OP-249 [Beekeeping Technology and Quality]
Honey composition 2.0 – comparison between traditional pollen analysis and modern DNA analyses

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Background
Honey is a highly regulated natural sweet substance, valued for its regional and floral origin, both of which influence its characteristics. The traditional accredited method to determine floral origin—and indirectly the region—is light microscopy pollen analysis.

Problem description
Traditional pollen analysis requires specialized expertise and extensive reference collections. Advances in DNA sequencing now offer a high-throughput, sensitive alternative. Even though the methodology to extract and sequence DNA from honey now exists, little is known of how the results of pollen analysis and DNA analysis correspond with each other.

Methodology
We compared pollen identification from light microscopy and DNA sequencing (ITS2, rbcL, trnL) of 65 honey samples collected in Finland (2022–2023).

Results and conclusion
Most plant families detected by microscopy were also found via DNA analysis. DNA analysis identified significantly more genera (218) than microscopy (82). Both methods consistently detected high proportions of Brassicaceae, Fabaceae, Rosaceae, and Salicaceae. However, microscopy showed higher proportions of Rosaceae, Trifolium sp., and Salix, while DNA analysis detected more Brassicaceae. Taxa abundance patterns differed between methods, but both approaches correlated significantly at the genus (Spearman's $r = 0.373$, $P = 0.001$) and family ($r = 0.406$, $P = 0.001$) levels. Given the substantial overlap in commonly detected taxa, DNA analysis provides a more comprehensive view of plant composition and is recommended for routine honey origin identification.

Practical implications for beekeepers
Beekeepers and the honey market now have a new option to choose when determining the floral origin of honey, in other words, the plant usage of honeybees. Moreover, DNA analysis is more sensitive and more precise than pollen microscopy, as any DNA from plant tissue in honey, not only pollen, can be identified. Through DNA analysis plants can be identified to genus or species level, while with pollen the plant origin can often only be distinguished at coarser level. Besides honey plant contents, DNA analysis may reveal many other factors, such as beneficial microbes or bee-related pathogens, which can tell about the regional origin of the honey, as well as the well-being of the bees.

Comparison between traditional pollen analysis and modern DNA analyses

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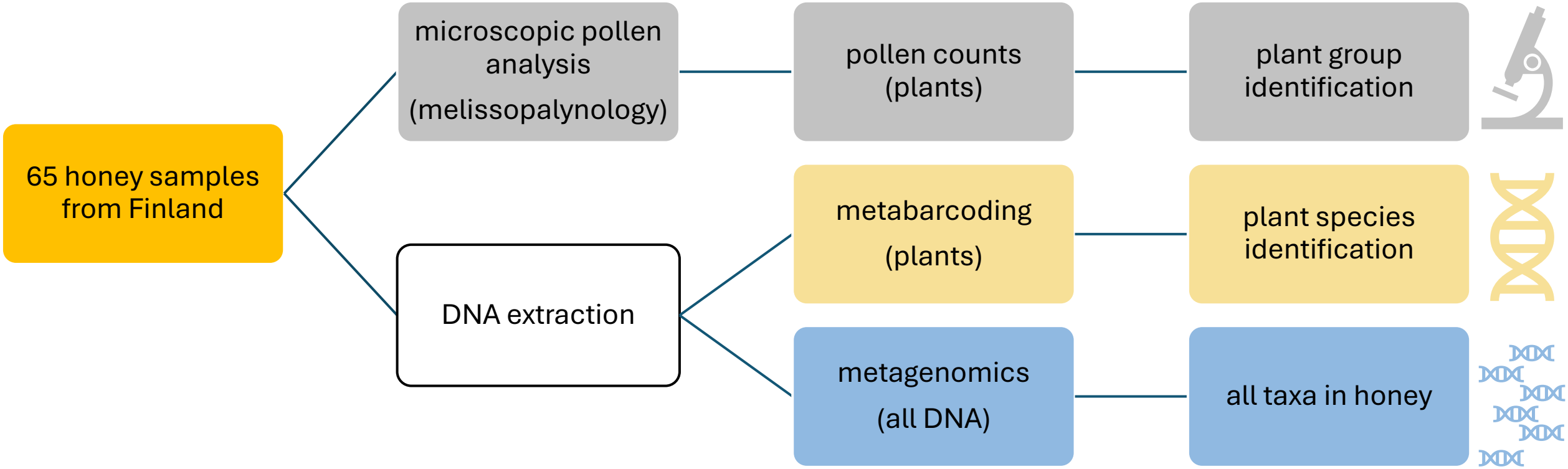
Background

