



RESEARCH PAPER

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Spermatogenesis and path of the spermatophores through the genitalia tracts of Côte D'ivoire brackish waters crab, *Callinectes amnicola* (Decapoda: Portunidae)

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Key words: *Callinectes amnicola*, Spermatogenesis, Path of spermatophores.

<http://dx.doi.org/10.12692/ijb/11.6.75-88>

Article published on December 12, 2017

Abstract

The present study aims to investigate spermatogenesis in *Callinectes amnicola*. Macroscopic observations indicate 7 stages in male sexual maturity. The testes are subdivided into lobes and numerous seminiferous lobules. Investigations with electron microscope after cytological treatment allowed following the different stages of spermatogenesis. Spermatogenesis comprises the sequence of events by which spermatogonium is transformed into spermatozoon. In the juvenile immature crabs, all the seminiferous lobules are occupied by spermatogonia that divide mitotically, proliferate to provide more spermatogonia. They become cluster of primary spermatocytes which enter in meiotic phase. Secondary spermatocytes are formed at the end of first meiotic division and spermatids result at the end of second meiotic division. The spermatid in maturing male is constituted by an electron dense nucleus and a large amount of cytoplasm bounded by a plasma membrane and contains Golgi apparatus, mitochondria and two centrioles. Spermatids during spermiogenesis undergo cytological transformations leading to the formation of aflagellate and immotile spermatozoa in mature male. These spermatozoa are packed up in the spermatophores at the level of the anterior vas deferens. Macroscopic, histological and cytological investigations through the vasa deferentia of the adult males and spermathecas of adult females allowed following the path of the spermatophores.

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Introduction

The crab *Callinectes amnicola* is Crustacean specie especially found in West Africa (Monod, 1956; Williams, 1974). As a matter of fact due to more fishing, the African swimming crab *Callinectes amnicola* which is the most exploited specie in Côte d'Ivoire, tends to be extinguished (Lhomme, 1994). In order to palliate this phenomenon, the rational management of the stock or the breeding becomes indispensable.

These aspects involve the mastering of structural organisation and physiology of the reproductive organs. Based on the literature Williams (1974) made studies of criteria for identifying the species of the genus *Callinectes amnicola*. Considering the economic importance of these crabs, studies were undertaken in order to know their biology. Study of the reproductive biology was conducted in Ghanaian lagoon (Kwei, 1978).

Similar investigations in two brackish waters of Côte d'Ivoire, Aby and Ebrié were undertaken by several authors such as Charles-Dominique and Hem (1981); d'Almeida (1999); d'Almeida *et al.* (2006a, 2006b, 2007, 2008, 2009 and 2010). Investigations made in Aby and Ebrié lagoons by Charles-Dominique and Hem (1981), Lhomme (1994) and Sankaré (2007) underlined the existence of a yearly reproductive cycle. To better understand this cycle, gametogenesis and particularly spermatogenesis which emerge from the microscopic study of the testes (D'Almeida *et al.*, 2007) is one of the parameters that should be followed.

In the majority of the animals, gametogenesis follows a process which is not generalizable because it presents alternatives according to the zoological groups. The present study aims to clarify the stages of *Callinectes amnicola* spermatogenesis. In *Callinectes amnicola*, the spermatozoa newly formed are packed up in the spermatophores (d'Almeida, 1999; d'Almeida *et al.*, 2007). The path of the spermatophores through male and female genitalia tracts was carried out.

Material and methods

Biologic material

Callinectes amnicola specimens used in present study were caught in the Aby and Ebrié lagoons in Côte d'Ivoire. In the laboratory, the animals are submitted to a first sorting in order to separate the males from the females. The identification parameters between the males and the females are based on the shape of the abdomen. Sixty three (63) males sorted out thereafter are classified according to the stage of the sexual maturity. Identification criteria used in this case is both sizes of the specimen and of the abdomen. The juveniles crabs sorted have abdomen attached to cephalothorax. The adult male's abdomen is detachable. The animals are frozen in a freezer (LIEBHERR) to consolidate and prevent the liquefaction or deterioration of the organs. The carapace is isolated from the exoskeleton and the testes located in the anterior part of the carapace are cleaned of the hepatopancreas that recover them. After whole observation, they are removed for the cytological study.

In order to follow the path of the spermatophores, differentiated vasa deferentia of adult males of the stage V as well as the spermathecas of the females of the stage IV, are appreciated. Twenty (20) males of the stage V sorted out and twenty five (25) adult females of the stage IV of the sexual maturity scale were selected. Macroscopic identification of the organs is made using a binocular magnifying glass (CETI) and by photographs. Observations and photographs of all the crabs were made with the camera MINOLTA AF 7000. Differentiated vasa deferentia and spermathecas are removed thereafter for the histological and cytological treatments.

Histological technique

Works of reference are those of Martoja and Martoja-Pierson (1967); Humason (1967); Gabe (1968); Nezelof *et al.* (1972); Locquin et Langeron (1978). To conduct histological studies, samples of vasa deferentia of the adult males of the stage V as well as the spermathecas of the adult females of the stage IV, were fixed by immersion in aqueous Bouin and dehydrated in ascending series of ethanol (70°, 95° and 100°).

