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Marine fungal diversity on the monocot woody litter in the intertidal region of southwest India

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ABSTRACT

Three hundred monocot woody litter collected from the three beaches of southwest of India were incubated in the laboratory in air-tight containers on sterile sand bed soaked with autoclaved seawater. On screening periodically up to 24 months, 31 sporulating species consisting of 15 ascomycetes, two basidiomycetes and 14 mitosporic fungi were recorded. Arenicolous ascomycetes were dominated similar to earlier observations on woody litter in the beaches of west coast of India. One of the arenicolous ascomycetes differed from the known species (*Arenariomyces* sp.), which is the fifth dominant species found in this survey. Long-term incubation of woody litter will reveal clear picture about the marine fungal composition. So-called terrestrial fungi were also found on the monocot woody litter (e.g. *Aspergillus flavus, A. fumigatus, A. niger* and *Fusarium oxysporum*). Some of them were also reported from the maritime habitats like salterns and the Dead Sea. Marine fungal composition on woody litter is may depends on the type of woody litter and survey of specific woody litter in maritime habitats reveals more precise information on the fungal dynamics.

Key words: Arenicolous fungi, beaches, driftwood, filamentous fungi, incubation period, species richness, substrates

1. INTRODUCTION

Marine fungi constitute are important biota in the oceans owing to their capability to degrade the organic matter and nutrient turnover (Hyde, 2002; Jones and Pang, 2012). They occupied several niches in marine habitats (e.g. intertidal, mangrove, saltmarsh, deep sea, coral reefs and islands) and colonize varied substrates (e.g. seaweeds, intertidal wood, floating wood, marine wooden structures, seagrass, calcareous substrates and sponges) (Raghukumar, 2012, 2017; Sabeena et al., 2018). A variety of woody substances are available for colonization by the marine fungi (e.g. drift wood, intertidal wood, bark, roots and seeds). Being sturdy, such woody debris are long

lasting substrates in marine habitats and colonization of such durable substrates is necessary for the filamentous fungi for their perpetuation. Either floating woody debris accumulated on the beaches or those woody debris firmly fixed in sand or boulders in intertidal habitats on incubation provide many marine filamentous fungi (Kohlmeyer and Kohlmeyer, 1979; Jones and Pang, 2012). If the woody debris or calcareous materials dried on the beaches for long duration, needs longer periods of incubation for development and maturity of fungal fruit bodies (Prasannarai and Sridhar, 1997; Ananda et al., 1998). Investigations on marine fungi in India have been reviewed recently by Borse and Sarma (2021). Several studies have focused on the marine fungal occurrence and diversity on the coastal regions of Indian subcontinent (e.g. Raghukumar, 1973; Chinnaraj, 1993; Borse, 1998; Prasannarai and Sridhar, 2001a; Raghukumar, 2017; Borse and Sarma, 2021). Majority of the studies in India focused on many woody debris accumulated on the beaches (Prasannarai and Sridhar, 2001a; Borse et al., 2005; Raveendran and Manimohan, 2007; Khan and Manimohan, 2011; Borse et al., 2012). Southwest coast of India embodies wild and cultivated woody monocots in different regions (e.g. *Areca catechu*, *Bambusa* spp., *Borassus flabellifer, Caryota urens, Cocos nucifera, Dendrocalamus* spp., *Elaeis* spp. and *Ochlandra travancorica*). Substantial amount of monocot woody debris are added to the ocean through the estuaries, mangroves, islands and beaches. This study intends to assess the species richness and diversity of marine filamentous fungi colonized on the monocot woody litter collected from the three beaches of southwest India.

