

ULTRASTRUCTURE OF THE SPERMATOZOON OF *AUSTRALOCARCINUS RIPARIUS* (CRUSTACEA: BRACHYURA: GONEPLACIDAE: TROGLOPLACINAE)

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The spermatozoon of the freshwater crab *Australocarcinus riparius* is heterotreme. However, absence of a recognizable acrosome ray zone is not a general heterotreme feature, though constant for thoracotremes. This zone is also unrecognizable in potamoids and in corystoids. The convex ring at the posterior end of the inner acrosome zone is similar to the xanthid ring, characteristic of the Xanthidae and Panopeidae, but homology is doubtful. Other xanthid features such as the accessory opercular ring and opercular overhang are absent from the sperm of *A. riparius* and relationship with xanthoids is not supported spermatologically. In its pointed shape, the perforatorium resembles that of corystoids but there the operculum is perforate. No special similarities to sperm of potamoids, which similarly have an obligatory freshwater existence at all stages of the life cycle, are apparent. The sperm of *A. riparius* shows no clear affinity with those of any other Heterotremata although confirming its inclusion in that group. In producing more than one spermatozoon per spermatophore, *A. riparius*, having marine relatives, may be less evolved along the path of lecithotrophy than are the potamoids, lacking marine cofamilials. □ *Australocarcinus riparius*, *Goneplacidae*, *Trogloplacinae*, spermatozoon, lecithotrophy.

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Davie (1988) described *Australocarcinus riparius* for a curious crab collected from a soft estuarine mud bank of the Murray River, NE Queensland. It has been found living in freshwater in rainforest (Davie & Guinot, 1996). This cryptic crab, excavating its own burrows, is remarkable in having direct development (the ovigerous female has only some 70 large eggs) and maternal care. Davie (1988) assigned it to the marine Goneplacidae. Guinot (1986) described *Trogloplax joliveti* from caves on New Britain. This specialised cavernicolous crab, with loss of pigmentation, thin cuticle, very long legs, and blind, nevertheless is a goneplacid for which Guinot (1986) erected the Trogloplacinae. Davie & Guinot (1996) describe 2 freshwater species of *Australocarcinus* (from New Caledonia and Palau) and show that the Australian epigean *Australocarcinus riparius* and the insular-subterranean hypogean *Trogloplax* are closely related in the Trogloplacinae. This, incidentally, furnishes additional evidence for the ancient faunal relationship of North Australia and southern Papua-New Guinea/ New Britain.

The Trogloplacinae have pleopod 2 longer than pleopod 1, a very large sternal plate, with all the sutures 4/5 to 7/8 incomplete, and a peculiar plate on sternite 8 which covers the penis at its origin

from the coxa of pereopod 5 (P5) until its final emergence in the middle of the lateral margin of sternite 8. The disposition of the male pores can be considered as coxo-sternal (Guinot, 1978, 1979a, b). The Trogloplacinae fall in that part of Heterotremata possessing a wide thoracic sternum. The Trogloplacinae, which have extant cofamilial marine relatives (Chasmocarciniinae) are derived from a former marine stock. They exemplify how true freshwater crabs (e.g. the potamoids), which lack contemporary marine cofamilials, might have originated from marine precursors during the Tertiary.

Classically the diverse Goneplacidae (Guinot, 1969), are intermediate between xanthids (or related forms) and other crabs such as grapsids.

The sperm of *Australocarcinus* is the first to be investigated in a goneplacid and in a heterotreme with a coxo-sternal disposition of the male genital pore and is here described as a first step in aiding resolution of the relationships within the Goneplacidae.

