



## **Assemblage and diversity of asexual fungi in 10 terrestrial damp leaf litters: Comparison of two incubation techniques**

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### **Abstract**

Freshwater and aero-aquatic hyphomycetes are also known to occur outside their usual aquatic habitats. Thus, this study aims to evaluate their assemblage and diversity in terrestrial damp leaf litters of 10 tree species in scrub jungles of southwestern India. Discs cut from the rinsed leaf litter collected during southwest monsoon were incubated in damp chambers (2 weeks) and bubble chambers (48 hours). Post-incubation at the damp chamber, discs were transferred to sterile, distilled water and shaken on a rotary shaker to release the conidia. Another set of leaf discs from rinsed leaf litter was incubated in sterile distilled water in cotton plugged conical flasks and bubbled through Pasteur pipettes. Released conidia in distilled water were filtered through Millipore filters, mounted on the microscope slides and stained with aniline blue to score the conidia. A total of 31 species of aquatic hyphomycetes were recorded in both methods of incubation. Even though the total number of species (21 vs 20 spp.) and exclusive species (11 vs 10 spp.) were almost equal in both methods, the species composition is varied. Among the nine core-groups (frequency of occurrence, >25%), *Flagellospora curvula*, *Lunulospora curvula* and *Triscelophous konajensis* were common in both methods. The species richness, as well as the conidial abundance were higher for *Acacia auriculiformis*, *Ficus benghalensis* and *Tectona grandis* in litters using both methods. For *Artocarpus hirsutus*, *Careya arborea* and *Terminalia paniculata*, the species richness was high but the conidial output was very low. Despite showing a higher number of species for *Artocarpus hirsutus*, *Eucalyptus tereticornis* and *Ficus religiosa* using both methods, the conidial richness was high only in damp incubation, while it was opposite for the *Anacardium occidentale*. The species diversity, as well as their equitability, were higher in the damp chamber than bubble chamber incubation. The rarefaction curves for the number of species vs. number of samples and the number of species vs. number of conidia were almost consistent in both methods. Damp chamber incubation, as well as bubble chamber incubation, could be improved to get a more complete picture of colonized mycota in damp leaf litter.

**Key words** – aquatic fungi – aero-aquatic fungi – bubble chamber – damp chamber – Ingoldian fungi – scrub jungles

### **Introduction**

Freshwater fungi consist of phylogenetically diverse groups of fungi belonging to Ascomycota, Chytridiomycota, Cryptomycota and Zygomycota in different habitats (Hyde &

Jeewon 2003, Vijaykrishna et al. 2005, Raja et al. 2018, Luo et al. 2019). The lifestyle of freshwater fungi widely differs (e.g. saprophytic, planktonic, endophytic, pathogenic and parasitic) and dependent on the specificity of their ecological niches (Zhang et al. 2008, Huang et al. 2018a, b). Ascomycetes are well adapted to freshwater habitats (Vijaykrishna et al. 2006). The diversity of freshwater fungi is rather high in the South East Asian region with many new species discovered recently (Luo et al. 2017, Bao et al. 2018, Su et al. 2018). Freshwater hyphomycetes (or Ingoldian fungi) have been characterized by their morphologically distinct staurosporus (multiradiate) and scolecosporus (sigmoid) conidia (Ingold 1975, Marvanová 1997, Gulis et al. 2005). However, some of them produce conidia of conventional shapes like spherical or oval or fusiform (e.g. *Dimorphospora*, *Tumularia* and *Vermispora*). The complexity of conidial shapes denotes functional traits in aquatic habitats especially floatation, impaction, sedimentation and dissemination.

Aquatic hyphomycetes have a worldwide distribution, and 335 morphospecies have been reported with preponderance in freshwaters of mid-latitudes (Duarte et al. 2016, Friggens et al. 2017). Although aquatic hyphomycetes have adapted to flowing waters, they also occur outside their preferred habitats, especially in several tree species or its detritus throughout the world (Chauvet et al. 2016). Colonization and sporulation of aquatic and aero-aquatic hyphomycetes have been reported from habitats such as stream banks, dew, canopy waters and tree holes (Sridhar & Kaveriappa 1987, Sridhar 2009, Sridhar & Bärlocher 1993). Typical aquatic and aero-aquatic hyphomycetes have been reported from the forest floors, forest canopies (tree holes, stemflow and throughfall) and as endophytes from the aerial plant parts (Chauvet et al. 2016). Aero-aquatic hyphomycetes are predominant in lotic habitats. They build their biomass in submerged conditions and produce conidia in the air-water interface. They are also the inhabitants of semi-aquatic habitats and moist forest litters (leaf, twigs and wood). *Helicoma*, *Helicoön*, *Helicosporium*, *Helicomycetes* and *Spirosphaera* are characterized by two or three-dimensionally coiled spores as a special adaption for flotation (Zhao et al. 2007, Luo et al. 2017). From the aquatic habitats, up to 90 aero-aquatic hyphomycete species (in 19 genera) have been reported worldwide (Shearer et al. 2007, Raja et al. 2018). Hence, the purpose of the present study is to assess the assemblage and diversity of aquatic and aero-aquatic hyphomycetes in the damp leaf litter of 10 tree species in the scrub jungles of southwestern India during southwest monsoon season.

