

The spermatozoon of *Calocarcinus africanus* (Heterotremata, Brachyura, Crustacea): ultrastructural synapomorphies with xanthid sperm

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Summary

Features of the spermatozoon of *Calocarcinus africanus* which are general for heterotremes and endorse its inclusion in the Heterotremata are: extension of the subacrosomal chamber almost to the anterior apex of the sperm, presence of an acrosome ray zone, and presence of a thickened ring where the capsule surrounds the base of the subacrosomal chamber. A feature shared with "higher" heterotremes is the restriction of cytoplasm to the periacrosomal region, the arms being nuclear only, in contrast with invasion of their chromatin with cytoplasm and microtubules in majids; and loss of a posterior median process, containing chromatin, which is present in majids as in raninids and homolids. The relationship of *Calocarcinus* with xanthids is unequivocally supported by (1) presence of a posterior circumperforatorial zone, the xanthid ring; (2) the precise form of the acrosome ray zone which is wide anteriorly and sends a long slender extension posteriorly to meet the ring; (3) the irregular external margin of the outer acrosome zone; (4) division of the operculum complex into a distinct upper zone and a lower, subopercular zone of lesser diameter; and (5) presence of an accessory ring around the operculum. Modification of the xanthid ring is typical of thoracotremes and partial modification in *Calocarcinus* may indicate origin of thoracotremes from a related xanthoid stock. A difference of *Calocarcinus* sperm from those of xanthids is the (plesiomorphic) presence of centrioles, also seen in some heterotremes and thoracotremes. No synapomorphies which are not common to other heterotremes are shared between *Calocarcinus* and trapeziid sperm. Trapeziid sperm (plesiomorphically?) lack the xanthid ring, the posterior extension of the acrosome ray zone and the irregular margin of the outer acrosome zone of *Calocarcinus* and xanthids. Apomorphic features of the *Calocarcinus africanus* sperm include a spiral configuration of the contents of the outer acrosome zone (autapomorphy?), as seen in cross-section, and presence of a periopercular rim. A well developed periopercular rim is known elsewhere only in *Potamonautes* (family Potamidae), but a rudiment occurs in some xanthids (e.g., *Etisus*). The periopercular rim is probably a true synapomorphy indicative of relationship of potamids to xanthoids (represented by *Calocarcinus*) which has been postulated elsewhere on morphological grounds.

Key words: Crustacea, phylogeny, spermatozoa, ultrastructure, *Calocarcinus*

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Introduction

Calocarcinus Calman, 1909, of which the type-species is *C. africanus*, is a rare genus which was monotypic until the addition of *C. habei* Takeda, 1980. Two further species have been included: *C. lewinsohni* Takeda and Galil, 1980, and *C. crosnieri* Galil and Clark, 1990. *Calocarcinus* was placed in the Xanthidae, close to *Trapezia* in the Trapeziinae by Balss (1957) or in the Trapeziidae by Guinot (1978). Takeda and Galil (1980) noted no major differences between *Calocarcinus* and other Trapeziidae. *Calocarcinus* appears to be the only putative trapeziid to occur in deep water (70–1100 m) but like most members of the family is associated with corals. A significant morphological difference of *Calocarcinus* from other trapeziids appears to be that the first and second sexual male pleopod are of equal length, whereas in *Trapezia* pleopod 2 is much shorter than pleopod 1, though it must be noted that the condition of the pleopods is not known in some trapeziids. Guinot (e.g., 1978) has argued that the proportions of pleopods 1 and 2 in the male are of evolutionary significance and considers that a shorter second pleopod, as in *Trapezia*, is plesiomorphic relative to the equal condition.

The ultrastructure of the spermatozoon has been shown to be of value in determining relationships within the Brachyura (see review by Jamieson, 1991). The Xanthidae are diagnosed spermatologically by a distinctive "xanthid ring" basal around the perforatorium. This structure is absent in the Trapeziidae which are considered basal to the xanthid-panopeid assemblage (Jamieson, 1992b). The present study examines the ultrastructure of the spermatozoon of *Calocarcinus africanus* for its interest per se and with a view to providing evidence of the relationships of the genus within the heterotreme-thoracotreme assemblage (see Guinot, 1977, 1978) and particularly with regard to trapeziids and xanthids.

