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Research

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Posted Date: August 18th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-820672/v1>

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Taxonomy and phylogeny of the *Absidia* (Cunninghamellaceae, Mucorales) introducing nine new species and two new combinations from China

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ABSTRACT

Absidia is ubiquitous and plays an important role in medicine and biotechnology. In the present study, nine new species were described from China in the genus *Absidia*, i.e. *A. ampullacea*, *A. brunnea*, *A. chinensis*, *A. cinerea*, *A. digitata*, *A. oblongispora*, *A. sympodialis*, *A. varians*, and *A. virescens*. Besides, two varieties *A. cylindrospora* var. *nigra* and *A. spinosa* var. *biappendiculata* were elevated to a specific rank as *A. nigra* comb. nov. and *A. biappendiculata* comb. nov., respectively. These new taxa were

proposed based on a comprehensive investigation of morphological traits (such as shape and size of sporangia, sporangiospores and projections on columellae), physiological feature (maximum growth temperatures), and multi-locus sequences (including internal transcribed spacer, large subunit D1-D2 domains of nuclear ribosomal DNA, partial translation elongation factor 1 alpha gene and actin gene). All species mentioned above are illustrated, and an identification key to all the known species of *Absidia* in China is included.

KEYWORDS: *Mucoromycota*, New taxa, Classification, Morphology, Molecular phylogeny

INTRODUCTION

The genus *Absidia* Tiegh. (Cunninghamellaceae, Mucorales) was established by van Tieghem (1876). *Absidia* members are ubiquitous, mostly from soil and sometimes also associated with animals and plants. For example, the ex-type of *A. spinosa* var. *biappendiculata* Rall & Solheim was isolated from the leaves of *Comandra pallida* (Rall and Solheim 1964), and the ex-type strains of *A. californica* J.J. Ellis & Hesselt. and *A. stercoraria* Hyang B. Lee, H.S. Lee and T.T.T. Nguyen both live on rat dung (Ellis and Hesseltine 1965; Li et al. 2016). Although several species are vital causative agents of mucormycosis, some species are capable of producing chitin, chitosan, chitooligosaccharides (Kaczmarek et al. 2019) and hydrocortisone (Chen et al. 2020). This genus is characterized by rhizoids, stolons bearing single or a bunch of sporangiophores, a septum close to the top of sporangiophores, pyriform apophysate deliquescent-walled sporangia, distinct apophyses, and typically appendaged zygospores (Hoffmann et al. 2007; Hoffmann 2010).

The circumscription and classification of the *Absidia* have long been disputed. Before 1964, seven generic names allied to *Absidia* were successively proposed according to morphology, *Tieghemella* Berl. & De Toni, *Mycocladius* Beauverie, *Proabsidia* Vuill., *Lichtheimia* Vuill., *Pseudoabsidia* Bainier, *Protoabsidia* Naumov

and *Gongronella* Ribaldi (Berlese and de Toni 1888; Beauverie 1900; Vuillemin 1903a, 1903b; Bainier 1903; Naumov 1935; Ribaldi 1952). However, after a study on hundreds of strains comprising these genera, Hesseltine and Ellis (1964) retained *Gongronella* and *Absidia* only. The *Gongronella* has a globose apophysis with a constriction showing the attachment of the sporangial wall. They further divided *Absidia* into two subgenera, namely subg. *Absidia* and subg. *Mycocladus* (Beauverie) Hesselt. & J.J. Ellis. The former produces appendages from the sespensors to envelop zygospores, whereas the latter does not produce any appendages. This classification framework was followed by Schipper (1990) who in turn recognized six groups in the subg. *Absidia*. However, with the advent of DNA-based molecular phylogenetics, the taxonomic classification of the genus has been refined. Hawksworth et al. (1995) did not accept the above morphology-based subgeneric delimitation, but synonymized all these allied genera with *Absidia* instead.

Recently, a comprehensive study of molecular phylogenetics, morphology and physiology has provided a more reliable delimitation among *Absidia* species (Hoffmann et al. 2007), where *Absidia* was classified into three groups: 1) the thermotolerant species with an optimal growth temperature of 37–45 °C; 2) the mesophilic species with an optimal growth temperature of 25–34 °C, which have been accepted up to now as *Absidia sensu stricto*; and 3) the mycoparasitic species, potential to parasitise other mucoralean hosts. Soon, the thermotolerant species were transferred into the genus *Lichtheimia* (Hoffmann et al. 2009a); and the mycoparasitic species were placed into a new genus *Lentamyces* Kerst. Hoffm. & K. Voigt (Hoffmann and Voigt 2008).

Currently, 39 species have been reported worldwide in *Absidia* (Bainier 1889; Hesseltine and Ellis 1961, 1964, 1966; Ellis and Hesseltine 1965, 1966; Index Fungorum <http://www.indexfungorum.org>, accessed on June 18, 2021). Among these species, 20 were proposed before 2001. Taxonomical research in *Absidia* seems to have stalled for a long time between 2002 and 2014. Since 2015, as many as 19 new species have been explosively described thanks to the strategy of combining morphology, physiology and phylogeny: *A. caatinguensis* D.X. Lima and A.L. Santiago, *A. cornuta*

D.X. Lima, C.A. de Souza, H.B. Lee and A.L. Santiago, *A. jindoensis* Hyang B. Lee and T.T.T. Nguyen, *A. koreana* Hyang B. Lee, Hye W. Lee and T.T. Nguyen, *A. multispora* T.R.L. Cordeiro, D.X. Lima, Hyang B. Lee and A.L. Santiago, *A. panacisoli* T. Yuan Zhang, Ying Yu, He Zhu, S.Z. Yang, T.M. Yang, Meng Y. Zhang and Yi X. Zhang, *A. pararepens* Jurjević, M. Kolařík and Hubka, *A. pernambucoensis* D.X. Lima, C.M. Souza-Motta and A.L. Santiago, *A. saloaensis* T.R.L. Cordeiro, D.X. Lima, Hyang B. Lee and A.L. Santiago, *A. stercoraria*, *A. terrestris* Rosas de Paz, Dania García, Guarro, Cano and Stchigel, *A. bonitoensis* C.L. Lima, D.X. Lima, Hyang B. Lee and A.L. Santiago, *A. ovalispora* H. Zhao and X.Y. Liu, *A. soli* V.G. Hurdeal, E. Gentekaki, Hyang B. Lee & K.D. Hyde, *A. edaphica* V.G. Hurdeal, E. Gentekaki, Hyang B. Lee & K.D. Hyde, *A. globospora* T.K. Zong & X.Y. Liu, *A. medulla* T.K. Zong & X.Y. Liu, *A. turgida* T.K. Zong & X.Y. Liu and *A. zonata* T.K. Zong & X.Y. Liu. (Ariyawansa et al. 2015; Li et al. 2016; Crous et al. 2018, 2020; Wanasinghe et al. 2018; Zhang et al. 2018; Cordeiro et al. 2020; Lima et al. 2020; de Lima et al. 2021; Hurdeal et al. 2021; Zhao et al. 2021; Zong et al. 2021). Totally, 13 species are distributed in China (Zheng and Liu 2018; Zhang et al. 2018; Zhao et al. 2021; Zong et al. 2021).

Recently, 16 strains of *Absidia* were isolated from China but could not be assigned to any described species. Morphological, physiological and molecular data (multi-gene locus included ITS, D1-D2 domains of LSU rDNA, TEF-1 α and *Act*) are presented herein to support them within the *Absidia sensu stricto*, including nine new species and two new combinations. In addition, the synoptic key to all the 23 known species of *Absidia* in China is revised.

MATERIALS AND METHODS

Isolation and Strains

Strains were isolated from the soil collected in China about 20 years ago or even earlier. Approximately 1 g of soil samples was suspended in 100 mL sterilized water and shaken vigorously. Then, 100 μ L of the suspension was added onto a potato dextrose agar (PDA: 20 g/L glucose, 20 g/L agar, 200 g/L potato, and 1000 mL distilled water)

with 100 mg / mL each of streptomycin sulfate and ampicillin. The plate was incubated upside down at 27 °C and examined once a day with a stereomicroscope (SMZ1500, Nikon Corporation, Japan). Upon the presence of cultures, a single colony was picked onto a new PDA plate. Living cultures were deposited in the China General Microbiological Culture Collection Center, Beijing, China (CGMCC). Dried cultures were preserved in the Herbarium Mycologicum Academia Sinica, Beijing, China (HMAS).

Morphology and Growth Experiments

Pure strains were cultivated with malt extract agar (MEA: 20 g/L malt extract, 20 g/L agar, and 1000 mL distilled water). For morphological observation, they were incubated at 27 °C for 4 – 7 d and daily examined under a microscope (Axio Imager A2, Carl Zeiss Microscopy, Germany). For determining maximum growth temperatures, they were initially incubated at 32 °C for 4 d, and then at higher temperatures until the colonies stopped growing. The color code of colonies was determined with the chromatography of Ridgway (1912).

DNA Extraction, PCR Amplification and Sequencing

Mycelia were grown at 27 °C for 5 d on PDA plates, and then cell DNAs were extracted with GO-GPLF-400 kit (GeneOnBio Corporation, Changchun, China). A span including the internal transcribed spacer (ITS) and large subunit (LSU) D1-D2 domain of rDNA were amplified with primer pair NS5M and LR5M (Wang et al. 2014). The partial translation elongation factor 1 alpha gene (*TEF-1 α*) was amplified with primer pair EF1-983F (Rehner and Buckley 2005) and *TEF1LL*Erev (Jaklitsch et al. 2005). The partial actin gene (*Act*) was amplified with primer pair Act-1 and Act-4R (Voigt and Wostemeyer 2000). The PCR procedure for all these gene loci was as follows: an initial temperature at 95 °C for 5 min (rDNA and *TEF-1 α*) or 3 min (*Act*), then 30 cycles of denaturation at 95 °C for 30 s (rDNA and *TEF-1 α*) or 60 s (*Act*), annealing at 55 °C for 60 s and extension at 72 °C for 60 s, and finally an extra extension at

72 °C for 10 min. PCR products were purified and sequenced at BGI Tech Solutions Beijing Liuhe Co., Limited, Beijing, China. All newly generated sequences were deposited at GenBank (Table 1).

Phylogenetic Analyses

The software platform Geneious 9.0.2 (<http://www.geneious.com>) was used to assemble and proofread DNA sequences. All the sequences were realigned using AliView version 3.0 (Larsson 2014). The sequence alignments and phylogenetic trees were deposited at TreeBase (submission ID 28429). Sequences of *Cunninghamella elegans* and *C. blakesleeana* retrieved from GenBank were used as outgroups in multi-gene analyses following Hoffmann et al. (2007).

Phylogenetic analyses were carried out using algorithms Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI). MP phylogenetic analyses followed Zhao and Wu (2017), and the tree construction was performed with PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using a heuristic search with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap analysis with 1,000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each maximum parsimonious tree generated.

ML phylogenetic analyses were conducted with raxmlGUI 2.0 beta (Edler et al. 2020). A general time reversible model was used with a gamma-distributed rate variation (GTR+G) and 1000 bootstrap replicates were carried out.

BI phylogenetic analyses were calculated with MrBayes 3.2.7a (Ronquist et al. 2012) by a general time reversible model with an estimate of the proportion of invariant sites and a gamma distribution for variable rates across sites (GTR+I+G). Four Markov chains were run simultaneously from random starting tree for 2,300,000 generations and the tree was sampled every 100 generations. The chains stopped once the average

standard deviation of split frequencies decreased lower than 0.01. The first one-fourth of generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches were considered as significantly supported if they received Maximum Likelihood bootstrap (MLB) $\geq 70\%$, Maximum Parsimony bootstrap (MPB) $\geq 70\%$, or Bayesian posterior probabilities (BPP) ≥ 0.95 (Hillis and Bull 1993; Huelsenbeck and Hillis 1993; Leaché and Reeder 2002).

RESULTS

Phylogenetic Analyses

The multi-gene dataset included sequences from 64 strains representing 44 species of *Absidia* and two outgroups. The dataset had an aligned length of 3402 characters, of which 1865 characters were constant, 370 were variable and parsimony-uninformative, and 1167 were variable and parsimony-informative. MP analyses yielded 135 equally parsimonious trees (TL = 8091, CI = 0.3297, HI = 0.6703, RI = 0.5653, RC = 0.1864). At the end of the inference, the average standard deviation of split frequencies was 0.009960 (BI). All BI, ML and MP phylogenetic trees resulted in similar topologies and consequently were integrated (Fig. 1).

Taxonomic Treatments

Absidia ampullacea T.K. Zong & X.Y. Liu, **sp. nov.** (Fig. 2)

MycoBank no.: XXXXXX (to add after review).

Holotype: **China**, Beijing (40°3'49"N, 116°1'26"E), from soil sample, 31 December 2019, Liu Xiaoyong (HMAS 350295, ex-type CGMCC 3.16054).

Etymology: *ampullacea* (Lat.) referring to the ampulliform-shaped swollen hyphae and sporangiophores.

Description: Colonies on MEA, irregularly zonate, attaining 46 mm diameter after 5 d at 27 °C, white at first and then Saccardo's olive (R16). Hyphae hyaline at first, becoming brown when mature, sometimes ampulliform-shaped swollen, 6.0–13.0(–16.5) μm wide. Stolons branched, hyaline, smooth, septate, 4.0–9.0 μm wide. Rhizoids

finger-like, mostly twice or more repeatedly, with a septum at the base. Sporangiophores erect or slightly bent, 1–6 in whorls, unbranched, simple, monopodial or sympodial, hyaline, with a septum 14.5–21.5 μm below apophyses, sometimes a swelling beneath sporangia, 30.0–320.0 \times 2.5–5.5 μm . Apophyses distinct, bell-shaped, slightly pigmented, 4.0–9.5 μm high, 2.5–6.0(–8.0) μm wide at the base, and 9.5–18.0(–20.0) μm wide at the top. Sporangia globose to pyriform, multispored, deliquescent-walled, 17.5–37.5 \times 17.5–45.0 μm . Columellae hemispherical, hyaline, smooth, occasionally with a 0.5–2.0 μm verrucous projection at the apex, (8.5–)10.0–22.5 \times (10.5–)14.0–24.0 μm . Collars distinct. Sporangiospores oval to subglobose, 3.0–4.0(–4.5) \times 2.5–3.5(–4.0) μm . Zygosporangia not observed. Chlamydospores absent. No growth at 30 °C.

Absidia brunnea T.K. Zong & X.Y. Liu, **sp. nov.** (Fig. 3)

Mycobank no.: XXXXXX (to add after review).

Holotype: **China**, Qinghai Province, Xining, Huangzhong County, Ta'er Monastery, from soil sample, 2 August 1999, Wang Long (HMAS 350296, ex-type CGMCC 3.16055).

Etymology: *brunnea* (Lat.) referring to the brown colony on MEA.

