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One of the most pressing issues facing the global apicultural sector is the frequent presence of honey on the market that does not meet regulatory requirements. This is evidenced by the numerous notifications of adulterated honey. The most common form of adulteration involves extending honey with sugar syrups. This malpractice presents enticing opportunities for fraudulent business operators due to the low cost of sugar syrup and the challenges associated with current analytical approaches in detecting them in honey. Other frauds include mislabeling the geographic, botanical and entomological origins of honey. Given the complexity of the issue and the extensive information required to detect adulterated honey and frauds in this production system, it is necessary to utilise a combination of analytical approaches and methodologies capable of examining different components and chemical-physical parameters of honey and foreign substances. In this study, we utilised a combination of various methodologies to analyse authentic, suspected and adulterated honey samples from different countries with various declared botanical origins. These methodologies included: 1) next-generation DNA sequencing techniques involving five plant and entomological metabarcoding analyses and whole DNA shot-gun sequencing, with advanced bioinformatic approaches for interpreting sequencing data and identifying multiple environmental DNA fingerprints in honey; 2) melissopalynological analysis to identify the pollen spectrum present in the samples; 3) standard chemical-physical parameters, such as humidity, acidity, hydroxymethylfurfural, diastase and pH; 4) standardized isotopic-based methods, including Elemental Analyzer (EA) for determining C-4 plant sugar in honey and Liquid Chromatography (LC) Coupled to Isotope Ratio Mass Spectrometry (LC-IRMS) method for determining the $\delta^{13}\text{C}$ ratios of mono-, di-, and trisaccharides in honey; 5) Liquid Chromatography High Resolution MS (LC-HRMS) in target mode to detect markers of the sugar syrups. The combination of results from these different methodologies provided a wealth of information that allowed us to effectively group honey samples in authentic or adulterated. Our pilot test suggests that using multiple methods is necessary to identify adulterated honey samples. We are working on constructing a reference database with multiple analytical information to further enhance our ability to identify counterfeit honey.



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Animal and Food Genomics Group

Toward the ultimate assay for honey authentication: combining multi-dimensional DNA sequencing data with multi-platform physical-chemical and isotopic d13C analyses

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APIMONDIA
SCANDINAVIA
2025
23-27 SEPTEMBER

COPENHAGEN





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Co-authors

Lifeanalytics



Samuele Bovo



Valeria Taurisano

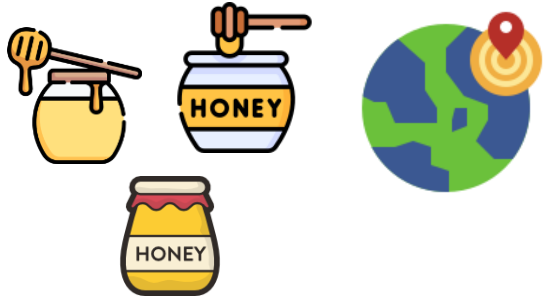


Anisa Ribani



Giancarlo Quaglia





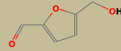
Honey samples

Sample ID	Declared botanical origin 🌸🌿🌳	Country	Sample ID	Declared botanical origin 🌸🌿🌳	Country
1	Polyfloral	Brazil 🇧🇷	19	Polyfloral	Hungary 🇭🇺
2	Polyfloral	Brazil 🇧🇷	20	Polyfloral	China 🇨🇳
3	Polyfloral	Brazil 🇧🇷	21	Acacia	Italy 🇮🇹
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12	Chestnut	Italy 🇮🇹	30	Polyfloral	China 🇨🇳
13	Polyfloral	Hungary 🇭🇺	31	Acacia	Italy 🇮🇹
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15	Acacia	Hungary 🇭🇺	33	Polyfloral	Europe 🇪🇺
16	Polyfloral	Ukraine 🇺🇦	34	Polyfloral	Argentina 🇦🇷
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18	Polyfloral	China 🇨🇳	36	Polyfloral	Europe 🇪🇺
37	Polyfloral	Chile 🇨🇱	38	na	Europe 🇪🇺

Analyses

1

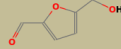
**Standard sensorial and
chemical-physical parameters:**
Sensorial, Humidity, Acidity, pH,
HMF, Diastase



Analyses

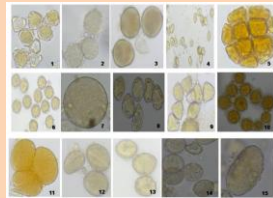
1

Standard sensorial and chemical-physical parameters:
Sensorial, Humidity, Acidity, pH,
HMF, Diastase



2

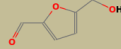
Melissopalynology



Analyses

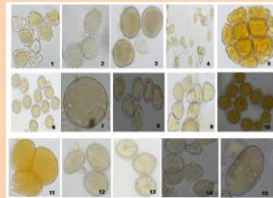
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Standard sensorial and chemical-physical parameters:
Sensorial, Humidity, Acidity, pH, HMF, Diastase



2

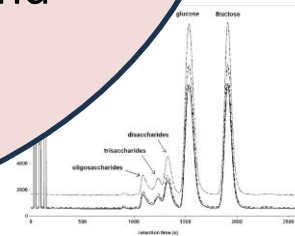
Melissopalynology



3

Standardized isotopic-based methods:

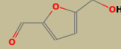
- Elemental Analyzer (EA) for determining C-4 plant sugar
- Liquid Chromatography (LC) Coupled to Isotope Ratio Mass Spectrometry (LC-IRMS) method for determining the $\delta^{13}\text{C}$ ratios of mono-, di-, and trisaccharides



Analyses

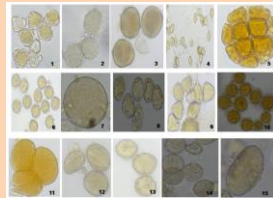
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Standard sensorial and chemical-physical parameters:
Sensorial, Humidity, Acidity, pH, HMF, Diastase



2

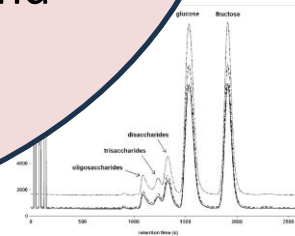
Melissopalynology



3

Standardized isotopic-based methods:

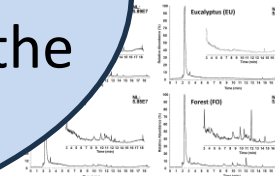
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4

Liquid Chromatography High Resolution MS (LC-HRMS):

- Detection of markers of the sugar syrups



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37	Polyfloral	Chile 🇨🇱	38	na	Europe 🇪🇺



5 - DNA analyses

End-point PCR

Next Generation Sequencing

Metabarcoding

Whole DNA
(Shot-gun sequencing)





5 - DNA analyses

End-point PCR

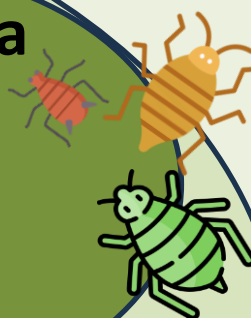
Next Generation Sequencing

Metabarcoding

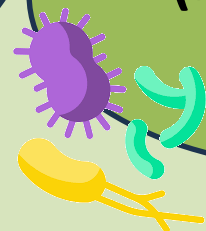
Plant markers



Hemiptera markers



Bacteria markers
(microbiota)

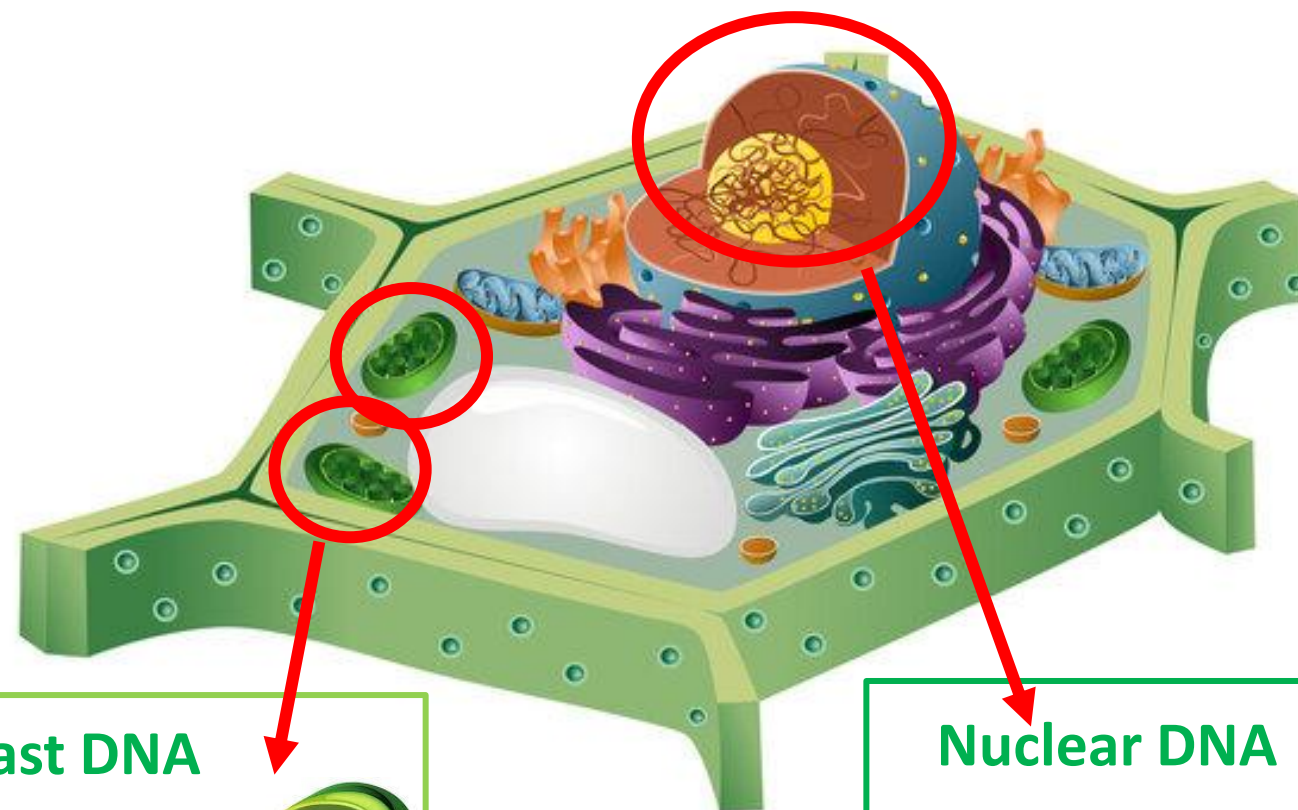
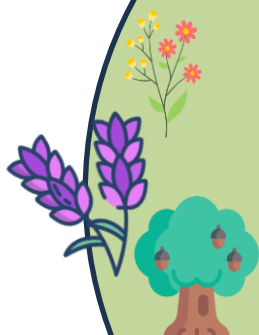


Whole DNA
(Shot-gun sequencing)



