

Euspermatozoa and paraspermatozoa in the volutid gastropod *Odontocymbiola magellanica*, Patagonia, Argentina, Southwestern Atlantic Ocean

Juliana Giménez

CONICET, Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

Keywords:

sperm ultrastructure, euspermatozoa, paraspermatozoa, Gastropoda, Volutidae

Accepted for publication:

6 August 2010

Abstract

Giménez J. 2010. Euspermatozoa and paraspermatozoa in the volutid gastropod *Odontocymbiola magellanica*, Patagonia, Argentina, Southwestern Atlantic Ocean. —*Acta Zoologica (Stockholm)*

The ultrastructure of mature spermatozoa and paraspermatozoa of *Odontocymbiola magellanica* is investigated. Euspermatozoa consist of: (1) a tall, conical acrosomal vesicle (with a short basal invagination, constricted anteriorly); (2) a rod-shaped, solid and highly electron-dense nucleus; (3) an elongate midpiece consisting of the axoneme sheathed by helical mitochondrial elements each exhibiting a dense U-shaped outer layer; (4) an elongate glycogen piece; (5) a dense annulus at the junction of the midpiece and glycogen piece; and (6) a short free-tail region. Paraspermatozoa of *O. magellanica* are vermiform and dimorphic. First type contain approximately 14–17 axonemes (arranged peripherally and interspersed with microtubules) and numerous oblong dense vesicles, numerous less dense (round) vesicles, and scattered mitochondria; the second type contains 38–45 axonemes peripherally arranged and closer to the core region of the cell and occasional mitochondria. Most of the euspermatozoal features of *O. magellanica* are also observed in many neotaenioglossans and neogastropods. However, the U-shaped outer layer of each mitochondrial element has only been previously reported in the Volutidae subfamily Zidoniinae. It is now reported here in the subfamily Odontocimbiolinae and may prove to be a diagnostic feature of the Volutidae family.

Juliana Giménez, CONICET, Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina. E-mail: jgimenez@bg.fcen.uba.ar

Introduction

The morphological diversity of spermatozoa in gastropods has been considered a guide to understanding phylogenetic and taxonomic relationships within the molluscs. The Volutidae family is highly diverse and abundant, rendering it particularly interesting, and it is among the many groups of marine gastropods that 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

some volutid species, which have provided much-needed base data on the timing and extent of reproduction (Penchaszadeh and De Mahieu 1976; Giménez and Penchaszadeh 2002, 2003; Giménez *et al.* 2004, 2005; Bigatti *et al.* 2008). Previous studies on spermatozoa in volutids have been conducted in *Zidona dufresnei* (Giménez *et al.* 2008), *Adelomelon ancilla* (Zabala *et al.* 2009) and *Adelomelon beckii* (Arrighetti and Giménez 2010), all of them are members of the Zidoniinae subfamily. As an extension of this work in the Volutidae family, we present the first ultrastructural study of sperm morphology in *Odontocymbiola magellanica* (Gmelin, 1791), which is included in Odontocimbiolinae subfamily. From these studies of two volutid subfamilies, comparisons are made with other caenogastropods, in particular with other species of the Neogastropoda, to identify possible diagnostic sperm features

of the Volutidae and within volutids. The structures of both morphological forms of sperm contribute to an understanding of the reproductive biology of these animals, and have also been useful in elucidating the taxonomic and phylogenetic relationships among them (Ponder *et al.* 2007).

These new ultrastructural descriptions may contribute to resolving some of the relationship of the Volutidae.

Material and Methods

Individuals of *O. magellanica* (Volutidae, Caenogastropoda) were captured from Golfo Nuevo, Puerto Madryn (42°43'S; 65°01'W). Seven mature males were taken by SCUBA, at depths of 5–20 m during low tide, in December of 2006. After removal from the shell, pieces of the testis (9 mm³) or sperm duct were fixed in modified Truby (3% glutaraldehyde in 0.1 M sodium phosphate buffer containing 0.1% CaCl₂) (for 4 h at 4 °C) and washed thoroughly either in sucrose-adjusted cacodylate buffer or in CaCl₂-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 ethanol (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. 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 Philips EM 301 (Netherlands) transmission electron microscopes operated at 75–80 kV. Gonadal tissue was fixed in Bouin's solution, dehydrated in a graded ethanol series, and embedded in methacrylate using standard procedures. Serial sections (5 µm thick) were stained with a modified Masson's trichrome and with the periodic acid-Schiff (PAS) stain for the demonstration of neutral glycoconjugates. Total sperm lengths were determined by viewing and photographing tissue squashes using a Zeiss Axiostar (Germany) light microscope.

