

Entomological signatures in honey: combining metabarcoding approaches to provide insights into plant-sucking insects that populate agricultural and forest landscapes

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Honeydew, primarily produced by plant-sucking insects of the order Hemiptera, is an important source of carbohydrates that complement the honey bee diet, especially when nectar is unavailable or does not fully meet the bees' dietary needs. Honeydew honey is a unique type of honey that comes from this prevalent source of sugar components. Honeydew contains valuable environmental DNA (eDNA) signatures that provide insights into the plant-sucking insect communities living in agricultural and forest landscapes. The presence and composition of these insect communities can indirectly indicate the botanical and geographical origin of honey, as many plant-sucking insects have host-plant specialization linked to specific ecosystems. In this study, we used DNA extracted from honey as a source of eDNA to determine its entomological fingerprinting, focusing specifically on honeydew-producing Hemiptera. We designed two primer pairs targeting mitochondrial DNA (mtDNA) genes (cytochrome b and COXI) of Hemiptera species to amplify the DNA extracted from 70 honey samples of various geographic and botanical origins, including several honeydew honey samples. We utilized a next-generation sequencing (NGS) metabarcoding approach, along with specially designed bioinformatics pipelines and a reference sequence database to analyse the amplicons obtained for the two targeted mtDNA regions. On average, 47 and 16.5 taxa per honey sample were identified with the cytochrome b and COXI primer pairs, respectively, demonstrating the high informativeness of this multiple metabarcoding approach. *Metcalfa pruinosa* was one of the most common hemipters identified, along with several other specialised aphids that provide an indirect signature of the botanical origin based on the specific insect community. Our results emphasize the potential of entomological metabarcoding as a robust tool for honey authentication and landscape characterization. This approach could provide new possibilities for verifying honey authenticity and analyzing the intricate entomological ecosystems of agricultural and forest landscapes, including the geographical identity and origin of honey.



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**Animal and Food
Genomics Group**

Entomological signatures in honey: combining metabarcoding approaches to provide insights into plant-sucking insects that populate agricultural and forest landscapes

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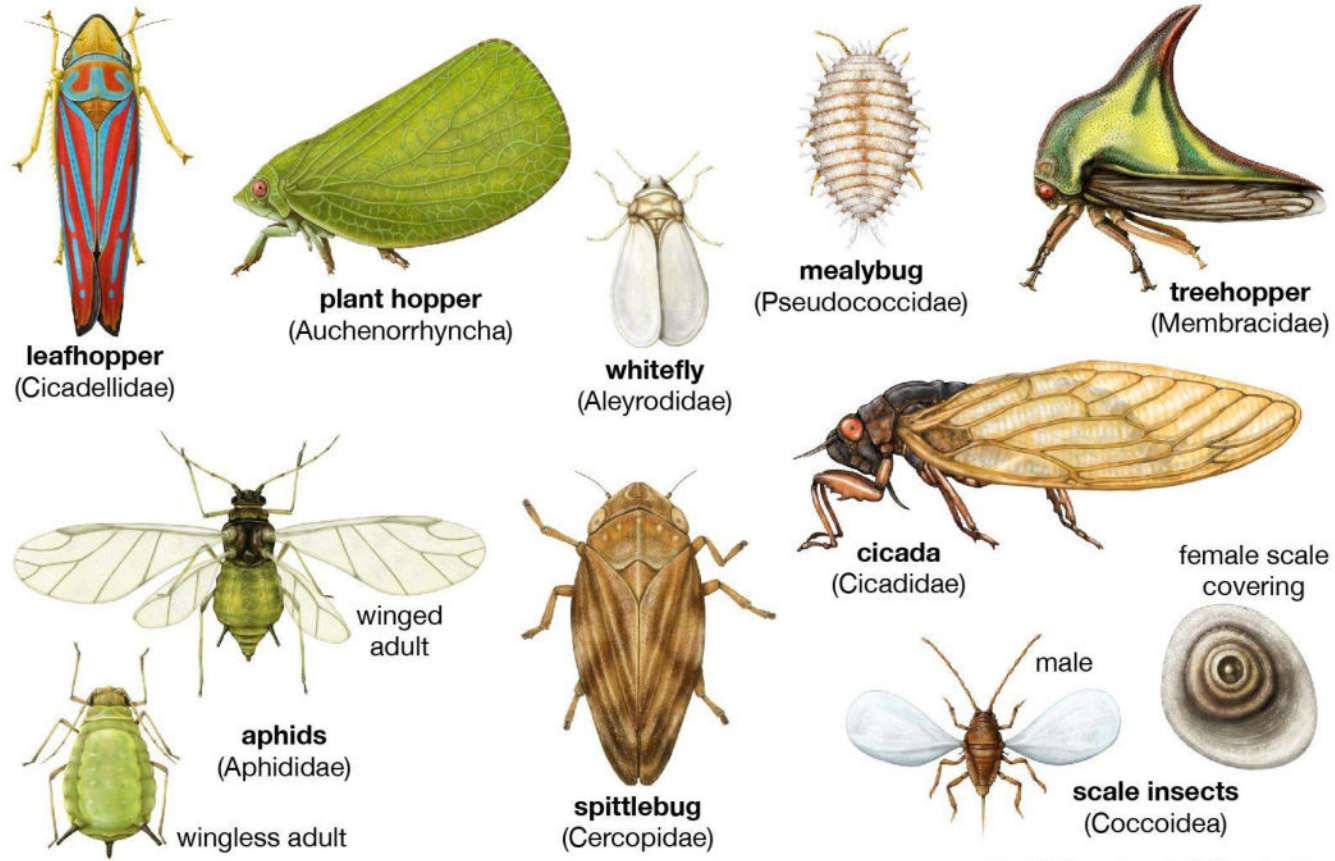


BIODLARNÄ
SINCE 1969

Honeydew in honey

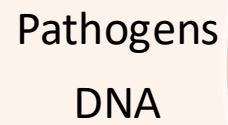
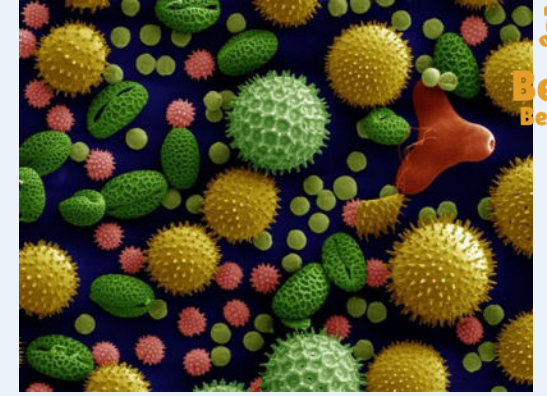
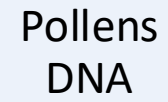


Plant-sucking insects



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Polyphagous
Oligophagous
Monophagous



Aphids/other
contacts DNA



Honeybee DNA



Previous work – 2018

Hemiptera metabarcoding



SCIENTIFIC REPORTS

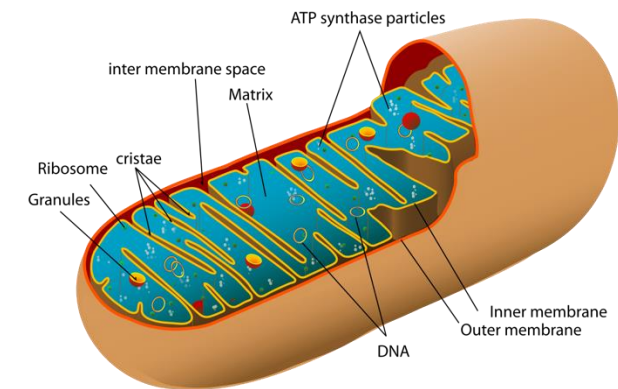
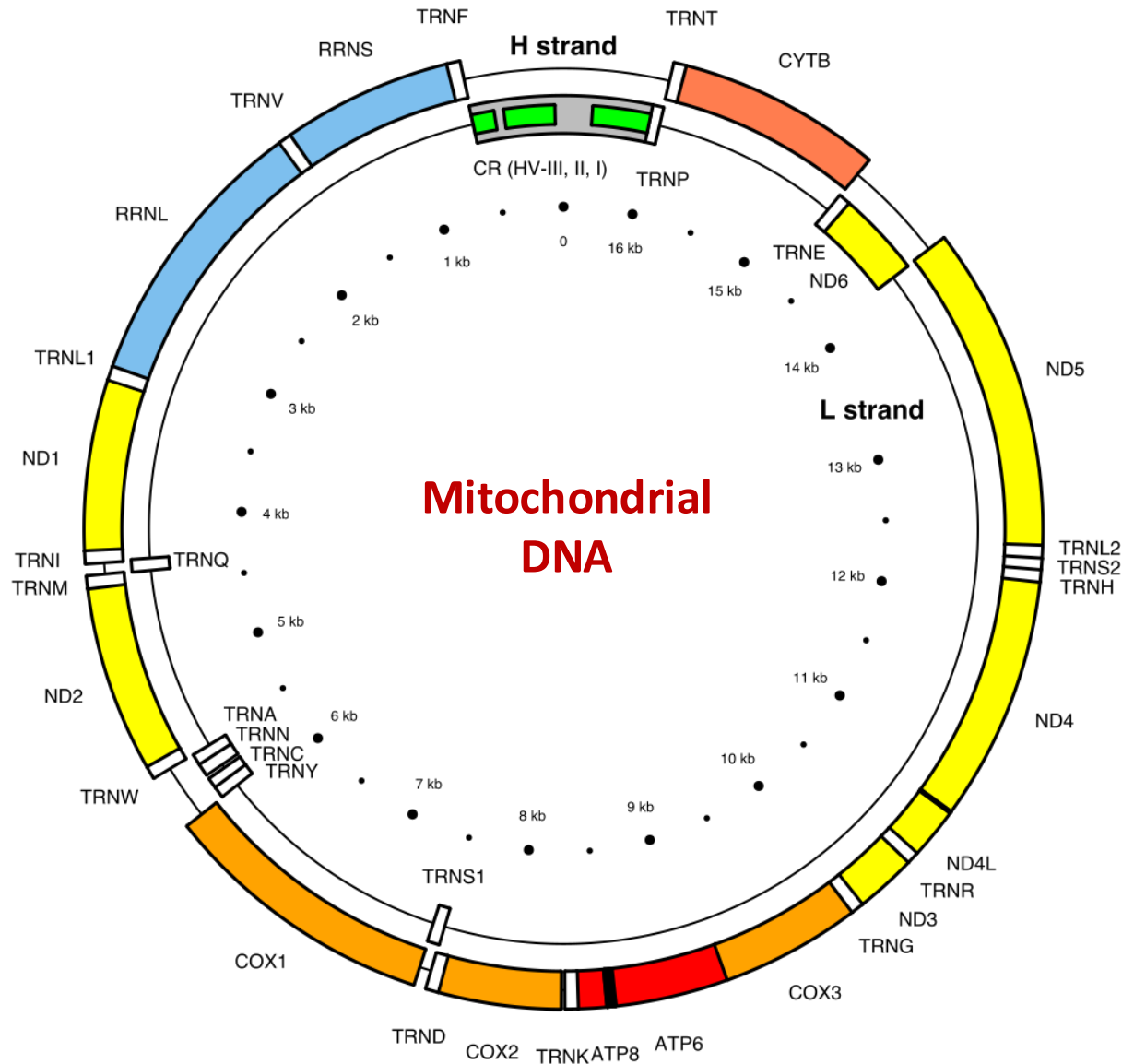
OPEN

Entomological signatures in honey: an environmental DNA metabarcoding approach can disclose information on plant-sucking insects in agricultural and forest landscapes

