Mycoscience VOL.62 (2021) 331–335

Mycoscience

AN OPEN ACCESS JOURNAL FOR GENERAL MYCOLOGY

The Mycological Society of Japan

Short communication

Absidia healeyae: a new species of *Absidia* (*Mucorales*) isolated from Victoria, Australia

Andrew S. Urquhart[a], Alexander Idnurm[a,*]

[a] *School of BioSciences, the University of Melbourne, VIC 3010, Australia*

ABSTRACT

Absidia healeyae is a new species described in the *Mucorales* genus *Absidia* after screening 16 strains of *Absidia* isolated from seven locations in the state of Victoria in Australia. After initial analysis of the large ribosomal subunit sequence, the genomes of representative strains from two clades were sequenced using short paired-reads. Additional taxonomic markers extracted from the genome sequencing data support the novelty of *A. healeyae*. The identification of a new species in the genus *Absidia*, from a relatively small collection of isolates, hints at an unexplored diversity in the early diverging lineages of fungi in Australia.

Keywords: *Mucoromycotina*, Southern Hemisphere, taxonomy, zygomycete

Article history: Received 18 May 2020, Revised 6 June 2021, Accepted 7 June 2021, Available online 20 September 2021.

The genus *Absidia* currently consists of approximately 25 species for which molecular data are available. With the advent of molecular techniques to identify variation between species, a clearer understanding of the composition of the genus has emerged through a series of phylogenetic studies, resulting in the transfer of many of the species formerly considered to be within *Absidia* (notably the animal pathogen *Absidia corymbifera* (Cohn) Sacc. & Trott.) into the genera *Lichtheimia* and *Lentamyces* (Hoffmann, 2010).

For most genera in the order *Mucorales*, in addition to the species considered in phylogenetic studies, there are a large number of validly published species for which no DNA sequence information or ex-type material exists. Given that many descriptions date from the 19th and early 20th centuries they often include insufficient detail to permit "rediscovery". How these names should be treated when describing a new species remains an open question (Dayarathne et al., 2016). Nevertheless, new *Absidia* species are being described, including *Absidia bonitoensis* C.L. Lima, D.X. Lima, Hyang B. Lee & A.L. Santiago (Lima et al., 2021), *Absidia pernambucoensis* D.X. Lima, C.M. Souza-Motta & A.L. Santiago, *Absidia cornuta* D.X. Lima, C.A. de Souza, H.B. Lee & A.L. Santiago (Lima et al., 2020), *Absidia multispora* T.R.L. Cordeiro, D.X. Lima, Hyang, B. Lee & A.L. Santiago, *Absidia saloaensis* T.R.L. Cordeiro, D.X. Lima, Hyang B. Lee & A.L. Santiago (Cordeiro et al., 2020), *Absidia pararepens* Jurjević, M. Kolařík & Hubka (Crous et al., 2020), *A. terrestris* Rosas de Paz, Dania García, Guarro, Cano & Stchigel (Crous et al., 2018), *A. panacisoli* T. Yuan Zhang, Ying Yu, He Zhu, S.Z. Yang, T.M. Yang, Meng Y. Zhang & Yi X. Zhang (Zhang et al., 2018), *A. jindoensis* Hyang B. Lee & T.T.T. Nguyen (Wanasinghe et al., 2018), *A. stercoraria* Hyang B. Lee, H.S. Lee & T.T.T. Nguyen (Li et al., 2016), *A. koreana* Hyang B. Lee, H.W. Lee & T.T.T. Nguyen and *A. caatinguensis* D.X. Lima & A.L. Santiago (Ariyawansa et al., 2015). However, thus far, no *Absidia* species have been discovered in Australia, which likely reflects our poor understanding of the early diverging fungi in this continent (Urquhart, Coulon, & Idnurm, 2017; Urquhart & Idnurm, 2020; Urquhart, Douch, Heafield, Buddie, & Idnurm, 2021). In this study we set out identify *Absidia* strains from the Australian state of Victoria. In a total of 16 strains (Table 1), we identified three species, at least one of them new and described here as *A. healeyae*.

