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Sperm ultrastructure in *Hemidonax pictus* (Hemidonacidae, Bivalvia, Mollusca): comparison with other heterodonts, especially Cardiidae, Donacidae and Crassatelloidea

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The systematic position and affinities of the marine bivalve genus *Hemidonax* (Heterodonta, Veneroida) are investigated using comparative sperm ultrastructure, with particular focus on the various groups to which this genus has been assigned [Donacidae (Tellinoidea), Cardiidae (Cardioidea) and Crassatellidae (Crassatelloidea)]. Ultrastructural examination (using transmission electron microscopy) reveals that *Hemidonax pictus* produces sperm of the aquasperm type, with a short, rounded-conical acrosomal vesicle, a short, barrel-shaped nucleus, a short midpiece (composed of two centrioles and four surrounding mitochondria) and a flagellum containing a conventional 9 + 2 pattern axoneme. The acrosomal vesicle exhibits a wedge-shaped, highly electron-dense, basal ring component, and less dense anterior component (including a thin, electron-lucent layer apically, which may prove to be a useful apomorphy for *Hemidonax*). A loose, granular deposit of subacrosomal material is located within a narrow invagination traversing most of the length of the vesicle. Comparison with sperm of other heterodont bivalves shows no close connection between *Hemidonax* and the Donacidae (Tellinoidea) or with the Crassatellidae (or other crassatelloideans). Although certain Veneridae (Veneroidea) and Cardiidae (Cardioidea, especially *Fragum*) show much better conformity in sperm morphology to that observed in *Hemidonax*, no complete match could be found among studied taxa. We conclude that *Hemidonax* should be retained in its own, previously introduced family Hemidonacidae, and the latter be placed *incertae sedis* within the Euheterodonta. *Zoological Journal of the Linnean Society*, 2008, **153**, 325–347.

ADDITIONAL KEYWORDS: Australian marine fauna – bivalve – gametes – molluscs – reproduction – systematics.

INTRODUCTION

The bivalve genus *Hemidonax* Mörch, 1871 contains five extant species, restricted to Australia (four endemics), Indonesia and the Philippines, in addition to one Australian fossil species (Ponder, Colman, Yonge & Colman, 1981). Although often found as beached valves, little is known of the habits or ecology of *Hemidonax* other than that the animals occur in shallow subtidal sandy habitats and are usually collected alive only through dredging. The absence of siphons and a pallial sinus and the presence of a hatchet-shaped foot indicate that they are shallow and probably active burrowers (Wilson, 1998). In terms of

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their shell valve profile, *Hemidonax* species show some resemblance to the tellinoid family Donacidae, and indeed the genus has often been included in that family (Lamy, 1917; Thiele, 1934; Keen, 1969; Vokes, 1980; Abbott & Dance, 1982). Boss (1971) drew attention to the early assignment of *Hemidonax* to the Cardiidae by von Vest (1875) as well as early placements within the Crassatellidae (as 'Crassatellitidae', Hedley, 1906, 1909, 1923) and (extinct) Tancrediidae (Fischer, 1887). Allan (1959: 319), although aware of the often disputed systematic position of *Hemidonax*, was confident that 'its anatomical structure places this genus in the family Cardiidae, rather than in those in which it has frequently been placed in the past.' She appears to be the first Australian author to have accepted a cardiid position for the genus. Iredale & McMichael (1962) introduced (without diagnosis) a new family name to accommodate *Hemidonax* – the Hemidonacidae (now credited to Scarlato & Starobogatov, 1971)* – that they placed between the Scintillidae and Cardiidae, and distant from the Donacidae and other tellinoidean families. Boss (1971) demonstrated that in terms of their anatomy, *Hemidonax* spp. lacked key tellinoidean features, most notably the paired, naked siphons and the cruciform muscle, and instead showed strong similarity to the Cardiidae. He considered that *Hemidonax* was not distinctive enough to warrant inclusion in its own family, and instead included it in the cardiid subfamily Hemidonacinae (a placement accepted by many including Kafanov & Popov, 1977; Keen, 1980). Perhaps the most unusual assignment of the Hemidonacidae was proposed by Scarlato & Starobogatov (1971, 1979), who grouped Hemidonacidae with the Donacidae and the extinct Tancrediidae in the Donacoidea, and associated with various galeommatoidean families into a suborder Erycina. Ponder *et al*. (1981) taxonomically reviewed the genus, and provided further details of anatomy in support of cardiid affinities of *Hemidonax*. However, they also listed several anatomical differences and some conchological differences between *Hemidonax* and the Cardiidae, and therefore maintained the need for a separate family Hemidonacidae to contain the genus – a view (generally) adopted in subsequent works (e.g. Kafanov & Popov, 1977; Boss, 1982; Vaught, 1989; Lamprell & Whitehead, 1992; Wilson, 1998). Schneider (1992) rekindled the debate concerning the relationship of *Hemidonax* to other Veneroida, preferring to regard the genus as *incertae sedis* and not a member of the Cardioidea or the tellinoidean Donacidae. Most recently, Schneider & Carter (2001) argued for a closer relationship between *Hemidonax* and the tellinoidean family Psammobiidae than with the Cardiidae, based on their comparative study of shell microstructure.

Comparative studies of bivalve sperm ultrastructure have shed new light onto higher relationships within the class (Popham, 1979; Healy, 1996a) and the systematics or phylogeny of several important taxa (e.g. Mytiloidea – Hodgson & Bernard, 1986; Kafanov & Drozdov, 1998; Palaeoheterodonta – Healy, 1989, 1996a, b; Pteriomorphia – Healy, Keys & Daddow, 2000; Crassatelloidea – Healy, 1995a, b; Galeommatoidea – Jespersen, Lützen & Morton, 2002; Veneroidea – Gharagozlou-Van Ginneken & Pochon-Masson, 1971; Healy, 1995b; Healy, Mikkelsen & Bieler, 2006). With this in mind, we have carried out a sperm ultrastructural study of a representative species of *Hemidonax* [using the type species *H. pictus* (Tryon, 1870)] in order to clarify the relationships of the genus to other Veneroida. In order better to assess the two strongest claims regarding *Hemidonax* affinities (i.e. with the Donacidae or with the Cardiidae) we also present data for both of these families, which will help to supplement the available literature (Donacidae – Hodgson, Bernard & Van der Horst, 1990; Sousa & Oliveira, 1994; Healy, 1995b; Cardiidae – Popham, 1979; Sousa & Azevedo, 1988; Healy, 1995b, 1996a; Sousa *et al*., 1998; Keys & Healy, 1999, 2000; Drozdov, Frolenko & Ferraguti, 2001). Aside from Pelseneer's (1911) statement that in *Hemidonax donaciformis* (Schröter, 1786) the sexes are separate, nothing appears to be known concerning the reproductive biology of *Hemidonax*. The present study represents the first contribution to knowledge of gamete morphology in the genus, and it is hoped that the results will stimulate further studies on this small but intriguing group.

MATERIAL AND METHODS

Hemidonax pictus (Tryon, 1870) was dredged from a depth of 20–30 m, approximately 1 km north-west of 'Yellowpatch' off the northern coast of Moreton Island, south-eastern Queensland, Australia (26°57.6′S, 153°24.6′E) on 3 April 2005 and trans-

*[Although Iredale & McMichael (1962) provided bibliographic references to both the genus *Hemidonax* Mörch, 1778 and to the type species of the genus [*H. pictus* (Tryon, 1870)], this cannot be considered a diagnosis of the family as it does not satisfy the requirements of Article 13.1 of the ICZN (1999) Code. The name Hemidonacidae Iredale & McMichael, 1962 is therefore a *nomen nudum* (see Bouchet & Rocroi, 2005: 7, for a recent discussion of this aspect of the Code). The first to provide a formal definition of Hemidonacidae (and therefore make the name available) were Scarlato & Starobogatov (before 7 July 1971, p. 16) who made no reference to Iredale & McMichael's checklist, but clearly stated 'Hemidonacidae Scarlato et Starobogatov, fam. nov.' As their work pre-dates that of Boss (20 July 1971, Hemidonacinae), Scarlato & Starobogatov (1971) must be credited with authorship of Hemidonacidae, despite later citing (see Scarlato & Starobogatov, 1979) Iredale & McMichael (1962) as the original authors of the family.]

ferred to holding tanks at the Moreton Bay Research Station (North Stradbroke Island, south-eastern Queensland). *Donax* (*Plebidonax*) *deltoides* (Lamarck, 1818) was collected from surf beaches near Point Lookout, North Stradbroke Island (27°28′S, 153°32′E). *Vasticardium vertebratum* (Jonas, 1844), *Fragum unedo* (Linnaeus, 1758) and *Lunulicardia hemicardium* (Linnaeus, 1758) were all collected from Myora, North Stradbroke Island, Moreton Bay, south-eastern Queensland (27°27′S, 153°26′E, 1986–1990). *Eucrassatella cumingii* (A. Adams & Angas, 1864) was dredged at a depth of 12 m, Banana Banks, Moreton Bay (27°32′S, 153°20′E, 1992). *Cardita muricata* Sowerby, 1832 was collected intertidally at Cockle Bay, Magnetic Island, northern Queensland (19°11′S, 146°49′E, 1991). *Papyridea semisulcata* (Gray, 1825) was collected from the Florida Keys, USA [station FK-720, 27 April 2004, Looe Key back reef, Monroe County, Florida Keys, 24°32.894′N, 81°24.360′W, rubble, sand and seagrass, snorkelling, 1.2–2.1 m, FLORIDAYS]. The following processing schedule was followed for most of the material examined. Small (2 mm^3) pieces of testicular tissue were taken from a ripe male and fixed in ice cold (2–4 °C) glutaraldehyde (3.5% in 0.1 M phosphate buffer containing 7.5% w/v sucrose) for 3 h. For *Papyridea semisulcata* the entire animal was fixed in cold 3.5% buffered glutaraldehyde (formula as above) in the refrigerator for 11 days, then shipped to Chicago (and later sent to Australia for subsequent processing). Tissue samples were subsequently rinsed in buffer (three 30-min changes), post-fixed in 1% osmium tetroxide (buffer as above) for 80 min, rinsed again in three changes of buffer, dehydrated in a graded series of ethanol and embedded in Spurr's epoxy resin. Samples were maintained at 2–4 °C (on ice) for all stages up until 70% ethanol, and thereafter at room temperature (25 °C). Semithin and silver–gold interface ultrathin sections were cut with Leica Ultracut T and LKB IV ultramicrotomes, collected on 200-square-mesh copper grids, stained according to the lead citrate–uranyl acetate–lead citrate 'sandwich stain' procedure of Daddow (1986) and examined using Jeol 101 or Hitachi 300 transmission electron microscopes operating at 75–80 kV or a Philips 300 transmission electron microscope at 60 kV. Voucher material of *Hemidonax pictus* used in the present study has been lodged with the Field Museum of Natural History (Registration number: FMNH 311639) and Queensland Museum (Brisbane, Australia) (QMMO 78087). Voucher material of other species of bivalves examined herein have been lodged with the Queensland Museum: *Vasticardium vertebratum* (QMMO 78083), *Fragum unedo* (QMMO 78084), *Lunulicardia hemicardium* (QMMO 78085), *Donax deltoides* (QMMO 78086), *Cardita muricata* (QMMO 53310), *Eucrassatella cumingii* (QMMO 53311), or the Field Museum of Natural History (Chicago, USA): *Papyridea semisulcata* (FMNH 311640).

