# Unveiling Species Diversity Within Early-Diverging Fungi from China VI: Four *Absidia* sp. nov. (*Mucorales*) in Guizhou and Hainan

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*Article*

# Unveiling Species Diversity Within Early-Diverging Fungi from China VI: Four *Absidia* sp. nov. (*Mucorales*) in Guizhou and Hainan

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**Abstract:** *Absidia* represents the most species-rich genus within the family Cunninghamellaceae, with its members commonly isolated from diverse substrates, particularly rhizosphere soil. In this study, four novel *Absidia* species (*A. irregularis* sp. nov., *A. multiformis* sp. nov., *A. ovoidospora* sp. nov., and *A. verticilliformis* sp. nov.) were discovered from south and southwestern Chinese soil samples through integrated morphological and molecular phylogenetic analyses. Phylogenetic analyses based on concatenated ITS, SSU, LSU, *Act*, and *TEF1α* sequence data reconstructed trees that strongly supported the monophyly of these four new taxa each. Key diagnostic features include *A. irregularis* (closely related to *A. oblongispora*) exhibiting irregular colony morphology, *A. multiformis* (sister to *A. heterospora*) demonstrating polymorphic sporangiospores, *A. ovoidospora* (forming a clade with *A. panacisoli* and *A. abundans*) producing distinctive ovoid sporangiospores, and *A. verticilliformis* (next to *A. edaphica*) displaying verticillately branched sporangiophores. Each novel species is formally described with comprehensive documentation, including morphological descriptions, illustrations, Fungal Names registration identifiers, designated type specimens, etymological explanations, maximum growth temperatures, and taxonomic comparisons. This work constitutes the sixth installment in a series investigating early-diverging fungal diversity in China, expanding the recognized *Absidia* species to 71. The findings enhance our understanding of mucoralean biodiversity in Asian tropical and subtropical ecosystems.

**Keywords:** Mucoromycota; soil-borne fungi; new species; taxonomy; molecular phylogeny

## 1. Introduction

The genus *Absidia* Tiegh. (Mucoromycota Doweld, Mucoromycetes Doweld, Mucorales Dumort., Cunninghamellaceae Naumov ex R.K. Benj.) was established in 1878 and typified with *A. reflexa* [1, 2]. It is currently the most numerous genus in the family Cunninghamellaceae, with 67 recognized species (https://www.catalogueoflife.org/data/taxon/LSM, accessed on 2 December 2024). Its taxonomic position has been controversial since its establishment. Initially, this genus was classified in Absidiaceae [3]. Then, Benny et al. transferred it to *Mucoraceae* in 2001 [4]. Finally, Ashton et al. accommodated it in Cunninghamellaceae in 2009 [5]. With the deepening of research, *Absidia* s.l. was divided into *Lichtheimia* Vuill. (thermotolerant, optimum growth temperature 37–45 ℃), *Absidia* s.s. (mesophilic, optimum growth temperature 25–34 ℃), and *Lentamyces* Kerst. Hoffm. & K. Voigt (parasitic on other mucoralean fungi, optimum growth temperature 14–25 ℃) [6-9].

Strains of *Absidia* are distributed worldwide, ubiquitous in soil, dung, leaf litter, food, air, etc [10]. In the GlobalFungi database (https://globalfungi.com/, accessed on 22 December 2024), *Absidia* members are recorded from soil (37,828 records, 44.52 % of all records), root (15,865, 18.67 %), shoot (11,740, 13.82 %), topsoil (6,923, 8.15 %), rhizosphere soil (2,530, 2.98 %), deadwood (2,253, 2.65 %), air (2,080, 2.45 %), water (1,549, 1.82 %), litter (1,406, 1.65 %), mosses (265, 0.41 %), lichen (217, 0.25

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%), coral (72, 0.08 %), dust (53, 0.06 %), fungal sporocarp (31, 0.04 %), and glacial ice debris (3, 0 %). In summary, soil and rhizosphere substrates account for approximately 74.32%.

*Absidia coerulea*, the most common species in this genus, plays an important role in bioengineering. It possesses the capability of transforming spirulina biomass into (-)-α-bisabolol [11]. It is able to catalyze the specific C-3 dehydrogenation for derivatives of ginsenoside-$Rg_1$, as well as hydroxylation at the 7β and 15α positions. And some metabolites in this species exhibit moderate reversal activity towards multidrug-resistant tumor cells [12].

Recently, eight fungal strains were isolated from soil in Hainan and Guizhou provinces, the south and southwest region of China. According to ITS-SSU-LSU-*Act*-*TEF1α* molecular phylogenetic analyses and morphological comparisons, these strains were classified into four new species of *Absidia* and described herein as *A. irregularis* sp. nov., *A. multiformis* sp. nov., *A. ovoidospora* sp. nov., and *A. verticilliformis* sp. nov. This is the sixth report of a serial work on diversity of Chinese early-diverging fungi [13-17].

## 2. Materials and Methods

### *2.1. Isolation and Morphology*

Soil samples were collected in Hainan province in April 2023 and Guizhou province in August 2023, following the methods by Li et al.and Zou et al. [18, 19]. Soil sample collection started with removal of surface contaminants using a sterilized stainless steel shovel. About 100 g of homogenized soil was put into sample bags, labeled with collection date, administrative location, GPS coordinates, and altitude. Strains were obtained from the soil samples by serial dilution spread plate and single spore isolation.

About 1 g of soil samples was mixed with 10 mL sterile water to prepare $10^{-1}$ soil suspension. One millilitre of the $10^{-1}$ suspension was transferred to 9 mL of sterile water to obtain a $10^{-2}$ soil suspension. In the same way, $10^{-3}$ and $10^{-4}$ soil suspensions were made. Approximately 0.2 mL of the final $10^{-4}$ soil suspension were dispersed evenly with sterilised coating rods on Rose-Bengal Chloramphenicol agar (RBC: peptone 5.00 g/L, glucose 10.00 g/L, $KH_2PO_4$ 1.00 g/L, $MgSO_4{\cdot}7H_2O$ 0.50 g/L, rose red 0.05 g/L, chloramphenicol 0.10 g/L, agar 15.00 g/L) [20], and then cultured in the dark at 25 ℃. Once visible, colonies were transferred and further cultured on Potato Dextrose Agar (PDA: glucose 20.00 g/L, potato 200.00 g/L, agar 20.00 g/L, pH 7) [14, 21].

Appropriately 0.1 g of soil samples were evenly sprinkled on the RBC medium with 0.06 mg/mL streptomycin and then incubated in darkness at 25 ℃ for 2–5 d. When sporangia formed, a sterilized inoculation needle was adopted to pick up a sporangium onto the PDA medium supplemented with 0.06 mg/mL streptomycin.

The maximum growth temperature was determined by a gradient method [13]. The strain was initially cultured at 25℃ for 2 d, and then increased by 1℃ per day until no growths.

Pure cultivation was applied for observing anamorphs, and pairing experiments were carried out for observing zygospores by adding 0.1 % lecithin to PDA and sealing Petri dishes to retain moisture. The morphological characteristics were observed with an optical microscope (Olympus BX53) and photographed with a high-definition colour digital camera (Olympus DP80). Each morphological character was statistically calculated against 30 measurements [22]. All strains were stored at 4 ℃ with 20 % sterilised glycerine. Cultures were deposited in the China General Microbiological Culture Collection Center, Beijing, China (CGMCC) and the Shandong Normal University, Jinan, Shandong, China (XG). Strains were deposited in the Herbarium Mycologicum Academiae Sinicae, Beijing, China (Fungarium, HMAS). Taxonomic information for the new taxa was registered in the Fungal Names repository (https://nmdc.cn/fungalnames/).