2. MATERIALS AND METHODS

Monocot woody litter fixed in sand or boulders of mid and upper intertidal region of three beaches of Karnataka (southwest coast of India) were collected during the summer season (March to May, 2019). The beaches chosen were Someshwara (sandy, rocky and boulder stretch) (12°47'N, 74°50'E), Tannirbhavi (sandy stretch) (12°53'N, 74°48'E) and Surathkal (sandy stretch) (12°59', 74°47'). One hundred monocot woody litter from each beach locations at a stretch of 3 km were collected brought to the laboratory (e.g. pieces of frond, roots, bark, inflorescence and culms) and rinsed in sterile seawater to eliminate the dirt and sand particles (Figure 1). They were cut into desired length (~10 cm) (remaining small segments were discorded) labelled and horizontally incubated individually in air-tight containers on sand bed soaked with sterile seawater at laboratory temperature (25–31°C). Incubation was continued up to 24 months with screening once in two months and re-incubated. Each wood was scanned for fungal structures using a stereomicroscope (Nikon, Model # ECLIPSE Ts2-FL 244472, Nikon Corporation, Tokyo, Japan). Mounts were prepared using lactophenol cotton blue (1%) for identification using monographs (e.g. Kohlmeyer, 1984; Ellis and Ellis, 1985; Kohlmeyer and Volkmann-Kholmeyer, 1991; Hyde and Sarma, 2000; Sarma and Hyde, 2000; Nagamani et al., 2006; Raveendran and Manimohan, 2007; Jones et al., 2009). Selected sporocarps and spores were photographed using a high power microscope (Nikon, Model # ECLIPSE Ni-U 941966, Nikon Corporation, Tokyo, Japan). Percent frequency of occurrence [(number of samples possess a specific fungus/total number of samples showed sporulating fungi) × 100] and percent relative abundance [(frequency of occurrence of a specific fungus/total frequency of occurrence) × 100] were calculated for fungal species found on woody litter. The diversity (Simpson and Shannon) and equitability (Pielou's) were calculated for fungi seen in each sampling location (Pielou 1975, Magurran 1988).

3. RESULTS AND DISCUSSION

This study documented a total of 31 species of fungi on monocot woody litter collected from the three beaches with highest number of fungi in Someshwara (24 spp.) followed by Tannirbhavi (21 spp.) and Surathkal (19 spp.) (Table 1). All the samples collected consist of sporulating fungi on incubation. The mean number species per woody litter was highest in Someshwara (1.8 sp.) followed by Tannirbhavi (1.2 sp.) and Surathkal (0.9 sp.). The total ascomycetes was highest in Someshwara (13 spp.) followed by Surathkal (10 spp.) and Tannirbhavi (8 spp.). The mitosporic fungi were highest in Tannirbhavi (13 spp.) followed by Someshwara (10 spp.) and Surathkal (9 spp.). Only two basidiomycetes were found in the samples of Someshwara. Among them, the spores of *Nia vibrissa* was found in the throughfall of trees of *Avicennia officinalis* in Nethravathi mangroves (Karamchand et al., 2021). Earlier one time study on the woody litter in the beaches of four islands of the Karwar (west coast) possess similar number of species (21-32 vs. 19-24 spp.) (Prasannarai and Sridhar, 2001b). The same trend was seen in another island adjacent to the Udupi (west coast) (Prasannarai and Sridhar, 1997). However, the number of ascomycetes were higher, while the mitosporic fungi were lower than the present study. The mean number of fungi per wood (0.9-1.8) also matches with other studies on the west coast locations (Prasannarai and Sridhar, 2001a, 2001b, 2003a, 2003b).

