FUSE

Fungal Systematics and Evolution

VOLUME 11
JUNE 2023
PAGES 85–93

doi.org/10.3114/fuse.2023.11.07

Freshwater ascomycetes from southern Australia*: Melanascomaceae fam. nov., Melanascoma panespora gen. et. sp. nov.,* and *Pleurothecium brunius sp. nov*.

S.C. Fryar[1*], D.E.A. Catcheside[1]

[1]College of Science and Engineering, Flinders University, G.P.O. Box 2100, Adelaide SA 5001, Australia

*Corresponding author: sally.fryar@flinders.edu.au

Key words:
Attractosporales
Diaporthomycetidae
freshwater fungi
new taxa
Pleurotheciaceae
Pleurotheciales
taxonomy

Abstract: During a survey of freshwater fungi in temperate southern Australia, two new taxa were found, *Melanascoma panespora* and *Pleurothecium brunius*. Morphological and molecular data place *Melanascoma panespora* in the *Diaporthomycetidae* representing a new genus. *Melanascoma*, along with *Proliferophorum* and *Paraproliferophorum*, form a new lineage and the family *Melanascomaceae* is introduced. Phylogenetic analyses using ITS, 28S, and 18S nrRNA gene sequences,, along with morphological examination revealed *Pleurothecium brunius* to be a new species of *Pleurothecium*, sister to *P. aquaticum*.

Citation: Fryar SC, Catcheside DEA (2023). Freshwater ascomycetes from southern Australia*: Melanascomaceae fam. nov., Melanascoma panespora gen. et. sp. nov.,* and *Pleurothecium brunius sp. nov*. *Fungal Systematics and Evolution* **11**: 85–93. doi: 10.3114/fuse.2023.11.07
Received: 26 April 2023; **Accepted:** 15 May 2023; **Effectively published online:** 25 May 2023
Corresponding editor: P.W. Crous

INTRODUCTION

Freshwater fungi are abundant and diverse in rivers, streams and lakes around the world (Jones *et al*. 2014). They include saprophytes, plant parasites, animal parsites, endophytes and some form mycorrhizae (Jones *et al.* 2014). Fungi form a rich tapestry of interactions with other organisms within freshwater ecosystems, playing critical roles such as decomposition of organic matter (Calabon *et al*. 2020).

A major impediment to full understanding of fungal ecology and conservation is the lack of taxonomic information (May *et al.* 2019). With only 3–8 % of fungal species described (Hawksworth & Lücking 2017), it is imperative to accurately describe and delimit many more fungi to allow ecologists, conservation, and evolutionary biologists to accurately study them and their interactions. During a survey of freshwater fungi on wood in streams in southern Australia, two distinctive and novel ascomycetes were collected.

The first ascomycete species found during our survey was morphologically similar to many species within the *Diaporthomycetidae* by having wide, septate paraphyses, unitunicate, cylindrical asci with a distinctive non-amyloid apical apparatus (Maharachchikumbura *et al.* 2015), but we were unable to assign it any known taxa. Maharachchikumbura *et al.* (2015) described the subclass *Diaporthomycetidae* (*Sordariomycetes*) and outlined the orders and families included in this new subclass. Hyde *et al.* (2021) further refined the phylogeny of this subclass including 21 orders.

The second species had morphological characters consistent with the genus *Pleurothecium* but did not fit the description of any of the previously described species. Höhnel (1919) established *Pleurothecium* for *P. recurvatum*, a species that is commonly found in freshwater habitats. Réblová *et al.* (2016) introduced *Pleurotheciales* and *Pleurotheciaceae* to accommodate *Pleurothecium* within the *Sordariomycetes*. There are currently eight accepted species of *Pleurothecium*, six of which occur in fresh water (Réblová *et al*. 2012, Hyde *et al*. 2017, Luo *et al.* 2018, 2019, Shi *et al.* 2021).

In this study, we provide morphological descriptions and phylogenies for these two novel taxa based on multigene analyses.

MATERIALS AND METHODS

Collection details and examination

Samples of submerged wood less than 5 cm diam were collected in Scott Creek Conservation Park, South Australia from two streams, approximately 50 cm deep with a muddy base, that flow only during winter. Samples were sealed into plastic bags for transport to the laboratory. The riparian vegetation is a mixture of native vegetation and invasive weeds.

Samples were incubated in sterile plastic containers and regularly examined for fungi over 6 months using a Leica MZ7s dissecting microscope. Any fungi observed were photographed, described, and transferred to a microscope slide with a drop of distilled water. A cover slip was added and the slide was examined using a Nikon Eclipse Ni with differential interference contrast. Photographs were taken using either a Sony RX-100 or Canon D6 camera.

Single spore isolation

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

Ascospores or conidia were transferred to a sterile 1.5 mL microtube with 20 µL of sterile water using a sterile micro needle. The mixture was agitated for several seconds, then transferred to a PDA plate using a pipette. Plates were incubated at room temperature and checked over 5 d for germinating spores using a Leica MZ7s dissecting microscope. Germinated spores were picked off the agar surface using a sterile needle and transferred to individual PDA plates which were incubated at room temperature.

