

Endophyte distribution and comparative analysis of diversity in wetlands showing contrasting geomorphic conditions

Young-Hyun You^{1,3} · Jong Myong Park^{2,3} · Jong-Han Park¹ · Jong-Guk Kim³

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Abstract The distribution and diversity of endophytic fungi in native hydrophytes from Korea were investigated. Fourteen hydrophytes (i.e., *Hydrocharis dubia*, *Iris pseudacorus*, *Miscanthus sacchariflorus*, *Miscanthus sinensis*, *Nymphoides peltata*, *Nymphaea tetragona*, *Nelumbo nucifera*, *Pistia stratiotes*, *Salvinia natans*, *Sagittaria trifolia*, *Trapa japonica*, *Typha orientalis*, *Vallisneria natan*, and *Zizania latifolia*) were sampled from freshwater marshes. A total of 216 fungal endophytes, isolated purely on the basis of morphological differences, were identified by ITS1-5.8S-ITS2 rDNA sequence similarity. The hydrophytes harbored a variety of endophytic fungi, most of which belonged to three phyla, seven classes, and 43 genera. Dramatic differences in diversity values were found among the six marshes, even within the same hydrophyte species. This distinguishing diversity or distribution of endophytes correlated with the specific environmental features but not the plant taxon. Several endophyte genera that have been

reported to have biological activity (PGPR, ISR) were identified. This study provided basic data on the symbiotic relationship between hydrophytes and their endophytic fungi.

Keywords Endophytic fungi · Freshwater marshes · Fungal diversity · Hydrophyte

1 Introduction

Freshwater marshes serve as natural habitats for various plants, animals, insects, and microbes. The ecological role of marshes is established by the material cycling that occurs as a result of the natural characteristics of the marshes (Arnold and Van 2012; Julie and Fennessy 2001; Kuczynska-Kippen 2007). Freshwater marshes may exhibit biological and microbial succession (Denny 1994). Furthermore, natural remediation, buffering capacity, flooding, storms, groundwater discharge, climate, and nutrients in freshwater marshes (Li et al. 2014a, b; Xiang et al. 2009) all contribute to their characterization as hot spots of biological and microbial diversity (Denny 1994). A wide variety of hydrophytes settle and grow in marshes (Arnold and Van 2012; Kong and David 1993). They carry out photosynthesis, affecting the dissolved oxygen, nitrogen, and phosphorus concentrations (Carpenter and Lodge 1986; Desmet et al. 2011; Yeh et al. 2011). Hence hydrophytes are important resources for purifying eutrophicated water which can induce salt stress in irrigated crops (Carpenter and Lodge 1986).

Endophytes in their host plant can provide growth-promoting activity (You et al. 2015; Varma et al. 1999; Waller et al. 2005). They may also induce systemic resistance (ISR) in their host against environmental stress and disease (Mack and Rudgers 2008; Mejia et al. 2008; Rodriguez et al. 2008; Vandenkoornhuysen et al. 2002;

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✉ Young-Hyun You
rocer@hanmail.net

✉ Jong-Guk Kim
kimjg@knu.ac.kr

¹ Horticultural & Herbal Crop Environment Division, National Institute of Horticultural & Herbal Science, Rural Development Administration, Wanju 55365, Republic of Korea

² Distribution Safety Team, Safety Center, LOTTE R&D Center, Seoul 07209, Republic of Korea

³ School of Life Science, Kyungpook National University, Daegu 41566, Republic of Korea

Varma et al. 1999). These positive roles may enable hydrophytes to be used for effective water purification (Redman et al. 2002). For this the endophyte diversity of potential water-purifying aquatic plants in freshwater marshes must be determined. However, the diversity of endophytic fungi such hydrophytes has not been well established (You et al. 2015).

The present study was carried out to elucidate the distribution and diversity of endophytic fungi from the dominant native hydrophytes in freshwater marshes of the Korean Peninsula.

2 Materials and methods

2.1 Plant sampling

A total of 14 hydrophyte species (30 individuals per species) were sampled from (1) the Pyeonggi wetland located at Hanam-gun (Kyungsdangnam-do), (2) the Junam wetland located at Changwon-si, (3) the Jungyang wetland located at Hapcheon-gun, (4) the Daegok wetland located at Changnyeong-gun, (5) the Daebong wetland located at Changnyeong-gun, and (6) the Hwapocheon wetland located at the Nakdong river, a state river in Korea (Table 1). The first five wetlands are marshes in lakes with stable environmental conditions that are suitable for the development of hydrophyte communities because they are not subjected to massive level changes. The Junam (2.85 km²) and Pyeonggi (0.033058 km²) wetlands are formed by very slow water flow from lakes, whereas the Jungyang (0.00004 km²), Daegok (0.44 km²), and Daebong (0.40 km²) wetlands formed around confined old lakes that are now changing into swamps. In contrast, the Hwapocheon wetland (138.38 km²) is a back marsh of a large river. Back marshes always suffer from seasonal or climatic changes (massive flooding, heavy rains, etc.); thus, their wetland features are not stable and are always changing.

Each of the six wetlands exhibited hydrophyte communities, particularly the Hwapocheon wetland. Generally, plant communities vary according to environmental factors. Each of the five wetlands exhibited different plant biota and the dominant hydrophyte from each wetland was sampled. These plants commonly flourish at the margin of the wetlands or were floating on or under the water surface. The hydrophytes can be categorized into groups based on their habitat (Table 2) (Arnold and Van 2012; Julie and Fennessy 2001). Emergent hydrophytes commonly colonize the edge of shallow freshwater marshes. Floating-leaved hydrophytes establish roots in the bed of freshwater marshes, with the leaves floating at the surface while the entire plant body of submerged hydrophytes is under the water. Finally, free-floating

hydrophytes are found at the water surface and have poorly developed roots.

The sampled native plants were hydrophytes known to purify eutrophicated water. To prevent any physiological changes, the plant samples were collected and transported along with their respective freshwater and some rhizosphere soil from the sampling site. Hydrophytes species from the five reservoir wetlands used in this study are as follows: *Iris pseudacorus*, *Nelumbo nucifera* and *Salvinia natans* (from the Pyeonggi marsh), *Hydrocharis dubia*, *Pistia stratiotes*, *S. natans* and *Trapa japonica* (from the Junam marsh), *H. dubia*, *Nymphaea tetragona*, *Sagittaria trifolia* and *Typha orientalis* (from the Jungyang marsh), *H. dubia*, *S. natans* (from the Daegok marsh), *H. dubia*, *S. natans*, and *Trapa japonica* (from the Daebong marsh). The hydrophyte used in this study from the river back marsh (Hwapocheon wetland) are as follows: *H. dubia*, *I. pseudacorus*, *T. orientalis*, *Nymphoides peltata*, *Zizania latifolia*, *Miscanthus sinensis*, *Miscanthus sacchariflorus*, and *Vallisneria natans*.

