Freshwater fungi from southern Australia: *Minivolcanus unicellularis* gen. et. sp. nov. and *Achrochaeta rivulata* sp. nov.

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Abstract

While conducting a survey of freshwater fungi in the temperate region of southern Australia, we came across two previously undiscovered anamorphic Ascomycota, a phoma-like coelomycete and a dictyochaeta-like hyphomycete. The coelomycetous fungus was classified in the family Morosphaeriaceae (Pleosporales) as a new genus, primarily supported by molecular data. We hereby introduce *Minivolcanus unicellularis*, the new genus and species, accompanied by both morphological and molecular evidence. Additionally, the dictyochaeta-like hyphomycete was placed in the genus *Achrochaeta* in the Chaetosphaeriaceae (Chaetosphaeriales) based on a combination of morphological characteristics and phylogenetic analyses utilising ITS, 28S, and TEF1 sequences. This newly identified species is proposed as *Achrochaeta rivulata*, the second species described within this genus. These findings expand our knowledge of fungal diversity in the region.

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Introduction

Freshwater fungi hold significant ecological importance in diverse freshwater ecosystems across the globe. These resilient organisms thrive in a variety of aquatic environments, including lakes, rivers, streams, and waterholes. They fulfil essential roles as decomposers, contributing to the breakdown of organic matter, and as endophytes, living within the tissues of plants and enhancing their health (Calabon et al. 2022, 2023). Furthermore, some species within this group act as pathogens, affecting both aquatic plants (Mazurkiewicz-Zapałowicz et al. 2017) and animals (Hill et al. 2019). Remarkably, a subset of freshwater fungi is known to form mycorrhizal associations, establishing symbiotic relationships with the roots of certain plants, thus playing a vital role in nutrient exchange (Neori and Agami 2017). The multifaceted functions of freshwater fungi...
underscore their significance in maintaining the balance and health of aquatic ecosystems worldwide. A total of 3870 freshwater species have been documented, primarily belonging to the Sordariomycetes and Dothideomycetes, with new discoveries continually expanding this number each year (Calabon et al., 2022). However, the total estimated diversity of freshwater fungal species globally is even more substantial, projected to be approximately 20000 (Gessner and Van Rysseghem 2003). This vast reservoir of undiscovered species highlights the need for ongoing exploration and recognition of the significant biodiversity that exists within freshwater environments.

Freshwater fungi encompass species that, either throughout their entire life cycle or during specific phases, depend on free-standing freshwater or utilize resources primarily of an aquatic or semi-aquatic nature as their substrate (Thomas 1996). It is important to note that this definition excludes spores or fungal DNA originating from terrestrial environments but found within freshwater habitats.

Coelomycete is a general term for anamorphs of Ascomycota and Basidiomycota which produce conidia within a cavity lined by fungal or fungal-host tissue (Nag Raj 1993). They are sometimes encountered in freshwater environments (Magaña-Dueñas et al. 2021a) and are most commonly classified within the Pleosporales (e.g. Acrocalymma aquatica (Zhang et al. 2012), Aquasubmersa miricensis (Zhang et al. 2012), Clohesomyces aquaticus (Zhang et al. 2012), Elongatopedicellata aquatica (Magaña-Dueñas et al. 2022), Halobyssothecium cohaiaense (Yang et al. 2023), Halobyssothecium kumingense (Calabon et al. 2021), Heterophoma polyopusiformis (Magaña-Dueñas et al. 2021a), and Neocurcibitoria aquadulcis (Magaña-Dueñas et al. 2021a)). However, there are also coelomycetes encountered in freshwater environments in other orders in the Dothideomycetes, (e.g. Capnodi-ales - Septoria linmamthi, Botryosphaeriales - Tiarosporella paludosa), as well as Leotiomycetes (e.g. Rhytismatales - Hypohelion scirpinum, Helotiales - Chaetomella raphigeri), and Sordariomycetes (e.g. Magnaporthales - Mycoleptodiscus terestris, Ampisphaera-ales - Pestalotiopsis submersus, Diaporthales - Phasectrostoma yomense) (Barbosa et al. 2013, Boonmee et al. 2021, Dong et al. 2020, Hyde 1993, Ingold 1954, Lamore and Goos 1978, Magaña-Dueñas et al. 2021a, 2021b, 2022, Révay and Gönczöl, 1990, Sati and Tiwari 1993, Shearer et al. 2015, Zhang et al. 2012).

Hyphomycetes are anamorphs of mostly Ascomycota and some Basidiomycota which form conidia on structures that are not enclosed (Seifert et al. 2011). Hyphomycetes are commonly found in freshwater environments (e.g. Bao et al. 2023, Fiuza et al. 2017, Fryar and Catcheside 2023a, 2003b, Wang et al. 2023, Yang et al. 2023).

During a survey of freshwater fungi on submerged wood in southern Australia, we encountered two undescribed fungi, a phoma-like coelomycete and a dictyo-ochaeta-like hyphomycete. The coelomycete forms pycnidial conidiomata with holoblastic, percurrently extending conidiogenous cells that produce single hyaline, aseptate conidia enclosed in a sheath. The observed morphology bears resemblance to the recognised anamorphs of the holomorphic genus Hongkongmyces in the Lindgomycetaceae (Pleosporales) (Tsang et al. 2014). Hongkongmyces, although initially described with H. pedis, an isolate originating from the foot of Homo sapiens, has since been extended to encompass other species thriving on submerged decaying wood, e.g. H. aquaticus (Dong et al. 2020) and H. brunniesporus (Zong-Long et al. 2021).

