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Monilochaetes and allied genera of the *Glomerellales*, and a reconsideration of families in the *Microascales*

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Abstract: We examined the phylogenetic relationships of two species that mimic *Chaetosphaeria* in teleomorph and anamorph morphologies, *Chaetosphaeria tulasneorum* with a *Cylindrotrichum* anamorph and *Australiasca queenslandica* with a *Dischloridium* anamorph. Four data sets were analysed: a) the internal transcribed spacer region including ITS1, 5.8S rDNA and ITS2 (ITS), b) nc28S (ncLSU) rDNA, c) nc18S (ncSSU) rDNA, and d) a combined data set of ncLSU-ncSSU-RPB2 (ribosomal polymerase B2). The traditional placement of *Ch. tulasneorum* in the *Microascales* based on ncLSU sequences is unsupported and *Australiasca* does not belong to the *Chaetosphaeriaceae*. Both holomorph species are nested within the *Glomerellales.* A new genus, *Reticulascus*, is introduced for *Ch. tulasneorum* with associated *Cylindrotrichum* anamorph; another species of *Reticulascus* and its anamorph in *Cylindrotrichum* are described as new. The taxonomic structure of the *Glomerellales* is clarified and the name is validly published. As delimited here, it includes three families, the *Glomerellaceae* and the newly described *Australiascaceae* and *Reticulascaceae*. Based on ITS and ncLSU rDNA sequence analyses, we confirm the synonymy of the anamorph genera *Dischloridium* with *Monilochaetes*. Consequently *Dischloridium laeënse*, type species of the genus, and three related species are transferred to the older genus *Monilochaetes*. The teleomorph of *D. laeënse* is described in *Australiasca* as a new species. The *Plectosphaerellaceae,* to which the anamorph genus *Stachylidium* is added, is basal to the *Glomerellales* in the three-gene phylogeny. *Stilbella annulata* also belongs to this family and is newly combined in *Acrostalagmus.* Phylogenetic analyses based on ncLSU, ncSSU, and combined ncLSU-ncSSU-RPB2 sequences clarify family relationships within the *Microascales*. The family *Ceratocystidaceae* is validated as a strongly supported monophyletic group consisting of *Ceratocystis, Cornuvesica*, *Thielaviopsis,* and the type species of *Ambrosiella*. The new family *Gondwanamycetaceae*, a strongly supported sister clade to the *Ceratocystidaceae*, is introduced for the teleomorph genus *Gondwanamyces* and its *Custingophora* anamorphs. Four families are accepted in the *Microascales*, namely the *Ceratocystidaceae*, *Gondwanamycetaceae*, *Halosphaeriaceae,* and *Microascaceae*. Because of a suggested affinity of a *Faurelina indica* isolate to the *Microascales*, the phylogenetic position of the *Chadefaudiellaceae* is reevaluated. Based on the results from a separate ncLSU analysis of the *Dothideomycetes*, *Faurelina* is excluded from the *Microascales* and placed in the *Pleosporales*.

Key words: *Australiasca*, *Australiascaceae*, *Ceratocystidaceae, Cylindrotrichum*, *Dischloridium*, *Gondwanamycetaceae*, *Reticulascus*, *Reticulascaceae*, phylogeny, *Plectosphaerellaceae*.

Taxonomic novelties: New order: *Glomerellales* Chadef. ex Réblová, W. Gams & Seifert, ord. nov. **New families**: *Australiascaceae* Réblová & W. Gams, fam. nov., *Ceratocystidaceae* Locq. ex Réblová, W. Gams & Seifert, fam. nov., *Gondwanamycetaceae* Réblová, W. Gams & Seifert, fam. nov., *Reticulascaceae* Réblová & W. Gams, fam. nov. **New genera**: *Reticulascus* Réblová & W. Gams, gen. nov., **New species**: *Australiasca laeënsis* Réblová & W. Gams, sp. nov., *Cylindrotrichum setosum* Seifert, sp. nov., *Reticulascus clavatus* Réblová & Fournier, sp. nov. **New combinations:** *Acrostalagmus annulatus* (Berk. & Broome) Seifert, comb. nov., *Hyalocylindrophora rosea* (Petch) Réblová & W. Gams, comb. nov., *Monilochaetes basicurvata* (Matsush.) Réblová & Seifert, comb. nov., *Monilochaetes camelliae* (Alcorn & Sivan.) Réblová, W. Gams & Seifert, comb. nov., *Monilochaetes laeënsis* (Matsush.) Réblová, W. Gams & Seifert, comb. nov., *Monilochaetes regenerans* (Bhat & W.B. Kendr.) Réblová & Seifert, comb. nov., *Reticulascus tulasneorum* (Réblová & W. Gams) Réblová & W. Gams, comb. nov.

INTRODUCTION

The genus *Chaetosphaeria* (*Chaetosphaeriaceae*, *Chaetosphaeriales*) is a cosmopolitan genus of nonstromatic, perithecial ascomycetes (Réblová 2000, Réblová & Winka 2000, Fernández *et al.* 2006). It is characterised by dark, opaque, usually subglobose to conical perithecia. The asci are unitunicate, short-stipitate with a distinct, inamyloid apical ring. The ascospores are hyaline, rarely bicolorous, 1- to several-septate, ellipsoidal to fusoid, sometimes cylindrical, and rarely fragment into part-spores. Periphyses and paraphyses are persistent, cylindrical, seldom branching, septate, and longer than the asci. The genus has been linked to 13 anamorph genera of phialidic dematiaceous hyphomycetes (Réblová 2000, 2004).

Several distantly related fungi mimic *Chaetosphaeria* in the morphology of perithecia, asci, ascospores, and phialidic, dematiaceous, hyphomycetous anamorphs. Recognising these species as distinct from *Chaetosphaeria* is difficult based purely on morphology. In most cases, their systematic placement can be ascertained by DNA sequence data, which suggest that the morphological similarities are a result of convergent evolution.

Chaetosphaeria tulasneorum was experimentally linked to its anamorph *Cylindrotrichum oligospermum* by Réblová & Gams (1999). Based on ncLSU rDNA sequence data, *Ch. tulasneorum* was separated from the core species of *Chaetosphaeria* in the *Chaetosphaeriaceae* (Réblová & Winka 2000) and tentatively placed in the *Microascales,* along with *Cylindrotrichum hennebertii*, a non-setose counterpart of *C. oligospermum*. *Chaetosphaeria tulasneorum* colonises decaying wood and forms minute, black perithecia containing unitunicate, short-stipitate asci with an inamyloid apical ring, 2–4-celled ellipsoidal to ellipsoidal-fusoid ascospores, and branching and anastomosing filiform paraphyses forming a "network" within the centrum. The reticulate paraphyses and the 1-septate, cylindrical conidia of the *Cylindrotrichum* anamorph are the only deviating morphological characters between *Ch. tulasneorum* and other core *Chaetosphaeria* species.

The phialidic, dematiaceous hyphomycete *Dischloridium laeënse*, described originally from dead leaves of *Musa paradisiaca* in Papua New Guinea (Matsushima 1971), is common on dead palm spathes in Australia. In some respects it is similar to species of *Chloridium,* a well-established anamorph genus associated with *Chaetosphaeria,* but the microscopic structures are much larger. On material from Australia and England, perithecia of *Australiasca* (Sivanesan & Alcorn 2002) were associated with fertile conidiophores of *D. laeënse*. This teleomorph was first reported from England by Kirk (1986) on stems of *Dicksonia antarctica*, but never described or illustrated. Sivanesan & Alcorn (2002) erected the monotypic ascomycete genus *Australiasca* including the type species, *A. queenslandica,* and named its anamorph *Dischloridium camelliae.* The fungus was isolated from leaves, stems, and branches of *Camellia sinensis* and the connection between the morphs was proven experimentally *in vitro*. They distinguished *D. camelliae* from *D. laeënse* by longer conidia and larger conidiophores. Sivanesan & Alcorn (2002) compared *Australiasca* with genera in the morphologically similar families *Chaetosphaeriaceae* and *Lasiosphaeriaceae*. At that time, no molecular data were available to confirm placement in either family.

The *Australiasca* teleomorph of *D. laeënse* is morphologically similar to species of *Chaetosphaeria* in perithecial and anamorph characters. *Dischloridium laeënse,* the type of its genus, produces effuse colonies of single to fasciculate, macronematous conidiophores with a stromatic base. The conidiophores are dark brown but paler towards the apex. The phialidic conidiogenous cells are terminally integrated bearing an indistinct collarette producing basipetal, broadly ellipsoidal, hyaline, nonseptate conidia with a slightly obtuse base produced in slime. Several of the 15 species described in *Dischloridium* are remarkably similar to *Monilochaetes* (Halsted 1890), recently revised and delimited from *Exochalara* and *Dischloridium* by Rong & Gams (2000) based on detailed morphology and cultivation studies.

To assess the higher level phylogenetic relationships of *Ch. tulasneorum* and related species of *Cylindrotrichum, Australiasca*, *Dischloridium,* and *Monilochaetes*, we analysed members from 19 orders or families of perithecial ascomycetes. We used DNA sequence data from the nuclear large (ncLSU rDNA) and small (ncSSU rDNA) subunits in independent analyses and combined these with the second largest subunit of RNA polymerase (RPB2) for a multigene analysis.

Based on the phylogenies presented here, several new and strongly supported families and orders are proposed. The order *Glomerellales* is phylogenetically well-defined and validated to include three families, the *Glomerellaceae* and the newly described *Australiascaceae* and *Reticulascaceae*. The internal transcribed spacer region (ITS including ITS1, 5.8S and ITS2) was used to further analyse the phylogenetic relationships among species of *Dischloridium* and *Monilochaetes*. Within the *Microascales,* we accept four families, *i.e. Ceratocystidaceae*, which is validated here, and the newly described *Gondwanamycetaceae*, *Halosphaeriaceae,* and *Microascaceae*. We discuss the family and order affinities of *Faurelina* attributed to the *Chadefaudiellaceae* of the *Microascales* by von Arx (1978) and by Tang *et al.* (2007). We examined authentic material, specifically the *in vitro* ex-type and another strain of *F. indica,* and analysed ITS and ncLSU sequence data. Based on results from a ncLSU analysis of the *Dothideomycetes*, *Faurelina* (*Chadefaudiellaceae*) is excluded from the *Microascales* and placed in the *Pleosporales* (*Dothideomycetes*).

MATERIAL AND METHODS

Morphological observations

All herbarium specimens examined and cultures studied are listed under each treated species. Dried specimens were rehydrated in water; material was examined with an Olympus SZX12 dissecting microscope and centrum material including asci, ascospores, and paraphyses was mounted in Melzer's reagent or 90 % lactic acid. Hand sections of the perithecial wall were studied. When present, conidiophores, conidiogenous cells, and conidia were examined in water, Melzer's reagent, or 90 % lactic acid. All measurements were made in Melzer's reagent. Means ± standard errors (S.E.) based on 25 measurements are given for ascospore, ascal, and conidial dimensions. Images were captured using differential interference (DIC) or phase contrast (PC) microscopy using an Olympus DP70 Camera operated by Imaging Software Cell* on an Olympus BX51 compound microscope or an Evolution MP digital camera operated by ImagePro v. 6.0 on an Olympus BX50 compound microscope. Conidia and conidiogenous cells of *Australiasca queenslandica* were photographed in the living state using an FEI Quanta 200 Environmental Scanning Electron Microscope (ESEM). A *ca.* 2 × 2 mm cube of agar with mycelium was observed at 20kV 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 (JT Manufacturing, Hudson, NH, USA). Images were processed with Adobe Photoshop CS4 Extended or Adobe Photoshop CS2.

Single-ascospore isolates were obtained from fresh material with the aid of a single-spore isolator (Meopta, Prague, Czech Republic). Isolates were grown on potato carrot agar (PCA), oatmeal agar (OA), and 2 % malt extract agar (MEA) (Gams *et al.* 1998). Colonies were examined after 7, 21, and 30 d at 25 °C in the dark and under near-UV light source (12 h light: 12 h dark). Two strains of *Faurelina indica* were grown on Blakeslee's malt extract agar (Gams *et al.* 1998) and OA and incubated under ambient room conditions for two mo to induce the arthroconidial anamorph. Cultures are maintained at BRIP (Plant Pathology Herbarium, Queensland, Australia), CBS (CBS Fungal Biodiversity Center, Utrecht, the Netherlands), DAOM (Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, Canada), and the Institute of Botany, Academy of Sciences, Průhonice, Czech Republic.

DNA extraction, amplification and sequencing

DNA was isolated with an UltraClean Microbial DNA Kit (MoBio Laboratories, Inc., Canada) using mycelium removed from PCA or MEA cultures following the manufacturer's protocol for filamentous fungi. All PCR experiments were carried out using a PTC-200 thermal cycler (MJ Research Inc., Watertown, MA, USA). PCR reactions containing 2–4 mM $MgSO_4$ were performed using Platinum *Taq* DNA polymerase High Fidelity (Invitrogen, Carlsbad, CA, USA) in 25 mL volume reactions. PCR conditions were as follows: for ncSSU 2 min at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 55 °C and 150–300 s at 68 °C; for ITS and ncLSU 2 min at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 55–60 °C, and 165–270 s at 68 °C; and for RPB2 2 min at 94 °C; 35 cycles of 30 s at 94 °C, 30 s at 55–61

°C, and 90–180 s at 68 °C; all amplifications were concluded by incubation for 10 min at 68 °C. Amplicons were purified using the UltraClean PCR Clean-up Kit (MoBio Laboratories, Inc., Canada) following the manufacturer's directions. All nucleotide sequences were obtained by the dideoxy chain-terminating method using automated DNA sequencers ABI PRISM 3100 or ABI PRISM 3130xl (Applied Biosystems, Foster City, CA, USA). For PCR reactions, the following primers were used: ncSSU, NSSU131-NS24 (Kauff & Lutzoni 2002, White *et al*., 1990); ncLSU, ITS5/NS5/LR0R-LR8 (White *et al*., 1990, Vilgalys unpublished: www.botany.duke.edu / fungi/mycolab); ITS NS5/ITS5-ITS4 (White *et al*., 1990); RPB2 fRPB2-5F-fRPB2-7cR (Liu *et al*., 1999). For sequencing reactions, the following primers were used: ncSSU, NSSU131, SR11R, SR7, SR7R, NSSU897R, NSSU1088, NSSU1088R, NS6, NS24 (White *et al*., 1990, Gargas & Taylor 1992, Spatafora *et al*., 1995, Kauff & Lutzoni 2002, Vilgalys unpublished: www.botany.duke.edu/fungi/mycolab); ncLSU LR0R, LR3R, LR6, LR7, LR16, LR5 (Vilgalys & Hester 1990, Rehner & Samuels 1994, Vilgalys & Sun 1994), JS7 and JS8 (Landvik 1996); ITS, ITS5 and ITS4 (White *et al.* 1990); and RPB2 fRPB2-5F, fRPB2-7cR, RPB2-980R and RPB2-1014F (Reeb *et al*. 2004). Sequences were edited using Sequencher v. 4.9 software (Gene Codes Corp., Ann Arbor, MI, USA).

Phylogenetic analyses

Accession numbers and isolate information for new ITS, ncLSU, ncSSU rDNA and RPB2 sequences are listed in Table 1. The new sequences were aligned with data retrieved from GenBank, mostly from studies published by Wingfield *et al*. (1999), Réblová & Winka (2000), Spatafora *et al*. (2006), and Zhang *et al*. (2006).

All sequences were manually aligned in BioEdit v. 7.0.9.0 (Hall 1999). Predicted models of the secondary structure of the ncLSU and ncSSU molecules of *Saccharomyces cerevisiae* (Gutell 1993, Gutell *et al.* 1993) were used to improve decisions on homologous characters. To assist with decisions on homologous characters in the ITS alignment, we used the predicted models of the secondary structure designed for species of *Chaetosphaeria* (Réblová & Winka 2000). They included a model for the whole ITS2 region and a model for long duplex structures located in the middle of the ITS1 region. The long duplex represents the most variable part of the ITS1 alignment because of its variable lengths and irregular occurrence of internal asymmetrical loops.

Phylogenetic relationships were examined using ncLSU, ncSSU, ITS rDNA, and RPB2 sequences of species from 19 orders or families of the *Sordariomycetes*. For all analyses rooting was accomplished by the outgroup method (Nixon & Carpenter 1993). Two outgroup taxa, *Leotia lubrica* and *Microglossum rufum* (*Leotiaceae, Helotiales*, *Leotiomycetes*), were used in the ncLSU, ncSSU, and the three-gene (ncLSU-ncSSU-RPB2) analyses of the *Sordariomycetes*; two outgroup taxa, *Vanderwaltozyma polyspora* and *Saccharomyces cerevisiae* (*Saccharomycetaceae, Saccharomycetales*, *Saccharomycetes*), were used for the ncLSU phylogeny of *Faurelina* in the *Dothideomycetes*; two *Chaetosphaeria* species (*Chaetosphaeriaceae, Chaetosphaeriales*, *Sordariomycetes*) were used as outgroups for the ITS phylogeny.

Maximum parsimony and Bayesian analyses were used to estimate phylogenetic relationships. Four alignments for ITS, ncLSU, ncSSU, and the combined set were constructed. The lengths of the alignments were determined after introduction of gaps. All characters in the ITS alignment were included. Bases 1–75 were excluded from analyses of the ncLSU and ncSSU alignments and bases 1–59 were excluded from the analysis of the RPB2 alignment, because of the incompleteness of the 5'-end of the majority of the available sequences. An additional 69 bases in the RPB2 part of the alignment, which were difficult to identify as homologous, were also excluded. All alignments are deposited in TreeBase (10538).

The three genes for the combined analysis (ncLSU-ncSSU-RPB2) were tested for heterogeneity among data partitions before combining them for the total evidence analysis. We used the partition homogeneity/incongruence-length difference test implemented in PAUP (Swofford 2002) to determine if different partitions of the data gave significantly different signals. Because combining data with value P > 0.01 generally improves phylogenetic accuracy (Cunningham 1997) and our data did not show significant heterogeneity (P = 0.01), the sequences were combined for further analysis.

Maximum parsimony analyses were conducted with PAUP v. 4.0b10 (Swofford 2002). A heuristic search was performed with the stepwise-addition option with 1 000 random taxon addition replicates and TBR branch swapping. All characters were unordered and given equal weight. Gaps were treated as missing data. Branch support was estimated on the recovered topologies by performing a heuristic search of 1 000 bootstrap replicates consisting of ten random-addition replicates for each bootstrap replicate.

Bayesian analysis was performed in a likelihood framework, as implemented by the MrBayes v. 3.0b4 software package, to reconstruct phylogenetic trees (Huelsenbeck & Ronquist 2001). 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 our sequence data sets. Bayesian searches using Metropolis-coupled Markov chain Monte Carlo sampling were conducted. One cold and three heated Markov chains were used in the analysis. Bayesian analyses were run for 5 M generations with trees sampled every 1 000 generations. The first 20 000 trees representing the "burn-in" phase were discarded. To estimate posterior probabilities (PP) of recovered branches (Larget & Simon 1999), 50 % majority rule consensus trees were produced from the remaining trees using PAUP.