Sixteen strains showing whorled sporangia consistent with the genus *Absidia* were obtained from leaf litter samples taken from seven nature reserves in the Australian state of Victoria (Supplemental Fig. S1). Approximately 7 g of leaf litter were suspended in sterile water to a total volume of 50 mL, and a range of aliquot volumes (50 µL to 2 mL) was spread onto potato dextrose agar (PDA) plates supplemented with cefotaxime (100 µg/mL) to inhibit bacterial growth. Each strain was purified by single spore isolation on PDA to ensure homogeneity. Cultures were maintained on PDA at 22 °C on a laboratory bench. Three distinct groups of isolates were immediately evident based on colony pigmentation (purple, brown or green).

Genomic DNA was extracted from lyophilised mycelium, using a 1% cetyl trimethylammonium bromide buffer incubated at 65 °C for 30 min before one chloroform extraction and precipitation with an equal volume of isopropanol (Pitkin, Panaccione, & Walton, 1996). A fragment of the large ribosomal subunit (LSU) was amplified from each strain using primers NL1 5′-GCATATCAATAAGC-

* Corresponding author.
School of BioSciences, the University of Melbourne, VIC 3010, Australia.
E-mail address: alexander.idnurm@unimelb.edu.au (A. Idnurm).

Table 1 Strains isolated in this study.

Strain name (s)	Species	Collection locality	Date collected[a]	GenBank accession (LSU)
UoMAU1 CBS 144487 NRRL 66765 JMRC:SF:013685	*Absidia healeyae*	Tarra-Bulga National Park	25/04/17	MT436027
UoMAU2 CBS 144488 NRRL 66766 JMRC:SF:013686	*A. healeyae*	Tarra-Bulga National Park	25/04/17	MT436026
UoMAU3 CBS 144489 NRRL 66767 JMRC:SF:013687	*A. healeyae*	Tarra-Bulga National Park	25/04/17	MT436025
UoMAU372	*A. glauca*	Wilson's Promontory National Park	27/10/18	MT436024
UoMAU373	*A. glauca*	Morwell National Park	19/05/18	MT436023
UoMAU374	*A. glauca*	Silvan Reservoir Park	20/05/18	MT436022
UoMAU375	*A. glauca*	Morwell National Park	19/05/18	MT436021
UoMAU377	*A. glauca*	Baluk Willam Nature Conservation Reserve	19/10/18	MT436020
UoMAU379	*Absidia* sp.	Silvan Reservoir Park	20/05/18	MT436019
UoMAU380	*A. healeyae*	Tarra-Bulga National Park	25/04/18	MT436018
UoMAU381	*A. glauca*	Morwell National Park	19/05/18	MT436017
UoMAU382	*A. healeyae*	Jack Cann Reserve	10/06/18	MT436016
UoMAU383	*A. glauca*	Jack Cann Reserve	10/06/18	MT436015
UoMAU384	*A. glauca*	Jack Cann Reserve	10/06/18	MT436014
UoMAU385	*A. healeyae*	Macedon Regional Park	10/06/18	MT436013
UoMAU386	*A. healeyae*	Jack Cann Reserve	10/06/18	MT436012

[a] Format is day, month, year (20##).

GGAGGAAAAG-3′ (Kurtzman & Robnett, 1997) and LR3 5′-GGTCCGTGTTTCAAGAC-3′ (Vilgalys & Hester, 1990) using ExTaq DNA polymerase (Takara, Otsu, Japan). PCR conditions were 94 °C for 2 min followed by 32 cycles of 94 °C for 20 s, 55 °C for 20 s and 72 °C for 1 min, in an Eppendorf Mastercycler PCR machine (Eppendorf South Pacific, Macquarie Park, Australia). These PCR products were sequenced using Sanger chemistry at the Australian Genome Research Facility.

