Ann Microbiol (2014) 64:1275–1290
DOI 10.1007/s13213-013-0772-0

ORIGINAL ARTICLE

Soil fungal diversity in three nature reserves of Jiuzhaigou County, Sichuan Province, China

You Zhou · Shuting He · Guoshu Gong · Shirong Zhang · Xiaoli Chang · Na Liu · Xiaofang Sun · Xiaobo Qi · Kunhao Ye · Yiyu Wang

Received: 3 July 2013 / Accepted: 8 November 2013 / Published online: 3 January 2014

Abstract Jiuzhaigou County is located at the southern transition zone of Sichuan Basin and the Qinghai–Tibet Plateau and is the site of three famous nature reserves, namely, the Jiuzhaigou Nature Reserve (JZNR), Baihe Nature Reserve (BHNR) and Wujiao Nature Reserve (WJNR). The soil fungal diversity in this region has not yet been investigated. In this study, we collected 25 soil samples from these three nature reserves. Soil fungi were isolated using the soil dilution plate technique and Rose Bengal agar medium. The culturable soil fungal density based on analysis of the 25 samples ranged from 2.18 log to 4.38 log CFU g^{-1} dry weight soil, with the fungal density being highest in samples from JZNR and lowest in those from BHNR. Based on morphological characters and the results of phylogenetic analysis of the internal transcribed spacer (ITS) of the rDNA operon, we identified 38 genera (two genera could not be identified) belonging to Ascomycota, Zygomycota and Basidiomycota. The dominant genera were *Penicillium*, *Humicola*, *Aspergillus* and *Trichoderma*. The species richness index S, biodiversity index H' and evenness index E of the 25 sampling sites were in the range 10–29, 1.96–3.05 and 0.74–0.95, respectively. The highest mean values of the S, H' and E indices were in soil samples from BHNR, where the values of these indices were 20.00, 2.66 and 0.90, respectively. These results indicate that the diversity of culturable fungi in these three nature reserves was high. Furthermore, a total 14 *Trichoderma* isolates were tested for their antagonism activity against mycelium growth of three pathogens: *Bipolaris maydis*, *Curvularia lunata*, *Rhizoctonia solani*. The results showed that six *Trichoderma* isolates had good antagonistic effects on the three pathogenic fungi.

Keywords Jiuzhaigou County · Nature reserve · Soil fungal diversity · Phylogenetic analysis

Introduction

It has been estimated that there are 712,000 extant fungal species worldwide (Schmit and Mueller 2007). Soil is an important fungal habitat, and the great majority of fungal species spend at least some part of their life cycles in the soil environment (Bridge and Spooner 2001). Fungi play a crucial role in the terrestrial ecosystem, and they are responsible for many key steps in the maintenance of ecosystem stability, particularly by recycling the soil organic matter and mineral elements (e.g. cellulose, lignin, carbon, nitrogen, phosphorus) (Gadd 2007; Barrico et al. 2010; Hollister et al. 2010), and their diversity and activity reflect soil health (Mueller et al. 2004; Wardle et al. 2006; Singh et al. 2007). Fungal functional diversity is intimately related to the taxonomic community structures (Zak and Visser 1996; Deacon et al. 2006). On the one hand, the structure of soil fungal communities vary depending on different ecological factors (Buee et al. 2011), including altitude (Laganà et al. 1999; Pan et al. 2009), climate (Persiani et al. 1998; Satish et al. 2007; McGuire et al. 2012), species and age of the vegetation (West and Jones 2000; Dong et al. 2004; Curlevski et al. 2010; Nie et al. 2012), soil nutrients (Lejon et al. 2005; Kara and Asan 2007; Thoms et al. 2010) and human disturbances (Cabello and Arambarri 2002; Bastias et al. 2006). On the other hand, they are significantly affected by fertilizer (Schneider et al.

You Zhou and Shuting He contributed equally to this work.

Y. Zhou · S. He · G. Gong (✉) · X. Chang · N. Liu · X. Sun · X. Qi · K. Ye · Y. Wang
Department of Plant Pathology, College of Agriculture, Sichuan Agricultural University, Chengdu 611130, People's Republic China
e-mail: guoshugong@126.com

S. Zhang
College of Resources and Environment, Sichuan Agricultural University, Chengdu 611130, People's Republic China

2010; Jirout et al. 2011) and tillage management (Wu et al. 2007, 2008; Entry et al. 2008; Wang et al. 2010; Zhang et al. 2011) of agricultural land. These findings suggest that soil fungal community structures are good tools for monitoring the changes in environmental conditions (Grishkan et al. 2008; Nie et al. 2012).

However, it is still difficult to isolate all soil fungi due to the limitations in available culture methods. The soil dilution plate method is a relatively reliable and widely employed approach by which to characterize the communities of culturable soil fungi (West and Jones 2000; Cabello and Arambarri 2002; Grishkan et al. 2006, 2009; Nesci et al. 2006; Pan et al. 2009; Wang et al. 2010; Arenz et al. 2011; Arenz and Blanchette 2011). Traditional fungal identification is mainly based on morphological criteria. However, some fungi do not form spores or fruiting body when cultured on/in an artificial substrate (Lim et al. 2005), causing great difficulties for identification purposes. Developments in biotechnology have resulted in new and improved methods for identifying fungi, including; these include the use of the internal transcribed spacer (ITS) of the rDNA operon, beta-tubulin gene and translation elongation factor 1 alpha gene (Alves et al. 2008; Phillips et al. 2008; Tanaka et al. 2009). The ITS region is widely used for identifying fungi and is regarded as the most powerful and reliable tool for the accurate identification of fungi (Henry et al. 2000; Anderson et al. 2003; Anderson and Parkin 2007; Ortega et al. 2008; Wang et al. 2008). However, nucleotide databases do not cover all fungal taxa, especially when the fungal sequences show only low similarity with sequences in nucleotide databases, thereby hampering the application of molecular identification techniques (Lim et al. 2005). It would therefore appear that the use of morphological characteristics coupled with molecular analysis can provide a more accurate identification of fungi.

The Jiuzhaigou Nature Reserve, Baihe Nature Reserve and Wujiao Nature Reserve are located in Jiuzhaigou County, at the southern edge of Sichuan Basin and the Qinghai-Tibet Plateau transition zone, China. Newly explored and unexplored habitats are important potential sites for discovering new fungal species (Hawksworth and Rossman 1997), and the soil fungal diversity in this region is noteworthy. Given that this area is characterized by its geographical location and low human disturbance, we have investigated the diversity of culturable soil fungi in the three nature reserves.

Materials and methods

Study site and soil sample collection

Jiuzhaigou County (area 5,290 km^2) is located at the southern edge of Sichuan Basin and the Qinghai–Tibet Plateau transition zone, China. Average temperature changes range between −3.7 °C and 16.8 °C (annual average 7.3 °C). Total annual rainfall is approximately 700–800 mm, with most precipitation occurring between May and September. Many of the sites are covered with forest, with an altitudinal variation ranging from mixed forest through coniferous forest to alpine meadows. The Jiuzhaigou Nature Reserve (hereinafter referred to as JZNR), Baihe Nature Reserve (hereinafter referred to as BHNR) and Wujiao Nature Reserve (hereinafter referred to as WJNR) are located in the southwest, northwest, and southeast parts of Jiuzhaigou County, respectively. JZNR is national nature reserve and famous for its State 4A-level scenery and its designations as a World Natural Heritage site and World Bio-sphere Reserve site. BHNR and WJNR are provincial nature reserves. All three nature reserves located far from industrial centers and human impact is low.

Soil samples were collected in July 2007 across the range of soil types, vegetation and altitudes of the three nature reserves. Global Positioning System technology (GPSMap76; Garmin Ltd., USA) was used to determine the sampling locations. We collected 25 soil samples in total (8 from JZNR, 8 from BHNR and 9 from WJNR). For each sample, five soil sub-samples were collected from the topsoil (depth 0–15 cm) from random positions of approximately 1.0 m^2 in size; these were then mixed to make up one sample for analysis.

After the removal of vegetation debris, approximately 300 g of the soil sampled from each sample site was immediately collected in sterile plastic bags, kept in the icebox, transported to the laboratory within 48 h and then stored at 4 °C. Details on the 25 soil sample sites are given in Table 1.

Fungal isolation and morphological identification

Soil fungi were isolated using the suspension plating method (Mueller et al. 2004). In brief, 10 g soil of each sample was added to 90 ml sterilized water, producing a soil slurry of 10^{-1} (w/v); this soil suspension was shaken for 15 min and diluted to final concentrations of 10^{-2} and 10^{-3}. Suspensions (1 ml) of different concentrations (10^{-1}, 10^{-2} and 10^{-3}) were placed in 90-mm diameter petri plate, and then the Rose Bengal agar medium (approximately 40 °C) was added and mixed evenly with the suspension. Sterilized water was used as the controls. The plates were kept in the dark at 25 °C for 5–7 days. Only plates containing 10–100 colony forming units (CFU) were used for counting CFU g^{-1} dry weight (DW) soil (Nesci et al. 2006; Wang et al. 2010). Three replicates were made for each concentration.

Single fungal colonies were transferred onto potato dextrose agar medium (PDA) for purification and then kept in tube slants of PDA for further taxonomic identification. All isolates were sub-cultured and initially grouped into

Table 1 The characteristics, biodiversity indices and density of 25 sampling sites

