

FUNGAL DIVERSITY IN FLORAL AND HONEYDEW HONEYS

D. MAGYAR¹, A. MURA-MÉSZÁROS² and F. GRILLENZONI³

¹Department of Air Hygiene and Aerobiology, National Public Health Centre
H-1097 Budapest, Gyáli út 2–6, Hungary; E-mail: magyar.donat@gmail.com

²Institute for Biology, Faculty of Veterinary Science, Szent István University
Budapest, Hungary

(Department of Cell and Molecular Biology, Leibniz Institute for Natural Product
Research and Infection Biology Hans Knöll Institute, Jena, Germany)

³Honeybee and Silkworm Research Unit, Consiglio per la Ricerca in Agricoltura e l'analisi
dell'economia Agraria, Bologna, Italy

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Studying fungal diversity in various environmental samples provides us with valuable knowledge about the occurrence of fungi of medical and ecological importance. Moreover, fungal composition may also characterise well the botanical and geographical source of food products, such as the origin of the spore enriched honeydew honeys. Thereby, we identified a wide spectrum of fungi found in 100 of honey samples from various geographical sources – most of them were from Italy, Greece and Hungary. Our honeydew honeys had a higher mean of the number of spore types found in them than floral honeys had. Statistically significant differences in diversity were found regarding the botanical source ($p = 1.29 \times 10^{-9}$) and the climatic classification ($p = 2.28 \times 10^{-2}$) according to Kruskal–Wallis rank sum tests. Most frequently encountered genera included ubiquitous saprotrophic species (*Alternaria*, *Cladosporium*, *Epicoccum nigrum*, *Stemphylium*), both in floral and honeydew honeys. On the other hand, certain sooty moulds like *Aureobasidium pullulans*, *Tripospermum* and *Capnobotrys* were rather present in different types of honeydew honeys. *Metschnikowia reukaufii*, the nectar inhabiting yeast reached considerably high quantities in floral honey samples. Present findings encourage further studies on quantifying the occurrence and the indicator value of specific fungal elements in honey, concerning its origin.

Key words: botanical origin, diversity, fungi, honey, honeydew

INTRODUCTION

The diversity of fungal species is an intensively studied subject because of ecological, phytopathological and pharmaceutical importance of fungi. Morphological identification of species is often used to detect various groups of fungi, e.g. allergenic and phytopathogenic fungi in air samples, Ingoldian fungi in stream water, fossilised spores in historical or forensic samples. Hon-

eys, especially those of honeydew origin are also rich in fungal spores (Dimou *et al.* 2006, Magyar *et al.* 2005, Pérez-Atanes *et al.* 2001, Seijo *et al.* 2011, Zander 1935). Spore content of honeys arose increasing interest, because it can help to determine their source. Knowing that fungi frequently live in a strong association with plants, different fungal species assemblages might be as typical of the honey as the spectrum of pollen grains.

Honeydew elements, namely fungal spores, hyphae fragments and algae are used as indicators of honeydew origin of honeys (Louveaux *et al.* 1978), due to their frequent accumulation in honeydew. Honeydew is the sugary secretion of phytophagous insects (i.e. Rinchota: Homoptera, Magyar *et al.* 2005). It is collected by bees mainly from late summer till September when there is a limited source of nectariferous flowers (Persano-Oddo *et al.* 2000). Honeydew honeys often reach a higher price than other types of honey – speaking mainly of Austria, Switzerland and Germany (Bogdanov and Martin 2002). In comparison with floral honeys, honeydew honeys have a higher mineral (González-Miret *et al.* 2005) and oligosaccharide content (Földházi 1994). Certain countries' total honey production, for example that of Greece, comes predominantly from honeydew honey (Thrasylvoulou and Manikis 1995). Thereby, the reliable identification of such honeys is of special commercial interest.

During traditional melissopalynological analysis, honeydew elements are quantified next to pollens. A careful differentiation between the pollen types of nectariferous and those of anemophilous plant species, as well as the ability to separate underrepresented elements from overrepresented ones, are important skills for an accurate honey validation process. As microscopical elements are direct biological indicators of the honey source and their analysis has a good reproducibility (Louveaux *et al.* 1978), their classification is surely useful. However, identification of fungal particles accounting for the majority of counted honeydew elements, is regrettably neglected. The lack of knowledge about fungal species occurring in honey samples, represented by their spores is a significant loss of information in honey analysis.

Therefore, our aim was to characterise fungal content in a collection of honey samples from various countries and botanical sources.

MATERIALS AND METHODS

We analysed a total of 100 samples (Table 1) from three continents (Fig. 1) and 21 countries (most of them were from Italy, Greece and Hungary). Thirty-one out of them were labelled as floral honey, while 62 of them as honeydew honey. Their botanical origin was previously identified using standard melissopalynological methods and guidelines described by Louveaux *et al.*

1978, Persano-Oddo and d'Albore 1989, Persano-Oddo *et al.* 2007, Sabatini *et al.* 2007 in the National Institute for Apiculture, Bologna. Seven samples were collected without pre-specified botanical origin.

