

Insect diversity and interactions revealed by environmental DNA

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The idea of environmental DNA (eDNA) lies on the assumption that all living organisms can release DNA in the environment in which they live and it was hypothesized that it would have been possible to study the presence of certain organisms looking for their genetic traces dispersed in environmental matrices, including faeces or undigested preys. Unlike the classical DNA barcoding approach, the characterization of communities from eDNA (i.e., eDNA metabarcoding), uses short loci to adapt the method to the greater degradation rate of DNA, compared to that extracted from fresh samples. Starting from these assumptions, the potential of eDNA metabarcoding was explored by applying it in three different case studies involving insects (and their interactions with a host or with the environment).

The first study included the diet analysis of three species of vespertilionid bats (*Hypsugo savii*, *Barbastella barbastellus*, and *Plecotus auritus*). Faecal samples were collected at three sites representative of two natural areas, the National Park of Vesuvio (one site) and two sites in the National Park of Abruzzo and Molise. These latter two sites are characterized by both disturbing events (cuts or reforestation) and a stable mature forest patches, whereas the Vesuvius park is a disturbed area (fires and urbanization). The second case study aimed at analysing the diet of two distinct populations of *Pyrrhocorax graculus*, an alpine passerine bird, starting from faecal pellets found in the field. The two sampling sites were the MontAvic Natural Reserve and the environmentally disturbed touristic site of Cervinia (both sites lie in Aosta Valley). For the identification of the prey contained in the faeces of these two case studies, fragments of two mitochondrial loci were sequenced with MiSeq Illumina sequencing technology. Specifically, the investigated loci were a region of the cytochrome oxidase 1 and a region of 16S rRNA. The third case study dealt with the analysis of a charismatic group of bioindicator insects belonging to odonates (dragonflies and damselflies) in northern Italy. Odonate diversity was inferred by analyzing the eDNA present in samples from different aquatic ecosystems.

The water sampled at each site (1L) was filtered with increasingly finer porosity (up to 0.22µm) to collect eDNA. After DNA extraction, two loci of the mtDNA COI region were sequenced with the same technology described above, to identify all the possible odonate species present in each water sample. To validate the method, we created an *ad-hoc* mock communities composed of 75 different species and tested the workflow, comparing the results with the environmental samples. After sequencing and bioinformatics analysis, more than 1000 of ASVs were obtained in the case of bats and birds faeces, that were assigned to several taxonomic invertebrate groups. Conversely, in the case of odonates, the number of molecular clusters was in line with the lower expected diversity. Our results allowed the validation of eDNA metabarcoding *in vitro* and natural environments, thanks to the development of a standardized workflow and the implementation of an *ad hoc* reference database. On the whole, these data demonstrate the potential of eDNA studies, highlighting the possibility of integrating new molecular approaches to classical ecology studies and biomonitoring activities, and also territorial management.

