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# COMPARATIVE ULTRASTRUCTURE OF HERMIT CRAB SPERMATOZOA (DECAPODA: ANOMURA: PAGUROIDEA)

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## ABSTRACT

Comparative sperm morphology of several species of hermit crabs is investigated using light and electron microscope techniques. Relationships postulated on the basis of sperm ultrastructure are compared with previously proposed phylogenetic relationships. Within the superfamily Paguroidea, the families Coenobitidae, Diogenidae, and Paguridae are shown to be united by a suite of ultrastructural sperm characters. Each of these families can, however, be distinguished by characteristic features. The Coenobitidae have spermatozoa which possess a large cylindrical to oblong-ovoid acrosomal vesicle, a columnar perforatorial chamber which is not divided into posterior bulb and anterior projection, a subopercular zone divided into two regions, and lateral microvillar projections in the perforatorial chamber. The spermatozoon of the sole investigated pagurid *Pagurus bernhardus* (L.) has a short, ovoid acrosomal vesicle with a perforatorial chamber which is divided into posterior bulb and anterior projection, supposedly no operculum at maturity, and no microvillar projections in the posterior bulb of the perforatorial chamber. The anatomically diverse family Diogenidae exhibits a range in sperm morphology intermediate between the Paguridae and Coenobitidae, suggesting that it may be a polyphyletic family. Diogenidae share (except *Dardanus*) the perforatorial shape with the Paguridae and the possession of microvillar projections with the Coenobitidae. *Diogenes custos* (Fabricius) shares an elongate acrosome shape with the Coenobitidae, but is unique in having a fibrillar acrosome core. Three species of the diogenid genus *Clibanarius* are distinct in possessing a dense ring around the perforatorial bulb.

Among the decapod Crustacea, the infraorder Anomura has undergone considerable revision and rearrangement since its introduction by MacLeay (1838). Classifications of the Anomura, such as those of Glaessner (1969) and Borradaile (1907), include the four superfamilies Thalassinoidea, Paguroidea, Galatheoidea, and Hippoidea. More recently, workers such as McLaughlin (1983) and McLaughlin and Holthuis (1985) have excluded the thalassinoids from the Anomura and redefined the constituent superfamilies as the Galatheoidea, Hippoidea, Lomoidea, and Paguroidea.

The superfamily Paguroidea consists of the Coenobitidae, Diogenidae, Paguridae, Parapaguridae, Pylochelidae (=Pomatochelidae of Miyake, 1978), and Lithodidae (see McLaughlin, 1983). All except the Lithodidae are grouped together as true hermit crabs (Bowman and Abele, 1982).

Of these five hermit crab families, only species of the Paguridae, Diogenidae, and Coenobitidae have been studied for sperm morphology. The representatives from each family are listed below.

Paguridae: *Pagurus angulatus* Risso, *P. prideaux* Leach (both as *Eupagurus* in Koltzoff, 1906), *P. bernhardus* (Linnaeus) (as *Eupagurus* in Chevaillier, 1966a, b, 1967, 1968, 1970; Pochon-Masson, 1963, 1965a, b, 1968; Retzius, 1909).

Diogenidae: *Clibanarius misanthropus* (Risso) (in Koltzoff, 1906), *Clibanarius longitarsus* (deHaan), *Diogenes miles* (Herbst) (both in Dhillon, 1964, 1968), *Dardanus arrosor* (Herbst) (as *Pagurus striatus* in Koltzoff, 1906), *Paguristes oculatus* (Fabricius) (as *P. maculatus* in Koltzoff, 1906).

Coenobitidae: *Coenobita clypeatus* (Herbst) (in Hinsch, 1980a, b), *Birgus latro* (Linnaeus) (in Tudge and Jamieson, 1991).

## MATERIALS AND METHODS

Collection sites and dates of the species investigated in this paper are as follows: *Coenobita spinosus* H. Milne Edwards, 1837 (possibly *C. spinosus* var. *variabilis* McCulloch, 1909), Darwin, Australia, May 1990. *Clibanarius taeniatus* (H. Milne Edwards, 1848), North Stradbroke Island, Australia, April 1990; *Clibanarius virescens* (Krauss, 1843), Hastings Point, Australia, April 1990; *Clibanarius corallinus* (H. Milne Edwards, 1848), One Tree Island, Great Barrier Reef, Australia, April 1988; *Dardanus crassimanus* (H. Milne Edwards, 1836), Moreton Bay, Australia, August 1990; *Diogenes custos* Fabricius, 1798, North Stradbroke Island, Australia, March 1990.

The male reproductive material was removed from fresh specimens and fixed in cold glutaraldehyde for

transmission electron microscopy. After the initial glutaraldehyde fixation and first phosphate buffer wash, the remainder of the fixation procedure (outlined below) was carried out in a Lynx® -el. Microscopy Tissue Processor (Australian Biomedical Corporation, Ltd., 96 Ricketts Rd., Mount Waverly, Victoria 3149, Australia).

Portions of the testis 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 an LKB 2128 UM IV microtome with a diamond knife. Sections were placed on carbon-stabilized colloidin-coated 200-μm mesh copper grids and stained with Reynold's lead citrate (Reynolds, 1963) for 30 s, rinsed in distilled water, stained in 6% aqueous uranyl acetate for 1 min, stained again with Reynold's lead citrate for 30 s, and further rinsed in distilled water. Micrographs were taken on a Hitachi 300 transmission electron microscope at 80 kV. All illustrations are by the author.

## Results

### Family Coenobitidae

The spermatozoa of *Coenobita spinosus* are 13 μm in length and 5 μm at their widest point across the nucleus. They are composed chiefly of a long, cylindrical acrosomal vesicle (approximately 10 μm long by 3 μm wide), a posterior, globular nucleus, and a cytoplasmic region (Figs. 1A, 5B).

