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Diversity of fungal spores in the canopy of two mangrove tree species of southwest India

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Abstract This study designed to assess the fungal spores present in throughfall, stemflow and water-filled tree holes (dendrotelmata) of two mangrove tree species (*Avicennia officinalis* and *Rhizophora mucronata*) in the Nethravathi mangroves of southwest India. Water samples were filtered through Millipore filters and stained immediately after filtration. The filters were scanned using a high power microscope for identification and enumeration of fungal spores. Physicochemical parameters of canopy region (air temperature and humidity) and water samples (temperature, pH, conductivity, salinity, dissolved oxygen and total dissolved solids) were assessed. Spores of 39 fungal species were recorded in the canopy water samples with 34 and 20 species in *R. mucronata* and *A. officinalis*, respectively. Spores of 15 species were common, while 19 and 5 species were confined to *R. mucronata* and *A. officinalis*, respectively. Fungal species with ≥5% contribution were the highest in *R. mucronata* than *A. officinalis* (12 vs. 10 spp.), while seven species of them were common to both trees. The number of fungal species were highest in the stemflow of both trees with the highest species richness as well as diversity. The spores in canopy waters composed of a blend of terrestrial, freshwater and marine fungi representing staurospores, scolecospores and helicospores. Owing to diverse known and unknown fungi in the mangrove canopies, further studies might facilitate to fill the gap of global fungal biodiversity pool.

Keywords: *Avicennia officinalis*, Dendrotelmata, *Rhizophora mucronata*, Stemflow, Throughfall, Tree holes

Introduction

Tree canopies are structurally complex habitats with aggregate of foliage, branches, twigs, epiphytes and atmosphere (Parker, 1995). Structurally canopy provides mechanical strength as well as harbors many non-vascular (bryophytes, mosses, lichens and algae) and vascular (non-parasitic plants, parasitic plants and ferns) epiphytes (Kress, 1986; Nadkarni *et al*., 2001). Tree

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canopies trap considerable quantity of autochthonous and allochthonous organic matter, it will be transformed into crown humus, which supports a variety of life forms. Canopy dwelling fauna include insects, annelids, gastropods, amphibians, reptiles and mammals (Ellwood and Foster, 2004). Leaf surface (phylloplane) and bark surface (cortiplane) also provide suitable niches for many microbiota and invertebrates. Water-filled tree holes (dendrotelmata) in the canopy are the specialized habitats provide suitable conditions for survival and activity of many biota (Nishadh and Das, 2014).

Fungi are ubiquitous owing to their distribution and adaptability to different ecological niches. Tree canopies provide excellent habitats for colonization, growth and perpetuation of fungi. Usual expectation is occurrence of plant pathogenic fungi in canopies those causing diseases in leaves, twigs, trunk and fruits. Several fungi have been accommodated mutualistically as endophytes in live tissues. A few studies are available on the fungal diversity in tree canopies (endophytic, pathogenic, phylloplane, lignicolous, aquatic, water-borne, aero-aquatic and dematiaceous fungi) (Sridhar, 2009; Chauvet *et al.*, 2016; Magyar *et al*., 2016; 2021). Fungi have been reported in canopies of 57 plant species in different parts of the world (Sridhar, 2009). Unterseher *et al*. (2005) reported up to 118 fungi in dominant tree species in Germany (*Acer, Fraxinus, Quercus* and *Telia*).

Mangroves are the second most productive ecosystems after coral reefs, consist of trees and shrubs in tropical and subtropical estuaries, backwaters, lagoons and deltas (Qasim and Wafar, 1990). Mangroves provide excellent niches for growth and perpetuation of marine fungi on the accumulated leaf and woody detritus (e.g. Maria and Sridhar, 2002; 2003; Ananda *et al*., 2008). Besides, mangroves harbor several endophytic fungi in leaves, stem and roots without causing disease symptoms (e.g. Ananda and Sridhar, 2002; Rajamani *et al*., 2019). Although some studies have been carried out on the fungal diseases of mangroves (e.g. Rafael and Clumpong, 2019), fungal diversity in mangrove tree canopies seems to be not been attempted so far. Therefore, the present study documents fungal spores present in water samples (throughfall, stemflow and tree holes) collected from two tree species (*Avicennia officinalis* and *Rhizophora mucronata*) of a mangrove stand in the southwest India.