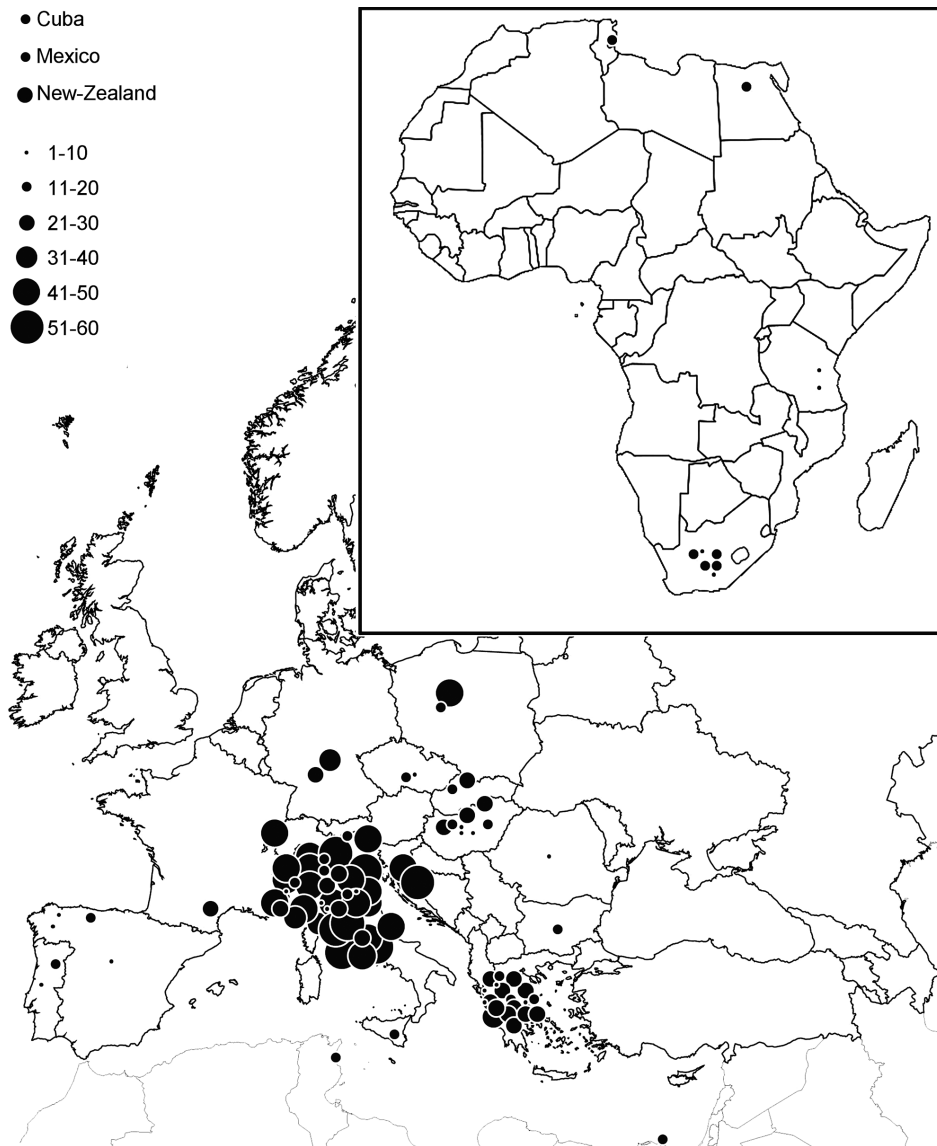


Fig. 1. Map showing all the locations, where samples were collected from. Samples are signified by spots and the diameter of each spot is proportional to the number of spore types found in the respective honey as indicated by the legend

Table 1

List and details of examined honey samples. Abbreviations: fl = floral, hd = honeydew, unk = unknown, WSC = warm summer continental, MT = maritime temperate, MTN = Mediterranean, WT = warm temperate, UD = undefined

Ref. no.	Honey type	Locality	Climate zone	Source of nectar or honeydew	Pollinator/producer of honeydew
1	fl	Africa	UD	unknown	<i>A. m.</i>
2	fl	Cuba	UD	unknown	<i>Melipona</i> sp.
3	fl	Egypt	Arid	unknown	<i>A. m.</i>
4	fl	Hungary: Oroszlány	WSC	<i>Phacelia tanacetifolia</i> Benth.	<i>A. m.</i>
5	fl	Hungary: Sárkeszi	WSC	<i>Foeniculum vulgare</i> Mill.	<i>A. m.</i>
6	fl	Hungary: Szentkirály	WSC	<i>Asclepias syriaca</i> L.	<i>A. m.</i>
7	fl	Italy	UD	<i>Hedysarum coronarium</i> L.	<i>A. m.</i>
8	fl	Central Italy	WSC	<i>Helianthus annuus</i> L.	<i>A. m.</i>
9	fl	N Italy	WSC	<i>Castanea sativa</i> Mill.	<i>A. m.</i>
10	fl	N Italy	WSC	<i>Tilia</i> sp.	<i>A. m.</i>
11	fl	Italy: Alps	WSC	<i>Rhododendron</i> sp.	<i>A. m.</i>
12	fl	Italy: Piemonte	WSC	<i>Taraxacum officinale</i> Weber	<i>A. m.</i>
13	fl	Italy: Sicily	MTN	<i>Citrus</i> sp.	<i>A. m.</i>
14	fl	Mexico	UD	unknown	<i>A. m.</i>
15	fl	New Zealand	MT	unknown	<i>A. m.</i>
16	fl	Poland	WSC	<i>Fagopyrum esculentum</i> Mill.	<i>A. m.</i>
17	fl	Portugal	MTN	<i>Rosmarinus officinale</i> L.	<i>A. m.</i>
18	fl	Portugal	MTN	unknown	<i>A. m.</i>
19	fl	South Africa	UD	<i>Acacia</i> sp.	<i>A. m.</i>
20	fl	South Africa	UD	unknown	<i>A. m.</i>
21	fl	South Africa	UD	<i>Citrus</i> sp.	<i>A. m.</i>
22	fl	South Africa	UD	unknown	<i>A. m.</i>
23	fl	South Africa	UD	unknown	<i>A. m.</i>
24	fl	South Africa	UD	unknown	<i>A. m.</i>
25	fl	Coastal N Spain	MT	<i>Eucalyptus</i> sp.	<i>A. m.</i>

Table 1 (continued)

Ref. no.	Honey type	Locality	Climate zone	Source of nectar or honeydew	Pollinator/producer of honeydew
26	fl	Spain	MT	<i>Citrus</i> sp.	<i>A. m.</i>
27	fl	NW Spain	MT	multifloral	<i>A. m.</i>
28	fl	NW Spain	MT	<i>Rubus</i> sp.	<i>A. m.</i>
29	fl	Tanzania	UD	unknown	<i>A. m.</i>
30	fl	Tanzania	UD	unknown	<i>A. m.</i>
31	fl	Tunisia	MTN	unknown	<i>A. m.</i>
32	hd	Bulgaria	WSC	unknown	<i>A. m.</i>
33	hd	Croatia	WT	unknown	<i>A. m.</i>
34	hd	Croatia	WT	unknown	<i>A. m.</i>
35	hd	Greece	MTN	<i>Abies alba</i> Mill.	<i>A. m.</i>
36	hd	Greece	MTN	<i>Abies alba</i> Mill.	<i>A. m.</i>
37	hd	Greece	MTN	<i>Abies alba</i> Mill.	<i>A. m.</i>
38	hd	Greece	MTN	<i>Abies alba</i> Mill.	<i>A. m.</i>
39	hd	Greece	MTN	<i>Abies alba</i> Mill.	<i>A. m.</i>
40	hd	Greece	MTN	<i>Abies alba</i> Mill.	<i>A. m.</i>
41	hd	Greece	MTN	<i>Abies alba</i> Mill.	<i>A. m.</i>
42	hd	Greece	MTN	<i>Abies alba</i> Mill.	<i>A. m.</i>
43	hd	Greece	MTN	<i>Abies alba</i> Mill.	<i>A. m.</i>
44	hd	Greece	MTN	<i>Abies alba</i> Mill.	<i>A. m.</i>
45	hd	Greece	MTN	<i>Abies alba</i> Mill.	<i>A. m.</i>
46	hd	Greece	MTN	<i>Pinus brutia</i> Tenore	<i>A. m.</i>
47	hd	Greece	MTN	<i>Pinus brutia</i> Tenore	<i>A. m.</i>
48	hd	Greece	MTN	<i>Pinus brutia</i> Tenore	<i>A. m.</i>
49	hd	Greece	MTN	<i>Pinus brutia</i> Tenore	<i>A. m.</i>
50	hd	Greece	MTN	<i>Pinus brutia</i> Tenore	<i>A. m.</i>
51	hd	Greece	MTN	<i>Pinus brutia</i> Tenore	<i>A. m.</i>
52	hd	Greece	MTN	<i>Pinus brutia</i> Tenore	<i>A. m.</i>
53	hd	Greece	MTN	<i>Pinus</i> sp.	<i>A. m.</i>
54	hd	Hungary	WSC	unknown	<i>A. m.</i>
55	hd	Hungary: JNSz County	WSC	unknown	<i>A. m.</i>
56	hd	Hungary: Keszthely	WSC	unknown	<i>A. m.</i>