Results

Odontocymbiola magellanica exhibits 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).

Euspermatozoa

Acrosomal complex. The acrosomal complex consists of a tall, conical, membrane-bound acrosomal vesicle, an axial rod and a basal plate (Fig. 1A). The acrosomal vesicle is approximately 9.2 µm long. 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,B). The acrosomal vesicle exhibits a very deep invagination (length 3.6 µm) within which is situated the axial rod (subacrosomal material) (Fig. 1A,C). This is the result of a constriction of the invagination. A short (1.49 µm) accessory membrane is closely associated with the base of the acrosomal vesicle (Fig. 1A). 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. 1A). The transverse profile of the acrosomal vesicle changes gradually from the flat in the region of the apical bleb (Fig. 1B), becoming compressed laterally at the anterior region of the invagination (Fig. 1C), until oval-shaped basally (Fig. 1D).

Nucleus. The nucleus is filiform (length from light microscopy 32 ± 2 µm), highly electron-dense and solid, with the exception of a short (5.8 ± 0.3 µm) invagination basally (Fig. 1H). The basal invagination contains a centriolar derivative (Fig. 1E,F) and is continuous with the initial portion of an axoneme composed of a 9 + 2 microtubular pattern (Fig. 1G,H).

Midpiece. Posterior to the nucleus the axoneme is enclosed in a mitochondrial sheath, forming the midpiece region (Fig. 1E). Oblique longitudinal sections through the midpiece clearly show that the sheath consists of 5–6 helically disposed mitochondrial elements around the axoneme (Fig. 2A). Longitudinal sections show that each element exhibits a U-shaped, bilaminar outer layer which is noticeably more electron dense than the remaining mitochondrial material (Fig. 2B). In transverse sections, the elements appear to blend into a continuous sheath (Fig. 2C).

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. 2A). The glycogen composition of these granules has been demonstrated cytochemically, with a PAS positive result (Fig. 2D). At the immediate junction of the midpiece and glycogen piece is an annular complex, consisting of a double ring attached to the inside surface of the plasma membrane (Fig. 2A).

End piece. The end piece succeeds the glycogen piece and consists of the continuing 9 + 2 microtubular-pattern axoneme and surrounding plasma membrane (Fig. 2F). In longitudinal sections, the end piece was not observed.

Paraspermatozoa

The paraspermatozoa of *O. magellanica* (length 50 ± 2 µm) 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 (Fig. 3A).



Fig. 1—Euspermatozoa of *Odontocymbiola magellanica*. —**A**. Longitudinal section (LS) through acrosomal complex and anterior portion of nucleus. Note dense membrane associated with plasma membrane in the basal portion of the acrosome. —**B–D**. Transverse section (TS) of the acrosomal complex from apical bleb region of the acrosomal vesicle. —**E–G**. TS nucleus anterior to centriolar fossa (E), centriolar fossa with axoneme (G). —**H**. 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). *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.

In the main body region of the first type of paraspermatozoa are observed: (1) 14–17 (15 ± 2 , $n = 11$) peripherally distributed axonemes lying close to or in contact with the inner surface of the plasma membrane (axonemes approximately equidistant from each other); (2) numerous very electron-dense (oblong) vesicles; (3) occasional, large round

vesicles of low to moderate electron-density (putative lipid vesicles); (4) numerous low electron-density small vesicles (possible mucoïd deposits); (5) occasional mitochondria (Fig. 3B–D).

Anteriorly only the axonemes and some cytoplasm persist in the apex, where each axoneme attaches to a granular

