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Absidia zygospora (Mucoromycetes), a new species from Nan Province, Thailand

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Abstract

Absidia is one of the most commonly isolated fungi among Cunninghamellaceae. The genus comprises saprobes isolated from soil, dung and other organic debris such as leaf litter. During a survey aimed at exploring the diversity of basal lineages of soil fungi, samples were collected from Nan province, Thailand. This led to the collection of a new *Absidia* isolate from soil. Characterization of the new isolate was based on morphological characters, colony growth and DNA sequence data. Phylogenetic analyses indicate that the new isolate comprises a lineage distinct from other described species. Morphological characterization showed that the isolate has smaller sporangia and columellae than its sister taxa. Furthermore, physiological data and genetic distance analysis supported the establishment of the new taxon. Hence, in this study, a new species of *Absidia* (*A. zygospora*) is introduced based on morphology, phylogeny and physiology.

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Introduction

Absidia, a genus belonging to the Cunninghamellaceae family, encompasses mesophilic organisms that thrive under moderate temperature conditions. These fungi exhibit optimal growth within a temperature range of 25 to 34 °C[1–7]. Previously, *Absidia* also included thermophilic species and mycoparasites[4,5,7]. However, advancements in molecular tools have brought more stability to the classification of *Absidia sensu lato*[4,5,7]. Phylogenetic analyses indicated that these genera did not belong to *Absidia*, but resided in Lichtheimiaceae instead. Hence, species of *Absidia sensu lato* were segregated into three distinct genera, *Absidia sensu stricto*, *Lichtheimia* and *Lentamyces*, based on phylogeny, physiology and morphology, which includes features such as the zygospores and their appendages[4,5,7]. Thermophilic species have since been reclassified under *Lichtheimia*, while mycoparasites now belong to *Lentamyces*[4,5,7].

Absidia species are usually isolated as saprobes in soil, but also on dung, and other organic debris[8–12]. The genus is ubiquitous with a broad distribution. They are found in temperate, tropical and subtropical countries such as Brazil, China, Thailand and South Korea[8–13]. *Absidia* species usually produce sporangiophores that are erect, arising singly or in whorls, with subsporangial septa (one or more). Sporangiophores are usually produced in whorls and bear a terminal columellate and apophysate pyriform sporangium. The columellae usually have apical projections distinct from other genera within the Cunninghamellaceae, and zygospores have finger-like appendages, usually produced on equal suspensors[9,11,13].

The taxon *Absidia* has experienced a rapid influx of new species in the last few years. Various novel taxa have been identified in Brazil, China, Thailand and South Korea[8–13]. Species are usually delineated using the ITS and LSU genetic markers. Some studies also include protein-coding genes such as actin (ACT) and translation elongation factor (EF-1α), which increases the reliability of the phylogenies[9,10]. However, it is well known, that obtaining the ITS rDNA sequence data and protein coding genes in this genus is extremely difficult and often cloning is required to obtain good quality DNA sequences[6,9].

In an attempt to explore the diversity of zygosporic fungi in northern Thailand, soil samples were collected from Nan Province. During the sampling process, an *Absidia* strain was isolated. We characterized this new isolate based on molecular phylogenetic analyses, and morphophysiological characteristics. The results revealed that the isolated strains differed significantly from known *Absidia* species. Consequently, we introduce this newly isolated strain as a novel species within *Absidia*, accompanied by a taxonomic diagnosis and photoplates. By characterizing and introducing this strain as a new species, we expand the taxonomic knowledge of the genus, and broaden our understanding of the evolutionary and ecological dynamics within this group of fungi. Furthermore, the identification of new species adds to the overall knowledge of fungal diversity, ultimately contributing to broader scientific research.

Materials and methods

Sample collection and isolation

Soil samples were collected from Nan province, Thailand in January 2020. During this time, the average temperature in Thailand ranges from 24–32 °C. Organic debris were manually removed from the surface of the soil prior to sampling. Sterile shovels and spoons were used to dig the surface layer (around 1–5 cm) and collect the soil. The samples were transferred to

zip lock bags and kept under ice until it was possible to store it at 4 °C.

