OP-115 [Bee Health]
The impact of pollen diet and microbiome on honey bee health

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In the last decade, drought has been identified as one of the most relevant climate change factors affecting ecosystem integrity across countries, affecting plant growth in agroecosystems, and leading to changes in the trophic potential of nectar and pollen. As a cascade effect, a deficit in the nutritional composition and availability of pollen can weaken pollinators, triggering additional threats to ecosystem stability. In this scenario, understanding the impact of trophic sources on honey bee health remains a significant gap that needs to be addressed. Our research aims to correlate pollen of different botanical and geographical origins, and therefore of different trophic potential, with selected honey bee markers: the abundance of core gut microbial taxa and proteins involved in the immune response detectable in the haemolymph. A holistic proteomic approach based on MALDI BeeTyping® and SDS-PAGE profiles, together with qPCR for the quantification of target microorganisms, was used to elucidate these interactions in bees fed with pollen of different botanical families. Briefly, newly-emerged caged honeybees were daily fed with a fresh suspension of pollen and sugar syrup (1:1 w/v) for a total of 40 monofloral and 13 polyfloral pollens belonging to 8 botanical families (Cistaceae, Asteraceae, Cornaceae, Boraginaceae, Fagaceae, Leguminosae, Rosaceae and Salicaceae) collected in 5 Italian regions. After 5 days, haemolymph and guts were extracted for the MALDI-BeeTyping, SDS-PAGE, and qPCR analysis after DNA extraction. Our results show that different pollens significantly affect the core gut microbiome composition but do not significantly affect the concentration and the total amount of small and large haemolymph proteins. Furthermore, the effect of different diets on the microbiome suggests an indirect effect on the immune system response by modulating and influencing the synthesis of some immune-related peptides. This research confirms the importance of gut symbionts in honey bee health also in a changing diet scenario. Finally, our results represent the first step in the understanding on the honeybees response to climate changes.

The impact of pollen diet and microbiome on honey bee health

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Frequently occurring (sad) situation

