Arboreal water-borne hyphomycetes on oak-leaf basket fern *Drynaria quercifolia*

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Fungi associated with the fern *Drynaria quercifolia* epiphytic on riparian trees of a coastal stream of southwest India were assessed. Bracket leaves, roots, rhizomes and trapped leaf litter yielded 16 water-borne hyphomycetes on aeration in sterile distilled water. The highest number taxa (15) as well as conidia (1780 g^{-1} dry mass) were produced from bracket leaves; both taxa (3) and conidia (15.4 g^{-1} dry mass) were lowest on rhizomes. The evenness of released conidia was lowest in trapped leaves due to dominance by *Flagellospora curvula*. As fern rhizomes are alive, the associated fungi are suspected to be epiphytic or endophytic. About 75 % of the taxa in the stream were found to be associated with the fern; however the conidial output was low and comparable with that from dry leaf litter on stream banks. The current study is compared with earlier investigations on the occurrence of water-borne hyphomycetes of canopies (tree leaves, tree holes, stem flow and tree snow).

Key words: aquatic hyphomycetes, diversity, tree fern, tree canopy, trapped litter.

Forest canopies have been recognized as a cradle of biological diversity (Erwin 1988, Hammond *et al.* 1997). Even though forest canopies constitute an interface between 90 % of earth's terrestrial biomass and atmosphere, it has become one of the most threatened terrestrial ecosystems (Ozanne *et al.* 2003). In India, canopy studies have not attracted much attention from scientists despite their global significance (Davy & Ganesh 2003). Canopies trap a considerable amount of organic matter (e.g. leaf litter, twigs, and inflorescence) and transform it into "crown humus", which supports a variety of floral and faunal resources. Trunks, branches, and leaves of canopy provide mechanical support for many epiphytes including tree ferns (Nadkarni *et al.* 2001). Ferns with their rosette crown-like overlapping fronds access nutrients through stem flow, throughfall, drips and act as "trash baskets". *Drynaria quercifolia* (Linn.)

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J. Sm. (Polypodiaceae), commonly known as "oak-leaf basket fern" is a widely distributed pteridophyte of forests of the Western Ghats and west coast of India (Fig. 1). This fern produces two kinds of leaves: nest leaves (slender, fragile, short-lived, turn brown on senescence shed on drying) and bracket leaves (plate-like, resembling oak leaves, tough, leathery, remain attached to creeping rhizome after senescence, decay as standing dead). The bracket leaves function as funnels to trap debris mainly from canopy water run-off.

Fig. 1. The epiphytic fern *Drynaria quersifolia* on the riparian tree trunks of Konaje stream showing prominent bracket leaves (stars) and remnants of rachis of nest leaves (arrows) attached to rhizomes (photographed on February 24, 2006).

Water-borne hyphomycetes are common on submerged dead leaves in streams and contribute to the energy flow between dead leaf litter and invertebrates (Ingold 1942, Bärlocher 1992). In addition to their existence in streams they are known from a variety of errestrial habitats such as soil, leaf litter, roots and leaf surfaces (Sridhar & Bärlocher 1993). The occurrence of typical water-borne hyphomycetes in tree canopies (stem flow, tree holes, tree gutters, throughfall) has been reported from Canada, Hungary, Japan, and Poland (e.g. Gönczöl 1976, Bandoni 1981, Ando 1984a, 1984b, Czeczuga & Orlowska 1994, 1998a, 1998b, Gönczöl & Révay 2003, 2004, 2005). Water-filled tree holes in canopies are natural continuous flow chambers, which trap leaf litter and support fungi (Gönczöl & Révay 2003). Many studies have been carried out on the terrestrial occurrence of water-borne hyphomycetes in tropics (e.g. Sridhar & Kaveriappa 1987, Sridhar & Bärlocher 1993); however, studies addressing their occurrence in tree canopies are rare. To complement the studies in temperate tree canopies, the current study aims to assess the association of water-borne hyphomycetes with the fern *Drynaria quercifolia* on the riparian trees of a coastal stream of Southwestern India.