MATERIALS AND METHODS

COLLECTIONS. The mature male specimen was collected by Peter Davie and John Short from freshwater rainforest habitat in the McIvor River

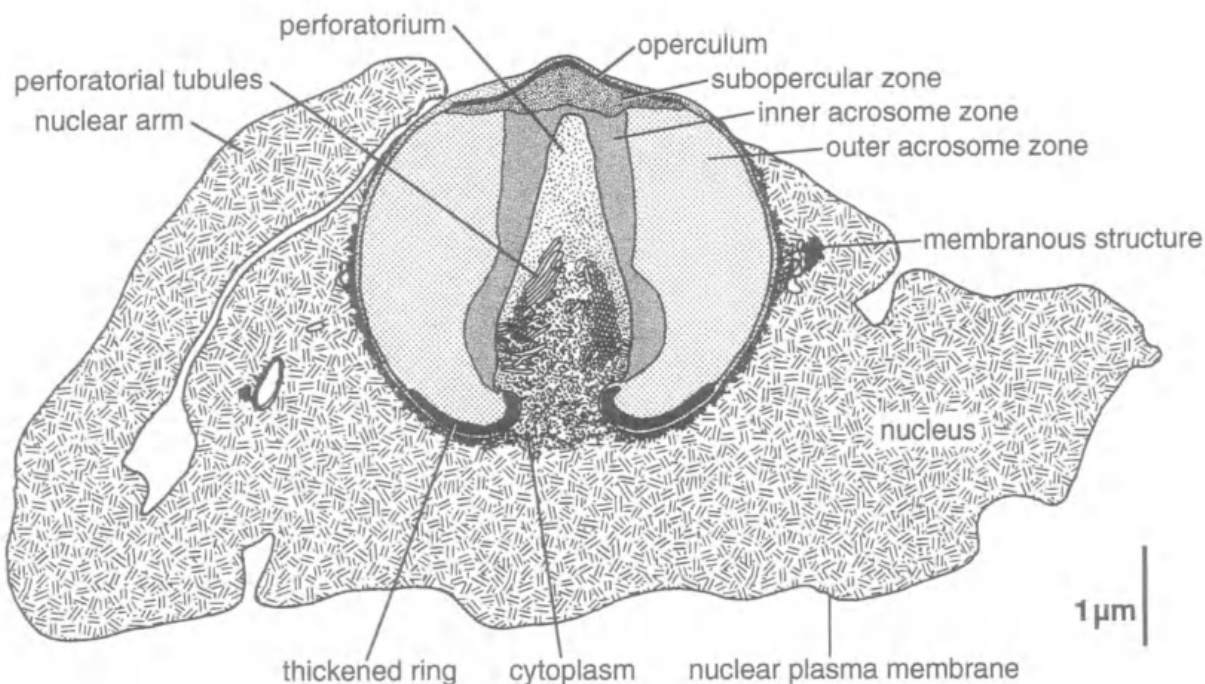


FIG. 1. *Australocarcinus riparius*, Semidiagrammatic sagittal section of a spermatozoon traced from a transmission electron micrograph.

at Isabella-McIvor road crossing, north Queensland in November 1992.

HISTOLOGY. The male reproductive material (both testes including the ducts of the vasa deferentia) was removed from the fresh crab and immediately fixed in cold glutaraldehyde for more than 2 hours at 4°. The gonad tissue was processed in the Zoology Department, The University of Queensland, by the 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).

Portions of the fixed testis (c.1 mm³) were rinsed in 0.2 M phosphate buffer (pH 7.2) (3 rinses 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, 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 s, rinsed in distilled water, then 6% aqueous uranyl acetate for 1 min,

lead citrate again for 30 s 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.

RESULTS

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

GENERAL MORPHOLOGY (Figs 1-3; Table 1). Each of the many spermatophores in the testes contains several to many spermatozoa. As such the spermatophores constitute coenospermia. An acrosome vesicle forms most of the volume. The acrosome is concentrically zoned but lacks the concentric lamellation of thoracotremes; it is capped apically by a dense operculum and is ensheathed in a thin cytoplasm which in turn is embedded in the nucleus. The acrosome vesicle is centrally penetrated by a cylindrical perforatorial column from the posterior end to immediately below the opercular complex. The nuclear material forms several marginal projec-

tions. The subspherical 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 surrounding the acrosome as in all other brachyurans. A thin layer of cytoplasm which intervenes between nucleus and acrosome as in other brachyurans, forms a small mass containing the centrioles at the posterior end of the perforatorial chamber. Cytoplasmic islets lateral to the acrosome and embedded in the chromatin (Figs 1, 2A, B, 3F) contain lamellae and bodies identifiable by homology with other crabs as degenerating mitochondria, although no cristae have been observed.