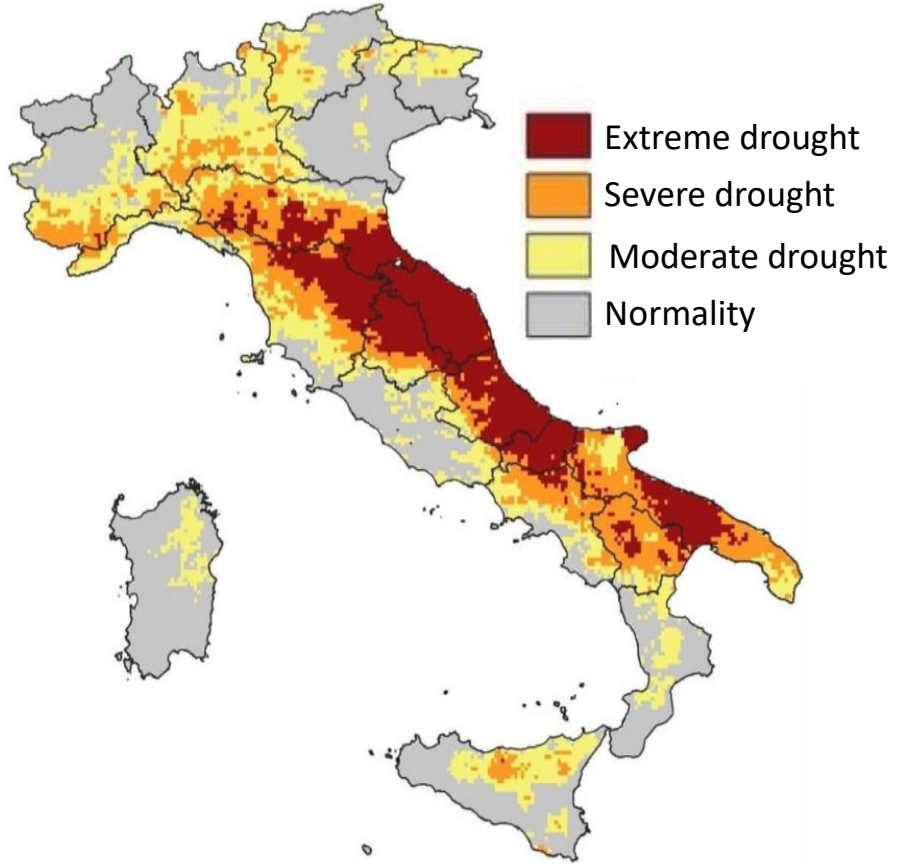
Extreme drought situation

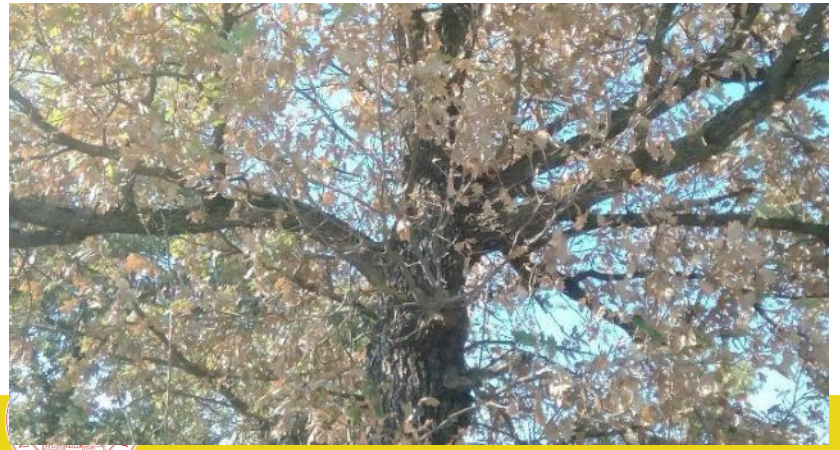
↓

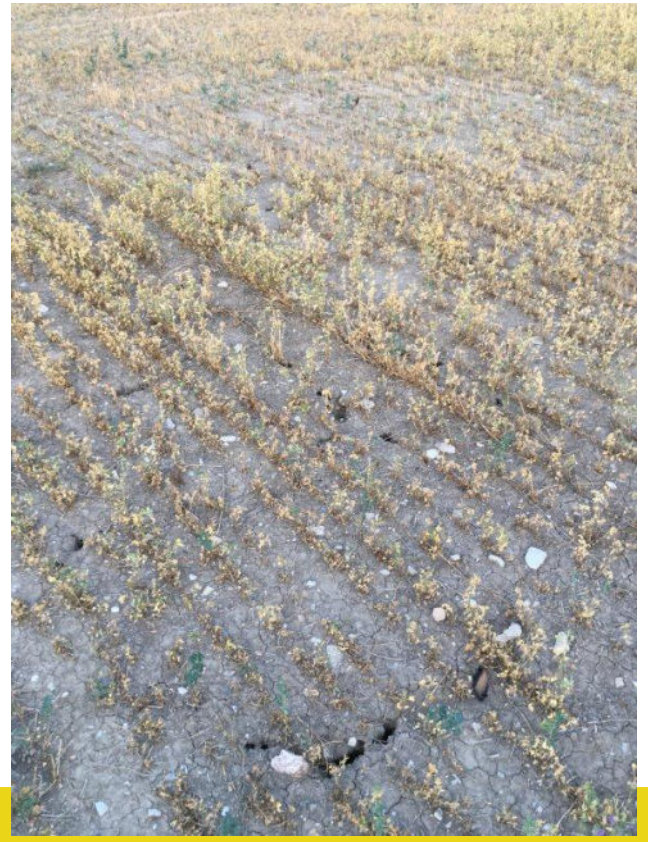
Poor health plant status

↓

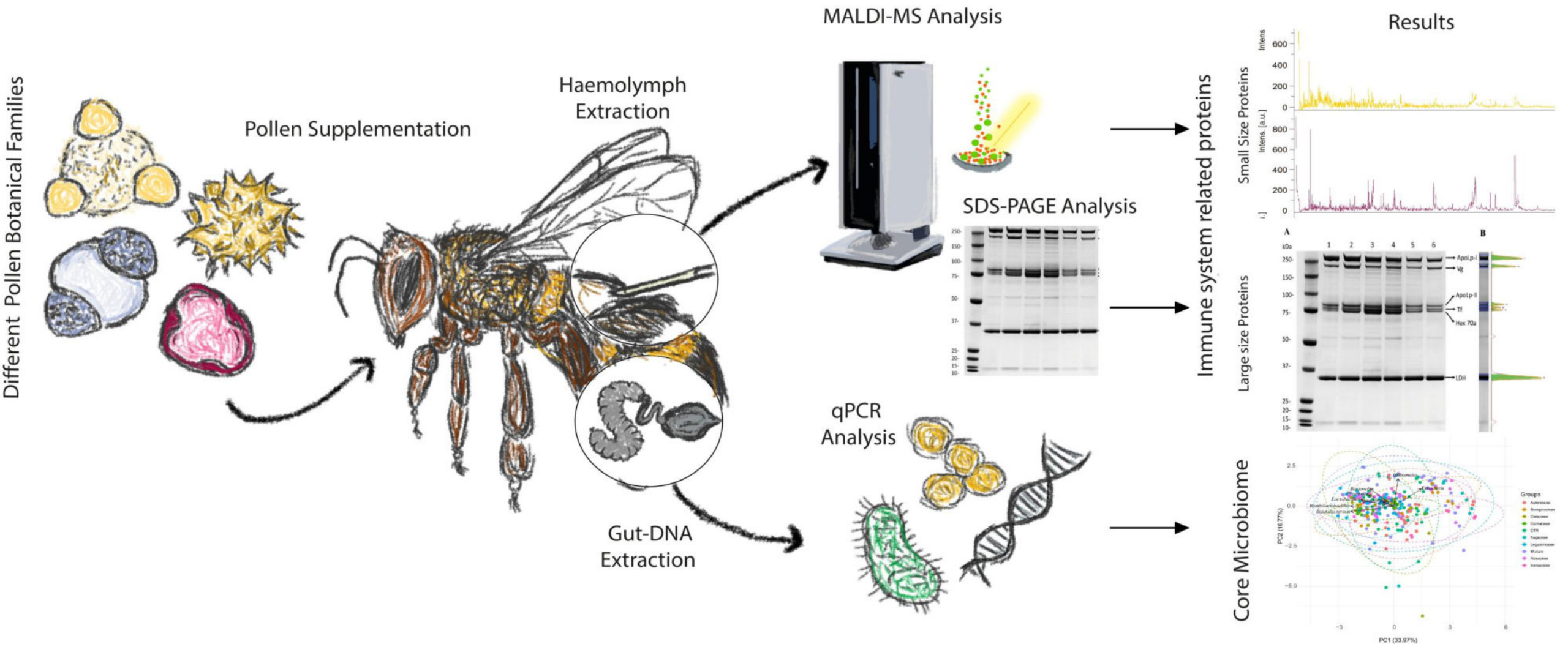
Pollen and nectar low quality
(when available!!)







Concept


Different Pollen Botanical Families
Pollen Supplementation
Haemolymph Extraction
Gut-DNA Extraction
MALDI-MS Analysis
SDS-PAGE Analysis
qPCR Analysis
Immune system related proteins
Results
Small Size Proteins
Large size Proteins
Core Microbiome

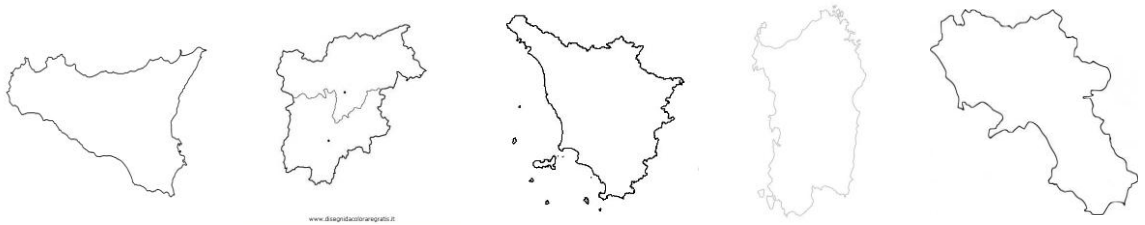
Pollen collection

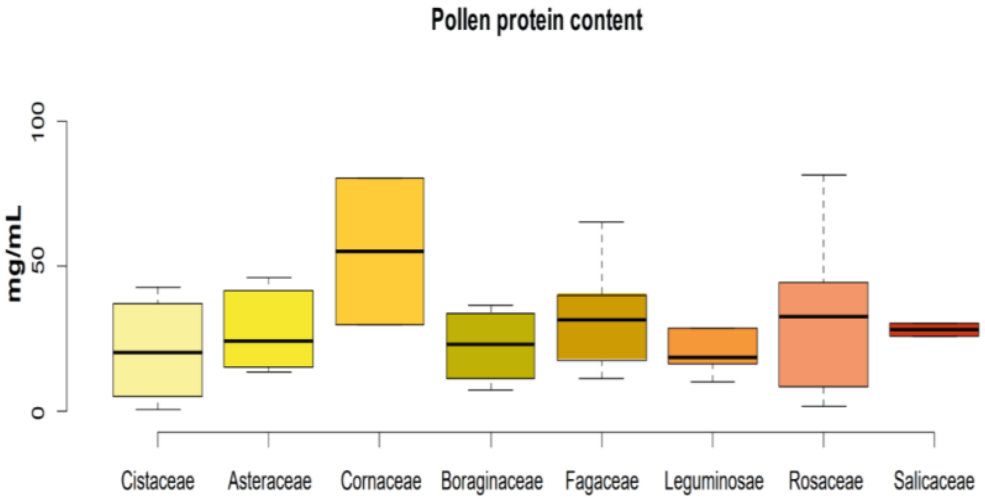
26 pollen species among
8 botanical families

