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Mucoralean fungi in Thailand: novel species of *Absidia* from tropical forest soil

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ABSTRACT
Species of *Absidia* Tiegh. (Mucorales) are commonly isolated from soil, dead plant materials and dung. The genus is of interest in industrial and medical fields due to the presence of active compounds and secondary metabolites. *Absidia* species are typically characterized by an arcuate stolon with rhizoids, zygospores with appendaged suspensors and columella with apical projections. Characterization and

description of novel *Absidia* species are unprecedented in Thailand. During our field visits to explore soil fungi in northern Thailand, topsoil samples from a tropical mixed forest in Chiang Mai were collected. Four strains of *Absidia* were isolated from the soil samples. Initial morphological characteristics revealed that four strains represent two new species. Phylogenetic analysis of combined ITS, SSU, LSU, and ACT-1 sequence data revealed that the two new species are phylogenetically distinct. Microscopic investigations indicated that the new species, *Absidia soli* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. produces larger sporangia (16-51 × 15-45.5 µm) and columella (7.5-12.5 × 9-24 µm) than *Absidia edaphica* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. (30.5-35.5 × 24-27 µm and 5-9.5 × 6.5-20 µm respectively). Both species have short cylindrical to cylindrical and hyaline sporangiospores. The sizes of the spores for *A. edaphica* sp. nov. and *A. soli* sp. nov. are 3.5-5.5 × 2-3.5 µm and 3-4.5 × 1.5-2.5 µm respectively. Mycelial growth in four media, namely malt extract agar (MEA), potato dextrose agar (PDA), corn meal agar (CMA) and yeast malt agar (YMA) were measured. Both species grow in the range of 4 to 30°C, but not at >37°C. DNA sequence analyses based on wider taxon sampling and LSU dataset revealed that there is a high phylogenetic diversity of species of *Absidia*.

KEY WORDS
Mucorales,
polyphasic approach,
phylogeny,
soil,
new species.

RÉSUMÉ
*Les microchampignons mucorales de Thaïlande : nouvelles espèces d'*Absidia *du sol de forêt tropicale.*
Les espèces d'*Absidia* Tiegh. (Mucorales) sont généralement isolées du sol, des matières végétales mortes et des excréments. Le genre présente un intérêt dans les domaines industriel et médical en raison de la présence de composés actifs et de métabolites secondaires. Les espèces *Absidia* sont typiquement caractérisées par un stolon arqué avec des rhizoïdes, des zygospores avec des suspenseurs appendus et des columelles avec des projections apicales. La caractérisation et la description des nouvelles espèces d'*Absidia* sont sans précédent en Thaïlande. Au cours de nos visites sur le terrain pour explorer les champignons du sol dans le nord de la Thaïlande, des échantillons de terre végétale ont été prélevés dans une forêt tropicale mixte à Chiang Mai. Quatre souches *Absidia* ont été isolées à partir des échantillons de sol. Les caractéristiques morphologiques initiales ont révélé que quatre souches représentent deux nouvelles espèces. L'analyse phylogénétique des données de séquences combinées ITS, SSU, LSU et ACT-1 a révélé que les deux nouvelles espèces sont phylogénétiquement distinctes. L'examen microscopique a indiqué que la nouvelle espèce *Absidia soli* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. produit des sporanges (16-51 × 15-45,5 µm) et des columelles (7,5-12,5 × 9-24 µm) plus grands que *Absidia edaphica* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. (30,5-35,5 × 24-27 µm et 5-9,5 × 6,5-20 µm respectivement). Les deux espèces ont des sporangiospores cylindriques courts à cylindriques et hyalines. Les tailles des spores pour *A. edaphica* sp. nov. et *A. soli* sp. nov. sont respectivement de 3,5-5,5 × 2-3,5 µm et de 3-4,5 × 1,5-2,5 µm. La croissance mycélienne a été mesurée dans quatre milieux, à savoir l'agar d'extrait de malt (MEA), l'agar dextrose de pomme de terre (PDA), l'agar de farine de maïs (CMA) et l'agar de malt de levure (YMA). Les deux espèces croissent entre 4 à 30°C, mais pas à > 37°C. L'analyse basée sur un échantillonnage plus large des taxons et sur l'ensemble des données LSU a révélé qu'il existe une grande diversité phylogénétique des espèces d'*Absidia*.

MOTS CLÉS
Mucorales,
approche polyphasique,
phylogénie,
sol,
espèces nouvelles.

INTRODUCTION

Mucoralean fungi are cosmopolitan saprobes inhabiting soil, dead plants and dung, but their diversity is poorly studied (Mousavi *et al.* 2017; Lima *et al.* 2018; Richardson & Rautemaa-Richardson 2019; Walther *et al.* 2019). A few mucoralean species have been repeatedly reported in soil samples (Lima *et al.* 2016, 2018; Oliveira *et al.* 2013; Santiago *et al.* 2013; Shainidze et al. 2019; Ziaee *et al.* 2016). Some are post-harvest pathogens of stored fruits and cereals, while others are plant pathogens, facultative fungal parasites and opportunistic human pathogens (Shtienberg 1997; Hoffmann *et al.* 2013). Few studies are available on fungi of seasonal tropical forest soils (Amma *et al.* 2018). So far, the majority of these focus on taxonomy and evolution of Dikarya, while other fungal groups have been mostly overlooked (Wijayawardene *et al.* 2018).

Cosmopolitan, species of *Absidia* Tiegh. have commonly been isolated in nature from soil (Zhang *et al.* 2018) (Table 1). The genus was described by Van Tieghem (1876) and is currently classified in the family Cunninghamellaceae Naumov ex R.K.Benj., order Mucorales Dumort., and subphylum Mucoromycotina Benny (Wijayawardene *et al.* 2018, 2020). The type species of the genus is *Absidia repens* Tiegh. (Hoffmann *et al.* 2010). Previously, *Absidia* included mesophilic, thermotolerant and mycoparasitic species. Later analysis of growth physiology, morphological characteristics and phylogeny revealed that taxa grouped into three distinct clades (Hoffmann *et al.* 2007). Thermotolerant species with non-appendaged zygospores grouped in one clade and thus were

transferred to *Mycocladus* Beauverie (later named *Lichtheimia* Vuill.). A second clade consisted of mycoparasites, *Absidia parricida* (Renner Muskat ex Hesselt. & J.J.Ellis) and *A. zychae* (Hesselt. & J.J.Ellis), both of which grouped in the genus *Lentamyces* Kerst. Hoffm. and K. Voigt (Hesseltine & Ellis 1964; O'Donnell et al. 2001; Hoffmann *et al.* 2007; Walther *et al.* 2019). Finally, the third clade included all mesophilic species, which are currently considered as *Absidia* (Hoffmann *et al.* 2007; Hoffmann 2010).

Arcuate stolons with rhizoids, pyriform sporangia, apophysis, and presence of appendaged suspensors during formation of zygospores are characteristics that are used to circumscribe this genus (Van Tieghem 1876; Hoffmann *et al.* 2007). All species have an apical projection on top of the columella. Sporangiospores are hyaline, unicelled and lack striations, while shapes vary from round, cylindrical to short cylindrical or short rod (Zhang *et al.* 2018). Species of *Absidia* are mesophilic showing rapid growth at temperatures ranging between 25°C to 34°C (Hoffmann *et al.* 2010). Since first introduced, the classification and circumscription of *Absidia* species have been contentious. Bainier (1882, 1889), Hagem (1908) and Lendner (1924) provided additional morphology-based studies in support to the initial description of the genus. With the advent of DNA based bioinformatics tools, taxonomic classification of the genus has been refined (Hoffmann *et al.* 2007; Hoffmann 2010; Walther *et al.* 2019).

During a survey of soil fungi in a tropical forest in northern Thailand, four strains of *Absidia* were discovered. A polyphasic approach, including morphological, phylogenetic, and physiological methods along with genealogical concordance phylogenetic species recognition (GCPSR) was used to support establishment of the new taxa. Herein, we introduce two new species of *Absidia*. In addition, we provide brief information on the geographic distribution, host/substrate information, and taxonomic insights of the genus *Absidia* (see Table 1).

MATERIAL AND METHODS

Collections and field sites

Soil samples were collected from the Mushroom Research Centre in Chiang Mai Province, Thailand on 11th August 2019. The site is an evergreen tropical forest dominated by *Castanopsis armata* (Roxb.) Spach, *Erythrina* sp., and *Dipterocarpus* sp. (Karunarathna *et al.* 2011). A square plot of approximately of 50 × 50 m was selected. Dead leaves and other organic matter were manually removed. A shovel was used to remove soil to a depth of 1-5 cm. From each corner of the plot, two sub-samples were collected, placed in zip lock bags and stored at 4°C until further use. Collected soil was dry, red with grainy appearance and small pores.

Isolation, culture and morphologicl studies

Soil samples were diluted to a ratio of 1:5 and 1:10 with sterilized distilled water. Mixtures were shaken for 2 hours at 25°C with a shaking incubator. 100 µl of the suspension was aseptically plated on MEA plates and YMA plates and spread using sterile a glass rod. Plates were sealed and incubated at 20°C for 3 days. Following incubation, plates were inspected for fungal colonies. Subsequently, sterile straws were used to cut fungal tips (with the agar) and sterile needles were used to transfer the plugs to fresh media plates. Cultures were observed using a compound microscope and images were taken with a Nikon DS-RI2 digital camera. Once grown, cultures were maintained at 4°C and in 15% glycerol. Type specimens are deposited at the Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand. Ex-type living cultures were deposited in the Mae Fah Luang culture collection (MFLUCC), Chiang Rai, Thailand. The photoplates and species description (taxonomy) were submitted to obtain the FacesofFungi (Jayasiri *et al.* 2015) and MycoBank numbers. New species are established following the guidelines outlined by Jeewon & Hyde (2016).

DNA extraction and PCR amplification

Total genomic DNA was extracted from fungal mycelia grown on MEA for 3-4 days at 25°C using Biospin Fungal Genomic DNA extraction kit (BioFlux, P.R China) following manufacturer's instructions. Polymerase chain reaction (PCR) was used to amplify partial fragments of internal transcribed spacer (ITS1-5.8S-ITS2), large subunit ribosomal RNA (LSU), small subunit ribosomal RNA (SSU), and actin (ACT-1). The primers used are listed in Table 2.

The PCR reagents consisted of 8.5 µL ddH2O, 12.5 µL 2× Easy Taq PCR Super Mix, 2 µL of DNA template, 1 µL of each forward and reverse primers (10 pM) accounting for a final volume of 25 µL. Amplification conditions for all gene regions were as follows: initial denaturation step of 5 minutes at 95°C (3 minutes for ACT-1) and a final elongation step of 10 minutes at 72°C. Conditions for the amplification of the LSU gene region consisted of 35 cycles, with a denaturation step of 1 minute at 94°C, annealing at 53°C for 50 seconds and elongation at 72°C for 1.30 minutes. For the amplification of the SSU region, 37 PCR cycles were used and consisted of a denaturation step at 94°C for 1 minute, annealing at 54°C for 50 seconds and elongation at 72°C for 1 minute. For ITS, the 35 cycles consisted of denaturation at 94°C for 1 minute, annealing at 50°C for 50 seconds and elongation at 72°C for 1.30 minute. The conditions for the protein coding gene ACT-1 consisted of 35 cycles, a denaturation step of 94°C for 1 minute, annealing at 55°C for 1 minute and elongation at 72°C for 1 minute.

Subsequent purification and sequencing of PCR products was carried out by Tsingke Sequencing Company (Kunming, China). Newly generated nucleotide sequences have been deposited in GenBank (Table 3).

PHYLOGENETIC ANALYSES

Raw reads were trimmed, merged and edited using the CLC Main Workbench (Qiagen). New sequences were used as queries to perform Blast search against the nucleotide database in GenBank to exclude contamination and to determine the closest taxa to the strains (https://blast.ncbi.

