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ORIGINAL PAPER

Ultrastructure and potential taxonomic importance of euspermatozoa and paraspermatozoa in the volutid gastropods *Zidona dufresnei* and *Provocator mirabilis* (Caenogastropoda, Mollusca)

Juliana Giménez · John M. Healy · Gladys N. Hermida · Fabiana Lo Nostro · Pablo E. Penchaszadeh

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Abstract The ultrastructure of mature spermatozoa is investigated for the first time in the Volutidae, based on the commercially significant South American species *Zidona dufresnei* (Donovan, 1823) (fresh material) and supplemented with observations on testicular (museum) material of the deep sea New Zealand species *Provocator mirabilis* (Finlay, 1926). Euspermatozoa of *Z. dufresnei* (*ex* sperm duct) consist of: (1) a tall-conical acrosomal vesicle (with short basal invagination, constricted anteriorly) which is flattened anteriorly and associated with an axial rod, centrally perforate basal plate and short accessory membrane; (2) a rod-shaped, solid and highly electron-dense nucleus (with short basal fossa containing centriolar complex and initial portion of a 9 + 2 axoneme); (3) an elongate midpiece consisting of the axoneme sheathed by 5–6 helical mitochondrial elements, each exhibiting a dense U-shaped outer layer; (4) an elongate glycogen piece (axoneme sheathed by nine tracts of putative glycogen granules); (5) a dense annulus at the junction of the midpiece and glycogen piece and (6) a short free tail region (axoneme surrounded only by plasma membrane). Paraspermatozoa of *Z. dufresnei* are vermiform and dimorphic: the first type contains approximately 14–20 axonemes (arranged peripherally and interspersed with microtubules) and numerous oblong dense vesicles, numerous less dense (round) vesicles, occasional, large lipid-like vesicles, and scattered mitochondria; the second type contains 25–31 axonemes (peripherally arranged, interspersed with microtubules), occasional mitochondria and extensive cytoplasm. Results obtained for *P. mirabilis* from testis material are essentially as observed in *Z. dufresnei*, although the euspermatozoan acrosome still has to achieve its compressed transverse profile. Observations on paraspermatozoa were limited by fixation quality of available (testis) tissues, but these cells are similar to the first type of *Zidona* paraspermatozoa. Although most of the euspermatozoal features are also observed in many neotaenioglossans and neogastropods, the U-shaped outer layer of each mitochondrial element has not previously been reported and may prove a diagnostic feature of the Volutidae, the subfamily Zidoniinae or possibly only the Zidonini (in which *Z. dufresnei* and *P. mirabilis* are currently placed).

J. Giménez (✉) · P. E. Penchaszadeh
Laboratorio de Invertebrados. Depto. de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pab. II, Buenos Aires C1428EHA, Argentina
e-mail: jgimenez@bg.fcen.uba.ar

J. Giménez · P. E. Penchaszadeh
CONICET and Museo Argentino de Ciencias Naturales, Av. Angel Gallardo 470, C1405DJR Buenos Aires, Argentina

J. M. Healy
Biodiversity Program, Queensland Museum, PO Box 3300, South Bank, Brisbane, QLD 4101, Australia

J. M. Healy
Department of Zoology, Field Museum of Natural History, Chicago, IL, USA

G. N. Hermida
Laboratorio de Histología Animal. Depto. de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pab. II, Buenos Aires C1428EHA, Argentina

F. L. Nostro
CONICET and Laboratorio de Embriología Animal. Depto. de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pab. II, Buenos Aires C1428EHA, Argentina

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Introduction

The morphological diversity of spermatozoa in gastropods has been considered as a guide to understand phylogenetic and taxonomic relationships within mollusc. The high diversity and abundance exhibited by the volutids render it particularly interesting. Among many groups of marine gastropods need a comprehensive revision. In contrast to many other species of the Neogastropoda, the reproductive biology of the Volutidae has not been intensively (or comparatively) examined, with most literature on this group still centring on systematics, (for example see Weaver and du Pont 1970; Novelli and Novelli 1982; Darragh 1988; Poppe and Goto 1992; Bondarev 1995; Bail and Poppe 2001; Bail et al. 2001). Recently however, detailed studies have been undertaken on the commercially significant species *Zidona dufresnei* which have provided the much needed database on the timing and extent of reproduction (Penchaszadeh and De Mahieu 1976; Giménez and Penchaszadeh 2002, 2003, Giménez et al. 2004, 2005). As an extension of this work, we present the first ultrastructural study of sperm morphology in the Volutidae based primarily on *Z. dufresnei* with supplementary observations on a second member of this group, the deep sea New Zealand species *Provocator mirabilis*. Comparisons are made with other caenogastropods, in particular with other species of the Neogastropoda to identify possible diagnostic sperm features of the Volutidae and to assess the relationships of volutids and other neogastropods.

We believe that these new ultrastructural descriptions may contribute to resolving some of the relationship of the Volutidae.