Analysis of the LSU sequences suggested two clades potentially distinct from previously described species of *Absidia*. A representative of each clade (strains UoMAU1 and UoMAU379) was chosen for genome sequencing, using Illumina sequencing at the Australian Genome Research Facility as 125 bp paired end reads. Sequences were assembled using Velvet (Zerbino & Birney, 2008) with the *k*-mer value set to 71 bp and a cut off minimum contig length of 500 bp. Assembly statistics are given in Table 2. The raw sequences and assemblies are available from the NCBI database under BioProjects PRJNA630489 (strain UoMAU1) and PRJNA630491 (strain UoMAU379).

Sequences for the internal transcribed spacer (ITS), translation elongation factor 1 alpha (TEF1) and actin were extracted from the genome assembly. Sequences were aligned using CLUSTAL Omega (Sievers et al., 2011). Phylogenetic analysis was then conducted using a maximum likelihood analysis in MEGA 10.2.4 (Kumar, Stecher, Li, Knyaz, & Tamura, 2018) using the Tamura-Nei model (Tamura & Nei, 1993) with gamma distribution.

The LSU tree grouped the 16 isolates into three clades (Fig. 1; TreeBASE accession URL: http://purl.org/phylo/treebase/phylows/study/TB2:S28061). The clades were congruent with the colony colours of the strains: UoMAU1, UoMAU2, UoMAU3, UoMAU380, UoMAU382, UoMAU385 and UoMAU386 were purple; UoMAU379 was brown; and the remaining strains were green. The clade consisting of the purple strains was distinct from LSU sequences previously deposited in GenBank while the green strains belonged to *A. glauca*. Green colony colour is a known characteristic of *A. glauca* (Ellis & Hesseltine, 1965). The third clade, containing only UoMAU379, is closely related to *Absidia inflata* NRRL 6591 (LSU sequence constructed from whole genome sequencing data, BioProject accession PRJNA519280) as well as unpublished sequences in GenBank (accessions LR606139 and LR606138). We hypothesize that these isolates may belong to the same species, however, further detailed taxonomic work will be required to confirm this. We have deposited the sequence of the region containing the 18S rRNA gene, 5.8S rRNA gene and 28S rRNA gene in GenBank (accession MT436029) along with those for actin and TEF1 (MW861732 and MW861734).

The three additional regions of the genome of strain UoMAU1 were examined and further support the separation from previously described species, and we thus named it as a new species *A. healeyae*. BLAST (Altschul, Gish, Miller, Meyers, & Lipman, 1990) comparison to the GenBank nr database revealed that ITS of *A. healeyae* was only 77% similar to the highest match, *Absidia* sp. SH1 accession EU816583.1, which is well below the expected similarity between strains of the same species (Vu et al., 2019). Similarly, the *A. healeyae* TEF1 and actin sequences were only 87% and 92% similar to the top BLAST results of *A. padenii* accession AF157238.1 (O'Donnell, Lutzoni, Ward, & Benny, 2001) and *A. repens* FSU4726 accession AY944763 (Hoffmann, Discher, & Voigt, 2007). While the molecular data presented in this paper clearly establish *A. healeyae* as a distinct species, our understanding of the phylogenetic struc-

Table 2 Genome assembly statistics for two strains in the genus *Absidia*.

Strain	Total length (bp)	Number of contigs (>500 bp)	Maximum contig (bp)	N50 (bp)	Coverage
UoMAU1	43,508,957	2,625	188,561	33,835	50X
UoMAU379	49,417,851	5,383	105,703	19,150	41X

