The Fine Structure of the Spermatozoa of Three Species of Land Snails Belonging to the Genus *Monacha* (Müller, 1774) in Egypt

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Abstract: The present study deals with the ultrastructure of the mature spermatozoa of three species belonging to the land snail genus Monacha (Müller, 1774). These snails are considered as agricultural pests. These species are M. cantiana, M. cartusiana and M. obstructa. They are morphologically closely similar to each other. The present investigation is a new trial to differentiate between them. The snails were collected from the field crops at Dakahlia governorate and brought alive to the laboratory where they were dissected and the gonads were isolated and fixed to be prepared until they were examined and photographed by the transmission electron microscope. Examination of the ultrastructure of the spermatozoa of the three species revealed that sperm of each of them composed of head (acrosome and nucleus), Neck region, midpiece and end-piece. The nucleus has nuclear fossa in which impregnated the components of the neck region. The midpiece contains the mitochondrial derivative and one glycogen helix containing glycogen granules. The end-piece contains only the axoneme surrounded by the plasma membrane. The axoneme has the typical 9+2 microtubule arrangement. There are some differences between them which can be summarized as follow; the nucleus in M. obstructa has a perinuclear sheath, the plasma membrane is convoluted in M. cantiana, the coarse fibers are present in the neck region in the three species but in M. obstructa they extend until the end of the midpiece. The midpiece can be differentiated into glycogen helix region and mitochondrial derivative region in M. cantiana and M. cartusiana but in M. obstructa it can be divided into glycogen helix region, middle region and posterior region. The mitochondrial derivative region in M. cartusiana contains cortical microtubules. In M. obstructa the anterior region of the midpiece has the glycogen helix; the middle region has the glycogen granules in a tubular form around the axoneme, while the posterior part contains only a condensation of glycogen granules around the axoneme. The glycogen helix region in M. obstructa contains cortical microtubules beneath the plasma membrane. According to the above descriptions of the ultrastructure of the spermatozoa of the three species of Monacha in Egypt and due to the presence of many differences between them, the present study recommend that they are actually three valid species representing this genus of land snails in Egypt.
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Keywords: Spermatozoa; Land Snail; Genus; *Monacha;* Egypt

Abbreviation: **A**: Acrosome, **AP:** Acrosomal pedestal, **AV:** Acrosomal vesicle, **Ax:** Axoneme, **CAxM**: Central pair of axonemal microtubules, , **CD:** Centriolar derivative, **Ch:** Chromatin, **CF:** Coarse fibers, **CM:** Cortical microtubules, **CMs:** Central microtubules, **CS:** Crystalline structure of mitochondria, **Cy:** Cytoplasm, **DAS:** Distal accessory sheath, **GG:** Glycogen granules, **GH:** Glycogen helix, **MD:** Mitochondrial derivative, **MM:** Mitochondrial material, **Mt:** Mitochondria, **MV:** Mitochondrial vesicle, **N:** Nucleus, **NF:** Nuclear fossa, **PAS:** Proximal accessory sheath, **PL:** Parallel layers, **PM:** Plasma membrane, **PNS:** Perinuclear sheath, **TGS:** Tubular glycogen structure, **VM:** Vesicular membrane.

1. Introduction

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&b).

The use of sperm ultrastructure for phylogeny and taxonomy was widely accepted and had been related to different aspects of reproductive biology (Franzen, 1955, 1970; Jamieson *et al*., 1991). The spermiogenesis and ultrastructure of the mature euspermatozoa of gastropods had been studied by many investigators (Morton and Young, 1964; Walker and Mac-Greogor, 1968; Franzen, 1970; Buckland-Nicks and Chia, 1976; Robertson, 1985; Healy, 1983b, 1988, 1994; Haszprunar, 1988; Hodgson and Bernard, 1989; Minniti, 1993; Al-Hajj and Attiga, 1995; Attiga and Al-Hajj, 1996; Suwanjarat and Suwaluk, 2003). The present study aims to describe for the first time the sperm ultrastructure of 3 species of land snails belonging to genus *Monacha* (Müller, 1774) living in Egypt.

2. Materials and method

As the land snails species belonging to the genus *Monacha* are considered as agricultural pests, adult individuals were handly collected from infested field crops and nurseries at Dakahlia governorate during spring and autumn seasons of 2008-2009. The collected snails were transfered to laboratory in the Faculty of Science, Mansoura University in cloth bags, kept under 20±2 ° c and 85±5% R.H and offered lettuce leaves for feeding.

Immediately after dissection, small pieces of gonads of the selected species were placed separately in freshly prepared 2.5% cold glutaraldehyde buffered in 0.1 M sodium cacodylate buffer with pH 7.2 at 4oC and rinsed overnight in the buffer at 4oC. The specimens were post-fixed in 1% cold osmium tetra-oxide buffered in 0.1 M sodium cacodylate at pH 7.2 for 3 h. Then, they were dehydrated through ascending grades of ethanol and embedded in resin. Ultrathin sections were stained in uranyl acetate and lead citrate and examined using JEOL 100 CX electron microscope, in Electron Microscope Unit at the Faculty of Science, Alexandria University, Egypt.

3. Results

It seems that the spermatogenesis in *Monacha cantiana, Monacha cartusiana* and *Monacha obstructa* takes place in all acini of the ovotestis. When the male cells complete maturation they are released to the lumen of the acini. The mature spermatozoa were observed alive in the acini of gonad under the binocular light microscope. After their maturation, the spermatozoa pass from the gonad into the hermaphroditic duct where they are stored. All observations presented herein were made on fully mature spermatozoa which are present in the lumen of acini of gonad and hermaphroditic duct. However, spermatozoa from the ovotestis showed no recognizable morphological difference from those of the seminal vesicle or hermaphroditic duct. Spermatophores of this animal were also observed, where their spermatozoa also do not differ morphologically from those stored within the hermaphroditic duct or inside the lumen of acini of gonad.

