

Discovering fungal communities in roots of *Zoysia japonica* and characterising novel species and their antifungal activities

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Abstract

Turf-grasses are economically important horticultural crops, which have been utilised by humans to improve the environment for more than a thousand years. Turf-grasses are widely distributed in landscapes, slopes and sport fields, such as golf courses. Endophytic fungi are a resource of unexplored fungal diversity with potential bioactive compounds. In this study, culture-independent ITS amplicon sequencing and culture-dependent isolation methods were used to reveal fungal community in roots of the turf-grass *Zoysia japonica*. A total of 317 OTUs were identified from root samples of *Z. japonica* by analysis of ITS amplicon reads. Fungal community was dominated by *Sordariales* (32.45%), followed by *Chaetothyriales* (18.16%), unknown taxa in *Sordariomycetes* (14.63%) and *Pleosporales* (12.48%). During isolation, 151 endophytic fungal strains were obtained from roots of *Z. japonica* and a variety of taxa were found by ITS amplification and sequencing. Moreover, 11 endophytic fungal species were further characterised in this study, based on morphological characterisation and multi-loci phylogenetic analysis, including *Niesslia dimorphospora*, a newly-recorded species in Korea and 10 novel species (*Dactylaria hwasunensis* **sp. nov.**, *Lophiostoma jeollanense* **sp. nov.**, *Magnaporthiopsis zoysiae* **sp. nov.**, *Poaceascoma endophyticum* **sp. nov.**, *P. koreanum* **sp. nov.**, *P. magnum* **sp. nov.**, *P. zoysiiadicicola* **sp. nov.**, *Stagonospora endophytica* **sp. nov.**, *Setophoma zoysiae* **sp. nov.** and *Pseudorhizophila poae* **sp. nov.**). Antifungal activities of these species were tested against the turf-grass brown patch pathogen *Rhizoctonia solani* AG2-2(IIIB), with *S. zoysiae* being the best antagonist. In addition, butanol extract from mycelia of *S. zoysiae* strongly inhibited *R. solani* AG2-2(IIIB) *in vitro* and *in planta*. The results of this study expand the biodiversity of endophytic fungi and revealed potential biological resources for future turf-grass management and bioactive compound exploitation.

Key words: Bioactivity, endophytic fungi, ITS amplicons, phylogeny, turf-grass



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Introduction

Zoysiagrasses (*Zoysia* spp. Willd.) are mat-forming perennial warm-season grasses belonging to the family *Poaceae*, native to the western Pacific Rim and the Indian Ocean (Tothill and Hacker 1983; Soreng et al. 2015). Zoysiagrasses gained popularity amongst growers for their low maintenance inputs and tolerance to multiple biotic and abiotic stresses. A total of 11 species are

reported in the genus *Zoysia*, but only *Z. japonica*, *Z. matrella* and *Z. pacifica* and their interspecific hybrids have been widely used as turf-grass including on golf courses, athletic fields, home lawns and other recreational sites (Chandra et al. 2017). *Zoysia japonica* is one of the most important turf-grass species distributed mainly in Asia, North and South America and Australia (Loch et al. 2017). Due to its popularity, some biotechnologies, such as conventional breeding and molecular transformation have been used to improve *Z. japonica* (Ge et al. 2006; Yang et al. 2023).

Endophytes colonising intra- or intercellular plant tissues play multifaceted roles in plant-microbe interactions, ranging from promotion of plant growth to biocontrol of pathogens and enhancement of biotic/abiotic tolerance (Sridhar 2019; Bharadwaj et al. 2020). Application of endophytes is increasingly envisioned to reduce the use of agrochemicals and lower production costs (Tiwari et al. 2023). In recent years, endophytic fungi have attracted great attention from researchers and are increasingly being investigated for their ability to secrete various bioactive compounds (secondary metabolites), simulating plant response to environmental stresses, facilitating nutrient uptake and acting as biocontrol agents (Poveda et al. 2020, 2021; Jha et al. 2023). For example, endophytic *Trichoderma* spp. are well known to enhance hosts' tolerance to multiple stresses and antagonise the growth of a wide range of plant pathogens (Ripa et al. 2019; Rajani et al. 2021). Xia et al. (2019) reported that endophytic fungal species *Aspergillus nidulans*, *Coniothyrium aleuritis*, *Fusarium oxysporum*, *Fusarium proliferatum*, *Pichia guilliermondii* and *Trichoderma spirale* significantly increased tomato fruit yield. According to Fávoro et al. (2012), the endophyte *Epicoccum nigrum* isolated from sugarcane (*Saccharum officinarum*) was able to increase root biomass and inhibit several pathogens *in vitro*. In turf-grass, the most frequently studied endophytes belong to the genus *Epichloë* (anamorphs in *Neotyphodium*) (Meyer et al. 2015). *Epichloë* sp. was reported to increase the root growth, metabolic activity and nutrient uptake of ryegrass (*Lolium perenne*), thereby improving its survival in less fertile soil (Chen et al. 2020). It was also found that ryegrass with the presence of the endophyte *Epichloë* showed increased resistance to pathogens *Drechslera siccans* and *Fusarium* spp. (Wiewióra et al. 2015). Currently, more than 80 commercial turf-grass cultivars contain endophytes and new grass-endophyte combinations are being developed to reduce pesticide use and lower turf maintenance input (Clay 1990; Wiewióra et al. 2015). However, endophytes have not been found in warm-season grasses (Held and Potter 2012).

Although many studies on endophytes have been carried out to capitalise on their potential applications in enhancing agricultural productivity, only a small fraction of endophytes have been isolated and studied and most endophytes remain largely unexplored. Thus, new approaches such as metagenomic and amplicon sequencing have provided more insights into the diversity of endophytes and simplified the analysis of endophytic microbial communities. For fungi, the internal transcribed spacer (ITS) region has been recognised as the standard barcode and either the ITS1 or the ITS2 region is used for high-throughput sequencing to analyse environmental fungal diversity (Monard et al. 2013). Using this technique, Porras-Alfaro et al. (2008) revealed root associated fungi (RAF) in a grass *Bouteloua gracilis*, which was dominated by dark septate fungi (DSF) in *Pleosporales*. Khidir et al. (2010)

found the presence of RAF in several grass species and a similar cohort of fungal dominants was shared by grasses in semi-arid landscapes. In addition to using a high-throughput sequencing technique alone, an increasing number of studies are incorporating culture-dependent isolation for improved results. For example, Abeywickrama et al. (2023) elucidated fungal community composition associated with *Astragalus sinicus* and *Vicia villosa* by culture-dependent methods and culture-independent amplicon analyses, which is meaningful for green manure practices. Manzotti et al. (2020) analysed the fungal root endophytes community structure of tomato with similar integrative methods and revealed pathogenicity of endophytic fungi by isolation and re-inoculation, suggesting that equilibrium and evenness within the microbiome community are essential for plant health.

Due to the significance of root-inhabiting endophytic fungi to turf-grasses and because little is known about the endophytes associated with the warm-season turf-grass *Z. japonica*, this study was designed to explore the structure of fungal community in roots of *Z. japonica* through culture-independent and culture-dependent approaches, describe unexplored species and investigate their potential antifungal activities (Fig. 1).

Materials and methods

Sampling and ITS amplicon sequencing

Healthy plants of *Z. japonica* were sampled from three different sites on a golf course located in Hwasun, South Jeolla Province, Korea. The three samples were washed of soil and the roots of each sample were cut with sterilised scissors. Then the roots were surface disinfected with 2% sodium hypochlorite for 2 min and rinsed three times in sterile distilled water. Genomic DNA from 100 mg of each root sample was extracted using a FavorPrep Plant Genomic DNA Extraction Mini Kit (Favorgen Biotech Corp, Ping-Tung, Taiwan) according to the manufacturer's instructions. Primer set ITS3 (5'-GCATCGATGAAGAACGCAGC-3')/ITS4

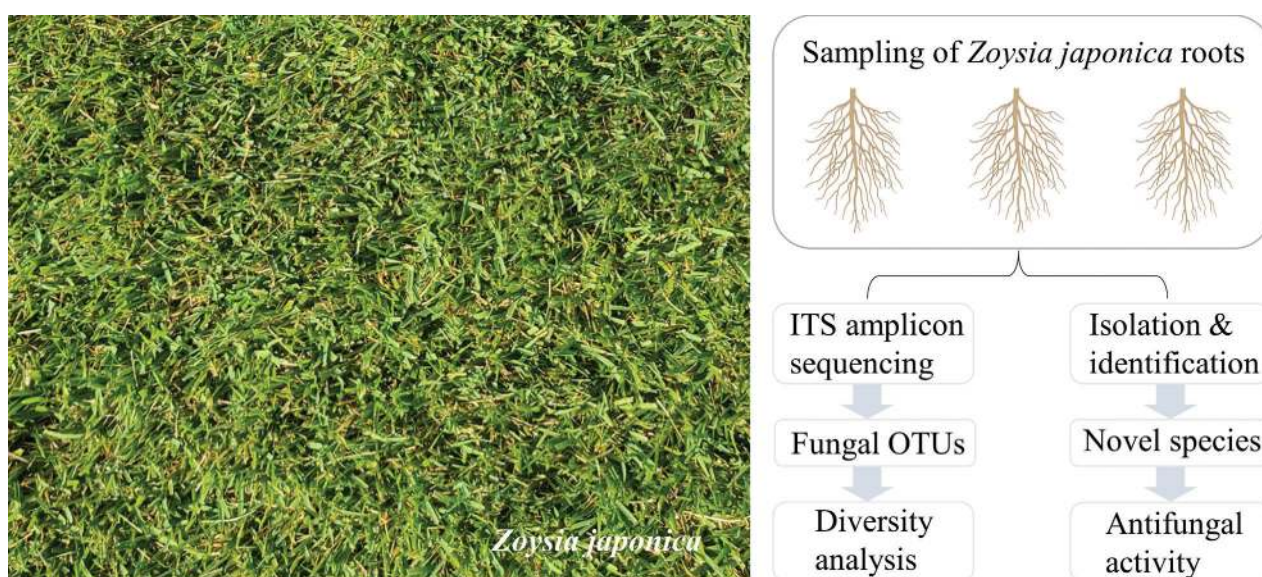


Figure 1. Sampling region of *Zoysia japonica* from a golf course (left) and schematic diagram of this study (right).

(5'-TCCTCCGCTTATTGATATGC-3') was used to amplify the ITS2 region of the internal transcribed spacer. Amplicon sequencing was performed using an Illumina MiSeq platform by the Life is Art of Science (LAS) Laboratory (Gimpo, Korea).

Analysis of ITS amplicon sequencing

Quality control of the raw reads from ITS amplicon sequencing of the three *Z. japonica* root samples was performed with FastQC v.0.12.1 (Andrews 2018). Primer sequences in the reads were removed by Cutadapt v.4.0 (Martin 2011). Resulting reads were further processed using a Vsearch v.2.22.1 pipeline (Rognes et al. 2016). First, paired-end reads were merged, truncated to an equal length (300 bp) and quality filtered. Chimeras were removed after *de novo* detection, followed by removal of singletons. Reads were subsequently clustered into operational taxonomic units (OTUs) with a threshold of 0.97 after full-length dereplication. Taxonomic annotation of the OTUs was conducted using a SINTAX algorithm (Edgar 2016) against the ITS reference database UNITE (Nilsson et al. 2019). OTUs annotated to *Zoysia* sp. were removed and only fungal OTUs were retained for data analysis.

The abundance distribution of fungal OTUs in the three root samples was summarised by alpha diversity (within-sample diversity) metrics including Shannon and Simpson indices. Composition of the fungal community in the samples was plotted, based on the relative abundance of OTUs at different taxonomic levels. Dominant fungal OTUs in each sample were identified by analysing the OTUs with relative abundance in the top 20 at the order and genus levels. Sequences of these OTUs were extracted and phylogenetic trees were constructed using the Maximum Likelihood method. The above data analysis and visualisation were performed using the packages vegan v.2.6-4 (Oksanen et al. 2022), phyloseq v.1.42.0 (McMurdie and Holmes 2013) and ggplot2 (Wickham 2011) in R v.4.2.2 software (R Core Team 2019).

Fungal isolation

The remaining root samples of *Z. japonica* were used for fungal isolation. Briefly, surface-disinfected roots were cut into small pieces (5 mm long) and placed on potato dextrose agar (PDA) amended with 50 µg ml⁻¹ of ampicillin and kanamycin. Plates were incubated in darkness at 25 °C for 3–7 days. Fungal colonies developed from plant tissues were sub-cultured on fresh PDA to obtain pure cultures. All the fungal strains were maintained in the Molecular Microbiology Lab., Department of Integrative Food, Bioscience and Biotechnology, Chonnam National University, Gwangju, Republic of Korea.

Fungal DNA extraction and polymerase chain reaction

Fungal genomic DNA was extracted by a modified CTAB method (Cubero et al. 1999) using fresh mycelia grown on PDA. Amplification of gene fragments of ITS, SSU, LSU, *TEF1*, *RPB1*, *RPB2*, *TUB2* and *MCM7* was performed using primer pairs of ITS4/ITS5 (White et al. 1990), NS1/NS4 (White et al. 1990), LR0R/LR5 (Vilgalys and Hester 1990), EF1-983F/EF1-2218R (Rehner and Buckley 2005), *RPB1*-Ac/*RPB1*-Cr (Stiller and Hall 1997; Matheny et al. 2002), *RPB2*-5f/

RPB2-7cR (Liu et al. 1999), T1/Bt2b (Glass and Donaldson 1995; O'Donnell and Cigelnik 1997) and MCM7-709/MCM7-1348 (Schmitt et al. 2009), respectively. The PCR products were purified with ExoSap-IT reagent (GE Healthcare, USA) before paired-end Sanger sequencing by Macrogen Inc. (Seoul, Korea).

Phylogenetic analysis

The resulting sequences of fungal strains in this study were subjected to BLASTn searches in the NCBI database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) with an additional option (sequences from type materials). Related reference sequences of different genera were accessed, based on recent relative publications (Cai et al. 2006; Zhang et al. 2011, 2023; Quaedvlieg et al. 2013; Luo et al. 2016; Thambugala 2017; Liu et al. 2019; Marin-Felix et al. 2020; Andreassen et al. 2021; Crous et al. 2022a; Wong et al. 2022; Tanney et al. 2023). Accession numbers of the reference strains were shown in Suppl. material 1. A newly-developed tool OFPT (One-click Fungal Phylogenetic Tool) by Zeng et al. (2023) was used for phylogenetic analysis in this study. Complete procedures including sequence retrieving, multi-sequence alignment, sequence trimming, sequence concatenating, model testing and construction of Maximum Likelihood (ML) and Bayesian trees were accomplished by this tool. Briefly, nucleotide substitution models of the datasets were tested by ModelFinder (Kalyaanamoorthy et al. 2017). For the ML analysis, 1,000 bootstrap replicates were performed by IQ-TREE (Nguyen et al. 2015) with the best-fit substitution model. For the Bayesian method, Markov Chain Monte Carlo (MCMC) analysis was performed by MrBayes 3.2.7 (Ronquist et al. 2012) with parameters of 50,000,000 generations and a sampling frequency of every 100th generation. The first 25% of the samples were discarded during the calculation of convergence diagnostics. Consensus BI trees with posterior probabilities (PP) were generated after the standard deviation of the runs fell below 0.01. Sequence alignments of phylogenetic analyses were shown in Suppl. material 2.

Morphological characterisation

Colonial characteristics of the fungal strains were recorded after incubation on PDA at 25 °C in darkness for 7 days. To stimulate sporulation, fungal strains were inoculated on malt extract agar (MEA) and oatmeal agar (OA) and incubated at 25 °C. Colonies of strains that had not sporulated after 7 days were scratched and exposed to UV light for 2 h and again incubated at 25 °C for further observation. Conidial morphology was observed under an optical microscope (Olympus, Tokyo, Japan) equipped with a differential interference contrast (DIC) module.

In vitro dual culture assay

Antifungal activity of fungal strains against turf-grass brown patch pathogen *Rhizoctonia solani* AG2-2(IIIB) KACC 40151 was tested by *in vitro* dual culture assay. Mycelia plugs (5 mm diameter) of endophytic fungi and pathogen *R. solani* AG2-2(IIIB) were placed on PDA (3 cm apart). PDA plates inoculated with only pathogen were used as control. The experiment was repeated twice. Mycelial growth of colonies of pathogens with (a) and without (b) endophytic fungi was

measured after incubation at 25 °C for 2 d. Inhibition rate of mycelia growth was calculated as follows: Inhibition rate (%) = $100 - (a/b \times 100)$. Endophytic fungus with the highest inhibition rate was chosen for further investigation of antifungal activity against pathogens *R. cerealis* KACC 40154, *R. solani* AG2-2(IV) KACC 40132, *Clariireedia jacksonii* CMML 20-31, *Pythium ultimum* KACC 40705, *Sclerotinia sclerotiorum* KACC 40457, *Botrytis cinerea* CMML 20-BC04, *Fusarium oxysporum* CMML 21-1 and *Colletotrichum gloeosporioides* KACC 40003.

Crude extraction of fungal metabolites

Mycelia of the selected endophytic fungus grown in potato dextrose broth (PDB) for 2 weeks were collected and used as raw material for metabolite extraction. First, a mycelial sample (2 g) was mixed with 8 ml of different solvents (methanol, ethyl acetate, hexane, acetone and butanol) and incubated in a shaker at room temperature for 24 h. Crude extracts were then obtained after centrifugation. Subsequently, antifungal activity against *R. solani* AG2-2(IIIB) was tested on each crude extract using the paper disc method. Briefly, agar plugs (5 mm) of *R. solani* AG2-2(IIIB) were inoculated on the centre of PDA plates, sterile paper discs were then loaded with 20 µl of the crude extracts, air-dried thoroughly and placed on the surface of pathogen-inoculated PDA plates. Mycelial growth inhibition was observed after incubation at 25 °C for 2 d. The experiment was performed three times.