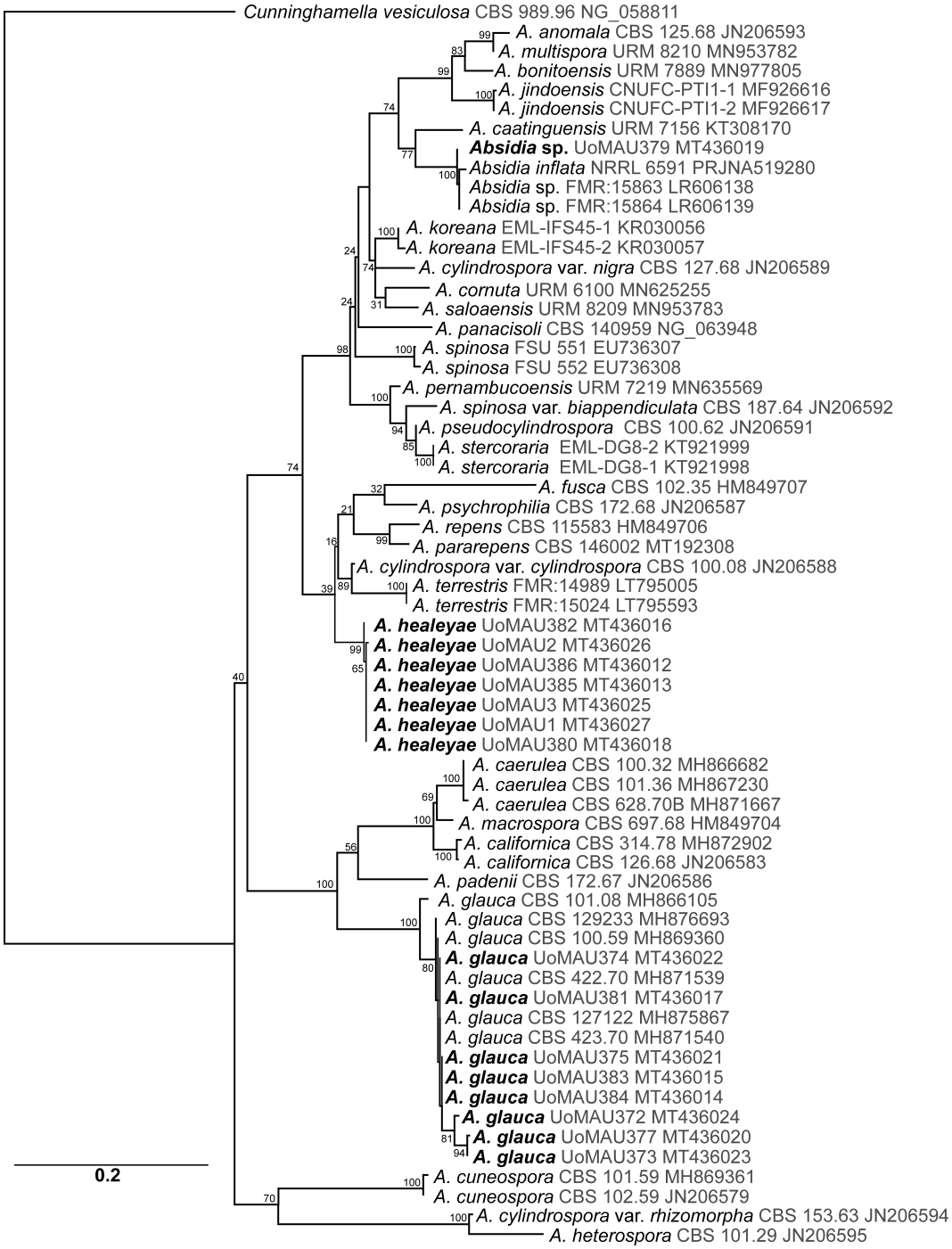

Fig. 1 – Phylogenetic tree based on the large ribosomal subunit showing the taxonomic placement of the 16 newly isolated *Absidia* strains (in bold) compared to previously described species (Lima et al., 2021; Lima et al., 2020; Cordeiro et al., 2020; Crous et al., 2020; Vitale et al., 2012; Walther et al., 2013; Vu et al., 2019; Crous et al., 2018; Zhang et al., 2018; Li et al., 2016; Hoffmann & Voigt, 2009; Ariyawansa et al., 2015; Wanasinghe et al., 2018). *Cunninghamella vesiculosa* P.C. Misra is used as the outgroup. Maximum likelihood tree generated in MEGA, bootstrap support values from 1,000 replicates are indicated.

ture of this genus is hampered by the limited number of regions available for phylogenetic studies. By providing whole genome sequencing data we hope that future researchers will be able to utilise a greater set of regions.

