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Contributed Paper

Diversity of Culturable Endophytic Fungi Associated with Bryophytes, Pteridophytes and Spermatophytes from Dawei Mountain Nature Reserve, China

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Abstract

Bryophytes, pteridophytes and spermatophytes are each plants with different evolutionary degrees. In the present study, the diversity and community structure of culturable endophytic fungi (EF) associated with bryophytes, pteridophytes and spermatophytes collected from Dawei Mountain in China were investigated. A total of 2230 EF were isolated from 1440 segments of nine plant species. The colonization rate (CR) of bryophytes, pteridophytes and spermatophytes were 97.92%, 98.75% and 98.13%, respectively ($P > 0.05$, LSD test). Based on the morphological characteristics, 18S rDNA and internal transcribed spacer (ITS) analysis, the isolates were identified to 61 taxa, of which *Colletotrichum* and *Xylaria* were the most dominant genera, and their relative frequencies were 40.00% and 23.68%, respectively. Only 21 taxa were common between bryophytes, pteridophytes and spermatophytes, whereas, some endophytes showed host specificity or tissue preference, for example, *Penicillium chrysogenum* and *P. daleae* were found only in bryophytes. In addition to *Colletotrichum* and *Xylaria*, *Trichoderma* and *Penicillium* were the dominant genera in bryophytes. However, in pteridophytes and spermatophytes, *Phomopsis* was the dominant genus. The Shannon indices (H') and the Sorenson's coefficient similarity indices (CS) of EF from bryophytes, pteridophytes and spermatophytes ranged from 2.02 to 2.92 and 0.64 to 0.74, respectively. It was found that H' of plants with different evolutional degree showed no significant difference ($P > 0.05$, LSD test). The CS of EF of two plant species with different evolutional degrees was not always lower than that of two plant species with the same evolutional degree.

Key words: bryophyte, pteridophyte, spermatophyte, evolutionary degree, endophyte

1. Introduction

The fungi causing asymptomatic infections in living plant tissues are known as endophytic fungi (EF) and they are a significant part of the plant microbiome [1]. They have been widely

studied in various geographic and climatic zones and are ubiquitous within a wide range of tissues of all examined plants with a rich diversity of species [2-3]. Various studies have demonstrated that endophytes can have profound effects on plant ecology, fitness and evolution, affecting the diversity and structure of the plant community [4-6]. In plant fossils formed 400 million years ago, endophytes have been found [7]. Thus, the interaction between host plants and endophytes, particularly the possible role of endophytes in the evolution of plants has gained considerable momentum. The study of diversity and community structure of EF associated with plants belonging to different evolutionary degrees is very important. Here, evolutionary degrees represent different taxonomic levels of phylogenetic classification systems. In this system the evolutionary relationships between the various organisms form the basis of classification. Most botanists consider bryophytes and pteridophytes to be the oldest living remnants of eukaryotic plants that colonized the land [8]. While bryophytes represent the basal clades, pteridophytes have been placed between bryophytes and spermatophytes (gymnosperm and angiosperm) in the subdivisions of the plant kingdom. Previous studies mainly focused on comparing the diversity and community structure of EF of the same plant species growing in different environments, or EF of different plant species growing in the same environment [9-10]. To our knowledge, the differences of EF community associated with different plant classes are still unknown.

Dawei Mountain Nature Reserve, which is located in Yunnan Province, Southwest China, is the most comprehensive region which has both evergreen and broad-leaved forests in China. And the climate type is a humid subtropical climate, with annual precipitation exceeding 1500mm. From tropical humid rainforest to seasonal rainforest, monsoonal evergreen broad-leaved forest, moss evergreen broadleaved forest and moss evergreen broad-leaved forest, there is a complete mountain forest ecosystem in the range of over 2000m above sea level. It was not affected by the "Quaternary Glacier" period [11], thus many old plant species have been retained, and the diversity of plant species is high [12-13], especially bryophyte, pteridophytes and spermatophytes. To compare the diversity and community structure of EF associated with bryophytes, pteridophytes and spermatophytes, nine plant species (three bryophytes, three pteridophytes and three spermatophytes) were collected from Dawei Mountain, and culturable endophytic fungi were studied in this work.

2. MATERIALS AND METHODS

2.1 Study Site and Sampling

Three species of bryophytes (*Marchantia polymorpha.*, Marchantiaceae; *Polytrichum commune,* Polytrichaceae; *Hylocomium splendens,* Hylocomiaceae), three species of pteridophytes (*Diacalpe aspidiodes,* Peranemaceae*; Coniogramme petelotii.,* Hemionitidaceae and *Plagiogyria maxima.,* Plagiogyriaceae) and three species of spermatophytes (*Taiwania cryptomerioides.*, Cupressaceae; *Embelia polypodioides.*, Mysinacease, and *Rhododendron irroratum.,* Ericaceae) were collected from Dawei Mountain (22°28'-22°45'N, 103°39'-103°51'E), Yunnan, southwest China in November 2012. For each plant species, 10 healthy individuals at least 30m apart from each other were chosen, and three healthy and separate branches were collected from each plant at random, and brought to the laboratory in sterile polythene bags and processed within 24 h.

