Polyphasic taxonomic study of *Absidia menglianensis* sp. nov. (Cunninghamellaceae, Mucorales) isolated from an avocado plantation in Yunnan, China

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Abstract

During our ongoing studies of microfungi associated with avocado trees in Yunnan Province of China, the samples were collected from Menglian Dai, Lahu, and Va Autonomous County, Pu'er Prefecture. Two new strains were isolated from soil samples in the avocado plantation and roots of avocado trees (*Persea americana*), respectively. Through morphological observation and multigene phylogenetic analyses of concatenated ITS and LSU sequence data, these two new isolates are conspecific and introduced as a new species of *Absidia*, namely *A. menglianensis*. *Absidia menglianensis* is closely related to *A. brunnea* and *A. soli* based on morphology and phylogeny. However, the new species differs significantly from *A. brunnea* and *A. soli* in sizes of sporangia, range of the septum below the apophysis, and maximum growth temperature. They presented sister relationships in the concatenated ITS-LSU phylogeny and can be distinct with high statistical supported values. Detailed descriptions, illustrations, updated phylogeny, as well as determination of maximum growth temperature are provided.

Introduction

Absidia was introduced by van Tieghem[1], with *A. reflexa* as the type species. Preliminarily, *Absidia* was accommodated in Absidiaceae as the type genus[2]. However, with DNA sequence techniques used in species identification, *Absidia* always formed a well-supported monophyletic clade with genera in Cunninghamellaceae (viz., *Chlamydoabsidia, Cunninghamella, Gongronella, Hesseltinella,* and *Halteromyces*)[3–7]. Hence, *Absidia* was hitherto classified in Cunninghamellaceae[8] whereas Absidiaceae was treated as a synonym of the highly polyphyletic family Mucoraceae by Kirk et al.[7]. Although, Hoffmann[9] highly recommended to resurrect the monophyletic Absidiaceae in accordance with Voigt et al.[10].

Since the genus *Absidia* was introduced[1], many different allied genera were subsequently established to accommodate absidia-like species based solely on morphological characteristics[11–16]. Considering the morphological distinctiveness, it may be challenging among the genera *Absidia, Gongronella, Lichtheimia, Mycocladus, Phycomyces, Proabsidia, Protoabsidia, Pseudoabsidia, Rhizopus,* and *Tieghemella,* because these genera are highly similar in morphology[17]. Nevertheless, Hesseltine & Ellis[17] accepted only *Absidia* and *Gongronella* as distinct genera. However, a morpho-molecular approach coupled with physiology significantly segregated these genera

into different families. Among them, only *Absidia* (synonyms: *Proabsidia, Pseudoabsidia,* and *Tieghemella*), and *Gongronella* were classified in Cunninghamellaceae whereas *Lichtheimia* (synonym: *Protoabsidia*) was classified in Lichtheimiaceae, *Mycocladus* in Mycocladaceae, *Phycomyces* in Phycomycetaceae, and *Rhizopus* in Rhizopodaceae[18].

A comprehensive study based on morphology, phylogeny, and physiology provided reliable species delineation of *Absidia*[19]. *Absidia* species were segregated into three groups *viz.* mesophilic species (optimal growth temperature 25–34 °C), mycoparasitic species on mucoralean fungi (optimal growth temperature 14–25 °C) and thermotolerant species (optimal growth temperature 37–45 °C)[19]. Consequently, the new genus *Lentamyces* was established to accommodate the mycoparasitic species[20], whereas the thermotolerant species were transferred to the genus *Lichtheimia*[21]. To date, only the mesophilic species (optimal growth temperature 25–34 °C) were accepted as *Absidia sensu stricto*[9,20,21]. Currently, species identification and classification for the genus *Absidia* are mainly based on morphological characteristics coupled with phylogeny and physiology (e.g., growth temperature)[9,19,20,22–26]. Based on evolutionary relationships, Zhao et al.[25] updated the classification framework of the phylum Mucoromycota and calibrated the taxonomic ranks with estimated divergence times using molecular dating. Among these, the estimated

divergence time of the phylum Mucoromycota was earlier than 617 Mya, the classes and orders earlier than 547 Mya, the families earlier than 199 Mya, and the genera earlier than 12 Mya. According to Zhao et al.[25], the divergence time of *Absidia* was estimated to be 327 Mya for the stem age and 135 Mya for the crown age, based on a time-scaled Bayesian maximum clade credibility phylogenomic tree inferred from ITS and LSU rDNA sequences[25].

Absidia is ubiquitous and distributed in a wide range of ecological niches worldwide, including air, animal dung, food, insect remains, leaf litter, and soil[24]. Notably, soil accounts for more than 50% of *Absidia* species[24]. China has been recognized as a region of high diversity for *Absidia* species, contributing ~20% to the origin of taxonomic types in this genus[24,27]. This genus is typically characterized by sporangiophores with columellae bearing one to several projections, rhizoids forming between the sporangiophores but never opposite them, and the development of pyriform, deliquescent-walled and apophysate sporangia[1,9,19,20,24,25,28–31]. Some species within *Absidia* such as *A. biappendiculata, A. chinensis, A. cinerea,* and *A. jiangxiensis*, produce zygospores within zygosporangia, with their opposite suspensory cells possessing appendages[9,19,25].

The secondary metabolites of *Absidia* have been relatively well-studied, particularly for their industrial implications[25,32–36]. For instance, *A. coerulea* is capable of biotransforming saponins, and regioselectivity to 20(S)-protopanaxatriol. Also, this species has been used in bio-transformation of the (−)-*α*-bisabolol, and in the hydroxylation of dehydroepiandrosterone (DHEA) to 7*α*-hydroxy-5-androstene-17-one (7*α*-OH-DHEA) and 7*β*-hydroxy-5-androstene-17-one (7*β*-OH-DHEA). These processes provide efficient pathways to enhance the yield of saponins, (−)-*α*-Bisabolol and 7*β*-OH-DHEA for the cosmetic and pharmaceutical industries[34–37]. Furthermore, *A. coerulea* and *A. glauca* have been used in the bio-transforming of flavones (chrysin, apigenin, luteolin, and diosmetin), flavanones (pinocembrin, naringenin, eriodictyol, and hesperetin) and 3-Oxo-Oleanolic acid into hydroxylated metabolites. These two species are also efficient producers of chitosan, which is widely used in food processing and antimicrobial products, as well as in the bio-transforming of steroid products[33,34]. *Absidia cylindrospora* has demonstrated the ability to act as a biosorption for cadmium, copper, and lead under controlled experimental conditions[34,38]. Additionally, *A. cylindrospora* and *A. fusca* have been used in bioremediation processes due to their capacity to degrade polycyclic aromatic compounds, such as hydrocarbons[34,39]. Moreover, during the bio-transformation of 20(R)-panaxadiol by *A. corymbifera*, six oxidized and hydroxylated products reported were identified as novel compounds[32]. Other *Absidia* species have also been explored for enzyme engineering and metabolomics in industrial contexts. For instance, *α*-galactosidase from *A. reflexa* has been used to produce rubusoside derivatives, *A. repens* has been utilized to yield chitosan and laccase from *A. spinosa* has been applied for the biotransformation of cresol red[25,40]. Given these findings, *Absidia* is a promising resource for applications in food processing, antimicrobial production, and biotransformation. Besides, new secondary metabolites are frequently discovered during microbial transformation involving *Absidia* species.

During a survey to collect microfungi associated with avocado in Yunnan Province, China, two isolates were obtained, one from the soil in an avocado plantation and another as a root endophyte of an avocado tree. Based on morphological, phylogenetic, and physiological analyses, these isolates were determined to be conspecific and represent a novel species of *Absidia*.