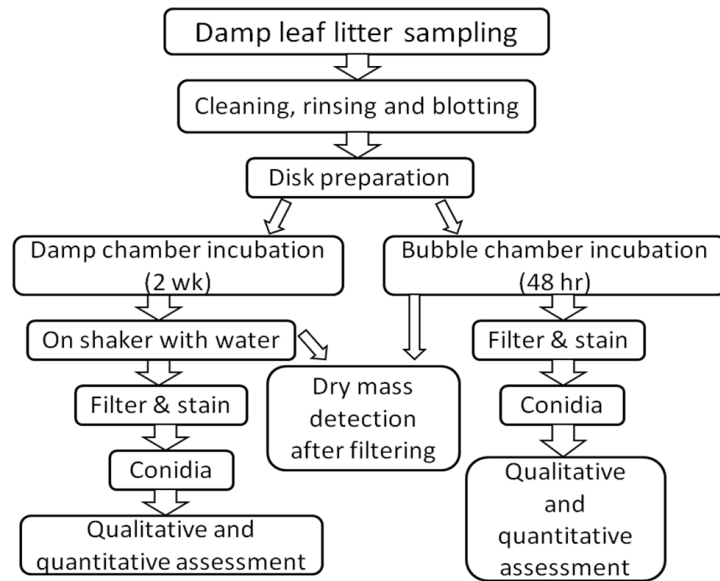
## Materials & Methods

### Location and sample collection

The geographic location chosen for the microfungus survey was Mangalore University Campus located ~5 km away from the Arabian Sea coast adjacent to the Mangalore City (Dakshina Kannada District, Karnataka State) (12°48'N, 74°55'E; 104–112 m asl). The vegetation in this undulating terrain (~150 ha) was initially grassland and developed patches of scrub jungles for the last five decades. The natural scrub vegetation builds up gradually and the plantation of cashew (*Anacardium occidentale*) was established in the early 1970s. Subsequently, the forest department has developed mixed plantations (e.g. *Acacia*, *Anacardium*, *Azadirachta*, *Casuarina*, *Cocos* and *Pongamia*). Several native tree species build up subsequently with minimum human interference (e.g. *Borassus*, *Careya*, *Caryota*, *Holigarna*, *Hopea*, *Macaranga*, *Sapium*, *Syzygium*, *Tamarindus* and *Terminalia*).

The survey of asexual fungi associated with damp leaf litters of 10 tree species was carried out in the southwest monsoon period (July 08–15, 2019). Schematic presentation of the study carried out has been given in Figure 1. The tree species selected in scrub jungles for leaf litter collection were *Acacia auriculiformis*, *Anacardium occidentale*, *Artocarpus heterophyllus*, *Artocarpus hirsutus*, *Careya arborea*, *Eucalyptus tereticornis*, *Ficus benghalensis*, *Ficus religiosa*, *Tectona grandis* and *Terminalia paniculata*. Humidity (beneath the canopy), air temperature (in shade below the canopy) (Mextech Digital Thermo Hygrometer M288CTHW, Mextech Technologies India Private Limited, Mumbai, Maharashtra, India) and soil temperature (mercury thermometer) below the canopy (8–10 cm depth) were measured during each day of sampling. The

damp leaf litter underneath the trees were collected (from three replicate trees) (Fig. 2) and rinsed in tap water followed by distilled water to remove the debris. They were cut into 1.2 cm disks using a cork borer. The leaf discs were again rinsed in distilled water followed by incubation.



**Fig. 1** – The schematic presentation of methodology followed to study aquatic hyphomycetes in terrestrial damp leaf litters.



**Fig. 2** – Sample collection sites. a View of a scrub jungle of southwestern India. b Accumulation of leaf litter in the basin of banyan (*Ficus benghalensis*). c Wild jack (*Artocarpus hirsutus*).

### Damp chamber incubation

Four leaf discs from four individual leaf litter samples of each tree species served as one replicate. Those leaf discs were placed on a folded paper towel in 10 cm Petri dishes. A small quantity of sterile distilled water was added to wet the paper towel periodically. This allows the leaf discs to receive water similar to the natural habitat (without submersion). Likewise, three replicate plates were prepared per tree species and incubated at room temperature ( $25\pm 3$  °C) up to two

weeks. After two weeks, four leaf-discs in each Petri plate were transferred to conical flasks (100 ml capacity) containing 25 ml sterile distilled water and shaken for about 15 min at 100 rpm to release the conidia of fungi developed on the leaf discs. Water in flasks was filtered through Millipore filters (5 µm; 3 cm diam.). The filters were stained with few drops of aniline blue in lactophenol and preserved in the dark until observation. Each disc was mounted on a microscope slide by addition of a few drops of lactic acid and examined to measure the conidia of hyphomycetes using low-power and high-power microscope (Nikon YS100, Nikon Corporation, Tokyo, Japan). Identification was carried out based on monographs and individual papers (Ingold 1975, Nawawi 1985, Marvanová 1997, Santos-flores & Betancourt-Lopez 1997, Gulis et al. 2005, Zhao et al. 2007). The dry mass of leaf discs in each Petri plate was determined gravimetrically by drying at 80 °C for 24 hr in a hot-air oven.

### **Bubble chamber incubation**

The leaf discs prepared as described above in damp chamber incubation were transferred to conical flasks (250 ml) containing 150 ml sterile distilled water. The discs were aerated using aquarium aerators through Pasteur pipettes up to 48 hours to allow the mycelia present in the leaf discs to develop and release the conidia. After aeration, the aerated water was filtered through Millipore filters (5 µm; 4.7 cm diam.), stained with aniline blue and screened for the presence of conidia. The dry mass of leaf discs in each flask was determined gravimetrically by drying at 80 °C for 24 hr in a hot-air oven.

### **Data analysis**

The frequency of occurrence (FO) of each species in the damp chamber and bubble chamber incubations were calculated as follows:

$$FO (\%) = \left( \frac{T_s}{T_a} \right) \times 100$$

(where  $T_s$ , Total number of samples that possess a specific species;  $T_a$ , Total number of samples assessed).

The relative abundance (RA) of each species in the damp chamber and bubble chamber incubations was calculated as follows:

$$RA (\%) = \left( \frac{F_s}{F_a} \right) \times 100$$

(where  $F_s$ , Frequency of occurrence of each species;  $F_a$ , Sum of frequency of occurrence of all species).

