

# ULTRASTRUCTURAL STUDY OF THE SPERMATOPHORES AND SPERMATOOZOA IN *UCA URUGUAYENSIS* (DECAPODA, BRACHYURA, OCYPODIDAE)

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## ABSTRACT

The present study shows that this species has a very simple spermatophore structure, consisting of a sperm mass embedded in seminal fluid and surrounded by a two layered acellular wall. Beneath the wall, there is a translucent zone lined by dense granules. These granules could be part of the spermatophore wall. The seminal fluid is composed by a viscous granular matrix and electron-dense granules with homogeneous aspect. The spermatophores were observed densely packed in the middle vas deferens (MVD) and laxly packed in the posterior vas deferens (PVD), suggesting an increment of the amount of seminal fluid. This increment would provide protection and maintenance to the spermatozoa. Mature spermatozoa have the typical structure of brachyuran crabs with a complex acrosome, cupped by the nucleus, and a thin cytoplasmic band between the former elements. Spermatozoa have spike-like arms with microtubules and chromatin, suggesting that they are extensions of the nucleus. The length to width rate of the acrosome is in the range observed for the rest of Thoracotremata. The acrosome of this species presents the bilayered appearance characteristic among brachyurans. The perforatorium has fibrous aspect attributed to the presence of tubular membranous structures. In summary, the spermatozoal features display the typical brachyuran characters, although the characteristic “onion ring” lamellation present in the thoracotremes acrosome was not observed.

**Keywords:** *Brachyura, Ocypodidae, spermatophore, spermatozoa.*

## RESUMO

Este trabalho apresenta uma caracterização morfológica do espermatóforo de *U. uruguayensis* e descreve o espermatozóide obtido do VDP (Vaso Deferente Posterior). O espermatóforo apresenta a estrutura mais simples existente entre os decápodos, consistindo de numa massa de espermatozoides embebida em fluido seminal e rodeada por uma parede composta por duas capas acelulares. No lado interno da parede observam-se grânulos electrodensos alinhados que formariam parte da mesma. O fluido seminal está composto por uma matriz granular viscosa com grânulos electrodensos de aspecto homogêneo. Os espermatozoides foram observados densamente agrupados no vaso deferente médio e dispostos de forma laxa no VDP, sugerindo um incremento de fluido seminal nesta zona. Este incremento proveria proteção e sustentação ao espermatozóide. Os espermatozoides maduros têm a típica estrutura dos caranguejos brachyuras, com um complexo acrossômico rodeado pelo núcleo e uma fina banda citoplasmática entre ambos. Os braços do espermatozóide (spike-like arms) se interpretam como extensões do núcleo, já que contêm microtúbulos e cromatina. A relação comprimento/largura do acrossoma está na categoria observada para o resto dos Thoracotrematas. O acrossoma desta espécie apresenta a típica estrutura bicapa dos brachyuras. O “perforatorium” tem aspecto fibroso atribuído à presença das estruturas membranosas tubulares. Em conclusão, o espermatozóide tem a estrutura característica dos brachyuras, ainda que careça da estrutura lamelar “onion ring” do acrossoma típico dos thoracotrematas.

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## INTRODUCTION

Based on the position of the genital pore and the nature of the spermatheca, brachyuran decapods have been divided into three sections: Podotremata, Heterotremata and Thoracotremata (GUINOT, 1978). Crabs from the Family Ocypodidae belong to the Thoracotremata because of the sternal location of both male and female genital pores and the development of the spermatheca as a diverticulum of the oviduct.

Numerous ultrastructural studies on decapod spermatozoa suggested that the particular structure of the spermatozoid presents species-specific variation evidencing importance of the spermatozoid features in taxonomic and phylogenetic studies (TUDGE, 1997). However, with the exception of a few studies they have not been adequately studied in Thoracotremata (ANILKUMAR et al., 1999).

