LM approaches to the imaging and taxonomical identification of pollen intake by *Chrysoperla carnea s.l.* adults.

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INTRODUCTION

Chrysoperla carnea s.l. (green lacewing) is an insect used in the biological control of pests affecting the olive tree in Spain. Larvae are active predators of other insects, which have been proposed to also use pollen grains to complement their nutrition. On the contrary, adults move to a glyco-palynophagous diet. Pollen content of the insect digestive tract may be used as a marker of the taxonomical preferences and requirements of this insect, in order to further promote growth of their populations and therefore their beneficial effects. However, the description of methods for LM visualization and species discrimination, and their compatibility with further molecular identification technologies are poorly described. We have developed three major methods to image pollen grains present in the individuals.

MATERIALS & METHODS

Materials

Chrysoperla carnea s.l. **adults emerged after formation of cocoon were feed with different sources of pollen grains, as well as with commercial bee pollen.**

1) Clearing/LM

C. carnea s.l. frozen individuals were treated with Hertwig solution (chloral hydrate, HCl, glycerol) at 60ºC for 24h (L1 and L2), or 11 days (L3).

2) Dissection of diverticula/ acetolysis

Individuals without pre-treatment were dissected using micro-scalpels and the diverticula isolated. Diverticula were crushed in a mortar and treated with a acetolysis mix (acetic and sulphuric acids) 120ºC, 5 min. Reaction was stopped with acetic acid and several washes with water. Pollen grains were centrifuged and supernatant discarded. Pollen preparations were mounted with glycerol.

3) Dissection and differential filtration

Isolated diverticula were subjected to disaggregation and filtration through meshes of different sizes, in order to isolate the individual pollen grains, and even separate pollens from different species according to their diameter.

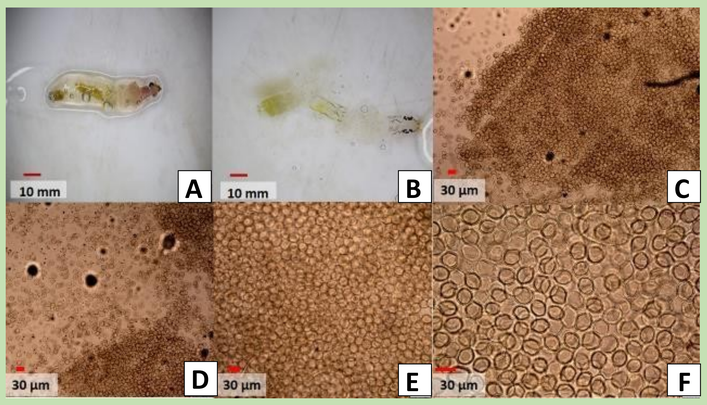

Figure 1. CLEARING METHOD. Images of *C. carnea* digestive tract of adult individuals after observation with a Leica M165FC stereomicroscope (A-B) and an inverted microscope Leica DMI600B, at different magnifications (C-F).

METHOD	DIFFICULTY	TIME REQUIREMENT	CHEMICAL AGGRESSIVITY	POLLEN LIFETIME	POSSIBILITY OF ADDITIONAL ANALYSES (i.e. PCR)
CLEARING/ LM	+	+	+++	++	+
DISSECTION/ LM	++	++	-	++	++++
DISSECTION/ ACETOLYSIS	+++	+++	++++	++++	-
DISSECTION/ FILTERING	++++	++++	-	++++	++++

Table 1. Main characteristics of the methods used.

