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Functional morphology of the male reproductive system in *Callichirus major* (Crustacea: Decapoda: Axiidea): Evidence of oocytes in the gonad

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Abstract

Callichirus major inhabits the intertidal region of marine ecosystems and it is frequently used as live bait for fishing. This study aimed to describe the functional anatomy of the male reproductive system by microscopic techniques. The animals were collected along the Corujão beach, Piuma—ES, Brazil, and, in laboratory, the males were classified into two phases: immature (IM) and developed (DE) based on the macroscopic characteristics of the gonads. The gonad and vas deferens were dissected for histological routine and histochemical tests. Histologically, it was noted that in both phases, the more distal region of gonads has ovarian characteristics, showing developing oocytes. Also, different male germ cells were identified: spermatogonium (SPG), spermatocytes I and II (SPTCI, SPTCII), initial and final spermatid (IS, FS) and sperm (SPZ). Accessory cells with spherical or pyramidal nuclei were also present inside the testicular lobules. According to the vas deferens structure, three regions can be characterized: proximal (PVD), middle (MVD) and distal (DVD). In the lumen of the vas deferens, a spermatophoric matrix highly reactive for histochemical tests was observed. The presence of female germ cells in males suggests the occurrence of intersexuality or hermaphroditism in this species.

KEYWORDS

ghost shrimp, histology, reproduction, spermatogenesis

1 | INTRODUCTION

Callichirus major, commonly known as ghost shrimp, is a crustacean of the order Decapoda, family Callianassidae, first described by Say in 1818. The species has an extensive geographic distribution, from the south coast of the USA to the south-eastern region of Brazil (Sakai 2005). The group comprising this organism plays an important functional role in marine ecosystems, as their burrowing habit, in addition to their interaction with the sediment, promotes bioturbation of the environment (Ziebis et al., 1996; Bird et al. 2000). Moreover, the species has economic value, being widely used as live bait for fishing (Souza and Borzone 2003).

Few studies are available regarding the biology of the genus *Callichirus*; however, it is known that the differentiation between male and female individuals in *C. major* follows the classical pattern for decapod crustaceans, with female and male gonopores located on the third and fifth pereiopod pairs, respectively. Moreover, the males of this species present asymmetrical chela growth (Alves-Junior et al., 2013). In this sense, some studies have already been carried out on the population dynamics in species of the genus *Callichirus*, including *C. major* (Souza and Borzone 1996; Souza et al. 1998; Botter-Carvalho et al., 2007; Hernáez and Wehrtmann, 2007), especially that of Lugon (2014), who evaluated the population structure of the species in the south coast of the state of Espírito Santo, Brazil.

With regard to reproduction, studies about *C. major* are still rare. It is known that the species has a continuous annual reproductive cycle, with reproduction peak and presence of ovigerous females between December and May and gender proportion of approximately 1:1 in the population (Botter-Carvalho et al., 2007).

Studies involving the morphology of the reproductive system and the spermatogenesis process have been described for various crab species, such as *Callinectes ornatus* (Nascimento and Zara 2013), *Callinectes danae* (Zara et al. 2012) and *Maja brachydactyla* (Simeó et al. 2009); crayfish and lobsters, like *Cherax quadricarinatus* (López-Greco et al., 2007), *Nephrops norvegicus* (Rottlant et al., 2012) and *Panulirus homarus* (Pillai et al. 2014); prawns, as *Sicyonia ingentis* (Shigekawa and Clark 1986), *Litopenaeus vannamei* (Alfaro-Montoya 2013); and carideans, such as *Exhippolysmata oplophoroides* (Nunes et al. 2010) and *Macrobrachium rosenbergii* (Poljaroen et al. 2010) among others. Nevertheless, there are no descriptions available for the functional morphology of the male reproductive system, including the spermatophore structure, in Axiidea.

Considering that information on the morphology of the reproductive system of *C. major*, as well as that of other species from the same genus, has not yet been reported, this study aimed to macro- and microscopically describe the structure of *C. major*'s male gonads, highlighting changes occurring during sexual maturation, as well as the vas deferens responsible for spermatozoa packaging in spermatophores and transport to the gonopore.

2 | MATERIAL AND METHODS

2.1 | Material collection

Callichirus major individuals were randomly captured in their galleries using a handmade suction pump made of PVC pipe, at Corujão beach, City of Piúma—ES, Brazil (20°50′41.6″S 40°44′15.7″W). The specimens were fixed in 10% buffered formaldehyde. At the laboratory, the gender of the animals was defined and their carapace length (CL) measured using a calliper.

