Marine Biology (1994) 119: 449–460

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Spermiogenesis and sperm structure in the shrimp *Parapenaeus longirostris* (Crustacea: Dendrobranchiata): comparative aspects among decapods

Received: 10 December 1993 / Accepted: 4 January 1994

Abstract Early spermatids of the dendrobranchiate shrimp *Parapenaeus longirostris* (Lucas, 1846) have a spherical nucleus with large patches of heterochromatin, surrounded by a cytoplasmic mass that contains the conspicuous proacrosomal vesicle. The highly polarized mid spermatid mainly consists of the nuclear region, displaying a discontinuous nuclear envelope, and a large proacrosomal vesicle located at the opposite side of the cell. The most recent spermiogenic transformations primarily concern elongation of the proacrosomal vesicle to form a tapering spike. This results in the typically tack-shaped sperm of natantian decapods. The initial steps of spermiogenesis in the two studied dendrobranchiates prove to be parallel to reptant spermiogenesis in some respects, namely rupture of the nuclear envelope, chromatin decondensation and differentiation of electron-dense regions within the proacrosomal vesicle content. Specifically, whereas the anteriormost condensation gives rise to the operculum in brachyurans, in dendrobranchiates it becomes the apical portion of the spike. Despite an unquestionable morphological similarity between the sperm of carideans and dendrobranchiates, spermiogenesis in both groups displays meaningful differences. Spermatids of caridean shrimps lack a distinct proacrosomal vesicle. In the course of spermiogenesis, the spike arises from aggregated cytosolic materials; hence it is not membrane-bound. Unlike in other decapods, caridean sperm do not undergo a conventional acrosome reaction, since exocytotic events are not involved in this process. The above arguments suggest that, in the Decapoda, separation into three sperm classes is more suitable than the two traditionally accepted classes. The dendrobranchiate and reptant sperm types share a number of spermiogenic and functional features, while the caridean sperm type appears to represent an independent evolutive line with regard to sperm development and function.

Communicated by S. M. Pérès, Marseille

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Introduction

The widely varying morphologies of decapod sperm have been classified into two gross categories according to different patterns of organization (Brown et al. 1977, Talbot and Summers 1978). The unistellate sperm, typical of the suborder Dendrobranchiata and the pleocyematan infraorder Caridea (both formerly grouped together in the Natantia), exhibits a single appendage or spike which is an extension of the acrosomal structure. Conversely, the multistellate sperm of the non-caridean Pleocyemata (comprising the assemblage of the older Reptantia) show several appendages or arms of nuclear or cytoplasmic origin, the acrosome lacking any outstanding prolongation. Abundant references to the sperm ultrastructure in "natantians" are available (see reviews by Jamieson 1987, 1991, and Felgenhauer and Abele 1991), including the Dendrobranchiata (Clark et al. 1973, 1981, 1984; Yudin et al. 1979; Kleve et al. 1980; Shigekawa and Clark 1986; Ogawa and Kakuda 1987; Clark and Griffin 1988; Griffin et al. 1988; Dougherty and Dougherty 1989; Demestre and Fortuño 1992) and the Caridea (Pochon-Masson 1968b, 1969; Arsenault et al. 1979, 1980; Koehler 1979; Dupré and Barros 1983; Lynn and Clark 1983a, b; Arsenault 1984; Felgenhauer et al. 1988; Pérez et al. 1991). Whereas caridean spermiogenesis has been reported for *Palaemonetes paludosus* (Koehler 1979), *Crangon septemspinosa* (Arsenault et al. 1979, 1980; Arsenault 1984) and *Palaemon serratus* (Papathanassiou and King 1984), the only previous ultrastructural account of dendrobranchiate spermiogenesis concerns the sicyoniid *Sicyonia ingentis* (Shigekawa and Clark 1986), the sperm of which clearly differs from those of the Penaeidea (Clark et al. 1973; Felgenhauer et al. 1988; Dougherty and Dougherty 1989; Felgenhauer and

Abele 1991) in possessing a more complex acrosomal structure.

In recent years, considerable phylogenetic value has been given to the spermatozoan ultrastructure (Burkenroad 1981; Jamieson 1987, 1991 a, b), although treatment of this character may be complicated, since among some taxa morphology is highly conservative, whereas in others great variations are present even at the genus level (Felgenhauer and Abele 1991). Such variations may reflect differentiation of sperm components that occur in late stages of spermiogenesis, which could lead to strong differences in the ultrastructure of fully mature spermatozoa from closely related species. Therefore, it appears clear that a sound knowledge of the origin and evolution of the sperm components is important for recognizing phylogenetic linkages among decapod taxa. Aware of the necessity of a more complete information on spermiogenesis and sperm ultrastructure in Dendrohranchiata, a series of studies on several species have been initiated at the Facultad de Ciencias del Mar, beginning with the shrimp *Parapenaeus longirostris*.

Materials and methods

Male shrimp, *Parapenaeus longirostris* (Lucas, 1846), were collected at a depth of ~130 m from waters of the Gulf of Cádiz (southern Spain) during the Expedition "ARSA 1992", organised by the IEO (Instituto Español de Oceanografía) (October 1992). Testes and vasa deferentia were cut into small fragments and fixed for 10 h at 4 °C in 2.5% glutaraldehyde buffered with filtered seawater (FSW). The tissue was then washed in FSW (2×30 min) and 0.1 *M* sodium cacodylate buffer (pH 7.25) (1×15 min), postfixed for 1.5 h at 4 °C in 1% osmium tetroxide in cacodylate buffer, rinsed in distilled water, dehydrated through a graded acetone series, and embedded in Spurr's resin (Spurr 1969). Thin sections were cut with glass knives, picked up on pioloform-coated or uncoated copper grids, contrasted with uranyl acetate and lead citrate, and examined on a Jeol JEM 1200 EX transmission electron microscope at an accelerating voltage of 80 kV.

Whole ampullae (terminal swollen segments of vasa deferentia) were fixed and postfixed as indicated above, cut through their longitudinal axis and dehydrated in acetones. Following critical-point drying with CO_2, the two halves of each ampulla were mounted on copper stubs, sputter-coated with 25 nm gold and viewed in a Jeol JSM 820 scanning electron microscope operated at 10 kV.

