Arquivos de Ciências do Mar

HISTOLOGICAL AND HISTOCHEMICAL STUDY OF THE ANDROGENIC GLAND OF TWO MANGROVE CRABS, Ucides cordatus (CRUSTACEA: OCYPODIDAE) AND Goniopsis cruentata (CRUSTACEA: GRAPSIDAE), FROM THE CEARÁ RIVER ESTUARY, BRAZIL

Histologia e histoquímica da glândula androgênica dos caranguejos *Ucides cordatus* (Decapoda: Ocypodidae) e *Goniopsis cruentata* (Decapoda: Grapsidae), do estuário do Rio Ceará, Brasil

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ABSTRACT

The androgenic gland has been shown to control sexual differentiation in decapods by secreting a hormone capable of determining the primary, secondary and behavioral aspects of the male. The objective of this paper was to make a histological and histochemical description of the androgenic gland of two mangrove crab species, Ucides cordatus *and* Goniopsis cruentata*, at the Ceará River estuary, Brazil, based on a forty-specimen sample of each species. Fragments of the vas deferens were preserved in Bouin's solution for 24 hours and submitted to routine histological analyses. The androgenic gland of the two species was observed microscopically to lie along the vas deferens, beginning at the location where the latter penetrates the musculature of the endofragmal skeleton. The gland consists of a sinuous string of irregularly shaped cells overlying a thick, circular and striated muscle layer, not restricted to the subterminal region. The morphology of the androgenic gland of* U. cordatus *and* G. cruentata *is similar, matching the general descriptions published for other decapod species.*

Key words: *androgenic gland,* Ucides cordatus, Goniopsis cruentata*, sexual differentiation.*

RESUMO

A glândula androgênica tem sido mostrada como controladora da diferenciação sexual nos decápodes, determinando a diferenciação das características primárias, secundárias e comportamentais dos machos. O objetivo deste trabalho foi realizar uma descrição histológica e histoquímica da glândula androgênica de Ucides cordatus *e* Goniopsis cruentata*. Quarenta indivíduos de cada espécie foram coletados no estuário de Rio Ceará. Fragmentos do vaso deferente foram fixados em mistura de Bouin por um período de 24 horas e submetidos à rotina histológica padrão. A glândula androgênica dos caranguejos estudados foi visualizada, microscopicamente, ladeando o vaso deferente, iniciando na região onde este órgão penetra na musculatura do esqueleto endofragmal. Esta glândula apresenta-se como um cordão sinuoso de células de formato irregular apoiado numa espessa camada muscular circular estriada não estando restrita à região subterminal. De modo geral, a morfologia da glândula androgênica dos caranguejos* Ucides cordatus *e* Goniopsis cruentata *é semelhante, seguindo o padrão descrito para outras espécies de Decapoda.*

Palavras-chaves: *glândula androgênica,* Ucides cordatus, Goniopsis cruentata*, diferenciação sexual.*

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INTRODUCTION

The androgenic gland was first described by Charniaux-Cottonn (1954) for the amphipod *Orchestia gammarellus*. This gland secretes a hormone involved in the differentiation of primary, secondary and behavioral aspects of the male (King, 1964). According to Adiyodi & Adiyodi (1970), this hormone is also involved in the differentiation of testicles and sperm ducts, and in the stimulation of spermatogenesis. In malacostraceans, the gland is generally located in the subterminal part of the vas deferens (King, 1964; Charniaux-Cotton *et al*., 1966; Ginsburger-Vogel, 1983; Taketomi, 1986; Lima, 1995; Fowler & Leonard, 1999).

The androgenic gland is of epithelial origin (Fingerman, 1987) and displays a highly variable morphology. According to Adiyodi (1988), in crabs it generally takes the form of simple, sinuous or even anastomosed strings of cells.

Similar structures have been observed under light microscopy in the lined shore crab, *Pachygrapsus crassipes* (King, 1964), in the ghost crab, *Ocypode platytarsis* (Thampy & John, 1970), in the red swamp crayfish, *Procambarus clarkii* (Taketomi, 1986; Taketomi *et al*., 1996b), in the yabby, *Cherax destructor* (Fowler & Leonard, 1999) and in the spiny lobster, *Panulirus laevicauda* (Lima, 1995). Ultrastructural descriptions have also been published for *P. crassipes* (King, 1964) and *P. clarkii* (Taketomi, 1986; Taketomi *et al*., 1996a).