Description: Colonies on MEA, obversely regularly concentric ring zonate, attaining 47 mm diameter after 5 d at 27 °C, white at first and then sayal brown to snuff brown (R29). Hyphae hyaline at first, becoming brown when mature, 6.0–17.5 μm wide. Stolons branched, hyaline, smooth, septate, 3.5–10.0 μm wide. Rhizoids branch-shaped, weakly branched, with a septum at the base. Sporangiophores erect or slightly bent, 1–7 in whorls but mostly 4–6, unbranched, simple or monopodial, rarely sympodial, hyaline, with a septum 11.0–17.0 μm below apophyses, sometimes a swelling beneath sporangia, occasionally branched at the swelling, 50.0–320.0(–420) \times 3.0–5.0(–6.5) μm . Apophyses distinct, funnel-shaped, slightly pigmented, 3.0–10.0 μm high, 2.5–7.5 μm wide at the base, and 10.0–17.5(–20.0) μm wide at the top. Sporangia globose to pyriform, multispored, deliquescent-walled, 17.5–31.0(–38.0) \times 19.0–30.0(–34.5) μm . Columellae hemispherical, hyaline, smooth, always with a 2.5–5.5(–6.0) μm clavate

projection at the apex, 7.0–16.0(–18.5) × 10.5–24.0 μm. Collars distinct. Sporangiospores two types, the smaller one cylindrical to oval, 3.0–4.0 × 2.0–2.5 μm, the larger one oval, with vacuole, 4.0–6.5(–7.0) × 3.0–5.0 μm, hyaline, smooth. Zygosporangia not observed. Chlamydospores absent. No growth at 35 °C.

Absidia chinensis T.K. Zong & X.Y. Liu, **sp. nov.** (Fig. 4)

MycoBank no.: XXXXXXX (to add after review).

Holotype: **China**, Yunnan Province, Xishuangbanna Dai Autonomous Prefecture, Mengla County, Xishuangbanna Tropical Botanical Garden, from soil sample, 4 July 1994, Chen Guiqing (HMAS 350297, ex-type CGMCC 3.16056).

Paratype: **China**, Sichuan Province, Tibetan Qiang Autonomous Prefecture of Ngawa, Jiuzhaigou County, Jiuzhaigou Valley Scenic and Historic Interest Area, from soil sample, 23 May 1994, Chen Guiqing (HMAS 350298, ex-type CGMCC 3.16057).

Etymology: *chinensis* (Lat.) referring to the country where the ex-type and ex-paratype strains both were isolated.

Description: Colonies on MEA, obversely regularly concentric ring zonate, attaining 50 mm diameter after 5 d at 27 °C, white at first and then drab gray (R46) to snuff brown (R29). Hyphae hyaline at first, becoming brown when mature, generally swollen, (6.0–)7.5–14.0(–15.5) μm wide. Stolons branched, hyaline, smooth, septate, 3.5–9.0(–11.0) μm wide. Rhizoids coralliform, mostly simple or two branched, with a septum at the base. Sporangiphores erect or slightly bent, 1–5 in whorls, mostly unbranched or simple, rarely monopodial, sympodial not observed, hyaline, with a septum 13.0–17.5(–22.0) μm below apophyses, generally a swelling beneath sporangia, sometimes branched at the swelling, 45.0–220.0 × (2.5–)3.0–5.0(–6.0) μm. Apophyses distinct, bell-shaped or hourglass-shaped, slightly pigmented, 3.5–6.5(–7.0) μm high, 2.5–5.5 μm wide at the base, and (6.0–)7.0–13.0(–15.5) μm wide at the top. Sporangia globose to pyriform, multispored, deliquescent-walled, 15.0–28.0(–39.5) × 15.0–28.5(–37.5) μm. Columellae spherical or hemispherical, hyaline, smooth, always with a 2.0–5.5 μm papillary projection at the apex, (5.0–)6.0–14.0(–15.5) × (7.5–)9.0–16.5(–17.5) μm. Collars present or absent, but distinct if present. Sporangiospores cylindrical to

oval, hyaline, smooth, $3.5\text{--}4.5 \times 2.0\text{--}2.5 \mu\text{m}$. Zygosporangia globose, brown to dark brown, rough, $(37.0\text{--})45.0\text{--}80.0(-90.0) \times (36.0\text{--})50.0\text{--}80.0 \mu\text{m}$. Suspensor unequal, parallel or nearly so, brown, the larger one $27.0\text{--}44.0 \mu\text{m}$ diameter, the smaller one mostly $20.0\text{--}33.0 \mu\text{m}$ diameter. Chlamydospores absent. No growth at $31 \text{ }^\circ\text{C}$.

Absidia cinerea T.K. Zong & X.Y. Liu, **sp. nov.** (Fig. 5)

Mycobank no.: XXXXXX (to add after review).

Holotype: **China**, Beijing ($40^\circ 21' 30''\text{N}$, $116^\circ 6' 27''\text{E}$), from soil sample, 31 December 2019, Liu Xiaoyong (HMAS 350303, ex-type CGMCC 3.16062).

Etymology: *cinerea* (Lat.) referring to the gray color of colonies on MEA.

Description: Colonies on MEA, regularly concentric ring zonate, attaining 44 mm diameter after 5 d at $27 \text{ }^\circ\text{C}$, white at first and then pale mouse gray (R51). Hyphae hyaline at first, becoming brown when mature, $5.5\text{--}14.5 \mu\text{m}$ wide. Stolons branched, hyaline, smooth, septate, $3.5\text{--}7.0 \mu\text{m}$ wide. Rhizoids branch-shaped and taper at the end, mostly two branches arising at a place, with a septum at the base. Sporangiphores erect or slightly bent, 1–7 in whorls, unbranched, simple or monopodial arising from stolons, mostly sympodial arising from aerial mycelium, hyaline, with a septum $11.0\text{--}17.0 \mu\text{m}$ below apophyses, $50.0\text{--}150.0 \times 2.5\text{--}5.0 \mu\text{m}$. Apophyses distinct, bell-shaped or funnel-shaped, slightly pigmented, $2.0\text{--}5.5(-7.5) \mu\text{m}$ high, $2.5\text{--}5.5 \mu\text{m}$ wide at the base, and $6.0\text{--}12.5 \mu\text{m}$ wide at the top. Sporangia globose to pyriform, multispored, deliquescent-walled, $(7.0\text{--})11.0\text{--}22.0(-27.5) \times (7.0\text{--})13.0\text{--}25.0 \mu\text{m}$. Columellae hemispherical, hyaline, smooth, always with a $1.3\text{--}5.0 \mu\text{m}$ rod-shaped or needle-like projection at the apex, $(5.0\text{--})5.5\text{--}12.0(-14.0) \times 7.0\text{--}15.0 \mu\text{m}$. Collars present or absent, but distinct if present. Sporangiospores cylindrical, slightly constricted in the center, hyaline, smooth, $4.5\text{--}6.0 \times 2.0\text{--}2.5 \mu\text{m}$. Zygosporangia globose, brown to dark brown, rough, $36.0\text{--}73.0(-81.0) \mu\text{m}$ diameter. Suspensor mostly one, rarely unequal two, brown, with 8–13 appendages on single suspensor, $17.0\text{--}33.0 \mu\text{m}$ diameter. Chlamydospores absent. No growth at $35 \text{ }^\circ\text{C}$.

Absidia digitata T.K. Zong & X.Y. Liu, **sp. nov.** (Fig. 6)

MycoBank no.: XXXXXX (to add after review).

Holotype: **China**, Xinjiang Uygur Autonomous Region, Ili Kazak Autonomous Prefecture, Qapqal Xibe Autonomous County, Wusun Mountain, from soil sample, 14 June 2002, Wang Xuewei (HMAS 350299, ex-type CGMCC 3.16058).

Etymology: *digitata* (Lat.) referring to the finger-like shape of rhizoids.

Description: Colonies on MEA, regularly flower-shaped zonate, attaining 66 mm diameter after 6 d at 27 °C, white at first and then snuff brown (R29). Hyphae hyaline at first, becoming brown when mature, sometimes swollen, 8.0–18.0 µm wide. Stolons branched, hyaline or light brown, smooth, septate, 4.0–8.0(–9.5) µm wide. Rhizoids finger-like, mostly twice or more repeatedly, with a septum at the base. Sporangiphores erect or slightly bent, 1–6 in whorls, unbranched, generally simple each sporangiphore in whorls, rarely monopodial or sympodial, hyaline or light brown, with a septum 14.5–23.5(–25.5) µm below apophyses, occasionally a swelling beneath sporangia, (60.0–)80.0–350.0(–470.0) × 3.5–7.0(–8.5) µm. Apophyses distinct, slightly pigmented, (5.5–)7.0–13.5 µm high, 4.5–7.5(–8.5) µm wide at the base, and 12.5–19.0(–21.0) µm wide at the top. Sporangia globose to pyriform, multispored, deliquescent-walled, (24.0–)27.0–47.0(–64.0) × 26.5–43.0(–48.5) µm. Columellae hemispherical, hyaline, smooth, sometimes with a 4.0–9.0 µm clavate projection at the apex, occasionally with two projections, slightly bulbous at the end, 10.5–22.0 × (11.5–)18.0–30.0 µm. Collars distinct if present. Sporangiospores globose, hyaline, smooth, non-uniform, 3.0–5.0 µm diameter. Zygosporangia not observed. Chlamydospores absent. No growth at 32 °C.

Absidia oblongispora T.K. Zong & X.Y. Liu, **sp. nov.** (Fig. 7)

MycoBank no.: XXXXXX (to add after review).

Holotype: **China**, Yunnan Province, Xishuangbanna Dai Autonomous Prefecture, Mengla County, Xishuangbanna Tropical Botanical Garden, from soil sample, 4 July 1994, Chen Guiqing (HMAS 350302, ex-type CGMCC 3.16061).

Etymology: *oblongispora* (Lat.) referring to the oblong sporangiospores.

Description: Colonies on MEA, slightly irregular concentric ring zonate, attaining

44 mm diameter after 5 d at 27 °C, white at first and then pale mouse gray (R51) to light drab (R46). Hyphae hyaline at first, becoming brown when mature, (5.5–)7.0–9.5(–11.5) µm wide. Stolons branched, hyaline, smooth, with few septa near the base of sporangiophores, 4.0–8.0(–9.5) µm wide. Rhizoids root-like, branched repeatedly, with a septum at the base. Sporangiophores erect or slightly bent, 1–5 in whorls, mostly unbranched, rarely simple or monopodial, sympodial not observed, hyaline, with a septum (9.5–)11.5–16.0 µm below apophyses, occasionally with a septum at the base, (33.0–)75.0–250.0(–300.0) × (3.0–)3.5–5.5 µm. Apophyses distinct, slightly pigmented, 3.5–6.5 µm high, 3.5–6.5(–7.5) µm wide at the base, and 7.0–16.5(–19.0) µm wide at the top. Sporangia globose, multispored, deliquescent-walled, (13.5–)19.5–29.5 × (14.5–)19.0–31.0 µm. Columellae mostly conical, rarely hemispherical, hyaline, smooth, always with a 3.0–7.5 µm bulbous projection at the apex, (7.0–)8.5–13.5(–15.0) × (8.5–)10.5–16.5 µm. Collars present or absent, but distinct if present. Sporangiospores cylindrical to oblong, hyaline, smooth, uniform, 3.5–4.5 × 2.5–3.0 µm. Zygosporangia not observed. Chlamydosporangia absent. No growth at 32 °C.

Absidia sympodialis T.K. Zong & X.Y. Liu, **sp. nov.** (Fig. 8)

MycoBank no.: XXXXXXX (to add after review).

Holotype: **China**, Shaanxi Province, Hanzhong, Foping County, from soil sample, 13 October 2002, Wang Xuewei (HMAS 350304, ex-type CGMCC 3.16064).

Paratype: **China**, Beijing (40°1'32"N, 116°8'54"E), from soil sample, 31 December 2019, Liu Xiaoyong (HMAS 350305, ex-type CGMCC 3.16063).

Etymology: *sympodialis* (Lat.) referring to the sympodial sporangiophores.

Description: Colonies on MEA, regularly zonate, attaining 76 mm diameter after 5 d at 27 °C, white at first and cedar green (R6). Hyphae hyaline at first, becoming light brown, (9.0–)10.5–19.0 µm wide. Stolons branched, hyaline, smooth, with few septa near the base of sporangiophores, 6.5–12.5 µm wide. Rhizoids fibrous-root-shaped, taper at the end, branched mostly repeatedly, with a septum at the base. Sporangiophores erect or slightly bent, 1–6 in whorls, unbranched, simple, monopodial or sympodial, hyaline or light brown, with a septum (7.5–)9.0–15.5(–17.5) µm below

apophyses, (90.0–)180.0–520.0(–630.0) × 4.5–9.0(–10.5) μm. Apophyses distinct, slightly pigmented, (6.0–)7.0–12.5(–13.7) μm high, 6.0–12.0(–14.1) μm wide at the base, and (12.0–)14.0–28.0(–31.0) μm wide at the top. Sporangia globose to pyriform, multispored, deliquescent-walled, (20.0–)23.5–55.0(–63.0) × (18.5–)22.5–53.0(–63.0) μm. Columellae spherical, oval to hemispherical or chestnut-shaped, hyaline, smooth, sometimes with a 2.0–4.0 μm papillary or pointed projection at the apex, (8.0–)10.5–30.5(–40.0) × (9.5–)13.5–35.0(–39.0) μm. Collars present or absent, but distinct if present. Sporangiospores globose, hyaline, smooth, uniform, 2.5–3.5 μm diameter. Zygosporangia not observed. Chlamydospores absent. No growth at 33 °C.

Absidia varians T.K. Zong, H. Zhao & X.Y. Liu, **sp. nov.** (Fig. 9)

MycoBank no.: XXXXXX (to add after review).

Holotype: **China**, Yunnan Province, Dehong Dai and Jingpo Autonomous Prefecture, Mangshi, Mangshi County G32 (24°32'36"N, 98°40'29"E), from soil sample, 30 April 2021, Zhao Heng (HMAS 350306, ex-type CGMCC 3.16065).

Etymology: *varians* (Lat.) referring to the various shape of projections on columellae.

Description: Colonies on MEA, regularly zonate, attaining 67 mm diameter after 4 d at 27 °C, white at first and then pale green-blue gray (R48). Hyphae hyaline to slightly pigmented, 8.5–12.0 μm wide. Stolons branched, hyaline, smooth, septate, 5.0–8.5 μm wide. Rhizoids root-like, multi-branched, no septa. Sporangiphores erect or slightly bent, 1–4 in whorls but mostly 2–3 borne from stolons, unbranched, simple, monopodial or sympodial, hyaline, with a septum 13.5–19.0 μm below apophyses, 100.0–380.0(–480.0) × 4.5–9.0 μm. Apophyses distinct, 6.0–12.0 μm high, 5.5–8.5(–11.0) μm wide at the base, and 10.5–22.5 μm wide at the top. Sporangia subglobose to elliptical, multispored, deliquescent-walled, 26.0–50.0(–58.0) × 22.5–45.0(–50.0) μm. Columellae spherical or hemispherical, hyaline, smooth, always with all kinds of projections at the apex, mostly one, occasionally two, bulbous swelling, papillary, clavate or spinous, 11.0–26.5(–30.5) × 12.5–30.0(–35.0) μm. Collars distinct if present. Sporangiospores globose to oval, hyaline, smooth, 3.0–3.5(–4.0) × 2.0–3.0 μm.