Mycelial viability

To test the viability of pathogen *R. solani* AG2-2(IIIB) with or without crude extracts of the selected endophytic fungus, mycelia were picked, placed on glass slides and stained with neutral red (0.1 µg ml⁻¹, DaeJung, Siheung, Korea) or Evans blue (0.5 µg ml⁻¹, Alfa Aesar, Haverhill, USA). After incubation for 5 min at room temperature, the mycelia were washed three times with sterile distilled water and examined under the microscope.

Pot assay

In planta antifungal activity against *R. solani* AG2-2(IIIB) was tested using butanol extract of the selected endophytic strain on creeping bentgrass. Mycelia of *R. solani* AG2-2(IIIB) grown in PDB for 3 d were collected and pulverised for inoculation. Approximately 2-week-old creeping bentgrass grown in pots was used for pathogen treatments. Butanol extract (5 ml) of the fungal strain was evaporated and re-dissolved in the same volume of distilled water, then sprayed on the pathogen treated pots. Distilled water was treated as a negative control and the same volume of the fungicide azoxystrobin (20 µg ml⁻¹) was treated as a positive control. Three pots were used for each treatment. All the pots were placed in a greenhouse at 25 °C with a light period of 16 h per day until disease was noted.

Statistical analysis

Mycelial growth of pathogens in dual culture assays was measured to calculate mycelial growth inhibition rates. Data were used for multiple comparison by the least significant difference (LSD) test ($P \leq 0.05$) in R software (R Core Team 2019).

Results

ITS amplicon analysis

A total of 317 fungal OTUs were obtained from *Z. japonica* root samples, based on amplicon sequencing of the ITS2 region. Sample size-based rarefaction curves show a saturated trend (Fig. 2a), indicating that the community diversity was adequately captured in the three samples. Alpha diversity measurements were similar amongst the samples, ranging from 2.16–2.61 and 0.76–0.88 for Shannon and Simpson indices, respectively (Fig. 2b, c).

Fungal OTUs from root samples of *Z. japonica* were classified into seven phyla, including *Ascomycota*, *Basidiomycota*, *Blastocladiomycota*, *Chytridiomycota*, *Glomeromycota*, *Monoblepharomycota* and *Rozellomycota*, with *Ascomycota* being the most abundant (94.15% on average) phylum (Fig. 3a). A total of 40 orders were found with different relative abundance, including *Agaricales*, *Annulatascales*, *Archaeosporales*, *Atractiellales*, *Atractosporales*, *Auriculariales*, *Blastocladales*, Branch06 (unclassified order in *Sordariomycetes*), *Cantharellales*, *Capnodiales*, *Chaetothyriales*, *Chytridiales*, *Cladochytriales*, *Cystobasidiales*, *Entrophosporales*, *Eurotiales*, *Exobasidiales*, *Glomerales*, *Glomerallales*, *Gyalectales*, *Helotiales*, *Hypocreales*, *Lecanorales*, *Magnaporthales*, *Malasseziales*, *Marthamycetales*, *Microascales*, *Monoblepharidales*, *Orbiliiales*, *Paraglomerales*,

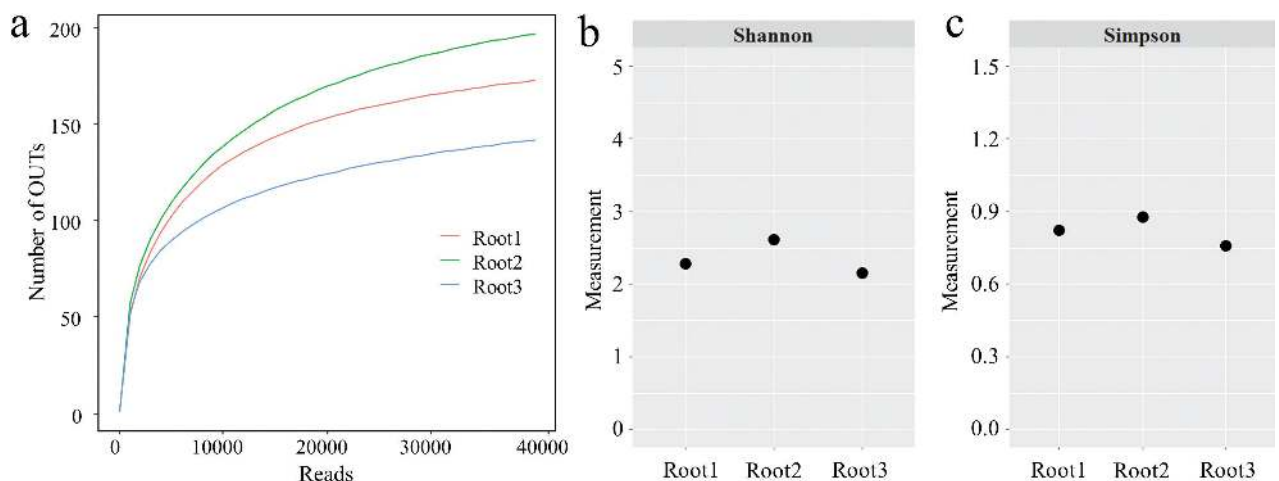


Figure 2. Alpha diversity of fungal communities in *Zoysia japonica* root samples **a** rarefaction curves for OTUs at different sequence reads **b** measurements of Shannon indices **c** measurements of Simpson indices.

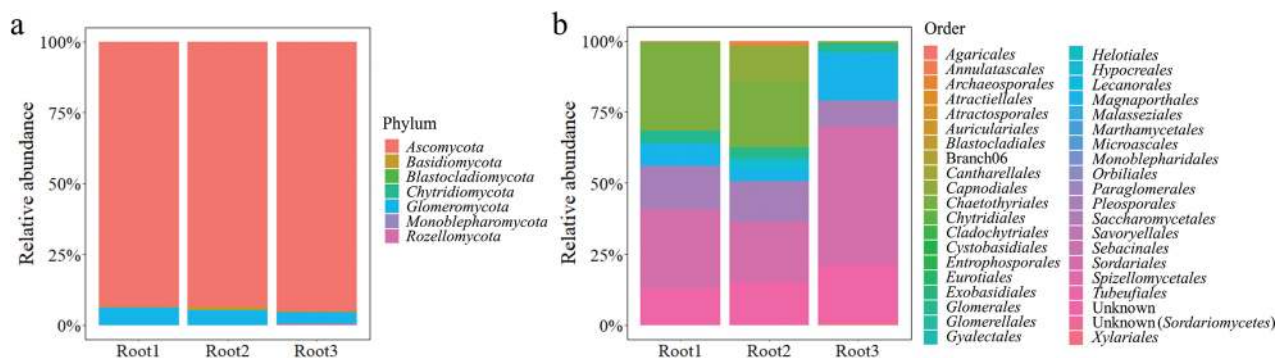


Figure 3. Fungal communities in *Zoysia japonica* root samples **a** relative abundance at phylum level **b** relative abundance at order level.

Pleosporales, *Saccharomycetales*, *Savoryellales*, *Sebacinales*, *Sordariales*, *Spizeliomycetales*, *Tubeufiales*, *Xylariales* and two unknown orders (Fig. 3b).

The composition of OTUs were similar in the three samples at the family and genus levels. A total of 49 families and 64 genera were found in the OTUs and unknown taxa in *Sordariales* dominated the fungal community at both the family and genus levels (Fig. 4). Other classified genera of the OTUs were *Ambispora*, *Atractiella*, *Biatora*, *Budhanggurabania*, *Cladophialophora*, *Colletotrichum*, *Cortinarius*, *Cucurbitinus*, *Curvularia*, *Dictyosporella*, *Dominikia*, *Entrophospora*, *Exophiala*, *Funneliformis*, *Fusarium*, *Fusidium*, *Glomus*, *Helicoma*, *Kamienskia*, *Lasiosphaeria*, *Lophiostoma*, *Macgarvieomyces*, *Magnaporthe*, *Magnaporthiopsis*, *Marthamyces*, *Melomastia*, *Microdominikia*, *Myrothecium*, *Naemacyclus*, *Paraglomus*, *Oliveonia*, *Phlyctis*, *Poaceascoma*, *Podospora*, *Pseudophialophora*, *Rhizophagus*, *Septoglomus*, *Serendipita*, *Slopeiomyces*, *Stagonospora*, *Tetraploa*, *Tirisporea* and *Wettsteinina*.

In the fungal OTUs, the top 20 relative abundance orders were *Sordariales* (32.45%), *Chaetothyriales* (18.16%), unknown order in *Sordariomycetes* (14.63%), *Pleosporales* (12.48%), *Magnaporthales* (9.34%), *Capnodiales* (4.14%), *Glomerales* (3.87%), *Hypocreales* (1.17%), unknown order in *Glomeromycota* (1.05%), *Annulatascales* (0.39%), *Tubeufiales* (0.32%), *Lecanorales* (0.26%), *Microascales* (0.25%), *Paraglomerales* (0.24%), *Xylariales* (0.20%), unknown order in *Sordariomycetes* (0.16%), *Auriculariales* (0.14%), *Atractiellales* (0.14%), unknown order in *Sebacinales* (0.13%) and unknown order in *Rozellomycota* (0.13%, Fig. 5).

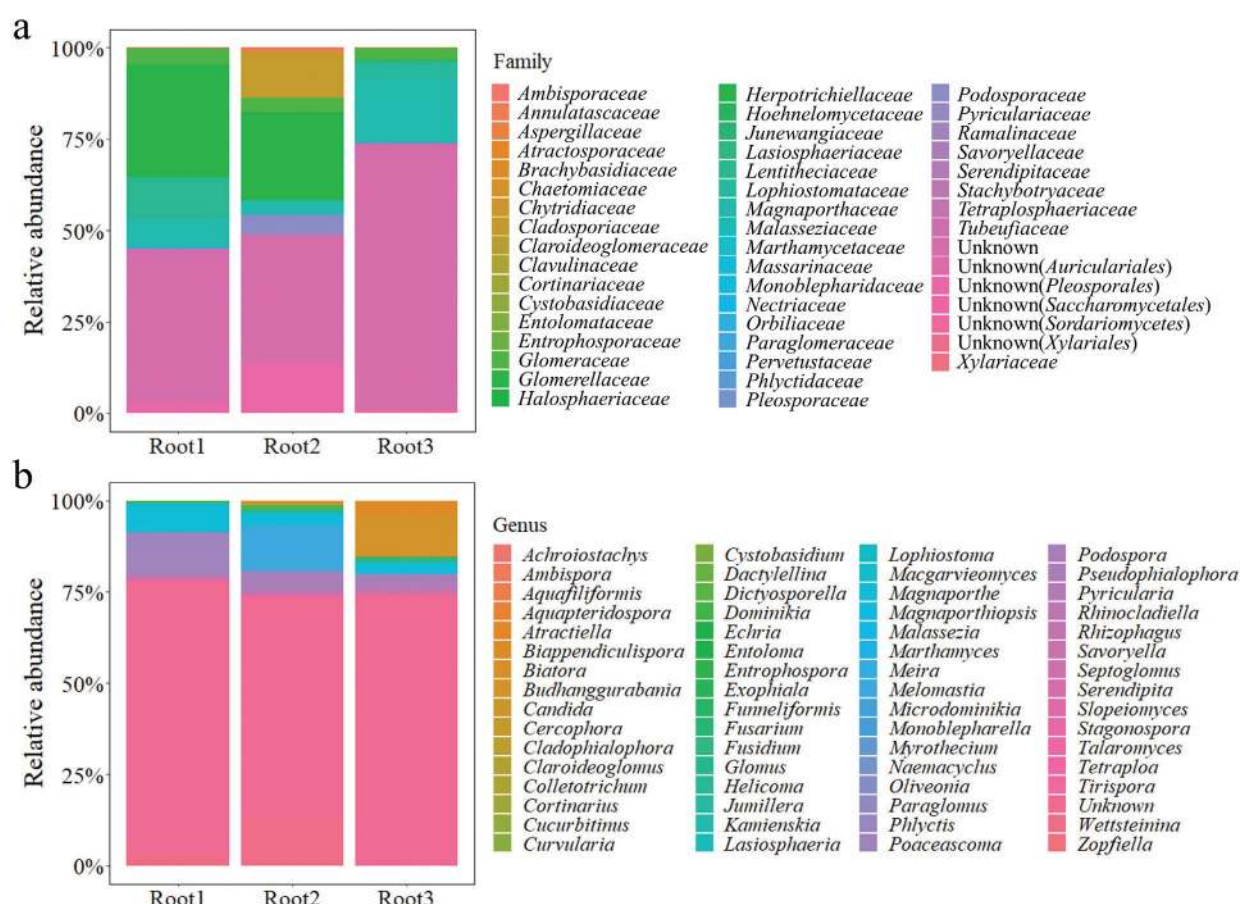


Figure 4. Fungal communities in *Zoysia japonica* root samples **a** relative abundance at family level **b** relative abundance at genus level.

Highest relative abundance was found in unknown genera in *Sordariales* (30.84%), followed by unknown genera in *Herpotrichiellaceae* (18.08%) and *Sordariomycetes* (14.63%). The remaining genera in the top 20 in relative abundance were *Wettsteinina* (5.39%), *Magnaporthe* (4.50%), *Melomastia* (4.14%), *Poaceasca* (4.06%), *Budhanggurabania* (3.76%), unknown genus in *Glomeraceae* (1.94%), *Podospira* (1.59%), *Rhizophagus* (1.42%), *Lophiostoma* (1.40%), unknown genus in *Pleosporales* (1.37%), unknown genus in *Hypocreales* (1.09%), unknown genus in *Glomeromycota* (1.05%), *Pseudophialophora* (0.98%), *Glomus* (0.4%), *Dictyosporella* (0.39%), *Helicoma* (0.32) and *Biatra* (0.26%, Fig. 6).

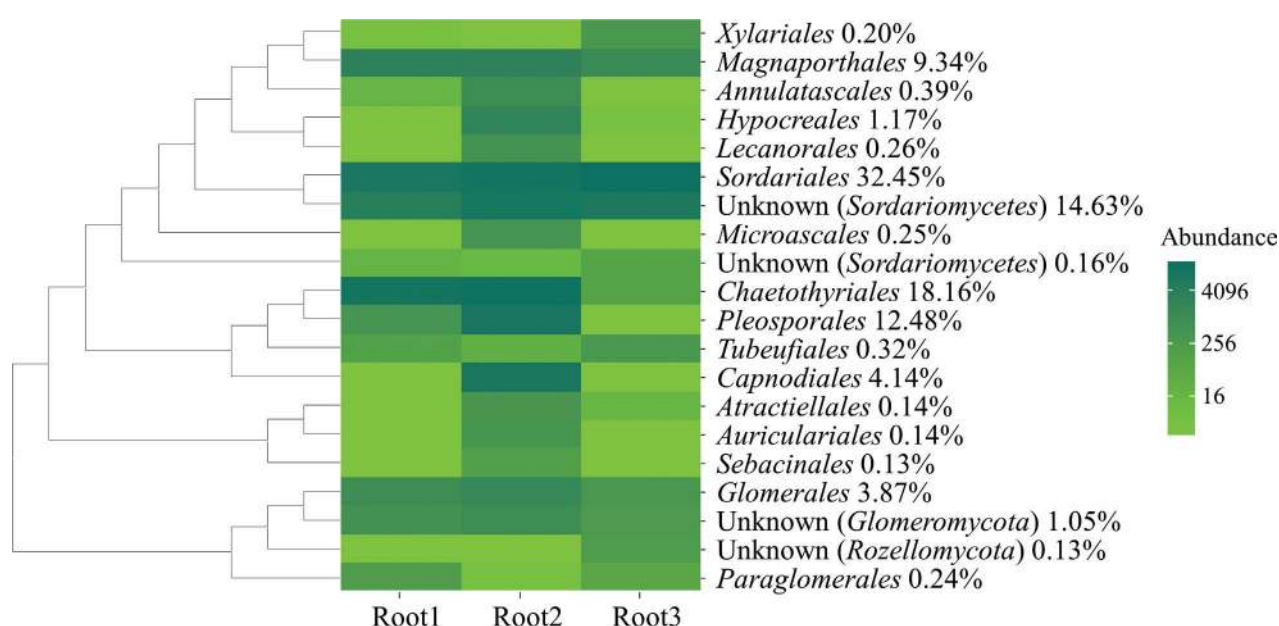


Figure 5. Top 20 abundance orders of fungal communities in *Zoysia japonica* root samples with their phylogenetic relationships.

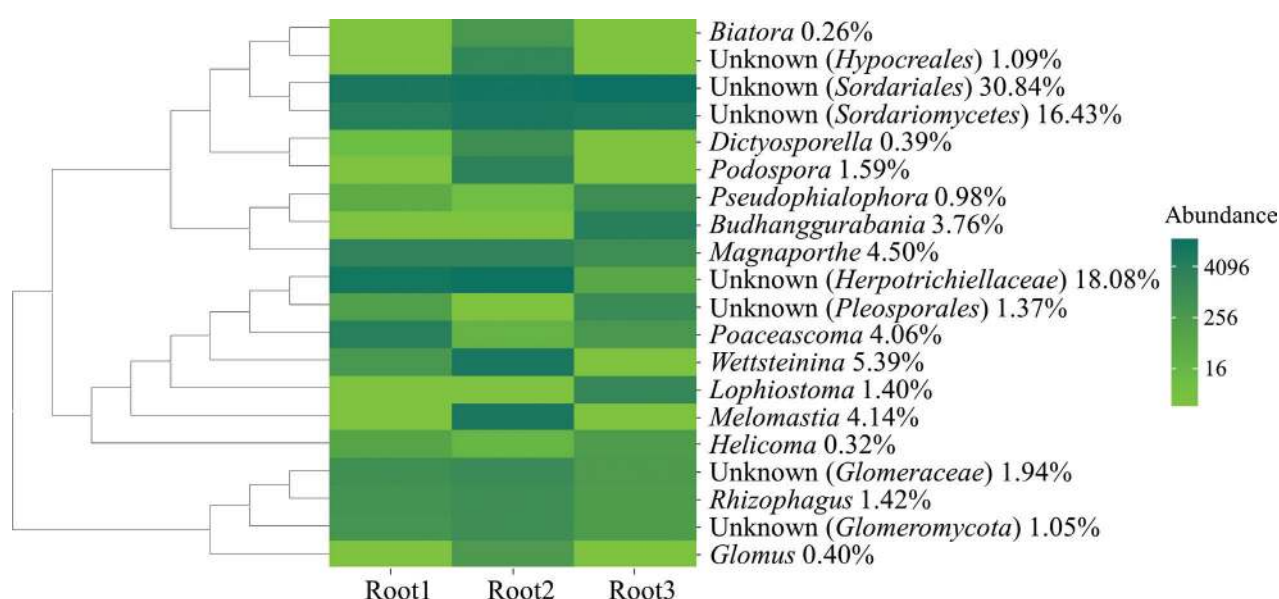


Figure 6. Top 20 abundance genera of fungal communities in *Zoysia japonica* root samples with their phylogenetic relationships.

