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Evidence of sexual intercourse involving giant sperm in Cretaceous ostracode revealed by holotomography

The micro-crustacean ostracods are the most abundantly preserved arthropods in the fossil record dating from at least the Ordovician (450 million years) (1). However, their body and appendages are only rarely preserved. One example of high quality soft parts preservation is *Harbinia micropapillosa* (Bate, 1972) (2) (Cyprididae, Cypridoidea) from the Cretaceous Santana Formation of Brazil. Because scanning electron microscopy (3) cannot view internal features we performed holotomography of five *H. micropapillosa* specimens, three males and two females, revealing the morphology of their internal reproductive system.

We compared sub-micron inline quantitative phase X-ray synchrotron tomography (holotomography) (4) on the beamline ID19 of the ESRF (Grenoble, France) on specimens of *H. micropapillosa* and of the extant species *Eucypris virens* (Jurine, 1820). Phase contrast based microtomographic techniques have been applied to non-destructively image microfossils (5), but application of phase retrieval on dense and complex fossils is a recent and powerful approach in paleontology (4). Extant cypridoidean ostracods possess an intricate reproductive system comprising about a third of the volume of the body. In both genders, the reproductive organs are divided into two separately functioning systems on both sides of the body. The males have two large sperm pumps (Zenker organs), modified from the posterior section of the vas deferens. The females

possess epithelial recipients (seminal receptacles) at the end of long ducts originating in the two vaginal openings. Empty seminal receptacles of virgin females are folded up; the volume of transferred sperm gives the receptacles their shape and size after mating. These specialized reproductive systems are required for maneuvering the giant sperm — aflagellate, but filiform sperm cells. Sperm lengths range across species from several hundred microns to millimeters and thus were often longer than the ostracods themselves. Their resistant sheaths allow fossil sperm to be recovered from 5000 years old ostracods from Neolithic excavations (6).

3D processing of holotomographic data revealed that three male specimens of *H. micropapillosa* contained paired hollow tubes in the posterior part of the body suggestive of Zenker organs. Two female specimens had paired cavities near the mid-point of the body, corresponding to the seminal receptacles in recent Cyprididae, which are only known from ostracods reproducing with giant sperm. The receptacles must have been filled with sperm in order to be preserved as two cavities. Thus, giant sperm developed in cypridoidean ostracods c. 100 million years ago.

Reproduction with giant sperm, mainly studied in *Drosophila* species, occurs in distinct groups scattered over the animal kingdom. The influence of different selection pressures on this character have been examined, but its long-term stability was unknown. In spite of potential costs, this trait appears to be long-lived even in geological time-scales. The wide occurrence of giant sperm in living members of Suborder Cypridocopina, coupled with our fossil evidence suggest that it evolved only once in the group, unlike in *Drosophila* (7). Given that sperm size provides an assay “for comparative analyses of the strength of sexual selection in (…) species without postmating parental investment” (8), the persistence of reproduction with giant sperm through geological time may add a criterion to test for the pressure of sexual selection with holotomography recovering data from the past through exceptionally preserved (micro-) fossils.

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