TABLE 1. — Geographic distribution of *Absidia* Tiegh. species, their reported hosts, countries and voucher number. The data was extracted from GenBank (accessed on September 2020). Countries and hosts where the type species were isolated are marked with asterisk (*).

Species name	Host/substrate	Country(ies)	Voucher no.
Absidia anomala Hesselt. & J.J.Ellis.	Cucurbita crown, soil*	United States, Cuba*	clone 2014_1570, CBS 125.68
Absidia caatinguensis D.X. Lima & A.L. Santiago	Invertebrates, soil*	China, Brazil*, India	URM 7156 isolate AP1.F9
Absidia caerulea Bainier	*Pinus sylvestris* wood, marinho, roots of stumps, *Abies alba*, *Pinus radiata*, *Lycopodium* spp, dung of rabbit, soil, *Lycopodium annotinum*, nuruks, roots of wheat, soil under Scots pine	Denmark, United States, Latvia, Russia, China, Poland, Spain, South Korea, United Kingdom	isolate P51, isolate 3A9, isolate PDW1-3, isolate PSJ2-2, HP141, isolate HP135, strain WA0000009402, CBS 101.28, CBS 102.28, KCCM60376
Absidia californica J.J.Ellis & Hesselt.	Dung of rat*, dung of mouse, poultry waste	United States*, India	CBS 126.68, Absid_ST8
Absidia cornuta D.X.Lima, C.A.de Souza, H.B.Lee & A.L.Santiago	soil*	Brazil*	URM 93639
Absidia cuneospora G.F. Orr & Plunkett	Sandy soil*, clay soil	United States*,	CBS 101.59, CBS 102.59
Absidia cylindrospora Hagem	Orchids, *Zostera marina*, Leaf, *Zingiber officinale Rosc*, rhizosphere of *MUnited States sapientum** (*Absidia cylindrospora* var. *rhizomorpha*), soil of pastured hardwood* (*Absidia cylindrospora* var. *nigra*)	Poland, United States*(*Absidia cylindrospora* var. *nigra*), India, Honduras* (*Absidia cylindrospora* var. *rhizomorpha*), Netherlands, Germany	isolate UWR_170, isolate CLE24, isolate Leaf1A-2013, strain KM6, CBS 153.63, CBS 127.68
Absidia fusca Linnem.	Soil of pine forest*, Myxomycete	Germany*, Netherlands	CBS 102.35, CBS 346.97
Absidia glauca Hagem	Manure in asparagus field (Host of type species is not available)	Germany (Country of origin of type species is not available)	CBS 100.48
Absidia heterospora Y. Ling	Soil of pine forest*	France*, China	CBS 101.29, strain SHTH021
Absidia jindoensis Hyang B. Lee & T.T.T. Nguyen	Rhizosphere soil of pine tree*	South Korea*	CNUFC-PTI1-1, CNUFC-PTI1-2
Absidia koreana Hyang B. Lee, Hye W. Lee & T.T.T. Nguyen	Soil*	South Korea*	EML-IFS45-1, EML-IFS45-2
Absidia macrospora Váňová	Soil*	Former Czechoslovakia*	CBS 697.68
Absidia panacisoli T.Yuan Zhang, Ying Yu, He Zhu, S.Z.Yang, T.M.Yang, MengY. Zhang & YiX.Zhang	Soil (rhizosphere of *Panax notoginseng*) *	China*	SYPF 7183, CBS 140959
Absidia pernambucoensis D.X.Lima, C.A.de Souza, H.B.Lee & A.L.Santiago	Soil*	Brazil*	URM 93638
Absidia pseudocylindrospora Hesselt. & J.J.Ellis	Virgin soil*, soil	Tanzania*, South Korea	CBS 100.62, EML-FSDY6-1, EML-FSDY6-2
Absidia psychrophilia Hesselt. & JJ.Ellis	G-jav rhizome-associated mycobiome, ambrosia beetle gland*, wine cellars, soil of pine forest	China, Canada*, Hungary	G-jav1-LSU2_OTU-0-354_2, CBS 128.68, voucher 714
Absidia repens Tiegh.	Wallpaper*, poultry dump site, cerrado soil, damp concrete, root of *Ambrosia artemisiifolia*, rhizoplane, soil, cave sediment	United Kingdom*, India, Brazil, Austria, Hungary, Pakistan, Brazil, Iran, Canada, Spain	CBS 115583, Absidar_ST9, isolate M54, strain Sz3_2H, LAHC-CMPK-M01, IBE5.1
Absidia spinosa Lendn.	*Comandra pallida* leaf*, soil of vineyard	United States, Germany	CBS 187.64, FSU549
Absidia stercoraria Hyang B. Lee, H.S. Lee & T.T.T. Nguyen	Rat dung*	South Korea*	EML-DG8-1, EML-DG8-2
Absidia terrestris Rosas de Paz, Dania García, Guarro, Cano & Stchigel	Soil*	Mexico*	FMR 14989, FMR 15024
Unclassified *Absidia*	Cheese, foil in soil, fruit syrup, grape marc, oil, plant canker, rhizosphere, root, soil, stem, wood	Brazil, Canada, China, Colombia, Croatia, Greece, Finland, Hungary, Iceland, India, Iran, Italy, Japan, New Zealand, Norway, Peru, Poland, Slovakia, Spain, South Korea, Thailand, United Kingdom, United States	Strain N20, strain T8 PDA8-f, strain T12 PDA2-f, FS-35, TU-GM13, strain K3, A1-C1-3, SAUFS3-5, isolate MM17-4, strain GBC-Fungus31
Environmental samples	Soil	United States, Australia	–

TABLE 2. — List of primers used in this study.

Primers	Sequence 5'-3'	Fragment size	References
ITS 4	TCC TCC GCT TAT TGA TAT GC	400-600 bp	White *et al*. 1990
ITS 5	GGA AGT AAA AGT CGT AAC AAG G		White *et al*. 1990
LR5	TCC TGA GGG AAA CTT CG	*c*. 1000 bp	Vilgalys & Hester 1990
LROR	ACC CGC TGA ACT TAA GC		Vilgalys & Hester 1990
NS1	GTA GTC ATA TGC TTG TCT C	*c*. 1000 bp	White *et al*. 1990
NS4	CTT CCG TCA ATT CCT TTA AG		White *et al*. 1990
ACT 1	TGG GAC GAT ATG GAI AAI ATC TGG CA	*c*. 950 bp	Voigt & Wostemeyer 2000
ACT 4	TCI TCG TAT TCT TGC TTI GAI ATC CAC AT		Voigt & Wostemeyer 2000

nlm.nih.gov) (Altschul *et al.* 1990). Individual datasets were constructed for SSU, ITS, LSU and ACT-1 separately. In addition, two concatenated datasets (a: ITS, LSU, SSU and ACT-1; b: LSU, SSU and ACT-1)) were used for phylogenetic analyses. A separate LSU dataset with a broader taxon sampling including 11 sequences from unclassified *Absidia* and seven from environmental sequences (Appendix 1) was also analyzed to assess the phylogenetic diversity of *Absidia*. Sequences of *Cunninghamella homothallica* Komin. & Tubaki (CBS 168.53), *C. phaeospora* Boedijn (CBS 692.68), and *C. bainieri* Naumov (FSU319) were used as outgroup taxa (Table 3). Sequences were aligned using MAFFT v.7 online server (Katoh & Toh 2008; https://mafft.cbrc.jp/alignment/server/) and trimmed with TrimAl [(v.1.0) Gappyout option] available on the online platform Phylemon2 (http://phylemon.bioinfo.cipf.es/) (Tárraga *et al.* 2007; Capella-Gutierrez *et al.* 2009).

Substitution models of molecular evolution were estimated independently for each gene region using MrModeltest 2.3 under the Akaike information criterion as implemented in PAUP 4.0. The best-fit model was GTR+G+I for all gene regions. Maximum parsimony (MP) analysis was done using PAUP 4.0 with the heuristic search option and 1000 replicates (Swofford 2002). Maximum likelihood (ML) analysis was performed using RAxML-HPC2 on XSEDE on the online CIPRES Portal (https://www.phylo.org/portal2) with bootstrap support obtained from 1000 pseudo replicates (Miller *et al.* 2010; Stamatakis 2014). Bayesian inference (BI) analysis was conducted using MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001). The Bayesian tree was constructed by running four simultaneous chains for 2 000 000 generations and a sampling frequency of 100. The first 1000 trees represented the burn-in phase and were discarded. Convergence was declared when the average standard deviation of split frequencies reached less than 0.01.

Genealogical concordance phylogenetic species recognition (GCPSR)analysis

New isolates along with phylogenetically related species (*Absidia koreana* Hyang B. Lee, Hye W. Lee & T.T. Nguyen (EML-IFS45-1, EML-IFS45-2), *Absidia jindoensis* Hyang B. Lee & T.T.T. Nguyen (CNUFC-PTI1-1, CNUFC-PTI1-2) and *Absidia anomala* Hesselt. & J.J.Ellis (CBS 127.68) were analyzed using GCPSR with a pairwise homoplasy index (PHI). Analysis was performed using SplitsTree version 4.15.1 (Huson 1998; Huson & Bryant 2005; Bruen *et al.* 2006) as described by Quaedvlieg *et al.* (2014) to assess presence of recombination between the new species and their closely related taxa. A concatenated dataset ITS-LSU-SSU-ACT-1 (with only close sister taxa) was used. The relationship between the species was visualized with Log-Det transformation and splits decomposition options. A value of $\Phi w < 0.05$ for the pairwise homoplasy index was considered as significant recombination within the dataset.

Genetic distance

Genetic distance of the new taxa and closely related groups were measured. Molecular Evolutionary Genetics Analysis Version X (MegaX) was used to compute genetic distances with the Kimura 2-parameter model as the substitution model with gamma distribution and pairwise deletion. Both untrimmed and trimmed datasets were used. The untrimmed/trimmed dataset contained 406/371 characters (with gaps) for the ITS region, 608/599 characters for LSU, 1019/1017 characters for SSU and 619/619 for ACT-1.

Measurement of mycellial growth

Mycelial growth in MEA, PDA, CMA, and YMA media was determined. Small blocks of mycelium from each strain were inoculated on plastic Petri plates. The plates were sealed and incubated at room temperature, 4, 20, 25, 30, 37 and 42°C. Length and width of colonies were documented every 24 or 48 hours. Due to fast growing colonies, the mycelial plug was placed near the margin of the agar plate. Measurement was taken from the point of inoculation to the outline of the colony.

Abbreviations

ACT-1 actin gene;
BYPP bayesian posterior probability;
CMA corn-meal agar;
ITS internal transcribed spacer;
LSU large subunit of the nuclear ribosomal RNA gene;
MEA malt extract agar;
ML maximum likelihood;
MP maximum parsimony;
PHI pairwise homoplasy index;
PDA potato dextrose agar;
SSU small subunit ribosomal RNA gene;
YMA yeast malt agar.

TABLE 3. — Data used for phylogenetic analysis in this study and their corresponding GenBank accession numbers. Type species are denoted by T. Sequences derived in this study are shown in **bold**.

