

RESEARCH PAPER

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Studies on the diversity of non-mycorrhizal fungi in bryophyte plants found in the Nilgiris, Tamil Nadu

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Abstract

Bryophytes create the most abundant microfungi in the Nilgiris Biosphere Reserve, while endophytic fungi infect healthy plant tissues without triggering any symptoms. Bryophytes form the most abundant micro fungal in the Nilgiri Biosphere Reserve, while endophytic fungi infect healthy plant tissues without triggering any symptoms. In this study, we investigated the endophytic fungal associated with diversity with bryophytes of a common Mosses. We examined fungal diversity and host assemblage in five species of bryophyte moss from the evergreen forest of NBR. A total of 37 endophyte species were isolated from 500 tissue segments of the five plants, with 37 species recovered from more than one host. We identified various groups of fungi, including Ascomycetes, Coelomycetes, Hyphomycetes, Zygomycetes, and others that are present in the evergreen forest. Additionally, fungal species diversity in plant tissues of mosses from bryophyte plants has a slightly different pattern. *Aspergillus, Cheatomium globosum, Fusarium oxysporum, Phomopsis, Rhizopus stolonifer, Sardaria fimicola* and *Xylaria* species were found to be most abundant in colonization and most common fungal species found in all plants. Our findings involve that the survival of several fungal species, even in small areas, and those bryophytes are critical to ensuring fungal diversity in this extreme climate.

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Introduction

Microorganisms such as bacteria, mycorrhizae and some macrofungi are linked to bryophytes, which include all three types of liverwort, moss, and hornwort. Numerous lichens, bacteria, and fungi can parasitize bryophytes (During and Tooren, 1990). A diversity of fungal species known as "fungal endophytes" occasionally inhabit living plants without visibly exhibiting signs of infection (Wilson, 1995). Endophytic fungi are frequently extremely varied. The term "endophyte" has been defined in various ways; in this context, we adopt the wide definition, which encompasses any microorganism that resides in healthy plant tissue (Stone et al., 2000). Endophytic fungi are found in various plant tissues (Tan and Zou, 2001), including root nodules, stems, leaves, roots, and seeds (Gaisen et al., 2017).

While some are classified as Basidiomycota (Koukol et al., 2012) and Zygomycota (Gazis and Chaverri, 2010), most are classified as Ascomycota. These fungi have a wide variety of hosts and habitats; they have been isolated from many different types of land plants as well as every terrestrial environment, from the dry tropics of Nilgiri Biosphere Reserve regions (Survanarayanan et al., 2018). Almost all plants and all parts investigated from bryophytes to vascular plants, have been discovered to have fungal endophytes (Hardoim et al., 2015; Venkatesan and Mahalakshmi, 2022), as well as chlorophytes and charophytes (Knack et al., 2015). However, there are insufficient reports on the biodiversity and potential of fungal endophytes associated with mosses and ferns (Kumaresan et al., 2013; Mahalakshmi and Venkatesan, 2024). Plant-fungus symbioses with fungi are common and have existed for ages. Fungal correlative may have aided plant ancestors in colonizing land, according to evidence from fossils, molecules, and physiology (Selosse et al., 2015). Bryophytes and microorganisms such as bacteria, fungi, and algae have crucial symbiotic relationships for the ecological processes and strategies for the survival of these plants. Plants also have important roles in the functioning of ecosystems (Poveda, 2020). For instance, nearly every branch of the plant

evolutionary tree has core genes that are functionally conserved, including those involved in symbioses with nitrogen-fixing bacteria and mycorrhizal fungi (Wang *et al.*, 2010). The present approach to understanding these cryptic organisms would be to study them at the plant community level. Many studies describe the distribution of endophytes in tropical forest trees, but none for non-vascular plants and endophytes in bryophytes. This study is motivated by an information gap from the Nilgiri Biosphere Reserve forest in Tamil Nadu.

Materials and methods

Study area

Bryophyte plants of Ceratodon purpureus, Leucobryum Pleurozium schreberi, glaucum, Polytrichum commune, and Syntrichia ruralis are mosses groups of the tropical herbs examined in endophytic fungi, are collected from Nilgiris district in the Western Ghats of Tamil Nadu. NBR is located at 11.345°N, 76.795°E and has an average elevation of 1,650 meters (5413 feet) above sea level. Because it is so high up, it has a subtropical highland climate. This results in a savanna, or tropical wet and dry, climate in the Nilgiris. All bryophyte plants were collected from individuals' species, and their fungal endophytes were isolated from 2023 to 2024 about once every six months throughout the year. For these studies, medicinally important plant species and members of the different family (Table 1) widely distributed around hill stations in the world. Each bryophyte plant sample was brought to the laboratory in sterile polythene bags and examined within 48 hours of collection.

