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RESEARCH ARTICLE

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Newly recognised lineages of perithecial ascomycetes: the new orders *Conioscyphales* and *Pleurotheciales*

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Key words

freshwater fungi
holoblastic conidiogenesis
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multigene analysis
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systematics

Abstract Phylogenetic analyses of DNA sequences from nuclear ribosomal and protein-coding loci support the placement of several perithecial ascomycetes and dematiaceous hyphomycetes from freshwater and terrestrial environments in two monophyletic clades closely related to the *Savoryellales*. One clade formed by five species of *Conioscypha* and a second clade containing several genera of uncertain taxonomic status centred on *Pleurotheciu*m, represent two distinct taxonomic groups at the ordinal systematic rank. They are proposed as new orders, the *Conioscyphales* and *Pleurotheciales*. Several taxonomic novelties are introduced in the *Pleurotheciales*, i.e. two new genera (*Adelosphaeria* and *Melanotrigonum*), three novel species (*A. catenata*, *M. ovale*, *Phaeoisaria fasciculata*) and a new combination (*Pleurotheciella uniseptata*). A new combination is proposed for *Savoryella limnetica* in *Ascotaiwania* s.str. based on molecular data and culture characters. A strongly supported lineage containing a new genus *Plagiascoma*, species of *Bactrodesmiastrum* and *Ascotaiwania persoonii*, was identified as a sister to the *Conioscyphales*/*Pleurotheciales*/*Savoryellales* clade in our multilocus phylogeny. Together, they are nested in a monophyly in the *Hypocreomycetidae*, significantly supported by Bayesian inference and Maximum Likelihood analyses. Members of this clade share a few morphological characters, such as the absence of stromatic tissue or clypeus, similar anatomies of the 2-layered ascomatal walls, thin-walled unitunicate asci with a distinct, non-amyloid apical annulus, symmetrical, transversely septate ascospores and holoblastic conidiogenesis. They represent the only fungi in the *Hypocreomycetidae* with apically free, filiform to cylindrical, persistent or partially disintegrating paraphyses. The systematic placement of two other dematiaceous hyphomycetes was resolved based on DNA sequences; *Phragmocephala stemphylioides* is a member of the *Pleurotheciales* and *Triadelphia uniseptata* is within the *Savoryellales*.

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INTRODUCTION

The subclass *Hypocreomycetidae* (*Sordariomycetes*) includes non-lichenised ascomycetes with perithecial and cleistothecial ascomata. Many species are parasitic on plants, insects and other fungi. Some are endophytes in plants or saprobes on decaying wood and herbs, and some are involved in obligate mutualism with wood-boring beetles. Based on DNA sequences from nuclear ribosomal and protein-coding loci, the *Hypocreomycetidae* was recognised as a strongly supported monophyletic clade encompassing five orders (Spatafora et al. 2007, Zhang et al. 2007), i.e. the *Coronophorales*, *Halosphaeriales*, *Hypocreales*, *Melanosporales*, *Microascales*, and one family not then placed in an order, the *Glomerellaceae*. The absence of paraphyses was used to delimit this subclass (Zhang et al. 2007). In the more recent classification, the *Hypocreomycetidae* comprises eight orders, i.e. the *Coronophorales*, *Falcocladiales*, *Glomerellales* including the *Plectosphaerellaceae* (Zare et al. 2007, Réblová et al. 2011), *Hypocreales*, *Melanosporales*, a revised *Microascales* (De Beer et al. 2013), *Savoryellales* (Boonyuen et al. 2011) and *Torpedosporales* (Schoch et al. 2007, Jones et al. 2014, 2015). Hamathecial elements in the *Hypocreomycetidae* comprise several types, i.e. apical, centripetal and lateral paraphyses, catenophyses, a reticulate network of filiform filaments attached at the top and bottom of the ascomatal cavity; sometimes interthecial filaments are lacking. The only group characterised by paraphyses, i.e. sterile filiform, apically free filaments emerging from the hymenium among asci and growing upwards, is the *Savoryellales*, placed in this subclass based on a combined analysis of six nuclear loci (Boonyuen et al. 2011).

The *Savoryellales* comprises three genera, *Ascotaiwania*, *Canalisporium*[5] and *Savoryella* from freshwater, brackish, marine and terrestrial habitats. They share a set of characters including non-stromatic, immersed, semi-immersed to superficial, dark, coriaceous ascomata, often lying horizontally to the host, unitunicate asci with a non-amyloid apical annulus, partly disintegrating paraphyses and fusiform to ellipsoidal, transversely septate ascospores with hyaline polar cells and brown middle cells. Asexual morphs were experimentally proven for two species of *Ascotaiwania* (as *Monotosporella*, Ranghoo & Hyde 1998, Sivichai et al. 1998) and one species of *Canalisporium* (with *Ascothailandia* sexual morph; Sri-indrasutdhi et al. 2010). The distant placement of *Helicoön farinosum*, the asexual morph of *Ascotaiwania hughesii* (Fallah et al. 1999), from members of the *Savoryellales* was revealed by rDNA data (Boonyuen et al. 2011, Réblová et al. 2012). The asexual morphs linked to the *Savoryellales* are dematiaceous hyphomycetes characterised by semi-macronematous conidiophores and monoblastic coni-

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[5] In keeping with the tenets of the new International Code on the Nomenclature of algae, fungi and plants, we hereafter routinely use the oldest generic name for holomorphs; in some cases, this was originally the name of an asexual morph.

diogenous cells producing brown, thick-walled, transversely septate or cheiroid, dictyoseptate macroconidia, rare characters in the *Hypocreomycetidae*. Although the asexual morphs of *Savoryella* are unknown (Boonyuen et al. 2011), dark brown, 3–5-septate macroconidia were obtained in living cultures derived from ascospore isolates of two of our specimens of *S. limnetica* (Chang et al. 1998) collected on wood submerged in freshwater in France. Identical conidia were also observed scattered among ascomata on the host.

Previous phylogenies inferred from sequences of the small and large subunit of nuclear ribosomal DNA (nuc18S and nuc28S rDNA) and the second largest subunit of RNA polymerase II (*rpb2*) revealed a close relationship among members of the *Savoryellales* and several terrestrial and freshwater genera of uncertain taxonomic status forming two clades, i.e. *Conioscypha* and a clade comprising *Phaeoisaria*, *Pleurotheciella*, *Pleurothecium* and *Sterigmatobotrys* (Réblová et al. 2012). However, relationships among these genera remained largely unresolved. They are characterised by non-stromatic, semi-immersed to superficial, brown, subhyaline to pale orange perithecial ascomata, paraphyses, unitunicate asci with a non-amyloid apical annulus and ellipsoidal to fusiform, hyaline to subhyaline, septate ascospores (Fernández et al. 1999, Réblová & Seifert 2004, 2011, Réblová et al. 2012). Their asexual morphs are hyphomycetes with dematiaceous or hyaline conidiophores, holoblastic, sympodial conidiogenous cells and conidia that are often formed on a short rachis on denticles. The conidiogenesis of *Conioscypha* is unique; brown, non-septate conidia are born in cyathiform to doliiform blastic conidiogenous cells surrounded by hyaline, cup-like collarettes with a multilamellar structure (Shearer & Motta 1973).

Preliminary analysis of DNA sequences of nuclear ribosomal and protein-coding loci of four undescribed ascomycetes revealed their close relationship with members of the *Savoryellales* and the clade mentioned above centred around *Pleurothecium*. Three of these unidentified fungi are perithecial ascomycetes that share with members of the *Pleurothecium* clade characters of ascomata, asci, paraphyses and ascospores. Five specimens of the first undescribed fungus were found on strongly decaying wood of *Quercus cerris* in the Czech Republic. Although no conidiophores were formed on the host, cultures derived from ascospore isolates yielded identical asexual morphs with oval to bean-shaped, 1-septate, brown conidia formed holoblastically on a short denticle on almost triangular conidiogenous cells. The second unidentified ascomycete was collected on decaying wood of *Fagus sylvatica* in the Czech Republic. A single collection of the third undescribed ascomycete was made on decaying wood of *Fraxinus excelsior* submerged in freshwater in southern France. Cultures of both fungi were derived from isolated ascospores. No conidiophores were observed on the host and none were formed in vitro; only brown, ellipsoidal to globose cells were formed blastically directly on vegetative hyphae in axenic culture. Based on the simple and nondescript sexual morphological characteristics, we could not conclusively attribute any of these three fungi to a known ascomycete genus.

Two morphologically similar specimens of a dematiaceous hyphomycete preliminary identified as *Phaeoisaria* sp. were made on decaying deciduous wood in Canada and the Czech Republic. They represent a fungus morphologically similar to *Ph. clematidis*, the type species of the genus, in producing non-septate, obovoid conidia holoblastically on short denticles on sympodially proliferating conidiogenous cells, but differ in the absence of well-developed synnemata on the host and in vitro (Von Höhnel 1909, Deighton 1974). In both strains, the conidiophores were arranged in fascicles and lacked a distinct stipe.

The aim of this study is to investigate phylogenetic relationships of the three unidentified perithecial ascomycetes, *Phaeoisaria* sp., and also *Dactylaria uniseptata* and *S. limnetica*, with members of the *Savoryellales* and *Pleurothecium* clade. The affinities of two dematiaceous hyphomycetes *Phragmocephala stemphylioides* and *Triadelphia uniseptata*, coincidentally discovered to be related to this clade, are also documented. We also investigate the relationships of taxa characterised by the presence of paraphyses in the subclass *Hypocreomycetidae*. Although not a part of the presentation of new taxa, our re-examination of this subclass allows further consideration of the *Conioscypha* clade, presently considered incertae sedis (Réblová & Seifert 2004, Zelski et al. 2014). In order to further clarify the systematic positions of the *Conioscypha* and *Pleurothecium* clades, we utilised DNA sequence characters from the nuc rDNA internal transcribed spacer barcode (ITS1-5.8S-ITS2), three protein-coding and two ribosomal nuclear loci.

MATERIALS AND METHODS

Herbarium material and fungal strains

Dry ascomata were rehydrated with water; material was examined with an Olympus SZX12 dissecting microscope and hand-sectioned centrum material (including asci, ascospores and paraphyses) was mounted in Melzer's reagent, Lugol, 90 % lactic acid, aqueous cotton-blue (1 mg/mL), Pelikan ink and blue or black Waterman ink. Hand sections of the ascomatal wall were studied in 3 % KOH or heated chloral-lactophenol. All measurements were made in Melzer's reagent. Means ± standard deviation (SD) based on 20–25 measurements are given for dimensions of asci and ascospores. Images were captured by differential interference (DIC) or phase contrast (PC) microscopy using an Olympus DP70 camera operated by Imaging Software Cell on an Olympus BX51 compound microscope. Conidia and conidiogenous cells were photographed in the living state using an FEI Quanta 200 Environmental Scanning Electron Microscope (ESEM). A c. 2 × 2 mm cube of agar with mycelium was observed at 20 kV after the sample chamber achieved local thermodynamic equilibrium: chamber pressure 200 Pa, sample temperature from -15 °C to -16 °C. A Gaseous Secondary Electron Detector (GSED) was used for signal detection. Cooling of the specimen in the chamber was achieved using a PC controlled Peltier cooling stage with external water chiller (made by JT Manufacturing, USA).

Multi-ascospore and multi-conidial isolates were obtained from fresh material with the aid of a spore isolator (Meopta, Prague, Czech Republic). Ascospores and asci were spread on water agar, ascospores and conidia germinated within 48 h. Germinating ascospores were transferred and isolates were grown on water agar, CMA (Difco), potato dextrose agar (PDA, Oxoid) and potato-carrot agar (PCA, Gams et al. 1998). Colonies were examined after 7, 21 and 30 d incubated at 25 °C in the dark. Ex-type and other cultures are maintained at the CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands (CBS) and Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, Canada (DAOMC). Type and other herbarium material is deposited in the Mycological Herbarium in the National Museum in Prague, Czech Republic (PRM) and Canadian National Mycological Herbarium, Ottawa, Canada (DAOM). The Online Auction Colour Chart (2004) was used as the colour standard.

DNA extraction, amplification and sequence alignment

Cultures used for DNA isolations were grown as previously described by Réblová et al. (2011) and DNA was extracted following the protocols of Lee & Taylor (1990). Procedures for amplifying and sequencing the internal transcribed spacer rDNA (ITS rDNA), small and large subunit nuclear ribosomal DNA (nuc18S rDNA, nuc28S rDNA), second largest subunit of

RNA polymerase II (*rpb2*) and DNA replication licensing factor (*mcm7*) were performed as described in Réblová et al. (2011, 2013). A fragment of the 5'-end of the β-tubulin gene region (exons 3 to 6) was amplified and sequenced using primers Bt2a/benA1and Bt2b (Glass & Donaldson 1995, Geiser et al. 1998). Sequences were edited using Sequencher v. 5.0 (Gene Codes Corp., Ann Arbor, MI, USA).

GenBank accession numbers for newly sequenced taxa and other homologous sequences of members of the *Savoryellales* and two new orders described in this study retrieved from GenBank are listed in Table 1. For detailed investigation of phylogenetic relationships within the *Sordariomycetes*, sequences of the three loci nuc28S, nuc18S and *rpb2* included in Réblová et al. (2015) were downloaded from GenBank and combined with those generated during the present study.

Table 1 A list of members of the *Conioscyphales*, *Pleurotheciales*, *Savoryellales* and other fungi, their isolate information and new sequences determined for this study and those retrieved from GenBank. Sequences with GenBank accession numbers in **bold** were generated for this study. Sequence nuc28S* published in Chew et al. (2010).