1. *Monacha cantiana* (Montagu, 1803)

Owing to the great total length of the spermatozoon of *Monacha cantiana*, it was not possible to observe it in its enterity by transmission electron microscopy (TEM). It is of the modified type. The spermatozoon is divided into the head, mid-piece and end piece. The mid-piece can be differentiated into neck region, helix region and posterior region.

I- Head

The head is occupied by or comprised of a nucleus and acrosome. Externally the head is surrounded by a convoluted plasma membrane which is in close contact with the nuclear envelope. The acrosome was clearly seen, but it seemed conical tapering and constructed of or contains dense material (Fig. 3). The acrosome lacks a subacrosomal space. The nucleus is dark, large, short and not straight where its anterior half is concaved with respect to the main axis of the spermatozoon (Figs. 1&3). From the longitudinal and transverse sections, the nucleus contains fibrous chromatin. The nuclear matrix is occupied by dark chromatin composed of filaments, which are longitudinally and transversally oriented (forming a clear network).This chromatin is much denser in the apical zone of the nucleus near the acrosome. Posteriorly, the nucleus has a shallow depression or invagination called nuclear fossa in which the centriolar complex is housed and from which the tail originates. (See nuclear structure in (Figs. 1-5).

II-Neck region

The mid-piece begins in the infolded basal nuclear zone or the nuclear fossa with the neck region. This composed of a central mass as a rod or centriolar derivative ensheathes the proximal portion of the central axonemal microtubules (Fig. 3). The centriolar derivative consists of a condensed homogeneous material. This amorphous material is not having a striated appearance and slightly less dense than the nucleus. Distally to the centriolar derivative, there is a well developed sheath of coarse fibers. The transverse sections revealed that these fibers are 9 in number, dark, alike and are radially arranged surrounding the centriolar derivative (Fig. 4).

III-Mid-piece

Glycogen helix region:

Immediately at the post nuclear region of the neck region, a single helix is present and ensheathed by the mitochondrial derivative. This helix is considered a lateral extension which gradually decreases in size as it spirals posteriorly along the mid-piece. It is also a vesicular structure, glycogen-filled, where it begins in close association with the basal nuclear region. Glycogen fills the cytoplasmic compartments of the mitochondrial helix and also found within the axoneme. The glycogen granules are more condensed in the compartments of mitochondrial sheath or helix than in the matrix of the axoneme. The axoneme is found to have the typical 9+2 microtubule arrangement (Figs. 1, 2 and

4-10).

Posterior region:

It is obvious that the mid-piece ends posteriorly with a distal portion which represents the longest part of it. This portion consists only of the axoneme which enclosed within the mitochondrial derivative and the plasma membrane (Figs. 5, 8, 9 and 12).

IV-End piece

The end piece seems to be very short. It represents the last portion of the spermatozoon and as revealed from the transverse sections, it consists of the axoneme surrounded by a single membrane (Fig. 12). The axoneme takes an approximatively central position for the whole length of the tail.

2- *Monacha cartusiana* (Müller, 1774)

The mature spermatozoon of *Monacha cartusiana* composed of a head, mid-piece and end-piece. The mid-piece can be differentiated into neck, glycogen helix and posterior region.

I- Head

The acrosome was not seen from the longitudinal and transverse sections. The nucleus is large, dark, short and lacking lateral projections or helical keels which had been described for many other stylommatophoran sperms. The chromatin appears fibrous, where the fibers are longitudinally oriented. The chromatin matrix contains lacunae occupied by lighter electron-dense material. Posteriorly, the nucleus is basally invaginated forming an implantation fossa (nuclear fossa) (Figs. 14-17)

II- Neck region

The neck region or the connecting piece is the area that fixes the mid-piece to the head region. It extends from the implantation fossa of the nucleus (nuclear fossa) up to the end of the distal accessory sheath (Fig. 15). The implantation fossa is filled by a conical plug of denser material called "proximal accessory sheath" or "centriolar derivative". The centriolar derivative ensheathes the proximal portion of the central microtubules of the axoneme. Also, in the nuclear fossa, distally to the conical plug of the denser material there are coarse fibers. These fibers are 9 in number and appear as a circular array that envelopes and masks the peripheral doublets of the axoneme. In cross section, The coarse fibres are similar in size and shape and are arranged in radial symmetry (Figs.18&19). These fibers are not accompanying the axonemal doublets throughout the mid-piece but it is found in the neck region only (Figs. 14-23).Also, there is a well developed distal accessory sheath surrounding the central pair of the axonemal microtubules. From the base of the distal accessory sheath to the nuclear fossa, the axonemal doublets are individually enveloped (and therefore are greately obscured) by their accompanying coarse fibers. The composite coarse fibers and enclosed doublets are continuous with the cortical region of the centriolar derivative. In contrast, the central pair of the axonemal microtubules attaches directly to the core of the centriolar derivative (Figs. 14-18 and 26-27).

III- Mid-piece

Glycogen helix region:

At the immediate post-nuclear region of the neck region, a single helix is present enclosed by the mitochondrial derivative. At this point, the helix is almost contains glycogen granules (Figs. 14, 15 and 20). In succeeding levels taken through the mid-piece, the mitochondrial derivative is reduced to a thin layer. The axoneme is seen to have the typical 9+2 microtubule arrangement. The shape of the mid-piece changes considerably throughout its length, such that the glycogen helix gradually becomes reduced in extent and eventually disappears altogether (Figs. 20-21).

