

Ultrastructure of the spermatozoa of *Corystes cassivelaunus* (Corystidae), *Platelistoma nanum* (Cancridae) and *Cancer pagurus* (Cancridae) supports recognition of the Corystoidea (Crustacea, Brachyura, Heterotremata)

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ABSTRACT: A combination of characters, not individually unique, possessed by the corystid, *Corystes cassivelaunus*, and the two cancrids, *Platelistoma nanum* and *Cancer pagurus*, defines a corystoid-type of spermatozoon: the basally bulbous, anteriorly narrowing perforatorium, the extent of this almost to the plasma membrane through a widely perforate operculum, and the simple inner acrosome zone, lacking an acrosome ray zone. The sperm of the two cancrids are closely similar, that of the corystid differing, for instance, in the less pointed, and less tapered, form of the perforatorium. This relative uniformity of spermatozoal ultrastructure in the cancrid+corystid assemblage so far investigated supports inclusion of the two families in the superfamily Corystoidea by Guinot (1978). The combination of perforation of the operculum and absence of an acrosome ray zone (at least in a clearly recognizable form) are features of the Potamidae which possibly indicate that the latter family, modified for a freshwater existence, is related to the cancrid+corystid assemblage. Some elongation of the centrioles, apparent at least in *Corystes*, may be a further link with potamids in which they are greatly elongated. The coenospermial spermatophores of cancridoids are a notable difference from the cleistospermia of potamids; but the latter is probably an apomorphic modification for fertilization biology.

INTRODUCTION

The Cancridea constitute one of the five major subdivisions of the Brachyura in the system summarized by Warner (1977). The Cancridae were retained with the Corystidae in a superfamily Corystoidea, within the Heterotremata, by Guinot (1978). We here investigate the ultrastructure of the spermatozoa of two cancrids, *Cancer pagurus* Linné, 1758, the type species of the genus *Cancer*, and *Platelistoma nanum* Davie, 1991, and a corystid, *Corystes cassivelaunus* (Pennant, 1777), the type and only species of the genus *Corystes*, for their interest per se and with a view to shedding further light on the rela-

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tionships of the two families with each other and with other heterotremes. *Platepistoma* Rathbun, 1906, was considered a subgenus of *Cancer* Linné, 1758, by Takeda (1977), but Davie (1991) restored it to generic rank.

The spermatozoon of *Cancer pagurus* has been briefly mentioned by Pochon-Masson (1968), and four additional *Cancer* species have been used in a combined account of spermiogenesis (chiefly of *Cancer borealis*) by Langreth (Langreth, 1965; Langreth, 1969). Tudge et al. (1994) and Tudge & Justine (1994) briefly described the ultrastructure of the sperm of *C. pagurus* in the course of immunofluorescence studies of actin and tubulin distribution in this cell.

MATERIALS AND METHODS

The collections

The specimen of *Corystes cassivelaunus* was collected by C. d'Udekem d'Acoz from coastal waters near Dinard, Brittany, France on April 8, 1993; *Platepistoma nanum* Davie, 1991, was collected by Dr B. Richer de Forges during the SMIB 8 Cruise on the R.V. "Alis" off New Caledonia between the 26th January and 3rd February 1993 and the mature male specimen of *Cancer pagurus* was purchased live from markets in Paris, France, in September 1992 by Dr J.-L. Justine.

Histological procedures

The male reproductive material (usually both testes including the ducts of the vasa deferentia) was removed from fresh crab specimens and immediately fixed in cold glutaraldehyde (see below), then posted to Brisbane at ambient temperature where the remaining fixation and embedding procedures for transmission electron microscopy were performed.

The gonad tissue was processed in the Zoology Department, The University of Queensland, by the standard fixation procedure (outlined below) for transmission electron microscopy. This was carried out in a Lynx -el. Microscopy Tissue Processor (Australian Biomedical Corporation, Ltd., Mount Waverley, Victoria, Australia), after the initial glutaraldehyde fixation and first phosphate buffer rinse.

