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## **VOLUME IX, PART A**

**Progress in Male Gamete Ultrastructure and Phylogeny**



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## 2. CNIDARIA AND CTENOPHORA

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### I. PHYLUM CNIDARIA: INTRODUCTION

Cnidarians are considered to be relatively primitive metazoans because of their simple anatomical structure and tissue level of organization (Hyman, 1940; Chapman, 1974). Despite their anatomical simplicity, cnidarians have effectively colonized most aquatic habitats, and some groups are ecologically very important. For example, scleractinian reef corals are primarily responsible for the construction of spectacularly diverse coral reef ecosystems, while some medusae possess nematocyst stinging cells capable of killing vertebrates including humans. These cnidae are a unique characteristic of cnidarians, and are among the most complex and largest intracellular secretory products known in the animal kingdom (Mariscal, 1974).

The phylum Cnidaria contains four classes: the Hydrozoa, Scyphozoa, Cubozoa and Anthozoa. It is estimated that there are more than 9,000 species of extant cnidarians comprising 2,700 species of hydrozoans, 200 species of scyphozoans, 17 species of cubozoans, and 6,200 species of anthozoans (Barnes and Harrison, 1991). Cnidarians exhibit a variety of reproductive patterns, and possess a surprisingly diverse range of spermatozoa. This chapter reviews current knowledge of the ultrastructure of cnidarian spermatozoa, phylogenetic implications, and some ecological aspects of sperm function. Emphasis is given to recent studies that were not available, or not emphasized, in the previous extensive review of cnidarian spermatogenesis and sperm function by Miller (1983), or other reviews of cnidarian reproduction (Campbell, 1974; Chapman, 1974; Fautin and Mariscal, 1991; and Thomas and Edwards, 1991).

In contrast to previous reviews, we have taken the unusual approach of first highlighting information from the Class Anthozoa. This decision stems from recent molecular studies and is also prompted by the greater range of spermatozoa now known to be present in anthozoans compared with other classes, and the larger number of anthozoan species studied to date (Table 1). In all anthozoan species examined for the gross structure of their mitochondrial DNA (mtDNA), this has been found to be a circular molecule as it is in ctenophores and other metazoans. In contrast, all species tested in the medusoid classes (Scyphozoa, Cubozoa, and Hydrozoa) have mtDNA in the form of two linear molecules (Bridge *et al.*, 1992), as previously shown for *Hydra* (Warrior and Gall, 1985). This appears to give unequivocal support for the notion that the Anthozoa, possibly a paraphyletic group, occupy a basal position within the Cnidaria (Bridge *et al.*, 1992). Similarly, a recent cladistic analysis of cnidarians (Schuchert, 1993) indicated that the Anthozoa were the most primitive group.

Sequence analysis of 18S ribosomal DNA (rDNA), mitochondrial 16S rDNA, the 5'-end of the 23S-like rDNA, and morphological characters are also unanimous in grouping the classes possessing a medusa stage, leaving the holobenthic Anthozoa basal within the phylum. These sequences also clearly resolved the Cnidaria from other lower Metazoa (Bridge *et al.*, 1995; Odorico and Miller, 1997).

Ultrastructural information on sperm is available for 138 cnidarian species (Table 1). Hexacorallian Anthozoa are comparatively well studied, with ultrastructural descriptions of sperm from 89 species, including 47 scleractinian coral species and 29 actiniarian anemone species. Hydrozoan hydroids are also relatively well studied, whereas there have been few studies on scyphozoan and octocorallian sperm. No information is available on the ultrastructure of sperm from three orders of octocorallian anthozoans, three orders of hydrozoans, or from the Class Cubozoa (Table 1).

## II. ANTHOZOA

### A. Introduction

Three major features distinguish the Anthozoa from the Hydrozoa, Scyphozoa and Cubozoa. Anthozoans are exclusively polypoid and lack any medusoid stage in their life cycle. Thus, the polyp is gametogenic, although in many species it also reproduces asexually (Fautin and Mariscal, 1991). A tube of tissue extends from the polyp mouth into the coelenteron (gastrovascular cavity) and constitutes the stomodaeum or pharynx which is absent in polyps of other classes. The coelenteron is subdivided radially by sheets of tissue (mesenteries) that grow in from the body wall, and some mesenteries contact the stomodaeum. Two groups of anthozoans are recognized: the subclass Octocorallia (Alcyonaria) possesses eight pinnate tentacles and eight mesenteries, while the subclass Hexacorallia (Zoantharia) possess cycles of six mesenteries and tentacles.

Table 1

Summary of ultrastructural descriptions of sperm within cnidarian orders.

Higher taxon	Number of species studied
<b>CLASS ANTHOZOA</b>	97
<b>Subclass Octocorallia</b> (Alcyonaria)	8
Order Stolonifera	—
Order Telestacea	—
Order Alcyonacea	3
Order Helioporacea	—
Order Gorgonacea	3
Order Pennatulacea	2
<b>Subclass Hexacorallia</b> (Zoantharia)	89
Order Ceriantharia	5
Order Scleractinia	47
Order Corallimorpharia	1
Order Actiniaria	29
Order Zoanthidea	5
Order Antipatharia	2
<b>CLASS HYDROZOA</b>	31
Order Hydroida	28
Order Milleporina	—
Order Stylasterina	—
Order Trachylina	1
Order Siphonophora	1
Order Chondrophora	—
Order Actinulida	1
<b>CLASS SCYPHOZOA</b>	10
Order Stauromedusae	1
Order Coronatae	1
Order Semaestomeae	5
Order Rhizostomeae	3
<b>CLASS CUBOZOA</b>	—
Order Cubomedusae	—

Recent molecular studies place the Anthozoa at the base of the Cnidaria and suggest that the medusa is a synapomorphy of the Hydrozoa+Scyphozoa. Paraphyly of the Anthozoa was inferred by Bridge *et al.* (1995) using 18S rDNA sequences, a gorgonian+pennatularian clade evolving before the remaining Cnidaria (and Placozoa), but the Anthozoa were monophyletic in a combined analysis based on 18S, mtDNA structure and morphological data.

Mitochondrial 16S rRNA gene sequencing supports the two-subclass system of the Anthozoa (Hexacorallia and Octocorallia) (France *et al.*, 1996). However, Song *et al.* (1994) claimed that 18S ribosomal RNA gene analysis supports the three-subclass scheme (Octocorallia, Hexacorallia, and Ceriantipatharia) while

indicating that the Anthozoa is a monophyletic group. Sequence analysis of the 5'-end of 28S rDNA indicates that the Ceriantharia (other 'Ceriantipatharia' were not examined) are most representative of the ancestral Anthozoa (Chen *et al.*, 1995), a view which agrees with spermatozoal evidence (Schmidt and Zissler, 1979).

### B. Spermatogenesis

Gonads of anthozoans are simply gametogenic areas in the mesenteries and lie central to the retractor muscle, if there is one (Fautin and Mariscal, 1991). In hermaphroditic species, gametes of both sexes may occur in the same mesentery, or in separate mesenteries. The earliest recognizable stages of anthozoan spermatogenesis are small groups of cells that occur in the gastrodermis of the mesenteries adjacent to the mesogloea (Larkman, 1980; Schmidt and Holtken, 1980; Harrison and Wallace, 1990). These cells are about 4  $\mu\text{m}$  in diameter and resemble enlarged interstitial cells (Delvoye, 1982), but are differentiated by the presence of a prominent pericentriolar complex (Schmidt and Holtken, 1980). The spermatogonia then enter, or are engulfed by, the mesogloea where they differentiate into primary and secondary spermatocytes, and then spermatids, within a spermatogenic cyst or loculus (Clark and Dewel, 1974; Schmidt and Zissler, 1979; Harrison, 1988a).

Most primary spermatogonia lack a flagellum, except for those of the corallimorpharian *Corynactis viridis* (Schmidt and Holtken, 1980). In the early developmental stages, the spermatogonia are attached to the mesogloea wall by cytoplasmic processes containing abundant mitochondria and lipid droplets (Schmidt and Zissler, 1979). Plasma membranes of scleractinian spermatocytes are highly irregular and are joined by desmosomes (Harrison, 1988a). Spermatids differentiate in groups of four cells joined by intercellular bridges (Dewel and Clark, 1972; Lyke and Robson, 1975; Larkman, 1980; West, 1980a; Miller, 1983). As spermiogenesis proceeds, the cytoplasm and nuclear chromatin condense, and a flagellum develops prior to the onset of meiosis (Clark and Dewel, 1974; Lyke and Robson, 1975; Schmidt and Zissler, 1979; West, 1980a; Miller, 1983; Harrison, 1988a).

Sperm maturation usually proceeds from the centre to the periphery of the sperm cysts until the central lumen is filled with flagella (Dewel and Clark, 1972; Larkman, 1980; Schmidt and Holtken, 1980; West, 1980a; Fautin and Mariscal, 1991). Maturation can occur synchronously within the spermary or asynchronously among loculi within each spermary (Harrison, 1988a). The final stages of spermiogenesis can proceed very rapidly in some anthozoans. For example, sperm head condensation is completed and flagellar motility increases rapidly in the week prior to spawning of scleractinian reef corals (Harrison *et al.*, 1984). During spawning of sea anemones, the mesogloea and gastrodermal layers rupture and the spermatozoa are released freely into the coelenteron and then pass to the exterior

via the mouth (Fautin and Mariscal, 1991). In most hermaphroditic scleractinian corals, spermatozoa are packaged into egg and sperm bundles just prior to spawning (reviewed by Harrison and Wallace, 1990).

### C. General Anthozoan Sperm Structure

Schmidt and Zissler (1979) provided a comprehensive ultrastructural and cladistic account of spermatogenesis and spermatozoa of 42 species of octocorallian and hexacorallian Anthozoa (Fig. 1). With information on sperm of a further 55 anthozoan species provided by other workers, it permits the following observations as to anthozoan sperm structure.

#### 1. General form

Anthozoan spermatozoa consist of a head, a midpiece and a single flagellum tail. They lack an acrosome and are therefore a modified form of the 'primitive' sperm type (*sensu* Franzén, 1956, 1970, 1977). In the terminology of Rouse and Jamieson (1987), they are ect-aquasperm and ent-aquasperm. In contrast to other cnidarian classes, the sperm of Anthozoa are very variable, with a number of cytological peculiarities.

##### (a) Nucleus

The shape of the sperm head is largely determined by the form of the nucleus which varies from conical, bullet-shaped to ovoid, or even spherical. The nuclear and cell membranes are usually in close proximity with little or no perinuclear cytoplasm, but in the Antipatharia (Fig. 1L) (Schmidt and Zissler, 1979) and some scleractinian corals (Steiner, 1991, 1993), these membranes are separated by electron-dense vesicles. In some scleractinian species, the chromatin at the tip of the sperm nucleus is less dense, and forms a rounded cap or a pointed apical body (Figs. 1D, 3A–D, 6C, 7) (Schmidt and Zissler, 1979; Harrison, 1985, 1988a, 1990; Steiner, 1991, 1993).

Electron-lucent spaces are frequently present in the nuclear chromatin. These are usually apical in pointed, but basal in round-headed, actiniarian sperm. In round-headed Type III actiniarian sperm (see below), these spaces form a ring around the base of the nucleus (Dewel and Clark, 1972; Schmidt and Zissler, 1979). A posterior nuclear fossa of varying form is present in many anthozoan groups, but is absent in other Cnidaria (Clark and Dewel, 1974; Lyke and Robson, 1975; Schmidt and Zissler, 1979; Larkman and Carter, 1980; Harrison, 1988a; Steiner, 1993).

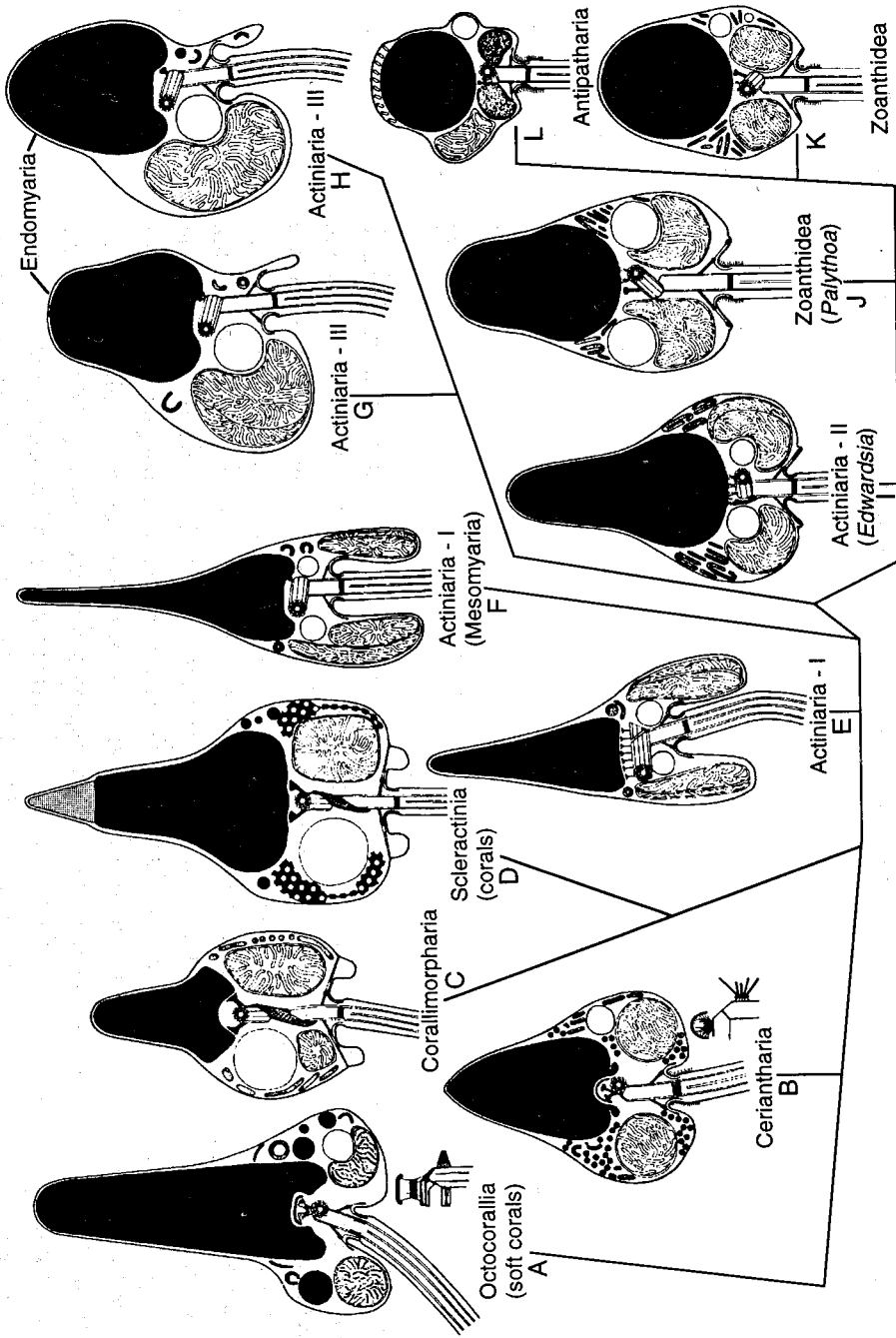


Fig. 1. Phylogeny of anthozoan sperm, especially the Hexacorallia. After Schmidt and Zissler (1979). *Zoologica (Stuttgart)* 44: 1-98. fig. 1. The Zoantharia *sensu* these authors are here termed the Zoanthidea.



(b) *Mitochondria and collar*

The number and arrangement of the mitochondria in the sperm midpiece varies among anthozoans. The primitive sperm pattern of four or five mitochondria (Franzén, 1977), which is found in many hydrozoans and scyphozoans, occurs in the Alcyonaria, Ceriantharia and in most zoanthids (Schmidt and Zissler, 1979). Varying degrees of mitochondrial fusion occur during spermiogenesis in other anthozoans, and in some cases the mitochondria fuse to form a single 'nebenkern' (*sensu* Schmidt and Zissler, 1979; see also Dewel and Clark, 1972; Larkman and Carter, 1980; West, 1980a; and Harrison 1988a, 1990). The nebenkern is usually crescentic and is curved around the midpiece, and may be displaced by the lipid body (Figs. 1, 3, 4). In some Actiniaria and probably the Corallimorpharia, the nebenkern forms a closed ring. In the Antipatharia, the nebenkern is situated around the lateral margin of the nucleus, whereas in some Type 1 actinarian sperm (Protantheae and Mesomyaria) it extends into a basal cytoplasmic collar to form a 'manchette' (Fig. 1E, F) (Schmidt and Zissler, 1979).

(c) *Lipid and Golgi derivatives*

The presence of one or more lipid spheres in the midpiece further distinguishes anthozoan sperm from those of other cnidarians (Figs. 1, 3–7). However, lipid-like inclusions occur in the perinuclear region of sperm of the hydroid *Eudendrium ramosum* (Summers, 1972a). A variety of electron dense, membrane bound bodies originating from the Golgi complex are present in the sperm of anthozoans and other Cnidaria.

(d) *Centrioles and flagellum*

The flagellum and a primary centriolar complex are already fully developed at the onset of meiosis in anthozoan sperm (as in some polychaetes and the insects). With the exception of the presence of a distal pericentriolar apparatus, the primary centriolar complex closely resembles the basal structures of normal cilia and flagella. Anthozoan spermatids have a microtubular nucleating satellite at the distal centriole of the primary centriolar complex, which is retained in a fully developed form only in the mature spermatozoa of primitive Octocorallia, and in a modified form, in the Ceriantharia (Schmidt and Zissler, 1979; Schmidt and Holtken, 1980).

In contrast with other cnidarian sperm, a secondary centriolar complex (Figs. 1, 3–7) connects the nucleus and flagellum of anthozoan sperm. The proximal centriole is connected with the basal nuclear membranes by processes that vary in number, electron density and shape in the different anthozoan sperm types. However, the pericentriolar processes surrounding the distal centriole are almost identical throughout the Cnidaria. In many anthozoan sperm, the proximal centriole

lies at right angles or obliquely to the distal centriole, and the two centrioles are virtually in contact. However, in sperm of Scleractinia and Corallimorpharia, the two centrioles are aligned along the longitudinal axis of the cell and are connected by a fibrillar intercentriolar ligament (Schmidt and Zissler, 1979; Harrison, 1985, 1988a, 1990; Steiner, 1991, 1993). A similar tandem arrangement of the centrioles occurs in some hydrozoan sperm, however in these sperm the centrioles are separate and are not connected by an intercentriolar ligament.

#### **D. Phylogeny and Sperm Ultrastructure**

A phylogeny for the Anthozoa based on spermatozoal ultrastructure was constructed by Schmidt and Zissler (1979). The scheme is, of course, hypothetical but their phylogram is presented (Fig. 1) because it retains its descriptive value for most anthozoan groups. Schmidt and Zissler (1979) regard the anthozoan condition as plesiomorphic because mutually perpendicular, interconnected centrioles and a cross striated rootlet, but not the satellite complex, are characteristics of somatic cells. The microtubular nucleating satellite at the distal centriole of Octocorallia and Ceriantharia sperm is regarded as plesiomorphic, whereas the intercentriolar ligament and centriolar arrangement in Scleractinia and Corallimorpharia sperm is seen as apomorphic, and clearly unifies these two orders. More recent ultrastructural studies (Harrison, 1985, 1988a, 1990; Steiner, 1991, 1993; Steiner and Cortes, 1996) have shown that scleractinian sperm are much more diverse than Schmidt and Zissler (1979) reported, and display a cytological diversity similar to that observed in the entire Anthozoa. The occurrence of prenuclear vesicles in some scleractinian sperm (Steiner, 1993), a characteristic originally thought to define the Antipatharia (Schmidt and Zissler, 1979), must now be considered homoplastic.

