


Article

Three New Species of *Absidia* (Mucoromycota) from China Based on Phylogeny, Morphology and Physiology

Heng Zhao ^{1,2} , Yong Nie ³, Tongkai Zong ⁴, Yucheng Dai ^{1,*} and Xiaoyong Liu ^{2,*}

¹ Institute of Microbiology, School of Ecology and Nature Conservation, Beijing Forestry University, Beijing 100083, China; zhaoheng181@mails.ucas.ac.cn

² College of Life Sciences, Shandong Normal University, Jinan 250014, China

³ School of Civil Engineering and Architecture, Anhui University of Technology, Ma'anshan 243002, China; lyly19851207@126.com

⁴ Key Laboratory for Forest Resources Conservation and Utilization in the Southwest Mountains of China, Ministry of Education, Southwest Forestry University, Kunming 650224, China; zongfungi@163.com

* Correspondence: yuchengdai@bjfu.edu.cn (Y.D.); liuxiaoyong@im.ac.cn (X.L.)

Abstract: Species of *Absidia* are distributed widely in the environment, while their diversity is insufficiently studied. Three new species, *A. frigida*, *A. gemella* and *A. longissima*, are proposed herein from Xinjiang and Yunnan in China based on phylogenetic, morphological and physiological evidence. According to maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) analyses, the phylogenetical results suggest that *A. frigida*, *A. gemella* and *A. longissima* are closely related to *A. psychrophilia*, *A. turgida* and *A. zonata* and *A. koreana*, respectively, based on ITS and LSU rDNA sequences. *Absidia frigida* is characterized by a lower growth temperature, which does not grow above 24 °C. It differs from *A. psychrophilia* by sporangiophores, sporangia, columellae, collars and projections. *Absidia gemella* is distinguished from *A. turgida* by hypha, sporangiospores, sporangia, projections and sporangiophores. *Absidia longissima* is discriminated from *A. zonata* and *A. koreana* by sporangiophores, columellae and collars. The three new species are described and illustrated in this article.



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Keywords: basal fungi; new taxa; molecular phylogeny; fungal diversity

1. Introduction

Absidia Tiegh. is a core genus of early-diverging fungi, belonging to Mucoromycota, Mucoromycotina, Mucoromycetes, Mucoromycetes, Mucorales and Cunninghamellaceae (www.indexfungorum.org, accessed on 27 December 2021). *Absidia* was erected in 1876 and typified by *A. reflexa* Tiegh. [1,2]. Members of *Absidia* are widely distributed in soils, plant residues, herbivorous dung, decaying substrates and air [2–10]. In particular, some species are found in the mycangia of ambrosia beetles (*A. psychrophilia*) [11,12], as well as the body surface of bats (*A. stercoraria*: <https://bccm.belspo.be/content/remarkable-fungal-biodiversity-northern-belgium-bats>, accessed on 27 December 2021). Species within *Absidia* possess important metabolites for industrial and medical applications, such as steroids, α -galactosidase, laccase, fatty acids and chitosan [13–18].

Species of *Absidia* produce stolons. Sporangiophores form on the middle part of the stolons, while rhizoids form at both ends. Sporangia are multi-spored, pyriform to globose, deliquescent-walled and apophysate [2–4,19]. Columellae are conical, suglobose to globose or applanate, commonly with one to several projections [2,4,20,21]. Zygospores are contained in zygosporangia, and their opposite suspensory cells are appendaged [13,20].

Some mycologists and taxonomists, such as Bainier, Hagemann, Lendner, Hessel-tine, Ellis and Schipper, not only described and classified the genus *Absidia* s.l. but also inferred its phylogeny based on morphological and physiological features [13,22–25]. Incorporating molecular data, morphological traits and growth temperatures, the traditional classification of *Absidia* s.l. has been revised and divided into three genera, namely,

Lentamyces (parasitic on other mucoralean fungi, optimum growth temperatures between 14 °C and 25 °C), *Absidia* s.s. (mesophilic, optimum growth temperatures between 25 °C and 34 °C) and *Lichtheimia* (thermotolerant, optimum growth temperatures between 37 °C and 45 °C) [13,20,21].

Currently, 43 species are described in *Absidia* (Table 1, www.indexfungorum.org, accessed on 13 December 2021), with type strains originating from 17 countries, including Australia, Brazil, Canada, China, Cuba, the Czech Republic, Egypt, France, Holland, India, Mexico, Pakistan, South Korea, Switzerland, Tanzania, Thailand and the USA. Until now, 14 species have been recorded in China [3,4,26], and more potential novel species are being illustrated [27]. In this paper, three new species, *A. frigida*, *A. gemella* and *A. longissima*, are described from soil in China according to phylogenetic, morphological and physiological evidence.

Table 1. The information of type strains of species in *Absidia*.

