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## Treehole fungal communities: aquatic, aero-aquatic and dematiaceous hyphomycetes

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Hyphomycete communities in water-filled treeholes, a microhabitat in woodland ecosystems are discussed. Thirteen treeholes in four mountainous and forested areas of Hungary were examined for hyphomycetes. Eleven treeholes were detected in beech (*Fagus sylvatica*) and one each in oak (*Quercus* sp.) and ash (*Fraxinus excelsior*). Forty-five hyphomycete species were detected in the treeholes. The species number per treehole ranged from 3 to 10, as seen after litter incubation, but reached 19 when membrane filtration of treehole-water was included. The most frequent species was *Alatospora acuminata* (over 80% of treeholes). Distribution of *Colispora cavincola* appears to be restricted to treeholes. Conidia belonging to lesser known taxa, such as *Arborispora*, *Dwayaangam*, *Trifurcospora* and *Trinacrium* are discussed and illustrated. Membrane filtration of treehole-water suggests *in situ* sporulation of some aquatic hyphomycete species. Treeholes in SW Hungary were re-examined after 25 years and results suggest that they are long-lasting rather than ephemeral fungal microhabitats in woodland ecosystems.

**Key words:** aero-aquatic, aquatic, dematiaceous hyphomycetes, treehole.

### Introduction

Aquatic hyphomycetes (Ingold, 1942) are important members of the mycota decomposing submerged leaf and wood litter in various types of streams. Although these fungi typically inhabit running waters they have also been reported from standing water bodies such as lakes (Suzuki and Nimura, 1961; Casper, 1965) or ponds (Marvanová and Marvan, 1963), as well as from terrestrial litter (Park, 1974; Webster, 1977; Bandoni, 1981; Gönczöl and Révay, 1983; Sridhar and Bärlocher, 1993). We know little, however, about their distribution and role in water-filled treeholes, which are specific aquatic habitats and an integral part of forest litter ecosystems.

Wet or water-filled holes in live trees generally contain a rich variety of bacteria, algae, protozoans, platyhelminths, rotifers, nematodes, crustaceans,

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insects and other organisms (Maguire, 1971). For decades intensive taxonomical, ecological and experimental studies have been made on various organisms, especially on invertebrate communities, inhabiting treeholes (e.g. Lackey, 1940; Maguire, 1971; Paradise, 1998, 1999). Kitching (1971) in an extensive study summarized some conclusions which may be useful for future work on any group of litter decomposing fungi.

At present, treeholes appear to be *terra incognita* in mycology. But wet or submerged litter, e.g. fallen leaves, twigs and wood, the most important substrates for aquatic and other fungi in larger lotic or lentic waters, are also common and abundant in treeholes. The neglect in the study of aquatic hyphomycetes in treeholes may be due to the false belief that such holes are an unsuitable habitat for these fungi.

During earlier investigations in SW Hungary (Zala County), *Alatospora acuminata*, *Articulospora tetracladia*, *Camposporium pellucidum*, *Colispora cavicola* (as *Dactylella submersa*), *Helicodendron paradoxum*, *Helicodendron westerdykiae*, *Tricladium castaneicola* (as *Tricladium* sp.), were repeatedly encountered in water-filled treeholes (Gönczöl, 1976). Although the number of species in a treehole was generally low, all of the treeholes in ca. ten trees monitored in beech woodlands in this area proved to contain aquatic and aero-aquatic hyphomycetes. Changes in the aquatic hyphomycete species composition in some of the treeholes from July to October were also noticed (Gönczöl, 1976). These preliminary investigations suggested that treeholes are unique aquatic microhabitats with alternate dominance of aquatic and aero-aquatic hyphomycete species. *Triadelphia hungarica* (Révay, 1987) and *Colispora cavicola* (Gönczöl and Révay, 1996) were later described from treeholes as new species. Other sporadic information on *Diplocladiella scalaroides*, *Oncopodiella trigonella* (Révay, 1986), *Triadelphia heterospora*, *T. uniseptata* (Révay, 1987) and *Pseudospiropes obclavatus* (Révay, 1988) from treeholes has also appeared. We have been, however, unable to find further data in the literature concerning aquatic, aero-aquatic or any other group of microscopic fungi in treeholes.

Our objectives in this study were: (i) to find further treeholes in different areas of Hungary and explore their hyphomycetes; (ii) to study *in situ* sporulation of aquatic hyphomycetes by membrane filtration of treehole-water; (iii) to examine, by means of different incubation techniques, fungal sporulation on different substrates collected from treeholes; and (iv) to re-examine the species composition of treeholes in SW Hungary which had been studied over 25 years ago.