The characteristic features of anthozoan sperm indicate that the Anthozoa are the sister group of the common ancestor of the Hydrozoa and Scyphozoa, which have relatively similar sperm. Based on synapomorphous sperm characters, the Octocorallia and Hexacorallia are considered to be derived from a common ancestor (Schmidt and Zissler, 1979). These relationships are supported by molecular studies (Bridge *et al.*, 1995; Odorico and Miller, 1997).

#### **E. Subclass Octocorallia (Alcyonaria)**

The Octocorallia consists of a diverse group of colonial cnidarians including the soft corals, sea fans, sea whips, red corals, sea pens, pipe corals and blue corals. Octocorals are either gonochoric or hermaphroditic and exhibit three basic patterns of sexual reproduction: broadcast spawning, internal brooding, or external surface brooding (Benayahu and Loya, 1986; Alino and Coll, 1989; Benayahu, 1991). Synchronized mass spawning by more than 20 soft coral and other octocoral species has been recorded on the Great Barrier Reef (Babcock *et al.*, 1986; Alino

and Coll, 1989), and these events generally coincide with mass spawning of scleractinian reef corals (see Scleractinia, below). Aspects of the ultrastructure of spermiogenesis or mature sperm have been described in the eight octocorallian species listed in Table 2.

Table 2

Ultrastructural studies of spermiogenesis and/or spermatozoa of the Octocorallia (Alcyonaria)

Higher taxon	Species	Reference
<b>Subclass Octocorallia</b>		
Order Alcyonacea	<i>Alcyonium digitatum</i>	Schmidt and Zissler, 1979
	<i>Alcyonium palmatum</i>	Schmidt and Zissler, 1979
	<i>Alcyonium glomeratum</i>	Schmidt and Zissler, 1979
Order Gorgonacea	<i>Eunicella cavolini</i>	Schmidt and Zissler, 1979;
		Schmidt and Holtken, 1980
	<i>Eunicella verrucosa</i>	Schmidt and Zissler, 1979;
		Schmidt and Holtken, 1980
Order Pennatulacea	<i>Corallium rubrum</i>	Schmidt and Zissler, 1979
	<i>Pteroeides spinosum</i>	Schmidt and Zissler, 1979
	<i>Veretillum cynomorium</i>	Schmidt and Zissler, 1979

### 1. Octocorallian sperm structure and phylogeny

Schmidt and Zissler (1979) concluded that the most primitive anthozoan sperm are present in the Alcyonacea, and that the Octocorallia constitute the most plesiomorphic group of Anthozoa. Alternatively, 18S rRNA analysis suggests that *Cerianthus* occupies this position in the Anthozoa (Song *et al.*, 1994).

#### (a) Nucleus

Alcyonacea (Fig. 1A) and Gorgonacea sperm have an elongate conical nucleus with a rounded or arched tip, whereas the elongate nucleus of Pennatulacea sperm narrows concavely towards a flattened tip. A similar elongate conical nucleus is also a feature of some Hydrozoa and Scyphozoa sperm. Schmidt and Zissler (1979) considered the nuclear shape of pennatulaceans to be an apomorphy relative to other octocorallians. A deep fossa is present at the base of the nucleus of the octocorallian sperm studied to date.

#### (b) Midpiece

The lateral region of the midpiece contains a variety of rod-shaped, circular and cup-shaped dense bodies; and dark and light lipid inclusions, one or two of which form lipid spheres (Fig. 1A). The structure of the electron-dense bodies of Pennatulacea sperm is apomorphic in comparison with those of other octocorallian

(Schmidt and Zissler, 1979). The proximal and distal centrioles are aligned at right angles, and processes originating from the proximal centriole connect with an electron-dense plate adjacent to the nuclear membranes within the basal nuclear fossa (Fig. 1B). The anchoring structures connecting the centriolar complex with the nuclear fossa are similar in the Ceriantharia and Alcyonaria, and Schmidt and Zissler (1979) regarded the basal plate as a synapomorphy between the two groups. A similar basal plate has subsequently been found in some scleractinian coral sperm (Steiner, 1993). A residual satellite apparatus remains associated with the distal centriole, and is connected with membrane-bound electron-dense bodies (Schmidt and Holtken, 1980). The pericentriolar apparatus and flagellum are similar to those in Octocorallia sperm. Schmidt and Zissler (1979) concluded that Ceriantharia sperm were the most primitive within the Hexacorallia.

### H. Order Scleractinia

Scleractinian corals are colonial or solitary anthozoans that secrete a complex calcium carbonate skeleton. Scleractinians are the most ecologically important group of cnidarians, at least in tropical and sub-tropical regions where reef-building (hermatypic) corals form the basis of coral reef ecosystems. The majority of scleractinian corals are hermaphroditic or gonochoric broadcast spawners with external fertilization, while some species have internal fertilization and brood larvae within their polyps (reviewed by Fadlallah, 1983; Harrison and Wallace, 1990; Richmond and Hunter, 1990). Among hermaphroditic broadcast spawners, gametes are usually packaged into egg-sperm bundles just prior to spawning, and released synchronously within each colony (Fig. 2B). These spawned bundles are positively buoyant and float to the sea surface where they break apart, releasing gametes for external fertilization (Harrison and Wallace, 1990). Most hermaphroditic coral species studied to date are self-sterile, although some species are capable of self-fertilization (reviewed in Harrison and Wallace, 1990). In gonochoric broadcast spawning species, sperm and eggs are usually spawned directly into the water column (Fig. 2A). Some brooding coral species produce asexually derived planula larvae (Stoddart, 1983; Ayre and Resing, 1986). In contrast, brooded planulae of other corals are products of sexual reproduction (Ayre and Resing, 1986), and in some coral species both broadcast spawning and brooding modes of development have been reported (Harrison and Wallace, 1990; Sakai, 1997). Brooded planulae tend to settle rapidly after release from the parent colony. This limits their dispersal and promotes localized larval recruitment (Harrison and Wallace, 1990; Harrison, 1993).

The discovery of the mass coral spawning phenomenon on the Great Barrier Reef (GBR), Australia (Harrison *et al.*, 1984; Willis *et al.*, 1985; Babcock *et al.*, 1986), heralded the recent renaissance of studies on the reproductive biology and ecology of reef corals (reviewed by Harrison and Wallace, 1990; Richmond and Hunter, 1990). Mass coral spawning involves the synchronous release of gametes

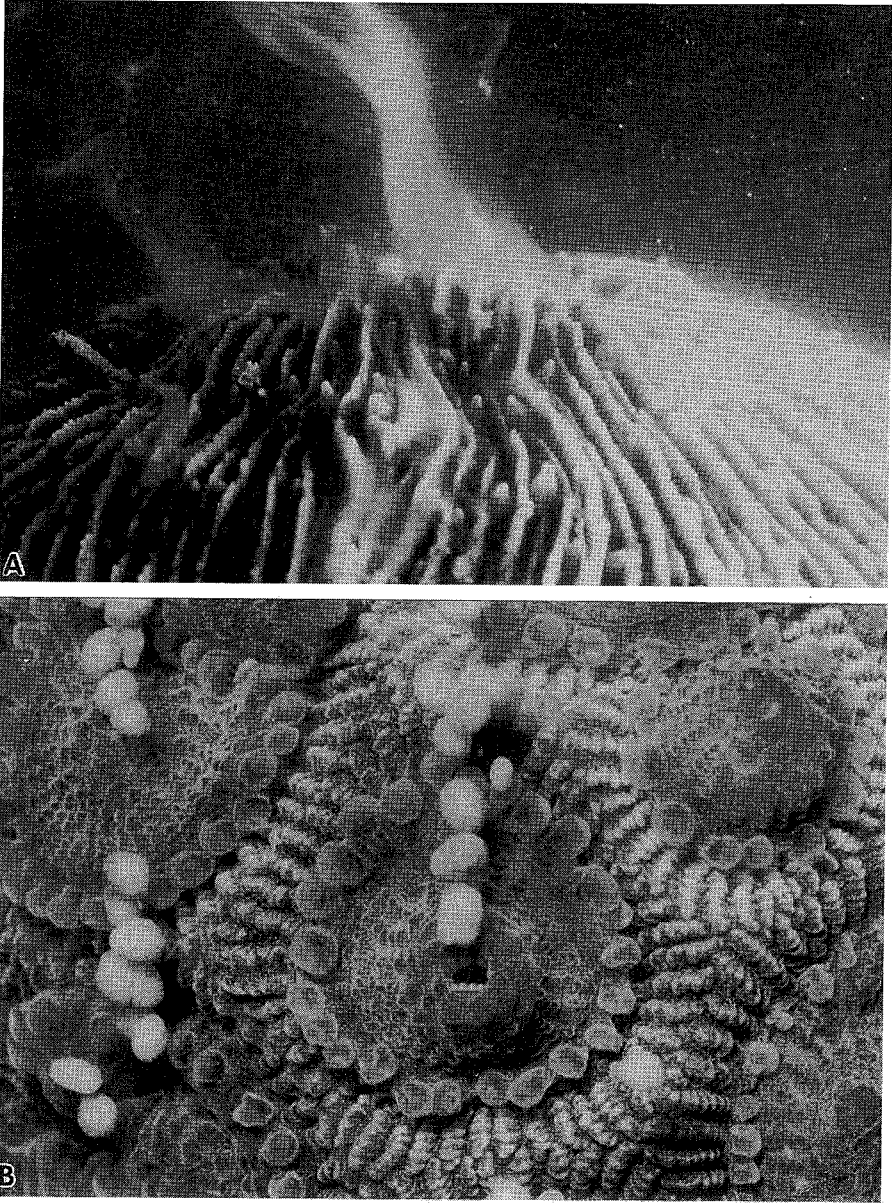


Fig 2. **A:** A male *Fungia fungites*, mushroom coral, releasing a cloud of sperm. **B:** Buoyant egg-sperm bundles spawned synchronously from polyps of the brain coral, *Favites abdita*. Photos: P. Harrison.

by many species of corals during a few predictable nights each year, and occurs during the week following full moons from October to December on the GBR (Harrison *et al.*, 1984; Willis *et al.*, 1985; Babcock *et al.*, 1986). More than 40

Table 4

## Ultrastructural studies of spermatozoa of the Scleractinia (Hexacorallia)

Family	Sperm type	Species, Reference
Pocilloporidae	pear-shaped and elongated	<i>Pocillopora damicornis</i> *, Steiner and Cortes, 1996 <i>P. elegans</i> *, Steiner and Cortes, 1996
Acroporidae	pear-shaped or ovoid, short to elongated	<i>Acropora latistella</i> , <i>A. tenuis</i> , <i>A. formosa</i> , <i>A. hyacinthus</i> , <i>A. valida</i> , <i>A. digitifera</i> , <i>A. millepora</i> , <i>A. pulchra</i> , <i>A. nobilis</i> , Harrison, 1988a; <i>A. microphthalma</i> , Harrison, 1988a, 1990; <i>A. elseyi</i> , Harrison, 1985, 1988a; <i>A. longicyathus</i> , <i>A. florida</i> , Harrison, 1988a; <i>A. cervicornis</i> *, Steiner, 1993; <i>Montipora digitata</i> , Harrison, 1985, 1988a; <i>M. angulata</i> , Harrison, 1988a
Poritidae	conical spherical	<i>Goniopora lobata</i> , Harrison, 1988a, 1990 <i>Porites astreoides</i> *, Steiner, 1993
Siderastreidae	spherical	<i>Siderastrea siderea</i> *, Steiner, 1991, 1993
Agariciidae	conical	<i>Pavona gigantea</i> *, Steiner and Cortes, 1996
Fungiidae	conical	<i>Fungia fungites</i> , <i>F. scabra</i> , Harrison, 1985, 1988a
Oculinidae	ovoid head and small midpiece	<i>Galaxea fascicularis</i> , Harrison, 1988a, 1988b, 1990; <i>Galaxea alta</i> , Harrison, 1988b, 1990
Rhizangiidae	conical	<i>Astrangia danae</i> , Hayes and Goreau, 1977; Szmant-Froelich <i>et al.</i> , 1980
Pectiniidae	pear-shaped	<i>Mycedium elephantotus</i> , Harrison, 1985, 1988a, 1990; <i>Pectinia alcornonis</i> , Harrison, 1988a, 1990
Meandrinidae	conical	<i>Dendrogyra cylindrus</i> *, Steiner, 1991, 1993
Mussidae	pear-shaped	<i>Mussa angulosa</i> *, Steiner, 1993
Faviidae	pear-shaped, short to elongated	<i>Caulastrea furcata</i> , Harrison, 1988a, 1990 <i>Favites abdita</i> , Harrison, 1988a, 1990; <i>Goniastrea aspera</i> , Harrison, 1985, 1988a, 1990; <i>G. favulus</i> , Harrison, 1988a, 1990; <i>Platygyra daedalea</i> , Harrison, 1988a, 1990; <i>Diploria strigosa</i> *, Steiner, 1991, 1993
	conical	<i>Cladocora caespitosa</i> , Schmidt and Zissler, 1979; Schmidt and Holtken, 1980; <i>Diploastrea heliopora</i> , Harrison, 1985, 1988a, 1990 <i>Montastrea annularis</i> *, Steiner, 1991, 1993; Harrison, unpublished
	spherical	<i>Favia fragum</i> *, Steiner, 1993
Caryophylliidae	conical	<i>Euphyllia divisa</i> , <i>Physogyra lichtensteini</i> , Harrison, 1988a, 1990
Dendrophylliidae	conical	<i>Turbinaria peltata</i> , Harrison, 1988a; <i>T. mesenterina</i> , Harrison, 1988a, 1990; <i>T. bifrons</i> , Harrison, 1988a; <i>Heteropsammia cochlea</i> , Harrison, 1988a, 1990

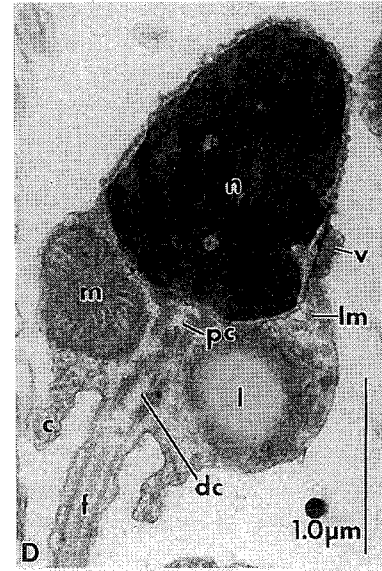
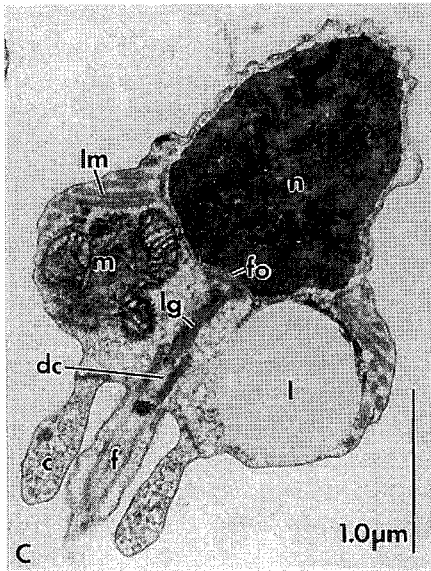
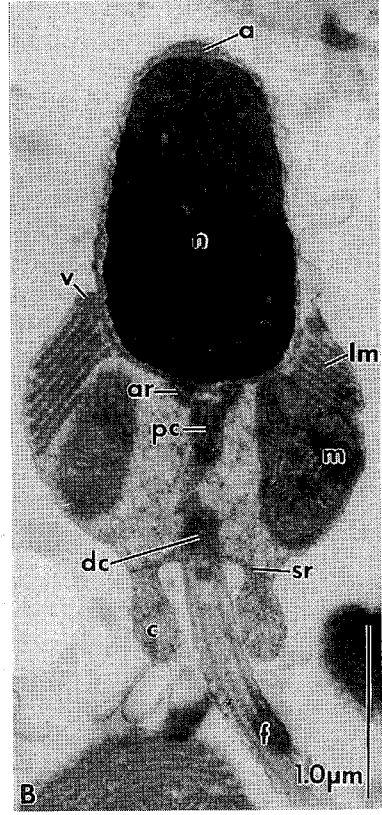
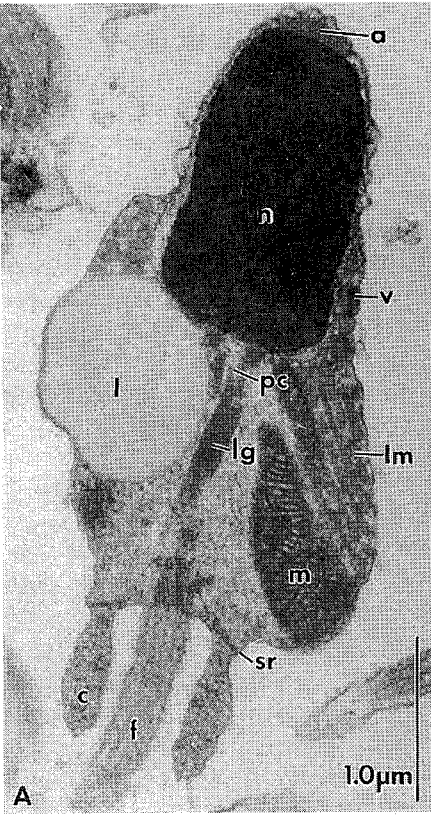
\* denotes maturing spermatids examined

1993) and three species from the eastern Pacific (Steiner and Cortes, 1996), clearly demonstrate that sperm structure within the Scleractinia is much more variable than these earlier studies indicated.

Three distinct types of spermatozoa have now been recorded in the Scleractinia (Figs. 3–6), and it seems likely that other types will be recorded when sperm from other families and additional brooding species with internal fertilization are studied. Among broadcast-spawning coral species, at least two fundamentally different types of ect-aquasperm can be recognized, although considerable variation occurs within each type (Harrison, 1988a, 1990; Harrison and Wallace, 1990). Variation in major components of the sperm (Steiner, 1993) is shown in Fig. 7. Some species produce conical sperm with a small, compact midpiece, whereas other species produce ovoid or pear-shaped sperm with an enlarged midpiece. In contrast, a smaller spherical sperm type is found in two brooding coral species with internal fertilization, and possibly in one species with external fertilization (Steiner, 1991, 1993).

### 1. Conical spermatozoa

Conical sperm with a pointed, sometimes elongated, apex and a small, compact midpiece occur in the families Poritidae, Agariciidae, Fungiidae, Rhizangiidae, Meandrinidae, Caryophylliidae and Dendrophylliidae, and in some species from the family Faviidae (Hayes and Goreau, 1977; Schmidt and Zissler, 1979; H. Schmidt, personal communication; Szmant-Froelich *et al.*, 1980; Harrison, 1985, 1988a, b, 1990; Steiner, 1991, 1993; Steiner and Cortes, 1996). The nuclear and cell membranes are closely applied to the nucleus and little or no perinuclear cytoplasm is present, hence the shape of the sperm head is determined by the form of the nucleus. The nucleus is conical and has a slightly rounded base, usually with a fossa of varying form (Fig. 3). In some species, a basal nuclear fossa appears to be lacking (Harrison, 1988a). Electron-lucent areas appear irregularly in the condensed nuclear chromatin. In most conical sperm studied, an anterior process of less electron-dense material extends from the distal portion of the nucleus. This varies from a short cone termed a 'spitzenkörper' by Schmidt and Zissler (1979; Fig. 1D), to a very prominent elongated spike in the dendrophylliids, *Turbinaria* spp. (Fig. 3D) and *Heteropsammia cochlea* and the faviid *Diploastrea heliopora* (Fig. 3B) (Harrison, 1985, 1988a, b, 1990; Harrison, unpublished). In *Goniopora lobata* sperm, the anterior process forms a flattened cap at the apex of the condensed chromatin, in *Fungia scabra* (Fig. 3A) and *Physogyra lichtensteini* (Fig. 3C) it forms a small rounded cap, whereas in *Fungia fungites*, only a very thin layer of less dense material is present at the tip of the nucleus (Harrison, 1988a). The function of the anterior process is unknown, but it is not likely to have an acrosomal function as it is contained within the nuclear membranes, and lacks obvious acrosomal features (Schmidt and Holtken, 1980; Harrison, 1990). A nuclear process of this type has not been reported in any other cnidarian or metazoan sperm,





although superficially similar fibrous material has been observed just anterior to the nucleus in some scyphozoan sperm (Hinsch and Clark, 1973; Hinsch, 1974). The less dense anterior process appears to be an apomorphic feature of scleractinian sperm.

