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Background/PURPOSE: Pollen, which is in the content of honey, helps identify the bee that makes the honey from which flowers and, therefore, will help detect the origin of honey. Plant families can be detected by the microscopic analysis of pollen in honey (Melissopalynological analysis). But, in recent years DNA isolation method for the determination of the origin of honey are regarded as faster and more reliable. So microscopic method and DNA Analysis method compared in this study.

METHODOLOGY: Honey samples were collected from various regions of Türkiye. The number of pollen analysis and botanical origin were determined by microscopically. DNA were isolated from these honey samples by Eurofin Genespin DNA extration kit. After DNA isolation, spectrophotometric and electrophoretic method were used to determine DNA quality. 18S rRNA gene amplification was performed to show also quality of DNA isolated from honey samples. The Next Generation Sequencing (NGS) device was used to create DNA barcodes. rbcL and psbA-trnH genetic regions were selected for NGS analyses.

Results and CONCLUSION: In the conclusion of this study, for the first time, honey samples collected from different geographical regions in Türkiye were barcoded using rbcL and trnH-psbA loci and compared using melissopalynology and NGS. Although there are some differences in the plants determined by both techniques, identified dominant floral components were shown high similarity. When the melissopalynological analysis and NGS analysis were compared in the study, it was shown that there were similar families according to the regions, and a higher number of families (%48) were determined in the NGS analysis. Results show that 53 families have an abundance of >20% when comparing the two methods. Of these, 29 taxa are found using both methods, giving 55% correspondence between DNA and microscopy. However, NGS analysis is disadvantageous compared to melissopalynological analysis, which is a cheaper and faster method, since plant reference information is scarce and it is an expensive method. So, more studies and comparisons between the two methods are needed.



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Comparison of Honey's Botanical and Geographical Origin Determination Using Melissopalynology and DNA Analysis Methods



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PALYNOLOGY

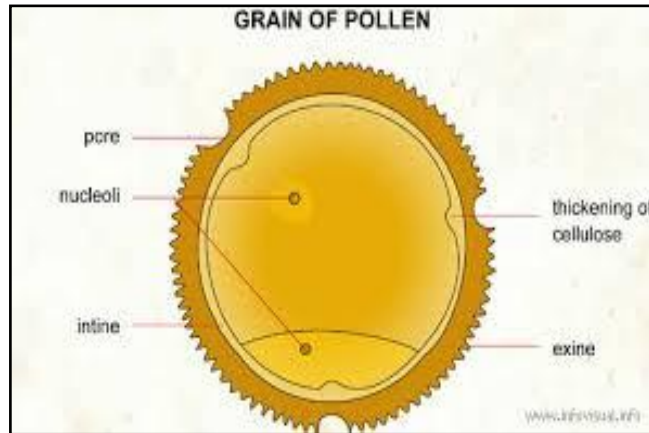
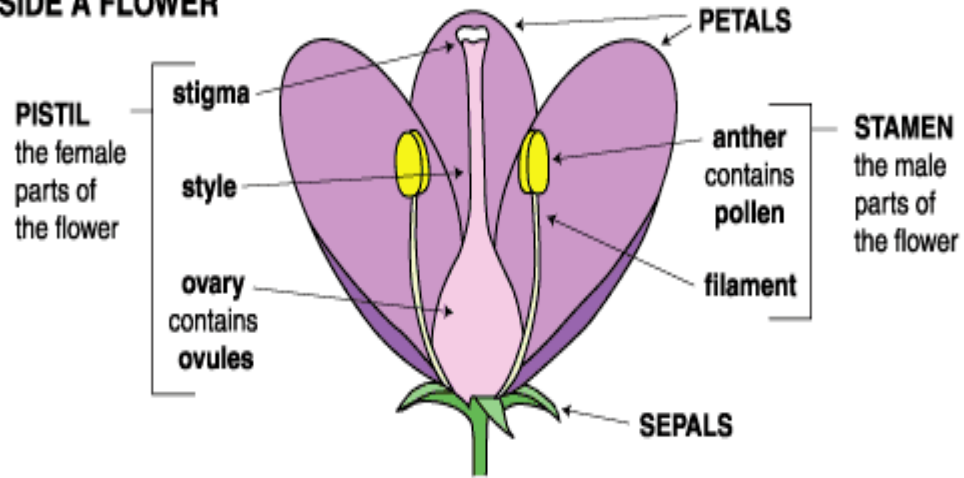


- Palynology is a branch of science that studies current and fossil pollen and spores.
- The term palynology was first used by Hyde and Williams in 1944.

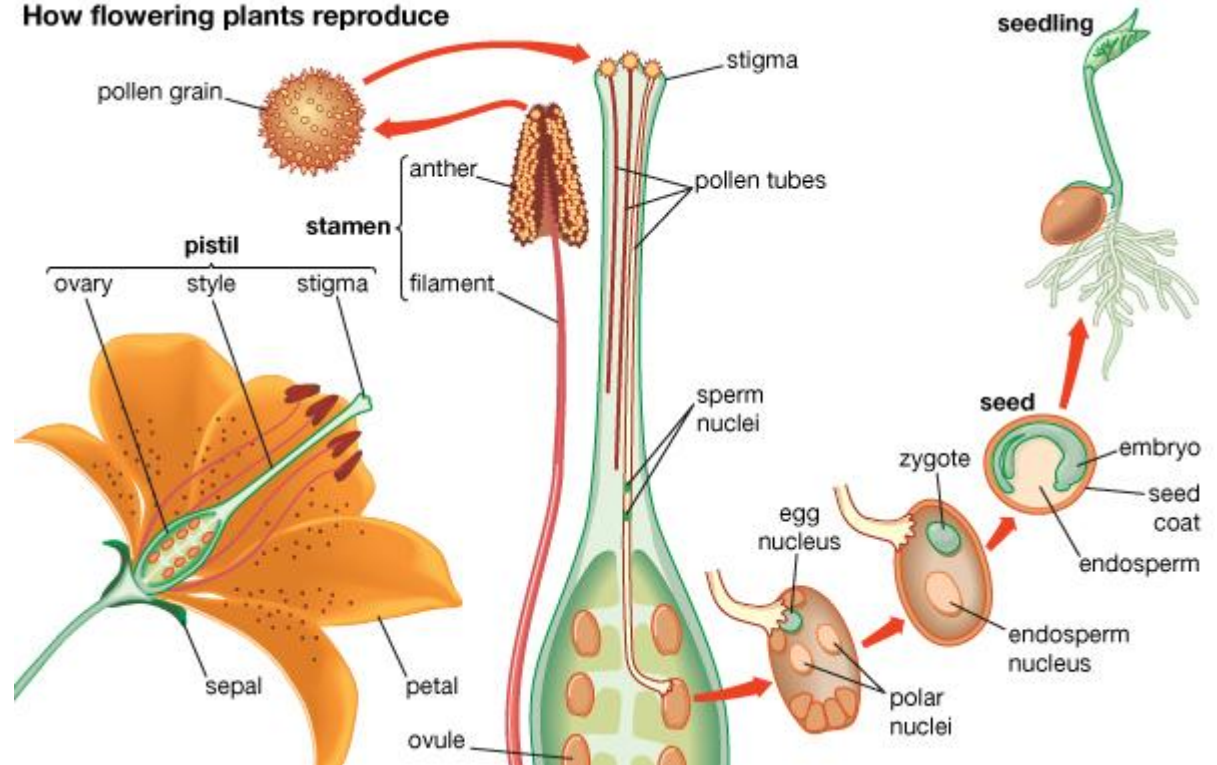
Palynology can be applied to problems in many scientific disciplines including GEOLOGY, BOTANY, PALEONTOLOGY, ARCHAEOLOGY, SOIL STUDY, PHYSICAL GEOGRAPHY ETC.

1. **Palynotaxonomy:** is the study of spore morphology of embryophytic plants and pollen morphology of seed plants.
2. **Aeropalynology:** is the study of palynomorphs found in the atmosphere.
3. **Melissopalynology (= melittopalynology):** is the study of pollen and spores present in honey.
4. **Forensic Palynology:** is the study of palynomorphs that are used as evidence in criminal cases and resolve other legal problems.
5. **Palaeopalynology:** fossil microorganisms such as pollen grains, spores, marine and fresh water algae, foraminifera and other planktonic forms’.
6. **Copropalynology:** Copropalynology is the study of palynomorphs present in coprolite (= fossilized excrement) of animals.
7. **Entomopalynology:** Entomopalynology is the study of pollen grains that are associated with insects.
8. **Palynodebris :** is the organic ‘junk’ = organic matter (“OM”) found in palynological preparations along with palynomorphs”
9. **Latropalynology:** is the ‘study of spores and pollen as applied to human health problems.

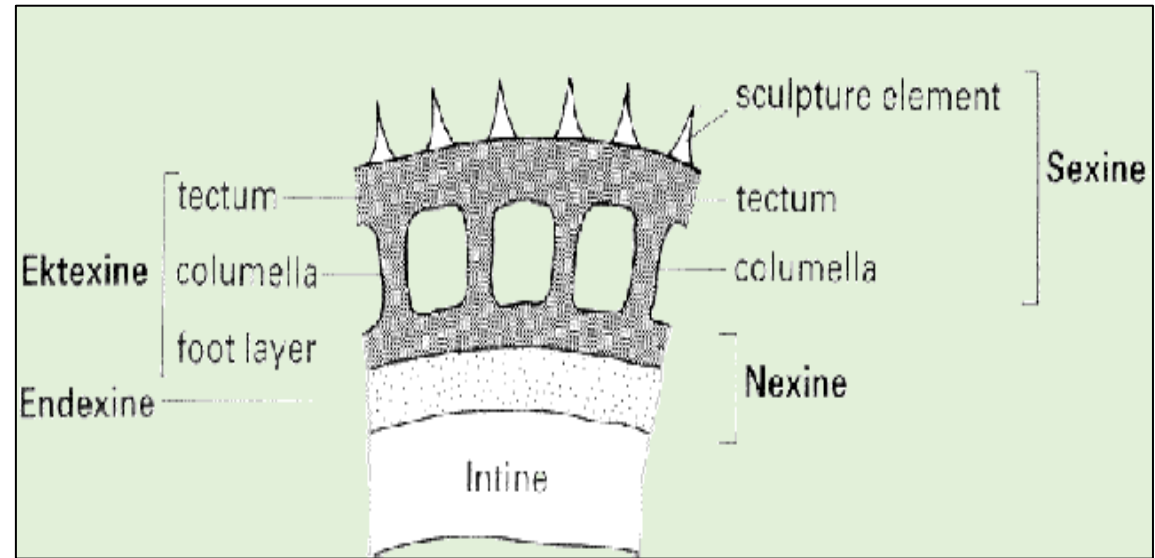
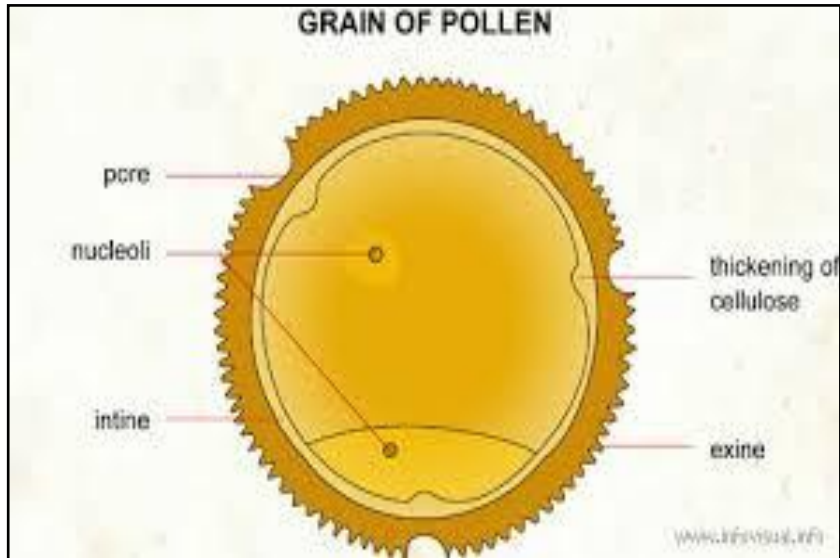
INSIDE A FLOWER