**Table 1.** The characteristics of treeholes in Hungary investigated for fungi.

Serial no.	Location	Date	Tree <sup>1</sup>	Condition of substrates	Water volume <sup>2</sup>	T <sup>3</sup>	pH	Cond. <sup>4</sup>
1	Bázakerettye	25 July 2001	Beech, living	Submerged	A	16	6.6	390
2	Bázakerettye	25 July 2001	Beech, living	Submerged	C	16	6.7	430
3	Bázakerettye	25 July 2001	Beech, living	Moist				
4	Bázakerettye	25 July 2001	Beech, living	Moist				
5	Csóványos	10 December 1979	Beech, living	Submerged	A			
6	Gálhegy	20 September 2001	Ash, living	Submerged	B	12	7.1	830
7	Gálhegy	30 July 2002	Oak, living	Moist				
8a	Királyrét	1 October 1993	Beech, living	Submerged	A	6	6.2	110
8b	Királyrét	27 August 2001	Beech, living	Submerged	A	20	5.9	390
8c	Királyrét	7 August 2002	Beech, living	Submerged	A	18	6.3	120
9	Királyrét	1 October 1993	Beech, living	Submerged	A	6	6.4	420
10	Királyrét	27 August 2001	Beech, dead	Moist				
11	Szelcepuszta	17 October 1990	Beech, living	Submerged	A	9	8.4	940
12	Velem	27 June 1990	Beech, living	Submerged	A			
13	Velem	25 August 1993	Beech, living	Submerged	A			

<sup>1</sup>ash: *Fraxinus excelsior*; beech: *Fagus sylvatica*; oak: *Quercus* sp.

<sup>2</sup>water volume in the treeholes: A < 1000 ml; B = 1000-2000 ml; C > 2000 ml.

<sup>3</sup>T = temperature (°C). <sup>4</sup>Cond. = conductivity (µS/cm).

## Materials and methods

Collections were made at the following sites in Hungary: Bázakerettye, Zala County, 25 July 2001; Csóványos, Börzsöny Mts., 10 December 1979; Gálhegy, Börzsöny Mts., 20 September 2001, 30 July 2002; Királyrét, Börzsöny Mts., 1 October 1993, 27 August 2001, 7 August 2002; Szelcepuszta, Borsod-Abaúj-Zemplén County, Aggtelek National Park, 17 October 1990; and Velem, Vas County, 27 June 1990, 25 August 1993. All the treeholes sampled were found in forested and mountainous areas and occurred in tree trunks at heights of 10 to 200 cm above ground level. Serial numbers and some characteristics of the treeholes are given in Table 1.

Decaying leaves, twigs and other detritus (e.g. cupules) from the treeholes were placed in plastic boxes. Water was added to those samples from water-filled treeholes. Samples were transported in a cooling box to the laboratory. After rinsing in tap water, substrates were incubated in a refrigerator (12°C) under the following conditions: (i) Leaves found in water-filled treeholes were placed in aerated distilled water for 1 week. Conidia were harvested by gently placing coverslips in contact with the water surface, before placing the coverslips on microscopic slides. Conidia were first examined, measured and photographed in water and then preserved in a drop of cotton blue with lactic acid. This method gave good information on both sporulating

species and conidial abundance of a given species. After these examinations the volume of water was reduced and the incubation resumed in damp chambers. (ii) Substrates collected from wet treeholes (without free water) were moist-incubated in Petri dishes. Twigs, leaves and cupules were examined under a dissecting microscope to detect fruiting structures. Incubation and examination was continued for several weeks. Semipermanent slide preparations were made and other herbarium specimens kept when possible.

Water was membrane filtered from some treeholes. With a hand-operated pump water was carefully extracted so as not to disturb the detritus. Depending on turbidity of the water 30-50 ml were passed through Whatman Isopore polycarbonate membrane filters (25 mm diam., 8  $\mu$ m pore size) using a pneumatic pump (Antlia Pressure Filtration System, Schleicher & Schuell, Dassel, Germany). The conidia trapped on the filters were stained, identified and counted.

Water temperature, pH and conductivity were measured in some of the water-filled treeholes.

## Results

Water temperature in the treeholes investigated was always lower (generally 6-7°C) than that of air, but in an extreme case the difference was 13°C. The pH ranged from 5.9 to 8.4, with the lowest value measured in treehole 8 after a day of heavy rain, suggesting that the rainwater was more acid.

The species encountered, their location and the methods by which they were detected are listed in Table 2. Conidia of 45 hyphomycete species were found. The most frequent was *Alatospora acuminata*, which occurred in more than 80% of the treeholes. Other frequently found species were *Hormiactis ontariensis* (69%), *Colispora cavincola* (62%), *Tricladium castaneicola* (62%), *Helicodendron triglitzense* (62%), *Helicodendron tubulosum* (54%) and *Beverwykella pulmonaria* (38%). Thirty-one out of the 45 species were detected with incubation, 20 species were recognized by membrane filtration and six species by using both techniques. Many catenate arthroconidia, seen as parts of chains and single conidia, were present in most of the samples but could not be identified even at the generic level. The exception was *Hormiactis ontariensis* (Figs. 20, 21).