Materials and methods

This study has been carried out in a mangrove stand of Nethravathi estuary located at the southwest Karnataka, India (12°50’N, 74°50E) during southwest monsoon season (July-August, 2021). Water samples were accessed from the throughfall, stemflow and water-filled tree holes (dendrotelmata) of

two mangrove tree species (*Avicennia officinalis* and *Rhizophora mucronata*) (Fig. 1). Three trees of each species about 300 m apart possessing tree hole/s were selected for the study. The air temperature and relative humidity near the trees were recorded using Mextech Digital Thermo Hygrometer (M288CTHW, Mumbai, India). The overall procedure followed to assess the fungal spores in canopy water samples is schematically represented in Figure 2.

Figure 1. Canopy water samples of tree species sampled for fungal spores in Nethravathi mangrove: *Avicennia officinalis* (a), its tree hole (b), *Rhizophora mucronata* (c) and its tree hole (d)

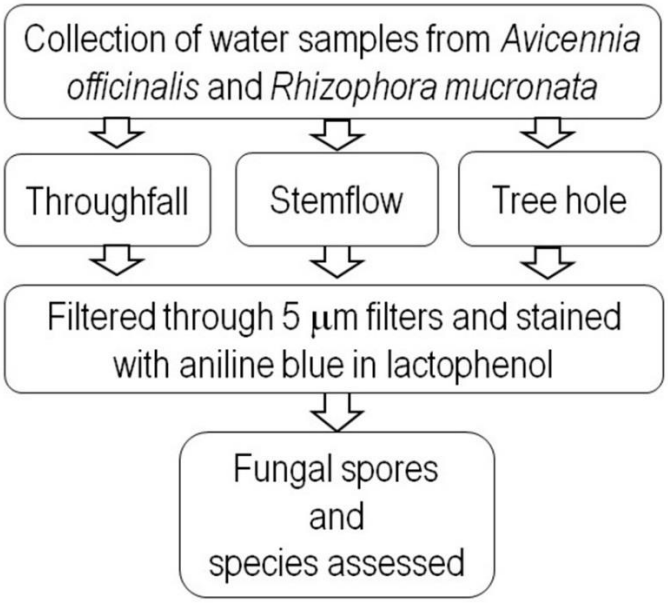

Figure 2. Schematic representation of procedures followed to assess the fungal spores in canopy water samples of two mangrove tree species

Water samples from throughfall (trickling under the canopy) were collected by spreading a clean plastic sheet. Water passing downwards though the trunk (stemflow or barkflow) were collected in clean polythene bags. Water samples from the tree holes were collected by immersing small clean bottles. Temperature, pH and conductivity of water samples collected were assessed on the sport, fixed water samples immediately after collection were assessed for dissolved oxygen in the laboratory (Winkler's method), while the salinity and total dissolved solids (TDS) in water samples were assessed in the laboratory (Water Analyzer 371, Systronics India Ltd., Ahmedabad, Gujarat, India; APHA, 1998).

Water samples (100 ml each) were filtered through Millipore filters (pore size, 5 μm) on the collection spot, each filter was transferred to clean Petri plate, stained with a few drops of aniline blue in lactophenol immediately after filtration, allowed to spread and brought to the laboratory. Sections of filters were mounted on the microscope slides with a few drops of lactic acid and scanned using the high power microscope. The stained spores on the filters were identified by monographs and the spore numbers were enumerated (Ingold, 1975; Nawawi 1985; Kohlmeyer and Volkmann-Kohlmeyer, 1991; Marvanová 1997; Gulis *et al*., 2020). The diversity of fungi in mangrove tree

canopy waters were assessed based on Magurran (1988) and the equitability by Pielou (1975).

Results

Physicochemical features

The mean air temperature of three sampling locations of the mangrove stand was 26.6°C, while the relative humidity was 87.5% (Table 1). The water temperature was lowest in the tree holes of both tree species, while it was the highest in the stemflow. The pH of water samples was highest in throughfall of *Avicennia*, while it was least in tree holes of both tree species. The conductivity was higher in the water samples of *Avicennia* than *Rhizophora*. The conductivity was highest in throughfall of *Avicennia*, whereas it was the lowest in throughfall of *Rhizophora*. The dissolved oxygen was highest in throughfall of *Rhizophora*, while it was least in tree holes. The salinity of water samples ranged from 0.06–0.10‰. The TDS was highest in throughfall of *Avicennia*, while it was least in tree holes.

