

**Fingerprinting chemical markers in the Mediterranean Orange Blossom Honey:
UHPLC-HRMS metabolomics study integrating melissopalynological analysis, GC-MS
and HPLC-PDA-ESI/MS**

Figure S1: Sampling locations of the PLANT-B case studies in Greece (Argolis, Peloponnese) Egypt (Al Shaeir Island), Italy (Sicily) (Images obtained from Google Earth)

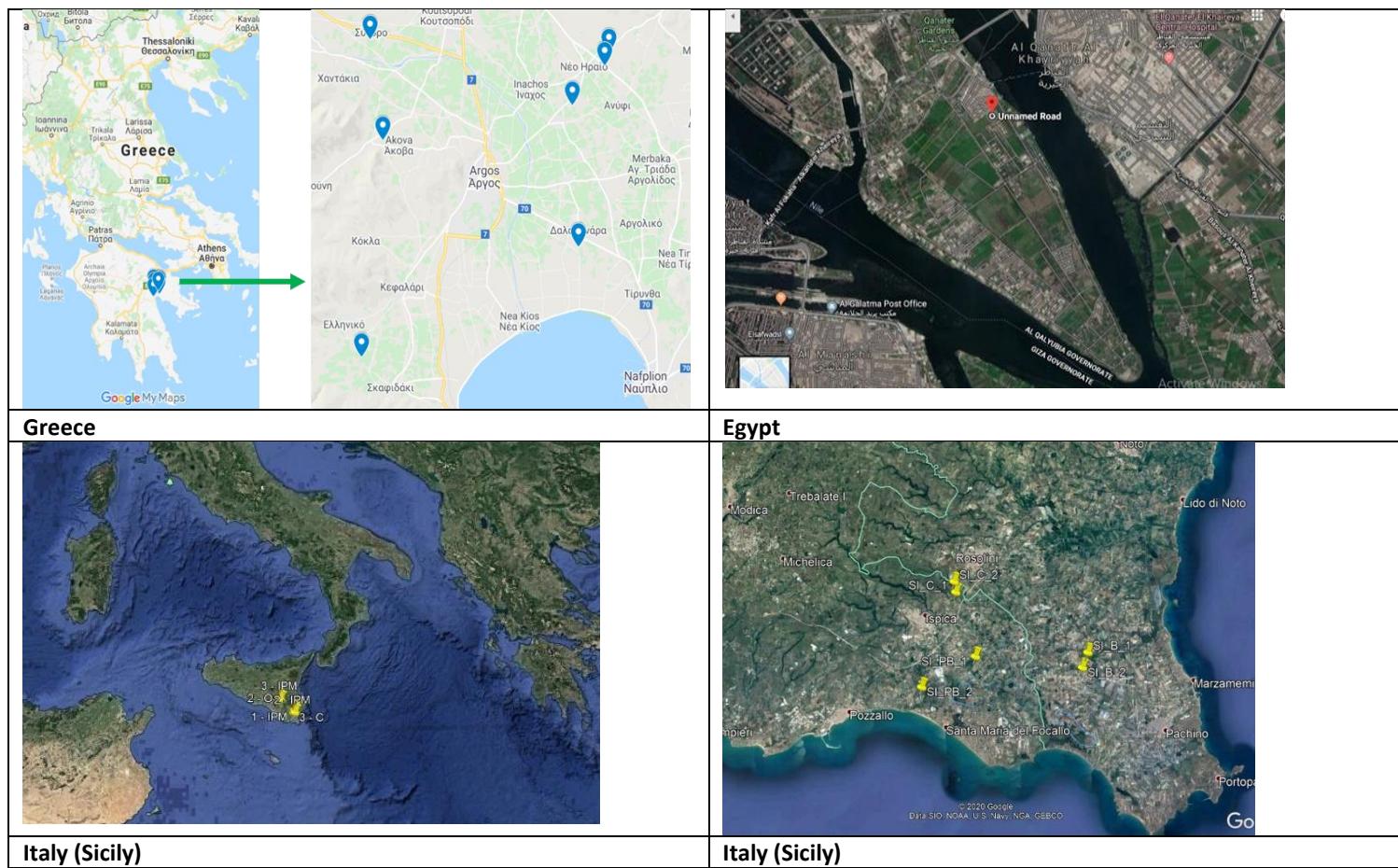


Table S1A: Egyptian case study honey samples

Samples	Crop	Date (m/y)	Place
Honey	Basil+ Citrus	5/ 2020	Al Shaeir Island
Honey	Citrus	5/ 2020	Al Shaeir Island

Honey	Clover	6/ 2020	Al Shaeir Island
Honey (Control)	Clover	6/ 2020	Al Shaeir Island
Honey (Citrus – Control)	Citrus	5/ 2020	Al Shaeir Island
Honey	Basil	2/ 2020	Al Shaeir Island
Honey	Basil+ <i>Borag+Coeiandrum+Anethum+Carum</i>	6/ 2020	Al Shaeir Island
Honey (AMP - Control)	AMPs	6/ 2020	Al Shaeir Island