2.2 Fungal Isolation, Culture and Identification

For isolation of endophytic fungi, 20 healthy leaves (or photosynthetic tissues) and 20 healthy stems (or rhizoid) were selected from each plant at random, washed under running tap water and processed as follows: the samples were cut into segments (about 5 × 5 mm) and

surface-sterilized by sequentially dipping into 0.5% sodium hypochlorite for 2 min, followed by 3 times washing with sterile distilled water, dipping into 70% ethanol for 2 min, rinsing 3 times with sterile distilled water, then drying on sterilized filter paper [14]. Then 80 leaf (or photosynthetic tissue) segments and 80 stem (or rhizoid) segments of each plant species were placed on a Petri dish containing potato dextrose agar (PDA) medium amended with 0.5 g/l streptomycin sulfate. The plates were incubated at 25°C and checked every other day for 45 d; the fungi growing out of the plant tissues were transferred to fresh PDA plates. The effectiveness of the surface sterilization was confirmed by making imprints of disinfected plant fragments on PDA plates from which no fungal growth was observed [15].

Fungal identification was based on morphology, mechanism of spore production and spore characteristics [16]. Some of the isolates, which posed difficulty in morphological identification, were further examined based on their ITS sequence analysis (The primers were ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3')), noting that sometimes matches in GenBank did not necessarily give correct names [17]. Sequences were submitted to GenBank and assigned the accession numbers were from KM357284 to KM357350 (Table 1). Sterile isolates were sorted into different groups on the basis of colony surface texture, hyphal pigmentation, margin shapes and growth rates. All of the isolates were deposited in the Medical School, Kunming University of Science and Technology under the assigned numbers in this report.

2.3 Statistical Analysis

The colonization rate (CR) was calculated as the total number of segments colonized by endophytic fungi (EF) divided by the total number of incubated segments [18]. The relative frequency (RF) was calculated as the number of isolates of one species divided by the total number of isolates [19].

The endophytic fungal diversity was evaluated using the Shannon index (H'), which has two main components, evenness and the number of species. The Shannon index was calculated according to the following formula:

$$H' = -\sum_{i=1}^{k} P_i \times \ln P_i \ ,$$

where k is the total species number of one plot and P_i is the relative abundance of endophytic fungal species in one plot [20].To evaluate the degree of community similarity of the EF between two treatments, Sorenson's coefficient similarity index (CS) was employed and calculated according to the following formula:

Table 1. The results of molecular identification.

Numbers	Isolate obtained in the present study	Accession no. of isolate obtained in the present study	Morphotype	Query coverage (%)	Identity (%)	Most closely related species (accesssion no.)
1	1X-38	KM357284	*Xylaria* sp.	97	99	*Xylaria* sp. (JQ862679.1)
2	2X-97	KM357285	*Colletotrichum gloeosporioides*	97	100	*Colletotrichum gloeosporioides* (HQ845102.1)
3	9Y-69	KM357286	*Colletotrichum boninense*	98	98	*Colletotrichum boninense* (EF221828.1)
4	Y1-29	KM357287	*Phomopsis* sp.	95	99	*Phomopsis* sp. (GQ250217.1)
5	3C2-O2	KM357288	*Trichoderma viride*	96	100	*Trichoderma viride* (GU934567.1)
6	10J-14	KM357289	*Xylaria* sp.2	97	99	*Xylaria* sp.2 (AY315405.1)
7	4S-3	KM357290	*Calonectria eucalyoti*	96	99	*Calonectria eucalyoti* (KF928290.1)

Note: Amplified primers were ITS1 and ITS4.

Table 1. The results of molecular identification. (continued)