Sites[a]	Geographical location	Altitude (m a.s.l.)	Vegetation type	Index (*S*)	Index (*H′*)	Index (*E*)	CFU[b]
JZNR1	33°02'59.47", 103°56'07.20"	3,052	Shrub	23	2.96	0.94	3.33±0.03
JZNR2	33°03'01.09", 103°56'44.58"	3,232	Mixed forest	20	2.53	0.84	3.49±0.03
JZNR3	33°03'44.17", 103°52'04.86"	3,177	Mixed forest	28	3.03	0.91	3.51±0.02
JZNR4	33°03'56.46", 103°51'55.14"	3,096	Mixed forest	13	2.25	0.88	4.34±0.06
JZNR5	33°04'03.92", 103°51'46.86"	3,058	Mixed forest	12	1.96	0.79	4.37±0.04
JZNR6	33°07'14.90", 103°52'05.34"	2,702	Grassland	26	2.83	0.87	3.66±0.02
JZNR7	33°07'17.65", 103°51'54.54"	2,759	Grassland	15	2.20	0.81	3.60±0.04
JZNR8	33°07'23.70", 103°51'47.94"	2,849	Mixed forest	18	2.55	0.89	4.38±0.04
BHNR1	33°13'59.01", 104°07'29.80"	2,197	Mixed forest	18	2.71	0.94	3.51±0.04
BHNR2	33°14'07.40", 104°06'56.20"	2,402	Mixed forest	15	2.43	0.90	3.47±0.04
BHNR3	33°14'11.17", 104°07'19.17"	2,159	Mixed forest	29	2.99	0.89	2.82±0.03
BHNR4	33°14'21.44", 104°07'02.20"	2,176	Broadleaf forest	28	3.05	0.92	3.64±0.03
BHNR5	33°14'22.72", 104°07'18.01"	2,123	Broadleaf forest	16	2.48	0.89	2.53±0.03
BHNR6	33°14'44.54", 104°07'26.72"	2,042	Mixed forest	20	2.65	0.88	3.49±0.03
BHNR7	33°15'05.41", 104°07'53.10"	1,907	Broadleaf forest	10	1.97	0.86	3.61±0.03
BHNR8	33°15'24.20", 104°08'06.30"	1,855	Broadleaf forest	24	3.01	0.95	3.72±0.02
WJNR1	32°54'06.60", 104°14'54.90"	2,772	Broadleaf forest	19	2.17	0.74	3.83±0.04
WJNR2	32°58'17.23", 104°12'50.32"	2,242	Mixed forest	12	2.17	0.87	3.33±0.03
WJNR3	32°58'32.21", 104°13'02.80"	2,172	Broadleaf forest	19	2.71	0.92	3.46±0.03
WJNR4	32°59'19.40", 104°10'04.30"	2,361	Broadleaf forest	14	2.37	0.90	3.58±0.03
WJNR5	32°59'28.31", 104°09'55.00"	2,445	Grassland	21	2.89	0.95	3.69±0.01
WJNR6	32°59'42.00", 104°09'35.10"	2,551	Broadleaf forest	17	2.48	0.88	2.38±0.05
WJNR7	33°00'06.59", 104°11'06.65"	2,177	Mixed forest	14	2.28	0.86	2.18±0.04
WJNR8	33°02'54.34", 104°05'26.86"	2,127	Broadleaf forest	21	2.86	0.94	4.37±0.04
WJNR9	33°03'07.60", 104°04'57.70"	2,160	Broadleaf forest	26	2.51	0.77	3.77±0.03

S Species richness index, *H′* the biodiversity (Shannon–Wiener) index, *E* Pielou's evenness index

[a] JZNR, Jiuzhaigou Nature Reserve; BHNR, Baihe Nature Reserve; WJNR, Wujiao Nature Reserve

[b] CFU: Log CFU g^{-1} dry weight (DW) soil ± standard error (SE)

morphotypes based on morphological characters. Those fungi which could produce fruiting bodies on PDA or sporulation induction media (such as Czapek's medium, Sabouraud's medium, Oat medium, water agar, etc.) were first identified to genus or species level based on the morphological characteristics reported in original taxonomic papers and relevant taxonomic keys (Wei 1979; Barnett and Hunter 1987; Bissett 1984, 1991a, b, c; Qi 1997; Zhang 2003; Domsch et al. 2007; Kong 2007; Visagie 2008; Jaklitsch 2009, 2011; Samson et al. 2011; Li et al. 2012; Jurjevic et al. 2012). The remaining non-sporulating isolates were identified based only on ITS sequence comparison.

DNA extraction, PCR amplification and sequencing analysis

Genomic DNA of the representative isolates of 111 morphotypes was extracted from pure cultures as described by Barnes et al. (2001). The fungal ITS region was amplified using the primers ITS1 and ITS4 (Tanaka et al. 2009). The PCR reaction mixtures (50 μl) contained 1 μl of genomic DNA (about 100 ng), 1 μl of each primer (10 mM), 22 μl of sterile deionized water, 25 μl of 2×Taq PCR Mastermix (0.05 U/μl Taq DNA polymerase, recombinant); 4 mM $MgCl_2$; 0.4 mM dNTPs) (Sangon Biotech, China). The PCR amplification program consisted of 94 °C for 3 min, followed by 35 cycles of 94 °C for 45 s, 58 °C for 45 s and 72 °C for 1 min, with a final extension of 72 °C for 10 min. The PCR products were sequenced by Sangon Biotech on an ABI-PRISM3730 automated sequencer (Applied Biosystems, USA).

The obtained ITS sequences were compared by BLAST on GenBank of NCBI. The sequences of all samples and their closest matches were aligned by ClustalX (ver. 1.7) with some other reference ITS sequences (Wang et al. 2007), but ambiguous regions on both sides were excluded from the analysis. The phylogenetic tree was inferred from the neighbor–joining algorithm by MEGA5 with 1,000 bootstrap replicates (Tanaka et al. 2009; Tamura et al. 2011).

Soil fungal diversity analysis

The density of soil fungi was denoted in units of CFU g^{-1} DW soil (here expressed in log CFU) (Wang et al. 2010). The following diversity indices were calculated at the species level: (1) species richness (S, the number of different species in a soil sample); (2) the biodiversity index (Shannon–Wiener) ($H' = -\sum_{I=1}^{s} pi \ln pi$, where pi is the proportion of total species I in a soil sample); (3) the Pielou's evenness index ($E=H'/H\max$, $H'\max = \ln S$); (4) relative abundance, which is the number of species (or genus)×100/number of total species (or genus).

Measure of the *Trichoderma* antagonism

To measure the inhibitory effect of dual culture, we tested 14 *Trichoderma* isolates (JZ-8, JZ-25, JZ-66, JZ-67, JZ-69, JZ-77, JZ-82, JZ-90, JZ-129, JZ-132, JZ-149, JZ-161, JZ-165, JZ-179) for antagonism activity against three pathogens, namely, *Bipolaris maydis*, *Curvularia lunata* and *Rhizoctonia solani*, which were provided by the Department of Plant Pathology, Sichuan Agricultural University, China. The experiment was carried out according to the method described by Patil et al. (2012) with some minor modifications. A 5-day-old mycelial disc (diameter 5 mm) was cut out from the margin of actively growing cultures, including pathogens and *Trichoderma* spp. The mycelial discs of *Trichoderma* strain and pathogen were placed opposite to each other at a distance of approximately 4.5 cm on a PDA petri plate. The plate with only pathogen was used as the control. Experiments were repeated at least three times. All plates were incubated at 26 °C, and the mycelial growth of pathogens was measured after 6 days or the growth of control treatment covered the petri plate, whichever came first. The percentage inhibition of mycelial growth was calculated according to the formula:

$$D(\%) = [(L1-L2)/L1] \times 100\%$$

where D = the percentage of growth inhibition; L1 = the radial growth measurement of pathogen in the control plate; L2 = the radial growth of the pathogen in the presence of *Trichoderma* spp.

The method of Galletti et al. (2008) was used to measure the inhibitory effect of the *Trichoderma* culture filtrates. Briefly, Seven 5-day-old mycelial discs (diameter 5 mm) of *Trichoderma* were inoculated into 100-ml sterilized potato dextrose broth and cultured for 9 days in the dark at 26 °C with shaking (180 rpm. The liquid cultures were then centrifuged at 3,500 rpm for 10 min and passed through a filter membrane (pore size 0.45 μm); 4 ml sterilized culture filtrate was then added to 16 ml PDA (final concentration of culture filtrate 20 %, v/v) and poured into a 9-cm-diameter petri plate. After the agar had solidified, an approximately 5-day-old mycelial disc (diameter 5 mm) of pathogen was placed on the center of the dish and the dish incubated at 26 °C. PDA plates with only pathogen were used as the control, and there were three replicates for each treatment and control. The mycelial growth of pathogens was measured after 6 days or the growth of control treatment covered the petri plate, whichever came first. The percentage inhibition was calculated using the following formula:

$$F(\%) = [(L1-L2)/L1] \times 100\%$$

where F = percentage of growth inhibition; L1 = colony growth on the control plate; L2 = colony growth on the treatment plate.

Results

Abundance of soil fungi

There were obvious differences in fungal density in 25 sampling sites of Jiuzaigou County, ranging from 2.18 log to 4.38 log CFU g^{-1} DW soil (Table 1; Fig. 1); the mean fungal density of the 25 sampling sites was 3.52±0.57 log CFU g^{-1} DW soil (Table 2). The highest and lowest values were found in site JZNR8 and site WJNR7, respectively (Table 1). The mean densities of soil fungi in the three nature reserves was ordered as follows: JZNR (3.84±0.45 log CFU g^{-1} DW soil) > WJNR (3.40±0.70 log CFU g^{-1} DW soil) > BHNR (3.35± 0.43 log CFU g^{-1} DW soil) (Table 2).

Identification and phylogenetic analysis

A total of 2,143 fungal isolates were obtained from top soils in three nature reserves. We grouped all isolates into 111 different morphotypes and then selected 111 representative isolates from the 111 morphotypes for morphological and molecular characterization. Based on their morphological characters and ITS sequences, 109 morphotypes were identified as 38 known genera. The other two morphotypes (representative isolates JZ-13, JZ-72) were non-spore forming types, and their closest BLASTN sequences were from unidentified fungi. Phylogenetic analysis of the ITS sequences demonstrated that JZ-13 and JZ-72 were classified into different genera of Ascomycota (Fig. 2). Therefore, a total of 40 genera (73 species) were obtained from the topsoil in Jiuzhaigou County (Tables 3, 4).

The phylogenetic relationships of the 111 isolates with their closest BLASTN matches and other reference nucleotide sequences were inferred from the neighbor-joining analysis. In Fig. 2, these ITS sequences are classified into three groups

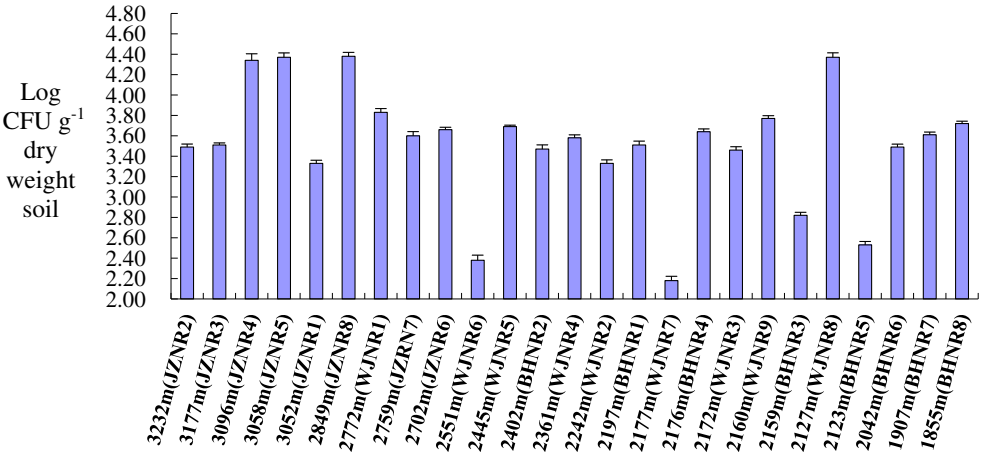

Fig. 1 Density of soil fungi in 25 soil samples taken from sampling sites at different altitudes (m a.sl.). *CFU* Colony forming units

(Ascomycota, Basidiomycota, Zygomycota). Except for JZ-70, all experimental sequences had strong affinities to their closest BLASTN sequences, which indicated that the phylogenetic tree coincided with the results based on morphological identification. Although the branch length of JZ-70 with *Absidia repens* (FJ849793) was >0.05 (Fig. 2), the morphological characteristics revealed that JZ-70 belonged to genus *Absidia* and that *Absidia glauca* (AY944880) also had a long-distance (>0.05) relationship with *Absidia repens* (FJ849793).

