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# FUNGAL DIVERSITY IN FLORAL AND HONEYDEW HONEYS

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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. Honeys, 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 (Thrasyvoulou 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.

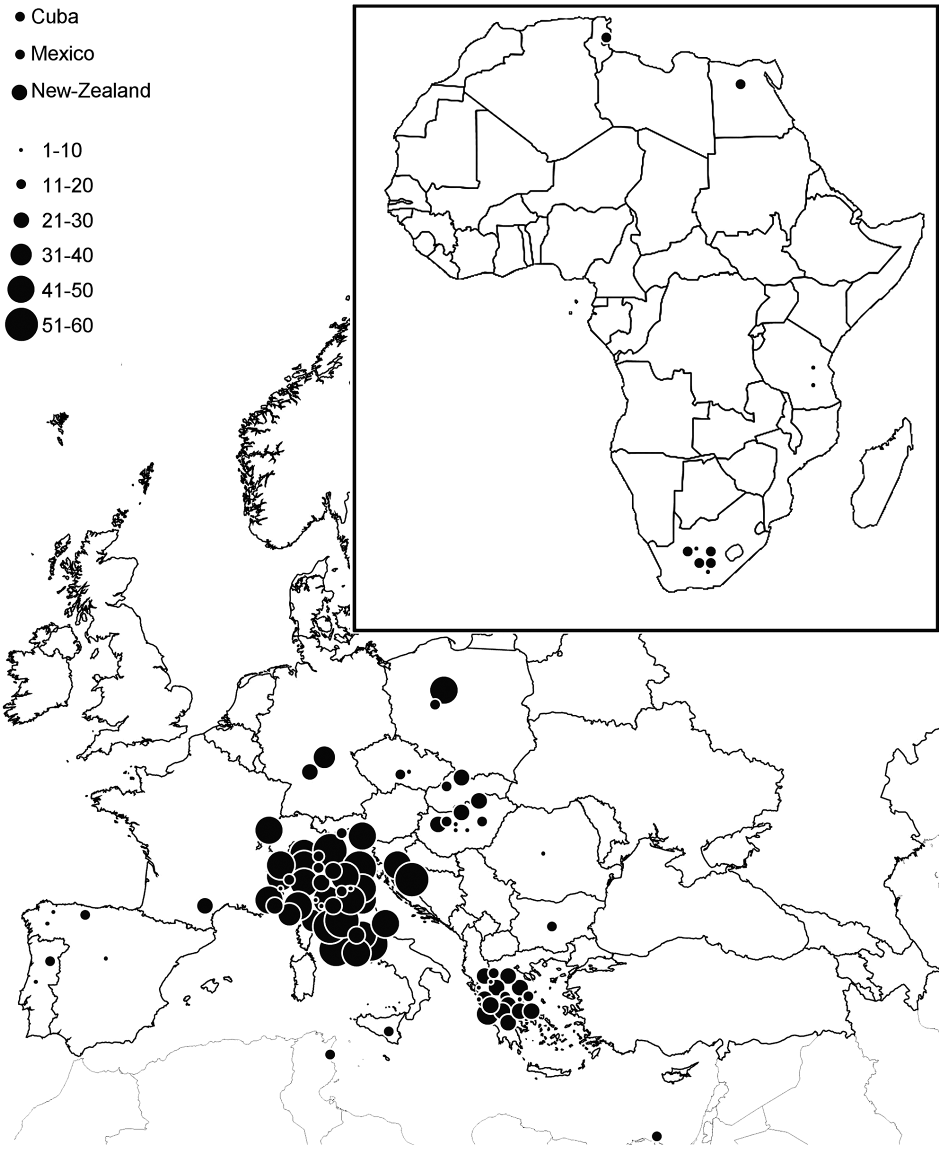


*Fig. 1.* Map showing all the locations, where samples were collected from. Samples are signified by spots and the diameter of each spot is proportional to the number of spore types found in the respective honey as indicated by the legend

