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Diversity of *Backusella* (*Mucoromycotina*) in south-eastern Australia revealed through polyphasic taxonomy

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Backusella
genome sequencing
invertase
Mucorales
new taxa
polyphasic taxonomy
zygospore

Abstract Here we explore the diversity of one morphologically distinguishable genus in the *Mucoromycotina*, *Backusella*, in south-eastern Australia. We isolated more than 200 strains from locations across the states of Victoria and Tasmania. Characterization of these strains using a combination of approaches including morphology, sucrose utilization and whole genome sequencing for 13 strains, revealed 10 new species. The genetic basis for interspecies variation in sucrose utilization was found to be the presence of a gene encoding an invertase enzyme. The genus *Backusella* is revised and a new key for species identification produced. Given that we have more than doubled the number of species in this genus, this work demonstrates that there may be considerable undiscovered species diversity in the early diverging fungal lineages.

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INTRODUCTION

Advances in DNA sequencing have increased the rate of discovery of new fungal species to over 2 000 species per year. However, despite their evolutionary and ecological significance, the basal fungal lineages not in the *Dikarya* represent only 1 % of this figure or just 24 species described in 2017 (Willis 2018). Of these lineages, species in the order *Mucorales* are significant decomposers in natural ecosystems, and some species are pathogens both to humans (Ribes et al. 2000) and other animals, such as *Mucor amphibiorum* of amphibians and platypuses (Obendorf et al. 1993). Many *Mucorales* species produce spores that are not optimized for dispersal in air, which might account for the development of greater endemic diversity compared to many ascomycetes and basidiomycetes. However, our understanding of the biogeography of these species is currently limited, and this hypothesis is largely untested.

Despite being considered a 'megadiverse' country (Williams et al. 2001) only a handful of *Mucorales* species have been described as being unique to Australia. After some initial work at the end of the 19th century by Cooke and Massee (discovery of *Spinellus gigasporus* (Cooke 1889) and *Pilobolus pullus* (Massee 1901)) the next productive period, in terms of describing diversity, was in the 1970–1980s (for example *Halteromyces radiatus* (Shipton & Schipper 1975), *Mucor amphibiorum* (Schipper 1978), *Umbelopsis ovata* and *U. fusiformis* (Yip 1986b), *U. swartii* and *U. westeae* (Yip 1986a), and *Mucor laxorrhizus* var. *ovalisporus* (Schipper 1989)). However, since this time new species reports have slowed, despite rapid progress elsewhere in fungal taxonomy. Only two new species, *Pilaira australis* and *Syncephalastrum contaminatum*, have been described, both from single specimens, in the last 30 yr (Urquhart et al. 2017, Urquhart & Idnurm 2020).

Two hypotheses might explain the lack of reported diversity in Australia. One is that the continent is depauperate in *Mucoromycotina* species and the second is the consequence of limited sampling. In this study we set out to address this dearth of knowledge by examining diversity in the genus *Backusella* in south-eastern Australia. *Backusella* is a convenient genus within the *Mucorales* to study because it can be readily distinguished in culture by its recurved juvenile sporangiophores (Walther et al. 2013). Currently, the genus consists of 14 species: *B. circina* (Ellis & Hesseltine 1969), *B. constricta* (Lima et al. 2016), *B. gigacellularis* (De Souza et al. 2014), *B. granulispora* and *B. johoriensis* (Loh et al. 2001), *B. lamprospora* (Benny & Benjamin 1975), *B. locustae* (Wanasinghe et al. 2018), *B. grandis*, *B. indica*, *B. oblongielliptica*, *B. oblongispora*, *B. recurva*, *B. tuberculispora*, and *B. variabilis* (Walther et al. 2013). It has previously been noted that *B. grandis* is likely to be a synonym of *B. variabilis* (Walther et al. 2013). The only report of *Backusella* in Australia is of *Backusella recurva* (strain CBS 673.75), isolated from north Queensland.

Here, through sampling from over 25 locations in south-eastern Australia more than 200 strains of *Backusella* were isolated and then analysed. Using a polyphasic approach integrating whole-genome-sequencing-based molecular phylogenies, morphology and physiology we identify 10 new species, one new combination (*Backusella dispersa*) and suggest synonymisation for two previously described species (*B. johoriensis* = *B. circina*; *B. variabilis* = *B. grandis*), and discuss how the taxonomy of *B. granulispora* does not conform to a modern morphological understanding of the genus. Collectively, the new species and refinements to existing taxa adjusts the total number of species in the genus from 14 to 23. As such, these findings provide an example in which diversity in a single genus is markedly increased by sampling in Australia, a potential indicator of a high level of diversity among Australian fungi.

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MATERIALS AND METHODS

Isolation of strains

Leaf litter and soil samples were collected from locations in the Australian states of Victoria and Tasmania under permits 10008557 (Victorian Department of Environment, Land, Water and Planning) or FL 18158 (Tasmanian Department of Primary Industries, Parks, Water and Environment). Samples of soil (c. 7 g each) were mixed with sterilized water and then plated onto potato dextrose agar (PDA) supplemented with cefotaxime (100 µg/mL) and chloramphenicol (100 µg/mL) to inhibit bacterial growth. After 2–4 d growth at ambient temperature, colonies displaying the characteristic recurved juvenile sporangia were selected and plated onto fresh medium. All isolates were purified by single spore isolation to ensure a homogeneous culture.

DNA extraction and amplicon sequencing

DNA was extracted from fungal material scraped from agar culture as described previously (Pitkin et al. 1996). *Taq* polymerase purified from the pTaq plasmid (Desai & Pfaffle 1995) was used for polymerase chain reaction (PCR) following standard procedures. The internal transcribed spacers (ITS) were amplified with primers ITS1 and ITS4 (White et al. 1990); the large subunit rRNA (LSU) was amplified with primers NL1 (Kurtzman & Robnett 1997) and LR3 (Vilgalys & Hester 1990); and a partial arginosuccinate lyase gene fragment (*argA*) was amplified using primers AP52 (5′ TGGGGAGGTCGYTTCTCC 3′) and AP53 (5′ TATCAGGRTTCTTCTTTTGAGG 3′), designed based on examination of the whole genome sequencing data described in the following sections. PCR products were purified with a gel purification kit (Qiagen) and Sanger-sequenced at the Australian Genome Research Facility (AGRF).

It was necessary in some cases to clone the ITS sequences before sequencing due to different versions occurring in the same strain. To achieve this, modified ITS1 and ITS4 primers were developed with the addition of *Sac*II restriction sites (ITS1*Sac*II 5′ AGACCGCGGTCCGTAGGTGAACCTGCGG 3′; ITS4*Sac*II 5′ CTCCGCGGTCCTCCGCTTATTGATATGC 3′). The PCR products were then cloned into plasmid pKLAC2 (New England Biolabs) linearized with *Sac*II. *Sac*II was chosen as it does not cut any previously obtained *Backusella* ITS sequences or any of the ITS sequences assembled from next generation sequencing (see below). Additionally, given that the *Sac*II recognition site is 100 % GC and the ITS sequences in *Backusella* are AT rich there is a low probability of cleaving the ITS DNA amplicons.

DNA extraction and next generation sequencing

Candidate isolates for next generation sequencing were chosen, based on LSU and ITS phylogenies, to represent putative species clades. DNA was extracted from pulverized lyophilized mycelia from 7-d-old liquid cultures using a buffer containing CTAB and incubation at 65 °C, before chloroform extraction and precipitation with an equal volume of 100 % isopropanol (Pitkin et al. 1996) and treatment with RNAse A. Sequencing was performed using 125 bp paired-end reads on an Illumina HiSeq 2500 instrument at AGRF. Assembly was conducted using Velvet (Zerbino & Birney 2008) with a *k*-mer length of 65. The completeness of each assembly was predicted using BUSCO (Simao et al. 2015).

Phylogenetic analyses

Published sequences were obtained from NCBI (O'Donnell et al. 2001, Shirouzu et al. 2012, Walther et al. 2013, De Souza et al. 2014, Lima et al. 2016, Wanasinghe et al. 2018, Vu et al. 2019). Gene sequences were aligned using MUSCLE (Edgar 2004) or in the case of the ITS region CLUSTAL W (Thompson et al. 1994) and phylogeny inferred using a Bayesian approach implemented through MrBayes (Huelsenbeck & Ronquist 2001) and by maximum likelihood implemented in MEGA v. 7.0.26. The species boundaries for the large ribosomal DNA region were poorly resolved using maximum likelihood and Bayesian approaches, with UPGMA trees implemented in Geneious v. 11.1.5 providing clearer phylogenetic insight. The UPGMA method is, however, limited in that it makes basic assumptions such as a constant rate of evolution. Thus, to confirm that these trees represent true phylogenetic relationships, we compared them to Bayesian inference trees generated using MrBayes (Huelsenbeck & Ronquist 2001) based on the single copy *argA* gene and whole genome sequencing.

Table 1 Gene regions used in the multigene phylogeny; ID refers to *B. circina* FSU 941.

Gene ID	Function based on homology
185987	WD40-repeat-containing subunit of the 18S rRNA processing complex
205947	GatB/YqeY domain-containing protein
216514	rRNA-processing protein FCF1
220627	Nucleolar ATPase Kre33
225083	Mitochondrial ribosomal protein
228697	DNA replication licensing factor
234491	Carbohydrate kinase
234892	Transport protein particle (TRAPP) complex subunit
235092	Mitochondrial DNA-directed RNA polymerase RPO41
237414	Argininosuccinate lyase
238565	WD40-repeat-containing subunit of the 18S rRNA processing complex
241757	Golgi SNAP receptor complex member
242843	WD40-repeat-containing
246934	Translocation protein sec63
249721	DUF323 domain-containing protein
251931	WD40-repeat-containing protein
252236	Ribonuclease III
252238	MIR motif-containing protein
252550	Molecular chaperone (ABC1)
257118	Dynein heavy chain
260240	WD40-repeat-containing subunit of the 18S rRNA processing complex
264113	PCI domain containing protein
268790	Cysteinyl-tRNA synthetase
272587	SAM-dependent methyltransferases
282013	Mevalonate pyrophosphate decarboxylase
282268	Dihydroorotate dehydrogenase
282537	Uridine 5′- monophosphate synthase/orotate phosphoribosyltransferase
283428	Lipoate-protein ligase
286046	Similar to bacterial dephospho-CoA kinase
286503	Histone acetyltransferase complex protein
288110	Ribonuclease H-like
291861	Ubiquinol-cytochrome C chaperone
295864	Transcription factor iws1
298931	Magnesium ion transporter
319532	3-ketoacyl-CoA reductase
321014	Conserved protein without annotated function
321666	Mitochondrial ribosomal protein L6
322419	Transmembrane protein
326705	DUF1014-domain-containing protein
331775	WD40-repeat-containing protein
334997	DNA replication licensing factor, MCM6 component
335403	Histidinol dehydrogenase
336212	Actin-related protein Arp2/3 complex
336277	GTP-binding protein
336874	DNA topoisomerase type II
337875	S-adenosyl-L-methionine-dependent tRNA 4-demethylwyosine synthase
338359	Ubiquinone biosynthesis protein
338761	ARM repeat-containing protein

Single copy genes in the whole genome assemblies were initially selected by examining MycoCosm (Grigoriev et al. 2014) for Markov Clustering (MCL) gene clusters present in single copy in *Backusella circina* FSU 941 and other *Mucorales* species. The list of genes was manually examined for those that showed sufficient conservation to allow unambiguous alignment and a subset of these was randomly selected for further analysis. The list of genes selected is given in Table 1. Partial gene sequences were aligned using MUSCLE and concatenated (Edgar 2004) into a final alignment of c. 50 kb.

Sucrose utilization and genetic testing of function by complementation of a Saccharomyces cerevisiae invertase mutant

The ability to utilize sucrose as a sole carbon source was assessed on yeast nitrogen base (YNB) agar (Sigma) supplemented with 5 g/L of either sucrose or glucose. A putative invertase sequence was identified in the strains via BLAST searches using *B. circina* protein ID 331483 as a query (Altschul et al. 1990, Grigoriev et al. 2014). We named this gene *sucB* after the *S. cerevisiae* homolog *SUC2*.

A *SUC2* deletion mutant of *S. cerevisiae* was generated by homologous recombination; *SUC2* null mutants of *S. cerevisiae* are unable to utilize sucrose (Carlson et al. 1981). The G418 resistance cassette of pFA6a-GFP(S65T)-kanMX6 (Bähler et al. 1998) was amplified with primers AP142 (5′ AAAAAGCTTTTCTTTTCACTAACGTATATGCGTACGCTGCAGGTCGAC 3′) and AP143 (5′ AAATAAAAAAGACAATAAGTTTTATAACCTATCGATGAATTCGAGCTC 3′) and transformed into *S. cerevisiae* strain BY4742 using a lithium acetate/polyethylene glycol method (Gietz & Schiestl 2007) with selection on G418. A gene replacement transformant was identified via PCR screening with primers AP148 (5′ GCCTATTACCATCATAGAGACG 3′) and AP149 (5′ AAATCATAAAGTTTTACATTCG 3′). A complementation construct carrying the *sucB* gene of *B. westeae* strain UoMAU4 was generated by amplifying the two exons of the gene with primer pairs AP144 (5′ CCAAGCATACAATCAACTCCAAGCTTATGGTATTCGTAAAATCAGG 3′) AP153 (5′ CCCCACGTCATATTGCCCCAGATTTGATCAAAAGGATTATGC 3′) and AP147 (5′ TAGCTTGGCTGCAGGTCGACGGATCCTTATTTCAAGGTTCTATCAAATGC 3′) AP152 (5′ GGGGCAATATGACGTGGGG 3′) off genomic DNA and combining them into the plasmid pTH19 (Harashima & Heitman 2005) linearized with EcoRI, using the NEBuilder DNA assembly cloning kit (New England Biolabs). This construct allows the yeast to grow on media without uracil and will express *sucB* under the control of a constitutive promoter. The construct and the empty plasmid pTH19 were transformed into the *S. cerevisiae SUC2* mutant with selection on medium lacking uracil. Growth of the *SUC2* mutant carrying either the *sucB* plasmid or empty pTH19 vector was compared on media containing either glucose or sucrose as the sole carbon source (YNB +histidine +leucine +lysine).

Mating

The mating type locus was identified via BLAST searches (Altschul et al. 1990) of the assembled genomes for the *sexP* and *sexM* homologs (Idnurm et al. 2008, Schulz et al. 2017). The mating type locus of *B. circina* FSU741 (+) has been identified (Schulz et al. 2017). Crosses were carried out on V8 medium (20 % Campbell's V8 juice, 2 % agar, 3.75 g/L $CaCO_3$; modified from Benny 2008) in the dark at ambient temperature for 4 wk between closely related strains to identify a representative mating pair for each species, where possible.

Morphological examinations

Colony characters were recorded at 3 d after inoculation of spores on PDA plates. Bright-field microscopy was performed on unstained samples immersed in water using either an Olympus BX51 or Leica DM6000 microscope. Measurements of asexual spore dimensions were from 30 spores. Spore quotient (Q) was calculated for each isolate by dividing the average spore length by average spore width. Air-dried fungal materials taken from culture plates were sputter-coated with gold using a Dynavac SC100 sputter coater and then examined with a Philips XL30 FEG scanning electron microscope.

RESULTS

Isolation of Backusella strains from south-eastern Australia

In total, 206 strains with a transiently-recurvate sporangium were isolated from a range of natural environments across the states of Victoria and Tasmania (Fig. 1, Table 2). Strains were preserved as living cultures in the Jena Microbial Resource Collection, Germany, and the type specimens at the National Herbarium of Victoria (MEL), Australia. Additionally, '*B. johorensis*' IMI 350574 was cultured from the IMI collection at CABI, UK.

Phylogenetic analysis of the argA, ITS and LSU regions revealed 13 Backusella species in Australia, 10 of them new

For delineating boundaries between species, the LSU region was found to be more practical than the ITS regions because variation between ITS copies within a single strain precluded direct sequencing of PCR products. In contrast to the ITS, the LSU could be directly sequenced after amplification in all cases. An LSU phylogeny was generated including all the isolates collected. This revealed 12 phylogenetic groups (Fig. 2). Given that the LSU is highly conserved, phylogenies based solely on LSU may miss some species diversity (Schoch et al. 2012, Vu et al. 2019). As such in addition to the ITS and LSU we sequenced a region encoding argininosuccinate lyase (*argA*) (Fig. 3, 4). The ITS and *argA* trees generally supported the same species groups as the LSU. Of the 12 clades, 10 were clearly distinct from previously described species. One clade was closely related to *B. tuberculispora* and we thus assigned these strains to that species. The final clade, which we named *Backusella* 'group X', showed close affinity to *B. lamprospora* CBS118.08; however, ITS similarity was only around 92 %, which is less than the typically accepted threshold for conspecificity (Vu et al. 2019). Thus, further detailed studies are required to resolve the taxonomy of this clade.

The LSU and ITS regions of '*B. johorensis*' strain IMI 350574 were sequenced (deposited to GenBank as MK966409 and MK958733, respectively). These regions showed 100 % similarity to *B. circina* strains, and hence we conclude that *B. johorensis* is a synonym of *B. circina.*

A highly resolved multigene phylogeny supports the Backusella species relationships inferred from the single gene trees

Representative isolates were selected based on the single gene phylogenies to be subjects for next generation sequencing. The aim was to produce whole genome sequencing information for subsequent highly resolved multi-gene phylogenies, which should better resolve the evolutionary relationships between the species. Between 15 and 21 M reads were generated from each strain, all of which were assembled into reasonably complete genomes, as assessed by BUSCO (Simao et al. 2015) (Table 3). Raw reads and assembled genomes are deposited in GenBank under BioProject PRJNA544350. The genome assemblies were between 44.5 and 48.5 Mb each except for

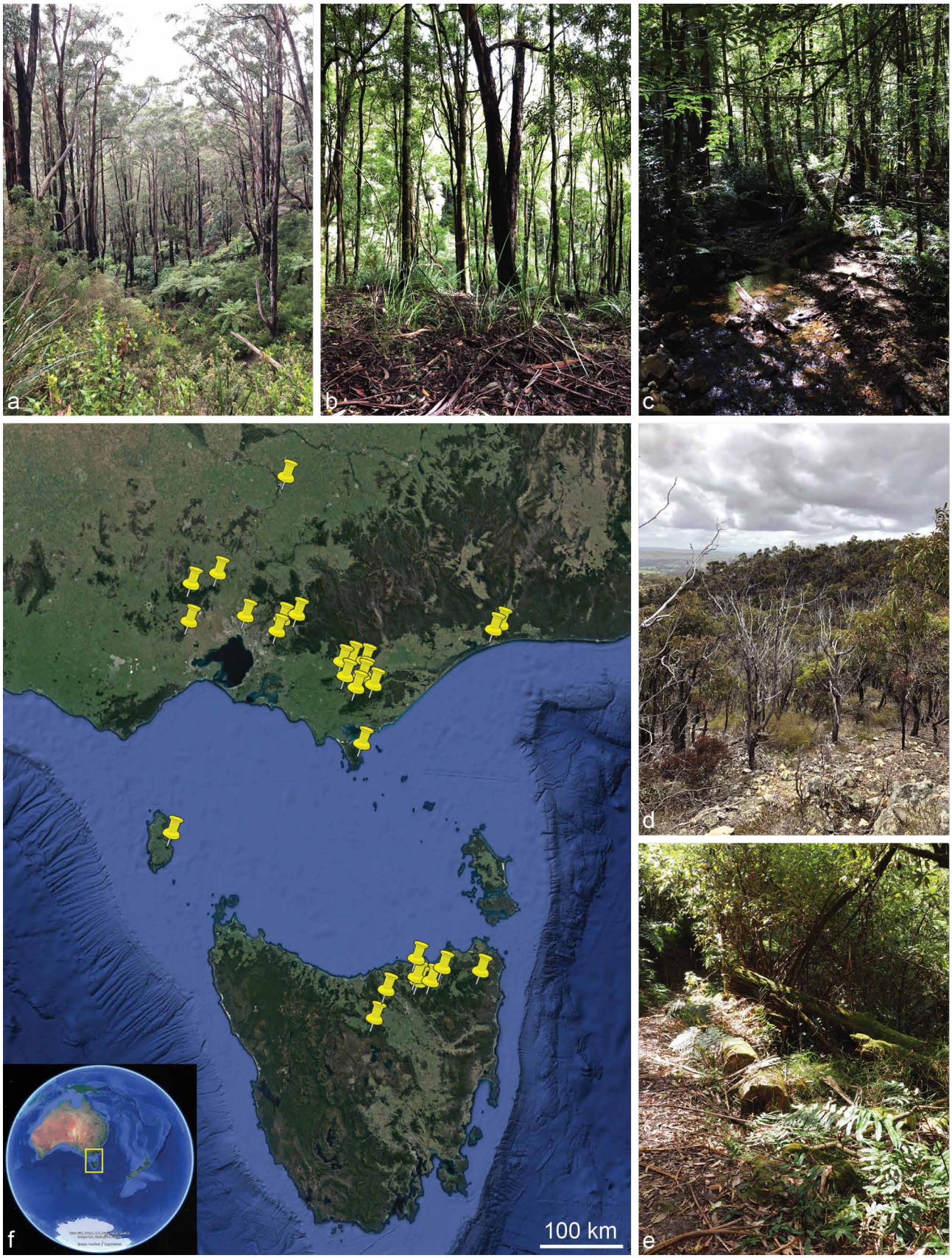

Fig. 1 Strains of *Backusella* were isolated across the south-eastern corner of Australia. Collections covered a range of habitats including wet sclerophyll forest in: a. Wilson's Promontory National Park; b. *Pittosporum undulatum* dominated warm temperate rainforest in Uralla Nature Reserve; c. *Nothofagus cunninghamii* dominated cool temperate rainforest in Toolangi State Forest; d. dry *Eucalyptus* woodland in the Brisbane Ranges National Park; e. damp *Eucalyptus* forest of Jack Cann Reserve. — f. Distribution of collection sites. Map data: Google, SIO, NOAA, U.S. Navy, NGA, GEBCO.

