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# Morphology of the Male Gonads of the Spiny Lobster *Panulirus laevicauda* (Latreille, 1817)

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## ABSTRACT

*The present study represented a contribution to the knowledge of the cytological and histological aspects of decapods' reproductive system, describing male germ cells of the spiny lobster* Panulirus laevicauda*. Seventy-one specimens of different sizes were caught off Fortaleza (Ceará, Brazil). Their testes were removed and fixed in Bouin solution, then, after 24 hours, dehydrated, cleared and embedded in the paraffin. Sections (4 µm thick) were stained with hematoxylin-eosin. The testes appeared macroscopically as a pair of long and highly convoluted tubes joined by a transversal commissure giving the organ an H-like shape. Microscopically, supporting cells and germ cells (spermatogonia I and II, spermatocytes, spermatids and spermatozoa) were seen in the testicular acini. Some of the acini showed signs of the spermotocytes and the spermatogonia degeneration. The spermatozoa were small cells with the peripheral nuclei and a lightly basophilic cytoplasm. They were nonmotile gametes and are characterized by the absence of a flagellum, but they had spikes radiating from the body. Three stages of follicular development in the mature individuals were observed: (a) predominance of spermatogonia I and II; (b) increasing numbers of spermatocytes I and II; and (c) spermatocytes I and II were prevalent. All the three stages of the spermatozoa were observed in the follicular lumen. These observations agreed with the published descriptions of other palinurid and homarid lobsters. A histochemical analysis of the testes showed that the main component of the tunic was collagenous fibers, that the seminal fluid contained plenty of glycoproteins and carboxyl-glycoconjugates and that the spherical bodies and spermatozoa contained glycoproteins and mucoproteins.*

**Key words*:*** Histology, male reproductive system, spiny lobster, *Panulirus laevicauda*

## INTRODUCTION

The crustaceans are abundant around the world and have been studied extensively. This includes the descriptions of the male reproductive system required to subsidize the stock management efforts. Particularly, the species of the Malacostraca have paired gonads concentrated in the cephalothorax. The testes typically line the anterior digestive tract and may reach as far as the abdomen (MacLaughlin, 1983). The histological studies of the lobster testes have revealed long, highly convoluted tubes, connected medially by a transversal commissure, resembling an 'H' (Mathews, 1951; Fielder, 1964; Mota-Alves and Tomé, 1966; Paterson, 1969). To our knowledge, no histochemical studies of lobster testes have been published so far. The objective of the present work was to contribute to the basic knowledge of the morphology, histology and cytology of the

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Decapods' reproductive system by providing a description of the male gonads of the spiny lobster, *Panulirus laevicauda*, including a histological examination of their structural components.

## MATERIAL AND METHODS

Seventy-one lobsters of varying sizes were collected off Fortaleza (Northeastern Brazil, state of Ceará). The reproductive system was dissected by mid-dorsal incision through the cephalothorax. The fragments representing different regions of the testes were excised and immersed in 4% formaldehyde, diluted in the sea water and neutralized with borax (Baker, 1944). The samples were dehydrated in gradual ethanol series, cleared in xylene and embedded in the paraffin. Sections (5µm thick) were stained with hematoxylin-eosin in order to identify the structures. The histochemical examinations were performed with Millon's reaction for the proteins, PAS for glycoproteins, alcian blue at pH 2.5 for glycoconjugates, and Azan-Heidenhain-Mallory for fibril composition, following the methodologies described by Chayen et al. (1973), Junqueira and Junqueira (1983), Pearse (1985) and Behmer et al.(2003). The sections were analyzed and photographed in a Zeiss light microscope.

## RESULTS

The male reproductive system of *P. laevicauda* is was "H" shaped structure placed in the mid-dorsal region of the cephalothorax. The paired testes originated anteriorly in the vicinity of the eye stalks, and ran along the digestive tract with the midpart lying below the heart. Posteriorly, they followed the muscles, lined the gut and finally emerged through an opening in the first, rarely the second, abdominal segment. A transversal commissure connected the testes medially (Fig. 1).