Zygosporangia not observed. Chlamydospores absent. No growth at 29 °C.

Absidia virescens T.K. Zong & X.Y. Liu, **sp. nov.** (Fig. 10)

MycoBank no.: XXXXXX (will add it after review).

Holotype: **China**, Yunnan Province, Xishuangbanna Dai Autonomous Prefecture, Jinghong, Mengyang Town, Xishuangbanna National Nature Reserve, from soil sample, 9 July 1994, Chen Guiqing (HMAS 350307, ex-type CGMCC 3.16067).

Paratype: **China**, Yunnan Province, Kunming, Yunnan Nationalities Village, from soil sample, 27 August 1995, Guo Yinglan (HMAS 350308, ex-type CGMCC 3.16066)

Etymology: *virescens* (Lat.) referring to the greenish colony on MEA.

Description: Colonies on MEA, regularly disc-shaped, attaining 63 mm diameter after 4 d at 27 °C, white at first and then ring elm green (R17). Hyphae hyaline at first, becoming brown when mature, (6.0–)7.0–11.5 µm wide. Stolons branched, hyaline, smooth, with few septa near the base of sporangiophores, 4.5–9.0 µm wide. Rhizoids root-like, branched mostly twice or three times, with a septum at the base. Sporangiophores erect or slightly bent, 1–4 in whorls, mostly unbranched or simple, rarely monopodial or sympodial, hyaline, with a septum (10.0–)12.0–19.5(–22.5) µm below apophyses, occasionally with a septum at the base, sometimes a swelling beneath sporangia, (75.0–)120.0–350.0(–480.0) × 5.5–10.5(–11.5) µm. Apophyses distinct, sometimes slightly pigmented, (5.5–)6.5–10.5(–14.0) µm high, 6.5–12.0(–15.5) µm wide at the base, and (12.5–)15.0–25.5 µm wide at the top. Sporangia globose to pyriform, multispored, deliquescent-walled, (15.5–)23.0–51.5 × (15.5–)20.5–45.5 µm. Columellae spherical or oval to hemispherical, hyaline, smooth, sometimes with a single or two 3.0–6.5 µm projection at the apex, (10.5–)13.0–31.5(–40.5) × (11.5–)16.5–37.5(–45.0) µm. Collars present or absent, but distinct if present. Sporangiospores globose to subglobose, hyaline, smooth, non-uniform, 3.0–5.0(–5.5) µm diameter, but mostly 3.0–3.5 µm diameter. Zygosporangia not observed. Chlamydospores absent. No growth at 32 °C.

Absidia nigra (Hesselt. & J.J. Ellis) T.K. Zong & X.Y. Liu, **comb. nov.** (Fig. 11)
MycoBank no.: XXXXXX (to add after review).

Basionym: *Absidia cylindrospora* var. *nigra* Hesselt. & J.J. Ellis, *Mycologia*, 56(4), 595. 1964. [MB no.: 353238]

Holotype: **United States**, Wisconsin, near Jefferson Junction, from the soil of pastured hardwood sample, 1940 (type NRRL 3060, isotype CBS 127.68, isotype HMAS 350309,)

Additional strains: **China**, Jilin Province, Jiaohe, State-owned Forest Protection Center of Forestry Experimental Area of Jilin Province, from soil sample, 1 September 1991, Bai Fengyan (HMAS 350300, living culture CGMCC 3.16059); Inner Mongolia Autonomous Region, Hinggan League, Arxan, from soil sample, 16 August 1991, Bai Fengyan (HMAS 350301, living culture CGMCC 3.16060).

Etymology: *nigra* (Lat.) adopting from basionym.

Description: Colonies on MEA, regularly zonate, attaining 62 mm diameter after 6 d at 27 °C, white at first and then light drab (R46). Hyphae hyaline at first, becoming brown when mature, sometimes swollen, 8.0–15.5(–18.5) µm wide. Stolons branched, hyaline, smooth, septate, 4.0–8.5 µm wide. Rhizoids finger-like, short, mostly twice or repeatedly, with a septum at the base. Sporangiphores erect or slightly bent, 1–5 in whorls, unbranched, simple, rarely monopodial or sympodial, hyaline, with a septum 11.5–21.0 µm below apophyses, sometimes a swelling beneath sporangia, 60.0–220.0(–380.0) × (2.5–)3.0–6.5 µm. Apophyses distinct, slightly pigmented, 3.5–8.5 µm high, 3.3–5.9 µm wide at the base, and 9.5–18.5 µm wide at the top. Sporangia globose to pyriform, multispored, deliquescent-walled, (17.0–)18.0–38.0(–46.5) × 16.5–38.5 µm. Columellae spherical or oval to hemispherical, hyaline, smooth, sometimes with a 1.5–5.0 µm clavate or slightly swollen projection at the apex, 8.5–19.0 × (9.5–)11.5–23.0 µm. Collars distinct if present. Sporangiospores two types, cylindrical or globose, hyaline, smooth, 3.0–4.0 × 2.0–2.5 µm when cylindrical, 4.5–8.0(–9.0) × 4.0–8.0 µm when globose. Zygosporangia not observed. Chlamydospores absent. No growth at 31 °C.

Absidia biappendiculata (Rall & Solheim) T.K. Zong & X.Y. Liu, **comb. nov.**

(Fig. 12)

Mycobank no.: XXXXXX (to add after review).

Basionym: *Absidia spinosa* var. *biappendiculata* Rall & Solheim, *Mycologia*, 56(1), 99. 1964. [MB no.: 348993]

Holotype: **United States**, Wyoming, Albany County, Medicine Bow Mountains, Dry Park near Fox Park, from the leaves of *Comandra pallida*, August 1960, G. Rall (NRRL 3033, isotype CBS 187.64, isotype HMAS 350310)

Etymology: *biappendiculata* (Lat.) adopting from basionym.

Description: Colonies on MEA, regularly zonate, attaining 75 mm diameter after 5 d at 27 °C, white at first and then pale mouse gray (R51). Hyphae hyaline to slightly pigmented, 5.5–11.0 µm wide. Stolons branched, hyaline, smooth, septate, 3.5–5.5 µm wide. Rhizoids root-like, taper at the end, with a septum at the base. Sporangiohores erect or slightly bent, 1–5 in whorls, unbranched or simple, monopodial and sympodial not observed, hyaline, with a septum 11.0–17.0 µm below apophyses, 65.0–180.0(–210.0) × 2.5–5.0 µm. Apophyses distinct, slightly pigmented, 3.5–7.0(–9.0) µm high, 2.5–4.5 µm wide at the base, and 8.0–14.0(–20.0) µm wide at the top. Sporangia globose to pyriform, multispored, deliquescent-walled, (16.0–)19.0–29.0 × (15.5–)18.5–27.0 µm. Columellae hemispherical to subglobose, hyaline, smooth, sometimes with a 3.0–7.0 µm spinous projection at the apex, occasionally two projections, or with a conical projection in some of smaller columellae, 7.5–15.0(–19.0) × 8.5–16.0(–20.0) µm. Collars distinct if present. Sporangiospores cylindrical to oval, slightly constricted in the center, hyaline, smooth, (3.5–)4.0–4.5 × 2.0–2.5 µm. Zygospores globose, brown to dark brown, rough, 33.0–66.0 µm diameter. Suspensor mostly two, nearly equal, parallel or nearly so, sometimes only one, hyaline or brown, 14.0–24.0(–27.0) µm diameter. Chlamyospores absent. No growth at 35 °C.

DISCUSSION

Phylogenetically, the multi-gene tree (**Fig. 1**) shows that nine new species and two new combinations all cluster in *Absidia*. Among these species, *Absidia ampullacea* is a sister group of *A. caatinguensis* with a 92% / 1.00 support value. However, *A. caatinguensis*

physiologically differs from *A. ampullacea* in maximum growth temperature (35 °C vs. 30 °C). They also morphologically differ in shape and size of sporangiospores and projection on columellae. In detail, cylindrical sporangiospores (5.0–7.0 × 2.5–3.7 µm) and bulbous projection (up to 5.7 µm in length) of *A. caatinguensis* are both longer than subglobose to oval sporangiospores (3.0–4.5 × 2.5–4.0 µm) and verrucous projection (up to 2.0 µm in length) of *A. ampullacea* (Ariyawansa et al. 2015).

Abisidia brunnea is most closely related to *A. soli* (100/100/1.00). However, physiologically, *A. soli* differs from *A. brunnea* in maximum growth temperature (37 °C vs. 35 °C). Morphologically, *A. soli* is characterized by its sporangiophores up to 6 in whorls, septa distantly below apophyses (21.5–37.5 µm), larger sporangia (16.0–51.0 × 15.0–45.5 µm, average 34.5 × 30.0 µm), and sporangiospores only cylindrical and without vacuole (Hurdeal et al. 2021).

Abisidia chinensis (100/100/1.00) clusters with *A. nigra* (100/100/1.00) but their sibling relationship does not receive a strong support. Physiologically, *A. chinensis* is similar to *A. nigra* in maximum growth temperature but morphologically they are different in sporangiospores, sporangiophores, rhizoids and apophyses. *A. chinensis* produces cylindrical sporangiospores only, swollen and branched sporangiophores, coralliform rhizoids and bell-shaped or hourglass-shaped apophyses, while *A. nigra* produces both cylindrical and globose sporangiospores, swelling but never branched sporangiophores, relatively short rhizoids and never hourglass-shaped apophyses (Hesseltine and Ellis 1964).

Abisidia cinerea appears to be phylogenetically basal to *A. pseudocylindrospora* Hesselt. & J.J. Ellis and *A. stercoraria*, although with relatively low support values. However, physiologically, *A. pseudocylindrospora* have a maximum growth temperature of 37 °C, but neither *A. cinerea* nor *A. stercoraria* can grow above 35 °C. Morphologically, *A. cinerea* differs in that it produces longer cylindrical sporangiospores (4.5–6.0 × 2.0–2.5 µm) and branch-shaped rhizoids with septa, whereas *A. pseudocylindrospora* and *A. stercoraria* both bear shorter sporangiospores, 2.5 × 3.5–5.0 µm and 4.0–5.0 × 2.0–3.0 µm, respectively. Rhizoids have rarely septa in *A. pseudocylindrospora* and are not well developed in *A. stercoraria*. Additionally,

sporangiophores of *A. cinerea* arise in whorls of up to 7, whereas those of *A. pseudocylindrospora* and *A. stercoraria* arise in whorls of up to 11 and 5, respectively (Hesseltine and Ellis 1961; Li et al. 2016).

Absidia digitata is most closely related to *A. turgida* with a high support value (99/100/1.00) and a similar maximum growth temperature (32 °C vs. 33 °C). However, morphologically, *A. turgida* differs from *A. digitata* in that it produces sporangiophores singly or in whorls of up to 4, only one projection on the columellae, and such variable sporangiospores as globose, cylindrical or irregular (Zong et al. 2021).

Absidia oblongispora is most closely related to *A. heterospora* Y. Ling, which is strongly supported with a high value of 100/100/1.00. At the same time, they have similar maximum growth temperatures physiologically (32 °C vs. 31 °C). But morphologically, *A. heterospora* differs from *A. oblongispora* in that it produces larger columellae (10.5–34 µm diameter) without projection and two types of sporangiospores (3.8–7.7 µm when globose and 3.3–6.6 × 2.2–4.4 µm when cylindrical to oval, Hesseltine and Ellis 1964), whereas *A. oblongispora* always presents projection on columellae and only smaller cylindrical to oval sporangiospores (3.5–4.5 × 2.5–3.0 µm).

Absidia sympodialis (100/100/1.00), *A. virescens* (100/100/1.00) and *A. varians* (100/100/1.00) are nested in the globospora clade, and they all producing green colonies. *A. sympodialis* and *A. virescens* are sister taxa and cluster with *A. globospora* (100/100/1.00), having similar sporangia. Their relationship is highly supported by the multi-gene tree, with a -/98/1.00 support value. However, physiologically and morphologically, *A. globospora* is different by no growth at 29°C, whereas the maximum growth temperature of *A. sympodialis* and *A. virescens* both are higher than 30 °C. In addition, the sporangiophores of *A. globospora* are in whorls up to 5, whereas those of *A. sympodialis* and *A. virescens* arise in whorls of up to 6 and 4, respectively. *A. virescens* produces sporangiospores of non-uniform in size and columellae with two projections, some projection up to 6.5 µm in length, whereas the sporangiospores of *A. sympodialis* and *A. globospora* are uniform in size, and both columellae with only one projection less than 5 µm in length. *A. sympodialis* differs from *A. virescens* and *A. globospora* in the shape of rhizoids and columellae. It has fibrous-root-shaped rhizoids,

differing from them in that both have root-like rhizoids. Additionally, columellae of *A. sympodialis* are hemispherical, oval and chestnut-shaped, unlike those hemispherical in *A. virescens* and *A. globospora* (Zong et al. 2021). *Absidia varians* (100/100/1.00) is phylogenetically next to *A. glauca* but morphologically similar to *A. repens* because they both have variable projections on columellae. *A. repens* is different from *A. varians* in that it having olive-gray colonies and two types of sporangiospores (Hesseltine and Ellis 1966).

Absidia nigra (100/100/1.00) and *A. biappendiculata* (100/100/1.00) were previously regarded as synonyms of *A. cylindrospora* Hagem and *A. spinosa* Lendn., respectively. These treatments of synonymization do not receive support either phylogenetically or morphologically. Phylogenetically, *A. nigra* is related to *A. chinensis* rather than *A. cylindrospora*, and *A. biappendiculata* clusters with *A. stercoraria*, *A. pseudocylindrospora* and *A. cinerea* (86/85/1.00) rather than *A. spinosa*. Morphologically, *A. nigra* differs from *A. cylindrospora* by not giving any mating reaction with any *A. cylindrospora* strains (Hesseltine and Ellis 1964). In addition, *A. nigra* produces cylindrical sporangiospores $3.0\text{--}4.0 \times 2.0\text{--}2.5 \mu\text{m}$ and globose sporangiospores $4.5\text{--}8.0(9.0) \times 4.0\text{--}8.0 \mu\text{m}$, differing from *A. cylindrospora* which produces only cylindrical sporangiospores $3.3\text{--}5.5 \times 2.2\text{--}3.5 \mu\text{m}$ (Hesseltine and Ellis 1964), as well as sporangiophores sometimes with a swelling beneath sporangia. *A. biappendiculata* is like *A. spinosa* in the appearance of sporangiospores, sporangia, and columellae (Rall et al. 1964). However, sporangiophores in *A. spinosa* are wider ($5\text{--}10.5 \mu\text{m}$) than those in *A. biappendiculata* (Rall et al. 1964). Additionally, *A. spinosa* produces only one projection on columellae (Hesseltine and Ellis 1964; Rall et al. 1964), unlike two projections in *A. biappendiculata*.