DNA extraction, amplification, and sequencing

Standard method

Approximately 50 mg of fungal mycelium was scraped from the surface of agar cultures with a sterile scalpel and the genomic DNA was isolated using a Qiagen DNeasy Plant Mini kit following the manufacturer's protocols. The final DNA extracts were eluted into 100 µL of buffer.

Primers LROR/LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1994) were used to amplify the sequences from the 28S nrRNA gene (28S), while ITS1/ITS 4 (White *et al.* 1990) were used to amplify the internal transcribed spacer regions and the intervening 5.8S nr RNA gene (ITS), and NS1/NS4 (White *et al.* 1990) were used to amplify the 18S nrRNA gene. Sequences from protein-coding gene, translation elongation factor 1-alpha (*TEF1*), were amplified with primers were EF1-983F and EF1-2218R (Rehner & Buckley 2005).

Reaction mixtures contained 5 µL buffer, 1 µL (10 mM each) dNTPs, 1 µL (10 µM) of each primer, 0.25 µL hotStart Taq DNA polymerase (New England Biolabs), 1 µL DNA template, and 16.75 µL sterile milliQ water.

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

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

Direct PCR method

Numerous attempts were made to culture specimens. When cultures were not obtained, conidiophores or ascomata were used for direct PCR. In these cases, Phire Plant Direct PCR Master Mix (Thermo Fisher Scientific) was used. Ascomata or conidiophores were placed into 20 µL of Phire dilution buffer at 4 °C, heated in a dry block to 98 °C for 10 min, then on ice for 5 min, vortexed briefly, then centrifuged for 1 min at 11 000 rcf.

Reaction mixtures for the Phire kit contained 7 µL sterile milliQ water, 10 µL master mix, 1 µL of each primer, 1 µL DMSO, and 1 µL DNA template. PCR cycling conditions for Phire kit extracts was 98 °C for 5 min; 35 cycles 98 °C for 5 s, 60 °C (ITS), 56.8 °C (28S) or 55.5 °C (18S) for 5 s, 72 °C for 20 s; followed by a final extension at 72 °C for 1 min.

For some samples using the Phire kit, a second band was persistent on electrophoresis gels, despite optimisation of PCR conditions. In these cases, the band-stab and re-amplification technique of Bjourson & Cooper (1992) was used. Re-amplification used the standard PCR protocol and primers described above. PCR primers, product clean up and sequencing were as described above for the standard method.

Phylogenetic analyses

The generated sequences for each gene were used in megablast searches (Zhang *et al.* 2000) to identify closely related sequences in NCBI's GenBank nucleotide database. Other sequences used in this study were derived from GenBank. Sequences were aligned in Geneious Prime v. 2023.0.4 (https://www.geneious.com) using MUSCLE. Ambiguously aligned regions were removed from the alignments using GBlocks v. 0.91b (Castresana 2000, Talavera & Castresana 2007) on the Phylogeny.fr platform (Dereeper *et al*. 2008). Alignments were imported into Mega X v. 10.2.6 (Stecher *et al.* 2020) to find the best substitution models for phylogenetic analyses. The best substitution models were K2+G (ITS) and TN93+G (28S and *TEF1*) for the first species and K2+G (ITS and 28S), K2 (18S) for the second species. However, RAxML and MrBayes do not use these models, so GTR+G+I was used for all analyses as recommended by Abadi *et al*. (2019). Genes were then concatenated and maximum-likelihood phylogenetic trees were constructed with partitions for each gene region using RAxML v. 8.2.11 (Stamatakis 2014) within Geneious using the GTR + I + G substitution model and branch support values were calculated with 1 000 rapid bootstrap inferences. The same alignment was analysed using Bayesian analysis with MrBayes using the GTR GAMMA I substitution model (v. 3.2.6; Huelsenbeck & Ronquist 2001) with partitions for each gene region within Geneious. All resulting trees were formatted in Geneious, then further edited in Adobe Illustrator v. 27.0.

RESULTS

Phylogenetic analyses

Phylogenetic trees based on multi-locus analyses (Figs 1, 2) show the relationships between the new species and other related taxa. Branch supports of Maximum Likelihood bootstrap ≥ 70 % and Bayesian PP value ≥ 0.90 are indicated above the branches.

Despite numerous attempts, we were unable to recover high-quality sequence data from the ITS region of either of the specimens of the first species. To place this undescribed perithecial species (AD291710 and AD219607) from our survey, the 28S and *TEF1* sequences were analysed with ITS, 28S and *TEF1* sequences of 30 species in five families in the *Sordariomycetes* (Fig. 1). The dataset comprised 1 670 characters: 321 bp for ITS, 506 bp for 28S, 843 bp for *TEF1*. The 18S sequences were mostly uninformative and were not included. The tree is rooted to *Cancellidium applanatum*, *C. cinereum* and *Obliquiminima*

Table 1. GenBank accession numbers of selected taxa from *Diaporthomycetidae* used for phylogenetic analyses. Newly generated sequences are shown in bold.