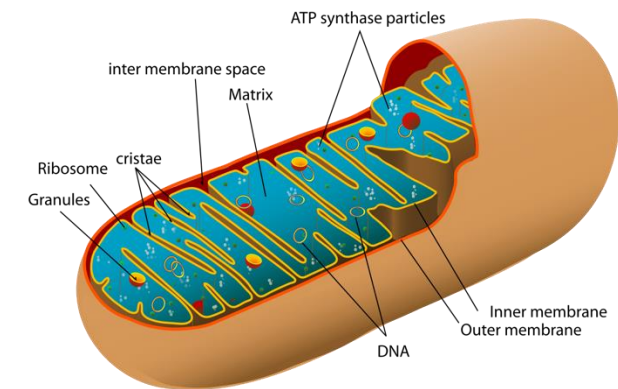
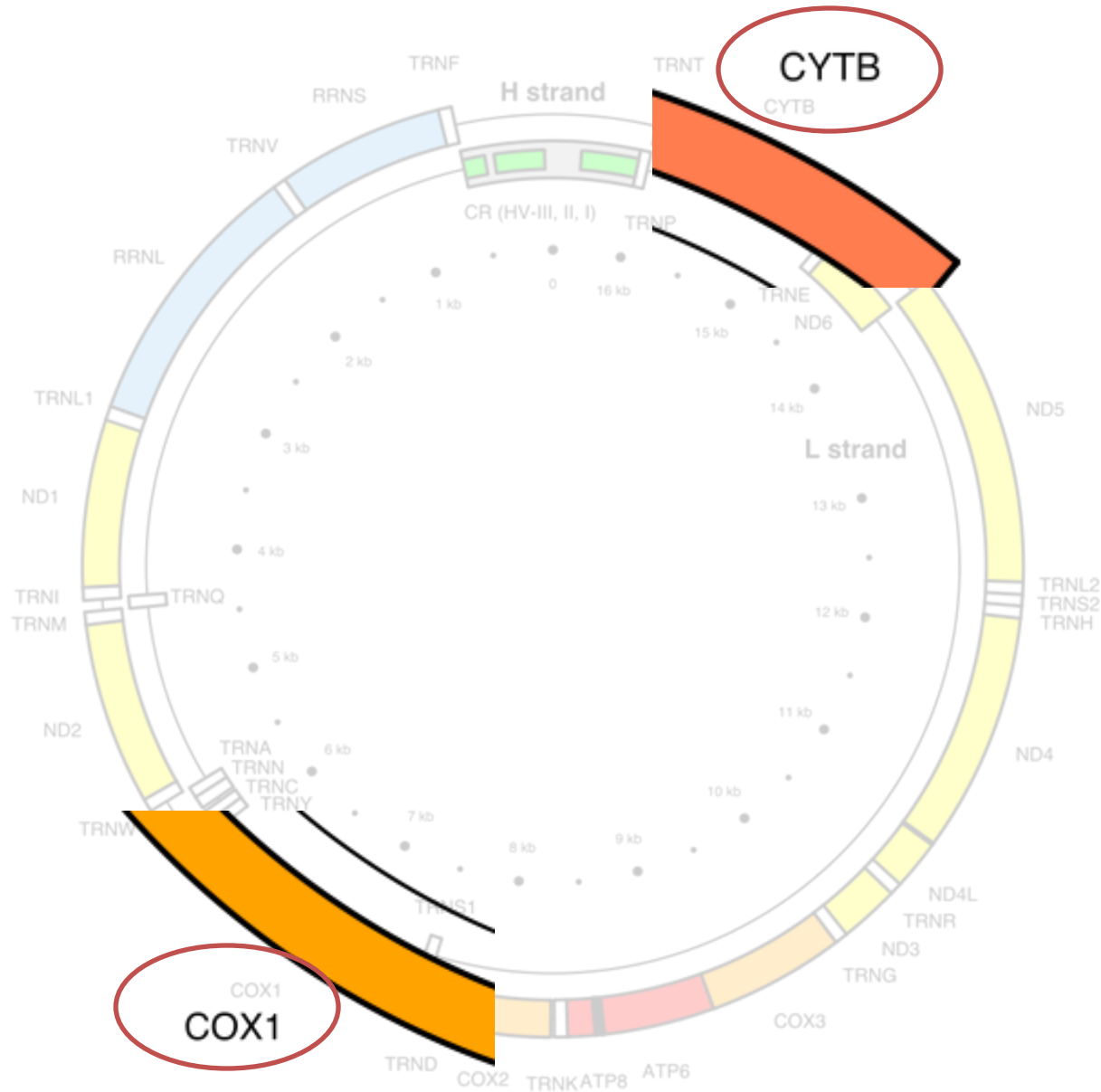
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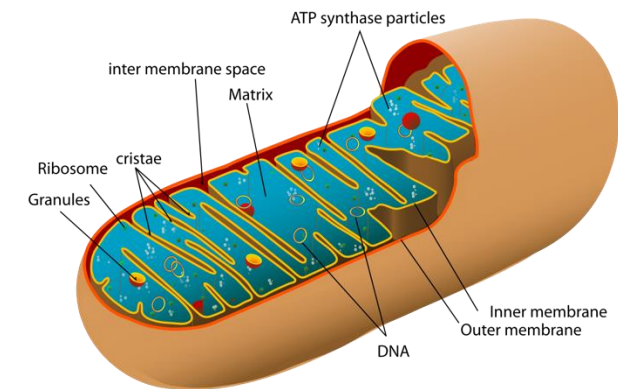
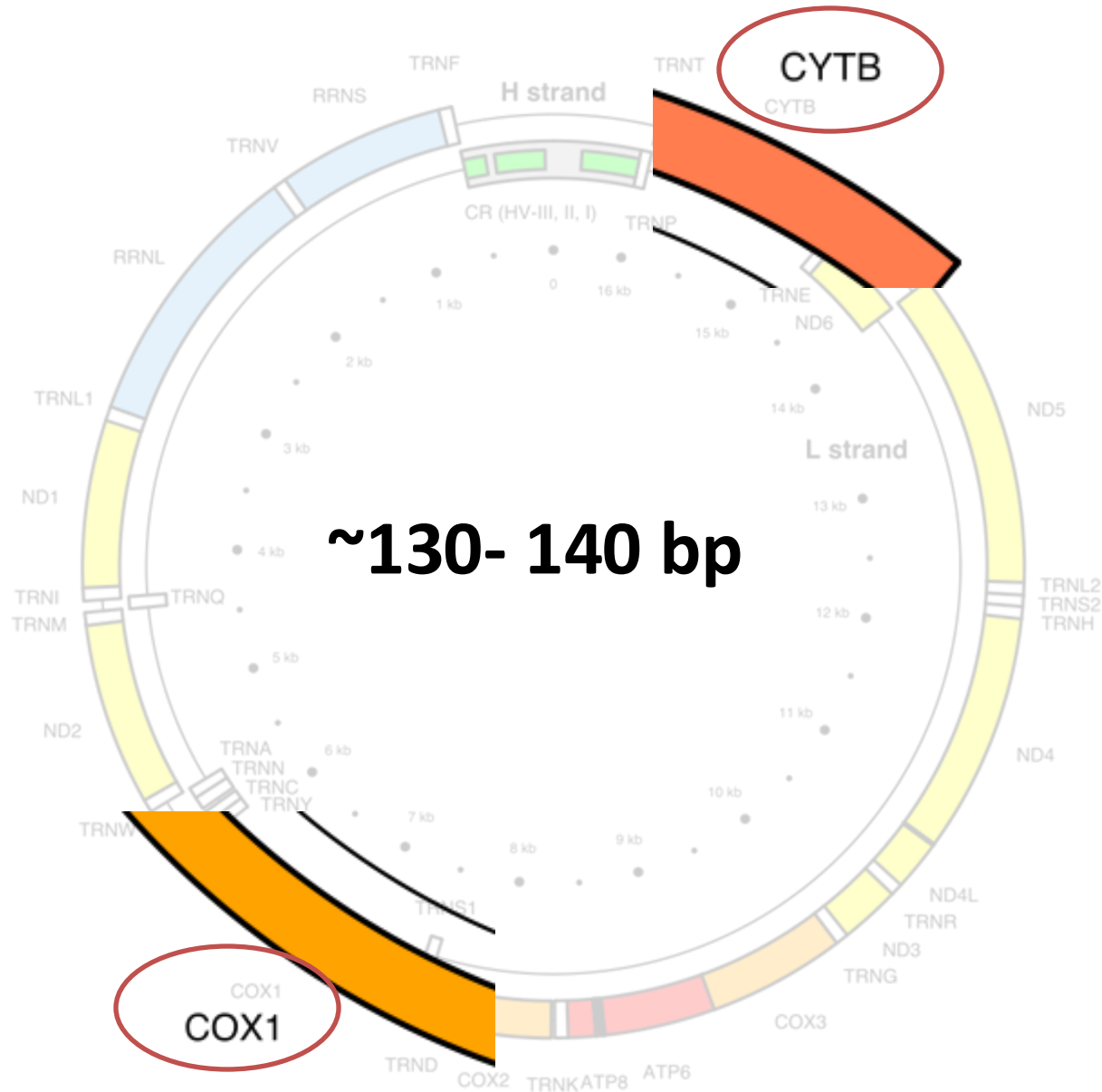
Hemiptera metabarcoding



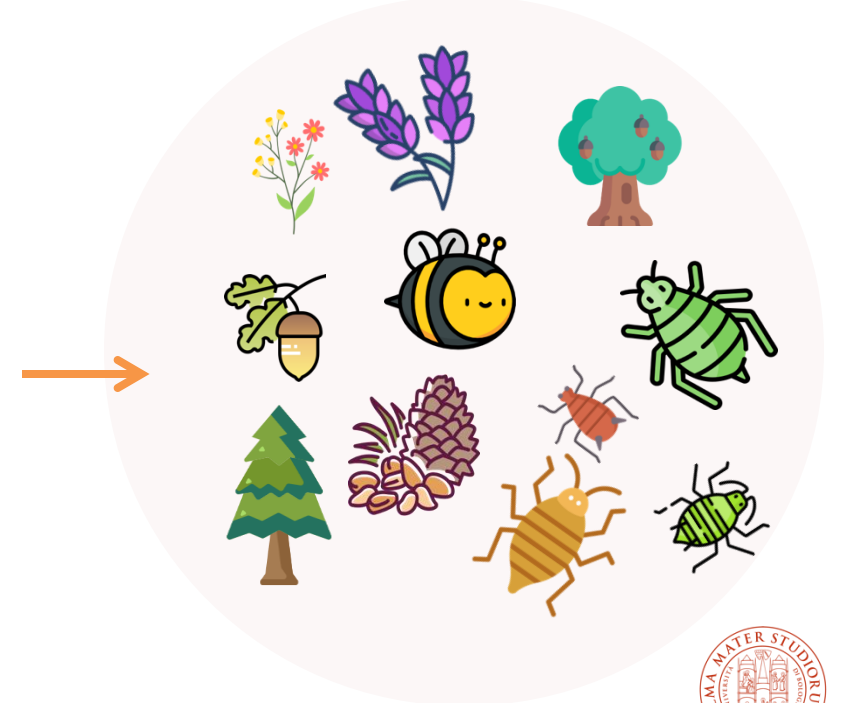
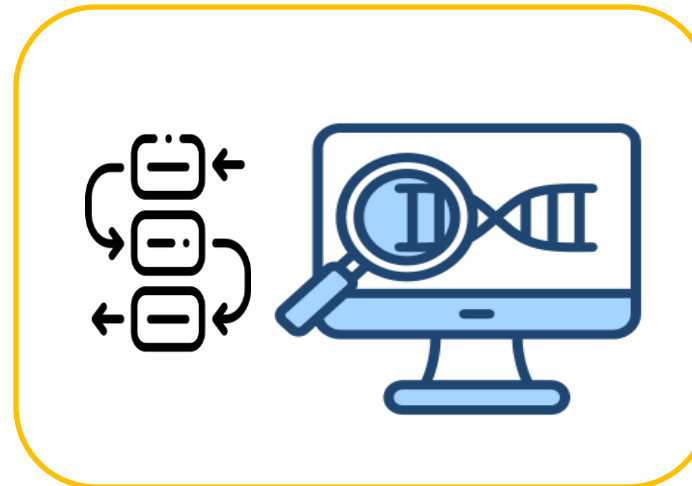
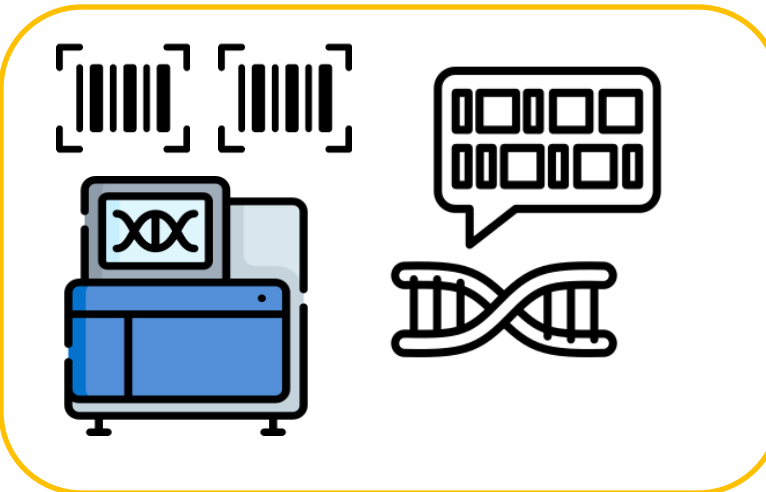
Hemiptera metabarcoding



Hemiptera metabarcoding



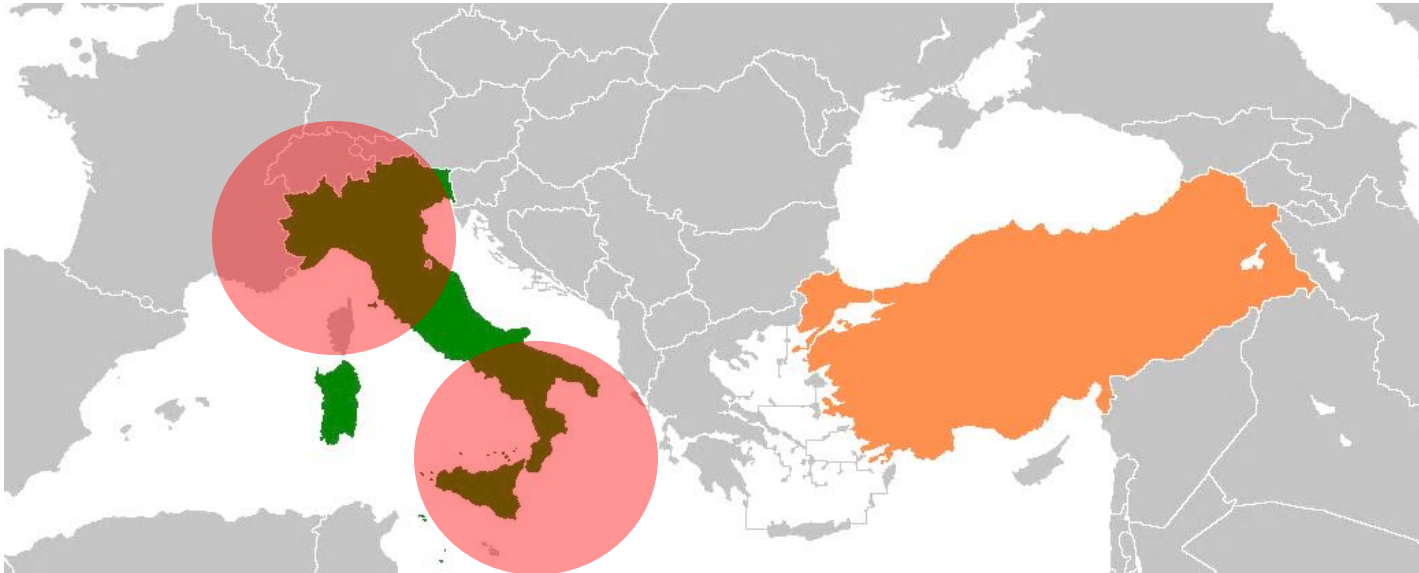
Workflow



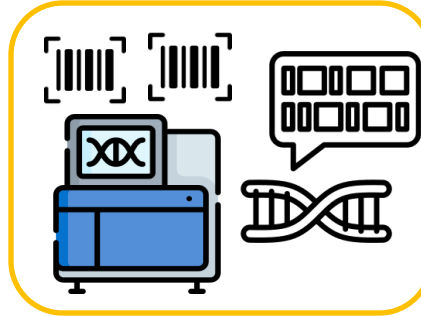
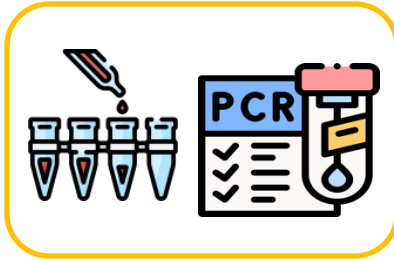
Workflow – samples collection



