

Plant biodiversity estimation through pollen spectra in Natura 2000 habitats: preliminary methodological steps for implementation of eDNA metabarcoding

Antonella Cristofori^{1,*}, Kleopatra Leontidou^{1,2}, Cristiano Vernesi¹

¹Department of Biodiversity and Molecular Ecology, Research and Innovation Centre, Fondazione Edmund Mach, Italy

²Department of Ecology, School of Biology, Aristotle University of Thessaloniki, Greece

*antonella.cristofori@fmach.it

Airborne pollens reflect differences in the species composition of the local flora and may capture the spreading of alien species. The air biomonitoring may detect the flowering season of anemophilous taxa as well as the reproductive response of plants to environmental changes at a temporal and spatial scale. Our study aims at characterizing the air biodiversity of different habitats - as coded by Natura 2000 and Corine Land Cover - through a DNA metabarcoding approach on environmental samples collected at ground level. Metabarcoding is promising for identifying airborne pollen from environmental samples, with potential advantages over microscopic methods. There is still no consensus in sample preparation and DNA extraction, especially for gravimetric pollen samplers. As a first step, we established protocols to process environmental samples for extracting DNA to be analyzed by metabarcoding, and to verify the efficacy of this approach for the taxonomic assignment of airborne pollens, collected by both gravimetric (Tauber trap) and volumetric samplers (Burkard spore trap). Protocols were tested across an increasing complexity of samples, from pure pollen to environmental samples, considering the variability in pollen grain structure, the mixture of different pollen species, the influence of adopted sampling medium. A short fragment (about 150 base pair of trnL P6 loop) of chloroplast DNA was amplified by universal primers for plants. Amplicons were Sanger-sequenced and taxonomic assignment was accomplished through comparison to a custom-made reference database - 46 families representing all the anemophilous plants occurring in the study area (Trentino, Italy, Eastern Italian Alps). By comparison to the classical morphological pollen analysis, it emerged that DNA metabarcoding is valid across a complexity of samples, from pure pollen of single species to environmental samples, provided that sample preparation, DNA extraction and amplification protocols are specifically optimized.