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Male external genitalia tracts of Côte d'Ivoire Brackishwaters crabs, *Callinectes amnicola*, (De Rochebrune, 1883; Decapoda: Portunidae).

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ABSTRACT
Objective: The crab *Callinectes amnicola* is Crustacean specie especially found in West Africa. Due to over fishing, the crab *Callinectes amnicola* that is the most exploited specie in Côte d'Ivoire, is facing extinction. In order to palliate this phenomenon, the management of the natural stock or breeding becomes indispensable. These aspects involve the mastering of structural organisation and physiology of the reproductive organs. The present study aims to clarify morphology and function of the external organs of the reproductive system.
Methodology and Results: Investigations were made by histological technique. The external reproductive tract is composed of paired penes and two pairs of pleopods (G1 and G2). Macroscopic observations indicate 7 stages in male sexual maturity. Investigations with light microscope after histological treatment, allowed following the evolution of these two organs. In the juvenile and mature males, the wall of penes is composed of a connective tissue layer, a muscular layer, an internal columnar and ciliated epithelium and relatively broad lumen. *Callinectes amnicola* possesses two types of gonopods, the tubular long first gonopods (G1) and the shorter second pleopods (G2). The first gonopod (G1) consists of coxopodite XIII, subterminal segment, and terminal segment or telopodite, which forms a tube. The tube or groove possesses basal and apical opening. A calcified wall surrounding bundles of striated muscle fibres and glandular areas bound Gonopods. The second gonopod (G2) consists of three segments, the coxopodite XIV, a medial portion and apical spine. It is inserted into the posterior foramen of the first pleopod and it forces semen and spermatophores through the first pleopod. Physiology of the external tract was carried out. Penis and first pleopods are mechanical organs of copulation.
Keys words: *Callinectes amnicola*, penes and pleopods, Morphology, histology.

INTRODUCTION
Due to the high demand of *Callinectes amnicola* in local and in Africa markets, they are heavily exploited in Côte d'Ivoire. This specie becomes a most foods, which command high price. 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 stock correlated with over fishing. Faced with that problem, researchers undertook investigations relating to the both sexes. The present results about external reproductive tract supplement studies of structures of internal reproductive system by d'Almeida (1999).About the genus, Williams (1974) made the synthesis of the previous taxonomic

studies. 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*. d'Almeida (1999) has investigated reproductive cycle of *Callinectes amnicola*. The sexual maturity scale of the male (d'Almeida, *et al...,* 2009) and the differentiation of the testis (d'Almeida *et al.*., 2007) were carried out. About the female, the sexual maturity (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*. To understand the histological aspects of the external reproductive system in *Callinectes amnicola*, the sexual maturity scale in the male was established (d'Almeida, 1999; d'Almeida *et al.*., 2009). That scale comprises 7 stages. The knowledge about the structures of the reproductive system is important to select the performing matured male for breeding. The present study is an attempt to explain physiology of the organs of the external tract. The gross morphology has allowed observations of the setting up, the localization and differentiation of the different organs (d'Almeida, 1999; d'Almeida *et al.*., 2009). In the present study, we aim to clarify form and function of the external genitalia tracts: paired penes and two pairs of pleopods (G1 and G2) in *Callinectes amnicola*.

MATERIAL AND METHODS

Biological material: Specimens of *Callinectes amnicola* used in this study were caught from the brackishwaters, Aby and Ebrié in Côte d'Ivoire. Sixty three (63) males sorted out thereafter are classified according to the stages of the sexual maturity. Identification parameters used in this case are both sizes of the specimen and of the abdomen whose shape remains invariable in the males. After their catching, the animals are cold anesthetized in a freezer (LIEBHERR). They were photographed with the camera MINOLTA AF 7000**.** The penes and the first pleopods (G1) are removed for microscopical investigations.