Materials and Methods

Specimens of *C. africanus* were collected by B. Richer de Forges during the BERYX 11 cruise on the R.V. "Alis", south of New Caledonia, on the guyots of the Norfolk Ridge (13–23 October 1992). Portions of the testes and male ducts were fixed in 3% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) with 3% sucrose at 4°C for 2 h and dispatched in the ivate to Brisbane for further processing. On receipt they were washed in buffer, post-fixed for 80 min in

similarly buffered 1% osmium tetroxide, washed in three 15 min changes of buffer, dehydrated through an ethanol series, and infiltrated and embedded in Spurr's epoxy resin. Sections were cut with diamond knives on an LKB 2128 UM IV microtome. Thin sections, 50–80 nm thick, were collected on carbon stabilized collodion-coated 200 mesh copper grids, rinsed in distilled water, stained for 30 s in Reynold's lead citrate, 1 min in 6% aqueous uranyl acetate and for a further 30 s in lead citrate before final rinsing. Electron micrographs were taken on an Hitachi 300 electron microscope at 75 kV and a JEOL 100 at 60 kV.

Results

The nomenclature for the various components of the brachyuran spermatozoon used in the account below follows Jamieson (1991).

General morphology

The spermatozoon of *C. africanus* is illustrated from transmission electron microscopy in a line drawing (Fig. 1) and in micrographs (Figs. 2A–G).

Each of the many spermatophores in the testes of *C. africanus* typically contains several to many spermatozoa. The spermatozoon (refer to line drawing, Fig. 1, throughout) is spheroidal but slightly depressed anteroposteriorly and, like all decapod sperm, lacks a flagellum (Fig. 2A). This simple form is modified by the presence of broadly based lateral projections or "arms". A plesiomorphic, chromatin-containing "posterior median process," seen in homolids, *Ranina* and some majids is absent. The nucleus consists of uncondensed, fibrous chromatin and forms a cup surrounding the acrosome as in all other brachyurans. The thin layer of cytoplasm intervening between nucleus and acrosome in most brachyurans is here virtually restricted to a small mass further posteriorly and islets lying lateral to the acrosome and embedded in the chromatin. These islets consist of membranous lamellae and subspheroidal bodies which are in places clearly attributable to degeneration of mitochondria (Figs. 2A–D).

Acrosome

The subspheroidal core of the *C. africanus* spermatozoon consists entirely of the concentrically zoned acrosome which is capped by, and includes, the oper-