Physiologically, as mentioned in the introduction, growth temperature has been an important characteristic to distinguish *Absidia s. str.* (optimal growth temperature of $25\text{--}34 \text{ }^\circ\text{C}$) and *Lichtheimia* species (optimal growth temperature of $37\text{--}45 \text{ }^\circ\text{C}$, Hoffmann et al. 2007). Most species of *Absidia s. str.* were within the temperature range, but it was also stated that *Absidia cuneospora* G.F. Orr & Plunkett, *A. idahoensis* var. *thermophile* G.Q. Chen & R.Y. Zheng, *A. koreana* and *A. pseudocylindrospora* were

able to grow above 37 °C (Orr and Plunkett 1959; Hesseltine and Ellis 1961; Chen and Zheng 1998; Ariyawansa et al. 2015). On the other hand, *Lichtheimia sphaerocystis* Alastr.-Izq. & Walther and *L. brasiliensis* A.L. Santiago, N. Lima & R.J.V. Oliveira were limited in growth at 40 °C (Alastruey-Izquierdo et al. 2010; André et al. 2014). This indicates that growth temperature does not accurately distinguish the species of *Absidia s. str.* and *Lichtheimia* (syn. *Absidia pro parte*). The maximum growth temperatures of the nine new species reported here are 29–35 °C, falling in the temperature range of *Absidia s. str.*

Morphologically, Hoffmann et al. (2007) found that *Absidia* usually have projections on the apex of columellae. The nine new species and two combinations described here also have this characteristic. Therefore, we believe that projection on columellae is a typical feature of the genus *Absidia*, except for the species *A. heterospora* (Hesseltine and Ellis 1964).

The *Absidia* genus was proposed to be divided into several groups distinguishable by their sporangiospores (Kwaśna et al. 2006; Hoffmann et al. 2007, 2009b; Hoffmann and Voigt 2008; Hoffmann 2010). However, with new species of *Absidia* were increasingly described, some of them were found to have two and more shapes of sporangiospores, such as *A. pararepens*, *A. repens*, *A. turgida*, etc (Fig. 1, Crous et al. 2020; Cordeiro et al. 2020; Zong et al. 2021). Therefore, the clades on the phylogenetic tree should be further divided, especially the clade previously defined as cylindrical spores. Additionally, *A. digitata* does not group within the globose spores clade, even though it has globose sporangiospores only, indicating that the shape of sporangiospores in *Absidia* might polyphyletic evolved.

Types in *Absidia* are mainly distributed in North America (e.g., Canada, Honduras, Mexico, United States), South America (e.g., Argentina, Brazil), Europe (e.g., Czech, Germany, Holland, United Kingdom, Switzerland), Africa (e.g., Tanzania) and Asia (e.g., China, India, South Korea, Thailand) (Fig. 13), but have never been reported in Oceania. Only type of *Absidia idahoensis* var. *thermophila* was from China before 1998 (Chen and Zheng 1998), suggesting an insufficient sampling by Chinese mycologists. In recent years, more and more new species, e.g. *Absidia globospora*, *A. medulla*, *A.*

ovalispora, *A. panacisoli*, *A. turgida* and *A. zonata*, have gradually been reported from China (Zhang et al. 2018; Zhao et al. 2021; Zong et al. 2021). This study further supplements nine new species, making the list of Chinese *Absidia* types as long as 16.

As many as eleven new taxa are placed in *Absidia*, and therefore it is necessary to update the synoptic key for the 23 species distributed in China as follows.

Key to the known species of *Absidia* in China

1. Sporangiospores obviously two or more types.....2
1. Sporangiospores always only one type.....7
2. Sporangiospores sometimes irregular in shape.....3
2. Sporangiospores always regular in shape.....5
3. With elliptical or very elongate sporangia; elliptical sporangia typically borne singly in great numbers along stolons.....*A. repens*
3. Without elliptical or elongate sporangia.....4
4. Hyphae without swelling, < 9 µm in width; sporangiophores sometimes simple, more often monopodial or verticillate; columellae sometimes with a short projection.....*A. idahoensis*
4. Hyphae occasionally swelling, > 9 µm in width; sporangiophores unbranched or sometimes simple; columellae always with a projection up to 9 µm in length.....*A. turgida*
5. Columellae without distinct apical projections.....*A. heterospora*
5. Columellae with apical projections.....6
6. Sporangiophores 1–7 in whorls; rhizoids relatively longer; sporangiospores never globose, the larger one oval with vacuole; no growth at 35 °C.....*A. brunnea*
6. Sporangiophores 1–5 in whorls; rhizoids relatively shorter; sporangiospores globose in the larger one, without vacuole; no growth at 31 °C.....*A. nigra*
7. Sporangiospores typically globose or nearly so.....8
7. Sporangiospores mostly cylindrical or oval.....13
8. Colonies brownish; rhizoids finger-like; with clavate projections on the columellae.....*A. digitata*
8. Colonies greenish; rhizoids root-like or other shapes.....9

9. Sporangia subglobose to elliptical; projections on the columellae variable in shape.....	<i>A. varians</i>	9
9. Sporangia globose to pyriform; projections on the columellae invariable in shape.....		10
10. Sporangiospores non-uniform in size; columellae sometimes with the single or two projections.....	<i>A. virescens</i>	10
10. Sporangiospores uniform in size; columellae with only one projection.....		11
11. Columellae sometimes chestnut-shaped; rhizoids fibrous-root-shaped.....	<i>A. sympodialis</i>	11
11. Columellae hemispherical but never chestnut-shaped; rhizoids root-like.....		12
12. Sporangiphores not reaching 10 µm in width; sporangia rarely reaching 55 µm diam; no growth at 29 °C.....	<i>A. globospora</i>	12
12. Sporangiphores reaching 12 µm in width; sporangia mostly 50–60 µm diam; no growth at 37 °C.....	<i>A. glauca</i>	12
13. Collar absent; sporangiospores ovoidal to ellipsoidal.....	<i>A. ovalispora</i>	13
13. Collar always present; sporangiospores typically cylindrical.....		14
14. Sporangiphores never in pairs or in whorls.....	<i>A. panacisoli</i>	14
14. Sporangiphores in pairs and in whorls.....		15
15. Sporangiphores as many as 7–11 in whorls.....		16
15. Sporangiphores no more than 6 in whorls.....		19
16. Rhizoids typically unseptate.....	<i>A. spinosa</i>	16
16. Rhizoids septate.....		17
17. Apophyses bell-shaped or funnel-shaped.....	<i>A. cinerea</i>	17
17. Apophyses never bell-shaped or funnel-shaped.....		18
18. Projections on columellae < 5 µm in length, taper at the top.....	<i>A. zonata</i>	18
18. Projections on columellae > 5 µm in length, rounded at the top.....	<i>A. pseudocylindrospora</i>	18
19. Maximum growth temperatures > 34 °C.....	<i>A. cylindrospora</i>	19
19. Maximum growth temperatures ≤ 34 °C.....		20
20. Hyphae and sporangiphores both swollen.....		21

20. Hyphae or sporangiophores never swollen.....22
21. Sporangiospores cylindrical to oval; rhizoids coralliform; papillary projections on columellae 2–5.5 μm in length.....*A. chinensis*
21. Sporangiospores oval to subglobose; rhizoids finger-like; verrucous projections on columellae up to 2 μm in length.....*A. ampullacea*
22. Sporangiohores 1–5 in whorls, no sympodial; columellae mostly conical, rarely hemispherical, with the bulbous projection up to 7.5 μm in length.....*A. oblongispora*
22. Sporangiohores 1–6 in whorls, sympodial; columellae mostly hemispherical, with the pointed projection up to 4.5 μm in length.....*A. medulla*

DECLARATIONS

Ethics approval and consent to participate

Not applicable.

Adherence to national and international regulations

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Details of the availability of the data and materials used in this study can be found within the text. DNA sequences were submitted to GenBank [<https://www.ncbi.nlm.nih.gov/>]. Scientific name and morphological description deposited at MycoBank [<https://www.mycobank.org>]. Alignments were deposited at TreeBase (submission ID: 28429, <https://www.treebase.org/treebase-web/>). Living Strains were deposited in the China General Microbiological Culture Collection Center, Beijing, China (CGMCC, <http://www.cgmcc.net/>). Dried Strains were deposited in the Herbarium Mycologicum Academiae Sinicae, Beijing, China (HMAS).

Competing interests

The authors declare no conflict of interest.

Funding

This research was funded by the National Natural Science Foundation of China, grant number 31970009 and the Yunnan Fundamental Research Project, Grant number 202001AS070043.

Authors' contributions

ZTK, ZCL and LXY contributed to conception and design of the study. ZTK wrote the draft of the manuscript. ZCL and LXY improved the manuscript. ZTK, ZH and LXY observed and described the morphology. LXL and RLY collected and analysed the molecular data. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

We thank Fengyan Bai, Long Wang, Xuwei Wang, Guiqing Chen, Yinglan Guo (Institute of Microbiology, CAS) for collecting soil samples.

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FIGURE LEGENDS

Fig. 1 The Maximum Parsimony strict consensus tree illustrating the phylogeny of nine new species and two new combinations of *Absidia* in Cunninghamellaceae based on multi-gene sequences. *Cunninghamella elegans* and *C. blakesleeana* serve as outgroups. Branches are labeled with Maximum Likelihood bootstrap values higher than 70 %, Maximum Parsimony bootstrap values higher than 70 % and Bayesian posterior probabilities more than 0.95. The lower left scale represents steps. Sporangiospore shapes are annotated.

Fig. 2 *Absidia ampullacea* CGMCC 3.16054. (A) obverse colony on MEA; (B) reverse colony on MEA; (C) sporangium; (D–F) columellae; (G–I) swelling on sporangiospores and hyphae; (J) sporangiospores; (K) rhizoids. Scale bars: (C–F, K) 10 μm ; (G–I) 20 μm ; (J) 5 μm .

Fig. 3 *Absidia brunnea* CGMCC 3.16055. (A) obverse colony on MEA; (B) reverse colony on MEA; (C) sporangium; (D, E) columellae; (F, G) swelling on sporangiospores; (H) sporangiospores; (I) rhizoids. Scale bars: (C–G, I) 10 μm ; (H) 5 μm .

Fig. 4 *Absidia chinensis* CGMCC 3.16056. (A) obverse colony on MEA; (B) reverse colony on MEA; (C) sporangium; (D–F) columellae; (G, H) swelling on sporangiospores; (I) sporangiospores; (J) rhizoids; (K–N) zygosporangia. Scale bars: (C–F, J–N) 10 μm ; (G, H) 20 μm ; (I) 5 μm .

Fig. 5 *Absidia cinerea* CGMCC 3.16062. (A) obverse colony on MEA; (B) reverse colony on MEA; (C) sporangium; (D–F) columellae; (G) sporangiospores; (H) rhizoids; (I) sporangiospores in whorls; (J–L) zygosporangia. Scale bars: (C–F) 10 μm ; (G) 5 μm ; (H–L) 20 μm .

Fig. 6 *Absidia digitata* CGMCC 3.16058. (A) obverse colony on MEA; (B) reverse

colony on MEA; (C) sporangium; (D–G) columellae; (H) swelling on hyphae; (I) sporangiospores; (J) rhizoids. Scale bars: (C–G) 10 µm; (H, J) 20 µm; (I) 5 µm.

Fig. 7 *Absidia oblongispora* CGMCC 3.16061. (A) obverse colony on MEA; (B) reverse colony on MEA; (C) sporangium; (D–F) columellae; (G) sporangiospores; (H) rhizoids. Scale bars: (C–F, H) 10 µm; (G) 5 µm.

Fig. 8 *Absidia sympodialis* CGMCC 3.16064. (A) obverse colony on MEA; (B) reverse colony on MEA; (C) sporangium; (D, E) columellae; (F) sporangiospores; (G, H) rhizoids; (I) sympodial sporangiospores. Scale bars: (C–E, G–I) 20 µm; (F) 5 µm.

Fig. 9 *Absidia varians* CGMCC 3.16065. (A) obverse colony on MEA; (B) reverse colony on MEA; (C, D) sporangia; (E–L) columellae; (M) sporangiospores; (N, O) rhizoids. Scale bars: (C–L) 10 µm; (M) 5 µm; (N, O) 20 µm.

Fig. 10 *Absidia virescens* CGMCC 3.16067. (A) obverse colony on MEA; (B) reverse colony on MEA; (C) sporangium; (D–G) columellae; (H) swelling on sporangiospores; (I) sporangiospores; (J, K) rhizoids; (L) monopodial sporangiospores. Scale bars: (C–H, J–K) 20 µm; (I) 5 µm; (L) 50 µm.

Fig. 11 *Absidia nigra* CGMCC 3.16059. (A) obverse colony on MEA; (B) reverse colony on MEA; (C) sporangia; (D, E) columellae; (F) swelling on sporangiospores; (G) sporangiospores; (H) rhizoids (I, J) swollen hyphae. Scale bars: (C–F, H) 10 µm; (G) 5 µm; (I, J) 20 µm.

Fig. 12 *Absidia biappendiculata* CBS 187.64. (A) obverse colony on MEA; (B) reverse colony on MEA; (C) sporangium; (D–G) columellae; (H) sporangiospores; (I) rhizoids; (K–L) zygospores. Scale bars: (C–G) 10 µm; (H) 5 µm; (I–L) 20 µm.

Fig. 13 The geographic distribution of *Absidia* types except for *A. glauca* and *A.*

cylindrospora (neither is available). Triangles represent nine new species and two combinations proposed in this study. Circles represent previously reported species.

Table 1 The list of species, strains, and GenBank accession numbers of sequences used in this study.