Species name	Voucher no.	ITS	LSU	SSU	ACT-1
Absidia anomala Hesselt. & J.J.Ellis T	CBS 125.68	NR_103626	NG_058562	-	-
Absidia caatinguensis D.X. Lima & A.L. Santiago T	URM7156	NR_154704	NG_058582	-	-
Absidia californica J.J.Ellis & Hesselt. T	CBS 126.68	-	NG_056998	-	-
Absidia caerulea Bainier	NRRLA9483	-	-	AF113406	-
Absidia caerulea	CBS 104.08	JN205811	MH866107	-	-
Absidia cornuta D.X.Lima, C.A.de Souza, H.B.Lee & A.L.Santiago T	URM 93639	MN625256	MN625255	-	-
Absidia cuneospora G.F. Orr & Plunkett	CBS 102.59	JN205819	JN206579	-	-
Absidia cylindrospora var. *cylindrospora* Hagem	CBS 100.08	JN205822	JN206588	-	-
Absidia cylindrospora var. *nigra* Hesselt. & J.J.Ellis T	CBS 127.68	-	NG_058560	-	-
Absidia cylindrospora var. *rhizomorpha* Hesselt. & J.J.Ellis T	CBS 153.63	-	NG_058563	-	-
***Absidia edaphica* sp. nov. T**	**MFLU 20-0416**	**MT396372**	**MT393987**	**MT394048**	**MT410739**
***Absidia edaphica* sp. nov.**	**MFLU 20-0415**	**-**	**MT393986**	**MT394047**	**MT410738**
Absidia fusca Linnem. T	CBS 102.35	NR_103625	NG_058552	-	-
Absidia glauca Hagem T	CBS 101.08	NR_111658	MH866105	-	-
Absidia heterospora Y. Ling	SHTH021	JN942683	JN982936	JQ004928	-
Absidia heterospora T	CBS101.29	-	NG_058564		-
Absidia jindoensis Hyang B. Lee & T.T.T. Nguyen T	CNUFC-PTI1-1	MF926622	MF926616	MF926626	MF926510
Absidia jindoensis	CNUFC-PTI1-2	MF926623	MF926617	MF926627	MF926509
Absidia koreana Hyang B. Lee, Hye W. Lee & T.T. Nguyen	EML-IFS45-2	KR030063	KR030057	KT321299	KR030059
Absidia koreana T	EML-IFS45-1	KR030062	KR030056	KT321298	KR030058
Absidia macrospora Váňová	FSU4746	AY944882	-	EU736276	-
Absidia macrospora T	CBS 697.68	-	NG_058549	-	-
Absidia panacisoli T.Yuan Zhang, Ying Yu, He Zhu, S.Z.Yang, T.M.Yang, MengY. Zhang & YiX.Zhang	SYPF 7183	MF522181	MF522180	MF522179	-
Absidia panacisoli T	CBS 140959	NR_159563	NG_063948	NG_065707	-
Absidia pernambucoensis D.X. Lima, C.A. de Souza, H.B. Lee & A.L. Santiago T	URM 93638	MN635568	MN635569		
Absidia pseudocylindrospora Hesselt. & J.J.Ellis	EML-FSDY6-2	KU923817	KU923814	KU923819	KU923815
Absidia pseudocylindrospora T	CBS 100.62	NR_145276	NG_058561	-	-
Absidia psychrophilia Hesselt. & J.J.Ellis	FSU4745	AY944874	EU736306	EU736279	AY944762
Absidia repens Tiegh.	FSU 4726	EU484288	-	EU484209	-
Absidia repens	NRRL1336	-	AF113448	AF113410	AJ287136
Absidia repens T	CBS 115583	NR_103624	NG_058551	-	-
***Absidia soli* sp. nov. T**	**MFLU 20-0414**	**MT396373**	**MT393988**	**MT394049**	**MT410740**
***Absidia soli* sp. nov.**	**MFLU 20-0413**	**MT396371**	**MT393985**	**MT394046**	**-**
Absidia spinosa Lendn.	FSU551	AY944887	EU736307	EU736280	EU736227
Absidia spinosa	FSU552	AY944888	EU736308	EU736281	AY944764
Absidia spinosa var. *biappendiculata* Rall & Solheim	CBS 187.64	-	MH870040	-	-
Absidia stercoraria Hyang B. Lee, H.S. Lee & T.T.T. Nguyen	EML-DG8-2	KU168829	KT921999	KT921997	KT922001
Absidia stercoraria T	EML-DG8-1	KU168828	KT921998	NG_065640	KT922000
Absidia terrestris Rosas de Paz, Dania García, Guarro, Cano & Stchigel T	FMR 1498	LT795003	LT795005		
Absidia terrestris	FMR 15024	LT795004	LT795006		
Chlamydoabsidia padenii Hesselt. & J.J.Ellis T	NRRL 2977	-	AF113453	NG_061008	-
Chlamydoabsidia padenii T	CBS 172.67	NR_153872	JN206586	-	-
Cunninghamella bainieri Naumov	FSU319	-	EU736313	EU736286	EU736232
Cunninghamella homothallica Komin. & Tubaki	CBS 168.53	MH857147	NG_058833	-	-
Cunninghamella phaeospora Boedijn	CBS 692.68	AF254934	NG_058812	-	-
Halteromyces radiatus Shipton & Schipper	NRRL6197	-	AF157192	AF157138	-
Halteromyces radiatus	CBS 162.75	NR_145293	NG_057938	-	-

RESULTS

PHYLOGENETIC ANALYSES

The ITS, LSU, SSU and ACT-1 concatenated dataset comprised 47 taxa including the two novel species, each represented by two strains. Initially, single gene trees were constructed followed by multigene trees. After removing unambiguous positions, final alignments contained 593 sites for ITS, 774 for LSU, 1720 for SSU, and 820 for ACT-1. Final single gene datasets were concatenated into two matrices, one with ITS and one without, to check the effect of the ITS gene region on the topology of the phylogenetic tree. The SSU-LSU-ACT-1 matrix contained 3314 characters, and the four-marker matrix (ITS, SSU, LSU and ACT-1) (Fig. 1) contained 3907 sites. The ITS, SSU, LSU and ACT-1 matrix contained 1303 distinct alignment patterns and 52.52% of undermined characters or gaps. Likelihood of the best scoring ML tree was -25734.267037. Tree topologies from all methods of analysis were almost similar. Phylogenetic analysis of the ITS, LSU, SSU and ACT-1 concatenated dataset indicates that the new taxa formed two separate sister clades with maximum statistical support each (100% ML/100% MP/1.00 BIPP) and showed a close phylogenetic affinity with *A. koreana* (EML-IFS45-1

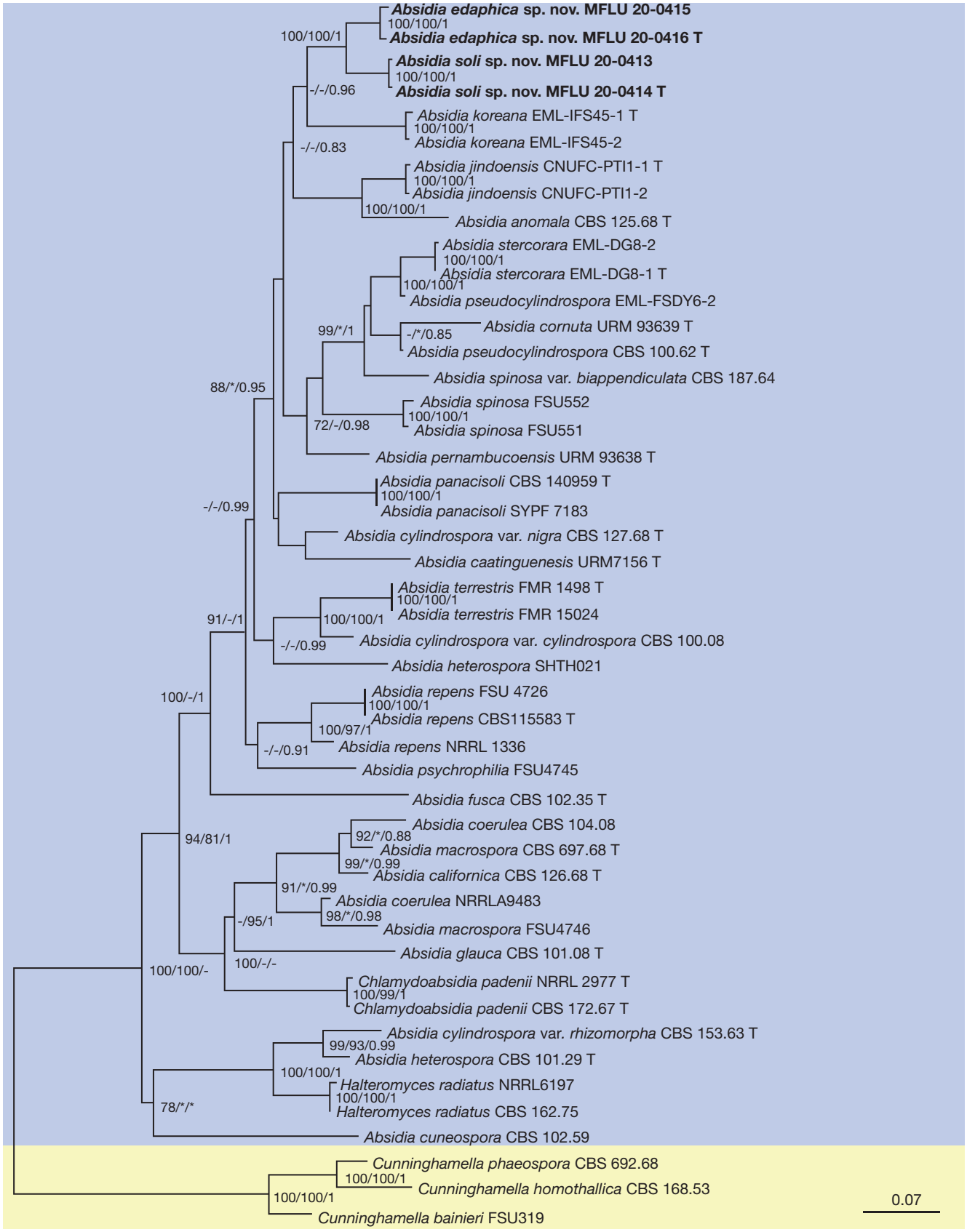


Fig. 1. — Maximum likelihood phylogram inferred from 47 taxa and 3907 characters based on ITS, LSU, SSU and ACT-1 matrix using GTR+G model. ML, MP bootstrap support (≥ 70%) and Bayesian posterior probability (≥ 0.70) are indicated above the branches or near the nodes as ML/MP/BYPP. Tree is artificially rooted using *Cunninghamella homothallica* Komin. & Tubaki (CBS 168.53), *C. phaeospora* Boedijn (CBS 692.68), and *C. bainieri* Naumov (FSU319). The new species are in **bold** and the type species in the dataset are indicated using T. (-) represent bootstrap support lower than 70% or for BYPP lower than 0.70. (*) indicates unrecovered branching.