Despite the morphological similarity to members of the Lindgomycetaceae preliminary analyses using molecular data indicated that our unknown coelomycete may be a member of the family Morosphaeriaceae (Pleosporales). Suetrong et al. (2009) introduced the Morosphaeriaceae based on molecular data and teleomorphs, placing Morosphaeria and Helicosus within this family. The family now has four additional genera: Aquihelicascus (Dong et al. 2020), Aquilomyces (Knapp et al. 2015), Clypeolocus (Tanaka et al. 2015), and Neohelicosus (Dong et al. 2020). Members of Morosphaeriaceae are mostly saprotrophs on wood in marine and freshwater habitats (Yang et al. 2023) and reproduce primarily sexually. The ascomata are immersed or superficial, subglobose, conical or lenticular, and are dark coloured. They have clavate to cylindrical, thick-walled, fissitunicate asci with an ocular chamber and a non-amyloid apical ring. Ascospores are hyaline to brown, septate and can be with or without a sheath or cap (Suetrong et al. 2009). Neohelicosus aquaticus and Aquilomyces met-rosideri are the only species within Morosphaeriaceae with an anamorph recorded (Crous et al. 2021, Zhang et al. 2013). Neohelicosus aquaticus was originally observed on wood in fresh water as a teleomorph, but pycnidia of this species were induced to form in culture using water agar and pine needles (Zhang et al. 2013). Aquilomyces met-rosideri was recently described from living tissue of Metrosideros sp. (Myrtaceae) in New Zealand (Crous et al. 2021).

The unknown hyphomycete, collected on submerged decaying wood and dead inflorescence of Doryanthus exelso in a terrestrial habitat, exhibits a striking resemblance to the holomorphic genus Achrochaeta in the Chaetosphaeriaceae (Chaetosphaeriales) (Réblová et al. 2021). These authors outlined its distinction from the genus Dictyochaeta (Spegazzini 1923) to include fungi forming only a single layer of dematiaceous, macronematous conidiophores lacking setae and/or taller setiform conidiophores and producing smaller, hyaline, aseptate, clavate to dactyloid phialidic conidia. Achrochaeta has been previously documented on decay-
ing wood in Australia and New Zealand (Hughes and Kendrick 1968, Réblová et al. 2021). The teleomorph of Achrochaeta closely resembles that of Dictyochaeta, featuring glabrous perithecial ascoma, unitunicate asci, and hyaline, transversely septate ascospores, making it challenging to differentiate between the two genera based solely on the teleomorphic structures.

In this study, we present comprehensive morphological descriptions, comparisons, and phylogenetic analyses, utilising a multi-gene approach, to elucidate the characteristics and relationships of these two newly identified taxa.

Methods

Sampling and fungal strains

Submerged wood samples between 5 mm and 5 cm in diameter were collected from Scott Creek Conservation Park, South Australia from two streams that flow only during winter and are approximately 50 cm deep with a muddy base. The riparian vegetation is a mixture of native vegetation and invasive weeds. Determining the source plant species of the wood was not possible due to the high biodiversity of the region. There are 492 plant species recorded for the area, at least 30 of which produce woody branches. However, the dominant tree species are Eucalyptus baxteri, E. obliqua (Myrtaceae) and a mixture of Acacia (Fabaceae), Allocasuarina (Casuarinaceae), Banksia (Proteaceae), Hakea (Proteaceae) and Melaleuca (Myrtaceae) species (NatureMaps 2023).

Following the methods of Luo et al. (2018), Raja and Shearer (2008), and Shearer (1993) samples were sealed in plastic bags for transport to the laboratory, incubated in sterile plastic containers and regularly examined for the occurrence of fungi using a Leica MZ7s dissecting microscope over six months.

Morphological studies

Morphological observations were conducted on fresh material. Conidiophores or pycnidia that were found using the dissecting microscope were photographed using a Sony RX100 camera, described, and measured, then transferred to a drop of distilled water on a microscope slide using fine forceps. A cover slip was placed on the specimen, then the slide was examined using a Nikon Eclipse Ni or Olympus BX51 light microscope with differential interference contrast. All measurements were taken in distilled water and means ± standard deviation (SD) were calculated for sizes of pycnidia, conidiogenous cells, conidiophores, setae, and conidia. Images were captured using a Canon 6D camera or Olympus DP70 camera with Imaging Software Cell^D mounted on the microscope.

Fungarium specimens were deposited in the State Herbarium of South Australia (AD), Adelaide, Australia.

Isolation

Potato dextrose agar (PDA, BD micro; potato starch 4 g/L, dextrose 20 g/L, agar 15 g/L) was autoclaved, cooled to 60 °C, 100 mg/L streptomycin and 70 mg/L of penicillin added as filter-sterilised stock solutions and 10 mL poured into each 60 mm diameter plate.

Fresh pycnidia were sliced open using a sterile razor blade and the contents scooped out using a sterile micro needle. These were then transferred to 20 mL of sterile water in an Eppendorf tube and agitated for several seconds to suspend them. To gather conidia from conidiophores, the sterile micro needle was first moistened with sterile water, then used to pick up masses of conidia directly from conidiophores. As with pycnidia contents, the conidia were then transferred to 20 mL of sterile water in an Eppendorf tube and agitated for several seconds to suspend them. The suspensions were then pipetted onto PDA plates. Plates were incubated at room temperature and checked over 5 days for germinating spores using a Leica MZ7s dissecting microscope with an underneath light source. Germinated spores were picked off the agar surface using a sterile needle and transferred to individual PDA plates which were incubated at room temperature.