Numbers	Isolate obtained in the present study	Accession no. of isolate obtained in the present study	Morphotype	Query coverage (%)	Identity (%)	Most closely related species (accesssion no.)
8	J6-76	KM357291	*Pestalotiopsis cocculi*	97	99	*Pestalotiopsis cocculi* (JQ266371.1)
9	1X-23	KM357292	*Nigrospora oryzae*	96	99	*Nigrospora oryzae* (EU918714.1)
10	2S-111	KM357293	*Colletotrichum acutatum*	97	99	*Colletotrichum acutatum* (KF541089.1)
11	2X-3	KM357294	*Mucor* sp.	95	94	*Mucor* sp. (AB638465.1)
12	J3-17	KM357295	*Xylaria* sp.3	97	94	*Xylaria* sp.3 (HM044135.1)
13	3X-33	KM357296	*Trichoderma asperellum*	96	99	*Trichoderma asperellum* (HQ671189.1)
14	J6-36	KM357297	*Nemania primolutea*	98	98	*Nemania primolutea* (EF026121.1)
15	G7-53	KM357298	*Ceratobasidium* sp.	98	94	*Ceratobasidium* sp. (HQ269823.1)
16	G7-69	KM357299	*Cylindrocladiella* sp.	93	99	*Cylindrocladiella* sp. (JN100602.1)
17	4X-22	KM357300	*Phoma herbarum*	98	99	*Phoma herbarum* (KF313118.1)
18	2S-13	KM357301	*Xylaria* sp.4	98	97	*Xylaria* sp.4 (AB511813.1)
19	3S-26	KM357302	*Mortierella* sp.	97	99	*Mortierella* sp. (GU985216.1)
20	Y2-64	KM357303	*Diaporthe* sp.	96	99	*Diaporthe* sp. (AB899786.1)
21	G6-41	KM357304	*Helotiales* sp.1	96	99	*Helotiales* sp.1 (JQ272327.1)
22	9J-79	KM357305	*Microdochium* sp.	97	98	*Microdochium* sp. (AB255278.1)
23	9G-33	KM357306	*Plectosphaerella cucumerina*	100	99	*Plectosphaerella cucumerina* (GU479908.1)
24	9G-7	KM357307	*Hypocrea semiorbis*	98	99	*Hypocrea semiorbis* (MH284743.1)
25	J3-95	KM357308	*Xylaria* sp.5	97	99	*Xylaria* sp.5 (AB449101.1)
26	8J-68	KM357309	*Helotiales* sp.2	87	98	*Helotiales* sp.2 (DQ914733.1)
27	5X-43	KM357310	*Umbelopsis isabellina*	94	80	*Umbelopsis isabellina* (JN206398.1)
28	J1-27	KM357311	*Humicola* sp.	98	99	*Humicola* sp. (HQ637378.1)
29	J2-73	KM357312	*Pezicula carpinea*	98	98	*Pezicula carpinea* (AF169306.1)
30	Y2-18	KM357313	*Guignardia mangiferae*	98	99	*Guignardia mangiferae* (JN791605.1)
31	G6-27	KM357314	*Phialocephala* sp.	86	96	*Phialocephala* sp. (AY606299.1)
32	3X-59	KM357315	*Trichoderma harzianum*	96	98	*Trichoderma harzianum* (KC576741.1)
33	10G-102	KM357316	*Bionectria rossmaniae*	97	92	*Bionectria rossmaniae* (AF210665.1)
34	G7-44	KM357317	*Beltrania rhombic*	91	98	*Beltrania rhombic* (GU797390.1)
35	Y7-49	KM357318	*Cercospora capsici*	98	100	*Cercospora capsici* (HQ700353.1)
36	4S-104	KM357319	*Arthrinium arundinis*	98	99	*Arthrinium arundinis* (KF693784.1)
37	1S-66	KM357320	*Muscodor albus*	98	98	*Muscodor albus* (AY555731.1)
38	J5-28	KM357321	*Xylaria* sp.6	97	99	*Xylaria* sp.6 (AB449101.1)
39	8G-21	KM357322	*Cladosporium tenuissimum*	98	100	*Cladosporium tenuissimum* (HM776419.1)
40	3X-29	KM357323	*Rhizomucor variabilis*	96	99	*Rhizomucor variabilis* (EU484249.1)
41	10C3-B-B1	KM357324	*Volutella consors*	95	97	*Volutella consors* (JQ693162.1)
42	4X-61	KM357325	*Chaetomium* sp.	98	99	*Chaetomium* sp. (JN168655.1)
43	J7-63	KM357326	*Chaetomium globosum*	98	99	*Chaetomium globosum* (JN582329.1)
44	1S-18	KM357327	*Penicillium manginii*	98	99	*Penicillium manginii* (JN617662.1)
45	G6-39	KM357328	*Trametes versicolor*	97	99	*Trametes versicolor* (KC176325.1)
46	Y4-48	KM357329	*Phomopsis amygdali*	98	99	*Daldinia eschscholtzii* (MF579574.1)
47	1X-92	KM357330	*Daldinia eschscholtzii*	97	99	*Daldinia eschscholtzii* (KC895542.1)
48	Y2-35	KM357331	*Ramichloridium apiculatum*	99	98	*Ramichloridium apiculatum* (JN850989.1)
49	5S-67	KM357332	*Chaetomium cupreum*	92	99	*Chaetomium cupreum* (JQ676206.1)
50	6A2'-b1	KM357333	*Mucor abundans*	96	97	*Mucor abundans* (KF305757.1)

Note: Amplified primers were ITS1 and ITS4

Table 1. The results of molecular identification. (continued)

Numbers	Isolate obtained in the present study	Accession no. of isolate obtained in the present study	Morphotype	Query coverage (%)	Identity (%)	Most closely related species (accesssion no.)
51	3S-41	KM357334	*Coprinellus disseminates*	98	97	*Coprinellus disseminates* (JN159560)
52	5X-30	KM357335	*Penicillium biourgeianum*	96	99	*Penicillium biourgeianum* (HM210835.1)
53	1X-46	KM357336	*Penicillium chrysogenum*	99	99	*Penicillium chrysogenum* (JF731255.1)
54	9G-95	KM357337	*Trichoderma pleuroticola*	97	99	*Trichoderma pleuroticola* (HM142362.1)
55	9B3'-Y-Y1	KM357338	*Penicillium daleae*	98	99	*Penicillium daleae* (AF033442.1)
56	8G-91	KM357339	*Penicillium expansum*	98	96	*Penicillium expansum* (HM469423.1)
57	1S-16	KM357340	*Penicillium dipodomyicola*	99	97	*Penicillium dipodomyicola* (FJ025172.1)
58	10G-54	KM357341	*Panellus stypticus*	98	85	*Panellus stypticus* (AB863032.1)
59	1X-61	KM357342	*Pestalotiopsis karstenii*	97	98	*Pestalotiopsis karstenii* (AY681473.1)
60	Y1-41	KM357343	unidentified 1	90	92	unidentified 1 (EU686126.1)
61	2S-13	KM357344	unidentified 2	97	98	unidentified 2 (KT957786.1)
62	8J-37	KM357345	unidentified 3	98	91	unidentified 3 (KF435314.1)
63	Y4-111	KM357346	unidentified 4	97	95	unidentified 4 (KR015700.1)
64	5S-63	KM357347	unidentified 5	99	98	unidentified 5 (KX722228.1)
65	1X-10	KM357348	*Fusarium* sp.	100	99	*Fusarium* sp. (AF178394)
66	2-J7-56	KM357349	*Melanconium* sp.	100	100	*Melanconium* sp. (KF572475)
67	J121	KM357350	*Phoma* sp.	100	99	*Phoma* sp. (AY10204)