Samples of the vas deferens were softened in a mixture of formic acid, formalin 37% and distilled water, before their dehydration. Without softening, the vas deferens fixed according to classical steps become very hard and friable, making satisfactory sectioning impossible. Afterwards samples were pre-impregnated in butanol. The impregnation and the embedding were carried out in paraplast (Paraplast Monoject scientific. Division of Sherwood Medical. Athy, CO. Kildare, Ireland). Sections of 7µm thickness were realized on a microtome REICHERT-JUNG or MICROM, and stained with hemalun and eosin. Observations and photographs were carried out on a light ZEISS microscope.

Cytological technique

Works of reference are those of Lewis and Knight (1977); Glauert (1978); Reid (1978). To conduct cytological studies, samples of the testes of the specimens of the seven stages of sexual maturity scale, samples of the vasa deferentia of adult males of the stage V and the spermathecas of adult females of the stage IV were fixed by immersion in 3% glutaraldehyde solution, washed with cacodylate buffer, postfixed in 1% osmium tetroxide solution and dehydrated in ascending series of ethanol (70°, 95°, and 100°), after one hour washing with cacodylate buffer. The dehydration is continued in different mixtures of absolute ethanol (100°) and propylene oxide. Samples were thereafter pre-impregnated in pure propylene oxide and after in Epon. Samples were embedded in Epon. The blocks are removed after polymerisation. Ultrathin sections were obtained with Diamond knife (Drukker International) on a microtome REICHERT-AUSTRIA and are contrasted with alcoholic uranyl acetate according to Echlin (1964) and by lead citrate according to Venable and Coggeshall (1965). Observations and photographs were carried out on a ZEISS EM 900 transmission electron microscope.

Results and discussion

Spermatogenesis

Macroscopic observations indicate 7 stages of the sexual maturity in the male. Its gonads consist of paired testes.

Spermatogenesis in *Callinectes amnicola* occurs in the testicular lobules (d'Almeida, 1999; d'Almeida *et al.*, 2007). In the Portunidae the testes characterized by multiple seminiferous lobules is a common feature observed in *Charybdis smithii*, Balasubramannian and Suseelan (2000), in *Clibanarius scolopetarius* Santos and Mantelatto (2011) in *Callinectes ornatus* do Nascimento and Zara (2013). In *Callinectes amnicola* development of male gametes from spermatogonia through primary spermatocytes, secondary spermatocytes and spermatids to spermatozoa takes place in the testicular lobules (d'Almeida, 1999; d'Almeida *et al.*, 2007).

In the immature crabs of the stages I and II, all the seminiferous lobules contain spermatogonia measuring 6 to 8µm and accessory cells which have an elongated nucleus (Fig. 1A) (d'Almeida, 1999; d'Almeida *et al.*, 2007). Spermatogonia have nucleus with 4 to 6µm diameter. The nucleoplasm is filled with heterochromatin, euchromatin and displays one or two nucleoli (Figs. 1A and 1B). Spermatogonium divides mitotically, following the classical mitotic stages that are prophase (Fig. 1C), metaphase (Fig. 1D) anaphase (Fig. 1E) and telophase (Fig. 1F). After proliferation phase, the spermatogonia become primary spermatocytes measuring 4 to 6µm.

Its cytoplasm is clear. Cluster of secondary spermatocytes with 2 to 4µm diameter are formed at the end of first meiotic division (Fig. 1G). Their nucleus is more condensed (Fig. 1G). The broad hyaloplasm is either dense or clear (Fig. 1G). Secondary spermatocytes are found in the testicular lobules of the juvenile crabs of the stage III (d'Almeida, 1999; d'Almeida *et al.*, 2007).

Spermatids with 1 to 2µm diameter result at the end of second meiotic division (Fig 1H). They have high electron density nuclei and large amount of cytoplasm and are found in the testicular lobules of adult maturing males of the stage IV (d'Almeida, 1999; d'Almeida *et al.*, 2007).

