the ridge is structurally thicker than the remainder of the spermatophore wall, this gap in the fibrous matrix is a weakness where splitting of the two ampullar halves occurs. A similar break in the spermatophore wall structure is seen in *Clibanarius virescens* (Fig. 3B), but an external

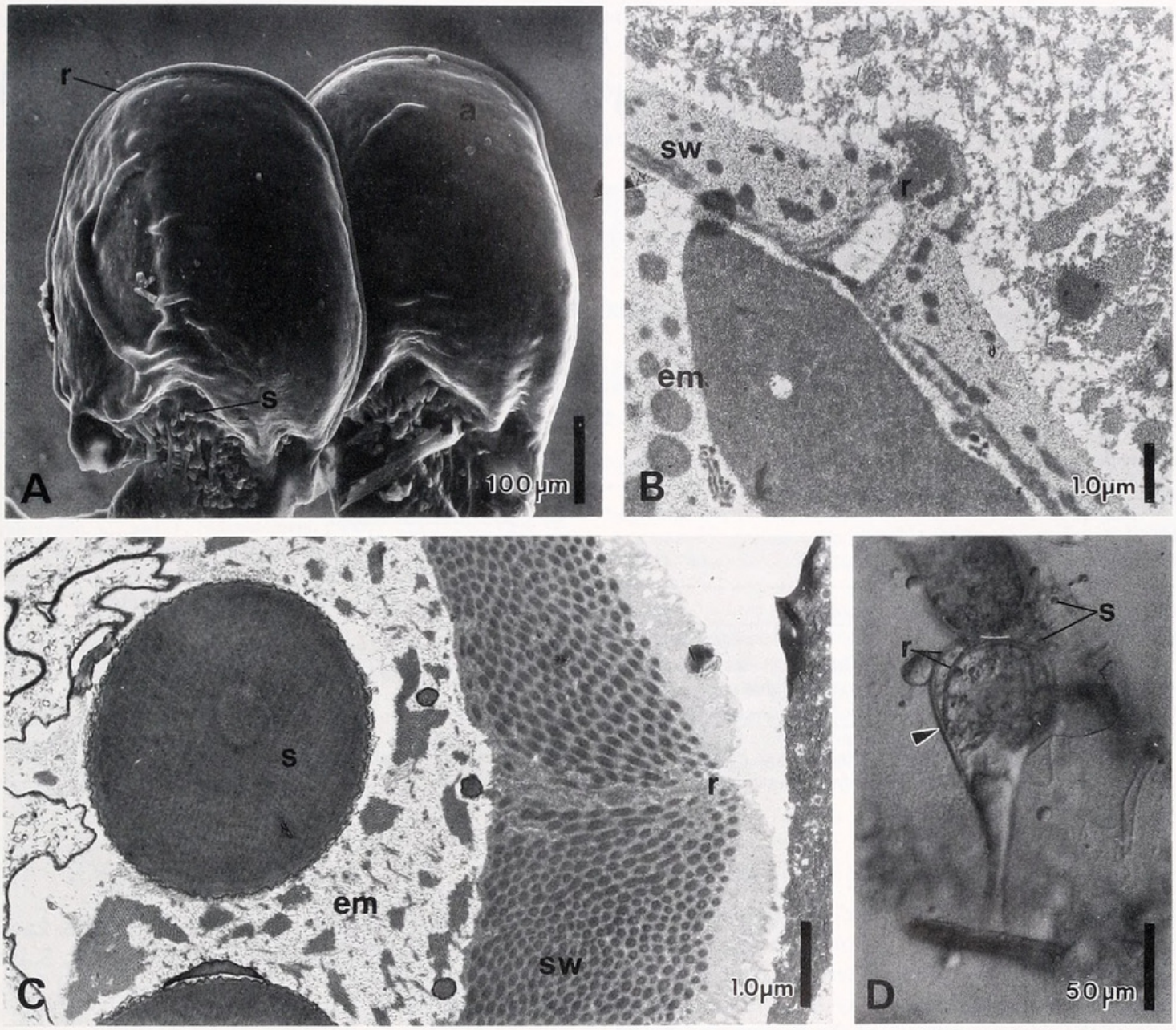

Figure 3. (A) *Birgus latro* (Coenobitidae). Scanning electron micrograph of two spermatophores showing conspicuous lateral ridge. (B and C) Transmission electron micrographs showing detail of the lateral ridge. (B) *Clibanarius virescens* (Diogenidae). Note the apparent plug of material in the lateral ridge. (C) *Coenobita spinosus* (Coenobitidae). (D) *Calcinus minutus* (Diogenidae). Light micrograph showing splitting of the spermatophore ampulla at the lateral ridge to release spermatozoa. (Arrow indicates the point of separation of the two ampullar halves). a; ampulla, em; extracellular matrix, r; lateral ridge, s; spermatozoa, sw; spermatophore wall.

plug appears to cement the two halves of the ampulla together in this species. Release of spermatozoa from the splitting lateral ridge of a spermatophore of *Calcinus minutus* is seen in Figure 3D.

