Stauro- and scolecoconidia in floral and honeydew honeys

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Forty-four samples of floral and honeydew honeys from Croatia, Greece, Italy, Mexico, New Zealand, Portugal, South Africa, Spain and Tanzania were microscopically examined for fungal spores. Most of the floral honeys were dominated by yeast cells of *Metschnikowia reukaufii* and contained very few conidia of hyphomycete species. By contrast, honeydew honeys contained scoleco- and stauroconidia belonging to more than 30 hyphomycete species, most of them previously reported from rainwater on living trees. Most belonged to the genera *Tripospermum, Retiarius* and *Trinacrium*. Their concentrations were highest in the honeydew honey from *Abies alba* and *Picea excelsa*. Conidia belonging to species of *Camposporium, Ceratosporium, Dwayaangam, Tricellula, Tricladium* and *Trifurcospora*, well-known litter-inhabiting fungi in terrestrial and/or aquatic habitats were encountered. Some other conidia probably belonged to species of *Articulospora, Curucispora, Gyoerffyella, Lemonniera* and *Varicosporium*, also well-known Ingoldian fungi from lotic ecosystems. The assemblages of fungal spores in honeydew honeys may provide important information on the geographical distribution of "canopy fungi". In addition, the results of this study support Carroll's theory on the existence of a fungal group termed "arboreal aquatic hyphomycetes" or "canopy fungi". Although their function in canopies is presently unknown, evidence accumulating in the literature suggests their widespread occurrence in the phyllosphere.

Key words: "canopy fungi", floral honey, honeydew honey, phyllosphere, stauro- and scolecoconidia.

Introduction

The existence of aquatic hyphomycetes in terrestrial forest litter has received little attention and even less so on living plants. There are, however, a

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few papers recording conidia of aquatic hyphomycetes in "unexpected places" (Carroll, 1981). Samples of throughfall from Douglas fir trees (*Pseudotsuga menziesii* (Mirb.) Franco) revealed numerous triradiate and tetraradiate conidia belonging to genera such as *Tripospermum*, *Tridentaria* and *Ceratosporium* amongst others. It was thought that these constituted a guild ("arboreal aquatic hyphomycetes" or "canopy fungi") which "may function in canopies much as classical aquatic hyphomycetes function in streams" (Carroll, 1981). Bandoni (1981) reported conidia of *Gyoerffyella biappendiculata, G. gemellipara* and *Tripospermum* spp. from stemflow of several trees in British Columbia, Canada. Ando and Tubaki (1984a, b) recorded conidia of aquatic hyphomycetes and described new species and genera from throughfall collected from various trees in Japan. They stated that these fungi "probably live usually on intact leaves as mycelia and can sporulate when leaves are moistened by mist, morning dew or rain". Ando (1992) later proposed the term "terrestrial aquatic hyphomycetes". Gönczöl (1976) reported an assemblage of mainly stauroconidia from foam also collected in an "unexpected place" i.e. on the trunk of a beech tree (*Fagus sylvatica*). In more recent studies Gönczöl and Révay (2003, 2004) identified conidia of 63 mostly hyphomycete species in stemflow and throughfall samples from living trees, and 45 hyphomycetes from treeholes in Hungary.

So far, rainwater is only known as a medium for accumulating and transporting these fungal spores on trees. During routine melissopalynological analyses of honeys kept in the National Institute for Apiculture, Bologna, Italy, fungal spores of unknown identity were encountered, mainly in honeydew honeys. Some conidia were very similar to those sometimes recorded from rainwater on live trees. This prompted the present study on whether these fungal spores occur in floral and honeydew honeys from different geographical regions.

Honeydew is an extract from piercing and plant-sucking insects (*Rinchota*: *Homoptera*, e.g. *Cinara cofinis* Koch, *C. pectinatae* Nordlinger, *C. pilicornis* Hartig, *Mindarus abietinus* Koch and *Physokermes piceae* Schrank), which suck phloem sap, which is rich in nutrients, especially amino acids. To satisfy their protein needs, these insects need large amounts of sap, which contains only 1-2% of proteins, though it is high in water content and sugars. In Italy honeydew is produced between July and September (Persano Oddo *et al*., 2000). When production is high on forest trees, honeydew drops fall to the ground. When the volume of honeydew on the leaves reaches a certain level, it is collected by honeybees (*Apis mellifera* L.). Honeybees then transport it to hives and process it into honeydew honey. Honeydew honey, often called

"forest honey", is commercially valuable. Those from silver-fir, oak-trees, wheat, citrus, etc. are marketed worldwide (Ricciardelli D'Albore, 1998).

Some algae and microscopic fungi, especially sooty moulds, develop in honeydew (Hughes, 1976). These fungi can thus be traced in the honeydew sediment (Ricciardelli D'Albore, 1998). In spite of such an interesting fungal spore content, little mycological analysis has been reported. In routine melissopalynological analyses fungal structures are only categorized as "spores" and "hyphae" (Fehlmann, 1911; Gontarski, 1951). Louveaux *et al*. (1978) mentioned, that honeydew elements (HDE) consisted of fungal spores and hyphae of sooty moulds. Especially, the forest honeys from *Pinus brutia* L. contain a great number of hyphae and spores (Ricciardelli D'Albore, 1998). The only study aimed at identifying the spores was carried out by Pérez-Atanes *et al*. (2001). A number of asco- and basidiospores together with conidia of some common hyphomycetes were recorded, but scoleco- and stauroconidia were not mentioned.

The main objectives of this study were: (a) to further explore the occurrence of fungal spores in floral and honeydew honeys with special attention to stauro- and scolecoconidia; (b) to determine if hyphomycete conidia which are known from aquatic habitats occur in honeys.

Materials and methods

We examined 19 floral honeys from *Acacia* sp., *Castanea sativa*, *Citrus* sp., *Eucalyptus* sp., *Helianthus annuus*, *Rhododendron* sp., *Rosmarinus officinalis*, *Rubus* sp., *Taraxacum officinale* and *Tilia* spp., one polyfloral honey and 25 honeydew honeys (Table 1). Some floral honey samples (nos. 14, 15, 17) were analysed in Spain by one of us (S-C) and the remainder were obtained from the National Institute for Apiculture, Bologna and analysed in Hungary.

Preparation of samples: 10 g were taken from 500 g of previously homogenised honey, dissolved in 20 ml of distilled water at 40^{o}, centrifuged for 5 s at 2,500 rpm 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^{o}. It was then mounted in glycerine-gelatine and covered (Louveaux *et al*., 1978). The entire surface of each preparation was scanned under phase contrast and fungal propagules were identified and counted. The detailed melyssopalynological description of the honeydew honey samples is given in Persano Oddo *et al*. (2000).

Table 1. Types of honeys examined for fungal spores.