The dilution plating method was used to isolate the fungus[14]. The sample was diluted to a ratio of 1:5 and 1:10 with sterile distilled water. The mixture was then shaken for 2 h at 25 °C. Subsequently, 100 µL of the supernatant was transferred to fresh media supplemented with chloramphenicol. The media used for inoculation were malt extract agar (MEA) (Himedia™), PDA and yeast malt extract (YMA) (yeast extract: 3 g; malt extract: 3 g; peptone: 5 g; glucose: 10 g; agar: 15 g; distilled water: 1 L). A flame sterilized glass spreader was used to spread the supernatant on the media. Once completed, the agar plates were wrapped in parafilm and kept at 20 °C. The inoculated plates were checked daily for fungal growth. Once growth (3 d post inoculation) was observed, fungal tips were transferred to fresh agar plates to acquire axenic cultures.

Morphological characters were observed using a compound microscope (Nikon Eclipse Ni) and images of fungal structures were captured using a Nikon DS-RI2 digital camera. The fungus was preserved in 15% glycerol and water. The ex-type culture was deposited in the Mae Fah Luang University Culture Collection (MFLUCC) and an inactive dried culture (on MEA and 2.5% glycerol) was deposited as the holotype in Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand. The new taxon was registered in Index Fungorum (2023).

DNA extraction and PCR amplification

Mature fungal cultures (grown for 3–5 d in MEA at 25 °C) were used for genomic DNA extraction. The total genomic DNA was extracted using the G-spin™ Total DNA Extraction Kit (Intron Biotechnology, South Korea) following the manufacturer's instructions. The partial fragments of ITS and LSU were amplified using polymerase chain reaction (PCR) using the primers ITS4/5 and LR0R/LR7, respectively[15,16]. The PCR conditions for both ITS and LSU were as follows, initial heat treatment for 5 min at 94 °C, 30 cycles with a denaturation step at 94 °C for 30 s, annealing at 52 °C for 45 s and an elongation step of 90 s at 72 °C and a final elongation period of 7 min at 72 °C.

The PCR products were purified using gel purification and subsequently with MEGAquick spin plus fragment DNA purification kit (Intron Biotechnology, South Korea). Sequencing was performed using an Applied Biosystems 3130XLDNA analyzer (Bionics, South Korea).

Phylogenetic analyses

The raw chromatograms were viewed using BioEdit to check the quality of the sequences and to remove ambiguous bases at both ends. Each sequence was subjected to a Blast search in GenBank to find the closest taxa and check for chimera and/or contamination. The forward and reverse reads were merged using SeqMan. The taxon sampling aimed to cover the genetic diversity of the genus. DNA sequence data were extracted from GenBank to build the dataset for phylogenetic analyses (Table 1). Individual ITS and LSU matrices were built and aligned using MAFFT on the online platform (https://mafft.cbrc.jp/alignment/server/)[17]. The alignment matrix was then trimmed to remove ambiguous bases using TrimAl 1.2[18].

Maximum likelihood (ML) phylograms were inferred using RAxML-NG 1.1.0 and IQ-tree in the online CIPRES Portal (www.phylo.org/portal2) and http://iqtree.cibiv.univie.ac.at/ respectively with bootstrap support obtained from 1,000 pseudo replicates[19,20]. Bayesian inference (BI) analysis was also

Table 1. Data used for phylogenetic analysis in this study and their corresponding GenBank accession numbers. Type species are denoted by ^{T}. Sequences derived in this study are shown in bold.