Name	Authors	Record in China	Year of Publication	Host	Location of Type Strains
<i>Absidia reflexa</i>	Tiegh.	no	1878	unknown	France
<i>A. repens</i>	Tiegh.	yes	1878	unknown	France
<i>A. dubia</i>	Bainier	no	1882	unknown	Unknown
<i>A. caerulea</i>	Bainier	no	1889	on wet bread	France
<i>A. spinosa</i>	Lendn	yes	1907	soil	Switzerland
<i>A. cylindrospora</i>	Hagem	yes	1908	unknown	Unknown
<i>A. glauca</i>	Hagem	yes	1908	unknown	Unknown
<i>A. heterospora</i>	Y. Ling	yes	1930	pine forest soil	Holland
<i>A. fusca</i>	Linnem	no	1936	unknown	Unknown
<i>A. egyptiaca</i>	R. Sartory, J. Mey. & Tawfic	no	1939	unknown	Egypt
<i>A. cuneospora</i>	G.F. Orr & Plunkett	no	1959	soil	USA
<i>A. pseudocylindrospora</i>	Hesselt. & J.J. Ellis	yes	1962	soil	Tanzania
<i>A. psychrophilia</i>	Hesselt. & J.J. Ellis	yes	1964	glands of <i>Curculionidae</i>	Canada
<i>A. anomala</i>	Hesselt. & J.J. Ellis	no	1964	soil	Cuba
<i>A. californica</i>	J.J. Ellis & Hesselt	no	1965	dung of <i>Rattus</i>	USA
<i>A. clavata</i>	B.S. Mehrotra & Nand	no	1967	dung of <i>Bos taurus</i>	India
<i>A. macrospora</i>	Váňová	no	1968	mountain forest soil	Czech Republic
<i>A. fassatiae</i>	Váňová	no	1971	soil	Czech Republic
<i>A. inflata</i>	J.H. Mirza, S.M. Khan, S. Begum & Shagufta	no	1979	soil	Pakistan
<i>A. narayanae</i>	Subrahm.	no	1990	on bat guano	India
<i>A. idahoensis</i>	Hesselt., M.K. Mahoney & S.W. Peterson	yes	1990	<i>Nomia melanderi</i>	USA
<i>A. caatingaensis</i>	D.X. Lima & A.L. Santiago	no	2015	soil	Brazil
<i>A. koreana</i>	Hyang B. Lee, Hye W. Lee & T.T. Nguyen	no	2015	soil	South Korea
<i>A. stercoraria</i>	Hyang B. Lee, H.S. Lee & T.T.T. Nguyen	no	2016	dung of rat	South Korea
<i>A. panacisoli</i>	T. Yuan Zhang, Ying Yu, He Zhu, S.Z. Yang, T.M. Yang, Meng Y. Zhang & Yi X. Zhang	yes	2018	rhizosphere of <i>Panax notoginseng</i>	China
<i>A. terrestris</i>	Rosas de Paz, Dania García, Guarro, Cano & Stchigel	no	2018	soil	Mexico
<i>A. jindoensis</i>	Hyang B. Lee & T.T.T. Nguyen	no	2018	rhizosphere soil of <i>Coniferae</i>	South Korea
<i>A. cornuta</i>	D.X. Lima, C.A. de Souza, H.B. Lee & A.L. Santiago	no	2020	soil	Brazil
<i>A. pernambucoensis</i>	D.X. Lima, Souza-Motta & A.L. Santiago	no	2020	soil	Brazil

Table 1. Cont.

Name	Authors	Record in China	Year of Publication	Host	Location of Type Strains
<i>A. multispora</i>	T.R.L. Cordeiro, D.X Lima, Hyang B. Lee & A.L. Santiago	no	2020	soil	Brazil
<i>A. saloensis</i>	T.R.L. Cordeiro, D.X Lima, Hyang B. Lee & A.L. Santiago	no	2020	soil	Brazil
<i>A. pararepens</i>	Jurjević, M. Kolařík & Hubka	no	2020	air	USA
<i>A. healeyae</i>	A.S. Urquhart & A. Idnurm	no	2021	leaf litter	Australia
<i>A. aguabelensis</i>	J.D. Leitão, T.R.L. Cordeiro, Hyang B. Lee & A.L. Santiago	no	2021	soil	Brazil
<i>A. montepascoalis</i>	L.W.S. Freitas, Hyang B. Lee, T.T.T. Nguyen	no	2021	soil	Brazil
<i>A. bonitoensis</i>	C.L. Lima, D.X. Lima, Hyang B. Lee & A.L. Santiago	no	2021	soil	Brazil
<i>A. ovalispora</i>	H. Zhao & X.Y. Liu	yes	2021	soil	China
<i>A. globospora</i>	T.K. Zong & X.Y. Liu	yes	2021	soil	China
<i>A. medulla</i>	T.K. Zong & X.Y. Liu	yes	2021	soil	China
<i>A. turgida</i>	T.K. Zong & X.Y. Liu	yes	2021	soil	China
<i>A. zonata</i>	T.K. Zong & X.Y. Liu	yes	2021	soil	China
<i>A. edaphica</i>	V.G. Hurdeal, E. Gentekaki, Hyang B. Lee & K.D. Hyde	no	2021	soil	Thailand
<i>A. soli</i>	V.G. Hurdeal, E. Gentekaki, Hyang B. Lee & K.D. Hyde	no	2021	soil	Thailand
<i>A. frigida</i> *	H. Zhao, Y.C. Dai & X.Y. Liu	yes	2021	soil	China
<i>A. gemella</i>	H. Zhao, Y.C. Dai & X.Y. Liu	yes	2021	soil	China
<i>A. longissima</i>	H. Zhao, Y.C. Dai & X.Y. Liu	yes	2021	soil	China

* Species proposed herein are shown in bold.