Taxon	Source	ex-type	GenBank accession numbers					
			ITS	nuc28S	nuc18S	*RPB2*	*MCM7*	*TUB2*
Adelosphaeria catenata	CBS 138679	T	**KT278721**	**KT278707**	**KT278692**	**KT278743**	**KT278733**	**KT278754**
Ascotaiwania lignicola	NIL 00005		–	HQ446364	HQ446284	HQ446419	–	–
Ascotaiwania limnetica	CBS 126576		–	–	**KT278689**	–	**KT278731**	–
	CBS 126792		–	–	**KT278690**	–	**KT278732**	–
Ascotaiwania mitriformis	HKUCC 3706		–	AF132324	–	–	–	–
Ascotaiwania sawadae	SS 00051		–	HQ446363	HQ446283	HQ446418	–	–
Ascotaiwania persoonii	A57-14C	T	–	AY094190	–	–	–	–
	A57-14C		–	AY590295	–	–	–	–
Bactrodesmiastrum obovatum	FMR 6482		–	FR870266	–	–	–	–
Bactrodesmiastrum pyriforme	FMR 10747		–	FR870265	–	–	–	–
	FMR 11931		–	HE646637	–	–	–	–
Brachysporiella setosa	HKUCC 3713		–	AF132334	–	–	–	–
Canalisporium caribense	SS 03683		–	GQ390269	GQ390254	–	–	–
Canalisporium elegans	SS 00895		–	GQ390271	GQ390256	HQ446425	–	–
Canalisporium exiguum	SS 00809		–	GQ390281	GQ390266	HQ446436	–	–
Canalisporium grenadoideum	BCC 20507	T	–	GQ390267	GQ390252	HQ446420	–	–
Canalisporium pulchrum	SS 03982		–	GQ390277	GQ390262	HQ446431	–	–
Conioscypha japonica	CBS 387.84	T	–	AY484514	JQ437438	JQ437438	–	–
Conioscypha lignicola	CBS 335.93	T	–	AY484513	JQ437439	JQ429260	–	–
Conioscypha minutispora	CBS 137253	T	–	KF924559	–	–	–	–
Conioscypha peruviana	ILL 41202	T	–	KF781539	–	–	–	–
Conioscypha varia	CBS 113653		–	AY484512	AY484511	JQ429261	–	–
Flammispora bioteca	BCC 13367	T	–	–	AY722100	–	–	–
Helicoön farinosum	DAOM 241947		JQ429145	JQ429230	–	–	–	–
	ILLS 53605		–	AY094189	–	–	–	–
	ILLS 53605		–	AY316357	–	–	–	–
Magnisphaera stevemossago	CBS 139776		–	**KT278704**	**KT278691**	**KT278740**	–	–
Melanotrigonum ovale	CBS 138742		**KT278723**	**KT278708**	**KT278695**	**KT278744**	–	**KT278756**
	CBS 138743	T	**KT278724**	**KT278709**	**KT278696**	**KT278745**	–	**KT278757**
	CBS 138744		**KT278725**	**KT278710**	**KT278697**	**KT278746**	–	–
	CBS 138815		**KT278722**	**KT278711**	**KT278698**	**KT278747**	–	**KT278755**
	M.R. 3685		**KT278726**	**KT278712**	–	**KT278748**	–	**KT278758**
Phaeoisaria clematidis	CBS 113340		EU552148	–	–	–	–	–
	DAOM 226789		JQ429155	JQ429231	JQ429243	JQ429262	–	–
Phaeoisaria fasciculata	CBS 127885	T	**KT278719**	**KT278705**	**KT278693**	**KT278741**	–	**KT278752**
	DAOM 230055		**KT278720**	**KT278706**	**KT278694**	**KT278742**	–	**KT278753**
Phaeoisaria sedimenticola	CGMCC 3.14949	T	JQ074237	JQ031561	–	–	–	–
Phaeoisaria sparsa	FMR 11939		–	HF677185	–	–	–	–
Phaeoisaria sp.	unknown		–	nuc28S*	–	–	–	–
Phragmocephala stemphylioides	DAOM 673211		**KT278730**	**KT278717**	–	–	–	–
Pisorisporium cymbiforme	CBS 127887		–	–	**KT278699**	**KT278750**	–	–
	CBS 127888		–	–	**KT278700**	**KT278751**	–	–
Plagiascoma frondosum	CBS 139031	T	–	**KT278713**	**KT278701**	**KT278749**	**KT278734**	–
Pleurotheciella centenaria	DAOM 229631	T	JQ429151	JQ429234	JQ429246	JQ429265	–	–
Pleurotheciella rivularia	CBS 125238	T	JQ429160	JQ429232	JQ429244	JQ429263	**KT278735**	**KT278759**
	CBS 125237		JQ429161	JQ429233	JQ429245	JQ429264	**KT278736**	**KT278760**
Pleurotheciella uniseptata	DAOM 673210	T	**KT278729**	**KT278716**	–	–	–	–
Pleurothecium obovoideum	CBS 209.95	T	EU041784	EU041841	–	–	–	–
Pleurothecium recurvatum	CBS 101581		JQ429148	AF261070	JQ429248	JQ429266	–	–
	CBS 138747		**KT278728**	**KT278714**	**KT278703**	–	–	–
	CBS 138686		**KT278727**	**KT278715**	**KT278702**	–	**KT278737**	–
	CBS 131646		JQ429150	JQ429236	JQ429250	–	–	–
	CBS 131272		JQ429149	JQ429237	JQ429251	JQ429268	–	–
Pleurothecium semifecundum	CBS 131271	T	JQ429159	JQ429240	JQ429254	JQ429270	–	–
	CBS 131482		JQ429158	JQ429239	JQ429253	–	–	–
Savoryella appendiculata	NF 00206		–	–	HQ446293	HQ446442	–	–
Savoryella aquatica	SS 03801		–	HQ446372	HQ446290	HQ446441	–	–
Savoryella lignicola	NF 00204		–	HQ446378	HQ446299	–	–	–
Savoryella longispora	SAT 00322		–	HQ446380	HQ446302	HQ446450	–	–
Savoryella paucispora	SAT 00866		–	HQ446381	HQ446303	HQ446451	–	–
Savoryella verrucosa	SS 00052		–	HQ446374	HQ446298	HQ446445	–	–
Sterigmatobotrys macrocarpa	PRM 915682		JQ429153	GU017317	JQ429255	–	**KT278739**	**KT278762**
	DAOM 230059		JQ429154	GU017316	–	JQ429271	**KT278738**	**KT278761**
Sterigmatobotrys uniseptata	FMR 11937		HF677178	–	–	–	–	–
Taeniolella rudis	DAOM 229838		JQ429152	JQ429241	JQ429256	JQ429272	–	–
Triadelphia uniseptata	DAOMC 250376		–	**KT278718**	–	–	–	–

Sequences were manually aligned in BioEdit v. 7.1.8 (Hall 1999). Nuclear ribosomal loci were aligned according to the secondary structure of *Saccharomyces cerevisiae* to improve the decisions on homologous characters and introduction of gaps (Gutell 1993, Gutell et al. 1993, www.rna.ccbb.utexas.edu). These procedures and alignment of the sequences of protein-coding genes were performed as described in Réblová & Réblová (2013).

The single-locus datasets were examined for topological incongruence among loci (ITS: 26 sequences and 616 characters; β-tubulin: 11 sequences and 500 characters; nuc28S: 126 sequences and 1 947 characters; nuc18S: 104 sequences and 1 792 characters; *rpb2* segments 5–7: 77 sequences and 1 216 characters; *mcm7*: eight sequences and 659 characters). The ITS and β-tubulin loci were generated only for members of the new order *Pleurotheciales*. Because only a few *mcm7* sequences were generated, they were not tested for topological conflicts among clades at familial or ordinal rank in the *Sordariomycetes*. For each individual partition, 500 bootstrap replicates were generated with RAxML-HPC v. 7.0.3 (Stamatakis et al. 2005, Stamatakis 2006) and compared visually for topological conflict among supported clades in phylogenetic trees. A conflict between two loci was assumed to occur when a clade appeared monophyletic with bootstrap support of ≥ 75 % in one tree, but was not supported as monophyletic in another (Mason-Gamer & Kellogg 1996). Individual, conflict-free alignments were concatenated to combine sequences for two subsequent phylogenetic analyses. The multiple sequence alignments are deposited in TreeBASE (Study no. 18187).

Phylogenetic analyses

Phylogenetic relationships of the unidentified fungi and other ascomycetes were resolved by two combined analyses of ITS, nuc18S, nuc28S, β-tubulin, *mcm7* and *rpb2* sequences of representatives of the *Sordariomycetes*. We analysed the whole ITS rDNA barcode, the first 2/3 of the 5' half of the nuc28S, the entire nuc18S, partial *mcm7*, exons 3–6 of β-tubulin and segments 5–7 of *rpb2*. Bases 1–155 of the nuc18S, 1–85 of the nuc28S and 1–58 of the *rpb2* alignments at the 5'-end and 1470–1947 of the nuc28S alignment at the 3'-end were excluded from analysis because of incompleteness of the majority of the available sequences. The coding regions (exons) 3, 4, 5 and partly 6 of the β-tubulin with a total length of 291 nucleotides were analysed, non-coding regions were excluded. The combined datasets were partitioned into several subsets of nucleotide sites, i.e. ITS, nuc28S, nuc18S, and first, second and third codon positions of β-tubulin, *mcm7* and *rpb2*. Two members of the *Leotiomycetes*, *Leotia lubrica* and *Microglossum rufum*, were used to root the two multilocus phylogenies.

The program MrModeltest2 v. 2.3 (Nylander 2008) was used to infer the appropriate substitution model that would best fit the model of DNA evolution for each sequence dataset and each partition of the combined datasets. Maximum likelihood (ML) and Bayesian inference (BI) analyses were used to estimate phylogenetic relationships. ML analysis was performed with RAxML-HPC v. 7.0.3 with a GTRCAT model of evolution. Nodal support was determined by non-parametric bootstrapping (BS) with 1 000 replicates.

Bayesian inference analysis was performed in a likelihood framework as implemented in MrBayes v. 3.0b4 to reconstruct phylogenetic trees (Huelsenbeck & Ronquist 2001). For the ITS, nuc18S, nuc28S, and *rpb2* dataset, we used for each partition the GTR+G+I substitution model. For β-tubulin we used HKY+G, F81 and SYM+G for the first, second and third codon position, and for *mcm7* we used HKY+G, GTR+G and GTR for the first, second and third codon position. Two Bayesian searches were performed using the default parameters. Analyses were run for 10 million generations, with trees sampled every 1 000 generations. Tracer v. 1.6.0. (Rambaut et al. 2013) was used to confirm convergence of trees and burn-in. The first 50 000 trees, which represented the burn-in phase of the analysis, were discarded. The remaining trees were used for calculating posterior probabilities (PP) of recovered branches (Larget & Simon 1999).

PHYLOGENETIC RESULTS

In the first analysis, 134 combined nuc18S, nuc28S and *rpb2* sequences were assessed for 120 species in 20 orders in the *Sordariomycetes*. The alignment had 2 767 distinct alignment patterns (ML analysis). In the ML tree shown in Fig. 1, a strongly supported monophyletic clade was resolved (100 ML BS/1.0 PP) in the *Hypocreomycetidae* with three nested clades. The *Savoryellales* (100/1.0) was associated with the *Conioscypha* clade (100/1.0) including five species and *Pleurothecium* clade (98/1.0) comprising eight genera and three other undescribed ascomycetes. They represent two new lineages of freshwater and terrestrial fungi and are introduced below as the orders *Conioscyphales* and *Pleurotheciales*. A strongly supported monophyletic lineage (100/1.0) containing *Ascotaiwania persoonii*, two species of the dematiaceous hyphomycetous genus *Bactrodesmiastrum* and one unidentified ascomycete is positioned as a sister to a clade containing *Conioscyphales*, *Pleurotheciales* and *Savoryellales*. Together they form a robust monophylum (100/1.0) in the *Hypocreomycetidae*, including *Flammispora bioteca* (BCC 13367) in a basal position.

Two undescribed perithecial fungi were nested within the *Pleurotheciales* and are described here as new genera, *Melanotrigonum* and *Adelosphaeria*. Two strains of *Phaeoisaria* sp. with fasciculate conidiophores were positioned in the strongly supported *Phaeoisaria* clade (100/1.0) of the *Pleurotheciales* as the sister taxon to *Phaeoisaria sparsa*. They are introduced as a new species. The third unidentified perithecial ascomycete from freshwater habitat was nested within the *Bactrodesmiastrum* clade on a separate branch and it is described as a new monotypic genus *Plagiascoma.*

Two strains of *Savoryella limnetica* and *Triadelphia uniseptata* were positioned in the *Ascotaiwania* clade (79/0.91) in the *Savoryellales*. The genus *Ascotaiwania* is polyphyletic in our phylogeny. *Helicoön farinosum*, the asexual state of *A. hughesii* is grouped within the *Pleurotheciales*. *Ascotaiwania lignicola*, the type species, and three other species are members of the *Savoryellales*, while *A. persoonii* is nested in the *Bactrodesmiastrum* clade. Two dematiaceous hyphomycetes, *Phragmocephala stemphylioides* and *Dactylaria uniseptata*, were grouped among members of the *Pleurotheciales*; the latter is transferred to *Pleurotheciella* below.

In the second phylogenetic analysis (Fig. 2), the combined ITS, nuc18S, nuc28S, β-tubulin, *mcm7* and *rpb2* dataset consisted of 60 sequences representing 18 species of the *Pleurotheciales*, five of the *Conioscyphales*, 15 species of the *Savoryellales* and four species of the *Bactrodesmiastrum* clade. The alignment had 2 370 distinct alignment patterns (ML analysis). The robust clade containing the three orders and the *Bactrodesmiastrum* clade (100/1.0) has identical topologies in the three- and six-gene phylogenies. Six terminal clades were identified in the *Pleurotheciales* and are labelled as Clade I to VI on Fig. 2. Clades I, V and VI are strongly supported monophyletic lineages representing genera *Melanotrigonum* (100/1.0), *Phaeoisaria* (100/1.0) and *Pleurothecium* s.str. (100/1.0). Clade II (72/0.97) is morphologically heterogeneous containing *Brachysporiella setosa*, *Helicoön farinosum*, *Phragmocephala stemphylioides*

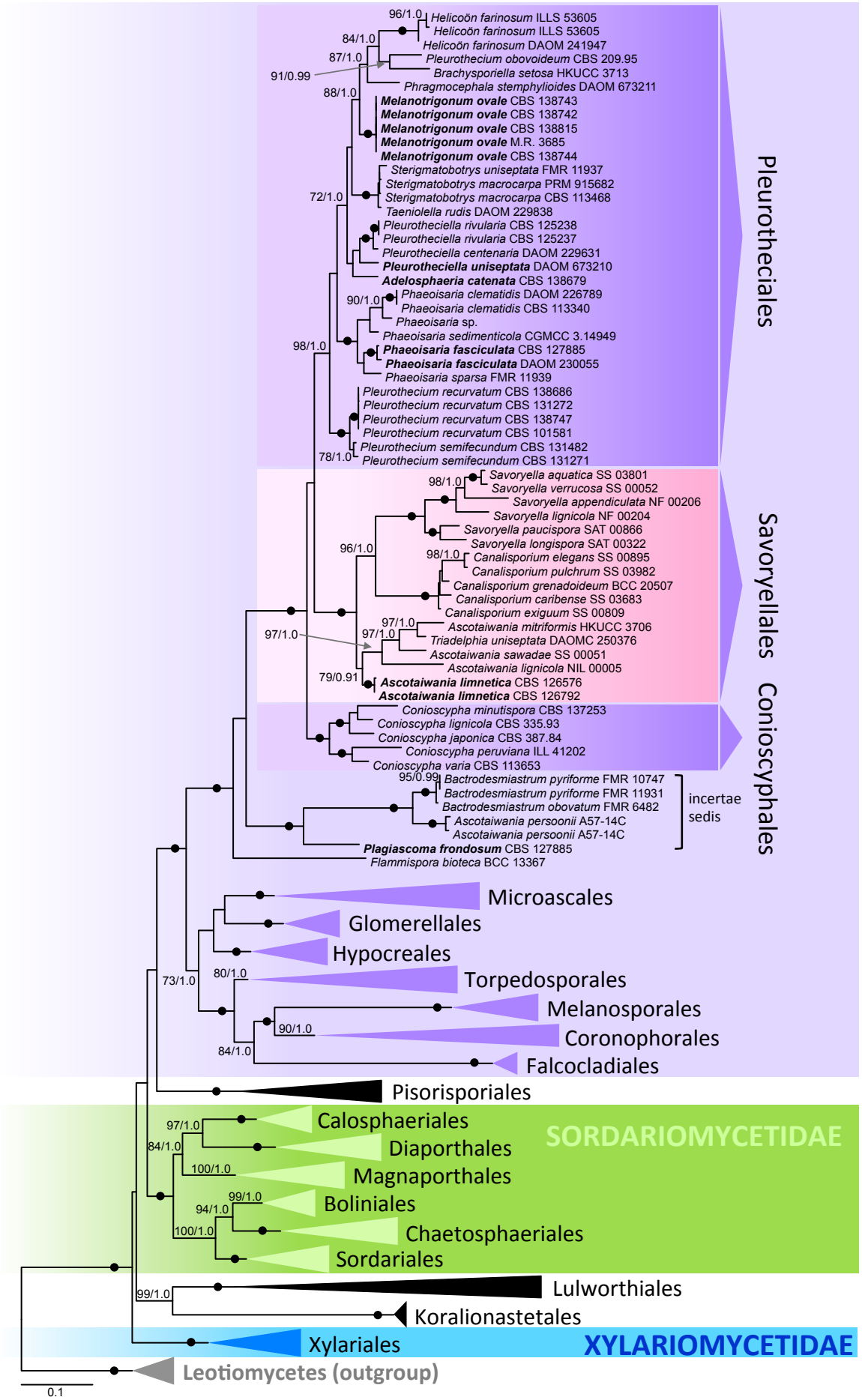

Fig. 1 Multilocus phylogenetic analysis of the nuc18S-nuc28S-*rpb2* sequences of the *Sordariomycetes* showing majority of the recognized ordinal lineages. Phylogram was inferred from the ML analysis with RAxML using a GTRCAT model of evolution. Only high branch support is shown at the nodes, maximum likelihood bootstrap support (ML BS) ≥ 70 % and Bayesian posterior probability (PP) ≥ 0.95. Symbol ● indicates nodes with 100 % ML BS and 1.0 PP. Taxa written in **bold** represent taxonomic novelties.