Species name	Voucher no.	GenBank accession number	
		ITS	LSU
Absidia abundans	CGMCC.3.16255^{T}	NR_182590	ON074683
Absidia aguabelensis	URM 8213^{T}	MW763074	MW762874
Absidia alpina	CGMCC 3.16104	OL678133	
Absidia ampullacea	CGMCC 3.16054	MZ354138	MZ350132
Absidia anomala	CBS 125.68^{T}	NR_103626	NG_058562
Absidia bonitoensis	URM 7889^{T}	MN977786	MN977805
Absidia brunnea	CGMCC.3.16055	MZ354139	MZ350133
Absidia caatinguensis	URM7156^{T}	NR_154704	NG_058582
Absidia californica	CBS 126.68^{T}	–	NG_056998
Absidia caerulea	NRRLA9483	–	–
Absidia caerulea	CBS 104.08	JN205811	MH866107
Absidia cornuta	URM 6100^{T}	NR_172976	MN625255
Absidia cuneospora	CBS 102.59	JN205819	JN206579
Absidia cylindrospora var. *cylindrospora*	CBS 100.08	JN205822	JN206588
Absidia cylindrospora var. *nigra*	CBS 127.68^{T}	–	NG_058560
Absidia cylindrospora var. *rhizomorpha*	CBS 153.63^{T}	–	NG_058563
Absidia edaphica	MFLU 20-0415	–	
Absidia edaphica	MFLU 20-0416^{T}	MT396372	MT393987
Absidia fusca	CBS 102.35^{T}	NR_103625	NG_058552
Absidia glauca	CBS 101.08^{T}	NR_111658	MH866105
Absidia heterospora	SHTH021	JN942683	JN982936
Absidia heterospora	CBS 101.29^{T}	–	NG_058564
Absidia jindoensis	CNUFC-PTI1-2	MF926623	MF926617
Absidia jindoensis	CNUFC-PTI1-1^{T}	MF926622	MF926616
Absidia koreana	EML-IFS45-2	KR030063	KR030057
Absidia koreana	EML-IFS45-1^{T}	KR030062	KR030056
Absidia macrospora	FSU4746	AY944882	–
Absidia macrospora	CBS 697.68^{T}	–	NG_058549
Absidia ovalispora	HMAS 249158	MW264133	MW264074
Absidia ovalispora	CGMCC 3.16018^{T}	MW264071	MW264130
Absidia panacisoli	SYPF 7183	MF522181	MF522180
Absidia panacisoli	CBS 140959^{T}	NR_159563	NG_063948
Absidia pseudocylindrospora	EML-FSDY6-2	KU923817	KU923814
Absidia pseudocylindrospora	CBS 100.62^{T}	NR_145276	NG_058561
Absidia psychrophilia	FSU4745	AY944874	EU736306
Absidia repens	FSU 4726	EU484288	–
Absidia repens	NRRL1336	–	AF113448
Absidia repens	CBS 115583^{T}	NR_103624	NG_058551
Absidia soli	MFLU 20-0413	MT396371	MT393985
Absidia soli	MFLU 20-0414^{T}	MT396373	MT393988
Absidia spinosa	FSU551	AY944887	EU736307
Absidia spinosa	FSU552	AY944888	EU736308
Absidia spinosa var. *biappendiculata*	CBS 187.64	–	MH870040
Absidia stercoraria	EML-DG8-2	KU168829	KT921999
Absidia stercoraria	EML-DG8-1^{T}	KU168828	KT921998
Absidia zygospora	RSPG 214	KC478527	-
Absidia zygospora	ANG28	DQ914420	-
Absidia zygospora	**MFLUCC 23–0061^{T}**	**OR104965**	**OR104992**
Absidia chinensis	CGMCC.3.16056	MZ354140	MZ350134
Absidia cinerea	CGMCC.3.16062	MZ354146	MZ350140
Absidia digitula	CGMCC 3.16058	MZ354142	MZ350136
Absidia globospora	CGMCC.3.16031	MW671537	MW671544
Absidia healeyae	UoMAU1	MT436028	MT436027

(to be continued)

Table 1. (continued)

Species name	Voucher no.	GenBank accession number	
		ITS	LSU
Absidia jiangxiensis	CGMCC 3.16105	OL678134	-
Absidia lobata	CGMCC 3.16256	ON074690	ON074679
Absidia medulla	CGMCC 3.16034	MW671542	MW671549
Absidia montepascoalis	CNUFC HT19001^{T}	MW473494	MW561560
Absidia multispora	URM 8210	MN953780	MN953782
Absidia oblongispora	CGMCC 3.16061	MZ354145	MZ350139
Absidia pararepens	CCF 6351	MT193670	MT192307
Absidia pernambucoensis	URM 7219	MN635568	MN635569
Absidia purpurea	CGMCC 3.16106	OL678135	-
Absidia radiata	CGMCC 3.16257	ON074698	ON074684
Absidia saloaensis	URM 8209^{T}	MN953781	MN953783
Absidia saloaensis	DXL2020	MN953781	MN953783
Absidia sichuanensis	CGMCC 3.16258^{T}	NR_182589	ON074688
Absidia sympodialis	CGMCC 3.16064	MZ354148	MZ350142
Absidia terrestris	FMR 15024	LT795004	LT795593
Absidia terrestris	FMR 14989^{T}	LT795003	LT795005
Absidia turgida	CGMCC.3.16032	MW671540	MW671547
Absidia varians	CGMCC.3.16065	MZ354149	MZ350143
Absidia virescens	CGMCC.3.16066	MZ354150	MZ350144
Absidia xinjiangensis	CGMCC.3.16107	OL678136	
Absidia yunnanensis	CGMCC 3.16259^{T}	NR_182591	NG_149054
Absidia zonata	CGMCC.3.16033	MW671541	MW671548
Chlamydoabsidia padenii	NRRL 2977^{T}	–	AF113453
Chlamydoabsidia padenii	CBS 172.67^{T}	NR_153872	JN206586
Cunninghamella bainieri	FSU319	–	EU736313
Cunninghamella homothallica	CBS 168.53	MH857147	NG_058833
Cunninghamella phaeospora	CBS 692.68	AF254934	NG_058812
Halteromyces radiatus	NRRL6197	–	AF157192
Halteromyces radiatus	CBS 162.75	NR_145293	NG_057938