Species	Strain	ITS	28S	*TEF1*
Acrodictys bambusicola	HSAUP myr9510	KU999973	KX033564	—
Acrodictys elaeidicola	HSAUP mj5528	KU999978	KX033569	—
	HSAUP mj5536	KU999977	KX033568	—
Acrodictys fluminicola	KUMCC 15-0240	MK828642	MK849786	—
Acrodictys globulosa	HSAUP myr4696	KU999970	KX033562	—
Acrodictys liputii	HSAUP myr7561	KU999974	KX033565	—
Acrodictys malabarica	HSAUP myr9509	KU999968	KX033560	—
Acrodictys peruamazonensis	HSAUP myr4694	KU999969	KX033561	—
Acrodictys porosiseptata	HSAUP myr4698	KU999967	KX033559	—
Ascobrunneispora aquatica	HKUCC 3708	AF177154	AF132326	—
Cancellidium cinereum	MFLUCC 18-0424	MT370353	MT370363	MT370488
Cancellidium atrobrunneum	MFLUCC 20-0100	MT422724	MT422740	MT436438
Fluminicola aquatica	MFLUCC 15-0962	MF374357	MF374366	MF370960
Fluminicola saprophytica	MFLUCC 15-0976	NR_153493	NG_069504	MF370956
	MFLUCC 15-0984	MF374359	MF374368	—
	MFLUCC 18-1244	MW286504	MW287778	MW396649
Fluminicola striata	MFLUCC 18-0990	MW286496	MW287770	—
Fluminicola thailandensis	MFLUCC 14-0037	MK828644	MK849789	MN194049
	MFLUCC 15-0984	MF374359	NG_069505	—
Melanascoma panespora	**AD291710**	—	**OQ789909**	—
	AD219607	—	OQ799385	**OQ870569**
Obliquiminima hyalina	MFLUCC 18-1401	MW286507	MW287781	MW396652
Papulosa amerospora	AFTOL-ID 748	—	DQ470950	DQ471069
Paraproliferophorum hyphaenes	CPC 40103	ON603770	ON603790	ON605632
Platytrachelon abietis	CBS 125235	—	NG_057957	—
Proliferophorum thailandicum	MFLUCC 17-0293	MK028344	MK028343	—
Pseudostanjehughesia aquitropica	MFLU 17-0857	MF077548	MF077559	MF135655
Pseudostanjehughesia lignicola	MFLUCC 15-0352	NR_168808	MK849787	MN194047
Wongia aquatica	MFLUCC 18-1607	MK828645	MK849788	MN194048
Wongia fusiformis	DLUCC:1767	MZ420746	MZ420761	—
Wongia griffinii	BRIP 60377	KU850472	KU850470	KU850466

hyalina. The undescribed ascomycete nested within the *Diaporthomycetidae* and formed a well supported clade (100/1) with *Proliferophorum thailandicum* and *Paraproliferophorum hyphaene*, which together form a new family (Fig. 1).

For the undescribed *Pleurothecium* (AD291629 and AD291640) the combined ITS, 18S and 28S sequences were assessed with the eight previously recognised species in *Pleurothecium* (Fig. 2). The dataset comprised 2 161 characters: 511 bp for ITS, 814 bp for 28S, 836 bp for 18S. The tree is rooted to *Phaeoisaria clematidis*. The two new specimens form a sister clade to *Pleurothecium aquaticum* and *Pleurothecium guttulatum,* representing a new species, *Pleurothecium brunius* (Fig. 2).

Taxonomy

Melanascomaceae Fryar & D.E.A. Catches, ***fam. nov.*** Index Fungorum IF 900400.

Asexual morph: Hyphomycetous. *Conidiophores* macronematous, mononematous, sub-cylindrical to cylindrical, unbranched or branched, erect, olivaceous brown to dark brown, light brown at the apex, septate, smooth or ornamented. *Conidiogenous cells* holoblastic, polyblastic, terminal, subhyaline to brown. *Conidia* fusiform to cylindrical, subhyaline to brown, 0–3-septate when mature, guttulate, smooth.

Sexual morph: *Ascomata* perithecial, non-stromatic, subglobose with a straight neck. *Ascomatal wall textura angularis*, dark brown. *Paraphyses* persistent, cylindrical, septate, unbranched. *Asci* unitunicate, eight-spored, apex with a non-amyloid apical ring. *Ascospores* ellipsoid, septate, ornamented, without appendages or sheath.

Type genus: *Melanascoma* Fryar & D.E.A. Catches

Table 2. GenBank accession numbers of selected taxa from *Pleurothecium* used for phylogenetic analyses. Newly generated sequences are shown in bold.

Species	Strain	ITS	28S	18S
Phaeoisaria clematidis	MFLUCC 18-1017	MW131990	MW132065	MW132063
Pleurothecium aquaticum	GZCC19-0546	MW133897	—	MW134679
	MFLU 21-0148	OM654775	OM654772	OM654807
	B-27	—	MK835854	MK834786
	MFLU 17-0922	NR_160597	NG_066197	—
Pleurothecium brunius	**AD291640**	**OQ799373**	**OQ799347**	**OQ799346**
	AD291629	OQ799378	OQ799377	OQ799376
Pleurothecium floriforme	MFLU 15-1163	NR_156614	NG_059791	—
Pleurothecium guttulatum	IFRD 9203	NR_176728	MT559115	NG_081395
Pleurothecium obovoideum	CBS 209.95	EU041784	EU041841	—
Pleurothecium pulneyense	MFLUCC 16-1293	—	MF399262	MF399228
Pleurothecium recurvatum	GZCC19-0441	MW133898	—	MW134680
	CBS 138686	KT278727	KT278715	KT278702
	CBS 138747	KT278728	KT278714	KT278703
Pleurothecium semifecundum	CBS 131271	NR_111710	NG_057951	NG_062854
	CBS 131482	JQ429158	JQ429239	JQ429253