Table 1 (continued)

Ref. no.	Honey type	Locality	Climate zone	Source of nectar or honeydew	Pollinator/producer of honeydew
57	hd	Hungary: Ózd	WSC	unknown	<i>A. m.</i>
58	hd	Hungary: Sárkeszi	WSC	unknown	<i>A. m.</i>
59	hd	Hungary: Solymár	WSC	unknown	<i>A. m.</i>
60	hd	Italy	UD	unknown	<i>A. m.</i>
61	hd	Italy	UD	unknown	<i>M. p. + A. m.</i>
62	hd	Italy	UD	unknown	<i>M. p. + A. m.</i>
63	hd	Italy	UD	<i>Abies alba</i> Mill.	<i>A. m.</i>
64	hd	Italy	UD	<i>Abies alba</i> Mill.	<i>A. m.</i>
65	hd	Italy	UD	<i>Abies alba</i> Mill.	<i>A. m.</i>
66	hd	Italy	UD	<i>Castanea sativa</i> Mill.	<i>A. m.</i>
67	hd	Italy	UD	<i>Castanea sativa</i> Mill.	<i>A. m.</i>
68	hd	Italy	UD	<i>Castanea sativa</i> Mill.	<i>A. m.</i>
69	hd	Italy	UD	<i>Castanea sativa</i> Mill.	<i>A. m.</i>
70	hd	Italy	UD	<i>Castanea sativa</i> Mill.	<i>A. m.</i>
71	hd	Italy: Abruzzo	WT	unknown	<i>A. m.</i>
72	hd	Italy: Friuli Venezia Lucia	WSC	unknown	<i>A. m.</i>
73	hd	Italy: Lazio	WSC	unknown	<i>A. m.</i>
74	hd	Italy: Liguria	WT	unknown	<i>M. p. + A. m.</i>
75	hd	Italy: Liguria	WT	unknown	<i>A. m.</i>
76	hd	Italy: Liguria	WT	unknown	<i>A. m.</i>
77	hd	Italy: Liguria	WT	unknown	<i>A. m.</i>
78	hd	Italy: Liguria	WT	unknown	<i>M. p. + A. m.</i>
79	hd	Italy: Liguria	WT	unknown	<i>M. p. + A. m.</i>
80	hd	Italy: Liguria	WT	unknown	<i>M. p. + A. m.</i>
81	hd	Italy: Liguria	WT	unknown	<i>A. m.</i>
82	hd	Italy: Liguria	WT	<i>Abies alba</i> Mill.	<i>A. m.</i>
83	hd	Italy: Lombardia	WSC	unknown	<i>A. m.</i>
84	hd	Italy: Piemonte	WSC	unknown	<i>A. m.</i>
85	hd	Italy: Toscana	WSC	unknown	<i>A. m.</i>
86	hd	Italy: Toscana	WSC	unknown	<i>A. m.</i>
87	hd	Italy: Toscana	WSC	unknown	<i>A. m.</i>

Table 1 (continued)

Ref. no.	Honey type	Locality	Climate zone	Source of nectar or honeydew	Pollinator/producer of honeydew
88	hd	Italy: Toscana	WSC	unknown	<i>A. m.</i>
89	hd	Italy: Trentino Alto Adige	WSC	unknown	<i>A. m.</i>
90	hd	Italy: Tusco-Emilian App.	WSC	<i>Abies alba</i> Mill. + <i>Picea excelsa</i> Link	<i>A. m.</i>
91	hd	Poland	WSC	unknown	<i>A. m.</i>
92	hd	Slovakia	WSC	unknown	<i>A. m.</i>
93	hd	Slovakia	WSC	unknown	<i>A. m.</i>
94	unk	Czech Republic	WSC	unknown	<i>A. m.</i>
95	unk	Czech Republic	WSC	unknown	<i>A. m.</i>
96	unk	France: Domaine St. Georges	MT	unknown	<i>A. m.</i>
97	unk	Germany: Altbulach	MT	unknown	<i>A. m.</i>
98	unk	Germany: Altensteig-Überberg	MT	unknown	<i>A. m.</i>
99	unk	Romania: Székelyudvarhely	WSC	unknown	<i>A. m.</i>
100	unk	Switzerland: Lesponts-de-Martel	WSC	unknown	<i>A. m.</i>

A. m. = *Apis mellifera* L.

M. p. = *Metcalfa pruinosa* Say

For each sample, 10 g were taken from 500 g of previously homogenised honey, dissolved in 20 ml of distilled water at 40 °C, centrifuged for 10 min at 560 g and allowed to settle. The sediment was recovered in 10 ml of distilled water and again centrifuged. The sediment was then collected with a Pasteur pipette and dried onto microscope slides at 40 °C. It was then mounted in glycerine-gelatine and covered (Louveaux *et al.* 1978). The entire surface of each preparation (18 mm × 18 mm) was scanned under 600× magnification of an Olympus CX 31 microscope. Identification of fungal spores was carried out both from experience and by means of scientific literature and monographs (Ellis and Ellis 1997, Hughes 1958, Ingold 1971, Kendrick 1990, Lacey and West 2006).

The samples were classified according to their locality into climate zones (Biondi and Baldoni 1994, Peel *et al.* 2007). If the geographical source of the honey was not known specifically enough, e.g. in the case of certain honeys

from Italy, the sample's climate zone was described as "undefined", because more than one zone is covered by the respective country.

Non-parametric comparative methods were used to test the difference between the spore type diversities in floral and honeydew honeys. Kruskal–Wallis rank sum tests were performed to see whether the botanical source or the climate of the locality affects this kind of diversity. The statistical tests were carried out in R v.3.0.2. Diagrams were generated in Microsoft Office Excel 2010.