1. Honey contents need to be analysed when commersialised
2. Current ”official” method is light microscopy (*melissopalynology*)
3. DNA offers a novel option for traditional methods
4. DNA is an entirely different tool that:
   - ➢ can detect all plant material in the sample
   - ➢ may also reveal other organisms
   - ➢ can be compared to microscopy, but we shouldn’t expect 1:1 results
   - ➢ DNA analysis can be **targeted** e.g. with PCR: **metabarcoding**
   - ➢ DNA can be sequenced **without targeting: metagenomics**

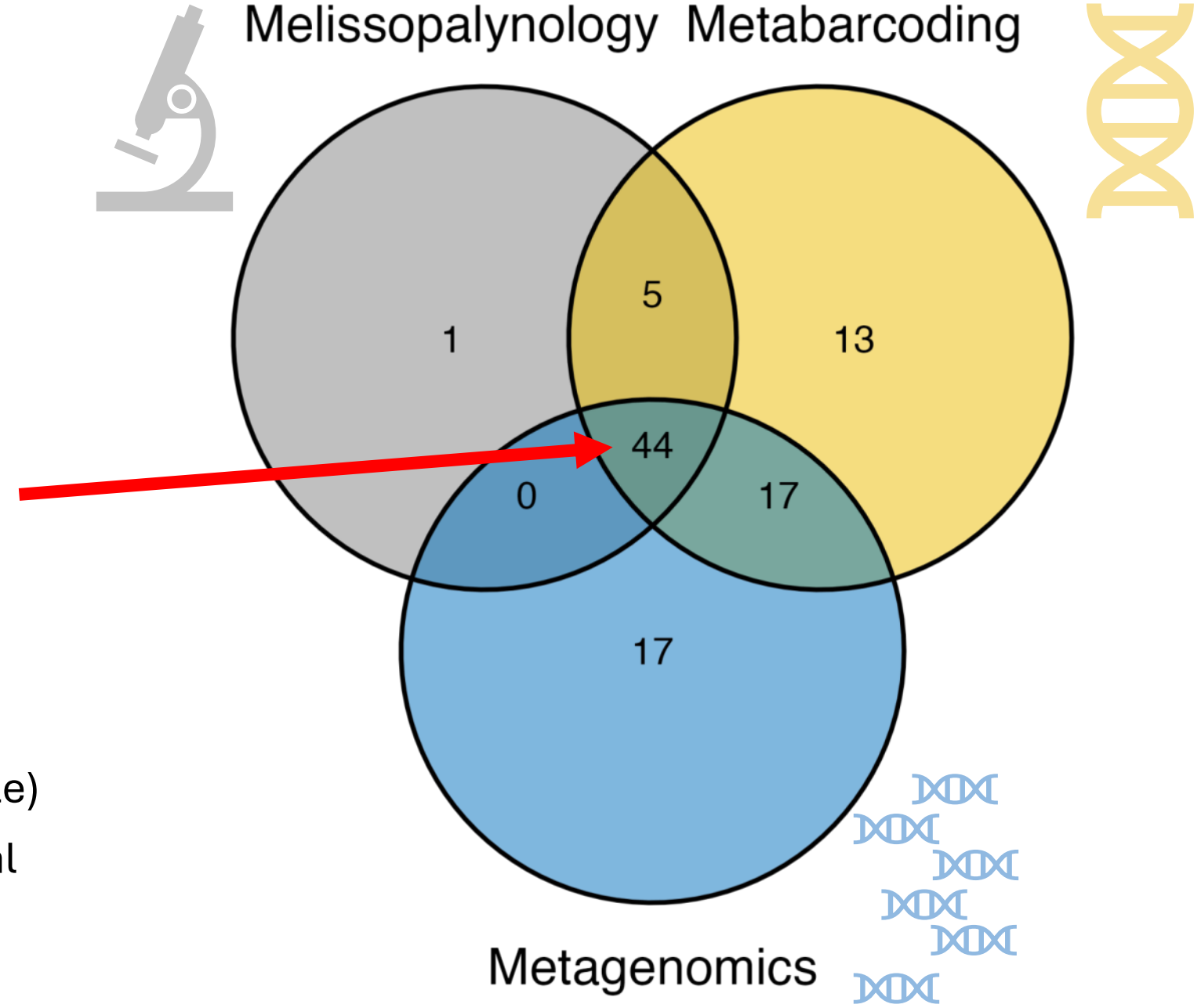
Our study



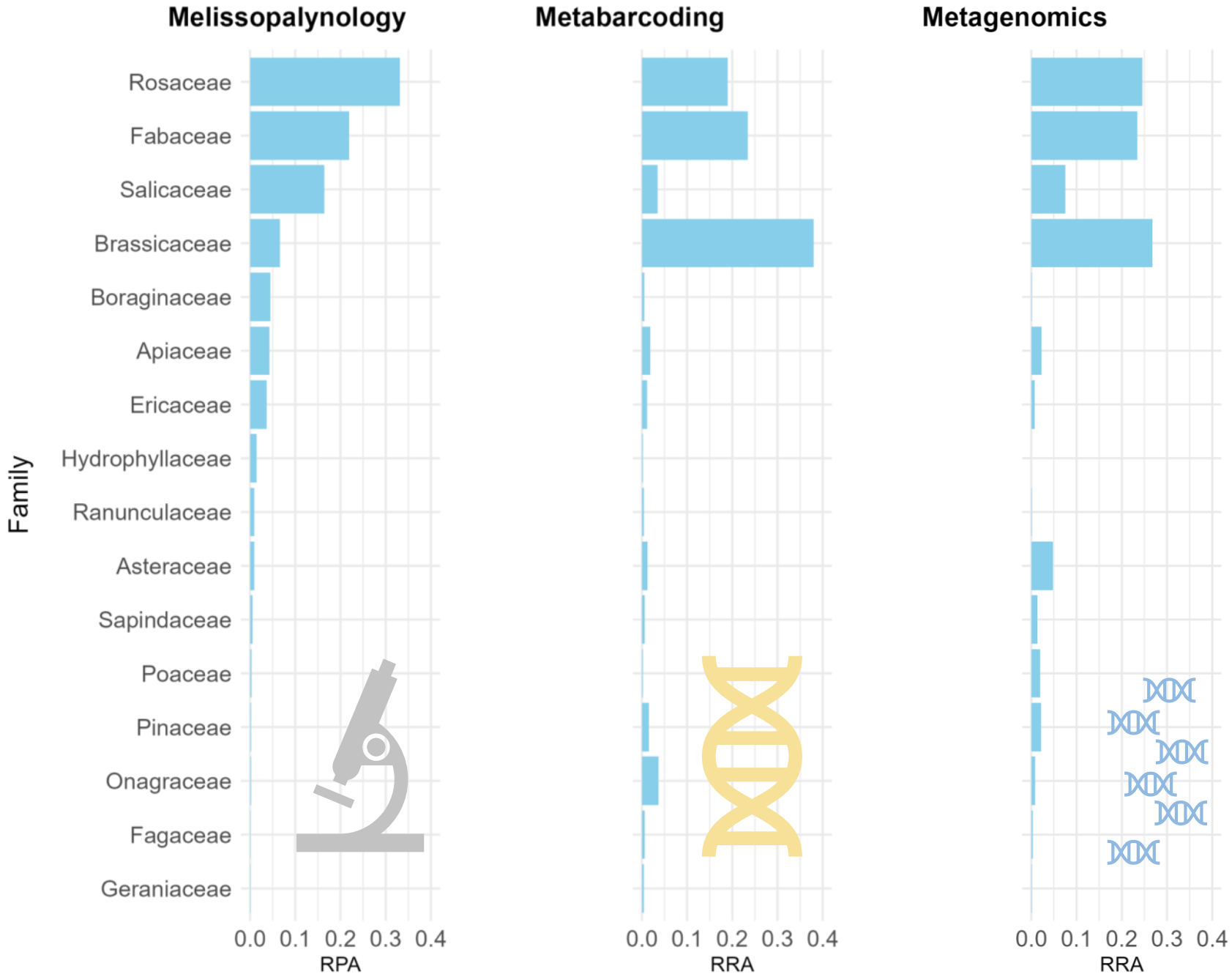
Melissopalynology: Von Der Ohe *et.al.* (2004) Harmonized methods of melissopalynology Apidologie, DOI: https://doi.org/10.1051/apido:2004050
DNA methods: Wirta H., Abrego N., Miller K. , and Vesterinen E. (2021) DNA traces the origin of honey by identifying plants, bacteria and fungi. Sci Rep 11, 4798. https://doi.org/10.1038/s41598-021-84174-0

