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Currently, inventories and monitoring of wild bees in France face significant methodological obstacles linked to a lack of entomologists and reliable, standardized methods allowing identifications down to the specific level. DNA barcoding and metabarcoding open new perspectives to study wild bee populations at large scales. However, such studies require reliable and complete reference databases. During the course of the CODABEILLES project, we collected samples from 2260 specimens representing approximately 650 species and processed them through DNA barcoding, targeting the standard COI-5P fragment. Our results produced DNA sequences for 1477 (65% success rate) specimens belonging to 561 French wild bee taxa. The specimens come from 16 academic or private collections; all of them were identified, photographed, and the associated metadata were integrated into the Barcode of Life Datasystems (BOLD). All sequences went through a curation and validation process including: the removal of contaminations, the correction of sequencing errors, the computation of genetic distances and the inference of distance trees. In cases of inconsistencies between the sequence and the initial identification, the specimens were re-examined by entomologists. Thanks to CODABEILLES and projects occurring in parallel, the proportion of French wild bee species with reference DNA barcodes increased from 63% to around 86% since 2021, and may even increase to 95%, depending on the sequencing success of additional samples currently in progress. This work contributed to expanding DNA barcode data for many species of the Mediterranean Basin that previously lacked references. Additionally, it highlights the high level of cryptic diversity that still exists among diversified groups in southern Europe, notably within the *Andrena* genus, underscoring the need for further extensive taxonomic investigations.

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2025



CODABEILLES

A Reliable Reference Library of COI DNA Barcodes for French Wild Bees Monitoring (Apoidea: Anthophila)

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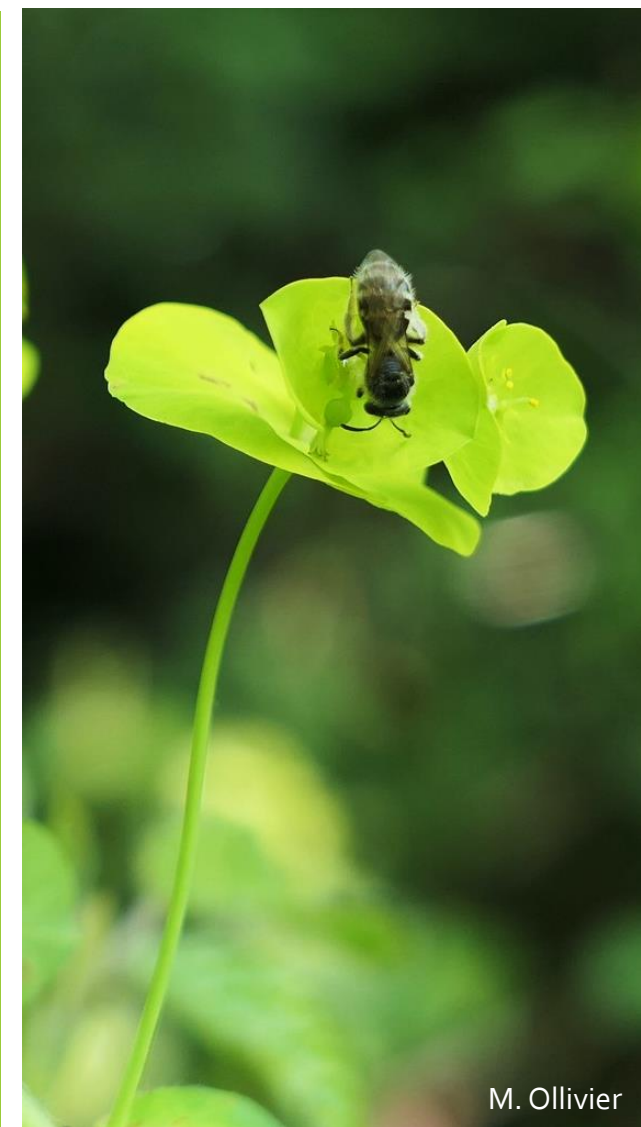
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⁵ Muséum national d'Histoire Naturelle, Sorbonne Universités, Institut de Systématique, Évolution, Biodiversité (ISYEB), UMR7205 – CNRS, MNHN, UPMC, EPHE-PSL, Paris, France.

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Members of the CODABEILLES Consortium: Emilie Andrieu, Emmanuelle Artige, Matthieu Aubert, Yvan Brugerolles, Alexandre Cornuel-Willermoz, Raphaël Da Silva Ropio, Adeline Dumet, David Genoud, Benoît Geslin, Laurent Guilbaud, Bernard Kaufmann, Lara Konecny, Anne-Laure Jacquemart, Danny Lebreton, Vincent Leclercq, Gabriel Nève, Annie Ouin, Christophe Philippe, Bertrand Schatz, Jean-Claude Streito, et Héloïse Vallod.



M. Ollivier

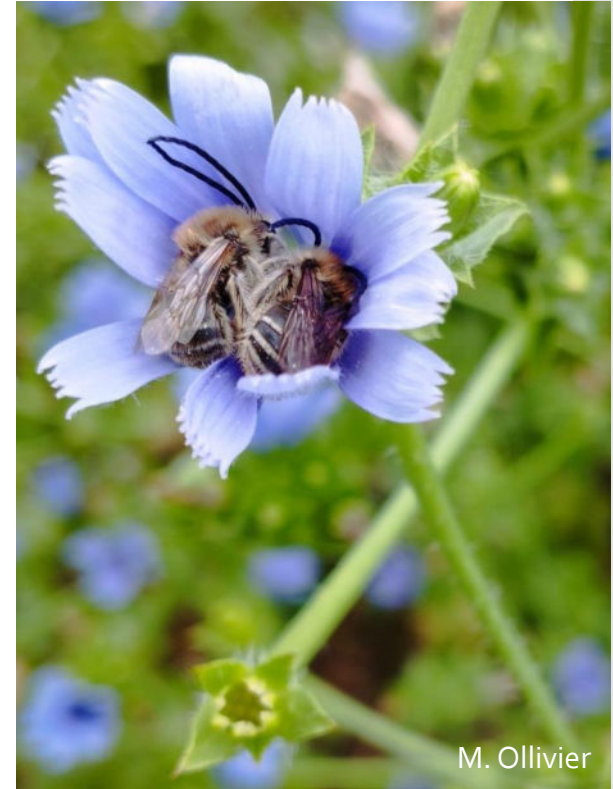
Funding



Background

Massive insect population decline in the Anthropocene (Wagner et al., 2021)

