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A dendrobranchiate, *Peisos petrunkevitchi* (Decapoda, Sergestidae), with reptant-like sperm: a spermiocladistic assessment

Marcelo A. Scelzo[1] and Antonio Medina[2]

[1]Departamento de Ciencias Marinas, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, CONICET, Funes 3350 (7600) Mar del Plata, Argentina; [2]Departamento de Biología, Facultad de Ciencias del Mar, Universidad de Cádiz, Avda. República Saharaui s/n, E-11510 Puerto Real, Cádiz, Spain

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Abstract

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The spermatozoon of *Peisos petrunkevitchi* differs significantly from those of any of the investigated dendrobranchiates in the anterior fusiform acrosome, lacking a spike, and embedded in (instead of capping) the nuclear region. In contrast, the position of the acrosome and the internal arrangement of its contents, as well as the apomorphic presence of a pair of centrioles (absent in all known dendrobranchiate spermatozoa) at the base of the acrosomal perforatorium, indicate a close affinity between this sperm plan and that found in reptants, especially anomurans and brachyurans. Based on the present and previous observations on decapod spermatozoal ultrastructure, we review the phylogeny of dendrobranchiate spermatozoa in the perspective of most recent phylogenetic analyses of malacostracan crustaceans.

Antonio Medina, Departamento de Biología, Facultad de Ciencias del Mar, Universidad de Cádiz, Avenida República Saharaui s/n, E-11510 Puerto Real, Cádiz, Spain. E-mail: antonio.medina@uca.es

Introduction

The application of electron microscopy to studies on comparative spermatology has proven useful in identifying phylogenetic relationships that escape somatic analyses in many animal phyla (Jamieson 1987, 1989, 1991a; Jamieson *et al.* 1995a). In particular, the crustacean spermatozoon can adopt a wide array of different forms, and the spermatozoal ultrastructure has become a useful tool in systematics (Felgenhauer and Abele 1991; Jamieson 1991b; Jamieson *et al.* 1995b; Medina 1995a,b; Tudge 1995; Jamieson and Tudge 2000; Martin and Davis 2001). The order Decapoda is an extensive and diverse taxon where internal relationships are still controversial and subject to investigation and revision. Sperm characters alone, or combined with nonspermatozoal characters, have been used in cladistic parsimony analyses of the Brachyura (Jamieson 1994; Jamieson *et al.* 1995b), and more recently, at a higher taxonomic level in the class Malacostraca (Richter and Scholtz 2001). It thus appears that the availability of an extensive data base on decapod spermatozoal ultrastructure would help in further phylogenetic and taxonomic research. Although the spermatozoal ultrastructure of pleocyemate crustaceans is well documented, comparable information is not available on dendrobranchiates, where most contributions deal with penaeids. Most penaeoids (namely penaeids, sicyoniids and solenocerids) share a sperm type characterized by an anterior acrosome consisting of a basal cap and a pointed, membrane-bound spike that projects forward (Kleve *et al.* 1980; Dougherty and Dougherty 1989; Medina 1994, 1995a; Medina *et al.* 1994a,b; Jamieson and Tudge 2000; Scelzo and Medina 2003). Unlike this single widespread dendrobranchiate sperm pattern, two different morphologies have been found in aristeids that are, in turn, discordant from the spermatozoal ultrastructure of the above penaeoid families (Medina 1995b). At present, the spermatozoal ultrastructure of the superfamily Sergestoidea is very poorly known because only one species, *Sergestes arcticus* Kroyer 1855, has been investigated (Medina 1995a). The anacrosomal spermatozoon of *S. arcticus* consists of a central, nonmembrane-bound nuclear region surrounded by a thin cytoplasmic band, and resembles in fine structure the nuclear region found in the spermatozoa of some euphausiids and aristeids, but markedly differs from the typical unistellate spermatozoa (endowed with a membrane-bound spike) of dendrobranchiates that is present in penaeids, solenocerids and sicyoniids.

Sergestid shrimps are pelagic crustaceans that represent an important food resource for other marine animals such as fish, whales and other crustaceans (Omori *et al.* 1972). The sergestid *Peisos petrunkevitchi* Burkenroad, 1945 is a typical neritic species, monospecific and endemic to warm-temperate coastal waters in the south-western Atlantic Ocean. Its distribution extends from south of Brazil (32°S), through Uruguay to Playa Unión, Chubut, Argentina (44°S) (Boschi *et al.* 1992). It is a filter-feeding species, consuming mainly detritus and phytoplankton, whose reproductive cycle occurs in coastal waters (Mallo and Boschi 1982). The presence, distribution and abundance of *P. petrunkevitchi* larvae in plankton samples were studied by Boschi and Scelzo (1969a,b), and the larval development was described from laboratory-reared specimens by Mallo (1986). With the exception of Burkenroad's descriptions of the reproductive system and secondary sexual characteristics (Burkenroad 1945), major aspects of the male reproductive biology of the species are almost unknown. In the present paper we describe the particular spermatozoal ultrastructure of the sergestid *P. petrunkevitchi* and review the evolution of dendrobranchiate spermatozoa in the context of recent phylogenetic analyses made in decapods.