RESULTS

Diversity of fungal taxa in samples

A total of 227 types of spores (Table 2) were found in 100 samples. We identified 94 of them on genus level, while 81 were specified on species level. The following types were present in more than half of all of our honey

Table 2
List of recorded spore types and the percentage of honeydew honey (HD) and floral honey (FL) samples where they were recorded

Ref. no.	Spore type	HD (%)	FL (%)
1	<i>Acroconidiella tropaeoli</i> (T. E. T. Bond) J. C. Lindq. et Alippi	0.00	12.90
2	<i>Acrodictys</i> sp.	6.45	6.45
3	Agaricomycetes	61.29	38.71
4	<i>Aglaospora profusa</i> (Fr.) De Not.	11.29	3.23
5	<i>Agrocybe</i> sp.	12.90	6.45
6	<i>Albugo</i> sp.	12.90	3.23
7	<i>Alternaria</i> ? <i>alternata</i> (Fr.) Keissl.	9.68	3.23
8	<i>Alternaria</i> sp.	90.32	80.65
9	<i>Ampulliferina persimplex</i> B. Sutton	1.61	0.00
10	<i>Anellophora</i> sp. ?	16.13	3.23
11	<i>Antennatula</i> sp.	14.52	3.23
12	<i>Anthostomella/Herpotrichiella</i> spp.	27.42	0.00
13	<i>Anthracoidea</i> sp.	8.06	0.00
14	<i>Arthrinium cuspidatum</i> (Cooke et Harkn.) Tranzschel	1.61	3.23
15	<i>Arthrinium</i> sp.	43.55	12.90
16	<i>Articulospora</i> sp.	3.23	0.00
17	<i>Ascobolus</i> sp.	4.84	0.00
18	<i>Aspergillus/Penicillium</i> spp.	4.84	12.90

Table 2 (continued)

Ref. no.	Spore type	HD (%)	FL (%)
19	<i>Aspergillus</i> sect. Nigri	1.61	0.00
20	<i>Asterosporium asterospermum</i> (Pers.) S. Hughes	1.61	0.00
21	<i>Atichia millardetii</i> Racib.	22.58	3.23
22	<i>Aureobasidium pullulans</i> (de Bary et Löwenthal) G. Arnaud	25.81	3.23
23	<i>Belemnospora verruculosa</i> P. M. Kirk	16.13	9.68
24	<i>Beltrania rhombica</i> Penz.	1.61	3.23
25	<i>Bipolaris</i> sp.	11.29	0.00
26	<i>Bipolaris spicifera</i> (Bainier) Subram.	14.52	6.45
27	Boletaceae	16.13	6.45
28	<i>Botrytis</i> sp.	30.65	6.45
29	<i>Bovista</i> sp.	1.61	0.00
30	<i>Caloplaca</i> sp.	29.03	0.00
31	<i>Camarosporium</i> sp.	14.52	3.23
32	<i>Camposporium</i> sp.	1.61	0.00
33	<i>Capnobotrys</i> sp.	16.13	3.23
34	<i>Ceratosporium cornutum</i> Matsush.	8.06	0.00
35	<i>Cercospora</i> sp.	4.84	9.68
36	<i>Cerebella andropogonis</i> Ces.	0.00	16.13
37	<i>Chaetomium</i> sp.	14.52	9.68
38	<i>Chaetosphaerella</i> sp.	9.68	0.00
39	<i>Cheiromycella microscopica</i> (P. Karst.) S. Hughes	24.19	0.00
40	<i>Cladosporium ? aecidiicola</i> Thüm.	1.61	0.00
41	<i>Cladosporium ? phlei</i> (C. T. Greg.) G. A. de Vries	25.81	3.23
42	<i>Cladosporium</i> spp.	69.35	48.39
43	<i>Clasterosporium</i> sp.?	37.10	3.23
44	<i>Clypeosphaeria notarisi</i> Fuckel	32.26	0.00
45	<i>Colletotrichum</i> sp.	4.84	0.00
46	<i>Coniosporium</i> sp.	11.29	3.23
47	<i>Coprinus ? niveus</i> (Pers.) Fr.	4.84	0.00
48	<i>Coprinus</i> sp.	37.10	22.58
49	<i>Cortinarius</i> sp.	22.58	3.23
50	<i>Corynespora</i> spp.?	9.68	6.45
51	<i>Coryneum</i> sp.	14.52	3.23

Table 2 (continued)

Ref. no.	Spore type	HD (%)	FL (%)
52	<i>Cucurbitaria</i> sp.	6.45	0.00
53	<i>Curucispora</i> sp.*	0.00	0.00
54	<i>Curvularia brachyspora</i> Boedijn	0.00	3.23
55	<i>Curvularia catenulata</i> S. M. Reddy et Bilgrami	0.00	3.23
56	<i>Curvularia geniculata</i> (Tracy et Earle) Boedijn	0.00	3.23
57	<i>Curvularia leonensis</i> M. B. Ellis	0.00	3.23
58	<i>Curvularia</i> spp.	9.68	25.81
59	<i>Dendryphion digitatum</i> Subram.	12.90	0.00
60	<i>Diapleella clivensis?</i> (Berk. et Broome) Munk.	11.29	0.00
61	Diatrypaceae	0.00	3.23
62	<i>Dicranidion</i> sp.	11.29	0.00
63	<i>Dictyopolyschema pirozynskii</i> M. B. Ellis	17.74	0.00
64	<i>Dictyosporium toruloides</i> (Corda) Guég.	1.61	6.45
65	<i>Diplocladiella scalaroides</i> G. Arnaud ex M. B. Ellis	1.61	3.23
66	<i>Diplodia frumenti</i> Ellis et Everh./ <i>Lasiodiplodia theobromae</i> (Pat.) Griffon et Maubl.	0.00	16.13
67	<i>Diplodia</i> spp.	30.65	9.68
68	<i>Discostroma corticola</i> (Fuckel) Brockmann	37.10	6.45
69	<i>Drechslera biseptata</i> (Sacc. et Roum.) M. J. Richardson et E. M. Fraser	3.23	3.23
70	<i>Dwayaangam dichotoma</i> Nawawi	1.61	0.00
71	<i>Dwayangaam</i> spp.	1.61	0.00
72	<i>Endophragmia bisby</i> B. Sutton	4.84	0.00
73	<i>Endophragmiella taxi</i> (M. B. Ellis) S. Hughes	1.61	0.00
74	<i>Epicoccum nigrum</i> Link	82.26	77.42
75	<i>Excipularia fusispora</i> (Berk. et Broome) Sacc.	45.16	6.45
76	<i>Excipularia narsapurensis</i> Subram.	0.00	3.23
77	<i>Exserohilum</i> sp.	3.23	3.23
78	<i>Flabellospora</i> sp.	4.84	3.23
79	<i>Fusarium</i> spp. (macroconidia)	16.13	6.45
80	<i>Fusicladium</i> sp.	9.68	0.00
81	<i>Ganoderma</i> sp.	58.06	6.45
82	<i>Gyoerffyella myrmecophagiformis</i> Melnik and Dudka	1.61	0.00

Table 2 (continued)