The present study provides a histological and histochemical description of the androgenic gland of two mangrove crab species, *Ucides cordatus* and *Goniopsis cruentata*, from the Ceará River estuary, Brazil.

MATERIAL AND METHODS

Forty specimens of *U. cordatus* and *G. cruentata* were collected from November, 2000 to June, 2001, and from October, 2002 to October, 2003, respectively, at the mangrove of the Ceará River estuary (3º45´S; 38º41'W).

Fragments of the *vas deferens* were preserved in Bouin's solution for 24 hours, then transferred to 70% alcohol and dehydrated in graded series. Samples were subsequently clarified with xylene and embedded in liquid paraffin at 60ºC. Histological sections measuring 5-7 µm were prepared with a rotating microtome and stained with hematoxylin-eosin (HE) (Junqueira & Junqueira, 1983). Glycoprotein compounds were detected histochemically with the periodic acid-Schiff (PAS) reaction method (Junqueira & Junqueira, 1983), while testing for total protein was performed with the bromophenol blue technique (Pearse, 1960) and Gomori's trichrome stain (Tolosa e*t al*., 2003).

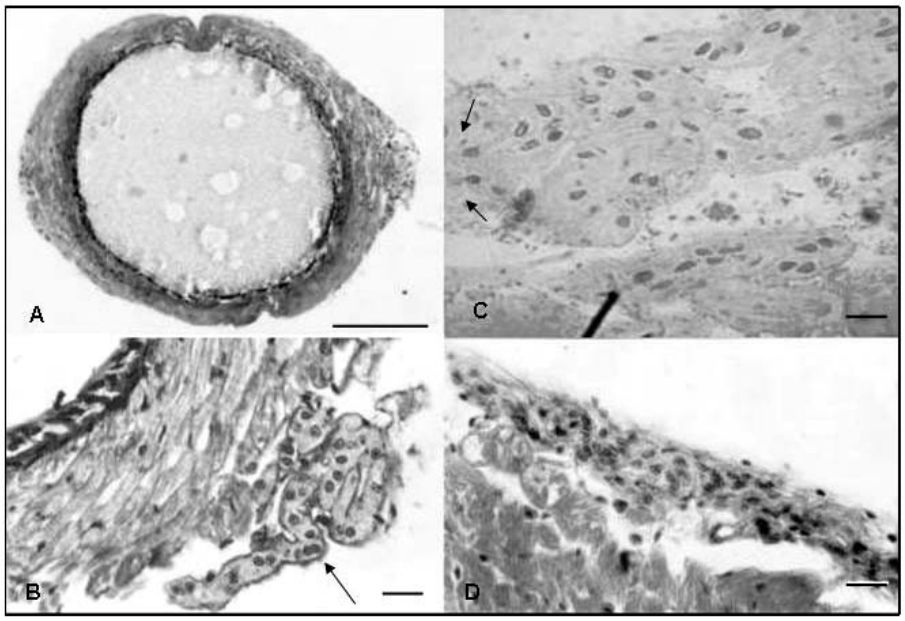

Figure 1 - Cross-section of Ucides cordatus:. A - overview of the vas deferens showing the androgenic gland (arrows) attached to the muscle layer; stain: HE (scale bar: 100 µm); B - androgenic gland structure; the arrow indicates the PAS-positive basal membrane (scale bar: 100 µm); C - longitudinal section of bromophenol blue-positive androgenic gland (scale bar: 100 µm); D - longitudinal section of androgenic gland structure; stain: HE (scale bar: 100 µm).

RESULTS

Ucides cordatus

The location and structure of the androgenic gland of *U. cordatus* are not observable by the naked eye. However, it was histologically identified as a cluster of cells attached to the thick muscle layer lining the external wall of the *vas deferens* (Figure 1A). Longitudinal sections showed the gland to cover most of the extension of the *vas deferens* (Figure 1-C).

The gland consists of groups of cells with round or ovoid nuclei surrounded by chromatin granules and containing clearly discernible basophilic nucleoli (Figure 1-B). The cells in this area present a homogenous, eosinophilic

cytoplasm without vacuoles or secretion. Cross-sections of the *vas deferens* show the androgenic gland to consist of a sinuous string of irregularly shaped cells (Figure 1-B). However, in longitudinal sections the gland appears as layered rows of cells (Figure 1-D).