Taxonomy

Absidia healeyae A.S. Urquhart & A. Idnurm, sp. nov. Fig. 2. MycoBank no.: MB 835491.

Description: Colonies 31 mm diam after 4 d at 22 °C on PDA, initially white with purple pigmentation apparent by 7 d. Growth inhibited at temperatures above 30 °C. Sporangiophores 3.5–5.2 μm, 4.3 μm on average, in width below sporangia, branching typically into whorls of short branchlets each bearing a terminal sporangium, with a single septum below the sporangium. Sporangia 25.7 μm × 32 μm to 30.4 μm × 39.1 μm, 34.8 μm × 26.9 μm on average, pyriform, apophysate. Sporangiospores spherical, 2.3–3.5 μm diam, 2.8 μm on average. Columellae hemispherical above the

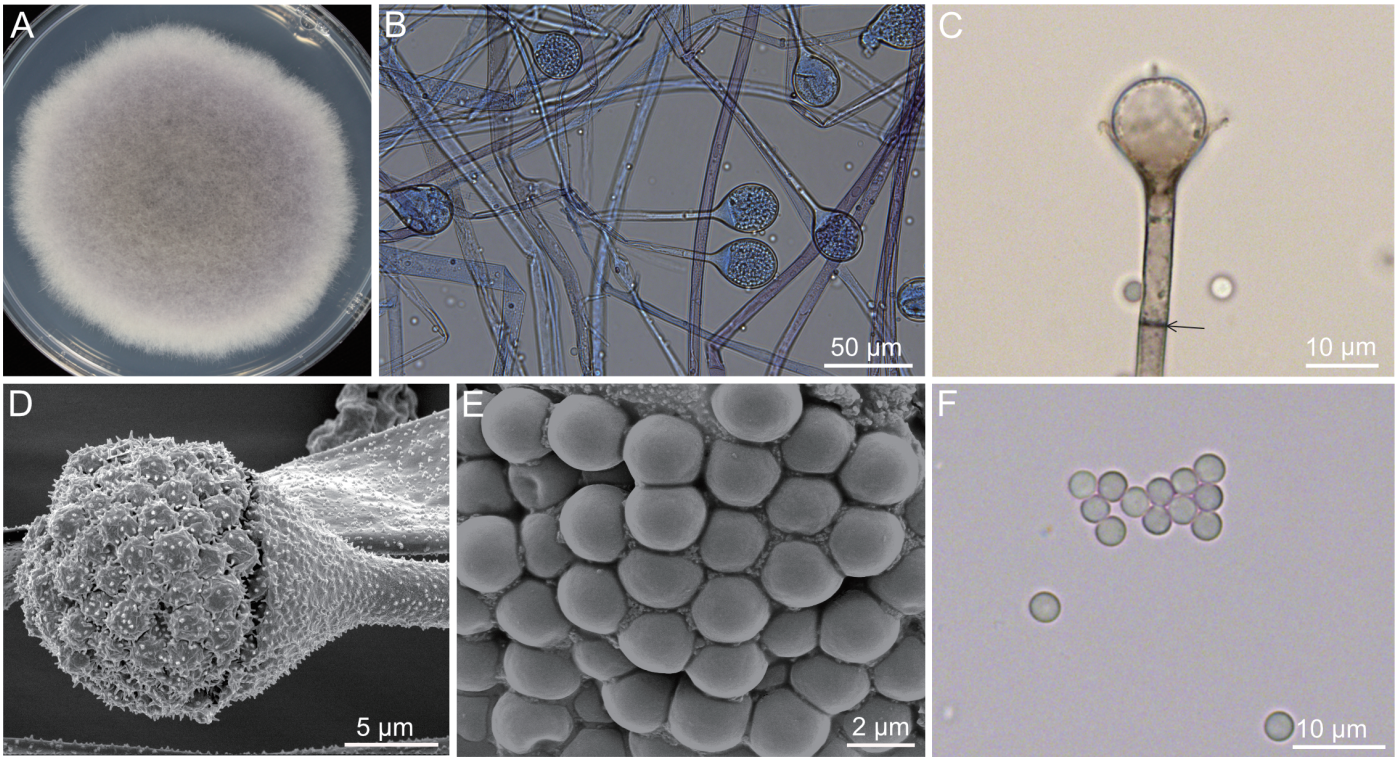

Fig. 2 – Morphological properties of *Absidia healeyae* (strain UoMAU1) cultured on potato dextrose agar. A: Growth on PDA after 6 d and 22 °C in a 9 cm diam Petri dish. B: Lactophenol cotton blue stained sporangia viewed under light microscope. C: Light microscopy of a columella, showing the placement of the septa (arrow) and other features such as the terminal protrusion and apophysis. D: Scanning electron micrograph of the sporangium showing the spikes that cover the sporangium and sporangiophore . E: Scanning electron micrograph of sporangiospores, conducted using a Philips XL30 scanning electron microscope on material sputter coated with titanium then gold (Xenosput apparatus, Dynavac Engineering, USA). F: Light microscopy of sporangiospores.

apophysis, sometimes with a small protrusion at the apex. Zygospores not observed.

Type: AUSTRALIA, Tarra-Bulga National Park, Victoria. A dried specimen from an in vitro culture on potato dextrose agar, preserved on Whatman® filter paper, comprising of mycelia, sporangia and sporangiospores (holotype in the National Herbarium of Victoria, Melbourne, Australia, MEL 2417184; ex-type cultures, UoMAU1 = CBS 144487 = JMRC:SF:013685 = NRRL 66765).

Genome sequence ex-holotype: BioProject accession PRJNA630489.

Gene sequences ex-holotype: MT436027 (LSU), MT436028 (18S rRNA gene, 5.8S rRNA gene, 28S rRNA gene, including ITS), MW861733 (TEF1), MW861731 (actin).

