

Ultrastructure of euspermatozoa and paraspermatozoa in the marine gastropod *Adelomelon beckii* (Caenogastropoda, Volutidae)

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Abstract The sperm morphology of *Adelomelon beckii* is described by optical and transmission electron microscopy. Both euspermatozoa and paraspermatozoa were found in the specimens studied. Euspermatozoa are filiform and have an elongate nucleus capped by an acrosome. A small basal plate lies between the base of the acrosome and the nucleus. The mid-piece consists of U-shaped mitochondria wrapped helically around the central axoneme. A dense annulus at the junction of the mid-piece and glycogen piece is found, ending in a short end-piece, composed of the axoneme surrounded by a plasma membrane. Two types of paraspermatozoa are found, both vermiform but differing internally with respect to the disposition and number of axonemes, as well as to the types of secretory vesicles. We suggest the use of paraspermatozoa as a systematic character to reveal phylogenetic relationships in this family.

Keywords Sperm ultrastructure · Parasperm · Giant snail · Reproduction · Mollusca

Introduction

Traditionally prosobranch sperm have been classified as typical or atypical depending on their capacity of fertilization. Healy and Jamieson (1981) proposed the terms euspermatozoa (typical) for those that contain genetic material and are capable of fertilization, and paraspermatozoa (atypical) for those lacking genetic material and being incapable of fertilization. The paraspermatozoa in some species are multifunctional (Hodgson 1997). The morphological diversity of both forms is an important tool that has been utilized for taxonomic and phylogenetic studies in molluscs (Koike 1985; Healy 1983, 1988a). The systematic position of some groups is based on sperm morphology (Healy 1988a, 1996a; Ponder and Lindberg 1997; Ponder et al. 2007).

Adelomelon beckii (Broderip, 1836) is one of the largest molluscan benthic carnivores, inhabiting sandy bottoms (40 and 70 m in depth) in the eastern south Atlantic coast. This species as well as some others of the family, is commercially exploited and recently detailed studies have been undertaken on its reproductive biology (Arrighetti 2009). The ultrastructure of eu- and paraspermatozoa of some species of the family Volutidae has also been described (Giménez et al. 2008; Zabala et al. 2009). However, a detailed description of both types of sperm has not been reported at ultrastructural level for *Adelomelon beckii*, except for some preliminary results on euspermatozoa (Giménez et al. 2009).

The purpose of the present paper is to provide a detailed description of the ultrastructure of eu- and paraspermatozoa in a member of the Volutidae, *Adelomelon beckii* (Broderip, 1833). Results are compared with those on other neogastropods, especially other volutids.

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Materials and methods

Reproductively mature male and female *Adelomelon beckii* (Broderip, 1836) (Volutidae, Caenogastropoda) were obtained from 30 to 50 m depth through commercial fishing outlets at Mar del Plata Harbour (57°37'W, 38°20'S) from September to December 2005. After shell removal, a smear of the interior part of the *bursa copulatrix* (where the male deposits its sperm during copulation) of each female ($N = 31$) was examined microscopically. For males, pieces of the testis (9 mm³) were fixed in Bouin's aqueous solution, dehydrated in a graded ethanol series and embedded in resin (Leica Histoplast) for light microscopy. Serial sections (5 µm thick) were stained with a hematoxylin-eosin and periodic acid Schiff reaction (PAS). Small pieces of testes were also fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer containing 0.1% CaCl₂ for 4 h at 4°C. Tissues were then washed in phosphate buffer. Subsequently, the tissue pieces were placed in a 1% solution of osmium tetroxide (in 0.1 M cacodylate) 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 in a 1:1 ethanol:propylene oxide solution for 15 min, and finally embedded in Araldite resin. Ultrathin sections, using either a Reichert or an LKB IV ultramicrotome, were stained with uranyl acetate and lead citrate. All sections were examined and photographed using Zeiss (Oberkochen, Germany) EM 109T, Hitachi 300 and Jeol 1,010 transmission electron microscopes operated at 75–80 kV. Total sperm lengths ($N = 10$, $n = 15$ per male) were determined by viewing and photographing tissue squashes using a Zeiss Axiostar light microscope.

Results

Euspermatozoon

The euspermatozoa are filiform and composed of an anterior acrosomal complex, nucleus, mid-piece, glycogen piece and end-piece.