*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 | *A. m.* |
| 2 | fl | Cuba | UD | unknown | *Melipona* sp. |
| 3 | fl | Egypt | Arid | unknown | *A. m.* |
| 4 | fl | Hungary: Orosz-lány | WSC | *Phacelia tanacetifolia* Benth. | *A. m.* |
| 5 | fl | Hungary: Sárkeszi | WSC | *Foeniculum vulgare* Mill. | *A. m.* |
| 6 | fl | Hungary: Szent-király | WSC | *Asclepias syriaca* L. | *A. m.* |
| 7 | fl | Italy | UD | *Hedysarum coronarium* L. | *A. m.* |
| 8 | fl | Central Italy | WSC | *Helianthus annuus* L. | *A. m.* |
| 9 | fl | N Italy | WSC | *Castanea sativa* Mill. | *A. m.* |
| 10 | fl | N Italy | WSC | *Tilia* sp. | *A. m.* |
| 11 | fl | Italy: Alps | WSC | *Rhododendron* sp. | *A. m.* |
| 12 | fl | Italy: Piemonte | WSC | *Taraxacum officinale* Weber | *A. m.* |
| 13 | fl | Italy: Sicily | MTN | *Citrus* sp. | *A. m.* |
| 14 | fl | Mexico | UD | unknown | *A. m.* |
| 15 | fl | New Zealand | MT | unknown | *A. m.* |
| 16 | fl | Poland | WSC | *Fagopyrum esculentum* Mill. | *A. m.* |
| 17 | fl | Portugal | MTN | *Rosmarinus officinale* L. | *A. m.* |
| 18 | fl | Portugal | MTN | unknown | *A. m.* |
| 19 | fl | South Africa | UD | *Acacia* sp. | *A. m.* |
| 20 | fl | South Africa | UD | unknown | *A. m.* |
| 21 | fl | South Africa | UD | *Citrus* sp. | *A. m.* |
| 22 | fl | South Africa | UD | unknown | *A. m.* |
| 23 | fl | South Africa | UD | unknown | *A. m.* |
| 24 | fl | South Africa | UD | unknown | *A. m.* |
| 25 | fl | Coastal N Spain | MT | *Eucalyptus* sp. | *A. m.* |

*Table 1* (continued)

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

*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 | *A. m.* |
| 58 | hd | Hungary: Sárkeszi | WSC | unknown | *A. m.* |
| 59 | hd | Hungary: Solymár | WSC | unknown | *A. m.* |
| 60 | hd | Italy | UD | unknown | *A. m.* |
| 61 | hd | Italy | UD | unknown | *M. p.* + *A. m.* |
| 62 | hd | Italy | UD | unknown | *M. p.* + *A. m.* |
| 63 | hd | Italy | UD | *Abies alba* Mill. | *A. m.* |
| 64 | hd | Italy | UD | *Abies alba* Mill. | *A. m.* |
| 65 | hd | Italy | UD | *Abies alba* Mill. | *A. m.* |
| 66 | hd | Italy | UD | *Castanea sativa* Mill. | *A. m.* |
| 67 | hd | Italy | UD | *Castanea sativa* Mill. | *A. m.* |
| 68 | hd | Italy | UD | *Castanea sativa* Mill. | *A. m.* |
| 69 | hd | Italy | UD | *Castanea sativa* Mill. | *A. m.* |
| 70 | hd | Italy | UD | *Castanea sativa* Mill. | *A. m.* |
| 71 | hd | Italy: Abruzzo | WT | unknown | *A. m.* |
| 72 | hd | Italy: Friuli Venezia Lucia | WSC | unknown | *A. m.* |
| 73 | hd | Italy: Lazio | WSC | unknown | *A. m.* |
| 74 | hd | Italy: Liguria | WT | unknown | *M. p.* + *A. m.* |
| 75 | hd | Italy: Liguria | WT | unknown | *A. m.* |
| 76 | hd | Italy: Liguria | WT | unknown | *A. m.* |
| 77 | hd | Italy: Liguria | WT | unknown | *A. m.* |
| 78 | hd | Italy: Liguria | WT | unknown | *M. p.* + *A. m.* |
| 79 | hd | Italy: Liguria | WT | unknown | *M. p.* + *A. m.* |
| 80 | hd | Italy: Liguria | WT | unknown | *M. p.* + *A. m.* |
| 81 | hd | Italy: Liguria | WT | unknown | *A. m.* |
| 82 | hd | Italy: Liguria | WT | *Abies alba* Mill. | *A. m.* |
| 83 | hd | Italy: Lombardia | WSC | unknown | *A. m.* |
| 84 | hd | Italy: Piemonte | WSC | unknown | *A. m.* |
| 85 | hd | Italy: Toscana | WSC | unknown | *A. m.* |
| 86 | hd | Italy: Toscana | WSC | unknown | *A. m.* |
| 87 | hd | Italy: Toscana | WSC | unknown | *A. m.* |