Etymology: *healeyae* in recognition of Kara Moana Healey, Victoria's first female national park ranger, of Tarra-Bulga National Park (Shingles, 2001).

Habitat: Leaf litter, wet native Australian forests.

Distribution: Isolates thus far obtained from three nature reserves in the Australian state of Victoria: Tarra-Bulga NP, Jack Cann Reserve and Macedon Regional Park. The distance between the farthest sites is approximately 220 km.

Notes – Eight other species of *Absidia* producing exclusively spherical-spores have been described. Five of these are available in culture collections and have corresponding molecular data: *A. glauca* Hagem, *A. caerulea* Bainier, *A. californica* J.J. Ellis & Hesselt. *A. padenii* (Hesselt. & J.J. Ellis) Milko and *A. macrospora* Váňová. *Absidia healeyae* is distinct from these species as the molecular data show a high degree of dissimilarity to these species across all four gene regions. Furthermore, *A. healeyae* can be distinguished from these species based on its unique combination of purple colony colouration and spherical spores, which are somewhat smaller than those produced by *A. caerulea* [3–5 µm, mostly about 3.5 µm (Ellis & Hesseltine, 1965)]. Three other species with spherical spores have been recognised as uncertain species since no ex-type strain is available (Ellis & Hesseltine, 1965): *A. septata* Tiegh., *A. scabra* Cocc. and *A. reflexa* Tiegh. *Absidia healeyae* can be distinguished from *A. septata* because *A. healeyae* is most likely heterothallic due to the lack of zygospore formation by individual strains and because analysis of the genome sequence indicates strain UoMAU1 contains only the *sexM* gene, positioned between homologs of *algL* and *sagA* that flanking the *sex* locus in other Mucorales species whereas *A. septata* is homothallic, from *A. scabra* because *A. healeyae* possesses a cross-wall in the sporagiophore below the apophysis, and from *A. reflexa* because the sporangiophores are not circinate in *A. healeyae*.