Materials and Methods

Adult specimens of *Peisos petrunkevitchi* were trawled from September 2001 to February 2002 off Mar del Plata, Buenos Aires, Argentina, at depths between 4 and 10 m, and transported alive to the laboratory. After dissection of the male reproductive system, small fragments of the testes and vasa deferentia were fixed for 3–4 h in 3% glutaraldehyde in 0.2 M phosphate-buffered saline (PBS; pH 7.2) containing 1–3% sucrose. Afterwards, the samples were washed in PBS (three rinses of 15 min each), post-fixed for 80 min in 1% osmium tetroxide, rinsed in PBS, and dehydrated through an ascending series of acetone. For scanning electron microscopy (SEM), fragments of the vasa deferentia were then critical-point-dried, sputter-coated with gold, and examined in a Jeol JSM 820 electron microscope. For transmission electron microscopy (TEM), following dehydration in acetones, samples of the reproductive system were infiltrated and embedded in Spurr's epoxy resin. Thin sections (80 nm) were mounted on copper grids, stained with uranyl acetate and lead citrate, and viewed in a Jeol JEM 1200 EX transmission electron microscope operated at 80 kV. Dimensions of whole spermatozoa were estimated from SEM micrographs, while measurements of the acrosome and other organelles were carried out on TEM micrographs. Data on sperm dimensions are expressed as means ± SD.

Results

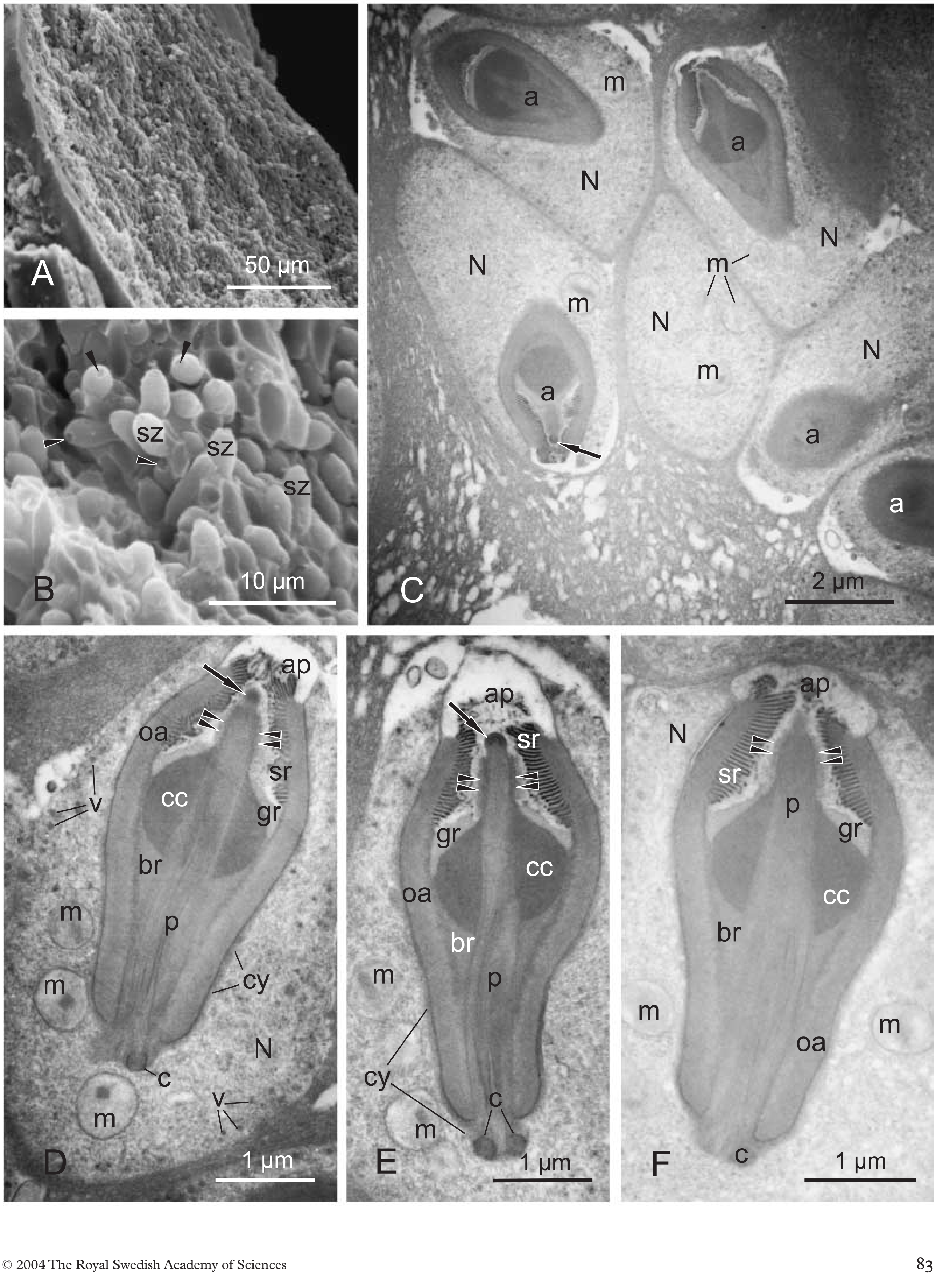
Under SEM, spermatozoa from the vasa deferentia of *Peisos petrunkevitchi* appear as ovoid or spindle-shaped cells that are embedded in a gelatinous matrix (Fig. 1A,B). The spermatozoon has a length of 6.21 ± 0.61 μm (range 5.31–7.00 μm, *n* = 10) and a maximum width of 2.37 ± 0.14 μm (range 2.13–2.50 μm, *n* = 10). The outer surface of the cell is smooth, with no apparent appendages, neither spikes nor lateral arms (Fig. 1B). A shallow circular depression is often visible at one end of the spermatozoon (Fig. 1B), which is presumed to be the pole occupied by the acrosome and here will be conventionally considered as the apical or anterior end of the sperm cell.