One living strain (CBS 148186) was obtained from Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, the Netherlands and was cultured on various nutrient media to evaluate colony characteristics, diffusible pigments, and growth patterns. These included cornmeal dextrose agar (CMD) (Oxoid Limited, containing 2% dextrose), malt extract agar (MLA), oatmeal agar (OA), potato-dextrose agar (PDA), and PCA (Crous et al. 2019). Colony characteristics were assessed in 4-week-old cultures grown in the dark at temperatures ranging from 20 to 23 °C. To induce sporulation, strains were also inoculated on cornmeal agar (CMA, Crous et al. 2019) supplemented with sterile stems of Urtica dioica.

Newly generated strains were deposited in the Queensland Plant Pathology culture collection (BRIP), Brisbane, Australia.

DNA extraction, amplification, and sequencing

Approximately 50 mg of fungal mycelium was transferred from the surface of agar cultures with a sterile scalpel and placed into a 1.5 mL tube. The tubes were then placed into a vacuum desiccator for 1 hour. A sterile 3 mm tungsten carbide bead (Qiagen) was inserted into each tube, then the tubes shaken in a bead beater (MP Fastprep-24 5G) for two sets of 40 s. Genomic DNA was isolated using a Qiagen DNeasy Plant Mini kit following the manufacturer’s protocols. The final DNA extracts were eluted into 100 μL of elution buffer provided with the kit.

Sequences from translation elongation factor 1-alpha (TEF1) were amplified with primers EF1-983F and EF1-2218R (Rehner and Buckley 2005). For nuclear ribo-
somal genes, primers ITS1/ITS 4 (White et al. 1990) were used to amplify ITS1, 5.8S and ITS2 and LR0R/LR5 (Vilgalys and Hester 1990; Rehner and Samuels 1994) to amplify the sequences from the 28S nrRNA gene. Reaction mixtures contained 1 μL (10 mM each) dNTPs, 1 μL (10 mM) of each primer, 0.25 μL hotStart Taq DNA polymerase (New England Biolabs), 1 μL DNA template, 5 μL buffer, and 16.75 μL sterile milliQ water to a final volume of 25 μL.

PCR amplification was performed in an Applied Biosystems 2720 Thermo Cycler. Cycling conditions for PCR were initial denaturation at 95 °C for 3 min; 35 cycles of denaturation at 95 °C for 1 min, annealing at 52 °C (ITS) or 54 °C (28S) for 50 s, and extension at 72 °C for 1 min; and a final extension at 72 °C for 10 min. Cycling conditions for TEF1 were 95 °C for 1 min; 35 cycles of 95 °C for 30 s, 57 °C for 50 s, and 68 °C for 1 min; followed by 68 °C for 5 min. PCR amplicons were visualised on 1.5% agarose electrophoresis gels stained with Gel Red (Gene Target Solutions).

PCR products were purified using a Qiagen QiAquick PCR Purification Kit and sequenced in both directions using the respective primers by the Australian Genome Research Facility. Raw sequence reads were assembled, examined, and edited using Sequencher v. 5.3 (Gene Codes Corporation). Newly generated sequences were submitted to NCBI GenBank under the accession numbers listed in tables 1 and 2.

**Phylogenetic analyses**

The generated sequences for each gene were subjected to megablast searches (Zhang et al. 2000) to identify closely related sequences in NCBI's GenBank nucleotide database.

Other sequences used in this study were derived from GenBank.

BLASTn searches indicated that the unknown coelomycete is a member of the Morosphaeriaceae (Pleosporales, Dothidiomycetes). In order to investigate the relationships between our unknown coelomycete and other representatives of the Morosphaeriaceae we constructed an alignment based on ITS, 28S and TEF1 loci using our two specimens from different wood samples along with representative sequences from each genus within the Morosphaeriaceae and morphologically similar species within the Pleosporales. Sequences of each gene were aligned in Geneious Prime v. 2023.0.4 (https://www.geneious.com) using MUSCLE then ambiguously aligned regions were removed from the alignments using GBLOCKS v. 0.91b (Castresana 2000, Talavera and Castresana 2007) on the Phylogeny.fr platform (Dereeper et al. 2008). Alignments of single gene sequences were then concatenated and a maximum-likelihood phylogenetic tree was constructed with partitions for each gene region using RAxML v. 8.2.11 (Stamatakis 2014) within Geneious and branch support values were calculated with 1000 rapid bootstrap inferences. The dataset including alignment gaps comprised 1990 characters: 341 for ITS, 822 for 28S, and 827 for TEF1. The outgroup strains were selected from members of the Jahnulales as they are within the Dothideomycetes, but not members of the Pleosporales.

In a megaBLAST search of ITS sequences, the dictyochaeta-like species was most similar to Achrochaeta talboti (strain ICMP 15161; ITS sequence MT4544480, Réblová et al. 2021) of the Chaetosphaeriaceae exhibiting 92% similarity. In order to resolve the relationships between the three strains of the new hyphomycete and other species within the Chaetosphaeriaceae we performed analyses of combined ITS, 28S and TEF1 sequences. The species included in the phylogenetic analyses comprised Achrochaeta and various members of morphologically similar genera such as Dictyochaeta, Brachydictyochaeta, and other closely related chloridi um-like genera, as per the study conducted by Réblová et al. (2023). The outgroup was selected from members of Chloridium, based on the known close relationship between the major clades of the family, as indicated by the findings of Réblová et al. (2023). The gene sequences, including those newly generated in this study and those of members of the Chaetosphaeriaceae retrieved from GenBank, underwent alignment using MAFFT v. 7.487 (Katoh and Standley 2013), a tool implemented within the CIPRES Science Gateway v. 3.3 (Miller et al. 2010). Where necessary, manual adjustments were applied using BioEdit v. 7.1.8 (Hall 1999). To refine the alignment, we utilized consensus secondary structure (2D) models previously developed for ITS1 and ITS2 regions in Chloridium and related taxa (Réblová et al. 2022). This enhancement involved comparing nucleotide positions within helices and loops. Additionally, the LSU alignment was improved through the incorporation of a predicted 2D model of the 23S RNA obtained from Saccharomyces cerevisiae (Gutell et al. 1993).