Results

As in *Penaeus setiferus* and *P. vannamei* (Chow et al. 1991), the testicles of *Parapenaeus longirostris* consist of paired separate lobes, each formed by highly convoluted seminiferous tubules. In transverse section, the seminiferous tubule displays a peripheral region containing spermatogonia and a lumen mainly occupied by spermatids and spermatozoa. The sperm cells produced in the testicles are emptied into two vasa deferentia, which end in terminal ampullae where the spermatophores are formed. For a complete description of spermiogenesis, three successive stages have been separated as follows:

Early spermatids (Figs. 1 A, B; 2)

Early spermatids are polarized cells measuring about 5.25 µm in diameter, which possess a cytoplasmic mass containing a voluminous proacrosomal vesicle (Figs. 1 A, B; 2 A, B). The nucleus shows extensive areas of condensed chromatin and is entirely separated from the cytoplasm by the nuclear envelope. The perinuclear cytoplasm includes numerous small vesicles, some of which seemingly coalesce with the large proacrosomal vesicle (Fig. 2 C). Thin cisternae of endoplasmic reticulum frequently form parallel concentric arrays around the nucleus (Fig. 2 D) or constitute large areas of membrane accumulations in close contact with the nucleus and proacrosomal vesicle (Fig. 2 E). Other organelles that are present in the perinuclear cytoplasm are granular ribosome-like particles and a few spherical mitochondria with irregular cristae (Fig. 2 D). Centrioles were seldom found (Fig. 2 F), but no trace of Golgi bodies or any other similar membranous organelles were observed.

Three cell membranes may be recognized at the level of the proacrosomal vesicle, from exterior to interior: the accessory cell plasma membrane, the spermatid plasma membrane, and the proacrosomal vesicle membrane (Fig. 2 G). Internally, a single membrane separates the contents of the proacrosomal vesicle from the perinuclear cytoplasm (Fig. 2 C). The substance filling the proacrosomal vesicle is heterogeneous. An apparently flocculent matrix envelops a conspicuous core of granulated material (herein referred to as the granule for consistency with the "anterior granule" described by Shigekawa and Clark 1986) that represents a considerable volume of the total proacrosomal vesicle content. The granule lies at the anterior end of the proacrosomal vesicle, extending almost up to its base (Fig. 2 B). Beneath the anterior membrane, the granule differentiates a more finely granular area of higher electron-density (Fig. 2 G).

Mid spermatids (Figs. 1 C; 3)

Mid spermatids are slightly elongated cells averaging ~6 µm in length by ~4.6 µm in width. The proacrosomal vesicle at this stage is dome-shaped, the nucleus has become uncondensed and the perinuclear cytoplasm is considerably reduced (Figs. 1 C; 3 A, B). The nucleus contains an evenly electron-lucent chromatin, which is finely granular in appearance. The nuclear envelope has ruptured, so that the nucleoplasm is mixed with the surrounding cytoplasm (Fig. 3 C–E). The perinuclear cytoplasmic band primarily consists of anastomosing tubular membranes (Fig. 3 E) and occasional mitochondria trapped between membranes (Fig. 3 D). This membranous mass preferentially accumulates laterally at the base of the proacrosomal vesicle, becoming thinner at the posterior region (Figs. 1 C, 3 D, E). At the anteriormost portion, the perinuclear cytoplasm forms an homogeneous, electron-lucent subacrosomal region, where only scanty vesicular membranes intervene between the nucleoplasm and the proacrosomal vesicle (Fig. 3 D). Centrioles are no longer visible at this stage.

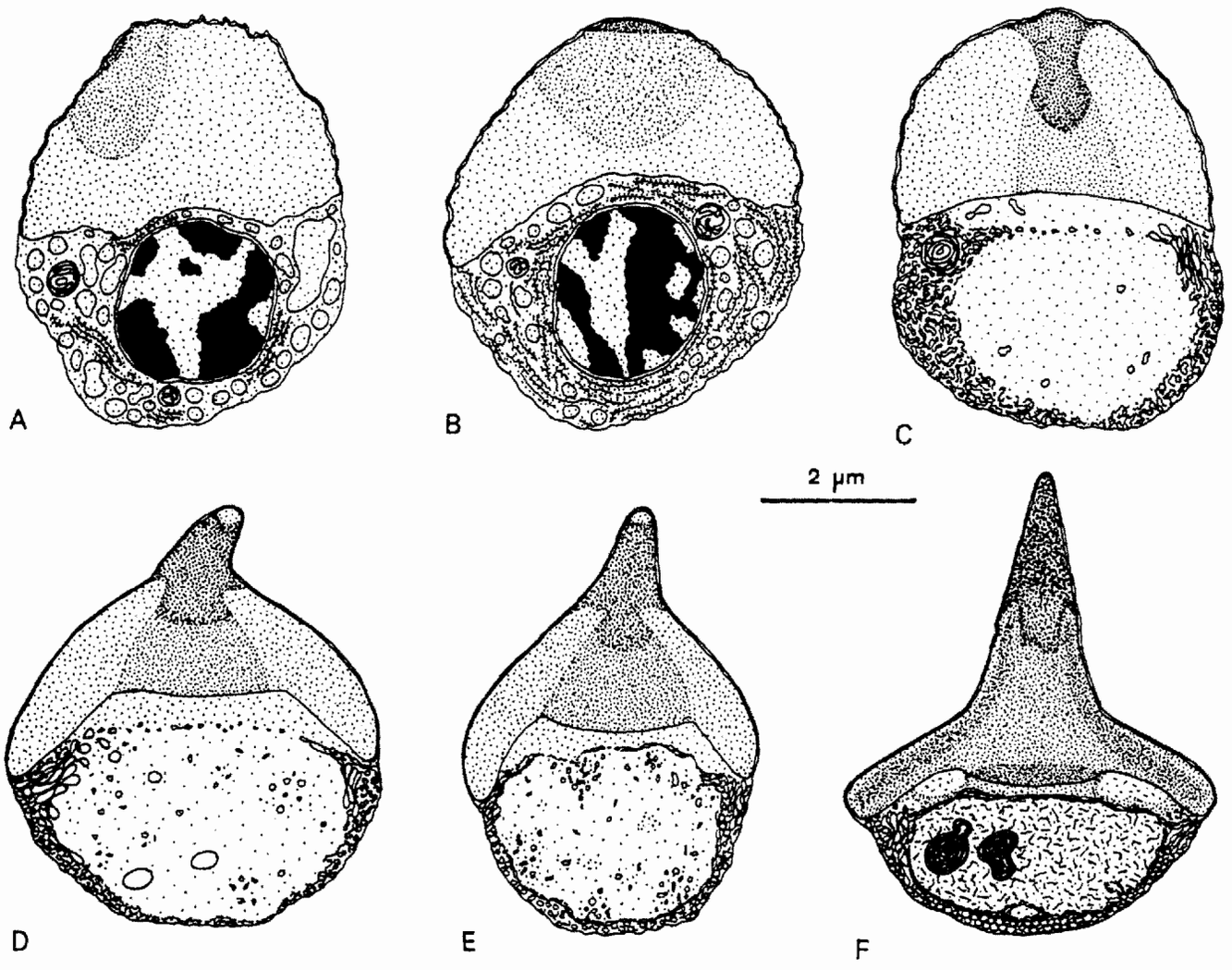

Fig. 1 *Parapenaeus longirostris*. Schematic drawing summarizing spermiogenic process. (A), (B) Early spermatids; (C) mid spermatid; (D), (E) late spermatids; (F) spermatozoon

Within the proacrosomal vesicle, the granule has formed a cone-like structure, the base of which settles on the posterior membrane whereas the tip extends into a short apical protuberance (Fig. 3C). Internally, this material maintains the two distinct regions described in the granule of early spermatids. While the basal portion is homogeneous, the anterior one is granulated and more dense. However, a small area is occupied by an electron-lucent substance just at the tip of the apical protuberance (Fig. 3F). The three tightly apposed membranes described for the preceding stage are still distinguishable at the anterior surface of the cell (Fig. 3F). The base of the proacrosomal vesicle is slightly concave, which contributes to creating a distinct subacrosomal region. The subacrosomal material is continuous with the chromatin (Fig. 3B–E).