2.2 | Morphological analyses

Approximately 20 male *C. major* specimens at different stages of gonadal development were analysed, with CL varying from 4.0 to 10.1 mm. The individuals, observed under a stereomicroscope Leica EZ4 HD, were classified as immature (IM) or developed (DE), based on chela size and characteristics of the gonad. Furthermore, averages and standard deviations of CL were calculated for each development stage. Their gonads were dissected and subjected to light microscopy (histology). The vas deferens was analysed histologically and histochemically.

2.3 | Light microscopy

The material, fixed in 10% buffered formaldehyde solution, was dehydrated in increasing solutions of ethyl alcohol (70-95%), subjected to embedding resin for 24 hr, and later included in moulds containing resin with catalyser. After polymerization, the blocks were sectioned into 5μm slices using a microtome Leica RM 2145. Next, the following techniques were applied:

2.3.1 | Histology

Sections were subjected to staining with Harris haematoxylin and eosin (H-E) for 5 and 10 min, respectively. The slides were stained in toluidine blue for 5 min and then washed in running water for removal of excess dye. After drying, the slides were mounted in Canada balsam.

2.3.2 | Histochemistry

2.3.2.1 | Bromophenol blue for detection of total proteins

The slides were stained with bromophenol blue for 1 hr, at room temperature. Next, the preparations were washed with 0.05% acetic acid and running water for 10 min, and subsequently mounted in Canada balsam.

2.3.2.2 | Periodic Acid–Schiff (PAS) for detection of neutral polysaccharides

The slides were placed in 0.4% periodic acid for 10 min and washed with distilled water. Next, the slides were maintained in Schiff's reagent for 1 hr in the dark and then washed with distilled water for 10 min. Immediately thereafter, the slides were dried and mounted in Canada balsam.

Image capture was accomplished with the program LEICA LAS EZ 3.0.0: Leica Microsystems, with calibration appropriate for the used objectives. The software ImageJ was used in the morphometric analysis to verify the largest diameter of the germ cells; the slides of at least three individuals were analysed, with 60 cells of each development stage being measured.

3 | RESULTS

The specimens of *C. major* classified as immature (IM) presented mean CL of 5.2 ± 1.2 mm, whereas developed ones (DE) had mean length of 8.7 ± 1.3 mm. The gonad of the latter individuals is located in the abdomen, dorsally to the hepatopancreas and the intestine, and is constituted by two

lobes extending from the caudal extremity, close to the uropod, up to the posterior region of the cephalothorax. Both IM and DE individuals present a translucent white colour in anterior region of the gonads while the posterior one has an orange coloration which intensifies as it develops and with ovarian characteristics (Figure 1a-d; 2a; 3a).

Histologically, it can be observed that this external connective tissue penetrates into the organ and linens the seminiferous tubules, forming lobes visible under light microscopy (Figure 2b). Each lobe is constituted of cells from the germ lineage at different maturation stages, besides accessory cells (Sertoli-like), which show pyramidal nucleus when located at the periphery of the lobe, and spherical when found among germ cells (Figure 2d and 3c-f).

Overall, the cell differentiation in the gonads is asynchronous, revealing lobes containing cells at different maturation stages. However, inside the lobes it is possible to observe cell polarization, with cells of same type always remaining grouped. The lobular regions presenting cells at less advanced development stage constitute the germinal zone (Figure 2b and 3b). Based on cytoplasmic, nuclear and morphometric characteristics (Mean ± Standard Deviation), it was possible to classify the different cell types of the spermatogenic lineage, as follows:

Spermatogonium (SPG)—Mean diameter of 9.57 ± 1.11 μm; the nucleus has a mean diameter of 7.41 ± 0.64 μm and presents typically homogeneous and intensely basophilic chromatin. This type represents the stem cell of the sperm lineage, being responsible for the maintenance of cell renovation. The cytoplasm forms a small basophilic band around the nucleus (Figure 2c-d and 3c-d).

Spermatocyte I (SPTCI)—Mean cell and nuclear diameter of 11.1 ± 0.85 μm and 8.83 ± 0.82 μm, respectively, with size similar to that of the spermatogonium. Differently, the nucleus presents chromatin organized in heterochromatic blocks. It is the cell type that continues in the process of sperm differentiation. Cytoplasm is scarce (Figure 2c-d and 3b-c, f).