2.2 Isolation of endophytic fungi

The harvested plant samples were washed with strong running sterile distilled water (SDW) and treated with sterilized 0.1 % Tween 80 solution (Sigma-Aldrich, St. Louis, MO, USA) for 10 min to eliminate suspended solids or microflora on the plant surfaces and were subsequently washed 3–4 times with SDW (Yamada et al. 2001). The samples were submerged in 1.0 % perchloric acid three times for 10 min each and subsequently washed 3–4 times with SDW. Residual surface moisture was removed with sterile gauze, and roots from plant samples were cut to a length of 3–4 cm (You et al. 2015). To exclude root bacteria, the treated samples were paced on Hagem minimal medium (You et al. 2012) containing 80 ppm streptomycin (Sigma-Aldrich) and incubated at 25 °C for 5–7 days (Hasan 2002; Khan et al. 2008; You et al. 2013). Subculture of any endophytic fungi to obtain pure cultures was done using potato dextrose agar (Difco, Detroit, MI, USA) under the same incubation conditions. Finally, isolates were selected for further study on the basis of morphological differences.

2.3 Extraction of genomic DNA and polymerase chain reaction (PCR)

Each isolate was inoculated into sterile potato dextrose broth medium and shake cultured at 25 °C and 120 rpm for 7 days. The mycobionts were then filtered and lyophilized for 2 days. Genomic DNA of the lyophilized mycobionts was extracted using a DNeasy Plant Mini Kit (Qiagen, Gaithersburg, MD, USA). The primers used to target the ITS regions for PCR were ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4

Table 1 Information of hydrophytes collected from various marshes in Korea

Geographical type of wetland	Sampling area	Scientific names	GPS information	Geographical position	Isolates number
Marsh of reservoir	Pyeonggi weland	<i>I. pseudacorus</i>	35°21'3.97"N/128°24'1.35"E	Gyeongsangnam-do	10
		<i>N. nucifera</i>	35°21'3.90"N/128°24'1.01"E	Haman	16
		<i>S. natans</i>	35°21'3.85"N/128°24'1.70"E		5
	Junam weland	<i>H. dubia</i>	35°18'25.48"N/128°40'28.67"E	Gyeongsangnam-do	5
		<i>P. stratiotes</i>	35°18'38.73"N/128°40'49.28"E	Changwon	14
		<i>S. natans</i>	35°18'25.47"N/128°40'28.79"E		11
		<i>T. japonica</i>	35°18'23.34"N/128°40'29.87"E		7
	Jungyang weland	<i>H. dubia</i>	35°33'12.08"N/128° 9'56.05"E	Gyeongsangnam-do	3
		<i>N. tetragona</i>	35°33'10.76"N/128° 9'56.80"E	Hapcheon	10
		<i>S. trifolia</i>	35°33'9.58"N/128° 9'58.04"E		2
	Daegok weland	<i>T. orientalis</i>	35°33'9.58"N/128° 9'58.04"E		10
		<i>H. dubia</i>	35°26'11.31"N/128°23'19.28"E	Gyeongsangnam-do	2
	Daebong weland	<i>S. natans</i>	35°26'17.04"N/128°23'24.04"E	Changnyeong	5
		<i>H. dubia</i>	35°26'25.23"N/128°27'6.45"E	Gyeongsangnam-do	2
	Back marsh of large river	Hwapocheon weland	<i>S. natans</i>	35°26'25.23"N/128°27'6.45"E	Changnyeong
<i>T. japonica</i>			35°26'26.41"N/128°27'12.23"E		3
<i>H. dubia</i>			35°18'47.55"N, 128°48'31.10"E	Pusan City,	16
<i>I. pseudacorus</i>			35°18'47.48"N, 128°48'30.84"E	Nakdong River	14
		<i>T. orientalis</i>	35°18'47.48"N, 128°48'30.84"E		19
		<i>N. peltata</i>	35°18'55.25"N, 128°48'3.17"E		17
		<i>Z. latifolia</i>	35°18'49.53"N, 128°47'36.11"E		13
		<i>M. sinensis</i>	35°18'49.95"N, 128°47'37.01"E		6
		<i>M. sacchariflorus</i>	35°18'42.34"N, 128°47'13.73"E		11
		<i>V. natans</i>	35°18'42.34"N, 128°47'13.73"E		10

(5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990). The PCR conditions were as follows: predenaturation (94 °C, 4 min), denaturation (94 °C, 1 min), annealing (55–58 °C, 1 min), and extension (72 °C, 2 min) for a total of 35 cycles, followed by a final extension (72 °C, 2 min) (You et al. 2015). The amplified PCR product was purified using an AccuPrep PCR Purification & Gel Extraction Kit (Bioneer, Daejeon, Korea) and sequenced on an ABI 3730xl DNA analyzer (Applied Biosystems, Carlsbad, CA, USA) (You et al. 2015).

2.4 Phylogenetic analysis and diversity

Using the BLASTn tool, ITS sequences of the fungal isolates were compared with the most similar sequences of fungal species published in the GenBank database of the National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov).

Phylogeny was performed using the MEGA program (Version 6.0) with sequence alignments prepared using the Clustal program (Tamura et al. 2013). The phylogenetic trees were inferred with the maximum-likelihood algorithm using

the Kimura 2-parameter model. The stability of relationships was evaluated by bootstrap analysis, with resampling repeated 1000 times (Tamura et al. 2013). To construct the trees, *Kluyveromyces lactis* Y-8279^T (NR 131273) was used as an outgroup.

2.5 Biodiversity

The diversity of endophytic fungi from each host hydrophyte species was deduced and compared. Diversity at the genus level was revealed using Margalef's richness index (Margalef 1958) and Menhinick's index (Whittaker 1977).

3 Results

3.1 Distribution of endophytic fungi

A total of 216 endophytic fungal strains were isolated from the six representative freshwater marshes (Supplementary Data 1). In total, 106 strains were isolated from the Hwapocheon