The filter membranes from treeholes 1 and 2 contained much fine particulate matter, which covered most of their surface, but some species could still be recognized among the detritus, i.e. *Alatospora acuminata*, *Tricladium castaneicola*, *Camposporium pellucidum*, *Triadelphia hungarica* and probably

a species of *Mirandina* (Fig. 15), suggesting *in situ* sporulation. We were, however, unable to quantify our results due to interference from the debris.

Relatively clean water was, however, found and sampled from treehole 8c (Börzsöny Mts., 7 August 2002). It had rained heavily in this area the previous day. This treehole contained *ca.* 300 ml of water in which just a few leaf fragments were found; 50 ml could be easily filtered. A total of 316 conidia were counted. We distinguished 16 taxa (Table 2) some of which may be noteworthy (Figs. 1-10). Four forms remain unidentified (Figs. 11-14). The sigmoid conidia (Figs. 12-14) are closely similar to those of some Ingoldian fungi. More than 100 conidia, however, were not registered because they belonged to widespread terrestrial hyphomycetes (*Alternaria*, *Cladosporium*, etc.) or coelomycetes (e.g. 26 conidia of *Asterosporium asterospermum* (Pers. ex Gray) S. Hughes, which also occur in stream foam or water).

The lowest number of species per treehole was 3 and the highest 19. However, the cumulative number of species in treehole 8, which was sampled on three occasions, was 24. This relatively high figure was probably due to repeated sampling as well as to the combination of different sampling techniques. Ignoring membrane filtration, the number seen per treehole ranged from 3 to 10. Three species of aquatic hyphomycetes were encountered. *Dimorphospora foliicola* and *Varicosporium elodeae* were recovered from water-filled treeholes only, while *Alatospora acuminata* occurred in both water-filled and wet treeholes. The number of aero-aquatic species was much higher. Among samples with the highest number of common species, four treeholes (8, 9, 12, 13) showed the highest similarity.

Treeholes 1 and 2, from a woodland in SW Hungary, which had been studied 25 years earlier, were re-examined, and a number of species were again detected.

### **Remarks on some species**

#### ***Alatospora acuminata s.l.***

Although this conidial form was the most frequently seen in the samples, there were differences between treeholes. For example, on leaves from treehole 1 many conidia were produced after 24 hours incubation, while on the leaves from treehole 2 there was very weak conidial production even after 2 weeks of incubation. These treeholes were close to each other and the leaves were similarly processed.

#### ***Alatospora acuminata s.s.***

(Fig. 25)

Submerged beech leaves from treeholes 8a and 9, after 1 week aerated incubation in shallow water, produced many one-branched and some

**Table 2.** Hyphomycete species found in treeholes.

Species	Treehole number															
	1	2	3	4	5	6	7	8a	8b	8c	9	10	11	12	13	
<i>Actinocladium rhodosporum</i> Ehrenb.						i										
<i>Alatospora acuminata</i> Ingold	if	if	i		i	i	i	i	i	if	i	i		i	i	
<i>Arborispora</i> sp.				i								i				
<i>Beverwykella pulmonaria</i> (Beverwijk) Tubaki	i	i	i				i							i		
<i>Cacumisporium capitulatum</i> (Corda) Hughes				i												
<i>Camposporium pellucidum</i> (Grove) Hughes	if			i							i	i				
<i>Camposporium</i> sp.												i				
<i>Candelabrum spinulosum</i> Beverwijk				i						i						
<i>Colispora cavincola</i> Gönczöl & Révay		i	i		i			i	i	if	i	i		i	i	
<i>Dactylaria obtriangularia</i> Matsush.													i			
<i>Dimorphospora foliicola</i> Tubaki		i														
<i>Diplocradiella scalaroides</i> Arnaud ex Matsush.							i			f						
<i>Dwayaangam cornuta</i> Descals											f					
<i>Dwayaangam</i> sp.											f					
<i>Helicodendron triglitzense</i> (Jaap) Linder	i	i				i	i	i	i	i	i		i	i		
<i>Helicodendron tubulosum</i> (Reiss) Linder	i		i			i		i	i				i	i		i
<i>Helicodendron westerdykiae</i> Beverwijk								i								
<i>Helicodendron</i> sp.								i								
<i>Helicomycetes bellus</i> Morgan								i								
<i>Hormiactis ontariensis</i> Matsush.			i	i		i	i	i	i		i		i	i	i	
<i>Isthmolongispora minima</i> Matsush.	i		i													
<i>Mirandina corticola</i> Arnaud								i		i						
<i>Mirandina</i> sp.	if														i	
<i>Monodictys putredinis</i> (Wallr.) Hughes			i													
<i>Oncopodiella trigonella</i> (Sacc.) Rifai										f						
<i>Phragmocephala glanduliformis</i> (Höhnel) Hughes															i	
<i>Pseudaegerita viridis</i> (Bayliss Elliot) Abdullah & Webster	i						i							i		i
<i>Spondylocladiopsis cupulicola</i> M.B. Ellis														i		i
<i>Sporidesmiella hyalosperma</i> P.M. Kirk														i		i
<i>Sporidesmium goidanichii</i> (Rambelli) Hughes										i						
<i>Titaea</i> sp.											f					
<i>Triadelphia hungarica</i> Révay	f	f														
<i>Tricladium castaneicola</i> Sutton	if		i					i	i		i	i	i	i	i	
<i>Trifurcospora</i> sp.											f					
<i>Trinacrium</i> sp. 1											f					
<i>Trinacrium</i> sp. 2											f					
<i>Trinacrium</i> sp. 3											f					
<i>Tripospermum myrti</i> (Lind) Hughes											f					
<i>Vargamyces aquaticus</i> (Dudka) Tóth								i						i		
<i>Varicosporium elodeae</i> Kegel						i			i	i		i				i