As seen in TEM images, the spermatozoa of *P. petrunkevitchi* are composed basically of an anterior elongate acrosome embedded in the nucleoplasm. The cytoplasm is scarce and its components appear scattered throughout the nuclear region (Figs 1C,D and 3). The inverted pear-shaped acrosome is 4.38 ± 0.31 μm long (range 3.90–4.90 μm, *n* = 9). It is widest at its anterior third (2.00 ± 0.07 μm; range 1.90–2.14 μm, *n* = 11), tapering at both ends, and the width at the posterior end is 1.16 ± 0.13 μm (range 0.97–1.38 μm, *n* = 9). These dimensions give a length to width ratio of 2.19.

The posterior membrane of the acrosomal vesicle invaginates to form an elongate perforatorium that penetrates throughout most of the length of the acrosome and terminates in a dome-shaped tip close to the anterior acrosome membrane (Figs 1D–F and 3). The columnar perforatorium is not completely straight, but describes a slightly sinuous course (Figs 1E and 3). It is somewhat wider at the base, where the perforatorial membrane is extremely folded and forms convoluted septa that are visible in both longitudinal (Figs 1D–F, 2B and 3) and transverse sections (Fig. 2F). These perforatorial membrane infoldings become less pronounced more anteriorly (Fig. 2G), and finally disappear (Figs 1D–F and 3), producing a circular profile in cross-section (Fig. 2H,I).

The highly heterogeneous contents of the acrosomal vesicle are arranged in several structurally distinct concentric regions that recall the inner organization of the acrosome of anomurans and brachyurans, from which much of the present terminology has been adopted. The most external

