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Comparative and phylogenetic studies on sperm ultrastructure and reproductive biology in the peracarids and lower malacostracan crustaceans

by

Gerald Kutish

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Graduate Faculty in Partial Fulfillment of
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INTRODUCTION - ORIGIN AND FUNCTION IN THE PERACARID SPERM

The peracarid crustacean spermatozoa (Retzius, 1909a,b) are very stereotyped in morphology and yet, they are uniquely divergent from the various other crustacean sperm morphologies (Grobben, 1878; Retzius, 1909a,b; Wilson, 1925).

For example, the peracarid orders Mysidacea (Labat, 1962), Cumacea, Amphipoda and Isopoda all have a very long (up to 2 mm) filiform tail with an anterior pendant nuclear region. Also, in ultrastructural studies of mysids, amphipods (Reger, 1966), and isopods (Fain-Maurel, 1966), the sperm tail shows a cross striated, highly structured wall modification instead of the familiar flagellar microtubular arrangement.

Very little resemblance exists between the morphology of these peracarid spermatozoa and the primitive metazoan sperm (c.f., Franzen, 1956; Baccetti, 1970; Baccetti and Afzelius, 1976), which has a short, round head containing the nucleus and acrosome, a middle piece, with mitochondria surrounding the centrioles, and the flagellum or tail. The only comparable region to the peracarid sperm is a supposed (Reger, 1966) anterior acrosomal vesicle.

In fact, the peracarid sperm do not even resemble the various other crustacean spermatozoa. The subclasses Mystacocarida (Brown and Metz, 1967), Cirripedia (Barnes et al., 1971), and Branchiura (Brown, 1970) have flagellated spermatozoa. The aflagellate filiform spermatozoa in the subclass Ostracoda (Reger, 1970) and the aflagellate spherical spermatozoa in the subclass Copepoda and Branchiopoda (Brown, 1969, 1970) are absolutely unique. In the subclass Malacostraca, which contains the division
Peracarida, aflagellate spheroid or stellate spermatozoa are found in the divisions Phyllocarida (Cannon, 1927), Hoplocarida (Kumé and Dan, 1958) and Eucarida (euphausids-Zimmer and Grunner, 1956).

Similarities between the peracarid and other crustacean spermatozoa become apparent only when the tail region is examined. Among the Malacostraca, the sperm tails of the Syncarida (Smith, 1909) and some of the tack-like spermatozoa in the Decapoda (Brown, 1966a; Pochon-Masson, 1969) may be homologous with the peracarid sperm tail. In other crustacean subclasses only the aflagellate tail of the primitive Cephalocarida (Brown and Metz, 1967) resembles the peracarid sperm tail.

Whatever the actual relationship among these various crustacean spermatozoa may be, the origin and function of the peracarid sperm morphology are unknown. Possibly the peracarid sperm pattern is an adaptation for spermatophore production, sperm transfer, sperm storage or fertilization. This follows Franzén’s (1956) idea that “a definite relation has been found between the morphology of the sperm and the biology of fertilization.” However, the peracarid spermatozoa represent a very extreme modification from the more conventional spermatozoa observed by Franzén (1956).

Nevertheless, it is possible to view the filiform peracarid sperm morphology and especially the tail as an adaptation for sperm transfer, sperm retention and for affecting the sperm-egg interaction. These adaptations were developed concurrently or prior to the acquisition of the peracarid brooding pattern. The filiform spermatozoa interacted with the lamellar setose brood plates (oostegites) and were retained until
ovoposition. Furthermore this elongated tail positioned the acrosomal region for the sperm-egg interaction. Finally, this view depicts the peracarid sperm as a very primitive pattern since the oldest caridoid fossils (Schram, 1969b, 1974a,b; Brooks, 1962a,b) exhibit a brooding pattern with oostegites on the legs. Also, the even more primitive Syncarida (Schram and Schram, 1974; Brooks, 1962c, 1969) are shown in this paper to have a similar sperm morphology.

In order to study the origin and function of the peracarid sperm six topics are examined: (1) sperm morphology in the lower malacostraca crustaceans; (2) sperm morphology and brood pouch structure in the mysid peracarids; (3) sperm morphology in the tanaid and cumacean peracarids; (4) reproduction and sperm morphology in isopod peracarids; (5) reproduction and sperm morphology in amphipod peracarids; and (6) brood pouch structure in amphipod peracarids.

The reasons for selecting these areas of investigation are twofold. Firstly, the origin of the peracarid sperm plan can be understood only if the common sperm features among the malacostraca are determined. Since the Peracarida arose from an early palaeozoic caridoid ancestor (Brooks, 1962a; Schram, 1969b, 1973, 1974c), it is worthwhile to examine the existing primitive malacostracans, i.e., Phyllocarida-Nebalia, Syncarida-Anaspides, Hoplocarida-Squilla, and Eucarida-Euphausia. These relict crustaceans show many primitive anatomical characters including perhaps sperm morphology (Calman, 1909; Siewing, 1963). Therefore, the lower malacostracans may give insight into the origin of the peracarid spermatozoa and bridge the gap between the sperm morphology of various crustacean groups.
Secondly, the peracarid sperm morphology and function can only be understood with relation to the peracarid pattern of marsupial incubation (c.f., Kaestner, 1970). The female must molt, presenting a fresh axenic surface on the brooding lamellae, before the spermatozoa are transferred to the brood cavity. Only then are the oocytes passed into the marsupium for fertilization. But considerable time (4 hours to 8 days) may pass before ovoposition, so sperm retention in the marsupium is necessary. This marsupial sperm retention is the key function for the unique peracarid sperm tail.

To test this hypothesis about peracarid sperm tail function, the mode of sperm transfer, marsupial structure and sperm egg interaction are examined because they represent the primitive caridoid condition which is ancestral to the other peracarids. Cumacea and Tanaidacea show trends toward the isopod condition. Also, the tanaids are unique for not sharing the stereotyped peracarid sperm plan. Isopoda represent one line of adaptive success while the Amphipoda represent the other major peracarid line of success.
PART I. SPERM MORPHOLOGY IN SELECTED PRIMITIVE MALACOSTRACAN CRUSTACEANS
An exact understanding of the sperm morphology in the primitive malacostracan groups is important because it sheds light on the origin of the peracarid sperm morphology. The peracarid crustaceans most certainly evolved during the mid-Palaeozoic from caridoid members of the dominant early palaeozoic leptostracans, anaspids and stomatopods. A substantial fossil record for the Leptostraca (Rolfe, 1969; Schram, 1969a), Anaspidae (Brooks, 1962c, 1969; Schram and Schram, 1974) and Stomatopoda (Brooks, 1969; Schram, 1969b, 1973) predates the peracarid fossil record (Schram, 1969b,c, 1974b). Therefore morphological relationships probably exist between the peracarid spermatozoa and the spermatozoa of the primitive malacostracan species.

Yet, the sperm morphology in the more primitive malacostracan orders Leptostraca, Anaspidae, Stomatopoda, and Euphausiacea, has not been re-examined recently. Earlier studies of Leptostraca (Claus, 1888b), Stomatopoda (Gilson, 1886; Grobben, 1876, 1878; Nichols, 1909; Komai, 1926; Balss, 1938) and Euphausiacea (Bargmann, 1937; Zimmer and Grunner, 1956) spermatozoa reveal simple spherical cells with a central nucleus. The euphausids and leptostracan spermatozoa also have cytoplasmic processes. Grobben (1878) believed that this simple sperm morphology, similar to a somatic cell, was the primitive malacostracan pattern. During the same period, Smith (1909) observed the filiform spermatozoa of the Anaspidae. He believed that the anaspid spermatozoa were similar to the peracarid spermatozoa.
This paper will examine the sperm morphology of four primitive malacostracans: (1) _Nebalia_ - a precaridoid malacostracan in the division Phyllocarida, extant since the Cambrian; (2) _Squilla_ - a caridoid malacostracan in the division Hoplocarida with an extensive early Palaeozoic fossil record; (3) _Anaspides_ - a caridoid malacostracan in the division Syncarida with an extensive mid-Palaeozoic fossil record; and (4) _Euphausia_ - a recent caridoid malacostracan in the division Eucarida. These species were chosen for two reasons: firstly, the historical phylogenetic interest (Claus, 1876, 1888b; Boas, 1883; Siewing, 1953, 1956, 1959, 1960, 1963) attached to these forms; and secondly, the presence of the primitive precaridoid and caridoid facies hypothesized to be the ancestral malacostracan condition (Calman, 1904; Burnette and Hessler, 1973).

Particular attention is given to the modification of malacostracan sperm features such as the acrosome, mitochondria, nucleus and tail structures. These sperm features are then compared with the peracarid sperm morphology.
MATERIALS AND METHODS

List of Species

Animals used in this study were obtained from the following sources and prepared for electron microscopic observation as indicated below.

Division Phyllocarida

Mature male specimens of Nebalia pugettensis (Clark, 1932) were hand collected under rocks and decaying seaweed (Fucus) at low tide from breeding populations during July at False Bay (Mar Vista Access), San Juan Island, Washington. The gonad was exposed dorsally (see description in Claus, 1888b), fixed in situ in 3% glutaraldehyde with 1% acrolein (0.2 M cacodylate buffer, pH 7.5) or 2.5% glutaraldehyde (0.07 M cacodylate buffer, pH 7.5 with 0.3 M sodium chloride), washed in the buffer, post-fixed with 1% osmium tetroxide in the buffer, dehydrated in ethanol and embedded in Spurr's resin (1969).

Division Eucarida

Male specimens of Euphausia pacifica Hansen (see Boden et al., 1955 for description) were hand collected at night with a suspended light in Friday Harbor, or collected with a one meter plankton net towed to a depth of 180 m in San Juan channel, San Juan Island, Washington during July. The gonad was exposed dorsally (see description in Raab, 1915; and Bargmann, 1937), fixed in 2.5% glutaraldehyde (0.2 M phosphate buffer, pH 7.5; 0.14 M sodium chloride), washed with the buffer plus 0.3 M sodium...
chloride, postfixed with 1% osmium tetroxide in the buffer, dehydrated
with ethanol and embedded in epon-araldite (Anderson and Ellis, 1965).

**Division Hoplocarida**

Male specimens of *Squilla empusa* Say (see Manning, 1969 for descrip-
tion) were hand collected from otter trawl catches at St. Andrews Bay,
Panama City and St. Joseph's Bay, Port St. Joe, Florida. For ultrastruc-
tural studies, the stomatopods were anesthetized in cold (2°C) sea water.
The gonad was exposed by dorsal dissection (see description in Grobben,
1876), fixed in situ with cold 2.5% glutaraldehyde (0.07 M cacodylate
buffer, pH 7.5; 0.5 M sodium chloride) for 15 minutes, dissected out and
placed in fresh fixative for 1 hour, washed in the cold buffer salt solu-
tion for 1 hour, postfixed in 1% osmium tetroxide (0.1 M cacodylate
buffer, pH 7.5), dehydrated with ethanol, and embedded in Spurr's (1969)
plastic.

Sections for ultrastructural examination of the spermatozoa and
spermatophores stored in the vas deferens were cut with glass or diamond
knives on a Porter Blum MT-2 ultramicrotome, mounted on uncoated grids,
stained with 5% uranyl acetate in 50% ethanol followed by lead citrate
(Venable and Coggeshall, 1965) and viewed with an Hitachi HU 11e-1 elec-
tron microscope.

**Division Syncarida**

Male specimens of *Anaspides tasmaniae* Thomson were collected from
streams at the base of Mount Wellington, Tasmania in July, 1974 by Dr.
P. S. Lake of the University of Tasmania, Hobart, Tasmania. The gonad was
exposed dorsally (see description in Nicholls and Sparro, 1932), immersed in a trialdehide fixative (Lake, 1973), partially dehydrated in 70% ethanol for shipment and then rehydrated prior to post-fixation with 1% osmium tetroxide in the buffer, dehydrated with ethanol and embedded in epon-araldite (Anderson and Ellis, 1965). Due to the storage in ethanol there was some unavoidable lipid extraction.

Mature spermatozoa were observed by two methods. Spermatozoa from spermatophores stored in the penis were collected by ejaculation. Penile stimulation with a dissecting needle and temperature elevation was used to cause spermatophore emission from the penis. Fixation also caused spermatozoa extrusion. Alternatively, spermatozoa from spermatophores stored in the seminal vesicles were examined. The male reproductive system was dissected from the body cavity and the seminal vesicles opened in sea water to release the spermatophores. In all cases, sperm suspensions in sea water were obtained by allowing spontaneous dissociation of the spermatophores or by squashing the spermatophores. The sperm morphology was examined with phase contrast and Nomarski differential interference optics. Samples were fluorochromed (0.01% acridine orange in sea water) for fluorescent differentiation of the nucleus (green), and acrosome (red). For cytochemical examination, the spermatozoa were stained with the Feulgen nuclear stain for DNA and the PAS method to indicate acrosomal regions (Humason, 1972).
RESULTS

The following observations were made: (1) mature sperm morphology; (2) immature sperm and late spermatid morphology; and (3) spermatophore morphology.

Division Leptostraca

Spermatozoa

The mature spermatozoa (Fig. 1) of Nebalia, packaged in spermatophores, are located in the lumen of the tesis from the fourth abdominal to the first thoracic segments and in the vas deferens exiting on the eighth thoracic segment. The spermatozoa, when released from the spermatophore by mechanical rupture, are spherical, 5-7 μm in diameter, with numerous cytoplasmic processes.

The ultrastructural morphology (Figs. 1-3) of these spermatozoa is very simple and compact. In general, the cytoarchitecture is formed from the inactive synthetic apparatus present at the end of spermiogenesis which was used to form the spermatophore wall, pigment granules, and cytoplasmic process. No motile apparatus or acrosome region is visible.

A central nucleus with densely packed and intensely staining chromatin is surrounded by a distinct nuclear membrane. Adjacent to the nucleus (Figs. 2-3) is a peripheral rim of dense cytoplasm. Four components are visible: membranes; ribosome aggregations; pigment granules; and cytoplasmic processes. Numerous compact membranous arrays around mitochondria fill the cytoplasm. These represent the endoplasmic reticulum and mitochondrial remnants of the spermatid synthetic apparatus for the spermatozoa.
phore wall secretion. Interspersed in the cytoplasm adjacent to the cell membrane are granular aggregations 1.5 μm in diameter. These packed granules (160 nm) are probably free ribosomes which filled large regions of the spermatid cytoplasm. No pattern of distribution is apparent.

Besides these inactive synthetic structures, the only specially elaborated sperm structures (Fig. 1) are the pigment granules and the cytoplasmic processes. The pigment granules are irregularly dispersed in the cytoplasm. These membranous bound densities, 200-250 nm in diameter, are formed during early spermiogenesis and do not represent autophagic vacuoles. Projecting from the surface are pointed cytoplasmic processes, 1.5 to 1.7 μm long. Six sets of filaments, closely applied to the cell plasma membrane support these projections.

Spermatid

Spermatids (Figs. 4-5) are distinguished in the testis by their location adjacent to the lumen; by the presence of developing cytoplasmic processes and pigment granules; and by the surrounding follicle cells. In general, 6 to 8 developing spermatids become surrounded by several follicle cells. These follicle cells secrete the outer spermatophore wall. The spermatid secretes the inner spermatophore wall as well as forms the cytoplasmic process and pigment granules.

These synthetic functions of the spermatid are suggested by the nuclear and cytoplasmic morphology. As was stated it is the remnants of this synthetic apparatus which persists in the cytoplasm of the mature sperm along with the elaborated pigment granules and cytoprocesses.
The nucleus has the morphology of a synthetically active cell. A nucleolar region is present, along with diffuse chromatin. Nuclear pores are very prominent in the nuclear membrane.

