

Water-borne conidial fungi inhabiting tree holes of the west coast and Western Ghats of India

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The present study focuses on water-borne conidial fungi associated with leaf litter in riparian tree holes of the west coast (Konaje) and Western Ghats (Sampaje) of India during dry (summer) and wet (monsoon) seasons. Out of a total of 34 fungal taxa recovered from leaf litter trapped in tree holes, 26 fully identified taxa constitute the first record for the tree hole habitat. Paired t-test revealed no significant difference in number of taxa ($P = 0.1177$) and conidial output ($P = 0.1816$) between Konaje and Sampaje tree holes, while they significantly differed between seasons of Konaje ($P = 0.0258$) and Sampaje ($P = 0.0206$). The Simpson and Shannon diversity of fungi were highest in Sampaje than Konaje tree holes. Tree-wise diversity of fungi revealed that banyan (*Ficus benghalensis*) tree holes are highly diverse during the summer and monsoon seasons in Konaje, but only during summer in Sampaje. In spite of a low conidial output, tree hole leaf litter of Konaje yielded 18 taxa against 20 taxa in the Konaje stream, whereas 29 taxa were recorded in Sampaje tree holes against 68 taxa in the Sampaje stream. The current study has been compared with earlier investigations on the occurrence of water-borne conidial fungi in tree canopies (e. g. intact leaves, leaf litter, tree holes, stemflow and throughfall).

Key words: tree holes, canopy, leaf litter, water-borne fungi, conidia, diversity.

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Studie je zaměřena na vodní konidiální houby osidlující listový opad v dutinách stromů na západním pobřeží Indie (lokalita Konaje) a v oblasti Západního Ghátu (Sampaje) během suchého letního a vlhkého monzunového období. Ze 34 zaznamenaných druhů hub představuje 26 přesně určených druhů jejich první nálezy v dutinách stromů. Dutiny na obou lokalitách se statisticky nelišily v počtu druhů, ale statisticky významně se lišily v závislosti na sezóně. Diverzita hub byla vyšší na lokalitě Sampaje. *Ficus benghalensis* měl velkou diverzitu hub během léta i monzunu na lokalitě Konaje, ale jen v létě na lokalitě Sampaje. Výsledky byly porovnány s dřívějšími studiemi o výskytu vodních konidiálních hub v korunách stromů.

INTRODUCTION

Forest canopies are well known for biological diversity (Erwin 1988) and constitute an interface between 90 % of the earth's terrestrial biomass and the atmosphere (Ozanne et al. 2003). They provide several niches for flora, fauna and microbes as they trap considerable amounts of organic matter (e. g. leaf litter, twigs, inflorescences) and transforms it into 'crown humus'. The mechanical support of trunks, branches and leaves provide shelter for many epiphytes including ferns (Nadkarni et al. 2001). Trees are also hosts for a variety of microorganisms in live tissues (as endophytes or symbionts), on surfaces (leaves, trunk, bark, root) and in tree holes. Rain water received by the canopies either evaporates directly, runs off the canopy (throughfall or stemflow) or reaches soil in intensive contact with tree surface (e. g. leaves, bark) (Schorch et al. 1999). Such contact of water with tree surfaces leads to an exchange of minerals and organic substances (Coxson et al. 1992). Minerals and organic substances of canopy-runoff are valuable nutrients to microorganisms including fungi. Despite the global significance of forest canopies, canopy studies have not drawn much attention of Indian scientists (Davy and Ganesh 2003).

