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Aquapteridospora rhizicola sp. nov., a novel root endophytic fungus at a mining site in Japan

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During a survey on root endophytes in mining areas, *Aquapteridospora* sp. was isolated from the roots of *Pinus densiflora* and *Miscanthus sinensis*. Morphological observation and molecular analysis revealed it to be an undescribed species. Here we provide its description as a novel taxon, *A. rhizicola*, with its phylogenetic position.

Key words: *Distoseptisporales*, dark septate endophyte, mining area, *Miscanthus sinensis*, *Pinus densiflora*.

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Během výzkumu kořenových endofytů v důlní oblasti byl izolován druh rodu *Aquapteridospora* z kořenů *Pinus densiflora* a *Miscanthus sinensis*. Morfologické pozorování a molekulární analýza odhalily, že se jedná o dosud nepopsaný druh. Zde je podán popis nového taxonu *A. rhizicola* a stanovena jeho fylogenetická pozice.

INTRODUCTION

Recovery of vegetation at abandoned mine sites is one of the critical issues in the world (Skousen et al. 2017). In particular, establishment of vegetation is markedly constrained on sedimentary clay deposits generated through the neutralisation

of acidic wastewater, owing to their increased concentrations of minerals and heavy metals and concomitant deficiency in bioavailable nutrients. However, such extreme habitats are sometimes preferred by pioneer plants, e.g. *Pinus densiflora* and *Miscanthus sinensis* (Hiroi 1974). These plants are expected to be candidates for greening abandoned mine sites. Although the tolerance mechanism of the plants growing in such extreme habitats are not always clear, many reports suggest that root endophytes are key players in the plant growth in these habitats (Jeffries et al. 2003, Colpaert et al. 2011, Li et al. 2011, Yamaji et al. 2016). Notwithstanding their importance, the biology and taxonomic positions of the root endophytes are not always fully understood.

During a survey of root endophytes in mining areas, we isolated a fungus from the root of *Pinus densiflora* seedlings and *Miscanthus sinensis* plants (Haruma et al. 2023). This fungus was characterised by polyblastic mononematous conidiogenous cells and fusiform conidia with a conspicuous sheath. Here we describe it as a new species of *Aquapteridospora*.

MATERIAL AND METHODS

Isolation. Details of sampling site, soil condition and isolation methods were described in Haruma et al. (2023). Roots of *M. sinensis* and *P. densiflora* were surface-sterilised with 70% ethanol, 7.5% hydrogen peroxide, rinsed with 70% ethanol and distilled water, dried and cut into 10 mm segments. These were placed on 1% malt extract agar (MA) plates and incubated. Growing mycelia were picked and transplanted onto 1% MA and established pure cultures. Representative isolates were deposited in the Biological Resource Center, NITE (NBRC), Japan. Also, each dried specimen was deposited in the herbarium of the Forestry & Forest Products Research Institute (TFM:FPH). Additional isolates examined in this study were kept in the private culture collection of K. Yamaji's laboratory at Tsukuba University, available upon request.

Morphological observation. Colony characters were taken from potato dextrose agar (PDA) and 1% MA media for 8 days at 28 °C. Sporulating structures of the fungus were abundantly produced on Spezieller Nährstoffarmer Agar (SNA) and 0.1% PDA under a blacklight blue (BL-B) lamp for 2 weeks. Morphological observation of the fungal structures was carried out on the SNA medium by using a Leica DM2500 stereomicroscope (Leica Microsystems, Wetzlar, Germany). Photographs of each character were taken using an MC170HD digital camera (Leica Microsystems) on a Leica DM2500.

Molecular analysis. Genomic DNAs were extracted from the mycelium using PrepMan™ Ultra Sample Preparation Reagent (Thermo Fisher Scientific Inc., Waltham, USA) following the manufacturer's instructions. ITS, LSU, EF 1-α, and RPB2 genes were amplified with primer pairs ITS1F and ITS4 (White et al. 1990), LR0R and LR5 (Rehner & Samuels 1995 and Vilgalys & Hester 1990, respectively), EF1 and EF2 (O'Donnell et al. 1998), and RPB 5F and RPB 7CR (Liu et al. 1999), respectively. The obtained amplicons were sequenced with BigDye Terminator kit v. 3.0 and ABI 3130 genetic analyser (Applied Biosystems). Sequence data were aligned using the MUSCLE algorithm (Edgar 2004) together with the dataset of Xu et al. (2024), and a Maximum Likelihood tree was inferred using the IQ-TREE programme (Nguyen et al. 2015) with the ModelFinder option (Kalyaanamoorthy et al. 2017). Two species of the genus *Pseudostanjehughesia* were used as the outgroup (Tab. 1). The obtained