Decapod spermatozoa are rather unusual in being non-motile and aflagellate, the sperm is enclosed in a sperm packet (spermatophore) with an acellular wall which is transferred to the female during mating (HARTNOLL, 1969). Inside the spermatophore, the spermatozoa are embedded in a matrix secreted by the middle and posterior region of the vas deferens (HINSCH, 1991). The family Ocypodidae has internal fecundation and in general the spermatophores are simple structures that degenerate after the reproductive season. Spermiogenesis occurs while germinal cells (spermatids) are passing along the different regions of the vas deferens (VD) (SAINT-MARIE ; SAINT-MARIE, 1999).

The Ocypodidae crab *Uca uruguayensis* Nobili 1901, inhabits the supra-littoral of Mar Chiquita lagoon (Argentina, 37° 45'S 57° 26'W); this decapod usually lives at the external margin of the crab community and borrows into the mud making galleries and caves. In this crab three regions are recognised in the vas deferens: anterior (AVD), medial (MVD), and posterior (PVD) (CUARTAS; PETRIELLA, 2004). The spermatophore formation begins at the base of the testicular lobes, when the spermatozoa pass to the vas deferens, and is completed at the distal vas deferens (CUARTAS; PETRIELLA, 2005). There is no evidence about the structure and characteristics of the spermatophore and spermatozoa of this species, although the mechanism of spermatophore formation was previously studied (CUARTAS; PETRIELLA, 2005).

The present work analyses and describes the characteristics of *U. uruguayensis*' spermatophore and the spermatozoa obtained from the vas deferens (VD). A introdução é a parte inicial do artigo, onde se expõe a delimitação do assunto que será abordado, os objetivos da pesquisa e demais fatores necessários para situar o artigo.

## MATERIAL AND METHODS

*U. uruguayensis*' adult males in reproductive activity were collected from Mar Chiquita lagoon (February 2003) and placed on ice. For histological

studies, the spermatophores were obtained from the vas deferens by cutting open the carapace through the dorsal side. The material was fixed in Davidson's fluid (BELL; LIGHTNER, 1988). Tissues were dehydrated in a series of graded ethanol and embedded in paraffin. Sections (4-5 µm) were stained with haematoxylin-eosin. Fresh smear preparations of spermatophores from the vas deferens were also observed.

For the ultrastructural study, the material was transferred to 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH = 7.2-7.4), during 24 hours at 4°C. Tissues were washed three times in the buffer. They were postfixed for 1h in 1% OsO<sub>4</sub>. Later the material was dehydrated through an ethanol series and embedded in Spurr resin. Semithin sections (1 µm) were stained with toluidine blue. Ultrathin sections were mounted on copper grids (400-mesh) and stained with lead citrate and uranyl acetate. TEM images were obtained with a JEOL JSM-100CX II transmission electron microscope.

## RESULTS

Fresh smear preparations, obtained from the posterior vas deferens (PVD), show that *U. uruguayensis*' spermatophores are simple oval structures embedded in a hyaline seminal matrix bounded by a translucent wall (Fig. 1).

Histological sections through the transition zone between collector ducts and the anterior vas deferens (AVD) evidence the spermatophores enclosing immature spermatozoa densely packed. Most of these immature spermatozoa present a central dense zone (acrosome) completely surrounded by the nucleus (Fig. 2).

In the middle vas deferens (MVD), the spermatophores contain differentiated spermatozoa with the acrosome completely structured and irregular nuclei (Fig. 3). The seminal fluid is composed by a viscous granular matrix (seminal plasma) and electron-dense granules with homogeneous aspect (Figs. 4-5). The spermatophores are densely packed in MVD and their walls are contiguous (Fig. 6), meanwhile in the PVD they are laxly included in the seminal fluid. In general, the seminal fluid is more electron-dense than the spermatophore fluid.

The spermatophore wall comprises two electron-dense layers, being the inner layer thicker than the outer one. Beneath the inner layer there is a translucent zone lined by dense granules (Figs. 5-6).

