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Cryo-SEM observations and imaging of minute lesser sclerotized insects

ABSTRACT

The study of minute cuticular details of small delicate insects is possible by slide mounting of the entire exoskeleton or part of it. This technique requires whole insect body clearing or tissue bleaching and washing. Lesser sclerotized insect body greatly suffers for such treatments and looses its natural body shape by shrinkage or by flattening, consequently. The aim of this study is to suggest an effective, fast and cheap technique to image less sclerotized insects that are prone to shrink or to wrinkle their bodies because of desiccation. Actual availability of desktop Cryo-SEM (Hitachi TM 3000 series) suggested us to experiment the opportunity to preserve natural body shape of the minute, delicate and lesser sclerotized insects in their living attitude. The technique bases on freezing the specimen, either living or previously EtOH-preserved but moved in water for the preparation, in the water down to -40°C on the SEM Cryo-stage and setting it for observation in SEM vacuum chamber. Once in the vacuum a proper T°C increase at about -28/-22°C allows external ice sublimation and exposes the frozen insect to direct SEM imaging. The technique appears promising because of the overall quality of results, the resolving power, the opportunity to measure the specimens. In fact, delicate specimens as *Phylloxera ilicis* Grassi (Hemiptera: Phylloxeridae, a representative of *Phylloxera quercus* Boyer de Fonscolombe group), the Italian grape mealybugs *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) and *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) maggots that are all usually ruined by desiccation during direct SEM observation, beautifully retain their natural body shape by this technique allowing the study and imaging of external morphology. As a further advantage, there is no need to critical point drying or metal coating, and the same sample can be submitted to conventional slide mounting later, after being studied by Cryo-SEM. Finally, we present a table of the running time/cost per observation of the proposed technique.

Keywords: electron microscopy, anatomy, gross & fine morphology.

We observed an adult female *Planococcus ficus* (Nedzelskii) by Cryo-SEM: from the dorsal view; from the ventral view; a mouthpart and first pair of legs; a left antenna; a detail of the posterior end of the body from dorsum; the abdominal circulus; the tip of the rostrum and last antennomere. The natural shape of the sample is well preserved, the mealy wax, the different grey shades on the antennomeres and rostrum and the antennomere sensilla are clearly visible.

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CRYO-SEM METHOD

We use a SEM Peltier stage (Coolstage) composed of the Peltier cooling group and the stub holder on which the sample will be placed. The first step is to put a drop of deionized water on the stub, then to dip the adult female of Planococcus ficus into the water drop. The excess of water is taken off by a piece of blotting paper. The sample in deionized water is ready to be frozen. The frozen sample, with part of the ventral abdominal sclerites out of the ice, is ready to be observed. The samples after observation are willing to be restored in 75% EtOH for future observation by light microscopy or further different techniques.

We also took a series of pictures of *Phylloxera ilicis* (Grassi) juvenile feeding on an inferior *Quercus ilex* leaf surface, at several laps in a total of observation time of 75 minutes. The gross body shape, the morphology, the structure of the sample and also the dorsal processes minute details are well preserved and remains coherent during the long observation. The leaf trichomes are cleary visible too.

We report the observation of the anterior part of the body of a *Drosophila suzukii* (Matsumura) maggot and the progressive sublimation of the ice embedding the adult female Planococcus ficus (Nedzelskii). The ice appears grey and the alloy stub, bright/white. The part of the ventral abdominal sclerites rests out of the ice eventually.

Running cost estimation on a four-year base experience. The mean SEM use is one hour per working day.

Cost per year, four year base comprehend planned annual and bi-annual plus breakdown mainteinance

Year	Gross cost
2013	36. 573, 00 €
2014	37. 598, 53 €
2015	36. 573, 00 €
2016	41. 269, 50 €
Four years total	152. 014, 03 €
Total/year	38. 003, 51 €
Cost per working hour	25, 34 €
disposable/obs.	10, 00 €
cost/minute	0, 42 €

Cost per observation Subject/h:min/Pictures	Cost
Plan. ficus/2:30/8	73, 34 €
Phill. Ilicis/1:15/5	41, 67 €
Ice Sub. /2:00/5	60, 67 €
Dros. suzukii/1:00/1	35, 34 €