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 first pleopods (G1) and penes were fixed by immersion in aqueous Bouin and dehydrated in ascending series of ethanol (70°, 95° and 100°). The calcified pleopods of adult were softened in a mixture of formic acid, formalin 37% and distilled water, before their dehydration. Without softening, samples fixed according to classical steps become very hard and friable. Pleopods are in this condition extremely hard after fixation, 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.

RESULTS

Macroscopic characters: In *Callinectes amnicola,* the body is fused head and thorax covered dorsally by a pentagonal carapace, which has two long lateral spines and several teeth on each side of the mouth field (Fig. 1A). In ventral view, the male present five fused segments forming the exoskeleton (Fig. 1A)**.** Five pairs of walking articulated appendages are fixed to the segments. The first is cheliped (Fig1A). Ventrally an abdomen is folded beneath the segmented exoskeleton (Fig.1A). The shape of the abdomen varies with the sex and maturity (Fig.1B). The adult male is distinguishable externally from the female. Its abdomen upset "T" shaped (Figs.1A, 1B and 1C), instead of triangular abdomen of the juvenile female and semi-circular abdomen of the mature one (Fig.1B). Juvenile male has his abdomen still attached to the thoracic sternum. Adult males have its abdomen and gonopods detachable.

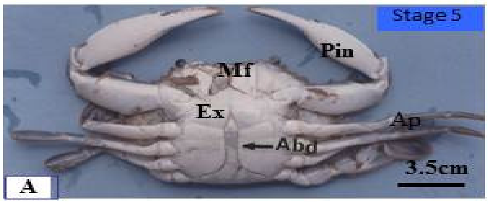

Figure 1 A : Ventral view of a male of stage V.
Five pairs of walking articulated legs or appendages (Ap) are fixed to fused segments **of exoskeleton**. The first is cheliped. Male abdomen (Abd) is "T" shaped, narrow and with a broad base.

Figure 1 B : External views of the adults male (a) and female (b) abdomen.
Figure 1 C : Internal views of the male abdomen showing Plp 1 and 2 (G1 andG2).
Figure 1 D : Morphology of first pleopods G1 (Plp1) and second pleopods G2 (Plp2) after removal.

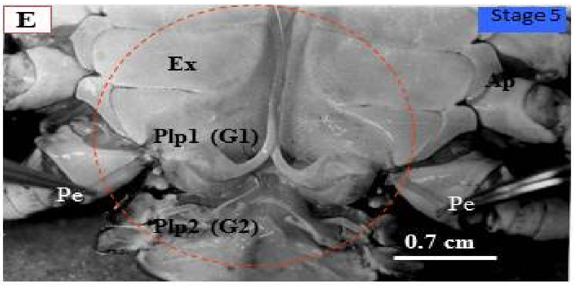

Figure 1 E : Ventral view of a male of the stage V. Two pairs of **pleopods 1 and 2 (G1 and G2)** are located at the base of the last abdominal segment of the exoskeleton.

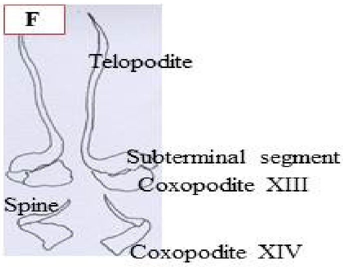

Figure 1 F : Diagrammatic representation of first pleopods (G1) and second pleopods G (2) after removal.

Ex : exoskeleton. ; Ap : Five pairs of walking articulated appendages ; Pin : pincers ; Abd : Abdomen upset "T" shaped ; Mf : mouth field ; Plp1 and Plp 2 : pleopods 1 and 2 (G1 and G2) ; Pe : Paired penes retained by pincers.

The male crabs possess paired penes (Fig. 1E) and two pairs of pleopods (G1 and G2) that constitute the external portion of the reproductive system. The "T" shaped abdomen of the male (Fig. 1C) covers and protects these organs (Figs. 1D, 1E, 1F and 2A).