The spermatozoa are irregular in shape and their size ranges between 3 and 5µm. At OM, each spermatozoid presents two well differentiated zones: a basophilic zone (acrosome) and a light basal zone (decondensed nucleus). Ultrastructurally, the acrosome is subspheroidal (1.1-1.2 length to width rate) and is encased by the acrosomal membrane. The acrosome presents two zones with different electron density and in the anteriormost portion there is a conical operculum. An electron-pale zone (periopercular ring) is observed encircling the periphery of the operculum. From the base of the acrosome, along the central core, and extending to

the subopercular region, there is a longitudinal groove called perforatorium by Anilkumar et al. (1999). The perforatorium is circular in cross section and is occupied by tubular formations which give it a fibrous appearance (Fig. 7). An electron-dense structure of membranous appearance is observed beneath the base of the acrosome. The nucleus is surrounded by the nuclear membrane, the chromatin material appears reticulate and microtubules are also observed (Fig. 8). Electron micrographs evidence projections in close vicinity of the spermatozoa, containing chromatin and microtubules. These structures are observed in cross, tangential and longitudinal sections (Figs. 7-8). The cytoplasm of the spermatozoid is restricted to a thin electron-dense area between the nucleus and the acrosome (Figs. 7-8).

## DISCUSSION

Like in other Thoracotremata, at mating *U. uruguayensis*' spermatophores are transferred from the male to the ventral surface of the female and are internalized until the time of oviposition (per. obs.). In agreement with KROL et al. (1992) and Subramonian (1993) these spermatophores provide protection and support for sperm during transfer to and storage by the female. Brachyurans present the simplest spermatophore among decapods (Krol et al., 1992), which contain varying number of sperm (HINSCH, 1988). *U. uruguayensis*' spermatophores consist of a sperm mass embedded in seminal fluid. In spite of the simplicity, these spermatophores comprise a two layered acellular wall. ANILKUMAR et al. (1999), working with a grapsid crab, found a translucent area between the both spermatophore layers where dense granules were also present. In contrast, in *U. uruguayensis*, this translucent zone is beneath the inner layer and is lined by dense granules. These granules could be part of the spermatophore wall, since the heterogeneity of the wall has been shown in other decapods (TUDGE, 1997; ANILKUMAR et al., 1999). However, in other brachyurans like *Libinia emarginata* Leach, 1815 (HINSCH; WALKER, 1974), *Uca pugilator* (Bosc, 1802) (HINSCH, 1991) and *Chionoecetes opilio* (O. Fabricius, 1788) (BENINGER et al., 1988), the spermatophore wall presents a single acellular layer.

In brachyuran crabs, different secretions are produced in each area of the vas deferens (KROL et al., 1992). Accordingly, in *U. uruguayensis* the seminal fluid is secreted by the epithelial cells of the vas deferens (CUARTAS; PETRIELLA, 2004) and is composed by a viscous granular matrix (seminal plasma) and electron-dense granules with homogeneous aspect. The role of the seminal fluid in decapods is not well known (BENHALIMA; MORIYATSU, 2000). Johnson (1980) suggested that the sperm plug of blue crabs could play the dual role of preventing loss of sperm and of providing a mechanical or chemical barrier to the entrance of harmful material. In the present study, the spermatophores were observed laxly packed in the PVD suggesting an increment in the amount of seminal fluid. This increment

of seminal fluid would provide protection and maintenance to the spermatozoa, as it was previously shown by other authors (SUBRAMONIAN, 1991).

Mature spermatozoa in this species have the typical structure of brachyuran crabs with a complex acrosome, cupped by the nucleus, and a thin cytoplasmic band between the former elements. The acrosome presents the bilayered appearance characteristic among the brachyurans and a length to width rate that is in the range observed for the rest of Thoracotremata. The occurrence of zonation in the contents of the acrosome is reported in several brachyurans and the layers have been termed the inner and the outer regions (FELGENHAUER; ABELE, 1991; ANILKUMAR et al., 1999). The "onion ring" lamellation of the outer acrosome zone mentioned for other Thoracotreme was not observed in the present study. The operculum, located in the most anterior portion of the acrosome, presents a variable morphology in the Thoracotremata (ANILKUMAR et al., 1999). In brachyuran species, the operculum can be flat, like in *U. tangeri* (Eydoux, 1835) (MEDINA; RODRÍGUEZ, 1992a) or conical, like in *Metopograpsus messor* (Forskål, 1775). (ANILKUMAR et al., 1999) and *Sesarma haematocheir* (de Haan) (HONMA et al., 1992), resembling our present observations. However, the periopercular ring is not so conspicuous in this species like in other brachyurans. The acrosomal longitudinal groove or perforatorium of *U. uruguayensis* has the general features described for the thoracotremes by Jamieson et al. (1996). The fibrous appearance of the perforatorium, observed in the present work, is attributed by other authors to the presence of tubular membranous structures (MEDINA; RODRÍGUEZ 1992b). The exact role of the perforatorium in fecundation is still unknown. HINSCH (1986) suggests that it is part of the mechanism of the spermatozoid movement. MEDINA; RODRÍGUEZ (1992a) propose that perforatorium tubules serve as a membrane source for male pronuclear formation during fertilisation.