The acrosomal complex is elongated and consists of an acrosomal vesicle, an axial rod and a basal plate (Fig. 1a, f). Apically, the plasma membrane of the acrosomal vesicle forms an apical bleb with a rounded shape. The acrosomal vesicle is invaginated posteriorly and constricted anteriorly (Fig. 1b–e). An axial rod lies within the posterior invagination (Fig. 1d). A basal plate is situated between the base of the acrosomal vesicle and the nucleus. In the basal region of the acrosome, there is an accessory membrane, parallel to the plasma membrane (Fig. 1f). Transverse sections of

the acrosomal complex show its conical form (0.43 µm in diameter anteriorly to 2.14 µm posteriorly).

The mature nucleus is long and very electron dense (length 26.67 ± 3.21 µm and width 2.30 ± 0.55 µm, $n = 20$) (Fig. 1a, f, g). The nucleus contains a basal invagination that includes a centriolar derivate and the proximal portion of the axoneme (Fig. 2a, b).

The mid-piece consists of the anterior region of the axoneme that is surrounded by a sheath of helically arranged mitochondria (Fig. 2c). From the analysis of longitudinal and transverse sections it appears that each mitochondrial element consists of a bilaminar, U-shaped, outer layer, which is more electron dense than other parts of the mitochondria (Fig. 2c, d).

Posteriorly the annulus complex, which consists of a double electron-dense ring attached to the inner surface of the plasma membrane, separates the mid-piece from a glycogen piece (Fig. 2c). Posterior to the annulus complex, the axoneme is associated with nine longitudinal and nine radiating tracts of dense granules (Fig. 2c–e). The granules of the glycogen piece stain positively with PAS, indicating that the granules contain neutral glycogen-conjugated elements.

Toward the end-piece the glycogen granules gradually disappear and the sperm diameter decreases from 1.7 ± 0.19 µm in the glycogen piece to 0.7 ± 0.06 µm in the end-piece. The last one consists of a continuous 9 + 2 microtubular pattern axoneme surrounded by a plasma membrane (Fig. 2f).

Paraspermatozoa

The paraspermatozoa of *Adelomelon beckii* are vermiform and 75.7 ± 3.2 µm in length. The apex regions are bunched in contact with the periphery of the tubule (Fig. 3a). Two morphological types can be recognized, differing in the number of internal axonemes. In the first type, the axonemes in the apical region of the paraspermatozoon terminate in basal bodies with rootlets that are embedded in an electron-dense cap (Fig. 3b, c). In the main body of the paraspermatozoa, the axonemes (22 ± 3 ; $n = 15$) are distributed peripherally (Fig. 3d, e). In addition, numerous very electron-dense vesicles lie close to, or in contact with, the inner surface of the plasma membrane. Occasionally, large round vesicles of low to moderate electron density (putative lipid vesicles), numerous less electron-dense small vesicles and often elongate mitochondria are observed (Fig. 3d, e).

The second type of paraspermatozoon shows 47 axonemes and 10 possible centriolar rootlets close to the apical region (Fig. 3f, g). The main body consists of 70 ± 5 axonemes ($n = 13$), distributed peripherally and in the core region of the cell (Fig. 3h).