Table 1. Physicochemical features of through fall, stemflow and tree holes of *Avicennia officinalis* and *Rhizophora mucronata* (n=3, mean)

	Avicennia officinalis			*Rhizophora mucronata*		
	Through-fall	**Stem-flow**	**Tree hole**	**Through-fall**	**Stem-flow**	**Tree hole**
Temperature (°C)	22.5	23.0	22.0	23.0	23.5	22.5
pH	7.1	6.9	6.1	6.5	6.5	5.7
Conductivity (µS/cm)	157.8	66.6	79.1	25.9	31.0	31.0
Dissolved oxygen (mg/l)	8.6	7.9	7.4	8.9	8.4	7.1
Salinity (‰)	0.10	0.08	0.10	0.06	0.07	0.09
Total dissolved solids (mg/l)	91.1	47.0	12.5	18.9	16.6	26.6

Fungal spores in Avicennia

Canopy samples of *Avicennia* was represented by the fungal spores of 20 species (Table 2). Representative spore photographs are given in Figure 3. The per cent contribution was highest by *Trinacrium subtile*, followed by *Flagellospora penicillioides, Fusarium*-like spores, *Mycocentrospora acerina* and *Dwayaangam cornuta*. Spores of four species were represented <1% (*Dwayaangam* sp. 2, *Retiarius bovicornutus*, *Synnematophora constricta* and *Tetraploa aristata*). Spores of the rest 10 species ranged from 1–9%. Five

species were found exclusively in *Avicennia* were *M. acerina*, *Dwayaangam* sp. 1, *Nia vibrissa, S. constricta* and *T. aristata*. Five fungal species were confined exclusively to *Avicennia*.

Table 2. Per cent contribution of fungal spores in through fall, stemflow and tree holes (100 ml each) in *Avicennia officinalis* (n=3, mean; *, exclusive species)

	Throughfall	Stemflow	Tree hole
***Trinacrium subtile* Riess (Fig. 3m)**	29	35	11
***Flagellospora penicillioides* Ingold (Fig. 3g)**	11	5	56
***Fusarium* spore-like**	43	-	-
****Mycocentrospora acerina* (R. Hartig) Deighton (Fig. 3i)**	-	37	-
***Dwyaangam cornuta* Descals (Fig. 3c)**	-	-	22
***Anguillospora longissima* (Sacc. & P. Syd.) Ingold (Fig. 3b)**	4	9	-
***Trinacrium robustum* Tzean & J.L. Chen (Fig. 3l)**	-	2	5
***Tripospermum myrti* (Lind) S. Huges**	-	6	-
***Isthmotricladia laeensis* Matsush. (Fig. 3h)**	5	-	-
***Lulworthia* sp.**	<1	-	5
****Nia vibrissa* R.T. Moore & Meyers (Fig. 3j)**	4	-	-
***Trinacirum* sp. 1**	4	-	-
***Dwayaangam dichotoma* Nawawi (Fig. 3d)**	-	2	-
***Alatospora acuminata* Ingold (Fig. 3a)**	-	1	-
****Dwayaangam* sp. 1 (Fig. 3e)**	-	1	-
***Isthmotricladia gombakiensis* Matush.**	-	1	-
***Dwayaangam* sp. 2 (Fig. 3f)**	<1	-	-
***Retiarius bovicornutus* D.L. Olivier**	<1	-	-
****Synnematophora constricta* K.R. Sridhar & Kaver.**	-	<1	-
****Tetraploa aristata* Berk. & Broome (Fig. 3k)**	-	<1	-

Fungal spores in Rhizophora

Canopy samples of *Rhizophora* was represented by the fungal spores of 34 species (Table 3). Representative spore photographs are given in Figure 4. The per cent contribution was highest by *Fusarium*-like spores, followed by *Anguillospora longissima, F. penicillioides, Helicosporium griseum,* unknown sp. 1, *Lulworthia* sp. and *Trinacrium subtile*. Spores of nine species were represented <1% (*Alatospora acuminata, Flabellospora* sp., *Condylospora spumigena, Dwayaangam* sp. 2, *Isthmotricladia gombakiensis, Tricladium* sp., *Trinacrium* sp. 2, *Verruculina enalia* and unknown sp. 4). Spores of the rest 18

species ranged from 2–7%. Nineteen Fungal species were confined exclusively to *Rhizophora.*