Posterior region:

It is the most distal portion of the mid-piece, where the axoneme is surrounded only by the matrix components of the mitochondrial derivative. The central axonemal doublet was seen well developed. In this region, the glycogen helix becomes absent or disappears. In this region, well developed microtubules were also seen beneath the plasma membrane (Figs. 23).

IV- End piece

It is the last portion of the spermatozoon. It consists of the axoneme surrounded by a single membrane (Figs. 22&25). The axoneme takes an approximately central position for the whole length of the tail of the spermatozoon.

3-*Monacha obstructa* (Pfeiffer, 1842)

The mature spermatozoa of *Monacha obstructa* when observed by normal light and phase-contrast microscopy appear identical and are typically aggregated by the apical zone and occupy the lumen of the acini of gonad and hermaphroditic duct.

Owing to the great total length of the spermatozoa, they were not possible to be observed in their entireties by the transmission electron microscope. This spermatozoon is of the modified type and is divided into the head, neck, midpiece and endpiece (Figs. 28-46).

I- Head:

The head is composed of acrosome and nucleus. The acrosome (Figs. 28, 29 and 34) consists of a membrane-bounded apical vesicle, which is a spherical structure partially embedded in the tip of the less-electron dense acrosomal pedestal. There is no subacrosomal space. The nucleus is extremely elongated with a tapering conical apical zone (Figs. 28 and 29). Externally, the nucleus is surrounded by the plasma membrane which is in close contact with an electron-dense layer of homogeneous material termed the perinuclear sheath (Figs. 28-30 and 33-34). The chromatin material appears in some spermatozoa as a vacuolated network (Figs. 28 and 31), while in some others appears homogeneously condensed (Figs. 32-35). In longitudinal sections, no lateral projections or helical keels were observed as has been described for many pulmonate stylommatophoran spermatozoa. Basally or posteriorly, the nucleus is invaginated to form a deep and elongated implantation or nuclear fossa (Figs. 30 and 31).

II- Neck region

The neck or connecting piece is the area that fixes the midpiece to the head. It extends from the nuclear fossa up to the end of the distal accessory sheath. The implantation fossa (Figs. 30 and 31) is filled with the proximal accessory sheath which ensheathes the proximal portion of the central microtubules of the axoneme. The proximal accessory sheath appears as a conical plug of denser material and follows posteriorly to the distal accessory sheath which is a tubular structure located between the coarse fibres and the central pair of axonemal microtubules. The coarse fibres start inside the nuclear fossa ensheathing the proximal and the distal accessory sheath. These fibres are nine in number and regularly surrounding the axoneme as each unit is present corresponding each doublet of the peripheral axonemal microtubules.

III- Midpiece

It is very long and contains the axoneme surrounded by the coarse fibres in its whole length. The axoneme is seen to have the typical 9+2 microtubule arrangement plus the nine coarse fibres and it is ensheathed by the mitochondrial derivatives. The midpiece of the spermatozoon of *M. obstructa* can be differentiated into three regions: anterior, middle and posterior regions (Fig.46). The anterior region or glycogen helix region of the midpiece starts immediately following the neck region where one glycogen helix originates containing glycogen granules. The glycogen helix is ensheathed by the outer paracrystalline layers of the mitochondrial derivatives. Posterior to the neck region, the matrix component of the mitochondrial derivative is organized in four to five layers (Figs. 36-42) but in succeeding levels (or regions of the midpiece) are changed into tubular structure containing glycogen granules in the middle region (Fig. 43) or as only condensations of large glycogen granules in the posterior region of the midpiece (Fig. 44) around the axoneme. The midpiece is also characterized by the presence of cortical microtubules beneath the plasma membrane in the glycogen helix region only (Figs. 37, 39 and 40).

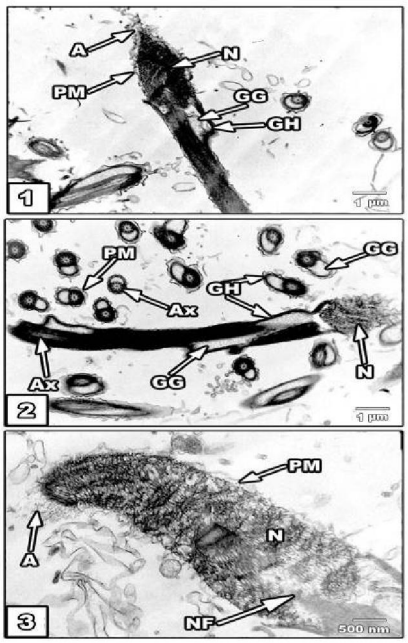
IV- Endpiece

It is the terminal posterior portion of the spermatozoon (Fig. 45). It is characterized by the absence of glycogen helix and mitochondrial derivative. It consists only of the axoneme surrounded by the plasma membrane. It was also observed that the cortical microtubules were absent but there are glycogen granules surrounding the axoneme and inside it (Fig 45).

4. Discussion

The study of molluscan classification has progressed a long way since the adaptation of the Linnean system of binominal nomenclature for all zoological names. Early preoccupation with shell morphology has now been tempered by recognition of the importance of soft part anatomy and radular teeth structure, though it should be added. Shells remain an extremely useful indicator of genetic relationships. General anatomy and shell structure/colour form the basis of modern taxonomic and evolutionary malacology, but increasingly with the assistance of more sophisticated techniques of analysis such as electron microscopy, electrophoresis (protein analysis) and genetic analysis (chromosome, DNA, RNA research). Moreover, comparative spermatozoan morphology in Mollusca using transmission electron microscopy being an attempt to unravel evolutionary relationships and also to determine the correct systematic placement of numerous "problems" (Healy. 1987).

