Euspermatozoon structure and euspermiogenesis in *Cerithidea cingulata* (Gmelin, 1791) (Caenogastropoda : Potamididae)

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Abstract

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Euspermatozoon structure and euspermiogenesis in *Cerithidea cingulata* (Gmelin, 1791) (Caenogastropoda : Potamididae)
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The ultrastructural characteristics of euspermatogenesis and mature euspermatozoa of *Cerithidea cingulata* are described. Euspermatogonia possesses a large round concentric nucleus with one or two nucleoli. The spermatocyte is characterized by the abundant cytoplasmic organelles and eccentric nucleus with chromatin distributed throughout as small granules. In the early spermatid, euspermiogenesis begins with the condensation of nucleus, the granular nuclear chromatin changes to fibrillar, lamellar and finally a homogenous and highly electron dense nucleus. The cytoplasm of the early spermatid contains a well developed Golgi complex with many vesicles and a prominent rough endoplasmic reticulum located between the connection of the two daughter cells. Acrosome formation starts with proacrosomal vesicle which usually appears close to the well developed Golgi complex. This proacrosomal vesicle differentiates into a pre-attachment acrosome which then moves anteriorly towards the nucleus and finally attaches to the nuclear apex. The features of euspermiogenesis and of the mature eusperm observed in *Cerithidea cingulata* are similar to many of those in Cerithioideans. The mature acrosome composes of a tapering acrosomal cone, an axial rod and a basal plate. The middle piece of the mature eusperm comprises four equal and non-helical mitochondrial elements around the axonemal microtubules. A dense ring structure separates the middle piece from the glycogen piece. The glycogen piece is the proximal part of the tail which consists of an axoneme surrounded

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by nine tracts of dense glycogen granules, while the end piece of the tail lacks glycogen. Euspermatozoa and euspermatogenesis of *Cerithidea cingulata*, though showing some differences between species, do share a number of basic structure features which distinguish potamidid snails from other relatively close families.

Key wards : *Cerithidea cingulata*, euspermatozoa, Potamididae, spermatogenesis, spermiogenesis

บทคัดย่อ

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โครงสร้างและการสร้างอสุจิยูสเปอร์มาโทซัวของหอยฝาเดียว *Cerithidea cingulata*

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ศึกษาโครงสร้างและการสร้างอสุจิชนิดยูสเปอร์มาโทซัว (euspermatozoa) ของหอยน้ำเค็มฝาเดียว สายพันธุ์ *Cerithidea cingulata* โดยบรรยายลักษณะโครงสร้างโดยละเอียดของเซลล์สืบพันธุ์ทุกระยะ ตั้งแต่เซลล์สืบพันธุ์เริ่ม ต้น ยูสเปอร์มาโทโกเนีย (euspermatogonia) ยูสเปอร์มาโทไซต์ (euspermatocyte) ยูสเปอร์มาติด (euspermatid) และยูสเปอร์มาโทซัว (euspermatozoa) รวมทั้งอธิบายขั้นตอนการเปลี่ยนสภาพ (differentiation) ของยูสเปอร์มาติด เป็นยูสเปอร์มาโทซัว จากการศึกษาครั้งนี้สรุปได้ว่า การสร้างอสุจิของ *C. cingulata* ประกอบด้วยลักษณะโครงสร้าง พื้นฐานคล้ายคลึงกับหอยฝาเดียวชนิดอื่น ๆ ในกลุ่ม Cerithioidean และอสุจิของ *C. cingulata* แสดงลักษณะร่วม ของหอยในวงศ์ Potamididae ซึ่งเป็นลักษณะที่สามารถจำแนกหอยฝาเดียววงศ์ Potamididae ออกจากวงศ์ที่มีลำดับ อนุกรมวิธานใกล้เคียงกันได้ การศึกษาครั้งนี้ได้รายงานลักษณะเฉพาะของหอยชนิดนี้ด้วย