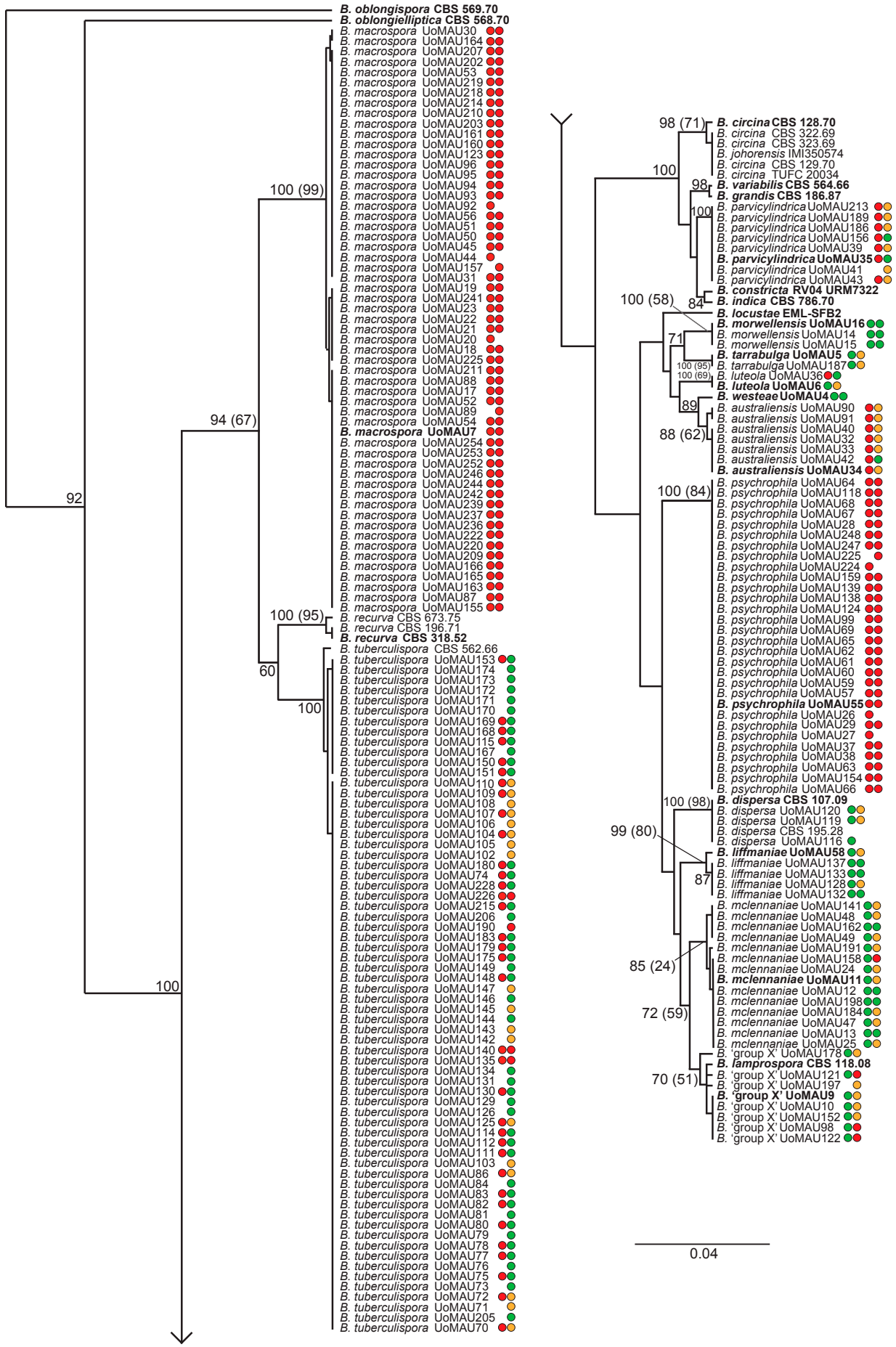

Fig. 2 UPGMA tree based on partial LSU sequence. Nodes are labelled with % bootstrap support from 1 000 replicates. The bootstrap support values derived from maximum likelihood analysis are given in parentheses based on 1 000 repeats for clades which were supported. Taxa in **bold** indicate sequences derived from type specimens. First dot represents growth on sucrose: green = utilizes sucrose, red = does not utilize sucrose. Second dot represents growth at 30 °C after 3 d. Red = no growth, amber = less than 2 cm growth, green = more than 2 cm growth.

Table 2 Strains isolated in this study.

Strain name	Species	Location*	State	GenBank accession numbers			Jena Microbial Resource Collection	MEL herbarium
				LSU	ITS	*argA*		
UoMAU4	*B. westeae*	Jack Cann Reserve	Victoria	MK958796	MK959061	MK982268	SF014021	2417242
UoMAU5	*B. tarrabulga*	Tarra-Bulga NP	Victoria	MK958804	MK959060	MK982263	SF014022	2446982
UoMAU6	*B. luteola*	Tarra-Bulga NP	Victoria	MK958795	MK959058	MK982265	SF014023	2446983
UoMAU7	*B. macrospora*	Tarra-Bulga NP	Victoria	MK958628	MK959107	MK982253	SF014024	2446984
UoMAU9	*Backusella* 'group X'	Silvan reservoir park	Victoria	MK958787	MK959098	MK982280	SF014025	2446985
UoMAU10	*Backusella* 'group X'	Silvan reservoir park	Victoria	MK958788	MK959096; MK959100	–	SF014026	2446986
UoMAU11	*B. mclennaniae*	Morwell NP	Victoria	MK958776	MK959077; MK959086; MK959088	MK982278	SF014027	2446987
UoMAU12	*B. mclennaniae*	Morwell NP	Victoria	MK958777	MK959087; MK959089	–	SF014028	2446988
UoMAU13	*B. mclennaniae*	Morwell NP	Victoria	MK958772	MK959081; MK959091	–	SF014029	2446989
UoMAU14	*B. morwellensis*	Morwell NP	Victoria	MK958806	–	–	SF014030	2446990
UoMAU15	*B. morwellensis*	Morwell NP	Victoria	MK958807	–	–	SF014031	2446991
UoMAU16	*B. morwellensis*	Morwell NP	Victoria	MK958808	MK959059	MK982267	SF014032	2446992
UoMAU17	*B. macrospora*	Tarra-Bulga NP	Victoria	MK958610	–	–	SF014033	2446993
UoMAU18	*B. macrospora*	Tarra-Bulga NP	Victoria	MK958602	–	–	SF014034	2446994
UoMAU19	*B. macrospora*	Macedon RP	Victoria	MK958609	–	–	SF014035	2446995
UoMAU20	*B. macrospora*	Macedon RP	Victoria	MK958604	–	–	SF014036	2446996
UoMAU21	*B. macrospora*	Macedon RP	Victoria	MK958605	–	–	SF014037	2446997
UoMAU22	*B. macrospora*	Macedon RP	Victoria	MK958606	–	–	SF014038	2446998
UoMAU23	*B. macrospora*	Macedon RP	Victoria	MK958607	–	–	SF014039	2446999
UoMAU24	*B. mclennaniae*	Morwell NP	Victoria	MK958778	MK959080	–	SF014040	2447000
UoMAU25	*B. mclennaniae*	Morwell NP	Victoria	MK958773	MK959082; MK959083	–	SF014041	2447001
UoMAU26	*B. psychrophila*	Wombat SF	Victoria	MK958748	–	–	SF014042	2447002
UoMAU27	*B. psychrophila*	Wombat SF	Victoria	MK958746	–	–	SF014043	2447003
UoMAU28	*B. psychrophila*	Wombat SF	Victoria	MK958766	–	–	SF014044	2447004
UoMAU29	*B. psychrophila*	Wombat SF	Victoria	MK958747	–	–	SF014045	2447005
UoMAU30	*B. macrospora*	Wombat SF	Victoria	MK958634	–	–	SF014046	2447006
UoMAU31	*B. macrospora*	Wombat SF	Victoria	MK958637	–	–	SF014047	2447007
UoMAU32	*B. australiensis*	Wombat SF	Victoria	MK958802	–	–	SF014048	2447008
UoMAU33	*B. australiensis*	Wombat SF	Victoria	MK958801	–	–	SF014049	2447009
UoMAU34	*B. australiensis*	Wombat SF	Victoria	MK958800	MK959062	MK982270	SF014050	2447010
UoMAU35	*B. parvicylindrica*	Jack Cann Reserve	Victoria	MK958727	MK959109	MK982259	SF014051	2447011
UoMAU36	*B. luteola*	Wombat SF	Victoria	MK958794	–	MK982266	SF014052	2447012
UoMAU37	*B. parvicylindrica*	Wombat SF	Victoria	MK958745	–	–	SF014053	2447013
UoMAU38	*B. parvicylindrica*	Wombat SF	Victoria	MK958744	–	–	SF014054	2447014
UoMAU39	*B. parvicylindrica*	Wombat SF	Victoria	MK958728	–	–	SF014055	2447015
UoMAU40	*B. australiensis*	Wombat SF	Victoria	MK958803	–	–	SF014056	2447016
UoMAU41	*B. parvicylindrica*	Wombat SF	Victoria	MK958725	–	MK982261	SF014057	2447017
UoMAU42	*B. australiensis*	Wombat SF	Victoria	MK958799	–	MK982272	SF014058	2447018
UoMAU43	*B. parvicylindrica*	Wombat SF	Victoria	MK958726	–	MK982262	SF014059	2447019
UoMAU44	*B. macrospora*	Wanderslore Sanctuary	Victoria	MK958638	–	–	SF014060	2447020
UoMAU45	*B. macrospora*	Wanderslore Sanctuary	Victoria	MK958639	–	MK982255	SF014061	2447021
UoMAU47	*B. mclennaniae*	Wanderslore Sanctuary	Victoria	MK958774	MK959066; MK959071	–	SF014063	2447023
UoMAU48	*B. mclennaniae*	Wanderslore Sanctuary	Victoria	MK958784	MK959057; MK959067	–	SF014064	2447024
UoMAU49	*B. mclennaniae*	Wanderslore Sanctuary	Victoria	MK958783	MK959068; MK959078	MK982279	SF014065	2447025
UoMAU50	*B. macrospora*	Toolangi SF	Victoria	MK958640	–	–	SF014066	2447026
UoMAU51	*B. macrospora*	Toolangi SF	Victoria	MK958641	–	–	SF014067	2447027
UoMAU52	*B. macrospora*	Jack Cann Reserve	Victoria	MK958630	–	MK982254	SF014068	2447028
UoMAU53	*B. macrospora*	Jack Cann Reserve	Victoria	MK958656	–	–	SF014069	2447029
UoMAU54	*B. macrospora*	Jack Cann Reserve	Victoria	MK958629	–	–	SF014070	2447030
UoMAU55	*B. psychrophila*	Jack Cann Reserve	Victoria	MK958749	MK959093	MK982283	SF014071	2447031
UoMAU56	*B. macrospora*	Jack Cann Reserve	Victoria	MK958642	–	–	SF014072	2447032
UoMAU57	*B. psychrophila*	Jack Cann Reserve	Victoria	MK958750	–	–	SF014073	2447033

Table 2 (cont.)

Strain name	Species	Location*	State	GenBank accession numbers			Jena Microbial Resource Collection	MEL herbarium
				LSU	ITS	*argA*		
UoMAU58	*B. liffmaniae*	Jack Cann Reserve	Victoria	MK958734	MK959065	MK982276	SF014074	2447034
UoMAU59	*B. psychrophila*	Jack Cann Reserve	Victoria	MK958751	–	–	SF014075	2447035
UoMAU60	*B. psychrophila*	Jack Cann Reserve	Victoria	MK958752	–	–	SF014076	2447036
UoMAU61	*B. psychrophila*	Jack Cann Reserve	Victoria	MK958753	–	–	SF014077	2447037
UoMAU62	*B. psychrophila*	Jack Cann Reserve	Victoria	MK958754	–	–	SF014078	2447038
UoMAU63	*B. psychrophila*	Black sugar loaf	Tasmania	MK958743	–	MK982285	SF014079	2447039
UoMAU64	*B. psychrophila*	Black sugar loaf	Tasmania	MK958739	–	–	SF014080	2447040
UoMAU65	*B. psychrophila*	Black sugar loaf	Tasmania	MK958755	–	–	SF014081	2447041
UoMAU66	*B. psychrophila*	Black sugar loaf	Tasmania	MK958742	–	–	SF014082	2447042
UoMAU67	*B. psychrophila*	Black sugar loaf	Tasmania	MK958767	–	MK982286	SF014083	2447043
UoMAU68	*B. psychrophila*	Jack Cann Reserve	Victoria	MK958768	–	–	SF014084	2447044
UoMAU69	*B. psychrophila*	Jack Cann Reserve	Victoria	MK958756	–	–	SF014085	2447045
UoMAU70	*B. tuberculispora*	Pegarah SF	King Island (Tasmania)	MK958680	–	–	SF014086	2447046
UoMAU71	*B. tuberculispora*	Pegarah SF	King Island (Tasmania)	MK958681	–	–	SF014087	2447047
UoMAU72	*B. tuberculispora*	Pegarah SF	King Island (Tasmania)	MK958682	–	–	SF014088	2447048
UoMAU73	*B. tuberculispora*	Pegarah SF	King Island (Tasmania)	MK958683	–	–	SF014089	2447049
UoMAU74	*B. tuberculispora*	Pegarah SF	King Island (Tasmania)	MK958723	–	–	SF014090	2447050
UoMAU75	*B. tuberculispora*	Pegarah SF	King Island (Tasmania)	MK958684	–	–	SF014091	2447051
UoMAU76	*B. tuberculispora*	Pegarah SF	King Island (Tasmania)	MK958685	–	–	SF014092	2447052
UoMAU77	*B. tuberculispora*	Pegarah SF	King Island (Tasmania)	MK958686	–	–	SF014093	2447053
UoMAU78	*B. tuberculispora*	Ross-Edwards Reserve	Victoria	MK958687	–	–	SF014094	2447054
UoMAU79	*B. tuberculispora*	Ross-Edwards Reserve	Victoria	MK958688	–	–	–	2447055
UoMAU80	*B. tuberculispora*	Ross-Edwards Reserve	Victoria	MK958689	MK959108	MK982250	–	2447056
UoMAU81	*B. tuberculispora*	Ross-Edwards Reserve	Victoria	MK958690	–	–	SF014097	2447057
UoMAU82	*B. tuberculispora*	Ross-Edwards Reserve	Victoria	MK958691	–	–	SF014098	2447058
UoMAU83	*B. tuberculispora*	Ross-Edwards Reserve	Victoria	MK958692	–	–	SF014099	2447059
UoMAU84	*B. tuberculispora*	Ross-Edwards Reserve	Victoria	MK958693	–	–	SF014100	2447060
UoMAU86	*B. tuberculispora*	Ross-Edwards Reserve	Victoria	MK958694	–	–	SF014101	2447062
UoMAU87	*B. macrospora*	Toolangi SF	Victoria	MK958611	–	MK982252	SF014102	2447063
UoMAU88	*B. macrospora*	Toolangi SF	Victoria	MK958631	–	–	SF014103	2447064
UoMAU89	*B. macrospora*	Toolangi SF	Victoria	MK958633	–	–	SF014104	2447065
UoMAU90	*B. australiensis*	Kalimna	Victoria	MK958797	MK959063; MK959064	MK982269	SF014105	2447066
UoMAU91	*B. australiensis*	Kalimna	Victoria	MK958798	–	MK982271	SF014106	2447067
UoMAU92	*B. macrospora*	Colquhoun SF	Victoria	MK958643	–	–	SF014107	2447068
UoMAU93	*B. macrospora*	Kalimna	Victoria	MK958644	–	–	SF014108	2447069
UoMAU94	*B. macrospora*	Kalimna	Victoria	MK958645	–	–	SF014109	2447070
UoMAU95	*B. macrospora*	Lake Tyers SP	Victoria	MK958646	–	–	SF014110	2447071
UoMAU96	*B. macrospora*	Lake Tyers SP	Victoria	MK958647	–	MK982258	SF014111	2447072
UoMAU98	*Backusella* 'group X'	Lake Tyers SP	Victoria	MK958789	MK959099; MK959101	–	SF014113	2447074
UoMAU99	*B. psychrophila*	Lake Tyers SP	Victoria	MK958757	–	–	SF014114	2447075
UoMAU102	*B. tuberculispora*	Blue Tier FR	Tasmania	MK958671	–	–	SF014116	2447077
UoMAU103	*B. tuberculispora*	Blue Tier FR	Tasmania	MK958695	–	–	SF014117	2447078
UoMAU104	*B. tuberculispora*	Blue Tier FR	Tasmania	MK958673	–	–	SF014118	2447079
UoMAU105	*B. tuberculispora*	Blue Tier FR	Tasmania	MK958671	–	–	SF014119	2447080
UoMAU106	*B. tuberculispora*	Blue Tier FR	Tasmania	MK958674	–	–	SF014120	2447081
UoMAU107	*B. tuberculispora*	Blue Tier FR	Tasmania	MK958675	–	–	SF014121	2447082
UoMAU108	*B. tuberculispora*	Blue Tier FR	Tasmania	MK958676	–	–	SF014122	2447083
UoMAU109	*B. tuberculispora*	Blue Tier FR	Tasmania	MK958677	–	–	SF014123	2447084
UoMAU110	*B. tuberculispora*	Blue Tier FR	Tasmania	MK958678	–	–	SF014124	2447085
UoMAU111	*B. tuberculispora*	Blue Tier FR	Tasmania	MK958696	–	–	SF014125	2447086
UoMAU112	*B. tuberculispora*	Myrtle Bank	Tasmania	MK958697	–	–	SF014126	2447087
UoMAU114	*B. tuberculispora*	Myrtle Bank	Tasmania	MK958698	–	–	SF014127	2447088

Table 2 (cont.)

