

SPERM CELL DEVELOPMENT OF THE RED-CLAWED MANGROVE-TREE CRAB, *Goniopsis cruentata* LATREILLE, 1803 (CRUSTACEA: DECAPODA: GRAPSIDAE)

Desenvolvimento das células espermáticas do caranguejo-aratu, *Goniopsis cruentata* Latreille, 1803 (Crustacea: Decapoda: Grapsidae)

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ABSTRACT

The red-clawed mangrove-tree crab, *Goniopsis cruentata*, constitutes an important economic resource in Northeastern Brazil. Male specimens were collected and submitted to histological routine tests. Four types of sperm cell were observed in the anterior region of the testis: spermatogonia, spermatocytes, spermatids and spermatozoa. The general development of sperm cells in *G. cruentata* follows the basic pattern described for decapods. To our knowledge, no description of sperm cell development in *G. cruentata* has been published previously.

Key words: sperm cell development, *Brachyura*, reproduction, *Goniopsis cruentata*.

RESUMO

O caranguejo-aratu, *Goniopsis cruentata*, constitui um importante recurso econômico no Nordeste do Brasil. Espécimes machos foram coletados e submetidos a testes histológicos de rotina. Quatro tipos de células germinativas foram observados na região anterior do testículo: espermatogônias, espermatócitos, espermátides e espermatozóides. O desenvolvimento das células espermáticas em *G. cruentata* segue o padrão básico descrito para os decápodes. A nosso conhecimento, nenhuma descrição do desenvolvimento espermático em *G. cruentata* foi publicada previamente.

Palavras-chaves: desenvolvimento de células espermáticas, *Brachyura*, reprodução, *Goniopsis cruentata*.

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INTRODUCTION

Most crustaceans are dioic (Ruppert & Barnes, 1994). The male reproductive organs of most malacostracans, including decapods, are located in the cephalothorax or thorax. In decapods, the male reproductive tract is typically composed of paired testes and genital ducts; each genital duct consists of a collecting tube, a vas deferens with regionally differing functions and an ejaculatory duct termed the seminal vesicle or, in some species, the terminal ampoule (Krol *et al.*, 1992).

Many aspects of crustacean testis morphology have been explored, but few studies have dealt with sperm cell development in crabs. These include light microscopy studies of the crab species *Callinectes sapidus* (Cronin, 1947; Johnson, 1980) and *Ucides cordatus* (Leite, 2002) and of other crustaceans such as the squat lobster, *Thenus orientalis* (Burton, 1995), the spiny lobster, *Panulirus laevicauda* (Lima, 1995) and the hermit crab, *Diogenes pugilator* (Manjón-cabeza & Raso, 2000).

A few ultrastructural studies of sex cell maturation are also available, including studies on *Cancer* (Langreth, 1969), *Callinectes sapidus* (Brown, 1966) and *Portunus pelagicus* (El-sherief, 1991), the hermit crab *Lithodes maja* (Tudge *et al.*, 1998), the spiny lobster *Panulirus* (Talbot & Summers, 1978), the crayfish *Pacifastacus leniusculus* (Dudenhause & Talbot, 1982) and the Hawaiian red lobster *Enoplometopus occidentalis* (Haley, 1986).

The present study describes the structure and development of the sperm cells of the red-clawed mangrove tree crab *Goniopsis cruentata* (Latreille, 1803) observed under light microscopy.

MATERIALS AND METHODS

Ten male specimens of *Goniopsis cruentata* were collected from the Ceará River mangrove (on

the outskirts of Fortaleza, Northeastern Brazil) in each month from October, 2002 to October, 2003. The dorsal carapace was raised to expose the internal organs and allow for excision of the anterior paired testes. The material was fixed in a cold Bouin solution and subsequently washed in a 70% ethanol bath until most of the picric acid had been eliminated. Segments of each testis were paraffin-embedded and cut serially in 5- μ m sections. The sections were mounted and stained using Alcian Blue, PAS, H.E. (Junqueira & Junqueira, 1983), Bromophenol Blue, Xylidine Ponceau (Pearse, 1960), Gomori Trichromic and Mallory Trichromic (Tolosa *et al.*, 2003).

