

## Aquatic hyphomycetes associated with leaves, leaf detritus and crown humus in palm canopies

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Aquatic hyphomycetes associated with attached dead leaves (autochthonous), accumulated leaf litter (allochthonous) and crown humus in canopies of wild palm (*Caryota urens*) and cultivated palm (*Cocos nucifera*) were assessed during wet and dry seasons by means of bubble chamber incubation. The canopy of *C. urens* trapped allochthonous leaf litter of seven tree species (*Alstonia scholaris*, *Artocarpus hirsutus*, *Ficus benghalensis*, *F. religiosa*, *Garcinia indica*, *Holigarna arnottiana* and *Mangifera indica*), while in the canopies of *C. nucifera* leaf litter of four tree species was found (*Acacia mangium*, *Delonix regia*, *Eucalyptus tereticornis* and *Polyalthia longifolia*).

Although the total number of species of aquatic hyphomycetes was almost identical during the dry season (17–18 spp.), in the wet season it was higher in *Caryota urens* than in *Cocos nucifera* (31 vs. 23 spp.). Based on conidium production, *Anguillospora crassa*, *Flagellospora curvula* and *Lunulospora curvula* were among the top five species during the wet and dry seasons in both palms. Shannon diversity was higher in the wet season than in the dry season in all samples of *C. urens*, while it was higher only in leaf samples of *C. nucifera*. Sørensen's similarity of aquatic hyphomycete communities between the samples was higher in *C. urens* than in *C. nucifera*. Three-way ANOVA revealed significant differences in species richness and conidium production between the seasons, palms and substrate assessed.

**Key words:** *Caryota urens*, *Cocos nucifera*, hyphomycetes diversity, abiotic factors, dry and wet season, India.

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Studie hodnotí výskyt vodních hyfomycetů na odumřelých listech plané palmy *Caryota urens* a pěstované *Cocos nucifera*, na zbytcích listů zanesených z jiných stromů a ve vrstvě humusu v korunách zmíněných palm. Houby ze vzorků nasbíraných v průběhu období dešťů a období sucha byly sledovány po inkubaci v provzdušňovaných nádobách. V korunách *C. urens* byl zjištěn cizorodý materiál pocházející ze sedmi druhů stromů (*Alstonia scholaris*, *Artocarpus hirsutus*, *Ficus benghalensis*, *F. religiosa*, *Garcinia indica*, *Holigarna arnottiana* a *Mangifera indica*), zatímco v korunách kokosové palmy byly zjištěny zbytky listů čtyř jiných dřevin (*Acacia mangium*, *Delonix regia*, *Eucalyptus tereticornis* a *Polyalthia longifolia*).

Během období sucha byl počet druhů vodních hyfomycetů takřka shodný v korunách obou palm (17–18 druhů), naopak v období dešťů byl výrazně vyšší u *Caryota urens* (31 oproti 23 druhům).

Mezi pěti nejhojnějšími druhy (bráno dle produkce konidií) byly na obou palmách v obou obdobích *Anguillospora crassa*, *Flagellospora curvula* a *Lunulospora curvula*. Na základě Shannonova indexu byla také v období dešťů vyšší diverzita ve všech vzorcích z *C. urens*, zatímco v případě *C. nucifera* byla průkazně vyšší jen ve vzorcích z autochtonních listů. Sørensenův index podobnosti společenstev vodních hyfomycetů v korunách konkrétních palm vychází vyšší u *Caryota urens*. Trojcestnou analýzou rozptylu byly zjištěny průkazné rozdíly v druhové bohatosti i produkci konidií mezi sledovanými obdobími, palmami i typy substrátu.

## INTRODUCTION

In forest ecosystems, the term ‘canopy’ refers to the upper layer or habitat formed by mature tree crowns, which encompasses a variety of organisms including epiphytes, lianas and arboreal animals. As complex ecosystems, tree canopies are endowed with a variety of organic matter (leaf litter, twigs, inflorescence and humus), which supports many organisms (e.g. invertebrates and fungi) (Ellwood & Foster 2004, Sridhar 2009). Although aquatic hyphomycetes prefer lotic ecosystems, their habitat extends beyond streams (Chauvet et al. 2016). Gönczöl (1976) was the first to report the occurrence of aquatic hyphomycetes in canopies, particularly in tree holes in Hungary. Subsequently, aquatic hyphomycetes have been reported from canopies in temperate and tropical regions (Chauvet et al. 2016). Niches (e.g. tree holes) and other habitats investigated for aquatic hyphomycetes include crown humus (product of decomposed organic matter), snow, honey (honey dew and floral honey), flowing water (stemflow and throughfall) and living parts of trees (leaves, needles, twigs, rhizomes and roots) (Sridhar 2009). Previous studies have revealed that the canopy ecosystem constitutes one of the major terrestrial habitats for aquatic hyphomycetes (Chauvet et al. 2016). Besides canopies of riparian trees, non-riparian tree canopies also harbour a variety of aquatic hyphomycetes (Karamchand & Sridhar 2009). Interestingly, these fungi have expanded their habitats beyond detritus decomposition in streams, as they are common in canopies (Sokolski et al. 2006, Sridhar 2009, Sridhar et al. 2013). There seems to be scanty information on the occurrence of aquatic hyphomycetes in detritus (autochthonous and allochthonous leaf litter) and crown humus in canopies (Sridhar et al. 2006, Karamchand & Sridhar 2008, Sudheep & Sridhar 2010). Studies on the occurrence of aquatic hyphomycetes in palm canopies are also lacking, although these are very common in the southwestern part of India. Unlike dicotyledonous trees, petiole junctions of palms provide ample room for accumulation of allochthonous material and humus.