Materials and Methods

The study was conducted in surroundings of the Konaje stream near Mangalore University Campus, Karnataka, India. The Konaje stream is a typical coastal seasonal stream bordered by riparian vegetation (*Artocarpus heterophyllus* Lam., *Ficus Benghalensis* Linn., and *Mangifera indica* Linn.). Five tree species associated with the fern *Drynaria quercifolia* were selected (Fig. 1). The distance between the stream and trees sampled for *Drynaria* was between 2 m and 50 m, while the height from ground to the fern was between 2 m and 20 meters. On 24 February 2006, pieces of rhizome with roots, partially skeletonized bracket leaves and trapped leaf litter were collected from each fern in separate sterile polythene bags and returned to the laboratory. Representative samples of bracket leaves, roots, rhizomes and trapped leaves were dried at 100 °C to constant weight to determine moisture gravimetrically.

Randomly excised tissues (5 – 6 leaf and rhizome pieces, 1 cm × 2.5 cm; 15 – 20 small root pieces) were rinsed in tap water to remove surface debris, suspended in sterile distilled water (150 mL in 250 mL conical flask) and aerated using a Pasteur pipette for 48 h to estimate conidium production by water-borne hyphomycetes. Aerated water was sucked through a Millipore filter (8 µm), and filters were stained in 0.1 % aniline blue in lactophenol. Each filter was cut, mounted with lactic acid on a microscope slide and the conidia were scored. The aerated tissues were dried (100 °C) to constant weight and dry mass was determined. Conidial output per gram dry mass of each tissue was determined.

The diversity and evenness of water-borne hyphomycetes was assessed with Simpson and Shannon diversity indices (Magurran 1988):

Simpson index, $D' = 1 \div \Sigma (p_i)^2$

Shannon index, $H' = -\Sigma (p_i \ln p_i)$

where, p_i is the proportion of individuals that taxa 'i' contribute to the total number of individuals.

The evenness (J') was expressed by:

$$J' = H' \div H'_{max}$$

where, H'_{max} is the maximum value of diversity for the number of taxa present (Pielou 1975).

Results

Figure 2 shows the number of taxa recovered and the conidial output of water-borne hyphomycetes along with moisture of tissues examined. In total 16 taxa of water-borne hyphomycetes were recovered (Tab. 1). The highest number of taxa was found in bracket leaves (total 15, mean 6.4, range 4 – 10) followed by trapped leaves (total 10, mean 4, range 2 – 7) and lowest in the rhizomes (total 3, mean 0.8, range 0 – 1) (Fig. 2). The conidial output per gram dry mass peaked in bracket leaves (1779 g^{-1}) followed by roots (343 g^{-1}) and was lowest in rhizomes (15.4 g^{-1}). The moisture was higher in the rhizomes (51.9 %) than in other tissues (11.3 % – 20 %) (Fig. 2). The Simpson and Shannon diversity were highest in bracket leaves, while the evenness was lowest in trapped leaves (Tab. 2).

Tab. 1. – Water-borne hyphomycetes recovered from tissues of *Drynaria* and trapped leaf litter (conidia g^{-1} dry mass, mean values, n = 5; absence is indicated by blank cells).

Taxon	Bracket leaf	Root	Rhizome	Trapped leaf
Anguillospora crassa Ingold	55.6	62.6	4.0	5.2
A. longissima (de Wild.) Ingold	301.4	83.4		2.0
Arborispora sp.	5.0			
Campylospora chaetocladia Ranzoni	18.4			
Cylindorcarpon sp.	20.0	152.6	9.6	1.2
Flabellospora crassa Alasoadura	42.2			
F. verticillata Alasoadura		5.0		1.2
Flagellospora curvula Ingold	319.0	17.2		78.4
F. penicillioides Ingold	722.8		1.8	4.4
Helicosporium sp.	8.4			
Retiarius bovicornutus Olivier	8.4			0.8
Tricladium sp.	18.0	4.6		2.6
Trinacrium subtile Riess	208.4			
Tripospermum inflacatum Ando & Tubaki	8.4			
Triscelophorus acuminatus Nawawi	35.6	12.6		2.4
T. konajensis Sridhar & Kaveriappa	8.4	4.6		3.2
Total number of taxa	15	8	3	10
Range (taxa)	4 – 10	2 – 4	0 – 1	2 – 7
Total number of conidia g^{-1} dry mass	1779.2	342.6	15.4	101.4
Range (conidia g^{-1} dry mass)	800 – 2369	46 – 800	0 – 48	4 – 376