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Prosobranch sperms have been classified as typical or atypical depending on whether they are capable of fertilization (Franzen, 1955). These two types of spermatozoa coexisting in the same species and in the same individuals. Healy and Jamieson (1981) introduced the term euspermatozoa and paraspermatozoa to represent the typical and atypical spermatozoa, respectively. The eusper-matozoon or typical spermatozoon is filiform and motile. It contains genetic material, and is capable of fertilization, while the paraspermatozoon or atypical spermatozoon is much larger, lacks genetic material, and is incapable of fertilization (Dohman, 1983; Franzen, 1955; Fretter, 1984; Maxwell, 1983). In many caenogastropod, based on ultrastructural studies of spermatogenesis and sperm morphology, there are also two types of sperm present (Healy, 1982a, 1983, 1986a). The use of sperm ultrastructure for phylogeny and taxonomy is now widely accepted and has been related to different aspects of reproductive biology (Franzen, 1955, 1970; Jamieson *et al.*,1991). Thus, the spermiogenesis and ultrastructure of the mature euspermatozoa of gastropods have been studied by many investigators (Al-Hajj and Attiga, 1995; Attiga and Al-Hajj,1996; Buckland-Nicks and Chia, 1976; Franzen, 1970; Haszprunar, 1988; Healy, 1983, 1988a, 1988b, 1988c, 1994; Hodson and Bernard, 1989; Morton and Young, 1964; Robertson,1985; Minniti, 1993; Walker and Mac-Gregor, 1968). Among these, the ultrastructure of mature euspermatozoa and the paraspermatozoa of some species within family Potamididae have also been described (Healy, 1982a ; 1983, 1986a; Healy and Jamieson 1981; Suwanjarat and Klepal, 2001). However, the detailed description of sper-matogenesis has not been reported at the ultra-structure level for *Cerithidea cingulata*.

Cerithidea cingulata (Gmelin, 1791) or the Girdled Horn Shell is a brackish small shelled

gastropod of the family Potamididae. Despite its role in the important food chain of the mangrove ecosystem, the population size of *Cerithidea cingulata* is an indicator of environmental pollution. In Pattani Bay, the Southern Gulf of Thailand, where *Cerithidea cingulata* is abundant, molluscs are used to monitor the level of pollution in coastal waters (Kan-atireklap *et al*, 1997 and Swennen *et al*, 2001). Thus, the study of the reproductive biology of this species is essential. The present work studies the spermiogenesis and the morphology of mature euspermatozoa in *Cerithidea cingulata* (Gmelin, 1791) that inhabit the muddy sands in the intertidal zone close to the mangrove forests of the western coast of Southern Thailand. This study not only provides useful information on the reproductive biology but also helps determining the taxonomic relationship between many other small shelled Cerithioideans.

Materials and Methods

Mature specimens of *Cerithidea cingulata* were collected from March-May 2001 in the intertidal mudflats of the mangrove forest along the coast of Satun province, Southern Thailand. The live specimens were taken to the laboratory of the department of Biology, Prince of Songkla University, Hat Yai. Small pieces of testes were fixed in 4% paraformaldehyde phosphate buffer pH 7.3 at 4°C, washed in phosphate buffer, postfixed in 1% osmium tetroxide for 1 hr, stained for 45 min in 2% uranyl acetate, dehydrated in an ethanol series and embedded in Epon 812. Semithin and ultrathin sections were cut with a ultramicrotome model MTXL, the ultrathin sections were collected on 200 mesh copper grids, stained with uranyl acetate and lead citrate. The sections were examined with a transmission electron microscope Zeiss EM 9S-2, at the Ultrastructure Research Unit, Institute of Zoology, University of Vienna, Austria.