5 - DNA analyses

Plant markers



Chloroplast DNA

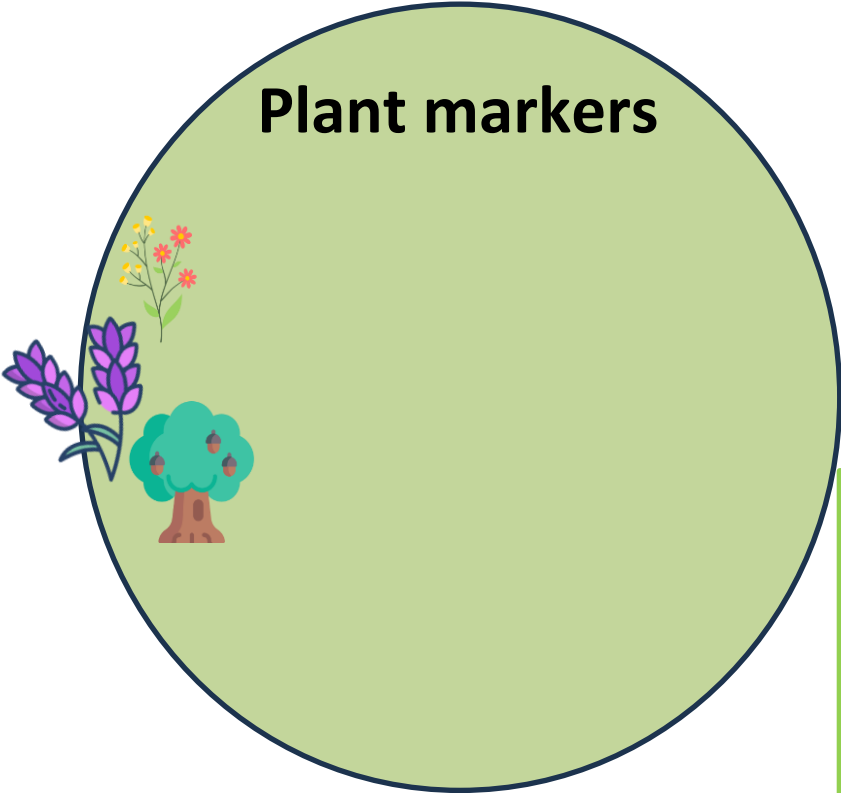
- *matk*
- *trnL_UAA*
- *rbcl* (163 bp)
- *rbcl* (213 bp)
- *psbA-trnH*



Nuclear DNA

- ITS2
- 18S



[illegible]

- *matk*
- *trnL_UAA*
- *rbcl* (163 bp)
- *rbcl* (213 bp)
- *psbA-trnH*





5 - DNA analyses

End-point PCR

Next Generation Sequencing

Metabarcoding

Plant markers

Chloroplast DNA

1. *matk*
2. *trnL_UAA*
3. *rbcl* (163 bp)
4. *rbcl* (213 bp)
5. *psbA-trnH*

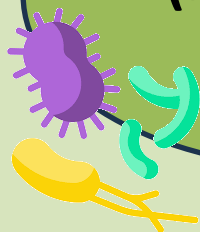
Nuclear DNA

1. ITS2
2. 18S

Hemiptera markers



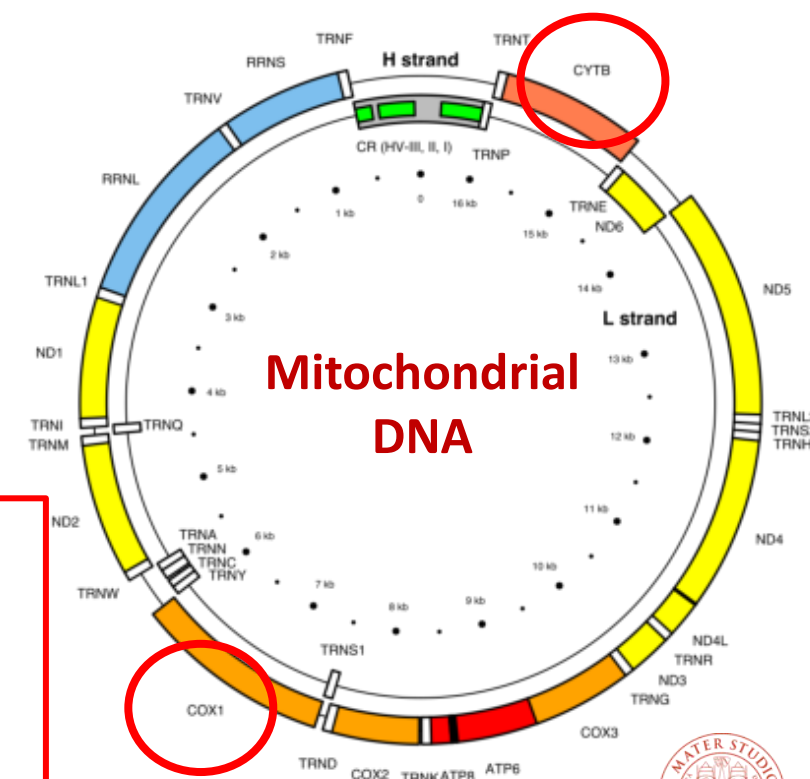
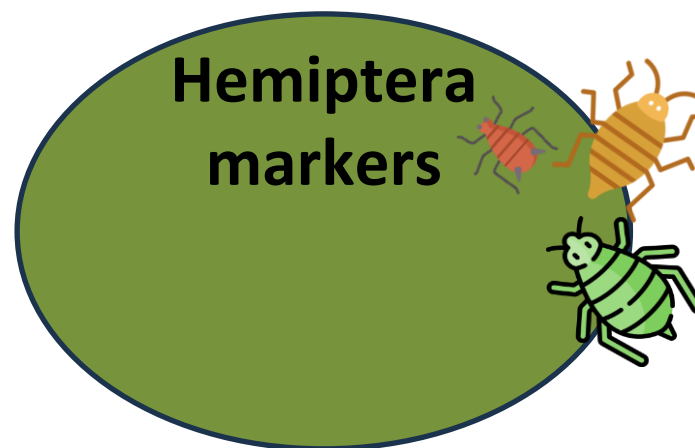
Bacteria markers (microbiota)



Whole DNA (Shot-gun sequencing)

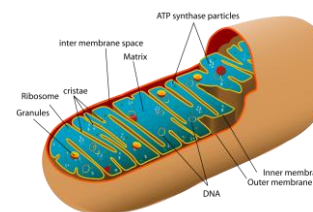


5 - DNA analyses



Mitochondrial DNA

- *cox1*
- *cytB*





5 - DNA analyses

End-point PCR

Next Generation Sequencing

Metabarcoding

Plant markers

Chloroplast DNA

1. *matk*
2. *trnL_UAA*
3. *rbcl* (163 bp)
4. *rbcl* (213 bp)
5. *psbA-trnH*

Nuclear DNA

1. ITS2
2. 18S

Hemiptera markers

Mitochondrial DNA

1. *cox1*
2. *cytB*

Bacteria markers (microbiota)

Whole DNA (Shot-gun sequencing)



5 - DNA analyses

End-point PCR

Next Generation Sequencing

Metabarcoding

Plant markers

Chloroplast DNA

1. *matk*
2. *trnL_UAA*
3. *rbcl* (163 bp)
4. *rbcl* (213 bp)
5. *psbA-trnH*

Nuclear DNA

1. ITS2
2. 18S

Hemiptera markers

Mitochondrial DNA

1. *cox1*
2. *cytB*

Bacteria markers (microbiota)

1. 16S V3
2. 16S V5

Whole DNA (Shot-gun sequencing)



5 - DNA analyses

End-point PCR

Next Generation Sequencing

Metabarcoding

Plant markers

Chloroplast DNA

1. *matk*
2. *trnL_UAA*
3. *rbcl* (163 bp)
4. *rbcl* (213 bp)
5. *psbA-trnH*

Nuclear DNA

1. ITS2
2. 18S

Hemiptera markers

Mitochondrial DNA

1. *cox1*
2. *cytB*

Bacteria markers (microbiota)

1. 16S V3
2. 16S V5

Whole DNA (Shot-gun sequencing)

1. Illumina platform
2. BGISEQ500 platform





5 - DNA analyses



End-point PCR

A. mellifera mtDNA

Next Generation Sequencing

Metabarcoding

Plant markers

Chloroplast DNA

1. *matk*
2. *trnL_UAA*
3. *rbcl* (163 bp)
4. *rbcl* (213 bp)
5. *psbA-trnH*

Nuclear DNA

1. ITS2
2. 18S

Hemiptera markers

Mitochondrial DNA

1. *cox1*
2. *cytB*

Bacteria markers (microbiota)

1. 16S V3
2. 16S V5

Whole DNA (Shot-gun sequencing)