RESULTS

In the germinal zone, the maturation stage was identified by means of the cell diameter and morphological characteristics of the nucleus and cytoplasm as evidenced by staining. According to these criteria, four different cell types were observed (Figures 1 and 2):

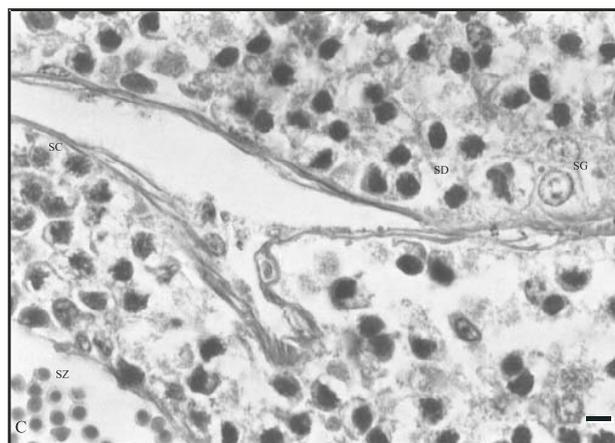


Figure 1 - Sperm cells in different stages of maturation: spermatogonia (SG), spermatocytes (SC), spermatids (SD) and spermatozoa (SZ). Stain: Mallory Trichromic (Scale bar: 10 μ m).

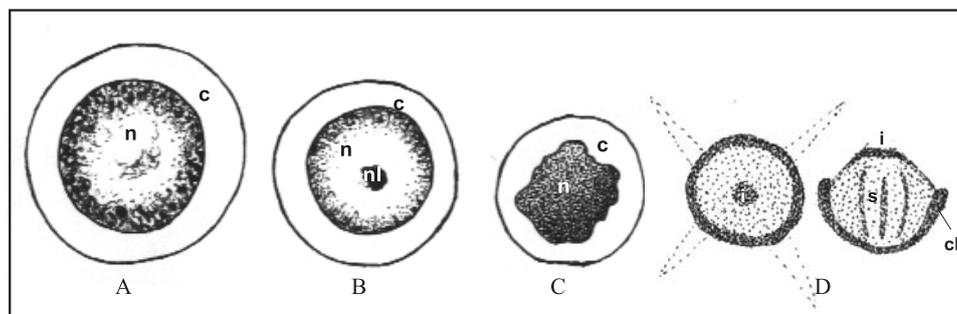


Figure 2 - Schematical drawing of germ cells development. Spermatogonium (A), Spermatocyte (B), Spermatid (C), Spermatozoa (D), nucleus (n), nucleolus (nl), cytoplasm (c), acrossoma (a), cell invagination (i), "spike-like structure"(s) and chromatin (ch).

1. Spermatogonia: nuclei measuring 15 µm across, staining pale blue with Mallory Trichromic. Condensed chromatin was observed on the internal surface of the cell membrane.

2. Spermatocytes: nuclei measuring approximately 13 µm, staining blue with Mallory Trichromic. Nucleoli were clearly visible.

3. Spermatids: nuclei measuring approximately 10 µm, staining bright pink with Mallory Trichromic. The nuclei are of irregular shape. No nucleoli were observed.

4. Spermatozoa: spherical cells with nuclei measuring 8 µm, staining bright pink with Mallory Trichromic. The cells are spiked and feature an acrosome in one of the cell poles. The results of all stains are on Table I.

DISCUSSION

The formation of the sex cells of the red-clawed mangrove-tree crab, *Goniopsis cruentata*, occurs in the germinal zone in the anterior region of the testis. Stages of cell maturation may be easily distinguished by staining with Mallory Trichromic, with the exception of the transitions between spermatid and spermatozoon and between primary and secondary spermatocytes, which present some difficulty due to the low resolution used in light microscopy.