The midpiece of conical sperm is relatively small and compact and exhibits a number of unusual features. The anterior region of the midpiece contains a series of densely-stained vesicles arranged asymmetrically around the lateral basal margin of the nucleus (Fig. 3). Similar vesicles occur in other anthozoan and cnidarian sperm (e.g. Hinsch and Clark, 1973; Schmidt and Zissler, 1979; Miller, 1983), and have been termed 'pro-acrosomal vesicles' because of their resemblance to vesicles arising from the Golgi apparatus that fuse to form the acrosome cap in sperm of other invertebrates (e.g. Afzelius and Franzén, 1971; Reunov and Kostina, 1991). Although ultrastructural examination of similar pro-acrosomal vesicles in the apical region of sperm of the hydromedusa *Cladonema uchidai* during fertilization, showed that these vesicles were not involved in any acrosomal reaction during sperm-egg interaction and membrane fusion, and were incorporated into the ooplasm intact (Yamashita, 1987), they were found to contribute to an acrosomal process in the leptomedusan *Clytia hemispherica* (Carré and Carré, 1992). Therefore, it remains to be determined whether these vesicles have an acrosomal function in scleractinian sperm.

The lateral margins of the midpiece are occupied by one or more thin lamellar sheets or bands that contain homogeneous material of moderate electron density (Fig. 3). In *Cladocora caespitosa* and *Turbinaria peltata* sperm, the lamellae form a netlike structure (Schmidt and Zissler, 1979; Harrison, 1988a). The lamellae lie adjacent to the mitochondria, which may indicate that they have an energy function (Harrison, 1990). Smaller lamellar structures are present in sperm from the orders Ceriantharia, Corallimorpharia, Actiniaria and Zoanthidea (Fig. 1) (Schmidt and Zissler, 1979), and some other cnidarians (Dewel and Clark, 1972; Hinsch and Clark, 1973; Clark and Dewel, 1974; Lyke and Robson, 1975). A ring of compressed mitochondria occupies most of the midpiece and reduces the volume of cytoplasm around the centriolar complex (Fig. 3). In some species, the mitochondria condense and partially fuse during spermatogenesis (Schmidt and Zissler, 1979; Harrison, 1988a; Steiner, 1993). A spherical lipid body is inserted among the mitochondria on one side of the midpiece, and causes a prominent asymmetrical bulge in some species (Harrison, 1988a, 1990). Steiner (1993) recorded a lipid body in maturing spermatids of some coral species, but concluded that a lipid body was absent in four species. Given that spermatozoa from all other coral species studied to date

Fig. 4. Mature pear-shaped, and *Galaxea*, scleractinian spermatozoa. A: *Acropora digitifera* (Acroporidae). B: *Montipora digitata* (Acroporidae). C: *Mycedium elephantotus* (Pectiniidae). D: *Galaxea fascicularis* (Oculinidae). a, Anterior process; ar, attachment ring; c, collar; dc, distal centriole; f, flagellum; l, lipid body; lg, intercentriolar ligament; lm, lamellae; m, mitochondria; n, nucleus; pc, proximal centriole; sr, pericentriolar network; v, vesicles. From Harrison (1988a). 'Comparative ultrastructure of scleractinian spermatozoa and its evolutionary implications', Ph.D. thesis, James Cook University of North Queensland, Townsville, 282 pp.



arrangement of microtubules. The partial fusion of the mitochondria, the presence of a lipid body, modified centriolar complex and cytoplasmic collar, and the asymmetrical arrangement of the midpiece represent apomorphic modifications of the 'primitive' sperm pattern (Franzén, 1970, 1977).

## 2. Ovoid and pear-shaped sperm

Spermatozoa with an ovoid or pear-shaped form and an enlarged midpiece (Figs. 4A–D) occur in the scleractinian families Pocilloporidae, Acroporidae, Pectiniidae, Mussidae, and in some species of Faviidae (Harrison, 1985, 1988a, 1990; Steiner, 1991, 1993; Steiner and Cortes, 1996). These sperm are distinguished from the conical sperm type principally by the generally rounded anterior of the head, the ovoid, bell-shaped or bullet-shaped nucleus, and the enlarged midpiece with more extensively developed lamellae (Figs. 4A–D, 5A; Harrison, 1990; Steiner, 1993). The bullet-shaped nucleus of acroporid sperm (Figs. 4A, B) has a domed cap of less dense material and a flattened base with slight indentations corresponding to the attachment site of the proximal centriolar ring. The length and shape of the nucleus varies in a species-specific manner among the *Acropora* species and *Montipora* species studied to date (Harrison, 1988a, 1990). A similar elongated, bullet-shaped nucleus has also been observed in spermatids of *Pocillopora elegans* and *P. damicornis* (Steiner and Cortes, 1996). It should be noted that although *P. damicornis* broods planulae within its polyps, electrophoretic evidence shows that these brooded planulae are derived asexually from parental tissues (reviewed in Harrison and Wallace, 1990), and Ward (1992) concluded that gametes from this species are spawned for external fertilization. Sperm ultrastructure provides additional evidence for broadcast spawning in this species, hence these sperm are considered ect-aquasperm. In Pectiniidae, Mussidae and Faviidae with pear-shaped sperm, the nucleus lacks a less dense cap and has a more prominent basal fossa (Fig. 4) (Harrison, 1985, 1988a, 1990; Steiner, 1991, 1993).

The midpiece is relatively large, and is characterized by extensive development of lamellae which occupy most of the anterior and lateral regions of the midpiece (Figs. 4, 5A). Up to nine layers of lamellae are stacked in parallel arrays to form an asymmetrical ring around the midpiece. A larger number of vesicles are present in these sperm compared with conical coral sperm. The extent of mitochondrial fusion varies considerably in different groups. Five to eight mitochondria are packed together but usually remain unfused in *Montipora* sperm, mitochondria are partly fused in some Pectiniidae and Faviidae sperm, whereas in *Acropora* sperm the mitochondria fuse to form a single mitochondrial mass or nebenkern (*sensu* Schmidt and Zissler, 1979) (Harrison, 1988a, 1990). The mitochondria and lamellate body are displaced by the large spheroidal lipid body present on one side of the midpiece. The intercentriolar ligament is much longer in these sperm compared with conical sperm with a shorter midpiece, and in some cases the ligament is bent in a manner suggesting that it may act to stabilize the centriolar

complex during flagellar movement (Harrison, 1988a). In *Acropora* sperm, the primary pericentriolar processes are connected by lateral tertiary processes, while the outer members of the secondary processes fuse to create a complex star pattern (Harrison, 1988a). The cytoplasmic collar is generally much longer in these sperm compared with conical sperm, and is most extensively developed in *Favites abdita* sperm, where it closely encircles and sometimes fuses with the flagellar membrane (Harrison, 1988a). In some *Acropora* sperm, the flagellar membrane expands laterally to form a winged profile, which may increase swimming efficiency (Harrison, 1988a).

### 3. *Galaxea* sperm

Although most of the sperm examined ultrastructurally from scleractinian broadcast spawning species can be categorized as either conical sperm or ovoid/pear-shaped types, sperm from the oculinid corals *Galaxea fascicularis* and *G. alta* have an intermediate form (Fig. 4D; Harrison, 1988b, 1990). These small ovoid sperm have an ovoid nucleus with a domed anterior and a shallow basal fossa, which is very similar to the nucleus of pear-shaped pectiniid (Fig. 4C) and some faviid sperm (Harrison, 1988a, 1988b, 1990). However, the midpiece is small, contains few lamellae and has a short cytoplasmic collar, like the midpiece of conical sperm (cf. Fig. 3).

### 4. Spherical sperm

Spermatids of the brooding corals *Porites astreoides* (Family Poritidae) (Fig. 5C) and *Favia fragum* (Family Faviidae) (Fig. 5D), and the broadcast spawning species *Siderastrea siderea* (Family Siderastreidae) (Fig. 5B) have a very similar ultrastructure (Steiner, 1991, 1993). They have a spherical nucleus with a shallow basal fossa and a single layer of small, electron-dense vesicles anterior to the nucleus (Steiner, 1993). Similar electron-dense vesicles are present above the nucleus of Antipatharia sperm (Fig. 1L) (Schmidt and Zissler, 1979; Schmidt and Holtken, 1980). The compact midpiece contains four or five mitochondria which are partially or fully fused to form a nebenkern in *S. siderea*, a single layer of electron-dense vesicles or lamellae that fuse to form a net-like structure in *Favia fragum*, and a large lipid body in *S. siderea*. The centriolar complex and flagellum are essentially identical to those described for conical and ovoid or pear-shaped ect-aquasperm, whereas these spermatids lack an obvious collar. Steiner (1993) considered that mature spermatozoa of these three species were likely to be spherical, however, spermatozoa of *Porites astreoides* have been recorded as conical (Chornesky and Peters, 1987), and Duerden (1902) described live spermatozoa of *Favia fragum* as pear-shaped. These spermatids are distinguished from other scleractinian sperm primarily by their spherical morphology, spherical nuclei and dense-staining vesicles at the anterior of the nucleus.

Steiner (1993) interpreted these spherical sperm as representing a third distinct type of sperm in scleractinians, and noted that their unusual structure was not consistent with brooding or spawning modes of development. However, an alternative interpretation is plausible if the spermatid samples recorded from *Siderastrea siderea* (Fig. 5B) were in fact collected from the closely related and morphologically very similar congener, *Siderastrea radians*, which is known to brood larvae (Duerden, 1902; Szmant, 1986). If these spermatids were from *S. radians*, the spherical sperm type could be regarded as ent-aquasperm (*sensu* Rouse and Jamieson, 1987), and would correlate with internal fertilization and a brooding mode of development.

### 5. Sperm structure in relation to reproductive biology

The spherical spermatids from brooding species in the disparate families Poritidae and Faviidae (and possibly Siderastreidae) (Figs. 5B–D), are distinct from the conical (Fig. 3) or pear-shaped (Fig. 4) sperm produced by broadcast spawning species in those families studied to date (Schmidt and Zissler, 1979; Harrison, 1985, 1988a, 1990; Steiner, 1991, 1993). If a spherical sperm type is subsequently found to be restricted to brooding coral species, it would indicate that internal fertilization has a major influence on sperm structure in scleractinians. This would agree with comparative studies of some other metazoan sperm, where species with internal fertilization have 'modified' spermatozoa, and sperm structure correlates better with the environment and site of fertilization than it does with systematic position (e.g. Franzén, 1970, 1977; Baccetti and Afzelius, 1976). This is not to deny the important contribution that comparative studies of sperm ultrastructure have made to phylogeny and taxonomy (e.g. Afzelius, 1979; Jamieson *et al.*, 1995).

Among broadcast-spawning corals, the distribution of conical sperm versus pear-shaped or ovoid sperm types was initially considered to be strongly correlated with sexual patterns (Harrison, 1985, 1988a, 1988b, 1990). Of the 41 coral species studied by Harrison (1988a, 1988b, 1990), the 24 species with pear-shaped or ovoid sperm are all hermaphroditic, whereas the 15 species with conical sperm are all gonochoric. Furthermore, the two *Galaxea* species with an intermediate sperm structure have an unusual intermediate 'pseudo-gynodioecious' sexual pattern consisting of female colonies and hermaphroditic colonies with functional sperm and infertile eggs (Harrison, 1988b). Harrison (1990) suggested that pear-shaped and ovoid sperm with an enlarged midpiece might have greater motility than conical sperm to enhance outcrossing and reduce self-fertilization in hermaphroditic species. However, more recent work has shown that conical sperm are produced by the hermaphroditic faviids *Cyphastrea serailia* (Harrison, unpublished) and *Montastrea annularis* (Steiner, 1991, 1993; Harrison, unpublished). Thus, sperm type is not rigidly correlated with the sexual pattern of corals. Therefore, differences in sperm structure among broadcast spawning corals probably have a phylogenetic

basis (Harrison, 1988a, 1990). Differences in sperm structure may also be related to differences in the external structure or fertilization properties of coral eggs (Harrison, 1990).

## 6. Sperm chemotaxis and hybridization

Mass multispecific spawning by many species of corals raises some intriguing questions as to how and why spawning is synchronized within and among species (reviewed by Harrison and Wallace, 1990). Synchronous spawning by congeneric species may also pose problems for recognition of conspecific gametes in the mass of spawned eggs and sperm that occurs during mass spawning episodes (Harrison *et al.*, 1984). Miller (1979, 1980, 1983) documented sperm chemotaxis among species of hydromedusae that exhibit multispecific spawning, and has subsequently recorded chemotactic turning behaviour in scleractinian sperm (Miller, personal communication). Coll and Miller (1991) recorded sperm attractants in some mass spawning corals, and Coll *et al.* (1994) found that the unsaturated fatty alcohol, dodeca-2,4-diynol, is a natural sperm attractant present in eggs of the coral *Montipora digitata*. Sperm of *M. digitata* are attracted by natural and synthetic versions of this compound, and by a mixture of this compound and two other unsaturated fatty alcohols derived from the eggs. Sperm of the related coral *M. crassituberculata* were not attracted by the compound, indicating that some degree of specificity exists in the sperm attractant of *M. digitata* (Coll *et al.*, 1994). Sperm of *M. digitata* were not attracted by the diterpene (-)-*epi*-thunbergol sperm attractant released by eggs of the alcyonacean soft coral *Lobophytum crassum* (Coll *et al.*, 1995).

If species-specific sperm chemotaxis is widespread among mass spawning corals, it would enhance reproductive success by counteracting sperm dilution problems following spawning, and might reduce the potential for hybridization. Oliver and Babcock (1992) examined the effects of sperm dilution on fertilization rates in three reef coral species, and found maximum fertilization rates at sperm concentrations of  $10^5$ – $10^6$  per mL, with reduced fertilization at both higher and lower sperm concentrations. Field studies showed that fertilization rates were high just after major synchronous spawning events, and that fertilization potential decreased over time, and on minor spawning nights (Oliver and Babcock, 1992). Thus, gamete dilution can play an important role in limiting reproductive success among corals. It is inferred, therefore, that there is strong selective pressure for synchronous spawning to ensure high gamete concentrations which would maximize the probability of fertilization and outcrossing.

Water quality also influences fertilization processes in coral gametes, and a variety of pollutants including oil hydrocarbons (Harrison, 1993), trace metals (Reichelt and Harrison, unpublished), nutrients (Harrison and Ward, unpublished) and stormwater runoff (Richmond, 1993) inhibit or reduce fertilization rates. The diterpenes, flexibilide and dihydroflexibilide, derived from the soft coral *Simularia*

*flexibilis*, inhibit the activity of *Acropora tenuis* and *Montipora digitata* sperm at a concentration of 5 ppm, resulting in reduced fertilization rates, and sperm ceased swimming after one hour exposure (Aceret *et al.*, 1995). UV radiation also reduces fertilization success in *Fungia scutaria* gametes, and Gulko (1995) found that UV exposure had a greater effect on spermatozoa than on eggs or planulae of *F. scutaria*. Susceptibility to UV radiation damage may explain why most corals spawn at night rather than during daylight (Harrison and Wallace, 1990; Gulko, 1995).

Mass multispecific coral spawning provides opportunities for hybridization because gametes from many congeneric species are spawned synchronously, the positively buoyant gametes are concentrated into a thin layer at the sea surface, and sperm and eggs remain viable for up to eight hours (Willis *et al.*, 1997). Experimental studies showed that hybridization occurred readily under laboratory conditions in 16 of 42 species pairs from the common coral genera *Acropora*, *Montipora* and *Platygyra* (Willis *et al.*, 1997), and among seven morphologically defined species of *Platygyra* (Miller and Babcock, 1997). Hybridization occurred most readily between species that are morphologically similar, and also between some species that are morphologically distinct, whereas breeding incompatibilities were found within some currently recognized species. Viability of larvae resulting from hybrid crosses was not significantly different from that of non-hybrid embryos, and hybrid larvae were able to settle successfully and grow (Willis *et al.*, 1997). Imprecision in the alignment of morphological and breeding boundaries may be a consequence of mating systems governed by interactions among gametes, where minor changes in egg-surface or sperm properties may lead to gamete incompatibility and reproductive isolation. Willis *et al.* (1997), like Veron (1995), concluded that a single species concept may not apply to scleractinian corals, and that hybridization introduces a reticulate nature to the evolution of corals, which has profound implications for understanding the phylogeny and evolutionary biology of scleractinians.

## 7. Systematic and phylogenetic implications for Scleractinia

The systematics and phylogeny of the Order Scleractinia are currently in a state of flux. Wells' (1956) classic evolutionary scheme for scleractinian corals has recently been revised by Veron (1995) who created a new suborder Poritiina for the Family Poritidae, and modified the systematic grouping of a number of other families. Molecular studies using ribosomal DNA have produced different interpretations of evolutionary relationships in the Scleractinia. Chen *et al.* (1995) and Veron *et al.* (1996) concluded that the Scleractinia were monophyletic based on analysis of the 5'-end of the 28S rDNA. Romano and Palumbi (1996) analysed mitochondrial 16S rDNA and found that coral families clustered into two major groups that do not correspond to morphologically based suborders. Romano and Palumbi (1996) concluded that the Scleractinia diverged before the appearance of coral skeletons

240 million years ago, and that there had been repeated evolution of the scleractinian skeleton. None of these recent analyses accords completely with systematic trends in scleractinian sperm structure and sexual patterns (Harrison, 1985, 1988a, 1990), however, some systematic groupings are evident.

The spermatozoa studied within the suborder Astrocoeniina have a similar bullet-shaped nucleus and elongated midpiece, and can be distinguished ultrastructurally from other pear-shaped sperm found in the suborder Faviina (Harrison, 1988a, 1990; Steiner and Cortes, 1996). This supports the separate systematic position of the Astrocoeniina within the Scleractinia (Wells, 1956; Veron, 1995; Veron *et al.*, 1996). The suborder Fungiina is regarded as polyphyletic (Veron *et al.*, 1996), and conical and spherical sperm have been recorded in this group (Harrison, 1985, 1988a, 1990; Steiner, 1991, 1993; Steiner and Cortes, 1996). Veron *et al.* (1996) concluded that the suborder Faviina should probably be retained as a monophyletic group, however conical, pear-shaped and spherical sperm have been recorded in the Faviina (Harrison, 1985, 1988a, 1990; Steiner, 1991, 1993; Steiner and Cortes, 1996), suggesting that it may be polyphyletic. Harrison (1988a) concluded that the presence of conical sperm and pear-shaped sperm within the Family Faviidae might indicate that the earlier subfamily division of the Faviidae was correct, as conical sperm have only been recorded from genera formerly grouped in the subfamily Montastreinae. Only conical sperm have been recorded in taxa studied from the suborders Caryophylliina and Dendrophylliina (Harrison, 1988a, 1990).