The cytoplasm (Fig. 4) has two distinct areas with the pigment dispersed within each. Regions of membranous arrays and irregular mitochondria are associated with the secretion of the inner spermatophore wall. The cisternal contents of the cytoplasmic membranes empty into large vesicles. These vesicles fuse to the external plasma membrane secreting the spermatophore wall. Also present are areas of free ribosomes in association with the developing cytoplasmic processes (Fig. 5). An underlying network of microfilaments extends from the processes to the ribosomal regions.

Spermatophores

In Nebalia, 5 to 8 spermatozoa are packaged in a spermatophore (Figs. 1, 4). These capsules are from 10 to 20 μm in diameter, tending toward an average of 17 μm. They fill the lumen of the testis and the vas deferens. No further packaging occurs to the individual spermatophores to form a single spermatophoric structure for transfer at copulation.

The coherency of the spermatozoa as a spermatophore is provided by an encapsulating wall, 2 μm thick. As was stated, due to the dual secretion of this wall by the spermatozoa and the surrounding follicle cells, two regions are visible. Outermost is a dense layer 0.4 μm thick; internally, a diffuse layer completely surrounds the spermatozoa. The spermatophore
wall is fairly resistant to sea water. No dissolution or size changes happen after 1 hour observation periods.

Division Eucarida

Spermatozoa

The spermatozoa (Fig. 6) of Euphausia, packaged in spermatophores, are located in the lumen of the vas deferens from the 6th thoracic segment to the gonopore in the 8th thoracic segment. When liberated in sea water from the spermatophores the spermatozoa are spherical, 8 to 16 µm in diameter. The surface appears to have numerous long (2 µm) thin fairly wide flabby projections.

The ultrastructural morphology (Fig. 6) of the spermatozoa is very simple. Each sperm secretes an external coat which accounts for the apparent flabby surface projections. This synthetic apparatus remains in the mature sperm. No motile apparatus, acrosomal region or special cytoplasmic structures are present.

In general the Euphausia sperm has a central nucleus, a peripheral rim of cytoplasm and a thick external cell coat. The central nucleus has a homogeneous, finely granular chromatin with no surrounding nuclear membrane apparent.

The cytoplasm has three distinct regions (Fig. 6). Free ribosomes are located in the perinuclear region. While at the periphery numerous vesicles are present. Some of these vesicles are secretory and fuse to the cell plasma membrane. At one cell pole, a crescent array of irregular tubular membranes and mitochondria are aggregated.
As was stated, the cytomorphology indicate that each cell secretes an external coat (Figs. 6, 7). However, by the time the spermatophore has been formed from the vas deferens secretions, this coat is well-developed, and the synthetic activity is reduced. The extensive, homogeneous dense coat is 0.5 to 1 µm thick and has a very dense limiting border 40 nm thick. Embedded within the coat are translucent granules. This coat forms the long flabby projections seen on the sperm surface.

Spermatids

Spermatids are distinguished in the reproductive system by their location in the vas deferens anterior to the 6th thoracic segment. Also, the spermatids are free, not being packaged into spermatophores yet. Structurally, these spermatids are engaged in the synthesis of the external cell coat (Fig. 9).

Each spermatid (not figured) is approximately twice the size (20 µm) of the spermatozoa in the spermatophores. The nucleus is active with a diffusely granular chromatin. Two regions are apparent in the peripheral cytoplasm. In the juxtanuclear area are located free ribosomes and mitochondria. At the outer rim the cytoplasm is actively synthesizing the cell coat. Tubular endoplasmic reticulum and secretory vesicles are present. No other specialized structures appear in the spermatids.

Spermatophores

The sperm in Euphausia are packaged (Figs. 6, 7, 8) with secretions from at least three different sources. Initially in the anterior vas deferens, the spermatozoa secrete individual coats. Later, vas deferens secretions are used to package 4 to 8 spermatozoa into a spermatophore.
These secretions form a distinctive encapsulating wall, 2 μm thick. The outer layer (25 nm) is densely consolidated while the inner wall has a striated appearance in cross section (Fig. 7) due to a hexagonal packaging posterior (Fig. 8).

Finally, the lower portion of the vas deferens in the 7th and 8th thoracic segments contribute several gelatinous secretions. These secretions are extruded with the spermatophores during copulation as the main spermatophoric mass. It is this ultimate structure which is usually called a spermatophore in the euphausids.

Division Hoplocarida

Spermatozoa

*Squilla* spermatozoa (Figs. 10, 11, 22) are spherical (10 μm in diameter) with an apical acrosome and rod complex, adjacent centrioles, subadjacent eccentric nucleus, and a basal crescent of cytoplasm. Mature spermatozoa are characterized by differentiated projections on the plasma membrane and a unique secreted envelope. Generally, the spermatozoa are found in the vas deferens from the penile ejaculatory duct to the convoluted ducts in the first abdominal segment.

The apical region contains the acrosome and a preformed subacrosomal rod which are closely appressed upon the nucleus (Figs. 10, 12). The membranous bound acrosome (1.7 μm in diameter, 0.3 μm thick) projects slightly from the cell surface presenting a button shaped appearance. Associated with the acrosomal membrane above the nuclear membrane is a density. No modifications are seen upon the apical acrosomal membrane.
which remains separated from the adjacent cell plasma membrane. Directly beneath the acrosome, a subacrosomal rod (90 nm diameter) extends into the nucleus (1.7 \mu m) but does not perforate the nuclear membrane (Figs. 10, 12, 13). Fibers and tubules are visible within the rod but there are no surrounding membranes. No acrosomal reaction has been observed but presumably the acrosome initially interacts with the egg surface followed by an acrosomal rod penetration of the egg envelope.

A small cytoplasmic pocket containing a pair of centrioles lies adjacent to the acrosome (Fig. 13). These centrioles are always equidistant (about 0.3 \mu m) from the acrosome, nucleus and cell plasma membrane. Sometimes, mitochondria and endoplasmic reticulum, remnants of spermiogenesis are present.

An eccentric nucleus, spherical in outline, lies immediately beneath the acrosome (Fig. 10). The only irregularity is an apical indentation caused by the acrosomal rod. In the periacrosomal region the nuclear membranes are closely applied or fused to the cell membrane so as to exclude all cytoplasm; while in the basal region, the nuclear membranes are occasionally fused with the surrounding endoplasmic reticulum. No nuclear pores are visible. The enclosed chromatin is uniformly flocculent in appearance similar to the spermatid nucleus, irrespective of the fixation technique. Within the nucleus, a densely compact nucleolus is centrally located.

The basal crescent of cytoplasm gives the impression of previous synthetic activity (Figs. 10, 16). Although no secretion vesicles, Golgi, lysosomes or microtubules are visible, numerous mitochondrial aggregations
and extensive areas of endoplasmic reticulum with dispersed ribosomes fill the entire basal cytoplasm. The oblong mitochondria are collected together in a single cluster, not dispersed, and have a dense matrix with shelf-like cristae. The cytomembranes with interspersed ribosomes form extensive stacks which extend to a perinuclear position between the nuclear and cell membranes. These cytomembranes do not contain a secretion and are sometimes present as myelin figures.

The cell plasma membrane has characteristic surface modifications, presumably for sperm-egg interaction (Figs. 13, 14, 15). Cytoplasmic processes (0.5 μm long) surround the acrosomal periphery while the perinuclear surface contains grooves. These indentations form concentric short slits (0.1-0.2 μm deep). No surface modifications are present below this region of grooves, along the basal cell membrane (Figs. 5, 10, 16).

An envelope or coat covers the entire cell surface and is secreted by the sperm (Figs. 10, 16). This envelope has a uniformly dense, finely granular appearance (0.25 μm thick) and is in contact with the cell membrane except in the acrosomal region where a small space exists. The external cell envelope apparently serves as a protective barrier to the sea water since the coat is not dissolved by sea water or salines. However, spermatozoa with incomplete envelopes exhibit osmotic swelling upon contact with sea water.

Spermatids
Final sperm development is a sequential process. Generally, in the posterior regions of the vas deferens, the spermatids elaborate the cytomembranes and cytoplasmic structures for acrosome and sperm envelope
formation (Fig. 17). While in the anterior portion of the vas deferens, the developing spermatozoa secrete the acrosome and the outer sperm envelope, assuming the mature form (Figs. 20, 21, 10).

The spermatids, newly released from the testis into the vas deferens, have the morphology of a cell engaged in synthetic activity (Figs. 18, 19). In general, the spermatids have the same size (8-9 µm diameter) as a mature sperm. The nucleus appears active in information release. All of the chromatin is dispersed with a uniform granular and filamentous texture found in the euchromatin of cells with synthetic activity in the cytoplasm. No peripheral clumps of inactive heterochromatin are visible. The very distinct nucleolus also appears active with the characteristic fibrillar zone and coarse surrounding granular zone (Fig. 18). Prominant nuclear pores are present in the nuclear membranes surrounding the nucleoplasm indicating information transfer to the cytoplasm. The thin rim of cytoplasm around the spermatid nucleus contains an irregular tubular endoplasmic reticulum, mitochondria and free ribosomes, all associated with synthesis. Along the external cell membrane pinocytotic vesicles are visible, indicating active uptake into the cell.

Within the spermatid previously described, the initial stages of acrosome formation are observed (Fig. 19). The acrosome appears first as a membranous bound vesicle in contact with the cell plasma membrane near the centrioles. Dense material from the surrounding endoplasmic reticulum accumulates gradually within the vesicle. No Golgi complex mediates the acrosome formation. Next, the material next to the basal acrosomal membrane condenses (Fig. 20). Directly beneath, a subacrosomal rod is formed
in contact with the basal acrosomal membrane (Fig. 21). The acrosome now appears as a bulge above the nucleus while the subacrosomal rod increases in length acquiring a surrounding dense matrix.

At this stage the spermatozoa synthesize and release the surrounding sperm envelope (not figured). The cytoplasm with the free ribosomes and cytomembranes, produces the envelope material which is released on the external sperm surface. A condensation gradually occurs around the sperm until a fuzzy coat appears on the sperm plasma membrane (Fig. 16).

Finally, the spermatozoa assume a mature form indicated by the general redistribution of the cytoplasmic organelles (Fig. 10). Acrosomal and envelope synthesis are complete. Maturation occurs as the nucleus and acrosome come into close contact so that the subacrosomal rod indents the nucleus. As a result, cytomembranes and mitochondria, are excluded in the apical region of the sperm (Fig. 12). Only the centrioles remain between the acrosome and the nucleus (Fig. 13). Next, the nuclear membranes and cell membranes come into close contact in the periacrosomal region. Any remaining cytomembranes are fused. Finally, the basal region contains the remaining cytoplasmic elements (Figs. 10, 16). The membranes which were previously involved in the synthesis of the acrosome and envelope, now form membranous arrays which may assume the form of myelin figures. Ribosomes are both attached to these membranes and free in the cytoplasm. The mitochondria are massed together.

**Spermatophores**

The spermatophores are composed of mature spermatozoa with their envelopes and the secretions of the vas deferens and terminal ejaculatory
duct (Fig. 10). The sperm cells in the middle vas deferens (first abdominal segment) exhibit the terminal stages of envelope secretion (not figured). The cytomembrane system of endoplasmic reticulum and vesicles around the nucleus and the basal cytoplasm synthesize a flocculent material which is released to coat the cell surface (Fig. 16). This material finally condenses; but it is not completely clear if the dense coat is secreted entirely by the sperm cell or if external materials already present are caused to condense upon the cell surface.

Secretions of the vas deferens epithelium surround and separate the individual sperm cells (Fig. 10). The ejaculatory duct epithelium also secretes a dense homogeneous spermatophore matrix which holds the sperm mass together (not figured). No distinct preformed spermatophores are present within the male genital tract. Only upon ejaculation is a coherent ropy spermatophore extruded from the penis and placed in the female seminal receptacle.

Division Syncarida

Spermatozoa

Mature Anaspides spermatozoa (Figs. 23, 24) are found in the vas deferens. They are filiform with a tail (length 70 μm) which projects posteriorly from a head region, 9 μm long and 5 μm wide. The head region contains an anterior acrosomal cap (PAS positive) and an underlying nucleus, shown by the Feulgen staining and acridine orange green fluorescence for DNA.
Since the gelatinous spermatophore was refractory to sectioning, late spermatids found in the lumen of the testis adjacent to the vas deferens were used for ultrastructural studies (Fig. 24). These spermatids or immature spermatozoa appear to have the same morphology and dimensions as mature spermatozoa. However, cytoplasmic organelles are present.

**Spermatids**

In general, the late spermatids in *Anaspides* contain an anterior acrosomal cap and an underlying cytoplasmic area containing the nucleus (Fig. 24). The posterior coiled tail extends through a nuclear groove to the base of the acrosome.

The cupula shaped acrosome is the most anterior spermatid structure (Fig. 24). It is membranous bound and contains a homogeneous matrix. A distinctive subacrosomal region is filled with a granular matrix. The anterior end of the tail projects through this subacrosomal region and is apposed to the subacrosomal membrane.

Underlying the acrosomal region is an area of cytoplasm containing the organelles associated with acrosomal synthesis. Rough endoplasmic reticulum and laminar or tubular membrane systems are present (Fig. 24). Some of these membrane systems still retain secretory vesicles connected with the acrosome. Also present are free ribosomes and large mitochondria with laminar cristae.

The nucleus is in a very condensed state, forming the posterior spermatid head region (Fig. 24). Only a few light granular chromatin regions are present within the nucleus. The nucleus is folded upon itself forming a groove through which extends the tail.
The anaspid tail does not have the features of a flagellum (Fig. 24). Instead, there is an outer wall, 50 nm thick, and a central amorphous core, 400 nm in diameter. The core extends to the acrosomal base. Closely packed microfilaments form the wall. Periodic striations (23 nm) are visible in longitudinal sections of the tail wall. At the level of the acrosomal base this wall material becomes diffuse, unconsolidated and expanded.

The tail extends posteriorly from the acrosomal base, through a nuclear groove. However, once beneath the nucleus, the tail coils upon itself two to three times. Some cytoplasm still surrounds the tail at this stage of spermiogenesis.

**Spermatophores**

The anaspid spermatophore is formed first from gelatinous secretions of the upper vas deferens (not figured). Then the outer spermatophore coat is secreted by the lower vas deferens. Within this inner spermatophore matrix, the spermatozoa are dispersed irregularly in packets of five to seven cells. Spermatids in the testis are packed closely together, so there exists a segregation accomplished upon release from the testis into the vas deferens. Also, the spermatozoa still retain their ring appearance, 12 μm diameter, with the tail coiled two times. The head region is on one side of the coiled tail.
DISCUSSION

Sperm Relationships with the Division Peracarida

Nebalia, Squilla, Euphausia, and Anaspides spermatozoa have unusual morphological features but three general conclusions are apparent when comparing their spermatozoa with peracarid and other aflagellate crustacean spermatozoa: (1) cytoplasmic processes with microfilamentous cores are widespread among aflagellate crustacean spermatozoa; (2) cellular synthesis and secretion of external sperm coats are widespread and account for the small change from a spermatid condition; and (3) distinct peracarid sperm features are found in Squilla and Anaspides spermatozoa.

Cytoplasmic Processes

Cytoplasmic processes are common among the various spermatozoa. Nebalia spermatozoa have cytoplasmic projections or arms supported by a packed array of microfilaments. The Anaspides sperm tail also has the same construction although restricted to one elongated process.