2. Materials and Methods

2.1. Sample Collection and Strain Isolation

Soil samples were collected from Yunnan and Xinjiang in China in September 2021. Then, strains were isolated according to the method in previous studies [3,4]. In brief, soil (1 g) was suspended in sterile water (100 mL), and then the suspension (100 µL) was spread on plates with potato dextrose agar (PDA: 200 g potato, 20 g glucose, 20 g agar and 1000 mL distilled water) supplied with streptomycin sulfate (100 mg/mL) and ampicillin (100 mg/mL). The plates were incubated in the dark at 20 °C and 25 °C. Colonies were purified and then deposited in the China General Microbiological Culture Collection Center, Beijing, China (CGMCC). Cultures were also dried and deposited in the Herbarium Mycologicum Academiae Sinicae, Beijing, China (HMAS).

2.2. Morphology and Maximum Growth Temperature

Morphological observations and maximum growth temperature tests followed the method by Zheng et al. [28–33]. In brief, malt extract agar medium [34] (MEA: malt extract 30 g, peptone 3 g, agar 20 g, 1000 mL distilled water), a stereomicroscope (SMZ1500, Nikon, Tokyo, Japan) and a microscope (Axio Imager A2, Carl Zeiss, Oberkochen, Germany) were used. For describing morphological characteristics, a range between the minimum and maximum sizes based on a statistic of more than 20 measurements was adopted. For maximum growth temperature tests, plates were firstly incubated at 20 °C for 2 d, and then the incubation temperature increased by a gradient of 1 °C until the colonies stopped growing.

2.3. DNA Extraction, Amplification and Sequencing

Colonies were grown at 20 °C and 25 °C on synthetic mucor agar medium (SMA: dextrose 20 g, asparagine 2 g, KH₂PO₄ 0.5 g, MgSO₄·H₂O 0.25 g, thiamin chloride 0.5 mg, agar 20 g, 1000 mL distilled water, pH7) for a week. Total cell DNAs were extracted with

a reagent kit (GO-GLP-400, GeneOnBio Corporation, Changchun, China). A fragment covering the entire internal transcribed spacer (ITS) and a partial large subunit of ribosomal DNA (LSU rDNA) were amplified with the primer pair NS5M and LR5M (5'-GGC TTA ATT TGA CTC AAC ACG G-3' and 5'-GCT ATC CTG AGG GAA ACT TCG-3', respectively). The polymerase chain reaction (PCR) program followed Zhao et al. [3]. Sanger sequencing for PCR products was conducted by an external company (BGI Tech Solutions Beijing Liuhe Co., Limited, Beijing, China). ITS and LSU rDNA sequences were assembled and proofread with Geneious 9.0.2 (<http://www.geneious.com>, accessed on 1 May 2021) and then submitted to GenBank under the accession numbers in Table 2; top hits of the BLAST search for ITS sequences are provided in Supplementary Table S1.

Table 2. The GenBank accession numbers of sequences used in this study.

Species *	Strains **	GenBank Accession nos.	
		ITS	LSU
<i>Absidia anomala</i>	CBS 125.68 ^T	NR_103626	NG_058562
<i>A. bonitoensis</i>	URM 7889 ^T	MN977786	MN977805
<i>A. caatinguensis</i>	URM 7156 ^T	KT308169	KT308171
<i>A. californica</i>	CBS 314.78	MH861141	MH872902
<i>A. californica</i>	FSU 4747	AY944872	EU736300
<i>A. californica</i>	FSU 4748	AY944873	EU736301
<i>A. coerulea</i>	CBS 101.36	MH855718	MH867230
<i>A. coerulea</i>	FSU 767	AY944870	AF113443
<i>A. cornuta</i>	URM 6100	MN625256	MN625255
<i>A. cuneospora</i>	CBS 101.59 ^T	NR_159602	NG058559
<i>A. cylindrospora</i>	CBS 100.08	JN205822	JN206588
<i>A. edaphica</i>	MFLU 20-0416	MT396372	MT393987
<i>A. frigida</i>	CGMCC 3.16201 ^T	OM108487	OM030223
<i>A. fusca</i>	CBS 102.35	NR103625	NG058552
<i>A. gemella</i>	CGMCC 3.16202 ^T	OM108488	OM030224
<i>A. glauca</i>	CBS 129233	MH865253	MH876693
<i>A. glauca</i>	CBS 101.08 ^T	NR_111658	NG_058550
<i>A. glauca</i>	FSU 660	AY944879	EU736302
<i>A. globospora</i>	CGMCC 3.16031 ^T	MW671537	MW671544
<i>A. globospora</i>	CGMCC 3.16035	MW671538	MW671545
<i>A. globospora</i>	CGMCC 3.16036	MW671539	MW671546
<i>A. heterospora</i>	SHTH021	JN942683	JN982936
<i>A. jindoensis</i>	CNUFC-PTI1-1 ^T	MF926622	MF926616
<i>A. koreana</i>	EML-IFS45-1 ^T	KR030062	KR030056
<i>A. longissima</i>	CGMCC 3.16203 ^T	OM108489	OM030225
<i>A. macrospora</i>	FSU 4746	AY944882	EU736303
<i>A. medulla</i>	CGMCC 3.16034 ^T	MW671542	MW671549
<i>A. medulla</i>	CGMCC 3.16037	MW671543	MW671550
<i>A. multispora</i>	URM 8210 ^T	MN953780	MN953782
<i>A. ovalispora</i>	CGMCC 3.16018 ^T	MW264071	MW264130
<i>A. panacisoli</i>	SYPF 7183 ^T	MF522181	MF522180
<i>A. pararepens</i>	CCF 6352	MT193669	MT192308
<i>A. pernambucoensis</i>	URM 7219 ^T	MN635568	MN635569
<i>A. pseudocylindrospora</i>	CBS 100.62 ^T	NR_145276	NG_058561
<i>A. pseudocylindrospora</i>	EML-FSDY6-2	KU923817	KU923814
<i>A. psychrophilia</i>	FSU 4745	AY944874	EU736306
<i>A. repens</i>	CBS 115583 ^T	NR103624	HM849706
<i>A. saloensis</i>	URM 8209 ^T	MN953781	MN953783
<i>A. soli</i>	MFLU 20-0414	MT396373	MT393988
<i>A. spinosa</i>	FSU 551	AY944887	EU736307
<i>A. stercoraria</i>	EML-DG8-1 ^T	NR_148090	KT921998
<i>A. terrestris</i>	FMR 14989 ^T	LT795003	LT795005
<i>A. turgida</i>	CGMCC 3.16032 ^T	MW671540	MW671547