### *2.2. DNA Extraction and Amplification*

Genomic DNA was extracted from mycelia using the CTAB method and GOMag™ Rapid Plant DNA Kit [21]. Information of the primers for PCR amplification is listed in Table 1. Amplification

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was performed in a final volume of 20 μL, containing 10 μL 2× Hieff Canace® Plus PCR Master Mix (Cat No.10154ES03; Yeasen Biotechnology, Shanghai, China), 0.5 μL of forward and reverse primers each (10 μM; TsingKe, Beijing, China), 1 μL template genomic DNA (1 μM) and 8 μL distilled deionised water. Molecular loci, PCR primers and programmes used in this study are listed in Table 1. PCR products were electrophoresed with 1 % agarose gel. DNA fragments were stained with TS-GelRed Nucleic Acid Gel Stain (10,000× in Water; TSJ002; Beijing Tsingke Biotech Co., Ltd.) and observed under ultraviolet light. Then a gel extraction kit (Cat# AE0101-C; Shandong Sparkiade Biotechnology Co., Ltd.) was used for gel recovery. Sanger sequencing was carried out by Biosune Co., Ltd. (Shanghai, China). Consensus sequences were assembled using MEGA v.7.0 [23]. Target sequences in some strains could not be obtained by conventional methods due to heterogeneous gene duplications, and thus they were extracted from genomic data [24]. The genomes were sequenced by Singke Biotech Co., Ltd. (Jinan, China). All sequences generated in this study were deposited at GenBank under the accession numbers in Table 2.

**Table 1.** Molecular loci, PCR primers and programmes used in this study.

| Loci | PCR primers | Sequence (5′ – 3′) | PCR cycles | References |
|---|---|---|---|---|
| *Act* | ACT-512F | ATG TGC AAG GCC GGT TTC GC | 95℃ 3 min; (95℃ 1 min, 55℃ 1 min, 72℃ 1 min) × 30 cycles; 72℃ 10 min | [25] |
| | ACT-783R | TAC GAG TCC TTC TGG CCC AT | | |
| ITS | ITS5 | GGA AGT AAA AGT CGT AAC AAG G | 95℃ 5 min; (95℃ 30 s, 55℃ 30 s, 72℃ 1 min) × 35 cycles; 72℃ 10 min | [26] |
| | ITS4 | TCC TCC GCT TAT TGA TAT GC | | |
| LSU | LR0R | GTA CCC GCT GAA CTT AAG C | 95℃ 5 min; (95℃ 50 s, 47℃ 30 s, 72℃ 1.5 min) × 35 cycles; 72℃ 10 min | [27] |
| | LR7 | TAC TAC CAC CAA GAT CT | | |
| SSU | NS1 | GTA GTC ATA TGC TTG TCT C C | 95 °C 5 min; (94 °C 60 s, 54 °C 50 s, 72 °C 60 s) × 37 cycles; 72 °C 10 min | [26] |
| | NS4 | CTT CCG TCA ATT CCT TTA AG | | |
| *TEF1α* | EF1-728F | CAT CGA GAA GTT CGA GAA GG | 95℃ 5 min; (95℃ 30 s, 55℃ 60 s, 72℃ 1 min) × 30 cycles; 72℃ 10 min | [28] |
| | EF2 | GGA RGT ACC AGT SAT CAT GTT | | |

**Table 2.** Information of strains used in this study.

| Species | Strains | GenBank accession numbers | | | | |
|---|---|---|---|---|---|---|
| | | ITS | LSU | *TEF1α* | *Act* | SSU |
| ***Absidia abundans*** | CGMCC 3.16255* | NR_182590 | ON074683 | n.a. | n.a. | n.a. |
| *A. abundans* | XY09274 | ON074696 | ON074682 | n.a. | n.a. | n.a. |

 doi:10.20944/preprints202504.1150.v1



| | | | | | | |
|---|---|---|---|---|---|---|
| *A. aguabelensis* | URM 8213* | NR_189383 | NG_241934 | n.a. | n.a. | n.a. |
| *A. alpina* | CGMCC 3.16104 | OL678133 | n.a. | n.a. | n.a. | n.a. |
| *A. ampullacea* | CGMCC 3.16054 | MZ354138 | MZ350132 | n.a. | n.a. | n.a. |
| *A. anomala* | CBS 125.68* | MH859085 | MH870799 | n.a. | n.a. | n.a. |
| *A. anomala* | FSU5798 | EF030523 | n.a. | n.a. | EF030535 | n.a. |
| *A. biappendiculata* | CBS 187.64 | MZ354153 | MZ350147 | MZ357420 | MZ357438 | |
| *A. bonitoensis* | URM 7889* | MN977786 | MN977805 | n.a. | n.a. | n.a. |
| *A. brunnea* | CGMCC 3.16055* | MZ354139 | MZ350133 | MZ357403 | MZ357421 | n.a. |
| *A. caatinguensis* | URM 7156* | NR_154704 | NG_058582 | n.a. | n.a. | n.a. |
| *A. caerulea* | CBS101.36* | MH855718 | MH867230 | n.a. | n.a. | n.a. |
| *A. californica* | CBS 314.78 | JN205816 | MH872902 | n.a. | n.a. | n.a. |
| *A. chinensis* | CGMCC 3.16057 | MZ354141 | MZ350135 | n.a. | MZ357422 | n.a. |
| *A. chinensis* | CGMCC 3.16056* | MZ354140 | MZ350134 | n.a. | n.a. | n.a. |
| *A. cinerea* | CGMCC 3.16062 | MZ354146 | MZ350140 | MZ357407 | MZ357427 | n.a. |
| *A. cornuta* | URM 6100* | NR_172976 | MN625255 | n.a. | n.a. | n.a. |
| *A. crystalloides* | CGMCC3.27496* | PP377803 | PP373736 | PP790574 | PP790582 | PP779723 |
| *A. crystalloides* | SAUCC693201 | PP377804 | PP373737 | PP790573 | PP790581 | PP779722 |
| *A. cuneospora* | CBS 101.59* | MH857828 | MH869361 | n.a. | n.a. | n.a. |
| *A. cuneospora* | FSU5890 | EF030524 | n.a. | n.a. | EF030533 | n.a. |
| *A. cylindrospora* | CBS 100.08 | JN205822 | JN206588 | n.a. | n.a. | n.a. |
| *A. digitula* | CGMCC 3.16058* | MZ354142 | MZ350136 | MZ357404 | MZ357423 | n.a. |
| *A. edaphica* | MFLUCC 20-0088 | NR_172305 | NG_075367 | n.a. | MT410739 | NG_074951 |

 doi:10.20944/preprints202504.1150.v1



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|---|---|---|---|---|---|---|
| *A. frigida* | CGMCC 3.16201* | NR_182565 | OM030223 | n.a. | n.a. | n.a. |
| *A. fusca* | CBS 102.35* | NR_103625 | NG_058552 | n.a. | n.a. | n.a. |
| *A. gemella* | CGMCC 3.16202* | OM108488 | OM030224 | n.a. | n.a. | n.a. |
| *A. glauca* | CBS 101.08* | MH854573 | MH866105 | n.a. | n.a. | n.a. |
| *A. glauca* | FSU660 | AY944879 | EU736302 | EU736248 | EU736225 | EU736275 |
| *A. globospora* | CGMCC 3.16031* | NR_189829 | MW671544 | MZ357412 | MZ357431 | n.a. |
| *A. globospora* | CGMCC 3.16036 | MW671539 | MW671546 | MZ357414 | MZ357433 | n.a. |
| *A. healeyae* | UoMAU1 | n.a. | MT436027 | n.a. | n.a. | n.a. |
| *A. heterospora* | SHTH021 | JN942683 | JN982936 | n.a. | n.a. | JQ004928 |
| ***A. irregularis*** | **CGMCC 3.27812 *** | **PQ306325** | **PQ289020** | **PV126019** | **PQ807209** | **PQ799254** |
| ***A. irregularis*** | **XG05674-7** | **PQ306326** | **PQ289021** | **PV126020** | **PQ807210** | **PQ799255** |
| *A. jiangxiensis* | CGMCC 3.16105* | OL678134 | PP780377 | n.a. | n.a. | n.a. |
| *A. jindoensis* | CNUFC-PTI1-1 | MF926622 | MF926616 | MF926513 | MF926510 | MF926626 |
| *A. koreana* | EML-IFS45-1* | KR030062 | KR030056 | KR030060 | KR030058 | KT321298 |
| *A. koreana* | XY00816 | OL620083 | ON123771 | n.a. | n.a. | n.a. |
| *A. lobata* | CGMCC 3.16256 | ON074690 | ON074679 | n.a. | n.a. | n.a. |
| *A. longissima* | CGMCC 3.16203* | NR_182566 | OM030225 | n.a. | n.a. | n.a. |
| *A. macrospora* | FSU4746 | AY944882 | EU736303 | EU736249 | AY944760 | EU736276 |
| *A. medulla* | CGMCC 3.16034 | NR_189832 | MW671549 | MZ357417 | MZ357436 | n.a. |
| *A. montepascoalis* | URM 8218 | NR_172995 | n.a. | n.a. | n.a. | n.a. |
| ***A. multiformis*** | **CGMCC 3.27807*** | **PQ306319** | **PQ803168** | **PV126021** | **PQ807203** | **PQ799260** |
| ***A. multiformis*** | **XG04016-3** | **PQ306320** | **PQ803169** | **PV126022** | **PQ807204** | **PQ799261** |