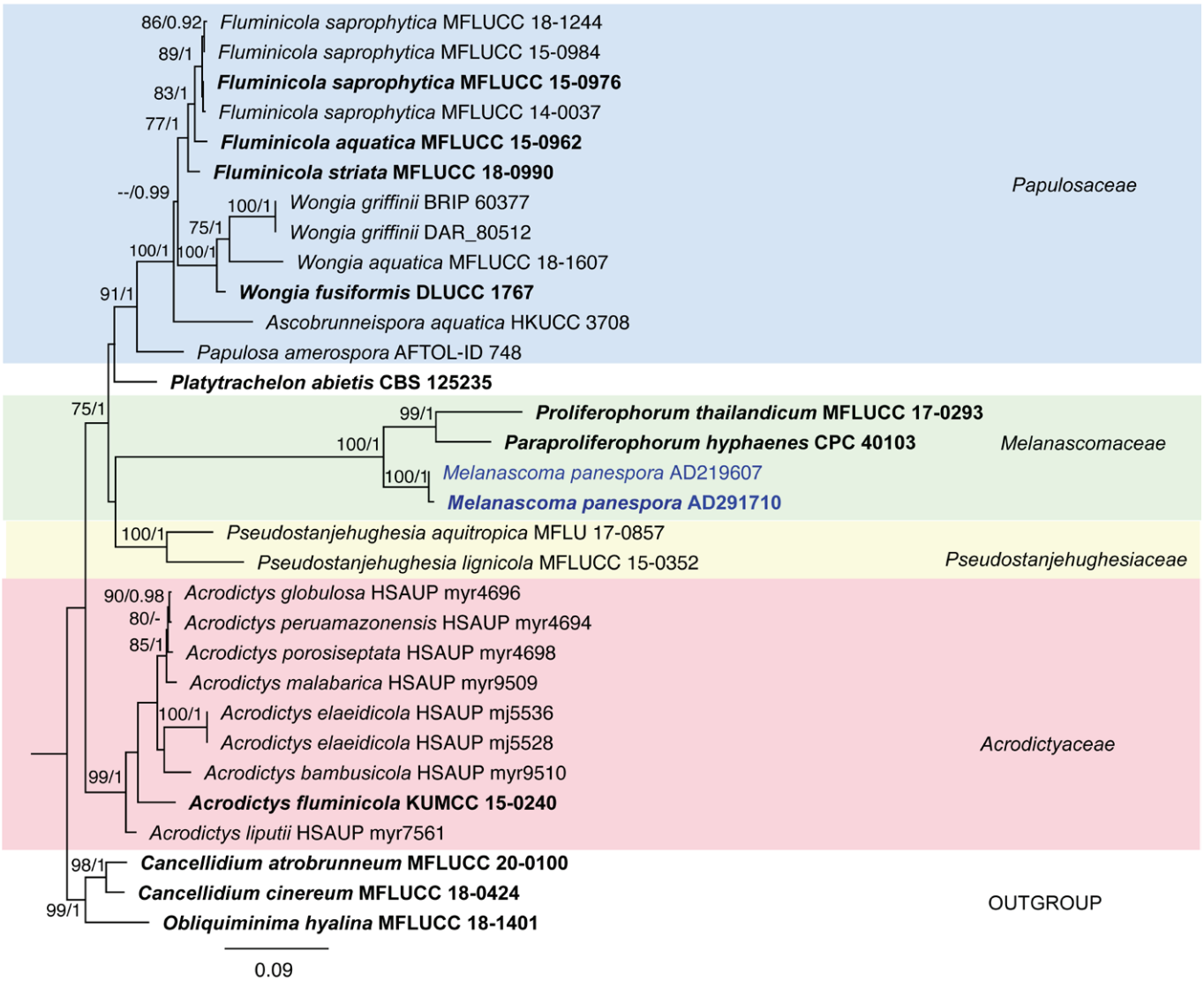

Fig. 1. Phylogram generated from maximum likelihood analysis based on combined ITS, 28S and *TEF1* sequence data of *Melanascoma panespora* and closely related taxa. Bootstrap values equal to or great that 70 % and Bayesian posterior probabilities equal to or greater than 0.90 are given above the nodes. Ex-type strains are shown in bold and newly generated sequences shown in blue.

