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Light Microscopic, Ultrastructure Analysis and Functional Morphology of *Cornu aspersum* Spermatozoa Contained in the Frozen Hermaphroditic Duct

Análisis de Microscopía de Luz, Ultraestructura y Morfología Funcional de Espermatozoides de *Cornu aspersum* Contenidos en el Conducto Hermafrodita Congelado

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SUMMARY: In this study we describe the functional morphology of *Cornu aspersum* (*Helix aspersa*), spermatozoa using light, scanning (SEM) and transmission electron (TEM) microscopies. The studies were performed with sperm located in the frozen hermaphroditic duct. Our results showed that the head presents an elongated conical shape slightly coiled in a corkscrew, with the nucleus partially covered by an acrosome, where an apical vesicle is located at the lateralized apex. This peculiar shape suggests the helical displacement movement of the spermatozoa. The head and the nucleus are slightly larger size compared to those of other gastropod species. The intermediate tract is surrounded by a mitochondrial complex and a glycogen helix. The glycogen helix is coiled helically along the intermediate tract, presenting at least five twists of glycogen helices. The complexity of both the mitochondrial complex and the glycogen helix suggests a high metabolic consumption considering the long period of time until fertilization occurs. Our findings on the detailed characterization of *Cornu aspersum* spermatozoa, obtained from a frozen hermaphroditic duct can contribute to a better understanding of the functional morphology of sperm and serve as a reference for future studies.

KEY WORDS: *Cornu aspersum*; **Spermatozoa; Frozen hermaphroditic duct; Transmission electron microscopy; Scanning electron microscopy.**

INTRODUCTION

Hermaphrodites usually possess sperm storage organs as a component of their reproductive systems (Michiels, 1998) playing an important role in sexual selection processes (Angeloni *et al*., 2003). Gastropod population densities are often so high that multiple mating are common before eggs are laid (Michiels, 1998), which provide an opportunity for sperm competition (Baur, 1998). The presence of allosperm obtained from successive copulations and stored in the spermathecal tubules (Tompa, 1984) increase such competition.

The great morphological diversity existing in the reproductive system of the family Helicidae (Beeman, 1977) makes the knowledge of the reproductive tract, and the sperm functional morphology of these gastropod interesting models to understand the mechanisms of post-copulatory sexual selection in hermaphrodites. As in many other animal taxa, there is vast amount of information available on the morphology of the reproductive system of land snails, although this knowledge has been used primarily in taxonomy and to infer phylogenetic relationships (Wágele & Willan, 2000; Dayrat & Gosliner, 2005; Alvim *et al*., 2011). The general morphology of the reproductive system of some species of helicids has been widely described (Rogers *et al*., 1980; Tompa, 1984; Gomez, 2001; Baur, 2010; Jarne *et al*., 2010; Lodi & Koene, 2016; Sales *et al*., 2019).

In *Cornu aspersum* also known as *Helix aspersa*, the genital tract has a hermaphroditic duct through which both sperm and oocytes circulate from a common gonad, the

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ovotestis. It empties into the fertilization chamber (which is located at the intersection of the hermaphroditic duct and the albumen gland) where sperm and eggs follow two different paths (ovispermiduct), one for the fertilized oocytes, the oviduct, and another for the sperm, the sperm duct. Both ducts end in a common cavity, the atrium (Rousselet, 1979; Tompa, 1984; Gomez, 2001; Baur, 2010; Jarne *et al*., 2010). The sperm duct diverges from the oviduct to form the vas deferens, which empties into the penis sac, which contains a copulatory organ that accesses the genital pore of the other individual (Lucarz, 1984; Blanc & Attia, 1992). The oviduct ends directly in the atrium next to the digitiform glands and the dart sac, which contains a calcareous dart (Koene & Chase, 1998). The concentration of sperm in the hermaphroditic canal of an individual of *Cornu aspersum* is on average 1,450,000 sperm/mm^3 depending on the sexual activity of the individual (Rogers & Chase, 2001; Baur, 2007).