Animal pollination: 75% of cultivated plants and the majority of wild plants (Klein et al., 2007)



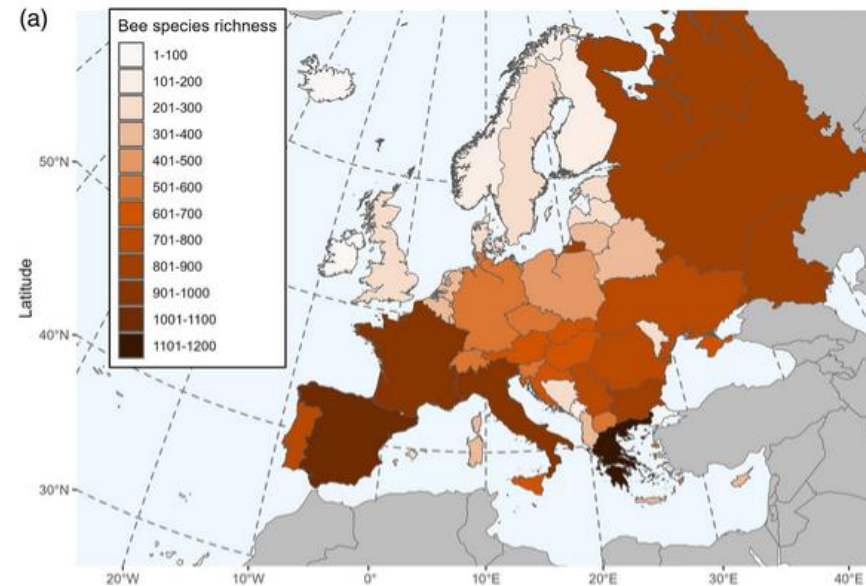
M. Ollivier

Eucera sp. ♂ in *Cichorium* sp. flower

Background

Massive insect population decline in the Anthropocene (Wagner et al., 2021)

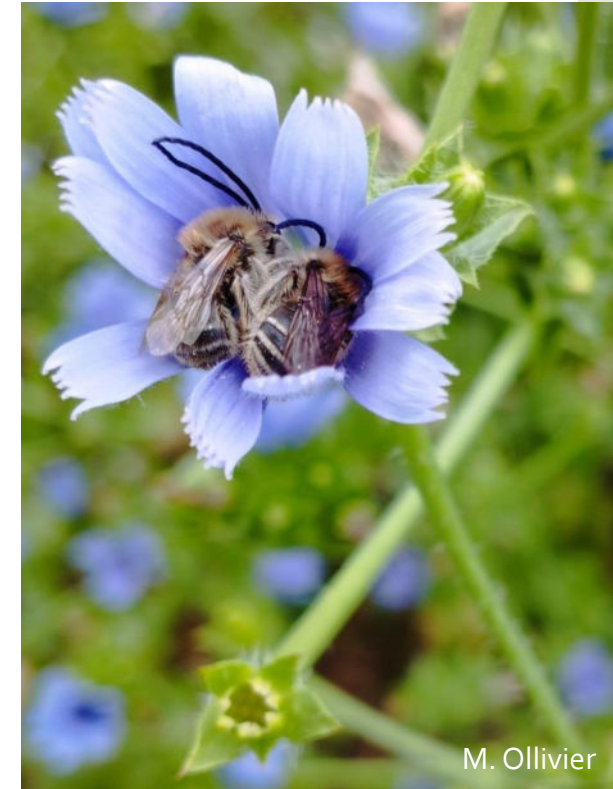
Animal pollination: 75% of cultivated plants and the majority of wild plants (Klein et al., 2007)



Bees (Apoidea: Anthophila) = 20 000 species worldwide, 2138 in Europe (Reverté et al., 2023)

The Mediterranean Bassin: a hotspot of bee diversity. **France** has the 4th most diversified fauna in Europe: **983 species** (Ropars et al., 2025)

Apimondia – Ollivier et al.



M. Ollivier

Eucera sp. ♂ in *Cichorium* sp. flower

Background

- Large-scale and long-term monitoring schemes: understand and counteract factors causing disruption to wildlife



Restoring Pollinator habitats across Europe

Needs:

- 1) Robust taxonomic framework
- 2) Rapid and cost-effective identification

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Restoring Pollinator habitats across Europe

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?

Morphological ID

Molecular ID (Ollivier et al., 2024)
= DNA barcoding

Image-based ID (Buschbacher et al., 2020)

