HONEY BEE-COLLECTED POLLEN FOR BOTANICAL IDENTIFICATION VIA ITS2 METABARCODING: A COMPARISON OF PRESERVATION METHODS FOR CITIZEN SCIENCE

Quaresma, Andreia[1,2,3]; Brodschneider, Robert[4]; Gratzer, Kristina[4]; Gray, Alison[5]; Keller, Alexander[6]; Kilpinen, Ole[7]; Rufino, José[8]; van der Steen, Jozef[9]; Vejsnæs, Flemming[5]; Pinto, M. Alice[1,2]

[1]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; [2]Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; [3]Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre, S/N, Edifício FC4, 4169-007, Porto, Portugal; [4]Institute of Biology, University of Graz, Graz, Austria; [5]Department of Mathematics and Statistics, University of Strathclyde, Glasgow, UK; [6]Cellular and Organismic Networks, Faculty of Biology, Ludwig-Maximilians-Universität Munich, Munich, Germany; [7]Danish Beekeepers Association, Sorø, Denmark; [8]Research Centre in Digitalization and Intelligent Robotics (CeDRI), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; [9]Alveus AB Consultancy, Oisterwijk, Netherlands

Framework:

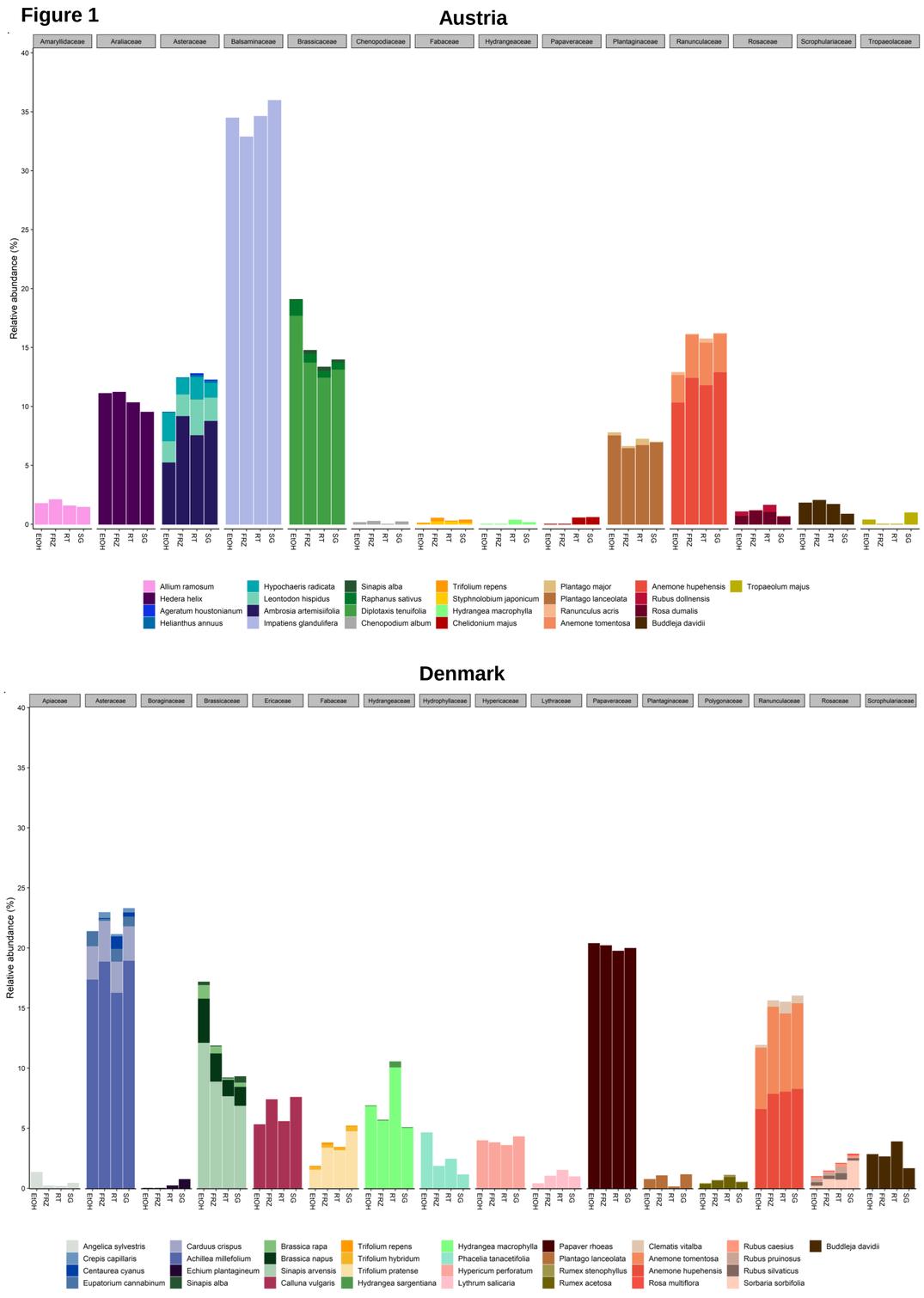
DNA metabarcoding is emerging as a powerful method for botanical identification of bee-collected pollen, allowing analysis of hundreds of samples in a single high-throughput sequencing run, therefore offering unprecedented scale in citizen science projects. Biases in metabarcoding can be introduced at any stage of sample processing and preservation is the first step of the pipeline. Hence, it is important to test whether the pollen preservation method influences metabarcoding performance. While in metabarcoding studies pollen has typically been preserved at −20°C, this is not the best method to be applied by citizen scientists. Here, we compared the freezing method (FRZ) with ethanol (EtOH), silica gel (SG) and room temperature (RT) in 87 pollen samples collected from hives in Austria and Denmark.