The Simpson's diversity ( $D'$ ), Shannon's diversity ( $H'$ ) (Magurran 1988) and Pielou's equitability ( $J'$ ) (Pielou 1975) indices for aquatic hyphomycetes on leaf discs, on the damp chamber and bubble chamber incubations were calculated as given below:

$$D' = \left( \frac{1}{\sum (p_i)^2} \right) \times 100$$

$$H' = -\sum (p_i \ln p_i)$$

(where,  $p_i$  is the proportion of individual that species  $i$  contributes to the total).

$$J' = \left( \frac{H'}{H'_{max}} \right)$$

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

To compare the richness of fungal species among the incubation techniques, rarefaction indices were calculated (Ludwig & Reynolds 1988). The expected number of fungal species [ $E(s)$ ] in a random of  $n$  samples taken from a total  $N$  samples was estimated:

$$E(s) = \sum_{i=1}^s \left\{ 1 - \left[ \frac{\binom{N-n_i}{n}}{\binom{N}{n}} \right] \right\}$$

(where,  $n_i$  is the occurrence of  $i$ th species in number of samples).

Similarly, the expected number of fungal species [ $E(s)$ ] in a random sample of  $n$  conidia from a total of  $N$  conidia was estimated (where,  $n_i$  is the number of conidia of  $i$ th species in a total number of conidia scored).

## Results

### Temperature and humidity

July is the peak period of rainfall during the southwest monsoon in southwest India. During the survey period in July, the soil temperature ranged from 25.3 °C (*Artocarpus hirsutus*) to 28 °C (*Acacia auriculiformis* and *Ficus benghalensis*) (Table 1). Air temperature ranged from 24.7 °C (*A. hirsutus* and *Careya arborea*) to 30.7 °C (*Eucalyptus tereticornis*). Humidity ranged from 66% (*Eucalyptus tereticornis*) to 88.7% (*A. hirsutus*). On 15<sup>th</sup> of July 2019, the least soil temperature, least air temperature and the highest humidity were reported beneath the trees of *A. hirsutus*. Similarly, the highest air temperature (30.7 °C) and least humidity (66%) was seen beneath the canopy of *E. tereticornis*. The mean soil temperature, air temperature and relative humidity were 26.9 °C, 27.3 °C and 77.5%, respectively.

**Table 1** Temperature and humidity during leaf litter sampling in the scrub jungles (n=3, mean).

	Code	Sampling date	Temperature (°C)		Humidity (%)
			Soil	Air	
<i>Acacia auriculiformis</i> A. Cunn. ex Benth.	ACAU	July 08, 2019	28.0	29.7	72.3
<i>Anacardium occidentale</i> L.	ANOC	July 04, 2019	27.8	26.7	85.7
<i>Artocarpus heterophyllus</i> Lam.	ARHE	July 10, 2019	26.0	26.3	79.0
<i>Artocarpus hirsutus</i> Lam.	ARHI	July 15, 2019	25.3	24.7	88.7
<i>Careya arborea</i> Roxb.	CAAR	July 15, 2019	26.0	24.7	83.7
<i>Eucalyptus tereticornis</i> Sm.	EUTE	July 13, 2019	27.2	30.7	66.0
<i>Ficus benghalensis</i> L.	FIBE	July 04, 2019	28.0	27.1	80.7
<i>Ficus religiosa</i> L.	FIRE	July 13, 2019	27.7	28.3	69.3
<i>Tectona grandis</i> L.f.	TEGR	July 10, 2019	26.0	26.0	78.0
<i>Terminalia paniculata</i> Roth	TEPA	July 08, 2019	27.3	29.1	71.7
Average			26.9	27.3	77.5
Range		July 04– 15, 2019	25.3– 28	24.7– 30.7	66– 88.7

### Damp chamber incubation

Damp chamber incubation resulted in the occurrence of 21 species of hyphomycetes (Table 2, Figs 3, 4). *Flagellospora curvula* showed the highest frequency of occurrence (52.8%) and *Dwayaangam* sp. and triradiate conidial fungus showed the least (6.6% each). Nine species belonged to core- group with the frequency of occurrence above 25% (26.4–52.8%). The leaf discs of *F. benghalensis* showed the highest mean number of hyphomycetes (8 spp.) followed by leaf

discs of *E. tereticornis* (6 spp.) and *A. heterophyllus* (5.7 spp.), while the leaf discs of *T. paniculata* showed the least (4 spp.) (Fig. 5a). The mean conidial output was the highest in *F. religiosa* and *T. grandis* (857 conidia/g) followed by *A. auriculiformis* (803 conidia/g), *E. tereticornis* (620 conidia/g) and *F. benghalensis* (610 conidia/g), while it was least in *A. occidentale* (143 conidia/g) (Fig. 5b). The observed species richness in damp incubation was 21 species, while the expected number of species [ $E_{(s110)}$ ] based on rarefaction was 30 species at 110 random samples (Table 3, Fig. 6a). Simpson's and Shannon's diversities were 0.942 and 4.232, respectively, and the Pielou's equitability was 0.963. The expected number of species [ $E_{(s500)}$ ] attained the highest (30 spp.) at 500 random counts of conidia (Fig. 7a).

**Table 2** Frequency of occurrence (FO) and relative abundance (RA) of aquatic hyphomycetes in leaf litter by damp chamber incubation (\*, Exclusive species; \*\*, Occurred also in bubble chamber incubation).