performed on the online CIPRES Portal (www.phylo.org/portal2) using MrBayes on XSEDE 3.2.7a[21]. The Bayesian tree was built by running four simultaneous chains of 2×10^6 generations and a sampling frequency of 100. The burn-in phase was estimated using Tracer software. The first 1,000 trees represented the burn-in and was herein discarded. Convergence was declared when the average standard deviation of split frequencies reached 0.01 or below. The substitution models of molecular evolution were estimated for each genetic marker using jModelTest2 on XSEDE in the CIPRES Portal. The best fit model for both the ITS and LSU was GTR+G+I under the Akaike information criterion.

Results

Phylogenetic analyses

The ITS and LSU concatenated dataset comprised 82 sequences including three outgroup taxa. The final trimmed alignment consisted of 1,401 sites: ITS: 449, LSU: 952. The final matrix contained 790 distinct alignment patterns and the likelihood of the best scoring ML tree was −21,271.48146. The topologies obtained from the ML and BI analyses were congruent, and similar to previous studies. In both phylogenetic analyses our isolate grouped with two unclassified strains namely *Absidia* sp. RSPG 214 and soil fungal sp. ANG28 with maximum support. This clade clustered separately from other known, and validly described *Absidia* species with a statistical support of 91/100/1 (ML/ML/PP). Together, they are sister to the clade formed by *A. jindoensis* and *A. jiangxiensis*.

The genetic distance in the trimmed ITS alignment of the new species and its sister taxa was computed. The genetic distance between our new species and *A. jindoensis* ranges from 11.5%–13.5%, and 24%–34.5% to *A. jiangxiensis*. This provides additional concrete evidence that validates the new species.

Absidia zygospora Hurdeal VG & Gentekaki E, sp. nov. Fig. 1, 2

Index Fungorum number: IF 900230

Etymology: named after its ability to produce sexual spores

Holotype: MFLU 23–0108

Asexual morph on MEA at 25 °C: Sporangiophores unbranched (mostly) or in whorls (2–4), initially hyaline, turning brown as the culture matures, up to 2.5 µm wide. Subsporangial septum present at the base of the sporangium ($\bar{x}$ = 18.5 µm from the apophysate line), below the apophysate line. Sporangia pale brown to brown, pyriform, globose to slightly elliptical, 10–16.5 × 12.5–19.5 µm ($\bar{x}$ = 12 × 16 µm, n = 30). Columellae hyaline, subglobose, pyriform, 3.5–6.5 × 4.5–8 µm ($\bar{x}$ = 5.5 × 6.5 µm, n = 30), with apical projection and collarette. Sporangiospores hyaline, short cylindrical to oblong, slightly curved at both ends, smooth-walled, 3.5–5 × 2–2.5 µm ($\bar{x}$ = 4 × 2 µm, n = 40). Chlamydospores not observed. Rhizoids present, poorly branched.

Sexual morph on MEA at 25 °C after 30 d: Homothallic. Zygosporangia globose to subglobose, 50–77.5 × 59.5–76.5 µm ($\bar{x}$ = 63 × 65.5 µm, n = 30), brown. Finger-like appendages from unequal suspensors.