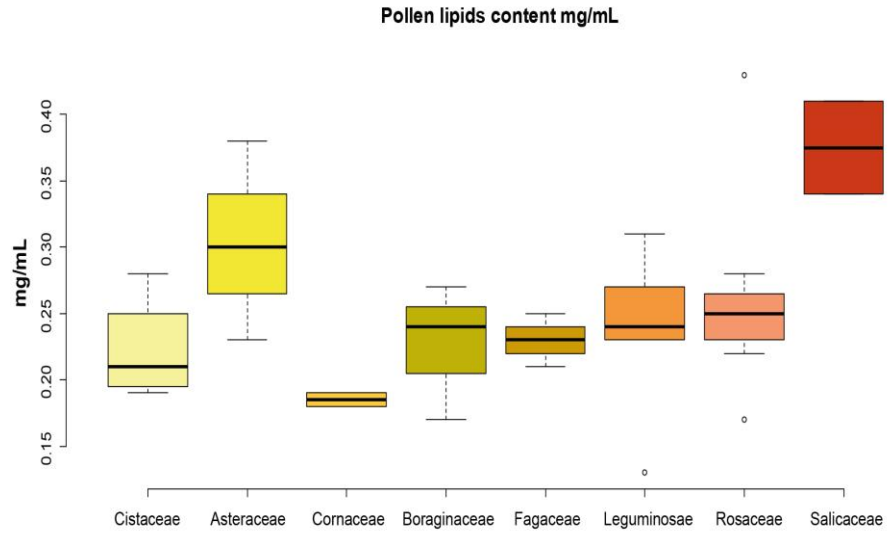
+

13 pollen mixture

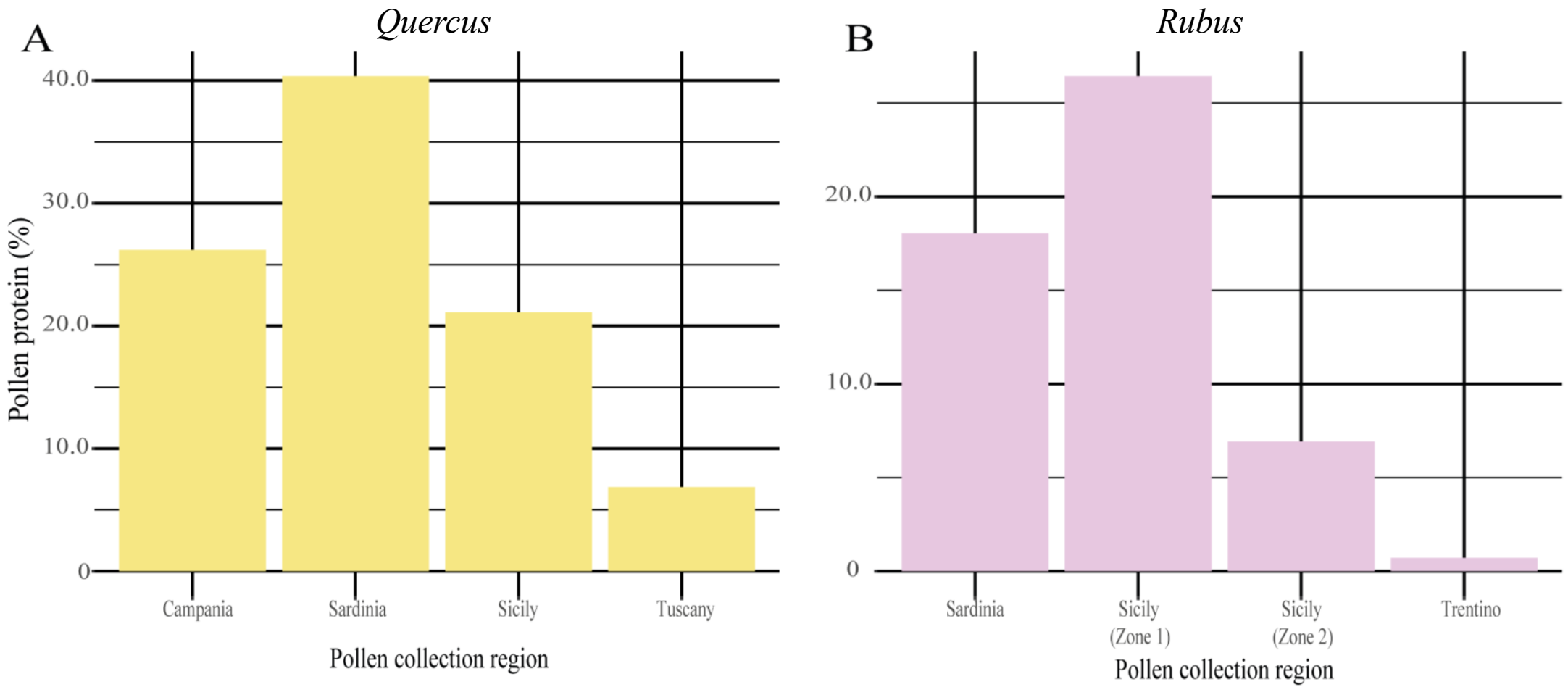
Botanical Family	Botanical Genera
Asteraceae	*Centaurea* sp., compositae T and S
Boraginaceae	*Borago* sp. and *Echium* sp.
Cistaceae	*Cistus* sp. and *Helianthemum* sp.
Cornaceae	*Cornus* sp.
Fagaceae	*Quercus* sp. and *Castanea* sp.
Leguminosae	*Hedysarum* sp., *Genista* sp., and *Trifolium* sp.
Rosaceae	*Rubus* sp., *Crataegus* sp., *Malus* sp., *Pyrus* sp., and *Sorbus* sp.
Salicaceae	*Salix* sp.