A maximum-likelihood phylogenetic tree was constructed with partitions for each gene region using RAxML within Geneious and branch support values were calculated with 1000 rapid bootstrap inferences. The dataset including alignment gaps comprised 3302 characters: 349 for ITS, 1761 for 28S, and 992 for TEF1. For both analyses the GTR+G+I substitution model was used for all partitions as recommended by Abadi et al. (2019). The same alignment was analysed with MrBayes v 3.2.6 (Huelsenbeck and Ronquist 2001) within Geneious with the default settings. Markov chain Monte Carlo (MCMC) parameters were a subsampling frequency of 200, chain length 1100000, 4 heated chains, and burn in length of 100000. The maximum-likelihood and Bayesian trees were compared visually for topology and support for clades. All resulting trees were formatted in Geneious, then further edited in Adobe Illustrator v. 27.0.
Table 1. GenBank accession numbers of strains used for phylogenetic analyses of *Minivolcanus unicellularis.* Newly generated sequences are shown in bold. T = holotype specimen.

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*Minivolcanus unicellularis*  

| AD291626 | OQ799384 | OQ799383 | OQ866586 |

*Minivolcanus unicellularis* T AD291633 OQ799382 OQ799391 OQ866585

Morospheria muthuputensis  
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Morospheria velatispora  
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Neohelicascus aquaticus  
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Neohelicascus galicus  
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Neohelicascus submersus  
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Periconia cyperacearum  
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Pheosphaeria sinensis  
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Pseudoasteromassaria aquatica  
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Pseudoasteromassaria spadicea  
MFLUCC 15-0973 NR_168162 NG_068792 -
Table 2. GenBank accession numbers of Chaetosphaeriaceae species used for phylogenetic analyses. Newly generated sequences are shown in bold. T = holotype specimen, E = epitype specimen.

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Results

Phylogenetic analyses

The initial phylogenetic analyses of newly generated ITS, 28S and TEF1 sequences positioned the two strains of our new coelomycete as a distinct lineage within a clade representing Morosphaericaeae (Pleosporales), adjacent to the subclade containing Aquilomyces and Clypeolocus (Fig. 1). The Morosphaericaeae form a well-supported clade (100/1) containing seven genera within the Pleosporales. Species within the Pleosporales which form coelomycetous anamorphs were included in the phylogram for comparison. To elucidate the relationships among three strains of the dictyochaeta-like species, we conducted a phylogenetic analysis using a data set comprising combined ITS, 28S and TEF1 sequences of 38 members of the Chaetosphaeriaceae. The three strains of this yet-unde-
Fig. 1 Phylagram generated from maximum likelihood analysis based on combined ITS, 28S and TEF1 sequence data of Minivolcanus unicellularis and closely related taxa within the Pleosporales. Jahnula dianchia (MFLUCC 16-1353) and Aliquandostipite khoaiensis (MFLUCC 21-0106) were used as outgroup taxa. Bootstrap values equal to or greater than 70% and Bayesian posterior probabilities equal to or greater than 0.95 are given next to the nodes.

scribed dictyochaeta-like species formed a well supported clade (95/1), grouping as a sister to Achrochaeta talbotti (Fig. 2). This finding indicates the presence of a new species within this group. Achrochaeta was nested in a well-supported clade, which also included Calicium, Caligospora, Crasperodidymum elatum, and Papillospora. The phylogenetic analysis affirmed the distinctiveness of Achrochaeta from genera like Dictyochaeta and Brachydictyochaeta, highlighting their separate lineages.

Discussion

Based on morphological differences and multi-gene phylogenies, we introduce the new genus Minivolcanus and the two new species Minivolcanus unicellularis and Achrochaeta rivulata.

Minivolcanus forms a well-supported clade within the Morosphaeriaceae based on phylogenetic analyses of combined ITS-28S-TEF1 sequences (Fig. 1). While coelomycetes are rare within Morosphaeriaceae, Pleosporales commonly have both coelomycetous and hyphomycetous anamorphs (Yu et al. 2022) with coelomycetous morphs being the most prevalent (Zhang et al. 2012). Phoma or phoma-like anamorphs are the most common anamorphs of Pleosporales (Zhang et al. 2012). Minivolcanus unicellularis has many similarities to these anamorphs such as pycnidia that are immersed to erumpent, ostiolate, dark-coloured, with annellidic conidiogenous cells and globose to ovoid, unicellular conidia (Kohlmeyer and Kohlmeyer 1979). The conidia of M. unicellularis are remarkably similar to those of Hongkongmyces species which initially led us to think that they were congeneric. Hongkongmyces aquaticus, H. khoaiensis and M. unicellularis all have globose to subglobose, hyaline, aseptate conidia with large oil globules. However, the conidia of H. aquaticus and H. Khoaiensis do not have a sheath and the conidiogenous
Fig. 2 Phylogram generated from maximum likelihood analysis based on combined ITS, 28S, and TEF1 sequence data of Achrochaeta rivulata and closely related taxa within the Chaetosphaeriaceae. Chloridium species were used as the outgroup taxa. Bootstrap values equal to or greater than 70% and Bayesian posterior probabilities equal to or greater than 0.95 are given next to the nodes.