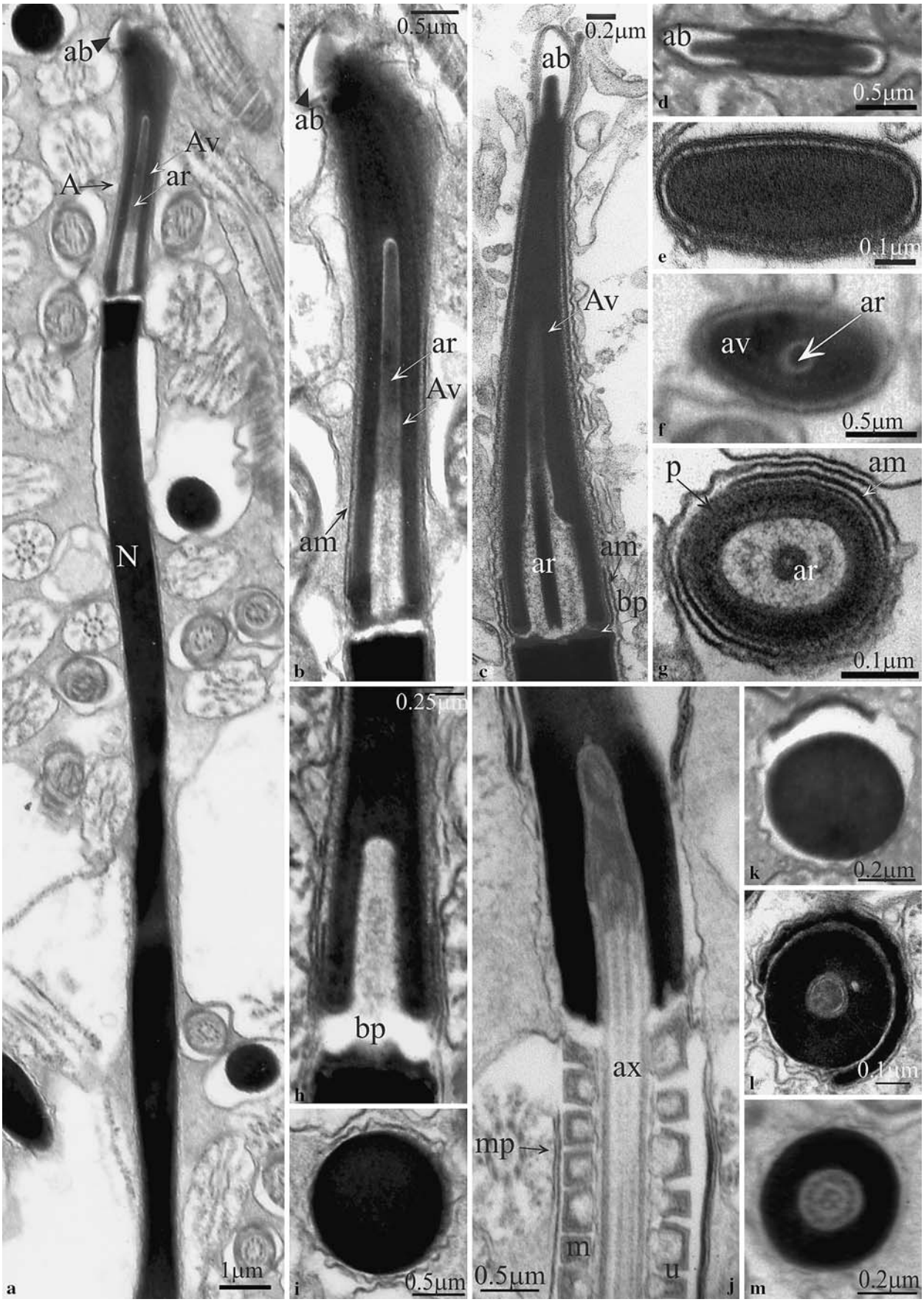
Materials and methods

Reproductively mature males of *Zidona dufresnei* (Donovan, 1823) (Volutidae, Caenogastropoda) were obtained through commercial fishing outlets at Mar del Plata Harbour from (57°37′W, 38°20′S) at depths from 40 to 60 m. After removal from the shell, pieces of the testis (9 mm^3) or sperm duct were fixed in modified Truby (3% glutaraldehyde in 0.1 M sodium phosphate buffer containing 0.1% $CaCl_2$) (for 4 h at 4°C) and subsequently washed thoroughly either in sucrose-adjusted cacodylate buffer or in $CaCl_2$-adjusted phosphate buffer. Subsequently the tissue pieces were placed in a 1% solution of osmium tetroxide (in 0.1 M cacodylate or phosphate buffer) for 1.5 h and again washed in buffer. Tissues were dehydrated using an ascending series of ethanols (from 20% to absolute ethanol), then placed first in a 1:1 ethanol:propylene oxide solution for 15 min and finally embedded in Araldite resin. For *P. mirabilis* (Finlay, 1926) (Volutidae, Caenogastropoda) testis samples were obtained from an Australian Museum (Sydney) specimen collected off Otago Heads, New Zealand (171°1′E, 45°50′S) from a depth of 540–590 m (Mu 70/45). This specimen was originally fixed in Bouin's solution (in 1977) and later transferred (for shelf storage) to sea water formalin (5% formalin in 95% sea water, buffered to pH 7.2). Tissues were dehydrated and observed in the Scanning electron microscopy. Tissues were transferred to phosphate buffer for 40 min, postfixed in 1% OsO_4 in 0.1 M sodium phosphate buffer for 1.5 h, again buffer-rinsed (40 min), ethanol-dehydrated and embedded in Spurr's resin. Ultrathin sections were cut using either a Reichert or an LKB IV ultramicrotome and stained with uranyl acetate and lead citrate. All sections were examined and photographed using Zeiss (Oberkochen, Germany) EM 109T, Hitachi 300 and Jeol 1010 transmission electron microscopes operated at 75–80 kV. Total sperm lengths were determined by viewing and photographing tissue squashes using a Zeiss Axiostar light microscope.

Fig. 1 Euspermatozoa of *Zidona dufresnei* **a** longitudinal section (LS) through acrosomal complex and anterior portion of nucleus. **b**, **c** LS showing detail of components of the acrosomal complex. Note in 1c the constriction of the acrosomal vesicle invagination (starting point indicated by *arrow*); the numbers correspond to transverse sections of **d**–**g**). **d–g** Series of transverse sections (TS) in series from apical bleb region of the acrosomal vesicle (**d**) to basal region (**g**). Note lateral flattening of the acrosomal complex beginning in anterior portion of the invagination (**d**–**f**), presence of radiating plates within the vesicle contents and accessory membrane (**g**). **h** LS showing base of acrosomal complex, in particular the centrally perforate basal plate lying on the nuclear apex. **i** TS nucleus anterior to centriolar fossa. **j** LS junction of nucleus (showing invagination and centriole/axoneme insertion) and anterior portion of midpiece. Note helical midpiece elements (defined by dense U-shaped profiles of periphery) and dense layer associated with plasma membrane (*arrow*). **k–m** TS nucleus with portion of dense membrane (**k**), anterior of centriolar fossa with extensive dense membrane (**l**), centrolar fossa with axoneme (**m**). *A* acrosomal complex, *ab* apical bleb, *ar* axial rod material, *Av* acrosomal vesicle, *am* accessory membrane, *ax* axoneme, *bp* basal plate, *m* mitochondrion, *mp* midpiece, *N* nucleus, *U* U-shaped defining edge of mitochondrial element

Results

Both *Z. dufresnei* and *P. mirabilis* exhibit two main types of spermatozoa: (1) euspermatozoa (fertile sperm composed of an acrosomal complex, nucleus, midpiece, glycogen piece and end piece with a single incorporated axoneme) and (2) paraspermatozoa (vermiform cells with multiple incorporated axonemes). In the following description, the text applies principally to *Z. dufresnei*, with supplementary observations on *P. mirabilis* (immature testicular euspermatozoa and paraspermatozoa) wherever relevant.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Euspermatozoa