Spermatocyte II (SPTCII)—Total and nuclear diameter of 8.4 ± 0.82 μm and 5.86 ± 0.45 μm, respectively—presents

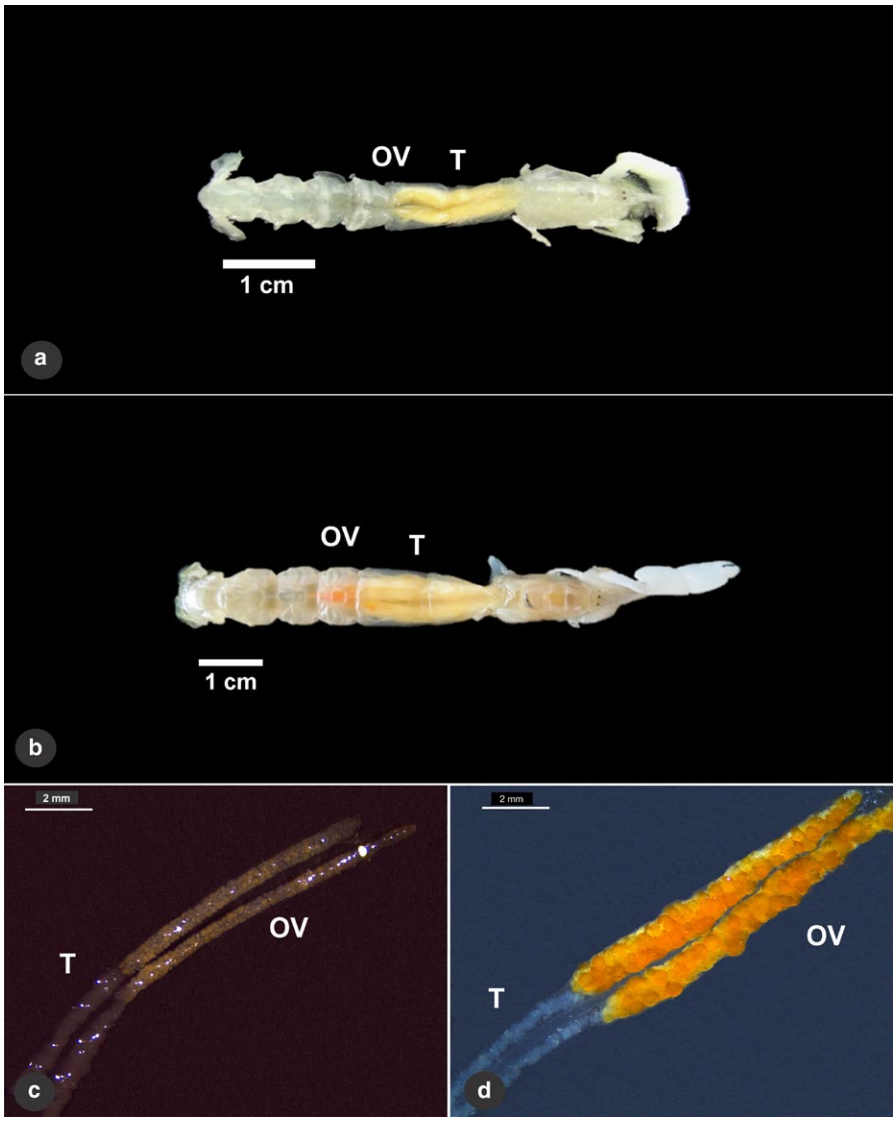

FIGURE 1 *Callichirus major* males at two different stages of gonadal development. (a, c): immature (IM) male. (b, d): developed (DE) male. Note in (c) the IM gonadal anterior translucent white region, and a posterior with a light orange colour. (d) Observe an intense colour in the posterior region of the DE gonad. Testicular region (T), Ovarian region (OV). Bars: (a, b) = 1 mm; (c, d) = 2 mm. [Colour figure can be viewed at wileyonlinelibrary.com]

