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Male internal reproductive tracts of Côte d'Ivoire brackishwaters crabs, *Callinectes amnicola*, (de Rochebrune; 1883; Decapoda: Portunidae).

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ABSTRACT
Objective: The study was aimed to investigate the macroscopic and the microscopic aspects of the vasa deferentia of *Callinectes amnicola (brackishwaters crabs)*
Methodology and results: Investigations with light and electron microscopes after respectively histological and cytological treatments, allowed following the differentiation of the internal tracts. A macroscopic observation has indicated 7 stages in male sexual maturity. Primary white vasa deferentia of the individuals of the stages II and early stage III are composed of an acellular area surrounded by an epithelium. The epithelial layer infolds centripetally fuses giving secondary ducts at the stage III. The gonoducts in the adults of stages IV to VII are subdivided in anterior vas (AVD), medial vas (MVD) and posterior vas (PVD); each one forms a package of secondary ducts. A secretory phenomenon appears in the ducts at the stage IV, and spermatophores are found among the vesicles of secretion. At the stages V to VII the secondary ducts of the anterior vas deferens (AVD) delineated by a columnar epithelium contain spermatophores in their lumen. The medial vas deferens (MVD) presents some secondary ducts containing pockets of secretion. Other ducts are invaded by spermatophores. In the posterior vas deferens (PVD), ducts contain spermatophores. Physiology of the tract was carried out.
Keywords: *Callinectes amnicola*, vasa deferentia, histology and cytology.

INTRODUCTION
With respect of the importance of *Callinectes amnicola* in African's foods, it is important to understand its biology to envisage its breeding to mitigate the decrease of natural stocks observed. The decrease of stocks correlated with population growth and more fisheries. *Callinectes amnicola* becomes a most popular food, which command high prices in the Côte d'Ivoire markets. Faced with that problem, researchers undertook investigations relating to the both sexes. Based on the literature Williams (1974) made the synthesis of the previous taxonomic studies of the genus trustworthy criteria for identifying species. Study of the reproductive biology of *Callinectes amnicola* in Africa was conducted in Ghanaian lagoon (Kwei, 1978). Charles-Dominique and Hem (1981) undertook investigations in *Callinectes amnicola* in brackish water Ebrié of Côte d'Ivoire. The distribution of individuals in the same lagoon was carried out by Pantousthier (1982). Lhomme (1994) studied the ecological and biological aspects of *Callinectes amnicola*. Beyond these studies, d'Almeida (1999) has investigated reproductive cycle of *Callinectes amnicola*. The sexual maturity scale of the male

(d'Almeida, *et al.*, 2009), the differentiation of the testis (d'Almeida *et al.*, 2007) were carried out. About the female, the sexual maturity scale (d'Almeida *et al.*, 2010), the ovogenesis (d'Almeida et *al.*, 2006a), the microscopical study of spermathecas (d'Almeida *et al.*, 2006b), and the study of the embryonic development (d'Almeida *et al.*, 2008) have been realised in *Callinectes amnicola*. In the literature, few works have associated the macroscopic and microscopic investigations of the reproductive system of Crustaceans of genus *Callinectes*. Cronin (1947) conducted both studies in the reproductive system of the male in *Callinectes sapidus*. Johnson (1980) carried out the microscopical study of some organs of *Callinectes sapidus*. Johnson and Otto (1981) undertook anatomical and histological studies of the reproductive organs of a gynandromorph crab, *Callinectes sapidus.* The knowledge about the male reproductive system is essential to select the matured male for breeding. To understand the histological and cytological aspects of the reproductive system in *Callinectes amnicola*, the sexual maturity scale of the male was established (d'Almeida, 1999; d'Almeida *et al.*, 2009). The present paper investigates the male genitalia tracts. This paper presents the macroscopical and the microscopical differentiation of the male internal reproductive structures of *Callinectes amnicola*.

MATERIAL AND METHODS

Biological material: Specimens of *Callinectes amnicola* used in this study were caught from the brackish waters, Aby and Ebrié in Côte d'Ivoire. Sixty three (63) males sorted out are classified according to the stage of the sexual maturity. Identification parameters used in this case are both sizes of the specimen and of the abdomen. After their catching, the animals were cold anesthetized in a freezer (LIEBHERR) to consolidate and prevent organs against any deterioration. The carapace is isolated from the exoskeleton and after whole observations of the vasa deferentia; animals were photographed with the camera MINOLTA AF 7000. The vasa deferentia are removed for microscopical investigations.