The fine structure of spermatozoa of different groups of mollusks have been extensively investigated (Buckland-Nicks *et al*., 1982; Healy & Willan, 1984; Healy, 1988, 1989; Healy & Jamieson, 1989; Jamieson & Hodgson, 1991; Ke & Li, 1992; Ponder & Lindberg, 1997; Bao *et al*., 1998). The spermatozoa morphological features can be used to determine taxonomic characteristics (*e.g.* Healy & Willan, 1984; Hodgson & Bernard, 1988) to delineate the evolutionary route (Justine, 1991) and to detect marine pollution (Ke & Li, 1992). Transmission electron microscopy (TEM) has been used in several studies in gastropods and other mollusks to describe the spermatogenesis process (Hodgson & Bernard, 1988; Healy, 1989) and the spermatozoa (Buckland-Nicks *et al*., 1982; Healy & Willan, 1984; Hodgson & Bernard, 1988; Healy & Jamieson, 1989; Cuezzo, 1994; Bojat *et al*., 2001; Fahey & Healy, 2003; Cuezzo, 2011; Mansour & Falla, 2012). Other studies have combined TEM with scanning electron microscopy (SEM) to complement the description of the spermatozoa (Atkinson, 1982; Buckland-Nicks & Howley, 1997; Vita *et al*., 2001; Prince & Cichocki, 2015) and spermatogenesis (Griffond *et al*., 1991).

To our knowledge, only some morphological aspects of *Cornu aspersum* sperm have been studied with light microscopy and TEM (Ritter & André, 1975; Healy & Jamieson, 1989) but SEM has not been widely used, apart from (Maxwell, 1975; Anderson & Personne, 1976), so that surface and three-dimensional morphology at high resolution remain poorly known. Otherwise, no previous study has examined sperm ultrastructure in land snails from a frozen hermaphrodite duct. The aim of this study is to describe the functional morphology of *Cornu aspersum* spermatozoa, obtained from frozen hermaphroditic ducts, using light, transmission, and scanning electron microscopies to delve into the characterization of the sperm as a reference for futures studies on ecological indicators, taxonomic characteristics, sexual selection among others.

MATERIAL AND METHOD

Animals. Colonies of frozen adults of *Cornu aspersum* were obtained directly from a snail farm located in Madrid, Spain. These colonies were transported to the Laboratory of Department of Physiology of the Complutense University of Madrid. Individuals were isolated and kept frozen at less than -18 ºC for 48 hours. Hermaphroditic ducts were extracted from 28 snails and fixed according to the purposes of the study.

Light microscopy (LM). Ten hermaphroditic ducts (6-10 mm thick) were fixed in 4 % paraformaldehyde (REF 15710; Electron Microscopy Sciences) and 2.5 % glutaraldehyde (REF 16210; Electron Microscopy Sciences) in phosphate buffered saline (PBS) 0.1 mol l-1 for 4 h at 4 ºC, washed in PBS and stored for 12 h at 4 ºC. Samples were then washed in distilled water and dehydrated in a graded acetone (REF UN1090; Panreac Applicchem) series and were subsequently pre-aggregated in agar solution to form blocks for paraffin embedding. Samples were sectioned into slices 4 µm thick and stained with hematoxylin and eosin. The slides were photographed using a Leica DML750 microscope equipped with a Leica DFC295 camera.

Transmission and Scanning Electron Microscopies (TEM and SEM). For transmission electron microscopy (TEM), ten hermaphroditic ducts (6-10 mm thick) were fixed in Karnovsky's fixative (4 % paraformaldehyde, 2.5 % glutaraldehyde) for 4 h at 4 ºC, and then washed three times (10 min each) in PBS and stored for 12 h at 4 ºC. Subsequently, samples were washed three times (10 min each) in PBS and postfixed in 1 % osmium tetraoxide (REF 19172; Electron Microscopy Sciences) for 1 h. Samples were then dehydrated by immersion in increasing concentrations of acetone and embedded in Spur resin (REF S024/D; Taab embedding materials spur resin embedding kit). Semithin sections (1 mm thick; stained with haematoxylin-eosin and Toluidine blue) and Ultrathin sections (50-70 nm thick) were obtained on a Reichert ultracut S microtome (Leica, Wetzlar, Germany) and stained with methylene blue azure and lead citrate-uranyl acetate, respectively. Sections were examined and photographed using a JEOL1010 TEM at the National Centre for Electron Microscopy, Complutense University of Madrid.

For scanning electron microscopy (SEM), eight hermaphroditic ducts (6-10 mm thick) were fixed as described for light and transmission electron microscopy. The ducts were then washed in distilled water and dehydrated in a graded acetone series until immersion in 100 % acetone. Samples were then dried at the critical point using CO_2 as intermediate, dissected to expose their contents, mounted on stubs, coated with gold, and analysed and photographed under a JSM6400 SEM at the National Centre for Electron Microscopy, Complutense University of Madrid.

RESULTS

Morphology of the hermaphroditic duct. The hermaphroditic duct is almost three centimeters long, coiled on itself like a spring, reducing its length to about 0.8 cm. It is located next to the hepatopancreas in the pallial cavity, is whitish in color and easily uncoils when is removed for processing.