Results

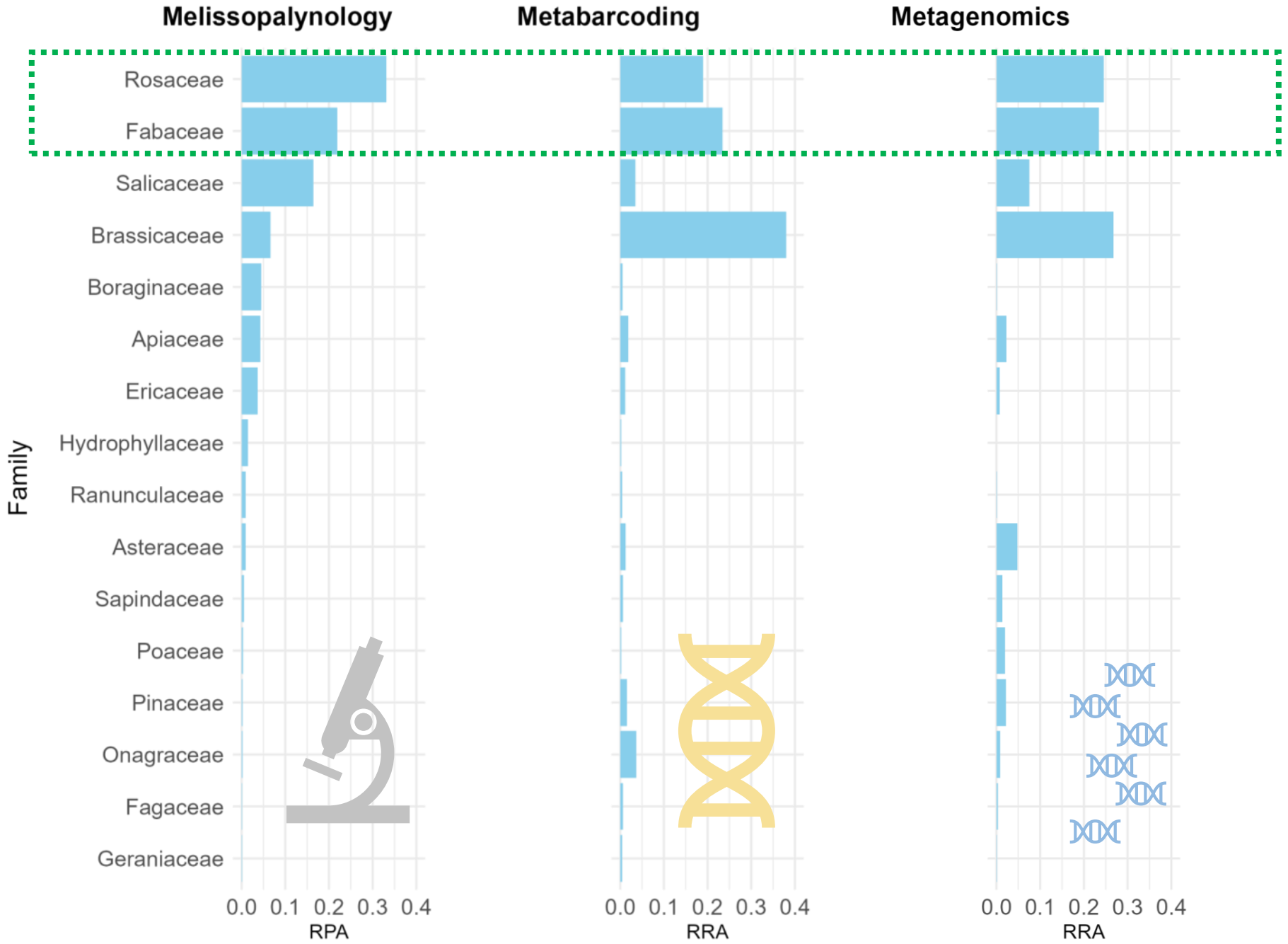
- Most plant families detected by all methods
- Only one family detected by microscopic analysis but NOT by DNA (Juncaceae, *Juncus*: one pollen grain in one sample)
- Both DNA methods reveal more families than microscopic analysis