Late spermatids (Figs. 1D, E; 4)

Transition from mid to late spermatids is mainly marked by modifications in the acrosomal volume and morphology (Fig. 1D, E). The spermatids at this stage become slightly more depressed anterio-posteriorly; their length decreases to ~5.6 µm including the elongating apical protuberance, whereas their width remains at ~4.6 µm. The membrane inclusions derived from the perinuclear cytoplasm, including some mitochondria, aggregate into a thin peripheral band bordering the slightly ovoid nuclear region, which becomes partly separated from a distinct subacrosomal region (Fig. 4A–C). Small fragments of membrane penetrate from the surrounding cytoplasmic band into the nucleoplasm (Fig. 4B–D), which remains largely uncondensed. Regularly sized, small vesicles occur at the posterior perinuclear cytoplasm (Fig. 4E). In advanced spermatids at this stage, the nuclear region is outlined by a dense thin band formed by accumulated membranes (Fig. 4C, E).

The proacrosomal vesicle becomes crescent-shaped, with an apical protuberance which tends to elongate progressively (Fig. 4A–C, F, G). The modified granule retains the same appearance as in the preceding stage, the dense apical portion being granular except, as seen for mid spermatids, at the very tip of the protuberance where a more homogeneous, electron-lucent material occurs (Fig. 4F, G). A dense coat covers the plasma membrane at the level

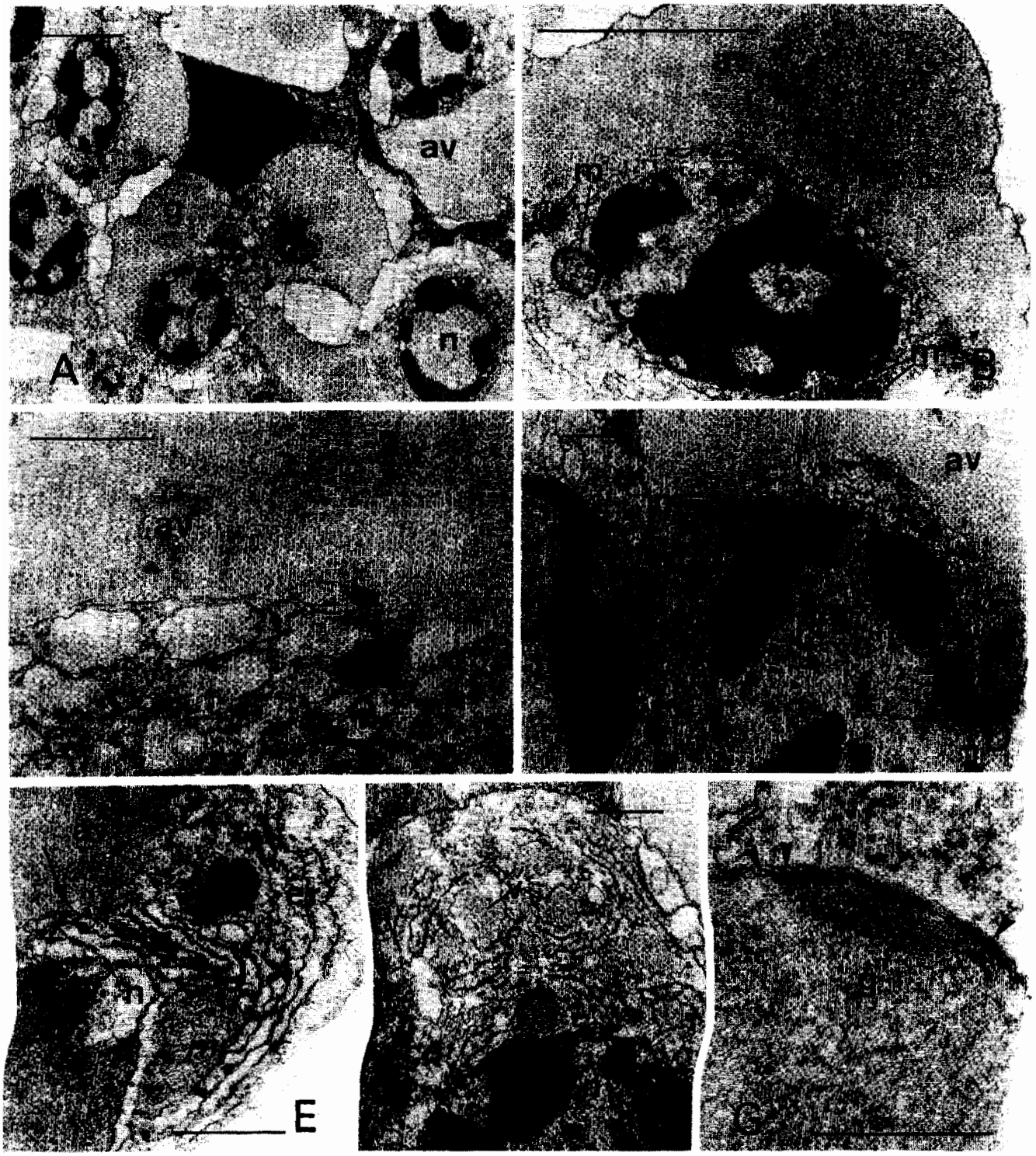

Fig. 2 *Parapenaeus longirostris.* Ultrathin sections of early spermatids. (A) Panoramic view of several spermatids in lumen of a seminiferous tubule. (B) Early spermatid showing a conspicuous granule and a few mitochondria. (C) Detail of large proacrosomal vesicle and adjacent small vesicles, one of which appears to coalesce with it (arrowed). (D) Nucleus and perinuclear region of a spermatid containing concentric arrays of endoplasmic reticulum. (E) Parallel membrane accumulations of endoplasmic reticulum closely associated with proacrosomal vesicle (arrows) and nucleus. (F) Portion of a spermatid displaying cytoplasmic vesicles and centriole. (G) Anterior surface of proacrosomal vesicle; apical condensation (asterisk) is overlaid by a triple membrane (arrowheads). av: proacrosomal vesicle; c: cytoplasm; ce: centriole; er: endoplasmic reticulum; g: granule; m: mitochondria; n: nucleus; v: cytoplasmic vesicles. Scale bars = (A), (B): 2 μm; (C)–(G): 500 nm