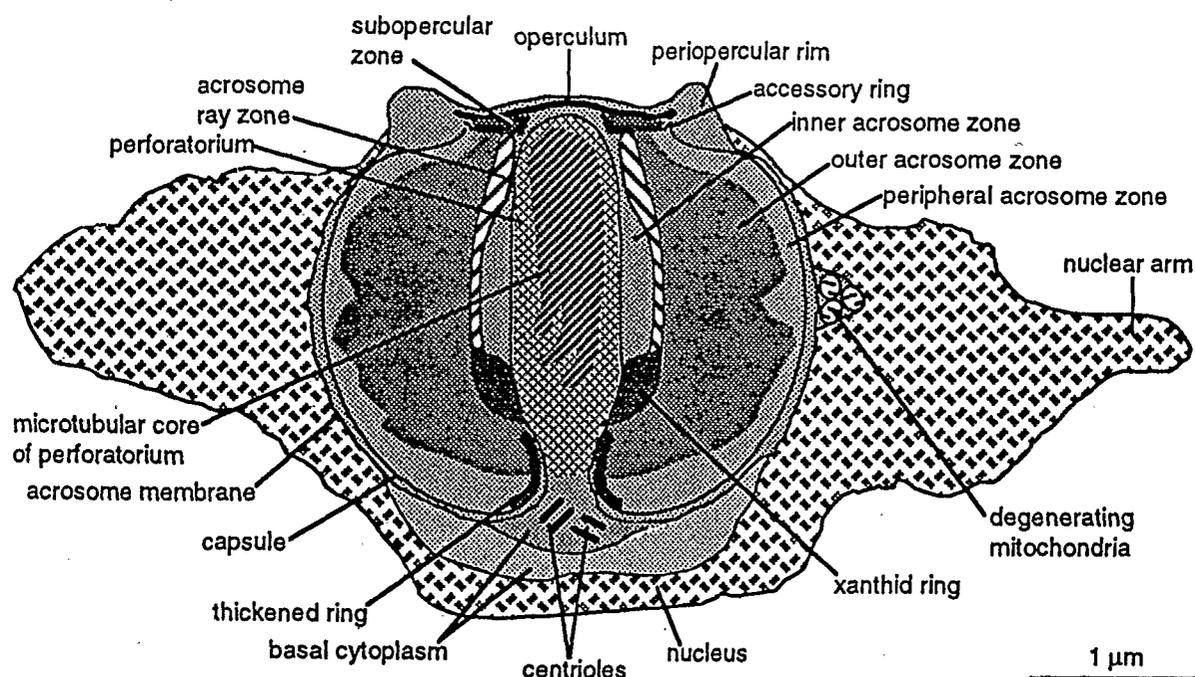


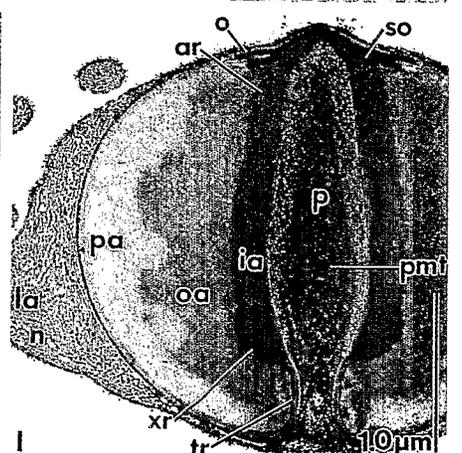
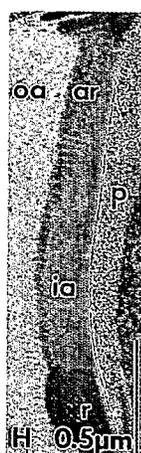
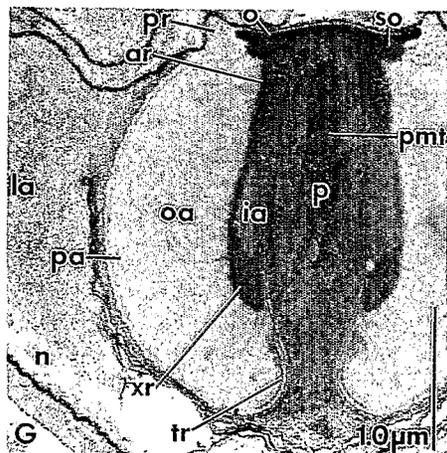
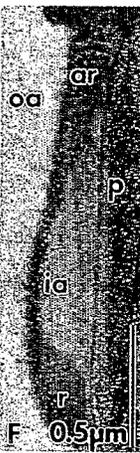
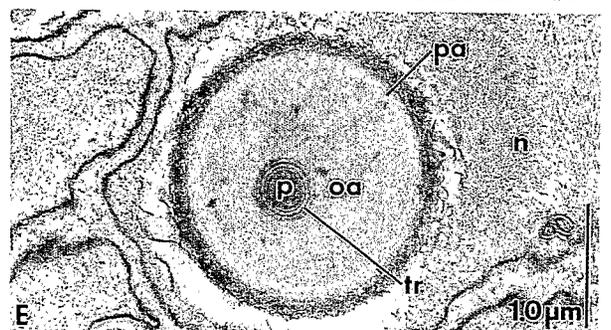