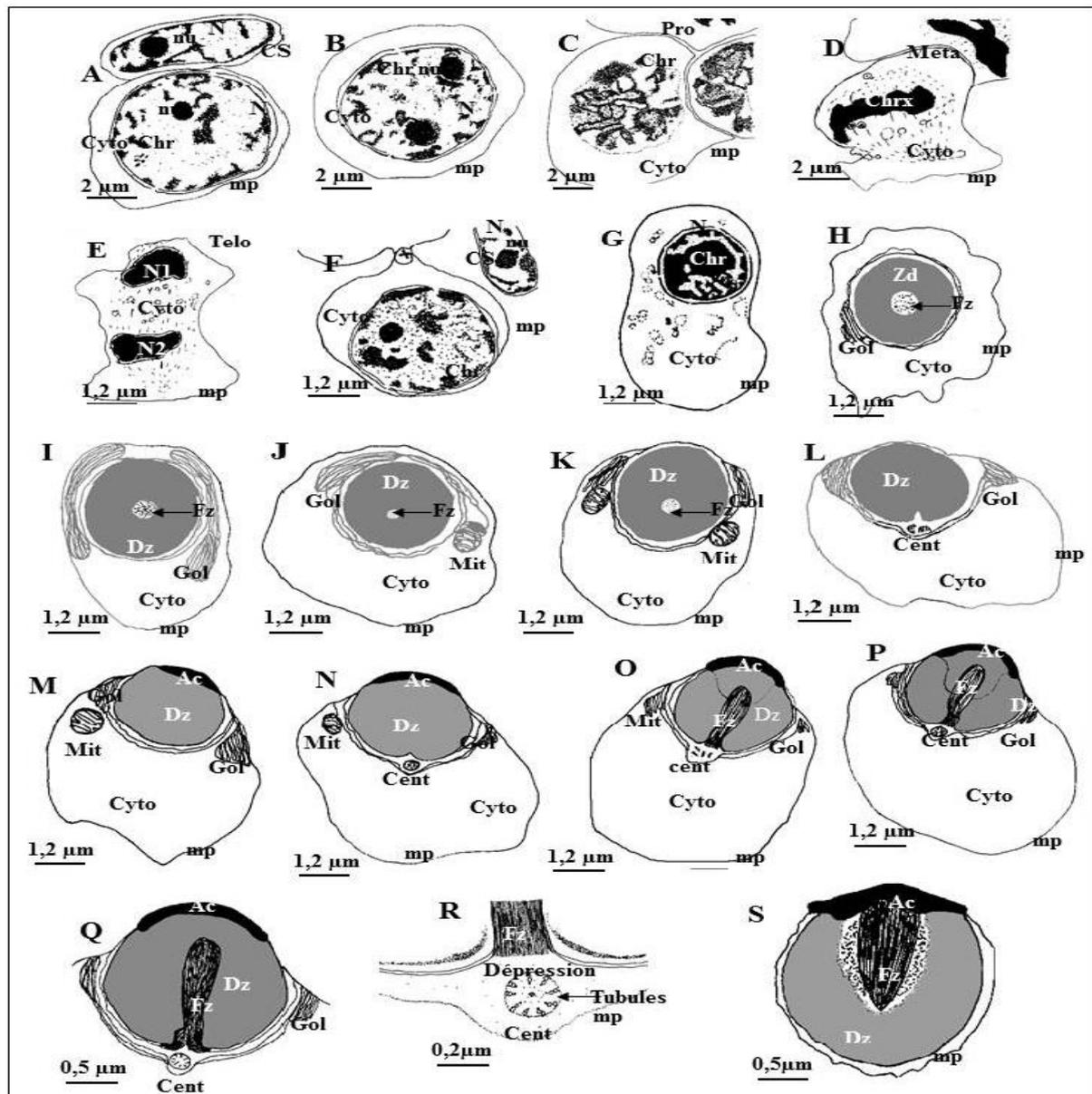


Fig. 1. Different stages of the spermatogenesis of *Callinectes amnicola*. A: Spermatogonium with one nucleolus in the nucleus. B: Spermatogonium with two nucleoli in the nucleus. C, D and E: Mitotic division of a spermatogonium. C: prophase; D: metaphase; E: anaphase; F: Telophase. The daughter cells are joined by a cytoplasmic bridge. G: Sagittal section through a secondary spermatocyte. Spermogenesis of *Callinectes amnicola* (Figure H to S) H: Transverse section through early spermatid constituted by plasma membrane, cytoplasm and nucleus. The nucleus with axial fibrillar area and peripheral dense zone is surrounded by Golgi saccules. I: Sagittal section through a young spermatid. The nucleus is in transverse section. J: Sagittal section through an intermediate spermatid. The nucleus in transverse section is near the plasma membrane. K: Sagittal section through an intermediate spermatid. Nucleus in transverse section reaches the plasma membrane. L: Sagittal section through intermediate spermatid. Nucleus in a parasagittal section is linked to the plasma membrane. Two half sections of the centrioles are located in the depression of the nucleus. M: Parasagittal section through late spermatid showing the beginning of the formation of the acrosome. The nucleus is in parasagittal Section. N: Parasagittal section through late spermatid. The acrosome at the cephalic pole forms a cap. The nucleus is in parasagittal section. One centriole in a transverse section is located in the depression. O: Late spermatid in sagittal section showing the acrosome in the cephalic pole above the fibrillar central zone. Nucleus in sagittal section is

surrounded by Golgi saccules. Two centrioles in a longitudinal section lying at right angles to each other are located under the fibrillar zone. P and Q: Sagittal sections through a young spermatozoon. The acrosome at the cephalic pole tops the fibrillar central zone. Nucleus in sagittal section is surrounded by Golgi saccules. The centriole is in transverse section under the fibrillar zone. R: In outline (inset), details of the cinetosome in the spermatozoon (Figure Q). The transverse section of the centriole shows nine doublets of peripherals tubules and central tubules. S: Sagittal section through old spermatozoon found in the testes of *Callinectes amnicola*. SC: accessory cell; N: nucleus; nu: nucleous; Cyto: cytoplasm; Chr: chromatin; pm: plasma membrane. Pro: prophase; Meta: metaphase; Chx: chromosome; Telo: telophase; N1 and N2: nwly nuclei; Cb: cytophasmic bridge; Dz: peripheral electron dense zone; Fz: fibrillar axial zone; Gol: Golgi saccules or Golgiapparatus; pm: plasma memberan e;mit: mitochondria; Cent: centrioles; Ac: acrosome.

Spermatogenesis is a continuous process and various stages of development of the spermatozoon are observed in the seminiferous tubules (Balinsky, 1975). During spermatogenesis, all the cells in a lobule undergo a synchronous maturation (d’Almeida, 1999;

d’Almeida *et al.*, 2007). In immature individuals of *Callinectes amnicola* and as mentioned (Do Nascimento and Zara, 2013) in *Callinectes ornatus*, the lobules are filled with spermatogonia and the presence of mitotic figures in common.

