

# Ultrastructure of euspermatozoa and paraspermatozoa in the volutid snail *Adelomelon ancilla* (Mollusca: Caenogastropoda)

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**Abstract** The ultrastructure of the euspermatozoa and the paraspermatozoa is investigated in *Adelomelon ancilla*, through histological section observed by transmission electron microscopy. Euspermatozoa of *A. ancilla* consists of: (1) a conical acrosomal vesicle (with a short basal invagination, constricted anteriorly) which is flattened at the apex and associated with an axial rod, a centrally perforated basal plate and a short accessory membrane, (2) a rod-shaped, solid and highly electron-dense nucleus (with a short basal fossa containing a centriolar complex and a 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 (where the axoneme is sheathed by nine tracts of glycogen granules), (5) a dense annulus at the junction of the midpiece and glycogen piece, and (6) a short free tail region (where the axoneme is surrounded only by plasma membrane). We observed a parasperm in *A. ancilla*. This is vermiform in shape and is composed of multiple axonemes and extensive cytoplasm

with numerous vesicles, and mitochondria are scattered inside the axonemes. Sperm of *A. ancilla* is characterized by the euspermatozoa type 2 and the paraspermatozoa morphology belongs to type 5. The U shaped electrodense mitochondrial element in the midpiece of the eusperm and the constriction in the acrosomal vesicle present in *A. ancilla* are exclusive. We suggest that these characteristics could have taxonomic importance, because these was observed in other volutids and have not been observed in the rest of caenogastropods studies. We consider that the morphology of paraspermatozoa in *A. ancilla* corresponds to the “lancet” type.

**Keywords** Euspermatozoa · Paraspermatozoa · Morphology · Gastropods · Volutidae

## Introduction

The morphological diversity of spermatozoa and the process of spermatogenesis are one of the tools that have been utilized for systematic and phylogenetic studies of gastropods, especially at or above the family level (Nishiwaki 1964; Giusti 1971; Melone et al. 1980; Healy 1982, 1983a, b, 1988, 1996a, b; Koike 1985; Thompson 1973). The systematic positioning and the construction of phylogenies of some groups are based on the ultrastructure of their spermatozoa. Sperm diversity reflects the environment of fertilization, as well as the systematic position and the phylogenetic relationships of the taxa that are being considered (Healy 1996a).

The production of dimorphic sperm is widespread among prosobranch mollusks, especially in caenogastropods (Melone et al. 1980; Nishiwaki 1964). This occurs when an organism produces both fertile sperm, the euspermatozoa,

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and sterile sperm, the paraspermatozoa (Buckland-Nicks et al. 2000). These two sperm types coexist in the same species as well as within the same individual (Suwanjarat and Suwaluk 2003).

Although the ultrastructure of caenogastropod sperm of has been studied in many families (Buckland-Nicks and Chia 1976; Buckland-Nicks et al. 2000; Healy 1982, 1986; Healy and Jamieson 1981) it has not been closely examined in the Volutidae. Only two species from this family have been studied: *Zidona dufresnei* (Donovan 1823) and *Provocator mirabilis* (Finlay 1926) (Giménez et al. 2008).

The marine snail, *Adelomelon ancilla* (Lightfoot 1786), inhabits shallow waters (5–15 m in depth) in the Patagonian littoral. Some species of the family Volutidae, such as *A. ancilla*, possess a importance as fisheries resource.

In the present study, we describe the ultrastructure of euspermatozoa and paraspermatozoa of *A. ancilla*. We might expect *A. ancilla* to have sperm ultrastructural similarities with other member of the Volutidae, such as *Z. dufresnei* and *P. mirabilis*. The ultrastructural descriptions in *A. ancilla* could utilize as comparative tools for systematic and may contribute to the understanding of the relationships among volutids and caenogastropods.