Culture characteristics – Colony grows faster in MEA than PDA. Within 3 d, the colony attains a diameter of 35 mm at 25 °C in PDA, while in MEA, it reaches 45 mm. At 15 and 20 °C, colony growth rate is similar from day 1 to day 3, in both MEA and PDA. The same is observed at temperatures 25 and 30 °C. Day-old cultures in MEA, at 25 °C, are white with a slight yellowish to pale brown tint around the inoculation plug. At this stage, only mycelial growth is observed, no reproductive structures, such as the sporangia, are produced. On day 2, the colony has a pale brown color and the outer most part of the colony is white (perimeter). Few sporangia on single sporangiophores are observed. On day 3, a pale brown colony with a white outer most layer persists. Production of sporangia and sporangiophores remain minimal, with most sporangia still immature (no colored sporangia). Most of the sporangiophores and sporangia are formed near the inoculation plug, hence in the older part of the culture. Whorls of sporangiophores which are typical of *Absidia* are not observed and if observed only whorls of 2 are seen. At the same temperature (25 °C), growth is quite slow and sporangiophore and sporangia formation is rare to none in PDA. However, by day 3, zygosporangia with finger-like appendages can be observed. More zygosporangia appeared to be produced on PDA than in MEA, in which only a few are observed. The fungus can grow at 15, 20, 25, 30 °C, with the optimal range being 25–30 °C. Growth was not observed at 10 and 37 °C.

Material examined: Thailand, Nan province, isolated from soil, on January 2020, collected and isolated by Vedprakash G. Hurdeal, ex-type culture, MFLUCC 23–0061; holotype: MFLU 23–0108.