sequences were deposited in the DDBJ database (https://www.ddbj.nig.ac.jp/) and this novel taxon was recorded in MycoBank (http://www.mycobank.org/). The obtained tree was visualised with TreeViewer (Bianchini & Sánchez-Baracaldo 2024).

Tab. 1. Strain and sequence accession numbers of the fungi used in the phylogenetic analysis. Newly generated sequences are highlighted in boldface, asterisks mark the outgroup.

Species	Country	Voucher / strain number	Sequence accession number			Reference
			LSU	ITS	EF 1-α	
Aquapteridospora aquatica	Thailand	MFLUCC17-2371	MW287767	MW286493	–	Dong et al. (2021)
Aquapteridospora bambusinum	Thailand	MFLUCC12-0850	KU863149	KU940161	KU940213	Dai et al. (2017)
Aquapteridospora bambusinum	Thailand	MFLUCC21-0027	MZ412526	MZ412514	MZ442688	Bao et al. (2021)
Aquapteridospora fusiformis	China	MFLUCC18-1606	MK849798	MK828652	MN194056	Luo et al. (2019)
Aquapteridospora guangxiensis	China	ZHKUCC24–1308	PV362583	PV362580	–	Shu et al. (2026)
Aquapteridospora hyalina	China	GZCC22-0072	ON527945	ON527937	ON533681	Ma et al. (2022)
Aquapteridospora hyalina	China	GZCC22-0073	ON527948	ON527940	ON533684	Ma et al. (2022)
Aquapteridospora jiangxiensis	China	JAUCC3008	MZ871502	MZ871501	MZ855767	Peng et al. (2022)
Aquapteridospora lignicola	Thailand	MFLUCC15-0377	KU221018	MZ868774	MZ892980	Yang et al. (2015)
Aquapteridospora linzhiensis	China	KUNCC10420	OQ970576	OP626343	OR597592	Xu et al. (2024)
Aquapteridospora linzhiensis	China	KUNCC10444	OQ970575	OQ847781	OR597591	Xu et al. (2024)
Aquapteridospora rhizicola	**Japan**	**NBRC116724**	**LC867025**	**LC867021**	**LC867023**	**This study**
Aquapteridospora rhizicola	**Japan**	**NBRC116727**	**LC867024**	**LC867020**	**LC867022**	**This study**
Aquapteridospora submersa	China	KUNCC10446	OQ970579	OQ847783	OR597595	Xu et al. (2024)
Aquapteridospora submersa	China	KUNCC10449	OQ970580	OQ970557	OR597596	Xu et al. (2024)
Aquapteridospora yadongensis	China	KUNCC10445	OQ970577	OQ847782	OR597593	Xu et al. (2024)
Aquapteridospora yadongensis	China	KUNCC10448	OQ970578	OQ970556	OR597594	Xu et al. (2024)
Distoseptispora atroviridis	China	GZCC20-0511	MZ868763	MZ868772	MZ892978	Yang et al. (2021)
Distoseptispora bambusae	China	MFLUCC20-0091	MT232718	MT232713	MT232880	Sun et al. (2020)
Distoseptispora euseptata	China	MFLU20-0568	MW081545	MW081540	MW084994	Li et al. (2021)
Distoseptispora fusiformis	China	GZCC20-0512	MZ868764	MZ868773	MZ892979	Yang et al. (2021)
Distoseptispora guizhouensis	China	GZCC21-0666	MZ474869	MZ474868	MZ501610	Hyde et al. (2021)
Distoseptispora hyalina	Thailand	MFLUCC17-2128	MZ868760	MZ868769	MZ892976	Yang et al. (2021)
Distoseptispora multiseptata	Thailand	MFLU17-0856	MF077555	MF077544	MF135652	Yang et al. (2018)
Distoseptispora rayongensis	Thailand	MFLUCC18-0415	MH457137	MH457172	MH463253	Hyde et al. (2020)
Distoseptispora rayongensis	Thailand	MFLUCC18-0417	MH457138	MH457173	MH463254	Hyde et al. (2020)
Distoseptispora rostrata	China	MFLUCC16-0969	MG979766	MG979758	MG988424	Luo et al. (2018)
Distoseptispora saprophytica	Thailand	MFLUCC18-1238	MW287780	MW286506	MW396651	Dong et al. (2021)
Distoseptispora verrucosa	China	GZCC20-0434	MZ868762	MZ868771	MZ892977	Yang et al. (2021)
Distoseptispora xishuangbannaensis	China	KUMCC17-0290	MH260293	MH275061	MH412768	Tibpromma et al. (2018)
Distoseptispora yunnanensis	China	MFLUCC20-0153	MW081546	MW081541	MW084995	Li et al. (2021)
Myrmecridium aquaticum	China	MFLUCC15-0366	MK849804	–	–	Luo et al. (2019)
Myrmecridium aquaticum	China	S-1158	MK849803	MK828656	MN194061	Luo et al. (2019)
Myrmecridium banksiae	Australia	CBS132.536	JX069855	JX069871	–	Crous et al. (2012)