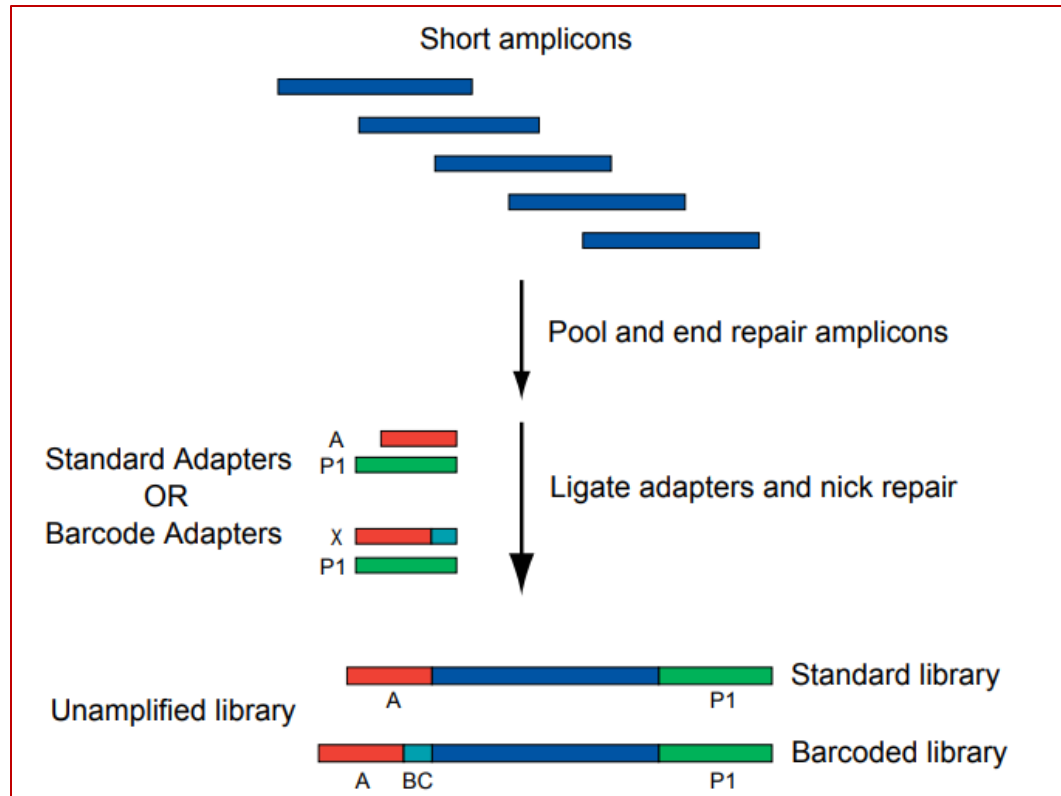
70 Honey samples: unifloral blossom honeys, polyfloral honeys, honeydew honeys



Workflow – PCR and NGS



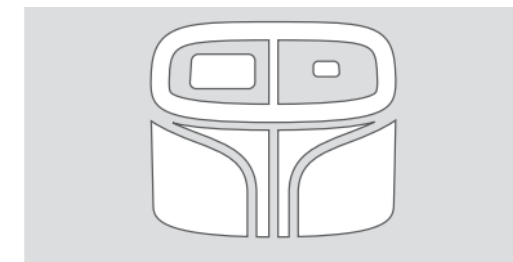
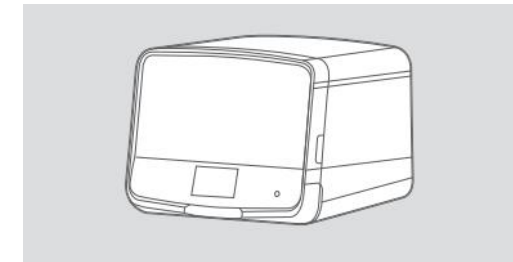
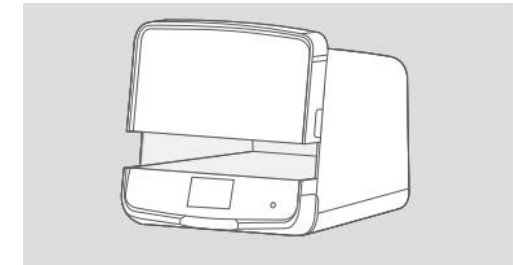
ThermoFisher
SCIENTIFIC



Library preparation

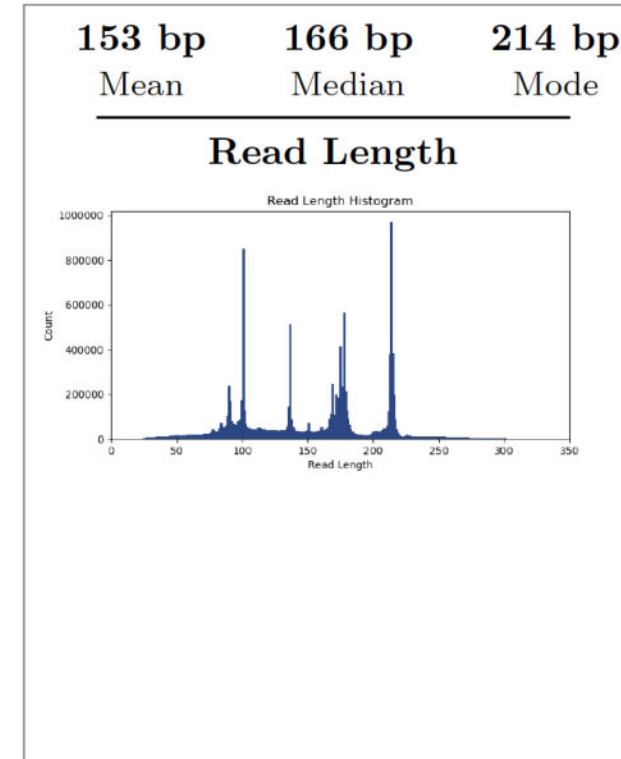
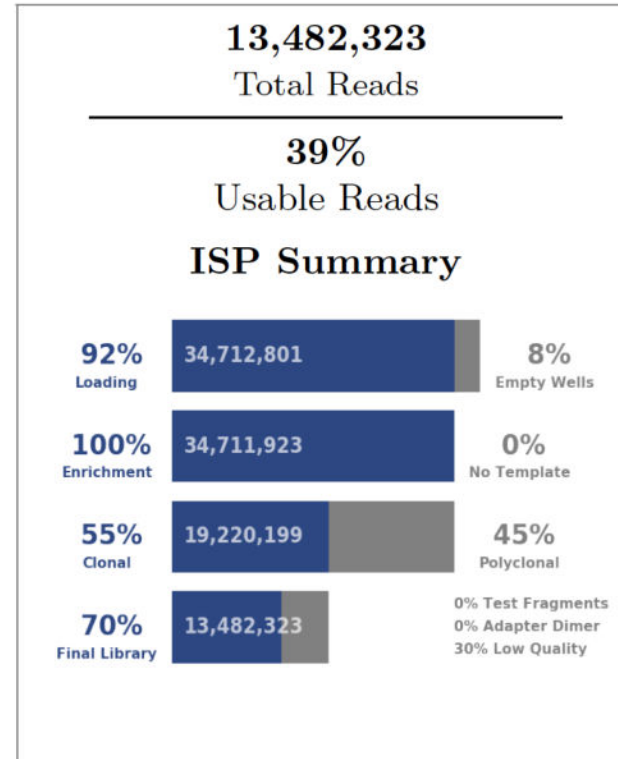
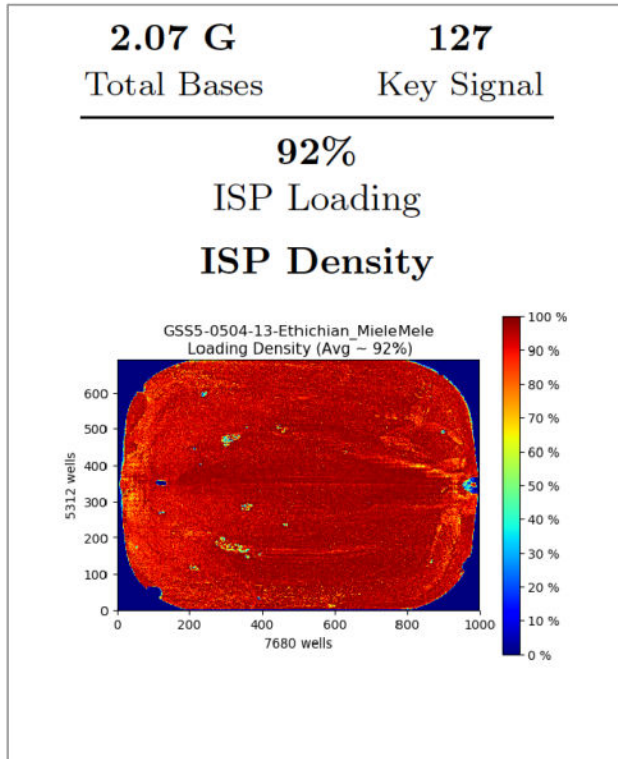
Chip loading

Sequencing



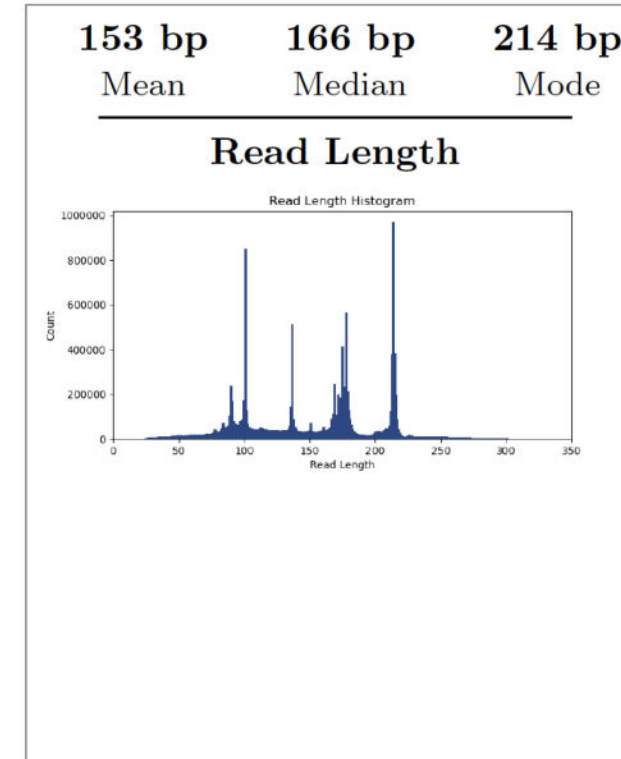
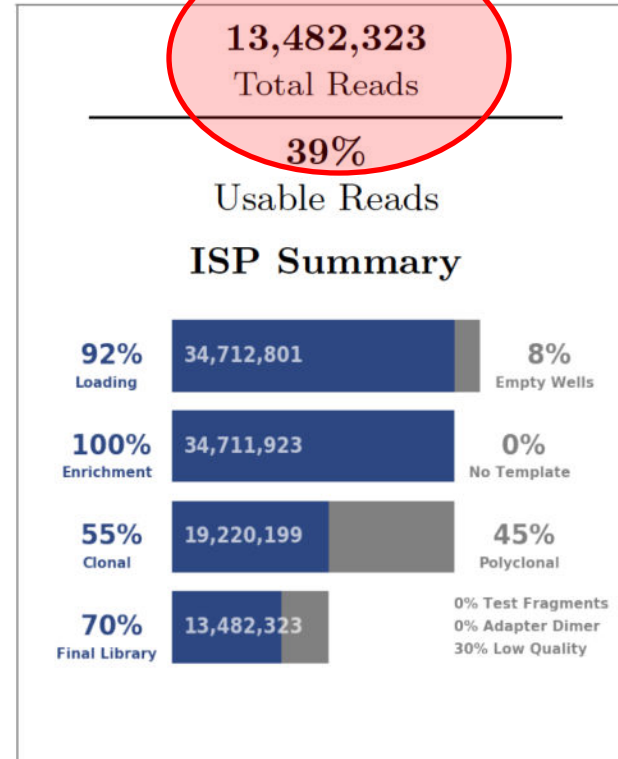
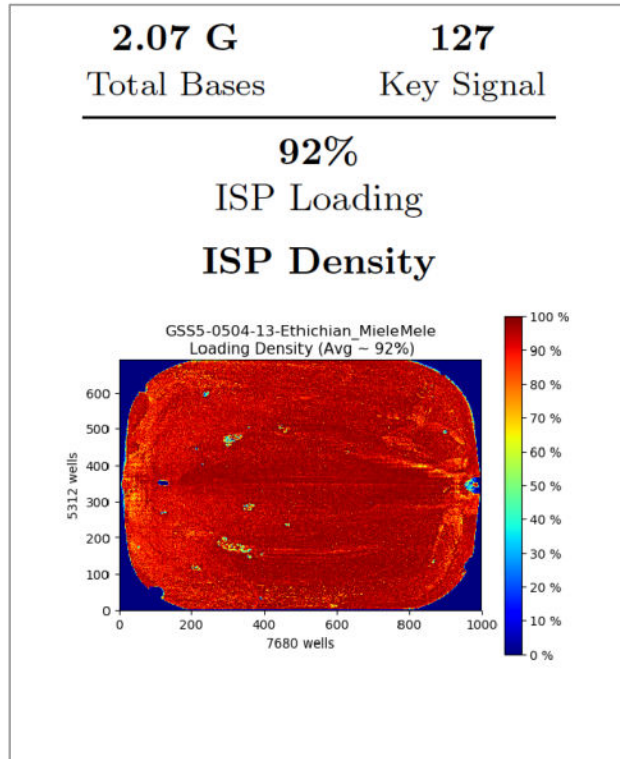
Sequencing results

