

Expanding the Known Diversity of Thai Fungus *Absidia* (Mucorales, Mucoromycota): Morphological and Molecular Evidence for *Absidia khaoyaiensis* sp. nov.

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Abstract

The genus *Absidia* (Mucorales, Mucoromycota) is globally recognized for its ecological, industrial, and medicinal significance, yet its diversity within Thai ecosystems remains understudied. We describe a novel soil-derived fungus, *Absidia khaoyaiensis* sp. nov. from the rhizosphere of an endemic plant *Polyalthia khaoyaiensis* in Khao Yai National Park, Thailand. A polyphasic approach was employed combining morphological data with phylogenetic analyses of concatenated internal transcribed spacers (ITS), large subunit (LSU), small subunit (SSU) ribosomal DNA, and α -actin (ACT) sequences. Phylogenetic analyses confirmed that *A. khaoyaiensis* forms a highly supported distinct lineage within the genus in the Mucorales. Morphologically, the novel species is distinguished from its phylogenetically closest relative, *A. hainanensis*, by its growth rate, sporangiophore, sporangia, columella measurements, and sporangiospore shape. Our results contribute to diversity of Thai *Absidia* and provide robust evidence for the establishment of *A. khaoyaiensis* as a new species.

Keywords: Mucorales, Novel taxon, Phylogeny, Soil fungus, Thailand

Introduction

The genus *Absidia* is a member of the Mucoromycota, specifically within the order Mucorales and the family Cunninghamellaceae (Index Fungorum, accessed July 25, 2025). The genus was originally described by van Tieghem, with *A. reflexa* as its type species [1]. Characteristic morphological features of this genus include erect or slightly bent sporangia with a septum below the columella [2]. The sporangia are multi-spored with thin deliquescent walls and are apophysate, ranging in shape from spherical to pyriform [3]. Sporangiospores arise singly or in whorls from the stolons, with rhizoids located at both ends [2-4]. The columella has an apical projection for all species, in

which can be conical, subglobose, or flattened [3,5]. Collars may also be evident. Sporangiospores are mostly hyaline, unicellular, and lack striations; their shapes differ for some species ranging from spherical, cylindrical, to short rod-like [6]. Zygospores and appendages have also been observed in some species [7,8].

The genus *Absidia* is abundant and globally distributed. It is predominantly found in diverse substrates such as soil, decaying plant materials, food products, herbivorous feces, ambrosia beetles, and air [4,5,9-12]. Many species are biotechnologically significant, particularly in biotransformation,

bioremediation, and enzyme production [13,14]. Furthermore, some species are causative agents of human mucormycosis, especially in immunocompromised individuals [15-17]. Recently, 66 species currently recorded by the latest record of Liu *et al.* [18]. The genus is commonly found in different regions worldwide, for example in Argentina, Australia, Brazil, China, India, South Korea, and Thailand [3]. The polyphasic taxonomy with integration of traditional morphological analysis, physiological characteristics and multi-gene phylogeny has significantly advanced our understanding of the genus's diversity and classification.

Over the last few years, the number of newly proposed *Absidia* species from diverse ecosystems has increased rapidly, with many new species reported from China. For instance, Zhao *et al.* [3] described three new species (*A. frigida*, *A. gemella*, *A. longissima*) from Xinjiang and Yunnan. Subsequently, with the use of multi-gene analyses, several studies from China have continuously followed this trend, with Luo *et al.* [19]; Tao *et al.* [20]; Ding *et al.* [21]; Ji *et al.* [2]; Wang *et al.* [11] collectively introducing 17 new species. Novel species have also been reported from other global locations. Nguyen *et al.* [22] described seven new species (*A. cheongyangensis*, *A. fluvii*, *A. kunryangriensis*, *A. paracylindrospora*, *A. tarda*, *A. variiprojecta* and *A. variispora*) from South Korea and Brazil, while Lim *et al.* [4] also reported *A. microsporangia* from South Korea. Additionally, Idnurm [23] described *A. urquhartii* from leaf litter in Australia, underscoring the genus's unexplored diversity. Recently, Liu *et al.* [18] unveiled four novel species from China: *A. brunneola*, *A. digitata*, *A. exilis* and *A. tumida*.

In Thailand, four *Absidia* species have been discovered mainly from soil including *A. edaphica*, *A. soli*, *A. zygospora* and *A. thailandica* [5,8,24]. This study, therefore, aims to describe a novel species of *Absidia*, isolated from the soil surrounding the roots of *Polyalthia khaoyaiensis*, a species endemic to the hill evergreen forests of Thailand, using morphological characterization and multi-gene phylogenetic analyses.

Materials and methods

Sample collection and fungal isolation

Ten g of soil surrounding the roots of *P. khaoyaiensis* were collected in Khao Yai National Park (14°42'42'' N, 101°25'18''E), Nakhon Ratchasima Province, Thailand. Samples were sealed in zipper storage bags and immediately placed into a cooler with ice. They were then transported to the National Center for Genetic Engineering and Biotechnology (BIOTEC) for further processing.

The serial dilution plating method was employed for fungal isolation, following the modified protocol of Hurdeal *et al.* [8]. Briefly, 1-gram aliquot of soil was suspended in 9 ml of sterile distilled water. A series of dilutions from 10^{-1} to 10^{-5} were then prepared. A 100 μ L volume from the 10^{-3} , 10^{-4} , and 10^{-5} dilutions was spread onto separate plates of Rose Bengal Agar Base (RBAB, Himedia, India) containing 50 mg/l penicillin G and 50 mg/l streptomycin sulfate. The inoculated plates were incubated at room temperature (25 - 28 °C) for a period of 7 - 14 days. Resulting fungal colonies were then purified by subculturing onto antibiotic-free PDA plates. Three axenic fungal strains, designated SL00078, SL00079, and SL00100, exhibiting consistent macro and microscopic morphological features were chosen for subsequent morphological examination and molecular phylogenetic analyses.