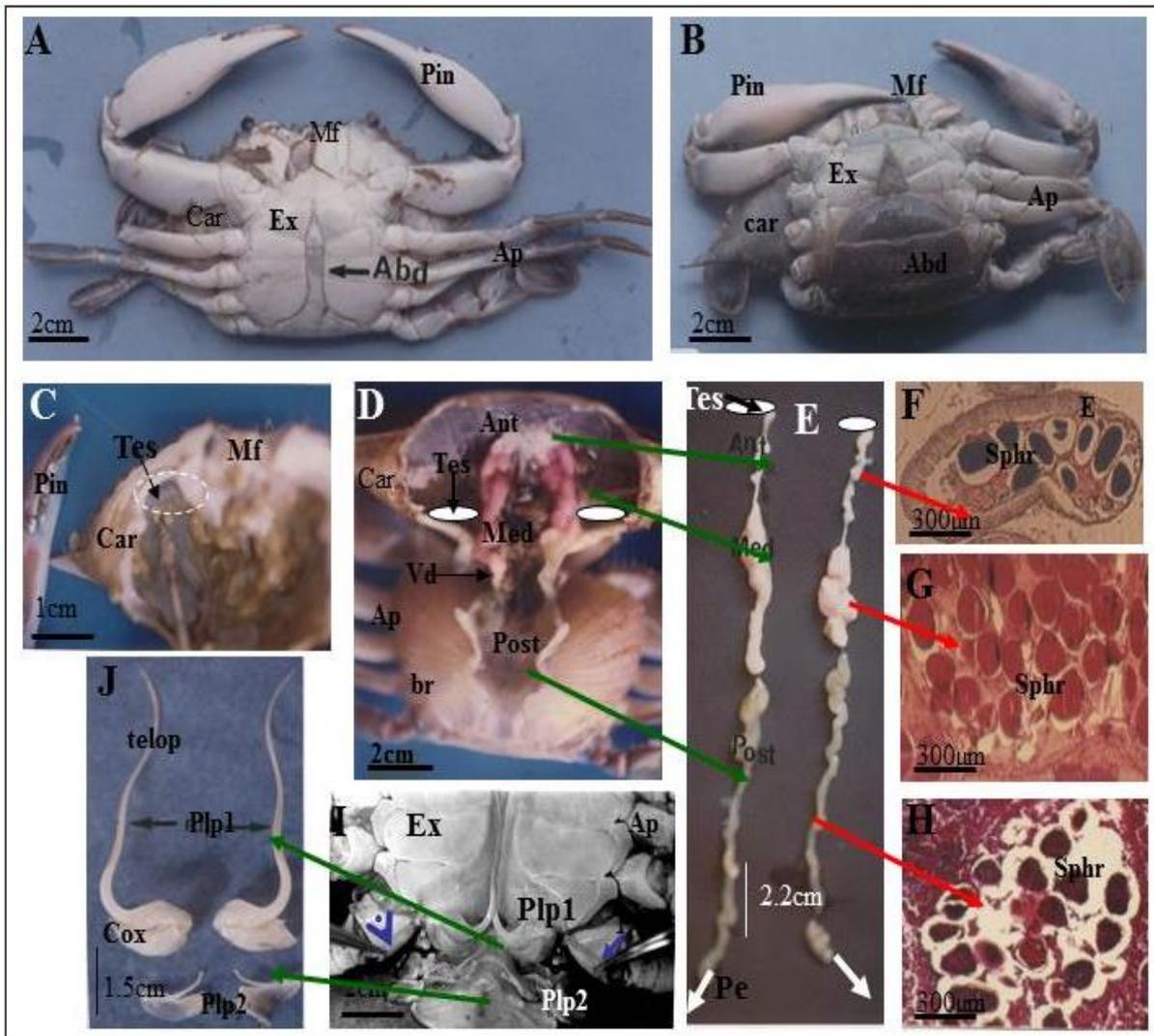


Fig. 2. Path of the spermatophores through genital tracts of the male and female in *Callinectes amnicola*. A: Ventral view of the adult male of the stage V. B: Ventral view of the adult female of the stage IV. C: Internal anatomy of the male of the stage I. The tip of the needle indicates the cleaner right testis. D: Internal anatomy of a

male of the stage V. The vasa deferentia are regionalized in three portions. E: Morphology of paired vasa deferentia of a male of the stage V, after removal and uncoiling. F: Section through the anterior portion of the vas showing spermatophores in the lumen of a secondary duct. H: Section through the posterior portion of the vas showing spermatophores in the lumen of secondary duct of this part. I: Ventral view of a male of the stage V showing two paired pleopods (P1p1 and PP12). Paired penes (Bleu arrow-Pe) retained by the pincers at the level of coxopodite of the last appendage. J: Paired articulated first pleopods (plp1) and paired second pleopods (P1p2) after removal. Ex: exoskeleton; Ap: Five pairs of walking articulated appendages. Pin: pincers; Abd: abdomen. Mf: mouth field; Car: carapace; Tes: localization of testes; Ant: white anterior vas; Med: medial bright pink vas; Post: translucent or greenish posterior vas; br: branchiae; Vd: vas deferens; Pe: localization of penes; Sphr: spermatophores; E: tall epithelium; Cox: coxopodite XIII; Telop: telopodite.