Spermatozoan ultrastructure has proven to be of importance for phylogenetic inferences in Mollusca (Thompson, 1973; Selmi *et al*., 1988; Healy, 1996a and Ponder and Lindberg, 1997). However, there are still too few data on spermatozoa in the stylommatophora to make any statement regarding the taxonomic potential within this large taxon (Healy, 1996a) and there are diverging openions about the degree of morphological diversity. Thompson (1973) emphasizes the great heterogeneity among the stylommatophora, while Giusti *et al*. (1991) stated a remarkable uniformity.

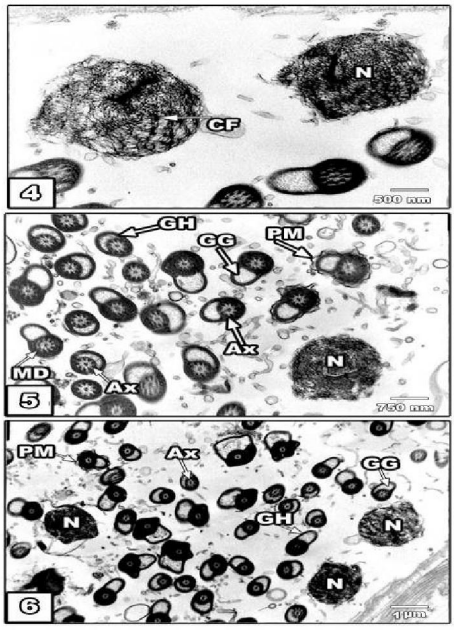


Figs. (1-3): Transmission Electron Micrographs of the mature spermatozoon of *Monacha cantiana* (Montagu, 1803)

1: Longitudinal section of the head region showing the nucleus (N and the acrosome (A).

2: Longitudinal and cross sections showing the glycogen helix (GH), glycogen granules(GG) and the axoneme (Ax).

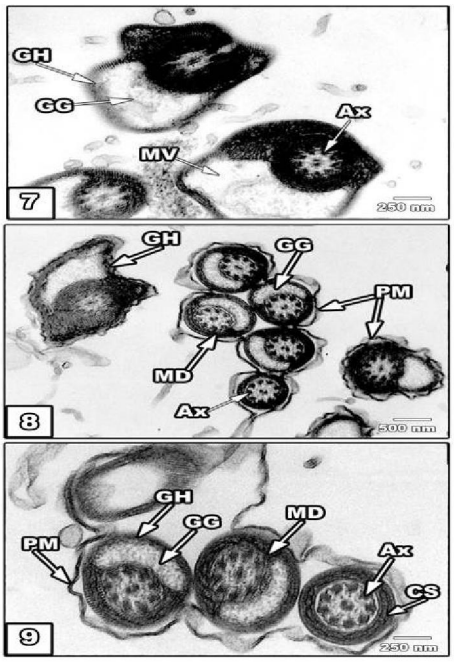
3: Enlarged head region showing the acrosome (A), the nucleus (N) and the nuclear fossa (NF).



Figs (4-6): Transmission Electron Micrographs of the mature spermatozoon of *Monacha cantiana* (Montagu, 1803)

4: Cross sections of the head region showing the fibrous chromatin and the coarse fibres (CF).

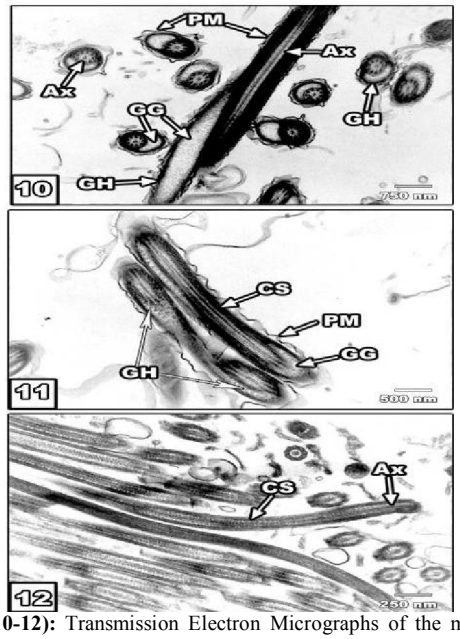
5&6: Cross sections of the sperm showing mainly the mid-piece which is characterized by the presence of a glycogen helix (GH) ensheathed by the mitochondrial derivative (MD) and filled with glycogen granules (GG).



Figs (7-9): Transmission Electron Micrographs of the mature spermatozoon of *Monacha cantiana* (Montagu, 1803)

7: Cross sections of the mid-piece of the sperm showing the mitochondrial vesicles (MV) of the glycogen helix (GH) filled with glycogen granules (GG).

8&9: Cross sections of the mid-piece showing the 9+2 arrangement of the axonemal microtubules (Ax), the convoluted membrane (PM) and the glycogen helix (GH).



Figs (10-12): Transmission Electron Micrographs of the mature spermatozoon of *Monacha cantiana* (Montagu, 1803)

10: Longitudinal and cross sections of the mid-piece showing the glycogen helix (GH) surrounding the axoneme (Ax).

11: Oblique section of the mid-piece with the glycogen helix (GH).

12: Longitudinal sections of the sperm showing the axoneme (Ax).