Table S1B: Italian case study honey samples

HONEY	CITRUS	Chiaramonte Gulfi (RG)	SICILY
HONEY	CITRUS	Chiaramonte Gulfi (RG)	SICILY
HONEYCOMB HONEY	CITRUS	Ispica (RG)	SICILY
HONEYCOMB HONEY	CITRUS	Ispica (RG)	SICILY
HONEYCOMB HONEY	CITRUS	Ispica (RG)	SICILY
HONEYCOMB HONEY	CITRUS	Ispica (RG)	SICILY
HONEYCOMB HONEY	CITRUS	Ispica (RG)	SICILY
HONEYCOMB HONEY	CITRUS	Chiaramonte Gulfi (RG)	SICILY

Table S1C: Greek case study honey samples and exact location

PLANT-B Case study fields (all citrus)	Lat	Long	Sample Number
PLANT-B - Rosemary	37,671857	22,782551	1
BIO Rosemary	37,676275	22,784508	2
PLANT-B - Savory	37,658812	22,768095	3
PLANT-B - Oregano	37,612032	22,770833	4
PLANT-B - Sage	37,57559	22,6732	5
CONTROL - 2	37,647203	22,682517	6
CONTROL - 1	37,680646	22,676654	7

Table S2. Flavonoids, phenolic and organic acids quantified by HPLC-PDA-ESI/MS in orange blossom honey from the three countries

Analyte	Mean content µg/g		
	Italy ^a	Greece ^b	Egypt ^c
Apigenin	0.12±0.03	0.10±0.02	0.07±0.01
Quercetin	0.39±0.09	0.34±0.10	0.24±0.07
Galangin	1.21±0.27	1.41±0.15	1.14±0.12
Luteolin	0.21±0.07	0.19±0.05	0.23±0.08

Pinobanksin	2.90 ± 0.25	3.71 ± 0.43	3.05 ± 0.27
Isosakuranetin	2.81 ± 0.24	2.99 ± 0.38	3.09 ± 0.52
Techtochrysin	0.16 ± 0.07	0.18 ± 0.07	0.14 ± 0.05
Protocatechuic acid	10.81 ± 1.09	12.82 ± 2.42	12.24 ± 1.78
Gallic acid	0.08 ± 0.03	0.09 ± 0.02	0.10 ± 0.02
Ferullic acid	1.22 ± 0.28	0.96 ± 0.21	0.65 ± 0.13
<i>trans</i> -cinnamic acid	0.15 ± 0.03	0.22 ± 0.06	0.14 ± 0.04
Syringic acid	12.23 ± 1.31	10.78 ± 0.68	10.01 ± 2.17
p-Coumaric acid	5.38 ± 0.45	6.71 ± 0.71	4.78 ± 0.43
Hesperetin	0.49 ± 0.20	0.19 ± 0.04	0.14 ± 0.04
Hesperidin	1.12 ± 0.16	3.78 ± 0.51	0.71 ± 0.17
Sakuranetin	0.09 ± 0.03	0.05 ± 0.02	0.04 ± 0.01
Chrysin	1.24 ± 0.18	0.98 ± 0.11	0.88 ± 0.09
Naringenin	4.26 ± 0.14	2.96 ± 0.24	0.97 ± 0.06
Pinocembrin	3.38 ± 0.41	2.63 ± 0.27	2.09 ± 0.31
Rutin	5.81 ± 0.35	4.21 ± 0.51	3.72 ± 0.41

a: n=8 , b: n=7, c: n=4

Figure S2. Loading plots of Melissopalynological parameters in the space of the two first principal components