Cultural and morphological characterization

To characterize cultural and micro-morphological features, all fungal strains were inoculated onto PDA, MEA (malt extract agar, Difco, USA) and OMA (oat meal agar, Difco, USA) and incubated at room temperature (25 - 28 °C) for a period of 7 - 14 days. Macroscopic characteristics of the cultures, including colony appearance, growth patterns, and sporulation, were observed under a stereo-zoom microscope (Olympus SZ51, Olympus Corporation, Japan). Microscopic examination of key asexual morphological structures including sporangia, columella, rhizoids, and sporangiospores, was performed under a compound light microscope (Olympus CX31; Olympus Corporation, Japan). The photographic images were taken with an Olympus BX51 compound light microscope, coupled with Olympus DP21 software (Olympus Corporation, Japan). Colony colors were

determined with the standard charts from the Methuen Handbook of Color [25].

The type strain of the novel species, *A. khaoyaiensis* sp. nov., were deposited at the Thailand Bioresource Research Center (TBRC; <https://www.tbrcnetwork.org>, accessed on September 12, 2025) under the accession numbers TBRC 21215 (SL00078), TBRC 21216 (SL00079), and TBRC 21217 (SL00100). Additionally, the holotype specimen, *A. khaoyaiensis* BBH 51616, was maintained in the BIOTEC Bangkok Herbarium (BBH; <https://www.nbt-microbe.org>, accessed on September 12, 2025). MycoBank numbers were assigned for the species: MB 860904 (<https://www.mycobank.org/>, accessed on September 12, 2025).

DNA extraction, PCR amplification, DNA sequencing

Genomic DNA was extracted through a modified method by O'Donnell *et al.* [26]. Four DNA regions were selected for PCR amplification and DNA sequencing: the internal transcribed spacers (ITS), large subunit (LSU), small subunit (SSU) ribosomal DNA, and α -actin (ACT) gene. The rDNA regions were amplified using primer pairs ITS5-ITS4 [27], LROR-LR5 [28], and NS1-NS4 [27]. For the ACT gene, the primer pair ACT1-ACT4 was used [29]. PCR conditions for ITS, LSU and SSU regions followed the protocol described by Preedanon *et al.* [30], while the conditions for ACT gene were based on Hurdeal *et al.* [5]. The PCR products were purified and sequenced by Macrogen (Seoul, South Korea) using the same PCR primers used for PCR amplification.

DNA sequence assembly and alignment

Newly generated sequences from each locus were checked for quality, trimmed and assembled using BioEdit 7.2.5 [31]. A nucleotide BLAST search (<https://blast.ncbi.nlm.nih.gov/Blast>) was performed to determine sequence homologies by comparing our sequences with those available in the GenBank database. Relevant DNA sequences were obtained from recent studies on the genus *Absidia* [2-4,8,11,18-24]. For phylogenetic analysis, representative taxa were primarily selected from type strains (Table 1). Individual single-locus datasets were separately aligned by BioEdit 7.2.5 [31] and AliView 1.28 [32].

Subsequently, a combined multi-locus dataset was created by concatenating the ITS, LSU, SSU, and ACT sequences. Finally, the alignment ends were trimmed to match the sequence lengths of our isolates, ensuring a consistent matrix for phylogenetic inference.

Phylogenetic analyses

Phylogenetic analyses were performed on individual single-locus datasets and a combined multi-locus dataset to infer evolutionary relationships. Maximum likelihood (ML) with the GTR + GAMMA substitution model and bootstrap analyses were performed on the RAxML 8.2.12 [33]. Bayesian posterior probabilities (BYPP) were estimated by MrBayes 3.2.6 [34]. The best-fitting model, GTR+I+G, was selected in MrModeltest 2.2 [35]. The analysis was run for 3 million generations with four Markov chains, sampling a tree every 100 generations. A burn-in value of 3,000 sampled trees was applied. The resulting consensus tree was visualized using the interactive Tree of Life (iTOL) [36]. The phylogenetic trees generated were manually edited using Microsoft Office PowerPoint 2021 (Microsoft Inc., USA). Photographic plates and phylogenetic tree images were converted to TIFF files using Adobe Photoshop CS6 software (Adobe Systems Inc., USA). All sequences obtained in this study were deposited in the GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>, accessed on September 5, 2025).

Results and discussion

Molecular phylogenetic analyses

Phylogenetic analyses were conducted using individual single-locus datasets and a combined multi-locus dataset to infer evolutionary relationships. Based on the single-locus analyses, all three strains of our novel species were monophyletic and well placed within the Cunninghamellaceae, order Mucorales. Phylogenetic analysis of the ITS gene showed that our new species formed a distinct lineage, exhibiting 97.2% nucleotide identity with two closely related *A. hainanensis* strains. The ACT gene phylogeny further supported this close relationship, placing our species near *A. oblongispora* (with 98.8% - 99.2% identity) and *A. hainanensis* (with 98.4% - 98.8% identity) (Figures S1 and S2).

The final multi-locus dataset for phylogenetic inference included the ITS, LSU, SSU, and ACT genes from 70 taxa, resulting in a sequence alignment of 3,249 characters. The DNA sequences lengths for each locus ranged from: ITS (1 - 600, 600 bp), LSU (601 - 1,592, 991 bp), SSU (1,593 - 2,619, 1,026 bp), and ACT (2,620 - 3,249, 629 bp). The outgroups chosen included *Cunninghamella homothallica* CBS 168.53 and *C. phaeospora* CBS 692.68. This dataset contained 2,025 constant characters, 882 parsimony-informative characters, and 342 parsimony uninformative characters. The maximum likelihood tree had a best score of -31409.936163 (ln L). Estimated base frequencies were: A = 0.260264, C = 0.205236, G = 0.242877, and T = 0.291624. The substitution rates were: AC = 0.949729, AG = 2.444079, AT = 1.453760, CG = 0.741994, CT = 3.924567, and GT = 1.00000.