Comparison between methods – only top families included



Comparison between methods – only top families included



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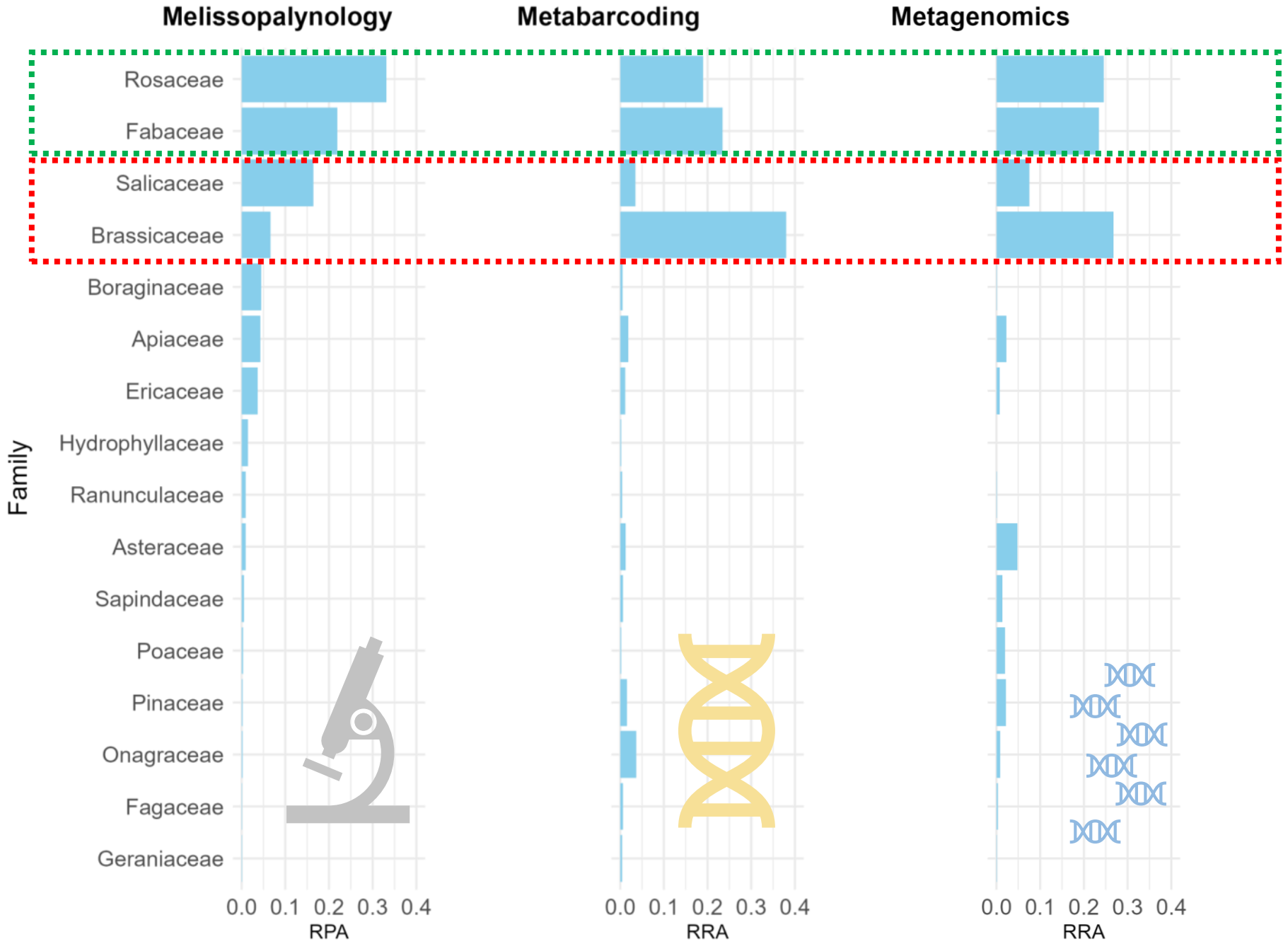


Results

	Melissopalynology
Number of families	50
Number of genera	73
Number of species	14

Results

	Melissopalynology	Metabarcoding
Number of families	50	79
Number of genera	73	199
Number of species	14	175