Anatomy and histology of penes: Penes are small slender tubes located at the level of the coxopodite of the last pair of locomotive appendages(Fig. 2A**).**They are short external extensions of the ductuse jaculatorius, the distal portion of the PVD (posterior vas deferens).Each posterior vas deferens (PVD) was connected with an ejaculatory duct leading into the penis. Each penis passes into the proximal foramen of the first pleopod. Sections of penes of juvenile specimens show a tissue whose structure is gradually modified from the base towards the apex (Figs.2B and 2C). The wall of the penes is constituted of connective tissue containing rare cells, bundles of striated muscle fibres (Figs.2B, 2C and 2E). The connective tissues contain ovoid nuclei (Fig. 2B and 2C). Muscle fibres are circular and some longitudinal bundles are observed (Fig. 2E). A columnar ciliated epithelium forming villi lines the lumen and tops all these structures (Figs.2B and 2C). Epithelial cells have basally located nuclei (Fig.2C). The penes of the adult specimen are enlarged (Fig.2D) and constituted of connective tissue containing few nuclei and muscular layers (Fig.2D). The villi have disappeared and a continuous ciliated columnar epithelium surrounds an enlarged lumen (Fig.2D). There is no indication of secretion in the lumen on the sections examined. The lumen contains only an acellular material (Fig.2D). In both the juvenile and adult males, towards the apex, a reduced connective tissue and muscular layers subsist and surround the lumen. Penes are evacuation ducts that discharge spermatophores in the first pleopod.

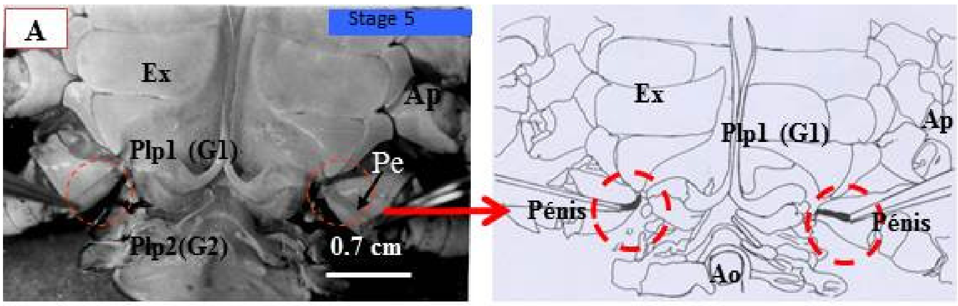

Figure 2 A : Ventral view of a male of stage V.

A pair of penes (Pe) localized at the level of coxa of last legs restraint by clamps.

Two pairs of pleopods (Plp1 and Plp2) are located at the base of the last abdominal segment of the exoskeleton.