Acrosomal complex

The acrosomal complex consists of a tall-conical, membrane-bound acrosomal vesicle, an axial rod and a basal plate (Fig. 1a–h). The acrosomal vesicle is approximately 4.3 µm long and its contents moderately electron-dense, with radially arranged plate-like structures set in a finely granular matrix (Fig. 1b–g). Apically the vesicle membrane separates from the vesicle contents and lies close to the plasma membrane to form an electron-lucent, balloon-like space, the apical bleb (Fig. 1a–d). The acrosomal vesicle exhibits a very deep invagination (length 4.1 µm) within which is situated the axial rod (subacrosomal material) (Fig. 1a–c, f, g). Although some longitudinal sections may suggest that the vesicle invagination is short (Fig. 1h), this is the result of a constriction of the invagination (compare with the true, axial plane of section shown in Fig. 1a–c). A short (0.5 µm) accessory membrane is closely associated with the base of the acrosomal vesicle (Figs. 1c, g, h). The centrally perforate, basal plate is positioned on the nuclear apex, and separated from the basal rim of the vesicle by a space of somewhat variable width (Fig. 1b, c, h). The transverse profile of the acrosomal vesicle changes gradually from oval (or near-so) basally (Fig. 1g) to compressed laterally at and above the anterior region of the invagination (Fig. 1d, e), to flat in the region of the apical bleb (Fig. 1d). The radiating plates of the acrosomal vesicle are conspicuously more electron-dense than the surrounding contents of the vesicle (Fig. 1g). Acrosomal morphology *P. mirabilis* (testis material only observed) resembles that of testicular (immature) euspermatozoa of *Z. dufresnei* (Fig. 3e) with very well defined radiating plates forming a highly electron-dense layer. The acrosomal vesicle (length approximately 3.5 µm) shows no evidence of the lateral compression observed in *Z. dufresnei* (Fig. 3a, c, d), but given that such compression occurs very late in spermiogenesis (J. Giménez et al., Paraspermatogenesis and spermatogenesis in the volutid *Zidona dufresnei* (Donovan, 1823) (Mollusca, Caenogastropoda), unpublished), this was to be expected. Our data show that, at least in *P. mirabilis*, the axial rod may exhibit transverse connectives to the invagination wall of the acrosomal vesicle, at least in the immature testicular euspermatozoa (Fig. 3c, d).

Nucleus

The nucleus is filiform (length from light microscopy 25 ± 3 µm), highly electron-dense and solid, with the exception of a short (2.1 ± 0.2 µm) invagination basally (Fig. 1a, i–m). The basal invagination contains a centriolar derivative which anchors, and is continuous with the initial portion of a 9 + 2 microtubular pattern axoneme (Fig. 1j, l, m). A dense layer was often observed associated with the posterior region portion of the nucleus (Fig. 1j, l) and the anterior region of the midpiece (Fig. 2a). This layer appears to be either a thickening or folding of the plasma membrane, and its status as a feature of the mature eusperatozoa remains uncertain, especially given its often irregular shape. Nuclear morphology in *P. mirabilis* is essentially as observed in *Z. dufresnei*, including the structure of the basal invagination, but the basal invagination is noticeably deeper than observed in *Z. dufresnei* (Fig. 3b). Length of nucleus in *P. mirabilis* was approximately 20 ± 2 µm.

Midpiece

Posterior to the nucleus, the axoneme is enclosed in a mitochondrial sheath to form the midpiece region (Figs. 1j, 2a–e). However, oblique longitudinal sections through the midpiece clearly show that the sheath consists of 5–6 helically disposed mitochondrial elements around the axoneme (Fig. 2a–d). In transverse section, the elements appear to blend into a continuous sheath, but oblique longitudinal sections show at least the denser, outer portion of each element to be distinct, although evidently devoid of discernible cristae (Fig. 2b–d). However, in all sections, it is apparent that each element exhibits a, U-shaped, bilaminar, outer layer which is noticeably more electron-dense than the remaining mitochondrial material. The dense membrane layer often observed in the nuclear region is also seen in the midpiece region (Fig. 2a). In *P. mirabilis*, the angular, U-shaped outer layer of the mitochondrial elements is clearly visible in museum material examined by us (unfortunately originally fixed in Bouin's solution—much inferior primary fixative than glutaraldehyde) indicating that it has greater structural durability than the mitochondrial matrix, and confirming its reality as a discrete sperm component (Fig. 3b). Fixation of the available material of *P. mirabilis* was not good enough to determine the presence or absence of the dense layer observed for *Z. dufresnei*.

Annular complex and glycogen piece

Beyond the midpiece the axoneme is associated with nine longitudinal and radiating tracts of dense granules (one tract per axonemal doublet) (Fig. 2e–h). Although not cytochemically tested by us, the glycogen composition of these granules has been demonstrated in several previous studies of gastropod euspermatozoa (e.g. Giusti 1969; Giusti and Mazzini 1973; Anderson and Personne 1970, 1976; Selmi and Giusti 1980; Wilson and Healy 2006) and it is safe to assume that this applies to the two volutid species examined herein. At the immediate junction of the