Table 2. Cont.

Species *	Strains **	GenBank Accession nos.	
		ITS	LSU
<i>A. zonata</i>	CGMCC 3.16033 ^T	MW671541	MW671548
<i>Cunninghamella blakesleeana</i>	CBS 782.68	JN205869	MH870950
<i>C. elegans</i>	CBS 167.53	JN205882	HM849700

* Sequences obtained herein are shown in bold. ** The “T” represents type strains.

2.4. Phylogenetic Analyses

For reconstructing phylogenetic trees, all the sequences were aligned with AliView (version 3.0) [35] and MAFFT (version 7, <https://mafft.cbrc.jp/alignment/server/>, accessed on 12 December 2021), and then manual proofreading was performed. Maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) analyses were all adopted for phylogenetic analyses as described in Nie et al. [36,37], using RAxML (version 8) with the GTRGAMMA substitution model [38], PAUP (version 4.0b10) [39] and MrBayes (version 3.2.7a) [40], respectively. Finally, sequence alignments and phylogenetic trees were deposited in TreeBase (submission ID SS29123).

3. Results

3.1. Taxonomy

In this study, we propose three new species of *Absidia* from Xinjiang and Yunnan, China (Tables 1 and 2; Figures 1–4). All these novel taxa were demonstrated by molecular sequences, morphology and physiology.