PHYLOGENETIC RESULTS

The first analysis was restricted to the ncLSU. The alignment consisted of the two first thirds of the ncLSU region for 99 sequences representing 91 species in 19 ascomycetous families and orders and 1 283 total characters: 615 constant, 140 not parsimony-informative, and 453 parsimony-informative. A maximum parsimony (MP) heuristic search produced 16 most parsimonious trees (MPTs) with a length of 3 303 steps (CI = 0.303, RI = 0.665, HI = 0.696). One of these trees is shown in Fig. 1. The GTR+I+G substitution model was selected for the Bayesian analysis. The order *Glomerellales* forms a monophyletic clade (82 % bootstrap support /0.7 posterior probability) with three families recognised, the *Australiascaceae* (90/1.0), *Glomerellaceae* (85/0.83), and *Reticulascaceae* (97/1.0). Within the *Reticulascaceae*, *Cylindrotrichum setosum* is sister to the *Reticulascus clavatus* clade (95/1.0), *R. tulasneorum* forms a well-supported clade (75/1.0), and *Kylindria peruamazonensis* and *Porosphaerellopsis* are nested at the base of the *Reticulascaceae* (97/1.0). The order *Microascales* as presently conceived appears to be polyphyletic. The monophyletic *Ceratocystidaceae* (100/1.0) and *Gondwanamycetaceae* (100/1.0) form a clade (92/1.0) as a

Table 1. Sources and accession numbers of isolates numbers of isolates analysed in this study. GU1806XX–GU1806YY are sequences newly generated in this study.

Teleomorph	Anamorph	M	Source*	Substrate and Locality	GenBank accession numbers **			
					ITS	LSU	SSU	RPB2
Australiasca laeënsis	*Monilochaetes laeënsis*	●	DAOM 226788	Australia, dead fronds of a tree fern	GU180623	GU180641	GU180610	–
	Monilochaetes laeënsis	●	PRM 915720	UK, stem of *Dicksonia antarctica*	GU180624	GU180642	–	–
Australiasca queenslandica	*Monilochaetes camelliae*	●	BRIP 24607a	Australia, branch of *Camellia sinensis*	HM237327	HM237324	–	–
	Monilochaetes camelliae	○	BRIP 24334c	Australia, branch of *Camellia sinensis*	HM237326	HM237323	–	–
Calosphaeria pulchella	*Calosphaeriophora pulchella*	●	CBS 115999	France, wood and bark of *Prunus avium*	–	AY761075**	AY761071**	GU180661
Ceratosphaeria lampadophora	*Harpophora*-like	●	CBS 117555	France, decayed wood	–	–	GU180618	–
Chaetosphaeria ciliata	*Menispora ciliata*	●	ICMP 18253	New Zealand, decayed wood	–	GU180637	GU180614	GU180659
Chaetosphaeria curvispora	*Chloridium*-like	●	ICMP 18255	New Zealand, decayed wood	–	GU180636	AY502933**	GU180655
Faurelina indica	*Arthrographis* sp.	●	CBS 126.78	India, dung of goat	GU291802	GU180653	–	–
	Arthrographis sp.	●	CBS 301.78	India, dung of cow	–	GU180654	–	–
Reticulascus clavatus	*Cylindrotrichum clavatum*	●	CBS 125296	France, submerged wood of *Alnus glutinosa*	GU180627	GU180643	GU180622	–
	Cylindrotrichum clavatum	○	CBS 125239	France, submerged wood of *Platanus* sp.	GU180633	GU180649	GU180615	–
	Cylindrotrichum clavatum	○	CBS 125297	France, submerged wood of *Fraxinus* sp.	GU180634	GU180650	–	–
	Cylindrotrichum clavatum	○	CBS 428.76	Sweden, decayed wood of *Ulmus scabra*	GU291799	–	–	–
Reticulascus tulasneorum	*Cylindrotrichum oligospermum*	○	CBS 561.77	Netherlands, twig of *Fraxinus excelsior*	GU291801	–	–	–
Reticulascus tulasneorum	*Cylindrotrichum oligospermum* (as *hennebertii*)	○	CBS 570.76	Germany, dead twig of *Symphoricarpos albus*	AF178560**	AF178560**	–	–
Reticulascus tulasneorum	*Cylindrotrichum oligospermum*	○	CBS 557.74	Czech Republic, wood of *Salix purpurea*	GU291798	–	–	–
	Cylindrotrichum oligospermum	●	CBS 101319	Czech Republic, wood of *Sambucus nigra*	AF178547**	AF178547 **	–	–
Togniniella acerosa	*Phaeocrella acerosa*	●	ICMP 18256 CBS 125298	New Zealand, decayed wood of *Nothofagus* sp.	–	AY761076**	AY761073**	GU180660
tu	*Acrostalagmus annulatus*	○	DAOM 212126	Germany, soil and roots	GU180632	GU180646	GU180611	GU180662
tu	*Cylindrotrichum gorii*	○	CBS 879.85	Sweden, dead stem of *Urtica dioica*	HM237328	HM237322	–	–
tu	*Cylindrotrichum setosum*	○	DAOM 229246	Australia, wood and bark mulch on the ground	GU180635	GU180652	GU180617	–
tu	*Custingophora olivacea*	○	CBS 335.68	Germany, compost	–	–	–	GU180665
tu	*Kylindria peruamazonensis*	○	CBS 838.91	Cuba, leaf litter of *Bucida palustris*	GU180628	GU180638	GU180609	GU180656
tu	*Kylindria peruamazonensis*	○	CBS 421.95	Cuba, leaf of *Bucida palustris*	GU291800	HM237325	–	–
tu	*Gibellulopsis nigrescens*	○	DAOM 226890	Canada, Ontario, soil	GU180631	GU180648	GU180613	GU180664
tu	*Monilochaetes guadalcanalensis*	○	CBS 346.76	Solomon Islands, leaf of *Musa*	GU180625	GU180640	–	–
tu	*Monilochaetes infuscans*	○	CBS 379.77	New Zealand, *Ipomoea batatas*	–	GU180645	GU180619	GU180658
tu	*Monilochaetes infuscans*	○	CBS 869.96	South Africa, *Ipomoea batatas*	GU180626	GU180639	GU180620	GU180657
tu	*Monilochaetes infuscans*	○	CBS 870.96	South Africa, *Ipomoea batatas*	–	GU180644	GU180621	–
tu	*Plectosporium tabacinum*	○	DAOM 229828	Canada, Ontario, soil	GU180630	GU180647	GU180612	GU180663
tu	*Stachylidium bicolor*	○	DAOM 226658	straw of *Oryza sativa* imported from India into Canada	–	GU180651	GU180616	–

* BRIP = Plant Pathology Herbarium, Queensland, Australia; CBS = Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; DAOM = Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa.

** These sequences were published elsewhere (Réblová & Winka 1999, Réblová & Seifert 2004, Réblová *et al.* 2004).

M: morph of material available: • = teleomorph, ◦ = anamorph.

tu = teleomorph unknown

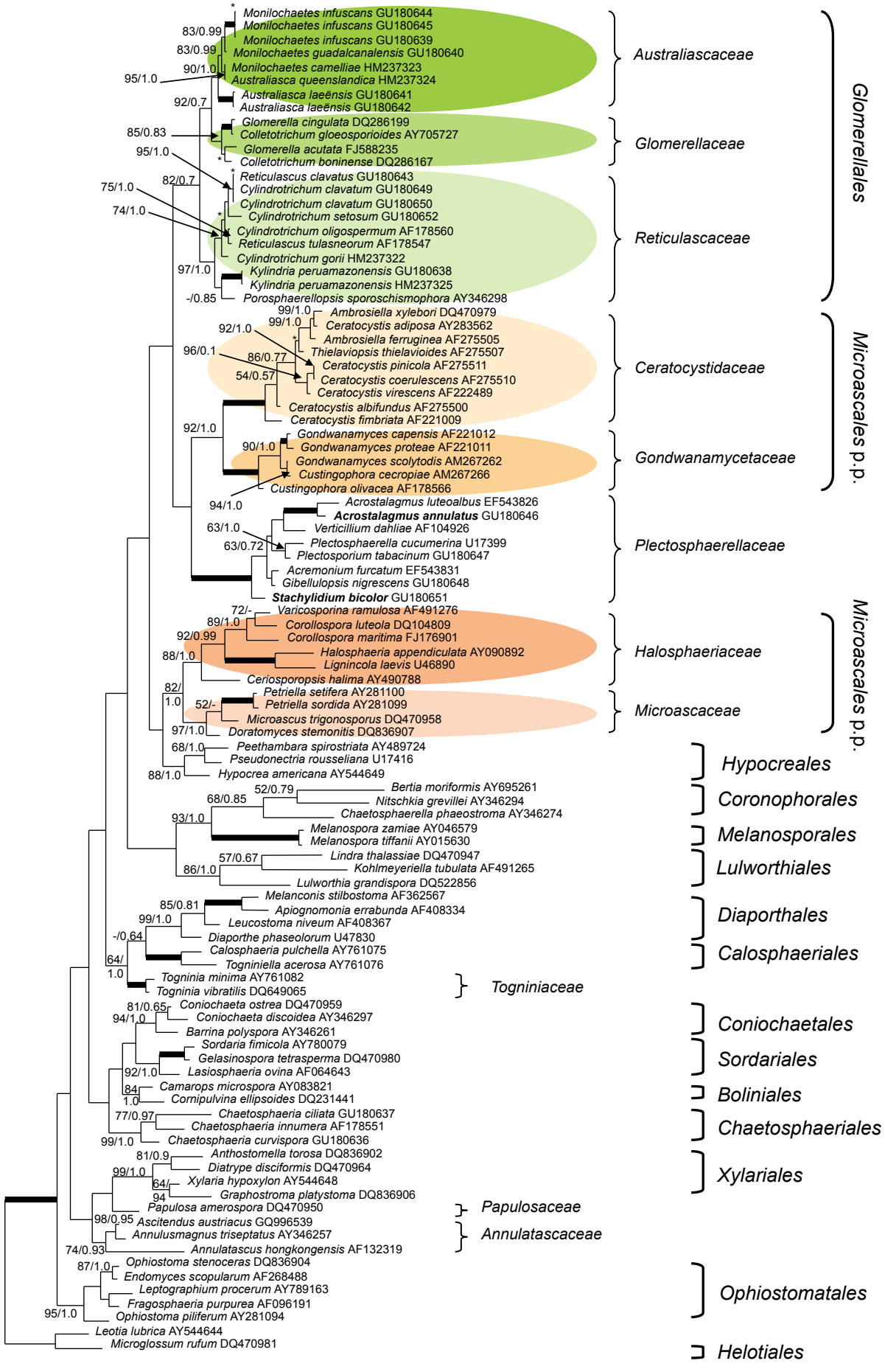

Fig. 1. One of 16 most parsimonious trees from a heuristic analysis of ncLSU rDNA sequences. Thickened branches indicate posterior probability values = 1.0 PP and 100 % bootstrap support. Bootstrap support values ≥ 50 % and Posterior probability values ≥ 0.5 are included at the nodes. Branch lengths are drawn to scale. An asterisk above or below a branch marks branches that collapse in the strict consensus tree.

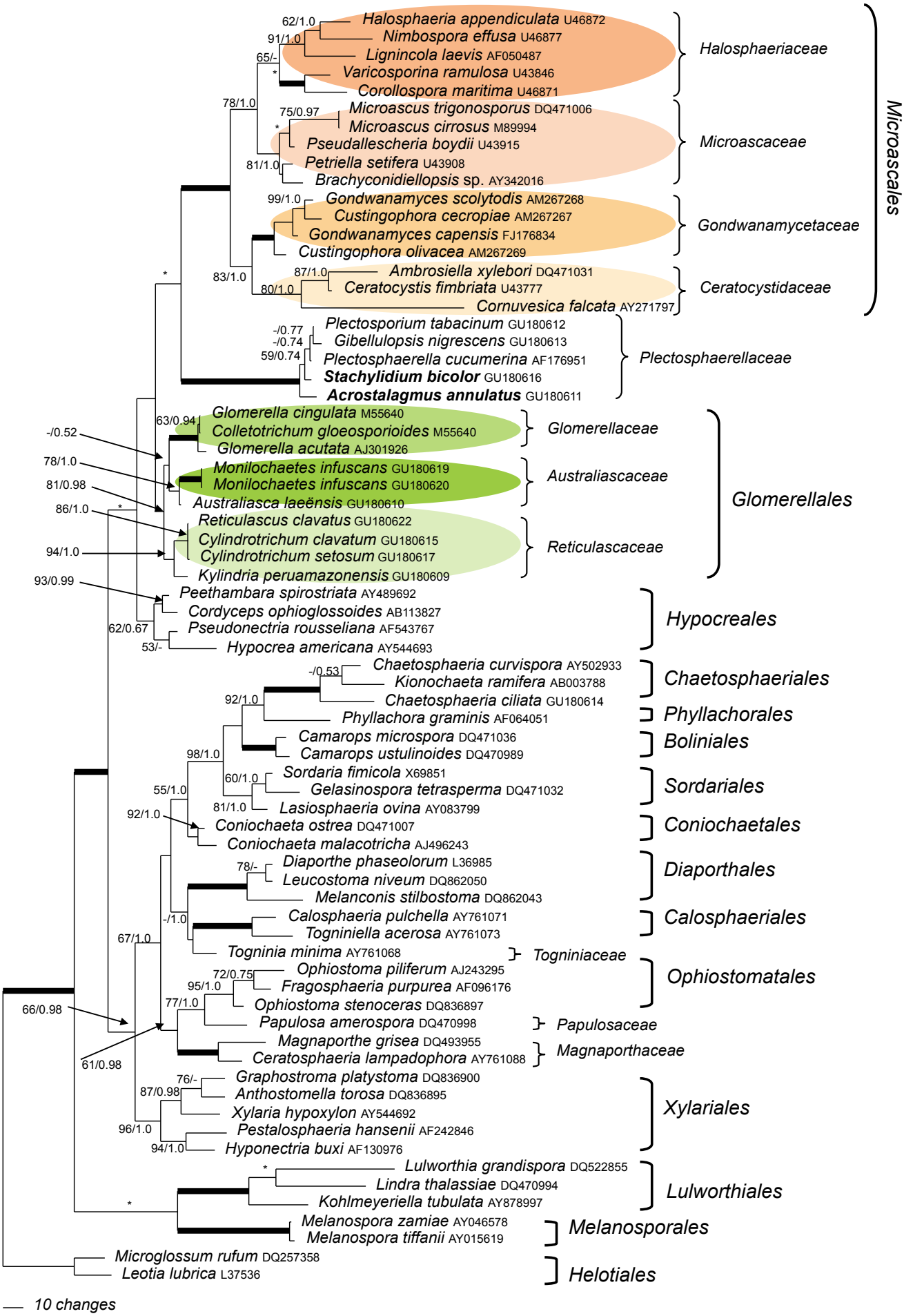

Fig. 2. One of the 14 most parsimonious trees from a heuristic analysis of ncSSU rDNA sequences. Details as in Fig. 1.

sister to the *Plectosphaerellaceae* (100/1.0). The other two families of the *Microascales* form a separate clade (82/1.0) containing the *Microascaceae* (97/1.0) and the *Halosphaeriaceae* (88/1.0). The second analysis was restricted to the ncSSU. The alignment consisted of the whole gene for ncSSU for 71 sequences representing 67 species in 19 ascomycetous orders and families and 1 777 total characters: 1 102 constant, 195 not parsimony-informative, and 405 parsimony-informative. A maximum parsimony heuristic search produced 14 MPTs with a length of 2 267 steps (CI = 0.390, RI = 0.697, HI = 0.609), one of which is shown in Fig. 2. For the Bayesian analysis, the GTR+I+G substitution model was inferred. The *Glomerellales* form a monophyletic, strongly supported clade (81/0.98) containing representatives of three families, the *Australiascaceae* (78/1.0), *Glomerellaceae* (100/1.0), and *Reticulascaceae* (94/1.0). The *Plectosphaerellaceae* form a separate, strongly supported clade (100/1.0) basal to the *Microascales*. The *Microascales* appear as a monophyletic clade (100/1.0) including two strongly supported subclades. The first subclade (78/1.0) contains the *Halosphaeriaceae* (65/-) and *Microascaceae* (81/1.0) and the second subclade (83/1.0) contains the *Ceratocystidaceae* (80/1.0) and *Gondwanamycetaceae* (100/1.0).

In the third analysis, a combination of the ncLSU and ncSSU data sets plus RPB2 sequences was assessed for 54 taxa representing 52 species in 18 ascomycetous orders and families. The alignment of the combined set of ncLSU-ncSSU-RPB2 DNA sequences consisted of 4 224 total characters: 2 148 constant, 314 not parsimony-informative, and 1 484 parsimony-informative. A maximum parsimony heuristic search produced two MPTs with a length of 11 688 steps (CI = 0.278, RI = 0.476, HI = 0.722); one is shown in Fig. 3. For the Bayesian analysis the GTR+I+G substitution model was selected. The *Glomerellales* are a monophyletic, well-supported clade (100/0.81) with the *Plectosphaerellaceae* as a sister group (100/1.0). The *Microascales* appear as a monophyletic, strongly supported clade (88/1.0), again with two subclades; the first (87/1.0) contains the *Halosphaeriaceae* (94/1.0) and *Microascaceae* (88/1.0) while the other (100/1.0) comprises the *Ceratocystidaceae* (100/1.0) and *Gondwanamycetaceae* (99/1.0).

The fourth analysis included ITS1, 5.8S, and ITS2 regions of species of the *Glomerellales* and *Plectosphaerellaceae*. The alignment consisted of 38 sequences representing 29 species in five families and 574 total characters: 277 constant, 75 not parsimony-informative, and 222 parsimony-informative. A maximum parsimony heuristic search produced nine MPTs with a length of 786 steps (CI = 0.592, RI = 0.821, HI = 0.407). One is shown in Fig. 4. The GTR+G substitution model was inferred for the Bayesian analysis. Following the results of our other analyses, two *Chaetosphaeria* species (*Chaetosphaeriales*) were used as outgroups. The order *Glomerellales* is a strongly supported monophylum (95/0.99) containing three strongly supported families, the *Australiascaceae* (99/1.0), *Glomerellaceae* (99/0.97), and *Reticulascaceae* (96/1.0). The *Plectosphaerellaceae* (100/1.0) appears as a strongly supported sister clade to the *Glomerellales*. Six strains represent the *Australiascaceae* in the analysis: two strains of *Australiasca queenslandica*, two strains of *A. laeënsis,* and one strain each of *Monilochaetes infuscans* and *M. guadalcanalensis*. The *Reticulascaceae* are represented by four strains of *Reticulascus clavatus* (anamorph *C. clavatum*), four strains of *R. tulasneorum* (anamorph *Cylindrotrichum oligospermum*), *C. setosum*, *C. gorii,* and two strains of *Kylindria peruamazonensis*. The two conidial (CBS 125239, CBS 125297) and single ascospore (ex-type strain CBS 125296) isolates of freshwater *R. clavatus* plus one terrestrial isolate (CBS 428.76) formed a strongly supported monophylum (100/1.0). Another strongly supported monophyletic clade (97/1.0) included one ascospore- (ex-type strain CBS 101319) and three conidial isolates of *R. tulasneorum* (CBS 557.74, CBS 561.77, ex-type strain of *C. hennebertii* CBS 570.76). The anamorphic *C. setosum* (ex-type strain DAOM 229246), *C. gorii* (CBS 879.85), and *K. peruamazonensis* (CBS 421.95, CBS 838.91) were basal to the rest of the clade on separate branches.