Since crown humus is derived from detritus (autochthonous and allochthonous), it is expected to accommodate a more diversified fungal community, different from that of canopy leaf litter and autochthonous palm leaves. Therefore, as an extension of a previous study by Ghate & Sridhar (2015) in India on rain-borne

fungi in palm canopies (stemflow and throughfall), the occurrence and diversity of aquatic hyphomycetes was assessed in attached dead leaves (autochthonous), accumulated leaf litter (allochthonous) and crown humus in canopies of two dominant palm species (*Caryota urens* and *Cocos nucifera*) during the wet and dry seasons in the southwestern region of India.

#### MATERIAL AND METHODS

**Abiotic factors.** During sampling, humidity and air temperature (in the shade) near each palm were determined (Thermo Hygro Clock TM-1, Mextech Digital Thermohygrometer, Mumbai, India; accuracy  $\pm 1\%$ ). Humus samples were diluted with distilled water (1:2.5 v/v) to assess pH and conductivity (Water Analysis Kit 304, Systronics, Ahmedabad, India).

**Samples from palms.** About 35–40 year old species of wild (*Caryota urens* L.) and cultivated (*Cocos nucifera* L.) palms grown in the lateritic belt of Mangalore University Campus (12°49' N, 74°55' E; 100 m a.s.l.) were chosen for the study (Fig. 1). Sampling was carried out during the wet season (post-monsoon: October 2014) and dry season (summer: April 2015) between 7 and 10 am. From three palms of each species, dead leaflets (dried and easily breakable) still attached in the crown were sampled as autochthonous leaves. Leaf litter of non-palm tree species trapped in the palm crowns was sampled as allochthonous leaf litter. Besides leaf samples, humus accumulated in the crown was also collected by means of scooping. Samples in triplicate from each palm tree were collected in separate sterile polythene bags and processed in the laboratory within 2–3 h (baiting of humus by means of sterile leaf disks was carried out after 24 h).

**Assessment of fungi in leaves.** Laminae of autochthonous leaves were rinsed in distilled water, assorted and cut into disks (1.5 cm) using a cork-borer. The disks were incubated in 150 ml of sterile distilled water in 250 ml conical flasks (5 disks/flask/sample: total 45 disks/palm species/season) and aerated with a Pasteur pipette for up to 48 h ( $23 \pm 2$  °C). The aerated water was filtered through Millipore filters (diameter 45 mm; porosity 5  $\mu\text{m}$ ) and stained with aniline blue in lactophenol (0.1%). Filters were mounted on microscopic slides with a few drops of lactic acid under a high-power microscope (Olympus CX 41 RF, Tokyo, Japan) for conidial assessment in the entire filter area (Iqbal & Webster 1973) and identification of aquatic hyphomycetes based on monographs (Ingold 1975, Carmichael et al. 1980, Webster & Descals 1981, Nawawi 1985, Marvanová 1997, Santos-Flores & Betancourt-López 1997, Gulis et al. 2005). The number of conidia released from leaf disks was calculated per mg dry mass on drying aerated leaf disks in an oven at 80 °C for 24 h.



**Fig. 1.** Representative palm species screened for aquatic hyphomycetes: *Caryota urens* (A – overall view; B – close-up of crown) and *Cocos nucifera* (C – overall view; D – close-up of crown).

Assessment of fungi in leaf litter. Allochthonous leaf litter was processed as indicated above to assess aquatic hyphomycetes during the dry and wet seasons. At each tree of the two palm species the same type of identified allochthonous leaf litter was sampled.

