




***Absidia urquhartii* (Mucoromycota) is a new species that is prevalent in Victoria, Australia**

ALEXANDER IDNURM^{1,2,*}

¹*School of BioSciences, the University of Melbourne, Victoria, 3010, Australia*

²  alexander.idnurm@unimelb.edu.au;  <https://orcid.org/0000-0001-5995-7040>

*Corresponding author:  alexander.idnurm@unimelb.edu.au

Abstract

A new species, *Absidia urquhartii*, was identified from four locations across Victoria, Australia, and is described. The species is related to a species commonly found around the world; *A. glauca*, but is a distinct species based on phylogenies derived from DNA sequences, the morphology of its zygospores and its reproductive isolation from *A. glauca*. The genome of the ex-type strain was sequenced using Illumina short read technology. Starting from the genome sequence, the mating type locus was characterised from strains of (+) and (–) mating types, which is consistent with a heterothallic mode of reproduction established from crosses between strains.

Key words: biodiversity, biological species concept, Cunninghamellaceae, Mucorales

Introduction

Species in the genus *Absidia* (Mucoromycota, Mucorales, Cunninghamellaceae) are readily identifiable in culture through the production of whorls of the sporangiophores that produce their asexual spores. With the availability of DNA sequencing information to guide phylogenetic analyses, the species within the genus that had been defined primarily on morphological features were re-examined from the mid 2000s, leading to revisions including the allocation of some species into two new genera (Hoffmann 2010; Hoffmann *et al.* 2007). While DNA sequencing is currently the predominant approach to infer new species in this genus, and many of the species within the Mucorales genera, some traits remain useful in supporting species delimitations, such as in *Absidia* species the colony pigmentation and asexual spore morphology (Urquhart & Idnurm 2021).

There are over 50 species of *Absidia*, half of which have been identified in the last five years, particularly from Asian countries (Htet *et al.* 2024; Hurdeal *et al.* 2023; Lim *et al.* 2024; Zhao *et al.* 2022a; Zhao *et al.* 2023; Zhao *et al.* 2022b; Zong *et al.* 2021) and Brazil (Cordeiro *et al.* 2020; de Freitas *et al.* 2022; Leitão *et al.* 2021). This also includes investigation from Australia, where the diversity of strains of *Absidia* isolated from the state of Victoria was explored, leading to the discovery of a new species *Absidia healeyae* (Urquhart & Idnurm 2021).

The recent discovery of many more species of *Absidia* suggests that there is still some distance to go before realising the full diversity within this genus, not to mention beginning to understand their ecological preferences and distributions. Here, as part of screening for new Mucoromycota species in 2023, a new *Absidia* was identified. Curiously, three additional strains of this species (or a close relative) had been isolated previously in 2018, but at that time assigned to a different *Absidia* species, and thus the new species was hidden in plain sight.

Material and methods

A sample of leaf litter, predominantly from *Eucalyptus tricarpa*, was collected in the Brisbane Ranges National Park on 17 September, 2023 (37.74061 °S 144.31439 °E; Figure 1A), as covered under a permit issued to the University of Melbourne from Parks Victoria. About 1 g was resuspended in 50 ml sterile water, and aliquots were plated onto

potato dextrose agar (PDA) supplemented with two antibacterials, rifampicin (10 µg/ml) and chloramphenicol (34 µg/ml). Putative *Absidia* species were identified by the whorled morphology of their sporangiophores, then purified via streaking out spores and isolating colonies derived from single spores. Additional strains of *Absidia* characterised in a previous study (Urquhart & Idnurm 2021) were used for comparison. To create a holotype, a disc of Whatman filter paper was placed on PDA, a strain inoculated in the middle, and after growth for seven days the sample was dried at room temperature using silica beads.

Colours of the colonies cultured on PDA were compared against a standard palette (Kornerup & Wanscher 1978). Radial growth rates were measured using incubators set at different temperatures ranging from 20°C to 37°C. The production of zygospores was assessed by placing two strains about 1 cm apart on V8 juice agar (10% cleared Campbell's V8 juice at pH 6, 2% agar), then culturing in darkness at 20°C. Microscopic features were examined using either Leica microscope models DM6000B or M205 FA.