A high diversity of ITS sequences occurred in *Penicillium* and *Trichoderma* (*Hypocrea*), and the close phylogenetic relationship between the experimental and reference sequences indicated that these *Penicillium* and *Trichoderma* (*Hypocrea*) isolates were correctly distinguished or identified. However, the ITS variation was low between *Penicillium griseofulvum*, *P. turbatum* and *P. chrysogenum* (Fig. 2), which is in agreement with previously reported results (Skouboe et al. 1999). Isolates JZ-10, JZ-11, JZ-43 and JZ-53 had a strong affinity to described species. However, the four *Penicillium* isolates were not clearly identified based on phenotype. As we know, the asexual state of all *Hypocrea* is *Trichoderma* (Chaverri et al. 2001), and this point was also supported by our molecular data. Surprisingly, JZ-27 and JZ-28 did not form sexual and asexual spores, and they showed no *Trichoderma* morphological characters; therefore the two isolates were identified using ITS sequences and termed as *Hypocrea* sp. (Fig. 2).

Based on observable morphological characters (e.g. the color of colonies or other cultural characteristics, conidia shape and their formation), we classified the ten *Aspergillus* isolates into three different species and the 11 *Humicola* isolates into three different species. The ITS sequences of the *Aspergillus* and *Humicola* isolates identified in our study formed six and four groups in the phylogenetic tree,

Table 2 The biodiversity indices and density of soil fungi in the three nature reserves

Region	Index (*S*)		Index (*H'*)		Index (*E*)		CFU[a]	
JZNR	Mean	19.38	Mean	2.54	Mean	0.87	Mean	3.84
	Min	12	Min	1.96	Min	0.79	Min	3.33
	Max	28	Max	3.03	Max	0.94	Max	4.38
	SD	5.95	SD	0.38	Std. dev	0.05	SD	0.45
BHNR	Mean	20	Mean	2.66	Mean	0.90	Mean	3.35
	Min	10	Min	1.97	Min	0.86	Min	2.53
	Max	29	Max	3.05	Max	0.95	Max	3.72
	SD	6.61	SD	0.37	Std. dev	0.03	SD	0.43
WJNR	Mean	18.11	Mean	2.49	Mean	0.87	Mean	3.40
	Min	12	Min	2.17	Min	0.74	Min	2.18
	Max	26	Max	2.89	Max	0.95	Max	4.37
	SS	4.37	SD	0.28	Std. dev	0.07	SD	0.70
Total[b]	Mean	19.12	Mean	2.56	Mean	0.88	Mean	3.52
	Min	10	Min	1.96	Min	0.74	Min	2.18
	Max	29	Max	3.05	Max	0.95	Max	4.38
	SD	5.49	SD	0.34	Std. dev	0.06	SD	0.57

SD, Standard deviation; Max, maximum; Min, minimum

[a] CFU: Log CFU g^{-1} dry weight soil

[b] Total: Jiuzhaigou County as a whole (25 soil samples)

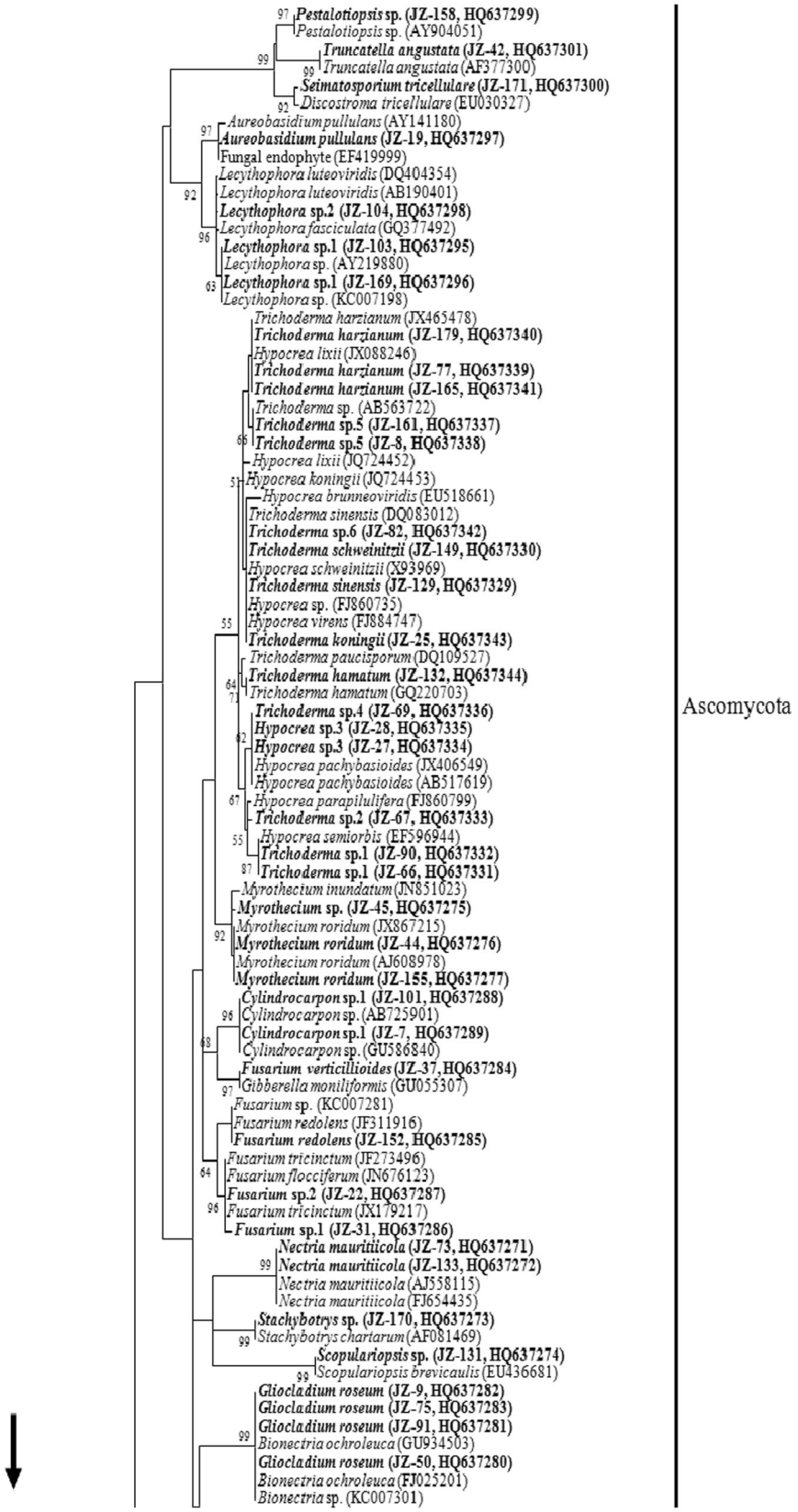

Fig. 2 Phylogenetic tree of 111 strains (*bold font*) with their closest BLASTN matches and other related taxa based on the internal transcribed spacer (ITS) sequence. *Numbers on branching points* ≥50 % bootstrap values of a bootstrap test of 1,000 runs, *number after species names* GenBank (ITS) accession number

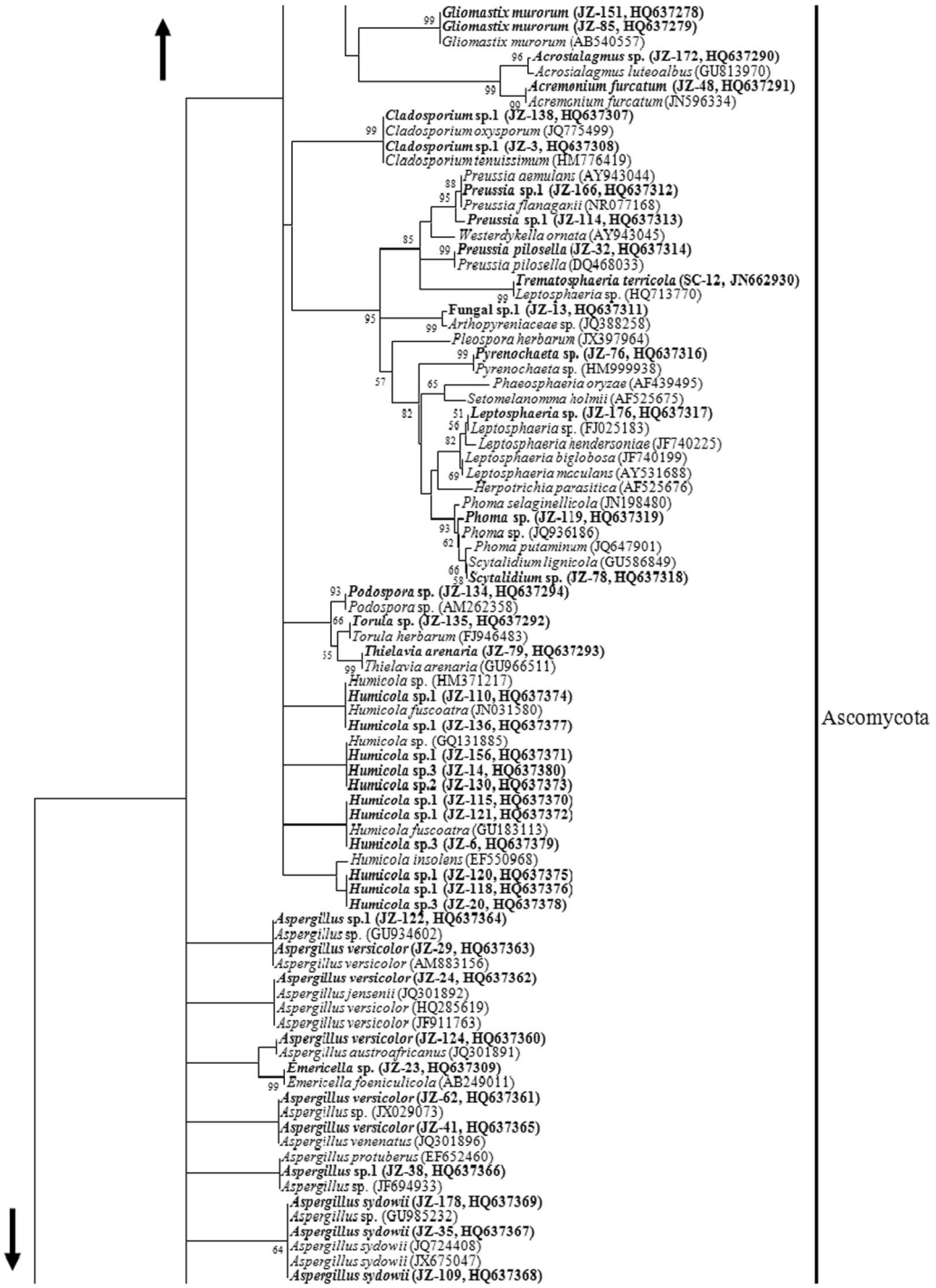

Fig. 2 (continued)