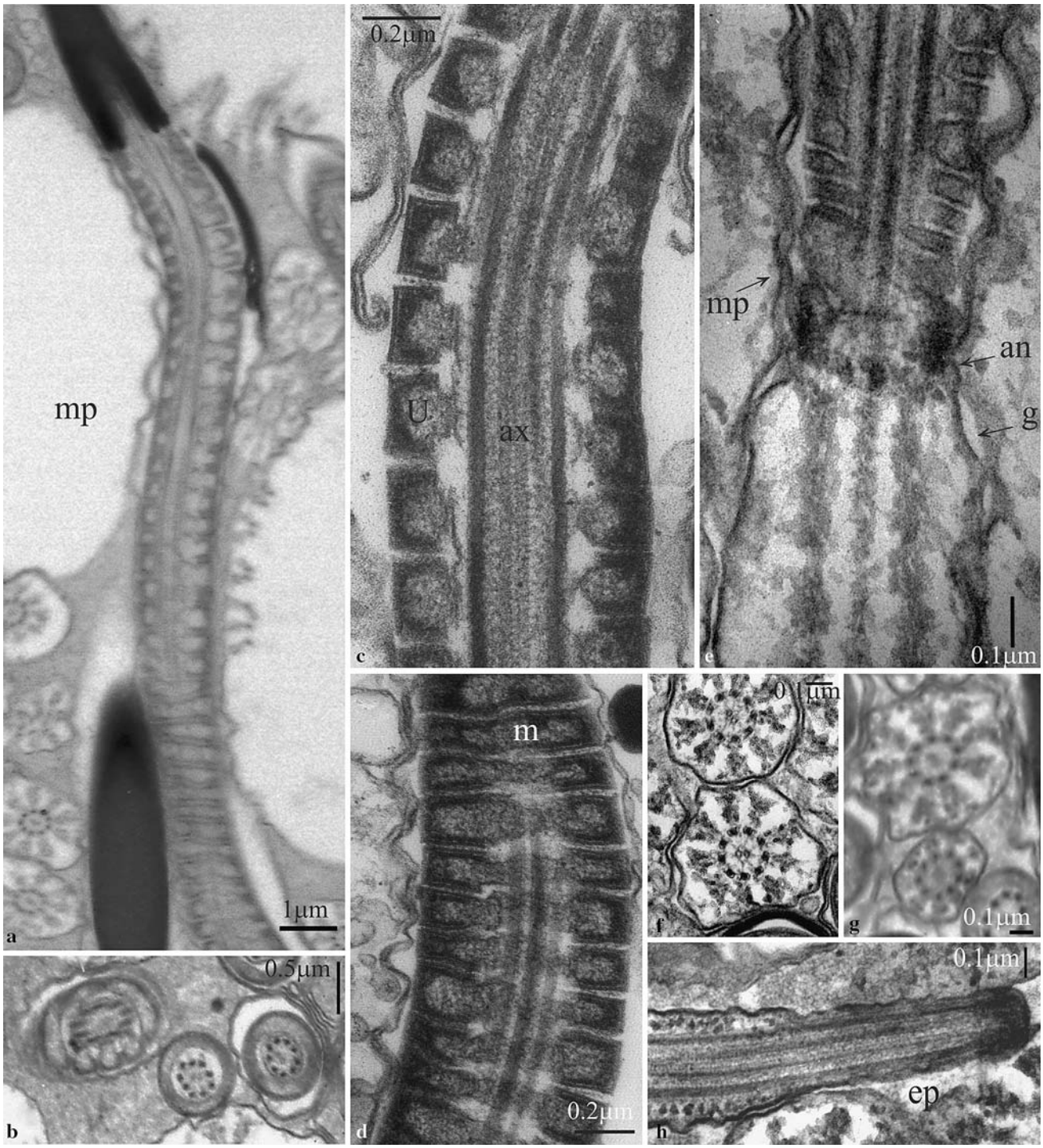


Fig. 2 Euspermatozoa of *Zidona dufresnei* **a** LS nucleus and anterior portion of midpiece. Dense layer (*arrow*) visible. **b** TS midpiece region showing dense periphery of midpiece elements and less dense matrix. **c**, **d** LS and oblique LS of midpiece showing internal structure of U-shaped outer layer, and spiralling of mitochondria around axoneme. **e** LS junction of midpiece and glycogen piece. Note annular complex. **f** TS glycogen piece showing radiating, longitudinal rows of putative glycogen granules. **g** TS showing decrease in diameter of glycogen piece towards posterior region of cell. **h** LS termination of glycogen piece and entire end piece region. *an* annulus; *ax* axoneme; e*p* end piece; *g* putative glycogen granules; *m* mitochondrion; *mp* midpiece; *n* nucleus; *U* U-shaped defining edge of mitochondrial element

midpiece and glycogen piece is an annular complex, consisting of a double ring attached to the inside surface of the plasma membrane (Fig. 2e). The annular complex and glycogen piece were not observable in *P. mirabilis* due to the inadequate fixation of these regions in our museum material.

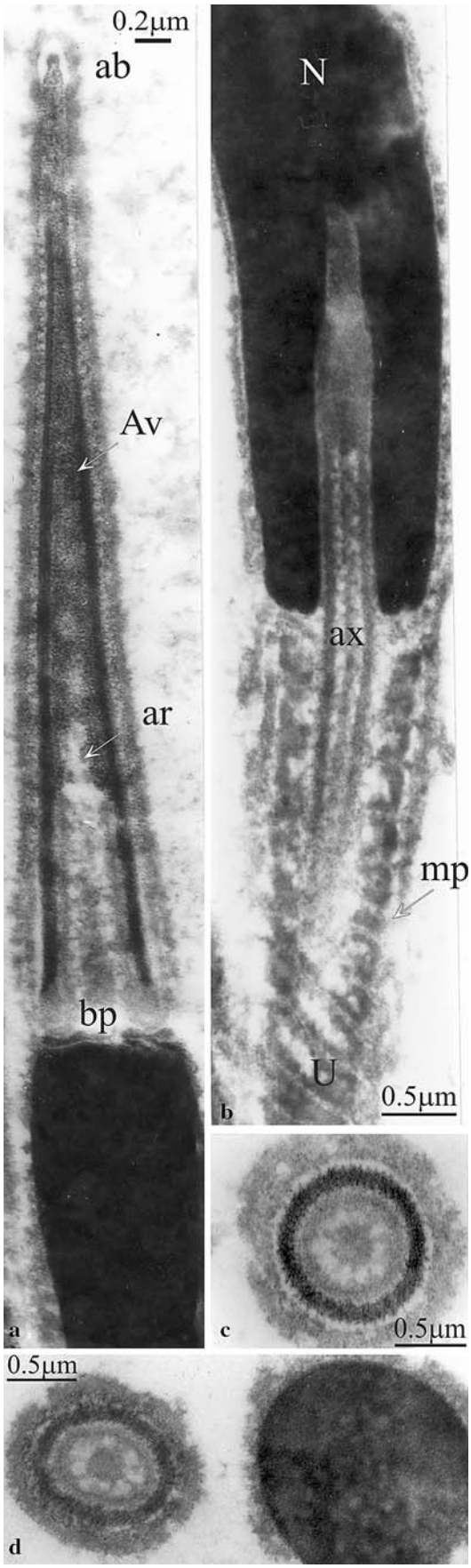


Fig. 3 **a–d** Testis euspermatozoa (immature) of *Provocator mirabilis* (Bouin's fixed material). **a** Highly electron-dense, internal layer (radiating plate layer) and axial rod with the basal invagination. **b** LS junction of nucleus (basal invagination containing centriolar derivative and initial portion of axoneme) and midpiece. Note the clearly marked U-shaped outer regions of each mitochondrial element persisting despite suboptimal fixation. **c** Transverse section (TS) showing radiating plates of acrosomal vesicle and apparent connectives between axial rod and acrosomal vesicle. **d** TS acrosomal vesicle and nucleus. *a* acrosomal complex; *ab* apical bleb; *ar* axial rod material; *Av* acrosomal vesicle; *ax* axoneme; *bp* basal plate; *mp* midpiece; *N* nucleus; *U* U-shaped defining edge of mitochondrial element

End piece

A very short end piece (length 0.75 ± 0.05 µm) succeeds the glycogen piece and consists of the continuing 9 + 2 microtubular pattern axoneme and surrounding plasma membrane (Fig. 2h). The structure and length of the end piece were not observable in *P. mirabilis*.