To generate mycelia for DNA extraction and subsequent sequencing, strains were cultured in potato dextrose broth for up to five days, whereby mycelia remained within the medium and without the formation of sporangia. The mycelia were frozen, lyophilized overnight and genomic DNA was extracted (Pitkin *et al.* 1996). Two of the rRNA regions were amplified from genomic DNA of strains [internal transcribed spacers + 5.8S, i.e. *ITS*, with primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and part of the ribosomal large subunit, i.e. *LSU*, with primers LR3 (5'-GGTCCGTGTTTCAAGAC-3') and NL1 (5'-GCATATCAATAAGCGGAGGAAAAG-3')] and then directly sequenced with Sanger chemistry at the Australian Genome Research Facility. Sequence of the *ITS* region of strain UoMAU377 indicated it contains variable copies. Therefore, the *ITS* amplicon was cloned into plasmid pCR2.1 using the TOPO cloning kit (Invitrogen, USA). The reaction was transformed into chemically-competent *Escherichia coli* strain NEB 10-beta, and plasmids were purified. Based on differences in restriction enzyme patterns, six plasmids of each copy were sequenced using Sanger chemistry to yield a consensus for the two copies. Phylogenetic analysis was used to examine the relationship between strains and the species of *Absidia* with available DNA sequences, using those previously analysed as the reference point (Zhao *et al.* 2022b). The closest relatives to the strains were selected, downloaded from GenBank and aligned with those generated here. Phylogenetic analyses used maximum likelihood (optimised for the Kimura-2 parameters for *ITS* and Tamura-3 parameters for *LSU*) and 100 bootstraps to assess support for relationships, in MEGA 11 (Tamura *et al.* 2021).

To obtain and provide additional DNA sequence information for future analyses, the whole genome of the strain UoMD23-19 was sequenced as 150 nucleotide paired-end reads on a NovaSeq 6000 sequencing system by the Victorian Clinical Genetics Services (VCGS), The Royal Children's Hospital, Parkville, Victoria. DNA regions of interest were extracted from the Illumina data and assembled using Geneious. In brief, over 36 million paired reads were generated and sequencing adapters removed by VCGS. A draft genome assembly was constructed using 12% of these data with the Velvet plug-in in Geneious using a *k*-mer set at 85. This draft genome was used for BLAST searches using homologous genes from *Absidia* species to identify the DNA regions. Once identified, the full set of Illumina reads were mapped to them individually.

From the genome sequencing information, the mating type locus of strain UoMD23-19 was identified based on sequence similarities to other such loci in the Mucorales. To define the region in a strain of opposite mating type, the equivalent region was amplified with primers (MAI0945 5'-TCCGTCAAGGCTAGATCTGC-3' and MAI0946 5'-CTATCAACATTCCAAAAGACCC-3') that were designed to be outside the idiomorphic region, from genomic DNA of strain UoMAU377 of the opposite mating type. The amplicon was then sequenced at the AGRF using Illumina technology. To define the mating types of *A. glauca* strains at a molecular level, the mating type specific transcription factors were identified by BLAST against the GenBank databases and then primers designed for amplification by PCR [MAI0972 5'-AGTACCGAGACCCAAGAACG-3' and MAI0973 5'-CCACGGTTCTTATGCGAGTG-3' for (-) and MAI0974 5'-CCGAGTAAAGCGACAGGATC-3' and MAI0975 5'-CTGCTGTATTTCTCTGATCC-3' for (+)]. PCR products were resolved on 1% agarose gels and visualised by staining with ethidium bromide.

Results

Two colonies that produced whorled sporangiophores, typical of the genus *Absidia*, were identified from the same leaf litter sample collected from the Brisbane Ranges National Park (Figure 1A). The *ITS* and/or *LSU* regions were sequenced and compared against the GenBank nr database. One of these strains is *Absidia glauca* (strain UoMD23-

11). The second strain, UoMD23-19, returned *ITS* matches with less than 88.8% identity to *Absidia* species with sequences in the GenBank database, thereby warranting additional investigation. However, the two *LSU* sequences present in strain UoMD23-19 were 98.8–100% identical to those from three strains previously isolated from Victoria that had been assigned as *A. glauca* (Urquhart & Idnurm 2021). The *ITS* regions of these three strains had not been previously sequenced, so here they were amplified and sequenced. Phylogenetic analysis presented of these *ITS* sequences in Figure 2 illustrates the distinct position of four strains from a clade that contains the species *A. glauca*, *A. globospora*, *A. sympodialis* and *A. virescens*. Analysis of the *LSU* regions revealed similar, but not identical, relationships (Supplemental Figure 1).