Light microscopy (LM). The hermaphroditic duct is lined mainly by simple cuboidal epithelium (Fig. 1). It is densely populated with spermatozoa, generally oriented longitudinally, forming waves and the aligned heads being perfectly visible. The morphology of the spermatozoa observed in the histological sections shows a long flagellum and a thin, very elongated, and densely stained head (Fig. 1). Each *Cornu aspersum* spermatozoa is formed by a very elongated head, followed by a long flagellum of 1.5 mm long, which cannot be differentiated from the intermediate tract under light microscopy but only under electron microscopy.

Ultrastructure of spermatozoa by TEM and SEM

Spermatozoa head. The head region has a very elongated conical shape slightly coiled in the shape of a corkscrew. The nucleus is partially covered by an acrosome. In the acrosome, an apical vesicle is located at the lateralized apex (spherical, 0.2-0.3 μm in diameter; Figs. 2A,G and 3A) and a less electron-dense acrosomal pedestal can be distinguished.

The nucleus presents a great elongation of the anterior-posterior axis that ends in a tip of approximate dimensions 7 μm long by 2 μm wide in its most basal area, which can be seen by both TEM (Fig. 2A) and SEM (Figs. 3A-G), and it is slightly coiled about the major axis. In addition, the nucleus presents an invagination in its basal area that covers the centriolar zone of the flagellum (this area includes the neck of the spermatozoa). The centriolar zone is shown as a less electron-dense compared to the nucleus. The head is completely covered by a helix-like perinuclear sheath (helical keel) as shown by TEM in transverse and longitudinal sections of the nucleus (Figs. 2A,B).

Spermatozoa intermediate tract (mild tract). The neck, as previously described, is located within the invagination at the base of the nucleus. The nucleus completely covers the nuclear fossa (Figs. 2C,D)describing a funnel shape where the centriolar bodies are located. At the flagellum initiation zone, there is a well-developed central sheath of 0.6 mm of length, surrounding the central pair of axonemal microtubules (Figs. 2 B,E,F and 3E). At the base of the central sheath, some electron-dense granules are observed (Fig.

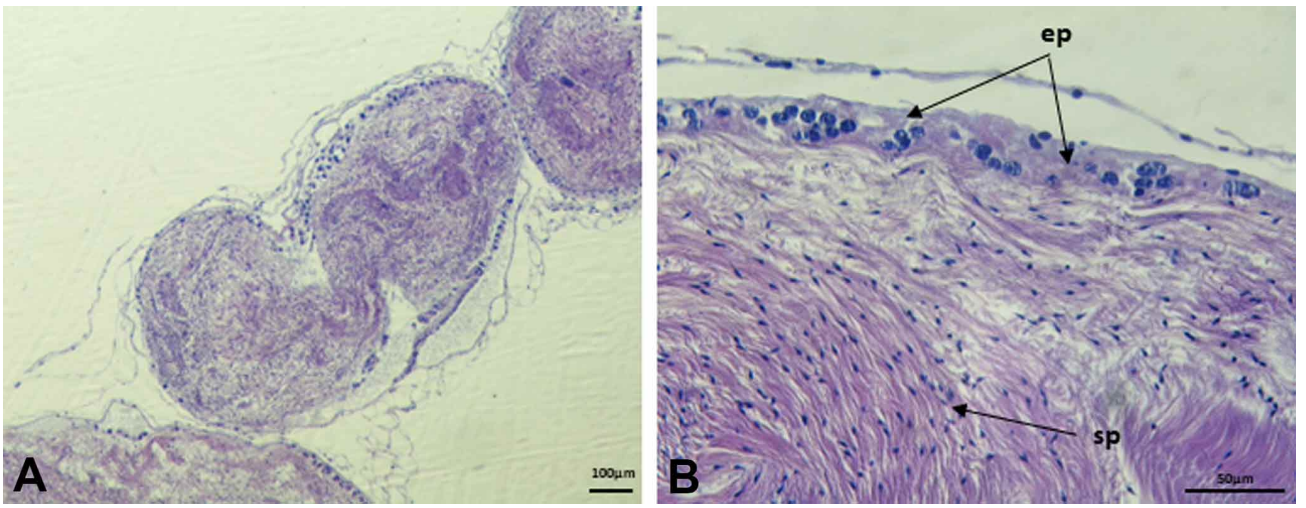

Fig. 1. Light microscopy of the hermaphroditic duct of *Cornu aspersum*. (A) Overview of a sagittal section of the hermaphroditic duct stained with Haematoxylin-Eosin. (B) Detail of the hermaphroditic duct showing the simple cuboidal epithelium (ep) and the presence of sperm (sp) in the lumen.