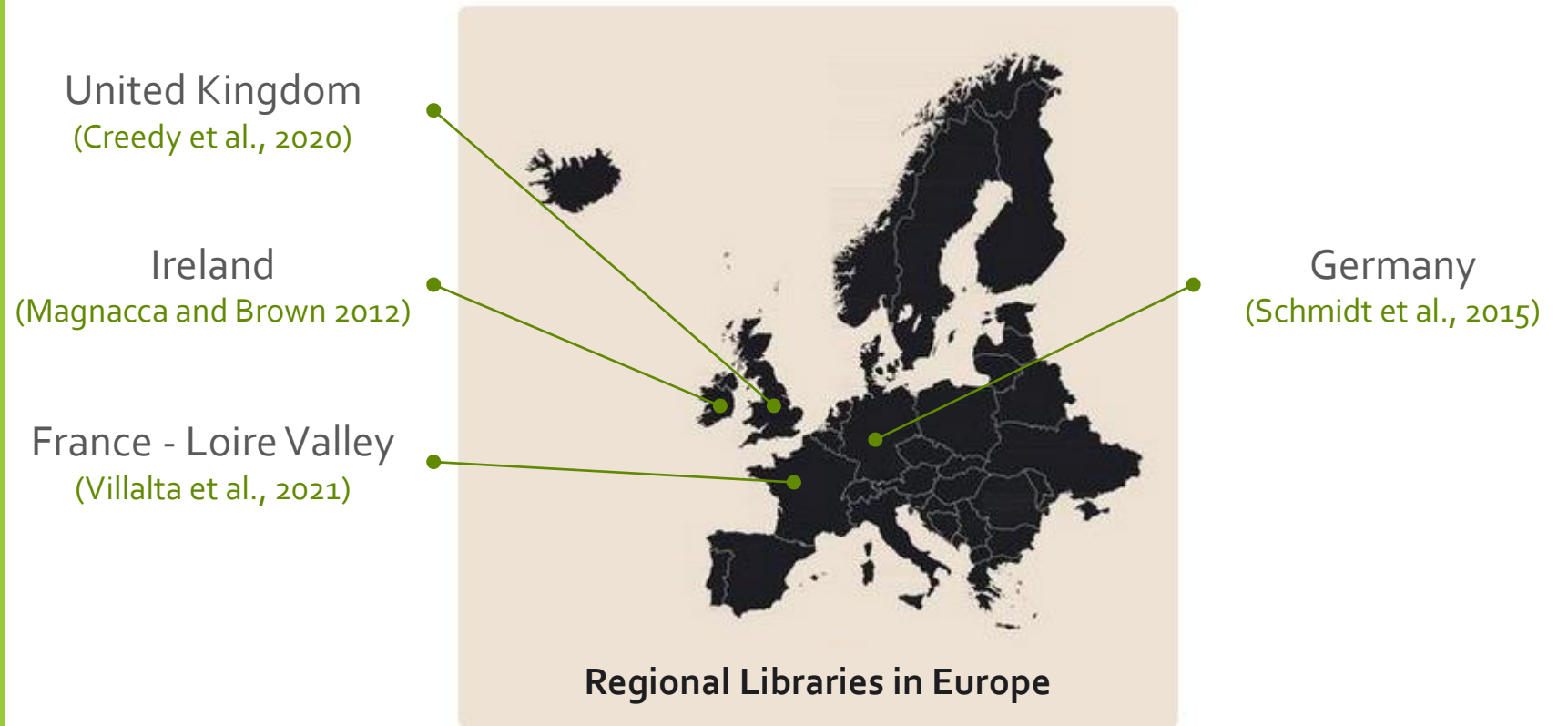
Comprehensive & reliable
DNA reference libraries



Reference barcode for wild bees?

Targeted gene:

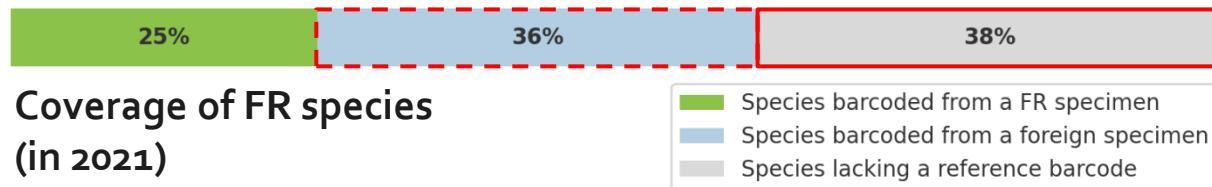
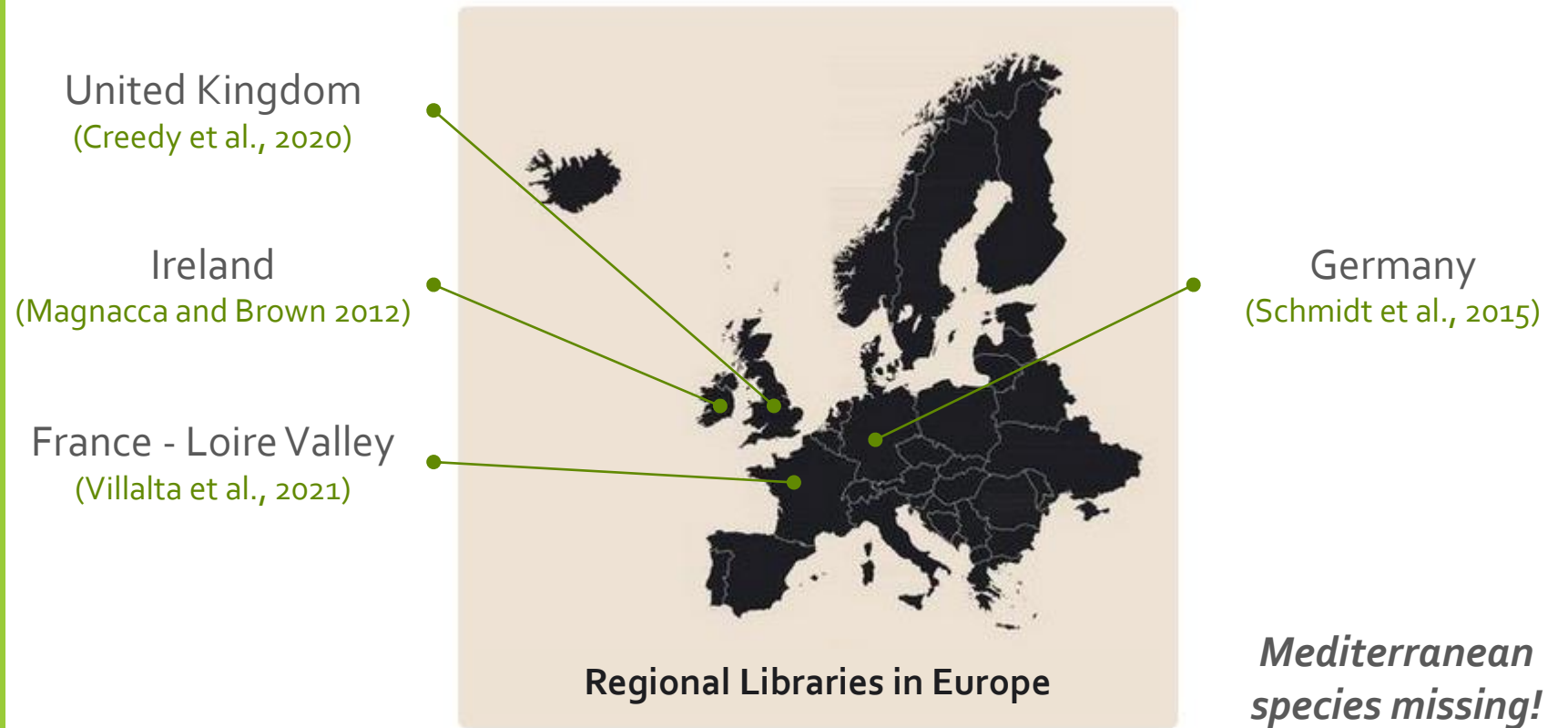
- Mitochondrial gene **CO1-5P** (658 bp) suitable for animal ID (Hebert et al., 2003)



Reference barcode for wild bees?