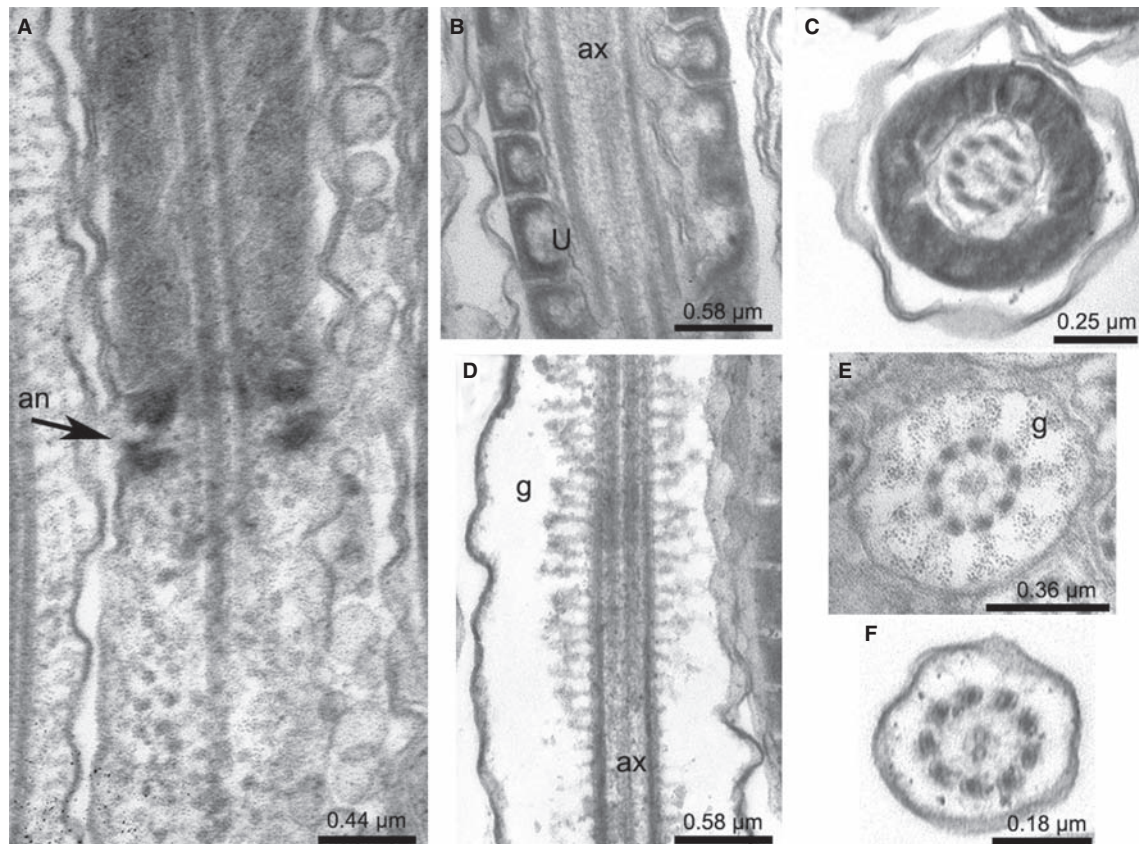


Fig. 2—Euspermatozoa of *Odontocymbiola magellanica*. —**A**. LS junction of midpiece and glycogen piece. Note annular complex with two rings. —**B**. LS midpiece showing internal structure of U-shaped outer layer. —**C**. TS midpiece spiralling of mitochondria around axoneme. —**D**. LS glycogen piece showing putative granules. —**E**. TS glycogen piece showing radiating, longitudinal rows of putative glycogen granules. —**F**. TS end piece region showing the axoneme pattern (9 + 2) close to the membrane. *an* annulus; *ax* axoneme; *ep* end piece; *g* putative glycogen granules; *m* mitochondrion; *mp* midpiece; *n* nucleus; *U* U-shaped defining edge of mitochondrial element.

deposit (Fig. 4A,B). In the second type of paraspermatozoa, the main body region consists of approximately 38–45 axonemes (42 ± 3 , $n = 12$), distributed peripherally and closer to the core region of the cell (Fig. 4C,D). 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. 4A,B). The apex was not observed in transverse sections. The posterior extremity of the paraspermatozoan appears to consist of decreasing numbers of axonemes accompanied by peripheral microtubules and occasional mitochondria (Fig. 4B,E).

Discussion

Most features of *O. magellanica* euspermatozoa can be observed in many other neogastropods and in the higher mesogastropods (Buckland-Nicks 1973; Healy 1982), as characterized by the second type of euspermatozoa described by Healy (1996). These shared characters include: (1) the morphology of the acrosomal complex (apical bleb, accessory

membrane, shape of the acrosomal vesicle and existence of the axial rod material); (2) a solid electron-dense nucleus; (3) a midpiece composed of the axoneme surrounded by multiple (6–10) helically coiled mitochondria; (4) a glycogen piece with nine tracts of granules associated with the axonemal doublets; and (5) the presence of a dense ring structure at the midpiece–glycogen piece junction.

