





Using Pollen DNA Metabarcoding to Assess the Foraging Preferences of Honeybees in Kastoria Region, Greece [†]

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Abstract: Identification of a plant's pollen components can be used to establish its geographical provenance, while also providing insights into the diet and foraging preferences of the honeybee (*Apis mellifera* L.). The diversity and amount of pollen represent crucial factors for pollinators. Here, we identified plant species visited by honeybees by analyzing the pollen pellets collected from honeybees in Kastoria, Greece. The results indicate that pollen from different periods was identified by means of floral composition. An interesting observation is that all identified plants belonged to different genera. Among the identified plants, native ones, such as the Macedonian pine, *Pinus peuce*, present a distinct foraging profile for local honeybees.

Keywords: honeybee; *Apis*; pollen; Kastoria; foraging preferences



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1. Introduction

Aside from their role in biodiversity retention, pollinators contribute to the European Union (EU) agricultural industry [1]. More specifically, pollinators are very important for the reproduction and preservation of a plethora of plants (e.g., medical herbal crops, agricultural crops, horticultural, and wild plants) [2–4]. Foraging behavior among pollinators can be affected from all available plants in the foraging area [5]. As the conservation of pollinators is a highly important issue, the identification of the foraging preferences of pollinators is considered a priority. However, most studies have used morphological data, where pollen identification constitutes a difficult task. In Western Macedonia and particularly in the Kastoria region, there are many endemic plant species [6]. Therefore, honeybees feeding on these species may provide honey with specific organoleptic characteristics, while at the same time informing us about its origin [7]. Thus, alternative methodologies, such as molecular techniques to identify the foraging preferences of honeybees, may be valuable for constructing a plant–honeybee interaction network. The main scope of the present study was to evaluate the preferences of the honeybee for plant species using pollen metabarcoding from the Kastoria region.

2. Materials and Methods

During summer 2021, six incoming forager bees with pollen loads were collected from the Kastoria region, Greece. The pollen pellets were collected from the hind legs of each bee and transferred to a sterile tube using disinfected tools. DNA extraction was performed using the kit Nucleospin tissue (Machery Nagel, Duren, Germany)

following the recommendations of the manufacturer. The concentration and purity of the DNA samples was evaluated using a Q5000 Microvolume spectrophotometer (Quawell Technology Inc., San Jose, CA, USA). PCR amplification of the trnL region was carried out using the primer set proposed by Kraaijeveld et al. 2015. The PCR products were resolved using electrophoresis on 1.5% *w/v* TBE agarose gels and purified using the NucleoMag kit. Amplicon libraries were prepared using the Ion Plus Fragment Library Kit (Cat. No. A28950, Thermo Fisher Scientific, Wilmington, DE, USA), following the instructions of the manufacturer. Sequencing was conducted using the Ion Torrent S5 system on a 530 chip (Cat No. A27764). The resulting reads were quality controlled, and then denoised into ASVs using the DADA2 plugin from the QIIME2 platform. A final taxonomic assignment was performed using BLAST against sequences of the trnL marker region procured from GenBank in 2018.

3. Results and Discussion

In total, we identified 32 plants at the species level, 10 at the genus level and the remaining 4 at the family level. An interesting observation is that all the identified plants belonged to different genera, while the most common families were Asteraceae, Thymelaeaceae, Fabaceae, Eupteleaceae, and Rosaceae. These results indicate a particularly rich flora diversity, in line with the high biodiversity observed in the Kastoria region. A percentage of up to 16.9% remained unassigned.

4. Conclusions

The above study revealed important information regarding the foraging preferences of honeybees across the Kastoria region from three different sampling dates. The use of DNA metabarcoding led us to identify the pollen composition of the samples, which is a more accurate technique in comparison to traditional microscopy methods. Apart from the foraging preferences of honeybees, pooled DNA metabarcoding can provide a powerful tool for rapid surveys on plant biodiversity [8]. For the region of Kastoria, which is a highly significant habitat due to its high biodiversity, there is a need for continuous monitoring. However, although we identified plenty of plant species, in two out of six pollen pellets, a high percentage (40–45%) of plants remained unassigned. From this observation, we can assume that these species may be native to this region, and as a result, they are missing from the databases. To conclude, with the use of pollen meta barcodes, we can assess the foraging preferences of the honeybees inhabiting this area and monitor the biodiversity of the areas, while at the same time, it can help us to provide honeybee colonies with their preferred plants.

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