# Refining molecular tools for the analysis of wild bee biodiversity

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**Abstract**:
The impact of human society is detectable in any habitat. For instance, land consumption has constantly been increasing over time, especially in Northern Italy where natural areas are replaced by industrial agriculture or urban settlements and roads at an alarming rate.

Across all types of environments, this widespread anthropogenic mark could alter the pollinator species occurrence, the interaction between plant and pollinators and other ecosystem services that are fundamental to preserve human health and wealth. Therefore, it is crucial to refine tools for the identifications of key species of pollinators, in order to define reliable conservation strategies to better support environmental management and sustainability favoring the pollination service.

Considering the Antophila bee clade, very little is known on their distribution and genetic variation especially in Italy. These limits could be further amplified by the constant reduction of expert taxonomists, the high number of species (more than 1000) that requires high expertise and the presence of sister species and cryptic ones that are often subtle to discriminate relying on morphological approaches only. Given these premises, to avoid a knowledge gap that could ultimately jeopardize bee conservation, an integrative strategy to reliable species recognition is urgent.

The aim of the study is to provide updated tools for characterizing the biodiversity of wild Italian bees (Anthophila) and to create a new regional dataset that can be expanded with the data from the whole country and that can be a base for future monitoring at regional and global level.

We collected hundreds of samples since 2019 belonging to the five most representative families (Andrenidae, Apidae, Colletidae, Halictidae, Megachilidae). Specimens from cryptic species groups and at least one for each putative species were processed, making a total of more than 400 samples. These specimens were identified integrating two approaches: morphological scrutinizes and biomolecular tools to delineate their taxonomy and outline their genetic variation on a continental scale. The biomolecular analysis was conducted through a standard DNA barcoding pipeline, by targeting the mtDNA 5'-end COI region (658bp) with different sets of primers depending on coamplificaiton of symbionts or aged specimens.

To characterize the genetic variation and the taxonomy, the sequences obtained were aligned with the ones belonging to NCBI and BOLD databases of the same taxon, including sister species. The results highlighted the presence of cryptic species and unexpected genetic variation in some genera.

This study will add substantial information on bee genetic variation and to create a database that can accelerate the identification of wild bees (e.g., DNA metabarcoding) for future studies and conservation policies for the biodiversity of pollinators.