 doi:10.20944/preprints202504.1150.v1



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|---|---|---|---|---|---|---|
| *A. multispora* | URM 8210* | MN953780 | MN953782 | n.a. | n.a. | n.a. |
| *A. nigra* | CBS 127.68* | NR_173068 | MZ350146 | MZ357419 | MZ357437 | n.a. |
| *A. nigra* | CGMCC 3.16060 | MZ354144 | MZ350138 | MZ357406 | MZ357425 | n.a. |
| *A. oblongispora* | CGMCC 3.16061 | MZ354145 | MZ350139 | n.a. | MZ357426 | n.a. |
| *A. ovalispora* | CGMCC 3.16019 | NR_176748 | MW264131 | n.a. | n.a. | n.a. |
| ***A. ovoidospora*** | **CGMCC 3.27811 clone1*** | **PQ306327** | **PQ803164** | **PV126015** | **PQ807207** | **PQ799256** |
| ***A. ovoidospora*** | **CGMCC 3.27811 clone2*** | **PV069753** | **PQ803165** | **PV126017** | **PV126023** | **PQ799257** |
| ***A. ovoidospora*** | **XG05673-3 clone1** | **PQ306328** | **PQ803166** | **PV126016** | **PQ807208** | **PQ799258** |
| ***A. ovoidospora*** | **XG05673-3 clone2** | **PV069754** | **PQ803167** | **PV126018** | **PV126024** | **PQ799259** |
| *A. pacifica* | CGMCC3.27497* | PP377802 | **PP373735** | PP839793 | PP790579 | PP779720 |
| *A. pacifica* | SAUCC413601 | PP377801 | PP373734 | PP839794 | PP790580 | PP779721 |
| *A. panacisoli* | SYPF 7183* | MF522181 | MF522180 | MF624251 | n.a. | MF522179 |
| *A. pararepens* | CCF 6352 | MT193669 | MT192308 | n.a. | n.a. | n.a. |
| *A. pateriformis* | CGMCC3.27495* | PP377805 | PP373738 | PP790575 | PP790583 | PP779724 |
| *A. pateriformis* | SAUCC634702 | PP377806 | PP373739 | PP790576 | PP790584 | PP779725 |
| *A. pernambucoensis* | URM<BRA>7219 | MN635568 | MN635569 | n.a. | n.a. | n.a. |
| *A. pseudocylindrospora* | EML-FSDY6-2 | KU923817 | KU923814 | n.a. | KU923815 | KU923819 |
| *A. pseudocylindrospora* | CBS 100.62* | NR_145276 | MH869688 | n.a. | n.a. | n.a. |
| *A. psychrophilia* | FSU4745 | AY944874 | EU736306 | EU736252 | AY944762 | EU736279 |
| *A. radiata* | CGMCC 3.16257 | ON074698 | ON074684 | n.a. | n.a. | n.a. |
| *A. radiata* | XY09330-1 | ON074699 | ON074685 | n.a. | n.a. | n.a. |

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|---|---|---|---|---|---|---|
| *A. repens* | CBS 115583* | NR_103624 | NG_058551 | n.a. | n.a. | n.a. |
| *A. saloaensis* | URM 8209* | MN953781 | MN953783 | n.a. | n.a. | n.a. |
| *A. sichuanensis* | CGMCC 3.16258* | NR_182589 | ON074688 | n.a. | n.a. | n.a. |
| *A. soli* | MFLU-20-0414* | MT396373 | MT393988 | n.a. | n.a. | MT394049 |
| *A. soli* | MFLU 20-0413 | MT396371 | MT393985 | n.a. | n.a. | MT394046 |
| *A. spinosa* | FSU551 | AY944887 | EU736307 | EU736253 | EU736227 | EU736280 |
| *A. stercoraria* | EML-DG8-1* | KU168828 | KT921998 | KT922002 | KT922000 | NG_065640 |
| *A. sympodialis* | CGMCC 3.16063* | MZ354147 | MZ350141 | n.a. | n.a. | n.a. |
| *A. sympodialis* | CGMCC 3.16064 | MZ354148 | MZ350142 | MZ357408 | n.a. | n.a. |
| *A. terrestris* | FMR 14989* | LT795003 | LT795005 | n.a. | n.a. | n.a. |
| *A. turgida* | CGMCC 3.16032* | NR_189830 | NG_241931 | MZ357415 | MZ357434 | n.a. |
| *A. varians* | CGMCC 3.16065* | MZ354149 | MZ350143 | MZ357409 | MZ357428 | n.a. |
| ***A. verticilliformis*** | **CGMCC 3.27810 clone1*** | **PQ306315** | **PQ803170** | **PV126011** | **PQ807205** | **PQ799262** |
| ***A. verticilliformis*** | **CGMCC 3.27810 clone2*** | **PV069755** | **PQ803171** | **PV126013** | **PV126025** | **PQ799263** |
| ***A. verticilliformis*** | **XG04088-4 clone1** | **PQ306316** | **PQ803172** | **PV126012** | **PQ807206** | **PQ799264** |
| ***A. verticilliformis*** | **XG04088-4 clone2** | **PV069756** | **PQ803173** | **PV126014** | **PV126026** | **PQ799265** |
| *A. virescens* | CGMCC 3.16066* | MZ354150 | MZ350144 | MZ357410 | MZ357429 | n.a. |
| *A. virescens* | CGMCC 3.16067 | MZ354151 | MZ350145 | MZ357411 | MZ357430 | n.a. |
| *A. xinjiangensis* | CGMCC 3.16107* | OL678136 | n.a. | n.a. | n.a. | n.a. |
| *A. yunnanensis* | XY09528 | ON074701 | ON074686 | n.a. | n.a. | n.a. |
| *A. yunnanensis* | CGMCC 3.16259* | NR_182591 | NG_149054 | n.a. | n.a. | n.a. |
| *A. zonata* | CGMCC 3.16033* | NR_189831 | MW671548 | MZ357416 | MZ357435 | n.a. |

 doi:10.20944/preprints202504.1150.v1



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|---|---|---|---|---|---|---|
| *A. zygospora* | RSPG 214 | KC478527 | n.a. | n.a. | n.a. | n.a. |
| *A. zygospora* | ANG28 | DQ914420 | n.a. | n.a. | n.a. | n.a. |
| *Cunninghamella blakesleeana* | CBS 782.68 | JN205869 | MH870950 | n.a. | n.a. | n.a. |

Notes: New species proposed in this study are in bold. Ex-type or ex-holotype strains are labelled with an asterisk "*". The abbreviation "n.a." stands for "not available".

Relative sequences were obtained by BLAST search against the NCBI GenBank nucleotide database. SSU, ITS, LSU, *Act* and *TEF1α* sequences both generated herein and retrieved from GenBank (Table 2) were individually aligned using MAFFT 7 online service. The aligned matrices were manually proofread and then jointly analyzed. The optimal evolutionary model was determined for each partition and included in the analysis using MrModelTest v.2.3 [29]. Phylogenetic history was reconstructed using maximum likelihood (ML) algorithm with RaxML-HPC2 on XSEDE (8.2.12) and Bayesian inference (BI) algorithm with MrBayes [30-32]. Maximum Likelihood analysis was performed using the best model with 1,000 bootstrap replications. The BI analysis consisted of five million generations with four parallel runs under stopping rules and a sampling frequency of 100 generations. The burn-in score was set to 0.25, and the posterior probability (PP) was determined from the remaining trees. Initial adjustments to the phylogenetic tree were made using FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and the finalization was performed using Adobe Illustrator CC 2019 (https://adobe.com/products/illustrator).