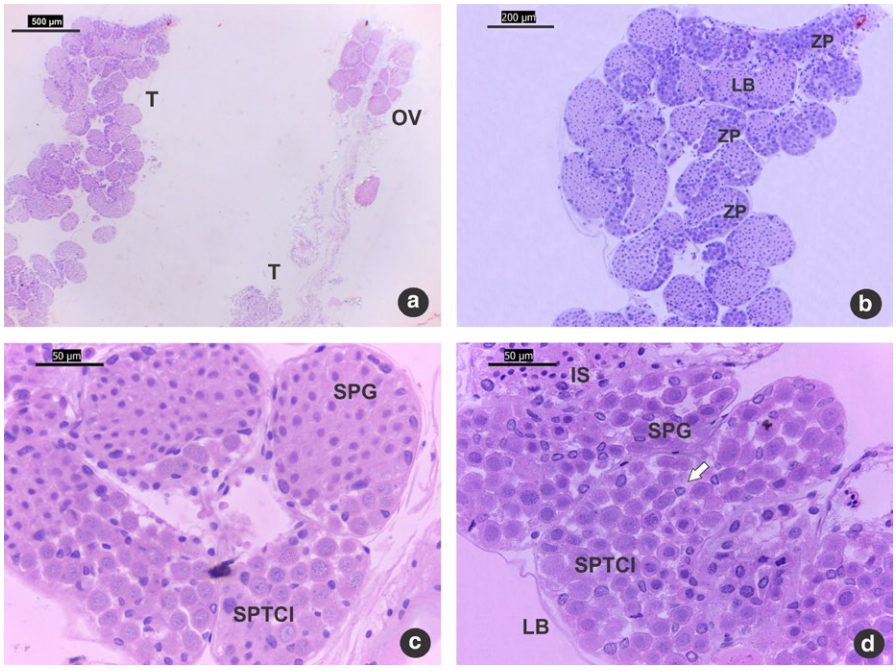

FIGURE 2 Morphology of *Callichirus major* male gonads in immature stage (IM). (a-b) General view. Note in (a), oocytes in the posterior ovarian region (OV) and (b), the lobes (LB) with germinal zone (ZP) of the testicular region (T). (c-d) Detail of lobes containing cells at different stages of differentiation and accessory cell (white arrow). Spermatogonia (SPG), Spermatocyte I (SPTCI), Initial Spermatid (IS). Bars: (a) = 500 µm; (b) = 200 cm; (c-d) = 50 µm. Haematoxylin–eosin Staining. [Colour figure can be viewed at wileyonlinelibrary.com]

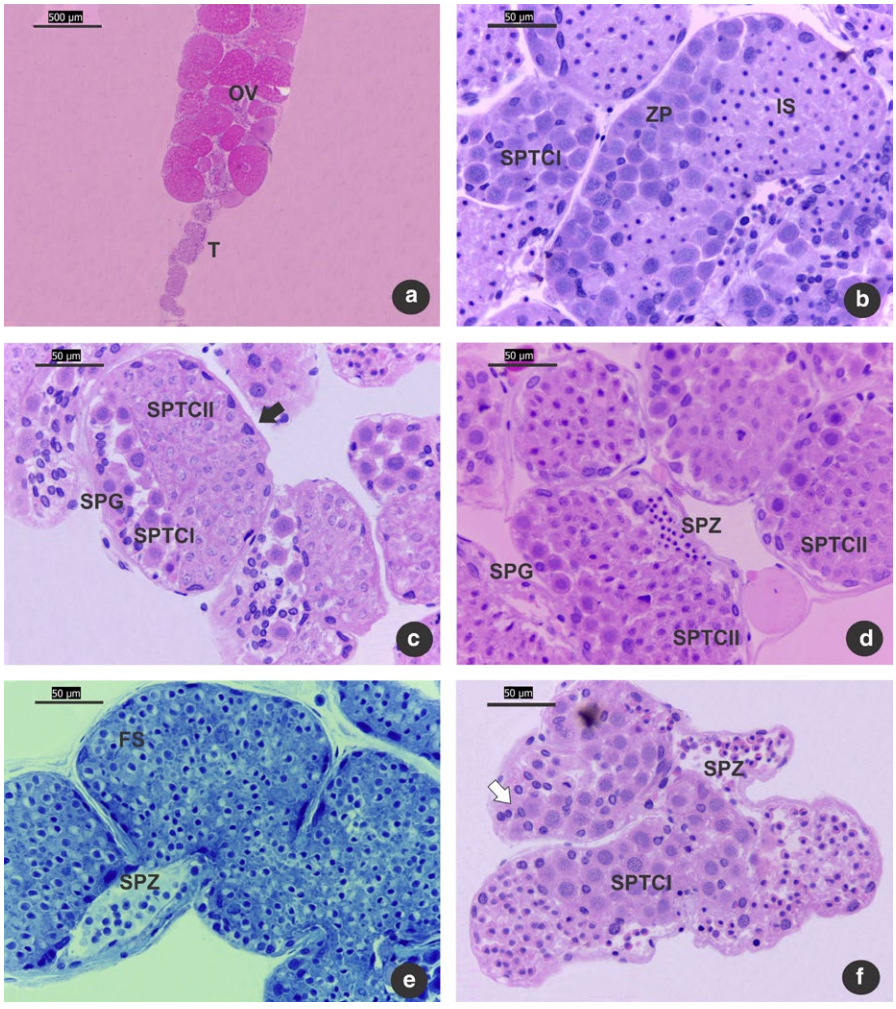

FIGURE 3 Morphology of *Callichirus major* male gonads in developed stage (DE). (a) Ovarian region (OV) located in the posterior portion of the gonad. (b-e) Observe the distribution of the different germ cells inside the lobes. The black arrow shows the pyramidal nucleus in accessory cells on periphery of the lobe. Note in (e) the polarization of final spermatid (FS) nuclei. (f) Sperm (SPZ) with an eosinophilic acrosome, in contrast to the nucleus. Observe the spherical shape when accessory cells are located among germ cells (white arrow). Germinal Zone (ZP), Spermatogonia (SPG), Spermatocytes I (SPTCI), Spermatocytes II (SPTCII), Initial Spermatid (IS), T = Testicular region. Bars: (a) = 500 µm. (b)-f = 50 µm. (a-d and f). Haematoxylin–eosin Staining, (e). Toluidine Blue Stain. [Colour figure can be viewed at wileyonlinelibrary.com]