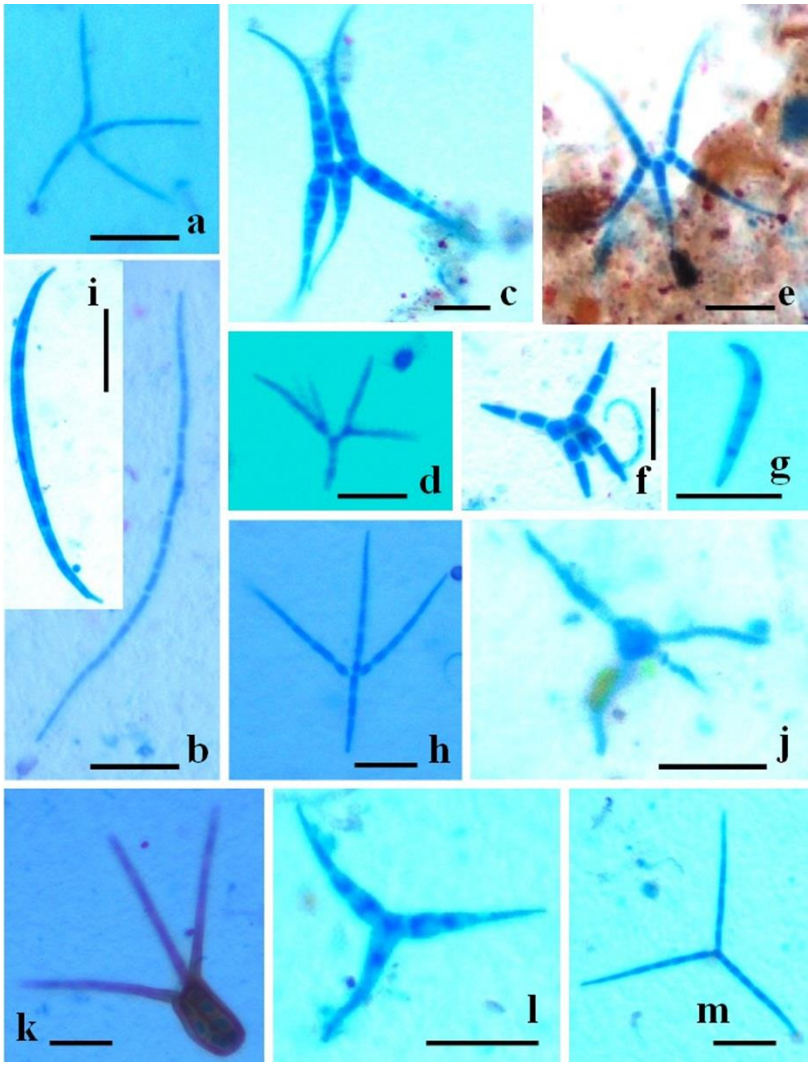

Figure 3. Fungal spores found in canopy waters of *Avicennia*: *Alatospora acuminata* (a), *Anguillospora longissima* (b), *Dwayaangam cornuta* (c), *Dwayaangam dichotoma* (d), *Dwayaangam* sp. 1 (e), *Dwayaangam* sp. 2 (f), *Flagellospora penicillioides* (g), *Isthmotricladia laeensis* (h), *Mycocentrospora acerina* (i), *Nia vibrissa* (j), *Tetraploa aristata* (k), *Trinacrium robustum* (l), *Trinacrium subtile* (m) (Scale bar, 20 μm)

Table 3. Per cent contribution of fungal spores in through fall, stemflow and tree holes (100 ml each) of *Rhizophora mucronata* (n=3, mean; *, exclusive species)