Water-borne conidial fungi are abundant on submerged leaf litter in streams and act as intermediaries of energy flow between leaf litter and invertebrates (Ingold 1942, Bärlocher 1992). Although they are aquatic or water-borne, they have been reported from a variety of terrestrial habitats (e. g. soil, leaf litter, roots, leaf surfaces) (Sridhar and Bärlocher 1993). Occurrence of typical aquatic and water-borne conidial fungi in tree canopies (throughfall, stemflow and tree holes) has been reported from temperate regions: Canada (Bandoni 1981), Hungary (Gönczöl 1976; Gönczöl and Révay 2003, 2004, 2006), Japan (Ando and Tubaki 1984a, 1984b) and Poland (Czeczuga and Orłowska 1994, 1998a, 1998b, 1999). Tree holes with litter and crown humus support a variety of bacteria, fungi, algae, protozoans, platyhelminths, rotifers, nematodes, crustaceans and insects (Maguire 1971, Gönczöl and Révay 2003). Tree holes with a substantial amount of detritus (e. g. leaves, twigs, wood) and continuous flow of nutrient-rich water in the rainy season is an ideal habitat for water-borne fungi. Although several studies have been carried out on the occurrence of water-borne hyphomycetes outside streams (Sridhar and Kaveriappa 1987, Sridhar and Bärlocher 1993), studies on their occurrence in tree canopies in India are scanty (Sridhar et al. 2006). Extensive rainfall during the southwest monsoon in Southern India (approx. 350–650 cm/annum) results in continuous wet canopies in trees of the Western Ghats and the west coast during June–September. Such aquatic or semi-aquatic conditions are likely to support the growth, sporulation and dissemination of water-borne conidial fungi in tree canopies. Therefore, the current study aims to examine the assemblage and diversity of water-borne conidial fungi in riparian tree holes of the west coast and Western Ghat region in India during the summer and monsoon seasons.

MATERIALS AND METHODS

The first sampling site, Konaje (12° 48' 50.79" N, 74° 55' 38.28" E) is adjacent to Mangalore University Campus at 70-85 m altitude located about 20 km from the coastal city Mangalore on the southwest coast of India. Tree holes of five riparian trees about 50–60 m apart, adjacent to the Konaje stream, were assessed (one tree each of *Ficus benghalensis* L., *Holigarna ferruginea* Marchand, *Macaranga peltata* (Roxb.) Müll.-Arg., *Mangifera indica* L. and *Syzygium caryophyllum* (L.) Alston). The second sampling site, Sampaje (12° 28' 59.83" N, 75° 32' 59.95" E) at 380 m altitude, located about 25 km from Madikeri City, which is at the peak of the Western Ghats. Tree holes of five riparian trees about 59–60 m apart, adjacent to the Sampaje stream, were sampled (two trees each of *Artocarpus hirsutus* Lam., *Elaeocarpus tuberculatus* Roxb. and one tree of *Ficus benghalensis* L.). The height of the tree holes in Konaje and Sampaje ranged between 2 and 5 m from the ground.

Partially decomposed leaf litter trapped in tree holes was collected in sterile polythene bags twice during summer (April 2006) and monsoon (July 2006). The leaves collected in the tree holes belong to either the same or another tree species. Unlike rainy season (July), the leaf litter samples derived from tree holes during summer (April) were dried. They were rinsed in tap water to remove surface debris, randomly cut into pieces, suspended in five replicates each in 150 ml sterile distilled water in a 250 ml Erlenmeyer flask and aerated with a Pasteur pipette for 48 hours. (23 ± 2 °C) to generate conidia from colonized fungi. Aerated water was filtered through a Millipore filter (pore size, 5 µm; diam. 47 mm) and the filters were stained (0.1 % cotton blue in lactophenol). Each filter was cut into half, mounted on a microscope slide with lactic acid and the conidia were identified (magnification 200–1000 ×) and enumerated (half a filter was scanned if the conidia were plentiful, the entire filter if they were sparse). The aerated leaf pieces were dried at 100 °C up to 24 hours and the dry mass was determined after attaining constant weight. The dry mass of aerated leaf litter ranged from 122 to 377 mg per replicate. The conidial output was expressed per gram dry mass of leaf litter.

During sampling in the monsoon, temperature, pH and conductivity of water from five tree holes were measured (Water Analyser 371, Gujarat, India). The water samples were fixed at the sampling site to estimate dissolved oxygen at the laboratory with Winkler's method (APHA 1995).