The mating type/*sex* locus was identified from the Illumina 150 nucleotide paired reads of strain UoMD23-19 and assembled (GenBank accession PP971769). Based on the presence of the *sexP* gene in this strain it would be designated as (+). By crossing and observing the production of zygosporangia (see details below), the mating type of other strains could be inferred. The mating type locus of a (–) strain, UoMAU377, was amplified and sequenced (GenBank accession PP971768). Comparing the sequences between the strains show they feature an idiomorphic region, with each containing a gene predicted to encode a high-mobility group domain protein (Figure 3). The (+) region also contains a second gene of unknown function. Analysis of the two genes flanking this locus in the minus mating type strain of *A. glauca* CBS 101.48 indicates they are conserved (Schulz *et al.* 2016). The *algL* gene is commonly observed adjacent to mating type loci in Mucorales species (Schulz *et al.* 2016). The locus in strain CBS 101.48 likely also contains an insertion of a Helitron-like transposable element.

Crosses were set up between *Absidia* strains. The four strains studied here were paired in all combinations and then paired with two strains of *A. glauca* that are able to mate with each other (Table 1). No mating was observed between these four strains and *A. glauca*. In contrast, UoMD23-19 formed zygosporangia in crossed with UoMAU373 and UoMAU377, and weakly interacted with UoMAU372.

TABLE 1. Strains examined. Strain names refer to a system at the University of Melbourne (‘UoM’) or that from the Jena Microbial Resource Collection (‘JMRC’).

Strain name(s)	<i>Absidia</i> species	Mating type	Details of isolation	GenBank accessions	Reference
UoMD23-19; JMRC:SF015714	<i>A. urquhartii</i>	(+)	Brisbane Ranges National Park, 17 September, 2023	<i>ITS</i> PP973115; <i>LSU</i> PP973118 and PP973119; <i>MAT</i> locus PP971769; Illumina genome sequencing PRJNA1117230	This study
UoMAU372; JMRC:SF015711	<i>A. urquhartii</i>	(–)	Wilson’s Promontory National Park, 27 October 2018	<i>ITS</i> PP973113; <i>LSU</i> MT436024	Urquhart & Idnurm 2021
UoMAU373; JMRC:SF015712	<i>A. urquhartii</i>	(–)	Morwell National Park, 19 May 2018	<i>ITS</i> PP973114; <i>LSU</i> MT436023	Urquhart & Idnurm 2021
UoMAU377; JMRC:SF015713	<i>A. urquhartii</i>	(–)	Baluk William Nature Conservation Reserve, 19 October 2018	<i>ITS</i> PP973116 and PP973117; <i>LSU</i> MT436020; <i>MAT</i> locus PP971768	Urquhart & Idnurm 2021
UoMD23-11	<i>A. glauca</i>	(–)	Brisbane Ranges National Park, 17 September, 2023	<i>LSU</i> PQ222915	This study
UoMAU381	<i>A. glauca</i>	(+)	Morwell National Park, 19 May 2018	<i>LSU</i> MT436017	Urquhart & Idnurm 2021
UoMAU384	<i>A. glauca</i>	(–)	Jack Cann Reserve, 10 June 2018	<i>LSU</i> MT436014	Urquhart & Idnurm 2021

Taxonomy

Absidia urquhartii A. Idnurm, *sp. nov.* (Fig. 1)

Mycobank:—MB 854137

Etymology:—the epithet *urquhartii* is in acknowledgement of the contributions of Andrew S. Urquhart in the pursuit of discovery of fungal biodiversity in Australia.

Holotype:—AUSTRALIA, Victoria, MELU F155166a preserved in the University of Melbourne Herbarium on Whatman filter paper and contains mycelium, sporangia and asexual spores.

Ex-type strains:—UoMD23-19 (University of Melbourne) = JMRC:SF015714 (Jena Microbial Resource Collection).

GenBank numbers:—*ITS* PP973115; two copies of *LSU* PP973118 and PP973119; genome sequencing reads PRJNA1117230.