Strain name	Species	Location*	State	GenBank accession numbers			Jena Microbial Resource Collection	MEL herbarium
				LSU	ITS	*argA*		
UoMAU115	*B. tuberculispora*	Pipers Brook	Tasmania	MK958663	–	–	SF014128	2447089
UoMAU116	*B. dispersa*	Scottsdale	Tasmania	MK958769	–	MK982274	SF014129	2447090
UoMAU118	*B. psychrophila*	Scottsdale	Tasmania	MK958740	–	–	SF014131	2447092
UoMAU119	*B. dispersa*	Scottsdale	Tasmania	MK958770	–	MK982275	SF014132	2447093
UoMAU120	*B. dispersa*	Scottsdale	Tasmania	MK958771	–	MK982273	SF014133	2447094
UoMAU121	*Backusella* 'group X'	Kalimna west	Victoria	MK958792	MK959103; MK959105	–	SF014134	2447095
UoMAU122	*Backusella* 'group X'	Kalimna west	Victoria	MK958790	–	–	SF014135	2447096
UoMAU123	*B. macrospora*	Lake Tyers SP	Victoria	MK958648	–	–	SF014136	2447097
UoMAU124	*B. psychrophila*	Lake Tyers SP	Victoria	MK958758	–	MK982287	SF014137	2447098
UoMAU125	*B. tuberculispora*	Pegarah SF	King Island (Tasmania)	MK958699	–	–	SF014138	2447099
UoMAU126	*B. tuberculispora*	Pegarah SF	King Island (Tasmania)	MK958700	–	–	SF014139	2447100
UoMAU128	*B. liffmaniae*	Pegarah SF	King Island (Tasmania)	MK958735	–	–	SF014140	2447101
UoMAU129	*B. tuberculispora*	Pegarah SF	King Island (Tasmania)	MK958701	–	–	SF014141	2447102
UoMAU130	*B. tuberculispora*	Pegarah SF	King Island (Tasmania)	MK958702	–	–	SF014142	2447103
UoMAU131	*B. tuberculispora*	Pegarah SF	King Island (Tasmania)	MK958703	–	–	SF014143	2447104
UoMAU132	*B. liffmaniae*	Pegarah SF	King Island (Tasmania)	MK958736	–	–	SF014144	2447105
UoMAU133	*B. liffmaniae*	Pegarah SF	King Island (Tasmania)	MK958737	–	–	SF014145	2447106
UoMAU134	*B. tuberculispora*	Pegarah SF	King Island (Tasmania)	MK958704	–	–	SF014146	2447107
UoMAU135	*B. tuberculispora*	Pegarah SF	King Island (Tasmania)	MK958705	–	–	SF014147	2447108
UoMAU137	*B. liffmaniae*	Pegarah SF	King Island (Tasmania)	MK958738	–	MK982277	SF014148	2447109
UoMAU138	*B. psychrophila*	Pegarah SF	King Island (Tasmania)	MK958759	–	–	SF014149	2447110
UoMAU139	*B. psychrophila*	Pegarah SF	King Island (Tasmania)	MK958760	–	MK982284	SF014150	2447111
UoMAU140	*B. tuberculispora*	Hollybank FR	Tasmania	MK958706	–	–	SF014151	2447112
UoMAU141	*B. mclennaniae*	W.A.G Walker Rhododendron Garden	Tasmania	MK958785	MK959069; MK959070	–	SF014152	2447113
UoMAU142	*B. tuberculispora*	Hollybank FR	Tasmania	MK958707	–	–	SF014153	2447114
UoMAU143	*B. tuberculispora*	Hollybank FR	Tasmania	MK958708	–	–	SF014154	2447115
UoMAU144	*B. tuberculispora*	Hollybank FR	Tasmania	MK958709	–	–	SF014155	2447116
UoMAU145	*B. tuberculispora*	Hollybank FR	Tasmania	MK958710	–	–	SF014156	2447117
UoMAU146	*B. tuberculispora*	Hollybank FR	Tasmania	MK958711	–	–	SF014157	2447118
UoMAU147	*B. tuberculispora*	Hollybank FR	Tasmania	MK958712	–	–	SF014158	2447119
UoMAU148	*B. tuberculispora*	Hollybank FR	Tasmania	MK958713	–	–	SF014159	2447120
UoMAU149	*B. tuberculispora*	Hollybank FR	Tasmania	MK958714	–	–	SF014160	2447121
UoMAU150	*B. tuberculispora*	Hollybank FR	Tasmania	MK958660	–	MK982249	SF014161	2447122
UoMAU151	*B. tuberculispora*	Hollybank FR	Tasmania	MK958661	–	–	SF014162	2447123
UoMAU152	*Backusella* 'group X'	Hollybank FR	Tasmania	MK958791	MK959102; MK959104	MK982281	SF014163	2447124
UoMAU153	*B. tuberculispora*	Uralla Reserve	Victoria	MK958659	–	MK982251	SF014164	2447125
UoMAU154	*B. psychrophila*	Morwell NP	Victoria	MK958741	–	–	SF014165	2447126
UoMAU155	*B. macrospora*	Morwell NP	Victoria	MK958612	–	–	SF014166	2447127
UoMAU156	*B. parvicylindrica*	Uralla Reserve	Victoria	MK958729	–	MK982260	SF014167	2447128
UoMAU157	*B. macrospora*	Uralla Reserve	Victoria	MK958636	–	–	SF014168	2447129
UoMAU158	*B. mclennaniae*	Uralla Reserve	Victoria	MK958779	MK959074; MK959075	–	SF014169	2447130
UoMAU159	*B. psychrophila*	Uralla reserve	Victoria	MK958761	–	–	SF014170	2447131
UoMAU160	*B. macrospora*	Morwell NP	Victoria	MK958649	–	–	SF014171	2447132
UoMAU161	*B. macrospora*	Morwell NP	Victoria	MK958650	–	MK982256	SF014172	2447133
UoMAU162	*B. mclennaniae*	Morwell NP	Victoria	MK958782	MK959072; MK959073	–	SF014173	2447134
UoMAU163	*B. macrospora*	Uralla Reserve	Victoria	MK958613	–	–	SF014174	2447135
UoMAU164	*B. macrospora*	Uralla Reserve	Victoria	MK958635	–	–	SF014175	2447136
UoMAU165	*B. macrospora*	Uralla Reserve	Victoria	MK958614	–	–	SF014176	2447137
UoMAU166	*B. macrospora*	Uralla Reserve	Victoria	MK958615	–	–	SF014177	2447138
UoMAU167	*B. tuberculispora*	Gillespies road, Nabowla	Tasmania	MK958662	–	–	SF014178	2447139
UoMAU168	*B. tuberculispora*	Gillespies road, Nabowla	Tasmania	MK958664	–	–	SF014179	2447140
UoMAU169	*B. tuberculispora*	Gillespies road, Nabowla	Tasmania	MK958665	–	–	SF014180	2447141

Table 2 (cont.)

Strain name	Species	Location*	State	GenBank accession numbers			Jena Microbial Resource Collection	MEL herbarium
				LSU	ITS	*argA*		
UoMAU170	*B. tuberculispora*	Gillespies road, Nabowla	Tasmania	MK958666	–	–	SF014181	2447142
UoMAU171	*B. tuberculispora*	Gillespies road, Nabowla	Tasmania	MK958667	–	–	SF014182	2447143
UoMAU172	*B. tuberculispora*	Gillespies road, Nabowla	Tasmania	MK958668	–	–	SF014183	2447144
UoMAU173	*B. tuberculispora*	Gillespies road, Nabowla	Tasmania	MK958669	–	–	SF014184	2447145
UoMAU174	*B. tuberculispora*	Gillespies road, Nabowla	Tasmania	MK958670	–	–	SF014185	2447146
UoMAU175	*B. tuberculispora*	Crinigan Road Reserve	Victoria	MK958715	–	–	SF014186	2447147
UoMAU178	*Backusella* 'group X'	Crinigan Road Reserve	Victoria	MK958786	MK959094; MK959095	MK982282	SF014189	2447150
UoMAU179	*B. tuberculispora*	Crinigan Road Reserve	Victoria	MK958716	–	–	SF014190	2447151
UoMAU180	*B. tuberculispora*	Crinigan Road Reserve	Victoria	MK958724	–	–	SF014191	2447152
UoMAU183	*B. tuberculispora*	Crinigan Road Reserve	Victoria	MK958717	–	–	SF014194	2447155
UoMAU184	*B. mclennaniae*	Mirboo North RP	Victoria	MK958775	MK959079; MK959085	–	SF014195	2447156
UoMAU186	*B. parvicylindrica*	Uralla Reserve	Victoria	MK958730	–	–	SF014197	2447158
UoMAU187	*B. tarrabulga*	Uralla Reserve	Victoria	MK958805	–	MK982264	SF014198	2447159
UoMAU189	*B. parvicylindrica*	Uralla Reserve	Victoria	MK958731	–	–	SF014200	2447161
UoMAU190	*B. tuberculispora*	Uralla Reserve	Victoria	MK958718	–	–	SF014201	2447162
UoMAU191	*B. mclennaniae*	Uralla Reserve	Victoria	MK958781	MK959076; MK959084	–	SF014202	2447163
UoMAU197	*Backusella* 'group X'	Crinigan Road Reserve	Victoria	MK958793	MK959097; MK959106	–	SF014208	2447169
UoMAU198	*B. mclennaniae*	Crinigan Road Reserve	Victoria	MK958780	MK959090; MK959092	–	SF014209	2447170
UoMAU202	*B. macrospora*	Mirboo North RP	Victoria	MK958657	–	–	SF014213	2447174
UoMAU203	*B. macrospora*	Mirboo North RP	Victoria	MK958651	–	MK982257	SF014214	2447175
UoMAU205	*B. tuberculispora*	Jackey's Marsh, Western Tiers	Tasmania	MK958679	–	–	SF014216	2447177
UoMAU206	*B. tuberculispora*	Jackey's Marsh, Western Tiers	Tasmania	MK958719	–	–	SF014217	2447178
UoMAU207	*B. macrospora*	Jeeralang Junction	Victoria	MK958658	–	–	–	2447179
UoMAU209	*B. macrospora*	Tarra-Bulga NP	Victoria	MK958616	–	–	SF014219	2447180
UoMAU210	*B. macrospora*	Edward Hunter Reserve	Victoria	MK958652	–	–	SF014220	2447181
UoMAU211	*B. macrospora*	Tarra-Bulga NP	Victoria	MK958632	–	–	SF014221	2447182
UoMAU213	*B. parvicylindrica*	Holey Plains SP	Victoria	MK958732	–	–	SF014223	2447184
UoMAU214	*B. macrospora*	Edward Hunter Reserve	Victoria	MK958653	–	–	SF014224	2447185
UoMAU215	*B. tuberculispora*	Edward Hunter Reserve	Victoria	MK958720	–	–	SF014225	2447186
UoMAU218	*B. macrospora*	Edward Hunter Reserve	Victoria	MK958654	–	–	SF014228	2447189
UoMAU219	*B. macrospora*	Edward Hunter Reserve	Victoria	MK958655	–	–	SF014229	2458421
UoMAU220	*B. macrospora*	Tarra-Bulga NP	Victoria	MK958617	–	–	SF014230	2447190
UoMAU222	*B. macrospora*	Morwell River Falls Reserve	Victoria	MK958618	–	–	SF014232	2447192
UoMAU224	*B. psychrophila*	University of Melbourne	Victoria	MK958762	–	–	SF014234	2447194
UoMAU225	*B. psychrophila*	Brisbane Ranges NP	Victoria	MK958763	–	–	SF014235	2458422
UoMAU226	*B. tuberculispora*	Baluk Willam Nature Conservation Reserve	Victoria	MK958721	–	–	SF014236	2447195
UoMAU228	*B. tuberculispora*	Baluk Willam Nature Conservation Reserve	Victoria	MK958722	–	–	SF014238	2447197
UoMAU236	*B. macrospora*	Wilson Prom NP	Victoria	MK958619	–	–	SF014243	2447202
UoMAU237	*B. macrospora*	Wilson Prom NP	Victoria	MK958620	–	–	SF014244	2447203
UoMAU239	*B. macrospora*	Wilson Prom NP	Victoria	MK958621	–	–	SF014245	2447204
UoMAU241	*B. macrospora*	Wilson Prom NP	Victoria	MK958608	–	–	SF014247	2447206
UoMAU242	*B. macrospora*	Wilson Prom NP	Victoria	MK958622	–	–	SF014248	2447207
UoMAU244	*B. macrospora*	Wilson Prom NP	Victoria	MK958623	–	–	SF014250	2447209
UoMAU246	*B. macrospora*	Wilson Prom NP	Victoria	MK958624	–	–	SF014251	2447210
UoMAU247	*B. psychrophila*	Wilson Prom NP	Victoria	MK958764	–	–	SF014252	2447211
UoMAU248	*B. psychrophila*	Wilson Prom NP	Victoria	MK958765	–	–	SF014253	2447212
UoMAU252	*B. macrospora*	Wilson Prom NP	Victoria	MK958625	–	–	SF014256	2447216
UoMAU253	*B. macrospora*	Wilson Prom NP	Victoria	MK958626	–	–	SF014257	2447217
UoMAU254	*B. macrospora*	Wilson Prom NP	Victoria	MK958627	–	–	SF014258	2447218
UoMAU255	*B. macrospora*	Wilson Prom NP	Victoria	MK958603	–	–	SF014259	2447219

* NP = National Park; SP = State Park; RP = Regional Park; SF = State Forest; FR = Forest Reserve.

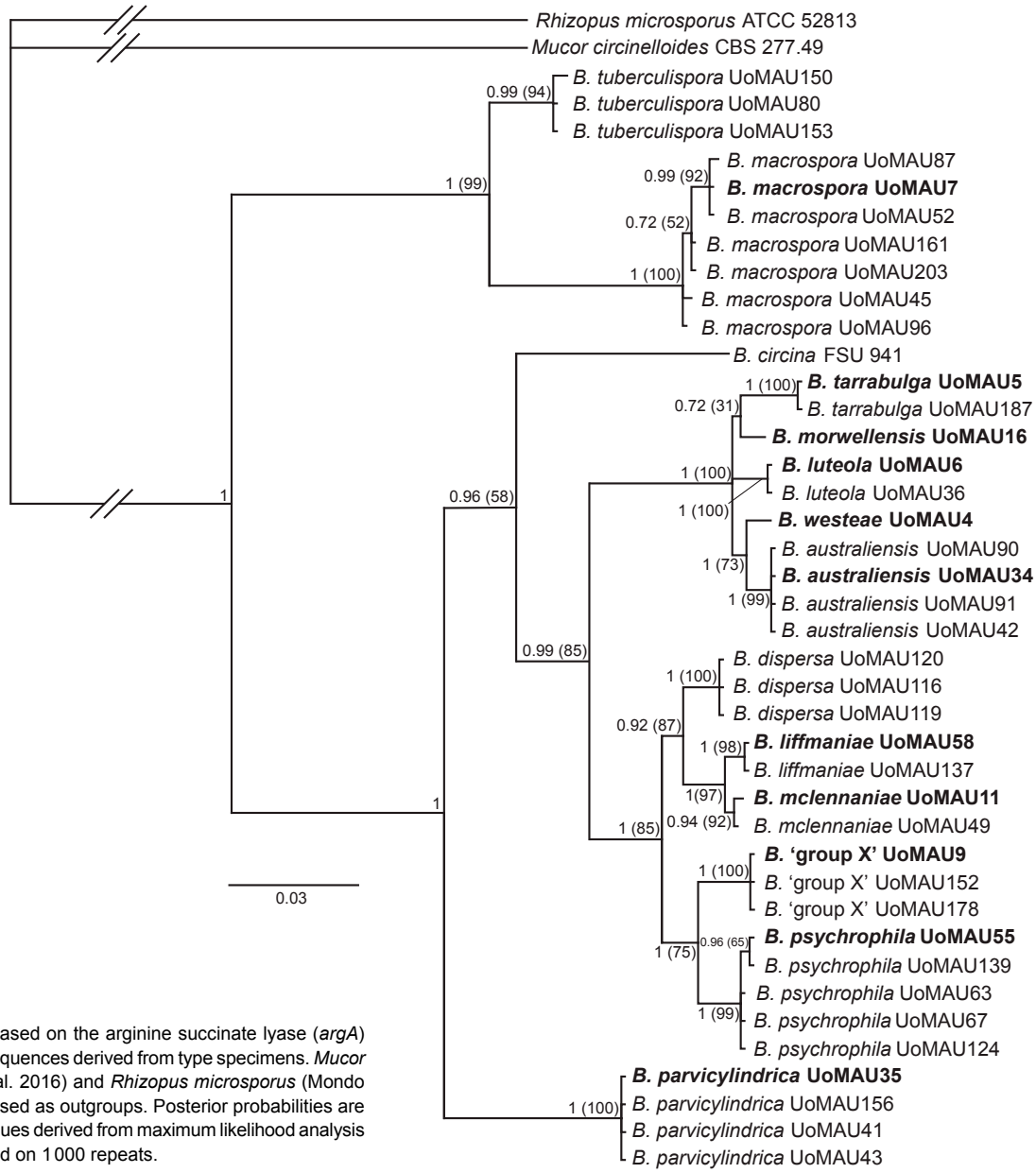

Fig. 3 Bayesian phylogeny based on the arginine succinate lyase (*argA*) region. Taxa in **bold** indicate sequences derived from type specimens. *Mucor circinelloides* (Corrochano et al. 2016) and *Rhizopus microsporus* (Mondo et al. 2017) sequences were used as outgroups. Posterior probabilities are indicated. Bootstrap support values derived from maximum likelihood analysis are given in parentheses based on 1 000 repeats.

the sister taxa *B. macrospora* and *B. tuberculispora*, which produced relatively large genome assemblies (> 56 Mb).

Forty-eight concatenated partial gene regions (Table 1), totalling c. 50 kb, were used to infer a highly-resolved phylogeny. Some multi-species clades shown in the single gene trees were strongly supported in the multiple gene tree. From this analysis, two major clades are resolved in *Backusella*, one that consists of *B. psychrophila*, *Backusella* 'group X', *B. liffmaniae*, and *B. mclennaniae* (Clade 1) and the another consists of *B. luteola*, *B. westeae*, *B. australiensis*, *B. morwellensis*, and *B. tarrabulga* (Clade 2) (Fig. 5).

Morphological characteristics support species differentiation of molecular phylogenies

To determine if the species designations based on molecular data were supported by morphology, measurements of spore size were made for each strain (Fig. 6). On a plot of length vs 'Q' (length/width) four species were distinct from all others (*B. luteola*, *B. tarrabulga*, *B. macrospora*, and *B. parvicylindrica*). *Backusella psychrophila* and *B. morwellensis* showed an overlapping distribution. The remaining species (*B. westeae*, *B. dispersa*, *B. australiensis*, *B. liffmanniae*, *Backusella* 'group X', *B. mclennaniae*, *B. parvicylindrica*, and *B. tuberculispora*) all have relatively small, globose spores.

