SPERM ULTRASTRUCTURAL FEATURES OF THE BATHYAL OCTOPOD GRANELEDONE GONZALEZI

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ABSTRACT. – The fine structure of the octopod Graneledone gonzalezi spermatozoa is described by electron microscopy. The acrosome is the longest ever found in Octopodidae. It consists of a long striated cone surrounded by a single helix, which is defined by a numerical expression. The nucleus is rod shaped and one of the largest in Octopodidae. The nuclear fossa reaches up to the fifth part of the nucleus acting as a flagellar root due to its connection with the axoneme-coarse fibres (ACF) via the centriole. Using the morphological characteristic of the sperm, the relationship of Graneledoninae within the family Octopodidae is discussed.

INTRODUCTION

The use of molecular techniques in cephalopod phylogeny has proved to be a useful tool in octopods (Carlini et al. 2001, Guzik et al. 2005). However, these attempts have led to some results that are not easily interpretable in light of other characters. On the other hand, an ideal description of a cephalopod will include morphological, meristic, ecological, ethological, and biochemical characters (Nixon 1998). Moreover, the importance of a broad comparative analysis of taxonomic and systematic characters to construct an accurate systematic, taxonomy and phylogeny of any cephalopod taxon has been recognized (Vecchione 1998).

Spermiogenesis and sperm ultrastructure have provided important clues in defining the taxonomic position and phylogenetic relationships between many groups of molluscs including cephalopods (e.g. Franzén 1955, Galangau & Tuzet 1968a, b, Longo & Anderson 1970, Hou & Maxell 1992, Healy 1988, 1989, 1990a, b, 1993, Selmi 1996, Zhu et al. 2005). The present paper is focused on a member of the subfamily Graneledoninae (Octopoda: Octopodidae). The diagnostic characters of this subfamily were summarized by Voss & Pearcy (1990). Unfortunately, it was not possible to include any reference to other examples of the subfamily’s sperm morphology, because no sperm data were available.

Graneledone gonzalezi Guerra, Gonzalez & Cherel, 2000 is a bathyal species, which inhabits the upper continental shelf (510-540 m) off the Kerguelen Islands (southwestern Indian Ocean). The aim of this paper is to describe the sperm morphology of this species and to compare it with that of other Octopodidae.

MATERIAL AND METHODS

Spermatophores were extracted from a preserved specimen of G. gonzalezi held in the collection of the Instituto de Investigaciones Marinas. The specimen (84 mm mantle length, 334 g body weight) was bottom trawled up by the vessel “Kerguelen de Tremarec” at 510-540 m depth off Kerguelen Islands, 43º13’-47º18’S and 69º09’-69º16’E, February 1994 (Guerra et al. 2000). The animal was frozen (-20 ºC) on board and fixed in 4 % buffered formalin in sea water for 24 hours, then preserved in 70 % ethanol.

For scanning electron microscopy (SEM), a piece of spermatophore was fixed during 4 h in 2.5 % glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.3 at 4 ºC) and washed for 30 min in the same buffer. The sample was then dehydrated in a series of ethanol, critical point-dried in CO₂ using a Polaron E3000 and sputter-coated in a Polaron SC500 using 60 % gold-palladium. Samples were then examined with a Philips XC30 SEM operating at 10-20 kV.

For transmission electron microscopy (TEM), sections of spermatophores were fixed in 3.0 % glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.2 for 12 h at 4 ºC, washed in the same buffer for 4 h at 4 ºC and then post-fixed in buffered 2.0 %
It has a head (nucleus and acrosome) of 44 µm, and a tail of 971 µm long. The mature spermatozoon is approximately 971 µm long.

Table I: Sperm cell measurements (in µm, unless stated otherwise) of *Graneledone gonzalezi*. Mean, standard deviation (SD) and number of spermatozoa measured (n).