A fifth analysis of the ncLSU rDNA sequences was run to determine the relationship of two strains of *Faurelina indica* with members of the *Dothideomycetes* and *Eurotiomycetes*. The alignment consisted of the first two thirds of the ncLSU for 68 sequences representing 66 species in 11 orders and families and 1 229 total characters: 716 constant, 76 not parsimony-informative, and 362 parsimony-informative. A maximum parsimony heuristic search produced 66 MPTs with a length of 1 593 steps (CI = 0.433, RI = 0.760, HI = 0.567). One is shown in Fig. 5. The GTR+I+G substitution model was selected for the Bayesian analysis. The two strains of *Faurelina* form a monophyletic clade (88/0.9), which is a sister to the *Didymellaceae* (96/1.0). The suggested relationship of *Faurelina* with the *Eremomycetaceae* and *Testudinaceae* could not be confirmed; the families grouped on separate branches with no close relationship to each other. *Faurelina* appears to be a member of the *Pleosporales* within the *Dothideomycetes* unrelated to the *Microascales*.

TAXONOMY

Glomerellales

Chadefaud (1960) proposed the order "Glomérellales" for a group of endophytic fungi and parasites of living plants with ascomata varying from endostromatal to apostromatal and ascospores that are often unicellular and hyaline. No Latin diagnosis was provided for the order. Within the order he suggested an evolution of the apical apparatus from an initial condition of the periocular thickening of the apical dome lacking a pronounced chitinoid or amyloid ring to derived conditions of either the apical thickening converted into an apical cushion reduced to a simple lens-shaped disc or with the initial of a chitinoid ring developing in the periocular thickening. According to the texture and pigmentation of the ascomata, he further divided the order into two groups: a) "Eu-Glomérellales", which included genera with a non-fleshy black stroma *i.e. Gibellina*, *Glomerella*, *Phyllachora*, and *Physalospora;* and b) “Polystigmatales” as “Glomérellales nectrioïdes”, which comprised one genus, *Polystigma*, with a orange to red, fleshy stroma. After this invalid introduction of the name *Glomerellales*, the order was also cited by Lanier *et al.* (1978) and later by Locquin (1984), when he listed the *Glomerellales* and *Polystigmatales* as separate orders, again without a Latin diagnosis. After the validation of the *Glomerellaceae* in Zhang *et al.* (2006), we validate here the phylogenetically delimited order *Glomerellales*, excluding the earlier validated but unrelated *Phyllachorales.*

Three families are accepted in the *Glomerellales*, namely the *Glomerellaceae*, *Australiascaceae*, and *Reticulascaceae*. The latter two families are newly described below based on cultural studies, detailed morphological comparisons of the holomorphs, and newly generated ITS, ncLSU, ncSSU, and RPB2 sequences.

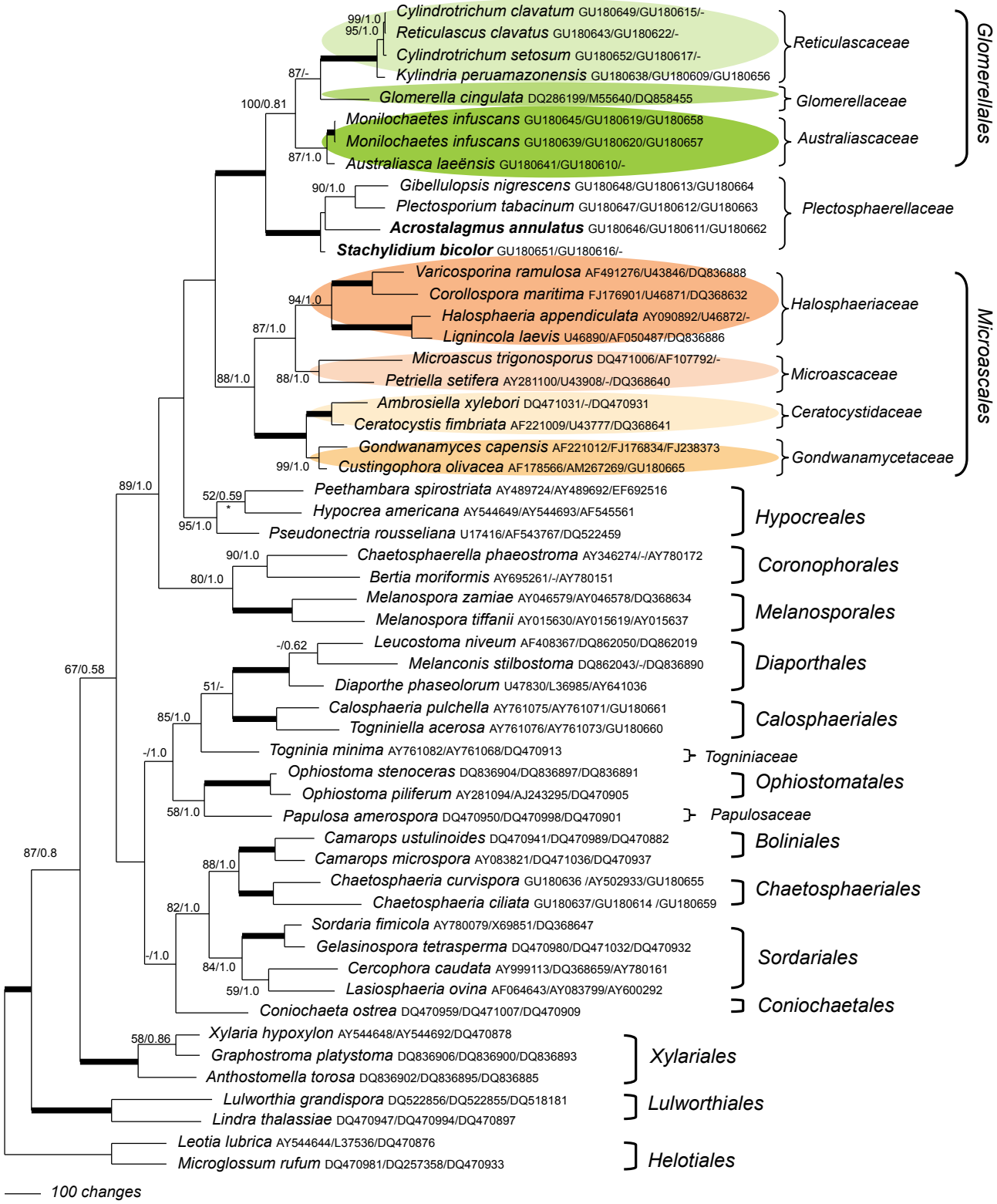

Fig. 3. One of the two most parsimonious trees from a heuristic analysis of the three-gene combined data set (ncLSU-ncSSU-RPB2). Details as in Fig. 1. The GenBank accession numbers given after the names are those of ncLSU/ncSSU/RPB2 genes. Missing sequences are indicated by "–".

Glomerellales Chadef. ex Réblová, W. Gams & Seifert, **ord. nov.** MycoBank MB515429.

Glomerellales Chadef., *Traité de botanique systématique*. Tome I, p. 613. 1960 (also in Lanier *et al.*, *Mycol. Pathol. Forest.* I: 292. 1978; Locquin, *Mycol. Gén. Struct.*, p. 170. 1984) nom. inval., Art. 36.

Ascomata perithecia, brunnea usque nigra, nonnumquam sclerotioidea, ostiolum periphysatum. Pariete ascomatum 2-3-stratoso. Hamathecium paraphyses verae. Asci unitunicati, brevi-stipitati, parte apicali iodo non reagente. Ascosporae hyalinae vel pallide pigmentatae, 0-pluri-cellulares. Anamorphe: conidia modo phialidico orientia.

Typus: *Glomerellaceae* Locq. ex Seifert & W. Gams, *Mycologia* 98: 1083. 2007 [2006].

Perithecia darkly pigmented, sometimes becoming ± sclerotial. *Perithecial wall* 2–3-layered, ostiolum periphysate. Interascal tissue of thin-walled, tapering paraphyses. *Asci* unitunicate, thin-

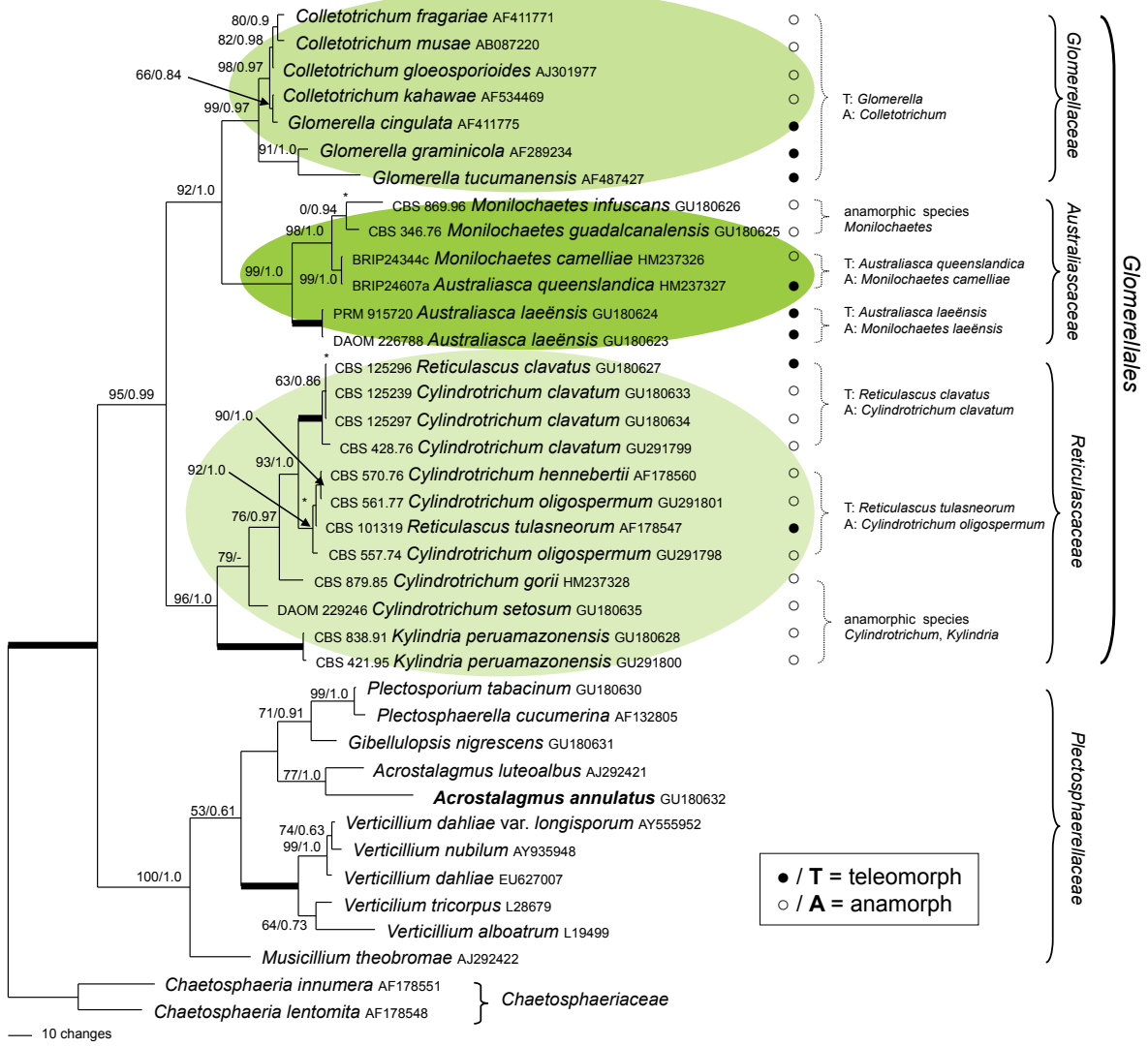

Fig. 4. One of the nine most parsimonious trees from a heuristic analysis of ITS rDNA operon of the *Glomerellales* and the *Plectosphaerellaceae*. The accession numbers of strains of the newly described *Australiascaceae* and *Reticulascaceae* are indicated. Details as in Fig. 1.

walled, ascal apex thickened without visible discharge mechanism or thin-walled with a distinct apical annulus, inamyloid, 8-spored. *Ascospores* hyaline or pigmented, 0–several-septate. Anamorphs with phialidic conidiogenesis.

Families: *Australiascaceae* Réblová & W. Gams, *Glomerellaceae* Locq. ex Seifert & W. Gams, and *Reticulascaceae* Réblová & W. Gams.

This order is phylogenetically distinct from the *Phyllachorales*, in which its members were formerly classified. The original classification proposed by Chadefaud (1960), who included *Phyllachora* in this order, is untenable based on our molecular data (Fig. 2). In the ncSSU phylogeny, the *Phyllachorales* represented by *Phyllachora graminis* (ncSSU rDNA sequence: AF064051, Winka & Eriksson 2000) are clearly separated from the *Glomerellales*; the former is nested within a clade (91/1.0) sister to the *Chaetosphaeriales* (100/1.0).

Glomerellaceae

This family accommodates the teleomorph genus *Glomerella* and its *Colletotrichum* anamorphs. For discussion and description refer to Zhang *et al.* (2006).

Glomerellaceae Locq. ex Seifert & W. Gams in Zhang *et al.*, Mycologia 98: 1083. 2007. [2006].

Australiascaceae Réblová & W. Gams, **fam. nov.** MycoBank MB515430.

Stromata absentia. Ascomata perithecia, brunnea usque nigra, ostiolum periphysatum. Pariete ascomatum fragili, 2-stratoso. Hamathecium paraphyses verae. Asci unitunicati, 8-spori, cylindraceo-clavati, annulo apicali iodo non reagente. Ascosporae hyalinae, septatae. Anamorphe *Monilochaetes*; conidiis 0(–3)-septatis, hyalinis modo phialidico orientibus.

Typus: *Australiasca* Sivan. & Alcorn, Aust. Syst. Bot. 15: 742. 2002.

Stroma absent. *Perithecia* brown to black, ostiolum periphysate. *Perithecial wall* 2-layered, fragile. *Interascal tissue* of thin-walled, tapering paraphyses. *Asci* unitunicate, 8-spored, cylindrical-clavate, apical ring distinct, inamyloid. *Ascospores* hyaline, septate. Anamorph: *Monilochaetes*; conidiogenesis phialidic, with hyaline 0(–3)-septate conidia, aggregated in slime or in chains.

The *Australiascaceae* accommodates the holomorphic genus *Australiasca* and anamorphic *Monilochaetes*. The molecular data for *Australiasca* (Figs 1–3) confirm that the genus is unrelated to the *Chaetosphaeriaceae* or *Lasiosphaeriaceae* as suggested by Sivanesan & Alcorn (2002). However, the *Australiascaceae*, like

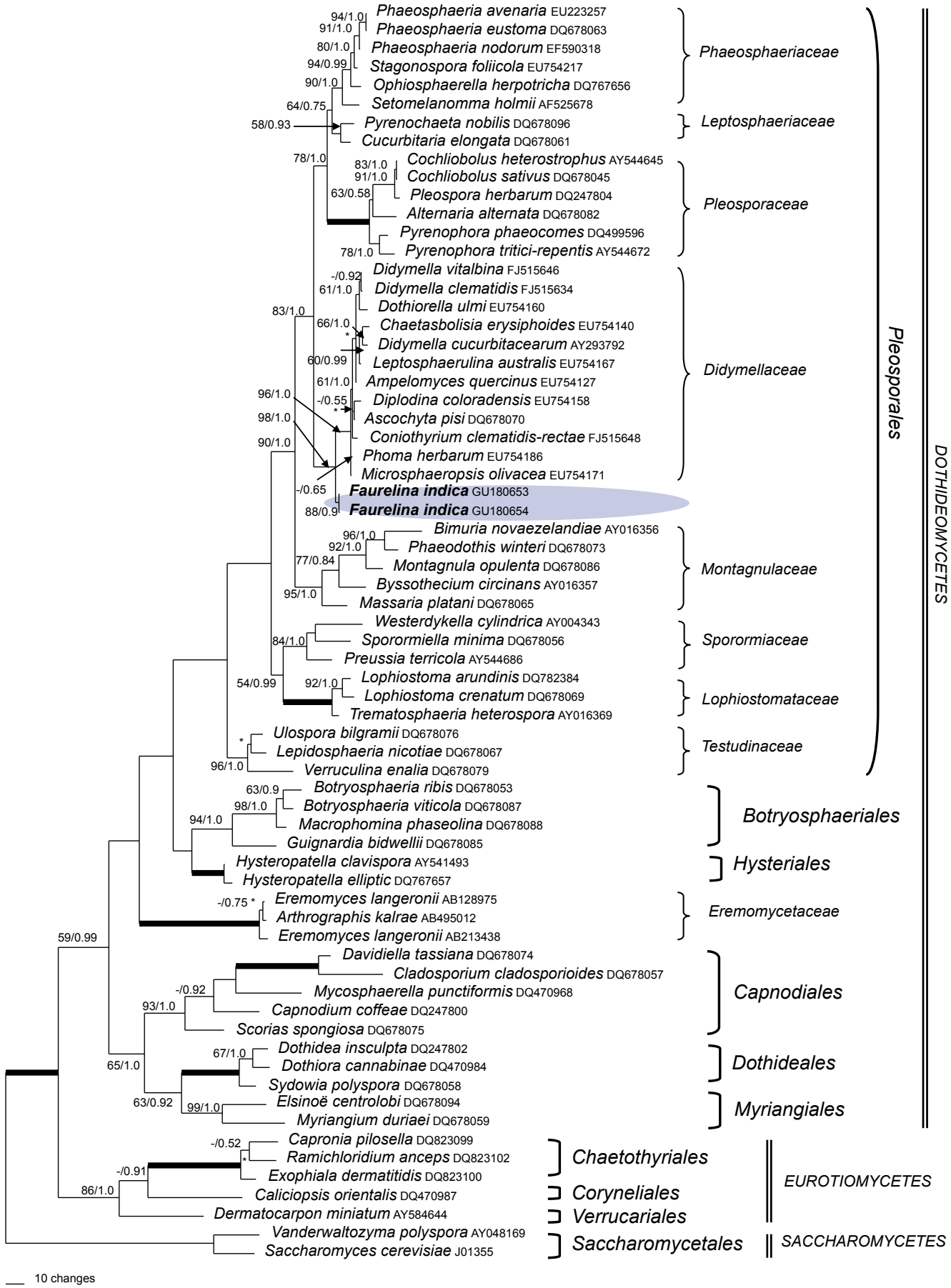

Fig. 5. One of 66 most parsimonious trees from a heuristic analysis of ncLSU rDNA of *Faurelina indica* and *Dothideomycetes*. Details as in Fig. 1.

the *Reticulascaceae*, accommodates teleomorphs that mimic *Chaetosphaeria* and which are almost indistinguishable from its perithecia on morphological grounds. The anamorphs are phialidic, dematiaceous hyphomycetes with hyaline, slimy conidia, which are also similar to anamorphs of *Chaetosphaeria*.