Species	Country	Voucher / strain number	Sequence accession number			Reference
			LSU	ITS	EF 1-α	
Myrmecridium schulzeri	Zaire	CBS100.54	EU041826	EU041769	–	Arzanlou et al. (2007)
*Pseudostanjehughesia aquitropica**	Thailand	MFLUCC16-0569	MF077559	MF077548	MF135655	Yang et al. (2018)
*Pseudostanjehughesia lignicola**	China	MFLUCC15-0352	MK849787	MK828643	MN194047	Luo et al. (2019)
Sporidesmium dulongense	China	MFLUCC17-0116	MH795817	MH795812	MH801191	Luo et al. (2019)
Sporidesmium lageniforme	China	DLUCC0880	MK849782	MK828640	MN194044	Luo et al. (2019)
Sporidesmium pyriformatum	Thailand	MFLUCC15-0620	KX710141	KX710146	MF135662	Hyde et al. (2016)
Sporidesmium thailandense	Thailand	MFLUCC15-0617	MF077561	MF077550	MF135657	Yang et al. (2018)
Sporidesmium thailandense	Thailand	MFLUCC15-0964	MF374370	MF374361	MF370957	Yang et al. (2018)

RESULTS

The obtained isolates did not produce any sporulating structures on any of the media used in this study in the dark, but production of conidiophores and conidia on the SNA and 0.1% PDA was induced under BL-B light. Conidiophores had pale brown stalks with polyblastic conidiogenous cells and fusiform conidia with a sheath. Similar characteristics were found in the genus *Aquapteridospora*. Phylogenetic analysis also showed that the fungus belongs to a distinct clade in the genus *Aquapteridospora* (Fig. 1). Their sequence data did not match those of the closest relatives [99.32% homology with *Sordariomycetidae* sp. (MN846256) on ITS, 98.78% with *A. lignicola* (KU221018) on LSU, 96.13% with *A. jiangxiensis* (MZ855767) on EF 1-α, 93.08% with *A. jiangxiensis* (MZ855768) on RPB2]. ITS sequence data of *Sordariomycetidae* sp. was available, but its strain and ecological information were not. As a result, we treat the fungus as an undescribed species and describe it as a new species as follows.

TAXONOMY

Aquapteridospora rhizicola Masuya, Ando, Haruma & Yamaji, **sp. nov.** Fig. 2

MycoBank: MB 860273

Etymology: The name refers to the root from which the fungus was isolated.

Diagnosis: *A. rhizicola* is easily distinguishable by its conidiophores without a well-defined base and conidia with a well-developed sheath from other *Aquapteridospora* having conidia with a sheath, such as *A. aquatica* X.D. Yu, W. Dong & H. Zhang, *A. jiangxiensis* J.E. Huang, H.Y. Song & D.M. Hu, and *A. lignicola* J. Yang & K.D. Hyde.

Type: Japan, Akita Pref., from roots of *Miscanthus sinensis* at a mining site, 12 August 2018, K. Noji (holotype TFM:FPH 11181 as a dried culture; ex-holotype: living culture NBRC 116724). DDBJ: LC867021 (ITS); LC867025 (LSU); LC867023 (EF 1-α); LC867027 (RPB2).

