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Morphology and histology of the male reproductive system of the mangrove land crab *Ucides cordatus* (L.) (Crustacea, Brachyura, Ocypodidae)

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Abstract

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This study provides a detailed description of the macro- and microscopic anatomy of the male reproductive system and the spermatogenesis of the mangrove land crab, *Ucides cordatus*. A total of 64 adult males were collected between December 2002 and February 2005 in mangroves of the Baía de Antonina, State of Paraná, Southern Brazil (25°25′08″S, 48°42′33″W). The reproductive system of this species is composed of the following paired symmetrical structures: testes, vasa deferentia (distal, medial and proximal portions), ejaculatory ducts and penises. During spermatogenesis, which takes place in the testes, the following developmental stages are observed: primary and secondary spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoids. Production of male gametes was continuous throughout the study period, indicating that males of this species are physiologically capable of reproducing all year long.

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Introduction

The mangrove land crab *Ucides cordatus* (L.), an ocypodid endemic to the western Atlantic, is an important fishery resource for low-income families, who rely on it for both food and commerce (Glaser 2003). The mangrove land crab is also an important component of mangrove ecosystems, playing a fundamental role in nutrient cycling (Schories *et al.* 2003; Wolff *et al.* 2000).

Although the external morphology of the male reproductive system of *U. cordatus* was first described by Mota-Alves (1975), there are very few subsequent studies on the subject. Matos *et al.* (2000) analysed the ultrastructure of the spermatozoids, and Dalabona (2001) assessed the amount of stored spermatozoa to determine the reproductive period.

Thus, the goal of the present study was to provide a detailed description of the macro- and microscopic anatomy of the male reproductive system and the spermatogenesis of the mangrove land crab, *U. cordatus*. The results indicate that the production of male gametes was continuous throughout the study period, suggesting that males of this species are physiologically capable of reproducing all year long.

Materials and Methods

Adult male *U. cordatus* were collected monthly between December 2002 and February 2005 in the mangroves of the Baía de Antonina, State of Paraná, southern Brazil (25°25′08″S, 48°42′33″W). All specimens were transported alive to the laboratory, where they were inspected to evaluate qualitatively their health status (e.g. presence of lesions, behavioural reactions to manipulation, etc.). Only macroscopically healthy individuals were processed further. The carapace width of each individual was measured using a calliper, such that only adult individuals were studied (i.e. carapace width > 59 mm; see Pinheiro and Hattori 2006).

The reproductive system of each specimen was completely dissected under a stereoscopic microscope, described with respect to its morphology, and preserved in Davidson's fixative for 24 h. The tissues were then dehydrated in an ethanol