The morphology of the paraspermatozoa of *O. magellanica* categorizes them with the 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 *O. magellanica* are shared with other caenogastropods (Healy 1983, 1996; Koike 1985). The mitochondrial elements and the constriction in the posterior acrosomal invagination, however, are

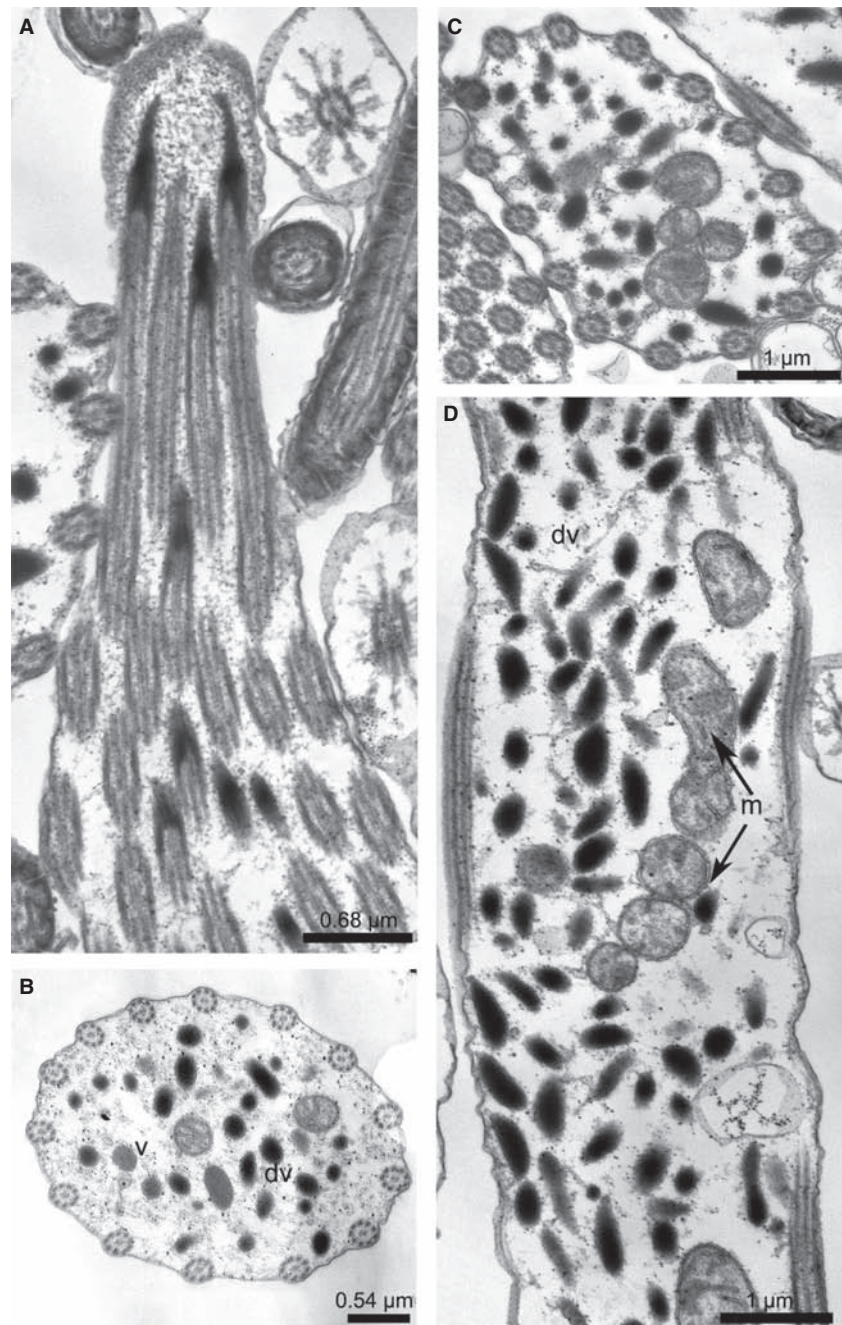


Fig. 3—Paraspermatozoa (Type 1) of *Odontocymbiola magellanica*. —**A**. Longitudinal section (LS) through apex showing axonemes and basal body/centriolar complexes. —**B–C**. TS main body region of paraspermatozoan, showing 14–15 peripheral axonemes closely adherent to the plasma membrane, dense vesicles and less dense vesicles. —**D**. LS main body region showing a part of peripheral axonemes, less dense vesicles and a elongate mitochondrion. *ax* axoneme(s); *dv* dense vesicles; *v* less dense vesicles; *m* mitochondrion; *cr* probable centriolar rootlet.

features shared only with other volutids (Giménez *et al.* 2008; Zabala *et al.* 2009; Arrighetti and Giménez 2010).

