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A novel spermatozoan ultrastructure in the shrimp *Hippolyte obliquimanus* Dana, 1852 (Decapoda: Caridea: Hippolytidae)

Mariana Terossi [a], Christopher Tudge [b c], Laura S. López Greco [d] & Fernando L. Mantelatto [a]

[a] Postgraduate Program in Comparative Biology, Laboratory of Bioecology and Crustacean Systematics, Department of Biology, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto (FFCLRP), University of São Paulo (USP), Av. Bandeirantes - 3900, CEP 14040-901, Ribeirão Preto (SP), Brazil

[b] Department of Biology, American University, Washington, DC 20016-8007, USA

[c] Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20013-7012, USA

[d] Biology of Reproduction and Growth in Crustaceans, Department of Biodiversity and Experimental Biology, University of Buenos Aires, Ciudad Universitaria, Pabellón II, C1428EGA Buenos Aires, Argentina

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A novel spermatozoan ultrastructure in the shrimp *Hippolyte obliquimanus* Dana, 1852 (Decapoda: Caridea: Hippolytidae)

Mariana Terossi[a], Christopher Tudge[bc], Laura S. López Greco[d] and Fernando L. Mantelatto[a]*

[a]*Postgraduate Program in Comparative Biology, Laboratory of Bioecology and Crustacean Systematics, Department of Biology, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto (FFCLRP), University of São Paulo (USP), Av. Bandeirantes – 3900, CEP 14040-901, Ribeirão Preto (SP), Brazil;* [b]*Department of Biology, American University, Washington, DC 20016-8007, USA;* [c]*Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20013-7012, USA;* [d]*Biology of Reproduction and Growth in Crustaceans, Department of Biodiversity and Experimental Biology, University of Buenos Aires, Ciudad Universitaria, Pabellón II, C1428EGA Buenos Aires, Argentina*

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The aim of this study was to describe and illustrate the morphology of the spermatozoon of the Western Atlantic shrimp, *Hippolyte obliquimanus*. Individuals were sampled from Itaguá Beach (Ubatuba, southern Brazil). The male reproductive system was dissected and morphological analysis was undertaken using a stereomicroscope, a light microscope, and transmission electron and scanning electron microscopes. When viewed from the nuclear or acrosomal poles, each spermatozoon has many translucent radiating arms (about 20) from a denser cell body, while laterally the cell body and arms resemble a ''cnidarian medusa'', with all the arms projecting away from the bell-like cell body. This sperm morphology is distinct from the ''thumbtack''-shaped spermatozoa observed in the majority of carideans but has similarities to the spermatozoa of *Rhynchocinetes* spp. The morphology of sperm of several species of the genus *Hippolyte* resembles the spermatozoon of *H. obliquimanus* with the presence of posterior nuclear arms, but it is necessary to study other *Hippolyte* species to place these arms in the context of the genus.

Keywords: spermatozoa; morphology; nuclear arms; acrosomal spike

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Introduction

Decapod spermatozoa are divided into a spherical body, with a nucleus, a cytoplasmic lamellar region, an acrosome, and a variable number of spikes (Jamieson 1991; Jamieson and Tudge 2000; Tudge 2009). In reptantians, multiple spikes project from the nucleus (Medina and Rodríguez 1992; Tudge and Jamieson 1996; Scelzo et al. 2006; Amadio and Mantelatto 2009; Santos and Mantelatto 2011), while in natantians, a single spike originates from the acrosomal region (Bauer 1976; Sellos and Le Gal 1981; Bauer and Min 1993).

The spermatozoa of Caridea are mostly uniform in their basic morphology. The mature spermatozoon, in most species, resembles a ''thumbtack'' and consists of a cell body, a cap region, and a spike (Felgenhauer and Abele 1991; Bauer 2004). Nevertheless, there are some exceptions reported in the literature, such as the spermatozoa of *Rhynchocinetes typus* H. Milne Edwards 1837 and *Rhynchocinetes uritai* Kubo 1942 (family Rhynchocinetidae) that have coplanar radial arms (11 and 9, respectively) in addition to the typical natantian spike (Dupré and Barros 1983; Bauer and Thiel 2011), and *Thor manningi* Chace 1972 (family Hippolytidae) that has a bullet-shaped spermatozoon (Bauer 1986).