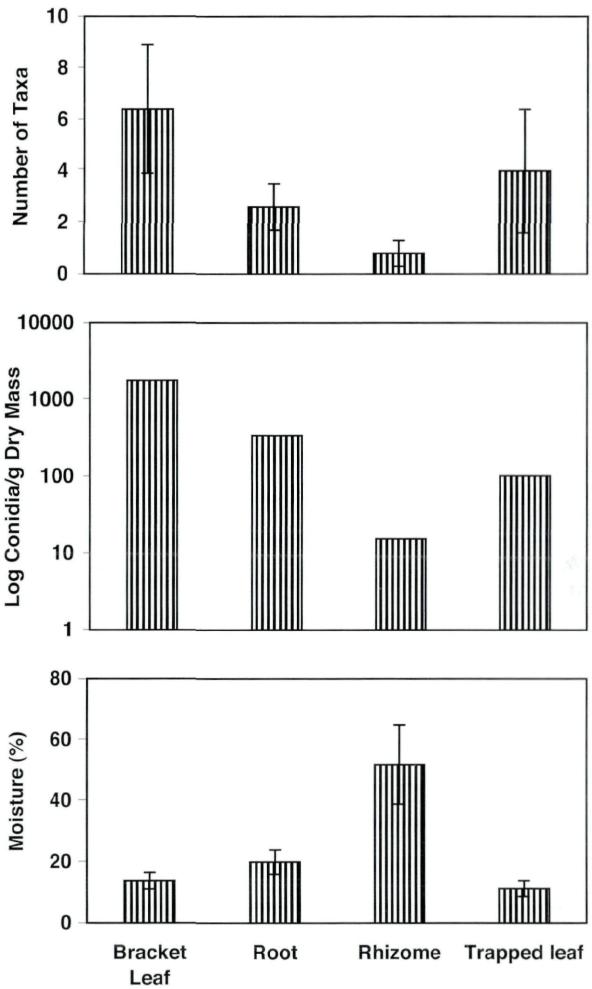

Fig. 2. Number of taxa and conidial output of water-borne hyphomycetes and moisture (%) of bracket leaves, roots, rhizomes *(Drynaria quersifolia)* and trapped leaf litter (n = 5; mean ± SD).

Tab. 2. – Diversity of water-borne hyphomycetes in tissues of *Drynaria* and trapped leaf.

Tissue	Diversity		Evenness	
	Simpson	Shannon	Simpson	Shannon
Bracket leaf	0.758	2.543	0.812	0.651
Root	0.705	2.112	0.805	0.704
Rhizome	0.530	1.292	0.795	0.815
Trapped leaf	0.395	1.442	0.439	0.434

Discussion

Colonization of stationary substrates in streams (roots, wood), along stream banks (wood) and in tree canopies (e.g. bark, tree holes, epiphytes) by water-borne hyphomycetes may help them overcome the risks of extinction due to unidirectional flow of water. Although woodland streams have been extensively investigated for the occurrence and ecological functions of water-borne hyphomycetes, our knowledge on their vertical distribution and role particularly in the woodland or riparian canopy is inadequate. Leaf litter in tree holes of Southwestern Hungary studied in the mid 1970s (Gönczöl 1976) has been re-examined recently (Gönczöl & Révay 2003). The results confirmed that tree holes of woodland ecosystems are the permanent habitats of water-borne hyphomycetes. The "aqueous film theory" predicts the movement of spores in an aqueous film on wet leaves or bark (Bandoni 1974, Bandoni & Koske 1974). In addition, tree hole inhabiting insects (Kitching 1971) might disseminate spores or propagules of fungi externally or through feces within or across canopies. Alternatively, the spores of sexual state of water-borne hyphomycetes (e.g. ascospores) might be dispersed through air.