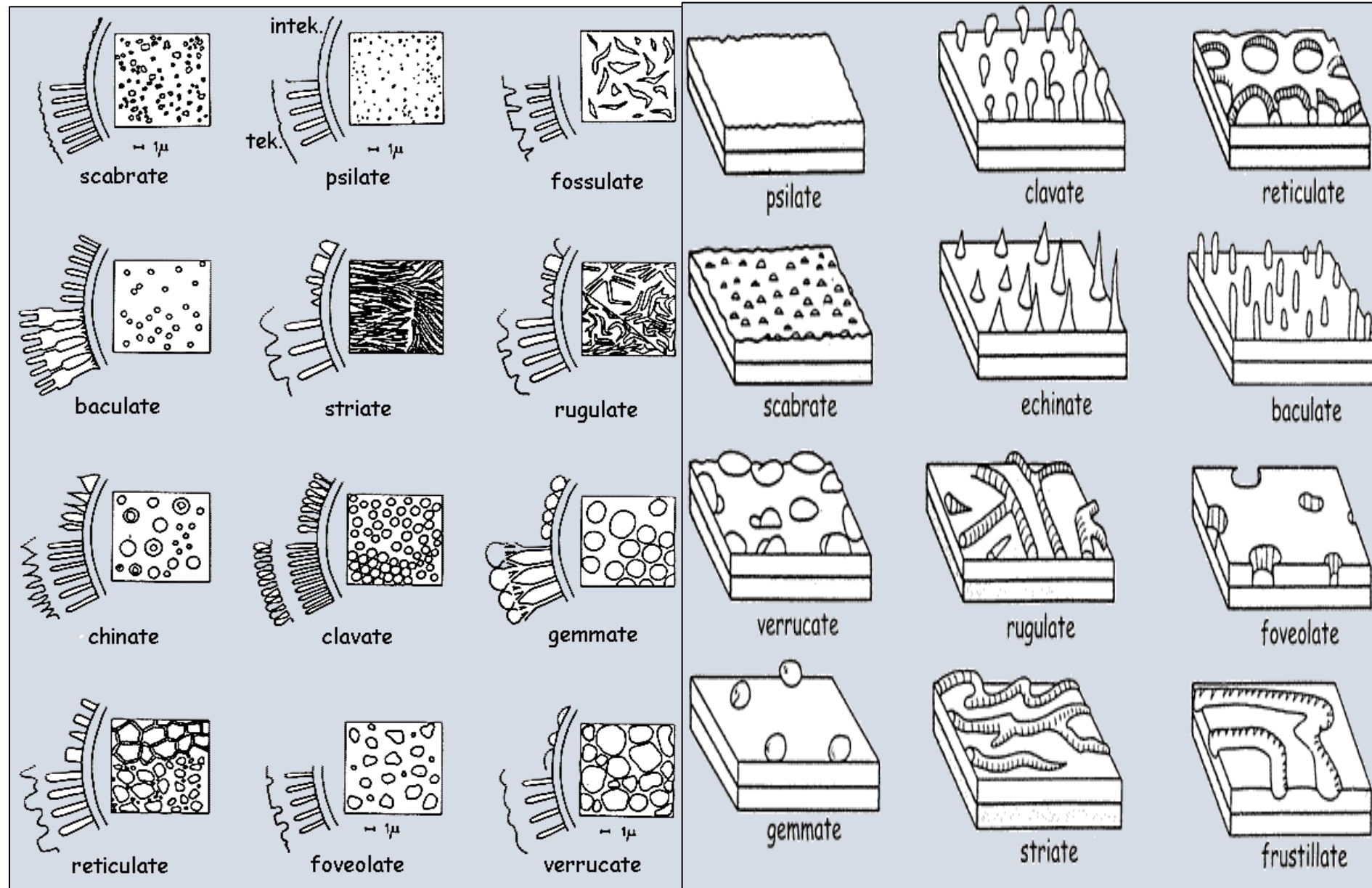
How flowering plants reproduce

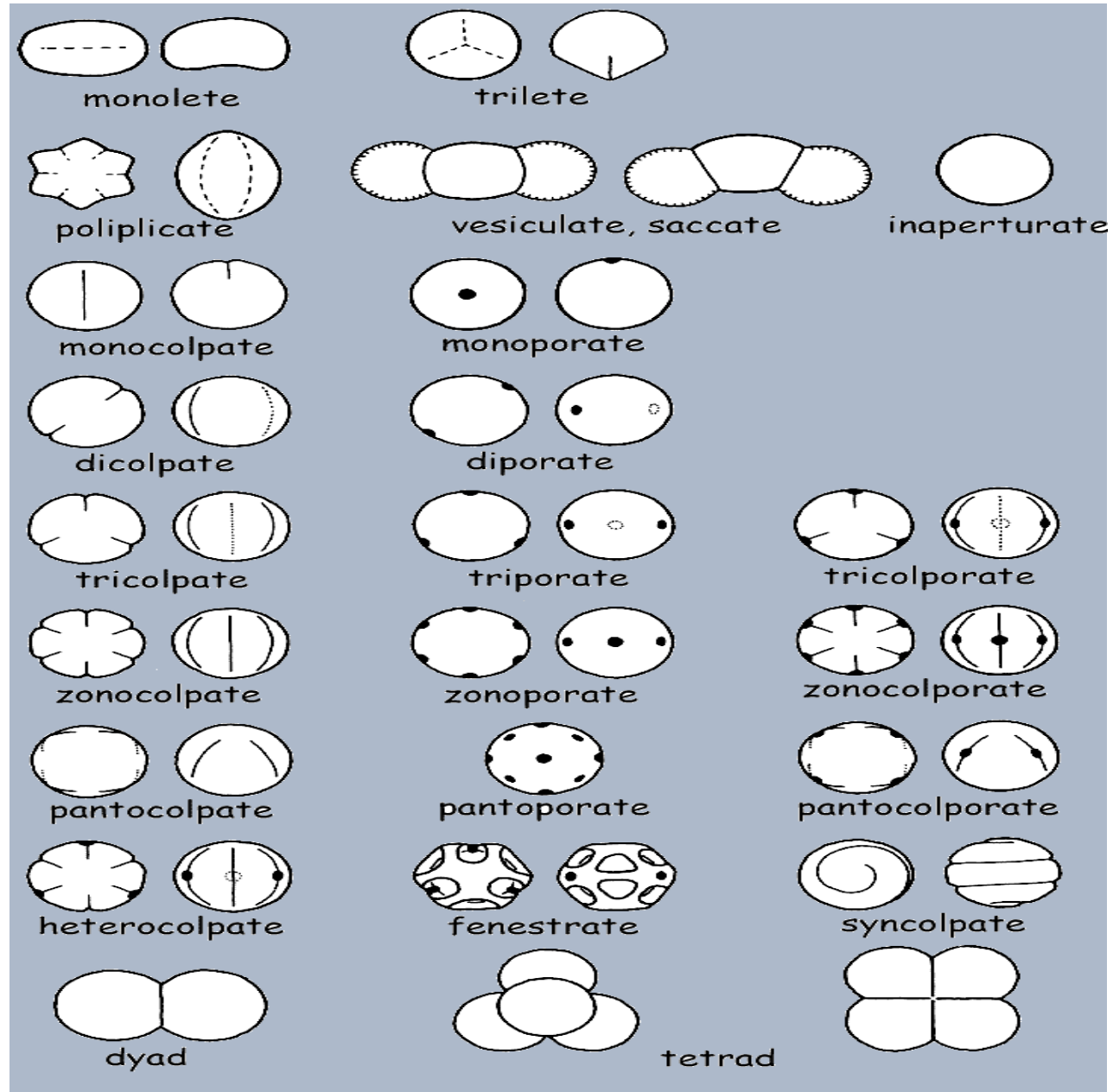


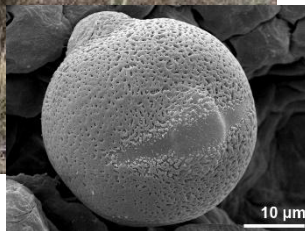
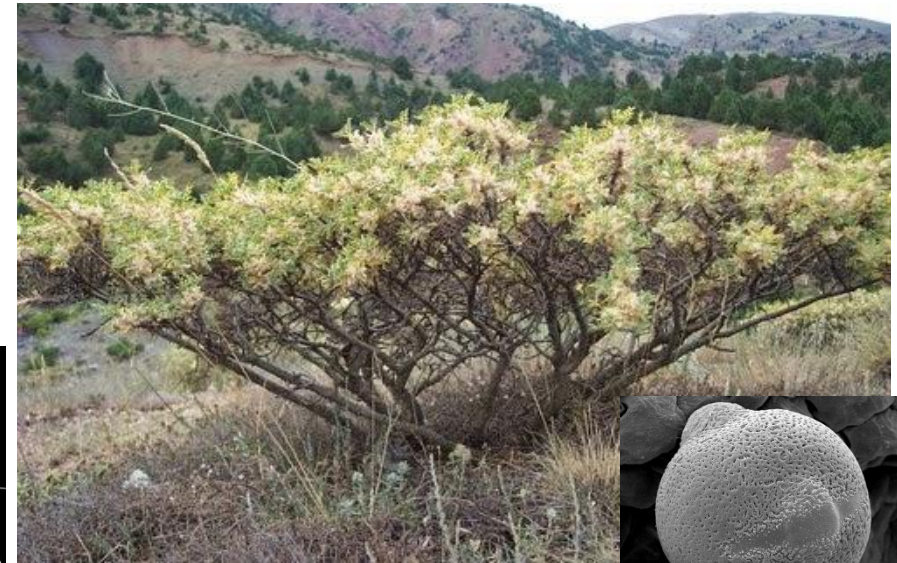
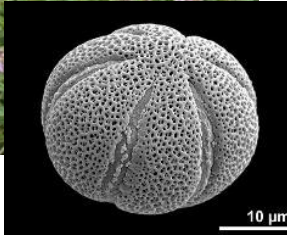
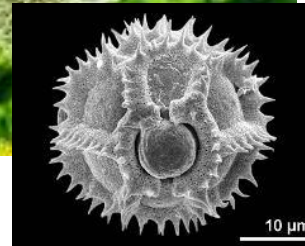
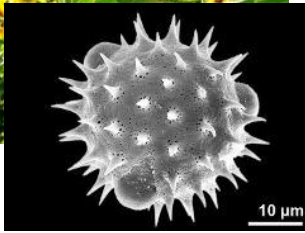
- Pollen is a powdery specialized structure that contains the male gametes of flowering plants and is responsible for their reproduction.