Difference in pollen protein content among same botanical families



Honey bee cage tests

26 pollen species among
8 botanical families

+

13 pollen mixture

Botanical Family	Botanical Genera
Asteraceae	*Centaurea* sp., compositae T and S
Boraginaceae	*Borago* sp. and *Echium* sp.
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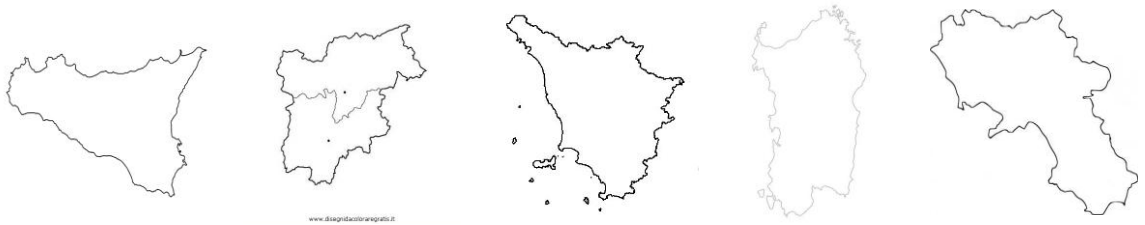
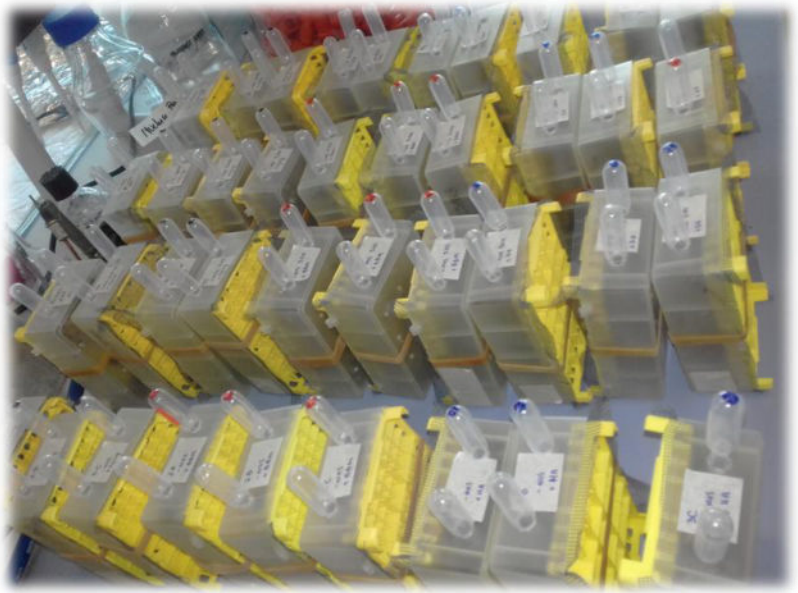
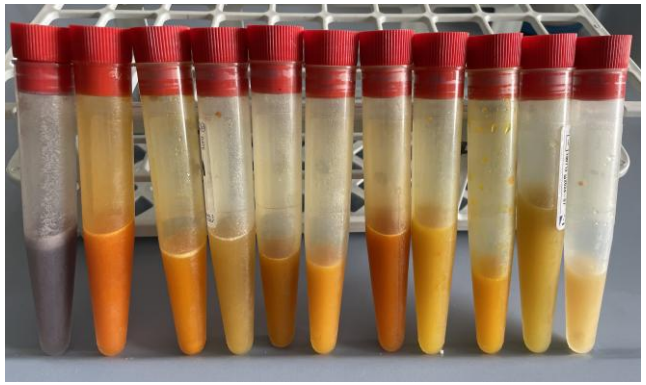
Salicaceae	*Salix* sp.



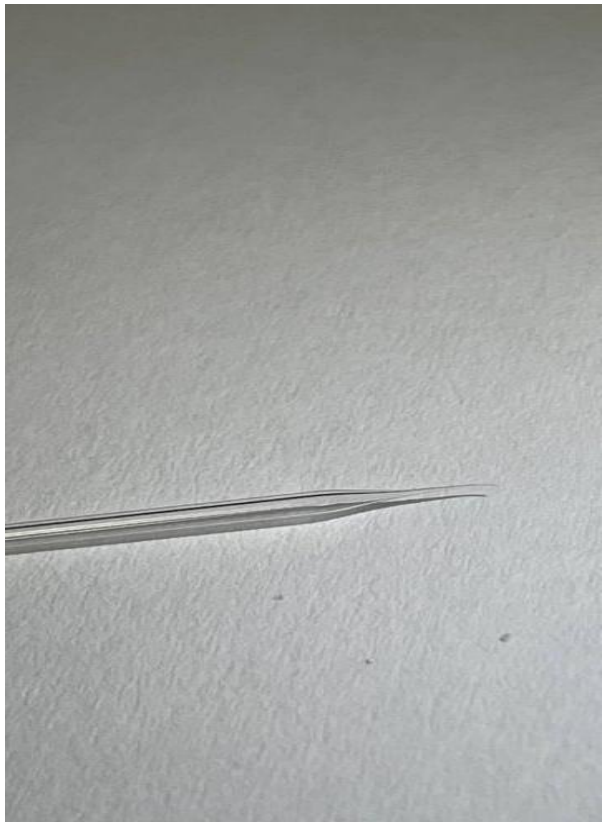
3 cages *x* 50 honey bees/pollen tested



1 g of pollen/ml



Honey bee haemolymph extraction

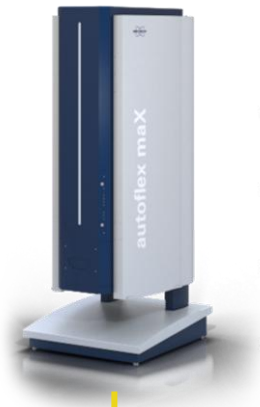


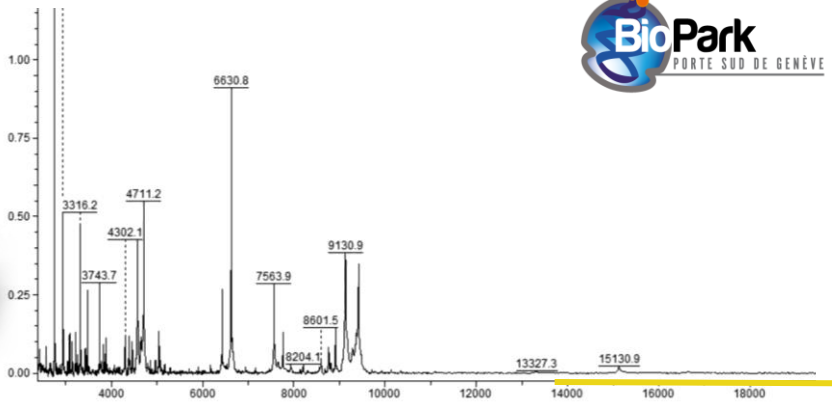
Materials & Methods: tools for haemolymph analysis

Small-size proteins

Matrix-Assisted Laser Desorption/Ionization Imaging – Mass Spectrometry

(MALDI-MS)