Based on the examination of water droplets (e.g. dews, rain drops) on intact living tree leaves in misty habitats, Ando (1992) and Ando and Kawamoto (1989) hypothesized that certain fungi with branched conidia and micronematous conidiophores evolved on trees rather than streams as "terrestrial aquatic hyphomycetes" (e.g. *Alatosessilispora, Arborispora, Ceratosporium, Curucispora, Dicranidion, Dwayaangum, Microstella, Ordus, Retiarius, Titaea, Titaeella, Tricladiella, Tridentaria, Trifurcospora, Trinacrium, Tripospermum, Trisculsporium*). An important feature of terrestrial aquatic hyphomycetes is their staurosporous conidia adapted to hold water around the conidium, what increases the possibility of quick germination. Sexual states of many aquatic hyphomycetes have been found in plant detritus from terrestrial habitats near streams (Webster 1992). Webster (1992) and Shearer (1992) are of the opinion that wood in terrestrial habitats, including emergent portions of riparian vegetation, will be rewarding in search of teleomorphs of water-borne hyphomycetes. It is likely that teleomorphs might also exist in the tree canopy and broadcast asexual propagules to streams or terrestrial habitats by throughfall, stem flow or invertebrates. Bandoni (1981) suspects that conidia and propagules of fungi formed in tree canopies are directly transferred into streams.

Forest trees contain living as well as dead parts (the latter transform into crown humus) and accommodate several life forms. Throughfall and stem flow supply a variety of nutrients (e.g. nitrogen, phosphorus, potassium, calcium, magnesium) (Schroth *et al.* 2001).

During the rainy season, depending on the extent of precipitation, fungi in canopy-trapped leaf litter receive additional nutrients. Water-borne hyphomycetes have been reported from stem flow, living leaves, trapped leaf litter, and canopy snow (Tab. 3). About 65 fully identified typical aquatic hyphomycetes and aquatic hyphomycete-like taxa have been recovered from the tree canopies of temperate regions (Canada, Hungary, Japan, and Poland). Several typical aquatic hyphomycetes were found in rainwater of roof gutters of buildings in Poland (Czeczuga & Orlowska 1999) (e.g. *Arbusculina irregularis, Clavariana aquatica, Colispora elongate, Lunulospora curvula*). Similarly, water drips through ornamental climbers of building canopies near Konaje stream during rainy season contain conidia of *Triscelophorus acuminatus* and *T. konajensis* (K.R. Sridhar, unpublished observations).

Tab. 3. – Aquatic hyphomycetes and aquatic hyphomycetes-like taxa recovered from tree canopy habitats. Reference numbers refer to: (1) Ando & Kawamoto 1986, (2) Ando & Tubaki 1984a, (3) Ando & Tubaki 1984b, (4) Bandoni 1981, (5) Czeczuga & Orlowska 1994, (6) Czeczuga & Orlowska 1998a, (7) Czeczuga & Orlowska 1998b, (8) Gönczöl 1976, (9) Gönczöl & Révay 2003, (10) Gönczöl & Révay 2004.

Taxon	Habitat	Reference
Actinospora megalospora Ingold	Tree leaves, Poland	5
Alatosessilispora bibrachiata Ando & Tubaki	Tree leaves, Japan	2
Alatospora acuminata Ingold	Tree holes, Hungary	8, 9
	Stem flow, Hungary	10
Anguillospora pseudolongissima Ranzoni	Tree leaves, Poland	5
	Tree snow, Poland	7
Arborispora palma Ando	Tree leaves, Japan	1
	Tree snow, Poland	7
A. paupera Marvanová and Baerlocher	Stem flow, Hungary	10
Articulospra proliferata Roldán & van der Merve	Tree leaves, Poland	6
A. tetracladia Ingold	Tree holes, Hungary	8
Brachiosphaera jamaicensis (Crane & Dumont) Nawawi	Tree leaves, Poland	6
Campylospora chaetocladia Ranzoni	Tree leaves, Poland	5
Ceratosporium cornutum Matsush.	Tree leaves, Japan	3
Clavariopsis brachycladia Tubaki	Tree leaves, Poland	5
Colispora cavincola Gönczöl & Révay	Tree holes, Hungary	9
Curucispora ombrogena Ando & Tubaki	Tree leaves, Japan	2
	Tree leaves, Poland	6
C. ponapensis Matsush.	Tree leaves, Japan	2
	Stem flow, Hungary	10
Dactylella submersa (Ingold) Nilsson	Tree leaves, Poland	5
	Tree holes, Hungary	8

Tab. 3 continued.

