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

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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.

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

Citation: Zhao, H.; Nie, Y.; Zong, T.-K.; Dai, Y.-C.; Liu, X.-Y. Three New Species of *Absidia* (Mucoromycota) from China Based on Phylogeny, Morphology and Physiology. *Diversity* **2022**, *14*, 132. https://doi.org/10.3390/d14020132

Academic Editors: Chang-Lin Zhao, Zai-Wei Ge and Michael Wink

Received: 28 December 2021
Accepted: 10 February 2022
Published: 13 February 2022

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 15 January 2022). *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). 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, Hesseltine, 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 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
Absidia reflexa	Tiegh.	no	1878	unknown	France
A. repens	Tiegh.	yes	1878	unknown	France
A. dubia	Bainier	no	1882	unknown	Unknown
A. caerulea	Bainier	no	1889	on wet bread	France
A. spinosa	Lendn	yes	1907	soil	Switzerland
A. cylindrospora	Hagem	yes	1908	unknown	Unknown
A. glauca	Hagem	yes	1908	unknown	Unknown
A. heterospora	Y. Ling	yes	1930	pine forest soil	Holland
A. fusca	Linnem	no	1936	unknown	Unknown
A. egyptiaca	R. Sartory, J. Mey. & Tawfic	no	1939	unknown	Egypt
A. cuneospora	G.F. Orr & Plunkett	no	1959	soil	USA
A. pseudocylindrospora	Hesselt. & J.J. Ellis	yes	1962	soil	Tanzania
A. psychrophilia	Hesselt. & J.J. Ellis	yes	1964	glands of *Curculionidae*	Canada
A. anomala	Hesselt. & J.J. Ellis	no	1964	soil	Cuba
A. californica	J.J. Ellis & Hesselt	no	1965	dung of *Rattus*	USA
A. clavata	B.S. Mehrotra & Nand	no	1967	dung of *Bos taurus*	India
A. macrospora	Váňová	no	1968	mountain forest soil	Czech Republic
A. fassatiae	Váňová	no	1971	soil	Czech Republic
A. inflata	J.H. Mirza, S.M. Khan, S. Begum & Shagufta	no	1979	soil	Pakistan
A. narayanae	Subrahm.	no	1990	on bat guano	India
A. idahoensis	Hesselt., M.K. Mahoney & S.W. Peterson	yes	1990	*Nomia melanderi*	USA
A. caatingaensis	D.X. Lima & A.L. Santiago	no	2015	soil	Brazil
A. koreana	Hyang B. Lee, Hye W. Lee & T.T. Nguyen	no	2015	soil	South Korea
A. stercoraria	Hyang B. Lee, H.S. Lee & T.T.T. Nguyen	no	2016	dung of rat	South Korea

