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# Spermatozoal ultrastructure of spiny oysters (Spondylidae, Bivalvia) including a comparison with other bivalves

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**Summary**

Sperm ultrastructure in three representative species of the marine bivalve family Spondylidae ("spiny or thorny oysters") is examined and compared with available data on other bivalves, especially other families of the subclass Pteriomorphia. Spondylid spermatozoa are of the externally fertilizing aquasperm type (ect-aquasperm). The acrosomal vesicle is conical with a deep basal invagination extending almost the full length of the vesicle. Vesicle contents are divisible into an inner, highly electron-dense anterior layer and a less dense posterior layer. The anterior layer is folded back on itself posteriorly and exhibits radiating plates (best developed peripherally). The vesicle rests on, and is partially embedded in, an extensive granular deposit of subacrosomal material at the nuclear apex. This deposit extends partly into acrosomal vesicle invagination and also fills a broad depression in the anterior of the nucleus. No pre-formed axial rod (perforatorium) is present. The nucleus is round-pyriform and its contents coarsely fibro-granular. At the base of the nucleus, four broad depressions partially accommodate the midpiece mitochondria. The midpiece consists the four spherical mitochondria and the proximal and distal centrioles. The centrioles are arranged at approximately 90° to each other, and each consists of nine, angularly-oriented, microtubular triplets embedded in a granular matrix. A short, periodically banded rootlet connects the proximal centriole to the nuclear fossa, whereas the distal centriole, which forms the basal body to the flagellar axoneme, is anchored to the plasma membrane by nine terminally forked satellite fibres. Extensive deposits of putative glycogen rosettes surround the centrioles and mitochondria. The flagellum consists of a 9+2 axoneme sheathed by the plasma membrane. Spondylid spermatozoa strongly resemble those of the Pectinidae, further confirming the traditional view (based on comparative anatomy and shell morphology) of a close relationship between the Spondylidae and the Pectinidae. Differences in acrosomal shape and dimensions were noted between the three species examined, indicating potential taxonomic utility for comparative sperm ultrastructure within the Spondylidae.

***Key words***: Bivalve, reproduction, sperm ultrastructure, Pteriomorphia, Mollusca

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## Introduction

Spondylids ("spiny or thorny oysters") are pectinoidean bivalves characterised in part by their spinose shells, strong ball-in-socket hinge dentition and, usually, partial or complete cementation of the left (lower) valve to the substratum (Hertlein and Cox, 1969; Boss, 1982; Lamprell, 1986). The family occurs in all oceans but is most diverse in subtropical and tropical regions especially in the Indo-West Pacific (Lamprell, 1986; Lamprell and Healy, 1998, 2001). Typically spondylids inhabit shallow water reef environments, but a number of species have been recorded from depths in excess of 550 m and some are found almost exclusively on muddy-sand sediments. Although spondylids are highly esteemed for the beauty of their shells and may form an important component of the benthos, very little is known about their reproductive biology, especially in comparison with the commercially harvested Pectinidae (scallops) [see Villalejo-Fuerte and García-Domínguez (1998) for a recent study of gonad indices and reproductive cycles in *Spondylus leucacanthus* Broderip].

Several studies have been published on sperm ultrastructure within the Bivalvia, and collectively these help to confirm the utility of sperm characters for taxonomic and phylogenetic analysis at and above the species level (e.g., Gharagozlou-Van Ginnekin and Pochon-Masson, 1971; Popham, 1974, 1979; Popham et al., 1974; Hodgson and Bernard, 1986a, 1986b; Eckelbarger et al., 1990; Hodgson et al., 1990; Healy, 1989, 1995a, 1995b, 1996; Reunov and Hodgson, 1994; Garrido and Gallardo, 1996; Kafanov and Drozdov, 1998; Healy et al., 2000). Among the Pectinoidea, only two species of Pectinidae (scallops) have been adequately investigated for sperm ultrastructure: *Pecten maximus* (Linnaeus) (Anderson and Personne, 1970a, 1970b, 1976; Dorange and Le Pennec, 1989) and *Gloripallium pallium* (Reeve) (Healy et al., 2000). Désilets et al. (1995) examined the events of fertilization using transmission electron microscopy in *Placopecten magellanicus* but provided only a brief description and no micrographs of the intact spermatozoon. Linck (1973a, 1973b) compared the microtubular substructure of cilia and sperm flagella in *Aequipecten irradians* but did not detail any other sperm features in this species. For the Spondylidae, sperm ultrastructural data are limited to brief observations on *Spondylus nicobaricus* (Schreibers) (Healy et al., 2000). The primary goals of the present study are to describe comparative sperm ultrastructure in representative species of Spondylidae and to compare the results obtained with available information on the Pectinidae, and more generally on other bivalves, especially other members of the subclass Pteriomorphia.

