Секция «Зоология беспозвоночных»

Upgrading Preparation of Marine Tardigrades for Scanning Electron Microscopy by Using Critical Point Drying (CPD) Technology

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Tardigrada is a group of microscopic widespread invertebrate water animals. Obtaining high-quality images of aquatic animals on a scanning electron microscopy requires experimental selection of optimal drying conditions. The most common for drying is the CPD technology. The greatest difficulties arise when working with tardigrades of miniature sizes. The goal of the study was to test the HCP-2 (HITACHI) unit with CO_2 as a transitional substance (critical point parameters are 72 kg/cm^2 and 31°C) to obtain high-quality samples for SEM close to the intravital state.

Marine tardigrades were fixed with standard protocol for SEM, dehydrated in ethanol and acetone; the samples were critical point dried with CO2 (HCP-2, Hitachi), sputter coated with a gold-palladium alloy and examined using a Thermo Fisher Scientific Quattro S.

If parameters for bypassing the critical point during the drying are violated, the intravital appearance of the tardigrade and the structure of the cuticle are disrupted. To improve the quality of the samples, we prepared a special device for dehydration procedures.

Upgrade 1: We hand-produced the special storage unit from eppendorfs and covered it from both sides with planktonic mesh. The device allowed us to dehydrate the tardigrade in ethanol gradient without contacting air, hence without loss of tardigrade samples. Our chamber easily holds transmission fluid, but when the chamber is transferred from one beaker to another beaker, the fluid remains inside the chamber and does not expose the surface of the object. The chamber has a tight lid, so retrieval of animals is not difficult, and losses are minimized. There was little or no loss from the chamber during drying CDP in the HCP-2.

Upgrade 2: We proposed to freeze the chamber's lid of HCP-2 and to close the chamber with a frozen plug. It causes the temperature in the HCP-2 chamber to drop and the CO_2 to stay in a liquid state during 3 cycles of washing with CO_2 to minimize the acetone concentration.

Studying tardigrades using the Thermo Fisher Scientific Quattro S SEM with these upgrades, we reached high-quality results. Marine tardigrades did not have compression on the body and had a life-time appearance. The cuticle had no defects or breaks on the surface, the limbs were straightened with clearly visible, undamaged claws.

The proposed upgrades of CPD technology led to 1) stable and visually pristine results approaching intravital appearance and 2) 10x improvement of success rate of dehydration process. Proposed improvements to CPD of tardigrades might also be suitable for other microscopic aquatic animals and can be used widely in marine biology and astrobiology.

Иллюстрации

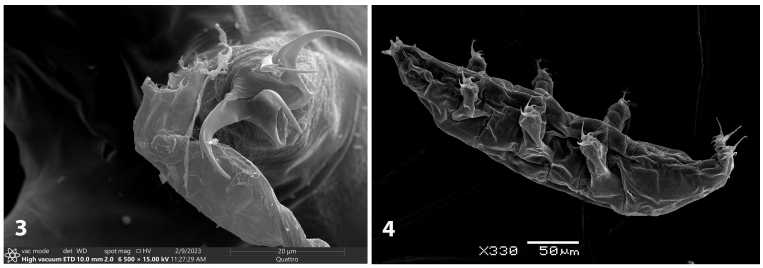

Рис. : Poor quality of tardigrade samples; the limbs is broken and the body is flattened, there are cuticle defects on the head and ventral surface.

Рис. : Marine tardigrades with thin smooth cuticle without defects on the lateral surface. Preparation with the storage unit and CDP in the HCP-2 with frozen plug. SEM Thermo Fisher Scientific Quattro S.

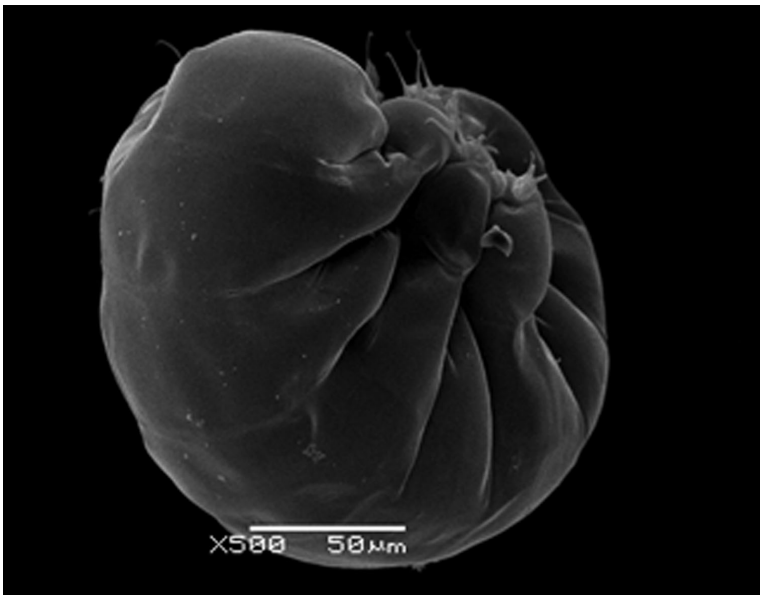

Рис. : Marine tardigrades with thin smooth cuticle without defects on the dorsal surface. Preparation with the storage unit and CDP in the HCP-2 with frozen plug. SEM Thermo Fisher Scientific Quattro S.

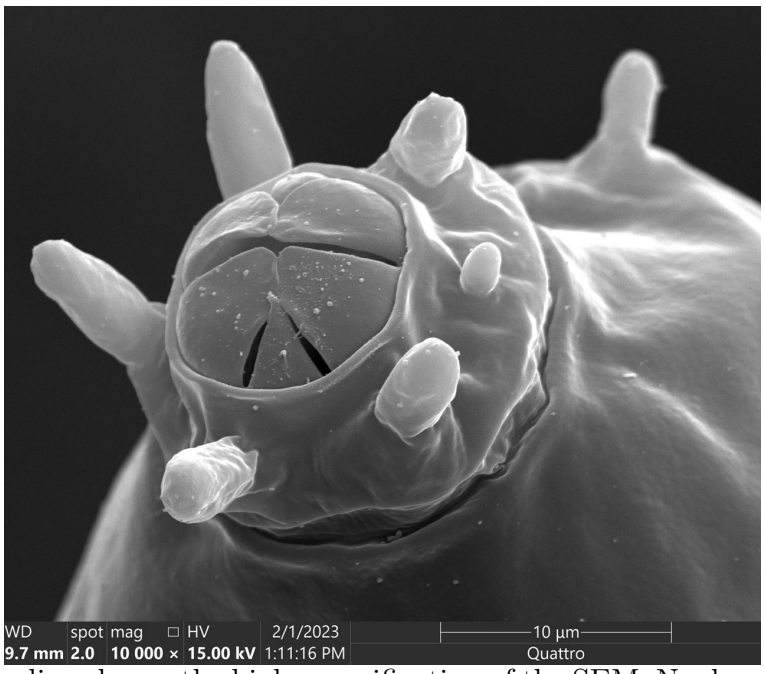

Рис. : Freshwater tardigrades on the high magnification of the SEM. No damages on the anterior sensory organs. Preparation with the storage unit and CDP in the HCP-2 with frozen plug. SEM Thermo Fisher Scientific Quattro S.