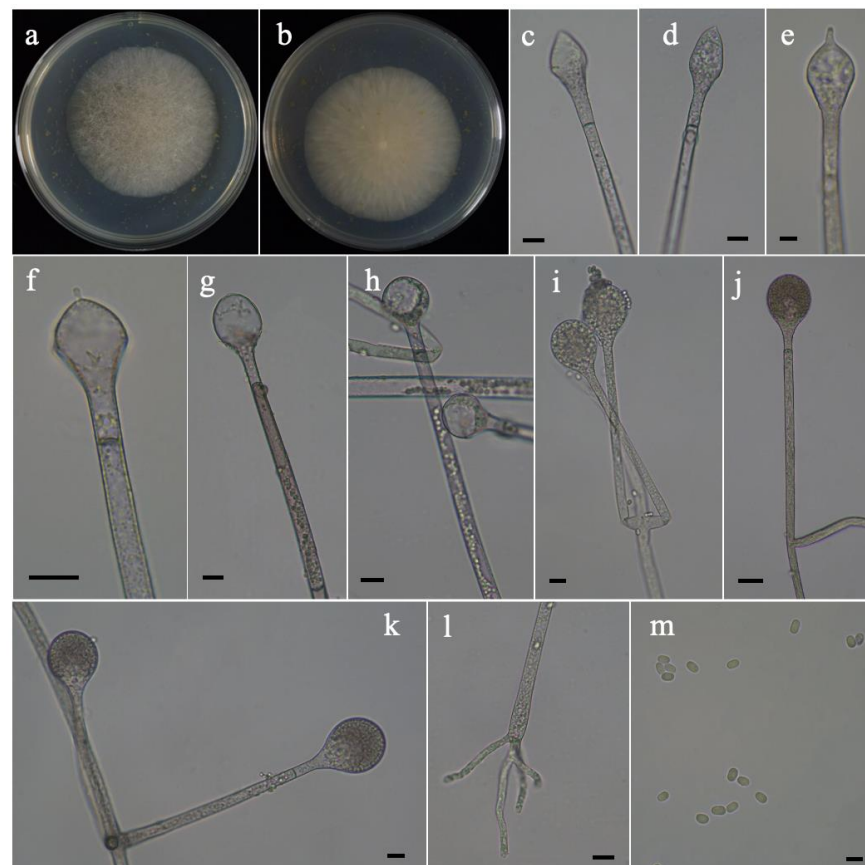


Figure 1. Morphologies of *Absidia frigida* ex-holotype CGMCC 3.16201. (a,b) Colonies on MEA, (a) obverse, (b) reverse; (c–h) columellae and projection; (i–k) sporangium; (l) rhizoids; (m) sporangiospores. Scale bars: (c–l) 10 μ m, (m) 5 μ m.



Figure 2. Morphologies of *Absidia gemella* ex-holotype CGMCC 3.16202. (a,b) Colonies on MEA, (a) obverse, (b) reverse; (c) monopodial sporangiophores; (d) sympodial sporangiophore; (e,f) columellae; (g–i) sporangium; (j,k) rhizoids; (l) substrate hyphae with swellings; (m) sporangiospores. Scale bars: (c) 20 μm , (d–l) 10 μm , (m) 5 μm .

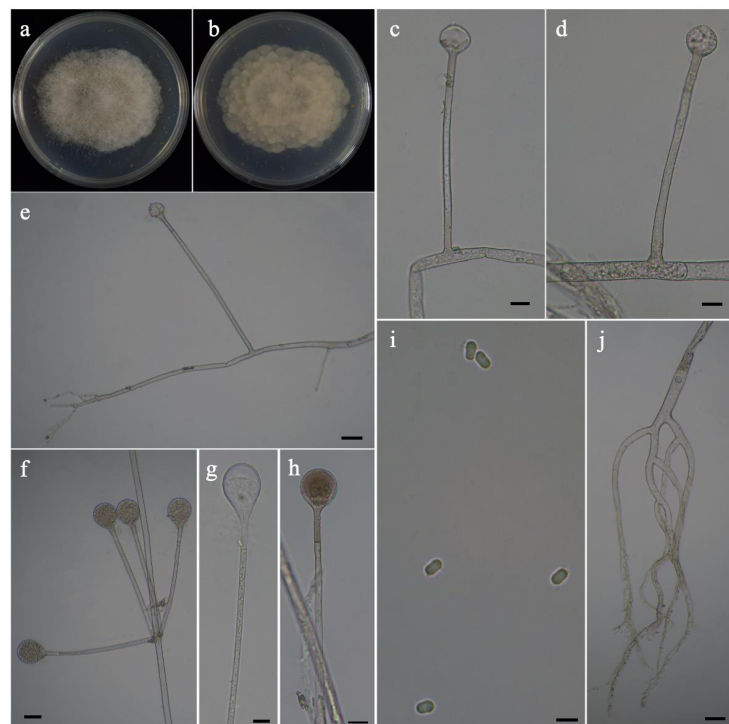


Figure 3. Morphologies of *Absidia longissima* ex-holotype CGMCC 3.16203. (a,b) Colonies on MEA, (a) obverse, (b) reverse; (c), (d) columellae and projection; (e) sporangiophores arising from stolons borne on rhizoids; (f–h) sporangium or sporangia; (i) sporangiospores; (j) rhizoids. Scale bars: (c,d,g,h) 10 μm , (e,f,j) 20 μm , (i) 5 μm .