Disclosure

The authors declare no conflicts of interest. The fungal material was collected under permit number 10007429 issued by the Department of Environment, Land, Water and Planning (State Government of Victoria).

Acknowledgments

Travis Heafield and Dr. Allison Van de Meene assisted with the electron microscopy.

References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic Local

Alignment Search Tool. *Journal of Molecular Biology*, *215*, 403–410.
Ariyawansa, H. A., Hyde, K. D., Jayasiri, S. C., Buyck, B., Chethana, K. W. T., Dai, D. Q., et al. (2015). Fungal diversity notes 111–252—taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity*, *75*, 27–274.
Cordeiro, T. R. L., Nguyen, T. T. T., Lima, D. X. da Silva, S. B. G., Lima, C. F., Leitão, J. D. A., Gurgel, L. M. S., Lee, H. B., & Santiago, A. L. C. M. A. (2020). Two new species of the industrially relevant genus *Absidia* (Mucorales) from soil of the Brazilian Atlantic Forest. *Acta Botanica Brasilica, 34,* 549–558.
Crous, P. W., Luangsa-ard, J. J., Wingfield, M. J., Carnegie, A. J., Hernandez-Restrepo, M., Lombard, L., et al. (2018). Fungal Planet description sheets: 785–867. *Persoonia*, *41*, 238–417.
Crous, P. W., Wingfield, M. J., Chooi, Y. H., Gilchrist, C. L. M., Lacey, E., Pitt, J. I., et al. (2020) Fungal Planet description sheets: 1042–1111. *Persoonia*, *44*, 201–459.
Dayarathne, M. C., Boonmee, S., Braun, U., Crous, P. W., Daranagama, D. A., Dissanayake, A. J., et al. (2016). Taxonomic utility of old names in current fungal classification and nomenclature: Conflicts, confusion & clarifications. *Mycosphere*, *7*, 1622–1648.
Ellis, J. J., & Hesseltine, C. W. (1965). The genus *Absidia*: globose-spored species. *Mycologia*, *57*, 222–235.
Hoffmann, K. (2010). Identification of the Genus *Absidia* (Mucorales, Zygomycetes): A Comprehensive Taxonomic Revision. In: Gherbawy T., Voigt, K. (Eds.), *Molecular Identification of Fungi* (pp. 439–460). Berlin: Springer.
Hoffmann, K., Discher, S., & Voigt, K. (2007). Revision of the genus *Absidia* (*Mucorales*, *Zygomycetes*) based on physiological, phylogenetic, and morphological characters; thermotolerant *Absidia* spp. form a coherent group, *Mycocladiaceae* fam. nov. *Mycological Research*, *111*, 1169–1183.
Hoffmann, K., & Voigt, K. (2009). *Absidia parricida* plays a dominant role in biotrophic fusion parasitism among mucoralean fungi (Zygomycetes): *Lentamyces*, a new genus for *A. parricida* and *A. zychae*. *Plant Biology*, *11*, 537–554.
Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution 35*, 1547–1549.
Kurtzman, C. P., & Robnett, C. J. (1997). Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5′ end of the large-subunit (26S) ribosomal DNA gene. *Journal of Clinical Microbiology*, *35*, 1216–1223.
Li, G. J., Hyde, K. D., Zhao, R. L., Hongsanan, S., Abdel-Aziz, F. A., Abdel-Wahab, M. A., et al. (2016). Fungal diversity notes 253–366: taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity*, *78*, 1–237.