Examination of colony pigmentation revealed support for the distinction between the Clade 1 and Clade 2. Species belonging to Clade 2 show only yellow colony pigmentation, while Clade 1 displayed a range of colony pigmentation (including a single case of pale yellow). This is most obvious after scraping fungal material from agar plate cultures (Fig. 7). Before scraping all of the Clade 1 species appear close to white but three of the species, i.e., *B. mclennaniae*, *Backusella* 'group X', and *B. liffmaniae*, darken to a greyish colour when scraped. *Backusella macrospora* is a salmon colour and *B. tuberculispora* is brownish (greyish brown before scraping). *Backusella parvicylindrica* shows no colour change upon scraping, i.e., remaining white-cream.

All Backusella species are presumably heterothallic

Earlier studies indicated that many previously described *Backusella* species are heterothallic (Schipper 1969, Stalpers & Schipper 1980). Given the absence of zygospore production in strains derived from single asexual spore cultures, we thus expected the newly isolated strains to also be heterothallic. This was

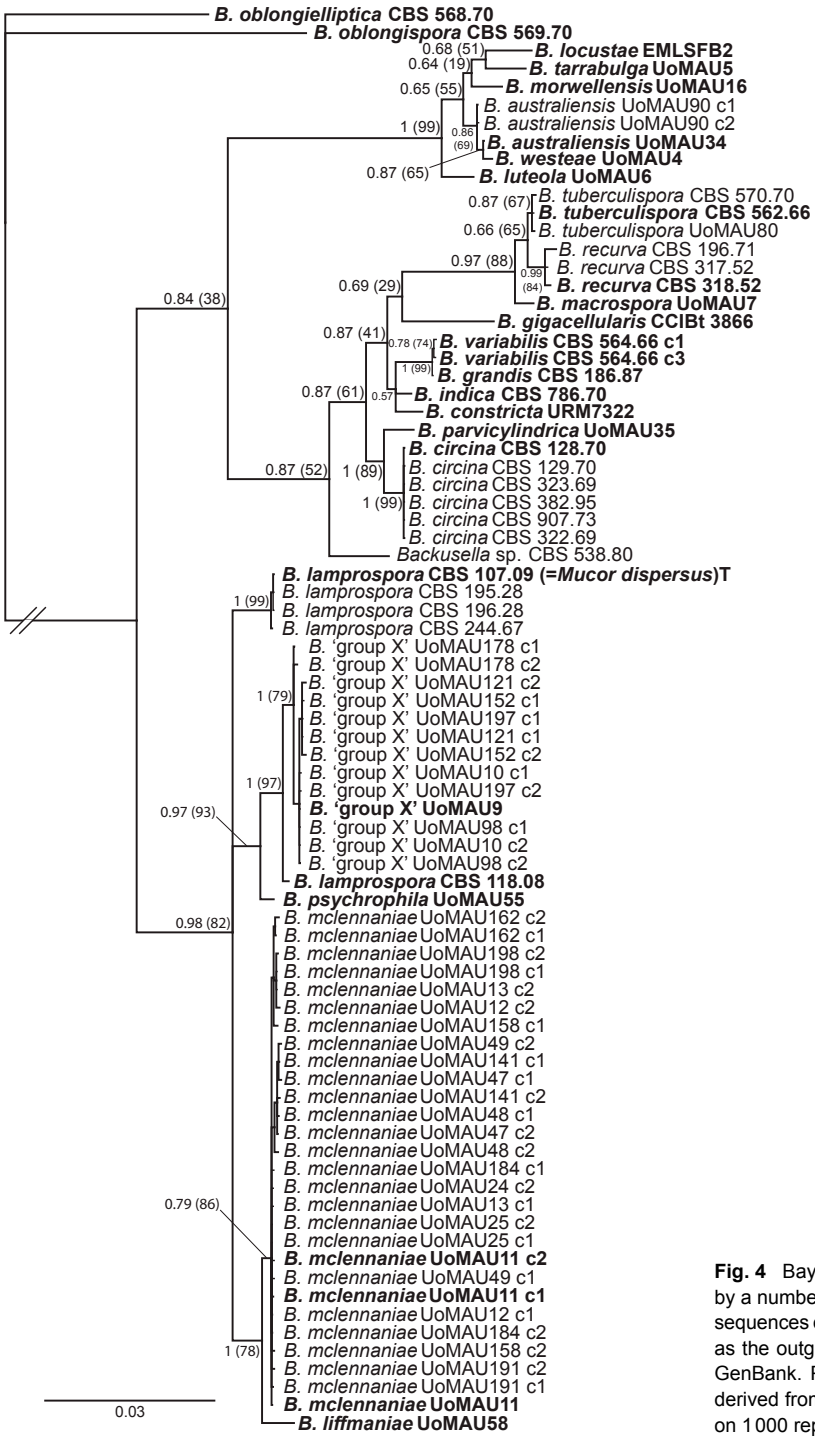

Fig. 4 Bayesian phylogeny based on the ITS region. The letter c followed by a number indicates a sequence from cloned DNA. Taxa in **bold** indicate sequences derived from type specimens. *Backusella oblongispora* was used as the outgroup. Sequences with accession numbers were obtained from GenBank. Posterior probabilities are indicated. Bootstrap support values derived from maximum likelihood analysis are given in parentheses based on 1 000 repeats.

Table 3 Details of genome assemblies of *Backusella* strains.

Strain	*Backusella* species	Number of reads	Coverage	Contigs	Size (bp)	BUSCO completeness
UoMAU4	*B. westeae*	17 536 088	45×	4 172	48 348 398	94.8 %
UoMAU5	*B. tarrabulga*	16 645 616	44×	2 526	47 344 639	87.3 %
UoMAU6	*B. luteola*	17 567 962	46×	3 540	47 953 259	85.9 %
UoMAU7	*B. macrospora*	17 543 504	39×	5 407	56 332 687	86.2 %
UoMAU9	*B.* 'group X'	18 173 802	49×	1 840	46 112 820	88.0 %
UoMAU11	*B. mclennaniae*	20 457 596	55×	2 119	46 408 055	84.1 %
UoMAU16	*B. morwellensis*	17 371 966	45×	2 949	48 384 723	89.0 %
UoMAU34	*B. australiensis*	19 936 302	52×	3 598	47 841 325	90.0 %
UoMAU35	*B. parvicylindrica*	20 679 958	54×	4 332	47 841 626	91.0 %
UoMAU55	*B. psychrophila*	15 493 552	43×	2 051	45 155 991	85.1 %
UoMAU58	*B. liffmaniae*	18 536 626	51×	3 206	44 962 020	84.5 %
UoMAU80	*B. tuberculispora*	18 564 934	40×	5 511	57 697 485	86.6 %
UoMAU90	*B. australiensis*	18 283 128	48×	3 192	47 594 588	88.0 %

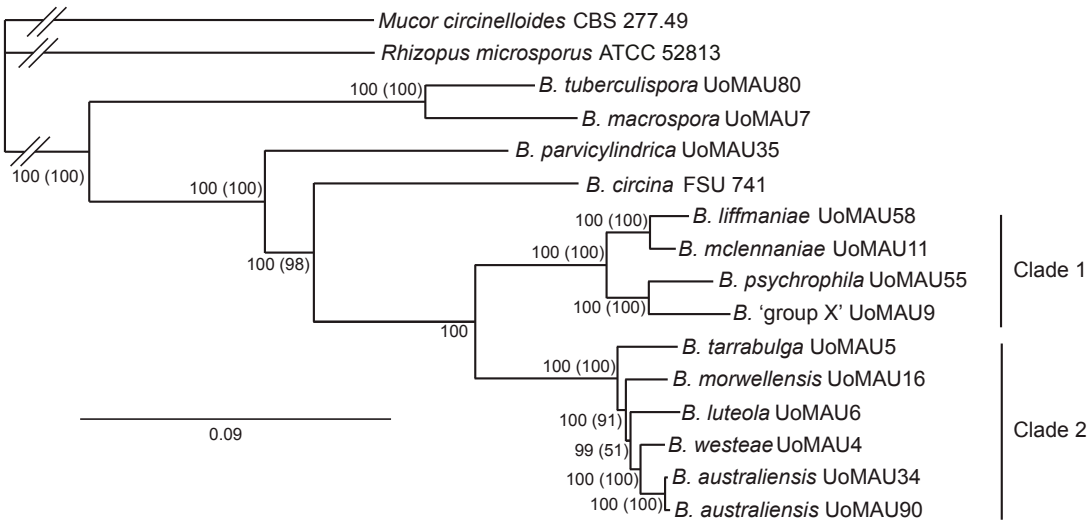

Fig. 5 A multi-locus Bayesian phylogenetic tree resolves two species groups within the genus *Backusella* in Australia. Branches are labelled with posterior probabilities (%). Bootstrap support values derived from a maximum likelihood analysis are given in parentheses based on 1 000 repeats.

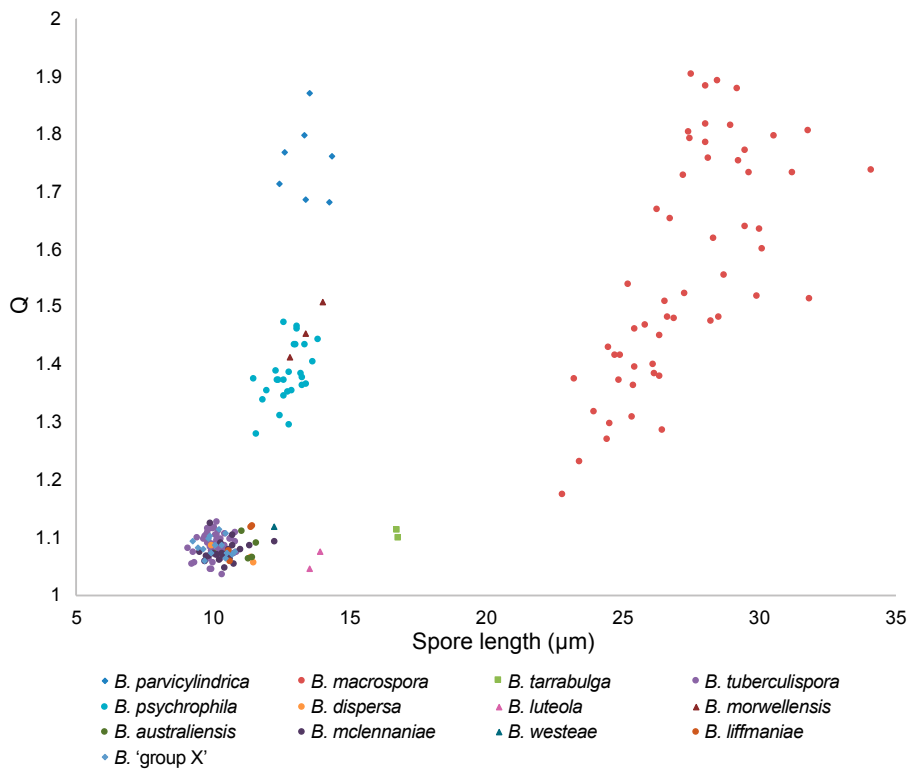

Fig. 6 Graph of spore dimensions for the strains of *Backusella* isolated in this study. Q represents the quotient of average spore length and width.

investigated using the genome sequencing data to identify the putative *sex* loci in these strains. A putative *sex* (mating type) locus, typical of heterothallic mating, was revealed in all sequenced strains.

Four strains had the *sexM* gene and nine had the *sexP* gene (Fig. 8a). The genes flanking the *sexM*/*sexP* genes (*algL*, *sagA* and *glrR*) were the same as those observed previously for *B. circina* and linked to the locus in other *Mucorales* species (Schulz et al. 2017). To confirm that the species were heterothallic, mating reactions were set up between the sequenced strains of known mating type with strains of the same species to identify a strong mating partner. Successful partners were identified for four of the species: *B. australiensis*, *B. luteola*, *B. mclennaniae*, and *B. parvicylindrica*. In agreement with previous studies (Stalpers & Schipper 1980), comparing the morphology of the zygospores produced by different species was less informative than the differences between the respective asexual reproductive structures (Fig. 8).

Sucrose utilization and thermotolerance provide evidence for physiological differences between Backusella species

After pilot studies examining carbon utilization using API® 50 CH strips (bioMérieux), sucrose utilization on defined medium was investigated as a species delineating trait. Some strains were unable to grow well on defined medium with either glucose or sucrose as the sole carbon source. The nutritional requirements of these strains would need to be further studied to be able to assess their ability to utilize sucrose. Nonetheless, most of the strains grew either well on both sucrose and glucose (indicating an ability to metabolize sucrose into its constitu-

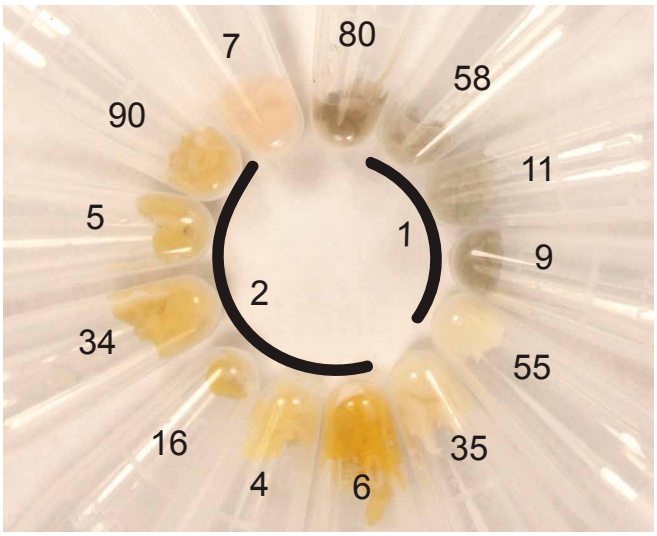

Fig. 7 Pigmentation of the whole genome sequenced strains of *Backusella* after scraping from potato dextrose agar culture. Strains are labelled with UoMAU numbers, with the Clade 1 and Clade 2 species indicated. All strains within each species had consistent colony pigmentation conforming to that of the representative stains shown here.

tive glucose and fructose monosaccharides) or only well on glucose (indicating a lack of the ability to use sucrose as a carbon source). The ability or inability to use sucrose appears to be stable between strains within a species. *Backusella liffmaniae*, *B. mclennaniae*, *Backusella* 'group X', *B. tarrabulga*, *B. westeae*, and *B. morwellensis* are able to utilize sucrose whereas *B. psychrophila*, *B. tuberculispora*, *B. australiensis*, *B. parvicylindrica*, and *B. macrospora* are not (Fig. 9a; *cf* Fig. 2).

Examination of the next generation sequencing data revealed variation in the presence of a putative gene (*sucB*) encoding invertase that was concordant with the ability to utilize sucrose (Fig. 9b). The strains UoMAU7, UoMAU35, UoMAU55, and UoMAU80 lack both a functional copy of this gene and the ability to grow on sucrose. The strains UoMAU4, UoMAU5, UoMAU6, UoMAU9, UoMA11, UoMAU16, and UoMAU56 both have a copy of *sucB* and the ability to utilize sucrose.

The exception to this generalization was the two sequenced *B. australiensis* strains that lack the ability to utilize sucrose but have a copy of invertase. However, examination of the *sucB* alleles revealed a large deletion in the promoter sequence of the invertase *sucB* gene in these strains when compared to the sister species *B. westeae* (Fig. 9d). This might affect expression of the gene.

To examine if the *sucB* gene does indeed encode a functional invertase, the gene was tested for its ability to complement a *S. cerevisiae* invertase mutant. The *SUC2* gene was mutated in

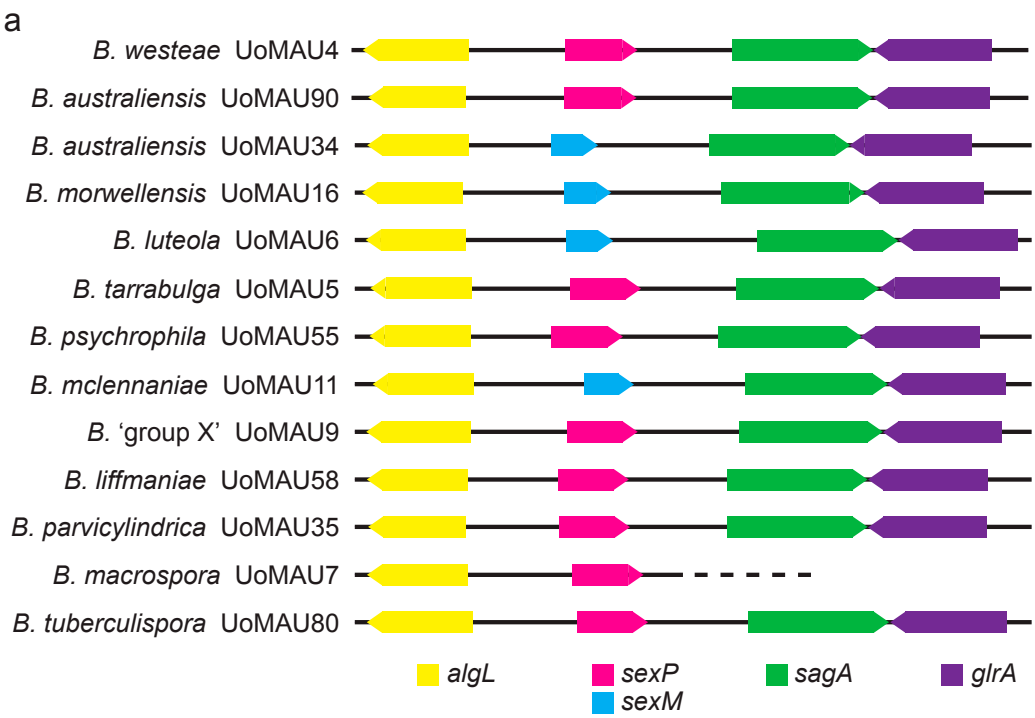

Fig. 8 Newly described *Backusella* species are heterothallic. a. Diagram of the mating type locus of the sequenced strains. The mating type of UoMAU7 was fragmented in the assembly, hence one flank is missing; b. SEM of zygospores for the four species for which mating partners were identified. — Scale bars = 20 µm.