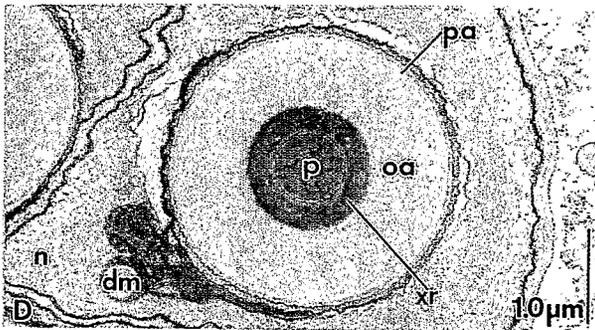
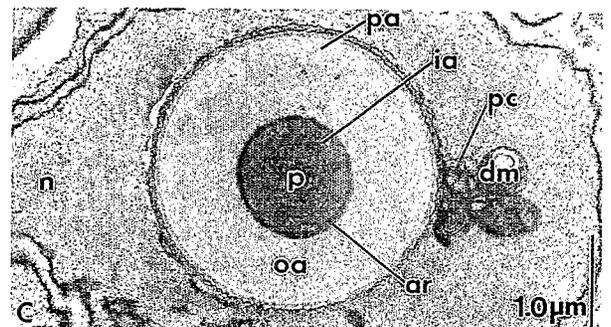
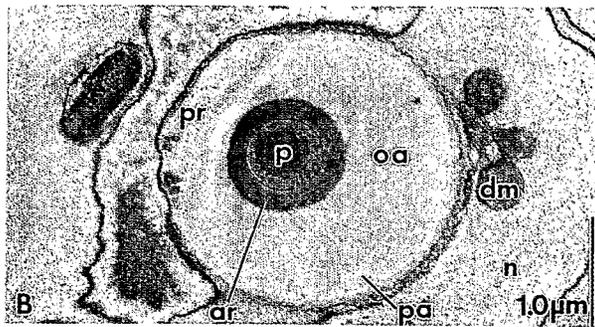
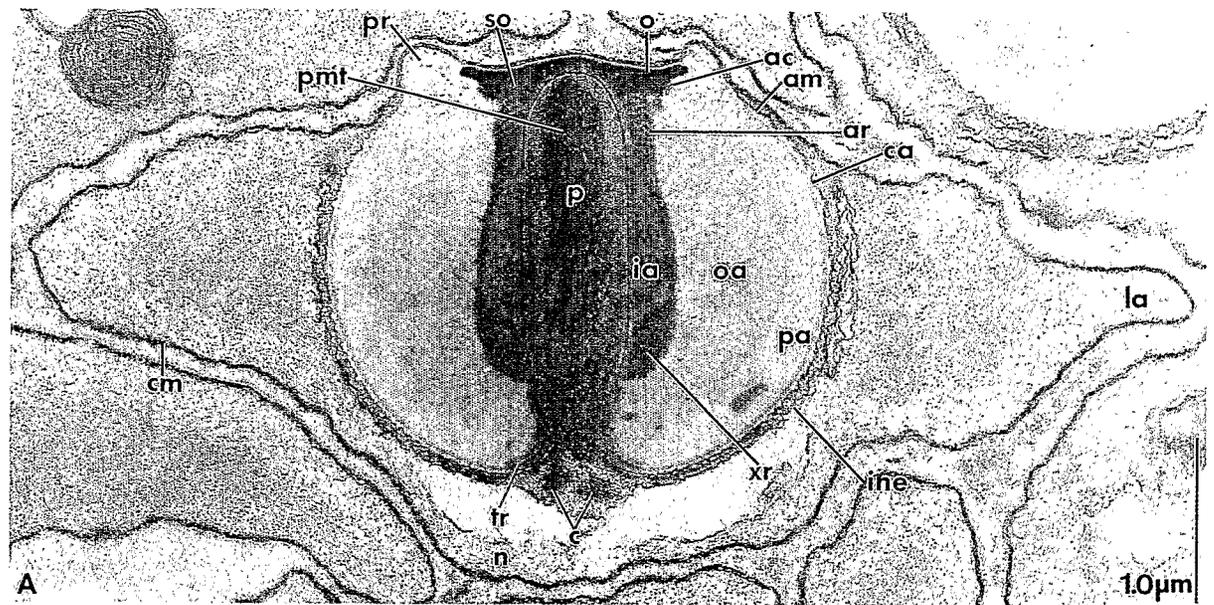
Fig. 1. *Calocarcinus africanus*. Diagrammatic representation of a longitudinal sagittal section of the spermatozoon.