Although all scleractinian sperm studied so far possess cytological features that deviate from the 'primitive' sperm pattern, the conical sperm type appears to be more primitive than pear-shaped and ovoid sperm which generally exhibit greater modifications of the midpiece and are structurally more diverse (Harrison, 1988a, 1990). As most coral species with conical sperm have a gonochoric sexual pattern, Harrison (1990) suggested that gonochorism may be the ancestral sexual pattern in the Scleractinia. Comparison of scleractinian sperm structure with that in other anthozoan orders indicates a close relationship between the Order Scleractinia and the Order Corallimorpharia, which is discussed below.

### I. Order Corallimorpharia

Corallimorpharians are solitary or colonial anthozoans that resemble scleractinian corals but lack a calcareous skeleton. Reproduction has been studied in very few corallimorpharian species, and these were found to be gonochoric broadcast spawners (Holts and Beauchamp, 1993). Spermatogenesis and spermatozoa ultrastructure has been studied only in the Mediterranean corallimorpharian *Corynactis viridis* (Schmidt and Zissler, 1979; Schmidt and Holtken, 1980).



### 1. Corallimorpharian sperm structure and phylogeny

The nucleus of *Corynactis viridis* sperm is concavely conical with a rounded anterior and a deep basal nuclear fossa (Fig. 1C) (Schmidt and Zissler, 1979). The midpiece is large relative to the nucleus, and contains a series of electron-dense bodies along the periphery. Four or five mitochondria are present, and in some sections these appear to be partly fused. A prominent lipid body is inserted on one side of the midpiece. The centriolar complex is very similar to that found in scleractinian sperm (Figs. 1C,D, 4-6). A series of dense processes originate in the proximal centriole and expand terminally to form an attachment ring connected to the nuclear membranes at the periphery of the basal nuclear fossa (Fig. 1C; Schmidt and Zissler, 1979; Schmidt and Holtken, 1980). The proximal and distal centriole are aligned or slightly tilted along the longitudinal axis of the sperm, and are interconnected by a spiral intercentriolar ligament. The distal centriole has a pericentriolar apparatus consisting of nine primary processes that branch into three secondary processes that give rise to an array of fibres that extend to the cell membrane (Schmidt and Zissler, 1979). A small cytoplasmic collar occurs at the base of the midpiece, and the flagellum has the usual 9+2 arrangement of microtubules.

Comparative sperm structure indicates that there is a close phylogenetic relationship between the Corallimorpharia, as represented by *Corynactis viridis*, and the Scleractinia. Sperm from both groups share unique features including centrioles aligned along the longitudinal axis of the cell, a similar proximal centriolar attachment ring and distal pericentriolar network, an intercentriolar ligament, and a cytoplasmic collar at the base of the midpiece (Schmidt and Zissler, 1979; Schmidt and Holtken, 1980; Harrison, 1988a, 1990; Steiner, 1993). The close relationship between scleractinians and corallimorpharians is also supported by comparative studies of nematocysts (Schmidt, 1974). However, Chen *et al.* (1995) found that analysis of rDNA sequence data resolved the Scleractinia as one group, and two other groups containing both corallimorpharians and actiniarians. Clearly, further comparative sperm studies and molecular studies are required to understand the evolutionary relationships among the Corallimorpharia, Scleractinia and Actiniaria.

### J. Order Actiniaria

Actiniarian sea anemones are solitary anthozoans that lack a skeleton. About 800 species are known (Barnes and Harrison, 1991), and some actiniarians provide essential habitats for anemone fish. Sea anemones are gonochoric or hermaphroditic and reproduce by broadcast spawning of gametes, or by brooding larvae internally or externally (reviewed by Chia, 1976; Fautin, 1991). Internally brooded larvae of some *Actinia* anemones are asexually produced (Ottaway and Kirby, 1975; Orr *et al.*, 1982; Fautin, 1991). Reproduction has been comparatively

well studied in actinurians, with ultrastructural information on spermatogenesis or mature spermatozoa, or both, available for the 29 species listed in Table 5.

### 1. Actinurian sperm structure and phylogeny

Schmidt and Zissler (1979) recognized three distinct types of sperm among the Actinurians. They suggested that the Actinurians are paraphyletic, as the

Table 5

Ultrastructural studies of spermatogenesis and/or spermatozoa of the Actinurians (Hexacorallia)

Higher taxon	Species	Reference
<b>Subclass Hexacorallia</b>		
Order Actinurians	<i>Actinia equina</i>	Larkman, 1980
	<i>Actinia equina</i> var. <i>mesembryanthemum</i>	Larkman and Carter, 1980
	<i>Actinothoe sphyrodeta</i>	Schmidt and Zissler, 1979
	<i>Adamsia palliata</i>	Schmidt and Zissler, 1979
	<i>Aiptasia diaphana</i>	Schmidt and Zissler, 1979;
		Schmidt and Holtken, 1980
	<i>Aiptasia pallida</i>	West, 1980a
	<i>Andresia partenopea</i>	Schmidt and Zissler, 1979
	<i>Anemonia sulcata</i>	Schmidt and Zissler, 1979
	<i>Anthopleura orientalis</i>	Reunov and Kostina, 1991
	<i>Anthopleura rubripunctata</i>	Schmidt and Zissler, 1979
	<i>Anthopleura</i> sp.	Schmidt and Zissler, 1979
	<i>Anthopleura stellula</i>	Schmidt and Zissler, 1979
	<i>Bunodactis verrucosa</i>	Schmidt and Zissler, 1979
	<i>Bunodosoma cavernata</i>	Dewel and Clark, 1971, 1972;
		Clark and Dewel, 1974
	<i>Calliactis parasitica</i>	Lyke, 1975; Lyke and Robson, 1975;
		Schmidt and Zissler, 1979
	<i>Cereus pedunculatus</i>	Schmidt and Zissler, 1979
	<i>Condylactis aurantiaca</i>	Schmidt and Zissler, 1979
	<i>Cribrinopsis crassa</i>	Schmidt and Zissler, 1979
	<i>Edwardsia callimorpha</i>	Schmidt and Zissler, 1979
	<i>Edwardsia timida</i>	Schmidt and Zissler, 1979
	<i>Gonactinia prolifera</i>	Lyke and Robson, 1975
	<i>Halcampa chrysanthellum</i>	Schmidt and Zissler, 1979
	<i>Hormathia coronata</i>	Schmidt and Zissler, 1979
	<i>Isoedwardsia lucifuga</i>	Schmidt and Zissler, 1979
	<i>Metridium senile</i>	Schmidt and Zissler, 1979
	<i>Metridium dianthus</i>	Hinsch and Clark, 1970a, 1973
		Clark and Dewel, 1974
	<i>Paracalliactis stephensoni</i>	Van-Praet, 1990
	<i>Protanthea simplex</i>	Lyke and Robson, 1975;
		Schmidt and Zissler, 1979
	<i>Tealia felina</i>	Schmidt and Holtken, 1980
	<i>Telmactactis forskali</i>	Schmidt and Zissler, 1979

Protantheae (Fig. 1E) appear in their cladogram to have been derived independently from the common ancestor of the Actiniaria and remaining Anthozoa (Figs. 1F–I).

(a) *Type I sperm*

The most primitive actiniarian sperm, designated Type I sperm, occur in the Protantheae (Figs. 1E, 8B), Boloceroidaria and Mesomyaria (Figs. 1F, 8A) (Schmidt and Zissler, 1979). This type of sperm has been recorded in *Protanthea simplex*, *Gonactinia prolifera*, *Aiptasia pallida*, *Aiptasia diaphana*, *Hormathia coronata*, *Adamsia palliata*, *Calliactis parasitica*, *Metridium senile*, *M. dianthus*, *Cereus pedunculatus*, *Telmatactis forskali*, *Actinothoe sphyrodeta*, and *Halcampa chrysanthellum* (Hinsch and Clark, 1973; Clark and Dewel, 1974; Lyke and Robson, 1975; Schmidt and Zissler, 1979; West, 1980a). These elongate pear-shaped sperm are characterized by an elongate conical head and an enlarged midpiece that forms a collar around the anterior region of the flagellum. Most Type I sperm examined have a long, concavely conical nucleus with a narrow, elongate tip, and a broad basal fossa (Figs. 1E,F, 8A,B). In *Calliactis* (Fig. 8A) and *Protanthea* (Fig. 8B) sperm the nucleus is about 3  $\mu\text{m}$  long and 0.8–1.2  $\mu\text{m}$  wide basally (Lyke and Robson, 1975). The narrow anterior region is about 1.5  $\mu\text{m}$  long and apically blunt. The posterior fossa is 400–800 nm wide and 120–200 nm deep. Electron-lucent spaces are often present near the tip of the nucleus (Clark and Dewel, 1974; Lyke and Robson, 1975; Schmidt and Zissler, 1979). In *Aiptasia pallida* sperm, the conical nucleus is shorter, and the chromatin is uniformly condensed (West, 1980a).

A limited number of small electron-dense doughnut-shaped or rod-shaped vesicles, 150–300 nm in diameter, surround the basal region of the nucleus and sometimes extend into the anterior region of the midpiece (Hinsch and Clark, 1973; Clark and Dewel, 1974; Lyke and Robson, 1975). These vesicles are derived from the Golgi complex during early maturation phases, and contain a carbohydrate moiety and proteins rich in basic amino acids (West, 1980a). Glycogen particles are present in the cytoplasm of the midpiece (Clark and Dewel, 1974; West, 1980a). Six to nine electron-dense or pale lipid spheres surround the centrioles and are in turn surrounded by the nebenkern. The nebenkern occupies most of the midpiece and consists of a ring of about 15 conjoined, but distinguishable, mitochondrion elements, which extend posteriorly during maturation to form a distinctive collar around the anterior portion of the flagellum (Figs. 1E,F, 8A,B) (Lyke and Robson, 1975; Schmidt and Zissler, 1979; West, 1980a). The mitochondrial collar is regarded as an apomorphic characteristic of these sperm (Schmidt and Zissler, 1979).

The proximal centriole lies within, or adjacent to, the basal nuclear fossa, and is nearly transverse to the longitudinal axis of the sperm. The proximal centriole is connected to the thickened nuclear membranes in the fossa by a series of electron-dense fibres that arise from the proximal side of the centriole (Figs. 1E,F, 8A,B). These centriolar processes are reduced in the Boloceroidaria and Mesomyaria, a

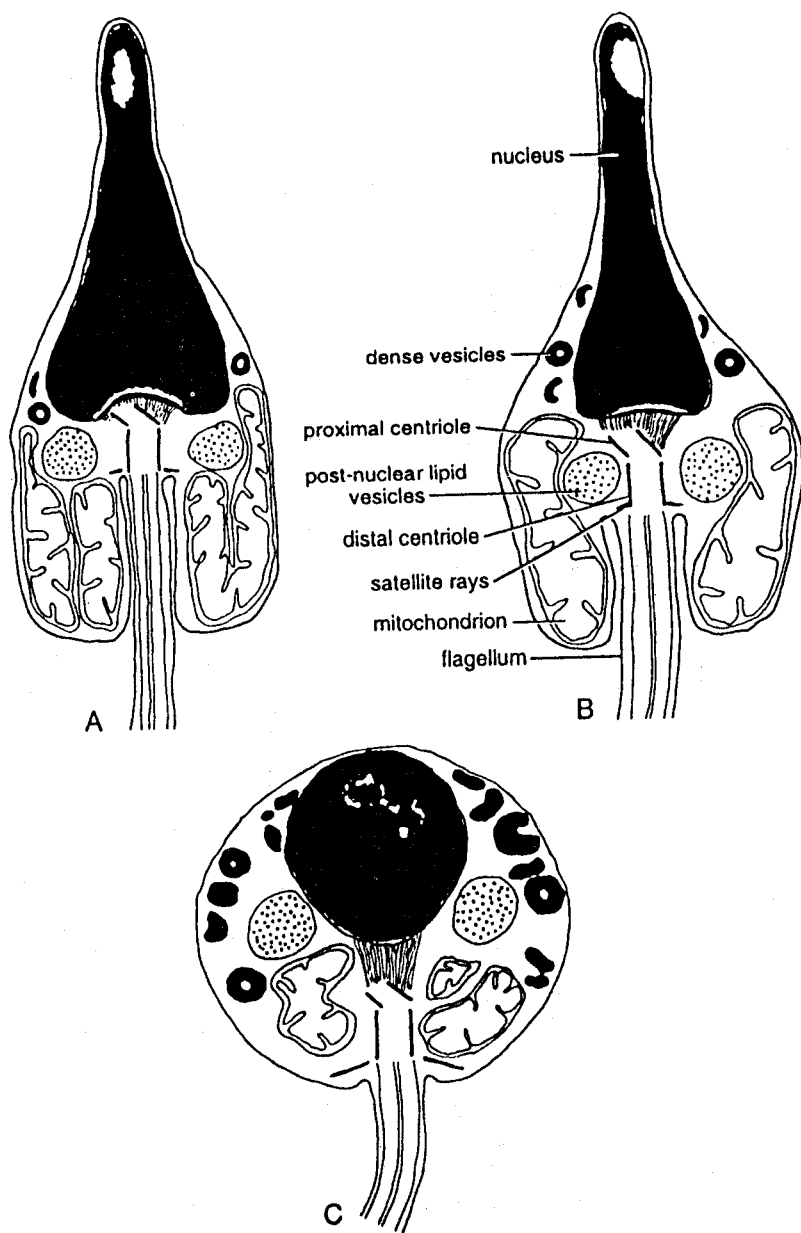


Fig. 8. Diagrams of fully formed spermatids in longitudinal section. A: *Calliactis parasitica* (Actiniaria). B: *Protanthea simplex* (Actiniaria). C: *Parazoanthus lucificum* (Zoanthidea). After Lyke and Robson (1975). *Cell and Tissue Research*, 157, 185–205, fig. 10.

synapomorphy with other Actiniaria (Schmidt and Zissler, 1979). The distal centriole is orientated longitudinally, and is connected by a fibre to the end of the overlying proximal centriole. Short pericentriolar processes extend from the matrix of the distal centriole and connect with the plasmalemma. *Aiptasia pallida* sperm have a simple pericentriolar apparatus that lacks secondary processes (West, 1980a). The flagellum of *Calliactis parasitica* spermatids is about 50  $\mu\text{m}$  long, and in some sections the flagellum membrane is continuous with the membrane of the mitochondrial collar (Lyke and Robson, 1975).

Schmidt and Zissler (1979) concluded that anemones with primitive Type I sperm gave rise to Actiniaria with Type II and Type III sperm. The remaining Anthozoa are considered to be the apomorph sister group of the Boloceroidea, and are divided into two branches. One branch includes the endomyarian Actiniaria (Figs. 1G,H). The other branch leads to the actiniarian *Edwardsia* (Fig. 1I), the Zoanthidea (their Zoantharia) (Figs. 1J,K), and the Antipatharia at the summit of the Anthozoa (Fig. 1L).

#### (b) Type II sperm

The sea anemones *Edwardsia callimorpha*, *E. timida* and *Isoedwardsia lucifuga* from the Family Edwardsiidae have a Type II actiniarian sperm (Schmidt and Zissler, 1979). Compared with Type I sperm, Type II sperm have a different nucleus, a shorter midpiece which is not extended around the flagellum as a collar, and a different arrangement of organelles in the midpiece (Fig. 1I). Type II sperm have a conical nucleus with a rounded anterior, and a rounded base with no fossa. The basal membrane of the nucleus has pores and is thickened (Schmidt and Zissler, 1979). *Isoedwardsia lucifuga* sperm are pear-shaped, with an elongate nucleus with concave sides and a broader rounded base (Fig. 1I). A series of electron-dense lamellar bodies extend around the lateral margins of the nucleus and into the upper region of the midpiece. Schmidt and Zissler (1979) regarded these lamellar structures and the absence of a basal nuclear fossa as synapomorphous with the Zoanthidea. Subsequent work has shown that some scleractinian sperm have similar lamellar structures in the midpiece, and a nucleus without a prominent basal fossa (Harrison, 1988a).

The midpiece is mainly occupied by a ring of partially fused mitochondria which is similar to the nebenkern of Type I sperm, but does not extend around the flagellum as a collar. Six to nine lipid spheres lie adjacent to the mitochondria and surround the centrioles at the base of the nucleus (Fig. 1I). The distal and proximal centrioles are orientated at an obtuse angle to each other, and are connected to the basal nuclear membranes by a series of electron-dense fibres. The distal centriole is aligned longitudinally, and gives rise to a pericentriolar apparatus that extends across the base of the midpiece adjacent to the plasma membrane. The absence, or extreme reduction, of a cytoplasmic collar in Type II sperm is apparently an apomorphy of the antipatharian line (Schmidt and Zissler, 1979).

(c) *Type III sperm*

Type III actiniarian sperm are characterized by having a relatively short nucleus, and a markedly asymmetrical midpiece (Figs. 1G,H). This type of sperm has been recorded in *Bunodosoma cavernata* (Dewel and Clark, 1972; Clark and Dewel, 1974), *Actinia equina* (Larkman, 1980; Larkman and Carter, 1980), *Tealia felina* (Schmidt and Holtken, 1980) and in eight species of the Endomyaria examined by Schmidt and Zissler (1979). The latter authors regarded the Endomyaria as monophyletic, with two branches; the first branch containing *Andresia* (Fig. 1G), and the other branch containing the remaining endomyarians (Fig. 1H).

In Type III sperm examined so far, the nucleus is short and has a prominent flat basal fossa, and in most cases, one or more small electron-lucent spaces are present within the condensed chromatin. The nucleus of *Andresia partenopea* sperm (Fig. 1G) has slightly concave sides and a broad rounded anterior (Schmidt and Zissler, 1979), while the nucleus of *Actinia equina* has a similar broadly rounded anterior and is 1  $\mu\text{m}$  in diameter and 1.25  $\mu\text{m}$  long (Larkman and Carter, 1980; Larkman, 1980). The nucleus of *Bunodosoma cavernata* and *Condylactis aurantiaca* sperm is short and broad with a domed anterior (Dewel and Clark, 1972; Clark and Dewel, 1974; Schmidt and Zissler, 1979). Spawned spermatozoa of *Tealia felina* have a more elongated nucleus with a narrow domed anterior (Schmidt and Holtken, 1980). Several types of curved or ring-shaped, membrane-bound vesicles occur around the lateral posterior margin of the nucleus and in the midpiece (Dewel and Clark, 1972; Schmidt and Zissler, 1979; Larkman and Carter, 1980).

The midpiece contains a single complex mitochondrion derived from fusion of smaller mitochondria during spermiogenesis, and consists of a central spherical region partly surrounded by a cup-shaped extension, or it has the form of a coiled or folded sheet (Dewel and Clark, 1972; Clark and Dewel, 1974; Schmidt and Zissler, 1979; Larkman and Carter, 1980). The mitochondrion causes a prominent asymmetrical bulge on one side of the midpiece, and is closely associated with a large spherical lipid body. Schmidt and Zissler (1979) regarded the marked asymmetry of these sperm as an autapomorphy of the Endomyaria.