A. panacisoli	T. Yuan Zhang, Ying Yu, He Zhu, S.Z. Yang, T.M. Yang, Meng Y. Zhang & Yi X. Zhang	yes	2018	rhizosphere of *Panax notoginseng*	China
A. terrestris	Rosas de Paz, Dania García, Guarro, Cano & Stchigel	no	2018	soil	Mexico
A. jindoensis	Hyang B. Lee & T.T.T. Nguyen	no	2018	rhizosphere soil of *Coniferae*	South Korea
A. cornuta	D.X. Lima, C.A. de Souza, H.B. Lee & A.L. Santiago	no	2020	soil	Brazil
A. pernambucoensis	D.X. Lima, Souza-Motta & A.L. Santiago	no	2020	soil	Brazil
A. multispora	T.R.L. Cordeiro, D.X Lima, Hyang B. Lee & A.L. Santiago	no	2020	soil	Brazil
A. saloaensis	T.R.L. Cordeiro, D.X Lima, Hyang B. Lee & A.L. Santiago	no	2020	soil	Brazil
A. pararepens	Jurjević, M. Kolařík & Hubka	no	2020	air	USA
A. healeyae	A.S. Urquhart & A. Idnurm	no	2021	leaf litter	Australia
A. aguabelensis	J.D. Leitão, T.R.L. Cordeiro, Hyang B. Lee & A.L. Santiago	no	2021	soil	Brazil
A. montepascoalis	L.W.S. Freitas, Hyang B. Lee, T.T.T. Nguyen	no	2021	soil	Brazil
A. bonitoensis	C.L. Lima, D.X. Lima, Hyang B. Lee & A.L. Santiago	no	2021	soil	Brazil
A. ovalispora	H. Zhao & X.Y. Liu	yes	2021	soil	China
A. globospora	T.K. Zong & X.Y. Liu	yes	2021	soil	China
A. medulla	T.K. Zong & X.Y. Liu	yes	2021	soil	China
A. turgida	T.K. Zong & X.Y. Liu	yes	2021	soil	China
A. zonata	T.K. Zong & X.Y. Liu	yes	2021	soil	China
A. edaphica	V.G. Hurdeal, E. Gentekaki, Hyang B. Lee & K.D. Hyde	no	2021	soil	Thailand
A. soli	V.G. Hurdeal, E. Gentekaki, Hyang B. Lee & K.D. Hyde	no	2021	soil	Thailand
A. frigida *	**H. Zhao, Y.C. Dai & X.Y. Liu**	**yes**	**2021**	**soil**	**China**
A. gemella	**H. Zhao, Y.C. Dai & X.Y. Liu**	**yes**	**2021**	**soil**	**China**
A. longissima	**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 uL) 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, KH_2PO_4 0.5 g, $MgSO_4 \cdot H_2O$ 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-GPLF-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 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
Absidia anomala	CBS 125.68 ^{T}	NR_103626	NG_058562
A. bonitoensis	URM 7889 ^{T}	MN977786	MN977805
A. caatinguensis	URM 7156 ^{T}	KT308169	KT308171
A. californica	CBS 314.78	MH861141	MH872902
A. californica	FSU 4747	AY944872	EU736300
A. californica	FSU 4748	AY944873	EU736301
A. coerulea	CBS 101.36	MH855718	MH867230
A. coerulea	FSU 767	AY944870	AF113443
A. cornuta	URM 6100	MN625256	MN625255
A. cuneospora	CBS 101.59 ^{T}	NR_159602	NG058559
A. cylindrospora	CBS 100.08	JN205822	JN206588
A. edaphica	MFLU 20–0416	MT396372	MT393987
A. frigida	**CGMCC 3.16201 ^{T}**	**OM108487**	**OM030223**
A. fusca	CBS 102.35	NR103625	NG058552
A. gemella	**CGMCC 3.16202 ^{T}**	**OM108488**	**OM030224**
A. glauca	CBS 129233	MH865253	MH876693
A. glauca	CBS 101.08 ^{T}	NR_111658	NG_058550
A. glauca	FSU 660	AY944879	EU736302
A. globospora	CGMCC 3.16031 ^{T}	MW671537	MW671544
A. globospora	CGMCC 3.16035	MW671538	MW671545
A. globospora	CGMCC 3.16036	MW671539	MW671546
A. heterospora	SHTH021	JN942683	JN982936
A. jindoensis	CNUFC-PTI1-1 ^{T}	MF926622	MF926616
A. koreana	EML-IFS45-1 ^{T}	KR030062	KR030056
A. longissima	**CGMCC 3.16203 ^{T}**	**OM108489**	**OM030225**
A. macrospora	FSU 4746	AY944882	EU736303

A. medulla	CGMCC 3.16034 T	MW671542	MW671549
A. medulla	CGMCC 3.16037	MW671543	MW671550
A. multispora	URM 8210 T	MN953780	MN953782
A. ovalispora	CGMCC 3.16018 T	MW264071	MW264130
A. panacisoli	SYPF 7183 T	MF522181	MF522180
A. pararepens	CCF 6352	MT193669	MT192308
A. pernambucoensis	URM 7219 T	MN635568	MN635569
A. pseudocylindrospora	CBS 100.62 T	NR_145276	NG_058561
A. pseudocylindrospora	EML-FSDY6-2	KU923817	KU923814
A. psychrophilia	FSU 4745	AY944874	EU736306
A. repens	CBS 115583 T	NR103624	HM849706
A. saloaensis	URM 8209 T	MN953781	MN953783
A. soli	MFLU 20-0414	MT396373	MT393988
A. spinosa	FSU 551	AY944887	EU736307
A. stercoraria	EML-DG8-1 T	NR_148090	KT921998
A. terrestris	FMR 14989 T	LT795003	LT795005
A. turgida	CGMCC 3.16032 T	MW671540	MW671547
A. zonata	CGMCC 3.16033 T	MW671541	MW671548
Cunninghamella blakesleeana	CBS 782.68	JN205869	MH870950
C. elegans	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.

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

Fungal Names: FN570961. Figure 1.