Aberrant euspermatozoa

In addition to euspermatozoa and paraspermatozoa, the present study detected euspermatozoa with two axonemes and irregularly arranged mitochondrial elements (Fig. 4a, c), disruption of the centriolar complex within the nuclear fossa (Fig. 4a), disruption of the axoneme within the glycogen piece (Fig. 4e, f) or occasionally showing absence of a glycogen sheath posterior to the annulus (Fig. 4b). Perhaps the most extreme example observed consisted of ten axonemes, each sheathed by a continuous layer of partially metamorphosed mitochondrial material, all enclosed by an outer membrane (Fig. 4f). As all of these cells were rare when compared to regular euspermatozoa and showed a variety of structural deviations (and no evidence of immaturity such as residual cytoplasmic droplets or cytoplasmic microtubules), we conclude that they are not a distinct sperm type (or a form of paraspermatozoa) but merely the result of spermiogenic abnormality within the euspermatozoan line.

Paraspermatozoa

The paraspermatozoa of *Z. dufresnei* show clear evidence of structural dimorphism based primarily on the number of internal axonemes present and types of vesicles contained. In both types, however, the cells are vermiform with tapered anterior and posterior extremities and contain no discernible nucleus or nuclear derivative.

In the main body region of the first type of paraspermatozoa are observed: (1) 14–20 (17 ± 3, $n = 15$) peripherally distributed axonemes lying close to or in contact with the inner surface of the plasma membrane (axonemes approximately equidistant from each other); (2) groups of microtubules

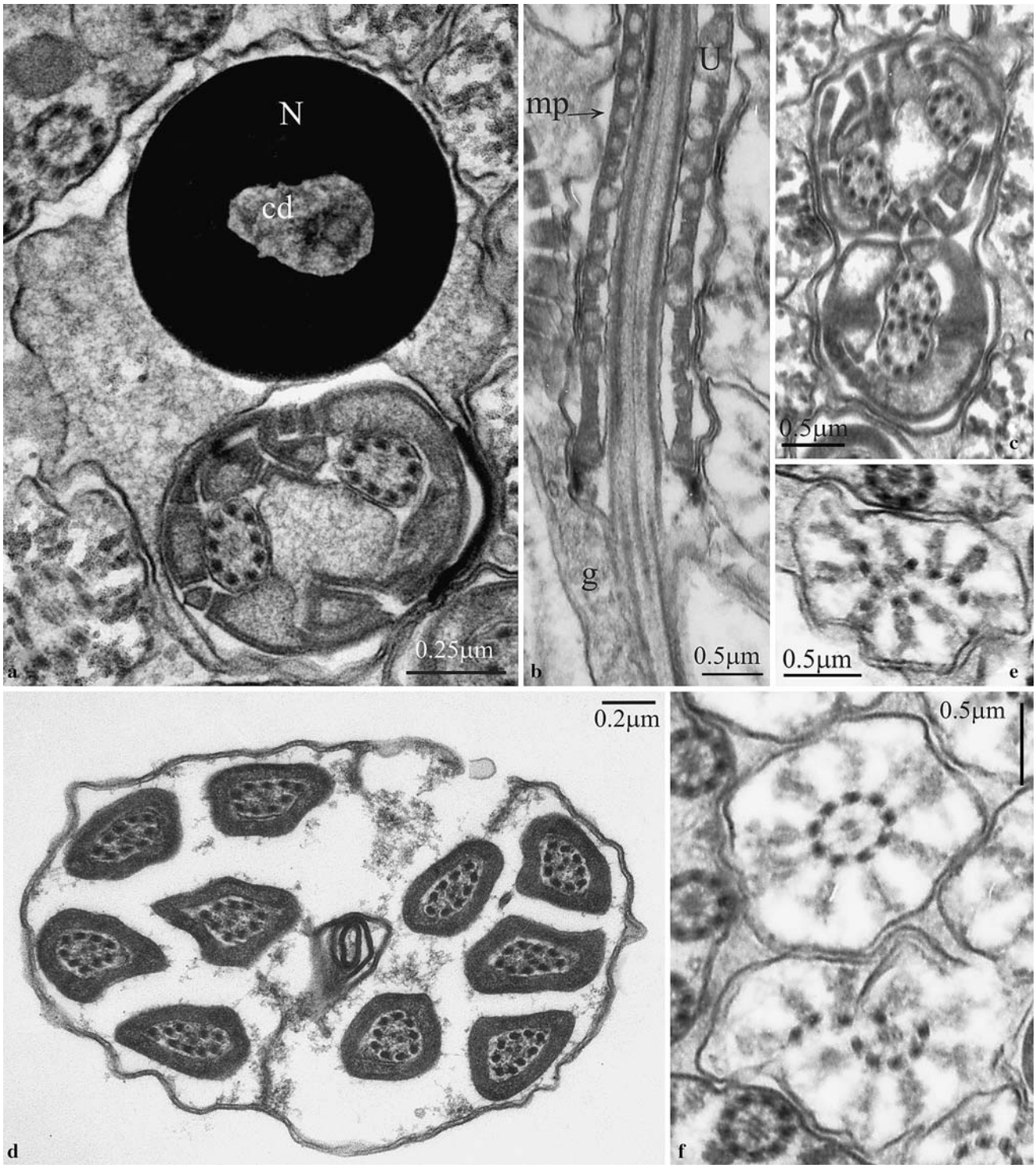


Fig. 4 Euspermatozoa of *Zidona dufresnei* (abnormal cells) **a** transverse sections (TS) through centriolar region of nucleus (with deformed centriolar complex) (*top*) and midpiece with duplicated axonemes and deformed mitochondrial elements; **b** longitudinal section (LS) posterior of midpiece region and anterior region of 'glycogen piece' the latter region consisting solely of the axoneme and plasma membrane (i.e. lacking its glycogen granule sheath). **c** TS midpiece region with duplicated axonemes and malformed mitochondrial elements. **d** TS multiaxonemal cell, with each axoneme with separate, fused mitochondrial sheath. **e**, **f** TS glycogen piece region with varying degrees of disruption to the axoneme. *ax* axoneme; *cd* centriolar derivative; *g* putative glycogen granules; *m* mitochondrion; *mp* midpiece; *N* nucleus; *U* U-shaped defining edge of mitochondrial element

occupying part of the space between adjoining axonemes; (3) numerous very electron-dense (oblong) vesicles; (4) occasional, large round vesicles of low to moderate electron-density (putative lipid vesicles); (5) numerous low electron-density small vesicles (possible mucoid deposits); (6) occasional, often elongate mitochondria (Fig. 5b–e). Anteriorly only the axonemes and some cytoplasm persist into the apex, where each axoneme attaches to a granular deposit (Fig. 5a). Posteriorly the axonemes initially become embedded in, and partly obscured by, a dense matrix (making this region noticeably more electron-dense than other regions), and the plasma membrane forms a more exaggerated pocket around each (externally showing as ridges) (Fig. 5f–i). In addition, the dense vesicles become closely pressed to each other, forming a loose reticulum (Fig. 5g). The posterior extremity is characterised by marked reduction in cell diameter (Fig. 5h, i) and degeneration of the axoneme into a bundle of disassociated doublets (Fig. 5i). For *P. mirabilis* we were only able to note that the paraspermatozoa (not here illustrated) are vermiform and contain approximately 14–20 peripherally distributed axonemes (internal cytoplasm and vesicle constituents not preserved in available testis samples).