Targeted gene:

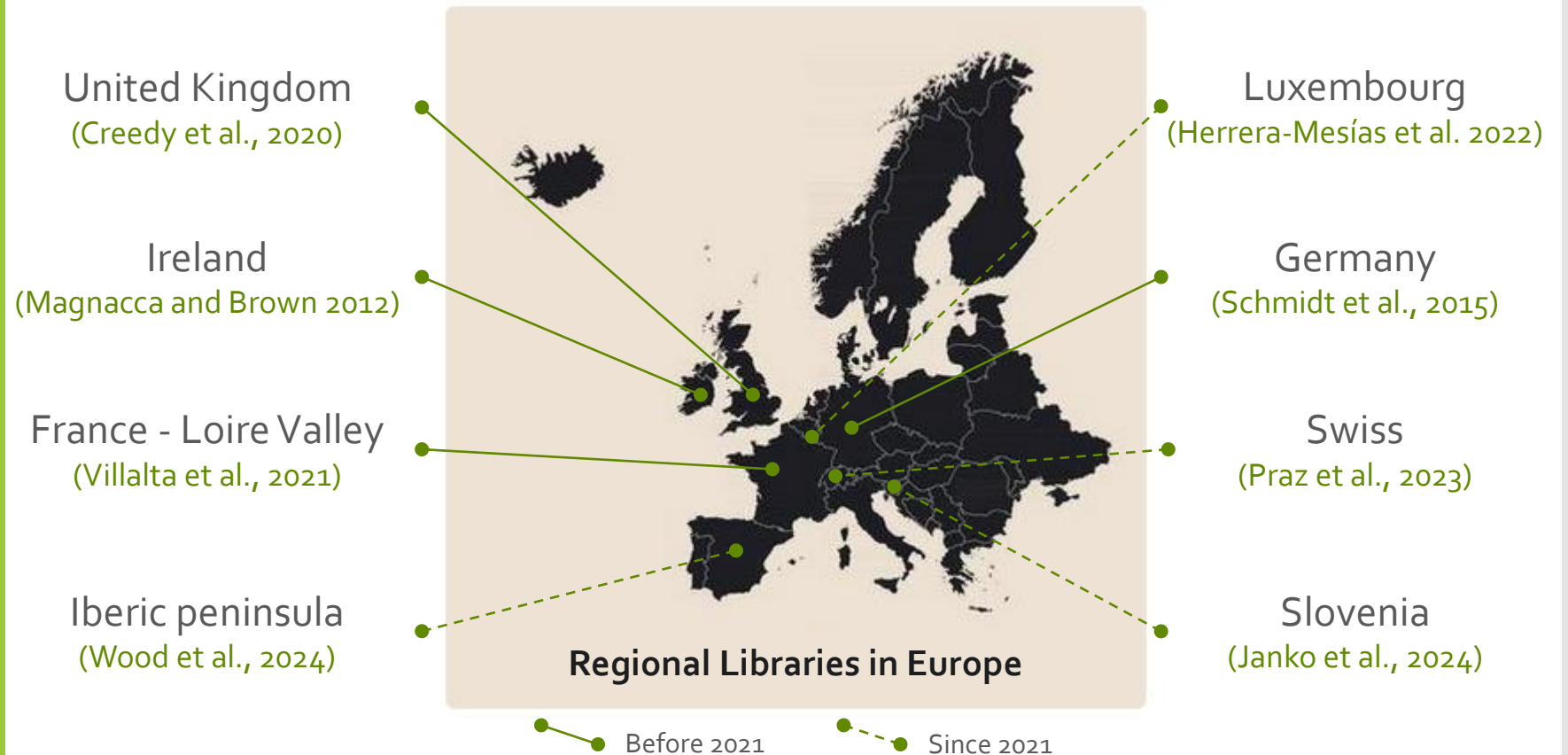
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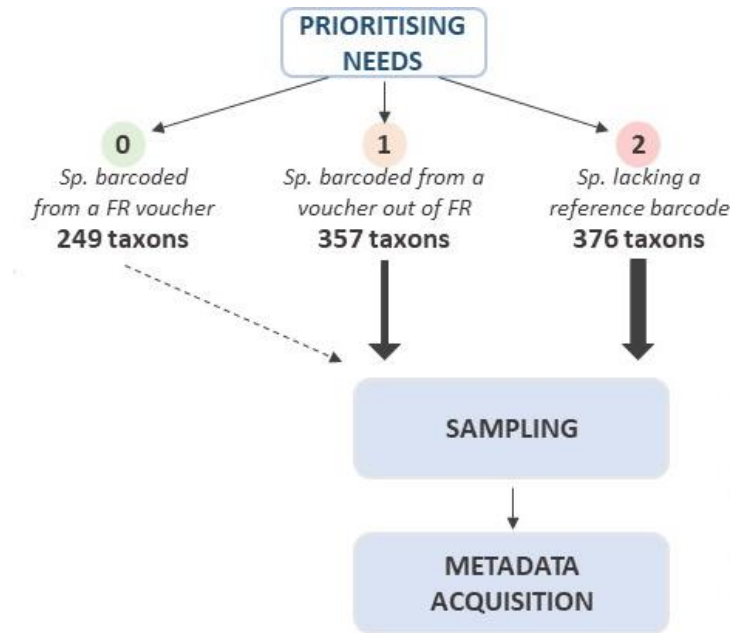
Reference barcode for wild bees?