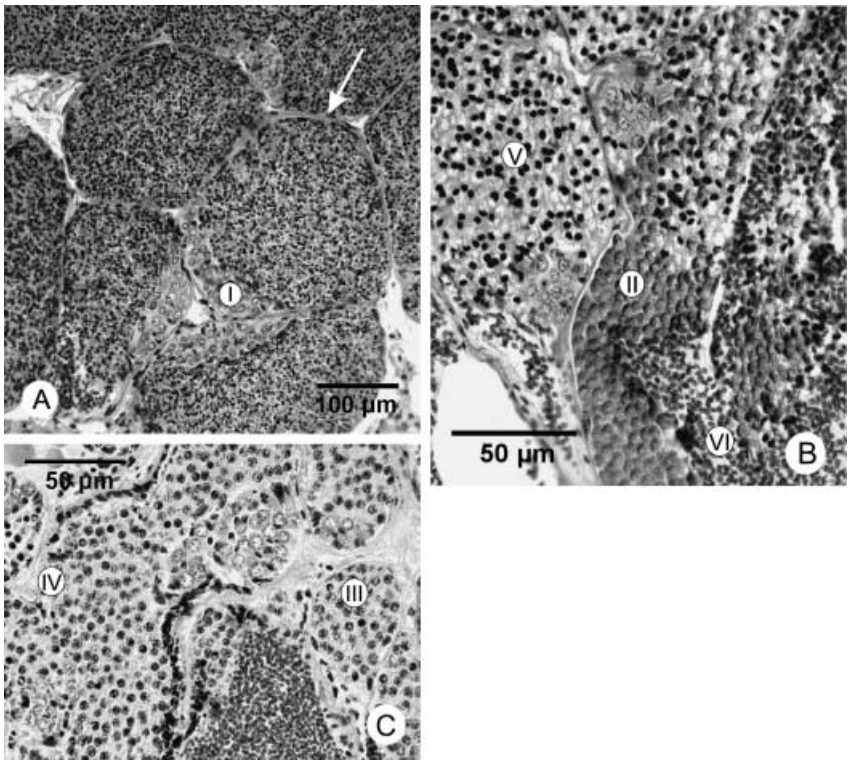

Fig. 1—Testis of *Ucides cordatus* in the process of spermatogenesis, in cross-section, stained with Harris' haematoxylin & eosin. —**A**. Testicular lobules (L) delimited by squamous epithelium indicated by the arrow. Germinative centres containing primary spermatogonia (I), magnification of 400×; —**B**. Testicular lobule containing secondary spermatogonia (II), spermatids (V) and spermatozoids (IV), magnification of 1000×; —**C**. Testicular lobules filled with primary and secondary spermatocytes (III and IV, respectively), magnification of 400×.

series, cleared in xylene, and embedded in blocks of histological paraffin wax. Sections (5 µm long) were prepared in a rotary microtome (Leica RM2125RT) and stained with haematoxylin & eosin, Mallory's trichromic stain, and the periodic acid–Schiff reaction (PAS) following the protocol of Behmer *et al.* (1976). Photographs were taken with a Leica DMLS optical microscope with an attached JVC TK-C1380 0.5 digital camera. All measurements are provided as means ± SD.

Results

Sixty-four adult males (average carapace width 66 ± 0.8.0 mm) were processed. The inner part of the male reproductive system of *U. cordatus* is a whitish, bilaterally symmetrical, 'H'-shaped structure composed of a pair of testes, vasa deferentia, ejaculatory ducts and penises. Each testis is formed by a whitish interwoven tubule located on the dorsal portion of the hepatopancreas, extending anterodorsally on the cephalothorax and continuing laterally to the stomach until connecting with the posterior portion of this organ. Testes are tubular organs, formed by microscopically visible lobules, where spermatogenesis takes place. They are surrounded by a simple squamous epithelium that encloses each testicular lobule. The spaces between the lobules are filled by haemolymph, which bathes this organ. The lobules are composed of germinative cells in one or more developmental stages, a pattern that was observed in all studied individuals.

The mature spermatozoids are transferred from the testes to the vasa deferentia through the seminiferous tubules, which are structures characterized by an irregular lumen composed of a simple squamous or cylindrical epithelium. The latter is mostly found near the portion proximal to the vasa deferentia. Seminiferous tubules can be found inserted laterally into the testicular lobules. The lateralization of the tubules was most commonly observed in the connection between the testes. Only mature spermatozoids are found within the seminiferous tubules.

Spermatogenesis consists of the differentiation of male germinative cells observed throughout the entire testis of *U. cordatus*. The histology of the testes indicated spermatogenesis was occurring in all the specimens studied throughout the study period.

The formation of spermatozoids begins at the germinative centres, which are agglomerates of primary spermatogonia usually located in the periphery of the lobules or near the seminiferous tubules. Primary spermatogonia are large cells possessing nuclei with granular chromatin (8.98 ± 1.72 µm, n = 84 cells) (Fig. 1A). Secondary spermatogonia are observed following the onset of differentiation, with cell sizes similar to those of primary spermatogonia (7.66 ± 0.83 µm, n = 130 cells), although their chromatin is more diffuse (Fig. 1B).

When secondary spermatogonia initiate the meiotic prophase, they transform into primary spermatocytes (7.23 ± 1.07 µm in diameter, n = 75 cells), which are cells with spherical nuclei and diffuse chromatin (Fig. 1C). These cells, in turn, give rise to secondary spermatocytes (5.82 ± 1.37 µm in diameter, n = 120 cells), which are characterized by nuclear reduction and intense cell division.