Note: Amplified primers were ITS1 and ITS4

$$CS = 2j/(a+b),$$

where j is the number of endophytic fungal species co-existing in two treatments, a is the total number of endophytic fungal species in one treatment, b is the total number of endophytic fungal species in another treatment [19]. The least significant difference (LSD) test was used to compare the difference in the CR and H' of endophytes between two types of plants, and the rejection level was set at $p< 0.05$. All data were analyzed by SPSS 17.0.

3. Results and Discussion

3.1 Colonization Rate of Endophytic Fungi

The colonization rate (CR) is an indication of the number of endophytic fungi in host plants and is known to vary with the altitude, humidity, precipitation, temperature and plant community [4,21,22]. In the present study, the CR of EF of bryophytes, pteridophytes and spermatophytes were 97.92%, 98.75% and 98.13%, respectively ($P> 0.05$, LSD test, Table 2), and the CR of EF from nine plant species ranged from 94.38% to 100%. The highest CR appeared in *H. splendens*, *C. petelotii*, *E. polypodioides* and *R. irroratum*, which was 100%, whereas the lowest was found in *T. flousiana*, with only 94.38%. The CR obtained was higher than those of bryophytes, pteridophytes and spermatophytes collected from other environments. For example, Davis and Shaw reported that the CR of three bryophytes from tropical and temperate ecosystems ranged from 60.9% to 96.6% [23]; Qian *et al.* found that the CR of seven pteridophytes from potassium mine areas ranged from 14.17% to 22.92% [24]; Gong and Guo found the CR of two spermatophytes from Jinghong city, Xishuangbanna ranged from 34% to 80% [25]. The results indicated that bryophytes, pteridophytes and spermatophytes from Dawei Mountain harboured more fungal endophytes than those from other environments.

A total of 2230 endophytic fungi (EF) were isolated from 1440 tissue segments of

Table 2. Number and colonization rate (CR) of endophytic fungi (EF) from nine plant species.

Host Plants		No.of segments plated (No.of segments colonized by EF)			No. of EF isolated			CR(%)		
		Leaf (Photosynthetic tissues)	Stem (Rhizoid)	Total	Leaf (Photosynthetic tissues)	Stem (Rhizoid)	Total	Leaf (Photosynthetic tissues)	Stem (Rhizoid)	Total
Bryophyte	*M.polymorpha*	80(80)	80(75)	160(155)	157	126	283	100a	93.75a	96.88
	P.commune	80(75)	80(80)	160(155)	118	69	187	93.75a	100a	96.88
	H.splendens	80(80)	80(80)	160(160)	127	81	208	100a	100a	100
Pteridophyte	*P.maxima*	80(80)	80(75)	160(155)	111	101	212	100a	93.75a	96.88
	D.aspidioide	80(80)	80(79)	160(159)	166	80	246	100a	98.75a	99.38
	C.petelotii	80(80)	80(80)	160(160)	173	146	319	100a	100a	100
Spermatophyte	*T.flousiana*	80(80)	80(71)	160(151)	113	124	237	100a	88.75a	94.38
	E.polypodioid	80(80)	80(80)	160(160)	165	147	312	100a	100a	100
	R.irroratum	80(80)	80(80)	160(160)	111	115	226	100a	100a	100
Total	Total	720(715)	720(700)	1440(1415)	1241	989	2230	99.31a	97.22a	98.27

Note: "a" means significant difference at $p< 0.05$ level.

nine plant species, the number of EF isolated from three bryophytes, three pteridophytes and three spermatophytes were 678, 777 and 775, respectively (Table 2). It was found that the number of EF from bryophytes was slightly lower than that of pteridophytes or spermatophytes. This may result from lower evolutionary degree of bryophytes: as they contain underdeveloped transport tissues and without real roots. Thereby, bryophytes cannot absorb and transport water and nutrients from the soil as efficiently as that of pteridophytes or spermatophytes, which affects the number of endophytes. The other possible reason may be that bryophytes are small and usually live under the shade of other plants, thus have less solar synthesis of carbohydrates to support more endophytes [26-27].

3.2 Composition of Endophytic Fungi

The EF from nine plant species were identified as belonging to 61 taxa (Table 3), the number of taxa found in bryophytes, pteridophytes and spermatophytes were 45, 42 and 39, respectively. Only 21 taxa were found co-existing in bryophytes, pteridophytes and spermatophytes, such as *Colletotrichum gloeosporioides*, *Trichoderma viride* and *Xylaria* sp. (Table 4). *Colletrichum* and *Xylaria* were the most dominant genera, because they were widely distributed in all nine plant species. The total RF value of them was 40.00% and 23.68%, respectively. Endophytic *Colletotrichum* and *Xylaria* were very common, and they have been reported as the dominant fungal endophytes of various plant species from diverse environments [28-30]. The results indicated that environmental condition is one of the most important factors affecting endophytes composition, especially the dominant endophytes.