Fig. 1—Electron micrographs of *Peisos petrunkevitchi* spermatozoa. —**A**, **B**. SEM micrographs of spermatozoa in the vas deferens showing a circular depression at the apical or acrosomal end (arrowheads). —**C**–**F**. TEM micrographs of sagittal sections of the spermatozoon. Arrows, spherical tip of the perforatorium; double arrowheads, dense coating of the anterior segment of the perforatorium. Abbreviations: a, acrosome; ap, apical protuberance; br, basal region of the inner acrosome zone; c, centrioles; cc, central core of the inner acrosome zone; cy, cytoplasmic layer around the basolateral acrosome surface; gr, granular region of the inner acrosome zone; m, mitochondrion; N, nuclear region; oa, outer acrosome zone; p, perforatorium (note internal septa); sz, spermatozoa; sr, striped region of the inner acrosome zone; v, dense cytoplasmic vesicle.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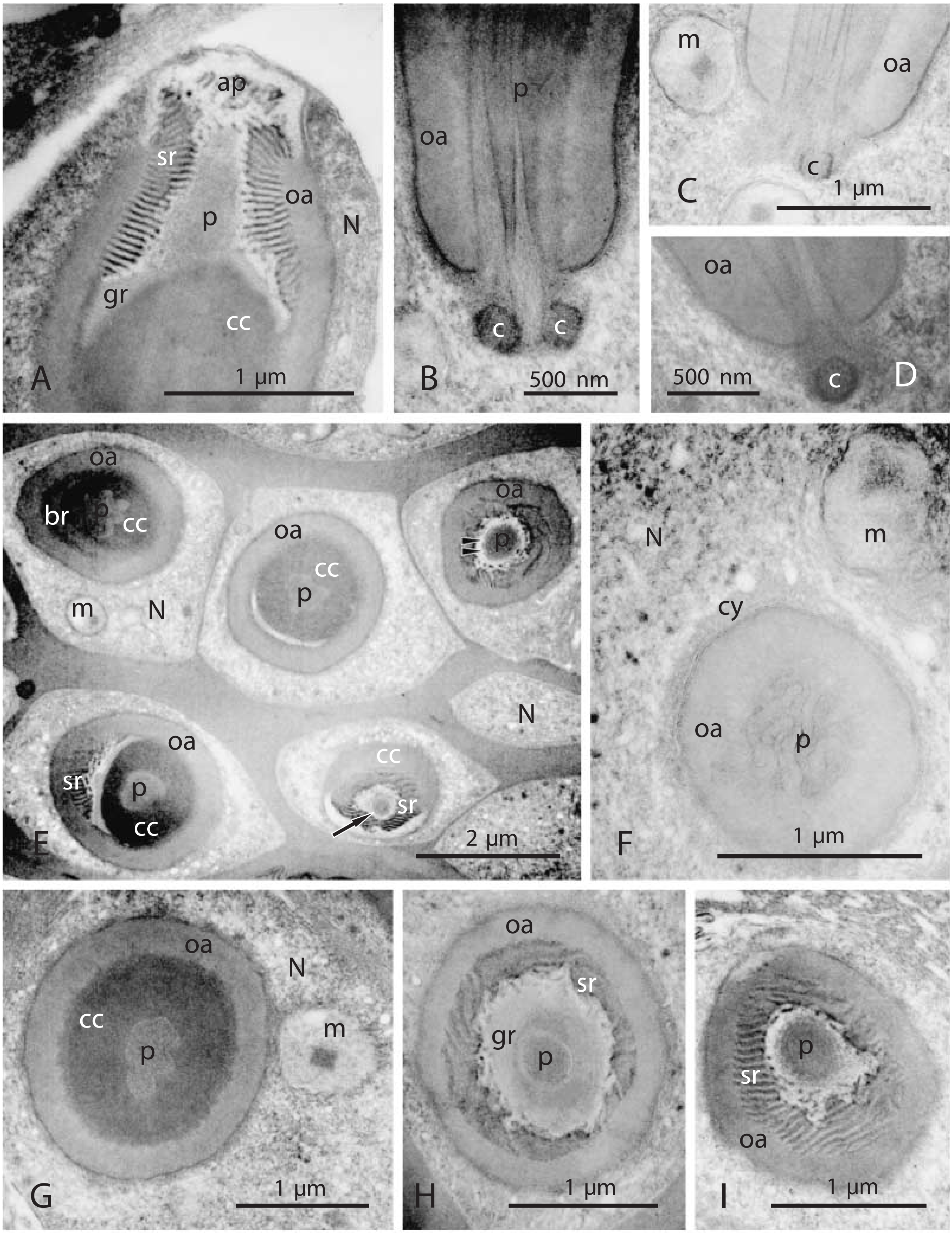
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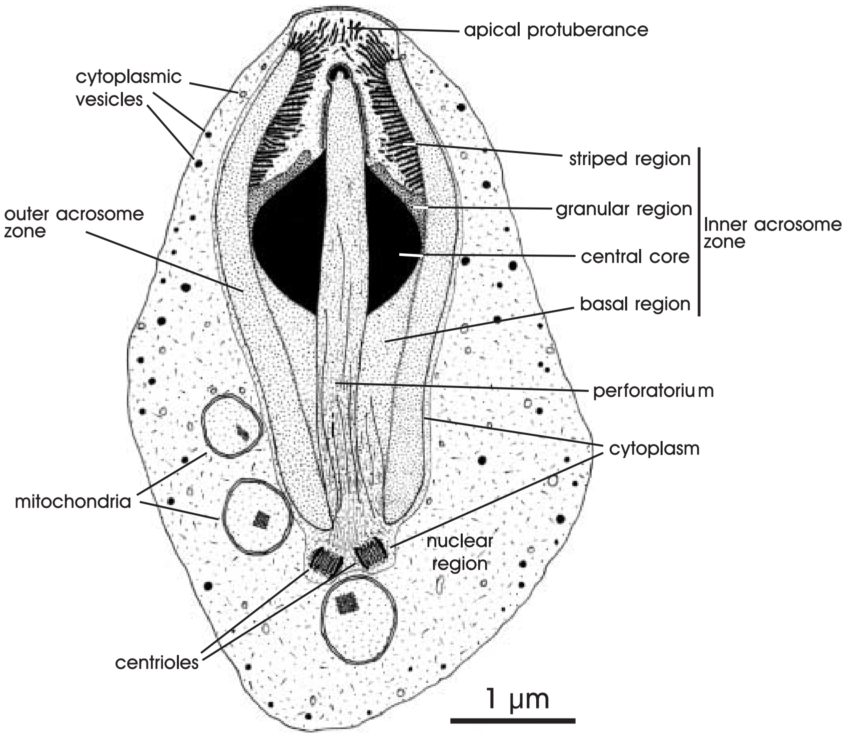