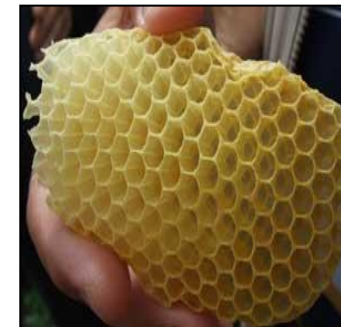
- Erdtman and Faegri&Iversen made the definitions about layers of pollen.
- Exine layer is resistant to high temperature and acids.
- Exine layer ornamentation and apertures are changing flower to flower.

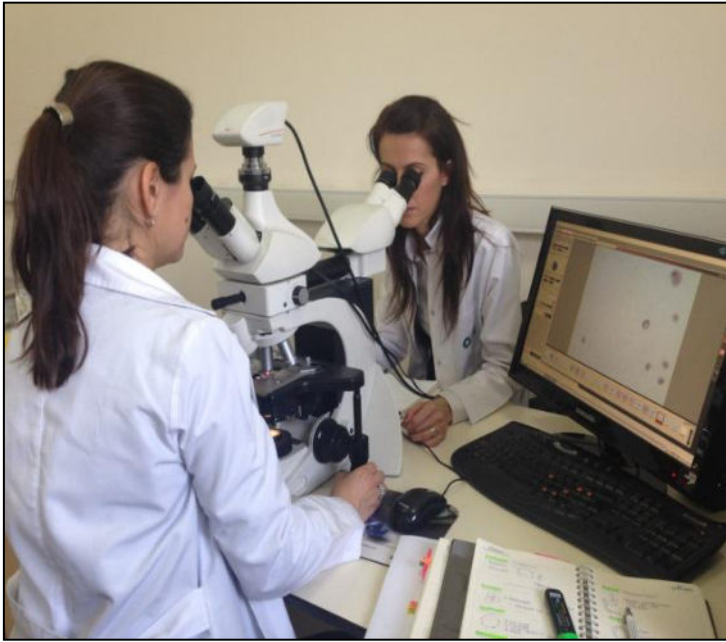




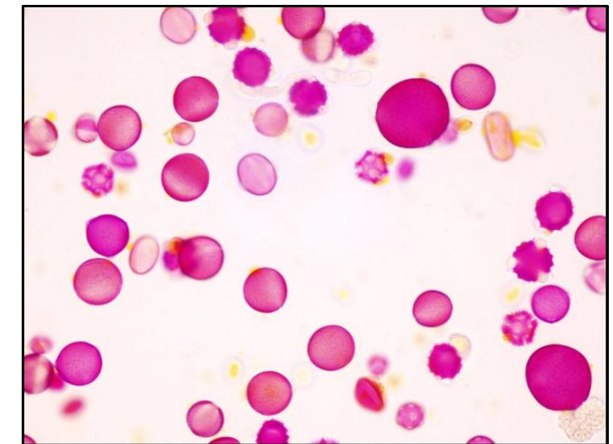
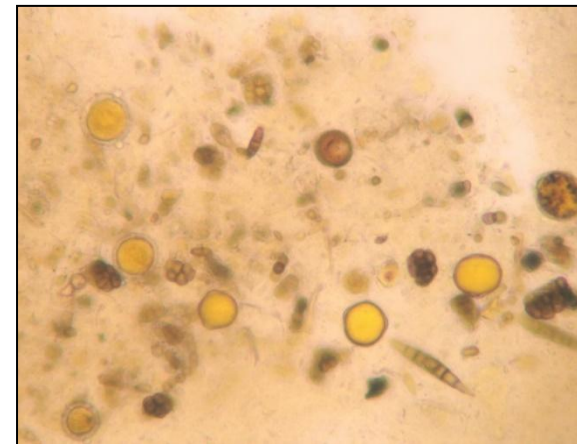


When we look at the pollen types in bee products, they show us which plants honey bees collect them from.





- Plant families can be detected by the microscopic analysis of pollen in honey (Melissopalynological analysis).





Analytical Methods

A DNA barcoding approach to identify plant species in multiflower honey



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Improving DNA isolation from honey for the botanical origin identification



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➤ But, in recent years DNA isolation method for the determination of the origin of honey are regarded as faster and more reliable.



Analytical Methods

Automated DNA extraction from pollen in honey



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ARTICLE INFO

Article history:
Received 15 March 2013

ABSTRACT

In recent years, honey has become subject of DNA analysis due to potential risks evoked by microorganisms, allergens or genetically modified organisms. However, so far, only a few DNA extraction pro-



Analytical Methods

Development of primer and probe sets for the detection of plant species in honey

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ABSTRACT



MATERIALS AND METHODS

Method

1- Collection of the samples

2-Melissopalynological Analysis

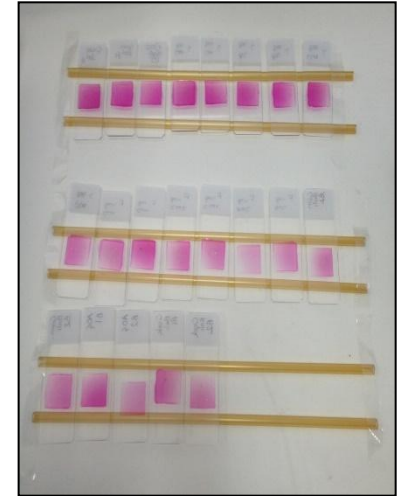
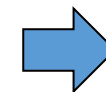
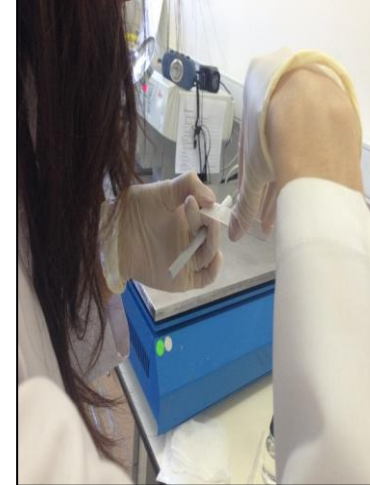
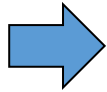
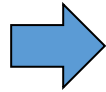
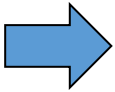
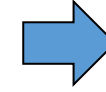
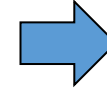
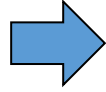
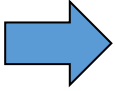
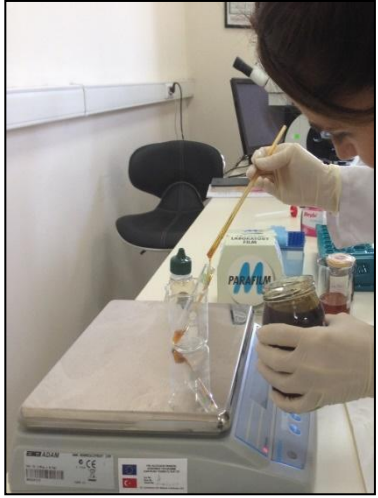
3-DNA Isolation Method and PCR

4-Next-generation DNA sequencing



- In this study total 75 honey samples, including sunflower, canola, chestnut, citrus, forest rose (Rhododendron-mad honey), clover, pine, oak, milkvetch, mullein, Anzer and Ardahan honeys were collected from various regions of Türkiye with the help of the Turkish Association of Beekeepers (TAB).

Microscopic Analysis Method



➤ The number of pollen analysis and botanical origin were determined by microscopically.

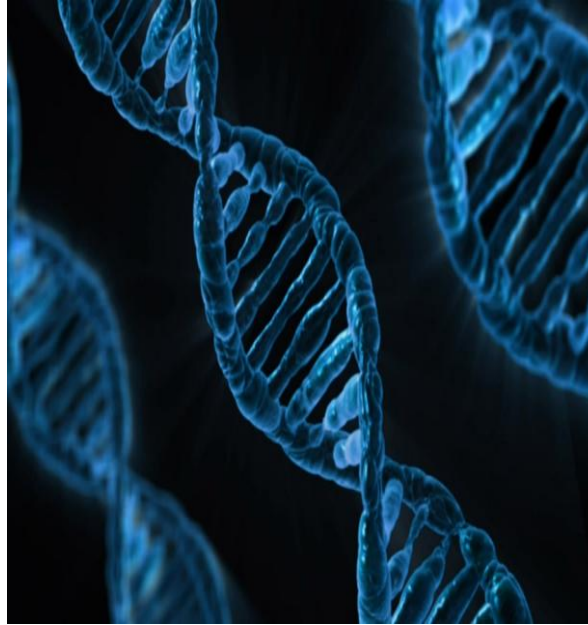
As a result of the melissopalynological analysis,

➤ 40 honey samples were selected for DNA Analysis because of the high number of pollen quality and unifloral origin.