The proximal centriole lies at a 45° angle to the sperm axis and is located in the basal fossa of the nucleus. Electron-dense fibres extend from the centrioles to the basal nuclear fossa (Schmidt and Zissler, 1979). The distal centriole is surrounded by a ring of nine pericentriolar processes that contact the plasma membrane of the cytoplasmic collar (Dewel and Clark, 1971; Larkman and Carter, 1980). In *Actinia equina* sperm, the cytoplasmic collar is up to 1  $\mu\text{m}$  long and has a layer of electron-dense material near the membrane, which may increase the rigidity of the collar (Larkman and Carter, 1980). The collar membrane fuses with the flagellar membrane in *Bunodosoma cavernata* sperm (Dewel and Clark, 1972; Clark and Dewel, 1974). The flagellar axoneme arises from the distal centriole, and consists of nine microtubule doublets, and two central tubules that appear

about 200 nm below the centriole (Larkman and Carter, 1980). Y-shaped fibrils extend from the outer doublets and contact the plasma membrane causing a scalloped appearance in the anterior portion of the flagellum (Dewel and Clark, 1972; Larkman and Carter, 1980).

## 2. Sperm-egg interaction

When sperm of *Metridium* sp. are exposed to eggs, the plasma membrane surrounding the posterior two thirds of the head sends out long finger-like protrusions (Clark and Dewel, 1974). In some instances the ring-shaped vesicles straighten out and extend into these projections, however, the role of these vesicles is unknown. During this reaction the anterior third of the head maintains its integrity (Clark and Dewel, 1974). Eggs of *Metridium* sp. and *Bunodosoma cavernata*, exposed to spawned spermatozoa, undergo a cortical reaction involving extensive reorganization of the cortical layer (Clark and Dewel, 1974; Dewel and Clark, 1974). Polyspermy has not been observed in fertilized eggs of these actinarians, and the massive cortical reaction in *B. cavernata* eggs may prevent polyspermy by forming an investing layer around the egg (Clark and Dewel, 1974).

## K. Order Zoanthidea

Zoanthids are colonial or solitary anemone-like anthozoans that lack a hard skeleton. They are particularly abundant in tropical regions. Zoanthids studied to date are hermaphroditic, gonochoric, or have a mixed sexual pattern, and spawn gametes for external fertilization and development (e.g. Fadlallah *et al.*, 1984; Ryland and Babcock, 1991). Sperm ultrastructure has been described for the five zoanthid species listed in Table 6.

Table 6

Ultrastructural studies of spermatozoa of the Zoanthidea (Hexacorallia)

Order	Species	Reference
Zoanthidea	<i>Epizoanthus couchi</i>	Schmidt and Zissler, 1979
	<i>Palythoa tuberculosa</i>	Schmidt and Zissler, 1979
	<i>Parazoanthus</i> sp.	Schmidt and Zissler, 1979
	<i>Parazoanthus axinellae</i>	Schmidt and Zissler, 1979
	<i>Parazoanthus lucificum</i>	Lyke and Robson, 1975

## 1. Zoanthidian sperm structure and phylogeny

Schmidt and Zissler (1979) recognized two types of sperm among the zoanthid species studied, and grouped these as either primitive (Fig. 1J) or derived (Fig.

1K). They regarded zoanthid sperm as synapomorphous with Type II *Edwardsia* sperm of actinarians because of their similar lamellar structures in the midpiece, and the absence of a basal nuclear fossa.

(a) *Palythoa* sperm

The primitive zoanthid sperm type is represented by *Palythoa tuberculosa* (Fig. 1J). The nucleus is ovoid with a domed anterior and slightly concave sides. The base of the nucleus is rounded and although reported to lack a fossa (Schmidt and Zissler, 1979), a slight indentation is present at the base of the nucleus in some sperm sections. This is similar to the shallow fossa observed in some scleractinian coral sperm (Harrison, 1988a, 1990). Numerous rod-shaped or ring-shaped electron-dense bodies and lamellar structures surround the proximal region of the nucleus and extend around the periphery of the midpiece (Fig. 1J). Similar lamellar bodies are present in Type II actinarian sperm (Schmidt and Zissler, 1979), and scleractinian sperm (Harrison, 1988a, 1990).

The greater part of the midpiece is filled with three to five mitochondria, and two or three lipid spheres are inserted among the mitochondria. The mitochondria and lipid spheres form a ring around the centriolar complex. The proximal centriole lies at an obtuse angle to the distal centriole and is connected to the basal nuclear membranes by numerous fine processes and one compact process that are united by terminal thickenings (Fig. 1J). The distal centriole is aligned along the longitudinal axis of the sperm head. The pericentriolar apparatus projects at an acute angle from the distal centriole and the nine primary processes have a thickened end from which three secondary processes arise. These terminate in a ring of fibres that extends to the plasma membrane at the base of the midpiece. The flagellum has the usual 9+2 arrangement of microtubules, and proximal Y-shaped fibrils extending to the flagellum membrane. A cytoplasmic collar is absent from most sections of mature *P. tuberculosa* sperm (Schmidt and Zissler, 1979).

(b) *Parazoanthus* sperm

The sperm of *Parazoanthus* spp. exemplifies the advanced zoanthid sperm type (Figs. 1K, 8C). The sperm head is ovoid with a round nucleus about 1.5  $\mu\text{m}$  in diameter, without a basal nuclear fossa (Lyke and Robson, 1975). Schmidt and Zissler (1979) regarded the round nucleus of these sperm as a synapomorphy with the Antipatharia. Recent work has demonstrated that some scleractinian coral sperm also have a round nucleus (Steiner, 1993). Electron-dense rod-shaped and ring-shaped bodies are present in spermatids of *Parazoanthus* spp., and these form lamellar structures around the proximal margin of the nucleus and anterior region of the midpiece in mature spermatozoa (Lyke and Robson, 1975; Schmidt and Zissler, 1979). The midpiece is dominated by a ring of short mitochondria or a



compact nebenkern, and a lipid body is present above the mitochondria (Figs. 1K, 8C). The proximal centriole is linked to the nuclear membrane by fibrillar structures, and lies at about  $45^\circ$  to the distal centriole which gives rise to the pericentriolar apparatus and a 35–40  $\mu\text{m}$  long flagellum (Lyke and Robson, 1975).

### L. Order Antipatharia

The Antipatharia are known as black corals due to their dense black or brown, thorny axial skeleton. Colonies can be gonochoric or hermaphroditic, and *Antipathes fiordensis* is a broadcast spawner (Parker *et al.*, 1997). Molecular studies indicate that the Antipatharia are highly divergent from the Ceriantharia (France *et al.*, 1996), and the Order Ceriantipatharia is not recognized here. Sperm ultrastructure has been described for the two species of Antipatharia listed in Table 7.

Table 7

Ultrastructural studies of spermatozoa of the Antipatharia (Hexacorallia)

Order	Species	Reference
Antipatharia	<i>Eucirripathes</i> sp.	Schmidt and Zissler, 1979
	<i>Stichopathes</i> sp.	Schmidt and Zissler, 1979

### 1. Antipatharian sperm structure and phylogeny

The Antipatharia have the most apomorphic anthozoan sperm (Fig. 1L). The nucleus is small and spherical, and in one spermatozoon section, the base of the nucleus has a small indentation adjacent to the proximal centriole (Schmidt and Zissler, 1979; Schmidt and Holtken, 1980). A single array of small, Golgi-derived, electron-dense vesicles are situated at the anterior region of the head, between the nucleus and cell membranes. Similar vesicles have since been demonstrated in spherical scleractinian sperm (Steiner, 1993). The mitochondria are fused into a single nebenkern which is curved around the base of the nucleus, and not around the centriolar complex, unlike other anthozoans (Fig. 1L). In cross sections of sperm, the nebenkern appears club-shaped owing to swelling of one end. A small lipid body is present near the lateral margin of the nucleus.

The most unusual characteristic of these sperm is the apomorphic ring-shaped body that surrounds the centriolar complex at the base of the midpiece (Fig. 1L) (Schmidt and Zissler, 1979). The ring-shaped body is formed by fusion of electron-dense granules, and consists of an outer net of electron-dense material, and an inner granular material of less electron density (Schmidt and Holtken, 1980). Supposedly similar structures known as centriolar adjuncts appear constantly or temporarily in sperm of some insects, pogonophores, and mammals (Schmidt and Holtken, 1980). The ring-shaped body is unique among cnidarians, and together with other characteristics of the sperm of Antipatharia, indicates that antipatharians are the most derived members of the phylum (Schmidt and Zissler, 1979).

The proximal centriole is at an angle of more than  $90^\circ$  to the distal centriole, and lies eccentrically and obliquely under the nucleus, to which it sends a small electron-dense process. The distal centriole is surrounded by a typical pericentriolar apparatus. Each of the nine primary pericentriolar processes divides into three secondary processes that terminate in a network of transversely striated rays which link with the base of the ring-shaped body. Below the distal centriole, the flagellum exhibits the usual 9+2 arrangement of microtubules.

### III. CLASS HYDROZOA

#### A. Introduction

Hydrozoans are polypoid or medusoid, or have complex life cycles with alternation of generations between sessile polypoid and planktonic medusoid stages. Hydromedusae are usually small and inconspicuous, unlike medusae of Class Scyphozoa. Most of the approximately 2,700 hydrozoan species are marine, while the few known freshwater cnidarians are also members of this class. Hydrozoans are characterized by having acellular mesogloea, cnidocytes restricted to the epidermis, and gametes that usually mature in the epidermis (Barnes and Harrison, 1991). Reproduction in Class Hydrozoa has been extensively reviewed by Hyman (1940), Campbell (1974), Miller (1983) and Thomas and Edwards (1991). Sexual reproduction usually involves the production of gametes in medusae which may be free-swimming or attached to the parent hydroid. In hydrozoans lacking a medusoid stage, the polyps bear gametes. Hydrozoans are either gonochoric or hermaphroditic, and fertilization may be external or internal.

#### B. Spermatogenesis

Hydrozoan gonads are accumulations of differentiating gametogenic cells that arise from interstitial cells, covered by unspecialized epidermis (Chapman, 1974; Campbell, 1974; Miller, 1983; Thomas and Edwards, 1991). There has been a vigorous debate in the literature as to whether gametes are derived from a subpopulation of interstitial cells constituting a distinct germ line, or whether interstitial cells are multipotent and capable of differentiating and dedifferentiating into both gametes and somatic lines (e.g. Burnett *et al.*, 1966; Tardent, 1974; Stagni, 1974b; see also reviews by Campbell, 1974; and Thomas and Edwards, 1991). Recent cloning and antibody experiments indicate that interstitial cells exist as discrete subpopulations, and that one subpopulation of these cells is specific for the spermatogenic line, and that differentiation of female stem cells is repressed by male stem cells (reviewed by Thomas and Edwards, 1991).

The spermatozoa develop from groups of interstitial cells in the ectoderm or in some cases in the endoderm, via a primary spermatogonium, secondary

spermatogonium, primary spermatocyte, secondary spermatocyte and spermatid (Miller, 1983). In *Hydra*, these cell types are distributed in an orderly sequence within the testis according to their chronological order of appearance, with primary spermatogonia nearest the mesogloea (Schincariol and Habowsky, 1967, 1972; Hanisch, 1970; Moore and Dixon, 1972; Zihler, 1972; Stagni, 1974a,b). The spermatogonia divide two or three times before entering the meiotic cycle (Miller, 1983). Early spermatocytes have a round nucleus with a distinct nucleolus that disappears at the beginning of meiosis, and a flagellum first appears at the primary spermatocyte stage. Nuclear division may proceed very rapidly, resulting in two to four nuclei being present in the undivided cytoplasm of the developing spermatids (Zihler, 1972). Cytoplasmic bridges link immature spermatogenic cells in *Hydra oligactis*, *Hydra fusca* (Schincariol *et al.*, 1967; Schincariol and Habowsky, 1972), and *Campanularia flexuosa* (Lunger, 1971). Similar cytoplasmic bridges have been observed between developing cnidoblasts (Fawcett *et al.*, 1959). Spermiogenesis involves condensation of the nuclear chromatin, shedding of cytoplasm and organization of the midpiece, and growth of the flagellum (reviewed by Miller, 1983).

### C. Order Hydroida

The majority of hydrozoans belong to the Order Hydroida, and are characterized by having a well developed sessile polypoid stage in their life cycle. The hydromedusa may be free-swimming, or commonly attached to the parent, or absent as in *Hydra*. Sexual reproduction has been well studied in the Hydroida, and ultrastructural descriptions of spermatogenesis and spermatozoa are available for the 29 hydroid species listed in Table 8.

#### 1. General sperm structure

The spermatozoa of the Hydroida are aquasperm that approximate the 'primitive' sperm type and have a small, compact midpiece. However, some tendency towards elongation of the sperm nucleus is seen in *Hydra vulgaris attenuata* (Stagni and Lucchi, 1970a,b; Stagni, 1974b), *H. carnea* (Honegger, 1981), *H. fusca* (Schincariol and Habowsky, 1972), *H. hymanae* (West, 1978), and *Pelmatohydra robusta* (Noda and Kanai, 1981) where the egg is extruded from the epidermis but is usually surrounded by a clear jelly layer; and in some species with internal fertilization, e.g. *Eudendrium racemosum* (Hanisch, 1970), *E. ramosum* (Summers, 1972a) (Fig. 9), and *Tubularia larynx* (Afzelius, 1971) (Fig. 10). West (1980b) concluded that sperm of hydrozoan species with internal versus external fertilization exhibited major differences in head shape and size. Sperm of *Tubularia crocea* and *Clava leptostyla* which have internal fertilization, have elongated cylindrical heads, whereas sperm of the externally fertilizing *Nemophis bachei* and *Bougainvillia supercilii* have short, bluntly conical heads (West, 1980b).

Table 8  
Ultrastructural studies of the spermatozoa of the Hydroida

Suborder	Species	Reference
Hydrida	<i>Hydra attenuata</i>	Stagni and Lucchi, 1970a,b; Stagni, 1974a,b; Zihler, 1972; Tardent, 1974
	(= <i>H. vulgaris attenuata</i> )	Honegger, 1981, SEM only
	<i>H. carnea</i>	Moore and Dixon, 1972
	<i>H. cauliculata</i>	Tardent, 1974
	<i>H. circumcincta</i>	Schincariol and Habowsky, 1967, 1972; Schincariol <i>et al.</i> , 1967; Tardent, 1974;
	<i>H. fusca</i>	West, 1977, 1978
	<i>H. hymanae</i>	Weissman <i>et al.</i> , 1969
	<i>H. littoralis</i>	Fawcett <i>et al.</i> , 1959
	<i>H. oligactis</i>	Tardent, 1974
	<i>H. ovata</i>	Burnett <i>et al.</i> , 1966; Tardent, 1974
	<i>H. viridis</i>	Noda and Kanai, 1981
	<i>Pelmatohydra robusta</i>	
Anthomedusae (Gymnoblastera)	<i>Bougainvillia superciliaris</i>	West, 1980b
	<i>Cladonema uchidai</i>	Yamashita, 1987
	<i>Clava</i> sp.	O'Rand, 1972a
	<i>Clava leptostyla</i>	West, 1980b
	<i>Cordylophora caspia</i>	Franzén, 1996
	<i>Eudendrium</i>	Dewel and Clark, 1972
	<i>Eudendrium racemosum</i>	Hanis, 1966, 1970;
	<i>Eudendrium ramosum</i>	Summers, 1972a; Hinsch, 1974
	<i>Hydractinia</i> sp.	Hinsch and Clark, 1970a, 1973; Hinsch, 1974; Kleve and Clark, 1975
	<i>Hydractinia echinata</i>	Kleve and Clark, 1976, 1980
	<i>Nemophis bachei</i>	West 1980b
	<i>Pennaria tiarella</i>	Summers, 1970; 1972b
	<i>Tubularia larynx</i>	Afzelius, 1971
	<i>Tubularia crocea</i>	West 1980b
	<i>Tubularia</i> sp.	Hinsch and Clark, 1970a, 1973; Hinsch, 1974
Leptomedusae (Calyptoblastera)	<i>Campanularia flexuosa</i>	Lunger, 1971; Hinsch, 1974; O'Rand, 1972a,b, 1974; O'Rand and Miller, 1974
	<i>Campanularia</i>	Miller, 1983
	<i>Clytia hemispherica</i>	Carré and Carré, 1992
	<i>Gonothyrea</i>	O'Rand, 1972a; Hinsch, 1974
	<i>Phialidium gregarium</i>	Szöllösi, 1964; Roosen-Runge and Szöllösi, 1965

(a) *Acrosome and anterior vesicles*

In many hydroids, small membrane-bound vesicles with electron-dense contents are present anteriorly and anterolaterally to the sperm nucleus, between the outer nuclear membrane and the plasma membrane (*Hydractinia*, *Tubularia*, Hinsch and Clark, 1970a, 1973; Afzelius, 1971; *Pennaria*, Summers, 1970; *Cladonema*,

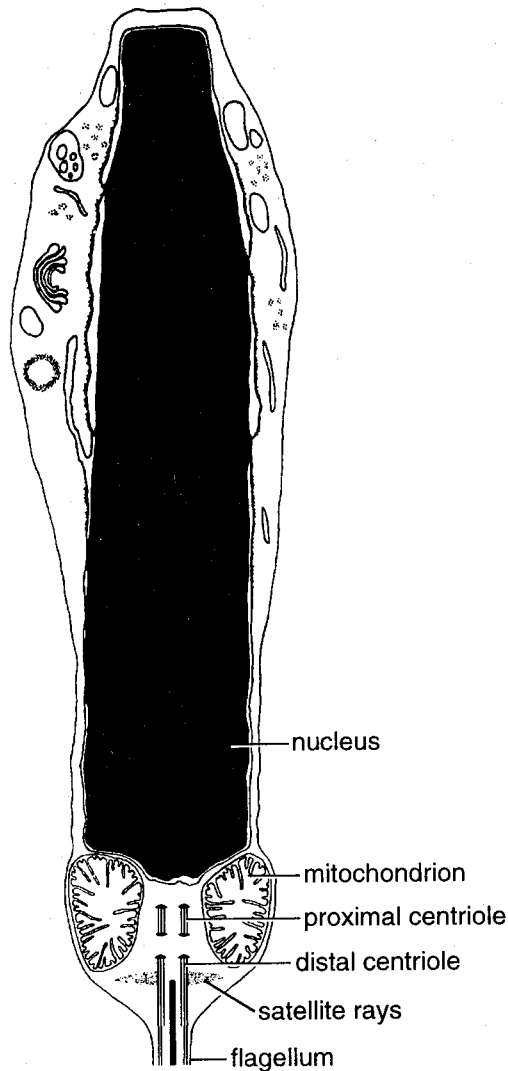


Fig. 9. *Eudendrium ramosum*. Semidiagrammatic representation of the composite ultrastructural features of the spermatozoon. After Summers (1972a). *Zeitschrift für Zellforschung und Mikroskopische Anatomie*, 132, 147–166, fig.122.