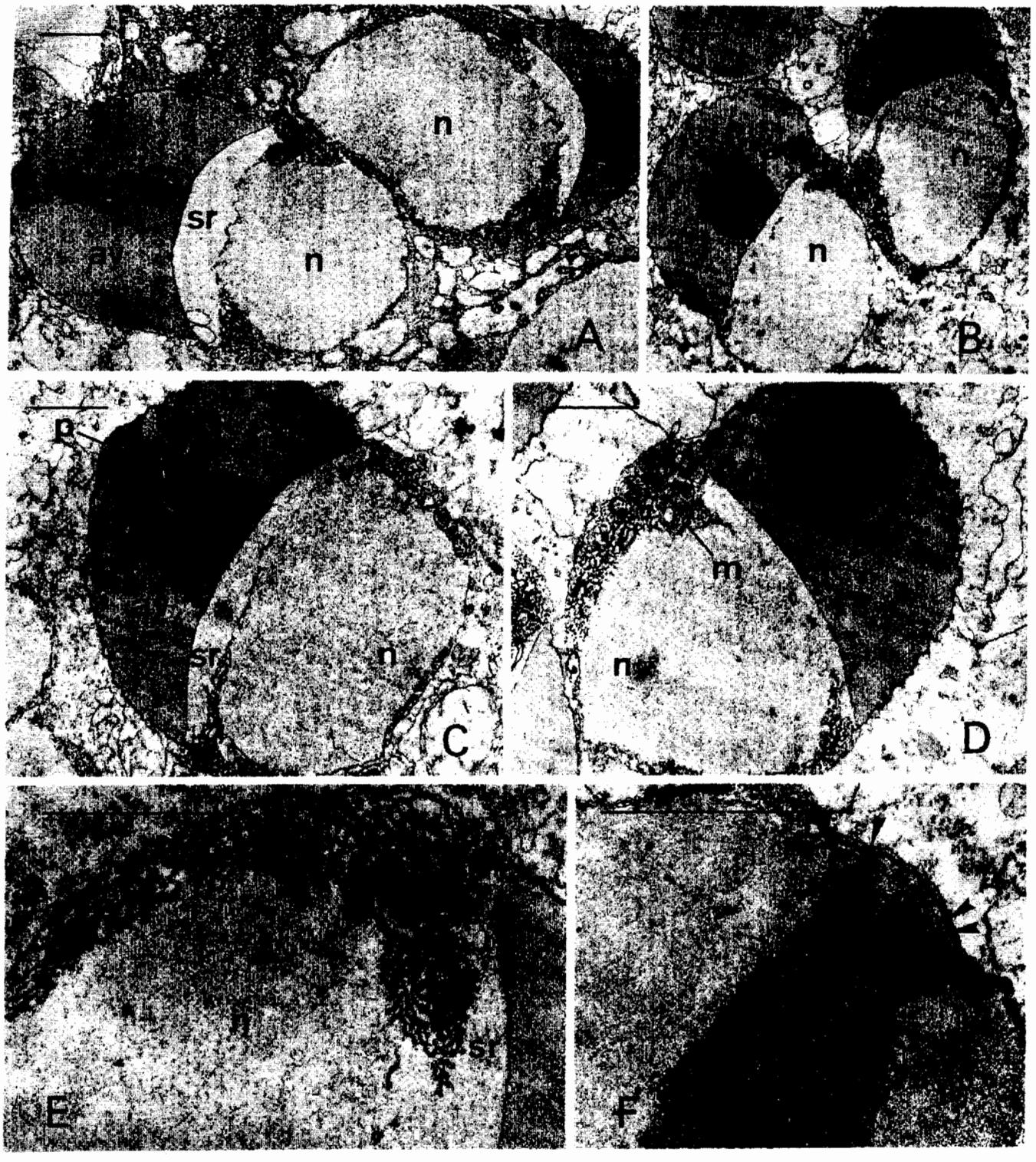

Fig. 3 *Parapenaeus longirostris*. Transmission electron micrographs of mid spermatids. (A), (B) Mid spermatids sectioned at various angles to show different perspectives of acrosomal vesicle and modified granule. (C), (D) Longitudinal sections of mid spermatids showing uncondensed, non-membrane-bound chromatin, dome-shaped proacrosomal vesicle, and perinuclear cytoplasm. (E) Detail of membrane-rich perinuclear cytoplasm. (F) Anterior portion of pro-acrosomal vesicle, showing triple membrane (arrowheads) and granulated structure of apical region of the modified granule; at tip of apical protuberance, a small area of electron-lucent substance (asterisk) is visible. av: proacrosomal vesicle; c: cytoplasm; g: granule; m: mitochondrion; n: nucleus; p: apical protuberance; sr: subacrosomal region. Scale bars = 1 µm