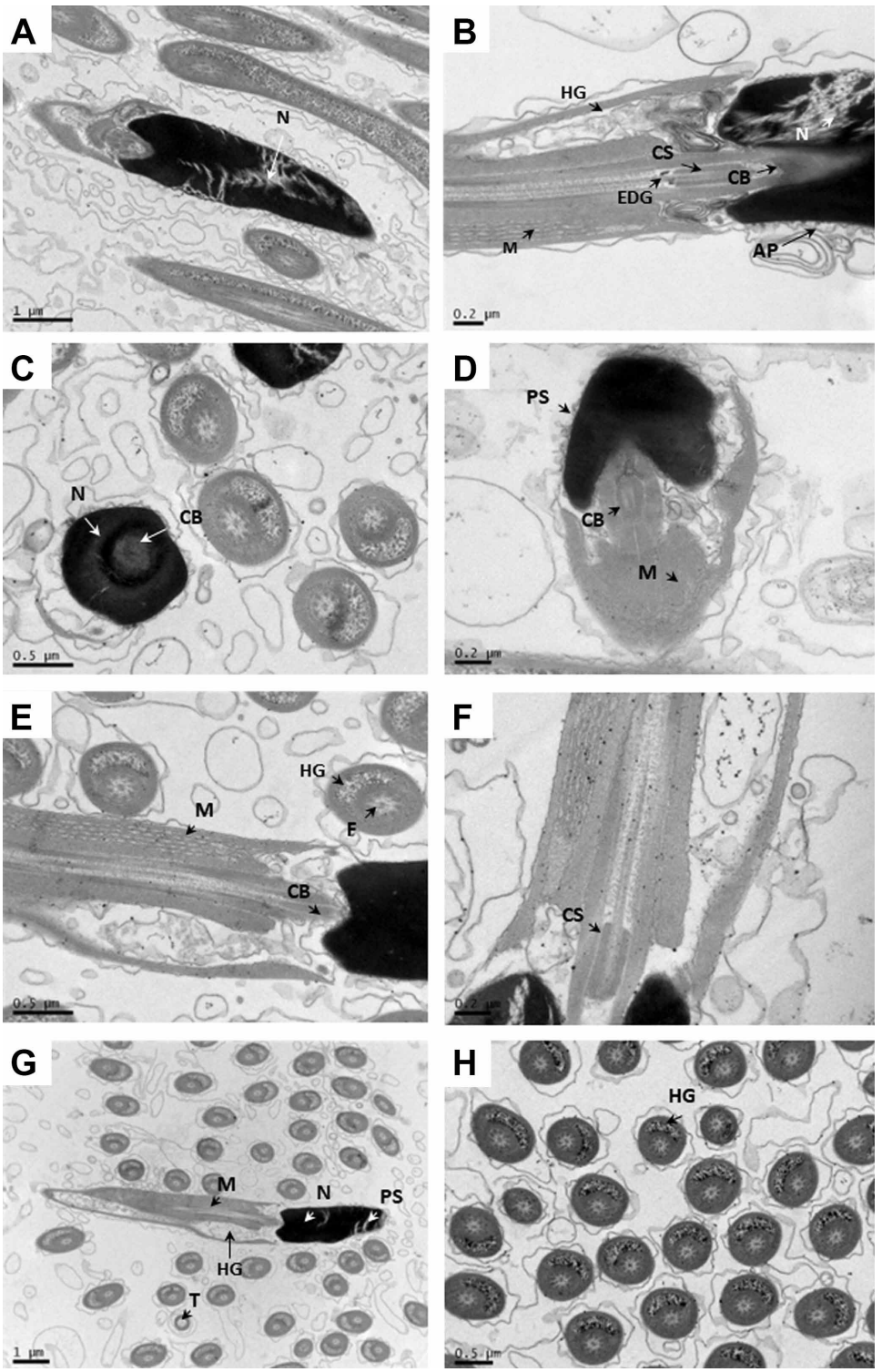

Fig. 2. TEM images showing longitudinal and transverse sections of *Cornu aspersum* spermatozoa. A: longitudinal section through the head, showing helix-like perinuclear sheath (helical keel). B: longitudinal section through the spermatozoa (scale bar = 0.2 µm), showing centriolar bodies inside of the nuclear invagination, electron-dense granules, and the central sheath. Besides, this section shows the begging of the helix of glycogen, mitochondria, and the perinuclear sheath. C: transverse section through the head (scale bar = 0.5 µm), showing the centriolar bodies inside of the nuclear invagination. D: transverse section through the spermatozoa (scale bar = 0.2 µm), showing acrosomal pedestals, centriolar bodies and the mitochondrial complex. E: longitudinal section through intermediate tract (scale bar = 0.5 µm), showing the mitochondria, and the centriolar bodies. Transverse section through the intermediate tract, showing the helix of glycogen and the flagellum. F: longitudinal section through intermediate tract (scale bar = 0.2 µm), showing the central sheath. G: longitudinal section through a complete spermatozoon (scale bar = 1 µm). Transverse section through the spermatozoa tail. H: transverse section through the intermediate tract of multiple spermatozoa (scale bar = 0.5 µm). AP, acrosomal pedestal; CB, centriolar bodies; CS, central sheath; EDG, electron-dense granules; F, flagellum; HG, helix of glycogen; M, mitochondria; N, nucleus; PS, perinuclear sheath; T, tail.

2B) that could act as a connection nodule between the axonemal doublets and the central sheath. The intermediate tract is more than 30 µm long and has a flagellum partially surrounded by mitochondria. In this assembly, a much thinner helix of glycogen is coiled (Figs. 3C,F). The tract is formed by a set of concentric structures that include: an axial filament, a crown of outer fibers, and a mitochondrial sheath. The axial filament originates from the distal centriole and extends to the end of the sperm tail, which consists of a bundle of longitudinal fibers, with a typical flagellar

structure. The crown, composed of nine external fibers, is arranged concentrically to the axial filament, and has a characteristic shape that varies with distance from the head. The axial filament and the crown of fibers maintain their rectilinear structure throughout the intermediate tract and posterior to the tail. The mitochondrial sheath externally surrounds the crown of fibers on one side (Fig. 2G), while a large accumulation of glycogen forming a helix, as previous described by other studies, twisting through at least five revolutions along the intermediate tract (Figs. 2G,H).

Spermatozoa tail. The tail is a very long and dense filamentous piece, with a length of about 1 mm, and contributes to the mobility of the sperm. It presents a structure similar to that described in the intermediate tract, of which it is a continuation apart from the absence of the mitochondrial sheath but presenting instead a fibrous protein sheath (Fig. 3G).