The ultrastructure of the spermatozoon of *Hippolyte* was first illustrated using transmission electron microscopy by Felgenhauer and Abele (1991) for the American species, *Hippolyte zostericola* (Smith 1873). In their review of decapod spermatozoa, they included two figures of a whole sperm cell and detail of the acrosomal spike (their figures 18.4a and b). Later, their brief description and figures of *H. zostericola* were used in the reviews of crustacean and decapod spermatozoa (Jamieson 1991; Jamieson and Tudge 2000). Sperm morphology of the Mediterranean species, *Hippolyte inermis* Leach 1815 and *Hippolyte niezabitowskii* d'Udekem d'Acoz 1996, was also recently briefly described and illustrated (Cobos et al. 2011; Manjón-Cabeza et al. 2011).

Thus, there is a lack of studies with complete descriptions of sperm ultrastructure in shrimps of the genus *Hippolyte*, especially considering the number of species described for this genus (32 species according to d'Udekem d'Acoz (1996, 2007)). In view of this

*Corresponding author. Email: flmantel@usp.br

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promising scenario for studying the sperm ultrastructure of *Hippolyte* species, the aim of this study is to describe and illustrate the morphology of the spermatozoa of *Hippolyte obliquimanus* Dana 1852. This species is endemic to the western Atlantic coast, and is the only species of *Hippolyte* that occurs in Brazilian shallow waters (Terossi and Mantelatto unpublished data).

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Material and methods

Males were collected during 2007/2008 at Itaguá Beach (Ubatuba, State of São Paulo, Brazil, 23°27′414″S, 45°03′047″W). Specimens were obtained in a water depth of approximately 1 m, from seaweed (*Sargassum* sp.), which was lifted by hand and stored in the plastic bags. After collection of the seaweed, males that were alive were transported to the Laboratory of Bioecology and Crustacean Systematics in Ribeirão Preto – University of São Paulo (USP). The specimens were anesthetized by chilling, and the carapace length (CL = maximum length from the posterior margin of the ocular orbit to the posterior margin of the carapace) was measured in order to know in what size the males begin to have spermatozoa in their vas deferens. Dissection of the vas deferens was made by means of a stereomicroscope. Spermatozoa were observed by means of a light microscope Leica DM1000 equipped with a digital camera C-7070 Olympus® to obtain photos. Details of the morphology of the spermatozoa were obtained by scanning electron microscopy (SEM) and transmission electron microscopy (TEM), in accordance with the standard methods of the Laboratory of Electronic Microscopy of the Department of Cellular and Molecular Biology and Pathogenic Bioagents, Faculty of Medicine (FMRP) – USP. For SEM, the samples (distal vas deferens) were fixed in 3% glutaraldehyde for 3 h, then transferred to phosphate-buffered saline and post-fixed in 1% OsO_4 in buffer solution. The material was dehydrated through ascending concentrations of ethanol (50–100%), and critical-point dried using ethanol as the transitional and CO_2 as the exchange fluids. Then, it was placed on stubs, sputter coated with gold, and viewed and photographed in a SEM EVO 50 (EHT = 20.00 kV) interfaced with a computer. For TEM, the procedures were similar, except that after dehydration the material was infiltrated and embedded in Epon-Araldite® resin, and thin sections were cut on an ultramicrotome Leica Ultracut S with diamond knife and then transferred to small copper grids. These were contrasted with lead citrate and uranyl acetate, viewed and photographed in a PHILIPS EM 208 TEM (EHT = 80.00 kV). Voucher specimens were deposited in the Crustacean Collection of the Biology Department of FFCLRP, USP, Brazil (CCDB/FFCLRP/USP, access numbers: 1825-1830).

In the description of the spermatozoon of *H. obliquimanus* presented here, the end of the sperm cell with the single acrosomal spike will be referred to as the acrosomal region or end, and the opposite end is called the nuclear region or end. The arms radiating out from the main cell body will be referred to as lateral.

Results

In total, 32 adult and mature males were analyzed (CL varied from 0.55 to 2.60 mm), but only shrimps larger than 0.75 mm CL had spermatozoa in their vas deferens.