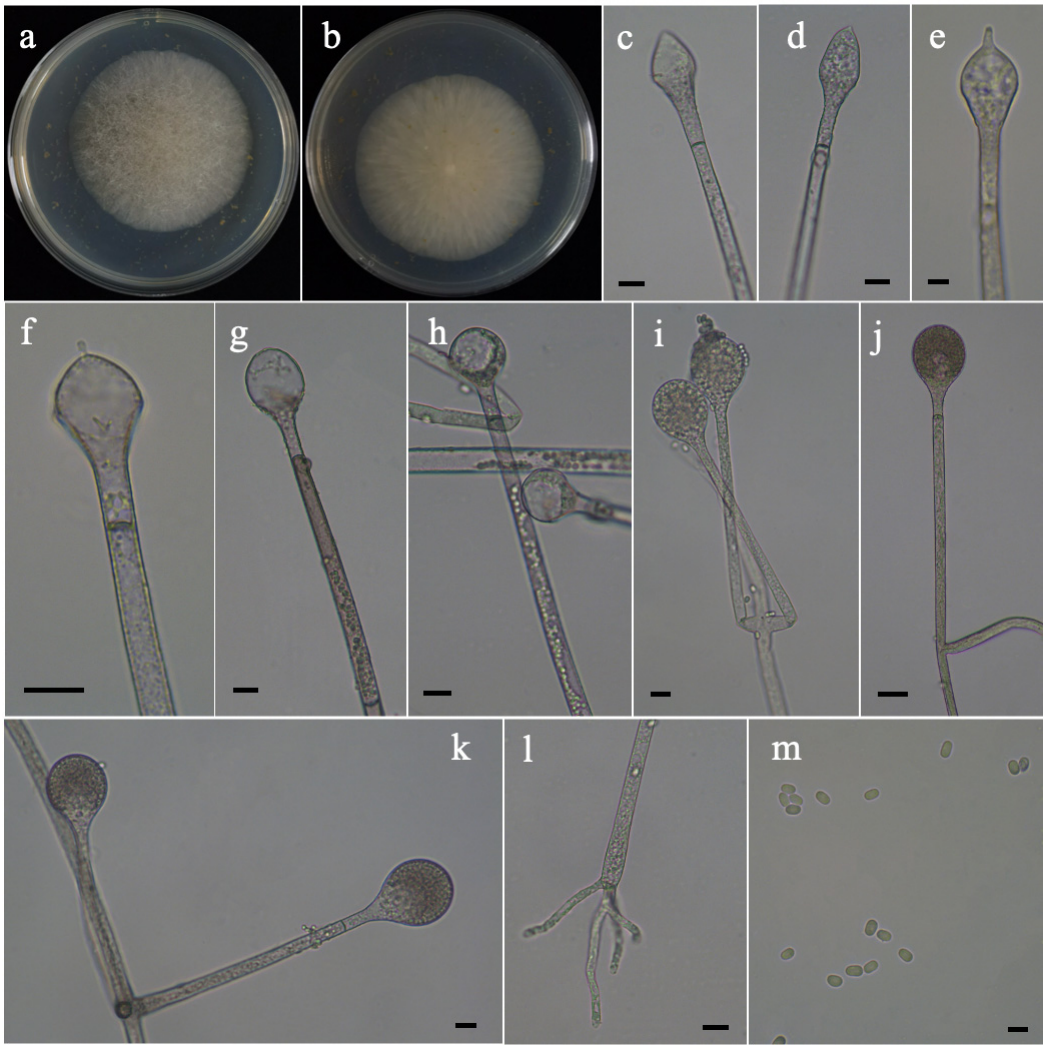

Figure 1. Morphologies of *Absidia frigida* ex-holotype CGMCC 3.16201. (**a,b**) Colonies on MEA, (**a**) obverse, (**b**) reverse; (**c–h**) columellae and projection; (**i–k**) sporangium; (**l**) rhizoids; (**m**) sporangiospores. Scale bars: (**c–l**) 10 μm, (**m**) 5 μm.

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. Sporangiophores 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. Zygospores 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.