Run Summary

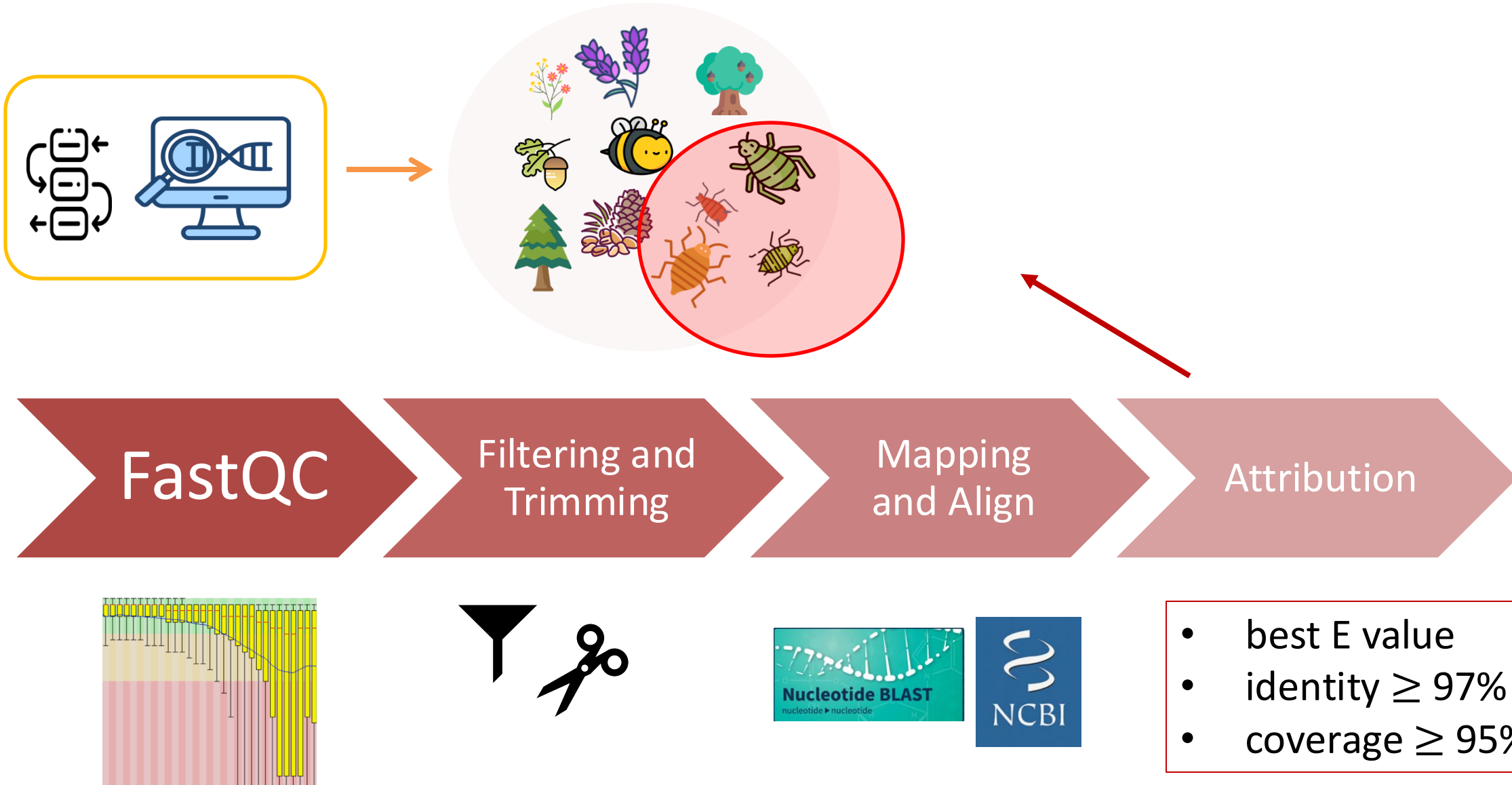


Sequencing results

Run Summary



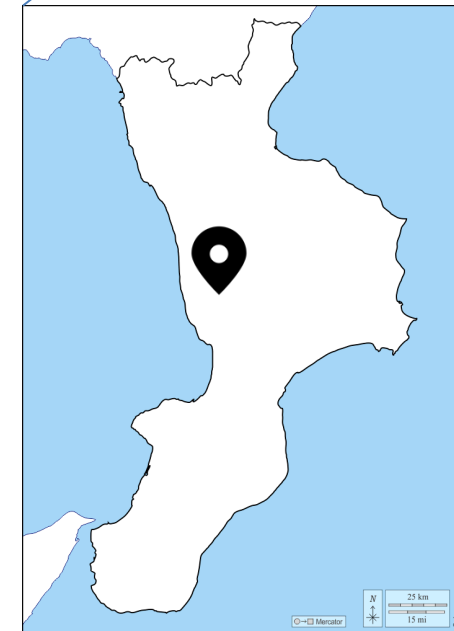
Workflow – Bioinformatic pipeline



Results – Citrus honey, Calabria region, Italy



BeSafe
BeeHoney



cox1

Aphis gossypii
29%

other
21%

Aphis sp.
50%

cytb

■ other ■ *Aphis sp.* ■ *Aphis gossypii*

Acyrtosiphon pisum
12%

other
23%

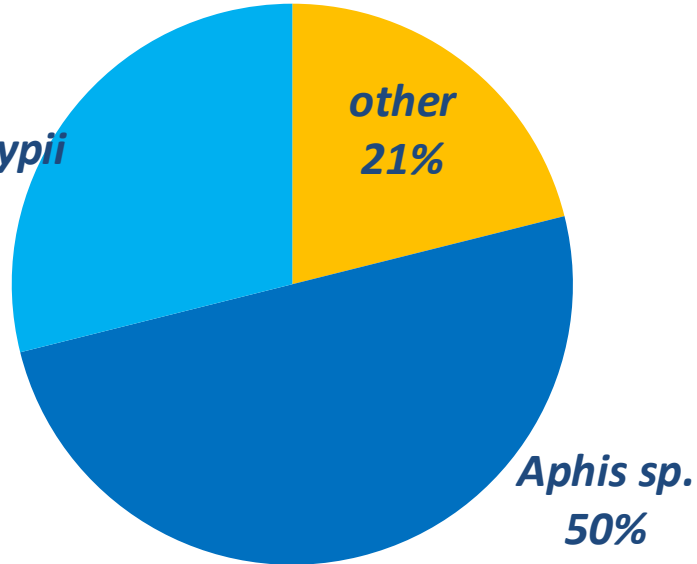
Aphis sp.
65%

■ other ■ *Aphis sp.* ■ *Acyrtosiphon pisum*

Results – Citrus honey, Calabria region, Italy

cox1

Aphis gossypii
29%

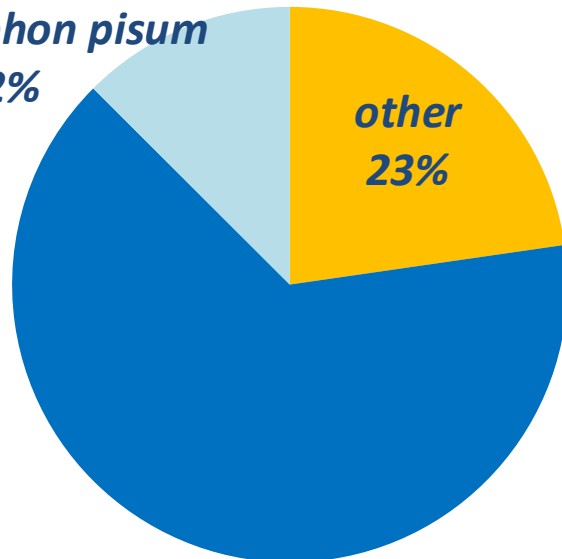


Aphis gossypii
Cotton aphid – global pest of several crops including *Citrus*

cytb

■ other ■ *Aphis sp.* ■ *Aphis gossypii*

Acyrtosiphon pisum
12%



Aphis sp.
65%



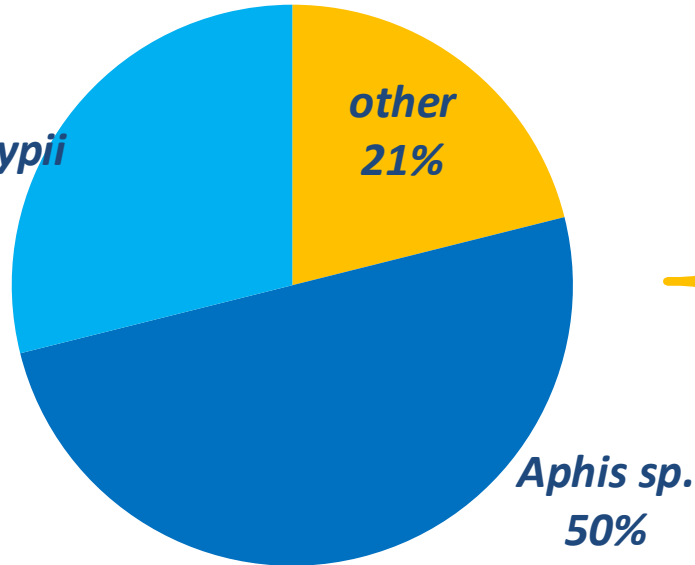
Acyrtosiphon pisum - pea
aphid – pest of legumes



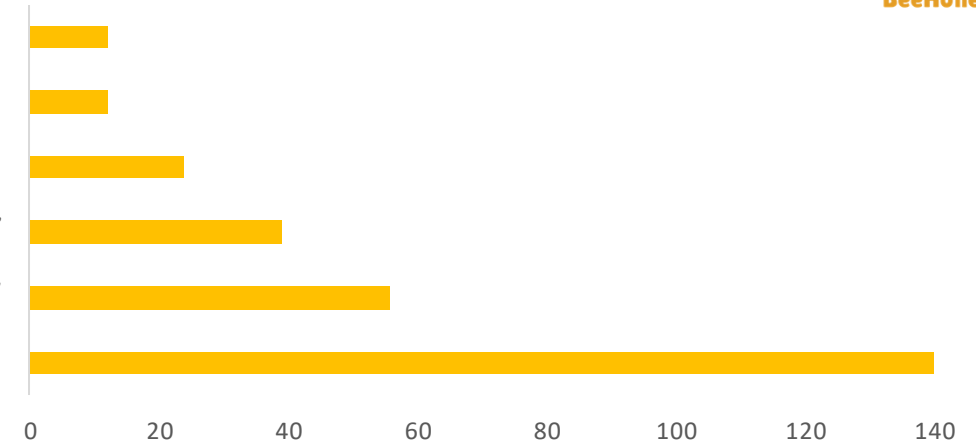
Results – Citrus honey, Calabria region, Italy

cox1

Aphis gossypii
29%



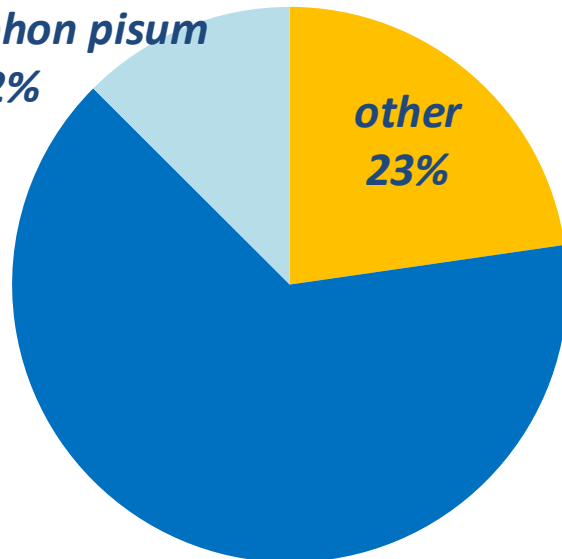
Hyalopterus sp.
Megoura sp
Chaitophorus sp.
Aphis lichtensteini
Brachycaudus helichrysi
Myzus persicae



cytb

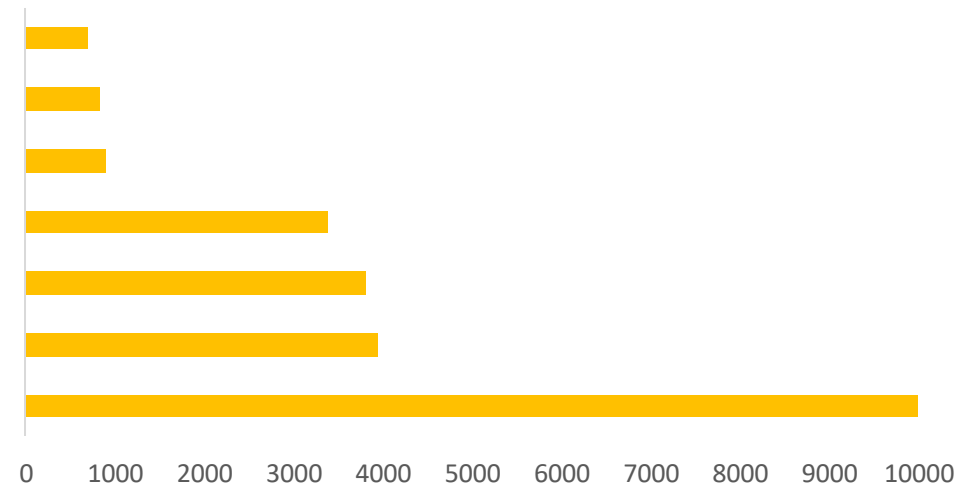
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Acyrtosiphon pisum
12%