Many neogastropods and some mesogastropods, like Potamididae, show uniformly electron-dense mitochondrial elements (Kohnert and Storch 1984; Koike 1985) other present a nonhelical arrangement of mitochondrial elements (Suwanjarat and Suwaluk 2003). In *O. magellanica*, I 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 the longitudinal section profile).

There was a spiraling of the mitochondrial elements, as found in *Z. dufresnei* and *Provocator mirabilis* (Giménez *et al.* 2008), *A. ancilla* (Zabala *et al.* 2009) and *A. beckii* (Arrighetti and Giménez 2010).

The glycogen piece of *O. magellanica* does not differ in any respect from the configuration shown to exist in many other caenogastropods (Healy 1983; Kohnert and Storch 1984; Koike 1985), as it shows the typical microtubular pattern (9 + 2) and has each pair of axonemes associated with glycogen granules. The junction between the midpiece and

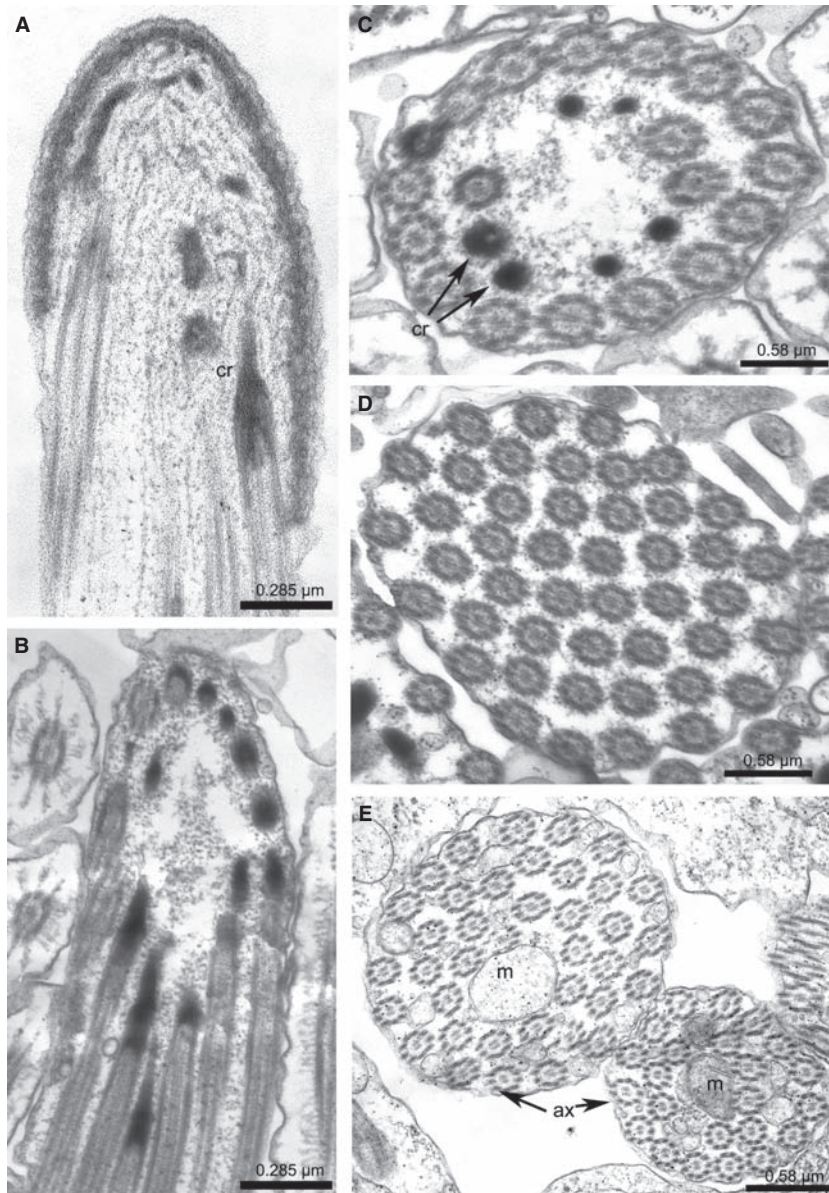


Fig. 4—Paraspermatozoa (Type 2) of *Odontocymbiola magellanica*. —**A**. LS through apex showing cap of granular material enclosing axonemes and basal body/centriolar complexes. —**B**. LS showing clustered basal bodies/centriolar complexes (some transitional to axonemes). —**C**. TS near apex showing mix of axonemes and dense structures (probable centriolar rootlets). —**D**. TS main body region of cell showing axonemes peripherally and within the cytoplasm. —**E**. TS possible posterior region of cell showing reduced number of axonemes. A mitochondrion is also visible. *ax* axoneme(s); *m* mitochondrion; *cr* probable centriolar rootlet.

glycogen piece exhibits a complex of ring elements attached to the plasma membrane, as in many caenogastropods (Buckland-Nicks *et al.* 1982a,b; Healy 1986, 1988; Healy and Jamieson 1993; Giménez *et al.* 2008; Zabala *et al.* 2009). However, the double-ring complex of *O. magellanica* as well as other members from Volutidae family differs from the annulus of other groups, which have a single ring, such as in the Cerithioidea family (Caenogastropoda) (Healy 1982, 1983).

Paraspermatozoa

Vermiform paraspermatozoa are present in most of the Neotaenioglossa and Neogastropoda (see Nishiwaki 1964;

Tochimoto 1967; Melone *et al.* 1980; Healy 1988; Hodgson 1997; Buckland-Nicks 1998). Buckland-Nicks *et al.* (1982a) 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 that never physically associates with euspermatozoa.

Odontocymbiola magellanica presents the vermiform lancet type of parasperm. The parasperm consist of a conical anterior region, where the axonemes decrease in number and are embedded in electron-dense material. This structure was observed in *Z. dufresnei* (Giménez *et al.* 2008), *A. ancilla* (Zabala *et al.* 2009) and *A. beckii* (Arrighetti and Giménez 2010), and other caenogastropods such as the Fusitriton and