When exposed to Mallory Trichromic, spermatogonia and spermatocytes stained blue, while spermatids and spermatozoa stained pink. These results show that the sex cells examined were engaged in different metabolic activities corresponding to different stages of maturation.

The nuclei of the spermatogonia of *G. cruentata* have a diameter of 15µm, staining pale blue with

Mallory Trichromic. Johnson (1980), Manjón-cabeza & Raso (2000) and Leite (2002) presented similar findings for the blue crab (*Callinectes sapidus*), the hermit crab (*Diogenes pugilator*) and *Ucides cordatus*, respectively. In another study, Lima (1995) classified the spermatogonia of the spiny lobster (*Panulirus laevicauda*) into (1) spermatogonia with large nuclei (10µm) and granules of condensed chromatin, and (2) spermatogonia with three evident and centered nucleoli. However, this pattern was not observed in our study.

Burton (1995) observed that spermatogonia form an adjacent, distinct group on the acinar epithelium. Cronin (1947) reported observing mitotic activity involving spermatogonia in the tubules. This activity was also observed in the present case.

Our description of the spermatocytes of *G. cruentata* (nuclei measuring 13µm, staining blue with Mallory Trichromic and presenting evident nucleoli) matches Leite's description of the spermatocytes of *U. cordatus*. However, the differentiation between the primary and the secondary stage could not be done as in Johnson (1980), Manjón-cabeza & Raso (2000) and Lima (1995).

The spermatids of *G. cruentata* are characterized by irregular nuclei measuring approximately 10 µm, staining bright pink with Mallory Trichromic, and the absence of nucleoli. In support of this finding, Johnson (1980) reports that when spermatids develop into spermatozoa, the nuclear chromatin is dislodged to one of the polar regions and the cytoplasm becomes PAS-positive. According to Lima (1995), spermatids are small with spherical nuclei measuring approximately 3 µm and the entire nuclear portion is dislodged to one side of the cell. Manjón-cabeza & Raso (2000) and Leite (2002) agree with this finding. However, though the chromatin

Table I - Stains results of the maturation stage identified. Cell average diameter (AD), Bromofenol Blue (BB), Xylidine Ponceau (XP), Gomori Trichromic (GT), Mallory Trichromic (MT), Nucleus (N) and Cytoplasm (C).

Germ cell	AD (µm)	Stain							
		BB		XP		GT		MT	
		N	C	N	C	N	C	N	C
Spermatogonium	22	+	+	+	+	+	-	+	-
						(purple)	(purple)	(blue)	(blue)
Spermatocyte	18	++	+	++	+	++	+	+++	+
						(purple)	(purple)	(blue)	(blue)
Spermatid	14	+++	++	+++	++	+++	+	+++	+
						(purple)	(purple)	(pink)	(pink)
Spermatozoa	12	++	+	++	+	+	-	+++	+
						(blue)	(blue)	(pink)	(pink)

was more condensed, no polarization was observed for *G. cruentata* in this stage.

In *G. cruentata*, the last stage of maturation of the sex cells, the spermatozoon, takes the form of spherical cells with nuclei measuring 8µm, staining bright pink with Mallory Trichromic. Spermatozoa are partly spiked and feature an acrosome in one of the polar regions. Johnson (1980), Lima (1995), Manjón-cabeza & Raso (2000) and Leite (2002) report similar findings.

No accessory cells were observed in the germinal zone of *G. cruentata*. Cronin (1947) reported accessory cells to be for the most part distributed in the periphery of the tubule. Johnson (op cite) describes them as cells with extended and irregular nuclei found in the lobes containing spermatids. Manjón-cabeza & Raso (2000), who termed them nutritional cells, suggest that since they are found in the vicinity of spermatids they may be involved in providing nourishment for these cells and in the formation of a lumen providing a passageway to the collecting tubes.