\*i = on incubated substrates; f = on membrane filter.

Table 2. (continued).

Species	Treehole number															
	1	2	3	4	5	6	7	8a	8b	8c	9	10	11	12	13	
<i>Ypsilina graminea</i> (Ingold <i>et al.</i> ) Descals, Webster & Marvanová							i									
Unknown sp. 1																f
Unknown sp. 2																f
Unknown sp. 3																f
Unknown sp. 4																f
Number of fungal species	10	6	9	4	3	8	9	7	8	19	7	9	9	7	8	8

unbranched conidia of this morphology (the acicular form seen in Marvanová and Descals, 1985, Fig. 2E) amongst the two-branched ones. Some of the leaves were moist-incubated and many acicular conidia were again observed. Few one-armed conidia could, however, be seen. During incubation the leaves were occasionally re-wetted but never submerged. Moist incubation was continued in a refrigerator (12°C) for *ca.* 3 months and masses of unbranched conidia were again observed several times. Some leaf fragments from treehole 8c were more recently collected and incubated in shallow unchanged standing water for 2 months. Moist-incubation was then continued and exclusively acicular conidia in great quantities were obtained. The predominance of acicular forms amongst the "normal" conidia has been rarely recorded by other workers. Bandoni (1972) also observed masses of unbranched conidia in cultures obtained from terrestrial litter. He concluded that "temperature and type of agar medium appear to be the critical factors controlling conidial formation in culture." Marvanová and Descals (1985) concluded that branch number may depend on conditions at sporulation.

Our observations suggest that incubation in unchanged standing water followed by moist-incubation may induce the production of greater quantities of acicular conidia. It is not known whether these incubation conditions have similar effects on conidial formation when the substrates are derived from running water. More precise experiments on incubation conditions and further collections from various habitats are needed to clarify the reasons for morphological variation in conidia of this species.

***Arborispora* sp.**

(Figs. 18, 19)

Several dozens of the characteristic and spectacular conidia of this fungus were observed on beech leaves from treeholes 4 and 10 after 1 week moist incubation. Both treeholes contained wet but unsubmerged leaves. Conidial dimensions varied (main axis 25-52 µm long) but conidia were constant in their structure. Conidia of the same species had been previously

found on terrestrial forest litter in Hungary and reported under the name *Magdalaenaea monogramma* Arnaud (Gönczöl and Révay, 1983). Marvanová and Bärlocher (2001) reported *Arborispora dolichovirga* K. Ando from Canada and were of the opinion that the earlier name for this species is probably *Magdalaenaea monogramma*. Our fungus is different in conidial shape and structure from *Arborispora dolichovirga*. The symmetrical, flabelliform conidia always have only two primary branches inserted on the basal cell of the axis and very near to the conidial base. Secondary and tertiary laterals are frequent. Thus the conidial shape contrasts with those of both the Japanese (Ando and Kawamoto, 1986) and the Canadian materials, which commonly bear more complicated structures and appear not to be in one plane. On the other hand, the conidia reported from England (Ingold and Ellis, 1952, Fig. 1H; Ingold, 1977, Fig. 1B); Malaysia (Nawawi, 1985, Fig. 76); Puerto Rico (Santos-Flores and Betancourt-López, 1997, Fig. 113) and India (Sati *et al.*, 2002) match ours.

***Colispora cavicola*** (Fig. 26)

Earlier studies (Gönczöl and Révay, 1996) and recent investigations on the species composition of fungal communities in treeholes suggest that this species is a common treehole resident. Its moderate sporulation underwater, which becomes massive on water films above the substrates, approaches this species to the aero-aquatic fungi. Its taxonomic position is problematic as it is close to *Colispora elongata* Marvanová and *Dactylella submersa* (Ingold) S. Nilsson. Its regular occurrence in treeholes suggests an evolutionary trend diverging from the stream species of Ingoldian fungi.