cular complex (Figs. 2A and 2G). The acrosome is invested by an acrosomal membrane underlain by a moderately electron dense sheath, the "capsule" (Fig. 2A). The length of the acrosome, from the apex of the operculum to the base of the capsule, is 2.37–2.75 μm (mean of 7 = 2.56 μm); the width is 2.98–3.21 μm (mean of 7 = 3.03 μm). The acrosomal membrane is separated by a very thin hyaline layer from the capsule and, like the capsule, is invaginated to cover a columnar subacrosomal chamber, the contents of which are the perforatorium (Figs. 2A–G). The anterior tip of this chamber extends almost to the anterior apex of the sperm. This central subacrosomal axis of the acrosome is surrounded by a moderately electron dense layer, the inner acrosome zone (Figs. 2A, C, F and G).

The inner acrosome zone is surrounded by a layer which consists of distinct but continuous anterior and posterior regions (Figs. 2A, B, C, F and G). The anterior region has a clearly defined substructure which identifies it as the acrosome ray zone typical of brachyuran and some anomuran sperm. The acrosome ray zone is widest adjacent to the anterior fifth of the perforatorial chamber which it almost abuts, thus almost occluding the inner acrosome zone in this region. Further posteriorly the acrosome ray zone narrows greatly and is separated from the perforatorial chamber by the full width of the inner acrosome

zone. Shortly behind the equator of the acrosome, the ray zone gives way to a short electron-dense periperforatorial zone, the xanthid ring, which curves medianly to almost abut the wall of the perforatorial chamber from which it is separated by only a very thin posterior extension of the inner acrosome zone (Figs. 2A, D, F and G). A thin outer layer of this dense zone (Fig. 2F) shows some resemblance to the acrosome ray zone.

The central core of the acrosome formed by the perforatorial chamber and its investing inner acrosome zone and acrosome ray zone is surrounded by a wide weakly electron dense layer, the outer acrosome zone. For about the posterior fourth of the acrosome this zone is almost in contact with the perforatorial chamber from which it is separated by only the invaginated capsule and the thickened ring (see below). The outer acrosome zone does not quite extend to the periphery of the acrosome, but, as in xanthids, is separated from this by an irregular ragged margin outside which is a narrow, paler, homogeneous peripheral acrosome zone. The substructure of the outer acrosome zone is of a type not reported in other brachyurans. It consists, as seen in transverse section of the spermatozoon, a radial striae twisted regularly in a turbine- or vortex-like configuration (Figs. 2B, C, D and E). The putative modified xanthid ring also shows narrow striations twisted in the same sense as



those of the outer acrosome zone but at a more acute angle and not continuous with the striation of the latter (Fig. 2D).

At the anterior pole of the *C. africanus* acrosome, there is a dense caplike structure, the operculum (Figs. 2A and G), which in *Calocarcinus* has a width of 1.27–1.45 μm (mean of 7 = 1.40 μm). It is impermeate and almost flat.

A subopercular zone is not identifiable with certainty. A lower tier of the opercular complex of slightly less diameter than the upper electron dense tier is here arbitrarily regarded as subopercular. A sharply distinguished plaque, as seen in a longitudinal section of the spermatozoon, represents a ring (accessory ring) latero-posterior to the subopercular zone (Figs. 1 and 2A). This separates the putative subopercular zone from the periopercular rim (see below).

At the opposite posterior pole the capsule is perforated by invagination of the acrosome membrane and capsule as a narrow orifice which opens into the columnar subacrosomal chamber. A thickening of the capsule or paralleling, this forms a "thickened ring" on each side of the subacrosomal invagination (Figs. 2A, E and G).

Periopercular rim

A remarkable feature of the spermatozoon of *C. africanus* is an electron pale rim encircling the periphery of the operculum (Figs. 2A, B and B). The rim is an expansion of the hyaline layer, immediately underlying the acrosomal membrane which overlies the capsule. The rim does not extend down the periphery of the concentric acrosome zones for the considerable distance seen in *Potamonautes*.

Subacrosomal region

The subacrosomal material consists of a highly differentiated stout perforatorium which extends from

the posterior perforation in the capsule to the vicinity of the operculum at the apex of the acrosome (Figs. 2A and G). The perforatorium is a stout cylinder, slightly narrowing anteriorly, with a domed tip and a posterior stalk constricted at the thickened ring. In cross-section the perforatorium is seen to contain many circular profiles resembling microtubules. These microtubules are distributed in a cirlet, albeit irregular, around a central core (Figs. 2A, B, C, D, E and G). Radial links (laterally directed microtubules) extend from the cirlet to the periphery of the perforatorium (Fig. 2D).

Cytoplasm

The thin layer of cytoplasm intervening between nucleus and acrosome in most brachyurans is virtually restricted in *Calocarcinus* to a small mass posterior to the perforatorial axis of the acrosome and containing the two centrioles, a structureless mass behind this, and islets containing lamellae and degenerating mitochondria, which lie lateral to the acrosome and are embedded in the chromatin (Figs. 2A–D). The structureless, hyaline, putatively cytoplasmic mass behind the centriolar enclave is surrounded by multiple membranes (Figs. 2A and G). No cytoplasm extends into the arms.