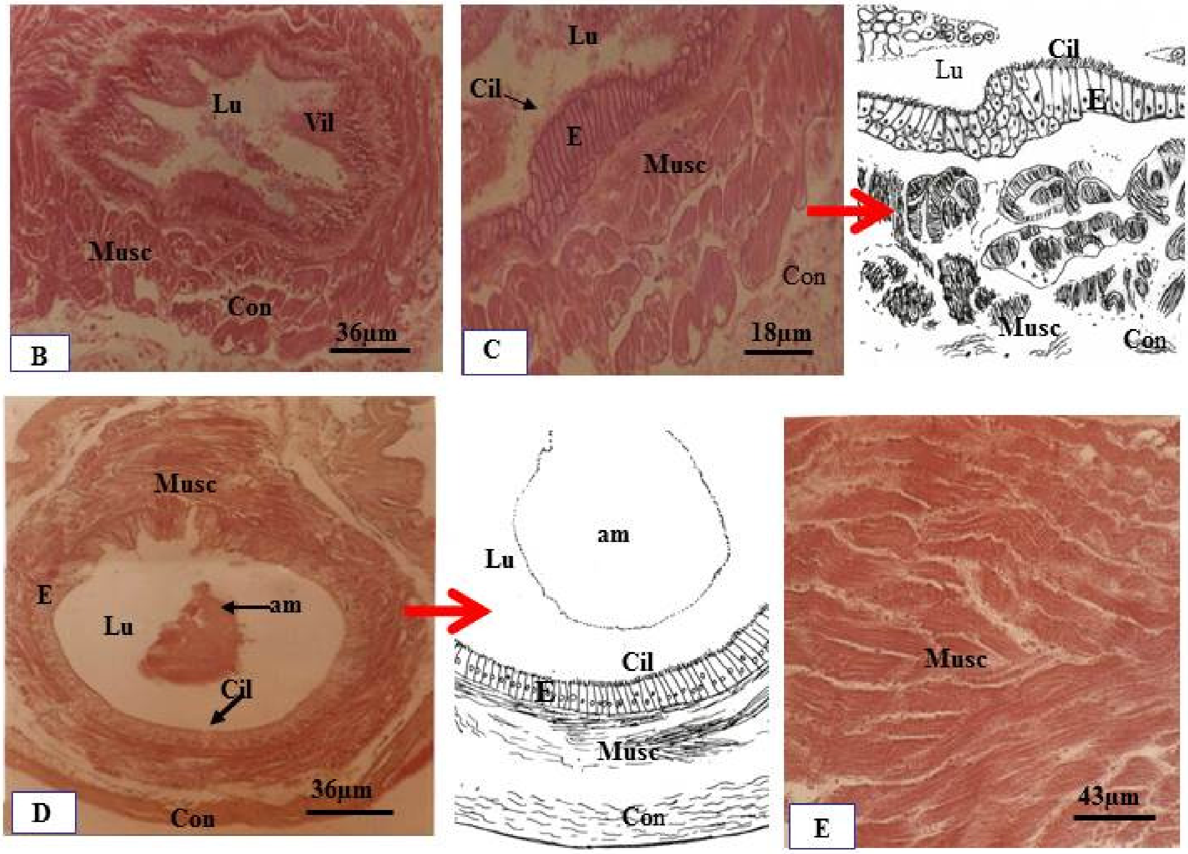

Figure 2 B to 2 E : Histology of the penes

B : Transverse section trough the penis of a juvenile male.

C : Detailed view of portion of Figure **(B) showing ciliated epithelial cells.**

D : Transverse section trough the penis of an adult male.

E : Detailed view of figure **D** showing muscles fibers .

Anatomy and histology of first pleopods: Gonopods (G1 and G2) are hidden underneath the folded abdomen (Figs.1A and 1C). The first gonopods (G1) are long and tubular in shape; the second pleopods (G2) are generally short. (Figs.1C, 1D, 1E, 1F and2A).

Gonopods (G1)*:* The first pleopods (G1) consist of three segments (Figs.1D, 1E and 1F): a broad articulated basal portion, the coxopodite XIII, a subterminal segment and frayed terminal segment, the telopodite (Figs.1E and 1F). The first pleopods are bounded by an outer calcified wall constituted of concentrical deposits layers of calcium (Fig.3A).

Coxopodite XIII. The calcified wall around the coxopodite XIII and the subterminal segment surrounds bundles of striated muscle fibres with variable size and glandular mucous areas (Fig.3B). The glandular zone forms acini or rosette glands composed of regrouped cells (Fig. 3C). A very small lumen is present in the centre of each gland (Fig.3C). Connective tissues and scattered cells were observed. No connection of the coxopodite XIII, the subterminal segment and the surface is established.