Results:

Relative Abundances (Fig. 1)

- ✓ 19 families were detected across methods and countries.
- ✓ Relative abundances are similar among the preservation methods in both countries.

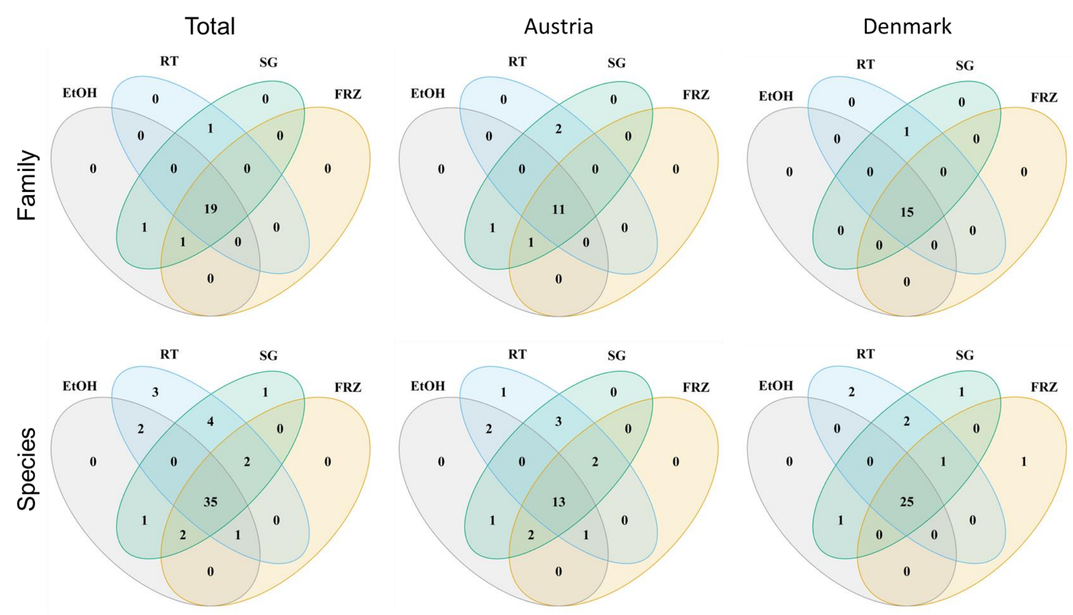
Figure 1



Taxa across storage methods (Fig. 2)