Taxon	Habitat	Reference
Dicranidion fissle Ando & Tubaki	Stem flow, Japan	3
Dimorphospora foliicola Tubaki	Tree leaves, Poland	5
	Tree holes, Hungary	9
Dwayaangam cornuta Descals	Tree holes, Hungary	9
D. dicotoma Nawawi	Stem flow, Hungary	10
D. yakuensis Matsush.	Tree leaves, Japan	2
	Stem flow, Hungary	10
Flabellospora crassa Alasoadura	Tree leaves, Japan	2
F. verticillata Alasoadura	Tree leaves, Japan	2
Gyoerffyella biappendiculata Marvanová	Stem flow, Canada	4
G. gemellipara Arnold	Stem flow and trapped leaves, Canada	4
G. tricapillata (Ingold) Marvanová	Tree leaves, Poland	5
Heliscus lugdunensis Sacc. & Théry	Tree leaves, Poland	5
Isthmologispora minima Matsush.	Tree holes, Hungary	9
Isthmotricladia laeensis Matush.	Tree holes, Hungary	10
Lateriramulosa uni-inflata Matsush.	Stem flow and tree holes, Hungary	10
Lemonniera aquatica de Wild	Tree leaves, Poland	5
L. cornuta Ranzoni	Stem flow, Hungary	10
L. terrestris Tubaki	Stem flow, Hungary	1
Lunulospora curvula Ingold	Tree leaves, Poland	5
Margaritispora aquatica Ingold	Tree leaves, Poland	5
Microstella pluvioriens Ando & Tubaki	Tree leaves, Japan	2
Mycocentrospora aquatica (Iqbal) Iqbal	Tree snow, Poland	7
Ordus tribrachiatus Ando & Tubaki	Tree leaves, Japan	2
	Tree leaves, Poland	6
Pyramidospora casuarinae Nilsson	Tree leaves, Poland	5
P. fluminea Miura and Kudo	Tree leaves, Poland	5
Retiarius bovicornutus Olivier	Stem flow, Hungary	10
Tetracladium marchalianum de Wild	Stem flow and tree holes, Hungary	10
T. maxilliforme (Rostrup) Ingold	Tree leaves, Poland	6
	Stem flow and tree holes, Hungary	10
Tetracladium setigerum (Grove) Ingold	Tree holes, Hungary	10
Titaea clarkeae Ellis & Everh.	Tree leaves, Poland	6
	Stem flow, Hungary	10
Tricellula aquatica Webster	Tree leaves, Poland	6
	Stem flow and tree holes, Hungary	10
T. aurantiaca (Haskins) Arx	Tree holes, Hungary	10

Tab. 3 continued.

Taxon	Habitat	Reference
T. inequalis van Beverwijk	Tree leaves, Poland	6
Tricladiella pluvialis Ando & Tubaki	Stem flow, Japan	3
	Tree leaves, Poland	6
Tricladium castaneicola Sutton	Tree holes, Hungary	9
T. patulum Marvanová and Marvan	Tree leaves, Poland	9, 10
Trifurcospora irregularis (Matsush.) Ando & Tubaki	Tree snow, Poland	7
	Tree leaves, Poland	6
	Stem flow, Hungary	10
Trinacrium robustum Tzean & Chen	Stem flow, Hungary	10
T. subtile Riess	Tree snow, Poland	7
Tripospermum acerinum (Sydow.) Spegazzini	Tree snow, Poland	7
T. camelopardus Ingold, Dunn & McDougall	Stem flow, Hungary	10
	Tree leaves, Poland	10
T. gardneri Hughes	Tree leaves, Poland	6
T. inflacatum Ando & Tubaki	Stem flow, Japan	3
	Tree leaves, Poland	6
T. myrti (Lind.) Hughes	Tree snow, Poland	7
	Tree leaves, Poland	6
	Tree holes, Hungary	9, 10
	Stem flow, Hungary	10
T. porosporiferum Matsushima	Tree leaves, Poland	6
Vargamyces aquaticus (Dudka) Tóth	Stem flow, Hungary	5
	Tree snow, Poland	7
	Tree holes, Hungary	9
Varicosporium elodeae Kegel	Trapped leaves, Canada	4
	Tree holes, Hungary	9, 10
	Stem flow, Hungary	10
Volucrispora aurentiaca Haskins	Tree leaves, Poland	6
Wiesneriomyces conjuctosporus Kuthubuth. & Nawawi	Tree leaves, Poland	6
Ypsilina graminea (Ingold *et al.*) Descals, Webster & Marvanová	Tree holes, Hungary	9