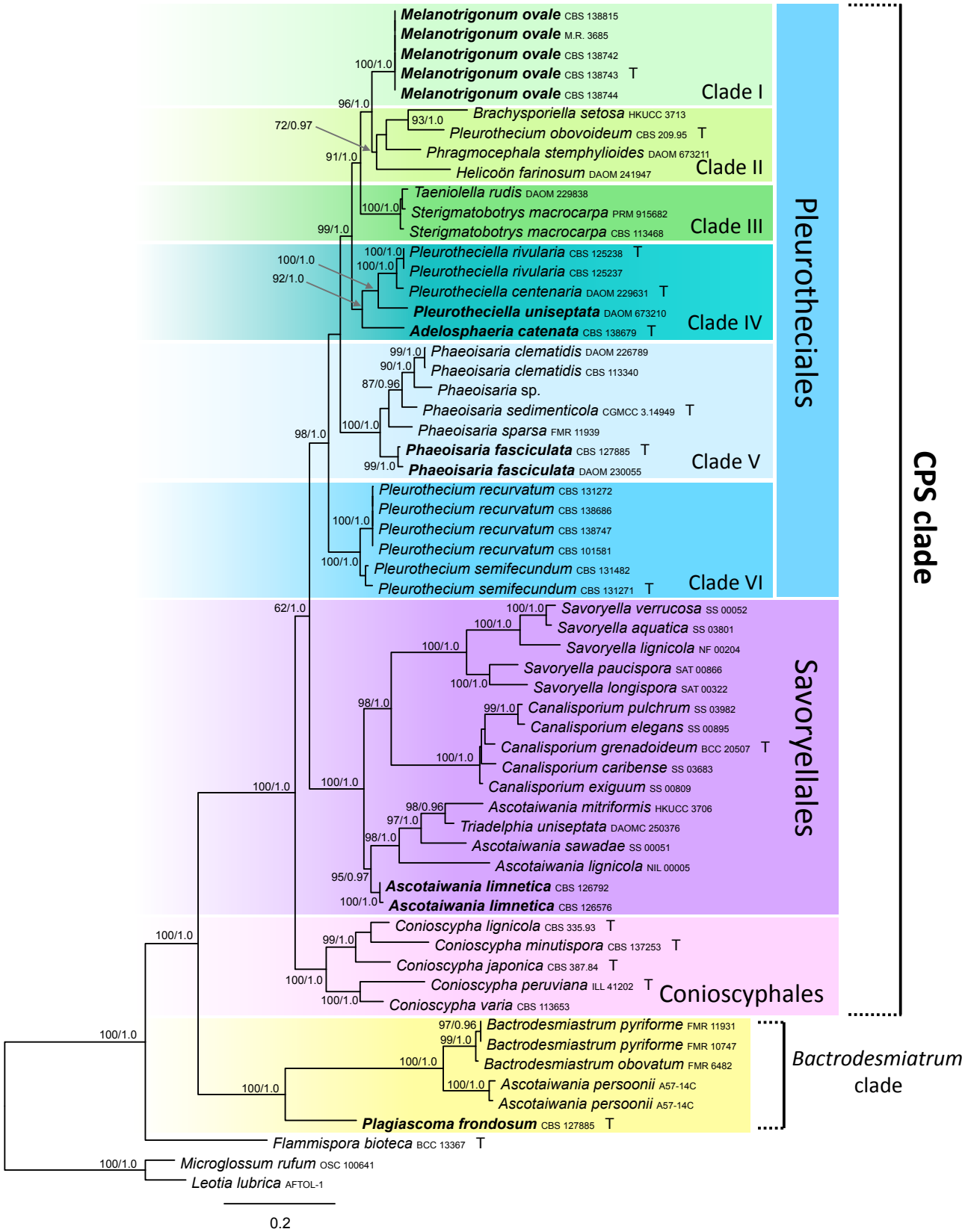

Fig. 2 Multilocus phylogenetic analysis of the ITS-nuc18S-nuc28S-β-tubulin-*rpb2*-*mcm7* sequences of the CPS (*Conioscyphales*, *Pleurotheciales*, *Savoryellales*) and *Bactrodesmiastrum* clades. Phylogram inferred from the ML analysis with RAxML using a GTRCAT model of evolution. Only high branch support is shown at the nodes, ML BS ≥ 70 %, PP ≥ 0.95. Taxa written in **bold** represent taxonomic novelties.

and *Pleurothecium obovoideum*. Clade III (100/1.0) includes *Sterigmatobotrys* and *Taeniolella rudis*. Clade IV (92/1.0) includes the monophyletic *Pleurotheciella* (100/1.0) and *Adelosphaeria catenata*.

TAXONOMY

Conioscyphales Réblová & Seifert, *ord. nov*. — MycoBank MB813226

Type family. *Conioscyphaceae* Réblová & Seifert.

Ascomata perithecial, non-stromatic. *Ostiole* periphysate. *Hamathecium* of paraphyses. *Asci* unitunicate, with a non-amyloid apical annulus. *Ascospores* hyaline, transversely multiseptate. *Conidiophores* micronematous, mononematous. *Conidiogenous cells* blastic, percurrently regenerating. *Conidia* brown, variable in shape; secession schizolytic. Saprobic on wood.

Conioscyphaceae Réblová & Seifert, *fam. nov*. — MycoBank MB813227

Type genus. *Conioscypha* Höhn., Ann. Mycol. 2: 58. 1904, emend. Shearer, Mycologia 65: 128. 1973.
= *Conioscyphascus* Réblová & Seifert, Stud. Mycol. 50: 100. 2004.

Ascomata perithecial, immersed to superficial, papillate or with elongated neck. *Ascomatal wall* leathery, waxy, comprising two layers. *Paraphyses* filiform, unbranched, longer than the asci. *Asci* unitunicate, persistent, 8-spored, with a pronounced non-amyloid apical annulus, cylindrical-clavate, stipitate. *Ascospores* fusiform to fusiform-navicular, hyaline, transversely multiseptate, lacking a mucilaginous sheath or appendages. *Conidiophores* micronematous, mononematous, hyaline. *Conidiogenous cells* blastic, cyathiform to doliiform. *Conidia* brown, non-septate, often with a basal pore, formed singly and successively by percurrent regeneration of the apex of the conidiogenous cell, liberating by apical rupture of the outer wall of the conidiogenous cell.

Pleurotheciales Réblová & Seifert, *ord. nov*. — MycoBank MB813228

Type family. *Pleurotheciaceae* Réblová & Seifert.

Ascomata perithecial, non-stromatic. *Ostiole* periphysate. *Hamathecium* of paraphyses. *Asci* unitunicate, with a non-amyloid apical ring. *Ascospores* hyaline or versicolorous with polar cells hyaline and middle cells brown, transversely multiseptate. *Conidiophores* macronematous or semi-macronematous, loosely fasciculate or aggregated in indeterminate synnemata. *Conidiogenous cells* producing conidia holoblastically, monoblastic or with sympodial extension, conidial secession rhexolytic or schizolytic. *Conidia* hyaline or brown or versicolorous, septate or non-septate. Saprobic on wood, rarely human pathogens causing keratomycosis.

Pleurotheciaceae Réblová & Seifert, *fam. nov*. — MycoBank MB 813229

Type genus. *Pleurothecium* Höhn., Ber. Deutsch. Bot. Ges. 37: 154. 1919.
= *Carpoligna* F.A. Fernández & Huhndorf, Mycologia 9: 253. 1999.

Ascomata perithecial, immersed to semi-immersed to superficial, papillate or with a central rarely eccentric neck. *Ostiole* periphysate. *Ascomatal wall* leathery to fragile, carbonaceous, brown, comprising two layers. *Paraphyses* abundant, sparsely branched, partially disintegrating, cylindrical. *Asci* unitunicate, 8-spored, with a pronounced non-amyloid apical annulus, cylindrical or cylindrical-clavate. *Ascospores* ellipsoidal to fusiform, hyaline or versicolorous with polar cells hyaline and middle cells brown, transversely multiseptate, lacking a mucilaginous sheath or appendages. *Conidiomata* present or absent, when present indeterminate synnemata or loose fascicles. *Conidiophores* macronematous or semi-macronematous, sometimes regenerating percurrently. *Conidiogenous cells* producing conidia holoblastically, conidial secession rhexolytic on short denticles or rachis on sympodially extending polyblastic conidiogenous cells, or schizolytic on monoblastic or solitary thallic conidiogenous cells. *Conidia* hyaline, sometimes with protracted maturation of the middle cells, which turn brown, or brown or versicolorous, septate or non-septate.

Adelosphaeria Réblová, *gen. nov.* — MycoBank MB813230

Type species. *Adelosphaeria catenata* Réblová.

Etymology. *Adelo-* (Gk), meaning unclear, referring to the difficulty of recognising this taxon among other morphologically similar fungi; *sphaera* (L) meaning globe, referring to ascoma.

Ascomata perithecial, non-stromatic, semi-immersed becoming superficial, subglobose, dark brown, papillate. *Ostiole* periphysate. *Ascomatal wall* leathery to fragile, 2-layered. *Paraphyses* abundant, persistent, septate. *Asci* unitunicate, cylindrical-clavate, stipitate, 8-spored, apex with a non-amyloid apical annulus. *Ascospores* ellipsoidal, slightly curved, hyaline, transversely septate. *Asexual morph* unknown.

Adelosphaeria catenata Réblová, *sp. nov.* — MycoBank MB813231; Fig. 3, 4

Etymology. *Cateniformis* (L), meaning chain-shaped, referring to the dark brown cells arranged in chains formed on vegetative hyphae in the axenic culture.

Ascomata perithecial, non-stromatic, semi-immersed, becoming superficial, solitary or in small groups; venter 200–280 µm diam, 300–360 µm high, subglobose, dark brown, glabrous, papillate, opening by a rounded pore. *Ostiole* periphysate. *Ascomatal wall* leathery to fragile, 20–30 µm thick, 2-layered; outer layer consisting of brown, polyhedral cells of *textura prismatica* with opaque walls, inner layer consisting of several rows of thin-walled, hyaline, flattened cells. *Paraphyses* abundant, persistent, septate, hyaline, sparsely branched, anastomosing, c. 3.5–5.0 µm wide, tapering to c. 2.5 µm. *Asci* (85–)93–105 µm long in the sporiferous part, 12.5–14.5 µm wide (mean ± SD = 199.7 ± 5.4 × 12.5 ± 1.2 µm), with a stipe 20–35(–50) µm long; cylindrical-clavate, broadly rounded apically to obtuse, 8-spored, apex with a flattened, non-amyloid apical annulus 3.0–3.5 µm wide, about 2.0 µm high. *Ascospores* 16.5–19.5(–20) × 5.0–5.5(–5.8) µm (mean ± SD = 17.8 ± 1.3 × 5.4 ± 0.2 µm), ellipsoidal, straight or slightly curved, hyaline, smooth, 3-septate, non-constricted at the septa, arranged 1–2-seriately in the sporiferous part.

Culture characteristics — Colonies slow growing reaching 12–15 mm diam on PDA after 21 d at 25 °C. Aerial mycelium dark brown (aoc735), paler brown (aoc723) towards the margin, mainly flat, felty, reverse brown (aoc734), with a marginal zone of dark brown (aoc734) submerged mycelium. Aerial and submerged hyphae 1.5–2.0 µm wide, smooth, subhyaline to pale brown, thin-walled, unbranched or sparsely branched. Sporulation absent. On vegetative hyphae are formed brown, globose to ellipsoidal cells 5.0–14.5 µm diam (mean ± SD = 10.3 ± 2.9 µm), with thick, often opaque walls, arranged in chains or arising laterally on another cell.

Specimen examined. Czech Republic, Southern Bohemia, Novohradské hory Mts, Dobrá voda, Hojná voda National nature monument, decorticated wood of a trunk of *Fagus sylvatica*, 4 Oct. 2012, *M. Réblová M.R. 3755* (holotype PRM 933853, culture ex-type CBS 138679).

Fig. 3 *Adelosphaeria catenata*. a, b. Ascomata; c. vertical section of the ascomatal wall; d–f. asci with ascospores; g, h. apical annulus; i. paraphyses (a–i. PRM 933853 holotype); d–f, h: DIC; g, i: PC. — Scale bars: a, b = 250 µm; c = 30 µm; d–f, i = 10 µm; g, h = 5 µm.

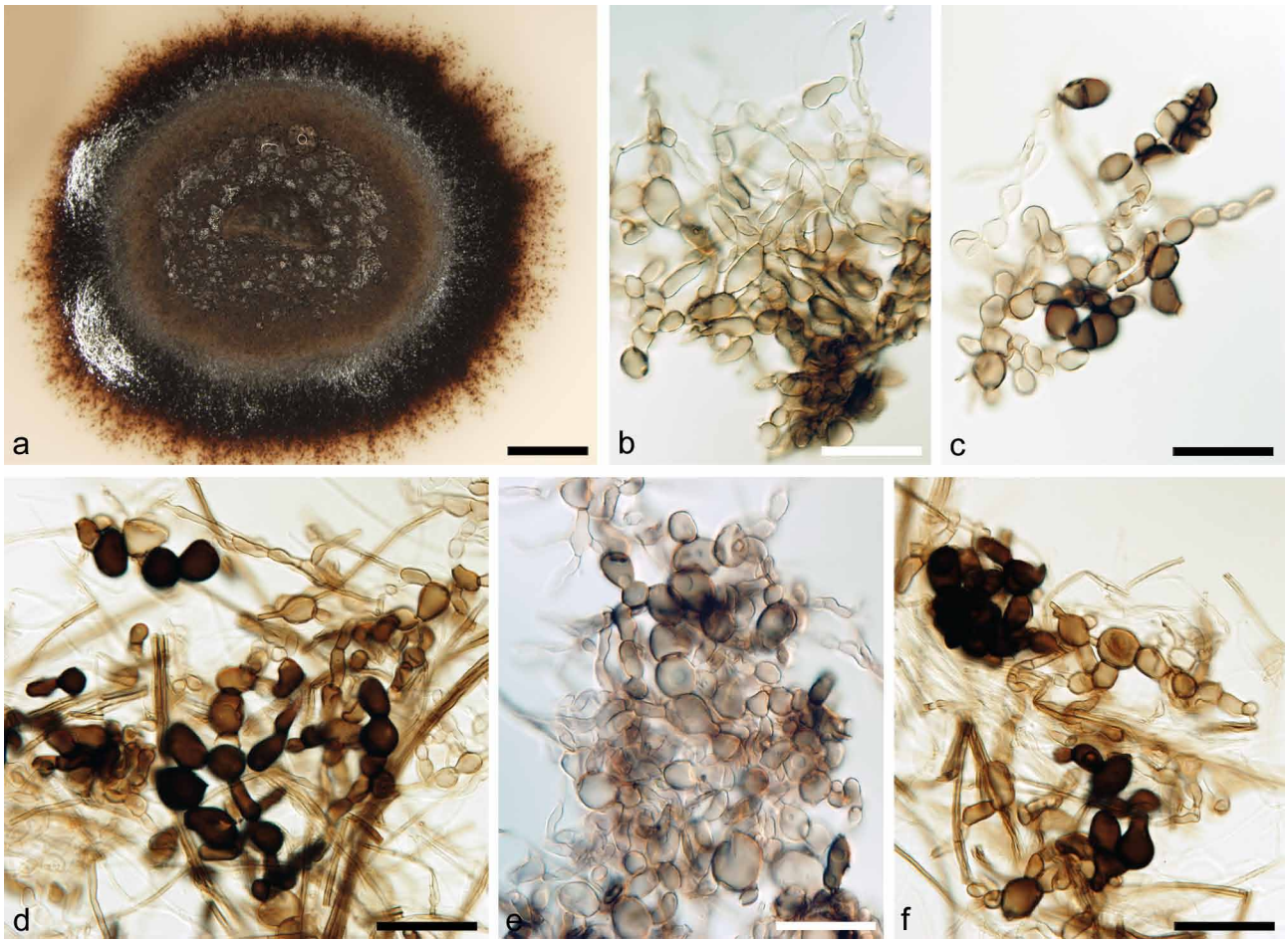

Fig. 4 Asexual morph of *Adelosphaeria catenata*. a. Colony on PCA; b–f. brown cells arising blastically from vegetative mycelium on PCA (a–f. CBS 138679, 21 d, 25 °C); b–f: DIC. — Scale bars: a = 4 mm; b–f = 20 µm.