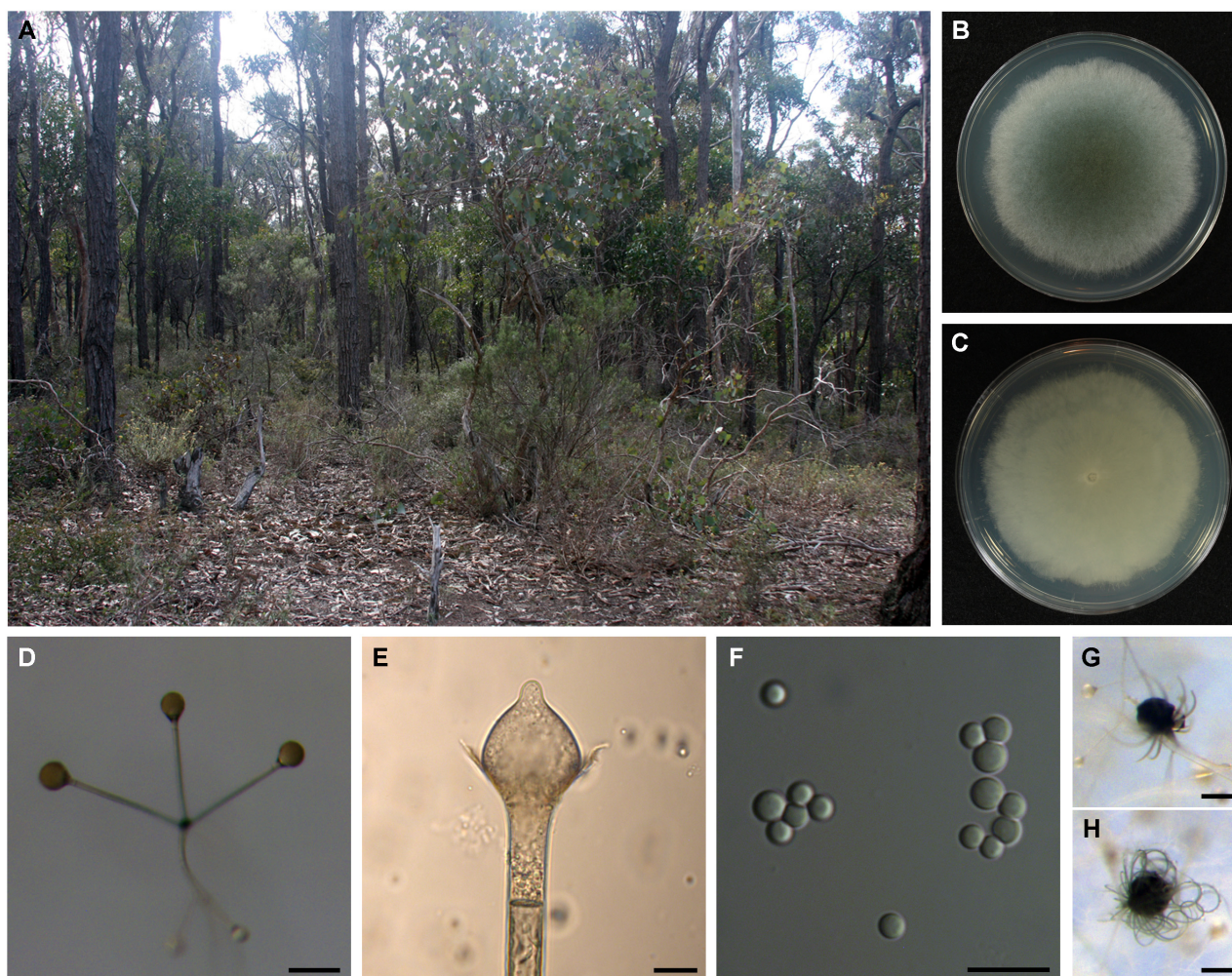


FIGURE 1. Features of *Absidia urquhartii* *sp. nov.*, strain UoMD23-19. A. Photograph of the site from where the type of the species was obtained. B. Culture on a PDA 9 cm diameter plate grown for 4 days at 22 °C from above, and C. Obverse of culture plate. D. Whorl of three sporangiophores terminating in sporangia. E. Columella. F. Asexual spores. G. Zygospore from a UoMD23-19 × UoMAU377 cross. H. Zygospore from a cross between two *A. glauca* strains. Scale bars: E, F = 10 µm, D, G–H = 100 µm.