As a considerable number of fungal taxa was observed in the tree holes, the diversity and evenness were calculated with the Simpson and Shannon diversity indices (Magurran 1998):

$$\text{Simpson index, } D' = 1 / \sum p_i^2$$

$$\text{Shannon index, } H' = - \sum (p_i \ln p_i)$$

where p_i is the proportion with which the i th taxon contributes to the total number of individuals.

The Simpson and Shannon evenness (J') were expressed by:

$$J' = H' / H'_{max}$$

where H'_{max} is the maximum value of diversity for the taxa present, if each individual is a different taxon (Pielou 1975).

Paired t-test was employed to assess the difference in number of taxa and conidial output from tree hole leaf litter between Konaje and Sampaje as well as between summer and monsoon seasons (Stat Soft Inc. 1995).

RESULTS AND DISCUSSION

Living as well as dead parts of forest tree canopies accommodate a variety of life forms including fungi. In spite of extensive studies on floristic and ecological functions of water-borne fungi in woodland streams, our knowledge of their significance, vertical distribution and ecological functions in riparian tree canopies is insufficient (Sridhar et al. 2006). Although these fungi are at risk of extinction due to the unidirectional flow of water in streams, they overcome such situation by colonizing stationary substrates in the stream column (e. g. roots, wood) and adjacent to stream banks (e. g. wood, leaf litter). Alternatively, tree canopy surfaces (e. g. leaves, bark) and habitats (e. g. tree holes, epiphytes) also serve as potential shelter for these fungi. Gönczöl and Révay (2003) re-examined the leaf litter in tree holes in forested mountains of Hungary studied about three decades ago (Gönczöl 1976) and confirmed that tree holes are the permanent habitats of water-borne conidial fungi. Carroll (1981) emphasized the role of conidial fungi of Douglas fir canopy and indicated that they constitute a guild in the canopy and serve as intermediaries of energy flow similar to aquatic hyphomycetes in streams.

Tab. 1. Water-borne conidial fungi recovered from tree hole trapped leaf litter (conidia/g dry mass; n = 25, mean) (*Recorded from tree hole habitats in Hungary by Gönczöl 1976; Gönczöl and Révay 2003, 2004).

Taxon	Konaje		Sampaje	
	Summer	Monsoon	Summer	Monsoon
* <i>Alatospora acuminata</i> Ingold	1400	422	5	5
<i>Anguillospora crassa</i> Ingold	3	3		8
<i>A. longissima</i> (Sacc. & Sydow) Ingold	412	34	49	69
<i>Arborispora palma</i> Ando	64			
<i>Campylospora chaetocladia</i> Ranzoni	2			2
<i>Campylospora filicladia</i> Nawawi				4
<i>Clavatospora tentacula</i> (Umphlett) Nilsson				88

Taxon	Konaje		Sampaje	
	Summer	Monsoon	Summer	Monsoon
<i>Cylindrocarpon</i> sp.				3
<i>Dendrospora erecta</i> Ingold	37			
<i>Flabellospora crassa</i> Alasoadura				9
<i>F. verticillata</i> Alasoadura	21			3
<i>F. multiradiata</i> Nawawi				1
<i>Flagellospora curvula</i> Ingold	402	36	14	49
<i>F. penicillioides</i> Ingold	8			10
<i>Helicosporium</i> sp.	21			
<i>Ingoldiella hamata</i> Shaw	5	5		5
<i>Isthmotricladia gombakiensis</i> Nawawi				132
* <i>I. laeensis</i> Matush.				8
<i>Lunulospora curvula</i> Ingold	28		11	10
<i>L. cymbiformis</i> Miura				48
<i>Phalangispora constricta</i> Nawawi & Webster	16		5	8
<i>Tetraploa aristata</i> Berk. & Br.	2			
<i>Tricladium angulatum</i> Ingold				1
<i>T. splendens</i> Ingold				5
<i>Trinacrium robustum</i> Tzean & Chen			47	
<i>T. subtile</i> Riess	2			3
<i>Tripospermum camelopardus</i> Ingold, Dunn & McDougall			18	
* <i>T. myrti</i> (Lind) Hughes			75	24
<i>Triscelophorus acuminatus</i> Nawawi			297	81
<i>T. konajensis</i> Sridhar & Kaveriappa	341	43	24	99
<i>T. monosporus</i> Ingold	46	9	33	9
* <i>Varicosporium elodeae</i> Kegel	31			
* <i>Ypsilina graminea</i> (Ingold, Dann & McDougall) Descals, Webster & Marvanová			4	
Unidentified (hyaline tetradiate conidia)			19	
Total taxa	18	7	13	25
Range of taxa	3–10	4–6	2–9	6–18
Total conidia/g leaf dry mass	2841	552	601	684
Range of conidia/g leaf dry mass	154–12241	177–1882	140–1366	240–1120