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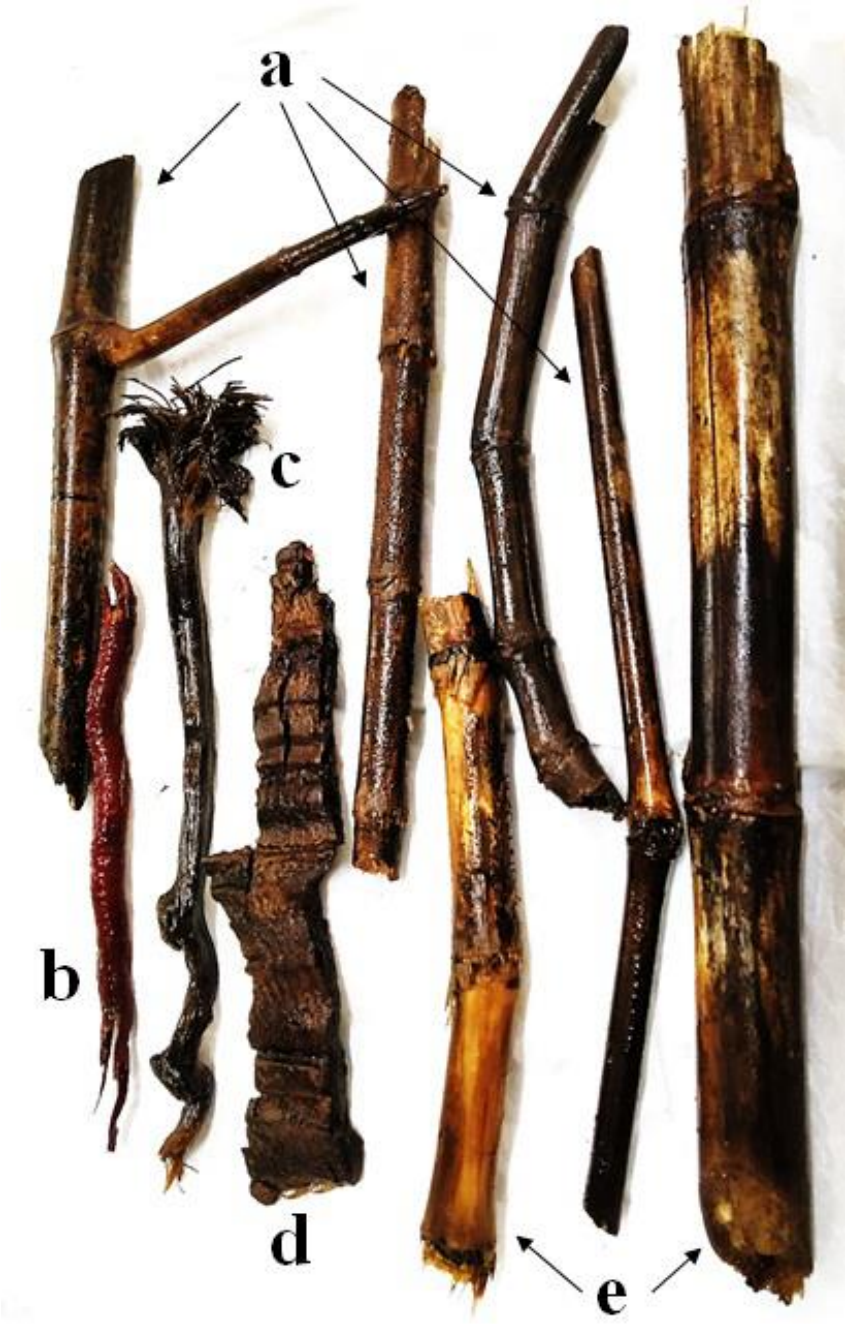

Figure 1. Representatives of monocot woody litter collected from the beaches of southwest India: bamboo wood pieces (a); root of coconut palm (b); inflorescence branch of a palm (c); bark of a palm (d); maize-like woody litter (e).

Table 1. Frequency of occurrence (FO) and relative abundance (RA) of marine fungi colonized on the monocot woody litter in three beaches of southwest India (B1, Someshwara; B2, Tannirbhavi; B3, Surathkal; TFO, total frequency of occurrence; *, exclusive species).