Materials and methods

Collection, fungal isolation, examination, and conservation

The two fungal strains were isolated from soil samples from an avocado plantation and fresh healthy roots of avocado trees, both of which were collected from Menglian County, Yunnan Province, China in September, 2023. Soil samples were collected from 10 cm below ground level, near the shallow roots of avocado trees using a sterilized auger boring tool (model XDB, China, total length 110 cm, drill outer diameter 57 mm, inner diameter 51 mm, single sampling length 20 cm). A portion of the soil (1 g) was mixed with 9 mL of sterile distilled H_2O. Serial dilutions of the mixtures were prepared from 10^{-1} to 10^{-4}. For each dilution, a 100 μL aliquot was plated onto potato dextrose agar (PDA; Qingdao Daily Water Biotechnology Co. Ltd. Shandong, China) with antibiotics (streptomycin sulfate, 100 mg/L, and ampicillin, 100 mg/L). The plates were incubated at 25–30 °C. The PDA plate was examined daily with an Olympus stereo-microscope series MoDELSZ2-ILST. The presence of colonies was transferred to new PDA plates. The isolation and induction of root endophytes from the root tissue of avocado tree were carried out following the guidelines as described in Senanayake et al.[41] and Mattoo & Nonzom[42]. Root samples were cleaned with tap water, and each root was cut into several small pieces (5 mm long). The root pieces were surface sterilized using 2% sodium hypochlorite (NaOCl) for 1 min, followed by three repeated washings with sterilized water. Furthermore, surface sterilization was carried out *via* washing with 75% ethanol for 30 s and three series of washing with sterile distilled water, and finally it was allowed to dry under a laminar flow cabinet. Then, the edges of the root pieces were trimmed, transferred onto plates, and incubated at 25–30 °C for 2–5 d. When individual hyphal tips grew out from root pieces, the mycelia were picked using sterilized needles, placed onto new PDA plates, and incubated at 25–30 °C under 12 h alternating light and dark conditions for one week to one month. After one month, the sporulation was observed. Dry culture materials were deposited in the herbarium of the Cryptogams Kunming Institute of Botany Academia Sinica (KUN-HKAS). The living cultures were conserved at the Kunming Institute of Botany Culture Collection (KUNCC). Index Fungorum number is provided for the newly described taxon.

Microscopic examination and growth temperature

Micromorphological features (sporangia, sporangiophores, sporangiospores, and columellae, etc.) were examined and captured by a Nikon compound microscope (model ECLIPSE Ni-U, Japan) connected with a Nikon DS-Ri2 camera using DIC microscopy. Measurements of these morphological features were made in Tarosoft® Image Frame Work software version 0.9.7. Photographic plates were edited and combined in Adobe Photoshop CS6 software (Adobe Systems Inc., USA). The determination of maximum growth temperature followed the method of Zhao et al.[24]. Four fungal blocks (5 mm × 5 mm)

were selected from a one-week-old colony growing on PDA and placed separately onto four new PDA plates, representing four replicates. These replicates were incubated at 30 °C for 2 d. Subsequently, the temperature was gradually increased in 2 °C increments (32, 34, and 36 °C), with incubation at each temperature for 2 d, until the colonies ceased to grow. When colony growth stopped (at 36 °C), the maximum growth temperature was confirmed by observing the mycelium growth at a reduced temperature (35 °C) at which the colonies also failed to grow.

DNA extraction, PCR amplification, and sequencing

The genomic DNA was extracted from fresh mycelia grown on PDA medium for two weeks using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, Hangzhou, China) following the manufacturer's instructions. Partial DNA sequences of the internal transcribed spacers (ITS1-5.8S-ITS2) and the 28S large subunit rDNA (LSU) were amplified by primers ITS5 and ITS4[43], and LR0R and LR5[44], respectively. The component of the PCR reaction mixture was performed in a 25 μl total volume containing 2 μl of DNA template, 1 μl of each forward and reverse primers (10 μM), 12.5 μl of 2× Power Taq PCR Master Mix (mixture of EasyTaqTM DNA Polymerase, dNTPs, and optimized buffer, Beijing Bio Teke Corporation (Bio Teke), China) and 8.5 μl of ddH$_2$O (double-distilled water). The thermal cycling of PCR amplification for ITS and LSU followed the method of Xu et al.[45] but the annealing temperature was adjusted to 52 °C. PCR products were sent to TsingKe Biological Technology (Beijing) Co., Ltd, China for purification and sequencing. Newly generated consensus sequences were deposited in GenBank under accession numbers provided in Table 1.

Sequence alignment and phylogenetic analyses

The nucleotide BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 10 August 2024) was applied to discover taxa closely related to the new isolates (KUNCC 24-18541 and KUNCC 24-18542). Similarity indices from the BLAST search indicated that KUNCC 24-18541 and KUNCC 24-18542 are conspecific and belong to *Absidia* (Cunninghamellaceae). Therefore, to reveal accurate phylogenetic placements of the new isolates, a concatenated ITS-LSU phylogeny of *Absidia* were analyzed based on maximum-likelihood and Bayesian inference criteria. Sequence datasets of representative taxa in *Absidia* and *Cunninghamella* (Table 1) were retrieved from recent publications[22–26,29–31], downloaded from GenBank (www.ncbi.nlm.nih.gov/nucleotide; accessed on 29 November 2024). Preliminarily individual DNA sequence matrixes were aligned via the online platform, MAFFT v. 7.511[46]. The ambiguous sites of the aligned sequence datasets were further trimmed and improved where necessary using BioEdit v. 6.0.7[47]. The complementation of the missing nucleotide bases at the start and end of the consensus sequence was trimmed. Individual gene datasets were prior analyzed by maximum likelihood to compare the congruence of tree topologies and further concatenated ITS-LSU sequence matrix was analyzed.

Maximum-likelihood (ML) analysis was performed *via* the online portal CIPRES Science Gateway v. 3.3[48], with RAxML-HPC v.8 on XSEDE (8.2.12) tool, using default settings but following the adjustments: the GAMMA nucleotide substitution model and 1000 rapid bootstrap replicates. The evolutionary model of nucleotide substitution for Bayesian inference (BI) analysis was selected independently for each locus using MrModeltest 2.3[49]. GTR + I + G was the best fit for ITS and LSU loci under the Akaike Information Criterion (AIC). BI analysis was performed *via* MrBayes v. 3.2.6[50]. Markov chain Monte Carlo sampling (MCMC) was used to determine posterior probabilities (PP)[51,52]. Six simultaneous Markov chains were run for 1,000,000 generations and trees were sampled every 100th generation. The 0.15 'temperature' value was set in the MCMC heated chain. All sampled topologies beneath the asymptote (20%) were discarded as part of a burn-in procedure and the remaining 8,000 trees were used for calculating posterior probabilities (PP) in the 50% majority rule consensus tree (when the standard deviation of split frequency is lower than 0.01).

The tree topologies generated in this study were visualized on FigTree v. 1.4.0[53]. The phylogram was edited and laid out using Microsoft Office PowerPoint 2016 (Microsoft Inc., Redmond, WA, USA) and converted to a tiff file in Adobe Photoshop CS6 software (Adobe Systems Inc., San Jose, CA, USA). The final alignment and phylogram were submitted to TreeBASE (submission ID: 31814, www.treebase.org, accessed on 10 December 2024).

Results

Phylogenetic analyses

The concatenated ITS-LSU sequence matrix comprised 102 strains of representative species in *Absidia*, with *Cunninghamella blakesleeana* (CBS 782.68) and *C. elegans* (CBS 167.53) as the outgroup taxa. The dataset consisted of 2,175 total characters, including gaps (ITS: 1–1,175 bp, LSU: 1,176–2,175 bp). The best-scoring ML tree was selected to represent the phylogenetic relationships of two new isolates with other representative taxa in *Absidia* (Fig. 1), with the final ML optimization likelihood value of –31,904.047878 (ln). All free model parameters were estimated by the RAxML GAMMA model of rate heterogeneity, with 1,431 distinct alignment patterns. Estimated base frequencies were as follows: A = 0.266550, C = 0.195987, G = 0.247413, T = 0.290050, with substitution rates AC = 1.040788, AG = 2.710746, AT = 1.633236, CG = 0.778485, CT = 5.127044, and GT = 1.000000. The gamma distribution shape parameter alpha = 0.336716 and the tree-length = 11.191212. The final average standard deviation of split frequencies at the end of the total MCMC generations was calculated as 0.009041 in BI analysis.

Tree topologies generated based on ML and BI analyses were similar in the present study and the ML phylogenetic backbone tree is shown in Fig. 1. In the present study, phylogenetic affinities of most *Absidia* species were well-resolved with significant support in ML and BI analyses (up to 70% MLBS and 0.95 BYPP). However, *A. aguabelensis*, *A. alpina*, *A. caatinguensis*, *A. cheongyangensis*, *A. healeyae*, *A. heterospora*, *A. longissimi*, *A. medulla*, *A. koreana*, *A. oblongispora*, *A. pernambucoensis*, *A. tarda*, and *A. variispora* are not phylogenetically well-resolved in the present study (remarkable as black circle on the tree). This lack of resolution may be attributed to limited taxon sampling and ambiguous sites in the ITS alignments. *Absidia caatinguensis* formed a well-resolved branch with significant support in the ML analysis (82% MLBS) but lacked significant support in BI analysis. This species formed an independent branch basal to *A. microsporangia*, *A. radiata*, *A. ampullacea*, *A. yunnanensis,* and *A. alpina*, respectively. *Absidia medulla* and *A. variispora* formed well-supported branches in the BI analysis (0.96 BYPP and 0.98 BYPP, respectively), though both species

Table 1. Taxa names, strain numbers, and GenBank accession numbers of taxa used for the present phylogenetic analyses.