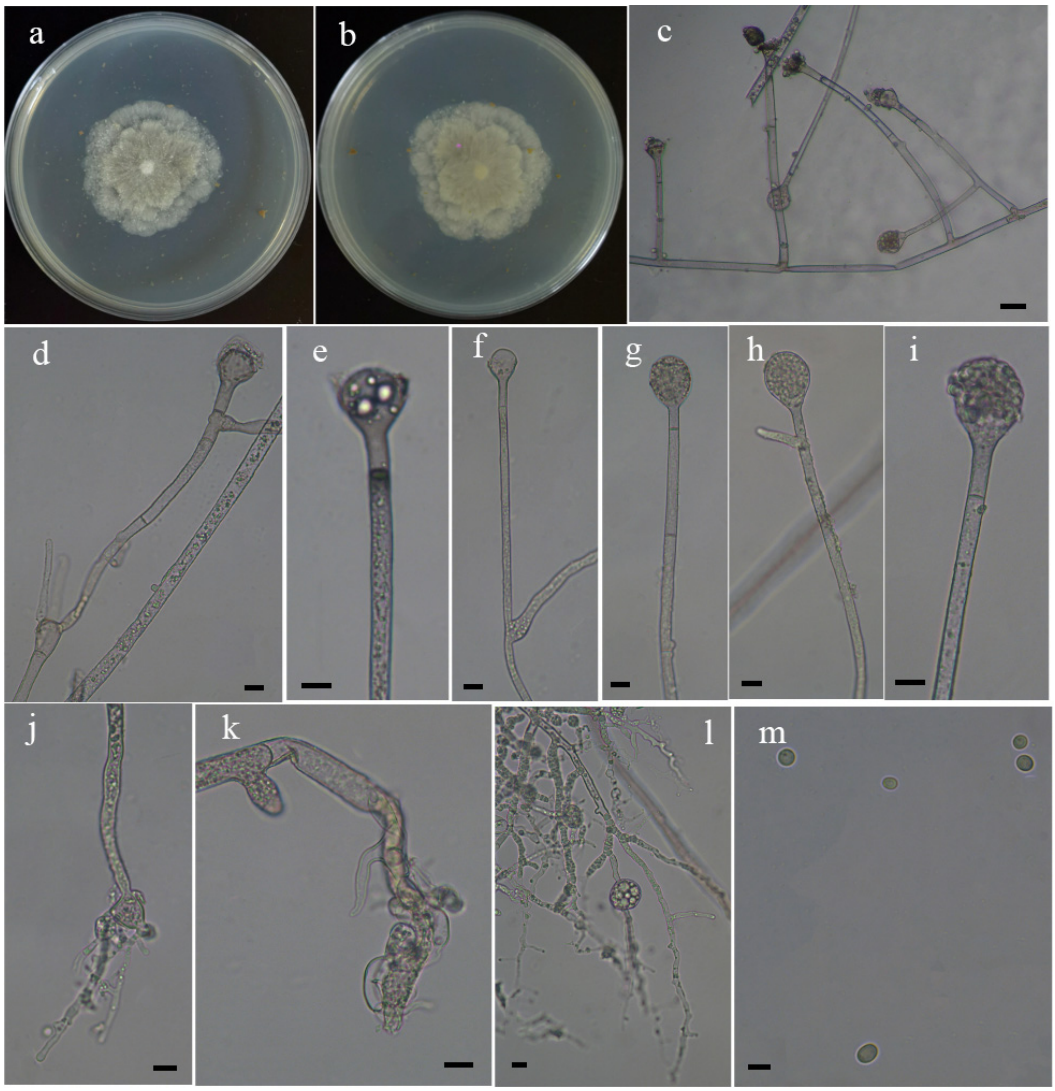

Figure 2. Morphologies of *Absidia gemella* ex-holotype CGMCC 3.16202. (**a**,**b**) Colonies on MEA, (**a**) obverse, (**b**) reverse; (**c**) monopodial sporangiophores; (**d**) sympodial sporangiophore; (**e**,**f**) columellae; (**g**–**i**) sporangium; (**j**,**k**) rhizoids; (**l**) substrate hyphae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

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. Sporangiophores 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. Zygospores 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.