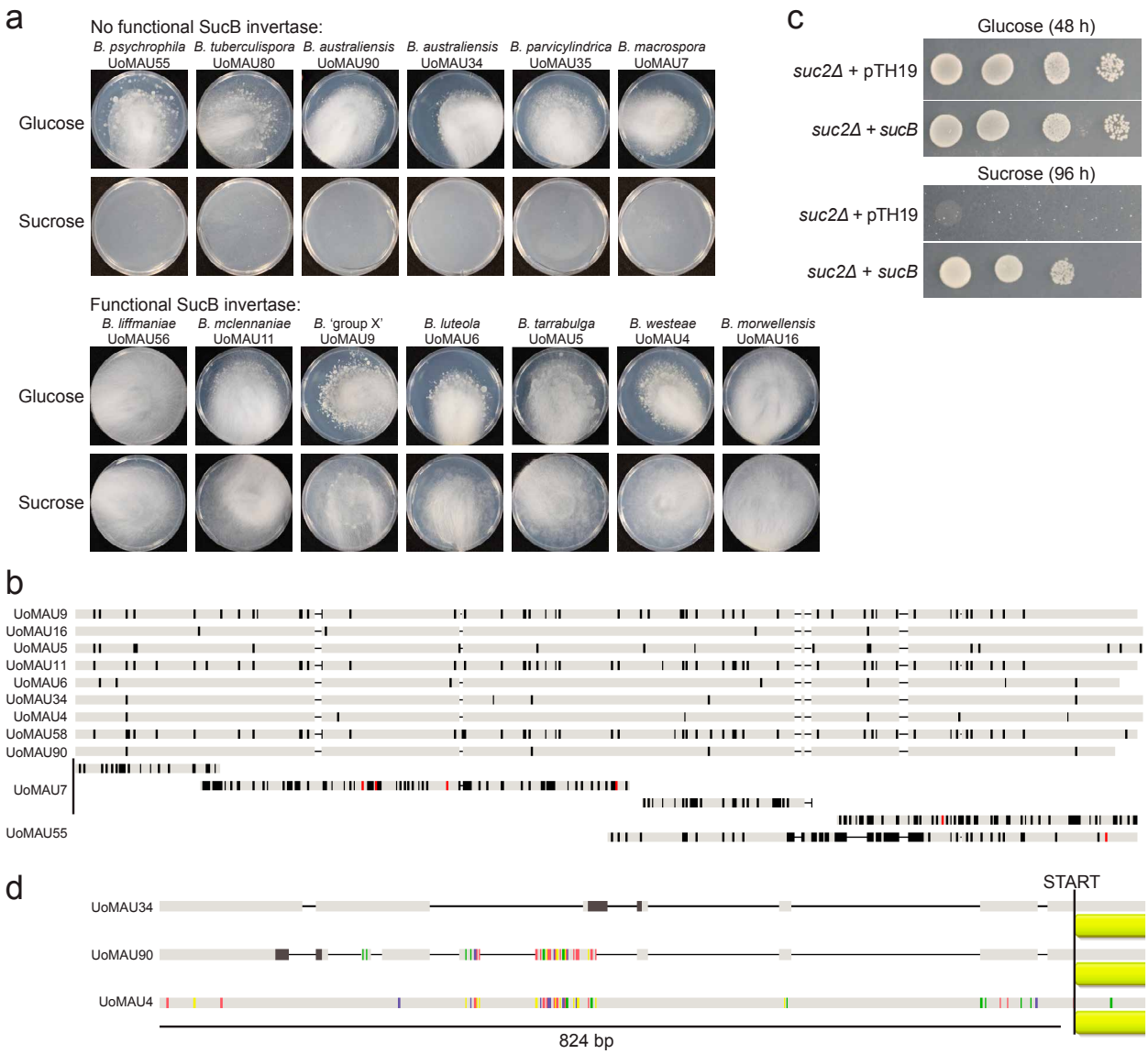

Fig. 9 Sucrose assimilation in *Backusella* corresponds to the presence of a functional *sucB* gene encoding invertase. a. Growth of strains on sucrose as the sole carbon source is variable between species; b. strains that are able to utilize sucrose have alleles of *sucB* with a full open reading frame. Shown is a translated nucleotide alignment with polymorphisms highlighted in black and stop codons in red in UoMAU7 and UoMAU55; c. the *sucB* gene from *B. westeae* confers the ability to utilize sucrose to a *Saccharomyces cerevisiae SUC2Δ* deletion mutant; d. two *B. australiensis* strains that are unable to grow on sucrose have a *sucB* allele. One possible explanation is that deletions in the promoter region are affecting expression of the gene, illustrated by the black lines in UoMAU34 and UoMAU90.

a *S. cerevisiae* strain by homologous recombination replacing the open reading frame with the KanMX selectable marker. The cDNA of the *sucB* gene was amplified from *B. westeae*, cloned into an expression vector, and this plasmid or the empty plasmid were transformed into the *S. cerevisiae SUC2*Δ mutant. The *B. westeae* gene was able to complement the loss of sucrose utilization in the *S. cerevisiae* mutant (Fig. 9c).

In addition to the ability to utilize sucrose we examined a second physiological trait: growth at restrictive temperatures. There was variation at both the inter-species and intra-species level for growth at 30 °C (Fig. 10). Two species showed either no growth or limited growth in all strains, these being *B. macrospora* (n = 53) and *B. psychrophila* (n = 29). Two species show strong growth at 30 °C in all strains, i.e., *B. westeae* (n = 1) and *B. morwellensis* (n = 3), but it should be noted that for both these species there are a limited number of strains available. The remaining strains showed variable thermotolerance; for example strains of *B. tuberculispora* showed a wide range of growth from very minimal growth (e.g., UoMAU108) to very strong growth (e.g., UoMAU80).

A third physiological trait, utilization of dulcitol, was tested in the three species *Backusella* 'group X', *B. mclennaniae*, and *B. liffmaniae* to provide a trait to distinguish *B. liffmaniae* from the former two species. Dulcitol was chosen based on preliminary data of carbon utilization capabilities obtained using API® 50 CH test strips. The *B. liffmaniae* strains grow less vigorously on dulcitol compared to *Backusella* 'group X' and *B. mclennaniae* (Fig. 11). The utilization of dulcitol has not been studied in the *Mucorales* and we have yet to identify the genetic basis for this trait.

TAXONOMY

Backusella australiensis Urquhart & Douch, *sp. nov.* — MycoBank MB831215; Fig. 12

Etymology. Referring to the country from which it was isolated.

Typus. Australia, Victoria, Morwell National Park, isolated from leaf litter (holotype MEL 2447010, dried culture on filter paper, National Herbarium of Victoria, Victoria, culture ex-type UoMAU34, JMRC SF014050).

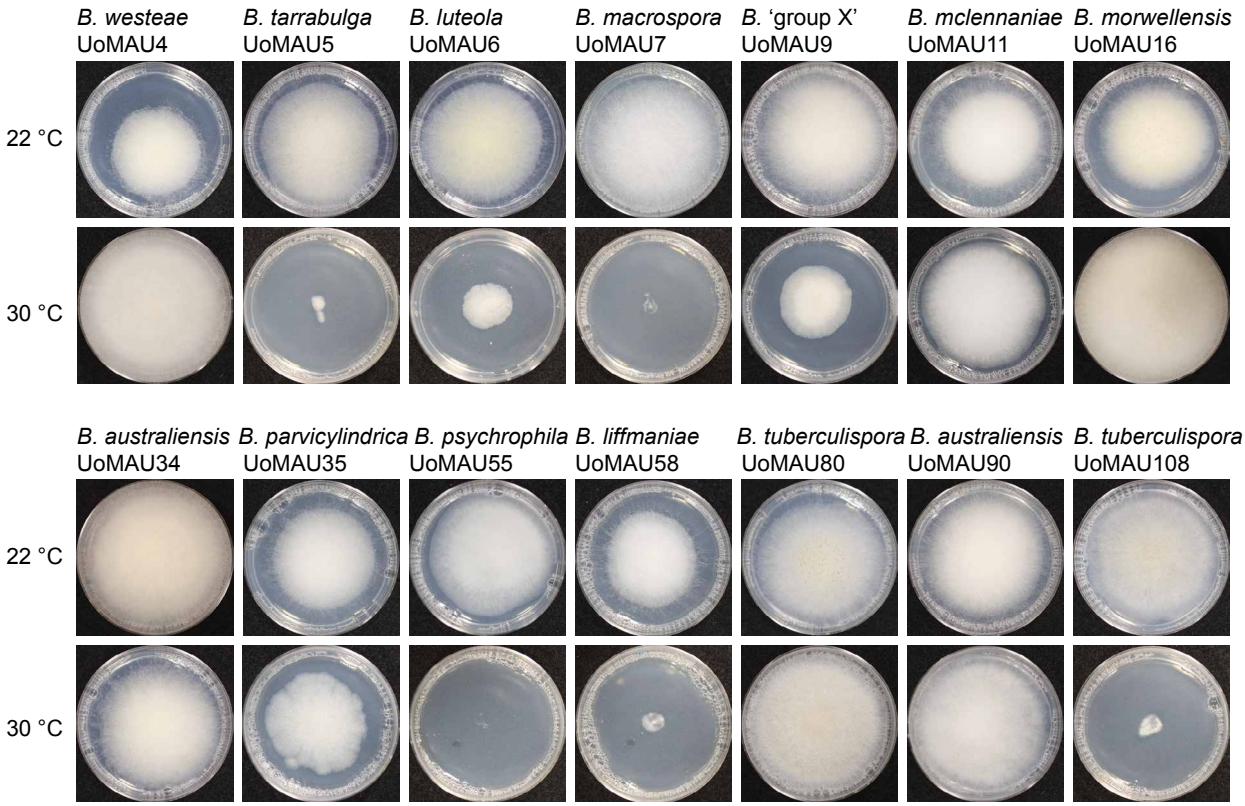

Fig. 10 *Backusella* strains have variable temperature dependent growth. Growth of representative strains at 22 °C and 30 °C. Note the wide intraspecific variation between *B. tuberculispora* UoMAU80 and *B. tuberculispora* UoMAU108.

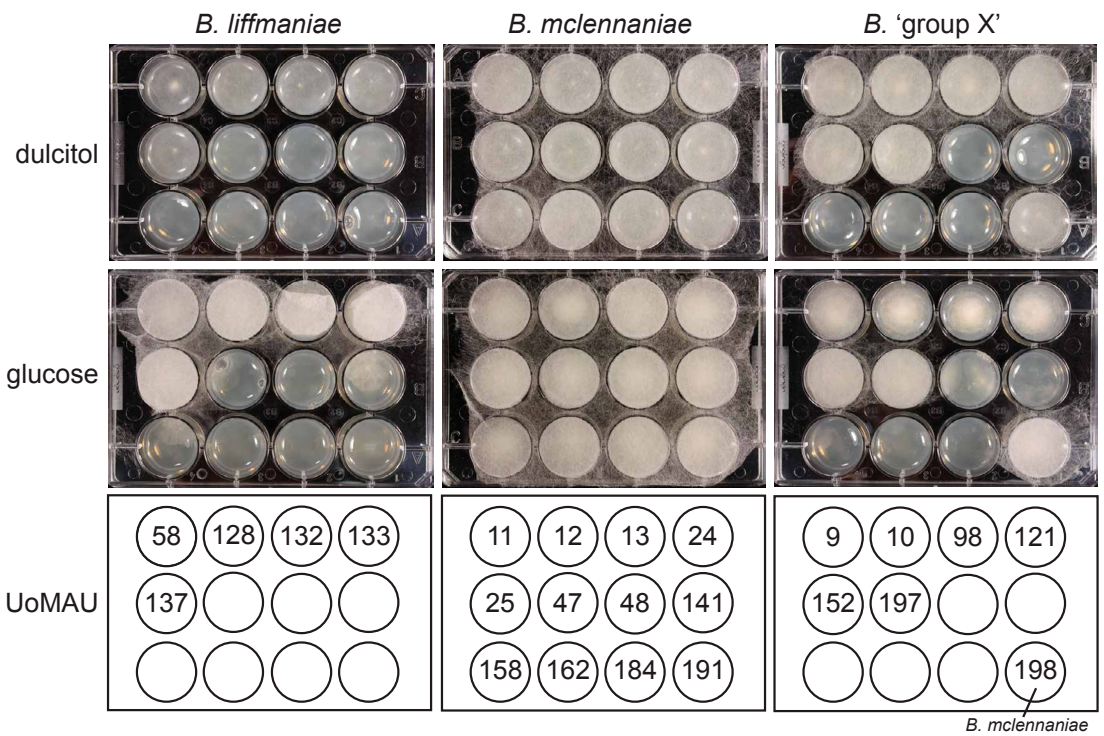

Fig. 11 Growth of *Backusella* 'group X', *B. mclennaniae* and *B. liffmaniae* on media containing either dulcitol or glucose as a sole carbon source.

Sporangiophores up to 12.3 µm diam, generally tapering very slightly towards sporangia, occasionally branched, hyaline, with or without pale yellow contents. *Sporangia* minutely spinulose, 22.9–95.0 × 21.7–92.2 (av ± SD = 57.7 ± 24.6 × 55.4 ± 23.8) µm, globose to subglobose (Q = 1.00–1.08 (av ± SD = 1.04 ± 0.02)). *Columellae* smooth-walled with pale yellow granular content, 18.8–32.2 × 15.9–29.9 (av ± SD = 24.1 ± 4.5 × 22.0 ± 4.6) µm, variably shaped globose, ellipsoid or applanate (Q = 1.02–1.21 (av ± SD = 1.10 ± 0.06)). Collars small and uncommon. *Sporangiospores* smooth-walled, 10.50–13.5 × 9.8–12.1 (av ± SD = 12.2 ± 1.2 × 10.8 ± 0.8) µm, subglobose to broadly ellipsoid (Q = 1.05–1.29 (av ± SD = 1.13 ± 0.07)). *Giant cells* and *chlamydospores* not observed. *Sporangiola* present.

Culture characteristics — Colony cottony in texture, reaching 41 mm diam and 19 mm height after 3 d growth on PDA at 22 °C. Obverse white, becoming yellow by 4 wk. Reverse pale yellow to yellow, becoming paler towards edges.

Notes — The species shows a close genetic and morphological similarity to its sister taxon *B. westeae*; however, based on whole genome sequencing of two independently isolated *B. australiensis* strains and the single *B. westeae* strain there is sufficient separation to warrant its treatment as a separate species. *Backusella australiensis* can be distinguished from *B. westeae* by physiological differences such as its inability to grow on sucrose as a sole carbon source.

Backusella circina J.J. Ellis & Hesselt., Mycologia 61: 865. 1969

Synonym. Backusella johorensis L.S. Loh et al., Mucoraceous Fungi from Malaysia: 70. 2001.

Notes — *Backusella johorensis* was reportedly unavailable for study (Lima et al. 2016) and no sequencing information is available. However, the original description of the species (Loh et al. 2001) cites an ex-type strain IMI 350574 deposited in the IMI collection at CABI, which is available as a living strain. LSU and ITS sequence information obtained from this strain indicates that *B. johorensis* is a synonym of *B. circina*.

Backusella dispersa (Hagem) Urquhart & Douch, *comb. nov*. — MycoBank MB831145; Fig. 13

Basionym. *Mucor dispersus* Hagem, Ann. Mycol. 8 (3): 271. 1910.

The following description is of UoMAU119 and is intended to illustrate the characteristics of the Australian collections, not replace Hagem's original diagnosis of the ex-type strain CBS 107.09.

Sporangiophores up to 11.3 µm diam, generally tapering very slightly towards sporangia, occasionally branched, hyaline, with or without pale yellow contents. *Sporangia* minutely spinulose, 30.2–47.5 × 28.3–46.1 (av ± SD = 37.5 ± 6.1 × 36.6 ± 6.2) µm, globose (Q = 1.00–1.06 (av ± SD = 1.03 ± 0.02)). *Columellae* smooth-walled with pale yellow granular content, 19.5–38.7 × 18.5–33.4 (av ± SD = 27.9 ± 6.8 × 25.4 ± 5.2) µm, variably shaped globose, ellipsoid or applanate (Q = 1.02–1.17 (av ± SD = 1.09 ± 0.05)). Collars small and uncommon. *Sporangiospores* smooth-walled, 8–12 × 7–10 (av ± SD = 9.5 ± 0.8 × 8.7 ± 0.9) µm, globose to broadly ellipsoid (Q = 1.00–1.29 (av ± SD = 1.09 ± 0.08)). Abundant *giant cells*. *Chlamydospores* not observed. *Sporangiola* present.

Culture characteristics — Colony cottony in texture, reaching 49 mm diam and 21 mm height after 3 d growth on PDA at 22 °C. Obverse white, becoming yellow by 4 wk. Reverse pale yellow, becoming paler towards edges.

Notes — All three phylogenetic trees indicate a clear separation between a clade consisting of three new collections (UoMAU116, UoMAU119, and UoMAU120) as well as several strains of '*B. lamprospora*' (CBS 224.67, 196.28, 107.09, and 195.28) which were originally identified as *Mucor dispersus* (Hagem 1910) from the ex-type strain of *B. lamprospora* (CBS

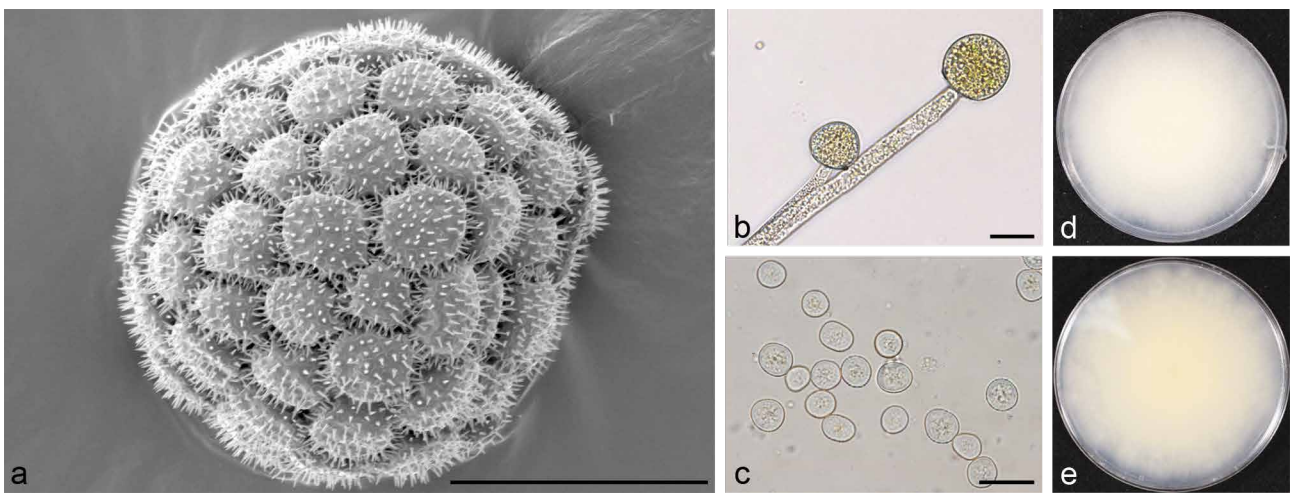

Fig. 12 Morphology of *Backusella australiensis* strain UoMAU34. a. SEM of sporangium; b. light microscope image of columella; c. light microscope image of sporangiospores; d, e. obverse and reverse of colony. — Scale bars = 20 µm.

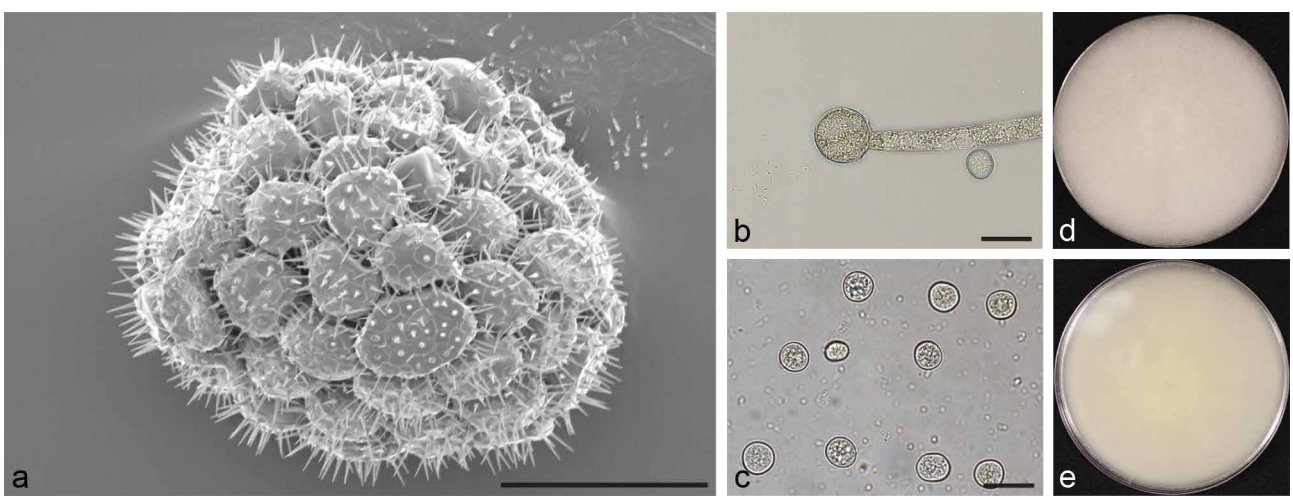

Fig. 13 Morphology of *Backusella dispersa* strain UoMAU119. a. SEM of sporangium; b. light microscope image of columella; c. light microscope image of sporangiospores; d, e. obverse and reverse of colony. — Scale bars = 20 µm.