Assessment of fungi in humus. Occurrence of aquatic hyphomycetes in humus (9 samples from 3 palms of each species) was determined by means of a leaf baiting technique. Humus from the same palm trees was processed during the dry and wet seasons. Briefly, humus samples were exposed to sterile banyan (*Ficus benghalensis*) leaf disks (5 disks/sample; total 45 disks/palm species/sea-

son). Pre-weighed humus samples (wet season: immediately after blotting; dry season: directly) of 300 mg dry mass (adjusted based on the moisture content) were transferred to 100 ml conical flasks containing 50 ml of sterile distilled water with five sterile banyan leaf disks (1.5 cm diameter, dry mass, 55–60 mg). The flasks were incubated on a rotary shaker (125 rpm) at laboratory temperature for up to seven days (wet season: 23–25 °C, 11 h light/13 h dark; dry season: 25–27 °C, 13 h light/11 h dark). Later, the leaf disks were harvested, rinsed in distilled water and aerated in bubble chambers (150 ml sterile distilled water in 250 ml conical flask) at laboratory temperature for up to 48 h to induce sporulation by aquatic hyphomycetes. After removal of the leaf disks, aerated water samples were sucked through Millipore filters, which were then processed as described for the fungal assessment in leaves.

Despite potential differences in life strategy of various fungi (some species may need a longer time for conidium production, some species may sporulate more abundantly in still water than in aerated water), bubble chamber incubation for 48 h was applied for all samples uniformly to obtain comparable results.

Data analysis. Total (in 900 mg dry mass) and mean (in 100 mg dry mass) numbers of aquatic hyphomycete species from leaves, leaf litter and humus of each palm species were calculated. Similarly, the total and mean conidial output of aquatic hyphomycetes was calculated. The percent contribution of each aquatic hyphomycete species was calculated based on the conidial output. Average values of abiotic factors were assessed for significant difference between palm species with the t-test. Also the Shannon diversity (Magurran 1988) and Pielou's equitability (Pielou 1975) for aquatic hyphomycetes were determined. Sørensen's similarity coefficient (%) was determined pair-wise for leaves, leaf litter and humus based on the presence or absence of each species (Chao et al. 2005). Three-way ANOVA (followed by Holm-Sidak's method) was applied to evaluate the influence of season (wet and dry), palm species (*C. urens* and *C. nucifera*) and substrates (leaves, leaf litter and humus) on species richness and conidium production of aquatic hyphomycetes (SigmaPlot, version 11, Systat Inc., San Jose, USA).

## RESULTS

### Habitat features

Abiotic features. As presumed, air temperature was significantly higher in the dry than in the wet season ( $P < 0.01$ ), while the opposite was true for humidity ( $P < 0.01$ ) (Tab. 1). The pH of humus was significantly lower during the dry season only in *Caryota urens* ( $P < 0.05$ ). The conductivity of humus was significantly higher during the dry than during the wet season ( $P < 0.001$ ).



**Tab. 1.** Humidity, air temperature (during sampling) and crown humus characteristics (pH and conductivity) of palm species (n = 9, mean ± SD; different letters across the seasons in the same palm species denotes significant difference: \* P < 0.01, \*\* P < 0.001; t-test).

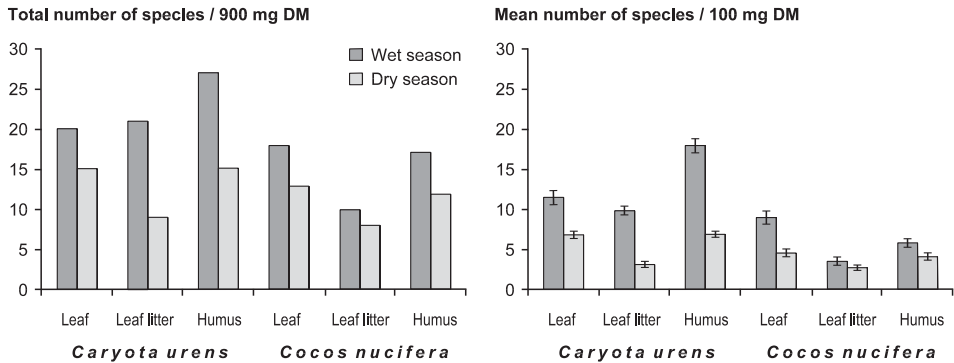
	Air		Humus	
	Humidity (%)	Temperature (°C)	pH	Conductivity (µS/cm)
<b>Wet season</b>				
<i>Caryota urens</i>	80.5 ± 1.5 <sup>a</sup>	26.4 ± 0.1 <sup>a</sup>	7.2 ± 0.02 <sup>a</sup>	81 ± 2.0 <sup>a</sup>
<i>Cocos nucifera</i>	84.0 ± 2.0 <sup>a</sup>	26.1 ± 0.2 <sup>a</sup>	7.1 ± 0.1 <sup>a</sup>	93 ± 1.5 <sup>a</sup>
<b>Dry season</b>				
<i>Caryota urens</i>	72.4 ± 1.6 <sup>b*</sup>	30.3 ± 0.2 <sup>b*</sup>	6.7 ± 0.1 <sup>b*</sup>	117 ± 3.0 <sup>b**</sup>
<i>Cocos nucifera</i>	69.3 ± 0.5 <sup>b*</sup>	32.7 ± 0.5 <sup>b*</sup>	6.9 ± 0.2 <sup>a</sup>	126 ± 2.0 <sup>b**</sup>