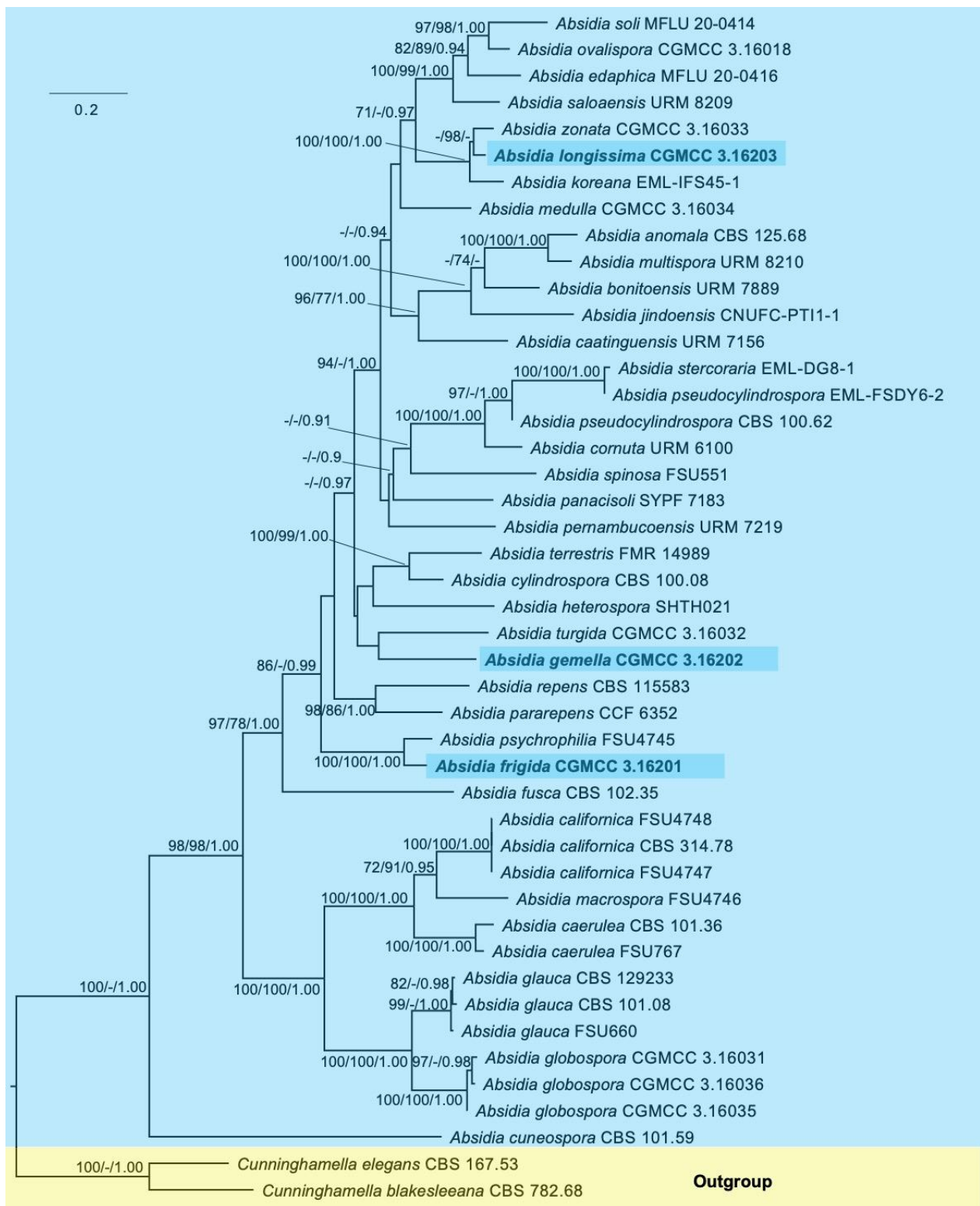


Figure 4. Maximum likelihood phylogenetic tree of *Absidia* based on ITS and LSU rDNA sequences, with *Cunninghamamella elegans* and *C. blakesleeana* as outgroups. The three new species, *A. frigida*, *A. gemella* and *A. longissimi*, are shaded. Maximum likelihood (ML) bootstrap values ($\geq 70\%$)/maximum parsimony (MP) bootstrap values ($\geq 70\%$)/Bayesian inference (BI) posterior probabilities (≥ 0.9) of each clade are indicated along branches. Scale bar in the upper left indicates substitutions per site.

3.1.1. *Absidia frigida* H. Zhao, Y.C. Dai & X.Y. Liu, sp. nov.

Fungal Names: FN570961. Figure 1.

Etymology: *frigida* (Lat.) referring to a relatively lower maximum growth temperature of no more than 24 °C.

Holotype: HMAS 351587.

Description: Colonies on MEA at 20 °C for 7 days, growing moderately slow, attaining 60 mm diameter, white at first, gradually becoming light brown, regularly at reverse. Hyphae branched, hyaline at first, sometimes brownish when mature, aseptate when juvenile, septate with age, 5.5–18.5 µm wide. Stolons branched, hyaline, brownish, smooth, with septa. Rhizoids finger-like, rarely present, always unbranched, rarely branched, few swollen at the top. Sporangioophores arising from rhizoids, or substrate hyphae, erect or slightly bent, 1–4 in whorls, unbranched or simple branched, while sympodial not observed, hyaline, sometimes with a septum 16.0–23.0 µm below apophyses, 21.0–300.0 µm or more in length and 3.0–7.0 µm in width. Sporangia subglobose to pyriform, deliquescent-walled, smooth, multi-spored, colorless when young, pigmented when elder, 13.0–33.0 µm long and 12.5–32.0 µm wide. Apophyses distinct, hyaline, subhyaline or slightly pigmented, 4.5–9.5 µm high, 3.5–7.0 µm wide at the base and 7.0–11.5 µm wide at the top. Collars absent. Columellae conical, ovoid, elliptical, subglobose to globose, hyaline or subhyaline, smooth, 16.0–23.0 µm long and 15.5–18.0 µm wide, or 13.0–20.5 µm in diameter. Projections present or absent, if present, always one only, rarely two, small, hyaline or subhyaline, 1.5–3.0 µm long. Sporangiospores cylindrical, hyaline to subhyaline, smooth, 2.5–4.5 µm long and 2.0–3.0 µm wide. Zygosporos not observed. Chlamydospores absent.

Maximum growth temperature: 24 °C.

Material examined: China, Xinjiang, Ili Kazak, Zhaosu County, 43°13'58" N, 81°10'45" E, altitude: 2219 m, from soil sample, 31 October 2021, Heng Zhao (holotype HMAS 351587, living ex-holotype culture CGMCC 3.16201).