Other examples of cytoplasmic processes fit these two patterns. A packed array of microfilaments support the numerous sperm cytoplasmic projections in the Arctic fairy shrimp, Branchinecta paludosa, subclass Branchiopoda (Kutish, personal observation). In contrast, Hutchinsoniella macracantha, subclass Cephalocarida (Brown and Metz, 1967) has a single immotile tail structure constructed from filaments. Similar immotile tail structures, formed from filaments, are found in some malacostracans, e.g., the decapod carid shrimp spermatozoa of Palaemonetes paludosus (Brown, 1966a), Palaemon elegans (Pochon-Masson, 1969) and Hippolyte californiensis.
(Kutish, personal observation). The short tails have an orderly filament arrangement forming a striated pattern in longitudinal section. In fact, from early light optical studies, tail structures are seen in many carid shrimp (Grobben, 1906; Spitschakoff, 1909).

Perhaps aflagellate cytoplasmic tail processes are more common crustacean sperm features than originally supposed. However, the exact functional significance for the cytoprocesses is obscure. Adaptation for sperm transfer and sperm retention is one possibility. But the Branchiopoda, i.e., Artemia (Wolfe, 1971), leptostracans (Claus, 1888b), and anaspid (Smith, 1909) transfer the spermatozoa in spermatophores for external fertilization. In the peracarids, e.g., mysids, have external fertilization and no spermatophores (Nair, 1939; Nouvel, 1940; Labat, 1954).

**Cellular synthesis**

Brown (1969) has remarked for the branchiopod fairy shrimp that "the Artemia sperm has changed comparatively little from a spermatid." He further states (Brown, 1970) that "Particularly unusual for mature spermatozoa is the large amount of cytoplasm found surrounding the nucleus." A similar observation is made for the Nebalia, Euphausia and Squilla spermatozoa. Large amounts of cytoplasm with mitochondria, free ribosomes and cytomembranes are present in the mature spermatozoa.

In fact this small change from the spermatid condition is seen in many crustacean spermatozoa. For example, the sperm cytoplasm of other fairy shrimp, e.g., Branchinecta paludosa, Chirocephalopsis bundyi, and Polyartemiella hazeni (Kutish, personal observation) have an abundant
synthetically active cytoplasm around the nucleus. And these spermatozoa are from copulatory males. Another example of a spherical sperm with various organelles and mitochondria in the cytoplasm is found in the copepod *Calanus* (Brown, 1970).

The common feature among these spermatozoa is cell synthesis of an external cell coat and sometimes cytoplasmic processes. The spermatid in *Nebalia, Euphausia* and *Squilla* are actively engaged in synthesis and secretion of an external cell coat. *Nebalia* also forms cytoplasmic arms during late sperm maturation. In other species, the copepod *Calanus* (Brown, 1970) and the fairy shrimp *Chirocephalopsis* (Kutish, personal observation) both have an external cell coat.

Following this sperm maturation activity synthesizing cell coats, etc., the cytoplasmic organelles or remnants are left in place. Thus, the mature spermatozoa appear like a spermatid or typical somatic cell.

**Peracarid relationships**

Two similarities are found between the peracarid spermatozoa and the primitive malacostracan spermatozoa: (1) the anaspid and peracarid sperm tails are identical; and (2) the stomatopod and peracarid acrosome develop in a similar fashion.

Smith (1909) first remarked that the anaspid (*Anaspides tasmaniae*) sperm tail resembled the filiform peracarid sperm tails. At the ultrastructural level both the anaspid and peracarid sperm tails are indeed equivalent.

Both tails originate in the subacrosomal region and extend posteriorly. In one peracarid case, *Archaeomysis* (Kutish, personal observation),
the nucleus wraps around the sperm tail as in Anaspides. Both tails have an amorphous core and a thick supporting wall. The walls are formed from closely packed filaments which appear striated in longitudinal section. The only difference between the peracarids and the anaspid spermatozoa is found in the acrosome. No subacrosomal rod and vesicle are found in the very large anaspid acrosome complex.

The stomatopod acrosomal morphology and development, however, are similar to the pattern found in peracarid spermatozoa. In Squilla, the acrosomal vesicle and density develop next to the centrioles and the nucleus but in close association with the cell membrane. A long subacrosomal rod develops beneath the acrosome and extends to the nucleus. Extensive areas of cytoplasm are located at the opposite cell pole from the developing acrosome.

A similar spermatid cellular appearance during development of the acrosome is also seen in peracarid spermatozoa. The sperm acrosomal region of three orders Mysidacea (Kluge, 1966), Amphipoda (Reger, 1966), and Isopoda (Reger, 1964a; Fain-Maurel, 1966), develops with exactly the same morphology. In fact, late Squilla spermatids appear like peracarid spermatids before the unique peracarid tail develops.

The mature Squilla sperm acrosomal region also resembles the peracarid sperm acrosome (Kutish, personal observation). Both have a small acrosome with a basal density. A subacrosomal rod extends posteriorly to the nucleus. The Squilla acrosomal complex caps the nucleus, adjacent to the centrioles, and in all respects resembles a peracarid sperm acrosomal complex.
Phylogenetic considerations

Since each crustacean group has unique sperm patterns some ideas are projected to account for the sperm diversity. The unusual morphological features found in the primitive malacostracan spermatozoa, i.e., nonmotile tails, spermatid condition of the mature spermatozoa, external cell coats, do not alter the fact that the primitive crustacean spermatozoa is flagellated. A flagellated sperm pattern is found in such diverse groups as the subclass Mystacocarida (Brown and Metz, 1967), Branchiura (Brown, 1970) and Cirripedia (Barnes et al., 1971). In these groups vague reproductive pressures have selected the retention of a flagellated spermatozoa.

However, in the remaining crustacean groups, the conservative restraint on sperm morphology has been released. Environmental pressures affecting reproduction have provided an evolution of many sperm types. Such pressures could select for (1) better sperm transfer and retention by developing immotile tails, (2) sperm protection by secreting cell coats, or (3) sperm attachment to the egg and penetration by elaborating the acrosomal region.

These sperm patterns were established early in crustacean evolution. Due to selective pressures on reproduction, some groups retained a sperm flagella while others developed a nonmotile tail. Still other groups lost the nonmotile sperm tail or the flagellum and modified the sperm for spermatid secretion of a protective coat. Some abbreviated spermiogenesis, packaging spermatids in spermatophores.
In summarizing the Malacostraca sperm patterns, all variations but the flagellated condition are seen. And these various sperm morphologies reflect selective pressures on the reproductive pattern. Nebalia spermatozoa have a spermatid condition with cytoplasmic processes, possibly for egg attachment, and a protective cell coat. Squilla spermatozoa have a spermatid condition with a protective cell coat and an acrosomal development like peracarid spermatozoa. Anaspides and the peracarid spermatozoa both developed immotile tails for sperm retention. However, the anaspids have shifted to spermatophores. Euphausia has a spermatid condition with a protective cell coat and spermatophores. And finally, decapods have unleashed all tendencies with elaborate acrosome, small tails and morphologies encountered nowhere else in the Crustacea (c.f., Brown, 1966a; Chevaillier and Maillet, 1965; Hinsch, 1969, 1971, 1973; Langreth, 1969; Pochon-Masson, 1968a,b).
Plate 1

Figs. 1-3. *Nebalia pugettensis* spermatozoa.

Fig. 1. Spermatophore with 3 spermatozoa. The spermatophore has an inner and an outer wall (SpH), both secreted by the enclosed spermatozoa. The individual spermatozoa are very dense with a central nucleus (N) and peripheral cytoplasm containing mitochondria (M), dense vesicles (V), ribosomal aggregations (Rb), and projecting arms (CP). Lumen wall of testis (Ep). X 11,500.

Fig. 2. Detail of the sperm cytoplasm. Each sperm has one prominent granular aggregation of ribosomes (Rb) per cross section. Also present are mitochondria (M) with lamellar cristae and membrane systems of endoplasmic reticulum (ER). The cytoplasmic matrix is very condensed. Peripheral vesicular indentations line the surface of the cell membrane (arrow). X 60,000.

Fig. 3. Detail of cytoplasmic processes and peripheral cytoplasm. Cytoplasmic arms or processes extend from the cell surface. A distinct trench surrounds each process (arrow). Granular aggregation of ribosomes (Rb), mitochondria (M), cytomembranes (ER), and vesicular indentations (*). X 60,000.
Plate 2

Figs. 4-5. *Nebalia pugettensis* late spermatid.

Fig. 4. Late spermatids (6) and developing spermatophore. The spermatids have a central nucleus (N) with nucleolus (Nu) and a cytoplasm engaged in synthesis of the spermatophore matrix. Secretory vesicles (V) with matrix material for the inner and outer spermatophore wall. Areas of free ribosomes (Rb), mitochondria (M), and dense bodies, lysosomes with pigment granules (Ly) fill the peripheral cytoplasm. Profiles of cytoplasmic arms (CP) are visible at the cell periphery. Follicle cell (Ep) contributing to the outer spermatophore wall secretions. x 8,000.

Fig. 5. Detail of a developing cytoplasmic process. Microfilaments provide a cytoskeletal support (arrow) for the process and extend back into the peripheral cytoplasm. Mitochondria (M), free ribosomes (Rb). x 28,000.
Plate 3

Figs. 6-9. *Euphausia pacifica* spermatozoa and spermatophores.

Fig. 6. Spermatophore with 4 spermatozoa. The spermatophore has 3 areas: (1) an individual sperm cell coat with flabby projections secreted by the sperm; (2) a surrounding matrix secreted by the spermatozoa; and (3) an outer spermatophore wall secreted by the vas deferens epithelium. This spermatophore wall is highly structured with striations (arrow) on its inner surface. Surrounding the spermatophore are other vas deferens secretions (*) which form the actual spermatophore transferred to the female. The spermatozoa contain a central nucleus (N), an extensive mitochondrial array (M) at one pole of the cell, and peripheral secretory vesicles (V). x 11,500.

Fig. 7. Detail of the spermatophore wall showing striations in cross section. The peripheral sperm cytoplasm is secreting the intermediate spermatophore matrix (V) which passes through the sperm cell coat (*). Nucleus (N). x 20,000.

Fig. 8. Tangential section of the outer spermatophore wall showing the hexagonal packing which appears as striations in cross section. x 27,500.

Fig. 9. Developing sperm cell coat being secreted by an early spermatid. Secretory vesicles with cell coat material (V). Vas deferens epithelium with microvillus secretory border (Vd) contributing to the general spermatophore secretions. x 27,500.
Plate 4

Fig. 10. *Squilla empusa* mature ejaculated sperm. The mature sperm is encased in an external cell envelope (SpE), secreted by the sperm following spermiogenesis. A surrounding spermatophore matrix (Sph) secreted by the vas deferens is still present. An apical-basal sperm axis is defined by the anterior button shaped acrosome (A) and subacrosomal rod (AR) which indents into the nucleus (N). Partially visible is a nucleolar region (Nu). The basal cytoplasm contains the vestiges of mitochondria (M), free ribosomes (Rb), and coalesced cytomembranes (ER). All of these structures were associated with the prior synthesis of the sperm envelope and acrosome. x 20,000.

Fig. 11. Spermatozoa ejaculated from the penis with nucleus (N) acrosome (A), and basal cytoplasm (Cy). Nomarski DIC, x 1500.
Plate 5

Figs. 12-16. *Squilla empusa* mature spermatozoa.

Fig. 12. Midsagittal section of the acrosome (A). The subacrosomal rod projects (AR) from a nuclear (N) indentation into the acrosomal membrane. Cytoplasmic processes (CP) project from the sperm surface adjacent to the acrosome. x 50,000.

Fig. 13. Subacrosomal rod (AR) in transverse section. The centrioles (C) lie adjacent to the acrosome and the nucleus. In this region the cell surface has numerous cytoplasmic grooves (G). x 50,000.

Fig. 14. Cytoplasmic grooves (G) in sagittal section. x 20,000.

Fig. 15. Apical sperm surface. The grooves (G) do not extend very far from the surface. x 30,000.

Fig. 16. Basal cytoplasm with well-defined mitochondria (M), endoplasmic reticulum (ER), and ribosomes (Rb). Sperm envelope (SpE). x 20,000.
Plate 6


Fig. 17. Spermatids in the vas deferens (VD) at the level of the 4th abdominal segment in front of the testis. The acrosome is forming in these spermatids (A). Methylene blue, x 700.

Fig. 18. Spermatid from the region shown in Fig. 17. At the stage of acrosomal synthesis, the nucleus (N) has a very dispersed chromatin and a well-developed nucleolar region (Nu). The peripheral rim of cytoplasm is scant but contains mitochondria (M) and cytomembranes. The centrioles (Ce) are already located by the developing acrosome (A). In this region the vas deferens (ER) is secreting some of the spermatophore matrix (SpM). x 8,000.

Fig. 19. Early acrosomal vesicle (A) being formed. x 20,000.

Fig. 20. Developing acrosome (A) in a more condensed state. The sub-acrosomal rod (AR) begins at this stage. x 30,000.

Fig. 21. Later stage in the acrosomal development. The acrosome now has the mature form with a basal dense plate, and the sub-acrosomal rod projecting back but not into a nuclear indentation. x 30,000.
Plate 7

Figs. 22-23. Diagrammatic representation of mature spermatozoa. Acrosomal rod (AR), acrosomal cap (A), cytoplasmic processes (CP), centrioles (Ce), core (C), endoplasmic reticulum (ER), grooves (G), mitochondria (M), nucleus (N), nucleolus (Nu), tail (T), wall (W).

Fig. 22. Squilla empusa. The spermatophore wall is not figured so as to emphasize the anterior periacrosomal cell membrane modifications into grooves and cytoplasmic processes. Also, the acrosome and subacrosomal rod complex are enlarged (not to scale).

Fig. 23. Anaspides tasmaniae. The tail core extends to the subacrosomal membrane; however, the wall separates into its component microfilaments in the subacrosomal region. Note that the tail lies in a nuclear groove.
Plate 8

Figs. 24-25. *Anaspides tasmaniae* mature sperm and spermatids.

Fig. 24. Mature sperm from a spermatophore, Feulgen nuclear stain. The tail (T) is coiled two times on itself before entering the head which contains the nucleus in the base and the acrosome at the apex. x 1000.

Fig. 25. Late spermatids (2) in the lumen of the testis at the entrance to the vas deferens. Each sperm head has an apical acrosome (A) and granular subacrosomal matrix (*). The posterior head region contains the nucleus (N). In between, the nucleus and acrosome are cytoplasmic remnants with mitochondria (M), cytomembranes and vesicles (ER) still contributing to the subacrosomal matrix. The tail (T) has an inner core (C) which extends to the acrosomal base and an outer wall (W). In the nuclear region, the tail lies in a nuclear groove. More posteriorly, the tail is coiled two times (arrow) within the spermatid cytoplasm. Intervening follicle cell (Fl) with extracted lipid droplets. x 11,500.
PART II. BROOD POUCH STRUCTURE AND SPERM MORPHOLOGY IN THE ORDER MYSIDACEA (PERACARIDA - ARCHAEMYYSIS, ACANTHOMYSIS, HETEROMYSIS)
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LITERATURE REVIEW

The filiform spermatozoa of the peracarid crustaceans are unique among animal spermatozoa (Wilson, 1925). A typical flagellated sperm consisting of an anterior head, middle connecting mitochondrial region, and posterior motile tail is not present in the peracarid crustaceans. In fact, the peracarid spermatozoa are different from all other crustacean spermatozoa (Calman, 1909; Retzius, 1909a,b; Brown, 1966a).