Telopodite: This terminal segment forms a tube that possesses basal and apical opening. It possesses a groove that extends to the tip of this terminal joint (Fig.3D). This groove is considerate as funnel to guide sperm. Longitudinal section through the telopodite indicates (Fig.3D) presence of continuous outer and internal calcified walls, longitudinal and circular muscle fibres (Fig. 3I), and glandular areas (Fig.3D). The internal calcified wall surrounds the central groove. In the apex of the telopodite that constitutes the spine, all the structures disappear, only the calcified wall persists. Cross section through the base of the telopodite showing the basal opening indicates the outer calcified wall, mucous and serous glands (Figs.3E, 3G and 3H). The telopodite is connected with the surface by a central groove (Figs. 3D, 3E, 3F and 3G) around which is glandular areas and an internal calcified wall (Figs.3E 3F, 3G and 3H). A layer of epithelial cells comes in between these two structures (Fig. 3G). One notes the presence of a furrow (Figs.3D and 3E) in which the second pleopods insert and push spermatophores through the lumen of the central groove (Figs.3D, 3E and 3F). In the male of *Callinectes amnicola,* the pleopods (G1) (Fig. 1E) constitute the intromittent organ used to conduct spermatophores into the female gonopores.

Gonopods (G2)*. :* The second gonopods (G2) consist also in three segments. They are composed of coxopodite XIV and spine and inserted under the first (Figs.1E and 1F). The second articulated gonopods (G2) are membranous and calcified organs (Figs. 1E and 1F). According to its structure, histological investigations were not carried out. The second pleopods force semen and spermatophores through the furrow of the first pleopods.

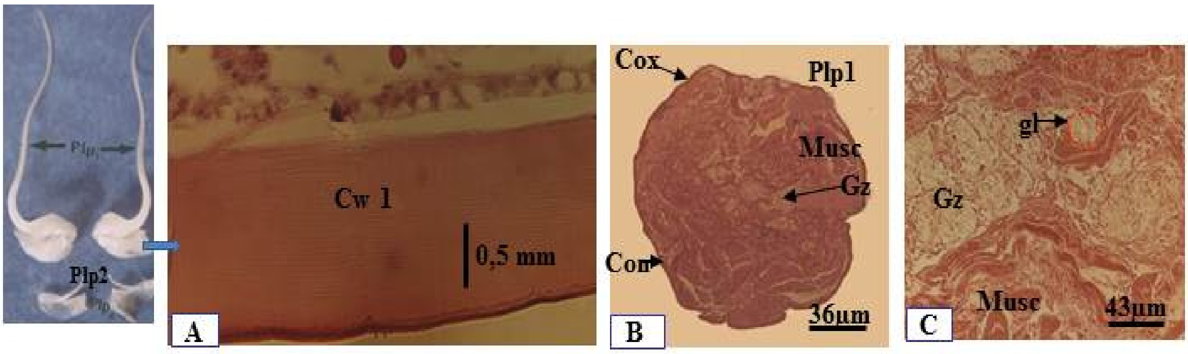

Figure 3 A to 3 C : Histology of the first pleopods (G1)
A : Transverse section through the external calcified wall enveloping the pleopods.
B : Transverse section through the coxopodite XIII of the first pleopod.
C : Detailed view of a portion of the figure. **B** showing glandular area.
Each glandular zone is constituted of rosette glands.