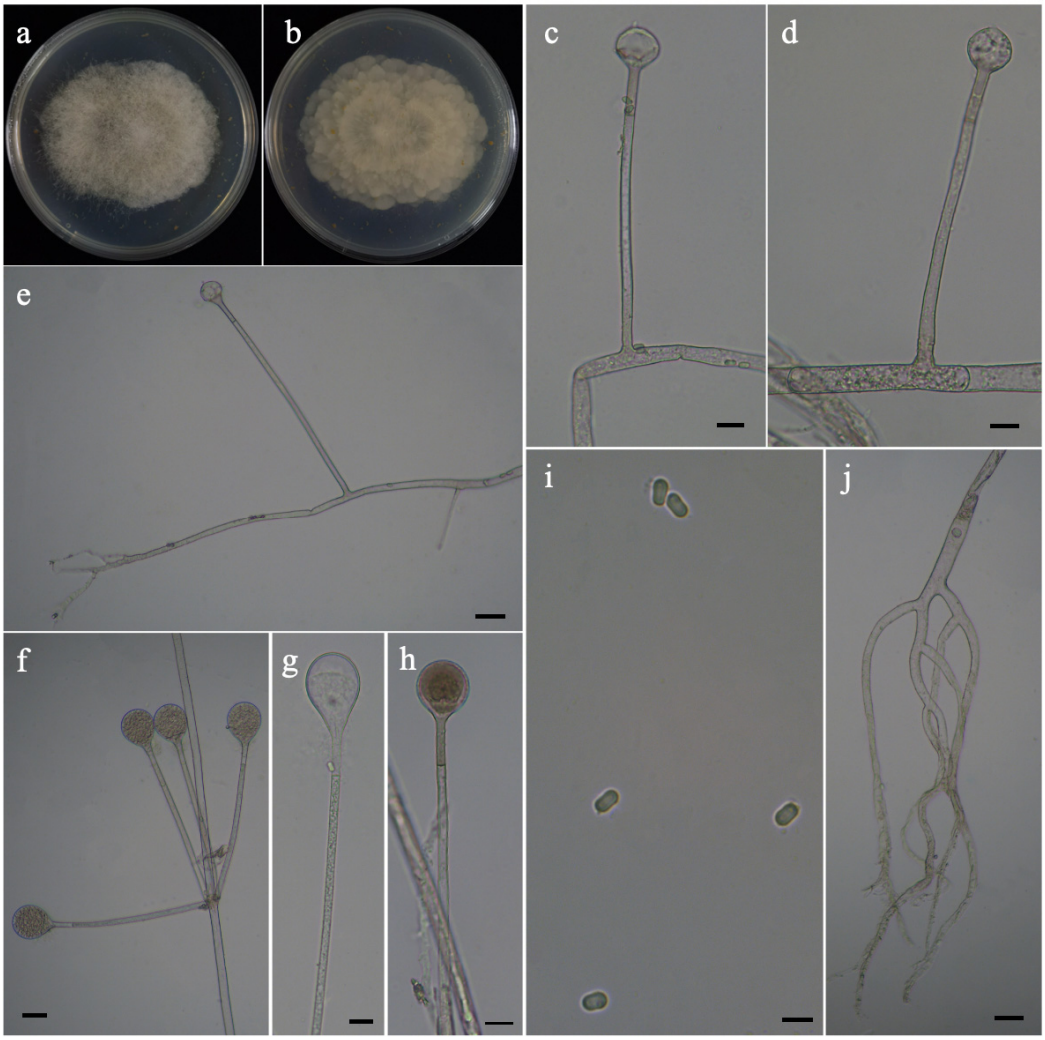

Figure 3. Morphologies of *Absidia longissima* ex-holotype CGMCC 3.16203. (**a**,**b**) Colonies on MEA,(**a**) obverse, (**b**) reverse; (**c**), (**d**) columellae and projection; (**e**) sporangiophores arising from

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.

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. Sporangiophores 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. Zygospores 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 and S1b, 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).