Species	Strains	GenBank accession no.			
		ITS	LSU	TEF-1 α	<i>Act</i>
<i>Absidia ampullacea</i>	CGMCC 3.16054 ^T	MZ354138	MZ350132		
<i>A. anomala</i>	CBS 125.68	NR_103626	NG_058562		
<i>A. anomala</i>	FSU 5798	EF030523			EF030535
<i>A. biappendiculata</i>	CBS 187.64 ^T	MZ354153	MZ350147	MZ357420	MZ357438
<i>A. bonitoensis</i>	URM 7889	MN977786	MN977805		
<i>A. brunnea</i>	CGMCC 3.16055 ^T	MZ354139	MZ350133	MZ357403	MZ357421
<i>A. caatinguensis</i>	URM 7156	KT308169	KT308171		
<i>A. californica</i>	CBS 314.78	MH861141	MH872902		
<i>A. californica</i>	FSU 4747	AY944872	EU736300	EU736246	AY944758
<i>A. californica</i>	FSU 4748	AY944873	EU736301	EU736247	EU736224
<i>A. chinensis</i>	CGMCC 3.16056 ^T	MZ354140	MZ350134		
<i>A. chinensis</i>	CGMCC 3.16057	MZ354141	MZ350135		MZ357422
<i>A. cinerea</i>	CGMCC 3.16062 ^T	MZ354146	MZ350140	MZ357407	MZ357427
<i>A. coerulea</i>	CBS 101.36	MH855718	MH867230		

<i>A. coerulea</i>	NRRL 1315	AY944870	AF113443	AF157226	AJ287133
<i>A. cornuta</i>	URM 6100	MN625256	MN625255		
<i>A. cuneospora</i>	CBS 101.59	NR_159602	NG058559		
<i>A. cylindrospora</i>	CBS 100.08	JN205822	JN206588		
<i>A. cylindrospora var. nigra</i>	CBS 127.68		NG_058560		
<i>A. cylindrospora var. rhizomorpha</i>	CBS 153.63		NG_058563		
<i>A. digitata</i>	CGMCC 3.16058^T	MZ354142	MZ350136	MZ357404	MZ357423
<i>A. edaphica</i>	MFLU 20-0416	MT396372	MT393987		MT410739
<i>A. fusca</i>	CBS 102.35	NR103625	NG058552		
<i>A. glauca</i>	CBS 129233	MH865253	MH876693		
<i>A. glauca</i>	CBS 101.08	NR_111658	NG_058550		
<i>A. glauca</i>	FSU 660	AY944879	EU736302	EU736248	EU736225
<i>A. globospora</i>	CGMCC 3.16031	MW671537	MW671544	MZ357412	MZ357431
<i>A. globospora</i>	CGMCC 3.16035	MW671538	MW671545	MZ357413	MZ357432
<i>A. globospora</i>	CGMCC 3.16036	MW671539	MW671546	MZ357414	MZ357433
<i>A. heterospora</i>	SHTH021	JN942683	JN982936		
<i>A. jindoensis</i>	CNUFC-PTI1-1	MF926622	MF926616	MF926513	MF926509

<i>A. koreana</i>	EML-IFS45-1	KR030062	KR030056	KR030060	KR030058
<i>A. macrospora</i>	FSU 4746	AY944882	EU736303	EU736249	AY944760
<i>A. medulla</i>	CGMCC 3.16034	MW671542	MW671549	MZ357417	MZ357436
<i>A. medulla</i>	CGMCC 3.16037	MW671543	MW671550	MZ357418	
<i>A. multispora</i>	URM 8210	MN953780	MN953782		
<i>A. nigra</i>	CGMCC 3.16059^T	MZ354143	MZ350137	MZ357405	MZ357424
<i>A. nigra</i>	CGMCC 3.16060	MZ354144	MZ350138	MZ357406	MZ357425
<i>A. nigra</i>	CBS 127.68	MZ354152	MZ350146	MZ357419	MZ357437
<i>A. oblongispora</i>	CGMCC 3.16061^T	MZ354145	MZ350139		MZ357426
<i>A. ovalispora</i>	CGMCC 3.16018	MW264071	MW264130		
<i>A. panacisoli</i>	SYPF 7183	MF522181	MF522180	MF624251	
<i>A. pararepens</i>	CCF 6352	MT193669	MT192308		
<i>A. pernambucoensis</i>	URM 7219	MN635568	MN635569		
<i>A. pseudocylindrospora</i>	CBS 100.62	NR_145276	NG_058561		
<i>A. pseudocylindrospora</i>	EML-FSDY6-2	KU923817	KU923814		KU923815
<i>A. psychrophilia</i>	FSU 4745	AY944874	EU736306	EU736252	AY944762
<i>A. repens</i>	CBS 115583	NR103624	HM849706		

<i>A. repens</i>	NRRL 1336		AF113448	AF157228	AJ287136
<i>A. saloaensis</i>	URM 8209	MN953781	MN953783		
<i>A. soli</i>	MFLU 20-0414	MT396373	MT393988		MT410740
<i>A. spinosa</i>	FSU 551	AY944887	EU736307	EU736253	EU736227
<i>A. spinosa var. biappendiculata</i>	CBS 187.64		MH870040		
<i>A. stercoraria</i>	EML-DG8-1	NR_148090	KT921998	KT922002	KT922000
<i>A. sympodialis</i>	CGMCC 3.16064^T	MZ354148	MZ350142	MZ357408	
<i>A. sympodialis</i>	CGMCC 3.16063	MZ354147	MZ350141		
<i>A. terrestris</i>	FMR 14989	LT795003	LT795005		
<i>A. turgida</i>	CGMCC 3.16032	MW671540	MW671547	MZ357415	MZ357434
<i>A. varians</i>	CGMCC 3.16065^T	MZ354149	MZ350143	MZ357409	MZ357428
<i>A. virescens</i>	CGMCC 3.16067^T	MZ354151	MZ350145	MZ357411	MZ357430
<i>A. virescens</i>	CGMCC 3.16066	MZ354150	MZ350144	MZ357410	MZ357429
<i>A. zonata</i>	CGMCC 3.16033	MW671541	MW671548	MZ357416	MZ357435
<i>Cunninghamella blakesleeana</i>	CBS 782.68	JN205869	MH870950	KJ156478	
<i>Cunninghamella elegans</i>	CBS 167.53	JN205882	HM849700	KJ156494	

*Sequences obtained herein are shown in bold. The “T” is abbreviated for ex-type strains.

Figures

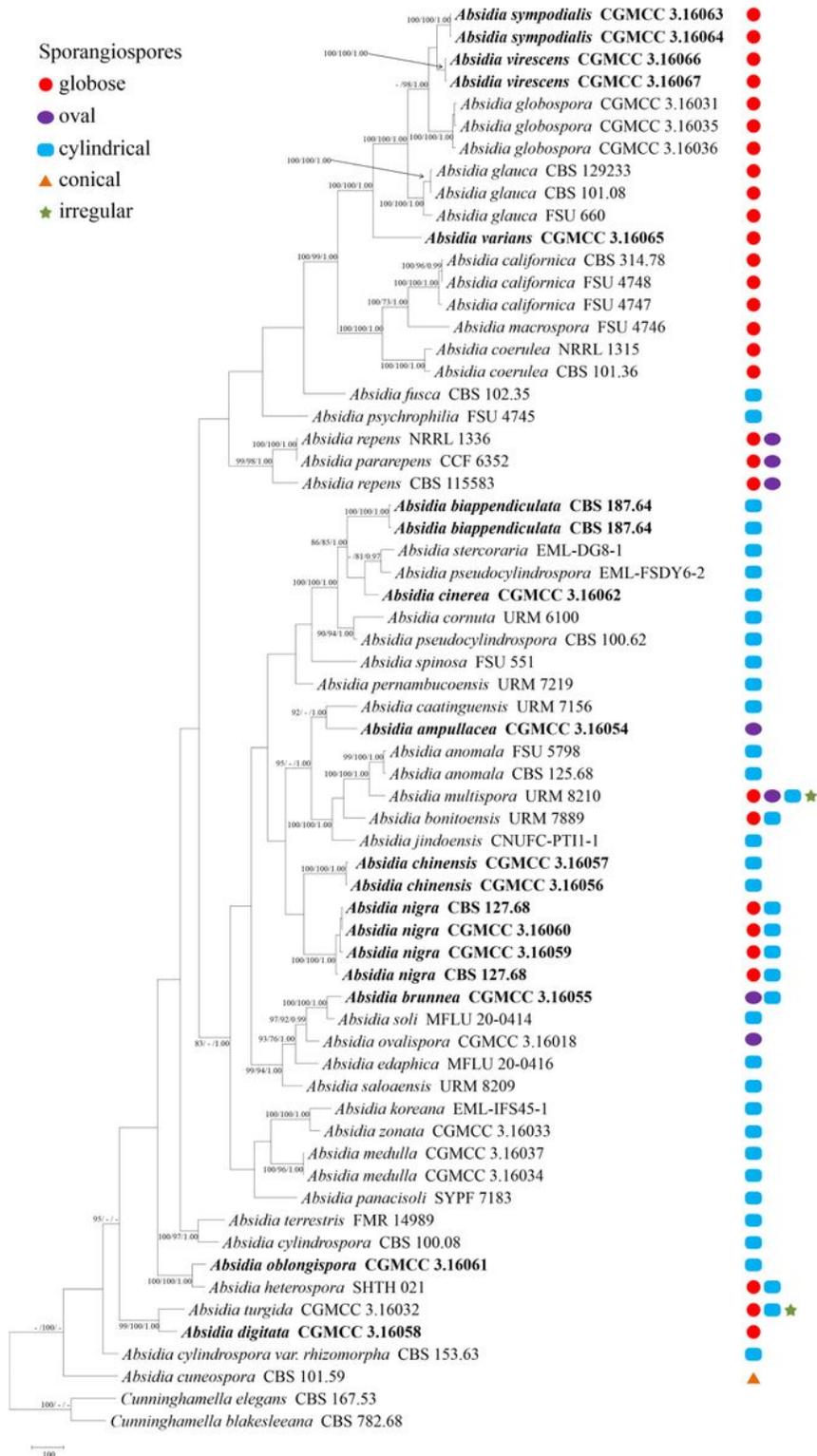


Figure 1

The Maximum Parsimony strict consensus tree illustrating the phylogeny of nine new species and two new combinations of *Absidia* in Cunninghamellaceae based on multi-gene sequences. *Cunninghamella elegans* and *C. blakesleeana* serve as outgroups. Branches are labeled with Maximum Likelihood

bootstrap values higher than 70 %, Maximum Parsimony bootstrap values higher than 70 % and Bayesian posterior probabilities more than 0.95. The lower left scale represents steps. Sporangiospore shapes are annotated.

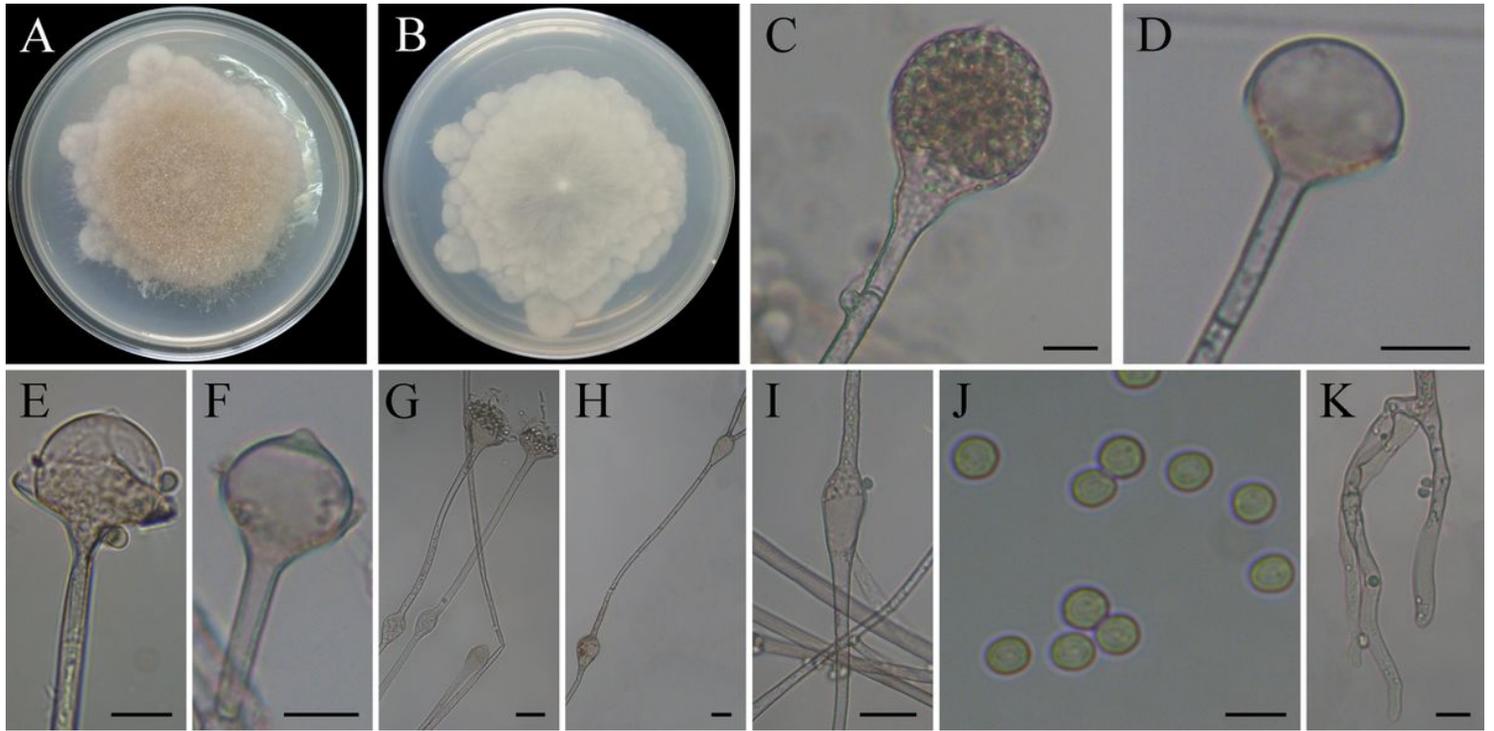


Figure 2

Absidia ampullacea CGMCC 3.16054. (A) obverse colony on MEA; (B) reverse colony on MEA; (C) sporangium; (D–F) columellae; (G–I) swelling on sporangiospores and hyphae; (J) sporangiospores; (K) rhizoids. Scale bars: (C–F, K) 10 µm; (G–I) 20 µm; (J) 5 µm.