## Materials and methods

Reproductively mature males of *A. ancilla* were collected in Punta Este, Golfo Nuevo, Chubut's province, Argentina (42°42'24.1"S and 65°06'20.9"W), at depths of 5–10 m by scuba diving. After removal of the shell pieces of the testis, (9 mm<sup>3</sup>) they were fixed in Bouin's aqueous solution, dehydrated in a graded ethanol series and embedded in methacrylate for light microscopy. Serial sections (5 µm thick) were stained with a modified Masson's trichrome, and the periodic acid Schiff reaction (PAS) was used for the demonstration of neutral glycoconjugates. For transmission electron microscopy (TEM), tissues were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 4 h at 4°C, and postfixed in 1% osmium tetroxide for 2 h. Semithin and ultrathin sections, cut using a Reichert ultramicrotome, were stained with uranyl acetate and lead citrate. The sections were examined and photographed using Philips EM 301 transmission electron microscopes operated at 75–80 kV. Total of 50 sperms lengths were determined in five males by viewing and photographing tissue squashes using a Zeiss Axiostar light microscope.

## Results

In *A. ancilla* the euspermatozoa and paraspermatozoa coexist within the same spermatogenic tubules. Euspermatozoa

are composed of an anterior acrosomal complex followed by a rod-shaped nucleus, an elongate midpiece, a glycogen piece and an end piece. Paraspermatozoa are vermiform and are composed of multiple incorporated axonemes peripherally; within the cell body, different types of vesicles and mitochondria are present.

### Euspermatozoa

#### Acrosomal complex

The anterior acrosomal complex consists of a tall, conical, membrane-bound acrosomal vesicle, an axial rod and a basal plate (Fig. 1a). The length of acrosomal vesicle is approximately  $1.49 \pm 0.04$  µm, and is electron-dense. Apically, the plasma membrane of the acrosomal vesicle is separated from the vesicle contents, forming the apical bleb (Fig. 1b). The acrosomal vesicle has a posterior invagination about 0.44 µm long and constricted anteriorly (Fig. 1c). An axial rod is situated inside the invagination.

A basal plate is located between the acrosomal complex and the nucleus. An accessory membrane (0.135 µm in length) is observed between the plasma membrane and the acrosomal vesicle, in the posterior region of the acrosomal complex (Fig. 1a). Transverse sections at different levels of the acrosomal complex show a gradual change in the shape: from oval at the base to more laterally compressed in the anterior region of the acrosomal complex (Fig. 1d–g).

#### Nucleus

The nucleus is filiform, composed of a dense and homogeneous material, with a short posterior invagination (0.43 µm in depth). The centriolar derivative is located within the invagination and is continuous with the initial portion of a 9 + 2 microtubular pattern axoneme (Fig. 1h, j, k).