The multi-gene phylogenetic analysis revealed that various *Absidia* species are positioned within several subclades (**Figure 1**). The three fungal isolates of *A. khaoyaiensis* sp. nov. (TBRC 21215, TBRC 21216, TBRC 21217) formed a well-supported monophyletic group. Our new fungus formed a distinct, well-supported lineage with *A. hainanensis*, *A. oblongispora*, *A. tarda*, *A. cheongyangensis*, *A. crystalloides*, and *A. heterospora* as sister taxa. Notably, our fungal strains branched off from the closely related taxon, *A. hainanensis*, with significant support from

both ML bootstrap values (97%) and Bayesian posterior probabilities (1.00).

Species delimitation within the genus *Absidia* relies on a polyphasic approach, integrating morphological characterization, physiological property, and multi-gene phylogenetic analysis. In this genus, physiological testing, specifically optimal growth media and maximum growth temperature, serve as critical diagnostic characters for distinguishing closely related taxa [2,11,12,19-22]. For molecular delimitation, the ITS rDNA region is the primary DNA barcode; however, protein-coding genes such as ACT and translation elongation factor 1-alpha (TEF1 α) are utilized as secondary markers to clarify species complexes [2,5,8,11,12,18-24]. While an explicit, universal sequence identity threshold for *Absidia* has not been clear established, Walther *et al.* [37] proposed a 99% ITS similarity cutoff as a general guideline for species delineation in Mucorales.

Our proposed species, *A. khaoyaiensis*, exhibits ITS sequence identity of 97.2% with *A. hainanensis*, which is well below the 99% threshold, suggesting clear genomic divergence. Furthermore, although the ACT sequence shows higher similarity (98.4% - 99.2%) with the closest species, *A. oblongispora*, our taxon forms a distinct, well-supported monophyletic lineage in the concatenated phylogeny. Thus, the polyphasic evidence collectively supports the establishment of this new species.

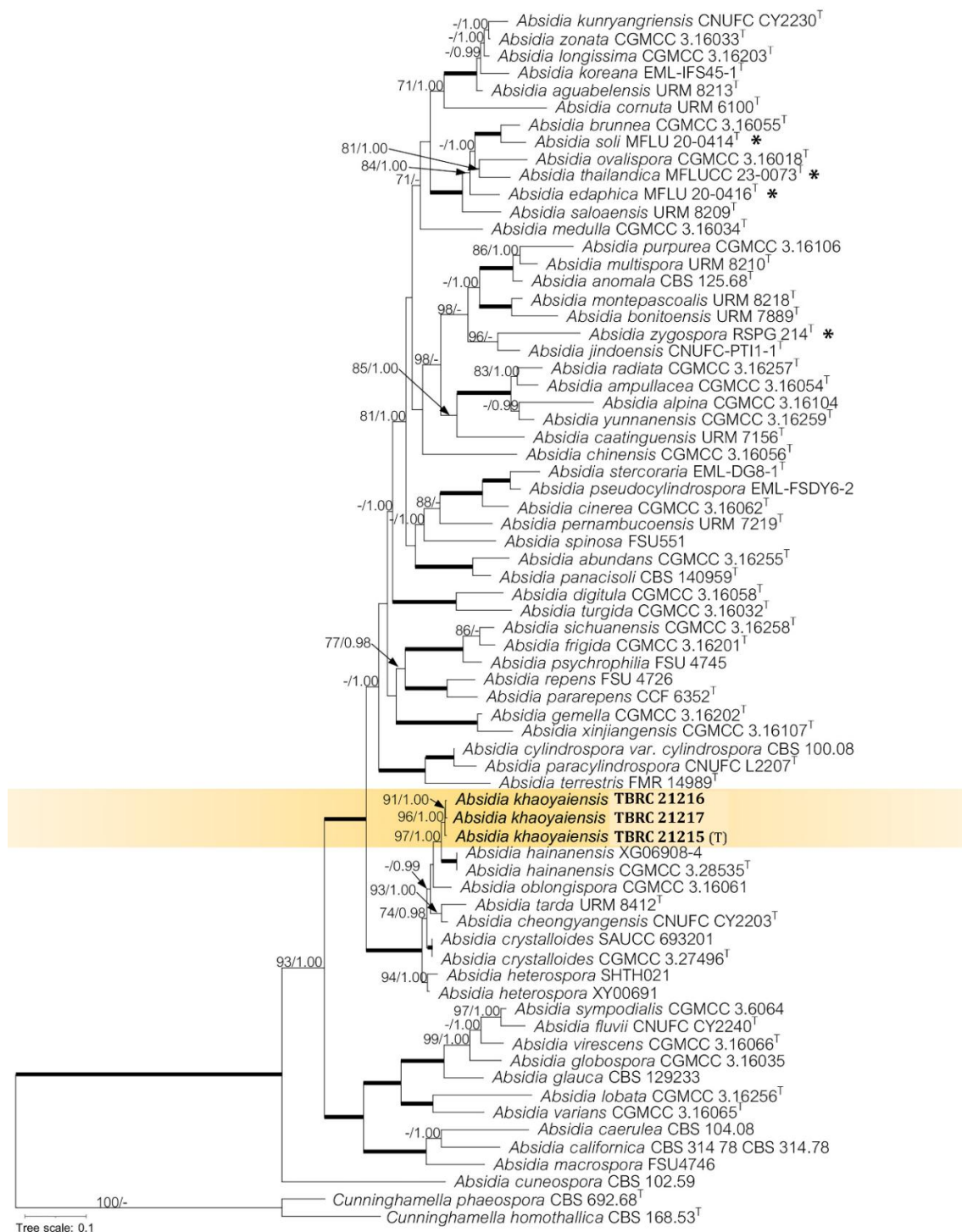


Figure 1 The RAxML phylogenetic tree of *Absidia khaoyaiensis* sp. nov. obtained from the combined ITS, LSU, SSU rDNA and ACT sequences. Maximum likelihood bootstrap values (left) $\geq 70\%$ and Bayesian posterior probabilities (right) ≥ 0.95 are shown above each branch. The nodes that are highly supported by bootstrap values (100%) and posterior probabilities (1.00) are shown in a thicker line. T = type strain. Asterisks (*) refer to species originally described from Thailand.