Ref. no.	Spore type	HD (%)	FL (%)
83	<i>Helicogermis</i> sp.	0.00	3.23
84	<i>Helicosporium</i> sp.	12.90	3.23
85	<i>Helicosporium</i> state of <i>Tubeufia palmarum</i> (Torrend) Samuels, Rossman et E. Müll.*	0.00	0.00
86	<i>Helminthosporium/Drechslera</i> spp.	48.39	32.26
87	<i>Hypoxylon fuscum?</i> (Pers.) Fr.	17.74	3.23
88	<i>Isthmologispora ampulliformis</i> (Tubaki) de Hoog et Hennebert	1.61	0.00
89	<i>Isthmospora spinosa</i> F. L. Stevens	1.61	0.00
90	<i>Isthmotricladia</i> sp.	8.06	0.00
91	<i>Lactarius/Russula</i> sp.	1.61	0.00
92	<i>Lasiosphaeria</i> spp.	3.23	0.00
93	<i>Lemonniera</i> sp.	8.06	3.23
94	<i>Leptosphaeria pleurospora</i> Niessl	1.61	0.00
95	<i>Leptosphaeria thurgoviensis</i> E. Müll.	1.61	0.00
96	<i>Leptosphaeria</i> spp.	50.00	19.35
97	<i>Lophiostoma vicinum</i> Sacc.	27.42	6.45
98	<i>Lylea tetracoila</i> (Corda) Hol.-Jech.	1.61	00.00
99	<i>Melampsoridium/Cronartium/Melampsora</i> spp.	62.90	22.58
100	<i>Metschnikowia reukaufii</i> Pitt. et M. W. Miller	27.42	58.06
101	<i>Microbotryum reticulatum</i> (Liro) R. Bauer et Oberw.	8.06	0.00
102	<i>Microbotryum violaceum</i> (Pers.) G. Deml et Oberw.	14.52	3.23
103	Mucorales	1.61	0.00
104	<i>Mycocentrospora</i> sp.	3.23	0.00
105	<i>Mycosphaerella</i> sp.	1.61	0.00
106	Myxomycetes	56.45	29.03
107	<i>Nakataea sigmoidea</i> (Cavara) Hara	14.52	3.23
108	<i>Neohendersonia kickxii</i> (Westend.) B. Sutton et Pollack	9.68	0.00
109	<i>Nigrospora</i> sp.	11.29	19.35
110	<i>Oidium</i> sp.	20.97	3.23
111	<i>Oncopodiella trigonella</i> (Sacc.) Rifai	6.45	0.00
112	<i>Ovulariopsis</i> sp.	1.61	0.00
113	<i>Paraphaeosphaeria michotii</i> (Westend.) O. E. Erikss.	17.74	3.23
114	<i>Parapyricularia</i> sp.?	8.06	0.00

Table 2 (continued)

Ref. no.	Spore type	HD (%)	FL (%)
115	<i>Passalora</i> sp.	20.97	0.00
116	<i>Periconia</i> sp.	61.29	35.48
117	<i>Periconia/Tilletia</i> sp.*	0.00	0.00
118	Peronosporaceae-type sporangia	22.58	9.68
119	<i>Pestalotiopsis</i> spp.	16.13	9.68
120	<i>Pestalotiopsis stevensonii</i> (Peck) Nag Raj	11.29	0.00
121	<i>Phragmidium</i> sp.	3.23	0.00
122	<i>Pithomyces? cynodontis</i> M. B. Ellis	1.61	6.45
123	<i>Pithomyces chartarum</i> (Berk. et M. A. Curtis) M. B. Ellis	50.00	25.81
124	<i>Pleospora rubelloides</i> (Plowr. ex Cooke) J. Webster	14.52	0.00
125	<i>Pleospora</i> sp.	33.87	12.90
126	<i>Pollaccia elegans</i> Servazzi	1.61	0.00
127	<i>Pollaccia</i> sp.	6.45	0.00
128	<i>Polythrincium trifolii</i> Speg.	45.16	6.45
129	<i>Psammia</i> sp.	0.00	0.00
130	Pucciniaceae spp. (teliospores)	3.23	0.00
131	Pucciniaceae spp. (uredospores)	59.68	48.39
132	<i>Pyrigemmula aurantiaca</i> D. Magyar et Shoemaker	22.58	0.00
133	<i>Rebentischia unicaudata</i> (Berk. et Broome) Sacc.	16.13	0.00
134	<i>Retiarius bovicornutus</i> D. L. Olivier	12.90	0.00
135	<i>Retiarius/Trinacrium</i> spp.	8.06	0.00
136	<i>Rhizopus</i> sp.	0.00	3.23
137	<i>Rhynchosporium</i> sp.	1.61	0.00
138	<i>Sarcostroma arbuti</i> (Bonar) M. Morelet	3.23	0.00
139	<i>Scopinella</i> sp.	1.61	0.00
140	<i>Scopulariopsis</i> sp.	1.61	0.00
141	<i>Seimatosporium</i> sp.	11.29	0.00
142	<i>Sirosporium/Acrodictys</i> sp.	8.06	0.00
143	<i>Sordaria</i> sp.	1.61	0.00
144	<i>Spegazzinia</i> sp.	4.84	29.03
145	<i>Sphaeropsis</i> sp. 1	9.68	0.00
146	<i>Sphaeropsis</i> sp. 2	1.61	0.00
147	<i>Spilocaea</i> spp.	37.10	6.45

Table 2 (continued)

Ref. no.	Spore type	HD (%)	FL (%)
148	<i>Splanchnonema</i> spp.	4.84	0.00
149	<i>Sporidesmium brachypus</i> (Ellis et Everh.) S. Hughes	4.84	0.00
150	<i>Sporidesmiella brachysporioides</i> T. Y. Zhang et W. B. Kendr.*	0.00	0.00
151	<i>Sporidesmiella hyalosperma</i> (Corda) P. M. Kirk	6.45	0.00
152	<i>Sporidesmium leptosporum</i> (Sacc. et Roum.) S. Hughes	1.61	0.00
153	<i>Sporidesmium macrotrichum</i> (Corda) S. Hughes	8.06	0.00
154	<i>Sporidesmium</i> spp.	25.81	3.23
155	<i>Sporormiella</i> sp.	8.06	3.23
156	<i>Stachybotrys</i> sp.	19.35	3.23
157	<i>Stegosporium</i> sp.	1.61	0.00
158	<i>Stemphylium solani</i> G. F. Weber	3.23	0.00
159	<i>Stemphylium</i> spp.	67.74	41.94
160	<i>Stenellopsis fagraeae</i> B. Huguenin	1.61	0.00
161	<i>Stigmia</i> sp.	1.61	0.00
162	<i>Taeniolella ? breviuscula</i> (Berk. et M. A. Curtis) S. Hughes	3.23	0.00
163	<i>Taeniolella</i> sp.	48.39	6.45
164	Telephoraceae	14.52	3.23
165	<i>Teloschistes/Xanthoria</i> sp.	1.61	3.23
166	<i>Tetraploa aristata</i> Berk. et Broome	1.61	3.23
167	<i>Thielaviopsis</i> sp.	1.61	0.00
168	<i>Thyrostroma negundinis</i> (Berk. et M. A. Curtis) A. W. Ramaley	4.84	0.00
169	<i>Tilletia</i> sp.	9.68	0.00
170	<i>Torula</i> sp.	48.39	54.84
171	<i>Toxosporium</i> sp.	0.00	0.00
172	<i>Tranzscheliella hypodytes</i> (Schltdl.) Vánky et McKenzie	11.29	3.23
173	<i>Tranzscheliella williamsii</i> (Griffiths) Dingley et Versluys	8.06	3.23
174	<i>Triadelphia heterospora</i> Shearer et J. L. Crane	9.68	0.00
175	<i>Triadelphia uniseptata</i> (Berk. et Broom) P. M. Kirk	19.35	3.23
176	<i>Tricellula</i> sp.	4.84	0.00
177	<i>Trichotecium roseum</i> (Pers.) Link	1.61	3.23
178	<i>Tricladium angulatum</i> Ingold*	0.00	0.00
179	<i>Trifurcospora</i> sp.	4.84	0.00
180	<i>Trimmatostroma salicis</i> Corda	25.81	0.00