	Throughfall	Stemflow	Tree hole
***Fusarium* spore-like**	40	47	44
***Anguillospora longissima* (Sacc. & P. Syd.) Ingold**	22	17	11
***Flagellospora penicillioides* Ingold**	-	4	11
****Helicosporium griseum* (Berk. & M.A. Curtis (Fig. 4e)**	11	-	-
***Unknown sp. 1 (tetraradiate spore) (Fig. 4j)**	11	-	-
***Lulworthia* sp.**	7	2	-
***Trinacrium subtile* Riess**	2	7	-
****Triscelophorus* sp.**	7	-	-
****Helicoma atroseptatum* Linder (Fig. 4b)**	-	-	6
***Unknown sp. 2 (tetraradiate spore with twist) (Fig. 4 k)**	-	-	6
***Trinacrium robustum* Tzean & J.L. Chen**	<1	4	2
***Isthmotricladia laeensis* Matsush.**	-	3	2
****Corollospora* sp.**	-	4	<1
***Dwayaangam cornuta* Descals**	-	-	4
****Helicoma* sp. 1 (Fig. 4c)**	-	3	-
***Tripospermum myrti* (Lind) S. Huges**	-	3	2
****Clavariopsis bulbosa* Anastasiou**	-	2	-
***Dwayaangam dichotoma* Nawawi**	-	2	-
****Helicoma* sp. 2 (Fig. 4d)**	-	-	2
****Spriropsis pedatospora* Tubaki**	-	-	2
***Retiarius bovicornutus* D.L. Olivier (Fig. 4f)**	-	-	2
****Triscelophorus acuminatus* Nawawi**	-	-	2
****Trisulcosporium* sp. (Fig. 4i)**	-	2	
***Unknown sp. 3 (sigmoid with central bulb) (Fig. 4l)**	-	-	2
***Trinacrium* sp. 1 (Fig. 4g)**	-	-	2
***Alatospora acuminata* Ingold**	<1	-	-
****Flabellospora* sp. (Fig. 4a)**	-	<1	-
****Condylospora spumigena* Nawawi**	<1	-	-
***Dwayaangam* sp. 2**	-	<1	-
***Isthmotricladia gombakiensis* Nawawi**	<1	-	-
****Tricladium* sp.**	<1	<1	-
****Trinacrium* sp. 2 (Fig 4h)**	<1	-	-
****Verruculina enalia* (Kohlm.) Kohlom. & Volk.-Kohlm.**	-	<1	-
***Unknown sp. 4 (appendaged ascospore) (Fig. 4m)**	-	<1	-