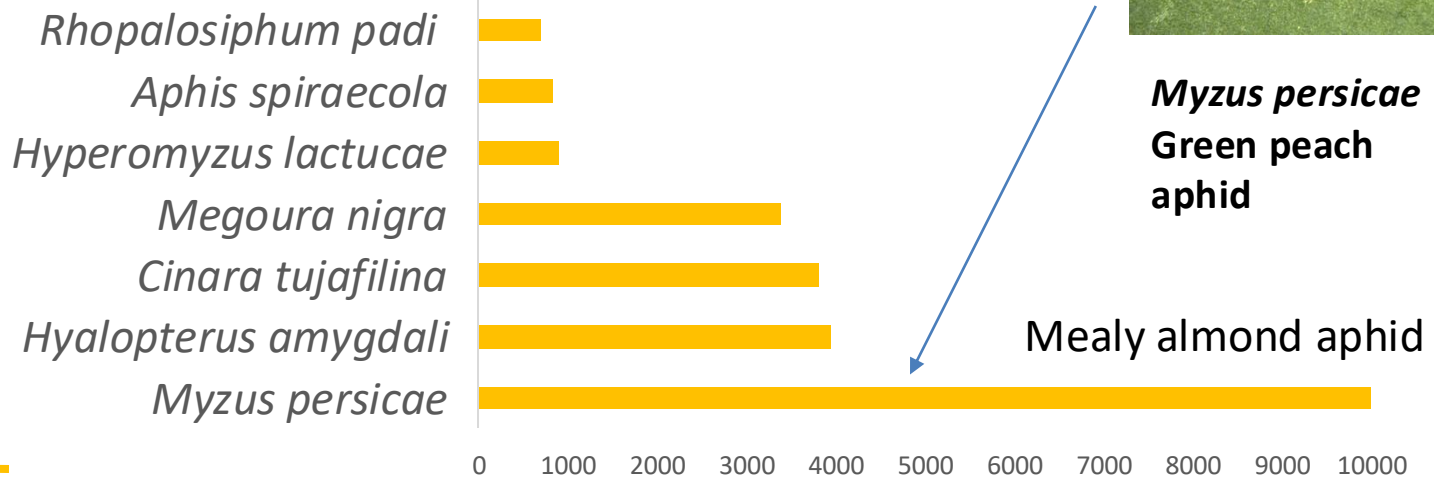
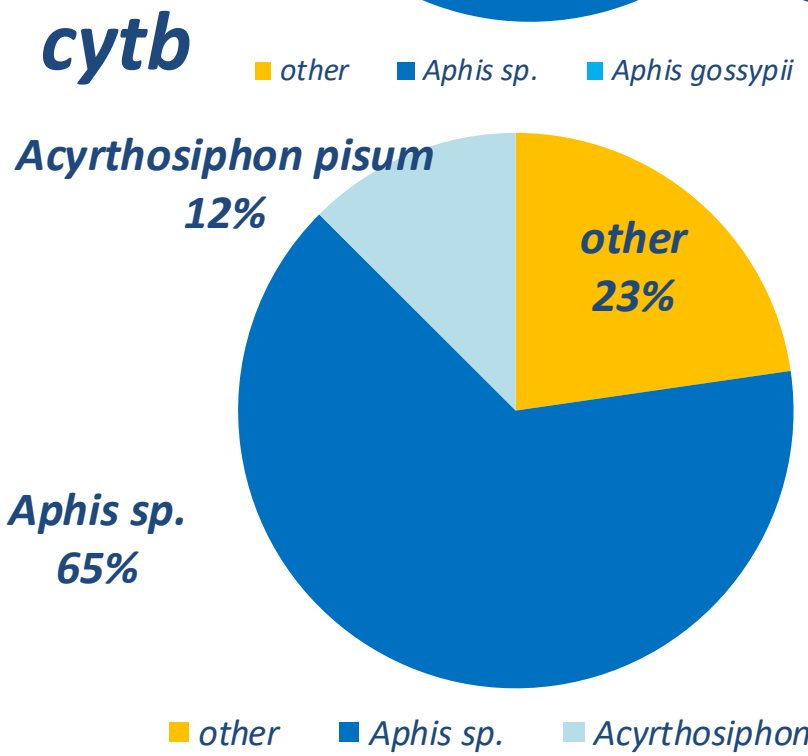
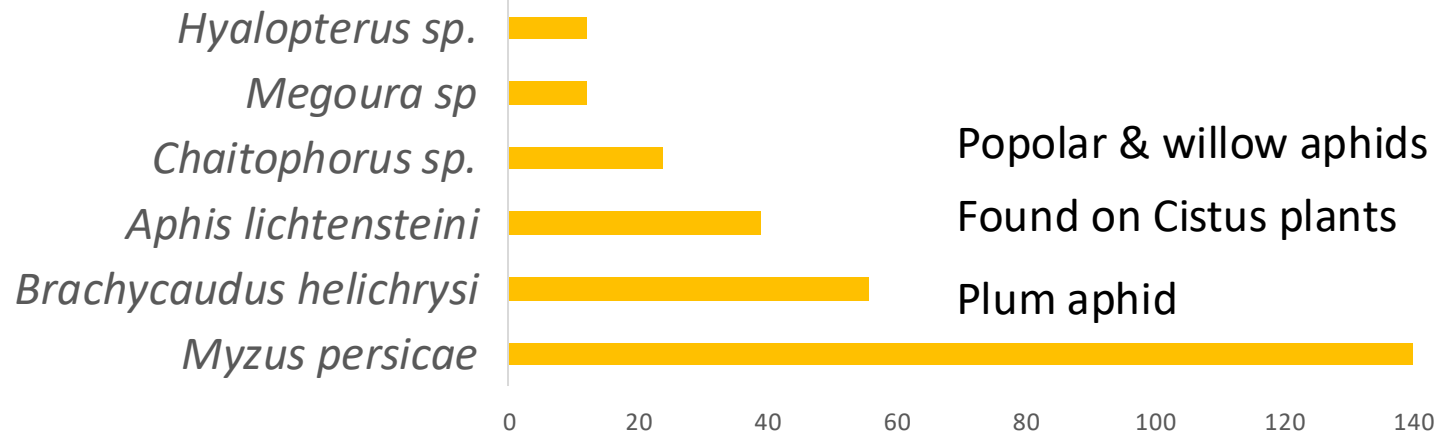
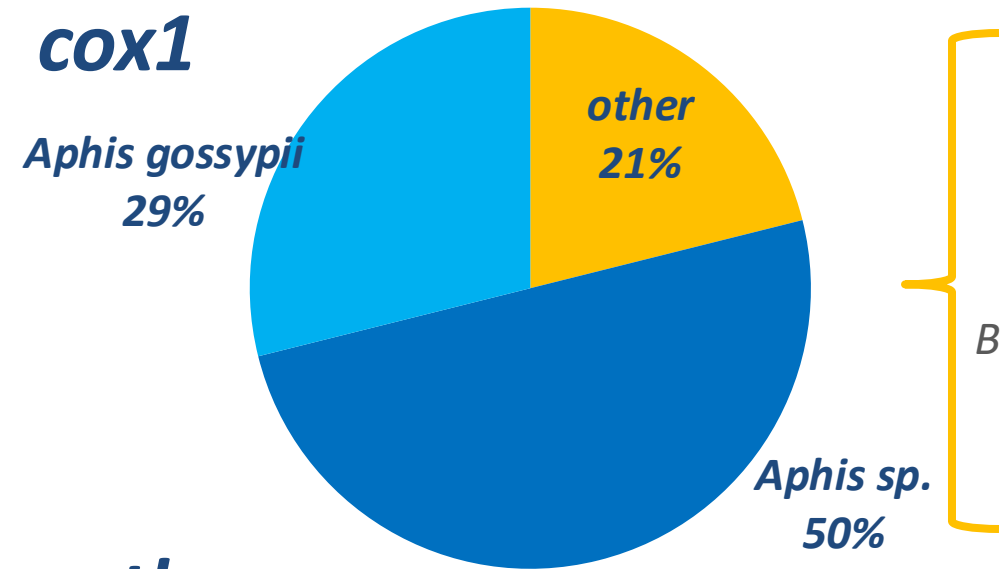
Aphis sp.
65%

Rhopalosiphum padi
Aphis spiraecola
Hyperomyzus lactucae
Megoura nigra
Cinara tujaefilina
Hyalopterus amygdali
Myzus persicae



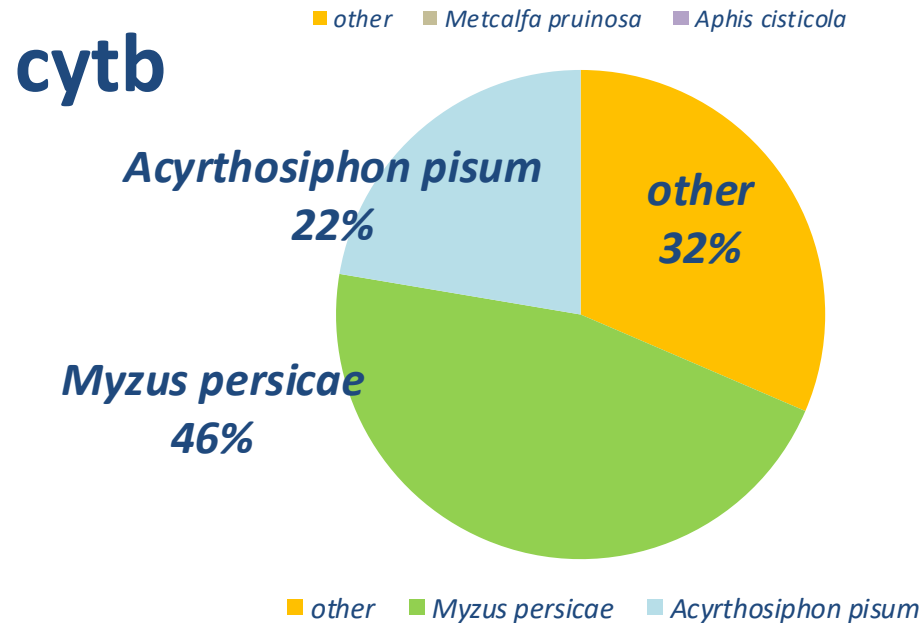
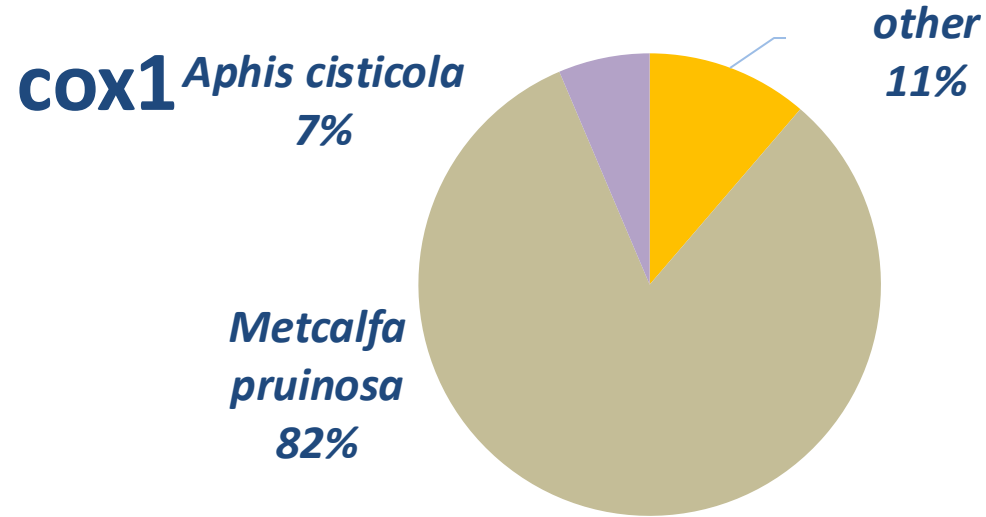
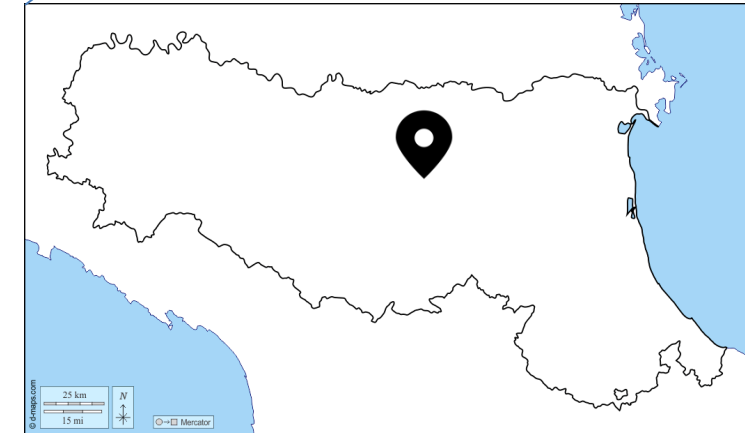
■ other ■ *Aphis sp.* ■ *Acyrtosiphon pisum*

Results – Citrus honey, Calabria region, Italy



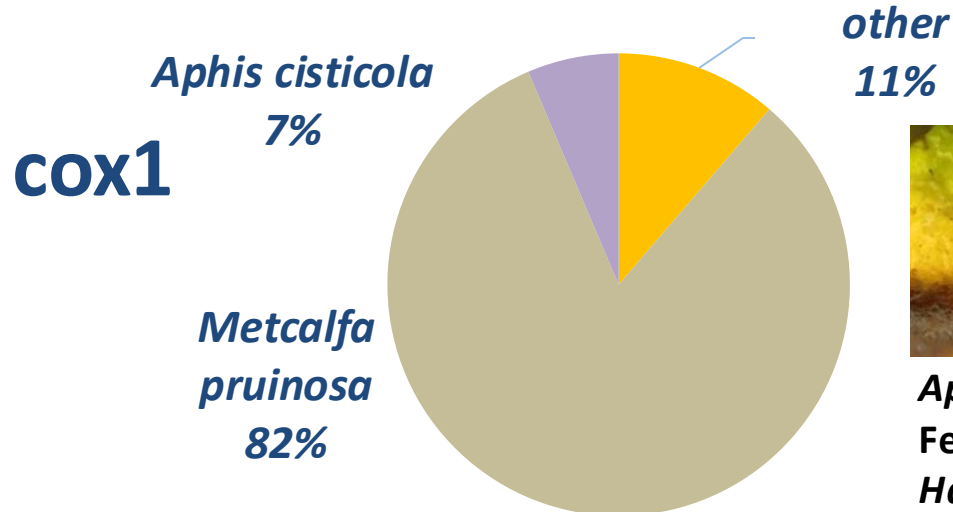
Myzus persicae
Green peach aphid

Results – Polyfloral Honey, Emilia-Romagna region, Italy





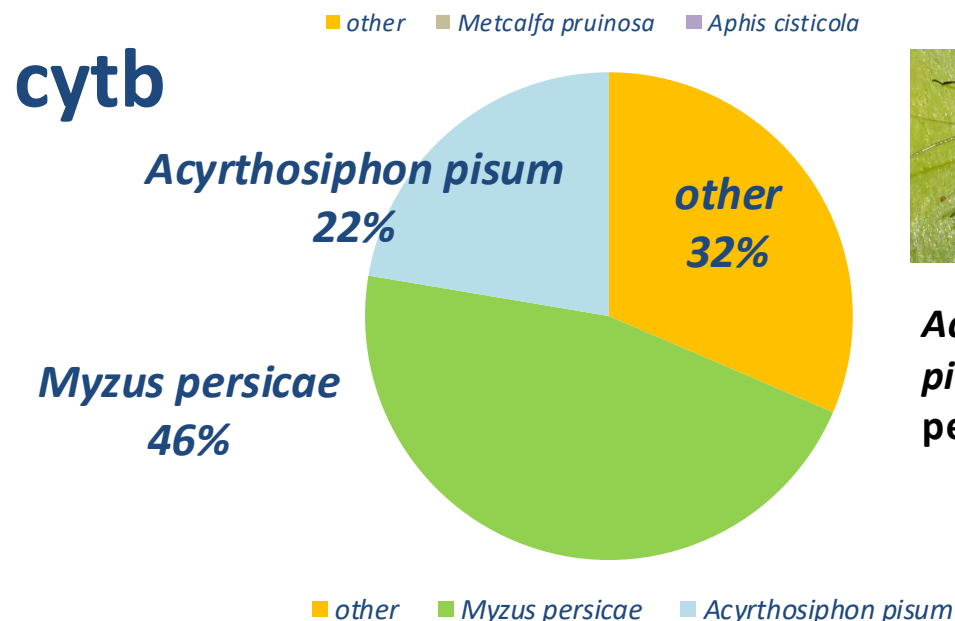
Results – Polyfloral Honey, Emilia-Romagna region, Italy