In the second type of paraspermatozoa the main body region consists of approximately 25–31 axonemes (28 ± 3, $n = 15$), most of which are distributed peripherally (associated with an outpocket of the plasma membrane) but some of which may be found closer to the core region of the cell (Fig. 6a–e). Aside from an extensive, granular cytoplasm and occasional mitochondria, the main body region appears to lack the dense oblong vesicles, the less-dense small vesicles and the large round (putative lipid) vesicles observed in type 1 paraspermatozoa (Fig. 6d). Microtubules are often observed between the peripheral axonemes (most prolifically towards the cell apex—Fig. 6b, c). Anteriorly, the axonemes become bunched, and toward the apex, each axoneme loses its central microtubules to form multiple basal bodies, centrioles and centriolar rootlets (Fig. 6c). The apex was not observed in longitudinal section. The posterior extremity of the paraspermatozoon appears to consist of decreasing numbers of axonemes accompanied by peripheral microtubules and occasional mitochondria (Fig. 6e). Peripheral axonemes are observed in the surface of the paraspermatozoon by scanning electronic microscope (Fig. 6f).

Fig. 5 Paraspermatozoa of *Zidona dufresnei* (Type 1) **a** Transverse sections (TS) through apex showing sheath of granular material enclosing axonemes and basal body/centriolar complexes. **b** TS (posterior to **b**) main body region of paraspermatozoon, showing 15 peripheral axonemes closely adherent to the plasma membrane, dense vesicles and less dense vesicles. Microtubules also occur between the axonemes (see also Fig. 6a). **c** Longitudinal section (LS) and TS main body region of cell. **d** LS main body region showing peripheral axonemes, dense vesicles, less dense vesicles and a large elongate mitochondrion. **e** LS putative lipid vesicle within main body region. **f** TS posterior region of cell showing 15 axonemes (embedded in a dense matrix) surrounding tightly packed dense vesicles. **g** LS posterior region of paraspermatozoon. Note dense vesicles forming a loose reticulum. **h** LS posterior region of paraspermatozoon showing marked decrease in cell diameter. **i** TS posterior extremity of paraspermatozoa. Note degeneration of axonemes into constituent doublets and singlet microtubules. *ax* axoneme(s); *bb* basal body; *dv* dense vesicles; *lv* putative lipid vesicle; *m* mitochondrion; *cr* centriolar rootlet; *v* less-dense vesicles

Discussion

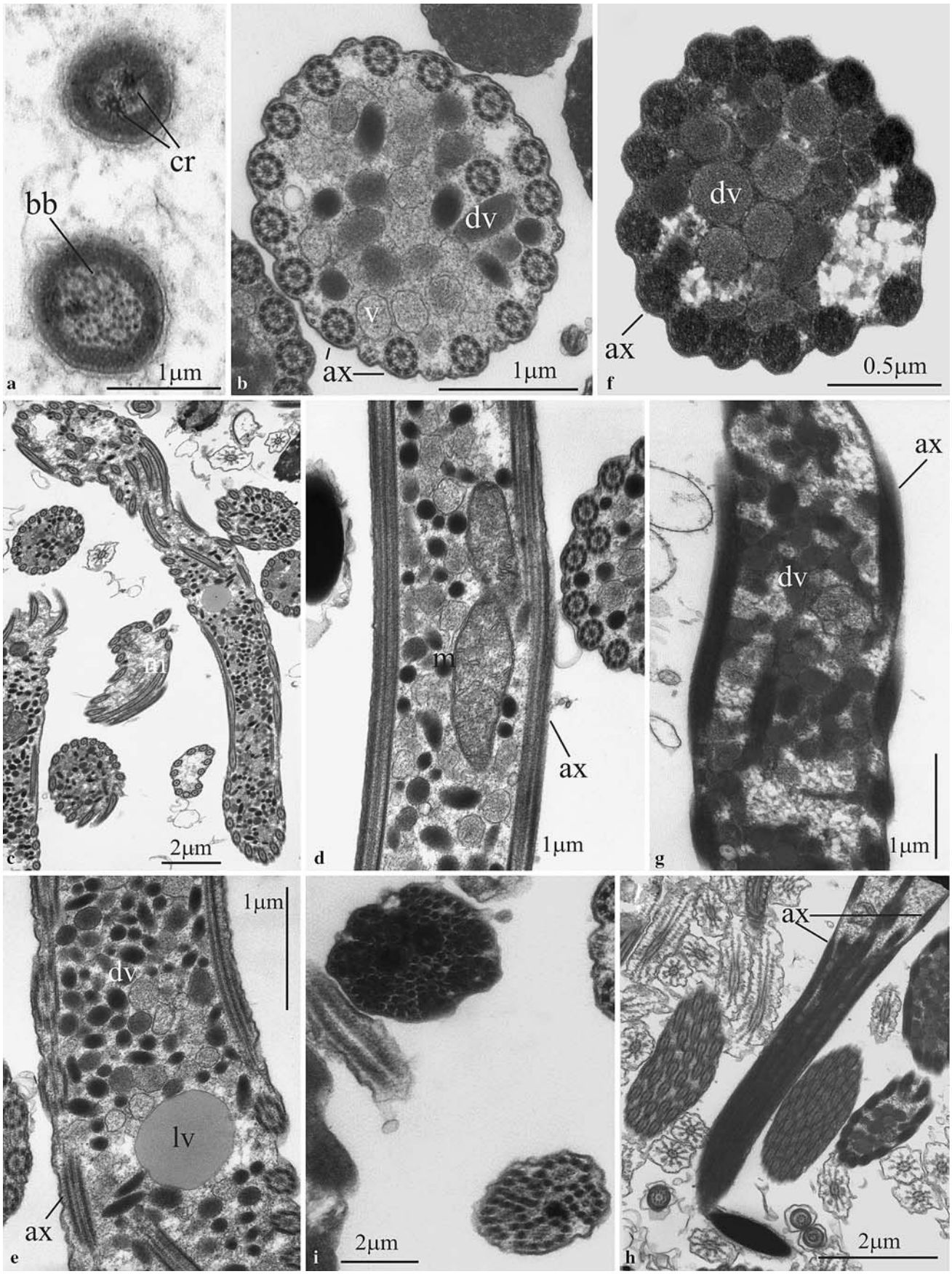
Comparison with other caenogastropoda

Euspermatozoa

Our results for *Z. dufresnei* clearly indicate that the Volutidae belong to that large group of caenogastropods which includes not only the Neogastropoda but also the so-called 'higher' mesogastropods (Neotaenioglossa of Ponder and Lindberg 1998). This group is characterised by the type 2 euspermatozoon of Healy (1996a) (acrosomal vesicle with apical bleb and accessory membrane; midpiece with 6–10 mitochondrial elements coiled helicaly around the axoneme) and type 5 paraspermatozoa of Nishiwaki (1964) (vermiform paraspermatozoa, exhibiting multiple peripheral axonemes, enclosed at maturity and bunched anteriorly; scattered mitochondria and small dense vesicles; total absence of nuclear material).