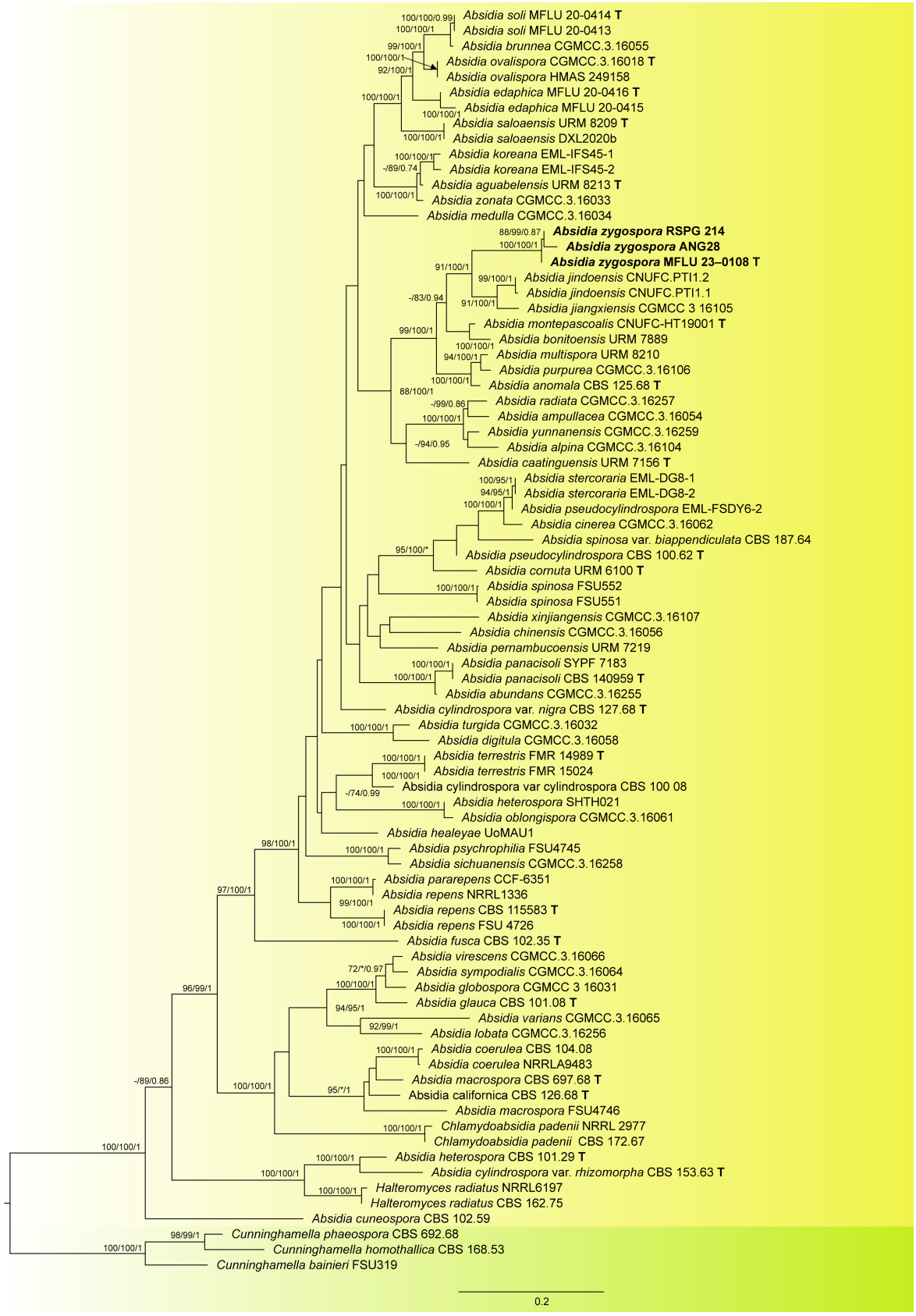

Fig. 1 Maximum likelihood phylogram inferred from 82 taxa and 1401 characters based on ITS, and LSU matrix using GTR+G+I model. ML bootstrap supports (≥ 70%) and Bayesian posterior probability (≥ 0.70) are indicated above the branches or near the nodes as ML/ML/PP. Tree is rooted using *Cunninghamella homothallica* (CBS 168.53), *C. phaeospora* (CBS 692.68), and *C. bainieri* (FSU319). Strains of the new species are in bold and the type species in the dataset are indicated using T. (–) represent bootstrap support lower than 70%.