Description:—Asexual morph: radial growth rate on PDA 9 mm per day at 22 °C, 5 mm per day at 30 °C; no growth at 37 °C. Colonies olive grey front side, greenish grey from the obverse of the plate. Asexual spore production is from sporangia, with the sporangiophores producing 3–4 branches. One branch elongates without the differentiation into sporangia, and that generates another whorl of sporangia-forming structures. Additional sporangiophores are produced laterally. Sporangiophores are up to 10 mm in height. Sporangia are globose, multi-spored, and increase in size and level of dark brown pigmentation depending on developmental age. Columellae are pear-shaped with

prominent apophyses and collars at their base, and below the collars clearly-defined septa. Columellae ($n = 9$) of width 19.35–28.46 μm (average 23.51 μm), length from tip to the septa, 42.26–51.66 μm (average 47.46 μm) and diameter of sporangiophore at the septa 6.22–10.17 μm (average 8.18 μm). Sporangiospores are globose, hyaline and smooth, and ($n = 32$) 3.12–5.29 μm (average 3.78 μm) in diameter.

Sexual morph: no production of zygospores when grown in isolation, and DNA sequence analysis supports a heterothallic life cycle. When crossed (e.g. UoMD22-19 \times UoMAU377), zygospores form that range in size during development up to ~ 110 μm . Gametangia are elongated and of equal size. One set of suspensor cells produces appendages that surround the zygospore.

Material examined:—single-spored *in vitro* culture for strain UoMD23-19 isolated from an environmental sample, collected on 17 September 2023, in the Brisbane Ranges National Park.

Additional specimens examined:—AUSTRALIA. Victoria: 27 Oct. 2018, *Urquhart* (UoMAU372); 19 May 2018, *Urquhart* (UoMAU373); 19 Oct. 2018, *Urquhart* (UoMAU377).

Habitat:—Isolated from leaf litter in Victoria, Australia.

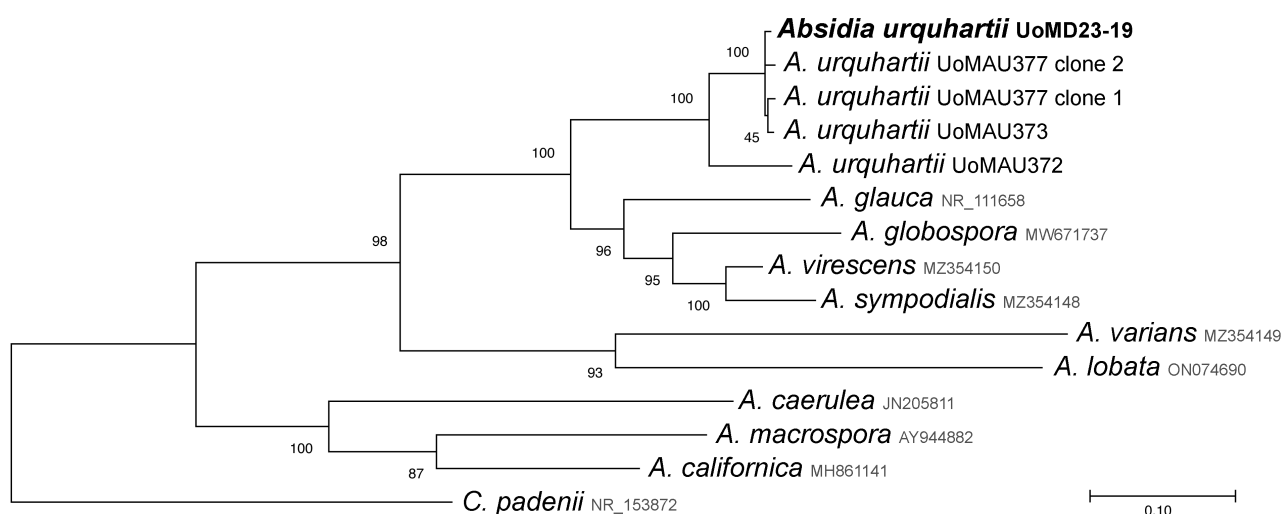


FIGURE 2. A species maximum likelihood phylogeny based on comparisons between the *ITS* regions of the *Absidia* species that are most closely related to *A. urquhartii*. For *A. urquhartii*, the strain names are provided, with GenBank accessions associated for them in table 1. For other *Absidia* species, GenBank accessions are in grey font. The tree is rooted with *Chlamydoabsidia* (= *Absidia*) *padenii*. The numbers near the nodes are percentage values from bootstrapping (100 replicates).