Yamashita, 1987; *Campanularia*, *Clava*, and *Gonothyrea*, Lunger, 1971; O'Rand, 1972a,b, 1974; Hinsch, 1974). These vesicles appear to arise in association with the Golgi apparatus of the spermatid (Summers, 1970; Lunger, 1971; Hinsch and Clark, 1973), and it has been suggested (Hinsch and Clark, 1973; Hinsch, 1974) that they may represent 'proacrosomal vesicles'. Similar electron-dense vesicles of Golgi

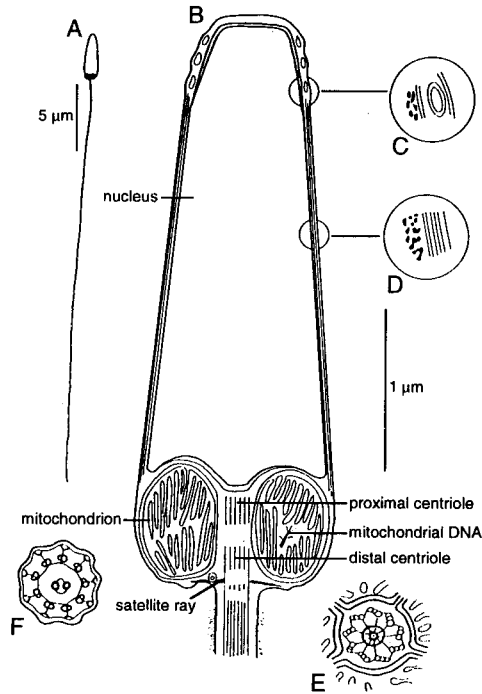


Fig. 10. *Tubularia larynx*. Semischematic diagram of the spermatozoon. A: Living sperm by phase contrast. B: Longitudinal section through the sperm-head, midpiece and anterior portion of tail. C: An anterolateral vesicle. D: Membrane layers covering the main portion of the nucleus. E: Cross section of a centriole. F: Cross section of tail at its most anterior level. After Afzelius (1971). *Journal of Ultrastructure Research*, **37**, 679–689, chart 1.

origin are also known from sperm of scyphozoans and many anthozoans. In *Tubularia larynx* sperm (Fig. 10), about 30 vesicles, each about  $0.05\ \mu\text{m}$  wide and  $0.1\ \mu\text{m}$  long, are arranged around the lateral margins of the nucleus (Afzelius, 1971). In *Pennaria tiarella* sperm, an aggregated mass of 30–40 vesicles cap the nucleus (Summers, 1970). The contents of these vesicles are electron dense after glutaraldehyde and osmium fixation, but are clearer after fixation with osmium alone. Summers (1970) noted that these vesicles do not fuse during development, and contact with the outer jelly layer of the egg does not result in breakdown or release of these vesicles or their contents. Similarly, the anterior vesicles in *Cladonema uchidai* sperm did not show any morphological changes during fertilization, which suggested that these vesicles did not function as an acrosome and do not play a significant role in fertilization (Yamashita, 1987). However, a true acrosome reaction appears to have been demonstrated for the leptomedusan *Clytia hemispherica* (Carré and Carré, 1992). In mature sperm of *C. hemispherica*, small dense vesicles form a crown around the anterior pole of the nucleus but are absent from the extreme anterior pole of the spermatozoon. These vesicles,  $0.1\ \mu\text{m}$

on average, are spherical or elongate, and are covered by a fine fuzzy coat. At fertilization the vesicles disappear and are replaced by a single, conspicuous, acrosomal process, 1.5  $\mu\text{m}$  long, which is covered by fibrous material containing longitudinally orientated filaments. It was hypothesized that a chemo-attractant and an inducer of the acrosomal reaction, both released at the animal pole of the eggs, were responsible for accumulation of spermatozoa and the formation of the acrosomal process. A test for actin, using NBD phalloidine, was negative (Carré and Carré, 1992).

In *Campanularia flexuosa* sperm, about 70 to 75 vesicles occur around the apex and lateral margins of the nucleus (O'Rand, 1972a; O'Rand and Miller, 1974; Miller, 1983). During sperm penetration into the somatic tissues of the female gonangium, a progressive reduction in numbers of vesicles has been recorded from 4.8–5.1 per sperm section outside the female to only 0.6 vesicles per section in five sperm observed close to the egg (O'Rand and Miller, 1974). Membranes of sperm near eggs appear inflated, and rodlike projections occur at the apex of the sperm. Similar changes have been recorded in sperm of the actinarian *Metridium* in response to contact with eggs (Clark and Dewel, 1974). It is suggested that these changes may indicate a capacitation-like reaction between *C. flexuosa* sperm and epithelial cells of the female gonangium which is required for fertilization (O'Rand, 1972b, 1974, 1979; O'Rand and Miller, 1974). The loss of vesicles from sperm attached to epithelial cells may indicate that their contents are used to loosen the attachment to allow sperm to move further down the gonangium (Miller, 1983). Vesicle loss may also provide lytic enzymes for penetration of the egg and protection against phagocytosis by funnel and epithelial cells enclosing the mature egg (O'Rand and Miller, 1974).

Such vesicles are absent from sperm of the freshwater genus *Hydra* (*H. fusca*, Schincariol *et al.*, 1969; *H. littoralis*, Weissman *et al.*, 1969; *H. attenuata*, Stagni and Lucchi, 1970a, 1970b; Zihler, 1972; *H. caudiculata*, Moore and Dixon, 1972). The anterior end of the sperm, nevertheless, shows specializations: the space between the nuclear envelope and the plasma membrane may have cross connections resembling septate desmosomes (*H. caudiculata*, Moore and Dixon, 1972) (Fig. 11) or may contain finely granular material (*H. hymanae*, West, 1978). West (1978) found no cytochemical or ultrastructural evidence for proacrosomal or acrosomal elements in *H. hymanae* sperm, but did not exclude the possibility that the anterior region may function in sperm-egg binding.

Although these vesicles are also reported to be absent from sperm of *Eudendrium ramosum* (Summers, 1972a) they appear to be present in micrographs (Hinsch, 1974). Summers (1972a) related the absence of an acrosome in these sperm to the presence of a micropyle-like structure in the ectoderm cells overlying the egg, and suggested that the absence of vesicles from the sperm of *Hydra* was correlated with the existence of an indentation in the egg at the site of sperm contact. This has been confirmed for *Pelmatohydra robusta* by Noda and Kanai (1981), and for *Hydra carnea* by Honegger (1981) where sperm penetrate the jelly layer and enter the egg

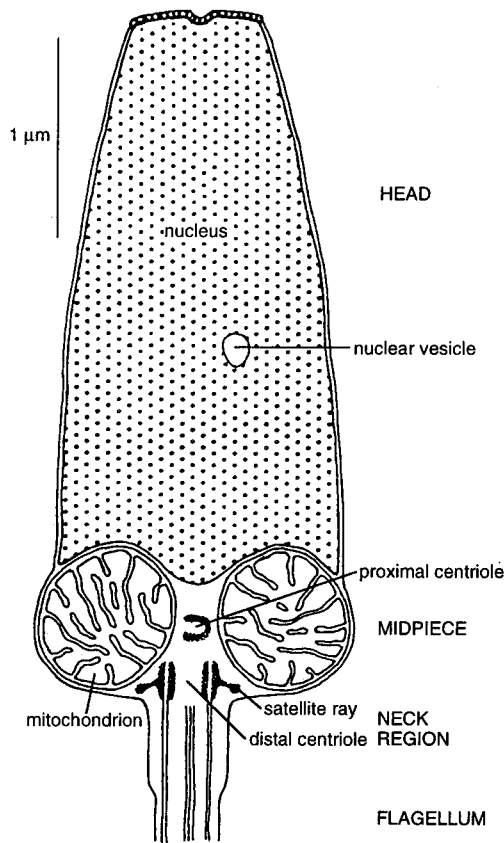


Fig. 11. *Hydra cauciculata*. Diagrammatic representation of the spermatozoon. After Moore and Dixon (1972). *Journal of Morphology*, 137, 483–502, fig. 12.

only at the base of a depression (fertilization pit) in the animal pole of the egg. This depression differs from the remaining surface of the egg in lacking microvilli, and the female pronucleus is located directly under the membrane at this site. A fast block to polyspermy clearly exists as, although many sperm enter the fertilization pit, the egg becomes incapable of fertilization by other sperm only seconds after the first has fused with the egg (Honegger, 1981). Experimental work with five other hydroid species has demonstrated that eggs can only be fertilized at the site of polar body formation adjacent to the female pronucleus (Freeman and Miller, 1982). The egg nucleus is also eccentric in the hydromedusan *Aequorea* (Freeman, 1996). In *Cladonema uchidai*, the sperm contact the egg near the animal pole where, alone, microvilli are absent and near which the egg nucleus is situated (Yamashita, 1987). Similar restriction of sperm-egg interactions to a specialized area of the egg is known also in an annelid and the frog *Discoglossus pictus* at the



'animal dimple' (Freeman and Miller, 1982). Sperm penetration and fertilization is restricted to the micropyle region in eggs of all cephalopods, insects and most fish that are surrounded by an impenetrable chorion. Treatment of *Aequorea* eggs with triticum or concanavalin A causes a marked reduction in the ability of these eggs to be fertilized, suggesting that sugar-containing moieties, to which the lectins bind, play a role in fertilization. Removal of sugars on these moieties with mannosidase or N-acetyl glucosaminidase, or the cleavage of the protein the sugars are attached to with trypsin, results in eggs that do not bind triticum or concanavalin A and also show a marked reduction in the ability to be fertilized. These experiments suggest that the lectin-binding moieties are glycoproteins (Freeman, 1996).

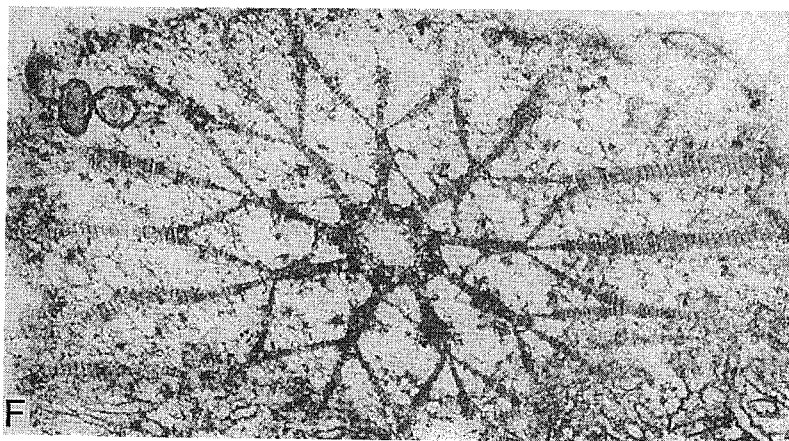
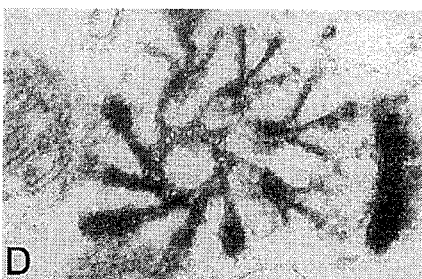
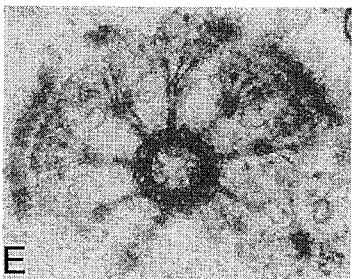
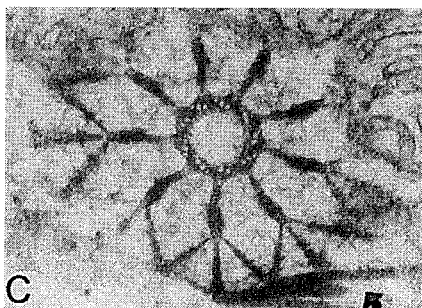
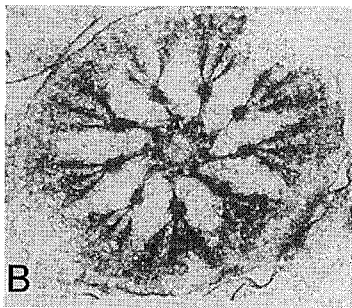
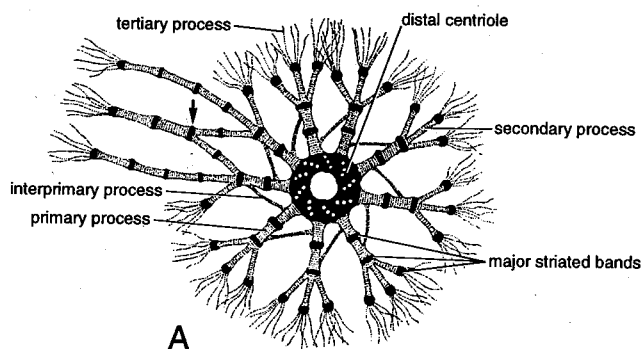
(b) *Other vesicles*

In *Clytia hemispherica*, bilateral symmetry of the sperm head is disturbed, in addition to asymmetry of the nucleus, by the presence, on one side, of 0.6  $\mu\text{m}$  vesicles with dense heterogeneous contents, and, on the same side, by the striated rootlet. These vesicles survive the acrosome reaction, accumulating around the pericentriolar apparatus, and are presumed to be have a storage role (Carré and Carré, 1992).

(c) *Nucleus*

The form of the nucleus varies among hydroid sperm. In *Nemopsis*, *Bougainvillia* and *Cladonema* sperm the nucleus is a short, truncated cone (West, 1980b; Yamashita, 1987), while the nucleus of *Pennaria* sperm is conical with an unusual 300 nm protrusion at its apex (Summers, 1970). Nuclear material projects apically through a discontinuity in the membranes and is capped by a dense derivative of the interrupted nuclear envelope. In *Hydra* sperm, the nucleus usually has a blunt, bullet-shaped form (e.g. Weissman *et al.*, 1969; Zihler, 1972; Moore and Dixon, 1972) or is an elongated cylinder (*H. hymanae*, West, 1978). *Tubularia*, *Clava* and *Eudendrium* sperm have a greatly elongated, cylindrical nucleus up to 4  $\mu\text{m}$  long (Hanisch, 1970; Afzelius, 1971; Summers, 1972a; West, 1980b). *Eudendrium ramosum* sperm appear to be unusual as a mass of cytoplasm and organelles are present at one side of the nucleus in mature spermatozoa (Summer, 1972a). In some sections of hydroid sperm, the nucleus and sperm head appear to be bent (e.g. Afzelius, 1971). In the leptomedusan *Clytia hemispherica*, the nucleus is asymmetrically conical (Carré and Carré, 1992).

The chromatin is strongly condensed, and there are often one or more intranuclear electron-lucent spaces, probably owing to incomplete condensation (*Pennaria tiarella*, Summers, 1970; *Tubularia*, Afzelius, 1971; *Hydra cauliculata*, Moore and Dixon, 1972). Similar spaces have been reported in the nucleus of many Anthozoa and Scyphozoa sperm. West (1978) concluded that the shape of the nucleus, and hence the sperm head, was the result of a genetically controlled pattern of chromatin condensation and aggregation. During maturation, the chromatin



and secondary branches show a distinct periodicity (e.g. Carré and Carré, 1992). Pericentriolar processes are present but appear unbranched in *Tubularia larynx* (Afzelius, 1971) and *Eudendrium* (Summers, 1972a), though in the former genus dichotomous rays occur at the beginning of the axonome. Processes have also been described for sperm of *Clava* and *Gonothyrea* (O'Rand, 1972b).

In *Clytia hemispherica*, one of the trifid processes is associated with a long striated rootlet that consists of a lamina, 0.3  $\mu\text{m}$  wide. The rootlet has a node at its posterior fourth, at which level the cross striation is interrupted, and terminates with an enlarged portion which is inserted on the plasma membrane. There are always 37 cross striations, at a periodicity of 22 or 27 nm, between the node and this termination (Carré and Carré, 1992). A similar rootlet has been demonstrated for *Hydractinia echinata* by Kleve and Clark (1980) and for the siphonophore *Muggiaea kochi* by Carré (1979).

In *Hydractinia echinata* sperm, the secondary processes give rise to many thin, filamentous tertiary processes that contact the plasma membrane, and additional non-striated interprimary processes interconnect adjacent primary processes (Fig. 12A) (Hinsch and Clark, 1973; Kleve and Clark, 1976, 1980). Four of the secondary processes are much longer than the others and induce asymmetry in the pericentriolar apparatus (Kleve and Clark, 1980). The pericentriolar complex forms a cradle around the mitochondria in the midpiece, and some elements extend anteriorly until they meet the nuclear membrane (Kleve and Clark, 1980).

In *Hydractinia*, it was claimed that the first and third secondary branches (here termed the 'lateral branches') of each primary process fuse with those of adjacent processes (Hinsch and Clark, 1973), as clearly shown for at least some levels (or conditions) of the pericentriolar apparatus of *Clytia hemispherica* (Carré and Carré, 1992) (Figs. 12 C,D), and by Afzelius and Franzén (1971), who recognize a 'nine-pointed star' formed by the fused lateral rays, for the scyphozoan *Nausithoe*. However, Kleve and Clark (1980), in an examination of the isolated (sonicated) centriolar apparatus of *Hydractinia echinata*, state that anastomosis occurs only between the two central long secondary processes (Fig. 12A). We consider it possible that sonication of the pericentriolar complex had disrupted connections, or contiguity, between the lateral secondary processes of adjacent processes or that contact and separation of the processes are alternative, dynamic states in the living spermatozoon, as suggested by the micrographs of Carré and Carré (1992) (Figs. 12 C,D). There

Fig. 12. Pericentriolar apparatus ('anchoring apparatus') of some cnidarians and a ctenophore, seen in transverse section of the distal centriole. A: *Hydractinia echinata* (Hydrozoa). After Kleve and Clark (1980). *Journal of Cell Biology*, **86**, 87-95, fig. 4. B: *Parazoanthus axinellae* (Anthozoa, Zoantharia). From Schmidt and Zissler (1979). *Zoologica (Stuttgart)*, **44**, 1-98, fig. 154. C, D: *Clytia hemispherica* (Hydrozoa), at different levels. From Carré and Carré (1992). *Canadian Journal of Zoology*, **70**, 866-873, figs. 7, 8. E: *Beroë ovata* (Ctenophora). From Franc (1973). *Journal of Ultrastructure Research*, **42**, 255-267, fig. 10. F: *Nausithoe* sp. (Scyphozoa). From Afzelius and Franzén (1971). *Journal of Ultrastructure Research*, **37**, 186-199, fig. 9.

### D. Order Trachylina

Members of the Order Trachylina are planktonic, medusoid hydrozoans lacking a polypoid stage in their life cycle, and this group includes perhaps the most primitive members of the Hydrozoa. Observations of maturing spermatids of *Liriope tetraphylla* by Chapman (1974) provide the only ultrastructural information on trachymedusan sperm. The short nucleus has a domed anterior end and basal indentations adjacent to the four mitochondria which dominate the midpiece. The mitochondria have tubular cristae, and one of them appears to be larger than the other three. The mitochondria form a ring around the proximal and distal centrioles.

### E. Order Siphonophora

Siphonophores are pelagic, colonial hydrozoans composed of modified polypoid and medusoid individuals. This group includes the remarkable *Physalia*, commonly known as the Portuguese man-of-war or blue bottle, which has a surface gas-filled float and long fishing tentacles containing many nematocysts that can cause painful or severe stings to swimmers.

Spermiogenesis, the mature spermatozoon, and the acrosome reaction have been described ultrastructurally in *Muggiaea kochi* (Calycophora) by Carré (1979, 1984) (Fig. 13). Carré (1979) also stated that in a rapid survey of various unnamed calycophore and physonect siphonophores, all species possessed the same type of spermatozoa as *M. kochi*. As in most calycophores, eudoxids of *M. kochi* each consisting of one unisexual gonophore, one bract and one gastrozoid with its fishing tentacle, are shed from and live independently of the hermaphroditic colony (Carré, 1979). Several generations of gonophores with irregular alternations of sex can be found in the same eudoxid. The gonocytes are ectodermal in origin, and gametes are shed into the sea water. The structure of the sperm corresponds to the 'primitive' sperm type except in the features described below (Carré, 1979).