## Materials and Methods

Material for this study was collected on the Great Barrier Reef, Queensland: *Spondylus squamosus* Schreibers, 1793 (Heron Is., Research Zone B, low tide, in coral rubble); *S. varius* Sowerby, 1827 (attached to slight coral overhang between Pioneer Bay and Hazard Bay, Orpheus Is., 10 m depth), *S. nicobaricus* Schreibers, 1793 (Lady Musgrave Island reef, 8 m depth). To supplement available data for the Pectinidae (for comparison with Spondylidae), *Gloripallium pallium* (Linnaeus, 1758) was also collected (under plate coral, Heron Island reef, 9 m depth). Small (1–2 $mm^3$) pieces of mature testis were excised and placed directly into ice cold 3% glutaraldehyde (prepared in 0.1 M sodium phosphate buffer containing w/v 10% sucrose) for 24 h, then rinsed in buffer (45 min) before being placed into a 1% solution of osmium tetroxide (buffer as for earlier steps) for 80 min. After a further rinse in buffer (45 min) tissues were dehydrated through a graded ethanol series before being gradually embedded in Spurr's epoxy resin. Semi-thin and ultra-thin sections were cut using an LKB 2428 Ultrotome IV, collected on uncoated 200 μm mesh copper grids and stained with lead citrate and uranyl acetate according to the contrast-enhancing method of Daddow (1986). Stained sections were examined and photographed using a Hitachi A 300 transmission electron microscope operated at an accelerating voltage of 75 kV. The specimens of *Spondylus squamosus*, *S. nicobaricus*, *S. varius* and *Gloripallium pallium* used in this study have been deposited in the Queensland Museum (Brisbane) as voucher lot numbers QMMO 68650, QMMO 66824, QMMO 67048, QMMO 66823).

## Results

Mature spermatozoa within the testis of spondylids are of the aquasperm (so-called "primitive" sperm) type. Each spermatozoon consists, in anterior-posterior sequence, of an acrosomal complex, a short nucleus, a short midpiece (centriolar complex surrounded by a ring of spherical mitochondria) and a flagellum (tail) (Fig. 1A). The ultrastructural features of each sperm region/component are described in detail for *Spondylus squamosus*, followed by a comparison with *S. varius*, *S. nicobaricus* and the pectinid *Gloripallium pallium*.

For all TEM dimensions, $n = 5$; for flagellar dimensions (light microscopy) $n = 10$.

**Spondylus squamosus**

*Acrosomal complex*

The acrosomal complex is positioned at the apex of the sperm nucleus and consists of a conical, membrane-bound, acrosomal vesicle and an extensive deposit of granular, subacrosomal material (Fig. 1A,B). The acrosomal vesicle measures 0.75±0.03 μm in length and has a maximum diameter, basally, of 0.45± 0.02 μm. Its contents are differentiated into a highly electron-dense, anterior (inner) layer and a less electron-dense posterior (outer) layer (Fig. 1B). The anterior layer is closely folded back on itself in the posterior half of the acrosomal vesicle (Fig. 1B). Transverse sections reveal evidence of fine, radiating plates within the periphery of the anterior layer (Fig. 1B, inset) but no other obvious internal structure in other vesicle contents. An invagination extends almost the full length of the acrosomal vesicle, becoming very narrow anteriorly. The deposit of finely granular subacrosomal material is extensive, filling not only a broad apical depression of the nucleus and the basal quarter of the acrosomal vesicle invagination but also separating the acrosomal vesicle from the nuclear surface by a distance of 0.1±0.03 μm (Fig. 1A,B). No perforatorial structure (such as an axial rod) is present within the subacrosomal material. Deep within the acrosomal vesicle invagination only scattered dense particles of subacrosomal material are observed.