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Mean, SD &amp; n</th>
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<tbody>
<tr>
<td>Sperm cell length 1</td>
<td>971.4 ± 52.6 (n = 8)</td>
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<tr>
<td>Head length 2</td>
<td>43.81</td>
</tr>
<tr>
<td>Tail length</td>
<td>927.59</td>
</tr>
<tr>
<td>Acrosome (Acr.) length 3</td>
<td>9.89 ± 0.46  (n = 15)</td>
</tr>
<tr>
<td>Acr. max width</td>
<td>0.53 ± 0.02  (n = 16)</td>
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<tr>
<td>Acr. min width</td>
<td>0.17 ± 0.01  (n = 16)</td>
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<tr>
<td>Acr. striation separation</td>
<td>56.8 ± 1.7 nm (n = 23)</td>
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<tr>
<td>Acr. sub-striation separation</td>
<td>28.4 ± 1.5 nm (n = 22)</td>
</tr>
<tr>
<td>Spire striation</td>
<td>26.5 ± 3.6 nm (n = 39)</td>
</tr>
<tr>
<td>Angle of spires</td>
<td>47.0° ± 2.62° (n = 22)</td>
</tr>
<tr>
<td>Nucleus length</td>
<td>33.92 ± 1.58 (n = 10)</td>
</tr>
<tr>
<td>Nucleus top width</td>
<td>0.53 ± 0.03  (n = 11)</td>
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<tr>
<td>Nucleus posterior width 1</td>
<td>0.85 ± 0.03  (n = 10)</td>
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<tr>
<td>Nuclear membrane</td>
<td>20.0 ± 2.1 nm (n = 20)</td>
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<tr>
<td>Nuclear fossa length</td>
<td>6.29 ± 0.34  (n = 9)</td>
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<tr>
<td>Neck length</td>
<td>1.58 ± 0.09  (n = 5)</td>
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<tr>
<td>Neck width</td>
<td>0.61 ± 0.05  (n = 10)</td>
</tr>
<tr>
<td>Middle piece length</td>
<td>7.38 ± 0.43  (n = 17)</td>
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<tr>
<td>Middle piece width</td>
<td>0.67 ± 0.04  (n = 15)</td>
</tr>
<tr>
<td>Annulus length</td>
<td>1.26 ± 0.04  (n = 5)</td>
</tr>
<tr>
<td>Annulus total width</td>
<td>0.64 ± 0.02  (n = 14)</td>
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<tr>
<td>Annulus constriction width</td>
<td>0.38 ± 0.07  (n = 4)</td>
</tr>
<tr>
<td>Axoneme-coarse fibres complex</td>
<td>0.33 ± 0.01 (n = 13)</td>
</tr>
<tr>
<td>Principal piece diameter</td>
<td>0.41 ± 0.01  (n = 11)</td>
</tr>
</tbody>
</table>

1 Measurements obtained from broken sperm cells
2 Acrosome length + Nucleus length
3 Measured at the level of the condensed chromatin

Table I. – Sperm cell measurements (in µm, unless stated otherwise) of *Graneledone gonzalezi*. Mean, standard deviation (SD) and number of spermatozoa measured (n).

osmium tetroxide for 4 h at the same temperature. After dehydration in a graded ethanol series, the fragments were embedded in Epon, sectioned with a diamond knife, double-stained with uranyl acetate and lead citrate, and observed in a JEOL 100CXII TEM operated at 80 kV.

Spermatozoa measurements were taken using an imaging data processor NIS-Elements D 2.30. In order to describe the helicoidal structure of the acrosome we measured the distance between spires, which correspond to a complete turn of the helix in 16 acrosomes. The distance between spires was compared with the number of spires using simple linear regression (Fig. 1).

RESULTS

Spermatozoa measurements are summarized in Table I. The mature spermatozoon is approximately 971 µm long. It has a head (nucleus and acrosome) of 44 µm, and a tail or flagellum of 927 µm. The acrosome is constituted by a long striated cone, surrounded by homogeneous material arranged in a single helix of almost 9 turns. The separation between spires significantly decreases towards the apex (Figs. 1, 2B). In longitudinal sections, the acrosome is composed of a highly compact and electron opaque substance, which is aggregated periodically to form dense striations oriented perpendicular to the long axis of the spermatozoon (Fig. 2C). Among these striations (Fig. 2D, arrowheads), exactly at the middle, there is a less electron dense double striation that we call sub-striation (Fig. 2D, arrow). Between the striations and the acrosome membrane there is a thin space filled with homogeneous material that constitutes the spires (Figs. 2C, D). The plasma membrane is tightly connected with the acrosome membrane leaving little space to allocate the cytoplasmic substance or periacrosomal material, which appears as a thin layer of electron dense granules (Fig. 2D). Longitudinal sections of the spires show parallel striations along its length (Fig. 2A arrowheads). The junction between the acrosome and the nucleus is flat, and a narrow cytoplasmic lacuna of electron lucent material can be observed (Fig. 2A, D).

The nucleus is rod shaped. The plasma membrane is electron dense and closely linked to the nuclear membrane (Figs. 2D, E). Cross sections at the nuclear fossa level, show three layers under the plasma membrane (Fig. 2E): i) an outer layer or nuclear membrane, encircling the nucleus; ii) the nucleus or chromatin layer which is optically very dense; and iii) the nuclear fossa, which is optically less dense than ii, surrounded by the nuclear membrane. The nuclear fossa extends from the anterior part of the centriolar fossa up to, approximately, the fifth part of the nucleus (Fig. 2A).