The spermatozoa of *H. obliquimanus* have a convex, acrosomal portion and a multi-stellate nuclear portion (Figure 1A). It is assumed that the domed end (with or without the acrosomal spike) is the apical end of the cell and the nuclear end has about 20 arms radiating from it (Figure 1A). When viewed from the nuclear or acrosomal pole, the spermatozoa have many translucent radiating arms from a denser cell body, but when viewed laterally, the cell body and arms resemble a ''cnidarian medusa'', with all the arms projecting posteriorly or laterally from the bell-like cell body (Figure 1A). The entire cell body is approximately 4–5 μm in width and 2–3 μm in depth (without the acrosomal spike), but the amorphous nature of the nucleus, with its many arms, make it almost impossible to obtain the exact measurements. Unusually, the characteristic single acrosomal spike of the caridean spermatozoa is not visible in the light microscope images, but is obvious when viewed under SEM (Figure 1B and C). When visible, this acrosomal spike is 3.0–3.2 μm in length, tapering anteriorly from a basal width of about 0.5 μm (Figure 1B and C).

Under SEM, the distinct anteriorly directed acrosomal spike is seen at the opposite pole to the numerous radiating arms. The arms are less stellate in appearance under SEM, instead appearing randomly arranged with most directed posteriorly or laterally (Figure 1B and C). The numerous arms are not straight and extend between 1 and 2 μm from the main cell body.

When viewed with TEM, the exact subcellular nature of this unusual sperm cell can be determined. The long acrosomal spike extends anteriorly from the center of the acrosomal pole of the cell (Figure 2A). It attenuates from its base with distance from the main cell body. The acrosomal spike is bounded by the plasma membrane, but does not appear to have another interior acrosomal membrane. Its contents are darkly staining and granular, with just the faintest

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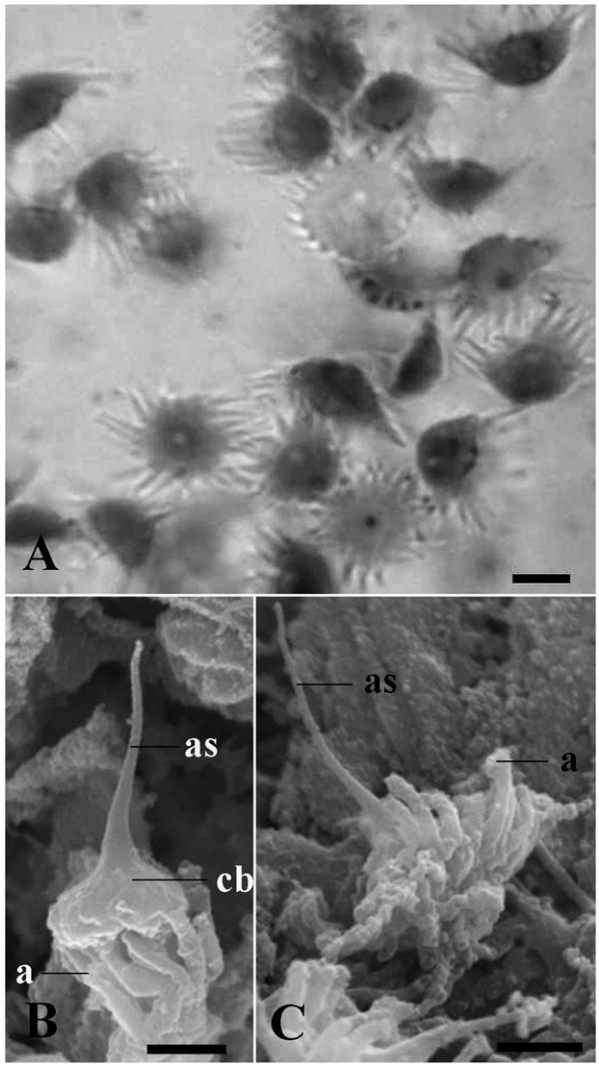

Figure 1. *Hippolyte obliquimanus* Dana 1852. Spermatozoa: (A) light microscopy; (B) and (C) SEM. Abbreviations: a, arms; as, acrosomal spike; and cb, cell body. Scale bars: A = 2 μm; B and C = 1 μm.

appearance of longitudinal striations in some sections (Figure 2B).