*Nucleus*

The nucleus is spheroidal-pyriform and has a length of 1.8±0.1 μm and maximum diameter of 2.0±0.1 μm (Fig. 1A). Its highly electron-dense contents consist of tightly packed dense fibres (diameter 20.0±1.0 nm) separated by finely granular, less dense material (Fig. 1B,C). Electron-lucent lacunae are sometimes observed within the nuclear contents (Fig. 1A). A broad, apical depression (depth 0.18 ± 0.02 μm), filled with subacrosomal material, occurs anteriorly while posteriorly four or depressions abut the four mitochondria of the midpiece. In addition to these indentations of the nuclear surface, a small posterior nuclear fossa occurs in the middle of the mitochondria-bearing depressions (Fig. 1A,C).

*Midpiece*

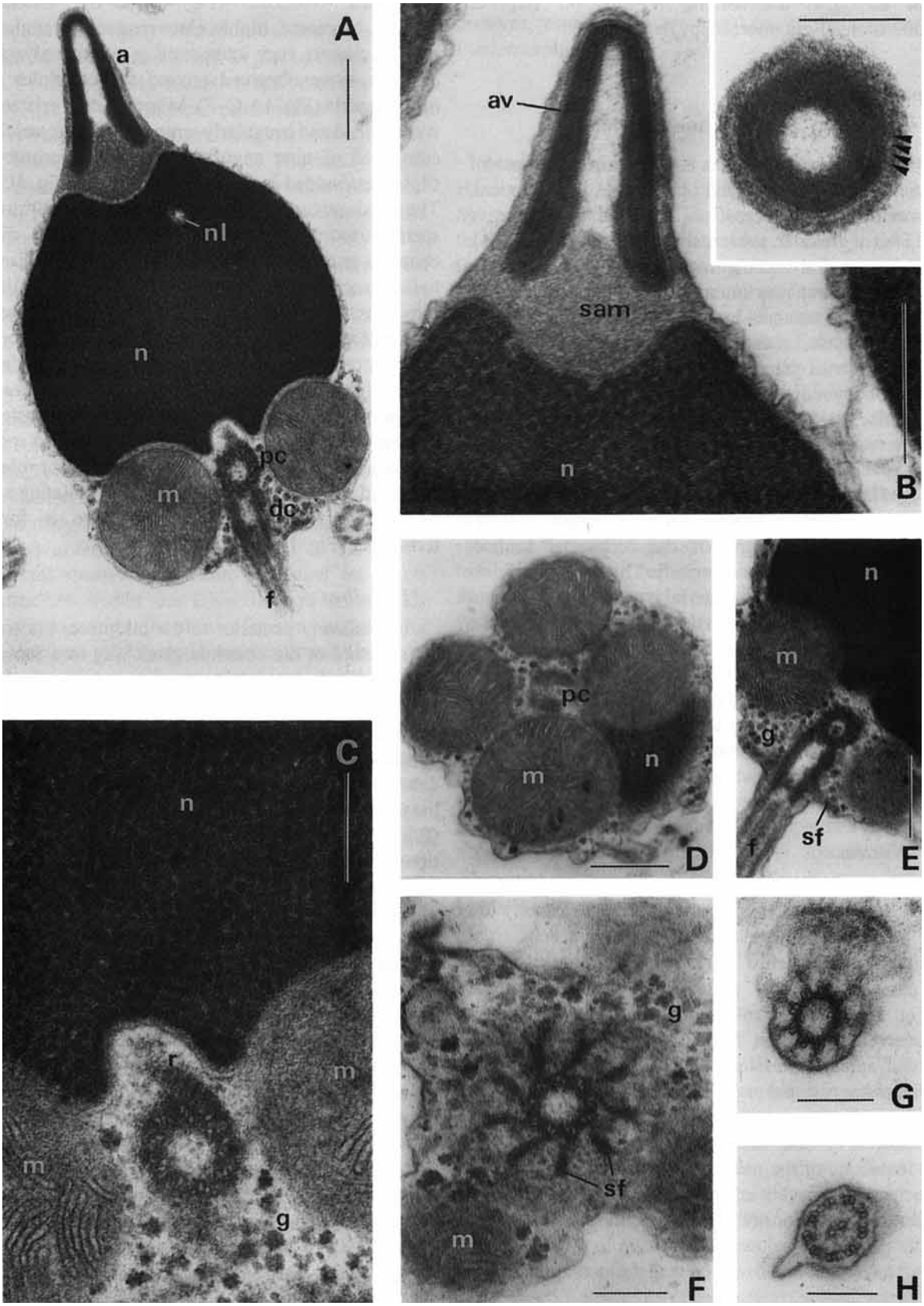
The midpiece region consists of four spherical mitochondria grouped in a ring around a pair of short, cylindrical centrioles (Fig. 1A,C–E). Extensive deposits of coarse, highly electron-dense granules or granule clusters, here interpreted as putative glycogen deposits, were observed around the centrioles and mitochondria (Fig. 1A,C–F). Mitochondrial cristae are well defined and irregularly arranged. Each centriole is composed of nine angularly disposed microtubular triplets embedded in a fine, dense matrix (Fig. 1C,F). The proximal centriole lies at approximately 90° to the spermatozoon's longitudinal axis while the distal centriole is aligned with this axis (Fig. 1A,E). Longitudinal sections show that the two centrioles lie in direct contact with each other and appear to be invested with a coating of finely granular material. Anchorage of each centriole to its adjacent region is achieved using different, but possibly homologous, structures — the proximal centriole is attached to the posterior nuclear fossa by a small, periodically banded rootlet (periodicity 27.0±1.0 nm); the distal centriole is anchored to the plasma membrane by a radiating array of satellite fibres. Each satellite fibre is forked terminally (Fig. 1F).