The dematiaceous hyphomycete genus *Monilochaetes* was described and illustrated for a single species, *M. infuscans* (Halsted 1890, Harter 1916), which causes scurf disease or soil stain of *Ipomoea batatas* (sweet-potato). Another saprobic species, *M. guadalcanalensis*, collected on leaves of *Musa* sp., originally described in *Catenularia,* was recently added (Rong & Gams 2000) and this classification is confirmed here by molecular data. *Monilochaetes* includes species with solitary, erect, sometimes curved or geniculate, macronematous conidiophores, darker near the base, becoming paler towards the apex, with prominently darkened septa, terminal, wide monophialides with a shallow collarette, and aseptate, rarely septate, hyaline conidia adhering in basipetal chains or heads. Rong & Gams (2000) distinguished *Monilochaetes* from the other two similar dematiaceous hyphomycete genera *Dischloridium* (Sutton 1976) and *Exochalara* (Gams & Holubová-Jechová 1976) by aspects of conidiophore branching and fasciculation and conidial shapes and dimensions. The present ITS and ncLSU phylogenies confirm that *Dischloridium* and the morphologically similar older genus *Monilochaetes*, which up to now was only known as asexual, are congeneric. Therefore, *Dischloridium laeënse*, type of the genus, is transferred to *Monilochaetes* and *Dischloridium* becomes a generic synonym of *Monilochaetes*.

The teleomorph-anamorph connections of *Australiasca queenslandica*, type species of the genus, with *M. camelliae* and of the newly described *A. laeënsis* with *M. laeënsis* were experimentally established (Sivanesan & Alcorn 2002, this study). The other four species accepted in *Monilochaetes* are presently only known to be anamorphic. A further species of *Monilochaetes* is described by Réblová *et al*. (2011)

KEY TO THE SPECIES OF *AUSTRALIASCA* AND *MONILOCHAETES* IN THE *AUSTRALIASCACEAE*

1. Conidia hyaline, ellipsoidal, aseptate, rarely 1–3-septate, longer than 26 µm 2
1. Conidia hyaline, ellipsoidal, aseptate, rarely 1-septate, shorter than 26 µm 3

2. Conidia ellipsoidal with an obtuse base, sometimes with laterally displaced hilum, aseptate, rarely 1–3-septate at maturity, 18–35 × 8–13 µm *in vitro*; 20.5–24(–26.5) × (10–)11–12(–13) µm on PCA; asci 65–140 × 12.5–17.5 µm; ascospores 18–31 × 7.5–10.5 µm ..... *A. camelliae* (anamorph *M. camelliae*)
2. Conidia cylindrical to ellipsoidal with an obtuse base, 25–38 × 12–16 µm *in vivo*; teleomorph unknown ..... *M. regenerans*

3. Conidia aseptate, ellipsoidal to oblong, usually in small clusters, aggregated in slimy droplets 4
3. Conidia 0–1-septate, rhomboid–ellipsoidal to obovoidal, usually forming chains 5

4. Conidia oblong, apically rounded, with an obtuse base, 9–25 × 3.5–6(–7) µm; teleomorph unknown ..... *M. basicurvata*
4. Conidia ellipsoidal to oblong with an obtuse base, 22–26 × 10–12 µm *in vivo*, (15.5–)18–22.5(–23.5) × 7.5–9(–10) µm *in vitro* (PCA); asci 130–148 × 12.5–17.5 µm; ascospores (20–)24.5–31.5(–33) × (7.5–)9–9.5 µm ..... *A. laeënsis* (anamorph *M. laeënsis*)

5. Conidia rhomboid–ellipsoidal to obovoidal on host, rarely 1-septate, ellipsoidal with an obtuse base in culture, 15–20 × 4–6 µm ..... *M. infuscans*
5. Conidia ellipsoidal with an obtuse base in culture, 18–21 × 6–9 µm ..... *M. guadalcanalensis*

Australiasca laeënsis Réblová & W. Gams, **sp. nov.** MycoBank MB518384, Fig. 6A–K.

Anamorph: ***Monilochaetes laeënsis*** (Matsush.) Réblová, W. Gams & Seifert, **comb. nov.** MycoBank MB515431.

Basionym: *Chloridium laeënse* Matsush., Bull. Natl. Sci. Mus. Tokyo 14: 462. 1971.

≡ *Dischloridium laeënse* (Matsush) B. Sutton, Kavaka 4: 47. 1976.

Etymology: Epithet from the anamorph species, originally derived from the type locality, Lae in Papua-New Guinea (Matsushima 1971).

Stromata absentia. Perithecia superficialia, gregaria vel solitaria, atra, conica usque obpyriformia, 200–320 µm diam, 340–450 µm alta, ostiolum periphysatum. Paries ascomatum fragilis, 2-stratosus. Paraphyses septatae, hyalinae, sursum angustatae, ascos superantes. Asci unitunicati, cylindraceo-clavati, 130–148 × 18–20 µm (in medio ± s.e. = 137.6 ± 5.3 × 19.3 ± 0.6 µm), 8-spori, brevi-stipitati, apice truncato. Ascosporae ellipsoideae usque ovoideae, 24.5–31.5(–33) × (8–)9–9.5 µm (in medio ± s.e. = 15.7 ± 0.2 × 4.4 ± 0.04 µm), hyalinae, 0–1-septatae. Anamorphe *Monilochaetes laeënsis*.

Perithecia 200–320 µm diam, 340–450 µm high, gregarious to solitary among conidiophores, superficial, base slightly immersed, conical to obpyriform, with a short beak, black, glabrous or with setae. Setae scanty, acute, thick-walled, septate, dark brown, paler to subhyaline towards apex, sometimes on upper half of perithecium, 90–155 × 5–7 µm; longer, thicker-walled setae, arising from base of perithecium, 300–420 × 10–11 µm. *Perithecial wall* 18–22 µm thick, becoming 45–54 µm thick towards base, fragile, 2-layered: outer layer of *textura prismatica* consisting of thick-walled, brick-like cells, cells becoming polyhedral towards base; inner layer of hyaline, compressed cells. *Paraphyses ca*. 2.5–3 µm wide, persistent, hyaline, septate, branching, longer than asci. *Asci* 130–148 × 18–20 µm (mean ± s.e. = 137.6 ± 5.3 × 19.3 ± 0.6 µm), unitunicate, cylindrical-clavate, short-stipitate, apex truncate, with a distinct, shallow annulus, *ca*. 6 µm wide, 1–1.5 µm high, 8-spored. *Ascospores* 24.5–31.5(–33) × (8–)9–9.5 µm (mean ± s.e. = 28.4 ± 0.6 × 8.9 ± 0.1 µm), ellipsoidal to oblong, apiculate at both ends, 1-celled, becoming transversely 1–3-septate after discharge, smooth, germinating with germ tubes at both ends, hyaline, irregularly 2-seriate in ascus.

Fig. 6.A–K. *Australiasca laeënsis*. A. Perithecium. B. Ascospores. C. Asci with ascospores, some ascospores with a developed median septum. D, E. Conidia and F–K. Conidiophores of the *Monilochaetes laeënsis* anamorph, in culture. A–C from PRM 915720; D–K DAOM 226788 (PCA, 14 d old). Scale bars: A = 100 µm; B, D–K = 10 µm; C = 50 µm. DIC: A, D, G–K; PC: B, C, E, F.

Colonies *in vivo* dark, hairy, effuse. *Conidiophores* 200–600 µm long, 6.5–9 µm wide, arising in small fascicles or small loose groups of 2–6 or solitary from a minute stromata, macronematous, percurrently proliferating, dark brown, 5–15-septate; base occasionally bulbous with smaller, thick-walled, adjacent pseudoparenchymatous cells forming stromatic tissue in substratum. *Conidiogenous cells* monophialidic, 50–70 × 4.5–8(–10) µm, terminal, cylindrical, hardly tapering at apex, subhyaline; collarette *ca.* 1–2 µm high, minute, conidiogenous locus located at base of collarette. *Conidia* 22–26 × 10–12 µm (mean ± S.E. = 23.2 ± 0.2 × 10.8 ± 0.2 µm), ellipsoidal to cylindrical-ellipsoidal, broadly rounded, sometimes obtuse at base, hyaline, basal scar 3.5–4 µm diam, smooth-walled.

Colonies *in vitro* after 14 d on PCA at 25 °C 15−20 mm diam, felty, stromatic tissue absent, aerial mycelium olive-brown, margin entire; reverse pale greyish-brown. Colonies readily sporulating, beginning

after 5 d on PCA at 25 °C under near-UV light (12 h light: 12 h dark). Conidiophores, phialides, and conidia morphologically identical to those on natural substratum. *Conidiophores* 40–160 × 7–8 µm, pale brown throughout, with none or 1 percurrent proliferation, 2–5-septate; in about 28 d, longer conidiophores developing, *ca.* 160-280 µm long, dark brown, subhyaline towards apex, with 1–4 percurrent proliferations, up to 2–15-septate. *Conidiogenous cells* monophialides 31–58 × (6–)7–9 µm, tapering to 4–6.5 µm just below collarette; collarette *ca*. 1.5 µm high and (5.5–)6–8 µm wide. *Conidia* (15.5–)18–22.5(–23.5) × 7.5–9(–10) µm (mean ± S.E. = 20.9 ± 1 × 8.2 ± 0.3 µm), ellipsoidal to cylindrical-ellipsoidal, broadly rounded, sometimes obtuse at base, hyaline, basal scar 2–3.5 µm diam, smooth-walled.

Specimens examined (anamorph and teleomorph): **Australia,** New South Wales, Blue Mountains, Mt. Tomah Botanical Garden, S 33 32.4, E 150 25.4, 1197 m alt., on dead stipes and spathes of a tree fern in a rain forest, 17 Aug. 1999, K.A. Seifert no. 884 and G.J. Samuels, DAOM 226788. **UK**, England, West Cornwall, Penjerrick House Gardens, 22 June 2000, dead stipes of *Dicksonia antarctica*, B. Candy, PRM 915720, **holotype** of *A. laeënsis*.

Notes: Based on the results from ITS and LSU rDNA phylogenies, *Australiasca laeënsis* and *A. queenslandica* are distinct species, although they are morphologically similar. *Australiasca queenslandica* and its *M. camelliae* anamorph were originally described and isolated into culture from leaves, stems and branches of *Camellia sinensis*; perithecia containing mature asci and ascospores formed *in vitro* (Sivanesan & Alcorn 2002). The ascospores released by *A. queenslandica* were often observed to be 1–3-septate, becoming dictyoseptate, and some produced phialides with hyaline microconidia *in vitro*. The recently collected material of *A. laeënsis* from England and Australia documents perithecia produced on the host associated with the conidiophores of its *M. laeënsis* anamorph. The ascospores were observed to be transversely 1–3-septate after discharge, but never became dictyoseptate or exhibited phialidic germination. *Australiasca laeënsis* is described here based on our observations on the host and the anamorph in culture.

The range of conidial lengths of *M. camelliae* and *M. laeënsis* overlap, but those of the former species are usually longer. *Monilochaetes camelliae* produces conidia 18–35 × 8–13 µm in culture on Sachs agar + maize leaves (Sivanesan & Alcorn 2002) or 20.5–24(–26.5) × (10–)11–12(–13) µm on PCA (this study). The conidia of *M. laeënsis* are 22–26 × 10–12 µm on the host and (15.5–)18–22.5(–23.5) × 7.5–9(–10) µm on PCA (this study). Therefore, the conidia of *M. camelliae* from Sachs agar overlap in length with conidia of *M. laeënsis*, but exceed its upper range by nearly 10 µm, while on PCA the conidia of *M. camelliae* are only slightly longer than those of *M. laeënsis*. The conidial dimensions for *M. laeënsis* from our collections correspond with measurements of the type and other specimens on host substrata from different localities, *e.g.* Sutton (1976, conidia 15–20 × 8–10 µm), Matsushima (1971, 17–26 × 8–12 µm), and Holubová-Jechová (1982, 14.5–24 × 6.5–10 µm). The conidia of *M. laeënsis* are hyaline, aseptate, and arise singly from the conidiogenous locus, usually in slimy heads. The conidia of *M. camelliae* were described as occasionally 1–3-septate, produced in heads or chains (Sivanesan & Alcorn 2002). The conidiophores of *M. camelliae* are also slightly longer, often swollen subapically (Sivanesan & Alcorn 2002).

Monilochaetes laeënsis has been collected on dead leaves in Papua New Guinea (Matsushima 1971), Sri Lanka (Sutton 1976, Bhat & Sutton 1985), and Cuba (Holubová-Jechová 1982), dead leaves or twigs and dead palm spathes in Australia, Ethiopia, India and Malaysia (Bhat & Sutton 1985), and dead fern stipes in the United Kingdom (Kirk 1986). Only the European and recent Australian material contained perithecia with mature asci and ascospores. Kirk (1986) noted that *Dischloridium* does not occur naturally in the British Isles but was probably introduced into gardens where it was found along with its host *Dicksonia antarctica*. He also suggested that the prevailing colder temperatures may have triggered sexual reproduction in nature; our own teleomorph specimen was collected in a cool, humid valley in the Australian winter.

Australiasca queenslandica Sivan. & Alcorn, Aust. Syst. Bot. 15: 742. 2002. Figs 7A–R, 8A–G.
Anamorph: ***Monilochaetes camelliae*** (Alcorn & Sivan.) Réblová, W. Gams & Seifert, **comb. nov.** MycoBank MB518385.
Basionym: *Dischloridium camelliae* Sivan. & Alcorn, Aust. Syst. Bot. 15: 743. 2002.

Colonies *in vitro* on MEA after 14 d at 25 °C with 22−25 mm radial growth, more or less planar, surface dark brown, covered with abundant, pale grey, lanose to cottony aerial mycelium, margin smooth and entire, reverse grey, sterile. Colonies on PCA after 14 d at 25 °C with 23–25 mm radial growth, planar, surface brown, covered with pale grey, lanose to cottony aerial mycelium, margin smooth and entire, reverse dark grey, sterile.

Colonies *in vitro* on PCA sporulating in 14 d at 25 °C in darkness. Setae absent. *Conidiophores* 200–720 µm long, 9–10(–10.5) µm wide near base and 6.5–7.5(–8.5) µm wide in middle, pale to dark brown, subhyaline towards apex, with none or 1 percurrent proliferation, up to 20-septate. *Conidiogenous cells* monophialidic, subhyaline, paler towards collarette, ampulliform to cylindrical, slightly swollen, 36–45(–60) µm long, 6.5–8(–9) µm wide at widest part, tapering to *ca*. 3–4 µm just below collarette; collarette 4.5–5.5 µm wide and *ca.* 1.5–2 µm high. *Conidia* 20.5–24(–26.5) × (10–)11–12 µm (mean ± S.E. 22.5 ± 0.3 × 11.8 ± 0.1), 0–1-septate, ellipsoidal to cylindrical-ellipsoidal, broadly rounded at end, obtuse at base, basal scar 3–3.5 µm diam, some conidia with a laterally displaced hilum, hyaline, smooth-walled.

After 6 mo on PCA at 25 °C in darkness, producing minute conidiophores with microconidia. Setae absent. *Conidiophores* more or less erect, arising from aerial mycelium, simple or sparingly branched, pale brown to subhyaline, 40–60 µm long and 2–2.5 µm wide, with terminally integrated or intercalary conidiogenous cells. *Conidiogenous cells* monophialidic, subhyaline to pale brown, usually paler towards apex, ampulliform to cylindrical, 8–20 µm long, 2.5–3.5 µm wide at widest part, tapering to *ca*. 1.5 µm just below collarette; collarette 2.5–3 wide, *ca*. 2 µm high. *Conidia* 4–5.5 × 3–3.5 µm (mean ± S.E. 4.5 ± 0.1 × 3.2 ± 0.1), aseptate, thick-walled, broadly ellipsoidal to subglobose, rounded at ends, base slightly tapering, obtuse with a minute abscission scar, accumulating in small, clear to whitish droplets, hyaline, smooth-walled. Chlamydospores not observed.

Specimens examined (anamorph only): **Australia,** Queensland, Malanda, isolated from branch of *Camellia sinensis*, 19 Feb. 1997, D. Steel M. 8982c, BRIP 24334c; Queensland, Brisbane, S 27 30, E 152 58, isolated from branch of *Camellia sinensis*, 10 July 1997, J.L. Alcorn, BRIP 24607a).

Notes: Two isolates of *M. camelliae* were examined and ITS and ncLSU sequences were generated (Table 1). One of these is an authentic, single-ascospore isolate listed among specimens examined in the protologue of *Dischloridium camelliae* (Sivanesan & Alcorn 2002).