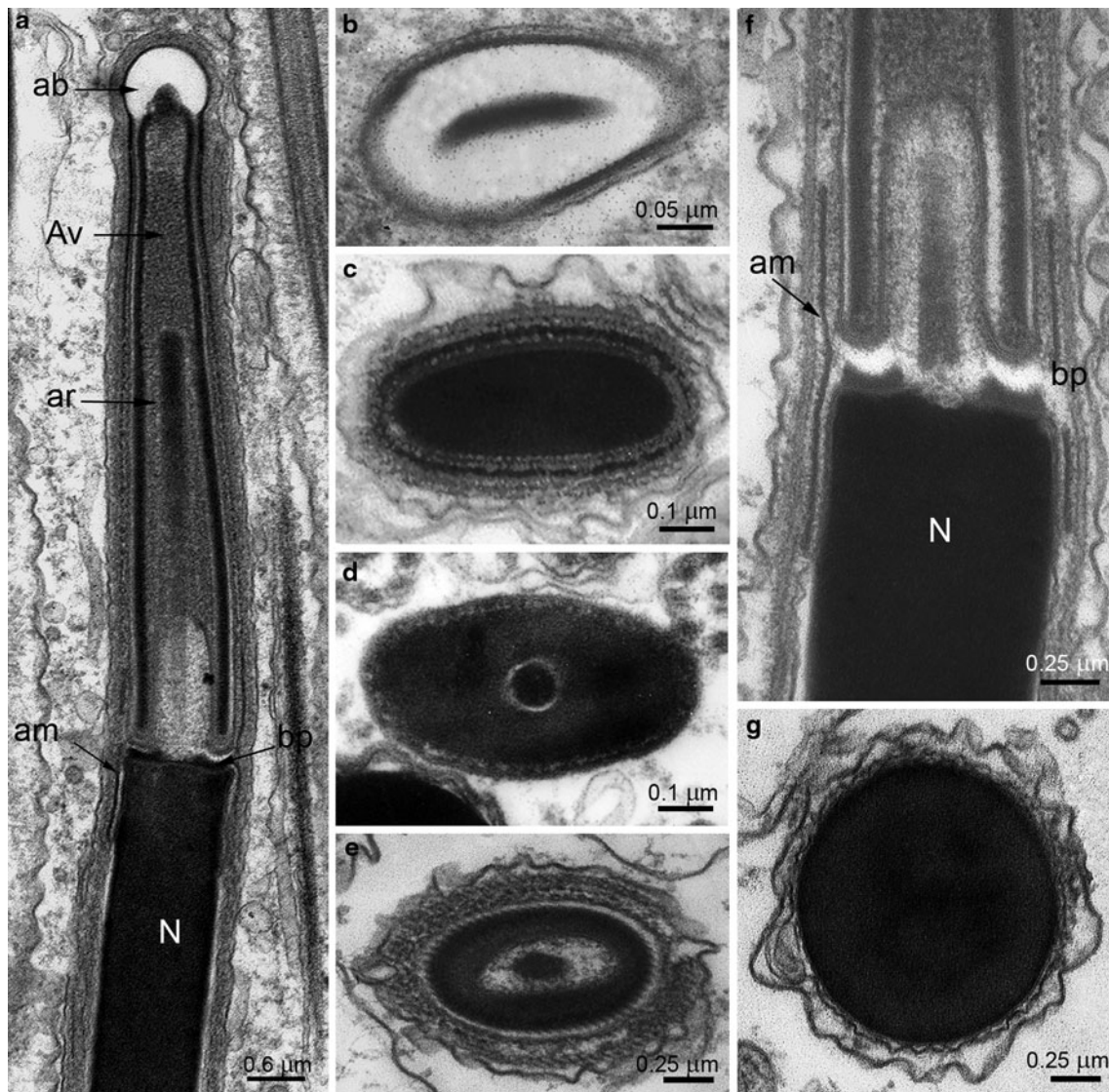


Fig. 1 Euspermatozoa of *Adelmelon beckii*. **a** Longitudinal section (LS) through the acrosomal complex and the anterior part of the nucleus. **b** Transverse section (TS) of the apical bleb region of the acrosomal vesicle. **c**, **d** Transverse sections through the acrosomal complex. **e** Anterior region of the acrosomal complex showing the

invagination and the axial rod material. **f** LS through the basal portion of the acrosomal complex, showing the junction with the nucleus. Note the basal plate and the accessory membrane. **g** TS of the nucleus. *ab* Apical bleb, *ar* axial rod material, *Av* acrosomal vesicle, *am* accessory membrane, *bp* basal plate, *N* nucleus

In the *bursa copulatrix* of females both cell types were observed, and paraspermatozoa were observed actively moving for the entire period.

Discussion

Euspermatozoon

As to the ultrastructure of euspermatozoa, two groups of caenogastropods can be distinguished (Healy 1996a). The structure of the euspermatozoa of *Adelmelon beckii* places this species in group 2.

The acrosome structure is similar to those found in other mesogastropods and neogastropods, except for the constriction present in the invagination of the acrosomal vesicle. This also was found in *Zidona dufresnei* and *Adelmelon ancilla* (Giménez et al. 2008; Zabala et al. 2009), both belonging to the subfamily Zidoninae.