Methods

Histological technique: Works of reference are those of Martoja and Martoja-Pierson (1967); Humason (1967); Gabe (1968); Nezelof *et al.* (1972; Locquin and Langeron (1978). To conduct histological studies, samples of gonoducts of the specimens of the stages II to VII of the sexual maturity, were fixed by immersion in aqueous Bouin and dehydrated in ascending series of ethanol (70°, 95° and 100°). Samples of the vas deferens of adult were softened in a mixture constituted of formic acid, formalin 37% and distilled water, before their dehydration. Without softening, samples of the vas deferens fixed according to classical steps become very hard and friable. 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: For the treatment of the samples, works of reference are those of Lewis and Knight (1977); Glauert (1978) and Reid (1978). To conduct cytological studies, samples of the gonoducts of specimens of the stages II to VII of the sexual maturity were fixed in 3% glutaraldehyde solution, washed in cacodylate buffer, post fixed in 1% osmium tetroxide solution, 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 812. The blocks are removed after polymerisation. The ultrathin sections were obtained with Diamond knife (of 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 ZEISS EM 900 transmission electron microscope.

RESULTS

Organisation and localisation of the internal reproductive system: The reproductive tracts are composed of internal and external organs. Male reproductive internal system is bilateral, symmetrical and composed of paired testes, paired vasa differentia located respectively in the cephalothorax and the central cavity. In

this present paper, morphology, histological and ultrastructural studies of the internal genitalia tracts or the vasa deferentia are reported (Figs.1A, 1B, 1C, 1D, 1E, 1F).

Anatomy and histology of the vasa deferentia: In *Callinectes amnicola*, gonoducts stretches from testes to penes. Differentiation of paired vasa deferentia occurs during the sexual maturity and their setting up starts at the stage II. In the juvenile of the stage II, gonoducts are two whitish masses (Fig. 1A). They become two parallel whitish vasa deferentia in the pubescent specimens of the stage III (Fig.1B). In these individuals, the vas deferens constitutes the primary vas and is composed of an acellular area surrounded by a cuboïdal epithelium (Fig.1B1). In the specimens of the stage III, the cells of the epithelial wall of the duct proliferate, follow mitotic stages and newly portion of the epithelium formed infolds centripetally (Fig.1 B2). The epithelial wall fuses and surrounds small areas of the acellular central zone, giving some secondary ducts (Fig.1B3). In the pubescent individuals of the last stage III, gonoducts are fragmented thereafter into secondary ducts and secretory activity occurs centripetally inside these (Fig.1B4). Longitudinal section of the vas deferens shows adjoined secondary ducts of variable length and size lined by connective tissue (Fig.1B5). The ducts are inter-connected and show confluence of their contents (Fig.1B5). The original vas deferens becomes a package of secondary ducts or tubules. In the stage IV adults, based on colour, three regions of the vas deferens are defined. The white anterior (AVD) and posterior (PVD) portions are separated by the pale-pink medial part (MVD) (Fig. 1C). In these specimens, each portion of the vas deferens is constituted of series of secondary ducts with variable size (Fig.1C1). Cuboïdal epithelial cells surrounding the primary vas become a columnar epithelium around the secondary ducts (Fig.1C2). In these specimens, finished sperms elaborated in the testis are embedded at the level of the AVD by a wall forming spermatophores that are scattered among vesicles of secretion at medial portion (MVD) level (Fig.1C3). At the stage V, the paired vasa deferentia are coiled and bound together by a thin membrane (Fig.1D). The rupture after freezing allows to separate and straight them after uncoiling (Fig.1D1). The vas deferens stretches from testes to penes (Fig.1D1). Three regions of the vas deferens were defined based on gross morphology, colour and content. The anterior vas (AVD) is whitish, the medial portion (MVD) is bright pink, the posterior vas (PVD) is translucent or greenish (Fig.1D1). The anterior vas (AVD) is the tight shortest part joined to the testes (Fig.1D1). The relatively long and medial bright pink vas deferens (MVD) is the strongly widened region inserted between the anterior and posterior portions (Fig. 1D1). The posterior vas deferens (PVD) is the longest part, extending from the medial vas to the base of the penis (Fig.1D1). The PVD is massive for its proximal part and its distal narrow part connected to the penis open to the ejaculatory duct. Under the fingers pressure, rod-shaped masses or spermatophores are released at the medial portion level. At the stage V adults, each portion of the vas deferens has its own histological features.

Anterior vas deferens (AVD): The spermatozoa accumulated in the testis are transferred to the vas deferens, through the seminiferous ducts, where they are packed in spermatophores. Secondary duct in this portion bears a tall columnar epithelium composed of a single layer of secretory columnar cells. Epithelial cells have basally located nuclei (Figs.1D2 and 1D3).Secretions are resorbed in the lumen and the ducts contain encapsulated spermatophores.