Nucleus

The nuclear material is located in the lateral arms and in the cup-shaped structure around both the acrosome and its — here negligible — cytoplasmic sheath (Figs. 2A, B, C, D, E and G). The chromatin is separated from the operculum by the periopercular rim (Fig. 2A and G). The envelope between the chromatin and the basal (here hyaline) cytoplasm is seen, in places at least, to consist of several loosely associated membranes (Fig. 2A).

The external basal surface of the cell is bounded by a dense membrane which may represent fused nuclear and plasma membranes, here termed the "cell membrane" (Fig. 2A). The general chromatin consists of electron dense filaments in a pale matrix as in other brachyurans (see Discussion).

Discussion

Some comparison of the sperm of *C. africanus* with those of other brachyurans and of anomurans is appropriate. The operculum is present at the anterior

Fig. 2. *Calocarcinus africanus*. Transmission electron micrographs of the spermatozoon. A, longitudinal sagittal section of spermatozoon; B, oblique TS passing through the periopercular rim and acrosome ray zone; C, TS through the equatorial line; D, TS through the xanthid ring; E, TS through the thickened ring; F, detail of longitudinal section through acrosome ray zone and xanthid ring. Note close similarity to configuration in the xanthid *Atergatis floridus* shown in H; G, longitudinal section of the acrosome showing setting of F and for comparison with *Atergatis floridus* in I; H and I, longitudinal sections through acrosome of the xanthid *Atergatis floridus*.

pole of the acrosome as in all other brachyurans and paguroids with the doubtful exception of *Pagurus bernhardus* (see Chevaillier, 1968). As in most heterotremes, the operculum is imperforate whereas in the dromiid, *Petalomera*, homolids, *Ranina* and the majid *Menaethus monoceros*, it is perforate apically (see Jamieson, 1991; Guinot et al., 1993) (it is also perforate but is closed by an apical button in most of the investigated thoracotremes; see Discussion). In *C. africanus* the operculum is almost flat whereas in other heterotremes it is more commonly domed (e.g., the xanthid *Pilodius areolatus*, the dorippid *Neodorippe astuta*, and the portunids *Portunus pelagicus*, *Caphyra laevis* and *C. rotundifrons*). It is intermediate, a low dome-shape, tending to flattening, in the xanthids *Atergatis floridus*, *Liagore rubromaculata* and *Etisus laevimanus*.

The lower tier of the opercular complex has here been arbitrarily regarded as subopercular, as in xanthids, rather than opercular, though it may well be homologous with the lower layer of the operculum as defined for *Potamonautes* (Jamieson, 1993a). The accessory ring also occurs in xanthids (e.g., *Etisus laevimanus*; *Pilodius areolatus*, pers. obs.).

The "thickened ring" on each side of the subacrosomal invagination occurs in other heterotremes though weakly if at all developed in *Potamonautes*. (It is absent in grapsid thoracotremes.)

The stout perforatorium extends from the posterior perforation in the capsule to the vicinity of the operculum, as in other heterotreme (and thoracotreme) Brachyura. The perforatorium contains, as in xanthids and portunids (Jamieson, 1991), many circular profiles resembling microtubules. The arrangement in a circlet is elsewhere reported in *Potamonautes* (see Jamieson, 1993a) and is here recognized in the xanthid *Etisus laevimanus*.

Occurrence of centrioles is a plesiomorphic condition seen in majids, parthenopids, calappids, portunids, dorippids, trapeziids, potamids and *Macrophthalmus* but unknown in xanthids and most thoracotremes (see Jamieson, 1991, 1993a, 1993b).

In *C. africanus*, unlike portunids, xanthids and *Potamonautes*, the envelope between the chromatin and the basal cytoplasm consists of several loosely associated membranes but does not form the regular hexalaminar or multilaminar membrane seen, for instance, in dorippids and trapeziids. As in portunids, dorippids, trapeziids, xanthids and potamids (Jamieson, 1991, 1992, 1993a, 1993b), no cytoplasm extends into the arms. The general chromatin consists of

electron dense filaments in a pale matrix, as in other brachyurans.