Portions of the testis (approximately 1 mm³) were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2), for a minimum of 2 h at 4 °C. They were rinsed in phosphate buffer (3 rinses, each 15 min), postfixated in phosphate buffered 1% osmium tetroxide for 80 min; similarly rinsed 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 (500–800 Å thick) were cut on a LKB 2128 UM IV microtome with a diamond knife. Sections were placed on carbon-stabilized collodion-coated 200 µm mesh copper grids and stained (according to Daddow, 1986) in Reynold's lead citrate for 30 sec, rinsed in distilled water, then 6% aqueous uranyl acetate for 1 min, rinsed, lead citrate again for 30 sec, and a final rinse in distilled water. Micrographs were taken on an Hitachi H-300 transmission electron microscope at 80 kV and a JEOL 100-S transmission electron microscope at 60 kV.

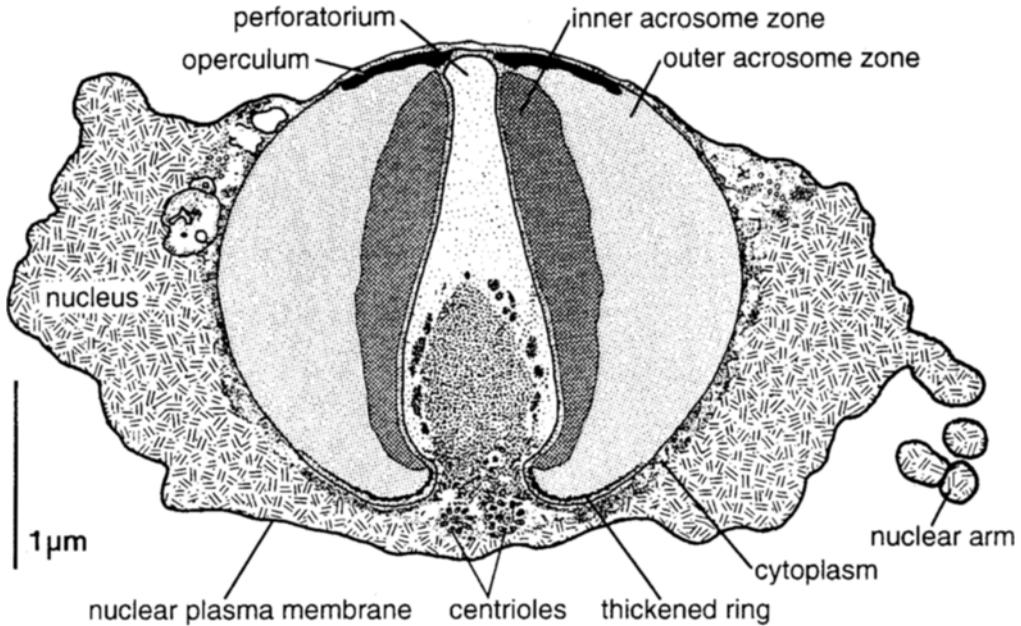


Fig. 1. *Corystes cassivelaunus*. Semidiagrammatic representation of the longitudinal section of mature spermatozoa traced from a micrograph

RESULTS

For a comparative account and explanation of the various components of the brachyuran spermatozoon see Jamieson (1991a, 1991b, 1994) and the "Discussion". The latter paper contains a diagram of these components.

General morphology

The spermatozoa of the corystid *Corystes cassivelaunus*, and the two cancrids, *Cancer pagurus* and *Platypistoma nanum*, are sufficiently similar to be described together and to be referred to a corystoid type. The spermatozoon of *Corystes cassivelaunus* is illustrated from transmission electron microscopy in a line drawing (Fig. 1) and in micrographs (Figs 2A–C, 3A–C). The spermatozoon of the cancrids is illustrated from transmission electron microscopy for *Platypistoma nanum* (Fig. 4A) and *Cancer pagurus* (Fig. 4B).

Each of the many spermatophores in the testes of all three species contains several to many spermatozoa. As such the spermatophores constitute coenospermia. The spermatozoa (Figs 1–4) are typically brachyuran in gross morphology. An acrosome vesicle forms most of the volume of the spermatozoon. The acrosome is concentrically zoned but lacks the concentric lamellation seen in thoracotremes; it is capped apically by a dense operculum and is ensheathed in a very thin layer of cytoplasm which in turn is embedded in the nucleus. The acrosome vesicle is centrally penetrated by a cylindrical perfo-