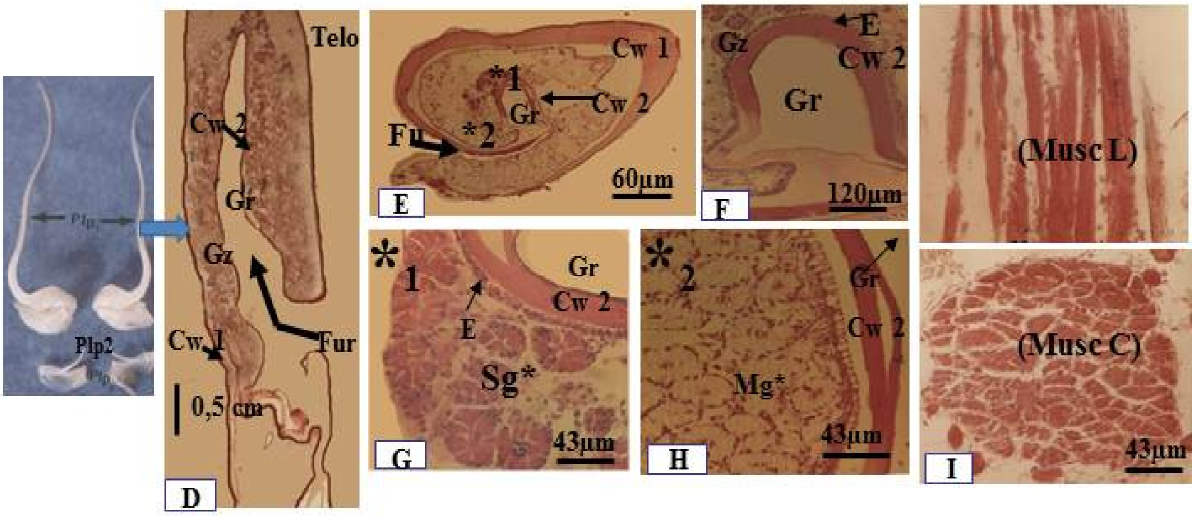

Figure 3 D to 3 I : Histology of the first pleopod (G1)
D : Longitudinal section through the telopodite of the first pleopod.
E : Transverse section at the basal part of the telopodite.
F: Detailed view of the central groove on the figure **E.**
G and H : Detailed views of a portion of the figure **E,** showing the structure of the mucous and serous glands.
I : Longitudinal and circular bundles of striated muscle fibers observed at the telopodite. Level.

Cw : calcified wall ; concentric deposited layers of calcium ; Cw 1 : external calcified wall ; Cw2 : internal calcified wall ; Cox : coxopodite ; Plp1 : First pleopods ; Plp2 : second pleopods ; Musc : bundles of muscle fibers ; Con : connective tissue ; Gz : glandular zone. ; Gl : gland ; Telo : telopodite ; Gr : central groove ; Fur : The arrow indicates the furrow of insertion of the second pleopod ; Sg*1 : serous glands ; Mg *2 : mucous glands ; E : epithelium. ; Musc L : Longitudinal bundles of muscle fibers ; Musc C : circular bundles of muscle fibers.

DISCUSSION

Macroscopic characters: Juvenile males are not able to reproduce successfully (d'Almeida, 1999; d'Almeida, *et al.,* 2009).Their attached abdomen does not allow copulation and the insertion of copulatory pleopods into the female gonopores. Adult males produce spermatophores, its abdomen and gonopods are detachable and they are able to copulate (d'Almeida, 1999; d'Almeida, *et al.*, 2009). In *Callinectes ornatus,* Do Nancimento and Zara (2013) reported similar findings. The male external reproductive system in *Callinectes amnicola* constituted by paired penes and two pairs of pleopods (d'Almeida, 1999; d'Almeida, *et al.,* 2009) is quite like that found in *Callinectes sapidus* (Cronin, 1947; Johnson, 1980; Johnson and Otto, 1981). The topographical distributions of the system as well as the morphology of these organs are similar in these two species. Similar findings have been described in the flower crab *Charybdis feriata* and the swimming crab *Portunus pelagicus* Soundarapandian *et al..* (2013a, 2013b). Becker *et al..* (2012) indicate similar copulatory system in the brachyuran.