ACROSOME. The subspheroidal core of the spermatozoon consists of the concentrically zoned acrosome which is capped by, and includes, the opercular complex (Figs 1, 2A, 3A). The acrosome is invested by an acrosomal membrane underlain by a moderately electron dense sheath, the capsule. The mean length of the acrosome, from the apex of the operculum to the base of the capsule is $3.83\ \mu\text{m}$ ($\text{SD}=0.10$, $n=4$); the mean width is $4.29\ \mu\text{m}$ ($\text{SD}=0.09$, $n=4$). The acrosomal membrane and capsule are invaginated to cover an elongate subacrosomal or perforatorial chamber, the contents of which are the perforatorium (Figs 1, 2A, B, 3A-D). The anterior tip of this chamber abuts on the posterior face of the subopercular zone of the opercular complex. The perforatorium has a pronounced anterior taper and has the outline, in longitudinal sagittal section of an elongate triangle with a rounded base (Figs 1, 2A, 3A). It consists of a moderately electron dense matrix and is chiefly remarkable for numerous microtubule-like structures, perforatorial tubules, which appear to arise near, if not from, the posterolateral walls of the perforatorial chamber and extend chiefly anteriorly (Figs 2A, 3A, D). These tubules are closely adpressed to each other and form large bundles in which the tubules, in transverse section (Fig. 3C,E), form a quasi-crystalline array, leaving a narrow central core of the perforatorium of matrix material.

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, B, 3A-E) which extends from the subopercular zone at the anterior end of the acrosome almost to the

posterior end of the acrosome, reaching the thickened ring. The inner acrosome zone tapers anteriorly, following the outline of the perforatorium. Approximately its posterior 1/3, immediately anterior to the thickened ring, is widened to form a convex ring around the perforatorium (Figs 1, 2A, 3D); the inner acrosome zone, anterior to this ring, narrows so that there is a deep constriction between it and the ring. In some micrographs the convex ring appears to be separate from, although overlapping with, the anterior inner acrosome zone. It is not possible to determine whether the convex ring is the homologue of a xanthid ring. Acrosome ray zone, typical of heterotreme sperm, absent.

An outer acrosome zone (Figs 1, 2A, B, 3A-E) 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 capsule. 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 circular, imperforate, cap-like operculum (Figs 1, 2A, 3B). In longitudinal section it has an almost flat base and a low triangular anterior surface. Only a very thin layer of the outer, anterior surface is strongly electron dense. The much thicker lower zone is the subopercular zone. The operculum has a mean width of $2.35\ \mu\text{m}$ ($\text{SD}=0.06$, $n=4$).

Accessory ring, present lateral to the operculum in xanthoids and, though differently orientated, in thoracotremes, is not present nor is there an opercular overhang. Periopercular rim absent.

At the posterior pole of the acrosome (Figs 1, 2A, 3A, D, G, H) 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 is strongly developed (Figs 1, 2A, 3A, D). It is more extensive on the posterior, peripheral aspect of the acrosome than where it skirts the inside of the base of the subacrosomal chamber.

CYTOPLASM. The cytoplasm of the sperm forms a thin, scarcely discernible layer of ir-