An ultrastructural investigation of spermatozoal morphology reveals that the acrosomal vesicle is divisible into several major areas. The electron-dense conical operculum is at the anteriormost end of the acrosomal vesicle. Immediately below this is a subopercular zone which can be divided into two regions of differing electron density and structure (Fig. 1A, B). The most anterior of the two subopercular zones is electron-pale, fibrillar in nature, and occupies the center of the conical operculum. This fibrillar section abruptly meets the posterior granular region just below the rim of the operculum. The granular region extends posteriorly, meeting the central core of the acrosomal vesicle (the inner acrosome zone), which in turn extends posteriorly to abut the invaginated perforatorial chamber. The inner acrosome zone is finely granular and of similar electron density to the most posterior subopercular zone (Fig. 1A).

The columnar perforatorial chamber extends from the open posterior end of the acrosomal vesicle to meet the inner acrosome zone. The perforatorial chamber is consistent in diameter for its full length, except for the narrower basal opening. In the posteriormost region of the perforatorial chamber the walls are modified into short microvillar projections, which extend laterally into the perforatorial chamber.

The remaining anterior region of the acrosomal vesicle forms a thick cylinder extending from the open end of the perforatorial chamber to the operculum. This cylinder is called the acrosome ray zone and in longitudinal section has the appearance of densely packed, radiating bands of alternating dark and light electron density. In transverse section the same zone appears as light tubules in a darker matrix (Fig. 1B).

The cytoplasmic material forms a thin cup which invests the posterior end of the acrosomal vesicle and slightly protrudes into the posterior opening of the perforatorial chamber. The cytoplasm is coarsely granular and contains numerous large mitochondria, remnants of membrane systems, and the bases of the three microtubular arms. No centrioles are seen in the cytoplasm and no distinct membrane separating the cytoplasm from the nuclear material is apparent.

The nuclear material is composed of diffuse chromatin fibers which are contained within a thick double membrane. This membrane is a combination of the nuclear membrane and the plasma membrane and is termed the nucleoplasma membrane (Fig. 1A).

### Family Diogenidae

The spermatozoon of *Clibanarius corallinus* is approximately 5.5 μm in length from the apex to the most posterior extremity of

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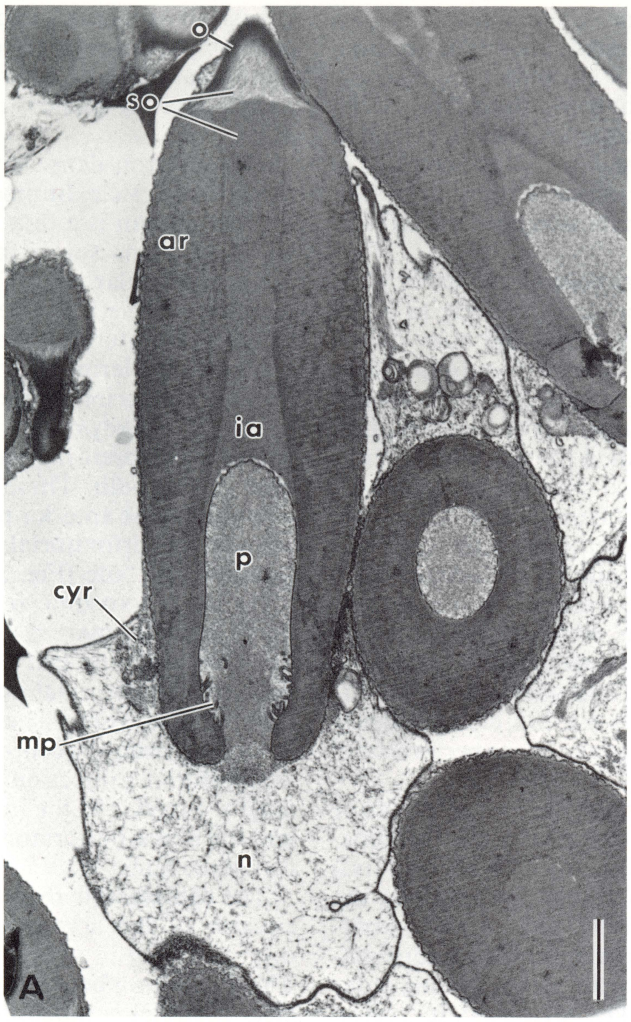
Fig. 1. *Coenobita spinosus*. A, Longitudinal section of spermatozoon; B, detail of longitudinal section of operculum and subopercular zone. Note the appearance of the acrosome ray zone in longitudinal and transverse section. ar, acrosome ray zone; cyr, cytoplasmic region; ia, inner acrosome zone; mp, microvillar projections; n, nuclear material; o, operculum; p, perforatorial column; so, subopercular zone. Scale bars = 1 μm.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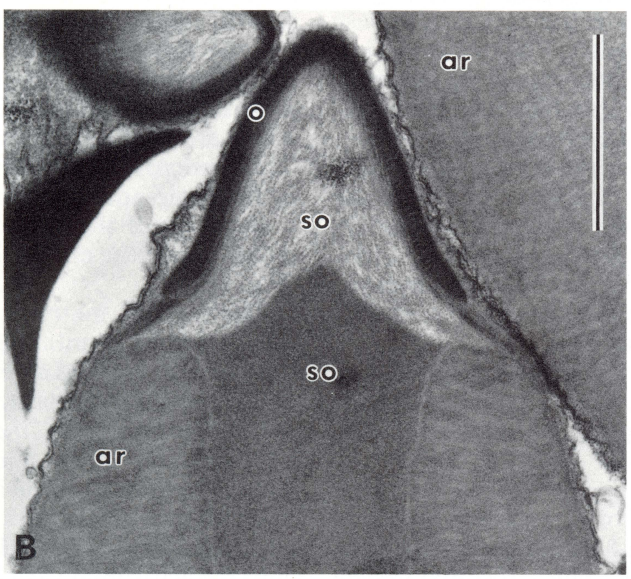
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the nucleus and approximately 4.5 μm across the nucleus. The almost spherical acrosome, 3.3 μm long and 2.9 μm across the equator, is broken at the base by the perforatorial invagination; the anterior pole is extended into a conical operculum. The operculum is an electron-dense dome with a thin subopercular zone immediately posterior (Figs. 2A, 5H).

