# Non-lethal Sampling Method for Studying Plant-Insect Interactions


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## Why?

Insect populations are declining, disrupting pollinator networks and threatening overall ecosystem stability[1+2]. Despite the importance of pollination, the foraging behavior of many pollinators remains poorly understood. A powerful tool to study these interactions is pollen metabarcoding, which traditionally involved killing the insect. Here we present a innovative, non-lethal technique to collect genetic material from both the pollinator (bumble bee, genus *Bombus*) for accurate species identification via barcoding and its pollen load for identification via metabarcoding.

## How?

21 bumble bees *(Bombus* sp.*)* were collected in a nature protected area in Cuxhaven. Barcoding for the insect as well as pollen metabarcoding analyses were condudcted.

**Field**
- Record flowering plants
- Collect pollen+tarsus

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**Wet lab**
- ITS2 region and Illumina sequencing for the pollen
- COI region and Sanger sequencing for the tarsus

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**Dry lab**
- Quality control
- Identification with reference database
- Data analysis
- Visualisation

**Fig.1.** *B. locurum* agg. foraging on *Trifolium repens*

## Tool development

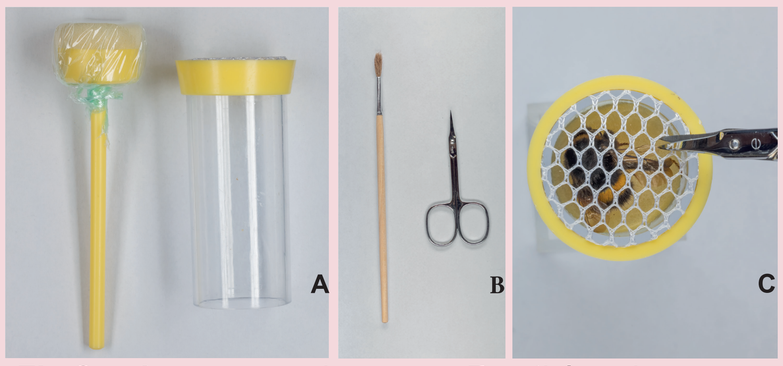


**Fig.2.** **A** queen-marker-cage, **B** cuticle scissors and fine art brush, **C** captured bumble bee (top), displaying the mesh's large holes

Bumble bees were captured using a specialized tool called a bee-marker-cage, designed for beekeepers. It consists of a tube with a punch on one end and a coarse mesh on the other. While confined, a fine art brush was used to remove pollen from the bumble bee's hind legs, and cuticle scissors to clip the tarsus of the mid leg - which does not hinder the bee's activities[3]. Afterwards, the bumble bee was released, and the collected sample was stored in a reaction tube for subsequent lab analysis. The cage was treated with a bleach solution before reuse.

## What we found

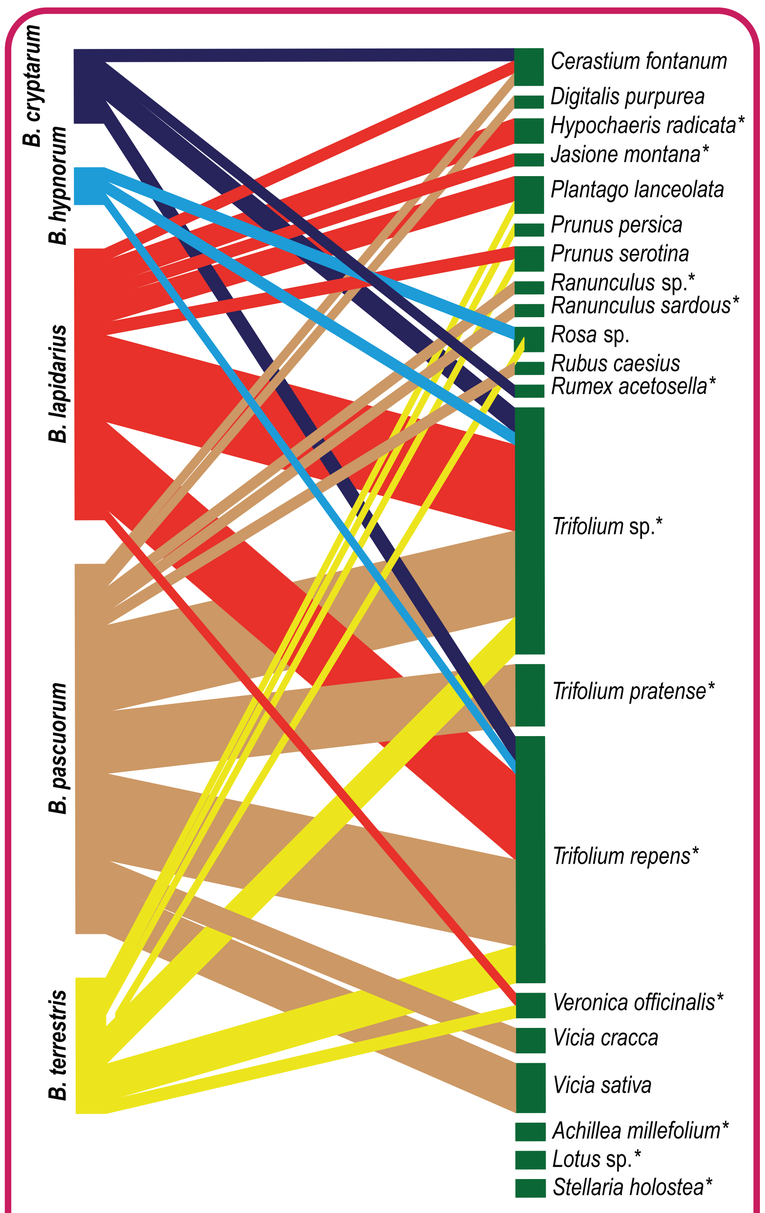


**Fig.3.** Interactions revealed by metabarcoding of pollen of 21 bumble bees and barcoding of insect tissue in a nature conservation area in Northern Germany on 13.06.2022. Detected plant species supported by phenology screening are marked with an asterisk.

Barcoding of the tarsus enabled accurate identification of worker species within the *B. locurum* agg., including *B. terrestris* and *B. cryptarum*, which are not distinguishable morphologically.

Metabarcoding results largely matched the phenology screening, with all other plants being reported as abundant in the nature protected area.

## What to do with it

This easy to implement and cheap technique enables a more frequent sampling without causing harm to the colony and allows the identification of cryptic species. This makes it possible to reconstruct changes in foraging preferences throughout the life cycle of a colony, information particularly important for understanding species interactions in protected areas.

## Literature


1 Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., ... & De Kroon, H. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PloS one, 12(10), e0185809.
2 Dicks, L. V., Breeze, T. D., Ngo, H. T., Senapathi, D., An, J., Aizen, M. A., ... & Potts, S. G. (2021). A global-scale expert assessment of drivers and risks associated with pollinator decline. Nature Ecology & Evolution, 5(10), 1453-1461.
3 Holehouse, K. A., Hammond, R. L., & Bourke, A. F. G. (2003). Non-lethal sampling of DNA from bumble bees for conservation genetics. Insectes Sociaux, 50, 277-285.