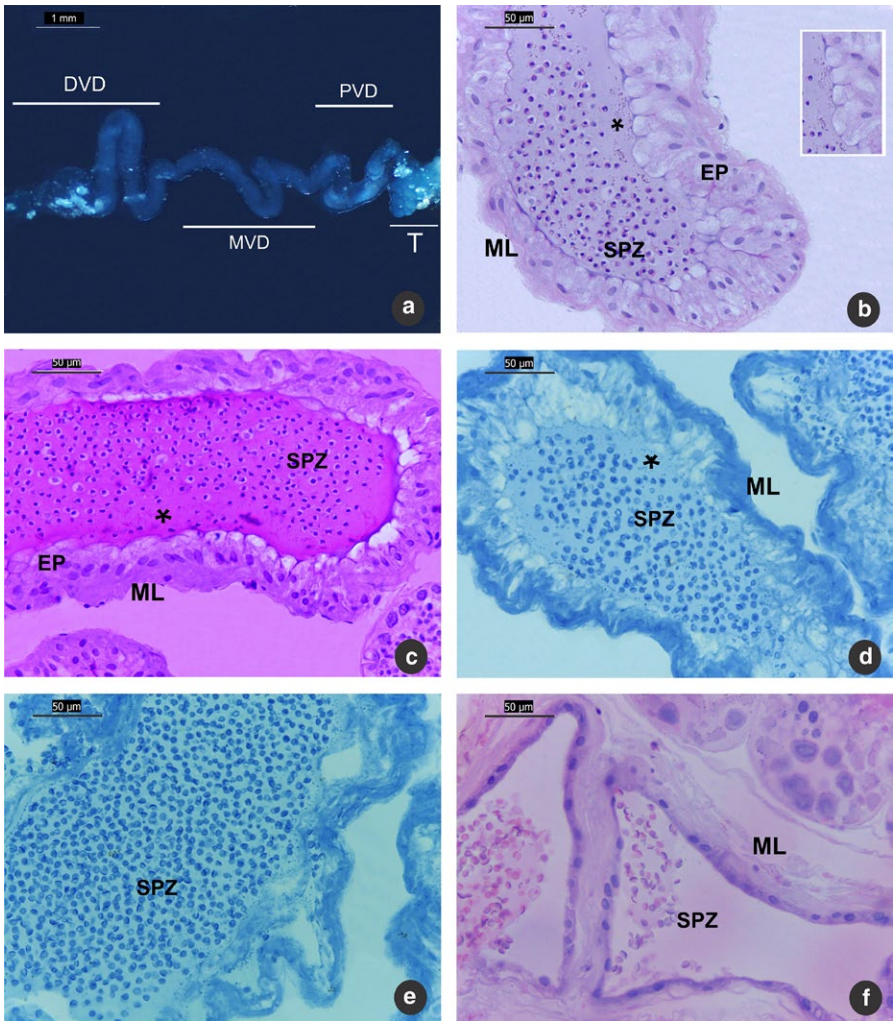

FIGURE 4 Vas deferens of *Callichirus major*. (a) View, under stereomicroscopy, of the vas deferens regions: proximal (PVD), middle (MDV) and distal (DVD). (b-f) Sections submitted to histological and histochemical techniques. (b-d) Proximal region. (e) Middle region. (f) Distal region. Testis (T), Secretory granules (*) shown in detail in B, Epithelium (EP), Sperm (SPZ), Muscle layer (ML). Bars: (a) = 1 mm; (b-f) = 50 µm. (b, f). Haematoxylin and eosin Staining; (c). PAS–Haematoxylin stain; (d, e). Bromofenol blue staining. [Colour figure can be viewed at wileyonlinelibrary.com]

small, euchromatic nucleus, being possible to observe a nucleolus. Cytoplasm is also scarce (Figure 3c-d).

Initial Spermatid (IS)—Total mean cell diameter of 7.23 ± 0.68 µm. The nucleus is small, appearing highly heterochromatic and condensed, with a mean diameter of 4.51 ± 0.44 µm. The cytoplasm is slightly basophilic and corresponds to about half of the cell volume (Fig. 2d and 3b).

Spermatid (FS)—Mean nuclear diameter of approximately 4.72 ± 0.44 µm. It has elliptical form, with flattened nucleus polarized at an extremity opposite to the cytoplasm (Figure 3e).

Sperm (SPZ)—Mean nuclear diameter of 2.8 ± 0.4 µm. The nucleus is extremely condensed and presents one flattened, eosinophil acrosome (Figure 3d-f).