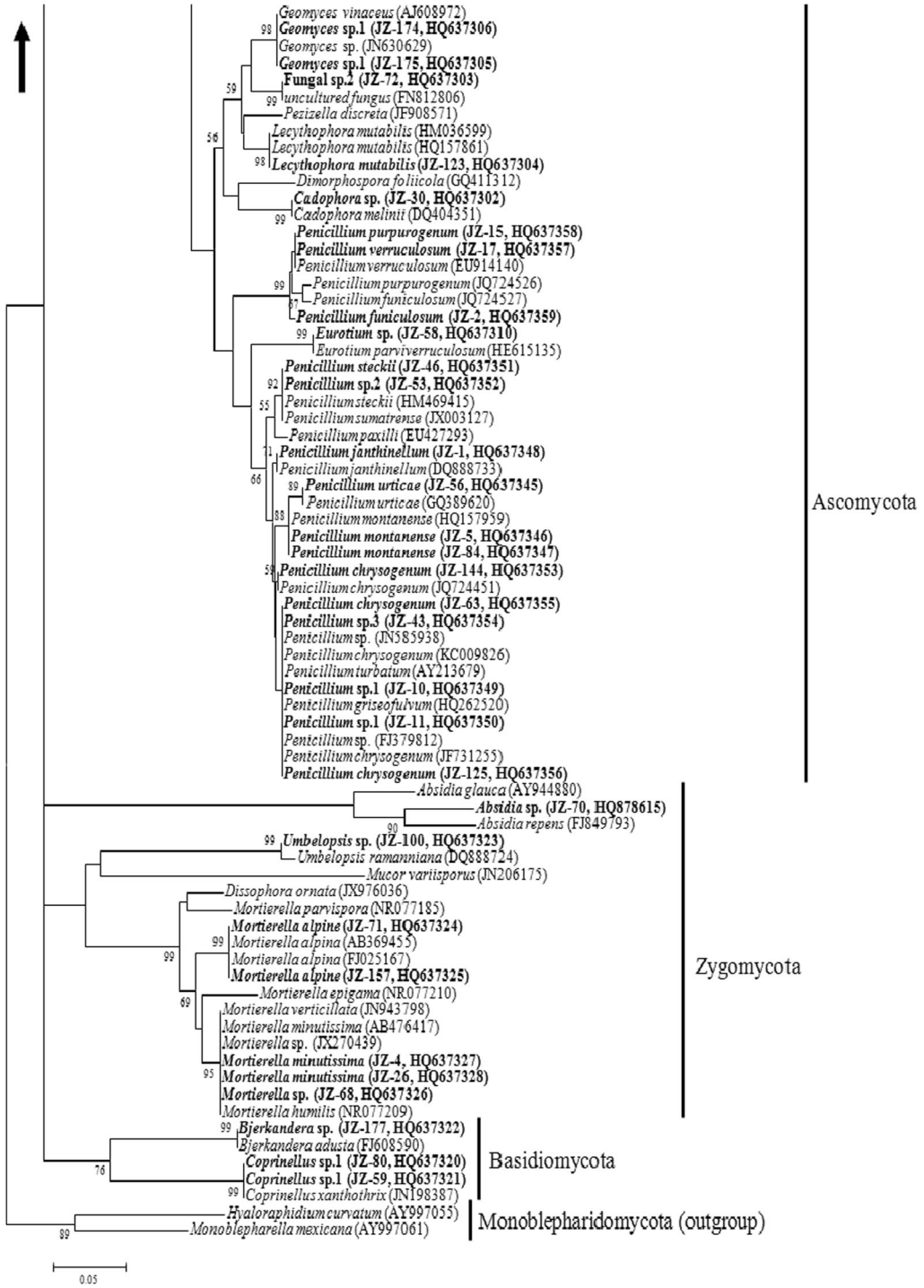

Fig. 2 (continued)

Table 3 The identification, closest BLASTN matches and relative abundance of isolated fungi

Representative isolates	Closest BLASTN matches	Sequence similarity (%)	Sequence coverage (%)	Species	Accession code	Relative abundance (%)		
						JZNR	BHNR	WJNR
JZ-73	*Nectria mauritiicola* (AJ558115)	99	97	*Nectria mauritiicola*	HQ637271	0.38	0.37	0.63
JZ-133	*Nectria mauritiicola* (FJ654435)	100	96	*Nectria mauritiicola*	HQ637272	0	0.86	0.38
JZ-170	*Stachybotrys chartarum* (AF081469)	99	98	*Stachybotrys* sp.	HQ637273	0	0.74	0.75
JZ-131	*Scopulariopsis brevicaulis* (EU436681)	98	97	*Scopulariopsis* sp.	HQ637274	0	1.36	2.25
JZ-45	Uncultured fungus (GU722059)	98	97	*Myrothecium* sp.	HQ637275	3.38	0.62	1.38
JZ-44	*Myrothecium roridum* (JX867215)	99	98	*Myrothecium roridum*	HQ637276	0	0.74	3.13
JZ-155	*Myrothecium roridum* (AJ608978)	99	98	*Myrothecium roridum*	HQ637277	0.75	0	1.88
JZ-151	*Gliomastix murorum* (AB540557)	99	98	*Gliomastix murorum*	HQ637278	1.88	2.22	0
JZ-85	*Gliomastix murorum* (AB540557)	99	98	*Gliomastix murorum*	HQ637279	0	0	4.88
JZ-50	*Bionectria ochroleuca* (FJ025201)	99	98	*Gliocladium roseum*	HQ637280	1.13	1.11	0
JZ-91	*Bionectria* sp. (KC007301)	99	98	*Gliocladium roseum*	HQ637281	0	0.37	1.38
JZ-9	*Bionectria ochroleuca* (GU934503)	99	98	*Gliocladium roseum*	HQ637282	0.38	0	0
JZ-75	*Bionectria ochroleuca* (HM113485)	99	96	*Gliocladium roseum*	HQ637283	0	0	0.88
JZ-37	*Gibberella moniliformis* (GU055307)	99	97	*Fusarium verticillioides*	HQ637284	0.38	1.36	0.50
JZ-152	*Fusarium* sp. (KC007281)	99	95	*Fusarium redolens*	HQ637285	1.88	0	0.38
JZ-31	*Fusarium flocciferum* (JN676123)	99	97	*Fusarium* sp.1	HQ637286	0	0	4.01
JZ-22	*Fusarium tricinctum* (JX179217)	99	97	*Fusarium* sp.2	HQ637287	0	4.56	0
JZ-101	*Cylindrocarpon* sp. (AB725901)	99	95	*Cylindrocarpon* sp.1	HQ637288	0	0	0.13
JZ-7	*Cylindrocarpon* sp. (GU586840)	99	92	*Cylindrocarpon* sp.1	HQ637289	0.19	0.12	0
JZ-172	*Acrostalagmus luteoalbus* (GU813970)	99	97	*Acrostalagmus* sp.	HQ637290	0.56	1.23	0.50
JZ-48	*Acremonium furcatum* (JN596334)	100	95	*Acremonium furcatum*	HQ637291	1.69	0.74	0
JZ-135	*Torula herbarum* (FJ946483)	99	98	*Torula* sp.	HQ637292	0.38	1.36	1.00
JZ-79	*Thielavia arenaria* (GU966511)	99	98	*Thielavia arenaria*	HQ637293	0.56	0.12	0.50
JZ-134	*Podospora* sp. (AM262358)	99	87	*Podospora* sp.	HQ637294	0	0	0.25
JZ-103	*Lecythophora* sp. (KC007198)	99	96	*Lecythophora* sp.1	HQ637295	0	0	0.50
JZ-169	*Lecythophora* sp. (AY219880)	98	94	*Lecythophora* sp.1	HQ637296	0.94	0.25	0
JZ-19	*Aureobasidium pullulans* (AY141180)	99	86	*Aureobasidium pullulans*	HQ637297	0.56	0.99	0.63
JZ-104	*Lecythophora luteoviridis* (DQ404354)	99	98	*Lecythophora* sp.2	HQ637298	1.50	0	0.88
JZ-158	*Pestalotiopsis* sp. (AY904051)	99	97	*Pestalotiopsis* sp.	HQ637299	1.31	0.12	0.88
JZ-171	*Discostroma tricellulare* (EU030327)	98	98	*Seimatosporium tricellulare*	HQ637300	0	0	0.38
JZ-42	*Truncatella angustata* (AF377300)	99	97	*Truncatella angustata*	HQ637301	0.38	1.11	0.25
JZ-30	*Cadophora melinii* (DQ404351)	99	95	*Cadophora* sp.	HQ637302	0.38	0.49	0.38
JZ-72	Uncultured fungus (FN812806)	99	96	Fungal sp.2	HQ637303	0.19	0	0
JZ-123	*Lecythophora mutabilis* (HQ157861)	99	98	*Lecythophora mutabilis*	HQ637304	0	0.74	0
JZ-175	*Geomyces vinaceus* (AJ608972)	99	98	*Geomyces* sp.1	HQ637305	1.88	0	0
JZ-174	*Geomyces* sp. (JN630629)	99	98	*Geomyces* sp.1	HQ637306	0	0.62	0
JZ-138	*Cladosporium oxysporum* (JQ775499)	99	92	*Cladosporium* sp.1	HQ637307	0.75	1.23	0.75
JZ-3	*Cladosporium cladosporioides* (HM776419)	99	99	*Cladosporium* sp.1	HQ637308	0	0	1.50
JZ-23	*Emericella foeniculicola* (AB249011)	100	96	*Emericella* sp.	HQ637309	0.38	1.73	0
JZ-58	*Eurotium parviverruculosum* (HE615135)	99	95	*Eurotium* sp.	HQ637310	1.13	0.86	0.88
JZ-13	Arthopyreniaceae sp. (JQ388258)	99	98	*Fungal* sp.1	HQ637311	0	0	0.63
JZ-166	*Preussia flanaganii* (NR_077168)	99	99	*Preussia* sp.1	HQ637312	0	0.37	0
JZ-114	*Preussia aemulans* (AY943044)	99	97	*Preussia* sp.1	HQ637313	0.75	0	0
JZ-32	*Preussia pilosella* (DQ468033)	98	90	*Preussia pilosella*	HQ637314	0	0	4.38
JZ-76	*Pyrenochaeta* sp. (FJ439593)	99	93	*Pyrenochaeta* sp.	HQ637316	0.19	0.62	0

Table 3 (continued)

