

## Short Paper

# Sperm–egg interaction in the palaemonid shrimp *Palaemonetes vulgaris*

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Sperm of the decapod crustaceans are non-motile, having no flagella and hence no ability to swim toward eggs. Ultrastructural studies have reported no apparent acrosomal structure in sperm of caridean shrimp.<sup>1,2</sup> In contrast, sperm of other decapod groups, such as Penaeidea, Astacidea, Palinura, Anomura and Brachyura, possess acrosomal structures,<sup>1,3–10</sup> and drastic structural changes of sperm morphology accompanied with acrosomal reaction have been observed upon sperm–egg contact.<sup>1,3,4,6</sup> Sperm–egg interaction in caridean shrimp has been observed only in the freshwater shrimp *Macrobrachium rosenbergii*,<sup>11,12</sup> where the ‘thumb tack-’ or ‘everted umbrella-’shaped sperm was observed to penetrate the egg membrane with its spike. In this report, we present several scanning electron microscope images of sperm–egg interaction in another palaemonid shrimp, the estuarine grass shrimp, *Palaemonetes vulgaris*.

Adult *P. vulgaris* were obtained from Goose Creek in Charleston County, South Carolina, USA. Female and male were held separately in 38 L aquaria at 25°C. An artificial photoperiod of 12 h light and dark was kept, and faint light was maintained in the dark period for observation. Females underwent the pre-spawning molt usually in the dark period, and they were transferred to an aquarium containing males to allow mating. Mated females were removed and isolated in another aquarium. Onset of spawning was observed as the initial flowing of eggs into the abdominal brood chamber. In caridean shrimp, contact between eggs and non-motile sperm apparently occurs as the eggs exit the ovipores and