In the IM phase, the gonad predominantly displays spermatogonia and spermatocytes, with spermatids and sperms being rarely observed in the lobes (Figure 2d). In the DE phase, all cell types of the spermatogenic lineage can be found frequently (Figure 3). Furthermore, oocytes in the posterior region can be found in both phases (Figure 2a and 3a).

The gametes already at final maturation stage in the gonadal lobe are found in the lumen of the seminiferous tubule, which is composed of squamous epithelium. In the anterior portion of the gonad, a short pair of vas deferens can be observed connecting the terminal region of the testicles to the sexual aperture, the gonopore, which is located on the base at the fifth pereiopod pair (Figure 4a).

The vas deferens has a translucent or whitish appearance, depending on the amount of spermatic material containing (Figure 4a). Although there are no significant variations in its structure at macroscopic level, three regions were identified in its histological study: proximal (PVD), middle (MVD) and distal (DVD). The vas deferens is formed by a secreting epithelium responsible for forming the spermatophoric mass (Figure 4b-f). The proximal region presents epithelium varying from pseudostratified to simple columnar. In this epithelium, cells presenting oval nucleus and vacuolated cytoplasm can be seen, especially in the apical portion (Figure 4b-d). Similarly to the distal portion, the middle region has predominantly squamous epithelium, but cubic cells may exist at some sites (Figure 4e-f). A muscle layer can be seen along the whole vas deferens (Figure 4b-f).

All along the vas deferens, the sperm are seen in the lumen and embedded in a matrix that composes the spermatophore.

The matrix appears strongly reactive to the PAS staining and moderately to bromophenol blue. Moreover, small secretion granules can be noticed in the lumen of the vas deferens (Figure 4b-d).

4 | DISCUSSION

Several studies have been accomplished on taxonomic and ecologic aspects of the population and reproductive biology of Axiidea species, including *C. major* (Botter-Carvalho et al. 2002, 2007; Hernaez et al. 2012; Sepahvand et al. 2012; Peiró et al. 2014;). However, thus far there have been no morphohistological descriptions of the reproductive organs of these animals, in particular for the male individuals.

The present results demonstrate that the reproductive system of *C. major* is composed of a pair of gonads and vas deferens. This system follows characteristics previously described for Decapoda, as seen in the lobster species *Panulirus laevicauda* (Lima and Gesteira 2008) and *Homarus gammarus* (Erkan and Ayun 2013), among others, and differently from the crayfish *Astacus leptodactylus*, which has trilobed testicles (Erkan et al. 2009). The localization of the reproductive system presented here is similar to that of the anomuran *Diogenes pugilator* (Manjón-Cabeza and Raso 2000). Nevertheless, it differs from most decapods, as the male gonad of *C. major* is found in the abdominal cavity, dorsally to the hepatopancreas, whereas in other species it is seen in the cephalothorax region.

During gonad maturation, no major differences in macroscopic morphology were found between IM and DE individuals, except for a clearly observable posterior ovarian region in the latter. This region, named as "part of the ovary in the testicles," is also found in *Upogebia major*, a species phylogenetically close to *C. major* (Kang et al. 2007). Nevertheless, there are no reports in the literature regarding the function of this structure in the male gonad or the possibility of sex change in this species. Further studies including morphological and morphometric analyses of the secondary sexual characters as well as mating experiments between DE males should be carried out to investigate a possible intersexuality or hermaphroditism in these species.

The division into development stages based on the macroscopic morphology of the male gonad in Decapoda is largely variable. Studying reproductive developmental aspects in the crab *C. ornatus*, Nascimento and Zara (2013) identified three stages for this species—juvenile, developing and mature adult—with a gradual increase in the weight of the testis. Lima and Gesteira (2008) also report variation in the colour of the testicles during the gonadal maturation process of *P. laevicauda*, ranging from transparent to yellowish. Colour changes were also detected in *C. major*, but arising from the accumulation of yolk in the oocyte region present in the male gonad of sexually developed individuals.

Varied terminology is employed to name the structure that clusters the spermatogenic lineage, which can be classified, for instance, as acini in the crayfish *Parastacus varicosus* (Silva-Castiglioni et al. 2008) and as seminiferous tubules in the brachyuran *M. brachydactyla* (Simeó et al. 2009). The histological analysis allowed us to verify that the gonad of this species is formed by lobes at macroscopic level, but show seminiferous tubules, which contain the male germ cells at different differentiation levels.