Fig. 3—Schematic drawing of the sperm of *Peisos petrunkevitchi* traced from electron micrographs of longitudinal sections.

zone of the acrosomal vesicle (outer acrosome zone) consists of a regular layer of a moderately electron-dense, homogeneous material that extends from the base to the apex of the acrosome (Figs 1D–F and 2A). The inner acrosome zone is made up of different layers that are arranged in four horizontal levels (Fig. 3). The anteriormost region (striped region) consists of a banded layer, subjacent and internal to the outer acrosome zone, which extends for the apical third of the acrosome and ends anteriorly close to the acrosome membrane (Fig. 2A). This layer shows a regular pattern in which parallel electron-dense bands, orientated towards the perforatorium, alternate with electron-lucent bands with a periodicity of about 40 nm (Figs 1D–F, 2A,I and 3). Such a regular pattern is not observed at the apex of the acrosomal vesicle, where the dense bands appear unordered. The apical surface of the acrosomal vesicle forms a slight protuberance and the acrosome membrane is overlain immediately by the plasma membrane (Fig. 2A). In no case did we find any depression or concavity at this zone as was observed in SEM images (Fig. 1B). Rather, in most sperm cells observed in TEM, a swelling (possibly artefactual) of the acrosomal membrane occurs, which appears to denote an extreme sensitivity to osmotic changes of this part of the acrosome (Fig. 1D–F). Interior to the striped layer is a narrow electron-lucent space, where a two-layered dense sheath can be observed to surround the anterior segment of the perforatorium below the spherical tip (Figs 1E and 2E). More posteriorly is the granular region of the acrosome (Figs 1D–F, 2A,H and 3), which is formed by coarse granules embedded in a more electron-lucent matrix and rests directly on the third region of the inner acrosome zone (here termed the central core). This central core consists of a conspicuous, electron-dense spherical or ovoid body (circular in cross-section) that roughly occupies the central third of the acrosome and is centrally penetrated by the perforatorium (Figs 1D–F, 2E,G and 3). The basalmost region of the inner acrosome zone is occupied by a homogeneous, finely granular content, which is similar in appearance and density to that of the outer acrosome zone, but is distinctly separated from it by a thin, electron-dense layer. This layer extends along the entire length of the outer acrosome zone and constitutes a distinct boundary on all the elements of the inner acrosome zone (Figs 1D–F, 2E and 3).

Most of the remainder of the sperm cell is occupied by the nuclear region (Figs 1C,D and 3), which is not separated

Fig. 2—**A**–**D**. Sagittal sections and —**E**–**I**. transverse sections at various levels of *Peisos petrunkevitchi* spermatozoa in TEM micrographs. Arrow, dome-shaped tip of the perforatorium; double arrowheads, dense coating of the anterior segment of the perforatorium. Abbreviations: ap, apical protuberance; br, basal region of the inner acrosome zone; c, centrioles; cc, central core of the inner acrosome zone; cy, cytoplasmic layer around the acrosome; gr, granular region of the inner acrosome zone; m, mitochondrion; N, nuclear region; oa, outer acrosome zone; p, perforatorium; sr, striped region of the inner acrosome zone.

from the cytoplasmic components by distinct membranes. This nucleoplasm consists of electron-lucent, filamentous and finely granular materials that are surrounded by the cell membrane (Figs 1D,E and 2E–G). In some regions the sperm membrane is double, which suggests that nuclear and plasma membranes join together to form the cell's outer boundary.