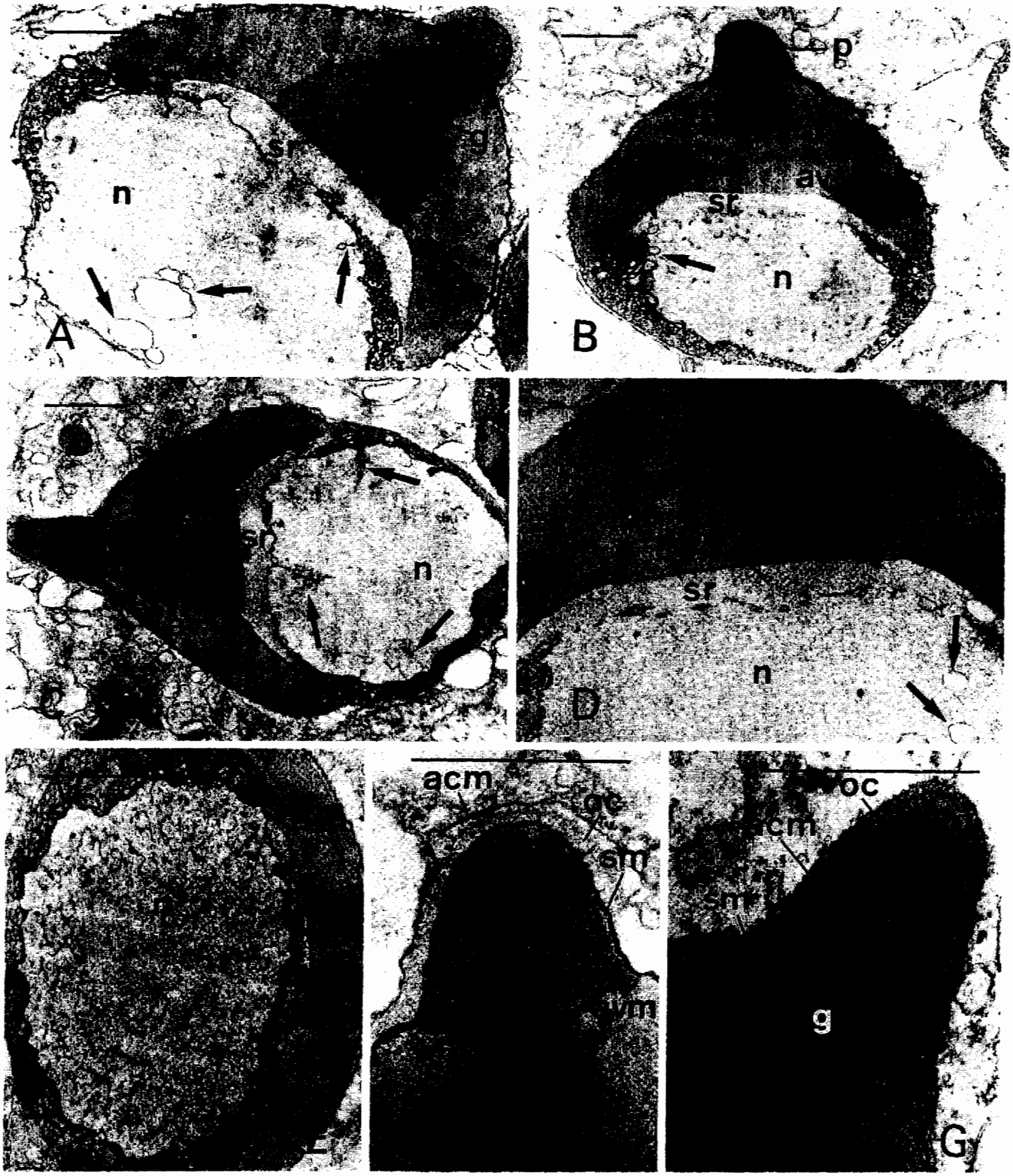

Fig. 4 *Parapenaeus longirostris.* Ultrathin sections of late spermatids. (A)–(C) Late spermatids at three successive stages of differentiation. (D) Enlargement of subacrosomal region and adjacent zones including part of proacrosomal vesicle, non-membrane-bound nucleoplasm and perinuclear cytoplasm. (E) Oblique section showing acrosomal vesicle, nuclear region bordered by dense membrane layer and perinuclear cytoplasm with small regular vesicles. (F), (G) Detail of apical protuberances in stages corresponding to those showed in (B) and (C), respectively; a dense coat is visible between plasma membranes of the spermatid and accessory cell. acm: accessory cell plasma membrane; av: proacrosomal vesicle; avm: membrane of proacrosomal vesicle; c: cytoplasm; g: modified granule; n: nucleus; oc: outer coat of protuberance; p: apical protuberance; sm: spermatid plasma membrane; sr: subacrosomal region; v: cytoplasmic vesicles; arrows: incorporation of cytoplasmic membrane vesicles into nucleoplasm; asterisks: electron-lucent substance at tip of apical protuberance. Scale bars = 1 μm