Substratum	Source of nectar	Pollinator	Locality	Honey ref. no.
Floral Honey	*Castanea sativa* Miller.	*Apis mellifera*	Italy (North)	1
Floral Honey	Unknown	*Apis mellifera*	South Africa	2
Floral Honey	Unknown	*Apis mellifera*	South Africa	3
Floral Honey	Unknown	*Apis mellifera*	South Africa	4
Floral Honey	Unknown	*Apis mellifera*	Africa	5
Floral Honey	Unknown	*Apis mellifera*	New Zealand Pohutukawa	6
Floral Honey	*Taraxacum officinale* Weber	*Apis mellifera*	Italy Piemonte	7
Floral Honey	*Acacia* sp.	*Apis mellifera*	South Africa	8
Floral Honey	*Citrus* sp.	*Apis mellifera*	Italy Sicilia	9
Floral Honey	*Helianthus annuus* L.	*Apis mellifera*	Italy (Middle)	10
Floral Honey	Unknown	*Apis mellifera*	Tanzania	11
Floral Honey	*Rosmarinus officinalis* L.	*Apis mellifera*	Portugal	12
Floral Honey	Unknown	*Apis mellifera*	Portugal	13
Floral Honey	*Eucalyptus* sp.	*Apis mellifera*	Coastal areas of NW Spain	14
Floral Honey	polyfloral	*Apis mellifera*	Spain (NW)	15
Floral Honey	*Rhododendron* sp.	*Apis mellifera*	Italian Alp	16
Floral Honey	*Rubus* sp.	*Apis mellifera*	NW Spain	17
Floral Honey	*Tilia* sp.	*Apis mellifera*	Italy (North)	18
Floral Honey	Unknown	*Apis mellifera*	Mexico	19
Honeydew H	*Abies alba* Mill.+ *Picea excelsa* Link	*Apis mellifera*	Italy Tusco-Emilian Appenines	20
Honeydew H	Unknown	*Apis mellifera*	Croatia	21
Honeydew H	Unknown	*Metcalfa pruinosa*	Italy Liguria	22
Honeydew H	Unknown	*Apis mellifera*	Italy Liguria	23
Honeydew H	*Abies alba*	*Apis mellifera*	Greece	24
Honeydew H	Unknown	*Apis mellifera*	Italy Trentino Alto Adige	25
Honeydew H	Unknown	*Apis mellifera*	Italy Liguria	26
Honeydew H	*Abies alba*	*Apis mellifera*	Greece	27
Honeydew H	Unknown	*Apis mellifera*	Greece	28
Honeydew H	*Abies alba*	*Apis mellifera*	Greece	29
Honeydew H	*Pinus* sp.	*Apis mellifera*	Greece	30
Honeydew H	Unknown	*Apis mellifera*	Italy Lombardia	31
Honeydew H	Unknown	*Apis mellifera*	Italy Lazio	32
Honeydew H	Unknown	*Apis mellifera*	Italy Friuli Venezia Giulia	33
Honeydew H	Unknown	*Apis mellifera*	Italy Abruzzo	34
Honeydew H	Unknown	*Apis mellifera*	Italy	35
Honeydew H	*Abies alba*	*Apis mellifera*	Italy	36
Honeydew H	*Abies alba*	*Apis mellifera*	Italy	37
Honeydew H	Unknown	*Apis mellifera*	Italy Piemonte	38

Table 1 continued. Types of honeys examined for fungal spores.

Substratum	Source of nectar	Pollinator	Locality	Honey ref. no.
Honeydew H	*Abies alba*	*Apis mellifera*	Greece	39
Honeydew H	'forest-type'	*Apis mellifera*	Italy	40
Honeydew H	*Abies alba*	*Apis mellifera*	Greece	41
Honeydew H	Unknown	*Metcalfa pruinosa*	Italy	42
Honeydew H	Unknown	*Apis mellifera*	Italy Liguria	43
Honeydew H	Unknown	*Metcalfa pruinosa*	Italy Liguria	44

Results and discussion

The species encountered and the numbers of spores in the honey samples are listed in Tables 2 and 3. Altogether 9 species were found in the floral honey (Table 2) and 35 in the honeydew honey samples (Table 3). The number of fungal species ranged from 0 to 3 in the floral honey samples and from 1 to 20 in the honeydew honey samples. The conidial concentration was highest in the honeydew honey of *Abies alba* and *Picea excelsa* (No. 20).

This study focused on the hyphomycete species with stauro- and scolecoconidia that had been previously seen in rainwater from trees. Over 30% of the floral honey samples and 100% of those from honeydew honey contained stauro- and scolecoconidia. Common hyphomycetes (e.g. *Alternaria*, *Botrytis*, *Cladosporium*, *Epicoccum*, *Stemphylium* etc.), widely distributed in the phyllosphere and in many other microhabitats, were omitted. Likewise, it was not the aim here to extend analyses to yeasts, although they were present in many samples.

Metschnikowia reukaufii (Fig. 1), a well-known nectar- and flower-inhabiting yeast, isolated frequently from nectars of different plants (Grüsz, 1927; Eisikowitch *et al*., 1990), was an exception because it was frequent mainly in floral honeys. *Metschnikowia reukaufii* was found in 68% of floral honeys and 39% of honeydew honeys, but 70% of the total number of its cell formations was counted in floral honeys. Although *M. reukaufii* proved to be frequent and generally distributed in floral honeys, it was absent in all those from South-Africa. The majority of the yeast cells were seen as "trident", "aeroplane" or "cross" formations, as commonly found in floral nectars (Pitt and Miller, 1968). The numbers of yeast cells in floral honeys varied greatly: an Italian and a Spanish sample had more than 400 cell formations per sample. The only floral honey sample from Mexico contained this yeast at a very high concentration, (615 cell colonies/pseudomycelia per sample). *Metschnikowia reukaufii* occurred rarely in honeydew honeys, however, in one sample (no. 33) an unexpectedly high number of cell formations was found, but their source