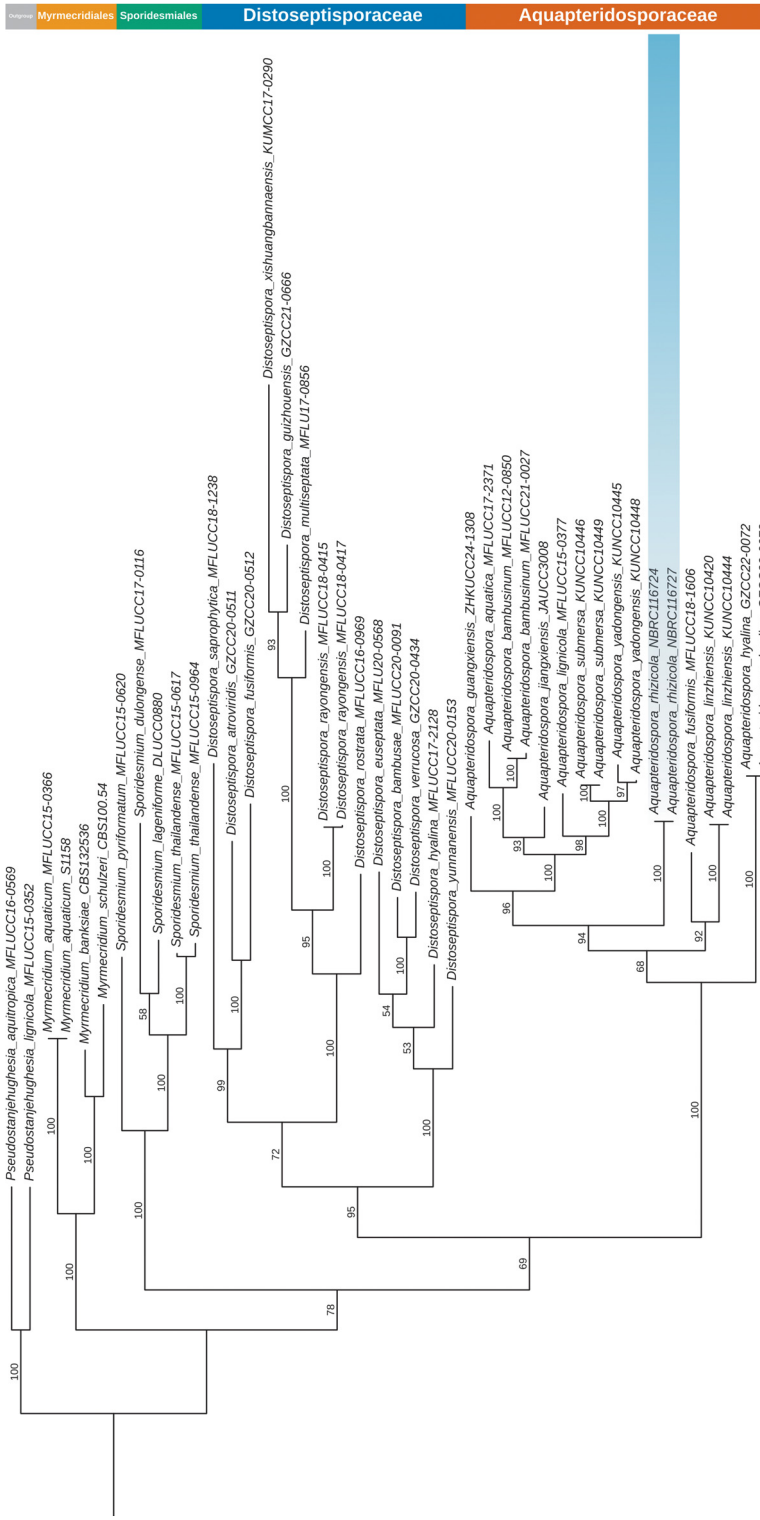

Fig. 1. Maximum Likelihood (ML) tree based on combined LSU, EF1-α, and ITS sequence data. ML bootstrap support values equal to or higher than 50% are shown above the branches. The tree is rooted with *Pseudostanjehughesia aquitropica* (MFLUCC 16-0569) and *P. lignicola* (MFLUCC 15-0352). The new species is indicated in pale blue.