The cytoplasm is scattered throughout and mixed with the chromatin of the nucleoplasm. A very thin layer of cytoplasm may be recognized apposed to the basolateral acrosome surface (Figs 1D–F, 2E,G and 3). It is most conspicuous under the perforatorial opening, where a pair of centrioles is visible (Fig. 2B–D). The centrioles are perpendicular to each other and are embedded in a dense material (Figs 1D–F and 2B–D). No microtubules (with the exception of those in the centrioles) are found in either the cytoplasmic or the nuclear regions. Mitochondrial derivatives are scattered in the nucleoplasm and number between two and four in sagittal sections of the spermatozoon. They are spherical, measuring about 0.75 μm in diameter, and show a central paracrystalline body that is more electron-dense than the mitochondrial matrix (Figs 1C–F, 2C,E–G and 3). Small vesicles, both electron-lucent and electron-dense, of likely cytoplasmic origin are also found dispersed in the chromatin. The dense vesicles are apparently more frequent at the periphery of the cell (Figs 1C,D and 3). No distinct peripheral cytoplasmic band like that observed in most penaeids is recognized.

Discussion

The crustacean decapod suborder Dendrobranchiata is divided into the superfamilies Penaeoidea and Sergestoidea. Until the present study, *Sergestes arcticus* was the only representative of the superfamily Sergestoidea whose spermatozoal ultrastructure had been described. Spermatozoa of *S. arcticus* have a central nuclear region surrounded by a peripheral cytoplasmic band, and lacking an acrosome (Medina 1995a). It resembles in overall morphology those of the dendrobranchiate aristeid *Aristaeomorpha foliacea* Risso, 1827 (Medina 1995b), the euphausiids *Euphausia* sp. (Jamieson 1991b) and *Meganyctiphanes norvegica* Sars, 1857 (Medina *et al.* 1998), and even the pleocyemate stenopid *Stenopus hispidus* Olivier, 1811 (Felgenhauer and Abele 1991). Nonetheless, possession of a simple ovoid sperm cell with central chromatin, and lacking an acrosome and appendages, may be the result of homoplasic developments (largely induced by the elimination of the acrosomal structure) rather than a real homology. The present contribution on *Peisos petrunkevitchi* shows notable disparity in spermatozoal features between the two known sergestid species. The spermatozoon of *Peisos* differs from that of *Sergestes* chiefly in the presence of a well-developed acrosome, but also in possessing a pair of centrioles at the base of the acrosome and an inconspicuous cytoplasm, many of whose components are scattered throughout the nuclear region instead of being pushed towards the cell periphery. These highly discordant sperm types denote the existence in the Sergestidae of at least two spermatozoal trends. This is congruent with Burkenroad's belief that within the subfamily Sergestinae there are two separate assemblages with close affinities between the genera *Sergia* and *Sergestes*, on the one hand, and between *Peisos* and *Acetes* on the other. The fifth genus *Petalidium* seems to be set apart from both by peculiarities of development (Burkenroad 1983). Further spermatozoal investigation, especially into the unstudied genera *Petalidium*, *Sergia* and *Acetes*, would be needed to assess sperm-based interrelationships in the group. The Luciferidae and Sergestidae are the two families that comprise the superfamily Sergestoidea (Burkenroad 1983; Pérez Farfante and Kensley 1997). The Luciferidae are included in the Dendrobranchiata even though they lack any of the features that are used to characterize the suborder, with the exception of hatching as free nauplii. Future research on sperm morphology and ultrastructure of other sergestid species might help to elucidate some of the present questions regarding the origin, evolution and speciation of the south-western Atlantic Sergestoidea.

Within the Penaeoidea, the families Penaeidae, Sicyoniidae and Solenoceridae (the Aristeidae excluded) form a congruent group in terms of spermatozoal structure. Their spermatozoa share an acrosomal vesicle (apomorphic among decapods) that caps the nuclear region anteriorly and prolongs into an elongate, membrane-bound spike (Medina 1995a). Clear apomorphies, such as the complex subacrosomal region of the sicyoniids (Kleve *et al.* 1980; Medina *et al.* 1994a), or the asymmetrical acrosome cap and separation of the plasma membrane and anterior acrosome membrane in the solenocerids *Solenocera membranacea* and *Pleoticus muelleri* (Medina 1995a; Scelzo and Medina, unpublished observations), unequivocally distinguish the sperm patterns of these three families. The spermatozoon of *Peisos petrunkevitchi*, with the anterior fusiform, spike-less acrosome, embedded in (instead of capping) the nuclear region, is not comparable to any of the investigated dendrobranchiates (Medina 1995a; Jamieson and Tudge 2000; references therein). Among the Dendrobranchiata, only the spermatozoon of the aristeid *Aristeus antennatus*, with the spherical anterior acrosome partially embedded in the supranuclear cytoplasm, displays a certain superficial resemblance, though the peculiar inner configuration of the acrosome in this species is completely unlike that of any other decapods (Demestre and Fortuño 1992; Demestre *et al.* 1993, 1997; Medina 1995b). The internal arrangement of the acrosome of the *P. petrunkevitchi* spermatozoon is very much like that found in reptant (mostly anomuran and brachyuran) species. A similarity between sergestoid and reptant sperm had been previously suggested by Burkenroad (1981), who briefly described (from light microscope observations) the spermatozoon of *Lucifer faxoni* Borradaile, 1915 as a superficially simple ellipsoid cell lacking external appendages, and likened it to that of the thalassinidean *Callichirus seilacheri* Bott, 1955 (as *Callianassa seilacheri*). Further investigation of thalassinidean sperm structure (in addition to Tudge 1995 and Jamieson and Tudge 2000) will provide a suitable background for sperm ultrastructure comparisons with sergestoids (and other decapod groups) to test possible affinities between taxa.