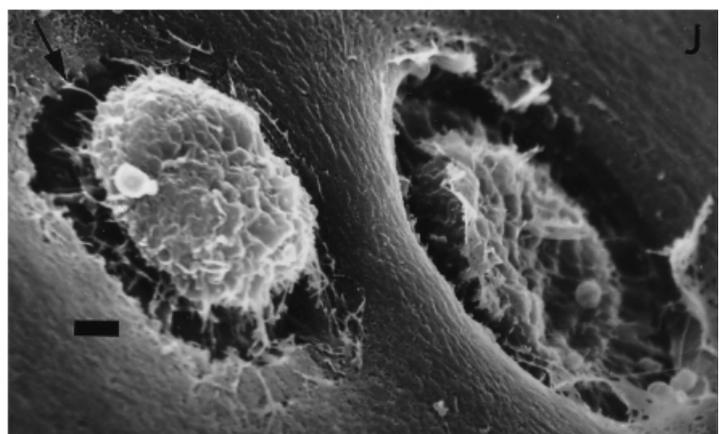
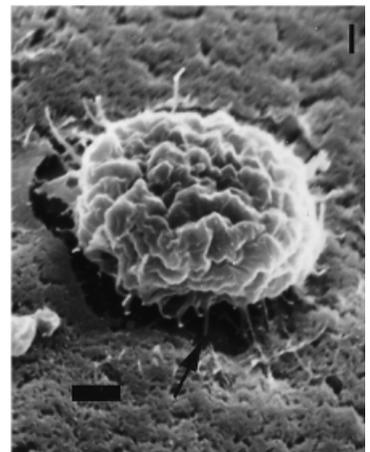
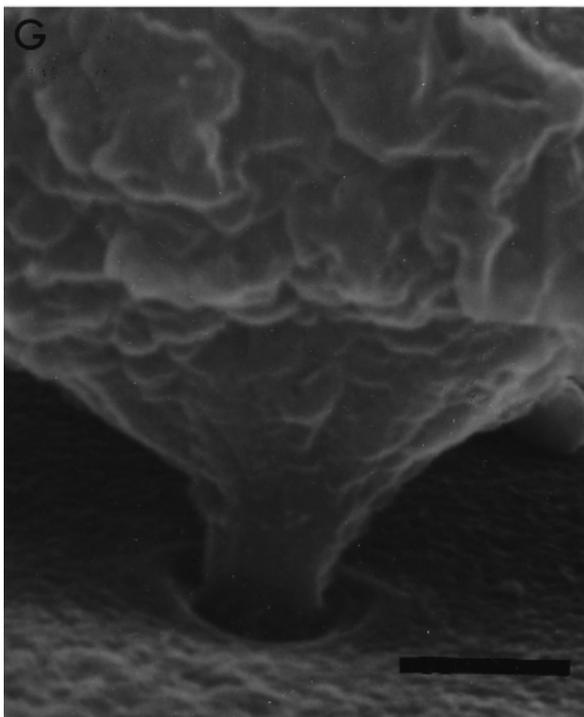
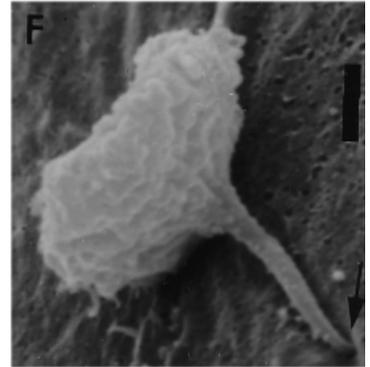
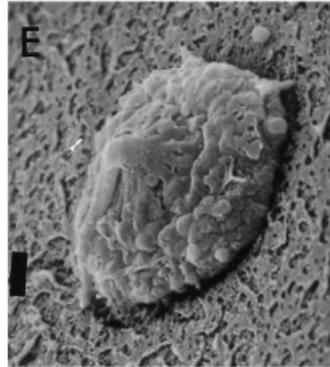
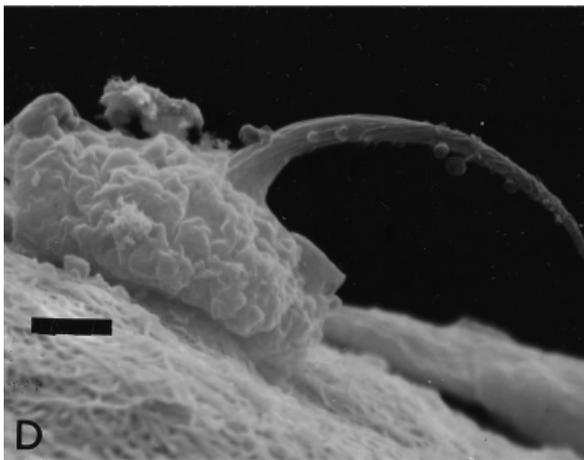
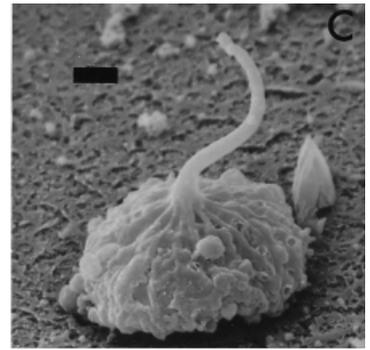
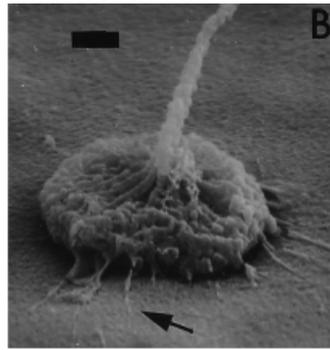
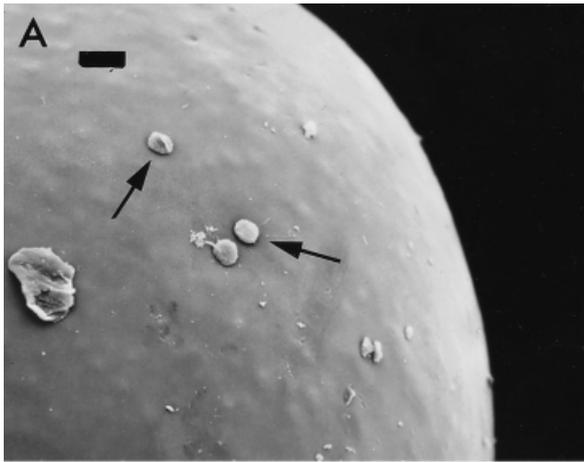
enter the narrow, closed channel and brood chamber formed by the female pleopods and pleura.<sup>2,11</sup> Samples of immediate post-spawning (30 s to 5 min) eggs were collected from three females. Each female was gently captured by a dip net and the eggs in its brood chamber were removed by a pipette. The eggs were prefixed in 5% glutaraldehyde-0.07 M sodium cacodylate solution (pH 7.4) at room temperature, kept for 4 to 12 h at 4°C and postfixed with 0.1% OsO<sub>4</sub> in 0.1 M phosphate buffer (pH 7.4) for 2 h at 4°C. The post-fixed eggs were rinsed in 0.1 M sodium cacodylate buffer (pH 7.4) containing 7% sucrose for a minimum of 1 day, dehydrated by alcohol series, and dried under critical point, followed by sputter-coating with gold for the scanning electron microscope observation. The eggs were observed by a scanning electron microscopy of a JEOL JSM 35C instrument at the Medical University of South Carolina.