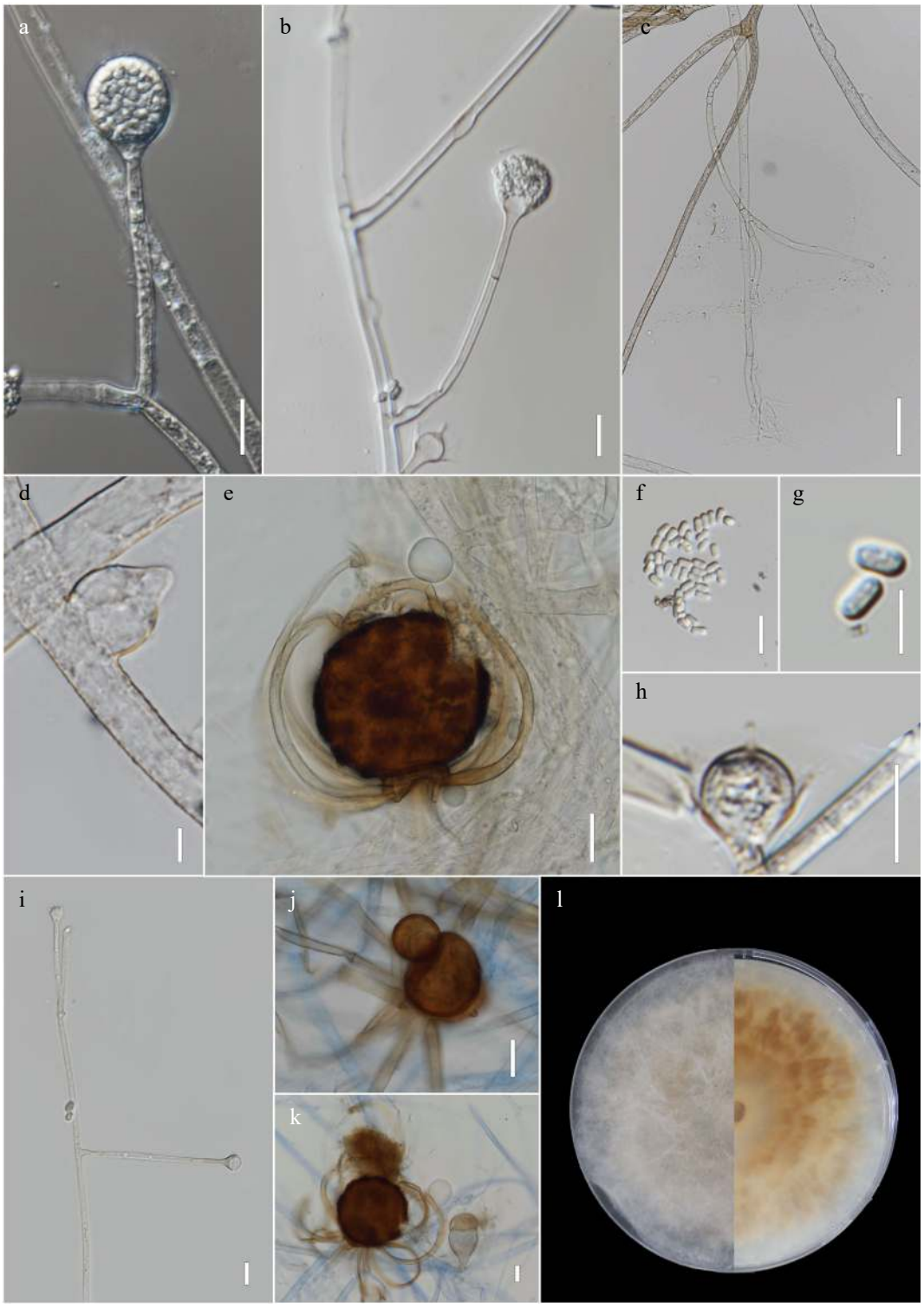

Fig. 2 *Absidia zygospora* MFLUCC 23–0061 (ex-type). (a) Developing sporangium. (b) Simple sporangiophore with sporangium, and subsporangial septation and columella with apical projection. (c) Rhizoids, (d) Swollen hyphae. (e), (j)–(k) Zygospores with unequal suspensors and finger-like appendages. (f)–(g) Sporangiospores. (h) Columella with apical projection. (i) Simple branching of sporangiophores. (l) Front and obverse images of culture in PDA. Scale bars: a–d, f, h, i = 10 μm, e, j–k = 20 μm, g = 5 μm.

Notes: In the phylogenetic analyses *A. zygospora* grouped as sister to the clade formed by *A. jindoensis* and *A. jiangxiensis* with high statistical support. Genetic distances between the new species and its sister taxa also provided further evidence for the introduction of the new taxon. Physiological data show that the new species has a lower growth rate than *A. jiangxiensis*. A more significant difference in rate of growth was seen between *A. zygospora* and *A. jindoensis* (45 mm vs 90 mm at 25 °C in MEA after 3 d). The new species produces smaller sporangia, and columella than sister taxa. The zygospores

produced by *A. zygospora* are within the range of the other species, and are produced on significantly unequal suspensors. Given that equal suspensors comprise a defining characteristic of *Absidia* an amendment in the genus description needs to be undertaken. The same phenomenon is seen in *A. jiangxiensis*. The production of the appendages is usually from the larger suspensor (also seen in *A. jiangxiensis*).

Discussion

Ecologically, *Absidia* species contribute to organic matter decomposition which is vital for recycling nutrients and have other functions such as in soil structure and aggregation. In this study, a new *Absidia* species, isolated from soil, is introduced based on morphological data, colony growth and phylogenetic analyses. The new species has smaller sporangia, columella and spores compared to the sister taxa (clade comprising of *Absidia jindoensis* CNUFC-PTI1-2, CNUFC-PTI1-1 and *Absidia jiangxiensis* CGMCC.3.16105). Inferred phylogeny indicates that our isolate is new with statistical support obtained by maximum likelihood (IQ-Tree and RAxML) analyses and Bayesian inference. Herein, phylogeny is inferred based on ITS and LSU genetic markers which are the most available sequences for this genus. ITS is generally considered the barcode for fungi. For *Absidia*, ITS can generally be used to differentiate species. However, cloning is often required to obtain the good quality ITS sequence data which renders the introduction and discovery of a new taxon difficult. As more taxa are introduced, the topology of the *Absidia* tree changes. This can be seen from the phylogenies of various taxonomic studies of this genus, highlighting the importance of taxon sampling[8–13]. Previously, spore shape was proposed as a taxonomically informative character of *Absidia*[7]. Our new species produces cylindrical spores and groups with *Absidia* species that produce similar spores. Hence, even with the establishment of more taxa, spore shape categorization is informative for initially identifying in which clade a species might be place.