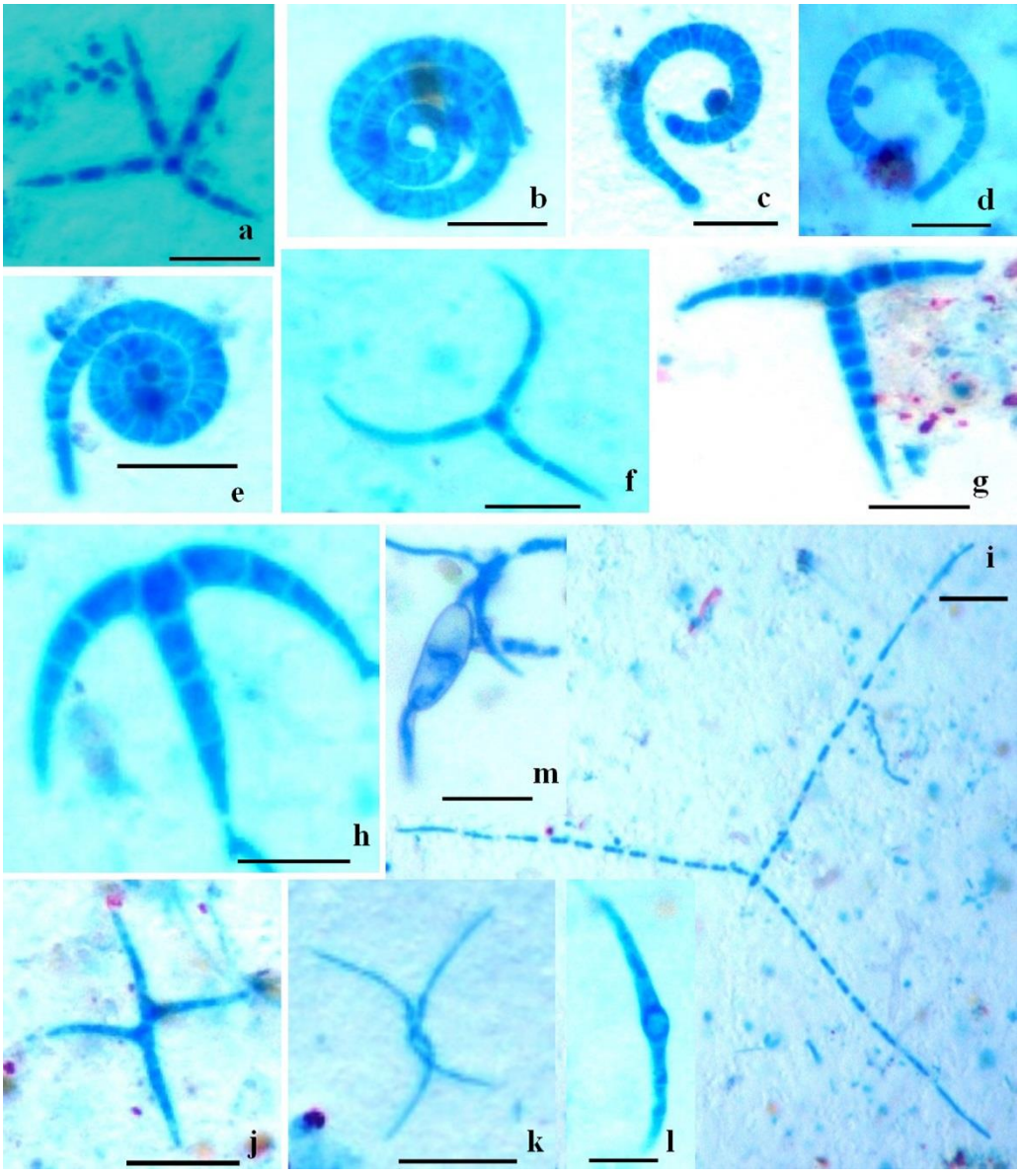

Figure 4. Fungal spores found in canopy waters of *Rhizophora*: *Flabellospora* sp. (a), *Helicoma atroseptatum* (b), *Helicoma* sp. 1 (c), *Helicoma* sp. 2 (d), *Helicosporium griseum* (e), *Retiarius bovicornutus* (f), *Trinacrium* sp. 1 (g), *Trinacrium* sp. 2 (h), *Trisulcosporium* sp. (i), unknown sp. 1 (tetraradiate spore) (j), unknown sp. 2 (tetraradiate spore with twist) (k), Unknown sp. 3 (sigmoid spore with central hub) (l), unknown sp. 4 (appendaged ascospore) (m) (Scale bar, 20 μm)

Comparison of Avicennia and Rhizophora

Spores of 39 fungal species were recorded in two tree species with 34 and 20 species in *Rhizophora* and *Avicennia*, respectively, while spores of 19 and 5 species (exclusive species) were confined respectively to these trees. Fungal species with ≥5% contribution were the highest in *R. mucronata* than *A. officinalis* (12 vs. 10 spp.), while seven fungal species with ≥5% contribution were common to these trees. The number of fungal species were highest in the stemflow of both tree species (12 vs. 18 spp.), while it was the lowest in tree holes of *Avicennia* and throughfall of *Rhizophora* (5 vs. 13 spp.). Species richness as well as the diversity (Simpson and Shannon) were highest in stemflow of both tree species followed by throughfall and tree holes (Table 4). Pielou's equitability was least in stemflow of *Avicennia*, while it was least in throughfall of *Rhizophora*. Although 39 species were identified in the canopies of two mangrove trees of Nethravathi River in this study, some of the spores were germinated and could not be identified even up to genus level.

Table 4. Species richness, diversity and equitability of fungal spores in throughfall, bark flow and tree holes of *Avicennia officinalis* and *Rhizophora mucronata*

	Avicennia officinalis			***Rhizophora mucronata***		
	Through-fall	**Stemflow**	**Tree hole**	**Through-fall**	**Stemflow**	**Tree hole**
Species richness	10	12	5	13	18	16
Simpson diversity	0.695	0.723	0.633	0.735	0.759	0.733
Shannon diversity	1.533	1.608	1.236	1.692	1.926	1.883
Pielou's equitability	0.666	0.647	0.768	0.660	0.666	0.679

Discussion

Tree canopies provide excellent habitats for colonization of fungi. Some of the niches in canopy occupied by fungi include foliage, twigs, roots, rhizomes, sediments, bark, honey dew and floral honey (Chauvet *et al*., 2016). Mangroves being highly productive detritus-based ecosystems, its fungal pool in the canopy seems to be unique to drive several ecosystem functions. Our study revealed the surrounding mangrove conditions (air temperature and humidity) and physicochemical features of canopy waters (temperature, pH, conductivity, dissolved oxygen, salinity and total dissolved solids) are suitable for fungal colonization and perpetuation of many terrestrial as well as aquatic fungi.