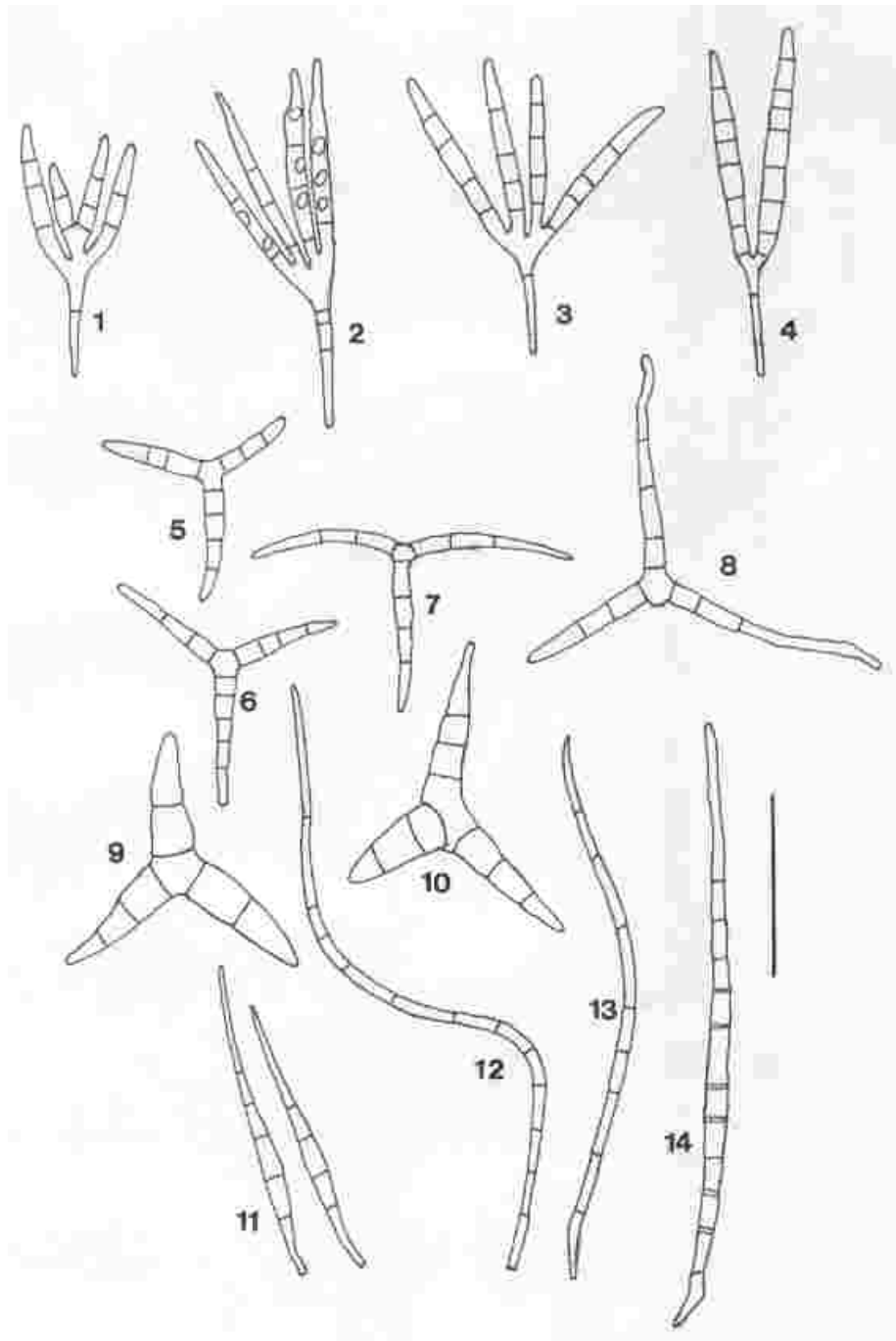
***Dwayaangam* sp.** (Figs. 1-4)

Four conidia with a clearly dichotomous branching structure were seen on the filter from treehole 8. Their main diagnostic characters agree with those of *Dwayaangam dichotoma* Nawawi (1985). We have found only one record of this species in later literature (Sati *et al.*, 2002). Descals (1998; Fig. 8K) illustrated a conidium bearing some resemblance to *D. dichotoma*, but the branching dichotomy is less obvious and the number of branches (8) is higher than that given for *D. dichotoma*.