Other species within the Pleosporales that form coelomycetous structures are both morphologically and phylogenetically different from M. unicellularis (Fig. 1). Acrocalymma (Acrocalymmaceae) species also have holoblastic conidiogenesis, but only A. fici has percurrent proliferation, and all have cylindrical conidia, often with appendages (Dong et al. 2020). Stagonospora haliclysta is also holoblastic like M. unicellularis but S. haliclysta has no proliferation of the conidiogenous cells.
and conidia that are fusiform and septate with appendages and no sheath (Kohlmeyer 1973). *Stagonospora halicysta* was not included in the phylogenetic analyses because there are no available molecular data for this species (GenBank 2023). *Closelyomycetes aquaticus* is also holoblastic and has unicellular, hyaline conidia with a sheath but the conidia do not have a large oil globule and are ellipsoid rather than globose to subglobose (Hyde 1993). *Aquasubmersa japonica* and *A. micrensis* are both holoblastic with hyaline, asceptate conidia, but they are ellipsoid, do not have a large oil globule and do not have percurrent proliferation (Aryawan et al. 2015; Zhang et al. 2012). *Halobyssotheicum bambusicola* and *Pseudoasteromassaria spadicea* have similarly shaped conidia to *M. unicellularis* with a large oil globule, but both lack a sheath, the conidiogenous cells are phialidic and do not proliferate (Calabon et al. 2021; Tibpromma et al. 2017). *Macrodiploidiopsis desmazeri* also has holoblastic conidiogenous cells with percurrent proliferation and a sheath on the conidia, but the conidia are brown, septate and ellipsoid to obovoid or clavate (Wijayawardene et al. 2014).

*Minivolcanus* is not unusual within Morosphaeriaceae in being from a freshwater habitat. A number of species from Morosphaeriaceae are found in fresh water including *Aquihelicascus thalassodi*eus, *A. yunnanensis*, *A. songklaensis* (Dong et al. 2020), *Clypeoloculus alitae*nsis, *C. hirosakiensis*, *C. microsporus*, *C. towadaensis* (Tanaka et al. 2015), *Helicascus alatus* (Zeng et al. 2018), *Neohelicascus aquaticus*, *N. aegyptiacus*, *N. chiangraiensis*, *N. elaterascus*, *N. gallicus*, *N. submersus* (Dong et al. 2020), *N. unilocularis* (Zhang et al. 2015), and *N. uniseptatus* (Luo et al. 2016).

The genus *Achrochaeta*, based on *A. talbotii*, has been recently erected for species segregated from the genus *Dictyochaeta* (R évlova et al. 2021) and characterised by cylindrical-clavate, non setulose conidia, mono- and polyphialidic conidiogenous cells with a single locus and conidiophores forming a single layer, sometimes accompanied by sterile, apically rounded setae. The existence of setae is newly confirmed in both species and the generic description of *Achrochaeta* is emended in this study. *Achrochaeta rivulata* is accepted as a second species in the genus. Both species are phenotypically very similar and their occurrence is confirmed only in the Southern hemisphere in Australia and New Zealand (Hughes and Kendrick 1968, Réblová et al. 2021, this study).

Members of *Achrochaeta* are also similar to *Codinaea clavulata* (Gams & Holubová-Jechová 1976) and *Chloridium codinaeoides* (Pirozynski 1972). *Codinaea clavulata* has taller conidiophores (up to 340 µm long), very shallow collarette and short sympodial elongations at the tip of the phialide. *Chloridium codinaeoides* can be distinguished by phialides only slightly tapering towards the collarette, a slightly widening or almost cylindrical collarette and narrowly fusiform to clavate, sometimes slightly allantoid conidia. *Brachydictyochaeta*, (Wu and Diao 2022) shares certain similarities with *Achrochaeta*. It features setiform conidiophores that terminate in a hyaline to subhyaline apical cell, which is broadly rounded and consistently sterile, resembling the setae of *Achrochaeta*. However, *Brachydictyochaeta* is distinguished by possessing setiform conidiophores with discrete phialides, sometimes with short branches, or solitary phialides located near the base of conidiophores. Additionally, its conidia are much longer and are characterized as falcate, lunate, or acerose, as documented by Castañeda Ruiz (1988) and further detailed by Wu and Diao (2022).

*Achrochaeta* forms a sister clade to *Papillospora* (Fig. 2), which is a monotypic genus (R évlova and Nekvindová 2023). While they exhibit similarities in both teleomorphic and anamorphic traits, they can be readily distinguished. Both genera have dark ascomata, cylindrical-clavate asci with eight fusiform, 3-septate, hyaline ascospores. The setae found alongside the conidiophores are erect, unbranched, brown, slightly inflated at the apex with a terminal hyaline to subhyaline cell. The conidiophores are macronematous, mononematous, erect, brown, and cylindrical. They terminate into phialides with narrow collarettes and produce non-septate, hyaline, nonsetulate, and smooth conidia. However, the conidiophores of *Papillospora* are sparsely branched whereas those of *Achrochaeta* are always unbranched. Phialides of *P. hebetiseta* are terminal, integrated or intercalary in comparison to *Achrochaeta* spp. with terminal phialides only that have a single apical opening or up to four lateral openings formed by successive sympodial elongation (in culture). In addition, the collarettes of *P. hebetiseta* are not flared compared to narrowly funnel-shaped collarettes of *Achrochaeta* (R évlova and Gams 2000, R évlova and Nekvindová 2023). Furthermore, the ascospores of *Papillospora* are finely verrucose.