RESULTS

HEMIDONAX PICTUS (FIG. 1)

Acrosomal complex

The acrosomal vesicle is conical, measuring 0.35 ± 0.02 μm ($N = 4$) in length and with a maximum diameter of approximately 0.4 ± 0.05 μm ($N = 4$) (Fig. 1A–D). Contents of the vesicle are differentiated into a highly electron-dense, faintly reticulate, basal ring sheathed by markedly less dense (and granular) material that also fills the anterior region of the vesicle (Fig. 1A, C, D). Associated with the vesicle apex is a broad, thin, electron-lucent layer, the extent of which is somewhat variable (Fig. 1A, C, arrows). A narrow basal invagination extends for most of the length of the acrosomal vesicle and is filled by a granular deposit of subacrosomal material (Fig. 1A–D). The plasma membrane forms the outermost sheath of the acrosomal complex, as it does in other regions of the spermatozoon (Fig. 1A, F) (this applies in all the species of bivalves examined herein).

Nucleus

The nucleus is short (length 1.8 ± 0.1 μm, $N = 4$) and barrel-shaped (tapers apically anteriorly), with a maximum diameter (near base) of 1.0 ± 0.1 μm ($N = 4$) (Fig. 1B). Nuclear contents are highly electron-dense, with scattered, irregularly shaped, electron-lucent lacunae. Broad, shallow depressions, present basally, contact the anterior surfaces of each midpiece mitochondrion (Fig. 1B, E). These depressions surround a smaller central recess filled with granular material loosely associated with the proximal centriole (Fig. 1E).

Midpiece

The midpiece is positioned at the base of the nucleus and consists of four spherical mitochondria surrounding a pair of orthogonally arranged centrioles (Fig. 1B, E, F). The entire midpiece region has a length of 0.75 ± 0.05 μm ($N = 3$) and a maximum diameter (of mitochondrial cluster) of approximately 1.44 ± 0.1 μm ($N = 3$). The centrioles lie in contact with each other and exhibit triplet microtubular structure which is largely obscured by a dense matrix (Fig. 1E–G). The distal centriole is connected by a series of nine satellite fibres each terminating in a Y-shaped fork attached to the plasma membrane (Fig. 1E, G).

Flagellum

The flagellum consists of a 9 + 2 microtubular configuration axoneme (nine microtubular doublets

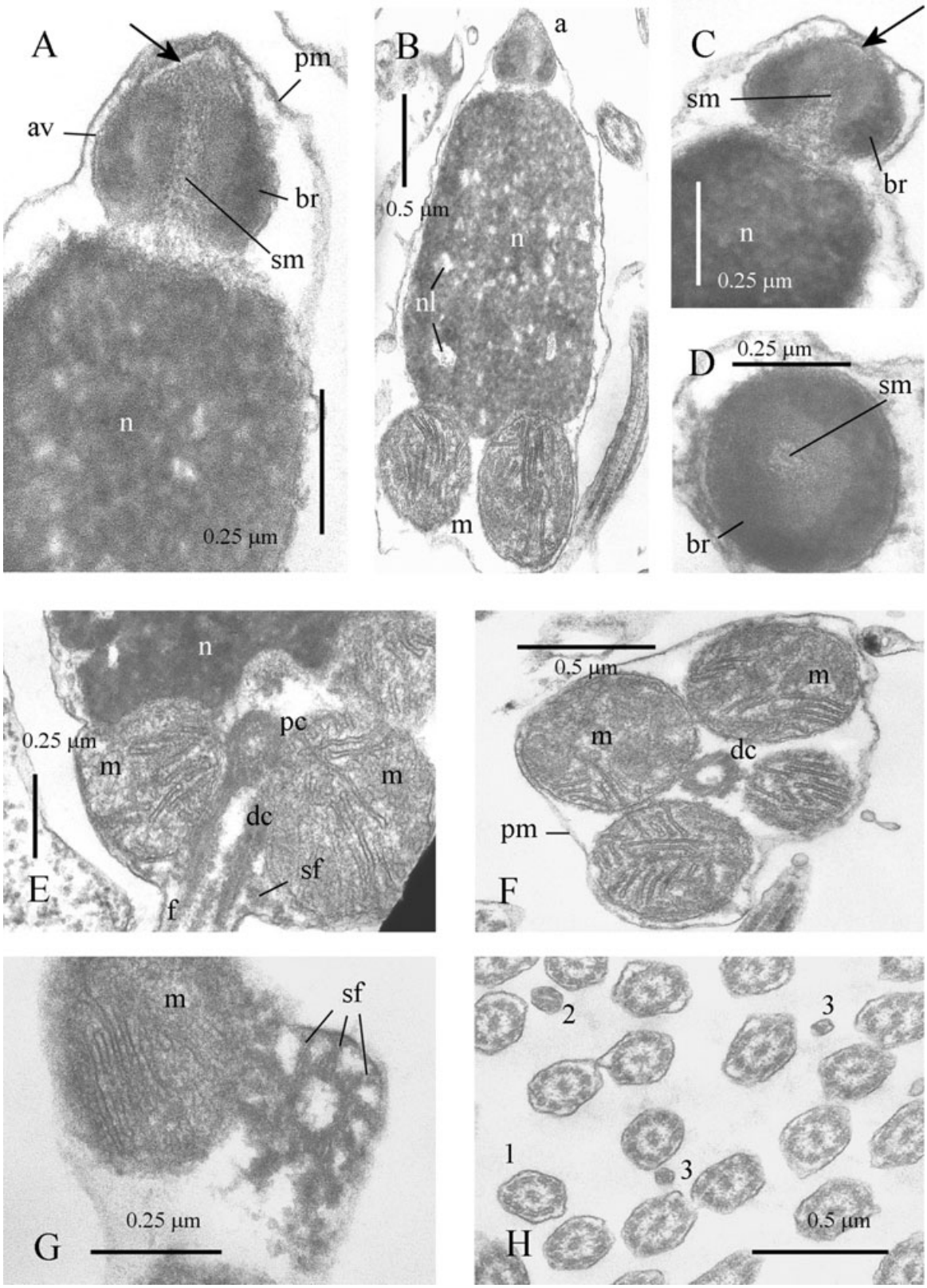

Figure 1. Ultrastructure (TEM) of spermatozoa of *Hemidonax pictus*. A, longitudinal section (LS) showing acrosomal complex (acrosomal vesicle + subacrosomal material) at apex of nucleus. Arrow indicates apical electron-lucent layer. B, LS showing size proportions of acrosomal complex, nucleus and midpiece mitochondria. C, LS of acrosomal complex. Arrow indicates apical electron-lucent layer. D, transverse section (TS) through mid-length region of acrosomal complex. Note granular subacrosomal material in narrow invagination of acrosomal vesicle, and also two components of vesicle contents (basal ring and less-dense anterior region). E, LS of midpiece at base of nucleus. Paired, connected centrioles are continuous with flagellar axoneme and closely surrounded by mitochondria. F, TS of midpiece at level of distal centriole showing four mitochondria. G, TS of posterior extremity of distal centriole (triplet microtubules), showing attachment, via Y-links, to plasma membrane. H, TS of flagellae showing intact axoneme (1) and successive (narrowed, degenerate) levels of terminal region (2, 3). Abbreviations: a, acrosomal complex; av, acrosomal vesicle; br, basal ring (of acrosomal vesicle contents); dc, distal centriole; f, flagellum (featuring axoneme); m, mitochondrion of midpiece; n, nucleus; nl, nuclear lacuna; pc, proximal centriole; pm, plasma membrane; sf, satellite fibres; sm, subacrosomal material.

surrounding a central pair of singlet microtubules) sheathed by the plasma membrane. The terminal region is characterized by a decreasing diameter and degeneration of the axoneme into singlet microtubules obscured by a dense matrix (Fig. 1H). From light microscopic observations, flagellar length is 51 ± 2.0 μm ($N = 10$).

CARDIIDAE

VASTICARDIUM VERTEBRATUM (FIG. 2)

Acrosomal complex

The acrosomal vesicle is dome-shaped, 0.28 ± 0.02 μm ($N = 4$) in length and with a maximum diameter of 0.46 ± 0.02 μm ($N = 4$). The anterior surface of the vesicle may be flat, slightly concave or, most commonly, slightly convex (Fig. 2A, B). Contents of the acrosomal vesicle are divisible into a very prominent, and markedly electron-dense basal ring, and less dense material anteriorly (Fig. 2B, C). The basal ring is separated from the vesicle membrane by an electron-lucent layer, and within the ring itself two components can be distinguished: a very electron-dense, internally reticulate outer layer and a less dense inner layer, both approximately 0.05–0.06 μm in thickness (Fig. 2B, C). A broad invagination extends almost the full length of the acrosomal vesicle, and is filled with granular subacrosomal material (Fig. 2B). Such material also occupies the thin gap between the base of the acrosomal vesicle and the smooth apex of the nucleus.

Nucleus

The nucleus is rod shaped, curved in its anterior half and 4.4 ± 0.1 μm long ($N = 5$) (Fig. 2A). Maximum diameter is approximately 0.7 ± 0.1 μm ($N = 5$) just anterior to the midpiece. Nuclear contents are highly electron dense with the exception of scattered, irregularly shaped lacunae, especially in the basal region (Fig. 2A, D, E).

Midpiece

The midpiece exhibits a pair of centrioles (arranged orthogonally) surrounded by four spherical, cristate mitochondria and scattered dense granules (putative glycogen deposits) (Fig. 2A, D–F). The centrioles are composed of triplet microtubules slightly obscured by dense material (Fig. 2D–F). The proximal centriole is attached to a thin layer of material lining a very shallow indentation of the nuclear base. Proximal and distal centrioles are attached to each other by dense material, the distal one also being attached to the plasma membrane by a series of nine satellite fibres (Fig. 2D, E). The entire midpiece region has a length of 0.58 ± 0.05 μm ($N = 4$) and a maximum diameter (of mitochondrial cluster) of 1.27 ± 0.05 μm ($N = 5$).

Flagellum

The flagellum consists of a 9 + 2 microtubular configuration axoneme sheathed by the plasma membrane (Fig. 2D–F). From light microscopic observations, flagellar length is 48 ± 2.0 μm ($N = 10$).

FRAGUM UNEDO (FIG. 3)

Acrosomal complex

The acrosomal vesicle is short (0.28 ± 0.02 μm, $N = 3$), broadly conical, with a maximum diameter of 0.7 ± 0.05 μm ($N = 3$) (at mid-length level) (Fig. 3A–C). It rests on the membranes of the flattened and slightly inclined nuclear apex. Contents of the vesicle are differentiated into a highly electron-dense basal ring (lower, outer portion of the vesicle) and less dense material (lower, inner portion of the vesicle and anteriorly) (Fig. 3C).