*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 | *A. m.* |
| 89 | hd | Italy: Trentino Alto Adige | WSC | unknown | *A. m.* |
| 90 | hd | Italy: Tusco-Emil-ian App. | WSC | *Abies alba* Mill. + *Picea excelsa* Link | *A. m.* |
| 91 | hd | Poland | WSC | unknown | *A. m.* |
| 92 | hd | Slovakia | WSC | unknown | *A. m.* |
| 93 | hd | Slovakia | WSC | unknown | *A. m.* |
| 94 | unk | Czech Republic | WSC | unknown | *A. m.* |
| 95 | unk | Czech Republic | WSC | unknown | *A. m.* |
| 96 | unk | France: Domaine St. Georges | MT | unknown | *A. m.* |
| 97 | unk | Germany: Altbulach | MT | unknown | *A. m.* |
| 98 | unk | Germany: Alten-steig-Überberg | MT | unknown | *A. m.* |
| 99 | unk | Romania: Székely-udvarhely | WSC | unknown | *A. m.* |
| 100 | unk | Switzerland: Les-ponts-de-Martel | WSC | unknown | *A. m.* |

*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 | *Acroconidiella tropaeoli* (T. E. T. Bond) J. C. Lindq. et Alippi | 0.00 | 12.90 |
| 2 | *Acrodictys* sp. | 6.45 | 6.45 |
| 3 | Agaricomycetes | 61.29 | 38.71 |
| 4 | *Aglaospora profusa* (Fr.) De Not. | 11.29 | 3.23 |
| 5 | *Agrocybe* sp. | 12.90 | 6.45 |
| 6 | *Albugo* sp. | 12.90 | 3.23 |
| 7 | *Alternaria* ? *alternata* (Fr.) Keissl. | 9.68 | 3.23 |
| 8 | *Alternaria* sp. | 90.32 | 80.65 |
| 9 | *Ampulliferina persimplex* B. Sutton | 1.61 | 0.00 |
| 10 | *Anellophora* sp. ? | 16.13 | 3.23 |
| 11 | *Antennatula* sp. | 14.52 | 3.23 |
| 12 | *Anthostomella/Herpotrichiella* spp. | 27.42 | 0.00 |
| 13 | *Anthracoidea* sp. | 8.06 | 0.00 |
| 14 | *Arthrinium cuspidatum* (Cooke et Harkn.) Tranzschel | 1.61 | 3.23 |
| 15 | *Arthrinium* sp. | 43.55 | 12.90 |
| 16 | *Articulospora* sp. | 3.23 | 0.00 |
| 17 | *Ascobolus* sp. | 4.84 | 0.00 |
| 18 | *Aspergillus/Penicillium* spp. | 4.84 | 12.90 |

*Table 2* (continued)

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

*Table 2* (continued)

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

*Table 2* (continued)

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

*Table 2* (continued)

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

*Table 2* (continued)

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

*Table 2* (continued)