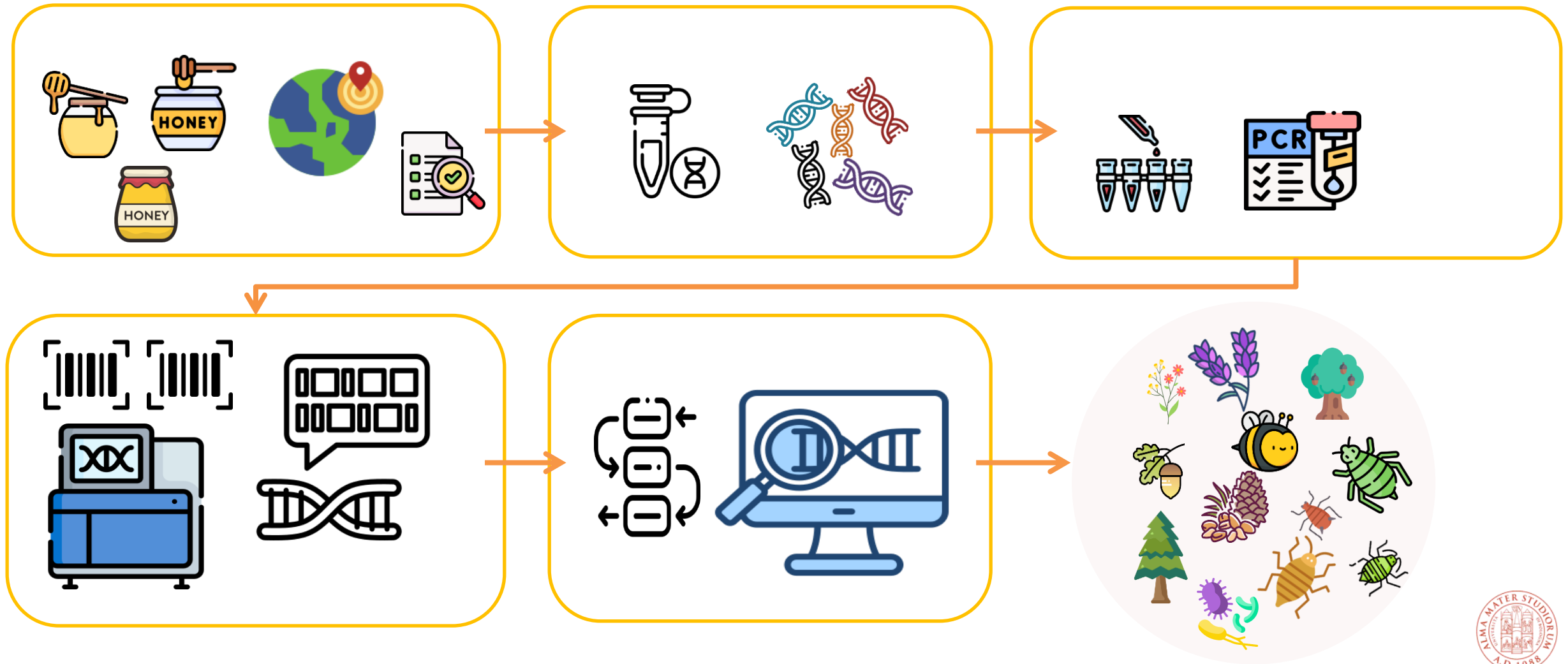
1. Illumina platform
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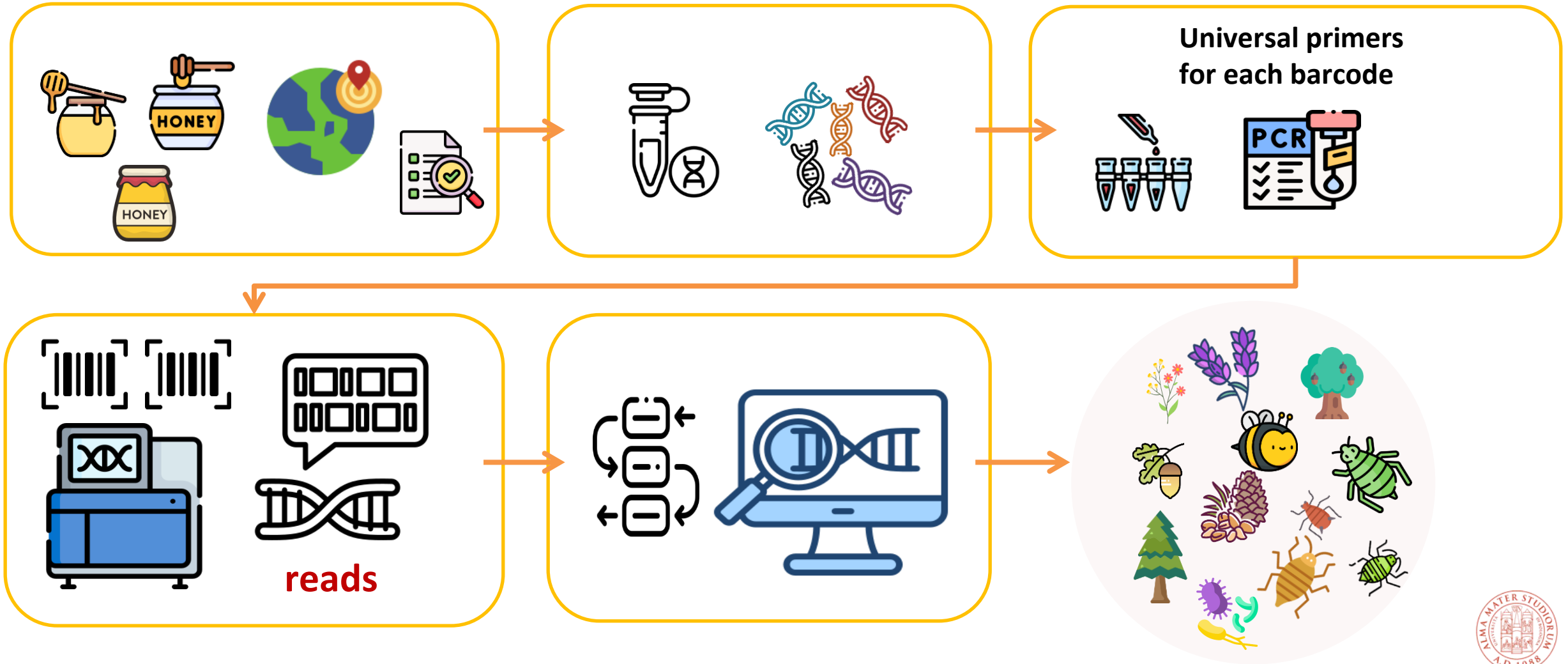
5 - DNA analyses

Workflow - Metabarcoding



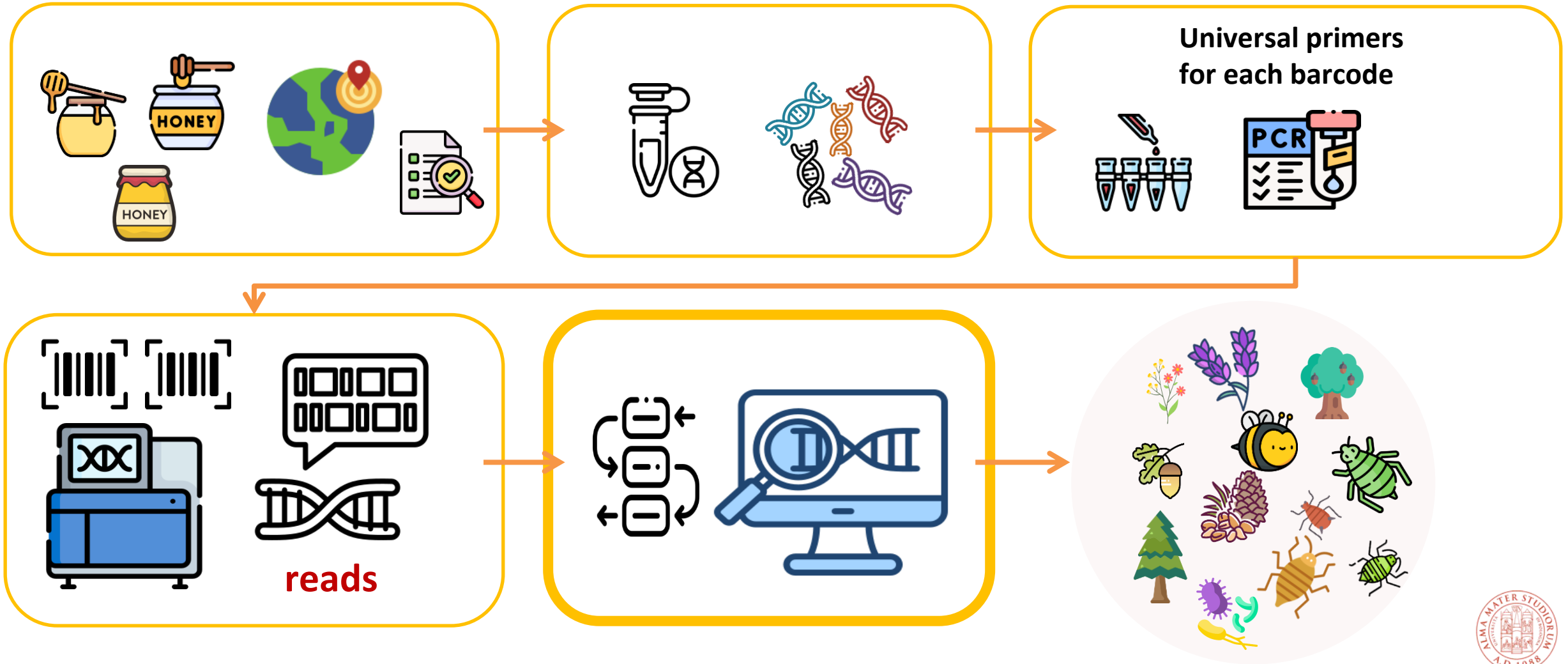
5 - DNA analyses

Workflow - Metabarcoding



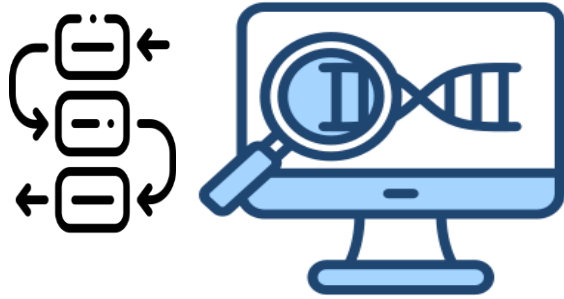
5 - DNA analyses

Workflow - Metabarcoding

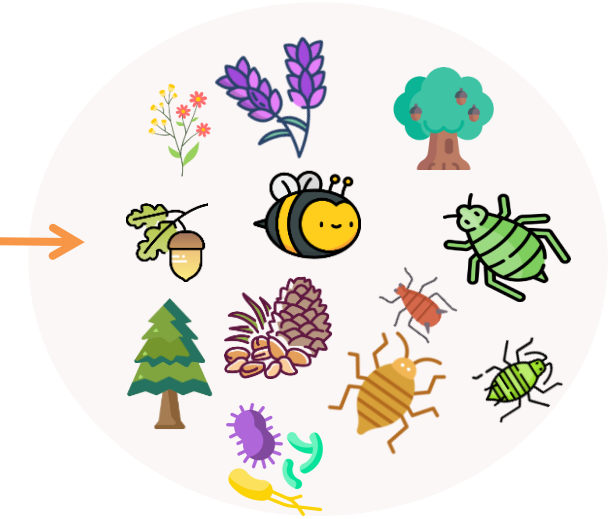


5 - DNA analyses

Metabarcoding - Bioinformatic pipeline



11
customized
databases



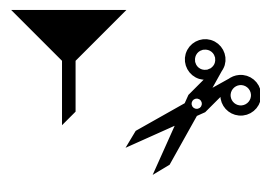
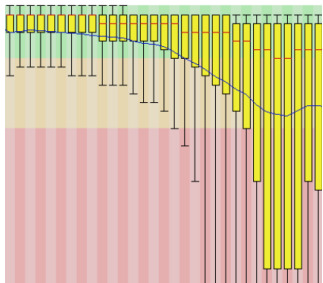
reads

FastQC

Filtering and
Trimming

Mapping
and Align

Assignment

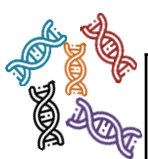


- best E value
- identity $\geq 97\%$
- coverage $\geq 95\%$





A few examples



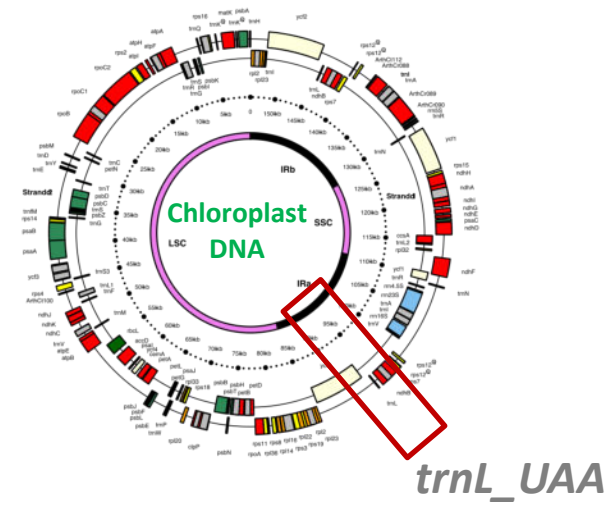
Sample ID	Declared botanical origin 🌸🌿🌳	Country	Sample ID	Declared botanical origin 🌸🌿🌳	Country
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Chloroplast DNA marker: *trnL_UAA*