The long acrosomal spike meets the main cell body at a subacrosomal region termed the subacrosomal cap (Figure 2A and B). This cap forms a central, circular plate that is located at the base of the acrosomal spike and is between 1.7 and 2.0 μm, with the acrosomal spike emerging from its center. This subacrosomal region is coarsely granular and less dense than the acrosomal spike and has several obvious inclusions, including some spherical electron lucent vesicles (Figure 2B) and possibly a pair of orthogonal centrioles. These centrioles are not always visible and generally are indistinct when they are (Figure 2A and C). The subacrosomal region is most likely cytoplasmic in origin and is loosely bounded from the more posterior nuclear material by a broken membrane (Figure 2A and B). Other cytoplasmic elements, namely large mitochondria, are visible lateral to the subacrosomal region at the ends of the anterior-most radiating arms. These mitochondria are generally spherical, contain visible cristae and are separated by a membrane from the nuclear material that constitutes the arms (Figure 2A and C).

The nuclear portion of the sperm cell has numerous radiating arms (both laterally and posteriorly directed) which are made up of the nuclear material. The highly crenulated outermost boundary of the sperm cell in this region has a clearly distinguishable double membrane, representing both the nuclear and the plasma membranes (Figure 2D). This nucleo-plasma membrane surrounds the nucleus of the sperm cell which is coarsely granular and of medium electron-density in the center becoming more electron-lucent near the cell boundary and into the radiating nuclear arms (Figure 2A, C, and D). In fact, the uniformly granular and dense central nuclear material becomes progressively transformed into longitudinally striated electron-lucent material inside the radiating arms (Figure 2D).

Discussion

This study provides a valuable fourth description of sperm morphology for the caridean shrimps of the genus *Hippolyte* and is only the second study for the genus in America, which is represented by eight species (d'Udekem d'Acoz 2007). The sperm morphology is unknown in more than 85% of the *Hippolyte* species, when considering 32 recognized species (d'Udekem d'Acoz 1996, 2007). This lack of information makes a detailed comparison within and among genera extremely limited.

The sperm morphology of *H. obliquimanus* is clearly of the distinct "thumbtack" shape described for other caridean genera, such as the hippolytids *Lysmata wurdemanni* (Gibbes 1850) (Bauer and Holt 1998) and *Exhippolysmata oplophoroides* (Holthuis 1948) (Nunes et al. 2010). The overall sperm cell morphology of *H. obliquimanus* is similar to that described for *H. zostericola* by Felgenhauer and Abele (1991), but the addition of the SEM images in this study allows a more detailed account of the acrosomal spike and numerous posterior nuclear arms. Under light microscopy, the spermatozoa of *Hippolyte williamsi* Schmitt 1924 from Chile are very similar to that of *H. obliquimanus* (L. López Greco and M. Terossi, personal observation). However, the recent descriptions of *H. inermis* and *H. niezabitowskii* spermatozoa differ from those mentioned above in which no posterior nuclear arms are described or illustrated (Cobos et al. 2011; Manjón-Cabeza et al. 2011). Perhaps, the sperm cells illustrated by these authors were not fully mature, or the presence of