Nucleus

The nucleus is short (2.38 ± 0.05 μm, $N = 3$), barrel-shaped (maximum diameter mid-nucleus of 1.35 ± 0.05 μm, $N = 3$) but slightly curved or twisted (Fig. 3A, B). Contents are highly electron-dense with the exception of scattered, electron-lucent lacunae (Fig. 3A, D). Apically the nuclear surface is flat and inclined due, in part, to nuclear curvature, whereas

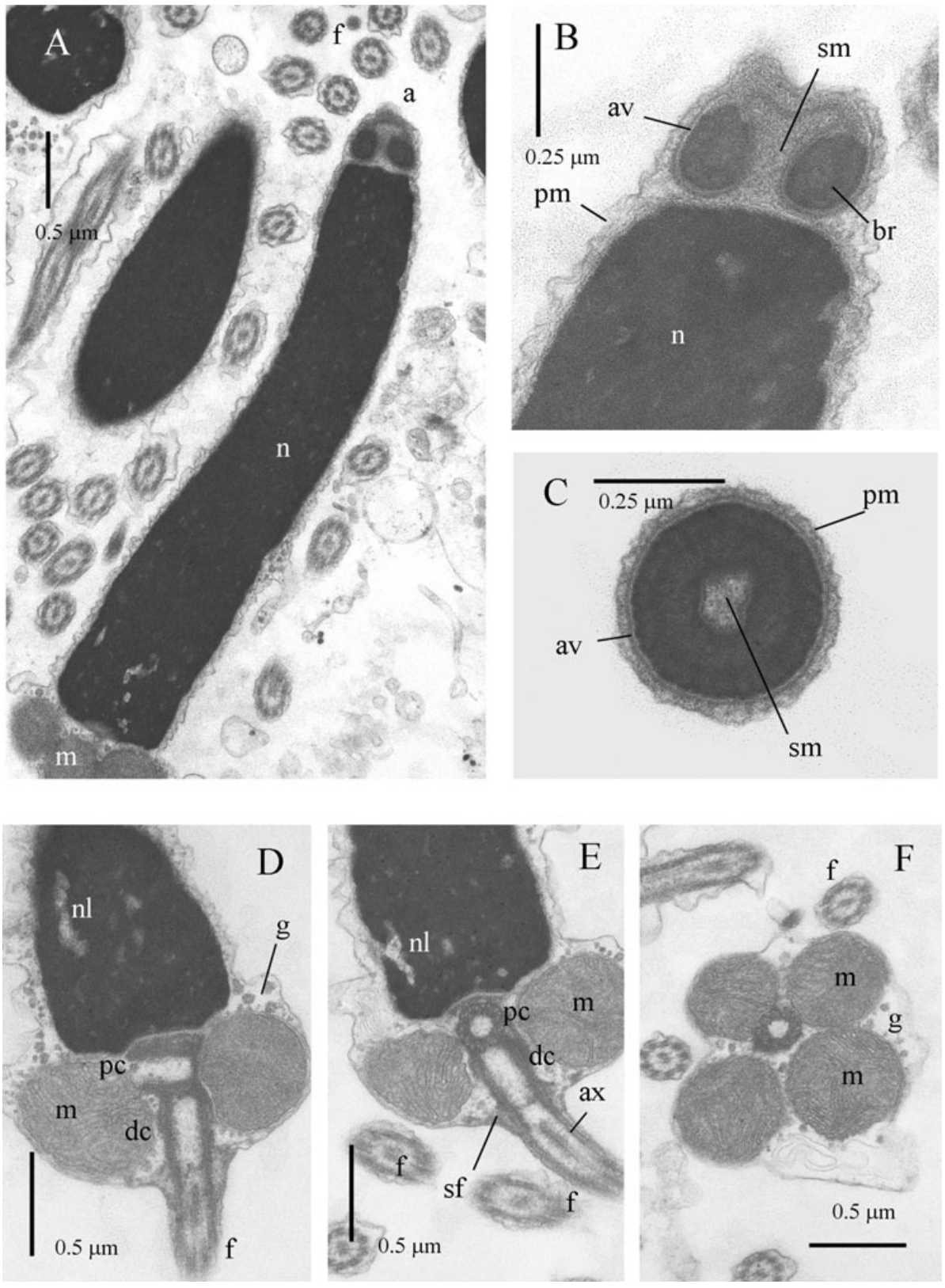

Figure 2. Ultrastructure (TEM) of spermatozoa of *Vasticardium vertebratum*. A, longitudinal section (LS) showing acrosomal complex, curved nucleus, portion of midpiece region. Note also several flagellae cut in transverse section (TS). B, LS of acrosomal complex (acrosomal vesicle + subacrosomal material) and nuclear apex. The degree of anterior dimpling is variable. C, TS at mid-level region of acrosomal complex. D, E, LS at base of nucleus, entire midpiece and proximal portion of flagellum. The proximal centriole shown in LS in D, in TS in E. Putative glycogen granules are also visible. F, TS of midpiece showing four mitochondria, distal centriole and putative glycogen granules. Abbreviations: a, acrosomal complex; av, acrosomal vesicle; ax, axoneme; br, basal ring (of acrosomal vesicle contents); dc, distal centriole; f, flagellum; g, putative glycogen granules; m, mitochondrion of midpiece; n, nucleus; nl, nuclear lacuna; pc, proximal centriole; pm, plasma membrane; sf, satellite fibres; sm, subacrosomal material.

basally, very shallow indentations are associated with the midpiece mitochondria and proximal centriole (Fig. 3A).

Midpiece

The midpiece contains four spherical mitochondria grouped around the centriolar pair (proximal and distal centrioles) (Fig. 3D, E). The centrioles are attached to each other, with the distal one anchored to the plasma membrane via nine satellite fibres. The entire midpiece region has a length of 0.75 ± 0.05 μm ($N = 3$) and a maximum diameter (of mitochondrial cluster) of 1.75 ± 0.08 μm ($N = 3$).

Flagellum

The flagellum consists of a 9 + 2 microtubular configuration axoneme sheathed by the plasma membrane. From light microscopic observations, flagellar length is 48 ± 2.0 μm ($N = 10$).

LUNULICARDIA HEMICARDIUM (FIG. 4)

Acrosomal complex

The acrosomal vesicle is short (0.24 ± 0.02 μm, $N = 2$) and dome-shaped (maximum diameter, at base, of 0.66 ± 0.01 μm, $N = 2$) (Fig. 4A, B). It rests on a shallow, circular groove of the nuclear apex (forming a shallow nuclear hump at the nuclear apex) (Fig. 4B). Contents of the vesicle are differentiated into a somewhat, dense, basal ring that has an angulate profile, and is sheathed by a less dense material that also fills the anterior half of the vesicle (Fig. 4A, B). The basal invagination of the vesicle is deep in comparison with vesicle length and very broad (maximum diameter at base 0.4 ± 0.02 μm, $N = 2$) and partially filled with a granular, but well-defined, deposit of subacrosomal material.

Nucleus

The nucleus is short (length 2.15 ± 0.05 μm, $N = 3$), barrel shaped (maximum diameter, at mid-length, of 1.65 ± 0.05 μm, $N = 3$) and slightly tapered anteriorly (Fig. 4A, B). Contents are highly electron dense with the exception of electron-lucent lacunae (the latter more pronounced posteriorly) (Fig. 4A). Apically, the nucleus projects slightly into the acrosomal vesicle invagination (Fig. 4A, B). Basally, very shallow indentations contact the surfaces of the midpiece mitochondria and also form an attachment point for the proximal centriole (Fig. 4A).

Midpiece

The midpiece consists of four or more rarely five spherical mitochondria and a pair of centrioles (Fig. 4A, C, D). The centrioles (proximal and centriole) lie in contact with each other and are arranged orthogonally. A thin layer of dense material connects the proximal centriole to a shallow indentation of the nucleus. Satellite fibres connect the distal centriole to the plasma membrane (Fig. 4A, C). The entire midpiece region has a length of 0.7 ± 0.05 μm ($N = 3$) and a maximum diameter (of mitochondrial cluster) of 1.8 ± 0.2 μm ($N = 3$).

Flagellum

The flagellum consists of a 9 + 2 microtubular configuration axoneme, sheathed by the plasma membrane (Fig. 4C, D). From light microscopic observations, flagellar length is 46 ± 3.0 μm ($N = 10$).

PAPYRIDEA SEMISULCATA (FIG. 5)

Acrosomal complex

The acrosomal vesicle is dome-shaped, with a flat or slightly domed apex, and measures approximately 0.55 ± 0.05 μm ($N = 5$) in length and 0.7 ± 0.05 μm ($N = 5$) in maximum diameter (Fig. 5A–D). Contents of the vesicle are differentiated into an extensive, very electron-dense basal ring, and a less dense (and granular) anterior component (Fig. 5B, D). A broad basal invagination extends for approximately half the length of the acrosomal vesicle and is filled with granular subacrosomal material (Fig. 5A–C). Discernible within the basal ring are two curved, very electron-dense layers and an innermost, electron-lucent layer (Fig. 5B, D).

Nucleus

The nucleus is short (length 1.45 ± 0.05 μm, $N = 3$) and barrel shaped, with a maximum diameter of