Aphis cisticola
Feeding *Cistus*,
Halimium, *Helianthus*



Metcalfa pruinosa

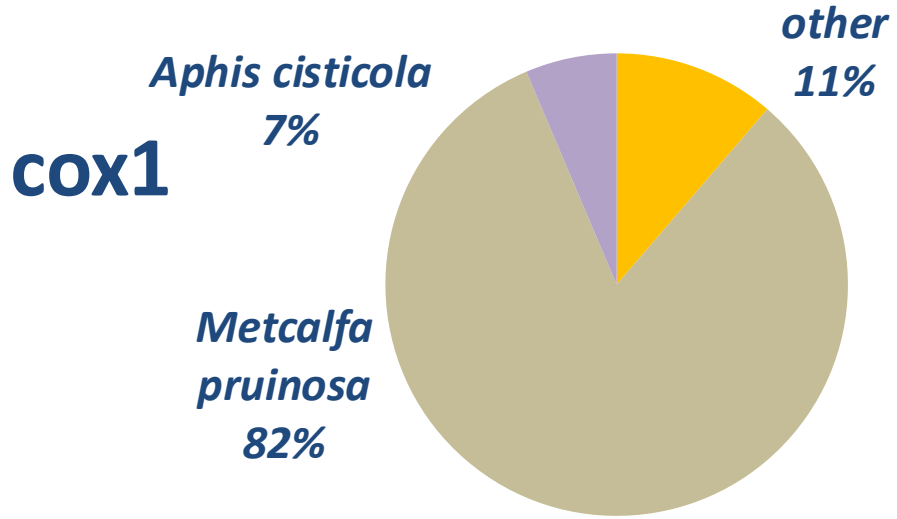


Acyrthosiphon
pisum - pea aphid
pest of legumes

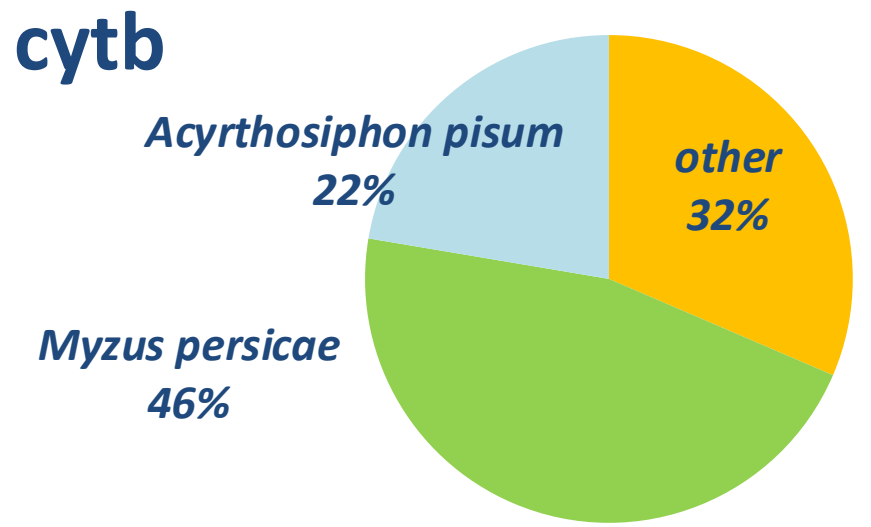
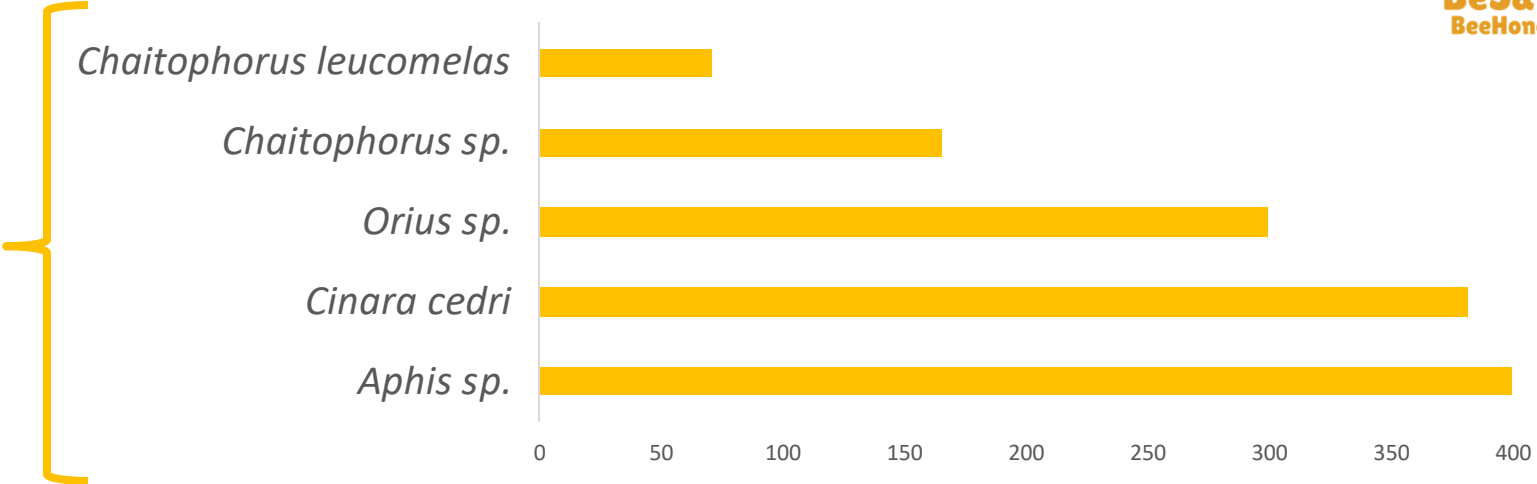


Myzus persicae
Green peach
aphid

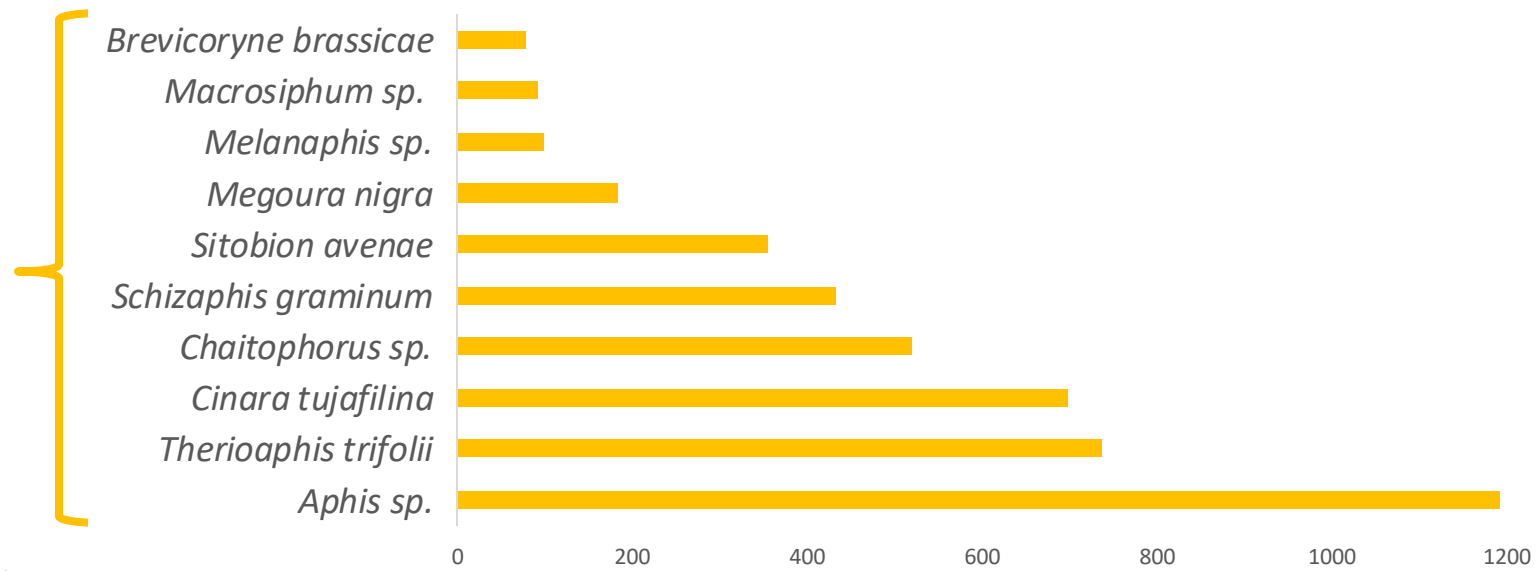
Results – Polyfloral Honey, Emilia-Romagna Region, Italy



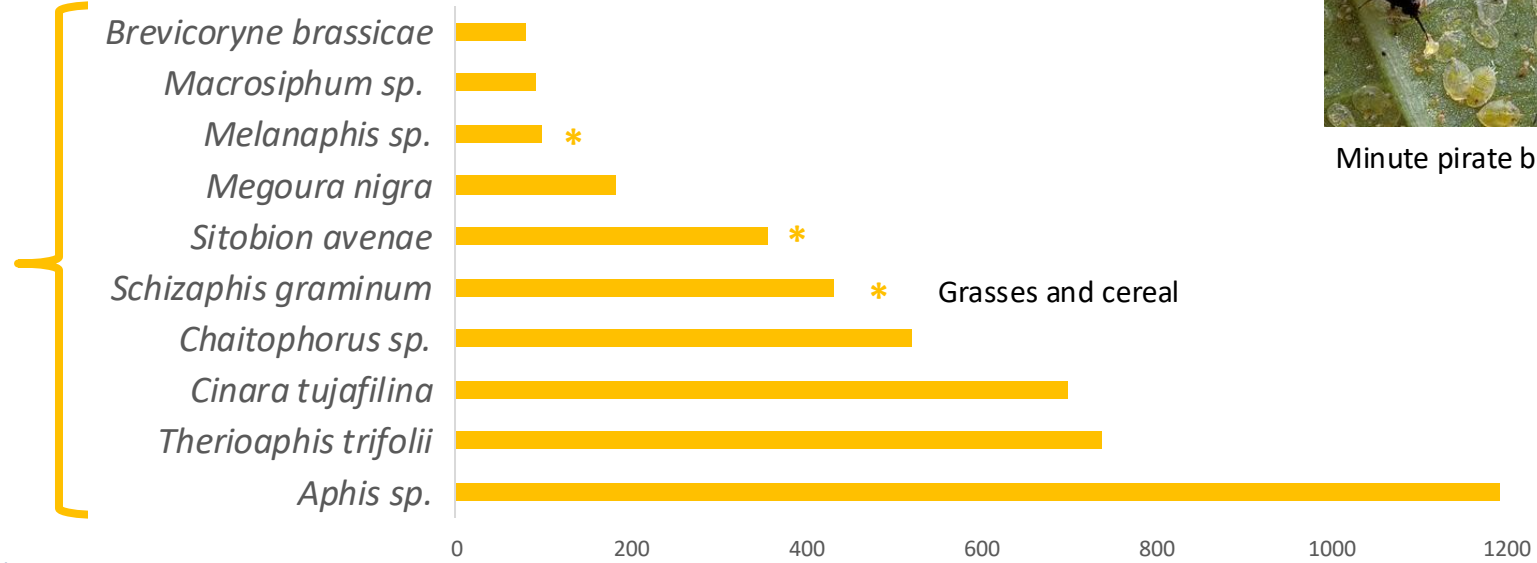
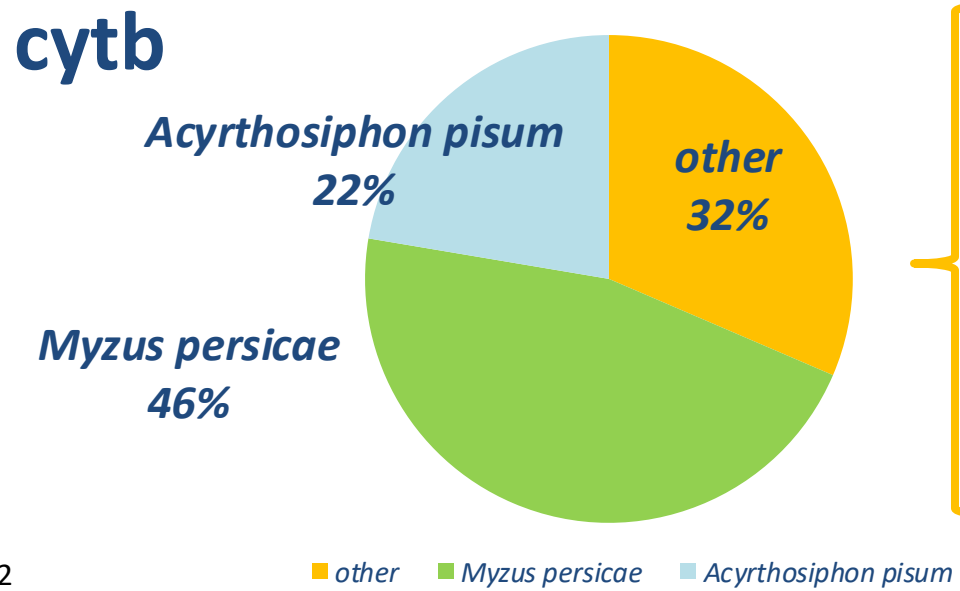
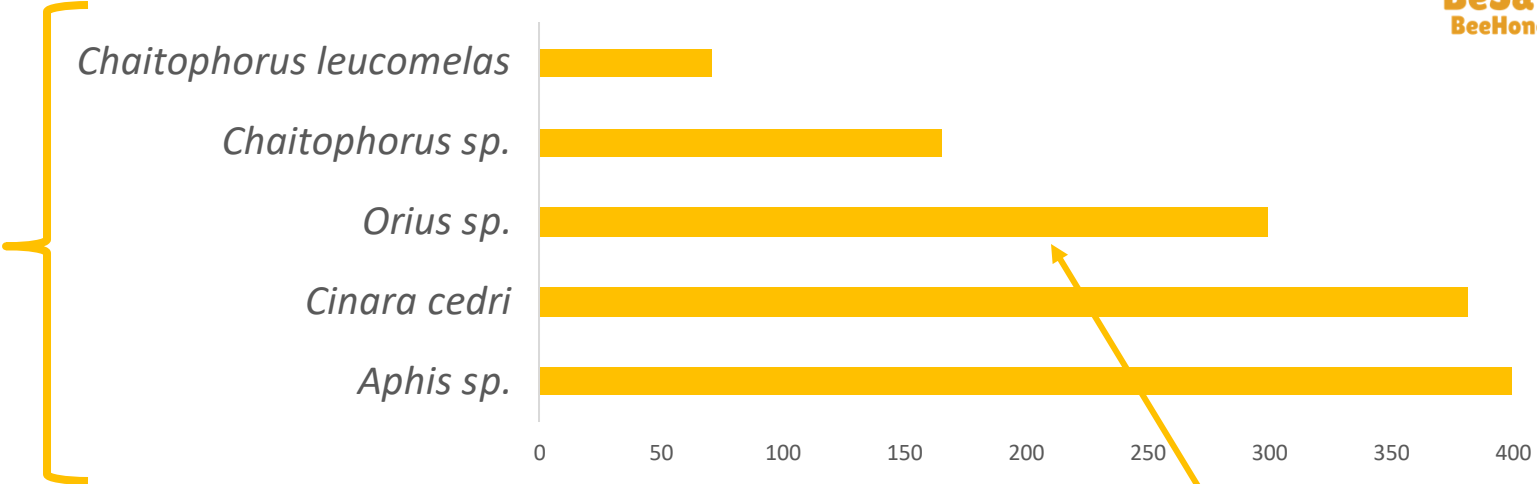
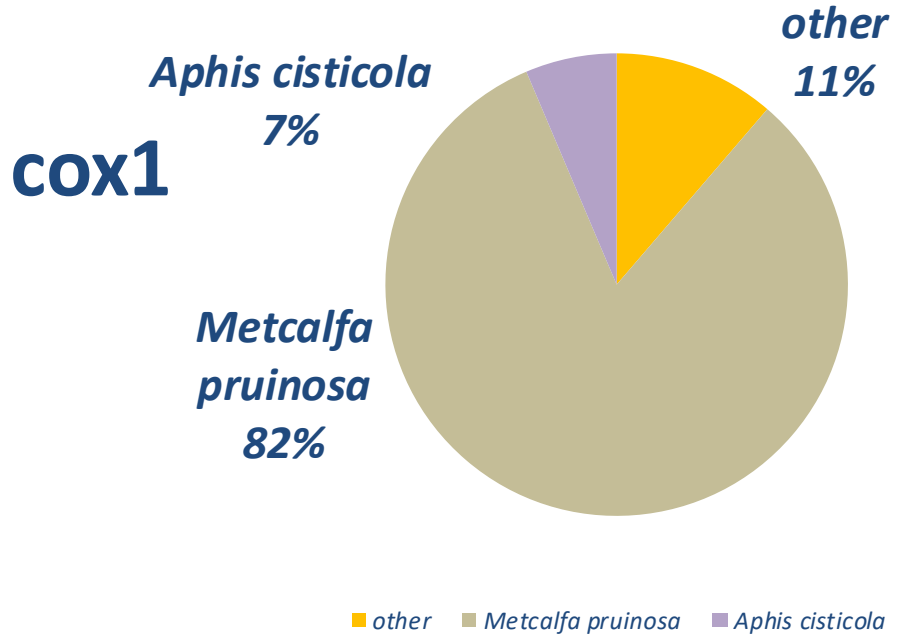
other Metcalfa pruinosa Aphis cisticola