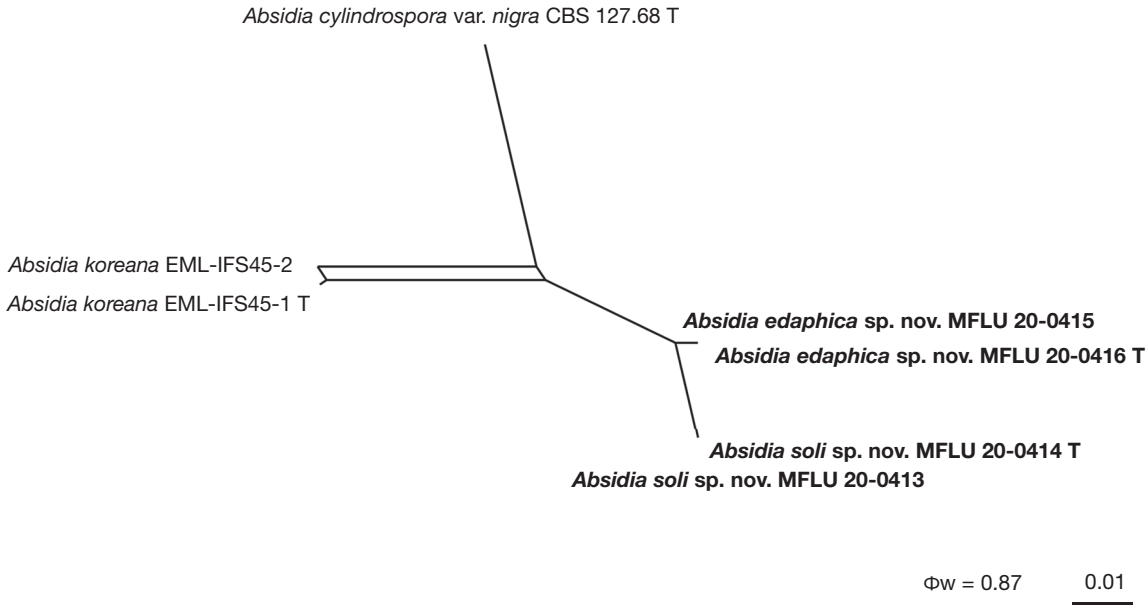


Fig. 2. — Pairwise homoplasy index (PHI) test for closely related species of *Absidia* Tiegh. using LogDet transformation and split decomposition. A PHI test result (Φw < 0.05) indicate significant recombination within the dataset.

and EML-IFS45-2) (Ariyawansa *et al.* 2015). When LSU, SSU and ACT-1 dataset (Appendix 3) was analyzed, *A. soli* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. and *A. edaphica* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. clustered together with high bootstrap support and *A. cornuta* D.X.Lima, C.A.Souza, H.B.Lee & A.L.Santiago groups in between our two new taxa (*A. soli* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. and *A. edaphica* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov.) with a well separated branch but without statistical support. However, *A. koreana* groups with *A. pseudocylindrospora* Hesselt. & J.J.Ellis, *A. stercoraria* Hyang B.Lee, H.S.Lee & T.T.T.Nguyen, *A. pernambucoensis* D.X.Lima, C.A.Souza, H.B.Lee & A.L.Santiago and *A. spinosa* var. *biappendiculata* Rall & Solheim with low bootstrap support rather than forming an independent clade (Appendix 3).

The genetic distance of the dataset (untrimmed/trimmed) of the new taxa and sister taxa was measured and listed in Table 4. The analysis using trimmed and untrimmed datasets gave the same results.

The PHI test gave a value of Φw = 1.0 indicating that there is no significant recombination between the new strains and their sister taxa (Fig. 2). This shows that these two species are different from each other and related species within *Absidia*.

The LSU alignment containing unclassified and environmental sequences consisted of 719 sites (Fig. 3). Of the 18 unclassified/environmental sequences, five grouped with known species of *Absidia*. The rest formed phylogenetically distinct clades, one of which is sister to the new species MFLU 20-0416 and MFLU 20-0415.

TAXONOMY

Family Cunninghamellaceae Naumov ex R.K.Benj.
Genus *Absidia* Tiegh.

Absidia edaphica V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov.

MycoBank number. — MB 836158.

Faces of fungi number. — FoF 07990.

Holotype. — MFLU 20-0416.

Etymology. — Referring to the substrate/host (soil) from which the species was first isolated.

Material examined. — **Thailand**. Chiang Mai Province, Mae Taeng District, Pa Pae sub district, Pha Deng village, isolated from soil, 11.VIII.2019, *Bhavesh Raghoonundon*, T19-0986 (holo-, MFLU [MFLU 20-0416]), ex-type living culture MFLUCC 20-0088, MFLUCC 20-0087.

Culture characteristics. — Colony exhibits high growth rate in YMA, PDA, and MEA (Figs 6; 7). Within two days, the colony attains a diameter of 55 mm (n = 10) at room temperature in PDA. Day-old cultures are white in all media changing to pale brown upon maturation (Fig. 7). Colours are changed from the center of the plate and progressing outwards. Initially, a pale brown colour is apparent only in PDA and YMA, as compared to MEA and CMA, where cultures are white or with a very light brown tint. Colony covers most of the surface of the agar plate within 4 days on PDA, MEA and YMA (when fungal plug is placed in the middle of the agar plate). Reverse of colony white and irregularly zonate. In YMA, the colony reverse gradually turns from white to pale yellow to pale brown. Specific to YMA, a distinct white zone is observed around

TABLE 4. — Genetic distance of the new taxa.

Species/ collection number	***Absidia edaphica* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. MFLU 20-0416**			
	ITS	LSU	SSU	ACT-1
A. soli sp. nov. MFLU 20-0414	11.2	4.7	0.2	4.3
A. koreana EML-IFS45-1	25.1	6.3	2.1	9.9
A. jindoensis CNUFC-PTI1-1	19.3	13.1	3.6	8.9
A. anomala CBS 125.68	23.6	14	-	-
A. cornuta URM 93639	36.6	6.1	-	-
	***Absidia soli* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. MFLU 20-0414**			
A. koreana EML-IFS45-1	25.7	5.4	2.1	11.7
A. jindoensis CNUFC-PTI1-1	21.6	11.1	3.6	8
A. anomala CBS 125.68	25.7	12.3	-	-
A. cornuta URM 93639	37.1	7.2	-	-

the colony with the zone being significantly larger at 20°C (Fig. 7). More concentric rings are observed in the colony reverse at 20°C than in 30°C in PDA. Colony is raised, undulate and can grow at 4, 20, 25 and 30°C. Growth at 30°C appears within the first 24 hours. No growth is observed at 37°C and 42°C, even after 7 days.

DESCRIPTION

Associated with soil.

Asexual morph

The measurements were taken from cultures grown in MEA.

Sporangiophore. Arised from the stolon singly or in whorls (1-6), are hyaline to pale brown, 2.5-5.4 µm wide and show occasional branching, erect. Septum consistently present below the apophysis, 2-4 µm long approximately 20-33.5 µm ($\overline{x}$ = 25.5 µm, n = 40), in length from the apophysate line.

Sporangium. Mature sporangia appear more quickly in PDA and YMA media, followed by MEA and CMA, are 30.5-35.5 × 24-27 µm ($\overline{x}$ = 33.5 × 25.5 µm, n = 30), globose to slightly elliptical. Immature sporangia beginning hyaline and later on turn to pale brown.

Columella. 5-9.5 × 6.5-20 µm ($\overline{x}$ = 7 × 12 µm, n = 40), globose, hyaline with apical projection and collarette as in all *Absidia* species (Fig. 4A-E, G, H).

Spores. Sporangiospores 3.5-5.5 × 2-3.5 µm ($\overline{x}$ = 4.5 × 2.5 µm, n = 30), numerous, short cylindrical to oblong, slightly curved at both end, hyaline, smooth-walled. Azygospores and zygospores not observed. Chlamydospores absent.

Rhizoids. Pale brown, aseptate and taper at the ends (Fig. 4F).

Sexual morph

Undetermined.

NOTES

Absidia edaphica V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. differs from *A. koreana* phylogenetically and morphologically. *Absidia edaphica* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. produces larger sporangia and columellae with the colony attaining a diameter 55 mm in PDA within two days but *A. koreana* reaches 39.5-41 mm by the fourth day at 25°C. Compared to *A. soli* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov., the sporangia and columella are smaller. The length from the apophysate line to the septum is shorter than *A. soli* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde sp. nov. (20-33.5 µm). Phylogenetic analysis provides strong statistical support to establish two different species (Figs 1; 3). This finding is also supported by GCPSR analyses where no significant recombination could be detected. The genetic distance between *A. edaphica* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. and *A. koreana* in the ITS regions is 25.1%, LSU 6.3% and SSU 2.1%. Compared to *A. soli* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov., genetic distance in the ITS regions was of 11.2%, LSU 4.7%, SSU region 0.2%/0.2% and in the ACT-1 gene region 4.3% (Table 4).

Absidia soli V.G.Hurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov.

MYCOBANK NUMBER. — MB 836159.

FACESOFFFUNGI NUMBER. — FoF 07991.

HOLOTYPE. — MFLU 20-0414.

ETYMOLOGY. — Referring to the substrate/host (soil) from which the species was first isolated.

MATERIAL EXAMINED. — **Thailand**. Chiang Mai Province, Mae Taeng District, Pa Pae sub district, Pha Deng village, isolated from soil, 11.VIII.2019, *Bhavesh Raghoonundon*, T19-0982 (holo-, MFLU [MFLU 20-0414]), ex-type living cultures MFLUCC 20-0089, MFLUCC 20-0086.

CULTURE CHARACTERISTICS. — Colony growth is high in YMA, PDA and MEA (Figs 6; 7). Young cultures are white gradually changing to pale greyish and grey brown/black (Fig. 7). Sporulation is evident by the second day, apparent by the pale greyish brown colour of the culture. At this stage, single sporangiophores are prominent. As colony matures, colour changes from the center to the extremities and there is usually a white margin at the outer border of the culture. Growth occurs both vertically and laterally, with culture mycelium reaching the top of the agar plate by day 3 or 4. At 30°C, colour development to greyish-black is faster, with a wider white outer margin due to more mature sporangia and pigmented hyphae,