Basally, the nucleus exhibits an invagination allowing the attachment for the tail (Figs. 2A, F). The first part of the invagination, which encircles the centriolar fossa is called the neck (Fig. 2F). The latter accommodates the proximal part of the flagellum and the centriole. The nuclear membrane coats the centriolar fossa and the nuclear fossa to its end (Figs. 2A, E). The centriole gives rise to the axoneme-coarse fibres complex (ACF), the axis of the flagellum (Fig. 2F).

The tail can be divided into three parts: middle, principal and end pieces. The middle piece is constituted by the ACF surrounded by the mitochondrial sheath, a fibrous sheath and the plasma membrane (Figs. 2F, G). A remarkable feature is the electron dense membrane that surrounds the ACF (Fig. 2G, arrow). The mitochondrial sheath is composed by 9 rounded, well-defined and elongated mitochondria that run parallel to the ACF axis (Figs. 2F, G). Plasma membrane and fibrous sleeve are folded in the distal region of the middle piece forming a cylindrical, optically dense and smooth structure, the annulus (Figs. 2F, H). At its apex, it has a constriction that limits the mitochondrial sheath (Fig. 2F, arrow). No mitochon-
Fig. 2. – A. Graneledone gonzalezi, a composite picture showing the sperm organization. Arrowheads: parallel striations along the spires. B. Scanning electron micrograph of the acrosome. C. Transmission electron micrograph (TEM) of the acrosome in longitudinal section showing the periodic striations. D. Longitudinal section of the acrosome-nucleus junction. Arrowheads: periodic striations, arrow: double sub-striations (TEM). E. Nucleus in cross section at the nuclear fossa level (TEM). F. Longitudinal section through the neck and middle piece. Arrow: annulus constriction (TEM). G. Middle piece in cross section. Note the membrane (arrow) that surrounds the axoneme-coarse fibres complex (TEM). H. Annulus in cross section (TEM). Abbreviations: A, acrosome; AM, acrosome membrane; ACF, axoneme-coarse fibres complex; An, annulus; Ce, centriole; CF, centriolar fossa; Cr, chromatin; FS, fibrous sheath; MP, middle piece; M, mitochondria; MS, mitochondrial sheath; Ne, neck; NF, nuclear fossa; NM, nuclear membrane; N, nucleus; PA, periacrosomal material; PM, plasma membrane; PP, principal piece; S, spires. Scale bars: A = 2 µm; B = 1 µm; C = 2 µm; D = 0.2 µm; E = 100 nm; F = 1 µm; G = 100 nm; H = 100 nm.
drial sheath is present at the principal piece (Figs. 2A, F). The principal piece’s diameter gradually diminishes towards the end piece, due to the reduction of the coarse fibres.

**DISCUSSION**

In the present paper, the acrosome helix was defined with a numeric expression (Fig. 1). In our view, this description provides an accurate tool to define the complexity of the acrosome.

Comparison of the acrosome lengths in the different Octopodidae studied to date (Galangau & Tuzet 1968, Longo & Anderson 1970, Martin et al. 1970, Maxwell 1974, Selmi 1996, Zhu et al. 2005, Roura et al. in press) reveals that the acrosome of *G. gonzalezi* is the longest ever reported. Morphologically, it resembles those of Octopodinae (Galangau & Tuzet 1968a, Longo & Anderson 1970, Martin et al. 1970, Zhu et al. 2005), because it is constituted by a single helix surrounding the acrosome. This feature distinguishes Graneledoninae from Bathypolypodinae, since the latter has a double helix surrounding the acrosome (Roura et al. in press). It can also be used to differentiate between Graneledoninae and Eledoninae, the latter showing an acrosome which is totally torsioned (Maxwell 1974, Selmi 1996). The extension of the inner cone to the top of the acrosome is a feature shared by Octopodinae, Bathypolypodinae and Graneledoninae. In contrast, the above extension is shorter in Eledoninae. Furthermore, the inner cone can be used as a character to distinguish between cirrates and incirrates. The incirrate octopods have an inner cone with striations oriented perpendicularly to the long axis of the spermatozoon (Galangau & Tuzet 1968a, Leik 1970, Longo & Anderson 1970, Healy 1989, Selmi 1996, Ribes et al. 2002, Zhu et al. 2005, Roura et al. in press). However, cirrate octopods do not have this inner cone, although they show few (two or three in *Opisthoteuthis persephone*) striations at the base of the acrosome (Healy 1993).

The nuclear length is a helpful character to discern between the different subfamilies of Octopodidae. The subfamily with the largest nucleus is Bathypolypodinae, where the nucleus reaches from 66 up to 86 µm (Roura et al. in press), followed by Eledoninae with nuclear lengths ranging from 37.5 up to 40 µm (Selmi 1996). Among the families studied, Graneledoninae, with 33.9 µm, and Octopodinae with lengths from 10 up to 21 µm, are the subfamilies studied to date, such as the radula, statoliths and beaks (Nixon 1998).

**REFERENCES**


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