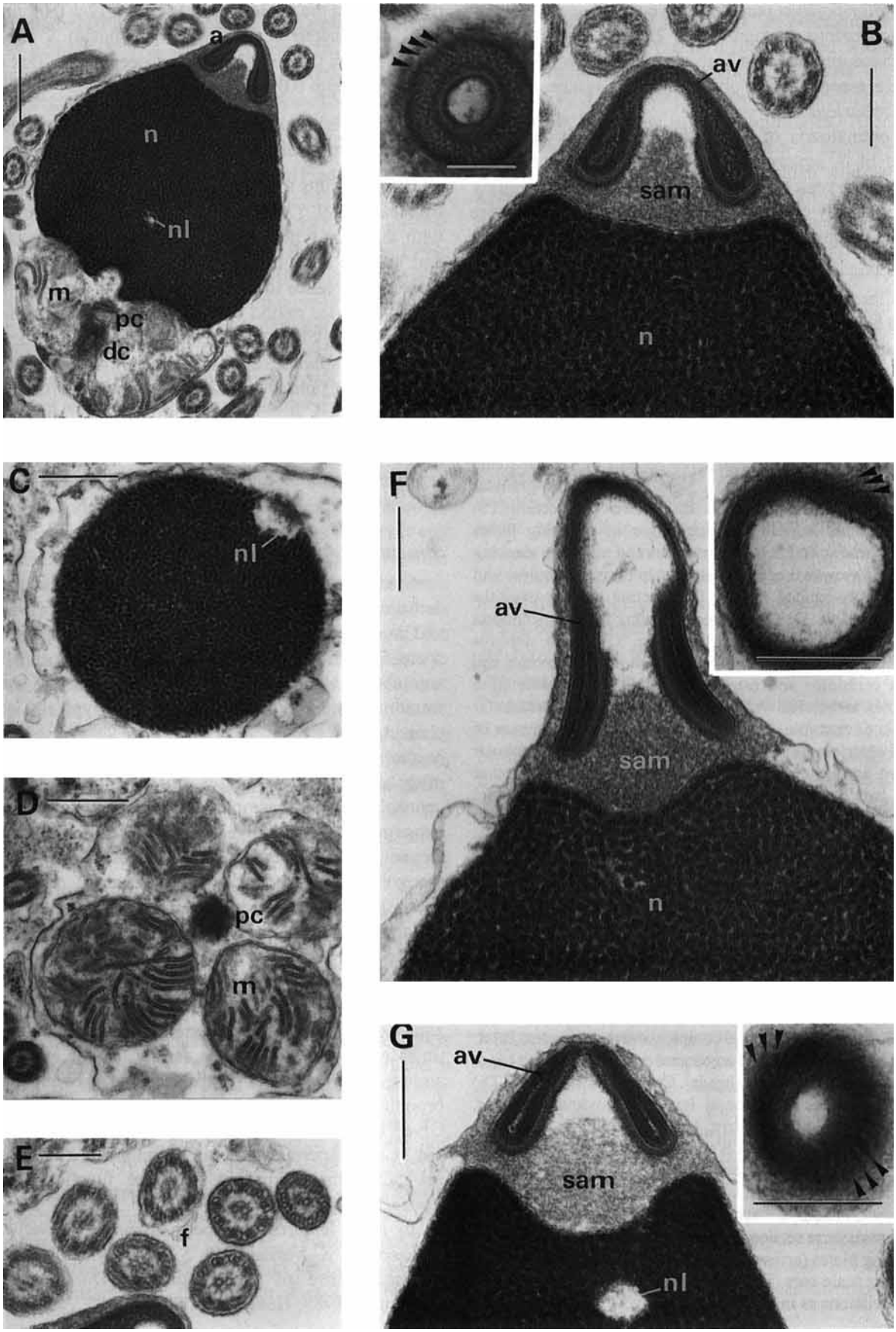
*Flagellum*

Immediately posterior to the midpiece, the triplet substructure of the centriole gives way to a standard 9+2 microtubular pattern axoneme (nine doublets surrounding a central pair of microtubules) (Fig. 1H). The transitional zone between centriole and axoneme consists of nine doublets connected to each other by a dense sheath on the inner aspect of the cylinder, and to the plasma membrane by short, terminally forked fibres (Fig. 1G). The plasma membrane shows no elaborations, although sometimes an extension of the membrane was observed which may be the equivalent of flange reported in certain other bivalve spermatozoa (Fig. 1H). Light microscopy gives a length of 50.0 ± 2.0 μm for the flagellum.

**Spondylus varius, S. nicobaricus**

Spermatozoa of *S. varius* differ from those of *S. squamosus* and *S. nicobaricus* in having a much shorter and basally wider acrosomal vesicle (length 0.45±0.02 μm; diameter at base 0.6±0.05 μm) and a very shallow apical depression of the nucleus (depth 0.09±0.01 μm) (Fig. 2A,B). The highly electron-dense anterior layer, characterised by radiating plates (Fig 2B and inset) is well developed and clearly shows its posteriorly folded profile (enclosing a coarsely granular matrix). The shape of the acrosomal vesicle in *S. varius* resembles more closely those of pectinids





(e.g. *Gloripallium pallium*) than either *S. squamosus* or *S. nicobaricus* (compare Figs 2B and 2 G). Nuclear, midpiece and flagellar features of *S. varius* (Fig. 2A–E) are essentially as observed in *S. squamosus* and *S. nicobaricus*.