The ESEM photographs of conidia of *M. camelliae* (Fig. 8A, B, F, G) demonstrate well that there is a continuum between conidial chains and slimy heads on the phialides. The osmolarity of the medium may influence the relative proportion of chains and slimy heads as seen particularly in *Chloridium,* where chains, cirrhi, and

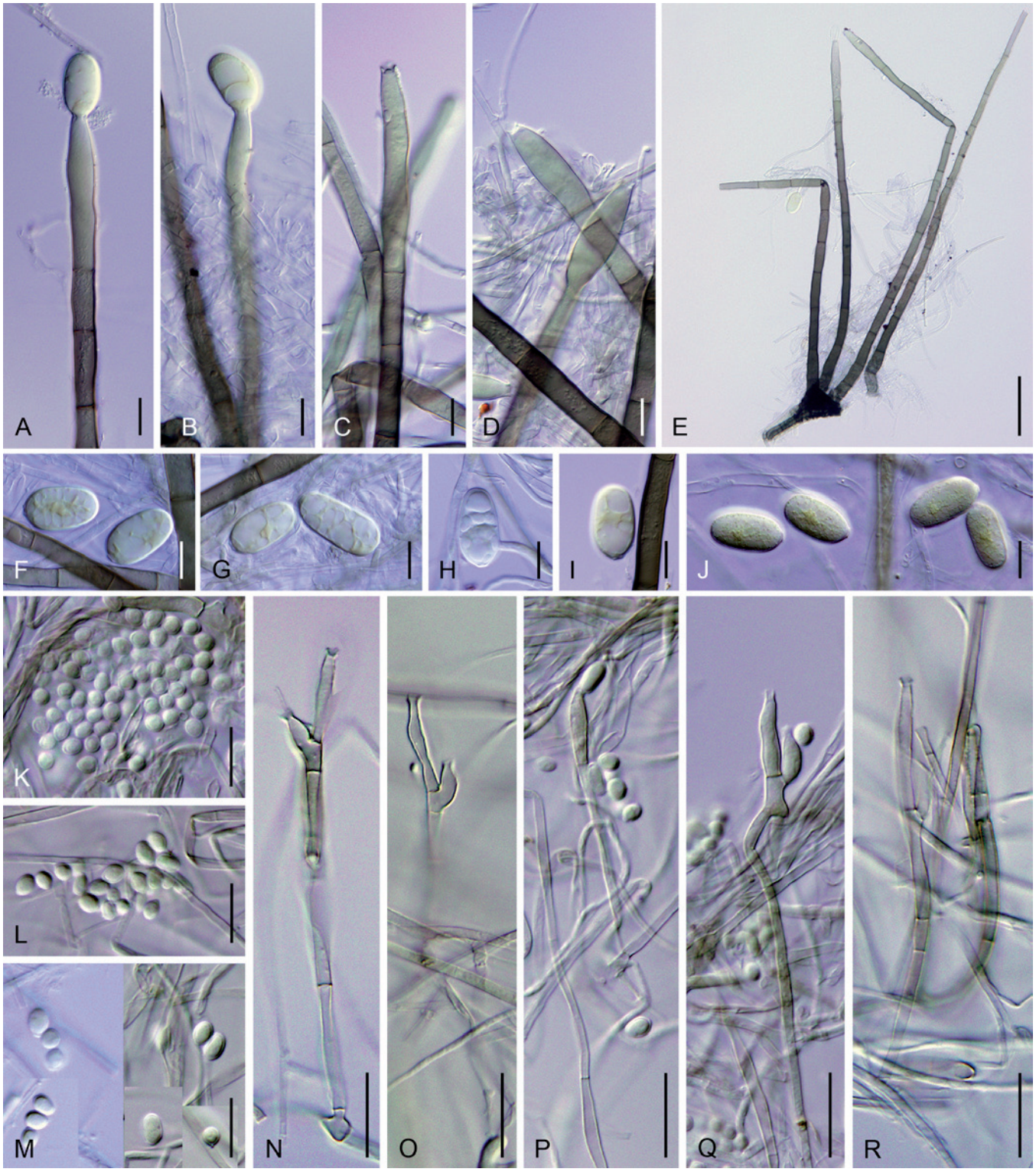

Fig. 7.A–R. *Monilochaetes camelliae* anamorph of *Australiasca queenslandica*. A–D. Conidiogenous cells. E. Conidiophores. F–J. Conidia. K–M. Microconidia. N–R. Minute conidiophores that produce microconidia. A–J from BRIP 24607 (PCA, 14 d old), K–R from BRIP 24334c (PCA, 6 mo old). Scale bars: A–D, F–J, K–R = 10 µm; E = 50 µm. A–R: DIC.

slimy heads are all observed in one genus or even one species (W. Gams, unpubl. data). The conidial chains of *M. camelliae* were difficult to observe in squash mounts from agar, but were visible directly in the Petri dish by light microscopy.

Additional species of *Monilochaetes*

Since its description the original generic concept of *Dischloridium* has been expanded with the addition of fifteen species having variable morphology of conidia and conidiophores including several species with brown, distoseptate conidia. To be consistent with the morphological delimitation of *Monilochaetes* indicated by phylogeny, we accept only two of the fourteen remaining species previously included in *Dischloridium* for transfer to *Monilochaetes,* namely *D. basicurvatum* and *D. regenerans.* Other species are newly transferred to or accepted in other hyphomycete genera, such as *Craspedodidymum*, *Hyalocylindrophora*, or *Paradischloridium*, and a few cannot presently be reassigned.

After revising type material, cultivation studies, and molecular data of *Exochalara longissima*, the type species of that genus, we

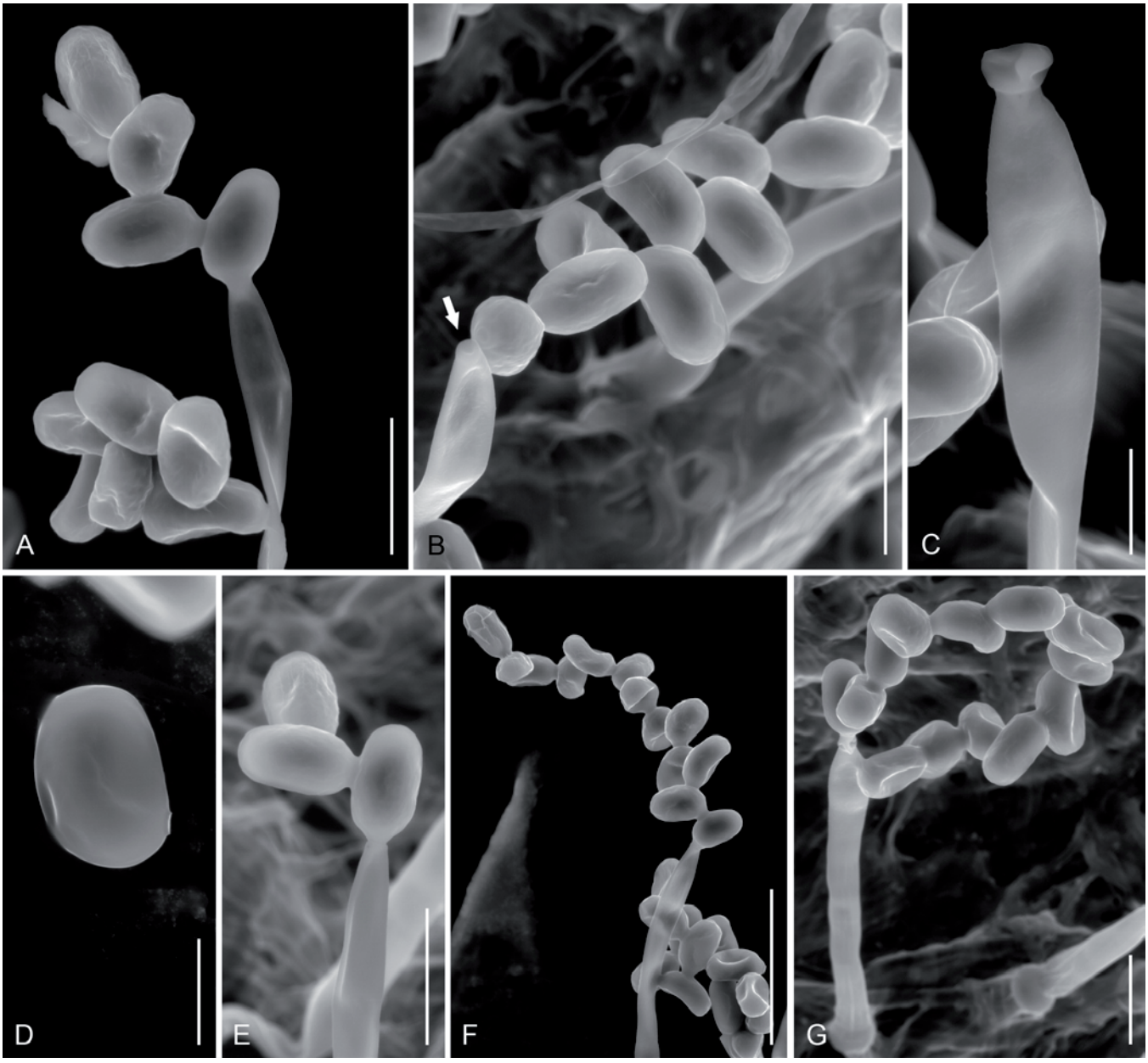

Fig. 8.A–G. Environmental Scanning Electron Microscopy photographs of *Monilochaetes camelliae* anamorph of *Australiasca queenslandica*. A, B, F, G. Chains of conidia; arrow indicates the tip with a porus of the conidiogenous cell after the liberation of the conidial chain. C. Conidiogenous cells with collarette. D. Conidium with laterally displaced hilum. E. Conidiogenous cell with conidia. A–G from BRIP 24607 (PCA, 14 d old). A, B, E, G = 20 µm; C, D = 10 µm; F = 50 µm.

confirm that the species is unrelated to *Monilochaetes* (material and isolates examined: IMI 18047 holotype of *Chalara longissima*; IMI 167413 holotype of *Catenularia piceae*; CBS 980.73, cited as the only strain in the description of *E. longissima* by Gams & Holubová-Jechová 1976, and CBS 393.82). The true relationship of the genus *Exochalara* lies with the *Helotiales* of the *Leotiomycetes* (Réblová *et al*. 2011). The strain studied by Rong & Gams (2000), CBS 662.82, with pronounced branching of the short conidiophores, is not conspecific with or related to *E. longissima.*

Monilochaetes Halst., New Jersey Agric. Exp. Stn. Bull. 76: 27. 1890.
= *Dischloridium* B. Sutton, Kavaka 4: 47. 1976.

Monilochaetes basicurvata (Matsush.) Réblová & Seifert, **comb. nov.** MycoBank MB515432.
Basionym: *Dischloridium basicurvatum* Matsush., Matsush. Mycol. Mem. 8: 18. 1995.

Monilochaetes guadalcanalensis (Matsush.) I.H. Rong & W. Gams, Mycotaxon 76: 455. 2000.
Basionym: *Catenularia guadalcanalensis* Matsush., Microfungi of the Salomon Islands and Papua New Guinea, Kobe, p. 10. 1971.
≡ *Exochalara guadalcanalensis* (Matsush.) W. Gams & Hol.-Jech., Stud. Mycol. 13: 58. 1976.

Monilochaetes infuscans Ellis & Halst., New Jersey Agric. Exp. Stn. Bull. 76: 27. 1890. Fig. 9A–I.
= *Dischloridium cylindrospermum* S.K. Srivast., Sydowia 39: 217. 1986.

Monilochaetes regenerans (Bhat & W.B. Kendr.) Réblová & Seifert, **comb. nov.** MycoBank MB515433.
Basionym: *Dischloridium regenerans* Bhat & W.B. Kendr., Mycotaxon 49: 48. 1993.

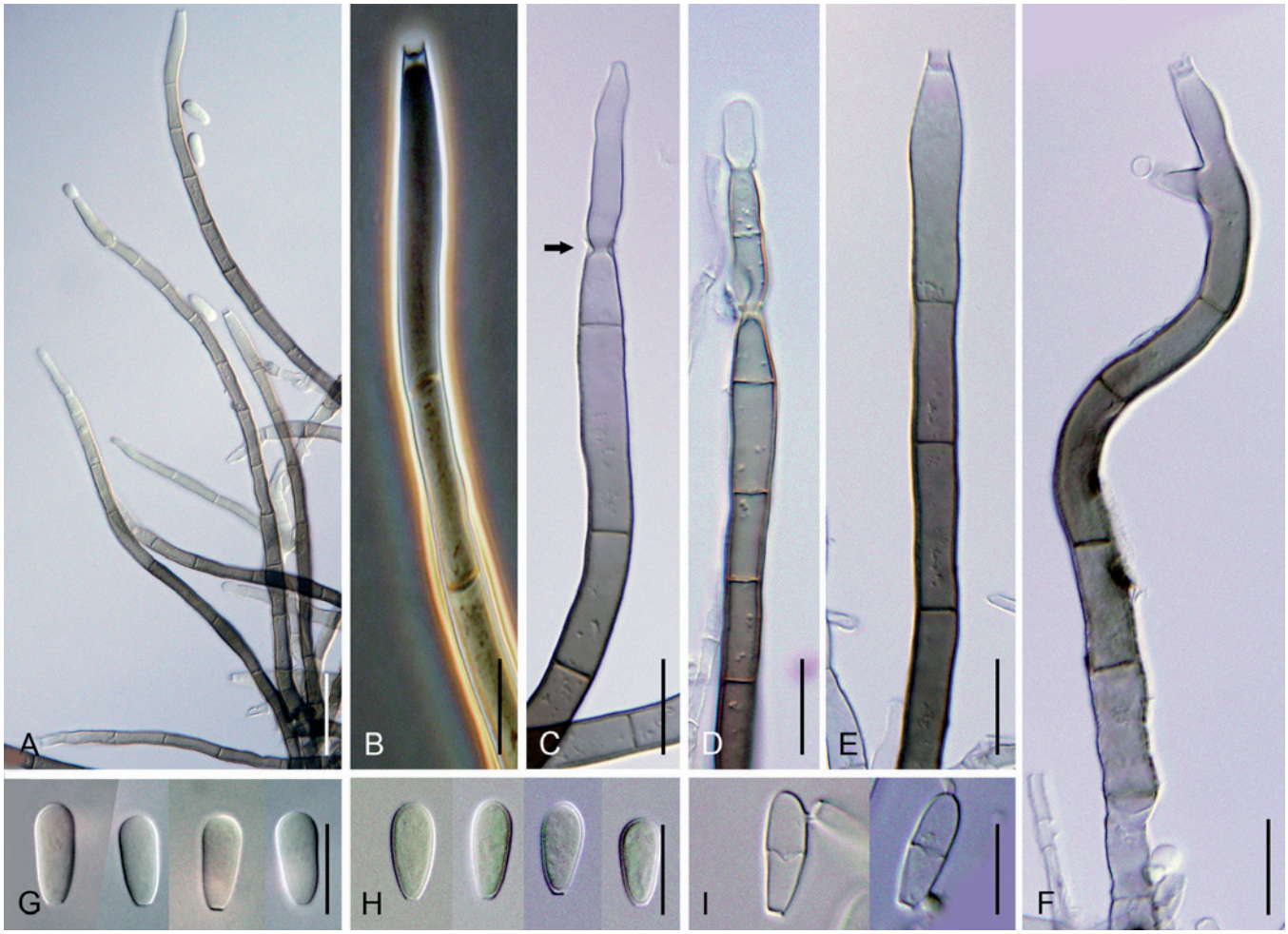

Fig. 9.A–I. *Monilochaetes infuscans*. A–F. Conidiophores; arrow indicates a percurrent regeneration of the conidiophore. G–I. Conidia. A–I from CBS 379.77 (PCA, 14 d old). Scale bars: A = 25 µm; B–I = 10 µm. DIC: A, C–I. PC: B.

Species excluded from *Dischloridium* and *Monilochaetes,* but not reclassified

Accepted names are printed in **bold**.

Dischloridium keniense P.M. Kirk, Mycotaxon 23: 30. 1985.
Basionym: *Craspedodidymum keniense* (P.M. Kirk) Bhat & W.B. Kendr., Mycotaxon 49: 37. 1993.

Dischloridium roseum (Petch) Seifert & W. Gams, Mycotaxon 24: 459. 1985.
Basionym: *Acremonium roseum* Petch, Ann. Royal Bot. Gard. Peradeniya 7: 317. 1922.
≡ ***Hyalocylindrophora rosea*** (Petch) Réblová & W. Gams, **comb. nov.** MycoBank MB515434
= *Hyalocylindrophora venezuelensis* J.L. Crane & Dumont, Canad. J. Bot. 56: 2616. 1978.
≡ *Dischloridium venezuelense* (J.L. Crane & Dumont) Bhat & B. Sutton, Trans. Brit. Mycol. Soc. 84: 725. 1985.

Notes: With this new combination the combining authors accept the argument by Holubová-Jechová (1990) that this hyaline species should not be considered congeneric with similar pigmented species. The species has not been cultured or sequenced.

Dischloridium triseptatum Hol.-Jech, Česká Mykol. 41: 110. 1987. Fig. 10A–J.
= ***Paradischloridium ychaffrei*** Bhat & B. Sutton, Trans. Brit. Mycol. Soc. 84: 723. 1985.

Specimens examined: **Cuba,** Oriente, Gran Piedra Mts., Nature Reserve Isabelica Norte, near Santiago de Cuba, on dead branches of an unidentified tree, 22 May 1985, V. Holubová-Jechová, PRM 842733, **holotype** of *D. triseptatum*.

Dischloridium venezuelense (J.L. Crane & Dumont) Bhat & B. Sutton, Trans. Brit. Mycol. Soc. 84: 725. 1985.
= ***Hyalocylindrophora rosea*** (Petch) Réblová & W. Gams (see above).

Dischloridium ychaffrei (Bhat & B. Sutton) Hol.-Jech., Česká Mykol. 42: 204. 1988.
Basionym: ***Paradischloridium ychaffrei*** Bhat & B. Sutton, Trans. Brit. Mycol. Soc. 84: 723. 1985. Fig. 10A–J.
= *Dischloridium triseptatum* Hol.-Jech, Česká Mykol. 41: 110. 1987.

Notes: *Paradischloridium* was erected for phialidic dematiaceous hyphomycetes reminiscent of *Dischloridium*, but with conidiophores that are not fasciculate and do not arise from stromatic tissue. The phialides lack even remnants of a collarette and conidia are brown with 3-distosepta (Bhat & Sutton 1985). The conidiogenesis of *P. ychaffrei* is particularly interesting. Fig. 10C–F show the conidiogenous locus sitting deeper in the venter of the cylindrical phialide than is typical for *M. laeënsis* or other *Monilochaetes* species; it is located more towards the bottom of the conidiogenous cells. Fig. 10C, D show young, hyaline conidia formed within the venter. In Fig. 10B, F, the top of a new phialide appears to be proliferating through the old phialide and collarette to form a new functional phialide. Similar phialidic structures and conidium ontogeny were described, for example, in species of *Catenularia*,

Fig. 10.A–J. *Paradischloridium ychaffrei*. A–H. Conidiophores. I. Conidia. J. Base of the conidiophore. K–T. *Dischloridium tenuisporum*. K–P. Conidiophores with conidia. Q. Conidia. R–T. Stromatic spots erumpent through the epidermis of the host bearing clusters of conidiophores. A–J from PRM 842733 (holotype of *Dischloridium triseptatum*), on the host; K–T from PRM 842727 (holotype), on the host. Scale bars: A–J, Q = 10 µm; K–P = 25 µm; R, T = 50 µm; S = 250 µm. DIC: A–J.

Chloridium, and *Sporoschismopsis* (Holubová-Jechová & Hennebert 1972). No living culture of *P. ychaffrei* was available to further assess the phylogenetic relationships of this genus.

Dischloridium species of uncertain status

A few *Dischloridium* species remain that cannot be transferred to *Monilochaetes* or other genera. Three of these form a group of morphologically similar taxa with features intermediate between *Monilochaetes* and *Colletotrichum*, *viz. Dischloridium*

gloeosporioides, *D. livistoniae,* and *D. tenuisporum.* The former two species were transferred into *Dischloridium* from *Cladosporium* and *Fusicladium* when Schubert & Braun (2005) observed terminal, monophialidic conidiogenous cells, stromata, and brown fasciculate conidiophores with paler tips. *Dischloridium gloeosporioides* was described from living stems and leaves, while the other two species were collected on dead leaves or petioles. All three produce well-delimited, subcircular to irregular spots, caused by emerging stromatic tissue that ruptures the epidermis (Fig. 10R–T), unlike the effuse colonies of *D. laeënse*. Conidia of *D. gloeosporioides* and *D. livistoniae* are obovoidal or ellipsoid-ovoidal, whereas conidia of *D. tenuisporum* are ellipsoidal to elongate-ellipsoidal, sometimes with a basal papilla. The single conidiogenous locus is at the base of the indistinct collarette. Although characters such as discrete stromatic tissue, fasciculate conidiophores, and terminal monophialides with hyaline conidia match the profile of dematiaceous hyphomycetes associated with the *Glomerellales*, transferring them to *Monilochaetes* or describing a new genus for them seems ill-advised until cultures and molecular data are available. Therefore, these species remain as *incertae sedis*.