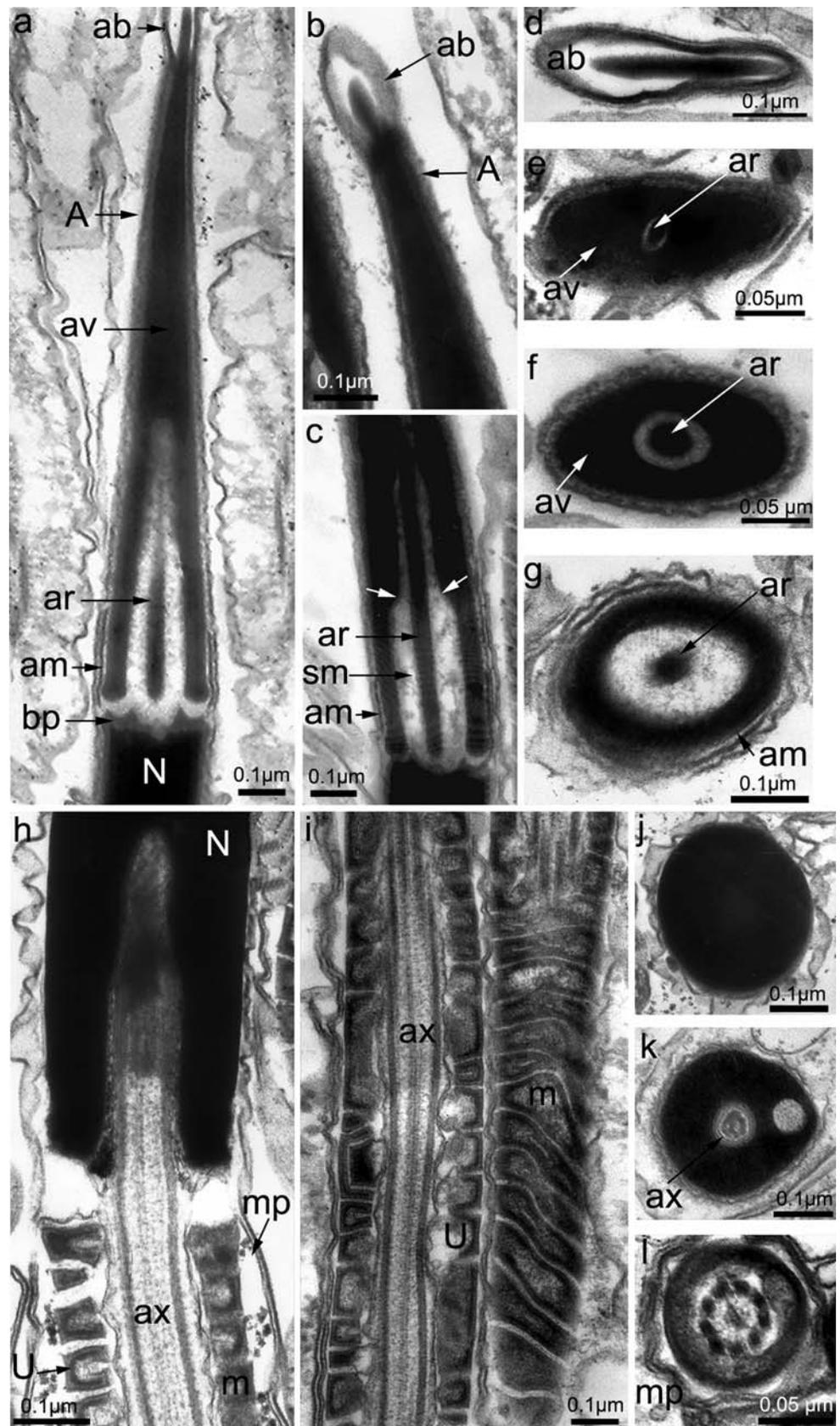
#### Midpiece

Continuous with and posterior to the nucleus, the axoneme (exhibiting the typical microtubular pattern 9 + 2) is sheathed by five to six helically arranged, elongate mitochondria. In longitudinal sections each mitochondrial element exhibits a U-shaped, bilaminar, outer layer that is notably more electro-dense than the rest of the mitochondrial material. In oblique longitudinal sections the mitochondrial elements are distinguished as a continuous sheath (Fig. 1i, l).