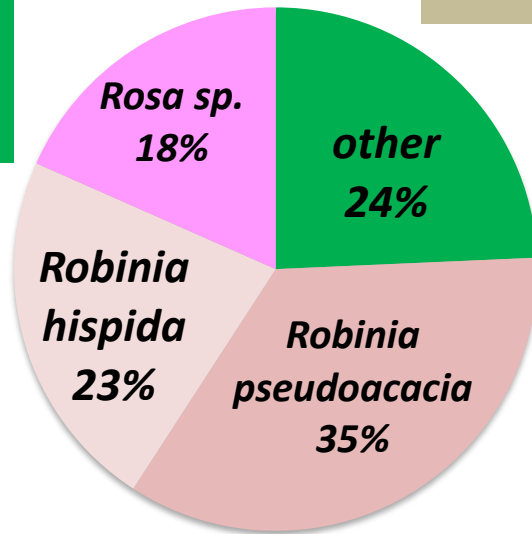
% of reads



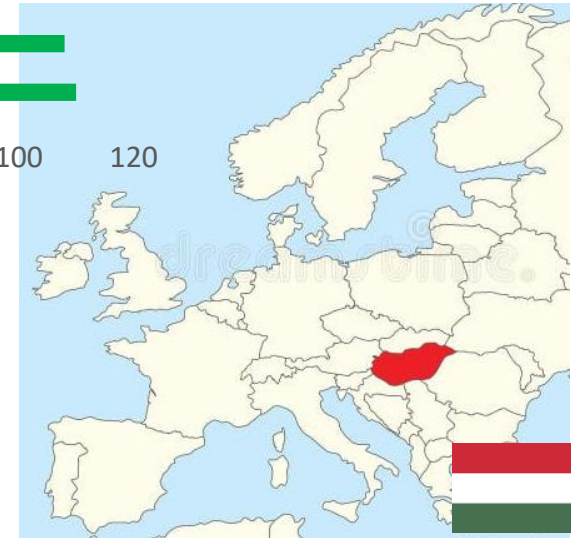
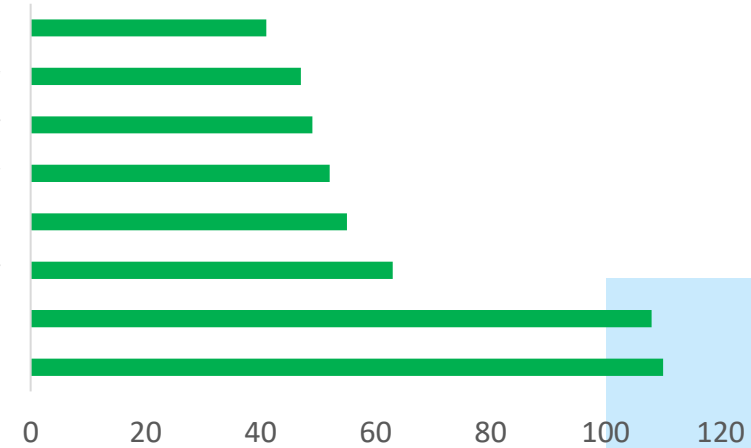


Chloroplast DNA marker: *trnL_UAA*

15 – Acacia
honey



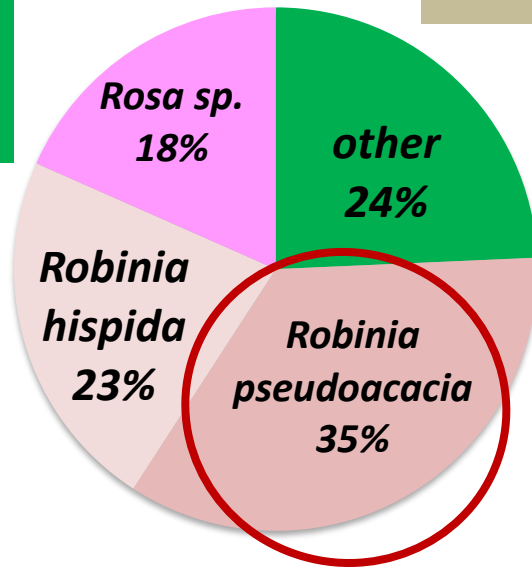
Quercus sp.
Meconopsis pseudohorridula
Papaver rhoeas
Amorpha roemeriana
Trifolium sp.
Quercus cerris
Prunus sp.
Acer sp.



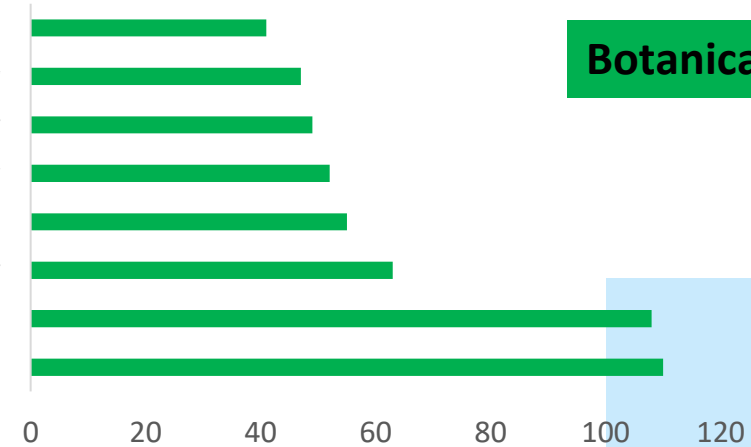


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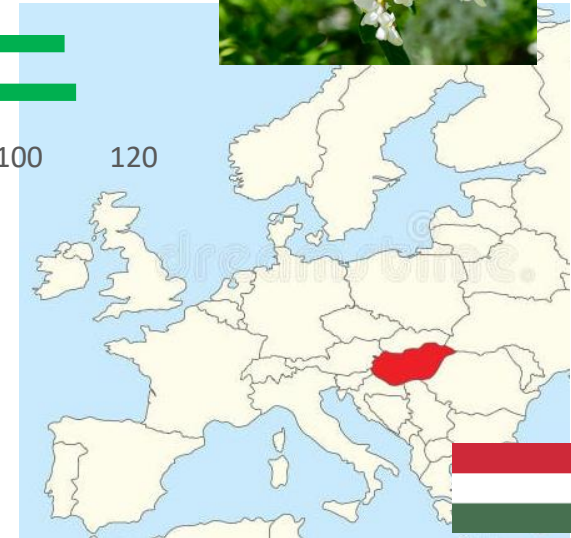
15 – Acacia
honey



Quercus sp.
Meconopsis pseudohorridula
Papaver rhoeas
Amorpha roemeriana
Trifolium sp.
Quercus cerris
Prunus sp.
Acer sp.



Botanical origin confirmed

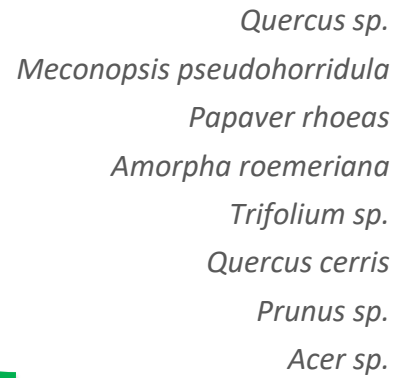
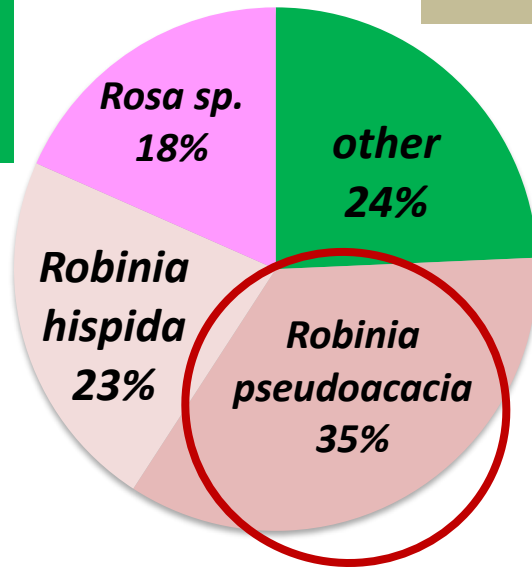


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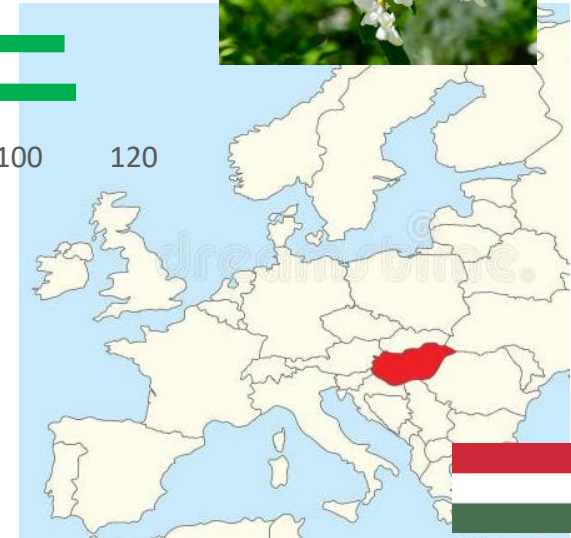


Chloroplast DNA marker: *trnL_UAA*

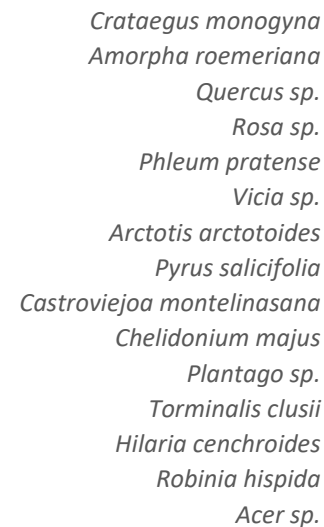
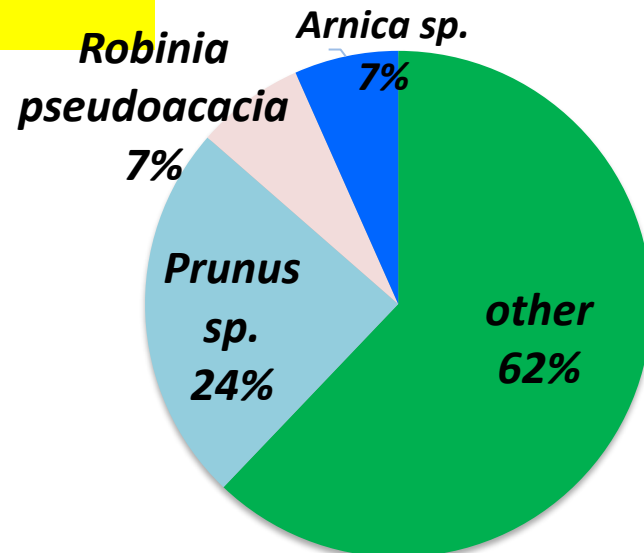
15 – Acacia honey