***Trifurcospora* sp.** (Fig. 8)

The triradiate conidial structure with a characteristic central body strongly suggest the generic identity of these conidia but, without knowledge of their mode of development, these characters are insufficient for safe identification at the species level. Our conidia may belong to either *T.*





**Figs. 1-14.** Conidia on membrane filters. 1-4. *Dwayaangam* sp. 5, 6. *Trinacrium* sp. 1. 7. *Trinacrium* sp. 2. 8. *Trifurcospora* sp. 9, 10. *Trinacrium* sp. 3. 11-14. Scolecoconidia of unknown species. Bar = 30  $\mu$ m.

*irregularis* (Matsush.) Ando & Tubaki or to *T. subsessilis* Matsush. & T. Matsush. All materials of *T. irregularis* collected and isolated by Ando (in Ando *et al.*, 1987) was obtained from rainwater draining from living trees, and this source is also suspected in our sample. The latter species was recently isolated and cultured from stream foam by Marvanová and Bärlocher (2001).

***Trinacrium* spp.** (Figs. 5-7, 9, 10)

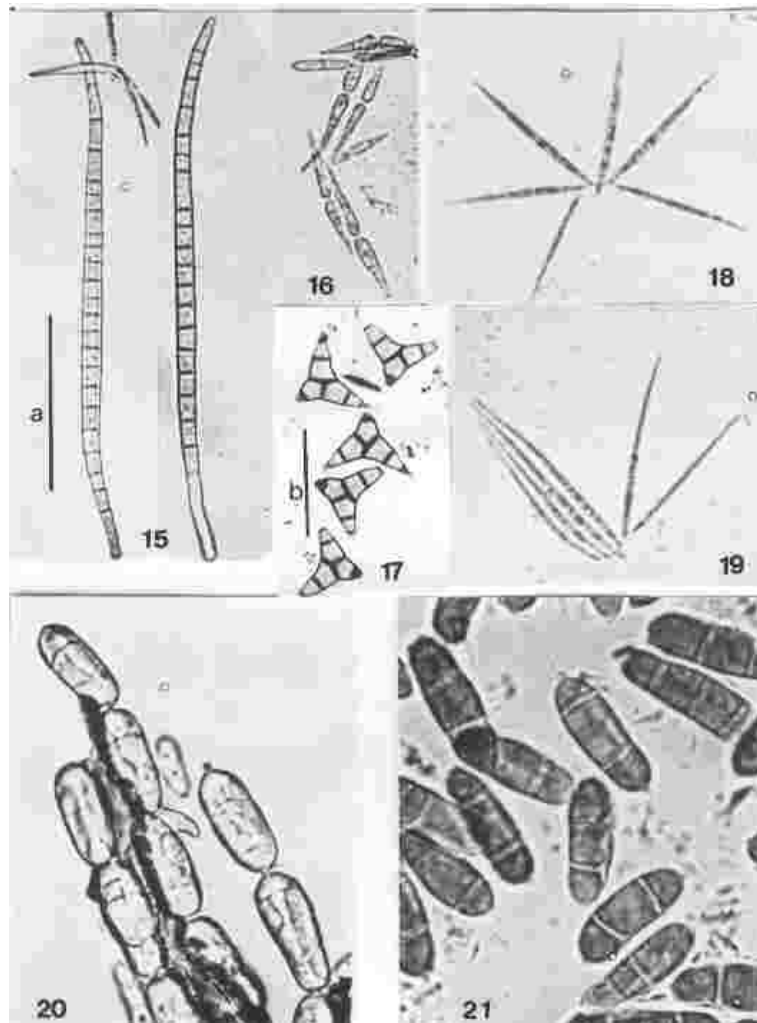
Nearly 30 characteristically triradiate conidia probably belonging to different species of *Trinacrium* were obtained in water filtered from treehole 8. We suspect, however, that they may also be transported by rainwater draining down the tree trunk, because treehole 8 was the only one filled by fresh rainwater on the day before sampling. We had previously observed the frequent occurrence of conidia of *Trinacrium* spp. in stemflow on other trees (unpublished obs.). Conidia in Figs. 5-7 (representing the most frequent forms on the filter) could not be identified to species. The shape, size and structure of the conidia shown in Figs. 9, 10, however, suggest *Trinacrium robustum* S.S. Tzean & J.L. Chen (1989) described on fallen, decaying stem from Taiwan.

## Discussion

Treeholes trapping water are reported from many parts of the world. In Western Europe most of the species of deciduous tree species have been reported to develop holes, and water-filled treeholes are important aquatic environments for insects and many other organisms (Kitching, 1971). Their role in the life-cycles of fungal species in the woodland ecosystem is not known.