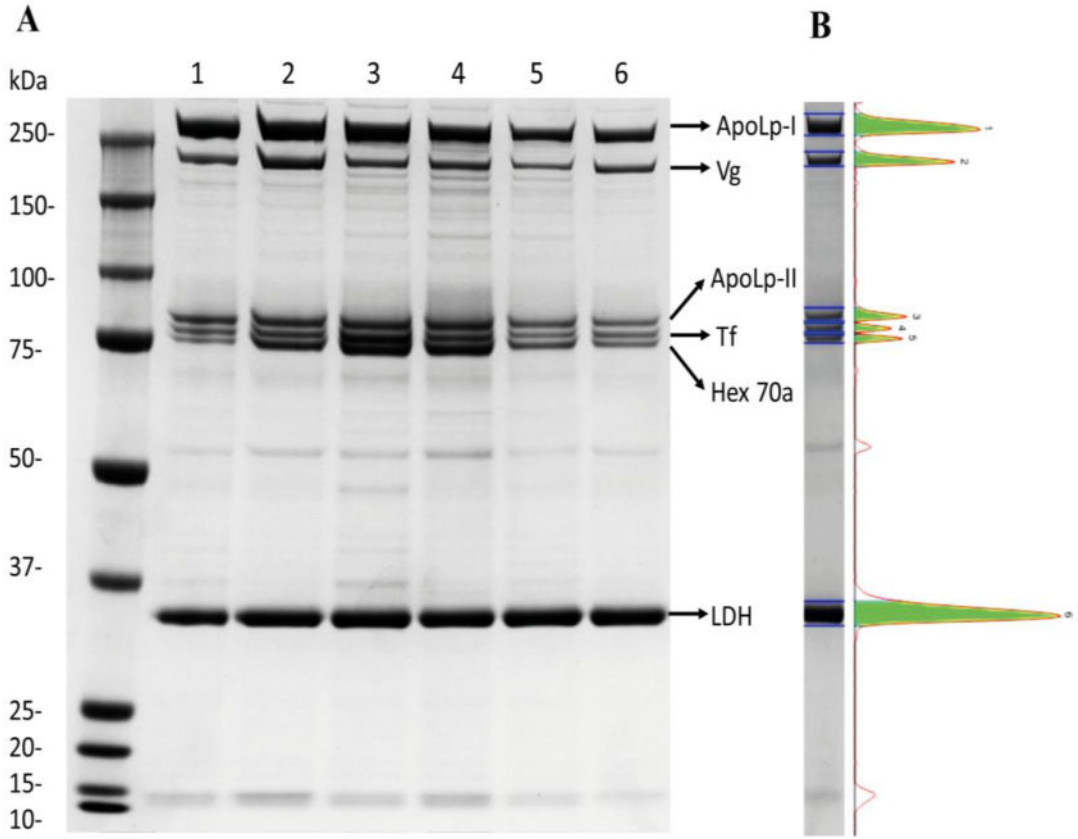
- Abaecin
- Apidaecin
- Defensin
- Hymenoptaecin

- Vitellogenin (Vg)
  (and others..)

Large-size proteins

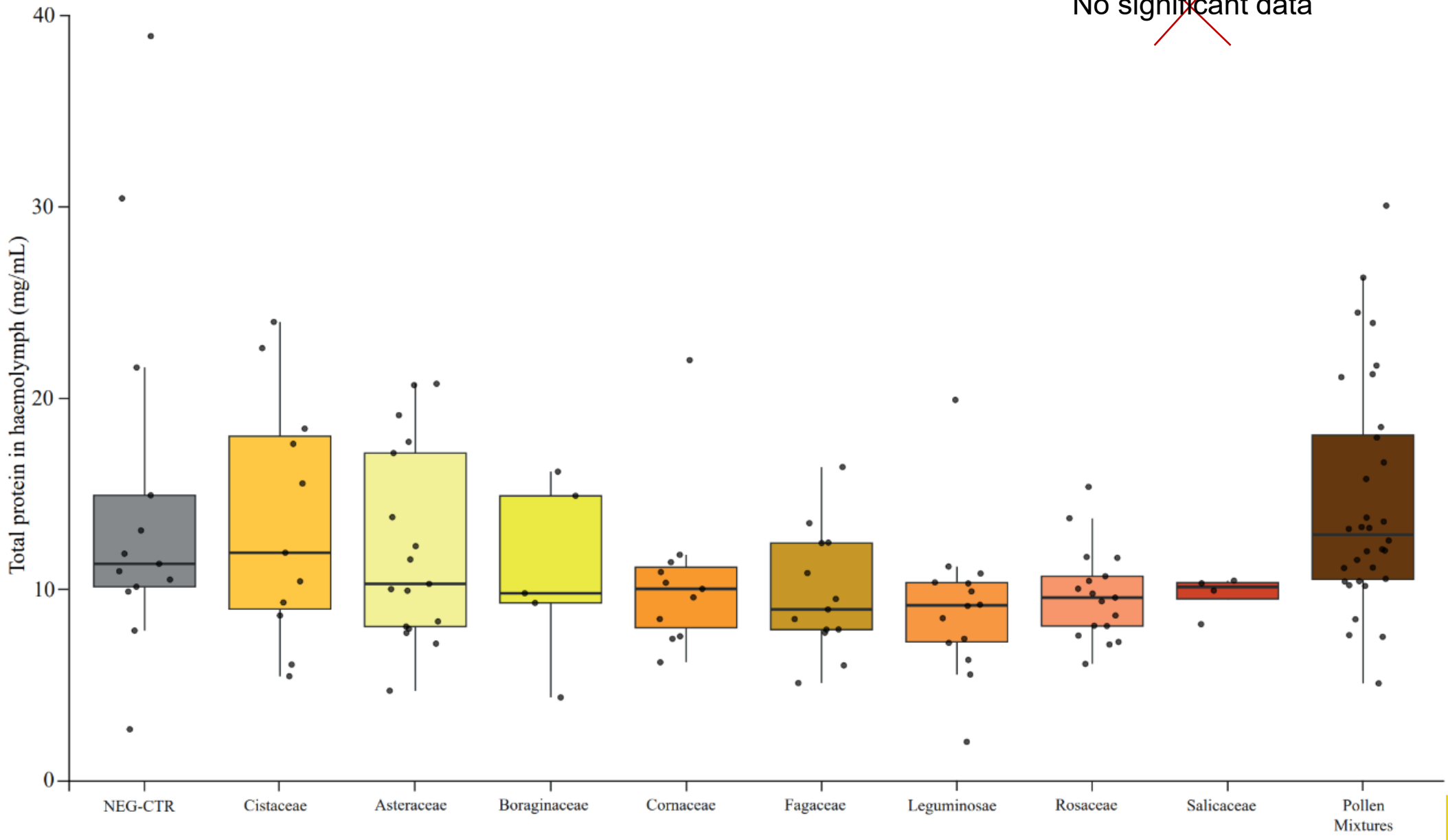
Sodium Dodecyl Sulphate PolyAcrylamide Gel Electrophoresis

