



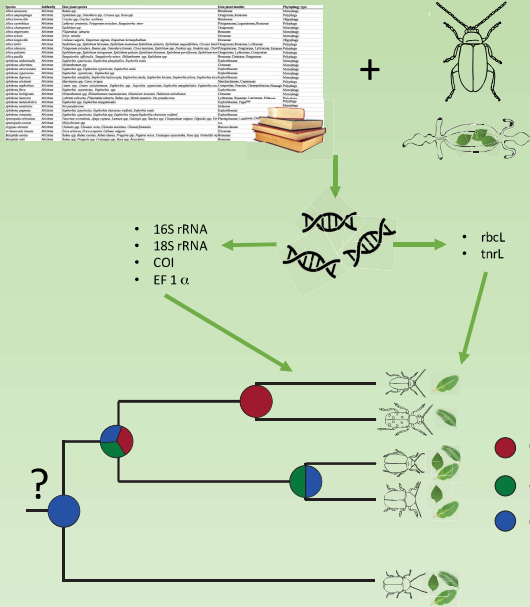
# Is monophagy an evolutionary dead end? Evidence from Euro-Mediterranean Chrysomelidae

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## IS EVOLUTION PATHWAY TOWARDS SPECIALIZATION? Experimental design



## INTRODUCTION

Leaf beetles (Coleoptera, Chrysomelidae) are a large family of phytophagous insects distributed worldwide. Due to their close relationship with the host plants, Chrysomelidae are considered interesting both from the evolutionary and the economic point of view (some species are agricultural pests). Species with different levels of specialization for the host plants exploitation are present within the family: monophagy; oligophagy; polyphagy are all represented. However, little is known about the distribution of these characters within the family and whether their evolution pathway is towards specialization or vice-versa.

## STUDY AIMS

The aims of this study are i) to define the types of phytophagy (i.e., monophagy, oligophagy and polyphagy) associated with 527 Euro-Mediterranean leaf beetles species using literature information, field observations on the host plants and insects gut content characterization; ii) to infer a phylogeny of these species using mitochondrial and nuclear markers (i.e., COI, 16S rRNA, EF-1α, 18S rRNA), by mining sequences from public repositories and *de novo* amplified; iii) to reconstruct the types of phytophagy across the leaf beetles phylogeny, in order to define if the evolutionary pathway is towards specialization (i.e. from polyphagy to monophagy) or monophagy occurred randomly.

## METHODS

Specialized literature on Chrysomelidae and field observations were used to create a database reporting host plants information for the species considered in the study. Processed samples were taken from C-BAR project (Barcoding of Chrysomelidae) ethanol collection. DNA extractions were performed starting from one hind leg of the specimens as reported in Magoga et al., 2016 or from the whole specimen using modified phenol-chloroform method (Meregetti et al., 2017). PCRs were performed for amplifying COI gene fragment of 568 bp length using LCO1490/HCO2198 and LepF1/LepR1 primers pairs. In order to amplify a segment of 18S rRNA and 16S genes 18SF2826/18SRW21847 and LRN13398/LRJ12887 primers pairs were used, respectively. Due to the low efficiency of the 16S primers used on the target region, new and more specific primer pairs were developed and tested. Positive amplicons were directly sequenced on both strands using the marker-specific primers from ABI technology (Applied Biosystems, Foster City, USA). Electropherograms correction was performed using Geneious R8 (Biomatters Ltd., Auckland, New Zealand). For each marker, some sequences were also mined from online databases using R packages ("BOLD" and "rentrez").

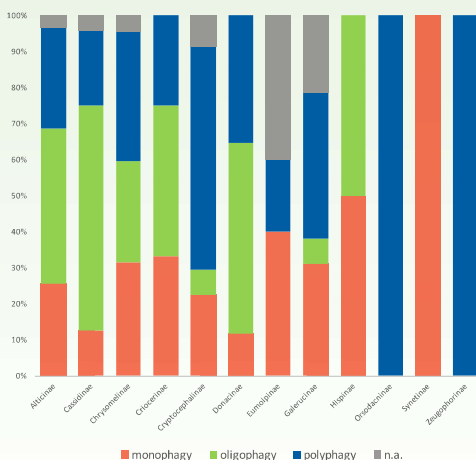


Fig 1. Histogram of relative abundance of monophagous, oligophagous and polyphagous species in each Chrysomelidae subfamily considered in the study.

## PRELIMINARY RESULTS AND FUTURE PROSPECTIVES

A database containing information about the host plants of 527 Chrysomelidae species was created; 25.2% of the species resulted to be monophagous, 28.7% oligophagous, 40% polyphagous, information are missing for the remaining species (Fig. 1). These missing information will be obtained by the analysis of the insect gut content through a metabarcoding approach (rbcL and trnL markers). For the 527 species, a dataset of COI sequences was obtained. Due to the fact that 18S rRNA marker is strongly conserved in phylogenetically close related species, a dataset composed of one 18S sequence for each subgenus of the selected species was built, amplifying or mining them from on-line databases. For 16S, 204 sequences were mined, and now DNA extractions and PCR are ongoing in order to complete the dataset with one sequence per species.

## REFERENCES

Magoga G et al. 2016. Barcoding Chrysomelidae: a resource for taxonomy and biodiversity conservation in the Mediterranean Region. In: Jolivet P, Santiago-Blay J, Schmitt M (Eds) Research on Chrysomelidae 6. *ZooKeys* 597: 27–38.  
 Meregetti V et al. 2017. Evidence for a conserved microbiota across the different developmental stages of *Plodia interpunctella*. *Insect Science*, DOI: 10.1111/1744-7917.12551.