On the west coast and in the Western Ghats of India, the southwest monsoon usually begins in late May or early June and lasts to as late as October-November. The extent of rainfall is very high during June-August and result in aqueous habitats on the tree canopies. Such conditions likely facilitate growth, sporulation and dissemination of fungi. Out of a total of 34 taxa of water-borne conidial fungi recovered from leaf litter of the tree holes in our study, 18 belong to Konaje (west coast) and 29 to Sampaje (Western Ghats) (Tab. 1). The leaf litter sampled during

summer consists of more taxa than that sampled in the monsoon in Konaje (total 18 vs. 7; mean 7.6 vs. 4.8), while it was reverse in Sampaje (total 13 vs. 25; mean 5.6 vs. 12) (Tab. 1, Fig. 1). The conidial output from the tree hole leaf litter was the highest in summer samples of Konaje (2841/g) followed by monsoon samples of Sampaje (684/g). The mean conidia released per species per gram dry mass of tree hole leaf litter were the highest in the summer samples of Konaje (354) and the least during monsoon in Sampaje (59) (Fig. 1). Although the Konaje tree holes contain fewer taxa than those in Sampaje, the mean conidia produced per taxa per gram dry mass was high in summer as well as monsoon (Fig. 1). The paired t-test revealed no significant difference in number of taxa ($P = 0.1177$) nor conidial output ($P = 0.1816$) between the Konaje and Sampaje tree holes, while the number of taxa significantly differed between summer and monsoon in Konaje ($P = 0.0258$) as well as Sampaje ($P = 0.0206$). The Simpson and Shannon diversity of tree hole conidial fungi were higher in Sampaje than Konaje (Tab. 2) and the evenness was low in summer and monsoon in Konaje due to dominance of a single species (*Alatospora acuminata*). It is likely that the water-borne fungal assemblage and diversity of tree canopies depends on the type of tree species. Banyan trees have been recognized as a keystone species as they provide niches for several species of flora and fauna. On assessing the tree-wise diversity of fungi, banyan (*Ficus benghalensis*) tree holes showed the highest diversity of all tree species in Konaje in both seasons and in Sampaje during summer (Tab. 2). The banyan leaf litter in the Konaje stream was also known to harbour more taxa of water-borne hyphomycetes than leaf litter of other tree species (Sridhar and Kaveriappa 1989a).

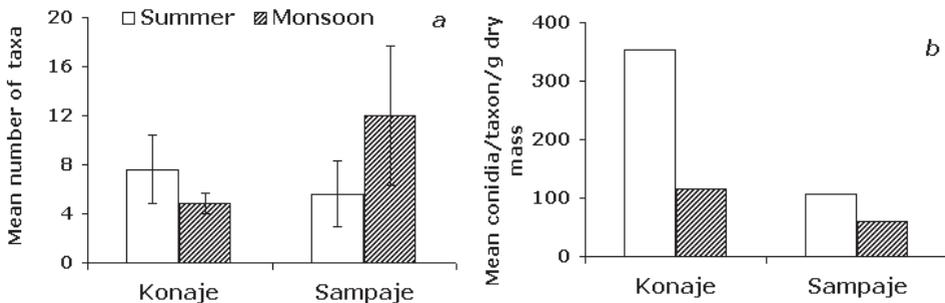


Fig. 1. Number of taxa (a) and mean number of conidia (b) per taxon per gram dry mass of tree hole leaf litter recovered from tree holes in Konaje and Sampaje during summer and monsoon.

