



METABARCODING APPROACHES FOR WILD BEE DIVERSITY ASSESSMENT IN FRENCH AGRICULTURAL LANDSCAPES

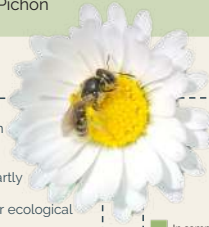
With COI and 16S markers

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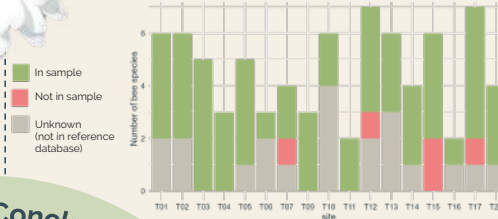
INTRODUCTION

- 90% of wild flowering plants and 70% of crops are pollination dependant¹
- Drastic decline of pollinator populations worldwide^{2,3,4}
- Land management and pesticides have been reported as partly responsible for that decline⁵
- Lack of entomologists represents a significant bottleneck for ecological studies^{6,7}
- Need to develop alternative high-throughput identification methods: molecular (metabarcoding, eDNA), acoustic-based, image-based,⁸
- Metabarcoding is widely used in pollinators studies, mainly with the COI (Cytochrome c oxidase subunit I) marker^{9,10,11} and other markers (16S, 18S...)^{12,13}
- Traditional metabarcoding, using bee tissue (legs, whole body...):
 - is destructive: need to conserve bee integrity for rare species or for morphological (re-)identification
 - only targets trapped bees, not traces of bees that escaped or were predated



PRELIMINARY RESULTS

Figure 1 : Number of morphologically identified bee species retrieved in metabarcoding 16S samples per site



- Between 2 and 7 wild bee species per site
- Missing species in 4/17 sites

QUESTIONS

- Is metabarcoding a suitable approach for wild bees ecological and conservation studies?
- Can metabarcoding of liquids be an alternative to the use of bee tissues?



Preliminary Conclusions

- 16S marker is performant for bee tissue metabarcoding
 - more efforts needed to complete databases
- Liquids capture less wild bee diversity than traditional metabarcoding with 16S marker
 - Not an alternative to destructive metabarcoding as is
 - Liquids containing only bee species could improve their detection
- Liquids detect non-bee taxa trapped in field
 - Can be used for multi-taxa studies
 - Need to design bee specific markers with available mitogenomes

What's Next?

- Continue previous efforts^{15,16} to complete COI and 16S databases
- 2024 COI metabarcoding (tissue, liquids) analysis + 2025 field sessions
- Investigate mismatches between morphologically identified specimens and metabarcoding results
 - Compare how agricultural practices influence bee biodiversity
- Environmental DNA analysis of flowers to detect traces of wild bees with 16S and COI markers

Table 1 : Proportions of bee species retrieved in 16S samples

	Bee legs	Sampling liquids	Storage liquids
In sample	67%	37%	30%
Not in database	27%	27%	27%
Not in sample	6%	37%	43%

- 67% of morphologically identified species found in bee legs samples
- 27% lacking barcode reference in database
- 2 species missing in bee legs samples due to:
 - Blast parameters (*L. politum*)
 - Same barcode sequence for *E. nigrescens* and *E. nigricans*
- 1 species unaccountably missing in T07 bee legs sample (*L. punctellae*)
- Liquid samples capture ~1/3 of trapped bee species

METHODS



Sampling :

- 17 sites in ZA PYGAR¹⁴ (France)
- Conventional vs organic crops
- 3 pan-traps / site, on crop edges, 3 days in April 2024



- Filtered insects transferred in ethanol and trapping liquids (water + detergent) stored at -20°C during 4 months



DNA Extraction :

- Trapping liquids (x57) and storage liquids (x51) filtration + DNA extraction per pan-trap
- Bee pinning for morphological identification (positive control)
- 114 bee legs sampling (31 species) + DNA extraction per site (x17)



Sequencing and analysis :

- PCR amplification with 250 bp 16S and 400 bp COI
- Aviti 2x300 bp sequencing at Get-PlaGe Platform
- Demultiplexing markers and trimming : cutadapt
- Denoising : Qiime2 denoise-paired
- Taxonomic assignment : BLASTn on Genbank nt database

Figure 2 :

A. *Trichodes* genus
B. Specimens in T14 sample



- Detection of non-bees species in liquid samples with the 16S marker
- Example : One of the most abundant sequence in T14 liquid samples blasts on the *Trichodes* genus, trapped in the field



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* *Trichodes aparius* (Linnaeus, 1758) Observed in Croatia by Ivica Pakrac, cropped vegetation ** *Xylcopa violacea* (Linnaeus, 1758) Observed in Austria by Andreas Pospisil, cropped background
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Comparing several metabarcoding approaches to assess wild bee diversity in contrasted agricultural landscapes using complementary COI and 16S markers

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DNA metabarcoding is widely used to study pollinator populations at large scales, as it enables the rapid identification of specimens without requiring taxonomic expertise. The most common approaches involve partially or completely destroying captured specimens to sequence a specific region of the COI gene, the most frequently used marker, allowing for their identification. However, since the method is destructive, it can be problematic when dealing with rare specimens, especially if they cannot be assigned to the species level due to the marker's low discriminatory power for these taxa, or amplification difficulties. To address this, we designed a study to evaluate DNA metabarcoding from trapping and storage liquids compared to destructive approaches, such as metabarcoding from bee leg tissue and morphological identification. Over three days, we deployed three colored pan traps at seventeen sites with contrasted agricultural landscapes and practices in ZA PYGAR, in the south-west of France. Trapping liquids were stored at -20°C, and captured specimens from each trap were preserved in ethanol at -20°C for five months. Bees were morphologically identified by an entomologist, with two legs being sampled from each specimen: one for individual barcoding and the other for site-level metabarcoding. Additionally, DNA was extracted and sequenced from both the storage and trapping liquids. To gain in taxonomic resolution and limit amplification failure for a given marker, we used a 400bp COI marker and a 250bp 16S marker, as reliable and extensive reference databases are available for both in our study area, and as previous studies suggest they are complementary. The use of liquids has emerged as a promising strategy to accelerate the metabarcoding workflow, render it non-destructive, and facilitate the identification of specimens that may have eluded capture.