In addition to *Colletotrichum* and *Xylaria*, *Trichoderma* and *Penicillium* were the dominant genera in bryophytes, too (RF are 18.29% and 10.77% respectively). However, in addition to *Colletotrichum* and *Xylaria*, the dominant generum in pteridophytes and spermatophytes was *Phomopsis*, and the RF were 5.66% and 10.84%, respectively (Table 4). In the study of Naik *et al.* [31] and Jankowiak *et al.* [32], they also found that *Penicillium* and *Phomopsis* were the dominant endophytes in shrubby medical plants and *Abies alba* seedlings, respectively.

Contrary to the dominant genera, some endophytic fungi were rare and showed host

Table 3. Number, taxa, relative frequency (RF) and Shannon index (*H'*) of endophytic fungi (EF) isolated from the nine plant species studied.

Taxa	No. of strains isolated from each plant species (RF%)									
	M.polymorpha	*P.commune*	*H.splendens*	*P.maxima*	*D.aspidioide*	*C.petelotii*	*T.flousiana*	*E.polypodioid*	*R.irroratum*	Total (RF%)
Arthriniumarundinis	–	–	–	–	–	2(0.63)	–	–	–	2(0.09)
Beltrania rhombic	2(0.71)	–	–	1(0.47)	–	1(0.31)	–	–	–	4(0.18)
Bionectriarossmaniae	1(0.35)	–	–	–	1(0.41)	3(0.94)	–	–	–	5(0.22)
Calonectriaeucalyoti	4(1.41)	3(1.60)	2(0.96)	–	3(1.22)	10(3.13)	–	–	1(0.44)	23(1.03)
Ceratobasidium sp.	–	–	1(0.48)	1(0.47)	–	–	–	–	–	2(0.09)
Chaetomiumcupreum	–	–	2(0.96)	–	–	–	–	–	2(0.88)	4(0.18)
Chaetomiumglobosum	–	2(1.07)	–	–	–	–	2(0.84)	–	2(0.88)	6(0.27)
Cladosporiumtenuissimum	–	–	–	–	1(0.41)	–	–	–	–	1(0.04)
Colletotrichumgloeosporioides	109(38.52)	20(10.70)	1(0.48)	94(44.34)	148(60.16)	161(50.47)	36(15.19)	146(46.79)	22(9.73)	737(33.05)
Colletotrichumacutatum	11(3.89)	–	–	7(3.30)	8(3.25)	3(0.94)	–	10(3.20)	9(3.98)	48(2.15)
Colletotrichumboninense	1(0.35)	1(0.53)	1(0.48)	5(2.36)	28(11.38)	46(14.42)	15(6.33)	7(2.24)	3(1.33)	107(4.80)
Coprinellus disseminates	–	1(0.53)	–	–	–	–	–	–	–	1(0.04)
Cylindrocladiellasp.	–	–	–	1(0.47)	–	–	–	–	6(2.65)	7(0.31)
Daldiniaeschscholtzii	–	–	–	–	–	–	–	–	1(0.44)	1(0.04)
Diaporthe sp.	–	–	–	–	–	–	1(0.42)	–	–	1(0.04)
Fusarium sp.	7(2.47)	–	2(0.96)	–	–	6(1.88)	–	–	–	15(0.67)
Nemaniaprimolutea	–	2(1.07)	–	9(4.25)	–	–	3(1.27)	–	6(2.65)	20(0.90)
Guignardiamangiferae	1(0.35)	–	–	–	–	1(0.31)	1(0.42)	–	1(0.44)	4(0.18)
Helotiales sp.	–	–	–	1(0.47)	–	–	–	–	–	1(0.04)
Helotiales sp. 2	1(0.35)	1(0.53)	–	–	1(0.41)	1(0.31)	–	–	1(0.44)	5(0.22)
Humicola sp.	2(0.71)	–	–	1(0.47)	1(0.41)	1(0.31)	4(1.69)	1(0.32)	–	10(0.45)
Microdochium sp.	–	2(1.07)	–	–	–	14(4.39)	–	–	–	16(0.72)
Mortierella sp.	–	8(4.28)	–	–	–	–	–	–	1(0.44)	9(0.40)
Mucorabundans	–	–	–	–	–	–	–	–	1(0.44)	1(0.04)
Mucorsp.	5(1.77)	6(3.21)	–	2(0.94)	–	2(0.63)	–	–	–	15(0.67)
Muscodoralbus	–	2(1.07)	–	–	–	–	1(0.42)	1(0.32)	–	4(0.18)
Nigrosporaoryzae	12(4,24)	12(6.42)	5(2.40)	5(2.36)	–	–	6(2.53)	11(3.53)	12(5.31)	63(2.83)
Penicilliumbiourgeianum	4(1.41)	9(4.81)	53(25.48)	–	–	2(0.63)	1(0.42)	–	1(0.44)	70(3.14)
Penicilliumchrysogenum	–	–	5(2.40)	–	–	–	–	–	–	5(0.22)
Penicilliumdaleae	1(0.35)	–	–	–	–	–	–	–	–	1(0.04)
Penicilliumexpansum	–	–	1(0.48)	1(0.47)	–	–	–	–	–	2(0.09)
Pestalotiopsiscocculi	–	–	–	1(0.47)	–	–	2(0.84)	1(0.32)	4(1.77)	8(0.36)
Pestalotiopsiskarstenii	13(4.59)	1(0.53)	–	1(0.47)	2(0.81)	4(1.25)	1(0.42)	–	2(0.88)	24(1.08)
Peziculacarpinea	–	–	–	–	3(1.22)	–	–	1(0.32)	–	4(0.18)
Plectosphaerellacucumerina	–	2(1.07)	–	1(0.47)	1(0.41)	2(0.63)	–	1(0.32)	1(0.44)	8(0.36)
Phialocephalasp.	–	–	–	1(0.47)	–	–	1(0.42)	–	–	2(0.09)
Phomaherbarum	9(3.18)	–	–	–	–	–	–	–	–	9(0.40)
Phoma sp.	–	–	–	–	–	–	4(1.69)	–	–	4(0.18)
Phomopsisamygdali	–	2(1.07)	–	–	1(0.41)	2(0.63)	–	1(0.32)	–	6(0.27)
Phomopsis sp.	7(2.47)	1(0.53)	–	12(5.66)	8(3.25)	21(6.58)	23(9.70)	42(13.46)	18(7.96)	132(5.92)
Ramichloridiumapiculatum	1(0.35)	1(0.53)	–	–	–	–	–	–	1(0.44)	3(0.13)
Rhizomucorvariabilis	–	7(3.74)	–	–	–	–	–	–	–	7(0.31)
Trametesversicolor	–	–	–	–	–	–	1(0.42)	–	–	1(0.45)
Trichodermaasperellum	14(4.95)	36(19.25)	5(2.40)	–	–	1(0.31)	–	–	–	56(2.51)