### ***Water-filled treeholes: flow chamber in nature?***

Possibly fluctuations in the amount of water in treeholes is one of the most important factors in fungal species composition and sporulation rates. The water level in the treehole decreases due to evaporation and perhaps to seepage into underlying wood. Then, due to incoming rainwater, the water level increases. In this way the plant litter is alternately submerged and exposed to air. Such effects may also influence the fungal species composition in lakes and ponds, but conditions in treeholes are obviously different. A water-filled treehole with its stagnant water appears to be a typical case of lentic waters but, this classification certainly is not adequate. Depending on the frequency and intensity of rain the stagnant water in treeholes may periodically become running water. We observed that during steady rain the rainwater draining from the tree trunk flowed into the treehole and, when it was filled, excess water



**Figs. 15-21.** Conidia from litter after laboratory incubation. **15.** *Mirandina* sp. **16.** *Isthmolongispora minima*. **17.** *Diplocladiella scalaroides*. **18, 19.** *Arborispora* sp. **20, 21.** *Hormiactis ontariensis*. Bars = 30  $\mu$ m; Fig. 17 with scale b and others with scale a.

flowed out. We believe that a treehole during heavy rains may work like a natural, open "flow chamber" (similar to that described in Descals *et al.*, 1976). Probably the current in a deep treehole may be very weak, if at all present. Still, these changing hydrological conditions are evidently preferred by some species. Such a renewal of treehole-water due to fresh rainwater may be a reasonable explanation as to why we have on several occasions found clean and odourless water in the holes. In the water-filled treeholes the skeletonized or partly decayed leaves were generally quite similar in their colour and condition to those in clean stream water. Nevertheless, in spite of the periodic

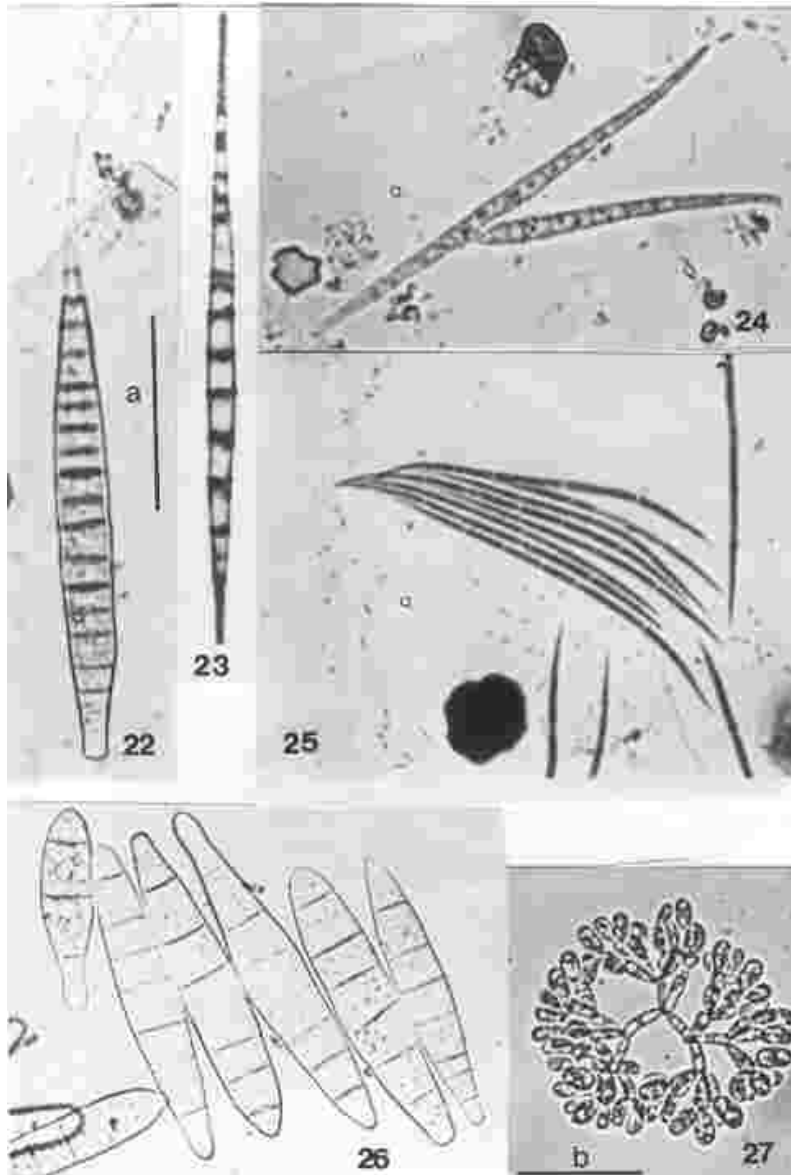
occurrence of these favourable conditions, water-filled treeholes may probably never become suitable habitats for the majority of the true aquatic hyphomycetes.

### ***Treehole inhabitant species***

Descals and Moralejo (2001) reviewed the names proposed in the literature for filamentous fungi found in continental aquatic habitats. Terms defining these groups of fungi, e.g. aquatic hyphomycetes, aero-aquatic hyphomycetes, amphibious hyphomycetes, terrestrial aquatic fungi and Ingoldian fungi, are based on fungal relationships with water and/or on taxonomical criteria. None of the above terms can alone be applied to fungi in treeholes found in this and earlier studies.