➤ These are;

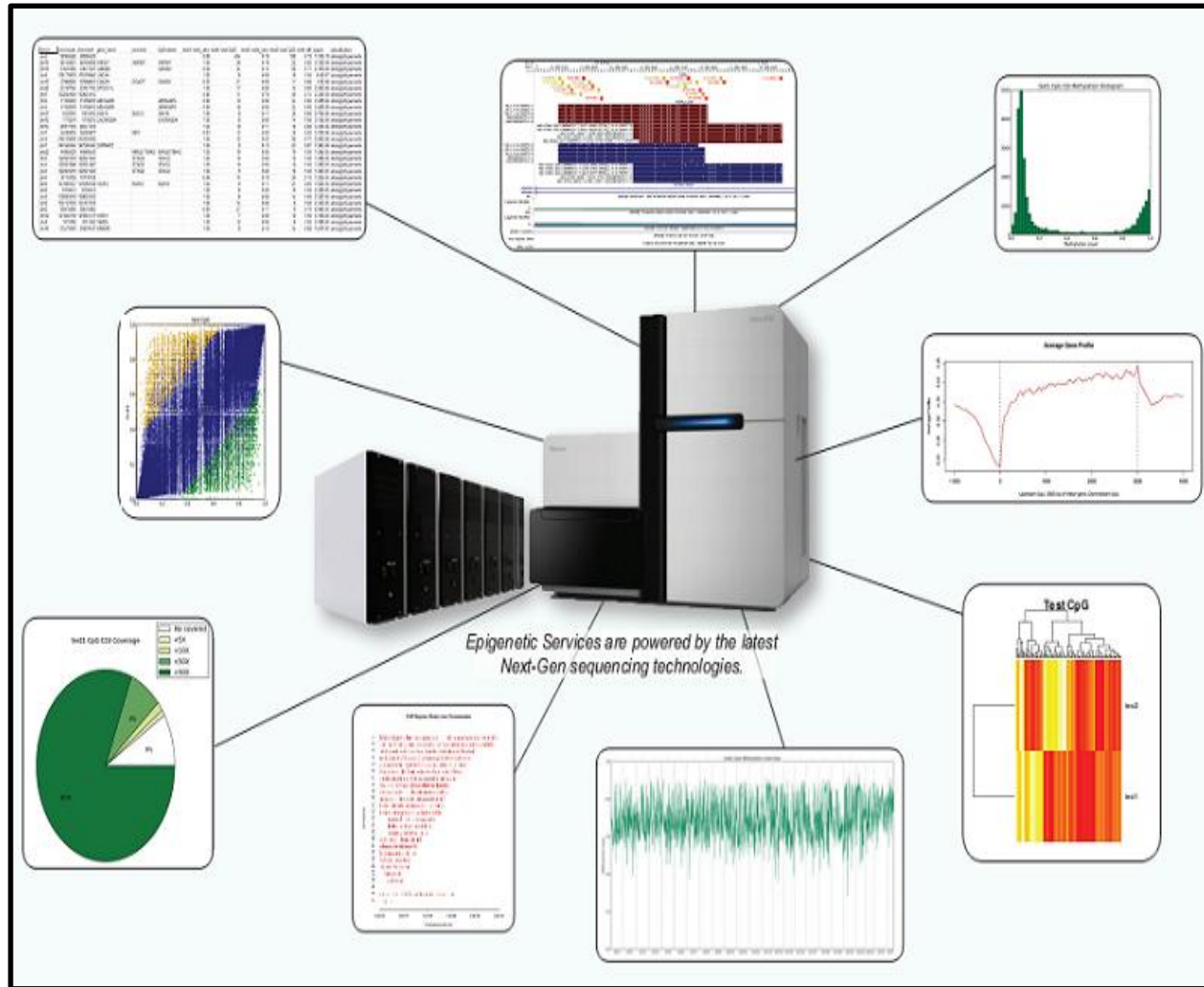
- Canola (3) – *Brassica napus*
- Chestnut (8)- *Castanea sativa*
- Citrus (3) - *Citrus* spp.
- Clover (5) - *Trifolium* spp.
- Forest rose (2) - *Rhododendron* spp.
- Milkvetch (1) - *Astragalus* spp.
- Mullein (1) - *Verbascum* spp.
- Oak (2) - *Quercus* spp.
- Pine (5)
- Sunflower (3) - *Helianthus annuus*
- Anzer (3)
- Ardahan (4)

DNA Analysis Method



- DNA were isolated from these honey samples by Eurofin Genespin DNA extration kit.
- After DNA isolation, spectrophotometric and electrophoretic method were used to determine DNA quality.
- 18S rRNA gene amplification was performed to show also quality of DNA isolated from honey samples.

NGS Analysis Method



- **Next Generation Sequencing (NGS) analysis** is a modern biotechnological method that allows DNA or RNA sequences to be read rapidly, in parallel, and at high throughput.
- In short, **NGS analysis** is a powerful method to comprehensively and accurately reveal genetic information in a sample.

NGS Analysis Method

Gene Region	Oligo Name	Sequence 5' → 3'
rbcL	<i>rbcLa</i> – F	ATGTCACCACAAACAGAGACTAAAGC
	<i>rbcLa</i> jf634 - R	GAAACGGTCTCTCCAACGCAT

Gene Region	Oligo Name	Sequence 5' → 3'
psbA	<i>psbA</i> - F	GTTATGCATGAACGTAATGCTC
	<i>trnH</i> - R	CGCGCATGGTGGATTCAACAATCC

- The Next Generation Sequencing (NGS) device was used to create DNA barcodes.
- *rbcL* and *psbA-trnH* universal genetic regions were selected for NGS analyses.



RESULTS

Melissopalynological Analysis Results

Sunflower honey

Sample no.	Total Pollen Number (TPN)	Pollen taxa	%*	Geographic al origin	Honey type
1	7651	<i>Helianthus annuus</i> : 94	75.2(D)	Edirne	Sunflower
		<i>Echium</i> spp.: 7	5.6(M)		
		<i>Linaria</i> spp.: 6	4.8(M)		
		Asteraceae: 4	3.2(M)		
		Poaceae: 4	3.2(M)		
		Rosaceae: 4	3.2(M)		
		Chenopodiaceae: 2	1.6(V)		
		<i>Eucalyptus</i> spp.: 2	1.6(V)		
		Apiaceae: 1	0.8(V)		
		Fabaceae: 1	0.8(V)		
2	12666	<i>Helianthus annuus</i> : 106	68.8(D)	Edirne	Sunflower
		Fabaceae: 10	6.5(M)		
		<i>Eucalyptus</i> spp.: 10	6.5(M)		
		Rosaceae: 8	5.2(M)		
		Poaceae: 11	7.14(M)		
		Asteraceae: 4	2.6(V)		
		Apiaceae: 1	0.65(V)		
		Chenopodiaceae: 4	2.6(V)		
3	17271	<i>Helianthus annuus</i> : 190	94.5(D)	Edirne	Sunflower
		<i>Eucalyptus</i> spp.: 6	3(M)		
		Fabaceae: 5	2.5(V)		

* ≥%45 Dominant (D), (%16-44) Sekonder (S), (%3-15) Minor (M), (<%3) Vestige (V)



Canola honey

Sam ple no.	Total Pollen Number (TPN)	Pollen taxa	%*	Geographical origin	Honey type
1	45898	<i>Brassica napus</i> : 60	61.8(D)	Tekirdağ	Canola
		<i>Eucalyptus</i> spp.: 6	6.18(M)		
		Fabaceae: 4	4.12(M)		
		Rosaceae: 15	15.4(M)		
		<i>Salix</i> spp.: 5	5.15(M)		
		<i>Helianthus annuus</i> : 4	4.12(M)		
		Liliaceae: 1	1.03(V)		
		Apiaceae: 1	1.03(V)		
		<i>Eleagnus angustifolia</i> : 1	1.03(V)		
2	73130	<i>Brassica napus</i> : 90	66.2(D)	Tekirdağ	Canola
		Rosaceae: 5	3.67(M)		
		<i>Salix</i> spp.: 20	14.7(M)		
		Lamiaceae: 9	6.61(M)		
		Ericaceae: 1	0.73(V)		
		Fabaceae: 3	2.20(V)		
		Poaceae: 1	0.73(V)		
		Geraniaceae: 2	1.47(V)		
		<i>Rumex</i> spp.: 1	0.73(V)		
		<i>Taraxacum</i> spp.: 1	0.73(V)		
3	63494	<i>Brassica napus</i> : 180	83.7(D)	Tekirdağ	Canola
		<i>Salix</i> spp.: 20	9.30(M)		
		Rosaceae: 10	4.65(M)		
		Fabaceae: 3	1.4(E)		
		Geraniaceae: 1	0.46(E)		
		Asteraceae: 1	0.46(E)		

* ≥%45 Dominant (D), (%16-44) Sekonder (S), (%3-15) Minor (M), (<%3) Vestige (V)



Chestnut honey

Sample no.	Total Pollen Number (TPN)	Pollen taxa	%*	Geographical origin	Honey type
1	58975	<i>Castanea sativa</i> : 195 Cistaceae: 3 Ericaceae: 2	97.5(D) 1.5(V) 1(V)	Şile/İstanbul	Chestnut
2	722419	<i>Castanea sativa</i> : 195 <i>Rumex</i> spp.: 2 Rosaceae: 2 Fabaceae: 1	97.5(D) 1(V) 1(V) 0.5(V)	Bartın	Chestnut
3	110844	<i>Castanea sativa</i> : 220 Brassicaceae: 4 Fabaceae: 1	97.7(D) 1.7(V) 0.4(V)	Bartın	Chestnut
4	511271	<i>Castanea sativa</i> : 195 Rosaceae: 3 <i>Rumex</i> spp.: 1 Asteraceae: 1	97.5(D) 1.5(V) 0.5(V) 0.5(V)	Bartın	Chestnut
5	398568	<i>Castanea sativa</i> : 197 Rosaceae: 1 Apiaceae: 1 <i>Rumex</i> spp.: 1	98.5(D) 0.5 (E) 0.5 (E) 0.5 (E)	Kastamonu	Chestnut

- $\geq 45\%$ Dominant (D), ($16-44\%$) Sekonder (S), ($3-15\%$) Minor (M), ($<3\%$) Vestige (V)
- Chestnut honey pollen content is overrepresented and should be 90% for unifloral honey.



Forest rose honey (Rhododendron-Mad honey)

Sample no.	Total Pollen Number (TPN)	Pollen taxa	%*	Geographical origin	Honey type
1	4778	Ericaceae: 9 Castanea sativa: 7 Poaceae: 2	50(D) 38.8(S) 11.1(M)	Bartın	Rhododendron
2	118658	Ericaceae: 80 Castanea sativa: 55 Salix: 25 Onobrychis: 2 Cistaceae: 1	48.4 (D) 33.3 (S) 15.15 (S) 0.6 (E)	Arit-Bartın	Rhododendron

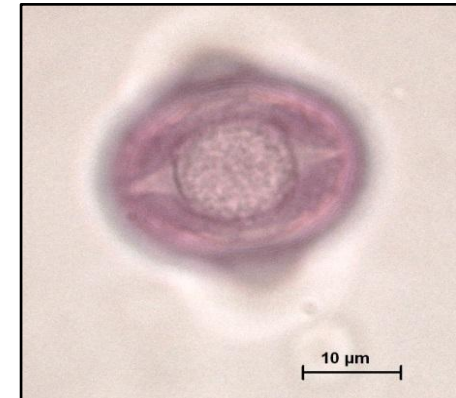
*≥%45 Dominant (D), (%16-44) Sekonder (S), (%3-15) Minor (M), (<%3) Vestige (V)



Clover honey

Sample no.	Total Pollen Number (TPN)	Pollen taxa	%*	Geographical origin	Honey type
1	136341	<i>Trifolium</i> spp.: 95 Poaceae: 3 Apiaceae: 1 Chenopodiaceae: 1	95 (D) 3 (M) 1 (E) 1 (E)	Bingöl	Clover
2	185141	<i>Trifolium</i> spp.: 95 Poaceae: 3 Apiaceae: 1 Rosaceae: 1	95 (D) 3 (M) 1 (E) 1 (E)	Bingöl	Clover
3	189662	<i>Trifolium</i> spp.: 95 Poaceae: 3 Rosaceae: 1 Ranunculaceae: 1	95 (D) 3 (M) 1 (E) 1 (E)	Bingöl	Clover
4	181237	<i>Trifolium</i> spp.: 94 Poaceae: 2 Asteraceae: 2 Brassicaceae: 2	94 (D) 2 (E) 2 (E) 2 (E)	Bingöl	Clover
5	206681	<i>Trifolium</i> spp.: 97 Poaceae: 2 Asteraceae: 1	97 (D) 2 (E) 1 (E)	Bingöl	Clover