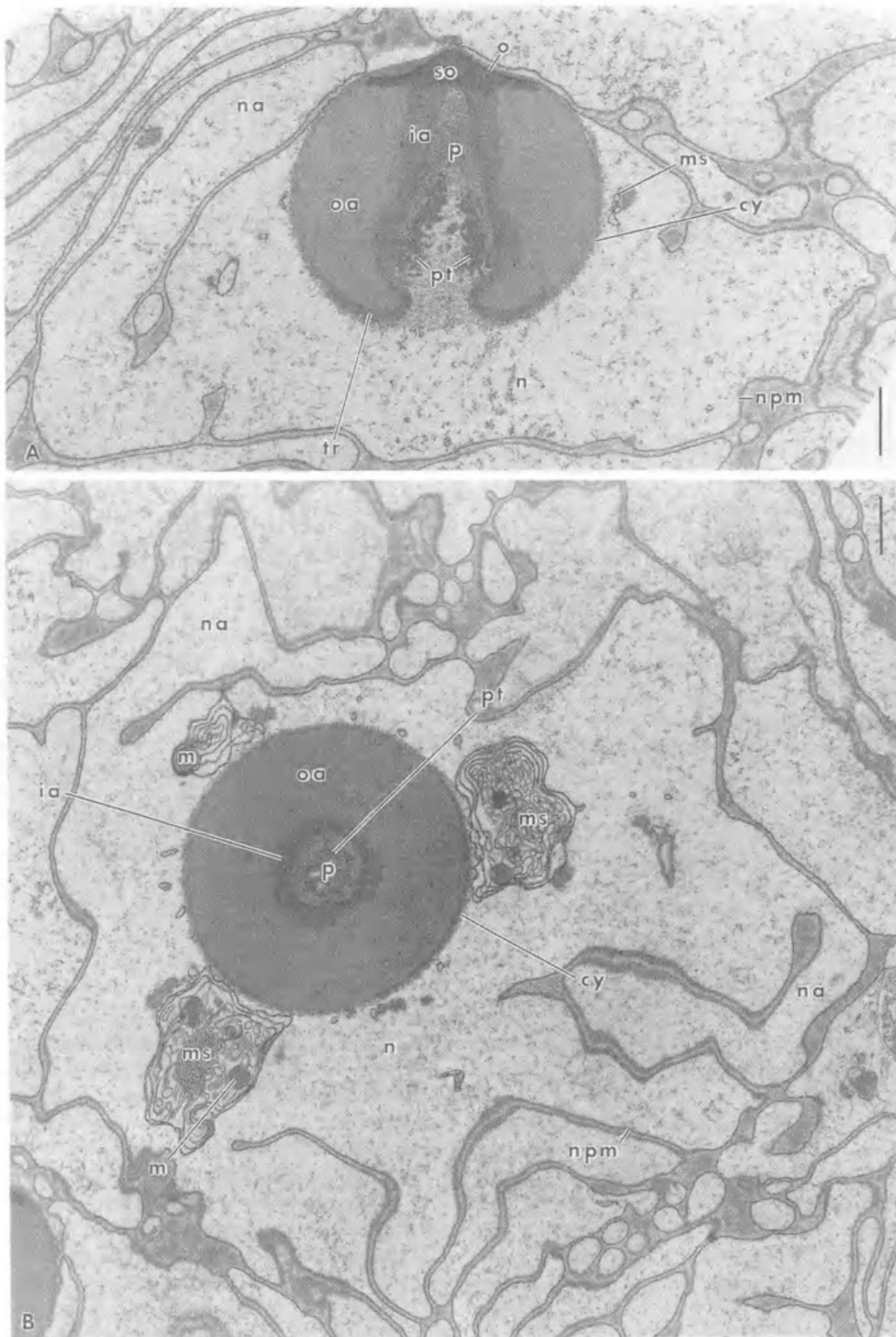


FIG. 2. *Australocarcinus riparius*, transmission electron micrographs of spermatozoa. A, sagittal section. B, transverse section. Abbreviations: ce=centriole; cy=cytoplasm; ia=inner acrosome zone; m=putative degenerating mitochondrion; ms=membranous structure; n=nucleus; na=nuclear arm; npm=nuclear plasma membrane; oa=outer acrosome zone; o=operculum; p=perforatorium; pt=perforatorial tubules; so=subopercular zone; tr=thickened ring.