There are many theories describing the transport of fungal propagules in different habitats. The 'aqueous film theory' explains the movement of spores in the water film on leaves or bark (Bandoni 1974, Bandoni and Koske 1974). Tree hole inhabiting insects (Kitching 1971) might also transfer fungal propagules within or across the canopies. Bandoni (1981) expressed the opinion that the fungal propagules formed in tree canopies are transferred directly into streams. Many teleomorphs of water-borne conidial fungi have been reported from plant detritus in terrestrial habitats adjacent to streams (Webster 1992). According to Shearer (1992) and Webster (1992), investigation of the emergent portions of riparian vegetation will be rewarding in search of teleomorphs of water-borne conidial fungi. Spores of the perfect state of water-borne conidial fungi usually disperse through the air but it is also possible that such spores persist in tree canopies and broadcast their asexual propagules to streams via throughfall, stemflow and invertebrates.

Tab. 2. Diversity and evenness of water-borne conidial fungi in tree holes of the west coast and Western Ghats in India (banyan tree hole in parenthesis).

Season	Diversity		Evenness	
	Simpson	Shannon	Simpson	Shannon
Konaje				
Summer	0.700 (0.847)	2.331 (2.941)	0.742 (0.941)	0.559 (0.885)
Monsoon	0.401 (0.723)	1.287 (2.111)	0.468 (0.873)	0.458 (0.871)
Sampaje				
Summer	0.720 (0.804)	2.579 (2.638)	0.780 (0.905)	0.697 (0.832)
Monsoon	0.888 (0.863)	3.574 (3.206)	0.925 (0.929)	0.770 (0.842)

Water-borne conidial fungi have been reported from a variety of canopy habitats such as throughfall, stemflow, intact leaves, trapped leaf litter and canopy snow (Czezug and Orłowska 1999, Gönczöl and Révay 2006, Sridhar et al. 2006). Ando (1992), based on an examination of dew and rain drops on intact tree leaves, hypothesized that some fungi with micronematous conidiophores (short or inconspicuous) bearing staurosporous conidia evolved on canopies rather than streams and termed them as 'terrestrial aquatic hyphomycetes' (e. g. *Alatosessilispora*, *Arborispora*, *Ceratosporium*, *Curucispora*, *Dicranidion*, *Dwayaangam*, *Microstella*, *Ordus*, *Retiarius*, *Tetraploa*, *Titaea*, *Titaeella*, *Tricladiella*, *Tridentaria*, *Trifurcospora*, *Trinacrium*, *Triposphermum*, *Trisulcosporium*). These fungi have been adapted to hold water around the conidium, which increases the chances of conidial germination.

Canopy water drain, such as throughfall and stemflow, supplies a variety of nutrients and minerals (e. g. nitrogen, phosphorus, potassium, calcium, magnesium) (Schroth et al. 2001). As seen in stream fungi, canopy fungi also meet their mineral requirements by intermittent water flow through the canopy. Depending on surface runoff, the fungal assemblage and diversity may also differ in tree species as seen in banyan and other tree species in the current study. The physicochemical features of tree hole water during monsoon revealed that the temperature in Sampaje was slightly lower than in Konaje, the pH was close to neutral, the dissolved oxygen was too low in both locations, while the conductivity is higher in Sampaje than Konaje (Tab. 3). The dissolved oxygen of the Konaje and Sampaje streams is higher than the tree hole water (7.9–10.9 vs. 1.14–1.33 ppm) (Sridhar and Kaveriappa 1989b) and the survival of fungi in tree holes indicates their tolerance to low oxygen tension. Interestingly, Field and Webster (1983) have confirmed the survival of aquatic hyphomycetes for limited periods under anaerobic conditions.

Tab. 3. Physicochemical characteristics of tree hole water during monsoon (n = 5, mean ± SD).