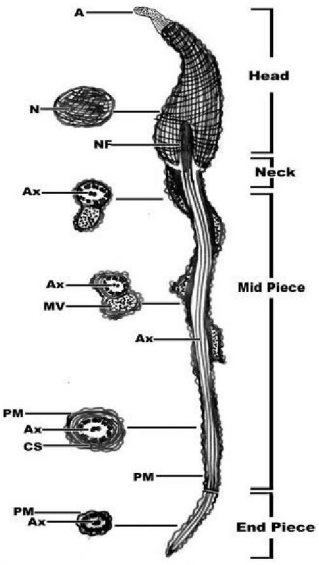
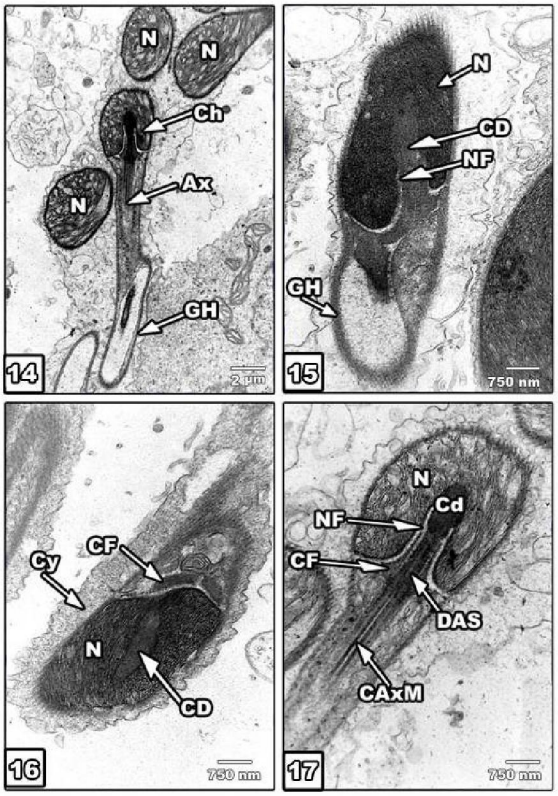


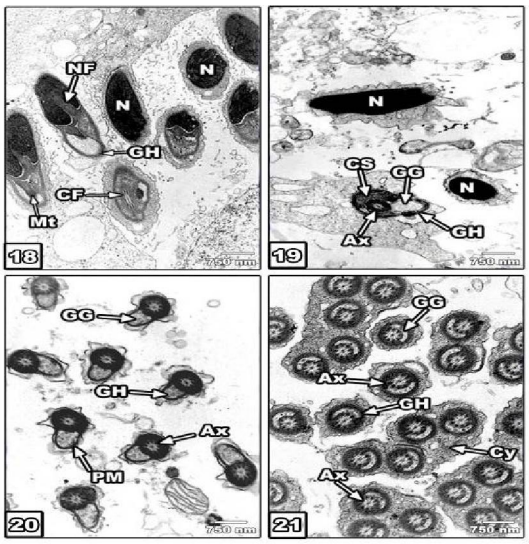
Fig.(13): Attempted reconstruction of the mature spermatozoon of *Monacha cantiana.*



Figs (14-17): Transmission Electron Micrographs showing the ultrastructure of the mature sperm of *Monacha cartusiana* (Müller, 1774)

14 : Longitudinal and cross sections of the head region showing the short nucleus (N), the acrosome (A) is not clearly seen.

15-17: Longitudinal sections of the head and neck regions showing the nucleus (N), the centriolar derivative (CD) and the nuclear fossa (NF).

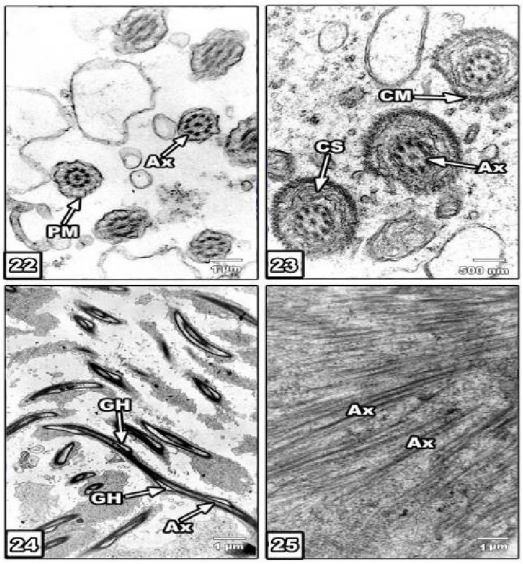


Figs (18-21): Transmission Electron Micrographs showing the ultrastructure of the mature sperm of *Monacha cartusiana* (Müller, 1774)

18&19: Cross and oblique sections of the head region showing the nucleus (N), coarse fibres (CF) and nuclear fossa (NF).

20: Cross sections of the anterior region of the helical region where the glycogen helix (GH) is ensheathed by the mitochondrial derivative (MD) and filled with glycogen granules (GG). The axoneme (Ax) appears to have the typical 9+2 microtubules arrangement.

21: Cross sections of the posterior part of the helical region where the glycogen helix (GH) decreases gradually in size.



Figs (22-25): Transmission Electron Micrographs showing the ultrastructure of the mature sperm of *Monacha cartusiana* (Müller, 1774)

22-23: Cross sections of the posterior region of the mid-piece where the axoneme is surrounded by the crystalline structure of mitochondria (CS). Cortical microtubules (CM) are present beneath the plasma membrane.

24: Longitudinal and oblique sections of the helical region with the glycogen helix (GH).

25: Longitudinal sections showing the axoneme of the sperm.

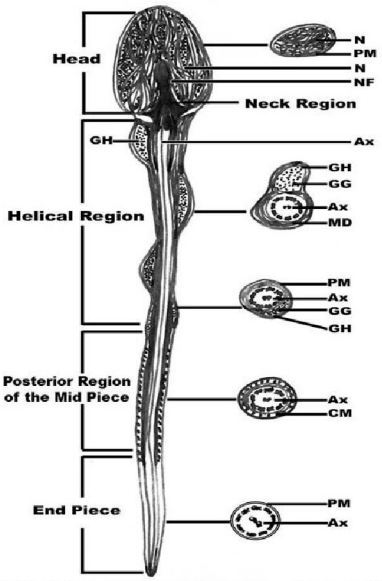


Fig.(26): Attempted reconstruction of the mature spermatozoon of *Monacha cartusiana*.