In *Callinectes ornatus* each lobe is filled with sexual cells at the same stage of maturation (Do Nascimento and Zara, 2013). Phenomenon is similar in immature crab *Charybdis smithii*, (Balasubramannian and Suseelan, 2000).

During spermatogenesis in *Callinectes amnicola* one distinguishes a diagram going from spermatogonia to spermatozoa. The same diagram was observed in *Charybdis smithii* (Balasubramannian and Suseelan, 2000), in *Callinectes ornatus* (Do Nascimento and Zara, 2013).

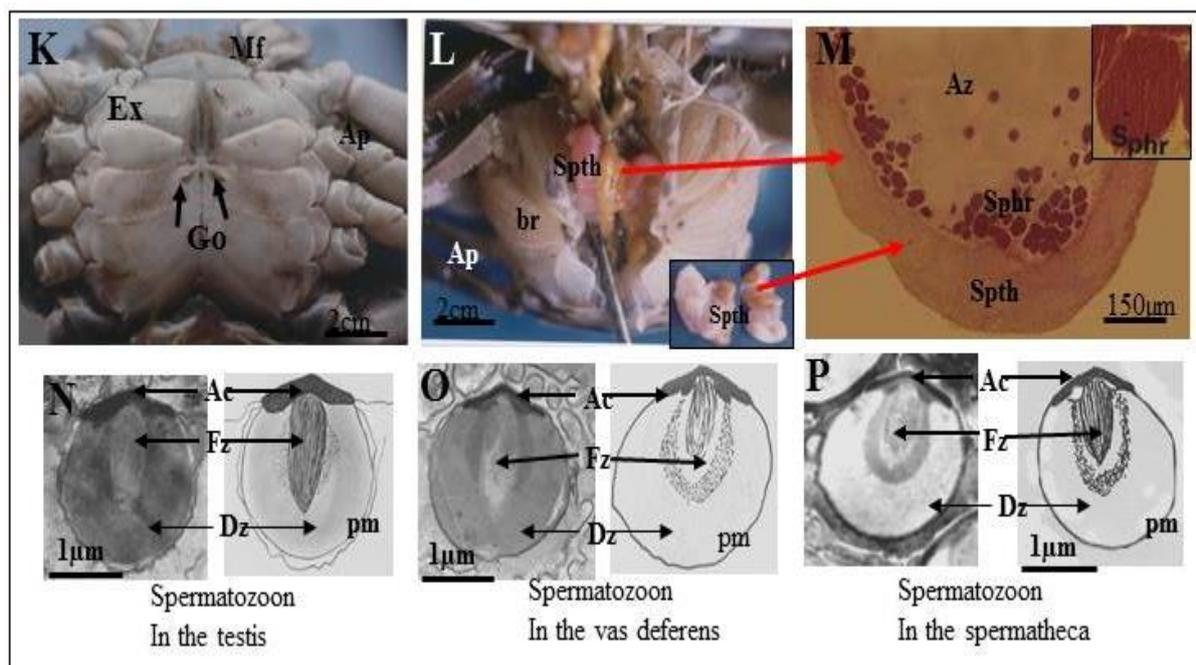


Fig. 2 (Continued). Path of the spermatophores through genital of the male and female in *Callinectes amnicola*. K: Ventral view of the female of the stage IV showing the gonopores (Go) on the 4th pair of the fused segments on the exoskeleton. L: Internal anatomy of the female of the stage IV. Paired enlarged bright pink spermathecas in the cavity. Inset shows paired removal spermathecas. M: Section through the spermatheca of the female of the stage IV. In the lumen of the spermatheca spermatophores are stored in an amorphous area. Inset shows a spermatophore. N: An old spermatozoon in sagittal section found in the testes. O: An old spermatozoon in sagittal section found in the vas deferens of the male. P: An old spermatozoon in sagittal section observed in the spermathecas of the female. Ex: exoskeleton; Ap: Five pairs of walking articulated appendages. Mf: mouth field; Az: amorphous zone; br: branchiae; Sphr: spermatophores; Spth: spermathecas; Go: gonopores; Dz: densezone; Fz: Clear fibrillar zone; Ac: acrosome; pm: plasma membrane.

Spermiogenesis

Spermiogenesis describes the events through which spermatids are transformed into spermatozoa. This process comprises nuclear and cytoplasmic phenomena. The spermatids evolve in three stages: early or young spermatids, intermediate or advanced stage and old or late spermatids stages. In *Callinectes amnicola*, the sequence of spermatid maturation, including three developmental stages, is very similar to that described in *Callinectes danae* by Zara and al. (2012) and in *Callinectes ornatus* by Do Nascimento and Zara (2013).

The process begins in the early spermatids by the formation of the axial fibrillar zone in the nucleus (Figs. 1H to 1Q). One distinguishes in the hyaloplasm of the young spermatid, nucleus, Golgi apparatus or dictyosome, (Figs. 1H to 1Q), mitochondria (Figs. 1J, 1K, 1M, 1N) and two centrioles forming diplosome (Figs. 1L, 1N, 1P, 1Q). The intermediate stage consists of the migration of the nucleus. During the late stage, acrosomal vesicle is differentiated. For this investigation, the differentiation of each organelle is described separately.