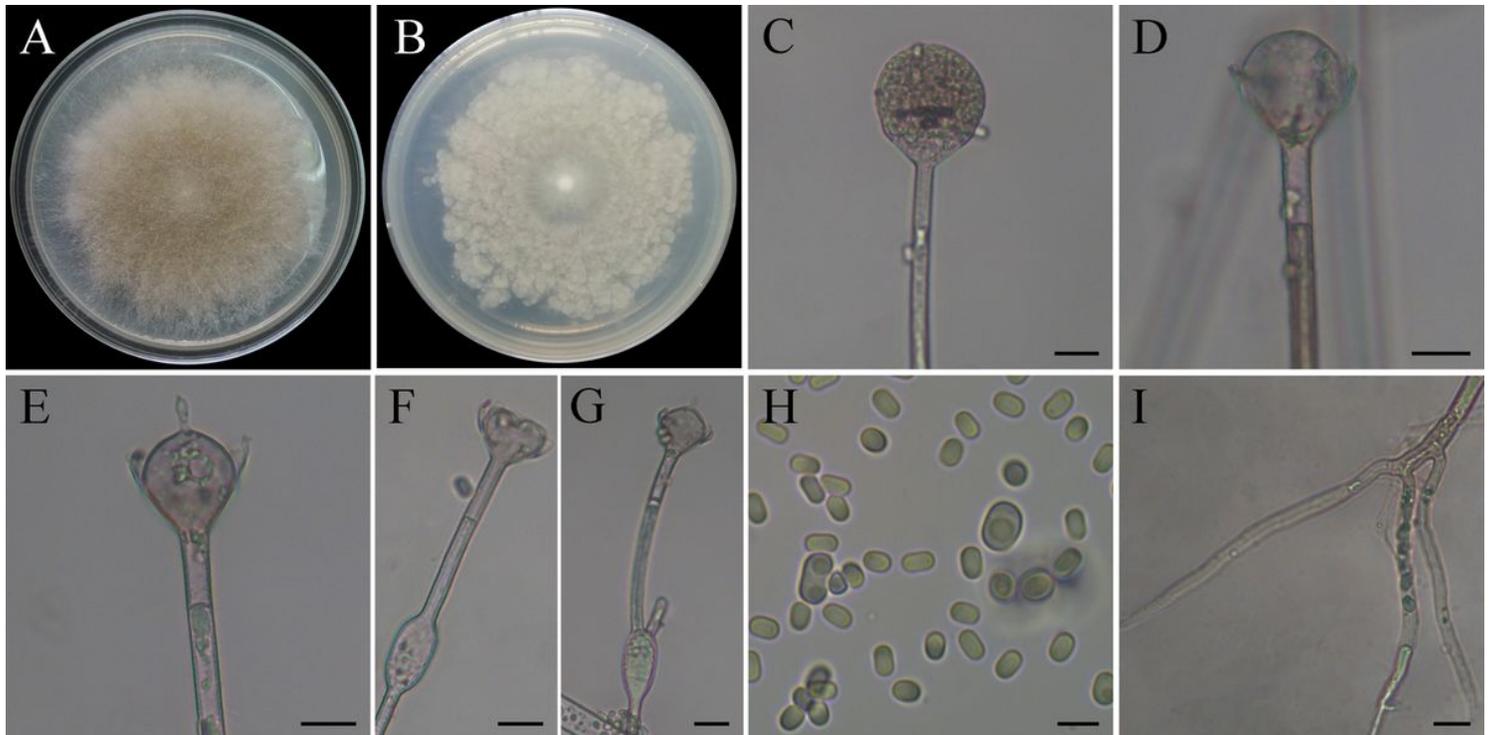


Figure 3

Absidia brunnea CGMCC 3.16055. (A) obverse colony on MEA; (B) reverse colony on MEA; (C) sporangium; (D, E) columellae; (F, G) swelling on sporangiospores; (H) sporangiospores; (I) rhizoids. Scale bars: (C–G, I) 10 μm ; (H) 5 μm .

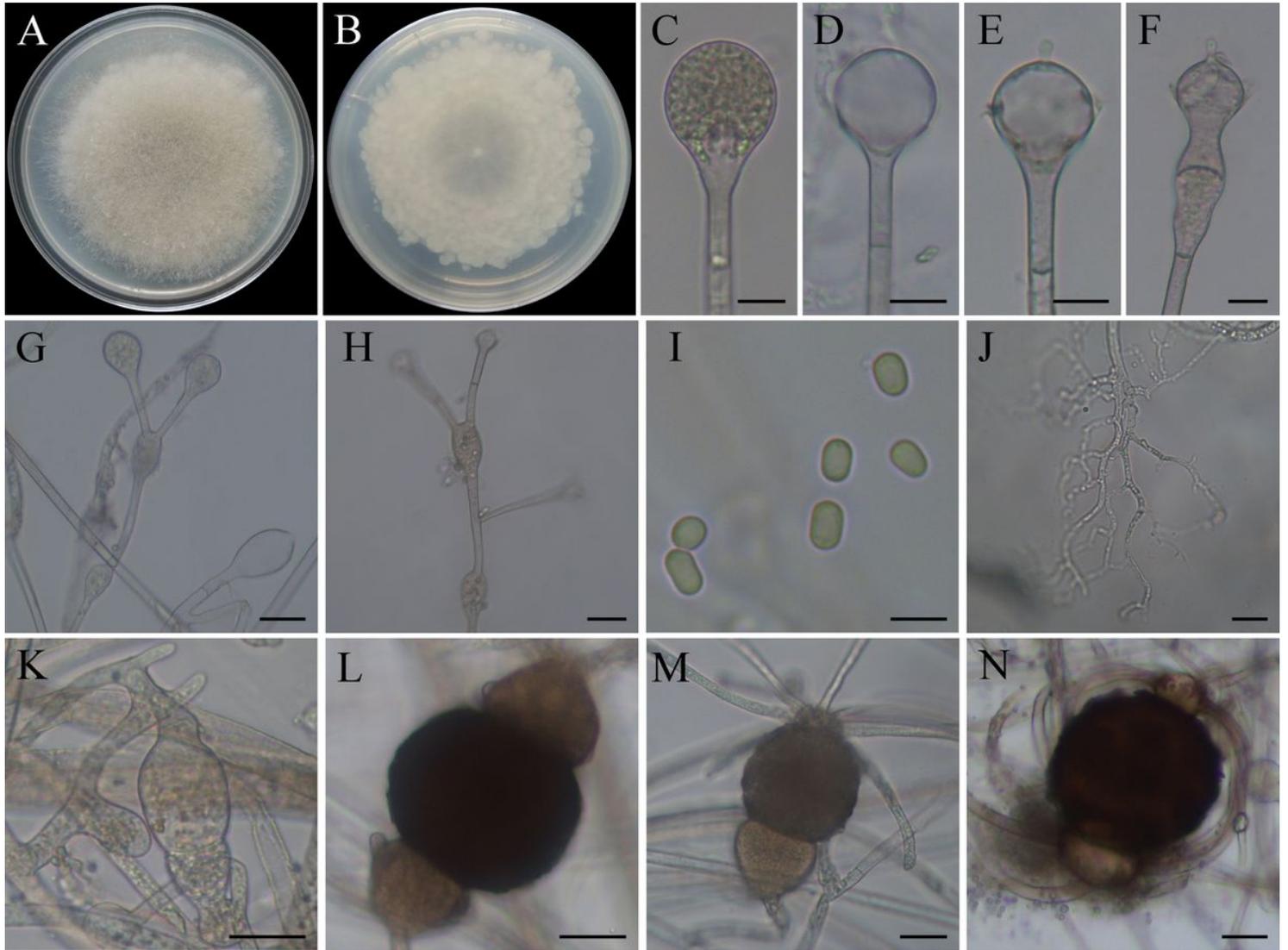


Figure 4

Absidia chinensis CGMCC 3.16056. (A) obverse colony on MEA; (B) reverse colony on MEA; (C) sporangium; (D–F) columellae; (G, H) swelling on sporangiospores; (I) sporangiospores; (J) rhizoids; (K–N) zygospores. Scale bars: (C–F, J–N) 10 μm ; (G, H) 20 μm ; (I) 5 μm .

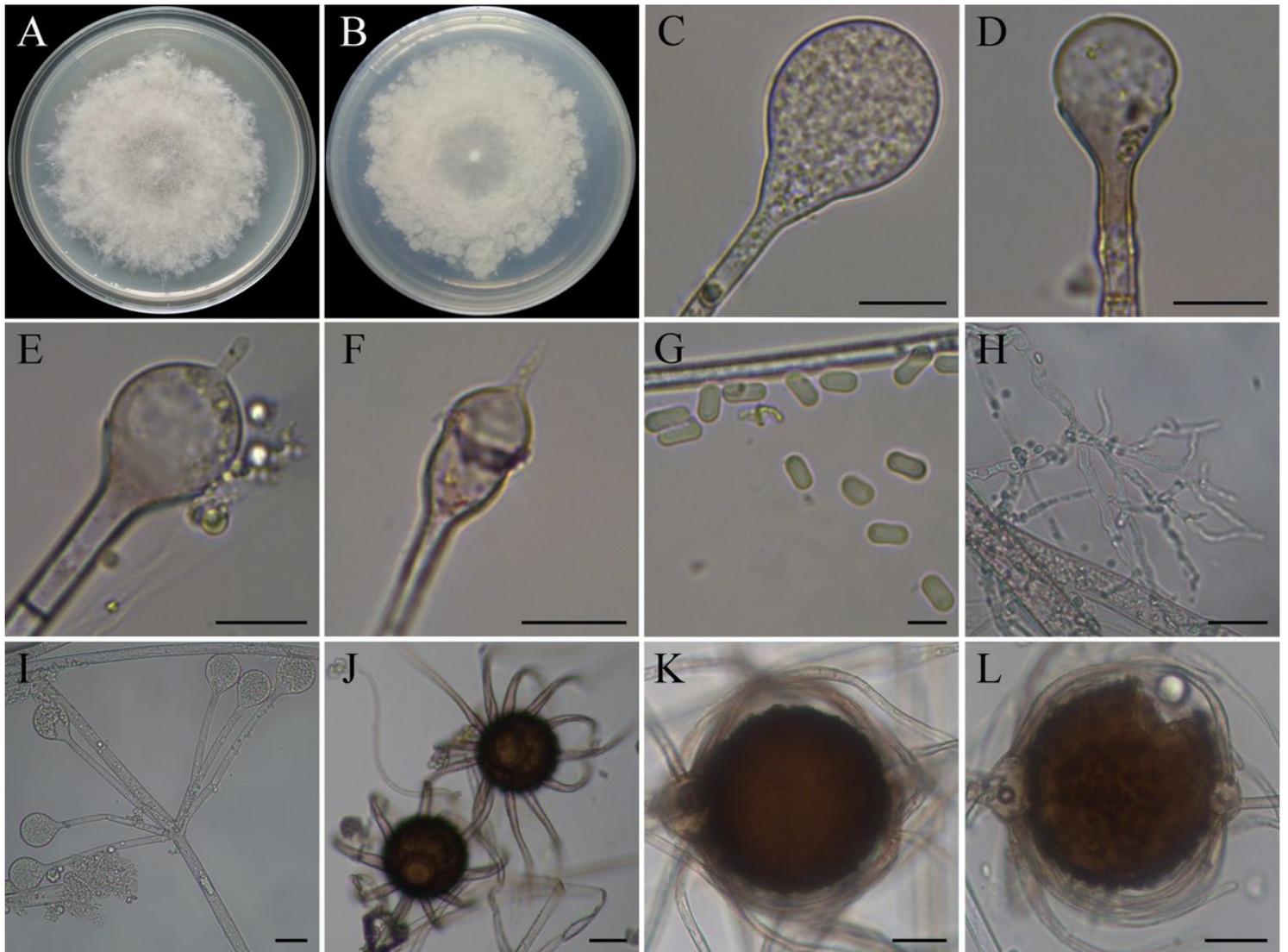


Figure 5

Absidia cinerea CGMCC 3.16062. (A) obverse colony on MEA; (B) reverse colony on MEA; (C) sporangium; (D–F) columellae; (G) sporangiospores; (H) rhizoids (I) sporangiophores in whorls; (J–L) zygospores. Scale bars: (C–F) 10 μm ; (G) 5 μm ; (H–L) 20 μm .

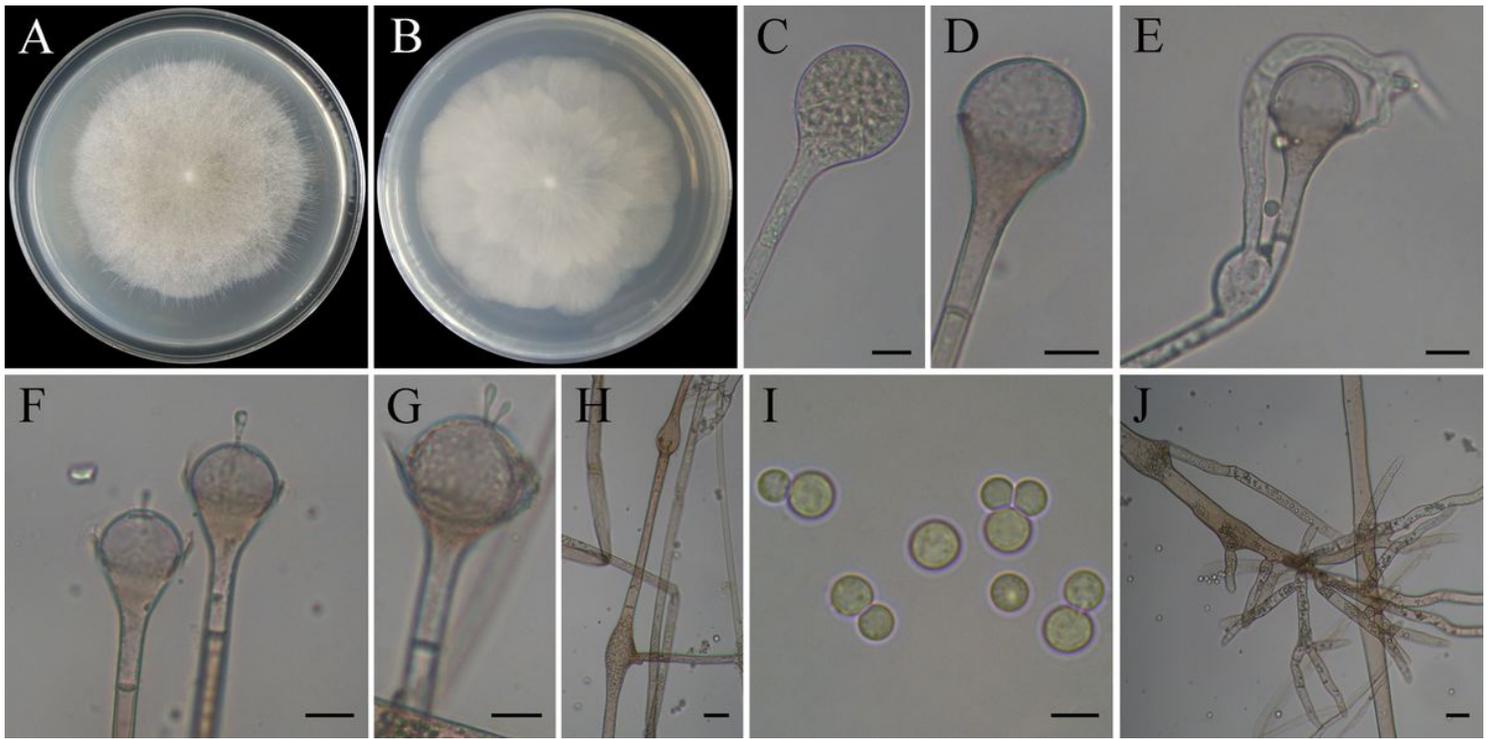


Figure 6

Absidia digitata CGMCC 3.16058. (A) obverse colony on MEA; (B) reverse colony on MEA; (C) sporangium; (D–G) columellae; (H) swelling on hyphae; (I) sporangiospores; (J) rhizoids. Scale bars: (C–G) 10 μm ; (H, J) 20 μm ; (I) 5 μm .