Cryo-SEM observations and imaging of minute lesser sclerotized insects

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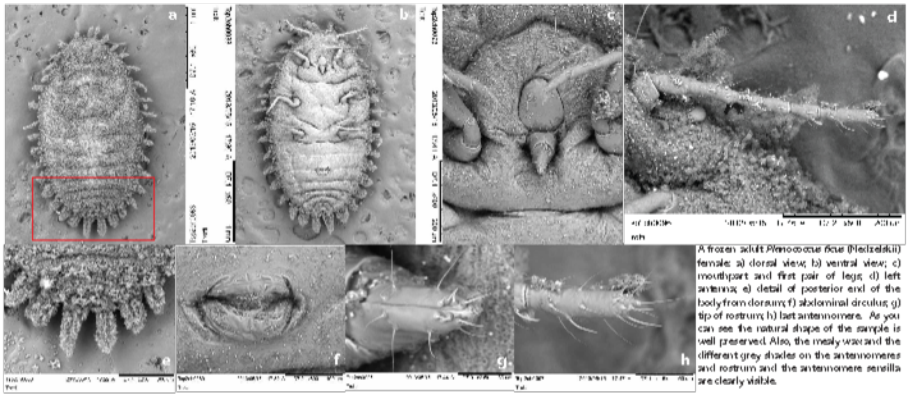
ABSTRACT: The study of minute cuticular details of small delicate insects is possible by slide mounting of the entire exoskeleton or part of it. This technique requires whole insect body clearing or tissue bleaching and washing. Lesser sclerotized insect body greatly suffers for such treatments and looses its natural body shape by shrinkage or by flattening, consequently. The aim of this study is to suggest an effective, fast and cheap technique to image less sclerotized insects that are prone to shrink or to wrinkle their bodies because of desiccation. Actual availability of desktop Cryo-SEM (Hitachi TM 3000 series) suggested us to experiment the opportunity to preserve natural body shape of the minute, delicate and lesser sclerotized insects in their living attitude. The technique bases on freezing the specimen, either living or previously EtOH-preserved but moved in water for the preparation, in water down to -40° C on the SEM Cryo-stage and setting it for observation in SEM vacuum chamber. Once in the vacuum a proper T°C increase set about -28/-22° C allows external ice sublimation and exposes the frozen insect to direct SEM imaging. The technique appears promising because of the overall quality of results, the resolving power, the opportunity to measure the specimens. In fact, delicate specimens as *Phylloxera illicis* Gaud. (Hemiptera: Phylloxeridae, a representative of *Phylloxera quercus* Boyer de Fonscolombe group), the Italian grape mealybugs *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) and *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) maggots that are all usually ruined by desiccation during direct SEM observation, beautifully retain their natural body shape by this technique allowing the study and imaging of external morphology. As a further advantage there is no need to critical point drying or metal coating and the same sample can be submitted to conventional slide mounting later, after being studied by Cryo-SEM. Finally, we present a table of the running time/cost per observation of the proposed technique.

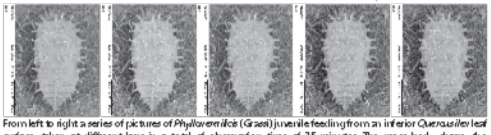
Keywords electron microscopy, anatomy, gross & fine morphology.

Acknowledgement: we recognize the help and competence of Pasquale Trotti, SELGE UPB laboratory responsible, for most of the SEM pictures shown on this poster.



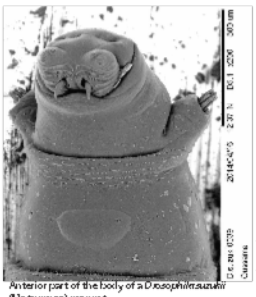
A frozen adult *Planococcus ficus* (Hemiptera) female: a) dorsal view; b) ventral view; c) mouthpart and first pair of legs; d) left antenna; e) detail of posterior end of the body from dorsum; f) abdominal circulus; g) tip of rostrum; h) last antennomere. As you can see the natural shape of the sample is well preserved. Also, the mealy wax and the different grey shades on the antennomeres and rostrum and the antennomere sensilla are clearly visible.

1) The SEM Peltier stage (Cool stage) is composed of the Peltier cooling group and the stub holder on which the sample will be placed; 2) A drop of deionized water is placed on the stub; first 3) One adult female of *Planococcus ficus* is dip into the water drop; 4. The excess of water is take off by a piece of blotting paper; 5. The sample in deionized water ready to be frozen; 6. The frozen sample with part of the ventral abdominal sclerites out of the ice (red arrow, see 6); 7. Two different samples after observation, ready to be restored in 75% EtOH for future observation by light microscope or further different techniques.



From left to right a series of pictures of *Phylloxera illicis* (Gaud.) juvenile feeding from an inferior *Quercus ilex* leaf surface, taken at different laps in a total of observation time of 75 minutes. The gross body shape, the morphology, the structure of the sample and also the dorsal processes minute details are well preserved and remains coherent during the long observation. The leaf trichomes are clearly visible too.

Progressive sublimation of the ice embedding the sample. The ice appears grey, the alloy stub, bright white. The red arrow points to part of the ventral abdominal sclerites that rest out of the ice (red arrow, see 6) eventually.



Anterior part of the body of a *Drosophila suzukii* (Matsumura) maggot.

Cost per year, four years base computed and planned around realistic and financial plausible down-sized revenues ..... Gross cost

year ..... 2013 ..... 36.573,00 €
year ..... 2014 ..... 37.994,80 €
year ..... 2015 ..... 36.573,00 €
year ..... 2016 ..... 41.265,60 €

Four years total ..... 152.691,40 €
Total/year ..... 38.003,51 €
Cost per working hour ..... 25,34 €
disposable/day ..... 1.900 €
cost/minute ..... 0,42 €

Cost per observation
Subject/Item/Pictures ..... Cost

Phyllox. leaf/25 ph 733,40
Phyll. leaf/15 ph 413,70
Ice 5+5/2.00 h 456,70
D. suzukii/1 ph/1 25,34

Running cost estimation on a four-year base experience. SEM mean use of one hour per working day.

Roberto R., Russo V., Diana L., Pellizzari G., Porcelli F. (2016) Cryo-SEM observations and imaging of minute lesser sclerotized insects. Proceedings of the XV International Symposium on Scale Insect Studies (ISSIS Bari, Italy), 19-21 Oct. 2016, Bari, Italy: 175-180. ISBN 978-88-6629-...