**Taxonomy**

*Minivolcanus* Fryar & D.E.A. Catches. gen. nov.

**Type species:** *Minivolcanus unicellularis* Fryar & D.E.A. Catches.

**Etymology:** *mini-* (Latin) meaning small and *vulcanus* (Latin) for volcano, referring to the volcano-like shape of the pycnidia on wood.

**Description:** *Conidiomata* pycnidial, immersed in substrate, semi-immersed, or superficial, globose to ellipsoid, black, ostiolate. *Conidiomatal wall* irregular thickness, made of indistinct dark brown cells and an inner layer of hyaline, ellipsoid, thick-walled cells. *Conidiogenous cells* hyaline, cylindrical, holoblastic, with per-
current proliferation. *Conidia* hyaline, appendages absent, sheath present.

**Minivolcanus unicellularis** Fryar & D.E.A.

*Catches. sp. nov.*

**MB848294**

**Typos:** AUSTRALIA, South Australia, Scott Creek Conservation Park (S35° 5' 45.90", E138° 40' 59.16) on submerged decaying wood in an ephemeral stream, S.C. Fryar, 26 August 2020 (holotype fungarium specimen AD291633, ex-holotype strain BRIP 76123). GenBank numbers: ITS - OQ799382; 28S - OQ799391; TEF1 - OQ866585.

**Fig. 3**

**Etymology:** *ūni-* derived from *ūnus* (Latin) one, *cellulāris* (Latin) cellular, referring to the aseptate conidia.

**Description:**

**Teleomorph** undetermined. **Anamorph:** On natural substrate. *Conidiomata* pycnidial, immersed in substrate, semi-immersed or rarely superficial, globose to ellipsoid, lying horizontal to substrate, scattered, black, ostiolate, (134-)335–480 x (135-)194–480 μm (x = 404.2 ±171.9 x 289 ± 138.2 μm). *Conidiomatal wall* made of indistinct dark brown cells, irregular thickness up to 80 μm and an inner layer of hyaline, ellipsoid, thick-walled cells, irregular thickness up to 50 μm. *Conidiogenous cells* hyaline, cylindrical, holoblastic, with conspicuous multiple percurrent proliferations, 11–35 x 2–4.5 μm (x = 19.1 ± 6.0 x 3.2 ± 0.7 μm). *Conidia* globose to subglobose, hyaline, aseptate, one large oil globule and multiple smaller oil globules, appendages absent, thin irregular sheath surrounds most conidia, 11–18 x 10–15 μm (x = 15.4 ± 1.6 x 12.4 ± 1.1 μm).

**Culture characteristics:** On **PDA** after 13 days 18 x 14 mm, irregular shape, 1 main colony, several satellite colonies, grey tinged with brown, margin white, 1 mm, slimy appearance, centre fluffy, Agar not discoloured. Reverse grey interior, white margin. Hyphae hyaline, twisted and intertwined, branched, septate, 2.5–3 μm wide. Aerial hyphae hyaline to dark grey, breaking easily, verrucose, 2–4 μm wide.

**Habitat and geographic distribution:** Saprobic on decaying wood in an ephemeral freshwater stream. Known from Australia.

Additional material examined: AUSTRALIA, South Australia, Scott Creek Conservation Park (S35° 5' 45.90", E138° 40' 59.16) on submerged decaying wood in an ephemeral stream, S. Fryar, 26 August 2020 (fungarium specimen AD291626, living culture BRIP 76128; *ibid.*, fungarium specimens AD291608, AD291689). GenBank numbers: (fungarium specimen AD291626, living culture BRIP 76128) ITS - OQ799384; 28S - OQ799383; TEF1 - OQ866586.

Notes: The majority of species within the Morosphaeriaceae are described based on their teleomorphs, making it challenging to conduct morphological comparisons when only anamorphic characters are available. However, the coelomycetous anamorphs of *Neo helicascus aquaticus* and *Aquilomyces metrosideri*, both members of the Morosphaeriaceae, are described (Zhang et al. 2013). *Minivolcanus unicellularis* differs from *N. aquaticus* in many aspects. The conidia of *M. unicellularis* are globose to subglobose compared with the ellipsoid to obovoid conidia of *N. aquaticus*, the conidiogenous cells of *M. unicellularis* are larger, and percurrently elongating, and the pycnidia are immersed and black, rather than superficial and brown. Both species have holoblastic conidiogenous cells. *Aquilomyces metrosideri* is also distinctly different to *M. unicellularis*. It has phialidic rather than holoblastic conidiogenous cells and smaller, subcylindrical conidia without a sheath (Crous et al. 2021).