| Ref. no. | Spore type | HD (%) | FL (%) |
|---|---|---|---|
| 181 | *Trimmatostroma scutellare* (Berk. et Broome) M. B. Ellis | 11.29 | 0.00 |
| 182 | *Trinacrium gracile* Matsush. | 1.61 | 0.00 |
| 183 | *Trinacrium incurvum* Matsush. | 1.61 | 0.00 |
| 184 | *Trinacrium robustum* Tzean et J. L. Chen | 1.61 | 0.00 |
| 185 | *Trinacrium* sp. 1 | 11.29 | 0.00 |
| 186 | *Trinacrium* sp. 2 | 1.61 | 0.00 |
| 187 | *Trinacrium subtile* Riess | 3.23 | 0.00 |
| 188 | *Tripospermum camelopardus* Ingold, Dann et P. J. McDougall | 16.13 | 0.00 |
| 189 | *Tripospermum* spp. | 70.97 | 12.90 |
| 190 | *Urocystis* sp. | 8.06 | 0.00 |
| 191 | *Ustilago bromivora* (Tul. et C. Tul.) A. A. Fisch. Waldh. | 24.19 | 12.90 |
| 192 | *Ustilago* sp. | 43.55 | 29.03 |
| 193 | *Valsaria* ? *insitiva* ( Tode) Ces. et De Not. | 9.68 | 0.00 |
| 194 | *Varicosporium elodeae* W. Kegel | 8.06 | 0.00 |
| 195 | Xylariaceae | 45.16 | 12.90 |
| 196 | *Zygophiala jamaicensis* E. W. Mason. | 14.52 | 6.45 |
| 197 | *Zygosporium geminatum* S. Hughes | 0.00 | 3.23 |
| 198 | *Zygosporium masonii* S. Hughes ? | 1.61 | 0.00 |
| 199 | unknown Ascomycetes (*Nectria*?) | 3.23 | 0.00 |
| 200 | unknown Ascomycetes (*Venturia*?) | 6.45 | 0.00 |
| 201 | unknown helicospore type 1 | 3.23 | 0.00 |
| 202 | unknown phaeoamerospore type 1 (*Mammaria*?) | 19.35 | 0.00 |
| 203 | unknown phaeoamerospore type 2 | 11.29 | 0.00 |
| 204 | unknown phaeodidymospore type 2 | 4.84 | 0.00 |
| 205 | unknown phaeophragmospore 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 *Atichia*-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 *Melampsoridium*/*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 Brunnel–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%. *Melampsoridium*/*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.

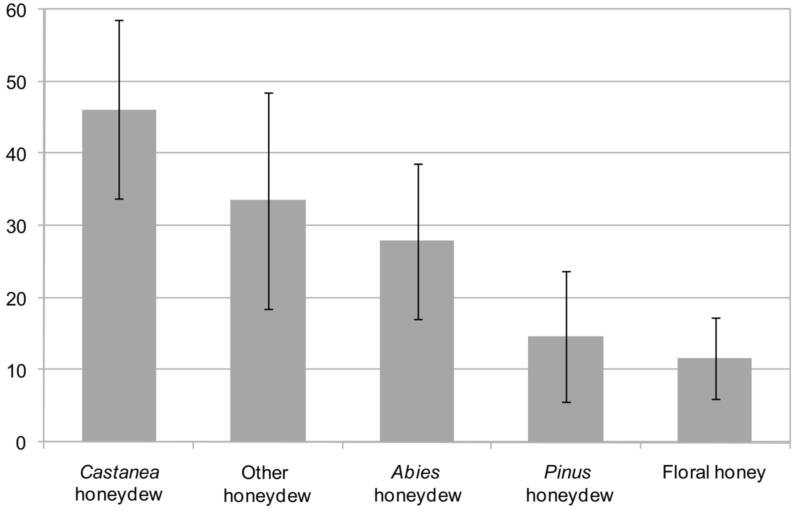


*Fig. 2.* The means of the number of spore types according to the botanical source of the honeys

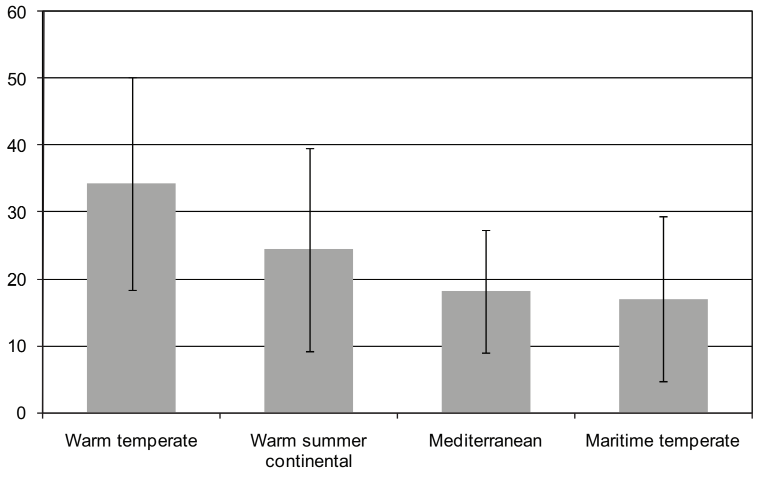


*Fig. 3.* The means of the number of spore types according to the climate zones of the source locations