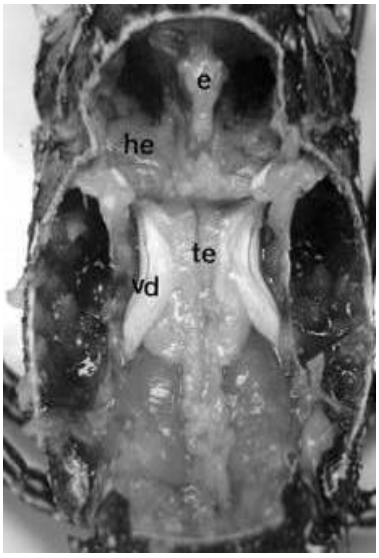


**Figure 1** - Dorsal view of cephalothorax showing the localization of reproductive tract: stomach (e), hepatopancreas (he), testes (te) and vas deferens (vd).

The gonad, which was transparent in the immature specimens, became yellowish in the mature ones. There were three stages of the follicular development in the mature individuals: (a) the predominance of spermatogonia I and II; (b) increasing numbers of spermatocytes I and II; and (c) spermatocytes I and II were prevalent. The testes had highly convoluted seminiferous tubules and collecting ducts throughout their extension. The anterior and posterior ducts communicated by a single, twisted duct in the mid-testicular region. A fine fibrous mantle enveloped the entire tubular structure; the interstitial space between the mantle and the tubules was filled by the connective tissue composed of several elements: an intercellular substance containing diffuse fibers, fibroblasts, scattered hemocytes and vessels with hemolymph (Fig. 2).

The seminiferous tubules were covered by a single fibrous layer containing the fibrocytes with the small, fusiform nuclei with strong basophilia, measuring 1-2 μm. The tubular lumen was filled with the seminal fluid, a basophilic substance of unknown origin. The internal wall between the fibrous layer and lumen consisted of the germ epithelium and supporting cells. The latter had rounded and strongly basophilic nuclei measuring 4-6 μm. Following the development of the germ cells, the supporting cells became more evident as they formed a sheath around the follicles, from their base to the tubular lumen (Fig. 3).

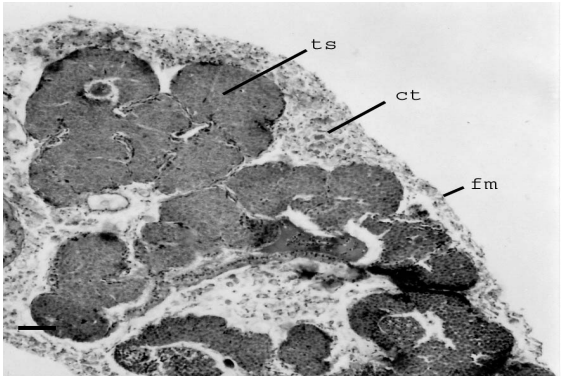


**Figure 2 -** Photomicrograph showing fibrous mantle (fm), seminiferous tubules (ts) and connective tissue (ct) (Scale bar: 2μm).

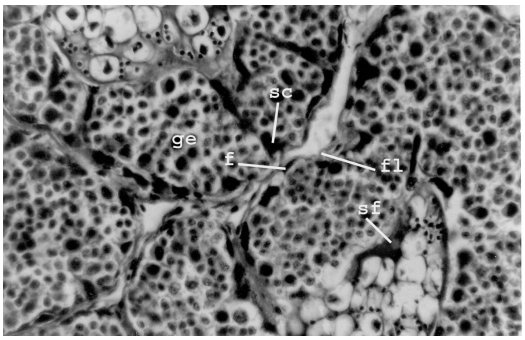


**Figure 3** - Photomicrograph showing fibrous layer (fl), fibrocytes (f), seminal fluid (sf), germinal epithelium (ge) and sustentacular cells (sc) (Scale bar: 0,5μm).

The germ cells were usually classified into four categories: spermatogonias (I and II), spermatocytes (I and II), spermatides and spermatozoa. In the first stage of the germinal development, the spermatogonias appeared near the follicular fibrous layer. Type I spermatogonias had large, round nuclei measuring 8-10 μm and displaying small knots of condensed chromatin scattered all over the nucleus. Type II spermatogonias were observed to have three centrally located nucleoli. Cytoplasm was scarce and difficult to observe in both types of cells (Fig. 4).