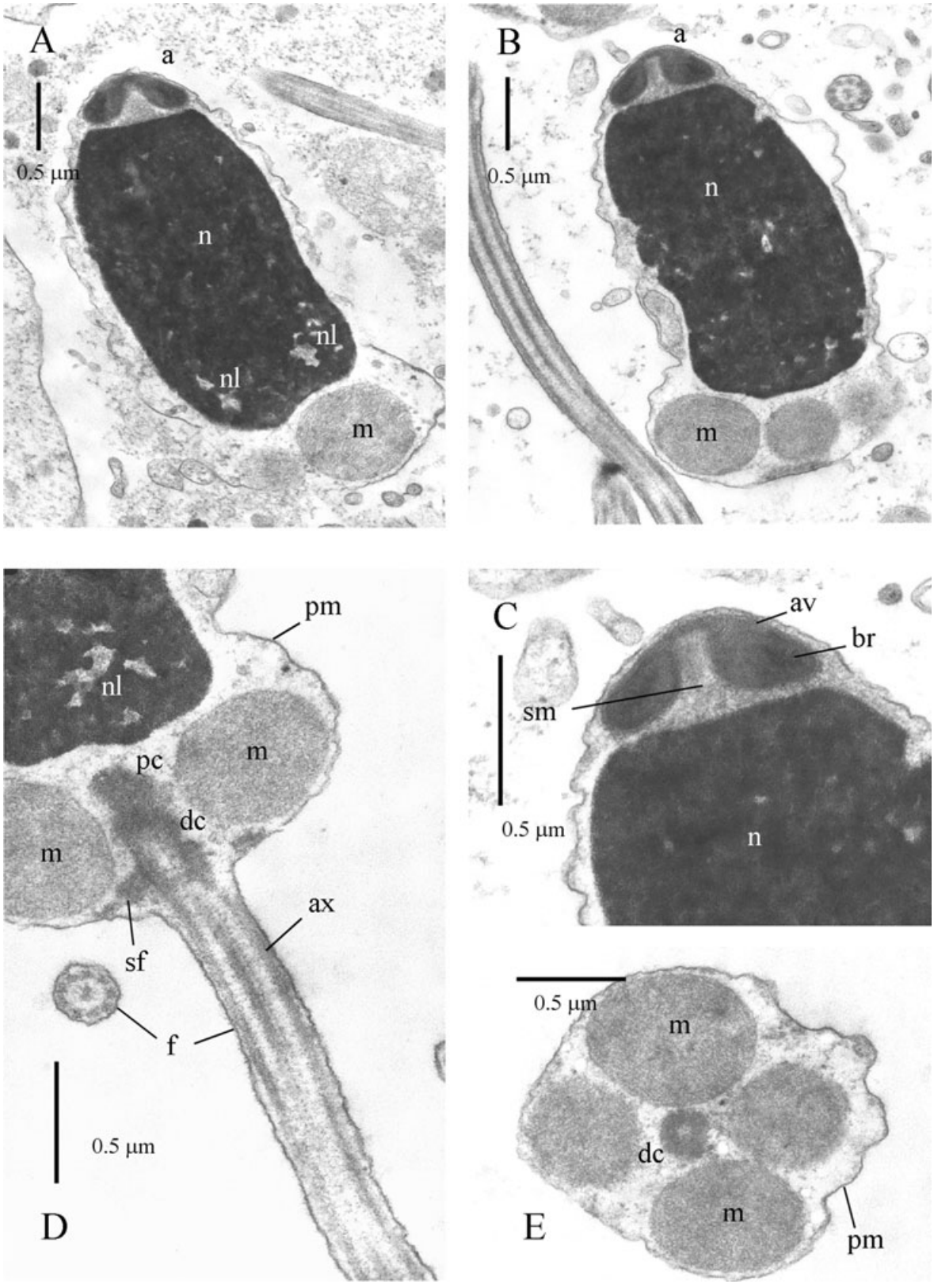

Figure 3. Ultrastructure (TEM) of spermatozoa of *Fragum unedo*. A, B, longitudinal sections (LS) showing acrosomal complex, nucleus (showing slight curvature) and portion of midpiece region. C, LS of acrosomal complex (acrosomal vesicle + subacrosomal material) and nuclear apex. Note narrow basal invagination of acrosomal vesicle. D, LS of flagellum projecting from distal centriole of midpiece. Note also nuclear lacunae and also satellite fibres attached to distal centriole and plasma membrane. E, transverse section (TS) of midpiece showing four mitochondria and distal centriole. Abbreviations: a, acrosomal complex; av, acrosomal vesicle; ax, axoneme; br, basal ring (of acrosomal vesicle contents); dc, distal centriole; f, flagellum; m, mitochondrion of midpiece; n, nucleus; nl, nuclear lacuna; pc, proximal centriole; pm, plasma membrane; sf, satellite fibres; sm, subacrosomal material.

1.45 ± 0.05 μm ($N = 3$) (at mid-length) (Fig. 5A). Nuclear contents are highly electron-dense, granulate, with numerous irregular lacunae (Fig. 5A, E). Apically the nuclear surface is straight or slightly concave, while posteriorly shallow indentations contact the anterior surfaces of each midpiece mitochondrion (Fig. 5A).

Midpiece

The midpiece consists of four spherical mitochondria (with well-developed cristae) surrounding a pair of centrioles (Fig. 5A, F). The entire midpiece region has a length of 0.7 ± 0.04 μm ($N = 3$) and a maximum diameter (of mitochondrial cluster) of 1.7 ± 0.1 μm ($N = 3$).

Flagellum

The flagellum consists of a 9 + 2 microtubular configuration axoneme sheathed by the plasma membrane. From light microscopic observations, flagellar length is 54 ± 2.0 μm ($N = 10$).

DONAX (*PLEBIDONAX*) *DELTOIDES* (FIG. 6)

Acrosomal complex

The acrosomal vesicle measures approximately 1.16 ± 0.02 μm ($N = 4$) in length, has a maximum diameter of 0.85 ± 0.05 μm ($N = 4$) (about one-third of the distance from the vesicle base) and rests within a broad depression of the nuclear apex (depth 0.24 ± 0.03 μm, $N = 4$) (Fig. 6A–C, E). A narrow invagination runs almost the full length of the vesicle and is filled with longitudinally fibrous subacrosomal material. Contents of the acrosomal vesicle can be differentiated into a very electron-dense, basal ring (curved-cylindrical in longitudinal profile) and homogeneous, less dense material – the latter enclosing the basal ring and filling the anterior region of the vesicle. The basal ring shows an internal structure of fine, parallel layers (approximately 25–35 in number) (Fig. 6A) that in transverse section (Fig. 6B) are arranged concentrically.

Nucleus

The nucleus is short [length 1.4 ± 0.1 μm ($N = 4$), inclusive of an overlap region with the acrosomal complex] and squat (maximum diameter 1.5 ± 0.04 μm, $N = 4$), with highly electron-dense contents (Fig. 6A, C–E). The apical surface is broadly indented to receive the basal portion (0.2 ± 0.05 μm, $N = 4$) of the acrosomal vesicle, while posteriorly, shallow indentations act as contact surfaces for the midpiece mitochondria and the anterior edge of the proximal centriole. Irregularly shaped electron-lucent lacunae are present throughout, though largest posteriorly (Fig. 6A, D).

Midpiece

The midpiece exhibits four, roughly spherical, mitochondria (showing prominent cristae) in addition to a pair of orthogonally arranged centrioles (Fig. 6A, E, F). Each centriole is composed of microtubular triplets set in a dense matrix (Fig. 6F). The proximal centriole is loosely connected via a diffuse deposit to a shallow indentation of the nuclear base, and is also attached to the distal centriole (Fig. 6A, E). The distal centriole is anchored to the plasma membrane via nine satellite fibres and is continuous with the doublets of the flagellar axoneme (Fig. 6A). The entire midpiece region has a length of approximately 0.6–0.7 μm and a maximum diameter (of mitochondrial cluster) of 1.6–1.8 μm.

Flagellum

The flagellum consists of a 9 + 2 microtubular configuration axoneme, sheathed by the plasma membrane (Fig. 6A). From light microscopic observations, flagellar length is 46 ± 3.0 μm ($N = 10$).

CRASSATELLOIDEA: *EUCRASSATELLA CUMINGII* (CRASSATELLIDAE), *CARDITA MURICATA* (CARDITIDAE)

Acrosomal complex

The acrosomal vesicle is elongate-conical (2.7 ± 0.1 μm *E. cumingii*, $N = 4$; length 1.6 ± 0.1 μm *C. muricata*, $N = 4$), sharply tapered anteriorly and almost completely invaginated (Fig. 7A, B). The vesicle has a maximum diameter (at base) of approximately 0.35 μm. Contents of the acrosomal vesicle are differentiated into a dense inner layer enveloped by markedly less dense material. (Fig. 7B, C) The subacrosomal material is organized as a well-defined

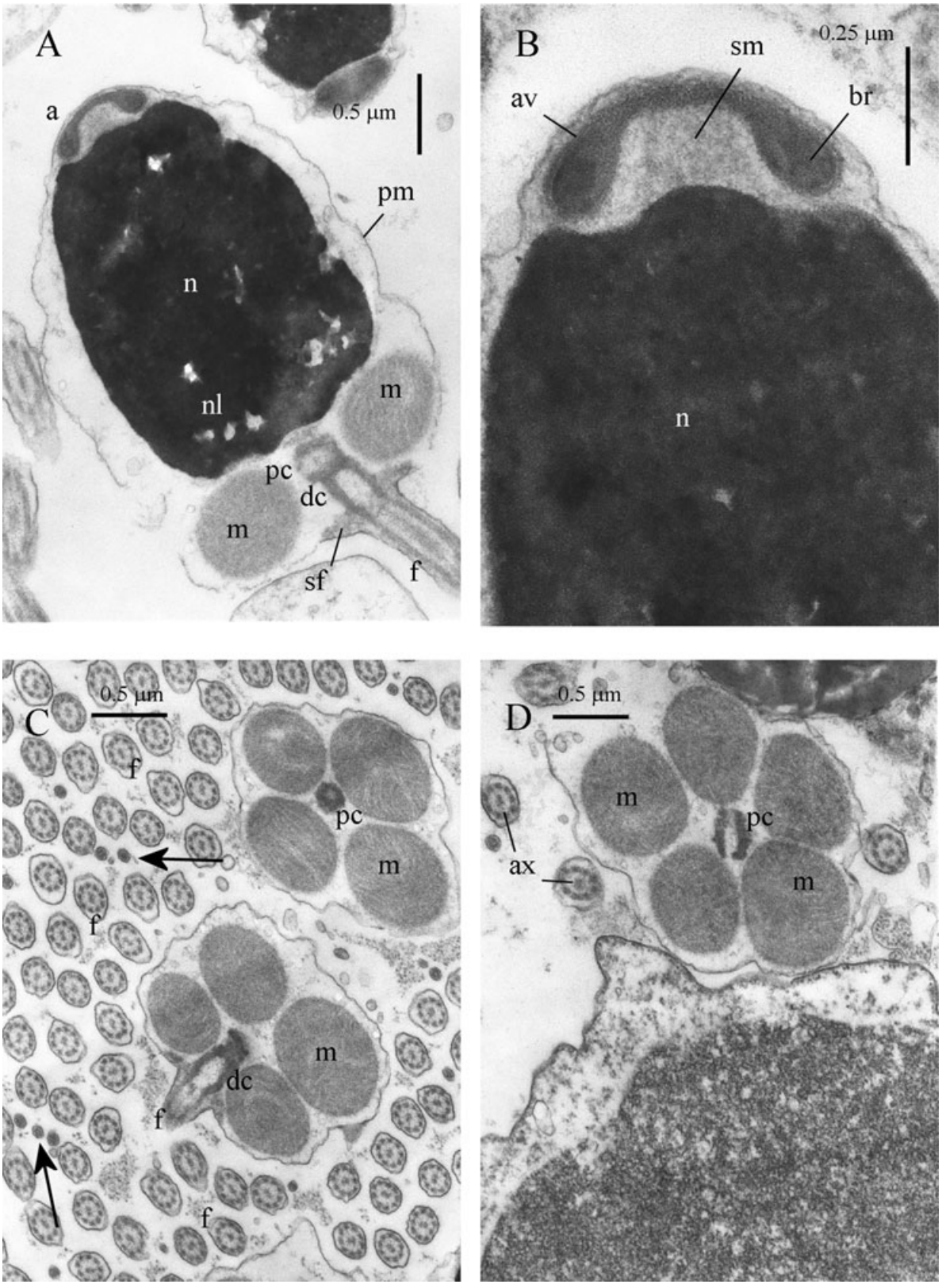

Figure 4. Ultrastructure (TEM) of spermatozoa of *Lunulicardia hemicardium*. A, longitudinal section (LS) showing acrosomal complex, nucleus (with nuclear lacunae), midpiece region (mitochondria surrounding proximal and distal centrioles) and proximal portion of flagellum. Note also satellite fibres linking distal centriole to plasma membrane. B, LS of acrosomal complex (acrosomal vesicle + subacrosomal material) and nuclear apex. Note broad but low nuclear projection. C, transverse section (TS) of midpiece of two spermatozoa and flagella of many others. Note four mitochondria. Arrows indicate terminal region of flagellum. D, TS of midpiece showing five mitochondria variant (proximal centriole shown in LS) and flagellae. Abbreviations: a, acrosomal complex; av, acrosomal vesicle; ax, axoneme; br, basal ring (of acrosomal vesicle contents); dc, distal centriole; f, flagellum; m, mitochondrion of midpiece; n, nucleus; nl, nuclear lacuna; pc, proximal centriole; pm, plasma membrane; sf, satellite fibres; sm, subacrosomal material.

axial rod that occupies not only the vesicle invagination but also a shallow apical invagination of the nucleus (Fig. 7B).