3.1.2. *Absidia gemella* H. Zhao, Y.C. Dai & X.Y. Liu, sp. nov.

Fungal Names: FN570962. Figure 2.

Etymology: *gemella* (Lat.) referring to producing two types of sporangiospores.

Holotype: HMAS 351588.

Description: Colonies on MEA at 27 °C for 7 days, growing slow, attaining 45 mm diameter, irregular zonate, white at first, become brown when older, irregular at reverse. Hyphae branched, hyaline at first, brownish when mature, aseptate when juvenile, septate with age, 4.5–17.0 µm wide, substrate hyphae well developed, always branched, sometimes formed elliptical, subglobose to globose swollen in the internal. Stolons branched, hyaline, brownish, smooth, septate. Rhizoids root-like, not well developed, always branched, rarely with a septum at the top. Sporangioophores arising from stolons, erect or slightly bent, often monopodial, simple branched, commonly sympodial, rarely 2–5 in whorls, sometimes a swelling beneath sporangium, hyaline, with one to several septa, 38.0 to more than 350.0 µm and 3.5–6.0 µm in width. Sporangia oval to pyriform, deliquescent-walled, smooth, multi-spored, colorless when young, pigmented when old, 16.0–25.5 µm long and 16.5–24.0 µm wide. Apophyses distinct, subhyaline to hyaline, 3.0–7.5 µm high, 3.5–7.0 µm wide at the base and 7.0–14.0 µm wide at the top. Collars present, small. Columellae globose, subglobose to elliptical, hyaline or pigmented, smooth or rough, 11.0–22.0 µm long and 12.5–17.5 µm wide. Projections absent. Sporangiospores two types, cylindrical, or subglobose to globose, hyaline or subhyaline, smooth, 3.0–4.5 µm long and 2.5–4.0 µm wide, or 2.5–3.5 µm in diameter. Zygosporos not observed. Chlamydospores absent.

Maximum growth temperature: 29 °C.

Material examined: China, Xinjiang, Altay City, Burqin County, 28°37'1" N, 87°2'58" E, altitude: 1330 m, from soil sample, 31 October 2021, Heng Zhao (holotype HMAS 351588, living ex-holotype culture CGMCC 3.16202).

3.1.3. *Absidia longissima* H. Zhao, Y.C. Dai & X.Y. Liu, sp. nov.

Fungal Names: FN570963. Figure 3.

Etymology: *longissima* (Lat.) referring to producing very long rhizoids.

Holotype: HMAS 351589.

Description: Colonies on MEA at 27 °C for 7 days, growing moderately fast, attaining 70 mm diameter, irregular concentrically zonate with ring, white at first, gradually becoming gray, irregular at reverse. Hyphae branched, hyaline at first, brownish when mature, aseptate when juvenile, septate with age, 3.5–11.5 µm wide. Stolons branched, hyaline, brownish, smooth, with septa. Rhizoids root-like, well developed, always branched. Sporangiphores arising from stolons, erect or slightly bent, 2–5 in whorls, monopodial, unbranched or simple branched, while sympodial not observed, hyaline, with a septum 12.5–19.5 µm below apophyses, 40.0–190.0 µm or more in length and 2.5–4.5 µm in width. Sporangia globose to pyriform, deliquescent-walled, smooth, multi-spored, colorless when young, brownish when old, 9.5–28.0 µm long and 10.0–28.0 µm wide. Apophyses small, sometimes not distinct, subhyaline to hyaline, slightly pigmented, 2.5–7.5 µm high, 3.0–6.0 µm wide at the base and 4.5–17.5 µm wide at the top. Collars absent. Columellae subglobose to globose, hyaline, smooth, 7.0–11.5 µm in diameter. Projections present or absent, if present, one only. Sporangiospores cylindrical, hyaline, smooth, slightly contracts in center, 3.0–4.0 µm long and 1.5–2.0 µm wide. Zygosporangia not observed. Chlamydospores absent.

Maximum growth temperature: 36 °C.

Material examined: China, Yunnan Province, Qujing, Huize County, from soil sample, 15 November 2021, Heng Zhao (holotype HMAS 351589, living ex-holotype culture CGMCC 3.16203).

3.2. Phylogenetic Analyses

The phylogenetic trees of individual ITS and LSU rDNA are provided in Supplementary Figure S1a,b, respectively. The concatenated ITS and LSU rDNA sequence dataset consists of 45 taxa, including 36 species of *Absidia* and 2 species of the outgroup *Cunninghamella*. A total of 1652 sites are composed of 619 constant, 775 parsimony-informative and 258 parsimony-uninformative characters. The maximum parsimony (MP) tree result shows that the tree length (TL), consistency index (CI), homoplasy index (HI), retention index (RI) and rescaled consistency index (RC) are 5600, 0.3493, 0.6507, 0.5326 and 0.1860, respectively. The best model of Bayesian inference (BI) is GTR + I + G, and the average standard deviation of split frequencies is no more than 0.01. The topology of the maximum likelihood tree (ML) was chosen to represent the phylogenetic relationship (Figure 4), since ML, MP and BI resulted in similar topologies. The results suggest that *Absidia frigida* is closely related to *A. psychrophilia* (100/100/1.00); *Absidia gemella* is next to *A. turgida*; and, finally, *A. longissima* is closely related to *A. zonata* and *A. koreana* (100/100/1.00).