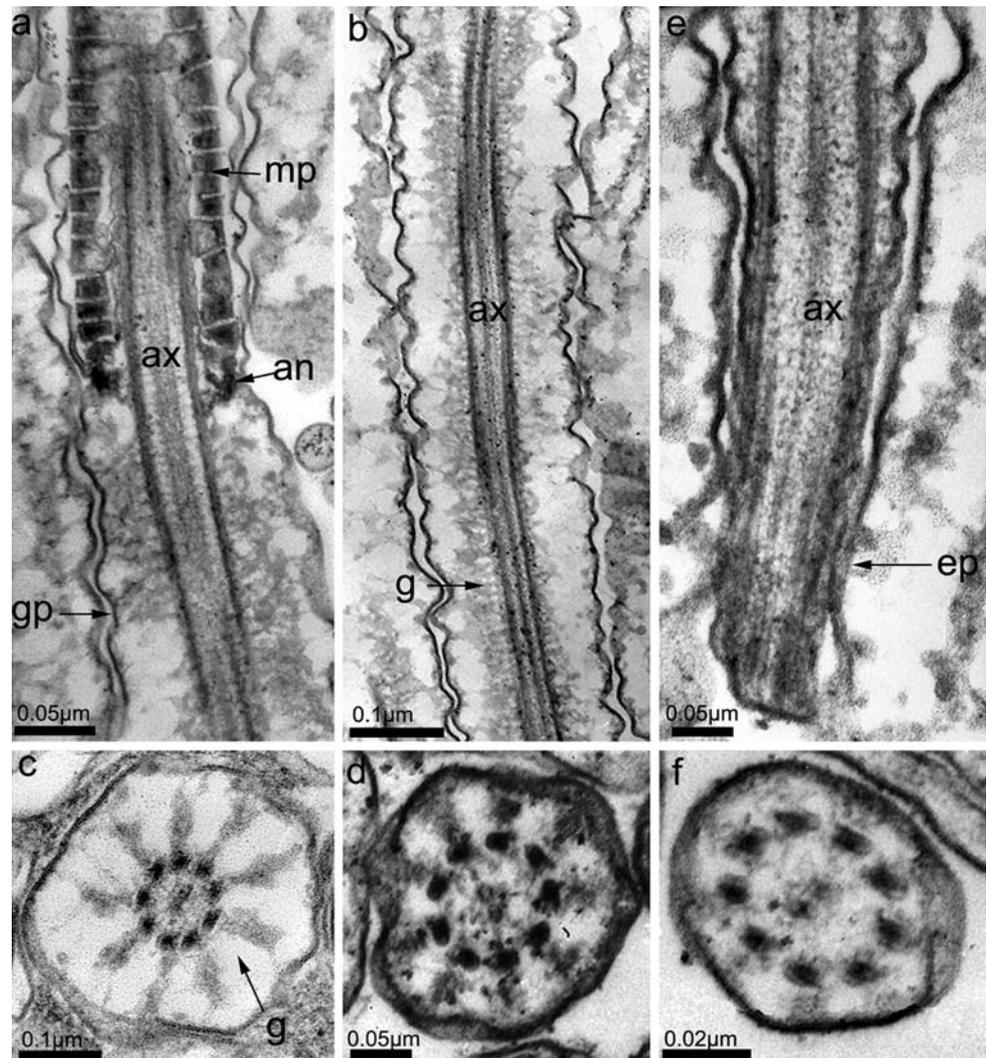
#### Annular complex and glycogen piece

Immediately following termination of the midpiece, the axoneme becomes enclosed by nine continuous tracts of

**Fig. 1** Euspermatozoa of *Adelomelon ancilla*. **a** Longitudinal section (LS) through acrosomal complex and anterior portion of nucleus. **b** LS of the acrosomal complex in the anterior region. Note the apical bleb. **c** LS of the acrosomal complex. Note the constriction of the posterior acrosomal invagination (starting point indicated by arrow). **d–g** Series of transverse sections (TS) from apical bleb region (**d**) to basal region (**g**) of the acrosomal vesicle. Note lateral flattening of the acrosomal complex beginning in anterior portion of the invagination (**d–f**) and the presence of the accessory membrane (**g**). **h** LS junction of nucleus (showing invagination and centriole/axoneme insertion) and anterior portion of midpiece. **i** LS of midpiece. Note helical mitochondrial elements (defined by dense U-shaped profiles of periphery). **j** TS of the nucleus in the middle region. **k** TS of the nucleus in posterior region, note centriolar fossa with axoneme. **l** TS of midpiece region, note the microtubular pattern (9 + 2). **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, **sm** subacrosomal material, **U** U-shaped defining edge of mitochondrial element



**Fig. 2** Euspermatozoa of *Adelomelon ancilla*. **a** LS of the junction of midpiece and glycogen piece. Note annular complex. **b** LS of glycogen piece showing radiating, longitudinal rows of putative glycogen granules. **c, d** TS showing decrease in the diameter of glycogen piece towards posterior region of cell. **e** LS termination of glycogen piece and entire end piece region **f** TS of the end piece. *an* annulus complex, *ax* axoneme, *ep* end piece, *g* putative glycogen granules, *gp* glycogen piece, *mp* midpiece



electron dense granules (Fig. 2c) and the plasma membrane to form the glycogen piece. In transverse sections each tract of granules is associated with one of the axonemal doublets, with the exception of the central pair (Fig. 2c, d). A dense double ring structure is present at the midpiece–glycogen piece junction (Fig. 2a, b). Transition to the end piece is gradual, with the diameter of granule sheath slowly diminishing until the axoneme is surrounded only by the plasma membrane (Fig. 2c–f). Using light microscopy we tested by cytochemical techniques that the glycogen piece is PAS positive.