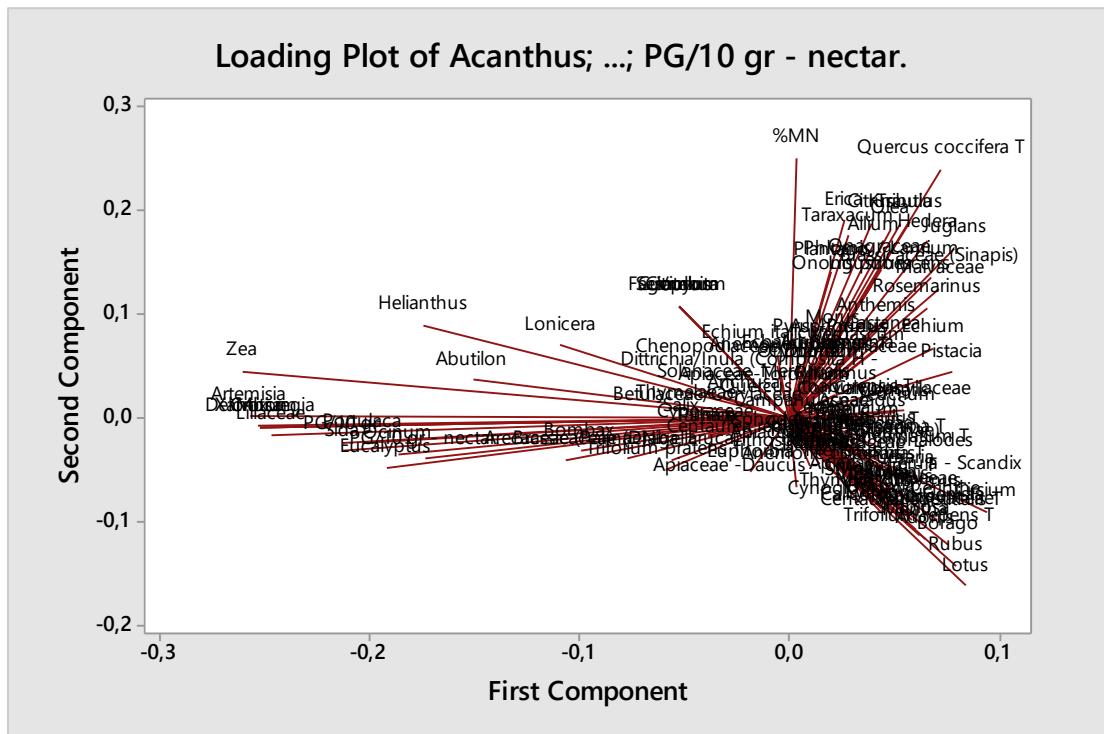


Table S3. The dominating flora during the citrus flowering season of experimental fields at Al-Qanater Al-Khairiya station – Alshaeir Island- Egypt [1,2]

No.	Family	Genus	Species
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1	<u>Rosaceae</u>	<i>Prunus</i>	<i>Prunus persica</i> L.
2	<u>Rosaceae</u>	<i>Prunus</i>	<i>Prunus armeniaca</i> L.
3	<u>Rosaceae</u>	<i>Prunus</i>	<i>Prunus domestica</i> L.
4	<u>Leguminosae</u>	<i>Vicia</i>	<i>Vicia faba</i> L.
5	<u>Leguminosae</u>	<i>Pisum</i>	<i>Pisum sativum</i> L.
6	<u>Boraginaceae</u>	<i>Borago</i>	<i>Borago officinalis</i> L.
7	<u>Lauraceae</u>	<i>Cinnamomum</i>	<i>Cinnamomum camphora</i> L.
8	<u>Oleaceae</u>	<i>Jasminum</i>	<i>Jasminum grandiflorum</i> L.
9	<u>Lamiaceae</u>	<i>Ocimum</i>	<i>Ocimum basilicum</i> L.
10	<u>Compositae</u>	<i>Matricaria</i>	<i>Matricaria chamomilla</i> L.
11	<u>Arecaceae</u>	= <i>Phoenix</i>	<i>Palma dactylifera</i> L.
12	<u>Rosaceae</u>	<i>Fragaria</i>	<i>Fragaria × ananassa</i> Duch
13	<u>Anacardiaceae</u>	<i>Mangifera</i> (<i>Mangifera indica</i> L.
14	<u>Rosaceae</u>	<i>Malus</i>	<i>Malus domestica</i> Borkh.
15	<u>Myrtaceae</u>	<i>Psidium</i>	<i>Psidium guajava</i> L.
16	<u>Umbelliferae/Apiaceae</u>	<i>Petroselinum</i>	<i>Petroselinum crispum</i> Mill.
17	<u>Umbelliferae/Apiaceae</u>	<i>Coriandrum</i>	<i>Coriandrum sativum</i> L.
18	<u>Lythraceae</u>	<i>Punica</i>	<i>Punica granatum</i> L.
19	<u>Myrtaceae</u>	<i>Eucalyptus</i>	<i>Eucalyptus camaldulensis</i> Dehn.
20	<u>Umbelliferae/Apiaceae</u>	<i>Anethum</i>	<i>Anethum graveolens</i> L.
21	<u>Verbenaceae</u>	<i>Lantana</i> (family).	<i>Lantana camara</i> L.
22	<u>Compositae</u>	<i>Lactuca</i>	<i>Lactus sativa</i> L.
23	<u>Umbelliferae/Apiaceae</u>	<i>Ammi</i> (family <u>Apiaceae</u>). <i>Ammi visnaga</i> L.	<i>Ammi visnaga</i> L.
24	<u>Solanaceae</u>	<i>Solanum</i> (family).	<i>Solanum melongena</i> L.
25	<u>Solanaceae</u>	<i>Capsicum</i>	<i>Capsicum</i> sp. L.
26	<u>Lamiaceae</u>	<i>Mentha</i>	<i>Menthe</i> sp. L.
27	<u>Cyperaceae</u>	<i>Cyperus</i>	<i>Cyperus rotundus</i> L.
28	<u>Amaryllidaceae</u>	<i>Allium</i>	<i>Allium cepa</i> L.
29	<u>Cyperaceae</u>	<i>Cyperus</i>	<i>Cyperus difformis</i> L.
30	<u>Convolvulaceae</u>	<i>Convolvulus</i>	<i>Convolvulus arvensis</i> L.
31	<u>Malvaceae</u>	<i>Corchorus</i>	<i>Corchorus olitorius</i> L.
32	<u>Amaranthaceae</u>	<i>Beta</i>	<i>Beta vulgaris</i> L.