Spermatozoa of *S. nicobaricus* closely resemble those of *S. squamosus*, with the exception that the acrosomal vesicle is longer (0.83 ± 0.02μm), slightly wider basally (0.56 ± 0.03μm) and with a much more inflated anterior region (Fig. 2F). In addition, the depression at the nuclear apex (depth 0.12 ± 0.02 μm) of *S. nicobaricus* is shallower than that of *S. squamosus* (depth 0.18±0.02 μm), but deeper than that of *S. varius* (depth 0.09 ± 0.01 μm) (Fig. 2F).

Fig. 1. Mature spermatozoa of *Spondylus squamosus* Schreibers. A. Longitudinal sections (LS) through (in anterior-posterior sequence) the acrosomal complex, nucleus, midpiece and proximal portion of the flagellum. Note putative glycogen deposits in midpiece region. B. LS through acrosomal complex and nuclear apex showing conical profile of acrosomal vesicle and extensive deposit of subacrosomal material (lacking axial rod). Note also the fibro-granular nature of the nuclear contents. Inset: transverse section (TS) of acrosomal vesicle showing edges of radiating plates (arrow heads). C. LS base of nucleus and midpiece showing detail of proximal centriole (note triplet microtubules) and periodically-banded rootlet. This section has bypassed the distal centriole. D. TS midpiece showing four mitochondria arranged around proximal centriole. Glycogen deposits shown. E. LS midpiece showing mitochondria, proximal and distal centrioles and satellite fibres. F. TS satellite fibre complex associated with distal centriole. Note microtubular triplets of centriole. G. TS satellite fibre complex at base of distal centriole. H. TS flagellum showing 9+2 microtubular pattern axoneme. Scale bars: A, D, E = 0.5 μm; all other scale bars (including inset for B) = 0.25 μm. a, acrosomal complex; av, acrosomal vesicle; dc, distal centriole; f, flagellum; g, glycogen granules; m, mitochondrion; n, nucleus; nl, nuclear lacuna; pc, proximal centriole; r, periodically banded rootlet; sam, subacrosomal material; sf, satellite fibres (connecting distal centriole to annulus and plasma membrane).