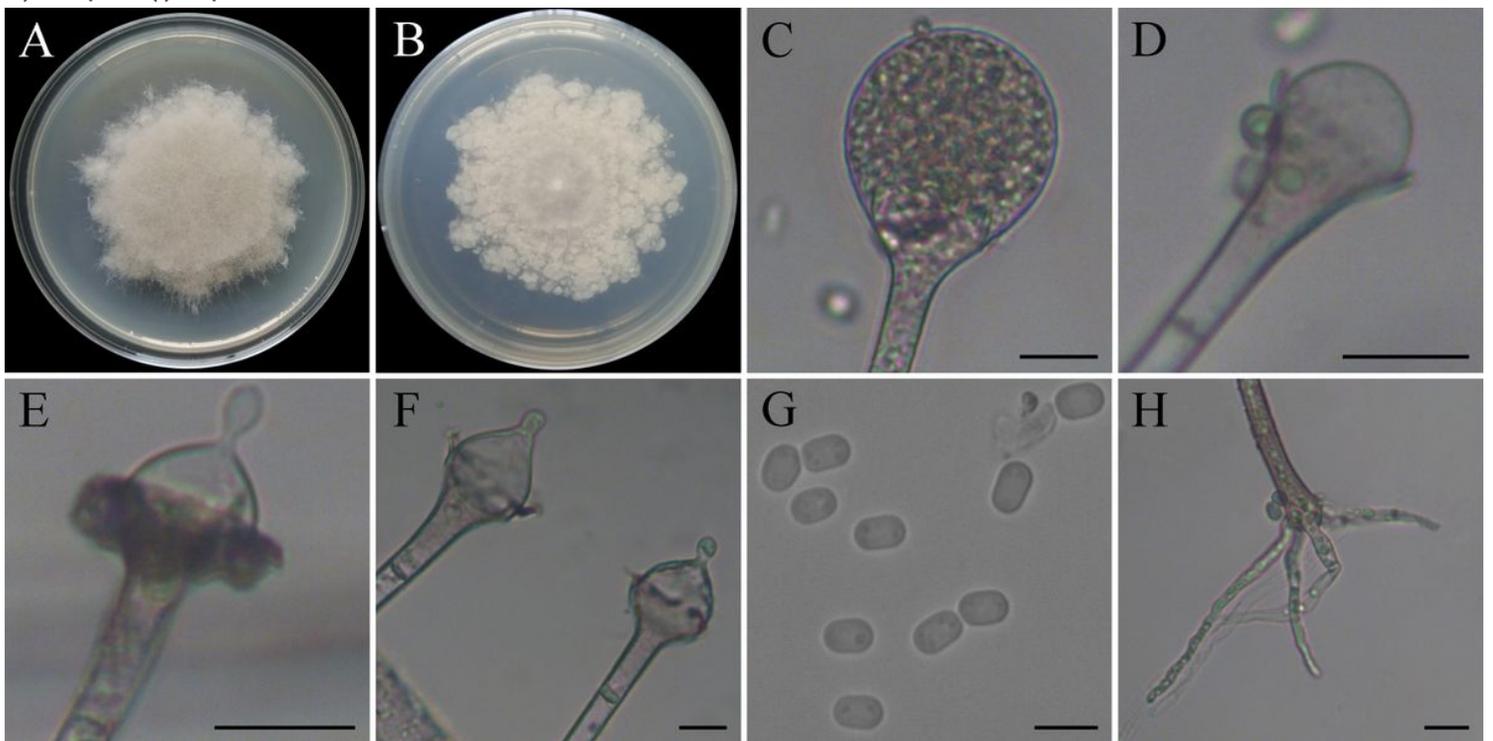


Figure 7

Absidia oblongispora CGMCC 3.16061. (A) obverse colony on MEA; (B) reverse colony on MEA; (C) sporangium; (D–F) columellae; (G) sporangiospores; (H) rhizoids. Scale bars: (C–F, H) 10 μm ; (G) 5 μm .

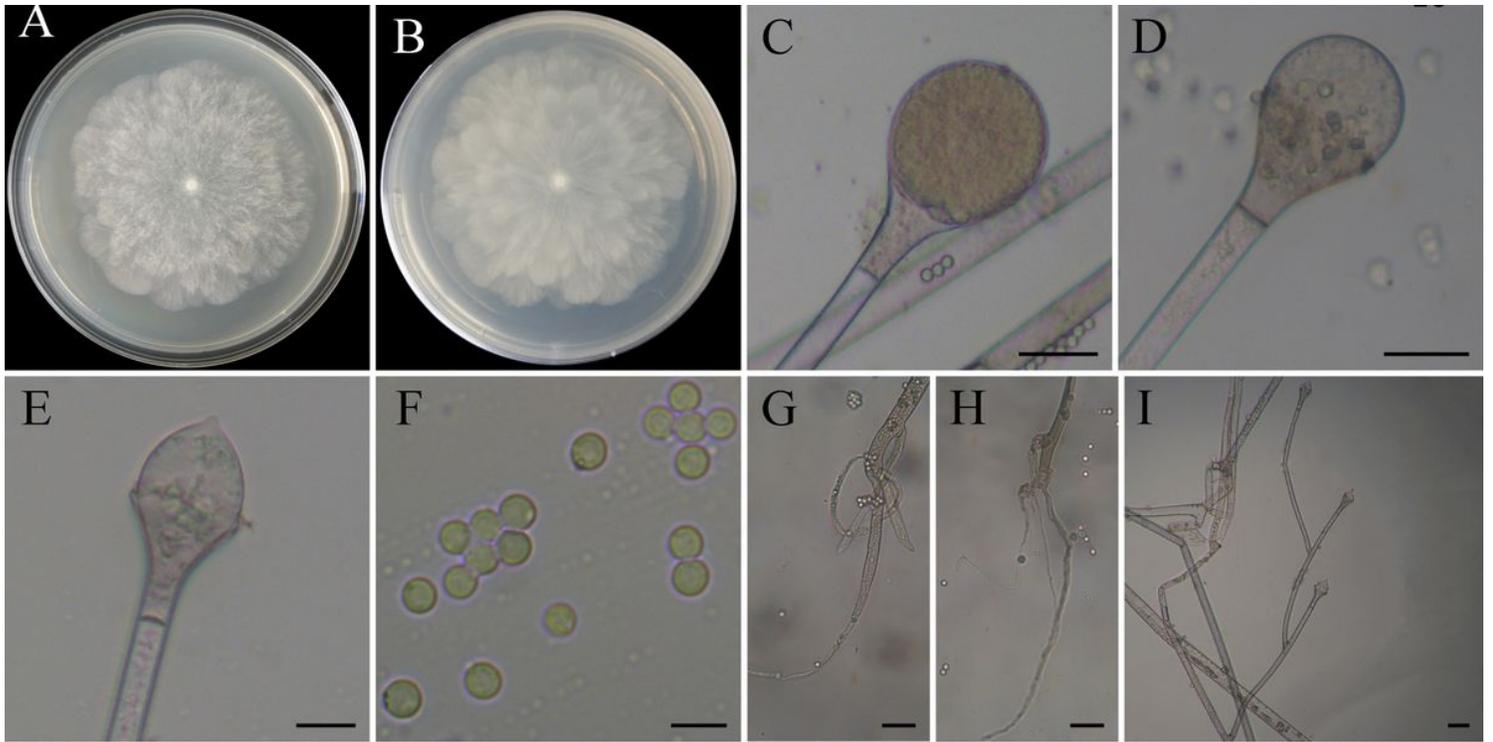


Figure 8

Absidia sympodialis CGMCC 3.16064. (A) obverse colony on MEA; (B) reverse colony on MEA; (C) sporangium; (D, E) columellae; (F) sporangiospores; (G, H) rhizoids; (I) sympodial sporangiospores. Scale bars: (C–E, G–I) 20 μm ; (F) 5 μm .

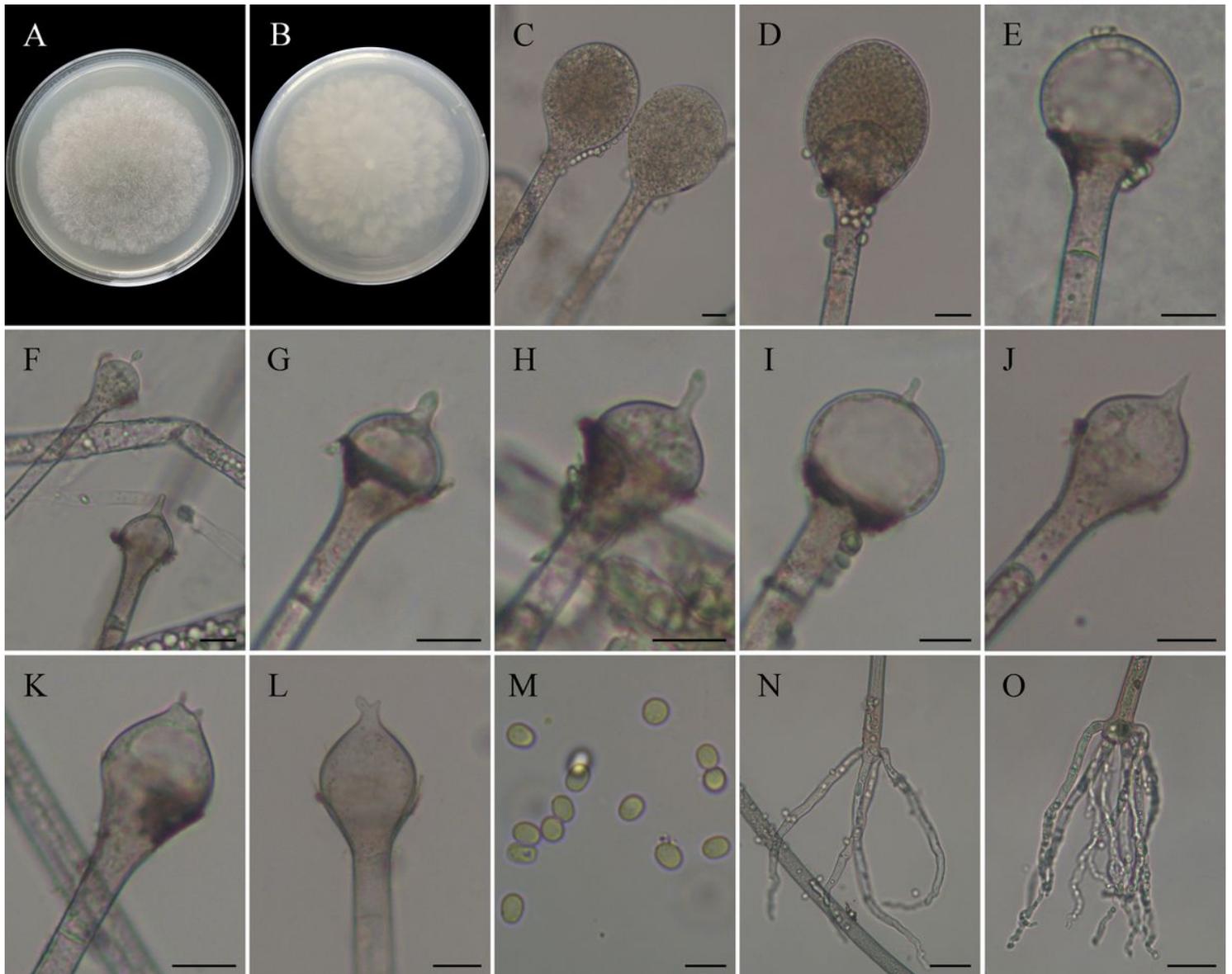


Figure 9

Absidia varians CGMCC 3.16065. (A) obverse colony on MEA; (B) reverse colony on MEA; (C, D) sporangia; (E–L) columellae; (M) sporangiospores; (N, O) rhizoids. Scale bars: (C–L) 10 μm ; (M) 5 μm ; (N, O) 20 μm .

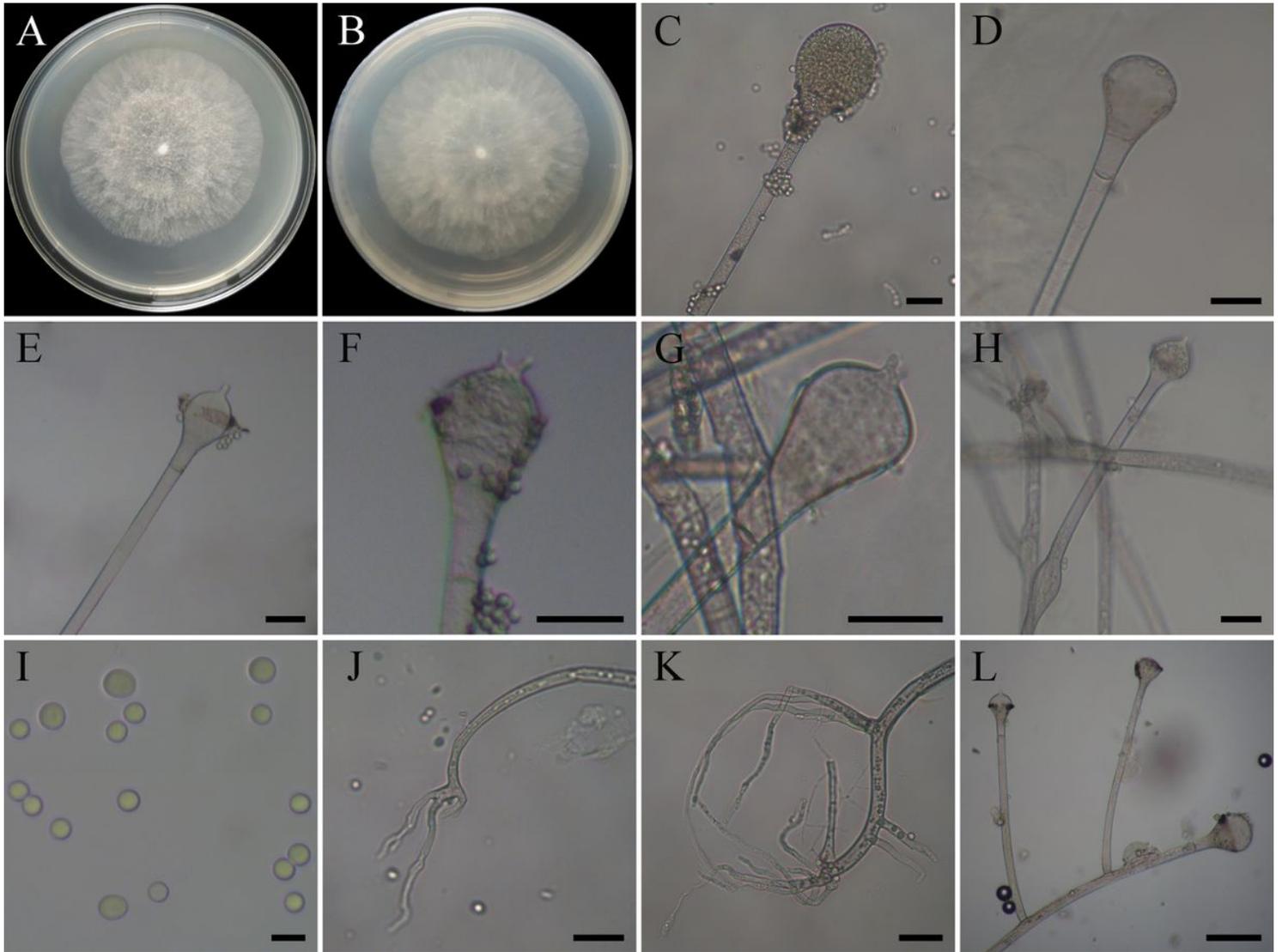


Figure 10

Absidia virescens CGMCC 3.16067. (A) obverse colony on MEA; (B) reverse colony on MEA; (C) sporangium; (D–G) columellae; (H) swelling on sporangiospores; (I) sporangiospores; (J, K) rhizoids; (L) monopodial sporangiospores. Scale bars: (C–H, J–K) 20 μm ; (I) 5 μm ; (L) 50 μm .