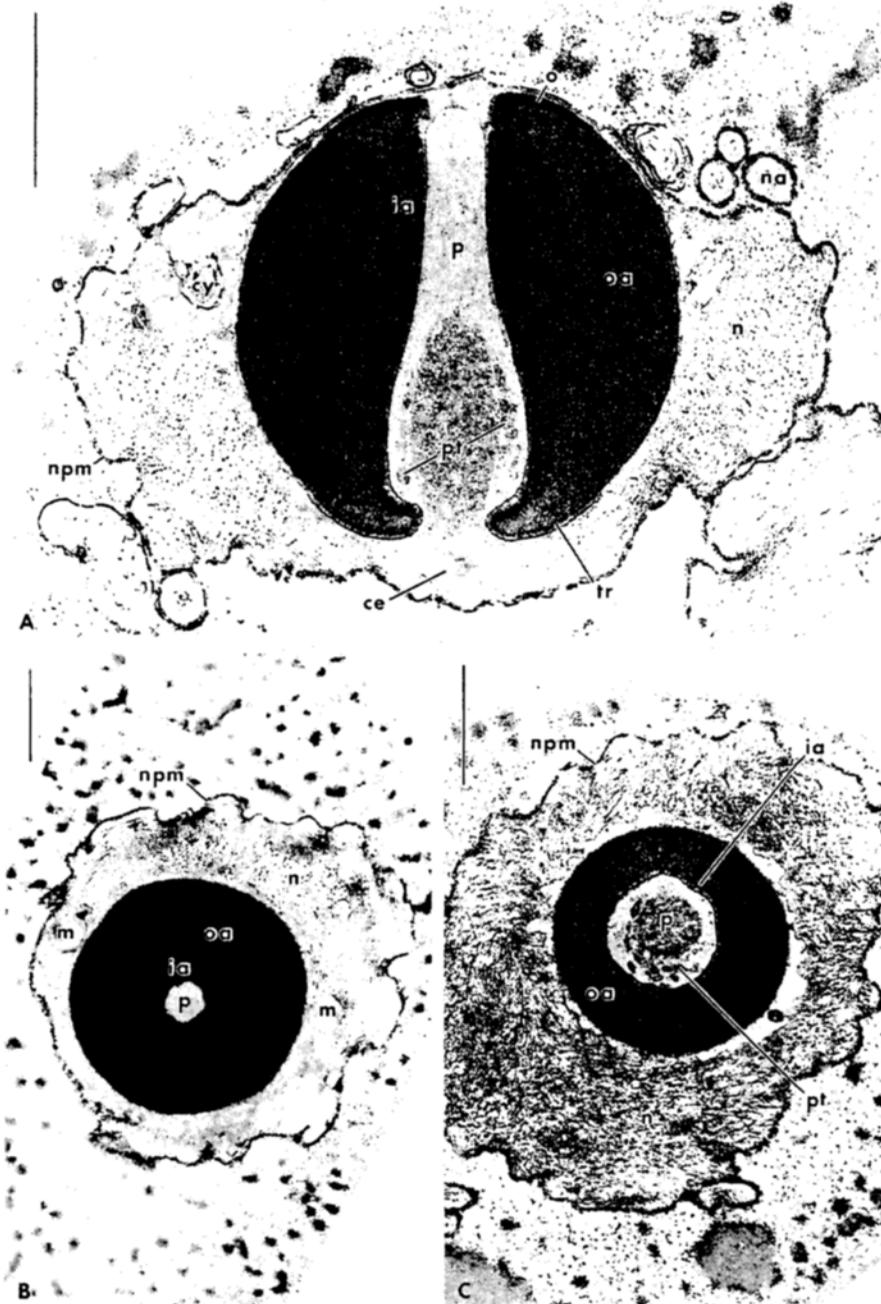


Fig. 2. *Corystes cassivelaunus*. Transmission electron micrographs. A: Sagittal longitudinal section of a spermatozoon. B: Transverse section (TS) above the equator of the acrosome. C: TS through the basal bulb of the perforatorium. Scale bars = 1 μm . ce – centriole; cy – cytoplasm; ia – inner acrosome zone; m – degenerating mitochondrion; ms – membranous structure; n – nucleus; na – nuclear arms; npm – nuclear-plasma membrane; o – operculum; oa – outer acrosome zone; p – perforatorium; pt – perforatorial tubules; tr – thickened ring