Scanning microscopic images are shown in Fig. 1. Multiple sperm cells were observed to attach to the egg outer investment coat (OIC) of almost all eggs (Fig. 1a). The sperm displayed the typical palaemonid morphology of a cupped base with a single spike and were associated with the OIC in variable orientations (Fig. 1b–f). Some sperm cells were observed to penetrate the OIC (Fig. 1g–j). Several phases of sperm–egg interaction were sometimes observed even on a single egg. The arrangement of our sequence of images of sperm–egg interaction for *P. vulgaris* followed that reported for *M. rosenbergii*.<sup>12</sup>

In *P. vulgaris*, when the cupped base of the sperm attached to the OIC, filamentous strands were observed to project from the sperm base, usually in association with the OIC (Fig. 1b). As reported for *M. rosenbergii*, the sperm spike then

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**Fig. 1** Scanning electron microscope images of sperm–egg interaction. (a) The egg surface, showing several sperm cells attaching to the egg investment (arrows). Bar = 20  $\mu\text{m}$ . (b–e) Sperm attaching to the egg investment by its base. Each bar = 2  $\mu\text{m}$ . (b) Several filamentous strands (arrow) are observed to associate with the egg investment. (c) Spike begins bending. (d) Spike shows prominent bend toward the egg investment. (e) The point of the spike reaches the egg investment. (f) The sperm base detaches from the egg investment, and the point of the spike penetrates into the egg investment (arrow). Bar = 2  $\mu\text{m}$ . (g–j) Sperm cells penetrating into the egg investment. Each bar = 2  $\mu\text{m}$ . (g) A spike is almost completely entered into the egg investment. (h) A depression is formed in the egg investment as sperm penetration continues. (i) A gaping hole is formed in the egg investment and filamentous strands (arrow) are associated with the hole. (j) Two sperm are penetrating at adjacent positions.

apparently began to bend (Fig. 1c,d) until the tip made contact with the OIC (Fig. 1e). The sperm base was partially detached from the OIC and the tip of the spike pointed to the OIC (Fig. 1f). The sperm base was then entirely detached from the OIC and lifted as the spike penetrated further into the OIC (Fig. 1g). As the sperm continued to penetrate the OIC, a depression (Fig. 1h) was formed in the OIC. This depression yielded a gaping hole in which the base of the sperm sank (Fig. 1i,j). Filamentous strands projected from the underside of the sperm base and appeared to associate with the gaping hole (Fig. 1i,j).

The penetration of individual eggs by multiple sperm (Fig. 1j), suggests that polyspermy might be functional or compensatory for ensuring fertilization in such non-motile sperm. Further, as noted previously for *M. rosenbergii*,<sup>12</sup> there was no apparent acrosomal reaction in *P. vulgaris*. In external morphology, the sperm of caridean and penaeid shrimp resemble each other. However, structurally they show significant dissimilarities. In particular, the spike of the penaeid sperm is believed to be an elaborate acrosome complex,<sup>7</sup> while the spike of caridean sperm consists of filaments.<sup>2</sup> Furthermore, the spike of the penaeid shrimp sperm is depolymerized upon sperm–egg contact,<sup>6</sup> and such reaction apparently does not occur in caridean sperm.<sup>12</sup> Upon contact with environmental water the OIC begins to hydrate,<sup>13</sup> and the sperm must enter the egg before entry is blocked by this coat.

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