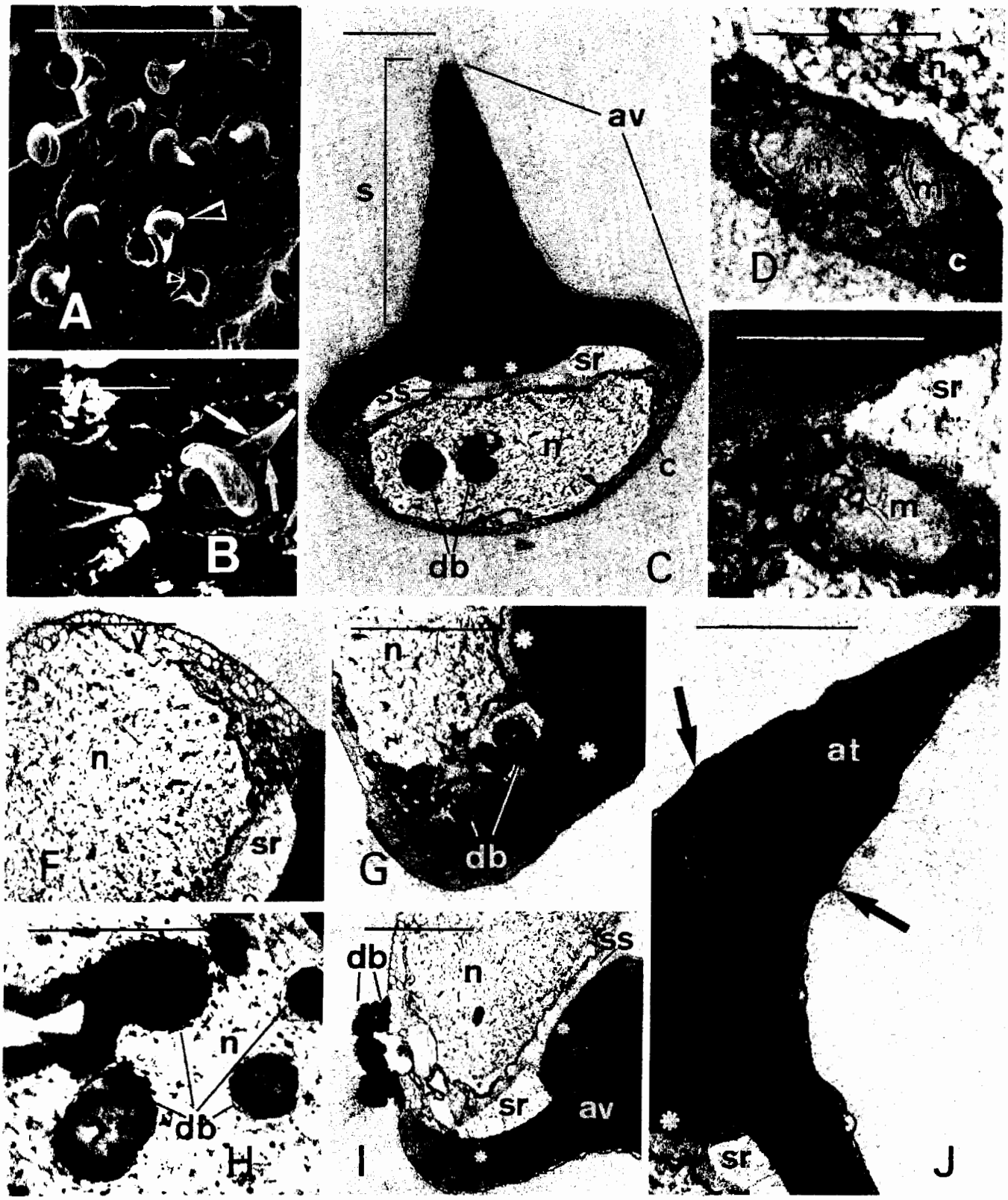

Fig. 5 *Parapenaeus longirostris.* Mature spermatozoa. (A), (B) Scanning electron micrographs of sperm embedded in gelatinous matrix in the ampulla; (C)–(J) transmission electron micrographs. (C) Longitudinal section of a spermatozoon; (D)–(I) details of different portions of the sperm, including perinuclear cytoplasm, acrosomal cap, subacrosomal and nuclear regions; (J) spike. ac: acrosomal cap; at: acrosomal tip, derived from anterior condensation of granule; av: acrosomal vesicle; c: perinuclear cytoplasm; db: dense bodies derived from membranes; m: mitochondria; n: nucleus; s: spike; sr: subacrosomal region; ss: subacrosomal substance; arrows in (B) and (J): transition from tip to base of spike; large arrowhead in (A): sperm cell; small arrowhead in (A): impression left in gelatinous matrix after removing sperm cell; asterisks: denser zones in acrosomal vesicle content. Scale bars = (A): 20 µm; (B): 5 µm; (C), (F), (G), (I), (J): 1 µm; (D), (E), (H): 500 nm

of the protuberance (Fig. 4F, G), sometimes extending beyond its base (Fig. 4G). The posterior membrane of the proacrosomal vesicle forms a concavity that is flat at the center, at the point where it meets the base of the modified granule (Fig. 4A, C).

Spermatozoa (Figs. 1F; 5)

Mature spermatozoa from the vasa deferentia show the typical tack-shaped morphology (Fig. 5A, B), with approximate dimensions of 5.5 µm total length by 4.2 µm width. The ~3 µm-long prolongation of the sperm (= spike) is constituted by a tapering extension of the acrosome (Fig. 5C). The main body of the sperm cell includes the basal part of the acrosomal vesicle (= acrosomal cap) along with the ovoidal nuclear region and the perinuclear cytoplasm (Figs. 1F; 5C). The chromatin assumes the usual fibrillar pattern of the decapod sperm. In the perinuclear cytoplasm, a conspicuous dense layer, derived from membrane systems, outlines the profile of the nuclear region, although a nuclear envelope proper is absent (Fig. 5C, D, F). The reduced perinuclear cytoplasm contains small regular vesicles that are especially abundant around the posterior nuclear region (Fig. 5F), and membrane accumulations that may include recognizable mitochondrial derivatives (Fig. 5D, E). Electron-dense bodies, originated from membranes in the spermatid cytoplasm, may be shed into the nuclear region, where they become surrounded by the filamentous chromatin strands (Fig. 5C, H), or lodged in the subacrosomal region (Fig. 5G). There is also the possibility that such waste-membrane remnants are shed outside at very late spermiogenesis (Fig. 5I), probably during passage through the collecting tubules of the testicles or vasa deferentia.

The acrosomal complex comprises the acrosomal vesicle (consisting of the spike and acrosomal cap, both derived from the proacrosomal vesicle), and the subacrosomal region. The ensemble of acrosomal cap and subacrosomal region would correspond to what Shigekawa and Clark (1986) termed the cap region. The outermost surface of the acrosomal vesicle is bound by a double membrane consisting of the plasma membrane and the membrane of the acrosomal vesicle proper. The spike is usually straight, although it may appear bent, most probably because of packaging of the sperm in the ampulla (Fig. 5A, J). As the sperm become detached from the accessory cells, the outer dense coat is lost from the leading edge of the acrosome. Internally, the tip of the spike is granular, retaining the inner arrangement of the apical portion of the granule, from which it derives (Fig. 5C, J). Behind the granular tip, the acrosomal content is homogeneous, although in certain sections of the acrosomal vesicle a more electron-dense material is visible at the base and at both sides, extending laterally throughout the cap (Fig. 5G, I, J). The transition from the tip to the base of the spike is clearly marked externally (Fig. 5B, J). The inner surface of the acrosomal cap is concave, exhibiting a central swelling coincident with the primitive base of the granule (Fig. 5C, G, I, J). At this level, the posterior membrane of the acrosomal vesicle is attached to the nuclear region by a moderately electron-dense subacrosomal substance (Fig. 5C, I). The rest of the subacrosomal region is fairly electron-lucent and filled with a loose filamentous or flocculent material (Fig. 5C, F, I, J).

Discussion and conclusions

A comparative examination of the relevant literature reveals that dendrobranchiate and reptant early spermatids share a similar ultrastructural arrangement and general morphology. The main components of these cells are the nucleus and a voluminous proacrosomal vesicle which occupy both cell poles (Pochon-Masson 1968a, Langreth 1969, Reger 1970, Haley 1986, McKnight and Hinsch 1986, Shigekawa and Clark 1986, Medina and Rodríguez 1992a, b), whereas the cytoplasmic mass is distributed around the nucleus (dendrobranchiates) or confined to a space adjacent to the nucleus and proacrosomal vesicle (reptants). From this stage, divergent evolution of sperm components, mainly affecting acrosomal structures, gives rise to two distinct sperm types. However, a distinctive key characteristic of caridean spermatids from the onset of spermiogenesis is the absence of an outstanding proacrosomal vesicle, and hence of a clear bicompartmentalization within the cell.

Differentiation of the main sperm components of *Parapenaeus longirostris* is comparatively discussed in detail below, concluding with an overview of decapod spermiogenesis and sperm structure.

Nucleus

The chromatin undergoes two morphologic changes during spermiogenesis in *Parapenaeus longirostris*. It forms large heterochromatic areas in early spermatids, then becomes uncondensed, constituting a finely granular nucleoplasm, and finally assumes the characteristic fibrillar pattern of the decapod spermatozoa at maturity. The initially continuous nuclear envelope breaks up in mid spermatids, thus allowing for free nucleocytoplasmic exchange and leading to a non-membrane-bound sperm nucleus. Rupture of the nuclear envelope and chromatin decondensation are also significantly concomitant events in spermatids of the other dendrobranchiate shrimp so far investigated, *Sicyonia ingentis* (Shigekawa and Clark 1986). These two features are generally shared by reptant spermatids, in which the chromatin evolves toward an uncondensed state and becomes incompletely bound by the nuclear envelope (Jamieson 1987, 1991b; Felgenhauer and Abele 1991). An odd exception to this general pattern in reptants is represented by the spiny lobsters *Panulirus argus* and *P. guttatus* which retains a continuous nuclear envelope in the mature spermatozoon (Talbot and Summers 1978). Unlike other decapods, the carideans maintain an intact nuclear

envelope throughout spermiogenesis (Arsenault et al. 1979; Arsenault 1984, Papathanassiou and King 1984).