- ✓ 3 of the 19 families were rare and were detected only in pollen preserved in (i) EtOH and SG (*Tropaeolaceae*), (ii) SG and RT (*Boraginaceae*), and (iii) EtOH, SG and FRZ (*Chenopodiaceae*).
- ✓ 17 species had very low abundances and were only identified in samples preserved by one (4 species), two (7 species) or three (5 species) methods.
- ✓ 8 rare species were only detected in RT and/or SG replicates.

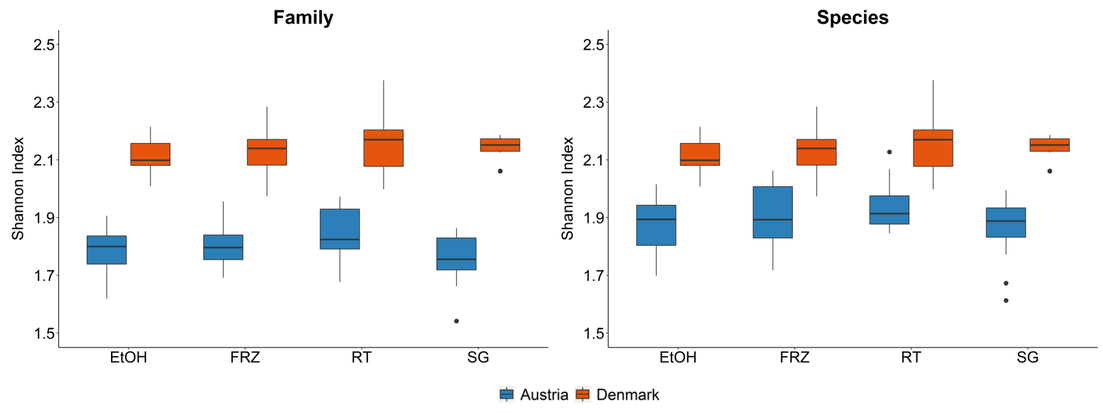
Figure 2



Shannon Index (Fig. 3)

- ✓ It showed close similarity of the flora diversity among preservation methods (Fig. 3).
- ✓ No differences were found for both Austria and Denmark, suggesting that bee-collected fresh pollen can be adequately preserved for ITS2 metabarcoding applications by any of the methods tested herein.

Figure 3



Final remarks:

- ✓ Methods involving desiccation can be used by the citizen scientist for medium-term pollen storage for downstream applications involving DNA metabarcoding.
- ✓ Relative humidity at room temperature may vary temporally and geographically, we recommend using silica gel for preserving bee collected fresh mixed pollen samples.
- ✓ The method is also easy to be applied by laymen, and therefore it is a robust option for widespread use in citizen science studies involving collection of pollen.

Methods:

- ✓ Homogeneous pollen solution was prepared in a magnetic stirrer using 2g of pollen sample and 4 mL of ultrapure water.
- ✓ DNA was extracted using the Macherey-Nagel NucleoSpin Food Kit, with an additional step were the pollen grains were ground in a bead mill.
- ✓ DNA metabarcoding was performed using a dual-indexed approach with the ITS2 barcode.

Acknowledgements:

AQ acknowledges the PhD scholarship (DFA/BD/5155/2020) funded by FCT. This study was funded by INSIGNIA “Environmental monitoring of pesticides use through honey bees” (SANTE/E4/SI2.788418-SI2.788452).

These results were published in the scientific journal Environmental Monitoring and Assessment (https://doi.org/10.1007/s10661-021-09563-4)