Results

Spermatogonia

The spermatogonia possess a large round, centrally located nucleus with small clumps of electron-dense chromatin. One or two nucleoli are also often found. The nuclear envelope is electron-dense, the cytoplasm contains numerous ribosomes, a Golgi body, endoplasmic reticulum, and a few small oval or round mitochondria (Figure 1).
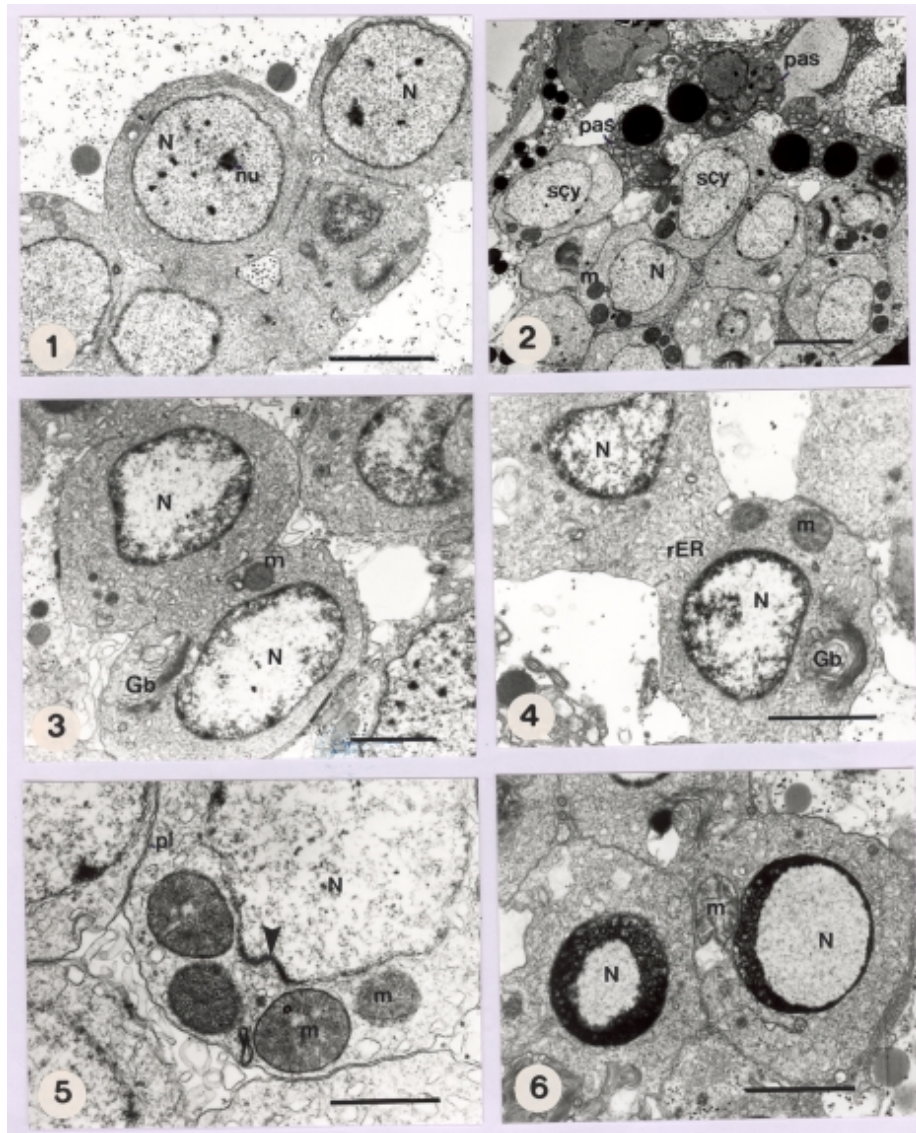
Spermatocytes

The eccentric nucleus of spermatocytes is smaller than that of spermatogonia. The nuclear chromatin is distributed as small granules throughout the nucleus. The cytoplasmic organelles are abundant with a small Golgi body, endoplasmic reticulum, and a number of round moderate sized mitochondria. Some paraspermatocytes or the atypical spermatocytes are found among spermatocytes (Figure 2). The paraspermatocyte is characterized by a large electron dense granule and the many dilated vesicles of the Golgi body, and the increased endoplasmic reticulum filling the entire cytoplasm. The primary and secondary spermatocytes are often difficult to distinguish. The cytoplasm of the late spermatocyte contains a smooth and rough endoplasmic reticulum. The Golgi complex is generally arranged in flattened cisternae close to the nucleus with more or less dilated vesicles in the peripheral regions of the cytoplasm. Some mitochondria are found scattering within the cytoplasm. Most developing spermatocytes are connected by intercellular bridge (Figure 3).

Spermiogenesis

During spermiogenesis, the spermatids undergo differentiation to become mature spermatozoa. The following major steps then take place: nucleus condensation; acrosome formation; midpiece development. These phenomena are described as follows:

Nuclear condensation: The nucleus of spermatid prior to spermiogenesis is spherical, eccentric with no apparent nucleolus. The cytoplasm of the early spermatid is characterized by a prominent well developed Golgi body and the rough endoplasmic reticulum is prominent in the cytoplasm between the two daughter cells (Figure

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Figure 1. Spermatogonia showing round nucleus (N) contains one or two nucleoli (nu). Scale bar = 4 μm.