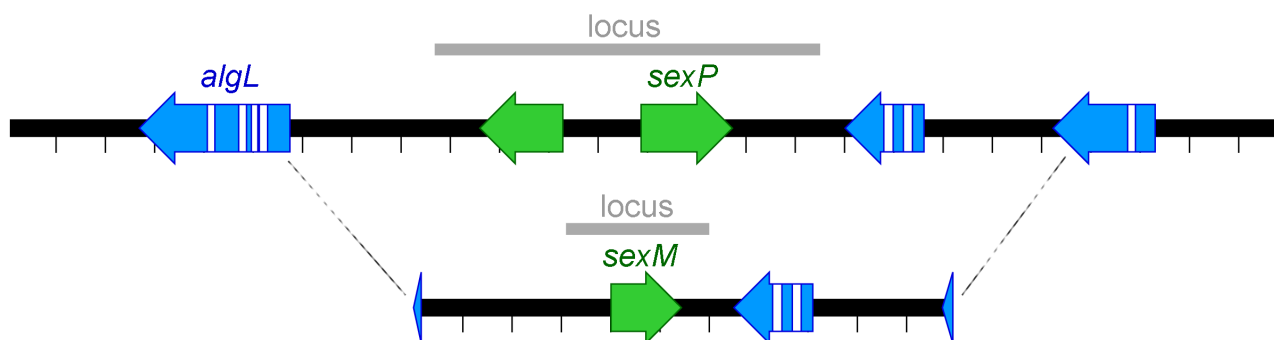


FIGURE 3. Diagram of the *A. urquhartii* mating type/sex locus. Tick marks indicate 500 bp spacing. The idiomorphic region is marked with a grey bar, with each strain containing within that region genes specific to each strain.

Notes:—The asexual spore and zygospore properties of *Absidia urquhartii* are consistent with other members in the genus. The basis for describing this as a new species is: i) the differences it shows in DNA sequences of diagnostic regions relative to other members in the genus, ii) the reproductive isolation from one of its closest relatives and iii) morphological differences. Based on the phylogenetic analyses, the four species that *A. urquhartii* is most closely related to are *A. glauca*, *A. globospora*, *A. sympodialis* and *A. virescens*. Morphologically it is distinct from all four. *Absidia glauca* produces more and longer appendages around its zygospores compared to *A. urquhartii*; furthermore, *A. glauca* produces unequally-sized suspensor cells while those of *A. urquhartii* are equally-sized. The columellae

of *A. urquhartii* have pronounced apophyses and collars, which are either not or are inconsistently observed in *A. globospora* and *A. sympodialis*. Likewise, collars are rarely or sparsely observed for *A. virescens*. The sporangiospores of *A. virescens* vary from globose to subglobose and are of a wider range of sizes than *A. urquhartii*. Furthermore, *A. globospora* cannot grow above 30 °C whereas *A. urquhartii* can.

Discussion

Previous research that isolated strains of *Absidia* from leaf litter samples from three different sites across the state of Victoria, Australia, suggested there is diversity in this genus that has not been described (Urquhart & Idnurm 2021). In this study, a new collection of leaf litter from a fourth site led to the isolation of an *Absidia* strain UoMD23-19 whose *ITS* could be sequenced cleanly and showed low percentage identity (<88.8%) to other *Absidia* species. Inferring new species based on differences in DNA sequences has challenges; nonetheless, this low level of similarity for *ITS* regions is certainly well under the recommended minimum of more than 1.5% differences as a possible indication of a new species (Jeewon & Hyde 2016). However, the *LSU* region from the new strain showed high similarity to three strains designated as *A. glauca* (i.e. UoMAU372, UoMAU373 and UoMAU377). Here, the *ITS* regions of these three strains were sequenced, including from UoMAU377 with its different copies, and used in phylogenetic analysis to place these with UoMD23-19 as a monophyletic group sister to a clade that includes *A. glauca*, *A. globospora*, *A. sympodialis* and *A. virescens* (Figure 2). This new species, *A. urquhartii*, shows reproductive isolation from *A. glauca*. Of the strains examined, it is worth noting that UoMAU372 (*ITS* <91.6% with the other three strains) formed a distinct branch in both the *ITS* and *LSU* phylogenetic trees and only reacted weakly to strain UoMD23-19 in initiating the first stages of mating, and may also represent an undescribed species.