* ≥%45 Dominant (D), (%16-44) Sekonder (S), (%3-15) Minor (M), (<%3) Vestige (V)



Citrus honey

Sample no.	Total Pollen Number (TPN)	Pollen taxa	%*	Geographical origin	Honey type
1	46501	Asteraceae: 102	43.03 (S)	Mersin	Citrus
		<i>Citrus</i> spp.: 50	21.09(D)*		
		Poaceae: 28	11.8 (M)		
		Apiaceae: 23	9.70 (M)		
		Plantago: 19	8.01 (M)		
		Fabaceae: 9	3.8 (M)		
		Brassicaceae: 4	1.68 (E)		
		Rosaceae: 1	0.42 (E)		
		Lamiaceae:1	0.42 (E)		
2	53961	Asteraceae: 100	48.3 (D)	Mersin	Citrus
		<i>Citrus</i> spp.: 35	17(D)*		
		Poaceae: 28	13.5 (M)		
		Plantago: 26	12.5 (M)		
		Fabaceae: 10	4.83 (M)		
		Apiaceae: 5	2.41 (E)		
		Rosaceae: 2	0.96 (E)		
		Brassicaceae: 1	0.48 (E)		
3	88124	Fabaceae: 45	39.13 (S)	Antalya	Citrus
		<i>Cistus</i> spp.: 30	26 (S)		
		Asteraceae: 20	17.4 (S)		
		<i>Citrus</i> spp.: 10	8.7 (M)		
		Ericaceae: 5	4.34 (M)		
		<i>Plantago</i> spp.: 3	2.60 (E)		
		Poaceae: 2	1.74 (E)		

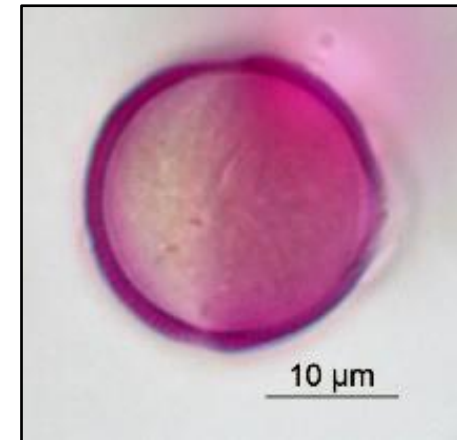
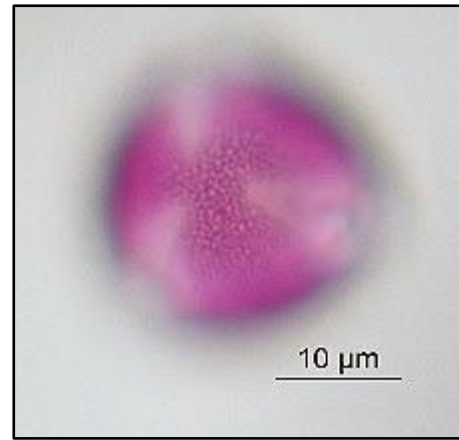
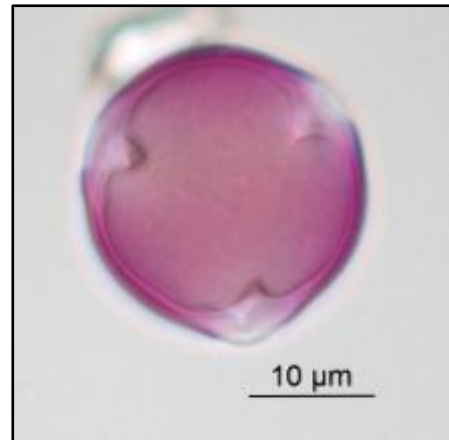
- ≥ 45 Dominant (D), (%16-44) Sekonder (S), (%3-15) Minor (M), (<%3) Vestige (V)
- Citrus honey pollen content is underrepresented and should be at least 10% for unifloral honey.



Mullein honey

Sample no.	Total Pollen Number (TPN)	Pollen taxa	%*	Geographical origin	Honey type
1	666795	<i>Verbascum</i> spp. Asteraceae Campanula	97(D) 2(E) 1(E)	Bingöl	Mullein

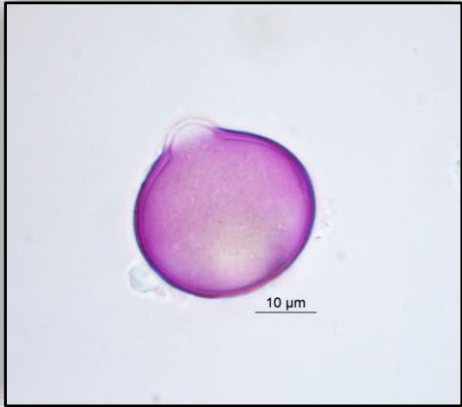
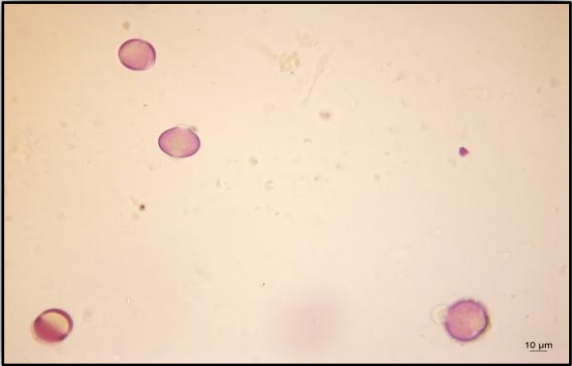
* ≥%45 Dominant (D), (%16-44) Sekonder (S), (%3-15) Minor (M), (<%3) Vestige (V)



Milkvetch honey

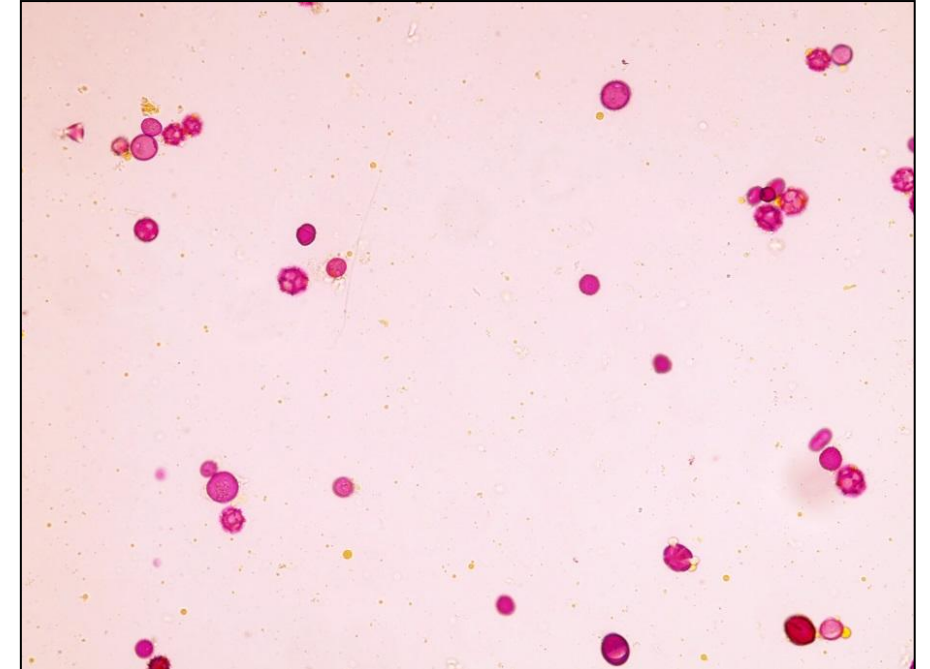
Sampl e no.	Total Pollen Number (TPN)	Pollen taxa	%*	Geographical origin	Honey type
1	184779		59 (D)	Tunceli	Milkvetch
		Astragalus spp.:	17 (S)		
		120	7.4		
		Trifolium spp.: 35	(M)		
		Vicia spp.: 15	3 (M)		
		Rosaceae: 6	2 (E)		
		Cistus: 4	2 (E)		
		Sanquisorba spp.: 4	2 (E)		
		Plantago spp.: 4	1.5		
		Castanea sativa: 3	(E)		
		Asteraceae: 2	1 (E)		
		Echium: 2	1 (E)		
		Poaceae: 2	1 (E)		
		Liliaceae: 2	1 (E)		
		Lamiaceae: 2	1 (E)		
		Brassicaceae: 1	0.5		
			(E)		

* ≥%45 Dominant (D), (%16-44) Sekonder (S), (%3-15) Minor (M), (<%3) Vestige (V)



Anzer Honey

Sam ple no.	Total Pollen Number (TPN)	Pollen taxa	%*	Geographic al origin	Honey type
1	14755	Cistaceae: 33	18.5(S)	Anzer	Anzer bali
		Fabaceae: 28	15.7(M)		
		Trifolium: 30	16.8(S)		
		Myosotis: 10	5.6(M)		
		Brassicaceae: 11	6.18(M)		
		Ericaceae: 3	1.68(V)		
		Medicago: 7	3.93(M)		
		Centaureae: 1	0.56(V)		
		Berberidaceae: 7	3.93(M)		
		Onobrychis: 7	3.93(M)		
		Betulaceae: 1	0.56(V)		
		Salix: 15	8.42(M)		
		Thymus: 4	2.24(V)		
		Rumex: 1	0.56(V)		
		Lamiaceae: 2	1.12(V)		
		Plantago: 3	1.68(V)		
		Epilobium: 1	0.56(V)		
		Caryophyllaceae:	2.80(V)		
		5	1.12(V)		
		Scabiosa: 2	0.56(V)		
		Asteraceae: 1	1.12(V)		
		Vicia: 2	1.68(V)		
		Teucrium: 3	0.56(V)		
		Rosaceae: 1			