The *U. uruguayensis*' mature spermatozoa have a decondensed nucleus as the most of brachyuran crabs; the decondensed sperm nucleus may provide the fluidity necessary to accommodate the unusual acrosomal reaction of this group in which the nucleus is rapidly thrust toward the oocyte when the acrosome everts (KROL et al., 1992). The electron-dense structure observed near the base of the acrosome of this species represents "the membrane lamellar complex" characteristic in the reptantia spermatozoa (BEACH; TALBOT, 1987).

Spermatozoa of some species present spike-like arms that appear to originate as extensions of the nucleus (FELGENHAUER; ABELE, 1991). However, in other species they appear to be extensions of the cytoplasm (HINSCH, 1986). The arms observed in *U. uruguayensis*' spermatozoa contain microtubules and chromatin, as in many other brachyurans, suggesting that they are extensions of the nucleus; the microtubules presumably provide support (FELGENHAUER; ABELE, 1991). In contrast, some species present chromatin but lack

microtubules while others only have microtubules in the spermatozoa arms (HINSCH, 1969). On the other hand, in the crab *Paratelpusa hydrodromous* (Herbst) the radial arms are absent (SUDHA DEVI; ADIYODI, 2001).

In summary, the spermatozoal features of *U. uruguayensis* display typical brachyuran and decapod characters. Most of the spermatozoa features, such as the length to width ratio of the acrosome, the concentric arrangement of the acrosome zones and the structure of the perforatorium represent thoracotreme characters, although the characteristic "onion ring" lamellation was not observed in the acrosome of this species' spermatozoa. Further work will be necessary to elucidate the possible morphological changes that take place in the spermatophore and the spermatozoa when they are transferred and stored in the spermatheca.

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Figures 1-3. Sperm and spermatophores of *Uca uruguayensis*.

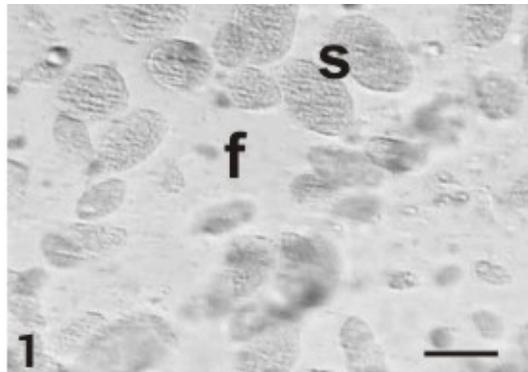


Figure 1. Photomicrograph of fresh-smear preparations from PVD lumen showing spermatophores. f: seminal fluid; s: spermatophores. Scale bar: 25µm

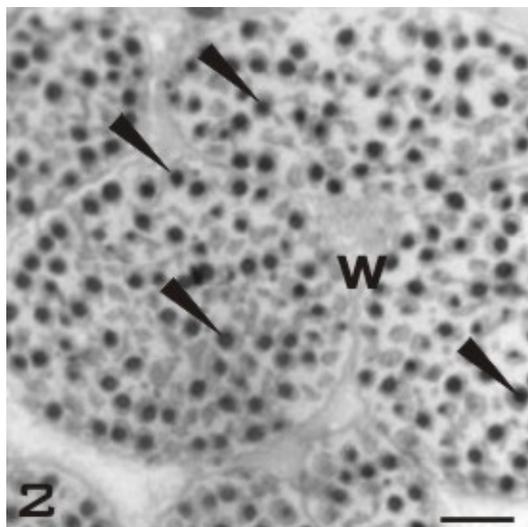


Figure 2. Cross section of the AVD showing immature spermatozoa (arrows) closely packed. w: spermatophore wall. Optical Microscopy (OM). Scale bar: 10 $\mu$ m