Type I spermatocytes are cells with ovoid, evenly stained nuclei measuring 6-8 μm, the chromatin of which is rather condensed. The cytoplasm is slightly basophilic. Spermatocytes I and II may only be distinguished by a difference in size. The nuclei of type II spermatocytes measure approximately 4 μm (4-5 μm). Some type I spermatocytes may not mature into type II and eventually degenerate presenting signs of nuclear and cytoplasmatic regression (Fig. 5).

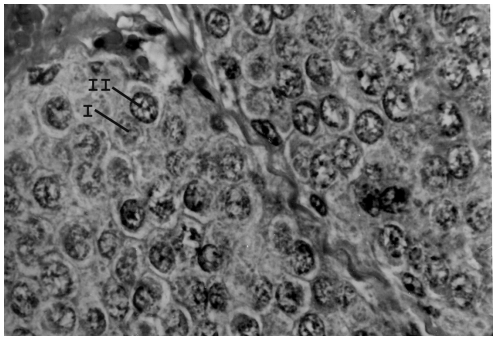


**Figure 4** - Photomicrograph showing type I spermatogonias (I) and type II spermatogonias (II) (Scale bar: 0,5µm).

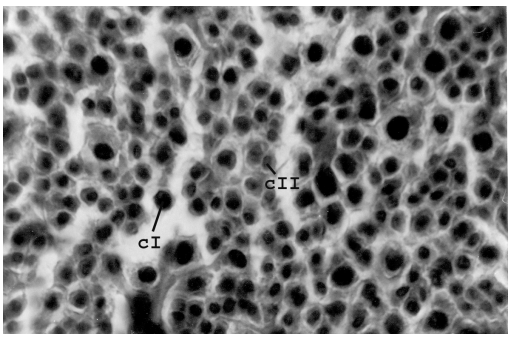


**Figure 5** - Photomicrograph showing type I spermatocytes (cI) and type II spermatocytes (cII) (Scale bar: 0,5µm).

The spermatides were small cells with the peripheral and evenly stained nuclei, measuring 3-4 µm. The entire nucleus was dislodged to one side of the cell making the cytoplasm slightly basophilic and rather evident (Fig. 6).The spermiogenesis and degenerating type I spermatocytes were observed simultaneously in the germ epithelium, suggesting that the latter were precursors of the structures termed spherical bodies that were seen enveloping the spermatozoa in the tubular lumen. Newly formed spherical bodies consisted of a membrane surrounding a central or peripheral substance half of which was acidophilic (possibly corresponding to the atresic cytoplasm) and half of which was basophilic, corresponding to the degenerated nucleus. An unstained region was observed surrounding the internal substance (Fig. 6).

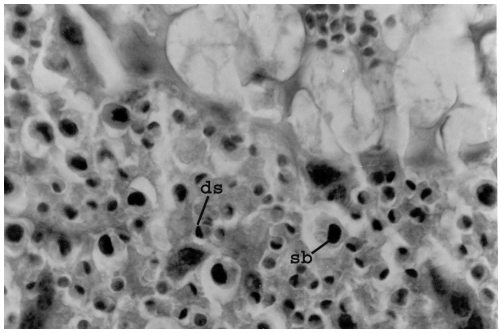


**Figure 6** - Photomicrograph showing spermatids (ds) and spherical bodies (sb) (Scale bar: 0,5µm).

By the end of the spermiogenesis and spherical body formation, the spermatozoa were found in the tubular lumen. Once in the tubular lumen, the internal substance of the spherical bodies acquired a flaky and diffuse appearance. The spermatozoa resembled spermatides, but were somewhat smaller. The nucleus measured 2-3 μm and was strongly basophilic and rather large compared to the cytoplasm, which appeared like a barely discernible peripheral ring (Fig. 7).