#### End piece

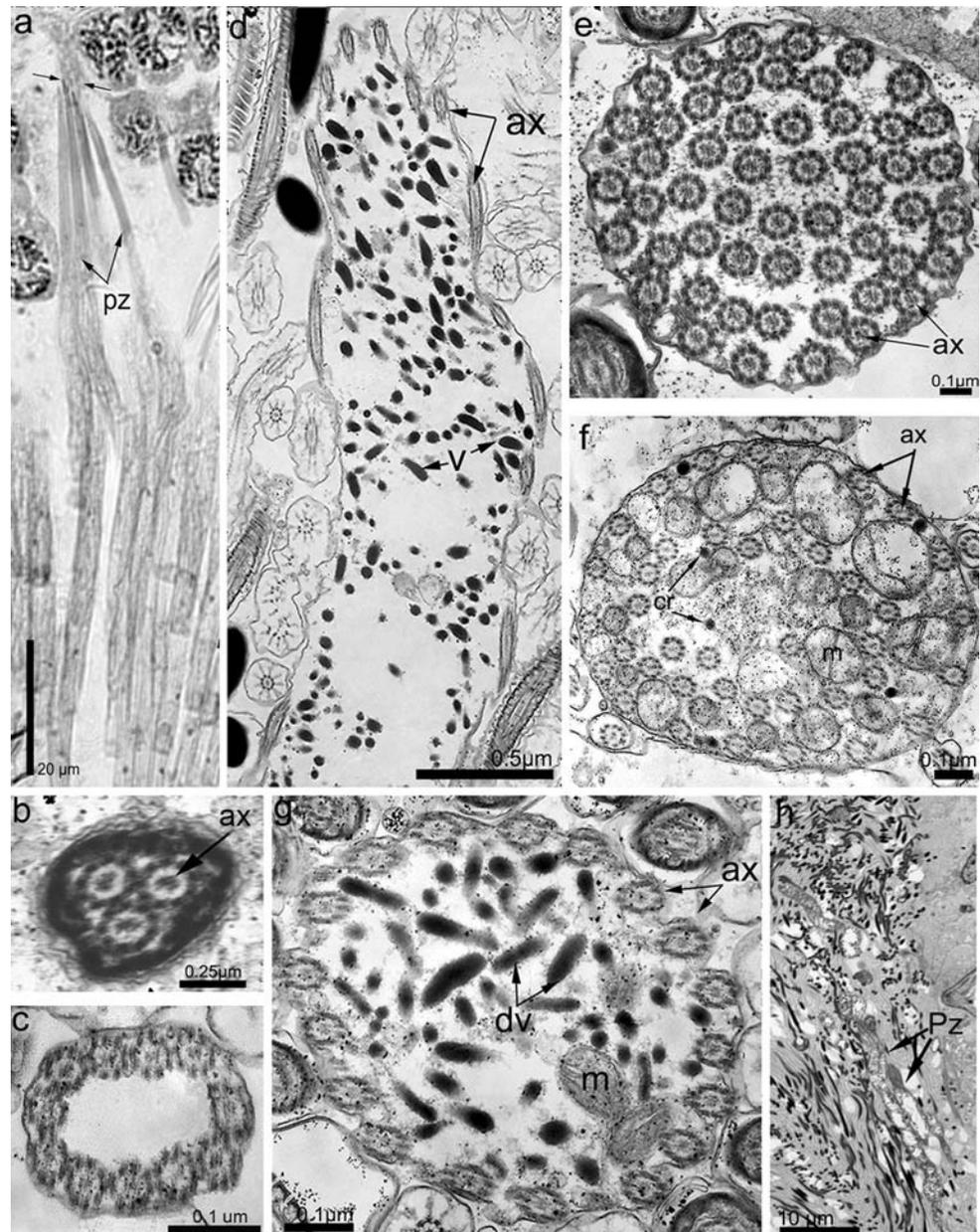
The final piece is continuous with the glycogen piece and consists of the continuing 9 + 2 microtubular pattern and is surrounded only by plasma membrane (Fig. 2e). The width measurement is approximately 0.08  $\mu\text{m}$  (Fig. 2f).