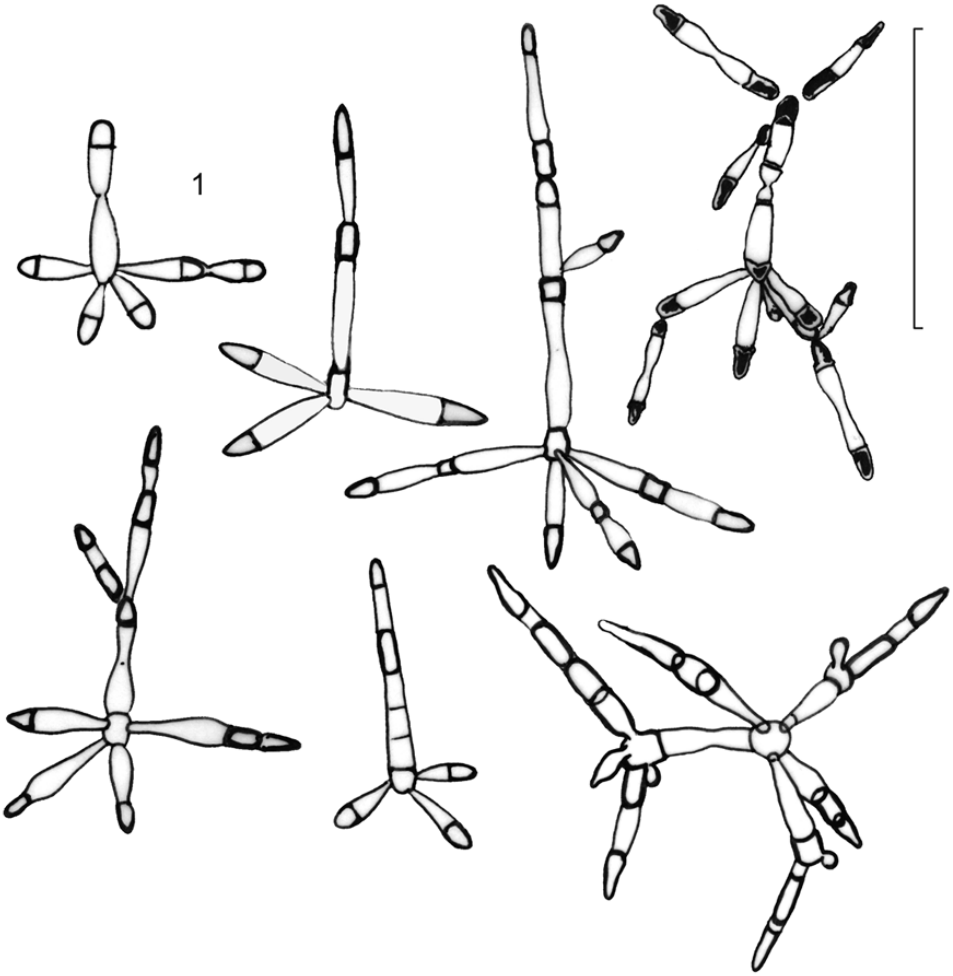


Fig. 1. Cell formations of *Metschnikowia reukaufii* as frequently seen in floral honeys. Bar = 50 µm.

remained unknown. The occurrence of stauro- and scolecoconidia indicates that floral honey contains some honeydew. Conversely, the presence of *Metschnikowia reukaufii* in honeydew honey may be of floral origin. The contamination of floral honeys with HDE occurs rarely, because the nectar harvesting period is earlier than that of honeydew (Sabatini *et al*., 2000).

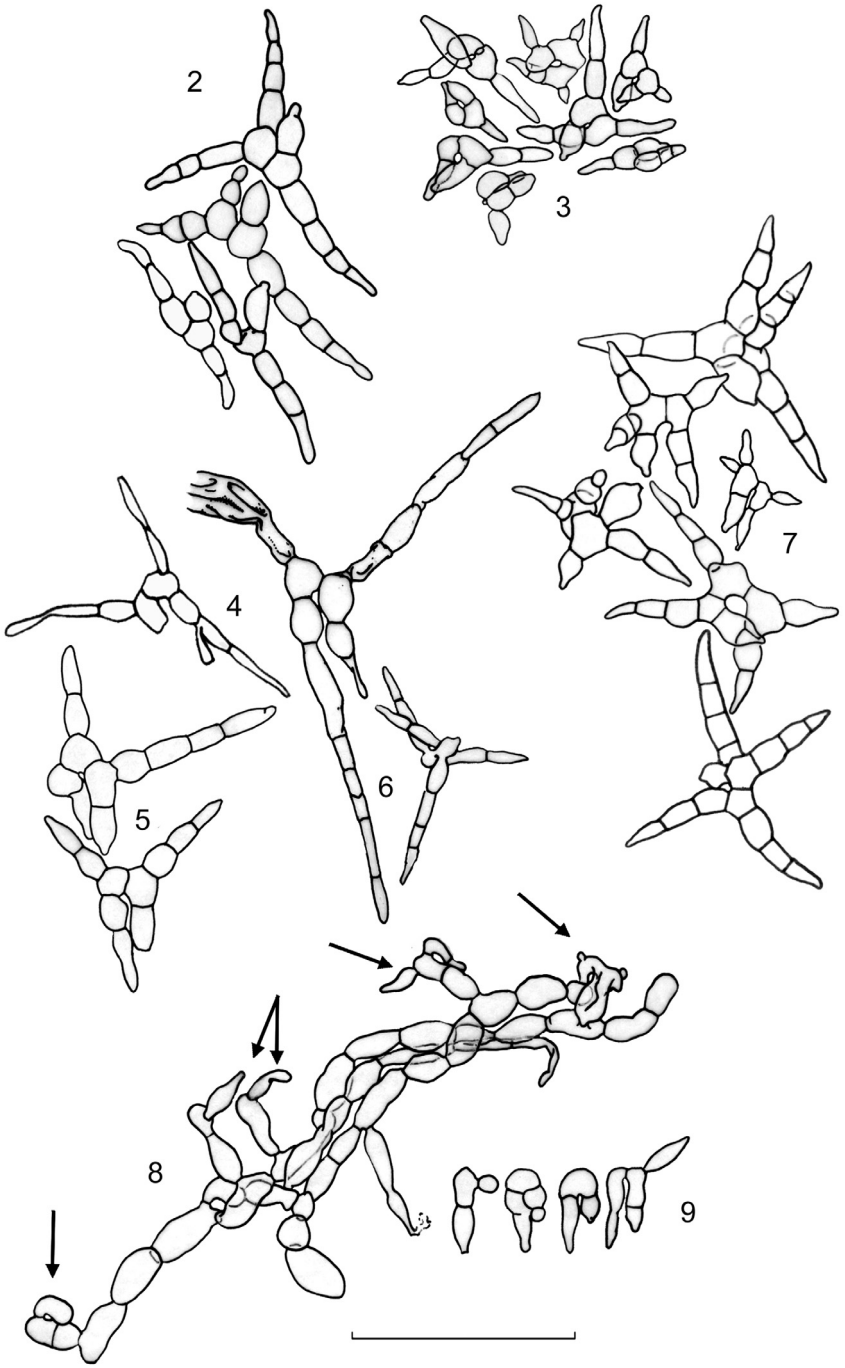
Very few other fungal species occurred with very low conidial numbers in floral honeys (Table 2). These included a single conidium of *Diplocladiella scalaroides* (Fig. 19) and conidia probably belonging to species of *Geniculospora* (Fig. 26) and *Lemonniera* (Fig. 24); as well as some conidia of *Tetraploa aristata* and *Tripospermum* spp. (Figs. 3-6) and two unknown staurospores (Figs. 33-34) were also found.

Table 2. Numbers of fungal spores found in floral honey samples. (Honey ref. No. see in Table 1., locality: A=Africa, I= Italy, M=Mexico, P=Portugal, S= Spain, Z= New Zealand.)