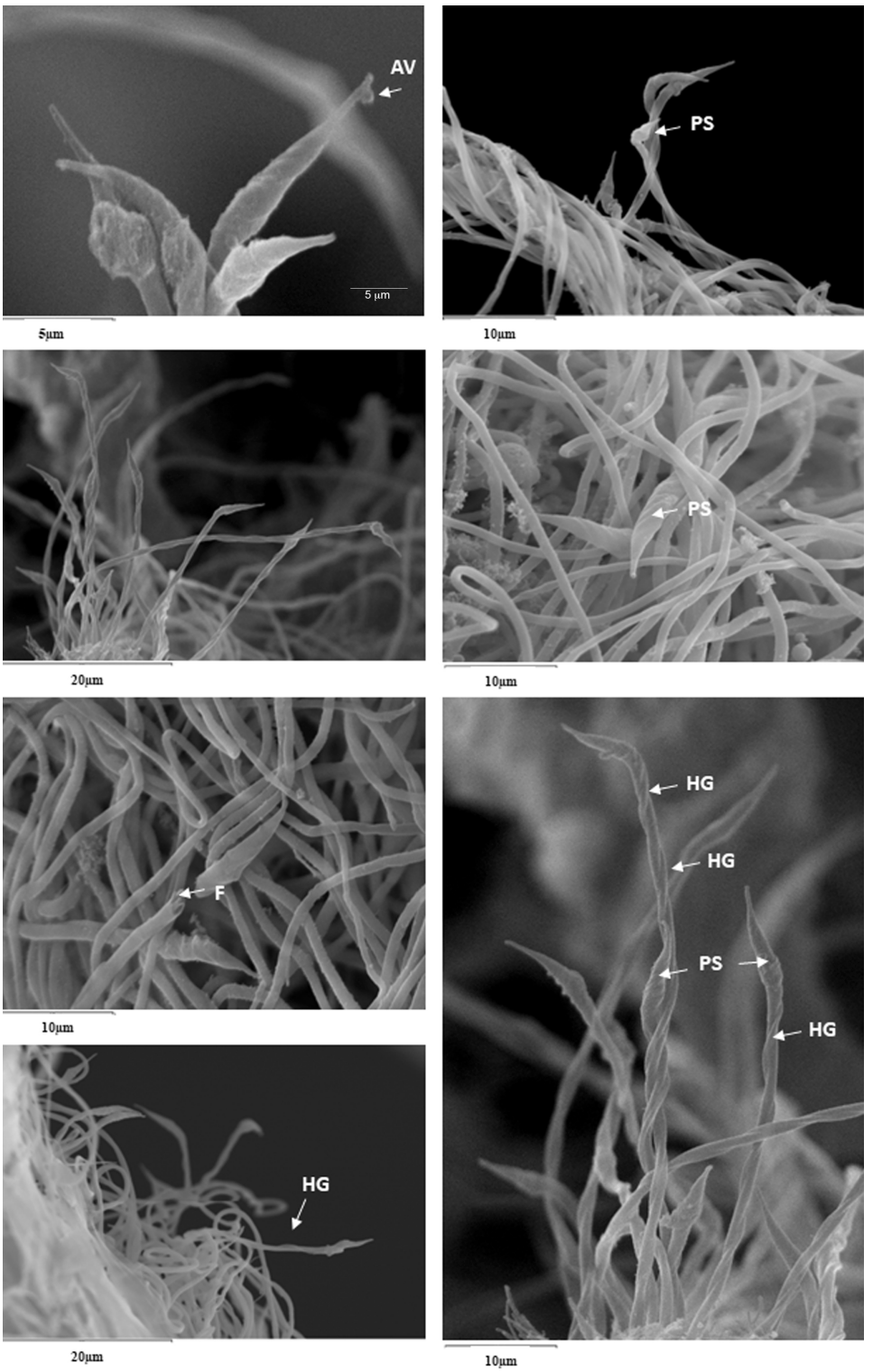

Fig. 3. SEM images of *Cornu aspersum* spermatozoa. A: Image showing the displacement of the apical vesicle. B: Image showing of one of the multiple spermatozoa. C: Image showing the helix of glycogen of multiple spermatozoa. D: Image showing the perinuclear sheath of the spermatozoa. E: Image showing a broken intermediate tract, where the flagellum is observed. F: Image showing that the glycogen helix coils helically along the intermediate tract. G: Image showing the helix of glycogen of the spermatozoa. AV, apical vesicle; F, flagellum; HG, helix of glycogen; PS, perinuclear sheath.

DISCUSSION

This study describes the morphology and ultrastructure of the sperm of *Cornu aspersum* obtained from the dissection of the hermaphroditic conduct. The morphological and functional characteristics of gastropods spermatozoa, as well as the anatomical structures of their reproductive system have been widely described, mainly based on the use of various microscopy techniques, as LM or TEM. In a recent study in sea slug (*Okaenia polycerelloides*) (Sales & Marian, 2020) the sperm-containing chambers were described by LM, TEM and SEM. However, the authors did not show the spermatozoa ultrastructure. Our findings in *Cornu aspersum* coincide with the study by Sales & Marian (2020) that large numbers of spermatozoa fill the sperm-chambers, with the heads of spermatozoa aligned.

The hermaphroditic duct of *Cornu aspersum* had a cuboidal epithelium as has been observed in previous studies in others gastropods such as *Physa fontinalis* (Duncan, 1958), and *Aplysia kurodaix* (Lee *et al*., 2015). In the latter, two kinds of epithelial cells were described: non-ciliated columnar epithelial cells and irregularly shaped capping cells with apical cilia. Ciliated cuboidal epithelium has also been reported in the hermaphroditic duct of other species such as *Retusa obtus* (Berry *et al*., 1992). However, we did not identify ciliated cells in any of the *Cornu aspersum* samples, which seems to be compatible with the function of the hermaphroditic duct, that is, a simple communication channel between the ovotestis and the fertilization chamber.

There is scarce information in the literature regarding the morphology and ultrastructure of *Cornu aspersum* spermatozoa (Ritter & André, 1975; Healy & Jamieson, 1989). By combining TEM and SEM some authors have focused on spermatogenesis Griffond *et al*., 1991) but the characterization of the spermatozoa has been poorly described by SEM (Maxwell, 1975; Anderson & Personne, 1976). However, there is a wide variety of studies on other species of snails and other mollusks (Healy & Willan, 1984; Wilson & Healy, 2002; Fahey & Healy, 2003).