The structure of the nucleus, with a basal invagination where the axoneme is inserted, is similar to that in most mesogastropods (Healy 1982, 1986a, b, 1994, 1996b) and some neogastropods (Giménez et al. 2008; Zabala et al. 2009).

In other species of the superfamily Muricoidea (West 1978; Jaramillo et al. 1986; Gallardo and Garrido 1989;

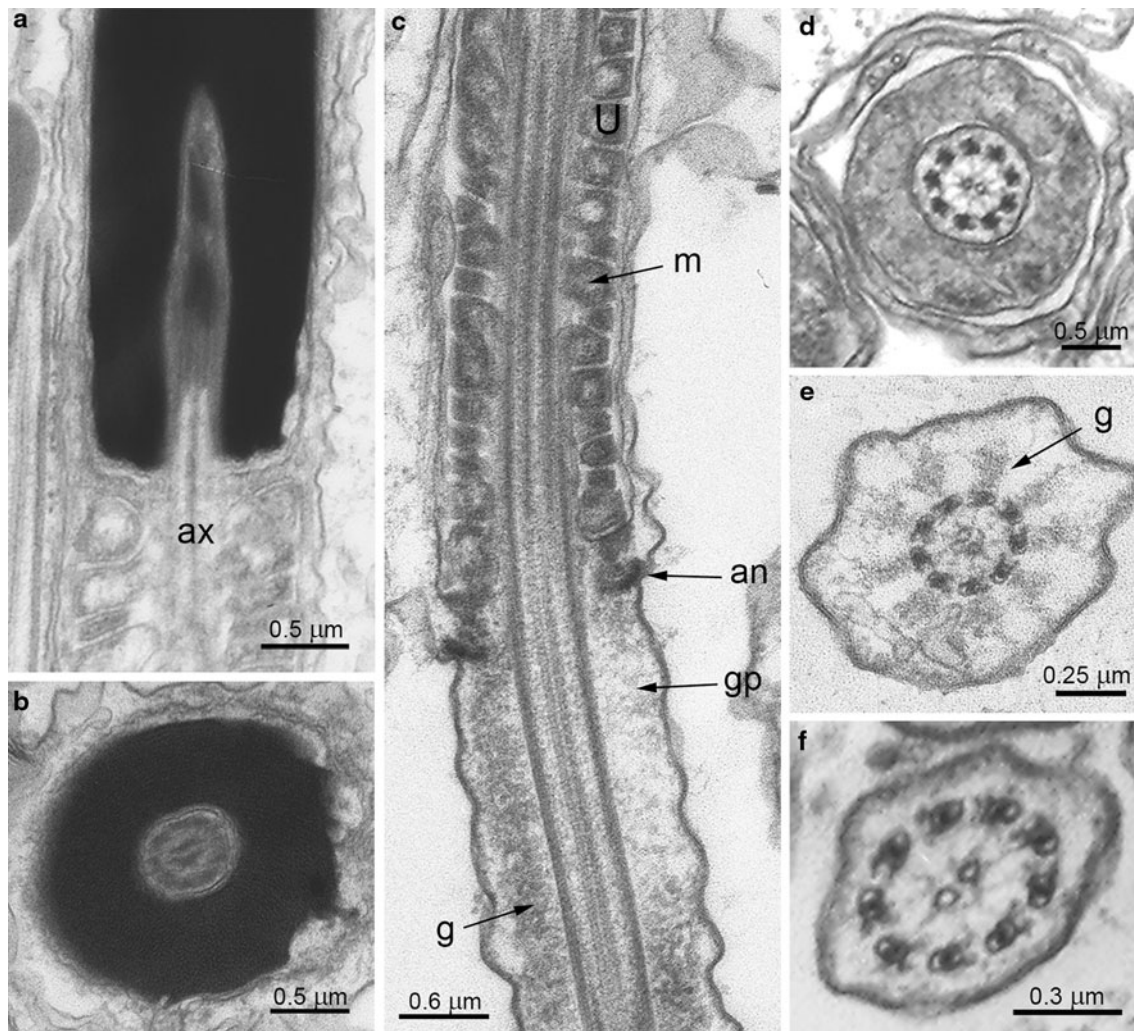


Fig. 2 Euspermatozoa of *Adelmelon beckii*. **a** LS of the junction of the nucleus (showing invagination and centriole/axoneme insertion) and anterior portion of mid-piece. **b** TS of the nucleus with centriolar fossa. **c** LS of the junction of mid-piece and glycogen piece. Note helical mid-piece elements (defined by dense U-shaped profiles) and the