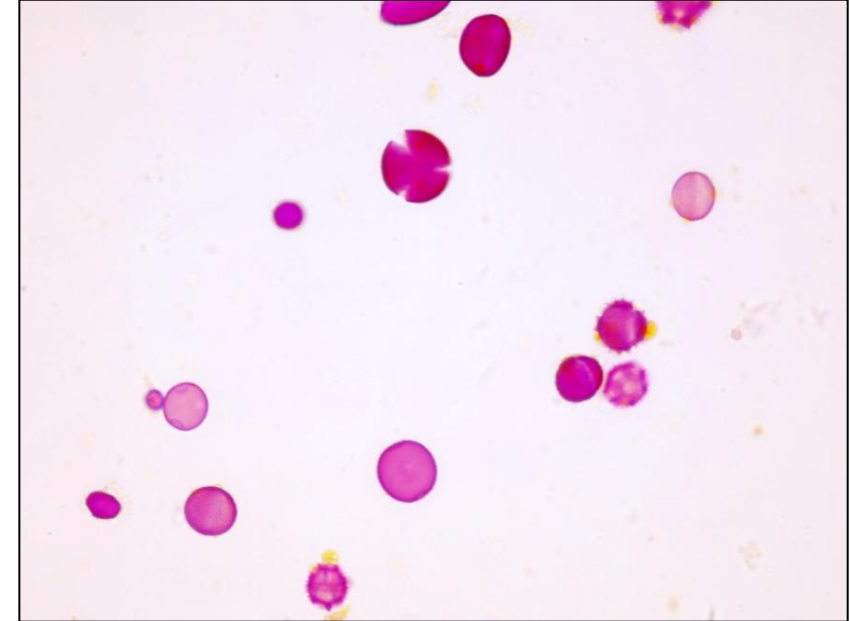


*≥%45 Dominant (D), (%16-44) Sekonder (S), (%3-15) Minor (M), (<%3) Vestige (V)

Ardahan Honey

Samp le no.	Total Pollen Number (TPN)	Pollen taxa	%*	Geographical origin	Honey type
1	10507	Cistaceae: 60	42(S)	Ardahan Posof- Bersumde Mevki	Ardahan
		Fabaceae: 28	19(S)		
		Scabiosa: 19	13.2(M)		
		Rosaceae: 8	5.5(M)		
		Campanula: 6	4.2(M)		
		Asteraceae: 5	3.5(M)		
		Lamiaceae: 4	2.77(V)		
		Echium: 4	2.77(V)		
		Taraxacum: 2	1.38(V)		
		Poaceae: 2	1.38(V)		
		Rosaceae: 1	0.70(V)		
		Brassicaceae: 1	0.70(V)		
		Apiaceae: 1	0.70(V)		
		Liliaceae: 1	0.70(V)		
		Onobrychis: 1	0.70(V)		
		Geraniaceae: 1	0.70(V)		
2	520206	Onobrychis: 110	43(S)	Ardahan Tunçoluk	Ardahan
		Echium: 50	19.6(S)		
		Trifolium: 50	19.6(S)		
		Liliaceae: 25	9.8(M)		
		Brassicaceae: 13	5(M)		
		Rosaceae: 2	0.8(V)		
		Taraxacum: 2	0.8(V)		
		Apiaceae: 1	0.4(V)		
		Lamiaceae: 1	0.4(V)		
		Poaceae: 1	0.4(V)		

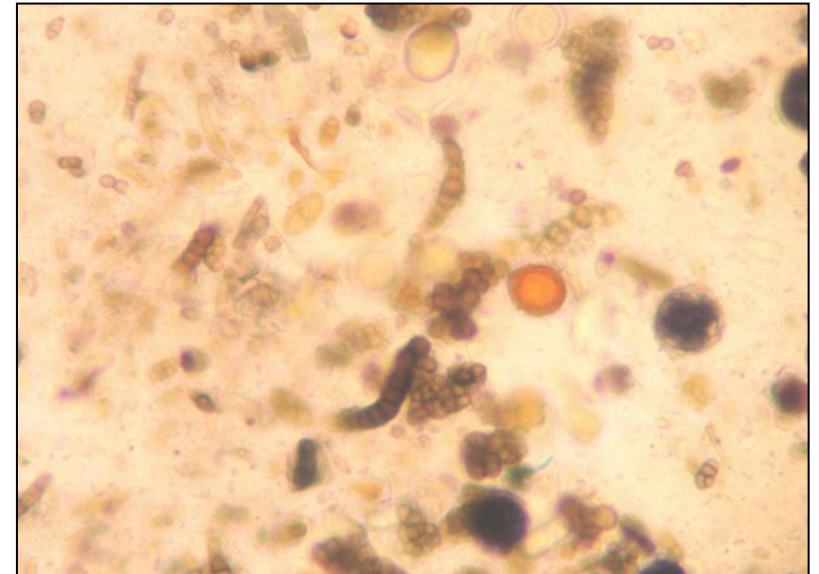
*≥%45 Dominant (D), (%16-44) Sekonder (S), (%3-15) Minor (M), (<%3) Vestige (V)



Pine Honey

Sample no.	Region	HDE	TPN	HDE/* TPN	Honey type
1	İzmir	218853	2188	100.02	Pine
2	İzmir	209562	2751	76.17	Pine
3	Muğla	123433	993.42	124.25	Pine
4	Muğla	541814.4	2 006.72	270	Pine
5	Çanakkale	941712.9	12754.58	73.83	Pine

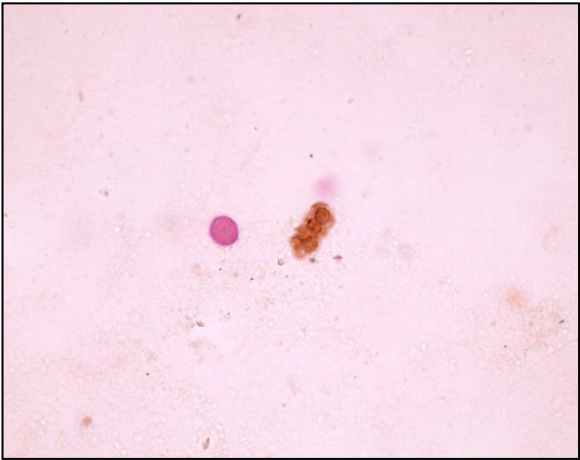
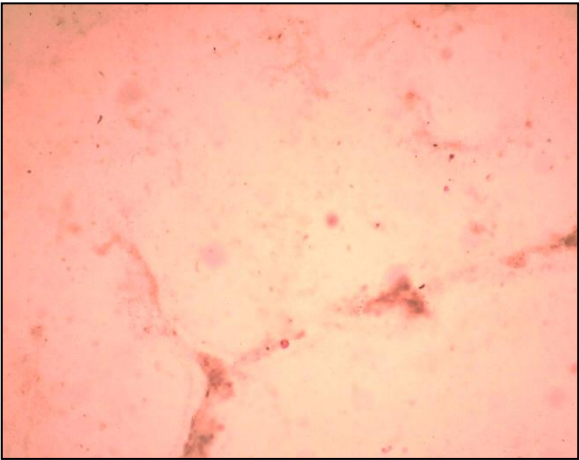
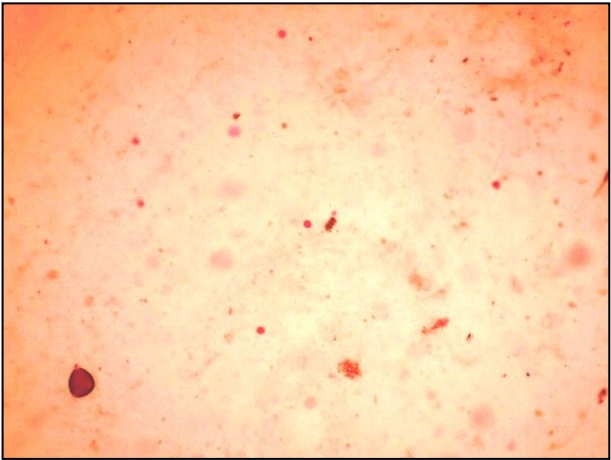
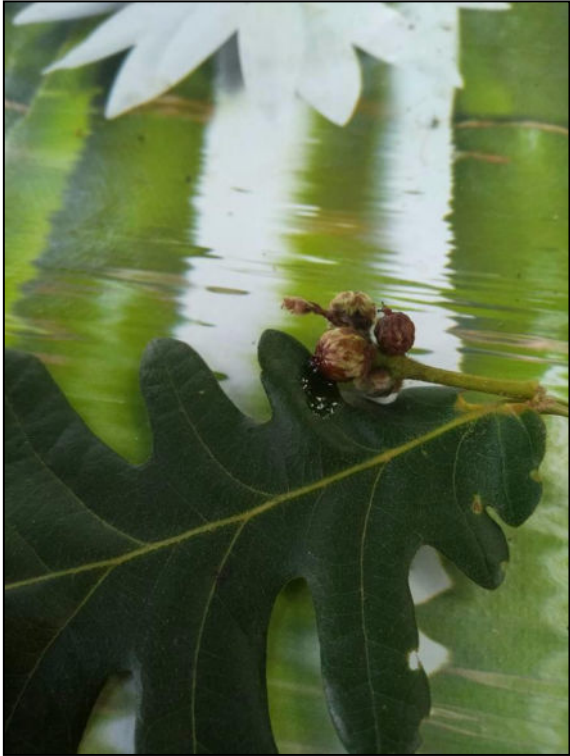
*HDE/TPN number >3 is pine honey



Oak Honey

Sample no.	Region	HDE	TPN	HDE/* TPN	Honey type
1	Kirklareli	37747	3653	10.33	Oak
2	Kirklareli	83061	15381	5.4	Oak