Honey ref. No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Locality	**I**	**A**	**A**	**A**	**A**	**Z**	**I**	**A**	**I**	**I**	**A**	**P**	**P**	**S**	**S**	**I**	**S**	**I**	**M**
Diplocladiella scalaroides Arnaud ex Matsush.	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
?*Geniculospora* sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Lemonniera sp. (?)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Metschnikowia reukaufii Pitt & Miller	15	-	-	-	-	4	-	-	53	1	4	195	10	461	233	417	23	14	615
Tetraploa aristata Berk. & Br.	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tripospermum myrti (Lind) Hughes	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tripospermum spp.	3	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-
Stauroconidia type 2	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stauroconidia type 3	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Number of species	2	1	0	2	0	1	1	2	1	1	1	1	1	1	1	1	1	1	3

By contrast, relatively high numbers of scoleco- and stauroconidia of various species were observed in honeydew honeys (Figs. 2-40). A rich variety of conidia of *Tripospermum* species dominated. Conidia were observed in various stages of development and colony fragments with developing conidia were sometimes also seen (Figs. 8-9). Conidia of *T. camelopardus* and *T. myrti* could be distinguished (Figs. 2 and 7). Conidia with very long branches (Fig. 4) exceeded the dimensions given for those of *T. camelopardus*. Many others could not be identified to species (Figs. 3-6 and 9) due to the great variability of form, which is a property of species in *Tripospermum*. Tubaki *et al*. (1985), for example, also concluded "that species of the genus are separated only after careful cultural observations".

Beside *Tripospermum* species, some 30 other forms, mostly stauro- and scolecoconidia, occurred in honeydew honeys from Italy and Greece and in the sole sample from Croatia. Relatively high species numbers occurred in some Greek (9-11 species) and even higher (8-20 species) in some Italian honeydew honeys. Conidial numbers in the majority of species were generally low (1-10 per sample). These numbers were, however, higher for other hyphomycete species. For example, 35 conidia of *Ceratosporium cornutum* (Fig. 14) and 32 conidia of *Retiarius bovicornutus* (Fig. 10) were counted in the samples of honeydew honey from *Abies alba* and *Picea excelsa* from the Tusco-Emilian Appenines, Italy. Matsushima (1975) described *Ceratosporium cornutum* from decaying plant material in a terrestrial habitat in Japan and he later collected this species on decaying wood from Alabama, USA (Matsushima, 1981). Ando and Tubaki (1984a) found its conidia in rainwater from *Zelkova serrata* in



Figs. 2-9. *Tripospermum* species. **2.** *Tripospermum camelopardus*. **3-6.** *Tripospermum* spp. **7.** *Tripospermum myrti*. **8.** Part of a *Tripospermum* colony with conidium initials (arrows: developing conidia). **9.** detached immature conidia of *Tripospermum* spp. frequently seen in honeydew honeys. Bar = 50 µm.

Table 3. Numbers of fungal spores identified in honeydew honey samples. (Honey ref. No. see in Table 1., locality: C=Croatia, G=Greece, I= Italy)

Honey ref. No.	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**
Locality	**I**	**C**	**I**	**I**	**G**	**I**	**I**	**G**	**G**	**G**	**G**	**I**	**I**	**I**	**I**	**I**	**I**	**I**	**I**	**G**	**I**	**G**	**I**	**I**	**I**
?*Articulospora* sp.	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Atichia sp.	70	-	-	-	6	-	-	21	18	1	-	-	-	-	-	-	-	-	-	5	-	7	-	-	-
Camposporium sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Ceratosporium cornutum Matsush.	35	-	-	-	-	5	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	1	-	-
?*Curucispora* sp.	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dicranidion sp.	-	1	1	1	-	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Diplocladiella scalaroides Arnaud ex Matsush.	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Dwayaangam dichotoma Nawawi	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dwayaangam sp.	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
?Geniculospora sp.	-	-	-	-	-	8	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	-	-	-
Gyoerffyella ? *myrmecophagiformis* Melnik & Dudka	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Helicosporium sp.	-	1	-	-	-	2	-	1	-	-	-	-	-	2	-	-	-	-	-	-	-	1	-	-	1
Isthmotricladia sp.	-	-	-	-	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
?*Lemonniera* sp.	1	-	-	-	-	1	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Metschnikowia reukaufii Pitt & Miller	-	-	-	-	1	3	-	-	3	2	-	-	2	891	-	-	-	1	-	-	-	1	5	-	-
Mycocentrospora sp.	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Oncopodiella sp.	-	-	-	-	-	-	-	-	-	-	-	2	-	-	1	-	-	-	-	-	-	-	-	-	-
Retiarius bovicornutus D.L. Olivier	32	-	-	-	1	6	-	-	-	-	-	-	-	6	-	1	-	1	-	-	-	1	-	-	-
Tricellula sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-
?Tricladium sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Trifurcospora sp.	2	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trinacrium ?parvisporum Matsush.	-	-	-	-	-	3	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trinacrium robustum Tzean & Chen	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trinacrium subtile Riess	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trinacrium sp.	8	-	-	-	-	13	-	-	-	-	-	-	1	1	-	-	-	-	-	1	-	-	-	-	-
Tripospermum camelopardus Ingold, Dann & McDougall	5	-	-	-	-	2	-	-	-	-	-	-	11	-	-	-	1	2	-	-	-	-	4	1	-
Tripospermum myrti (Lind) Hughes	-	31	14	8	-	47	4	-	-	-	-	-	118	6	1	8	17	5	66	1	23	1	14	2	3
Tripospermum spp.	191	9	9	5	3	54	3	7	5	-	1	2	32	5	-	12	7	11	8	2	6	-	10	5	4
?*Varicosporium* sp.	5	-	-	-	2	-	-	1	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-
Unknown helicoconidia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-