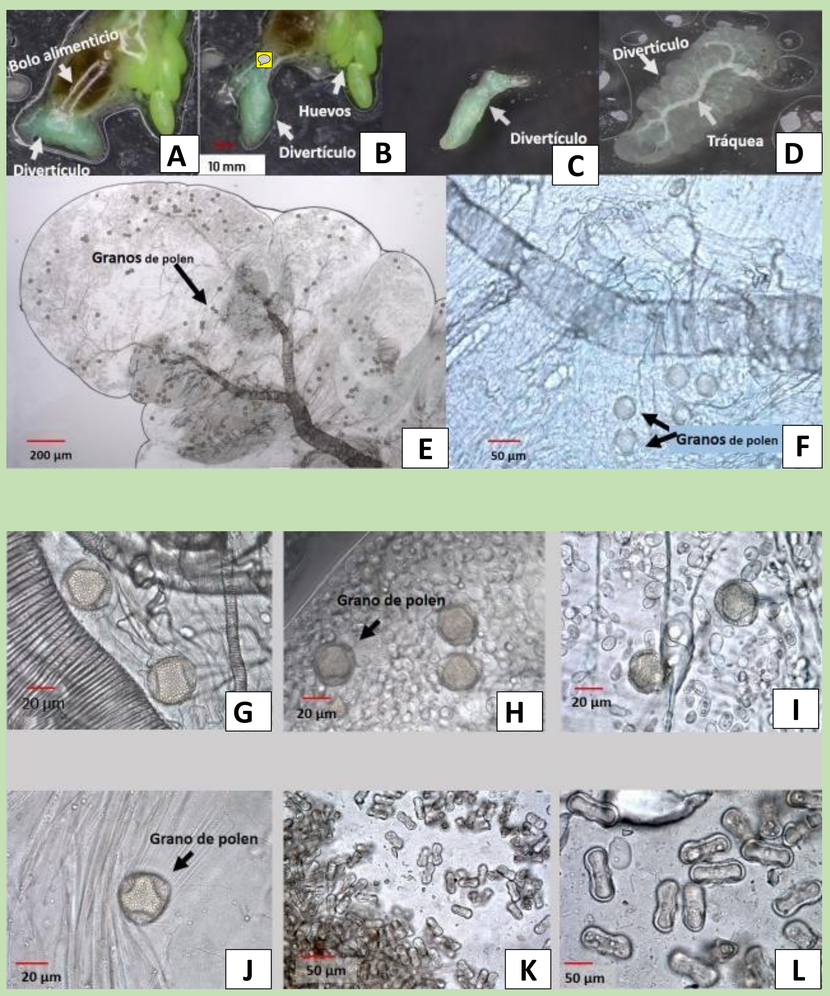

Figure 2. DIVERTICLE DISSECTION (A-D), and further observation after mounting with gycerol and visualization with a Leica DMI600B inverted microscope.

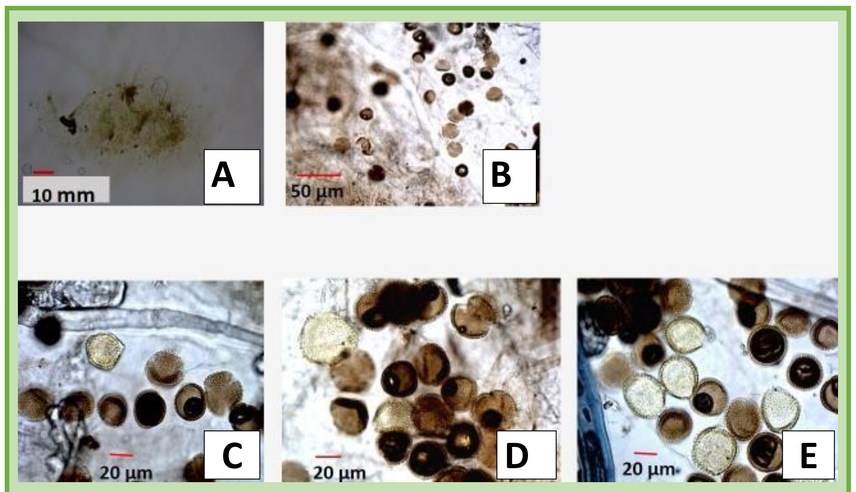

Figure 3. ACETOLYSIS. Images of pollen grains isolated from *C. Carnea* diverticula and subjected to acetolysis and observation after mounting with glycerol and visualization with a Leica DMI600B inverted microscope.

Figure 4. DISSECTION/FILTERING. Images of pollen grains isolated from *C. Carnea* diverticles and subjected to differential filtering through meses of 200, 100 and 50 µm. Observation after mounting with gycerol and visualization with a Leica DMI600B inverted microscope.

CONCLUSIONS

a) **Clearing methods are appropriate for further populational studies on *C. carnea* as per their simplicity.**
b) **Dissection of diverticula/acetolysis is the method of choice for deep taxonomical analysis of the pollen content, because of the higher resolution obtained. In addition, most palynological databases have been built after using this preparation method. As the counterpart. Isolated pollen grains subjected to the procedure can not be further processed to analyse biochemical markers due to the strength of the treatment.**
c) **In order to proceed with further biochemical/molecular analyses of the pollen content, diverticula isolation and separation of pollen grains by filtering is the preferred method, as it combines a good LM resolution with the maintenance of marker-compatible conditions.**

Acknowledgements

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