Raw reads of ITS amplicon sequencing data in this study were deposited into the sequence read archive (SRA) in NCBI with BioProject number PRJ-NA1165193.

Diversity of culturable endophytic fungi

A total of 151 fungal strains were isolated from roots of *Z. japonica*. Based on colony morphology on PDA, 54 strains with different colony morphology (shape, colour, texture etc.) were preliminarily selected for ITS amplification and sequencing. Resulting sequences of these strains were then used for BLASTn search against NCBI database to obtain reference sequences with high similarities. Phylogenetic analysis revealed that these strains belonged to genera *Curvularia*, *Setophoma*, *Poaceascoma*, *Preussia*, *Lophiostoma*, *Stagonospora*, *Niesslia*, *Purpureocillium*, *Fusarium*, *Collectotrichum*, *Pseudorhizophila*, *Magnaporthiopsis*, *Nemania*, *Xylaria*, *Cladosporium*, *Cutaneotrichosporon*, *Irpex*, genera in *Tubeufiaceae*, *Magnaporthales* and two unknown taxa. (Fig. 7). Amongst these strains, 17 strains (31.48%) were detected as *Curvularia* spp. and 10 strains (18.51%) were identified as *Poaceascoma* spp. Strains with relative low similarities against GenBank were used for separate multi-loci phylogenetic analyses.

Taxonomy

***Niesslia dimorphospora* (W. Gams) W. Gams & Stielow (2019).**

Fig. 8

Culture characteristics. Colony reaching 53.82 mm diam. after 7 days in darkness at 25 °C on PDA, surface initially floccose, later slimy, white on front and reverse sides (Fig. 8a).

Description from living culture CMML 20-40. Sexual morph: undetermined. Asexual morph: Sporulation abundant on MEA. **Phialides** 40–75 µm long, 1.5–2.3 µm wide, thick-walled. **Conidia** smooth-walled, globose, 4.5–6.5 µm diam., or ellipsoidal, slightly curved, 6.5–10.5 × 2.2–3.8 µm (Fig. 8b–k).

Type. Korea • South Jeolla Province, Hwasun, isolated from roots of *Zoysia japonica*, October 2020, H. Liu and H. Sang, living cultures CMML 20-40, CMML 20-41 and CMML 20-42.

Notes. *Niesslia dimorphospora* typically produce dimorphic conidia (globose and ellipsoidal). In multi-loci phylogenetic analysis using gene sequences of ITS, *TEF1*, *TUB2* and *RPB2*, three strains (CMML 20-40, CMML 20-41 and CMML 20-42) were clustered into a clade containing ex-type strain of *N. dimorphospora* (CBS 785.69) and representative strain CBS 361.76 with high statistical support (100%/1.00) (Fig. 9). This is the first record of *N. dimorphospora* associated with *Z. japonica* in Korea.

***Dactylaria hwasunensis* H. Liu & H. Sang, sp. nov.**

Mycobank No: MB857258

Fig. 10

Etymology. Name refers to Hwasun County in Korea, where it was isolated.

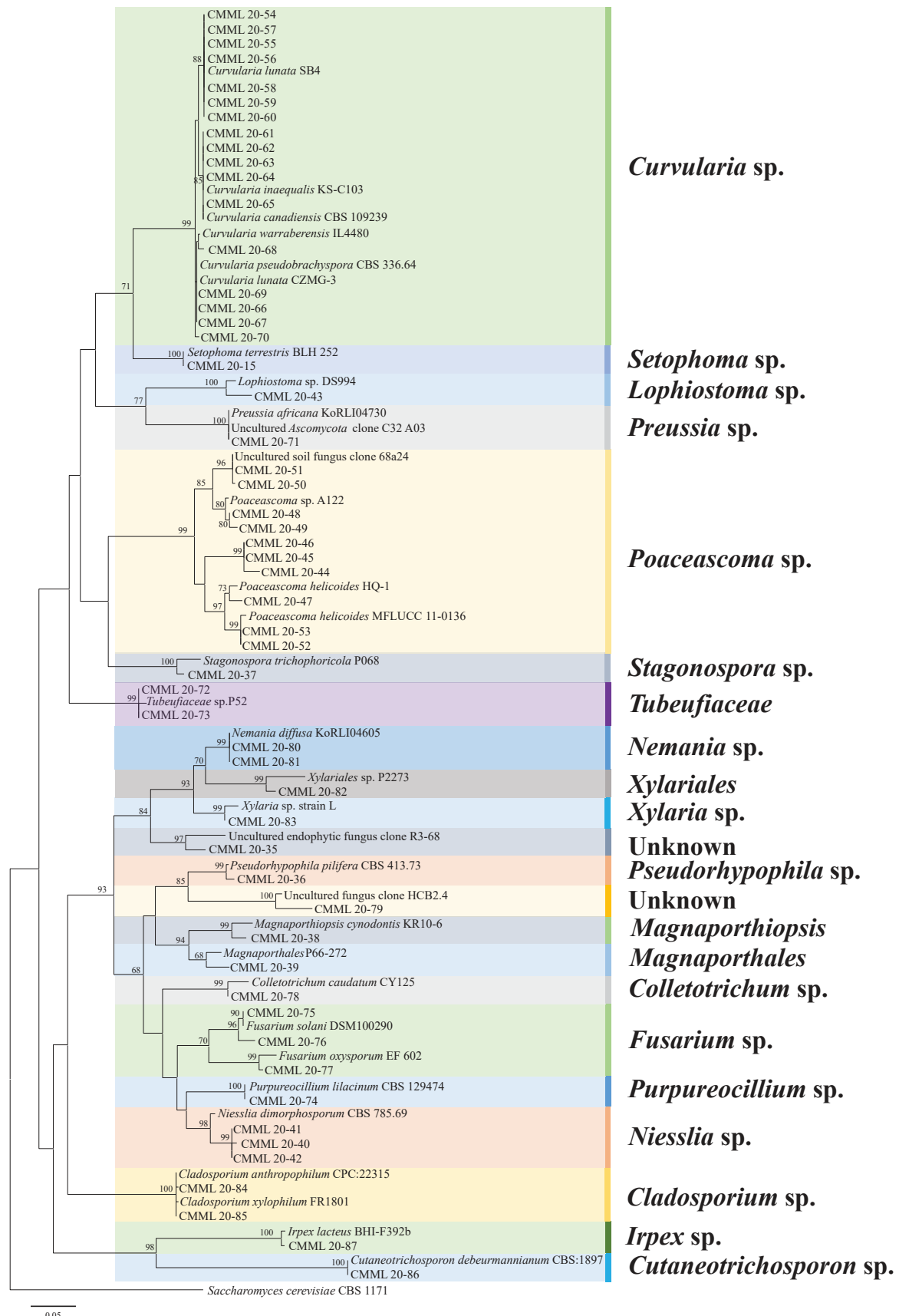


Figure 7. Phylogenetic analysis of fungal strains isolated from *Zoysia japonica* root samples, based on ITS sequences using Maximum Likelihood method. Bootstrap values (BS) are given at the nodes. *Saccharomyces cerevisiae* (CBS 1171) was used as the outgroup taxon.



Figure 8. *Niesslia dimorphospora* (CMML 20-40) **a** front and reverse sides of colony on PDA **b–d** globose conidia on a conidiophore **e–i** ellipsoidal conidia on a conidiophore **j** globose conidia **k** ellipsoidal conidia. Scale bars: 10 µm.

Description from living culture CMML 20-35. Sexual morph: undetermined. Asexual morph: Sporulation abundant on MEA. **Conidiophore** erect, mironematous to macronematous, aseptate or septate, hyaline, 6–35 µm in length, 2.2–2.8 µm in width. **Conidiogenous cells** terminal, integrated, hyaline 2–2.8 µm wide. **Conidia** clavate, hyaline, blunt end, 1–5 septate, 10–60 × 2.2–2.8 µm (Fig. 10b–h).

Type. Korea • South Jeolla Province, Hwasun, isolated from roots of *Zoysia japonica*, October 2020, H. Liu and H. Sang, holotype CMML 20-35H (permanently preserved in a metabolically inactive state), ex-holotype CMML 20-35, ex-isotype CMML 20-88.

Culture characteristics. Colony reaching 31.81 mm diam. after 7 days in darkness at 25 °C on PDA, white to yellowish, surface smooth, cracked (Fig. 10a).

Notes. In phylogenetic analysis of genus *Dactylaria* using sequence data of LSU, the strains used in the present study CMML 20-35 and CMML 20-88 formed a distinct clade sister to clade containing representative strain of *D. fragilis* (P057) and ex-type strain of *D. acaciae* (CPC 29771) with a high statistical support (84%/0.95) (Fig. 11). Based on nucleotide sequences, ex-holotype strain of *D. hwasunensis* (CMML 20-35) differed from ex-type strain of *D. acaciae* (CPC 29771): LSU sequence identities = 806/818 (98.53%). *D. hwasunensis* (CMML 20-35) also differed from representative strain of *D. fragilis* (P057): LSU sequence identities = 844/857 (98.48%). Morphologically, conidial dimensions

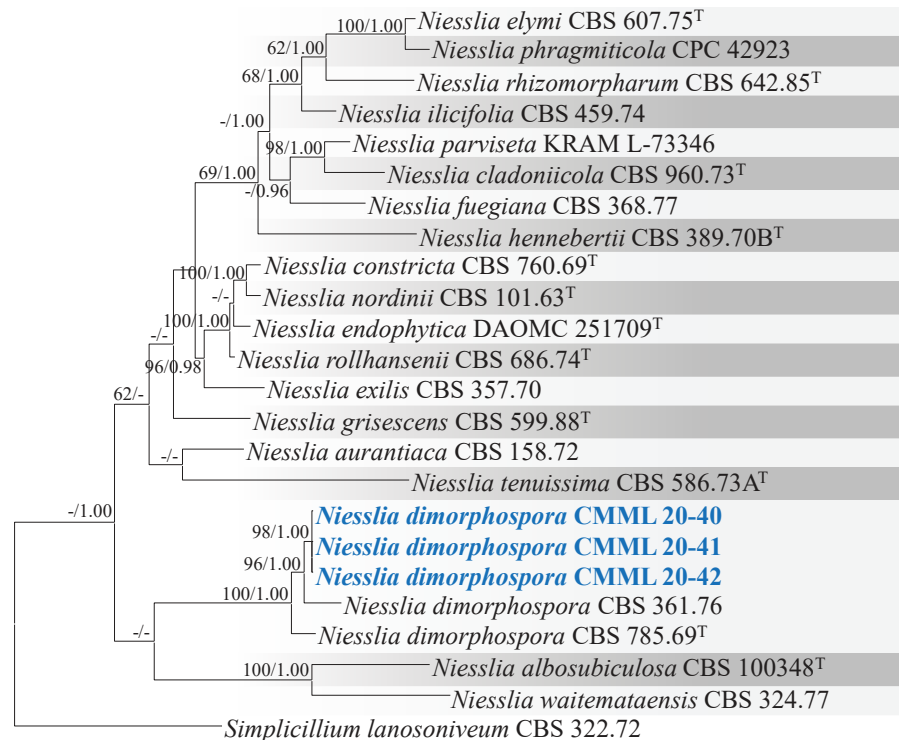


Figure 9. Maximum Likelihood phylogenetic tree, based on combined sequences of ITS, LSU, *RPB2* and *TUB2* from *Niesslia* species. Bootstrap values (BS) and Bayesian posterior probabilities (PP) are given at the nodes (BS/PP). Strains obtained from this study are in bold blue. Ex-type isolates are marked with ^T. *Simplicillium lanosoniveum* (CBS 322.72) was used as the outgroup taxon.

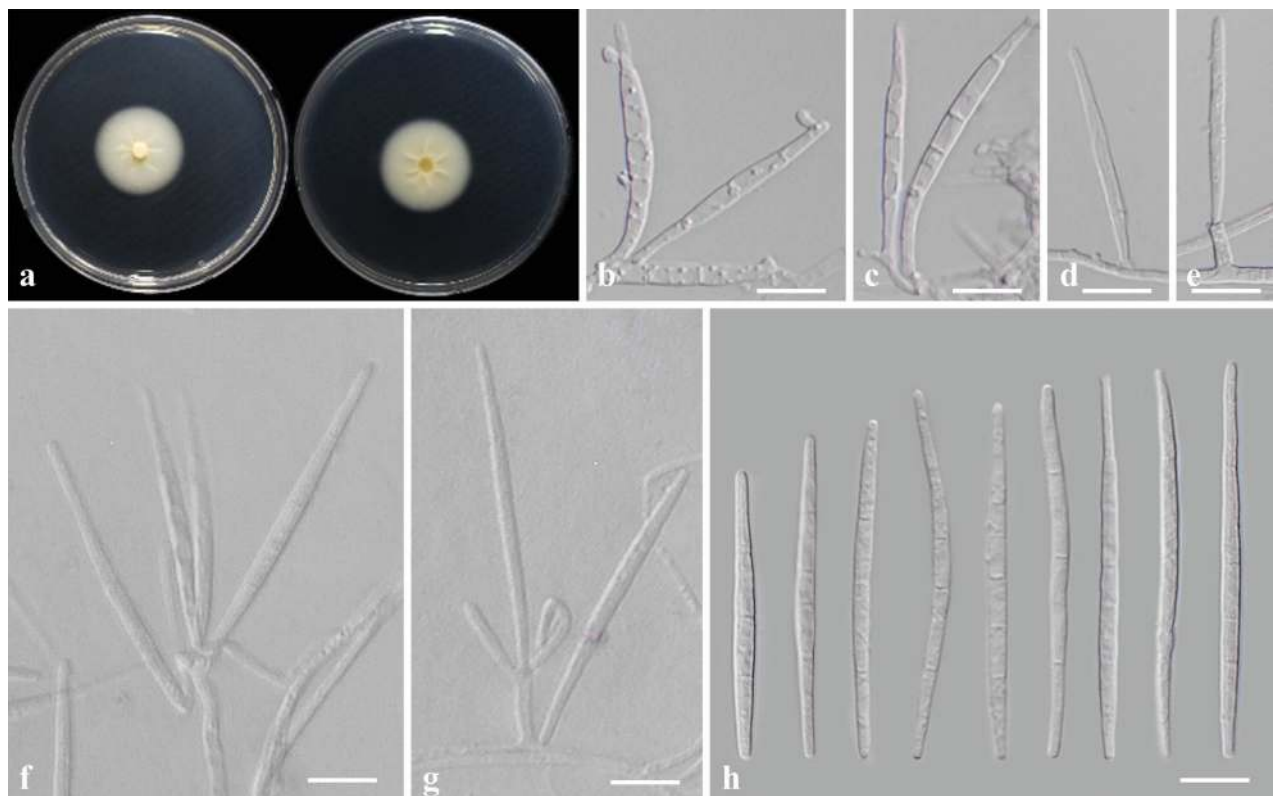


Figure 10. *Dactylaria hwasunensis* sp. nov. (CMML 20-35) **a** front and reverse sides of colony on PDA **b–g** conidiophores and conidia **h** conidia. Scale bars: 10 μm.