In *Sicyonia ingentis*, blebbing of the outer nuclear membrane has been regarded as being directly involved in proacrosomal vesicle formation (Shigekawa and Clark 1986). A similar mechanism has also been reported to contribute to the cytoplasmic membrane system in reptant spermatids (Kaye et al. 1961; Moses 1961; Pochon-Masson 1968 a). Instead of blebbing, extensions of the outer nuclear membrane into the acrosomal zone of the spermatid have been beautifully illustrated by Arsenault (1984) in the caridean *Crangon septemspinosa*, and recently described by Demestre et al. (1993) in the dendrobranchiate *Aristeus antennatus*. In contrast to the above examples, no extraordinary activity of the nuclear envelope has been detected morphologically in *Parapenaeus longirostris*. Therefore, a direct origin of the proacrosomal vesicle from the nuclear envelope can be ruled out in this species.

A most notable feature in *Parapenaeus longirostris* sperm is the presence of intranuclear lipid-like droplets derived from membrane remnants, which incorporate into the chromatin fibre network from the surrounding cytoplasm. Such inclusions markedly resemble in appearance and location the intranuclear pigment granules described for cultured *Penaeus vannamei* by Dougherty and Dougherty (1989). Degenerating lamellar bodies are also shed into the nucleoplasm in late spermatids of *Sicyonia ingentis* (Shigekawa and Clark 1986).

Cytoplasm

Because of its special interest, differentiation of the acrosomal vesicle will be discussed separately in the next subsection.

Caridean and dendrobranchiate sperm share the feature of possessing a cytoplasmic band that surrounds the nuclear region posteriorly, whereas in reptant spermatozoa the cytoplasmic mass lies between the acrosomal vesicle and the nucleus, the free edge of which is invested directly by the plasma membrane (for reviews see Felgenhauer and Abele 1991 and Jamieson 1991 b). A plausible explanation for the existence of these two patterns would be that the relative position of the acrosome would determine the placement of the cytoplasmic mass. If this were the case, the spiked condition (acrosomal structure capping the nucleus) would result in a perinuclear cytoplasm, whereas the spikeless condition (acrosomal vesicle cupped by the nucleus) would lead to a subacrosomal location of the cytoplasmic mass.

The perinuclear cytoplasm of early spermatids from *Parapenaeus longirostris* is first vesiculated, and subsequently accumulates concentric arrays of endoplasmic reticulum membranes. The vesicles are thought to derive from endoplasmic reticulum cisternae; some could coalesce with the large proacrosomal vesicle, contributing flocculent material to its contents. Membrane complexes located close to both the nucleus and the developing acrosomal structure are universally present in spermatids of decapod crustaceans (Moses 1960, 1961; Yasuzumi 1960; Kaye et al. 1961; Anderson and Ellis 1967; Pochon-Masson 1968 a; Langreth 1969; Reger 1970; Pearson and Walker 1975; Arsenault et al. 1979; Koehler 1979; Arsenault 1984; Papathanassiou and King 1984; Haley 1986; McKnight and Hinsch 1986; Shigekawa and Clark 1986; Medina and Rodríguez 1992 a). In brachyurans, degenerating membranes from the narrow band of subacrosomal cytoplasm are incorporated into the perforatorium (Reger 1970;, Medina and Rodríguez 1992 a). Similarly, in *P. longirostris* part of the degenerative membrane bodies formed in the perinuclear cytoplasm migrate to the subacrosomal region.

After reduction in volume, the perinuclear cytoplasm forms a thin band around the nuclear region, where mitochondrial remnants and membrane accumulations predominate. As centrioles degenerate at early spermiogenesis, they do not represent constitutive elements of the mature sperm of *Parapenaeus longirostris*. Microtubules were not observed at any time throughout spermiogenesis. Centrioles appear to be generally absent from mature spermatozoa of the Dendrobranchiata (Clark et al. 1973; Kleve et al. 1980; Shigekawa and Clark 1986; Felgenhauer et al. 1988; Dougherty and Dougherty 1989), although in caridean sperm single or double centrioles are commonly encountered (Pochon-Masson 1968 b, 1969; Arsenault et al. 1979; Dupré and Barros 1983; Lynn and Clark 1983 b; Papathanassiou and King 1984). Centrioles may or may not be present in reptant spermatozoa (Jamieson 1991 b).

From the mid spermatid stage onwards, a distinct subacrosomal region becomes evident. Perhaps the most remarkable difference in the sperm ultrastructure between the Sicyoniidae and Penaeidae investigated is the arrangement of the subacrosomal region, which is sophisticated in the former (Kleve et al. 1980;, Shigekawa and Clark 1986), and fairly simple in the latter (Clark et al. 1973; Felgenhauer et al. 1988; Dougherty and Dougherty 1989; Felgenhauer and Abele 1991; present study).

(Pro)acrosomal vesicle

A large proacrosomal vesicle is clearly visible from early spermiogenic stages in *Parapenaeus longirostris*. Within its flocculent matrix the granule, a dense granulated core, soon becomes apparent under the anterior membrane of the vesicle. In the numerous cytoplasmic vesicles assumed to contribute material to the proacrosomal vesicle, no differentiation of the content has been found. In contrast, Shigekawa and Clark (1986) reported that in *Sicyonia ingentis* the acrosomal vesicle derives from coalescence of several membrane-bound areas, each containing a dense spherical body. All individual dense bodies aggregate together to form a single anterior granule. The ultrastructural appearance of such dense bodies is fairly similar between both penaeid species at the onset of acrosome formation, but their further evolution shows a slight difference. In *Parapenaeus longirostris*, as the granule forms in early spermatids it immediately differentiates an anterior dense zone

subjacent to the anterior membrane of the proacrosomal vesicle (equivalent to what Shigekawa and Clark called the "spike primordia"), whereas the bulk of the granule extends to reach the posterior membrane. In *S. ingentis* spermatids, once the anterior granule has gained its definitive position adjacent to the posterior acrosomal vesicle membrane, the spike primordia differentiates independently under the anterior vacuolar membrane, so that the granule and spike primordia are separate entities. Unexpectedly a priori, this situation much resembles that observed in early spermiogenesis of the brachyuran *Uca tangeri* (Medina and Rodríguez 1992 a). The two condensations of the early proacrosomal vesicle (from which, in this crab, the operculum and thickened ring later originate) are similar in both appearance and location to the primordia spike and granule of *S. ingentis*, respectively. Similarly, the proacrosomal vesicle in many other reptants differentiates distinct electron-dense regions prior to formation of the definitive acrosomal structure (Yasuzumi et al. 1960; Moses 1961; Anderson and Ellis 1967; Pochon-Masson 1968 a; Langreth 1969; Reger 1970; Haley 1986; McKnight and Hinsch 1986; Medina and Rodríguez 1992 a).