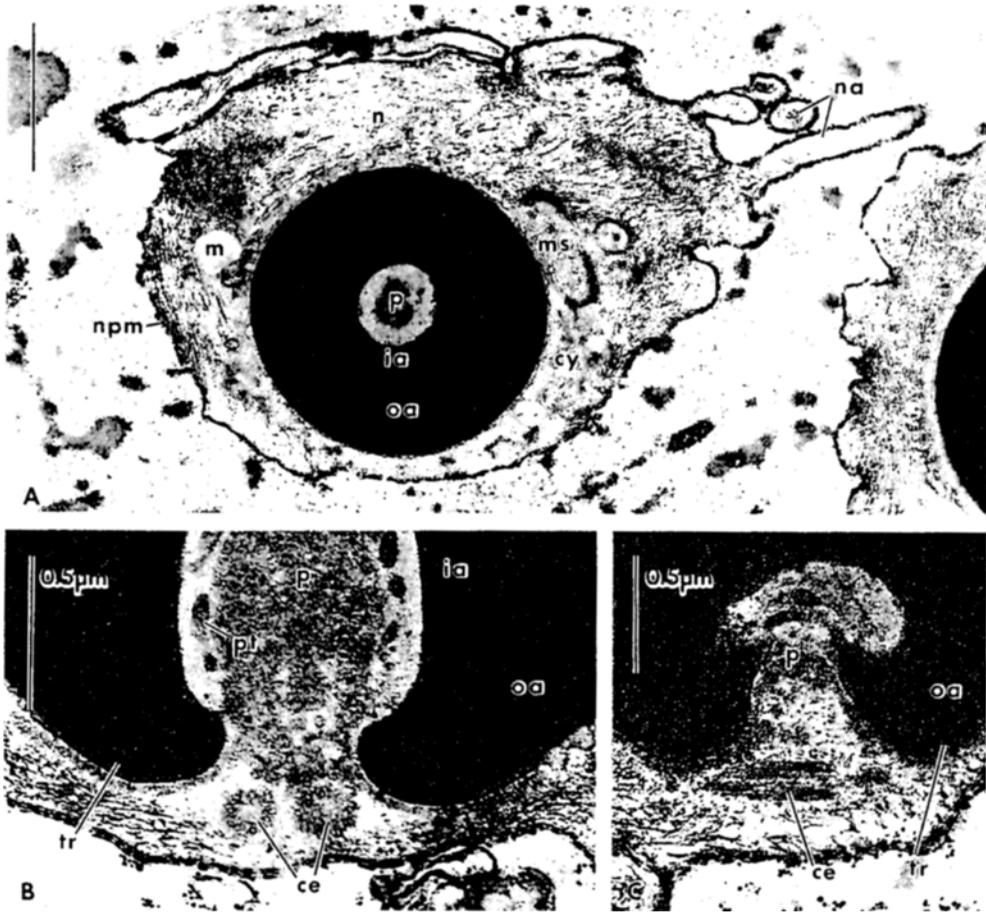


Fig. 3. *Corystes cassivelaunus*. Transmission electron micrographs. A: TS approximately through the equator of the spermatozoon. B: Sagittal longitudinal section through the base of the perforatorial chamber. C: Slightly oblique section through the same region. Scale bar = 1 μ m unless stated otherwise. Abbreviations as for Fig. 2

ratorial column, in these species being unusual in extending anteriorly almost to the plasma membrane. The nuclear material forms several marginal projections or 'arms'. The spherical, or only slightly depressed, form of the acrosome is typical of the Eubranchyura (Heterotremata + Thoracotremata).

A chromatin-containing 'posterior median process' of the nucleus, seen in homolids, *Ranina* and some majids is absent. The nucleus consists of uncondensed, fibrous chromatin, and forms a cup around the acrosome as in all other investigated eubranchyuran sperm. A thin layer of cytoplasm which intervenes between nucleus and acrosome, as in other brachyurans, forms a small mass containing the centrioles (not definitely identified in *Platypistoma nanum*) projecting at the posterior end of the perforatorial chamber. Cyto-