Figure 2. Primary spermatocytes (scy) and paraspermatocytes (pas). N: nucleus; m: mitochondria. Scale bar = 6 μm

Figure 3. Late spermatocyte connected by a cytoplasmic bridge. Note the Golgi body (Gb) with the flattened cisternae. m: mitochondria. Scale bar = 3 μm

Figure 4. Early spermatid with the thickening of the nuclear chromatin. Gb: Golgi body; m: mitochondria; N: nucleus; rER: rough endoplasmic reticulum. Scale bar = 3 μm

Figure 5. Early spermatid showing the gathering enlarged mitochondria at the nuclear base (arrow head). m: mitochondria; N: nucleus. Scale bar = 1.5 μm

Figure 6. Spermatid showing beginning of chromatin condensation. N: nucleus; m: mitochondria. Scale bar = 3 μm

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4). The large mitochondria developed by the fusion of the small ones, are clustered close together at the presumptive posterior pole of the nucleus (Figure 5). The chromatin granules of this stage are condensed at the periphery of the nucleus, forming the thick layer beneath the nuclear envelope (Figure 6). The posterior nuclear pole is marked by the attachment of large spherical mitochondria and the implantation fossa with the axoneme insertion (Figure 7). As spermiogenesis proceeds, the granular nuclear chromatin which has been thickening at the periphery begins to accumulate at the posterior nuclear pole (Figures 8-10). Then, the nucleus begins to expand laterally (Figure 11). During the middle spermatid stage the nuclear chromatin transforms from a dispersed state to fine fibrillar threads and latterly to thick fibrillar threads, which are elongated and orientated longitudinally along the nuclear vertical axis. At the next step, the chromatin fibrils thicken to form lamellae (Figures 12-13). During further development the nucleus lengthens and the chromatin lamellae continue to become thicker (Figure 14), coupled with axonemal growth, and eventually increase the
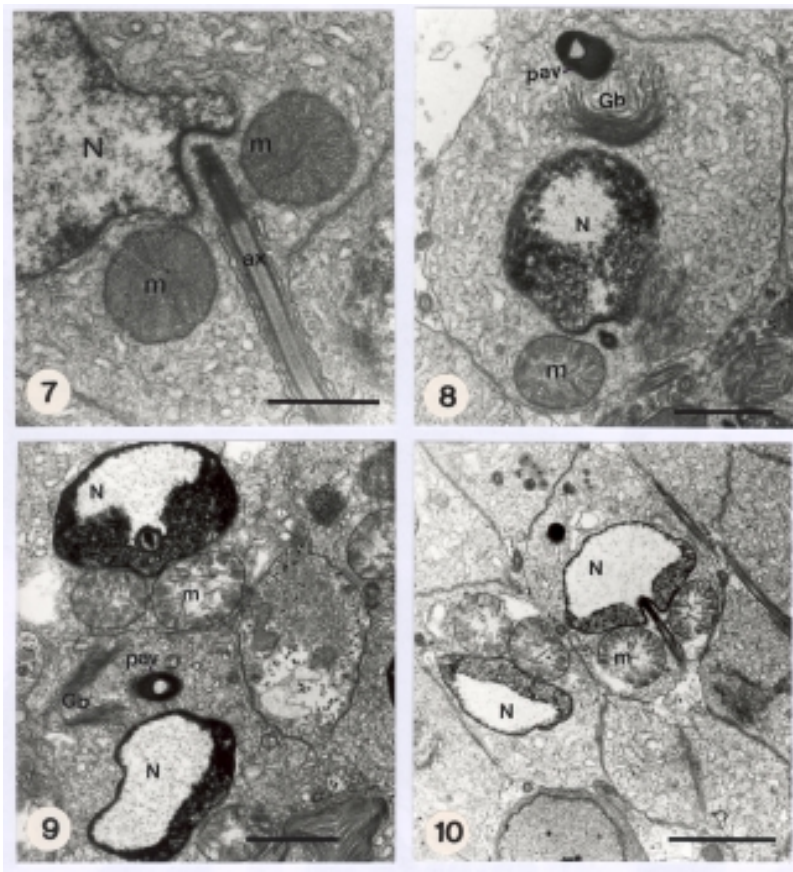