Notes — *Adelosphaeria catenata* resembles species of *Pleurothecium* and *Pleurotheciella* because of its hyaline, 3-septate ascospores, cylindrical-clavate asci and brown semi-immersed ascomata. *Pleurothecium recurvatum* is easily distinguishable from *A. catenata* by its slender ascospores, pronounced apical annulus, setose ascomata and macronematous conidiophores bearing hyaline, polyblastic, denticulate conidiogenous cells elongating in a sympodial pattern and 3-septate, hyaline conidia with protracted maturation of the middle cells, which turn brown. It is more difficult to separate *Adelosphaeria* from *Pleurotheciella*, because species of both genera share similar morphological characters of ascospores, asci and ascomata. The only conspicuous difference lies in morphology of their asexual states; *Pleurotheciella* can be easily distinguished by *Dactylaria*-like, hyaline to subhyaline conidiophores and conidia.

Melanotrigonum Réblová, *gen. nov.* — MycoBank MB813232

Type species. *Melanotrigonum ovale* Réblová.

Etymology. *Melas-* (Gk), meaning dark, referring to the brown conidia; *Trigonon* (Gk) meaning triangle, referring to conspicuous triangle-like conidiogenous cells of the asexual morph.

Ascomata perithecial, non-stromatic, immersed to semi-immersed, subglobose to broadly conical, dark brown, papillate. *Ostiole* periphysate. *Ascomatal wall* leathery to fragile, 2-layered. *Paraphyses* abundant, persistent, septate. *Asci* unitunicate, cylindrical, stipitate, 8-spored, apex with a conspicuous, non-amyloid apical annulus. *Ascospores* ellipsoidal, hyaline, transversely septate. *Asexual morph* a dematiaceous hyphomycete, conidiophores semi-micronematous, conidiogenous cells producing brown, 1-septate conidia holoblastically on short denticles.

Melanotrigonum ovale Réblová, *sp. nov.* — MycoBank MB813233; Fig. 5, 6

Etymology. *Ovalis* (L), referring to the oval shape of conidia.

Ascomata perithecial, non-stromatic, immersed to semi-immersed, gregarious, occurring in small to large groups; venter 320–480 µm diam, 400–500 µm high, subglobose to broadly conical, brown, glabrous, papillate, opening by a rounded pore. *Ostiole* periphysate. *Ascomatal wall* leathery to fragile, 23–30 µm thick, 2-layered; outer layer consisting of brown, polyhedral cells of *textura prismatica* with opaque walls; towards the exterior grading into polyhedral to angular cells of *textura angularis*; towards the interior grading into pale-brown, elongated cells. Inner layer consisting of several rows of thin-walled, hyaline, flattened cells. *Paraphyses* abundant, persistent, septate, anastomosing, hyaline, sparsely branched, c. 3.0–4.5 µm wide, tapering to c. 3.0 µm, longer than the asci. *Asci* (105–)115–128(–142) µm long in the sporiferous part, (8.5–)9.0–11.5 µm wide (mean ± SD = 122.8 ± 7.4 × 11.0 ± 5.2 µm) with a stipe 32–50 µm long; cylindrical, obtuse apically, 8-spored, apex with a large, conspicuous non-amyloid apical annulus 4.5–5.0 µm wide, 3.5–4.0 µm high. *Ascospores* (17–)18–21(–21.5) × 5.0–6.0(–6.5) µm (mean ± SD = 19.4 ± 1.5 × 5.8 ± 0.3 µm), ellipsoidal, straight to slightly curved, hyaline, smooth, 3-septate, non-constricted at the septa, arranged obliquely uniseriate, sometimes 2-seriate only in the upper part of the ascus.

Culture characteristics — Colonies slow growing, reaching 8–10 mm diam on PDA after 21 d at 25 °C. Aerial mycelium beige in the centre of the colony and on the inoculum block, white towards the margin, felty, centre elevated, later with a moist appearance, bent into deep folds, reverse dark beige.

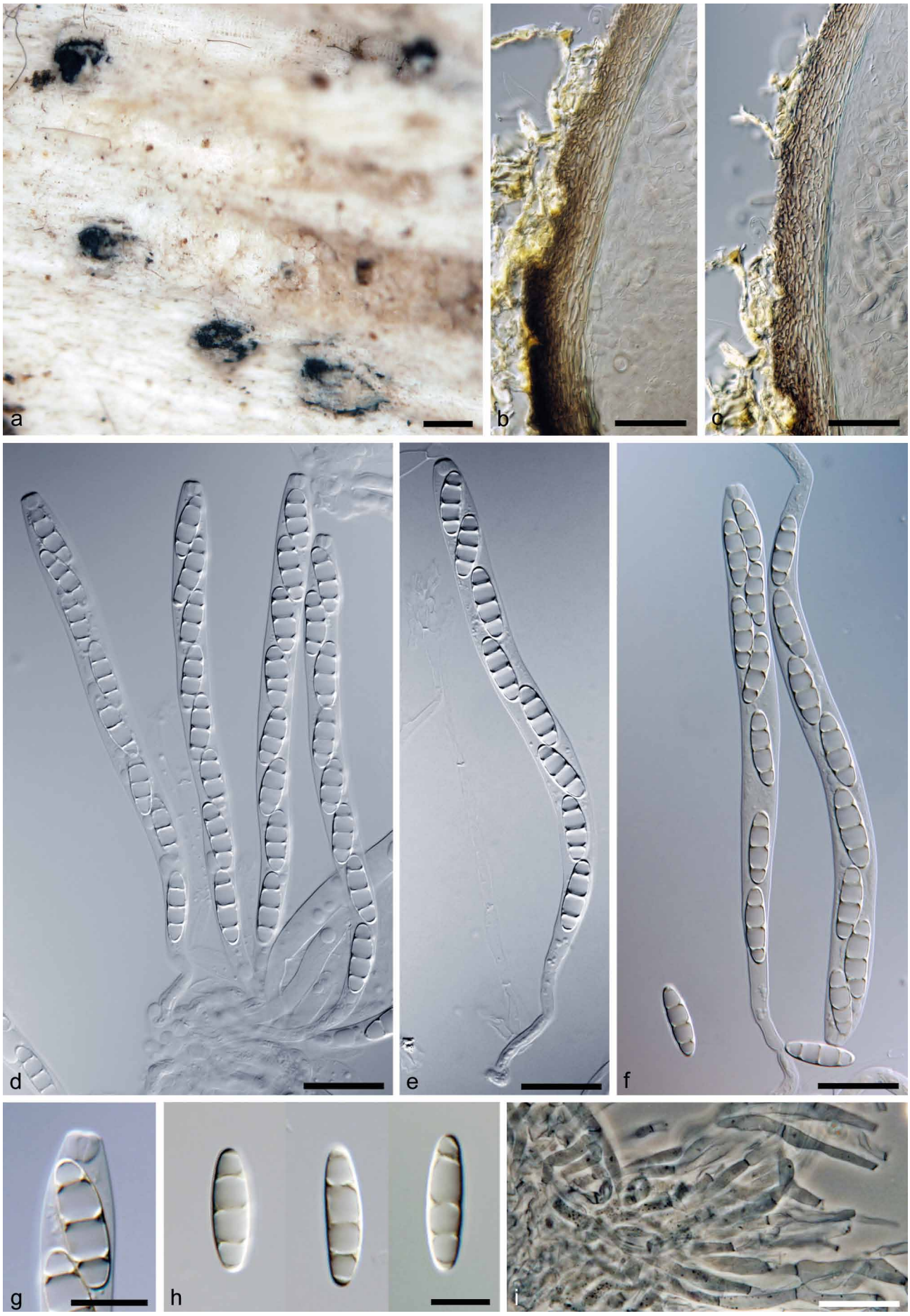

Fig. 5 *Melanotrigonum ovale*. a. Ascomata; b, c. vertical sections of the ascomatal wall; d–f. asci with ascospores; g. apical annulus; h. ascospores; i. paraphyses (a–i. PRM 933852 holotype); b–h: DIC; i: PC. — Scale bars: a = 250 µm; b, c = 25 µm; d–f, i = 20 µm; g, h = 10 µm.

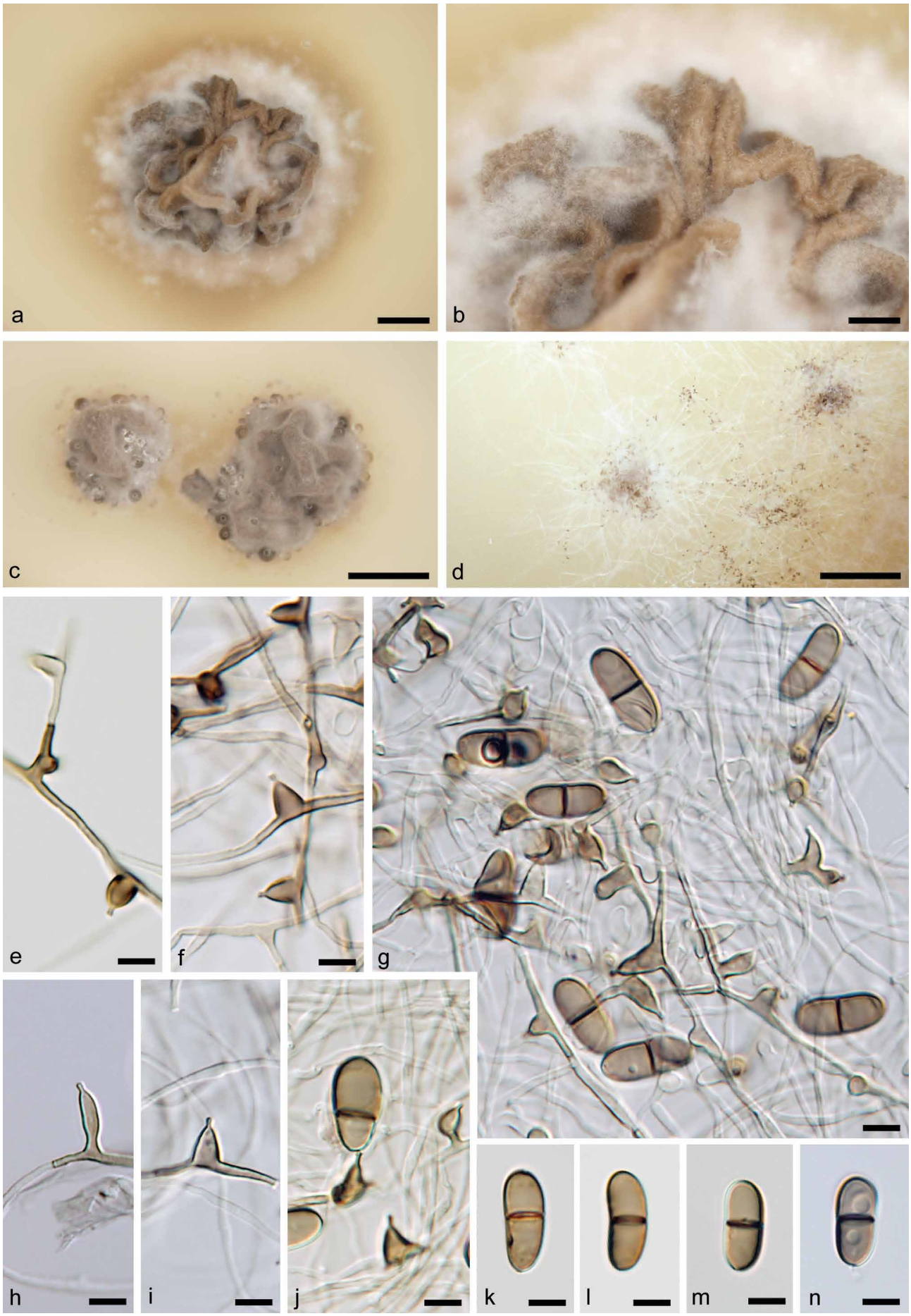

Fig. 6 Asexual morph of *Melanotrigonum ovale*. a, b. Colony on PDA (5 mo, 25 °C); c, d. colony on PDA (1 mo, 25 °C); e, f, h, i. conidiogenous cells on PCA; g, j. conidiogenous cells with conidia borne on a denticle on PCA; k–n. conidia (a, b, g–i, k–n. M.R. 3685; c, d–f, j. CBS 138742; e–j. 21 d, 25 °C); e–n: DIC. — Scale bars: a = 2.5 mm; b = 5 mm; c, d = 10 mm; e–n = 5 µm.

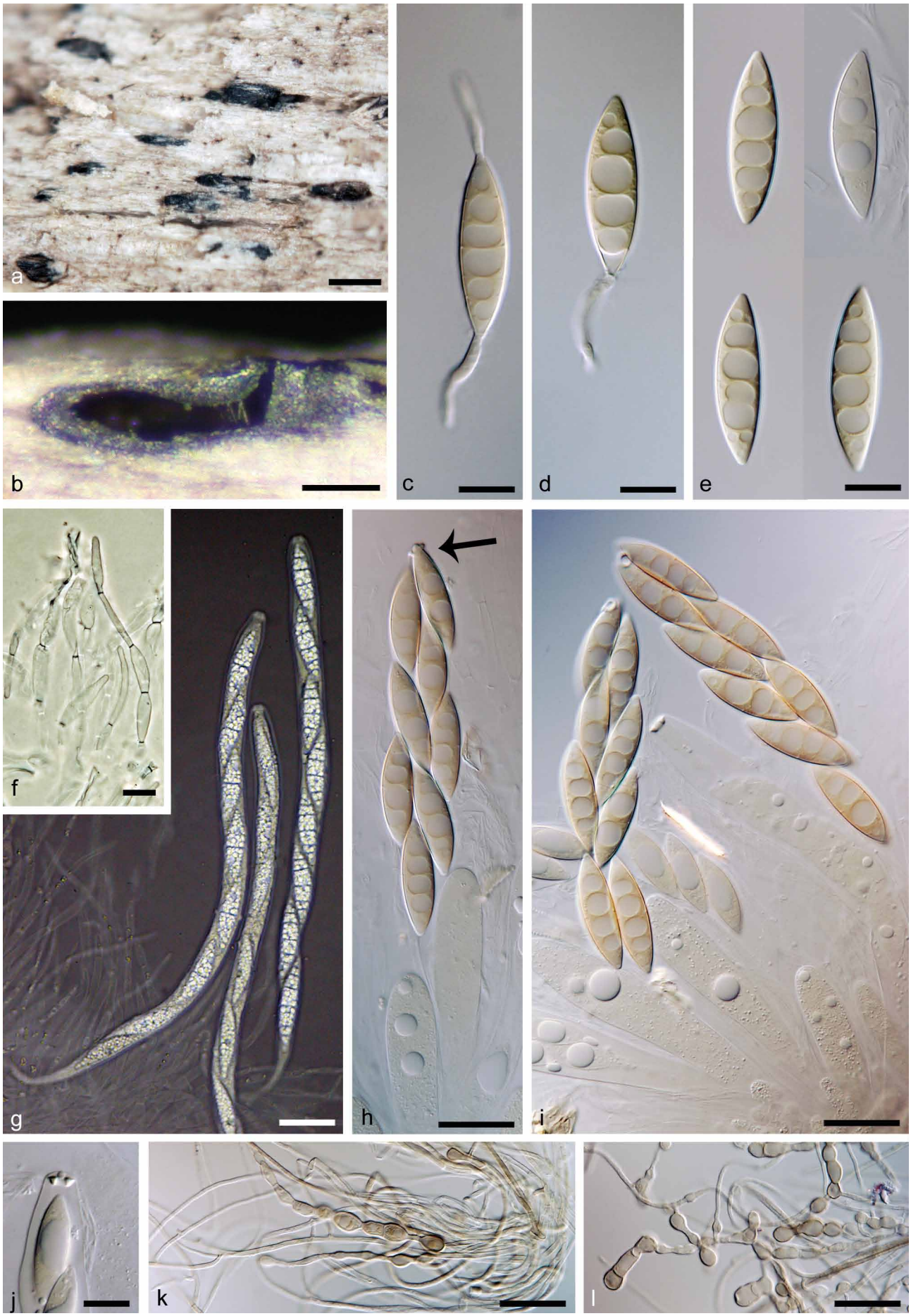

Fig. 7 *Plagiascoma frondosum*. a. Ascomata; b. vertical section of the ascomatal wall; c, d. germinating ascospores; e. ascospores; f. paraphyses; g. asci in freshly collected material in Pelikan ink; h, i. asci from air dried herbarium material, arrow indicates apical annulus; j. apical annulus; k, l. pigmented cells formed in vitro on vegetative hyphae on PCA (a–j. PRM 933854 holotype; k, l. CBS 139031, 21 d, 25 °C); c–e, h–l: DIC; f, g: PC. — Scale bars: a = 500 µm; b = 250 µm; c–f, j = 10 µm; g = 25 µm; h, i, k, l = 20 µm.