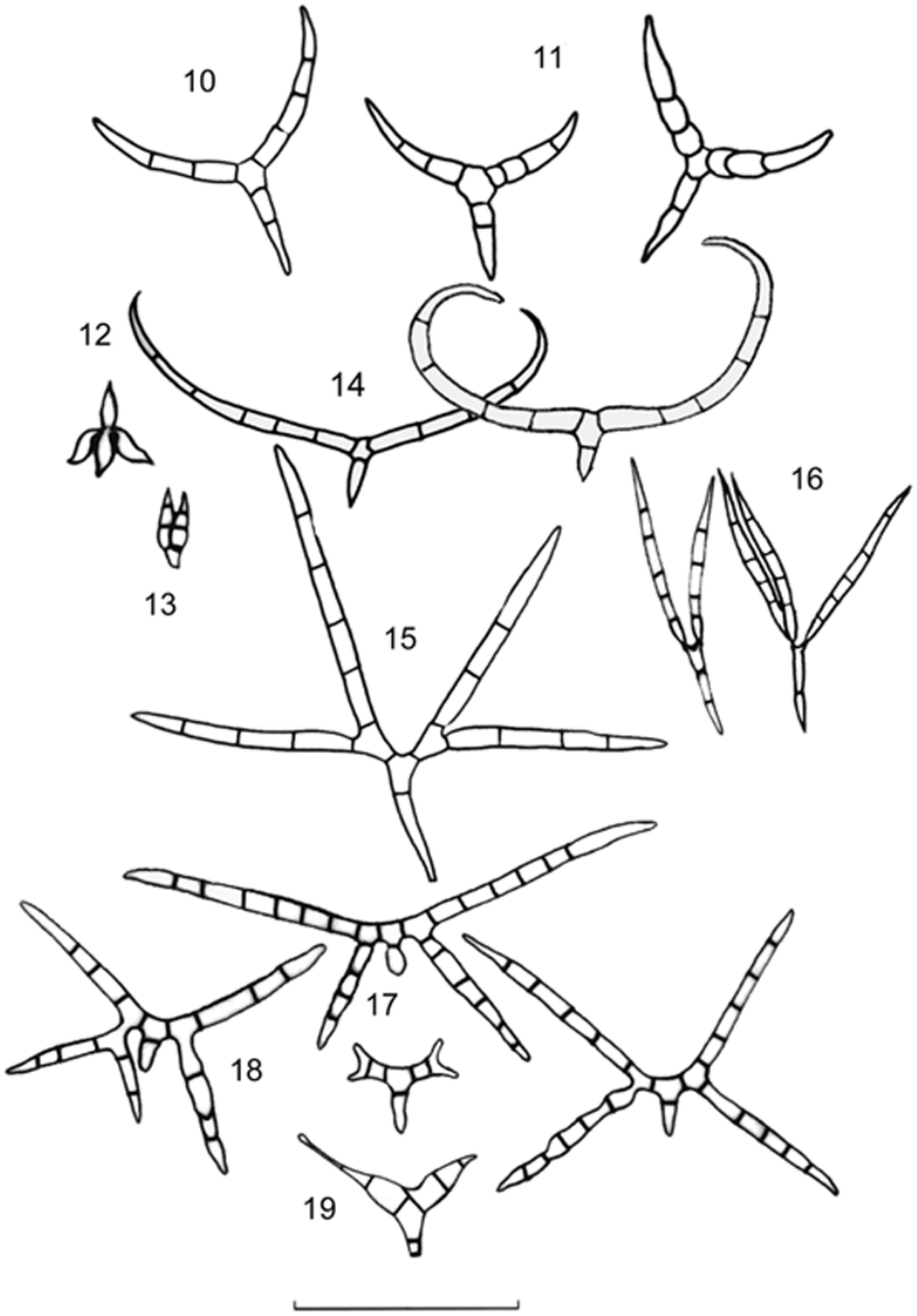
Table 3 continued. Numbers of fungal spores identified in honeydew honey samples.

Honey ref. No.	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
Locality	**I**	**C**	**I**	**I**	**G**	**I**	**I**	**G**	**G**	**G**	**G**	**I**	**I**	**I**	**I**	**I**	**I**	**I**	**I**	**G**	**I**	**G**	**I**	**I**	**I**
Scolecoconidia type 1	551	2	2	-	8	89	-	61	91	10	-	-	-	6	-	-	-	11	-	14	6	27	22	-	-
Scolecoconidia type 2	-	-	-	-	1	-	-	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Scolecoconidia type 3	-	-	-	-	-	8	-	4	6	-	-	-	-	2	-	-	-	-	-	1	-	4	-	-	-
Stauroconidia type 1	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
Stauroconidia type 4	-	-	-	-	-	4	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Number of species	12	6	4	3	7	20	4	11	9	4	1	2	8	14	2	3	3	7	2	7	3	11	6	4	4

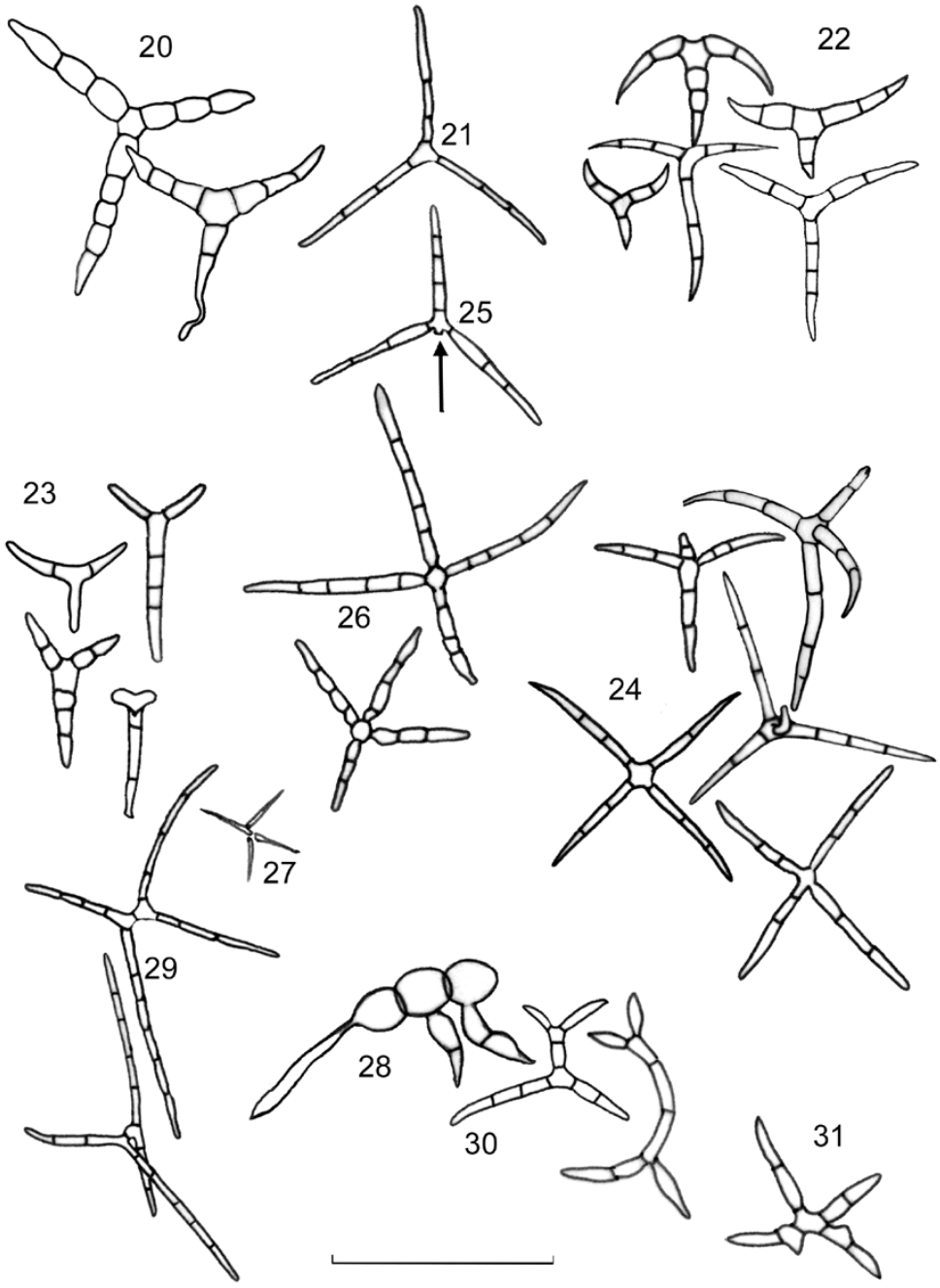
Japan. *Retiarius bovicornutus* was originally described from S. Africa as a pollen-capturing fungus in the phyllosphere (Olivier, 1978). It was also isolated from rainwater on *Pinus densiflora* in Japan by Ando and Tubaki (1984c), who believe that it may grow on intact leaf surfaces and produce "conidia under the impetus of fresh water in the form of rain, morning dew or mist". They considered this fungus to be a member of the "arboreal aquatic hyphomycetes". Our present findings, i.e. the occurrence of many conidia of these two species in honeydew honey from coniferous trees, also support their wide distribution in canopies, and the possibility of getting conidia into aquatic habitats from trees.