## 3. Results

### *3.1. Phylogenetic Analyses*

The sequence matrix included 87 strains in 64 species of Absidia, with Cunninghamella blakesleeana CBS 782.68 as outgroup. A total of 4,656 characters comprised ITS rDNA (1–975), SSU rDNA (976–2,017), LSU rDNA (2,018–3,030), Act (3,031–3,689) and TEF1α (3,690–4,656). As many as 2,737 characters were constant, while 706 and 1,213 among the variable characters were parsimony-uninformative and informative, respectively (Supplementary File S1). MrModelTest suggested that the Dirichlet fundamental frequency and GTR-I-G evolution pattern for all partitions were adopted in Bayesian Inference. The topology of the Bayesian tree, consistent with that of the ML tree, was used as a representative to summarise the evolutionary history within the genus Absidia (Figure 1), exhibiting the phylogenetic placement of the four new species. A. irregularis is related to A. oblongispora with full supports (MLBV = 100, BIPP = 1.00), A. ovoidrospora is closely related to A. panacisoli and A. abundans with full supports (MLBV = 100, BIPP = 1.00). A. multiformis is most closely related to A. heterospora with high supports (BIPP = 0.99). And A. verticilliformis is closely related to A. edaphica with robust supports (MLBV = 94, BIPP = 1).

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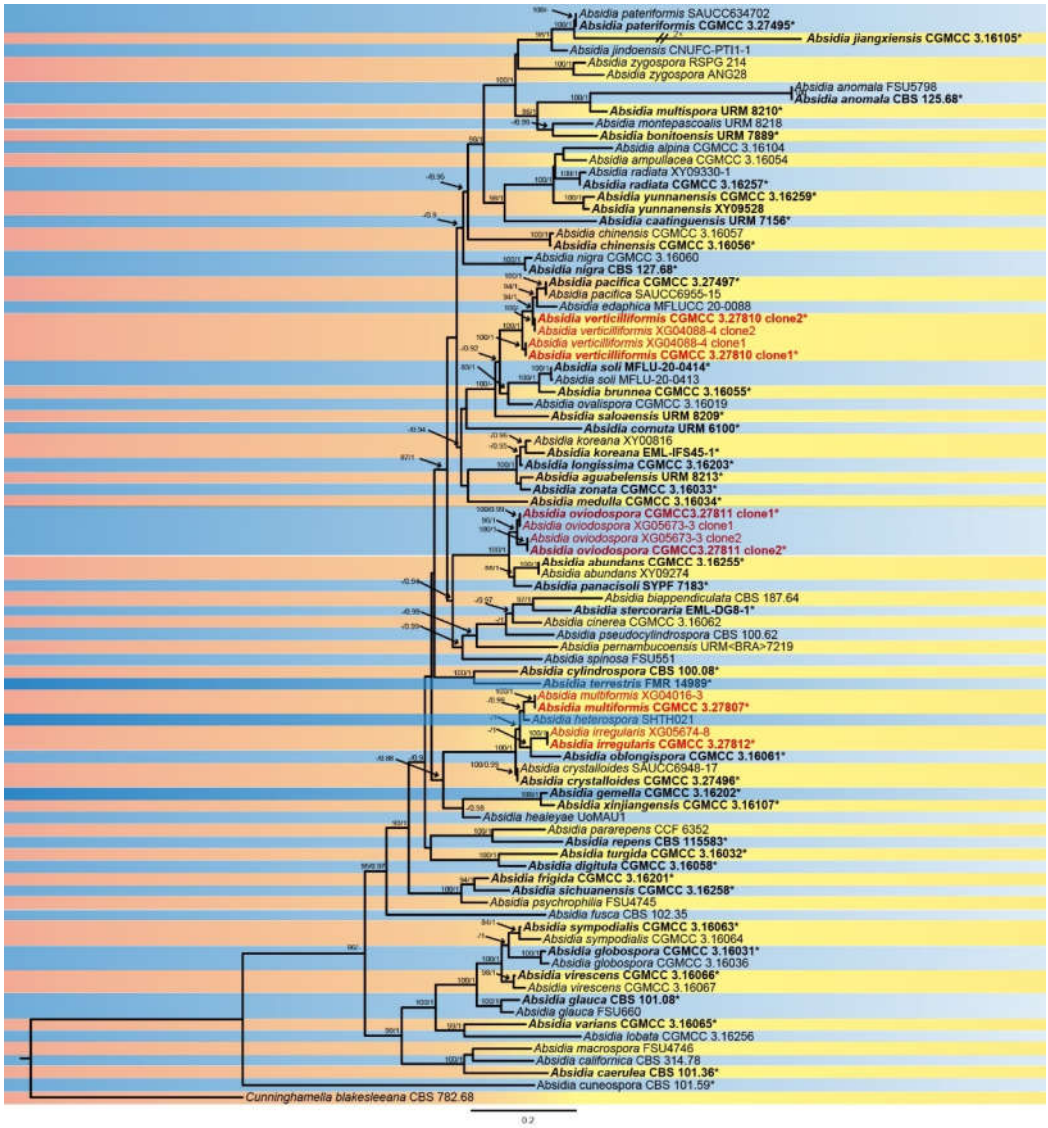


**Figure 1.** The Beyesian phylogenetic tree of Absidia based on ITS-SSU-LSU-Act-TEF1$\alpha$ sequences, with Cunninghamella blakesleeana CBS 782.68 as outgroup. The maximum likelihood bootstrap value (MLBV) ≥ 75, and the Bayesian inference posterior probability (BIPP) ≥ 0.85 are shown at the first and second positions and separated by a slash "/" on relevant nodes. The ex-types or ex-holotypes are in bold and marked with an asterisk "*", and strains involved in this study are in red. Branches shortened to fit the page are represented by double slashes "//" and folds "×". The scale at the bottom centre indicates 0.2 substitutions per site.

*3.2. Taxonomy*

***Absidia irregularis* Yi Xin Wang & X.Y. Liu, sp. nov. Figure 2**

Fungal Names—FN 572283

Etymology—The epithet "*irregularis*" (Latin) pertaining to irregular colonies.

Type—China, Hainan, Changjiang Li Autonomous County, Bawangling National Forest Park, 19.0859333° N, 109.122752° E, altitude 745.3 m, from soil sample, August 9, 2023, Yi-Xin Wang (Holotype HMAS 353186, ex-holotype strain CGMCC 3.27812 = XG05674-6)

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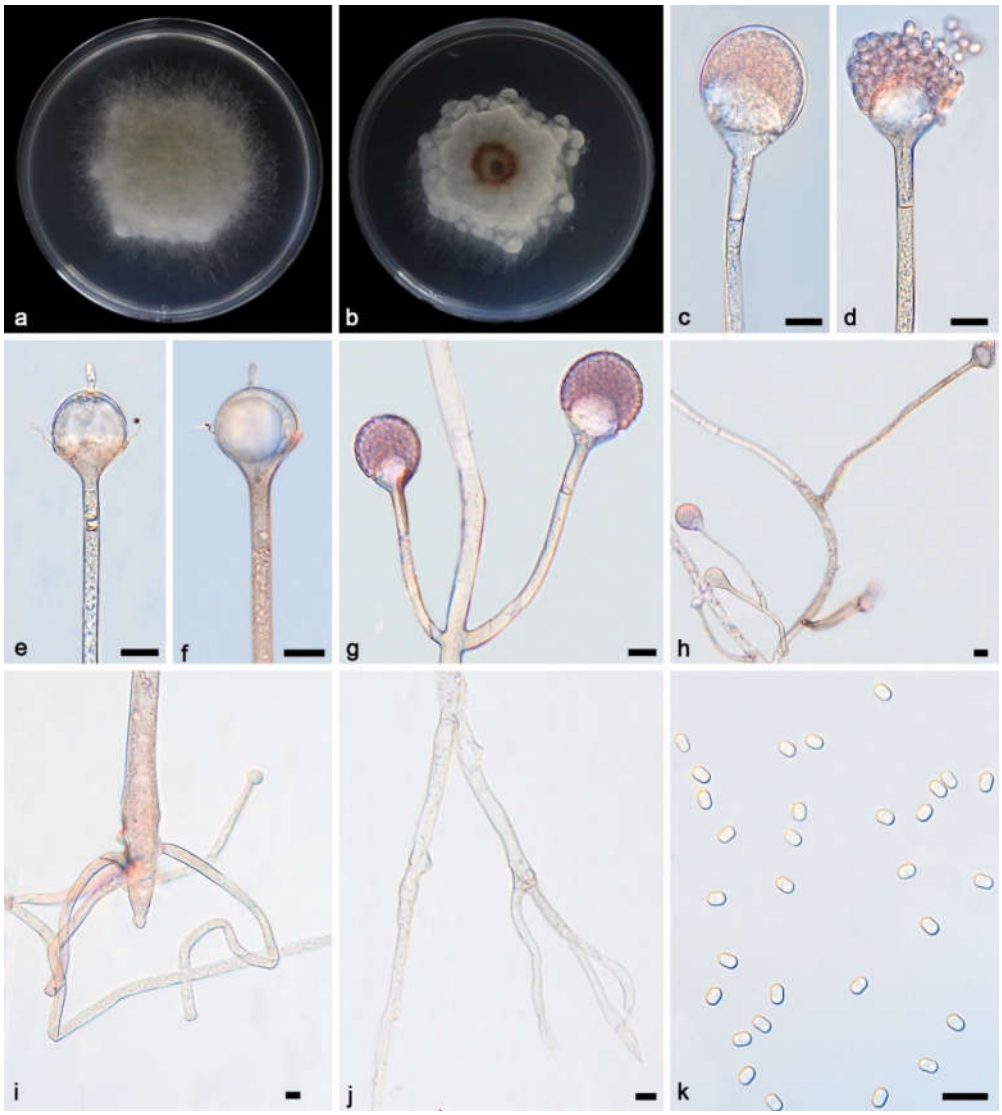