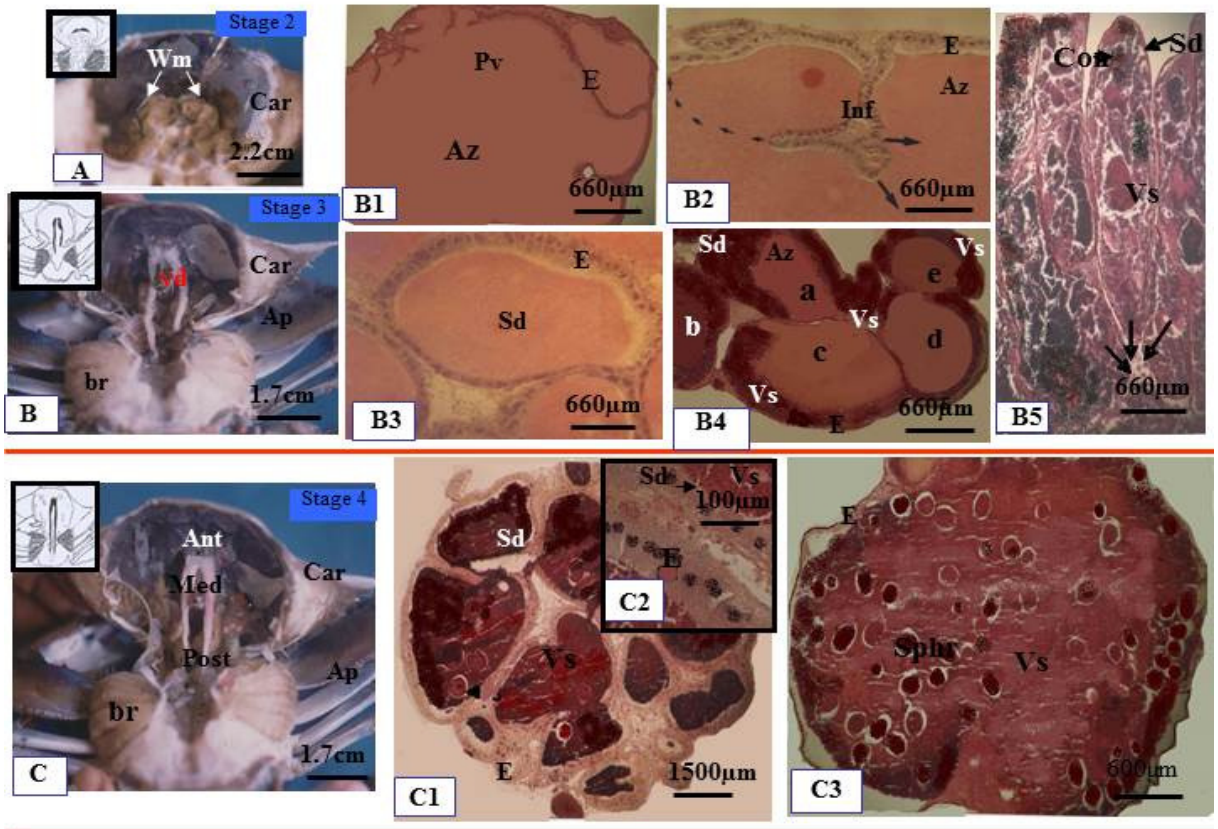

Figure 1 : Anatomy and histology of the vasa deferentia.

A : Internal anatomy of a juvenile male of the stage II. gonoducts are two whitish masses **B** : Internal anatomy of a pubescent male of the stage III. gonoducts become two parallel whitish vasa deferentia. **B1 :** Transverse section trough the primary vas deferens of specimens of the stages II and III . **B2 :** Section of the primary vas deferens of specimens of the stages III. Arrows indicate future fusion of the epithelial wall. **B3 :** Section trough the vas deferens of specimens of the stages III after the formation of the secondary ducts. **B4 :** The view shows five (5) secondary ducts (a, b, c, d and e) in which secretory activity starts centripetally. **B5 :** Longitudinal section trough the vas deferens of a specimen of the last stage III. **C** : Internal anatomy of an adult male of the stage IV. **C1** : Transverse section trough the vas deferens of specimens of the stage IV. Secondary ducts are entirely filled by vesicles of secretion. **C2 (Inset) :** Detailed view of epithelium of two joined secondary ducts. **C3** : Section trough the medial vas. Some rare spermatophores have reached this portion.

Wm-arrows : two white masses ; Car : carapace ; Vd : vas deferens ; br : branchiae ; Ap : appendages ; Pv : primary vas ; E : cuboïdal epithelium ; Az : acellular zone ; Inf : infolded epithelium ; Sd : secondary ducts ; Vs : Vesicles of secretion ; Con : Connective tissue ; Ant : anterior vas ; Med : medial vas ; Post : posterior vas ; Sphr : spermatophores.