The perforatorial chamber extends from the base of the acrosomal vesicle to a position immediately below the apex of the conical operculum. The perforatorial chamber is divided into a posterior perforatorial bulb and an anteriorly projected column. The column is termed the anterior perforatorial projection and contains longitudinally directed parallel fibers. The posterior perforatorial bulb contains homogeneous granular material but is characterized by convolution of the walls to form microvillus-like projections which extend into the bulb. Immediately exterior to the posterior perforatorial bulb, and adjacent to the microvillar projections, is a thin, electron-dense investing layer called the dense perforatorial ring (Fig. 2A).

Extending anteriorly from the posterior perforatorial bulb to the subopercular zone, and surrounding the anterior perforatorial projection, is a columnar region called the inner acrosome zone. This zone is composed of a fine, granular, homogeneous matrix which is similar to the remaining outer acrosome zone. The cytoplasmic region forms an extensive collar around the base of the acrosomal vesicle. The cytoplasm appears to be composed mostly of electron-pale, granular vesicles which may be degenerate mitochondria, although none appears to be membrane-bound. An occasional section of the bases of the three microtubular arms is seen, but the remainder of the cytoplasm is a heterogeneous granular matrix. The cytoplasmic–nuclear boundary is easily recognized, but no definite membrane separates the two areas.

The nuclear material, homogeneous and granular in nature, is surrounded by a thick, combined nucleoplasma membrane (Fig. 2A).

The sperm cells of *Clibanarius taeniatus* are approximately 5 μm long and approximately 4.5 μm wide. The acrosomal vesicle is basically spherical, except for the conical anterior portion, and the cytoplasm and microtubular arms lie between the acrosomal vesicle and the nuclear material (Figs. 2B, 5G).

The electron-dense operculum caps the acrosomal vesicle. Immediately posterior to this operculum is a thin subopercular zone which contains subtle radiating striations, the subopercular rays. The base of the acrosomal vesicle is broken by the entrance of the perforatorial chamber, divided into a swollen posterior perforatorial bulb, and a thinner anterior perforatorial projection. Microvillus-like projections extend laterally from the perforatorial bulb wall into the lumen of the bulb. These microvilli do not extend into the anterior projection. An electron-dense perforatorial ring surrounds the perforatorial bulb (Fig. 2B).

An inner acrosome zone, composed of a finely granular matrix, occurs as a cylinder from the anterior end of the perforatorial bulb to a subterminal position beneath the subopercular zone. The axis of the inner acrosome zone is occupied by the anterior perforatorial projection for nearly its full length. Exterior to the inner acrosome zone and the dense perforatorial ring is the outer acrosome zone.

The cytoplasmic region extends from the equator of the acrosomal vesicle posteriorly, as a cup in which the acrosome lies. The distinction between cytoplasm and nuclear material is indefinite and the two appear to intermingle. Some bodies, possibly degenerating mitochondria, though not membrane-bound, are present in the cytoplasm along with bundles of microtubules, which are sections of the microtubular arms. These microtubular arms pass through the cytoplasmic-nuclear boundary and leave the main body of the sperm, retaining a thin collar of nuclear material for a short length (Fig. 2B).

The nuclear material, surrounded by a nucleoplasma membrane, has a finely granular appearance and a very similar electron density to the cytoplasmic region.

The sperm cell of *Clibanarius virescens* is approximately 6 μm long and 6 μm wide. The spherical acrosome is capped by a thin operculum and features a well-developed perforatorial chamber. The nuclear region cups the acrosomal vesicle from the equator