Species names	Strain numbers	GenBank accession numbers	
		ITS	LSU
Absidia abundans	XY09265	ON074697	ON074681
Absidia abundans	**CGMCC 3.16255**	NR_182590	ON074683
Absidia abundans	XY09274	ON074696	ON074682
Absidia aguabelensis	**URM 8213**	NR_189383	NG_241934
Absidia alpina	**CGMCC 3.16104**	OL678133	/
Absidia ampullacea	**CGMCC 3.16054**	NR_191130	NG_242502
Absidia anomala	**CBS 125.68**	NR_103626	NG_058562
Absidia biappendiculata	**CBS 187.64**	NR_190243	MZ350147
Absidia bonitoensis	**URM 7889**	MN977786	MN977805
Absidia brunnea	**CGMCC 3.16055**	NR_191131	NG_242503
Absidia caatinguensis	**URM 7156**	NR_154704	NG_058582
Absidia caerulea	CBS 101.36	MH855718	MH867230
Absidia caerulea	XY00608	OL620081	/
Absidia californica	**CBS 126.68**	NR_077169	NG_056998
Absidia californica	FSU4748	AY944873	EU736301
Absidia cheongyangensis	**CNUFC CY2203**	PP844904	PP852788
Absidia chinensis	CGMCC 3.16057	MZ354141	MZ350135
Absidia chinensis	**CGMCC 3.16056**	NR_191132	NG_242504
Absidia cinerea	**CGMCC 3.16062**	NR_191134	NG_242506
Absidia coerulea	FSU767	AY944870	/
Absidia coerulea	XY00729	OL620082	/
Absidia cornuta	**URM 6100**	NR_172976	MN625255
Absidia crystalloides	**CGMCC3.27496**	PP377803	PP373736
Absidia crystalloides	SAUCC693201	PP377804	PP373737
Absidia cuneospora	**CBS 101.59**	MH857828	NG_058559
Absidia cylindrospora	**CBS 153.63**	**/**	NG_058563
Absidia cylindrospora	XY00313	ON123744	ON123765
Absidia cylindrospora var. *cylindrospora*	CBS 100.08	JN205822	JN206588
Absidia digitula	**CGMCC 3.16058**	MZ354142	MZ350136
Absidia edaphica	**MFLU 20-0416**	NR_172305	NG_075367
Absidia fluvii	**CNUFC CY2240**	PP844891	PP852703
Absidia frigida	**CGMCC 3.16201**	NR_182565	NG_149032
Absidia fusca	**CBS 102.35**	NR_103625	NG_058552
Absidia gemella	**CGMCC 3.16202**	OM108488	NG_149033
Absidia glauca	CBS 129233	MH865253	MH876693
Absidia glauca	**CBS 101.08**	NR_111658	NG_058550
Absidia glauca	FSU660	AY944879	EU736302
Absidia globospora	**CGMCC 3.16031**	NR_189829	NG_241930
Absidia globospora	CGMCC 3.16035	MW671538	MW671545
Absidia globospora	CGMCC 3.16036	MW671539	MW671546
Absidia healeyae	**UoMAU1**	MT436028	MT436027
Absidia heterospora	SHTH021	JN942683	JN982936
Absidia jiangxiensis	**CGMCC 3.16105**	OL678134	/
Absidia jiangxiensis [as *jindoensis*]	XY000810	ON123748	ON123770
Absidia jindoensis	**CNUFC-PTI1-1**	MF926622	MF926616
Absidia koreana	**EML-IFS45-1**	KR030062	KR030056
Absidia kunryangriensis	**CNUFC CY2230**	PP844905	PP956882
Absidia lobata	**CGMCC 3.16256**	ON074690	ON074679
Absidia longissima	**CGMCC 3.16203**	NR_182566	OM030225
Absidia macrospora	FSU4746	AY944882	EU736303
Absidia medulla	**CGMCC 3.16034**	NR_189832	NG_241932
Absidia menglianensis	KUNCC 24-18541	PQ594927	PQ594929
Absidia menglianensis	**KUNCC 24-18542**	PQ594928	PQ594930
Absidia microsporangia	KUNF-22-121A	OQ868364	OQ868366
Absidia microsporangia	KUNF-22-126A	OQ868365	OQ868367
Absidia microsporangia	**KUNF-22-316**	PP264375	PP264469
Absidia montepascoalis	**URM 8218**	MW473494	MW561560
Absidia multispora	**URM 8210**	MN953780	MN953782
Absidia nigra	CGMCC 3.16060	MZ354144	MZ350138
Absidia nigra	CGMCC 3.16059	MZ354143	MZ350137
Absidia nigra	**CBS 127.68**	NR_173068	NG_058560

(to be continued)

Table 1. (continued)

Species names	Strain numbers	GenBank accession numbers	
		ITS	LSU
Absidia oblongispora	**CGMCC 3.16061**	NR_191133	NG_242505
Absidia ovalispora	**CGMCC 3.16019**	NR_176748	MW264131
Absidia pacifica	**CGMCC3.27497**	PP377802	PP373735
Absidia pacifica	SAUCC413601	PP377801	PP373734
Absidia panacisoli	**SYPF 7183**	NR_159563	NG_063948
Absidia paracylindrospora	**CNUFC L2207**	PP844907	PP956883
Absidia pararepens	**CCF 6352**	MT193669	NG_242483
Absidia pateriformis	**CGMCC3.27495**	PP377805	PP373738
Absidia pateriformis	SAUCC634702	PP377806	PP373739
Absidia pernambucoensis	**URM 7219**	MN635568	MN635569
Absidia pseudocylindrospora	**CBS 100.62**	NR_145276	NG_058561
Absidia psychrophilia	FSU4745	AY944874	EU736306
Absidia psychrophilia	**CBS 128.68**	NR_154667	NG_058544
Absidia purpurea	**CGMCC 3.16106**	NR_191153	/
Absidia radiata	**CGMCC 3.16257**	ON074698	ON074684
Absidia radiata	XY09330	ON074699	ON074685
Absidia repens	**CBS 115583**	NR_103624	NG_058551
Absidia saloaensis	**URM 8209**	MN953781	MN953783
Absidia sichuanensis	**CGMCC 3.16258**	NR_182589	ON074688
Absidia soli	**MFLU 20-0414**	NR_172306	NG_075368
Absidia sp. [as *pseudocylindrospora*]	EML-FSDY6-2	KU923817	KU923814
Absidia spinosa	FSU551	AY944887	EU736307
Absidia stercoraria	**EML-DG8-1**	KU168828	KT921998
Absidia sympodialis	CGMCC 3.16063	MZ354147	MZ350141
Absidia sympodialis	**CGMCC 3.16064**	NR_191135	NG_242507
Absidia tarda	**URM 8412**	PP844911	PP956884
Absidia terrestris	**FMR 14989**	LT795003	LT795005
Absidia thailandica	**MFLUCC 23-0073**	OR606547	OR606546
Absidia turgida	**CGMCC 3.16032**	NR_189830	NG_241931
Absidia varians	**CGMCC 3.16065**	NR_191136	NG_242508
Absidia variiprojecta	**URM 8620**	PP844913	PP956885
Absidia variispora	**URM 8720**	PP844915	PP956886
Absidia virescens	CGMCC 3.16066	MZ354150	MZ350144
Absidia virescens	**CGMCC 3.16067**	NR_191137	NG_242509
Absidia xinjiangensis	**CGMCC 3.16107**	OL678136	/
Absidia yunnanensis	XY09528	ON074701	ON074686
Absidia yunnanensis	**CGMCC 3.16259**	NR_182591	NG_149054
Absidia zonata	**CGMCC 3.16033**	NR_189831	NG_242490
Absidia zygospora	**MFLUCC 23-0061**	OR104965	OR104992
Cunninghamella blakesleeana	CBS 782.68	JN205869	MH870950
Cunninghamella elegans	CBS 167.53	MH857146	HM849700