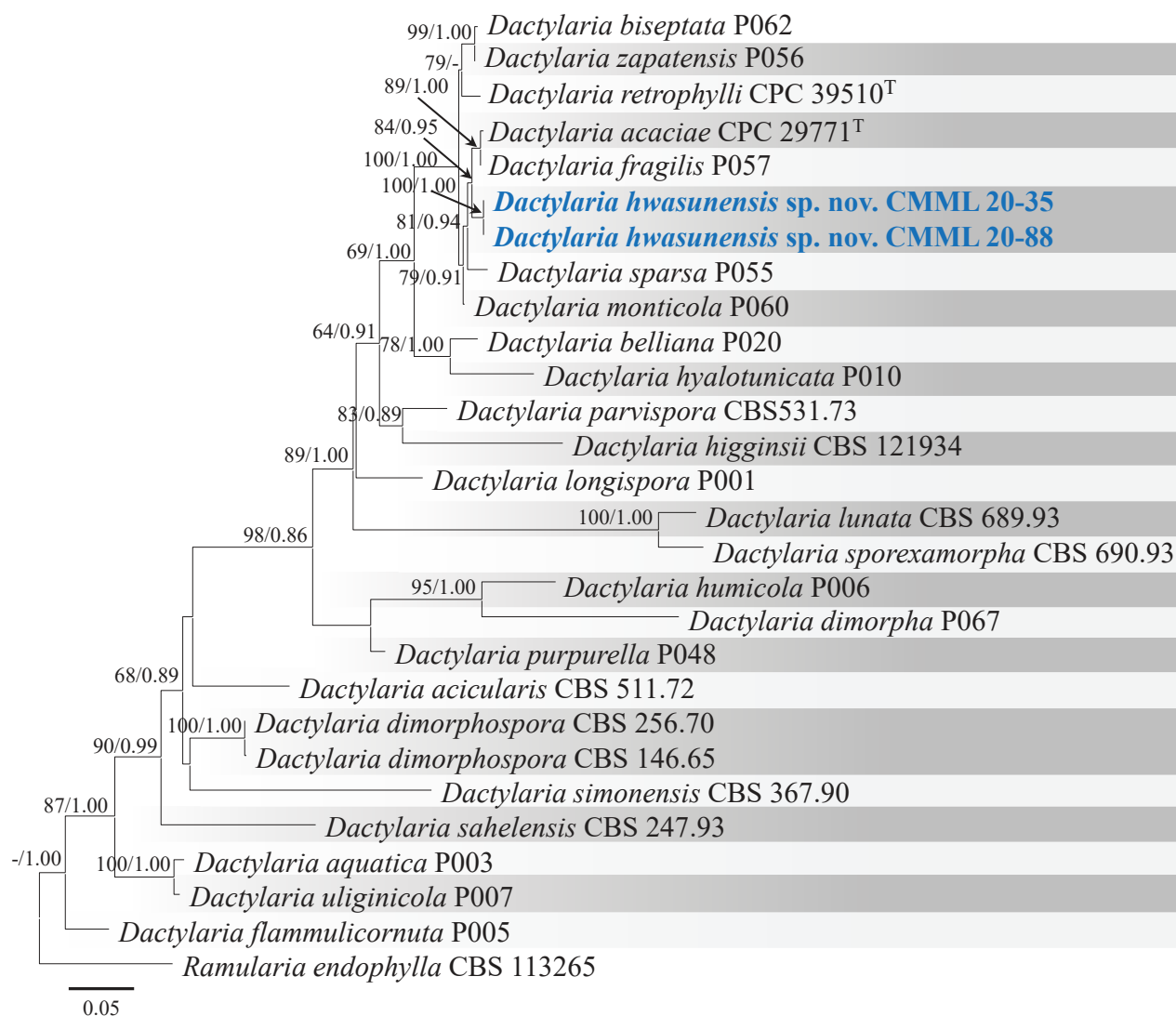


Figure 11. Maximum Likelihood phylogenetic tree, based on LSU sequences from *Dactylaria* species. Bootstrap values (BS) and Bayesian posterior probabilities (PP) are given at the nodes (BS/PP). Strains obtained from this study are in bold blue. Ex-type isolates are marked with ^T. *Ramularia endophylla* (CBS 113265) was used as the outgroup taxon.

of *D. hwasunensis* are larger than *D. acaciae* (16–37 × 2–2.5 µm; Crous et al. (2016)) and *D. fragilis* (18–26 × 1.5 µm; De Hoog (1985)). Therefore, *Dactylaria hwasunensis* sp. nov. was introduced in this study to accommodate CMML 20-35 and CMML 20-88 in the genus *Dactylaria*.

***Magnaporthiopsis zoysiae* H. Liu & H. Sang, sp. nov.**

Mycobank: MB857259

Fig. 12

Etymology. Name refers to its host *Zoysia japonica*.

Description from living culture CMML 20-39. Sexual morph: undetermined. Asexual morph: Sporulation observed on OA media. **Conidiophores** hyaline, single or sometimes branched, septate. **Conidiogenous cells** erect or curved, hyaline, 2.5–4 µm in width. **Conidia** ovoid or cylindrical, hyaline, slightly curved, 5.5–14.5 × 3.0–5.2 µm (Fig. 12b–h).



Figure 12. *Magnaporthiopsis zoysiae* sp. nov. (CMML 20-39) **a** front and reverse sides of colony on PDA **b–e** sporulation pattern on OA media **f** conidia. Scale bars: 3 µm.

Type. Korea • South Jeolla Province, Hwasun, isolated from roots of *Zoysia japonica*, October 2020, H. Liu and H. Sang, holotype CMML 20-39H (permanently preserved in a metabolically inactive state), ex-holotype CMML 20-39, ex-isotype CMML 20-92.

Culture characteristics. Colony reaching 31.81 mm diam. after 7 days in darkness at 25 °C on PDA, centre dark, margin white, mycelia frizzy (Fig. 12a).

Notes. In phylogenetic analysis of *Magnaporthiopsis*, based on sequences of six genes (ITS, SSU, LSU, *RPB1*, *TEF1* and *MCM7*), the strains used in the present study CMML 20-39 and CMML 20-92 fell into a distinct clade with a high statistical support (100%/1.00) (Fig. 13), sister to clades of species *M. cynodontis*, *M. agrostidis* and *M. meyeri-festucae*, which are all turf-grass-associated species. Morphologically, the conidial size of these two strains is larger than those of *M. agrostidis* (4–6 × 1 µm; Crous et al. (2015)) and *M. meyeri-festucae* (3–5 × 1–2.5 µm; Luo et al. (2017)). Conidia of these two strains is slightly longer than *M. cynodontis* (7–13 × 2–6.5 µm; Vines et al. (2020)). Therefore, based on phylogenetic analysis and morphological characteristics, *Magnaporthiopsis zoysiae* sp. nov. was introduced in this study.

***Setophoma zoysiae* H. Liu & H. Sang, sp. nov.**

Mycobank No: MB857260

Fig. 14

Etymology. Name refers to its host genus *Zoysia*.

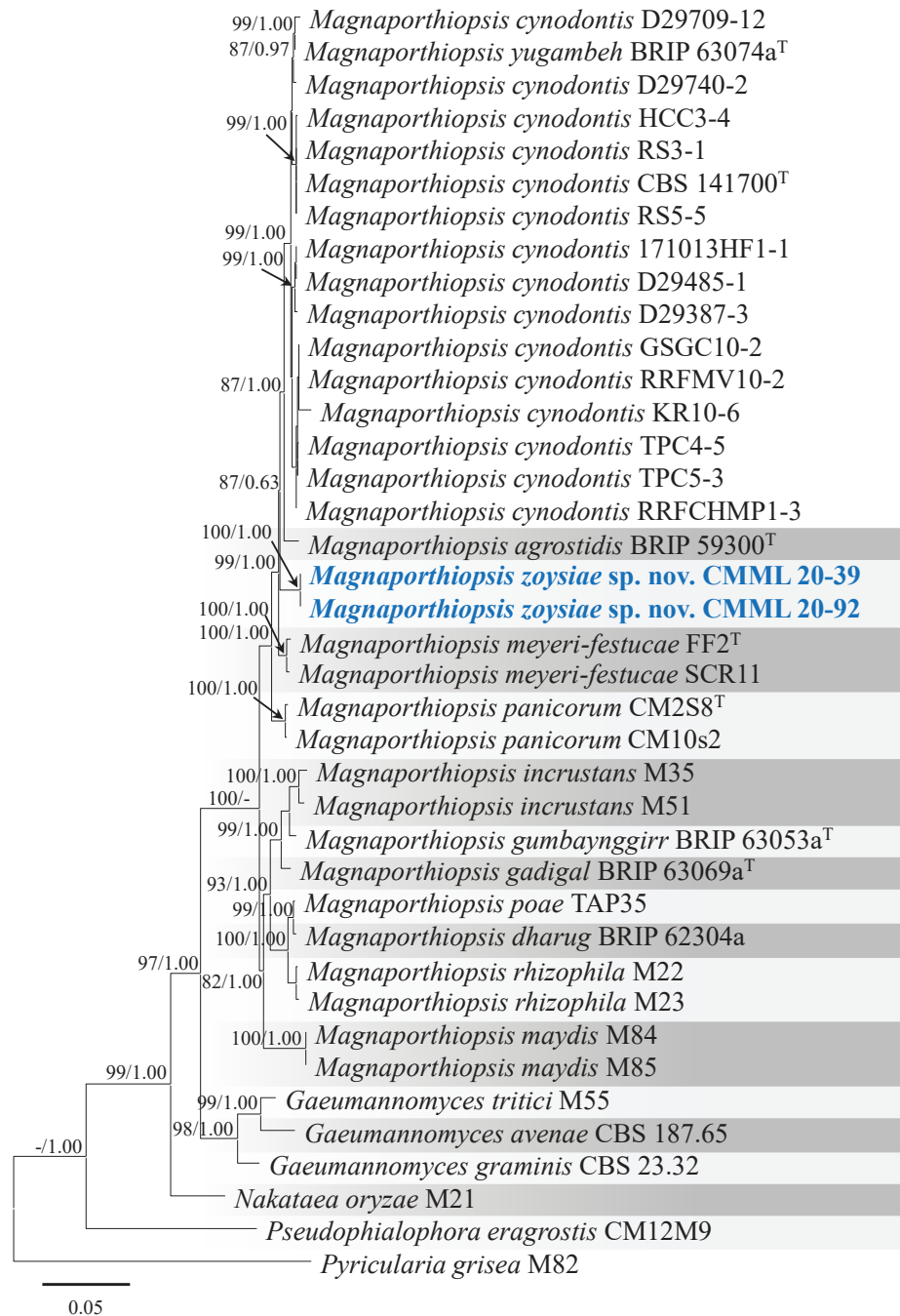


Figure 13. Maximum Likelihood phylogenetic tree, based on combined sequences of ITS, SSU, LSU, *RPB1*, *TEF1* and *MCM7* from *Magnaporthiopsis* species. Bootstrap values (BS) and Bayesian posterior probabilities (PP) are given at the nodes (BS/PP). Strains obtained from this study are in bold blue. Ex-type isolates are marked with †. *Pyricularia grisea* (M82) was used as the outgroup taxon.

Description from living culture CMML 20-14. Sexual morph: undetermined. Asexual morph: Sporulation observed on OA media (Fig. 14b–f). **Conidiomata** produced on surface of colonies (Fig. 14b, c). **Conidia** ellipsoidal to subcylindrical, aseptate, $3.0\text{--}4.6 \times 1.8\text{--}2.5 \mu\text{m}$ (Fig. 14d–f).

Type. Korea • South Jeolla Province, Hwasun, isolated from roots of *Zoysia japonica*, October 2020, H. Liu and H. Sang, holotype CMML 20-14H (permanently preserved in a metabolically inactive state), ex-holotype CMML 20-14, ex-isotype CMML 20-15.

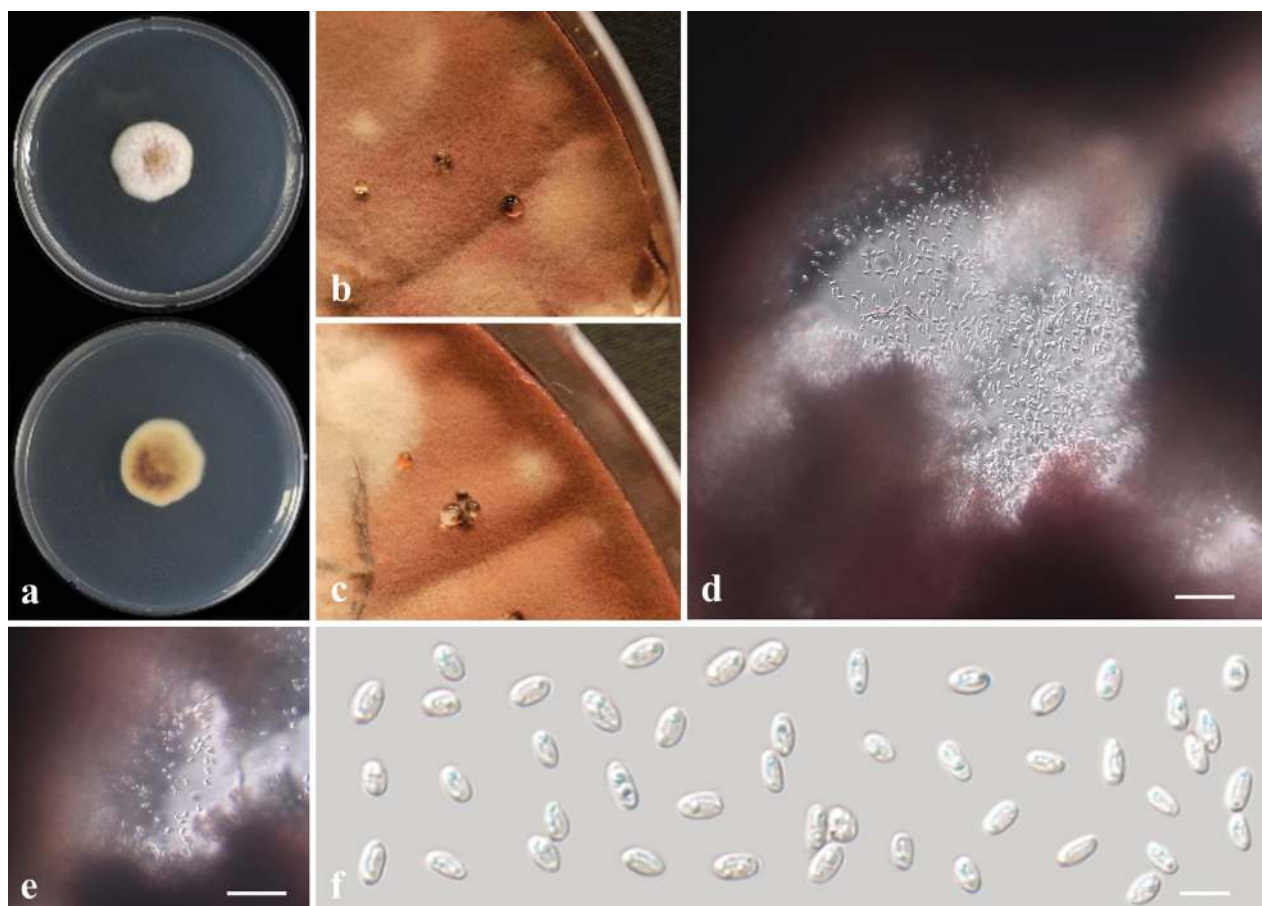


Figure 14. *Setophoma zoysiae* sp. nov. (CMML 20-14) **a** front and reverse sides of colony on PDA **b, c** conidiomata on OA media **d–f** conidia. Scale bars: 30 μ m (**d, e**); 4.5 μ m (**f**).

Culture characteristics. Colony reaching 28.12 mm diam. in darkness after 7 days at 25 °C on PDA, front side white to light pink, reverse side yellow to sandy brown, mycelia dense (Fig. 14a).

Notes. Phylogenetic analysis was conducted using dataset from combined sequences of ITS, LSU, *TEF1*, *RPB2* and *TUB2*. The strains CMML 20-14 and CMML 20-15 formed a distinct single branch in the genus *Setophoma*, supported with a high statistical support (100%/1.00) (Fig. 15), sister to clade containing ex-type strain (CBS 335.29) and representative strains (CBS 335.87, CBS 377.52 and CPC 18417) of *S. terrestris*. However, conidia of these two strains are smaller than those of *S. terrestris* (previously *Phoma terrestris*, 4.5–5.5 $\times$ 1.8–2.3 μ m; Hassen (1929)). Hence, *Setophoma zoysiae* sp. nov. was introduced in this study to accommodate CMML 20-14 and CMML 20-15 in the genus *Setophoma*.

***Stagonospora endophytica* H. Liu & H. Sang, sp. nov.**

Mycobank No: MB857261

Fig. 16

Etymology. Name refers to endophyte.

Description from living culture CMML 20-37. Sexual morph: undetermined. Asexual morph: Sporulation observed on MEA. **Conidiomata** globose, dark brown, 73–105 μ m diam. (Fig. 16b–f). **Conidiophores** reduced to conidiogenous

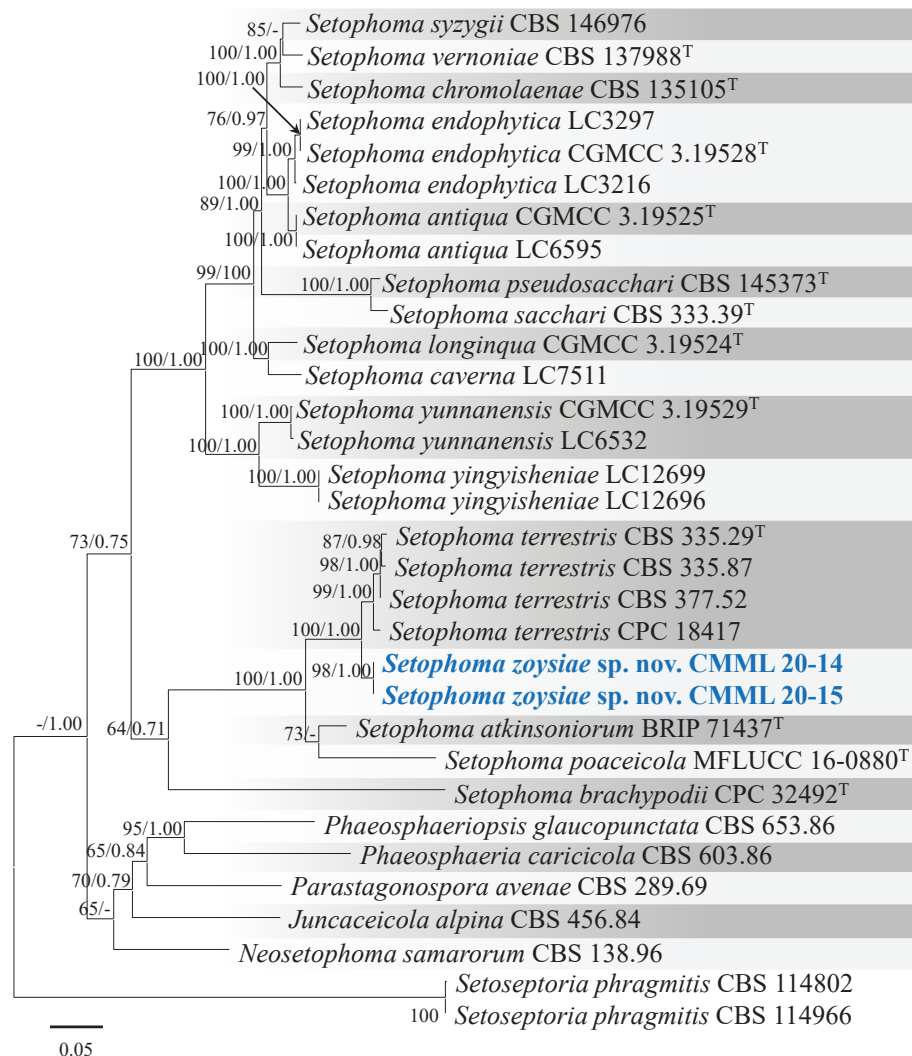


Figure 15. Maximum Likelihood phylogenetic tree based on combined sequences of ITS, LSU, *TEF1*, *RPB2* and *TUB2* from *Setophoma* species. Bootstrap values (BS) and Bayesian posterior probabilities (PP) are given at the nodes (BS/PP). Strains obtained from this study are in bold blue. Ex-type isolates are marked with ^T. *Setoseptoria phragmitis* (CBS 114802 and CBS 114966) was used as the outgroup taxon.

cells. **Conidiogenous cells** 5–8.5 × 4–7.5 µm, hyaline, smooth, ampulliform, produced from the inner wall of conidiomata (Fig. 16g). **Conidia** smooth, 1–3 septate, globose or ellipsoidal with obtuse ends, constricted at septa, 15–22 × 7–9 µm (Fig. 16h–l).