Note: '-' indicates that the taxa were not found in plants.

Table 3. Number, taxa, relative frequency (RF) and Shannon index (*H'*) of endophytic fungi (EF) isolated from the nine plant species studied. (continued)

Taxa	No. of strains isolated from each plant species (RF%)									
	M.polymorpha	*P.commune*	*H.splendens*	*P.maxima*	*D.aspidioide*	*C.petelotii*	*T.flousiana*	*E.polypodioid*	*R.irroratum*	Total (RF%)
Trichodermaharzianum	25(8.83)	20(10.70)	–	–	–	–	–	–	10(4.42)	55(2.47)
Trichodermaviride	22(7.77)	2(1.07)	–	5(2.36)	2(0.81)	–	–	1(0.32)	13(5.75)	45(2.02)
Umbelopsisisabellina	–	6(3.21)	2(0.96)	–	–	1(0.31)	–	–	–	9(0.40)
Xylariales sp.	12(4.24)	–	–	1(0.47)	–	1(0.31)	–	–	–	14(0.63)
Xylaria sp. 1	2(0.71)	5(2.67)	87(41.83)	28(13.21)	21(8.54)	24(7.52)	98(41.35)	49(15.71)	59(26.11)	373(16.73)
Xylaria sp. 2	–	1(0.53)	–	2(0.94)	2(0.81)	3(0.94)	–	–	–	8(0.36)
Xylaria sp. 3	10(3.53)	3(1.60)	10(4.81)	27(12.74)	3(1.22)	1(0.31)	15(6.33)	26(8.33)	20(8.85)	115(5.16)
Xylaria sp. 4	–	–	–	1(0.47)	–	–	2(0.84)	1(0.32)	4(1.77)	8(0.36)
Xylaria sp. 5	–	–	–	1(0.47)	–	1(0.31)	2(0.84)	2(0.64)	4(1.77)	10(0.45)
Unidentified 1	–	29(15.51)	10(4.81)	–	1(0.41)	1(0.31)	1(0.42)	1(0.32)	7(3.10)	50(2.24)
Unidentified 2	–	–	9(4.33)	3(1.42)	1(0.41)	1(0.31)	8(3.38)	10(3.21)	12(5.31)	44(1.97)
Unidentified 3	–	–	7(3.37)	–	3(1.22)	–	–	–	1(0.44)	11(0.49)
Unidentified 4	3(1.06)	–	5(2.40)	–	–	1(0.31)	–	–	1(0.44)	10(0.45)
Unidentified 5	–	–	–	–	–	–	9(3.80)	–	–	9(0.40)
Unidentified 6	–	–	–	–	7(2.85)	2(0.63)	–	–	–	9(0.40)
Unidentified 7	2(0.71)	2(1.07)	–	–	–	–	–	–	–	4(0.18)
Unidentified 8	2(0.71)	–	–	–	–	–	–	–	–	2(0.09)
Total	283(100)	187(100)	208(100)	212(100)	246(100)	319(100)	237(100)	312(100)	226(100)	2230(100)
H'	2.41	2.7	1.89	2.04	1.6	1.91	2.1	1.74	2.7	2.67

Note: '-' indicates that the taxa were not found in plants.