The ex-type strains are in bold and newly sequences in the present study are indicated in blue. Abbreviations: CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; CCF: Culture Collection of Fungi, Charles University in Prague; CGMCC: China General Microbiological Culture Collection Center, Beijing, China; CNUFC: Chonnam National University Fungarium, Gwangju, South Korea; FMR: Facultad de Medicina, Reus, Tarragona, Spain; KUNCC: Kunming Institute of Botany Culture Collection, Kunming, China; MFLU: Herbarium of Mae Fah Luang University, Chiang Rai, Thailand; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; NRRL: USDA-Agricultural Research Service Culture Collection, US; SAUCC: Shandong Agricultural University Culture Collection (SAUCC), China; URM: University Recife Mycology Culture Collection of the Universidade Federal de Pernambuco, Brazil.

showed weak support in the ML analysis. *Absidia medulla* formed an independent branch positioned as a sister to *A. nigra*. Conversely, *A. variispora* was basal to a subclade comprising *A. tarda*, *A. cheongyangensis*, *A. crystalloides*, *A. oblongispora*, *A. heterospora*, *A. paracylindrospora*, *A. cylindrospora* var. *cylindrospora,* and *A. terrestris*. *Absidia tarda* was identified as a sister to *A. cheongyangensis*, while *A. oblongispora* was sister to *A. heterospora*. These four species formed a weakly supported monophyletic subclade with *A. crystalloides*. *Absidia aguabelensis* formed a separated, weakly supported branch with *A. koreana* and clustered with *A. longissimi*, *A. zonata,* and *A. kunryangriensis*, supported by significant values (97% MLBS and 1.00

BYPP). *Absidia healeyae* was closely related to *A. gemella* and *A. xinjiangensis*, forming a basal branch clustered with these two species. *Absidia pernambucoensis* clustered as basal to a group comprising *Absidia* sp. [as *pseudocylindrospora*] strain EML-FSDY6-2, *A. stercoraria*, *A. cinerea*, *A. biappendiculata*, *A. pseudocylindrospora*, *A. cornuta*, and *A. spinosa*. The two new isolates (strains KUNCC 24-18541 and KUNCC 24-18542) formed a robust subclade (100% MLBS and 1.00 BYPP), and is sister to *A. brunnea* (82% MLBS and 0.99 BYPP), and *A. soli* (100% MLBS and 1.00 BYPP) within *Absidia*. Hence, *Absidia menglianensis* (strains KUNCC 24-18541 and KUNCC 24-18542) is introduced as a novel species.

Taxonomy

Absidia menglianensis H.B. Jiang, C.Y. Luo & Phookamsak, sp. nov.

Index Fungorum number: IF 902073; Fig. 2.

Etymology: The specific epithet 'menglianensis' refers to the location, Menglian, Yunnan Province of China, where the new species was collected.

Holotype: KUN-HKAS 144524.

Endophytic on roots of avocado trees (*Persea americana*) and living in the soil of an avocado plantation. *Hyphae* hyaline at first, becoming brown when mature, 5–14.5 μm wide. *Stolons* branched, hyaline or light brown, smooth, with few septa near the base of sporangiophores, 3–8.5 μm wide. *Rhizoids* brown to dark brown, rarely branched. *Sporangiophores* 110–185 × 2.5–4.5 μm ($\bar{x}$ = 150 × 3.6 μm, n = 20), arising from stolons, or substrate hyphae, monopodial, erect, mostly unbranched, 2–4 in whorls, initially hyaline, brown to dark brown at maturity, with a septum 16–21.5 μm below apophyses, sometimes a swelling flask-shaped structure beneath sporangia. *Sporangia* 13–25 × 10–22 μm ($\bar{x}$ = 19 × 16 μm, n = 20), brown, globose to pyriform, multi-spored, smooth, deliquescent-walled. *Apophyses* distinct, hyaline or subhyaline, 3–5.5 μm high, gradually widened upwards, 3–6.5 μm wide at the base, and 8–13.5 μm wide at the top. *Columellae* 10–15 × 8–13 μm ($\bar{x}$ = 12 × 10.5 μm, n = 20), hyaline, hemispherical to pyriform, smooth, bearing a collarette and a 2–3 μm long projection at the apex. *Sporangiospores* 3–3.6 × 2–2.5 μm ($\bar{x}$ = 3.3 × 2.3 μm, n = 20), hyaline, subglobose to ovoid, smooth-walled. *Chlamydospores* absent.

Maximum growth temperature: 36 °C.

Culture characteristics: *Colony* grows fast on PDA, reaching 30 mm in 3 d at room temperature (25–30 °C) under normal
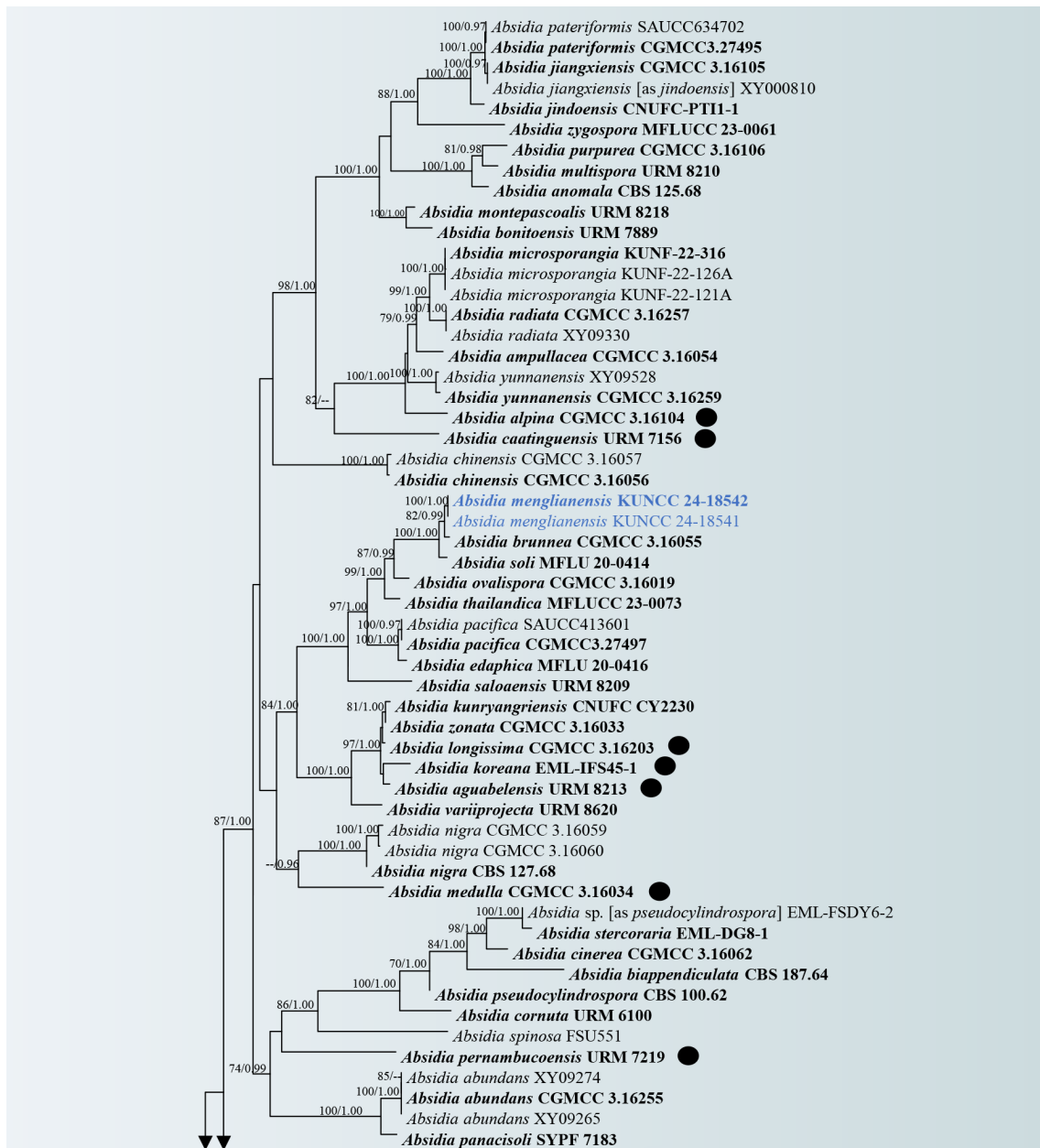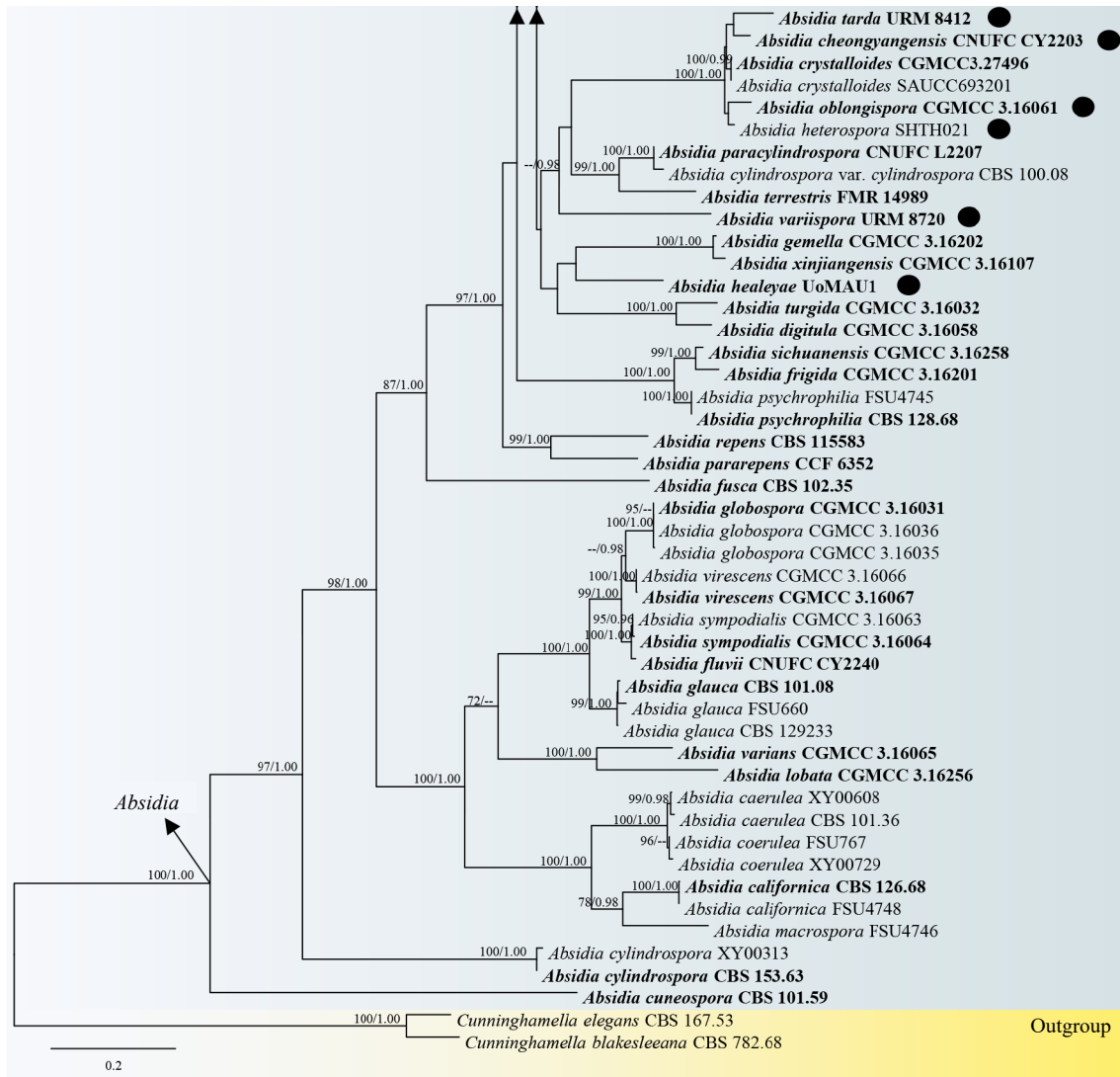