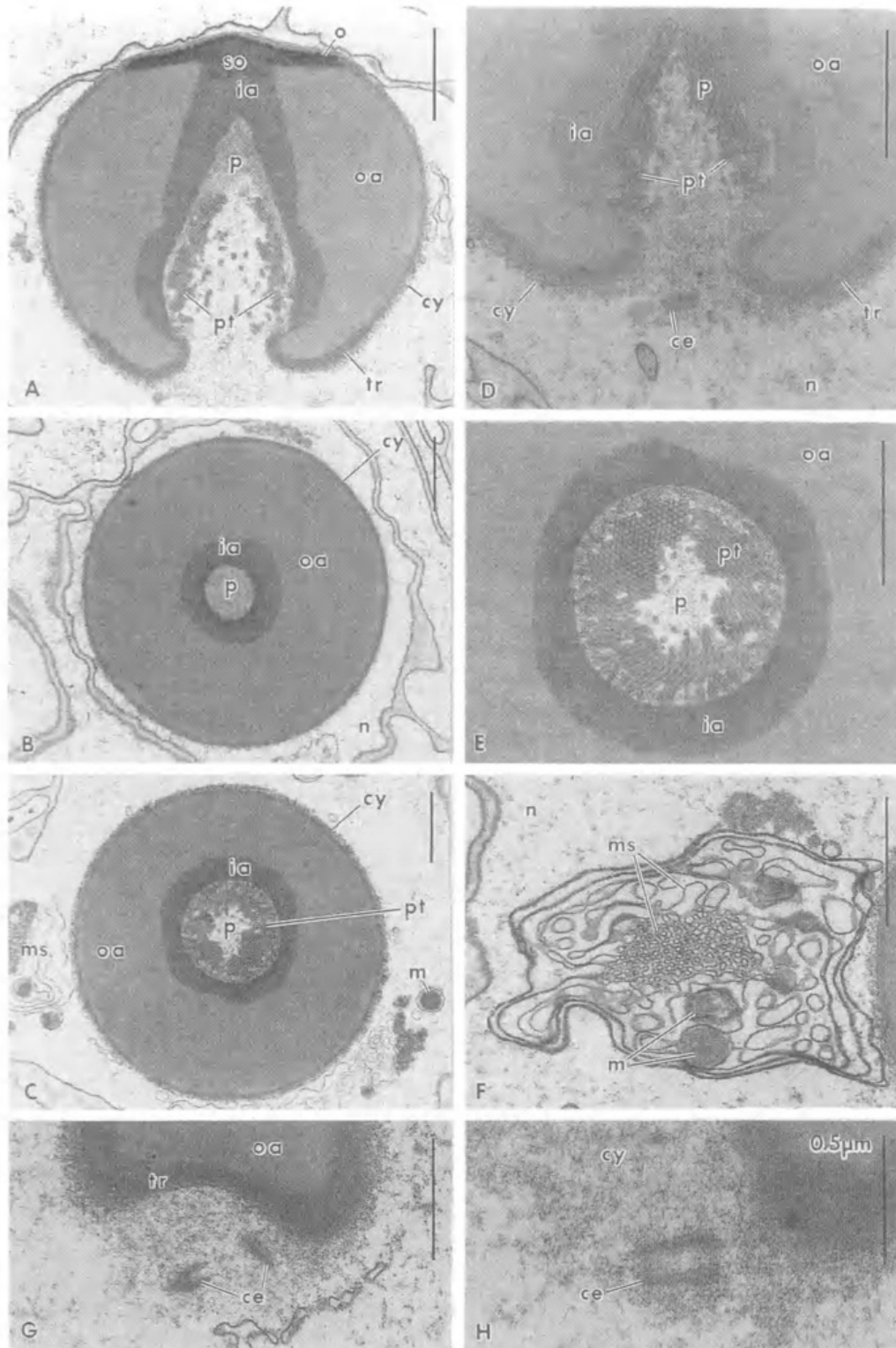


FIG. 3. *Australocarcinus riparius*, transmission electron micrographs of spermatozoa. A, sagittal section (LS). B, transverse section (TS) through the anterior region of the perforatorium. C, TS through the base of the inner acrosome zone proper, showing the quasi-crystalline array of perforatorial tubules. D, LS through the perforatorial invagination, showing the basal origins of the perforatorial tubules. E, TS through the convex ring, showing the quasi-crystalline array of perforatorial tubules. F, TS, showing a large membranous (lamellar) structure. G, Oblique section at the base of the perforatorium, showing two mutually perpendicular centrioles. H, longitudinal section of a centriole. Abbreviations as in Fig. 2.

regular thickness, ensheathing the acrosome excepting its opercular region (Figs 1, 2A, B, 3A, 4A, B). Membrane complexes and putative degenerating mitochondria which extend as islands far into the nucleus material are presumably derived from the cytoplasm but continuity of the large membranous structures of this type (Figs 2B, 3F) with the periacrosomal cytoplasmic layer has not been demonstrated. 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 a cytoplasm. No cytoplasm extends into the nuclear arms.