Parameter	Konaje	Sampaje
Temperature (°C)	28.40 ± 0.41	26.68 ± 0.84
pH	6.84 ± 0.05	6.98 ± 0.50
Conductivity (µS/cm)	113.4 ± 17.42	148.1 ± 81.29
Dissolved oxygen (ppm)	1.33 ± 0.33	1.14 ± 0.10

Our study demonstrated that the litter accumulated in riparian tree holes in the west coast and Western Ghats in India constitutes an excellent niche for a variety of water-borne conidial fungi (Fig. 2). So far, 19 fully identified typical conidial water-borne fungal taxa had been reported from tree holes in Hungary (Gönczöl 1976; Gönczöl and Révay 2003, 2004) (Tab. 4). The current study has added 26 identified taxa from India. They persist in dry tree holes during summer and the tree holes constitute a temporary refuge for their survival as seen on dried leaf litter on stream banks for several months (Sanders and Webster 1978, Sridhar and Kaveriappa 1987). Gönczöl and Révay (2003) suspected that the water-borne fungi in stemflow have either an epiphytic or an endophytic origin.

Western Ghat streams are more diverse in water-borne hyphomycetes than the west coast streams (Sridhar et al. 1992). About 20 taxa have been reported from the Konaje stream on the west coast (Sridhar et al. 1992). Examination of tree hole litter of riparian trees along the Konaje stream in this study yielded 18 taxa in spite of a lower conidial output (which is comparable to dry leaf litter on stream banks) than submerged leaf litter in streams (Sridhar and Bärlocher 1993). Similarly, 29 taxa have been recovered from riparian tree holes against 68 taxa in the Sampaje stream of the Western Ghats (Raviraja et al 1998). Among the 34 taxa