Type. Korea • South Jeolla Province, Hwasun, isolated from roots of *Zoysia japonica*, October 2020, H. Liu and H. Sang, holotype CMML 20-37H (permanently preserved in a metabolically inactive state), ex-holotype CMML 20-37, ex-isotype CMML 20-93.

Culture characteristics. Colony reaching the edge of the PDA plates (90 mm) after 7 days in darkness at 25 °C, front side white to yellowish, centre brown, reverse side faint yellow (Fig. 16a).

Notes. Phylogenetic analysis of *Stagonospora* was performed using sequences of ITS, SSU, LSU, *RPB2* and *TUB2*. Strains in the present study CMML 20-37 and CMML 20-93 fell into a distinct single clade, supported by a high statistical support (100%/1.00) (Fig. 17), sister to clade comprising ex-type strain of *S. tauntonensis* (BRIP 70573) and representative strains of *S. tauntonensis*

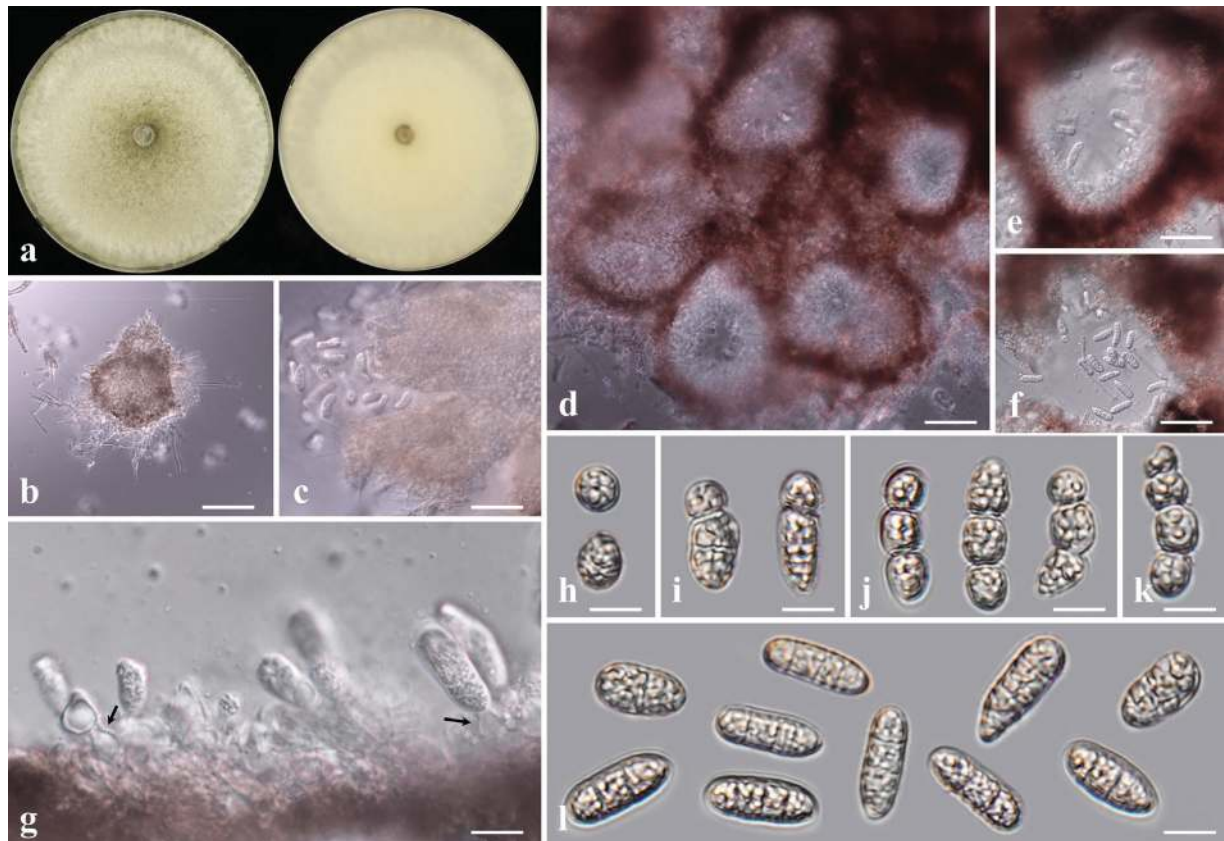


Figure 16. *Stagonospora endophytica* sp. nov. (CMML 20-37) **a** front and reverse sides of colony on PDA **b, c** conidiomata produced on MEA **d–f** section of conidiomata **g** conidiogenous cell (indicated by arrows) with developing conidia **h–l** conidia. Scale bars: 40 μ m (**b–d**); 20 μ m (**e**); 50 μ m (**f**); 12 μ m (**g**); 10 μ m (**h–l**).

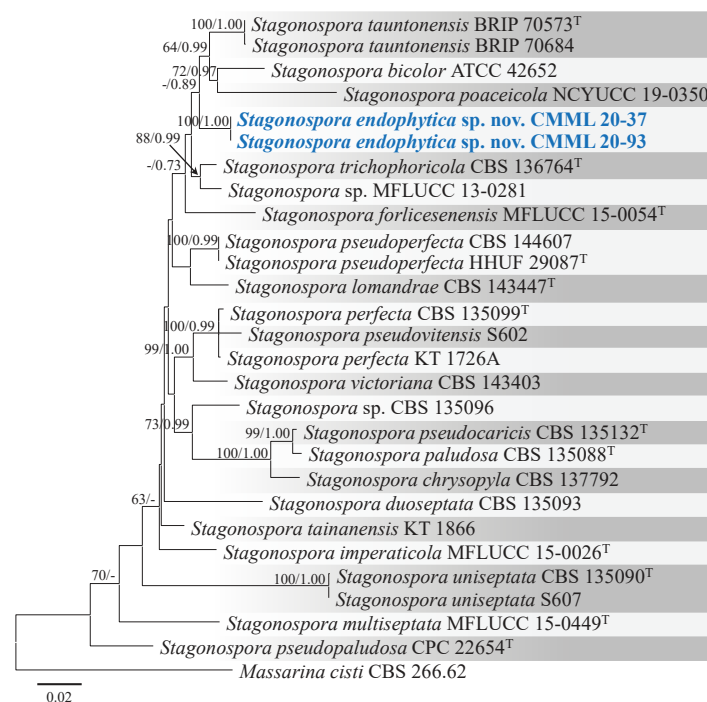


Figure 17. Maximum Likelihood phylogenetic tree, based on combined sequences of ITS, SSU, LSU, *RPB2* and *TUB2* from *Stagonospora* species. Bootstrap values (BS) and Bayesian posterior probabilities (PP) are given at the nodes (BS/PP). Strains obtained from this study are in bold blue. Ex-type isolates are marked with ^T. *Massarina cisti* (CBS 266.62) was used as the outgroup taxon.

(BRIP 70684), *S. bicolor* (ATCC 42652) and *S. poaceicola* (NCYUCC 19-0350). In morphology, these two strains differ from *S. tauntonensis* (Crous et al. 2022b) and *S. bicolor* (previously *Leptosphaeria bicolor*; Kaiser et al. (1979)) in having globose conidia and visible contraction at septa of conidia. For *S. poaceicola*, sexual morph was described and asexual morph of this species remains undetermined. Thus, based on phylogenetic analysis and morphological characteristics, *Stagonospora endophytica* sp. nov. was introduced in this study.

***Pseudorhizophila poae* H. Liu & H. Sang, sp. nov.**

Mycobank No: MB857267

Fig. 18

Etymology. Name refers to its host family *Poaceae*.

Description from living culture CMML 20-36: Sexual morph: undetermined. Asexual morph: Sporulation abundant on MEA. **Conidiophore** erect, 1.5–2.5 μm in width, **Conidia** solitary or in clusters, pyriform, obovoid or triangular, 4.2–5.6 $\times$ 2.5–4.5 μm (Fig. 18b–e).

Type. Korea • South Jeolla Province, Hwasun, isolated from roots of *Zoysia japonica*, October 2020, H. Liu and H. Sang, holotype CMML 20-36H (permanently preserved in a metabolically inactive state), ex-holotype CMML 20-36, ex-isotype CMML 20-89.

Culture characteristics. Colony reaching 82.88 mm diam. on PDA after 7 days in darkness at 25 °C, white to buff in both front and reverse sides (Fig. 18a).

Notes. The genus *Pseudorhizophila* was recently introduced by accommodating four species including *Triangularia mangelotii*, *Zopfiella marina*, *Z. pilifera* and *Z. submersa* (Harms et al. 2021). In phylogenetic analysis using

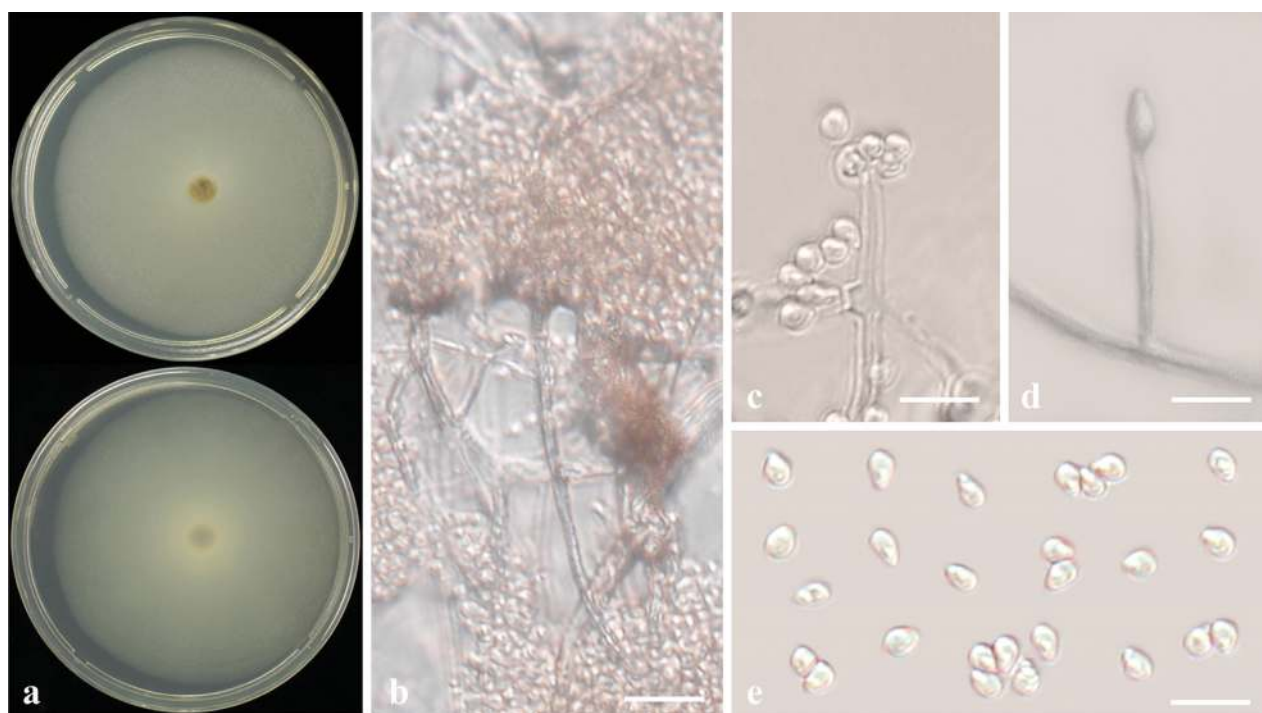


Figure 18. *Pseudorhizophila poae* sp. nov. (CMML 20-36) **a** front and reverse sides of colony on PDA **b–d** conidiophores and conidia **e** conidia. Scale bars: 10 μm (**b–d**); 8 μm (**e**).

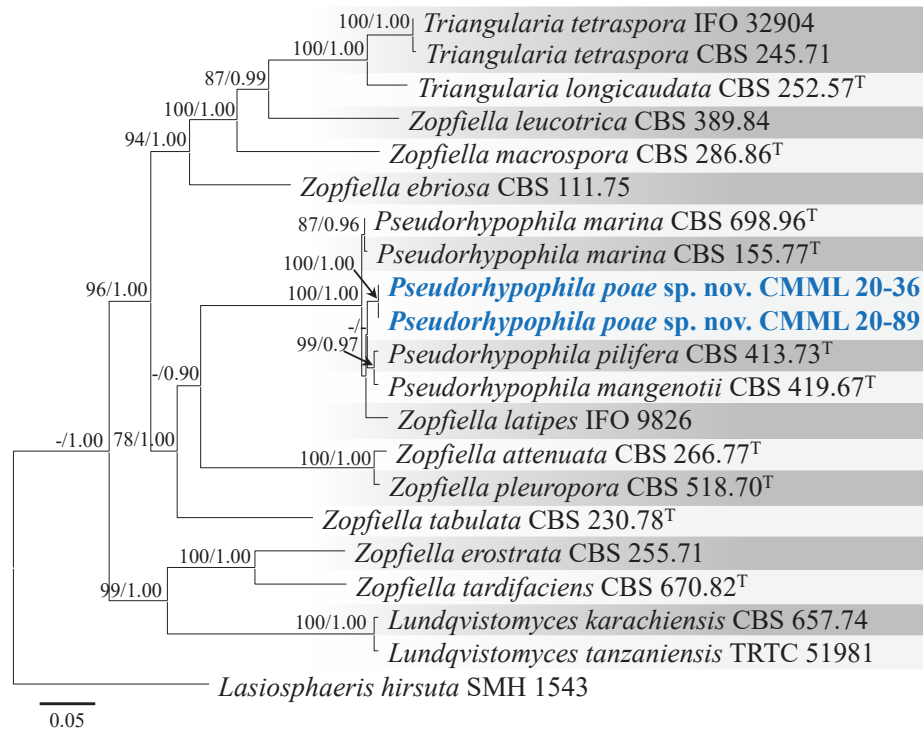


Figure 19. Maximum Likelihood phylogenetic tree based on combined sequences of ITS, LSU, *RPB2* and *TUB2* from *Pseudorhizophila* and relevant genera. Bootstrap values (BS) and Bayesian posterior probabilities (PP) are given at the nodes (BS/PP). Strains obtained from this study are in bold blue. Ex-type isolates are marked with ^T. *Lasio-sphaeria hirsuta* (SMH 1543) was used as the outgroup taxon.

combined sequences of ITS, LSU, *RPB2* and *TUB2*, strains in the present study CMML 20-36 and CMML 20-89 formed a single clade in the genus *Pseudorhizophila* supported with a high statistical support (100%/1.00) close to clade comprising ex-type strains of *P. pilifera* (CBS 413.73) and *P. mangelotii* (CBS 419.67) (Fig. 19). However, both *P. pilifera* and *P. mangelotii* produce sexual morph, which was not observed in strains CMML 20-36 and CMML 20-89. In addition, these two strains differ from *P. mangelotii* in producing conidia singly or in clusters, whereas the latter produces conidia singly (Harms et al. 2021). Therefore, based on phylogenetic analysis and morphological characteristics, *Pseudorhizophila poae* sp. nov. was introduced in this study.

***Lophiostoma jeollanense* H. Liu & H. Sang, sp. nov.**

Mycobank No: MB857262

Fig. 20

Etymology. Name refers to Jeolla Province in Korea, the place it was isolated from.

Description. *Lophiostoma jeollanense* differs from its closest phylogenetic neighbour, *L. japonicum* (KT573) by unique fixed alleles in three loci: ITS positions 25 (A), 26 (G), 31 (indel), 40 (indel), 70 (C), 91 (C), 93 (G), 114 (G), 132 (T), 134 (A), 136 (C), 138 (T), 142 (G), 364 (G), 365 (A), 368 (T), 383 (T), 407 (C); LSU positions 41 (T), 43 (C), 155 (T), 614 (C); *TEF1* positions 42 (C), 127 (T), 128 (C), 129 (C), 162 (T), 222 (C), 225 (C), 240 (T), 249 (T), 318 (T), 336 (C), 342 (C), 351 (T), 372 (C), 399 (T), 405 (C), 408 (T), 442 (G), 465 (C), 477 (G), 492 (C), 528 (C), 537 (C), 663 (T), 669 (C), 672 (G), 693 (C), 705 (C), 708 (T), 735 (T), 748 (G), 780 (G), 792 (C).