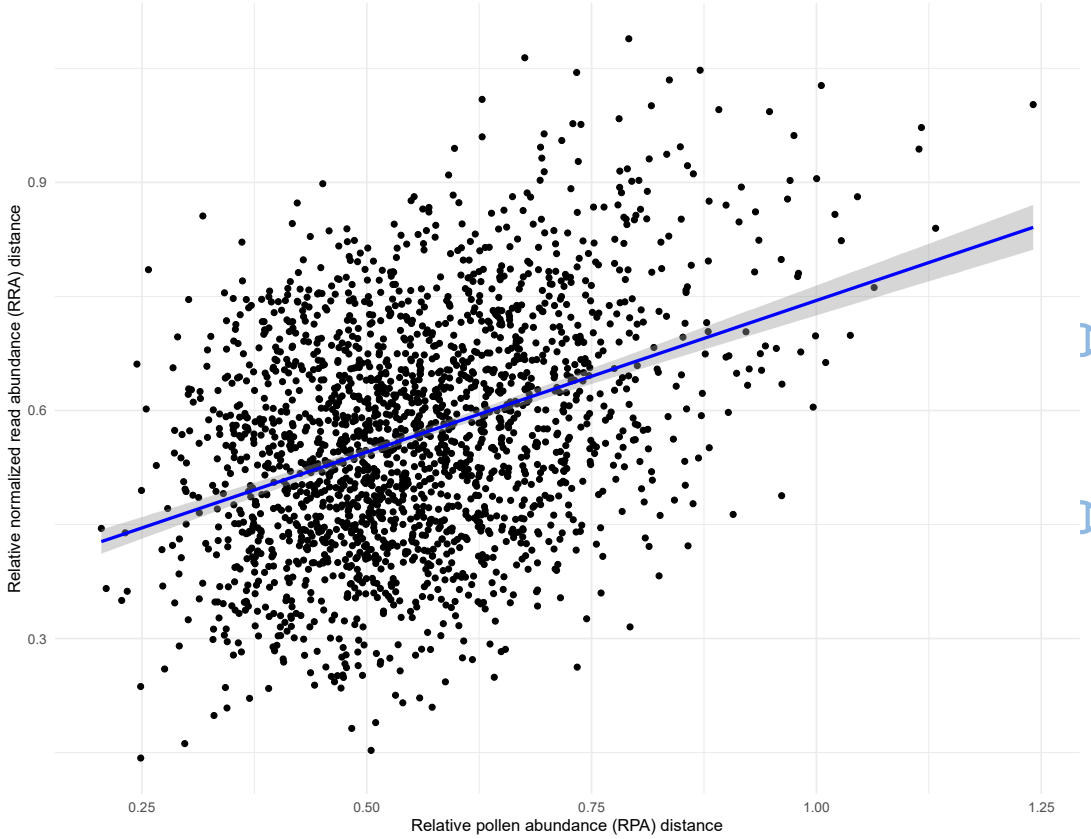
Results

	Melissopalynology	Metabarcoding	Metagenomics
Number of families	50	79	78
Number of genera	73	199	206
Number of species*	14	175	227

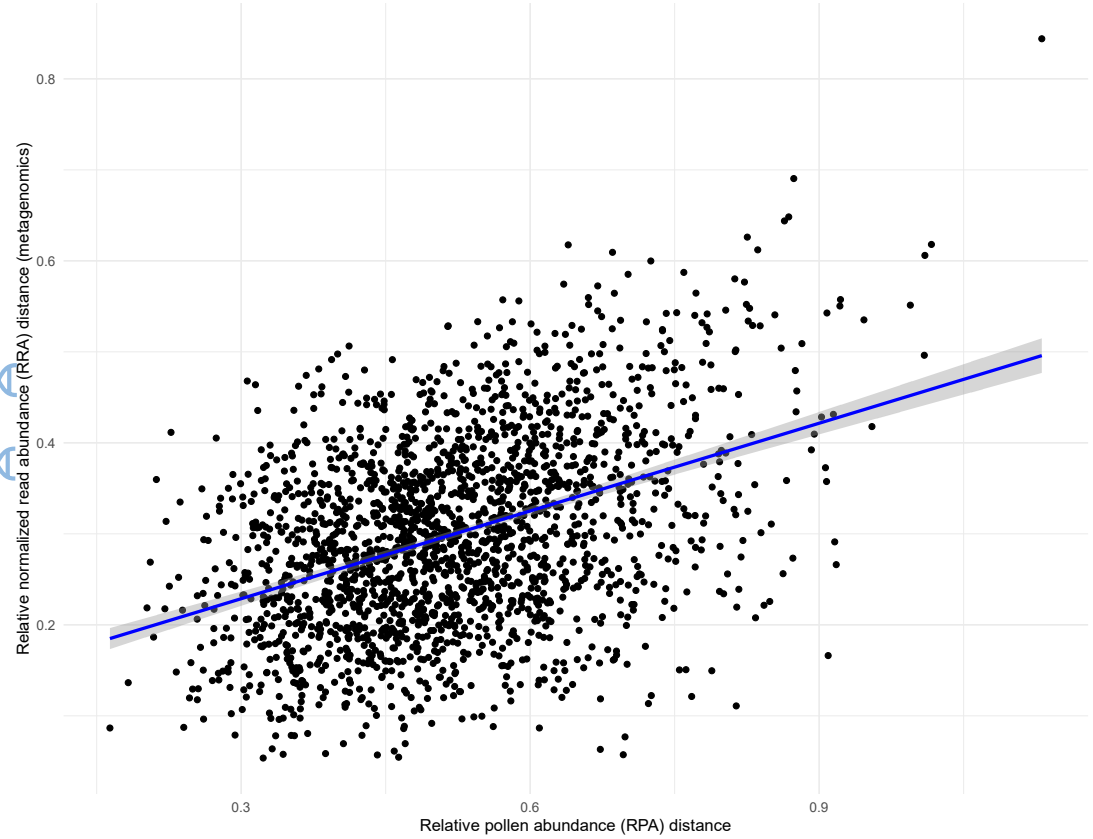
*These were identifed to species level with species name, and for this reason the number of genera may be larger than the number species.