Representative isolates	Closest BLASTN matches	Sequence similarity (%)	Sequence coverage (%)	Species	Accession code	Relative abundance (%)		
						JZNR	BHNR	WJNR
JZ-176	*Leptosphaeria* sp. (FJ025183)	100	96	*Leptosphaeria* sp.	HQ637317	2.44	0.25	0
JZ-78	*Scytalidium lignicola* (GU586849)	99	97	*Scytalidium* sp.	HQ637318	0.75	0.86	0.63
JZ-119	*Phoma* sp. (JQ936186)	99	96	*Phoma* sp.	HQ637319	1.69	0.37	0.25
JZ-80	*Coprinellus xanthothrix* (JN198387)	99	98	*Coprinellus* sp.1	HQ637320	0.19	0	0
JZ-59	*Coprinellus xanthothrix* (JN198387)	99	95	*Coprinellus* sp.1	HQ637321	0.19	0	0
JZ-177	*Bjerkandera adusta* (FJ608590)	98	98	*Bjerkandera* sp.	HQ637322	0.19	0.12	0.63
JZ-100	*Umbelopsis ramanniana* (DQ888724)	98	95	*Umbelopsis* sp.	HQ637323	0.19	0	0
JZ-71	*Mortierella alpine* (AB369455)	99	96	*Mortierella alpine*	HQ637324	0	0.86	0
JZ-157	*Mortierella alpine* (FJ025167)	99	97	*Mortierella alpine*	HQ637325	0.38	0.62	0
JZ-68	*Mortierella* sp. (JX270439)	99	95	*Mortierella* sp.	HQ637326	2.81	0	0
JZ-4	*Mortierella minutissima* (AB476417)	99	97	*Mortierella minutissima*	HQ637327	0	0	2.50
JZ-26	*Mortierella minutissima* (AB476417)	99	97	*Mortierella minutissima*	HQ637328	2.06	2.96	1.50
JZ-129	*Trichoderma sinensis* (DQ083012)	99	94	*Trichoderma sinensis*	HQ637329	1.50	0.25	0
JZ-149	*Hypocrea schweinitzii* (X93969)	99	93	*Trichoderma schweinitzii*	HQ637330	0	0.12	0
JZ-66	*Hypocrea semiorbis* (EF596944)	99	96	*Trichoderma* sp.1	HQ637331	0.19	0	0.25
JZ-90	*Hypocrea semiorbis* (EF596944)	99	96	*Trichoderma* sp.1	HQ637332	0.56	0	0
JZ-67	*Hypocrea virens* (FJ884747)	97	98	*Trichoderma* sp.2	HQ637333	5.44	0	0
JZ-27	*Hypocrea pachybasioides* (JX406549)	99	96	*Hypocrea* sp.3	HQ637334	0	0	0.63
JZ-28	*Hypocrea pachybasioides* (AB517619)	98	98	*Hypocrea* sp.3	HQ637335	1.13	0	0
JZ-69	*Hypocrea pachybasioides* (GU934589)	99	97	*Trichoderma* sp.4	HQ637336	0	0	1.63
JZ-161	*Trichoderma* sp. (AB563722)	99	97	*Trichoderma* sp.5	HQ637337	3.00	1.11	0
JZ-8	*Trichoderma* sp. (AB563722)	99	97	*Trichoderma* sp.5	HQ637338	0	0.86	0.88
JZ-77	*Hypocrea lixii* (JX088246)	100	97	*Trichoderma harzianum*	HQ637339	0.19	0	1.25
JZ-179	*Hypocrea lixii* (JQ724452)	99	100	*Trichoderma harzianum*	HQ637340	0.94	0	0
JZ-165	*Trichoderma harzianum* (JX465478)	99	98	*Trichoderma harzianum*	HQ637341	5.07	2.34	0.63
JZ-82	*Hypocrea* sp. (FJ860735)	99	95	*Trichoderma* sp.6	HQ637342	3.00	0.99	0.50
JZ-25	*Hypocrea koningii* (JQ724453)	99	100	*Trichoderma koningii*	HQ637343	2.81	1.11	1.50
JZ-132	*Trichoderma hamatum* (GQ220703)	99	98	*Trichoderma hamatum*	HQ637344	0	2.59	0
JZ-56	*Penicillium urticae* (GQ389620)	99	96	*Penicillium urticae*	HQ637345	0	0.37	1.38
JZ-5	*Penicillium montanense* (HQ157959)	99	99	*Penicillium montanense*	HQ637346	0	2.96	0
JZ-84	*Penicillium montanense* (HQ157959)	99	98	*Penicillium montanense*	HQ637347	2.44	0	0
JZ-1	*Penicillium janthinellum* (DQ888733)	98	98	*Penicillium janthinellum*	HQ637348	0	0	2.88
JZ-10	*Penicillium* sp. (JN585938)	99	98	*Penicillium* sp.1	HQ637349	6.57	2.84	0.88
JZ-11	*Penicillium* sp. (FJ379812)	99	98	*Penicillium* sp.1	HQ637350	4.32	6.17	0
JZ-46	*Penicillium steckii* (HM469415)	99	98	*Penicillium steckii*	HQ637351	0	2.34	0
JZ-53	*Penicillium sumatrense* (JX003127)	99	96	*Penicillium* sp.2	HQ637352	2.63	4.19	0
JZ-144	*Penicillium chrysogenum* (JQ724451)	99	100	*Penicillium chrysogenum*	HQ637353	0.38	1.97	4.38
JZ-43	*Penicillium griseofulvum* (HQ262520)	99	98	*Penicillium* sp.3	HQ637354	2.44	0	0
JZ-63	*Penicillium chrysogenum* (KC009826)	99	98	*Penicillium chrysogenum*	HQ637355	0	5.06	3.88
JZ-125	*Penicillium chrysogenum* (JF731255)	99	98	*Penicillium chrysogenum*	HQ637356	0	3.95	1.63
JZ-17	*Penicillium verruculosum* (EU914140)	99	95	*Penicillium verruculosum*	HQ637357	0.75	0	2.13
JZ-15	*Penicillium purpurogenum* (JQ724526)	99	100	*Penicillium purpurogenum*	HQ637358	1.50	1.36	2.88
JZ-2	*Penicillium funiculosum* (JQ724527)	99	100	*Penicillium funiculosum*	HQ637359	0	0.25	0.63
JZ-124	*Aspergillus versicolor* (AM883156)	100	98	*Aspergillus versicolor*	HQ637360	0.94	0	2.63
JZ-62	*Aspergillus versicolor* (AM883156)	100	98	*Aspergillus versicolor*	HQ637361	0	0	1.50
JZ-24	*Aspergillus versicolor* (JF911763)	100	98	*Aspergillus versicolor*	HQ637362	0	1.73	0

Table 3 (continued)

Representative isolates	Closest BLASTN matches	Sequence similarity (%)	Sequence coverage (%)	Species	Accession code	Relative abundance (%)		
						JZNR	BHNR	WJNR
JZ-29	*Aspergillus versicolor* (GU934602)	99	98	*Aspergillus versicolor*	HQ637363	0	3.45	0
JZ-122	*Aspergillus* sp. (JF694933)	99	98	*Aspergillus* sp.1	HQ637364	1.50	0.62	4.26
JZ-41	*Aspergillus* sp. (JX029073)	99	98	*Aspergillus versicolor*	HQ637365	0	0	2.13
JZ-38	*Aspergillus versicolor* (HQ285619)	99	98	*Aspergillus* sp.1	HQ637366	1.69	4.19	3.13
JZ-35	*Aspergillus sydowii* (JX675047)	99	99	*Aspergillus sydowii*	HQ637367	3.94	0	0
JZ-109	*Aspergillus* sp. (GU985232)	99	99	*Aspergillus sydowii*	HQ637368	0	0.12	1.00
JZ-178	*Aspergillus sydowii* (JQ724408)	99	100	*Aspergillus sydowii*	HQ637369	0	0.49	0
JZ-115	*Humicola* sp. (GQ131885)	97	98	*Humicola* sp.1	HQ637370	3.56	0	0.63
JZ-156	*Humicola* fuscoatra (GU183113)	97	97	*Humicola* sp.1	HQ637371	0	0	4.13
JZ-121	*Humicola* sp. (HM371217)	97	99	*Humicola* sp.1	HQ637372	0	3.58	0
JZ-130	*Humicola* sp. (GQ131885)	97	96	*Humicola* sp.2	HQ637373	0	1.73	0
JZ-110	*Humicola fuscoatra* (GU183113)	97	97	*Humicol*a sp.1	HQ637374	0	0.37	1.88
JZ-120	*Humicola fuscoatra* (JN031580)	98	94	*Humicola* sp.1	HQ637375	1.13	0.86	0
JZ-118	*Humicola fuscoatra* (GU183113)	97	96	*Humicola* sp.1	HQ637376	0	0.37	2.88
JZ-136	*Humicola* sp. (GQ131885)	97	98	*Humicola* sp.1	HQ637377	1.31	0	1.38
JZ-20	*Humicola fuscoatra* (GU183113)	98	95	*Humicola* sp.3	HQ637378	0	2.10	0.25
JZ-6	*Humicola fuscoatra* (GU966514)	97	98	*Humicola* sp.3	HQ637379	0.38	2.59	0
JZ-14	*Humicola fuscoatra* (GU183113)	97	97	*Humicola* sp.3	HQ637380	2.06	0.49	2.50
JZ-70	*Absidia repens* (FJ849793)	95	33	*Absidia* sp.	HQ878615	3.75	3.45	1.50
SC-12	*Leptosphaeria* sp. (HQ713770)	99	87	*Trematosphaeria terricola*	JN662930	0.19	0	0

respectively. The bootstrap values among these groups were relatively low, indicating that the genetic diversity in these *Aspergillus* or *Humicola* species is quite low. According to microscopic analysis, the different species of *Aspergillus* or *Humicol*a were clustered together into a clade by the neighbor-joining phylogenetic analysis (Fig. 2), which is in accordance with the clustering obtained in earlier studies (Zhao et al. 2008; Varga et al. 2010).

Fungal community composition

Forty genera with 73 species were collected from Jiuzaigou County and classified as Ascomycota, Zygomycota and Basidiomycota at a ratio of relative abundance of 219.65:17.44 :1, respectively. Thirty–five genera were classified into Ascomycota with 66 species, three genera into Zygomycota with five species and two genera into Basidiomycota with two species.

The relative abundance of Ascomycota, Zygomycota and Basidiomycota in JZNR was 90.28, 9.19 and 0.57 %, respectively. In BHNR and WJNR, the relative abundance of Ascomycota was 91.98 and 93.88 %, respectively. The relative abundance of Ascomycota colonies in JZNR was 9.82- and 158.39-fold higher than that of Zygomycota and Basidiomycota colonies, respectively. This pattern was broadly similar in BHNR and WJNR, in which the ratios of Ascomycota:Zygomycota:Basidiomycota were 766.50:65.75:1 and 149.02:8.75:1, respectively.