Taxonomy and description of a new species

Absidia khaoyaiensis Preedanon & Sakay., sp. nov. MycoBank: 860904. Etymology: The specific epithet refers to “Khao Yai National Park”, where the type strain was first collected. Typification: Thailand, Nakhon Ratchasima Province, Khao Yai National Park, Mo Sing To permanent plot, soil, December 14, 2022, Sita Preedanon, Supicha Saengkaewsuk, Charisa Srihom, Prasert Srikitikulchai and Sarunyou Wongkanoun, culture, Sita Preedanon, SL00078 (holotype BBH 51616, ex-type strain TBRC 21215). GenBank accession numbers for holotype strain: ITS = PX277501, LSU = PX277504, SSU = PX277507, ACT = PX512134

Colonies on PDA at room temperature (25 - 28 °C) attaining 85 mm in diameter after 14 days, fascicular, cottony to woolly entire margin, aerial mycelia

abundant, white (1A1). Reverse grey (1D1). Colonies on MEA attaining 85 mm in diameter after 14 days, fluffy with raised margin, grey (1D1). Reverse greyish yellow (4B3). Colonies on OMA attaining 85 mm in diameter after 14 days, fascicular, cottony to woolly, aerial mycelia abundant, grey (1C1). Reverse grey (1C1).

Sporangiophores arising from stolon, hyaline, with 1 septum below apophyses, $1.14 - 5.06 \times 12.73 - 89.03 \mu\text{m}$ ($\bar{x} = 2.54 \times 43.36 \mu\text{m}$, $n = 30$). Sporangia hyaline, subglobose, smooth, $4.34 - 17.66 \times 4.66 - 17.91 \mu\text{m}$ ($\bar{x} = 9.47 \times 9.91 \mu\text{m}$, $n = 30$). Columellae hyaline, subglobose, smooth-walled, $8.48 - 19.1 \times 6.97 - 25.04 \mu\text{m}$ ($\bar{x} = 9.81 \times 12.71 \mu\text{m}$, $n = 30$), with collarete and apical projection. Sporangiospores hyaline, oblong, smooth, $1.26 - 2.01 \times 3.46 - 4.59 \mu\text{m}$ ($\bar{x} = 1.56 \times 3.84 \mu\text{m}$, $n = 30$). Sexual morph not determined (**Figure 2**).

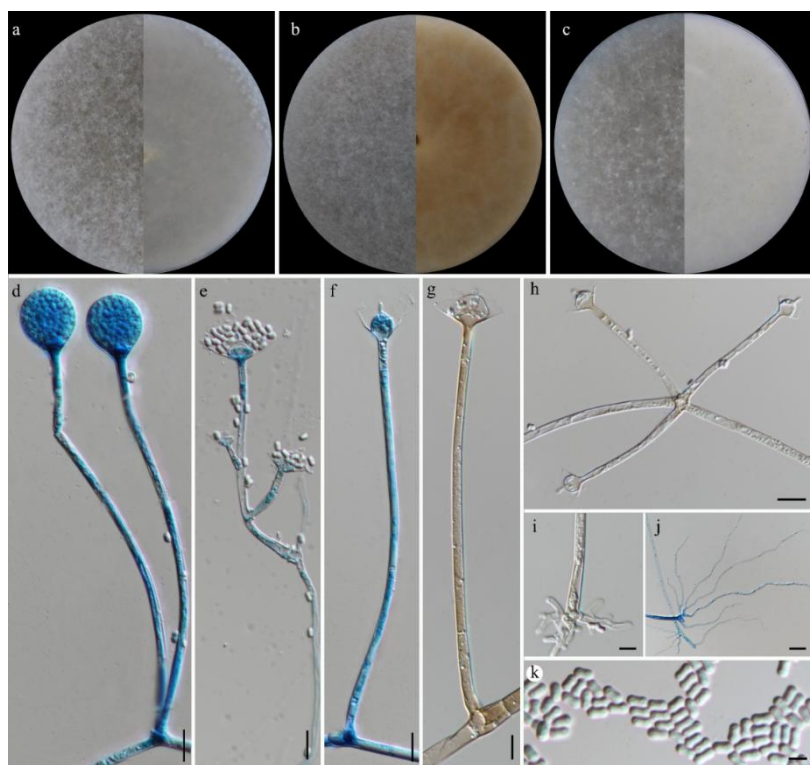


Figure 2 *Absidia khaoyaiensis* sp. nov. TBRC 21215 (ex-type strain), (a-c) Obverse and reverse views of 14-day cultures on PDA, MEA and OMA grown at room temperature (25 - 28 °C), respectively; (d) sporangiophores bearing sporangia; (e) branching sporangiophores with columella; (f,g) columella with collarete, apical projection and septum below the apophysis; (h) branching sporangiophores from the stolon; (i,j) rhizoids; (k) oblong cylindrical sporangiospores. Scale bars: (d-g) = 10 μm , (h-k) = 4 μm .