#### Paraspermatozoa

*A. ancilla* present a vermiform parasperm at least 120  $\mu\text{m}$  in length by light microscopy. The apex region is acidophilic and the paraspermatozoa cluster in cohorts close to, or in contact with, the tubular membrane (Fig. 3a). We tested by cytochemical techniques that the main body region of the paraspermatozoa is PAS positive. In transverse section the anterior region shows an electron-dense material around the axonemes peripherally, like a cap, a deposit of dense granular material is observed, and vesicles are not visible. Towards the apex of the paraspermatozoa the diameter decreases (Fig. 3b). On the other hand, the posterior region of parasperm, the axonemes, are situated peripherally (Fig. 3c).

One of the sections shows a main body region composed by: (1) peripherally distributed axonemes with the ( $17 \pm 2$ ,  $n = 20$ ) lying close to or in contact with the plasma mem-

**Fig. 3** Paraspermatozoa of *Adelomelon ancilla*. **a** Light microscope image. Modified Masson's trichrome. General view of the paraspermatozoa showing a vermiform aspect. Note the acidophilic area in the apex. **b** Transverse sections (TS) through apex showing electron-dense material enclosing axonemes. **c** TS posterior section showing peripheral axonemes. **d** LS main body region of paraspermatozoa with dense vesicles present. **e** TS of paraspermatozoa showing 52 axonemes. Note the spiral disposition of the axonemes. **f** TS main body region contains dense vesicles and a large mitochondrion, showing 18 peripheral axonemes. **g** TS main body region contains dense vesicles, mitochondrion, and axonemes in internally and peripherally. **h** Seminal vesicle, paraspermatozoa join to euspermatozoa *ax* axoneme, *m* mitochondrion, *pz* paraspermatozoa, *dv* dense vesicles



brane (axonemes approximately equidistant from each other), (2) numerous vesicles containing dense granular material but no obvious internal structure, (3) a few elongate mitochondria, and (4) a diffuse cytoplasmic matrix (Fig. 3d, g).

Two different transverse sections of parasperm are observed: one the disposition of axonemes distributed internally and peripherally ( $52 \pm 2$ ,  $n = 18$ ) (Fig. 3e); the other one, the axonemes distributed peripherally ( $18 \pm 2$ ,  $n = 18$ ) and internally ( $21 \pm 2$ ,  $n = 10$ ). Vesicles and mitochondria are observed inside it (Fig. 3f).

Semithin sections by light microscope paraspermatozoa were found in the seminal vesicle, join to euspermatozoa (Fig. 3h).

## Discussion

Structural comparison, euspermatozoa and paraspermatozoa

Most features of *A. ancilla* euspermatozoa can be observed in many other neogastropods and in the higher mesogastropods (Buckland-Nicks and Chia 1973; Healy 1982) characterized by the euspermatozoa type 2 described by Healy (1996b). These shared characters include: (1) morphology of the acrosomal complex (apical bleb, accessory membrane, shape of the acrosomal vesicle and the axial rod material), (2) a solid electron-dense nucleus, (3) midpiece composed of the axoneme surrounded by multiple (6–10)

helically coiled mitochondria, (4) glycogen piece with nine tracts of granules associated with the axonemal doublets, and (5) presence of a dense ring structure at midpiece-glycogen piece junction.