Genera (or species) with a relative abundance of >5.0 % were considered to be dominant (Wang et al. 2010). In JZNR, among the 35 genera (56 species) isolated, the range in relative abundance of species was 0.19 to 6.57 %, and only three species showed values of >5.0 %, whereas ten species showed the lowest values (Table 3). The dominant genera detected were *Trichoderma*, *Penicillium*, *Humicola*, *Aspergillus* and *Mortierella* (Table 4). In BHNR, among the 33 genera (54 species) isolated, the relative abundance of species was 0.12–6.17 %, with two *Penicillium* species having a relative abundance of >5.0 % and six species having the lowest percentages (Table 3). The dominant genera were *Penicillium*, *Humicola*, *Aspergillus*, *Trichoderma*, and *Fusarium* (Table 4). In WJNR, 31 genera (50 species) were isolated, and the relative abundance of all species ranged from 0.13 to 4.88 %, with no species having a relative abundance of >5.0 %, and six species having a relative abundance of >4.0 % (Table 3). The main genera detected were *Penicillium*, *Aspergillus*, *Humicola*, *Trichoderma*, and *Myrothecium* (Table 4).

For Jiuzhaigou County as a whole (all three nature reserves, 25 soil samples), the most dominant genus was

Table 4 Relative abundance of isolated genera

Genus	JZNR	BHNR	WJNR	Total[a]
Acremonium spp.	1.69	0.74	0	0.70
Aspergillus spp.	8.07	10.6	14.64	11.48
Aureobasidium spp.	0.56	0.99	0.63	0.75
Cadophora spp.	0.38	0.49	0.38	0.42
Cladosporium spp.	0.75	1.23	2.25	1.49
Cylindrocarpon spp.	0.19	0.12	0.13	0.14
Emericella spp.	0.38	1.73	0	0.75
Eurotium spp.	1.13	0.86	0.88	0.93
Fusarium spp.	2.25	5.92	4.88	4.62
Geomyces spp.	1.88	0.62	0	0.70
Gliocladium spp.	1.50	1.48	2.25	1.77
Gliomastix spp.	1.88	2.22	4.88	3.13
Humicola spp.	8.44	12.08	13.64	11.76
Lecythophora spp.	2.44	0.99	1.38	1.49
Leptosphaeria spp.	2.44	0.25	0	0.70
Myrothecium spp.	4.13	1.36	6.38	3.92
Nectria spp.	0.38	1.23	1.00	0.93
Penicillium spp.	21.01	31.44	20.65	24.83
Pestalotiopsis spp.	1.31	0.12	0.88	0.70
Phoma spp.	1.69	0.37	0.25	0.65
Podospora spp.	0	0	0.25	0.09
Preussia spp.	0.75	0.37	4.38	1.96
Pyrenochaeta spp.	0.19	0.62	0	0.28
Seimatosporium spp.	0	0	0.38	0.14
Scopulariopsis spp.	0	1.36	2.25	1.35
Scytalidium spp.	0.75	0.86	0.63	0.75
Stachybotrys spp.	0	0.74	0.75	0.56
Thielavia spp.	0.56	0.12	0.5	0.37
Torula spp.	0.38	1.36	1.00	0.98
Trichoderma spp.	23.83	9.37	7.26	12.18
Truncatella spp.	0.38	1.11	0.25	0.61
Trematosphaeria spp.	0.19	0	0	0.05
Verticillium spp.	0.56	1.23	0.5	0.79
Bjerkandera spp.	0.19	0.12	0.63	0.33
Coprinellus spp.	0.38	0	0	0.09
Umbelopsis spp.	0.19	0	0	0.05
Absidia spp.	3.75	3.45	1.50	2.80
Mortierella spp.	5.25	4.44	4.01	4.48
Genus 1 (Fungal sp.1, JZ-13)	0	0	0.63	0.23
Genus 2 (Fungal sp.2, JZ-72)	0.19	0	0	0.05

[a] Total: Jiuzhaigou County as a whole (25 soil samples)

Penicillium, with 11 species accounting for 24.83 % of isolates, followed by *Trichoderma*, with 11 species accounting for 12.18 %, *Humicola* with three species accounting for 11.76 % and *Aspergillus* with three species accounting for 11.48 % (Table 4). The other three main genera were *Fusarium* (4.62 %), *Mortierella* (4.48 %) and *Myrothecium* (3.92 %) (Table 4). The seven genera represented together 73.27 % of total abundance. All seven of the main genera were found in all three nature reserves where they represented the main populations.

Diversity characteristics

For the 25 sampling sites, the range in values for species richness (S), the biodiversity index (H') and evenness (E) were 10–29, 1.96–3.05 and 0.74–0.95, respectively (Tables 1, 2). Among the three sites, site BHNR3 showed a larger S value (29), and sites WJNR5 and BHNR8 had the highest E value (0.95), whereas site BHNR4 showed the higher H'' value (Table 1).

Among the three nature reserves, the highest S value was found in the BHNR (20.00), followed by JZNR (19.38) and WJNR (18.11). Similarly, the highest H' value was observed in BHNR (2.66), followed by JZNR (2.54) and WJNR (2.49). The highest evenness (E) value was also found in BHNR (0.90). This means that the culturable fungal species of BHNR were better distributed than those of other regions, with the higher contribution to biodiversity (H').

Trichoderma antagonism

A total 14 *Trichoderma* isolates were tested for their antagonism activity against three pathogens (*Bipolaris maydis*, *Curvularia lunata*, *Rhizoctonia solani*). Table 5 shows the inhibition effect of three pathogens for six *Trichoderma* isolates which gave inhibition values of >20 %; the other eight *Trichoderma* isolates showed little effect to the three pathogens according to the dual culture or culture filtrate tests.

For the dual culture tests, the inhibition values of mycelium growth of the three pathogens by these six *Trichoderma* isolates were all >22 % (Table 5). These results indicate that the six *Trichoderma* isolates can clearly inhibit the growth of *B. maydis*, *C. lunata* and *R. solani*. In particular, JZ-77 (*T. harzianum*) showed the highest inhibition value to *R. solani* (62.44 %) and *C. lunata* (56.97 %), and the highest inhibition value of *B. maydis* was induced by *T. koningii* (40.13 %).

In terms of the inhibitory effect of the *Trichoderma* culture filtrates, growth inhibition ranged from 20.28 to 52.14 %, from 27.13 to 70.44 % and from 27.15 to 67.79 % for *B. maydis*, *C. lunata* and *R. solani*, respectively (Table 5). These values demonstrate that the highest inhibition values of *C. lunata* and *R. solani* were induced by *T. koningii* (70.44 and 67.79 %, respectively) and that the highest inhibition value of *B. maydis* was induced by *T. hamatum* (52.14 %).

Table 5 Effect of six *Trichoderma* species on mycelial growth inhibition of three pathogens according to dual culture and *Trichoderma* culture filtrates on potato dextrose agar medium

Trichoderma species	*Bipolaris maydis*		*Curvularia lunata*		*Rhizoctonia solani*	
	D[a]	F[b]	D[a]	F[b]	D[a]	F[b]
Trichoderma harzianum (JZ-77)	34.39±0.78	31.57±1.37	56.97±1.70	53.19±3.33	62.44±2.75	58.42±1.99
Trichoderma hamatum (JZ-132)	28.53±0.94	52.14±2.08	43.37±1.69	27.13±1.46	43.20±1.88	30.16±1.71
Trichoderma koningii (JZ-25)	40.13±0.98	29.37±2.19	47.67±2.97	70.44±1.79	56.89±2.04	67.79±1.94
Trichoderma sinensis (JZ-129)	25.44±1.53	35.74±1.47	31.05±1.81	41.07±2.01	34.91±2.45	27.15±2.12
Trichoderma sp.2 (JZ-67)	22.37±2.54	20.28±2.04	51.32±3.92	40.18±2.60	61.09±2.80	51.06±1.99
Trichoderma sp.5 (JZ-8)	31.76±2.17	50.75±1.93	45.75±3.78	37.66±2.04	49.17±3.04	38.18±2.60

Data are given as a percentage ± SD. Each value is the mean of three replicates

[a] Percentage of growth inhibition (%) as assessed by the dual culture tests

[b] Percentage of growth inhibition (%) as assessed by the 20 % (v/v) concentration of*Trichoderma* culture filtrates

Discussion

In this study, a conventional method (soil dilution plate method) and Rose Bengal agar medium were used to assess the diversity of culturable soil fungi in three nature reserves of Jiuzhaigou County. Among the 25 sampling sites, a sharp change in fungal density appeared in two pairs at two nearby sites (BHNR1 and WJNR7; BHNR5 and WJNR8) (Fig. 1) which showed the same vegetation and little change in altitude (≤20 m) (Table 1; Fig. 1). On the contrary, some sites showed significant differences in vegetation and altitude (e.g. JZNR7 and BHNR7; WJNR5 and BHNR4; BHNR2 and WJNR3), but little change in fungal density (<0.05) (Table 1; Fig. 1). It is therefore difficult to draw clear inferences of the relationship among fungal density in terms of vegetation or altitude. Although we could not rule out the existence of differences of micro-environmental characteristics within each sampling site, previous research revealed that quantitative changes in plant rhizodeposition, geographical environment, pH and carbon availability may affect fungal abundance (Grishkan et al. 2006, 2009; Kara and Asan 2007; Hannula et al. 2010; Jirout et al. 2011).

For culturable fungi, the ranges in the values of species richness (S), biodiversity index (H') and evenness (E) of the 25 sampling sites were 10–29, 1.96–3.05 and 0.74–0.95, respectively (Table 1). Sites BHNR3 and BHNR4 showed the highest value of S (29) and H' (3.05), respectively. The highest E value (0.95) was found in BHNR8 and WJNR5. However, because of the isolation technique used, the S, H' and E of the 25 sampling sites only depend upon their culturable fungal fraction. Thus, to ensure a reliable assessment of soil fungal diversity characteristics, a wider range of research techniques should be employed in further studies.

It has been estimated that there are approximately 3,150 species of soil fungi, many of which have a worldwide distribution (Gams 2007). The culturable fungal community of the 25 sites analyzed in our study was dominated by Ascomycota, accounting for 92.25 % of isolates, with 66 species (35 genera), followed by Zygomycota, accounting for 7.33 %, with five species (3 genera), and Basidiomycota, which were only rarely isolated (0.42 %). These results confirm those reported previously on soil fungal communities (Grishkan et al. 2009; Arenz and Blanchette 2011). Some isolates belong to ubiquitous genera which have been reported earlier to be soil fungi (e.g., *Absidia*, *Aspergillus*, *Fusarium*, *Mortierella*, *Penicillium*, *Trichoderma* and *Geomyces*). In our study, *Penicillium*, *Trichoderma*, *Humicola* and *Aspergillus* species comprised a higher proportion of the fungal isolates and were particularly common (representing 24.83, 12.18, 11.76 and 11.48 % of total isolates, respectively); this result is similar to that previously reported and supports the notion that these species can utilize soil organic matter more widely than other species and inhabit various soil environments (Grishkan et al. 2009).

Compared to past research, our data differ with respect to the occurrence of some genera; for example, culturable isolates belonging to *Alternaria* spp., *Botrytis* spp., *Rhizopus* spp., *Mucor* spp., *Phialocephala* spp. among others, were not identified in our study. In addition, although the environments among the three nature reserves are similar, we found significant differences among fungal species and relative abundance: for example, *Umbelopsis* sp., *Coprinellus* sp., *Trematosphaeria* sp. and Fungal sp.2 (strain JZ-72) were isolated only from JZNR sites, *Podospora* sp., *Seimatosporium* sp. and Fungal sp.1 (strain JZ-13) were not observed at JZNR and BHNR sites and a sharp increase of *Myrothecium* genus relative abundance (mainly due to *M. roridum*) was observed at WJNR sites, possibly indirectly providing a significant signal of plant disease caused by this pathogen. Overall, different soil characteristics (e.g. pH, aeration, water content, plant litter quality input, among others) would appear to influence the existence and culturability of fungal species in the different regions.