The interfollicular collecting ducts were composed of an external fibrous layer and a single internal epithelial layer of the cuboidal cells. These cells had strongly basophilic, spherical and centralized nuclei measuring 4-6 μm without evident nucleoli. The fibrous layer resembled that described for the follicles. The ducts contained the seminal fluid, scattered spermatozoa and spherical bodies from the tubular lumen of fully mature follicles (Fig. 8).

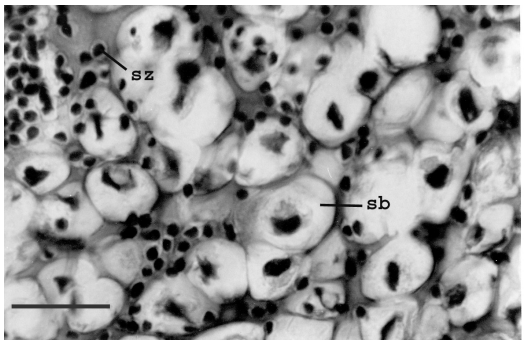


**Figure 7** - Photomicrograph of tubular lumen showing spermatozoa (sz) and spherical bodies (sb) (Scale bar: 0,5μm).

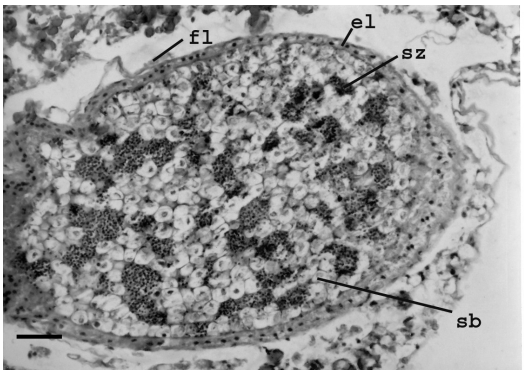


**Figure 8 -** Photomicrograph of interfollicular collecting duct showing fibrous layer (fl), epithelial layer (el), spermatozoa (sz) and spherical bodies (sb) (Scale bar: 1μm).

Near the middle of the testicle the ducts opened into a large duct, called the convoluted collecting duct, covered by the thick layer of fibers and fibrocytes and protected by a single epithelial layer of the tall cells with the basal nuclei. The nucleus measured 4-6 μm with uncondensed chromatin and a central nucleolus. The convoluted duct connected with the first region of the proximal vas deferens and was filled with the free spermatozoa and spherical bodies as well (Fig. 9).

The histochemical tests indicated that the follicular mantle was mostly collagenous. The seminal fluid was rich in glycoproteins and acid glycosaminoglycans. The spermatozoa and spherical bodies contained mostly the glycoproteins, but tested moderately positive for the mucoproteins and weakly positive for the glycoconjugates (Table I).

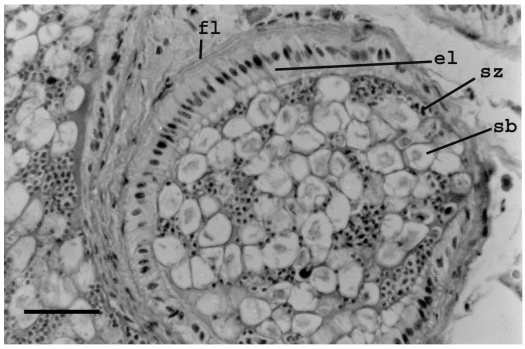


**Figure 9** - Photomicrograph of convoluted duct showing fibrous layer (fl), epithelial layer (el), spermatozoa (sz) and spherical bodies (sb) (Scale bar: 1µm).

**Table 1 -** Histochemical characterization of some of the components of the testicles of the spiny lobster (*Panulirus laevicauda*).

| Components | Pas | Alcian Blue | Millon | Azan Mallory |
|---|---|---|---|---|
| Follicular mantle | ++ | ++ | - | +++ |
| Seminal fluid | +++ | +++ | - | - |
| Spherical bodies | +++ | + | - | - |
| Spermatozoa | +++ | + | - | - |

+++= strongly positive; ++= moderately positive; += weakly positive; - = negative.