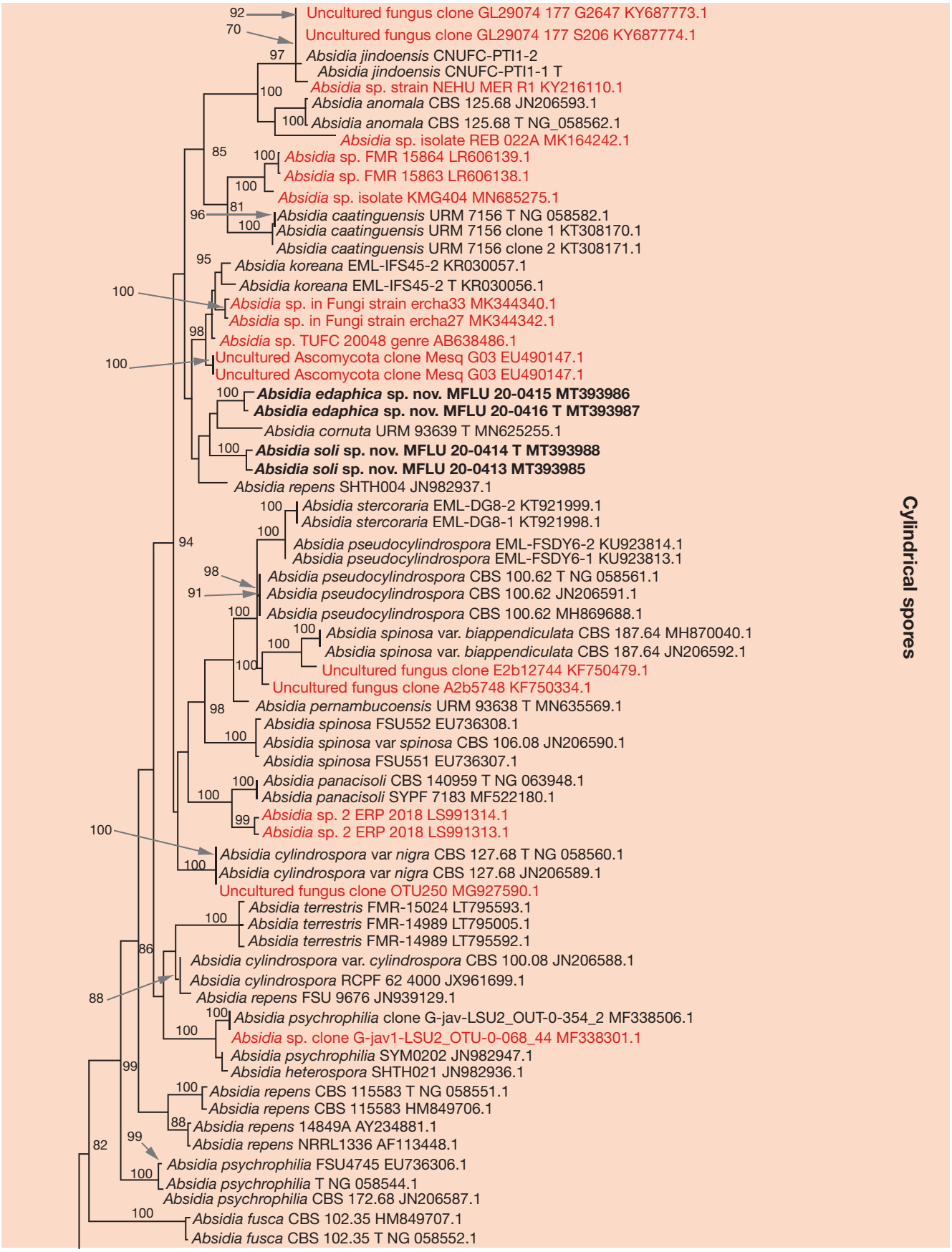
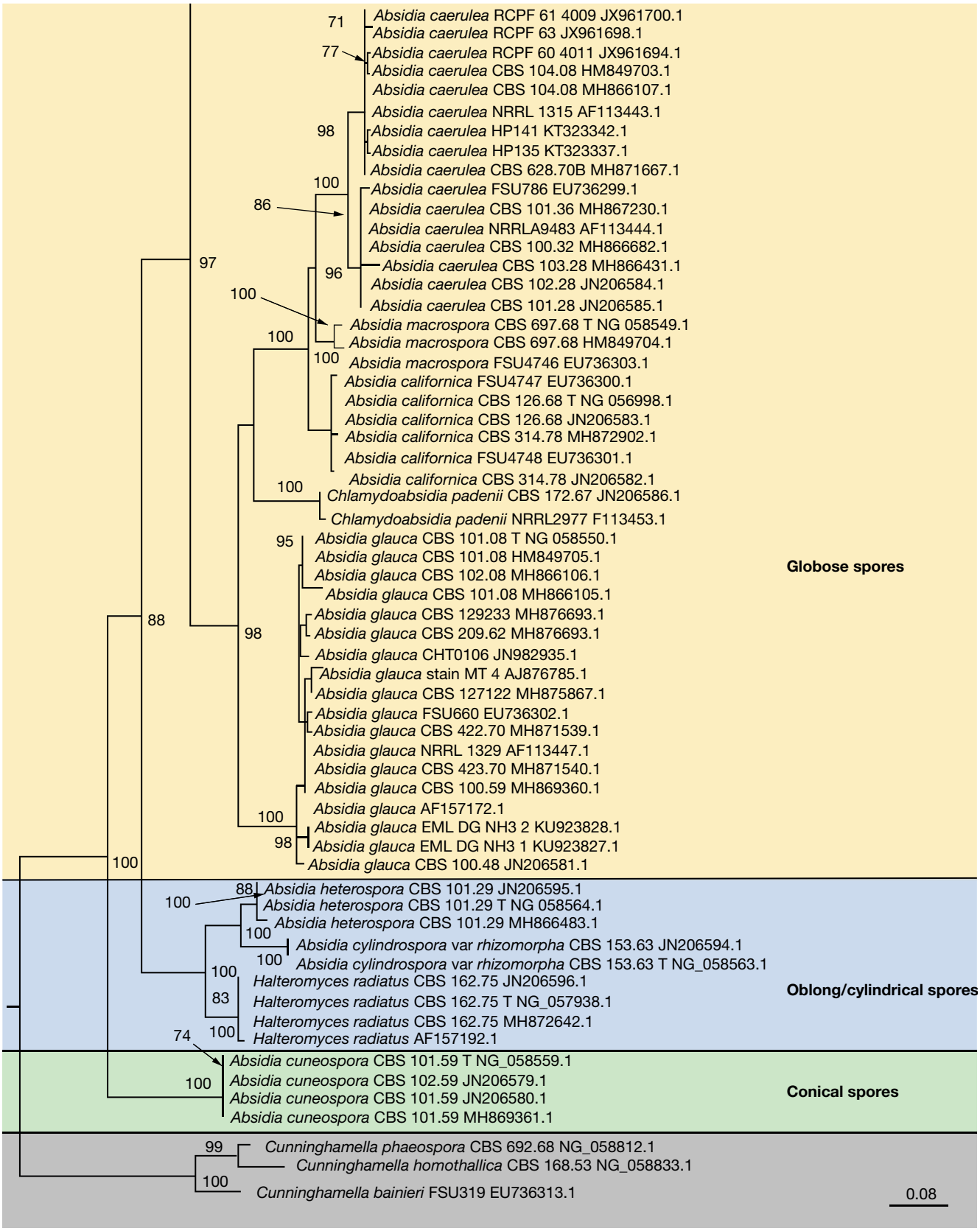


FIG. 3. — Maximum likelihood tree based on LSU data. The tree is inferred from 129 taxa consisting of 719 characters and using GTR+G model. ML bootstrap support (≥70%) are indicated above the branches or near the nodes. Tree is rooted using *Cunninghamella homothallica* Komin. & Tubaki (CBS 168.53),



C. phaeospora Boedijn (CBS 692.68), and *C. bainieri* Naumov (FSU319). Environmental samples and unclassified *Absidia* are marked in **red**. Type species are indicated using T.

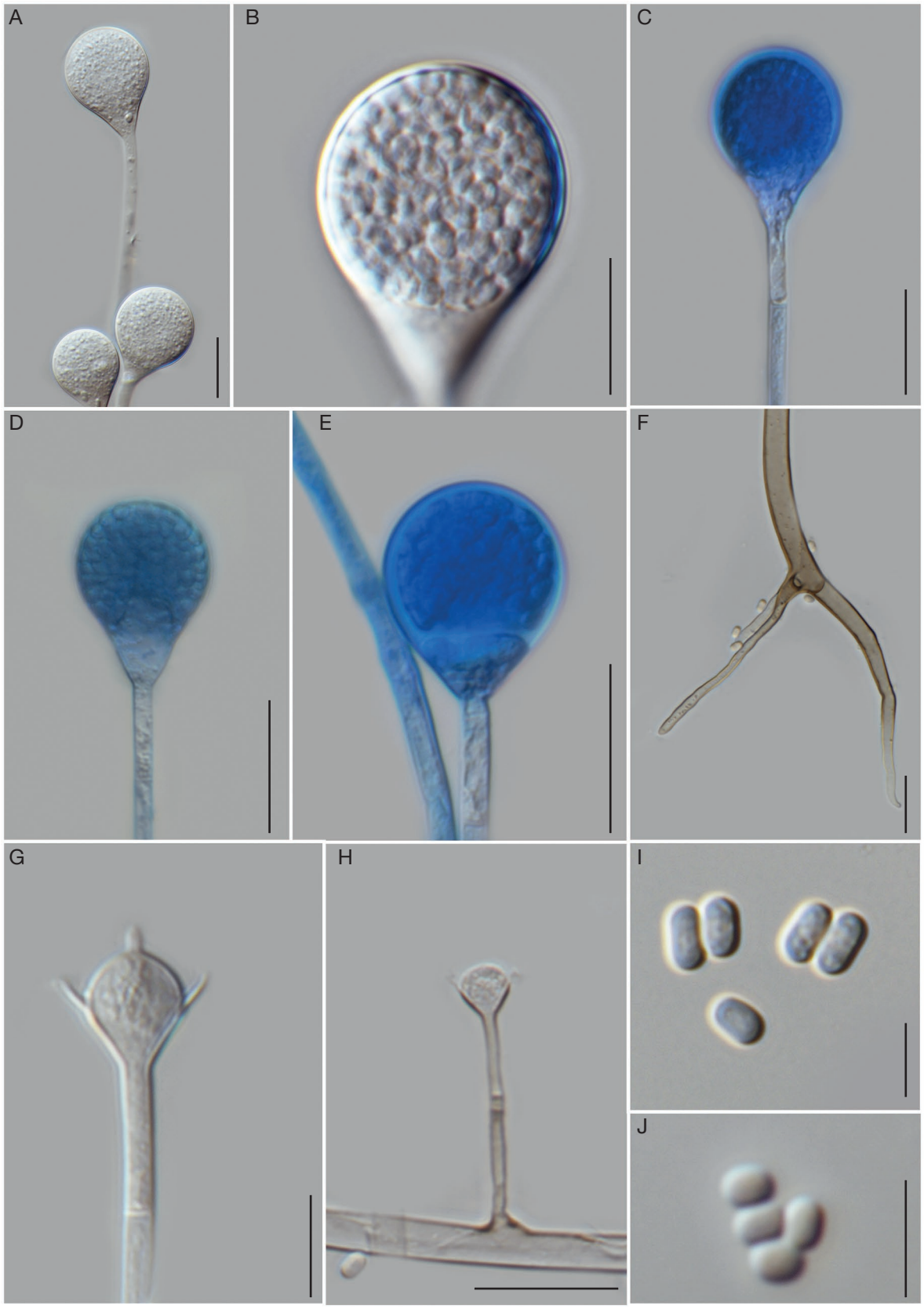


FIG. 4. — *Absidia edaphica* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. (MFLU 20-0416, holotype) (Cultures grown at room temperature for 3-5 days): **A**-**E**, different stages of sporangia development (**C**-**E**, sporangium stained with Lacto-phenol cotton blue reagent); **F**, rhizoids; **G**, columella with collarette, apical projection and septum below the apophysis; **H**, single sporangiophore originating from the stolon; **I**, sporangiospores in CMA; **J**, sporangiospores produced in MEA. Scale bars: A-F, H, 20 µm; G, 10 µm; I-J, 5 µm.