A heterogeneous group of fungi appears to dominate treehole habitats. Its components are:

1. Fungi such as *Alatospora acuminata*, *Articulospora tetracladia*, *Dimorphospora foliicola* and *Varicosporium elodeae*, which are regularly recorded from stream waters. *Dimorphospora foliicola* and *V. elodeae* during this study and *Articulospora tetracladia* in an earlier one (Gönczöl, 1976) were recovered only from water-filled treeholes, while *Alatospora acuminata* occurred in both water-filled and moist treeholes. In the present study, underwater sporulation was observed in all these species during laboratory incubation. Despite their frequent occurrence in streams, several observations suggest that they are terrestrial rather than typically aquatic species, or alternatively they may have more than one biotype (Nilsson, 1964; Bandoni, 1972; Sanders and Webster, 1978; Descals and Moralejo, 2001).
2. A relatively high number of species in treeholes are helicosporous and other aero-aquatic fungi, which are typically also found in stagnant surface waters. On the basis of their sporulating behaviour (i.e. their conidia are only produced, often in great masses, if the submerged and/or wet substrates are exposed to the air), *Colispora cavicola*, *Hormiactis ontariensis*, *Mirandina corticola* and *Tricladium castaneicola* could be classified as aero-aquatic. It seems likely that all these species occupy a similar ecological niche.
3. Some species among the dematiaceous forms, such as *Camposporium*, *Diplocladiella*, *Triadelphia* and *Vargamyces* are regularly collected on moist woody litter from or near streams, but little is known on their sporulation underwater (Descals and Moralejo, 2001).
4. Other dematiaceous species, e.g. *Sporidesmium* and *Phragmocephala*, where sporulation is normally aerial, probably inhabit treeholes only incidentally.



**Figs. 22-27.** Conidia from litter after laboratory incubation. **22.** *Camposporium* sp. **23.** *Mirandina corticola*. **24.** *Ypsilina graminea*. **25.** Group of acicular conidia of *Alatospora acuminata*. **26.** *Colispora cavincola*. **27.** *Dimorphospora foliicola*, conidiophores with conidia. Bars = 30  $\mu$ m; Fig. 27 with scale b and others with scale a.

5. Some species, e.g. *Trinacrium* and *Trifurcospora*, known primarily from rainwater draining from tree trunks (Ando, 1992), may also be components of the treehole fungal communities. Further analysis is needed to clarify their sporulation behaviour and role here.

The probably widespread and in some cases (treeholes 1, 2, 8) repeated occurrence of *Alatospora acuminata* s.l. and s.s., *Colispora cavincola*, *Hormiactis ontariensis*, *Helicodendron* spp., *Tricladium castaneicola* and *Varicosporium elodeae* imply that they play an important role in treehole communities. So far only *Colispora cavincola* appears to be restricted to treeholes. These species are probably able to maintain or renew their biomass in treeholes from year to year, or even for decades, as was demonstrated in the Börzsöny Mts. and in SW Hungary.

Inevitably the question arises, not only in the case of fungi but in that of many other treehole-inhabiting biota: "how did these forms first enter treeholes" (Lackey, 1940). Aerially dispersed spores can obviously enter treeholes, but how about hydrochorous (water dispersed) spores? Some forty years before Bandoni (1974) proposed his aqueous film theory, Lackey (1940) mentioned that "upward migration of some forms in the film of water along the bark in wet seasons may also be taken into account" for explaining the terrestrial distribution of several aquatic organisms. The occurrence, activity and distribution of aquatic fungal species in non-aquatic habitats (e.g. terrestrial litter) have been discussed in several papers addressing this problem (Park, 1974; Webster, 1977; Bandoni, 1981; Gönczöl and Révay, 1983; Sridhar and Bärlocher, 1993). The horizontal distribution of spores in aqueous films in the litter layer (Bandoni, 1974; Bandoni and Koske, 1974) is plausible, but it is less acceptable for upward distribution to treehole litter or even less so for dispersal between treeholes. The "splashing water theory" of Kitching (1971) may also suitably explain the escape of spores from treeholes rather than their entry into treeholes from soil or terrestrial litter. Spores or other fungal elements may also have a chance to find entrance into treeholes in or on wet or dried leaves or leaf fragments (Sanders and Webster, 1978; Sridhar and Kaveriappa, 1987). Rainwater running down the tree trunk (in many papers referred to as 'stemflow') is also apparently a means for propagules to enter treeholes. Obviously in this case another question arises: where do the spores in this rainwater come from? The endophytic and/or epiphytic origin of some of these species may also be suspected but all of these questions require further analysis. Additionally studies on fungal composition in treeholes in other regions would also be warranted.

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