Medial vas deferens (MVD): Secondary ducts are bounded by relatively tall columnar epithelium (Fig 1D5). The vas deferens presents some heterogeneity (Fig.1D4). In the lumen of some secondary ducts, secretion product is fragmented in pockets of secretion with a diameter varying between 80 and 250µm and free enveloping membrane (Figs.1D4 and 1D5). Spermatophores with a diameter varying between 150 to 300µm (Fig.1D4 and 1D5), lined by a membrane, invade other secondary ducts. The sperm masse is stored inside this coiled medial portion. At the end of this process, one observes adjoined secondary ducts containing pockets of secretion or fluid and others containing spermatophores (Fig.1D5).

Posterior vas deferens (PVD): The adjacent structure around the posterior vas is an epithelium or connective tissue (Figs.1D6 and 1D7). Ducts delineated by epithelial

cells are observed in the transitional portion between the medial and the posterior vas deferens. In the main part of the posterior vas deferens, secondary ducts free in vesicles of secretion contain essentially spermatophores (Figs.1D6 and 1 D7) and all the ducts are in confluence and are inter-connected (Figs.1D6 and 1D7). The spermatophores present in the vas deferens are enveloped by a membrane, which differentiates them from the fluid pockets observed in the medial portion. At the stages VI and VII, gonoducts present the same subdivisions and colour as at the stage V. At these stages, histological features are similar as at the stage V.

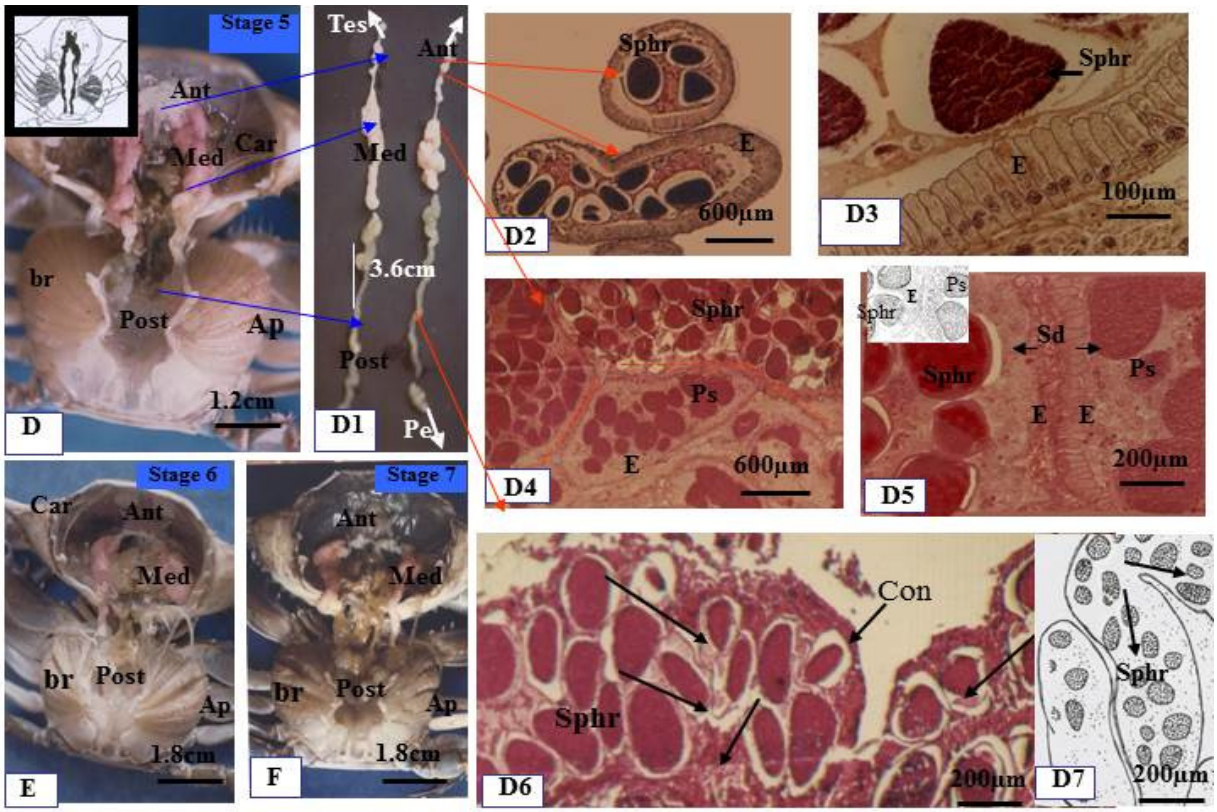

Figure 1 . Anatomy and histology of the vasa deferentia.