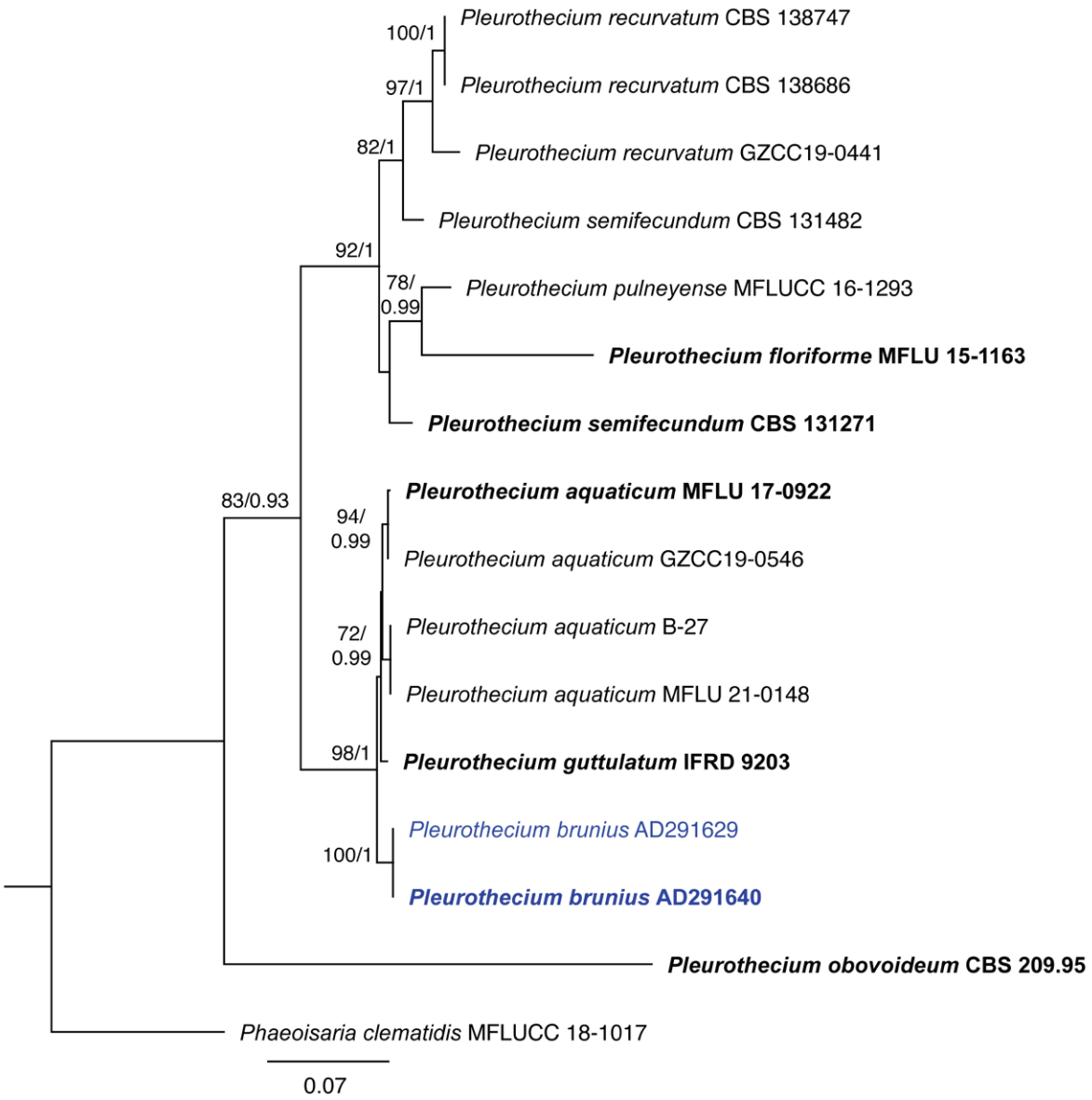

Fig. 2. Phylogram generated from maximum likelihood analysis based on combined ITS, 28S and 18S sequence data of *Pleurothecium* species. Bootstrap values equal to or great than 70 % and Bayesian posterior probabilities equal to or greater than 0.90 are given above the nodes. Ex-type strains are shown in bold and newly generated sequences shown in blue.

Melanascoma Fryar & D.E.A. Catches, ***gen. nov.***, Index Fungorum IF 900396.

Etymology: The prefix "Melan-" means darkly coloured. *Melanascoma* refers to the darkly coloured ascomata.

Asexual morph: undetermined. *Sexual morph*: *Ascomata* perithecial, non-stromatic, immersed, subglobose with a straight neck, dark brown. *Ascomatal wall textura angularis*, dark brown. *Paraphyses* persistent, cylindrical, septate, unbranched. *Asci* unitunicate, pedicellate, eight-spored, apex with a non-amyloid apical ring. *Ascospores* ellipsoid, hyaline, septate, ornamented, without appendages or sheath.

Type: *Melanascoma panespora* Fryar & D.E.A. Catches

Melanascoma panespora Fryar & D.E.A. Catches, ***sp. nov.*** Fig. 3. Index Fungorum IF 900395.

Typus: **Australia**, South Australia, Scott Creek Conservation Park (S35°5'45.90", E138°40'59.16), on submerged decaying wood in an ephemeral stream, 30 Aug. 2020, *S.C. Fryar* (**holotype** AD219607; GenBank sequences: 28S - OQ799385; 18S - OQ799375).

Etymology: The specific epithet refers to the appearance of the ascospores, like Vienna bread loaves.

Sexual morph: *Perithecia* immersed, subglobose, solitary or gregarious, 300–432 × 216–312 µm. *Ascomatal wall* dark brown, *textura angularis* (35–50 µm thick). *Neck* long, cylindrical, black, apex hyaline, (469–)536–771 × (67–)80–87 µm. *Paraphyses* hyaline, septate, unbranched, constricted at septa up to 4 µm wide, tapering to rounded ends 2 µm wide. *Asci* cylindrical, uniseriate, inamyloid apical ring, 4–5 µm wide, (1.5–)2 µm high, 8-spored, pedicellate, slowly dissolving in water, (155–)160–165(–178) × (12–)15–18 µm. *Ascospores* hyaline, ellipsoid, 1–3-septate, very slightly constricted at the septa, finely verrucose 18–22(–24) × 7–9 µm. *Asexual morph* undetermined.