	FO (%)			TFO	RA
	B1	B2	B3	(%)	(%)
Ascomycetes					
Corollospora pulchella Kohlm., I. Schmidt & N.B. Nair	89.9	72.5	55.1	72.3	8.4
Lindra sp.	55.1	66.7	69.6	63.8	7.9
Corollospora quinqeseptata Nakagiri	66.7	40.6	49.3	52.2	7.1
Caryosporella rhizophorae Kohlm.	55.1	37.7	43.5	45.3	5.3

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Arenariomyces sp.	26.1	31.3	49.3	35.6	4.2
Lindra crassa (Kohlm.) Kohlm. & Volkm.-Kohlm.	49.3	-	43.5	30.9	3.5
Phaeosphaeria spartinicola Leuchtm.	40.6	26.1	23.2	30.0	3.4
Leptosphaeria orae-maris Linder	43.5	31.9	-	25.1	2.9
Remispora maritima Linder	49.3	-	20.3	23.2	2.6
Corollospora indica Prasannarai, Ananda & K.R. Sridhar	20.3	26.1	-	15.5	1.8
**Savoryella paucispora* (Cribb & J.W. Cribb) J. Koch	31.9	-	-	10.6	1.2
**Corollospora intermedia* I. Schmidt	26.1	-	-	9.0	1.0
**Arenariomyces parvulus* Jørg. Koch	20.3	-	-	6.8	0.8
**Halotthia posidoniae* (Durieu & Mont.)	-	-	20.3	6.8	0.8
**Halosphaeria maritima* (Linder) Kohlm.	-	-	17.4	5.8	0.7
Basidiomycetes					
**Nia vibrissa* R.T. Moore & Meyers	11.6	-	-	3.9	0.4
**Halocyphina villosa* Kohlm. & E. Kohlm.	8.7	-	-	2.9	0.3
Mitosporic fungi					
Doratomyces microsporus (Sacc.) F.J. Morton & Sm.	78.3	55.1	66.7	66.7	7.6
Cirrenalia macrocephala (Kohlm.) Meyers & R.T. Moore	72.5	66.7	55.1	64.8	7.5
Zalerion maritima (Linder) Anastasiou	49.3	43.5	43.5	45.4	5.2
Drechslera poae (Mart.) Sacc.	-	66.7	46.4	37.7	4.4
Fusarium sp.	34.8	29.0	40.6	34.8	4.0
Aspergillus niger Tiegh.	37.7	31.9	26.1	31.9	3.6
Aspergillus fumigatus Fresen.	31.9	26.1	29.0	29.0	3.3
Aspergillus oryzae (Ahib.) Cohn	-	37.7	46.4	28.0	3.2
Fusarium oxysporum Schltdl.	43.5	31.9	-	25.1	2.9
**Aspergillus flavus* Link	-	49.3	-	16.4	1.9
Zalerion varia Anastasiou	14.5	20.3	-	11.6	1.3
**Doratomyces* sp.	-	23.2	8.7	10.6	1.2
**Cumulospora marina* I. Schmidt	23.2	-	-	7.7	1.0
**Cirrenalia fusca*	-	14.5	-	4.8	0.6
Total species	24	21	19	31	
Mean species per wood	1.8	1.2	0.9	1.4	
Total ascomycetes	13	8	10	15	
Total basidiomycetes	2	-	-	2	
Total mitosporic fungi	10	13	9	14	

Considering all the beaches, the most dominant ascomycetes (≥50%) were *Corollospora pulchella* (72.3%) followed by *Lindra* sp. (63.8%) and *Corollospora quinqeseptata* (52.2%). Individual beaches showed a slight difference in species dominance. In Someshwara *Caryosporella rhizophorae, Corollospora quinqeseptata, C. pulchella* and *Lindra* sp. were the top four species. In Tannirbhavi, the top two species include *C. pulchella* and *Linra* sp. In Surathkal the *Lindra* sp. was most dominant followed by *C. pulchella*. This study corroborates with the earlier studies that the arenicolous fungi dominates on the woody litter in the beaches of southwest coast of India (Prasannarai and Sridhar, 2001a, 2001b, 2003a, 2003b). Among the mitosporic fungi, the most dominant species (≥50%) include *Doratomyces microspores* (66.7%) and *Cirrenalia macrocephala* (64.8%). The same dominance was seen in Someshwara and Surathkal, while in Tannirbhavi, *C. macrocephala* showed dominance followed by *D. microsporus* and *Drechslera poae*. However, such dominant mitosporic fungi were not seen in the earlier studies on beach woody litter (Prasannarai and Sridhar, 2001a, 2001b, 2003a, 2003b).