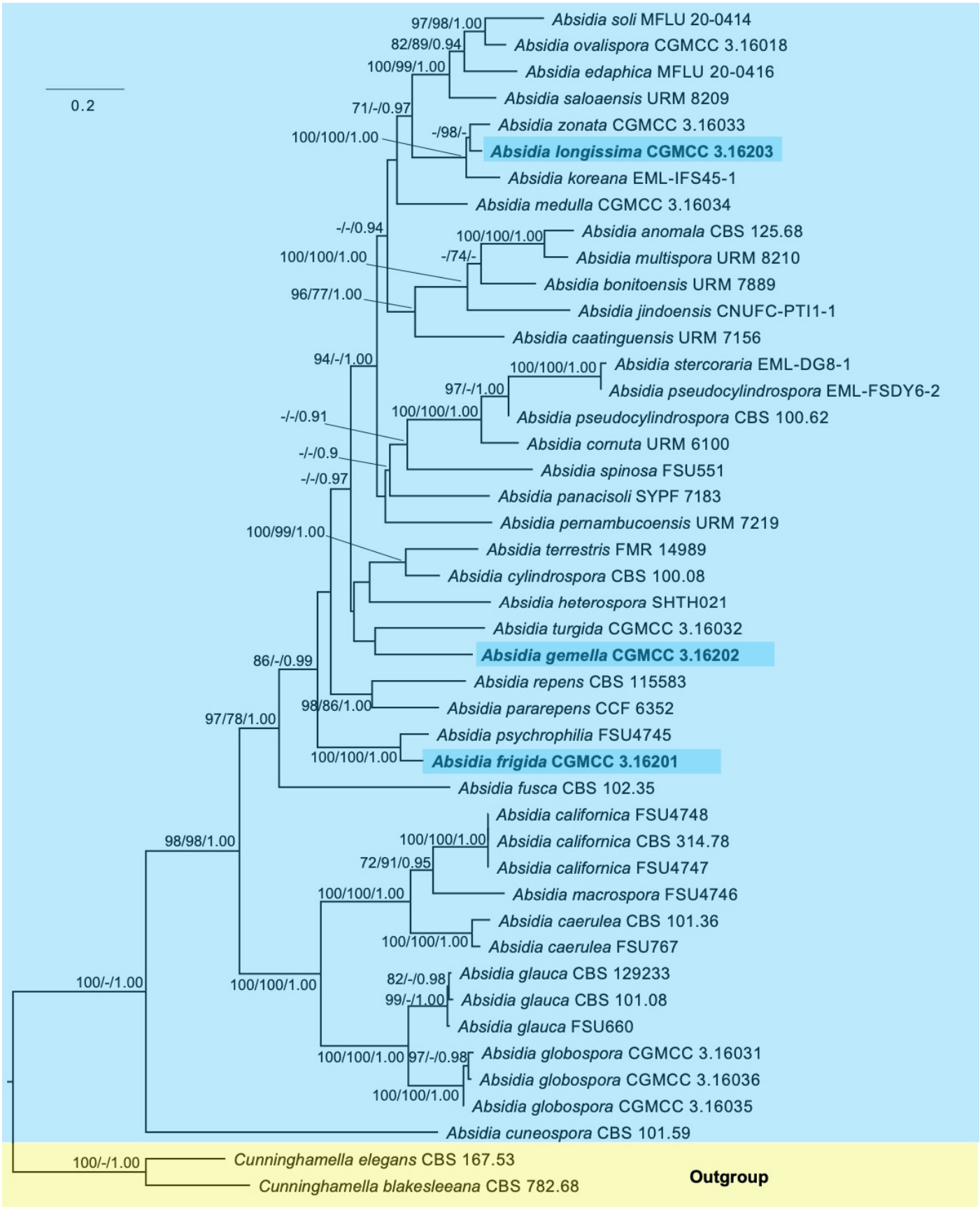

Figure 4. Maximum likelihood phylogenetic tree of *Absidia* based on ITS and LSU rDNA sequences, with *Cunninghamella elegans* and *C. blakesleeana* as outgroups. The three new species, *A. frigida*, *A. gemella* and *A. longissimi*, are shaded. Maximum likelihood (ML) bootstrap values

(≥70%)/maximum parsimony (MP) bootstrap values (≥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.

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 sporangiophores (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 15 January 2022). 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 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**.**

Author Contributions: Data curation, H.Z.; formal analysis, Y.-C.D.; funding acquisition, Y.-C.D. and X.-Y.L.; investigation, H.Z.; methodology, Y.N. and T.-K.Z.; project administration, X.-Y.L.; software, Y.N. and T.-K.Z.; writing—original draft, H.Z.; writing—review and editing, Y.-C.D. and X.-Y.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Second Tibetan Plateau Scientific Expedition and Research Program (STEP), Grant No. 2019QZKK0503, and the National Natural Science Foundation of China, Grant Nos. 31970009 and 32170012.

Institutional Review Board Statement: Not applicable.

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

Acknowledgments: Many scholars are thanked for their help with sampling and depositing, and they are Bao-Kai Cui, Shun Liu, Chang-Ge Song and Tai-Min Xu (Beijing Forestry University); Ze-Fen Yu and Min Qiao (Yunnan University); and Ke Wang, Zhuo Du and You-Zhi Wang (Institute of Microbiology, Chinese Academy of Sciences).

Conflicts of Interest: All authors declare no conflict of interest.

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