Nucleus

The nucleus is rod-shaped (length 10.0 ± 0.2 μm *E. cumingii*; 7.4 ± 0.2 μm *C. muricata*) ($N = 5$), tapering (in each species) from a diameter of 1.0 ± 0.1 μm ($N = 6$) near the base to 0.4 ± 0.05 μm ($N = 6$) at the apex (Fig. 7A, B, G, E). Aside from the apical invagination (accommodating part of the subacrosomal material), the nucleus exhibits a short invagination for the centriolar rootlet and slight concavities that contact the mitochondria (Fig. 7E, G). Irregularly shaped electron-lucent lacunae are present throughout, though largest and most conspicuous posteriorly (Fig. 7G).

Midpiece

The midpiece exhibits eight (occasionally seven or nine) mitochondria tightly packed around a dense rootlet plus distal centriole complex (Fig. 7D–H). Profiles of the mitochondria are angular in transverse section, with the contacting surfaces of each being flattened. Although the distal centriole shows no modifications, the proximal one has been transformed into a portion of the rootlet attached anteriorly to a shallow indentation at the base of the nucleus and posteriorly to the distal centriole (Fig. 7E–H). The distal centriole is anchored to the plasma membrane via a radial array of nine satellite fibres (Fig. 7D), and is continuous with the doublets of the flagellar axoneme (Fig. 7E). The entire midpiece region (in each species) has a length of approximately 0.65 ± 0.05 μm ($N = 8$) and a maximum diameter of 1.7 ± 0.1 μm ($N = 8$).

Flagellum

The flagellum consists of a 9 + 2 microtubular configuration axoneme, sheathed by the plasma membrane (Fig. 7F). From light microscopic observations, flagellar length for both species is 47 ± 3.0 μm ($N = 10$ per species).

DISCUSSION

Comparison of *Hemidonax* sperm morphology with other heterodont bivalves

Spermatozoa of *Hemidonax pictus* are of the simple, aquasperm type characteristic of molluscs showing aquatic fertilization, especially the Bivalvia (Franzén, 1955, 1983; Popham, 1979; Hodgson *et al*., 1990; Healy, 1996a), and also seen in a number of other invertebrate groups (e.g. cnidarians, polychaete annelids, brachiopods, echiurids, sipunculans – Franzén, 1956; Baccetti & Afzelius, 1976; Jamieson & Rouse, 1989). Rouse & Jamieson (1987) differentiated aquasperm into those that fertilize eggs in the ambient water (ect-aquasperm) and those that fertilize aquatically but within a protected space such as a worm tube or a molluscan mantle cavity (ent-aquasperm). In the absence of any information on the fertilization biology of *H. pictus*, it is impossible to characterize the type of aquasperm in this species. However it is worth noting that in shipworms (Teredinidae, Pholadoidea) Popham (1974) found that species fertilizing within the mantle cavity had smaller acrosomes than those that fertilized externally (i.e. in the ambient seawater). If such a correlation holds among the Bivalvia in general, then the small size of the acrosome of *H. pictus* compared with most investigated heterodonts (see Figs 8, 9) would suggest the likelihood of ent-aquatic (mantle cavity) fertilization in this species and probably in other species of *Hemidonax*.

Whereas it is true to state that spermatozoa of *Hemidonax pictus* do not exhibit any unique or new features, the precise combination of features is distinctive and presumably characteristic of the genus as a whole. Like other heterodonts, *H. pictus* shows a well-developed basal ring component of the acrosomal vesicle (see Fig. 8, also for comparative figures and extensive literature see Healy, 1995b, 1996a). Substantial diversity exists among heterodont taxa in the shape of the acrosomal vesicle (and the shape and internal structure of the basal ring), as well as the size and length of the nucleus (short or long, straight, curved or helical) and the number of midpiece mitochondria (four or five, sometimes

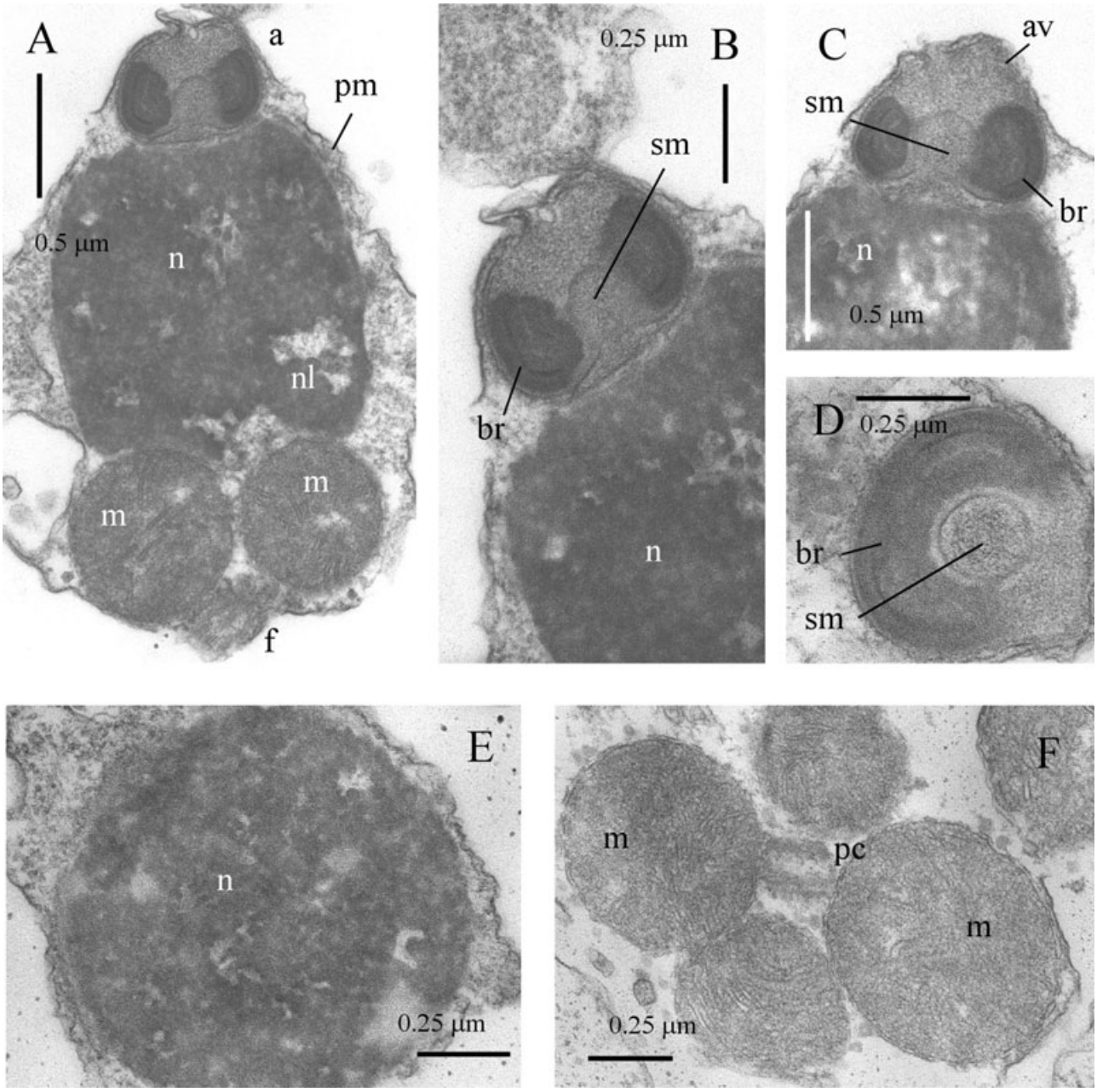

Figure 5. Ultrastructure (TEM) of spermatozoa of *Papyridea semisulcata*. A, longitudinal section (LS) showing acrosomal complex, nucleus, midpiece region (tangential, not through centrioles) and emergent flagellum. B, C, LS of acrosomal complex (acrosomal vesicle + subacrosomal deposit) and nuclear apex. Note internal differentiation of basal ring material. D, transverse section (TS) at posterior region (basal ring region) of acrosomal complex. Note layers of differing electron density within basal ring. E, TS of nucleus. F, TS of midpiece showing four mitochondria and proximal centriole (the latter shown in LS profile). Abbreviations: a, acrosomal complex; av, acrosomal vesicle; br, basal ring (of acrosomal vesicle contents); f, flagellum; m, mitochondrion of midpiece; n, nucleus; nl, nuclear lacuna; pc, proximal centriole; pm, plasma membrane; sm, subacrosomal material.

more, but always with a predominating number). As there are a number of differing opinions relating to the placement and affinities of *Hemidonax* among the Heterodonta, the following part of the discussion will deal with each of these, including a brief resumé of group features then progressing to a comparison with *Hemidonax* results.

HEMIDONAX AND THE DONACIDAE (TELLINOIDEA)

To date, five species of Donacidae have been examined in detail for sperm ultrastructure (the South African *Donax serra* Dillwyn, 1817, *D. madagascariensis* Wood, 1828, *D. sordidus* Hanley, 1845 – see Hodgson *et al*., 1990; the eastern Atlantic/Mediterranean *Donax trunculus* – see Sousa & Oliveira, 1994; the Australian *D. deltoides* Lamarck, 1818 – this study, see also Healy, 1995b). In all of these species (see Figs 8, 9), with the exception of *D. trunculus*, the acrosomal vesicle is large, with prominent, parallel layers within the basal ring, the nucleus is barrel-shaped and the midpiece contains the centrioles and four mitochondria. In *D. trunculus* the nucleus is appreciably longer than in other investigated species of *Donax*, but four midpiece mitochondria are observed (as also in other species). More significantly, no layering within the basal ring of the acrosomal vesicle was reported by Sousa & Oliveira (1994) nor can any trace of it be seen in their micrographs of mature or developing sperm (that all appear to show a homogeneously textured basal ring). Surprisingly, Sousa & Oliveira (1994) did not comment on the difference in acrosomal morphology between *D. trunculus* and the species examined by Hodgson *et al*. (1990). Healy (1995b) noted the difference and suggested the possible taxonomic utility of sperm in future studies of the Donacidae, as has already been demonstrated by Hodgson *et al*. (1990) for the three South African species examined by them (the close sperm similarity between *D. madagascariensis* and *D. sordidus* being linked to reports of hybridization in areas where these species occur together – see Discussion in Hodgson *et al*., 1990). A sixth species of *Donax*, the Indian Ocean *D. lubricus* Hanley, 1845 was examined briefly by Pal (1992, 1996), but the descriptions and micrographs provide incomplete data. As in other examined species of *Donax*, the acrosomal vesicle of *D. lubricus* is seated in a broad anterior depression of the nucleus, and the subacrosomal material is organized as an axial rod. However, in contrast to other species of *Donax,* but similar to groups such as the Mactroidea (Longo & Anderson, 1969; Hylander & Summers, 1977; Healy, 1995b; J. M. Healy, P. M. Mikkelsen & R. Bieler, unpubl. data), the anterior portion of the acrosomal vesicle appears to be largely devoid of contents. A more detailed study of this species would seem warranted.