**Achrochaeta Rébllová & Hern.-Restr.**

**MB835573**

**Type species:** *Achrochaeta talbotii* (S. Hughes, W.B. Kendr. & Shoemaker) Rébllová & Hern.-Restr.

**Emended description:** Colonies effuse, hairy, gray to black on the natural substrate, composed of conidiophores and/or ascomata. *Ascomata* perithecial, nonstromatic, superficial, subglobose, papillate, dark brown to black, glossy, glabrous or clothed with conidiophores of the anamorph. *Ostiole* periphysate. *Ascomatal wall* fragile, carbonaceous, two-layered. *Paraphyses* hyaline, septate, persistent, longer than the asci. *Asci* uniseriately, 8-spored, cylindrical to cylindrical-fusiform, short-stipitate, ascal apex with a nonamyloid apical annulus. *Ascospores* ellipsoid to ellipsoidal-fusiform, straight or inequilateral, transversely septate, hyaline, 2-seriate or obliquely 1-seriate within the ascus. *Setae*, when present, unbranched, upright, straight or flexuous, dark brown towards the base, paler towards the apex with a hyaline subhyaline, broadly rounded to inflated, always sterile apical cell. *Conidiophores* macroconamous, mononematous, unbranched, upright, straight or flexuous, cylindrical, brown. *Conidiogenous cells* phialidic, terminal, integrated, with an apical opening or several lateral openings formed by successive sympodial elongation. *Collarettes* narrowly funnel-shaped, hyaline. *Conidia* cylindrical-clavate to ellipsoid or dacyroid, straight or slightly curved, nonseptate, nonsetulate, hyaline, smooth, accumulating in slimy, colorless droplets on the conidiogenous cells.

**Accepted species:** *Achrochaeta rivulata, A. talbotii*

**Habitat and geographical distribution:** The genus includes species that are saprobes on decaying wood and bark in terrestrial and freshwater habitats. Known only from the southern hemisphere (Hughes and Kendrick 1968, Rébllová et al. 2021, this study).

Notes: The description is extended by the existence of setae that occur on the natural substrate (observed only
Fig. 3 Minivolcanus unicellularis. (holotype AD AD291633). a Culture of Minivolcanus unicellularis (holotype, AD291633) on 60 mm PDA plate after 13 days. b–c Pycnidia on host surface. d Cross section through a pycnidium showing pycnidal wall, conidiogenous cells and conidia. e–f Conidiogenous cells (percurrent proliferation shown in e). g–h conidia. Scale bars: a = 1 cm, b–c = 100 μm, d = 10 μm, e–h = 5 μm
in A. rivulata) and they also develop in vitro (observed in both species), and by additional shapes of conidia.

**Achrochaeta rivulata** Fryar, D.E.A. Catches. & Rébélová sp. nov.

MB848295

**Typus:** AUSTRALIA, Scott Creek Conservation Park (S35° 5' 46", E138° 40' 59"), on decaying wood submerged in an ephemeral stream, 26 August 2020, S.C. Fryar (holotype: fungarium specimen AD291612, ex-holotype culture BRIP 76129). GenBank numbers: ITS - OQ799381; 28S - OQ799389; TEF1 - OQ866587

**Fig. 4, 5 & 6**

Etymology: derived from *rivulatus* (Latin), inhabiting a small stream or rivulet.

**Description:** **Teleomorph:** Undetermined.  **Anamorph:** On natural substrate.  **Conidiophores** mononematous, macronematous, 77–150(-170) × (2.5–)3–4 µm (x = 110 ± 23.8 x 3.1 ± 0.3 µm), simple, dark brown, hyaline or subhyaline at the apex, septate, single or branched at the base into 3, straight or slightly flexuous, cylindrical.  **Setae** 70–155 × 3(-3.5) µm (x = 108.9 ± 28.7 x 3.0 ± 0.2 µm), scattered among conidiophores, rounded, slightly bulbous apex, dark brown at base graduating through to subhyaline at the apex, septate.  **Conidiogenous cells** 17–37 × 3–3.5 µm (x = 24 ± 4.9 x 3.1 ± 0.2 µm), monophialidic, tapering towards the apex, hyaline to subhyaline.  **Collarettes** funnel shaped, 1.5–2.5 × 1.5–2 µm (x = 1.8 ± 0.3 x 1.8 ± 0.2 µm). **Conidia** (6–)7–10 × 2–3 µm (x = 7.8 ± 0.9 x 2.4 ± 0.3 µm), hyaline, aseptate, without appendages, straight or curved, no ornamentation or sheath, smooth, ellipsoid, one end rounded, one end pointed.

Culture characteristics: **On PDA:** Colonies on PDA after 4 weeks 12–15 mm diam., margin undulate, finely furrowed, velvety, dark grey in patches, white in patches, white outer zone, slightly convex (indentated), reverse dark grey, with white margin. **On CMD:** Colonies 28–29 mm diam., flat, margin entire, velvety, white, reverse ivory. **On MLA:** Colonies 44–45 mm diam., convex, margin entire, velvety, mucoid towards the margin, white with paler zones of sparse growth, zonate, reverse pale ochre. **On OA:** colonies 48–50 mm diam, flat, margin entire, felty, white, with whitish-grey zones of sporulating conidiophores, reverse white. **On PCA:** colonies 40–43 mm diam., flat, margin entire, very similar to those on OA, aerial mycelium is better developed, lanose, floccose, mucoid towards the margin, whitish centrally, becoming whitish-grey due to sporulating conidiophores, reverse white-beige.