annular complex. **d–f** TS through the mid-piece until the end-piece. **e** TS of the glycogen piece. Note the putative granules. **f** TS of the end-piece, the axoneme is close to the membrane. *an* Annulus, *ax* axoneme, *g* putative glycogen granules, *m* mitochondrion, *U* U-shaped defining edge of mitochondrial element

Giménez et al. 2008; Zabala et al. 2009), the mid-piece is formed by mitochondrial elements helically arranged around the axoneme. In *A. beckii*, in particular, the outer layer of each mitochondrial element is very electron dense, with a U-shape profile in transverse section, coinciding with the other volutids studied (Giménez et al. 2008; Zabala et al. 2009).

The annulus of *A. beckii* is similar to that found in other neogastropods and in many neotaenioglossans (Buckland-Nicks et al. 1982a, b; Koike 1985; Healy 1986a, 1988b; Healy and Jamieson 1993) and differs from the single ring annulus observed in basal caenogastropods (Healy 1982, 1983). The glycogen piece and the end-piece do not differ from the configurations shown in other caenogastropods (Koike 1985).

Paraspermatozoa

Our observations confirm the presence of two types of parasperm, a phenomenon that has previously been demonstrated in five caenogastropod families (Nishiwaki 1964; Buckland-Nicks et al. 1982a, b; Healy 1986b; Giménez et al. 2008). Buckland-Nicks et al. (1982a) differentiated two types of vermiform paraspermatozoa in the ranellid *Fusitriton oregonensis* (Redfield, 1848), a bulkier ‘carrier’ type, which bears numerous attached euspermatozoa and contains vary large-dense vesicles and approximately 112 axonemes, and a smaller type, the ‘lancet’, which never physically associates with euspermatozoa and contains small vesicles and only 16 axonemes. The first type is similar to the ‘lancet type’ while the second type is similar to