Table S4. The flora of experimental fields in Sicily, Italy

Family	Genus	Species
Pinaceae		
	Pinus	<i>p. halepensis</i>
	Pinus	<i>pinca l.</i>
Cupressaceae		
	Cupressus	<i>sempervirens</i>
Ephedraceae		
	Ephedra	<i>fragilis desf.</i>
Alismataceae		
	Alisma	<i>plantago – aquatica l.</i>
Liliaceae		
	Asphodelus	<i>fistulosus l.</i>
	Asphodelus	<i>microcarpus salzm.</i>
	Allium	<i>roseum l.</i>
	Allium	<i>neapolitanum cyr.</i>
	Allium	<i>palleas l. ssp. <i>siciliense stearn</i></i>
	Allium	<i>nigrum l.</i>
	Scilla	<i>autumnalis l.</i>
	Scilla	<i>sicula tia.</i>
	Ornithogalum	<i>narbonense l.</i>
	Leopoldia	<i>comosa parl.</i>
	Asparagus	<i>albus l.</i>
	Asparagus	<i>acutifolius l.</i>
	Asparagus	<i>aphyllus l.</i>
	Asparagus	<i>stipularis</i>
Amaryllidaceae		
	Sternbergia	<i>lutea</i>
Agavaceae		
	Agave	<i>americana l.</i>
Iridaceae		
	Crocus	<i>longiflorus rafin</i>
Orchidaceae		
	Ophrys	<i>lutea cav.</i>
	Ophrys	<i>fusca link</i>
Salicaceae		
	Populus	<i>alba l.</i>
	Populus	<i>nigra l.</i>
	Salix	<i>alba l.</i>
Juglandaceae		
	Junglans	<i>regia l.</i>
Fagaceae		
	Castanea	<i>sativa miller</i>
	Quercus	<i>ilex l.</i>
	Quercus	<i>suber l.</i>
Ulmaceae		
	Celtis	<i>australis l.</i>
Moraceae		
	Morus	<i>alba l.</i>
	Ficus	<i>carica l.</i>
Urticaceae		
	Urtica	<i>dioica l</i>
	Urtica	<i>rupestris guss.</i>
	Urtica	<i>urens l.</i>

Chenopodium		
	Beta	<i>vulgaris l.</i>
	Chenopodium	<i>album l.</i>
Portulacaceae		
	Portulaca	<i>oleracea l.</i>
Caryophyllaceae		
	Stellaria	<i>media (l.) vill.</i>
	Silene	<i>fuscata link</i>
Lauraceae		
	Laurus	<i>nobilis l.</i>
Papaveraceae		
	Papaver	<i>rhoeas l.</i>
Brassicaceae		
	Cardaria	<i>draba desv.</i>
	Sisymbrium	<i>irio l.</i>
	Sinapsis	<i>arvensis l.</i>
	Sinapsis	<i>alba l.</i>
	Diplotaxis	<i>crassifolia dc.</i>
	Diplotaxis	<i>crucooides dc.</i>
	Brassica	<i>inaca ten.</i>
	Brassica	<i>nigra</i>
Capparidaceae		
	Capparis	<i>spinosa l.</i>
Rosaceae		
	Pyrus	<i>pyrasyer</i>
	Pyrus	<i>amygdaliformis vill.</i>
	Malus	<i>sylvestris miller</i>
	Mespilus	<i>germanica (l.)</i>
	Crataegus	<i>azalorus l.</i>
	Rubus	<i>ulmifolius schott</i>
	Fragaria	<i>vesca l.</i>
	Sanguisorba	<i>minor scop.</i>
	Rosa	<i>canina l.</i>
	Rosa	<i>sempervireas l.</i>
	Prunus	<i>avium l.</i>
	Prunus	<i>spinosa l.</i>
Fabaceae		
	Cercis	<i>siliquastrum l.</i>
	Ceratonia	<i>siliqua l.</i>
	Anagyris	<i>foelida l.</i>
	Lupinus	<i>angustifolius</i>
	Lupinus	<i>albus l.</i>
	Onomis	<i>natrix l. ssp. ramosissima</i>
	Trigonella	<i>maritime l.</i>
	Trigonella	<i>corniculata l.</i>
	Medicago	<i>lupulina l.</i>
	Medicago	<i>sativa l.</i>
	Medicago	<i>scutellata miller</i>
	Medicago	<i>rugosa</i>
	Medicago	<i>marina l.</i>
	Medicago	<i>arabica hudson</i>
	Melilotus	<i>italic lam.</i>
	Melilotus	<i>indica all.</i>
	Melilotus	<i>infesta guss.</i>