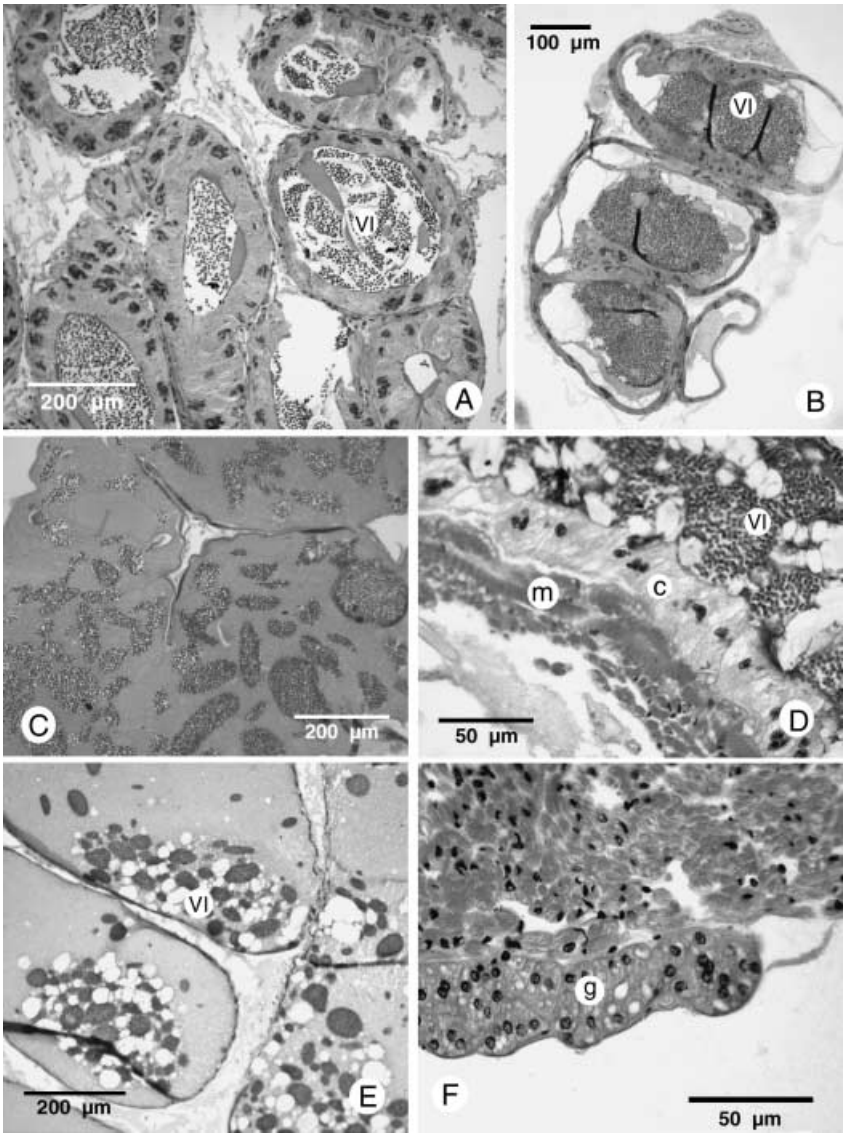

Fig. 2—Male reproductive system of *Ucides cordatus* in cross-section, magnification of 400×. —**A**. Anterior region of the PVD. Spermatozoids (VI) are present in the lumen. —**B**. Medial region of the PVD, showing an increase in the diameter of the lumen and the presence of spermatozoids (VI). —**C**. Posterior region of the PVD, indicating the formation of spermatophores (VI). —**D**. MVD showing spermatophores in its lumen (VI) and lined with columnar epithelium (c) and muscle tissue (m). —**E**. DVD with spermatophores in its lumen (VI). —**F**. Ejaculatory duct with glandular tissue in its wall (g).

The meiosis II of the secondary spermatocytes gives rise to the spermatids (5.33 ± 0.71 µm in diameter, n = 111 cells) (Fig. 1C). These cells have a small, lateralized, elliptical nucleus with great condensation of the chromatin. Following the formation of spermatids, the germinative cells differentiate into spermatozoids without cell division (3.19 ± 0.41 µm in diameter, n = 161 cells) (Fig. 1B), a process known as spermiogenesis. Spermatozoids of *U. cordatus* are composed of a protruding acrosome, a polymorphic nucleus, and a cytoplasm harbouring a variety of organelles, as reported by Matos *et al.* (2000). During the process of spermatogenesis, there is a sharp reduction in cell diameter, resulting in spermatozoids about 36% the size of a primary spermatogonium.

The vas deferens is divided into distal, medial and proximal portions, each showing distinct macro- and microanatomical features and functional roles. The proximal portion of the vas deferens (PVD) originates in the testes and is located in the mediodorsal region of the cephalothorax. The PVD has an interwoven aspect and is composed of a pair of whitish tubules of reduced width (when compared to the testes and the medial portion of the vas deferens) that are themselves divided into three regions based on the presence and diameter of the seminiferous tubules (in which spermatophores are formed). The first region (closer to the testis) is formed by the seminiferous tubules, covered by a layer of muscle fibres with longitudinal and transversal orientation. A columnar epithelium is found underneath this muscle layer, with irregularly shaped nuclei that are positioned medially or basally. Mature spermatozoids without spermatophores are present in the lumen of the tubules (Fig. 2A). The second region (in the medial portion) is characterized by an increase in the diameter of the tubules and an associated thinning of its walls.