D : Internal anatomy of an adult male of the stage V. The vas deferens presents three portions : white anterior vas, bright pink medial vas, and translucent or greenish posterior vas. **D1 : V**as deferens after removal and partial uncoiling. **D2 : S**ection trough the anterior vas deferens of an adult male of the stages V. **D3 :** Detailed view of a portion of the figure **D2. D4 : S**ection trough the medial portion of the vas deferens of the specimen of the stages V showing secondary ducts lined by an epithelium containing in the lumen spermatophores or pockets of secretion. **D5 :** Detailed view of a portion of the figure **D4.** The left secondary duct contains spermatophores embedded in a membrane. The right one contains pockets of secretion. **D6 :** Section trough the posterior portion of the vas deferens of the specimen of the stage V. Ducts contain spermatophores. Arrows show confluence of their contents. **D7 :** Diagrammatic representation of longitudinal section of a portion of posterior vas deferens of an adult. Arrows indicate confluence of the lumens.

E : Internal anatomy of adult male of the stage VI. **F** : Internal anatomy of adult male of the stage VII. The vasa deferentia are flattened and reddish.

Ant : anterior vas ; Med : medial vas ; Post : posterior vas ; Car : carapace ; br : branchiae ; Ap : appendages ; Tes : localization of testes ; Pe : localization of penes ; E : columnar epithelium ; Sd : secondary ducts ; Ps : pockets of secretion ; Con : Connective tissue; Sphr : spermatophores.

Cytology of the vasa deferentia

Ultrastructure of the gonoducts: In juvenile individuals of the stage II and the pubescent crabs of the stage III, the lumen of the primary vas deferens and the newly formed secondary ducts contains an acellular substance (Fig2A). A single cuboïdal epithelial layer lines the ducts. In those of the last stage III (Fig.2B) and at the beginning of the stage IV (Fig.2C), epithelium secretes a substance of variable electron density, which forms vesicles of secretion. Secretions aggregate in the lumen of the medial vas deferens. Some are clear, others have high electron density. The amount of vesicles of secretion is more important at the stage V (Fig. 2I). The epithelium of the secondary duct is constituted by cells with variably shaped nuclei (Figs.2B and 2I). The cell organelles observed are essentially the granular endoplasmic reticulum, free ribosome and mitochondria (Fig.2D). In stage V mature crabs, the presence of two types of secondary ducts in the medial vas is confirmed. At the medial portion level can be noted the presence of ducts containing encapsulated spermatophores (Figs.2E and 2F) and others containing vesicles of secretion (Figs.2H and 2I). It appears that in the mature crabs of the stage V, cytological study confirms the histological observations. In stages VI and VII adults specimens, who have already copulated, reproductive system structures observed are similar as at the stage V but contain less secretion and spermatophores. The spermatophores contain spermatozoa morphologically similar to those observed in the testis (Figs.2F and 2G). *Callinectes amnicola* spermatozoa are aflagellate, and immotile. They are composed of an acrosome, above a nucleus constituted by fibrillar axial zone and a dense peripheral high electron density zone. Spermatozoa are surrounded by a narrow cytoplasm and a plasma membrane (Fig.2G).