Aerial hyphae 2.0–3.0 µm wide, smooth, hyaline, thin-walled, sparsely branched. Submerged hyphae 2.0–2.5 µm wide, smooth, hyaline. Sporulation appears later on the youngest aerial hyphae at the margin of the colony. Conidiophores semi-micronematous, reduced to a conidiogenous cell, arising vertically from hyphae, unbranched, smooth, tapering towards the apex. Conidiogenous cell 4.5–8.0(–10.0) µm long, 2.0–3.0 µm wide in the broadest point (mean ± SD = 6.9 ± 1.1 × 2.8 ± 0.4 µm), integrated, intercalary, almost triangular to ampulliform, tapering towards the apex, pale brown, with a single, rarely two, pale brown to subhyaline denticle 1.0–2.0 µm long. *Conidia* (10–)11.5–13.5(–14) × 5.0–6.0 µm (mean ± SD = 12.3 ± 0.6 × 5.4 ± 0.5 µm), 1-septate, oval to bean-shaped, straight or slightly curved, leaving a pore when detached, smooth, rounded at both ends or slightly tapering towards the base, brown, darker at the septum, non-constricted, sometimes cells asymmetrical.

Specimens examined. Czech Republic, Southern Moravia, Břeclav distr., Valtice, Rendezvous Valtice National nature monument, decaying wood of a trunk of *Quercus cerris*, 17 Nov. 2012, *M. Réblová M.R. 3698* (holotype PRM 933852, culture ex-type CBS 138743); ibid., *M.R. 3685*, *M.R. 3688A* (culture CBS 138742), *M.R. 3688B* (culture CBS 138744), *M.R. 3699* (culture CBS 138815).

Notes — Five strains of *M. ovale* were collected on soft, strongly decaying wood of several fallen trunks of *Quercus cerris*, the remains of old growth trees that were more than hundred years old. Specimen *M.R. 3685* has asci shorter in the sporiferous part (100–105 µm long) and generally smaller ascospores, (15–)16–17.5(–19.5) × 4.5–5.5 µm.

Melanotrigonum ovale is similar to *Pleurotheciella rivularia* in characters of ascospores, asci and ascomata, but differs from it by the ascal apex with a conspicuous and larger apical annulus, 4.5–5.0 × 3.5–4.0 µm (w × h) vs 2.5–3.5 × 1.5 µm in *P. rivularia*. Both species produce conidia on denticulate conidiogenous cells. In *Melanotrigonum*, the conidiogenous cells are almost triangular to ampulliform, tapering towards the apex with a single or rarely two denticles, while conidiogenous cells of *Pleurotheciella* are cylindrical, elongate sympodially with one to several denticles.

Plagiascoma Réblová & J. Fourn., *gen. nov.* — MycoBank MB813234

Type species. Plagiascoma frondosum Réblová & J. Fourn.

Etymology. *Plágios* (Gk), meaning slanting, oblique, sideways, referring to the flattened ascomata arranged horizontally to the host.

Ascomata perithecial, non-stromatic, immersed gradually erumpent to semi-immersed, conical, dark brown, lying obliquely to horizontally, papillate or with a neck. *Ostiole* periphysate. *Ascomatal wall* fragile, 2-layered. *Paraphyses* abundant, persistent, septate. *Asci* unitunicate, cylindrical to cylindrical-fusiform, stipitate, 8-spored, apex with a non-amyloid apical annulus. *Ascospores* fusiform, hyaline, transversely septate. *Asexual morph* unknown.

Plagiascoma frondosum Réblová & J. Fourn., *sp. nov.* — MycoBank MB813235; Fig. 7

Etymology. *Frondosus* (L), meaning leaf-bearing, referring to a deciduous tree as a host.

Ascomata non-stromatic, immersed, gradually erumpent to semi-immersed, solitary or in small groups or in rows; venter 200–280 µm diam, 450–550 µm high, conical, dark brown, glabrous, slightly pinched laterally, lying obliquely to horizontally, papillate or with a beak 30–120 µm high, conical, lateral, opening by a rounded pore. *Ostiole* periphysate. *Ascomatal wall* fragile, 24–30 µm thick, becoming thicker in the beak up to c. 35 µm, 2-layered; outer layer consisting of brown, polyhedral, flattened cells of *textura prismatica* with opaque walls. Inner layer consisting of several rows of thin-walled, hyaline, flattened cells. *Paraphyses* abundant, persistent, septate, hyaline, c. 4.0–6.0(–7.0) µm wide, tapering to c. 3.5 µm. *Asci* in fresh material 225–240 µm long in the sporiferous part, 13–15 µm wide, with a stipe 30–52 µm long, cylindrical, with ascospores arranged obliquely uniseriate; upon drying asci 100–160 µm long in the sporiferous part, 15–20 µm wide with a stipe 53–73 µm long, cylindrical-fusiform, with ascospores arranged 2-seriately; obtuse to broadly rounded apically, 8-spored; apex with a non-amyloid apical annulus 4.5–5.5 µm wide, 1.5–2.5 µm high. *Ascospores* (28.5–)30–34.5(–36) × 7.5–8.5(–9.0) µm (mean ± SD = 32.8 ± 1.9 × 8.4 ± 0.6 µm), fusiform, tapering towards the ends, hyaline, smooth, 3–5-septate, non-constricted at the septa, with a large guttule in each cell.

Culture characteristics — Colonies slow growing, 10–15 mm diam on PDA after 21 d at 25 °C. Aerial mycelium brown near the centre of the colony and on the inoculum block, pale brown to beige (oac800) towards the margin, felty, reverse brown (oac734). Aerial and submerged hyphae 1.5–2.5 µm wide, smooth, subhyaline, thin-walled, sparsely branched. Sporulation absent. On aerial hyphae arise ellipsoidal cells 5.5–9.0 µm diam (mean ± SD = 7.0 ± 1.0 µm), pale brown to subhyaline, thick-walled, intercalar, terminal or arranged in a short chain.

Specimen examined. France, Midi-Pyrénées, Ariège, Rimont, valley of La Maille brook, c. 550 m asl, submerged decorticated wood of *Fraxinus excelsior*, 9 May 2014, *J. Fournier J.F. 14044* (holotype PRM 933854, culture ex-type CBS 139031).

Notes — The examination of fresh material of *P. frondosum* revealed asci over 200 µm long and 13–15 µm wide, with uniseriate ascospores arranged obliquely (Fig. 7g). Upon drying, the arrangement of ascospores changes and they became biseriate within the ascus. The asci in dry herbarium material are shorter in the sporiferous part, 100–160 µm long, and wider 15–20 µm with almost twice the stipe length (Fig. 7h, i). No sheath or appendages were observed on immature or mature ascospores. Freshwater perithecial ascomycetes often have ascospores enclosed in a hyaline sheath or have appendages to facilitate their attachment on moist woody substrates. Interestingly, this is largely true for species from Asia, America and Australia but not in Europe, where many of the most widespread freshwater species lack these structures.

The fusiform, hyaline, 3–5-septate ascospores of *P. frondosum* resemble multiseptate ascospores of some species of *Annulatascus*, e.g. *A. nilensis* (Abdel-Wahab et al. 2011) and *A. tropicalis* (Tsui et al. 2002). In our multilocus phylogeny, *P. frondosum* is positioned in the strongly supported *Bactrodesmiastrum* clade.

Phaeoisaria fasciculata Réblová & Seifert, *sp. nov*. — MycoBank MB813236; Fig. 8

Etymology. *Fasciculus* (L), meaning fascicle or bundle, referring to conidiophores arranged in fascicles and lacking a distinct stipe.

Colonies in vivo effuse, dark grey, whitish to beige when sporulating. *Sexual morph* not observed. *Synnemata* absent, conidiophores forming fascicles. *Conidiophores* 25–65 × 3.0–3.5 µm (mean ± SD = 41.7 ± 14.2 × 3.3 ± 0.3 µm), macronematous, arising from brown, thick-walled cells, cylindrical, pale brown, subhyaline towards the apex, unbranched, smooth-walled. *Conidiogenous cells* 10–29(–36) × 2.5–3.5 µm (mean ± SD = 20.2 ± 6.7 × 3.1 ± 0.5 µm), integrated, terminal, cylindrical, tapering towards tip, pale brown to subhyaline near base, hyaline towards apex, smooth-walled, polyblastic, forming conidia sympodially on conspicuous denticles 1.0–1.5 µm long, about 0.5 µm wide, scattered or clustered in the apical region. *Conidia*

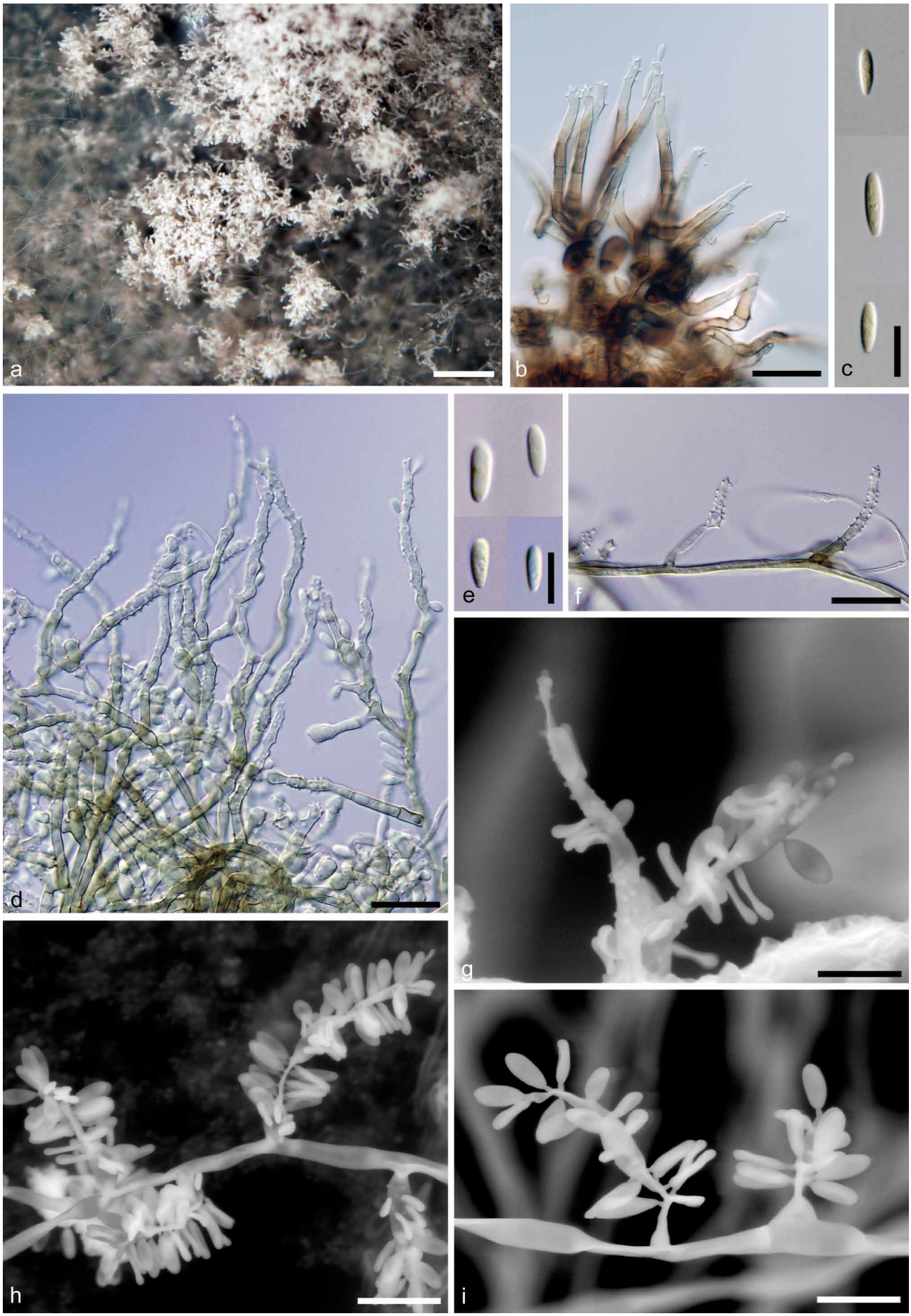

Fig. 8 *Phaeoisaria fasciculata*. a. Colony on PCA; b. conidiophores in vivo; c. conidia in vivo; d, f, g–i. conidiophores on PCA; e. conidia on PCA (a, d–f. CBS 127885; b, c. PRM 933855 holotype; g–i. DAOM 230055; a, d–i. 21 d, 25 °C); b–f: DIC; g–i: ESEM. — Scale bars: a = 50 µm; b = 250 µm; b, d, f = 20 µm; c, e = 10 µm; g–i = 10 µm.

6.0–8.0(–9.0) µm long, about 2.0 µm wide (mean ± SD = 7.3 ± 1.2 × 2.0 ± 0.1 µm), ellipsoidal to obovoid, straight, rounded at the apex, obtuse and tapering towards base, hyaline, non-septate, smooth-walled.

Culture characteristics — Colonies reaching 12–18 mm diam on PCA after 21 d at 25 °C. Aerial mycelium beige to pale brown (oac662), at first smooth, later cottony, reverse brown (oac640). Aerial and submerged hyphae 2.0–3.0 µm wide, hyaline to pale brown, sparsely branched, smooth-walled. Sporulation appears first in the centre of the colony, later present over the whole colony or in isolated patches; sporulating colony beige (oac809) with a powdery appearance. Synnemata absent. Conidiophores 20–75 × 2.5–3.5 µm (mean ± SD = 45.3 ± 17.0 × 3.0 ± 0.3 µm), macronematous to semi-macronematous reduced to a single conidiogenous cell, arising from aerial hyphae, cylindrical, slightly tapering towards the apex, pale brown, subhyaline towards the apex, unbranched, smooth-walled. Conidiogenous cell 10–29(–36) × 2.5–3.5(–4.0) µm (mean ± SD = 20.2 ± 6.7 × 3.1 ± 0.5 µm), integrated, terminal and intercalar, cylindrical, tapering towards tip, pale brown to subhyaline, hyaline towards apex, smooth-walled, polyblastic, with numerous conspicuous denticles 1.0–1.5 µm long, c. 0.5 µm wide, scattered along the whole length of intercalar conidiogenous cell and clustered in the apical region. Conidia 5.5–7.5(–8.5) × 2.0–2.5(–3.0) µm (mean ± SD = 6.7 ± 0.9 × 2.5 ± 0.3 µm), ellipsoidal to obovoid, straight, rounded at the apex, obtuse and tapering towards base, hyaline, non-septate, smooth-walled.

Specimens examined. Canada, Ontario, Goulbourn Twp., Stittsville, bark on branch on ground, 8 Oct. 2001, *Keith A. Seifert K.A.S. 1433* (DAOM 230055). – Czech Republic, Southern Moravia, Břeclav distr., Milovice, Milovická stráň Nature Reserve, north slopes of Mt Špičák, 293 m asl, decorticated wood of *Sambucus nigra*, 18 Nov. 2009, *M. Réblová M.R. 3084* (holotype PRM 933855, culture ex-type CBS 127885).

Notes — *Phaeoisaria fasciculata* is easily distinguished from other species of the genus by its conidiophores, which grow in fascicles on the host, while typical indeterminate synnemata are not formed. The ellipsoidal to obovoid, non-septate conidia of *Ph. fasciculata* resemble those of *Ph. caffera*, the *Ph. clematidis* species complex and *Ph. magnifica*. *Phaeoisaria caffera* differs from the new species by longer, pale yellowish brown conidia. The conidia of *Ph. clematidis* and *Ph. magnifica* are subhyaline to very dilute olivaceous and generally wider.

Ascotaiwania limnetica (H.S. Chang & S.Y. Hsieh) Réblová & J. Fourn., *comb. nov.* — MycoBank MB813237; Fig. 9, 10

Basionym. *Savoryella limnetica* H.S. Chang & S.Y. Hsieh, Mycol. Res. 102: 715. 1998.