other Myzus persicae Acyrthosiphon pisum



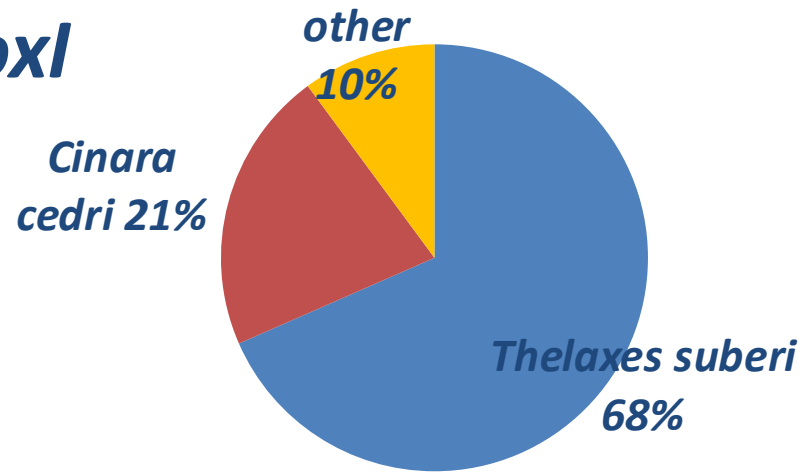
Results – Polyfloral Honey, Emilia-Romagna Region, Italy



Minute pirate bug

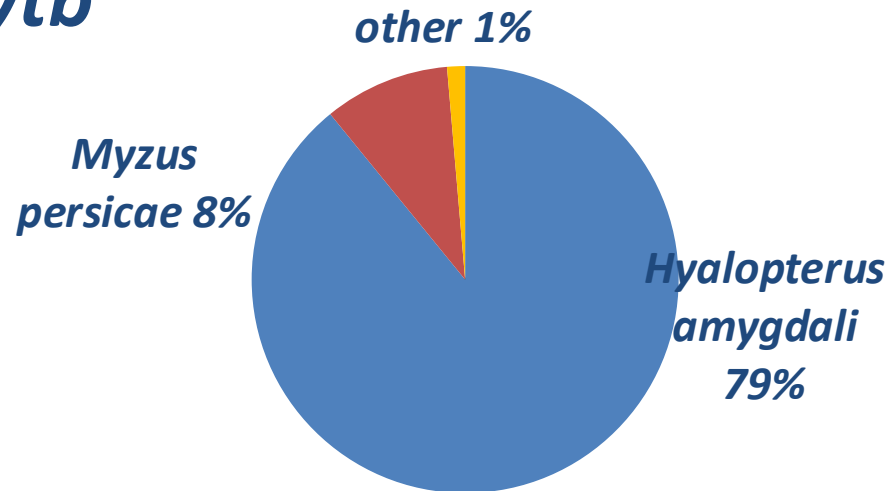
Results – Carob honey, Aegean region, Turkey

coxI

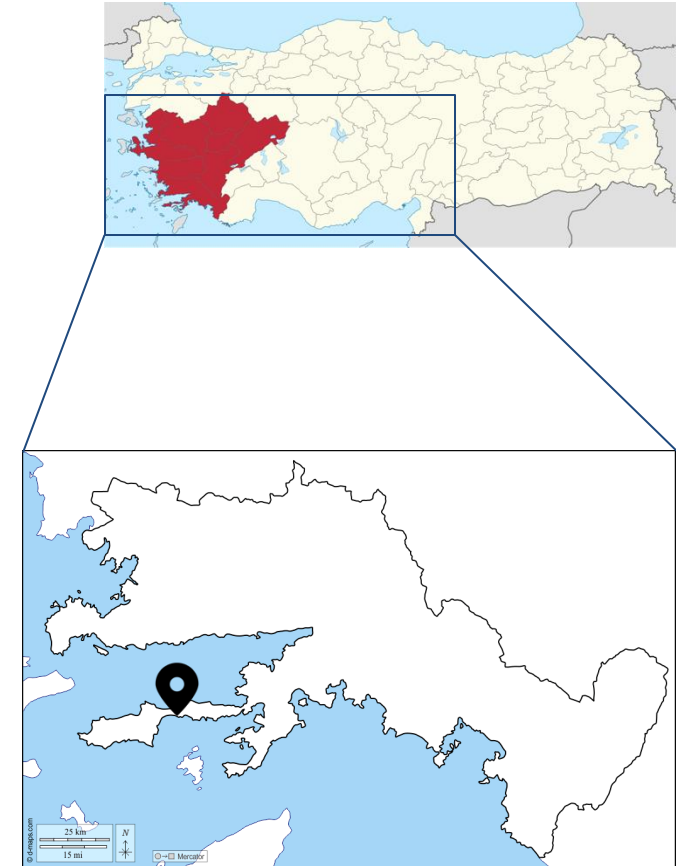


■ *Thelaxes suberi* ■ *Cinara cedri* ■ other

cytb

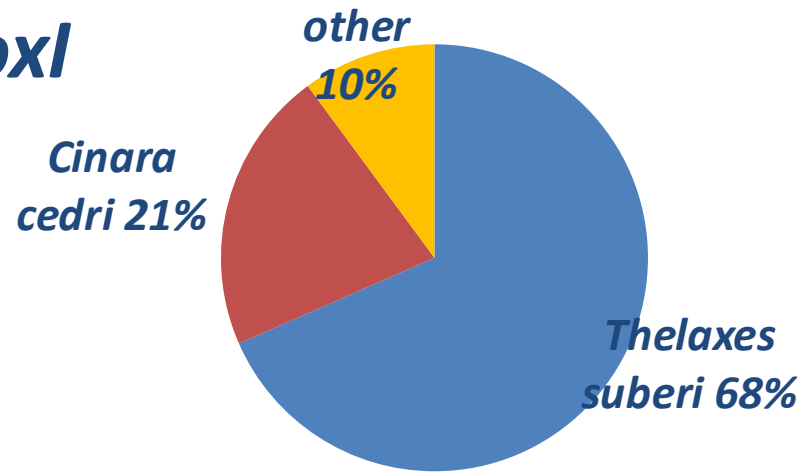


■ *Hyalopterus amygdali* ■ *Myzus persicae* ■ other



Results – Carob honey, Aegean region, Turkey

coxI



■ *Thelaxes suberi* ■ *Cinara cedri* ■ other



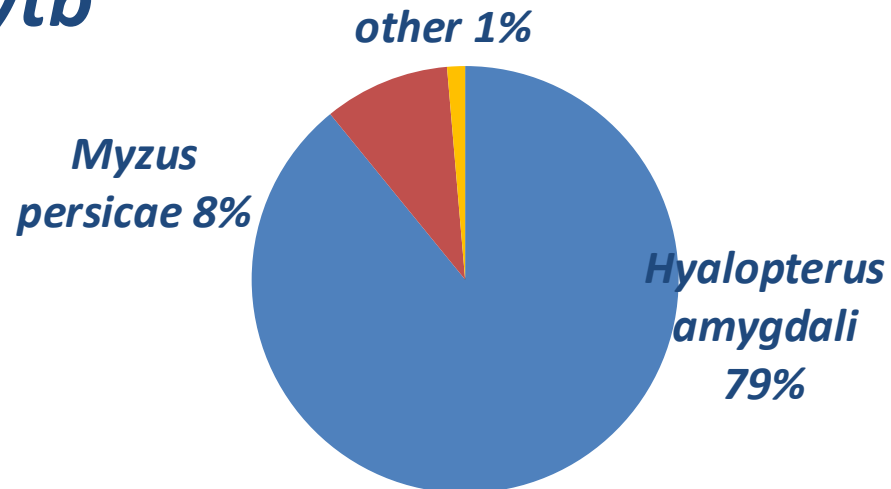
Cinara cedri



Thelaxes suberi
Oak aphid



cyt b



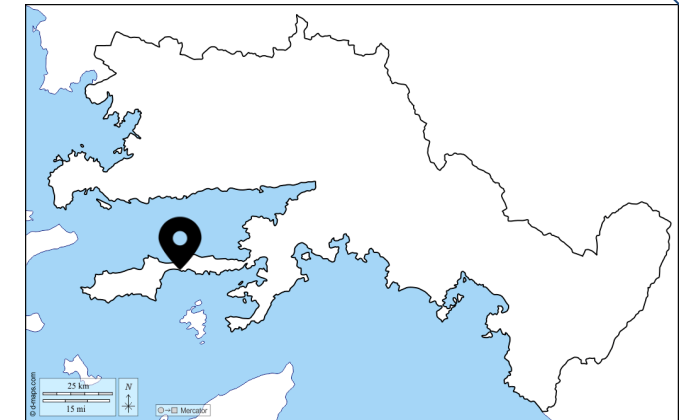
■ *Hyalopterus amygdali* ■ *Myzus persicae* ■ other