	Trifolium	<i>strictum l.</i>
	Trifolium	<i>repens l. ssp.</i>
	Trifolium	<i>nigrescens viv. ssp.</i>
	Trifolium	<i>pratense l.</i>
	Robinia	<i>pseudoacacia l.</i>
	Astragalus	<i>boeticus l.</i>
	Vicia	<i>villosa roth</i>
	Vicia	<i>sativa l.</i>
	Lathyrus	<i>odoratus l.</i>
	Pisum	<i>sativum l.</i>
Oxalidaceae		
	Oxalis	<i>acetosella l.</i>
	Oxalis	<i>pes – caprae l.</i>
Simaroubaceae		
	Alianthus	<i>altissima (miller) swingle</i>
Euphorbiaceae		
	Euphorbia	<i>peplis l.</i>
Anacardiaceae		
	Pistacia	<i>lentiscus l.</i>
	Rhus	<i>coriaria l.</i>
Vitaceae		
	Vitis	<i>vinifera l.</i>
Malvaceae		
	Marva	<i>sylvestris l.</i>
Cactaceae		
	Opuntia	<i>ficus indica miller</i>
Punicaceae		
	Punica	<i>granatum l.</i>
Myrtaceae		
	Myrtus	<i>communis l.</i>
	Eucalyptus	<i>sp. pl.</i>
Araliaceae		
	Hedera	<i>helix l.</i>
Apiaceae		
	Eryngium	<i>campestre l.</i>
	Daucus	<i>carota l.</i>
Plumbaginaceae		
	Plumbago	<i>europea l.</i>
Oleaceae		
	Olea	<i>europea l. sylvestris</i>
	Olea	<i>europea l. europaea</i>
Convolvulaceae		
	Convolvulus	<i>siculus l.</i>
	Convolvulus	<i>arvensis l.</i>
Boraginaceae		
	Borago	<i>officinalis l.</i>
	Cerinthe	<i>major l.</i>
Verbenaceae		
	Verbena	<i>officinalis l.</i>
Lamiaceae		
	Rosmarinus	<i>officinalis l.</i>
	Sideritis	<i>romana l.</i>
	Salvia	<i>viridis</i>
	Origanum	<i>vulgare l.</i>

	Thymus	<i>capitatus l.</i>
	Mentha	<i>longifolia hudson</i>
Solanaceae		
	Solanum	<i>nigrum l.</i>
	Datura	<i>stramonium l.</i>

Table S5A: Qualitative Melissopalynological analysis for Greece (EL), Italy (IT), and Egypt (EG)

This treemap visualization displays the relative pollen frequencies (%) for various plant families and genera across different pollen sources and market/honeycomb categories. The data is organized into four main sections: GREECE, EL_FIELD, hw = honey wax, and ITALY/CH-IT-SI.

Legend:

- Color Scale:** Blue for GREECE, EL_FIELD, and hw = honey wax; Orange for ITALY, CH-IT-SI, m = market, and HC = honeycomb.
- Frequency Labels:** Numerical values representing pollen frequency percentages, with '<1' indicating values below 1%.

Section Headers:

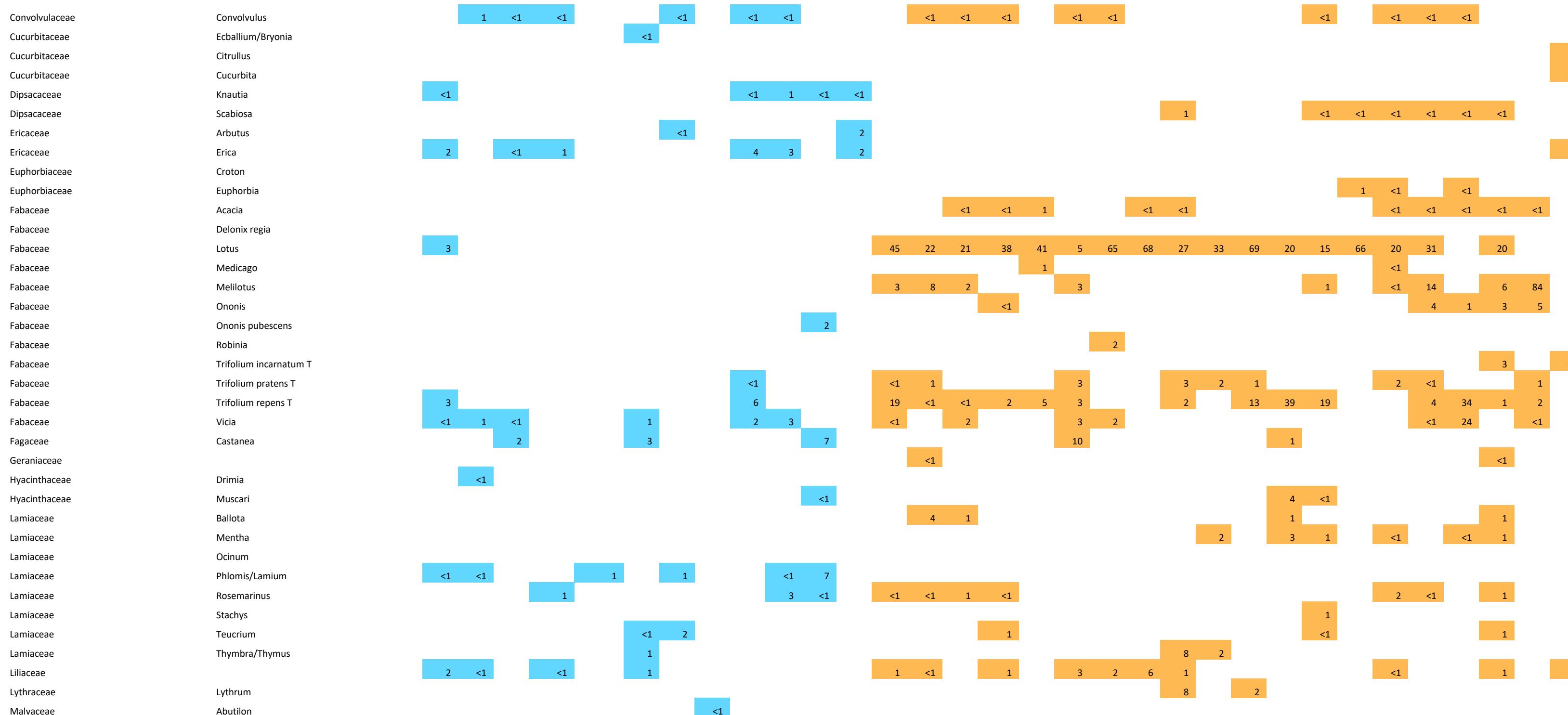
- Relative pollen frequencies (%)**
- GREECE**
- EL_FIELD**
- hw = honey wax**
- ITALY**
- CH-IT-SI**
- m = market**
- HC = honeycomb**

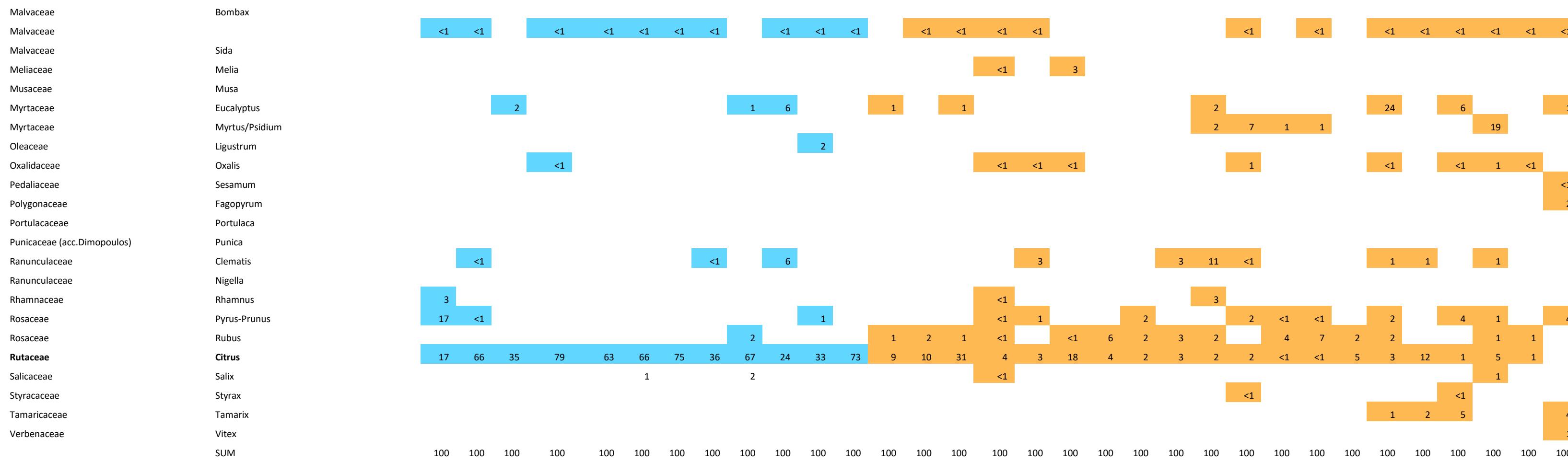
Family Groupings:

- NECTARIFEROUS PLANTS (N)**
- FAMILY**
- GENUS/SPECIES type**

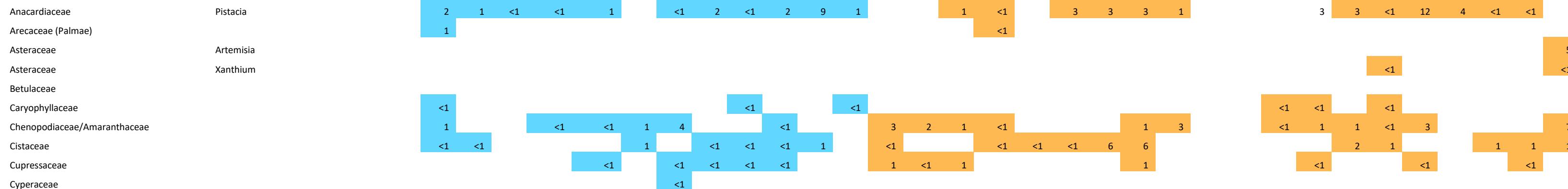
Data Summary:

The visualization shows a wide distribution of pollen frequencies across all categories. Notable high frequencies include Echium (46%) in the honey wax section, and various species in the Asteraceae family (e.g., Centaurea, Taraxacum) across most sections. The honeycomb section (orange) generally shows higher frequencies for many genera compared to the market section (m).





NECTARLESS PLANTS (NL)



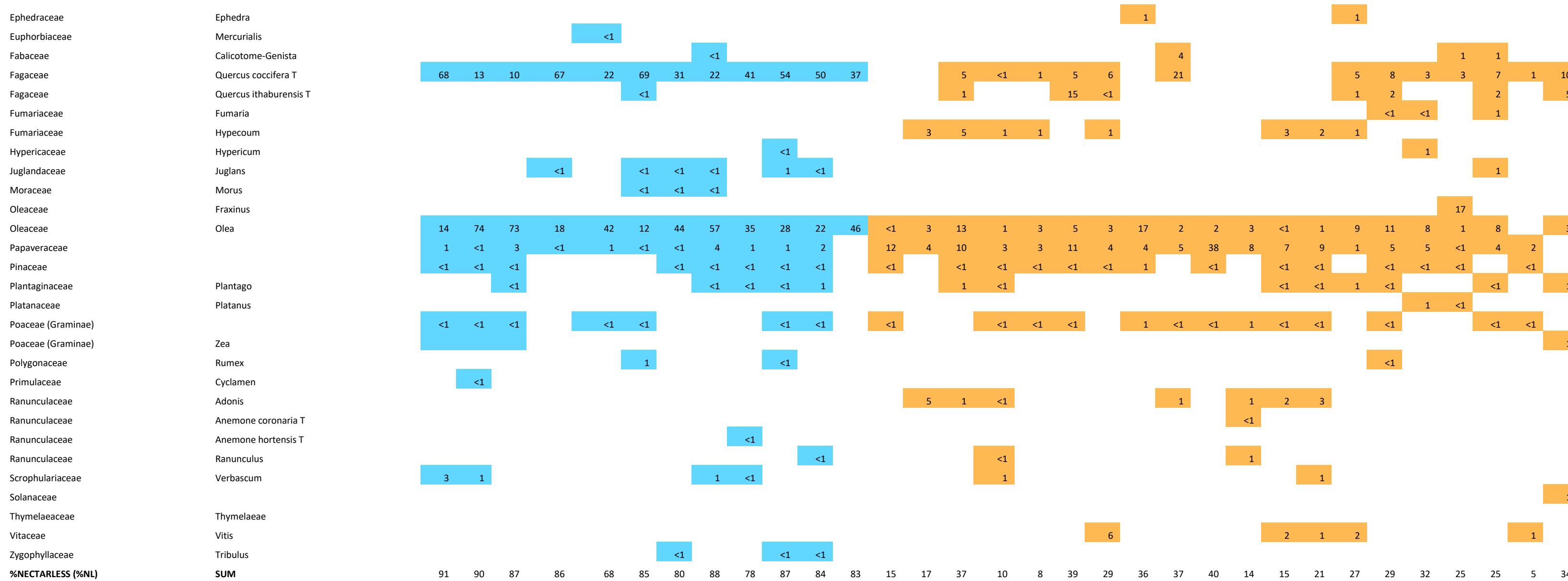


Table S5B: Quantitative Melissopalynological Analysis for Greece (EL), Italy (IT), and Egypt (EG)

GREECE	EL_FIELD											
1_1	2_1	2_2	3_1	3_2	4_1	4_2	5_1	5_2	6_1	7_1	7_2	
27100	62600	68000	359200	28500	49100	28500	63600	15400	37500	18800	54900	
2500	6300	8600	51400	9000	7600	5500	7800	3400	4900	2900	9100	

	ITALY	CH_IT_SI	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Sample No			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
PG/10 gr honey	4600		6700		10900		29600		11700		6200		4600		5200		6300		6400		29500		109900
PG nectariferous/10 gr honey	3900		5500		6800		26700		10700		3800		3300		3300		3900		3900		25400		93200

