

## Short communication

A peculiar new virus-spermatozoon association in the bug *Raphigaster nebulosa* (Poda) (Heteroptera-Insecta)

David Mercati, Romano Dallai\*

Department of Life Sciences, University of Siena, via Aldo Moro 2, 53100 Siena, Italy

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## ABSTRACT

The sperm of the heteropteran bug *Raphigaster nebulosa* (Poda) are of two types, differing in length and size of their flagella. The thicker sperm are shorter than the thinner ones and have large mitochondrial derivatives. The presence of virus particles associated with the plasma membrane of thinner sperm is described for the first time; thicker sperm are immune to virus infection. The fact that virus particles are present on thinner sperm only initiates considerations on the transmission of virus.

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## 1. Introduction

Pentatomidae are one of the largest families of Heteroptera. As in several other insect orders, i.e. Diptera, Hemiptera, Lepidoptera and Coleoptera (Jamieson et al., 1999; Swallow and Wilkinson, 2002; Araújo et al., 2010), sperm polymorphism exists among pentatomid species (Araújo et al., 2011) with two types of sperm differing in the length and size of their heads and flagella (Bowen, 1922; Schrader and Leuchtnberger, 1950; Jamieson et al., 1999; Araújo et al., 2010).

During an ultrastructural study on sperm structure in the pentatomid *Raphigaster nebulosa* (Mercati et al., 2009) we found two sperm types, one having larger mitochondrial derivatives. We also found numerous virus particles associated with the plasma membrane of the thinner sperm type. Here we describe the characteristics of these viruses for the first time.

## 2. Material and methods

Ten males and two females of *R. nebulosa* (Poda) were collected on flowers of *Ferula* sp. (Umbelliferae) in the Siena area (Mt. Amiata). The material was dissected in 0.1 M phosphate buffer containing 3% sucrose (PB) and fixed in 2.5% glutaraldehyde in PB

overnight at 4 °C. After thorough rinsing, it was post-fixed in osmium tetroxide for 2 h at 4 °C. Dehydration in an ethanol series was followed by embedding in Epon-Araldite resin. Part of the material was fixed according to Dallai and Afzelius (1990) using tannic acid and omitting osmium fixation. Ultrathin sections, obtained with a Reichert ultramicrotome, were stained with uranyl acetate and lead citrate and observed with a Philips CM 10 electron microscope, operating at 80 kV.