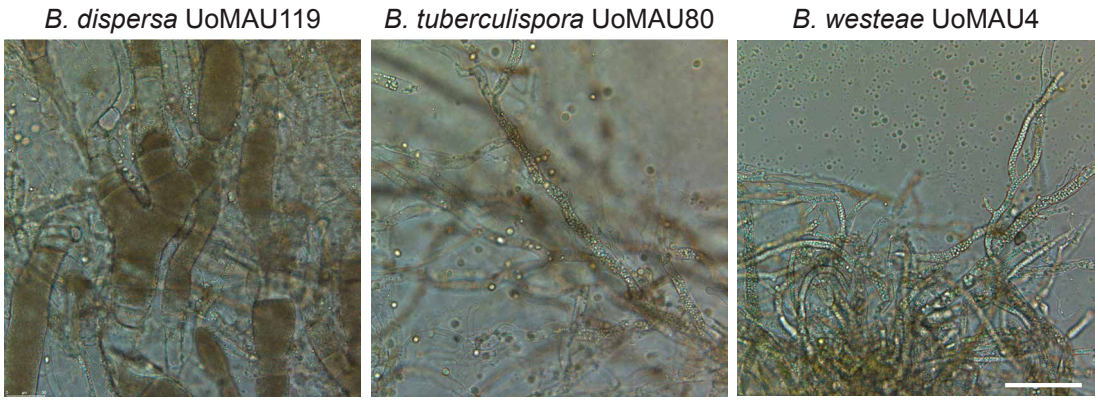

Fig. 14 *Backusella dispersa* strains produced abundant giant cells in their substrate mycelia that are distinct from the inflated droplet filled hyphal regions seen in other strains like *B. tuberculispora* and *B. westeae.* — Scale bar = 100 µm.

118.08) isolated by Lendner (Lendner 1908). *Mucor dispersus* was subsequently synonymized with *Mucor lamprospora* (Schipper 1969) prior to the transfer of *Mucor lamprospora* to the genus *Backusella* (Benny & Benjamin 1975). Hence these strains are currently considered to be *B. lamprospora.* However, synonymizing *Mucor dispersus* with *Mucor lamprospora* was not universally agreed upon, with different authors giving different weight to morphological differences vs the ability to form zygospores in interspecific crosses (Schipper 1969, Mehrotra et al. 1974, Benny & Benjamin 1975). The key morphological difference supporting the separation of *M. dispersus* from *B. lamprospora* is the presence of giant cells (Hagem 1910, Sarbhoy 1968, Ellis & Hesseltine 1969, Mehrotra et al. 1974). We therefore examined our strains for the presence of giant cells (Fig. 14). We found that these could be readily observed in the three strains which grouped with *M. dispersus* in the molecular phylogenies (and not in other species). These cells closely resemble those illustrated in the original description of

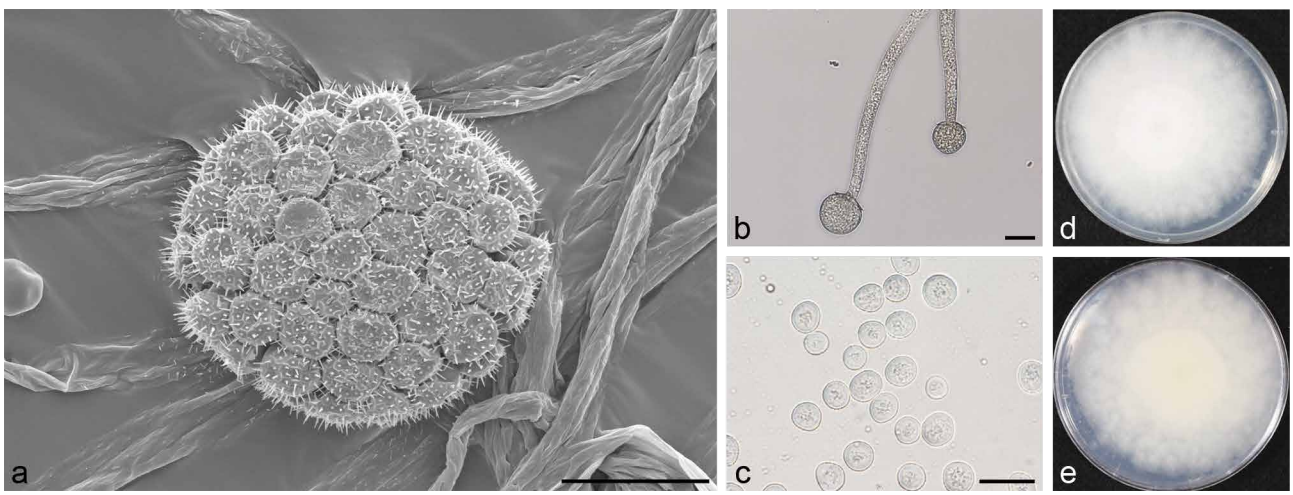

Fig. 15 Morphology of *Backusella liffmaniae* strain UoMAU58. a. SEM of sporangium; b. light microscope image of columella; c. light microscope image of sporangiospores; d, e. obverse and reverse of colony. — Scale bars = 20 µm.

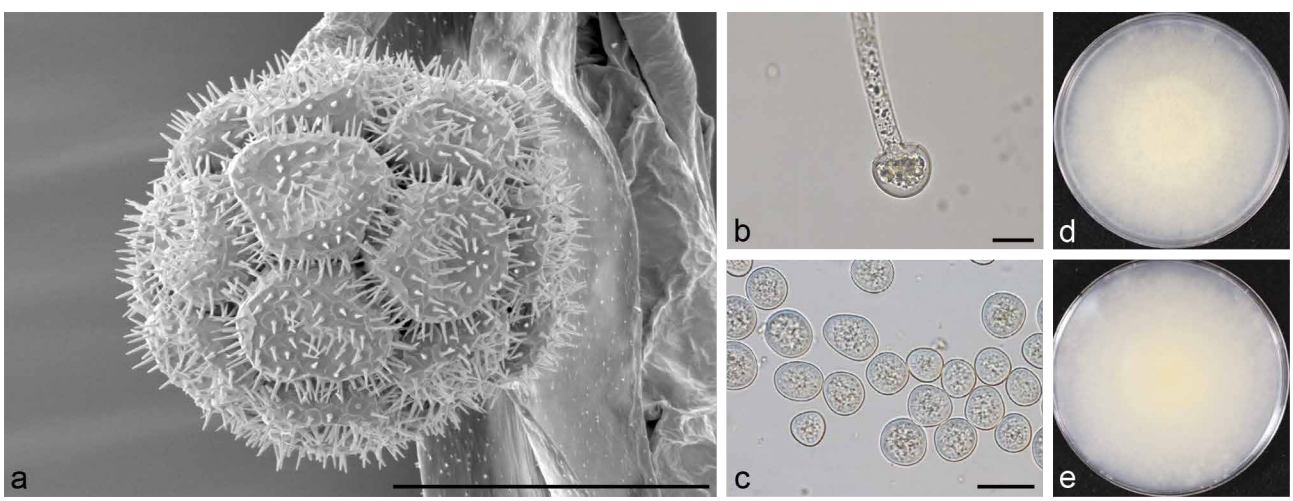

Fig. 16 Morphology of *Backusella luteola* strain UoMAU6. a. SEM of sporangium; b. light microscope image of columella; c. light microscope image of sporangiospores; d, e. obverse and reverse of colony. — Scale bars = 20 µm.

M. dispersus (Hagem 1910) and are distinct from the inflated droplet-filled hyphal region that have been observed more widely among *Backusella* species (e.g., *B. tuberculispora* and *B. westeae,* Fig. 14). Another species, *B. gigacellularis*, was recently reported to produce 'giant cells' (De Souza et al. 2014), however, these interesting cells appear to represent an unrelated cell type. In light of the clear molecular and morphological differences between *M. dispersus* and *B. lamprospora,* it is our opinion that they should be considered separate, despite the formation of zygospores in crosses between these two species. The new combination *Backusella dispersa* is proposed.

Backusella granulispora (Loh et al. 2001)

Notes — The ex-type strain cited by species (Loh et al. 2001) is not in the IMI collection at CABI in Egham, UK. The herbarium component of the IMI collection was transferred to the herbarium at the Royal Botanic Gardens Kew; however, the specimen is not available (Begoña Aguirre-Hudson pers. comm.). Thus, type material for this species is unavailable. The description of *B. granulispora* states that the species does not have recurved juvenile sporangia and therefore it does not conform to the current morphological understanding of the genus (Loh et al. 2001, Walther et al. 2013). The issues presented when dealing with so-called 'old names' in taxonomy, i.e., those without an available type or sufficient description have been discussed previously (Dayarathne et al. 2016). While it is clear that this species is not a true member of the genus *Backusella*, future research will hopefully clarify the true taxonomy of this species.

Backusella liffmaniae Urquhart & Douch, *sp. nov.* — MycoBank MB831151; Fig. 15

Etymology. Recognition of the contribution made by Patricia Liffman in protecting the natural environment where the ex-type strain was isolated (Liffman 2016).

Typus. Australia, Victoria, Jack Cann Reserve, isolated from leaf litter (holotype MEL 2447034, dried culture on filter paper, National Herbarium of Victoria, Victoria, culture ex-type UoMAU58, JMRC SF014074).

Sporangiophores up to 13.6 µm diam, generally tapering very slightly towards sporangia, occasionally branched, hyaline, with or without pale yellow contents. Sporangia minutely spinulose, 26.5–55.2 × 26.0–54.1 (av ± SD = 38.5 ± 10.8 × 36.9 ± 10.6) µm, globose to subglobose (Q = 1.02–1.12 (av ± SD = 1.04 ± 0.03)). *Columellae* smooth-walled with pale yellow granular content, 14.8–26.3 × 14.8–24.4 (av ± SD = 20.8 ± 3.7 × 19.4 ± 3.2) µm, variably shaped globose, ellipsoid or applanate (Q = 1.00–1.24 (av ± SD = 1.07 ± 0.07)). Collars small and uncommon. *Sporangiospores* smooth-walled, 9.0–12.9 × 8.6–12.0 (av ± SD = 11.4 ± 1.3 × 10.4 ± 1.2) µm, globose to broadly ellipsoid (Q = 1.01–1.17 (av ± SD = 1.09 ± 0.06)). *Giant cells* and *chlamydospores* not observed. *Sporangiola* present.

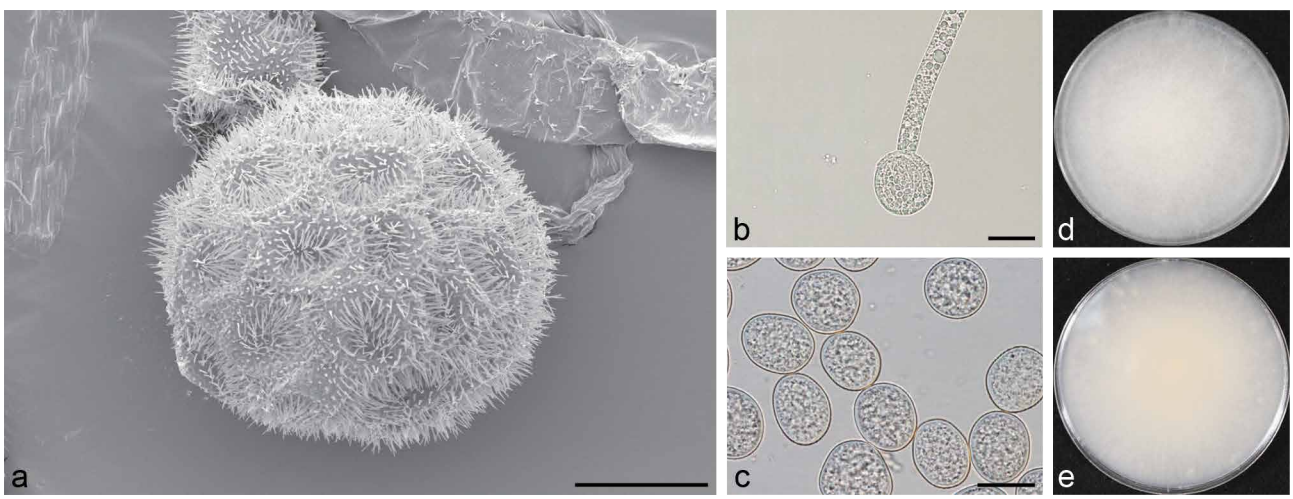

Fig. 17 Morphology of *Backusella macrospora* strain UoMAU7. a. SEM of sporangium; b. light microscope image of columella; c. light microscope image of sporangiospores; d, e. obverse and reverse of colony. — Scale bars = 20 µm.

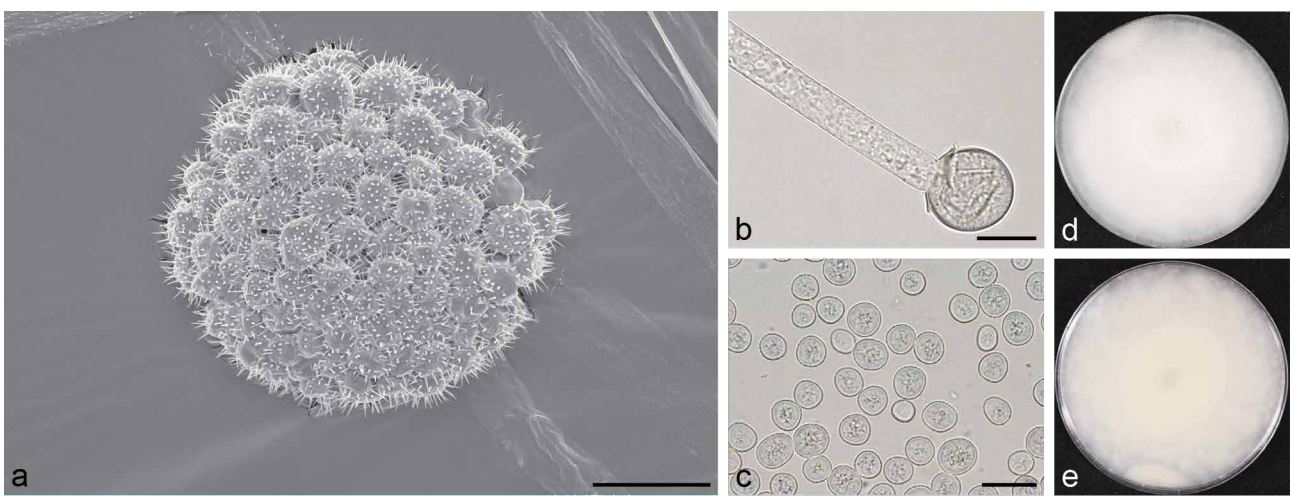

Fig. 18 Morphology of *Backusella mclennaniae* strain UoMAU11. a. SEM of sporangium; b. light microscope image of columella; c. light microscope image of sporangiospores; d, e. obverse and reverse of colony. — Scale bars = 20 µm.

Culture characteristics — Colony cottony in texture, reaching 32 mm diam and 17 mm height after 3 d growth on PDA at 22 °C. Obverse white, becoming yellowish grey by 4 wk. Reverse creamy white, becoming paler towards edges.

Notes — Can be distinguished from *Backusella* 'group X' and *B. mclennaniae* by its inability to efficiently utilize dulcitol as a sole carbon source.

Backusella luteola Urquhart & Douch, *sp. nov.* — MycoBank MB831149; Fig. 16

Etymology. Referring to the yellow colony pigmentation, which is a trait in common with other Clade 2 species.

Typus. Australia, Victoria, Jack Cann Reserve, isolated from leaf litter (holotype MEL 2446983, dried culture on filter paper, National Herbarium of Victoria, Victoria, culture ex-type UoMAU6, JMRC SF014023).

Sporangiophores up to 9.7 µm diam, generally tapering very slightly towards sporangia, occasionally branched, hyaline, with or without pale yellow contents. *Sporangia* minutely spinulose, 25.8–59.2 × 22.9–55.0 (av ± SD = 41.6 ± 10.8 × 38.0 ± 10.2) µm, globose to ellipsoid (Q = 1.01–1.43 (av ± SD = 1.10 ± 0.12)). *Columellae* smooth-walled with pale yellow granular content, 16.9–26.4 × 14.7–23.4 (av ± SD = 22.0 ± 3.4 × 18.2 ± 2.6) µm, variably shaped globose, ellipsoid or applanate (Q = 1.12–1.52 (av ± SD = 1.21 ± 0.13)). Collars small and uncommon. *Sporangiospores* smooth-walled, 12.9–20.1 × 12.3–16.7 (av ± SD = 16.2 ± 2.2 × 14.8 ± 1.4) µm, globose to ellipsoid (Q = 1.00–1.34 (av ± SD = 1.10 ± 0.10)). *Giant cells* and *chlamydospores* not observed. *Sporangiola* present.

Culture characteristics — Colony cottony in texture, reaching 42 mm diam and 38 mm height after 3 d growth on PDA at 22 °C. Obverse very pale yellow, becoming brownish yellow by 4 wk. Reverse pale yellow, becoming paler towards edges.

Notes — Known from two independent collections taken 225 km apart in Victoria. Both isolates have similar spore dimensions which are unique from all other species isolated in this study (Fig. 6).

Backusella macrospora Urquhart & Douch, *sp. nov.* — MycoBank MB831143; Fig. 17

Etymology. Referring to the large sporangiospores.

Typus. Australia, Victoria, Tarra-Bulga National Park, isolated from leaf litter (holotype MEL 2446984, dried culture on filter paper, National Herbarium of Victoria, Victoria, culture ex-type UoMAU7, JMRC SF014024).

Sporangiophores up to 10.5 µm diam, generally tapering very slightly towards sporangia, occasionally branched, hyaline, with or without pale yellow contents. *Sporangia* minutely spinulose, 37.8–76.4 × 34.3–70.7 (av ± SD = 60.6 ± 12.2 × 54.3 ± 10.4) µm, globose to ellipsoid (Q = 1.01–1.40 (av ± SD = 1.12 ± 0.12)). *Columellae* smooth-walled with pale yellow granular content, 16.8–30.4 × 13.0–27.3 (av ± SD = 25.0 ± 4.3 × 20.9 ± 3.9) µm, variably shaped globose, ellipsoid or applanate (Q = 1.00–1.48 (av ± SD = 1.20 ± 0.15)). Collars small and uncommon. *Sporangiospores* smooth-walled, 21.4–33.3 × 19.6–26.8 (av ± SD = 27.7 ± 3.8 × 22.6 ± 2.5) µm, globose to ellipsoid (Q = 1.00–1.39 (av ± SD = 1.20 ± 0.13)). *Giant cells* and *chlamydospores* not observed. *Sporangiola* present.

Culture characteristics — Colony cottony in texture, reaching 42 mm diam and 14 mm height after 3 d growth on PDA at 22 °C. Obverse white, becoming cream by 4 wk. Reverse white to very pale salmon, becoming paler towards edges.

Notes — Can be distinguished from all other species isolated in this study by its large sporangiospores, the shape of which is variable between isolates.