The cells of the androgenic gland are enclosed by a thin, reddish-pink PAS-positive tissue layer (Figure 1-B), which appears to be related to the physical support of the cells. While the cytoplasm did not react strongly in the histochemical test, gland cells tested positive for total protein with the bromophenol blue dye (Figure 1-C). The cytoplasm was homogeneous, though more weakly stained than the nucleus. In some cells the nucleoli were strongly stained.

Goniopsis cruentata

The androgenic gland of *G. cruentata* is located below the muscles of the coxopodite of the last pair of legs. It consists of long strings of epithelial cells attached to the muscle layer lining the external wall of the *vas deferens* posteriorly (Figure 2). In spite of being adjacent to the *vas deferens*, no communication between the gland and the vas deferens was found.

Two cell types were observed in the androgenic gland of *G. cruentata*: type-A cells with nuclei measuring approximately 5 μm, a clear homogeneous cytoplasm and densely concentrated chromatin staining strongly with HE; and type-B cells with larger nuclei (approximately 10 μm), clearly discernible nucleoli, a clear homogeneous cytoplasm and peripheral chromatin granules. The cells formed groups in a repetitive pattern (Figure 2).

Both cell types were encased in a fine sheath which stained deep green with Gomori Trichromic dye (Figure 2). Despite the presence of gland cells, no secretion was observed in the cytoplasm.

DISCUSSION

As observed under a microscope, the androgenic gland of both *U. cordatus* and *G. cruentata* lies alongside the *vas deferens*, beginning at the location where the latter penetrates the musculature of the endofragmal skeleton. Similar findings were reported for the red swamp crayfish, *P. clarkii* (Taketomi, 1986), for the lined shore crab, *P. crassipes* (King, 1964), for the

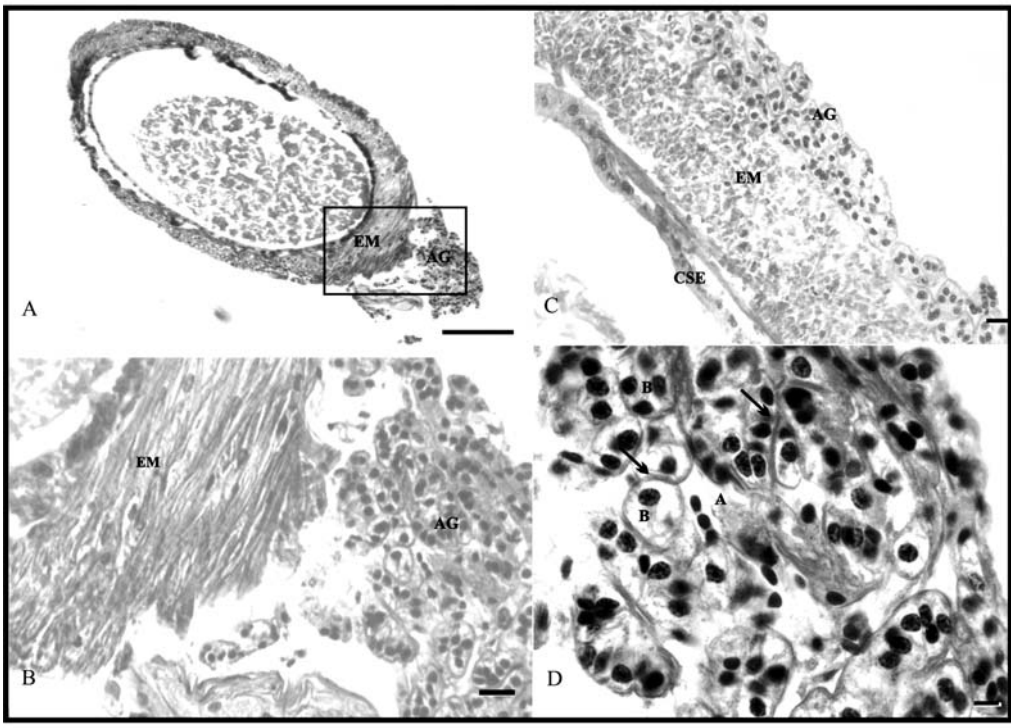

Figure 2 - Cross-section of *Goniopsis cruentata*: A - androgenic gland (AG) attached to the external muscle (EM) of the vas deferens; stain: Gomori Trichromic (scale bar: 100 μm); B - detail of the androgenic gland (AG) adjacent to the vas deferens; stain: Gomori Trichromic (scale bar: 20 μm); C - photomicrograph of longitudinal section with overview of the androgenic gland (AG), the external muscle (EM), the simple cuboid epithelium (CSE) and the collagen fibers (CF) of the basal membrane; stain: Gomori Trichromic (scale bar: 20 μm); D - detail of the androgenic gland with grouped cells (B) and dispersed cells (A). The thin sheath is deep stained (arrows); stain: Gomori Trichromic (scale bar: 10 μm).

ghost crab, *O. platytarsis* (Thampy & John, 1970) and for *C. destructor* (Fowler & Leonard, 1999). In contrast, Lima (1995) found the androgenic gland of the spiny lobster *P. laevicauda* to be connected to the subterminal region of the distal *vas deferens*.