Statistical comparison: Mantel test

Mantel correlation: 0.323, p-value: 0.001



Mantel correlation: 0.37, p-value: 0.001



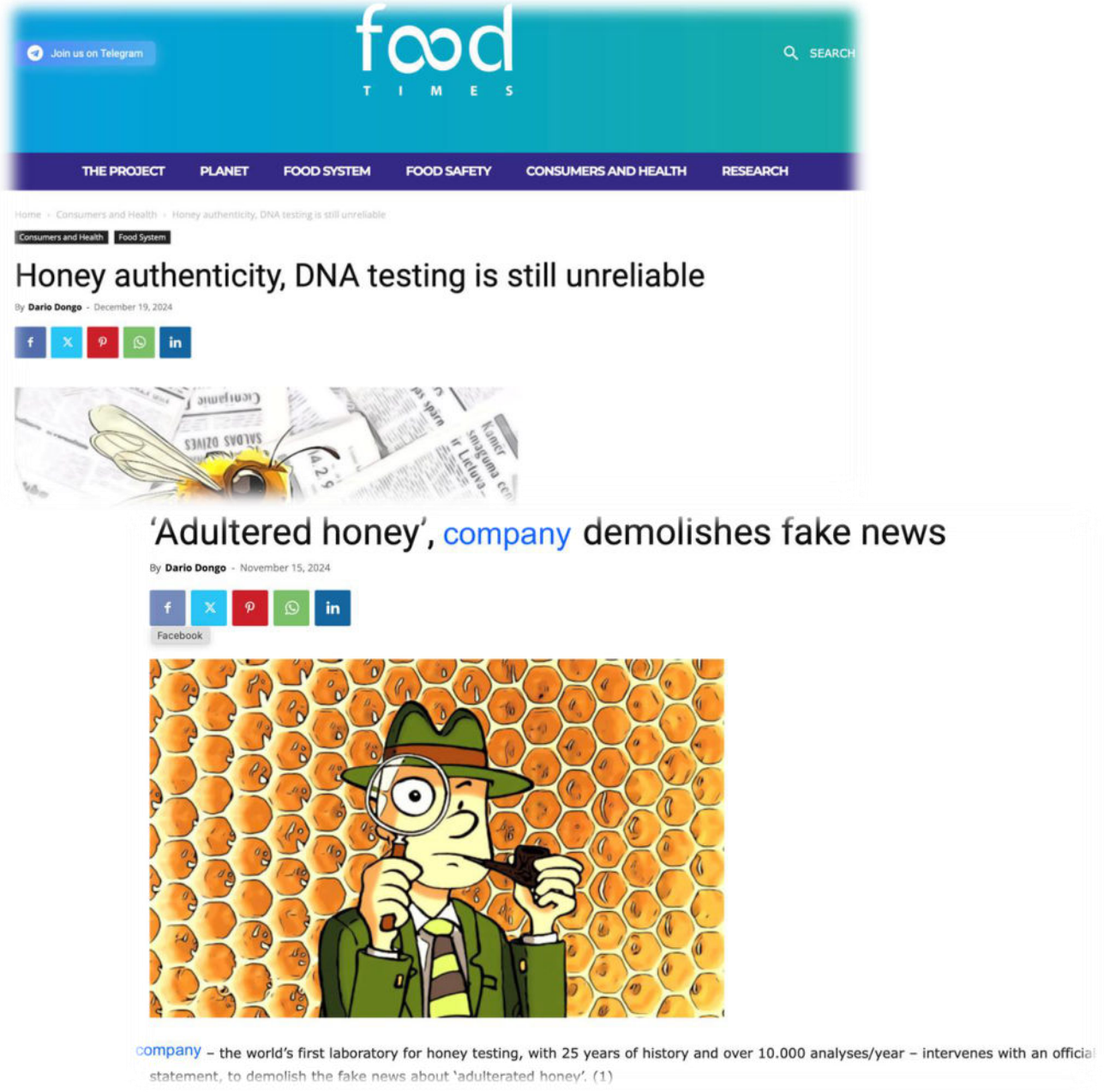
Conclusions

1. DNA gives comparable results with microscopic analysis
2. DNA is more sensitive, revealing more plant taxa
3. Generally, DNA is a very promising tool for honey producers
4. Honey DNA analysis requires accrediation before used “for real”
5. Also, the determination of honey origin requires more work
   → more honey samples from all over the world
6. Honey adulteration detection using DNA might be possible, **but requires extensive expertise** → one should not jump into conclusions too quickly!