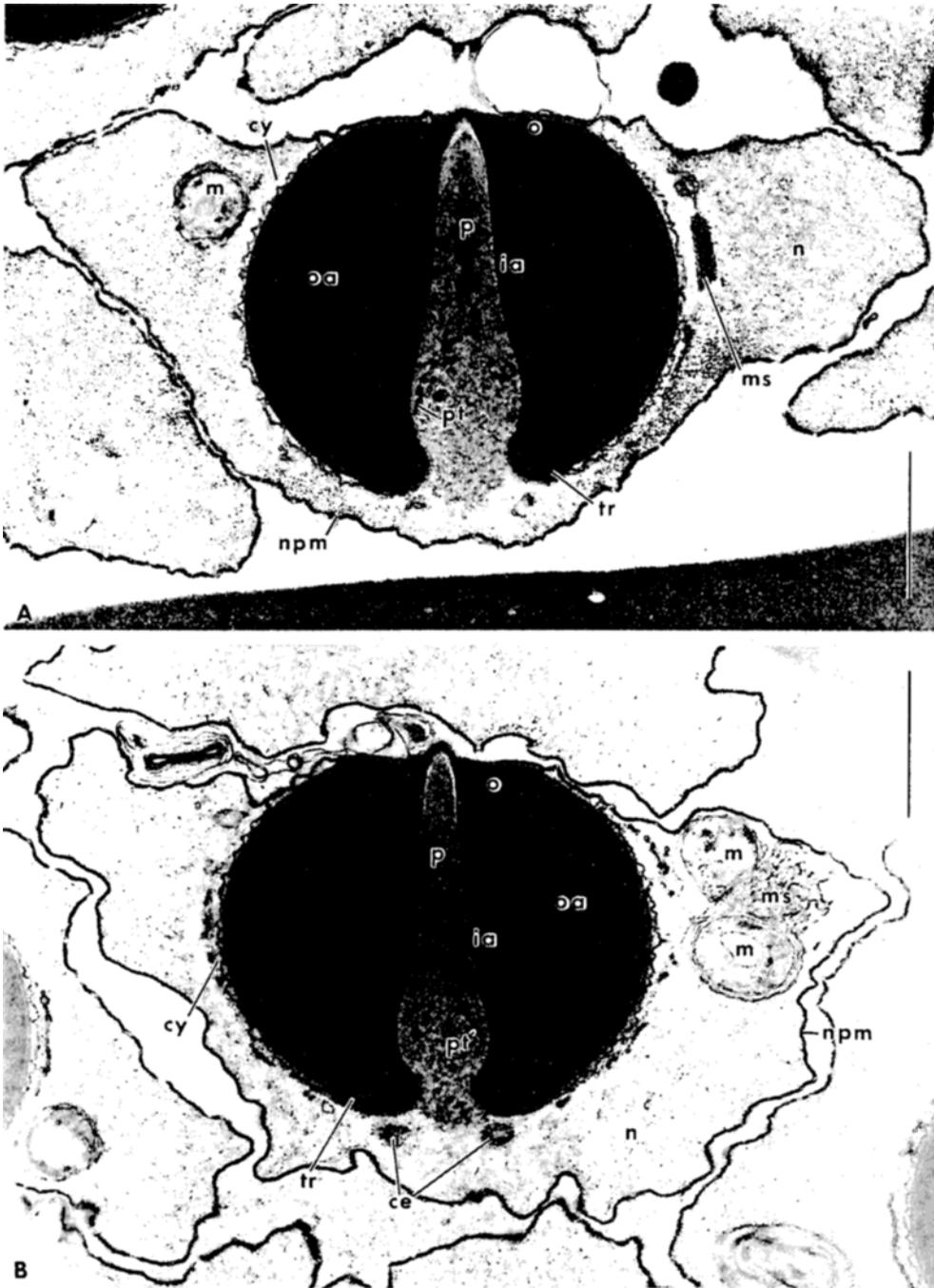


Fig. 4. Transmission electron micrographs of sagittal longitudinal sections through a spermatozoon. A: *Platepistoma nanum*. B: *Cancer pagurus*. Scale bars = 1 μ m. Abbreviations as for Fig. 2

plasmic islets are present lateral to the acrosome and embedded in the chromatin (Figs 1, 2A, 3A, 4A, B); they contain lamellae and bodies identifiable by homology with other crabs as degenerating mitochondria, although no cristae have been observed.

A combination of characters, not individually unique, defines the crystoid-type of spermatozoon possessed by these three species: the basally bulbous, anteriorly narrowing perforatorium, the extent of this almost to the plasma membrane through a widely perforate operculum, and the simple inner acrosome zone, lacking an acrosome ray zone.