	FO (%)	RA (%)
** <i>Flagellospora curvula</i> Ingold (Fig. 3d)	52.8	10.0
* <i>Tumularia aquatica</i> (Ingold) Descals & Marvanová (Fig. 4h)	46.2	8.8
** <i>Lunulospora curvula</i> Ingold (Fig. 3j)	42.9	8.2
** <i>Lateriramulosa uni-inflata</i> Matsush. (Fig. 3i)	36.3	6.9
* <i>Speiropsis pedatospora</i> Tubaki (Fig. 4a)	29.7	5.6
** <i>Wiesneriomyces laurinus</i> (Tassi) P.M. Kirk (Fig. 4i)	29.7	5.6
* <i>Anguillospora crassa</i> Ingold	26.4	5.0
* <i>Helicosporium</i> sp. 1 (Fig. 3e)	26.4	5.0
** <i>Triscelophorus konajensis</i> K.R. Sridhar & Kaver. (Fig. 4f)	26.4	5.0
* <i>Helicosporium</i> sp. 2 (Fig. 3f)	23.1	4.4
* <i>Helicosporium</i> sp. 3	23.1	4.4
* <i>Tripospermum camelopardus</i> Ingold, Dann & P.J. McDougall (Fig. 4b)	23.1	4.4
** <i>Triscelophorus monosporus</i> Ingold (Fig. 4g)	23.1	4.4
** <i>Phalangispora constricta</i> Nawawi & J. Webster (Fig. 3k)	19.8	3.8
** <i>Triscelophorus acuminatus</i> Nawawi (Fig. 4e)	19.8	3.8
** <i>Anguillospora longissima</i> (Sacc. & P. Syd.) Ingold	16.5	3.1
** <i>Flabellospora verticillata</i> Alas. (Fig. 3c)	16.5	3.1
* <i>Flagellospora penicillioides</i> Ingold	13.2	2.5
* <i>Anguillospora angulata</i> (R.H. Petersen) Wolfe	13.2	2.5
* <i>Dwayaangam</i> sp. (Fig. 3b)	6.6	1.3
*Unknown (triradiate conidia) (Fig. 4d)	6.6	1.3