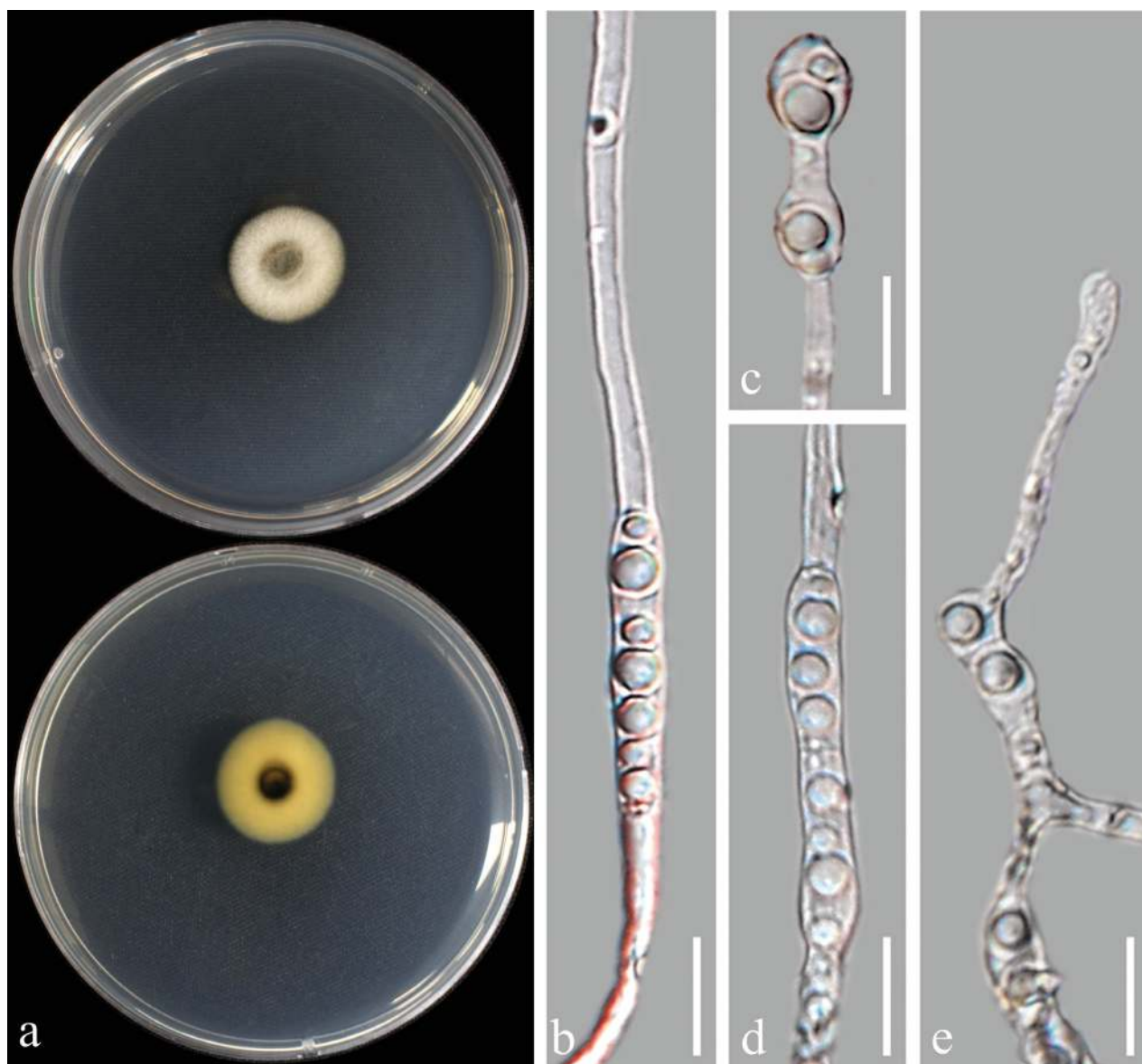


Figure 20. *Lophiostoma jeollanense* sp. nov. (CMML 20-43) **a** front and reverse sides of colony on PDA **b–e** chlamydospore-like structures on OA. Scale bars: 10 µm.

Type. Korea • South Jeolla Province, Hwasun, isolated from roots of *Zoysia japonica*, October 2020, H. Liu and H. Sang, holotype CMML 20-43H (permanently preserved in a metabolically inactive state), ex-holotype CMML 20-43, ex-isotype CMML 20-90.

Culture characteristics. Colony reaching 22.24 mm diam. on PDA after 7 days in darkness at 25 °C, surface white to light brown, reverse side yellow, mycelia dense (Fig. 20a).

Notes. *Lophiostoma jeollanense* did not sporulate on synthetic media. Chlamydospore-like structures within mycelia were observed on OA after two weeks (Fig. 20b–e). In phylogenetic analysis of the genus *Lophiostoma*, based on combined sequences of ITS, LSU, *TEF1* and *RPB2*, strains CMML 20-43 and CMML 20-90 formed a distinct single clade with a high statistical support (96%/1.00) (Fig. 21), sister to clade comprising an ex-type strain (KT573) and representative strains (KT 686-1, UESTCC 23.0040 and MFLUCC 17-2450) of

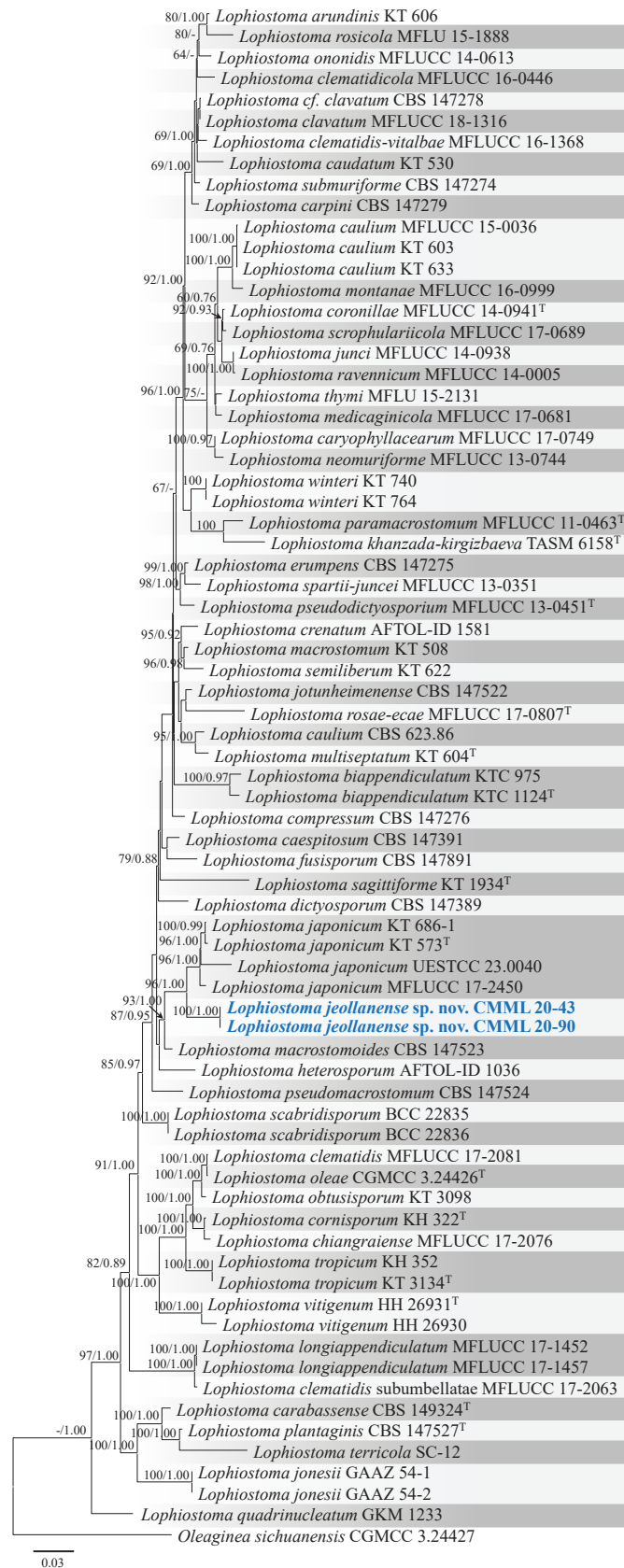


Figure 21. Maximum Likelihood phylogenetic tree based on combined sequences of ITS, LSU, *TEF1* and *RPB2* from *Lophiostoma* species. Bootstrap values (BS) and Bayesian posterior probabilities (PP) are given at the nodes (BS/PP). Strains obtained from this study are in bold blue. Ex-type isolates are marked with ^T. *Oleaginea sichuanensis* (CGMCC 3.24427) was used as the outgroup taxon.

L. japonicum. Based on nucleotide sequences of three loci, ex-holotype strain of *L. jeollanense* (CMML 20-43) was different from the ex-type strain of *L. japonicum* (KT573): ITS sequence identities = 494/513 (96.30%), gaps = 2; LSU sequence identities = 851/855 (99.53%); TEF sequence identities = 835/868 (96.20%). The species *L. japonicum* (previously *Biappendiculispora japonica*) was found as a saprophyte on dead stems of unknown herbaceous plants with its sexual morph (Thambugala et al. 2015), whereas strains CMML 20-43 and CMML 20-90 were isolated from roots of *Z. japonica* as a potential endophyte and only chlamydospore-like structures were observed in these strains. Therefore, *Lophiostoma jeollanense* sp. nov. was introduced in this study to accommodate CMML 20-43 and CMML 20-90 in the genus *Lophiostoma*.

***Poaceascoma magnum* H. Liu & H. Sang, sp. nov.**

Mycobank No: MB857263

Fig. 22

Etymology. Name refers to the character of large chlamydospores produced by this fungus.

Description. Chlamydospores 10–85 µm in length and 15–23 µm in width, hyaline to dark, clavate, sometimes dumb-bell-shaped or gourd-shaped, straight or sometimes curved. *Poaceascoma magnum* differs from its closest phylogenetic neighbour, *L. lochii* (BRIP 71546) by unique fixed alleles in two loci: ITS positions 49 (G), 57 (G), 65 (C), 67 (C), 70 (C), 71 (A), 73 (G), 76 (T), 77 (C), 79 (C), 95 (C), 133 (T), 137 (A), 143 (C), 152 (T), 157 (C), 158 (A), 162 (G), 163 (indels), 169 (A), 184 (C), 190 (T), 192 (G), 194 (A), 376 (indels), 440 (C), 444 (G), 446 (T), 474 (C), 480 (T), 481 (G), 482 (T), 483 (A), 511 (T), 512 (G), 515 (indel), 528 (A), 542 (T), 549 (indel), 560 (T); LSU positions 99 (G), 138 (G), 206 (G), 208 (A), 291 (T), 693 (C), 695 (C), 696 (indel).

Culture characteristics. Colony reaching 22.02 mm diam. on PDA after 7 days in darkness at 25 °C, white to grey at the edge, centre tawny, reverse side yellow brown (Fig. 22a).

Type. Korea • South Jeolla Province, Hwasun, isolated from roots of *Zoysia japonica*, October 2020, H. Liu and H. Sang, holotype CMML 20-47H (permanently preserved in a metabolically inactive state), ex-holotype CMML 20-47, ex-isotype CMML 20-91.

Notes. Sporulation was not observed during culture on synthetic media. On MEA, strains CMML 20-47 and CMML 20-91 produced large (10–85 × 15–23 µm), clavate, hyaline to dark, intercalary or terminal chlamydospores (Fig. 22b–k). Phylogenetic analysis using multi-loci of ITS, LSU, SSU and *TEF1* revealed that strains CMML 20-47 and CMML 20-91 formed a single clade within the genus *Poaceascoma* with a strong statistical support (100%/1.00) basal to clade containing ex-type strains of *P. lochii* (BRIP 71546), *P. helicoides* (MFLUCC 11-0136), *P. herbaceum* (GZCC 19-0046) and representative strain of *P. helicoides* (MFLU 11-0172) (Fig. 26). In comparison of nucleotide sequences of ITS and LSU, ex-holotype strain of *P. magnum* (CMML 20-47) differed from ex-type strain of *P. lochii* (BRIP 71546): ITS identities = 491/526 (93.35%), 38 gaps; LSU identities = 889/896 (99.22%). In addition, *P. magnum* (CMML 20-47) differed from ex-type strain of *P. helicoides* (MFLUCC 11-0136) in four loci:



Figure 22. *Poaceascoma magnum* sp. nov. (CMML 20-47) **a** front and reverse sides of colony on PDA **b–k** chlamydospores on MEA. Scale bars: 10 µm.

ITS identities = 435/469 (92.75%), 71 gaps; SSU identities = 914/916 (99.78%); LSU identities = 786/794 (98.99%); *TEF1* identities = 879/924 (95.13%). *P. magnum* (CMML 20-47) also differed from ex-type strain of *P. herbaceum* (GZCC 19-0046) in these loci: ITS identities = 390/420 (92.86%), 69 gaps; SSU identities = 1021/1025 (99.61%); LSU identities = 891/900 (99.00%); *TEF1* identities = 883/924 (95.56%). Morphologically, this fungus differs from other *Poaceascoma* spp. by producing large and sometimes dark chlamydospores. Therefore, *Poaceascoma magnum* sp. nov. was introduced in this study to accommodate CMML 20-47 and CMML 20-91.

***Poaceascoma endophyticum* H. Liu & H. Sang, sp. nov.**

Mycobank No: MB857264

Fig. 23

Etymology. Name refers to endophyte.

Description. *Poaceascoma endophyticum* differs from its closest phylogenetic neighbour, *P. halophilum* (MFLUCC 15-0949) by unique fixed alleles in two loci: LSU positions 84 (T), 88 (indel), 280 (C), 484 (C), 534 (T), 654 (T), 691 (T), 766 (indel), 800 (indel); SSU position 174 (indel), 972 (indel).

Culture characteristics. Colony reaching 29.33 mm diam. on PDA after 7 days in darkness at 25 °C, white ring at the edge, centre brownish, reverse side dark brown with a white edge, mycelia dense (Fig. 23a).



Figure 23. *Poaceascoma endophyticum* sp. nov. (CMML 20-48) **a** front and reverse sides of colony on PDA **b–i** chlamydospore-like structures on MEA. Scale bars: 10 µm.

Type. Korea • South Jeolla Province, Hwasun, isolated from roots of *Zoysia japonica*, October 2020, H. Liu and H. Sang, holotype CMML 20-48H (permanently preserved in a metabolically inactive state), ex-holotype CMML 20-48, ex-isotype CMML 20-49.

Notes. Strains CMML 20-48 and CMML 20-49 did not sporulate on synthetic media. Only chlamydospore-like structures were observed on MEA after two weeks, mostly elliptic or oval in shape and 5.5–12.5 µm in width (Fig. 23b–i). In phylogeny, based on multi-loci of ITS, LSU, SSU and *TEF1*, strains CMML 20-48 and CMML 20-49 clustered into a distinct clade, sister to clade comprising ex-type strains of *P. halophilum* (MFLUCC 15-0949), *P. zoysiiradicicola* (CMML 20-50) and representative strain CMML 20-51. Based on nucleotide sequence, ex-holotype strain of *P. endophyticum* (CMML 20-48) differed from ex-type strain of *P. halophilum* (MFLUCC 15-0949) in LSU sequence (identities = 830/836, 99.28%). *P. endophyticum* (CMML 20-48) also differed from *P. zoysiiradicicola* (CMML 20-50 and CMML 20-51) in three different loci: ITS identities = 523/558 (93.73%), 40 gaps; LSU identities = 828/838 (98.81%); *TEF1* identities = 824/849 (97.06%). According to (Hyde et al. 2017), colonies of *P. halophilum* on PDA reaches 20–30 mm diameter after 4 weeks, indicating a slower vegetative growth rate than *P. endophyticum*. In addition, *P. endophyticum* differs from *P. zoysiiradicicola* in producing larger chlamydospore-like structures. Thus, *Poaceascoma endophyticum* sp. nov. was introduced in this study to accommodate CMML 20-50 and CMML 20-51 in the genus *Poaceascoma*.

***Poaceascoma koreanum* H. Liu & H. Sang, sp. nov.**

Mycobank No: MB857265

Fig. 24

Etymology. Name refers to Korea, the country from where it was isolated.

Description. *Poaceascoma koreanum* differs from its closest phylogenetic neighbour *P. lochii* (BRIP 71546) by unique fixed alleles in two loci: ITS positions 13 (A), 16 (C), 19 (G), 20 (T), 21 (C), 22 (G), 28 (G), 29 (indels), 41 (C), 42 (C), 44 (C), 45 (T), 46 (C), 47 (G), 50 (T), 51 (T), 52 (C), 58 (G), 60 (C), 68 (C), 84 (T), 98 (C), 107 (indel), 109 (C), 112 (indels), 114 (G), 116 (C), 124 (G), 125 (A), 127 (C), 130 (C), 131 (T), 132 (C), 136 (A), 137 (G), 140 (T), 141 (T), 144 (A), 153 (indel), 155 (G), 156 (T), 157 (A), 158 (C), 165 (C), 166 (G), 168 (A), 176 (A), 350 (indels), 388 (C), 391 (T), 397 (G), 404 (T), 410 (A), 420 (C), 435 (C), 440 (C), 443 (G), 447 (C), 449 (G), 450 (A), 469 (T), 475 (G), 476 (T), 481 (T), 489 (T), 495 (A), 497 (G), 500 (A), 502 (C); LSU positions 100 (G), 104 (indel), 138 (C), 141 (G), 143 (G), 145 (G), 205 (C), 206 (C), 210 (C), 488 (C), 550 (T), 700 (C), 705 (C), 755 (G), 907 (T).

Culture characteristics. Colony reaching 39.72 mm diam. on PDA after 7 days in darkness at 25 °C, front side greyish-yellow, reverse side black-brown, margins burr-like (Fig. 24a).