In DE individuals, the gonad additionally shows oocytes. In the testicular lobes, interspersed with the germ lineage, accessory (Sertoli-like) cells can be found, which act as helpers during the spermatogenesis process. Studies with these cells demonstrate that they have cytoplasmic extensions that keep contact, through desmosomes and gap junctions, with the spermatogonia and spermatocytes (Hinsch 1993a). In the areas of contact between these cells and maturing spermatids, phagocytic vesicles can be visualized during spermiogenesis, consisting of cytoplasmic remnants absorbed during the formation of mature sperm (Hinsch 1993b), and therefore considered to play an important role during gamete formation.

In the isopod *Saduria entomon*, Sertoli-like cells are classified in two types: type A, which occupies the part of the tubule that contains spermatogonia and spermatocytes, becoming associated with these cell types; and type B, abundantly present and emitting long cytoplasmic projections, making contact with the differentiating spermatids (Gabała 2006). The presence of Sertoli cells, with different nuclear morphologies and distribution in the gonad's lobe, allows this classification to be applied to *C. major* as well, ascribing them distinct functions.

The spermatogonia are located in the periphery, constituting the so-called proliferation zone, where the initial cells of the germ lineage are found. This observation is also made for the brachyurans *Libinia spinosa* (Gavio et al. 2010) and *M. brachydactyla* (Simeó et al. 2009). Differently, for the lobster *N. norvegicus* this region is not seen composed exclusively of one cell type; also spermatocytes I are present (Rottlant et al., 2012), having a constitution similar to that found in *C. major*. In this species, the other cells are found in differentiation phase in the lobe, as in *L. spinosa* (Gavio et al., 2010). Simeó et al. (2009) denominate the region where the spermatocytes, spermatids and sperms are located as transformation region.

Spermatocytes I are typically larger than the spermatogonia and present heterochromatic blocks in the nucleus. In *N. norvegicus*, these cells exhibit a central nucleus containing two to three nucleoli, with morphology similar to that of the spermatocytes II found in *C. major* (Rottlant et al., 2012). On the other hand, Nunes et al. (2010) characterize

only one type of spermatocyte for the caridean *E. oplophoroides*, consisting of one cell with large nucleus and disperse, decondensed chromatin, differing from the results found in the present study.

During spermiogenesis, the process through which the spermatid produces the sperm, several morphostructural and physiological modifications occur. In the crab *Eriocheir sinensis*, for instance, the enzyme acid phosphatase, which is found spread across various cell sites, becomes concentrated in the acrosomal tubule (Guirong et al. 2007), where it will be important during oocyte fertilization. Here, modifications were verified in this phase which are similar to those described in the literature for other species, especially in the morphology, with polarization of the nucleus. This has been reported for the anomuran *D. pugilator* (Manjón-Cabeza and Raso 2000), the lobster *N. norvegicus* (Rottlant et al., 2012) and the caridean *E. oplophoroides* (Nunes et al., 2010). In the shrimp *M. rosenbergii*, three marked phases are reported during spermiogenesis: initial, middle and late spermatid (Poljaroen et al., 2010). However, for *C. major* only two marked stages could be distinguished: initial and final.

At light microscopy level, it could be verified that the sperm of *C. major* possesses a condensed and spherical nucleus, and the acrosome, an acidophilic structure, was seen covering a small nuclear portion. Manjón-Cabeza and Raso (2000) describe the sperm of *D. pugilator* as having ovoid morphology, with polarization of the nucleus and cytoplasm, the latter being highly elongated and prominent, which differs from the findings in *C. major*. The sperm morphology can be very diverse among the groups. In *E. oplophoroides* and *M. rosenbergii*, for instance, this cell possesses discoid nucleus and presents a single spike (Nunes et al., 2010; Poljaroen et al., 2010). In turn, in the decapods *Astacus astacus*, *Callinectes sapidus* and *Panulirus argus* the gametes are considered multistellate, showing several spikes in their structure (Krol et al. 1992; Holdich 2002). According to these structural variations, the male gametes have been widely employed as phylogenetic tools (Tudge 2009).

The formed sperm proceed to the vas deferens, where formation of the spermatophore occurs, an important structure for fertilization of the female (López-Greco et al. 2007; Zara et al. 2012). In *C. major*, the proximal region of this organ (PVD) was seen as a typically secreting epithelium, presenting apical vacuoles, which indicates that it is active in the secretion of the spermatophoric matrix, similarly to the structure observed in *M. brachydactyla* (Simeó et al. 2009). In the ultrastructural study of the cells composing this region, Simeó et al. (2009) observed that the cytoplasm is filled with rough endoplasmic reticulum and Golgi complex units, and that various electron-dense vesicles are released into the lumen through the apical region. Among the wall cells of the vas deferens, some basal cubic ones with round nucleus were also seen in *C. major*, suggesting that they may be responsible for cell reposition in this epithelium. Pillai (2007) suggests that this initial portion has a nutritional function during sperm maturation. Moreover, in decapods it is suggested that the PVD may present absorptive function (Adiyodi and Anilkumar 1988).