Table 4. Number, taxa, relative frequency (RF) of endophytic fungi (EF) from bryophytes, pteridophytes and spermatophytes.

Taxa	No. of strains isolated from bryophytes, pteridophytes and spermatophytes (RF%)			
	Bryophytes	Pteriodophytes	Spermatophytes	Total (RF%)
Arthriniumarundinis	–	2(0.26)	–	2(0.09)
Beltrania rhombic	2(0.29)	2(0.26)	–	4(0.18)
Bionectriarossmaniae	1(0.15)	4(0.51)	–	5(0.22)
Calonectriaeucalyoti	9(1.33)	13(1.67)	1(0.13)	23(1.03)
Ceratobasidium sp.	1(0.15)	1(0.13)	–	2(0.09)
Chaetomiumcupreum	2(0.29)	–	2(0.26)	4(0.18)
Chaetomiumglobosum	2(0.29)	–	4(0.52)	6(0.27)
Cladosporiumtenuissimum	–	1(0.13)	–	1(0.04)
Colletotrichumgloeosporioides	130(19.17)	403(51.87)	204(26.32)	737(33.05)
Colletotrichumacutatum	11(1.62)	18(2.32)	19(2.45)	48(2.15)
Colletotrichumboninense	3(0.44)	79(10.67)	25(3.22)	107(4.80)
Coprinellus disseminates	1(0.15)	–	–	1(0.04)
Cylindrocladiella sp.	–	1(0.13)	6(0.77)	7(0.31)
Daldiniaeschscholtzii	–	–	1(0.13)	1(0.04)
Diaporthe sp.	–	–	1(0.13)	1(0.04)
Fusarium sp.	9(1.33)	6(0.77)	–	15(0.67)

Note: '-' indicates the taxa were not found in plants.

Table 4. Number, taxa, relative frequency (RF) of endophytic fungi (EF) from bryophytes, pteridophytes and spermatophytes. (continued)

Taxa	No. of strains isolated from bryophytes, pteridophytes and spermatophytes (RF%)			
	Bryophytes	Pteriodophytes	Spermatophytes	Total (RF%)
Nemaniaprimolutea	2(0.29)	9(1.16)	9(1.16)	20(0.90)
Guignardiamangiferae	1(0.15)	1(0.13)	2(0.26)	4(0.18)
Helotiales sp.	–	1(0.13)	–	1(0.04)
Helotiale ssp. 2	2(0.29)	2(0.26)	1(0.13)	5(0.22)
Humicola sp.	2(0.29)	3(0.39)	5(0.65)	10(0.45)
Microdochium sp.	2(0.29)	14(1.80)	–	16(0.72)
Mortierella sp.	8(1.18)	–	1(0.13)	9(0.40)
Mucorabundans	–	–	1(0.13)	1(0.04)
Mucor sp.	11(1.62)	4(0.51)	–	15(0.67)
Muscodoralbus	2(0.29)	–	2(0.26)	4(0.18)
Nigrosporaoryzae	29(4.28)	5(0.64)	29(3.74)	63(2.83)
Penicilliumbiourgeianum	66(9.73)	2(0.26)	2(0.26)	70(3.14)
Penicilliumchrysogenum	5(0.74)	–	–	5(0.22)
Penicilliumdaleae	1(0.15)	–	–	1(0.04)
Penicilliumexpansum	1(0.15)	1(0.13)	–	2(0.09)
Pestalotiopsiscocculi	–	1(0.13)	7(0.90)	8(0.36)
Pestalotiopsiskarstenii	14(2.06)	7(0.90)	3(0.39)	24(1.08)
Peziculacarpinea	–	3(0.39)	1(0.13)	4(0.18)
Plectosphaerellacucumerina	2(0.29)	4(0.51)	2(0.26)	8(0.36)
Phialocephala sp.	–	1(0.13)	1(0.13)	2(0.09)
Phomaherbarum	9(1.33)	–	–	9(0.40)
Phoma sp.	–	–	4(0.52)	4(0.18)
Phomopsisamygdali	2(0.29)	3(0.39)	1(0.13)	6(0.27)
Phomopsis sp.	8(1.18)	41(5.28)	83(10.71)	132(5.92)
Ramichloridiumapiculatum	2(0.29)	–	1(0.13)	3(0.13)
Rhizomucorvariabilis	7(1.03)	–	–	7(0.31)
Trametesversicolor	–	–	1(0.13)	1(0.45)
Trichodermaasperellum	55(8.11)	1(0.13)	–	56(2.51)
Trichodermaharzianum	45(6.64)	–	10(1.29)	55(2.47)
Trichodermaviride	24(3.54)	7(0.90)	14(1.81)	45(2.02)
Umbelopsisisabellina	8(1.18)	1(0.13)	–	9(0.40)
Xylariales sp.	12(1.77)	2(0.26)	–	14(0.63)
Xylariasp. 1	94(13.86)	73(9.40)	206(26.58)	373(16.73)
Xylariasp. 2	1(0.15)	7(0.90)	–	8(0.36)
Xylariasp. 3	23(3.39)	31(3.99)	61(7.87)	115(5.16)
Xylariasp. 4	–	1(0.13)	7(0.90)	8(0.36)
Xylariasp. 5	–	2(0.26)	8(1.03)	10(0.45)
Unidentified 1	39(5.75)	2(0.26)	9(1.16)	50(2.24)
Unidentified 2	9(1.33)	5(0.64)	30(3.87)	44(1.97)
Unidentified 3	7(1.03)	3(0.39)	1(0.13)	11(0.49)
Unidentified 4	8(1.18)	1(0.13)	1(0.13)	10(0.45)
Unidentified 5	–	–	9(1.16)	9(0.40)

Note: '-' indicates the taxa were not found in plants.