Nucleus

During spermiogenesis the nucleolus disappears. The size of the spherical nucleus decreases from the spermatogonium stage progressively to the spermatid stage. The spermatogonium and spermatocyte I nucleus shrinks inducing the concentration of the nuclear material (Figs. 1A, 1B, 1F). The chromatin becomes closely packed into a small volume and the nucleus becomes homogenous dense mass in the secondary spermatocyte (Fig. 1G) and in the spermatid (Fig. 1H). Spermatid nucleus at the three stages show high electron density and its centre becomes bright and fibrillar (Figs. 1H to 1K). Fibrils lie down according to the main axis of the nucleus. It is particularly apparent in the spermatozoon (Figs. 1O, 1P, and 1Q). The nucleus in central position in the intermediate spermatid migrates progressively towards a pole of the cell by rolling and turning over. The nucleus revolves and adopts variable positions in the cytoplasm. Appearance of nucleus in maturing and mature spermatids is variable according the plane of section.

The nucleus with an initial central position becomes during its ascension, eccentric (Figs. 1H to 1L). At the end of the migration, the nucleus links to the plasma membrane (Figs. 1H to 1L). The point of contact of the nucleus and the plasma membrane marks the future anterior or cephalic pole of the early spermatozoon.

The reduction of the diameter of the nucleus has also been observed in *Callinectes sapidus*, in *Portunus pelagicus*, in *Callinectes danae* and in *Callinectes ornatus* (Johnson, 1980; Castilho *et. al.*, 2008; Stewart *et. al.*, 2010; Ravi *et. al.*, 2012; Zara *et. al.*, 2012; Do Nascimento and Zara, 2013).

Spermatozoa nucleus in many species has variable shape: spherical, ovoid, and elongated. In *Callinectes amnicola*, spermatozoa nucleus is spherical. Its organisation is similar to those of some Insects. The presence of a fibrillar zone in the centre of the nucleus echoes what Chapman (1969) indicates in Insects. In Insects some filaments of chromosomes in the centre of the nucleus are aligned more or less parallel with the main axis. From observations made in Insects, one can deduce that filaments in the central zone of the spermatozoon nucleus in *Callinectes amnicola* could be anastomosed chromosomes. The nucleus described above is observed in the spermatozoa found in the testes, and in the vasa deferentia of the male (d'Almeida, 1999; d'Almeida *et al.*, 2007).

Golgi apparatus

Following shrinking of the nucleus, in the perinuclear area appears a network of Golgi apparatus arranged concentrically around the nucleus (Figs 1 H to 1Q). Dictyosome consists of a series of saccules (Figs. 1H to 1L). One of these saccules generates the acrosome (Figs. 1M to 1Q). The remainder Golgi saccules undergo a gradual regression, become residual bodies which will be eliminated (Figs. 1Q and 1S).

Acrosome

Acrosome is formed at the late spermatid stage. The acrosome of the spermatozoon is derived from a Golgi saccule which in intermediate spermatid appears in the point of contact between the nucleus and the plasma membrane (Figs. 1J to 1L).

The point of its adherence marks the future anterior tip of the spermatozoon (Fig. 1L). Thereafter, this sacculle secretes a material which becomes concentrated afterwards (Fig. 1M). The acrosome vesicle newly formed becomes adherent to the outer part of the nuclear envelope (Figs. 1M to 1Q). The acrosome formed and applied to the tip of the nucleus makes protrusion in the intercellular zone (Figs. 1M to 1Q). It spreads on the nucleus and constitutes the cap of the spermatozoon (Figs. 1M to 1Q, 1S). Its central zone rises up and acrosome displays pyramidal shape in the old spermatozoon (Fig. 1S). The pyramidal shaped acrosome is located above the central fibrillar zone observed in the nucleus (Fig. 1S).

In *Callinectes amnicola* as in many animals, the acrosome derives from Golgi apparatus. The role of the Golgi apparatus in the formation of the acrosomal vesicle was proposed by Bowen (1924). The cytological study of the formation of the acrosome was carried out for many species in the Vertebrates as well as in the Invertebrates (Burgos and Fawcett, 1958, 1965; Sawada, 1957; Hortsmann, 1961; Prokofjeva-Belgovskaya and Tchzai Tchzhun-Khe, 1961; Fawcett and Hollenberg, 1963; Hopsu and Arstila, 1965; Fawcett and Ito, 1965; Gardner, 1966; Idelman, 1968; Sandoz, 1968, 1969, 1970; Foliot, 1969).

In the majority of Vertebrates and as underlined by Porter and Bonneville (1968) the formation of the acrosome passes by the formation of proacrosomal granule in young spermatid. It is not the case in *Callinectes amnicola*. At the beginning, the acrosomal vesicle is spread and covers the anterior tip of the nucleus. It starts in this vesicle, the accumulation of an enzymatic material which is used to dissolve the membrane of the ovum. In the Vertebrates, during the fertilization, take place acrosomal reactions (Porter and Bonneville, 1968). According to Balinsky (1975) these reactions are hardly observable in the Invertebrates which have an internal fertilization, but must follow the same process. It is the case in *Callinectes amnicola*. In the majority of case the acrosome derives from Golgi apparatus. (Grassé, 1994) indicates that the acrosome in some Crustaceans derives from the endoplasmic reticulum.