*HDE/TPN number >3 is pine honey



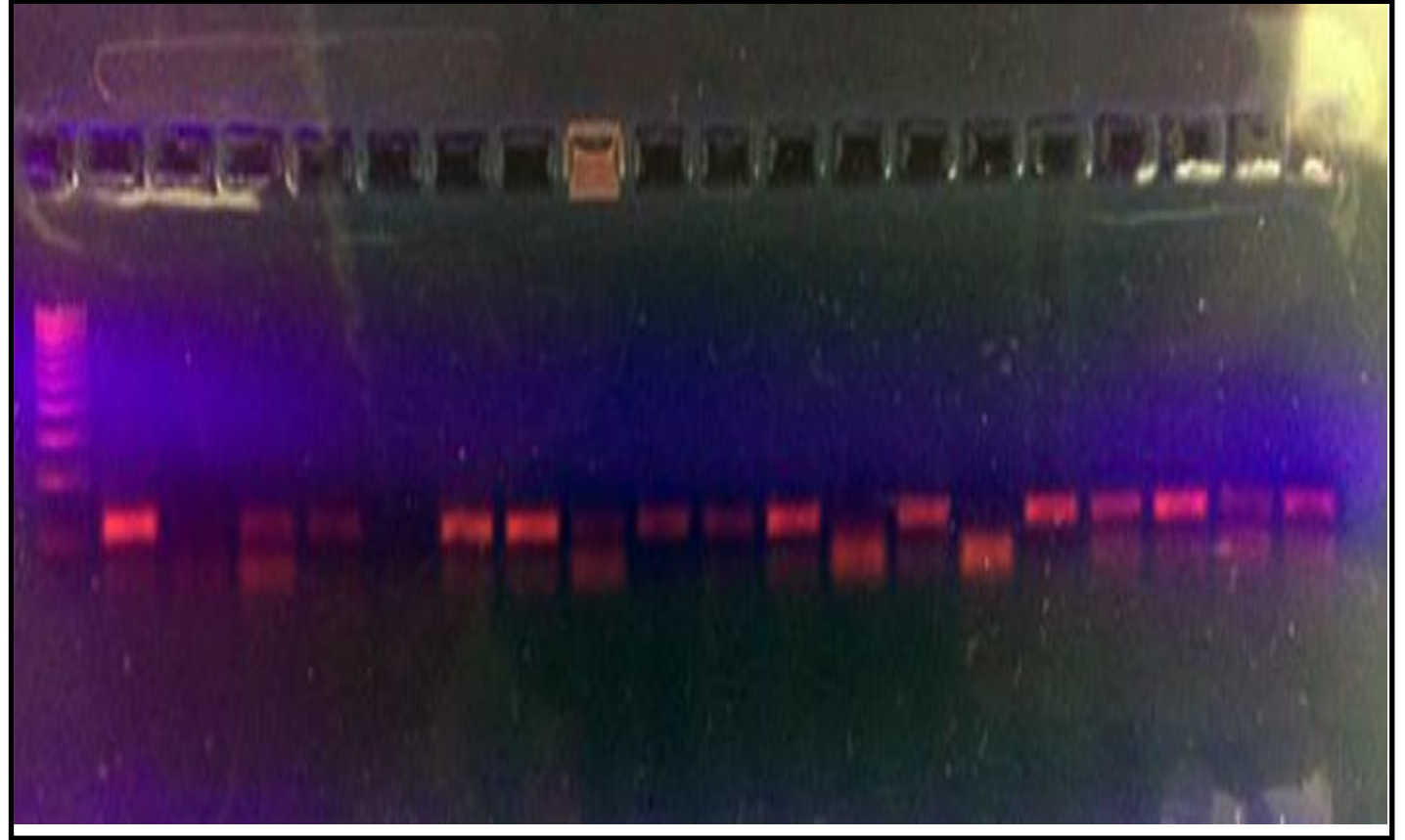
DNA Analysis Results

No	Honey Type	TPN or HDE/TPN	DNA Concentration (ng/μl)	Purity (260/280nm)
1	Sunflower	7651	38.02	0.86
2	Sunflower	12666	31.79	2.05
3	Sunflower	17271	57.23	1.7
4	Canola	45898	10.11	2.26
5	Canola	7313	4.19	1.68
6	Canola	63494	41.7	1.99
7	Chestnut	722419	50.39	1.87
8	Chestnut	511271	18.01	1.4
9	Chestnut	37626	36.36	1.79
10	Chestnut	292364	57	1.37
11	Chestnut	58975	15.79	2.3
12	Chestnut	398568	10.23	1.55
13	Chestnut	162796	7.68	1.99
14	Chestnut	63113	6.60	1.33
15	Rhododendron	118658	27.41	1
16	Rhododendron	4778	19.83	2
17	Mullein	666795	30	1.65
18	Clover	136341	7.96	1.71
19	Clover	185141	14.62	1.54
20	Clover	189662	10.83	1.56
21	Clover	181237	10.42	1.81
22	Clover	206681	54.34	2
23	Milkvetch	184779	21.31	1.75
24	Citrus	46501	10.7	1.49
25	Citrus	53961	9.75	1.68
26	Citrus	88124	10.15	1.35
27	Anzer	48161	41.16	2.01
28	Anzer	26781	24.38	1.91
29	Anzer	32609	21.23	1.68
30	Ardahan	8345	28.68	1.73
31	Ardahan	330442	11.99	1.72
32	Ardahan	20068	13.12	1.41
33	Ardahan	520206	16.74	1.93
34	Pine	100.02	10.69	1.55
35	Pine	76.17	5.15	1.37
36	Pine	124.25	6.89	1.51
37	Pine	270	23.76	2.07
38	Pine	73.83	2.62	1.76
39	Oak	10.33	191.03	1.64
40	Oak	5.4	24.55	1.93

- Nucleic acids give maximum absorbance at 260 nm, while proteins, phenol and other contaminants absorb at 280 nm. It is possible to comment on the purity of the isolated DNA by looking at the ratio of 260/280. The ratio of **260/280 ~ 1.8** is considered to be pure. This ratio, up or down, indicates the presence of RNA or protein contamination in the environment, respectively.
- The second important parameter after isolation is the amount of isolated DNA. Approximately **10-100 ng of DNA** (25 microliters volume) is used as a template for a standard PCR reaction. The use of template DNA at lower concentrations affects the success of amplification in the negative direction.

DNA Analysis Results

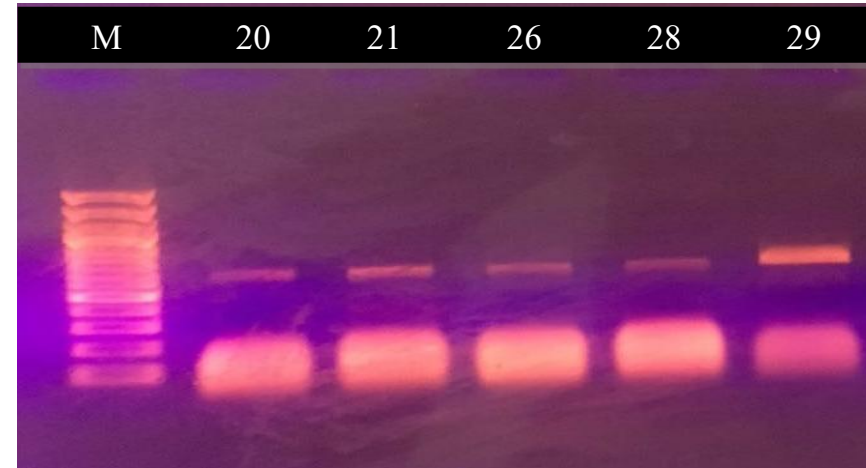
- The 18S rRNA gene was amplified to test the availability of the isolated DNA samples for PCR reactions. PCR results show that DNA isolations were carried out successfully.
- One of the main aims of the project was to isolate DNA from honey in a quality that could be used in new-generation DNA analyses. In this context, the project has achieved its goal and is ready for the Next Generation Sequencing (NGS) device to create DNA barcodes.



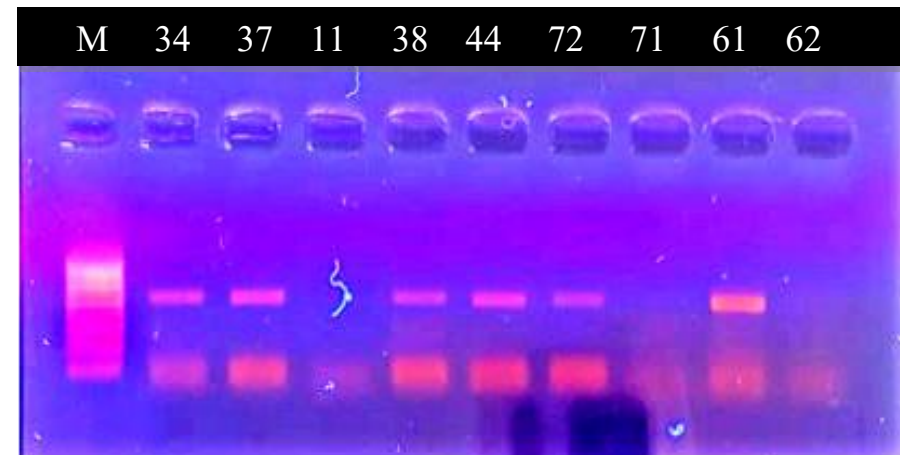
Amplification of the 18S rRNA gene

NGS Analysis Results

- PCR experiments were completed with universal primers used in barcoding studies for the *rbcl* and *trnH-psbA* gene regions.
- A total of 35 samples belonging to the *rbcl* and 31 *trnH-psbA* gene regions were prepared for NGS analysis.
- NGS analysis of 35 *rbcl* and 31 *trnH-psbA* gene region samples, whose PCR was completed at a quality that can be entered into NGS, was successfully completed.



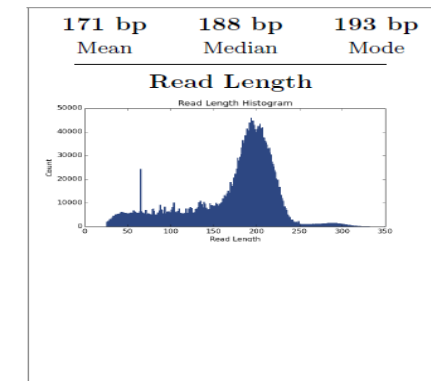
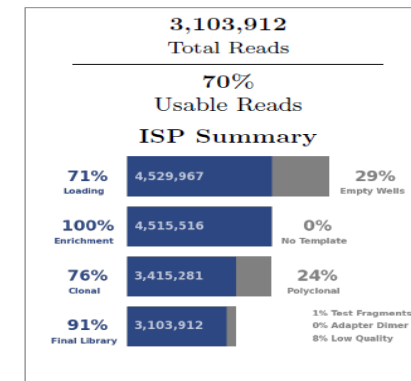
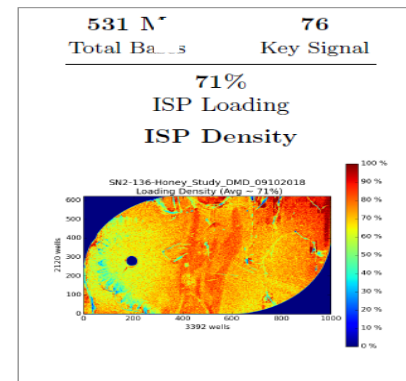
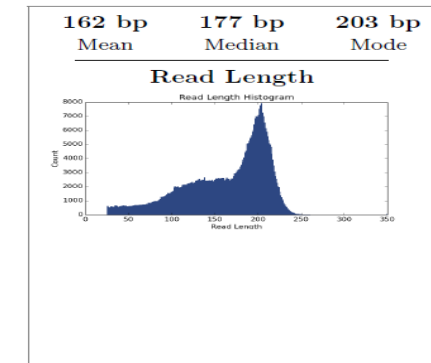
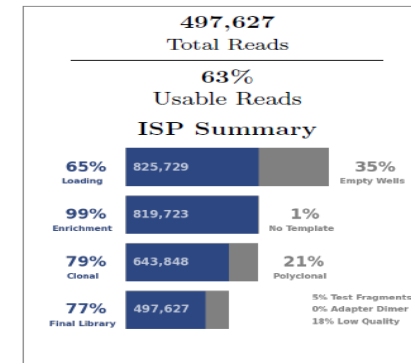
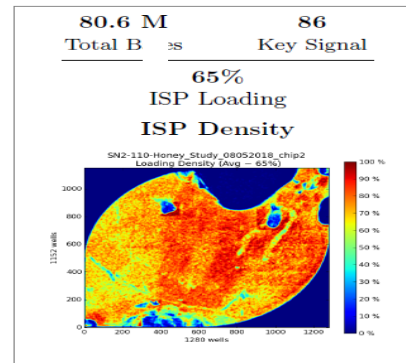
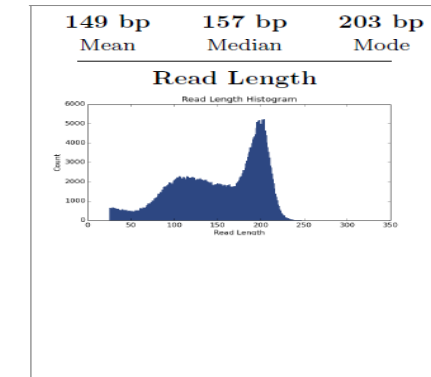
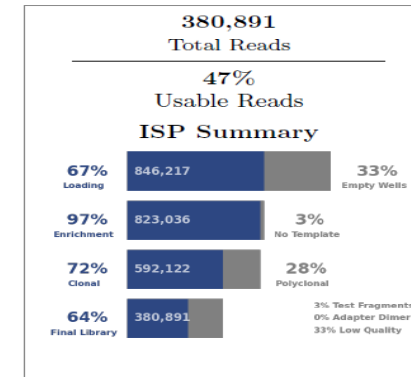
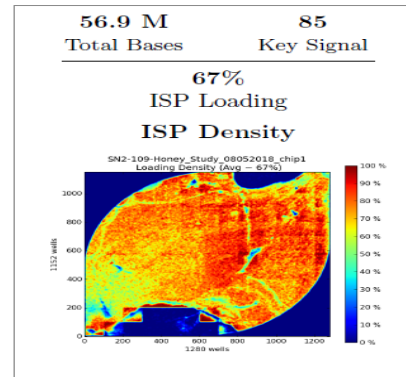
rbcl gene PCR results [M: Marker, 20-21: Anzer honey, 26-28-29: *Castanea sativa* (chestnut)]