Despite the spermatozoal differences between the two apparent 'groups' of *Donax*, all *Donax* sperm show two consistent differences from *Hemidonax pictus*: (1) the acrosomal vesicle is seated in a marked depression of the nuclear apex (*Hemidonax*: apex flat or showing minimal depression); and (2) the subacrosomal material is organized into a well-formed axial rod, often showing longitudinal-fibrous structure (*Hemidonax*: subacrosomal material consisting of diffuse granules). Further work on other species of Donacidae seems warranted, if only to reach a better appreciation of the range of sperm morphologies within this family (and possibly to assess the monophyletic status of the family). Available sperm ultrastructural data thus do not support the decision of some authors to allocate *Hemidonax* to the Donacidae (Lamy, 1917; Thiele, 1934; Keen, 1969; Vokes, 1980; Abbott & Dance, 1982) or Donacoidea (Scarlato & Starobogatov, 1979). The rather fragmentary sperm ultrastructural data for other Tellinoidea (see Sousa, Corral & Azevedo, 1989; Healy, 1995b; Sousa *et al*., 1998) likewise suggest no connection with Tellinidae, Semelidae or Scrobiculariidae. The suggestion by Schneider & Carter (2001) of a close relationship between *Hemidonax* and the Psammobiidae (Tellinoidea) also does not seem likely, at least based on the light microscopic results of Guerra, Campos & Esponda (1994) showing an elongate, rod-shaped nucleus in *Gari solida* (Gray, 1828). Certainly this hypothesis needs to be tested using TEM of psammobiid sperm.

HEMIDONAX AND THE CRASSATELLOIDEA (CRASSATELLIDAE, CARDITIDAE, ASTARTIDAE)

Sperm ultrastructure has been examined in three species of Crassatellidae, one species of Carditidae and one species of Astartidae (Healy, 1995a, b, 1996a; present study: Fig. 7). The crassatelloidean spermatozoon differs profoundly from those of *Hemidonax* in almost all features: (1) the acrosomal vesicle is long and attenuate and the subacrosomal material is developed as a thick axial rod (*Hemidonax*: acrosomal vesicle short and squat; subacrosomal material diffuse and granular); (2) the nucleus is narrow and rod-shaped (*Hemidonax*: nucleus short and wide); (3) the proximal centriole transforms into an electron-dense rod connecting the distal centriole (and axoneme) to the nuclear base (*Hemidonax*: proximal centriole unmodified); and (4) the midpiece consists of eight (occasionally seven or nine) wedge-shaped mitochondria, tightly packed around the centriolar complex (*Hemidonax*: midpiece exhibits four round, unmodified mitochondria). The idea of a crassatelloidean affinity for *Hemidonax* (e.g. Hedley, 1906, 1909, 1923) or for that matter a link between the Carditidae and *Hemidonax* + Cardiidae (e.g. Schneider & Carter, 2001) can safely be rejected purely on spermatological grounds.

HEMIDONAX AND THE CARDIIDAE (CARDIOIDEA)

A number of species of Cardiidae (*sensu* Schneider, 1992, 1995; now inclusive of Tridacnidae as subfamily

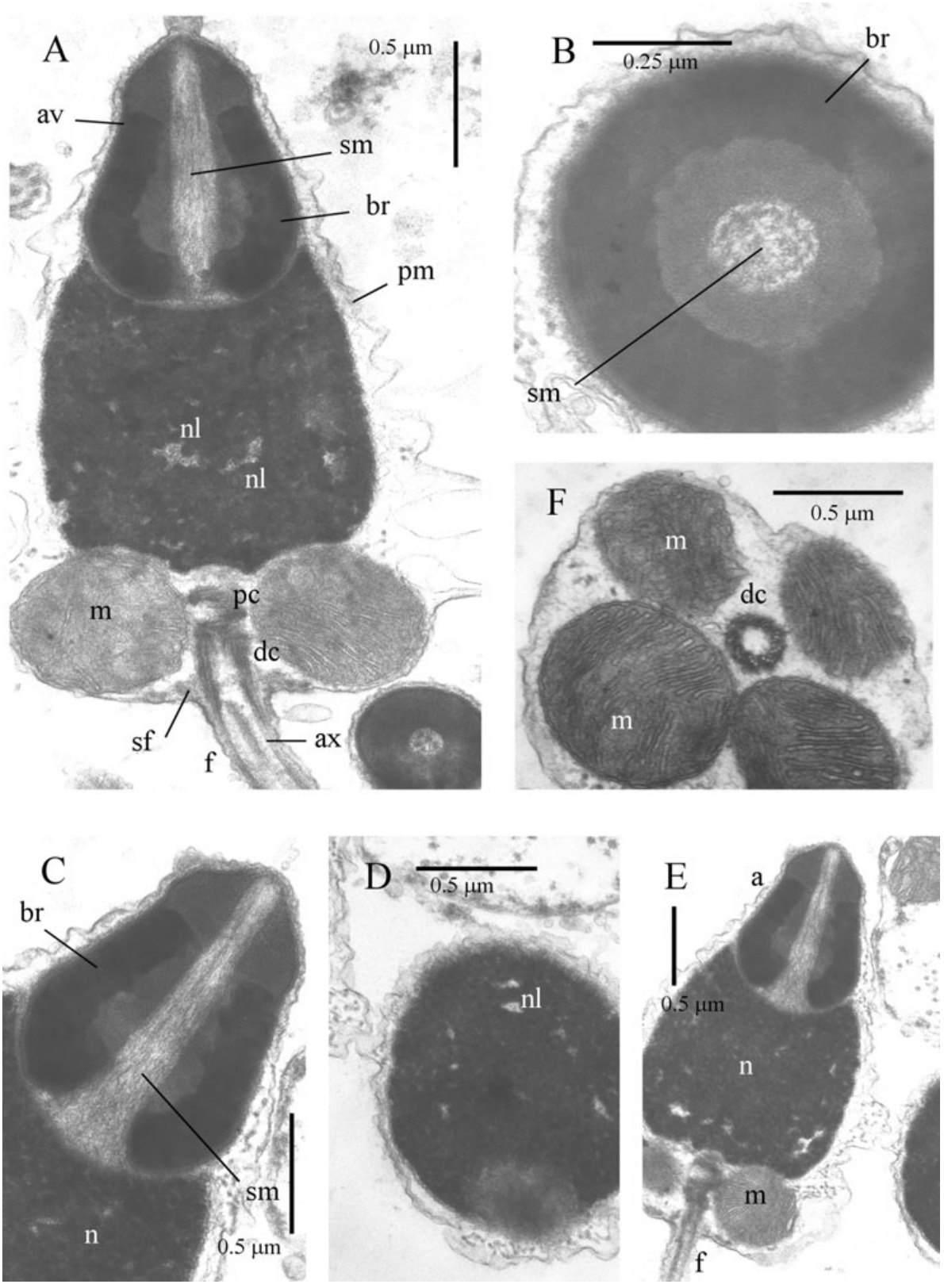

Figure 6. Ultrastructure (TEM) of spermatozoa of *Donax* (*Plebidonax*) *deltoides*. A, longitudinal section (LS) showing acrosomal complex, nucleus, midpiece (with proximal and distal centrioles) and proximal portion of flagellum. Note prominent depression at nuclear apex accommodating base of acrosomal complex. B, transverse section (TS) through acrosomal complex, showing parallel internal layers within basal ring, and homogeneous (less electron-dense) inner material. C, LS of acrosomal complex. Note longitudinally fibrous nature of subacrosomal material. D, TS of nucleus. E, LS of acrosomal complex, nucleus, midpiece and proximal portion of flagellum. F, TS of midpiece showing four mitochondria and triplet structure of distal centriole. Abbreviations: a, acrosomal complex; av, acrosomal vesicle; ax, axoneme; br, basal ring (of acrosomal vesicle contents); dc, distal centriole; f, flagellum; m, mitochondrion of midpiece; n, nucleus; nl, nuclear lacuna; pc, proximal centriole; pm, plasma membrane; sf, satellite fibres; sm, subacrosomal material.

Tridacninae) have been examined for sperm ultrastructure (Popham, 1979; Sousa & Azevedo, 1988; Healy, 1995b; Sousa *et al*., 1998; Keys & Healy, 1999, 2000; Drozdov *et al*., 2001; present study) and even though many genera and even some subfamilies await investigation, enough is known to provide a meaningful comparison with *Hemidonax pictus* (see Figs 8, 9). Sperm morphology in the Cardiidae varies widely between taxa, to such an extent in fact that no distinctive, family-defining characters are yet apparent. However, it can be stated that in all investigated species: (1) the acrosomal vesicle is never elongate (usually with a rounded or truncated apex); (2) the acrosomal complex is never seated in a depression of the nuclear apex; (3) the subacrosomal material is never formed into a well-defined rod (herein 'axial rod' or 'perforatorium'; although this does not preclude the generation of such a structure via polymerization of the subacrosomal material during the acrosome reaction); and (4) the midpiece almost always features four mitochondria (five occurring occasionally as a variant, but not predominating). Comparison of *H. pictus* with the range of sperm morphologies encountered in the Cardiidae to date (see Figs 8, 9) reveals that only *Fragum unedo* (Fragiinae) shows any degree of similarity to *H. pictus*, although the acrosomal vesicle is larger and more compressed and the nuclear profile is curved in longitudinal section (see Fig. 3). The basal invagination of the acrosomal vesicle of *H. pictus* and *F. unedo* is narrow within the anterior half of the vesicle, and the wedge-shaped basal ring profile is comparable between the two taxa. In almost all other investigated species of Cardiidae (including *Lunulicardia hemicardium* of the Fragiinae) the anterior region of the invagination is either as wide or wider than the basal region. A narrow invagination also appears present in *Cerastoderma* spp. (published micrographs are, unfortunately, few in number and lacking in much detail – see Sousa & Azevedo, 1988; Sousa *et al*., 1998; Drozdov *et al*., 2001), but, unlike that in *H. pictus* or *F. unedo*, and like certain species of Tridacninae (Keys & Healy, 1999, 2000), the nuclear apex projects deeply into this invagination (see Fig. 8). *Cerastoderma* spp. also exhibit marked helical coiling of the nucleus, a feature long ago recorded by Retzius (1905) and one also observed in the enigmatic cardioidean (?cardiid) genera *Monodacna*, *Didacna* and *Adacna* (see comparative light microscopic account by Karpevich, 1961). An apparent difference between *H. pictus* and the Cardiidae (based on available data) is the electron-lucent layer underlying the curved apex observed in the former. A similar layer has been seen by us in at least one venerid species (*Lioconcha annettae* Lamprell & Whitehead, 1990) (R. Bieler, P. M. Mikkelsen & J.M. Healy, unpubl. observ.) but we are hesitant to attach undue significance to it, as the apical region of the acrosomal vesicle is the most structurally unstable component of the acrosomal complex. If the electron-lucent layer is not an artefact of fixation, and occurs in other *Hemidonax* species, it may prove a useful genus-defining or even family-defining feature.