Fig. 1 (to be continued)

Fig. 1 Maximum likelihood phylogenetic tree generated by RAxML based on a concatenated ITS and LSU sequence data. The tree is rooted to *Cunninghamella blakesleeana* (CBS 782.68) and *C. elegans* (CBS 167.53). Maximum likelihood bootstrap values (MLBS) greater than 70% and Bayesian posterior probabilities (BYPP) greater than 0.95 are noted above the nodes as MLBS/BYPP. Ex-type strains are in bold and newly generated sequences are in blue.

light conditions, colonies cottony to floccose, irregular, raised, pale brown to brown in the center, white at the margin. *Mycelium* superficial or immersed in agar medium, with brown to dark brown, branched, septate, smooth hyphae. The fungus can grow at room temperature, 32, 34, and 35 °C and cannot be grown at 36 °C (Fig. 3).

Material examined: China, Yunnan Province, Pu'er Prefecture, Menglian Dai, Lahu, and Va Autonomous County, Mangxin Town, isolated from soil in the avocado plantation (22°10′34″ N, 99°33′42″ E, altitude 1,078.6 m), 15 September 2023, H.B. Jiang, dried herbarium culture, MLAS011 (KUN-HKAS 144524, holotype), ex-holotype living culture, KUNCC 24-18542.

Other materials: China, Yunnan Province, Pu'er Prefecture, Menglian Dai, Lahu, and Va Autonomous County, Mangxin Town, associated with the root of avocado trees (22°10′34″ N, 99°33′42″ E, altitude 1,078.6 m), 15 September 2023, H.B. Jiang, root 2-17 (KUN-HKAS 144523); living culture, KUNCC 24-18541.

Notes: Based on NCBI nucleotide blast search of ITS region, *Absidia menglianensis* (strains KUNCC 24-18541 and KUNCC

24-18542) showed the closest similarity with *A. brunnea* strain CGMCC 3.16055 with 98.17% similarity (Identities = 536/546 bp, with two gaps), and *A. soli* strain MFLU 20-0413 (96.45% similarity, Identities = 380/394 bp, with five gaps) and the type strain of *A. soli* (MFLUCC 20-0089) with 95.50% similarity (Identities = 467/489 bp, with six gaps). *Absidia menglianensis* has a close relationship with *A. brunnea* and *A. soli* based on morphology and phylogeny. These three species are morphological resemblance in forming monopodial, erect sporangiophores with a septum below apophyses, brown, globose sporangia at the maturity, sometimes with a swelling beneath sporangia, funnel-shaped apophyses, hyaline columellae bearing collarette and apical projection, and hyaline sporangiospores (this study)[25,54]. However, *A. menglianensis* can be distinguished from *A. brunnea* and *A. soli* in size of sporangia, range of the septum below the apophyses, sporangiospore shape, and maximum growth temperature. *Absidia brunnea, A. menglianensis,* and *A. soli* presented sister relationships in the concatenated ITS-LSU phylogeny and can be distinct with high
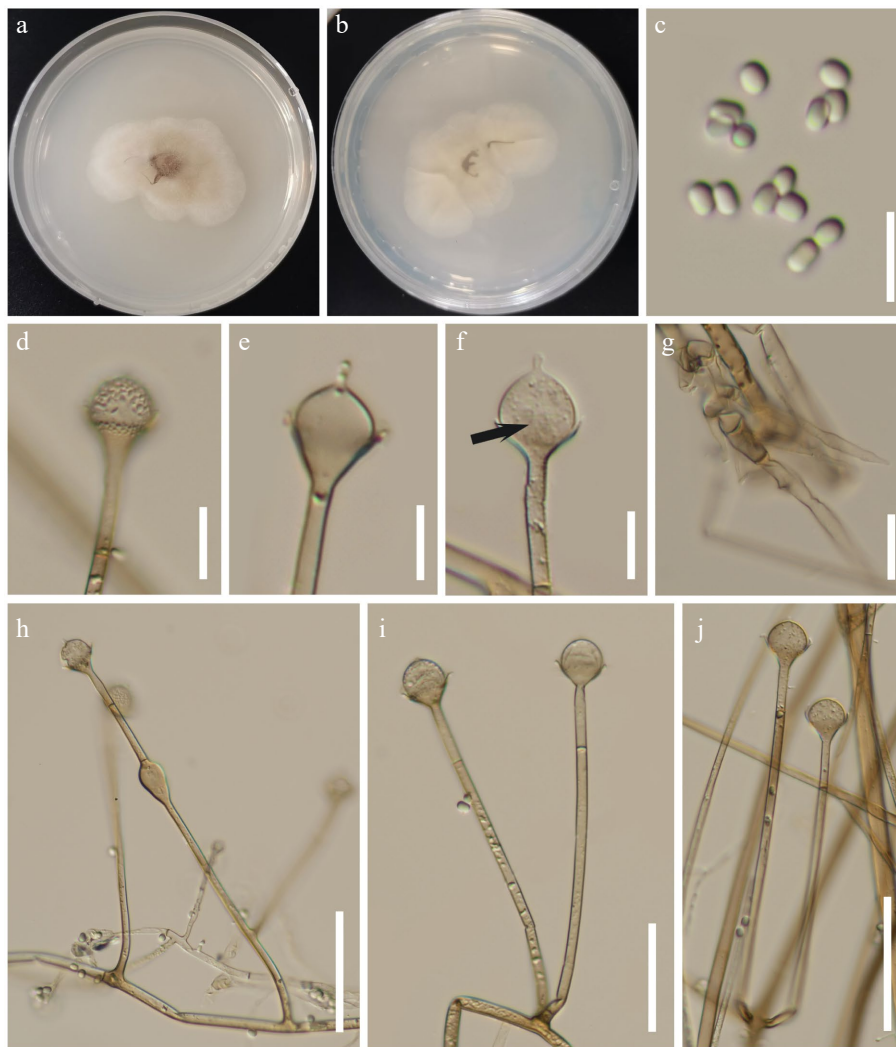
Fig. 2 *Absidia menglianensis* (KUN-HKAS 144524, holotype). (a), (b) Culture from above and reverse; (c) Sporangiospores; (d) Sporangium; (e), (f) Sporangia with columellae (arrow); (g) Rhizoids; (h) Swelling on sporangiophores; (i), (j) Sporangiophores. Scale bars: (c), (f) = 10 μm, (d) = 15 μm, (e) = 7 μm, (g) = 20 μm, (h), (j) = 50 μm, (i) = 30 μm.