**Figure 2.** *Absidia irregularis* ex-holotype CGMCC 3.27812. **a**, **b**, Colonies on PDA (**a**, obverse; **b**, reverse); **c, d**, An unbranched sporangiophore with a sporangium; **e**, **f**, Columellae, collars, sporangiospores and apophyses; **g**, Branched sporangiophores with sporangia; **h**, Branched sporangiophores with columellae, collars, sporangia and apophyses; **i**, **j**, Rhizoids; **k**, Sporangiospores; Bars: **c–k** 10 μm.

Description—Colonies growing moderately on PDA in darkness at 25 ℃ for 7 d, reaching 43.2–54.8 mm in diameter, initially white, soon becoming gray to brown, irregular and scaly at edge, cottony, reversely white to gray. Hyphae hyaline at first, brownish when mature, branched, irregular, 10.2–14.5 μm in diameter. Stolons hyaline, branched, and smooth. Rhizoids hyaline, branched, irregular or root-like. Sporangiophores on aerial mycelia, hyaline, erect or slightly curved, unbranched or slightly branched, swollen usually below sporangia, umbellately or sympodially branched, often with a septum below apophyses, 26.5–148.1 × 3.4–5.3 μm. Sporangia oval to subglobose, 27.2–32.4 × 26.5–28.9 μm, hyaline at first and then brown, deliquescent-walled, leaving a collar after releasing sporangiospores. Apophyses hyaline, smooth, bowl-shaped, 2.9–9.6 × 8.6–19.0 μm. Collars distinct. Columellae hyaline or brown, hemispherical, 10.0–19.8 × 9.8–21.0 μm, with a protruding (3.5–6.3 × 1.4–2.9 μm) at the top. Protrudings always slightly contracted in the middle. Sporangiospores hyaline, smooth, not uniform, mostly cylindrical, 3.6–4.6 × 2.3–2.9 μm. Chlamydospores present. Zygospores not observed.

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Additional strain examined—China, Hainan, Changjiang Li Autonomous County, Bawangling National Forest Park, 19.0859333° N, 109.122752° E, altitude 745.3 m, from soil sample, August 9, 2023, Yi-Xin Wang (living culture XG05674-8).

GenBank accession numbers—CGMCC 3.27812 (ITS, PQ306325; LSU, PQ289020; *Act*, PQ807209; SSU, PQ799254; *TEF1α*, PV126019), XG05674-7 (ITS, PQ306326; LSU, PQ289021; *Act*, PQ807210; SSU, PQ799255; *TEF1α*, PV126020).

Maximum growth temperature—CGMCC 3.27812 (32 ℃), XG05674-7 (32 ℃).

Notes—Based on phylogenetic analyses of ITS-SSU-LSU-*Act*-*TEF1α* sequences, the two isolates of the new species *Absidia irregularis* formed an independent clade with full supports (MLBV = 100, BIPP = 1; Figure 1), which is closely related to *A. oblongispora* (BIPP = 1; Figure 1). This new species differs morphologically from *A. oblongispora* in apophysis and columella [33]. The new species is bigger than *A. oblongispora* in apophysis, 2.9–9.6 × 8.6–19.0 μm vs. 3.5–6.5 × 3.5–7.5 μm. The new species is also bigger than *A. oblongispora* in columellae, 10.0–19.8 × 9.8–21.0 μm vs. 7.0–15.0 × 8.5–16.5 μm. Combining the morphological and molecular phylogenetic analyses, the two isolates were classified as a new taxon, allied to *A. oblongispora*.

***Absidia multiformis*** **Yi Xin Wang & X.Y. Liu, sp. nov. Figure 3**

Fungal Names—FN 572285

Etymology—The epithet " *multiformis* " (Latin) pertaining to polymorphic sporangiospores.

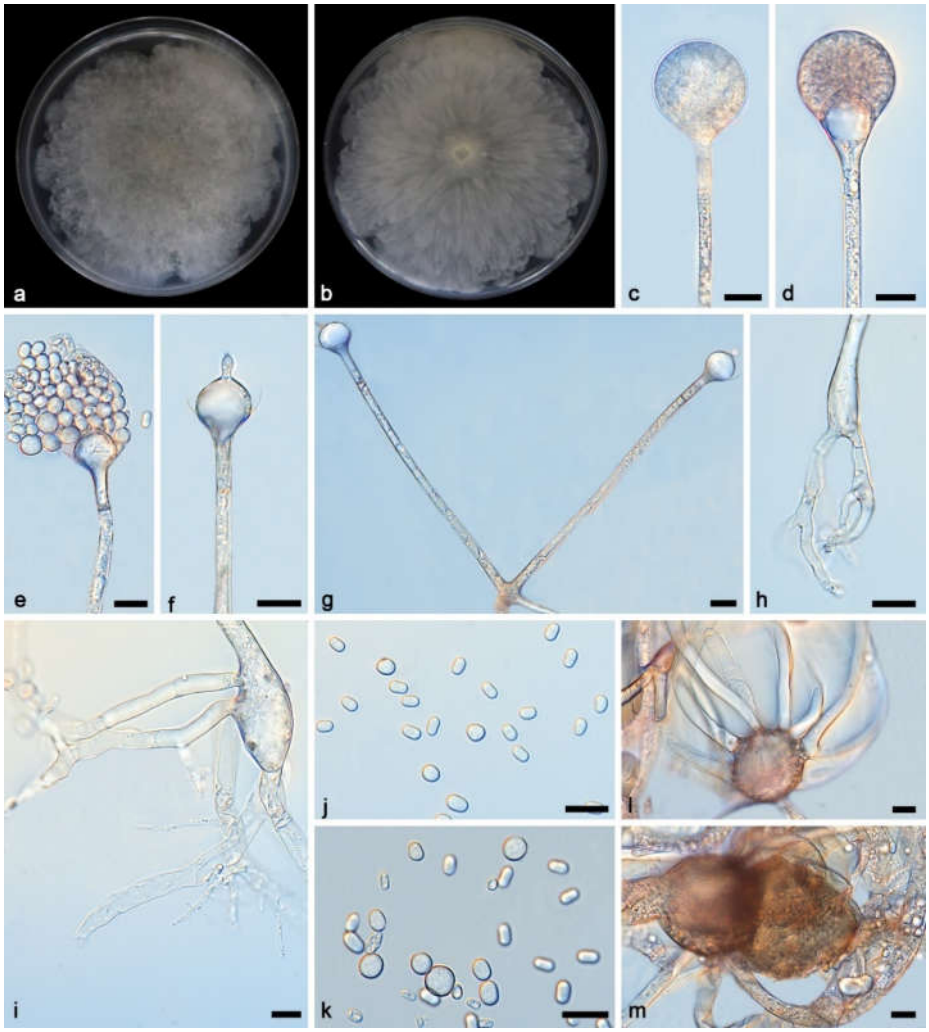


**Figure 3.** *Absidia multiformis* ex-holotype CGMCC 3.27807. **a**, **b**, Colonies on PDA (**a**, obverse; **b**, reverse); **c**, **d**, An unbranched sporangiophore with a sporangium; **e**, **f**, Columellae, collars, sporangiospores and apophyses;

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**g**, Branched sporangiophores with columellae, collars, sporangium and apophyses; **h**, **i**, Rhizoids; **j**, **k**, Sporangiospores; **k**, **m**, Zygospores; Bars: **c–m** 10 μm.