Conidia of three species of *Trinacrium*, namely *T. parvisporum* (Fig. 23), *T. robustum* (Fig. 20) and *T. subtile* (Fig. 21) were identified, though with some doubts. Most other conidia, however, could only be identified to genus (Fig. 22). In a recent study a great variety and a relatively great abundance of conidia belonging to different species of *Trinacrium* occurred in rainwater from living trees and treeholes in Hungary throughout the year (Gönczöl and Révay, 2003, 2004). These findings support the theory that most conidia of *Trinacrium* spp. found in foam or stream water may be from canopies.

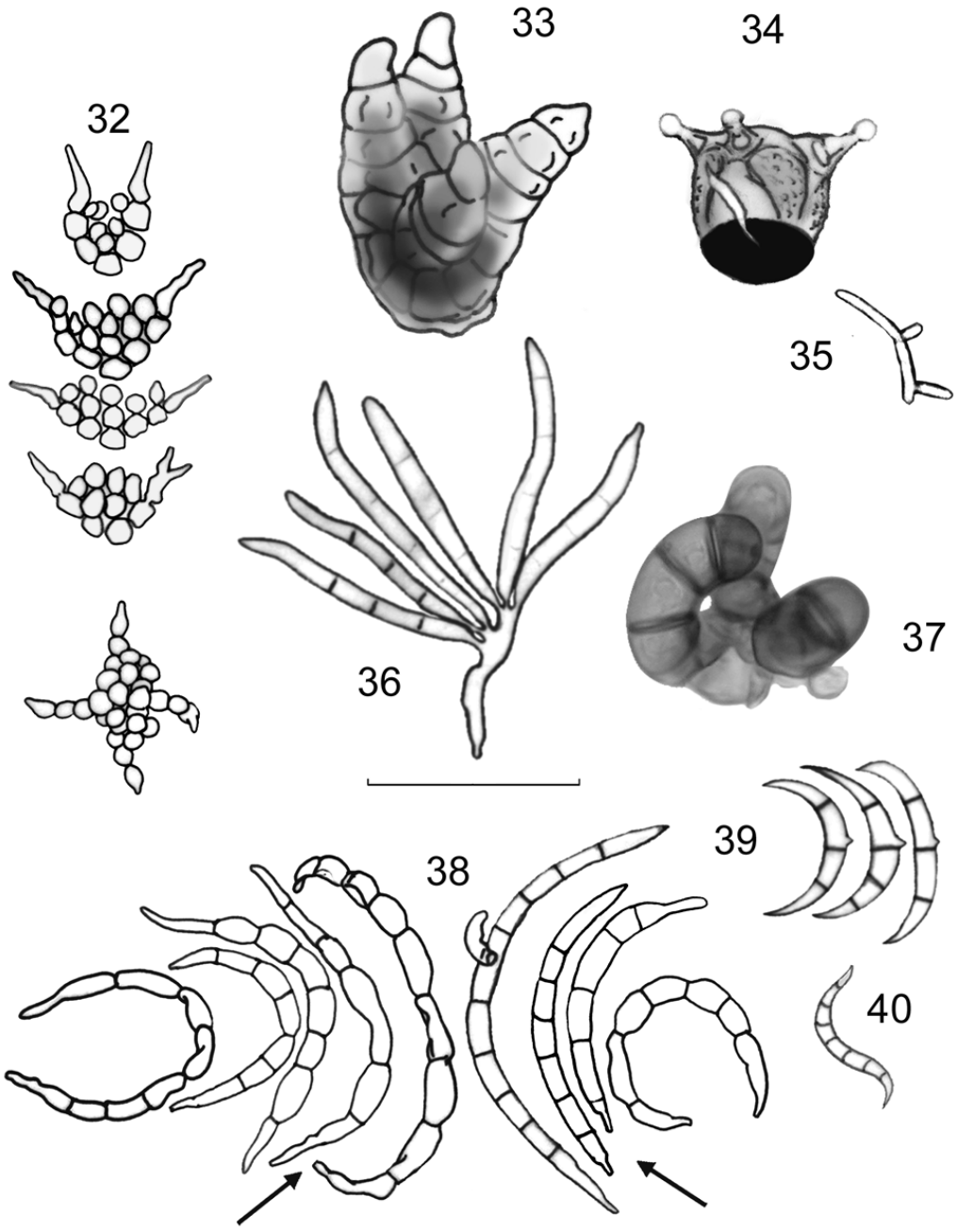
A conidium with characteristic, dichotomous branching was identified as *Dwayaangam dichotoma* (Nawawi, 1985) (Fig. 15). Other forms also resembled *Dwayaangam* (Figs. 17-18). Some tetraradiate conidia resembled *Lemonniera* spp. (Fig. 24). Some conidia of a *Trifurcospora* (probably *T. irregularis*) (Fig. 25) were seen in two samples from Italy. Together with our findings, increasing evidence suggests that *T. irregularis* is widely distributed on living plants, mostly trees. Conidia were also found in rainwater from *Commelina communis* and *Pinus densiflora* in Japan (Ando *et al*., 1987). Czeczuga and Orlowska (1999) recorded conidia of this species in rainwater from *Abies*, *Acer*, *Fraxinus* and *Tilia* spp. All stemflow samples from beech (*Fagus sylvatica*) in a Hungarian forest also contained many conidia of this species (Gönczöl and Révay, 2004). Stemflow samples collected from different