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-

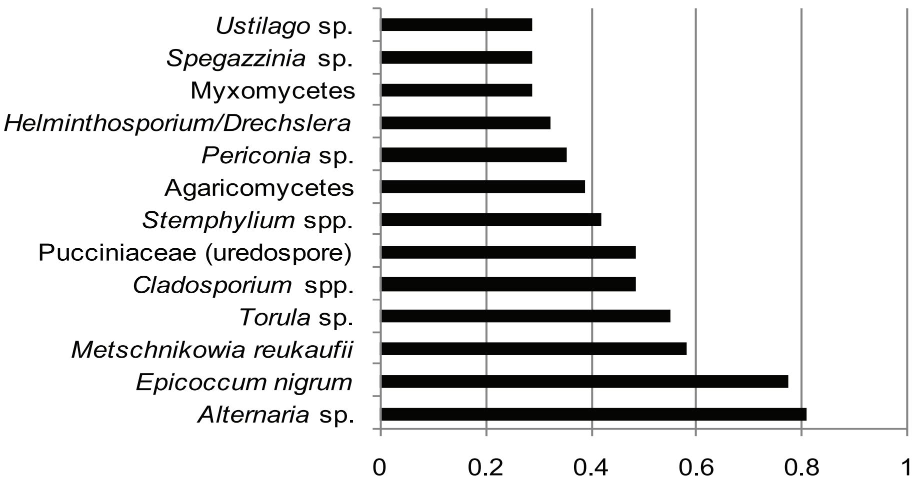


*Fig. 4.* The 13 most frequent spore types found in floral honeys. The scale corresponds to the ratio of honey samples, which contained the respective spore type

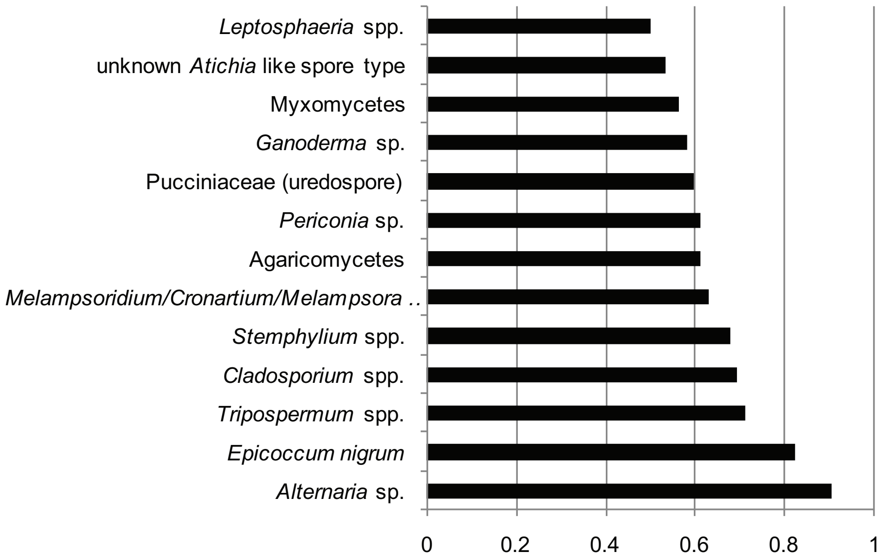


*Fig. 5.* The 13 most frequent spore types found in honeydew honeys. The scale corresponds to the ratio of honey samples, which contained the respective spore type

