

## Buzzing through the unknown: uncovering wild bee molecular diversity in southern Italy

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### Abstract:

The Mediterranean region and Italy are recognized as major biodiversity hotspots, hosting a large number of endemic species or local DNA variants thanks to their diverse geo-climatic conditions. This biodiversity is responsible for several ecosystem services, where pollination covers a key role as shown by increasing scientific insight as well as a broader public and institutional awareness. In contrast, pollinators are declining and, within the Anthophila bee clade, knowledge about species distribution and genetic variation remains scarce, highlighting the need for improved tools to identify key pollinator species. The aim of this study is to characterize wild bee DNA variants in southern Italy and to test an efficient sample-preparation method that enables fast and high-quality DNA extraction. This will improve the reliability of subsequent genetic analyses and significantly extend the knowledge in an otherwise largely unexplored context.

The samples were collected from 2017 to 2025 in southern continental Italy and islands (Calabria, Puglia, Basilicata, Abruzzo, Marche, Sicilia, Sardegna, Pantelleria, Ventotene, Capri, Ischia and S. Stefano), and then identified using an integrated method, combining molecular approaches (DNA barcoding) with morphological identification. This study was performed within the National Biodiversity Future Center (PNRR) and the EU Horizon projects “Butterfly” and “ProPollSoil”. For the first time, a test compared three different methods for preparing the specimen leg for the DNA extraction: mincing with a scalpel to expose tissue, leg incubation in an oven and whole-leg lysis. Samples were extracted, amplified targeting the 5' end of the mitochondrial COI region (658 bp). Different primer sets were tested to determine which ones performed most effectively across a broader range of bee genera and families.

From the total collected specimens, 324 barcode sequences were generated. Experimental test revealed that sequence outcomes varied according to the primers used for amplification, while no major differences were observed among the leg-preparation methods, thus allowing faster processing with good results. As part of this work, haplotype networks were constructed, enabling the identification of different haplotypes and providing deeper insights into the genetic variability and population structure of target pollinator species for the very first time, an essential step for biodiversity characterization. To a larger extent, this study may provide a robust foundation for the development of biodiversity monitoring and protection of pollinators, which could even trigger new insights for sustainability and human well-being via ecosystem services.