Type. South Korea • South Jeolla Province, Hwasun, isolated from roots of *Zoysia japonica*, October 2020, H. Liu and H. Sang, holotype CMML 20-44H (permanently preserved in a metabolically inactive state), ex-holotype CMML 20-44, ex-isotype CMML 20-45; CMML 20-46.

Notes. No conidiogenous structures or sexual morph were observed in strains CMML 20-44, CMML 20-45 and CMML 20-46. On MEA, chlamydospore-like structures (4.5–10.5 µm in width) in hyphae were observed after incubation for two weeks (Fig. 24b–g). In multi-loci phylogeny, based on ITS, LSU, SSU and *TEF1*, these three strains formed a distinct single clade within the genus *Poaceascoma* sister to clade comprising strain of *P. magnum* (ex-type CMML 20-47 and ex-isotype CMML 20-71), *P. herbaceum* (ex-type GZCC 19-0046), *P. helicoides* (ex-type MFLUCC 11-0136 and representative strain MFLU 11-0172) and *P. lochii* (ex-type BRIP 71546) (Fig. 26). Based on nucleotide sequence comparison, *P. koreanum* (CMML 20-44) differed from ex-type strain of *P. lochii* (BRIP 71546) in two loci: ITS identities = 456/518 (88.03%), 40 gaps; LSU identities = 882/896 (98.44%). *P. koreanum* (CMML 20-44) differed from ex-type strain of *P. helicoides* (MFLUCC 11-0136) in four loci: ITS identities = 381/428 (89.02%), 41 gaps; SSU identities = 915/916 (99.89%); LSU identities = 779/793 (98.23%); *TEF1* identities = 896/958 (93.53%). Compared to ex-type of *P. herbaceum* (GZCC 19-0046), nucleotide sequences were different in these loci: ITS identities = 335/380 (88.16%), 41 gaps; SSU identities = 1021/1025 (99.61%); LSU identities = 890/904 (98.45%); *TEF1* identities = 897/958 (93.63%). *P. koreanum* (CMML 20-44) also differed from ex-holotype strain of *P. magnum* (CMML 20-47): ITS identities = 433/500 (86.60%), 45 gaps; SSU identities = 1028/1032 (99.61%); LSU identities = 884/900 (98.22%); *TEF1* identities = 874/924 (94.59%). All of these species were originally found on herbaceous plants. Specifically, *P. lochii* was found on leaves of turf-grass *Zoysia matrella*, *P. helicoides* and *P. herbaceum* were saprophytes on dead stem of *Digitaria sanguinalis* and dead culm of unidentified herbaceous plants, respectively (Phookamsak et al. 2015; Hyde et al. 2017; Tan et al. 2021).

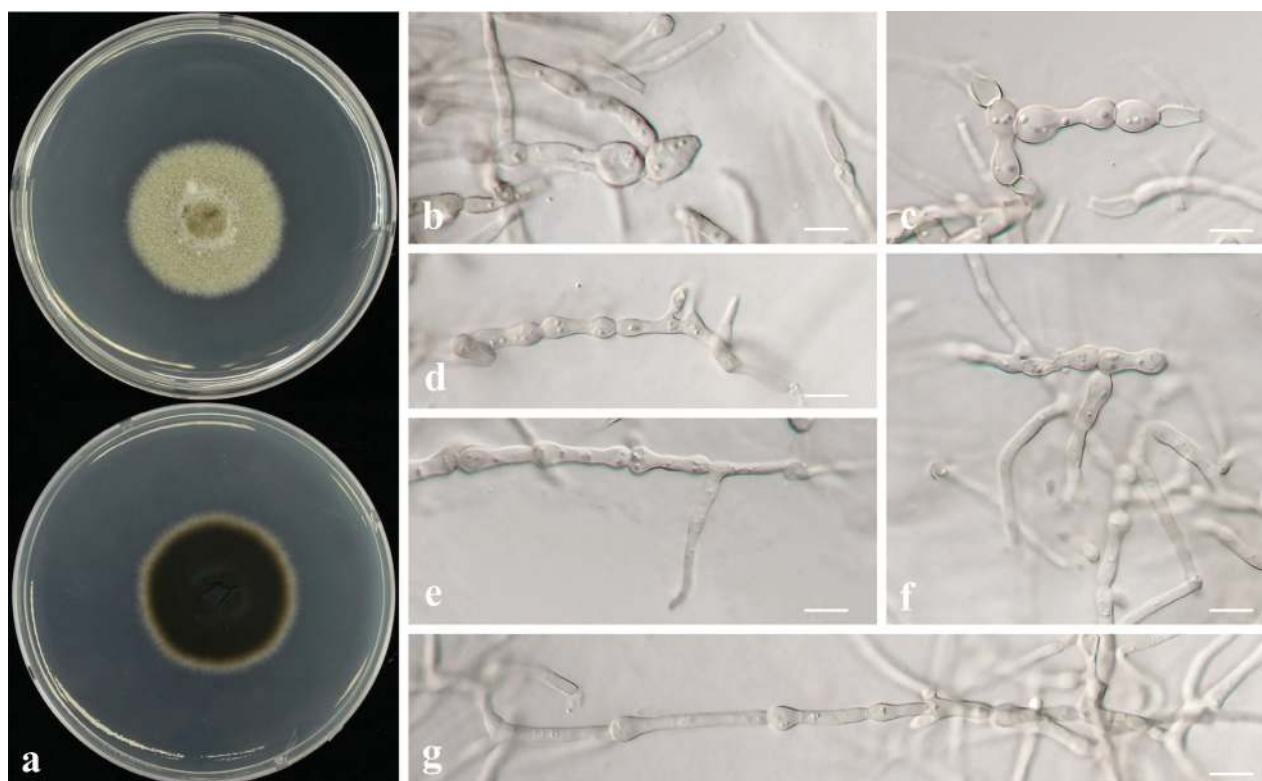


Figure 24. *Poaceascoma koreanum* sp. nov. (CMML 20-44) **a** front and reverse sides of colony on PDA **b–g** chlamydospore-like structures on MEA. Scale bars: 10 µm.

Morphologically, *P. herbaceum*, *P. helicoides* and *P. lochii* were described, based on their sexual morph, while only chlamydospore-like structures were observed in strains CMML 20-44, CMML 20-45 and CMML 20-46. Additionally, *P. magnum* differs from these strains in producing large chlamydospores. Therefore, *Poaceascoma koreanum* sp. nov. was introduced in this study.

***Poaceascoma zoysiiradicicola* H. Liu & H. Sang, sp. nov.**

[Mycobank No: MB857266](#)

Fig. 25

Etymology. Name refers to roots of *Zoysia japonica*.

Description. *Poaceascoma zoysiiradicicola* differs from its closest phylogenetic neighbour *P. halophilum* (MFLUCC 15-0949) by unique fixed alleles in two loci: LSU positions 2 (T), 48 (T), 49 (T), 52 (indel), 144 (T), 359 (T), 458 (T), 655 (T), 730 (indel), 764 (indel), 866 (A); SSU positions 171 (indel), 969 (indel).

Culture characteristics. Colony reaching 44.23 mm diam. on PDA after 7 days in darkness at 25 °C, front side reseda green, reverse sides crineous to dark, margin white on both sides (Fig. 25a).

Type. Korea • South Jeolla Province, Hwasun, isolated from roots of *Zoysia japonica*, October 2020, H. Liu and H. Sang, holotype CMML 20-50H (permanently preserved in a metabolically inactive state), ex-holotype CMML 20-50, ex-isotype CMML 20-51.

Notes. No sporulation was found on synthetic media in this fungus. However, chlamydospore-like structures in hyphae were observed on MEA after two weeks,



Figure 25. *Poaceascoma zoysiiradicicola* sp. nov. (CMML 20-50) **a** front and reverse sides of colony on PDA **b–g** chlamydospore-like structures on MEA. Scale bars: 10 µm.

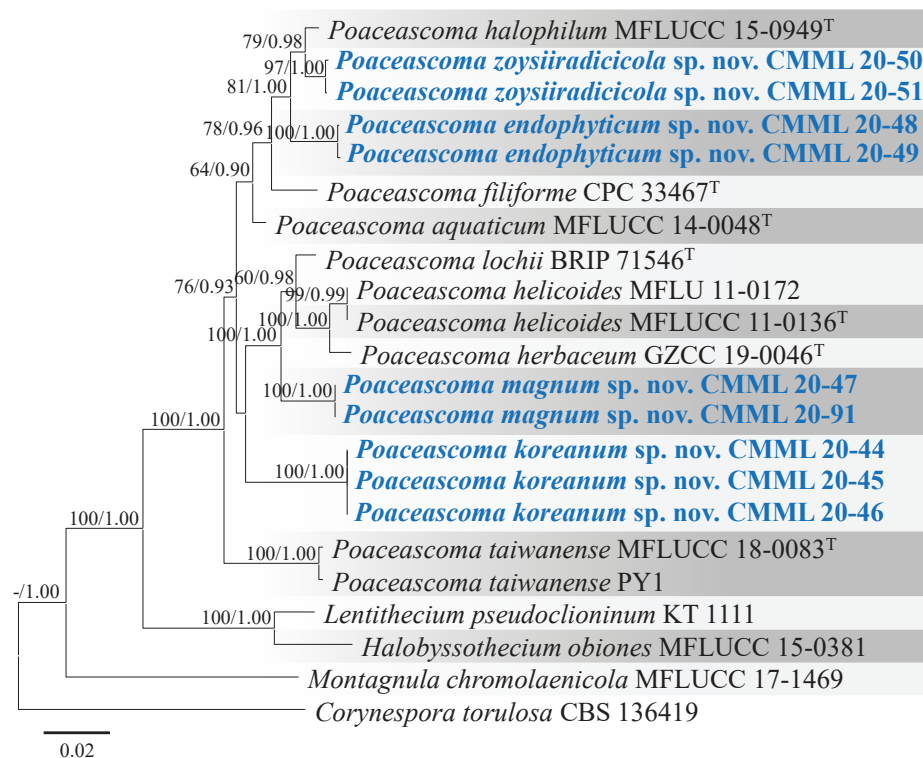


Figure 26. Maximum Likelihood phylogenetic tree based on combined sequences of ITS, LSU, SSU and *TEF1* from *Poaceascoma* species using Maximum Likelihood method. Bootstrap values (BS) and Bayesian posterior probabilities (PP) are given at the nodes (BS/PP). Strains obtained from this study are in bold blue. Ex-type isolates are marked with ^T. *Corynespora torulosa* (CBS 136419) was used as the outgroup taxon.

4–6.5 μm in width (Fig. 25b–g). In phylogenetic analysis, based on multi-loci of ITS, LSU, SSU and *TEF1*, strains CMML 20-50 and CMML 20-51 formed a separate clade sister to ex-type strain of *P. halophilum* (MFLUCC 15-0949) (Fig. 26). Based on nucleotide sequences of LSU and SSU, ex-holotype strain of *P. zoysiiradicicola* (CMML 20-50) was different with the ex-type strain of *P. halophilum* (MFLUCC 15-0949): LSU sequence identities = 855/863 (99.07%), gaps = 3; SSU sequence identities = 1038/1038 (100%), gaps = 2. *Poaceascoma halophilum* was found as a saprophyte on a decaying bamboo stick and its asexual morph is undetermined (Hyde et al. 2017). In terms of culture characteristics, *P. halophilum* differs from *P. zoysiiradicicola* in the slower vegetative growth rate on PDA (Hyde et al. 2017). Therefore, *P. zoysiiradicicola* sp. nov. was introduced in this study to accommodate CMML 20-50 and CMML 20-51 in the genus *Poaceascoma*.

In vitro antifungal activity against *Rhizoctonia solani* AG2-2(IIIB)

Antifungal activities of the species described above were tested by *in vitro* dual culture against *R. solani* AG2-2(IIIB), the casual pathogen of turf-grass brown patch disease. Different antifungal activities were observed amongst these species (Fig. 27a–l). Mycelial growth inhibition rates were 37.97% for *D. hwasunensis* CMML 20-35, 23.15% for *L. jeollanense* CMML 20-43, 40.35% for *M. zoysiae* CMML 20-39, 37.90% for *N. dimorphospora* CMML 20-40, 21.53% for *P. endophyticum* CMML 20-49, 21.51% for *P. zoysiiradicicola* CMML 20-51, 7.95% for *P. koreanum* CMML 20-46, 35.37% for *P. magnum* CMML 20-47, 51.68% for *S. zoysiae* CMML 20-15, 41.07% for *S. endophytica* CMML 20-37 and 15.12% for *P. poae* CMML 20-36 (Fig. 27m). *Setophoma zoysiae* CMML 20-15 showed a significantly higher inhibition to *R. solani* AG2-2(IIIB) and was therefore selected for further antifungal activity assays.

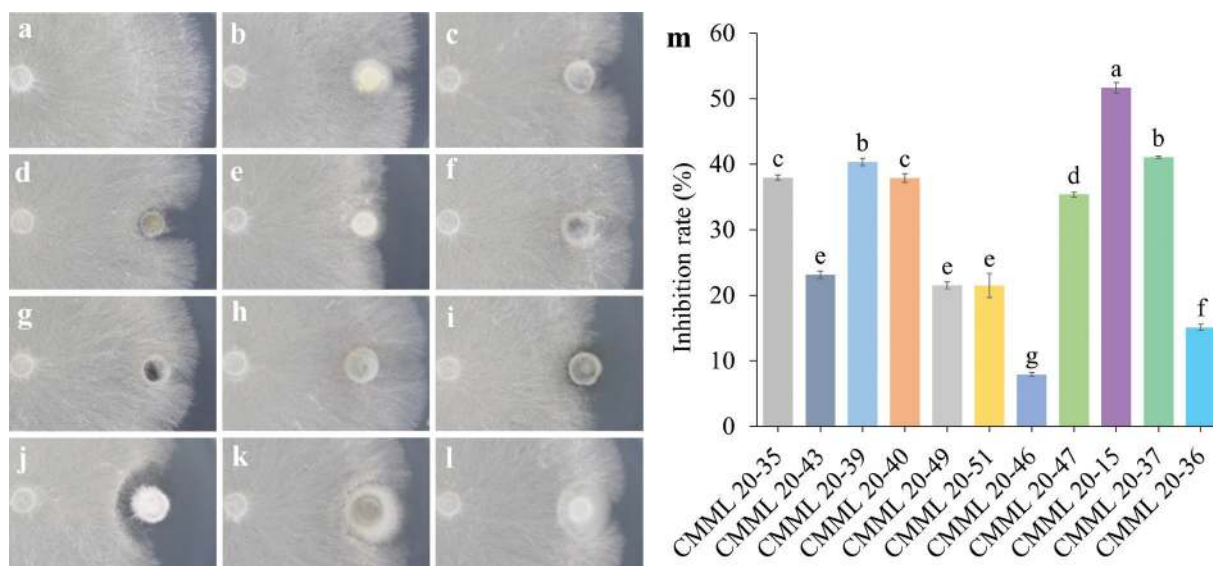


Figure 27. Dual culture of endophytic fungi against turfgrass brown patch pathogen *Rhizoctonia solani* (AG-2-2 (IIIB)) **a** control **b** *Dactylaria hwasunensis* (CMML 20-35) **c** *Lophiostoma jeollanense* (CMML 20-43) **d** *Magnaporthiopsis zoysiae* (CMML 20-39) **e** *Niesslia dimorphospora* (CMML 20-40) **f** *Poaceascoma endophyticum* (CMML 20-49) **g** *P. zoysiiradicicola* (CMML 20-51) **h** *P. koreanum* (CMML 20-46) **i** *P. magnum* (CMML 20-47) **j** *Setophoma zoysiae* (CMML 20-15) **k** *Stagonospora endophytica* (CMML 20-37) **l** *Pseudorhizophila poae* (CMML 20-36) **m** Inhibition rates of each fungal strains. Error bars indicate the standard errors of the means.

***In vitro* antifungal activity of *Setophoma zoysiae* (CMML 20-15) against eight phytopathogens**

To test the antifungal spectrum of *S. zoysiae* (CMML 20-15), eight different agriculturally important phytopathogens were used for dual culture assay. Mycelial growth inhibition rates were 50.65% for *R. cerealis* (KACC 40154), 48.86% for *R. solani* (AG2-2(IV) KACC 40132), 54.70% for *Clariireedia jacksonii* (CMML 20-31), 50.96% for *Pythium ultimum* (KACC 40705), 60.82% for *Sclerotinia sclerotiorum* (KACC 40457), 46.57% for *Botrytis cinerea* (CMML 20-BC04), 21.68% for *Fusarium oxysporum* (CMML 21-1) and 33.56% for *Colletotrichum gloeosporioides* (KACC 40003) (Fig. 28).

Antifungal activities of mycelial crude extracts of *Setophoma zoysiae* (CMML 20-15)

Mycelial crude extraction of *S. zoysiae* (CMML 20-15) was conducted using five different solvents (methanol, ethyl acetate, hexane, acetone and butanol). Antifungal activities of the five crude extracts against *R. solani* (AG2-2(IIIB)) were tested using a paper disc assay. No mycelial growth inhibition was found in methanol, ethyl acetate, hexane and acetone crude extracts, while dramatic growth inhibition was found in butanol extract (Fig. 29).