**Allochthonous leaves.** Allochthonous leaves of different tree species situated in the surroundings were trapped in the canopy of palms. However, some leaves could not be identified with certainty as they were crumpled or decayed. The canopies of *Caryota urens* were found to trap leaf litter from surrounding tree species like *Alstonia scholaris*, *Artocarpus hirsutus*, *Ficus benghalensis*, *F. religiosa*, *Garcinia indica*, *Holigarna arnottiana* and *Mangifera indica*. The canopies of *Cocos nucifera* possessed leaf litter of *Acacia mangium*, *Delonix regia*, *Eucalyptus tereticornis* and *Polyalthia longifolia*. Besides, nested leaves of oak-leaf basket fern (*Drynaria quercifolia*), a common epiphyte on *C. nucifera*, were also found.

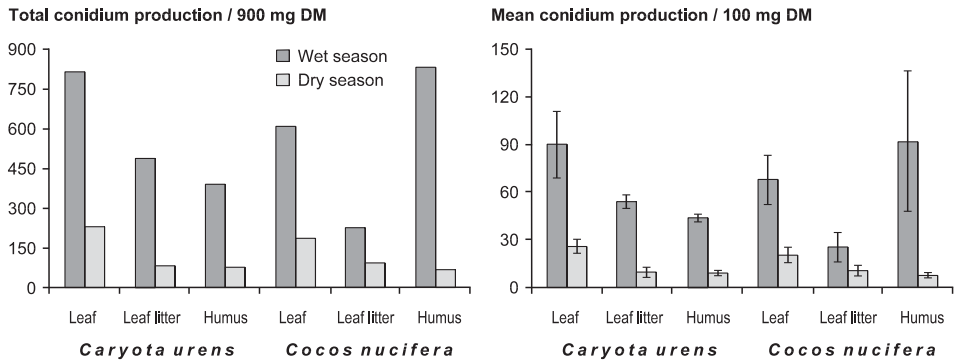
### Species richness and conidium production

In all palm samples (leaf, leaf litter and humus), total number of species as well as mean number of species were higher in the wet than in the dry season (Fig. 2). The total number of species and mean number of species were highest in humus samples of *Caryota urens* during the wet season, while it was the lowest in leaf litter of *Cocos nucifera*. In the dry season, the highest counts were in leaf samples as well as humus samples of both palms. Just like species richness (10–27 vs. 8–15 spp. in individual samples), total conidium production (6–354 vs. 1–46 / 100 mg DM) and mean conidium production in association with both palm species were higher in the wet than in the dry season (Fig. 3). Leaves of *C. urens* and humus in *C. nucifera* in the wet season showed the highest conidium production. In the dry season, the highest and mean values of conidium production were found in leaves of both palm species.

During the wet season, a higher species richness of aquatic hyphomycetes was found in association with *C. urens* than with *C. nucifera* (31 vs. 23 species)



**Fig. 2.** Total number of species and mean number of species ( $n = 9$ , mean  $\pm$  SD) in leaves, allochthonous leaf litter and crown humus collected from palms (*Caryota urens* and *Cocos nucifera*).



**Fig. 3.** Total conidium production and mean conidium production ( $n = 9$ , mean  $\pm$  SD) in leaves, allochthonous leaf litter and crown humus collected from palms (*Caryota urens* and *Cocos nucifera*).

(Tab. 2). Fifteen and seven species of aquatic hyphomycetes, respectively, occurred in all samples from *C. urens* and *C. nucifera*. A total of seven species of aquatic hyphomycetes was confined to humus of *C. urens* (*Dactylella submersa*, *Dendrospora* sp., *Lemonniera aquatica*, *Phalangispora constricta*, *Trifurcospora irregularis*, *Trinacrium incurvum* and *Isthmotricladia laeensis*), while a number of four species was confined to leaves of *C. nucifera* (*Dendrospora juncicola*, *Flabellospora verticillata*, *Hydrometrospora symmetrica* and *Trifurcospora irregularis*). Of the top five species of aquatic hyphomycetes based on conidial output, *Anguillospora crassa*, *Flagellospora curvula*, *Lunulospora curvula* and *Triscelophorus acuminatus* were common in association with both palm species.

**Tab. 2.** Percent contribution of aquatic hyphomycetes in leaf (autochthonous), leaf litter (allochthonous) and crown humus from *Caryota urens* and *Cocos nucifera* during the wet season (arranged in descending order).