Figure 7.   Details of mitochondrial spheres surrounding the axoneme (ax). N: nucleus; m: mitochondria. Scale bar = 1.5 μm

Figure 8-9. Spermatid showing nucleus with the accumulating of granular chromatin and attachment of mitochondrial spheres. Gb: Golgi body; m: mitochondria; N: nucleus; pav: proacrosomal vesicle. Scale bar = 4 μm

Figure 10.   Spermatid showing the Golgi body (Gb). N: nucleus; m: mitochondria. Scale bar = 2.5 μm

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nuclear length. Toward the late spermatid stage, the nuclear chromatin continues to condense and finally the nucleus appears homogeneous and electron dense.

Acrosome formation: In the earliest stage of acrosomal development, electron-dense granules arising from the Golgi apparatus are obvious in the cytoplasm of the early spermatid (Figure 8). This structure form the proacrosomal vesicle. The proacrosomal vesicle elongates and differentiates into pre-attachment acrosome. This pre-attachment acrosome moves anterioly towards an apical depression of the condensing fibrous phase of the nucleus (Figure 13), then the large dense granule attaches to the nuclear apex, and flattens to form a basal plate. The basal plate with the acrosomal cone placed upon is set in the apical depression of the lamella phase of the nucleus (Figure 14). The body of the acrosome begins to elongate, and the dense internal supporting structures appear within it (Figure 14). In the semimature sperm, the acrosomal cone fuses over the anterior half of its length, thus constricting the subacrosomal space with an acrosomal rod arising in its center (Figure 18).

Midpiece development: The development of the middle piece is closely associated with mitochondria and centrioles. In the early spermatid, the mitochondria which are distributed throughout the cytoplasm begin to aggregate at the developing posterior pole of the condensing nucleus to form the four large spherical mitochondria. These mitochondria cluster around the proximal region of the axoneme which is initially inserted into the implantation fossa at the base of the nucleus (Figure 7). The elongation of the four giant mitochondria was obvious in the cytoplasm of the mid spermatid (Figure 15), and was eventually evident in the form of the four mature midpiece elements surrounding the axoneme (Figure 16).
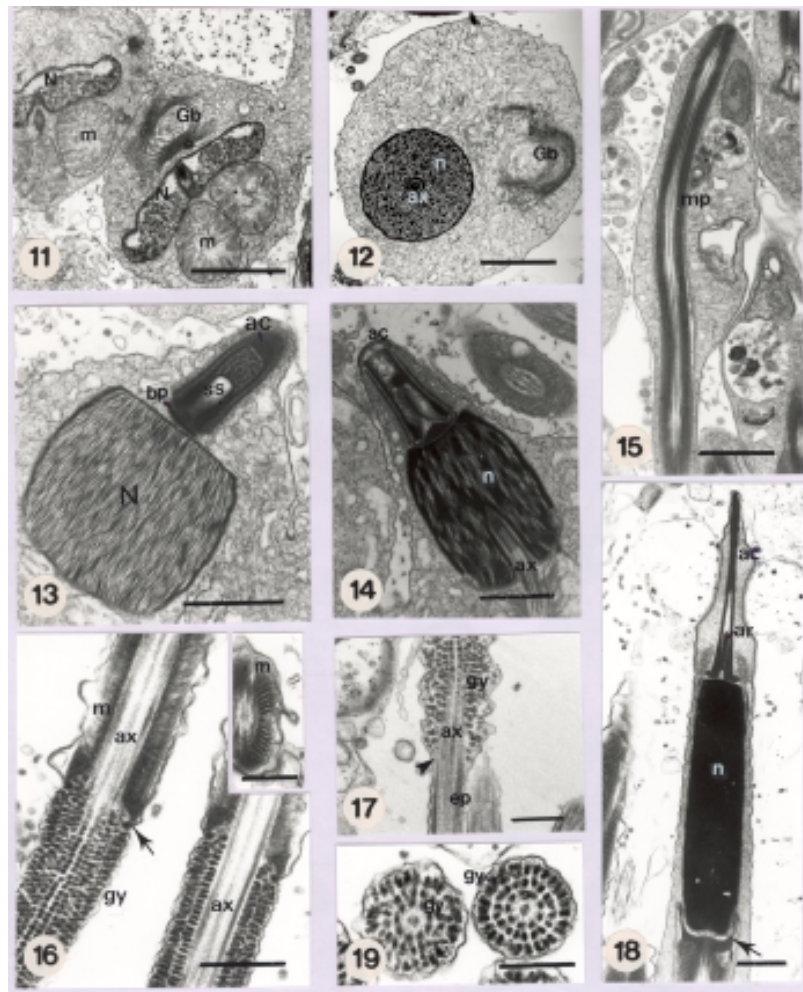
Mature euspermatozoa