Botanical origin confirmed



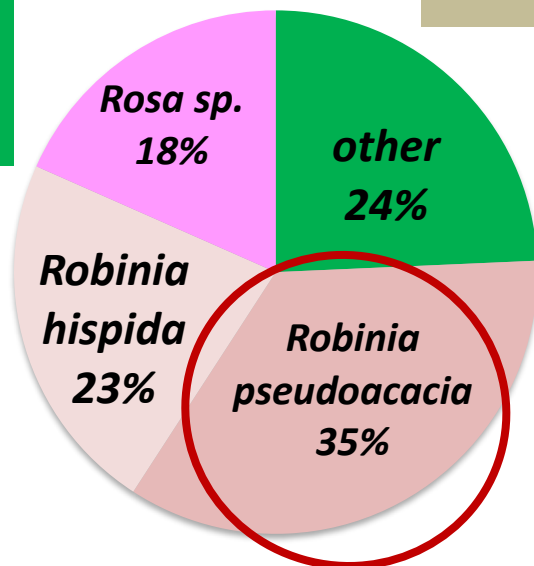
29 – Acacia honey



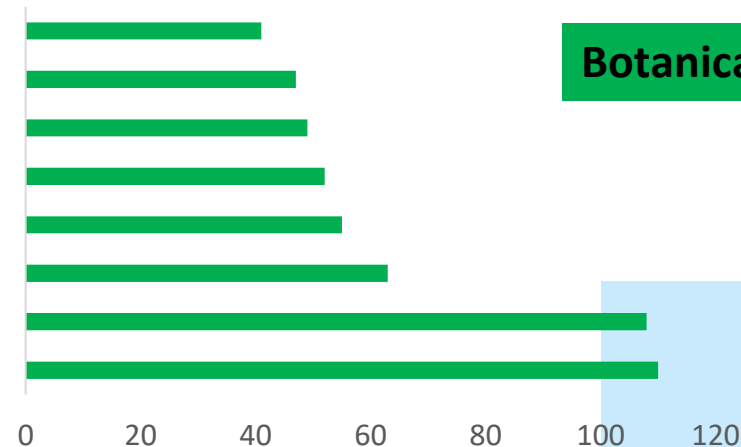


Chloroplast DNA marker: *trnL_UAA*

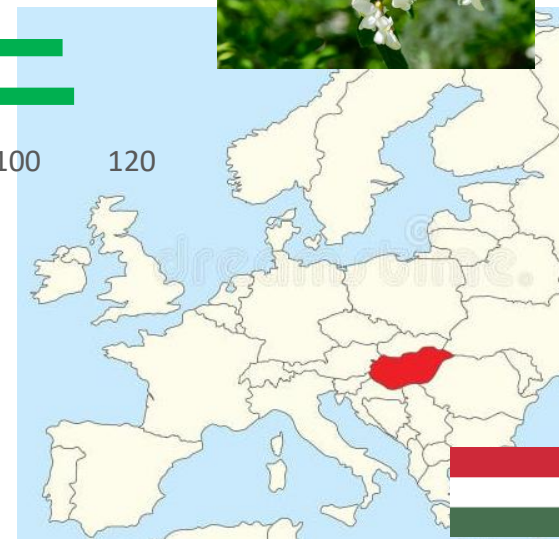
15 – Acacia honey



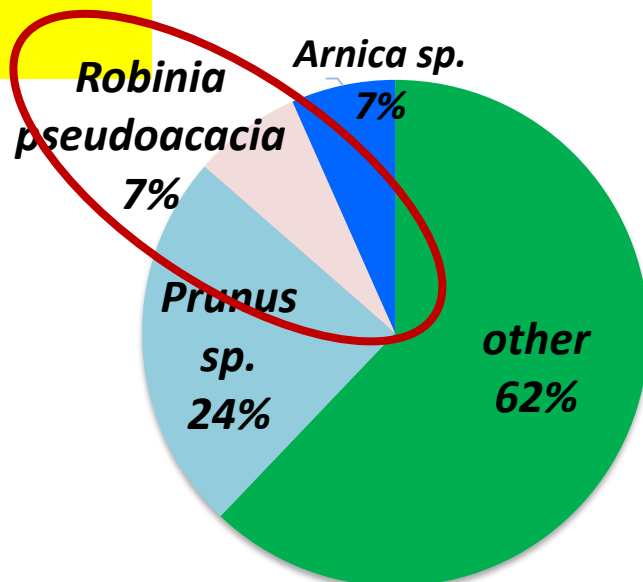
Quercus sp.
Meconopsis pseudohorridula
Papaver rhoeas
Amorpha roemeriana
Trifolium sp.
Quercus cerris
Prunus sp.
Acer sp.



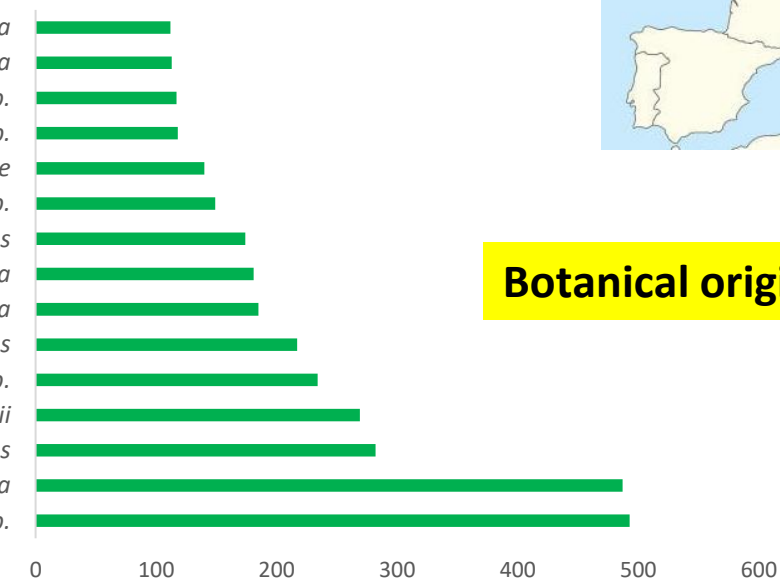
Botanical origin confirmed



29 – Acacia honey



Crataegus monogyna
Amorpha roemeriana
Quercus sp.
Rosa sp.
Phleum pratense
Vicia sp.
Arctotis arctotoides
Pyrus salicifolia
Castroviejoa montelinasana
Chelidonium majus
Plantago sp.
Torminalis clusii
Hilaria cenchroides
Robinia hispida
Acer sp.



Botanical origin NOT confirmed





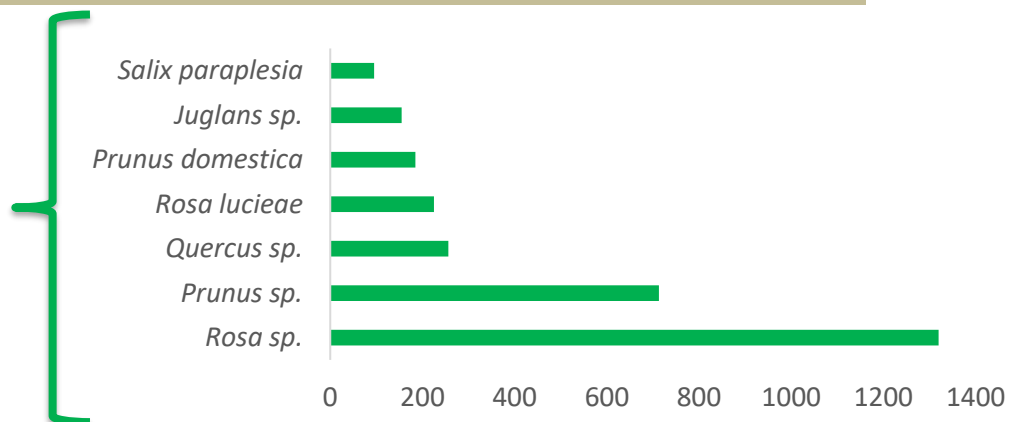
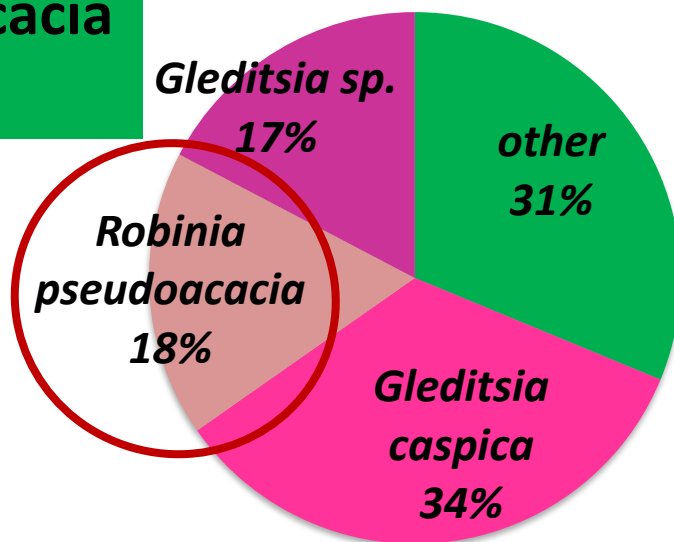
% of reads





Chloroplast DNA marker: *matk*

15 – Acacia
honey



Botanical origin confirmed

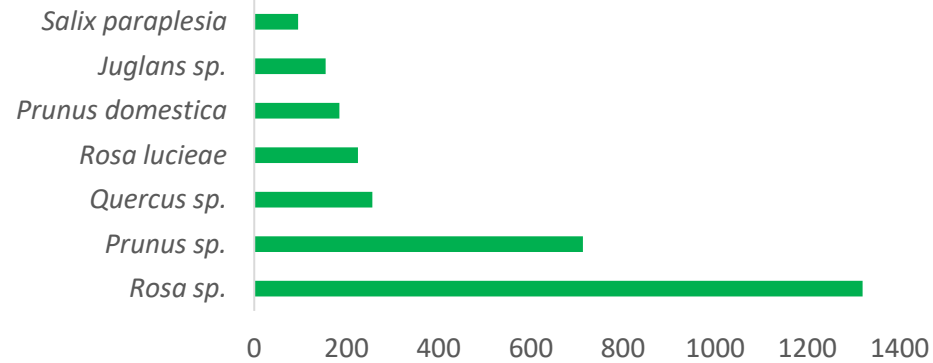
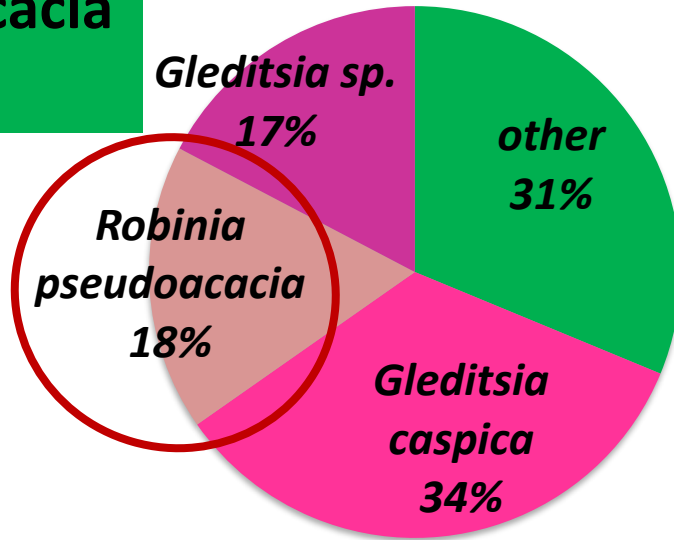


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Chloroplast DNA marker: *matk*