Ascomata perithecial, non-stromatic, semi-immersed, gradually erumpent to almost superficial, scattered or clustered in small groups of 2–3, upright, obliquely oriented or lying horizontally on the host; venter 210–260 µm diam, 220–250(–300) µm high, black, subglobose with a flattened base and a broadly conical apex, often laterally flattened, flask-shaped when lying horizontally, with a papilla or short neck, broadly conical or cylindrical, apically truncate, central, eccentric or lateral, oriented upwards when ascomata lie horizontally. *Ostiole* periphysate. *Ascomatal wall* fragile, 9–15 µm thick, thicker at the apex up to 20 µm, 2-layered; outer layer consisting of dark brown, polyhedral, flattened cells of *textura prismatica* with opaque walls and sparse pores, outwards grading into small protruding cells, inner layer consisting of several rows of thin-walled, hyaline, flattened cells. *Asci* 125–150 × 11–14 µm (mean ± SD = 137 ± 9.4 × 12.6 ± 1.2 µm), cylindrical, short-stipitate, broadly rounded apically to obtuse, with a non-amyloid, discoid apical annulus 4.5–5.5 µm wide, 1.0–2.0 µm high. *Paraphyses* sparse, partially disintegrating at maturity, septate, branching, anastomosing, 4.0–9.5 µm wide. *Ascospores* (17.5–)19.5–23.5(–24) × (6.3–)7.0–8.5 µm, (mean ± SD = 21.4 ± 1.4 × 7.7 ± 0.5 µm), ellipsoidal, equilateral, straight, versicolorous, middle cells olivaceous brown to brown, containing numerous small guttules, polar cells smaller, hyaline, smooth, unequally 3-septate, slightly constricted at the septa, without sheath or appendages, arranged obliquely uniseriately in the ascus. *Colonies* in vivo diffuse, visible only as single scattered macroconidia arising from short, hyaline conidiogenous cells on vegetative mycelium near ascomata. *Conidia* (30–)33–41 × 15–17.5 µm, ellipsoidal, broadly rounded at the apex, tapering basally, dark brown, opaque, basal cell subhyaline to pale brown, (3–)5–6-septate, septa obscured by a darker band.

Culture characteristics — Colonies slow growing, reaching c. 8–10 mm diam on PDA after 21 d at 25 °C. Aerial mycelium brown (oac639), pale brown (aoc661) in the centre of the colony and on inoculum block, velvety, reverse brown (oac733). Aerial hyphae smooth, thin-walled, sparsely branched, hyaline to subhyaline 1.5–2.0 µm, submerged hyphae sometimes pale brown 2.0–3.0 µm wide. Conidiophores reduced to a monoblastic conidiogenous cell. Conidiogenous cells 4.5–7.0 × 5.0–8.0 µm, usually with several subtending cells, integrated, hyaline to subhyaline with a single conidiogenous locus. Conidia 32–36(–39) × (14.5–)16–17.5(–18.5) µm (mean ± SD = 34.5 ± 2.2 × 17.0 ± 1.0 µm), ellipsoidal to obovoid, straight or slightly curved, smooth, dark brown, 3–5-septate, with darker bands obscuring the septa, non-constricted at the septa, basal cell subhyaline 3.0–4.5 µm wide tapering to 2.5–3.0 µm.

Specimens examined. France, Midi-Pyrénées, Ariège, Rimont, valley of the Peyrau brook, c. 400 m asl, 23 Feb. 2008, on submerged wood, *J. Fournier J.F. 08011* (PRM 933849, culture CBS 126792); ibid., 22 May 2009, submerged wood of *Alnus glutinosa*, *J. Fournier J.F. 09127* (PRM 933851, culture CBS 126576); ibid., 19 Apr. 2010, submerged wood of *Fraxinus excelsior*, *J. Fournier J.F. 10014*; ibid., Vernajoul, Vernajoul brook, Pont Fagé, c. 350 m asl, on unidentified submerged wood, 2 July 2007, *J. Fournier J.F. 07123* (PRM 933850).

Notes — *Savoryella limnetica* was originally collected on decaying wood submerged in freshwater in Taiwan and assigned to the genus based on its 3-septate ascospores and flattened apical apparatus (Chang et al. 1998). This species was recently repeatedly collected on submerged deciduous wood in southern France. Two living cultures were successfully obtained from isolated ascospores from fresh material.

Savoryella and *Ascotaiwania* are closely related, morphologically similar genera and their delimitation is based primarily on ascospore septation, morphology of the apical apparatus of the ascus and width of the paraphyses (see Discussion). The transfer of *S. limnetica* to *Ascotaiwania* is supported by molecular data and culture characters. The majority of *Ascotaiwania* species have 5–7-septate ascospores and only few are characterised by ascospores with three septa, i.e. *A. hughesii*, *A. palmicola* and *A. sawadae*. *Helicoön farinosum* and its sexual morph described as *A. hughesii* (Fallah et al. 1999), is a member of the *Pleurotheciales*. *Ascotaiwania palmicola* differs from *A. limnetica* by terrestrial habitat and affiliation to palm wood, asci with a conspicuous apical apparatus 4 × 5 µm and slender ascospores, 17.5–20 × 5.0–6.5 µm with polar mucilaginous appendages (Hyde 1995). *Ascotaiwania sawadae* can be compared to *A. limnetica* by ascomatal morphology, but differs by asci with a less flattened apical apparatus and larger and inequilateral ascospores 25–30 × 7.5–10 µm (Sivichai et al. 1998).

When observed in Congo red, the asci of *A. limnetica* revealed a conspicuous flattened apical annulus that stains deep red (Fig. 9k).

Fig. 9 *Ascotaiwania limnetica*. a, b. Ascomata, arrow indicates ascospores aggregated at the top of the neck; c, d. vertical sections of the ascomatal wall; e. asci with ascospores in Pelikan ink; f. asci with ascospores; g–i. paraphyses; j. apical annulus, arrow indicates the tip of ascal apex, when ascospore is released through the annulus; k. asci with apical annulus in Congo red (a–e, k. PRM 933850; f, g, i, j. PRM 933851; h. PRM 933849); c–f, j, k: DIC; g–i: PC. — Scale bars: a, b = 150 µm; c, j = 10 µm; d = 100 µm; c, e = 10 µm; e, f, k = 50 µm.

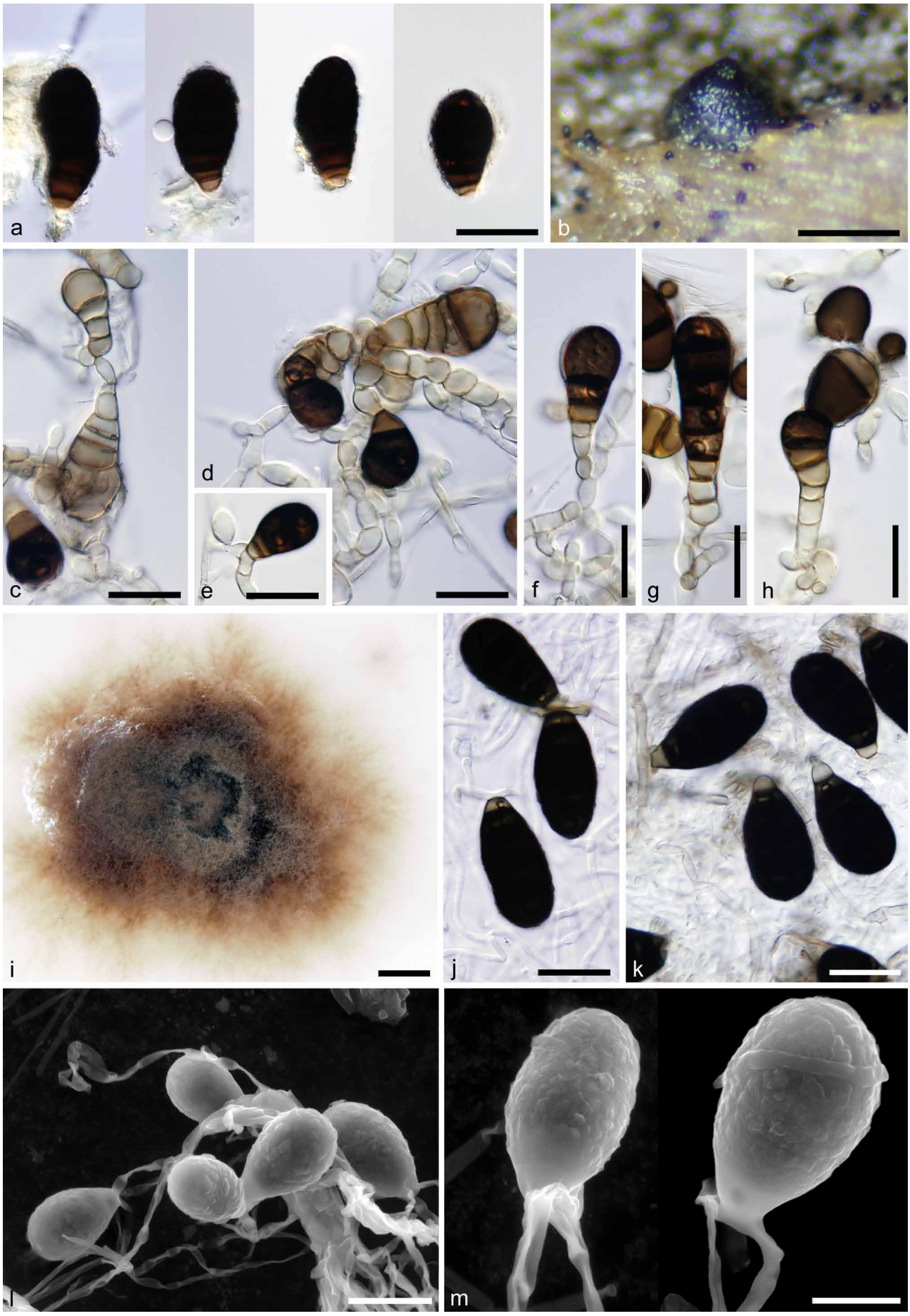

Fig. 10 Asexual morph of *Ascotaiwania limnetica*. a. Conidia in vivo; b. ascoma with macroconidia scattered on wood surface; c–h. conidia and conidiogenous cells on PDA; i. colony on PCA; j–m. conidia on PCA (a, c–h. CBS 126576; b. PRM 933850; j–m. CBS 126792; a, c–m. 21 d, 25 °C); a, c–h, j, k: DIC; l, m: ESEM. — Scale bars: a, c–h, j, k, l = 20 µm; b = 250 µm; i = 5 mm; m = 10 µm.

Pleurotheciella uniseptata (Matsush.) Seifert, *comb. nov.* — MycoBank MB813238; Fig. 11

Basionym. *Dactylaria uniseptata* Matsush., Microfungi of the Solomon Islands and Papua-New Guinea: 19. 1971.

Colonies in vivo effuse, visible as solitary to 4–5 caespitose dark brown conidiophores with dry, whitish to greyish conidia. *Sexual morph* not observed. *Conidiophores* mostly 100–150 µm tall, 4.5–5.0 µm wide at the base, tapering to 3.0–4.0 µm wide, macronematous, unbranched, straight or sinuous, dark brown at the base, with walls up to 1.0 µm thick near the base, thinner towards the apex, cylindrical, smooth-walled or slightly granular or roughened, usually with a terminal node of denticles, but rarely extending through the original node with a new extension of the conidiophore. *Conidiogenous cell* 15–32 µm long, 2.5–3.5 µm wide at the base, 2.0–3.0 µm wide below the fertile zone, integrated, terminal, cylindrical or tapering towards tip, pale brown to subhyaline near base, hyaline towards apex, smooth-walled or slightly granular, polyblastic, forming conidia sympodially on conspicuous denticles 1.0–2.0 µm long, about 0.5 µm wide, sometimes slightly broader at base, occluded, fertile zone at first just a few denticles, but can expand into a node-like zone that is cylindrical to ellipsoidal in outline, usually with compact clusters of 4–15 denticles but sometimes extended, rarely geniculate, up to 5.0–9.0 × 3.0 µm, wide, or be constricted down to 1.5 µm, up to 15 denticles seen. *Conidia* 12.5–16.5 × 2.0–4.0 µm (mean ± SD = 14.1 ± 0.9 × 2.9 ± 0.5 µm), fusoid or slightly clavate, straight, rounded at the apex, obtuse and tapering towards base, hyaline, 1-septate with an inconspicuous central septum, often with 1–2 large guttules in each cell, smooth-walled, remains of denticle sometimes attached to seceded conidium.

Culture characteristics — Colonies reaching 8–10 mm diam on CMA after 21 d at 25 °C. Aerial mycelium absent, colony and reverse inconspicuous to white. Submerged hyphae 1.5–2.0 µm wide, hyaline, smooth-walled. Sporulation appears first on the inoculum of the colony, and later is sparsely present on the older parts of the new growth. Conidiophores 50–85 × 3.5–4.0 µm wide, slightly swollen at base to about 4 µm, semi-macronematous, pale brown, subhyaline towards the apex, unbranched, smooth-walled. Conidiogenous cells and conidia similar to those produced in vivo.

Specimen examined. Canada, Ontario, Arnprior, MacNamara Trail, on decaying wet wood, 12 Oct. 2011, *K.A. Seifert & G. White K.A.S. 4459* (DAOM 673210, culture DAOMC 250294).

Notes — *Pleurotheciella uniseptata* is known only from its asexual morph. Its occurrence on water saturated decayed wood is consistent with the ecology of the other two species now classified in this genus. Its conidia are of a similar length

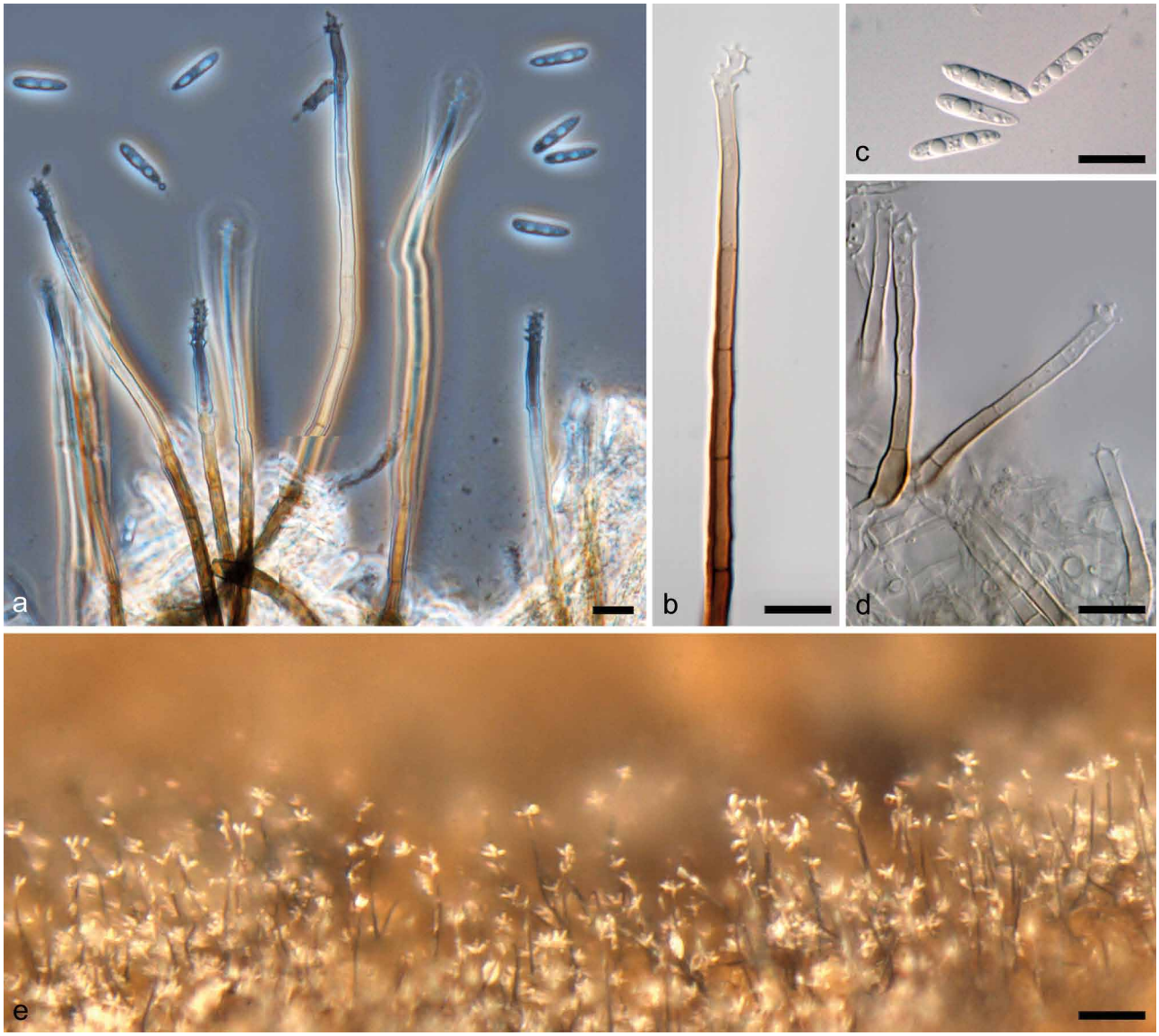

Fig. 11 *Pleurotheciella uniseptata*. a, b. Conidiophores in vivo; c. conidia in vivo; d. conidiophores on CMA; e. colony in vivo (a–c. DAOM 673210, d. DAOMC 250294, 21 d at 25 °C); a: PC; b–d. DIC. — Scale bars: a–d = 10 µm; e = 100 µm.

and septation to those of *P. rivularia*, but narrower and more uniformly fusiform rather than the often obovoidal shape of the latter species. The conidia of *P. centenaria* are also fusoid, but longer than the other two species and consistently 3-septate. Our specimen from Canada fits the description and illustration of *Dactylaria uniseptata* by Matsushima (1971) well, considering that the protologue was based on a culture grown on banana leaf agar. We note that De Hoog (1985) failed to obtain the holotype of *D. uniseptata* and we did not attempt to obtain it here. We have resisted the temptation to epitypify a Japanese species with a Canadian specimen and culture. Lectotypification with the drawings from the protologue would be a precondition to epitypification if the holotype is truly unavailable.