Fungal species	Percent contribution		
	Leaf	Leaf litter	Humus
<b><i>Caryota urens</i></b>			
<i>Flagellospora curvula</i> Ingold	14.4	16.0	6.3
<i>Anguillospora crassa</i> Ingold	15.3	9.1	8.1
<i>Triscelophorus acuminatus</i> Nawawi	4.6	10.7	18.8
<i>Lunulospora curvula</i> Ingold	–	17.0	16.9
<i>Anguillospora longissima</i> (Sacc. & P. Syd.) Ingold	10.5	6.6	1.9
<i>Triscelophorus monosporus</i> Ingold	8.4	4.6	6.3
<i>Triscelophorus konajensis</i> K.R. Sridhar & Kaver.	5.4	3.5	8.6
<i>Flabellospora crassa</i> Alas.	8.9	0.9	4.1
<i>Isthmotricladia gombakiensis</i> Nawawi	4.6	6.0	2.7
<i>Flagellospora penicillioides</i> Ingold	4.7	6.2	1.6
<i>Campylospora chaetocladia</i> Ranzoni	3.9	5.1	0.1
<i>Dwayaangam cornuta</i> Descals	4.3	1.4	1.5
<i>Alatospora acuminata</i> Ingold	3.6	1.8	2.3
<i>Condylospora spumigena</i> Nawawi	2.5	0.3	3.3
<i>Cylindrocarpon</i> sp.	–	4.9	2.6
<i>Flabellospora verticillata</i> Alas.	3.5	0.7	0.4
<i>Heliscella stellata</i> (Ingold & V.J. Cox) Marvanová	1.3	0.9	1.3
<i>Lemonniera</i> sp.	–	1.9	1.9
<i>Ypsilina graminea</i> (Ingold, P.J. McDougall & Dann) Descals, J. Webster & Marvanová	1.1	–	0.7
Unidentified sp. 1 (sigmoid conidia)	1.3	0.3	–
<i>Dendrospora nana</i> Descals & J. Webster	0.1	2.0	–
<i>Lemonniera aquatica</i> de Wild.	–	–	2.3
<i>Flabellospora multiradiata</i> Nawawi	0.8	–	0.3
<i>Dendrospora juncicola</i> S.H. Iqbal	1.0	–	–
<i>Trifurcospora irregularis</i> (Matsush.) K. Ando & Tubaki	–	–	1.6
<i>Phalangispora constricta</i> Nawawi & J. Webster	–	–	1.4
<i>Dactylella submersa</i> (Ingold) Sv. Nilsson	–	–	1.2
<i>Dendrospora</i> sp.	–	–	0.9
<i>Trinacrium incurvum</i> Matsush.	–	–	0.7
<i>Hydrometrospora symmetrica</i> J. Gönczöl & Révay	–	0.3	–
<i>Isthmotricladia laeensis</i> Matsush.	–	–	0.2
<b><i>Cocos nucifera</i></b>			
<i>Triscelophorus acuminatus</i> Nawawi	7.4	38.3	78.5
<i>Flagellospora curvula</i> Ingold	13.6	11.7	12.2
<i>Lunulospora curvula</i> Ingold	22.9	1.8	1.1
<i>Triscelophorus monosporus</i> Ingold	8.2	25.6	1.4



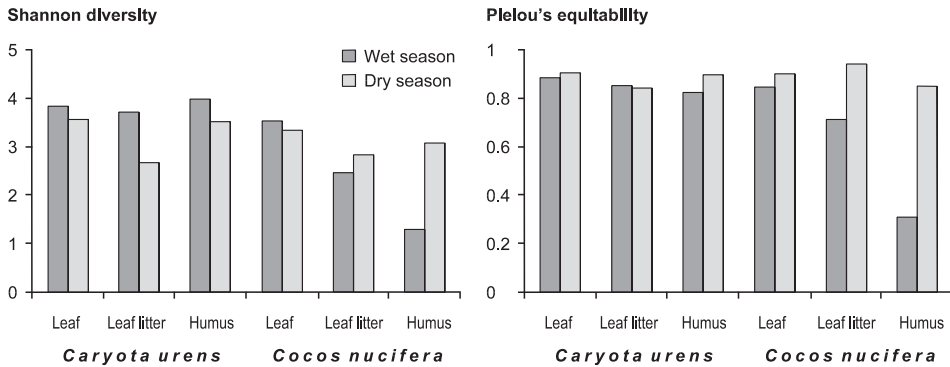
Fungal species	Percent contribution		
	Leaf	Leaf litter	Humus
<i>Anguillospora crassa</i> Ingold	11.2	11.3	1.9
<i>Flabellospora crassa</i> Alas.	6.2	–	0.2
<i>Isthmotricladia gombakiensis</i> Nawawi	6.2	–	0.2
<i>Condylospora spumigena</i> Nawawi	5.7	–	0.2
<i>Dactylella submersa</i> (Ingold) Sv. Nilsson	4.6	–	1.0
<i>Anguillospora longissima</i> (Sacc. & P. Syd.) Ingold	3.0	3.5	0.4
<i>Alatospora acuminata</i> Ingold	3.7	–	0.1
<i>Flagellospora penicillioides</i> Ingold	–	2.5	1.7
<i>Dendrospora juncicola</i> S.H. Iqbal	2.2	–	–
<i>Dwayaangam cornuta</i> Descals	1.7	0.4	0.1
<i>Trifurcospora irregularis</i> (Matsush.) K. Ando & Tubaki	1.5	–	–
<i>Lemonniera aquatica</i> de Wild.	–	3.2	–
<i>Ypsilina graminea</i> (Ingold, P.J. McDougall & Dann) Descals, J. Webster & Marvanová	0.2	–	0.5
<i>Flabellospora verticillata</i> Alas.	0.7	–	–
<i>Triscelophorus konajensis</i> K.R. Sridhar & Kaver.	–	–	0.4
<i>Hydrometrospora symmetrica</i> J. Gönczöl & Révay	0.4	–	–
<i>Phalangispora constricta</i> Nawawi & J. Webster	–	0.7	–
<i>Isthmotricladia laeensis</i> Matsush.	–	–	0.1
Unidentified sp. 1 (sigmoid conidia)	–	–	< 0.1