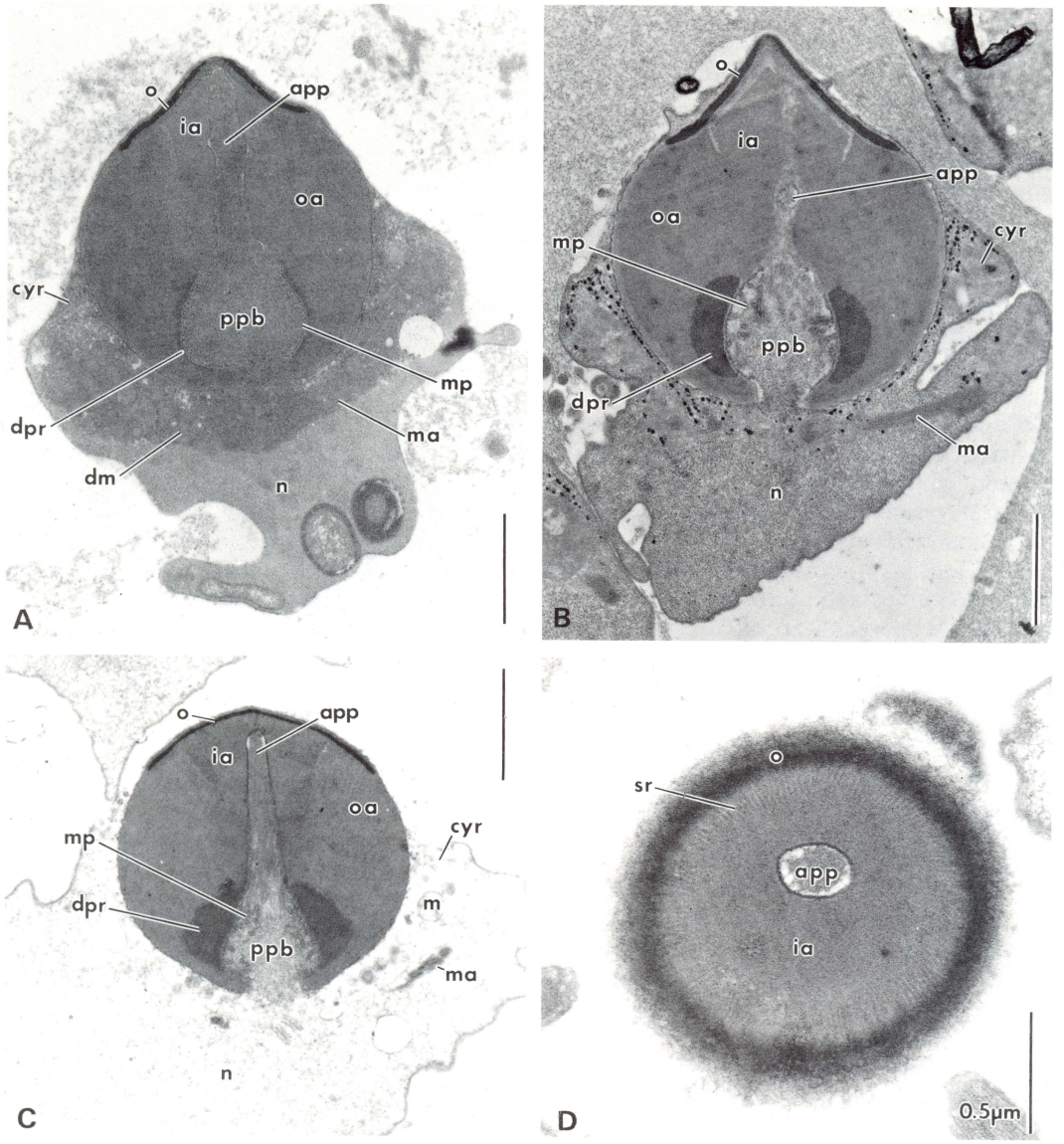


Fig. 2. *Clibanarius* spp. A, Longitudinal section of spermatozoon of *Clibanarius corallinus*; B, longitudinal section of spermatozoon of *Clibanarius taeniatus*; C, longitudinal section of acrosome vesicle of spermatozoon of *Clibanarius virescens*. D, Transverse section of opercular region of spermatozoon of *Clibanarius virescens* showing subopercular rays and anterior end of perforatorial chamber. app, anterior perforatorial projection; cyr, cytoplasmic region; dm, degenerating mitochondria; dpr, dense perforatorial ring; ia, inner acrosome zone; m, mitochondrion; ma, microtubular arm; mp, microvillar projections; n, nuclear material; o, operculum; oa, outer acrosome zone; ppb, posterior perforatorial bulb; sr, subopercular rays. Scale bars = 1 μm, except where indicated.

of the acrosome sphere posteriorly and a thin cytoplasmic area is incorporated between the acrosome and the nucleus (Figs. 2C, 5F). The three microtubular arms emerge from the cytoplasmic-nuclear junction.

Immediately posterior to the operculum is an area of thin radiating subopercular rays (Fig. 2D). The spherical appearance of the vesicle is broken only by the entrance of the perforatorial chamber, which is divided into two distinct regions. The posterior perforatorial bulb has its walls pinched into many conspicuous microvillus-like projections, which extend laterally into the perforatorial bulb. The thin anterior perforatorial pro-

jection contains longitudinally directed, parallel, fibrillar structures. The perforatorial chamber terminates before reaching the operculum (Fig. 2C).

The contents of the acrosomal vesicle can be divided into three areas of differing electron density. A dense perforatorial ring lies adjacent to the posterior perforatorial bulb and extends anteriorly to the junction between the perforatorial bulb and the perforatorial projection. Anterior to this dense perforatorial ring is a hollow column of homogeneous acrosomal material. This central cylinder is regarded as the inner acrosome zone and extends to a subopercular position, at which point it increases in diameter. The remaining portion of the acrosomal vesicle constitutes the outer acrosome zone (Fig. 2C).

The cytoplasm remains as a thinly spread cup which invests the acrosomal vesicle, excepting the opercular area. The cytoplasmic region contains some loose membrane assemblages, mitochondria and mitochondrial remnants, and unidentified vesicles. No centrioles were seen in the cytoplasm. No distinct nuclear-cytoplasmic membrane separates the two regions, although a series of vesicles along the boundary may be remnants of a membrane. Immediately posterior to this cytoplasmic-nuclear boundary can be found the longitudinally arranged bundles of microtubules which are the bases of the microtubular arms (Fig. 2C). The three microtubular arms create a characteristic "triad" pattern around the base of the acrosomal vesicle when seen in cross section.

The nuclear material is diffuse, granular, relatively electron-lucent, and bounded externally by a combined nucleoplasma membrane.

The spermatozoa of *Dardanus crassimanus* are approximately 13 $\mu$m long and 6 $\mu$m wide. The acrosomal vesicle is 6.5 $\mu$m long and 4.4 $\mu$m wide and is ovoid with a perforatorial invagination penetrating into the center of the vesicle. A domed opercular region caps the acrosomal vesicle. Microtubular arms originate from the base of the acrosome where the cytoplasm and nuclear material are situated (Figs. 3A, 5D).

Occupying the space beneath the operculum is the subopercular zone, which is coarsely granular and extends posteriorly to meet the central column or inner acrosomal zone. This latter zone is less electron-dense than most of the acrosomal vesicle and posteriorly invests the perforatorial chamber as a thin envelope. The perforatorial chamber is columnar in shape and extends anteriorly for one-third of the length of the acrosomal vesicle (Fig. 3A). The wall of the perforatorial chamber forms microvillus-like projections (Fig. 3B). The acrosome ray zone forms a cylinder (around the perforatorial chamber and the inner acrosomal zone) and is composed of radiating light and dark tubules (Fig. 3B). External to this acrosome ray zone is a very thin outer acrosomal zone which invests the entire acrosomal vesicle except for the operculum.

The cytoplasm occurs as a ring around the base of the acrosomal vesicle and is continuous with the perforatorial chamber and the nuclear region, although a disrupted cytoplasmic-nuclear membrane is present (Fig. 3A). The main components of the cytoplasm are mitochondria, reticulate lamellar bodies, the bases of the three microtubular arms, and, in this species, a single centriole (Fig. 3B).