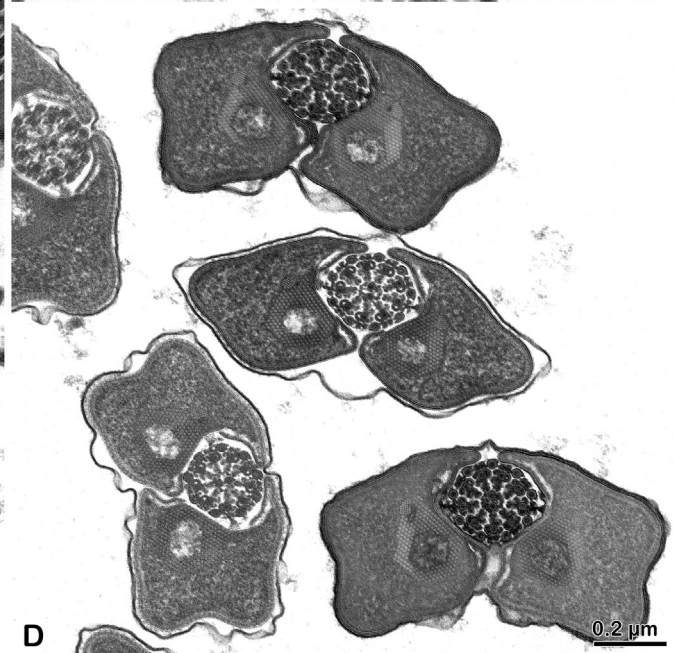
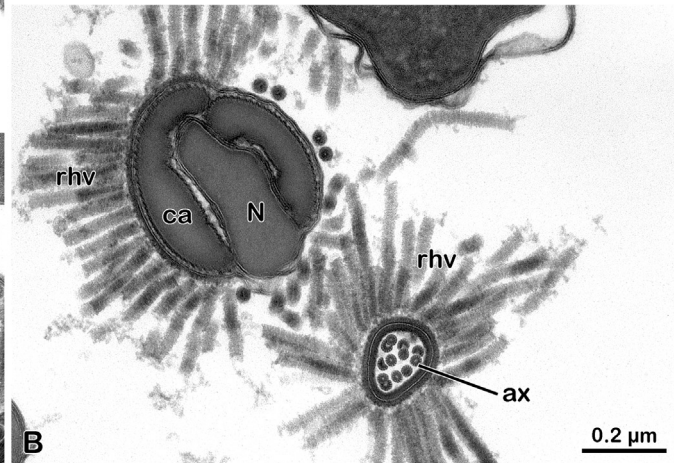
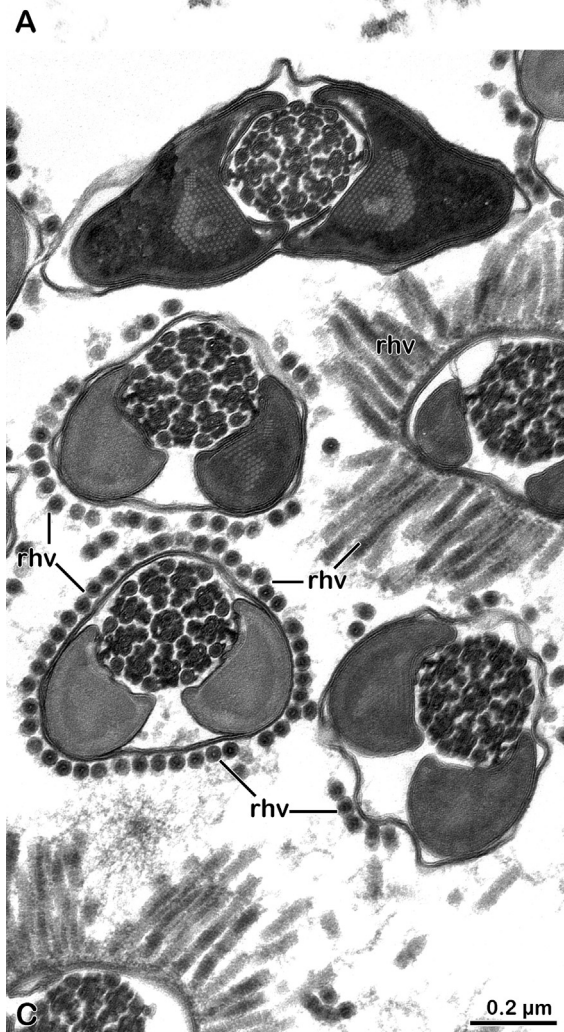
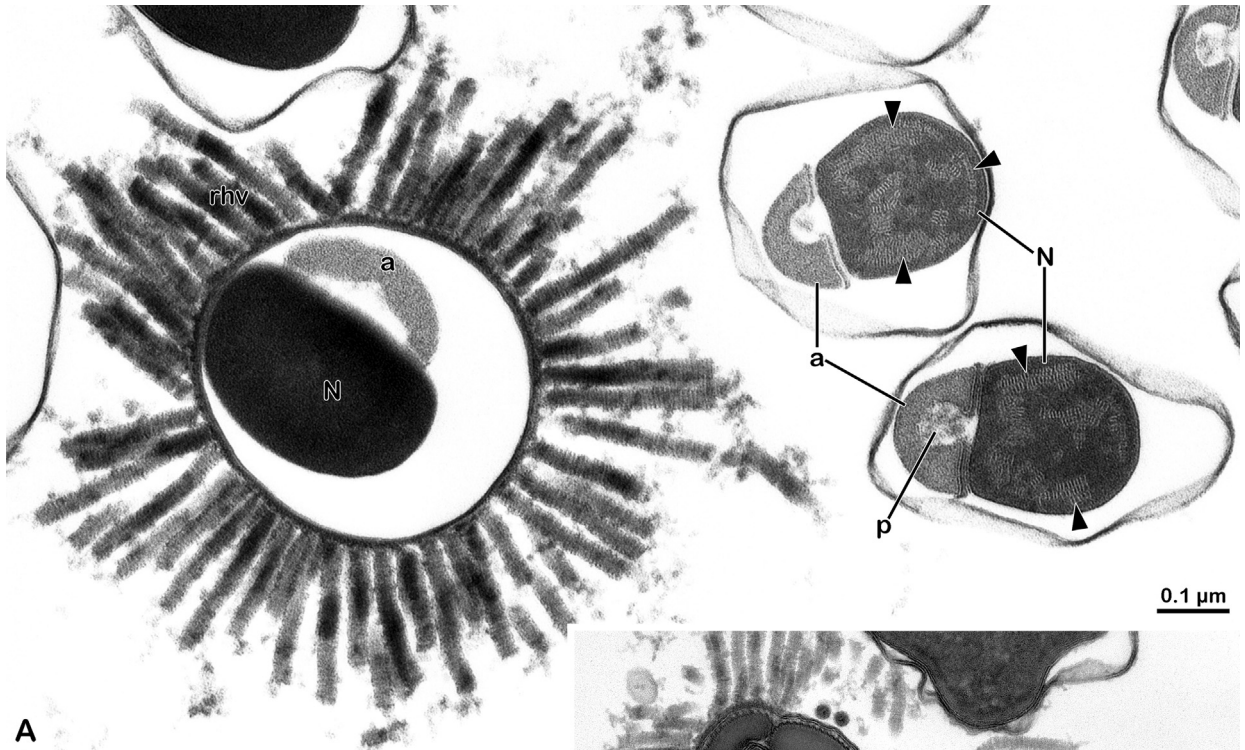
## 3. Results

Testes and seminal vesicles of *R. nebulosa* contained bundles of sperm, which showed the features of sperm of other Heteroptera (Jamieson et al., 1999; Mercati et al., 2009). Two types of sperm were distinguished, one about twice the width of the other (Fig. 1). Thinner sperm were about 240 µm long and about 0.65 µm wide at cross-section of the flagellum; thicker sperm were about 215 µm long and 1.30 µm wide. A few sperm showed a flagellum cross-section as wide as 3.1 µm. While the cross-section of the thinner sperm flagellum was of quite uniform shape, consisting of moon-like mitochondrial derivatives embracing the central axoneme, the appearance of the thicker sperm varied along the length of the flagellum: in the anterior region the whole section had a rectangular outline while towards the posterior region the section was slightly flat (Fig. 1A, C, D).

Two specimens of the ten males examined showed evidence of virus infection (Figs. 1 and 2). Virus shape and structure suggested a rhabdovirus species (Kitajima and Costa, 1979; Francki et al., 1981;

\* Corresponding author. Tel.: +39 0577 234412.

E-mail addresses: [david.mercati@unisi.it](mailto:david.mercati@unisi.it) (D. Mercati), [romano.dallai@unisi.it](mailto:romano.dallai@unisi.it) (R. Dallai).



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