HEMIDONAX AND THE VENEROIDEA

Several non-cardioid members of the Veneroida have been examined for sperm ultrastructure (but still only a fraction of the known genera) especially among the Veneridae (Pochon-Masson & Gharagozlou, 1970; Gharagozlou-Van Ginneken & Pochon-Masson, 1971; Nicotra & Zappata, 1991; Reunov & Hodgson, 1994; Sousa *et al*., 1998; Healy *et al*., 2006). As part of a ongoing survey of sperm ultrastructure within the Veneroidea (and especially Veneridae), we have also examined many genera (J. M. Healy, P. M. Mikkelsen & R. Bieler, unpubl. data) and include results for a few previously unstudied examples in this account for comparison with *Hemidonax*. *Lioconcha annettae* and *Antigona chemnitzii* (Hanley, 1844) show similar acrosomal dimensions to *Hemidonax pictus*, but in both, the acrosomal complex rests in an anterior depression of the nucleus and is angularly tilted, and in neither of these venerids is the acrosomal vesicle invagination narrow anteriorly (see Figs 8, 9). In addition, there are nuclear differences (narrow and curved in both) and at least in *L. annettae* five rather than four mitochondria. As mentioned previously in this discussion, *L. annettae* exhibits an electron-lucent layer at the apex of the acrosomal vesicle,

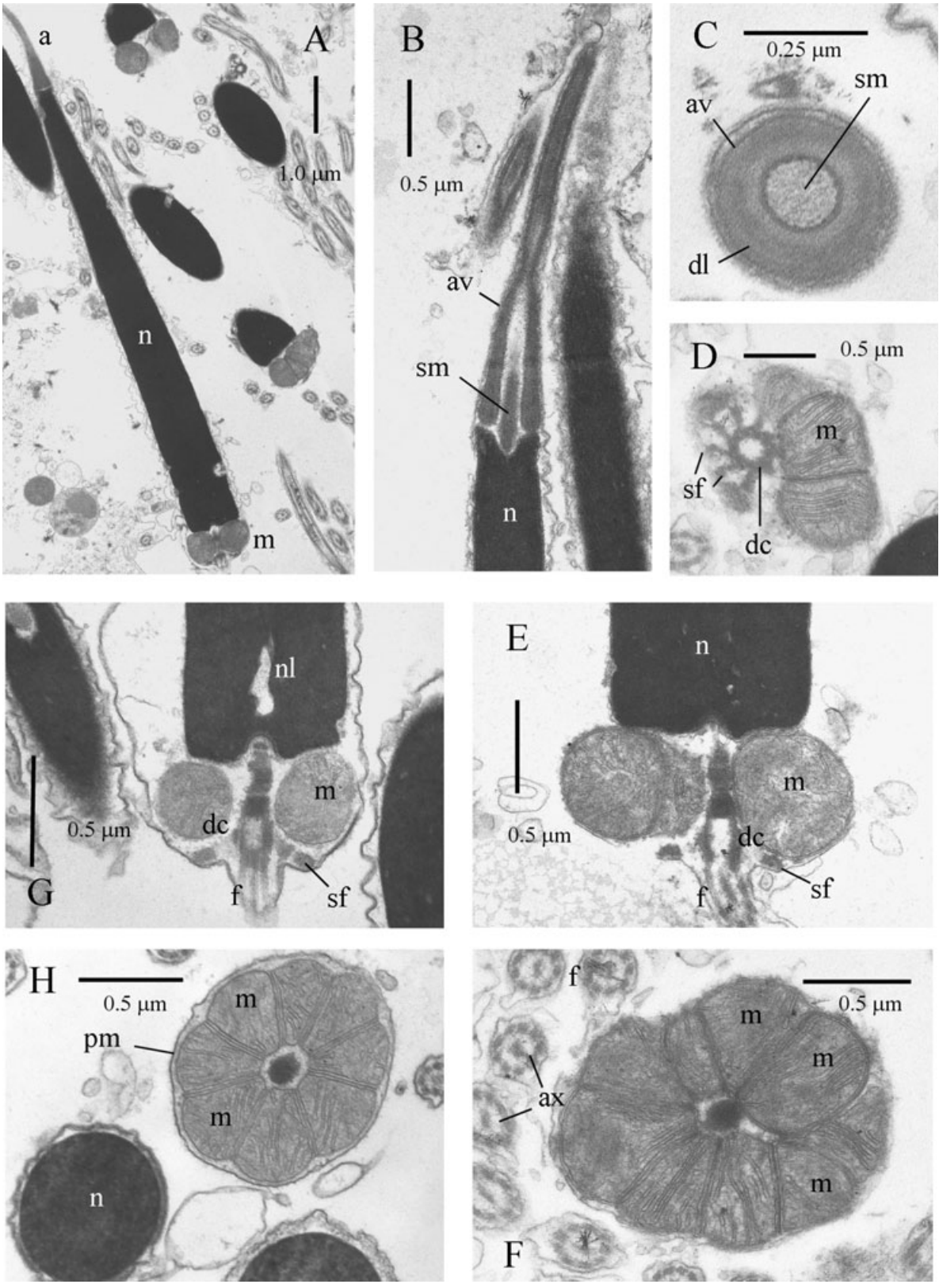

Figure 7. Ultrastructure of spermatozoa of two species of Crassatelloidea: *Eucrassatella cumingii* (Crassatellidae) (A, B, D–F) and *Cardita muricata* Lamarck, 1818 (Carditidae) (C, G, H) (TEM). A, longitudinal section (LS) of acrosomal complex, nucleus and midpiece. B, LS of acrosomal complex (subacrosomal material organized as axial rod) and nuclear apex (note anterior depression). C, transverse section (TS) of acrosomal complex. D, TS of posterior portion of midpiece showing distal centriole (composed of microtubular triplets) with associated radial array of satellite fibres. E, LS of base of nucleus, midpiece and proximal portion of flagellum. Note rod-like structure formed from the transformed proximal centriole. F, TS midpiece showing eight angular mitochondria surrounding the rod formed from the proximal centriole and rootlet. G, LS of base of nucleus (note nuclear lacuna) and midpiece. H, TS of nucleus and midpiece. Eight mitochondria surround the dense rod (transformed from proximal centriole). Abbreviations: a, acrosomal complex; av, acrosomal vesicle; dc, distal centriole; dl, dense core layer; f, flagellum; m, mitochondrion of midpiece; n, nucleus; nl, nuclear lacuna; pm, plasma membrane; sf, satellite fibres; sm, subacrosomal material.

similar to that observed in *H. pictus*. While this may prove significant, the lability of this region of the acrosomal complex (i.e. acrosome reaction stages) may be a factor and we reserve our judgement on the nature of this layer. In *Dosinia nedigna* (Iredale, 1930) the nucleus and midpiece are essentially as observed in *H. pictus*, but the acrosomal vesicle is tilted and exhibits a wide invagination (see Figs 7, 8). In most other investigated venerids, acrosomal, nuclear or midpiece features (usually a combination of these) do not closely tally with results obtained for *H. pictus* (see Pochon-Masson & Gharagozlou, 1970; Gharagozlou-Van Ginneken & Pochon-Masson, 1971; Reunov & Hodgson, 1994; Sousa *et al*., 1998). Only in *Callista chione* (Linnaeus, 1758) does the acrosomal vesicle approach that of *H. pictus* in shape, size and narrowness of the invagination, but even in this species, an axial rod is present within the subacrosomal material, the nucleus is slightly elongate and strongly curved, and the midpiece exhibits five mitochondria (see Nicotra & Zappata, 1991). However, despite various sperm differences between investigated Veneridae and *Hemidonax*, it remains impossible, at least at this stage, to rule out unequivocally a relationship between these two taxa, especially in view of the large number of venerid genera awaiting sperm study.

Taxonomic and phylogenetic considerations: the affinities of *Hemidonax*

'After examination and comparison of the shell and of the anatomy (both external and internal) of *Hemidonax* to both cardiids and donacids, I cannot justify placing *Hemidonax* as a member of the Cardioidea. However, neither can I place *Hemidonax* within the Donacidae. Instead, I favor placing *Hemidonax* as *incertae sedis* within the order Veneroida, until a phylogenetic analysis of the Veneroida is undertaken' (Schneider, 1992: 145). With those words, Jay Schneider – a recognized authority on cardioid systematics and phylogeny (see also Schneider, 1995, 1998a, b) – effectively reopened the debate concerning the affinities of *Hemidonax.* His rejection of a relationship between *Hemidonax* and the Donacidae (based on his own anatomical observations) was in accordance with the views of Boss (1971) and Ponder *et al*. (1981), and it can be said with confidence that all available sperm ultrastructural data (Hodgson *et al*., 1990; Pal, 1992, 1996; Sousa & Oliveira, 1994; Healy, 1995a; present study) likewise argue strongly against any connection between these two taxa. The same conclusion was reached by Schneider & Carter (2001) based on comparative shell microstructure. Schneider's other conclusion – that *Hemidonax* is non-cardioidean – is all the more remarkable when it is considered that he was not persuaded by the arguments of either Boss (1971) or Ponder *et al*. (1981), who strongly supported cardioid affinities for *Hemidonax*. Certainly, in terms of its anatomy, *Hemidonax* shows a number of features not consistent with placement in the Cardiidae as outlined in some detail by Ponder *et al*. (1981; who argued for retention of a separate family Hemidonacidae). Perhaps significantly, neither Boss (1971) nor Ponder *et al*. (1981) identify any affiliations between *Hemidonax* and any cardioidean genus, lending some degree of credence to Schneider's (1992) decision to leave *Hemidonax* as *incertae sedis* among the Veneroida. It is of interest to note that Keen (1980), while accepting Boss's (1971) subfamily Hemidonacinae within the Cardiidae, did not choose to discuss the relationships of *Hemidonax* [being evidently influenced by Wilson & Stevenson's (1977) decision not to include the genus in their review of the Western Australian Cardiidae]. Keen, however, grouped the Fraginae and Hemidonacinae in her tabulation of cardiid shell features and also in her taxonomic keys, and by so doing perhaps was hinting at the possibility of some relationship between the two subfamilies. Most recently, Schneider & Carter (2001) have argued, largely on the basis of comparative shell microstructure, that *Hemidonax* shows much closer affinity with the tellinoidean family Psammobiidae than with the Cardiidae (or any other