4. Discussion

The ITS and LSU rDNA tree (Figure 4) shows the phylogenetic positions of the three new species in the genus *Absidia*. In detail, *A. frigida* is closely related to *A. psychrophilia* with sufficient support values (100/100/1.00). However, *A. frigida* physiologically differs from *A. psychrophilia* by maximum growth temperature (25 °C vs. 28 °C) [11]. Additionally, morphologically, *A. frigida* is distinguished from *A. psychrophilia* by less whorls of sporangiphores (four vs. eight), smaller sporangia (13.0–33.0 µm long and 12.5–32.0 µm wide vs. 20.0–50.0 µm in diameter), smaller columellae (16.0–23.0 µm long and 15.5–18.0 µm wide, or 13.0–20.5 µm in diameter vs. 6.5–30.0 µm in diameter), no collars (absent vs. indistinct) and smaller projections (1.5–3.0 µm vs. 6.5 in length) [11].

Absidia gemella clusters with *A. turgida*, though their sibling relationship does not obtain a strong support. Physiologically, the maximum growth temperature of *A. gemella* is lower than that of *A. turgida* (30 °C vs. 33 °C). Morphologically, *A. gemella* is differentiated from *A. turgida* by narrower hyphae (4.5–17.0 µm wide vs. 9.0–23.0 µm wide),

narrower sporangiospores (3.5–6.0 μm in width vs. 4.5–11.0 μm in width), different shape of sporangia (oval to pyriform vs. globose to pyriform), smaller sporangia (16.0–25.5 μm long and 16.5–24.0 μm wide vs. 20.5–42.5 μm long and 20.0–41.5(–46.0) μm wide) and no projections (absent vs. 9.5 μm in length) [4]. Moreover, sporangiophores in *A. gemella* are monopodial, simple branched, commonly sympodial and rarely 2–5 in whorls, while in *A. turgida*, sporangiophores are 1–4 in whorls, unbranched or sometimes simple [4].

Phylogenetically, *Absidia longissima* is most closely related to *A. zonata* (-/98/-) and *A. koreana* (100/100/1.00). Physiologically, *A. longissima*, *A. zonata* and *A. koreana* also possess similar maximum growth temperatures: no growth at 37 °C, no growth at 38 °C and restricted growth at 37 °C, respectively [4,41]. However, *A. longissima* morphologically differs from *A. zonata* and *A. koreana* by whorls of sporangiophores (2–5 in whorls vs. 2–5(–8) in whorls vs. 2–6 in whorls), shape of columellae (subglobose to globose vs. hemispherical vs. globose), smaller columellae (7.0–11.5 μm in diameter vs. 9.5–19.0 μm long and (6.0–)7.5–14.5(–16.5) μm wide vs. 11.4–19.0 μm long and 11.0–17.0 μm wide) and collars (absent vs. absent or present vs. present around each columella) [4,41].

Morphologically, sporangiospores and projections play essential roles in distinguishing *Absidia* from other genera [13,20,21]. However, as the numbers of species in *Absidia* gradually increased, some species were found to possess two or more different shapes of sporangiospores, such as *A. gemella*, *A. multispora*, *A. pararepens*, *A. repens* and *A. turgida* [4,5,10], which suggested more characters should be adopted. In addition, the majority of species form projections on the apex of columellae, except *A. heterospora* [11]. The new species *A. gemella* does not produce projections on MEA plates even after ten days. Therefore, we believe that the morphological delimitation of the genus *Absidia* should be revised, especially for the character of projections.

Since 2015, a total of 22 species of *Absidia* have been proposed, outnumbering those described in the last century (Table 1). Remarkably, in the past two years, seven and five new species have been described in Brazil and China, respectively. At present, *Absidia* is already the second largest genus in the phylum Mucormycota (www.indexfungorum.org, accessed on 27 December 2021). Taking into account the 3 new species reported herein, 46 species are accepted from all around the world, among which 16 are recorded in China.

Currently, an increasing number of studies focus on fungal diversity and ecological distribution [3,4,42–48], which provides a foundation to deeply understand the kingdom Fungi. Although a total of 46 species are described in *Absidia*, their ecological distribution has still not been unraveled. A few of these species were found in dung, insects, leaf litter, etc., while most species were collected from soil (Table 1), which suggested that they may have complex ecological habits or hosts. In this study, the maximum growth temperature of the new species *A. frigida* was 24 °C, implying that some species of *Absidia* have adapted to low-temperature environments, and that maybe more potential species will be hidden in extreme habitats.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/d14020132/s1>, Figure S1: Maximum likelihood phylogenetic tree of *Absidia* based on ITS and LSU rDNA sequences, respectively, with *Cunninghamella elegans* and *C. blakesleeana* as outgroup, Table S1: Top hits for the new species based on BLAST search for ITS sequences from type materials.

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Data Availability Statement: Sequences and trees have been deposited in GenBank (Table 2) and TreeBase (number: SS29123), respectively.

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