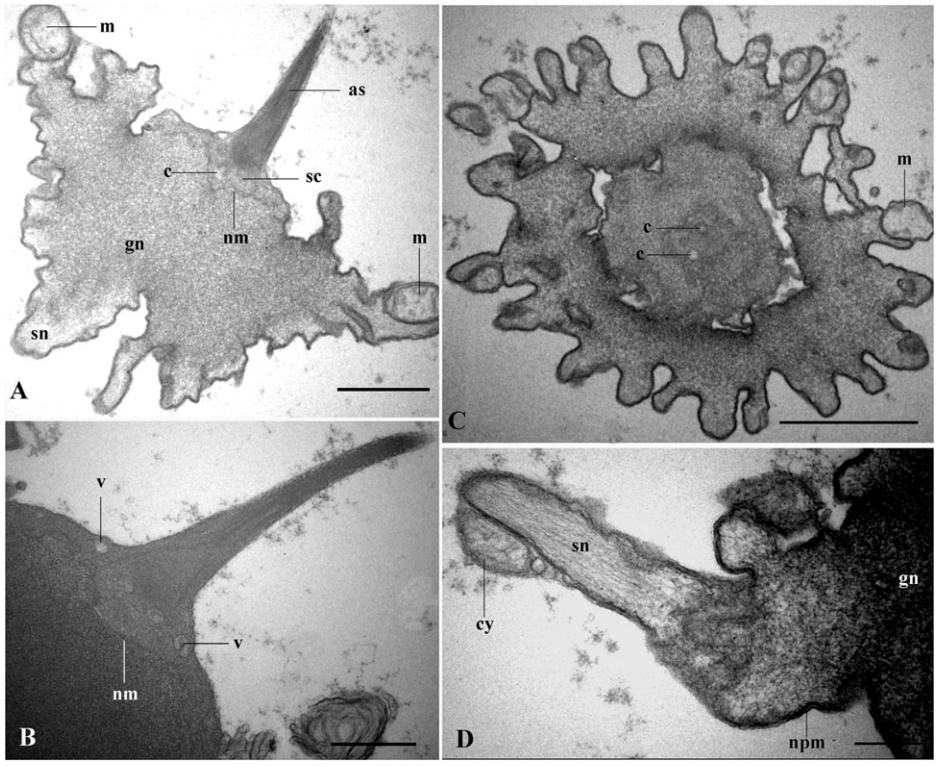

Figure 2. *Hippolyte obliquimanus* Dana 1852. Transmission electron micrographs of spermatozoa: (A) longitudinal section of a spermatozoon; (B) detail of acrosome zone; (C) transverse section of the spermatozoon; and (D) detail of the arm. Abbreviations: as, acrosomal spike; v, vesicle within the acrosomal spike; c, centriole; cy, cytoplasm; gn, granular nucleus; m, mitochondrion; nm, nuclear membrane; npm, nucleo-plasma membrane; sc, subacrosomal cap; and sn, striated nucleus. Scale bars: A and C = 1 μm; B and D = 0.5 μm.

posterior nuclear arms is not a pattern for the genus *Hippolyte*. In order to confirm these hypotheses, it is necessary to analyze the sperm cell ultrastructure of these species (*H. inermis* and *H. niezabitowskii*) and other *Hippolyte* species. The works of Cobos et al. (2011) and Manjón-Cabeza et al. (2011) confirm the presence of a single anterior acrosomal spike in this genus.

In an extensive review of decapod spermatozoa, Jamieson and Tudge (2000) provided an overview of the typical caridean shrimp sperm cell and list all caridean taxa investigated to date for spermatozoal ultrastructure. They noted the following points about caridean sperm cells: (1) the entire sperm cell generally appears as a ''tack'' shape with the single anterior acrosomal spike and a rounded or concave posterior, nuclear region. The trend is considered to be from a rounded nuclear region to the more concave nuclear shape, with the development of radiating nuclear arms as a further derivation in the nematocarcinoid *Rhynchocinetes* and some *Hippolyte* species; (2) the acrosomal spike may also be conspicuously cross-striated in some carideans (notably *Macrobrachium*, *Palaemonetes*, and *Palaemon*), but lacks striations in *Crangon*, *Paratya*, and here in *Hippolyte*; (3) most carideans have a distinct nuclear membrane separating the subacrosomal region from the nucleus, but in *Palaemonetes* and here in *H. obliquimanus* the nuclear membrane at this point is rather disrupted and discontinuous; (4) mitochondria mostly occur lateral to the nucleus but it may also be adjacent to the subacrosomal cap region; (5) centrioles have been previously observed in the subacrosomal cap region in other carideans, such as *Crangon*, *Palaemon*, and now here in *Hippolyte*; (6) the only other carideans, besides *Hippolyte*, to be recorded with the nucleus extended into radiating arms (as is common in many higher decapods) are *R. typus* and *R. uritai*, but there are only 11 and 9 arms, respectively, in these species, as compared to more than 20 seen in *Hippolyte*. *Rhynchocinetes typus* and *R. uritai* have 11 and 9, respectively, radiating nuclear arms symmetrically arranged in the same plane and they are all consistently the same size and shape (Barros et al. 1986; Perez et al. 1991; Bauer and Thiel 2011). These differ from the less symmetrical arrangement of the shorter, more amorphous arms in *Hippolyte*.

This study confirms that the sperm morphology of *H. obliquimanus* has characteristics expected in the genus and family Hippolytidae, and has morphological characteristics found in congeneric species, as well as other carideans like *Rhynchocinetes* spp. However, this

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is a large genus with 32 species worldwide (d'Udekem d'Acoz 1996, 2007), so the available knowledge concerning the morphology of the spermatozoa in only four species (including this study) does not represent its complete biodiversity. Thus, future studies are encouraged to improve our understanding of the phylogeny and evolution of members of this diverse genus.

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