The morphology of paraspermatozoa of *A. ancilla* belongs to type 5 paraspermatozoa of Nishiwaki (1964) (vermiform paraspermatozoa, exhibiting multiple peripheral axonemes, enclosed at maturity and bunched anteriorly; scattered mitochondria and small dense vesicles; and total absence of nuclear material).

#### Euspermatozoa

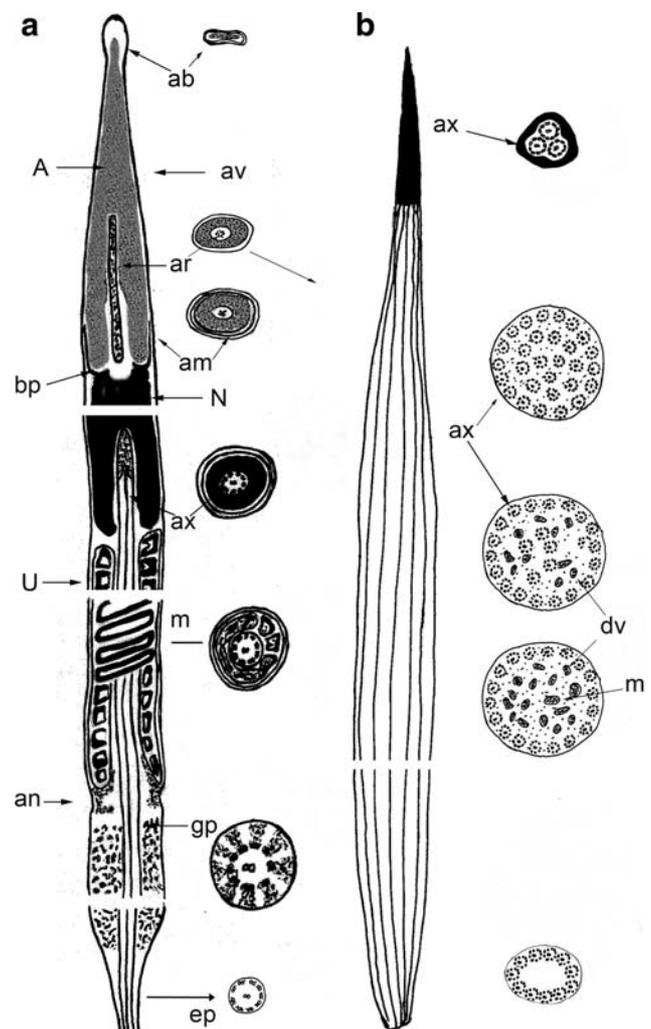
General features of the acrosomal complex in *A. ancilla* are shared with other caenogastropods (Healy 1983a, 1996b; Koike 1985), except for mitochondrial element and the constriction in the posterior acrosomal invagination (Fig. 4a), which only is shared with *Z. dufresnei* (Giménez et al. 2008).

Many neogastropods and some mesogastropods, like Potamididae, show the mitochondrial elements uniformly electron-dense (Kohnert and Storch 1984; Koike 1985) or present a non-helical arrangement of mitochondrial elements (Suwanjarat and Suwaluk 2003), respectively. In *A. ancilla* we found the outer layer of each mitochondrial element considerably more electron-dense than the remainder of the matrix component (which shows a U-shape in longitudinal section profile), the number of mitochondria was taken from previous stages of spermatogenesis (data unpublished) and there was a spiraling of the mitochondrial elements, as in *Z. dufresnei* and *P. mirabilis* (Giménez et al. 2008).

The glycogen piece of *A. ancilla* does not differ in any respect from the configuration shown to exist in many other caenogastropods (Healy 1983a; Kohnert and Storch 1984; Koike 1985), showing the typical microtubular pattern (9 + 2) associated with glycogen granules for each pair. The junction between the midpiece and glycogen piece presents a complex of ring elements attached to the plasma membrane, as in many caenogastropods (Buckland-Nicks and Chia 1973; Buckland-Nicks et al. 1982a, b; Healy 1986, 1988; Healy and Jamieson 1993; Giménez et al. 2008). This complex structure of double ring differs from the annulus of other groups, which have a single ring, as Cerithioidea family (Caenogastropoda) (Healy 1982, 1983a).