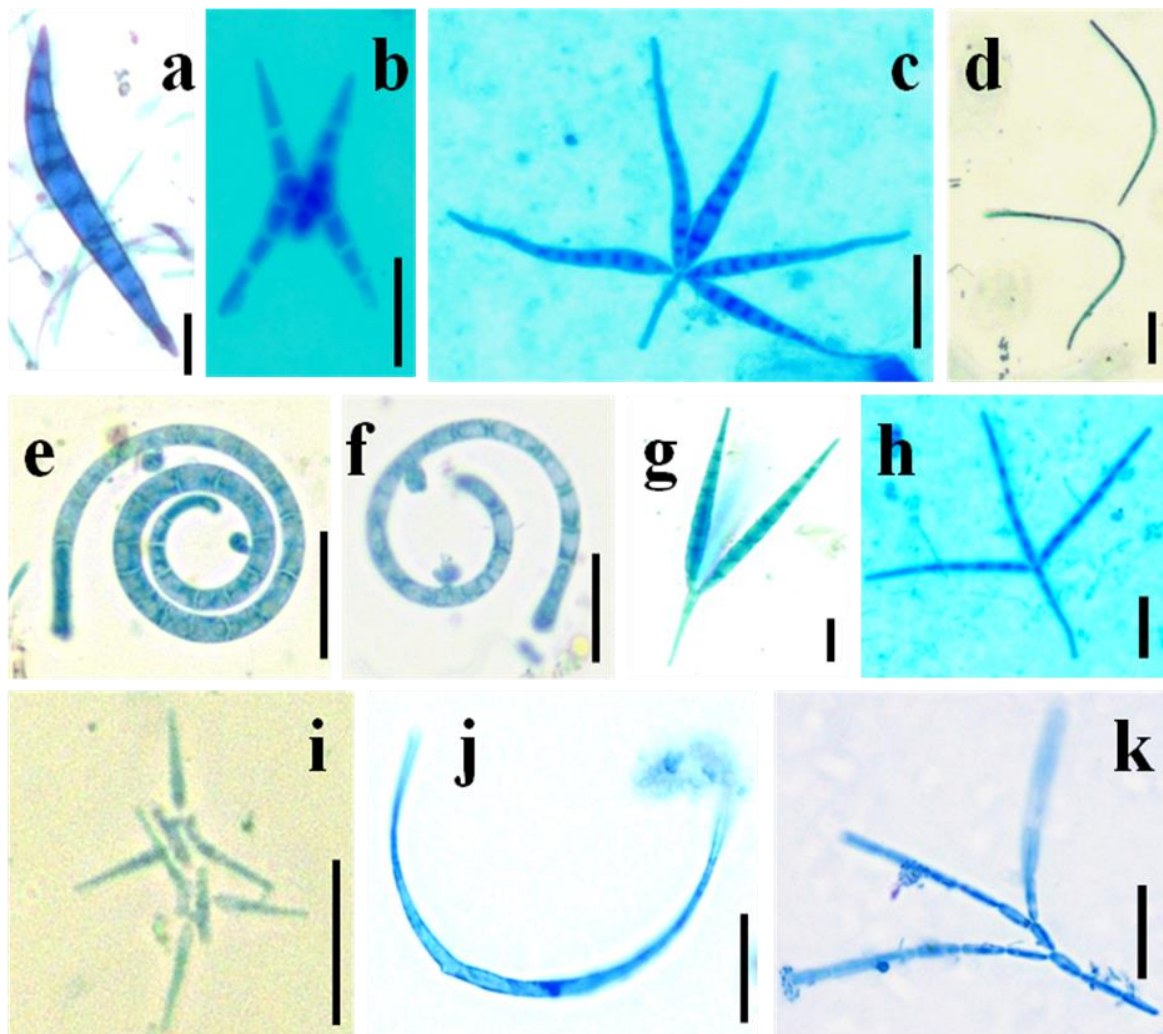
### Bubble chamber incubation

The bubble chamber incubation of leaf discs resulted in the occurrence of 20 species of aquatic hyphomycetes (Table 4, Figs 3, 4). *Triscelophorus acuminatus* showed the highest frequency of occurrence (83.3%) and *Lateriramulosa uni-inflata* and *Tripospermum myrti* showed the least (3.3% each). Nine species were core-group species with the frequency of occurrence above 25% (26.7–83.3%). The leaf discs of *A. hirsutus* showed the highest mean number of aquatic hyphomycetes (8.3 spp.) followed by *F. benghalensis* (8 spp.), *C. arborea* (7.3 spp.) and *A. auriculiformis* (7 spp.), while it was least in *F. religiosa* (4 spp.) (Fig. 5a). The mean conidial output was highest in the discs of *F. benghalensis* (987 conidia/g) followed by *A. occidentale* (627 conidia/g) and *T. grandis* (543 conidia/g), while it was least in *A. heterophyllus* (107 conidia/g) (Fig. 5b). The observed species richness in damp incubation was 20 species, while the expected number of species [ $E_{(s110)}$ ] based on rarefaction was 30 species at 110 random samples (Table 2,

Fig. 6b). The Simpson's and Shannon's diversities were 0.672 and 2.686, respectively, and the Pielou's equitability was 0.622. The expected number of conidia species attained maximum at 110 samples (30 spp.) and saturated at 180 samples. The expected number of species [ $E_{(s,500)}$ ] attained the highest (30 spp.) at 500 random counts of conidia (Fig. 7b).

**Table 3** Species richness, diversity and evenness of aquatic hyphomycetes in two methods of incubation.

	Damp chamber incubation	Bubble chamber incubation
Observed species richness among 30 samples	21	20
Expected number of species in samples [ $E_{S(s,110)}$ ]	30	30
Expected number of species in conidia [ $E_{S(s,500)}$ ]	30	30
Simpson's diversity	0.942	0.672
Shannon's diversity	4.232	2.686
Pielou's equitability	0.963	0.622

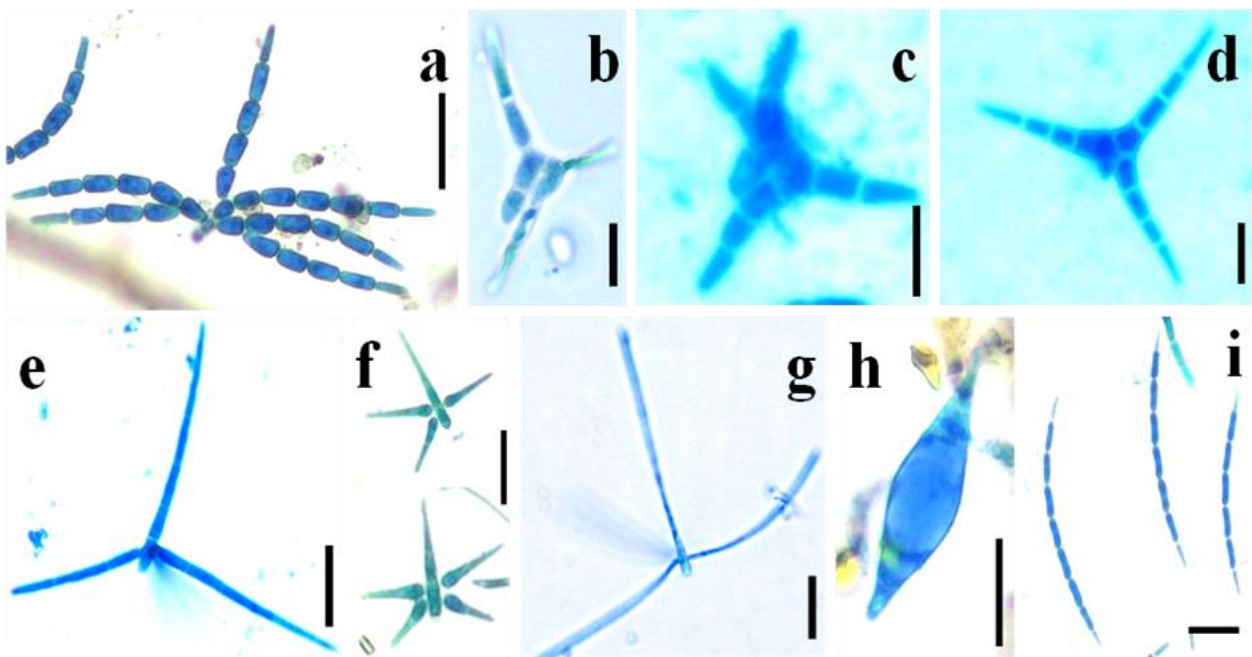


**Fig. 3** – Conidia of: a *Anguillospora* sp. b *Dwayaangam* sp. c *Flabellospora verticillata*. d *Flagellospora curvula*. e *Helicosporium* sp. 1. f *Helicosporium* sp. 2. g *Isthmotricladia gombakiensis*. h *Isthmotricladia laeensis*. i *Lateriramulosa uni-inflata*. j *Lunulospora curvula*. k *Phalangispora constricta*. Scale bar: 20  $\mu$ m.

### Comparison of incubation methods

A total of 31 species of aquatic hyphomycetes were recorded using two incubation methods (Tables 3, 4). The total number of species (21 vs 20 spp.) and the exclusive species (11 vs 10 spp.) were almost similar in both methods of incubation. However, species composition and the conidial output differs among the methods of incubation (Figs 5a, b). The species and conidial richness were high in leaf litters of *A. auriculiformis*, *F. benghalensis* and *T. grandis* in both methods. Even though the species richness was higher in leaf litters of *A. hirsutus*, *C. arborea* and *T. paniculata* was higher in both methods, the conidial output was very low. In spite of high species richness in both methods of incubation of leaf litters of *A. hirsutus*, *E. tereticornis* and *F. religiosa*, the conidial richness was higher only in damp incubation, while it was opposite for the *A. occidentale*.