A well-developed perforatorium (deep basal invagination of the acrosomal vesicle) is a typical feature of reptantian (notably anomuran and brachyuran) spermatozoa that is found also in *Peisos*. In *Peisos* the perforatorium resembles that of some anomurans in the highly folded membrane forming septa at the perforatorial base, while at the apical portion the membrane is smooth and transverse sections of the perforatorium show a circular profile. Infoldings of the perforatorial membrane are common in anomuran spermatozoa, but the membrane invaginations usually form microvillar, digitiform, or tubular structures, while robust septa have only been observed in the posterior portion of the perforatorial chamber of some anomurans, such as the porcellanids *Petrolisthes armatus* Gibbs, 1850 and *P. lamarcki* Leach, 1820, several species of *Pagurus*, a species of *Solitariopagurus* (as *Porcellanopagurus*), and the galatheids *Allogalathea* and *Munida* (Tudge 1995; Tudge and Jamieson 1996; Jamieson and Tudge 2000).

As in many reptants, mainly anomurans and brachyurans, the acrosome vesicle contents of *P. petrunkevitchi* spermatozoa are arranged concentrically around the perforatorium into an inner acrosome zone and an outer acrosome zone. The outer zone is quite homogeneous, whereas the more elaborate inner zone is subdivided into several distinct regions arranged in four consecutive horizontal levels. The apical portion of the acrosome resembles that of the homolid brachyuran *Latreillopsis gracilipes* Guinot & Richer de Forges, 1981 (Jamieson 1994; Jamieson and Tudge 2000) in the subopercular protuberance (similar to the apical protuberance of *Peisos*), and in the digitiform projections of the operculum into the subopercular material, which recall the dense bands of the striped region in *Peisos*. Although the striped region is not likely to be homologous with the operculum of reptantian sperm, since unlike most opercula this layer extends posterior to the outer acrosome zone, structural and positional resemblances suggest a similar functional role.

A further typically reptant feature found in *Peisos* is the apomorphic presence of a pair of centrioles (absent in all other investigated dendrobranchiates) located at the base of the perforatorium. The microtubule bundles frequently seen in reptants radiating from the centriolar region and prolonging into radial arms are not seen in the mature spermatozoon of *Peisos*. Jamieson (1991b) suggested that the presence of well-developed nuclear arms is a synapomorphy of nondromid Brachyura, Palinura, Astacidea and Anomura, while its absence is a symplesiomorphy of other Malacostraca. It is difficult to ascertain at the present time whether similarities in the spermatozoal ultrastructure (particularly the acrosomal complex) actually reflect phylogenetic proximity between Sergestidae and the assemblage Anomura–Brachyura (the Meiura of Scholtz and Richter 1995) or, as appears more plausible, they are otherwise the result of independent evolution in adaptation to a similar gamete physiology. Therefore, a much wider comparative investigation on sergestid sperm structure is needed to trace down reliable evolutionary spermatozoal relationships.

It was previously argued (Medina 1995a) that the absence of an acrosome in euphausiids and several decapod species was indicative of the formerly assumed sister-group relationship between both taxa, and that ancestral eucarids must possess anacrosomal spermatozoa. However, recent phylogenetic analyses of the Malacostraca suggest that the Eucarida is not a monophyletic group and the Euphausiacea are more closely related to peracarids than they are to decapods (Jarman *et al.* 2000; Richter and Scholtz 2001).