(SDS-PAGE)



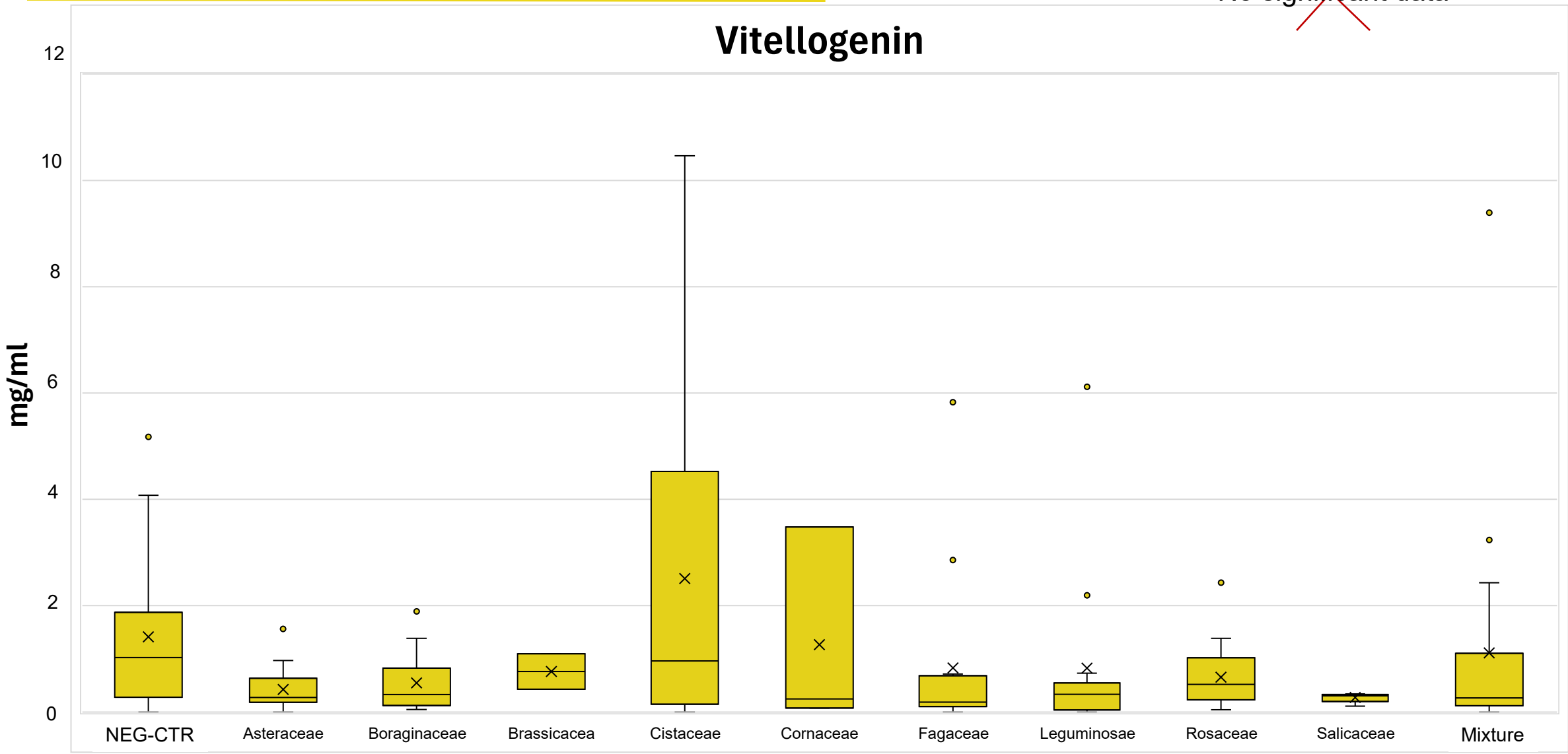
Total proteins in haemolymph

No significant data



SDS-PAGE results on Large-size proteins

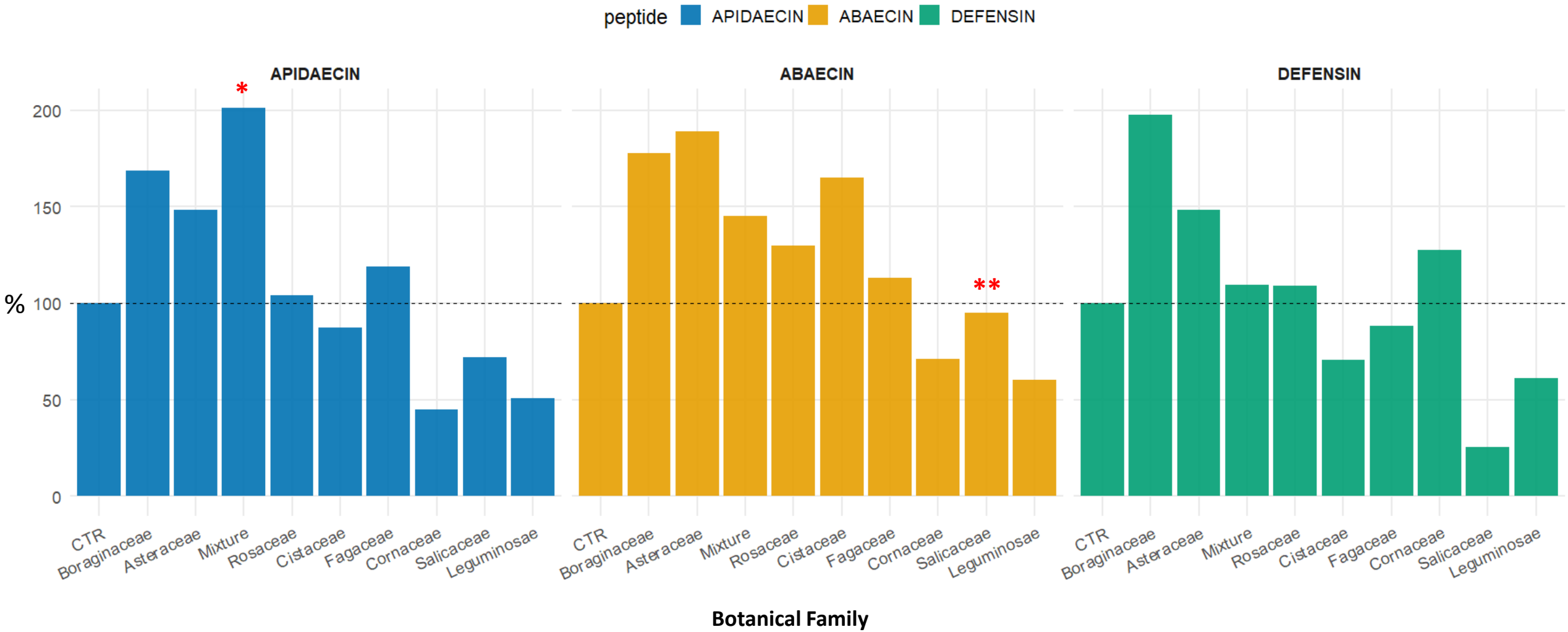
No significant data



MALDI-MS results on Small-size proteins

~~Hymenoptaecin~~