Fig. 2. Mature spermatozoa of Spondylidae (A–F) and Pectinidae (G). *Spondylus varius* Sowerby (A–E). A. Longitudinal section (LS) of acrosomal complex, nucleus and midpiece. B. LS acrosomal complex and nuclear apex. Inset: transverse section (TS) of acrosomal complex showing edges of radiating plates (arrow heads). C. Transverse section (TS) nucleus. D. TS midpiece at level of proximal centriole, showing four mitochondria. E. TS flagella. F. *Spondylus nicobaricus* Schreibers. LS acrosomal complex and nuclear apex. Inset: transverse section (TS) of acrosomal complex showing radiating plates (arrow heads). G, *Gloripallium pallium* Linnaeus. LS acrosomal complex and nuclear apex. Inset: transverse section (TS) of acrosomal complex showing radiating plates (arrow heads). Scale bars: A,C,D = 0.5 μm; all other scale bars (including insets for B,F,G) = 0.25 μm. Abbreviations as in Fig. 1.

### Gloripallium pallium

Sperm results for this pectinid are reported in detail by Healy et al. (2000a), but for ease of comparison with the spondylids examined herein, the acrosomal and nuclear apex profiles are shown in Fig. 2G. A close resemblance to spondylid sperm is evident: the narrow anterior region of the acrosomal invagination and the extent of the nuclear depression are similar to that of *S. squamosus* whereas the length of the acrosomal vesicle is similar to that of *S. varius*. The radiating plates within the anterior layer of the acrosomal vesicle are especially well developed (Fig. 2G, inset).

## Discussion

### *Structural comparison*

Spermatozoa of spondylids conform to the aquatic or "aquasperm" type of Rouse and Jamieson (1987) and Jamieson (1987), often referred to as "primitive sperm" (Franzén, 1955, 1956). Aquasperm occur in animals which release spermatozoa into the surrounding water (Franzén, 1956) and typically are characterised by a conical acrosomal vesicle, a short nucleus, a midpiece composed of a ring of spherical mitochondria surrounding a pair of triplet structure centrioles and a simple flagellum (9+2 pattern axoneme sheathed only by the plasma membrane). Rouse and Jamieson (1987) and Jamieson (1987) recognised two categories of aquasperm: ect-aquasperm (sperm fertilising eggs in ambient water) and ent-aquasperm (sperm fertilising eggs within a tube or mantle cavity). Among the Bivalvia there are several described examples of both of these categories (e.g., ect-aquasperm — giant clams, Tridacnidae; ent-aquasperm — freshwater mussels, Unionoidea) sometimes even within the same family, as for example among the rock oysters [Ostreidae; free spawning and brooding species (Boss, 1982; Slack-Smith, 1998)]. Given that the Spondylidae are free spawners (Boss, 1982), their spermatozoa can therefore be classed as ect-aquasperm according to the function-based Rouse/Jamieson system.

In most features the spermatozoa of *Spondylus squamosus*, *S. varius* and *S. nicobaricus* closely resemble those of the Pectinidae (Dorange and Le Pennec, 1989; Healy et al., 2000).

Within the Bivalvia, considerable variation exists in the internal structure of the acrosomal vesicle contents, and perhaps this is best illustrated by the subclass Pteriomorphia (see Healy, 1996; Healy et al., 2000). The degree of differentiation of the acrosomal vesicle contents varies widely among pteriomorphians ranging from homogeneous [as in the Arcoidea and Anomioidea (Popham, 1979; Reunov and Hodgson, 1994)], to limited anterior differentiation [often as layers or whorls — Ostreoidea (Daniels et al., 1971; Brandriff et al., 1978; Healy and Lester, 1991; Bozzo et al., 1993; Eckelbarger and Davis, 1996; Gwo et al., 1996)] to multiple zones of differentiation (Pectinoidea, Mytiloidea, Pterioidea) (Niijima and Dan, 1965a, 1965b; Bourcart et al., 1965; Endo, 1976; Hodgson and Bernard, 1986b; Kafanov and Drozdov, 1998) (for a recent survey of pteriomorphian sperm see Healy et al., 2000). This difference in acrosomal complexity is a reflection of the phylogenetic affinities of each group (and between species, genera and families within each group) and also suggests variation in the acrosome reaction and in other structural aspects of sperm-egg contact within the Pteriomorphia. Compared to other pteriomorphians (and more generally other bivalves), the acrosomal vesicle of spondylids and pectinids (Pectinoidea) most closely approach those of the Pinnoidea and Pterioidea, especially in relation to vesicle shape and the radial-plate internal structure of the vesicle contents (Thielley et al., 1993; Healy et al., 2000). Much coarser radial plates have been observed in the Ostreoidea and Limoidea but presumably these are still homologous with those of the Pectinoidea, Pinnoidea and Pterioidea. Interestingly, in the Mytiloidea, the acrosomal vesicle contents show distinctive concentric internal structure but no radial plates (for comparative figures see Healy et al., 2000).