Acrosome

The subspheroidal core of the spermatozoon consists entirely of the concentrically zoned acrosome which is capped by, and includes, the opercular complex (Figs 1, 2A–C, 3A, 4A, B). The acrosome is invested by an acrosomal membrane underlain by a moderately electron-dense sheath, the 'capsule' (Figs 2A, 3A, 4A, B). The mean length of the acrosome, from the apex of the operculum to the base of the capsule, in *Corystes cassivelaunus*, is 2.38 μm (SD = 0.13, n = 6); the mean width is 2.63 μm (SD = 0.16, n = 6). In *Platelistoma nanum*, the mean length is 2.90 μm (SD = 0.22, n = 7), with a mean width of 3.41 μm (SD = 0.27, n = 7). In *Cancer pagurus*, the mean length is 2.93 μm (SD = 0.40, n = 8), with a mean width of 3.17 μm (SD = 0.29, n = 8). The acrosomal membrane is separated by a very thin, hyaline layer from the capsule and, like the capsule, is invaginated to cover an elongate subacrosomal or perforatorial chamber, the contents of which are the perforatorium (Figs 1, 2A–C, 3A–C, 4A, B). The anterior tip of this chamber extends closer to the apex of the sperm than is usual in Eubrachyura. It is covered anteriorly only by the apposed acrosome and plasma membrane in *Platelistoma nanum* (Fig. 4A) and *Cancer pagurus* (Fig. 4B), in both of which its anterior end is sharply pointed. In *Corystes cassivelaunus* (Figs 1, 2A), its blunt anterior end is separated from the anterior pole of the spermatozoon only by a small amount of hyaline, presumably acrosomal, material. In all three the perforatorium is wide or (cancriids) bulbous posteriorly. In the cancriids it has a very pronounced anterior taper, being obelisk-like in longitudinal section anterior to the bulbous region (Fig. 4A, B). In *C. cassivelaunus* it is only slightly tapered anterior to the basal bulb (Fig. 2A). The perforatorium, or the contents of the perforatorial chamber, in *C. cassivelaunus* (Figs 1, 2A, C, 3A, B), have a dense core surrounded by a few tubule like structures in the wider basal portion, and are pale and fairly uniform further anteriorly. In *P. nanum* (Fig. 4A) and *C. pagurus* (Fig. 4B), the contents of the perforatorial chamber are of more even consistency, with a few scattered tubules. The central, subacrosomal axis of the acrosome formed by the perforatorial chamber is surrounded by a moderately electron-dense layer, the inner acrosome zone (Figs 1, 2A–C, 3A, B, 4A, B) which extends from the operculum at the anterior end of the acrosome almost to the posterior end of the acrosome, reaching the thickened ring. The inner acrosome zone is slightly convex outwardly in *C. cassivelaunus* (Fig. 2A) and *P. nanum* (Fig. 4A), but is narrowest shortly anterior to its bulbous base in *C. pagurus* (Fig. 4B). There is no xanthid ring or modification of this. No acrosome ray zone, typical of heterotreme sperm, is recognizable (an absence also noted in potamid sperm, see "Discussion").

An outer acrosome zone (Figs 2A–C, 3A, B, 4A, B) surrounds the inner acrosome zone and the base of the perforatorial chamber, being several times wider than the inner zone. This outer zone extends to the convex margin of the acrosome, being bounded by the cap-

sule. It is uniform in structure and moderately electron-dense, though paler than the inner acrosome zone, and, like other heterotreme sperm, does not display the concentric lamellae which are characteristic of thoracotreme sperm.

At the anterior pole of the acrosome, as in all other brachyurans and in paguroids, there is a dense caplike structure, the operculum (Figs 1, 2A, 4A, B). It is unusual for heterotremes (see "Discussion") in being perforate, having a wide central orifice (Figs 1, 2A, 4A, B). The operculum has a mean width of 1.47 μm (SD = 0.10, n = 6) in *Corystes cassivelaunus*, 1.81 μm (SD = 0.15, n = 7) in *Platepistoma nanum* and 1.51 μm (SD = 0.16, n = 9) in *Cancer pagurus*. The operculum is also unusual in consisting of only a single electron-dense layer (Figs 1, 2A, 4A, B), whereas in many heterotremes it has an underlying less dense layer, often termed the subopercular zone. It is fairly thin in *C. cassivelaunus* but is thick in *Platepistoma nanum* and especially in *Cancer pagurus*. Unlike thoracotremes, there is no apical button.

- No accessory ring, present lateral to the operculum in xanthoids and, differently orientated, in thoracotremes, is present nor is there an 'opercular overhang'. There is no trace of a periopercular rim in any of the three species.