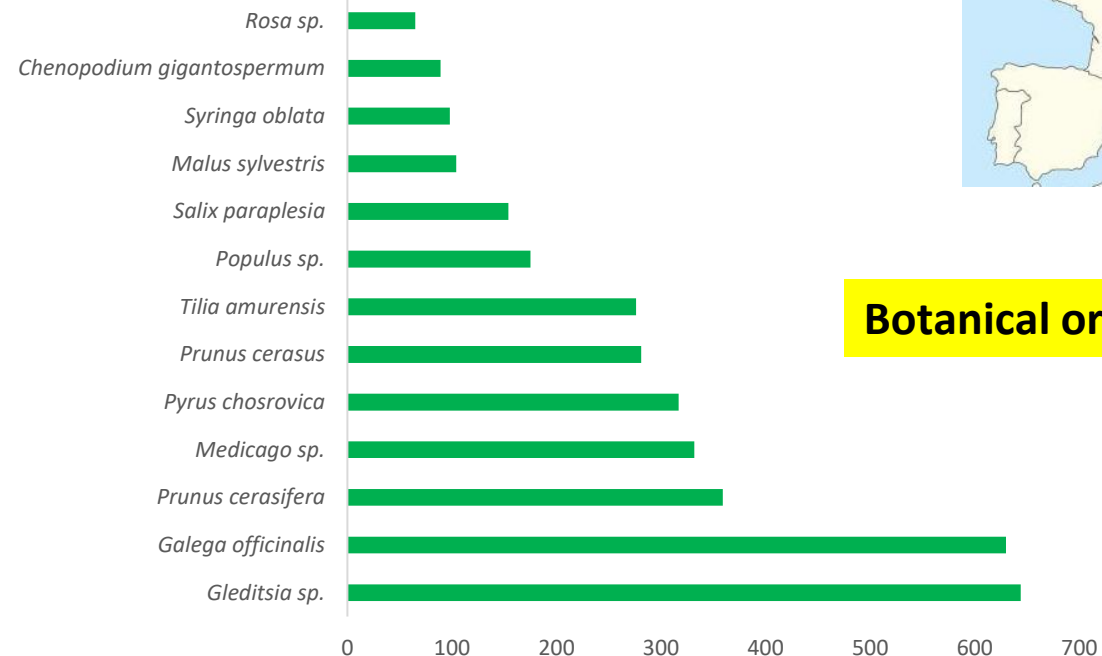
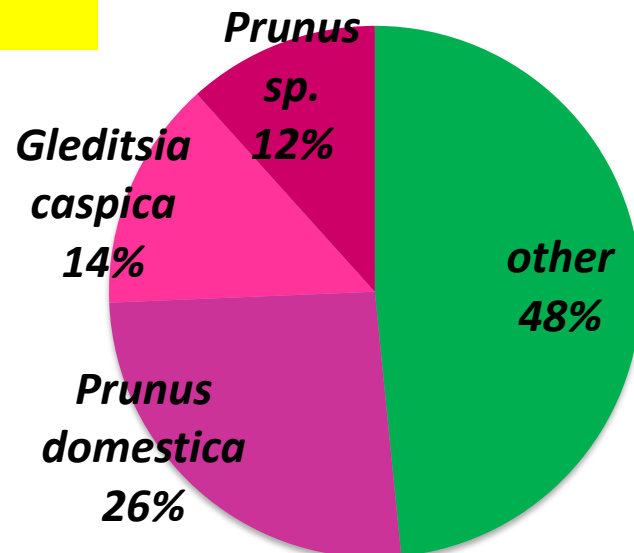
15 – Acacia honey



Botanical origin confirmed



29 – Acacia honey

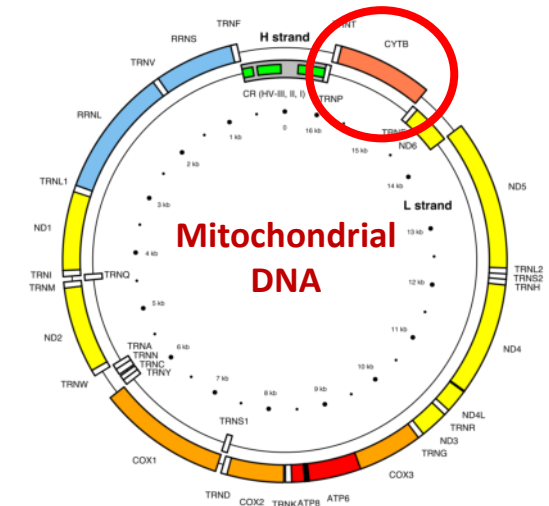


Botanical origin NOT confirmed



Hemiptera mtDNA marker: *cytB*

% of reads



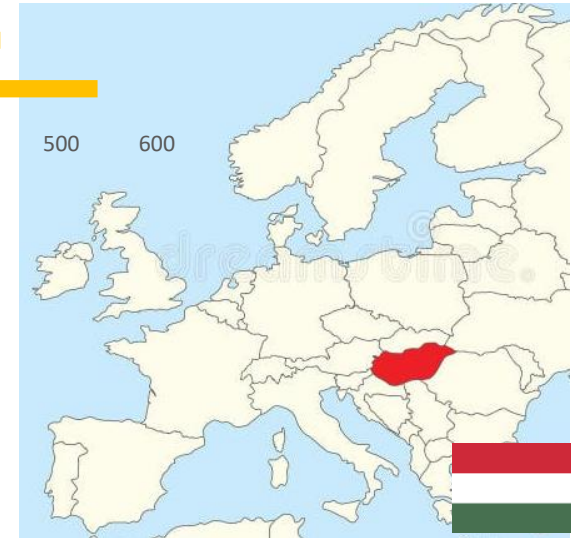
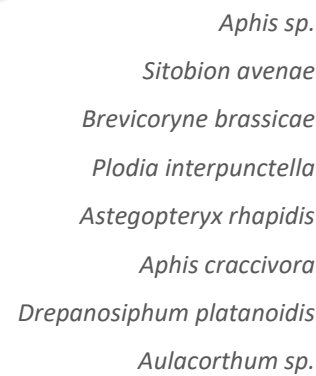
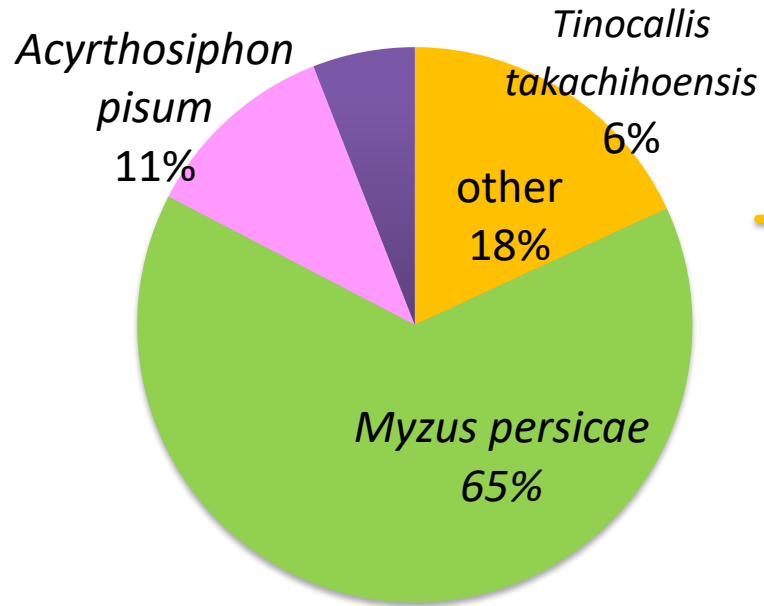


Hemiptera mtDNA marker: *cytB*

15 – Acacia honey



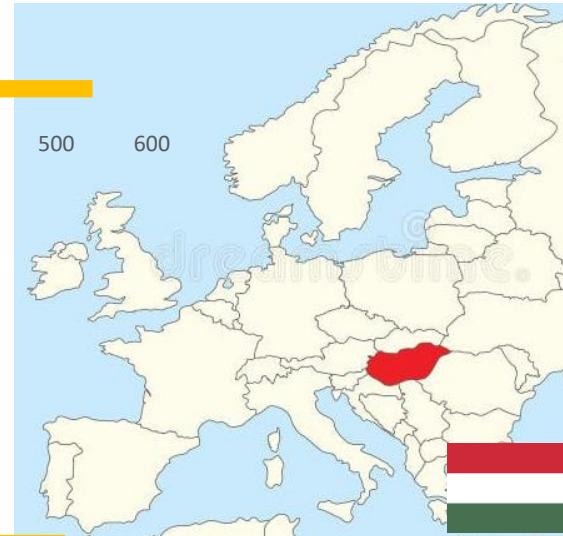
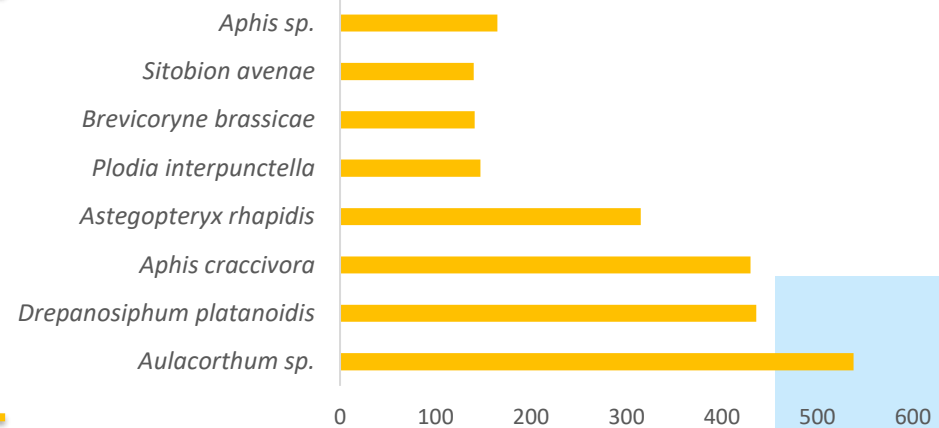
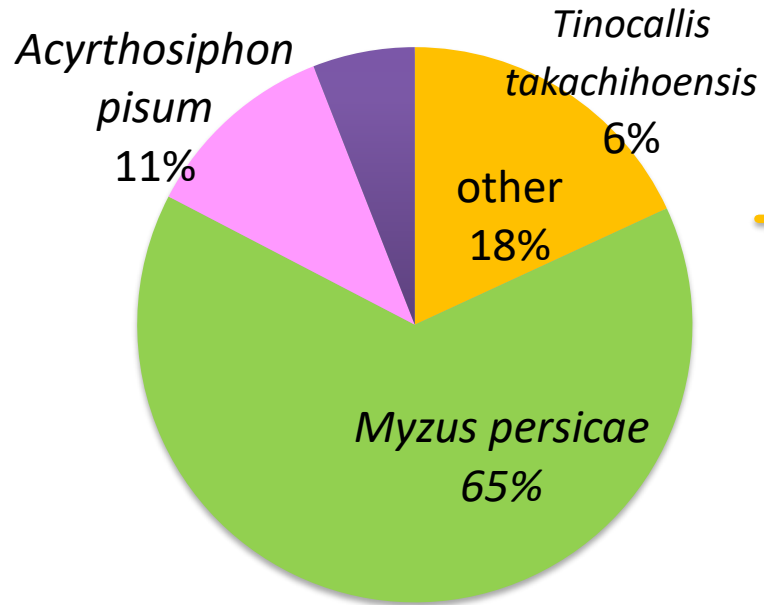
29 – Acacia honey