In spondylids and pectinids (Pectinoidea) the extensive subacrosomal deposit keeps the acrosomal vesicle well separated from the nuclear apex (Dorange and Le Pennec, 1989; Healy et al., 2000; present study). This condition also occurs in some other pteriomorphians such as the Arcoidea (Popham, 1979; Reunov and Hodgson, 1994; Healy et al., 2000) and in some deep-sea Mytiloidea [*Bathymodiolus* spp. (Le Pennec and Beninger, 1997; Eckelbarger and Young, 1999)], in the Protobranchia (Popham and Marshall, 1977; Franzén, 1983; Morse and Zardus, 1997) and certain veneroidean and galeommatoidean heterodonts (Gharagozlou-Van Ginnekin and Pochon-Masson, 1971; Eckelbarger et al., 1990). Typically in bivalves the acrosomal vesicle lies very close to, or in direct contact with, the nuclear apex (see Popham, 1979; Franzén, 1983; Hodgson et al., 1990; Healy, 1989, 1996, Healy et al., 2000) . In pteriomorphians, the occurrence of a pre-formed axial rod within the subacrosomal material appears to be linked with the presence of a very deep anterior invagination of the nucleus. It is absent in the Pectinoidea, Arcoidea, Limoidea, Anomioidea, Pterioidea and certain Mytiloidea, but present in the Ostreoidea and several Mytiloidea (Mytiliinae) (for comparative figures or micrographs see Popham, 1979; Hodgson and Bernard, 1986a, 1986b; Tilney et al., 1987; Gwo et al., 1996; Kafanov and Drozdov, 1998; Healy et al., 2000). By contrast, in heterodont bivalves, no such correlation exists, there being many examples of an axial rod being well developed in sperm lacking a deep nuclear invagination (e.g., see Gharagozlou-Van Ginnekin and Pochon-Masson, 1971; Popham, 1974; Popham et al., 1974; Hodgson et al., 1990; Healy, 1995a, 1995b, 1996).

According to Dan and Wada (1955, light microscopy), spermatozoa of *Spondylus cruentus* Lischke, when exposed to "egg-water" (seawater in which mature oocytes have been stored), produce a 15-μm-long "acrosomal filament" during the acrosome reaction. Most of the other bivalves studied by Dan and Wada belong to genera which, through TEM studies, are now known to exhibit a pre-formed axial rod (Niijima and Dan, 1965a, 1965b; Tilney et al., 1987). Presumably *S. cruentus*, like other pectinoideans (Dorange and Le Pennec, 1989; Healy et al., 2000; present study), lacks a pre-formed axial rod. Although the production of a long perforatorial process during the acrosomal reaction has been found to not reflect a natural sperm-egg response (the "super-elongation" phenomenon [for discussion see Hylander and Summers, 1977)], it is nevertheless interesting to note that the generation of such a long process can occur irrespective of whether a pre-formed axial rod is present or absent.

The morphology of the nucleus and midpiece of *S. squamosus* is essentially as observed in many other Bivalvia. The coarsely granulo-fibrous nature of the nuclear contents appears to be a very common pattern among bivalves, but is especially well developed in most of the subclass Pteriomorphia (Arcoidea, Limopsoidea, Pterioidea, Pinnoidea Pectinoidea and Ostreoidea (Anderson and Personne, 1970a, 1970b; Dorange and Le Pennec, 1989; Bozzo et al., 1993; Sousa and Oliveira, 1994; Gwo et al., 1996; Healy et al., 2000; present study). Presence of a well developed anterior invagination of the nucleus (as seen in *Spondylus* and *Pecten*) is widespread throughout the