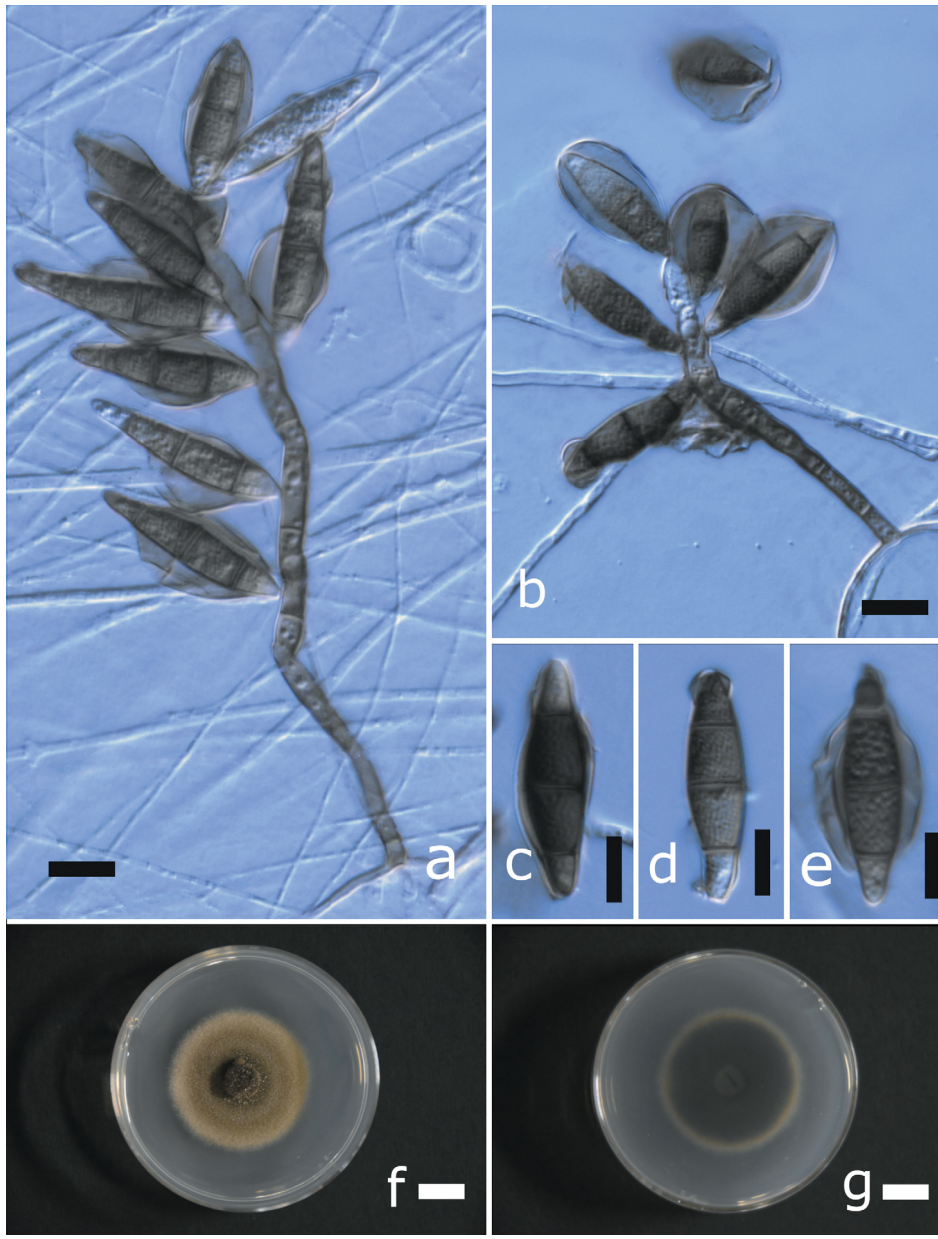

Fig. 2. *Aquapteridospora rhizicola* (TFM:FPH 11181, holotype). **a, b** – conidiophore, conidiogenous cells with conidia; **c–e** – variation of conidia; **f, g** – culture on PDA: f – surface view, g – reverse view. Scale bars: 10 µm (a–e); 1 cm (f, g). Photo: Hayato Masuya.