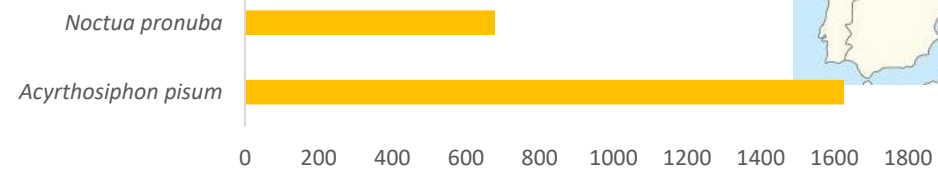
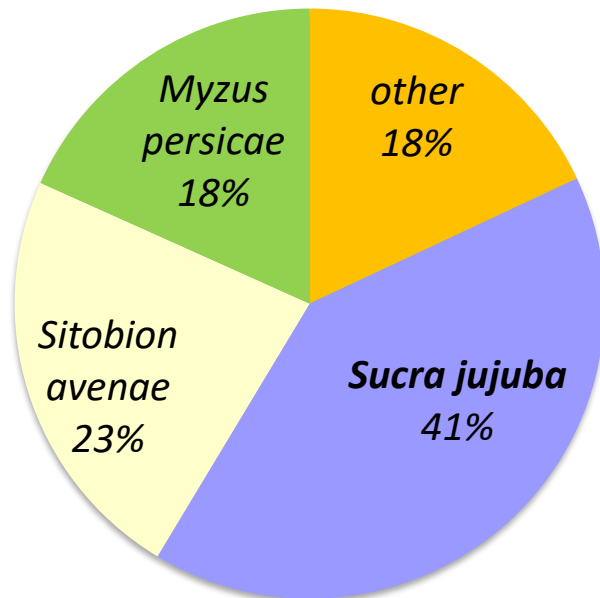


Hemiptera mtDNA marker: *cytB*

15 – Acacia honey



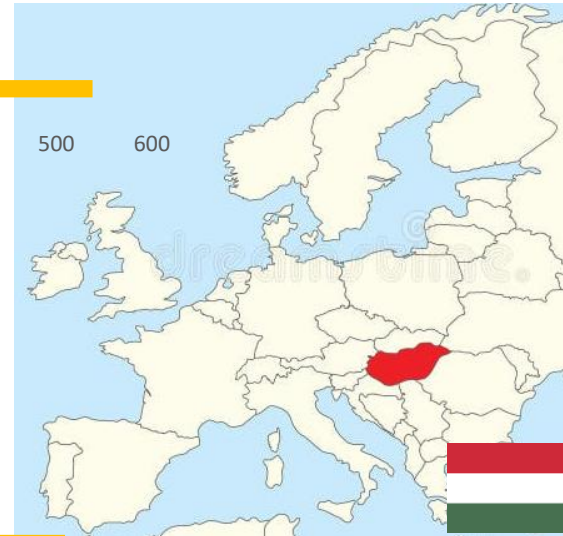
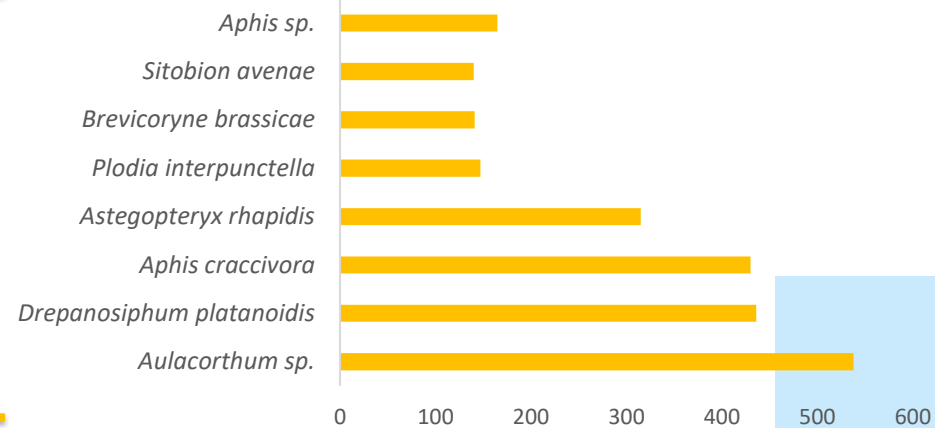
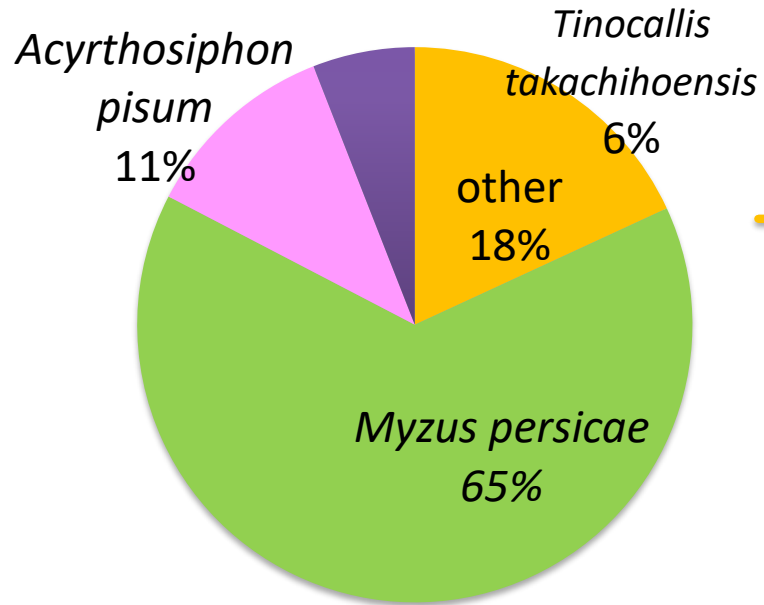
29 – Acacia honey



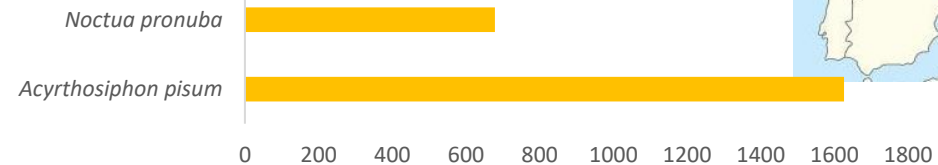
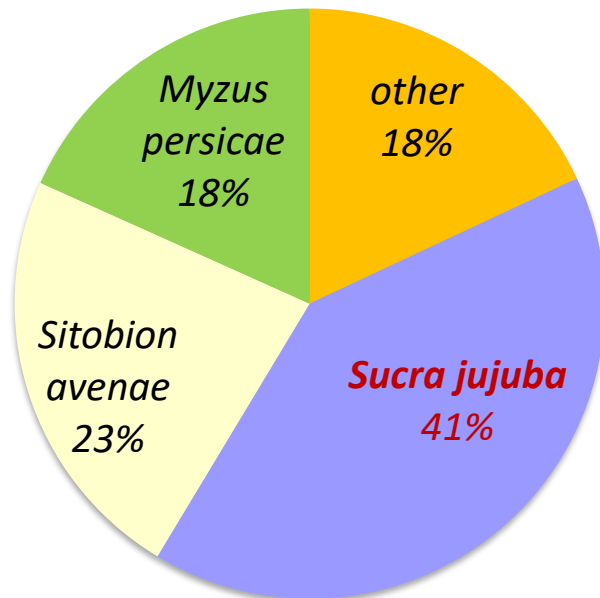


Hemiptera mtDNA marker: *cytB*

15 – Acacia honey

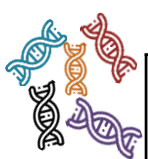


29 – Acacia honey



Sucra jujuba (Lepidoptera) → native to and distributed only in northern China, pest of Chinese jujube trees (*Ziziphus jujuba*)





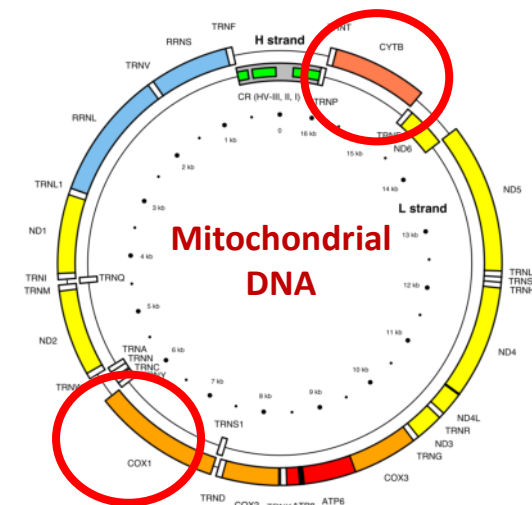
Sample ID	Declared botanical origin 🌸🌿🌳	Country	Sample ID	Declared botanical origin 🌸🌿🌳	Country
1	Polyfloral	Brazil 🇧🇷	19	Polyfloral	Hungary 🇭🇺
2	Polyfloral	Brazil 🇧🇷	20	Polyfloral	China 🇨🇳
3	Polyfloral	Brazil 🇧🇷	21	Acacia	Italy 🇮🇹
4	Polyfloral	Hungary 🇭🇺	22	Polyfloral	China 🇨🇳
5	Polyfloral	Moldova 🇲🇩	23	Polyfloral	China 🇨🇳
6	Polyfloral	Hungary 🇭🇺	24	Polyfloral	China 🇨🇳
7	Polyfloral	Hungary 🇭🇺	25	Polyfloral	China 🇨🇳
8	Polyfloral	Argentina 🇦🇷	26	Polyfloral	China 🇨🇳
9	Polyfloral	China 🇨🇳	27	Polyfloral	China 🇨🇳
10	Polyfloral	Argentina 🇦🇷	28	Polyfloral	China 🇨🇳
11	Polyfloral	Argentina 🇦🇷	29	Acacia	Hungary 🇭🇺
12	Chestnut	Italy 🇮🇹	30	Polyfloral	China 🇨🇳
13	Polyfloral	Hungary 🇭🇺	31	Acacia	Italy 🇮🇹
14	Polyfloral	Italy 🇮🇹	32	Polyfloral	Argentina 🇦🇷
15	Acacia	Hungary 🇭🇺	33	Polyfloral	Europe 🇪🇺
16	Polyfloral	Ukraine 🇺🇦	34	Polyfloral	Argentina 🇦🇷
17	Polyfloral	Hungary 🇭🇺	35	Polyfloral	Argentina 🇦🇷
18	Polyfloral	China 🇨🇳	36	Polyfloral	Europe 🇪🇺
37	Polyfloral	Chile 🇨🇱	38	na	Europe 🇪🇺





(Hemiptera) mtDNA markers: *cox1* and *cytB*

% of reads



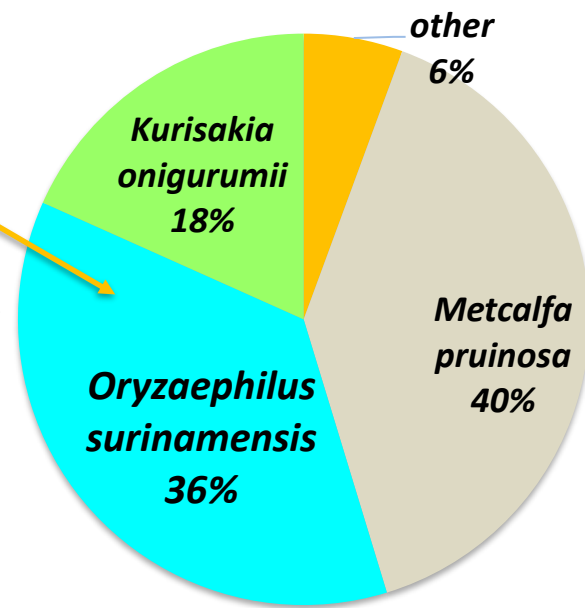


(Hemiptera) mtDNA markers: *cox1* and *cytB*

cox1



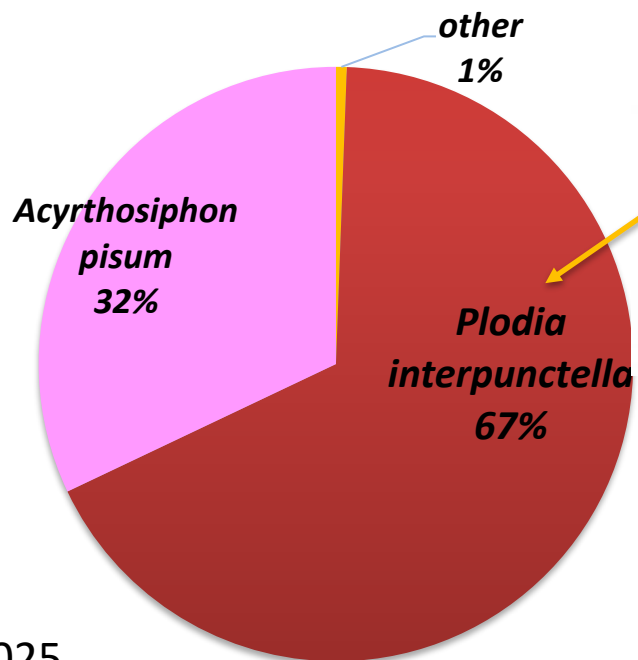
Coleoptera -
pest of
grains and
stored food
products