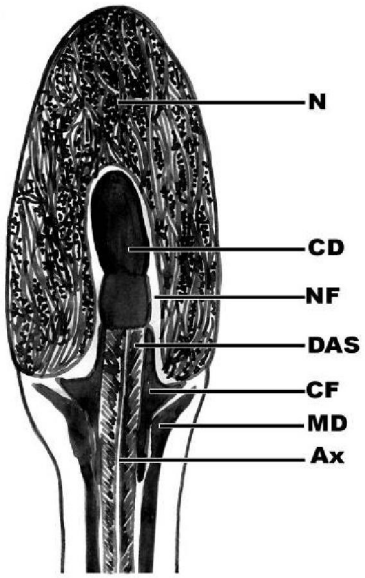
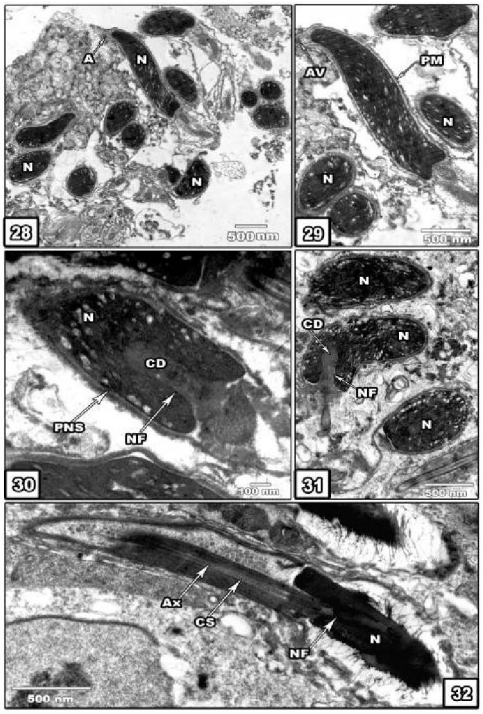


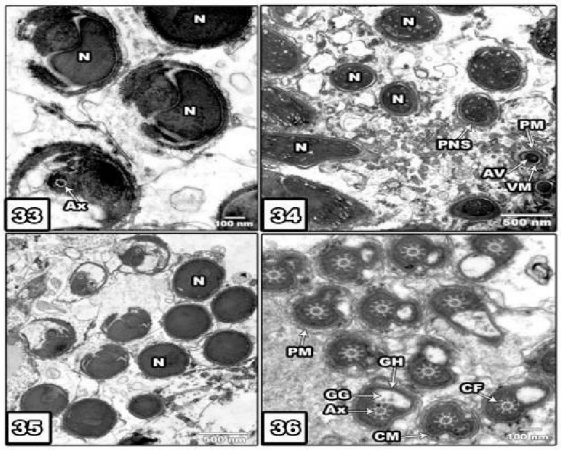
Fig.(27): Diagrammatic reconstruction of the neck region of spermatozoon of *Monacha cartusiana*.



Figs (28-32): Transmission Electron Micrographs showing the ultrastructure of the mature sperm of *Monacha obstructa* (Pfeiffer, 1842).

28-29: Longitudinal and transverse sections in the head region showing the nucleus (N) and the acrosome (A).

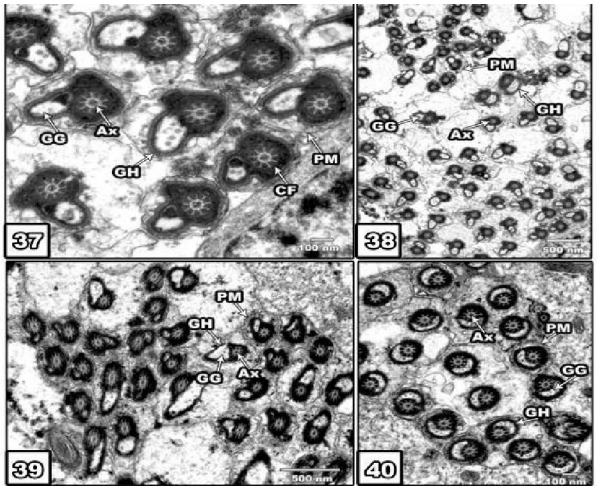
30-32: Longitudinal sections in the head region showing the nuclear fossa (NF).



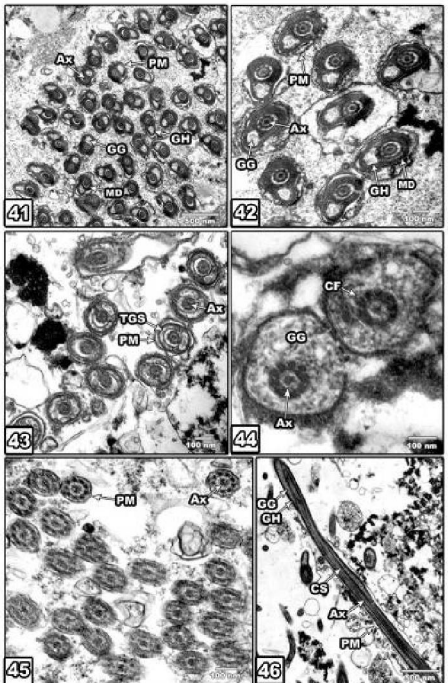
Figs (33-36):Transmission Electron Micrographs showing the ultrastructure of the mature sperm of *Monacha obstructa* (Pfeiffer, 1842).