In both ~~our~~ species the androgenic gland was found to adhere to a thick, circular and striated muscle layer, not restricted to the subterminal region, unlike descriptions of other decapod species published in the literature. Since the androgenic gland could not be observed macroscopically in the present study, it is likely that ultrastructural analyses will shed more light on its location and extension.

Cross-sections of the *vas deferens* show the androgenic gland of both *U. cordatus* and *G. cruentata* as a sinuous string of irregularly shaped cells, matching descriptions of brachyurans by Adiyodi & Adiyodi (1970). According to these authors, the cell strings of the androgenic gland may be sinuous or convoluted. Longitudinal sections showed the gland to be composed of cells disposed in layered rows. Both two cell types observed, type-A and type-B, were supported by a basal membrane staining strong green with Gomori's trichrome dye.

The cells of the androgenic gland of *U. cordatus* had round or ovoid nuclei with eosinophilous cytoplasm. Future and more detailed studies will help determine whether the difference in nucleus shape indicates a simple variation or the occurrence of two distinct cell types.

According to Taketomi (1986), the androgenic gland of *P. clarkii* resembles a milky-white cluster of grapes. The gland features a string with two cell types, type A and B, presenting alongated and spherical nuclei, respectively. More recently Taketomi *et al.* (1996*a*) confirmed these findings and added that the number of cells seems to be nearly constant throughout the year, although the ratio between type A and type B may vary with the season.

Lima (1995) found evidence of degeneration in the androgenic gland cells of the spiny lobster, but no signs of on-going or past secretion. In comparison, in the present study no degeneration or secretion was observed in the cells of the androgenic gland of *U. cordatus* and *G. cruentata*.

Our histochemical analyses were positive for total protein in both nuclei and cytoplasm of the androgenic gland of *U. cordatus* and *G. cruentata*, indicating the presence of macromolecules in these cell regions, as observed earlier for the lined shore crab (King, 1964). According to this author, the androgenic hormone may be a protein or polypeptide, since histochemical tests indicate the presence of proteins and lipids in the cytoplasm. Further, King's ultrastructural analysis has shown the cell membranes to be highly interdigitated and the cytoplasm to contain abundant granular endoplasmic reticulum.

However, the use of light microscopy alone, as in the present study, does not allow to make consistent inferences regarding the metabolic activity of the cells of the androgenic gland or regarding the chemical nature of the hormone it produces.

King (1964) described the cells of the androgenic gland as having ovoid nuclei and a moderate amount of cytoplasm and as being encased in a clear and thin, PAS-positive sheath. Charniaux-Cotton & Payen (1988) found no secretion from the gland, but they did observe an unidentified substance in the space between the cells, possibly corresponding to the basal membrane observed in the present study. The PAS-positive layer enveloping the cells in the androgenic gland of *G. cruentata* and *U. cordatus* is possibly a basal membrane made of connective tissue, matching findings published by Ross *et al.*, (1993). According to these authors, the fact that the basal membrane is PAS-positive, with the appearance of a well-defined pink layer, shows it to be composed of glycoprotein. The basal membrane appears to be related to the physical support of the cells.

Charniaux-Cotton & Payen (1985) observed relatively well-developed connective tissue among the cell groups that make up the androgenic gland. Fowler & Leonard (1999) found the androgenic gland to be surrounded by connective tissue and to be connected to the *vas deferens* by an extension of this tissue. Lima (1995) reported the androgenic gland of the spiny lobster to consist of a mass of cells attached to the posterior part of the vas deferens and enveloped by connective tissue.

The androgenic glands of *U. cordatus* and *G. cruentata*, respectively, are not visible to the naked eye, but were found to be generally similar upon histological examination. This similarity could be evidence of an evolutionary link between the families Ocypodidae and Grapsidae.

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