Targeted gene:

- Mitochondrial gene **CO1-5P** (658 bp) suitable for animal ID (Hebert et al., 2003)



Sequence data acquisition & analysis



Bee images: Y. Brugerolles A. Perrard, M. Ollivier

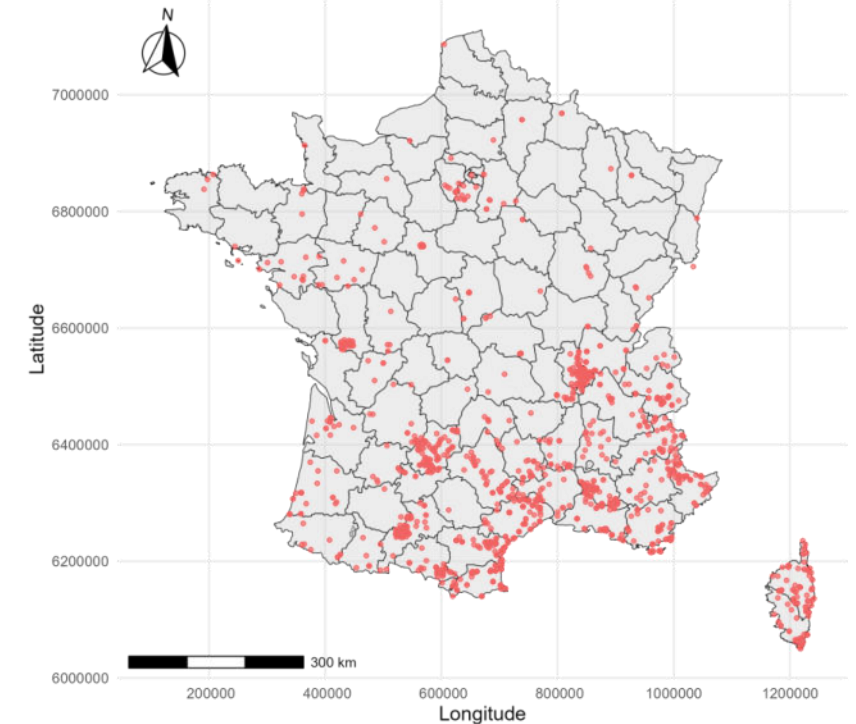


Halictus carinthiacus

Thyreus histrionicus

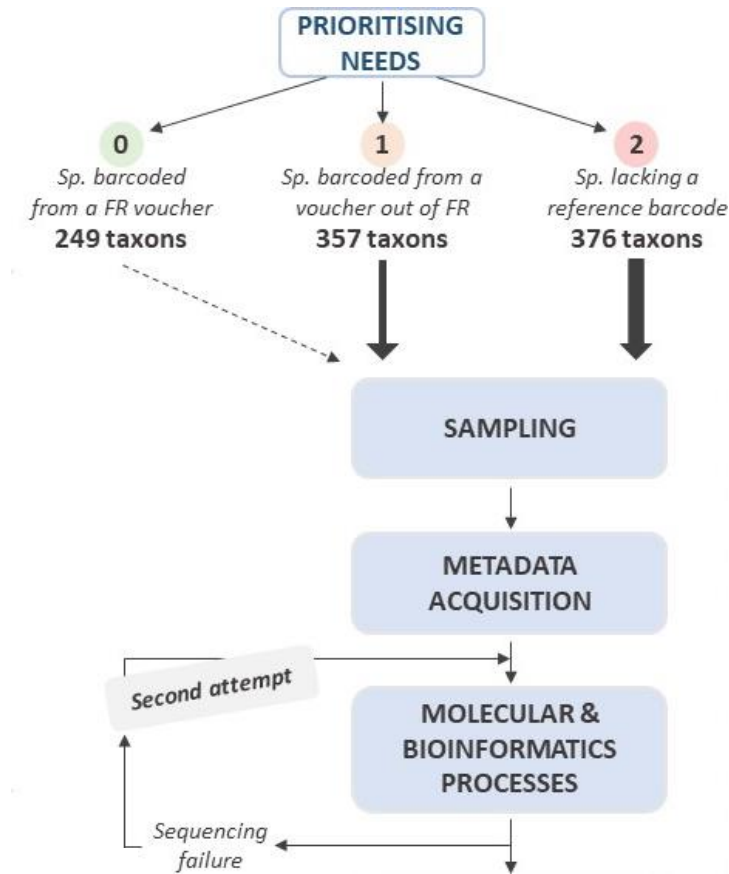
Anthidium cingulatum

Sampling locations of the specimens processed in CODABEILLES



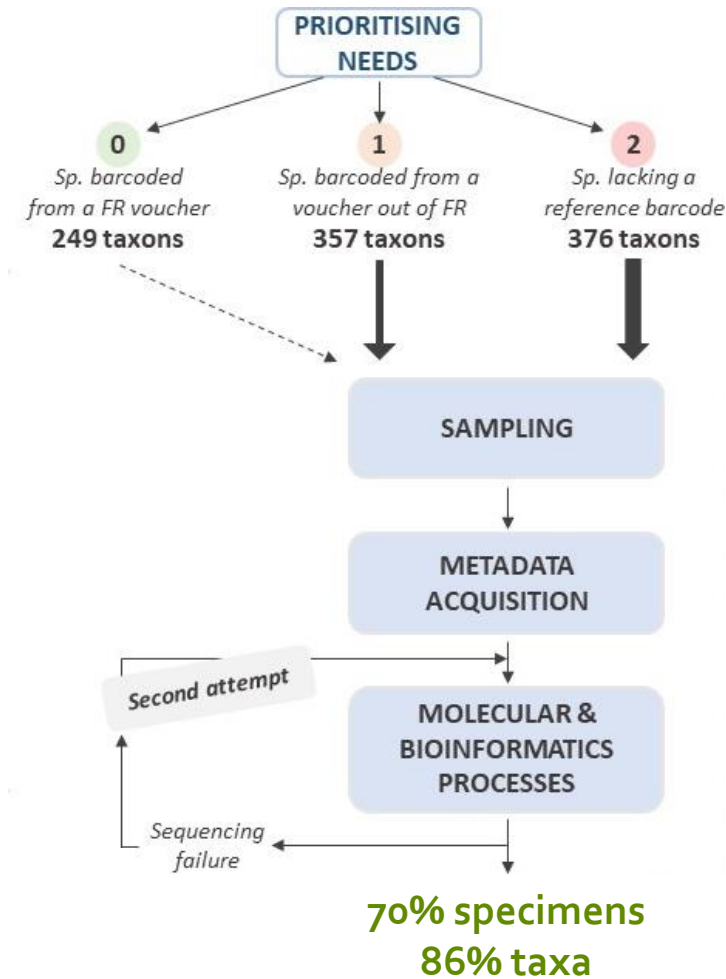
16 Institutional and private collections
Sampling of one fore or hind leg
3 specimens from different locations
➤ **2260 specimens, 649 taxa**

Sequence data acquisition & analysis



DNA extraction, amplification CO1-5P
(primer cocktail LCO1490/HCO2198 + LepF1/LepR1)
SMRT sequencing PacBio Sequel
mBrave Bioinformatic analyses

Sequence data acquisition & analysis



- Sequencing success varied :
- Accross taxonomic groups
 - Accross collections (storage conditions)

Apimondia – Ollivier et al.