Absidia species can share morphological features, such as the globular shape of their asexual spores (Ellis & Hesseltine 1965), which may be indicative of a common ancestor. Of the three *Absidia* species that produce spherical spores and without DNA sequence information available, these can be eliminated as candidates for this new species. That is, *A. scabra* does not produce a septum within the sporangiophore below the apophysis, *A. septata* is homothallic, and the sporangiophores are circinate in *A. reflexa* (Hoffmann 2010; Urquhart & Idnurm 2021).

Species in the genus *Absidia* have undergone a number of revisions, and the species are often most easily distinguished based on difference in DNA sequences (Hoffmann 2010). *Absidia urquhartii* can be considered an example of finding a ‘cryptic species’ even when DNA sequence information was available. The definitions of cryptic species varies, but all mention the species are morphologically indistinguishable. In the fungi and other microbes, where numbers of traits for comparison may be fewer or more difficult to measure than macroscopic organisms, the identification of cryptic species has largely been driven through the phylogenetic analysis of DNA sequencing information (Chethana *et al.* 2021). Of the four strains examined here, three had previously been assumed to be *A. glauca* based on DNA sequence-based phylogeny. Hence, this illustrates the importance of selecting suitable barcodes in trying to use those for discovering cryptic species (Jeewon & Hyde 2016). It also supports the power of using the classical ‘biological species concept’ as a mechanism to distinguish species. Strikingly, to date in two separate environmental samples *A. glauca* and *A. urquhartii* have both been co-isolated (i.e. from Jack Cann Reserve in 2018 and Brisbane Ranges National Park in 2023, table 1), supporting the concept of reproductive boundaries between species.

Acknowledgements

This research was supported by the Hermon Slade Foundation (HSF22060) and Caitlin Hull helped culture strains. Thanks are extended to the teaching team of subject BOTA20002 ‘Plant Biodiversity’ for arranging the field trip to isolate strains.