In our previous publication (Zhou et al. 2013), the isolate SC-12 (JN662930) was identified as a new *Trematosphaeria* species and named as *Trematosphaeria terricola*, which is the first *Trematosphaeria* species from alpine soil in China. As we know, this study represents the first record of the presence of *Podospora* sp. and *Seimatosporium* sp. in soil of China. It is interesting that some of the species isolated in our study were obtained from different hosts or habitat and showed saprophytic or parasitic character in comparison to results of earlier studies. This suggests that fungi can shift their ecological role with changes in ecological features. Some isolated fungi are important plant pathogens, such as, *Stachybotrys* sp., *Fusarium* sp., *Myrothecium* sp., *Cladosporium* sp., *Phoma* sp., *Aspergillus* sp., *Penicillium* sp., *Scopulariopsis* sp., *Cylindrocarpon* sp., among others. To the contrary, *Aspergillus* spp., *Penicillium* spp., *Acremonium* spp. and *Trichoderma* spp. are among the best known fungal agents of bio-control and perhaps play an important role by controlling or preventing soil-borne fungal diseases. In fact, some *Trichoderma* isolates did show bio-control potential against pathogens in our test. The antagonistic mechanisms used by *Trichoderma* species to control plant pathogens include competition, antibiosis, mycoparasitism and so on (Sánchez et al. 2007; Patil et al. 2012). In addition, there are reports of other fungi playing an important role in ecological functioning. For example, *Mortierella* sp. can solubilize soil immobile phosphorus (Osorio and Habte 2001), *Humicola* sp. is a strongly cellulolytic microfungi (Deacon et al. 2006) and *Bjerkandera* sp. is regarded as the white rot fungi because of its lignin degradation function (Dorado et al. 2001). Therefore, these species are possible important factors for ecological balance in the three nature reserves.

The ITS sequences of JZ-103, JZ-104, JZ-123 and JZ-169 are most closely related to the BLASTN sequence of *Lecythophora* sp. (AY219880), *Lecythophora luteoviridis* (DQ404354) and *L. mutabilis* (HM036599) (Table 3), while these sequences appeared to be clustered into two clades with a more distant relationship (Fig. 2). These results suggest that *Lecythophora* species are polyphyletic even though they showed a similar morphology. This phenomenon has been observed in other genera, such as *Lulworthia* (Campbell et al. 2005), *Lignincola* (Pang et al. 2003) and *Lophiostoma* (Zhang et al. 2009). Although the ITS data play an important role in current mycological taxonomy, the phylogeny inferred from any gene may not really reflect the evolution history of organisms. According to Uilenberg et al. (2004), the polyphasic taxonomy should include genotypical and phenotypical characteristics. Therefore, the JZ-103, JZ-104, JZ-123 and JZ-169 were identified as *Lecythophora* spp. *Penicillium* has been characterized using molecular techniques in many studies, but the data from these are insufficient too provide a statistical solution for classifying infrageneric *Penicillium* (Samson et al. 2004). Therefore, morphological features were the main appraisal method for *Penicillium* species.

Many studies have used molecular techniques to detect fungal diversity by direct DNA extraction from environmental samples and separation of amplicons obtained by different methods, including denaturing gradient gel electrophoresis, terminal restriction fragment length polymorphism and single–strand conformation polymorphism (Viaud et al. 2000; Jumpponen 2003; Anderson and Cairney 2004; Jeewon and Hyde 2007). Although these methods are able to analyze the diversity independently of culturability, their results may still show biases due to the processes of sample DNA extraction or PCR amplification (Kirk et al. 2004).

The number of isolated fungal species is clearly related with the isolation method and culture medium (Cabello and Arambarri 2002). According to Bridge and Spooner (2001), only about 17 % of known fungal species can be cultured using conventional microbiological culture techniques, and most remaining fungi are missed. The conventional cultural techniques only detect a small fraction of soil fungi—those which can grow and sporulate on the isolation medium used (Cabello and Arambarri 2002; Jeewon and Hyde 2007), and the selective culture medium is a major determining factor in the isolation of soil fungal species (Zhang et al. 2013). Thus, it is impossible to accurately detect fungal diversity in soil samples using the single isolation method and medium, and soil fungal diversity might be greatly underestimated when based only on the traditional cultural techniques used in this study. Consequently, there are limitations to the techniques used in this study for the detection of the true diversity in any chosen environment. However, we were able to provide a basic diversity measurement of soil fungi which can readily grow on the culture medium used, as well as obtain the pure cultures for further study. It is clear that a combination of traditional and molecular approaches will provide a comprehensive picture of fungal diversity in sampling sites because many species of "unculturable" fungi have already been detected using molecular techniques (Jeewon and Hyde 2007).

Acknowledgments This study was funded by a key project from the Educational Department of Sichuan Province (2004-A004) and the Two-Way Support Project of Sichuan Agricultural University. We wish to thank anonymous reviewers for good proposals to improve this manuscript and Jia Yao, Jianghu Cui, Zhixiang Chen, Liwan Chen and Shian Shen for supporting the research work.

References

Alves A, Crous PW, Correia A, Phillips AJL (2008) Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. Fungal Divers 28:1–13

Anderson IC, Cairney JW (2004) Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. Environ Microbiol 6(8):769–779

Anderson IC, Parkin PI (2007) Detection of active soil fungi by RT-PCR amplification of precursor rRNA molecules. J Microbiol Methods 68(2):248–253

Anderson IC, Campbell CD, Prosser JI (2003) Prosser Diversity of fungi in organic soils under a moorland-Scotspine (*Pinus sylvestris* L.) gradient. Environ Microbiol 5(11):1121–1132

Arenz BE, Blanchette RA (2011) Distribution and abundance of soil fungi in Antarctica at sites on the Peninsula, Ross Sea Region and McMurdo Dry Valleys. Soil Biol Biochem 43(2):308–315

Arenz BE, Held BW, Jurgens JA, Blanchette RA (2011) Fungal colonization of exotic substrates in Antarctica. Fungal Divers 49:13–22

Barnes I, Roux J, Wingfield MJ, Coetzee MPA et al (2001) Characterization of *Seiridium* spp. associated with cypress canker based on β-tubulin and histone sequences. Plant Dis 85:317–321

Barnett HL, Hunter BB (1987) Illustrated genera of imperfect fungi. Macmillan, New York

Barrico L, Rodríguez-Echeverría S, Freitas H (2010) Diversity of soil basidiomycete communities associated with *Quercus suber* L. in Portuguese montados. Euro J Soil Biol 46(5):280–287

Bastias BA, Huang ZQ, Blumfield T, Xu ZH, Cairney JWG (2006) Influence of repeated prescribed burning on the soil fungal community in an eastern Australian wet sclerophyll forest. Soil Biol Biochem 38(12):3492–3501

Bissett J (1984) A revision of the genus *Trichoderma*. I. Section Longibrachiatum sect. nov. Can J Bot 62(5):924–931

Bissett J (1991a) A revision of the genus *Trichoderma*. II. Infrageneric classification. Can J Bot 69(11):2357–2372

Bissett J (1991b) A revision of the genus *Trichoderma*. III. Section Pachybasium. Can J Bot 69(11):2373–2417

Bissett J (1991c) A revision of the genus *Trichoderma*. IV. Additional notes on Section Longibrachiatum. Can J Bot 69(11):2418–2420

Bridge P, Spooner B (2001) Soil fungi: diversity and detection. Plant Soil 232(1–2):147–154

Buee M, Maurice JP, Zeller B, Andrianarisoa S, Ranger J et al (2011) Influence of tree species on richness and diversity of epigeous fungal communities in a French temperate forest stand. Fungal Ecol 4(1):22–31

Cabello M, Arambarri A (2002) Diversity in soil fungi from undisturbed and disturbed *Celtis tala* and *Scutia buxifolia* forests in the eastern Buenos Aires province (Argentina). Mycol Res 157(2):115–125

Campbell J, Volkmann-kohlmeyer B, Grafenhan T, Spatafora JW, Kohlmeyer J (2005) A re-evaluation of Lulworthiales: relationships based on 18S and 28S rDNA. Mycol Res 109(5): 556–568

Chaverri P, Samuels GJ, Stewart EL (2001) *Hypocrea virens* sp. nov., the teleomorph of *Trichoderma virens*. Mycologia 93(6):1113–1124

Curlevski NJA, Xu ZH, Anderson IC, Cairney JWG (2010) Converting Australian tropical rainforest to native Araucariaceae plantations alters soil fungal communities. Soil Biol Biochem 42(1):14–20

Deacon LJ, Janie PME, Frankland JC, Bainbridge BW, Mooreet PD et al (2006) Diversity and function of decomposer fungi from a grassland soil. Soil Biol Biochem 38(1):7–20

Domsch KH, Gams W, Anderson TH (2007) Compendium of soil Fungi, 2nd edn. APS Press, New York

Dong AR, Lu GZ, Wu QY, Song RQ, Song FQ (2004) Diversity of soil fungi in Liangshui natural reserve, Xiaoxing'anling forest region. J Northeast Forestry Univ 32(1):8–10

Dorado J, van Beek TA, Claassen FW, Sierra-Alvarez R (2001) Degradation of lipophilic wood extractive constituents in Pinus sylvestris by the white-rot fungi *Bjerkandera* sp. and *Trametes versicolor*. Wood Sci Technol 35(1–2):117–125

Entry JA, Mills D, Mathee K, Jayachandran K, Sojka RE, Narasimhan G (2008) Influence of irrigated agriculture on soil microbial diversity. Appl Soil Ecol 40(1):146–154

Gadd GM (2007) Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. Mycol Res 111(1):3–49

Galletti S, Burzi PL, Cerato C, Marinello S, Sala E (2008) Trichoderma as a potential biocontrol agent for Cercospora leaf spot of sugar beet. BioControl 53:917–930

Gams W (2007) Biodiversity of soil-inhabiting fungi. Biodivers Conserv 16(1):69–72

Grishkan I, Zaady E, Nevo E (2006) Soil crust microfungi along a southward rainfall gradient in desert ecosystems. Eur J Soil Biol 42(1):33–42

Grishkan I, Tsatskin A, Nevo E (2008) Diversity of cultured microfungal communities in surface horizons of soils on different lithologies in Upper Galilee, Israel. Eur J Soil Biol 44(2):180–190

Grishkan I, Tsatskin A, Nevo E (2009) Comparative mycobiotic and edaphic analyses of two neighboring soil profiles on different lithologies in Upper Galilee, Israel. Eur J Soil Biol 45(4):341–350