Family	Nb of sampled specimens	Success rate (%)
Andrenidae	480	49
Apidae	799	69
Colletidae	133	58
Halictidae	402	64
Megachilidae	401	81
Melittidae	45	67

Species	Nb of sampled specimens	Success rate (%)
<i>Lasioglossum bimaculatum</i>	12	0
<i>Andrena tenuistriata</i>	10	0
<i>Lasioglossum prasinum</i>	10	0
<i>Chelostoma florissomne</i>	9	0
<i>Andrena aeneiventris</i>	7	0
<i>Bombus jonellus</i>	7	0
<i>Andrena strohmei</i>	6	0
<i>Megachile pilicrus</i>	5	0
<i>Andrena agilis</i>	13	8

DNA extraction, amplification CO1-5P
(primer cocktail LCO1490/HCO2198 + LepF1/LepR1)
SMRT sequencing PacBio Sequel
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Species delimitation?

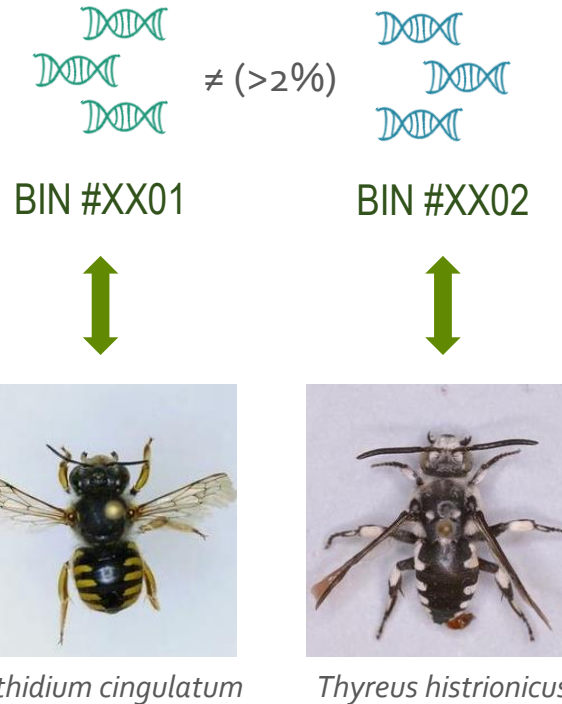
Is CO1-5P relevant for bee species delimitation?

Congruence morpho vs molecular classifications: 1051/1477 sequences
(each BIN was assigned to a single species & each species corresponded to a single BIN)

BIN (cluster of seqs) = putative species

COI DNA barcoding would be a valuable approach for the identification of specimens and the delineation of a majority of bee species -> **437 species**

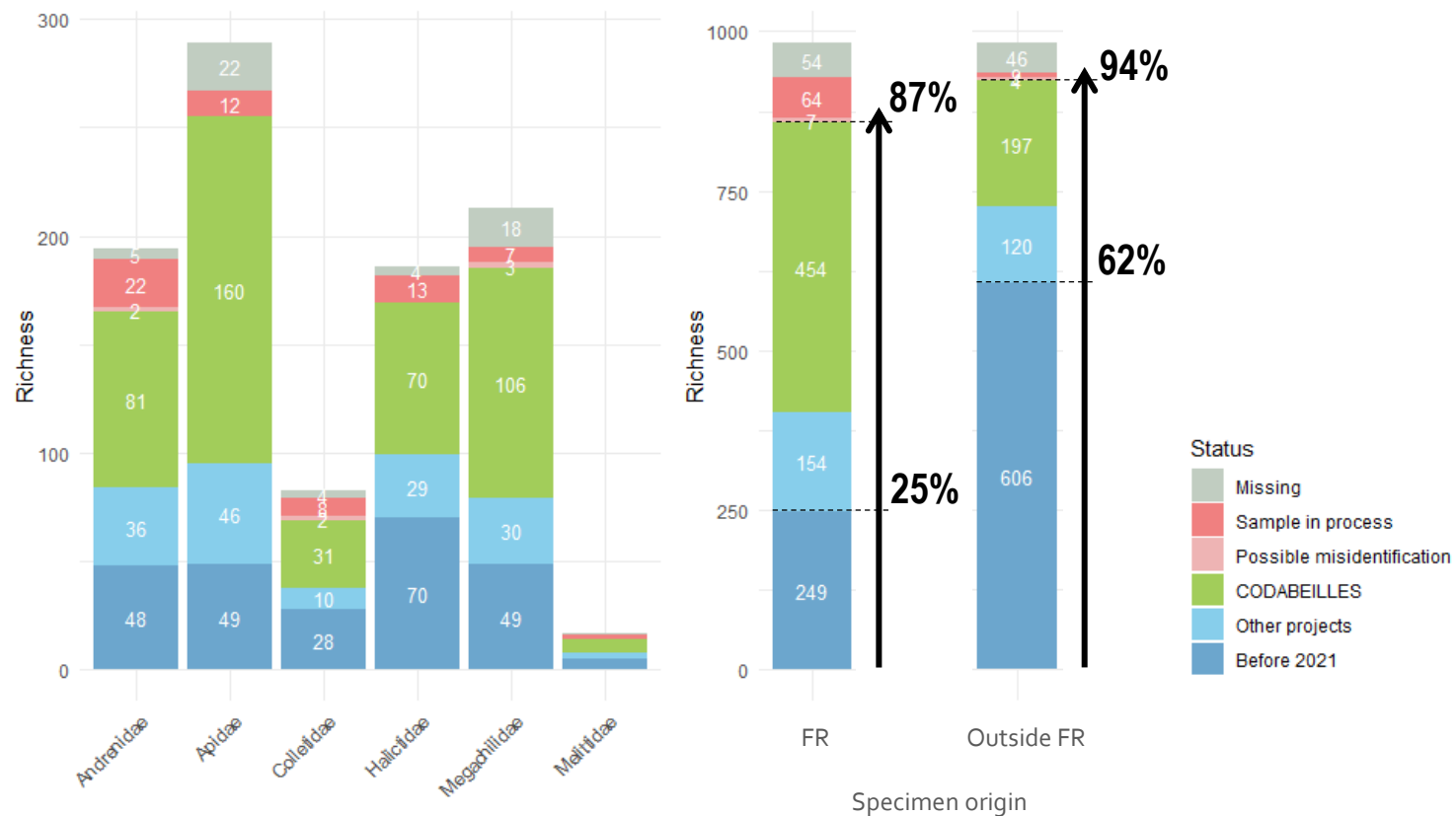
In line with Schmidt et al. 2015; Villalta et al. 2021; Wood et al. 2024