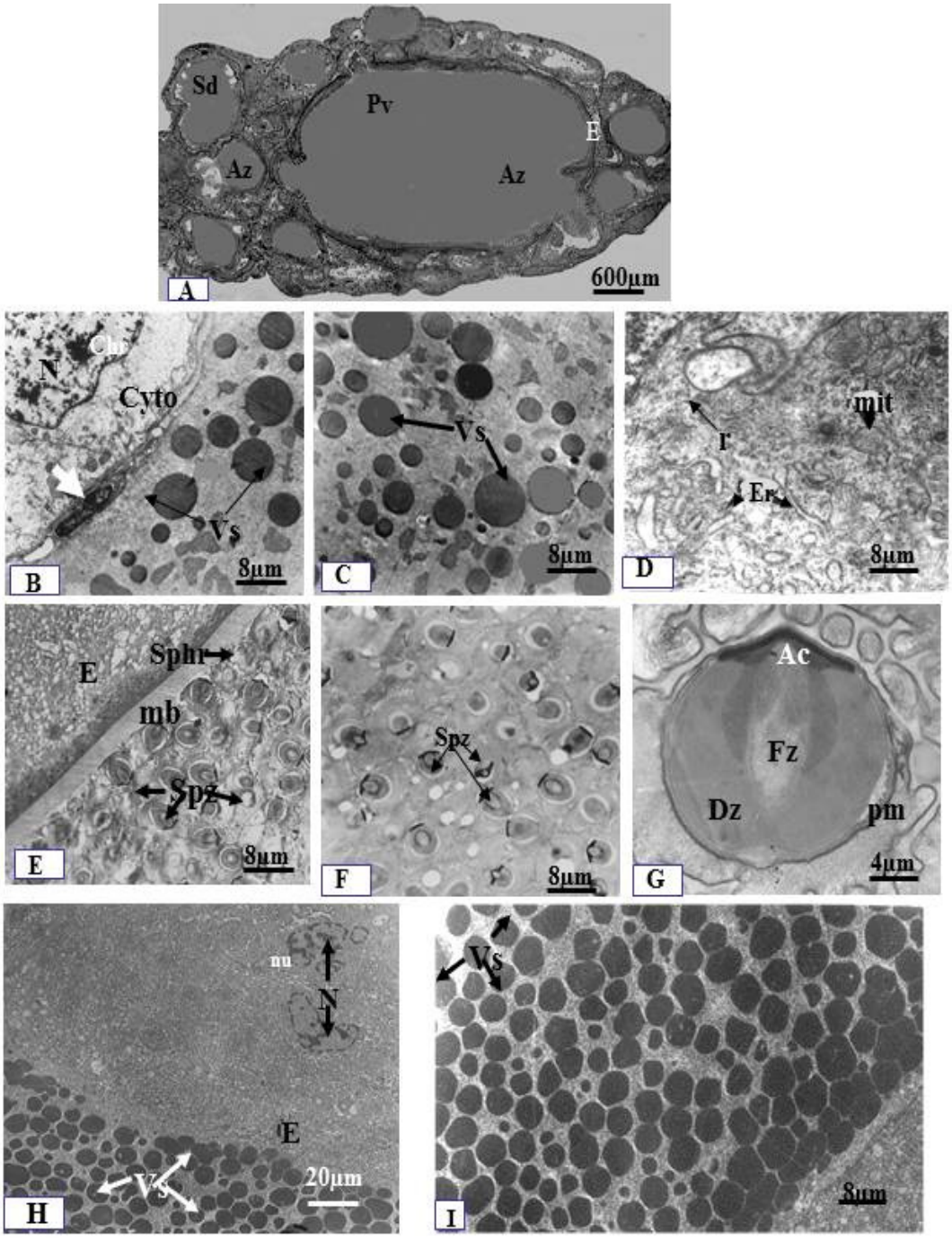

Figure 2 : Cytological study of the vasa diferentia of the crab *Callinectes amnicola*

A : Transverse section trough the vas deferens of the juvenile specimens of the stages II and III. Several small secondary ducts round the central primary vas. **B :** Portion of secondary duct of the specimen of the stage III. The epithelial cell is lined by a syncytium (Arrows). **C :** Portion of secondary duct of the specimen of the stage IV showing vesicles of secretion. **D :** Epithelial wall of secondary duct of the specimen of the stage IV showing cell organelles. **E :** Portion of secondary duct in the medial vas of the specimen of the stage V containing spermatophores. **F : Portion of figure (E)**. Detailed view of a portion of area of spermatozoa. **G :** Ultrastructure of the spermatozoon found in the medial vas deferens of an adult specimen of the stage V. **H :** Detailed view of the area of secretion lined by epithelial wall in secondary duct of specimen of stage V. I : Electronmicrography of of a portion ot the area of the pocket of the secretion in the secondary duct of specimen of stage V.

Sd : secondary ducts ; Pv : primary vas ; E : epithelial cells ; Az : acellular zone ; Vs : vesicles of secretion ; N : nucleus ;

DISCUSSION

Organisation and localisation of the internal reproductive system: Crabs of the genus *Callinectes* are represented in Côte d'Ivoire by two species. *Callinectes amnicola* is one of the commercially important specie. The present results supplement the work undertaken by Charles-Dominique and Hem (1981) in *Callinectes amnicola.* Male reproductive internal system is bilateral, symmetrical and composed of paired testes, paired vasa differentia (d'Almeida, 1999; d'Almeida *et al.*, 2009). Previous results about the testes (d'Almeida, *et al.,* 2007) are not presented. The male reproductive system in *Callinectes amnicola* is quite like that found in *Callinectes sapidus* (Cronin, 1947; Johnson and Otto, 1981), in the blue swimming crab, *Portinus pelagicus* (Stewart *et al*., 2010), in *Paguristes eremita* (Tirelli *et al*., 2010), in hermit crab *Clibanarius sclopetarius,* (Santos and Mantelatto, 2011), in Pea Crabs (Becker *et al*., 2013), in *Callinectes ornatus* (Do Nascimento and Zara, 2013). Soundarapandian *et al*. (2013a, 2013b) indicate similar organs in the two species, *Charybdis feriata* and *Portunus pelagicus.* The topographical distributions of the system as well as the morphology of these organs are similar in these species. With the exception of the hermit crabs, *Calcinus tibicen* which have their reproductive system allocated in the pleon (McLaughlin, 1983; Amadio and Mantelatto, 2009).