Table 2 summarizes the species richness, exclusive species, diversity and equitability. More number of species were confined to only Someshwara beach, while two species each were confined to Tannirbhavi and Surathkal. The Simpson's as well as Shannon's diversities were higher, while the Pielou's equitability was lower in Someshwara compared to Tannirbhavi and Surathkal. The diversity and equitability of marine fungi in three beaches almost corroborates with the earlier studies on the woody litter in the west coast (Prasannarai and Sridhar, 2003a, 2003b).

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Table 2. Species richness, diversity and equitability of marine fungi in three beaches of southwest India

	Beaches			All beaches
	Someshwara	Tannirbhavi	Surathkal	
Species richness	24	21	19	31
Exclusive species	6	2	2	-
Simpson's diversity	0.947	0.944	0.938	0.950
Shannon's diversity	3.045	2.959	2.850	3.163
Pielou's equitability	0.876	0.918	0.910	0.763

Three ascomycetes (Are*nariomyces parvulus, Corollospora intermedia* and *Savoryella paucispora*), two basidiomycetes (*Halocyphila villosa* and *Nia vibrissa*) and one hyphomycete (*Cumulospora marina*) were restricted to the Someshwara beach. Two mitosporic fungi were found only in Tannirbhavi (*Aspergillus flavus* and *Cirrenalia fusca*), while two ascomycetes (*Halosphaeria maritima* and *Halotthia posidoniae*) were confined to Surathkal. The arenicolous fungi usually grow on sand grains in the intertidal zones, they were dominated especially on the bamboo stem inside the hollow core. *Corollospora indica* was recorded on the calcareous substrates accumulated on the beaches of the southwest coast (Mangalore and Goa) (e.g. shells, cuttlefish bone, crab exoskeleton and feathers) (Prasannarai et al., 2000). It was also found on the monocot woody litter of Someshwara as well as Tannirbhavi in the our study over 20% frequency of occurrence (20.3-26.1%) (Figure 2). Another arenicolous fungus *C. quinqueseptata* was the second dominant ascomycete in Someshwara (66.7%) and found in other two locations (66.7-69.6%) (Figure 3). It was recorded from the woody litter in Anjadweep Island near Karwar and the mainland beaches (Kaup and Someshwara) (Prasannarai and Sridhar, 2001b, 2003b). Besides calcareous substrates, the arenicolous fungi were also found on the leaf as well as woody litter in two mangroves of the southwest coast (Nethravathi and Udyavara) (Ananda et al., 1998; Ananda and Sridhar, 2004). Dominance of arenicolous fungi in our study possibly due to undisturbed conditions in the hollow core in some monocot woody litter (e.g. bamboo stem).