The large nuclear region appears loosely granular and generally electron-opaque and is bounded by a nucleoplasma membrane (Fig. 3A).

The spermatozoa of *Diogenes custos* are composed of a large cylindrical acrosomal vesicle anterior to a globular cytoplasmic and nuclear region, from which three microtubular arms originate. The entire sperm cell is approximately 10 $\mu$m long and 3.5 $\mu$m wide. The cylindrical acrosome (8.5 $\mu$m long and 2.9 $\mu$m wide) is capped anteriorly

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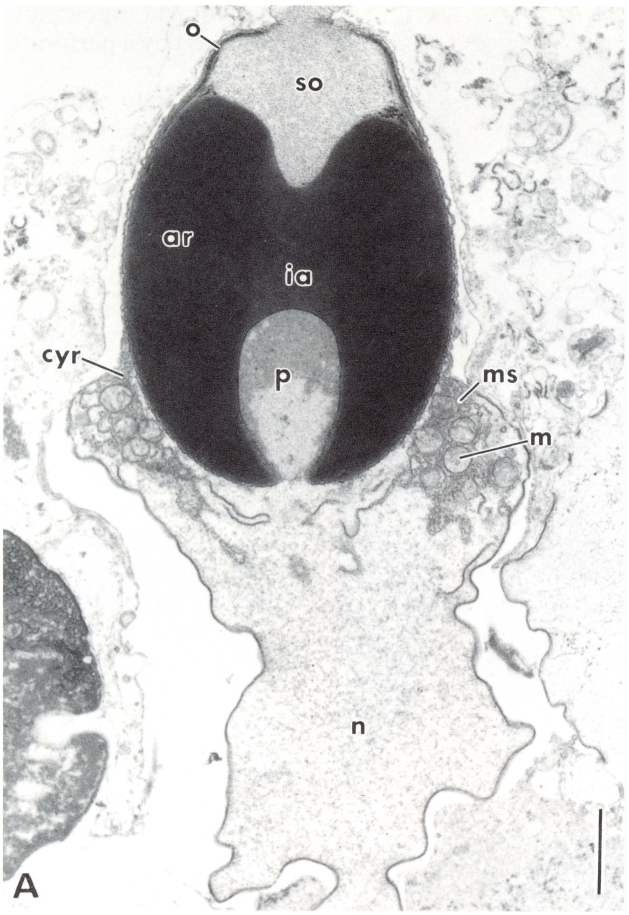
Fig. 3. *Dardanus crassimanus*. A, Longitudinal section of spermatozoon; B, detail of longitudinal section of posterior end of perforatorial chamber showing microvillar projections and single centriole. ar, acrosome ray zone; c, centriole; cyr, cytoplasmic region; ia, inner acrosome zone; m, mitochondrion; mp, microvillar projections; ms, membrane system; n, nuclear material; o, operculum; p, perforatorial column; so, subopercular zone. Scale bars = 1 $\mu$m, except where indicated.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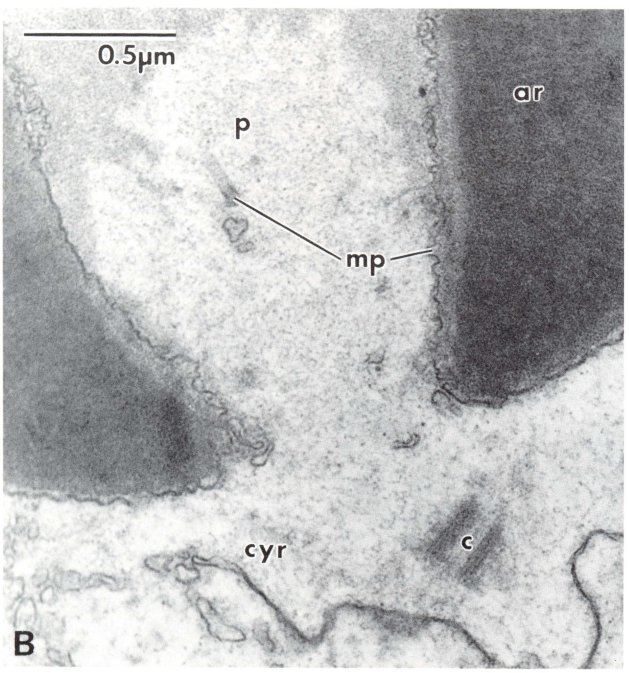
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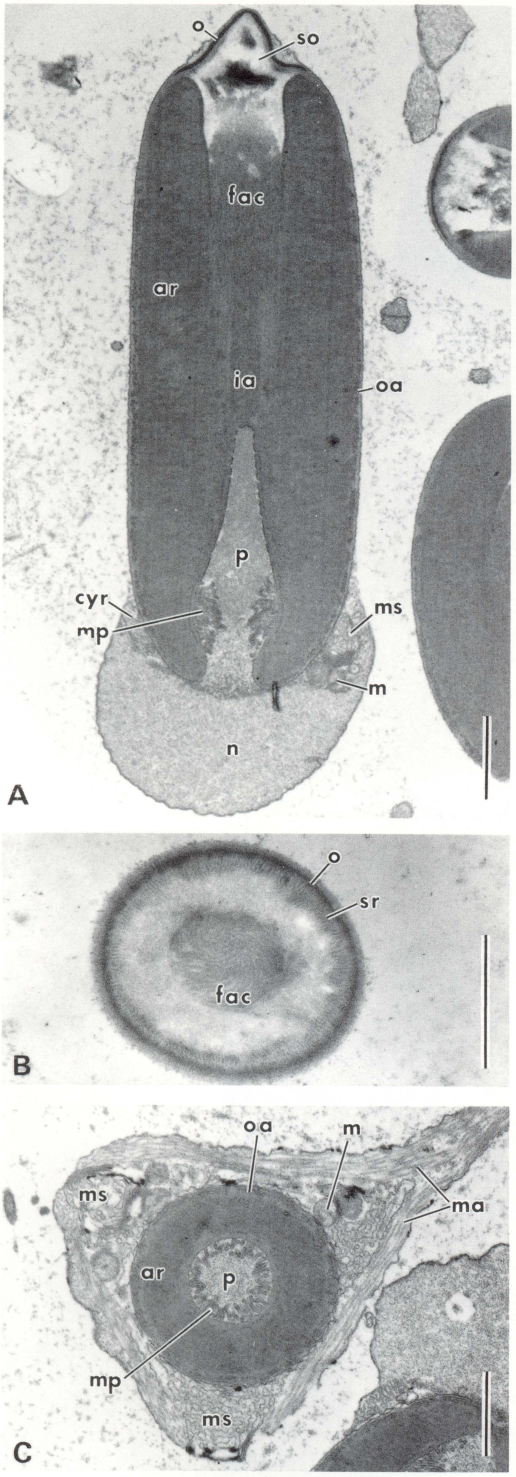