REFERENCES

- Brown, G.G. Ultrastructural studies of sperm-egg interaction in the decapod *Callinectes sapidus*. *J. Ultrastr. Res.*, v.14, p.425-440, 1966.
- Burton, T.E. The spermatid pathway and associated reproductive structures of squat lobster *Thenus orientalis* (Lund, 1793). *Invert. Reprod. Develop.*, v.28, n.1, p.53-61, 1995.
- Cronin, L.E. Anatomy and histology of the male reproductive system of *Callinectes sapidus*, Rathbun. *J. Morphol.*, v.81, n.2, p.209-239, 1947.
- Dudenhause, E.E. & Talbot, P. An ultrastructural analysis of mature sperm from the crayfish *Pacifastacus leniusculus*, Dana. *Invert. Reprod. Develop.*, v.5, p.149-159, 1982.
- El-Sherief, S.S. Fine structure of the sperm and spermatophores of *Portunus pelagicus* (L.) (Decapoda, Brachyura). *Crustaceana*, v.61, n.3, p.171-179, 1991.
- Haley, S.R. Ultrastructure of spermatogenesis in the Hawaiian red lobster *Enoplometopus occidentalis* (Randall). *J. Morphol.*, v.190, p.81-92, 1986.
- Johnson, P.T. *Histology of the blue crab Callinectes sapidus: a model for decapoda*. Praeger Publishers, 440 p., New York, 1980.
- Junqueira, L.C. & Junqueira, L.M.M.S. *Técnicas básicas de citologia e histologia*. Livraria e Editora Santos, 123 p., São Paulo, 1983.
- Krol, R.M.; Hawkins, W.E. & Overstreet, R.M. Reproductive components, p. 295-343, in Harrisson, F.W. & Humes, A.G. (eds.), *Microscopic anatomy of invertebrates*. Wiley-Liss Inc., v.10, 1992.
- Langreth, S.G. Spermiogenesis in *Cancer* crabs. *J. Cell Biol.*, v.43, p.575-603, 1969.
- Leite, M.M.L. *Estudo morfológico do sistema reprodutor masculino do caranguejo-uçá Ucides cordatus Linnaeus, 1763 (Crustacea: Decapoda: Ocypodidae)*. Monografia de Graduação em Ciências Biológicas, Departamento de Biologia, Universidade Federal do Ceará, 66 p., Fortaleza, 2002.
- Lima. A.V.P. *Estudo do Sistema Reprodutivo de machos da lagosta verde Panulirus laevicauda (Latreille, 1817) (Decapoda: Palinuridae)*. Dissertação de Mestrado em Ciências Biológicas, Universidade Federal da Paraíba, 200 p., João Pessoa, 1995.
- Manjón-Cabeza, M.E. & Raso, J.E.G. Morphological reproductive aspects of males of *Diogenes pugilator* (Roux, 1829) (Crustacea, Decapoda, Anomura) from southern Spain. *Sarsia*, v.85, p. 95-202, 2000.
- Pearse, A.G.E. *Histochemistry theoretical and applied*. Jet. Churchill Ltd., v.2, 965 p., 1960.
- Ruppert & Barnes. *Invertebrate Zoology*. 6th edition, 1056 p., 1994.
- Talbot, P. & Summers, R.G. The structure of sperm from *Panulirus*, the spiny lobster, with special regard to the acrosome. *J. Ultrastr. Res.*, v.64, p.341-351, 1978.
- Tudge, C. C.; Jamieson, B.G.M.; Sandberg, L. & Erséus, C. Ultrastructure of the mature spermatozoon of the king crab *Lithodes maja* (Lithodidae, Anomura, Decapoda): further confirmation of a lithodid-pagurid relationship. *Invert. Biol.*, v.117, n.1, p.57-66, 1998.
- Tolosa, E.M.C.; Rodrigues, C.J.; Behmer, O.A. & Freitas-Neto, A.G. *Manual de técnicas para histologia normal e patológica*, 331 p., 2003.