At the opposite, posterior, pole of the acrosome (Figs 1, 2A, 3B, C, 4A, B) the capsule is interrupted, as in all brachyurans, by invagination of the acrosome membrane and capsule as an orifice which opens into the columnar subacrosomal chamber. A 'thickened ring' which is visible on each side of the subacrosomal invagination in most heterotremes and many thoracotremes (see "Discussion") is moderately well developed (Figs 1, 2A, 3B, C, 4A, B). In *P. nanum* it is rather thick and is approximately semicircular in longitudinal section (Fig. 4A). In *C. cassivelaunus* (Figs 1, 2A, 3B), in which it is beaded (Fig. 2A) and *Cancer pagurus* (Fig. 4B) it is, however, somewhat compressed antero-posteriorly, as is the region of invagination, and it is more extensive on the posterior, peripheral aspect than where it skirts the base of the subacrosomal chamber.

Cytoplasm

The cytoplasm in the sperm of the three species forms a thin layer, of irregular thickness, ensheathing the whole of the acrosome excepting its opercular region (Figs 1, 2A-C, 3A, 4A, B). In places it expands, sometimes far, into the nuclear material as islets (sometimes detached?) which contain putative mitochondrial remnants and groups of membranes (Figs 2A, B, 3A, 4A, B). The periacrosomal cytoplasm is continuous with a mass lying at the posterior pole of the perforatorial chamber and the material within the posterior perforatorial chamber may also be regarded as cytoplasm. No cytoplasm extends into the nuclear arms.

Centrioles

The basal cytoplasm in the perforatorial chamber contains two centrioles in *C. cassivelaunus* (Fig. 3B); these are seen each to consist of doublets and to lie approximately parallel to each other and to be somewhat more elongate than is typical of centrioles (Fig. 3C). Two centrioles are confirmed for *C. pagurus* (Fig. 4B). They have not been demonstrated in *P. nanum* but there seems no reason to doubt their presence.

Nucleus

In the three species, as in other brachyurans, the nuclear material is located in the lateral arms and in the cup-shaped structure around both the acrosome and its cytoplasmic sheath; there is a smaller amount of nuclear material posterior to the acrosome than in some other heterotremes (Figs 1, 2A, 3B, 4A, B). The nuclear membrane around the acrosome, and its cytoplasmic sheath, is unusually indistinct, and separation of the nuclear material from the cytoplasm is, indeed, often indiscernible (Figs 1, 2 A, B, 3A–C, 4A, B). In *Cancer pagurus* short arcs, seen in vertical section of the sperm, of small dark vesicles (Fig. 4B) represent the disrupted nuclear envelope but there is less indication of the envelope in the other species. There is no hexalaminar or multilaminar nuclear membrane as seen in some heterotremes (for instance, in dorippids, and trapeziids). Doubtless the conservation of the membrane varies dynamically in the corystoid-type sperm.

The external surface of the cell is bounded by a moderately dense or (*C. cassivelaunus*) dense membrane which may represent fused nuclear and plasma membranes, the nuclear plasma membrane (Figs 1, 2A–C, 3A–C, 4A, B). The general chromatin consists of a diffuse network of electron-dense filaments in a pale matrix as in other brachyurans.

In *C. cassivelaunus*, the arms, which are clearly numerous, can be seen encircling the nucleus for a considerable distance or projecting directly from it (Fig. 3A).

DISCUSSION

We have considered, in this study, the spermatozoa of the corystid, *Corystes cassivelaunus*, with those of the two cancrids *Cancer pagurus* (the type-species) and *Platepistoma nanum* because of their general similarity. A combination of characters, not individually unique, possessed by these three species defines what we have termed a corystoid-type of spermatozoon: the basally bulbous, anteriorly narrowing perforatorium, the extent of this almost to the plasma membrane through a widely perforate operculum, and the simple inner acrosome zone, lacking an acrosome ray zone. The sperm of the two cancrids are closely similar, that of the corystid differing, for instance, in the less pointed, and less tapered, form of the perforatorium. This relative uniformity of spermatozoal ultrastructure in the cancrid+corystid assemblage so far investigated supports retention of the two families in the superfamily Corystoidea by Guinot (1978).

All three species have the unusual condition, for heterotremes, of perforation of the operculum and in this respect resemble majid sperm. The operculum is an imperforate cap in other heterotremes, although a small opercular perforation is questionably present in the bythograeid *Austinograea alayseae* (Jamieson et al., unpubl.). Perforation is also general for podotremes, excepting the cyclodorippoid *Cymonomus*. In most investigated thoracotremes a narrow orifice at its tip is plugged by an apical button, though it is imperforate and lacks the button in *Macrophthalmus crassipes* (see Jamieson, 1994; Jamieson et al., 1995).