The observed species richness was almost the same in both incubation methods (20–21 spp.). The Simpson's diversity, Shannon's diversity and Pielou's equitability were higher in damp chamber incubation than bubble chamber incubation (Table 2). The rarefaction curves for species richness based on the samples as well as conidia were almost similar in both methods (Figs 6, 7).



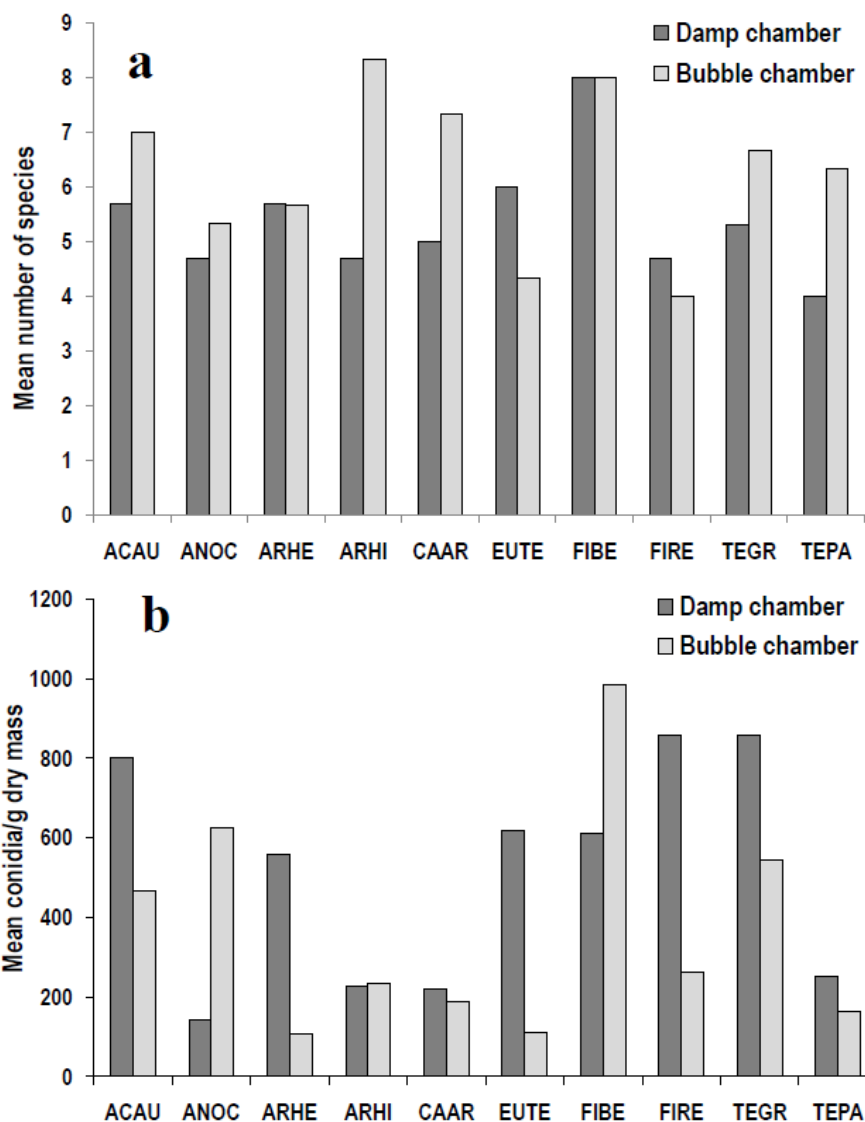
**Fig. 4** – Conidia of: a *Speiropsis pedatospora*. b *Tripospermum camelopardus*. c *Tripospermum myrti*. d unknown triradiate conidia. e *Triscelophorus acuminatus*. f *Triscelophorus konajensis*. g *Triscelophorus monosporus*. h *Tumularia aquatica*. i *Wiesneriomyces laurinus*. Scale bar: 20  $\mu$ m.

**Table 4** Frequency of occurrence (FO) and relative abundance (RA) of aquatic hyphomycetes in leaf litter by bubble chamber incubation (\* Exclusive species; \*\* Occurred also in damp chamber incubation).

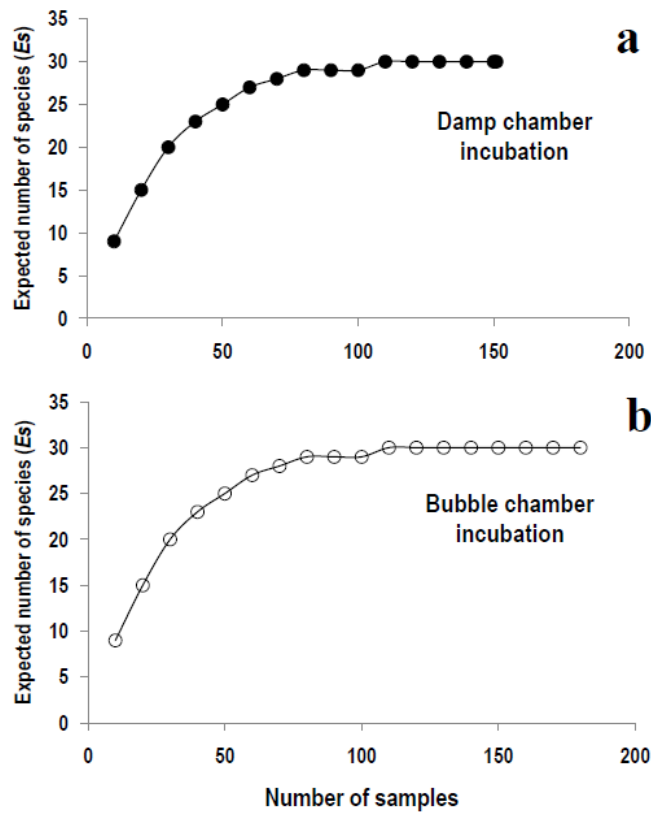
	FO (%)	RA (%)
** <i>Triscelophorus acuminatus</i> Nawawi (Fig. 4e)	83.3	14.0
** <i>Triscelophorus konajensis</i> K.R. Sridhar & Kaver. (Fig. 4f)	76.7	12.9
** <i>Flagellospora curvula</i> Ingold (Fig. 3d)	70.0	11.7
** <i>Triscelophorus monosporus</i> Ingold (Fig. 4g)	60.0	10.1
** <i>Lunulospora curvula</i> Ingold (Fig. 3j)	46.7	7.8
*Unknown (sigmoid conidia)	40.0	6.7
** <i>Phalangispora constricta</i> Nawawi & J. Webster (Fig. 3k)	30.0	5.0
** <i>Flabellospora verticillata</i> Alas. (Fig. 3c)	30.0	5.0