Description in culture: **Teleomorph:** Not observed.  **Anamorph:** Setae, conidiophores, conidiogenous cells and conidia similar to those on the natural, although conidiophores can be sometimes reduced and setae absent.  **On PDA:** Vegetative hyphae hyaline, septate, branching, 2.5–4 µm wide. **Sheath** 8–18 × 7–10 µm, on some of the darker hyphae, often near the base of the conidiophores. **Conidiophores** 12–54 × 2.5–4 µm, semimacronematous, tapering towards the apex, subhyaline to brown, often reduced to single conidiogenous cells, which are monophialidic, collarette funnel-shaped 1–2 x 1–2 µm. **Conidia** 5–13 × 2–3 µm, hyaline, mostly straight, sometimes curved, aseptate, without sheaths or appendages, ellipsoidal-clavate to dacyroid, tapering towards the base, broadly rounded apically. **On MLA:** Colonies effuse, hairy, vegetative hyphae 1.5–3 µm diam., branched, septate, hyaline or pale brown, smooth.  **Setae** 48–96 × 3–3.5(-4) µm (x = 68 ± 14.6 x 3.5 ± 0.2 µm) are sometimes present among conidiophores, solitary, erect, straight, cylindrical, septate, brown, paler towards the apex with a hyaline to subhyaline, broadly rounded to inflated apical cell, always sterile. **Conidiophores** (25–)42–124 × 2.5–3.5 µm (x = 73.8 ± 25.1 x 2.9 ± 0.2 µm), macronematous, solitary or crowded, erect, straight or gently curved, cylindrical, unbranched, dark brown, paler towards the apex, occasionally reduced to single conidiogenous cells. **Conidiogenous cells** (11–)19–25 × 2.5–3.5 µm (x = 18.9 ± 4.2 x 3.1 ± 0.3 µm), tapering upwards and abruptly constricted to ca. 1(-1.5) µm below the collarette, integrated, terminal, with a single conidiogenous locus, monophialidic, extending percurrently, rarely polyphialidic, extending sympodially with 1–2 lateral openings, subcylindrical, pale brown, paler towards the apex; collarettes 3–3.5 µm wide, ca. 2.5 µm deep (x = 3.2 ± 0.3 x 2.5 ± 0.1 µm), flaring, narrowly funnel- to vase-shaped, hyaline, partially disintegrating. **Conidia** (5–)5.5–7.5 × 1.5–2(-2.5) µm (x = 6.6 ± 0.8 x 1.9 ± 0.2 µm), cylindrical-clavate, straight to slightly curved, obtuse at the apical end, gradually tapering towards the basal end, aseptate, non-setulate, hyaline, smooth, adhering in slimy white heads. Chlamydospores absent.

Habitat and geographic distribution: **Saprobic** on decaying wood in an ephemeral freshwater stream and on dead inflorescence. Known only from Australia.

Additional material examined: AUSTRALIA, South Australia, Scott Creek Conservation Park (S35° 5' 46", E138° 40' 59"), on decaying wood submerged in a stream, 26 August 2020, S.C. Fryar (fungarium specimens AD291619, AD291630, AD291604, AD291618, AD291619, AD291604, AD291618, AD291605). AUSTRALIA, New South Wales, near Kalnura on Springhill Rd., on dead inflorescence of *Doryanthes excelsa*, 23 August 1999, Keith A. Seifert K.A.S. 1199 (living culture CBS 148186).

GenBank numbers: (fungarium specimen AD291619, living culture BRIP 76130) ITS - OQ799348, 28S - OQ799390, TEF1 - OQ866588; (CBS 148186) ITS - OR286508, 28S - OR286551, TEF1 - OR326680.

The two species of *Achrochaeta* share many characters such as unbranched, brown, septate conidiophores with hyaline phialidic conidiogenous cells, and funnel-
Fig. 4 *Achrochaeta rivulata* (holotype AD291612). a Conidiophores on natural substrate. b Seta. c Conidiogenous cell. d–f Conidiophores. g–j Conidia. Scale bars: a = 500 μm, b = 20 μm, c–j = 5 μm

shaped collarettes and hyaline, aseptate, asymmetrical conidia. *Achrochaeta rivulata* closely resembles *A. talbotii* but differs from it by producing conidia that are ellipsoid-clavate instead of cylindrical-clavate and by form-
ing, in nature, exclusively phialides with a single apical opening (although sometimes with lateral openings in culture). On the other hand, *A. talbotii* (ICMP 15161) predominantly forms monophialides and rarely polyphialides with several lateral openings. In *A. talbotii* (ICMP 15161), setae have not been yet observed on material from nature (Hughes and Kendrick 1968, Réblová et al. 2021). However, Réblová et al. (2021) noted that some of the conidiophores appeared sterile, shorter, and ended in a subhyaline, rounded apical cell. These structures, scattered among regular conidiophores and observed only *in vitro*, are consistent with setae observed in *A. rivulata* on material from nature and in culture.

*Achrocheata talbotii* was originally described as *Chaetosphaeria talbotii* (Hughes and Kendrick 1968). The original collection of *C. talbotii* by Hughes and Kendrick was in Kuitpo Forest, South Australia, which is 20 km from the collection site of *A. rivulata*.

There are substantial differences in morphology of *A. rivulata* when grown on different substrates. On PDA, the conidiophores are shorter than when grown on MLA
Fig. 6 Culture of Achrochaeta rivulata (CBS 148186). a Colonies on 100 mm CMA plate with Urtica dioica stem after 4 weeks. b Sporulating conidiophores. c, d, f Conidiophores. e Setae. g-i Conidiophores reduced to single conidiogenous cells. k Diversity of colony morphology on 50 mm plates of CMD, MLA, OA, and PCA, respectively (from left to right) after 4 weeks. Scale bars: a, k = 1 cm, b = 500 μm, c-j = 10 μm
or water. There are setae present when grown on wood and MLA, but not on PDA. The setae growing on MLA are also shorter than those growing on wood. The col-larettes are larger in MLA than on wood and PDA, and the conidia are much smaller on MLA compared with those on PDA and wood. Also, sympodial extensions were observed on conidiogenous cells grown on MLA, but not PDA or wood.

Disclosures

The authors declare that there are no competing interests.

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