Table 2 Phylogenetical, ecological classification of each hydrophyte

Sampling area	Scientific names	Plant taxon		Life cycle of hydrophytes	Ecological types
		Phylum	Order		
Pyeonggi wetland	<i>I. pseudacorus</i>	Phanerogamae	Liliales	Perennial	Emerged plant
	<i>N.nucifera</i>	Angiospermae	Ranunculales	Perennial	Floating leaf
	<i>S. natans</i>	Pteridophyta	Polypodiales	Annual	Floating plant
Junam wetland	<i>H. dubia</i>	Angiospermae	Psammophytes	Perennial	Floating plant
	<i>P. stratiotes</i>	Angiospermae	Arales	Perennial	Floating plant
	<i>S. natans</i>	Pteridophyta	Polypodiales	Annual	Floating plant
	<i>T. japonica</i>	Phanerogamae	Memecylaceae	Annual	Floating leaf
Jungyang wetland	<i>H. dubia</i>	Angiospermae	Psammophytes	Perennial	Floating plant
	<i>N. tetragona</i>	Phanerogamae	Nymphaeales	Perennial	Floating leaf
	<i>S. trifolia</i>	Phanerogamae	Alismatales	Perennial	Emerged plant
	<i>T. orientalis</i>	Angiospermae	Pandanales	Perennial	Floating leaf
Daegok wetland	<i>H. dubia</i>	Angiospermae	Psammophytes	Perennial	Floating plant
	<i>S. natans</i>	Pteridophyta	Polypodiales	Annual	Floating plant
Daebong wetland	<i>H. dubia</i>	Angiospermae	Psammophytes	Perennial	Floating plant
	<i>S. natans</i>	Pteridophyta	Polypodiales	Annual	Floating plant
	<i>T. japonica</i>	Phanerogamae	Memecylaceae	Annual	Floating leaf
Hwapocheon wetland	<i>H. dubia</i>	Angiospermae	Psammophytes	Perennial	Fmerged plant
	<i>I. pseudacorus</i>	Phanerogamae	Liliales	Perennial	Fmerged plant
	<i>T. orientalis</i>	Angiospermae	Memecylaceae	Perennial	Floating leaf
	<i>N. peltata</i>	Phanerogamae	Nymphaeales	Perennial	Floating leaf
	<i>Z. latifolia</i>	Angiospermae	Poales	Perennial	Emerged plant
	<i>M. sinensis</i>	Angiospermae	Poales	Perennial	Emerged plant
	<i>M. sacchariflorus</i>	Angiospermae	Poales	Perennial	Emerged plant
<i>V. natans</i>	Angiospermae	Alismatales	Perennial	Submerged plant	

wetland, 34 strains were isolated from the Pyeonggi wetland, 37 strains were isolated from the Junam wetland, 25 strains were isolated from the Jungyang wetland, seven strains were isolated from the Daegok wetland, and seven strains were isolated from the Daebong wetland. The most isolates were from the Hwapocheon wetland reflecting the greater diversity of hydrophytes at this site (Table 3). A total of eight hydrophyte species were dominant groups in the Hwapocheon wetland (i.e., *H. dubia*, *I. pseudacorus*, *T. orientalis*, *N. peltata*, *Z. latifolia*, *M. sinensis*, *M. sacchariflorus*, and *V. natans*), whereas at the other sites there were fewer: in Pyeonggi (*I. pseudacorus*, *N.nucifera*, and *S. natans*), in Junam (*H. dubia*, *P. stratiotes*, *S. natans*, and *T. japonica*), in Jungyang (*H. dubia*, *N. tetragona*, *S. trifolia*, and *T. orientalis*), in Daegok (*H. dubia* and *S. natans*), and in Daebong (*H. dubia*, *S. natans*, and *T. japonica*) (Table 4). A diversity of endophyte fungi were found in these native Korean hydrophytes (Table 1). The number and distribution of fungal isolates from the dominant plants was differed between marshes. There were also variations in isolate numbers

among the marshes, even within the same species of hydrophyte.

3.2 Molecular identification and phylogenetic analysis

The 216 endophytic fungal strains isolated from the 14 host plant species were categorized into three phyla, seven classes, and 43 genera. Most of isolates belonged to the phylum Ascomycota; however, three strains belonged to Basidiomycota and one strain belonged to Zygomycota. This demonstrated the numerical dominance (98.1 %) of Ascomycota as hydrophytic endophytes in native to freshwater wetland environments.

The following classes were confirmed: Sordariomycetes (76 strains, 35.1 %), Dothideomycetes (70 strains, 32.4 %), Saccharomycetes (30 strains, 13.8 %), Eurotiomycetes (20 strains, 9.2 %), Leotiomycetes (13 strains, 6.0 %), Ustilaginomycetes (three strains, 1.3 %), and Mucoromycotina (one strain), as shown in Fig. 1a. Thus, the classes Sordariomycetes, Dothideomycetes, and

Table 3 Diversity index of endophytic fungi distributed in each freshwater marshes

Fungal taxon	Hwapocheon wetland	Junam wetland	Pyeonggi wetland	Jungyang wetland	Daegok wetland	Daebong wetland
<i>Acephala</i>		1	1			
<i>Alternaria</i>	15	1		1		
<i>Anguillospora</i>	3					
<i>Aspergillus</i>			1			
<i>Calonectria</i>		1				1
<i>Cephalosporium</i>	1		1			
<i>Cladosporium</i>	18	9		2	1	
<i>Clavariopsis</i>	1					
<i>Colletotrichum</i>	2	1				
<i>Curvularia</i>	2					
<i>Dimorphospora</i>	0		2			
<i>Epicoccum</i>	1	2	1	1		
<i>Filospora</i>		7				
<i>Fusarium</i>	13	2	7	16	1	2
<i>Geomyces</i>			1			
<i>Gibberella</i>	7					
<i>Ilyonectria</i>	1					
<i>Leptosphaerulina</i>	1					1
<i>Massarina</i>	3		1			
<i>Microsphaeropsis</i>	2					
<i>Mucor</i>	1					
<i>Nectria</i>	1					
<i>Neopestalotiopsis</i>		1	1			
<i>Paraconiothyrium</i>		1				
<i>Paraphaeosphaeria</i>	5	1	3	2		
<i>Paraphoma</i>	5					
<i>Penicillium</i>			4			
<i>Pestalotiopsis</i>	2	3		1		
<i>Phaeosphaeria</i>			1			
<i>Phoma</i>	7	4			3	1
<i>Plectosphaerella</i>	2			1		
<i>Pseudeurotium</i>			1			
<i>Pseudozyma</i>	2			1		
<i>Robillarda</i>	1					
<i>Sarcopodium</i>			2			
<i>Setophaeosphaeria</i>	1					
<i>Stagonosporopsis</i>						
<i>Talaromyces</i>	3	2	7		2	1
<i>Tetraplophaeria</i>						
<i>Thielavia</i>	1					
<i>Trichoderma</i>	1	1				1
<i>Torula</i>	1					
<i>Westerdykella</i>	3					
Total	106	37	34	25	7	7
S	29	15	15	8	4	6
Margalef's richness	6.004	3.877	3.970	2.175	1.542	2.569
Menhinick's index (Dmn)	2.817	2.466	2.572	1.600	1.512	2.268

Table 4 Diversity index of endophytic fungi isolated from each hydrophyte species