Anatomy, histology and cytology of the vasa deferentia: Differentiation of paired vasa deferentia occurs during the sexual maturity (d'Almeida, 1999; d'Almeida *et al.*, 2009). The vasa deferentia in *Callinectes amnicola* evolve during the sexual maturity from the primary stage to the secondary stage. Cronin (1947) has observed similar configuration in the posterior vas of *Callinectes sapidus* and regarded them as lateral pockets resulting from one or two central cavities. In the adult male of *Callinectes amnicola,* the whole vas deferens is subdivided in several secondary ducts. Cronin (1947) carried out similar structures in the anterior vas deferens and described them as numerous lobule-like subdivisions containing spermatophores. The vasa deferentia of others adult crabs are divided into ducts or tubules. In *Pinnotheres pisum* and *Nepinnotheres pinnotheres*, the anterior vas deferens is composed of coiled tubules (Becker *et al*., 2013). In *Charybdis feriata* and *Portunus pelagicus* the vasa deferentia are pair coiled tubules (Soundarapandian *et al*.., 2013a, 2013b). According to Beninger *et al*. (1988), in *Chionoecetes opilio* the anterior vas deferens (AVD) is composed of a complex network of ducts. The primary white vas deferens of juvenile specimens in *Callinectes amnicola* does not contain any spermatophores and it specificity is the accumulation of luminar acellular or agranular substance in the secondary ducts. Similar phenomenon was mentioned in the anterior vas of *Portunus pelagicus* (Soundarapandian *et al*.., 2013b). In contrast, Do Nascimento and Zara (2013) reported that in *Callinectes ornatus,* all stages, juvenile, developing and mature adult males produce sperm and spermatophores. However the juveniles are not able to reproduce since they have abdomen attached to the cephalothoracic sternum. The vasa deferentia of adult crabs are most frequently divided into three sections (George, 1963 ; Hinsch and Walker, 1974 ; Hartnoll, 1975 ; Hinsch, 1988a ; Martin Garcia and Fietosa Silva, 2006 ; Erkan *et al.*, 2009 ; Simeo *et al*., 2009 ; Amadio and Mantelatto, 2009 ; Becker *et al.*, 2013 ; Soundarapandian *et al*., 2013a, 2013b). Cronin (1947), Johnson and Otto (1981) mentioned the regionalization of the vasa deferentia according to its tinctorial aspect in the adults of *Callinectes sapidus*. In *Callinectes ornatus* the male reproductive system was classified macroscopically by the colour and the size (Do Nascimento and Zara, 2013). Moreover, according to Johnson and Otto (1981), the pink colour of the medial portion attests the presence of spermatophores, which confer to them this colour. This assumption is supported by Cronin (1947). Thus in *Callinectes amnicola,* as well as in *Callinectes sapidus* (Cronin, 1947), each vas is divisible by both anatomy and physiology into three portions. Microscopical investigations reveal physiology of the vas deferens. In the juvenile individuals, the vas deferens is surrounded by a cuboïdal epithelium that evolves in columnar one at the stage V to VII. The epithelial modifications in the secondary ducts are equally carried out in the specie *Callinectes sapidus* (Cronin, 1947 ; Johnson and Otto, 1981), in *Charybdis smithii*, (Balasubramanian and Suseelan, 2000) in *Calcinus tibicen* (Amadio and Mantelatto, 2009), in *Pinnotheres pisum* and *Nepinnotheres pinnotheres*, (Becker *et al.*, 2013) and in *Portunus pelagicus* (Soundarapandian *et al.*, 2013b). Do Nascimento and Zara, (2013) described similar modification in *Callinectes Ornatus.* At the stage V adults, each portion of the vas deferens has its own histological features.

Anterior vas deferens (AVD): In the adults male, although the anterior portion of the vas deferens does not present pink coloration, contains essentially spermatophores as mentioned by Cronin (1947) in *Callinectes sapidus.* In *Callinectes ornatus,* the main function of the AVD is packing the spermatozoa into spermatophores Do Nascimento and Zara (2013).