Honey authenticity

DNA approach for honey needs to be:

- independently tested for its robustness and accuracy
- compared with several laboratories in comparative analyses
- based on a wide and confirmed database



This is how to achieve the requirements

A. Detailed investigation at the honey sample level
B. Experimental testing for honey adulteration
C. Examples of real, authentic honey
D. Geographic coverage from all over the world

Shortly: detection of honey adulteration using DNA requires more work.

To progress with honey DNA analyses, **many more honey samples need to be processed**!

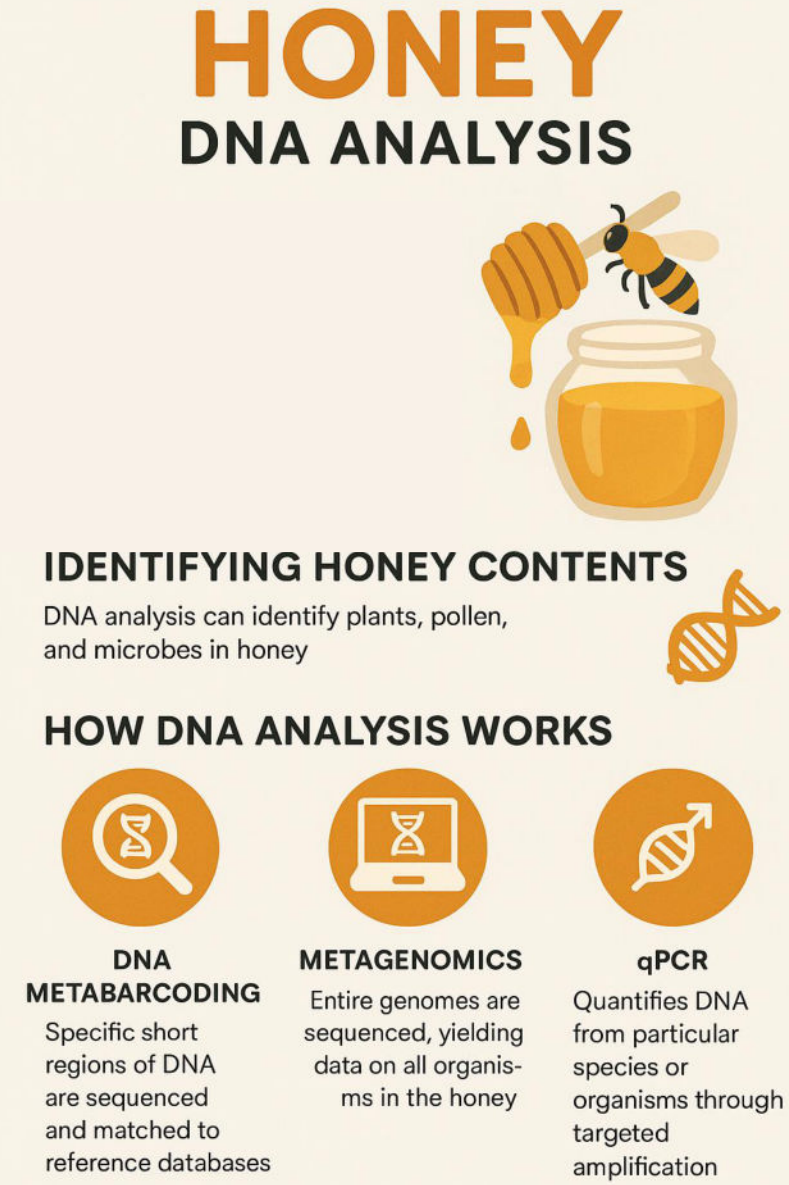
How to sample more honey?

1) Register your honey sample here:

https://forms.cloud.microsoft/r/eVKa5d1fFx

2) You may send your honey sample to our laboratory or give it to me during Apimondia

3) Anyone can participate



UNIVERSITY OF TURKU

BioName

DNA analyses as turnkey solutions

Thank you!

1. Register your honey sample

2. If you want your own results, then:

order DNA analysis results

50% sale during Apimondia 2025

Extra information revealed by DNA approach

Disease	Causative agent	Pathogen	Samples
American foulbrood	Bacterial	*Paenibacillus larvae*	6
Chalkbrood	Fungal	*Ascosphaera apis*	8
Varroasis	Mite	*Varroa destructor*	14
Nosema disease	Protozoan	*Vairimorpha ceranae*	4