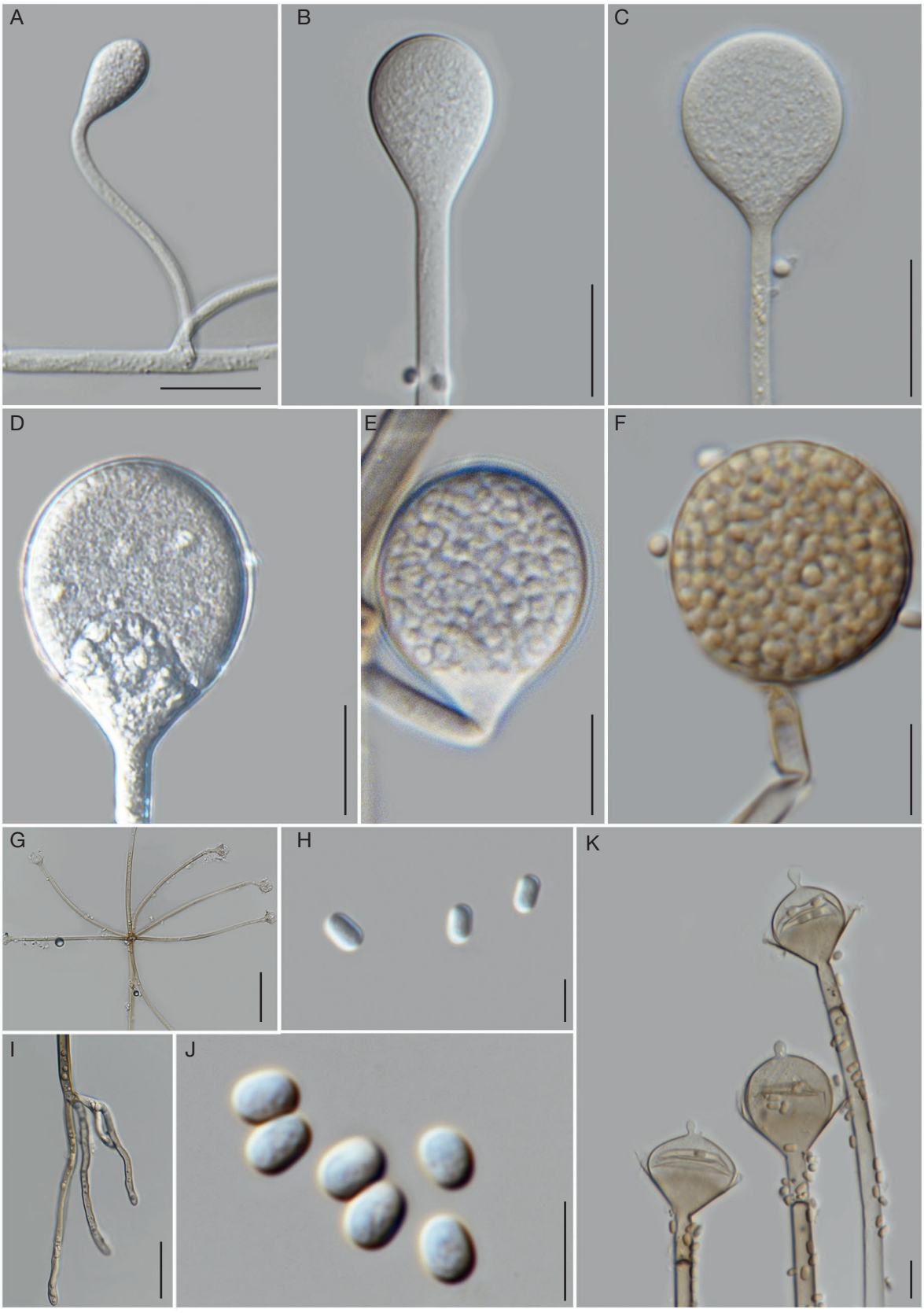


FIG. 5. — *Absidia soli* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. (MFLU 20-0414, holotype) (Cultures grown at room temperature for 3-5 days): **A-F**, different stages in development of sporangium; **D**, sporangium mounted with 5% potassium hydroxide; **F**, mature sporangium; **G**, branching of sporangiophores from a single point of the stolon (whorls); **H**, sporangiospores produced when cultured in PDA; **I**, rhizoids; **J**, sporangiospores from culture grown in MEA; **K**, columella with apical projection, collarette and septum below the apophysis. Scale bars: A-D, I, 20 µm; E-F, K, 10 µm; H, J, 5 µm; K, 60 µm.

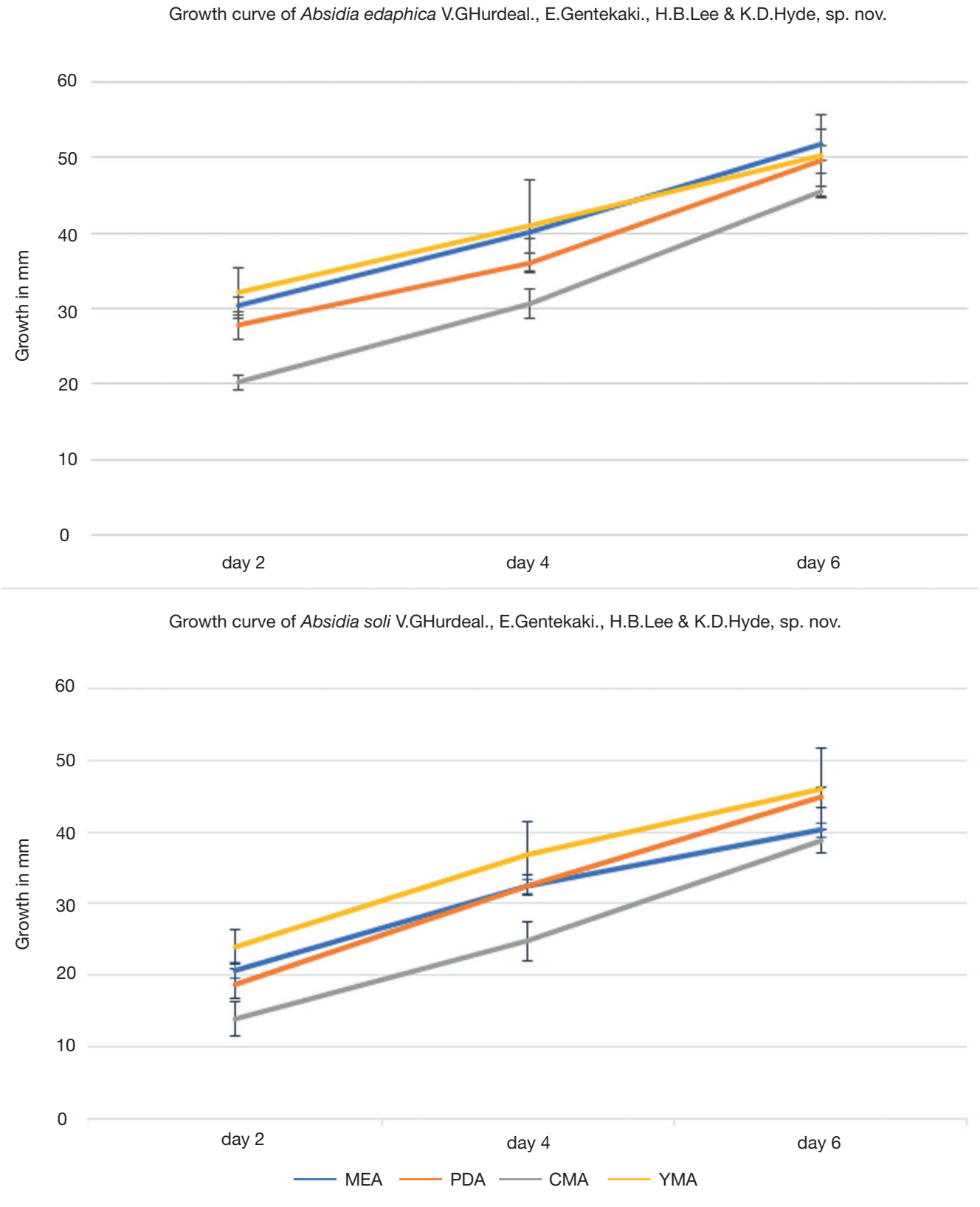


Fig. 6. — Mycelial growth of *Absidia edaphica* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. and *Absidia soli* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. in different media at 25°C.

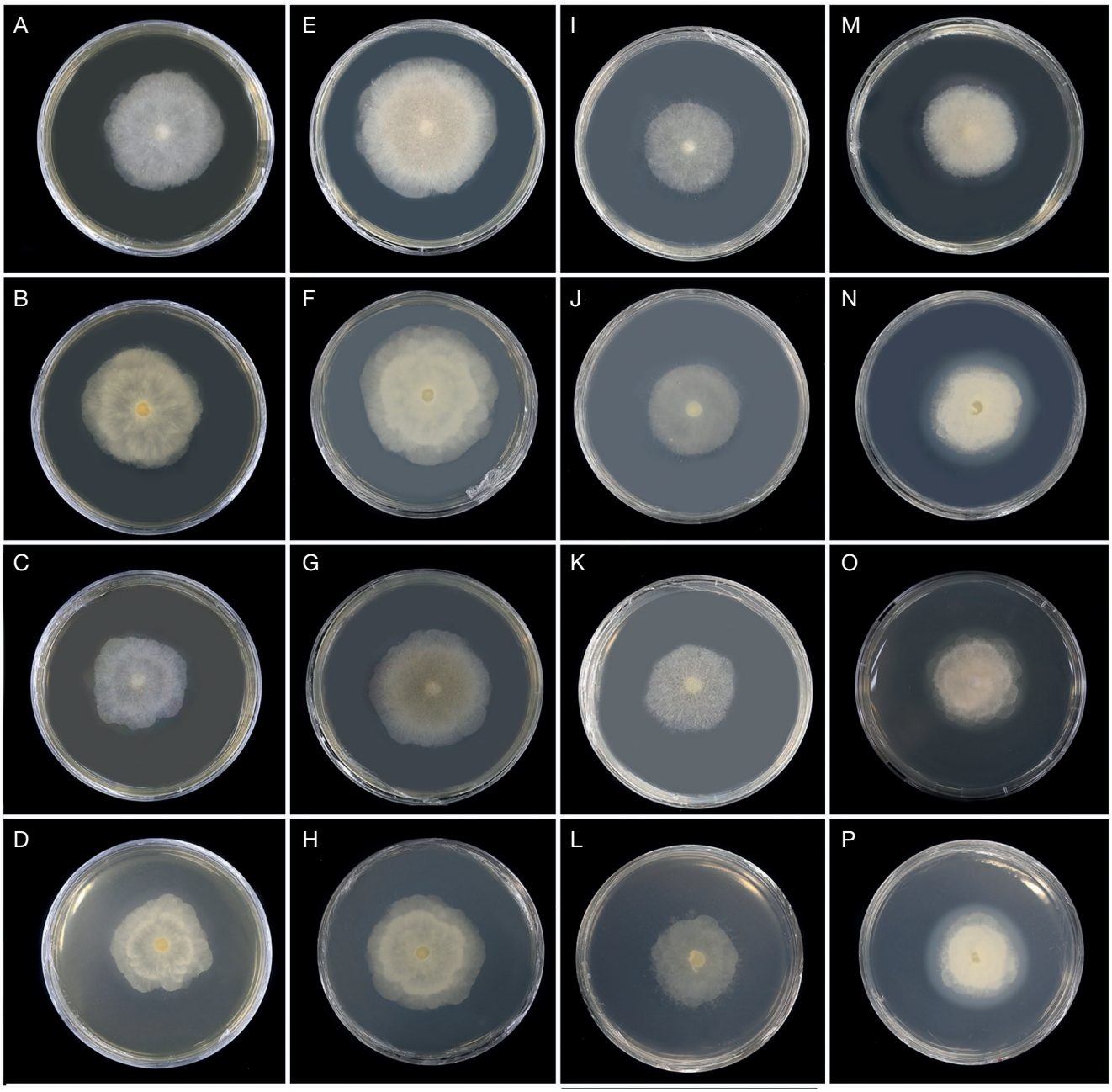


FIG. 7. — Mycelial growth of *A. edaphica* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. (MFLUCC 20-0088, ex-type) and *A. soli* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. (MFLUCC 20-0086, ex-type) in various media at room temperature (around 26°C to 27°C) after two days: **A-D**, colonies on MEA; **E-H**, colonies on PDA; **I-L**, colonies on CMA; **M-P**, colonies on YMA. The first two rows represent colonies of of *A. edaphica* sp. nov. and the bottom two rows *A. soli* sp. nov (obverse (first and third rows) and reverse (second and fourth rows).

than at 20°C. Colony reverse white and irregularly zonate. Most concentric rings observed in the colony reverse at 30°C. In YMA, colony forms a distinct white zone around the colony. Reverse of colony white with irregular zonation. The fungi grew at 4, 20, 30°C but not at 37 and 42°C.

DESCRIPTION

Associated with soil.

Asexual morph

The measurements were taken from cultures grown in MEA.

Sporangiophores. Arise singly or in whorls (1-6) are initially hyaline and turn into brown as the culture matures measuring 2.5-5.5 µm wide. Septum present at the base of the sporangium below the apophysis and measures 21.5-37.5 µm ($\overline{x}$ = 28.5 µm, n = 40) below the apophysate line (Fig. 5).

Sporangia. 16-51 × 15-45.5 µm ($\overline{x}$ = 34.5 × 30 µm, n = 30), mature sporangia brown, globose to slightly ellipsoidal.

Columellae. 7.5-12.5 × 9-24 µm ($\overline{x}$ = 10 × 15.5 µm, n = 40), globose, hyaline to pale brown, bearing collarette and apical projection.

Spores. Sporangiospores 3-4.5 × 1.5-2.5 µm ($\overline{x}$ = 4 × 2.5 µm, n = 30), numerous, short cylindrical to oblong, slightly curved at both end, hyaline, non-striated and smooth walled. Azygospores and zygospores not observed. Chlamydospores absent.

Rhizoids. Pale brown, aseptate and taper at the ends (Fig. 5I).

Sexual morph
Undetermined.