	EGYPT	(Citrus, Clover, AMPS, Basil)	1	2	3	4	5	6	7	8												
Sample No			1	2	3	4	5	6	7	8												
PG/10 gr honey	20500		128700		92800		26500		3600000		135400		246900		2024000							
PG nectariferous/10 gr honey	14000		96700		87800		23600		2289200		115900		245600		729700							

Table S6: Parameters from OPLS-DA analysis for each comparison, using the ropls R Bioconductor package. For the analysis the standard scaling method was used after log10 data transformation

Greece vs Italy -Negative ion mode

	R2X	R2X(cum)	R2Y	R2Y(cum)	Q2	Q2(cum)	Signif.
p1	0.1740	0.174	0.6220	0.622	0.5190	0.519	R1
o1	0.3600	0.533	0.2240	0.224	0.1970	0.197	R1
o2	0.1020	0.635	0.1010	0.325	0.0605	0.258	R1
o3	0.0814	0.716	0.0290	0.354	0.0504	0.308	R1
o4	0.0287	0.745	0.0165	0.371	0.0112	0.319	R1

R2X(cum) R2Y(cum) Q2(cum) RMSEE pre ort pR2Y pQ2
 Total 0.745 0.992 0.838 0.0476 1 4 0.005 0.005

Greece vs Egypt -Negative ion mode

	R2X	R2X(cum)	R2Y	R2Y(cum)	Q2	Q2(cum)	Signif.
p1	0.1700	0.170	0.505	0.505	0.3890	0.389	R1
o1	0.4800	0.650	0.3290	0.329	0.3280	0.328	R1
o2	0.0853	0.735	0.1130	0.442	0.0579	0.385	R1
o3	0.0540	0.789	0.0328	0.475	0.0462	0.432	R1
o4	0.0246	0.814	0.0158	0.491	0.0520	0.484	R1

R2X(cum) R2Y(cum) Q2(cum) RMSEE pre ort pR2Y pQ2
 Total 0.814 0.996 0.873 0.0352 1 4 0.005 0.005

Italy vs Egypt -Negative ion mode

	R2X	R2X(cum)	R2Y	R2Y(cum)	Q2	Q2(cum)	Signif.
p1	0.1290	0.129	0.7320	0.7320	0.5340	0.534	R1
o1	0.3610	0.490	0.0982	0.0982	0.1990	0.199	R1
o2	0.0800	0.570	0.1200	0.2180	0.0501	0.249	R1
o3	0.0922	0.662	0.0225	0.2410	0.0402	0.289	R1
o4	0.0293	0.692	0.0215	0.2620	0.0277	0.317	R1

R2X(cum) R2Y(cum) Q2(cum) RMSEE pre ort pR2Y pQ2
 Total 0.692 0.994 0.851 0.0384 1 4 0.005 0.005

Greece vs Italy -Positive ion mode

	R2X	R2X(cum)	R2Y	R2Y(cum)	Q2	Q2(cum)	Signif.
p1	0.1490	0.149	0.3980	0.398	0.3320	0.332	R1
o1	0.4000	0.548	0.3470	0.347	0.2560	0.256	R1
o2	0.1010	0.649	0.1500	0.496	0.1110	0.367	R1
o3	0.0575	0.707	0.0601	0.556	0.0525	0.420	R1
o4	0.0400	0.747	0.0296	0.586	0.0188	0.439	R1
o5	0.0279	0.774	0.0101	0.596	0.0208	0.460	R1

R2X(cum) R2Y(cum) Q2(cum) RMSEE pre ort pR2Y pQ2
 Total 0.774 0.994 0.792 0.0437 1 5 0.005 0.005

Italy vs Egypt -Positive ion mode

	R2X	R2X(cum)	R2Y	R2Y(cum)	Q2	Q2(cum)	Signif.
p1	0.0928	0.0928	0.5250	0.525	0.3060	0.306	R1
o1	0.4070	0.5000	0.2530	0.253	0.2990	0.299	R1
o2	0.0697	0.5700	0.1390	0.392	-0.0225	0.276	NS
o3	0.0614	0.6310	0.0554	0.448	0.0568	0.333	R1
o4	0.0448	0.6760	0.0195	0.467	0.0260	0.359	R1

R2X(cum) R2Y(cum) Q2(cum) RMSEE pre ort pR2Y pQ2
 Total 0.676 0.992 0.665 0.0465 1 4 0.005 0.005

References

- [1] Gazala, N. and Nowar, E., 2014. Survey of different pollen sources gathering by honey bee at Qunatir Al-khiria, Qaluobia Governorate. *Journal of Plant Protection and Pathology*, 5(6), pp.755-771.
- [2] Hassanien, Mai M., Microscopic and chemical analysis of honey and bee bread at certain apiaries in Qalyubia Governorate and available honey in local market, Egypt."MSc thesis, Ain Shams University, Egypt 2018