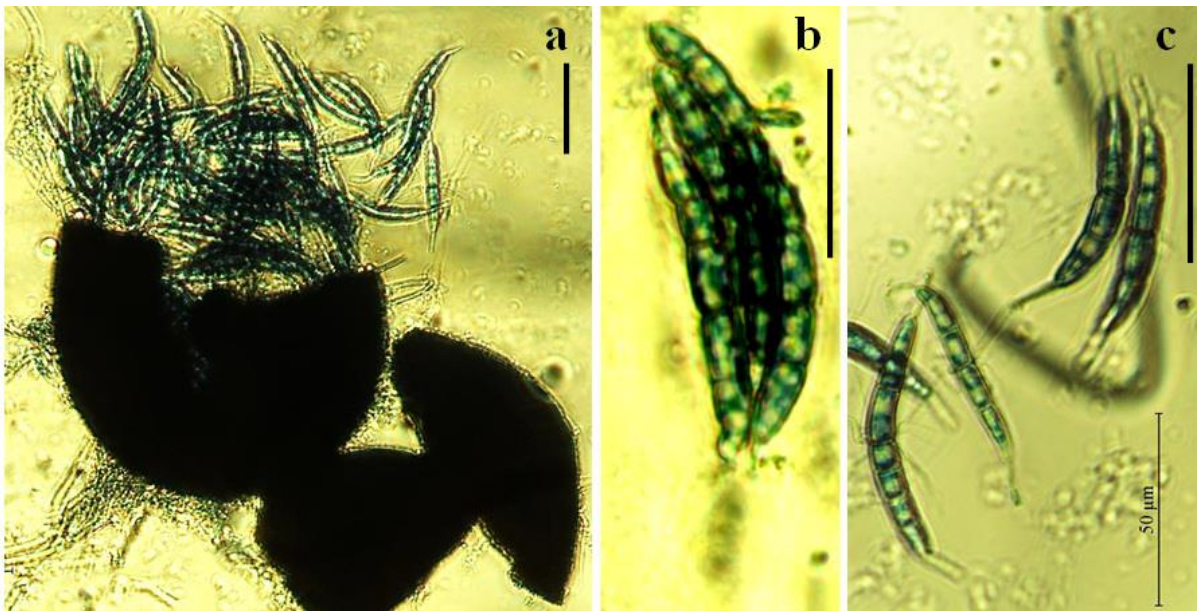

Figure 2. *Corollospora indica*: crushed carbonaceous ascocarp (a); ascus (b); multiseptate ascospores with median and terminal appendages (c) (Scale bar, 50 µm).

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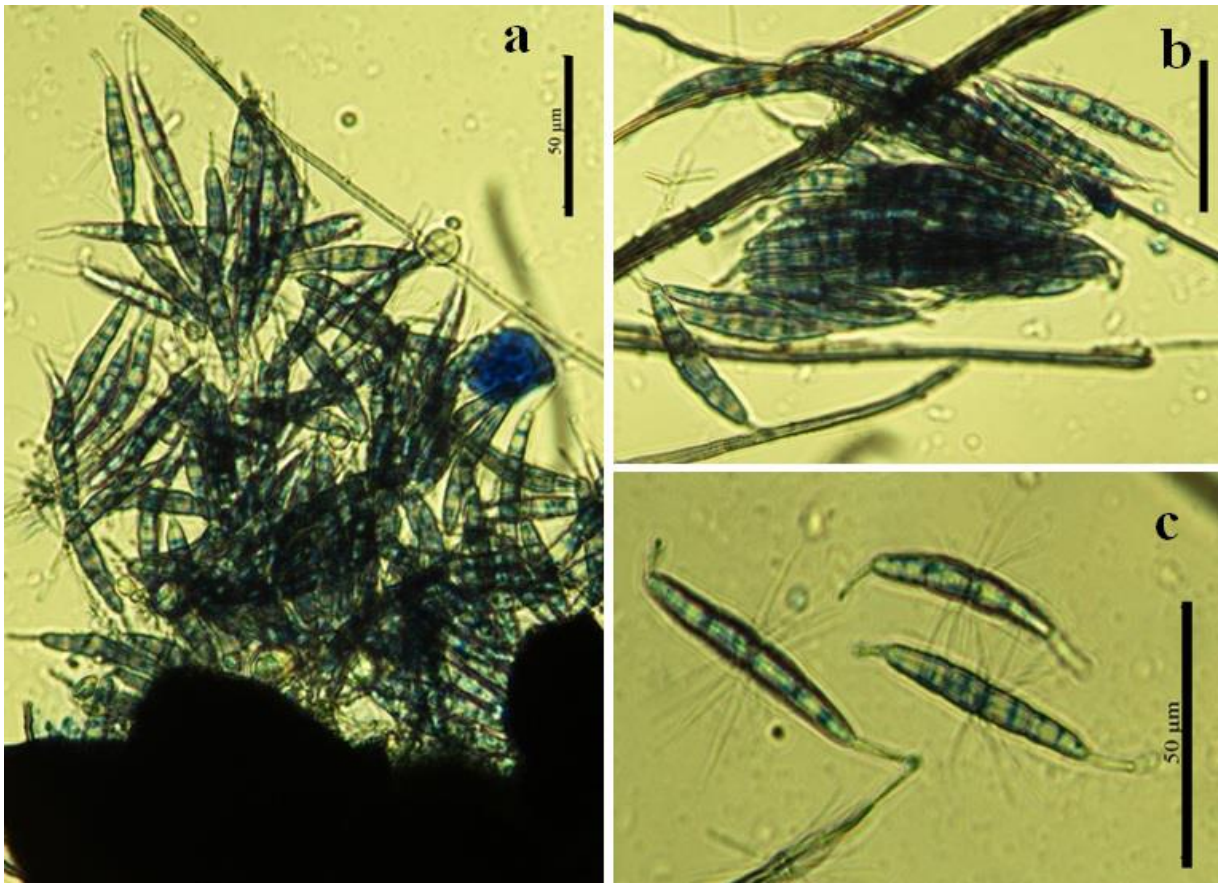