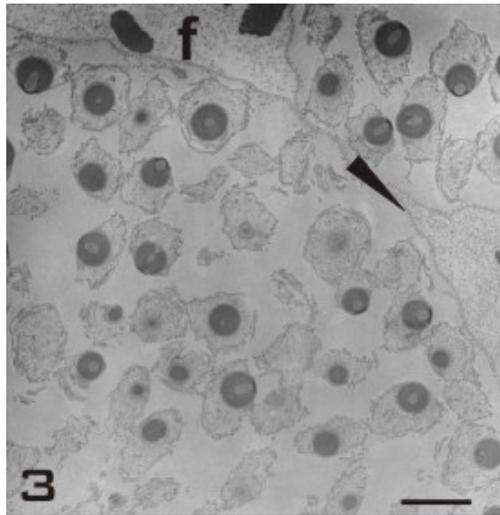


Figure 3. TEM of cross section of MVD. Spermatophore wall (arrow). f: seminal fluid. Scale bar: 3 $\mu$ m

Figure 4-6. Ultrastructure of the spermatophore wall from *U. uruguayensis*' MVD.

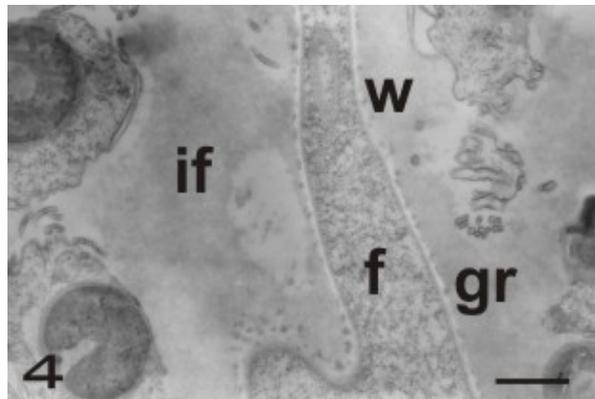


Figure 4. Neighbouring spermatophores immersed in heterogeneous seminal fluid. f: seminal fluid; gr: dense granules; if: internal fluid, w: spermatophore wall. Scale bar: 1 $\mu$ m

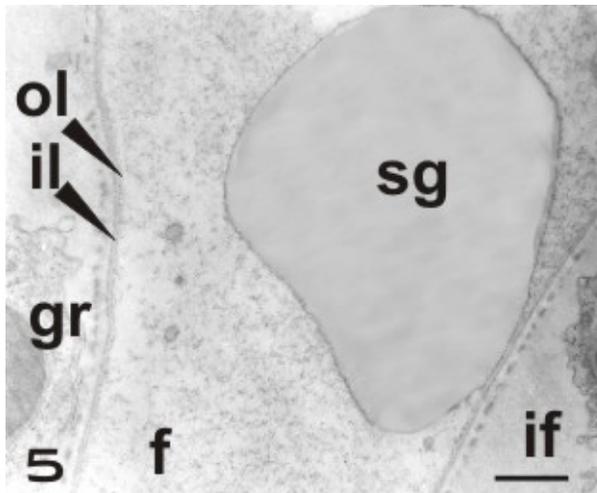


Figure 5. Neighbouring spermatophores immersed in heterogeneous seminal fluid with homogeneous seminal granules. f: seminal fluid; gr: dense granules; if: internal fluid; il: inner layer; ol: outer layer; sg: seminal granule. Scale bar: 0.5µm