Summarizing features of the spermatozoon of *C. africanus* which are general for heterotremes, there are: extension of the subacrosomal chamber almost to the anterior apex of the sperm, whereas it reaches only to the approximate equator of the acrosome in the archaeobrachyuran *Ranina*, presence of an acrosomal ray zone (albeit with indistinct substructure in *Potamonautes*) flanked by an outer acrosomal zone; presence of a thickened ring (weakly developed in *Potamonautes*) where the capsule surrounds the base of the subacrosomal chamber; and absence of the apical button which occurs above a central perforation of the operculum in most investigated thoracotremes. A feature shared with "higher" heterotremes is absence of cytoplasm and of microtubules in the nuclear arms. Peculiar to *C. africanus* relative to other heterotremes (and paguroid anomurans) so far studied is the turbine- or vortex-like configuration of the contents of the outer acrosome zone.

The occurrence of a periopercular rim in *Calocarcinus* and in the freshwater crab *Potamonautes perlati sidneyi* Rathbun, 1905 (Potamonautidae) (see Jamieson, 1993a) may well represent a true synapomorphy, confirming a xanthoid-potamoid relationship, in view of our opinion that *Potamonautes* is related to the South American Trichodactylidae H. Milne Edwards, 1853, which in turn we can derive from xanthoids near the Eriphiidae or Menippidae. These, like *Potamonautes*, have a pair of second sexual pleopods of the same length as the first pair. On the other hand, we hypothesize that the other group of South American freshwater crabs, the Pseudothelphusidae, is derived from the mud crabs, i.e., the Panopaeidae Ortmann, 1893 (Guinot, 1967, 1969), partly on the grounds that Pseudothelphusidae and Panopaeidae share the same type of first pleopod, with a similar complex apex. A rudimentary periopercular rim seen in *Etisus laevimanus* (pers. obs., see Fig. 2C, Jamieson, 1989) appears to support origin of the rim within the Xanthidae.

The relation of *Calocarcinus* with xanthids is supported by the irregular external margin of the outer acrosome zone internal to a peripheral acrosome zone. The posterior circumperforatorial dense zone is here regarded as a modification of the xanthid ring known to be characteristic of xanthids and Panopeids rather than a posterior extension of the acrosome ray zone. This interpretation is supported by the similarity to the xanthid condition (compare Figs. 2H and I

for the xanthid *Atergatis floridus* with Figs. 2F and G for *Calocarcinus*). The relationship of *Calocarcinus* with xanthids is therefore supported by: (1) presence of a xanthid ring, (2) the precise form of the acrosome ray zone which is wide anteriorly and sends a long slender extension posteriorly to meet the ring, (3) the irregular external margin of the outer acrosome zone, (4) division of the opercular complex into a distinct upper zone and a lower subopercular zone of lesser diameter, and (5) presence of an accessory ring around the operculum. Modification of the xanthid ring in *Calocarcinus* from the thick crescentic form in other xanthids is typical of thoracotremes, and partial modification (here as an inverted comma-shape) in *Calocarcinus* may indicate origin of thoracotremes from a related xanthid stock. Alternatively, the presence of acrosome rays in the *Calocarcinus* ring could be a plesiomorphic condition indicating origin of the xanthid ring from a specialized posterior region of the acrosome ray zone. A difference of *Calocarcinus* sperm from those of xanthids, as so far investigated (see Jamieson, 1991), is the (plesiomorphic) presence of centrioles, also seen in some heterotremes, including *Trapezia*, and some thoracotremes (Jamieson, 1993b). No synapomorphies are shared between *Calocarcinus* and trapeziid sperm which are not also seen in other heterotremes. Trapeziid sperm (Jamieson, 1993b) lack the xanthid ring, the posterior extension of the acrosome ray zone and the irregular margin of the outer acrosome zone of *Calocarcinus* and xanthids and differ further in that the lower zone of operculum is approximately the same width as the upper. It is uncertain which of these trapeziid states is plesiomorphic or apomorphic. Jamieson (1993b) has suggested that the absence in *Trapezia* of the xanthid ring is plesiomorphic but that restriction of the acrosome ray zone to an anterior location is apomorphic. The unequal lengths of the first and second pleopods in *Trapezia* (pleopod 2 much shorter than pleopod 1) is regarded by Guinot (pers. comm.) as advanced relative to the condition in *Calocarcinus* in which both pleopods are equal in length. Apomorphic features of the *C. africanus* sperm include a spiral configuration of the contents of the outer acrosome zone (autapomorphy?) as seen in cross-section, and the presence of a periopercular rim.

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