Cerastostoma (Buckland-Nicks 1998). Bigatti *et al.* (2008) called two types of cells they found in *O. magellanica* parasperm based on external characteristics observed with a scanning electron microscope. Posteriorly, the number of axonemes increases and centriolar rootlets are present. Transverse sections through the parasperm show the increase in the number 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 *O. magellanica*, the same clustering of parasperm was observed, but 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 researchers (Buckland-Nicks and Chia 1977) consider the function to be the provision of nutrients to and/or the stimulation of euspermatozoa by the products released upon breakdown of the paraspermatozoa. Fretter and Graham (1962) suggest the role of parasperm is to provide 'transportation' for euspermatozoa during and after sperm transfers from male to female, via mobile associations with euspermatozoa. Buckland-Nicks (1998) suggests paraspermatozoa may be involved in creating a hostile prefertilization 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 *O. magellanica* may be involved in supplying nutrition to or stimulation of the eusperm, as euspermatozoa and paraspermatozoa were found in the seminal vesicle together. Still, more intensive studies are needed to determine if paraspermatozoa are present in the *bursa copulatrix*.

Systematic considerations

We consider the U-shaped electron-dense mitochondrial elements in the midpiece of the eusperm present in *O. magellanica*, *A. ancilla*, *A. beckii*, *Z. dufresnei* and *P. mirabilis* and the constriction in the posterior acrosomal invagination in *O. magellanica*, *A. ancilla*, *A. beckii*, *Z. dufresnei* and *P. mirabilis* to be diagnostic at least to the family level. These characters was not evident in *Olivancillaria deshayesiana*, a member of Olividae family (Giménez *et al.* 2009). We suggest that these characteristics could have taxonomic importance because they have not been observed in other caenogastropods or in the close family Olividae, which shares the same superfamily (Muricoidea) with Volutidae. The volutid subfamilies Zidoniinae and Odontocymbiolinae share these characteristics and is possible to confirm that the characters are shared with the rest of the Volutidae family. The content of axonemes in the

paraspermatozoa number varies in each species of the Volutidae family, and this could be considered as a systematic character of this group.

Acknowledgements

Carlos Sanchez Antelo and Gregorio Bigatti generously helped with collection of the samples, Natalio de Vicenzo for his help with the TEM. Jennifer Antonides for her help in English version and improvement of this manuscript. Financial support for this project was provided by UBACyT X171 and CONICET PIP 2788.