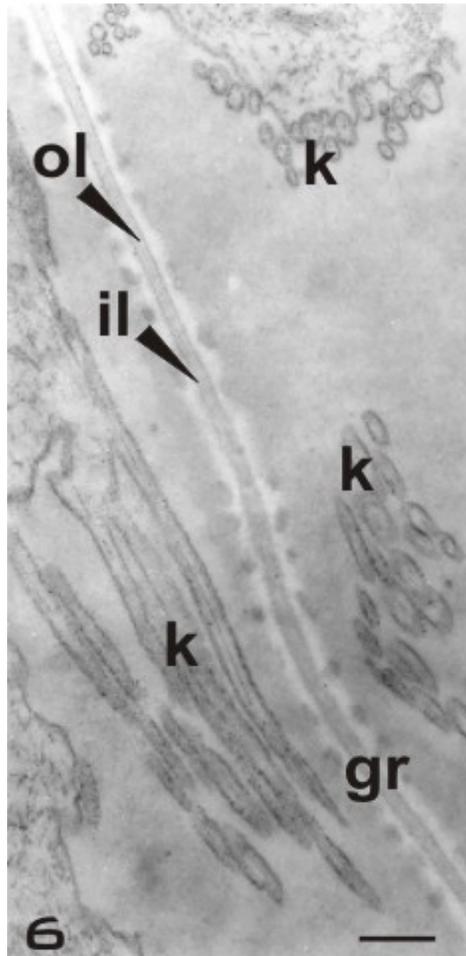


Figure 6. Contiguous spermatophores walls evidencing the inner and outer layers, the electrolucent area and dense granules. See the spike like arms in longitudinal and cross section. gr: dense granules; il: inner layer; k: spike like arms; ol: outer layer. Scale bar: 0.1µm

Figures 7-8. TEM micrograph of spermatozoa from *Uca uruguayensis*' PVD.

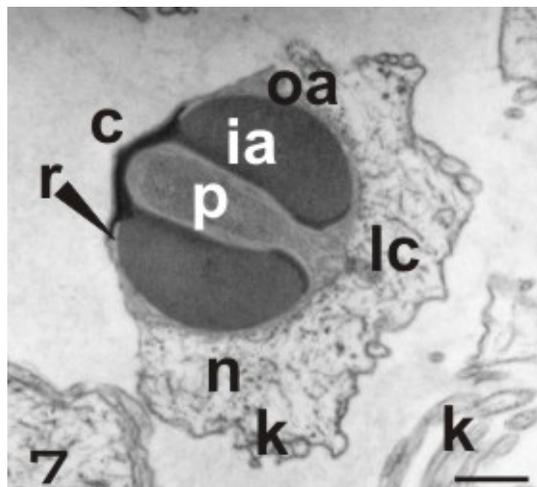


Figure 7. Longitudinal section of a spermatozoid. Note de conical apical operculum. c: operculum (cap); ia: acrosomal internal area; lc: membranous lamellar structure; k: spike like arms; n: nucleus; oa: acrosomal outer area; p: perforatorium; r: periopercular rim. Scale bar: 0.6 $\mu$ m.

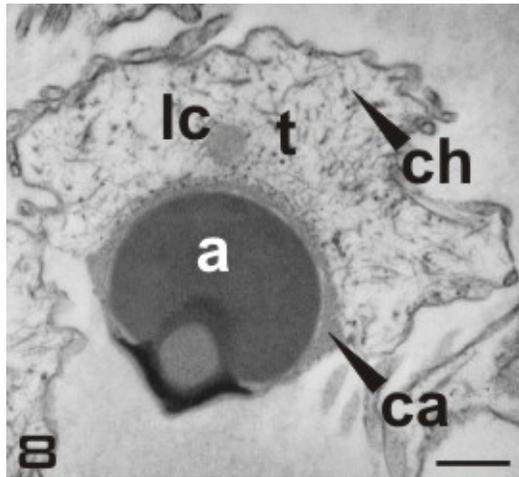


Figure 8. Tangential section of a spermatozoid. Note the thin cytoplasmic area between the acrosome and the nucleus. a: acrosome; ca: cytoplasmic area; ch: chromatin; lc: membranous lamellar structure; t: microtubules. Scale bar= 0.6 $\mu$ m