In the dry season, the total number of species of aquatic hyphomycetes in association with palms was almost identical (18 vs. 17 species; 14 of them were common) (Tab. 3). Eight and six species of aquatic hyphomycetes, respectively, were present in all samples from *C. urens* and *C. nucifera*. Five and seven species of aquatic hyphomycetes, respectively, were confined to only one of the samples from *C. urens* and *C. nucifera*. *Anguillospora crassa*, *A. longissima*, *Flagellospora curvula*, *Lunulospora curvula* and *Triscelophorus monosporus* were the top five species in association with the two palm species.

It should be taken into account that there need not always be a direct relation between abundance of species and abundance of conidia (some species produce a huge amount of conidia, whereas other ones never sporulate abundantly). Nevertheless the top five species concept is based on the occurrence in three substrates representing the entire habitat range in the canopy. Sporulation of aquatic hyphomycetes in dry season samples in bubble chamber incubation for 48 h was low, which is likely due to a low mycelial biomass.

**Tab. 3.** Percent contribution of aquatic hyphomycetes in leaf (autochthonous), leaf litter (allochthonous) and crown humus from *Caryota urens* and *Cocos nucifera* during the dry season (arranged in descending order).

Fungal species	Percent contribution		
	Leaf	Leaf litter	Humus
<b><i>Caryota urens</i></b>			
<i>Anguillospora crassa</i> Ingold	15.2	16.7	14.5
<i>Triscelophorus monosporus</i> Ingold	8.6	38.4	10.0
<i>Flagellospora curvula</i> Ingold	13.8	6.3	15.3
<i>Lunulospora curvula</i> Ingold	16.3	–	12.5
<i>Anguillospora longissima</i> (Sacc. & P. Syd.) Ingold	7.4	8.9	8.4
<i>Triscelophorus acuminatus</i> Nawawi	9.5	–	10.4
<i>Triscelophorus konajensis</i> K.R. Sridhar & Kaver.	5.2	10.3	1.6
<i>Condylospora spumigena</i> Nawawi	3.0	7.3	6.4
<i>Campylospora chaetocladia</i> Ranzoni	3.3	8.0	2.0
<i>Flabellospora crassa</i> Alas.	4.7	–	2.8
<i>Isthmotricladia gombakiensis</i> Nawawi	5.1	–	1.2
<i>Dwayaangam cornuta</i> Descals	3.2	2.3	2.0
<i>Flagellospora penicillioides</i> Ingold	–	–	9.2
Unidentified sp. 1 (sigmoid conidia)	2.1	–	–
<i>Alatospora acuminata</i> Ingold	1.3	1.9	–
<i>Ypsilina graminea</i> (Ingold, P.J. McDougall & Dann) Descals, J. Webster & Marvanová	1.2	–	–
<i>Lemonniera</i> sp.	–	–	2.0
<i>Flabellospora verticillata</i> Alas.	–	–	1.6
<b><i>Cocos nucifera</i></b>			
<i>Anguillospora crassa</i> Ingold	25.7	18.7	4.6
<i>Flagellospora curvula</i> Ingold	10.5	21.5	27.3
<i>Anguillospora longissima</i> (Sacc. & P. Syd.) Ingold	14.3	12.8	11.6
<i>Triscelophorus monosporus</i> Ingold	7.4	7.8	13.4
<i>Lunulospora curvula</i> Ingold	4.6	18.1	1.4
<i>Triscelophorus acuminatus</i> Nawawi	3.6	6.9	19.9
<i>Campylospora chaetocladia</i> Ranzoni	7.2	11.1	–
<i>Cylindrocarpon</i> sp.	8.3	–	0.9
<i>Isthmotricladia gombakiensis</i> Nawawi	5.7	–	5.1
<i>Condylospora spumigena</i> Nawawi	4.3	–	2.3
<i>Flagellospora penicillioides</i> Ingold	–	–	11.1
<i>Alatospora acuminata</i> Ingold	3.4	–	–
<i>Triscelophorus konajensis</i> K.R. Sridhar & Kaver.	3.1	–	–
<i>Flabellospora crassa</i> Alas.	–	3.2	–
<i>Ypsilina graminea</i> (Ingold, P.J. McDougall & Dann) Descals, J. Webster & Marvanová	1.8	–	–
<i>Heliscella stellata</i> (Ingold & V.J. Cox) Marvanová	–	–	1.4
<i>Trifurcospora irregularis</i> (Matsush.) K. Ando & Tubaki	–	–	0.9



**Fig. 4.** Shannon diversity and Pielou's equitability in leaves, allochthonous leaf litter and crown humus collected from palms (*Caryota urens* and *Cocos nucifera*).