Characteristically, the peracarids have a fusiform nuclear containing head region pendant on the anterior end of an extremely long immotile tail (Retzius, 1909a). This stereotyped sperm pattern is reinforced when the tail ultrastructure is examined (Reger, 1966; Reger et al., 1970; Fain-Maurel, 1966). Only an amorphous core and solid wall are seen in cross section (Kasaoka, 1974; Reger and Rain-Maurel, 1973). In longitudinal section, the sperm tail wall shows only a repeating striated pattern (Blanchard et al., 1961). There is no resemblance between the peracarid sperm tail and the microtubular arrangement of a typical sperm flagellum.

In spite of the unique morphology compared to other crustaceans, peracarid orders, i.e., Mysidacea (Kasaoka, 1974), Cumacea (Reger and Fain-Maurel, 1973), Isopoda (Fain-Maurel, 1966) and Amphipoda (Reger, 1966), have a similar morphology. Only the order Tanaidacea, which have a spherical sperm containing an enormous decapod-like acrosome and numerous cytoplasmic processes, do not retain the peracarid sperm pattern (Kutish, personal observation).

Such a morphological unity for the peracarid spermatozoa implies a common function pattern for the spermatozoa among the various orders; and
a common origin for this reproductive pattern. The very peculiar tail and pendant head region must be adapted for some aspect of fertilization in the peracarid marsupium. Perhaps, this adaptation is for spermatophore production, sperm transfer or sperm storage in the marsupium prior to ovulation. Kluge (1966) suggests that the filiform tail is a supporting structure which positions the sperm for initial sperm-egg interaction.

Whatever the peracarid sperm function is, its origin is even more obscure and perhaps ancient. Peracarid crustaceans appear as a major taxon during the lower Mississippian and were the principal caridoid crustaceans during the late Paleozoic (Schram, 1969b). Since these fossil peracarids had marsupiums (Brooks, 1962a,b), the unique filiform sperm morphology may have been present also. The assumption is that the peracarid sperm morphology is adapted to fertilization in a marsupium and developed as the marsupial incubation behavior developed. However most peracarid sperm studies do not consider the entire reproductive process (Reger, 1964a,b, 1966; Fain-Maurel, 1966). As such, no satisfactory explanation is available for the peracarid filiform sperm morphology.

This study examines the order Mysidacea which retain most completely the primitive peracarid reproductive pattern, the primitive "caridoid facies," and which most resemble the oldest caridoid fossils (Caiman, 1909; Tattersall and Tattersall, 1951; Siewing, 1956; Schram, 1974c). In particular three ecologically and morphologically diverse mysids in the family Mysidae were selected: (1) a primitive mysid in the subfamily Gastroscaccinae, Archaeomysis grebnitzkii, which lives intertidally buried in the sand; and two morphologically advanced mysids in the very large
subfamily Mysinae, (2) Acanthomysis sculpta, in the tribe Mysini, which
live in kelp beds, and (3) Heteromysis formosa in the tribe Heteromysini,
which is a littoral scavenger. A broad view of the peracarid reproductive
process, including sperm and spermatophore morphology, the penis, and the
female marsupium of these mysids will be examined in order to gain a func­
tional understanding of the peracarid sperm morphology. It will be shown
that specific marsupial structures exist which promote retention of the
filiform sperm until ovoposition has occurred.
MATERIALS AND METHODS

List of Species

Breeding mysids were obtained from the following three sources:

Suborder Mysida

Family Mysidae

Subfamily Gastroscincinae


Subfamily Mysinae


Identification was based upon descriptions in Tattersall (1932a,b, 1951), Tattersall and Tattersall (1951), and Ii (1964). Mysids were maintained in wide mouth one gallon pickle jars, filled with sand 4 cm deep, and covered with nylon screening, 2 mm mesh. The pickle jars were aerated to increase water circulation and placed under running sea water or in an Instant Ocean aquarium. A 10°C water temperature and a light/dark cycle of 16 and 8 hours were used. The mysids were fed pieces of ocean catfish.

Mature spermatozoa were observed by two methods. Spermatozoa from spermatophores stored in the penis were collected by ejaculation. Penile stimulation with a dissecting needle and elevation of the ambient temperature were used to cause spermatophore emission from the penis. Alterna-
tively, spermatozoa from spermatophores stored in the seminal vesicles were examined. The male reproductive system was dissected from the body cavity and the seminal vesicles opened to release the spermatophores. In both cases, sperm suspensions in sea water were obtained by allowing spontaneous dissociation of the spermatophores. The sperm morphology was examined with phase contrast and Nomarski differential interference optics. Samples were also fluorochromed (0.01% acridine orange in sea water) for fluorescent differentiation of the nucleus (green), tail and acrosome (both red).

The gross morphology of the marsupium, oostegites, and penis was viewed with a dissecting microscope. More detailed examinations of dissected tissues were made with bright field, phase contrast, and Nomarski optics. The tissues were viewed in the following preparations: (1) freshly dissected in sea water; (2) fixed in 10% neutral formalin; (3) cleared in lactic acid; or (4) stained in acetocarmine.

Spermatozoa and the marsupium were prepared for transmission and scanning electron microscopic observation as follows. Spermatozoa stored in the penis were used for ultrastructural studies. Males were anesthetized in cold (2°C) sea water. The penis was removed with forceps and immersed in the fixative. For marsupial observations, females were cold anesthetized, immersed in the fixative and then cut in half along the midsagittal axis. In this way, an internal view was possible of the intact marsupium.

Tissue samples prepared for transmission and scanning electron microscopic examination were fixed in (a) 3% glutaraldehyde with 1%
acrolein in 0.2 M sodium cacodylate buffer (pH 7.5) or (b) 3% glutaraldehyde with 0.15 M sodium chloride in 0.2 M phosphate buffer (pH 7.5) for 2 hours at room temperature. The tissues were then washed briefly in buffer, and postfixed with 1% osmium tetroxide in the appropriate buffer for 2 hours at room temperature.

For transmission electron microscopy, the tissues were dehydrated with ethanol and embedded in Spurr's (1969) plastic or epon (Luft, 1961). Sections were cut on a Sorvall Porter-Blum MT-2 microtome with a diamond knife or tungsten coated glass knives, stained with uranyl acetate and lead citrate (Venable and Coggeshall, 1965) and viewed with a Hitachi Hu-lle-l electron microscope.

For scanning electron microscopy, the tissues were dehydrated with 2,2-dimethoxypropane (Muller and Jacks, 1975), washed in methanol-acetone (1/1, v/v) and critical point dried with carbon dioxide. The dried specimens were mounted on stubs with silver conducting paint, coated by vacuum deposition with gold-paladium (60/40) and viewed with a JEOLCO JSM-l scanning electron microscope.

List of Terms

Terms used in this study include the following:

Oostegites - brood lamellae extending ventrally from the coxae of the thoracic legs. Primitively there are seven oostegites. The 6th oostegite carries the genital orifice.

Marsupium - ventral brood cavity beneath the sternum; formed by the imbrication of the oostegites. Spermatozoa are retained in the marsupium by special acanthus setae on the oostegites.

Plueral plate - ventral extension of the lateral abdominal body wall; a primitive character.
OBSERVATIONS

The following mysid reproductive features were observed: (1) sperm morphology; (2) spermatophores; (3) penis; (4) marsupium. Some brief observations are made also about feeding function in relation to the brooding function of thoracic legs.

Sperm Morphology

Comparisons

The mysid spermatozoa in Archaeomysis grebnitzkii, Acanthomysis sculpta, and Heteromysis formosa exhibit the same morphological and structural features for the acrosome, nucleus, and tail (Figs. 25, 26, 27). Only two variable features are observed: 1) the manner of tail attachment to the nuclear containing head region; and 2) the occasional persistence from spermiogenesis of mitochondria and membranes.

In A. sculpta and H. formosa, the spermatozoa have a uniform appearance. On the anterior end of the long filiform nonmotile tail is a fusiform nuclear containing region or head. At the apex of the head and tail lies the acrosome and subacrosomal rod. The only singular difference in A. grebnitzkii involves the tail which penetrates the nucleus as an anterograde intussusception. This distal invagination passes through the nucleus to the anterior acrosomal end.

The structural features, common to all three mysids, include an apical acrosomal vesicle, subacrosomal rod and vesicle, a more posterior pair of centrioles and nucleus. Size variations are the only differences. The apical acrosomal vesicle is small and clavate (A. grebnitzkii),
conical with a laminar extension along the subacrosomal rod (*A. sculpta*), or lanceolate (*H. formosa*). Immediately posterior is a subacrosomal vesicle containing a retrograde invagination for the microtubules of the subacrosomal rod.

Similarly, the tail has a uniform construction in all three mysids. Only an amorphous core and solid wall is seen in cross section. The wall is formed from numerous closely packed microfilaments which are aligned longitudinally to the tail plasma membrane. Because of the alignment, these microfilaments appear striated, with a repeating pattern in longitudinal section. The core is also attached to the wall in a regular, repeating manner.

As was mentioned, the tail-head attachment has two patterns. In *A. sculpta* and *H. formosa*, the tail narrows anteriorly, attaching beneath the acrosome by means of a short anterograde intussusception or invagination of the lateral head plasma membrane. However, in *A. grebnitzkii*, the intussusceptive tail passes through a distal invagination in the head. The resultant anterograde indigitation extends through the nucleus and along the subacrosomal vesicle to the acrosomal base. Only then does the tail plasma membrane form a intussusceptum by continuing into the head plasma membrane.

So far only a comparison of similar features has been made, which indicates a common plan within the mysids. However, there are specific patterns which serve to distinguish the three spermatozoa even at the ultrastructural level.
Archaeomysis grebnitzkii

In the light microscope, the sperm of *Archaeomysis* (Figs. 43, 46) is easily identified by the clavate acrosome, large subacrosomal vesicle, short nucleus and tail (350 μm). See Table 1 for dimensions. However, the most unique feature is the intussusception of the tail through the nucleus. Because of this, the head is not pendant from the tail as it is in the other mysids.

**Acrosome** The acrosomal region (Figs. 49, 67) occupies the most anterior portion of the sperm and has three distinct components: an apical acrosome, a subacrosomal vesicle, and an acrosomal rod. Both the spherical acrosome (1 μm diameter) and the cylindrical subacrosomal vesicle (10 μm long by 1 μm) are membranous bound (Figs. 52, 53, 54). However, the subacrosomal vesicle (Fig. 25) contains a retrograde invagination to the distal end of the acrosomal rod (140 nm diameter). About 26 distinct microtubules (10 nm diameter) are contained within the rod matrix (Fig. 52).

Occasionally, spontaneous acrosomal reactions were observed with the light microscope (Fig. 44). These consisted of an anteriorly projecting rod, 5 μm long. Concurrently, the bulge containing the subacrosomal vesicle disappeared, leaving a concave indentation in the sperm head. No tail response was observed. The number of acrosomal reactions was not sufficient to be examined with the electron microscope.

**Tail** The entire tail length (Figs. 49, 50, 51) presents an invariant structure. In cross section, the tail contains a central core (300 μm diameter), a surrounding wall (70 nm thick), and an outer plasma
Table 1. Mysid sperm dimensions

<table>
<thead>
<tr>
<th>Structure</th>
<th>Archaeomysis</th>
<th>Acanthomysis</th>
<th>Heteromysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tail periodicity</td>
<td>79 nm</td>
<td>87 nm</td>
<td>76 nm</td>
</tr>
<tr>
<td>Tail length</td>
<td>350 μm</td>
<td>600 μm</td>
<td>1200 μm</td>
</tr>
<tr>
<td>Nuclear length</td>
<td>30 μm</td>
<td>150 μm</td>
<td>90 μm</td>
</tr>
<tr>
<td>Acrosome</td>
<td>0.5 μm</td>
<td>10 μm</td>
<td>14 μm</td>
</tr>
<tr>
<td>Subacrosomal vesicle</td>
<td>8 μm</td>
<td>8 μm</td>
<td>1.5 μm</td>
</tr>
<tr>
<td>Tail/head</td>
<td>12</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>Sperm-spermatophore</td>
<td>120±5</td>
<td>101±1</td>
<td>13±3</td>
</tr>
</tbody>
</table>

membrane (Figs. 54, 55, 56). Numerous closely packed longitudinal filaments form the wall. An orderly parallel arrangement of filaments (6 nm each) is visible in longitudinal sections (Fig. 50) as periodic (79 nm) striations. The core has no specific structure except that it is attached to the largest electron dense repeating band in the wall (Fig. 50).

The overall dimensions of these tail components (Table 2) vary with the region. Starting beneath the acrosome, the tail is solid for a short distance. Then a core appears. In general, as the tail courses through the nuclear invagination, the core increases in diameter (Fig. 50). Posteriorly, the core decreases in thickness (Fig. 56). At all regions, the supporting wall has the same thickness and striated structure. In this way the tail tapers at both ends.
Table 2. Mysid sperm tail dimensions

<table>
<thead>
<tr>
<th>Genus</th>
<th>Tail level</th>
<th>Tail diameter</th>
<th>Wall thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Archaeomysis</td>
<td>Anterior</td>
<td>150 nm</td>
<td>70 nm</td>
</tr>
<tr>
<td></td>
<td>Middle-head</td>
<td>330</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Posterior</td>
<td>500 to 150</td>
<td>70</td>
</tr>
<tr>
<td>Acanthomysis</td>
<td>Anterior</td>
<td>220 nm</td>
<td>70 nm</td>
</tr>
<tr>
<td></td>
<td>Middle-head</td>
<td>600, 1000 (oval)</td>
<td>70, 150</td>
</tr>
<tr>
<td></td>
<td>Posterior</td>
<td>600 to 150</td>
<td>70</td>
</tr>
<tr>
<td>Heteromysis</td>
<td>Anterior</td>
<td>250 nm</td>
<td>40 nm</td>
</tr>
<tr>
<td></td>
<td>Middle-head</td>
<td>410</td>
<td>70 nm</td>
</tr>
<tr>
<td></td>
<td>Posterior</td>
<td>520 to 240</td>
<td>70</td>
</tr>
</tbody>
</table>

A peculiar feature of Archaeomysis spermatozoa is the apparent nuclear perforation by the tail (Fig. 57). Actually, the tail enters the nuclear head region and attaches beneath the acrosome by an intussusception (Fig. 49). This tail arrangement is most obvious posteriorly in longitudinal section where the external plasma membrane infolds upon itself and the tail enters the invagination (Fig. 51). Beneath the acrosome, the tail plasma membrane turns back on itself and is continuous with
the invaginated head plasma membrane (Fig. 49). Therefore, an extracellular space bounds the sperm tail within the head region (Fig. 55).

**Head** The nucleus extends posteriorly from beneath the acrosome for 30 μm, wrapping around the subacrosomal vesicle and the tail invagination (Figs. 53-57). Because the tail extends in this invagination from the distal to the proximal and of the head, the nucleus appears to have a donut topology. However, careful study of the nuclear membranes shows that the nucleus definitely wraps around the tail and thus has a spherical topology. Sometimes two nuclear plasma membranes are visible. But more often these membranes are fused with the cellular plasma membrane. A characteristic filamentous chromatin is present within the nucleus irrespective of the fixation technique.

**Acanthomysis sculpta**

The spermatozoa of *Acanthomysis* are characterized by a filiform tail with a long (150 μm) pendant nuclear containing head (Fig. 48). At the apex is a conical acrosome. Unique features (Fig. 46) include the persistence of membranes and mitochondria adjacent to the nucleus; the attachment of the tail beneath the acrosome and adjacent to the large subacrosomal vesicle by means of a short invagination; and a laminar extension of the acrosome along the subacrosomal vesicle.