Bivalvia, although among the Pteriomorphia, the deepest invaginations occur in those taxa which also possess a very elongate axial rod [e.g., the Ostreidae, Mytiliinae and Musculinae of the Mytilidae (see Gwo et al., 1996; Healy, 1996; Kafanov and Drozdov, 1998)]. Mitochondrial number may show substantial variation between genera of some pteriomorphians families, as in the Mytilidae (4–16, depending on genus [for discussion and further references see Kafanov and Drozdov (1998)], but typically the number varies from four to five, as is usual for molluscan aquasperm (Popham, 1979; Healy, 1996). Glycogen deposits surrounding the centrioles and mitochondria have been reported for several species of bivalves, including pectinids and ostreids (Anderson and Personne, 1970a, 1970b, 1976), teredinids (Popham and Dickson, 1975) and veneriods (Gharagozlou Van Ginneken and Pochon-Masson, 1971; Eckelbarger et al., 1990; Hodgson et al., 1990) and are thought to represent an endogenous energy reserve (Anderson and Personne, 1976). The orthogonal arrangement of the proximal and distal centrioles (and their triplet microtubular structure) and the terminally-forked satellite fibre complex observed in *Spondylus* spp. are essentially as observed in most other bivalves, and indeed most other aquatically fertilising molluscs (for comparisons see Popham, 1979; Franzén, 1983; Healy, 1996; Healy et al., 2000 and references contained therein). A rootlet is often reported in studies of molluscan aquasperm, but relatively few authors have demonstrated or described periodic banding similar to that demonstrated here for *Spondylus* [in other Bivalvia: Ostreoidea, Ostreidae (Healy and Lester, 1991; Gwo et al., 1996); Limopsoidea, Glycymerididae (Healy et al., 2000); Arcidae (Popham, 1979)].

### *Taxonomic considerations*

Ultrastructurally, spermatozoa of the Spondylidae are very similar to those of the Pectinidae, based on all available data (Anderson and Personne, 1970; Dorange and Le Pennec, 1989; Healy et al., 2000; present study). While this result clearly supports the traditional view of a close relationship between the Spondylidae and Pectinidae (Newell, 1965; Yonge, 1973; Boss, 1982; Allen, 1985; Waller, 1978, 1998), it has not yielded any sperm characters to separate these families. Given the relatively small number of anatomical and conchological differences between spondylids and pectinids (Boss, 1982; Slack-Smith, 1998), a close similarity in sperm structure between the two groups was to be expected. Some authors, including Dakin (1928) and Thiele (1935), have regarded spondylids as forming merely a genus or at best a subfamily of the Pectinidae. Nevertheless, spondylids have a relatively long geological history [since the Jurassic (Hertlein and Cox, 1969)], and the features which separate them from the Pectinidae appear to be consistent (Slack-Smith, 1998). If the available sperm results for the Spondylidae and Pectinidae are truly representative of these families, then it is likely that variation in sperm morphology within and between the many proposed genera and subgenera of the Pectinidae will be relatively small. However, it is worth noting that in a number of bivalve families, including the Mytilidae (Garrido and Gallardo, 1996; Kafanov and Drozdov, 1998), Cardiidae (Keys and Healy, 1999, 2000), Donacidae (Hodgson et al., 1990; Sousa and Oliveira, 1994) and Veneridae (Gharagozlou Van Ginneken and Pochon-Masson, 1971), significant variation in acrosomal and nuclear shape have been noted between and within genera. Certainly the differences in acrosomal length and/or shape reported in this study for *S. squamosus*, *S. varius* and *S. nicobaricus* are encouraging signs that sperm ultrastructural studies within the Pectinoidea will prove taxonomically rewarding. It is noteworthy that *S. varius* alone among spondylids produces water-filled shell chambers [water-traps or camerae (see Healy et al., 2001)], and that the acrosomal vesicle of this species differs substantially in longitudinal profile from those of *S. squamosus* and *S. nicobaricus*. Instead the acrosomal vesicle resembles more closely that of investigated Pectinidae (Dorange and Le Pennec, 1989; Healy et al., 2000), thereby offering support for Dakin's (1928) pectinid origins of the Spondylidae. Within the Spondylidae, sperm studies have only dealt with *Spondylus sensu stricto*, and it is hoped that future work can be directed toward representatives of the subgenera *Eltopera* Iredale and *Corallospondylus* Monterosato if only to confirm a valid basis for these taxa. Sperm ultrastructural information for the unstudied families Propeamussiidae and Entoliidae (Syncyclonemidae) are now required to test the monophyletic status of the Pectinoidea and also familial relationships within the group.

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