Hyalopterus amygdali
almond

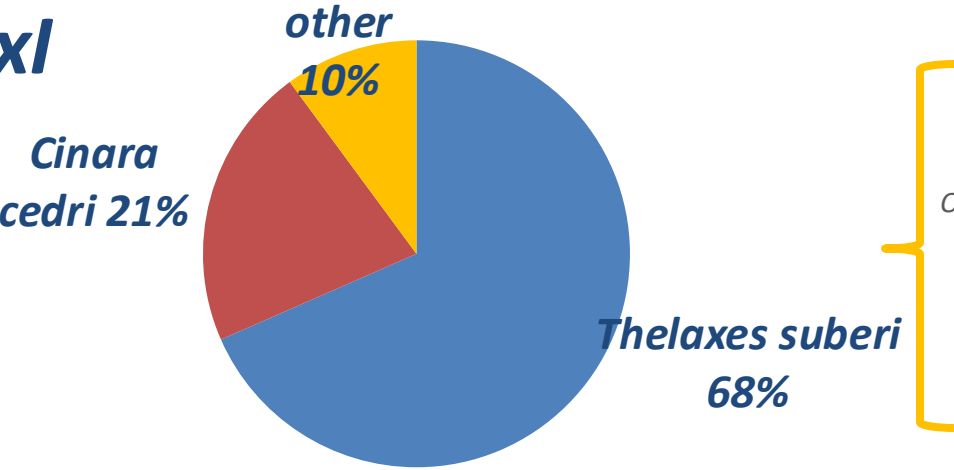


Myzus persicae
Green peach aphid

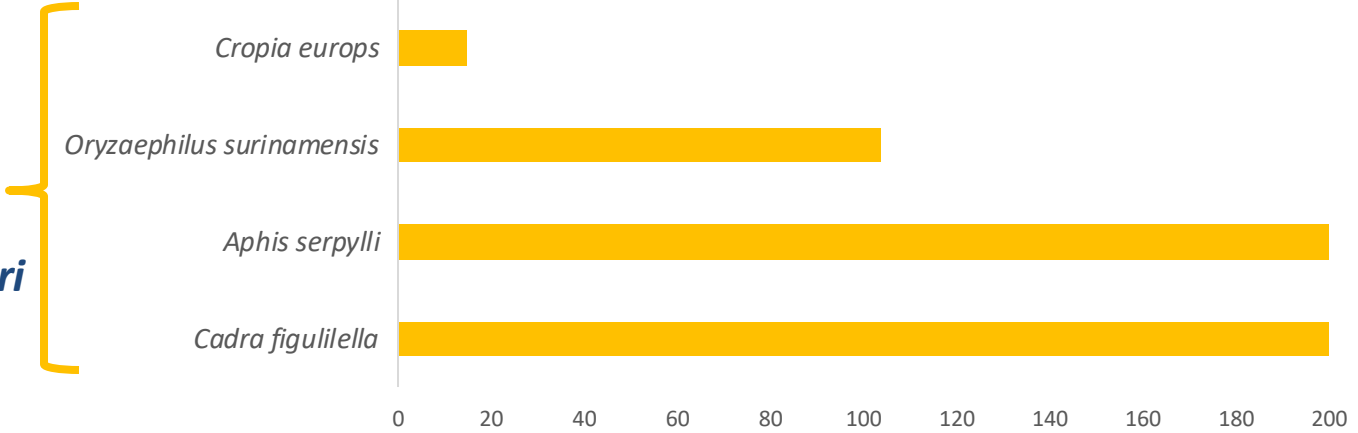


Results – Carob honey, Aegean region, Turkey

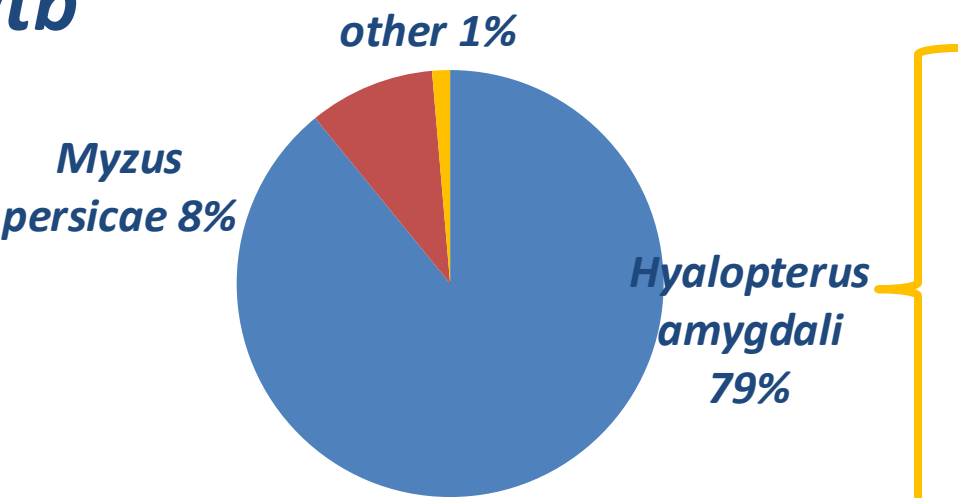
coxI



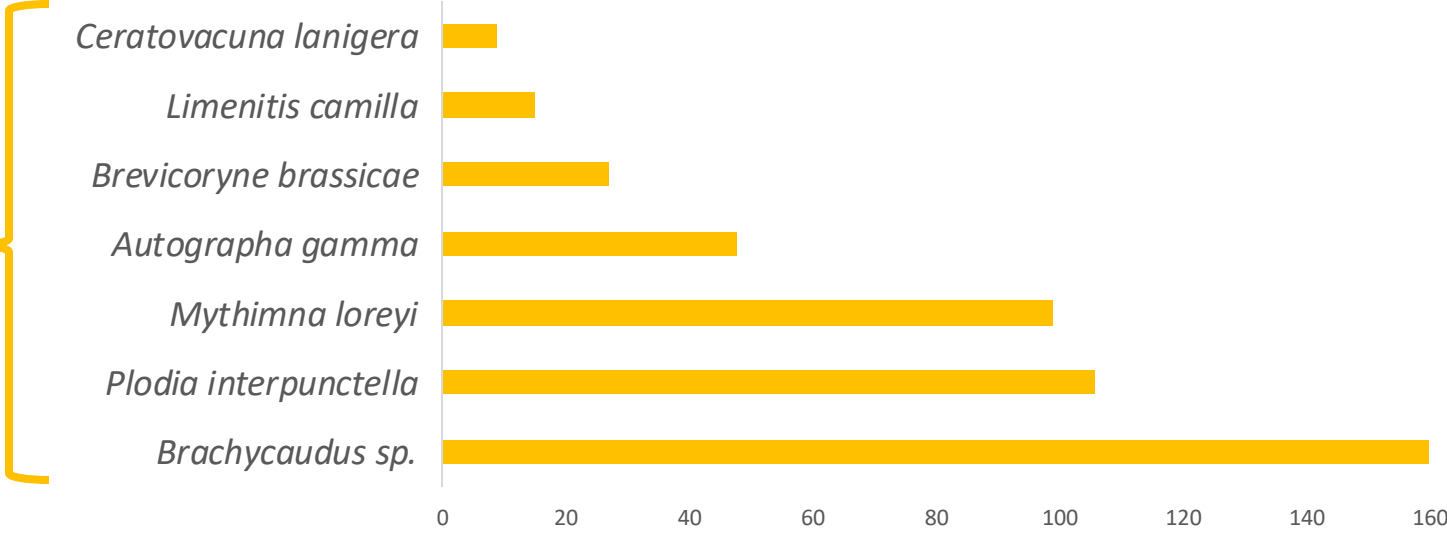
■ *Thelaxes suberi* ■ *Cinara cedri* ■ other



cytb

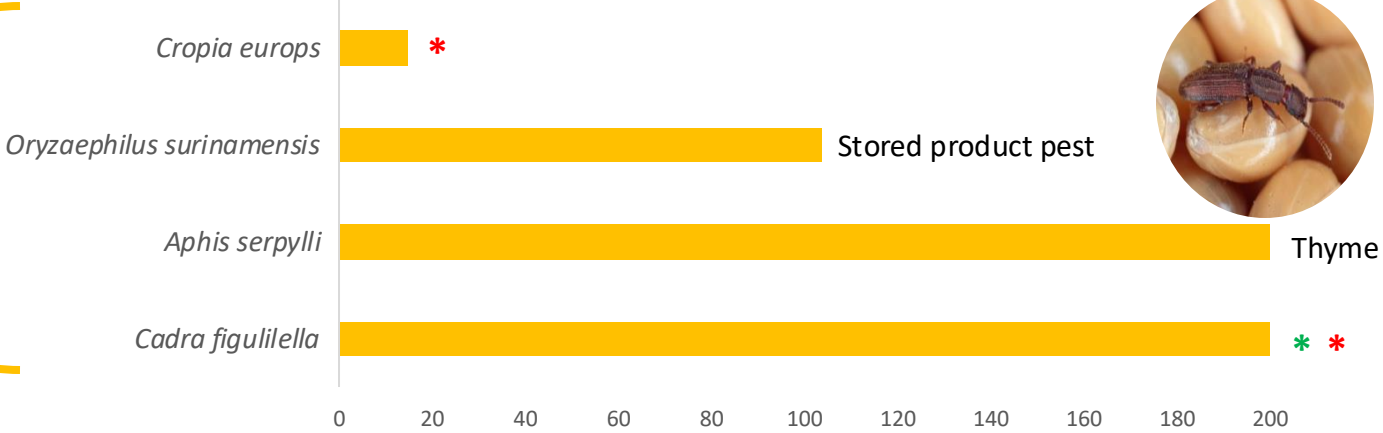
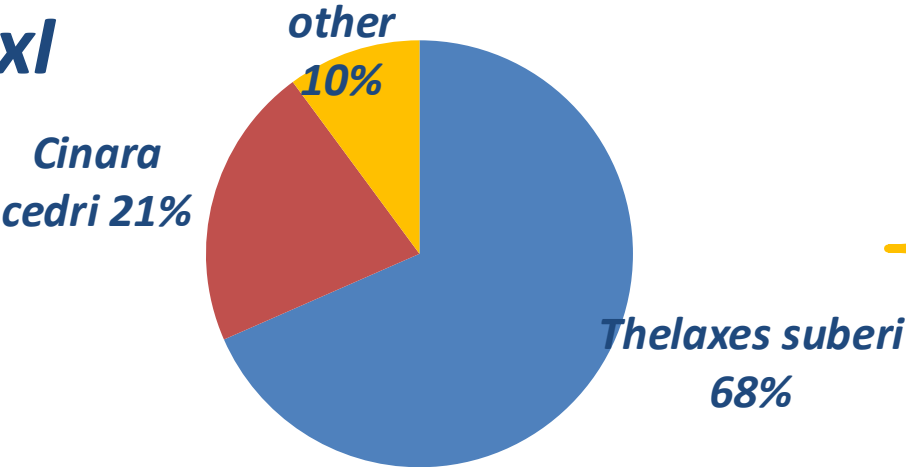


■ *Hyalopterus amygdali* ■ *Myzus persicae* ■ other

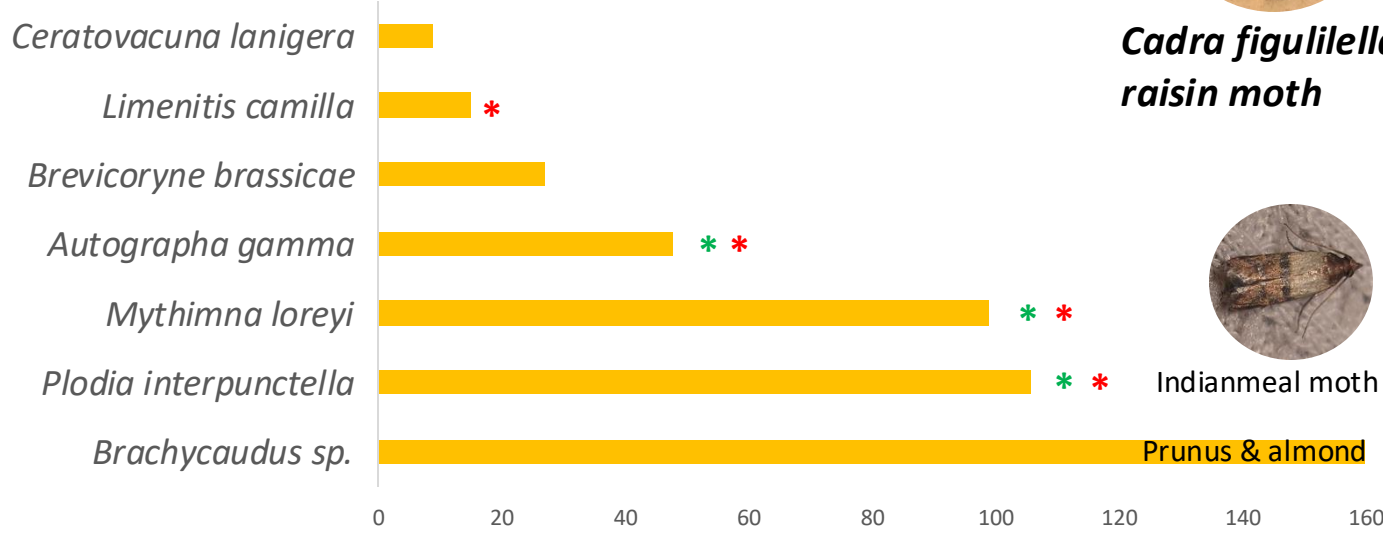
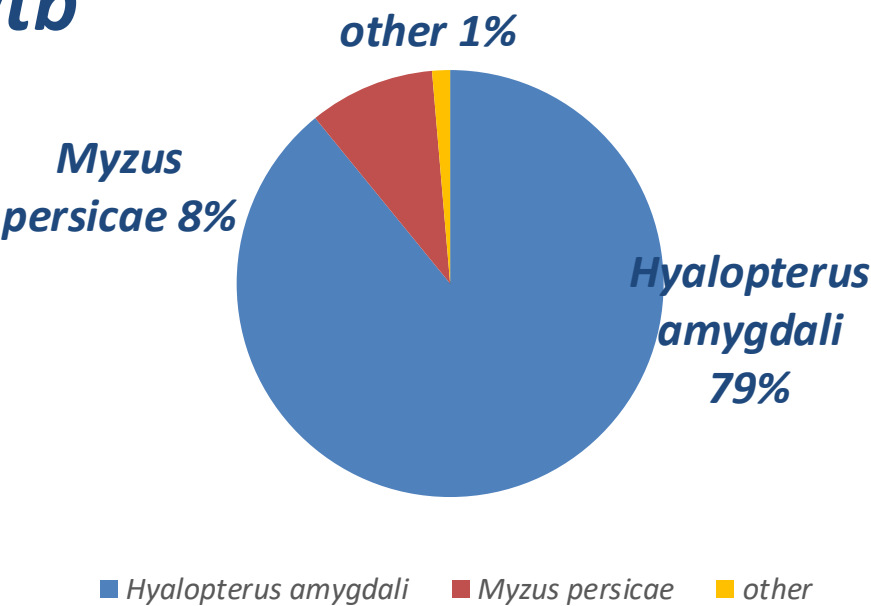


Results – Carob honey, Aegean region, Turkey

coxI



cytb



Conclusions

Indirectly indicating the geographic origin of the honey



Aphis pomi
Green apple Aphid



Cinara pectinatae
Silver fir Aphid



Lachnus roboris
Oak/Chestnut



Aphis aurantii
Citrus



Conclusions

- The combination of honey bee foraging ability, environmental DNA in honey, and advanced sequencing technologies provides powerful insights into honey authentication.
- This study demonstrates the effectiveness of an aphid-based eDNA metabarcoding approach, which can be further strengthened by integrating complementary botanical data to verify the geographical origin of honey.

Animal and Food Genomics Group



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Junior Assistant Professor



Francesca Bertolini
Associate Professor



Maria Letizia Calabri
PhD Student
Molecular genetics



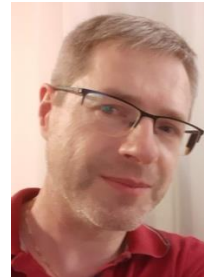
Matteo Bolner
PostDoc
Bioinformatics



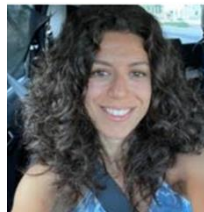
Giuseppina Schiavo
Junior Assistant Professor



Samuele Bovo
Junior Assistant Professor



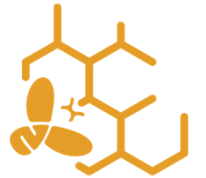
Luca Fontanesi
Full Professor



Dalal Sami
Lab Technician



Valerio Joe Utzeri
Post-Doc
External collaborator



**BeSafe
BeeHoney**

CA22105

www.besafebeehoney.eu



ItaHoneyDNA



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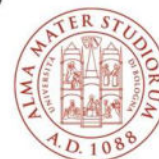
11th European Conference of Apidology

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

<https://www.eurbee2026.com/>



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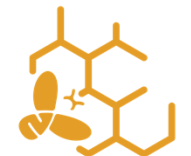
Entomological signatures in honey: combining metabarcoding approaches to provide insights into plant-sucking insects that populate agricultural and forest landscapes

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