Spermatophore morphology and modes of sperm transfer provide some clarification of systematic problems at familial and generic levels (Schaller, 1979; Bauer, 1986) and sometimes even at the specific level (Matthews, 1953, 1956). The phylogenetic value of spermatophore form and sperm transfer mode has been demonstrated in pseudoscorpions by Weygoldt (1966). One has to be cautious, however, in drawing homologies between species based on spermatophore form and modes of sperm transfer before the modes of formation, chemical nature, and ultrastructural morphology of the spermatophores in question have been studied in depth. Similarly, caution has to be exercised when phylogenetic hypotheses are formulated on limited data sets and small numbers of representatives from the selected taxa. Both Clark (1981) and Mann (1984) state that particular modes of sperm transfer used by organisms may be more directly influenced by the habitat of those organisms than the phy-

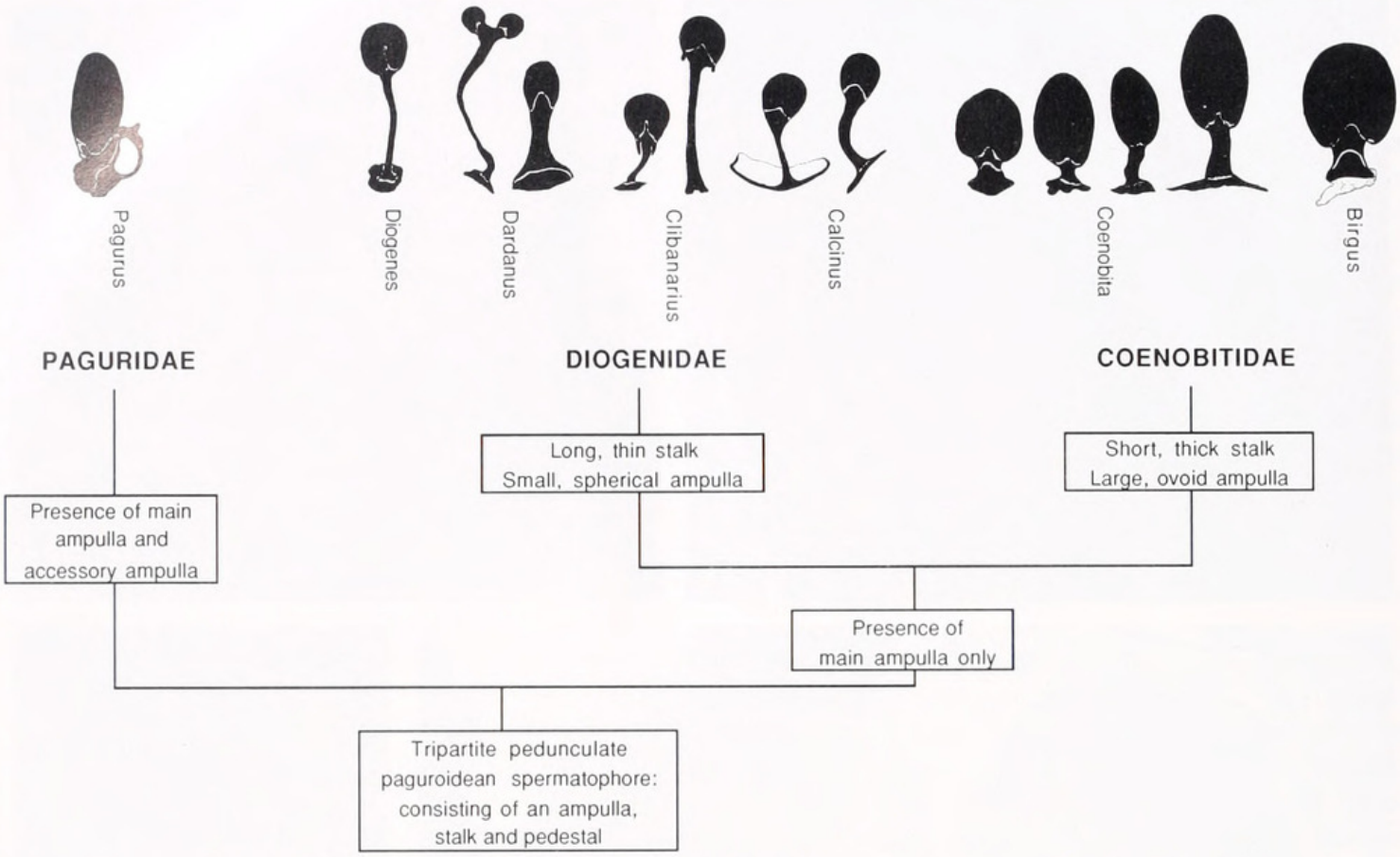

Figure 4. A branching key to classify the investigated hermit crabs into their respective family based on the structure of their spermatophores. Spermatophores not to scale.

logenetic relationships. It seems very likely that both habitat and phylogeny influence spermatophore morphology of any particular taxa. For example, in the Coenobitidae, the move to a semi-terrestrial or terrestrial lifestyle from a marine one may have influenced the shape of the spermatophores of the species concerned, but the over-all tripartite, pedunculate spermatophore plan is phylogenetically set.

In the Paguroidea, light microscope observations of spermatophores can be used successfully to distinguish the three separate families and possibly even apply at the generic level, but more species will have to be studied to be confident at a specific level. Some exceptions have been recorded, as in the case of *Cestopagurus timidus* (Mouchet, 1931, as *Eupagurus timidus*), in which a pagurid was recorded as possessing a diogenid type spermatophore morphology. As spermatophore studies widen to include more species and even other families, further confirmation or contradiction to this system will undoubtedly occur. A simple key (not intended as a phylogeny), in branching form (Fig. 4), is presented here and can be used to classify the currently investigated hermit crabs to the family level based on gross spermatophore morphology.

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