statistically supported values (Fig. 1). Compared to the size of sporangia, *A. brunnea* (17.5–38 × 19–34.5 μm)[25] and *A. soli* (16–51 × 15–45.5 μm)[54] have larger sporangia than *A. menglianensis* (13–25 × 10–22 μm). The septum below the apophysis presents different measurement ranges among *A. brunnea* (11–17 μm)[25], *A. menglianensis* (16–21.5 μm), and *A. soli* (21.5–37.5 μm)[54]. Besides, *A. menglianensis* and *A. soli* formed only cylindrical to ovoid sporangiospores[54], while *A. brunnea* formed ovoid sporangiospores with vacuoles[25]. Moreover, from physiology, the maximum growth temperature of *A. menglianensis* (36 °C on PDA) differs from *A. brunnea* (35 °C on MEA)[25] and *A. soli* (37 °C on YMA and PDA)[54]. Although they are slight differences in maximum growth temperature (1–2 °C), the limitation of maximum growth temperature range is a significant feature in delineating species in this fungal group. Furthermore, the nucleotide pairwise comparison of the ITS region demonstrated that *A. menglianensis* (strains KUNCC 24-18541 and KUNCC 24-18542) differs from *A. brunnea* (CGMCC 3.16055, ex-type strain) in 9/523 bp (1.72%) and differs from *A. soli* (MFLUCC 20-0089, ex-type strain) in 22/489 bp (4.5%). Hence, the species is justified as a new species.

Discussion

Avocado (*Persea americana*) is one of the important economic crops in the Yunnan Province of China. However, there is relatively little knowledge about fungi associated with this plant as well as soil fungi in its plantation. Therefore, the present study attempted to explore the fungal diversity on avocado as well as soil fungi in the land-use for avocado plantations. In the present study, the new species *Absidia menglianensis* was found as a root endophyte as well as a soil fungus in an avocado plantation, which confirmed that *Absidia* is ubiquitous in a wide range of hosts and habitats in various ecological niches. In accordance with soil fungi that are usually recognized as saprobes in nature; however, many soil fungi are also known as soil borne pathogens, taking part in regulating soil health and managing interactions between soil and economic crops[54–56]. Interestingly, *A. menglianensis* can act either as a root endophyte or a soil fungus. This demonstrates the possibility of horizontal propagation of *A. menglianensis*. Unfortunately, a neighboring avocado plantation is facing root rot disease, where the causal agent was identified as *Phytophthora*
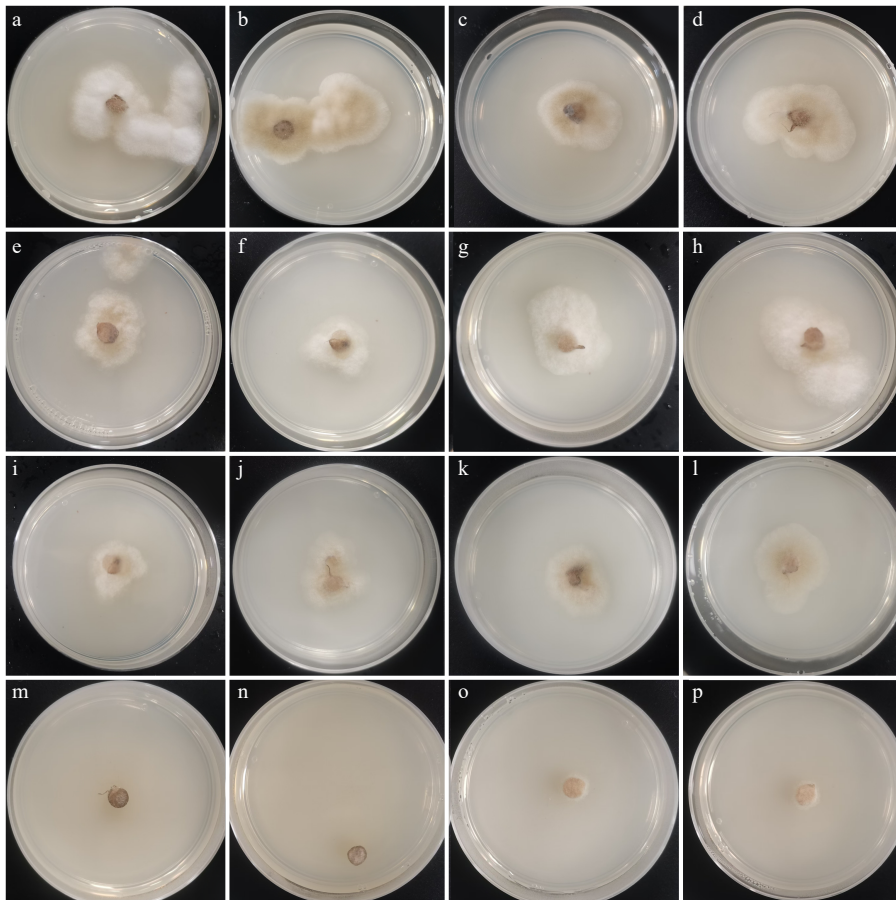
Fig. 3 Mycelial growth of *Absidia menglianensis* in PDA at different temperatures (32, 34, 35, 36 ℃) after 3 d. (a)–(d) Colonies at 32 ℃; (e)–(h) Colonies at 34 ℃; (i)–(l) Colonies at 35 ℃; (m)–(p) Colonies at 36 ℃.

sp. Whether *A. menglianensis* as a basal fungus joins the pathogenic process of root rot disease on avocado or as an endophyte helps the resistance to *Phytophthora* sp., the correlation between the two basal fungi and how they influence the pathogenic process of root rot disease in avocado plantations requires further study.

Studies on fungi associated with avocado plants are underrepresented in China, with only 15 fungal species reported from avocados in this region[27]. In the present study, a new species *Absidia menglianensis* is introduced, thereby enriching the number of fungi associated with the avocado. This is also the first report of an *Absidia* species associated with the avocado in China, with implications for expanding this knowledge globally. This novel discovery contributes significantly to fungal research on economic plants, such as the avocado, by providing insights into the diversity and polyphasic taxonomy of fungi associated with this host plant. Furthermore, it offers potential strategies for crop management aimed at reducing avocado diseases caused by fungi. It is likely that more fungal species will be discovered in avocado plantations in China as further sampling and investigations are conducted.

