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Technical dissection aspects for obtaining giant sperm

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The length of ostracod cypridoidean spermatozoa is exceptional, in both absolute and relative values. However, only a small number of sperm measurements have actually been done in the past (e.g., Müller 1884; Bauer 1940; Gupta 1968; Wingstrand 1988; Matzke-Karasz 2005) and have ever since been referred to in the literature. In order to provide a broad overview on cypridoidean sperm lengths, we aimed at dissecting a large number of different species.

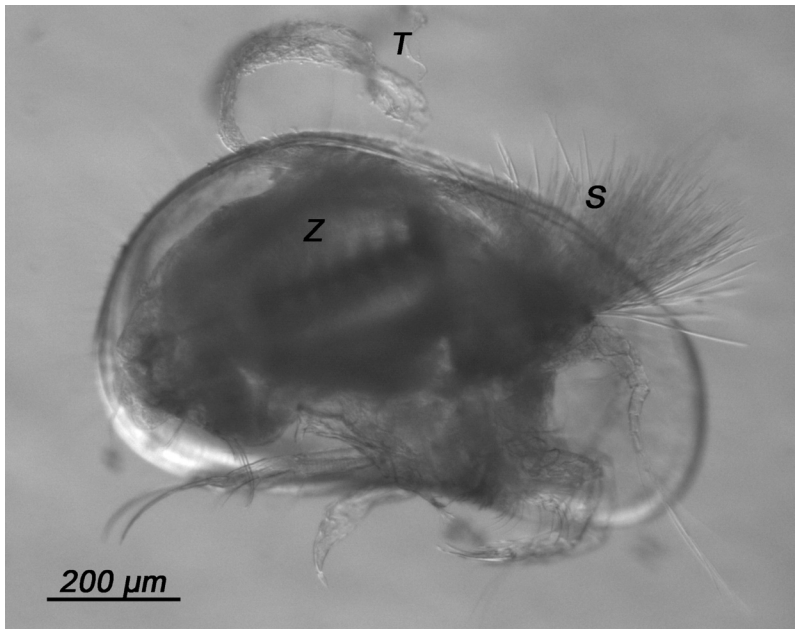

Fig. 1: Dissection of a male *Pseudocandona marchica*, right valve removed. The storage area for mature sperm cells is located anterio-dorsally in the male animal, in a distal loop of one of the spermducts behind the naupliar eye. In this dissection, one sperm duct has been opened and dozens of spermatozoa (S) protrude from the duct. T: Testes from right body side. Z: Zenker organ.

We found that particularly in species with very long sperm, it is very important to optimize the dissection techniques rather than apply the routine dissection mode used for species identification.

In male cypridoidean ostracods, the giant sperm are stored in the anterior-dorsal body area, housing the part of the sperm duct that lies in front of the sperm pump, or Zenker organ. Here, mature sperm are accumulated prior to insemination of females. Only from this part of the ostracod body can mature giant sperm be obtained in larger amounts.

Dissected ostracods were fixed in ethanol, but dissections were carried out in distilled water. After opening the carapaces with etched tungsten needles, valves were stored dry, appendages dissected in Hydromatrix® (mounting media by Micro-Tech-Lab) separately, and sperm were subsequently treated in water. Sperm separation was done on chrome-alum-gelatine coated glass slides, which provide a high water surface tension and thus a high water drop (good for needle dissection) and a sticky surface (good for sperm attachment). In order to obtain isolated sperm cells in a single focus level, we aimed to separate the cells without damage and finally let the preparation air dry to make the cells attach firmly to the slide. Cover slips were then mounted with Hydromatrix®.

In some taxa, the sperm form a loose aggregate that can easily be teased apart with finest dissection needles. However, in other taxa, the long filamentous sperm are stored in a densely packed bundle, forming a ring. Sometimes, the sperm are firmly attached to their neighbours at their posterior ends. Since single, non-folded or crossed sperms are required for measurements, such “sticky” sperm had to be separated: we tested digestion in weak KOH solution and application of ultrasound.

Following to the application of these techniques, either as stand-alone treatments, or in combination, we conclude that the technique of dissection to obtain complete sperm has to be adjusted to each species (or sometimes genus). Only by doing so, the optimal dissection result, in terms of isolated and thus measurable sperm cells, can be achieved. Our poster will show different dissection results and discuss problems and solutions in detail (Fig. 1).

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