Fungal taxonomic	Hwapocheon wetland					Junam wetland						
	<i>H. dubia</i>	<i>I. pseudacorus</i>	<i>T. orientalis</i>	<i>N. peltata</i>	<i>Z. latifolia</i>	<i>M. sinensis</i>	<i>M. sacchariflorus</i>	<i>V. natans</i>	<i>H. dubia</i>	<i>P. stratiotes</i>	<i>S. natans</i>	<i>T. japonica</i>
<i>Acephala</i>												
<i>Alternaria</i>	2		4	5	1		2	1	1		1	
<i>Anguilospora</i>	2							1				
<i>Aspergillus</i>								1				
<i>Calonectria</i>								1				
<i>Cephalosporium</i>												
<i>Cladosporium</i>		5	5	5	2	1	1		2	2	5	
<i>Clavariopsis</i>	1											
<i>Colletotrichum</i>	2				1							1
<i>Curvularia</i>	1											
<i>Dimorphospora</i>												
<i>Epicoccum</i>				1						1	1	
<i>Filosporella</i>										4		
<i>Fusarium</i>			5	1	1	3	3					3
<i>Geomyces</i>												2
<i>Gibberella</i>			2	1	1	1	2					
<i>Ilyonectria</i>						1						
<i>Leptosphaerulina</i>												1
<i>Massarina</i>	1							2				
<i>Microsphaeropsis</i>	1							1				
<i>Mucor</i>												
<i>Nectria</i>								1				
<i>Neopestalotiopsis</i>								1				
<i>Paraconiothyrium</i>									1		1	
<i>Paraphaeosphaeria</i>	2	1			1	1			1			
<i>Paraphoma</i>	1	1			1	1	1					
<i>Penicillium</i>												
<i>Pestalotiopsis</i>			2						1	1	1	
<i>Phaeosphaeria</i>	1											
<i>Phoma</i>	1		1	4	1						2	1
<i>Plectosphaerella</i>	1	1										
<i>Pseudeurotium</i>												
<i>Pseudozyma</i>		1									1	
<i>Robillarda</i>											1	
<i>Sarcopodium</i>												
<i>Setophaeosphaeria</i>	1											
<i>Stagonosporopsis</i>												
<i>Talaromyces</i>		1			1	1	1			1	1	
<i>Tetraplospora</i>		1										
<i>Thielavia</i>												1
<i>Trichoderma</i>												
<i>Torula</i>					1		1					
<i>Westerdykella</i>		3										

Table 4 (continued)

Fungal taxonomic	Hwapocheon wetland					Junam wetland														
	<i>H. dubia</i>	<i>I. pseudacorus</i>	<i>T. orientalis</i>	<i>N. peltata</i>	<i>Z. latifolia</i>	<i>M. sinensis</i>	<i>M. sacchariflorus</i>	<i>V. natans</i>	<i>H. dubia</i>	<i>P. stratiotes</i>	<i>S. natans</i>	<i>T. japonica</i>								
Total	16	14	19	17	13	6	11	10	6	14	11	7								
S	12	8	6	6	12	4	7	9	4	10	6	4								
Margalef's richness	3.967	2.652	1.698	1.765	4.289	1.674	2.502	3.474	1.674	3.410	2.085	1.542								
Mehnick's index	3.000	2.138	1.376	1.455	3.328	1.633	2.111	2.846	1.633	2.673	1.809	1.512								
Fungal taxonomic	Pyeonggi wetland					Jungyang wetland					Daegok wetland					Daebong wetland				
	<i>I. pseudacorus</i>	<i>N.nucifera</i>	<i>S. natans</i>	<i>H. dubia</i>	<i>N. tetragona</i>	<i>S. trifolia</i>	<i>T. orientalis</i>	<i>H. dubia</i>	<i>S. natans</i>	<i>H. dubia</i>	<i>S. natans</i>	<i>T. japonica</i>								
<i>Acephala</i>	1																			
<i>Alternaria</i>				1																
<i>Anguillospora</i>																				
<i>Aspergillus</i>	1																			
<i>Catonectria</i>												1								
<i>Cephalosporium</i>	1																			
<i>Cladosporium</i>				2																
<i>Clavariopsis</i>																				
<i>Colletotrichum</i>																				
<i>Curvularia</i>																				
<i>Dimorphospora</i>																				
<i>Epicoccum</i>	1				1															
<i>Filosporella</i>																				
<i>Fusarium</i>	3	1	3		9	1	6	1				2								
<i>Geomyces</i>																				
<i>Gibberella</i>	1																			
<i>Ilyonectria</i>																				
<i>Leptosphaerulina</i>											1									
<i>Massarina</i>	1																			
<i>Microsphaeropsis</i>																				
<i>Mucor</i>																				
<i>Nectria</i>																				
<i>Neopestalotiopsis</i>				1																
<i>Paraconiothyrium</i>																				
<i>Paraphaeosphaeria</i>	1											1								
<i>Paraphoma</i>																				

Table 4 (continued)

Fungal taxonomic	Pyeonggi wetland		Jungyang wetland			Daegok wetland		Daebong wetland	
	<i>I. pseudacorus</i>	<i>N. nucifera</i>	<i>S. natans</i>	<i>H. dubia</i>	<i>N. tetragona</i>	<i>S. trifolia</i>	<i>T. orientalis</i>	<i>H. dubia</i>	<i>S. natans</i>
<i>Penicillium</i>		1							
<i>Pestalotiopsis</i>							1		
<i>Phaeosphaeria</i>			1						
<i>Phoma</i>								3	1
<i>Plectosphaerella</i>							1		
<i>Pseudeurotium</i>	1								
<i>Pseudozyma</i>							1		
<i>Robillardia</i>									
<i>Sarcopodium</i>	1		1						
<i>Setophaeosphaeria</i>									
<i>Stagonosporopsis</i>									
<i>Talaromyces</i>	2	5						2	1
<i>Tetraplophaeria</i>									
<i>Thielavia</i>									1
<i>Trichoderma</i>									
<i>Torula</i>									
<i>Westerdykella</i>									
Total	11	17	6	3	10	2	10	2	2
S	7	10	3	2	2	2	5	2	2
Margalef's richness	2.502	3.177	1.116	0.910	0.434	1.443	1.737	1.443	1.443
Mehinick's index	2.111	2.425	1.225	1.155	0.632	1.414	1.581	1.414	1.414