Backusella mclennaniae Urquhart & Douch, *sp. nov.* — MycoBank MB831152; Fig. 18

Etymology. For Australian mycologist Ethel Irene McLennan (Ducker 2012).

Typus. Australia, Victoria, Morwell National Park, isolated from leaf litter (holotype MEL 2446987, dried culture on filter paper, National Herbarium of Victoria, Victoria, culture ex-type UoMAU11, JMRC SF014027).

Sporangiophores up to 8.7 µm diam, generally tapering very slightly towards sporangia, occasionally branched, hyaline, with or without pale yellow contents. *Sporangia* minutely spinulose, 25.6–39.6 × 24.2–39.1 (av ± SD = 31.8 ± 4.3 × 30.2 ± 4.7) µm, globose to subglobose (Q = 1.01–1.10 (av ± SD = 1.06 ± 0.03)). *Columellae* smooth-walled with pale yellow granular content, 13.0–25.3 × 9.1–23.8 (av ± SD = 18.8 ± 3.8 × 16.5 ± 4.5) µm, variably shaped globose, ellipsoid or applanate (Q = 1.00–1.42 (av ± SD = 1.1 ± 0.14)). Collars small and uncommon. *Sporangiospores* smooth-walled, 9.6–13.6 × 9.2–12.0 µm (av ± SD = 11.4 ± 1.3 × 10.3 ± 0.8) µm, globose to ellipsoid (Q = 1.02–1.32 (av ± SD = 1.10 ± 0.09)). *Giant cells* and *chlamydospores* not observed. *Sporangiola* present.

Culture characteristics — Colony cottony in texture, reaching 42 mm diam and 44 mm height after 3 d growth on PDA at 22 °C. Obverse white, becoming yellowish grey by 4 wk. Reverse white sometimes with grey zones. becoming paler towards edges.

Notes — See *Backusella* 'group X'.

Backusella morwellensis Urquhart & Douch, *sp. nov.* — MycoBank MB831148; Fig. 19

Etymology. Referring to Morwell National Park, the origin of the type specimen.

Typus. Australia, Victoria, Morwell National Park, isolated from leaf litter (holotype MEL 2446992, dried culture on filter paper, National Herbarium of Victoria, Victoria, culture ex-type UoMAU16, JMRC SF014032).

Sporangiophores up to 10.9 µm diam, generally tapering very slightly towards sporangia, occasionally branched, hyaline, with or without pale yellow contents. *Sporangia* minutely spinulose, 23.5–71.6 × 23.2–57.2 (av ± SD = 37.0 ± 14.8 × 34.1 ± 10.9) µm, globose to ellipsoid (Q = 1.01–1.25 (av ± SD = 1.07 ± 0.09)). *Columellae* smooth-walled with pale yellow granular content, 18.6–29.8 × 16.1–28.0 (av ± SD = 23.5 ± 3.7 × 20.9 ± 3.5) µm, variably shaped globose, ellipsoid or applanate (Q = 1.01–1.30 (av ± SD = 1.13 ± 0.08)). Collars small and uncommon. *Sporangiospores* smooth-walled, 9.4–17.4 × 7.7–13.0 µm (av ± SD = 13.2 ± 2.6 × 9.9 ± 1.8) µm, broadly ellipsoid to ellipsoid (Q = 1.15–1.55 (av ± SD = 1.33 ± 0.14)). *Giant cells* and *chlamydospores* not observed. *Sporangiola* present.

Culture characteristics — Colony cottony in texture, reaching 45 mm diam and 23 mm height after 3 d growth on PDA at 22 °C. Obverse very pale yellow becoming brownish yellow by 4 wk. Reverse yellow, becoming paler towards edges.

Notes — Spore dimensions overlap those of *B. psychrophila*. Despite the similar spore morphology, molecular data show that these two species are not closely related. *Backusella morwellensis* can be readily distinguished from *B. psychrophila* by the ability of *B. morwellensis* to utilize sucrose.

Backusella parvicylindrica Urquhart & Douch, *sp. nov.* — MycoBank MB831150; Fig. 20

Etymology. From the Latin *parvus* meaning small and *cylindrica* from the Greek *kylindros* meaning a roller or cylinder, referring to the dimensions of the sporangiospores.

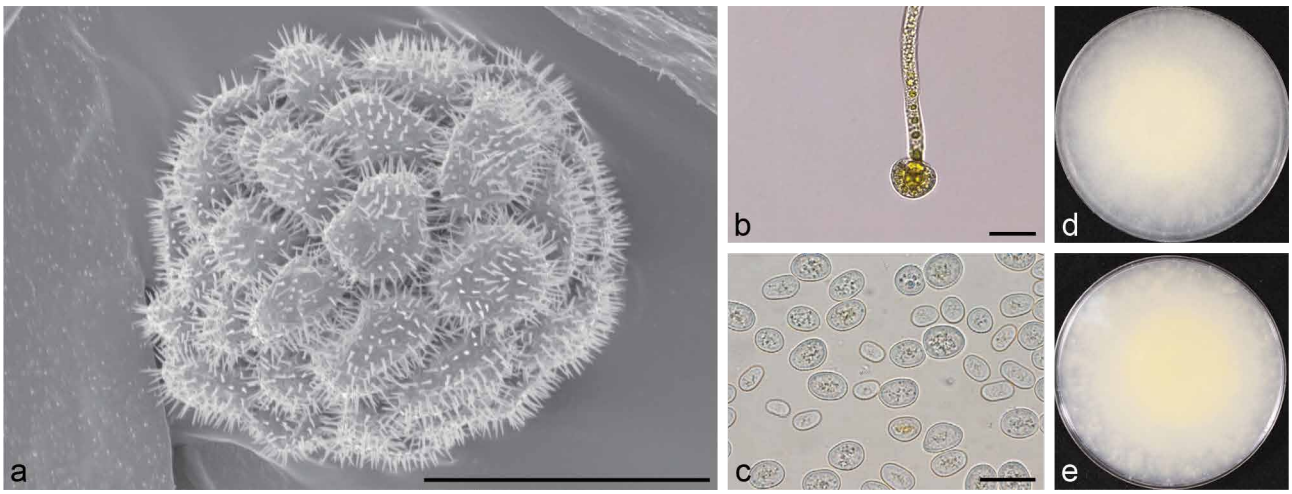

Fig. 19 Morphology of *Backusella morwellensis* strain UoMAU16. a. SEM of sporangium; b. light microscope image of columella; c. light microscope image of sporangiospores; d, e. obverse and reverse of colony. — Scale bars = 20 µm.

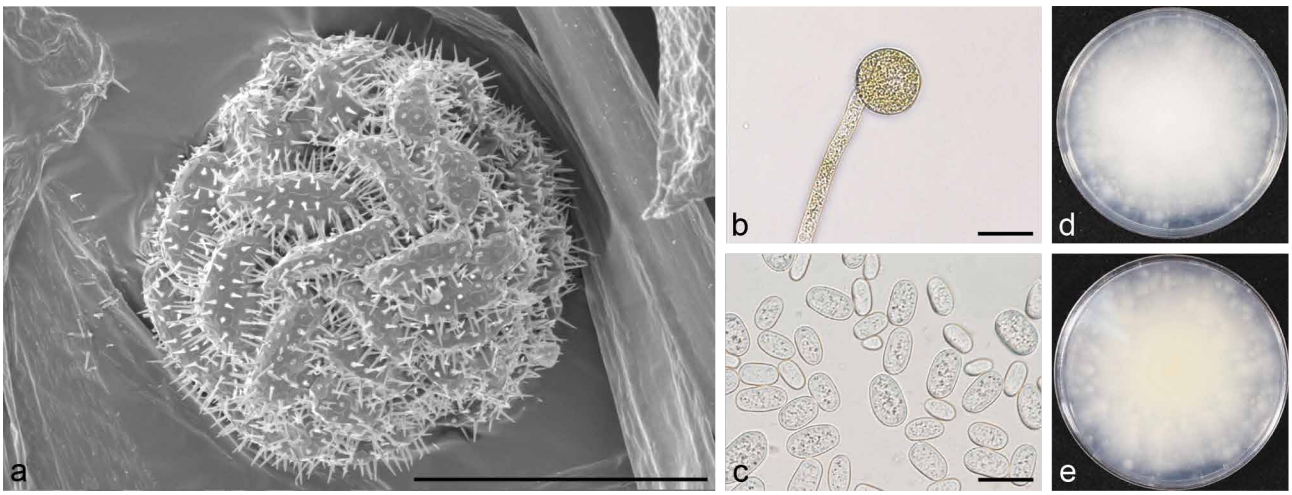

Fig. 20 Morphology of *Backusella parvicylindrica* strain UoMAU35. a. SEM of sporangium; b. light microscope image of columella; c. light microscope image of sporangiospores; d, e. obverse and reverse of colony. — Scale bars = 20 µm.

Typus. Australia, Victoria, Jack Cann Reserve, isolated from leaf litter (holotype MEL 2447011, dried culture on filter paper, National Herbarium of Victoria, Victoria, culture ex-type UoMAU35, JMRC SF014051).

Sporangiophores up to 11.8 µm diam, generally tapering very slightly towards sporangia, occasionally branched, hyaline, with or without pale yellow contents. *Sporangia* minutely spinulose, 23.5–47.2 × 23.1–45.9 (av ± SD = 33.9 ± 7.5 × 32.4 ± 7.4) µm, globose to subglobose (Q = 1.02–1.09 (av ± SD = 1.05 ± 0.03)). *Columellae* smooth-walled with pale yellow granular content, 23.3–29.6 × 19.6–25.4 (av ± SD = 26.8 ± 2.3 × 22.4 ± 2.0 µm), variably shaped globose, ellipsoid or applanate (Q = 1.06–1.44 (av ± SD = 1.20 ± 0.10)). Collars small and uncommon. *Sporangiospores* smooth-walled, 10.2–17.6 × 5.9–9.9 (av ± SD = 13.7 ± 2.0 × 7.8 ± 1.2) µm, ellipsoid to cylindric (Q = 1.57–2.31 (av ± SD = 1.78 ± 0.24)). *Giant cells* and *chlamydospores* not observed. *Sporangiola* present.

Culture characteristics — Colony cottony in texture, reaching 40 mm diam and 22 mm height after 3 d growth on PDA at 22 °C. Obverse white, becoming pale yellow by 4 wk. Reverse white to creamy white.

Notes — Can be morphologically distinguished from all other species isolated in this study by its unique spore dimensions that are on average less than 15 µm long and have a width/length ratio of less than 0.6.

Backusella psychrophila Urquhart & Douch*, sp. nov.* — MycoBank MB831154; Fig. 21

Etymology. Referring to the inability of all strains to grow above 30 °C.

Typus. Australia, Victoria, Jack Cann Reserve, isolated from leaf litter (holotype MEL 2447031, dried culture on filter paper, National Herbarium of Victoria, Victoria, culture ex-type UoMAU55, JMRC SF014071).

Sporangiophores up to 14.1 µm diam, generally tapering very slightly towards sporangia, occasionally branched, hyaline, with or without pale yellow contents. *Sporangia* minutely spinulose, 28.1–43.5 × 28.1–40.5 (av ± SD = 34.8 ± 5.9 × 33.5 ± 4.9) µm, globose to subglobose (Q = 1.00–1.13 (av ± SD = 1.03 ± 0.04)). *Columellae* smooth-walled with pale yellow granular content, 9.3–25.8 × 8.4–19.6 (av ± SD = 18.7 ± 4.6 × 15.7 ± 3.0) µm, variably shaped globose, ellipsoid or applanate (Q = 1.04–1.39 (av ± SD = 1.18 ± 0.11)). Collars small and uncommon. *Sporangiospores* smooth-walled, 10.6–16.9 × 9.1–11.7 (av ± SD = 14.2 ± 1.9 × 1.0 ± 0.9) µm, broadly ellipsoid to ellipsoid (Q = 1.17–1.57 (av ± SD = 1.42 ± 0.11)). *Giant cells* and *chlamydospores* not observed. *Sporangiola* present.

Culture characteristics — Colony cottony in texture, reaching 47 mm diam and 20 mm height after 3 d growth on PDA at 22 °C. Obverse white becoming brownish yellow by 4 wk. Reverse pale yellow to cream.

Notes — See *B. morwellensis*.

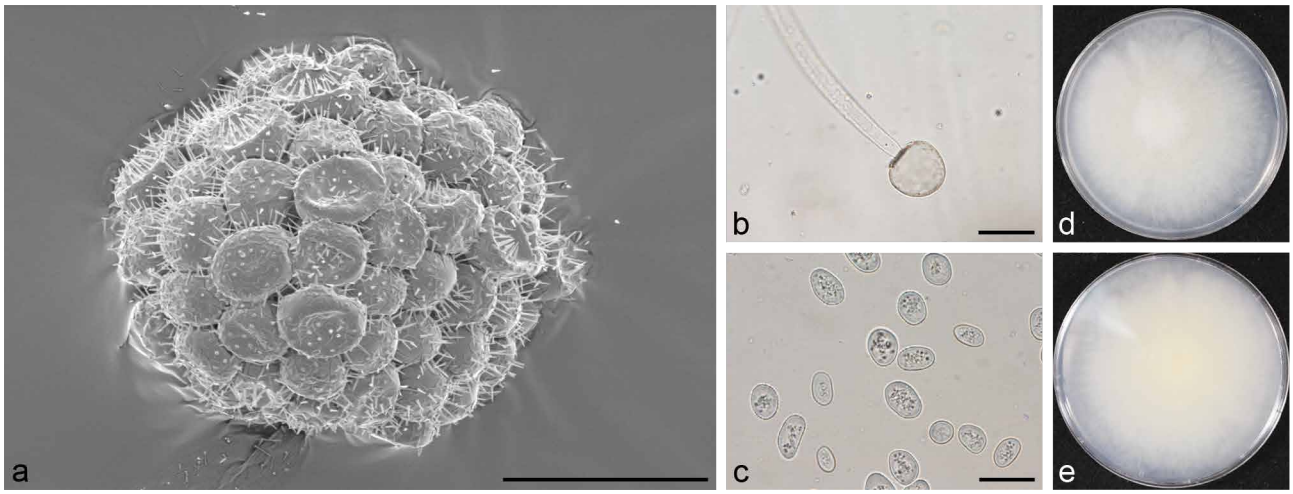

Fig. 21 Morphology of *Backusella psychrophila* strain UoMAU55. a. SEM of sporangium; b. light microscope image of columella, c. light microscope image of sporangiospores; d, e. obverse and reverse of colony. — Scale bars = 20 µm.

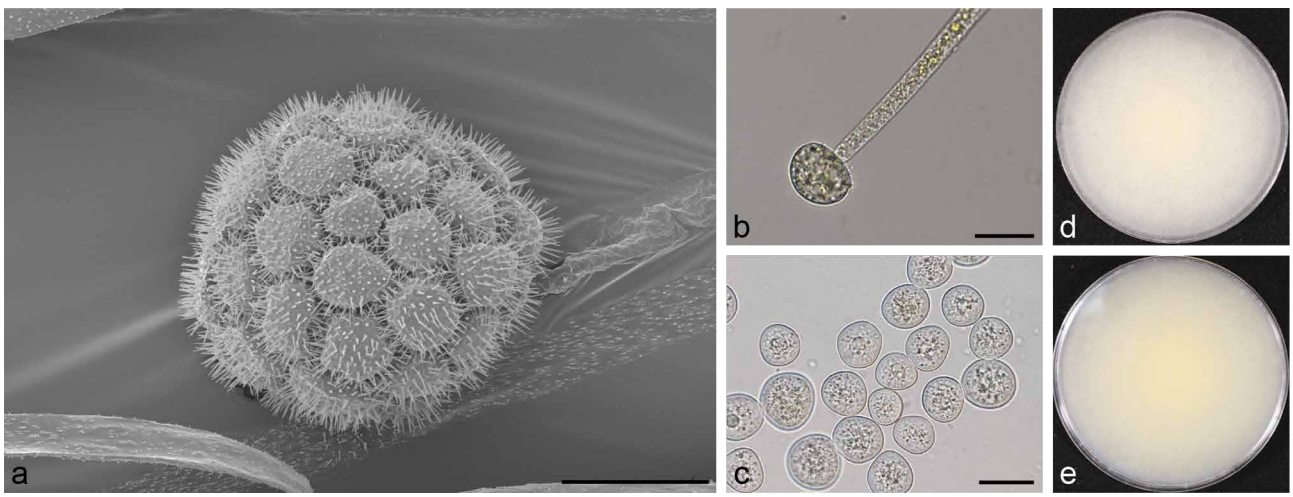

Fig. 22 Morphology of *Backusella tarrabulga* strain UoMAU5. a. SEM of sporangium; b. light microscope image of columella; c. light microscope image of sporangiospores; d, e. obverse and reverse of colony. — Scale bars = 20 µm.

Backusella tarrabulga Urquhart & Douch*, sp. nov.* — MycoBank MB831147; Fig. 22

Etymology. Derived from Tarra-Bulga, the name of the National Park where it was collected.

Typus. Australia, Victoria, Tarra-Bulga National Park, isolated from leaf litter (holotype MEL 2446982, dried culture on filter paper, National Herbarium of Victoria, Victoria, culture ex-type UoMAU5, JMRC SF014022).

Sporangiophores up to 8.4 µm diam, generally tapering very slightly towards sporangia, occasionally branched, hyaline, with or without pale yellow contents. *Sporangia* minutely spinulose, 29.7–48.0 × 24.2–41.8 (av ± SD = 36.8 ± 6.1 × 34.0 ± 6.5) µm, globose to ellipsoid (Q = 1.01–1.24 (av ± SD = 1.09 ± 0.08)). *Columellae* smooth-walled with pale yellow granular content, 20.4–34.0 × 16.2–27.4 (av ± SD = 25.3 ± 4.1 × 19.8 ± 3.4) µm, variably shaped globose, ellipsoid or applanate (Q = 1.16–1.51 (av ± SD = 1.28 ± 0.10)). Collars small and uncommon. *Sporangiospores* smooth-walled, 12.2–23.4 × 11.9–20.1 (av ± SD = 17.0 ± 3.1 × 15.8 ± 2.6) µm, globose to broadly ellipsoid (Q = 1.00–1.17 (av ± SD = 1.08 ± 0.06)). *Giant cells* and *chlamydospores* not observed. *Sporangiola* present.

Culture characteristics — Colony cottony in texture, reaching 41 mm diam and 21 mm height after 3 d growth on PDA at 22 °C. Obverse white, becoming brownish yellow by 4 wk. Reverse pale yellow, becoming paler towards edges.

Notes — Only two strains of *B. tarrabulga* have been obtained from two independent sites c. 40 km apart in eastern Victoria. Both these strains showed very similar spore morphology (av. length = 17 µm) which make *B. tarrabulga* unique among the *Backusella* species isolated in this study (Fig. 6).

Backusella tuberculispora G. Walther & de Hoog, Persoonia 30: 41. 2013 — Fig. 23

Basionym. *Mucor tuberculisporus* (Schipper 1978).

The following description is based on strain UoMAU80 to illustrate the morphology of the Australian collections.

Sporangiophores up to 13.8 µm diam, generally tapering very slightly towards sporangia, occasionally branched, hyaline, with or without pale yellow contents. *Sporangia* brown minutely spinulose, length 32.8–58.6 × width 32.7–57.9 (av ± SD = 47.7 ± 9.5 × 46.9 ± 9.1) µm, globose (Q = 1.00–1.05 (av ± SD = 1.02 ± 0.01)). *Columellae* smooth-walled with pale yellow granular content, 19.6–40.1 × 19.5–39.3 (av ± SD = 33.4 ± 6.1 × 31.8 ± 6.1) µm, variably shaped globose, ellipsoid or applanate (Q = 1.01–1.13 (av ± SD = 1.05 ± 0.04)). Collars small and uncommon. *Sporangiospores* smooth-walled, 7–11 × 7–10 (av ± SD = 8.8 ± 0.9 × 8.2 ± 0.8) µm, globose to ellipsoid (Q = 1.00–1.38 (av ± SD = 1.08 ± 0.11)). *Giant cells* and *chlamydospores* not observed. *Sporangiola* present.