Table 2 (continued)

Ref. no.	Spore type	HD (%)	FL (%)
181	<i>Trimmatostroma scutellare</i> (Berk. et Broome) M. B. Ellis	11.29	0.00
182	<i>Trinacrium gracile</i> Matsush.	1.61	0.00
183	<i>Trinacrium incurvum</i> Matsush.	1.61	0.00
184	<i>Trinacrium robustum</i> Tzean et J. L. Chen	1.61	0.00
185	<i>Trinacrium</i> sp. 1	11.29	0.00
186	<i>Trinacrium</i> sp. 2	1.61	0.00
187	<i>Trinacrium subtile</i> Riess	3.23	0.00
188	<i>Tripospermum camelopardus</i> Ingold, Dann et P. J. McDougall	16.13	0.00
189	<i>Tripospermum</i> spp.	70.97	12.90
190	<i>Urocystis</i> sp.	8.06	0.00
191	<i>Ustilago bromivora</i> (Tul. et C. Tul.) A. A. Fisch. Waldh.	24.19	12.90
192	<i>Ustilago</i> sp.	43.55	29.03
193	<i>Valsaria ? insitiva</i> (Tode) Ces. et De Not.	9.68	0.00
194	<i>Varicosporium elodeae</i> W. Kegel	8.06	0.00
195	Xylariaceae	45.16	12.90
196	<i>Zygothiala jamaicensis</i> E. W. Mason.	14.52	6.45
197	<i>Zygosporium geminatum</i> S. Hughes	0.00	3.23
198	<i>Zygosporium masonii</i> S. Hughes ?	1.61	0.00
199	unknown Ascomycetes (<i>Nectria?</i>)	3.23	0.00
200	unknown Ascomycetes (<i>Venturia?</i>)	6.45	0.00
201	unknown helicospore type 1	3.23	0.00
202	unknown phaeoamerspore type 1 (<i>Mammaria?</i>)	19.35	0.00
203	unknown phaeoamerspore type 2	11.29	0.00
204	unknown phaeodidymospore type 2	4.84	0.00
205	unknown phaeophragmspore type 1	1.61	0.00
206	unknown cheirospore type 1	0.00	0.00
207	unknown scolecospore type 1	33.87	0.00
208	unknown scolecospore type 2	4.84	3.23
209	unknown scolecospore type 3	16.13	0.00
210	unknown scolecospore type 4	3.23	0.00
211	unknown scolecospore type 5	1.61	0.00
212	unknown scolecospore type 6	8.06	0.00
213	unknown scolecospore type 7	1.61	0.00

Table 2 (continued)

Ref. no.	Spore type	HD (%)	FL (%)
214	unknown <i>Atichia</i> -like spore type	53.23	3.23
215	unknown staurospore type 1	3.23	0.00
216	unknown staurospore type 3	1.61	3.23
217	unknown staurospore type 4	1.61	0.00
218	unknown staurospore type 5	1.61	0.00
219	unknown staurospore type 6	4.84	0.00
220	unknown staurospore type 7	1.61	0.00
221	unknown staurospore type 8	1.61	0.00
222	unknown staurospore type 9	1.61	0.00
223	unknown staurospore type 10	1.61	0.00
224	unknown staurospore type 11*	0.00	0.00
225	unknown staurospore type 12*	0.00	0.00
226	unknown staurospore type 13*	0.00	0.00
227	unknown staurospore type 14*	0.00	0.00

*Spore type that only occurred in samples without pre-specified botanical origin (not classified as honeydew or floral honey)

samples: *Alternaria* (88%), *Epicoccum* (81%), *Cladosporium* (65%), *Stemphylium* (59%), Pucciniaceae uredospores (57%), miscellaneous Agaricomycetes basidiospores (55%). *Tripospermum* occurred in 53% of samples. Precisely half of the honey samples contained spores belonging to *Melampsorium/Cronartium/Melampsora* group, *Torula* spp. and *Aspergillus/Penicillium* type conidia.

In this study, means of the number of identified spore types differed between honeydew (30.27 ± 15.21) and floral honeys (11.87 ± 5.64). Non-parametric methods showed significant difference between these two main groups of samples ($p = 2.002 \times 10^{-16}$ for Kolmogorov–Smirnov test, $p = 1.301 \times 10^{-13}$ for Brunel–Munzel test). The mean of the number of spore types (46.20 ± 12.46) was outstanding in the case of *Castanea sativa* honeys ($N = 5$). *Abies* ($N = 16$) samples had a mean of 27.94 ± 10.74 while in honeydew honeys from *Pinus* trees ($N = 8$) averagely 14.75 ± 9.02 types of spores were present (Fig. 2).

Honeys from the maritime temperate zone ($N = 8$, 17.00 ± 12.40) and from the Mediterranean regions ($N = 23$, 18.17 ± 9.25) contained averagely a lower number of spore types than honeys from other categories (Fig. 3). Samples from warm temperate regions ($N = 12$) had the highest mean (34.25 ± 15.88). At the same time, honeys coming from the warm summer continental zone ($N = 33$, 24.44 ± 15.18) reached an intermediate value.

Based on Kruskal–Wallis rank sum tests, differences in the number of spore types found are statistically significant regarding the botanical source ($p = 1.29 \times 10^{-9}$) and the climatic categories ($p = 2.28 \times 10^{-2}$) as well.

Comparison between the most occurring taxa in honeydew and floral honeys

The 13 most frequent taxa of honeydew honeys and floral honeys were also compared (Figs 4–5). The genera *Alternaria* and *Epicoccum* were the most encountered in both main kinds of honey. However, they were found in a higher percentage of honeydew honeys, as the ratio of *Alternaria*-containing samples was 9.68% higher and the ratio of *Epicoccum*-containing honeys was 4.84% higher among honeydew honeys. The third most frequent taxa were, however, different. *Metschnikowia reukaufii* was found in 58.06% of all floral honey samples, while *Tripospermum* spp. were present in 70.97% of honeydew honeys.

Although *Cladosporium* genus was found in 69.35% of honeydew honeys, only 48.39% of floral honeys contained its spores. Uredospores of family Pucciniaceae were just as frequently encountered in floral honeys as conidia of *Cladosporium*, but in honeydew honeys, they are ranked ninth with a percentage of 59.67%. *Melampsorium/Cronartium/Melampsora* spp. spores that were counted separately from those of other rust fungi were among the most frequently encountered in honeydew honeys with 62.90%, but they were not even in the first ten most occurring taxa of floral honeys.