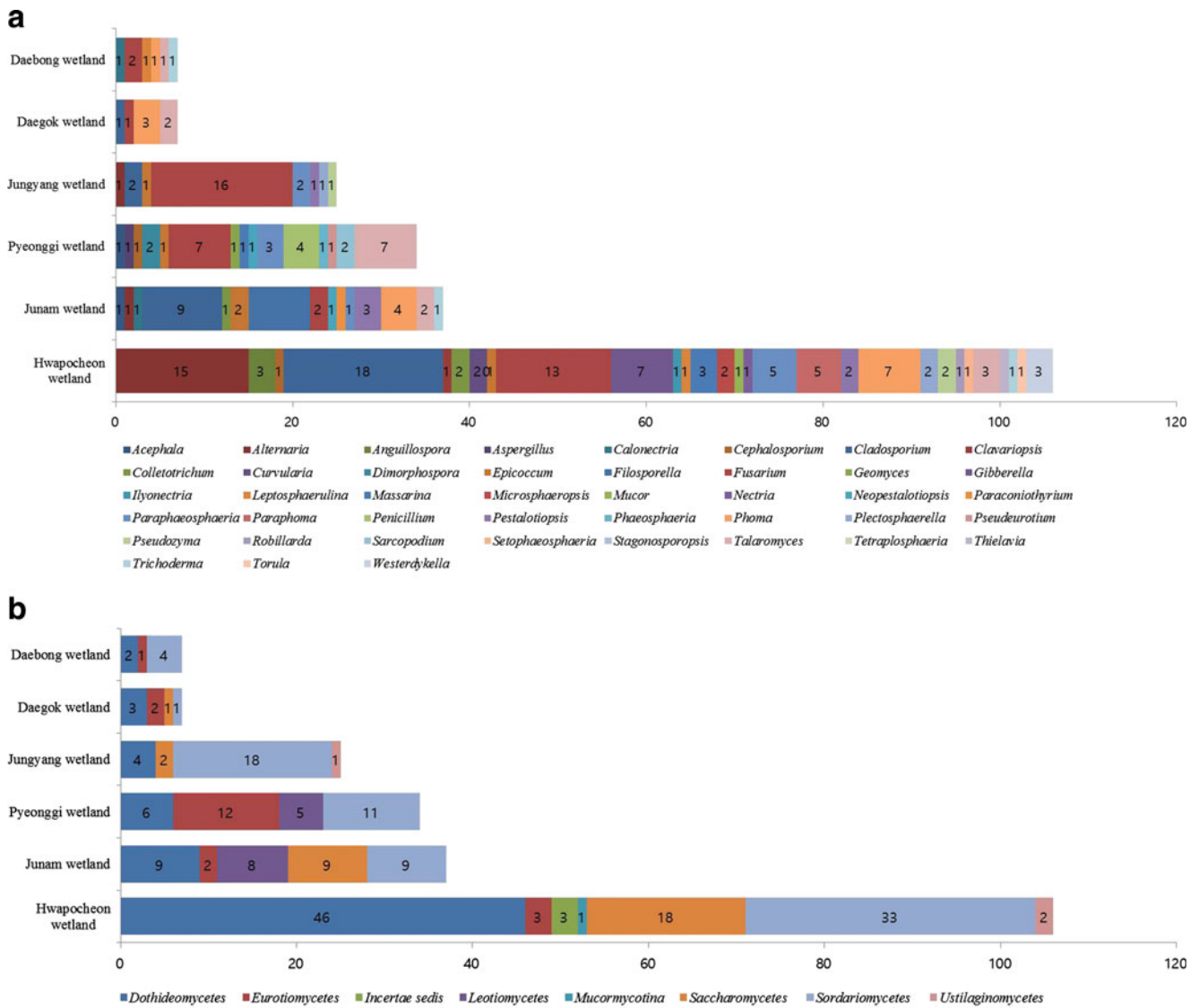


Fig. 1 The distribution of fungal isolates in different different marshes at the classes (a) and genera (b) levels

Saccharomycetes were the most dominant endophyte group in these Korean hydrophytes. However, Eurotiomycetes were dominante in the Pyeonggi wetland, and this is may be explained by the emergence of a high proportion of *Talaromyces* from the hydrophyte *N. nucifera*. Incidentally, this plant has been known as water-purifying hydrophyte, and several species of *Talaromyces* have been shown to have biological activity for PGPR (plant growth-promoting rhizobacteria) or ISR (Li et al. 2014a, b; Murray et al. 1977; Soltani and Hosseyni Moghaddam 2015). Studies are required to characterize better some of the isolates belonging to *Talaromyces*.

At the genus level, there were no variations in dominant endophyte genera among wetlands or host hydrophyte species (Fig. 1b). However, within the same species, fungal distribution or diversity varied according to the wetlands they inhabited. Endophytic biotas therefore seem to be affected

more by the specific freshwater wetland environment than by the plant species. The nucleotide sequences of the endophytic fungal strains recovered have been registered at the NCBI GenBank database. The phylogenetic trees of the endophytic fungi isolated from the roots of hydrophytes native to each marsh were constructed. To construct the trees, *Bacillus altitudinis* 41KF2b^T (AJ831842) was used as an outgroup (Fig. 2).

3.3 Biodiversity

The biodiversity of the fungal flora was assessed on the basis of the results from each hydrophyte species native to each marsh. Based on the genera count by plant sample, the diversity value was calculated (Table 4). In the Hwapocheon

wetland, the fungal biota from each hydrophyte exhibited the following diversity values:

H. dubia, 3.967; *I. pseudacorus*, 2.652; *T. orientalis*, 1.698; *N. peltata*, 1.765; *Z. latifolia*, 4.289; *M. sinensis*, 1.674; *M. sacchariflorus*, 2.502; and *V. natans*, 3.474 (Margalef's richness, Dmg) or *H. dubia*, 3.000; *I. pseudacorus*, 2.138; *T. orientalis*, 1.376; *N. peltata*, 1.455; *Z. latifolia*, 3.328; *M. sinensis*, 1.633; *M. sacchariflorus*, 2.111; and *V. natans*, 2.846 (Menhinick's index, Dmn). *Z. latifolia* (4.289 and 3.328) had the highest diversity values according to both the Margalef's richness (Dmg) and Menhinick's index (Dmn). This result was likely due to the relatively low dominance of fungal genus (*Cladosporium*) and the higher richness. The reason that *T. orientalis* had the lowest diversity values for both indices may be the dominance of the specific genera (*Fusarium*, *Cladosporium*) and low species richness. The thicknesses of the root epidermis and root cap differ among hydrophyte species and may have an influence on the fungal biotas (Arnold and Van 2012; Julie and Fennessy 2001).

The biodiversity of fungal endophytes was also assessed for each marsh (Table 3). In terms of genus richness, each marsh had the following Margalef's richness indices: Hwapocheon wetland, 6.004; Pyeonggi wetland, 3.97; Junam wetland, 3.877; Jungyang wetland, 2.175; Daegok wetland, 1.542; and Daebong wetland, 2.569. The genus richness according to Menhinick's index was 2.572 for the Pyeonggi wetland, 2.466 for the Junam wetland, 1.600 for the Jungyang wetland, 1.512 for the Daegok wetland, 2.268 for the Daebong wetland, and 2.817 for the Hwapocheon wetland.