Type—China, Hainan, Lingshui Li Autonomous County, 18.6958768° N, 109.9407998° E, altitude 151.6 m, from soil sample, April 23, 2023, Yi-Xin Wang (Holotype HMAS 353194, ex-holotype strain CGMCC 3.27807 = XG04016-2).

Description—Colonies growing moderately on PDA in darkness at 25 ℃ for 7 d, reaching 82.2–90.0 mm diameter, initially white, soon becoming gray to brown, irregular at edge, cottony, reversely white to gray. Hyphae hyaline at first, brownish when mature, branched, irregular, 4.2–12.3 μm in diameter. Stolons hyaline, branched, and smooth. Rhizoids hyaline, branched, irregular or root-like. Sporangiophores on aerial mycelia, hyaline, erect or slightly curved, unbranched or slightly branched, swollen usually below sporangia, often with a septum below apophyses, 49.9–125.4 × 2.9–3.7 μm. Sporangia oval to subglobose, 20.1–36.1 × 23.1–36.3 μm, hyaline at first and then brown, deliquescent-walled, leaving a collar after releasing sporangiospores. Apophyses hyaline, smooth, shallow mouth bowl shaped, 2.5–7.9 × 8.0–19.8 μm. Collars distinct. Columellae hyaline or brown, hemispherical, 5.3–19.4 × 8.3–22.9 μm, with long or short cylindrical protrusions at top, 1.7–6.4 × 1.1–4.0 μm. Sporangiospores hyaline, smooth, not uniform, mainly cylindrical, 4.5–6.1 × 2.5–4.4 μm, some ovoid, 4.4–5.8 × 3.9–6.1 μm, occasionally subglobose, 4.9–7.2 μm. Chlamydospores present. Zygospores present, not uniform.

Additional strain examined—China, Hainan, Lingshui Li Autonomous County, 18.6958768° N, 109.9407998° E, altitude 151.6 m, from soil sample, April 23, 2023, Yi-Xin Wang (living culture XG04016-3).

GenBank accession numbers—CGMCC 3.27807 (ITS, PQ306319; LSU, PQ803168; *Act*, PQ807203; SSU, PQ799260; *TEF1α*, PV126021), XG0 4016-3 (ITS, PQ306320; LSU, PQ803169; *Act*, PQ807204; SSU, PQ799261; *TEF1α*, PV126022).

Maximum growth temperature—CGMCC 3.27807 (32 ℃), XG0 4016-3 (32 ℃).

Notes—Based on phylogenetic analyses of ITS-SSU-LSU-*Act*-*TEF1α* sequences, the two isolates of the new species *Absidia multiformis* formed an independent clade with full supports (MLBV = 100, BIPP = 1; Figure 1), which is closely related to *A. heterospora* (BIPP = 0.99; Figure 1). This new species differs morphologically from *A. heterospora* in sporangiophore, sporangium, apophysis and columella [34]. It differs from *A. heterospora* by narrower and shorter sporangiophores, 49.9–125.4 × 2.9–3.7 μm vs. 230–1700 × 4.3–10.0 μm. It is smaller than *A. heterospora* in sporangia, 20.1–36.3 μm vs. 15.0–55.0 μm. In columellae, it differs from *A. heterospora* by more shapes and smaller size. In detail, it is hemispherical and 5.3–19.4 × 8.3–22.9 μm, while *A. heterospora* is regularly dorsiventrally flattened and 10.5–34 μm in diameter. Combining morphological and molecular phylogenetic analyses, the two isolates were classified as a new taxon, allied to *A. heterospora*.

***Absidia ovoidospora* Yi Xin Wang & X.Y. Liu, sp. nov. Figure 4**

Fungal Names—FN 572284

Etymology—The epithet "*ovoidospora*" (Latin) pertaining to ovoid sporangiospores.

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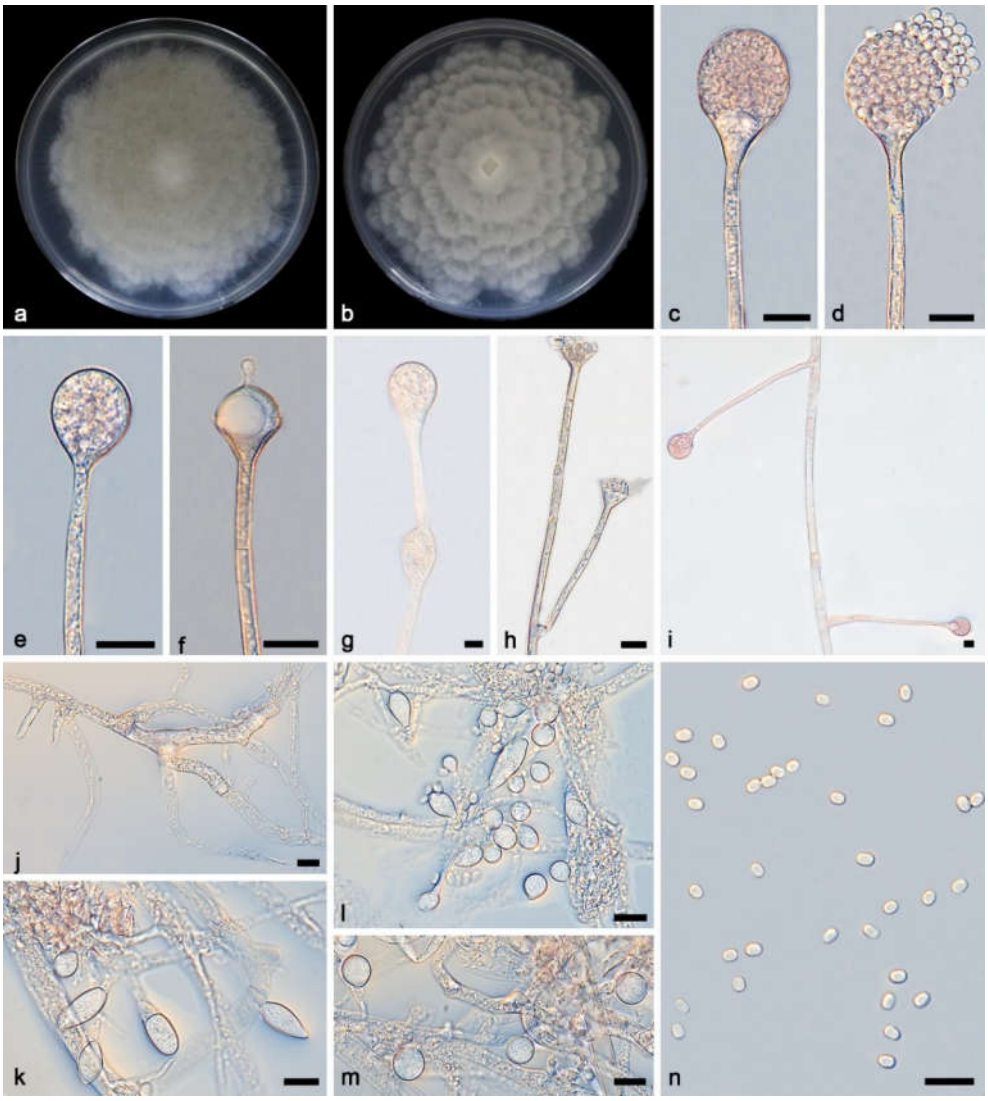


**Figure 4.** *Absidia oviodospora* ex-holotype CGMCC 3.27811. **a**, **b**, Colonies on PDA (**a**, obverse; **b**, reverse); **c–e**, An unbranched sporangiophore with a sporangium; **f**, Columellae, collars, sporangiospores and apophyses; **g**, Unbranched sporangiophores with swelling and sporangium; **h**, Branched sporangiophores with columellae, collars and apophyses; **i**, Branched sporangiophores with sporangia; **j**, Rhizoids; **k–m**, Giant cells; **n**, Sporangiospores; Bars: **c–n** 10 μm.

Type—China, Hainan, Changjiang Li Autonomous County, Bawangling National Forest Park, 25.905722° N, 107.279063° E, altitude 745.3 m, from soil sample, August 9, 2023, Yi-Xin Wang (Holotype HMAS 353185, ex-holotype strain CGMCC 3.27811 = XG05673-2).