Figure 3. *Corollospora quinqeseptata*: crushed carbonaceous ascocarp (a); ascus (b); multiseptate ascospores with median and terminal appendages (c) (Scale bar, 50 µm).

In our study, sporulation of mitosporic fungi was seen in the initial stages of incubation (2 months onwards), followed by rest of the fungi (6 months onwards). The arenicolous fungi were found at the later stage of incubation (10 months onwards). Such pattern of succession in sporulation was found in earlier studies on woody as well as calcareous substrates in the beaches and mangroves of southwest coast (Prasannarai and Sridhar, 1997; Ananda et al., 1998; Ananda and Sridhar, 2004). One of the interesting observations of this study is the record of a probable new species in the genus *Arenariomyces* as the fifth dominant species among ascomycetes (35.6%) (Figure 4). It was found in all three locations (26.1-49.3%) of survey and with the highest frequency of occurrence in Surathkal (49.3%). It differs in morphology of the known species of *Arenariomyces*. The carbonaceous ascocarps are spherical with hairy outgrowths and ascocarps measures 100-200 µm. the ascospores are bi-celled with deep constriction at the septa, measures 20-30 × 8-12 µm and possess antenna-like four polar appendages (two at each pole) characteristically curled outwards like moustache (20-25 × 1-1.5 µm).