The present study enriches the diversity of *Absidia* species that improves an accurate species number of the genus within the country and globally. Currently, about 33 *Absidia* species have been reported in China including the novel species introduced herein[25,57]. Consequently, China could be considered as a hotspot for species richness of *Absidia*, which currently

accounts for more than 1/3 of the species number of total *Absidia* globally[24,25,29–31]. In a large-scale geography of China, species of *Absidia* were mostly studied in western China, particularly Yunnan Province, which has been reported to contain 14 species (including *A. menglianensis*)[57].

Through the phylogenetic analyses in the present study, some strains of *Absidia* species that are available in GenBank, were misidentified. For instance, the unpublished strain XY000810 was identified as *A. jindoensis*, of which sequence data are currently only known on ITS and LSU in the GenBank database without other relevant information such as collection site, host information, morphological illustration, etc. However, the nucleotide blast search of the ITS region indicated that the strain is identical to *A. jiangxiensis* (CGMCC 3.16105), occurring with the phylogenetic results that strain XY000810 shared the same branch length with *A. jiangxiensis* (CGMCC 3.16105). Therefore, the strain XY000810 was identified as *A. jiangxiensis* herein temporarily based on the present phylogeny. Furthermore, *A. pseudocylindrospora* strain EML-FSDY6-2 formed a robust subclade (100% MLBS and 1.00 BYPP; Fig. 1) with *A. stercoraria* (EML-DG8-1) and was distant from type strain of *A. pseudocylindrospora* (CBS 100.62) (Fig. 1), of which the results are similar to those of Zhao et al.[25]. The nucleotide blast search of the ITS region indicated *A. pseudocylindrospora* strain EML-FSDY6-2 is closest in similarity to *A. stercoraria* (EML-DG8-1) with 98.97% similarity. Besides, a comparison between *A. pseudocylindrospora* strain EML-FSDY6-2 and ex-type strain CBS

100.62 demonstrated their morphological resemblances. However, they are different in size of columellae (9.5–19.5 µm vs 9–26 µm[58]). Hence, the *A. pseudocylindrospora* strain EML-FSDY6-2 was treated as *Absidia* sp. in the present study and the conspecific status of *Absidia* sp. strain EML-FSDY6-2 and *A. stercoraria* (EML-DG8-1) require further clarification and study. These findings should be taken into account for further resolving taxonomic ambiguities occurring in the genus *Absidia*, providing a better taxonomic resolution in the genus and extending to the higher taxonomic rank.

In the current state of species number in *Absidia*, there is confusion on the taxonomic classification and a variable of species number in different fungal repositories. Index Fungorum[57] lists 88 species of *Absidia*, nine varieties, and one form under *A. ramosa* var. *ramosa*, whereas Species Fungorum[59] accepted only 78 species of *Absidia* without any varieties or forms. In contrast, there are 83 species of *Absidia* and seven varieties accommodated in MycoBank[60]. Therefore, the accurate species number of *Absidia* requires further refinement and a solution to this problem needs to be found. Otherwise, there will be continuous confusion on the extant species in this fungal group for subsequent research. Among these, 73 *Absidia* species were clarified based on their molecular phylogeny.

Author contributions

The authors confirm contribution to the paper as follows: conceptualization, data curation, and formal analysis: Luo C, Chen F, Jiang H, Phookamsak R; funding acquisition: Xu J, Jiang H; investigation, methodology, and writing – original draft: Luo C, Chen F, Jiang H; project administration: Jiang H, Phookamsak R; supervision: Xu J, Jiang H, Phookamsak R; writing – review and editing: Jiang H, Sun F, Phookamsak R. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The data generated and analyzed during this study are available in this article. DNA sequence data are available in the GenBank database, and the accession numbers are provided in Table 1. Specimens and living cultures of new species have been deposited in a herbarium. The final alignment and phylogram were deposited in TreeBASE (submission ID: 31814). The introduced novel species, *Absidia menglianensis*, has been registered in Index Fungorum (Index Fungorum number: IF 902073).

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. van Tieghem P. 1878. Troisième mémoire sur les Mucorinées. *Annales des Sciences Naturelles Botanique* 4:313−99

2. von Arx JA. 1982. On Mucoraceae s. str. and other families of the Mucorales. *Sydowia* 35:10−26

3. Voigt K, Cigelnik E, O'donnell K. 1999. Phylogeny and PCR identification of clinically important Zygomycetes based on nuclear ribosomal-DNA sequence data. *Journal of Clinical Microbiology* 37:3957−64

4. Voigt K, Wöstemeyer J. 2001. Phylogeny and origin of 82 zygomycetes from all 54 genera of the Mucorales and Mortierellales based on combined analysis of actin and translation elongation factor EF-1α genes. *Gene* 270:113−20

5. O'donnell K, Lutzoni FM, Ward TJ, Benny GL. 2001. Evolutionary relationships among mucoralean fungi (Zygomycota): Evidence for family polyphyly on a large scale. *Mycologia* 93:286−297

6. Cannon PF, Kirk PM. 2007. *Fungal Families of the World*. Wallingford, UK: CABI. doi: 10.1079/9780851998275.0000

7. Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008. *Dictionary of the Fungi*. 10th Edition. Wallingford, UK: CABI. doi: 10.1079/9780851998268.0000

8. Voigt K. 2012. Chytridiomycota. In: *Syllabus of plant families–A. Engler's Syllabus der Pflanzenfamilien. Part 1/1: Bluegreen algae, Myxomycetes and Myxomycete-like organisms, Phytoparasitic protists, Heterotrophic Heterokontobionta and Fungi*, ed. Frey W. Stuttgart: Borntraeger Verlag. pp. 106−29

9. Hoffmann K. 2010. Identification of the genus *Absidia* (Mucorales, Zygomycetes): a comprehensive taxonomic revision. In *Molecular identification of fungi*, eds. Gherbawy Y, Voigt K. 1st Edition. Berlin: Springer. pp 439−60. doi: 10.1007/978-3-642-05042-8_19

10. Voigt K, Hoffmann K, Einax E, Eckart M, Papp T, et al. 2009. Revision of the family structure of the Mucorales (Mucoromycotina, Zygomycetes) based on multigene genealogies: phylogenetic analyses suggest a bigeneric Phycomycetaceae with *Spinellus* as sister group to *Phycomyces*. In *Current advances in molecular mycology*, eds. Gherbawy Y, Mach RL, Rai M. New York, USA: Nova Science Publishers, Inc. pp. 313−32

11. Berlese AN, De Toni JB. 1888. Phycomyceteae. In *In Sylloge Fungorum series 7*, ed. Saccardo PA. pp. 181−322

12. Beauverie J. 1900. *Mycocladus verticillatus* (gen. nov. sp. nov.). *Annales de l'Université de Lyon* 3:162−80

13. Vuillemin P. 1903. Importance taxonomique de l'appareil zygosporé des Mucorinées. *Bulletin de la Société Mycologique de France* 19:106−18

14. Bainier M. 1903. Sur quelques espèces de Mucorinées nouvelles ou peu connues. *Bulletin de la Société Mycologique de France* 19(2):153−72

15. Naumov NA. 1935. *Opredelitel Mukorovykh (Mucorales)*. Akademia Nauk SSSR. 136 pp.

16. Ribaldi MS. 1952. Sopra un interessante Zigomicete terricolo: *Gongronella urceolifera* n. gen. et n. sp. *Rivista di Biologia* 49(1):157−66

17. Hesseltine CW, Ellis JJ. 1964. The genus *Absidia*: *Gongronella* and cylindrical-spored species of *Absidia*. *Mycologia* 56:568−601

18. Wijayawardene NN, Hyde KD, Dai DQ, Sánchez-García M, Goto BT, et al. 2022. Outline of Fungi and fungus-like taxa – 2021. *Mycosphere* 13:53−453

19. Hoffmann K, Discher S, Voigt K. 2007. Revision of the genus *Absidia* (Mucorales, Zygomycetes) based on physiological, phylogenetic, and morphological characters; thermotolerant *Absidia* spp. form a coherent group, Mycocladiaceae fam. nov. *Mycological Research* 111:1169−83

20. Hoffmann K, Voigt K. 2009. *Absidia parricida* plays a dominant role in biotrophic fusion parasitism among mucoralean fungi (Zygomycetes): *Lentamyces*, a new genus for *A. parricida* and *A. zychae*. *Plant Biology* 11:537−54

21. Hoffmann K, Telle S, Walther G, Eckart M, Kirchmair M, et al. 2009. Diversity, genotypic identification, ultrastructural and phylogenetic characterization of zygomycetes from different ecological habitats and climatic regions: limitations and utility of nuclear ribosomal DNA barcode markers. In *Current Advances in Molecular Mycology*, eds. Gherbawy Y, Mach R, Rai M. New York, USA: Nova Science Publishers. pp 263–312.