Sterilization and culture Protocols

The host plant studied was collected from Nilgiri, Tamil Nadu, and South India. These stages of plant leafy thallus were collected for the investigation. Leafy thallus samples were collected from healthy plants. In these plants, leaves were randomly collected and their one hundred tissue segments were cut from one hundred and fifty leaves. However, sterilization techniques were followed before cutting these segments.

Sterilization and culture Protocols

The host plant studied was collected from Ooty, Tamil Nadu, and South India. Leafy thallus samples were collected from healthy plants. However, sterilization techniques were followed before cutting these segments. They were thoroughly washed under running tap water, after the surface sterilization, the leaves were cut approximately into 0.5 cm segments/with some plant leafy thallus in each corner/depending on the growth/size of the plants. The samples were washed in running water, dipped in 70% ethanol for 60 seconds, immersed in 2.5% NaOCl (Sodium hypochlorite) for 90 seconds, and then immersed in sterile water for 10 seconds (Venkatesan, (2025) some changes were made to the sterilization methods described by Suryanarayanan et al. (1998) or three times and washed. The sterilized samples were placed on the PDA medium amended with antibiotics (Chloramphenicol 150 mg/l) contained in Petri dishes. The Petri dish was sealed with Parafilm[™] and incubated in a light chamber at 26±°C for 7 to 21 days (Bills and Polishook, 1992; Suryanarayanan et al., 1998). The light regimen was 12 hours of light followed by 12 hours of darkness. Fungi grew from the segments and were periodically observed, and endophytes were identified.

Morphological identification of isolated endophytic fungi

Preliminary identification was done by studying the fungi's cultural characteristics, such as colony growth, colour, shape, etc. The morphological characters were examined by growing cultures on PDA plates for 7–21 days. Microscopic observations of conidiophores, conidia, and mycelia characters were carried out by preparing slides stained with cotton blue and Congo red and observed under the compound microscope.

Statistical analysis

Relative percentage of occurrence of each group of fungi (RPO)

The relative percentage of occurrence (RPO) of each group (viz., Ascomycetes, Coelomycetes, Hyphomycetes, Zygomycetes and Sterile-like forms) of fungal species in each plant species was calculated as follows: Tedersoo *et al.* (2018).

RPO = {(Total colonization frequency of one group)/ (Total colonization frequency for all the groups of fungi)}×100

Diversity index

(Fisher's α) the diversity index was calculated using the method of Fisher *et al.* (1943).

Species evenness index and species richness index (E5, R1)

The species evenness (E5, modified Hill's ratio) and species richness (R1, Margalefs index) were calculated as described by Ludwig and Reynolds (1998) using the software provided by John Wiley and Sons, SPDIVERS.BAS.

Results

Ceratodon purpureus, Leucobryum glaucum, Pleurozium schreberi, Polytrichum commune and Syntrichia ruralis are mosses groups of the tropical terrestrial plant belonging to Bryophyte in the (Ditrichaceae, Leucobryaceae, Hylocomiaceae, Polytrichaceae and Pottiaceae families was studied for their foliar structure in endophyte assemblages in fungi during 2023-2024 (Tables 1-5; Fig. 1-4). This research aimed to have a systematic understanding of the endophytic position in tropical plants. Fungal endophytes were isolated from these plants' thallus and leaves during that period. The presence of fungal endophytes has been identified in one hundred leaf segments of five different bryophyte mosses. The segments were cut from the entire portion of the basal leaves. Surface sterilized using ethanol and sodium hypochlorite (Sterilization and Culture Protocols) and screed as mentioned under Materials and Methods. A total of 37 endophyte species from moss bryophytes were isolated from 1000 tissue segments from five plants. In most cases, each tissue segment was infected by more than one fungal species (multiple infections), substantiating the view that tropical plants have high rates of endophyte colonization (Suryanarayanan et al., 2002).