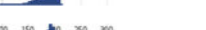
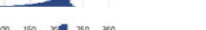
trnH-psbA gene PCR results

NGS Analysis Results

- The samples prepared for NGS experiments were analyzed in 3 sets.



NGS Analysis Results

Barcode Name	Sample	Bases	$\geq Q20$	Reads	Mean Read Length	Read Length Histogram	Barcode Name	Sample	Bases	$\geq Q20$	Reads	Mean Read Length	Read Length Histogram
No barcode	none	3,286,237	2,966,974	23,051	142 bp		IonXpress_049	rbcl.034	6,647,413	6,480,830	38,627	172 bp	
IonXpress_049	rbcl.20	4,728,633	4,539,812	31,855	148 bp		IonXpress_050	rbcl.22B	7,411,463	7,236,596	41,851	177 bp	
IonXpress_050	rbcl.21	3,997,556	3,830,299	28,383	140 bp		IonXpress_051	rbcl.70	8,122,141	7,905,549	47,285	171 bp	
IonXpress_051	rbcl.44	6,507,483	6,257,535	48,633	133 bp		IonXpress_052	rbcl.38	11,550,303	11,244,980	65,020	177 bp	
IonXpress_052	trn.6	4,911,720	4,371,402	34,532	142 bp		IonXpress_053	rbcl.63	9,956,579	9,693,428	56,870	175 bp	
IonXpress_053	trn.9	7,789,593	7,159,021	48,003	162 bp		IonXpress_054	rbcl.009	6,321,360	6,164,452	35,907	176 bp	
IonXpress_054	trn.29	4,786,972	4,433,632	29,198	163 bp		IonXpress_055	rbcl.28	8,175,298	7,926,226	45,681	178 bp	
IonXpress_055	trn.30	4,510,226	4,124,671	29,648	152 bp		IonXpress_056	rbcl.008	6,018,545	5,858,099	35,908	167 bp	
IonXpress_056	trn.33	5,956,940	5,424,996	35,740	166 bp		IonXpress_057	rbcl.011	5,245,847	5,113,539	29,725	176 bp	
IonXpress_057	trn.34	5,545,241	5,067,175	37,199	149 bp		IonXpress_058	rbcl.054	7,618,986	7,409,997	41,323	184 bp	
IonXpress_058	trn.37	4,841,738	4,421,616	34,435	140 bp		IonXpress_059	rbcl.61	6,413,836	6,235,440	35,143	182 bp	
IonXpress_059	trn.38	6,454,643	5,943,620	40,873	157 bp		IonXpress_060	rbcl.37	7,679,852	7,465,942	39,966	192 bp	
IonXpress_060	trn.61	3,208,003	2,959,804	19,261	166 bp		IonXpress_061	77M	6,916,636	6,542,967	38,288	180 bp	
IonXpress_061	trn.62	10,343,261	9,497,003	57,093	181 bp		IonXpress_065	rbcl.010	7,117,471	6,919,968	40,688	174 bp	
IonXpress_062	trn.63	9,290,415	8,591,661	60,562	153 bp		IonXpress_066	rbcl.16	11,339,406	10,994,940	58,223	194 bp	
IonXpress_063	trn.70	8,449,035	7,771,274	49,680	170 bp		IonXpress_067	rbcl.26	17,175,847	16,676,780	89,523	191 bp	
IonXpress_064	trn.72	13,255,136	11,962,992	82,322	161 bp		IonXpress_068	rbcl.32	16,709,486	16,260,147	85,067	196 bp	
IonXpress_065	trn.73	7,472,747	6,876,570	46,532	160 bp		IonXpress_069	rbcl.64	10,422,958	10,127,951	51,867	200 bp	
IonXpress_066	trn.77	8,012,819	7,405,219	51,825	154 bp		IonXpress_070	rbcl.65	10,927,338	10,632,735	55,239	197 bp	
IonXpress_067	trn.78	9,623,381	8,901,365	62,735	153 bp		IonXpress_071	rbcl.71	7,669,392	7,469,898	50,333	152 bp	
IonXpress_001	rbcl.67	6,761,428	6,578,687	36,048	187 bp		IonXpress_072	rbcl.73	14,498,343	14,088,722	71,974	201 bp	
IonXpress_002	rbcl.69	7,592,837	7,399,183	42,060	180 bp		IonXpress_073	trnh.16	16,073,308	15,031,315	78,403	205 bp	
IonXpress_003	rbcl.62	4,723,329	4,598,258	24,394	193 bp		IonXpress_074	trnh.22	18,960,077	17,826,958	98,287	192 bp	
IonXpress_004	rbcl.006	8,940,504	8,690,255	47,104	189 bp		IonXpress_075	trnh.28	15,410,518	14,336,011	77,520	198 bp	
IonXpress_005	rbcl.007	9,538,807	9,262,020	51,015	186 bp		IonXpress_076	trnh.32	18,496,742	17,148,362	92,432	200 bp	
IonXpress_006	rbcl.030	5,414,951	5,269,712	28,673	188 bp		IonXpress_077	trnh.54	12,214,454	11,508,489	64,409	189 bp	
IonXpress_007	rbcl.033	4,999,649	4,859,255	26,790	186 bp		IonXpress_078	trnh.64	9,284,225	8,684,879	57,448	161 bp	
IonXpress_008	rbcl.77	7,345,138	7,137,875	39,943	183 bp								
IonXpress_009	rbcl.72	8,521,682	8,293,658	45,249	188 bp								
IonXpress_010	44	11,081,681	10,550,238	58,609	189 bp								
IonXpress_011	65	6,879,421	6,548,991	38,636	178 bp								
IonXpress_012	26	9,110,631	8,706,636	50,592	180 bp								
IonXpress_013	67	6,319,871	6,159,704	35,991	175 bp								
IonXpress_014	rbcl.79	13,451,714	12,776,621	73,031	184 bp								
IonXpress_015	trnh.79	10,544,365	10,263,768	56,909	185 bp								
IonXpress_016	rbcl.78	2,555,533	2,484,226	14,403	177 bp								

➤ Read length, read sequence length and histograms of NGS-analyzed samples



Conclusion

- In the conclusion of this study, for the first time, honey samples collected from different geographical regions in Türkiye were barcoded using *rbcl* and *trnH-psbA* loci and compared using melissopalynology and NGS.
- Although there are some differences in the plants determined by both techniques, identified dominant floral components were shown high similarity.
- When the melissopalynological analysis and NGS analysis were compared in the study, it was shown that there were similar families according to the regions, and a higher number of families (%48) were determined in the NGS analysis.
- Results show that 53 families have an abundance of >20% when comparing the two methods. Of these, 29 taxa are found using both methods, giving 55% correspondence between DNA and microscopy.
- However, NGS analysis is disadvantageous compared to melissopalynological analysis, which is a cheaper and faster method, since plant reference information is scarce and it is an expensive method. So, more studies and comparisons between the two methods are needed.
- This study is particularly relevant for revealing Türkiye's unique and diverse flora, with the potential to be used as a reference atlas for honey bee floral resources.
- Furthermore, studies with NGS can be possible patenting of honeys' according to the provinces, geographical indication, determining GMO, endemic, and toxic plants' pollen in honey.



Comparing the melissopalynological and next generation sequencing (NGS) methods for the determining of botanical origin of honey

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ARTICLE INFO

Keywords:

DNA

Honey

Melissopalynology

NGS

rbcl

trnH-psbA

ABSTRACT

This study aims to compare the melissopalynological method with Next-Generation Sequencing (NGS) to determine honey's botanical and geographical origin. For NGS analysis, 40 honey samples in the collected 74 honey samples were selected by melissopalynological methods considering the total number of pollen, honeydew elements, and dominance pollen status. The quality of the isolated DNAs was determined by spectrophotometric and electrophoretic methods. DNA specimens extracted from the 40 honey samples yielded between 2 and 190 ng/μL. We compared NGS data using the rbcl and trnH-psbA DNA barcode marker with melissopalynological analysis. With NGS analysis, 3,020,963 sequence reads and 526,746,371 bases were obtained for 61 amplicons. NGS analysis detected 48% more plant families for all honey samples than the melissopalynological method, showing that NGS is a powerful and robust method for detecting honey provenance. In the conclusion of this study, for the first time, honey samples collected from different geographical regions in Turkey were barcoded using rbcl and trnH-psbA loci and compared using melissopalynology and NGS. Although there are some differences in the plants determined by both techniques, identified dominant floral components were shown high similarity. Results show that 53 families have an abundance of >20% when comparing the two methods. Of these, 29 taxa are found using both methods, giving 55% correspondence between DNA and microscopy. This study is particularly relevant for revealing Turkey's unique and diverse flora, with the potential to be used as a reference atlas for honey bee floral resources.

A. ÖZKÖK Et Al. , "Comparing the melissopalynological and next generation sequencing (NGS) methods for the determining of botanical origin of honey," Food Control , vol.148, 2023.

- This work was carried out with the project of Hacettepe University Scientific Research Projects (BAP) Coordination Unit FHD-2015-8179 and
- The continuation of this work is carried out with the project number TAGEM-16 / AR-GE / 31 by the General Directorate of Agricultural Researches (TAGEM).

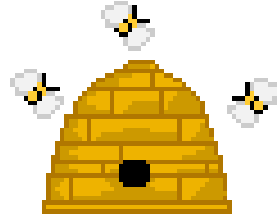




APIMONDIA
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COPENHAGEN



Thank you for your attention

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