#### Paraspermatozoa

Vermiform paraspermatozoa are present in most of Neotaenioglossa and Neogastropoda (see Nishiwaki 1964; Tochimoto 1967; Melone et al. 1980; Healy 1988; Hodgson 1997; Buckland-Nicks 1998). Buckland-Nicks et al. (1982a)



**Fig. 4** Diagram of spermatozoa of *Adelomelon ancilla*. **a** Internal view of eusperm. TEM. Note the constriction of subacrosomal invagination and the U-shape of electron-dense mitochondrial elements in the midpiece. **b** External and general view of parasperm. Light microscopy. Transversal sections observed by TEM. Note the TS of the apical, medial, and final region. A acrosomal complex, ab apical bleb, ar axial rod material, av acrosomal vesicle, am accessory membrane, an annulus, ax axoneme, bp basal plate, ct acrosomal vesicle constriction, dv dense vesicles, ep endpiece, g putative granules, gp glycogen piece, m mitochondria, mp midpiece, N nucleus, U U-shaped defining edge of mitochondrial element. Not drawn exactly to scale

describe two types of vermiform paraspermatozoa: the bulkier “carrier” type which has many euspermatozoa attached and contains very large, dense vesicles and approximately 112 axonemes, and the smaller “lancet” type which never physically associates with euspermatozoa.

*Adelomelon ancilla* present the vermiform lancet type of parasperm (Fig. 4b). The parasperm consist of a conical anterior region, where the number of axonemes decrease and they are embedded in electron-dense material in the anterior region. This structure was observed in another

caenogastropods as *Fusitron* and *Ceratostoma* (Buckland-Nicks 1998) and recently in the volutid *Z. dufresnei* (Giménez et al. 2008). Posteriorly, the numbers of axonemes increase and centriolar rootlets are present. Transversal section through the parasperm shows the increment of vesicles and decrease in the number of axonemes.

In other caenogastropods, paraspermatozoa are clustered in cohorts of either four or eight parasperm linked by cytoplasmic bridges (Buckland-Nicks 1998). In *A. ancilla* this disposition of parasperm was observed; however, the cytoplasmic bridges were not observed.

#### Functional considerations of paraspermatozoa

Alternative theories have been advanced in an attempt to answer the role of paraspermatozoa in the reproductive cycle. Many workers (Buckland-Nicks and Chia 1976) consider the function of nutrition and/or stimulation of euspermatozoa by the products of paraspermatozoan breakdown. Fretter and Graham (1962) suggest the ‘transportation’ of euspermatozoa during and after sperm transfers from male to female, via mobile associations of euspermatozoa. Buckland-Nicks (1998) suggest paraspermatozoa may be involved in creating a hostile pre-fertilization environment for rival sperm. In this context, lancet parasperm form a sperm plug in the *bursa copulatrix* that binds eusperm in the lumen.

These processes are poorly understood in snails but are central to our understanding of the role of parasperm in reproduction (Buckland-Nicks 1998).

The function of paraspermatozoa in *A. ancilla* may be involved in nutrition or stimulation of the eusperm; euspermatozoa and paraspermatozoa were found in the seminal vesicle together. Despite this more intensive studies are needed, as to determinate if the paraspermatozoa are present in the *bursa copulatrix*.

#### Systematic considerations

We consider that the U shaped electron-dense mitochondrial elements in the midpiece of the eusperm present in *A. ancilla*, *Z. dufresnei* and *P. mirabilis* and the constriction in the posterior acrosomal invagination in *A. ancilla* and *Z. dufresnei* are diagnostic at list to the family level. We suggest that these characteristics could have taxonomic importance, because these have not been observed in other caenogastropods. Furthermore, there are not enough investigations of sperm ultrastructure in Volutidae. Just knowing the ultrastructure of these species, we cannot assure that these characters are comparable for the Volutidae or Zidoninae. Further studies in sperm ultrastructure are needed in other volutids such as Odontocymbiolinae to confirm this hypothesis.

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