This is the first species of *Pleurotheciella* for which the conidiophores have been observed on the natural substrate. The protologue of the genus suggested that the conidiophores were dactylaria-like, but in *P. uniseptata* the conidiophores are macronematous and much more similar to those of *Pleurothecium* species. However, the conidiophores of *P. uniseptata* produced in culture lack dark basal cells and are rather similar to those produced by *P. rivularia* and *P. centenaria* in vitro (Fig. 11d). It seems possible that the conidiophores or all *Pleurotheciella* species would be macronematous in vivo. Morphologically, there are few if any characters to distinguish between the asexual morphs of *Pleurotheciella uniseptata* and some species classified in the hyphomycete genus *Pleurophragmium.* The two genera are clearly phylogenetically distinct, with *Pleurophragmium parvisporum*, the type of that genus, classified in the *Papulosaceae*, *Sordariomycetes* by Réblová (2009). A great morphological diversity of species are classified in *Pleurophragmium* (see key in D'Souza & Bhat 2012) and it is unlikely to be phylogenetically homogeneous.

DISCUSSION

The CPS (Conioscyphales/Pleurotheciales/Savoryellales) clade

The combined three- and six-gene phylogenetic analyses of the newly described genera *Melanotrigonum* and *Adelosphaeria* with members of the *Savoryellales* and other taxa related to *Conioscypha* and *Pleurothecium* revealed a robust monophylum in the *Hypocreomycetidae* (Fig. 1). It contains three nested monophyletic clades significantly supported by BI and ML methods, namely i) the *Savoryellales*; ii) a clade containing five species of *Conioscypha*; and iii) another clade that comprises several genera centred around *Pleurothecium*. The two latter clades represent distinct taxonomic groups at the ordinal systematic level and are introduced as the *Conioscyphales* and *Pleurotheciales* above. A sister relationship was revealed between the CPS (*Conioscyphales*/*Pleurotheciales*/*Savoryellales*) clade and a monophyletic strongly supported lineage of uncertain systematic position containing *Ascotaiwania persoonii*, *Bactrodesmiastrum* and the new genus *Plagiascoma*.

Members of the CPS and *Bactrodesmiastrum* clades share a few morphological features such as the absence of stromatic tissue or clypeus, similar anatomies of the ascomatal walls, thin-walled unitunicate asci with a distinct, non-amyloid apical annulus, paraphyses and symmetrical, transversely septate ascospores. The known asexual morphs are dematiaceous hyphomycetes with holoblastic conidiogenesis. Although the morphology of sexual morphs is more or less uniform and rather nondescript within each order, the observed variability in extension of conidiogenous cells and conidial morphology is characteristic of each order. In the CPS clade, pleomorphism is commonly observed, i.e. the ability of fungi to reproduce sexually and asexually and form independent spore-stages in the life cycle. All known life-histories discussed here were established experimentally, i.e. *Ascotaiwania* (Ranghoo & Hyde 1998, Sivichai et al. 1998, this study), *Canalisporium* (Sri-indrasutdhi et al. 2010), *Conioscypha* (Réblová & Seifert 2004, Zelski et al. 2014), *Helicoön farinosum* (Fallah et al. 1999), *Pleurothecium* (Fernández et al. 1999), *Pleurotheciella* (Réblová et al. 2012), *Sterigmatobotrys* (Réblová & Seifert 2011) and the new genera described in this study.

At the base of the monophyletic clade with the nested CPS and *Bactrodesmiastrum* clades, *Flammispora bioteca* is positioned on a separate branch (Fig. 1, 2). This species was collected on submerged leaves of the peat swamp palm *Licuala longecalycata* and is characterised by non-stromatic, black, immersed ascomata, clavate deliquescent asci without an apical annulus, subcylindrical to elongate-fusiform ascospores with a polar appendage and absence of paraphyses (Pinruan et al. 2004). Its asexual morph is unknown.

The Bactrodesmiastrum clade

Bactrodesmiastrum, based on *B. obscurum*, was described by Holubová-Jechová (1984) for dematiaceous hyphomycetes characterised by schizolytic conidial secession and a formation of conidiogenous cells related to the maturation of brown, septate conidia. When the conidium matures at the tip of the conidiogenous cell, a new monoblastic conidiogenous cell is borne near the previous one on repent basal hyphae, followed by formation of other conidiogenous cells in the same manner. No DNA sequences are available of the type species of *Bactrodesmiastrum*. The sexual state of *Bactrodesmiastrum* is unknown (Holubová-Jechová 1984, Hernández-Restrepo et al. 2013, 2015) and no conidia or conidiogenous cells were observed on the type and other herbarium material of its closest ascoma-producing sibling *A. persoonii* (Fallah et al. 1999). We prefer to avoid proposing a new genus for *A. persoonii* or its new combination in *Bactrodesmiastrum*, based on current DNA sequence data, until similarities in the life histories of these two taxa are proven or disproven experimentally.

Conioscyphales

The *Conioscyphales* comprises a single genus *Conioscypha* with 12 species from freshwater and terrestrial habitats. *Conioscyphascus* based on *C. varius* was originally proposed for fungi with *Conioscypha* asexual morphs by Réblová & Seifert (2004). *Conioscypha* exhibits a unique mode of conidiogenesis with multiple, conspicuous collarettes forming a multilamellar structure around the blastic conidiogenous locus of the intercalary conidiogenous cells (Shearer & Motta 1973). It is characterised by inconspicuous perithecial ascomata that are typically immersed to semi-immersed, hyaline, subhyaline to pale orange with a papilla or long upright neck, coriaceous, waxy ascomatal wall, cylindrical-clavate stipitate asci with a pronounced non-amyloid apical annulus, filiform paraphyses and fusiform to fusiform-navicular, septate, hyaline ascospores.

Nine species are known as apparently asexual (Von Höhnel 1904, Shearer 1973, Matsushima 1975, 1993, 1996, Kirk 1984, Udagawa & Toyazaki 1983, Chen & Tzean 2000, Crous et al. 2014) and only two have experimentally proven link between the sexual and asexual morphs, i.e. *C. varia* (Réblová & Seifert 2004) and *C. peruviana* (Zelski et al. 2014). A third sexually reproducing species, *Conioscyphascus gracilis*, was recently transferred to *Conioscypha* (Zelski et al. 2014).

Pleurotheciales

Six monophyletic clades that include species of eleven genera were nested in the clade that we describe above as the *Pleurotheciales* (Fig. 2). Members of the *Pleurotheciales* share dark,

papillate, glabrous or sparsely setose perithecia, upright or lying horizontally to the host, asci with a distinct non-amyloid apical annulus, filiform paraphyses that disintegrate partially at maturity and fusiform to ellipsoidal, septate, hyaline ascospores. Only ascospores of the sexual morph of *Helicoön farinosum* are versicolorous with brown middle cells and hyaline polar cells.

The variation in the details of holoblastic conidiogenesis correlates with clades recovered within the order. Rhexolytic conidial secession either on short denticles or rachis on sympodially proliferating conidiogenous cells occurs in *Helicoön farinosum*, *Phaeoisaria*, *Melanotrigonum*, *Pleurothecium*, *Pleurotheciella* and *Sterigmatobotrys*. This type of conidiogenesis is characteristic of Clades I, IV, V, VI and partially occurs in Clades II and III. Schizolytic conidial secession on a single locus on percurrently regenerating conidiogenous cells is characteristic of *Brachysporiella* sensu Ellis (Ellis 1959). The same type of secession but on monoblastic or solitary thallic conidiogenous cells is typical of *Phragmocephala*. Both latter genera are positioned in Clade II. In *Taeniolella*, a sister of *Sterigmatobotrys* in Clade III, the dark brown macroconidia are formed on monoblastic conidiogenous cells in dry, acropetal chains, while the apex of the conidium may develop into a fertile penicillate head with sympodially elongating conidiogenous cells similar to *Sterigmatobotrys* (see further under *Taeniolella*).

The nondescript morphology of sexual characters of members of the *Pleurotheciales* makes their correct placement in the *Sordariomycetes* difficult and significantly hinders their identification and even distinction from each other. Without cultivation and/or molecular data their correct systematic placement is challenging. The presence of conspicuous asexual morphs in intimate juxtaposition to ascomata on the natural substratum helps identification of several genera only. Some species of *Pleurotheciella* do not form conidiophores in vivo, only reduced, hyaline to subhyaline conidiophores in the axenic culture. Genera like *Adelosphaeria* and *Plagiascoma*, the latter is positioned in the *Bactrodesmiastrum* clade outside the *Pleurotheciales*, do not even form typical asexual morphs. They produce brown, ellipsoidal to globose, non-septate cells arising blastically from vegetative hyphae or other cells in the axenic culture.

Members of *Chaetosphaeria* (*Chaetosphaeriales*) are morphologically similar to *Pleurothecium*, *Pleurotheciella* and *Sterigmatobotrys* of the *Pleurotheciales*, especially species with *Menispora* asexual morphs, e.g. *C. ciliata*, *C. ovoidea*, *C. pulviscula* or *C. tortuosa* (Holubová-Jechová 1973, Réblová et al. 2006, Réblová & Seifert 2008). They possess brown, upright, papillate ascomata, fusiform, 3-septate, hyaline ascospores in cylindrical-clavate asci with distinct apical annulus and their phialidic asexual morphs are often absent on the host. Several freshwater genera such as *Aquaticola*, *Annulatascus* and *Annulusmagnus* (Ho et al. 1999, Hyde 1992a, Campbell & Shearer 2004) can be compared with *Adelosphaeria*, *Melanotrigonum*, *Pleurothecium*, *Pleurotheciella* and *Sterigmatobotrys* based on morphology of ascomata, asci, ascospores and paraphyses. Species of *Aquaticola* have miniature, coriaceous ascomata lying horizontally to the host, asci with inconspicuous non-amyloid apical annulus and septate or non-septate, hyaline ascospores (Ho et al. 1999, Tsui et al. 2003). *Annulatascus* and *Annulusmagnus* are easily distinguished by asci with a conspicuous, non-amyloid apical annulus and relatively large, septate, fusiform ascospores with a sheath or appendages in the former taxon, arranged 1-seriately or obliquely 1-seriately in the ascus. Their asexual morphs are unknown and when isolated from ascospores, sterile mycelium, or in the case of *Annulusmagnus triseptatus* abundant fertile ascomata (M. Réblová, pers. obs.) are formed in vitro. *Phomatospora*, whose taxonomic placement in the *Sordariomycetidae* is uncertain (Lumbsch & Huhndorf 2010), is another perithecial ascomycete that can be compared with genera of the *Pleurotheciales*. Its species are distinguishable by occurrence primarily on submerged herbaceous stems, rarely on wood in freshwater and marine habitats, immersed ascomata with thickened wall surrounding the ostiolum and hyaline, longitudinally striate non-septate ascospores enclosed in mucilaginous sheath or with bipolar appendages (e.g. Hyde 1988, 1992b, Fallah & Shearer 1998, Fournier & Lechat 2010). Only *Phomatospora berkeleyi*, the type species, and *P. arenaria* produce sporothrix-like asexual morphs with holoblastic denticulate conidiogenesis in axenic culture (Rappaz 1992).

Savoryellales

The *Savoryellales* was placed in the *Hypocreomycetidae* based on DNA sequences of six ribosomal and protein-coding loci (Boonyuen et al. 2011). It forms a well-supported lineage that includes saprobic, lignicolous species from terrestrial, marine, brackish and freshwater environments and water-cooling towers (e.g. Jones & Eaton 1969, Minoura & Muroi 1978, Hyde & Jones 1988, Chang et al. 1998, Ranghoo & Hyde 1998). Although Ranghoo (1998) introduced the family *Savoryellaceae* as a member of the *Halosphaeriales* in her PhD Thesis, a valid description was never published. The family was formally introduced recently as *Savoryellaceae* (Jaklitsch & Réblová 2015).

As now delimited, the *Savoryellales* comprises three genera, *Ascotaiwania*, *Canalisporium* and *Savoryella*. *Ascotaiwania* is polyphyletic in our analyses, although the genus appeared monophyletic in three previous studies (Campbell & Shearer 2004, Hernández-Restrepo et al. 2013, 2015). The latter results were inadvertently distorted by the inclusion of species of *Ascotaiwania* that only represent the CPS and *Bactrodesmiastrum* clades on a small scale. In our multilocus phylogenies (Fig. 1, 2) the core of *Ascotaiwania* in the *Savoryellales* is centred around the type species *A. lignicola* (Sivanesan & Chang 1992) and three other species. *Helicoön farinosum* (as *A. hughesii*, Fallah et al. 1999) is nested in the *Pleurotheciales*, while *A. persoonii* (Fallah et al. 1999) is in a strongly supported monophyletic clade with *Bactrodesmiastrum* and *Plagiascoma* basal to the CPS clade.

Genera of the *Savoryellales* share a similar morphology of dark, minute perithecial ascomata with elongated, dark or subhyaline neck, often oblique or lying horizontally on the host with the neck facing upwards, asci with a non-amyloid apical annulus, partly deliquescing paraphyses and ellipsoidal to fusiform, transversely septate, versicolorous ascospores. The generic delimitation of *Ascotaiwania* and *Savoryella* is narrow and for two decades was based predominantly on ascospore septation, and the morphologies of paraphyses and the ascal apex, i.e. size and shape of the apical annulus and presence or absence of apical thickening. The ascal apex of *Savoryella* was variously interpreted in different studies, by authors studying different species. In the protologue of the type species *S. lignicola*, the ascal apex was described as apically thickened with a pore (Jones & Eaton 1969). Sivanesan & Chang (1992) separated *Ascotaiwania* from *Savoryella* by an unthickened ascal apex with a distinct apical annulus and ascospores with more than three septa, while delimiting *Savoryella* for species lacking an apical ring and having 3-septate ascospores. Later, several other species were introduced to the genus, e.g. *S. aquatica* (Hyde 1993) and *S. limnetica* (Chang et al. 1998), characterised by a thickened ascal apex containing apical annulus with a pore. Read et al. (1993) based their distinction of *Ascotaiwania* and *Savoryella* on ultrastructural observations and used the term 'apical apparatus' to describe the complex structure of the ascal apex of these fungi. They characterised species of *Ascotaiwania* by ascal apical apparatus comprising an annulus with a protrusion (pendant) and plugged pore, whereas in species of

Savoryella the ascus apex was described as thickened with a pore, but lacking a pendant-like protrusion. Chang et al. (1998) also used characters of paraphyses to delimit the genera, i.e. narrow, filiform, early deliquescing filaments up to 2 µm wide in *Ascotaiwania* vs filaments consisting of broad, partially disintegrating cells up to 8 µm wide in *Savoryella*. Sri-indrasutdhi et al. (2010) introduced another morphologically similar genus, *Ascothailandia*, as the sexual state of *Canalisporium* and distinguished it from *Savoryella* by its conspicuous apical annulus. Recently, Boonyuen et al. (2011) modified the generic concept of *Savoryella* and accepted species with 3-septate ascospores and comparatively flattened apical ring.