Dischloridium gloeosporioides (G.F. Atk.) U. Braun & K. Schub., Fung. Diversity 20: 189. 2005.
Basionym: *Cladosporium gloeosporioides* G.F. Atk., Cornell Univ. Sci. Bull. 3: 39. 1897.

Dischloridium inaequiseptatum (Matsush.) Hol.-Jech., Česká Mykol. 41: 111. 1987.
Basionym: *Endophragmia inaequiseptata* Matsush., Icones Microfungorum a Matsushima lectorum, Kobe, p. 69. 1975.

Notes: This species is not accepted in *Monilochaetes* because of its 3-septate, cylindrical, slightly curved conidia with a subhyaline basal cell and the remainder of the conidial cells dark brown. Conidiophores are fasciculate without stromatic tissue; a collarette is absent or very indistinct.

Dischloridium livistoniae (P. Karst.) U. Braun & K. Schub., Fung. Diversity 20: 192. 2005.
Basionym: *Fusicladium livistoniae* P. Karst., Hedwigia 30: 302. 1891.

Dischloridium microsporum R.F. Castañeda & W.B. Kendr., Univ. Waterloo Biol. Ser. 35: 50 (fig. 29). 1991.

Specimen examined: **Cuba,** La Estrella, Buey Arriba, Granma, on dead leaves of *Trophis racemosa*, 14 Mar. 1991, R.F. Castañeda, INIFAT C91/98-2, **holotype**, ex-type strain CBS 498.92.

Notes: The ex-type strain (CBS 498.92) looks *Acremonium*-like; it formed a *Nectria*-like teleomorph *in vitro* (W. Gams, unpubl. data).

Dischloridium tenuisporum Hol.-Jech., Česká Mykol. 41: 31. 1987. Fig. 10K–T.

Specimen examined: **Cuba,** Habana province, Jaruco, Loma de la Coca, south from Campo Florido, 142 m a. s. l., on dead leaves of *Clusia rosea*, 13 Feb. 1981, V. Holubová-Jechová, PRM 842727, **holotype**.

Reticulascaceae

This family contains two holomorph genera, *Reticulascus* and *Porosphaerellopsis.* Although these genera differ morphologically, ontogeny and morphology of the centrum and interthecial filaments unite them and partially define the family. The interthecial tissue is formed of filiform branching and anastomosing filaments, forming a "network" among the asci. They are attached to the hymenium and to the top of the ascomatal wall. This structure was first described and illustrated by Samuels & Müller (1978) for *Porosphaerellopsis sporoschismophora* and is documented here for *Reticulascus tulasneorum* and *R. clavatus*. The second species in the genus *Porosphaerellopsis, P. bipolaris* (Ranghoo *et al*. 2001), collected on submerged wood in a stream in China, does not form this "network", and has paraphyses that are wider and simple. The link between *P. bipolaris* and a *Sporoschismopsis* anamorph suggested by Ranghoo *et al.* (2001) has not been established convincingly.

Reticulascaceae Réblová & W. Gams, **fam. nov.** MycoBank MB515435.

Stromata minuta nonnumquam formata. Ascomata perithecia, fusca usque nigra, ostiolum periphysatum. Pariete ascomatum 2-stratoso. Hamathecium paraphyses verae; paraphyses septatae, hyalinae, ramosae, anastomosantes, sursum angustatae, ascos superantes. Asci unitunicati, cylindraceo-clavati, 8-spori, annulo apicali iodo non reagente. Ascosporae hyalinae vel atrobrunneae, ellipsoideae usque fusiformes, septatae, nonnumquam utrinque poro praeditae. Anamorphae: *Cylindrotrichum*, *Sporoschismopsis*; conidia modo phialidico formata.

Typus: *Reticulascus* Réblová & W. Gams.

Stromata minute, sometimes present. *Perithecia* brown to black. Ostiolum periphysate. *Perithecial wall* 2-layered. Interascal tissue of thin-walled, tapering, branching and anastomosing paraphyses. *Asci* unitunicate, 8-spored, cylindrical-clavate, apical ring inamyloid. *Ascospores* hyaline or dark brown, ellipsoid to fusiform, sometimes with end pores. Anamorphs: *Cylindrotrichum*, *Sporoschismopsis*; conidiogenesis phialidic.

Reticulascus Réblová & W. Gams, **gen. nov.** MycoBank MB515436.

Etymology: from the Latin *ascus* and *reticulum*, referring to the network of interthecial filaments.

Stromata absentia. Perithecia superficialia, solitaria vel aggregata, fusca, venter subglobosus usque conicus, ostiolum periphysatum. Paries perithecii fragilis, bistratosus. Paraphyses septatae, hyalinae, filiformes, ramosae, anastomosantes, reticulum formantes, sursum angustatae, ascos superantes. Asci unitunicati, cylindraceo-clavati, 8-spori, brevi-stipitati. Ascosporae ellipsoideae usque fusiformes, hyalinae, septatae. Anamorphe *Cylindrotrichum*.

Typus: *Reticulascus tulasneorum* (Réblová & W. Gams) Réblová & W. Gams.

Stroma absent. *Perithecia* superficial, solitary, or gregarious, brown, venter subglobose to conical. Ostiolum periphysate. *Perithecial wall* fragile, 2-layered. *Paraphyses* septate, hyaline, filiform, forming a branching and anastomosing "network". *Asci* unitunicate, cylindrical-clavate, 8-spored, short-stipitate. *Ascospores* ellipsoidal to fusiform, hyaline, septate. Anamorph *Cylindrotrichum*.

Notes: Based on the results of our ITS, ncLSU, ncSSU analyses, and the combined three-gene phylogeny, the new holomorph genus *Reticulascus* is introduced below for two holomorph species. *Chaetosphaeria tulasneorum* with the anamorph *Cylindrotrichum oligospermum* including *C. hennebertii* as a synonym, is recombined as the type of *Reticulascus*; the second species, *R. clavatus,* is introduced as a new species with *C. clavatum* as its anamorph.

Several anamorphic species are related to this clade. The delimitation of *Cylindrotrichum,* typified by *C. oligosporum,* and morphologically similar genera of dematiaceous hyphomyces has been controversial, with varying concepts proposed by Gams & Holubová-Jechová (1976), DiCosmo *et al*. (1983), Rambelli & Onofri (1987), Arambarri & Cabello (1989), and Holubová-Jechová (1990). The *Cylindrotrichum* anamorphs of *Reticulascus* species generally resemble the dematiaceous, phialidic hyphomycetous anamorphs linked with *Chaetosphaeria* (Réblová 2000, 2004), but the presence of cylindrical, 1-septate conidia seems to be a deviating character. The conidia are formed from conspicuously sympodially proliferating, terminally integrated phialides within shallow collarettes (Gams & Holubová-Jechová 1976: 48, figs 23, 24; Réblová & Gams 1999: 34, fig. 16). Based on a ncLSU phylogeny, Réblová & Winka (2000) showed that several species included in *Cylindrotrichum* by Gams & Holubová-Jechová (1976) belong to the *Chaetosphaeriaceae* (*Chaetosphaeriales*), but others are phylogenetically unrelated with a possible affinity with the *Microascales*. Our molecular analyses of ITS, ncLSU, ncSSU, and the combined data set of three genes (Figs 1–4) confirm that *Reticulascus tulasneorum/C. oligospermum*, *R. clavatus/C. clavatum*, the newly described anamorphic *C. setosum,* previously known species *C. gorii*, *Kylindria peruamazonensis,* and *Porosphaerellopsis* (anamorph *Sporoschismopsis*) group outside the *Chaetosphaeriales* and *Microascales*. They form a monophyletic group that we recognise as a new family within the *Glomerellales*.

The dematiaceous hyphomycete genera *Cylindrotrichum*, *Kylindria,* and *Sporoschismopsis*, linked as anamorphs with the *Reticulascaceae*, possess conidia that vary in shape, colour, and size, and, although conidiogenesis is phialidic, the position of the conidiogenous locus within the collarette also varies. In *Sporoschismopsis,* the first and a few subsequent conidia arise endogenously and are formed in basipetal succession from the apical portion of the phialide from deep-set conidiogenous loci within a deep collarette. After formation of several conidia, the phialide proliferates through the collarette to form a new functional phialide (Holubová-Jechová & Hennebert 1972: 385, fig. 1). Similar conidium ontogeny also occurs in *Catenularia* and *Chloridium* anamorphs of *Chaetosphaeria* species and in species of *Cadophora* or *Phialophora*.

The remaining 18 species previously classified in *Cylindrotrichum*, including those transferred to *Kylindria* (13 species) and *Xenokylindria* (3 species) by DiCosmo *et al*. (1983), are putative members of the *Chaetosphaeriales* for which the name *Kylindria* was given preference by Réblová (2000). In fact, the *Cylindrotrichum*-like anamorphs linked with *Chaetosphaeria* are variations on the *Chloridium* theme and do not represent a unique or unusual pattern within the *Chaetosphaeriaceae*. If future analyses confirm the placement of *K. triseptata* in the *Reticulascaceae*, *Kylindria* will be excluded from the anamorphs linked with *Chaetosphaeria* and separated from *Xenokylindria*.

Kylindria peruamazonensis did not group in the same clade as the four *Cylindrotrichum* species, rather it formed a poorly supported branch with *Porosphaerellopsis* at the base of the *Reticulascus/Cylindrotrichum* clade (Figs 1, 4). This species is discussed and illustrated below and is the only typical representative of the genus *Kylindria* included in our analysis. Unlike *Cylindrotrichum* species of *Kylindria* have oblong, longer, and wider, 1-several-septate, often asymmetrical conidia and wider and shorter conidiophores terminating with a monophialide swollen in its upper part with or without a collarette. The phialides occasionally elongate above the collarette with several percurrent extensions. These characters contrast with *Cylindrotrichum* having 1-septate, symmetrical, cylindrical conidia and narrower, longer, and often seta-like conidiophores with cylindrical mono- or polyphialides that never elongate above the collarette. Because of the morphological characters distinguishing *Kylindria* and *Cylindrotrichum* and results from the ITS and ncLSU phylogenetic analyses, we prefer to keep these anamorph genera separate.

Reticulascus tulasneorum (Réblová & W. Gams) Réblová & W. Gams, **comb. nov.** MycoBank MB515437. Fig. 11.

Basionym: *Chaetosphaeria tulasneorum* Réblová & W. Gams, Czech Mycol. 51: 32. 1999.

Anamorph: *Cylindrotrichum oligospermum* (Corda) Bonord., Handb. Allg. Mykol. p. 88. 1851.

= *Cylindrotrichum hennebertii* W. Gams & Hol.-Jech., Stud. Mycol. 13: 50. 1976.

For a full description and more information, refer to Réblová & Gams (1999).

Specimen examined: **Czech Republic**, South-western Bohemia, Javornická hornatina Mts., Strašín near Sušice, on dead branch of *Sambucus nigra*, 21 Oct. 1997, M. Svrček, PRM 842978, **holotype** of *Chaetosphaeria tulasneorum*, ex-type strain CBS 101319.

Notes: *Reticulascus tulasneorum* produces minute, black, nonstromatic ascomata growing on decaying wood. The ascospores are hyaline, narrowly ellipsoidal, 1- to rarely 3-septate, and glabrous at maturity, similar to those of *R. clavatus* having slightly verruculose ascospores. In the features of asci, interthecial filaments, and perithecial wall, these species are indistinguishable. The morphological characters of the associated anamorphs are diagnostic. The teleomorph is known from only one locality (Réblová & Gams 1999).

Cylindrotrichum hennebertii (ex-type strain CBS 570.76) groups with *R. tulasneorum* including its anamorph *C. oligospermum*. The former taxon was described for specimens with only a short layer of conidiophores (Gams & Holubová-Jechová 1976: 50, fig. 24), contrasting with the development of two strata of conidiophores for the latter species. The layering of the conidiophores described in the protologue of *C. hennebertii* seems to be quite variable depending on substrate and age of the material. With the further evidence of their identical ITS sequences, *C. hennebertii* is now regarded as a synonym of *C. oligospermum*, the anamorph of *Reticulascus tulasneorum*.

Reticulascus clavatus Réblová & Fournier, **sp. nov**. MycoBank MB515652. Figs 11F–M, 12A–F.

Anamorph: *Cylindrotrichum clavatum* W. Gams & Hol.-Jech., Stud. Mycol. 43: 54. 1976.

Etymology: Epithet taken from that of the anamorph species, derived from the shape of conidia.

Perithecia 150–170 µm alta, 120–200 µm diam, superficialia, solitaria, subglobosa vel conica, minute papillata, ostiolata, glabra. Canalis ostiolaris periphysatus. Paries perithecii fragilis, ad latus et apicem sclerotialis, deorsum attenuatus; paries lateralis 15 µm crassus, bistratosus. Paraphyses copiosae, filiformes, septatae, ramosae, anastomosantes, reticulum formantes, hyalinae, 1.5 µm latae, ultra ascorum apices protrudentes. Asci 87–108 × 7–8.5 µm (in medio ± s.e. = 95.5 ± 0.2 × 7.5 ± 0.2 µm), cylindrici vel clavati, breviter stipitati. Ascosporae 14–18(–19) × 4–4.5 µm (in medio ± s.e. = 15.7 ± 0.2 × 4.4 ± 0.04 µm), fusiformes, bi- vel quadri-cellulares, verruculosae, hyalinae, 1–2-seriatae in asco.

Perithecia 150–170 µm high, 120–200 µm diam, scattered among conidiophores, superficial, solitary, subglobose to conical, with

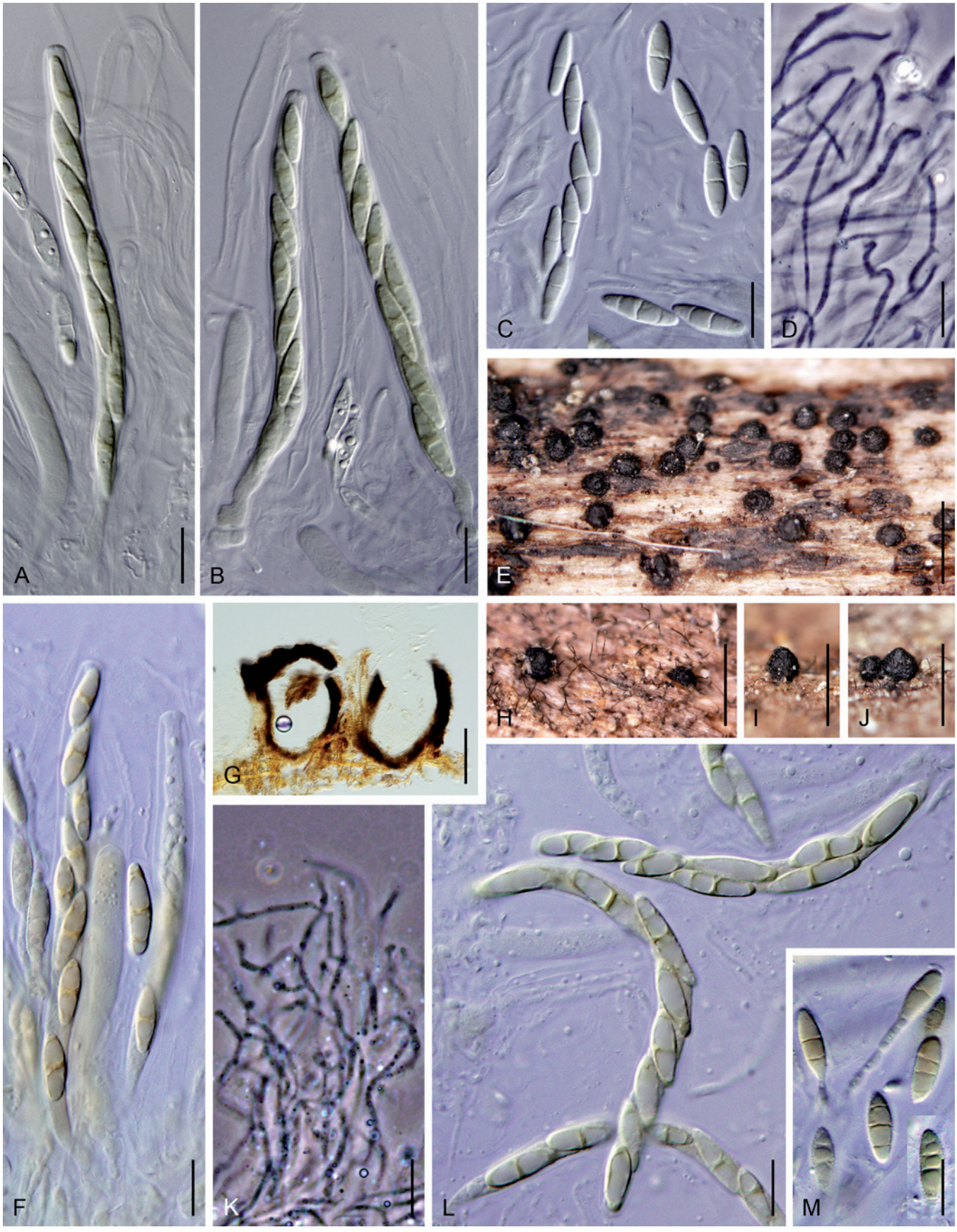

Fig. 11.A–E. *Reticulascus tulasneorum*. A, B. Asci containing ascospores. C. Ascospores. D. Interthecial filaments. E. Perithecia on the host. F–M. *Reticulascus clavatus*. F, L. Asci with ascospores. G. Vertical section of the perithecial wall. H–J. Perithecia with conidiophores of the anamorph on the host. K. Interthecial filaments. M. Ascospores. A–E from PRM 842978 (holotype); F–M from PRM 915717 (holotype). Scale bars: A = A–D, F, K–M = 10 µm; E, H–J = 250 µm; G = 50 µm. DIC: A–C, E–J, L, M; PC: D, K.

minute papilla, glabrous, ostiolum lined with periphyses. *Perithecial wall* brittle, heavily sclerotised in upper part, sclerotisation weakens towards base. Lateral wall *ca.* 15 µm thick, 2-layered: outer layer of thin-walled, dark brown, brick-like cells; inner layer of flattened, elongated hyaline cells. *Paraphyses ca.* 1.5 µm wide, copious, filiform, sparsely septate, not constricted at septa, forming a network, hyaline, longer than asci. *Asci* 87–108 × 7–8.5 µm (mean ± S.E. = 95.5 ± 0.2 × 7.5 ± 0.2 µm), cylindrical to clavate, slightly