#### 1. General sperm structure

##### (a) Acrosome

A structure which appears to be a true functional acrosome has been described in *Muggiaea kochi* sperm by Carré (1979, 1984). However, peculiarities of this acrosome lend support to the isolated position of the Cnidaria relative to other metazoans. The acrosome of *M. kochi* is unlike that of other sperm, and has two components: a large Golgi-derived vesicle located asymmetrically in the curvature of the nucleus; and a chain of smaller Golgi-derived saccules anterior and perpendicular to the nucleus that migrate along the terminal part of a long, striated rootlet. The rootlet is derived from the pericentriolar processes of the distal centriole

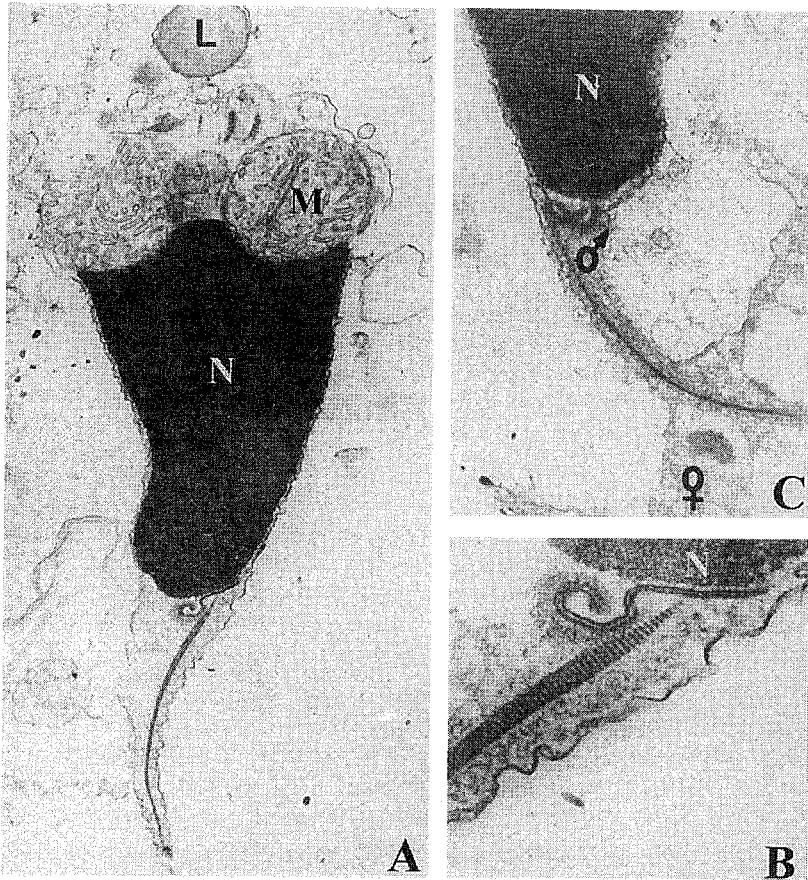


Fig. 13. *Muggiaea kochi*. A: Free sperm before the acrosome reaction. Note the long striated rootlet and the bilateral symmetry imposed by this and the large Golgi vesicle. B: Detail of the base of the striated rootlet. C: Sperm-egg fusion involving the acrosomal process which is supported by the striated rootlet. After Carré (1984). *International Journal of Invertebrate Reproduction and Development*, 7, 95–103, figs. 9–11.

(Carré, 1979). When the sperm is liberated, the Golgi vesicle contains heterogeneous material absent at an earlier stage. The anterior pole of the nucleus is always capped by a slender saccule which seems to be a remnant of the nuclear envelope (Carré, 1984).

#### (b) Acrosome reaction

*In vivo* interference contrast studies indicate that only a few, sometimes only one, of the spermatozoa produces an anterior prolongation, and this occurs only if the

sperm is near the predetermined site of insemination of an egg (Carré, 1984). It is inferred that an acrosome reaction inducer is localized or liberated at this site.

Ultrastructurally, three stages occur in the reaction: orientation of the terminal part of the striated rootlet perpendicular to the axis of the nucleus; dehiscence of the large Golgi vesicle accompanied by median rupture of the rootlet, if not previously accomplished; and formation of an acrosomal prolongation at the anterior pole of the nucleus, the axis of which is formed by the terminal part of the striated rootlet (Fig. 13) (Carré, 1984). The acrosomal nature of this tube-like prolongation is confirmed by the sperm-egg fusion.

By the end of this reaction, the morphology of the sperm has profoundly changed. Lipid inclusions initially lodged in the curvature of the nucleus are relocated close to the mitochondria. In the mid-region, the Golgi secretions and apparatus have disappeared and only the nucleus persists, immediately bordered by the plasma membrane. At the anterior pole, the saccule capping the nucleus has coiled under one of its borders and is hook-shaped in cross section (Carré, 1984).

Apart from morphological peculiarities of this acrosome complex and its reaction, the reaction is physiologically atypical. This reaction could not be induced by an increase in pH, addition of calcium to the ambient sea water, or addition of ionophore A23187. However, an acrosomal tube was formed rapidly in all spermatozoa in two trials using normal sea water in the absence of eggs.

The term acrosome has been applied by Carré (1984) to the large Golgi vesicle and the elongated saccule bordering the rootlet. Although exocytosis of the large vesicle appears to be a stage of the acrosome reaction essential for the products which it liberates and for deployment of the terminal part of the rootlet, the vesicle does not participate directly in formation of the anterior process. Rather, the membrane of the anterior process seems to be formed by the plasma membrane of the sperm and by the membrane of the long Golgi saccule which borders the rootlet. Carré (1984) noted that it was difficult to determine the origin of the apical membrane of the anterior tube which is the sole region fusing with the egg membrane, or sometimes accidentally with another sperm.

The role of the products of exocytosis during the course of the acrosome reaction is enigmatic. The eggs of siphonophores are devoid of a chorion and other secondary membranes. It is possible that the products of exocytosis seen to be liberated from the acrosomal vesicles in the vicinity of the predetermined fertilization site remove the delicate vitelline membrane and enable sperm-egg fusion. The terminal part of the striated rootlet, originating from the pericentriolar apparatus, is nevertheless considered homologous with perforatoria in other sperm. The perforatoria in sperm of *Limulus*, echinoderms and others, is known to contain actin filaments. Similarly, Kleve and Clark (1980) demonstrated that actin was present in the striated pericentriolar complex of *Hydractinia* sperm. Carré (1984) reported contraction of the acrosome of *Muggiaea kochi*, consummate with this observation, in the seconds before sperm-egg fusion. Carré (1984) questioned

whether other Cnidaria have lost a pre-existing acrosome, and whether the striated pericentriolar processes of *Hydractinia* represent a vestige of, or a tentative development towards, an acrosome. Ehlers (1993) concluded that the specialized acrosome of *M. kochi* represents a derived condition and cannot be the ground pattern of the Cnidaria.

(c) *Nucleus*

The nucleus is at first spherical during spermatogenesis, but becomes conical and slightly curved (Fig. 13). In micrographs, its contents appear electron-dense but not strongly condensed, being fairly coarsely and uniformly granular. Ribosomes are associated with the nuclear membrane which is thickened at the anterior pole (Carré, 1979). Carré (1984) stated that spawned sperm lacked a nuclear membrane, but a membrane is present in micrographs. The base of the nucleus is indented adjacent to the mitochondria.

(d) *Golgi apparatus*

A persistent Golgi apparatus that disappears during the acrosome reaction, occurs in the midregion of the head beside the nucleus. By maturity this has secreted the large anterior vacuole and small saccules which come to lie along the terminal portion of the rootlet. Two or three putative lipid inclusions are also present near the nucleus (Carré, 1979).

(e) *Midpiece and pericentriolar apparatus*

At maturity there are often six mitochondria packed closely together around the two centrioles. The proximal centriole lies at a slight angle relative to the axially aligned distal centriole. The pericentriolar apparatus arises from the dense matrix of the distal centriole, and consists of nine cross-striated terminally tripartite processes, similar to those described in other Cnidaria. However, in *Muggiaea kochi* there is a much longer, striated submembranous rootlet which runs along the spermatozoon and ends at the anterior pole, against the nuclear membrane (Fig. 13A) (Carré, 1979). The striated rootlet is a lamina, 0.35  $\mu\text{m}$  wide, which enlarges slightly at the anterior pole. It is cross-striated by dark bands with a major periodicity of 25 nm. In favourable profiles, two dark bands, one thick, the other thin, are visible. It appears that the rootlet is not continuous throughout the sperm head, and is broken medially, either before or during the acrosome reaction (Carré, 1984). This rootlet is possibly the equivalent of the spur described for *Nausithoe* by Afzelius and Franzén (1971).

## 2. Chemotaxis

The sperm of *Muggiaea kochi* are reported to perform one of the most spectacular examples of chemotaxis between sperm and eggs in the animal kingdom (Carré, 1979; Carré and Sardet, 1981). The bilateral symmetry of the sperm, determined by the long striated rootlet, curvature of the nucleus and by the Golgi apparatus was correlated with their chemotactic behaviour by Carré (1979). Similarly, Kleve and Clark (1976, 1980) suggested that the asymmetry of the pericentriolar apparatus in *Hydractinia* sperm may facilitate chemotaxis.

## F. Order Actinulida

The Order Actinulida contains unusual, minute, polypoid hydrozoans that are ciliated and motile, and live interstitially among sand grains (Barnes and Harrison, 1991). A recent paper by Ehlers (1993) on *Halammohydra schulzei* provides the only ultrastructural information on spermatozoa of this group, and discusses the phylogenetic significance of acrosomal structures within the Eumetazoa. Male specimens of *H. schulzei* have testicular areas formed within the somatic gastrodermal cells of the gastric tube, and spermatogenesis is not strongly synchronized.

### 1. Sperm structure and phylogeny

Spermatids have considerable amounts of perinuclear cytoplasm and a Golgi complex lying in a bulge in this cytoplasm. The perinuclear cytoplasm is reduced to form a thin cytoplasmic rim in mature spermatozoa. The anterior region of the spermatozoon contains a series of small vesicles of varying electron density located between the nuclear and plasma membranes. The nucleus is conical with a domed anterior, and is indented basally adjacent to the mitochondria. The midpiece contains four large mitochondria surrounding the proximal centriole, which is orientated perpendicularly to the long axis of the sperm. The distal centriole is axially aligned, and gives rise to the flagellar axoneme, and a pericentriolar complex consisting of nine striated primary processes which branch into secondary anchoring fibres. These sperm correspond to the primitive sperm type, and are not obviously modified with respect to the interstitial habitat of *Halammohydra* (Ehlers, 1993).

Ehlers (1993) considered the phylogenetic implications of the presence of the Golgi-derived apical vesicles in cnidarians and other metazoans, and concluded that the sperm cell pattern with small apical vesicles represents an autapomorphy of the Metazoa. This pattern has been inherited by the stem lineages of the Porifera, and the Cnidaria and other Eumetazoa, and an acrosomal vesicle without a perforatorium has evolved secondarily within some Porifera. Ehlers (1993) concluded that the development of an acrosome and perforatorium (dark



subacrosomal substance) are evolutionary novelties (autapomorphies) of the Ctenophora+Bilateria, hence comparative sperm ultrastructure does not support the earlier hypothesis for the monophyly of the Cnidaria+Ctenophora, which were originally grouped as the Coelenterata. It is here considered, however, that a sister-group relationship of Cnidaria and Ctenophora cannot yet be dismissed.

#### IV. CLASS SCYPHOZOA

##### A. Introduction

Scyphozoans have a medusa as the dominant and conspicuous stage of the life cycle, and the polypoid scyphistoma stage is small and larval (Barnes and Harrison, 1991). Young motile medusae (ephyrae) are budded from the scyphistoma and develop rapidly into sexually mature scyphomedusae that are usually larger than hydromedusae, lack a velum, and exhibit tetramerous internal organization. The mesogloea is cellular, and gametes originate from the gastrodermis. Comparatively little information is available on reproduction in the Scyphozoa (reviewed by Campbell, 1974; Miller, 1983; Lesh-Laurie and Suchy, 1991). Scyphozoans are either gonochoric or hermaphroditic, and fertilization is internal (Widersten, 1965). Development proceeds to the blastula or gastrula stage before embryos leave the genital sinus, chiefly via ciliary currents. Spermatozoal ultrastructure has been examined in the 10 scyphozoan species listed in Table 9, which include representatives from all four orders (Afzelius and Franzén, 1971; Hinsch and Clark, 1970a, 1973; Hinsch, 1974; Hedwig and Schäfer, 1986). Remarkably, the sperm of the Cubomedusae (now usually placed in a separate class, the Cubozoa) have not been described. The Cubozoa are seen to be closer to the Scyphozoa than to any other cnidarian class in the molecular study of Bridges *et al.* (1995).

##### B. Spermatogenesis

Scyphozoan gonads develop from groups of cells that separate from the gastrodermis and migrate into the mesogloea immediately adjacent to the gastric filaments. It has been shown for *Cyanea*, *Aurelia*, *Chrysaora* and *Rhizostoma* that sperm develop in follicles which form as cells migrate into the genital mesogloea from the genital epithelium adjacent to the gastric pockets of the medusa (Widersten, 1965). A connection between the follicle cavity and the genital sinus permits egress of sperm from the follicle into the genital sinus where sperm complete their development. Spermatozoa are released into the sea water and are transported by ciliary currents through the female gastrovascular cavity to the ovarian genital sinus and fertilize eggs in the ovarian mesogloea (Widersten, 1965; Lesh-Laurie and Suchy, 1991).

Table 9

Ultrastructural studies of spermatozoa of the Scyphozoa

Order	Species	Reference
Stauromedusae	<i>Craterolophus tethys</i>	Hedwig and Schäfer, 1986
Coronatae	<i>Nausithoe</i>	Afzelius and Franzén, 1971
Semaestomeae	<i>Aurelia aurita</i> <i>Aurelia</i> sp.	Hinsch, 1974; Hedwig and Schäfer, 1986 Hinsch and Clark, 1970a, 1973; Hinsch, 1974
	<i>Chrysaora hysoscella</i>	Hedwig and Schäfer, 1986
	<i>Cyanea</i>	Hinsch and Clark, 1970a; Hinsch, 1974
	<i>Dactylometra</i>	Hinsch, 1974
	<i>Pelagia</i>	Hinsch, 1974
Rhizostomae	<i>Cassiopea</i>	Hinsch, 1974
	<i>Rhizostoma octopus</i>	Hedwig and Schäfer, 1986
	<i>Rhizostoma pulmo</i>	Hedwig and Schäfer, 1986

### C. Comparative Sperm Structure and Phylogeny

Spermatozoa of *Craterolophus tethys* (Stauromedusae) have a short conical nucleus, the base of which forms a protrusion that extends to the distal centriole. In the midpiece there are five mitochondria and only the distal centriole is present, which lacks a pericentriolar complex. The basal nuclear protrusion and the loss of the pericentriolar complex and proximal centriole are probably apomorphies for the Stauromedusae (Hedwig and Schäfer, 1986). The sperm of the Discomedusae (Semaestomeae and Rhizostomeae) have an elongate conical nucleus, electron-dense vesicles in the head and midpiece, always four mitochondria, and a proximal and distal centriole, the latter with a pericentriolar complex; the midpiece forms a collar enveloping the proximal part of the flagellum (Hinsch and Clark, 1973; Hinsch, 1974; Hedwig and Schäfer, 1986). Some sperm of the Semaestomeae have a shallow basal nuclear fossa (Hedwig and Schäfer, 1986). *Nausithoe* (Coronatae) sperm, investigated by Afzelius and Franzén (1971), share characters with the Discomedusae such as almost identical organelles in the midpiece, and with the Stauromedusae in having a relatively short, conical nucleus, and absence of a collar. Hedwig and Schäfer (1986) concluded that comparative sperm structure does not support the concept of Werner (1973a) that the Coronatae represent the most primitive Scyphozoa; rather, the Coronatae are probably intermediate in systematic position between the Stauromedusae and Discomedusae, as proposed by Thiel (1966).

A rootlet-like process or 'spur' proximal to the pericentriolar apparatus (Afzelius and Franzén, 1971; Hinsch, 1974) and the terminal ring-like structure in which each pericentriolar process ends, have been considered to distinguish the pericentriolar apparatus of scyphozoans from that of most hydrozoans (Hinsch and Clark, 1973). The rootlet has a spatial origin different from that of the

elongate rootlet in the hydroid *Hydractinia* (Kleve and Clark, 1975, 1976, 1980), the well developed rootlet in the leptomedusan *Clytia hemispherica* (Carré and Carré, 1992) and the very extensive rootlet in the siphonophore *Muggiaea kochi* (Carré, 1979, 1984).

#### D. Order Stauromedusae

In the Stauromedusae, the adult has the organization of a sessile ephyra that is derived directly from the benthic scyphistoma without budding.

##### 1. *Craterolophus* sperm

The ultrastructure of *Craterolophus tethys* sperm has been described by Hedwig and Schäfer (1986).

###### (a) Nucleus

The nucleus is short and conical, and has an unusual distal protuberance (nuclear peg) that extends far into the midpiece down to the distal centriole. Only a thin layer of cytoplasm intervenes between the nuclear and plasma membranes. The chromatin is compact, homogeneous and electron-dense with a single small space.

###### (b) Midpiece

The midpiece is dominated by five (rarely four) cristate mitochondria that surround and indent the base of the nucleus, giving the peg a five-rayed appearance. A proximal centriole is absent. The close approximation of the nuclear peg to the single centriole in *Craterolophus* appears to compensate for the absence of a pericentriolar anchoring apparatus. A fine electron-dense filament projects from the nucleus into the lumen of the distal centriole. A very slight protuberance of the distal surface of the midpiece is possibly a rudimentary cytoplasmic collar.

###### (c) Flagellum

The flagellum has the usual 9+2 axoneme which narrows distally and the number of microtubules decreases (Hedwig and Schäfer, 1986).

#### E. Order Coronatae

##### 1. *Nausithoe* sperm

The sperm of *Nausithoe* have been described in detail by Afzelius and Franzén (1971). These sperm are of the 'primitive' type with a bluntly conical head, a small

midpiece, and a 65  $\mu\text{m}$  long flagellum (Figs. 14A, G). The form of the head and nucleus resembles those in some hydrozoan and anthozoan sperm, and is unlike the elongate pointed head seen in sperm of the semaeostomes (Hinsch and Clark, 1973; Hinsch, 1974; Hedwig and Schäfer, 1986).

*(a) Anterior region*

The anterior region of the sperm does not contain an acrosome cap, but between the nucleus and the apical cell membrane there is a distinctive cytoplasmic layer (Fig. 14B). It is about 0.2  $\mu\text{m}$  thick, with moderately electron-dense cytoplasm and contains several flattened vesicles, estimated at about 20 per sperm. The vesicles have a larger dimension of about 0.2  $\mu\text{m}$ , and a lighter core slightly less than 0.1  $\mu\text{m}$  in diameter. Both the cores and the vesicles are limited by a triple-layered membrane (Afzelius and Franzén, 1971).

*(b) Nucleus*

The conical nucleus (Fig. 14A) is 3.4  $\mu\text{m}$  high and basally 2.2  $\mu\text{m}$  wide, with an estimated volume of about 7  $\mu\text{m}^3$ . The anterior is domed, while the base is flattened and indented adjacent to the mitochondria. The chromatin is condensed but contains a few electron-lucent spaces. Relatively prominent lateral cytoplasm contains unidentified membranous material.

*(c) Midpiece*

The four large mitochondria are closely apposed with their mutual borders in planes parallel to the long axis of the sperm. Commonly, one or more of the mitochondria contains a smaller mitochondrion indicative of formation of the larger mitochondria by fusion of smaller ones. Posterior to the mitochondria, the midpiece may contain a Golgi apparatus, multivesicular bodies and lysosomes; electron-dense glycogen granules are common here and between the mitochondria.