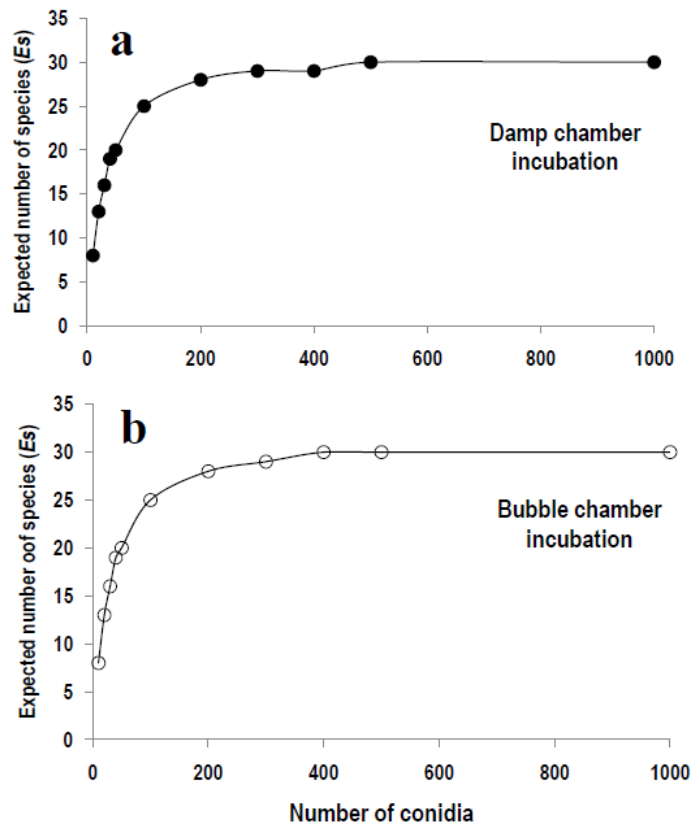
	FO (%)	RA (%)
* <i>Isthmotricladia laeensis</i> Matsush. (Fig. 3h)	26.7	4.5
* <i>Tricladium</i> sp.	20.0	3.4
* <i>Alatospora acuminata</i> Ingold	16.7	2.8
* <i>Ingoldiella hamata</i> D.E. Shaw	16.7	2.8
* <i>Anguillospora</i> sp. (Fig. 3a)	16.7	2.8
** <i>Wiesneriomyces laurinus</i> (Tassi) P.M. Kirk (Fig. 4i)	16.7	2.8
* <i>Isthmotricladia gombakiensis</i> Nawawi (Fig. 3g)	13.3	2.2
** <i>Anguillospora longissima</i> (Sacc. & P. Syd.) Ingold	10.0	1.7
* <i>Campylospora chaetocladia</i> Ranzoni	10.0	1.7
* <i>Campylospora parvula</i> Kuzuha	6.7	1.1
** <i>Lateriramulosa uni-inflata</i> Matsush. (Fig. 3i)	3.3	0.6
* <i>Tripospermum myrti</i> (Lind) S. Hughes (Fig. 4c)	3.3	0.6



**Fig. 5** – Mean number of species and mean conidia produced by the aquatic hyphomycetes in damp and bubble chamber incubations of 10 leaf litters (n=3, mean) (Tree species: ACAU, *Acacia auriculiformis*; ANOC, *Anacardium occidentale*; ARHE, *Artocarpus heterophyllus*; ARHI, *Artocarpus hirsutus*; CAAR, *Careya arborea*; EUTE, *Eucalyptus tereticornis*; FIBE, *Ficus benghalensis*; FIRE, *Ficus religiosa*; TEGR, *Tectona grandis*; TEPA, *Terminalia paniculata*).



**Fig. 6** – Rarefaction curves of expected number of species [ $E_{(s,110)}$ ] of aquatic hyphomycetes among 110 random samples by damp chamber and bubble chamber incubations.



**Fig. 7** – Rarefaction curves of expected number of species [ $E_{(s,500)}$ ] of aquatic hyphomycetes among 500 random conidia by damp chamber and bubble chamber incubations.

## Discussion

### Damp chamber incubation

The damp chamber incubation is one of the common methods to assess the fungal colonization on plant materials. The conidia present on the surface of damp leaves were removed by rinsing in distilled water. Damp incubation method facilitates the constant supply of water sufficient for growth and sporulation of colonized hyphomycetes. Among the 11 exclusive species, four species were typical aquatic hyphomycetes (*Anguillospora angulata*, *A. crassa*, *Flagellospora penicillioides* and *Tumularia aquatica*), while the rest were aero-aquatic hyphomycetes (three *Helicosporium* spp., *Speiropsis pedatospora*, *Triposermum camelopardus*, *Dwayaangam* sp. and unknown triradiate conidia). Unlike bubble chamber incubation, the damp chamber incubation showed uniformity in the frequency of occurrence as well as their percent conidial contribution.