**Acrosome** The acrosome occupies the same position as in *Archaeomysis* and has the same three components (Figs. 58, 59). Apically above the tail insertion, the acrosome is conical (2 μm long) and extends posteriorly as a lamina along the periphery of the subacrosomal vesicle. Between this laminar extension and the tail lies a cylindrical (1 μm by 10
10 µm) acrosomal vesicle (Fig. 60). As in Archaeomysis, there is a retrograde invagination which contains the acrosomal rod (40 nm diameter). Numerous microtubules are in the acrosomal rod matrix. Beneath, a pair of centrioles are located. More posteriorly is the nuclear containing head region.

**Head** The nucleus extends below the acrosome for 150 µm and fills the streamer shaped head (Figs. 48, 60, 63). Throughout the nucleus is a finely dispersed chromatin. A double nuclear membrane is not always apparent, sometimes being fused to the cell plasma membrane. Only elongate (250 µm) mitochondria with tubular cristae fill the perinuclear space. These mitochondria are not embedded inside the nucleus.

**Tail** For the entire length, the tail shows a uniform construction as in Archaeomysis. Numerous closely packed filaments 4-5 nm thick line the tail plasma membrane to form a supporting wall (Fig. 61). In longitudinal sections, periodic striations (87 nm repeat) are visible (Fig. 62). The core has no specific structure except that it is always attached to the same repeating band of the wall.

The overall dimensions of the core and wall vary (Table 3). Also, there is a distinct kidney shaped bilateral asymmetry in the tail adjacent to the nucleus (Figs. 60, 61, 63). Starting beneath the acrosome, the tail is round in cross section with a core continuous into the head region. However, in the midregion, the tail becomes ellipsoidal with one wall being twice the thickness of the other wall (Figs. 60, 61). Posteriorly below the head, the tail is again round in cross section but the bilateral wall asymmetry is still visible (Fig. 63). One side of the wall
is slightly thicker than is the other side. This asymmetry is aligned in all of the tails within each spermatophore.

**Heteromysis formosa**

The spermatozoa of *Heteromysis*, as in *Acanthomysis*, are characterized by a very long, 1.2 mm, filiform tail, with a pendant nuclear containing head (Fig. 47). At the apex is a long, 10 μm, unique lanceolate acrosome. Unique features include a very small subacrosomal vesicle and rod (Fig. 27); the attachment of the tail beneath the subacrosomal vesicle by means of a short intussusception; and an extensive array of perinuclear membranes.

**Acrosome** The apical end of the sperm, as in *Acanthomysis*, contains the lanceolate acrosome above the tail (Figs. 47, 64-67). The distal portion of this vesicle lies adjacent to a small membrane bound subacrosomal vesicle. An acrosomal rod (60 nm diameter by 1.1 μm long) fills an apical retrograde invagination of the subacrosomal vesicle. Within this invagination are numerous microtubules (20 nm diameter). Beneath the acrosome and adjacent to the tail insertion are 2 centrioles.

**Head** As in *Acanthomysis*, the nucleus of *Heteromysis* extends beneath the acrosome and fills the spindling head (Figs. 47, 64, 68). A finely dispersed chromatin is present and bounded by 2 distinct nuclear membranes. Surrounding the nucleus are up to 15 pair of membranes. These perinuclear membrane arrays also entirely fill the posterior region of the head (Fig. 68).

**Tail** The tail is uniformly constructed (Figs. 68, 69). As in the other mysids, there is an outer plasma membrane, a supporting wall and an
amorphous core. Closely packed filaments line the wall. Their alignment gives the wall a cross striated appearance (76 nm repeat) in longitudinal section. An amorphous core fills the center and is attached to the wall at the periodicity for the entire tail length.

As in Archaeomysis, the overall tail dimensions vary (Table 2). Near the insertion beneath the acrosome, the wall is very thin (Fig. 64). However, for the remaining length, the tail wall is uniform in thickness. Only the core diameter varies.

Spermatophores

The spermatophore morphology and manner of storage are the same in all three mysids examined (Fig. 45). In general, a specific number of spermatozoa are aligned parallel to each other and in register. The heads are peripheral while the tails are central (Fig. 63). Parallel to the spermatozoa are numerous extracellular filaments (45 nm diameter). These filaments extend from the most anterior acrosomal region to the most posterior region of the spermatozoa in the spermatophore (Fig. 61). For any cross sectional level, at least one layer of filaments separate the spermatozoa from each other.

Spermatophores are stored in the seminal vesicle region of the efferent duct and also in the penis. Each of the regions represent dilations of the ejaculatory duct leading from the testis. Depending on the reproductive condition of the male, as many as 100-200 spermatophores may be seen in cross sections of the two ejaculatory duct regions. In both
cases the numerous spermatophores are oriented so that the acrosomal region is directed towards the genital orifice.

Upon ejaculatory release from the penis, the individual spermatophores separate but remain initially intact (Fig. 45). In sea water the propelling seminal fluid does not coagulate or encase the spermatophores but disperses. Dissolution of the spermatophores occurs slowly (30 min) until the spermatozoa are completely liberated into a homogeneous distribution (Fig. 46).

Specific spermatophore features are as follows. Archaeomysis has the largest spermatophores (0.5 mm long, 0.1 mm diameter) with approximately 120 spermatozoa (Fig. 45). The encasing filaments are 45 nm in diameter (Fig. 54). Acanthomysis and Heteromysis have small spermatophores. In Acanthomysis, approximately 10 spermatozoa are packaged in an individual spermatophore, 0.7 mm long and 10 μm in diameter (Fig. 63). The encasing filaments are 40 nm in diameter (Fig. 61). A distinctive feature of these spermatophores is the alignment of the tail asymmetry within the spermatophore. Heteromysis has about 13 spermatozoa per spermatophore which measures 1.3 mm long by 10 μm in diameter. The extracellular filaments are 47 nm in diameter (Fig. 67).

Penile Structure

Comparisons

The penis is a long cuticular papilla (0.3-1 mm for 7-15 mm long mysids) projecting medially from the coxae of the 8th thoracic legs (Figs. 28, 29, 30). At the apical end is the genital orifice. In all three
mysids, the penis has an ejaculatory function, spermatophore storage function and a specific cuticular sculpture. All of these are associated with sperm transfer. Also the penis closes off the posterior end of the food groove.

Located within the penis is an extension of the hemocoel and the terminal end of the vas deferens (not figured). This region of the vas deferens is very muscular and expels the numerous spermatophores during sperm transfer. An extrinsic muscular system, associated with the cuticle, gives the penis limited movement. But most penile movement is caused by leg movement.

Spermatophore storage is also accomplished in the penis. This storage is one factor accounting for the large penile size.

The most diverse penile feature is the armature. Each mysid has a specific sculpture of flaps and lobes associated with the distal genital orifice. The margins may be covered by stout sensory setae, spines, plumose setae or it may be naked. This external variation of the penis serves to distinguish the three species.

Nonreproductive functions of the penis, involve closing the posterior food groove. The medial penile borders are in apposition and the lateral borders are setose in Archaeomysis and Acanthomysis. These two mysids are filter feeders. Heteromysis, which is a large particle scavenger with little filter feeding, lacks the border setation. Only the apical ornamentation associated with sperm transfer remains in Heteromysis.

Specific penile features are described as follows.
**Archaeomysis**  The external penile sculpture of *Archaeomysis* is the most highly ornate of the mysids examined (Fig. 28). These oblate cuticular papilla (0.45 mm diameter, 1.1 mm long in adults) project from the posterior 8th coxa and extend ventrally to the ischial article of the endopodite. Medial apposition of the right and left penes closes the food groove posteriorly.

An ornate sculpture of the distal lobes and lateral spines are characteristic. The lateral border has 8 stout setae with the basal 5 being pinate. On the distal end of the penis are 2 lobes. The genital orifice lies between the lobes on the anterio-medial border. The lateral lobe is simple while the medial lobe has an excavation. When closed on each other, these lobes control the ejection and placement of the spermato­phores.

**Acanthomysis**  This penis contains two lobes, similar to *Acanthomysis*, but with a variety of setation (Fig. 29). More particularly, the penis is a straight tube, triangular in cross section (0.1 mm across by 0.7 mm long) and extends ventrally from the posterior border of the 8th coxa. Nine short stout setae adorn the lateral border and one stout seta projects from the medial border. Characteristic for *Acanthomysis* is the row of 5 stout setae projecting over the genital orifice and between the two lobes.

**Heteromysis**  This penis is the simplest of the three mysids, retaining only some distal ornamentation around the genital orifice (Fig. 30). In relation to the body size (5 mm) the penis is enormous (25 μm diameter by 500 μm long). It extends ventrally from the 8th coxa to the
ischial article of the endopodite. Three lobes project over the genital orifice on the distal end. The anterior lobe has one stout seta; the posterior lobe is asetose; and the lateral lobe extends over the anterior lobe, ending with a nodular double callus.

Marsupium

Comparisons

To understand the derived marsupial pattern in Archaeomysis, Acanthomysis and Heteromysis, it is first necessary to review the more primitive pattern. The marsupium in the more primitive mysids, e.g., Lophogaster, is formed from medially directed coxal brood plates or oostegites on the 2nd to the 8th thoracic legs. Their setose borders interdigitate to maintain a protective incubatory space which is separated from the external environment during fertilization and embryo development. The setation and imbrication functions to prevent the exit of spermatozoa, eggs or embryos.

These functions persist in the three mysids examined. However, the number of anterior oostegites is reduced. The oostegite reduction is more in Archaeomysis (Figs. 31, 32, 34) than in Acanthomysis (Figs. 33, 35) or Heteromysis (Fig. 36). Reduction in oostegite number allows the separation of the anterior filter feeding from the posterior brooding function. Acanthomysis and Heteromysis both retain vestigial oostegites (Figs. 38, 39) on the genital appendage (6th leg) and some of the anterior legs (Figs. 35, 36). The acanthus setae (Fig. 74) of these oostegites project into the marsupial cavity and presumably retain the spermatozoa until
ovoposition (Figs. 35, 36). The last two oostegites (7th and 8th legs) serve to form the actual brood pouch or marsupium (Fig. 33). Plumose setae on these oostegites interlock in the midline to keep the brood plates together. In general the marsupia are similar in morphology.

Archaeomysis has a very different and extremely reduced marsupium (Figs. 31, 32, 34). Only a vestigial asetose oostegite (Fig. 37) is associated with the genital orifice and coxa (6th leg). Acanthus setae (Figs. 70, 71) associated with sperm retention are present on the posterior region of the 7th oostegite, instead of on the more anterior oostegites as in the other two mysids. Also, the marsupium is formed solely from the 8th oostegite (Fig. 31).

Specific marsupial features are described as follows.

Archaeomysis

In general, the marsupium is composed of setiferous plates or oostegites which project medially from the 7th and 8th coxa (Fig. 31). The 6th oostegite, covering the genital orifice, is vestigial (Fig. 37). Also, included is an enlarged plural plate from the 1st abdominal segment which gives lateral support to the 8th oostegite (Fig. 34).

Functionally, the pair of oval 8th oostegites form the entire marsupial cavity (Figs. 31, 34). They extend from the 1st abdominal to the 6th thoracic segment. For an adult female 2 cm long with 40 embryos, the cavity measures 4 mm wide, 5 mm long and 1 mm deep. Pinate setae, 300 μm long and 30 μm apart, line the ventral medial margin (Fig. 72). Setal interdigitation joins together medially the right and left oostegites. Covering the posterior dorsal surface and medial border are long (1 mm)
acanthus setae (Fig. 71). These very distinct setae fill the posterior marsupial cavity (Fig. 34). Sometimes sperm tails adhere to these setae.

The shoe horn shaped, lamellar 7th oostegite fills the anterior and middle portion of the marsupial cavity with setae and it also serves as an egg guide (Figs. 32, 40, 70). An anterior arched portion of the 7th oostegite covers the 6th oostegite and genital orifice (Fig. 32). In this region the margins bear short (0.7 mm) plumose setae, 30 μm apart. The posterior linquiform portion of the 7th oostegite lies apposed to the sternum. Here, as many as 40 long (1 mm) acanthous setae project into the marsupial cavity (Fig. 70).

The 6th oostegite is vestigial (Fig. 37) and serves as an egg guide. This asetose flap (100 μm diameter) covers the genital orifice on the posterior medial coxa. Both the 6th oostegite and the genital orifice are covered by an anterior extension of the 7th oostegite.

The 6th and the 7th oostegites also serve as egg guides (Figs. 32, 70). During ovoposition, the eggs are directed posteriorly by the 6th oostegite into an arched, vaulted part of the 7th oostegite. Then the eggs pass onto the setated surface of the 7th oostegite, and through the sperm laden setae to be fertilized. Thus these two oostegites direct the eggs posteriorly to the spermatozoa.

Acanthomysis

The marsupium in Acanthomysis (Figs. 33, 35) is similar to Heteromysis (Fig. 36) both in oostegite morphology and in construction but is distinctly different from Archaeomysis (Fig. 34). The marsupium is composed of oostegites on the 4th to the 8th legs. Only the 8th and 7th
oostegites are large and laminar, whereas the 6th oostegite covering the
genital orifice is a reduced flap and the 5th and 4th are diminutive
setose crests.

In Acanthomysis, the marsupium covers most of the ventral sternal
surface from the 1st abdominal to the 2nd thoracic segments (Fig. 33). As
such it severely obstructs the anterior portion of the food groove. Over­
all, the marsupium measures 1.2 mm wide, 1.5 mm long and 0.6 mm deep for a
10 mm female incubating 35 embryos.

Functionally, only the 8th and 7th oostegites form the marsupium,
while portions of the 7th to the 4th oostegites have special setae (Fig.
35). The 8th oostegite provides support for the lateral posterior and
ventral portions of the marsupium. This oostegite also supports the
posterior region of the 7th oostegite. In the anterior marsupial region,
the 7th oostegite provides support. A setated lobe on the posterior of
the 7th oostegite fills the posterior marsupial cavity with acanthus
setae (Figs. 41, 74). Acanthus setae from the 4th to 6th oostegites also
fill the anterior marsupial cavity. Presumably, these special setae re­
tain spermatozoa in the marsupium. Other plumose interlocking border
setae on the 7th and 8th oostegites keep the medial borders in apposition.

Specific morphological features of the 8th to 4th oostegites in
Acanthomysis are as follows. The 8th oostegite (Fig. 33) has the form of
a scoop shaped boat, posteriorly broad and anteriorly accuminate. Ex­
tending from the posterior coxal surface, this last oostegite covers all
of the other oostegites. Pinate setae, 200 nm long and spaced 20 nm
apart, line the margins. Setae from opposite borders interdigitate along the medial line.

The 7th oostegite (Figs. 35, 41) is a narrow semilunar lamina extending from the 8th to the 3rd thoracic sternite. Posteriorly, the border is deeply incised, forming a digiform lobe (Fig. 41). A keel is formed by the interdigitation of the anterior medial borders on the right and left oostegites. Lining the anterior border are short pinate setae 300 \( \mu m \) long, and 30 \( \mu m \) apart. Quite distinct are the remaining setae extending from the posterior margin and digiform lobe. These acanthus setae (Fig. 74) are very long (up to 500 \( \mu m \), 30 \( \mu m \) apart) and fill the posterior marsupial cavity (Fig. 35).