In comparison with the closest species, *A. hainanensis*, our new species is slow-growing, reaching a diameter of 85 mm in 14 days on PDA, whereas *A.*

hainanensis grows faster, reaching 75 mm in 5 days. The sporangiophores of *A. khaoyaiensis* sp. nov. are significantly shorter ($1.14 - 5.06 \times 12.73 - 89.03 \mu\text{m}$) than

those of *A. hainanensis* (1.9 - 3.7×18.8 - 159.2 µm). The sporangia of our new species are smaller (4.34 - 17.66×4.66 - 17.91 µm) compared to those of *A. hainanensis* (15.1 - 35.2×14.3 - 29.4 µm). Moreover, the columella of *A. khaoyaiensis* sp. nov. is larger (8.48 - 19.1×6.97 - 25.04 µm) than that of *A. hainanensis* (3.9 - 13.9×8.6 - 19.5 µm). While the sporangiospore measurements of both species are in a similar range (1.26 - 2.01×3.46 - 4.59 µm vs. 1.9 - 2.5×3.1 - 3.7 µm), however, their shapes differ. *Absidia khaoyaiensis* sp. nov. possesses oblong-cylindrical sporangiospores, whereas *A. hainanensis* has ovoid to cylindrical spores (**Table 2**).

Absidia hainanensis, the closest relative to our novel species, was originally described from Hainan, China [2]. Hainan is characterized primarily by lowland tropical rainforests influenced by island biogeography. Although Hainan is a recognized hotspot for *Absidia* and other mucoralean species [2,11,12,20], its geography and climate differ significantly from those of Khao Yai National Park. Khao Yai National Park features of the hill-dry evergreen and mixed deciduous tropical rainforests [38]. It is a significant site for novel fungal discovery in Thailand. Since 2000, several new fungal species across various ecological groups including basidiomycetes, freshwater lignicolous fungi, and entomopathogenic fungi, have been described from this location [39-42]. The unique geography of Khao Yai National Park results in a high accumulation of leaf litter, including the endemic plant *P. khaoyaiensis*, which might serve as the organic substrates required by our novel species.

Traditionally, species delineation within the genus *Absidia* has relied on morphological characters, including variations in asexual morph structures such as sporangiophore, sporangia, and sporangiospore dimensions [5,8,19,43]. Notably, Hoffmann *et al.* [43] proposed that sporangiospore shape was a key taxonomic criterion and later a classification corroborated by the emergence of phylogenetically distinct clades: globose, cylindrical, and conical. This morphological-molecular congruence is supported by the recent discoveries, such as *A. zygospora*, which is characterized by its cylindrical spores and phylogenetically placed in the cylindrical clade [8]. In the present study, *A. khaoyaiensis* aligns with this morphological group, exhibiting oblong to cylindrical

spores—a feature shared with *A. heterospora* [44], *A. oblongispora* [45], *A. cylindrospora* [46], *A. crystalloides* [19], and its closest relative, *A. hainanensis* [2].

Diversity of *Absidia* in Thailand

Over the last 5 years, there has been a dramatically significant in the discovery of *Absidia* species across Asia, with a particular concentration of new taxa reported from China. Studies investigating species from China's diverse climatic zones have demonstrated a clear correlation between geographical origin and physiological traits, most notably optimal growth temperatures [2,3,19]. A prominent example is the contrast between the psychrotolerant *A. frigida*, which has a maximum growth temperature of only 24 °C, and the mesophilic *A. hainanensis*, which thrives at temperatures up to 34 °C [2,19]. This underscores the importance of exploring understudied tropical ecosystems, such as those in Thailand, where environmental pressures may have shaped unique physiological adaptations in novel lineages.

Diversity knowledge of the genus *Absidia* in Thailand remains limited. To date, only 5 species have been documented including the present study. All of which were predominantly isolated from soil. Hurdeal *et al.* [5] originally explored Thai *Absidia* from soil samples, describing *A. soli* and *A. edaphica* based on the dimensions of their sporangia, sporangiospores, and columellae. Subsequently, Hurdeal *et al.* [8] further expanded the known diversity with *A. zygospora*, identified through a combination of morphological, physiological, and rDNA sequence data. This species is particularly notable for producing a sexual morph in culture and possessing smaller sporangia and columellae than its sister taxa. More recently, Htet *et al.* [24] described *A. thailandica*, distinguished by the unique morphology of its rhizoids, sporangiospores, and columellae (**Table 3**). Therefore, four previously described species, alongside our discovery of *A. khaoyaiensis* sp. nov., represent distinct phylogenetic lineages within the genus (**Figure 1**), underscoring the significant hidden diversity of soil fungi in Thailand.

The discovery of *A. khaoyaiensis* sp. nov. is ecologically significant, representing the first record of a rhizosphere fungus associated with the endemic plant *P. khaoyaiensis* [47]. This plant is characterized by its

restricted geographical distribution and high ecological value within Thai evergreen forests [47]. In tropical forest ecosystems, soil-dwelling fungi play a fundamental role in nutrient cycling and plant nutrient uptake, forming symbiotic relationships that are vital for host survival and growth [48-50]. Given that species within the genus *Polyalthia* are recognized as rich sources of bioactive compounds [51], the unique microhabitat of their root systems likely harbors

specialized microbial communities. This hypothesis is supported by previous findings, such as the isolation of the endophyte *Trichoderma polyalthiae* from *P. debilis* [52] and studies on the phytochemical-microbiome associations of *P. longifolia* [53]. Consequently, further research is warranted to characterize the secondary metabolites of *A. khaoyaiensis* and to define its specific ecological contributions within this unique rhizosphere.

Table 1 GenBank accession numbers of sequences used in this study, (T) = Type species.