As we have described by TEM, *Cornu aspersum* spermatozoa had a slightly displaced apical vesicle at an angle of 45º at the apex of the head followed by less electron-dense acrosomal pedestals and a perinuclear sheath that covers the entire head. Although our morphological findings are in line with those previously described by Healy & Jamieson (1989) in *Cornu aspersum* and by Bojat *et al*. (2001) in *Aranta arbustorum* by TEM, the SEM analysis allowed us to add more interesting information about, for example, the shape (spherical), the length (0.2-0.3 μm in diameter) and the location of the apical vesicle. The vesicle located just at the edge of the acrosome, slightly displaced to the side as described in a previous study in *Aranta arbustorum* (Bojat *et al*., 2001). Although the apical vesicle has been described by TEM in up to five species of the nudibranch gastropods (Wilson & Healy, 2002) as well as in *Epiphragmophora tucumanensis*, *Cochlicella acuta* (Mansour & Falla 2012), and in up to six species of orthalicid gastropods (Cuezzo, 2011), the displacement of the vesicle was not mentioned. Subsequent TEM studies in other three species (*Actinocyclus verrucosus*, *Hallaxa iju* and *Hallaxa indecora*) of nudibranch (Wilson, 2005), described an ovoid-shape centered on the edge of the acrosome. In addition, in *Actinocydas verrucosus*, an ovoid apical vesicle was shown to measure 0.11 μm in its major axis (Wilson, 2005), a very low data compared with our study in *Cornu aspersum*. Parallel data have been obtained for other species of molluscan nudibranch (Fahey & Healy, 2003), indicating a mean size of the apical vesicle of 0.18 μm, similar to that of *Anguispira alternata* (Atkinson, 1982), which was 0.1 μm of diameter. In conclusion, the apical vesicle of *Cornu aspersum* shows a slightly larger size than that of the described nudibranch species, which seems to have no correlation with any functional difference. The presence of an apical vesicle has not always been described in mollusks, as for instance in different species of patellid limpet (Hodgson & Bernard, 1988) where, instead, a gap between the subcellular membrane and the acrosomal membrane was described. This vesicle is a constant finding in most gastropods' spermatozoa, but its physiological function is not defined in the literature consulted. We suggest that it could rather be a remnant of the perinuclear sheath.

As we have shown in our study, the spermatozoa´s head of *Cornu aspersum* is covered by a perinuclear sheath and acrosomal pedestals, which are said to support the apical vesicle (Healy & Jamieson 1989). We observed by SEM a spiral coiling that gives the head a corkscrew appearance, the head being 7 μm long and 2 μm wide at the base. Compared to analysis of the spermatozoa´s head in other species, such as patellid limpets (Hodgson & Bernard, 1988) and *Notaspidean opisthobranchs* (Healy & Willan, 1984), our data show very high numbers, which means a larger size of the spermatozoa´s head in *Cornu aspersum*. The presence of acrosomal pedestals has also

been described in studies in nudibranchs, showing a conical shape of 0.22μm long and periodic striations in a transverse plane (Wilson & Healy, 2002). We have shown similar findings in *Cornu aspersum*. The nucleus of the nudibranchs *Actinocydes verrucosa* (Wilson, 2005) presented a helical shape like *Cornu aspersum* that is longer than the nucleus of *Actinocydes verrucosa* (length of 4.63μm), and the nucleus of another nudibranch of the genus Halgerda (length of 7.57 μm) (Fahey & Healy, 2003) and other gastropods species (Cuezzo, 1994; Bojat *et al*., 2001; Cuezzo, 2011; Mansour & Falla, 2012). Therefore, these *Cornu aspersum* sperm head structures are slightly larger compared to other gastropod species, as we have yet described for other spermatozoa structures.

Regarding the structure called the spermatozoa "neck" by some authors (Atkinson, 1982; Wilson & Healy, 2002), our findings do not show such an independent structure, at least it seems that the "neck" does not have enough morphological entity since it is difficult to observe since it is included within the nuclear invagination. The latter agrees with previous studies on *Cornu aspersum* (Healy & Jamieson, 1989) and other species such as opisthobranchs (Healy & Willan, 1984).

We have shown that in *Cornu aspersum*, the intermediate sperm tract is surrounded by a mitochondria complex (Healy & Jamieson, 1989) and a glycogen helix. The latter turned out to be more developed than that of other gastropods (Healy & Willan, 1984). In *Limax* species, the glycogen helix has not been reported (Reger & Fitzgerald, 1982). We found that the glycogen helix coils helically along the intermediate tract, getting thinner and thinner until it disappears, in agreement with previous studies in nudibranchs (Fahey & Healy, 2003). Wilson (2005) was the only author who specifically described the presence of two revolution of the glycogen helix in gastropod nudibranchs, unlike other authors who did not indicate any number. Our findings in *Cornu aspersum* by SEM show morphological complexity with respect to the number of revolutions of the glycogen helix. We found a higher number of glycogen helix revolutions, at least five of them. Atkinson (1982) in a stylomatophore pulmonate gastropod (*Anguispira alternata*) denominated the glycogen helix as striations of the coarse fibers. Fahey & Healy (2003) in nudibranchs referred to the glycogen helices as outer layers of paracrystalline material, which depending on the section plane may appear as continuous layers. Whatever name it is given to it, the presence of the glycogen helix in the spermatozoa of gastropods is well known, and it has been reported to be required for sperm metabolism (Healy, 1988; Healy & Jamieson, 1989). The complexity of the glycogen helix shown in *Cornu aspersum* may be reflecting a high metabolic consumption considering that a couple of months may elapse from sperm formation in the sexual gland until fertilization occurs.