NOTES
Absidia soli V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. produces larger sporangia and columella than *A. koreana* and *A. edaphica* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. The length of the septum below the apophysate line is longer than *A. edaphica* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. (21.5- 37.5 µm). The genetic distances of *A. koreana* and *A. cornuta* to *A. edaphica* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. are provided in Table 4.

MYCELIAL GROWTH
Mycelial growths of both species were tested and measured on PDA, MEA, YMA and CMA at 25°C (Fig. 6). *Absidia edaphica* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. shows high growth in all media used. In YMA, the margin of the colony is irregular with part of the colony growing faster. This resulted in high standard deviation (Fig. 6). In YMA and PDA media, *A. soli* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. exhibited a mycelial growth of 46 mm and 45 mm respectively within the first six days and *A. edaphica* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. 50 mm. When the limits of temperature of the new taxa were tested, both of them could grow at a low temperature of 4°C, but no growth was detected for temperatures above 37°C.

DISCUSSION

Absidia edaphica V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. and *A. soli* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. are introduced as new species. Based on multi-gene analysis, the species are sister taxa and cluster with *A. koreana* (Ariyawansa *et al.* 2015). The two new taxa are different by having different size of sporangium and columella, phylogenetic and genetic distance analyses differences. In phylogenetic analysis, the two new species form two different clades with high bootstrap support and have genetic distance differences in the ITS, LSU, SSU, and ACT-1 gene regions (Fig. 1 and Table 4). These differences support the establishment of new taxa according to proposed guidelines (Jeewon & Hyde 2016). Additional analysis using GCPSR further supported this finding by indicating no significant recombination between the novel species or other closely related species. However, it is known that this method is sensitive to taxon sampling. Hence, when using GCPSR, taxon sampling should represent the breadth of phylogenetic diversity (Laurence *et al.* 2014). In this study, only two strains of the new taxa were isolated and nine sequences were used for the analysis. Due to the low taxon sampling, a concrete conclusion cannot be made based solely on GCPSR analysis.

Hoffmann *et al.* (2007) proposed three groups of *Absidia* according to spore shape. These groups also constitute phylogenetically distinct clades (Fig. 3): 1) the globose-shaped spore clade included *A. caerulea* Bainier, *A. glauca* Hagem, *A. macrospora* Váňová and *A. californica* J.J.Ellis & Hesselt.; 2) the cylindrical-spored clade included *A. caatinguensis* D.X.Lima & A.L.Santiago, *A. anomala* Hesselt. & J.J.Ellis, *A. koreana*, *A. repens*, *A. stercoraria*, *A. pseudocylindrospora*, *A. panacisoli* T.Yuan Zhang, Ying Yu, He Zhu, S.Z.Yang, T.M.Yang, MengY. Zhang & Yi X. Zhang, *A. cylindrospora*, *A. psychrophilia* Hesselt. & J.J. Ellis, *A. terrestris* Rosas de Paz, Dania García, Guarro, Cano & Stchigel, *A. heterospora* Y. Ling and *A. fusca* Linnem.; and 3) the conical spored clade included only *A. cuneospora* G.F.Orr & Plunkett (Fig. 3). In this study, all three clades were recovered. The two new species both of which produce short cylindrical spores indeed fall within the cylindrical spore forming clade (Hoffmann *et al.* 2007). However, a fourth clade, including *A. heterospora*, *A. cylindrospora* var. *rhizomorpha* Hesselt. & J.J.Ellis and *Halteromyces* Shipton & Schipper was also recovered. *Halteromyces* produces oblong/cylindrical spores and *A. heterospora* and *A. cylindrospora* var. *rhizomorpha* produce cylindrical spores. Molecular data for these taxa were not available when spore shape was proposed as a phylogenetically significant character. This indicates the need for additional studies which takes into account all described *Absidia* species.

In our phylogenetic analyses using all datasets, *Absidia* is not monophyletic. *Chlamydoabsidia* and *Halteromyces* nest within *Absidia sensu stricto* in agreement with previous studies (Fig. 1) (Hoffmann *et al.* 2007; Hoffmann 2010; Walther *et al.* 2013, 2019). Specifically, *Chlamydoabsidia* clustered together with *A. glauca*, while *Halteromyces* formed an independent clade.

Both *A. soli* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. and *A. edaphica* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. have been isolated from forest soil and are saprobic in nature. Many *Absidia* inhabit soil but are not limited to it (Table 1) (Hoffmann *et al.* 2007; Hoffmann 2010). Species of *Absidia* have been isolated from various healthy parts of several plants (*Phragmites australis* (Cav.) Trin. ex Steud., and *Avicennia officinalis* L.) denoting them as endophytic (Mishra *et al.* 2019). *Absidia* have also been found in insects and mammals. For other Mucorales taxa, pivotal data to accurately infer the ecological niche or geographic distribution of *Absidia* is lacking (Walther *et al.* 2019). Compiled data from GenBank indicate that *Absidia* has a worldwide distribution having been obtained from Europe, Americas, Africa and Asia, in tropical, subtropical temperate and subarctic countries (Table 1).

Currently 28 species of *Absidia* have been reported according to Species Fungorum (2020). We constructed a maximum likelihood tree incorporating all known species with LSU molecular data along with 11 unclassified *Absidia* and seven environmental sequences. Of the 18 unclassified/environmental sequences, only five grouped with known *Absidia* species. The rest formed a minimum of nine clades. The genetic distances between these clades and their nearest sister taxa were also measured in the trimmed LSU region (Appendix 2). Results from the phylogenetic tree (Fig. 3) and genetic distance of 2-10% (Appendix 2) may indicate potential new species but more concrete evidence is needed. *Absidia cornuta* groups between *A. edaphica* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. and *A. soli* V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. similar to the phylogenetic tree without ITS (Fig. 3). The genetic distance between the two new taxa introduced to *A. cornuta* is 6.1% and 7.2% in the trimmed LSU gene region and 36.6% and 37.1% in the ITS region respectively. With the inclusion of one more taxon, the establishment of the new species is reinforced. Collectively, these findings reveal high diversity of *Absidia* that has yet to be officially documented. In many biodiversity studies using next generation sequencing, *Absidia* is often not detected or incorrectly identified (Zhu *et al.* 2018; Frickmann *et al.* 2019). This could be due to preferential primer affinities to only certain microorganisms, which are known issues in amplification-based diagnostic approaches especially with ribosomal genes (Jeewon *et al.* 2017, 2018; Frickmann *et al.* 2019). Designing specific primers for non-Dikarya taxa might be an option to overcome these challenges.

The ITS, SSU, LSU, and ACT-1 genes are used to infer phylogeny of *Absidia*. Use of the ITS region, the universal DNA barcode marker for fungi has been debated in the case of *Absidia*. Previous research suggests a highly polymorphic ITS region and its possible unsuitability for use in single gene phylogenetic analysis (Zhang *et al.* 2018). Herein, the topology of trees with and without the ITS differed, indicating that further work is needed to investigate ITS as a reliable taxonomic marker for *Absidia*.

Herein, we provide insight into the taxonomy of *Absidia* and phylogenetic diversity of the genus. At the same time, we have outlined some problematic issues. Future investigations should focus on documenting diversity of this understudied genus.

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APPENDICES

Appendix 1. — Data used for phylogenetic analysis of the LSU gene and their corresponding GenBank accession numbers.

Species Name	CODE	GenBank Accession number
Absidia anomala	CBS 125.68	JN206593.1
Absidia anomala	CBS 125.68 T	NG_058562.1
Absidia caatinguensis	URM 7156 T	NG_058582.1
Absidia caatinguensis	URM 7156 clone 2	KT308171.1
Absidia caatinguensis	URM 7156 clone 1	KT308170.1
Absidia caerulea	CBS 628.70B	MH871667.1
Absidia caerulea	CBS 101.36	MH867230.1
Absidia caerulea	CBS 100.32	MH866682.1
Absidia caerulea	CBS 103.28	MH866431.1
Absidia caerulea	CBS 104.08	MH866107.1
Absidia caerulea	RCPF 61/4009	JX961700.1
Absidia caerulea	RCPF 63	JX961698.1
Absidia caerulea	RCPF 60/4011	JX961694.1
Absidia caerulea	HP141	KT323342.1
Absidia caerulea	HP135	KT323337.1
Absidia caerulea	CBS 101.28	JN206585.1
Absidia caerulea	CBS 102.28	JN206584.1
Absidia caerulea	CBS 104.08	HM849703.1
Absidia caerulea	FSU786	EU736299.1
Absidia caerulea	NRRLA9483	AF113444.1
Absidia caerulea	NRRL1315	AF113443.1
Absidia californica	CBS 314.78	MH872902.1
Absidia californica	CBS 126.68 T	NG_056998.1
Absidia californica	CBS 126.68	JN206583.1
Absidia californica	CBS 314.78	JN206582.1
Absidia californica	FSU4748	EU736301.1
Absidia californica	FSU4747	EU736300.1
Absidia cuneospora	CBS 101.59	MH869361.1
Absidia cuneospora	CBS 102.59	JN206579.1
Absidia cuneospora	CBS 101.59 T	NG_058559.1
Absidia cuneospora	CBS 101.59	JN206580.1
Absidia cylindrospora var. *rhizomorpha*	CBS 153.63 T	NG_058563.1
Absidia cylindrospora var. *rhizomorpha*	CBS 153.63	JN206594.1
Absidia cylindrospora	RCPF 62/4000	JX961699.1
bsidia cylindrospora var. *nigra*	CBS 127.68	JN206589.1
bsidia cylindrospora var. *nigra*	CBS 127.68 T	NG_058560.1
Absidia cylindrospora var. cylindrospora	CBS 100.08	JN206588.1
Absidia fusca	CBS 102.35 T	NG_058552.1
Absidia fusca	CBS 102.35	HM849707.1
Absidia glauca	CBS 129233	MH876693.1
Absidia glauca	CBS 127122	MH875867.1
Absidia glauca	CBS 423.70	MH871540.1
Absidia glauca	CBS 422.70	MH871539.1
Absidia glauca	CBS 209.62	MH869730.1
Absidia glauca	CBS 100.59	MH869360.1
Absidia glauca	CBS 102.08	MH866106.1
Absidia glauca	CBS 101.08	MH866105.1
Absidia glauca	FSU660	EU736302.1
Absidia glauca	CBS 101.08 T	NG_058550.1
Absidia glauca	EML-DG-NH3-2	KU923828.1
Absidia glauca	EML-DG-NH3-1	KU923827.1
Absidia glauca	CBS 100.48	JN206581.1
Absidia glauca	CHT0106	JN982935.1
Absidia glauca	CBS 101.08	HM849705.1
Absidia glauca		AF157172.1
Absidia glauca	strain MT 4	AJ876785.1
Absidia glauca	NRRL1329	AF113447.1
Absidia heterospora	CBS 101.29	MH866483.1
Absidia heterospora	CBS 101.29 T	NG_058564.1
Absidia heterospora	CBS 101.29	JN206595.1
Absidia heterospora	SHTH021	JN982936.1
Absidia koreana	EML-IFS45-2	KR030057.1
Absidia koreana	EML-IFS45-1	KR030056.1
Absidia macrospora	CBS 697.68 T	NG_058549.1
Absidia macrospora	CBS 697.68	HM849704.1
Absidia macrospora	FSU4746	EU736303.1
Absidia panacisoli	CBS 140959 T	NG_063948.1

Appendix 1. — Continuation.