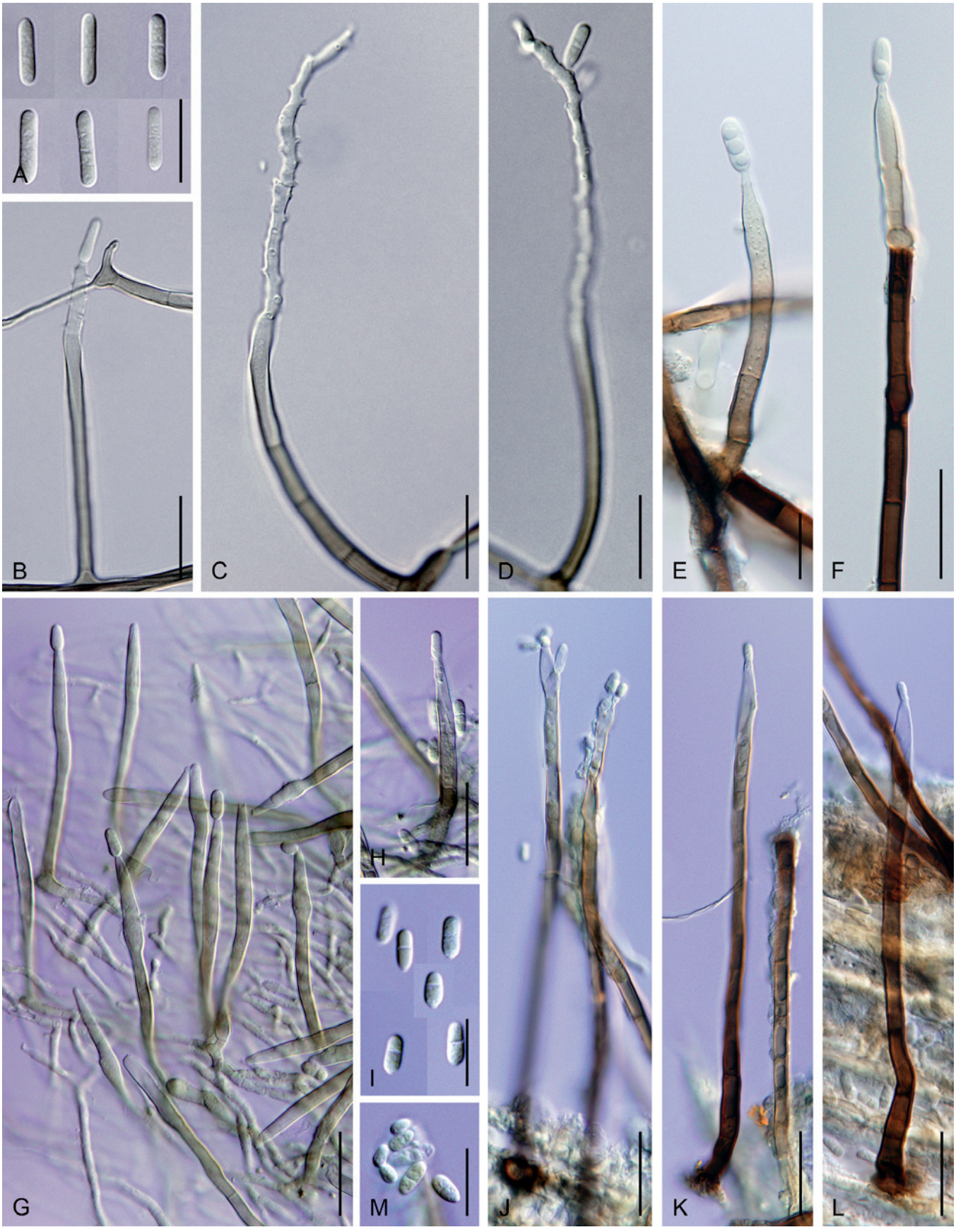

Fig. 12.A–F. *Cylindrotrichum clavatum* anamorph of *Reticulascus clavatus*. A. Conidia. B–D. Conidiophores of the lower layer (shorter conidiophores) with sympodially extending sporiferous apices, in culture. E–F. Conidiophores ending into a monophialide on the host. A–C from ex–type strain CBS 125296 (PCA, 14 d old), E–F from PRM 915717 (holotype). G–M. *Cylindrotrichum gorii*. G, H. Conidiophores, in culture. I. Conidia, in culture. J–L. Conidiophores, on the host. M. Conidia, on the host. G–M from CBS 879.85 (PCA, 14 d old). Scale bars: A = 10 µm; B–F = 20 µm; G, H, J–L = 20 µm; I, M = 10 µm. DIC: A–F, G–M.

truncate to broadly rounded at apex, short–stipitate, ascal apex with inamyloid apical annulus, 3–3.5 µm wide, 1–1.5 µm deep, 8-spored. *Ascospores* 14–18(–19) × 4–4.5 µm (mean ± S.E. = 15.7 ± 0.2 × 4.4 ± 0.04 µm), fusiform, 2–4-celled, with a delayed formation of second and third septa, slightly constricted at septa, mature ascospores finely verruculose, 1–2-seriate in ascus.

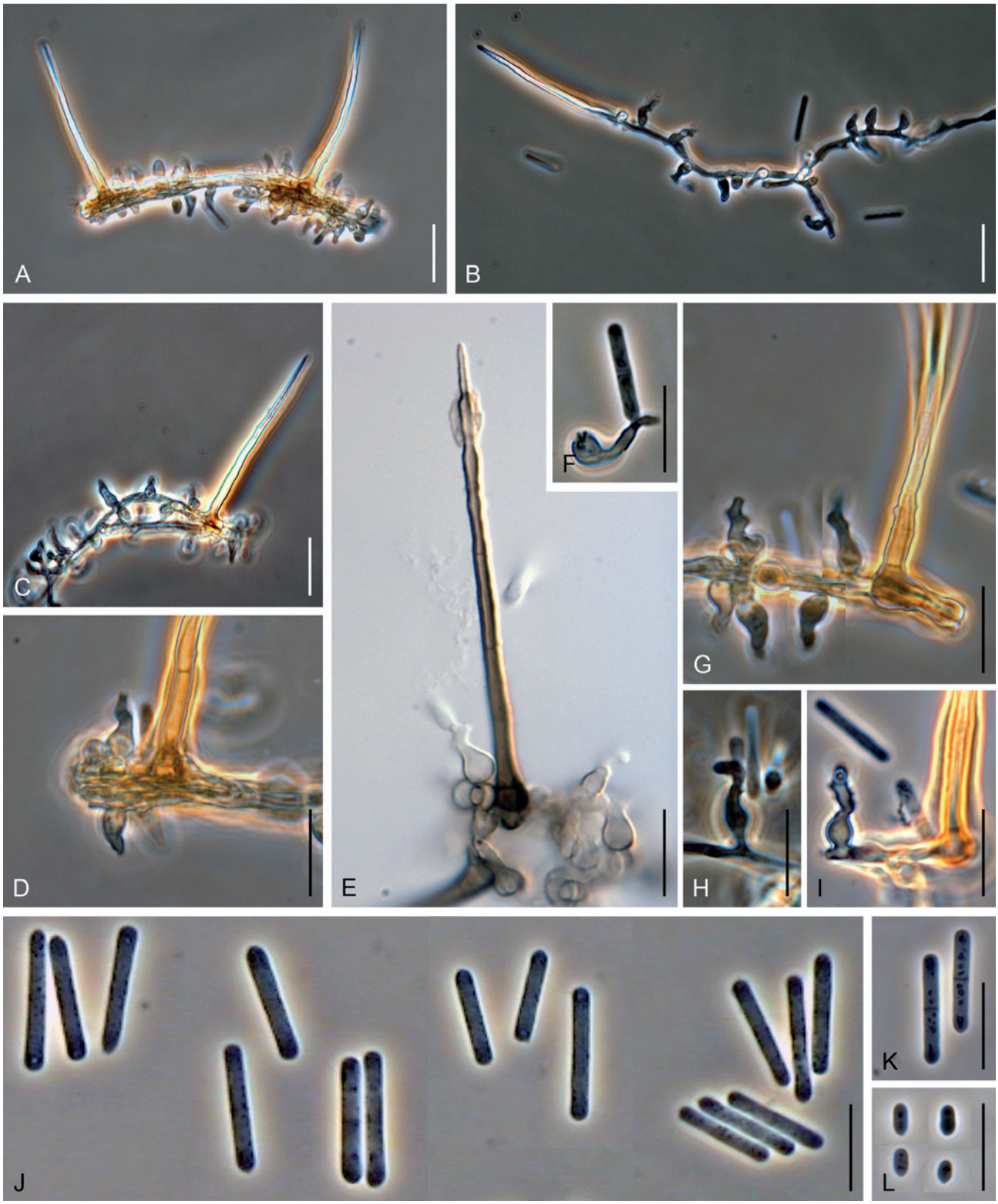

Fig. 13.A–L. *Cylindrotrichum setosum*. A–C. Conidiophores with setae and polyphialidic conidiogenous cells. D, F–I. Polyphialides. E. Seta with unbranched basal conidiogenous cells. J, K. Macroconidia. L. Microconidia. A–L from ex-type strain DAOM 229246 (OA, 3wk old). Scale bars: A–C = 20 µm; D–I = 10 µm. DIC: all PC except E, DIC.

Colonies *in vivo* brown to black, hairy, effuse. Setae absent. *Conidiophores* macronematous, mononematous, cylindrical, straight, forming two layers. *Conidiophores* of lower layer shorter, 60–135 × 4.5–5 µm, pale brown, subhyaline towards apex, 2–5-septate; longer conidiophores forming an upper layer, 200–360 × 5–5.5 µm, mid to dark brown, subhyaline towards apex, up to 10-septate; conidiophores of both layers ending in a monophialide or polyphialide. *Conidiogenous cells* 25–37 × 3.5–5 µm, usually monophialidic, rarely polyphialidic with up to two lateral openings; collarette hyaline to subhyaline, 1.5–2 µm wide, *ca.* 1.5 µm high. *Conidia* 10.5–11 × 4–4.5 µm (mean ± S.E. = 10.2 ± 0.2 × 4.2 ± 0.04 µm), cylindrical, rounded at apex, slightly tapering, obtuse at base, 1-septate, not constricted at septum, hyaline, smooth.

Colonies *in vitro* after 14 d on PCA at 25 °C 14–17 mm diam, cushion–like, aerial mycelium greyish brown, margin entire, reverse dark brown. Colonies sporulating after 7–10 d

on PCA at 25 °C in darkness. *Conidiophores* macronematous, mononematous, solitary, erect, forming two layers: conidiophores of lower layer 50–100 × 2.5–3 µm, cylindrical, straight or slightly flexuous, 2–10-septate, pale brown, subhyaline to hyaline towards apex; conidiophores of upper layer up to 260 µm long, 3.5–4 µm wide, mid brown, subhyaline towards apex. *Conidiogenous cells* integrated, terminal or intercalary, with up to 30 lateral phialidic openings arising from sympodial elongation, fertile apices 15–70 µm long; collarettes hyaline to subhyaline, 1–1.5 µm wide, *ca.* 1.5 µm high. *Conidia* 9–12.5(–13.5) × 2.5–3 µm (mean ± S.E. = 11.6 ± 0.3 × 2.7 ± 0.05 µm), cylindrical, rounded at apex, slightly tapering, obtuse at base, 1-septate, not constricted at septum, hyaline, smooth. In PDA culture, conidia slightly smaller, 8.5–10.5 × 2.5(–3) µm (mean ± S.E. = 9.3 ± 0.1 × 2.6 ± 0.03 µm).

Specimens examined: **France**, Haute Garonne, Mancioux, along road D635 on the way to Frechet, on submerged wood of *Alnus glutinosa*, 28 Feb. 2009, J. Fournier no. J.F. 09009, PRM 915717, **holotype**, ex-type strain CBS 125296; Rimont, Le Baup, on submerged wood of *Fraxinus* sp., 12 June 2009, J. Fournier no. J.F. 09154, PRM 915718, living culture CBS 125297; Ariège, Rimont, road D18, 1.5 km south of the village, Le Baup, 500 m a. s. l., on submerged wood of *Platanus* sp., associated with *Achroceratosphaeria potamia*, *Cosmospora* sp., *Savoryella limnetica*, 23 May 2008, J. Fournier & M. Delpont no. J.F. 08139, PRM 915719, living culture CBS 125239.

Notes: *Reticulascus clavatus* is a common dweller of submerged wood in lotic sites in France. The anamorph does not always occur on freshly collected material, although fertile conidiophores usually appear after incubation in a moist chamber for 1–2 wk (J. Fournier, unpubl. data).

Reticulascus clavatus differs from the closely related *R. tulasneorum* and its *C. oligospermum* anamorph by verruculose mature ascospores, absence of setae among the conidiophores, which terminate with a monophialide *in vivo* and only rarely a polyphialide. In axenic culture (PCA, PDA) of *R. clavatus,* the lower layer of conidiophores terminates in polyphialides with up to 30 lateral openings (Fig. 12C, D).

Cylindrotrichum setosum Seifert, **sp. nov.** MycoBank MB515589. Fig. 13A–L.

Coloniae in agaro farina avenae confecto post 20 dies radium 6–7 mm attingentes, in agaro maltoso 4–5 mm. Conidiophora simplicia vel raro ramosa, stipite subhyalino vel dilute brunneo ad 200 µm longo, 1.5–2.5 µm lato, vel cellulae conidiogenae ex hyphis fasciculatis dilute brunneis, 3.5–8.5 µm latis singulae vel acervatae oriundae; setae seu conidiophora superantes seu ex hyphis aggregatis perpendiculariter oriundae, 45–80 µm longae, simplices, brunneae vel fuscae, aciculares, in parte inferiore 3.5–4 µm latae, sursum acutatae. Cellulae conidiogenae mono- vel polyphialides, subhyalinae vel dilute brunneae, ampulliformes vel subulatae, 6–13 µm longae, parte inferiore ellipsoidea, 3.5–7 × 2–4 µm, rachide recta vel geniculata ad 7 × 1.5–2.5 µm, 1–6 foramina conidiogena sessilia vel < 3 µm longa ferente, collare inconspicuum, vix periclinaliter inspissatum. Conidia cylindrica, 1-septata, 12–16.5 × 1.5–2.5 µm; microconidia rara, continua, ellipsoidea vel oblonge ellipsoidea, 3.5–5 × 1.5–2.5 µm.

Colonies on OA after 3 wk about 6–7 mm radial growth, more or less planar, surface pale yellow, with little aerial mycelium but diffusely hirsute because of setae and conidiophores, margin smooth, entire, reverse unpigmented, lacking soluble pigments. Colonies on Blakeslee's malt-peptone agar after 3 wk 4–5 mm radial growth, convex, surface dark brown to black, covered with pale grey, lanose to cottony aerial mycelium that gives the central part of the colony a greyish aspect, with embedded small droplets of black exudates, with some sporulation but setae not seen, reverse grey, margin somewhat uneven.

Conidiophores on OA more or less erect, usually undulating, unbranched or sparingly branched, subhyaline to pale brown with stipes up to 200 µm long, 1.5–2.5 µm wide, or conidiogenous cells directly on erect or lax fascicles of pale brown hyphae 3.5–8.5 µm wide, more or less at right angles, single, in pairs or clusters, sometimes on a metula, with setae either terminating conidiophores or emerging from fascicles more or less at right angles. Setae 45–80 µm long, unbranched, brown to dark brown, acicular, 3.5–4 µm wide at base, slightly thick-walled, with 3–7 septa and acute apex, sometimes terminating with a monophialide. *Conidiogenous cells* monophialidic or polyphialidic, subhyaline to pale brown, concolourous with subtending hypha or conidiophore, usually ampulliform, sometimes subulate, 6–13 µm long, with an ellipsoidal base about 3.5–7 × 2–4 µm, slightly thick-walled, and then a narrower neck 1.5–2.5 µm wide, up to 7 µm long, straight or becoming geniculate with proliferation; lateral conidiogenous apertures usually sessile, but sometimes up to 3 µm long, with 1–6 conidiogenous openings about 1 µm wide with minute frills and inconspicuous periclinal thickening. *Conidia* 12–16.5 × 1.5–2.5 µm (mean ± S.E. 14.4 ± 0.2 × 2.1 ± 0.1), L/W 5.5–8, 1-septate, cylindrical, straight or rarely slightly bent, with rounded ends, base sometimes with an inconspicuous papilla-like abscission scar, accumulating in small, clear to whitish droplets. Microconidia rare, less than 1 % abundance, 3.5–5 × 1.5–2.5 µm, l/w ~1.7–3, aseptate, ellipsoidal to oblong-ellipsoidal, with a minute basal papilla, hyaline, with no obvious abscission scar.

Specimen examined: **Australia,** New South Wales, Mt. Annan Botanical Garden, S 34° 05.118', E 150° 45.438', 315 m alt., on wood and bark mulch on the ground, 26 Aug. 1999, K.A. Seifert no. 1228, DAOM 229246 **holotype**, ex-type strain in CCFC.

Notes: *Cylindrotrichum setosum* is unique in the genus because of the physical separation of the conidiogenous cells and the setae. In other species, the conidiophores are seta-like and have a terminal phialide or polyphialide at the apex. In *C. setosum*, the conidiogenous cells tend to be clustered at the base of the setae in a manner reminiscent of species of *Circinotrichum* or *Gyrothrix*. However, the proliferation of the conidiogenous cells and the morphology of the 1-septate conidia resemble other species of *Cylindrotrichum*. Unlike *C. setosum,* microconidia have not been reported in other *Cylindrotrichum* species.

KEY TO ACCEPTED SPECIES OF *CYLINDROTRICHUM*

1. Conidia cylindrical to slightly clavate, usually wider than 2.5 µm; conidiophores seta–like but sterile setae absent 2
1. Conidia cylindrical, not wider than 2.5 µm, usually 1.5–2.5 µm *in vivo*; setae present or absent 3

2. Conidia longer than 9 µm; 8.5–13 × 3–4 µm *in vivo* and 9–12.5(–13.5) × 2.5–3 µm *in vitro*; teleomorph *R. clavatus* ..... *C. clavatum*
2. Conidia shorter than 9 µm; (5–)5.5–7.5(–9) × 3–3.5 *in vivo* and 7–9(–9.5) × 3–3.5 µm *in vitro*; teleomorph unknown ..... *C. gorii* (Lunghini 1979)

3. Setae sterile, pointed, distinct from the ampulliform to subulate conidiogenous cells; teleomorph unknown ..... *C. setosum*
3. Conidiophores often seta-like, with a terminal mono- or polyphialide at the apex; teleomorph *R. tulasneorum* ..... *C. oligospermum*

Species phylogenetically related to *Cylindrotrichum*

Kylindria peruamazonensis Matsush., Matsush. Mycol. Mem. **7**: 56. 1993. Fig. 14.

Specimens examined: **Cuba**, Ciénaga de Zapata Matanzas, on leaf litter of *Bucida palustris*, Dec. 1991, R.F. Castañeda, INIFAT C91/111, living culture CBS 838.91; Sancti Spiritus. Las V, on leaf of *B. palustris*, 25 Aug. 1994, R.F. Castañeda, INIFAT C94/84R, living culture CBS 421.95.