Fig. 1. Moss bryophyte plants pictures 1. *Leucobryum* glaucum, 2. *Pleurozium* schreberi, 3. *Polytrichum* commune, 4. *Syntrichiya* ruralis



Fig. 2. Number of endophytic fungi and species isolated from five moss bryophyte



Fig. 3. Relative percentage of occurrence of each group of fungi (RPO) from five moss bryophyte plants

Sardaria fimicola showed the highest colonization frequency from the mosses group of bryophyte then *Phomopsis* spp, *Xylaria* species, *Cheatomium* species, *Aspergillus* species, *Fusarium* species, *Rhizopus* stolonifer showed the maximum colonization frequency of bryophytes. Also, they were found in all plants. In general, more species of Hyphomycetes were found among fungi, followed by Ascomycetes, Coelomycetes and Zygomycetes. In the bryophyte moss, a few sterile forms and unknown fungal strains were discovered. Based on the results of several research reports, we propose that fungal species and diversity of fungi isolated from higher plants and lower plants show only some variation from plant tissues.



Fig. 4. A histogram graph of the number of fungi is plotted

There are many reports of similar studies (Umesha et al., 2016; Venkatesan and Mahalakshmi, 2022). Several studies have reported the presence of Aspergillus species, Cladosporium species, Mucor species, Trichoderma species, Penicillium species in multiple environments around the world, such as air, land, water, plants and animals. In addition, it can be found in unfriendly location such as deserts, highly salty and soils contaminated (Sterflinger et al., 2012). Sordaria and Sporormiella species are common fungus that grows on dung, decaying matter in soil (Suryanarayanan et al., 1998).

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Sl	Name of the bryophytes	Plants initials	Family
1	Ceratodon purpureus Hedw.	CP	Ditrichaceae
2	Leucobryum glaucum Hedw.	LG	Leucobryaceae
3	Pleurozium schreberi (Willd. ex Brid.) Mitt.	PS	Hylocomiaceae
4	Polytrichum commune Hedw.	PC	Polytrichaceae
5	Syntrichiya ruralis (Hedw.) F.Weber.	SR	Pottiaceae

Table 1. A list of bryophytes plants was examined for endophytic fungus

Table 2. Number of endophytic fungi isolated from five Moss bryophyte plants

	Name of plants Initials					
PC LG CP SR	PS	_				
ASCOMYCETES						
1 Botruis cinera 1 1		2				
2 Cheatomium globosum 9 9		18				
3 Cheatomium sp.1 4 2		6				
4 Culindrocladiella sp. 1 1		1				
5 Pestalotionsis cocculi 4 1 2		7				
6 Sardaria finicola 15 41 22 20	37	135				
7 Verticillium sp. 1 1 1	0/	2				
8 Xularia sp. 1 2 1 6 2	10	21				
$0 Xularia \text{sp. } 2 \qquad 1 \qquad 8$	1	10				
COFLOMYCETES	-	10				
10 Colletatrichum aleosporioides 5		5				
$\begin{array}{cccccccccccccccccccccccccccccccccccc$		17				
12 Phomonsis sn 2 6 10 2 10	1	22				
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	5	10				
HYPHOMYCETES	5	10				
14 Asperaillus flavines 15		15				
15 Asperailly niger 6 3 1 4	1	15				
16 Asperailly sp. 1 1 1 1	-	-0 -2				
17 Asperallus sp 2 1 1 2 1	2	5				
18 Alternaria alternata 2 A 1	-	7				
19 Alternaria sp. 1 1 1 4 1		7				
20 Alternaria sp 2 1 3		1				
21 Cladosporium herbarum 2 1 2	1	7				
22 Curringlaria lunata 6		6				
23 Fusarium moniliforme 1		1				
23 Fusarium oxisporium 4 2 1 2	2	12				
25 Niarosnora oruzze 2 1 1	5	12				
25 Projectium sp 1 1	1	- + 2				
27 Penicillium sp 2 1 1		2				
2/ Foncinitian sp. 2 I I		1				
20 Trichoderma viride 1 1 1		2				
29 Tripoderma sp 1 1 1		2				
ZVGOMVCETES		2				
21 Muorsp 1 1 4 2	1	0				
22 Rhizonus stolonifer 2 2 1 2	10	17				
22 Sincephalastrum racemosim 1 1	10	2				
STERILE FORMS		-				
34 Sterile form 1 1 1		Q				
35 Sterile form 2 2 1		ວ ຊ				
36 Sterile form 3 1 2 1 2		6				
27 Sterile form 4 2		0				
Total no of Species 28 20 22 22	19	ა ⊿07				
Total no. of Colonies 26 26 25 25 Total no. of Colonies 96 80 84 71	76					