In this study, the genetic distance in the ITS between *A. zygospora* MFLUCC T20-0309 and *A. jindoensis* ranges from 11.5%–13.5 %, and 24%–34.5 % to *A. jiangxiensis*, which seems in this case to meets the criterion for the establishment of a new species[22,23].

Our isolate forms a clade with the unclassified strains *Absidia* sp. RSPG 214 and soil fungal sp. ANG28, which are therefore referred to as *A. zygospora*. This supplements evidence to the introduction of the new species by providing additional information in terms of rDNA sequences and increasing taxon sampling. With several strains isolated from various places of the globe, important ecological information can be deducted in terms of the distribution. Interestingly, *Absidia* sp. RSPG 214 was also found in Thailand, but in Surat Thani, a southern province providing clues on the distribution of this species in the country. Meanwhile, ANG28 was isolated from soil in the United States.

Currently, few reports on the sexual morph of *Absidia* species has been published. In this study, we provide a description based on both the sexual and asexual stages of *A. zygospora*. The sexual stage is typical of the genus with finger-like appendages, but with unequal suspensors. The presence of unequal suspensors is in conflict with the generic description whereby, the genus is said to produce zygospore strictly on equal suspensors. Hence, the generic description of *Absidia* is amended to include zygospores produced on both equal and unequal suspensors. This finding underscores our limited knowledge of this genus and emphasizes the necessity for further taxonomic investigations, including the discovery of more species. Moreover, accurate taxonomic identification is essential for comprehending the biodiversity and distribution patterns of *Absidia* species, including the newly isolated species from the Nan Province in Thailand.

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Conflict of interest

The authors declare that they have no conflict of interest.

Dates

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References

1. 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
2. Wijayawardene N, Hyde KD, Al-Ani LKT, Tedersoo L, Haelewaters D, et al. 2020. Outline of Fungi and fungus-like taxa. *Mycosphere* 11:1160–456
3. Voigt K, James TY, Kirk PM, Santiago ALCM de A, Waldman B, et al. 2021. Early-diverging fungal phyla: taxonomy, species concept, ecology, distribution, anthropogenic impact, and novel phylogenetic proposals. *Fungal Diversity* 109:59–98
4. 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
5. Hoffmann K, Walther G, Voigt K. 2009. *Mycocladus* vs. *Lichtheimia*: a correction (*Lichtheimiaceae* fam. nov., Mucorales, Mucoromycotina). *Mycological Research* 113:277–8
6. Hoffmann K. 2010 Identification of the genus *Absidia* (Mucorales, Zygomycetes): a comprehensive taxonomic revision. In *Molecular identification of fungi*, eds. Gherbawy Y, Voigt K. Berlin, Heidelberg: Springer. pp. 439-60. https://doi.org/10.1007/978-3-642-05042-8_19
7. 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
8. Zhang T, Yu Y, Zhu H, Yang S, Yang T, et al. 2018. *Absidia panacisoli* sp. nov., isolated from rhizosphere of *Panax notoginseng*. *International Journal of Systematic and Evolutionary Microbiology* 68:2468–72

9. 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
10. Zong T, Zhao H, Liu X, Ren L, Zhao C, et al. 2021. Taxonomy and phylogeny of four new species in *Absidia* (Cunninghamellaceae, Mucorales) from China. *Frontiers in Microbiology* 4:12
11. Lima DX, Cordeiro TRL, De Souza CAF, De Oliveira RJV, Lee HB, et al. 2020. Morphological and molecular evidence for two new species of *Absidia* from Neotropic soil. *Phytotaxa* 446:61–71
12. Zhao H, Nie Y, Zong T, Dai Y, Liu X. 2022. Three new species of *Absidia* (Mucoromycota) from China based on phylogeny, morphology and physiology. *Diversity* 2:132
13. 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
14. 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* 11:2678–754
15. White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics. In *PCR Protocols*, eds. Innis MA, Gelfand DH, Sninsky JJ, White TJ. Academic Press. pp. 315–22. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
16. 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
17. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215:403–10
18. Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. 2009. TrimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25:1972–73
19. 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*. pp. 1–8. https://doi.org/10.1109/GCE.2010.5676129
20. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32:268–74
21. Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–55
22. Jeewon R, Hyde KD. 2016. Establishing species boundaries and new taxa among fungi: Recommendations to resolve taxonomic ambiguities. *Mycosphere* 7:1669–77
23. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, et al. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *PNAS* 109:6241–46