4 Discussion

Since the prehistoric age, marshes have been present in the Korean Peninsula and been used as sources of irrigation water (Kong and David 1993). Because of this, hydrophytes have adapted to specific marshes that have distinct environmental characteristics. Based on the results of our molecular identification and phylogenetic analysis, there are fungal genera that have positive interactions with their host plants. The genera *Theobroma*, *Penicillium*, *Pseudozyma*, *Paraphaeosphaeria*, *Anguillospora*, *Microsphaeropsis*, *Gibberella*, *Talaromyces* have been reported to promote plant growth, induce resistance to environmental stress, and produce antimicrobial agents (Bezerra et al. 2015; Buxdorf et al. 2013; Hossain et al. 2007; Hussain et al. 2015; Khan et al. 2011; Khan et al. 2013; Mejía et al. 2008; Nicoletti and Stefano 2012; Sati and Singh 2014). Thus there are potential biotechnological applications for endophytes from hydrophytes. If the endophytes identified in this study can improve the growth or salt tolerance of water-purifying hydrophytes, they might represent

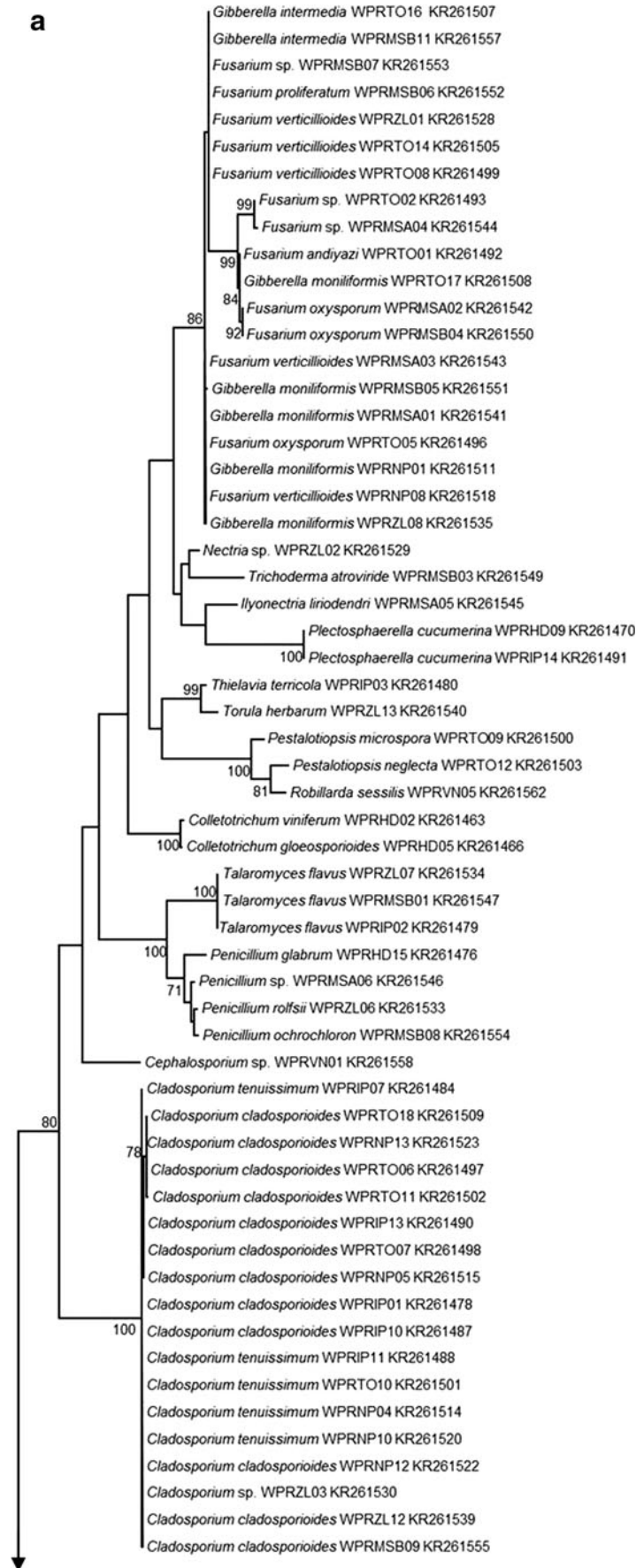
Fig. 2 Phylogenetic analysis of endophytic fungi isolated from various marshes of Korea. This phylogenetic tree was constructed by using the maximum likelihood (1000 bootstrap replications). Bootstrap values (70 %) are indicated at relevant nodes. Dendrogram of endophytic fungi isolated from the hydrophytes in **a** Hwapocheon Wetland, **b** Junam marsh, **c** Pyeonggi marsh, **d** Jungyang marsh, **e** Daegok marsh and **f** Daebong marsh. Scale bar: 0.1

new types of microbial agents that can be used to cope with water pollution or eutrophication.

The endophyte diversity from of the Hwapocheon wetland was higher than that from the other wetlands, even within the same hydrophyte species. In the case of *H. dubia*, the diversity values were 3.967/3.000 in the Hwapocheon wetland, but were only 1.674/1.633 in the Junam wetland, 0.910/1.155 in the Jungyang wetland, and 1.443/1.414 in the Daegok wetland. In case of *I. pseudacorus*, the diversity values were 2.652/2.138 in the Hwapocheon wetland, but 2.502/2.111 in the Pyeonggi wetland. Furthermore, at the marsh level, the Hwapocheon wetland exhibited higher diversity values than the other five reservoir wetlands for both of the diversity indices. Among the five reservoir wetlands, the Pyeonggi and Junam marshes showed higher diversity values and broader distributions of fungal genera than the Jungyang, Daegok, and Daebong marshes. Overall, the Hwapocheon wetland had the most isolates and the highest endophyte diversity at the hydrophyte individual and wetland levels. This may have been caused by the specific geographical characteristics of each wetland.

The endophyte distribution and diversity of mesophytes can vary according to the host plant species because of many factors including the thickness of the root epidermis or root cap. Therefore, we attempted to determine the correlation between the microbial distribution and the characteristics of the plant (Arnold and Van 2012). Perennial plants were found to have relatively higher diversity values for endophytes than those of annual plants within the same marsh land (Tables 1 and 2). Thus, perennial plants appeared to have a dominant position over annual plants for establishing endosymbiotic relationships with fungi. The genetic diversity of endophytes also seemed to be affected by the life cycle of hydrophytes, interacting with the environment over time. However, this appeared not to apply to isolated from the Hwapocheon wetland. This is may be because back marshes of large rivers exhibit constant changes in environmental factors as a result of flooding, precipitation, or drought. Therefore, endophytes within such environments do not differentiate between annual or perennial plants. Nevertheless, the diversity values and isolate numbers were higher in this wetland which may reflect geological factors.