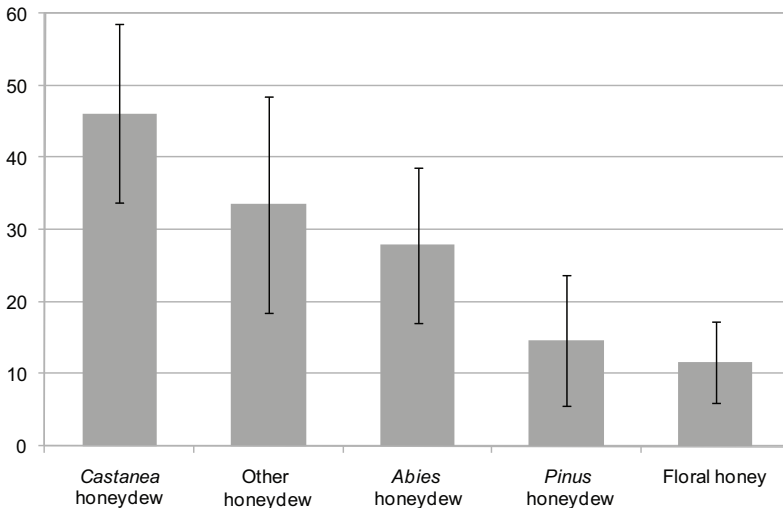


Fig. 2. The means of the number of spore types according to the botanical source of the honeys

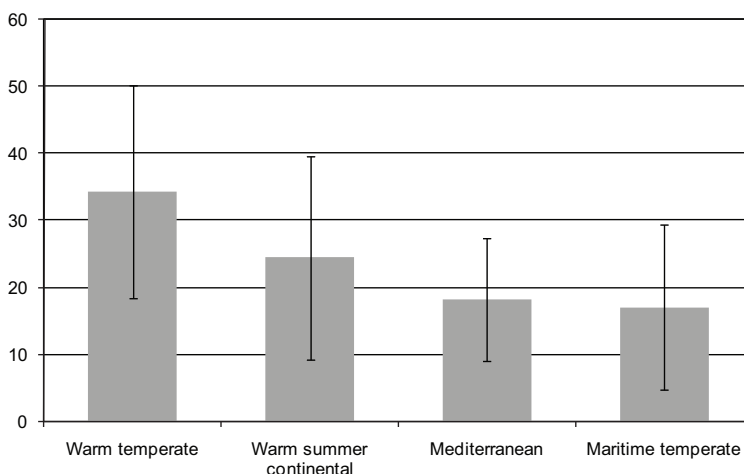


Fig. 3. The means of the number of spore types according to the climate zones of the source locations

The median of the first thirteen percentages in honeydew honeys is 61.29% that corresponds to *Periconia* and to the category of miscellaneous Agaricomycetes. The median is 41.94% in the case of floral honeys, belonging to *Stemphylium*. Myxomycetes, *Ustilago* and *Spegazzinia* were found in 29.03% of floral honeys, while Myxomycetes were present in 56.45% of honeydew hon-

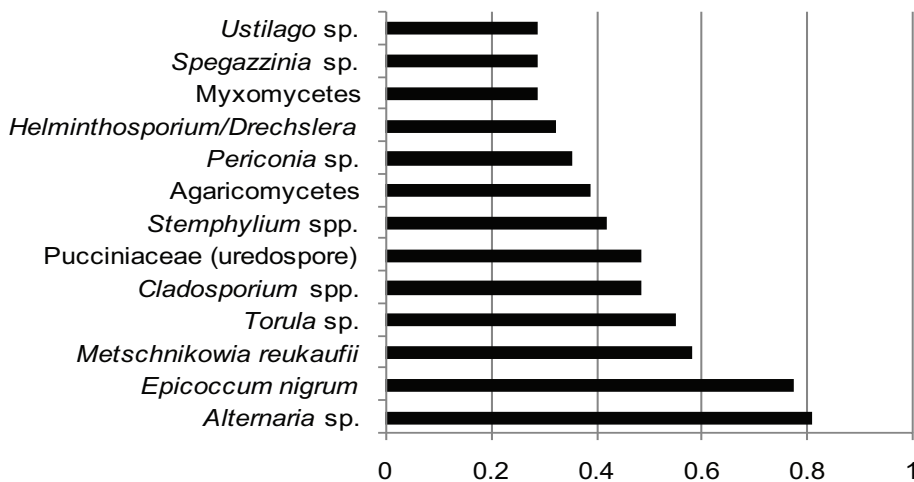


Fig. 4. The 13 most frequent spore types found in floral honeys. The scale corresponds to the ratio of honey samples, which contained the respective spore type

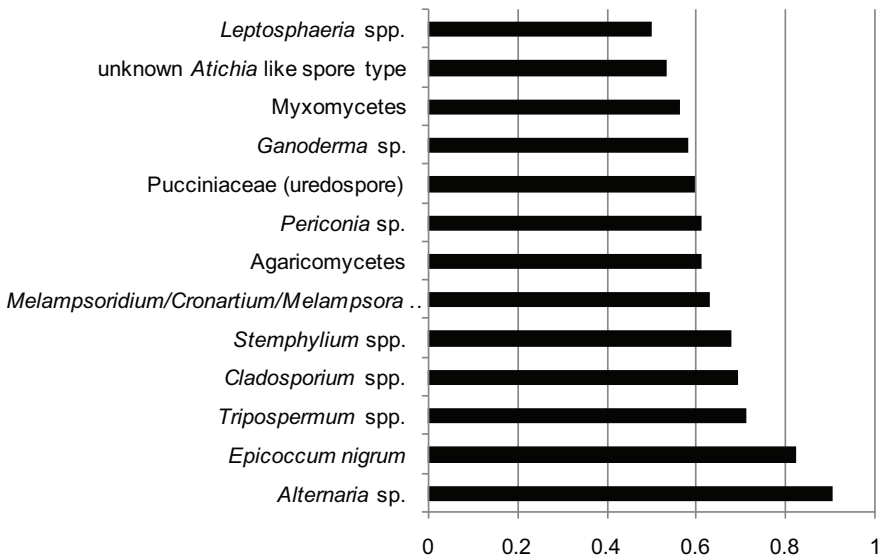


Fig. 5. The 13 most frequent spore types found in honeydew honeys. The scale corresponds to the ratio of honey samples, which contained the respective spore type

eyes. *Ustilago* and *Spegazzinia* were encountered in less than 50% of non-floral honey samples. In honeydew honeys, *Leptosphaeria* was the thirteenth most occurring with 50%. Between Myxomycetes and *Leptosphaeria*, an unknown *Atichia*-like spore type is also ranked with 53.23% in honeydew honeys.

Honey samples, especially those of honeydew origin had rich content of stauroid spores. Examples of the stauroid fungal spores found are shown in Figure 6.