Trichoderma sp., *Penicillium* sp., isolated from the Antarctic moss *Bryum argenteum* (Bradner *et al.*, 2000). When compared with earlier reports in bryophytes mosses, the endophytic fungi were isolated into classes of Ascomycetes, Coelomycetes, Hyphomycetes, and Zygomycetes from Victoria Land, Jubany on King George Island (Möller and Dreyfuss, 1996, Tosi *et al.*, 2002). On the reported of Thormann *et al.* (2001) common saprobes are among Ascomycota: *Sporormiella* sp., *Sordaria* sp., Zygomycota: *Mucor* sp., Anamorphic fungi: *Cladosporium* sp., *Aspergillus* species etc. These endophytic fungal strains belonging to Ascomycota (9), Coelomycota (4), Hyphomycota (17), Zygomycota (3), and others (4) species were obtained from mosses plants (Table 2-4). According to Davis *et al.* (2003) and Kauserud *et al.* (2008), endophytic Xylariaceae

have been observed in bryophytes in several habitats. The endophytic flora of the *Aspergillus* and *Penicillium* spores have been shown to occur commonly in soil and air samples (Mitchell *et al.*, 2016; Umesha *et al.*, 2016; Subhashini, 2018).

Table 3. Similarity coefficients between the number of species, relative percentage of occurrence (RPO), species richness (R1), species evenness (E5) and species diversity (Fisher's α) of the endophyte assemblages of the bryophyte plants in mosses

Sl	Statistical analysis	Ceratodon purpureus	Leucobryum glaucum	Pleurozium schreberi	Polytrichum commune	Syntrichiya ruralis
1.	Species	28	20	23	23	12
2.	Individuals	96	80	84	71	76
3.	Total no. of segments	100	100	100	100	100
5.	R1(Margalef's)	2.91	1.98	2.51	2.57	1.73
6.	E5 (Hill's ratio)	0.84	0.42	0.66	0.65	0.58
7.	Fisher's alpha	13.29	8.56	10.45	11.81	4.01

Table 4. Number of endophytic fungi isolated and each group of fungi from five Moss bryophyte plants

Sl	Plant Names	A	All group of fungi (Total 37 species)					
		AM	CM	HM	ZM	SF	_	
1	Ceratodon purpureus	5	3	10	2	3	23	
2	Leucobryum glaucum	4	2	10	2	2	20	
3	Pleurozium schreberi	3	2	5	2	0	12	
4	Polytrichum commune	8	4	11	3	2	28	
5	Syntrichiya ruralis	5	2	11	2	3	23	

Table 5. Relative percentage of occurrence of each group of fungi (RPO) from five moss bryophyte plants

Sl	Plant name	AM	СМ	HM	ZM	SF	Percentage (%)
1	Ceratodon purpureus	21.73	13.04	43.47	8.69	13.04	99.97
2	Leucobryum glaucum	20.00	10.00	50.00	10.00	10.00	100.0
3	Pleurozium schreberi	25.00	16.66	41.66	16.66	0.00	99.98
4	Polytrichum commune	28.57	14.28	39.28	10.71	7.14	99.98
5	Syntrichiya ruralis	21.73	8.69	47.82	8.69	13.04	99.97

Discussion

The plant parts of leaf tissues were found to harbuor various endophytic fungal species with different colonization isolated. The diversity of fungi in species of bryophyte mosses *Polytrichum commune*, *Ceratodon purpureus*, *Leucobryum glaucum*, *Pleurozium schreberi*, and *Syntrichia ruralis* are distinguished and the statistics analysis (PCO, E5, H1). We think the present work is completely new in this area, and many explanations and some examples may be for the first time. It was observed that there were significant differences in endophyte colonization among individuals.