The mature euspermatozoon of *Cerithidea cingulata* is a filiform cell, consisting of an acrosome and a nucleus as a head region; a midpiece region; and a tail region which is subdivided into a glycogen piece and an endpiece. The nucleus of mature spermatozoa is elongated with homogeneous electron dense chromatin, the nucleus has a short posterior invagination, accommodating the proximal portion of the axoneme. The apex of the nucleus is situated by the tapering acrosome which comprises of an acrosomal cone, the acrosomal rod or axial rod, and the basal plate. Behind the nucleus is the middle piece with an electron-dense plate or midpiece flange structure at the junction (Figure 18). The midpiece of the euspermatozoon is characterized by the four equal and non-helical mitochondrial elements around the axonemal microtubules. Each mitochondrial element is constructed of multiple, curved, paralleled cristal plates (Figure 16). There is a circular dense band around the axoneme separating the mitochondrial portion or midpiece from the glycogen piece (Figure 16). The proximal part of the tail or the glycogen piece consists of an axoneme surrounded by the tracts of dense glycogen granules, while the distal region or the end piece of the tail lacks glycogen (Figure 17). The transverse section of glycogen piece shows the 9+2 pattern of microtubular arrangement surrounded by a sheath of nine tract glycogen granules limited by the plasma membrane (Figure 19).

Discussion

The *Cerithidea cingulata* reproduces using internal fertilization and its sperm shows the characteristic features of modified euspermatozoa of caenogastropods as described by Maxwell (1983) and Voltzow (1994). The euspermatogenesis and mature sperm of *Cerithidea cingulata* includes many common features that were reported in all other cerithioidians (Attiga and Al-Hajj, 1996; Healy, 1982a, 1982b, 1983, 1986a, 1986b, 1994; Suwanjarat and Klepal, 2001). The ultrastructure of *Cerithidea cingulata* mature sperm has been described by Healy (1983) and confirmed herein with more details on spermatogenesis. As in numerous species studied (Buckland-Nicks and Chia, 1976; Hodson and Bernard, 1989), the spermatogonia of *Cerithidea cingulata* possess a large

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Figure 11. Spermatid with the antero-posteriorly compressed nucleus (N) and enlarged mitochondrial spheres (m). Gb: Golgi body. Scale bar = 4 μm

Figure 12. Transverse section of spermatid showing the lamella nucleus (N). ac: acrosome; ax: axoneme; m: mitochondria. Scale bar = 2 μm

Figure 13. Spermatid shows the longitudinal arrangement of the thickening fibrils in the nucleus and the elongated acrosome. ac: acrosomal cone; bp: basal plate; N: nucleus; ss: subacrosomal space. Scale bar = 2 μm

Figure 14. Transverse section through elongated acrosome. Note the invagination in the basal pole of the nucleus (n). ax: axoneme. Scale bar = 2 μm

Figure 15. Spermatid showing longitudinal section of midpiece (mp). Scale bar = 1.5 μm

Figure 16. Longitudinal section through midpiece (m) and gylcogen piece (gy) showing dense structure (arrow) at the junction. ax: axoneme; m: mitochondria. Inset: cross section of mitochondrial elements. Scale bar = 0.6 μm

Figure 17. Longitudinal section through glycogen piece (gy) and tail. Note the junction of glycogen piece and end piece (arrow head). ax: axoneme; ep: end piece. Scale bar = 0.5 μm

Figure 18. Longitudinal section through head region of euspermatozoon. Note the electron dense plate (arrow). ac: acrosome; ar: axial rod; n: nucleus. Scale bar = 1 μm

Figure 19. Transverse section through glycogen piece (gy). Scale bar = 0.5 μm

round nucleus with one or two nuclei and the cytoplasmic organelles are poorly developed.