Hannula SE, Boer WD, Veen JAV (2010) In situ dynamics of soil fungal communities under different genotypes of potato, including a genetically modified cultivar. Soil Biol Biochem 42(12):2211–2223

Hawksworth DL, Rossman AY (1997) Where are all the undescribed fungi? Phytopathology 87(9):888–891

Henry T, Iwen PC, Hinrichs SH (2000) Identification of Aspergillus species using internal transcribed spacer regions 1 and 2. J Clin Microbiol 38(4):1510–1515

Hollister EB, Schadt CW, Palumbo AV, Ansley RJ, Boutton TW (2010) Structural and functional diversity of soil bacterial and fungal communities following woody plant encroachment in the southern Great Plains. Soil Biol Biochem 42(10):1816–1824

Jaklitsch WM (2009) European species of Hypocrea Part I: the green-spored species. Stud Mycol 63(1):1–91

Jaklitsch WM (2011) European species of Hypocrea part II: species with hyaline ascospores. Fungal Divers 48(1):1–250

Jeewon R, Hyde KD (2007) Detection and diversity of fungi from environmental samples: traditional versus molecular approaches. In: Varma A, Oelmüller R (eds) Advanced techniques in soil microbiology. Springer, Berlin, pp 1–15

Jirout J, Šimek M, Elhottová D (2011) Inputs of nitrogen and organic matter govern the composition of fungal communities in soil disturbed by overwintering cattle. Soil Biol Biochem 43(3):647–656

Jumpponen A (2003) Soil fungal community assembly in a primary successional glacier forefront ecosystem as inferred from rDNA sequence analysis. New Phytol 158(3):569–578

Jurjevic Z, Peterson SW, Horn BW (2012) *Aspergillus* section Versicolores: nine new species and multilocus DNA sequence based phylogeny. IMA Fungus 3(1):59–79

Kara Ö, Asan A (2007) Microfungal community structure from forest soils in Northern Thrace Region, Turkey. Ann Microbiol 57(2):149–155

Kirk JL, Beaudette LA, Hart M, Moutoglis P, Klironomos JN, Lee H, Trevors JT (2004) Methods of studying soil microbial diversity. J Microbiol Methods 58:169–188

Kong HZ (2007) *Penicillium* et teleomorphi cognati. Flora fungorum sinicorum 35. Science Press, Beijing

Laganà A, Loppi S, Dominicis VD (1999) Relationship between environmental factors and the proportions of fungal trophic groups in forest ecosystems of the central Mediterranean area. For Ecol Manag 124(2–3):145–151

Lejon DP, Chaussod R, Ranger J, Ranjard L (2005) Microbial community structure and density under different tree species in an acid forest soil (Morvan, France). Microb Ecol 50(4):614–625

Li QR, Tan P, Jiang YL, Hyde KD, Mckenzie EHC et al (2012) A novel *Trichoderma* species isolated from soil in Guizhou, *T. guizhouense*. Mycol Prog 12(2):167–172

Lim YW, Kim JJ, Chedgy R, Morris PI, Breuil C (2005) Fungal diversity from western redcedar fences and their resistance to β-thujaplicin. Antonie Van Leeuwenhoek 87:109–117

McGuire KL, Fierer N, Bateman C, Treseder KK, Turner BL (2012) Fungal community composition in neotropical rain forests: the influence of tree diversity and precipitation. Microb Ecol 63(4): 804–812

Mueller GM, Bills GE, Foster MS (2004) Biodiversity of fungi: inventory and monitoring methods. Elsevier Academic Press, Burlington, pp 280–302

Nesci A, Barros G, Castillo C, Etcheverry M (2006) Soil fungal population in preharvest maize ecosystem in different tillage practices in Argentina. Soil Tillage Res 91(1–2):143–149

Nie M, Meng H, Li K, Wan JR, Quan ZX et al (2012) Comparison of bacterial and fungal communities between natural and planted pine forests in subtropical China. Curr Microbiol 64(1):34–42

Ortega A, Suárez-Santiago VN, Reyes JD (2008) Morphological and ITS identification of *Cortinarius* species (section Calochroi) collected in Mediterranean Quercus woodlands. Fungal Divers 29:73–88

Osorio NW, Habte M (2001) Synergistic influence of an arbuscular mycorrhizal fungus and a P solubilizing fungus on growth and P uptake of *Leucaena leucocephala* in an oxisol. Arid Land Res Manag 15(3):263–274

Pan HQ, Zhang TY, Huang YH, Xia HB, Yu JF (2009) Diversity and niche of soil moniliaceous hyphomycetes in TaibaiMountain. Chin J Appl Ecol 20(2):363–369

Pang KL, Vrijmoed LLP, Kong RYC, Jones EBG (2003) *Lignincola* and *Nais*, polyphyletic genera of the Halosphaeriales (Ascomycota). Mycol Prog 2(1):29–36

Patil A, Laddha A, Lunge A, Paikrao H, Mahure S (2012) In vitro antagonistic properties of selected *Trichoderma* species against tomato root causing *Pythium* species. Int J Environ Sci Te 4(1):302–315

Persiani AM, Maggi O, Casado MA, Pineda FD (1998) Diversity and variability in soil fungi from a disturbed tropical rain forest. Mycologia 90(2):206–214

Phillips AJL, Alves A, Pennycook SR, Johnston PR, Ramaley A et al (2008) Resolving the phylogenetic and taxonomic status of dark-spored teleomorph genera in the Botryosphaeriaceae. Persoonia 21: 29–55

Qi ZT (1997) *Aspergillus* et teleomorphi cognati. Flora fungorum sinicorum 5. Science Press, Beijing

Samson RA, Seifert KA, Kuijpers AFA, Houbraken JAMP, Frisvad JC (2004) Phylogenetic analysis of *Penicillium* subgenus *Penicillium* using partial β-tubulin sequences. Stud Mycol 49:175–200

Samson RA, Peterson SW, Frisvad JC, Varga J (2011) New species in *Aspergillus* section Terrei. Stud Mycol 69(1):39–55

Sánchez V, Rebolledo O, Picaso RM et al (2007) In vitro antagonism of *Thielaviopsis paradoxa* by *Trichoderma longibrachiatum*. Mycopathologia 163:49–58

Satish N, Sultana S, Nanjundiah V (2007) Diversity of soil fungi in a tropical deciduous forest in Mudumalai, southern India. Current Sci 93(5):669–677

Schmit JP, Mueller GM (2007) An estimate of the lower limit of global fungal diversity. Biodivers Conserv 16(1):99–111

Schneider S, Hartmann M, Enkerli J, Widmer F (2010) Fungal community structure in soils of conventional and organic farming systems. Fungal Ecol 3(3):215–224

Singh BK, Munro S, Potts JM, Millard P (2007) Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. Appl Soil Ecol 36(2–3):147–155

Skouboe P, Frisvad JC, Taylor JW, Lauritsen D, Boysen M et al (1999) Phylogenetic analysis of nucleotide sequences from the ITS region of terverticillate *Penicillium* species. Mycol Res 103(7):873–881

Tamura K, Peterson D, Peterson N, Stecher G (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28(10):2731–2739

Tanaka K, Hirayama K, Yonezawa H, Hatakeyama S, Harada Y et al (2009) Molecular taxonomy of bambusicolous fungi: Tetraplosphaeriaceae, a new pleosporalean family with *Tetraploa*-like anamorphs. Stud Mycol 64:175–209

Thoms C, Gattinger A, Jacob M, Thomas FM, Gleixner G (2010) Direct and indirect effects of tree diversity drive soil microbial diversity in temperate deciduous forest. Soil Biol Biochem 42(9):1558–1565

Uilenberg G, Thiaucourt F, Jongejan F (2004) On molecular taxonomy: what is in a name? Exp Appl Acarol 32(4):301–312

Varga J, Frisvad JC, Samson RA (2010) Samson. Polyphasic taxonomy of *Aspergillus* section Sparsi. IMA Fungus 1(2):187–195

Viaud M, Pasquier A, Brygoo Y (2000) Diversity of soil fungi studied by PCR-RFLP of ITS. Mycol Res 104(9):1027–1032

Visagie CM (2008) Biodiversity in the genus *Penicillium* from coastal Fynbos soil. MSc thesis. Stellenbosch University Press, Stellenbosch

Wang HK, Aptroot A, Crous PW, Hyde KD, Jeewon R (2007) The polyphyletic nature of Pleosporales: an example from *Massariosphaeria* based on rDNA and RBP2 gene phylogenies. Mycol Res 111(11):1268–1276

Wang L, Zhang WM, Hu B, Chen YQ, Qu LH (2008) Genetic variation of *Cordyceps militaris* and its allies based on phylogenetic analysis of rDNA ITS sequence data. Fungal Divers 31:147–155

Wang Y, Xu J, Shen JH, Luo YM, Scheu S, Ke X (2010) Tillage, residue burning and crop rotation alter soil fungal community and water-stable aggregation in arable fields. Soil Tillage Res 107(2):71–79

Wardle DA, Yeates GW, Barker GM, Bonner KI (2006) The influence of plant litter diversity on decomposer abundance and diversity. Soil Biol Biochem 38(5):1052–1062

Wei JC (1979) Handbook of identification Fungi. Shanghai Science and Technology Press, Shanghai

West L, Jones RH (2000) Responses of understory tree seedlings to alteration of the soil fungal community in mid-and late-successional forests. For Ecol Manag 134(1–3):125–135

Wu TH, Chellemi DO, Martin KJ, Graham JH, Rosskopf EN (2007) Discriminating the effects of agricultural land management practices on soil fungal communities. Soil Biol Biochem 39(5):1139–1155

Wu TH, Chellemi DO, Graham JH, Rosskopf EN (2008) Assessment of fungal communities in soil and tomato roots subjected to diverse land and crop management systems. Soil Biol Biochem 40:1967–1970

Zak JC, Visser S (1996) An appraisal of soil fungal biodiversity: the crossroads between taxonomic and functional biodiversity. Biodivers Conserv 5(2):169–183

Zhang ZY (2003) *Cladosporium, Fusicladium, Pyricularia*. Flora fungorum sinicorum 14. Science Press, Beijing

Zhang Y, Wang HK, Fournier J, Crous PW, Jeewon R et al (2009) Towards a phylogenetic clarification of *Lophiostoma/Massarina* and morphologically similar genera in the Pleosporales. Fungal Divers 38:225–251

Zhang ZY, Lin WX, Yang YH, Chen H, Chen XJ (2011) Effects of consecutively monocultured *Rehmannia glutinosa* L. on Diversity of Fungal Community in Rhizospheric Soil. J Integ Agri 10(9): 1374–1384

Zhao LR, Desjardin DE, Soytong K, Hyde KD (2008) A new species of bird's nest fungi: characterisation of *Cyathus subglobisporus* sp. nov. based on morphological and molecular data. Persoonia 21:71–76

Zhou Y, Gong GS, Zhang SR, Liu N, Wang JJ et al (2013) A new species of the genus *Trematosphaeria* from China. Mycol Prog. doi:10.1007/s11557-013-0889-3