Species	Strain number	GenBank accession numbers			
		ITS rDNA	LSU rDNA	SSU rDNA	ACT
<i>A. abundans</i>	CGMCC 3.16255 (T)	NR182590	ON074683	-	-
<i>A. aguabelensis</i>	URM 8213 (T)	MW763074	MW762874	-	-
<i>A. alpina</i>	CGMCC 3.16104	OL678133	-	-	-
<i>A. ampullacea</i>	CGMCC 3.16054 (T)	MZ354138	MZ350132	-	-
<i>A. anomala</i>	CBS 125.68 (T)	NR103626	NG058562	-	EF030535
<i>A. bonitoensis</i>	URM 7889 (T)	MN977786	MN977805	-	-
<i>A. brunnea</i>	CGMCC 3.16055 (T)	MZ354139	MZ350133	-	MZ357421
<i>A. caatinguensis</i>	URM 7156 (T)	NR154704	NG058582	-	-
<i>A. caerulea</i>	CBS 104.08	MH854575	MH866107	-	-
<i>A. californica</i>	CBS 314.78	MH861141	MH872902	-	-
<i>A. cheongyangensis</i>	CNUFC CY2203 (T)	PP844904	PP852788	-	PP893196
<i>A. chinensis</i>	CGMCC 3.16056 (T)	MZ354140	MZ350134	-	-
<i>A. cinerea</i>	CGMCC 3.16062	MZ354146	MZ350140	-	MZ357427
<i>A. cornuta</i>	URM 6100 (T)	MN625256	MN625255	-	-
<i>A. crystalloides</i>	SAUCC 693201	PP377804	PP373737	PP779722	-
<i>A. crystalloides</i>	CGMCC 3.27496 (T)	PP377803	PP373736	PP779723	-
<i>A. cuneospora</i>	CBS 102.59	JN205819	JN206579	-	-
<i>A. cylindrospora</i> var. <i>cylindrospora</i>	CBS 100.08 (T)	JN205822	JN206588	-	-
<i>A. digitula</i>	CGMCC 3.16058 (T)	MZ354142	MZ350136	-	-
<i>A. edaphica</i>	MFLU 20-0416 (T)	MT396372	MT393987	MT394048	MT410739
<i>A. fluvii</i>	CNUFC CY2240 (T)	PP844891	PP852703	-	PP893201
<i>A. frigida</i>	CGMCC 3.16201 (T)	NR182565	OM030223	-	-
<i>A. gemella</i>	CGMCC 3.16202 (T)	OM108488	OM030224	-	-
<i>A. glauca</i>	CBS 129233	MH865253	MH876693	-	-
<i>A. globospora</i>	CGMCC 3.16035	MW671538	MW671545	-	-
<i>A. hainanensis</i>	CGMCC 3.28535 (T)	PQ610537	PQ605108	PQ605118	PQ613283
<i>A. hainanensis</i>	XG06908-4	PQ610538	PQ605109	PQ605119	PQ613284

Species	Strain number	GenBank accession numbers			
		ITS rDNA	LSU rDNA	SSU rDNA	ACT
<i>A. heterospora</i>	SHTH021	JN942683	JN982936	JQ004928	-
<i>A. heterospora</i>	XY00691	ON123747	ON123768	-	-
<i>A. jindoensis</i>	CNUFC-PTI1-1 (T)	MF926622	MF926616	MF926626	MF926510
<i>A. khaoyaiensis</i> sp. nov.	TBRC 21215, BBH 51616 (T)	PX277501	PX277504	PX277507	PX512134
<i>A. khaoyaiensis</i> sp. nov.	TBRC 21216, BBH 51617	PX277502	PX277505	PX277508	PX512135
<i>A. khaoyaiensis</i> sp. nov.	TBRC 21217, BBH 51618	PX277503	PX277506	PX277509	PX512136
<i>A. koreana</i>	EML-IFS45-1 (T)	KR030062	KR030056	-	KR030058
<i>A. kunryangriensis</i>	CNUFC CY2230 (T)	PP844905	PP956882	-	PP893198
<i>A. lobata</i>	CGMCC 3.16256 (T)	ON074690	ON074679	-	-
<i>A. longissima</i>	CGMCC 3.16203 (T)	NR182566	OM030225	-	-
<i>A. macrospora</i>	FSU4746, CBS 696.68	AY944882	EU736303	EU736276	AY944760
<i>A. medulla</i>	CGMCC 3.16034 (T)	MW671542	MW671549	-	MZ357436
<i>A. montepascoalisi</i>	URM 8218 (T)	NR172995	MW561560	-	-
<i>A. multispora</i>	URM 8210 (T)	MN953780	MN953782	-	-
<i>A. oblongispora</i>	CGMCC 3.16061	MZ354145	MZ350139	-	MZ357426
<i>A. ovalispora</i>	CGMCC3.16018 (T)	MW264071	MW264130	-	-
<i>A. panacisoli</i>	CBS 140959 (T)	NR159563	NG063948	MF522179	-
<i>A. paracylindrospora</i>	CNUFC L2207 (T)	PP844907	PP956883	-	PP893204
<i>A. pararepens</i>	CCF 6352 (T)	MT193669	MT192308	-	-
<i>A. pernambucoensis</i>	URM 7219 (T)	MN635568	MN635569	-	-
<i>A. pseudocylindrospora</i>	EML-FSDY6-2	KU923817	KU923814	KU923819	KU923815
<i>A. psychrophilia</i>	FSU 4745	AY944874	EU736306	EU736279	AY944762
<i>A. purpurea</i>	CGMCC 3.16106	OL678135	-	-	-
<i>A. radiata</i>	CGMCC 3.16257 (T)	ON074698	ON074684	-	-
<i>A. repens</i>	FSU 4726	EU484288	-	-	-
<i>A. saloaensis</i>	URM 8209 (T)	MN953781	MN953783	-	-
<i>A. sichuanensis</i>	CGMCC 3.16258 (T)	ON074692	ON074688	-	-
<i>A. soli</i>	MFLU 20-0414 (T)	MT396373	MT393988	MT394049	MT410740
<i>A. spinosa</i>	FSU551	AY944887	EU736307	EU736280	EU736227
<i>A. stercoraria</i>	EML-DG8-1 (T)	KU168828	KT921998	NG065640	KT922000
<i>A. sympodialis</i>	CGMCC 3.16064	MZ354148	MZ350142	-	-
<i>A. tarda</i>	URM 8412 (T)	PP844911	PP956884	-	PP893199
<i>A. terrestris</i>	FMR 14989 (T)	LT795003	LT795005	-	-
<i>A. thailandica</i>	MFLUCC 23-0073 (T)	OR606547	OR606546	-	-
<i>A. turgida</i>	CGMCC 3.16032 (T)	MW671540	MW671547	-	MZ357434
<i>A. varians</i>	CGMCC 3.16065 (T)	MZ354149	MZ350143	-	-