Although the limited number of ultrastructural data available for dendrobranchiates does not allow the construction of consistent phylograms from sperm characters alone, the spermatozoal features can be useful for phylogenetic analyses. Figure 4 shows a phylogenetic tree that attempts to make compatible sperm characters with relationships proposed by recent studies. In the lineage of the Pleocyemata the reptants usually exhibit a large ovoid or spherical acrosome showing a marked basal invagination that in brachyurans and anomurans forms a conspicuous perforatorium. A first offshoot in the pleocyematan lineage leads to the Caridea, which possess an acrosomal spike that is similar but not homologous to the penaeoid spike (Medina 1995a). A second emerging branch corresponds to the Stenopodidea, which are characterized by the loss of the acrosome. The close affinity between the spermatozoa of *P. petrunkevitchi* and those of many reptants could suggest that ancestral decapods possessed spermatozoa with an ovoid acrosome – probably originating from the endoplasmic reticulum as a supposedly malacostracan plesiomorphy (Jamieson 1991b; Medina 1995a) – and diffuse filamentous chromatin lacking (partially or fully) a nuclear envelope. However, the current phylogenetic evidence contravenes a close relationship between sergestids and reptants, hence the acrosomal similarity between both taxa appears to be the result of convergent evolution. Absence of the acrosome from sperm of many phylogenetically distant decapod taxa could also be envisaged as a plesiomorphy (Medina 1995a). Within the Dendrobranchiata, some Sergestoidea (*Sergestes arcticus*) show an acrosome-less sperm pattern, whereas others (*Peisos petrunkevitchi*) would have evolved a spermatozoon endowed with a well-developed acrosome. The membrane-bound acrosomal spike present in all the investigated nonaristeid Penaeoidea is a clear synapomorphy among the Decapoda. Within the Aristeidae two different sperm morphologies are found, one (*Aristaeomorpha foliacea*) characterized by the absence of the acrosome and the peripheral distribution of the cytoplasmic components, and the other (*Aristeus antennatus*) depicting a spherical protruding acrosome that is ultrastructurally unique, and a cytoplasmic collar intervening between it and the basal nucleus. This highly divergent pattern suggests a derived spermatozoal structure in *A. antennatus*.

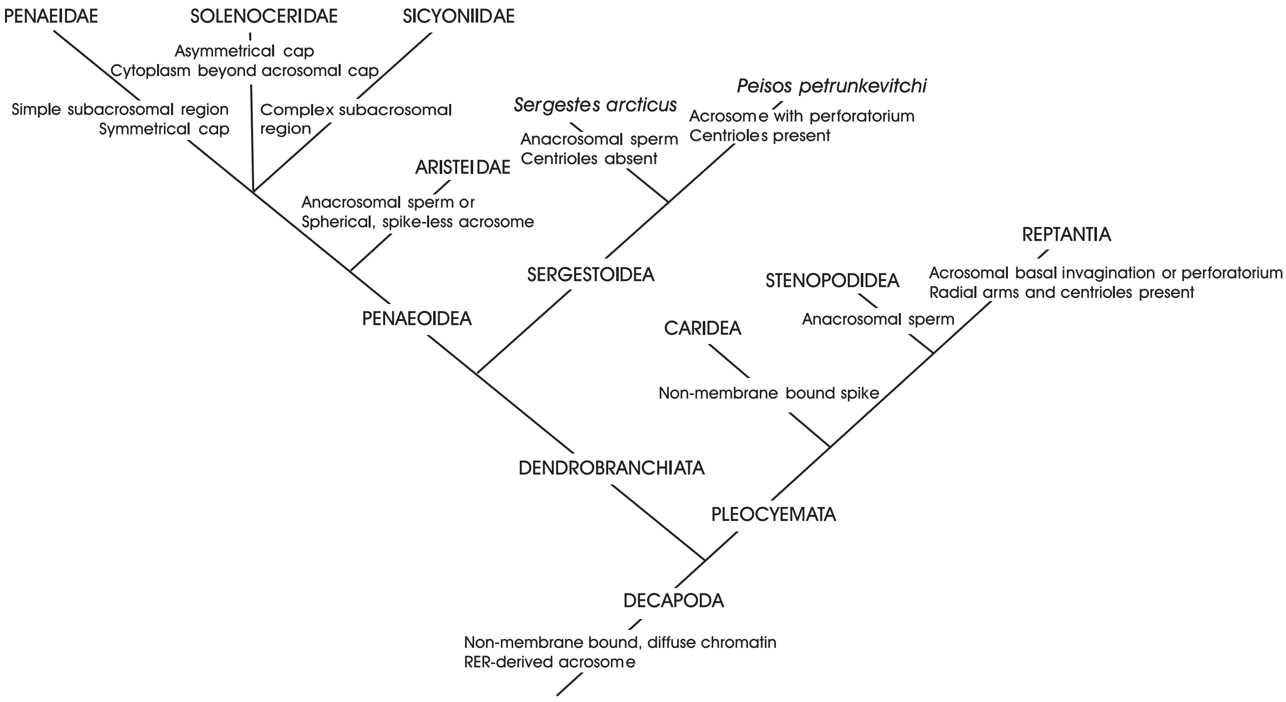

Fig. 4—Diagram representing a dendrobranchiate phylogeny based on spermatozoal evidence and recent cladistic analyses of morphological characters (see Discussion). Significant sperm features are included that may be useful to infer evolutionary transformations of sperm characters.

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