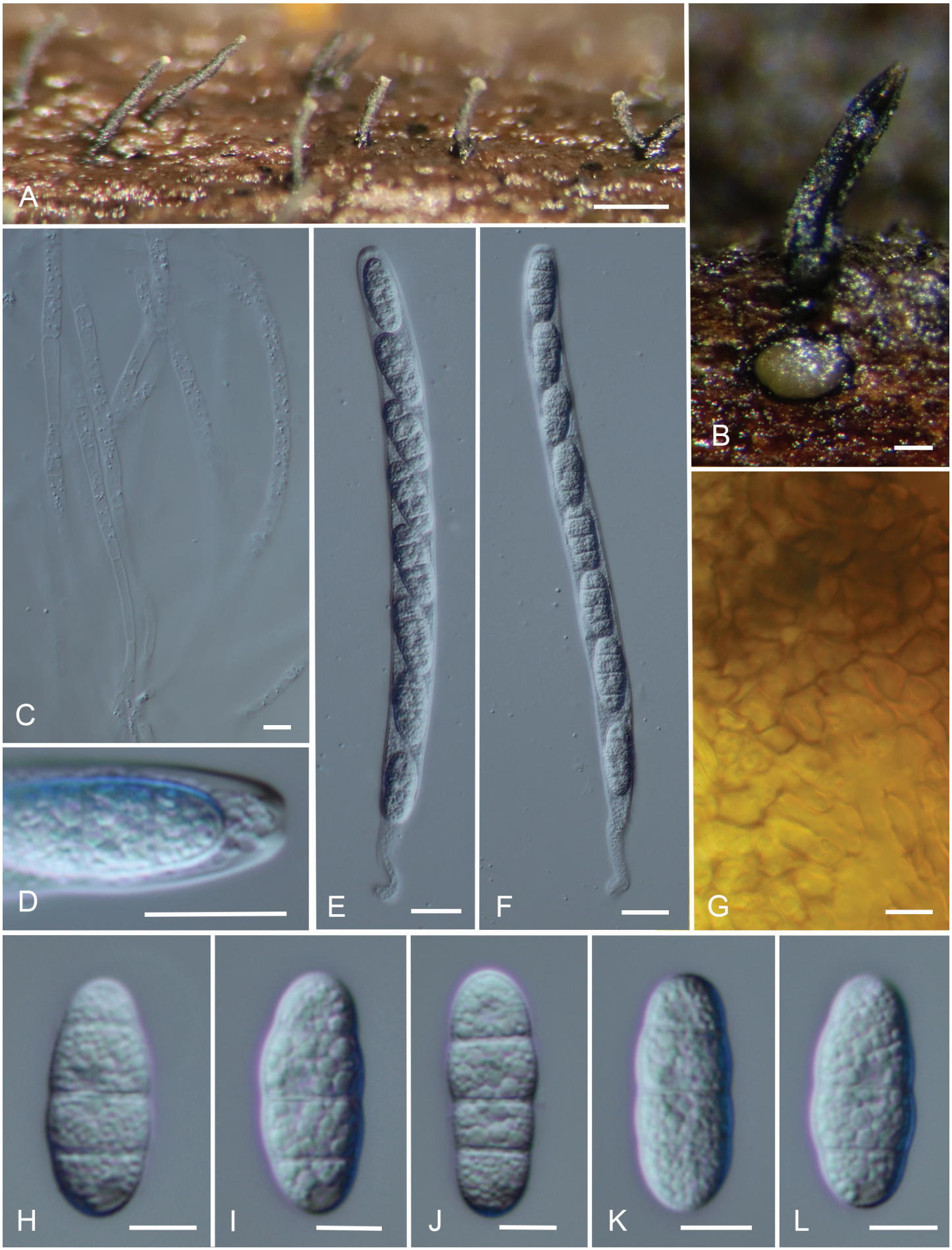

Fig. 3. *Melanascoma panespora* (holotype AD291710). **A.** Necks on wood from fresh water. **B.** Sectioned perithecium in wood. **C.** Paraphyses. **D.** Ascus tip. **E, F.** Asci. **G.** Peridium. **H–L.** Ascospores. Scale bars: A = 400 µm; B = 200 µm; C–L = 5 µm.

Fig. 4. *Pleurothecium brunius* (holotype AD291640). **A.** Conidiophores on wood from fresh water. **B–D.** Conidiophores and conidiogenous cells. **E–J.** Conidia. Scale bars: A = 100 µm; all others = 5 µm.

Additional material examined: **Australia**, South Australia, Scott Creek Conservation Park (S35°5'45.90", E138°40'59.16), 30 Aug. 2020, on submerged decaying wood in an ephemeral stream, *S. Fryar* (**paratype** AD219607; GenBank sequences: 28S - OQ789909; 18S - OQ789908; *TEF1* - OQ870569).