This results in an evenness index for the endophyte assemblages. In comparing the endophyte

assemblages of the host, the endophyte diversity was slightly different for mosses due to a higher mean for all bed plants. However, the diversity index was almost identical for all plants. However, other studies indicate that tropical forests do not support high endophyte diversity (Suryanarayanan et al., 2002; 2003). Earlier studies involving a few individual plant species revealed that snowfall, precipitation, and endophyte colonization of leaf tissues are positively correlated (Suryanarayanan et al., 1998). Already studies revealed that Ascomycetes, Colomycetes, Sterile forms Hyphomycetes, and invariably constitute the endophyte assemblages of trees (Suryanarayanan et al., 2018); Basidiomycetes and Oomycetes are rarely encountered (Petrini, 1986). To our knowledge, no study of endophyte fungi on

bryophytes has been done so far in the Nilgiri region. In this tropical forest, mosses of bryophytes and fungi groups are differentiated by a relative percentage of occurrences (RPO). In this investigation, numerous recognized but infrequently isolated funguses were among the prevalent endophytes, including species of Cladosporium, Curvularia, Alternaria, and Colletotrichum. These findings are consistent with previous research conducted on tropical endophytic fungi (Prathyusha et al., 2015). Cladosporium, Fusarium, Colletotrichum, Verticillium, Curvularia, Nigrospora, Guignardia, and Phoma are among the fungi that have been discovered as endophytes that may also be pathogens (Cui et al., 2021).

Recent research has identified a few endophytic isolates from the genus Trichoderma as novel species (Rodriguez et al., 2021; Zheng et al., 2021). This could be the consequence of the genus's rapid speciation following adaptation to new ecological niches (Chaverri et al., 2015), or it's more likely that these researchers concentrated mainly on endophytes. The foliar-dominating endophytic fungi belonging to Sardaria fumicola species were found in mosses and liverworts in Colletotricum gloeosporioide species in the current study on mosses and liverwort plants. Aspergillus flavipes., Fusarium sp., Colletotricum gloeosporioide, and Cheatomium globosum. Phomopsis and Trichoderma sp. were the second-most dominant endophytes in this plant. Several previous studies have reported the presence of Colletotricum gloeosporioide, Phomopsis sp., and Phyllosticta capitalensis; these fungal species are a dominant group of endophytes residing in association with different medicinal plants (Prabha Toppo et al., 2024); many trees from Nilgiri tropical forests (Suryanarayanan et al., 2018); and other reports the dominant fungi were the same for groups; Colletotrichum gloeosporioides and Phomopsis have shown that more endophyte isolates occur in leaves screened in the Adiantum capillus and Nephrolepis cordifolia plants (Mahalakshmi and Venkatesan, 2024). The number of isolated species described in each plant fungus is compared to those recorded in the species richness. Endophytic fungal genera such

as *Colletotrichum gloeosporioides* and *Phomopsis* species had higher abundance rates and frequencies in both groups. Hyphomycetes have more fungal diversity in both groups. We observed several species in groups of Zygomycetes. Endophytes can alleviate abiotic and biotic stressors such as drought, salinity, heavy metals, and other toxic compounds introduced by the environment, floods, extreme temperatures, predators, and pathogens (Clay *et al.*, 1999; Su *et al.*, 2021).

Soil and airborne fungal spore concentrations and their diversity vary with the season of the year, geographical region, soil, air, meteorological parameters, presence of local resources, and vegetation. A few fungi that failed to sporulate were designated as "mycelia sterile" and can be identified later with different incubations, such as sporulation in UV light, so for colony characteristics, the mycelia were transferred to PDA agar media.

Conclusion

Investigation of a few bryophyte plants for the first time. The practical method to estimate the global diversity of endophytic fungi species-richness such as thallus fungi is investigation from C. purpureus, L. glaucum, S. ruralis, P. schreberi and P. commune of the tropical forests in the Nilgiri Biosphere Reserve. The present investigation suggested that the endophyte diversity in tropical forests depends on precipitation and that certain endophytes are ubiquitous, maybe reflecting their adaptation to exploiting the internal tissues of plants as a habitat niche. Ascomycetes, coleomycetes, hyphomycetes, zygomycetes, and sterile forms unidentified were present as endophytes. A few ascomycetes, hyphomycetes members coleomycetes, and contributed to the endophyte assemblage of bryophyte plants. In the previous reports and current studies, the most promising several are endophytic fungi. These are becoming an important source of bioactive compounds for many applications in industry, agriculture, and medicine. In the current work, fungal endophytes were isolated, identified, and characterized using morphological explants of the

bryophytes groups of plant mosses and liverworts. In the past few decades, many researchers have mainly focused on the investigation of fungal endophytes for diversity and their relationships with their higher plants. Because of this, we investigated the fungus in the plant tissues of a few significant and widely utilized plants from lower plants of crypto-bryophytes plant species of the tropical herb studied by endophytic fungi and found a huge diversity of fungal species.

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