### Bubble chamber incubation

The bubble chamber incubation is one of the common methods followed to induce sporulation of aquatic hyphomycetes on submerged leaf litter in streams. The fine jet of air bubbles leads to mechanical stimulation and supply oxygen for sporulation for the colonized fungi (Iqbal & Webster 1973). Usually, 48 hours of aeration is sufficient to induce conidia of the colonized fungi on the substrate. Among the 10 exclusive species, many were typical aquatic hyphomycetes (*Alatospora acuminata*, *Anguillospora longissima*, *Anguillospora* sp., *Campylospora chaetocladia*, *C. parvula*, *Ingoldiella hamata*, *Isthmotricladia gombakiensis*, *I. laeensis* and *Tricladium* sp.), while the *Tripospermum myrti* and unknown sigmoid conidial fungus seem to be aero-aquatic hyphomycetes.

### Other studies

Similar to the present study, many surveys were carried out to screen the aquatic and aero-aquatic hyphomycetes present in several tree species (canopy leaf litter, stemflow and throughfall) (Sridhar et al. 2006, Karamchand & Sridhar 2008, 2009, Sudheep & Sridhar 2010, Ghate & Sridhar 2015a, 2016). As many aquatic and aero-aquatic hyphomycetes occur in the canopy, they will be transferred to the leaf litter beneath the canopy as the inocula. However, the occurrence of hyphomycetes as endophytes in the canopy substrates (e.g. live leaves, twigs and bark) cannot be ruled out.

Among the various leaf litters present in the streams, banyan leaf litter (*Ficus benghalensis*) served as an ideal substrate for the colonization of aquatic hyphomycetes (Sridhar & Kaveriappa 1989). In the present study also, the leaf litter of *F. benghalensis* served as a superior substrate for fungi. In addition, leaf litter of *A. auriculiformis* and *T. grandis* were also worth considering as good substrates as they supported more species and high conidial output. Probably, the texture, nutrient composition of the above leaf litter and physicochemical conditions of scrub jungles prevailing in southwestern India seem to be ideal for the colonization, survival and perpetuation of aquatic hyphomycetes.

### Conidial output

According to earlier reports, aquatic hyphomycetes under optimum conditions (e.g. substrate quality and physicochemical features) produce up to 8 million conidia per gram dry mass of leaf litter per day (Gessner 1997). In the present study, up to 1850 conidia were produced in two days per gram of dry mass from the preferred leaf litter of *Ficus benghalensis*. It corresponds to about 0.012% compared to the leaf litter which exists in the streams. This is obvious because the natural damp conditions underneath the canopy are not equivalent to stream habitats for aquatic hyphomycetes. However, the scrub jungles of the southwest of India provide almost continuous dampness for a long period during southwest monsoon (June–November). Interestingly, the aquatic hyphomycetes in naturally dried leaf litter present in the tree holes also produced conidia during the summer in the Western Ghats and west coast of India (602–2841 and 552–684 conidia/g dry mass,

respectively) (Sridhar et al. 2006, Karamchand & Sridhar 2008, Sudheep & Sridhar 2010). Similarly, aquatic hyphomycetes were found in dried bracket leaves of the epiphytic fern (*Drynaria quersifolia* (L.) J. Sm.) during summer (Karamchand & Sridhar 2009).

### **Niche duality**

Niche duality of aquatic fungi is one of the interesting concepts in fungal ecology (Colwell and Rangel 2009, Sélosse et al. 2018). Although aquatic fungi mainly adapted to flowing waters, their occurrence or distribution outside their usual habitat (e.g. terrestrial damp litter and tree holes) is helpful to replenish their conidia or propagules to the streams having unidirectional flow (Sridhar et al. 2013, Chauvet et al. 2016). The route of transport from terrestrial to aquatic or vice versa will be interesting. It may happen through wind, insects, foam and input of live or dead aerial parts of riparian tree species colonized by endophytic/saprophytic hyphomycetes. The nearest stream to the investigation region of this study (Konaje stream), which showed richness ranging from 10–22 species by following any one the methods of assessment (Sridhar & Kaveriappa 1984, 1989, Sridhar et al. 2013, Ghate & Sridhar 2015b). In the present study, the richness ranged from 20–21 species in both methods of incubation. However, the species composition and the conidial output varied between the methods, probably due to the substrates and environmental factors. The role of aquatic hyphomycetes in terrestrial habitats in catering to the nutritional needs of terrestrial or semi-terrestrial fauna needs further study. The role of these fungi in carbon cycling in regions receiving heavy rains will be an interesting topic to investigate.

### **Conclusions**

Based on the two methods of incubation, 31 species of aquatic fungi were recorded. Among them, 10 species were common to both incubation methods; 11 and 10 species were exclusive to damp and bubble chamber incubation methods, respectively. Hence, both methods of incubation will be necessary to get a better picture of colonized mycota in damp leaf litter. According to the present study, based on species richness and conidial output, leaf litter of *Acacia auriculiformis*, *Ficus benghalensis* and *Tectona grandis* are the ideal substrates for colonization of aquatic hyphomycetes. Among the 10 core-group hyphomycetes found in both methods, *Flagellospora curvula*, *Lunulospora curvula* and *Triscelophorus konajensis* were common. Hence, they deserve special attention and need to assess their role in damp leaf litter. Although the extent of aquatic fungi in terrestrial habitats is not up to the extent of flowing waters, the roles played by them in terrestrial habitats is one of the important issues in fungal ecology. Further studies are necessary to ascertain the presence of loose conidia on the leaf litter during the summer season, their viability and recolonization of leaf litter on the onset of monsoon.

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