Fig. 4. *Diogenes custos.* A, Longitudinal section of spermatozoon; B, transverse section of opercular region showing subopercular rays and spiral arrangement of fibrillar acrosome core; C, transverse section through perforatorial chamber showing "triad" arrangement of bases of three microtubular arms and cytoplasmic organelles. ar, acrosome ray zone; cyr, cytoplasmic region; fac, fibrillar acrosome core; ia, inner acrosome zone; m, mitochondrion; ma, microtubular arm; mp, microvillar projections; ms, membrane system; n, nuclear material; o, operculum; oa, outer acrosome zone; p, perforatorial column; so, subopercular zone; sr, subopercular rays. Scale bars = 1 $\mu$m.

by a conical operculum, and posteriorly penetrated by a perforatorial chamber (Figs. 4A, 5E).

Immediately below the operculum is an area of fine, radiating striations, the subopercular rays (Fig. 4B). The wall of the perforatorial chamber, at its widest point, features numerous microvillus-like extensions which extend laterally into the center of the perforatorial chamber (Fig. 4A, C). Closely investing the perforatorial chamber is the inner acrosome zone. This zone extends anteriorly to meet the subopercular area but undergoes a change in form anteriorly. The granular form near the perforatorial chamber is transformed into a longitudinally aligned fibrous column (Fig. 4A). It is here termed the fibrillar acrosome core; the fibrillar nature is more apparent closer to the operculum, where the hollow fibers assume a spiral arrangement (Fig. 4B).

External to the perforatorial chamber and the fibrillar acrosome core, the acrosomal vesicle is divided into two zones. Most of this column is composed of the acrosome ray zone, with a thin outer acrosome zone investing all other layers. The outer acrosome zone extends from the posterior opening of the perforatorial chamber, around the periphery of the acrosomal vesicle, to the posterior rim of the operculum (Fig. 4A, C).

The cytoplasmic region envelopes the base of the cylindrical acrosomal vesicle as a ring, although a thin layer of cytoplasm passes under the vesicle and appears continuous with the open perforatorial chamber (Fig. 4A). The cytoplasmic organelles include numerous mitochondria associated with extensive reticulate, membrane systems, and the typical "triad" arrangement of the bases of the microtubular arms (Fig. 4C). No centrioles were seen in the cytoplasmic region of *Diogenes custos.*

The nuclear material appears diffuse but loosely granular, and is bounded by a char-

acteristic combined nucleoplasma membrane.

## Discussion

Like the crustaceans themselves, their spermatozoa are very diverse in their morphology (Jamieson, 1989c, 1991). It is, therefore, difficult to designate sperm features that characterize the entire class. Nevertheless, sperm data are extremely useful in determining relationships between crustacean taxa. Except for some crustacean groups, all in the Maxillopoda, such as the Ascothoracica (see Grygier, 1982), the Cirripedia (see Healy and Anderson, 1990), and the Remipedia (see Yager, 1989), most crustaceans have aflagellate, immotile sperm. The noncaridean, pleocyemate decapods, of which the Anomura are members, all share a common sperm form consisting of an often large acrosomal vesicle (which can be multilayered), a posterior nucleus of variable density, intervening cytoplasm containing mitochondria, microtubules, lamellar structures, and sometimes centrioles, and a variable number (from three to many) of arms or spikes. The arms may be composed of nuclear material (higher Brachyura) or microtubules (Anomura) or both (Majidae).

The basic sperm morphology among the Anomura is similar to that found in the Brachyura (Jamieson, 1989a, b, 1990; Jamieson and Tudge, 1990; Felgenhauer and Abele, 1991). Among other differences, including opercular shape and acrosome zonation, the major difference between anomurans and brachyurans is that the spherical acrosomal vesicle in brachyuran sperm is never elongated (Jamieson, 1991).

The sperm of the two genera in the Coenobitidae, *Coenobita* and *Birgus,* have been extensively studied ultrastructurally. That of the coconut crab *Birgus latro* (Fig. 5A) was ultrastructurally described by Tudge and Jamieson (1991), and two species of the genus *Coenobita, C. clypeatus* (see Hinsch, 1980a, b) and *C. spinosus* (present study) (Fig. 5B, C) have been studied. The Coenobitidae appear to have a general sperm type that combines the following ultrastructural characters: (1) a large cylindrical to oblong-ovoid acrosomal vesicle with a conical operculum and a fairly deeply invaginated columnar perforatorium, which is not divided into a posterior bulb and anterior projection, (2) the posterior walls of the perforatorium extend into the lumen as long, microvillar projections, the subopercular zone is divided into two distinct regions with the posteriormost region extending down the center of the acrosomal vesicle to abut against the inner acrosome zone, (3) three long microtubular arms enter the cytoplasmic region, midway between the nucleus and the acrosomal vesicle, and join around the base of the vesicle in a triad, and (4) mitochondria and lamellae are prominent components of the cytoplasm, and, with the exception of *C. clypeatus* (see Hinsch, 1980a), no centrioles have been reported in the mature sperm.