CENTRIOLES. The basal cytoplasm of the perforatorial chamber at the level of the posterior end of the acrosomal capsule contains centrioles (Fig. 3D, G, H). Two centrioles, at right angles (Fig. 3G) are each of normal length (Fig. 3H) and not elongate as in potamoids.

NUCLEUS. 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. C.1/3 of the length of the spermatozoon consists of the nuclear material which lies posterior to the acrosome (Figs 1, 2A). A nuclear envelope is not present between the chromatin and the periacrosomal cytoplasmic sheath, but some scattered vesicles (Fig. 3G) and components of the membranous structures possibly represent residues of the nuclear envelope.

The external, surface of the cell is bounded by a moderately dense membrane which may represent fused nuclear and plasma membranes, here termed the nuclear plasma membrane (Figs 1, 2A, B, 3A). The general chromatin consists of a diffuse network of electron dense filaments in a pale matrix as in other brachyurans. In longitudinal (Fig. 2A) and transverse sections (Fig. 2B), the nucleus is deeply and irregularly incised by what are presumed to be the bases of the nuclear arms but discrete nuclear arms have not been observed.

DISCUSSION

Varuna litterata and *Cardisoma carnifex* return to brackish or marine water to breed, and during larval growth, and therefore might be expected to differ in fertilization biology from *A. riparius*, which has no marine stages though, as a goneplacid, having extant cofamilial marine relatives. One or other of these species might also differ from freshwater potamoids which have

neither marine stages nor contemporary cofamilial marine relatives. Sperm morphology might reflect different fertilization biology while any unusual similarities between the sperm of *Australocarcinus* and potamoids which differed from those of crabs with marine or estuarine stages might be related to the internal physiological environment of nonmarine crabs. However, such correlates, if they exist, are not apparent.

It is presumably coincidental that all 5 genera in Table 1 lack a recognizable acrosome ray zone as, although absence is an unusual feature for heterotremes (in the first 3 genera), absence is an ancestral feature (thoracotreme synapomorphy) in *Varuna* and *Cardisoma*. The acrosome ray zone is absent in all thoracotremes (a predominantly marine group), in which (in contrast with true freshwater crabs) freshwater or terrestrial species always have contemporary relatives in the sea. Absence of the acrosome ray zone in corystoids (pers. obs.), a marine group, as in thoracotremes, suggests that absence cannot simply be attributed to a freshwater existence. Further lack of correlation of absence of an acrosome ray zone with the mode of life is seen in *Varuna litterata*, lacking this zone and reproducing in estuarine and marine conditions whereas *A. riparius*, and the potamoids, also lacking it, reproduce in freshwater. It is difficult to be certain whether acrosome rays are present or absent, as in *Potamonautes perlatus* (Jamieson, 1993).

The convex ring at the posterior end of the inner acrosome zone in *A. riparius* is not seen in other freshwater crabs. It is similar to the xanthid ring, of the Xanthidae and Panopeidae, but homology is doubtful. Other xanthid features such as the accessory opercular ring and the opercular overhang are absent from the sperm of *A. riparius* and relationship with xanthoids cannot be supported spermatologically. In its pointed shape, the perforatorium resembles that of corystoids but there the operculum is perforate.

An opercular perforation in *Potamon*, *Potamonautes* (homoplastic with majids) and in *Cardisoma* (where it is an ancestral, synapomorphic, thoracotreme condition), but not in *Varuna*, or the wholly nonmarine *Australocarcinus*, again cannot be attributed to a nonmarine habitat.