## DISCUSSION

The description of the male gonads of *Panulirus laevicauda* as being H-shaped corroborated the findings of Mathews (1951), Fielder (1964), Paterson (1969), Radha and Subramonian (1985), Nakamura (1990), for *P. penicillatus*, *Jasus lalandii*, *J. novaehollandiae*, *P. homarus* and *P. japonicus*, respectively and Garcia and Silva (2006) for the crab *Goniopsis cruentata.* According to Mathews (1951), the length of the testicles was variable. The histological findings of the present study confirmed the observations made by the authors above. Mota-Alves and Tomé (1966) described the testes of *P. laevicauda* as being externally covered by a layer of the epithelial tissue, overlying a layer of connective tissue, but provided no further details. The positive PAS reaction and the results of staining with Azan/Mallory confirmed the presence of a fine fibrous mantle probably made of collagenous fibers and glycoproteins. The connective tissue found underlying the mantle was found in almost all the subclasses of Decapoda and has been reported by a number of authors, some of whom observed the vessels, hemocytes, fibers and a hemolymph-like in this tissue (Mathews, 1954; Silberbauer, 1971; Jonhson, 1980), supporting the results of the present study. Sagristá and Durfort (1990) observed loose connective tissue with the collagenous fibers within certain organs and suggested that it might serve as a barrier to the pathogens while aiding in the exchange of the fluid (metabolites and nutrients) between the organ structures and hemolymph. The seminiferous tubules inside the testes of *P. laevicauda* were surrounded by the loose connective tissue and covered by the follicular mantle, a fine layer of the collagenous fibers. The composition of these fibers was confirmed by staining with the Azan/Mallory. This layer was not mentioned by Mota-Alves and Tomé (1966), but was included in the descriptions of *Penaeus setiferus* (King, 1948) and *Callinectes sapidus* (Jonhson, 1980)**.** The layer might also appear as an agglomerate of the collagenous fibers of the connective tissue surrounding the seminiferous tubules. The fusiform-nucleated cells observed were most likely the fibrocytes