The remaining 4th, 5th, and 6th oostegites are small rudimentary ventrally directed flaps from the medial coxal face which decrease in size anteriorly (Fig. 35). In size, the 6th oostegite, which covers the genital orifice, is 100 \( \mu m \) in diameter (Fig. 38), while the remaining oostegites are setose crests. The margins have long (300-400 \( \mu m \)) pinate to acanthus setae. Eight to ten setae extend from the 6th oostegite with less on the more anterior oostegites. These setae fill the anterior marsupial cavity in distinction to the setae of the 7th oostegite which fill the posterior marsupial cavity. Presumably spermatozoa are retained by the setae until ovoposition occurs.

**Heteromysis formosa**

The oostegite morphology and marsupial construction of *Heteromysis* (Fig. 36) are very similar to that of *Acanthomysis* (Fig. 35). The only difference is the absence of the vestigial 4th and 5th oostegites. Even
the digiform posterior lobe on the 7th oostegite is present in *Heteromysis* (Fig. 42).

The marsupium covers most of the ventral sternal surface from the 1st abdominal to the 2nd thoracic segments in ovigerous females (not figured). When empty, the marsupium extends only to the 4th thoracic segment, obstructing most of the anterior food groove.

Functionally, as in *Acanthomysis*, only the 8th and 7th oostegites provide support for the marsupium (Fig. 36). The 6th oostegite and part of the 7th oostegite fill the marsupial cavity with setae. Lateral, posterior and ventral enclosure is provided by the 8th oostegite. This oostegite also supports the posterior region of the 7th oostegite. Anteriorly, the 7th oostegite provides support and covers the 6th oostegite. As in *Acanthomysis*, a setated lobe (Fig. 42) and the margin on the 7th oostegite fill the marsupium with acanthus setae (not figured). The anterior marsupial cavity is filled with acanthus setae from the 6th oostegite. Presumably, these special setae retain the spermatozoa in the marsupium. The marginal setae restrain the oostegite borders in medial apposition.

Specific morphological features of the 8th to the 6th oostegites are as follows. The 8th oostegite is a scoop shaped plate on the inner coxal face of the 8th leg (Fig. 36). Its anterior extension in the food groove prevents the apposition of all but the food filtering setae on the bases of the 2nd to the 4th legs. Two deep incisions, forming two rounded stoutly setated lobes, adorn the anterior end (Fig. 36). The medial margins are closely setated (150 μm long, 40 μm apart). Also, they are
turned dorsally, partially dividing the marsupium into right and left chambers. The right and left marginal setation interdigitated.

The laminar 7th oostegite is acuminate anteriorly and extends from the 7th coxal face to the 3rd thoracic leg (Fig. 36). As in Acanthomysis, the posterior border is deeply incised forming a falcate lobe (Fig. 42). Border setation is similar to the 8th oostegite, interdigitating anteriorly. Setation on the falcate lobe and posterior margin fills the posterior marsupial cavity.

The 6th oostegite is a small rudimentary flap (100 μm diameter) on the posterior medial coxal face (Fig. 39). It covers the genital orifice. As in Acanthomysis, the margins have 6 long acanthus setae (0.2 mm) which fill the anterior marsupial cavity.
DISCUSSION

Sperm Transfer and Marsupial Fertilization - a Peracarid Model

A comparison of the mysid spermatozoa, spermatophores, penes, and marsupial structures points to three conclusions concerning peracarid reproductive biology: (1) the mysid sperm structure has a typical peracarid sperm morphology and serves as a model for the other peracarid sperm patterns; (2) special marsupial structures aid sperm retention; and (3) mysid sperm transfer for fertilization in the marsupium represents an original peracarid condition.

Peracarid sperm structure

The Mysidacea were chosen since they are the most primitive peracarid order compared to the Cumacea, Tanaidacea, Isopoda, and Amphipoda. Therefore, the mysid spermatozoa should show the relationships among the peracarid sperm morphologies. In general, the mysid spermatozoa serve as a model for the other peracarid orders except the tanaids; and there are some variations in mysids sperm morphology which point to interesting phylogenetic relationships among malacostracan spermatozoa.

Peracarid sperm model

The mysid spermatozoa of Archaeomysis, Acanthomysis and Heteromysis are constructed with the same plan as the amphipod (Reger, 1966), isopod (Fain-Maurel, 1966), and cumacean (Reger and Fain-Maurel, 1973) spermatozoa. In each, the typical sperm has an apical acrosomal vesicle, subacrosomal rod and vesicle, and centrioles above the pendant nucleus. A filiform tail attaches to the head beneath the acrosomal complex.
The most consistent feature of the peracarid sperm plan is the tail ultrastructure. Closely packed microfilaments line the tail plasma membrane, forming a rigid supporting wall. The orderly arrangement of the filaments accounts for the repeating striated tail appearance in longitudinal section. Centrally, in the tail has an amorphous core. The anterior continuation of the tail core and plasma membrane serve as the articulating connection between the tail and the head. Only the tanaid family, Paratananidae, does not follow this filiform model for the peracarid spermatozoa.

**Sperm variations** Although the above description is the peracarid model, there are some interesting variations between the peracarid orders and also within the mysids. Firstly, of all the peracarid orders the Mysidacea have the thinnest supporting tail wall (1/5th the tail diameter). In the isopods, amphipods and cumaceans 1/2 to 4/5ths of the tail diameter is formed by the tail wall (Reger and Fain-Maurel, 1973). There is a distinct trend for increased tail rigidity by increasing the supporting wall thickness.

Secondly, there is a distinct difference between the head-tail connection in the order Amphipoda and the orders Mysidacea, Cumacea, and Isopoda (Retzius, 1909a). In amphipods, the head freely articulates 180 degrees with the tail, whereas in the mysids and isopods the tail articulation is restricted. This restricted movement is due to the tail entering an invagination in the head before actually attaching to the head. Such a head invagination is not present in the amphipods. The
unrestricted head movement about the tail in amphipods probably allows a more effective sperm-egg interaction.

Within the mysids many more variations occur than exist in the other peracarid orders. All previous studies have examined mysid spermatozoa from two closely related genera, *Mysis* (Frey and Leuchart, 1847; Sars, 1867; Gilson, 1886; Retzius, 1909a; Holmquist, 1959; Labat, 1962; Reger and Fain-Maurel, 1973; Reger et al., 1970) and *Neomysis* (Kasaoka, 1974; Kluge, 1966). These are advanced mysids in the subfamily Mysinae (Tattersall and Tattersall, 1951). When the distantly related species in the subfamily Mysinae (*Acanthomysis* and *Heteromysis*) are compared with the more primitive subfamily Gastrosaccinae (*Archaemysis*) variations are seen in the mysid sperm acrosome and tail. *Archaemysis* and *Acanthomysis* have relatively large acrosomes, subacrosomal rods and vesicles compared with the diminutive acrosomal complex in *Heteromysis*. The acrosomal complex of *Heteromysis* is entirely above the tail insertion, while the acrosomal cap in *Acanthomysis* extends along the subacrosomal rod, below the tail insertion.

However, the most important sperm variation occurs in the primitive mysid, *Archaemysis*. The pendant head morphology, typical of other peracarid is not present. Instead, there is a ventral head invagination where the tail extends anteriorly through the head, attaching beneath the acrosome. Also, the nucleus is wrapped around the tail, as is found in the sperm of the related division Syncarida, i.e., *Anaspides* (Kutish, personal observation). The sperm morphology in *Archaemysis* is unexpected and an examination of the more primitive mysid genera may provide more
insight into peracarid sperm morphology and phylogenetic relationships with other malacostracan crustaceans.

**Phylogenetic relationships** However, the mysid sperm morphology which appears so unique, does share two features with other malacostracan crustacean spermatozoa: (1) the tail and (2) the acrosomal morphology. The unique peracarid sperm tail appears very similar to the syncarid (Anaspides) sperm tail (Kutish, personal observation). Both have a supporting tail wall made of microfilaments which form a repeating striated pattern in longitudinal section. An amorphous core is present. Also, the sperm nucleus in Anaspides, as in Archaeomysis, wraps around the tail. In both species the tail originates in the subacrosomal region.

Another peracarid sperm tail resemblance, perhaps superficial, is with decapod sperm in the primitive section Caridea. Carid shrimp (Palmaeon, Pochon-Masson, 1969; Palaemonetes, Brown, 1966a) have a posterior spike protruding from the nuclear region. The spike or short tail has a supporting microfilamentous wall, striated in longitudinal section, associated with the plasma membrane. This is similar to the peracarid sperm tail wall.

Finally, the peracarid acrosomal morphology and development during spermiogenesis superficially resembles the stomatopod (Squilla) sperm. Squilla has a caping acrosomal vesicle, subacrosomal rod and adjacent centrioles above the nucleus as does the peracarid sperm. Also the stomatopod acrosomal region develops (Kutish, personal observation) in the same way as in the isopods (Fain-Maurel, 1966) amphipods (Reger, 1966) and mysids (Kluge, 1966).
Marsupial sperm retention

Another purpose of this study was to relate marsupial morphology with fertilization in the marsupium. If a substantial time difference occurs between sperm transfer to the marsupium and egg passage to the marsupium, then there must be a mechanism for sperm retention. The filiform sperm tails are attached to some marsupial structures.

In the three mysids examined, *Archaeomysis*, *Acanthomysis*, and *Heteromysis*, special acanthus setae project from the oostegites and fill the marsupium cavity. In a few cases (*Archaeomysis*, *Acanthomysis*) the filiform sperm tails are entangled on these setae.

The acanthus setae and the marsupia have two patterns which are widespread in the Mysidae. In the subfamily Mysinae, including *Acanthomysis* and *Heteromysis*, acanthus setae occur in two places. Reduced or vestigial oostegites fill the anterior marsupial cavity with acanthus stae. And the digiform lobe on the 7th oostegite fills the posterior marsupial cavity with acanthus setae. The literature is replete with descriptions of digiform lobes (called baling lobes) and setated vestigial oostegites (Sars, 1870, 1872, 1879a; Zimmer, 1926a, 1927; Tattersall, 1932a,b, 1951; Ii, 1936, 1964; Bacescu, 1955). These authors do not mention that the setae fill the marsupial cavity and probably aid sperm retention.

A second pattern is seen in *Archaeomysis* (subfamily Gastrosaccinae). Acanthus setae on the surface of the 7th and 8th oostegites fill respectively the anterior and posterior portions of the marsupial cavity. Other related genera in the Gastrosaccinae (Sars, 1876, 1885; Bacescu, 1955;
Tattersall, 1951; Hansen, 1910) show similar oostegite morphology. Again the setae probably aid sperm retention in the marsupium.

Generally, setae fill the marsupium in all mysids from the primitive families Lophogastridae (M. Sars, 1862; Fage, 1940, 1941, 1942) and Eucopidae (Fage, 1940, 1941; Faxon, 1895) to the more advanced family Mysidae (Sars, 1870, 1872, 1877, 1879a, 1885; Willemøes-Suhm, 1873; Bacescu, 1955; Tattersall, 1932b, 1951; Ii, 1934, 1964; O. Tattersall, 1955; Zimmer, 1926a, 1927, 1909). A careful study of the setae within the mysid marsupium after copulation should show the exact nature of sperm retention. Most likely, the sperm tail interacts with the setae, holding the spermatozoa within the marsupium until fertilization. This is the function of the filiform peracarid sperm tail.

Peracarid fertilization pattern

The sperm retention feature is important to the mysids. It points first to the original pattern of peracarid sperm transfer and fertilization and secondly to the reason for the filiform sperm morphology.

Peracarid model The original peracarid condition for sperm transfer and fertilization in the marsupium is represented by the mysid pattern. A sexually receptive female is held by the male in a precopulatory amplexus until a reproductive molt liberates the oostegites, forming the marsupium (Nouvel, 1937, 1940; Nouvel and Nouvel, 1939; Nair, 1939; Labat, 1954). Then during the copulatory amplexus, spermatozoa are placed in the marsupium. The male releases the female. Setae inside the marsupium retain the spermatozoa until after an interval of several hours to 24 hours the eggs pass into the marsupium. Fertilization occurs when the eggs
pass across the spermatozoa entangled in the setae. Therefore the function of the filiform tail is to aid sperm retention in the marsupium.

The molting and sperm transfer pattern are seen in all peracarids except for slight modifications in isopods and amphipods (Kaestner, 1970). In amphipods, the spermatozoa are placed on a special setated region by the genital orifice (Koster, 1909). The isopods place the spermatozoa in the oviduct and have fertilization in the ovary (Schöbl, 1880).

Sperm-egg interaction is probably similar to the amphipod case since the peracarid sperm morphology is uniform except for the tanaids (Kutish, personal observation). The flexible head region, articulating on the tail, allows correct acrosomal attachment to the egg coat. Acrosomal rod penetration of the egg coat and membrane fusion between egg and sperm result in a typical sperm egg interaction found in many crustaceans, e.g., Callinectes (Brown, 1966a,b).

Filiform sperm origin The second purpose of this study is to speculate on the origin of the peracarid sperm morphology. Most likely, the filiform morphology originated as part of a more efficient peracarid reproductive strategy. To examine this idea, the overall primitive crustacean reproductive pattern is first reviewed and then the peracarid reproductive pattern is examined to see where a filiform sperm offers advantages.

The primitive crustacean reproductive strategy was the "shot-gun" approach (Kaestner, 1970) seen in so many marine polychaets, mollusks, and echinoderms (Pianka, 1976). Numerous gametes, eggs and spermatozoa, are shed into the sea for external fertilization. The free eggs develop and
hatch as a dispersing planktonic or benthic larvae. There is a gradual or anamorphic development to a sexually mature adult. Modern crustaceans use a more effective sperm transfer pattern, e.g., spermatophores, but the primitive larval stage (nauplius) and development remain (Kaestner, 1970).

The peracarids have the other extreme in reproductive strategies, a rifle approach (Kaestner, 1970). Much energy is expended on producing a few successful offspring. Only a few gametes, yolky eggs and spermatozoa, are produced. Fertilization is carefully controlled for efficient gamete use. A few eggs and spermatozoa are brought together by the female with either external or internal fertilization. A complete or epimorphic development of the egg is associated with brood care. The young hatch as miniature adults while the adult brooding female acts as the dispersal stage.

With this peracarid reproductive strategy, spermatozoa are adapted for sperm retention as follows. Originally, brood care was like the very primitive division Phyllocarida, e.g., Nebalia (Cannon, 1927). Setae between the thoracic legs hold the embryos during brooding. Locomotion is with the abdominal pleopods. The thoracic limbs cannot be used for locomotion or feeding. In this case a filiform sperm retained by the thoracic leg setae following sperm transfer favors a selective reproductive advantage over a motile flagellated sperm. The eggs are passed subsequently for external fertilization and brooding.

As seen in the primitive lophogastrid mysids and peracarid fossils (Schram, 1969b, 1974b) the ancient peracarids developed oostegites from modified epipodites to hold the filiform sperm and eggs during
fertilization and incubation. This allows simultaneous brooding, feeding and the high reproductive potential seen in the present peracarid orders.

In summary, the mysids sperm structure represents a typical peracarid sperm morphology with a filiform tail, apical acrosome, and pendant head. The filiform tail is associated with sperm retention in the marsupium and offers a selective advantage for the peracarid brooding strategy and fertilization pattern.
Figs. 25-27. Diagrammatic representation of mature sperm, longitudinal view. Acrosomal cap (AC), acrosomal rod and vesicle (AR, AV), nucleus (N), tail (T) and connecting region to head (*), mitochondria (M), membranes (ER), centrioles (C).

Fig. 25. Archaeomysis grebnitzkii. A singular mysid sperm morphology. The tail enters a canal formed by an invagination of the plasma membrane, perforates the nucleus and attaches to the head beneath the acrosomal cap. A prominent acrosomal rod and vesicle lie adjacent to the tail. No cytoplasmic remnants of spermiogenesis remain.