Spermatozoon devoided of acrosome exists and is observed in some fishes as *Pomadasys jubelini* (Fantodji, 1987), *Mormyrops anguilloides*, *Marcusenius ussheri* and *Schilbe mandibularis* (Ouattara, 2000), and *Heterobranchus longifilis* (Otémé, 2001). The eggs fertilized by those types of spermatozoa must have a system of fertilization (Legendre and *al.*, 1996). These eggs show a surface pattern with furrows that may function as sperm guidance system (Riehl and Patzner, 1993).

Mitochondria

In the hyaloplasm of early and intermediate spermatids, mitochondria are very few and are located in the perinuclear zone (Fig. 1J, 1K, 1M and 1N). In *Callinectes amnicola*, they do not form a mitochondrial mantle.

Middle piece designed mitochondrial mantle is mainly constituted in the flagellated spermatozoon by the base of the flagellum and around it the mitochondria (Balinsky, 1975; Bloom and Fawcett ; 1975; Girod and Czyba, 1977). In *Callinectes amnicola* mitochondria do not give rise to mitochondrial mantle. Mitochondria disappear naturally. Observations related to the absence of mitochondrial mantle in *Callinectes amnicola* support those made by Balinsky (1975). He advanced that the absence of mitochondrial mantle in Decapod is due to the fact that these spermatozoa do not need any energy to move.

Centrioles

The spermatid contains two centrioles forming a diplosome which lie at right angle to each other (Figs. 1L and 1P). In the intermediate and late spermatids, both centrioles move just behind the nucleus and are located in a depression formed in the posterior region of the nucleus at the opposite side of the acrosome (Figs. 1L, 1N, 1O, 1P, 1Q, 1R). Transverse sections through the centrioles show organization of the ciliary derivatives. It consists of two central single tubules surrounded by nine (9) doublets (Fig. 1R). The centrioles do not forms flagellum. It remains vestigial organ and disappears subsequently.

Ultrastructural investigation of decapods spermatozoa indicates that they do not have centrioles (Balinsky, 1975). The absence of centrioles in spermatozoa of *Callinectes amnicola* corroborates observations made by (Balinsky, 1975). But as Mammals (Balinsky, 1975; Bloom and Fawcett, 1975), two centrioles are observed also at the spermatid stage in *Callinectes amnicola* and possess the classical organization of the ciliary derivatives respectively described by Novikoff and Holtzman (1970), Bloom and Fawcett (1975), Berkaloff *et. al.* (1993), Mallet (1995).

These centrioles are located precisely at the ideal place to give rise to a flagellum. But these vestiges are eliminated thereafter and do not differentiate any flagellum. The real role of this organelle is arguable. Nevertheless they have played essential role during mitotic and meiotic phases. As previously mentioned, spermatozoa of *Callinectes amnicola* are aflagellate. This result support observations realized by some authors. Waterman and Chace (1960) affirmed that spermatozoa in certain zoological groups could be flagellate. In the Decapoda, spermatozoa largely immotile are aflagellate. According to Balinsky (1975) spermatozoa in Crustaceans Decapods as *Callinectes amnicola* do not have any flagellum and are unable to swim.

Cytoplasm and plasma membrane

An abundant hyaloplasm is spread around the nucleus of the early spermatids (Fig. 1H). Thereafter the cytoplasm flows away in opposite direction of the migration of the nucleus (Figs. 1G to 1P). The flowing of the cytoplasm starts at the secondary spermatocyte stage (Fig. 1G) and is achieved when the nucleus is linked to the plasma membrane at the intermediate spermatid stage (Figs. 1L to 1P). The bulk of cytoplasm is displaced behind the caudal pole of the nucleus (Figs. 1L to 1O). Afterwards residual hyaloplasm is constituted (Fig. 1P). The process of transformation and elimination of the cytoplasm seems similar in most groups of animals (Balinsky, 1975; Czyba *et. al.*, 1975; Houillon, 1978).

The spermatozoon (Fig. 1S) resulting from all these transformations presents an external plasma membrane, a fairly narrow cytoplasm. Its nucleus has a peripheral high electron dense area, a fibrillar axial zone, a pyramidal shaped acrosome above the fibrillar zone. All this morphological and cytological evolution occurs in the testes (d'Almeida, 1999; d'Almeida *et al.*, 2007). Spermatozoa are found in the testicular lobules of adult males of stages V to VII (Fig. 2N) (d'Almeida, 1999; d'Almeida *et al.*, 2007). Biochemical maturation of the spermatozoon will take place during its migration in the vas deferens of the male (Fig. 2O) and in the spermathecas of the female (Fig. 2P) (d'Almeida, 1999).

Spermatozoa of *Callinectes amnicola* present morphological similarity with those of *Carcinus maenas* (Grassé, 1994), *Calcinus tibicen* (Amadio and Mantelatto, 2009), *Charybdis smithii* (Balasubramanian and Suseelan, 2000).

The spermatozoal ultrastructure of *Callinectes amnicola* is similar of those of *Pinnotheres pisum* and *Nepinnotheres pinnotheres* Becker and *al.* (2013) and resembles that of the pinnotherid *Pinnixa* sp (Reger, 1970; Krol and *al.*, 1992). In hermit crab *Calcinus tibicen* the spermatozoa have as those of *Callinectes amnicola* three main regions (the acrosomal vesicle, the nucleus and the cytoplasm) Amadio and Mantelatto (2009).