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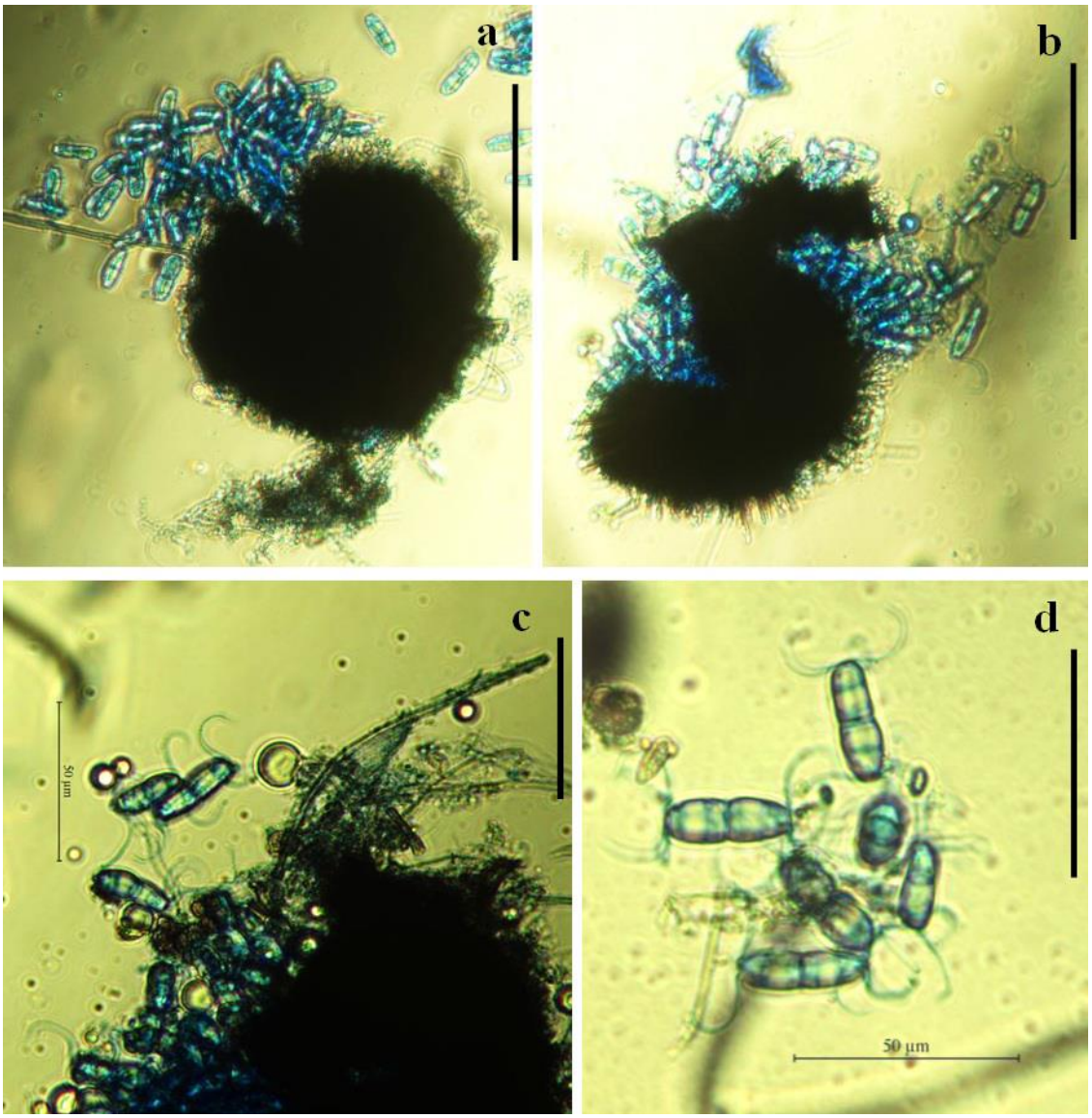

Figure 4. *Arenariomyces* sp.: crushed spherical carbonaceous ascocarps with hairy surface (a–c); uniseptate ascospores with deep constriction at the septa with curled polar appendages (c & d) (Scale bar: a & b, 100 µm; c & d, 50 µm).

So-called terrestrial fungi are also common in marine habitats (e.g. woody litter, coral reefs and deep sediments) (e.g. Raghukumar and Raghukumar, 1991). Many of them might have adapted to marine habitats. For instance, some of the fungi found in our study were also reported as most frequent in the salterns across the globe (e.g. *Aspergillus flavus, A. fumigatus* and *A. niger*) as well as in the waters of the Dead Sea in Jordan (Oren and Gunde-Cimerman, 2012; Zajc et al., 2012). The latter two fungi were also found in the deep-sea sediments, while *Fusarium oxysporum* was reported from the oxygen-deficient marine environment (Raghukumar, 2017).

4. CONCLUSION

This study showed that there will be difference in the marine fungal species composition, richness and diversity on different types of woody litter. Observation of naturally deposited unidentified woody litter gives different picture in species composition and dominance compared to the identified woody substrates. The pattern of occurrence, species richness, diversity and dominance of marine fungi seem to be dependent on the type of substrate. Marine fungi on woody litter are influenced by the environment variables such as temperature, pH, continuous submersion, intermittent submersion and long exposure to terrestrial conditions. Long-term incubation of woody litter will be advantageous to follow the whole range of colonized fungi. Further studies need to

focus on the other long-lasting known woody litters (e.g. seeds and roots) accumulating in the maritime habitats to have clear picture on the dynamics of marine fungi.

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Ethical approval

The ethical guidelines are followed in the study for collection & identification of Specimens with the help of Dr. Ka-Lai Pang, National Taiwan Open University, Taiwan.

Authors' contributions

All authors contributed equally.

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This study has not received any external funding.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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