responsible for the production of collagenous fibers. In the present study, the walls of the tubules were found to be lined with the germinal epithelium composed of the cells at different stages of the maturity: spermatogonias I and II, spermatocytes I and II, spermatides and spermatozoa. The classification of the spermatogenic cells into types I and II was based on the presence and absence, respectively, of nucleoli. Type I represented a stage of intense mytotic activity, while type II corresponded to the period of the transition to the following stage characterized by strong protein synthesis activity. A similar description of the spermatogonias could be found in Mota and Tomé (1965) for *P. argus*, Silberbauer (1971) for *Jasus lalandii* and Haley (1984) for the Hawaiian reef lobster, *Enoplometopus occidentalis*. The spermatocytes I originated from the spermatgonias II, when migrated towards the lumen, began to display a distinct cell morphology as a result of the first meiotic division and turned into the relatively smaller cells. The findings agreed with those for *P. argus*, *P. laevicauda*, *Portunus sanguinolentus*, *J. lalandii*, *E. occidentalis*, and *P. laevicauda* (Mota and Tomé, 1965; 1966; Ryan, 1967; Silberbauer, 1971; Haley, 1984; Silva, 1993)**.** According to Mathews (1951), the first heterotypic division of the spermatocytes of *P. penicillatus* resulted in type II spermatocytes and a number of degenerated cells unable to reach the next stage. The desintegration products of these cells might serve as the nutrients during the spermiogenesis. Fielder (1964) also observed this degeneration but, like Mathews, made no references to the structures resembling the spherical bodies observed in this study. In a study on the species *P. interruptus*, Martin et al. (1987) observed the cavities that resembled the spermatozoa with regard to the intercellular components, but no explanation was given for the origin of these structures. In *P. laevicauda*, the spherical bodies released into the lumen together with the spermatozoa might stem from the degenerate spermatocytes, while the substance observed inside these bodies presumably served as the nutrients for the spermatozoa.The spermatides are the result of the second meiotic division of the type II spermatocytes. Mota-Alves and Tomé (1966) sugeted the presence of theses cells in *P. laevicauda***,** but Jonhson (1980) found them in the lumen of the seminiferous tubule and described them as having the nucleus on one side of the cell and the cytoplasm on the other, as confirmed by the present study. Part of the cytoplasm was probably lost during the spermiogenesis. Decapods' spermatozoa are considered atypical because they are nonmotile gametes and without flagellum. They usually have different number of spikes. Throughout the study, the spermatozoa were always found inside the lumen, with clearly visible nuclei, although the spikes could not be seen due to the juxtaposition of spermatozoa and spherical bodies. The spermatozoa were PAS positive like those of *P. guttatus* and *P. argus* (Talbot and Summers, 1978) and *Homarus americanus* (Talbot and Chanmanon, 1980; Kooda-Cisco and Talbot, 1982 and Gesteira, 1989). As explained by these authors, it was the acrosome region inside the cell nucleus that reacted with PAS, thereby proving to be rich in the glycoprotein. The supporting cells observed in *P. laevicauda* became more evident as the spermatogenesis proceeded, suggesting an increase in size as a result of the accumulated nutrients. Mota and Tomé (1965; 1966) did not find these cells in *P. argus* and *P. laevicauda*. In a study involving the latter species, Silva (1993) only referred to the existence of such structures. However, the cuboidal or cylindrical cell epithelium lining the collecting ducts in the present study was similar to that described by Haley (1984) for *E. occidentalis*. The present observation that the follicles were formed by the epithelium itself on one side and by the duct epithelium on the other suggested the existence of the regions releasing the spermatozoa from the follicles directly into the collecting ducts. In Mathews (1954), *Parribacus antarcticus* was described as having stratified or multinucleated duct epithelium. Mota-Alves and Tomé (1966) did not describe the presence of the collecting duct epithelium in *P. laevicauda.* The seminal fluid found in the tubular lumen of *P. laevicauda* was composed of the glycoproteins and acid mucopolysaccharides. According to Ryan (1967), this fluid supplied the spermatozoa inside the spermatophores with the nutrition. In a study on *Artemia*, Wolfe (1971) suggested the seminal fluid observed inside the tubules might stem from the sustentacular cells. According to Adiyodi (1985), the fluid consisted of glycogen, glycoproteins, high molecular weight proteins (tirosine in small concentrations) and small amounts of the lipids. The results of this work showed that *P. laevicauda* male gonads presented the same pattern of other lobster species already described.

## RESUMO

O presente trabalho tem como objetivo ampliar os conhecimentos na área de histologia e citologia do sistema reprodutivo dos Decapoda, descrevendo as células germinativas do macho da lagosta *Panulirus laevicauda*. Animais com tamanhos variados foram coletados em diferentes épocas do ano no litoral de Fortaleza, Estado do Ceará - Brasil, totalizando 71 machos. Os testículos foram retirados e fixados em bouin e processados. Cortes de 5µm foram corados em hematoxilina - eosina. Seguindo observações macroscópicas, os testículos são longos tubos pares altamente convolutos unidos por uma comissura transversal na porção mediana, apresentando uma forma de H. As análises microscópicas dos folículos testiculares mostraram células sustentaculares e germinativas (espermatogônias I e II, espermatócitos I e II, espermatócitos I em degeneração, espermátides e espermatozóides). Os espermatozóides são células pequenas com núcleo periférico e citoplasma levemente basofílico. Três estágios foliculares para indivíduos maduros foram identificados: tipo **a**, onde predominam espermatogônias I e II; tipo **b** com maior incidência de espermatócitos I e II e no tipo **c** foi observado principalmente espermátides e espermatócitos I em degeneração. Espermatozóides podem ser encontrados no lúmen dos folículos nos três estágios de desenvolvimento. Os resultados obtidos seguem o padrão encontrado na bibliografia especializada em outras espécies de lagostas palinurídeas e homarídeas. Testes histoquímicos realizados em alguns componentes dos testículos mostraram que a túnica é rica em fibras colágenas, o fluido seminal é formado por glicoproteinas e glicosaminoglicanas, os corpos esféricos e espermatozóides são compostos de glicoproteinas e mucoproteinas.

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