In *Callinectes amnicola* as in the majority of groups of animals, the old spermatozoon presents only a fairly hyaloplasm around the nucleus and the head is embedded in a plasma membrane (Balinsky, 1975; Czyba *et. al.*, 1975; Houillon, 1978; Otémé, 2001).

According to Dollander and Fenart (1979), to assume sexual function, the whole organisation of the spermatozoa generally observed in most animals consists of the following parts:

The head

The components of the head are: acrosome, nucleus, cytoplasm and plasma membrane.

The middle piece

This portion contains the base of the flagellum and is surrounded by mitochondria. They supply the flagellum with energy for the propulsion of the spermatozoon.

The flagellum

Its movements allow the spermatozoon to swim towards the ovum.

This morphology of gametes was described in the Mammals by Fawcett (1958, 1965), in Birds and Batrachians Dollander and Fenart (1979). Chapman (1969) shows it in the Insects. In Crustaceans, this description cannot be generalized because some important modifications have occurred during the spermatogenesis. All the different steps of this process were not completely observed in *Callinectes amnicola*. The morphology of the spermatozoon attests it.

Path of the spermatophores through the genitalia tracts. This study is carried out in the adult male of the stage V (Fig 2A), and in the adult female of the stage IV (Figure 2B).

In the mature male, paired testes (Fig. 2D) are joined to the paired complex coiled vasa deferentia subdivided in tree colored regions (Figs. 2D and 2E). The proximal anterior region is a white coiled tube (Fig. 2E). The medial region is large and bright pink (Fig. 2E). The posterior distal part of the vas deferens (Fig. 2E) is translucent or greenish. Its final portion is a slender tube connected with the penis.

The female possesses paired spermathecas connected externally to paired gonopores (Fig. 2K) (d'Almeida, 1999; d'Almeida *et al.*, 2006b, 2010). The size and the color of the receptacle vary with the sexual maturity. The spermathecas of the juvenile females are white and do not contain spermatophores. The mature females have large bright pink receptacles during period following copulation (Fig. 2L) and contain consequently spermatophores (Fig. 2M) which confer the pink color (d'Almeida, 1999; d'Almeida *et al.*, 2006b, 2010). The spermathecas shrink and turn white when spermatophores are delivered.

The path consists in the production of the spermatozoa in the testes (Figs. 2C and 2D) and their progress towards the genital tracts of the male (Figs. 2 D, 2E) and the female (Figs. 2 L and 2M). Spermatozoa (Fig. 2 N) are packed up in the spermatophores (Figs. 2. F, 2G and 2H) which are evacuated in the various portions of the vasa deferentia: anterior (Fig. 2F), median (Fig. 2G), and posterior (Fig. 2H) portions. The penises serve as duct carrying the spermatophores towards the first pleopods (Fig. 2I). The first pleopods (Figs. 2I, 2J) constitute the copulatory organ that acts during copulation (Fig. 2M) through the gonopores (Fig. 2K). The second pleopods (Figs. 2I, 2J) push the spermatophores through the groove of these latter. The spermathecas play the role of spermatophores storage (Figs. 2L and 2M).

In adult males of *Callinectes sapidus* spermatozoa newly formed are packed up in spermatophores Cronin (1947); Johnson and Otto (1981). In *Carcinus maenas*, Spalding (1942) has observed spermatophores. In *Callinectes amnicola* spermatophores are evacuated towards the internal and external reproductive system in the male, are transported into the spermathecas of the female (d'Almeida, 1999; d'Almeida *et al.*, 2006a). The path of the spermatophores corroborates observations carried out by Cronin (1947) in *Callinectes sapidus* in the hermit crab, *Clibanarius vittatus* (Hess and Bauer 2002) and in *Clibanarius sclopetarius* (Santos and Mantelatto, 2011).

Conclusion

The present study compared to those made in Mammals and fishes indicates that spermatogenesis has specificities according to the zoological group and even species. The morphology of the spermatozoon is different from the common gametes observed in the Vertebrates and the Invertebrates. The main different parts of this latter are the head, the middle piece and the flagellum.

The spermatozoon in *Callinectes amnicola* do not have mitochondrial mantle and is aflagellate. Only the head constitutes the whole spermatozoon.

Spermatozoa elaborated in the testes are encapsulated in the spermatophores at the anterior vas level. The path of the spermatophores consists of the following diagram. Spermatophores are carried towards the internal reproductive system in the male. The posterior vas discharges capsules in the penes which pass them into the furrow at the base of the first pleopods. Gonopods are used to deliver spermatophores into the female's spermathecas through the gonopores.

Acknowledgements

The author expresses her gratefulness to GTZ (Germany collaboration Organization in Côte d'Ivoire) donor of electron microscope. She thanks the staff of the ex-GERME (Groupe d'Etude et de Recherche en Microscopie Electronique) or (Study and research group in electron microscopy). They are, TANOH Koffi, KOUAKOU N'Goran, GNANGO Ahou Celestine†, GBA Felix and ABLOH Salomon † for their collaboration. The author thanks N'Guessan Gbaka Gutembert and SEWA Yves René and KOUAKOU Koffi for their contribution.

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