Species Name	CODE	GenBank Accession number
Absidia panacisoli	SYPF 7183	MF522180.1
Absidia pseudocylindrospora	CBS 100.62	MH869688.1
Absidia pseudocylindrospora	CBS 100.62 T	NG_058561.1
Absidia pseudocylindrospora	EML-FSDY6-2	KU923814.1
Absidia pseudocylindrospora	EML-FSDY6-1	KU923813.1
Absidia pseudocylindrospora	CBS 100.62	JN206591.1
Absidia psychrophilia	clone G-jav1-LSU2_ OTU-0-354_2	MF338506.1
Absidia psychrophilia	TYPE material	NG_058544.1
Absidia psychrophilia	CBS 172.68	JN206587.1
Absidia psychrophilia	SYM0202	JN982947.1
Absidia psychrophilia	FSU4745	EU736306.1
Absidia repens	CBS 115583 T	NG_058551.1
Absidia repens	SHTH004	JN982937.1
Absidia repens	FSU 9676	JN939129.1
Absidia repens	14849A	AY234881.1
Absidia repens	CBS 115583	HM849706.1
Absidia repens	NRRL1336	AF113448.1
Absidia spinosa var. *biappendiculata*	CBS 187.64	MH870040.1
Absidia spinosa var. *biappendiculata*	CBS 187.64	JN206592.1
Absidia spinosa var. *spinosa*	CBS 106.08	JN206590.1
Absidia spinosa	FSU552	EU736308.1
Absidia spinosa	FSU551	EU736307.1
Absidia stercoraria	EML-DG8-2	KT921999.1
Absidia stercoraria	EML-DG8-1	KT921998.1
Absidia sp. 2	ERP-2018	LS991314.1
Absidia sp. 2 ERP-2018	ERP-2018	LS991313.1
Absidia sp.	RVO_2019 culture URM_BRA_7219	MN635569.1
Absidia sp.	RVO-2019	MN625255.1
Absidia sp.	isolate KMG404	MN685275.1
Absidia sp.	clone G-jav1-LSU2_ OTU-0-068 44	MF338301.1
Absidia sp.	FMR:15864	LR606139.1
Absidia sp.	FMR:15863	LR606138.1
Absidia sp.	isolate REB-022A	MK164242.1
Absidia terrestris	FMR:15024	LT795593.1
Absidia terrestris	FMR:14989	LT795592.1
Absidia terrestris	FMR:14989	LT795005.1
Absidia sp. (in: Fungi)	strain ercha27	MK344342.1
Absidia sp. (in: Fungi)	strain ercha33	MK344340.1
Absidia sp.	strain NEHU.MER-R1	KY216110.1
Absidia jindoensis	isolate CNUFC-PTI1-2	MF926617.1
Absidia jindoensis	isolate CNUFC-PTI1-1	MF926616.1
Absidia sp. FS-35		LC026071.1
Absidia sp. BAB-88		KF946131.1
Absidia sp. CL098		AY234876.1
Absidia sp. SW097		AY234875.1
Absidia sp. CL143		AY234874.1
Absidia sp. TUFC 20048 gene		AB638486.1
Cunninghamella phaeospora	CBS 692.68	NG_058812.1
Cunninghamella bainieri	FSU319	EU736313.1
Cunninghamella homothallica	CBS 168.53	NG_058833.1
Halteromyces radiatus	CBS 162.75	MH872642.1
Halteromyces radiatus	CBS 162.75	JN206596.1
Halteromyces radiatus		AF157192.1
Halteromyces radiatus	CBS 162.75 T	NG_057938.1
Chlamydoabsidia padenii	CBS 172.67	JN206586.1
Chlamydoabsidia padenii	NRRL297	F113453.1
Uncultured fungus	clone A2b5748	KF750334.1
Uncultured fungus	clone E2b12744	KF750479.1
Uncultured *Ascomycota*	clone Mesq_G03	EU490147.1
Uncultured fungus	clone GL29074_177_ S206	KY687774.1
Uncultured fungus	clone GL29074_177_ G2647	KY687773.1
Uncultured fungus	clone_OTU250	MG927590.1
Uncultured *Ascomycota*	clone Mesq_G03	EU490147.1

APPENDIX 2. — Genetic distance in the LSU gene region.

Absidia anomala CBS 125.68 JN206593						
Absidia anomala CBS 125.68 T NG_058562	0.000					
Absidia sp. strain NEHU MER-R1 KY216110	0.072	0.072				
Absidia jindoensis CNUFC-PTI1-2 MF926617	0.083	0.083	0.009			
Absidia jindoensis T CNUFC-PTI1-1 MF926616	0.093	0.093	0.009	0.005		
Uncultured fungus clone GL29074-177 S206 KY687774	0.083	0.083	0.009	0.000	0.005	
Uncultured fungus clone GL29074-177 G2647 KY687773	0.083	0.083	0.009	0.000	0.005	0.000

Absidia sp. FMR-15864 LR606139 580 bp					
Absidia sp. FMR-15863 LR606138 580 bp	0.000				
Absidia caatinguensis URM 7156 T NG_058582 580 bp	0.101	0.101			
Absidia caatinguensis URM 7156 clone 2 KT308171 580 bp	0.098	0.098	0.002		
Absidia caatinguensis URM 7156 clone 1 KT308170 580 bp	0.101	0.101	0.000	0.002	
Absidia sp. isolate KMG404 MN685275 580 bp	0.027	0.027	0.096	0.094	0.096

Absidia koreana EML-IFS45-2 640 bp						
Absidia koreana EML-IFS45-1 640 bp	0.016					
Absidia sp. in Fungi strain ercha27 MK344342 640 bp	0.024	0.019				
Absidia sp. in Fungi strain ercha33 MK344340 640 bp	0.024	0.019	0.000			
Absidia sp. TUFC-20048-gene AB638486 640 bp	0.026	0.024	0.018	0.018		
Uncultured *Ascomycota* clone-Mesq-G03 EU490147 640 bp	0.031	0.029	0.026	0.026	0.021	
Uncultured *Ascomycota* clone-Mesq-G03 EU490147 640 bp	0.031	0.029	0.026	0.026	0.021	0.000

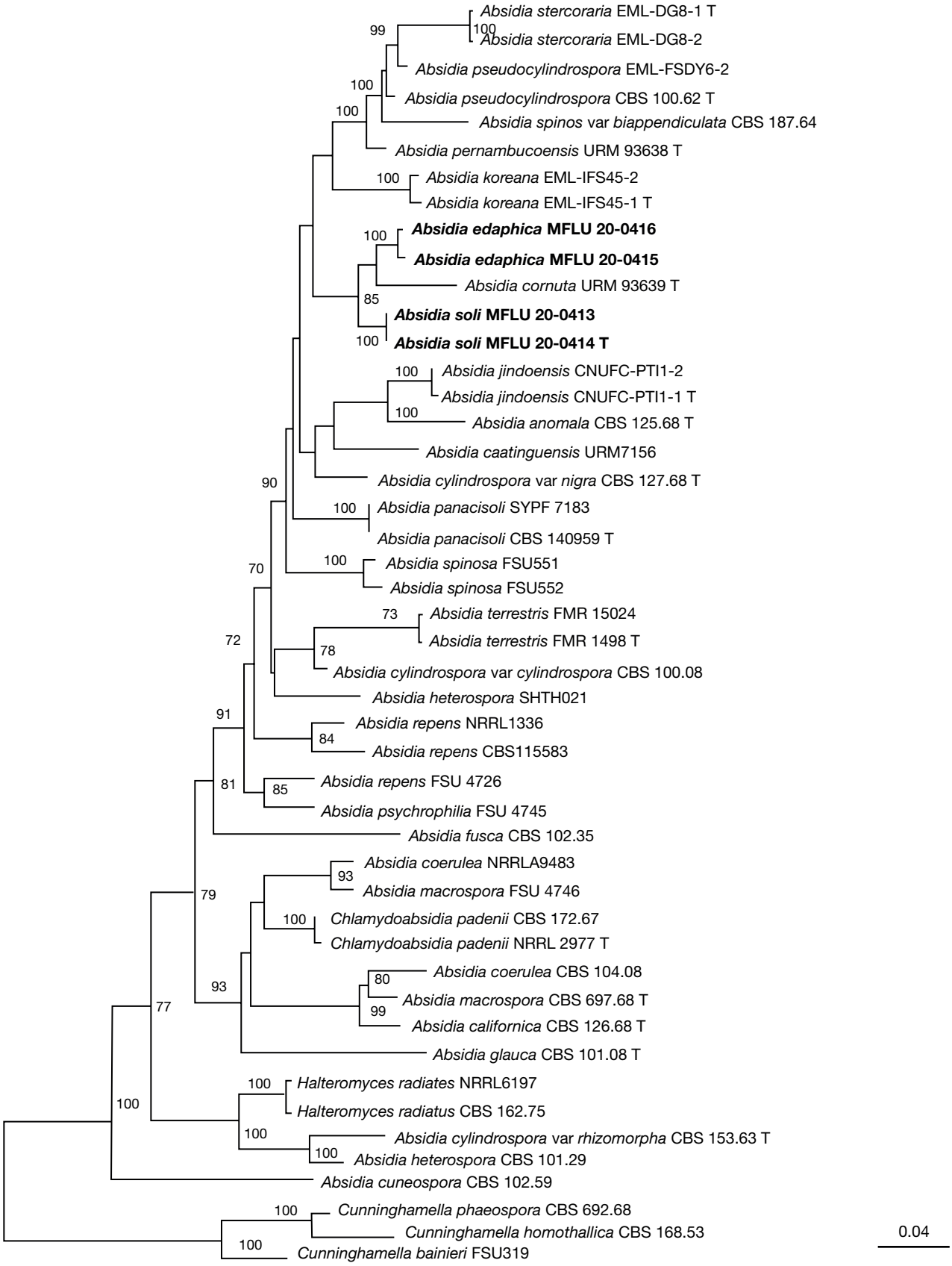
Absidia spinosa var. *biappendiculata* CBS 187.64 MH870040 699 bp					
Absidia spinosa var. *biappendiculata* CBS 187.64 JN206592 699 bp	0.000				
Uncultured fungus clone A2b5748 KF750334 699 bp	0.066	0.048			
Uncultured fungus clone E2b12744 KF750479 699 bp	0.034	0.020	0.060		
Absidia sp. RVO 2019 culture-URM-BRA 7219 MN635569 699 bp	0.076	0.059	0.056	0.078	

Absidia panacisoli CBS 140959 T NG_063948 671 bp				
Absidia panacisoli SYPF 7183 MF522180 671 bp	0.000			
Absidia sp. 2 ERP 2018 LS991314 671 bp	0.051	0.051		
Absidia sp. 2 ERP 2018 LS991313 671 bp	0.055	0.055	0.009	

Absidia sp. RVO-2019 MN625255 630 bp					
Absidia edaphica V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. MFLU 20-0416 630 bp	0.080				
Absidia soli V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. MFLU 20-0413 630 bp	0.109	0.071			
Absidia edaphica V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. MFLU 20-0415 630 bp	0.070	0.010	0.058		
Absidia soli V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. MFLU 20-0414 630 bp	0.109	0.071	0.000	0.058	

APPENDIX 2 (continuation). — Genetic distance in the ITS gene region of the two new species to *Absidia* sp. RVO 2019 MN625255.1.

Absidia sp. RVO-2019 MN625255 630 bp				
Absidia edaphica V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. MFLU 20-0416 630 bp	0.080			
Absidia soli V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. MFLU 20-0413 630 bp	0.109	0.071		
Absidia edaphica V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. MFLU 20-0415 630 bp	0.070	0.010	0.058	
Absidia soli V.GHurdeal., E.Gentekaki., H.B.Lee & K.D.Hyde, sp. nov. MFLU 20-0414 630 bp	0.109	0.071	0.000	0.058



Appendix 3. — Maximum likelihood phylogram inferred from 47 taxa and 3314 characters based on LSU, SSU and ACT-1 matrix using GTR+G model. ML bootstrap support (≥ 70%) are indicated above the branches or near the nodes. Tree is artificially rooted using *Cunninghamella homothallica* (CBS 168.53), *C. phaeospora* (CBS 692.68), and *C. bainieri* (FSU319). The new species are in black bold and the type species in the dataset are indicated using T. (-) represent bootstrap support lower than 70%. (*) indicates unrecovered branching.