Table 4. Number, taxa, relative frequency (RF) of endophytic fungi (EF) from bryophytes, pteridophytes and spermatophytes. (continued)

Taxa	No. of strains isolated from bryophytes, pteridophytes and spermatophytes (RF%)			
	Bryophytes	Pteriodophytes	Spermatophytes	Total (RF%)
Unidentified 6	–	9(1.16)	–	9(0.40)
Unidentified 7	4(0.59)	–	–	4(0.18)
Unidentified 8	2(0.29)	–	–	2(0.09)
Total	678(100)	777(100)	775(100)	2230(100)
H'	2.92	2.02	2.34	2.67

Note: '-' indicates the taxa were not found in plants.

specificity or tissue preference. For example, *Penicillium chrysogenum* and *P. daleae* were found only in bryophytes, *Cladosporium tenuissimum* and fungi belonging to Helotiales order were found only in pteridophytes, while *Mucorabundans* was found only in *R. irroratum*, *Trichoderma harzianum* and *Pezicula carpinea* were found only in the stems of spermatophytes, and *Diaporthe* sp. was only reported in the leaves of spermatophytes (Table 4). The same phenomenon was found in previous studies [33-34]. These results suggest that the colonization and distribution of endophytic fungi may be influenced by the texture, physiology and chemistry of the plant/tissue [35-36].

3.3 Diversity and Similarity of Endophytic Fungi

The *H'* of EF from bryophytes, pteridophytes and spermatophytes were 2.92, 2.02 and 2.34, respectively (Table 4), and it showed no significant difference ($P > 0.05$, LSD test), which was slightly higher than that of other plants, such as 1.25-2.70 of five plant species from Baima Snow Mountain [33]; 1.60-2.31 of *Cinnamomum bejolghota* from Northern Thailand [36=7].

The *CS* of EF from bryophytes, pteridophytes and spermatophytes ranged from 0.64 to 0.74, and the *CS* of EF from nine plant species ranged from 0.37 to 0.68 (Table 5). Among them, the *CS* of EF from three bryophytes ranged from 0.44 to 0.58, which were higher than those of three bryophytes from Antarctic region (0.18-0.40) [38]. While, the *CS* of EF from three pteridophytes (0.52-0.68) were close to those of seven pteridophytes from potassium mine areas (0.50-0.87) [23], and the *CS* of EF from three spermatophytes (0.58-0.63) were lower than those of seven spermatophytes from five places of Yunnan province (0.95-1.91) [39]. In addition, it was found that the highest *CS* was found between *C. petelotii* (pteridophyte) and *M.*

Table 5. The Sorenson's coefficient similarity indices of fungal endophytes from nine plant species.

Plant	*M.polymorpha*	*P.commune*	*H.splendens*	*P.ma Xima*	*D.aspidioides*	*C.petelotii*	*T.flousiana*	*E.polypodioides*
P.commune	0.58							
H.splendens	0.44	0.44						
P.maxima	0.5	0.45	0.37					
D.aspidioides	0.5	0.53	0.41	0.52				
C.petelotii	0.68	0.6	0.51	0.56	0.68			
T.flousiana	0.4	0.47	0.39	0.58	0.41	0.46		
E.polypodioides	0.4	0.48	0.39	0.65	0.67	0.51	0.63	
R.irroratum	0.56	0.62	0.5	0.58	0.55	0.54	0.6	0.58

polymorpha (bryophyte). The results indicated that the *CS* of EF of two plant species with different evolutional degrees was not always lower than that of two plant species with the same evolutional degree.

4. Conclusion

The results of this study revealed a total of 2230 EF isolated from 1440 segments of nine plant species (*M. polymorpha*, *P. commune*, *H. splendens D. spidiodes, C.petelotii*, *P. maxima T. flousiana*, *E. polypodioides* and *R. irroratum*). The colonization rate (CR) of bryophytes, pteridophytes and spermatophytes were 97.92%, 98.75% and 98.13%, respectively. The EF were identified as belonging to 61 taxa, of which *Colletotrichum* and *Xylaria* were the most dominant genera. In addition, 21 taxa were found both in bryophytes, pteridophytes and in spermatophytes. The *H′* and the *CS* of EF from bryophytes, pteridophytes and spermatophytes ranged from 2.02 to 2.92 and 0.64 to 0.74, respectively. The *CS* of EF of two plant species with different evolutional degree was not always lower than that of two plant species with the same evolutional degree. Our results indicate that the diversity and community structure of culturable endophytic fungi associated with bryophytes, pteridophytes and spermatophytes are different. The present study is the first report on the difference of community structure of EF associated with plants belonging to different evolutionary degrees. This appears to be a consequence for the possible role of endophytes in plants evolution process. Further investigations on the endophytes in plants evolution are necessary for the future.

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