Barcode coverage?

How has barcode coverage improved since 2021?

- New reference data for the species from the Mediterranean Basin



Improvement in barcode coverage for French wild bees since 2021

Perspectives



❖ CODABEILLES:

- Second attempt of amplification for failing samples (~700 specimens)
- Addition of barcode sequences for rare species (**ANR IDMYBEES**, A. Perrard)
- Re-examination of specimens to address complex situations (combination of several markers, sequencing of chromosome-length genomes)

❖ Metabarcoding of wild bee communities in vineyards

- **FLAVI Project** (2023 – 2026): PhD thesis on ecosystem services provided by inter-row plant cover in vineyards.
Collab: CEFE, ANSES, DYNAFOR (E. Kazakou, G. Fried, M. Ollivier).



❖ Development of non-lethal approaches for the monitoring of wild bee species: eDNA from flowers

- **BEETRACK** (Plan Ecophyto. 2024-2027). Collab: GENPHYSE, DYNAFOR (A. Marquisseau, A. Vignal, K. Tabet, M. Pichon, M. Ollivier)
- **MASSIF** (PEPR FORESTT. 2025-2029). Automated Monitoring and Smart Surveillance of Insect Biodiversity in French Forest Ecosystems.
Collab: DYNAFOR, CBGP, URZF, EFNO, iEES, MECADEV, SETE, LAAS, LNEF



Thank you!

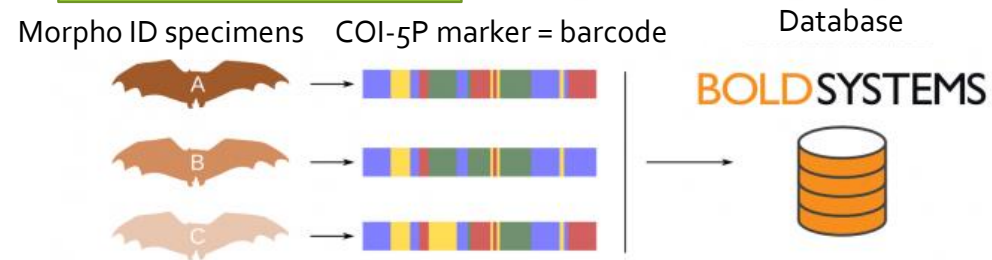
Read the CODABEILLES article
Ollivier et al., 2025. *BMC Ecology and Evolution*