Fig. 26. Acanthomysis sculpta. A typical mysid sperm. The elongate nuclear containing head region is pendant next to the tail which attaches to the head beneath the small acrosomal cap and adjacent to the acrosomal rod and vesicle. Numerous mitochondria are interposed between the nucleus and cell membranes.

Fig. 27. Heteromysis formosa. A typical mysid sperm similar to A. sculpta. However, there is a very extensive region of perinuclear cytomembranes. The tail penetrates a moderately long plasma membrane invagination before attaching to the head beneath the short acrosomal rod and long acrosomal cap.

Figs. 28-30. Right penis, posterior view. Genital orifice (G).

Fig. 28. A. grebnitzkii. A distinctive notched medial lobe (arrow) extends above the genital orifice. Opposite, a lateral flap covers the distal penile surface. Projecting from the lateral border is a row of stout setae.

Fig. 29. A. sculpta. A conspicuous row of stout setae (arrow) arch over the genital orifice. The lateral border of this elongate, prismatic penis is setated.

Fig. 30. H. formosa. This long digiformed penis ends with three conspicuous lobes (arrow) projecting over the genital orifice. The anterior flap has a single stout seta while the lateral flap ends in a bifurcated polyp.
Plate 10

Fig. 31. *Archaeomysis grebnitzkii*. Ventral view of marsupium. Posterior position of oostegites (T7-8) under the abdomen (Abl) does not obstruct the food groove between the thoracic legs (T2-6). Carapace (Cp), pleural plate (*) of 1st abdominal segment supports the lateral marsupial wall.

Fig. 32. *A. grebnitzkii*. Ventral view of female. Eggs pass (arrows) from genital orifice of 6th leg (T6) onto 7th oostegite surface (T7) and contact the sperm bearing setae, cf., Fig. 70, before entering the posterior marsupial cavity.

Fig. 33. *Acanthomysis sculpta*. Ventral view of marsupium. Anterior position of oostegites (T7-8) and reduced oostegites on the thoracic limbs (T4-6) obstruct the food groove. Acanthus setae of reduced oostegites and digiform lobe on 7th oostegite (arrow) retain sperm in marsupium until ovulation.

Fig. 34. *A. grebnitzkii*. Lateral view of marsupium. Acanthus setae, cf., Fig. 70-71, of 8th oostegite (*) and 7th oostegite (arrow) fill marsupial cavity.

Fig. 35. *A. sculpta*. Lateral view of marsupium. Acanthus setae, cf., Fig. 74, of reduced oostegites (*) of 4th to 6th legs and the marsupial cavity. The serrate border setae couple the 7th and 8th oostegite medial margins, cf., Fig. 73.

Fig. 36. *Heteromysis formosa*. Lateral view of marsupium. Acanthus setae of reduced oostegite on 6th leg (*) and digiform lobe on 7th oostegite (arrow) fill marsupial cavity. Marsupium extends to the 2nd leg obstructing the food groove.

Fig. 37. *A. grebnitzkii*. Left 6th thoracic leg. Asetose reduced oostegite (arrow) covers genital orifice. The basis (B) retains numerous plumose filter setae (*) and unobstructed food groove. Ischium (I), preischium (PI), coxa (Cx), exopodite (Ex).

Fig. 38. *A. sculpta*. Left 6th thoracic leg. Acanthus setose oostegite covers genital orifice and obstructs sparse filtering plumose setae on basis (B).

Fig. 39. *H. formosa*. Left 6th thoracic leg. Acanthus setae (arrow) obstruct weak setae on basis and food groove.

Fig. 40. *A. grebnitzkii*. 6th thoracic oostegite and acanthus setae, cf., Figs. 70-71.

Figs. 41-42. *A. sculpta* and *H. formosa*. Acanthus setae on digiform lobe of 6th oostegite.
Plate 11

Fig. 43. Archaeomysis grebnitzkii. Unreacted spermatozoa. Sperm head with acrosomal cap (AC), acrosomal vesicle (AV), tail (T) penetrating the nucleus (N). Nomarski Differential Interference Contrast x 1500.

Fig. 44. A. grebnitzkii. Acrosomal reaction with projecting acrosomal rod (AR). Nomarski DIC x 1500.

Fig. 45. A. grebnitzkii. Two intact spermatophores from penis showing sperm alignment. Phase x 200.

Fig. 46. A. grebnitzkii. Free spermatozoa from spermatophore showing the head (H) region is not pendant from the tail (T). Phase x 200.

Fig. 47. Heteromysis formosa. Sperm head (H), long anterior acrosomal cap (AC) and short acrosomal vesicle (AV) pendant from the tail (T). Nomarski DIC x 800.

Fig. 48. Acanthomysis sculpta. Sperm with long nuclear (N) region, acrosomal vesicle (AV) pendant from the tail (T) with an anterior acrosomal cap (AC). Phase x 200.
Plate 12

Figs. 49-57. *Archaeomysis grebnitzkii* sperm. Arrows on Figs. 49-51 indicate the level of cross sections for other figures.

Fig. 49. Long. sec. of anterior end with very short mushroom acrosomal cap (AC), cf., Fig. 25, 43. The tail (T) perforates the nucleus (N) and lies adjacent to the very large acrosomal rod and vesicle (AV). x 40,000.

Fig. 50. Sperm head, longitudinal section. Tail core (T) showing periodic connections (arrows) to striated tail wall. Extracellular space (*) between nucleus (N) and tail. x 40,000.

Fig. 51. Sperm posterior end of head (*), long. sec. with tail entering extracellular indentation (arrows) through nucleus. x 40,000.

Fig. 52. Apical end of sperm in cross section with microtubules in the acrosomal rod (AR) and surrounding vesicle (AV). x 85,000.

Fig. 53. Mid-acrosomal region showing microtubules (AR) with nucleus surrounding both the long acrosomal vesicle (AV) and the tail (T). x 40,000.

Fig. 54. Posterior end of the acrosome in cross section. x 40,000.

Fig. 55. Sperm head in cross section below the acrosome with the nucleus (N) surrounding an extracellular space (*) containing the tail (T). x 40,000.

Fig. 56. Sperm tails (1, 2) below the nucleus. In cross section. x 40,000.

Fig. 57. Anterior end of sperm in long. sec. with very short acrosomal cap (AC), large acrosomal vesicle (AV), and long acrosomal rod (AR), cf., 25, 43. The tail (T) extends through an indentation, penetrates the nucleus (N) and attaches beneath the acrosomal cap. x 10,000.
Plate 13

Figs. 58-63. *Acanthomysis sculpta* sperm. Arrows on Fig. 58 indicate the level of cross sections for other figures.

Fig. 58. Long. sec. of anterior end with very short conical acrosomal cap (AC) which has a laminar extension peripheral to the large acrosomal vesicle (AV) and rod, cf., 26,48. The tail is attached to the nuclear containing head region above the acrosomal vesicle. x 30,000.

Fig. 59. Anterior end of sperm in cross section. Acrosomal rod (AR) and laminar acrosomal cap (AC), tail (T). x 50,000.

Fig. 60. Posterior region of acrosomal rod and vesicle (AV), with adjacent laminar acrosomal cap (AC). The tail (T) is round in the acrosomal region; however, in the nuclear region beneath the acrosome, the tail becomes asymmetric, and ellipsoidal with one wall increasing in thickness (*). Nucleus (N) with perinuclear mitochondria (M). x 35,000.

Fig. 61. Cross section of asymmetric tail showing the microfilaments which form the wall. x 135,000.

Fig. 62. Tail in long. sec. with periodic (arrows) attachment of core (*) to the striated wall. The microfilamentous nature of the wall is apparent. x 250,000.

Fig. 63. Three spermatophores showing tail alignment (arrows 1, 2) and central position of filaments. The head region contains the nucleus with heterochromatin areas (N) and euchromatin areas surrounding mitochondria (M). x 8000.
Plate 14

Figs. 64-69. *Heteromysis formosa* sperm. Arrows on Fig. 64 indicate the level of cross section for other figures.

Fig. 64. Long. sec. of anterior end with very long acrosomal cap (AC) and short acrosomal rod (AR), cf., Figs. 27, 47. The tail (T) is attached to the pendant, nuclear containing (N) head beneath the acrosomal rod. Centriole (C), filaments (F) of spermatophore matrix. x 30,000.

Fig. 65. Spatulate acrosomal cap in cross section, cf., Figs. 43, 47, 48. x 50,000.

Fig. 66. Anterior end of acrosomal rod and vesicle (AR) in cross section. x 50,000.

Fig. 67. Posterior region of acrosomal rod and vesicle (AV), with adjacent acrosomal cap (AC). Note tubules in acrosomal vesicle. x 50,000.

Fig. 68. Nuclear containing head region (N) in cross section with perinuclear membranes (ER). Filaments (F). x 50,000.

Fig. 69. Tail in long. sec. with periodic (arrows) attachment of core (*) to the striated wall (T). x 120,000.
Plate 15

Fig. 70. *Archaeomysis grebnitzkii*. Right 7th oostegite (Ot7) projecting into posterior marsupial cavity ventral to the abdomen (Ab). Numerous ventrally directed setae (*) retain spermatophores within the marsupium. The ovulated eggs pass from the genital orifice, across the oostegite surface (arrow), to be fertilized by spermatozoa held on these setae. Left 7th sternite and adjacent coxa (T7) with oostegite removed. x 100.

Fig. 71. *A. grebnitzkii*. Surface of oostegite setae and acanthus setuli (*), projecting into the marsupium. x 2400.

Fig. 72. *A. grebnitzkii*. Plumose setae, from border of oostegite, which do not project into marsupium. Phase x 400.

Fig. 73. *Acanthomysis sculpta*. Border plumose setae of 7th oostegite, with serrated setuli (arrow), which do not project into marsupium. x 2000.

Fig. 74. *A. sculpta*. Surface of 6th oostegite setae, and acanthus setuli (*), projecting into marsupium, cf., Fig. 71. x 5000.
PART III. COMPARISON OF THE SPERM MORPHOLOGY IN THE ORDERS TANAIDACEA AND CUMACEA (PERACARIDA)
Cumacean and tanaidacean peracarid crustacean sperm ultrastructure has never been examined. Light optical studies of cumacean spermatozoa show a typical peracarid filiform sperm (Retzius, 1909a,b) with a pendant head extending from the tail (Dohrn, 1870; Sars, 1864, 1900). But the tanaid spermatozoa are radically different. Spherical sperm cells (Müller, 1864; Claus, 1888a; Blanc, 1884; Roubault, 1937; Bückle-Ramírez, 1965) and even flagellated spermatozoa (Lang, 1953a; Gardiner, 1972) have been reported in the Tanaidacea. If these reports are true then the tanaid sperm pattern is completely outside the typical peracarid sperm plan, as exemplified by the cumaceans.

The tanaid sperm morphology is more perplexing because the tanaids are closely related to isopods (Siewing, 1953; Dennell, 1937; Lang, 1953b) and the isopods have a typical peracarid sperm morphology. In fact, the orders Cumacea (Dennell, 1934; Monod, 1922; Siewing, 1952; Calman, 1911) and Tanaidacea form a direct line of peracarid evolution from the order Mysidacea to the very successful order Isopoda.

The question arises if the tanaid sperm actually is different from the typical peracarid sperm found in the cumaceans. To answer this question the sperm morphology is examined in two cumaceans, Cumella vulgaris and Oxyurostylis pacifica, and in two tanaidaceans, Leptochelia savignyi and Hargeria rapax.
MATERIALS AND METHODS

List of Species

Animals used in this study were obtained from the following sources.

Order Cumacea

Family Cumellidae

Family Oxyurostylidae

Order Tanaidacea

Suborder Dikonophora

Family Paratanaidae

Hargeria rapax (Harger) - St. Andrews Bay, Panama City, Florida and St. Joseph's Bay, Port St. Joe, Florida. Hand picked from ascidians (Mogula occidentalis and Styela plicata); ascidians were cooled to 3°C causing the tanaids to leave their tubes. October 1973, December 1974, March 1975.

The cumaceans were identified using the descriptions of Hart (1930) and Zimmer (1936). Tanaidaceans were identified using the descriptions of Shiino (1965) and Lang (1973). The ascidians with epifaunal tanaids were maintained in Instant Ocean aquaria at 15°C.
Morphologically mature male cumaceans with the antenna extending beyond the uropods (Kaestner, 1970) were prepared for electron microscopic examination of the spermatozoa. The gonad was exposed dorsally by removing the carapace (see Sars, 1900 for description) and the entire animal fixed in 2.5% glutaraldehyde (0.2 M phosphate buffer, pH 7.6; 0.14 M sodium chloride), washed in the buffer plus 0.3 M sodium chloride, postfixed with 1% osmium tetroxide in the buffer, dehydrated with ethanol and embedded in epon-araldite (Anderson and Ellis, 1965). Alternatively, some animals were fixed in 2.5% glutaraldehyde (0.07 M cacodylate buffer, pH 7.6 with 0.3 M sodium chloride), washed in the buffer salt solution, and postfixed in 1% osmium tetroxide (0.1 M cacodylate buffer, pH 7.6). Sections were cut with a diamond knife on a Porter Blum MT 2 ultramicrotome, mounted on uncoated grids, stained with 5% uranyl acetate in 50% ethanol followed by lead citrate (Venable and Coggeshall, 1965) and examined with an Hitachi HU ile-l electron microscope.

Morphologically mature male tanaidaceans with strong sexual dimorphism including enlarged chelipeds, reduced mouth parts, genital papilla, and definite testis containing spermatozoa in the seminal vesicles (Gardiner, 1972) were prepared for ultrastructural examination of the spermatozoa as follows. The gonads were prepared by immersing the tanaids in cold fixative and removing the legs along with the abdomen. Specimens were fixed and processed as described for the cumaceans or with Karnovsky's (1965) fixative, dehydrated with ethanol and embedded in Epon (Luft, 1961) or Spurr's (1969) resin.
The marsupium and oostegite morphology was examined with light optical and the scanning electron microscope. Mature copulatory females, brooding embryos, were cold anesthetized (2°C) in sea water. The 3rd to 6th thoracic legs bearing oostegites were grasped at the coxae with forceps and removed. Oostegites were examined with phase contrast and Nomarski Differential Interference Contrast optics. Some oostegites were stained with aceto-orcein prior to observation. For SEM observation, the thoracic legs with attached oostegites were fixed and dehydrated as described for ultrastructural examination. Then the specimens were critical point dried with carbon dioxide, mounted on stubs with silver conducting paint, coated by vacuum deposition with gold-paladium 60/40 and viewed with a JEOLCO JSM-S1 scanning electron microscope.

Mature spermatozoa were observed as follows. The reproductive system was dissected from the body cavity and seminal vesicles opened in sea water to release the spermatozoa. Some squash preparations (aceto-orcein) of the seminal vesicles were also made. The spermatozoa were observed with phase contrast and Nomarski Differential Interference Contrast Optics.
OBSERVATIONS

The following observations were made: (1) the sperm and spermatoaphore morphology in the cumaceans Cumella and Oxyurostylis and (2) the sperm morphology in the Tanaids Leptochelia and Hargeria.

Cumacean Sperm Morphology - Cumella and Oxyurostylis

Spermatozoa

The cumacean spermatozoa (Figs. 78, 79, 80) in Cumella and Oxyurostylis exhibit the same morphology. Each has a long tail, an anterior acrosome, and a trailing fusiform pendant head region which contains the nucleus. The tail is 450 µm long while the apical acrosome is 0.5 µm in both species. Oxyurostylis has a very short head region (15 µm long) when compared to the head (45 µm) of Cumella. But the nuclear region is about the same diameter (650 nm) in both species.