Prior to being classified into an expanded Muricoidea (see Ponder 1974), the superfamily Volutoidea included the Volutidae, Marginellidae, Mitridae, Olividae, Turbinellidae (formerly Vasidae) and Harpidae. Unfortunately sperm ultrastructural data for these groups are very fragmentary (or missing, as in the case of the Harpidae), but it can be noted that the Volutidae and Turbinellidae both exhibit a solid, rod-shaped eusperm nucleus, and that the Olividae and Marginellidae show a long, tubular nucleus (Healy 1984; Kohnert and Storch 1984; Koike 1985; present study).

In other species of the Gastropoda which possess the type 2 euspermatozoon (other neogastropods and most neotaenioglossans), the mitochondrial elements which spiral around the axoneme are uniformly electron dense and usually show some form of cristae (irregular, sometimes tubular or plate-like; Kohnert and Storch 1984; Koike 1985). In *Z. dufresnei* and *P. mirabilis* the outer layer of each mitochondrial element is considerably more electron-dense than the remainder of the matrix component (this dense layer having an angular, U-shaped profile in longitudinal section) and no recognisable cristae are observed. Although oblique longitudinal sections show the helical spiralling of the mitochondria, transverse sections suggest that the dense layer forms an almost loose division of a collective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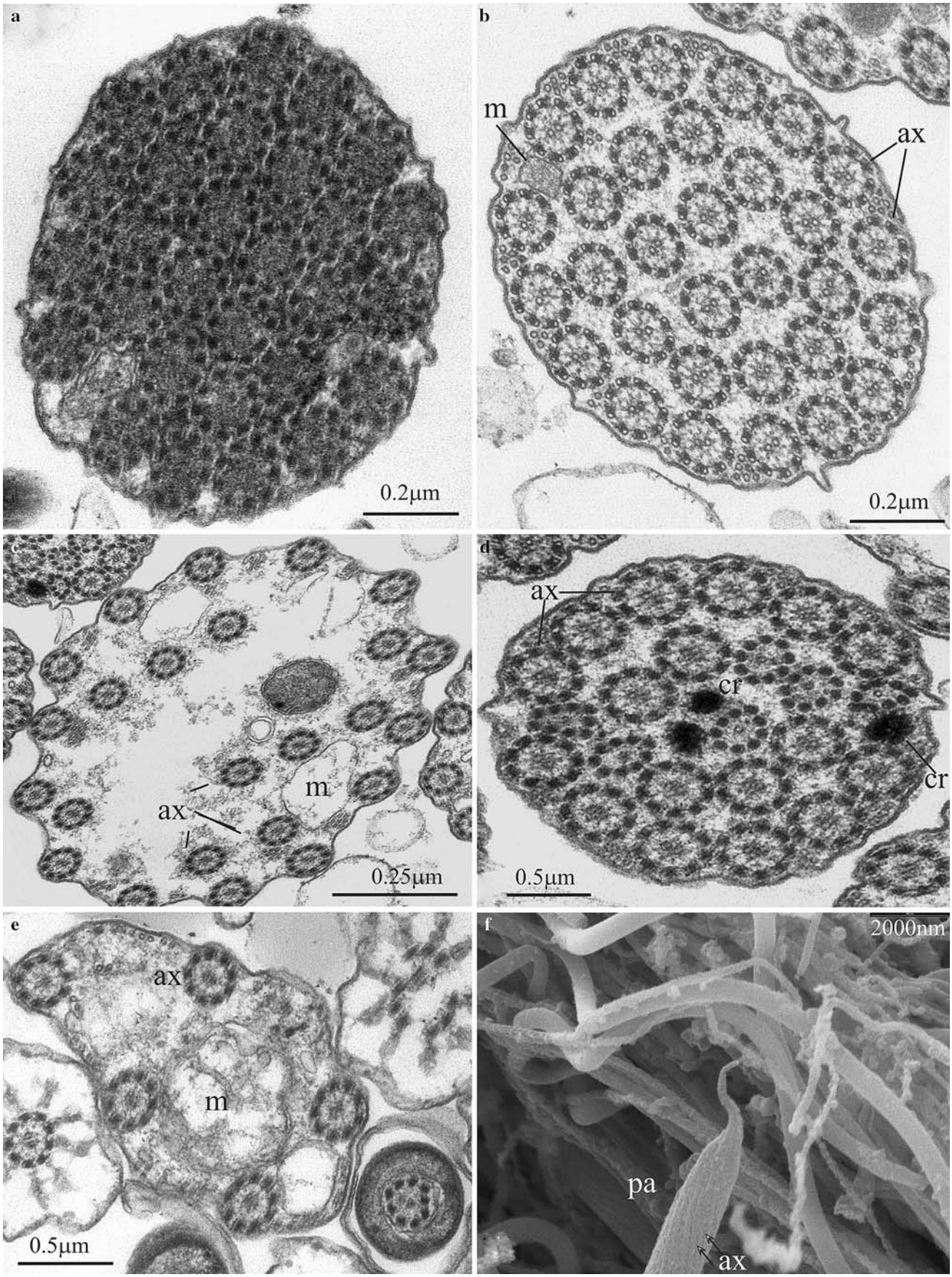
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◀ **Fig. 6** Paraspermatozoa of *Zidona dufresnei* (Type 2 a-b) **a** TS near apex of cell showing 29 clustered basal bodies (some transitional to axonemes). **b** TS anterior region of cell, posterior to **a** and **b**, showing 31 9 + 2 axonemes. Microtubules are visible peripherally. **c** TS near apex showing mix of axonemes, basal bodies and dense structures (probable centriolar rootlets). Microtubules also visible peripherally. **d** TS main body region of cell showing axonemes peripherally and within the cytoplasm. A mitochondrion is also visible. **e** TS possible posterior region of cell showing reduced number of axonemes, peripheral microtubules and one mitochondrion. **f** Body region of the paraspermatozoide showing axonemes peripherally. *ax* axonema(s); *dv* dense vesicles; *m* mitochondrion; *cr* probable centriolar rootlet; *pa* paraspermatozoide

mitochondrial matrix. Possibly the outer layer, with its bilaminar, precise appearance, represents a partial 'crystallization' of the mitochondrial elements, analogous to that occurring in certain rissoidean caenogastropods (see Healy 1983b).