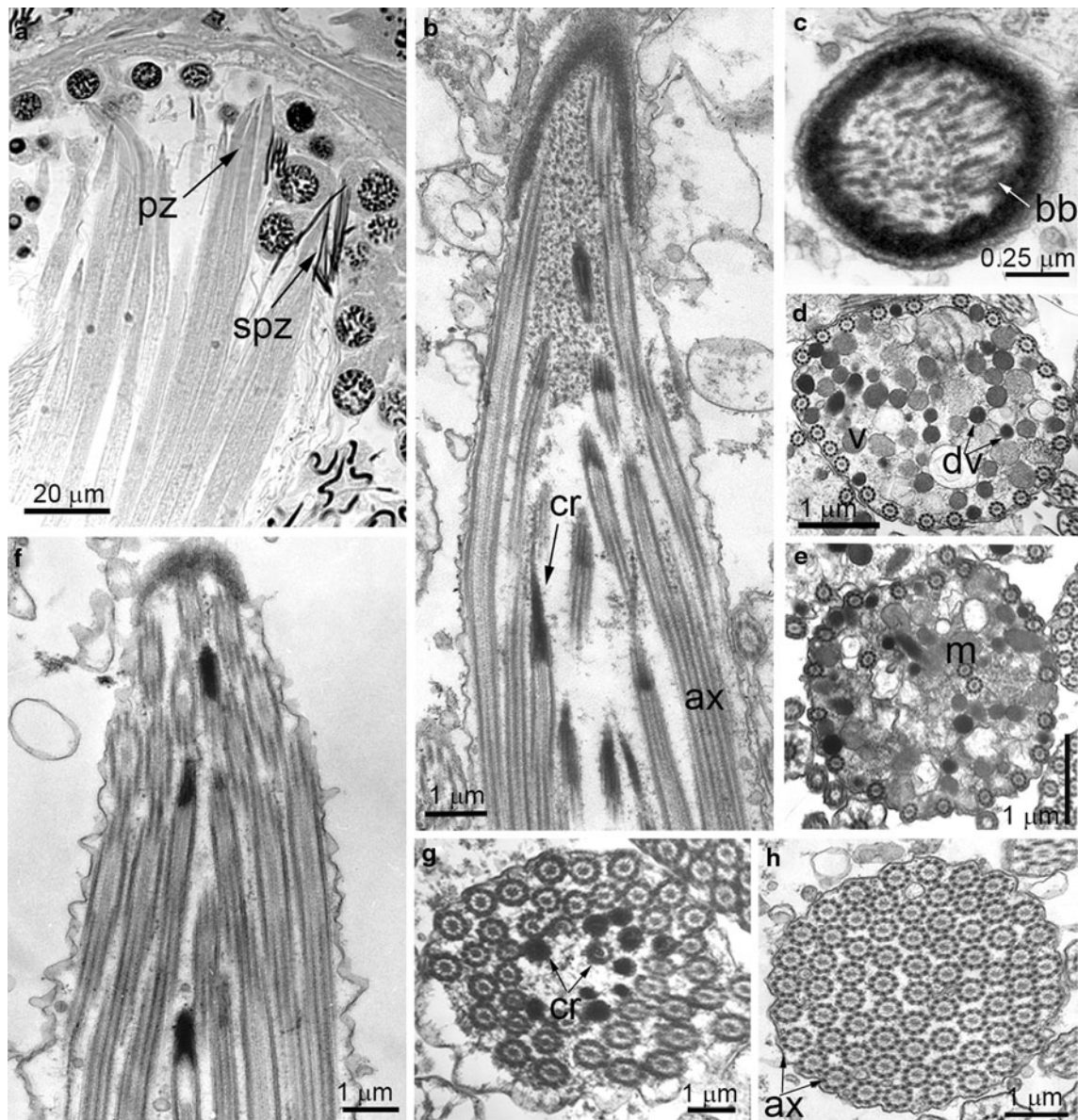


Fig. 3 Paraspermatozoa of *Adelmelon beckii*. **a** Detail of cohorts of paraspermatozoa showing vermiform shape. Paraspermatozoa Type 1: **b** LS of the apical region showing electron-dense material as a cap. Axonemes are ending in the granular material beneath the dense cap. **c** TS through the apex showing sheath of granular material enclosing axonemes and basal body/centriolar complexes. **d, e** TS of the main body region showing the 22 peripheral axonemes, dense vesicles, less-dense vesicles, some axonemes and a mitochondrion embedded in a

dense matrix. **e** TS of the body region showing 22 peripheral axonemes, dense vesicles, less-dense vesicles and mitochondria. Paraspermatozoa Type 2: **f** LS of the apical region showing electron-dense material as a cap. **g** TS near apex of cell showing 47 axonemes and 10 centriolar rootlets. **h** TS anterior region of cell showing 70 (9 + 2) axonemes. *ax* Axoneme(s), *bb* basal body, *cr* centriolar rootlet, *dv* dense vesicles, *v* less-dense vesicles

the ‘carrier type’ described by Buckland-Nicks et al. (1982b). We did not observe any physical association of euspermatozoa and paraspermatozoa in the testis and in the sperm duct of *A. beckii*.

We suggest the use of paraspermatozoa characteristics as an important taxonomic tool in the family Volutidae. The number of axonemes differs markedly among the members of this family: *Z. dufresnei* has 16 axonemes in the paraspermatozoa of type 1 and 28 axonemes in those of type 2

(Giménez et al. 2008), while *A. beckii* has 22 and 70 axonemes, respectively.

The presence of very active paraspermatozoa and active euspermatozoa in the *bursa copulatrix* of females was observed in vivo by light microscopy. Further studies are necessary to confirm a possible association of the two cell types in the *bursa copulatrix* after copulation. Arrighetti (2009) observed the presence of live euspermatozoa and paraspermatozoa in the *bursa copulatrix*. Our observation

could give us an idea about the function of these structures in the *bursa copulatrix*. Buckland-Nicks (1998) suggest paraspermatozoa may be involved in creating a hostile pre-fertilization environment for rival sperm. We propose that paraspermatozoa could also contribute to the movement of the euspermatozoa, facilitating their motility in the female after copulation.

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