### Statistical assessment

**Diversity and similarity.** Shannon diversity was higher in the wet season than in the dry season in all samples of *Caryota urens*, while it was higher only in leaves of *Cocos nucifera* (Fig. 4). The diversity was lowest in humus of *C. nucifera* during the wet season. Sørensen's similarity of aquatic hyphomycete species between the samples was higher in those from *C. urens* than from *C. nucifera* (66.7–82.9% vs. 59.3–74.3%) (Tab. 4).

**Tab. 4.** Sørensen's similarity (%) of aquatic hyphomycetes in samples from *Caryota urens* and *Cocos nucifera* during the wet and dry seasons.

<i>Caryota urens</i> (wet season)			<i>Cocos nucifera</i> (wet season)		
	Leaf litter	Humus		Leaf litter	Humus
Leaf	82.9	72.3	Leaf	57.1	74.3
Leaf litter		75.0	Leaf litter		59.3

<i>Caryota urens</i> (dry season)			<i>Cocos nucifera</i> (dry season)		
	Leaf litter	Humus		Leaf litter	Humus
Leaf	75.0	80.0	Leaf	66.7	72.0
Leaf litter		66.7	Leaf litter		60.0

**Analysis of variance.** Three-way ANOVA revealed significant differences in species and conidium production between seasons, palms and samples ( $P < 0.001$ ) (Tab. 5). Holm-Sidak's method showed highly significant differences: i) in the overall conidium production between the wet and dry seasons in both palm species; ii) between leaf and leaf litter in the wet season; iii) between hu-

mus and leaf samples in wet and dry seasons. There was also a significant difference in species richness: i) between leaf litter in wet and dry seasons; ii) leaf litter samples between palms; iii) between leaf litter of *C. urens* in the wet and dry seasons; iv) between leaf litter samples from palm species in the wet season; v) between humus samples of *C. urens* in the wet and dry seasons; vi) of humus samples in the wet as well as dry seasons between palms.

**Tab. 5.** Three-way ANOVA of the impact of season (wet and dry), palm species (*Caryota urens* and *Cocos nucifera*) and substrate (leaf, leaf litter and humus) on richness of species and conidium production of aquatic hyphomycetes (df – degrees of freedom; F – ratio of two mean square values; P – level of significance).

Treatment	Species richness			Conidium production		
	df	F	P	df	F	P
Season	1	240.999	< 0.001	1	29.766	< 0.001
Palm	1	187.812	< 0.001	1	0.0249	0.875
Substrate	2	57.109	< 0.001	2	2.297	0.058
Season × Palm	1	64.288	< 0.001	1	0.0008	0.977
Season × Substrate	2	7.169	0.001	2	1.141	0.324
Palm × Substrate	2	22.748	< 0.001	2	1.957	0.147
Season × Plant × Substrate	2	19.392	< 0.001	2	1.933	0.150

## DISCUSSION

Tree canopies represent a mosaic of tree organs (foliage, twigs, fine branches and epiphytes) in close contact with the atmosphere (Parker 1995). Habitats in the canopy serve as cradles for organic matter of autochthonous and allochthonous origin (e.g. tree holes, branch junctions and funnel/basket/humus epiphytes) (Nadkarni et al. 2001, Sridhar et al. 2006). Besides autochthonous input, storms and precipitation deposit organic matter into canopy habitats. Such organic matter is processed by a variety of microbes and fauna, primarily by bacteria, fungi and invertebrates, similar to other terrestrial habitats. It is known that wet tropical forests develop a vertical gradient of temperature and relative humidity facilitating distribution and stratification of a variety of fungi (Hedger 1985).

Conidial fungi in canopies serve as a guild to mediate energy flow (Carroll 1981) and are likely to contain many undescribed species, which meets at least partially the challenge of reaching the estimated 5.1 million species proposed by Blackwell (2011). A striking estimate was provided by Carroll et al. (1980); he suggested that twig and needle surfaces of an old-growth Douglas fir forest canopy sustain up to 450 kg of microfungus biomass/ha/yr. Such a huge biomass of fungi has several major functions to play in the forest ecosystem (e.g. decomposition, nutrient turnover/acquisition/supply and interaction with fauna).