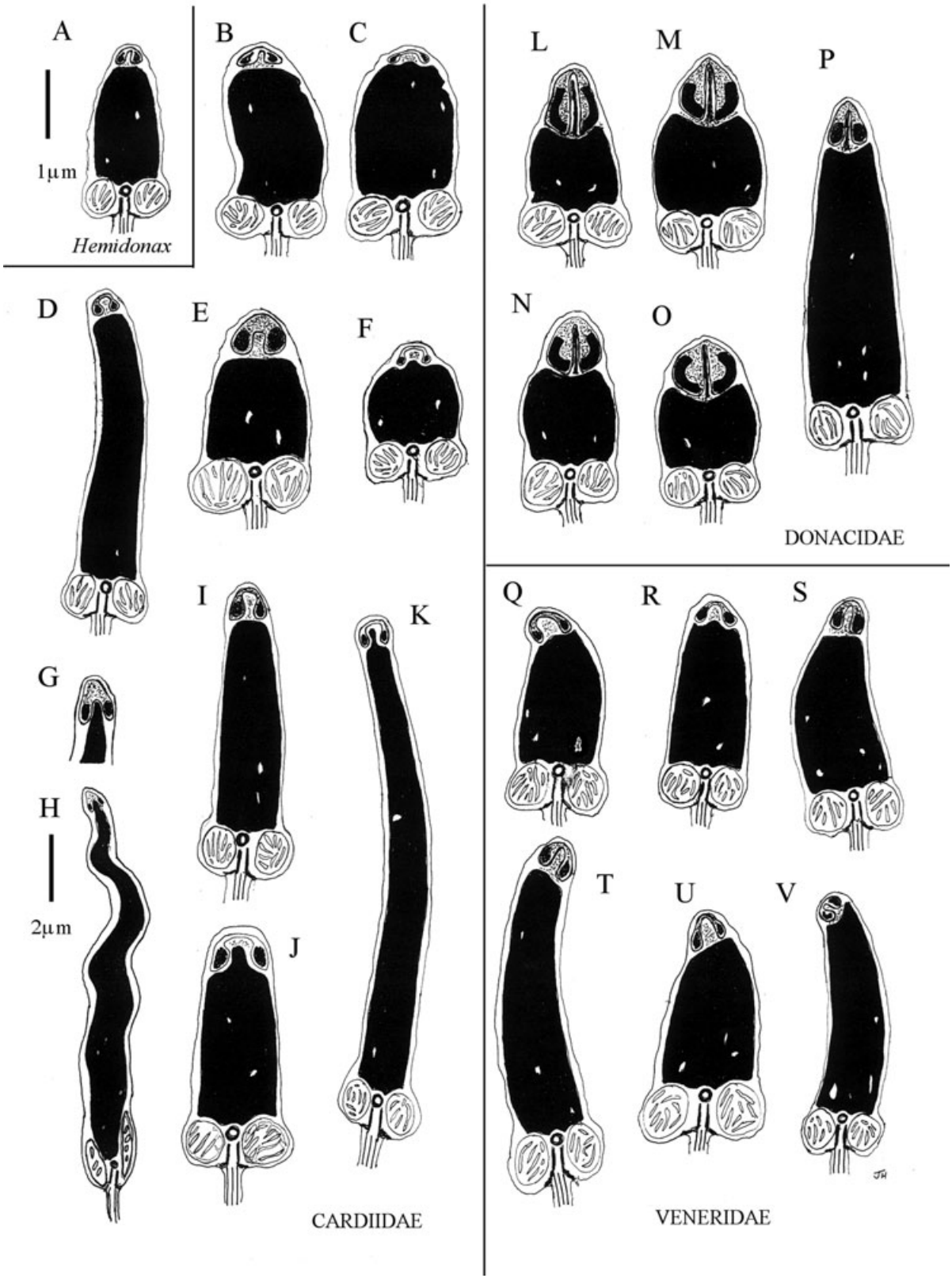

Figure 8. Diagram showing comparative sperm ultrastructure (TEM) (longitudinal sections through head, midpiece and proximal portion of flagellum) of *Hemidonax pictus* (Tryon, 1870) (A), Cardiidae (B–K), Donacidae (L–P) and selected Veneridae (Q–V). B–K, Cardiidae. B, *Fragum unedo* (Linnaeus, 1758); C, *Lunulicardia hemicardium* (Linnaeus, 1758); D, *Vasticardium vertebratum* (Jonas, 1844); E, *Papyridea semisulcata* (Gray, 1825); F, *Fulvia tenuicostata* (Lamarck, 1819); G, H, *Cerastoderma* spp.; I, *Hippopus hippopus* (Linnaeus, 1758); J, *Tridacna gigas* (Linnaeus, 1758); K, *Tridacna maxima* (Röding, 1798). L–P, Donacidae. L, *Donax deltoides* Lamarck, 1818; M, *Donax madagascariensis* Wood, 1828; N, *Donax sordidus* Hanley, 1845; O, *Donax serra* Röding, 1798; P, *Donax trunculus* Linnaeus, 1758. Q–V, Veneridae. Q, *Venerupis aurea* (Gmelin, 1791); R, *Venerupis corrugata* (Gmelin, 1791); S, *Petricola lapicida* (Gmelin, 1791); T, *Antigona chemnitzii* (Hanley, 1844); U, *Dosinia nedigna* (Iredale, 1930); V, *Lioconcha annettae* Lamprell & Whitehead, 1990. Scale bar for *Hemidonax pictus* (= 1.0 μm) applies to all other figures except H (= 2.0 μm). Sources of data: A–E, L, S–V (this paper; J. M. Healy, P. M. Mikkelsen & R. Bieler, unpubl. data); F (Popham, 1979); G, H [composite reconstruction based on *Cerastoderma lamarcki* (Reeve, 1844) from Drozdov *et al*., 2001 and *Cerastoderma edule* (Linnaeus, 1758) from Sousa & Azevedo, 1988 and Sousa *et al*., 1998]; I–K (Keys & Healy, 1999, 2000); M–O (Hodgson *et al*., 1990); P (Sousa & Oliveira, 1994); Q, R (Pochon-Masson & Gharagozlou, 1970; Gharagozlou-Van Ginneken & Pochon-Masson, 1971).

group of heterodonts). Whereas it is true that shell microstucture has proven a very valuable source of characters for phylogenetic analysis, Schneider & Carter (2001) have not offered any explanation for the key anatomical differences between *Hemidonax* and the Tellinoidea, particularly the absence of the cruciform muscle (its presence is a synapomorphy of the Tellinoidea – see also Boss, 1971, 1982). In this connection it is worth noting that *Pharus* and the Novaculininae (both originally included in Solecurtidae) were often cited as tellinoidean taxa lacking a cruciform muscle (Yonge, 1949, 1959; Ponder *et al*., 1981; Boss, 1982) but are now both placed within the Solenoidea (e.g. Morton, 1984; von Cosel, 1990; Willan, 1998).

CONCLUSIONS

In the present account we have examined the features of the mature gonadial spermatozoa of *Hemidonax pictus*, and provided comparisons with other heterodonts, especially the two most favoured affiliates, the Donacidae (Tellinoidea) and the Cardiidae (Cardioidea). In addition we have drawn attention to important sperm similarities (some albeit of a rather broad nature) with the Veneridae.

We conclude, based on the available data, that the spermatozoan features of *Hemidonax pictus* do not show a close match to those of the investigated 13 species of Cardioidea, with *Fragum unedo* being the most similar. If Keen (1980) did intend to suggest a connection between the Fragiinae and Hemidonacinae, then it would appear that sperm morphology supplies some supporting evidence for this stance (i.e. a connection with *Fragum*) and some against (e.g. differences between *Hemidonax* and *Lunulicardia*). There are also very interesting sperm similarities (especially acrosomal) to various members of the Veneridae, although no species examined to date completely matches our results for *H. pictus*. Finally, we can find no sperm ultrastructural evidence to indicate that a Hemidonacidae + Cardiidae group either arose from or is otherwise related to the Carditidae or other crassatelloideans (cf. Schneider & Carter, 2001). Spermatozoa of crassatelloideans are very distinctive (Healy, 1995a, b; present study), and given the antiquity of families such as the Astartidae, have long had their own evolutionary pathway.

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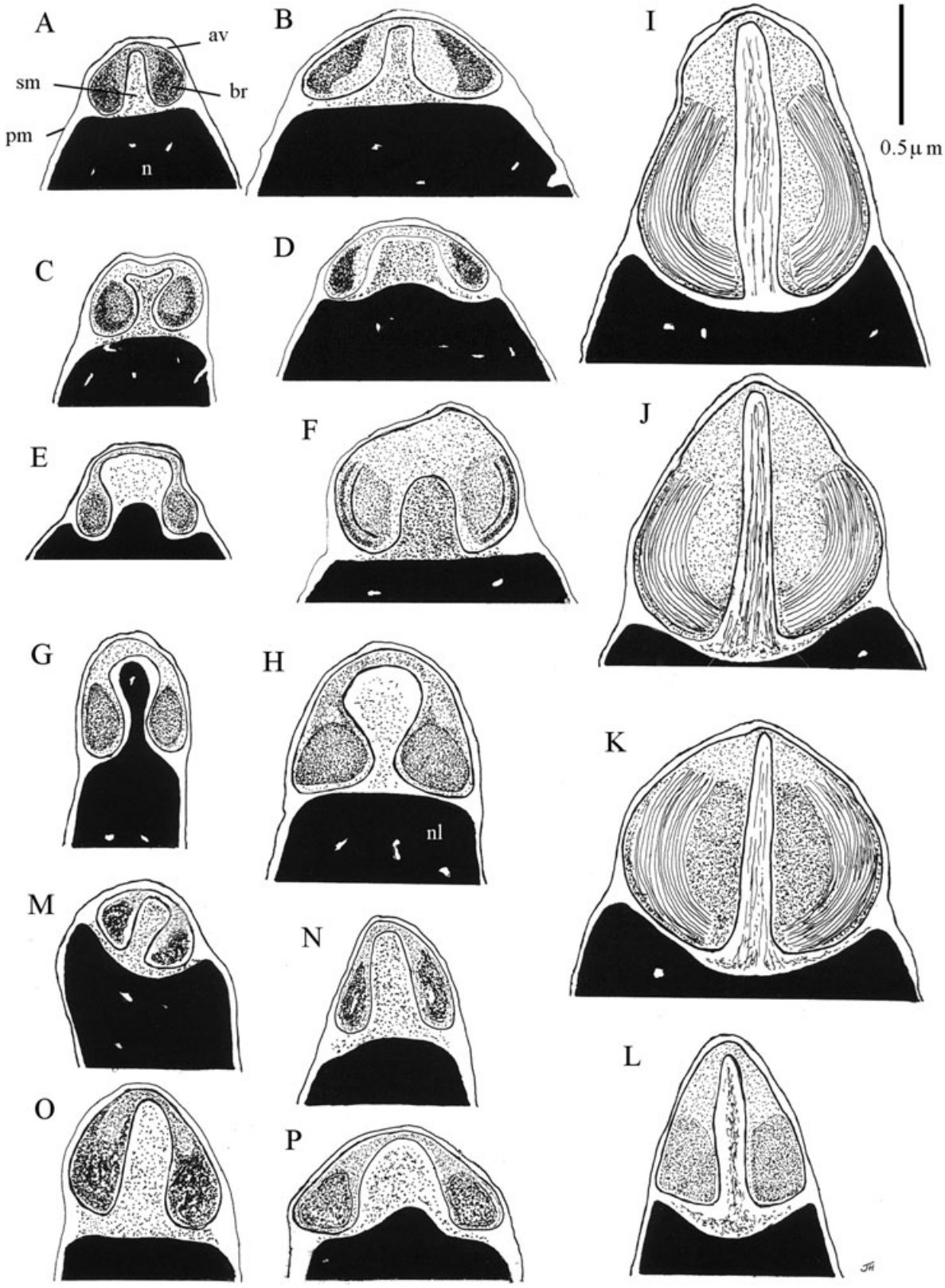

Figure 9. Diagram showing comparative acrosomal complex ultrastructure (TEM) of *Hemidonax pictus* (A), Cardiidae, Donacidae and selected Veneridae. B–H, Cardiidae: B, *Fragum unedo*; C, *Vasticardium vertebratum*; D, *Lunulicardia hemicardium*; E, *Fulvia tenuicostata*; F, *Papyridea semisulcata*; G, *Tridacna maxima*; H, *Hippopus hippopus*. I–L, Donacidae: I, *Donax deltoides*; J, *Donax madagascariensis*; K, *Donax serra*; L, *Donax trunculus*. M–P, Veneridae: M, *Lioconcha annettae*; N, *Dosinia nedigna*; O, *Antigona chemnitzii*; P, *Venerupis corrugata*. Scale bar (1.0 μm) applies to all figures. Sources of data: A–D, F, I, M–O (this paper; J. M. Healy, P. M. Mikkelsen & R. Bieler, unpubl. data); E (Popham, 1979); G, H (Keys & Healy, 1999, 2000); P (Gharagozlou-Van Ginneken & Pochon-Masson, 1971); J, K (Hodgson *et al*., 1990); L (Sousa & Oliveira, 1994).

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