Spermatozoids without spermatophores are also present in its lumen (Fig. 2B). The third region of the PVD is composed of a tube of distended epithelium, with an increase in eosinopholic secretion in its lumen and the formation of spermatophores (Fig. 2C).

The medial portion of the vas deferens (MVD) is composed of a pair of milky-white meandering tubules of a higher calibre than both the testes and the PVD. The MVD originates in the posterior region of the pair of PVD, being located at the mediodorsal region of the cephalothorax. It is surrounded by two layers of muscle fibres, an outer transverse layer and a longitudinal inner layer (Fig. 2D). The lumen of the MVD is lined with a cylindrical epithelium of predominantly basal nuclei (although medial and apical nuclei are also observed) and harbours numerous ovoid spermatophores (average size of 54.20 ± 17.84 μm by 30.04 ± 11.77 μm) immersed in a PAS-reactive substance.

The distal portion of the vas deferens (DVD) originates in the final portion of the MVD and is characterized by a large number of evaginations in the duct walls. The DVD is located posteroventrally to the cephalothorax. It has a whitish coloration and is surrounded by two layers of muscle tissue. The cylindrical epithelium with irregular nuclei of the DVD has evaginations filled with a secretion positive to the PAS reaction, indicating the presence of polysaccharides (Fig. 2E). The presence of spermatophores in its lumen is infrequent in relation to both PVD and MVD.

One ejaculatory duct originates from the end of each DVD. The ejaculatory duct is formed by thin, posteroventral tubules located underneath the thoracic musculature of the fifth pair of pereiopods. Each ejaculatory duct leads the spermatophores to the corresponding penis. It is surrounded by a thick layer of transverse muscle fibres. A pseudo-stratified epithelium is located immediately underneath the muscle layer and lines the duct. The lumen is filled with spermatophores, as well as with a substance that is positive to the PAS reaction. Glandular tissue is found discontinuously around the muscle tissue of the ejaculatory duct, with spherical nuclei and basophylic, abundant, vacuolized cytoplasm (Fig. 2F).

The penises are conically shaped structures located at the base of the fifth pair of pereiopods, related to the sternite of the eighth thoracic segment. Histologically, the penis is formed by a thick layer of tissue represented by a cuboid epithelium coated with a chitin cuticle. A loose conjunctive tissue is located underneath the epithelium, followed by striated muscle tissue and a squamous epithelium, with the latter lining the lumen of the penis. Regions of glandular tissue are found near the base of the penis, between the loose connective tissue and the muscle tissues.

Discussion

The present study expands on the pioneer work of Mota-Alves (1975) by providing detailed information on the spermatogenesis of *U. cordatus*, as well as thoroughly describing the anatomical and histological features of the male reproductive system of this species. Mota-Alves (1975) suggested a classification of maturation stages of the vas deferens based on the presence of spermatozoids in the seminiferous tubules and MVDs. Spermatozoids could be absent (stage I), plentiful (stage II), or few (stage III). This classification was not adopted in the present study, given that there were few empty spaces between the spermatophores and the MVDs, with a constant presence of spermatozoids. Indeed, the examination of specimens collected over more than two years reveals that the testes were in a constant process of spermatogenesis, without any apparent seasonal changes. The difference between our results and those of Mota-Alves (1975) might reflect the fact that the present study only included adult individuals.

The macroscopic morphology of the male reproductive system of *U. cordatus* observed in the present study is similar to that found in other brachyurans, consisting of an H-shaped structure with spermatogenesis taking place at its anterior portion (Bond-Buckup *et al.* 1991; Garcia and Silva 2006). The presence of paired testes and vas deferentia has also been reported in other decapod crustaceans (e.g. Krol *et al.* 1992; Almeida and Buckup 1999).

The glandular epithelium, described in the present study as being located on the wall of the ejaculatory duct of *U. cordatus*, is similar to the androgenic glands described by Charniaux-Cotton (1960) in *Carcinus maenas* and by Garcia and Silva (2006) in *Goniopsis cruentata*. Although the function of the glandular tissue observed in *U. cordatus* was not investigated in the present study, if it indeed has a function that is similar to that described by Charniaux-Cotton (1960), this gland might be responsible for the induction of the development of primary and secondary sexual traits, as reported in the crab *Carcinus maenas* and the amphipod *Orchestia gammarellus.*

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