A remarkably distinctive aspect of the spermatozoa of carideans in comparison with the other decapods is that the acrosomal structures (spike and cap) are non-membrane-bound (Pochon-Masson 1968 b, 1969; Dupré and Barros 1983; Lynn and Clark 1983 b; Felgenhauer et al. 1988; Pérez et al. 1991), since they do not derive from any membrane-bound precursor. Instead, the spike forms from sheets of material originated between membrane stacks associated with the nuclear envelope (Arsenault et al. 1979; Koehler 1979; Arsenault 1984; Papathanassiou and King 1984; Felgenhauer et al. 1988). These observations strongly suggest that the caridean sperm spike is not homologous to the dendrobranchiate sperm spike.

General conclusions

The spermatozoon of *Parapenaeus longirostris* is of the unistellate sperm type, since it possesses a single appendage (spike) of acrosomal nature. The mature sperm of the aristeid *Aristeus antennatus* appear to be unique among the Dendrobranchiata because, as illustrated by Demestre and Fortuño (1992), it lacks a distinct spike and, although nuclear arms are absent, it resembles a "reptant sperm" rather than a "natantian sperm". In fact, the spike-less acrosomal cap is shown to be spherical and appears to be partly surrounded by the nucleus at its base, as typically occurs in reptant spermatozoa (Chevaillier and Maillet 1965; Langreth 1965; Brown 1966; Pochon-Masson 1968 a, b; Hinsch 1969, 1973, 1980, 1986, 1988, 1991; Talbot and Summers 1978; Talbot and Chanmanon 1980 a; López-Camps et al. 1981; Dudenhausen and Talbot 1982; McKnight and Hinsch 1986; Jamieson 1987, 1989 a, b, 1990, 1991 b; Jamieson and Tudge 1990; Felgenhauer and Abele 1991; Tudge and Jamieson 1991; Tudge 1992). This may create considerable confusion when applying criteria of sperm morphology to phylogenetic approaches. Indeed, further research on the evolution of the sperm components throughout spermiogenesis in *A. antennatus* would be of much assistance in the establishment of possible phylogenetic links between this and other species of decapods.

The similarity observed in the general morphology of the sperm from *Aristeus antennatus* and reptant decapods may be related to a certain parallelism in the spermiogenic process between dendrobranchiates and reptants, especially concerning acrosomal differentiation. In both cases, the large proacrosomal vesicle initially shows two distinct condensations, which then further evolve in different ways in each group. Thus, the anteriormost condensation originates the operculum in the brachyuran *Uca tangeri* (Medina and Rodríguez 1992 a), whereas it represents the anlage of the spike in dendrobranchiates (Shigekawa and Clark 1986, and present study). On the other hand, the posterior condensation in *U. tangeri*, which gives rise to the thickened ring of the acrosomal vesicle (Medina and Rodríguez 1992 a), could be similar to the anterior granule of Shigekawa and Clark (herein referred to as the granule), which eventually comes into contact with the posterior membrane of the acrosomal vesicle.

In my view, classification of the varying decapod sperm morphologies into two phylogenetic categories (natantian and reptantian sperm types) merely on the basis of a superficial consideration of the mature sperm structure may be an unreliable simplification. An illustrative example of this assessment is provided by the above-mentioned dendrobranchiate *Aristeus antennatus*, whose spermatozoon resembles reptantian rather than natantian sperm. In the same connection, there would be no sound justification for grouping the sperm of carideans and dendrobranchiates (the former Natantia) within the same phylogenetic category, despite their unquestionable morphological resemblance. Burkenroad (1981) was aware of this when he claimed that the supposed uniformity of spermatozoa among "Natantia" does not in fact exist. Throughout the process of spermiogenesis, many ultrastructrual differences between carideans and dendrobranchiates, mainly concerning differentiation of the acrosomal structure, reveal that homologies can barely be traced. Furthermore, apart from differences in the genesis of the acrosome between carideans and dendrobranchiates, the manner in which the acrosome functions during fertilization also differs. Thus, the acrosome reaction in carideans, extremely well illustrated by Lynn and Clark (1983 a) and Barros et al. 1986), simply consists of direct penetration of the egg vitelline envelope by the spike, a mechanism that does not involve exocytotic events. In contrast, in the dendrobranchiate *Sicyonia ingentis* a more conventional acrosome reaction takes place, consisting of exocytosis of the acrosomal vesicle followed by formation of an acrosomal filament (Yudin et al. 1979; Clark et al. 1981, 1984; Clark and Griffin 1988; Griffin et al. 1988; Griffin and Clark 1990). The acrosome reaction in reptants also proceeds in two phases, namely exocytosis of the acrosomal vesicle contents and exposure of the perforatorium (Binford 1913; Pochon-Masson 1965, 1968 a, b; Brown 1966; Hinsch 1971; Talbot and Chanmanon 1980 b; Goudeau 1982;

Talbot et al. 1991; Medina 1992; Medina and Rodríguez 1992 b). Unfortunately, ultrastructural studies on the acrosome reaction in dendrobranchiates other than *S. ingentis* have not been attempted. Therefore, generalisations on this subject should await future research.

To sum up, the above arguments suggest that the various decapod sperm morphologies be grouped into three categories instead of the two classically acknowledged categories. The unistellate *caridean sperm* have a non-membrane bound, fibrous spike. In contrast, the spiked acrosome of the unistellate *dendrobranchiate sperm* is membrane-bound, as is the spike-less acrosomal vesicle of the multistellate *reptantian sperm*. Although very different in gross morphology, the two latter sperm types share a number of ultrastructural features regarding spermiogenesis and acrosome reaction. Within the Decapoda, despite an apparent superficial similarity to the dendrobranchiate sperm, the caridean sperm type seems to represent a rather independent evolutive line in terms of sperm differentiation and function. These data are in apparent disagreement with the phylogenetic dendrograms presented by Burkenroad (1981) and more recently by Kim and Abele (1990), whose trees showed the first branch leading to the Dendrobranchiata, followed by a node leading to the remaining decapods. Accordingly, the carideans would be closer to reptants than dendrobranchiates. My results do not absolutely disagree with this view. They suggest that the original spermatozoon possessed a membrane-bound acrosome, which has been retained in dendrobranchiates and reptants, but not in carideans.

Acknowledgements Thanks are due to I. Sobrino and I. González for supplying the specimens, to Dr. A. Rodríguez and A. Santos for invaluable help during preparation of the manuscript, and to O. Aliseda, J. González and J. M. Geraldía for skillful technical assistance. Electron microscope observations and micrographs were made in the Servicio de Microscopía Electrónica de la Universidad de Cádiz.

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