The current study clearly demonstrates that dead or living tissues of epiphytic fern *Drynaria* and trapped leaf litter are colonized by aquatic and water-borne hyphomycetes, which may persist until the rainy season and then reproduce, as they are known to survive on dry leaves for several months (Sanders & Webster 1978, Sridhar & Kaveriappa 1987). The fern rhizome in our study yielded a low number of conidia produced by *Anguillospora crassa, Cylindrocarpon* sp., and *Flagellospora penicillioides* (15.4 g^{-1} dry mass). This suggests that water-borne hyphomycetes exist in rhizomes as endophytes or epiphytes. Gönczöl & Révay (2003) also suspected that the water-borne hyphomycetes in stem flow have an epiphytic or endophytic origin. The fern roots screened in our study were partially alive, thus, the number of taxa and conidial output were lower than those from bracket leaves and trapped leaves. In total, there are approximately 20 taxa of water-borne hyphomycetes in the Konaje stream (Sridhar *et al.* 1992). In the current study, a single survey of ferns during the dry season revealed about 75% of these taxa in spite of conidial output below that of submerged leaf litter. But, the conidial output was comparable to that of dry leaf litter on stream banks (Sridhar & Bärlocher 1993). Assessment of dead and live tissues of *Drynaria* during the wet season may reveal additional information on species richness, biomass, and conidial output of water-borne hyphomycetes.

Among the fungi recorded in the present study, *Campylospora chaetocladia, Flabellospora crassa, F. verticillata, Retiarius bovicornutus,* and *Trinacrium subtile* are known from tree canopies (Tab. 3). The genera *Anguillospora, Flagellospora,* and *Triscelophorus* are not common in tree canopies, however, in our study, *Anguillospora crassa, A. longissima, Flagellopsora curvula, F. penicillioides, Triscelophorus acuminatus,* and *T. konajensis* were present in tissues of *Drynaria* and trapped leaf litter. *Cylindrocarpon* and *Helicosporium* species found in *Drynaria* were not previously known from tree canopies, while *Tricladium* were also uncommon canopy fungi. *Arborispora* with its characteristic multiradiate conidia is common in intact leaves, tree holes, stem flow and tree snow (Tab. 3). *Trinacrium* is primarily known from rain water, stem flow, throughfall, and tree holes (Ando 1992, Gönczöl & Révay 2003, 2004). *Tripospermum* grows, abundantly sporulating, on surfaces of living leaves and is very common in tree canopies (Ando 1992, Gönczöl & Révay 2004).

Carroll (1981) discussed the role of arboreal aquatic hyphomycetes in the Douglas fir canopy and opined that they constitute a guild in the canopy and function similar to classical Ingoldian fungi in streams. Based on the occurrence of several aquatic hyphomycetes in tree canopy, Gönczöl & Révay (2003, 2005) suspect the existence of several biotypes. The majority of spores found in the tree canopy are multiradiate (Tab. 3). These conidia might have adapted to canopy habitats and their branched nature resist total removal from the leaf or bark surface as in streams. In the Western Ghats and the west coast of India, the rainy season usually begins in late May and extends until October to November. The extent of precipitation is severe during June to August and creates aquatic or semi-aquatic habitats in the tree canopies. Such conditions are ideal for growth, sporulation and dispersal of water-borne hyphomycetes. Further studies on the pattern of fungal colonization on leaf litter and leaf litter decomposition in canopies (e.g. treeholes, junctions of branches, canopy-trapped dead leaf litter) in the wet season may reveal a better understanding of the biology of fungi. For instance, the banyan tree *(Ficus benghalensis)* has wide branch junctions and accommodates huge amounts of leaf litter as well as water. Like tree bark, rachides of bracket leaves and nest leaves of *Drynaria* are more persistent than pinna, thus constituting an ideal substrate to study the occurrence of teleomorphs of water-borne hyphomycetes. For more detailed information on the evolution of canopy fungi, teleomorph and anamorph connections and their relationship with typical stream hyphomycetes molecular techniques are indispensable.

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