Niche duality is one of the common phenomena in fungi (Colwell and Rangel, 2009; Sádosse *et al*., 2018). Such niche duality is also found in freshwater fungi as they occur outside their usual aquatic habitats such as terrestrial leaf litter and canopy (throughfall, stemflow and tree holes) (Sridhar *et al*, 2013; Ghate and Sridhar, 2015; 2016; Chauvet *et al*., 2016; Magyar *et al*., 2021). Similarly, mutualistic fungi in mangroves and coastal sand dunes are also a complex assemblage of endophytic, saprophytic, plant pathogenic and entomopathogenic fungi (e.g. Ananda and Sridhar, 2002; Seena and Sridhar, 2004). In the palms of coastal region of southwest India, the canopy fungi consist of mosaic of true aquatic, pseudo-aquatic and aero-aquatic hyphomycetes (Ghate and Sridhar, 2015). In our study in the mangrove canopy also a variety of terrestrial, aquatic, aero-aquatic and marine fungal spores were found. It is not surprising to find typical freshwater hyphomycetes in mangrove canopies as 16 species were recorded in a seasonal study of decaying leaf litter in the brackish waters of Nethravathi estuary (Sridhar and Kaveriappa, 1988).

Presence of typical marine fungal spores such as *Corollospora* sp., *Lulworthia* sp. *Nia vibrissa* and *Verruculina enalia* indicates their input into the canopy by different routes. For instance, marine detritus feeders (e.g. crabs and snails) may disseminate such spores or mycelia from the detritus accumulated on the mangrove flour or stable decomposing parts of the mangrove trees. Dissemination of fungi from terrestrial and freshwater habitats to canopy may take place through various routes like mechanical, wind, aerosols, foam, insects, birds and bats (Magyar *et al*., 2016). It is also possible that some fungal spores adhere to the mangrove tree parts during the high tide conditions owing to sticky appendages. The conidial architecture in palm canopies of southwest India was a mixture of staurospores, scolecospores and helicospores (Ghate and Sridhar, 2015). In the present study also such categories of complex spores were recorded. Some of the fungi in mangrove tree canopies are of special interest owing to their preponderance: *Trinacrium* (triradiate), *Dwayaangam* (pentaradiate), *Helicoma* or *Helicosporium* (helicosporous) were represented by four species each.

Although tree canopies serve as fungal guild, nearly 15–20% were unidentified up to the genus or species level (Carroll, 1981; Sridhar, 2009; Chauvet *et al*., 2016). In our study, among the spores of 39 fungi in mangrove canopy waters, 10% of spores were not identified. Several spores are germinated in our study posed difficulties in identification. Presence of germinated spores in canopy waters reveals their definite role of fungal saprophytism in canopies similar to the terrestrial or aquatic habitats. Such observations was also made in an earlier study in palm canopies by Ghate and Sridhar (2015). Tree canopies being rich in various unknown fungi, there are

possibilities to fill the gap of global estimate 2.2−3.8 million fungi (Blackwell, 2011; Hawksworth and Lücking, 2017).

It concluded that mangrove canopies occupied by mixture of terrestrial, freshwater and marine fungi. Continuous exposure of mangrove canopy fungi (terrestrial and freshwater fungi) to the mangrove or marine conditions might be accountable for their adaptability to the marine habitats which resulted in niche duality. It is interesting to study the pattern zonation of fungal occupation in the mangrove tree canopies. Occurrence of several unidentified or unknown fungi in the mangrove canopies may fill the gaps in our knowledge on the global fungal diversity estimate. Adapting suitable molecular approaches to assess the fungal composition in the tree canopies might answer many questions pertain to their adaptability and functions.

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