A comparisons of the wetlands based on plant taxon (Julie and Fennessy 2001). revealed that there were no distinguishing patterns related to diversity of gymnosperms, angiosperms, and pteridophyta (Tables 1 and 2). A



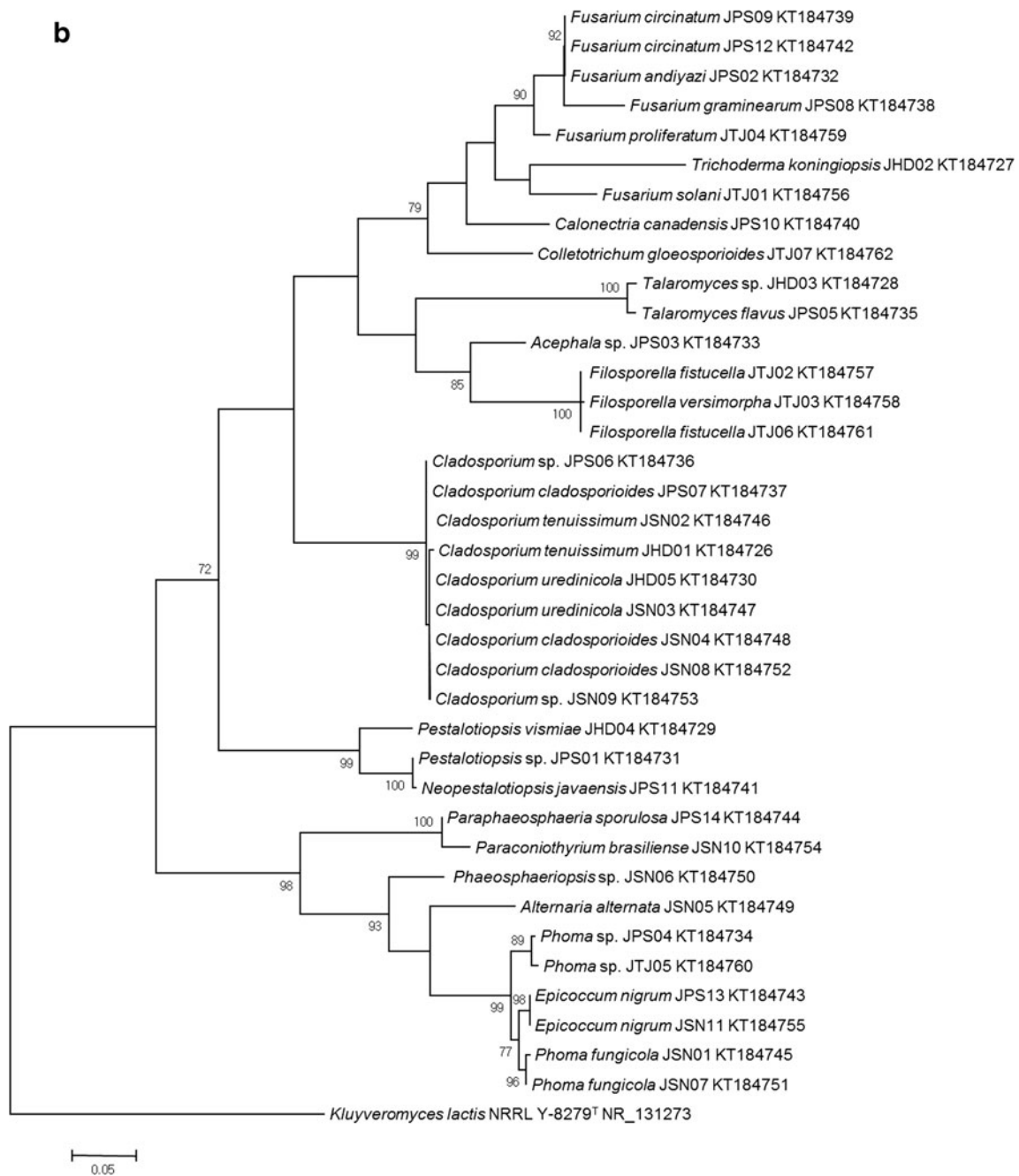


Fig. 2 (continued)

comparisons based on plant lifestyle in the aquatic environment (Arnold and Van 2012), revealed that floating plants had higher diversity values than emerged plants or floating-leaved plants (Tables 1 and 2). Thus the environmental characteristics of each wetland seemed to affect the diversity or distribution of endophytes to a greater extent than plant life style or plant taxon.

The geomorphological features of each wetland modified by the indigenous topographical characteristics of the Korean Peninsula (Kong and David 1993), influence the fungal

diversity. The Pyeonggi marsh (approximately 250 m in diameter) and the Junam marsh (2.9 km in length) have been separated from the main river so there is no chance of freshwater exchange. However, the Jungyang marsh (1 km length) is located between two branches of the river and is not separated from the river so large amounts of freshwater can flow in or out. In the Daegok and Daebong marshes (both approximately 300–400 m in diameter), water flows in from upstream, originating from the surrounding mountains and then flows out. It would be expected that wetlands not linked to the