33-35: Cross sections in the head region with the nucleus (N), the acrosome (A) and the perinuclear membrane (PnM).

36: Cross sections in the anterior region of the midpiece the glycogen helix (GH) ensheathed by the mitochondrial derivative (MD) and filled with glycogen granules (GG).



Figs (37-40): Transmission Electron Micrographs **of c**ross sections in the anterior region of the midpiece of the mature sperm of *Monacha obstructa* (Pfeiffer, 1842) showing the glycogen helix (GH) ensheathed by the mitochondrial derivative (MD) and filled with glycogen granules (GG).



Figs (41-46): Transmission Electron Micrographs showing the ultrastructure of the mature sperm of *Monacha obstructa* (Pfeiffer, 1842).

41-42: Cross sections in the anterior region of the midpiece the glycogen helix (GH) ensheathed by the mitochondrial derivative (MD) and filled with glycogen granules (GG).

43: Cross sections in the middle region of the midpiece showing the tubular structure containing glycogen granules (GG).

44: Cross sections in the posterior region of the midpiece.

45: Cross sections in the endpiece of the sperm. The axoneme shows the typical (9+2) microtubules arrangement.

46: Longitudinal section showing the midpiece with the glycogen helix (GH) and the axoneme (Ax).

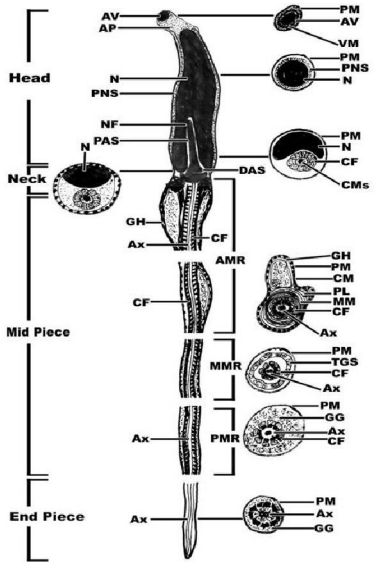


Fig.(47):Attempted reconstruction of the mature spermatozoon of *Monacha obstructa*.

In Gastropoda, spermatozoan morphology has been useful in determining the subclass position. Sperm morphology also clearly shows that the subclass Pulmonata (most land snails, some marine and mangrove snails) is very closely related to the subclass Opisthobranchia, supporting the view of some scientists that these two subclasses could be amalgamated into a single category. Spermatozoa of these two subclasses are long, thread-shaped with one or more spiral keels (glycogen helices) and are among the most complex of all animal spermatozoa (Healy, 1983a and Healy, 1987).

The spermatozoa of mollusks are of two types, i.e., primitive type and modified type. Generally, the mollusk spermatozoan has a head with variable length, a simple mitochondrial midpiece and a tail with a "9+2" arrangement of microtubules. The proximal and distal centrioles separate the head from the mid-piece and the end piece (Franzen, 1983; Hodgson and Bernard, 1988; Bao *et al*., 1998 and Ying *et al*., 2004). The last authors stated that the morphology of the acrosome and nucleus are the most important features for taxonomy. This is insufficient because the other structures of the spermatozoa are very important for the differentiation between sperms of different species, such as presence or absence of coarse fibres and their length of existence across the tail of the sperm, components of mitochondrial derivatives and the number of the outer and inner paracrystalline layers, number of glycogen helices in the mid-piece and presence or absence of glycogen piece.

In the present study, the ultrastructure of the

mature spermatozoa of three species of the genus *Monacha*, namely *M. cantiana* (Montagu, 1803), *M. cartusiana* (Müller, 1774) and *M. obstructa* (Pfeiffer, 1842) was studied. Spermatozoa of the three species, like most euthyneuran spermatozoa possess the following general characters: (1) an acrosome composed of an apical vesicle (usually round) and an acrosomal pedestal (variously shaped depending on taxon, ften columnar), (2) helically-keeled nucleus (either distinct from or partially intertwined with the axoneme and mitochondrial derivative), (3) mid-piece composed of a complex mitochondrial derivative -with one or more incorporated glycogen helices- which surrounds the axoneme and associated coarse fibres, and (4) ordered (usually 9+2) rows of intraaxonemal glycogen granules throughout midpiece and, if present (absent in *Monacha*), the glycogen piece. While exceptional character states occur in spermatozoa of some undisputed euthyneurans (for example, the absence of coarse fibres in spermatozoa of the opisthobranch *Tornatina sp*. as recorded by Healy (1982b). The above listed features readily distinguish opisthobranch and pulmonate spermatozoa from the euspermatozoa of meso- and neogastropod prosobranchs.

The acrosome was not recognized in *M. cartusiana*, while it was present in the others, as it was conical, tapering and constructed of or contains dense material in *M. cantiana* and well-developed composed of a membrane-bounded apical and spherical vesicle and a less-electron dense pedestal in *M. obstructa*. According to Healy (1983a), the acrosome consists of two main elements: a small spherical acrosomal vesicle and a prominent subacrosomal cone (acrosomal pedestal). This scheme is common to all euthyneuran spermatozoa, clearly differs from the structure of the prosobranch sperm acrosome (Walker and MacGreogor, 1968; Garreau de Loubresse, 1971; Buckland-Nicks, 1973; Buckland-Nicks and Chia, 1976; Griffond, 1980; Azvedo, 1981; Healy, 1982 a & b and 1983b; Buckland-Nicks *et al*., 1982; Azvedo *et al*., 1986 and Griffond *et al*., 1991).

The nuclei in the three species of the present study of the genus *Monacha* are similar, where they have fibrous chromatin network, without helical keels characterizing many stylommatophoran species, but the nucleus of *M. obstructa* has an electron-dense perinuclear sheath. Also, the chromatin network of this species appears vacuolated.