Notes: *Melanascoma panespora* forms a clade with *Proliferophorum thailandicum* and *Paraproliferophorum hyphaenes* (Fig. 1) both of which form monotypic genera. They are described from asexual morphs, with sexual morphs unknown, therefore morphological comparisons with these genera are not possible at this stage. This clade is sister to *Papulosaceae* and forms a larger clade with *Pseudostanjehughesiaceae* and *Acrodictyaceae*. Further analysis and data will be required to decide which of these clades represents a new order within the *Diaporthomycetidae*.

Morphologically *M. panespora* resembles other species within the subclass *Diaporthomycetidae* in having globose to subglobose brown to black ascomata, wide, septate paraphyses, unitunicate, cylindrical asci with a distinctive non-amyloid apical apparatus (Maharachchikumbura *et al.* 2015). *Rhamphoria* is similar to *Melanascoma* but has dictyosporous ascospores (Réblová *et al*. 2018). *Atractospora* is also similar but has fusiform ascospores and lateral necks (Réblová *et al*. 2016).

Melanascoma panespora shares some characteristics with *Rivulicola* species (Hyde *et al.* 1997, Raja *et al.* 2009, Ranghoo *et al.* 2000). The asci of *M. panespora* resemble those of *Rivulicola* species having uniseriate asci with large inamyloid apical rings and a pedicel. The ascospores of *Rivulicola* are ellipsoid, hyaline, and septate as in *M. panespora* but *Rivulicola* has ascospores with a mucilaginous sheath and without constriction at the septa. In addition, the ascomata and necks of *Rivulicola* are hyaline to pale brown compared with the dark brown ascomata and necks of *Melanascoma*. There are currently no available sequences for *Rivulicola* in GenBank for phylogenetic comparison.

Hyde *et al.* (2021) noted that, in their analyses, *Proliferophorum* diverged from *Platyrachelon* around 76 MYA, which falls within the family range (50–130 MYA). In our analysis the clade including *Melanascoma*, *Proliferophorum* and *Paraproliferophorum* is sister to *Platyrachelon*, forming a distinct lineage. We therefore introduce the new family *Melanascomaceae* to accommodate these three genera.

Pleurothecium brunius Fryar & D.E.A. Catches, ***sp. nov.***, Index Fungorum IF 900397. Fig. 4.

Etymology: The specific epithet refers to the brown colour of the conidia.

Typus: **Australia**, South Australia, Scott Creek Conservation Park (S35°5'45.90", E138°40'59.16) on submerged decaying wood in an ephemeral stream, *S.C. Fryar* (**holotype** AD291640; GenBank sequences: ITS - OQ799373; 28S - OQ799347; 18S - OQ799346).

Asexual morph: *Conidiophores* macronematous, mononematous, straight or slightly flexuous, septate, smooth, unbranched, dark brown, paler towards the apex, apex hyaline, (60–)80–100(–115) × 3–3.5 µm. *Conidiogenous cells* integrated, terminal, polyblastic, denticulate, hyaline, swollen, with 6–8 cylindrical denticles, swollen part 3–7 × 3–5 µm, denticles (2–)2.5–4 µm long, 1 µm wide. *Conidia* hyaline to dark brown, ellipsoid, 1–3-septate with conspicuous septa, not constricted at the septa, straight to slightly curved, smooth-walled (14–)16–19 × 5–6 µm. *Sexual morph* undetermined.

Distribution: Found in South Australia, Australia.

Additional material examined: **Australia**, South Australia, Scott Creek Conservation Park (S35°5'45.90", E138°40'59.16), on submerged decaying wood in an ephemeral stream, *S.C. Fryar* (**paratype** AD291629; GenBank sequences: ITS - OQ799378; 28S - OQ799377; 18S - OQ799376; *TEF1* - OQ784578).

Notes: *Pleurothecium brunius* forms a clade with *P. aquaticum* and *P. guttulatum* (Fig. 2). Morphologically it is different to *P. aquaticum* in having dark brown, shorter conidia, and longer conidiophores. *Pleurothecium brunius* is different to *P. guttulatum* by having dark brown, septate conidia, and a bulbous apex.

ACKNOWLEDGEMENTS

Financial support from the Australian Biological Resources Survey (ABRS) is gratefully acknowledged.

Conflict of interest: The authors declare that there is no conflict of interest.

REFERENCES

Abadi S, Azouri D, Pupko T, *et al*. (2019). Model selection may not be a mandatory step for phylogeny reconstruction. *Nature Communications* **10**: 934.

Bjourson AJ, Cooper JE (1992). Band-stab PCR: a simple technique for the purification of individual PCR products. *Nucleic Acids Research* **20**: 4675–4675.

Calabon M, Hyde K, Jones E (2020). www.freshwaterfungi.org, an online platform for the taxonomic classification of freshwater fungi. *Asian Journal of Mycology* **3**: 419–445.

Castresana J (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* **17**: 540e552.

Crous PW, Boers J, Holdom D, *et al*. (2022). Fungal Planet description sheets: 1383–1435. *Persoonia* **48**: 261–371.

Dereeper A, Guignon V, Blanc G, *et al*. (2008). Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research* **36**: W465eW469.