Description—Colonies growing fast on PDA in darkness at 25 ℃ for 7 d, reaching 90 mm diameter, initially white, soon becoming gray to brown, irregular at edge, cottony, reversely white to gray. Hyphae hyaline at first, brownish when mature, branched, irregular, 5.8–13.7 μm in diameter. Stolons hyaline, branched, and smooth. Rhizoids hyaline, branched, irregular or root-like. Sporangiophores on aerial mycelia, hyaline, erect or slightly curved, unbranched or slightly branched, swollen usually present below sporangia, umbellately or sympodially branched, often with a septum below apophyses, 45.3–355.3 × 2.5–4.0 μm. Sporangia oval to subglobose, 13.6–29.0 × 13.3–28.5 μm, hyaline at first and then brown, deliquescent-walled, leaving a collar after releasing sporangiospores. Apophyses hyaline, smooth, bowl shaped and long funnel shaped, 4.1–6.9 × 8.6–10.9 μm. Columellae hyaline or brown, hemispherical with a short or long protruding at the top, 7.9–12.9 × 9.0–11.6 μm. Protrudings always slightly contracted in the middle, 1.9–4.6 × 1.7–2.6 μm.

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Sporangiospores hyaline, smooth, not uniform, mostly ovoid, 3.2–3.8 × 2.4–3.1 μm, some cylindrical, 3.4–4.6 × 2.2–3.0 μm μm. Chlamydospores present. Zygospores not observed.

Additional strains examined—China, Hainan, Changjiang Li Autonomous County, Bawangling National Forest Park, 25.905722° N, 107.279063° E, altitude 745.3 m, from soil sample, August 9, 2023, Yi-Xin Wang (living culture XG05673-3).

GenBank accession numbers—CGMCC 3.27811 clone1 (ITS, PQ306327; LSU, PQ803164; *Act*, PQ807207; SSU, PQ799256; *TEF1α*, PV126015), CGMCC 3.27811 clone2 (ITS, PV069753; LSU, PQ803165; *Act*, PV126023; SSU, PQ799257; *TEF1α*, PV126017), XG05673-3 clone 1 (ITS, PQ306328; LSU, PQ803166; *Act*, PQ807208; SSU, PQ799258; *TEF1α*, PV126016), XG05673-3 clone 2 (ITS, PV069754; LSU, PQ803167; *Act*, PV126024; SSU, PQ799259; *TEF1α*, PV126018).

Maximum growth temperature—CGMCC 3.27811 (30 ℃), XG05673-3 (30 ℃).

Notes—Based on phylogenetic analyses of ITS-SSU-LSU-*Act*-*TEF1α* sequences, the new species *Absidia ovoidospora* formed two sister clades with high supports (MLBV = 96, BIPP = 1; Figure 1), which are closely related to *A. panacisoli* and *A. abundans* with full supports (MLBV = 100, BIPP = 1; Figure 1). These two clades were resulted from two clones. This new species differs morphologically from *A. oblongispora* in sporangiophore and sporangium [35]. The new species differs from *A. panacisoli* by wider sporangiophore, 2.5–4.0 μm vs. 1.7–2.8 μm. In sporangia, the new species is bigger than *A. panacisoli*, 13.6–29.0 × 13.3–28.5 μm vs. 13.0–21.5 × 9.4–15.5 μm. This new species differs morphologically from *A. abundans* in sporangiophore, sporangium and columella [9]. The new species differs from *A. abundans* by bigger sporangiophores, 45.3–355.3 × 2.5–4.0 μm vs. 35.0–170.0 × 2.0–3.5 μm. The new species is bigger than *A. abundans* in sproangia, 13.6–29.0 × 13.3–28.5 μm vs. 8.0–16.5 × 8.5–16.0 μm. In columellae, the new species is bigger than *A. abundans*, 7.9–12.9 × 9.0–11.6 μm vs. 4.5–10.0 × 3.5–8.0 μm. Combining morphological and molecular phylogenetic analyses, the two were classified isolates as a new taxon, allied with *A. panacisoli and A. abundans*.

***Absidia verticilliformis*, Yi Xin Wang & X.Y. Liu sp. nov. Figure 5**

Fungal Names—FN 572287

Etymology—The epithet "*verticilliformis*" (Latin) pertaining to verticillate branches of sporangiophores.

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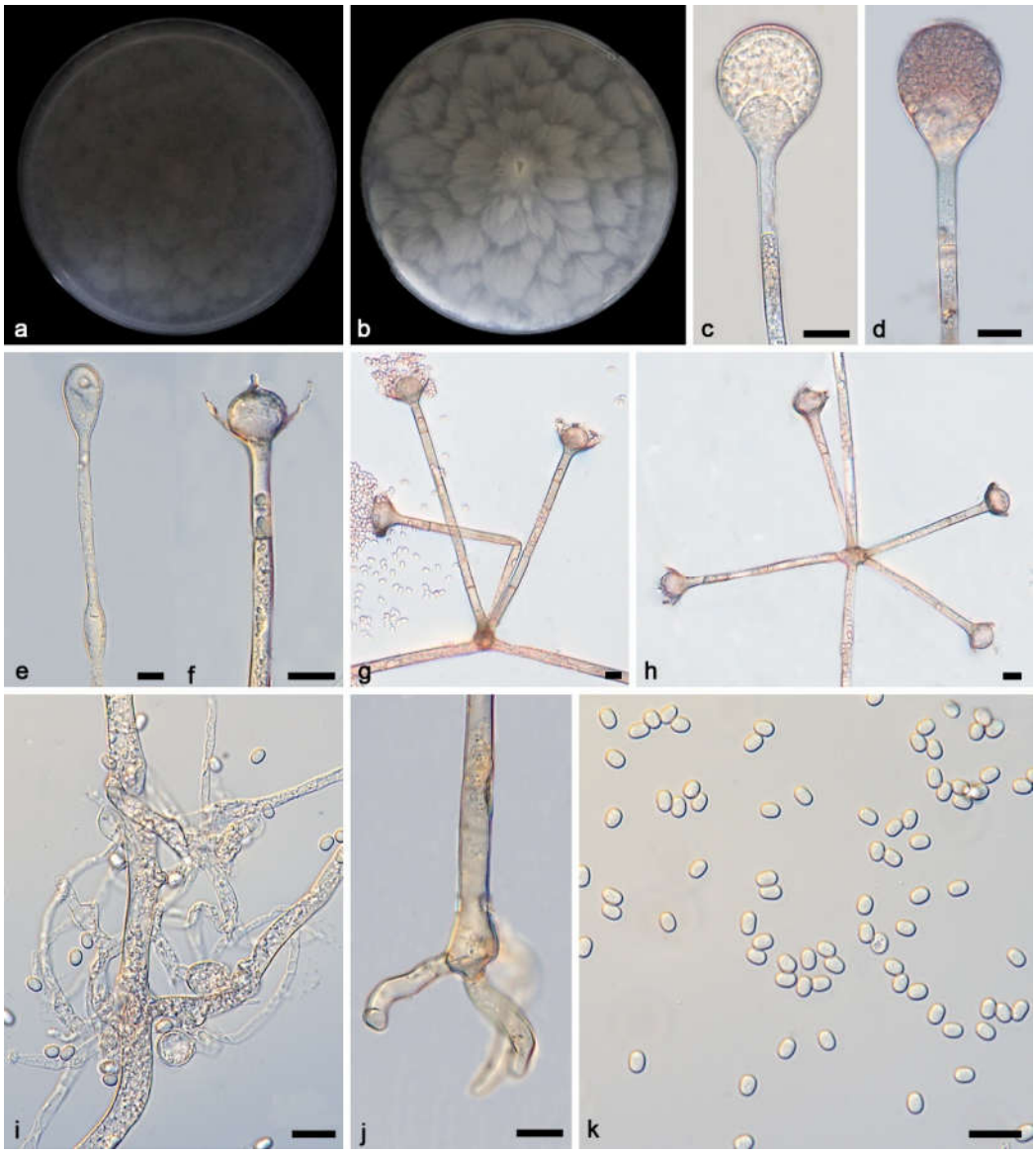


**Figure 5.** *Absidia verticilliformis* ex-holotype CGMCC 3.27810. **a**, **b**, Colonies on PDA (**a**, obverse; **b**, reverse); **c–e**, An unbranched sporangiophore with a sporangium; **f**, Columellae, collars, sporangiospores and apophyses; **g**, **h,** Branched sporangiophores with columellae, collars, sporangiospores and apophyses; **i**, **j**, Rhizoids; **k**, Sporangiospores; Bars: **c–k** 10 μm.