The mitochondrial complex of the *Cornu aspersum* spermatozoa was described by TEM on transverse sections of the intermediate tract (Ritter & André, 1975) but no morphological description of sperm ultrastructure was made. There is only one study (in *Fusitrion oregonensis*) that has described the origin of the mitochondrial complex (Buckland-Nicks & Howley, 1997), indicating that it is derived from eight mitochondria of the spermatid. Our observations on the mitochondrial complex in *Cornu aspersum* are in line with the findings described by other authors (Healy & Willan, 1984; Healy & Jamieson, 1989). In fact, the mitochondrial complex is a common structure described in many other mollusks.

Although sperm from mollusks such as oysters (Ostreidae), and mussels (Mytilidae) can be cryopreserved without altering sperm ultrastructure (Di Matteo *et al*., 2009), no previous studies have examined sperm ultrastructure in land snails that have been frozen. This study describes that the results obtained are similar to those obtained with fresh sperm (Healy & Willan, 1984; Healy & Jamieson, 1989).

Taking our results together, we see that the general shape and the morphology of the structures described in the head and the intermediate tract of the *Cornu aspersum* spermatozoa, give it a corkscrew-like appearance. This peculiar shape suggests the helical displacement movement of the spermatozoa, which would be necessary given its great length, in opposition to smaller spermatozoa of other species that move through an undulating movement (Freitas *et al*., 2017; Bondarenko & Cosson, 2019).

In conclusion, this study showed the detailed morphology and ultrastructure of *Cornu aspersum* sperm. TEM results supported and enhanced previous findings whereas by SEM, we have been able to verify the shape and the size of some important spermatozoa structures such as the apical vesicle, the perinuclear sheath, and the glycogen helix. This detailed characterization of *Cornu aspersum* spermatozoa can contribute to better understand the functional morphology of sperm and serve as a reference for future studies on physiology, ecology, and snail production industry.

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FUERTES-RECUERO, M.; ROJO, C.; GARCÍA-CUENCA, I. & FONTANILLAS, J. C. Análisis de microscopía de luz, ultraestructura y morfología funcional de espermatozoides de *Cornu aspersum* contenidos en el conducto hermafrodita congelado. *Int. J. Morphol., 41(4)*:1219-1227, 2023.

RESUMEN: En este estudio describimos la morfología funcional de *Cornu aspersum* (*Helix aspersa*), espermatozoides utilizando microscopías de luz, barrido (SEM) y electrónica de transmisión (TEM). Los estudios se realizaron con espermatozoides localizados en el conducto hermafrodita congelado. Nuestros resultados mostraron que la cabeza presenta una forma cónica alargada ligeramente enrollada en un tirabuzón, con el núcleo parcialmente cubierto por un acrosoma, donde se ubica una vesícula apical en el ápice lateralizado. Esta peculiar forma sugiere el movimiento de desplazamiento helicoidal de los espermatozoides. La cabeza y el núcleo son de un tamaño ligeramente mayor en comparación con los de otras especies de gasterópodos. El tracto intermedio está rodeado por un complejo mitocondrial y una hélice de glucógeno. La hélice de glucógeno se enrolla helicoidalmente a lo largo del tracto intermedio, presentando al menos cinco giros de hélices de glucógeno. La complejidad tanto del complejo mitocondrial como de la hélice de glucógeno sugiere un alto consumo metabólico considerando el largo período de tiempo hasta que ocurre la fecundación. Nuestros hallazgos sobre la caracterización detallada de los espermatozoides de *Cornu aspersum*, obtenidos de un conducto hermafrodita congelado, pueden contribuir a una mejor comprensión de la morfología funcional de los espermatozoides y servir como referencia para futuros estudios.

PALABRAS CLAVE: *Cornu aspersum***; Espermatozoide; Conducto hermafrodita congelado; Microscopio de transmisión por electrones; Microscopía electrónica de barrido.**

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