22. Leitão JDA, Cordeiro TRL, Nguyen TTT, Lee HB, Gurgel LMS, et al. 2021. *Absidia aguabelensis* sp. nov.: a new mucoralean fungi isolated from a semiarid region in Brazil. *Phytotaxa* 516:83−91

23. Lima CLF, Lima DX, Cordeiro TRL, Lee HB, Nguyen TTT, et al. 2021. *Absidia bonitoensis* (Mucorales, Mucoromycota), a new species isolated from the soil of an upland Atlantic forest in Northeastern Brazil. *Nova Hedwigia* 112:241−251

24. Zhao H, Nie Y, Zong TK, Wang YJ, Wang M, et al. 2022. Species Diversity and ecological habitat of *Absidia* (Cunninghamellaceae, Mucorales) with emphasis on five new species from forest and grassland soil in China. *Journal of Fungi* 8:471

25. Zhao H, Nie Y, Zong TK, Wang K, Lv ML, et al. 2023. Species diversity, updated classification and divergence times of the phylum Mucoromycota. *Fungal Diversity* 123:49−157

26. Hurdeal VG, Jones EBG, Gentekaki E. 2023. *Absidia zygospora* (Mucoromycetes), a new species from Nan Province, Thailand. *Studies in Fungi* 8:15

27. Farr DF, Rossman AY. 2024. *Fungal Databases*. U.S. National Fungus Collections, ARS, USDA. https://fungi.ars.usda.gov/ (Accessed 17 October 2024)

28. Benny GL, Humber RA, Morton JB. 2001. Zygomycota: Zygomycetes. In *Systematics and evolution. The Mycota (a comprehensive treatise on fungi as experimental systems for basic and applied research)*, eds. McLaughlin DJ, McLaughlin EG, Lemke PA. Berlin/Heidelberg, Germany: Springer. Volume 7A. pp. 113–46. doi: 10.1007/978-3-662-10376-0_6

29. Zong TK, Zhao H, Liu XL, Ren LY, Zhao CL, et al. 2021a. Taxonomy and phylogeny of the *Absidia* (Cunninghamellaceae, Mucorales): Introducing nine new species and two new combinations from China; Research Square: Durham, NC, USA. doi: 10.21203/rs.3.rs-820672/v1

30. Zong TK, Zhao H, Liu XL, Ren LY, Zhao CL, et al. 2021b. Taxonomy and phylogeny of four new species in *Absidia* (Cunninghamellaceae, Mucorales) from China. *Frontiers in Microbiology* 12:2181

31. Zhao H, Zhu J, Zong TK, Liu XL, Ren LY, et al. 2021. Two new species in the family Cunninghamellaceae from China. *Mycobiology* 49:142−50

32. Chen G, Ge H, Li J, Li J, Zhai X, et al. 2016. Microbial transformation of 20(R)-panaxadiol by *Absidia corymbifera* AS 3.3387. *Journal of Molecular Catalysis B: Enzymatic* 123:154−59

33. Sordon S, Popłoński J, Tronina T, Huszcza E. 2019. Regioselective *O*-glycosylation of flavonoids by fungi *Beauveria bassiana*, *Absidia coerulea* and *Absidia glauca*. *Bioorganic Chemistry* 93:102750

34. Cordeiro TRL, Nguyen TTT, Lima DX, da Silva SBG, de Lima CF, et al. 2020. Two new species of the industrially relevant genus *Absidia* (Mucorales) from soil of the Brazilian Atlantic Forest. *Acta Botanica Brasilica* 34:549−58

35. Park J, Han F, Lee IS. 2022. Biotransformation of (−)-α-Bisabolol by *Absidia coerulea*. *Molecules* 27:881

36. Song M, Fu R, Cai S, Jiang X, Wang F, et al. 2023. 7α and 7β hydroxylation of dehydroepiandrosterone by *Gibberella* sp. and *Absidia coerulea* biotransformation. *Catalysts* 13:272

37. Chen G, Yang M, Lu Z, Zhang J, Huang H, et al. 2007. Microbial transformation of 20(*S*)-protopanaxatriol-type saponins by *Absidia coerulea*. *Journal of Natural Products* 70:1203−6

38. Albert Q, Leleyter L, Lemoine M, Heutte N, Rioult JP, et al. 2018. Comparison of tolerance and biosorption of three trace metals (Cd, Cu, Pb) by the soil fungus *Absidia cylindrospora*. *Chemosphere* 196:386−92

39. Guiraud P, Bonnet JL, Boumendjel A, Kadri-Dakir M, Dusser M, et al. 2008. Involvement of *Tetrahymena pyriformis* and selected fungi in the elimination of anthracene, and toxicity assessment of the biotransformation products. *Ecotoxicology and Environmental Safety* 69:296−305

40. Kristanti RA, Fikri Ahmad Zubir, Hadibarata T. 2016. Biotransformation studies of cresol red by *Absidia spinosa* M15. *Journal of Environmental Management* 172:107−11

41. Senanayake IC, Rathnayaka AR, Marasinghe DS, Calabon MS, Gentekaki E, et al. 2020. Morphological approaches in studying fungi: Collection, examination, isolation, sporulation and preservation. *Mycosphere* 11:2678−54

42. Mattoo AJ, Nonzom S. 2022. Investigating diverse methods for inducing sporulation in endophytic fungi. *Studies in Fungi* 7:16

43. White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*, eds. Innis MA, Gelfand DH, Sninsky JJ, White TJ. Volume 18. San Diego, CA, USA: Academic Press. pp. 315–22. doi: 10.1016/b978-0-12-372180-8.50042-1

44. Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172:4238−46

45. Xu S, Phookamsak R, Jiang HB, Tibpromma S, Yang JB, et al. 2022. First report of *Occultibambusa jonesii* on para grass (*Brachiaria mutica*) in Yunnan, China. *Chiang Mai Journal of Science* 49:581−97

46. Katoh K, Rozewicki J, Yamada KD. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20:1160−66

47. Hall T. 2004. Bioedit Version 6.0.7. www.mbio.ncsu.edu/bioedit/bioedit.html

48. Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the cipres science gateway for inference of large phylogenetic trees. *2010 Gateway Computing Environments Workshop (GCE), New Orleans, LA, USA, 14 November 2010*. New York, USA: IEEE. pp. 1–8. doi: 10.1109/GCE.2010.5676129

49. Nylander JAA. 2008. *MrModeltest2 v. 2.3 (Program for Selecting DNA Substitution Models Using PAUP*)*. Uppsala, Sweden: Evolutionary Biology Centre. https://github.com/nylander/MrModeltest2

50. Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572−74

51. Rannala B, Yang Z. 1996. Probability distribution of molecular evolutionary trees: A new method of phylogenetic inference. *Journal of Molecular Evolution* 43:304−11

52. Zhaxybayeva O, Gogarten JP. 2002. Bootstrap, Bayesian probability and maximum likelihood mapping: Exploring new tools for comparative genome analyses. *BMC Genomics* 3:4

53. Rambaut A. 2012. *FigTree Version 1.4. 0*. Edinburgh: Institute of Evolutionary Biology, University of Edinburgh. http://tree.bio.ed.ac.uk/software/figtree/ (Accessed January 10, 2024)

54. Hurdeal VG, Gentekaki E, Lee HB, Jeewon R, Hyde KD, et al. 2021. Mucoralean fungi in Thailand: novel species of *Absidia* from tropical forest soil. *Cryptogamie Mycologie* 42:39−61

55. Lahlali R, Ezrari S, Radouane N, Kenfaoui J, Esmaeel Q, et al. 2022. Biological control of plant pathogens: A global perspective. *Microorganisms* 10:596

56. Muhammad M, Basit A, Ali K, Li WJ, Li L, et al. 2024. Endophytic fungi as potential bio-control agents of soil-borne pathogen. *Journal of Crop Health* 76:617−36

57. Index Fungorum. 2024. www.indexfungorum.org/Names/Names.asp

58. Nguyen TTT, Lee SH, Bae S, Jeon SJ, Mun HY, et al. 2016. Characterization of two new records of zygomycete species belonging to undiscovered taxa in Korea. *Mycobiology* 44:29−37

59. Species Fungorum. 2024. www.speciesfungorum.org/Names/Names.asp

60. MycoBank. 2024. www.mycobank.org/quicksearch.aspx