Type—China, Hainan, Sanya City, Jiyang District, G224 Haiyu Middle Line, 18.391817° N, 109.641068° E, altitude 193.0 m, from soil sample, April 24, 2023, Yi-Xin Wang (Holotype HMAS 353183, ex-holotype strain CGMCC 3.27810 = XG04088-3)

Description—Colonies growing fast on PDA in darkness at 25 ℃ for 7 days, reaching 90 mm diameter, initially white, soon becoming gray to brown, irregular at edge, cottony, reversely white to gray, growing outward in a petal shape. Hyphae hyaline at first, brownish when mature, branched, 4.4–8.9 μm in diameter. Stolons hyaline, branched, and smooth. Rhizoids hyaline, branched, irregular or root-like. Sporangiophores on aerial mycelia, hyaline, erect or slightly curved, unbranched or slightly branched, mostly umbellately branched, swollens usually present below sporangia, often with a septum below apophyses, 22.3–368.7 × 3.3–4.6 μm. Sporangia oval to subglobose, 15.7–29.0 × 17.5–26.0 μm, hyaline at first and then brown, deliquescent-walled, mostly leaving a collar after releasing sporangiospores. Apophyses hyaline, smooth, shallow mouth bowl shaped, 4.5–14.4 × 11.1–

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20.4 μm. Collars distinct. Columellae hyaline or brown, hemispherical, tip with short or long cylindrical protrusions, 12.6–25.6 × 14.7–26.2 μm. Protrudings 3.1– 6.3× 1.8–2.2 μm. Sporangiospores hyaline, smooth, not uniform, mostly ovoid, 3.4–4.3 × 2.3–3.1 μm, some cylindrical, 3.9–4.4 × 2.1–3.0 μm. Chlamydospores present. Zygospores not observed.

Additional strain examined—China, Hainan, Sanya City, Jiyang District, G224 Haiyu Middle Line, 18.391817° N, 109.641068° E, altitude 193.0 m, from soil sample, April 24, 2023, Yi-Xin Wang (living culture XG04088-4)

GenBank accession numbers—CGMCC 3.27810 clone 1 (ITS, PQ306315; LSU, PQ803170; *Act*, PQ807205; SSU, PQ799262; *TEF1α*, PV126011), CGMCC 3.27810 clone 2 (ITS, PV069755; LSU, PQ803171; *Act*, PV126025; SSU, PQ799263; *TEF1α*, PV126013), XG04088-4 clone1 (ITS, PQ306316; LSU, PQ803172; *Act*, PQ807206; SSU, PQ799264; *TEF1α*, PV126012), XG04088-4 clone2 (ITS, PV069756; LSU, PQ803173; *Act*, PV126026; SSU, PQ799265; *TEF1α*, PV126014).

Maximum growth temperature—CGMCC 3.27810 (34 ℃), XG04088-4 (34 ℃).

Notes—Based on phylogenetic analyses of ITS-SSU-LSU-*Act*-*TEF1α* sequences, the two isolates of the new species *Absidia verticilliformis* formed two sister clades with full supports (MLBV = 100, BIPP = 1; Figure 1), which is closely related to *A. edaphica* (MLBV = 94, BIPP = 1; Figure 1). This new species differs morphologically from *A. edaphica* in sporangium, columella and sporangiospore [36]. The new species is smaller than *A. edaphica* in sporangia, 15.7–29.0 × 17.5–26.0 μm vs. 30.5–35.5 × 24–27 μm. In columellae, the new species is bigger than *A. edaphica*, 12.6–25.6 × 14.7–26.2 μm vs. 5–9.5 × 6.5–20 μm. The new species is smaller than *A. edaphica* in sporangiospores, 3.4–4.4 × 2.1–3.1 μm vs. 3.5–5.5 × 2–3.5 μm. Combining morphological and molecular phylogenetic analyses, the two isolates were classified as a new taxon, allied to *A. edaphica*.

## 4. Discussion

The genus *Absidia* was established nearly 150 years ago. Between 1878 and 2010, the taxonomic status of the genus was changed several times, and the species of the genus were divided according to the optimum growth temperature. In 2007, Kerstin Hoffmann et al. divided *Absidia* s. l. into *Absidia* (Absidiaceae s. str., mesothermal type) and *Mycocladus* (Mycocladiaceae, thermophilic type) [7]. In 2009, *A. parricida* and *A. zychae* were separated from the genus *Absidia* by Hoffmann and Voigt and reassigned to a newly described genus *Lentamyces* [8]. In 2010, Hoffmann et al. investigated and evaluated *Absidia* s. l., and finally divided it into three genera, namely *Absidia* s. s., *Lichtheimia* and *Lentamyces* [6]. From 2011 to 2019, the discovery of *Absidia* stagnated, only six new species have been described (https://www.indexfungorum.org/, accessed on 7 February 2025; [35, 37-40]). Since 2020, taxonomic studies on this genus have been extensively carried out, with a total of 49 new species described (https://www.indexfungorum.org/, accessed on 7 February 2025; [9, 10, 13, 33, 36, 41-50]). Nowadays, it is the most species-rich genus in the family Cunninghamellaceae. Together with the four new species proposed in this study, the world diversity of *Absidia* reaches 71 recognized species.

*Absidia* species predominantly inhabit soil environments across tropical, subtropical, and temperate climatic zones. In accordance with this ecological distribution pattern, the four novel species described in this study were successfully isolated from soil samples collected in tropical and subtropical geographic regions of China.

Prior to 2020, the phylogenetic tree of this genus was predominantly reconstructed utilizing the combination of ITS and LSU sequences. In 2021, Hurdeal et al. added SSU and *Act* sequences for reconstructing the phylogenetic [36]. Subsequently, in 2024, Tao et al. employed a more comprehensive approach, utilizing SSU, ITS, LSU, *Act*, and *TEF1α* to reconstruct the phylogenetic tree, yielding results that were largely in line with previous findings [13]. In this study, the phylogenetic history was inferred using ITS-SSU-LSU-*Act*-*TEF1α*, and newly isolated strains were grouped into four individual clades with high supports, namely *Absidia irregularis* sp. nov., *A. multiformis* sp. nov., *A. ovoidospora* sp. nov., and *A. verticilliformis* sp. nov.

*Absidia irregularis* is related to *A. oblongispora* with high supports (MLBV = 100, BIPP = 1; Figure 1), while distinguished by bigger apophyses and columellae. *Absidia multiformis* is most closely

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related to *A. heterospora* with high support (BIPP = 0.99; Figure 1). Morphologicaly, *A. multiformis* has narrower and shorter sporangiophore, smaller sporangia size, different columellae shape and size. *Absidia ovoidospora* is closely related to *A. panacisoli* and *A. abundans*. However, compared with *A. panacisoli*, *A. ovoidospora* had wider sporangiophore width and larger sporangium. At the same time, compared with *A. abundans*, the microscopical measurements of the sporangiophore, sporangium and columella of *A. oviodrospora* were larger. *A. verticilliformis* is closely related to *A. edaphica*. *A. verticilliformis* differs from *A. edaphica* by smaller sporangia, bigger columellae and smaller sporangiospores.

In this study, thermal tolerance thresholds of fungal strains were determined using a temperature gradient cultivation technique. Growth characterization revealed distinct maximum growth temperatures for the four *Absidia* species: *A. ovoidospora* (30℃), *A. irregularis* (32℃), *A. multiformis* (34℃), and *A. verticilliformis* (34℃). These thermal parameters align with the established physiological profile of *Absidia* s. s. (mesophilic genus with optimal growth at 25-34℃).

In summary, the molecular phylogenetic and morphological results both support the identification of the four new species. These findings further enhance our understanding of mucoralean biodiversity in Asian tropical and subtropical ecosystems.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/doi/s1, File S1: ITS-SSU-LSU- *Act-TEF1a*.

**Author Contributions:** Y.-X.W. took charge of the microscopy, DNA sequencing, data analyses and manuscript draft; Z.-Y.D. and Y.-X.W. collected samples and isolated living cultures; X.-Y.J. and Z.-Y.D. made dry cultures; Z.M. revised the manuscript; X.-Y.L. contributed to new species proposal, manuscript revision and financial support; funding acquisition. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The sequences were deposited in the GenBank database.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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