Penes: Penes of *Callinectes amnicola* are short external extensions of the distal portion of the PVD (d'Almeida, 1999; d'Almeida, *et al.,* 2009). This configuration is characteristic of the genus *Callinectes* (Cronin, 1947; d'Almeida, 1999; d'Almeida *et al.,* 2009). In the swimming crab *Portunus pelagicus* also, the posterior vas deferens (PVD) shows similar connection, Soundarapandian *et al..* (2013b). In all these species, spermatophores and fluid pockets elaborated in the internal organs take devious route through the vas deferens and reach the penes. In *Carcinus maenas* (Tixier and Gaillard, 1969), in the genus *Callinectes* (Cronin, 1947; d'Almeida, 1999; d'Almeida *et al.,* 2009), in *Charybdis smithii,* Balasubramanian and Suseelan (2000), the penes is slender tube and constitute evacuation ducts that discharge spermatophores in the first pleopod and do not have any function in the copulation as in the Mammals. The wall of the penis is composed of 3 layers: a connective tissue layer, a muscular layer and an internal epithelium around a central lumen. Similar findings have been described in *Portunus pelagicus,* Soundarapandian *et al..* (2013b). Histological structures observed at the penis level in *Callinectes amnicola* and in *Portunus pelagicus,* Soundarapandian *et al..* (2013b) correspond to those described by Cronin (1947) in *Callinectes sapidus*. During copulation, penes insert into the tube or furrow of the pleopods (G1). Beninger *et al..* (1991); Orensanz *et al..* (1995); Brandis *et al.* (1999) have mentioned similar observations. According to Ewers-Saucedo *et al..* (2015) male mating products are moved through the penis into G1 by capillary action.

Pleopods: As underlined Ewers-Saucedo *et al.* (2015) male crabs use two pairs of gonopods to deliver mating production during copulation. Commonly the second pair is shorter than the first. However, there are several morphological features within the copulatory system that show distinct variation, such as the length of gonopods. They have described two species of box crabs *Calappula saussurei* and *Calappa pelii* with second pleopods, which are longer than the first. In *Callinectes amnicola* the gross morphology of the first pleopods is quite like that found in *Callinectes pallidus* and *Callinectes sapidus* (Cronin, 1947; Williams, 1974; Johnson and Otto, 1981).They are articulated and consist in three segments. The pleopods of *Callinectes amnicola and Callinectes pallidus* have the same localizations differing probably only in proportions. The examined males of the two species *Calappula saussurei* and *Calappa pelii* show similar structure of G1 (Ewers-Saucedo *et al.*., 2015). In contrast, Soundarapandian *et al..* (2013a and 2013b) mentioned in other hand that in the flower crab *Charybdis feriata* and the swimming crab *Portunus pelagicus,* the first pleopods is made up of two segments.

Coxopodite : Histology of the first pleopods in *Callinectes amnicola* corroborates the observations made in *Callinectes sapidus*, Cronin (1947). This author has also observed aciniform glands. The real function of the various glands observed in the gonopods is unknown. This remark echoes what mentioned Cronin (1947). Nevertheless, Cronin (1947) supposed that rosette glands at coxopodite XIII level in *Callinectes* probably produce cuticle. No connection of the coxopodite XIII and the surface is established Cronin (1947).