Ultrastructural observations were made only with Cumella (Figs. 81, 82). Three morphological regions are identifiable within the sperm. An apical acrosomal region connects the head with the filiform tail.

Acrosome In this cumacean, the acrosome (Fig. 75) occupies the most anterior portion of the sperm (Fig. 81) and has three distinct regions: an apical acrosomal vesicle, a subacrosomal vesicle and subacrosomal rod. Both the acrosome and the subacrosomal vesicle are membranous bound. However, the subacrosomal vesicle contains a retrograde invagination which contains the subacrosomal rod. This rod is formed from...
microtubules (not figured). Beneath the acrosome complex is a pair of centrioles.

**Tail** The entire tail (Figs. 81, 82) has a uniform construction which follows the general peracarid sperm pattern. However, this cumacean tail is still readily distinguishable from other peracarids by the very small core size and thick wall (Fig. 82). In cross section, the tail contains a core (20 nm diameter, a surrounding wall (100 nm thick) and an outer plasma membrane. Overall, the tail is uniform in diameter (200 nm). Numerous closely packed long filaments (6 nm diameter) form the wall (Fig. 82). An orderly parallel arrangement of these filaments is visible in longitudinal sections creating a periodic striated pattern (repeat 70 nm).

**Head** The nucleus extends posteriorly from beneath the acrosome for 40 μm (Fig. 81). Two nuclear membranes are present, often apposed onto the cell plasma membrane. A characteristic finely dispersed chromatin unlike other peracarids is present in the nucleus. Only a few membrane bound vesicles are present between the nucleus and the cell plasma membrane.

**Spermatophores**

The spermatophore morphology and manner of storage are the same in both *Cumella* and *Oxyurostylis* (Figs. 79, 80, 81). In general, about 60 spermatozoa are aligned parallel to each other and in register. The heads are peripheral while the tails are central. Parallel to the spermatozoa are numerous extracellular tubules (40 nm diameter) composed of about 9 subunits (Fig. 82). These tubules extend throughout the spermatophore.
Spermatophores are stored in the seminal vesicle region of the vas deferens. About 10 large spermatophores (600 μm long) are stored in each seminal vesicle region of *Oxyurostylis*.

**Tanaidacean Sperm Morphology - *Leptochelia* and *Hargeria***

**Spermatozoa**

The tanaid sperm morphology as seen by light microscopy and electron microscopy is identical in both species (*H. rapax* and *L. savignyi*). The spermatozoa are spherical cells, 7 μm in diameter, with projecting cytoplasmic arms, 3 to 5 μm in length (Fig. 76). An acrosome, subacrosomal rod, and a central nucleus are also visible. Spontaneous acrosomal reactions were seen in sperm suspensions. Attempts to induce acrosomal reactions or affect the sperm by using alkaline sea water, calcium free sea water or double strength sea water were unsuccessful. In all cases, spermatozoa taken from the seminal vesicles, vas deferens or posterior testicular duct were free with no evidence of spermatophore formation.

Under electron microscopic observation (Fig. 83) the tanaid sperm display four very distinctive regions: (1) an acrosome with invaginated core containing a subacrosomal rod; (2) a central nucleus with an indentation containing the centrioles; (3) a peripheral cytoplasm filled with membranes and mitochondria; and (4) a surface membrane modification with projecting cytoplasmic processes.

**Acrosome**

The acrosome (Fig. 83) is a membranous bound sphere, about 4 μm in diameter. Apically, the acrosomal membrane is appressed onto the inner surface of the sperm plasma membrane. Basally, the
acrosomal membrane indents, projecting about one-half the way into the acrosome center. The indentation contains part of the subacrosomal rod.

Within the acrosome (Figs. 76, 83) three regions are visible; a matrix, basal density and subacrosomal rod. The matrix is uniformly granular in appearance and contains numerous small, 70 μm, membranous bound densities. Basally, a thin saucer shaped density covers the inner surface of the acrosomal membrane, while more centrally there is a thick donut shaped density. Perforating both densities is a subacrosomal rod which is surrounded by a membranous indentation, 1.7 μm by 0.5 μm, and extends into the acrosomal center. Inside the indentation are microtubules and a dense vacuolar matrix. This matrix expands basally and covers the external acrosomal membrane surface.

Nucleus The nucleus (Fig. 83) lies centrally in the sperm at the base of the acrosome. Characteristically, the nuclear membrane retains a smooth outline which follows the acrosomal contour. Also, nuclear pores are present in the nuclear membrane. A pair of centrioles lies between the nucleus and the acrosome.

A nucleolar region is present within the nucleus. The surrounding chromatin is either granular with peripheral clumps or finely homogeneous depending on the fixation. In general, the nuclear appearance suggests activity because a nucleolus, nuclear pores, and dispersed finely clumped chromatin are still present in the sperm.

Cytoplasm Three very distinctive and large areas of cytoplasm are found in the tanaid sperm (Fig. 83). Each has a specific architecture and occupies a specific region, i.e., either periacrosomal, perinuclear or
peripheral. In each case, however, mitochondria are evenly dispersed. The periacrosomal cytoplasm contains no membranes and has many mitochondria, e.g., three to five per section. The cytoplasm has numerous clumped granules similar to ribosomal aggregations. The perinuclear cytoplasm contains extensive (5-20) stacks of laminar membranes following the nuclear contour. These membranes are very close together. The peripheral cytoplasm is most distinctive because of the pervasive vesicular endoplasmic reticulum. The tubular membranes are appressed to the inner surface of the cell plasma membrane and continue into the stacks of laminar endoplasmic reticulum. Usually, the tubular membranes are in contact with each other, excluding the cytoplasmic ground substance. There is no secreted material with the cisternae of these irregular membranes.

Cytoplasmic processes The external cell plasma membrane is differentiated into a region of cytoplasmic processes (Fig. 83). Numerous straight tubular membranous extensions (120 nm thick by 5 \( \mu \)m long) cover the cell surface except for the apical region covering the acrosome, and are evenly spaced about 5 \( \mu \)m apart. A tubular membranous extension (30 \( \mu \)m across) from the peripheral vesicular endoplasmic reticulum fills the center of each process and extends to the tip.
DISCUSSION

Two general conclusions are apparent when comparing cumacean spermatozoa with tanaid spermatozoa: (1) cumacean spermatozoa have a generalized peracarid sperm morphology; and (2) tanaid spermatozoa have a unique morphology outside the peracarid sperm plan.

No Surprises in the Cumacean Spermatozoa - Cumella and Oxyurostylis

Both cumacean species, Cumella and Oxyurostylis, show the typical peracarid sperm pattern and a mysid spermatophore pattern. Generally, cumacean spermatozoa resemble the mysid and isopod patterns rather than the amphipod sperm pattern (Dohrn, 1870; Sars, 1864, 1900; Retzius, 1909a). As in the mysids Acanthomysis and Archaeomysis, Cumella has a very large acrosome, subacrosomal rod and surrounding vesicle. The centrioles lie below the subacrosomal rod and above the nucleus as in all peracarid spermatozoa. Also characteristic for cumacean spermatozoa, along with mysids and isopods, is a restricted head movement about the tail. The amphipod sperm head moves through a 180° angle with respect to the tail. Finally, the tail in Cumella is very similar to the isopod sperm tail. Both have a very thick wall, filling three-fourths of the tail diameter. Mysid and amphipod sperm tails exhibit a very thin wall, representing one-fourth of the tail diameter.

The cumacean spermatophore morphology, storage and formation are the same as mysids. A large number of spermatozoa form a discrete tear drop
spermatophore in *Cumella* and *Oxyurostylis* similar to mysid (*Arachaeomysis*) spermatophores. Common in all peracarids, the spermatophores develop during spermiogenesis. In *Cumella*, spermatids, developing together in the testis, are released into the seminal vesicles already aggregated with extracellular tubules.

Probably the large cumacean spermatophores and filiform spermatozoa aid sperm retention in the marsupium, similar to mysids. The cumacean marsupium is well-formed from large oostegite plates on the 3rd to 6th thoracic limbs (Sars, 1864, 1871, 1879a, 1900; Zimmer, 1908). Somewhat different from the mysids (Sars, 1876), the rudimentary 2nd oostegite fills the marsupium with setae (Fage, 1944, 1951; Zimmer, 1920). The events of sperm transfer are comparable in both orders. Cumaceans have a precopulatory amplexus (Foxon, 1936; Bacescu, 1951; Gnewuch, 1972). Next the female molts releasing the functional brooding oostegites (Forsman, 1933). Then in a copulatory amplexus, the male transfers the spermatophores to the marsupium of the female (Sars, 1900; Zimmer, 1926b; Fage, 1944). The long sperm tail and large spermatophore size probably retain the spermatozoa inside the marsupium until the eggs pass to the marsupium. Fertilization then occurs in the marsupium which is the original peracarid pattern.
A Unique Tanaid Sperm Morphology Outside the Peracarid Sperm Plan

In contrast to the typical peracarid sperm plan of cumaceans, the tanaid sperm ultrastructure is exceptional. No morphological features are shared between the two orders.

*Leptochelia savignyi* and *Hargeria rapax* have no filiform sperm tail but they do have an extremely large acrosome. Unlike any other peracarid sperm acrosome, the tanaid acrosome fills almost half the spherical sperm cell. No subacrosomal vesicle is present. Finally, numerous cytoplasmic membranes persist in the tanaid sperm and the surface is covered with cytoplasmic processes.

Whether the spherical tanaid sperm morphology is characteristic for the entire order is not known. In the family Paratanainaiedae, including *Leptochelia savignyi* and *Hargeria rapax*, a spherical sperm morphology has been reported for the genera *Heterotanaiais* (Blanc, 1884; Bückle-Ramírez, 1965) and *Leptochelia* (Müller, 1864; Roubault, 1937). However, filiform spermatozoa (100-200 μm long) are found in the two primitive tanaid families Apseudidae, *Apsuedes* (Lang, 1953a), and Neotanaidae, *Neotanaiais* (Gardiner, 1972). The spermatozoa in the light optical descriptions have a globular head region instead of the typical pendant head of the other peracarid spermatozoa. But confusing the entire situation, Claus (1888a) reports a spherical sperm in the Apseudidae, *Apsuedes*, in contrast to Lang.

Possibly the Paratanainaiedae generally represent a deviation from the typical peracarid plan while the more primitive tanaids, Apseudidae (Lang,
1956) and Netotanaidae (Gardiner, 1972) still retain a filiform sperm. However, the reproductive advantage for a spherical sperm morphology is not obvious, because the tanaids have a reproductive molting pattern similar to isopods and cumaceans (Lang, 1953b) but a sperm transfer pattern like the mysids (cf., Nouvel, 1940; Nair, 1939; Labat, 1954). During a precopulatory amplexus the female tanaid has a parturial molt releasing the oostegites and forming the marsupium (Forsman, 1956; Salvat, 1967; Shiino, 1937; Wolff, 1956). The male transfers the spermatozoa to the marsupium where fertilization occurs (Buckle-Ramírez, 1965). Finally at the end of the brooding period a postparturial molt forms reduced oostegites and a nonbrooding female morphology as in isopods (Lang, 1953b). This tanaid reproductive pattern with precopulatory amplexus, parturial molt, copulatory amplexus, and postparturial molt is found in the closely related cumaceans and isopods which have a filiform sperm pattern. Also, the marsupia of Leptocheilia, Hargeria and other tanaids (Apseudes, Moers-Messmer, 1936) are very tightly formed and compact. But no sperm retention structures are present on the inner oostegite surface as in amphipods. Nor are there setae projecting into the marsupium as in the mysids. Perhaps the sperm cytoprocesses aid sperm retention in the tanaids. Or these processes may interact with the egg prior to acrosomal reaction.

The tanaid spermatozoa in Leptocheilia and Hargeria are most likely modified spermatids. In fact except for the large size of the acrosome, the tanaid spermatozoa appear similar to peracarid spermatids before the tail is formed (Fain-Maurel, 1966; Reger, 1966; Kluge, 1966). Only the
more primitive tanaids in the families Apseudidae and Neotanaidae (Gardiner, 1972) seem to retain the filiform peracarid sperm pattern.

In summary the cumacean spermatozoa do not deviate from the peracarid pattern while the tanaid spermatozoa in the family Paratanainidae have a unique morphology outside the peracarid filiform sperm pattern.
Fig. 75. Diagrammatic representation of mature sperm in the cumacean *Cumella vulgaris* from the seminal vesicles showing a typical peracarid sperm morphology. Anterior acrosomal cap (AC), subacrosomal rod with vesicle (AR) and pair of centrioles (CE) at the base of the nucleus (N). The tail is more broadly connected to the head than in most peracarids which restricts articulation between the head and tail (T). Membranous bound vesicles (V).

Fig. 76. Diagrammatic representation of mature sperm in the tanaids *Hargeria* and *Leptochelia*. Large acrosome (AC) with apical membrane fused to the cell surface. Prominent subacrosomal rod (AR) penetrates a ventral invagination of the acrosome. Pair of centrioles (CE) lie a nuclear (N) depression. Cytoplasm filled with vesicular (ERV) and laminar (ERL) membranes and mitochondria (M). Prominent cytoplasmic processes (CP) project from the cell surface. This sperm morphology is not found in other peracarid orders.
Plate 17

Figs. 77-82. Cumacean spermatozoa and spermatophores.

Fig. 77. *Oxyurostylis* spermatozoa from a spermatophore in the seminal vesicles. Head (H) and anterior acrosomal region (AC) attach to the tail (T) in the typical peracarid pattern. Nomarski DIC, x 1500.

Fig. 78. *Oxyurostylis* spermatophore stored in seminal vesicles. A very compact and discrete structure with the sperm head (H) anterior and the tails (T) posterior. Several spermatophores are stored in the seminal vesicles. Nomarski DIC, x 90.

Fig. 79. *Oxyurostylis* spermatophore dissociating in sea water. Eventually, the spermatozoa are liberated. Head (H) and tail (T). Phase contrast, x 180.

Fig. 80. *Cumella* spermatophore from the seminal vesicles. A less compact and discrete structure, but *Cumella* is only 3 mm in body length compared to a body length of 12 mm *Oxyurostylis*. Phase contrast, x 180.

Fig. 81. *Cumella* spermatozoa from the seminal vesicles. The nucleus has a very finely dispersed chromatin (N). An apical region contains a membranous bound acrosome (AC). Part of the subacrosomal rod (AR) is present in a basal invagination of the acrosome. The anterior ends of the tail (T) in this spermatophore cross section have the characteristic cumacean small core and thick wall. Numerous extracellular tubules are present around the spermatozoa. x 30,000.

Fig. 82. *Cumella* sperm tails in detail. The tail has a plasma membrane and adjacent outer wall (W) made of distinct filaments typical for the peracarids. A discrete small core (C) characteristic of cumaceans is present. Extracellular tubules (arrow) with subunits. x 220,000.
Fig. 83. *Leptochelia* sperm from the seminal vesicle from a copulatory male. The large apical acrosome (AC) has a basal density (*) which is perforated by an invagination containing the subacrosomal rod (AR). Between the acrosome and the nucleus (N) is a pair of centrioles (Ce). Surrounding the nucleus are laminar and vesicular membrane systems (ER) with a few interspersed mitochondria (M). The periacrosomal cytoplasm is devoid of membrane systems. Cytoplasmic processes (CP) extend from the cell membrane except in the region of the acrosome. Each process has a microtubular core. This sperm type is not seen in any other peracarid order. x 20,000.