The spermatozoon of *Birgus latro* (Fig. 5A) differs from the representatives of the genus *Coenobita* (Fig. 5B, C) in that the acrosomal vesicle is more oblong-ovoid than cylindrical and the nuclear material is drawn out into filamentous extensions or arms (in addition to microtubular arms), although a tendency toward these nuclear extensions is also seen in *C. clypeatus* (see Hinsch, 1980a).

The three investigated genera in the Diogenidae, *Diogenes, Dardanus,* and *Clibanarius* (Fig. 5D–H), can be distinguished from each other on various sperm features. *Diogenes* spp. (Dhillon, 1968; present study) have sperm which possess some of the characteristics of the coenobitid type. These include the large cylindrical acrosome, acrosome ray zone, and columnar perforatorium with microvillar projections. However, the perforatorium possesses a slightly bulbous posterior region. In *Diogenes custos* (Fig. 5E) the inner acrosome zone is reduced posteriorly to a thin perforatorial covering, while anteriorly it is modified into a fibrillar acrosome core.

*Clibanarius* spp. (Fig. 5F–H) (Dhillon, 1968; present study), on the other hand, have a smaller, spherical acrosome with a perforatorial column that is bulbous posteriorly and forms a thin anterior projection. This distinctive perforatorial shape is reminiscent of that seen in *Pagurus bernhardus* (Fig. 5I) (Chevaillier, 1970; Pochon-Masson, 1965b), but, unlike *P. bernhardus,* small microvillar extensions are present in the posterior bulb of the perforatorium. This last feature is shared with *Dardanus crassimanus* (Fig. 5D), *Diogenes custos* (Fig. 5E), *Coenobita* spp. (Fig. 5B, C), and *Birgus latro*

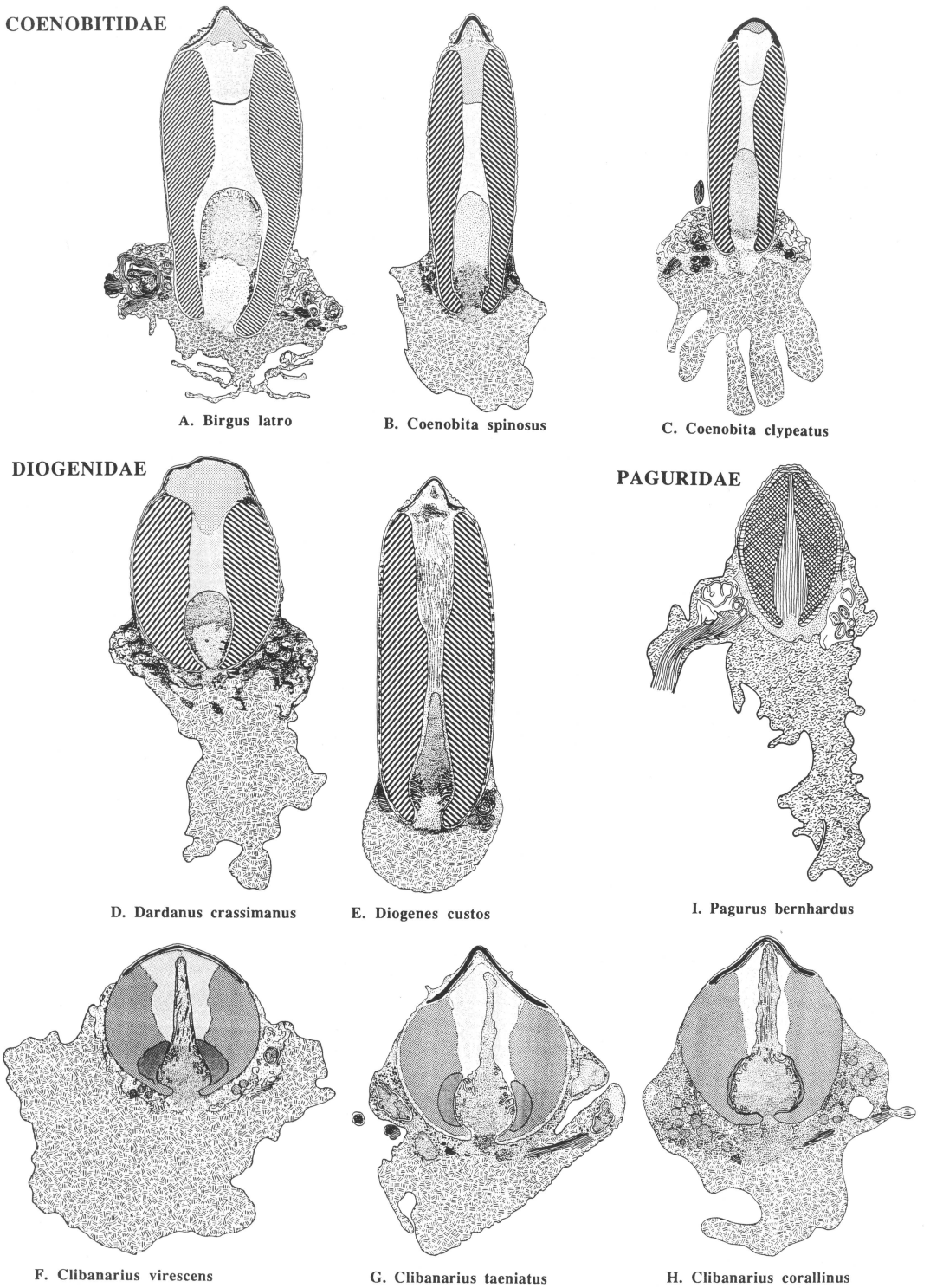


Fig. 5. Semidiagrammatic representations of longitudinal sections of hermit crab spermatozoa. A–C, Coenobitidae (A, Redrawn from Tudge and Jamieson, 1991) (C, Drawn from micrographs in Hinsch, 1980a.); D–H, Diogenidae; I, Paguridae. (I. Redrawn from Chevaillier, 1970.) Drawings not to scale.