Telopodite: The telopodite of first gonopods (G1) in *Calappula saussurei* and *Calappa pelii* possesses a groove (Ewers-Saucedo *et al.*., 2015). These authors hypothesized that the groove acts in the guidance of sperm. This hypothesis is in concordance with description reported in the present work. In *Chionoecetes opilio,* the first pleopod acts as funnel to collect the emitted spermatophore**s** Beninger *et al.* (1988)**.** The telopodite is specialized in the genus *Callinectes* (Cronin, 1947 ; d'Almeida, 1999 ; d'Almeida *et al.*., 2009) and in the two species *Calappula saussurei* and *Calappa pelii* (Ewers-Saucedo *et al..,* 2015). It forms a coniform tube. The first gonopods (G1) of true crabs is always tubular in shape and the tube possesses basal and apical openings (Hartnoll, 1968). As in the genus *Callinectes* the first gonopods in pinnotherid (Becker *et al*.., 2013) has the ejaculatory canal with both basally and distally openings. According to Cronin (1947) and as observed in the male of *Callinectes amnicola,* the assigned role with the first and second pleopods is confirmed in this work. In pinnotherid (Becker *et al*.., 2013) the long first gonopods transfer the sperm mass into the female genital duct. This function is confirmed by the presence of the furrow and the groove. The telopodite is practically composed by the same elements as the coxopodite. They have muscle fibres and aciniform glands. There are two types of glands, mucous and serous. In *Callinectes amnicola,* the gross morphology or anatomy of the second pleopods is quite like that found in *Callinectes pallidus* and *Callinectes sapidus* (Cronin, 1947 ; Williams, 1974 ; Johnson and Otto, 1981). The second gonopods (G2) is also articulated and consist in three segments in the two species *Calappula saussurei* and *Calappa pelii* (Ewers-Saucedo *et al*., 2015). The second pleopods (G2) of true crabs are generally short (Hartnoll, 1968) and force semen and spermatophores through the furrow of the first pleopods. According to Soundarapandian *et al.*(2013a, 2013b), in the flower crab *Charybdis feriata* and the swimming crab *Portunus pelagicus* the second pleopod (G2) helps in passing the seminal fluids from the penis into the first pleopod. The role is similar in *Chionoecetes opilio* (Beninger *et al*.., 1988). The copulatory system of true crabs appears to have a general pattern of both organ morphology and copulatory function. While the penis injects the sperm mass, the second pleopod functions in the transport of spermatozoa (spermatophores) inside the ejaculatory canal toward distal opening of the gonopods (G1) (Becker *et al*.., 2012). It is not the case in *Calappula saussurei* and *Calappa pelii* (Ewers-Saucedo and al., 2015). The second pleopods in these species are longer than the first. In this case, Ewers-Saucedo *et al.* (2015) propose that second gonopods deliver male mating products directly into the spermathecas of the female.

Transfer of spermatophores in the spermathecas of the female: The external reproductive tract function in *Callinectes amnicola* and *Callinectes sapidus*is sperm transfer (Cronin, 1947 ; Johnson and Otto, 1981). The function is similar in the flower crab *Charybdis feriata,* in the swimming crab *Portunus pelagicus,* (Soundarapandian *et al*.., 2013a, 2013b), and in box crabs, *Calappula saussurei* and *Calappa pelii* (Ewers-Saucedo *et al*., 2015). The mechanism of the process of spermatophores transfer and related to the shape of the second gonopods is G2 shorter than G1. According to Hartnoll (1969) the tip of G1 is in close contact with the female gonopores during this process. Male crabs use these two pairs of gonopods to deliver mating production during copulation. The second pair is shorter than the first. The first gonopods transfer the sperm and the second pleopod helps in passing the seminal into the first pleopod (Cronin, 1947; d'Almeida, 1999; d'Almeida *et al*.., 2009; Soundarapandian *et al*.., 2013a, 2013b; Becker *et al*.., 2013).Asin the genus *Callinectes* (Cronin, 1947; d'Almeida, 1999; d'Almeida *et al*.., 2009), adult male in *Callinectes ornatus* produced sperm into the female spermathecas through the gonopores (Do Nancimento and Zara, 2013).

CONCLUSION

The copulatory system or external reproductive tract is formed by paired penes and two pairs of abdominal appendages, the gonopods (G1 and G2). Thus male crabs use two pairs of gonopods to deliver mating products during copulation. As underlined Becker *et al..,* (2013) paired penes and two pairs of gonopods form a functional unit to transfer the sperm mass into the paired female gonopores. The tubular long first gonopods(G1) transfer the sperm mass to the female, while the shorter second pleopod (G2) works like a piston inside the (G1) to transport the sperm mass within the ejaculatory canal. The differentiation of gonoducts, pleopods (G1) and penes seems to be features of the genus. The microscopical study of reproductive system allowed a better understanding of its physiology. This physiology consists of the formation of gametes in the testes (d'Almeida, *et al..,* 2007), their transportation through the gonoducts and their evacuation by the external reproductive organs. The assigned role of all the organs of the genitalia tracts mentioned in *Callinectes sapidus* by Cronin (1947) is confirmed in the present work. The differentiation of all these organs would be considered as features of the genus.

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