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Comparison of different fixation methods for the acoel worm Symsagittifera roscoffensis

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Symsagittifera roscoffensis GRAFF (1882) belongs to the class of the acoela, which are marine worm-like animals without gut or anus. Recently several studies on genomic sequences of acoela showed that this group may be the sister taxon to all remaining bilateria [1]. Some species (e.g. *S. roscoffensis*) can form symbioses with green algae, which allows them to live like photoautotroph organisms. The larvae of *S. roscoffensis* acquire their algae (*Tetraselmis convolutae*) shortly after their hatching, and there is no vertical transfer of the symbionts via the egg cytoplasma [2]. Without this symbiosis the larvae cannot reach the adult stage. After infection the symbionts arrange dircectly under the epidermis, where they are exposed to the light.

Recently *S. roscoffensis* was established as a model for basic CNS-organisation [3], regeneration [4], or the relationship between an invertebrate and symbiotic algae [5].

For such studies a good ultrastructural analysis is necessary. In the present study we tested different fixation methods on *S. roscoffensis*. At first we compared conventional chemical fixation (3,5 % glutaraldehyde in 50 mM HEPES- buffer with 3 % NaCl and 8 % sucrose [6], figure 1A) and sample processing with high pressure freezing (HPF, figure 1 C,D) followed by freeze substitution. After high pressure freezing the tissue is dense and packed with organelles and vesicles in contrast to the samples after conventional sample processing, where vesicles are extracted.

For samples that cannot be cultured and must be collected in the field, high pressure freezing is not practicable. For such samples a combination of chemical fixation followed by a freeze-substitution (see figure 1 B) is advisable. The results of this procedure are comparable to those of the HPF-samples. Chemical fixation and freeze substitution may also be a good alternative for samples that cannot be frozen properly.

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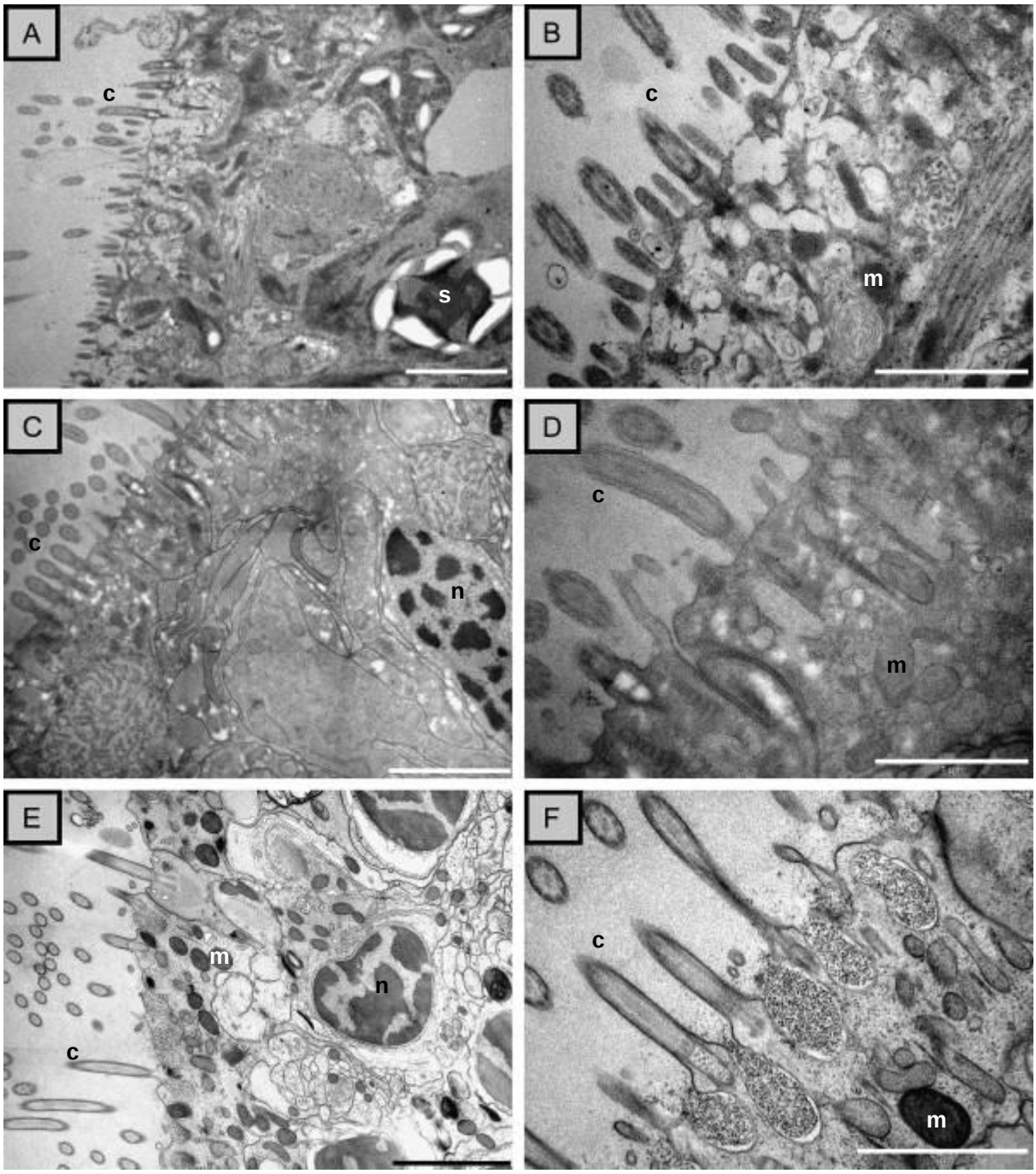

Figure 1: Comparison of different fixation methods for *Symsagittifera roscoffensis*, all pictures show the epidermis of adult worms; a) chemical fixation with 3,5 % glutaraldehyde in 50 mM HEPES- buffer containing 3 % NaCl and 8 % sucrose, scale bar 2 µm; b) chemical fixation as in A, details, scale bar 1 µm; c) chemical fixation as in A followed by freeze-substitution, scale bar 2 µm; d) chemical fixation as in A followed by freeze-substitution, details, scale bar 1 µm; e) high pressure freezing (HPF) followed by freeze substitution, scale bar 2 µm; f) HPF, details, scale bar 1 µm; c, cilia; m, mitochondrium; n, nucleus; s, symbiont.