Lima, C. L. F., Lima, D. X., Cordeiro, T. R. L., Lee, H. B., Nguyen, T. T. T, Gurgel, L. M. S., & Santiago, A. L. C. M. A. (2021). *Absidia bonitoensis* (Mucorales, Mucoromycota), a new species isolated from the soil of an upland Atlantic forest in Northeastern Brazil. *Nova Hedwigia, 112,* 241–251.
Lima, D. X., Cordeiro T. R. L., de Souza, C. A. F., de Oliveira R. J. V., Lee H. B., Souza-Motta C. M., & Santiago, A. L. C. M. A. (2020). Morphological and molecular evidence for two new species of *Absidia* from Neotropic soil. *Phytotaxa, 446,* 61–71.
O'Donnell, K., Lutzoni, F. M., Ward, T. J., & Benny, G. L. (2001). Evolutionary relationships among mucoralean fungi (Zygomycota): Evidence for family polyphyly on a large scale. *Mycologia, 93,* 286–296.
Pitkin, J. W., Panaccione, D. G., & Walton, J. D. (1996). A putative cyclic peptide efflux pump encoded by the *TOXA* gene of the plant-pathogenic fungus *Cochliobolus carbonum*. *Microbiology*, *142*, 1557–1565.
Shingles, R. (2001). Triumph over the heartbreak hills: personal reminiscences from the 1880s to the present. JJB Publishing, Victoria.
Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J. D., & Higgins, D. G. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Molecular Systems Biology*, *7*, 539.
Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, *10*, 512–526.
Urquhart, A. S., Coulon, P. M. L., & Idnurm, A. (2017). *Pilaira australis* sp. nov. (Mucorales, Mucoromycota) isolated from emu faeces in Australia. *Phytotaxa*, *329*, 277–283.
Urquhart, A. S., & Idnurm, A. (2020). *Syncephalastrum contaminatum*, a new species in the Mucorales from Australia. *Mycoscience*, *61*, 111–115.
Urquhart, A. S., Douch, J. K., Heafield, T. A., Buddie, A. G., & Idnurm, A. (2021). Diversity of *Backusella* (*Mucoromycotina*) in south-eastern Australia revealed through polyphasic taxonomy. *Persoonia*, *64*, 1–25.
Vilgalys, R., & Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology*, *172*, 4238–4246.
Vitale, R. G., de Hoog, G. S., Schwarz, P., Dannaoui, E., Deng, S. W., Machouart, M., et al. (2012). Antifungal susceptibility and phylogeny of opportunistic members of the order Mucorales. *Journal of Clinical Microbiology*, *50*, 66–75.
Vu, D., Groenewald, M., de Vries, M., Gehrmann, T., Stielow, B., Eberhardt, U., et al. (2019). Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. *Studies in Mycology*, 135–154.
Walther, G., Pawlowska, J., Alastruey-Izquierdo, A., Wrzosek, M., Rodriguez-Tudela, J. L., Dolatabadi, S., et al. (2013). DNA barcoding in Mucorales: an inventory of biodiversity. *Persoonia*, *30*, 11–47.
Wanasinghe, D. N., Phukhamsakda, C., Hyde, K. D., Jeewon, R., Lee, H. B., Jones, E. B. G., et al. (2018). Fungal diversity notes 709-839: taxonomic and phylogenetic contributions to fungal taxa with an emphasis on fungi on *Rosaceae*. *Fungal Diversity*, *89*, 1–236.
Zerbino, D. R., & Birney, E. (2008). Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Research*, *18*, 821–829.
Zhang, T. Y., Yu, Y., Zhu, H., Yang, S. Z., Yang, T. M., Zhang, M. Y., et al. (2018). *Absidia panacisoli* sp. nov., isolated from rhizosphere of *Panax notoginseng*. *International Journal of Systematic and Evolutionary Microbiology*, *68*, 2468–2472.