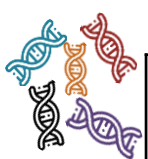
20 – Polyfloral honey



cytB

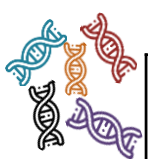


Lepidoptera –
a common pest of stored foods



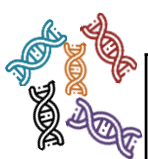
Sample ID	Declared botanical origin 🌸🌿🌳	Country	Sample ID	Declared botanical origin 🌸🌿🌳	Country
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3	Polyfloral	Brazil 🇧🇷	21	Acacia	Italy 🇮🇹
4	Polyfloral	Hungary 🇭🇺	22	Polyfloral	China 🇨🇳
5	Polyfloral	Moldova 🇲🇩	23	Polyfloral	China 🇨🇳
6	Polyfloral	Hungary 🇭🇺	24	Polyfloral	China 🇨🇳
7	Polyfloral	Hungary 🇭🇺	25	Polyfloral	China 🇨🇳
8	Polyfloral	Argentina 🇦🇷	26	Polyfloral	China 🇨🇳
9	Polyfloral	China 🇨🇳	27	Polyfloral	China 🇨🇳
10	Polyfloral	Argentina 🇦🇷	28	Polyfloral	China 🇨🇳
11	Polyfloral	Argentina 🇦🇷	29	Acacia	Hungary 🇭🇺
12	Chestnut	Italy 🇮🇹	30	Polyfloral	China 🇨🇳
13	Polyfloral	Hungary 🇭🇺	31	Acacia	Italy 🇮🇹
14	Polyfloral	Italy 🇮🇹	32	Polyfloral	Argentina 🇦🇷
15	Acacia	Hungary 🇭🇺	33	Polyfloral	Europe 🇪🇺
16	Polyfloral	Ukraine 🇺🇦	34	Polyfloral	Argentina 🇦🇷
17	Polyfloral	Hungary 🇭🇺	35	Polyfloral	Argentina 🇦🇷
18	Polyfloral	China 🇨🇳	36	Polyfloral	Europe 🇪🇺
37	Polyfloral	Chile 🇨🇱	38	na	Europe 🇪🇺





Sample ID	Declared botanical origin 🌸🌿🌳	Country	Sample ID	Declared botanical origin 🌸🌿🌳	Country
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6	Polyfloral	Hungary 🇭🇺	24	Polyfloral	China 🇨🇳
7	Polyfloral	Hungary 🇭🇺	25	Polyfloral	China 🇨🇳
8	Polyfloral	Argentina 🇦🇷	26	Polyfloral	China 🇨🇳
9	Polyfloral	China 🇨🇳	27	Polyfloral	China 🇨🇳
10	Polyfloral	Argentina 🇦🇷	28	Polyfloral	China 🇨🇳
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16	Polyfloral	Ukraine 🇺🇦	34	Polyfloral	Argentina 🇦🇷
17	Polyfloral	Hungary 🇭🇺	35	Polyfloral	Argentina 🇦🇷
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37	Polyfloral	Chile 🇨🇱	38	na	Europe 🇪🇺

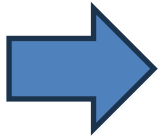
Lepidoptera and coleoptera species & ...





Other presentations of the Animal and Food Genomics Group at Apimondia2025

Sept. 26th



Hall A1 – Symposium / Beekeeping Technology & Quality

15.15-15.30 - Entomological signatures in honey: combining metabarcoding approaches to provide insights into plant-sucking insects that populate agricultural and forest landscapes. **Valeria Taurisano**

Sept. 24th - 25th

PP-294



Multiple-metabarcoding assays provides botanical and entomological DNA fingerprints that can reveal the geographic origin of Turkish honey. **Valeria Taurisano**

Sept. 27th

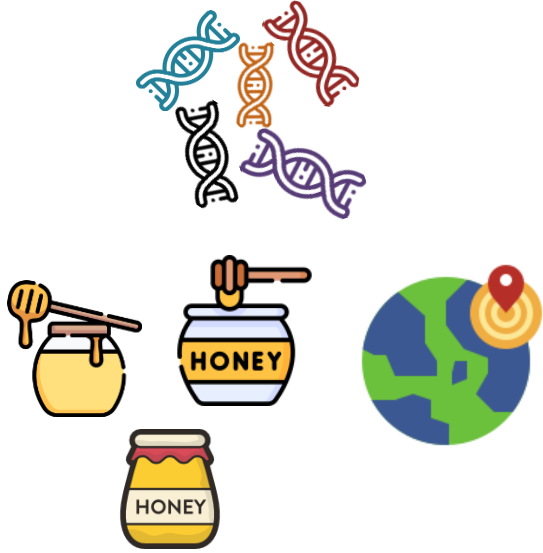


Hall A2 – Symposium / Bee Biology

11.45-12.00 - A dynamic picture of the diffusion of *Apis mellifera* mitochondrial DNA lineages in Italy over the last few decades utilizing honey as a source of honey bee population genetic information. **Luca Fontanesi**



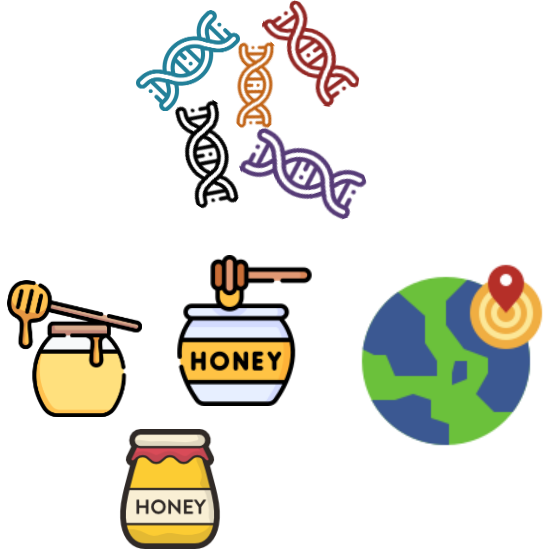
Conclusions



- This is one of the first study that combined information from multi-analytical approaches to identify frauded honey



Conclusions



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- Only multi-analytical approaches can disclose elusive frauds and track potential sources of adulterated honey

Conclusions



- This is one of the first study that combined information from multi-analytical approaches to identify frauded honey
- Only multi-analytical approaches can disclose elusive frauds and track potential sources of adulterated honey
- We are working to translate multiple DNA level information into routine practise for honey authentication



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Co-authors

Lifeanalytics



Samuele Bovo



Giancarlo Quaglia



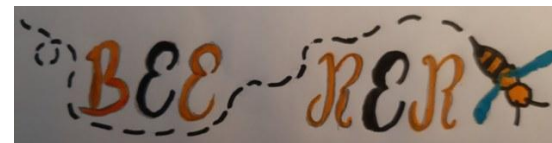
Valeria Taurisano



Anisa Ribani



ItaHoneyDNA



Regione Emilia-Romagna



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UNIVERSITÀ DI BOLOGNA

Animal and Food Genomics Group @ DISTAL (UNIBO)



L. Fontanesi
PI, Full Professor



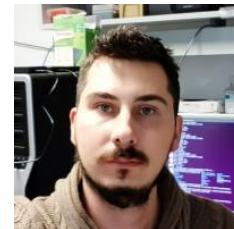
F. Bertolini
Associate Professor



S. Dall'Olio
Associate Professor



P. Zambonelli
Associate Professor



S. Bovo
Assistant Professor



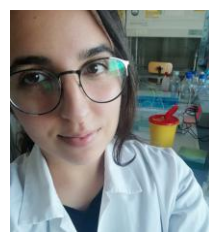
A. Ribani
Assistant Professor



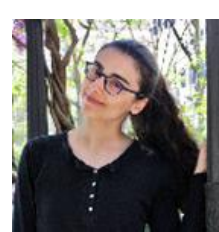
G. Schiavo
Assistant Professor



M. Bolner
Post doc



V. Taurisano
Post doc



M. L. Calabri
Research Fellow



D. Sami
Lab. technician



**Animal and Food
Genomics Group**



<https://site.unibo.it/animal-and-food-genomics/en>



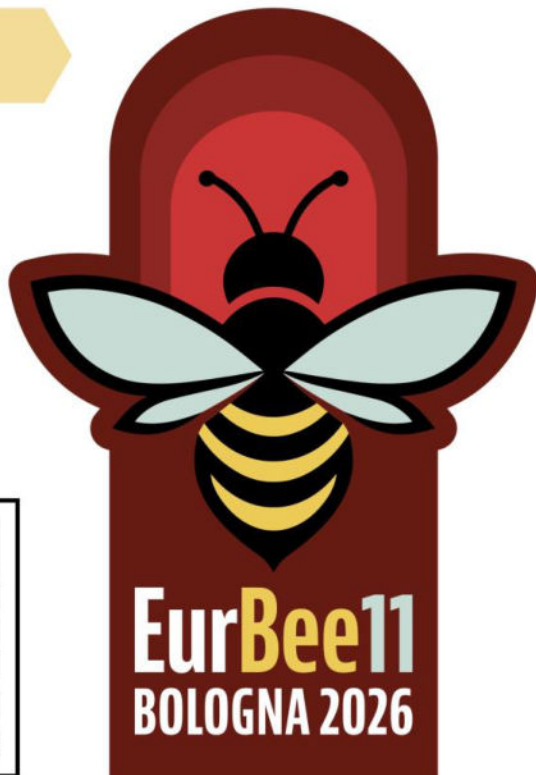
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UNIVERSITÀ DI BOLOGNA

11th European Conference of Apidology

7-10 September 2026 · Bologna, Italy

**LIKE FORAGING BEES, WE SEEK NOVELTY,
WE NEED COOPERATION AND WE PROFIT
FROM DIVERSITY**

The conference is jointly organized by CREA-AA – Research Centre for Agriculture and Environment and the Alma Mater Studiorum – University of Bologna



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Animal and Food Genomics Group

Toward the ultimate assay for honey authentication: combining multi-dimensional DNA sequencing data with multi-platform physical-chemical and isotopic d13C analyses

Luca Fontanesi

Animal and Food Genomics Group
Department of Agricultural and Food Sciences
University of Bologna, Italy

luca.fontanesi@unibo.it



APIMONDIA
SCANDINAVIA
2025
23-27 SEPTEMBER

COPENHAGEN