References

- Arrighetti, F. and Giménez, J. 2010. Ultrastructure of euspermatozoa and paraspermatozoa in the marine gastropod *Adelomelon beckii* (Caenogastropoda, Volutidae). – *Helgolander Marine Research* **64**: 143–148.
- Bail, P. and Poppe, G. T. 2001. A taxonomic introduction to the recent Volutidae. *A Conchological Iconography*. ConchBooks, Hackenheim.
- Bail, P., Limpus, A. and Poppe, G. T. 2001. The genus *Amoria*. *A Conchological Iconography*. ConchBooks, Hackenheim.
- Bigatti, G., Marzinelli, E. and Penchaszadeh, P. E. 2008. Seasonal reproduction and sexual maturity in *Odontocymbiola magellanica* (Caenogastropoda, Volutidae). – *Invertebrate Biology* **127**: 314–326.
- Bondarev, I. 1995. A phylogenetic classification of Australian Volutidae. – *La Conchiglia* **276**: 25–38.
- Buckland Nicks, J. A. and Chia, F. S. 1977. On the nurse cell and the spermatozeugma in *Littorina sitkana*. – *Cell and Tissue Research* **179**: 347–356.
- Buckland-Nicks, J. A. 1973. The fine structure of the spermatozoan of *Littorina* (Gastropoda: Prosobranchia), with special reference to sperm motility. – *Zeitschrift für Zellforschung* **144**: 111–129.
- Buckland-Nicks, J. A. 1998. Prosobranch parasperm: sterile germ cells that promote paternity? – *Micron* **29**: 267–280.
- Buckland-Nicks, J. A., Williams, D., Chia, F. S. and Fontaine, A. 1982a. The fine structure of the polymorphic spermatozoa of *Fusitriton oregonensis* (Mollusca: Gastropoda), with notes on the cytochemistry of the internal secretions. – *Cell and Tissue Research* **227**: 235–255.
- Buckland-Nicks, J., Williams, D., Chia, F. S. and Fontaine, A. 1982b. Studies on the polymorphic spermatozoa of a marine snail. I – genesis of the apyrene sperm. – *Biologie Cellulaire* **44**: 305–314.
- Darragh, T. A. 1988. A revision of the tertiary Volutidae (Mollusca: Gastropoda) of south-eastern Australia. – *Memoirs of the Museum of Victoria* **49**: 195–307.
- Fretter, V. and Graham, A. 1962. British prosobranch molluscs: their functional anatomy and ecology. *The Ray Society Series*, **144**, pp. 755. Ray Society, London.
- Giménez, J. and Penchaszadeh, P. E. 2002. Reproductive cycle of *Zidona dufresnei* (Caenogastropoda: Volutidae) from the south-western Atlantic Ocean. – *Marine Biology* **140**: 755–761.
- Giménez, J. and Penchaszadeh, P. E. 2003. Size at first sexual maturity in *Zidona dufresnei* (Caenogastropoda: Volutidae) of the south-western Atlantic Ocean (Mar del Plata, Argentina). – *Journal of the Marine Biological Association of the United Kingdom* **83**: 293–296.
- Giménez, J., Brey, T., Mackensen, A. and Penchaszadeh, P. E. 2004. Age, productivity and mortality of the prosobranch snail *Zidona*