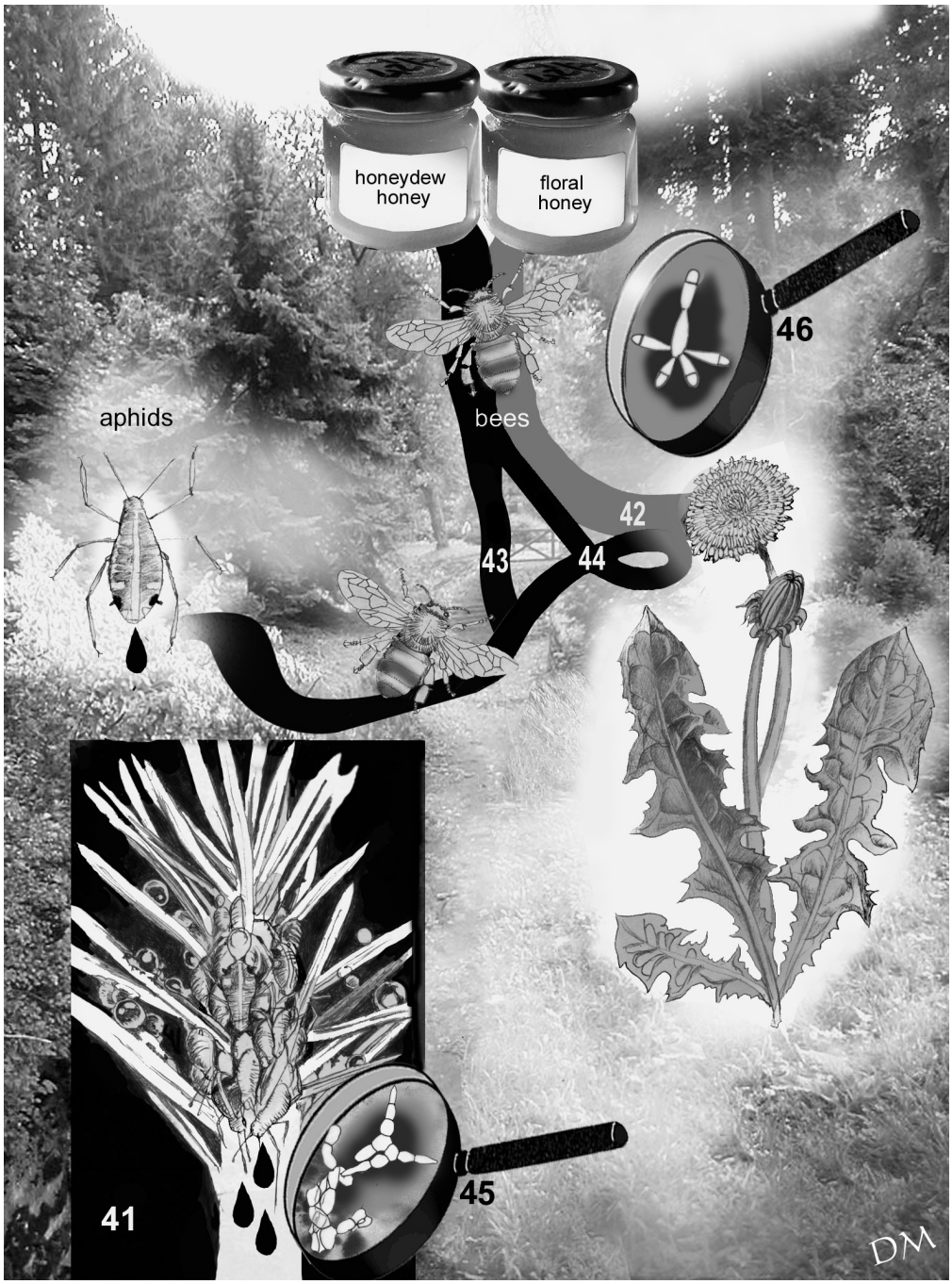
Figs. 10-19. Stauroconidia in honeydew honeys. **10-11.** *Retiarius bovicornutus*. **12.** *Tricellula* sp. **13.** *Dicranidion* sp. **14.** *Ceratosporium cornutum*. **15.** *Dwayaangam dichotoma*. **16.** *Isthmotricladia* sp. **17-18.** Young and mature conidia of *Dwayaangam* sp. **19.** *Diplocladiella scalaroides*. Bar = 50 µm.



Figs. 20-31. Stauroconidia in honeydew honeys. **20.** *Trinacrium robustum*. **21.** *Trinacrium subtile*. **22.** *Trinacrium* spp. **23.** *Trinacrium parvisporum*. **24.** *Lemonniera* spp. **25.** *Trifurcospora* sp. **26.** *Geniculospora* sp. **27.** Stauroconidia type 4. **28.** *Gyoerffyella* cf. *myrmecophagiformis*. **29.** *Curucispora* sp. **30.** *Articulospora* sp. **31.** *Tricladium* sp. Bar = 50 µm.



Figs. 32-40. Conidia in floral honeys and honeydew honeys. **32.** *Atichia* sp. **33.** Stauroconidia type 2. **34.** Stauroconidia type 3. **35.** *Varicosporium* sp. **36.** Stauroconidia type 1. **37.** Unknown helicoconidium. **38.** Scolecoconidia type 1 (arrows: short basal extensions on some conidia). **39.** Scolecoconidia type 3. **40.** Scolecoconidia type 2. Bar = 50 µm.



Figs. 41-46. Hypothetical process of accumulation of spores of filamentous fungi and of colonies of *Metschnikowia reukaufii* in honeydew honey and floral honey. **41.** Honeydew producing aphids in the phyllosphere. **42.** Floral nectar-collecting bees. **43.** Honeydew-collecting bees. **44.** Mixing of honeydew and floral nectar. **45.** Ingoldian-like fungi in the phyllosphere. **46.** Nectar-inhabiting *Metschnikowia reukaufii*.

tree species in the Black Forest region, Germany, likewise contained conidia of *T. irregularis* (Gönczöl, unpubl. obs.).

Unidentified scolecospores (type 1, Fig. 38) were found in unusually high numbers in two honeydew honey samples from Italy and in one from Greece. All were poorly resolved under the microscope, due to unsuitable techniques or mounting medium. Most of them probably belonged to several hyphomycete species. Basal extensions like those on *Filosporella* and *Anguillospora* were clearly seen on some. Others were seen as broken in the middle or with a central constriction. Their concentration was especially high (551 conidia) in the sample from the Tusco-Emilian Appenines. This finding is interesting because most of the studies on these "canopy fungi" have almost exclusively reported staurospores.

Various conidia of *Atichia* sp. (Fig. 32) were encountered in an Italian honeydew sample and five in a Greek one. *Atichia* spp. have been reported as epiphytic fungi on various trees and shrubs in both tropical and temperate regions (Meeker, 1975). However, a search in the literature suggests that "the members of *Atichiaceae* are true saprophytes living on honeydew like the members of the *Capnodiaceae* and that they therefore should be included in the sooty moulds group" (Fraser, 1936). The high conidial concentration in honeydew honey from *Picea excelsa* and *Abies alba* in the Italian sample and occurrence of conidia in almost all Greek honeydew honeys from *Abies alba* also support the above hypothesis.

We also observed some microorganisms other than fungi. Some individuals of *Vorticella* spp. in the floral honeys of *Castanea*, *Citrus*, *Eucalyptus*, *Rhododendron* and *Tilia* and *Vorticella*, *Euglena*, *Paramecium* and diatoms (e.g. *Cyclotella*, *Cymbella*, *Merismopedia* and *Nitzschia* spp.) in honeydew honeys were often seen. Whether the occurrence of these microorganisms reflects the existence of wet or aquatic microhabitats in canopies where some of the aquatic hyphomycetes may find adequate conditions for growth and sporulation requires more studies.