Medial vas deferens (MVD): In MVD the epithelium gradually becomes less columnar. In *Portunus pelagicus* similar features are mentioned (Soundarapandian *et al.*, 2013b). The MVD region produces seminal fluid and stores the spermatophores. The heterogeneity of the medial vas deferens carried out in the present paper corroborates those made in *Callinectes sapidus* by Cronin (1947), in the golden crab *Geryon fenneri* by Hinsch (1988a), in *Pinnotheres pisum* and *Nepinnotheres pinnotheres* by (Becker *et al.*, 2013) in *Callinectes ornatus* by Do Nascimento and Zara (2013) and in *Portunus pelagicus* by (Soundarapandian *et al.*, 2013b). The medial vas deferens involved in the storage of a heterogeneous substance Becker *et al*. (2013). These materials are spermatophores and fine fluid droplets synthesized to maintain for a long time in life the spermatozoa. In *Pinnotheres pisum* and *Nepinnotheres pinnotheres*, spermatophores are present in the medial and distal vasa Becker *et al*. (2013). Spermatophores and fluid pockets reach the posterior vas deferens.

Posterior vas deferens (PVD): In the main part of the posterior vas deferens, secondary ducts free in vesicles of secretion contain essentially spermatophores and all the ducts are in confluence and are inter-connected. This phenomenon was supported by Cronin (1947) in *Callinectes sapidus*. Although not presenting pink coloration, the histology reveals in the posterior portion also the presence of some spermatophores. The presence of spermatophores in the anterior and posterior portions echoes what carried out Cronin (1947) and (Soundarapandian *et al*., 2013b). The spermatophores present in the vas deferens are enveloped by a membrane, which differentiates them from the fluid pockets observed in the medial portion. These observations corroborate those made in *Callinectes sapidus,* (Cronin, 1947; Johnson and Otto, 1981), in *Carcinus maenas* (Spalding, 1942). The encapsulated spermatophores were describe by Amadio and Mantelatto (2009); Becker *et al*. (2013); Do Nascimento and Zara (2013); Soundarapandian *et al*. (2013b). In the snow crab *Chionoecetes opilio* Beninger *et al*. (1988) and in hermit crab *Calcinus tibicen*, Amadio and Mantelatto (2009) spermatophores are surrounded by a pellicle that may act as a safeguard against dehiscence. *Paguristes eremita* presents spermatophores enveloped by protective layers, (Tirelli *et al*., 2010). The gonoducts serve in driving spermatophores toward the external organs. The vasa deferentia of the adult male contain spermatophores (d'Almeida, 1999; d'Almeida *et al.*, 2006b). According to Santos and Mantelatto (2011) in *Clibanarius sclopetarius,* the testes produce sperm, which are packaged in spermatophores and brought along the vas deferens to be transferred to females. Similar observation is made in marine crustaceans (Subramoniam, 1993) and in the hermit crab, *Clibanarius vittatus* (Hess and Bauer, 2002).Spermatophores take devious route through the vas deferens and reach the external portion of the reproductive system, which includes the penes and the pleopods. Spermatozoa of *Callinectes amnicola* and *Carcinus maenas* present some similitude (Grassé, 1994). Spermatozoa of *Calcinus tibicen* (Amadio and Mantelatto, 2009) present morphological similarity with those of *Callinectes amnicola*. Balasubramanian and Suseelan (2000) described similar spermatozoa in *Charybdis smithii*. The spermatozoal ultrastructure of *Callinectes amnicola* resembles that of *Pinnotheres pisum* and *Nepinnotheres pinnotheres* (Becker *et al..,* 2013) and of *pinnotherid Pinnixa* sp (Reger, 1970; Krol *et al.*, 1992). All the features of this reproductive system could be extrapolated to the genus.

CONCLUSION

The differentiated vas deferens evolves from a primary stage to a secondary stage with formation of secondary ducts. In adult male of *Callinectes amnicola,* paired vasa deferentia constitute the internal tract and serves in driving spermatophores toward the external organs, which are penes and pleopods. Primary white vas of the juvenile crabs are not functional and do not contain spermatophores. The whole organisation of the vasa differentia of the mature crabs is adapted to assume assigned function. Three regions of the vas deferens were defined based on gross morphology, colour and content. The anterior vas (AVD) is whitish, the medial portion (MVD) is bright pink and the posterior vas (PVD) is translucent or greenish. The function of the AVD is packing the spermatozoa into spermatophores. The medial vas deferens involved in the storage of spermatophores and fine fluid droplets synthesized to maintain in life the spermatozoa. The posterior vas contains essentially spermatophores. The present paper and previous results about the (d'Almeida *et* testes *et al.,* 2007) showed the path of spermatophores through the reproductive tracts. It consists of the formation of gametes in the gonads (d'Almeida *et al.*, 2007), their transportation through the gonoducts and their evacuation toward the external reproductive organs. All the secondary ducts along the vas are interconnected. Spermatophores and fluid pockets take devious route through these ducts and reach the external portion (Cronin, 1947).The

differentiation of all these organs could be considered as features of the genus.

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