- dufresnei* (Donovan, 1823) in the Mar del Plata area, SW Atlantic Ocean. – *Marine Biology* **145**: 707–712.
- Giménez, J., Lasta, C., Bigatti, G. and Penchaszadeh, P. E. 2005. Exploitation of the volute snail *Zidona dufresnei* in Argentine waters, Southwestern Atlantic Ocean. – *Journal of Shellfish Research* **24**: 4.
- Giménez, J., Healy, J. M., Hermida, G. N., Lo Nostro, F. and Penchaszadeh, P. E. 2008. Ultrastructure and potential taxonomic importance of euspermatozoa and paraspermatozoa in the volutid gastropods *Zidona dufresnei* and *Provocator mirabilis* (Caenogastropoda, Mollusca). – *Zoomorphology* **127**: 161–173.
- Giménez, J., Arrighetti, F., Teso, V., Hermida, G. N., Zabala, S. and Penchaszadeh, P. E. 2009. Sperm morphology of two marine neogastropods from the southwestern Atlantic Ocean (Caenogastropoda: Volutidae and Olividae). – *The Nautilus* **123**: 166–171.
- Healy, J. M. 1982. An ultrastructural examination of developing and mature euspermatozoa in *Pyrazus ebeninus* (Mollusca, Gastropoda, Potamididae). – *Zoomorphology* **100**: 157–175.
- Healy, J. M. 1983. Ultrastructure of euspermatozoa of cerithiacean gastropods (Prosobranchia: Mesogastropoda). – *Journal of Morphology* **178**: 57–75.
- Healy, J. M. 1986. An ultrastructural study of euspermatozoa, paraspermatozoa and nurse cells of the cowrie *Cypraea errones* (Gastropoda, Prosobranchia, Cypraeidae). – *Journal of Molluscan Studies* **52**: 125–137.
- Healy, J. M. 1988. Sperm morphology and its systematic importance in the Gastropoda. In Ponder, W. F. (Ed.): *Prosobranch Phylogeny*. – *Malacological Review* **4**: 251–266.
- Healy, J. M. 1996. An ultrastructural study of euspermatozoa in *Bembicium auratum* including a comparison with other caenogastropoda, especially Littorinoidea. – *Journal of Molluscan Studies* **62**: 57–63.
- Healy, J. M. and Jamieson, B. G. M. 1993. Euspermatozoa, paraspermatozoa and spermatozeugmata of *Littorina (Palustorina) articulata* (Prosobranchia: Caenogastropoda) with special reference to the pseudotrich. – *Acta Zoologica* **74**: 321–330.
- Hodgson, A. N. 1997. Paraspermogenesis in gastropod molluscs. – *Invertebrate Reproduction and Development* **31**: 31–38.
- Kohnert, R. and Storch, V. 1984. Vergleichend-ultrastrukturelle Untersuchungen zur Morphologie eupyrener Spermien der Monotocardia (Prosobranchia). – *Zoologischer Jahrbucher* **111**: 51–93.
- Koike, K. 1985. Comparative ultrastructural studies on the spermatozoa of the Prosobranchia (Mollusca: Gastropoda). – *Science Report of the Faculty of Education, Gunma University* **34**: 33–153.
- Melone, G., Lora Lamia Donin, D. and Cotelli, F. 1980. The paraspermatic cell (atypical spermatozoon) of Prosobranchia: a comparative ultrastructural study. – *Acta Zoologica* **61**: 191–201.
- Nishiwaki, S. 1964. Phylogenetical study on the type of the dimorphic spermatozoa in Prosobranchia. – *Science Reports of the Tokyo Kyoiku Daigaku, Section B* **11**: 237–275.
- Novelli, R. and Novelli, A. 1982. Algumas consideracoes sobre a subfamilia Zidoninae e notas sobre a anatomia de *Adelomelon brasiliense* (Lamarck, 1811), Mollusca, Gastropoda, Volutidae. – *Atlântica, Rio Grande* **5**: 23–34.
- Penchaszadeh, P. E. and De Mahieu, G. C. 1976. Reproducción de gasterópodos prosobranquios del Atlántico Surccidental. – *Volutidae Physis A* **35**: 145–153.
- Ponder, W. F., Colgan, D. J., Healy, J. M., Nützel, A., Simone, R. L. and Strong, E. E. 2007. Caenogastropoda. In: Ponder, W. F. and Lindberg, D. L. (Eds): *Molluscan Phylogeny*, pp. 331–383. University of California Press, Los Angeles.
- Poppe, G. T. and Goto, Y. 1992. *Volutes*. Mostra Mondiale: Cupra Marittima, Ancona, Italy.
- Suwanjarat, J. and Suwaluk, S. 2003. Euspermatozoa structure and euspermogenesis in *Cerithidea cingulata* (Gmelin, 1791) (Caenogastropoda: Potamididae). – *Songklanakarinn Journal of Science and Technology* **25**: 413–422.
- Tochimoto, T. 1967. Comparative histochemical study on the dimorphic spermatozoa of the Prosobranchia with special reference to polysaccharides. – *Science Report of the Tokyo Kyoiku Daigaku* **13**: 75–109.
- Weaver, C. S. and du Pont, J. 1970. *The Living Volutes*. Delaware Museum of Natural History, Greenville.
- Zabala, S., Hermida, G. N. and Giménez, J. 2009. Ultrastructure of euspermatozoa and paraspermatozoa in the volutid snail *Adelomelon ancilla* (Mollusca: Caenogastropoda). – *Helgoland Marine Research* **63**: 181–188.