A DNA-based methodology for airborne pollen identification in complex environmental samples

K. Leontidou[1], C. Vernesi[1], D. Vokou[2], J. De Groeve[1], F. Cristofolini[1], A. Cristofori[1]

[1]*Department of Biodiversity and Molecural Ecology, Fondazione Edmund Mach, San Michele all' Adige, Trento, Italy*; [2]*Department of Ecology, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece*

Metabarcoding is a promising DNA-based method for identifying airborne pollen from environmental samples, with potential advantages over microscopic methods. This method requires several preparatory steps of the samples, with the extraction protocol being of fundamental importance to obtain optimal DNA yield. Currently, there is no clear consensus in sample preparation and DNA extraction, especially for gravitational pollen samplers. Here, we present a DNA-based method to analyse environmental samples collected by both volumetric (Burkard spore trap) and gravitational samplers (Tauber trap). Results obtained are compared to those from microscopic analysis. DNA extraction was tested for three variables (extraction kit, bead beating, lysis) on pure pollen (single species) and the best combination was applied to environmental samples (pollen mixtures of different taxa). For the environmental samples, an improved protocol for the preparation of pollen pellets was established: DNA was extracted from the pollen and a short fragment of chloroplast DNA (cpDNA) was amplified by universal primers for plants (trnL). After PCR amplification, 30 amplicons were Sanger-sequenced and taxonomic assignment was accomplished through comparison to a reference custom-made database, including important widespread anemophilous taxa from the study area (Eastern Italian Alps). Results of metabarcoding with the trnL primers were consistently similar to those with microscopic analyses. For the environmental samples collected by volumetric trap, 75% of the taxa identified with the microscope were also identified by molecular analysis, which proved more efficient in identifying taxa even at the species level. We plan to apply a semi-quantitative analysis by Next Generation Sequencing in order to assess the pollen spectra of different Natura 2000 habitats in the Alps.

Keywords: trnL metabarcoding, taxonomic identification, next-generation sequencing.