To evaluate the mycelial viability of *R. solani* (AG2-2(IIIB)), Evans blue and neutral red were used to stain the mycelia from PDA plates with or without butanol extract of *S. zoysiae* (CMML 20-15). Staining results were examined under a microscope. Mycelia from the control groups (without exposure to butanol extract) were not stained by Evans blue, but were stained red by neutral red. On the contrary, mycelia exposed to butanol extract were stained blue by Evans

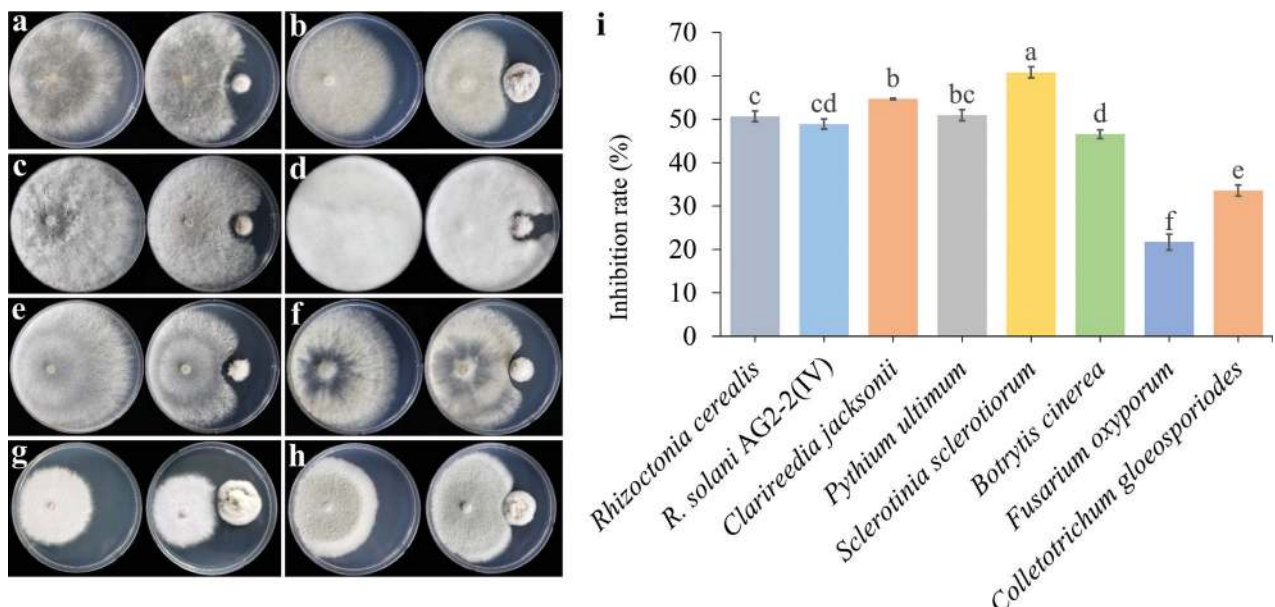


Figure 28. Dual culture of *Setophoma zoysiae* (CMML20-15) against eight different plant pathogens **a** *Rhizoctonia solani* (AG-2-2(IV) KACC 40132) **b** *R. cerealis* (KACC 40154) **c** *Clariireedia jacksonii* (CMML 20-31) **d** *Pythium ultimum* (KACC 40705) **e** *Sclerotinia sclerotiorum* (KACC 40457) **f** *Botrytis cinerea* (CMML 20-BC04) **g** *Fusarium oxysporum* (CMML 21-1) **h** *Colletotrichum gloeosporioides* (KACC 40003) **i** mycelial growth inhibition rates of each pathogen. Error bars indicate the standard errors of the means.

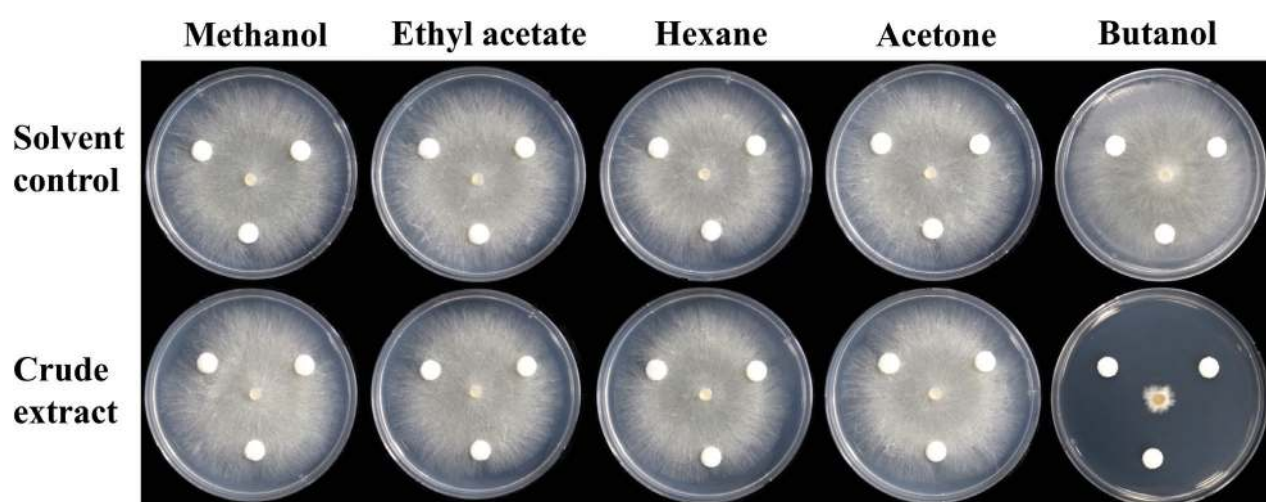


Figure 29. Antifungal activities of five crude extracts (methanol, ethyl acetate, hexane, acetone and butanol) of *Setophoma zoysiae* (CMML20-15) against *Rhizoctonia solani* (AG2-2(IIIB)) on PDA media. Paper discs were moistened with 20 μ l of the crude extracts. Only solvent was treated in the control groups.

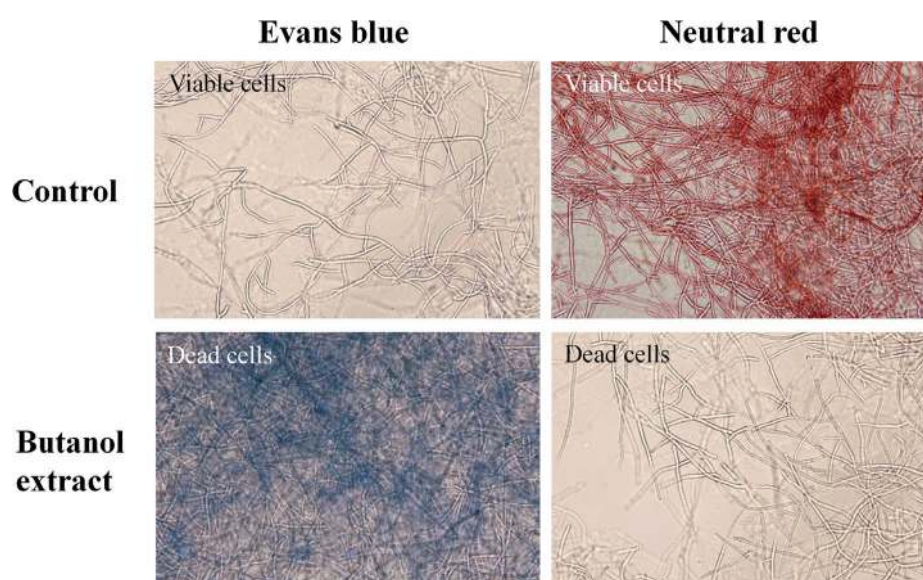


Figure 30. Mycelia of *Rhizoctonia solani* (AG2-2(IIIB)) with or without exposure to butanol extract of *Setophoma zoysiae* (CMML20-15) were stained with Evans blue and neutral red. Dead cells stained blue; viable cells, red.

blue, while remaining unstained by neutral red (Fig. 30). This revealed that cell death of *R. solani* (AG2-2(IIIB)) occurred under the treatment of butanol extract of *S. zoysiae* (CMML 20-15).

***In planta* brown patch disease control**

Butanol extract of *S. zoysiae* (CMML 20-15) was re-dissolved in sterile distilled water and used for brown patch control on creeping bentgrass in pots, with fungicide azoxystrobin used for the positive control. Symptoms of severe disease were observed on creeping bentgrass treated with only the pathogen *R. solani* (AG2-2(IIIB)). Fungicide azoxystrobin showed complete control of brown patch disease and only minor disease symptoms occurred on butanol extract-treated creeping bentgrass. No symptoms were observed in the sterile water-treated control group (Fig. 31).

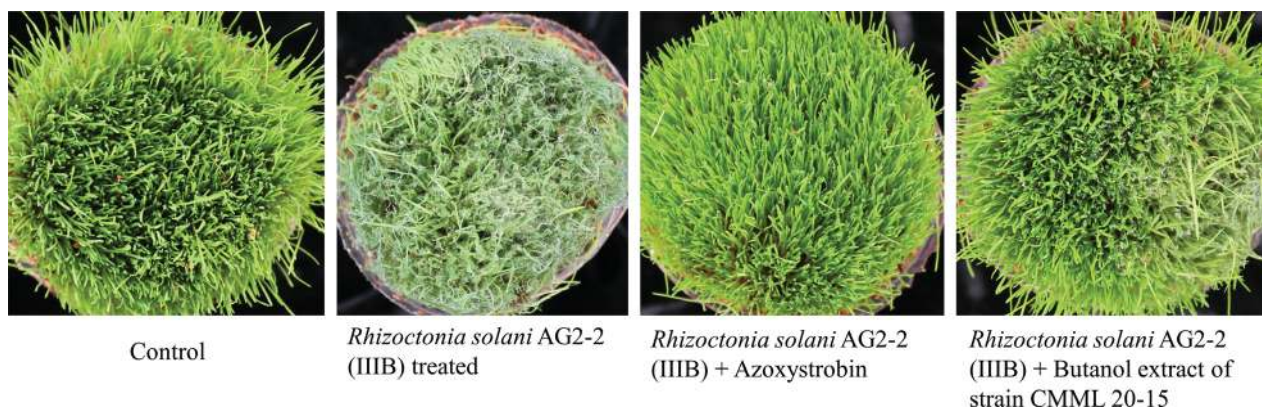


Figure 31. *In planta* brown patch control of creeping bentgrass by butanol extract of *Setophoma zoysiae* (CMML20-15), with a comparison to a commercial fungicide azoxystrobin. Control group was treated with sterile water.

Discussion

In this study, both culture-independent and -dependent methods were used to determine endophytic fungal diversity associated with roots of *Z. japonica*. Novel species were identified based on morphological characterisation and multi-loci phylogenetic analyses. In addition, antifungal activities of the described species were tested against *R. solani* (AG2-2(IIIB)) with *S. zoysiae* (CMML20-15) being the best antagonist. Butanol crude extract of this strain was applied to control *R. solani* (AG2-2(IIIB)) by *in vitro* mycelial growth inhibition and *in planta* brown patch control. This is the first comprehensive work revealing fungal community on *Z. japonica* with an attempt for exploration and application of biological resources.

Through analysis of ITS amplicon reads, abundant OTUs were identified in roots of *Z. japonica*, dominated by members of the class *Sordariomycetes*. Fungi in this class have also been reported to dominate epiphytic and endophytic samples of tomato (Dong et al. 2021). The functions of endophytic fungi in this class and their roles in interacting with host plants deserve to be investigated. However, the classification of the most abundant taxa at the family and genus levels have remained unknown, suggesting that a large number of endophytic fungi in *Z. japonica* are remain unidentified. This supports the fact that endophytic fungi constitute a major part of unexplored fungal diversity (Rajamanikyan et al. 2017).

A variety of endophytic fungi were obtained in roots of *Z. japonica* by isolation in this study. Taxa in genera *Collectotrichum*, *Fusarium*, *Curvularia*, *Lophiostoma*, *Magnaporthiopsis*, *Poaceascoma* and *Stagonospora* were detected by both culture-independent and culture-dependent methods. Amongst these taxa, *Biappendiculispora* (currently *Lophiostoma*) and *Poaceascoma* were in the top 20 most abundant genera in ITS amplicon sequencing analysis and *Poaceascoma* spp. were also detected with high portions (18.52%) during isolation. Therefore, *Poaceascoma* spp. may have potential functions in the root-inhabiting mycobiome of *Z. japonica*.

Combining morphological characterisation and phylogenetic analysis represents a reliable strategy for fungal taxonomy. Based on this approach, a newly-recorded species (*Niesslia dimorphospora*) in Korea and 10 new species (*Dactylaria hwasunensis*, *Lophiostoma jeollanense*, *Magnaporthiopsis zoysiae*, *Poaceascoma endophyticum*, *P. koreanum*, *P. magnum*, *P. zoysiiradicicola*,

Setophoma zoysiae, *Stagonospora endophytica* and *Pseudorhizophila poae*) were identified. Some genera such as *Magnaporthiopsis*, *Poaceascoma* and *Stagonospora* have been reported to be associated with grasses. Specifically, *Magnaporthiopsis* spp., such as *M. dharug*, *M. gadigal*, *M. gumbaynggirr* and *M. yugambeh*, were isolated from diseased turf-grass species in Australia (Wong et al. 2022). *Magnaporthiopsis meyeri-festuca* was associated with a summer patch-like disease of fescue turf-grasses in New Jersey (Luo et al. 2017). *Magnaporthiopsis zoysiae* was isolated from healthy roots of turf-grass *Z. japonica* in this study, it being genetically different from other *Magnaporthiopsis* spp. The genus *Poaceascoma* was established with type species *P. helicoides*, which is isolated as a saprophyte from dead stems of a grass in *Poaceae* (Phookamsak et al. 2015). Herein, four novel species *P. endophyticum*, *P. koreanum*, *P. magnum* and *P. zoysiiradicicola* were isolated as endophytes from turf-grass *Z. japonica*. Species in *Stagonospora* are commonly associated with grasses (Thambugala 2017) as pathogens and *S. endophytica* was supplemented as an endophyte in this study.

In previous studies, most of the endophytic fungi have not sporulated during culture. For example, Lin et al. (2007) reported that 48.9% of the obtained endophytic taxa from *Camptotheca acuminata* were non-sporulating fungi. Tejesvi et al. (2011) isolated 87 endophytic fungi from asymptomatic leaf tissues of *Rhododendron tomentosum* and most of the isolates were unable to sporulate. Therefore, it is difficult to identify endophytic non-sporulating fungi, based on morphological characters and sequence-based molecular tools can be used to achieve their classification (Promputtha et al. 2005; Huang et al. 2009; Tejesvi et al. 2011; Selim 2012). In the present study, species *L. jeollanense*, *P. endophyticum*, *P. koreanum*, *P. magnum* and *P. zoysiiradicicola* did not sporulate on synthetic media – only chlamydospores were produced. Similarly, sporulation was not observed in endophytic fungi, such as *Cyanoderma asteris*, *Nemania aquilariae*, *N. yunnanensis*, *Batnamyces globulariicola*, *Endopandanicola thailandica*, *Endomelanconiopsis freycinetiae*, *Diaporthe pandanicola* and *Mycoleptodiscus endophyticus*, which were proposed as novel species recently (Jahn et al. 2017; Tibpromma et al. 2018, 2021; Noumeur et al. 2020).

Endophytic fungi are also an important source of novel and potential bioactive compounds (Strobel and Daisy 2003; Tomita 2003; Rajamanikyan et al. 2017). Application of endophytic fungi as biocontrol agents has increased considerably. To date, endophytic fungi have been reported to control a variety of plant pathogens, ranging from pathogenic fungi, bacteria, oomycetes to plant-parasitic nematodes (Latz et al. 2018; Huang et al. 2020; Poveda et al. 2020; Hapida et al. 2021; Simamora et al. 2021). Brown patch caused by *R. solani* is a major disease of turf-grasses worldwide and its control relies heavily on fungicide applications (Daniels and Latin 2013). To reduce the use of fungicides, biocontrol agents have been applied for brown patch control, such as *Bacillus velezensis* (GH1-13) (Lee et al. 2023), *Paenibacillus ehimensis* (KWN38), *Stenotrophomonas maltophilia* (C3) (Yuen and Zhang 2001) and *Trichoderma harzianum* (Guerrero et al. 2008). In this study, antifungal activities against *R. solani* (AG2-2(IIIB)) were observed amongst the tested endophytic fungi and *S. zoysiae* (CMML20-15) could serve as an antagonist for brown patch management. Additional dual culture assay suggested that *S. zoysiae* (CMML20-15) was potentially able to control the turf-grass

large patch pathogen *R. solani* (AG2-2 (IV)), yellow patch pathogen *R. cerealis*, dollar spot pathogen *C. jacksonii*, Pythium blight pathogen *P. ultimum* and non-turf-grass pathogens *S. sclerotiorum*, *B. cinerea*, *F. oxysporum* and *C. gloeosporioides*.

In conclusion, this study provides insights from the mycobiome diversity in *Z. japonica* to an application of a biocontrol agent in controlling pathogens of turf-grass, which will be valuable for future management of *Z. japonica*. Identifying the functions of other root-colonizing fungi of *Z. japonica* and searching for their bioactive compounds warrant future investigation.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Adherence to national and international regulations

All the fungal strains used in this study have been legally obtained, respecting the Convention on Biological Diversity (Rio Convention).

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Author contributions

Haifeng Liu: Methodology, Investigation, Data curation, Writing – Original Draft. Hyeonju Choi: Methodology, Investigation. Narayan Chandra Paul: Conceptualisation, Methodology. Hiran A. Ariyawansa: Conceptualisation, Writing – review and editing. Hyunkyu Sang: Conceptualisation, Writing – review and editing, Supervision, Project administration.

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Data availability

The raw reads of ITS amplicon sequencing generated in this study were deposited in NCBI (BioProject no. PRJNA1165193). Nucleotide sequences of species described in this study were submitted to GenBank with accession numbers shown in Suppl. material 1.

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Supplementary material 1

Genbank accession numbers for the strains used for phylogenetic analysis in this study

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Data type: xlsx

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Link: <https://doi.org/10.3897/imafungus.16.138479.suppl1>

Supplementary material 2

Sequence alignments

Authors: Haifeng Liu, Hyeongju Choi, Narayan Chandra Paul, Hiran A. Ariyawansa, Hyunkyu Sang

Data type: zip

Explanation note: Sequence alignments (fas.-files in ZIP.archive).

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