The variation in the height of the epithelium from PVD to middle (MVD) and distal (DVD) regions in the genital duct of *C. major* is apparently related to the decrease in secretory activity, as pointed out by Simeó et al. (2009). Furthermore, when comparing these regions, a reduction in the lumen of the vas deferens in the distal portion can be noted, being related to an increase in the thickness of the muscle layer. The thick muscle layer in the distal region is also observed in the crayfish *C. quadricarinatus* (López-Greco et al., 2007). In *C. major*, the muscle layer may help in the transportation of gametes to the outside, that is, shows an ejaculatory function, being responsible for expelling the sperm mass during mating, as in the lobster *P. homarus* (Pillai 2007).

No differences were observed regarding the histochemical characteristics of the vas deferens regions, all reacting moderately for bromophenol blue and PAS. However, Zara et al. (2012) reported that there are acid–base differences in the secretions that compose the spermatophoric matrix of *C. danae*, being classified as secretions of type I and II, both arising from the proximal region of the vas deferens. According to these authors, the type I secretion is reactive only to acid polysaccharides, not being observed in any of the other regions. Differently, the type II secretion appeared strongly positive for neutral polysaccharides and proteins. Small basophilic granules reactive to PAS and bromophenol blue were noted throughout the vas deferens, especially in the proximal region, consisting of substances released by the epithelial cells to compose the spermatophore. These small structures are also observed in *C. danae*, demonstrating that the matrix, composed mainly of neutral polysaccharide and protein elements, is a mixture of granular and fluid components (Zara et al., 2012).

In males of *C. danae*, the nutrients are mobilized from the hepatopancreas during gonadal development, having direct relation to the vas deferens (Zara et al., 2012). Moreover, there is evidence that the elements needed in spermatophore formation also originate from this organ in *C. major*, as this is its main function in the decapods. Ultrastructural aspects of *M. brachydactyla* reveal that the basal region of vas deferens epithelial cells presents many interdigitations and mitochondria, being related to a high rate of ionic exchange (Simeó et al., 2009). This way, it is possible that the components used to constitute the spermatophore matrix are derived from the haemolymph. Spermatophore formation does not follow the same characteristics in all decapods. The structure can be spherical, as reported in Brachyura for *C. danae* (Zara et al., 2012) and *Goniopsis cruentata* (Garcia and Silva 2006), or

may present more complex shapes, such as the pedunculate spermatophore of the hermit crab *Calcinus tibicen* (Amadio and Mantelatto 2009). However, in *C. major* this structure is composed of a gelatinous mass that does not present such elaborate morphotypes in the lobster *P. homarus*, for instance (Pillai 2007). This variation in spermatophore formation occurs depending on the sperm transfer and mating method employed by each species (Subramoniam 1984).

The presence of spermatogonia at different phases, as well as spermatids and sperm simultaneously, indicates that the process of sperm production by the gonads is continuous, at least in this population. This confirms the information found in the literature that points to a continuous reproduction of this species (Botter-Carvalho et al. 2007). The presence of cells at advanced maturation stage also in IM individuals, even though at low frequency, in addition to the observation of asynchronous spermatogenesis helps understand the reproductive biology of *C. major*. This continuous formation of gametes has great importance for the maintenance of reproduction throughout the year and population stability of the species.

However, there are no reports in the literature that females of *C. major* possess some specialized structure for the storage of sperm, contrary to crabs (Mc Lay and López-Greco 2011). The absence of this organ has implications for the reproduction. This way, a female can be fertilized by more than one male, as observed in *Callichirus islagrande*, characterizing a species whose offspring may present multiple paternity (Bilodeau et al. 2005). Mating with more than one male becomes an important strategy for the population, as the descendants will present greater genetic diversity, contributing for the maintenance of the species.

The present study analysed the gonadal structure and classified the cell types found during spermatogenesis in the ghost shrimp *C. major*, advancing the understanding on the reproduction of the species. Furthermore, the presence of mature oocytes in the posterior region of the gonad of DE individuals indicates that this species may present intersex or hermaphrodite individuals.

Cases of sex change occurs in carideans, for example, *Lysmata wurdemanni* (Bauer, 2002) and *Exhippolysmata oplophoroides* (Nunes et al. 2010), and these species were classified as simultaneous protandric hermaphrodite. In this sexual system, juveniles first mature into functional males and then turn into simultaneous hermaphrodites with ovotestis (Baeza et al. 2007; Baeza, et al. 2010).

Silva-Castiglioni et al. (2008) analysed the sexual pattern of the *Parastacus varicosus* and found intersex individuals with oocytes in the gonad, which suggests a case of hermaphroditism. However, due to the few data on the reproductive biology of *C. major,* further studies being required to investigate the intersexuality or possible sex change in this specie.

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