The thickened ring is exceptionally well developed in *Australocarcinus*. Reduction of the thickened ring occurs in *Potamon* and *Potamonautes* and is extreme in grapsids (though moderately developed in *Varuna*) and in *Cardisoma*. Although reduction probably relates to

TABLE 1. Spermatozoal characters of *Brachyura* as in freshwater (*) and terrestrial crabs (#).

	* <i>Australo- carcinus riparius</i> Goneplacidae (This study)	* <i>Potamonautes perlatus</i> Potamidae (Jamieson, 1993)	* <i>Potamon fluviale</i> Potamidae Guinot et al. in press	* <i>Varuna litterata</i> Grapsidae	# <i>Cardisoma carnifex</i> Gecarcinidae
1. Acrosome length/width	0.9	0.9	0.8	0.8	0.9
2. Acrosome zonation	concentric	concentric	concentric	concentric	concentric
3. Operculum	imperforate	imperforate	perforate	imperforate but indented	perforate with apical button
4. Opercular projections into suboperculum	absent	absent	absent	absent	absent
5. Operculum-capsule continuity	discontinuous	discontinuous	discontinuous	discontinuous	discontinuous
6. Operculum thickness	thin	moderate	moderate but thicker	moderate	moderate
7. Opercular width	moderately wide	moderately wide	moderately wide	not wide	extremely wide
8. Periopercular rim	absent	well developed	weakly developed	absent	weak
9. Accessory opercular ring	absent	absent	absent	absent	
10. Subopercular protuberance	absent	absent	absent	absent	absent
11. True acrosome ray zone	absent	presence uncertain	absent	absent	absent
12. Outer acrosome zone	not ragged	not ragged	not ragged	not ragged	not ragged
13. Anterolateral pale zone	absent	absent	absent	absent	absent
14. Flangelike lower zone	absent	absent	absent	absent	absent
15. Xanthid ring	absent?	absent	absent	absent	modified elongate
16. Perforatorium	preequatorial	preequatorial	preequatorial	preequatorial	preequatorial
17. Head of perforations	noncapitate	noncapitate	noncapitate	noncapitate	noncapitate
18. Corrugations of perforatorial chamber	absent	absent	absent	absent	absent
19. Lateral arms	several	several	several	several	several
20. Lateral arms	nuclear only	nuclear only	nuclear only	nuclear only	nuclear only
21. Centrioles	not elongate	elongate	elongate	present	not seen
22. Posterior median process of nucleus	absent	absent	absent	absent	absent
23. Thickened ring	well developed	vestigial	vestigial	present (reduced)	absent
24. Concentric lamellae	absent	absent	absent	lateral vesicle	absent
25. Capsular chambers	absent	absent	absent	absent	absent
26. Capsular projections	absent	absent	absent	absent	absent
27. Capsular flange	absent	absent	absent	absent	absent
28. Spiral acrosome zone	absent	absent	absent	absent	absent
29. Opercular overhang	absent	absent	present?	absent?	present
30. Spermatophores	coenospermia	cleistospermia	cleistospermia	coenospermia	coenospermia

peculiarities of the acrosome reaction (Medina, 1992; Medina & Rodriguez, 1992) it is not a common feature of freshwater crabs.

A. riparius produces large eggs and is suspected to have maternal care but its spermatophores contain several to many spermatozoa, the coenospermial condition. This contrasts with the

production of spermatophores with single spermatozoa, cleistospermia, in *Potamon* and *Potamonautes*. This contrast need not invalidate the hypothesis (Guinot et al., in press) that the cleistospermial condition is an adaptation to peculiar features of fertilization biology in potamoids and, particularly, to the large size and

small numbers of eggs, which correlates with their known lecithotrophic, direct development. It was considered possible that sperm from potamoid spermathecae are delivered singly to the eggs. Production of single-sperm spermatophores (cleistospermia) was conjectured to be a device preventing polyspermy in individuals in which wastage of the small numbers of large eggs (and incidentally of spermatozoa) has to be minimized. Possibly *A. riparius*, having marine relatives, is less evolved along the path of lecithotrophy than the potamoids, lacking marine cofamilials.

Sperm of *A. riparius* shows no clear affinity with those of other Heterotremata although confirming its inclusion in that group.

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