If the perforate condition of the operculum of the corystoid-type spermatozoon were regarded as plesiomorphic, the cancrids and corystids could reasonably be deduced to be plesiomorphic crabs as no other features of the sperm appear particularly advanced. However, in a cladistic analysis (Jamieson, 1994), perforation of the operculum appeared to be a synapomorphy of the Podotremata independently developed in majids. It cannot

therefore be said with any certainty, at least pending cladistic analysis, that spermatozoal ultrastructure places cancrids and corystids near the base of the Heterotremata, nor is this refuted. Some indications of relationships of the two families relative to other genera can tentatively be recognized, however. First, they appear to be closely related to each other in spermatozoal and general morphology. Second, the absence of a xanthid ring gives no reason for associating them with at least that section of the Xanthoidea represented by the Xanthidae or Panopeidae (Jamieson, 1991b; Jamieson, 1994; Jamieson et al., 1995). Furthermore, the combination of perforation of the operculum and absence of an acrosome ray zone (at least in a clearly recognizable form) are features of the Potamidae sensu lato. These were demonstrated in two species of *Potamon*, *P. fluviatile* and *P. ibericum* by Guinot et al. (1997), though the operculum is imperforate in *Potamonautes perliatus sidneyi* (see Jamieson, 1993), and conceivably indicate that the Potamidae, modified for a freshwater existence, are related to the cancrid+corystid assemblage. Some elongation of the centrioles, apparent at least in *Corystes*, may be a further link with potamids, in which they are very elongate. The coenospermial spermatophores of corystoids are a notable difference from the cleistospermia of potamids but the latter is probably an apomorphic modification for fertilization biology involving lecithotrophic eggs (Guinot et al., 1997; Jamieson, 1993).

With regard to previous accounts of cancrid sperm (Langreth, 1965, 1969; Pochon-Masson, 1968; Tudge & Justine, 1994; Tudge et al., 1994), these are largely confirmed by the present account. In the mature sperm of *C. borealis* illustrated by Langreth (1965), the large, dense operculum is craterlike and centrally perforate but Jamieson (1991b), summarizing Langreth's work, considered that, as the pointed tip of the perforatorium protrudes through it, perforation of the operculum might indicate that the acrosome reaction has commenced. A similar condition is here shown for *Cancer pagurus*, and, as normal fixation and processing were carried out in the present study (see also Tudge & Justine, 1994; Tudge et al., 1994, for the same material), it now seems unlikely that an acrosome reaction had commenced. The slightly protuberant perforatorium may therefore be a normal condition of cancrid sperm. Nevertheless, penetration of the 'cap' (operculum) appears to occur only at maturity (Langreth, 1965) and presumably indicates readiness for reaction. Tudge et al. (1994) observed two concentric rings of actin fluorescence which they considered to coincide with the concentric zonation of the acrosome contents. Actin became evident in the perforatorium only when the acrosome reaction took place but was then chiefly located in the nucleocytoplasm immediately subjacent to the perforatorial column and in an apical perforatorial projection. Correlating with the absence of microtubules in the arms, Tudge & Justine (1994) detected no alpha or beta tubulin in the sperm of *C. pagurus*. However, they showed that the antibody anti-tubulin 16D3 produced some weak fluorescence.

Other features of the *Cancer* sperm demonstrated by Langreth (1965) and reviewed by Jamieson (1991b), using current terminology, were an inner dense zone differentiated externally as an acrosome ray zone (presence of the acrosome ray zone was not confirmed in the present work); a large, electron-lucent, outer acrosome zone; a conspicuous thickened ring in continuity with the thinner capsule; presence of DNA throughout the length of the rather short arms; absence of a posterior median process; and a shape of the perforatorial column, similar to that reported here, widest at its posterior fourth and tapering almost straight to a pointed tip.

Tudge et al. (1994) recognized a very narrow peripheral acrosome zone, in addition to the inner and outer acrosome zones. However, although a pale layer has been observed under the capsule in the present examination it is continuous with the thickened ring and is not here distinguished as a peripheral acrosome zone comparable with the wide layer seen, for instance, in xanthid sperm (Jamieson, 1989, 1991a, b).

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