In early spermatids, the presence of a well developed Golgi complex suggested that it could possibly be involved in acrosome and midpiece development during spermiogenesis. Many of the spermiogenesis features observed in *Cerithidea cingulta* appear to be common for caenogastropods in general. These include substructural changes in the nucleus. In *Cerithidea cingulata*, the pattern of nuclear condensation passing through the granular, fibrillar and lamellar phase is similar to *Cerithidea obtusa* (Suwanjarat and Klepal, 2001), as well as to those reported in other prosobranchs (Al-Hajj and Attiga, 1995; Attiga and Al-Hajj, 1996; Buckland-Nicks and Chia, 1976; Healy, 1982b, 1986a 1986b, 1988b; Walker and MacGregor, 1968). However, this slightly differs from *P. ebeninus*, the potamidid snail that the early condensation is reticular (Healy, 1982a). Mature eusperm ultrastrucure of *Cerithidea cingulata* basically consists of a rod shaped nucleus with posteriorly invagination, which according to Healy (1983) is standard to all Potamidid spermatozoa.

In Potamididae, the acrosomal cone is elongate and it varies in shape from truly conical to flat. In *Cerithidea cingulata*, the structure of the mature acrosome in general, is similar to those of other mesogastropod and neogastropod euspermatozoa which consist of an acrosomal cone, axial rod, and basal plate. However, species-specific features of the acrosome have been seen with comparison of *Cerithidea obtusa*. The axial rod of the mature acrosome in *Cerithidea cingulata* is clearly seen and the acrosomal cone is oval in the basal region and was become flattened further anteriorly, while the acrosomal cone of *Cerithidea obtusa* is less flattened or oval-shaped anteriorly in transverse section and the axial rod is absent (Suwanjarat and Klepal, 2001). The acrosomal development in *Cerithidea cingulata* is consistent with that observed in *Cerithidea obtusa* of Potamididae (Suwanjarat and Klepal, 2001), *Clypeomorus bifasciata* and *Clypeomorus tuberculatus* of Cerithiidae (Attiga and Al-Hajj,1996).

The association between the Golgi complex and the developing acrosome is clearly seen in *Cerithidea cingulata*. Moreover, the appearance of the internal and external supporting structure within the acrosomal cone during development is similar to many species of cerithiidae such as: *Cerithium caeruleum* (Al-Hajj and Attiga,1995), *Clypeomorus bifasciata* and *Clypeomorus tuberculatus* (Attiga and Al-Hajj, 1996), and in some other Caenogastropods (Buckland-Nicks and Chia, 1976). This structure is supposed to be microtubules that provide the frame and shaping of acrosomal cone. Healy (1983) divided Potamididae into two subgroups due to the pronounced differences on the basis of euspermatozoon midpiece and acrosome structure, existing between the two subfamilies of the potamididae. *Cerithidea cingulata* has been classified into the subgroup that the acrosomal cone is elongated and the midpiece composed of four non-helical equaled size midpiece elements. The non-helical arrangement of mitochondrial elements around the axoneme which are found in *Cerithidea cingulata* are considered a primitive structure compared to the helical mitochondrial sheath seen in other mesogastropods and neogastropods (Giusti, 1969; Walker and MacGregor,1968). The midpiece elements of *Cerithidea cingulata* mitochondria show parallel crystal plates which are a common feature of the superfamily Cerithioidea (Healy, 1983). The process of forming the glycogen piece was not traced, the glycogen piece of mature *Cerithidea cingulata* euspermatozoa does not differ in any respect from the configuration shown to exist in many other Caenogastropod (Healy, 1983).

In conclusion, euspermatozoa and euspermatogenesis of *Cerithidea cingulata*, though showing some differences between species, do share a number of basic structural features which distinguish potamidid snails from other relatively close families likes cerithiidid snails. Although the early stages of paraspermatozoa have been seen no evidence of mature paraspermatozoa could be found. In order to obtain the rudimentary information of *Cerithidea cingulata*, the further study on parasperm of this species should be conducted.

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