Dong W, Hyde KD, Jeewon R, *et al*. (2021). Towards a natural classification of annulatascaceae-like taxa II: introducing five new genera and eighteen new species from freshwater. *Mycosphere* **12**: 1–88.

Hawksworth DL, Lücking R (2017). Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiology Spectrum* **5**: FUNK–0052-2016.

Huelsenbeck JP, Ronquist F (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754–755.

Hyde KD, Bao D-F, Hongsanan S, *et al*. (2021). Evolution of freshwater *Diaporthomycetidae* (*Sordariomycetes*) provides evidence for five new orders and six new families. *Fungal Diversity* **107**: 71–105.

Hyde KD, Norphanphoun C, Abreau VP, *et al*. (2017). Fungal Diversity Notes 603–708: taxonomic and phylogenetic notes on genera and species. *Fungal Diversity* **87**: 1–235.

Hyde KD, Read SJ, Jones EBG, *et al*. (1997). Tropical Australian freshwater fungi. XII *Rivulicola incrustata gen. et sp. nov.* and notes on *Ceratosphaeria lampadophora*. *Nova Hedwigia* **64**: 185–196.

Jones EBG, Hyde KD, Pang K-L (2014). Freshwater fungi: and fungal-like organisms: de Gruyter.

Luo Z-L, Hyde, KD, Bhat DJ, *et al.* (2018). Morphological and molecular taxonomy of novel species *Pleurotheciaceae* (*Pleurotheciales*) from freshwater habitats in Yunnan, China. *Mycological Progress* **17**: 511–530.

Luo Z-L, Hyde KD, Liu J-K, *et al.* (2019). Freshwater *Sordariomycetes*. *Fungal Diversity* **99**: 451–660.

Maharachchikumbura SSN, Hyde KD, Jones EBG, *et al.* (2015). Towards a natural classification and backbone tree for *Sordariomycetes*. *Fungal Diversity* **72**: 199–301.

May TW, Cooper JA, Dahlberg A, *et al*. (2019). Recognition of the discipline of conservation mycology. *Conservation Biology* **33**: 733–736.

Phookamsak R, Hyde KD, Jeewon R*, et al.* (2019). Fungal Diversity Notes 929–1035: taxonomic and phylogenetic contributions on genera and species of fungi. *Fungal Diversity* **95**: 1–273.

Raja HA, Ferrer A, Shearer CA (2009). Freshwater Ascomycetes: A new genus, *Ocala scalariformis gen. et sp. nov.*, and two new species, *Ayria nubispora sp. nov*., and *Rivulicola cygnea sp. nov*. *Fungal Diversity* **34**: 79–86.

Ranghoo V, Hyde KD, Wong S, *et al*. (2000). *Vertexicola caudatus gen. et sp. nov*., and a new species of *Rivulicola* from submerged wood in freshwater habitats. *Mycologia* **92**: 1019–1026.

Réblová M, Seifert KA, Fournier J, *et al.* (2012). Phylogenetic classification of *Pleurothecium* and *Pleurotheciella gen. nov.* and its dactylaria-like anamorph (*Sordariomycetes*) based on nuclear ribosomal and protein-coding genes. *Mycologia* **104**: 1299–1314.

Réblová, M, Seifert KA, Fournier J, *et al*. (2016). Newly recognised lineages of perithecial ascomycetes: the new orders *Conioscyphales* and *Pleurotheciales*. *Persoonia* **37**: 57–81.

Réblová M, Fournier J, Stepánek V (2016). Two new lineages of aquatic ascomycetes: *Atractospora gen. nov.* and *Rubellisphaeria gen. et sp. nov*., and a sexual morph of *Myrmecridium montsegurinum sp. nov*. *Mycological Progress* **15**: 1–18.

Réblová M, Štěpánek V (2018). Introducing the *Rhamphoriaceae, fam. nov*. (*Sordariomycetes*), two new genera, and new life histories for taxa with *Phaeoisaria*- and *Idriella*-like anamorphs. *Mycologia* **110**: 750–770.

Rehner SA, Buckley E (2005). A *Beauveria* phylogeny inferred from nuclear ITS and *EF1-α* sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* **97**: 84–98.

Rehner SA, Samuels GJ (1994). Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. *Mycological Research* **98**: 625–634.

Shi L, Yang H, Hyde KD, *et al*. (2021). Freshwater *Sordariomycetes*: new species and new records in *Pleurotheciaceae*, *Pleurotheciales*. *Phytotaxa* **518**: 143–166.

Stamatakis A (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.

Stecher S, Tamura K, Kumar S (2020). Molecular evolutionary genetics analysis (MEGA) for macOS. *Molecular Biology and Evolution* **37**: 1237–1239.

Talavera G, Castresana J (2007). Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* **56**: 564e577.

Vilgalys R, Hester M (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* **172**: 4238–4246.

von Höhnel F (1919). Fünfte vorläufige mitteilung mycologische Ergebnisse (Nr. 399–500). *Berichte der Deutschen Botanischen Gesellschaft* **37**: 153–161.

White TJ, Bruns T, Lee S, *et al.* (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: a guide to methods and applications* (Innis MA, Gelfand DH, Sninsky JJ, *et al*., eds). Academic Press, San Diego (California): 315–322.

Zhang Z, Schwartz S, Wagner L*, et al.* (2000). A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology* **7**: 203–214.