Relative % compared to CTR



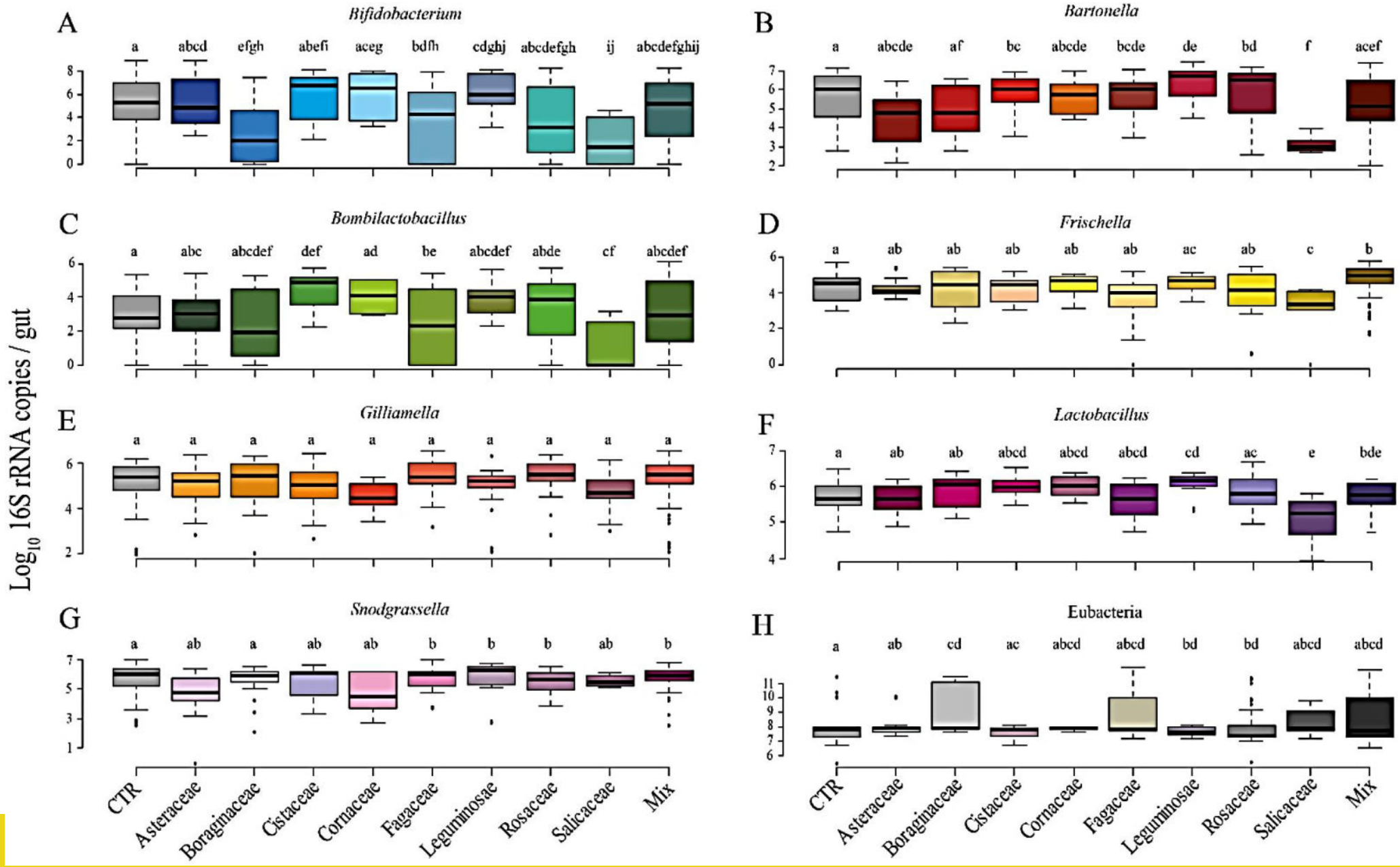
Materials & Methods: gut microbiome analysis

Gut microbiome core taxa

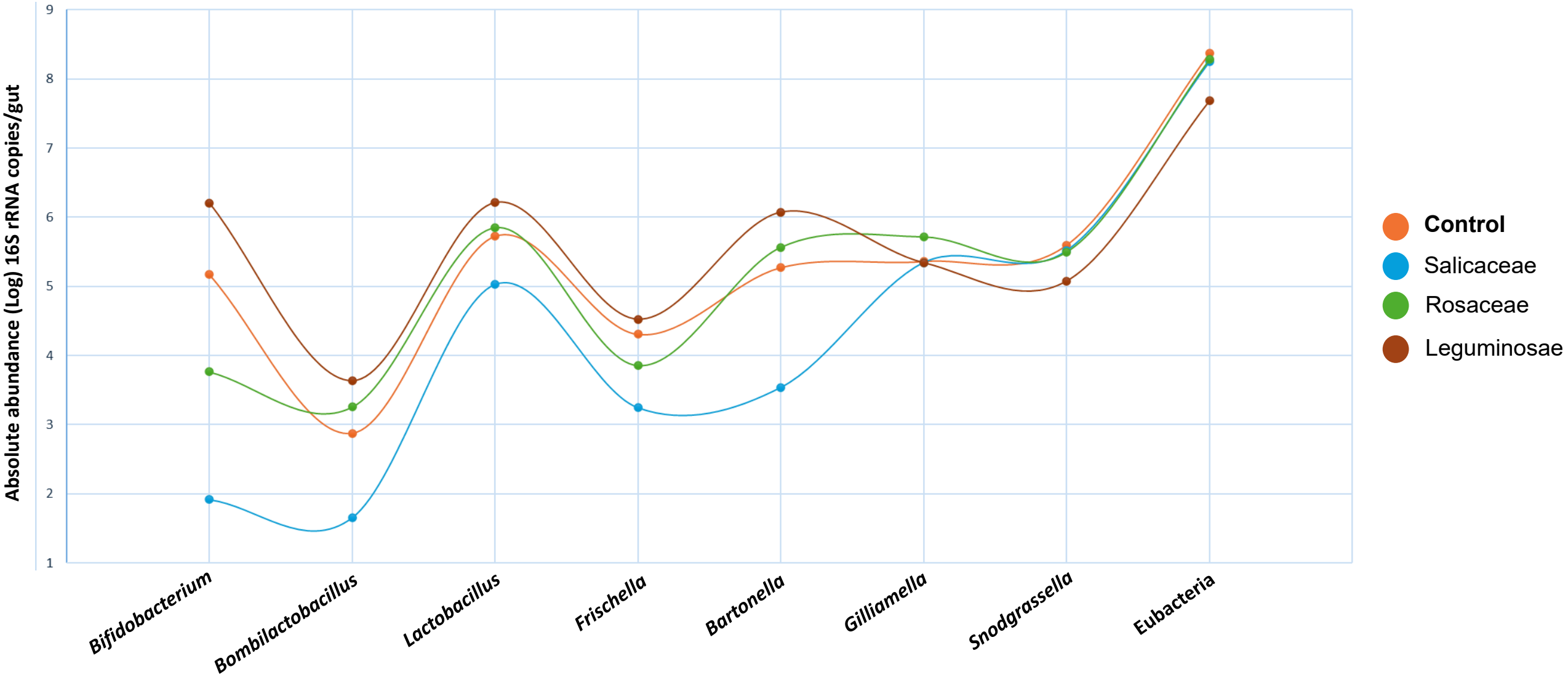
Bifidobacterium
Bombilactobacillus
Lactobacillus
Frischella
Bartonella
Gilliamella
Snodgrassella