*(d) Centrioles and anchoring apparatus*

The short proximal centriole (Fig. 14A), lacking attachments, is orientated perpendicular to the long axis of the sperm and to the distal centriole. The centrioles are separated from the nucleus by a distance of about 1  $\mu\text{m}$ , which is considered a departure from usual primitive sperm pattern. The distal centriole forms the basal body of the flagellum, and gives rise to a highly developed pericentriolar apparatus (Fig. 14A). The apparatus, shown also as a micrograph (Fig. 12F), consists of nine striated radiating primary processes, each of which branches into three secondary fibres. The middle of the secondary processes is a continuation of the main fibre; the two lateral ones join the neighbouring secondary

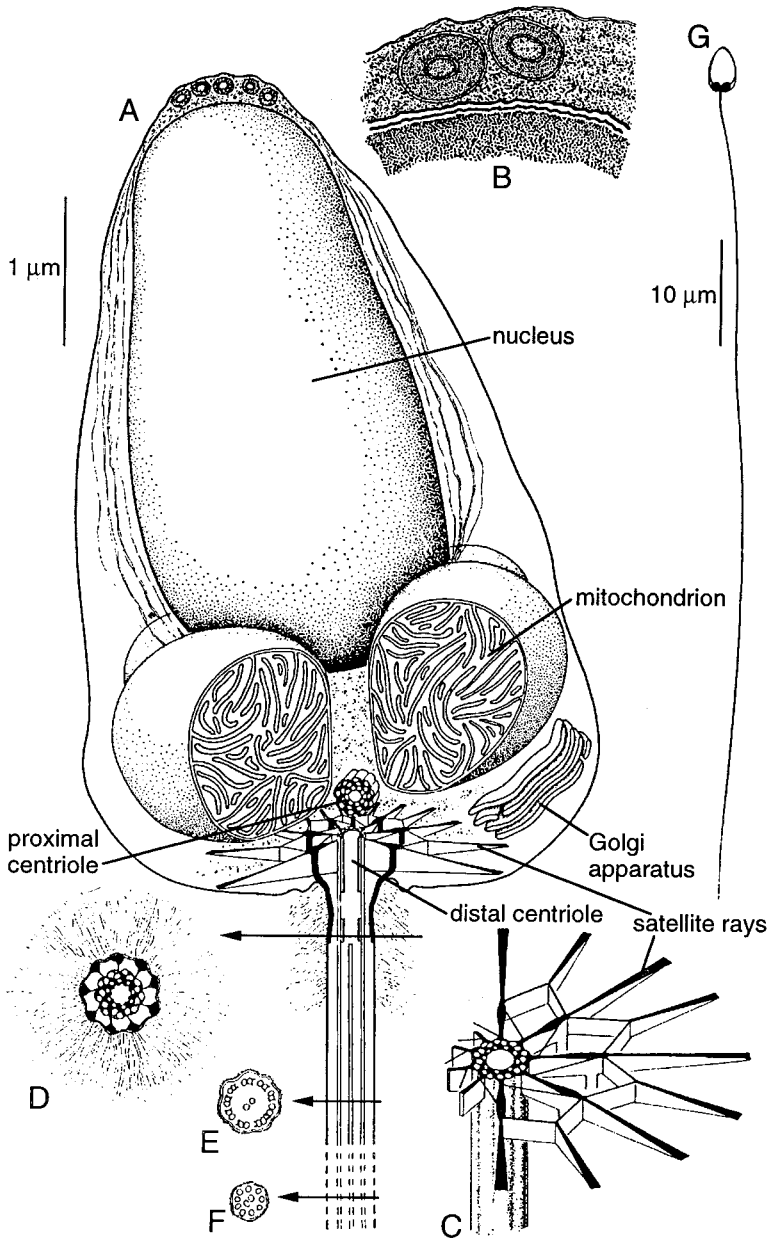


Fig. 14. *Nausithoe* sp. A: Semischematic diagram of the spermatozoan. B: Anterior end of the sperm at higher magnification. C: The anchoring apparatus and distal centriole. D: Cross section of the tail at the level of the hairy coat. E: Cross section of the mainpiece of the tail. The surface is covered with short projections forming an apparent glycocalyx. F: Cross section of the end piece. G: Optical drawing of a living sperm. After Afzelius and Franzén (1971). *Journal of Ultrastructure Research*, 37, 186–199, chart 1.

processes and then continue radially. Each primary process has a pronounced thickening,  $0.1\ \mu\text{m}$  from the centriolar surface. At this level another set of thin fibres emanate, each fibre joining the neighbouring, next-clockwise primary process. Thus, the pericentriolar process forms a nine-pointed star with a diameter of  $1\ \mu\text{m}$ , excluding the outermost radial processes. The cell membrane has electron-dense thickenings where the 18 secondary processes are attached to it. The central branch, a continuation of the primary ray, appears in each case to continue as a well developed cross-striated process.

The distal centriole also has a rootlet or 'spur' (not here illustrated), which is an elongate, blunt lateral projection from the centriole just proximal to the pericentriolar apparatus. This rootlet is cross-striated with a periodicity similar to that of the pericentriolar processes. Microtubules sometimes visible in the vicinity of the distal centriole are questionably features of the mature sperm.

#### (e) *Flagellum*

At its origin from the midpiece and extending for a distance of about  $0.8\ \mu\text{m}$ , the tail is covered by a brush of fine fibrils, of unknown origin and function, each about  $30\ \text{\AA}$  wide and  $0.2\ \mu\text{m}$  long (Fig. 14D). This coat extends completely around the tail and is distinct from structures coating the flagellum in some algae or the sponge choanocyte (Afzelius and Franzén, 1971). In longitudinal section this portion of the tail is narrower than the remainder. Each axonemal filament is connected to the plasma membrane by Y-shaped fibrils at this level, and the external fibrils are most numerous at this attachment. The two central singlets commence just distal to this zone (Fig. 14E).

Doublets 5 and 6 of the  $9+2$  axoneme are joined by the outer dynein arm of 5 connecting to a ridge on the B tubule of 6. In the endpiece (Fig. 14F), the B microtubules, arms and spokes disappear first, and the profiles of the A microtubules may become triangular. The central singlets extend furthest posteriorly (Afzelius and Franzén, 1971).

### F. Superorder Discomedusae — Orders Semaestomeae and Rhizostomae

The order Semaestomeae includes many common scyphomedusae with bowl- or saucer-shaped bells having a scalloped margin and four large oral arms. The order Rhizostomae has scyphomedusae in which the bell lacks tentacles and the oral arms are branched and folded to form many secondary mouths. Ultrastructural features of the sperm of the eight semaestome and rhizostome species studied to date are considered together.

### 1. Nucleus

The sperm head and nucleus in the Discomedusae may be more (*Aurelia*, *Chrysaora*) or less (*Rhizostoma*) elongated than in stauromedusans. In *Aurelia*, *Pelagia* and *Cassiopea* especially, the nucleus is very elongated and sharply conical, whereas in other species the nucleus is shorter (Hinsch and Clark, 1973; Hinsch, 1974; Hedwig and Schäfer, 1986). The number of spaces within the condensing chromatin decreases during development. A posterior extension of the nucleus, seen in the Stauromedusae, is absent. In *Aurelia*, the nucleus has a basal indentation or fossa adjacent to the mitochondria in the midpiece (Hedwig and Schäfer, 1986).

### 2. Anterior region

At the apex of the sperm, above the nucleus, there is an accumulation of granular to slightly fibrous material which, in *Aurelia*, is superficially similar to the anterior nuclear process seen in conical scleractinian sperm (Schmidt and Zissler, 1979; Harrison, 1985, 1988a, 1990). In *Aurelia* and *Cassiopea* sperm, lying among slightly helical microtubules, are several membrane bound vesicles which have ring or doughnut-shaped profiles and are filled with an electron-dense material; at least some of these appear to arise in association with the Golgi apparatus (Hinsch and Clark, 1973; Hinsch, 1974; Hedwig and Schäfer, 1986). Electron-dense vesicles are also found between the nuclear and plasma membranes in *Chrysaora* and *Rhizostoma* sperm, and these vesicles are also present in the midpiece of *Chrysaora* (Hedwig and Schäfer, 1986). In *Cassiopea* sperm, an electron-dense structure lies along one side of the nucleus (Hinsch, 1974).

### 3. Midpiece and collar

Four mitochondria dominate the midpiece at the base of the nucleus. They are radially arranged and in close contact, being conjoined by electron-dense plates. In all Discomedusae, as in many anthozoans but unlike *Nausithoe*, the posterior region of the midpiece extends as a long sleeve or cytoplasmic collar around the anterior region of the flagellum.

### 4. Centrioles and anchoring apparatus

The proximal centriole is orientated perpendicular to the distal centriole which is aligned along the longitudinal axis of the sperm. As in *Nausithoe*, the distal centriole is just posterior to the mitochondria (Hinsch and Clark, 1973; Hinsch, 1974; Hedwig and Schäfer, 1986). The distal centriole has nine triplets embedded in a dense matrix from which arise the nine primary pericentriolar processes which terminate in thickened tips. The general arrangement of the pericentriolar processes in Discomedusae resembles that in *Nausithoe*, and while in both *Aurelia* and *Cyanea*

this anchoring apparatus is slightly reduced, the primary processes divide into three branches (Hinsch, 1974; Hedwig and Schäfer, 1986).

## 5. Flagellum

All Discomedusae sperm studied have the usual 9+2 pattern of microtubules in the flagellum. In *Chrysaora*, the axoneme is thickened for its proximal 0.4µm, and there is a transverse electron-dense plate in the first third of this region, a feature not seen in the Stauromedusae or Coronatae (Hedwig and Schäfer, 1986). This plate is probably identical with the ring of dense material lying anterior to the two central singlets and within the ring of doublets in the axoneme of *Aurelia*; in the region where Y-shaped fibrils extend to the plasma membrane (Hinsch and Clark, 1973; Hinsch, 1974).

# V. PHYLUM CTENOPHORA

## A. Introduction

Ctenophores are marine, planktonic, or sometimes epibenthic organisms, with biradial symmetry, a gastrovascular cavity, and gelatinous mesenchymal mesogloea with associated muscle cells. More than 50 species of Ctenophora are known (Barnes and Harrison, 1991). The principal distinguishing feature is the occurrence of eight radially arranged rows of cilia, the ctenes or combs (Hernandez-Nicaise, 1991). These comb rows are coordinated through an apical sense organ which contains a statocyst (Barnes and Harrison, 1991). Unlike cnidarians, the tentacles are not arranged in circlets around the mouth, and the pair of tentacles contain special adhesive cells, the colloblasts. The colloblasts are not like nematocysts of cnidarians, and ctenophores do not generally possess nematocysts. However, one genus possesses exogenous nematocysts like some nudibranch molluscs, which are incorporated into carrier endodermal cells (Hernandez-Nicaise, 1991).

Despite the presence of a mesogloea, and other superficial similarities with cnidarians, ctenophores have a bilateral rather than a radial symmetry, and their histology is substantially different to that of cnidarians, and similar to that of higher metazoans (Hernandez-Nicaise, 1991; Barnes and Harrison, 1991). Preliminary molecular evidence suggests that the Ctenophora are the sister taxon of Cnidaria, but this is equivocal because insufficient phyla were included in the analyses which were concerned primarily with internal cnidarian relationships (Bridge *et al.*, 1995; Odorico and Miller, 1997). Other molecular phylogenetic studies, based on 28S ribosomal RNA sequencing, indicate that within the diploblastic Metazoa, Ctenophora have been evolving independently from Cnidaria for a considerable period (Hernandez-Nicaise, 1991).



Reproduction in ctenophores has been reviewed by Dunlap-Pianka (1974), Sardet *et al.* (1990) and Hernandez-Nicaise (1991). Most ctenophores are hermaphroditic, although two species are reported to be gonochoric (Sardet *et al.*, 1990). Gonadal tissues of ctenophores are sufficiently differentiated and specialized to be regarded as organs, and gametes are endodermal in origin (Dunlap-Pianka, 1974). As in higher metazoans the gametes are released through true gonopores, and cleavage is determinate (Hernandez-Nicaise, 1991; Barnes and Harrison, 1991). Development usually produces a cydippid larva with the same general features as the adult (Sardet *et al.*, 1990).

### B. Sperm of Ctenophora

Spermatogenesis and spermatozoa have been studied in detail ultrastructurally only in *Beroe ovata* (Franc, 1973), with additional ultrastructural information on fertilization provided by Carré *et al.* (1991). As this ctenophore is a member of the unusual Class Nuda (Order Beroida), it remains to be determined whether these sperm are representative of other Ctenophora. Dunlap-Pianka (1974) made brief reference to the ultrastructure of the spermatocytes of *Bolinopsis* and noted that earlier light microscope studies recorded filiform sperm in some other ctenophores.

The spermatozoon of *Beroe* (Fig. 15) is of the 'primitive' ect-aquasperm type but differs from cnidarian sperm in possessing a true acrosome. It has a rounded head, 4  $\mu\text{m}$  in diameter, in which the nucleus, although very dense and homogeneous, remains finely granular. In some sections, the nucleus possesses a small peripheral mass at the centre of a paler area. The acrosomal vesicle forms by the confluence of Golgi vesicles, initially in the vicinity of the flagellum, and becomes diametrically opposed to the flagellum in the mature spermatozoon. It differentiates into a conical cap, in contact with a corresponding protuberance of the plasma membrane, and an underlying dense mass. This mass resembles subacrosomal material but appears to lie within the bounding membrane of the original acrosomal vesicle. A peculiar large body, the paranuclear body, which lies alongside the nucleus, originates from a stack of endoplasmic reticulum cisternae on the external nuclear membrane. During the last stage of maturation, numerous large, 50 nm dense granules occupy the periphery of the cytoplasm (Franc, 1973).

The mitochondria fuse to form a single large mitochondrial mass which may lie anterolaterally to the nucleus. The Golgi apparatus persists in the mature spermatozoon. The flagellar tail, 50  $\mu\text{m}$  long, has a classical 9+2 axoneme. This tail develops early in spermiogenesis and is attached to an anchoring pericentriolar apparatus (Fig. 15) developed from the distal centriole. The pericentriolar apparatus (Fig. 12E) consists of nine radial rays each of which is terminally digitate. An endoplasmic cisterna always caps the distal centriole and sends a short projection into this. The proximal centriole shows no special differentiation and is orientated perpendicular to the distal centriole (Franc, 1973).

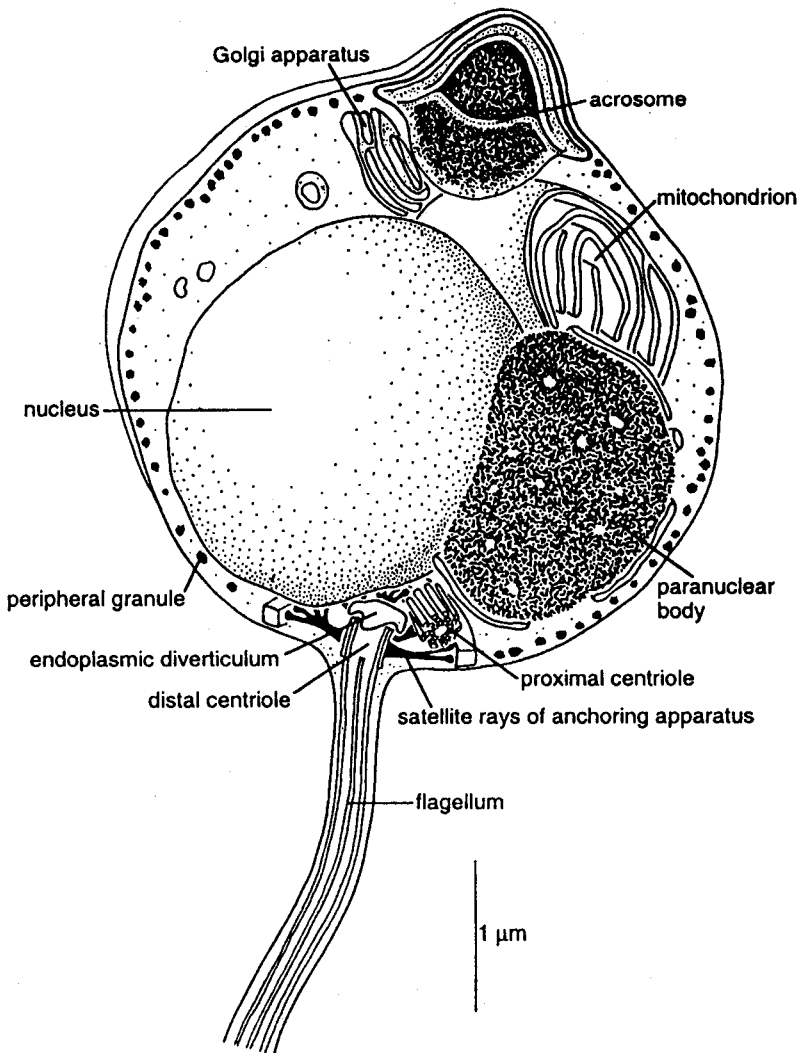


Fig. 15. *Beroë ovata*. Semi-diagrammatic reconstruction of the spermatozoon. After Franc (1973). *Journal of Ultrastructure Research*, 42, 255–267, schema 1.

### C. Sperm-egg Interaction and Fertilization

A detailed description and analysis of the events during fertilization in *Beroë ovata* are provided by Carré *et al.* (1991). The spherical sperm head becomes modified when in contact with the egg. A flange forms in the anterior region of the sperm, probably due to an acrosomal reaction, and the flagellum becomes rigid. This

probably corresponds to the onset of sperm-egg fusion, and the egg surface subsequently contracts around the site of sperm entry. Radiating membrane folds develop in a 70  $\mu\text{m}$  zone around the sperm during the first minute after sperm fusion, and then subside during the following minute. This is followed by the formation of a large fertilization cone (20–30  $\mu\text{m}$ ) which disappears after about 20 minutes. The sperm nucleus and mitochondria enter the egg cortex at a speed of 2  $\mu\text{m min}^{-1}$ , and mitochondria accumulate around the sperm nucleus. The female pronucleus migrates beneath the surface of the egg to the sperm entry site, and fuses with the male pronucleus. This is followed by a visible decondensation of the male chromatin within the zygote nucleus, and the onset of mitosis (Carré *et al.*, 1991).

## VI. CONCLUSIONS

Studies of sperm ultrastructure in cnidarians and ctenophores have great potential for elucidating functional aspects of sperm structure and phylogenetic patterns in simple metazoans. Although sperm ultrastructure has been studied in 138 species of cnidarians, this represents only 1.5 per cent of the known extant cnidarian species, hence it is very likely that other types of sperm remain to be discovered. Aspects of sperm structure are relatively well documented in scleractinian corals, sea anemones and hydroids. However, sperm structure is poorly documented in most other cnidarian and ctenophore groups, and no information is available for the Class Cubozoa and six other cnidarian orders. Studies of Cubozoa sperm are likely to yield particularly interesting information, as at least some species transfer sperm in spermatophores to female medusae which have sperm storage structures (Werner, 1973b; Hartwick, 1991; Matsumoto, 1995).

Anthozoans exhibit a greater range of sperm structure than Hydrozoa and Scyphozoa studied to date. However, caution is needed when interpreting these trends because the greater diversity in sperm structure observed in Anthozoa is correlated with a much larger number of species studied. Furthermore, the relationship between sperm morphology and fertilization biology in cnidarian remains uncertain, although there is some indication that brooding species may have modified spermatozoa. Comparative studies of congeneric brooding and broadcast-spawning species are needed to determine the relative importance of reproductive mode and phylogeny on sperm structure. These comparative studies would be most efficiently completed using scleractinian, actiniarian and perhaps hydroid taxa where substantial information on the timing and mode of reproduction is available. Collaborative studies involving molecular studies and ultrastructural analyses of sperm in important taxa are likely to provide the most useful data for resolving the uncertain phylogenetic relationships among the Cnidaria and Ctenophora.

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