The transfer of *S. limnetica* to *Ascotaiwania* proposed above is based on molecular evidence and an experimentally proven life history. The micromorphological characters of *S. limnetica*, i.e. flattened apical annulus, cylindrical, septate, disintegrating paraphyses 4.0–9.5 µm wide and 3-septate ascospores, do not fit well with the long-held morphology-based concepts of either genus. Stable delimitation of *Ascotaiwania* and *Savoryella* will require re-evaluation of all sexual and asexual morphological characters and concentrated sampling filtered through the optics of multigene phylogenetics.

Asexual morphs associated with the *Savoryellales* were described for *Canalisporium grenadoideum* (as *Ascothailandia grenadoidea* sexual morph, Sri-indrasutdhi et al. 2010) and three species of *Ascotaiwania* were linked with *Brachysporiella*-like dematiaceous hyphomycetes, *A. mitriformis* and *A. sawadae* (as *Monotosporella*, Ranghoo & Hyde 1998, Sivichai et al. 1998) and *A. limnetica* (this study). With some reservations *Acarocybiopsis* was suggested as another suitable genus for asexual morphs of *Ascotaiwania* (Réblová & Seifert 2004). They are characterised by semi-macronematous conidiophores often reduced to conidiogenous cells with a single locus and brown macroconidia. Conidia are either cheiroid, dictyoseptate with pores between cells and conidiogenous cells arise from sporodochia in *Canalisporium*. The asexual morphs of *Ascotaiwania* produce aleuroconidium-like, transversely septate macroconidia with darker bands around septa and a few rhizoids arising from subtending cells beneath the monoblastic conidiogenous cell.

In our analysis, the dematiaceous hyphomycete *Triadelphia uniseptata* nested within the monophyletic *Ascotaiwania* clade as a sister to *A. mitriformis*. *Triadelphia*, based on *T. heterospora*, was introduced for fungi from freshwater and brackish environments and characterised by conidiophores reduced to subglobose, subhyaline to dematiaceous conidiogenous cells, schizolytic conidial secession and conidia produced blastically from a single locus (Shearer & Crane 1971). Conidia of species of *Triadelphia* are brown or versicolorous often with one or two polar cells paler than the middle ones, septate, usually with darker bands obscuring several septa. Although *T. heterospora* was described with two morphologically distinct types of conidia, currently eight types are known (Constantinescu & Samson 1982), but these other asexual morphs have never been formally named. The gregarious to caespitose, globose to subglobose to ampulliform conidiogenous cells borne directly on vegetative hyphae are the hallmark of *Triadelphia*. They are also remarkably similar to cylindrical to lageniform aggregated conidiogenous cells of *Bactrodesmiastrum*. The morphology of larger, broad to ellipsoidal, brown, septate conidia of *T. heterospora* and their conidiogenesis illustrated in the protologue (Shearer & Crane 1971: f. 9c, f, g) resembles conidia and conidiogenesis of *A. limnetica* (Fig. 10c–h) that we observed in axenic culture on PDA. The ampulliform conidiogenous cells are absent and conidia arise directly from mycelium or a small monoblastic conidiogenous cell with several supporting cells. *Triadelphia* comprises 17 species, but phylogenetic placement of its type species is unknown. The only available ITS and nuc28S rDNA sequences in the GenBank belong to *T. pulvinata* and they show affinity with members of the *Microascales* (Edathodu et al. 2013). The position of *T. uniseptata* in the *Savoryellales* shown here demonstrates that the present concept of *Triadelphia* is polyphyletic, and that the application of this generic name, and the redisposition of its species, requires much improved sampling.

The ascoma centrum in the Hypocreomycetidae

In members of the *Hypocreomycetidae*, the centrum consists of several types of interthecial filaments. The other two subclasses of the *Sordariomycetes*, *Sordariomycetidae* and *Xylariomycetidae* include either only paraphyses and periphyses in the ostiolum or paraphyses are lacking in some groups. Apical, lateral and centripetal paraphyses occur in members of the *Hypocreales* (e.g. Samuels 1973, Mhasker & Rao 1976, Jaklitsch 2009, Jaklitsch & Voglmayr 2014). Filaments consisting of wide, inflated, early disintegrating cells interspersed among the asci occur in the *Bertiaceae* and *Chaetosphaerellaceae* of the *Coronophorales* (Réblová 1999, Huhndorf et al. 2004). A hamathecium consisting of catenophyses, i.e. pseudoparenchymatous cells that break up to form chains of large, thin-walled, early dissolving cells interspersed among asci or the pseudoparenchyma may completely disappear in mature ascomata, is typical of members of the *Halosphaeriaceae* of the *Microascales* (Spatafora et al. 1998, Sakayaroj et al. 2011). A pseudoparenchymatous centrum occurs in the *Melanosporales* (Goh & Hanlin 1994, Samuels & Blackwell 2001). A reticulate network of filiform, branching and anastomosing filaments attached at the top and bottom of the cavity uniquely characterises the *Reticulascaceae* of the *Glomerellales* while in members of other two families, *Australiascaceae* and *Glomerellaceae*, sparse septate filaments occur (Samuels & Müller 1978, Sivanesan & Alcorn 2002, Réblová et al. 2011). Numerous unbranched filaments attached to the top and bottom of the ascomatal cavity occur in members of the *Torpedosporales* except for *Marinokulati chaetosa*, where the filaments are apically free (Jones et al. 2014, 2015). In some groups, a hamathecium is lacking, e.g. in the *Scortechiniaceae* and *Nitschkia* of the *Coronophorales* (Huhndorf et al. 2004) or in some members with cleistothecial ascomata of the *Microascales*. The presence of periphyses in genera of the *Coronophorales* is variable and depends on how the apex of ascomata is formed, whether it contains a Quellkorper (Nannfeldt 1975) and whether it is ostiolate or non-ostiolate (Huhndorf et al. 2004).

Members of the CPS clade represent the only three orders in the *Hypocreomycetidae* defined by the presence of apically free paraphyses in the ascomatal centrum. These sterile, filiform, septate filaments emerge from the subhymenium either interspersed among the asci, e.g. in *Ascotaiwania*, *Conioscypha*, *Melanotrigonum*, *Pleurotheciella*, *Savoryella* and *Sterigmatobotrys*, or form separate tuft-like structures, e.g. *Pleurothecium*. Paraphyses are usually longer than the asci and may disintegrate at maturity; for example in some species of *Savoryella* or *Ascotaiwania* they disintegrate rapidly and are difficult to observe.

Recently, the new order *Pisorisporiales* was introduced for predominantly aquatic fungi, which morphologically mimic members of the *Annulatascaceae* in ascoma and ascospore characters, and the *Amphisphaeriaceae* in a conspicuous, amyloid apical annulus and non-stromatic ascomata (Réblová et al. 2015). The order is isolated on a separate branch as a sister to the *Hypocreomycetidae* but without statistical support. The *Pisorisporiales* represents another group related to this subclass and characterised by filiform, septate, partly disintegrating paraphyses interspersed among asci, but densely branching

and anastomosing above their apices in the ascoma cavity. Although the two species of *Pisorisporium*, *P. cymbiforme* and *P. glaucum*, were described from wood submerged in freshwater, several recent collections of *P. cymbiforme* were made in terrestrial habitats in the Czech Republic, suggesting that the fungus might be widespread. The new nuc18S rDNA and *rpb2* sequences of terrestrial strains are listed in Table 1.

Pleurotheciales: The polyphyletic genera Helicoön, Phaeoisaria, Pleurothecium and Taeniolella

Helicoön

Several genera now classified in the *Pleurotheciales* appear polyphyletic based on molecular phylogenies. *Helicoön farinosum*, which has hyaline, coiled, septate conidia formed holoblastically on short denticles, is the only representative with helicosporous conidia in the *Pleurotheciales* and in the whole CPS clade. It was experimentally linked with its sexual state *Ascotaiwania hughesii* (Fallah et al. 1999) and in our phylogeny it is nested in Clade I as a sister to *Brachysporiella setosa*. We confirmed the phylogenetic position of *H. farinosum* (DAOM 241947) with collections, cultures and sequences made in Canada (Réblová et al. 2012). Although the correct species epithet for this holomorphic fungus would be '*farinosum*', whether the generic assignment should be *Helicoön* is unclear pending confirmation of the phylogenetic placement and classification of the type species *H. sessile*. The genus *Helicoön* sensu Goos et al. (1986) was shown to be polyphyletic with DNA sequences of two nuc rDNA loci by Tsui & Berbee (2006), but *H. sessile* was not included. The only available ITS rDNA sequence of this species (U72605, Pfister et al. 1997) shows 99 % similarity with the ITS sequence of *Sarocladium kiliense* of the *Hypocreales* (KP132606, Irinyi et al. 2015), an unlikely relationship suggestive of a mislabelled or contaminated culture. Other species of *Helicoön* were placed in the *Pleosporales*, *Tubeufiales* and *Venturiales* of the *Dothideomycetes* (Tsui & Berbee 2006).

Phaeoisaria

Phaeoisaria is a dematiaceous hyphomycete genus with species producing indeterminate synnemata with septate or non-septate ellipsoidal, obovoidal, fusiform-cylindrical or falcate conidia formed on a sympodially extending rachis, occurring on decaying wood, plant debris or soil sediments (e.g. Sutton 1973, Deighton 1974, Castañeda et al. 2002, Seifert et al. 2011, Mel'nik 2012, Cheng et al. 2014, Crous et al. 2015). The genus was proposed by Von Höhnel (1909) with the only species *Ph. bambusae*. It was originally described as an asexual state of *Neopeckia bambusae*, inferred from the intimate juxtaposition of synnemata and ascomata. Based on his revision of type and herbarium material, Deighton (1974) considered *Ph. bambusae* a synonym of *Ph. clematidis*. He compiled an extensive synonymy of the latter species, distinguishing it from morphologically similar *Ph. magnifica*, which has broader conidia. Deighton's concept of *Ph. clematidis* seems to represent a complex of several phylogenetic species.

Phaeoisaria now includes 19 species, five of which were analysed in our study. The sampled species form a strongly supported monophyletic clade in the *Pleurotheciales* that includes species with synnemata and conidiophores formed in fascicles. In our analysis, *P. clematidis* is represented by two strains isolated from bark and senescent flower heads of *Protea*.

Phaeoisaria curvata is the only described mononematous species; it was isolated from leaves of *Parinari capense* and its wild type is unknown (De Hoog & Papendorf 1976). The nuc28S sequence of the ex-type strain CBS 153.72 (sequence in the CBS strain database) shows affinity with taxa of the *Sordariomycetidae*.

Although the majority of *Phaeoisaria* species are asexual, including all species in our analyses, several perithecial ascomycetes have been linked with *Phaeoisaria*-like asexual states. In the *Sordariomycetes*, *Lentomitella* and *Rhamphoria* produce sparsely branched, mononematous conidiophores with aseptate conidia borne on a short rachis in culture (Müller & Samuels 1982, Réblová 2006). Two genera of the *Diatrypaceae*, *Eutypella* (as *Peroneutypella*, Deighton 1974) and *Pareutypella* (Ju & Rogers 1995), were linked with *Phaeoisaria*-like synnematous asexual states. For these connections, the morphologically similar synnematous genus *Harpographium*, typified by the asexual state of *Eutypella scoparia*, should be considered.

Although *Phaeoisaria* is usually considered non-pathogenic to human beings, two cases of inflammation of the eye's cornea called keratitis were attributed to *Phaeoisaria* sp. (Chew et al. 2010) and *Ph. clematidis* (Guarro et al. 2000). The former pathogenic strain *Phaeoisaria* sp. was included in our analysis and is a sister taxon to two saprobic strains of *Ph. clematidis* with strong branch support.

Pleurothecium

Pleurothecium includes fungi with dematiaceous, macronematous, unbranched conidiophores and holoblastic, hyaline to subhyaline, sympodially extending conidiogenous cells with a conspicuous rachis of denticles and hyaline, septate conidia. The sexual morph is known only for the very common *P. recurvatum*, the type species (as *Carpoligna pleurothecii*, Fernández et al. 1999). Of the eight species assigned to the genus, only three have been studied with DNA sequence data. *Pleurothecium recurvatum* and *P. semifecundum* represent the core of the genus and form a strongly supported monophyletic clade in the *Pleurotheciales*, while *P. obovoideum* is nested within another clade and sister to *Brachysporiella setosa*. The asexual morph of *P. semifecundum* lacks macronematous conidiophores in culture and sporulates sparsely; whether its wild type would better match the distinctive conidiogenous apparatus of *P. recurvatum* remains unknown.

Pleurothecium obovoideum, originally described in *Ramichloridium*, is known only from culture and it was isolated from a dead leaf of *Pasania edulis* (Matsushima 1975). It is characterised by reduced, septate conidiophores, sympodially proliferating conidiogenous cells with a short rachis giving rise to 2–3 denticles and ellipsoidal to obovate, pale brown, non-septate conidia formed singly or in short chains. The morphology is rather nondescript and we prefer to avoid introducing a new genus for this species, until either the wild type is collected or relationship with other morphologically similar taxa is revealed. Based on its morphology, *P. obovoideum* is similar to *Rhinocladiella mackenziei* (*Chaetothyriales*), a pathogen causing severe cerebral phaeohyphomycosis in humans (Sutton et al. 1998). It also resembles members of *Subramaniomyces* (*Xylariales*, Crous et al. 2007) and *Pterygosporopsis* (Kirk 1983), whose phylogenetic placement is unknown.

Taeniolella

Taeniolella exilis, the type of the genus, is commonly found on decaying wood and bark of *Betula* (Hughes 1958, Ellis 1971). During a revision of the type material of *T. exilis* by Jones et al. (2002), a penicillately branched conidiophore was observed as an extension of the terminal macroconidia. A similar penicillate conidiophore was observed in two other species, *T. longissima* and *T. rudis* (Hughes 1980, Jones et al. 2002). The latter taxon was shown to be closely related to *Sterigmatobotrys macrocarpa* of the *Pleurotheciales*, whose asexual state is characterised by similar penicillate conidiophores with several series of branches and metulae terminating macronematous conidiophores (Réblová & Seifert 2011). However, brown, sep-

tate *Taeniolella* macroconidia were not observed in axenic cultures obtained from conidia or ascospores of *S. macrocarpa*.

Several other species of *Taeniolella* are positioned in distantly related groups. Taeniolella-like conidia were obtained in a culture derived from ascospores of the freshwater ascomycete *Chaetorostrum quincemilensis*, tentatively placed in the *Annulatascaceae* (Zelski et al. 2011). Shearer et al. (2009) showed the strain of *T. alta* (CBS 488.80) nested in a clade with *Diaporthe angelicae* and *Phomopsis* sp., and the ex-type strain of *T. typhoides* (CCM F-10198) in the *Lingdomycetaceae* of the *Pleosporales*. A taeniolella-like fungus was isolated from the rhizosphere soil of strawberry, producing a phialophora-like asexual state on vegetative hyphae or directly on macroconidia in vitro, and described as *T. phialosperma* (Watanabe 1989, 1992). The ITS sequences of two non ex-type strains of *T. phialosperma* deposited in GenBank (KF703925, GU966521, unpubl.) indicate a relationship with members of the *Sordariales*. Finally, the sexual morph of a *Taeniolella* sp. with ascolocular ascoma development was classified as *Mytilinidion gemmigenum* (*Mytilinidiales*, Minter & Holubová-Jechová 1981). These inconsistencies suggest that the generic concept of *Taeniolella* requires increased taxon sampling and investigation with molecular methods.

A case of human subcutaneous phaeohyphomycosis caused by *T. exilis* species was reported by Alonso et al. (1993). While the pathogenic strain of *T. exilis* isolated from a human skin lesion (strain IP 2199.93) was shown closely related to *Ochrocladosporium elatum* (CBS 146.33) of the *Pleosporales* by Masclaux et al. (1995), the placement of the wood-inhabiting strain of *T. exilis* resembling *T. rudis* (*Pleurotheciales*) has yet to be confirmed with molecular sequence data.

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