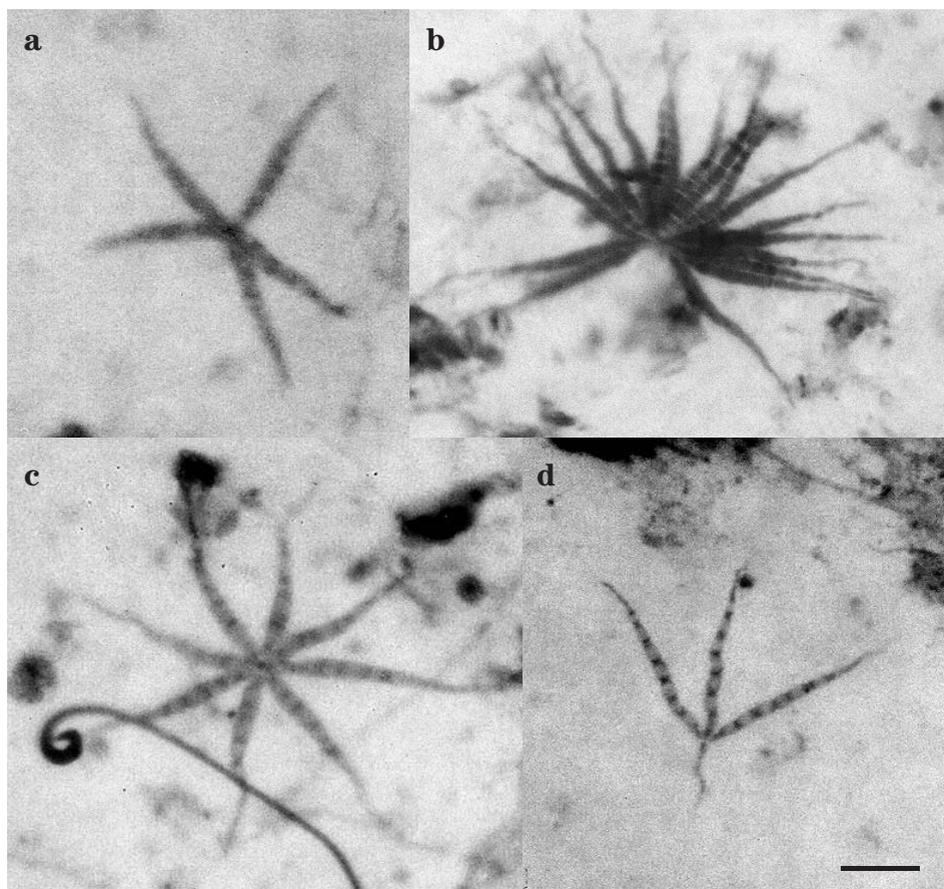


Fig. 2. Banyan tree hole conidial fungi stained with cotton blue in lactophenol on Millipore filters: *Flabellospora crassa* (a), *Flabellospora multiradiata* (b), *Flabellospora verticillata* (c), *Isthmotricladia gombakiensis* (d) (bar = 20 μ m).

found in our study, five were known from tree holes in Hungary (*Alatospora acuminata*, *Isthmotricladia laeensis*, *Tripaspermum myrti*, *Varicosporium elodeae* and *Ypsilina graminea*) (Tabs. 1, 4). The majority of conidia recovered from tree canopies are multiradiate (Sridhar et al. 2006) and probably resist total removal from the leaf or bark surface in contrast to streams (Sridhar and Kaveriappa 1987). Our study also yielded more multiradiate than sigmoid conidia in tree holes (Tab. 1, Fig. 2). The fungal genera producing sigmoid conidia recorded in this study were *Anguillospora*, *Flagellospora* and *Lunulospora*. The former two genera and the tetra-radiate spore producer *Triscelophorus* were usually uncommon in tree canopies (Sridhar et al. 2006). Surprisingly, all four genera mentioned above were the most common in tree hole litter in this study (Tab. 1).

Tab. 4. Water-borne conidial fungi recovered from mountain-forest tree holes in Hungary (* recorded in the present study).

Taxon	Reference
* <i>Alatospora acuminata</i> Ingold	Gönczöl 1976, Gönczöl and Révay 2003
<i>Articulospora tetracladia</i> Ingold	Gönczöl 1976
<i>Colispora cavicola</i> Gönczöl & Révay	Gönczöl and Révay 2003
<i>Dactylella submersa</i> (Ingold) Nilsson	Gönczöl 1976
<i>Dimorphospora foliicola</i> Tubaki	Gönczöl and Révay 2003
<i>Dwayaangam cornuta</i> Descals	Gönczöl and Révay 2003
<i>Isthmologispora minima</i> Matsush.	Gönczöl and Révay 2003
* <i>Isthmotricladia laeensis</i> Matsush.	Gönczöl and Révay 2004
<i>Lateriramulosa uni-inflata</i> Matsush.	Gönczöl and Révay 2004
<i>Tetracladium marchalianum</i> de Wild.	Gönczöl and Révay 2004
<i>T. maxilliforme</i> (Rostrup) Ingold	Gönczöl and Révay 2004
<i>T. setigerum</i> (Grove) Ingold	Gönczöl and Révay 2004
<i>Tricellula aquatica</i> Webster	Gönczöl and Révay 2004
<i>T. aurantiaca</i> (Haskins) Arx	Gönczöl and Révay 2004
<i>Tricladium castaneicola</i> Sutton	Gönczöl and Révay 2003
* <i>Tripopermium myrti</i> (Lind) Hughes	Gönczöl and Révay 2003, 2004
<i>Vargamyces aquaticus</i> (Dudka) Tóth	Gönczöl and Révay 2003
* <i>Varicosporium elodeae</i> Kegel	Gönczöl and Révay 2003, 2004
* <i>Ypsilina graminea</i> (Ingold, Dann & McDougall) Descals, Webster & Marvanová	Gönczöl and Révay 2003

This is the first report on riparian tree hole fungi of the Indian subcontinent. Future studies have to focus on the occurrence of tree hole fungi in different tree species, habitats and seasons, with emphasis on tree hole water chemistry. Such studies help to understand the fungal assemblage, diversity and ecological functions, which complement the investigations carried out in terrestrial and aquatic habitats. The pattern of fungal colonization on baited leaf litter and their decomposition in tree holes may reveal more about the lifestyle, evolution and relationship between tree hole fungi and terrestrial and stream fungi.

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