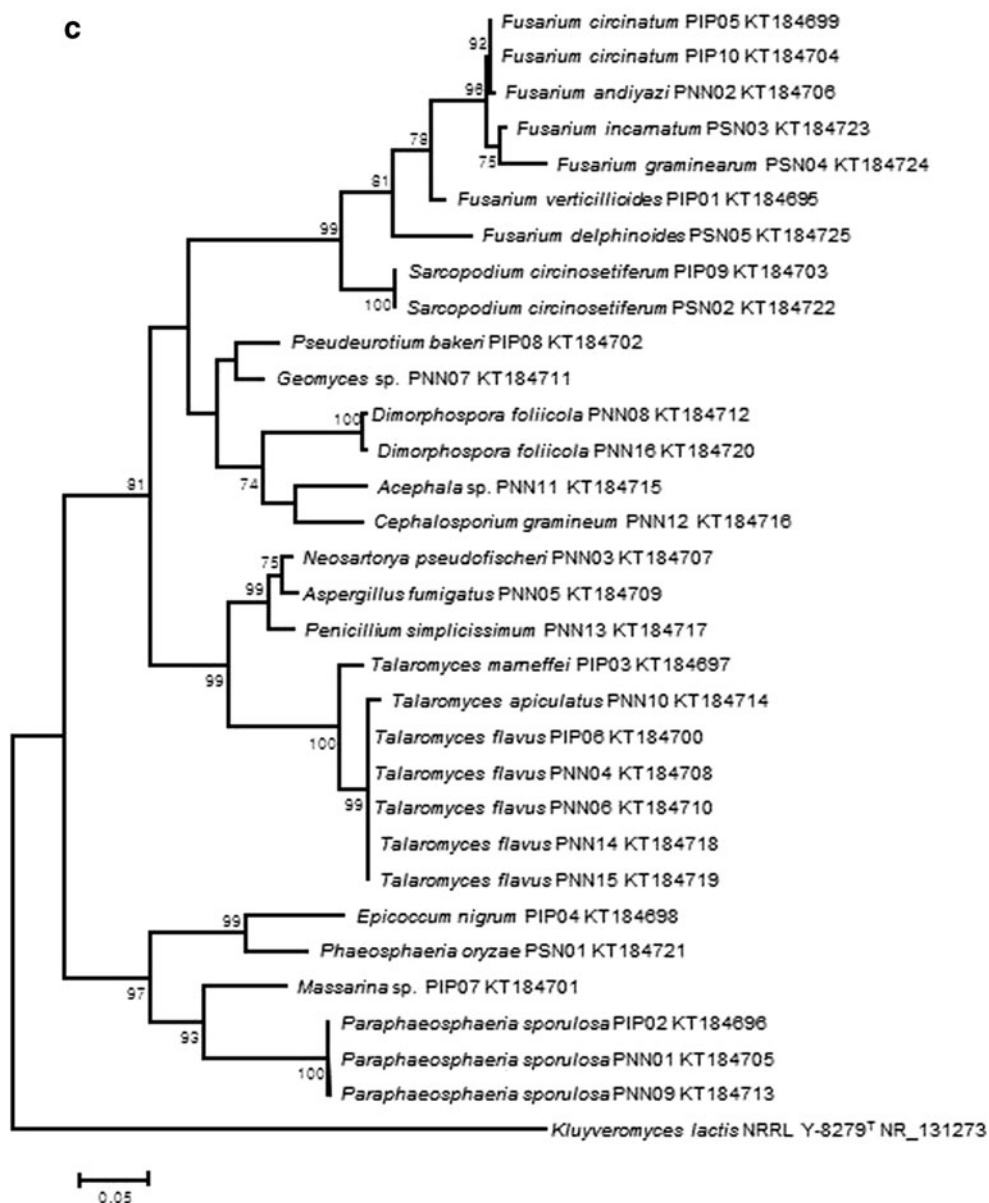


Fig. 2 (continued)

main river might show a higher diversity of endophytic fungi (Table 3) because of the opportunity for stable interactions between the fungus and host hydrophyte. However, at least in the early stages of wetlands developing in reservoirs, this does not seem to apply (Kong and David 1993). Indeed, the Hwapocheon wetland periodically is subject to major water exchanged due to flooding and heavy rain but still exhibits the highest diversity values for endophytes.

Salvinia natans and *H. dubia*, are known to control eutrophication (Xiang et al. 2009). and had a higher diversity of endophytic fungi at the Pyeonggi and Junam marshes than at the Jungyang, Daegok, and Daebong marshes. Endophyte isolates from water-purifying plants native to marshes separated

from their main river have been assumed to be potential sources of microbes for promoting the growth such plants where water quality is low. This is based on the observation that *Nelumbo nucifera* from the Pyeonggi wetland harbors a higher density of *Talaromyces*, which have been shown to have biological activity related to associated plant growth-promoting rhizobacteria (PGPR) or ISR, as described above (Li et al. 2014a, b; Murray et al. 1977; Soltani and Hosseini Moghaddam 2015). Our finding that the stagnant freshwater marshes, Pyeonggi and Junam which can be easily eutrophicated, had high endophyte diversity values may reflect the importance of these organisms to the success of hydrophytes growing there.

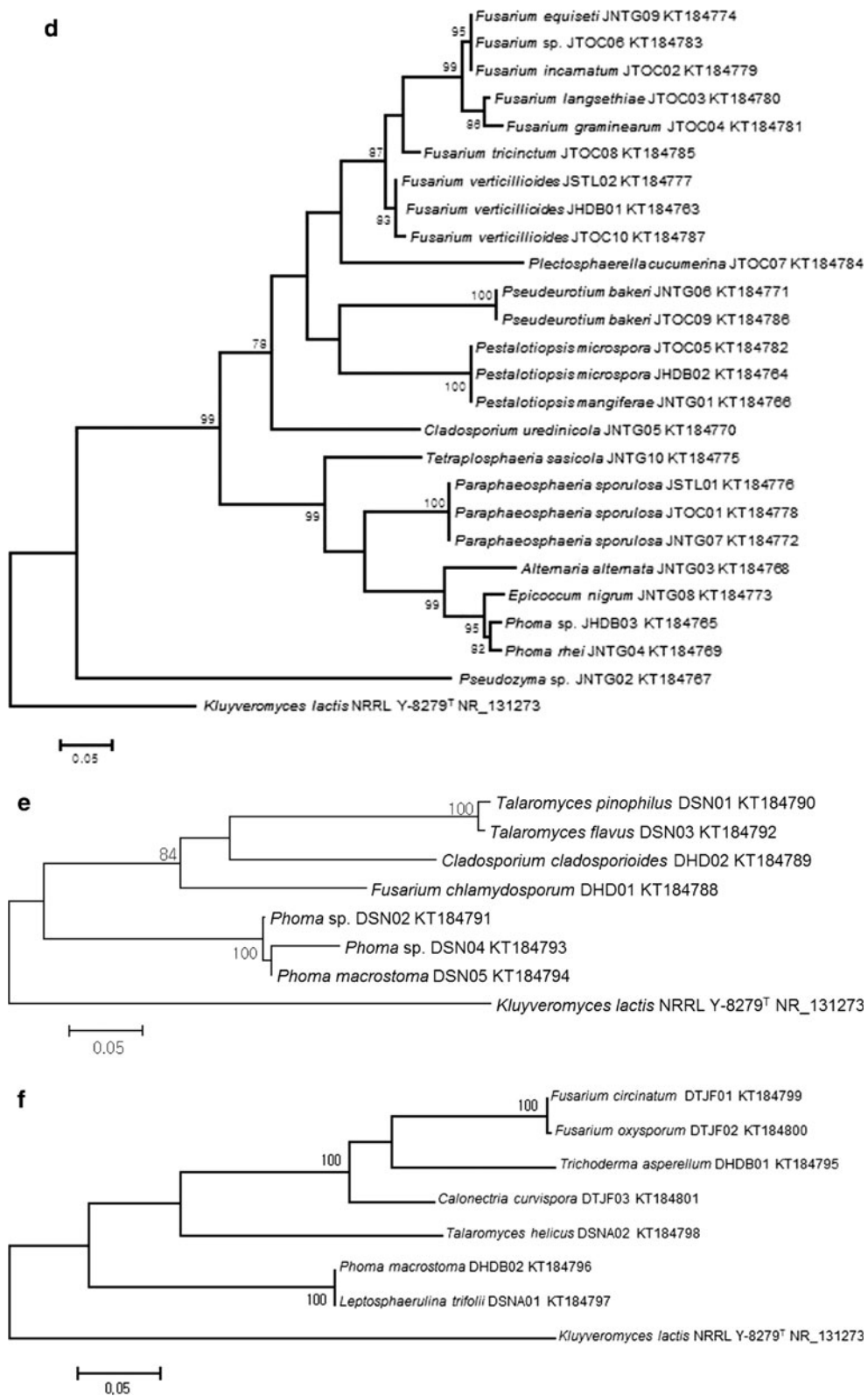


Fig. 2 (continued)

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