Species	Strain number	GenBank accession numbers			
		ITS rDNA	LSU rDNA	SSU rDNA	ACT
<i>A. virescens</i>	CGMCC 3.16066 (T)	MZ354150	MZ350144	-	MZ357429
<i>A. xinjiangensis</i>	CGMCC 3.16107 (T)	OL678136	-	-	-
<i>A. yunnanensis</i>	CGMCC 3.16259 (T)	NR182591	NG_149054	-	-
<i>A. zonata</i>	CGMCC 3.16033 (T)	MW671541	MW671548	-	MZ357435
<i>A. zygospora</i>	RSPG 214 (T)	KC478527	-	-	-
<i>Cunninghamella homothallica</i>	CBS 168.53 (T)	MH857147	NG058833	-	-
<i>C. phaeospora</i>	CBS 692.68 (T)	AF254934	NG058812	-	-

Table 2 Morphological comparison of a new species described from the present study and *Absidia* phylogenetically closest species.

Species	Colonies	Sporangiophores	Sporangia	Columellae	Sporangiospores	Ref.
<i>Absidia khaoyaiensis</i> sp. nov.	PDA: 28 °C, 14 days, 85 mm in diameter	arising from stolon, with 1 septum below apophyses, 1.14 - 5.06×12.73 - 89.03 µm ($\bar{x}$ = 2.54 ± 1.05×43.36 ± 20.73 µm, n = 30)	subglobose, smooth, 4.34 - 17.66×4.66 - 17.91 µm ($\bar{x}$ = 9.47 ± 4.55×9.91 ± 4.57 µm, n = 30)	subglobose, smooth-walled, 8.48 - 19.1×6.97 - 25.04 µm ($\bar{x}$ = 9.81 ± 4.06×12.71 ± 5.31 µm, n = 30), with collarette and apical projection	oblong-cylindrical, 1.26 - 2.01×3.46 - 4.59 µm, ($\bar{x}$ = 1.56 ± 0.21×3.84 ± 0.31 µm, n = 30), sexual morph undetermined	This study
<i>A. hainanensis</i>	PDA: 27 °C, 5 days, 75 mm in diameter	erect or slightly bent, unbranched or branched, monopodial or sympodial, single or 2 - 4 in whorls, 1.9 - 3.7×18.8 - 159.2 µm ($\bar{x}$ = 75.4×2.8 µm, n = 15)	spherical to subspherical, smooth, 15.1 - 35.2×14.3 - 29.4 µm ($\bar{x}$ = 24.2×22.1 µm, n = 15)	oval, 3.9 - 13.9×8.6 - 19.5 µm, ($\bar{x}$ = 6.3×10.7 µm, n = 15), projections absent or present, hyaline, single	ovoid to cylindrical, 1.9 - 2.5×3.1 - 3.7 µm, ($\bar{x}$ = 3.6×2.2 µm, n = 20), sexual morph undetermined	[2]
<i>A. oblongispora</i>	MEA: 27 °C, 10 days, 90 mm in diameter	mostly unbranched, rarely simple or monopodial, single or 2 - 5 in whorls, with a septum, 9.5 - 16.0 µm below apophyses	globose, 13.5 - 31.0 µm in diameter	conical, hemispherical, 7.0 - 15.0×8.5 - 16.5 µm, always with bulbous projection at the apex	oblong, 3.5 - 4.5×2.5 - 3.0 µm, sexual morph undetermined	[12]

Table 3 Comparative morphological characteristics of soil-derived *Absidia* species described from Thailand.

Species	Sporangiophore	Sporangia	Columellae	Sporangiospore	Sexual morph	Ref.
<i>A. soli</i>	arise singly or 1 - 6 in whorls, hyaline, one septum below the apophysis,	mature sporangia brown, globose to slightly ellipsoidal	globose, hyaline to pale brown, bearing collarette and apical projection	short cylindrical to oblong, slightly curved at both end, hyaline, non-striated and smooth walled	undetermined	[5]
<i>A. edaphica</i>	arise singly or 1 - 6 in whorls, hyaline to pale brown, one septum below the apophysis	globose to slightly elliptical, hyaline to pale brown	globose, hyaline with apical projection and collarette	short cylindrical to oblong, slightly curved at both end, hyaline, smooth-walled	undetermined	[5]
<i>A. zygospora</i>	mostly unbranched or 2 - 4 in whorls, hyaline to brown, one septum below the apophysis	brown, pyriform, globose to slightly elliptical	hyaline, subglobose, pyriform with apical projection and collarette	hyaline, short cylindrical to oblong, slightly curved at both ends, smooth-walled	zygospores found	[8]
<i>A. thailandica</i>	single, in pairs or groups on stolons, bearing apophysate	pyriform with a deliquescent wall	conical with apical projection and collarette	cylindrical, smooth-walled	undetermined	[24]

Conclusions

We report the discovery of *Absidia khaoyaiensis* sp. nov., a novel species of the Mucoromycota isolated from the rhizosphere of the endemic Thai plant *P. khaoyaiensis*. Our polyphasic analysis, combining morphological data with multi-locus phylogeny, confirms its status as a new species. *Absidia khaoyaiensis* is distinguished from its phylogenetic closest species by its growth rate and the unique morphology of sporangiophores, sporangia, and columellae. This finding contributes to the diversity of the soil-derived fungi in the tropical ecosystem in Thailand.