eys. *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

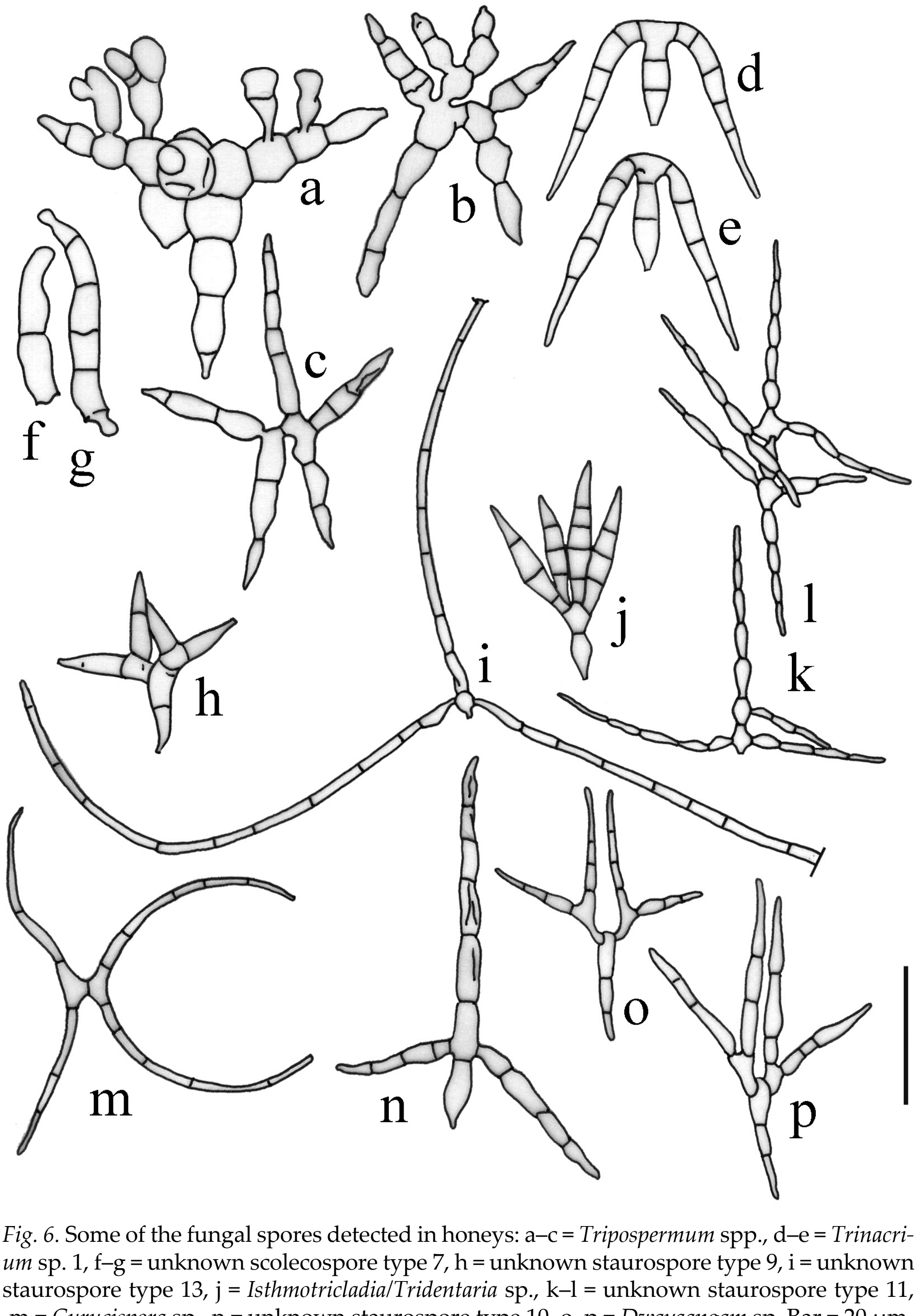


*Fig. 6.* Some of the fungal spores detected in honeys: a–c = *Tripospermum* spp., d–e = *Trinacrium* sp. 1, f–g = unknown scolecospore type 7, h = unknown staurospore type 9, i = unknown staurospore type 13, j = *Isthmotricladia*/*Tridentaria* sp., k–l = unknown staurospore type 11, m = *Curucispora*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

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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