It is well known that honeydew serves as a nutrient for the sooty moulds (e.g. metacapnodiaceous fungi) (Reynolds, 1975; Hughes, 1976). However, we believe that honeydew acts only as a trap for the hyphomycete conidia found in the present study and it is not known if these can utilize honeydew as sooty moulds do. Due to the collecting activities of insects (primarily honeybees) the spores trapped in the honeydew will therefore accumulate in honeydew honey. This process is proposed in Figs. 41-46. We believe that honeydew, as with rainwater, merely washes spores (and sometimes other fungal elements, see Fig. 8) off tree surfaces. Rainwater possibly due to its much higher volumes and displacement, gathers fungal spores on aerial plant surfaces more

efficiently than honeydew. On the other hand honeydew honeys can preserve fungal spores (probably in inactive state) for a long time. Therefore honeydew honeys bear important mycological information on the source region.

One of the most intriguing questions is where do these fungi with stauro- and scolecoconidia, occurring regularly in rainwater and honeydew honey, live on trees. Living trees offer various habitats for saprotrophs, endo- or epibionts and parasitic fungi where they can find adequate conditions for short or long-term existence. Consequently rainwater or any other liquid (e.g. honeydew) on plant surfaces may contain a great variety of spore assemblages. However, we have little evidence of the active presence of "aquatic hyphomycete" species in canopies.

Numerous attempts have been made to find an appropriate term (e.g. arboreal aquatic hyphomycetes, canopy fungi, terrestrial aquatic hyphomycetes, terrestrial ingoldian hyphomycetes, terrestrial aquatic fungi) for this fungal group. To create a really adequate term we need to culture them, to establish their correct taxonomic position and to study their growth habits in nature. The same is true for traditional aquatic hyphomycetes: more data are needed to confirm whether they are actually able to exist on aerial, intact or dead parts of canopies. Experiments are also needed to better understand their physiological relationship with free water and possibly with some other environmental factors.

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References

Ando, K. (1992). A study of terrestrial aquatic hyphomycetes. Transactions of the Mycological Society of Japan 33: 415-425.

Ando, K. and Tubaki, K. (1984a). Some undescribed hyphomycetes in the rain drops from intact leaf-surface. Transactions of the Mycological Society of Japan 25: 21-37.

Ando, K. and Tubaki, K. (1984b). Some undescribed hyphomycetes in rainwater draining from intact trees. Transactions of the Mycological Society of Japan 25: 39-47.

Ando, K. and Tubaki, K. (1984c). Materials for the fungus flora of Japan. Transactions of the Mycological Society of Japan 25: 395-398.

Ando, K., Tubaki, K. and Arai, M. (1987). *Trifurcospora*: a new generic name for *Flabellospora irregularis*. Transactions of the Mycological Society of Japan 28: 469-473.

Bandoni, R.J. (1981). Aquatic hyphomycetes from terrestrial litter. In: *The Fungal Community*. *Its Organization and Role in the Ecosystem* (eds. D.T. Wicklow and G.C. Carroll), Marcel Dekker, New York: 693-708.

Carroll, G.C. (1981). Mycological inputs to ecosystem analysis. In: *The Fungal Community*. *Its Organization and Role in the Ecosystem* (eds. D.T. Wicklow and G.C. Carroll). Marcel Dekker, New York: 25-35.

Czeczuga, B. and Orlowska, M. (1999). Hyphomycetes in rain water, melting snow and ice. Acta Mycologica 34: 181-200.

Eisikowitch, D., LaChance, M.A., Kevan, P.G., Willis, S. and Collins-Thompson, D.L. (1990). Effect of the natural assemblage of microorganisms and selected strains of the yeast *Metschnikowia reukaufii* in controlling the germination of pollen of the common milkweed *Asclepias syriaca*. Canadian Journal of Botany 68: 1163-1165

Fehlmann, C. (1911). Beiträge zur mikroskopischen Untersuchung des Honigs. Mitteilungen der Schweizerischen Gesundheitsamt 2: 179-221.

Fraser, L. (1936). Notes on the occurrence of the Trichopeltaceae and Atichiaceae in New South Wales, and on their mode of nutrition, with a description of a new species of *Atichia*. The Proceedings of the Linnean Society of New South Wales 61: 277-284.

Gontarski, H. (1951). Zur Analise der Formbestandtteile des Waldhonigs. Zeitschrift für ienenforschung 1: 33.

Gönczöl, J. (1976). Ecological observations on the aquatic Hyphomycetes of Hungary II. Acta Botanica Academiae Scientiarum Hungaricae 22: 51-60.

Gönczöl, J. and Révay, Á. (2003). Treehole fungal communities: aquatic, aero-aquatic and dematiaceous hyphomycetes. Fungal Diversity 12: 19-34.

Gönczöl, J. and Révay, Á. (2004). Fungal spores in rainwater: stemflow, throughfall and gutter conidial assemblages. Fungal Diversity 16: 67-86.

Grüsz, J. (1927). Genetische und gärungsphysiologische Untersuchungen an Nektarhefen. Jahrbuch für wissenschaftliche Botanik 66: 109-82

Hughes, S.J. (1976). Sooty Moulds. Mycologia 68: 693-820.

Louveaux, J., Maurizio, A. and Vorwohl, G. (1978). Methods of melissopalynology. Bee World 59: 139-157.

Matsushima, T. (1975). Icones Microfungorum a Matsushima Lectorum. Published by the Author, Kobe.

Matsushima, T. (1981). Matsushima Mycological Memoirs No.2. Published by the Author, Kobe.

Meeker, J.A. (1975). Revision of the Seuratiaceae. I. Morphology of *Seuratia*. Canadian Journal of Botany 53: 2462-2482.

Nawawi, A. (1985). Some interesting Hyphomycetes from water. Mycotaxon 34**,** 217-226.

Olivier, D.L. (1978). *Retiarius* gen. nov.: Phyllosphere fungi which capture wind-borne pollen grains. Transactions of the British Mycological Society 71: 193-201.

Pérez-Atanes, S., Del Carmen Seijo-Coello, M. and Méndez-Álvarez, J. (2001). Contribution to the study of fungal spores in honeys of Galicia (NW Spain). Grana 40: 217-222.

Persano Oddo, L., Sabatini, A.G., Accorti, M., Colombo, R., Marcazzan, G.L., Piana, M.L., Piazza, M.G. and Pulcini, P. (2000). I mieli uniflorali italiani- Nuove Shede di Caracterizzare. Ministero delle Politiche Agricole e Forestali.

Pitt, J.I. and Miller, M.W. (1968). Sporulation in *Candida pulcherrima*, *Candida reukaufii* and *Chlamydozyma* species: their relationships with *Metschnikowia*. Mycologia 60: 663-685.

Reynolds, D.R (1975). Observations on the growth forms of sooty mould fungi. Nova Hedwigia 26: 179-183.

Ricciardelli D'Albore, G. (1998). Mediterranean melissopalynology. Universita degli Studi, Perugia.
Sabatini, A.G., Piana, M.L. and Grillenzoni, F.V. (2000). I mieli della Emilia-Romagna. Instituto Nazionale di Apicoltura, Bologna.
Tubaki, K., Tokumasu, S. and Ando, K. (1985). Morning dew and *Tripospermum* (Hyphomycetes). Botanical Journal of the Linnean Society 91: 45-50.

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