We attach no importance to the occasional eupermatozoon of *Z. dufresnei* found with multiple axonemes and/or duplicated mitochondria or centriolar abnormalities. These were only rarely encountered and almost certainly can be considered aberrant end-products of euspermiogenesis. Such sperm have also been observed by one of us in other caenogastropods and in certain heterobranchs (Healy 1984), but they are always rare and show varying degrees of malformation. The euspermatozoa with duplicated axonemes should not be confused with the paired (so-called 'conjugated') euspermatozoa observed in the Turritellidae (Caenogastropoda, Cerithioidea) (see Afzelius and Dallai 1983). Such a phenomenon, although rare in the Mollusca, is not an abnormality and therefore not comparable to the situation observed in *Z. dufresnei*.

The glycogen piece and end piece of *Z. dufresnei* (no observations possible for *P. mirabilis*) are essentially as observed in other caenogastropods, which, with few exceptions (e.g. the rissooidean *Stenothyra* sp—see Healy 1983b), show the nine tract configuration of glycogen granules associated with the axonemal doublets. The annulus of *Z. dufresnei*—a complex of two ring elements attached to the plasma membrane—is similar to that recorded in other neogastropods and in many neotaenioglossans (Buckland-Nicks et al. 1982a, b; Kohnert and Storch 1984; Koike 1985; Healy 1986a, 1988b, 1992; Amor and Durfort 1990; Healy and Jamieson 1993). This differs from the single ring annulus observed in basal caenogastropods such as the Cerithioidea (Healy 1982, 1983a).

Paraspermatozoa

The phenomenon of paraspermatozoal dimorphism, as here noted for *Z. dufresnei*, has previously been demonstrated in at least four other caenogastropod families (Nishiwaki 1964; Tochimoto 1967; Nishiwaki and Tochimoto 1969; Buckland-Nicks 1973, 1982a, b; Healy 1986b, c). Vermiform paraspermatozoa (type 5 of Nishiwaki 1964) appear to be characteristic of a significant proportion of the Neotaenioglossa and most of the Neogastropoda (see Nishiwaki 1964; Tochimoto 1967; Melone et al. 1980; Healy 1988a; Hodgson 1997; Buckland-Nicks 1998). Buckland-Nicks et al. (1982a) differentiated two types of vermiform paraspermatozoa in the ranellid *Fusitriton oregonensis* (Redfield, 1848) a bulkier 'carrier' type which bear numerous attached euspermatozoa and contains vary large dense vesicles and approximately 112 axonemes (the latter generally centrally positioned) and a smaller type, the 'lancet' which never physically associates with euspermatozoa (or for that matter, the 'carrier' paraspermatozoa) and contains small vesicles and only 16 axonemes. Although we have not observed any physical association of euspermatozoa and paraspermatozoa in *Z. dufresnei* (or in *P. mirabilis*, based on limited material), both of our *Zidona* paraspermatozoa correspond most closely to the 'lancet type' of Buckland-Nicks et al. 1982b, although the type with larger numbers of axonemes shows a bunching of axonemes anteriorly more close resembling the 'carrier' type of Buckland-Nicks et al. 1982b. At this stage we cannot rule out the possibility that paraspermatozoa never physically interact with euspermatozoa in *Z. dufresnei* (or *P. mirabilis*) after sperm transfer, but at least within the sperm duct (and testis) we are confident that spermatozeugma-formation does not occur in *Z. dufresnei*, nor has it been recorded for any other neogastropod. Regarding the chemical composition of the various vesicles observed in type 1 *Zidona* paraspermatozoa, the dense vesicles are presumably homologous with those of other caenogastropod paraspermatozoa, and likely contain glycoprotein or possibly yolk (although we did not observe yolk-type crystalloids in these vesicles). The small, less dense, round vesicles of type 1 appear to correspond with those observed by Buckland-Nicks et al. 1982a, and termed by them 'mucoid vesicles'. Similarly the occasional large, round vesicle of type 1 is morphologically very similar to lipid vesicles.

Although the ultrastructure of the anterior extremity of vermiform caenogastropod paraspermatozoa has been demonstrated on a number of occasions (e.g. Melone et al. 1980; Buckland-Nicks 1998; Buckland-Nicks et al. 1982a, b; Healy 1986a; Hodgson 1993), the posterior region has been virtually ignored with the exception of a single micrograph of Buckland-Nicks et al. (1982a, b) for *Fusitriton oregonensis* which showed peripheral microtubules and scattered vesicles. Our observations on *Z. dufresnei* demonstrate that at least in the first type of 'lancet' paraspermatozoa (the 14–20 axonemes type), the axonemes continue posteriorly in their peripheral position, but then become embedded in dense material (markedly increasing the

electron-density of this region) and ultimately degenerate into disorganised doublets and singlet microtubules. We suspect that further (hopefully comparative) studies will show that these structural changes also occur in some other caenogastropod vermiform paraspermatozoa—at least those conforming to the 'lancet type' of Buckland-Nicks et al. (1982a). For example, Nishiwaki (1964), using light microscopy, illustrates vermiform parasperm with dark posterior regions in certain species of Cypraeidae.

Sperm ultrastructure and the Volutidae: systematic considerations

The taxonomic and phylogenetic utility of sperm ultrastructure has been well demonstrated within the Caenogastropoda (Giusti 1971; Giusti and Selmi 1982; Healy 1983a, 1996b; Kohnert and Storch 1984; Koike 1985) and results of such work have been incorporated into recent classifications (see Ponder and Lindberg 1997). Although our observations on *P. mirabilis* are limited somewhat by fixation deficiencies and cytological maturity of the available material [museum (testis) material fixed in Bouin's solution and stored in sea water formalin], the preservation of key euspermatozoan components is good enough to demonstrate close similarity with *Z. dufresnei*. In particular the dense, angulate, U-shaped profile of each mitochondrion is very distinctive, and to our knowledge this feature has not been demonstrated or noted in any previous study of caenogastropod euspermatozoa. Currently *P. mirabilis* and *Z. dufresnei* are the only two members of the Volutidae examined for sperm ultrastructure, and hence it is impossible to state whether the U-profiles are characteristic of the entire taxon, or just the subgroup Zidoninae or only the Zidonini, consisting of *Zidona*, *Provocator* and *Harpovoluta* (see Bail and Poppe 2001). Sperm data for other subfamilies of the Volutidae are now required to test this.

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