DISCUSSION

Hereby, we attempted to investigate the widest possible spectrum of fungal spores in a hundred of honey samples collected from diverse geographical localities. Our results suggest that the most frequent fungal taxa in floral and honeydew honeys belong to ubiquitous species of fungi (*Alternaria*, *Cladosporium*, *Epicoccum*, *Stemphylium*). Therefore, it seems probable that these types of spores come from various sources and are not typical of the botanical or geographical origin of honey. On the other hand, less frequent fungi are apparently more specific. According to previous findings, floral honeys contained nectar-inhabiting *Metschnikowia* cells (Magyar *et al.* 2005, Seijo *et al.* 2011). The fungi found in honeydew honey samples represent phyllosphere fungi, including sooty moulds, and fungi with airborne or unknown origin. Sooty

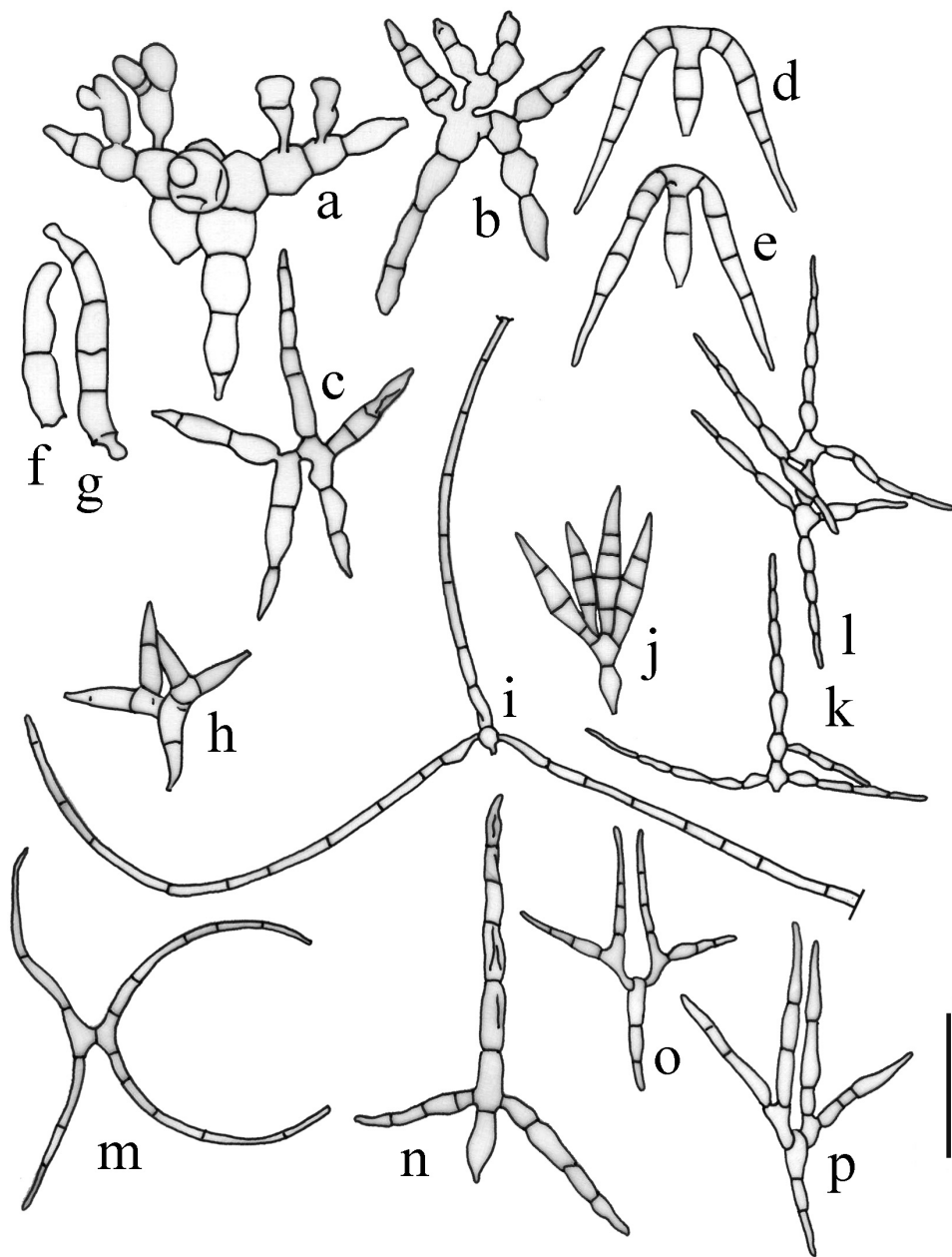


Fig. 6. Some of the fungal spores detected in honeys: a–c = *Tripospermum* spp., d–e = *Trinacrium* sp. 1, f–g = unknown scolecospore type 7, h = unknown staurospore type 9, i = unknown staurospore type 13, j = *Isthmotricladia/Tridentaria* sp., k–l = unknown staurospore type 11, m = *Curucispora* sp., n = unknown staurospore type 10, o–p = *Dwayaangam* sp. Bar = 20 μ m

moulds are a heterogeneous group including *Antennatula*, *Aureobasidium*, *Cladosporium*, and *Tripospermum* as well. Most of them are primarily growing on plant surfaces covered with honeydew that is secreted by sucking-piercing insects, e.g. aphids. These fungi are characterised by dark coloured hyphae and spores, because their pigmentation becomes intense due to exposure to sunlight. Other phyllosphere fungi belong to two major groups, namely to plant pathogens and to a group of saprotrophs not dependent on the presence of honeydew (e.g. lignin and hemicellulose decomposers). Some of them are more or less host specific (e.g. *Pollaccia elegans* on *Populus* spp.) and because of this, the presence of their spores in honeydew honey may be worthy to note, when the aim is to determine the botanical origin of the sample.

Other phyllosphere fungi may indicate honeydew origin from woody plants. For instance bark inhabiting fungi (*Excipularia*, *Oncopodiella*, *Pyrigemmula* spp.) might be more abundant in these samples. Such fungi are living on the bole, the branches and on the twigs of the trees, but not only at the bark surface. A hidden habitat of less known groups of fungi was found under the bark surface (between horizontal layers of the bark, Magyar 2008, Magyar and Révay 2009, Magyar *et al.* 2011). Underbark fungi are transported to and from the surface via cracks and fissures on the bark (Magyar 2008). Some fungi found in honeys are considered to be non-specific to the host, being common saprotrophs, e.g. *Alternaria* and *Cladosporium*. They are not only common on honeydew, but on leaf litter, decaying plant materials as well. The majority of the above mentioned fungi could be trapped in the honeydew by being in contact with the fungal colony. Spontaneous spores (originated from sources other than the host plant or from contamination during harvest and processing) could also be detected in honey samples, and their non-specific nature becomes evident when identified (e.g. basidiospores of *Cortinarius*, or the spores of coprophilous fungi, e.g. *Ascobolus*).

Identification of fungi in honey samples could therefore be a useful tool when it serves the purpose to determine the botanical origin of the honey or to prove its originality. Although the qualitative (presence or absence) data of fungi could be used for that sake, the quantitative data may be even more useful, and merit further studies. More research is therefore needed to calculate the indicator value of these fungi.

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