Selmi *et al*., (1988) recorded that the spermatozoa from the first hermaphrodite duct (corresponding to a seminal vesicle) constantly appear with uncondensed chromatin. This phenomenon is apparently independent of fixation techniques and is found not only in the genus *Monacha* but also in some primitive pulmonates such as *Siphonaria algesirae* and *Siphonaria japonica* (Siphonariidae) (Sumikawa and Funakoshi, 1984; Azevedo and Corral, 1985). A similar phenomenon has also been described in spermatozoa from seminal receptacles of the female tract of the opisthibranch *Archidoris pseudargus* (Thompson, 1973). The spermatozoa from the gonad or ovotestis showed no recognizable morphological difference from those of the seminal vesicles (Healy, 1983b and Cuezzo, 1994).

The nuclear base has a deep fossa which is completely filled by the centriolar portion of the axoneme, as has been described in other cases of euthyneuran spermatozoa (Bloch, 1969; Subirana *et al*., 1973; Healy, 1986; Selmi *et al*., 1988; Cuezzo, 1994; Hodgson and Healy, 1998; Nenad *et al*., 2002 and Winik *et al*., 2009).

In the neck region, the present study has not been able to find out the lamellar body described in *Agriolimax* (Bayne, 1970) or the cote described in *Helix* (Anderson and Personne, 1967). However, a cone of amorphous material exists at the anterior end of the axoneme, as in *Discus* (Maxwell, 1976) and *Anguispira* (Atkinson, 1982). The coarse fibres observed in the neck region of the three species of *Monacha* demonstrate periodicity surrounding the axoneme as observed in many other species such as *Radix japonica* (Ohsako, 1972); *Discus rotundus* and *Helix* (Maxwell, 1976); *Arion rufus* (Pastisson and Lacorre, 1996).

In these species of *Monacha* as in many other pulmonates (Maxwell, 1976; Hodgson and Healy, 1998 and Winik *et al*., 2009), the coarse fibres and the central doublet of the axoneme are the only connecting structures between the head and the flagellum.

In the middle piece, the mitochondria fuse in a continuous sheath around the axoneme and the mature spermatozoon possesses a highly modified mitochondrial derivative which is partly composed of a paracrystalline compartments. The three species of *Monacha* have one helically coiled channel passing through the paracrystalline structure. This is called glycogen helix, where it is filled with a large quantity of glycogen as many stylommatophoran mollusks (Anderson and Personne, 1967 and 1970; Bayne, 1970; Thompson 1973; Tackaichi, 1975; Maxwell, 1980; Atkinson, 1982; Pastisson and Lacorre, 1996 and Winik *et al*., 2009).

Glycogen storage in the middle piece seems to be a widespread characteristic in the spermatozoa of fresh water and terrestrial pulmonate gastropods (Pastisson and Lacorre, 1996). Glycogen granules are too detected in the matrix of the axoneme in the present study as observed by Healy and Willan, 1984

and Pastisson and Lacorre, 1996. These granules are also found within the tail piece of spermatozoa of all the freshwater pulmonate gastropods studied by Anderson and Personne, 1970 (*Lymnaea, Helisomia* and *Planorbis*) and in other groups, such as Hirudinea (Pastisson, 1966) and Annelids (Anderson *et al*., 1967). The glycogen granules might be a substrate reserve for flagellar metabolism (Maxwell, 1980), and an important source of glycolyzable material for the production energy (Pastisson and Lacorre, 1996).

Moreover, Pastisson and Lacorre (1996) stated that the number of compartments containing glycogen varies with the species , but in all cases, when they are present, each compartment run as helical coarse from the neck to the tail piece (end piece). It must be noticed that in the stylommatophoran mollusks, the glycogen helix is lacking in the tail complex, while the axoneme is the only structure staying in the posterior part of this tail piece.

The very long middle piece containing the complex mitochondrial derivative is a main reason for systematic affinities (Selmi *et al*., 1988).

The mature spermatozoa of *M. obstructa, M. cartusiana* and *M. cantiana* are similar in morphology, but there are special characterstics in each one not found in the others. *M. obstructa* has a well-developed perinuclear sheath; the coarse fibres extend from the neck region until a distance in the glycogen helix region; the mid-piece is divided into anterior part containing one glycogen helix, middle part containing tubular structure with glycogen granules inside and posterior part with large quantity of glycogen granules around the axoneme, and generally the glycogen granules in the species are obviously larger than in the others. *M. cantiana* has a convoluted plasma membrane and doesn't have cortical microtubules. *M. cartusiana* has a nucleus with fibrous chromatin matrix containing lacunae occupied by lighter electron-dense material. The coarse fibres in the neck region only in *M. cantiana* and M. cartusiana and the mid-piece in the two species can be differentiated only into glycogen helix region and posterior region. The cortical microtubules are present in the posterior region of mid-piece of *M. cartusiana*.

It is clear from the study of the ultrastructure of the mature spermatozoa that any difference present in this structure must be taken into consideration to aid in the differentiation between the closely similar species. In this respect, for example the mature spermatozoa of *Bulinus africanus* and *B. globosus* were studied by Applenton and Brackenbury (1997) and they said that the mature spermatozoa of *B. africanus* and *B. globosus* are very similar in morphology with only one taxonomically useful difference being observed between them, viz. the extent of the "frilliness" of the plasma membrane covering the mid-piece. It is more extensive in *B. africanus* than in *B. globosus*. From this point of view, the present study agrees that the species of the land snails Belonging to the genus *Monacha* living in Egypt are three valid species, namely *M. obstructa, M. cantiana* and *M. cartusiana.*

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