References

- Chethana, K.W.T., Manawasinghe, I.S., Hurdeal, V.G., Bhunjun, C.S., Appadoo, M.A., Gentekaki, E., Raspé, O., Promputtha, I. & Hyde, K.D. (2021) What are fungal species and how to delineate them? *Fungal Diversity* 109: 1–25.
<https://doi.org/10.1007/s13225-021-00483-9>
- Cordeiro, T.R.L., Nguyen, T.T.T., Lima, D.X., da Silva, S.B.G., de Lima, C.F., Leitão, J.D.A., Gurgel, L.M.S., Lee, H.B. & Santiago, A.L.C.M.d.A. (2020) Two new species of the industrially relevant genus *Absidia* (Mucorales) from soil of the Brazilian Atlantic Forest. *Acta Botanica Brasílica* 34: 549–558.
<https://doi.org/10.1590/0102-33062020abb0040>
- de Freitas, L.W.S., Cruz, M.O., Nguyen, T.T.T., Lee, H.B., dos Santos, F.R.S. & Santiago, A.L.C.M.d.A. (2022) *Absidia variicolumellata*, sp. nov., a new species of mucoralean fungi isolated from Atlantic Forest in Bahia state (Brazil). *Sydowia* 75: 75–86.
<https://doi.org/10.12905/0380.sydowia75-2022-0075>
- Ellis, J.J. & Hesseltine, C.W. (1965) The genus *Absidia*: globose-spored species. *Mycologia* 57: 222–235.
<https://doi.org/10.1080/00275514.1965.12018205>
- Hoffmann, K. (2010) Identification of the genus *Absidia* (Mucorales, Zygomycetes): a comprehensive taxonomic revision. In: Gherbawy, Y. & Voigt, K. (Eds.) *Molecular Identification of Fungi*. Springer-Verlag, Berlin Heidelberg, pp. 439–460.
https://doi.org/10.1007/978-3-642-05042-8_19
- 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.
<https://doi.org/10.1016/j.mycres.2007.07.002>
- Htet, Y.M., Yasanthika, W.A.E., Hurdeal, V.G. & de Farias, A.R.G. (2024) *Absidia thailandica* sp. nov., an addition to the diversity of soil fungi from Thailand. *Phytotaxa* 646: 157–168.
<https://doi.org/10.11646/phytotaxa.646.2.5>
- Hurdeal, V.G., Jones, E.B.G. & Gentekaki, E. (2023) *Absidia zygospora* (Mucoromycetes), a new species from Nan Province, Thailand. *Studies in Fungi* 8: 15.
<https://doi.org/10.48130/SIF-2023-0015>
- Jeewon, R. & Hyde, K.D. (2016) Establishing species boundaries and new taxa among fungi: recommendations to resolve taxonomic ambiguities. *Mycosphere* 7: 1669–1677.
<https://doi.org/10.5943/mycosphere/7/11/4>
- Kornerup, A. & Wanscher, J.H. (1978) *Methuen Handbook of Colour* (3rd ed.). London: Erye Methuen.
- Leitão, J.D.A., Cordeiro, T.R.L., Nguyen, T.T.T., Lee, H.B., Gurgel, L.M.S. & Santiago, A.L.C.M.d.A. (2021) *Absidia aguabelensis* sp. nov.: A new mucoralean fungi isolated from a semiarid region in Brazil. *Phytotaxa* 516: 83–91.
<https://doi.org/10.11646/phytotaxa.516.1.6>
- Lim, S.-K., Das, K., Lim, G.-J., Choi, J.-S., Lee, S.-Y. & Jung, H.-Y. (2024) Molecular identification of a new species of *Absidia* (Cunninghamellaceae, Mucorales) isolated from soil in Korea. *Mycobiology* 52: 201–206.
<https://doi.org/10.1080/12298093.2024.2366047>
- 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.
<https://doi.org/10.1099/13500872-142-6-1557>
- Schulz, E., Wetzell, J., Burmester, A., Ellenberger, S., Siegmund, L. & Wöstemeyer, J. (2016) Sex loci of homothallic and heterothallic Mucorales. *Endocytobiosis and Cell Research* 27: 39–57.
- Tamura, K., Stecher, G. & Kumar, S. (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution* 38: 3022–3027.
<https://doi.org/10.1093/molbev/msab120>
- Urquhart, A.S. & Idnurm, A. (2021) *Absidia healeyae*: a new species of *Absidia* (Mucorales) isolated from Victoria, Australia. *Mycoscience* 62: 331–335.
<https://doi.org/10.47371/mycosci.2021.06.001>
- Zhao, H., Nie, Y., Zong, T., Dai, Y. & Liu, X. (2022a) Three new species of *Absidia* (Mucoromycota) from China based on phylogeny, morphology and physiology. *Diversity* 14: 132.
<https://doi.org/10.3390/d14020132>
- Zhao, H., Nie, Y., Zong, T.-K., Wang, K., Lv, M.-L., Cui, Y.-J., Tohtirjap, A., Chen, J.-J., Zhao, C.-L., Wu, F., Cui, B.-K., Yuan, Y., Dai, Y.-C. & Liu, X.-Y. (2023) Species diversity, updated classification and divergence times of the phylum Mucoromycota. *Fungal*

Diversity 123: 49–157.

<https://doi.org/10.1007/s13225-023-00525-4>

Zhao, H., Nie, Y., Zong, T.-K., Wang, Y.-J., Wang, M., Dai, Y.-C. & Liu, X.-Y. (2022b) Species diversity and ecological habitat of *Absidia* (Cunninghamellaceae, Mucorales) with emphasis on five new species from forest and grassland soil in China. *Journal of Fungi* 8: 471.

<https://doi.org/10.3390/jof8050471>

Zong, T.-K., Zhao, H., Liu, X.-L., Ren, L.-Y., Zhao, C.-L. & Liu, X.-Y. (2021) Taxonomy and phylogeny of four new species in *Absidia* (Cunninghamellaceae, Mucorales) from China. *Frontiers in Microbiology* 12: 677836.

<https://doi.org/10.3389/fmicb.2021.677836>

Supplementary Materials. The following supporting information can be downloaded at the DOI landing page of this paper:

SUPPLEMENTAL FIGURE 1. A species maximum likelihood phylogeny based on comparisons between the *LSU* regions of the *Absidia* species that are most closely related to *A. urquhartii*. For *A. urquhartii*, the strain names are provided, with their GenBank accessions in table 1. For other *Absidia* species, GenBank accessions are in grey font. The tree is rooted with *Chlamydoabsidia* (= *Absidia*) *padenii*. The numbers near the nodes are percentage values from bootstrapping (100 replicates).