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Declaration of Generative AI in Scientific Writing

The authors acknowledge the use of generative AI tool (Gemini) during manuscript preparation for English editing and grammar correction. No content generation or data interpretation was performed by AI. The authors take full responsibility for the content and conclusions of this work.

CRediT Author Statement

Sita Preedanon: Conceptualization; Methodology; Software; Validation; Formal analysis; Investigation; Data curation; Writing-original draft preparation; Writing-review and editing; Resources; Visualization. **Pornpimon Wongsaman:** Molecular study. **Supicha Saengkaewsuk:** Investigation. **Charisa Srihom:** Investigation; Morphological study. **Kitsada Siwaaurai:** Morphological study. **Rathasart Somnuk:** Investigation. **Anuttara Nathalang:** Resources; Supervision. **Satinee Suetrong:** Writing-review and editing; Project administration; Funding acquisition. **Jariya Sakayaroj:** Methodology; Software; Validation;

Formal analysis; Investigation; Writing-original draft preparation; Writing-review and editing; Visualization; Funding acquisition.

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Supplementary Materials

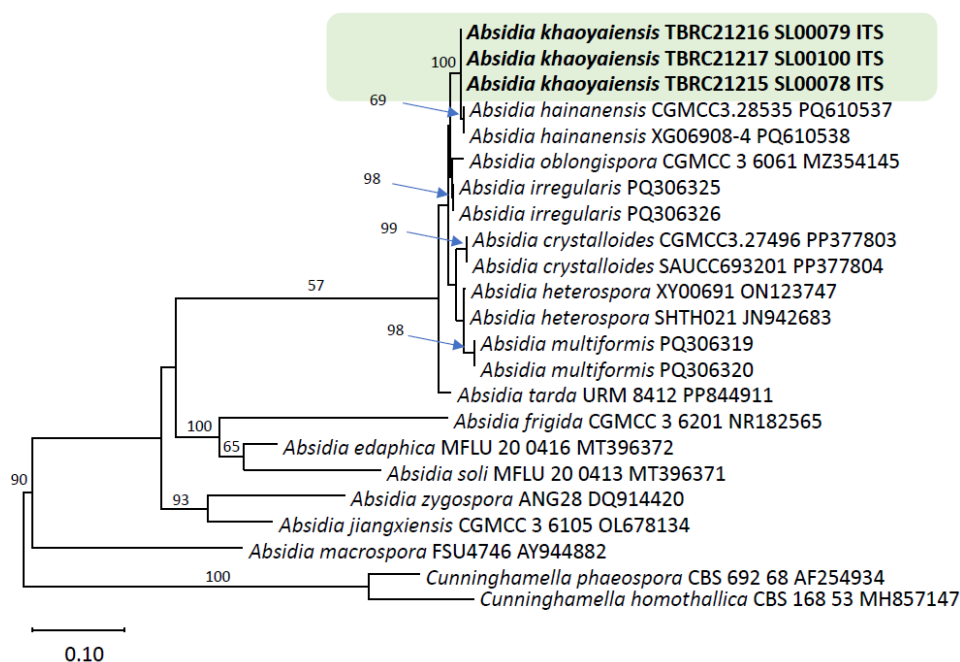


Figure S1 The phylogenetic tree of *Absidia khaoyaiensis* sp. nov. obtained from the ITS rDNA sequences. Maximum likelihood bootstrap values $\geq 70\%$ is shown above each branch.

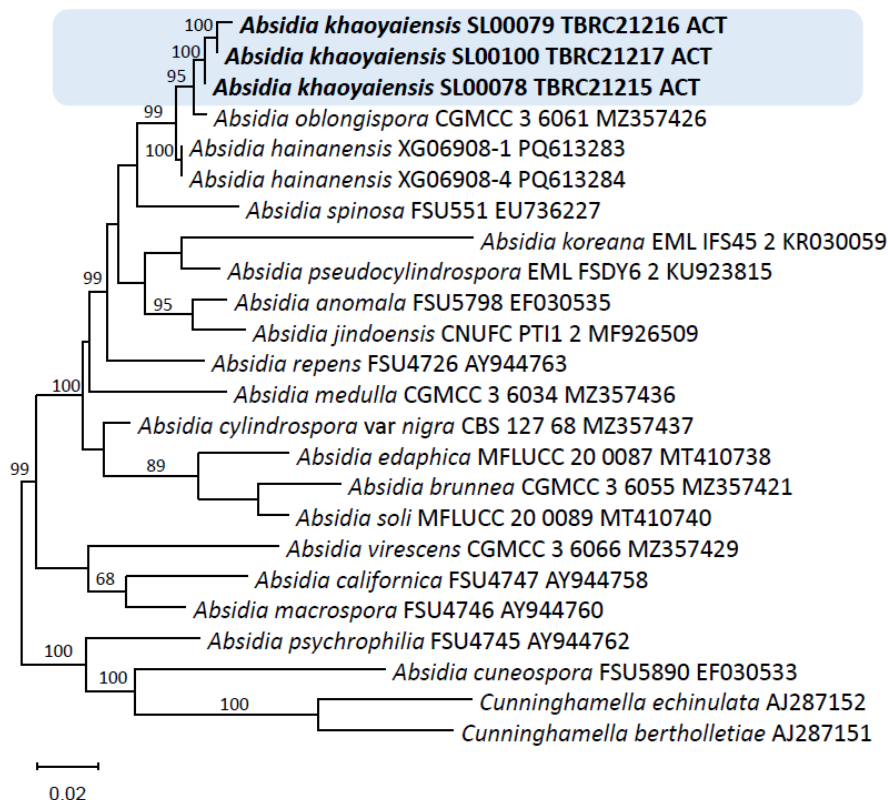


Figure S2 The phylogenetic tree of *Absidia khaoyaiensis* sp. nov. obtained from the ACT DNA sequences. Maximum likelihood bootstrap values $\geq 70\%$ is shown above each branch.