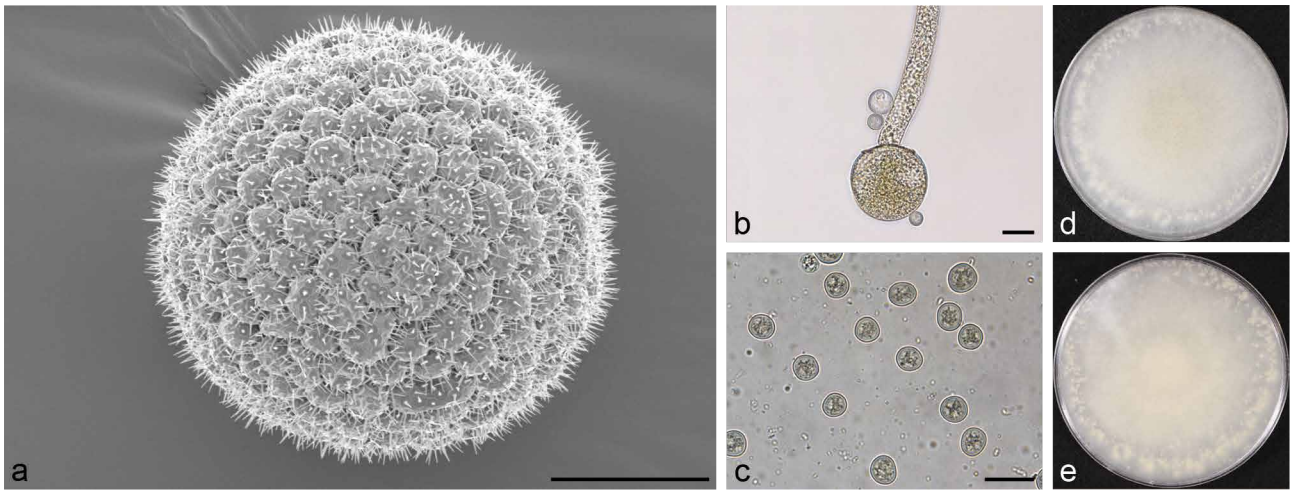

Fig. 23 Morphology of *Backusella tuberculispora* strain UoMAU80. a. SEM of sporangium; b. light microscope image of columella; c. light microscope image of sporangiospores; d, e. obverse and reverse of colony. — Scale bars = 20 µm.

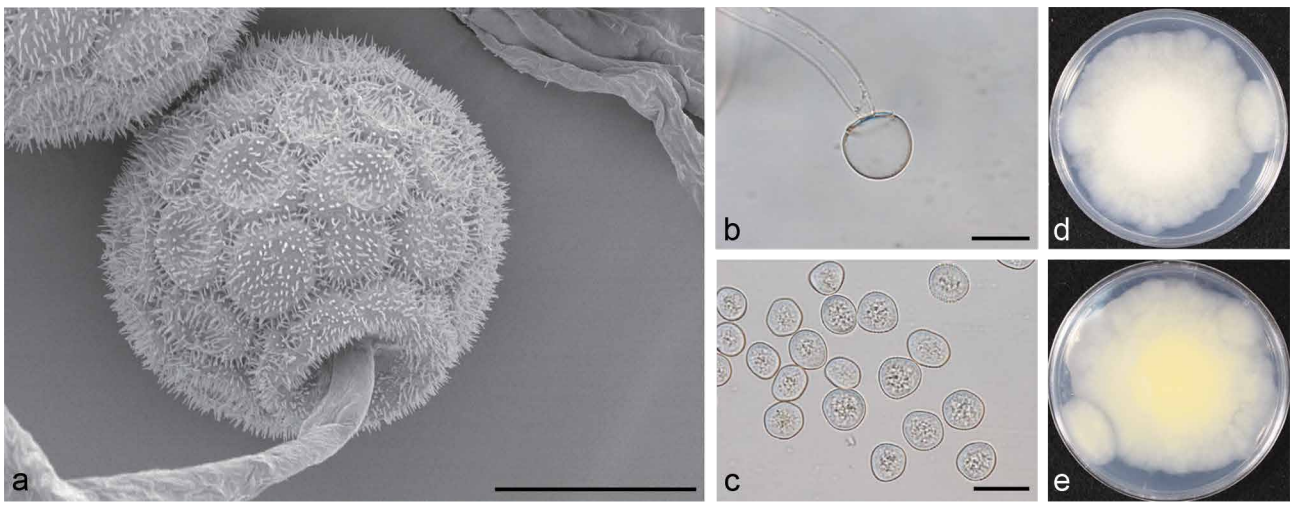

Fig. 24 Morphology of *Backusella westeae* strain UoMAU4. a. SEM of sporangium; b. light microscope image of columella; c. light microscope image of sporangiospores; d, e. obverse and reverse of colony. — Scale bars = 20 µm.

Culture characteristics — Colony cottony in texture, reaching 56 mm diam and 17 mm height after 3 d growth on PDA at 22 °C. Obverse light grey due to darkly coloured sporangia, becoming dark brown by 4 wk. Reverse creamy white.

Notes — The *B. tuberculispora* strains isolated in this study are clearly distinguished by their darkly pigmented sporangia. These give the colonies a darker appearance than any of the other Australian species. This is consistent with descriptions of the ex-type strain CBS 562.66 which is light grey on PDA. However, the eponymous rounded projections on the sporangiospores have not been observed despite being reported in the type strain on a number of media including PDA (Baijal & Mehrotra 1965, Schipper 1978). Despite this difference, given the supporting evidence, we believe that these strains should be considered as *B. tuberculispora.*

Backusella variabilis (A.K. Sarbhoy) G. Walther & de Hoog, Persoonia 30: 41. 2013

Synonyms. Mucor grandis Schipper & Samson, Mycotaxon 50: 479. 1994. *Backusella grandis* (Schipper & Samson) G. Walther & de Hoog, Persoonia 30: 41. 2013.

Notes — Previous authors have noted the close relationship between *B. variabilis* and *B. grandis* (Walther et al. 2013) and suggested the possibility of future synonymisation of these species. While there are morphological differences between the species, we believe that in light of the more detailed phylogenetic understanding of the genus presented here, the sequence similarity between these species justifies the formal synonymisation of these two species.

Backusella westeae Urquhart & Douch*, sp. nov.* — MycoBank MB831155; Fig. 24

Etymology. In honour of mycologist Gretna Weste (Linden 2007).

Typus. Australia*,* Victoria, Tarra-Bulga National Park, isolated as a contaminant during attempts to culture *Laccaria* species from freshly collected sporocarps (holotype MEL 2417242, dried culture on filter paper, National Herbarium of Victoria, Victoria, culture ex-type UoMAU4, JMRC SF014021).

Sporangiophores up to 9.5 µm diam, generally tapering very slightly towards sporangia, occasionally branched, hyaline, with or without pale yellow contents. *Sporangia* minutely spinulose, 26.2–64.0 × 22.4–58.4 (av ± SD = 47.1 ± 11.5 × 42.5 ± 10.5) µm, globose to broadly ellipsoid (Q = 1.03–1.23 (av ± SD = 1.11 ± 0.07)). *Columellae* smooth-walled with pale yellow granular content, 10.3–25.8 × 8.9–22.3 (av ± SD = 17.5 ± 4.8 × 15.3 ± 4.6) µm, variably shaped globose, ellipsoid or applanate (Q = 1.02–1.38 (av ± SD = 1.16 ± 0.11)). Collars small and uncommon. *Sporangiospores* smooth-walled, 10.5–13.5 × 8.7–13.3 (av ± SD = 12.6 ± 1.0 × 11.2 ± 1.7) µm, globose to ellipsoid (Q = 1.00–

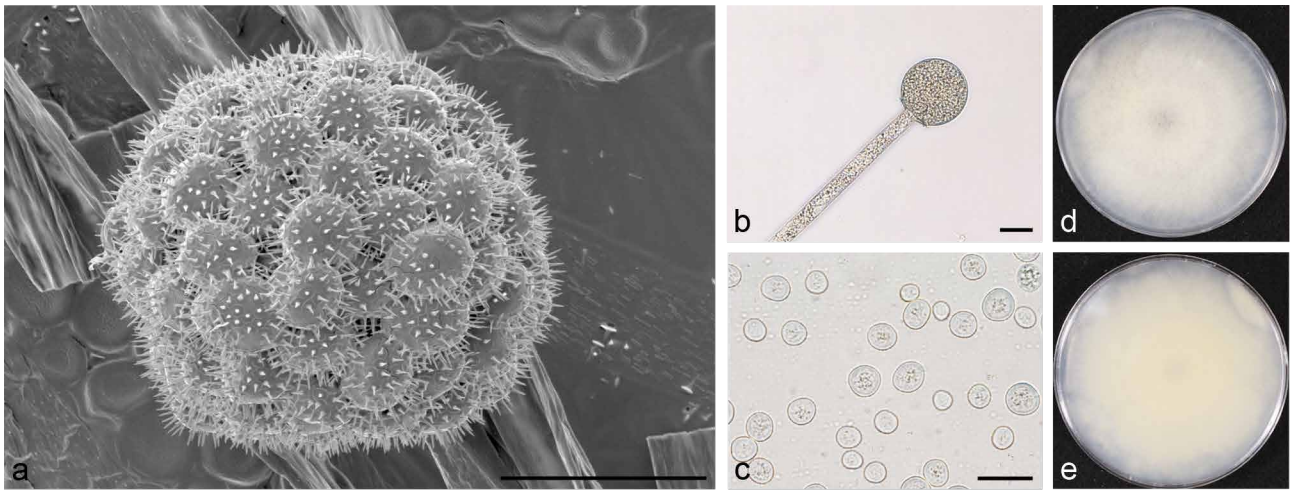

Fig. 25 Morphology of *Backusella* 'group X' strain UoMAU9. a. SEM of sporangium; b. light microscope image of columella; c. light microscope image of sporangiospores; d, e. obverse and reverse of colony. — Scale bars = 20 µm.

1.41 (av ± SD = 1.14 ± 0.13)). *Giant cells* and *chlamydospores* not observed. *Sporangiola* present.

Culture characteristics — Colony cottony in texture, reaching 35 mm diam and 20 mm height after 3 d growth on PDA at 22 °C. Obverse white, becoming brownish yellow by 4 wk. Reverse yellow, becoming paler towards edges.

Notes — See *B. australiensis.*

***Backusella* 'group X'** — Fig. 25

Sporangiophores up to 8.1 µm diam, generally tapering very slightly towards sporangia, occasionally branched, hyaline, with or without pale yellow contents. *Sporangia* minutely spinulose, 23.6–56.0 × 23.1–55.2 (av ± SD = 38.2 ± 11.4 × 37.6 ± 11.3) µm, globose (Q = 1.00–1.04 (av ± SD = 1.02 ± 0.01)). *Columellae* smooth-walled with pale yellow granular content, 17.9–34.1 × 16.8–29.2 (av ± SD = 25.0 ± 4.8 × 23.3 ± 4.0) µm, variably shaped globose, ellipsoid or applanate (Q = x = 1.00–1.17 (av ± SD = 1.07 ± 0.06)). Collars small and uncommon. *Sporangiospores* smooth-walled, 9.3–12.1 × 7.6–11.3 (av ± SD = 10.4 ± 1.0 × 9.7 ± 1.1) µm, globose to broadly ellipsoid (Q = 1.00–1.22 (av ± SD = 1.07 ± 0.06)). *Giant cells* and *chlamydospores* not observed. *Sporangiola* present.

Culture characteristics — Colony cottony in texture, reaching 48 mm diam and 22 mm height after 3 d growth on PDA at 22 °C. Obverse white, becoming brownish yellow by 4 wk. Reverse creamy white. Grey zones sometimes visible close to substrate.

Notes — Morphologically and physiologically similar, *Backusella* 'group X' and *B. mclennaniae* cannot yet be discriminated based on the characters examined but show clear separation in the molecular data. ITS similarity between *Backusella* 'group X' and the type of *B. lamprospora* CBS 118.08 is only around 92 %, however, further taxonomic work will be required to determine whether 'group X' should be included in *B. lamprospora* or described as a separate species.

KEY TO THE BACKUSELLA SPECIES OF SOUTH-EASTERN AUSTRALIA

1. Spore length av = 22–35 µm . . . . . *B. macrospora*
1. Spore length av ~ 17 µm; Q ~ 1.1 . . . . . *B. tarrabulga*
1. Spore length av ~ 14 µm; Q ~ 1.05 . . . . . *B. luteola*
1. Spore length av = < 15 µm long; Q > 1.6 *B. parvicylindrica*
1. Spore length av = < 15 µm long; Q 1.2–1.51. 2
1. Spore length av = < 13 µm long; Q < 1.15. 3
2. Utilizes sucrose as sole carbon source . . *B. morwellensis*
2. Does not utilize sucrose as sole carbon source . . . . . *B. psychrophila*
3. Giant cells present . . . . . *B. dispersa*
3. Giant cells absent 4
4. Utilizes sucrose as sole carbon source 5
4. Does not utilize sucrose as sole carbon source 6
5. Reverse colony colour typically yellow . . . . . *B. westeae*
5. Colony colour typically white, sometimes showing black pigmentation close to substrate 7
6. Colony colour typically yellow, especially after 'scraping' . . . . . *B. australiensis*
6. Darkened sporangia giving colony a grey appearance . . . . . *B. tuberculispora*
7. Strong growth on dulcitol . . . . . *B. liffmannii*
7. Weak growth on dulcitol. . . . . *Backusella* 'group X'; . . . . . *B. mclennaniae**

* *Backusella* 'group X' and *B. mclennaniae* are distinguished by analysis of DNA sequences.

DISCUSSION

Globally, the discovery rate of new, non-Dikarya fungal species is remarkably low. In contrast to 2017, when 24 non-Dikarya species were identified (Willis 2018), here we report 10 new species in the genus *Backusella*. These species were shown to be genetically, morphologically, and physiologically distinct. Phylogenies based on comparing the sequences of DNA regions between different strains revealed clear separation between species (Fig. 2–5). Additionally, we describe a group of species which we tentatively refer to as *B.* 'group X' with affinity to *B. lamprospora*, the taxonomy of which requires further study, and may potentially be another novel species. The most phylogenetically informative morphological trait that we examined was spore dimensions, which strongly support the species groups made apparent by phylogenetic trees based on DNA sequences.

Both the discovery and then analysis of the *Mucorales* has been hampered by features of their genomes that are less commonly encountered in the *Ascomycota* and *Basidiomycota*. In particular, the genomes of *Mucorales* species can contain whole and/or segmental genome duplications (Corrochano et al. 2016), potentially confounding phylogenies based on what are single genes in other fungi (e.g., the gene encoding actin).

To circumvent such problems, we turned to whole genome sequencing to provide a far more substantial set of DNA information, and identified the *argA* gene as one example of a single copy gene that could potentially be adopted more widely to explore relationships between species and genera in the *Mucorales*. The *argA* gene encodes a putative argininosuccinate lyase, which breaks down argininosuccinate into arginine and fumarate. Being essential for the production of arginine, it is well conserved between plants, fungi and animals. Examining the MCL cluster data available through MycoCosm shows that of 54 genomes of *Mucoromycotina* species, 52 have a single copy *argA* homolog (Grigoriev et al. 2014). The two exceptions are *Rhizopus microsporus* var. *chinensis* CCTCCM201021 which carries two copies as a result of a large duplicated region (Wang et al. 2013) and *Endogone* sp. FLAS 5907 which lacks an *argA* homolog, this might reflect either an incomplete assembly or the interesting biology of this species (Chang et al. 2019).

With decreasing costs in genome sequencing, reporting a draft genome sequence could become a mandatory requirement for the description of new fungal species, providing future investigators with a far more comprehensive gene set from which to choose regions that may establish relationships, thereby providing the resolution of multi-gene phylogenetic inferences.

In addition to DNA sequences and morphology, we also examined two physiological traits – utilization of sucrose and growth at different temperatures. Sucrose was found to be consistently utilized by the strains corresponding to some species but not others. This is in keeping with previous work suggesting carbon source utilization can sometimes discriminate between species (Scholer & Müller 1966, Schwarz et al. 2007, Pawłowska et al. 2019). Sucrose is known to be broken-down by the enzyme invertase in fungi, including some *Mucorales* (Watanabe & Oda 2008, Dong et al. 2018). Examination of the genome sequences revealed a putative invertase gene that was present in the genomes of the sucrose-utilizing species but absent or mutated from those which lack this ability. The ability of *B. westeae sucB* DNA to complement the *S. cerevisiae SUC2* deletion mutant phenotype confirms the prediction that *sucB* is a functional invertase. The appearance of invertase-producing species in two places on the tree implies that the ability to utilize sucrose has been lost multiple times during the evolution of the genus. This hypothesis is supported by the presence of independently-derived non-functional alleles in UoMAU7 and UoMAU55 (stop codons within the reading frame); and UoMAU34 and UoMAU90 (with large deletions in the promoter region). The fact that these species are apparently under different selection pressures in regard carbon source utilization suggests that there may be niche separation between the species based on their ability to utilize different carbon sources. More generally, the discovery of sucrose utilization as a potentially taxon-discriminating character provides an example of how implementation of polyphasic taxonomy can link morphological or physiological taxonomic traits backed by DNA sequence analysis.

The impacts of climate change on soil biodiversity have been considered previously (Classen et al. 2015). Given the different capacity of *Backusella* strains to withstand increased temperature (Fig. 2), there is a possibility that a warming climate will disturb the species composition (selecting against those species which appear to be uniformly heat sensitive) or in the case of those species with variable tolerance shifting population structures. Compared to sucrose utilization, thermotolerance is a more complex trait likely involving the contributions of many genes. Further studies at the population-level, particularly in the case of *B. tuberculispora,* might help us to understand the genetic basis for thermotolerance in this genus and allow us to predict the evolutionary effects of climate change on it.

The biological species concept is one system by which species may be deliminated. While mating reactions could be a powerful tool to understand taxon boundaries, interpretation of mating reactions in the *Mucorales* is complicated by the production of azygospores in some interspecific crosses that morphologically resemble zygospores and the frequent lack of interaction, even between closely related strains (Schipper 1978, Stalpers & Schipper 1980). Furthermore, zygospore dormancy, which is found in other *Mucorales*, may hamper the ability to resolve post-zygotic isolation, which requires reliable production of progeny from compatible crosses.

A limitation of this study was that we were unable to directly study a number of non-Australian isolates, in large part due to quarantine restrictions. We hope that future research will result into detailed observations of these strains, particularly their ability to utilize sucrose so that this information can be integrated with that described here. The study of the non-Australian isolate CBS 118.08 will be particularly important to clarify the relationship between this species and the *Backusella* 'group X' strains that we isolated.

In summary, this study has uncovered a considerable and previously unexplored diversity of one *Mucorales* genus, *Backusella*, in south-eastern Australia. The low degree of overlap between the species isolated in this study and those isolated internationally in the last decade (*B. gigacellularis* and *B. constricta* from Brazil and *B. locustae* from South Korea) provides initial evidence that different geographical areas may possess unique *Backusella* flora. This work highlights how understudied the mucoralean flora of Australia are, and will stimulate other researchers to focus greater efforts on understanding other basal genera in both this region and around the world.

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