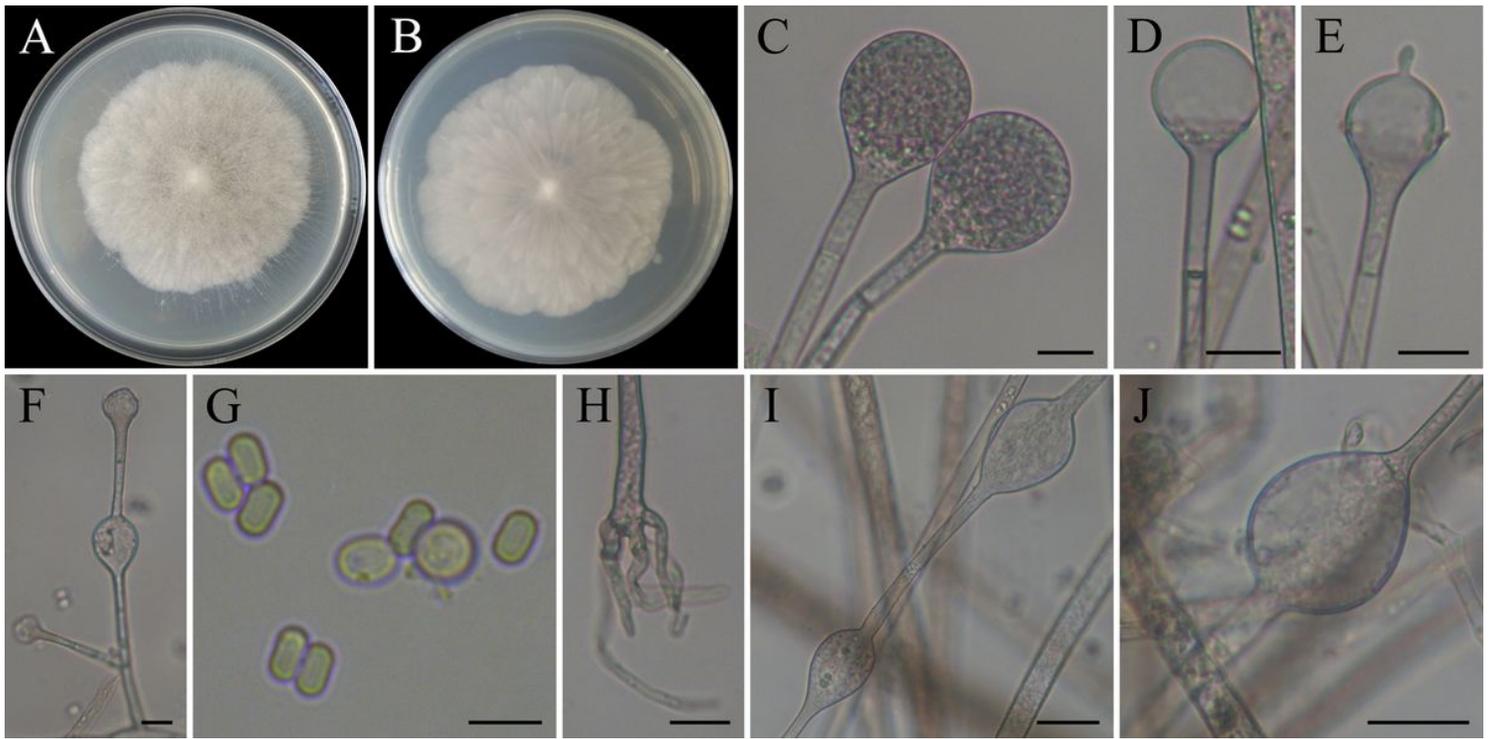


Figure 11

Absidia nigra CGMCC 3.16059. (A) obverse colony on MEA; (B) reverse colony on MEA; (C) sporangia; (D, E) columellae; (F) swelling on sporangiospores; (G) sporangiospores; (H) rhizoids (I, J) swollen hyphae. Scale bars: (C–F, H) 10 μm ; (G) 5 μm ; (I, J) 20 μm .

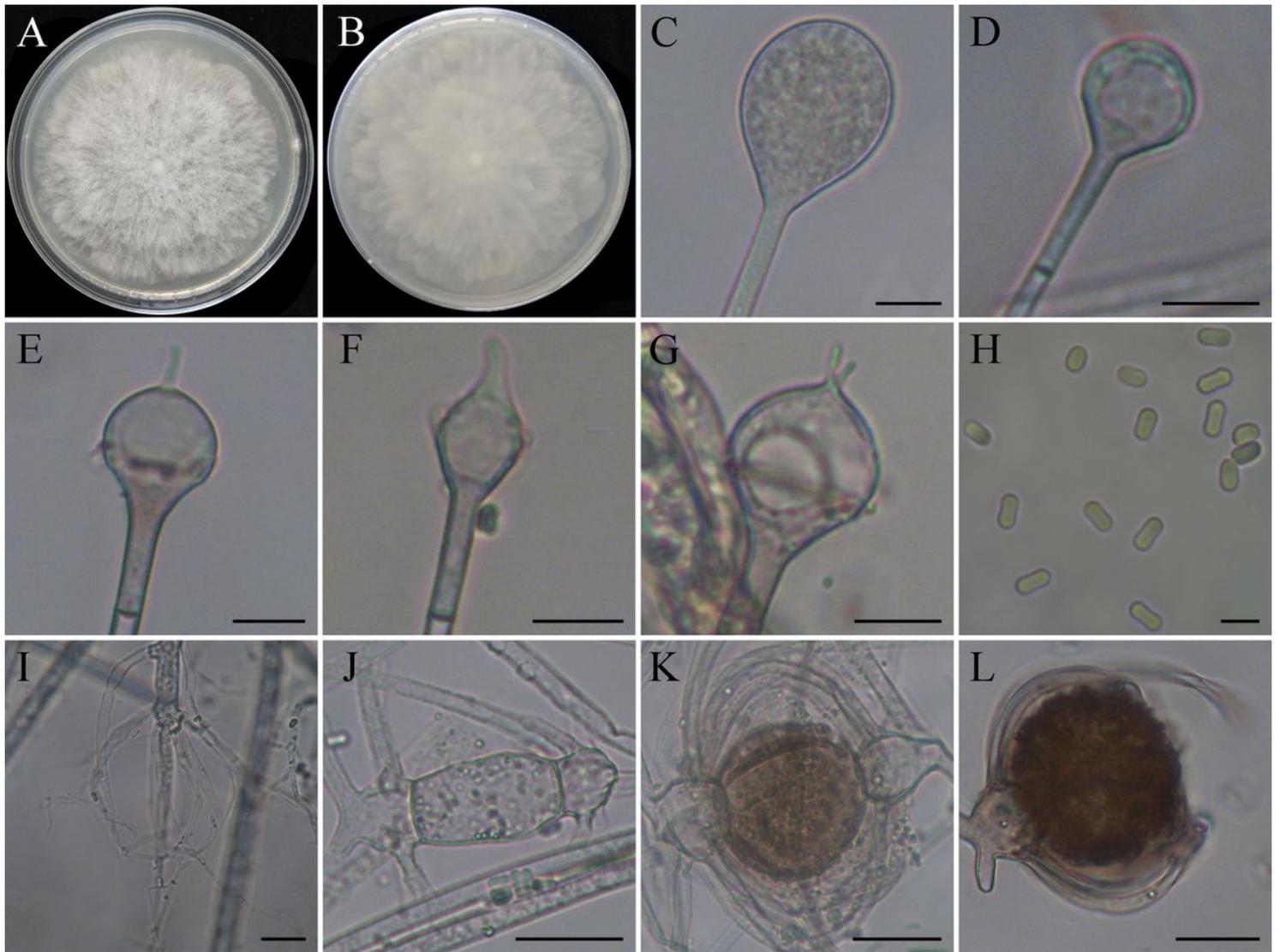
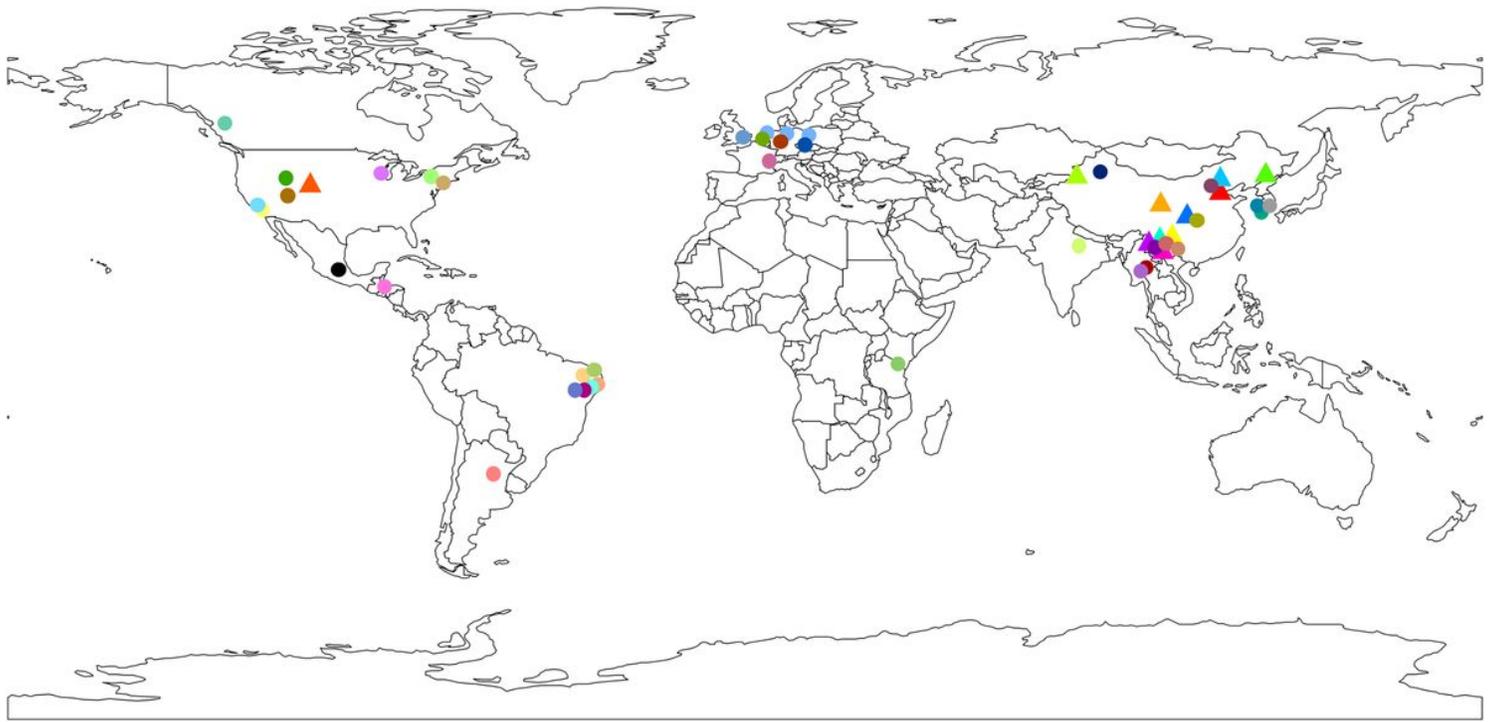


Figure 12

Absidia biappendiculata CBS 187.64. (A) obverse colony on MEA; (B) reverse colony on MEA; (C) sporangium; (D–G) columellae; (H) sporangiospores; (I) rhizoids; (K–L) zygosporangia. Scale bars: (C–G) 10 μm ; (H) 5 μm ; (I–L) 20 μm .



- | | | | | |
|-----------------------------|---------------------------|---|---------------------------------|---------------------------|
| ▲ <i>A. ampullacea</i> | ● <i>A. anomala</i> | ● <i>A. cylindrospora</i> var. <i>nigra</i> | ● <i>A. koreana</i> | ● <i>A. psychrophilia</i> |
| ▲ <i>A. biappendiculata</i> | ● <i>A. bonitoensis</i> | ● <i>A. cylindrospora</i> var. <i>rhizomorpha</i> | ● <i>A. macrospora</i> | ● <i>A. repens</i> |
| ▲ <i>A. brunnea</i> | ● <i>A. caatinguensis</i> | ● <i>A. edaphica</i> | ● <i>A. medulla</i> | ● <i>A. saloensis</i> |
| ▲ <i>A. chinensis</i> | ● <i>A. californica</i> | ● <i>A. fusca</i> | ● <i>A. multispora</i> | ● <i>A. soli</i> |
| ▲ <i>A. cinerea</i> | ● <i>A. clavata</i> | ● <i>A. glauca</i> | ● <i>A. ovalispora</i> | ● <i>A. spinosa</i> |
| ▲ <i>A. digitata</i> | ● <i>A. coerulea</i> | ● <i>A. globospora</i> | ● <i>A. panacisoli</i> | ● <i>A. stercoraria</i> |
| ▲ <i>A. nigra</i> | ● <i>A. comuta</i> | ● <i>A. heterospora</i> | ● <i>A. pararepens</i> | ● <i>A. terrestris</i> |
| ▲ <i>A. oblongispora</i> | ● <i>A. cuneospora</i> | ● <i>A. idahoensis</i> | ● <i>A. pemambucoensis</i> | ● <i>A. turgida</i> |
| ▲ <i>A. sympodialis</i> | ● <i>A. cylindrospora</i> | ● <i>A. jindoensis</i> | ● <i>A. pseudocylindrospora</i> | ● <i>A. zonata</i> |
| ▲ <i>A. varians</i> | | | | |
| ▲ <i>A. virescens</i> | | | | |

Figure 13

The geographic distribution of *Absida* types except for *A. glauca* and *A. cylindrospora* (neither is available). Triangles represent nine new species and two combinations proposed in this study. Circles represent previously reported species.