Access the data on BOLD
<https://portal.boldsystems.org/recordset/DS-CODABo1>

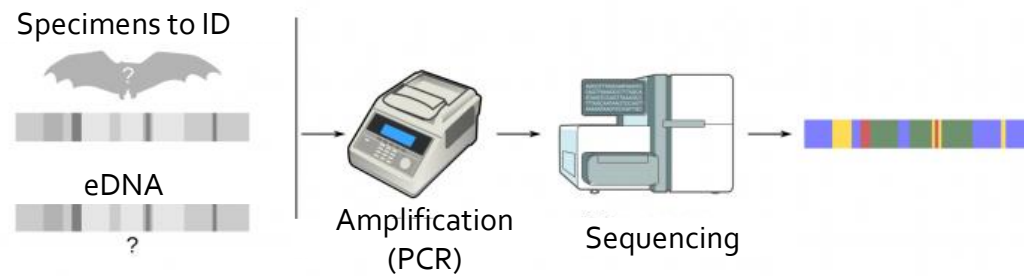


DNA Barcoding

1. Database construction



2. Extract, amplify and sequence target sequence

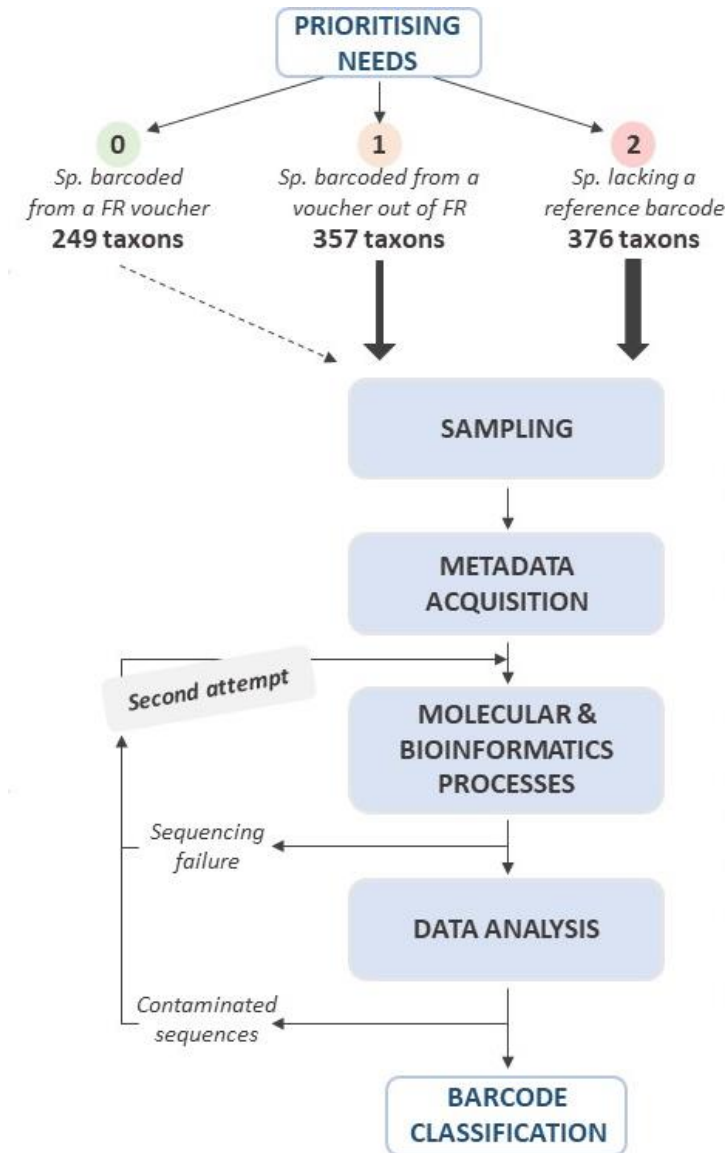


3. Compare the target sequence with database



- Adapted from planet-vie.ens.fr, Pascal Combemorel

Sequence data acquisition & analysis



Is CO1-5P relevant for bee species delimitation?

What is the extent of the barcode gap in French bee species?

How has barcode coverage improved since 2021?

- Neighbor joining (NJ) tree, K2P model
 - Barcode gap analysis (intra vs interspecific distances)
- Congruence between BIN assignment and morphological ID

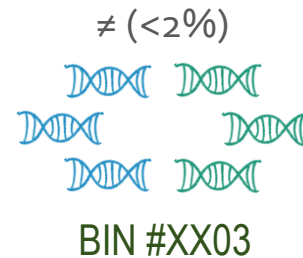
Species delimitation?

Is CO1-5P relevant for bee species delimitation?

Mismatches observed between morphological and molecular species-level identifications
-> *Andrena* and *Nomada* genera.

Different species merging into a single BIN

- Still exhibiting low intraspecific divergence
-> barcoding can serve as a diagnostic tool



Nomada alboguttata
Apimondia – Ollivier et al.



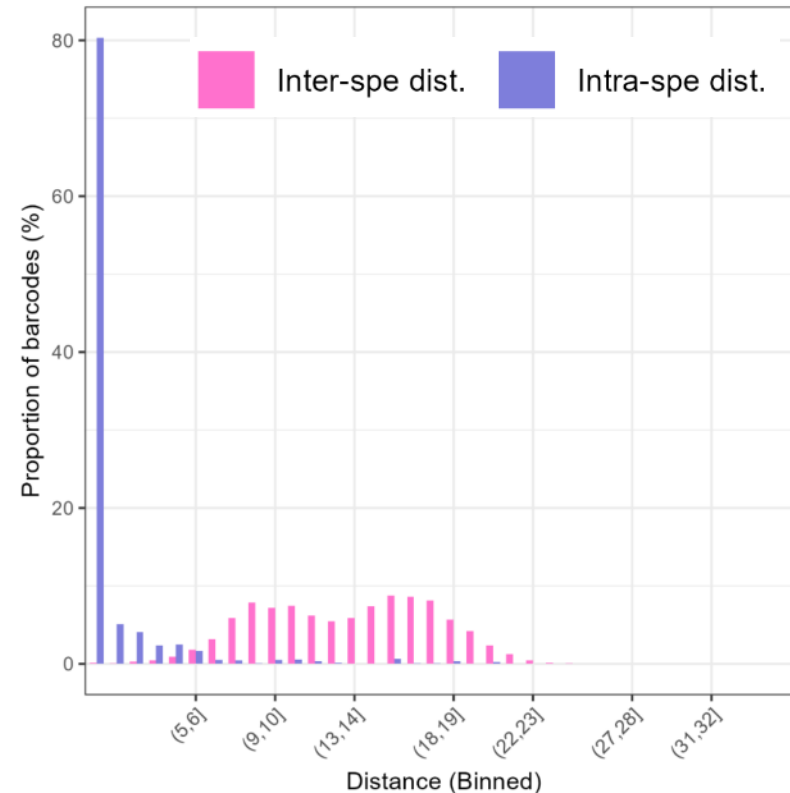
Nomada baccata

Species		BIN
<i>Nomada alboguttata</i>		BOLD:AAC8572
<i>Nomada baccata</i>		
<i>Nomada fabriciana</i>		BOLD:AAE5753
<i>Nomada gransassoi</i>		
<i>Nomada flava</i>		BOLD:AAC8809
<i>Nomada glabella</i>		
<i>Nomada leucophthalma</i>		
<i>Nomada illustris</i>		BOLD:AEN8381
<i>Nomada numida</i>		
<i>Nomada nigrovaria</i>		BOLD:AAP1578
<i>Nomada sheppardana</i>		
<i>Nomada piccioliana</i>		BOLD:AAF3496
<i>Nomada zonata</i>		
<i>Nomada striata</i>		BOLD:ABY7961
<i>Nomada villosa</i>		BOLD:AAO6546
<i>Nomada distinguenda</i>		BOLD:ACY0250
		BOLD:ACE0147
<i>Nomada fulvicornis</i>		BOLD:ACF5896

Mismatches observed between morphological and molecular species-level identifications within *Nomada* genera. The thickness of the link is proportional to the number of specimens

Barcode gap?

What is the extent of the barcode gap in French bee species?



Divergence histogram of the CODABEILLES barcodes: distribution of normalized divergence at both species and genus levels. (Kimura 2-parameter distance model)

Barcoding gap: the intraspecific genetic divergence is an order of magnitude smaller than the interspecific genetic divergence.

Recent studies reported the absence of an arbitrary universal barcode gap across all Anthophila.

- Barcode gap specific to bee genus
- Uneven barcoding efforts
- Biased distance estimates (erroneous data)

Marquisseau et al. 2025; Janko et al. 2024; Gibbs 2018; Magnacca and Brown 2012

Barcode coverage?

How has barcode coverage improved since 2021?

Which species are missing?

- Species recently discovered on the French territory or recently described as new to science (16)
- Rare taxa such as cleptoparasitic bees (16)
- Sequencing or identification issues with the sampled specimen (13)