(Fig. 5A). The distinctive feature of sperm of *Clibanarius* spp. is the presence of a dense perforatorial ring around the posterior perforatorial bulb. The extent of this dense ring, differences in opercular shape, and the extent of the cytoplasmic region distinguish the three species of *Clibanarius* from one another.

*Dardanus crassimanus* (Fig. 5D) has an acrosomal vesicle which is larger than that of the three species of *Clibanarius,* but not as cylindrical as that of the coenobitids or *Diogenes custos.* The perforatorial chamber shape, differentiation of perforatorial chamber matrix, and extent of the inner acrosomal zone are similar to those seen in *Birgus latro* and *Coenobita spinosus,* but the subopercular zone in *Dardanus crassimanus* does not appear to be divided into two areas of differing electron density (Figs. 3A, 5D).

Within the Diogenidae, there appears to be a mixture of sperm types approaching the pagurid and coenobitid types. This combination of spermatozoal characters may explain the discrepancy seen in the positioning of these three pagurid families in the phylogenetic study of Martin and Abele (1986). The Diogenidae are more closely allied with the Paguridae in one tree and with the Coenobitidae in another. This latter arrangement is also advocated by McLaughlin (1983). If a similar discrepancy in somatic morphology occurs, as with sperm morphology, between genera in the Diogenidae, then the relationship will change according to which genera are used for comparison. Perhaps the family Diogenidae contains genera that are representatives from other families, which are close enough in somatic morphology to appear cohesive. The Diogenidae may be a polyphyletic family with some of the genera being more closely allied to the other hermit crab families than to each other.

Since only a single species has been studied for the Paguridae, it is difficult to draw general conclusions about the type of sperm morphology present within that family. Both Pochon-Masson (1963, 1965a, b, 1968) and Chevaillier (1966a, b, 1967, 1968, 1970) undertook light and transmission electron microscope studies of the sperm of *Pagurus bernhardus* (Fig. 5I). The acrosomal vesicle is ovoid and fairly short, the posterior nuclear material is diffuse and extensive, and the cytoplasmic region contains mitochondria, lamellae, centrioles, and the contiguous bases of the three prominent microtubular arms. Chevaillier reported that an electron-dense cap (or operculum) on the apex of the acrosome disappeared at maturity, but Pochon-Masson (1965b) showed

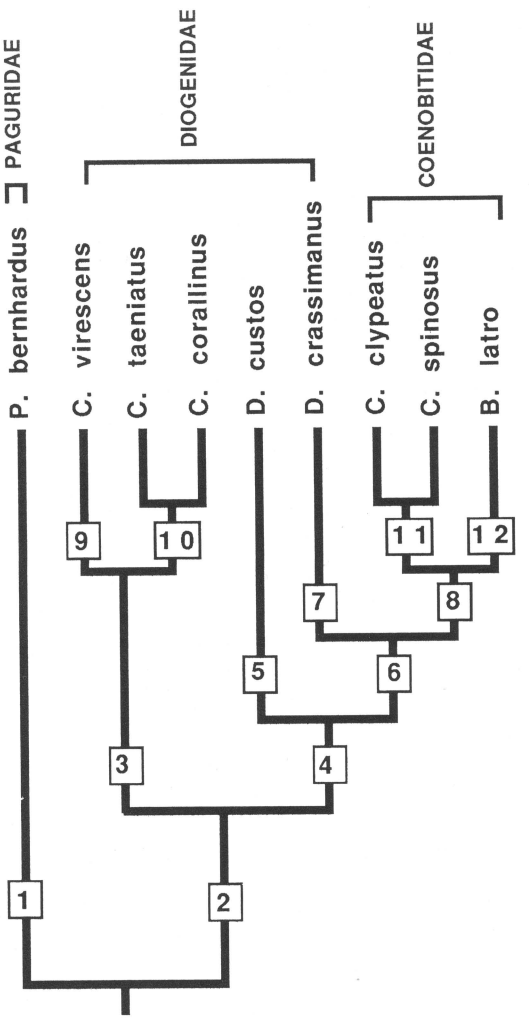


Fig. 6. Branching tree of hermit crab spermatozoa. Characters are: 1, operculum absent and no microvillus-like extensions in perforatorial chamber; 2, operculum present and microvillus-like extensions in perforatorial chamber; 3, dense perforatorial ring present and acrosome ray zone absent; 4, dense perforatorial ring absent and acrosome ray zone present; 5, perforatorial chamber slightly bulbous posteriorly; 6, perforatorial chamber columnar; 7, subopercular zone uniform; 8, subopercular zone divided; 9, domed operculum; 10, conical operculum; 11, cylindrical acrosome; 12, oblong-ovoid acrosome.

an apparent operculum in one of her micrographs of sperm of *P. bernhardus*. Neither author indicated the presence of microvillar extensions of the acrosomal vesicle into the posterior chamber of the perforatorium. Both of these ultrastructural characters are present in the Diogenidae and Coenobitidae. The presence of the centriole in the cytoplasm at the posterior end of the perforatorial column in *P. bernhardus* has been recognized in only two other hermit crabs, *Coenobita clypeatus* (Fig. 5C) (Hinsch, 1980a) and *Dardanus crassimanus* (Fig. 3B) (present study).

Using the combination of spermatozoal characters exhibited by hermit crabs, a branching tree is presented showing proposed relationships between the nine species (Fig. 6). *Pagurus bernhardus,* representing the Paguridae, is shown to be distinct from the other species and similarly the three species in the Coenobitidae group together. The diverse sperm features of the Diogenidae tend to split the three genera involved and ally *Diogenes* and *Dardanus* with the coenobitids, while the three species of *Clibanarius* are collectively distinctive.

The interfamilial distinction between these three families of hermit crabs is supported by recent work on spermatophore morphology (Tudge, 1991). Representatives from the Coenobitidae, Diogenidae, and Paguridae can be classified into their respective family on the basis of light microscope observations of gross spermatophore structure.

Sperm ultrastructural studies of other species within these families, and especially the two unstudied families, Parapaguridae and Pylochelidae, may help unravel the phylogenetic relationships within the Paguroidea.

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