⊕ Eubacteria

Effect of pollen source on gut microbiome population

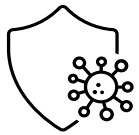


qPCR results on gut microbiome population



Linear Mixed-Effect Model results

Gut microbiome



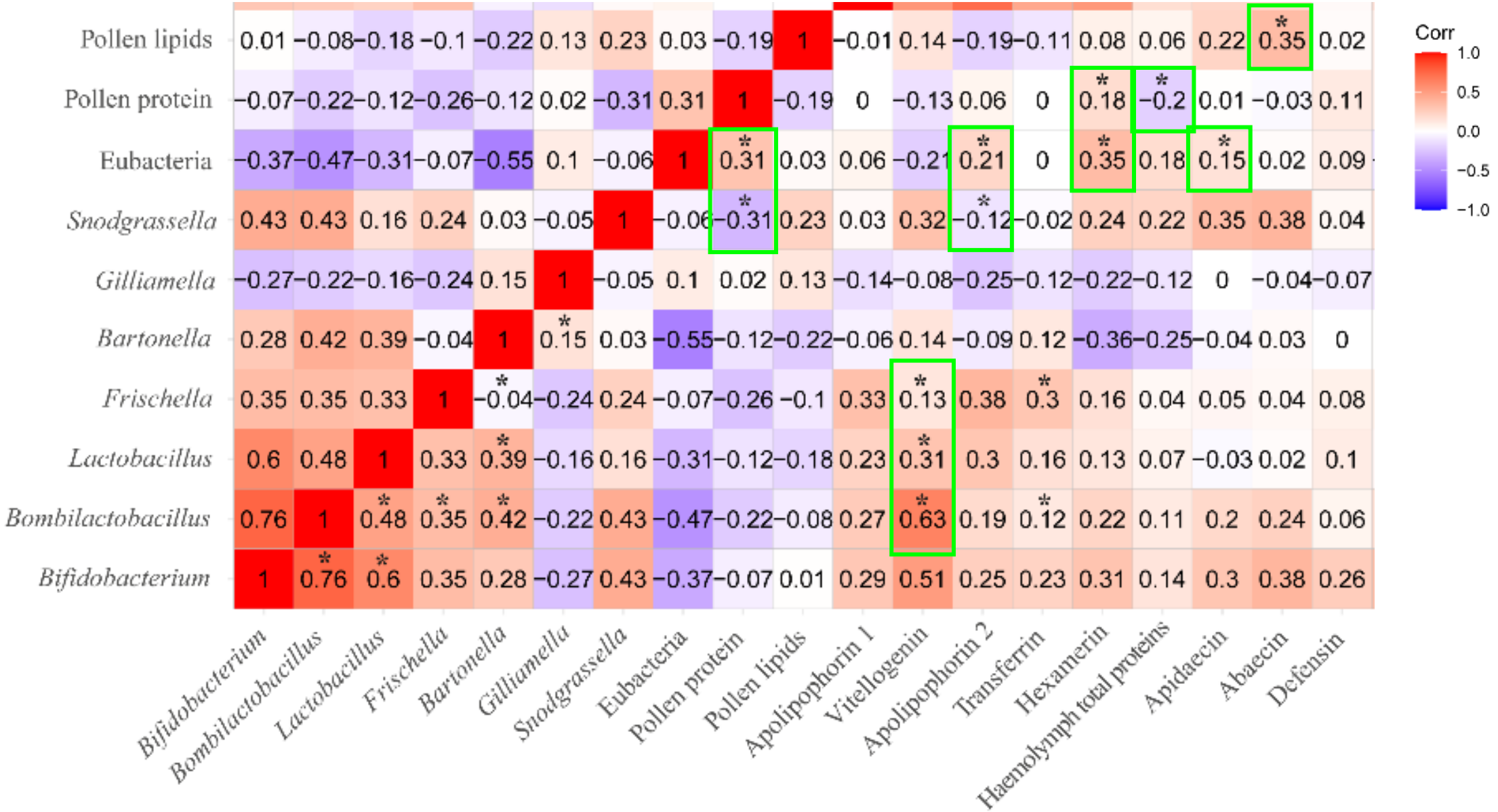
IMMUNE SYSTEM

Large-size proteins

Small-size proteins

	All treatment vs. all treatment
	Botanical family
Bifidobacterium	**1.15E-05**
Bombilactobacillus	**0.019**
Lactobacillus	**1.97E-07**
Frischella	**0.003**
Bartonella	**0.0001**
Gilliamella	0.700
Snodgrassella	**0.012**
Eubacteria	0.093
Apolipophorin-I	**0.044**
Vitellogenin	0.948
Apolipophorin-II	**0.0006**
Transferrin	0.113
Hexamerin	**0.043**
Total protein hemolymph	0.083
Apidaecin	**0.0001**
Abaecin	**0.025**
Defensin	0.217
AMCI-1	0.191
AMCI-2	0.232

Correlation between gut microbiome population and AMPs

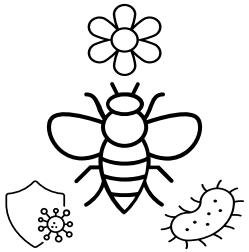


Conclusions

Pollens botanical family do not affect the total amount of haemolymph proteins

Pollens botanical family significantly impact the core microbiome composition

Core microbial taxa correlate with the synthesis of immune system peptides



Trophic sources indirectly affect the immune system through the gut microbiome

Thank you for your attention

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