Besides terrestrial fungi, typical water-borne hyphomycetes have also been reported from canopies of temperate and tropical regions (Chauvet et al. 2016). The question remains what their ecological functions are in canopies which are away from aquatic habitats.

The extent of deposition and retention of organic matter seems to be dependent on the canopy structure. For example, palms have a canopy architecture which differs from dicotyledonous trees, and petiole junctions of palms provide ample space for accumulation of organic matter (leaves, leaf litter and humus). There seems to be only one study on the occurrence of rain-borne hyphomycetes in palm canopies (Ghate & Sridhar 2015).

Nearly 20 wild and cultivated palms have been located in and around Mangalore University Campus. The wild palm *Caryota urens* has a wide geographic distribution and *Cocos nucifera* is a common cultivated palm in southwestern India. Ghate and Sridhar (2015) reported a variety of aquatic hyphomycetes in stemflow and throughfall in these palms, their origin probably being from crown substrates (autochthonous/allochthonous leaves and crown humus). Considering the extent of rainfall, these palms serve as potential sources of inoculum for nearby terrestrial (soil) as well as aquatic (streams) habitats. As palms retain a considerable amount of moisture in detritus and crown humus trapped in their canopies even during the dry season, they provide better opportunities for survival and activity of these fungi than dry terrestrial habitats. In our study, the richness of species and conidia of aquatic hyphomycetes were demonstrated to be significantly palm-, substrate- and season-dependent.

Sampaje stream has been considered an outlier among the streams of the Western Ghats, as the highest number of aquatic hyphomycetes (76 species) was reported from here. When stream (water, leaf litter and foam; Sridhar & Kaveriappa 1989, Sridhar et al. 1992, Raviraja et al. 1998), riparian canopies (tree holes and ferns; Karamchand & Sridhar 2008, 2009, Sridhar 2009) and root endophytes (Ghate & Sridhar 2016) are included, this number comes close to 100. This raises the question whether fungi in the canopies of riparian trees of the Sampaje stream are also much more diverse than in the canopies of other regions in the Western Ghats and elsewhere.

Species belonging to the genera *Anguillospora*, *Flagellospora*, *Lunulospora* and *Triscelophorus* were consistently well represented in streams, tree holes, epiphytic ferns and drain water from canopies (Sridhar & Kaveriappa 1984, 1989, Sridhar et al. 1992, 2006, 2013, Karamchand & Sridhar 2008, 2009, Sridhar & Karamchand 2009, Sudheep & Sridhar 2010). These genera are ubiquitous in warm climates and also dominant as root endophytes in the Sampaje and Virajpet-Badaga streams of the Western Ghats (Ghate & Sridhar 2016). However, the overall diversity and extent of conidial output are considerably lower in tree hole litter, epiphytic ferns, and trapped litter in tree canopies than on leaf litter in

streams. Species richness and diversity of aquatic hyphomycetes in attached dead leaves (or epiphytic ferns) are higher than in allochthonous leaf litter, possibly because they are stationary for extended periods (Sridhar et al. 2006, Karamchand & Sridhar 2009). However, crown humus obtained from both autochthonous and allochthonous organic matter is a potentially more complex substrate than just canopy leaves or trapped leaf litter, sustaining a more diverse fungal community in *Caryota urens*, as seen in the present study (Fig. 4).

Canopy studies have revealed numerous morphologically complex stauro- and scolecosporous conidia of hyphomycetes (15–20%) similar to those found in aquatic habitats, which have not been identified to genus or species level (e.g. Sridhar & Kaveriappa 1992, Gönczöl & Révay 2004, 2006, Magyar et al. 2005, Chauvet et al. 2016). Blackwell's (Blackwell 2011) recent global estimate of 5.1 million fungal species is mainly based on molecular methods. In addition to time-consuming conventional methods, it will be essential to apply molecular techniques to gain deeper and more comprehensive insight into fungal diversity. Such approaches will also help us characterise fungal distribution in different ecological niches and their functional attributes.

The dual lifestyle of aquatic hyphomycetes in tree canopies and streams needs to be compared more thoroughly to understand specific pathways of dissemination, colonisation, decomposition and energy flow (Seena & Monroy 2016). The presence of viable conidia in water draining through canopies (stemflow and throughfall) indicates that these fungi have a functional role and are not merely using canopies as a temporary refugium.

Is the composition of these fungi in canopies similar in different geographic regions? Some studies have been carried out on the southwest coast and in the Western Ghats of India. Those regions receive heavy precipitation during the southwest monsoon and moderate rains during the northeast monsoon. How do other vegetation types (e.g. virgin forests and monocrop plantations) and extremely wet or extremely xeric geographic locations differ in fungal composition? Forest canopies of tropical regions have been substantially modified in the recent past due to cultivation of exotic tree species (e.g. *Acacia*, *Grevillea* and *Eucalyptus*). Under such altered conditions of native forests, it is pertinent to understand the assemblage, diversity and ecological functions of fungi in canopies.

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