Description in culture. Sexual morph unknown. Asexual morph hyphomycetous. Mycelium septate, hyaline, partly immersed and partly superficial on the medium. Conidiophores macronematous, mononematous, cylindrical, erect, sometimes flexuous, smooth, 3–10-septate, unbranched, hyaline to pale olive in the middle and below, pale brown above, thick-walled, with small guttulae, not swollen at the base, 25–205 × 4–7 µm (mean = 104.3 × 4.7 µm, n = 50). Conidiogenous cells polyblastic, integrated, terminal or intercalary, pale brown to brown, smooth, subclavate to subcylindrical, with several sympodial proliferations, bearing conspicuous, rounded, pale brown to brown scars. Conidia acrogenous or lateral, fusiform to subclavate, rounded to almost pointed at the apex, base truncate, straight to slightly curved, 3-septate, with medium to dark brown central cells and pale to medium brown end cells, with abundant small smooth-walled guttulae, often with well-developed thick sheath, 0.5–7.5 µm in width, 18.5–33.0 × 4.5–8.5 µm (mean = 27 × 6.5 µm without sheath, n = 50).

Culture characteristics. Colonies grown on PDA circular, flat, reaching 18 mm diam. after 8 days of incubation at 28 °C, light grey at the entire margin, brown in the centre, reverse side grey to pale brown at the entire margin, dark brown in the centre. Conidiophores produced on the surface of SNA and 0.1% PDA media under BL-B light.

Ecology. The species was isolated from roots of living *Pinus densiflora* and *Miscanthus sinensis* without any symptoms in a mining area.

Material examined

Japan. Akita Pref., from roots of *Pinus densiflora* at a mining site, 12 August 2018, T. Haruma (TFM:FPH 11182; living culture NBRC 116727); ibid., from roots of *Miscanthus sinensis* at a mining site, 12 August 2018, K. Noji (TFM:FPH 11181; ex-holotype: living culture NBRC 116724, additional living cultures Aq544, Aq3202).

DISCUSSION

Aquapteridospora rhizicola is distinguished by short conidiophores without a well-defined base, as well as conidia with well-developed sheaths. The conidia with developed sheaths in the genus are also found in three other species, *A. aquatica* (Dong et al. 2021), *A. jiangxiensis* (Peng et al. 2022), and *A. lignicola* (Yang et al. 2015). However, a distinguishing feature of *A. rhizicola* is the short conidiophore without a swollen base, a characteristic that sets it apart from the other *Aquapteridospora* species. The genus *Aquapteridospora* is distinguished as freshwater fungi, and all reported species have been found to inhabit dead plant remains in freshwater environments (Yang et al. 2015, Dai et al. 2017, Luo et al. 2019, Bao et al. 2021, Dong et al. 2021, Ma et al. 2022, Peng et al. 2022, Xu et al. 2024,

Shu et al. 2026). By contrast, *A. rhizicola* is the sole species isolated from living roots growing in soil which is neither temporarily nor permanently waterlogged, i.e. without any connection to an aquatic environment. Phylogenetic analysis further substantiates its distinct classification differing from other *Aquapteridospora* species, supporting its description as a new species.

Aquapteridospora rhizicola was reported as *Aquapteridospora* sp., one of the dark septate endophytes (DSEs) associated with *M. sinensis* and *P. densiflora* in a mining area by Haruma et al. (2023). At this site, the infection rates of arbuscular mycorrhiza (AM) and DSEs in the *M. sinensis* roots were reported to be 1.9% and 39.3%, respectively. However, neither ectomycorrhiza nor AM were observed in the *P. densiflora* seedlings, which are known to be ectomycorrhizal trees. *Aquapteridospora rhizicola* was among the three most frequent species isolated from roots of both plants, the frequency of occurrence of *A. rhizicola* in *M. sinensis* and *P. densiflora* being 7.2% and 1.2%, respectively (Haruma et al. 2023). At this site, DSEs including *A. rhizicola* are thought to be the main symbionts in the roots of *P. densiflora* and *M. sinensis*. Haruma et al. (2023) proposed that these specific DSEs may facilitate adaptation of *P. densiflora* to harsh environments, thereby contributing to vegetation succession. However, the precise mechanisms underlying this symbiotic relationship between the fungus and its plant hosts remain to be fully elucidated.

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