Notes: Two strains identified as *Kylindria triseptata* were analysed (CBS 838.91, 421.95), but neither matches the fungus described by Matsushima (1975) as *Cylindrotrichum triseptatum* Matsush. in the morphology of conidiogenous cells and conidial dimensions. Our cultural observations suggest that the conidiophores, conidiogenous cells, and conidia of these strains match the description of *Kylindria peruamazonensis*. The apex of the phialide terminates with a funnel-shaped collarette unlike that of *C. triseptatum*, in which the monophialides lack a collarette and may elongate slightly and possess apical densely annellate proliferations. Previously such proliferations were observed in *Cacumisporium capitulatum*, the anamorph of *Chaetosphaeria decastyla*, and in the so-called *Cylindrotrichum* anamorph of *Ch. acutata* (Réblová & Gams 1999); these were considered a diagnostic character of *Xenokylindria* (DiCosmo *et al*. 1983). *Kylindria ellisii* also has 3-septate hyaline conidia, but differs from *K. peruamazonensis* by a hardly visible collarette and symmetrical, 3-septate conidia rounded at both ends, without an apiculus.

Unlike *K. peruamazonensis*, cylindrical to oblong, septate conidia with a tapering, obtuse to papillate base with a laterally displaced hilum are typical of several *Kylindria* species, namely *K. excentrica*, *K. pluriseptata,* and *K. triseptata*. *Kylindria excentrica* has 3-septate conidia, but differs from *K. peruamazonensis* in absence of a collarette and much larger conidia (27.5–35 × 7.5–8 µm; Bhat & Sutton 1985). *Kylindria peruamazonensis* is probably the species morphologically most similar to *K. triseptata*; it differs from the latter by the presence of a collarette, either lacking or with a very short elongation of the phialides above the collarette, with several percurrent proliferations, the unusual formation of imbricate conidial chains, and production of a microconidial form *in vitro* (macroconidia 12.5–23 × 4–7.5 µm; Matsushima 1993). Sympodial proliferation of the apex of the conidiogenous cell was not observed in cultures of *K. peruamazonensis* and *K. triseptata* (Matsushima 1975, 1993). Unlike *K. peruamazonensis, K. pluriseptata* has 6–8-septate and much longer conidia (35–40 × 5–6 µm; Castañeda 1987).

Additional anamorph species affiliated with the *Plectosphaerellaceae*

Our phylogenetic analyses place the anamorph species *Stachylidium bicolor* (DAOM 226658) in a basal position in the family *Plectosphaerellaceae* (Figs 1–3). Several anamorph genera in this family have verticillate conidiophores such as *Acrostalagmus* and *Verticillium*. *Stachylidium bicolor*, the type of its genus, produces erect, roughened, verticillate conidiophores, often with additional verticillate axes emerging from the main stipe; this results in a more complex conidiophore than in other similar genera. As with the species of the other genera, the conidiogenous cells are phialidic but taper strongly near the tip, and the conidia are oblong-ellipsoidal and accumulate in slime. We consider *S. bicolor* sufficiently distinct both morphologically and phylogenetically from *Acrostalagmus* and *Verticillium* to continue to be recognised as a distinct genus.

The phylogenetic analyses demonstrate that the common tropical hyphomycete described and illustrated by Seifert (1985) as *Stilbella annulata* is a member of the *Plectosphaerellaceae* and a sister species to *Acrostalagmus luteoalbus*, the type of the genus. Both *S. annulata* and *A. luteoalbus* produce ameroconidia in bright orange to reddish slimy masses; in both species the reddish pigmentation sometimes also colours the phialides. The conidiophore branching of *S. annulata* lacks the regular verticillate aspect of *A. luteoalbus*, being intermediate between verticillate and penicillate. The synnemata of *S. annulata* and their conspicuously lobed marginal hyphae are also deviating characters from the present generic concept of *Acrostalagmus*. Given the well-supported phylogenetic relationship between these two species, it seems preferable to focus on the similarities between these species rather than the differences and to transfer *S. annulata* to *Acrostalagmus* rather than propose a new genus. This modifies the generic concept of *Acrostalagmus* to include synnematous species:

Acrostalagmus annulatus (Berk. & Broome) Seifert, **comb. nov.** MycoBank MB518663.

Basionym: *Stilbum annulatum* Berk. & Broome, Grevillea 3: 63. 1874. (holotype: no. 6045, on Brassica sp., Car. Inf., herb. Berkeley, 1879, K.

≡ *Stilbella annulata* (Berk. & Broome) Seifert, Stud. Mycol. 27: 58. 1985

Note: For full synonymy and examined material, refer to Seifert (1985).

MICROASCALES

Kirk *et al*. (2008) and Cannon & Kirk (2007) included four families in the *Microascales*, *i.e. Ceratocystidaceae*, *Chadefaudiellaceae*, *Halosphaeriaceae,* and *Microascaceae*, although the *Ceratocystidaceae* is not validly published and was not listed among accepted fungal families by Hawksworth & David (1988). On the basis of our results from ncSSU rDNA and three-gene phylogenies (Figs 2, 3), the following families are accepted in the order, *Microascaceae*, *Halosphaeriaceae*, *Ceratocystidaceae*, which is validated here, and *Gondwanamycetaceae* fam. nov. We accept the *Halosphaeriaceae* as a family of the *Microascales* (Kirk *et al.* 2008), although they are often placed separately in their own order (Spatafora *et al.* 1998).

Recent studies by Spatafora *et al.* (1998), Kong *et al.* (2000), and Zhang *et al.* (2006) suggested that the *Microascales* may prove to be paraphyletic or polyphyletic. In our study, the cladogram based on ncLSU rDNA sequences (Fig. 1) provided no support for any of the backbone branches of the four families that we accept in the order. In the phylogenies based on ncSSU rDNA and the combined ncLSU-ncSSU-RPB2 data sets (Figs 2, 3), the *Microascales* appear as a monophyletic grouping of four families, all with high branch support. In both phylogenies the *Microascales* are divided into two major subclades, one containing the *Halosphaeriaceae* and *Microascaceae* and a second subclade with the *Ceratocystidaceae* and *Gondwanamycetaceae*. The ncSSU and the three-gene phylogeny did not support the putative para- or polyphyly of the *Microascales*.

The family *Microascaceae* and order *Microascales* were introduced by Luttrell (1951) and were later validated with Latin descriptions by Malloch (1970) and Benny & Kimbrough (1980),

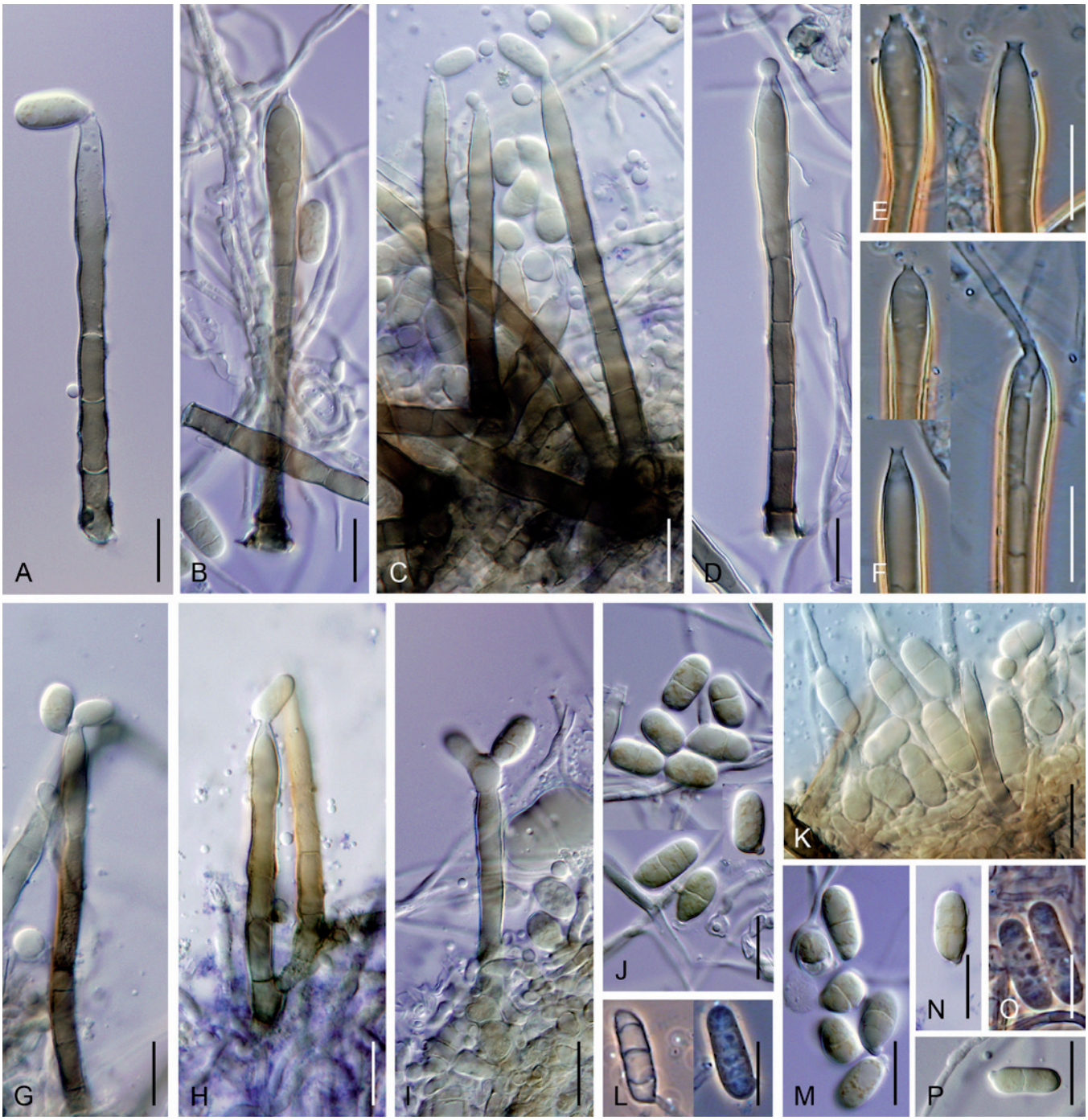

Fig. 14.A–P. *Kylindria peruamazonensis*. A–I. Conidiophores, in culture. J–P. Conidia, in culture. A–D, G–K, M, N, P from CBS 421.95 (PCA, 14 d old); E, F, L, O from CBS 838.91 (PCA, 3 wk old). Scale bars: A–D, G–K, M, N, P = 10 µm; E, F, L, O = 15 µm. DIC: A–D, G–K, M, N, P; PC: E, F, L, O.

respectively. Luttrell (1951) described the *Microascaceae* for taxa with beaked ascomata and evanescent, nonstipitate asci disposed irregularly throughout the filamentous centrum. Corlett (1963, 1966) confirmed the observations of Luttrell (1951) and described the asci of *Microascus* and *Petriella* as developing directly from the cells of the ascogenous hyphae and not from croziers. Members of the *Microascaceae* appear to have evolved away from a hymenial configuration; in the microascaceous centrum a peripheral layer of paraphysoidal elements develops that grows inward towards the ascogenous hyphae (Benny & Kimbrough 1980). Malloch (1970) redefined the *Microascaceae* to include both ostiolate and nonostiolate taxa; ascocarps are darkly pigmented, usually hairy, rarely glabrous; asci arise singly or in chains, without croziers, evanescent, irregularly disposed throughout the centrum; ascospores are reddish brown to copper-coloured with germ pores, dextrinoid when young and smooth. The genera of the *Microascaceae* differ in the manner of ramification of ascogenous hyphae and the formation of asci among the interthecial elements. The associated anamorphs are of the annellidic type, *e.g. Cephalotrichum* and *Scopulariopsis*. Aleurioconidia as in *Petriella* and arthroconidia as in *Kernia* also occur (Malloch 1970, 1971).

Ceratocystidaceae

The family level classification of *Ceratocystis* has been discussed since the genus was removed from the *Ophiostomatales* (Barr 1990, Samuels 1993). In recent literature the genus has sometimes been placed in the *Chadefaudiellaceae*, while other authors placed it in its own family, the *Ceratocystidaceae,* as proposed by Locquin (1972, as "*Ceratocystaceae*"). The name *Chadefaudiellaceae* predates

the *Ceratocystidaceae*, but these families are phylogenetically distinct (see below). The *Ceratocystis* clade is a monophyletic group centred on species of *Ceratocystis* or anamorphic species of the *Chalara*-like genus *Thielaviopsis*. *Ambrosiella xylebori*, type of this anamorph genus, occurs in a monophyletic clade together with *Ceratocystis,* now separated from similar anamorphs of the *Ophiostomatales* that are classified in *Raffaelea* (Cassar & Blackwell 1996, Jones & Blackwell 1998, Harrington *et al.* 2010).

The teleomorph genus *Cornuvesica* shares similar characters of centrum ontogeny, ascospore morphology, evanescent asci, and associated anamorphs with *Ceratocystis*, and may belong to the same clade. Because there are no available ncLSU sequences for *Cornuvesica*, its relationship to *Ceratocystis* and *A. xylebori* could only be explored with the ncSSU rDNA phylogeny (Fig. 2). *Cornuvesica falcata,* with a *Chalara*-like anamorph (Viljoen *et al.* 2000), falls in a basal position with these taxa in a monophyletic clade.

These four genera, *Ambrosiella, Ceratocystis*, *Cornuvesica,* and *Thielaviopsis*, constitute a family of their own, which has no valid name. The family name *Ceratocystidaceae* (as "*Ceratocystaceae*") proposed by Locquin (1972) was never validly published. It is phylogenetically well-established and is validated here.

Ceratocystidaceae Locq. ex Réblová, W. Gams & Seifert, **fam. nov.** MycoBank MB515438.

Ceratocystaceae Locq., Rev. Mycol., Supplément, 1 Table. 1972, *nom. inval.*, Art. 36.

Stromata absentia. Ascomata perithecia, fusca usque nigra, saepe aggregata, collo longo angustato et hyphis ostiolaribus protrudentibus, divergentibus praedita. Paries tenuis. Structura interascalis nulla. Asci unitunicati, catenati, saccati, evanescentes, 8-spori. Ascosporae hyalinae, forma variabiles, 0–1-septatae, saepe pariete partim inspissato vel lamina superficiali circumdatae. Anamorphe: aleurioconidia vel conidia modo phialidico orientia; *Thielaviopsis* vel *Chalara* similia.

Typus: *Ceratocystis* Ellis & Halst., New Jersey Agric. Coll. Exp. Sta. Bull. 76: 14. 1890.

Stromata absent. *Perithecia* dark brown to black, often aggregated, long-necked, usually with divergent ostiolar setae. *Perithecial wall* thin. *Interascal tissue* absent. *Asci* unitunicate, formed in chains, saccate, evanescent, 8-spored. *Ascospores* hyaline, varied in shape, 0–1-septate, often with eccentric wall thickening or sheaths. Anamorphs with phialidic conidiogenesis and aleurioconidia; *Thielaviopsis* and *Chalara*-like.

The holomorph taxa of the *Ceratocystidaceae* share common diagnostic characters of centrum ontogeny, evanescent catenate asci, ascospores, and associated anamorphs that are referred to *Thielaviopsis* (Fig. 1) or as *Chalara*-like, producing ameroconidia from phialides and in some cases also aleurioconidia. Ascospores are hyaline, often with eccentric wall thickening or sheaths, aseptate or 1-septate, hat-shaped in *Ceratocystis* or acicular in face view and falcate in side view with a hyaline sheath in *Cornuvesica*. Parguey-Leduc (1977) described the ontogeny of asci of this group with the examples of *Ceratocystis*, *Faurelina,* and *Sphaeronaemella,* Asci arise from the basal hymenium and, as the ascogenous hyphae ramify upward, asci differentiate, dissolve basally from the ascogenous hyphae, and become free within the centrum. Interascal tissue is lacking.

Faurelina and *Chadefaudiella* (*Chadefaudiellaceae*) are discussed below.

Gondwanamycetaceae

Species of *Gondwanamyces* and their *Custingophora* anamorphs form a strongly supported monophyletic clade (Figs 1–3) that is sister to the *Ceratocystidaceae*. The diagnostic characters of this clade include the apparent absence of interascal filaments in the ascomatal centrum and hyaline, allantoid ascospores with a hyaline sheath giving the spore a falcate to lunate appearance. The teleomorphs, described either from infructescences of *Protea* (Wingfield *et al.* 1988, Marais *et al.* 1998) or from sapwood associated with *Scolytidae* (bark beetles) (Bright & Torres 2006, Kolařík & Hulcr 2008), produce dark, globose perithecia with a long, filiform neck, evanescent asci, and hyaline, fusiform ascospores with or without a gelatinous sheath.

Detailed observations on the ontogeny of asci and centrum of *Gondwanamyces* are lacking. Based on the phylogenetic position of the genus, it is likely to be similar to that of the *Ceratocystidaceae*. The morphology of the anamorphs of *Gondwanamyces* is distinctive. The conidiophores are erect, darkly pigmented, paler towards the apex, and either monoverticillate, sometimes with a terminal vesicle or divergently penicillate with whorls of phialides producing hyaline conidia. The conidiogenous locus is located at the base of the shallow collarette. The terminal vesicle was not observed in the anamorph of *Gondwanamyces scolytodis* and *Custingophora cecropiae*, both associated with bark beetles in *Cecropia* (Kolařík & Hulcr 2008). The conidiogenesis of *Custingophora* (as *Knoxdaviesia proteae*), the anamorph of *Gondwanamyces proteae*, observed with fluorescence microscopy, TEM, and SEM, was illustrated by Mouton *et al.* (1993). After discharge, conidia adhere in slimy droplets on the phialide apices. In contrast, the phialidic conidia of species of the *Ceratocystidaceae* are formed in long chains deep within the venter of the cylindrical phialide.

The taxonomic relationships of the anamorph genera *Knoxdaviesia* and *Custingophora,* both phylogenetically related to this family, have been discussed by others, *e.g.* Viljoen *et al.* (1999), Kolařík & Hulcr (2008). Although the genera appear morphologically identical as originally described, they differ in their ecological behaviour. Species of *Custingophora* occur in compost, whereas species of *Knoxdaviesia* associated with *Gondwanamyces* were first observed in infructescences of *Protea* spp. infested by insects (Wingfield *et al.* 1988, Marais *et al.* 1998). The fact that some recently described species of *Gondwanamyces* and *Custingophora* are associated with *Scolytidae* (bark beetles) (Bright & Torres 2006, Kolařík & Hulcr 2008) raises the possibility that the originally reported ecological distinction might have been an artifact of intense sampling of *Protea* in a relatively narrow geographical area in the Western Cape Province of South Africa. Based on molecular and morphological features, Kolařík & Hulcr (2008) considered *Knoxdaviesia* and *Goidanichiella* to be synonyms of *Custingophora*. We prefer to recognise *Goidanichiella* as distinct because of the *Aspergillus*-like vesicles on the conidiophores of the only species, *G. barronii*.

We recognise this clade as a distinct family in the *Microascales*, proposed here as the *Gondwanamycetaceae*.

Gondwanamycetaceae Réblová, W. Gams & Seifert, **fam. nov.** MycoBank MB515439.

Stromata absentia. Ascomata perithecia, nigra, collo comparate longo praedita, apicem versus angustata, ostiolum hyphis divergentibus praeditum. Paries ascomatum fragilis. Filamenta interthecialia nulla. Asci unitunicati, evanescentes. Ascosporae hyalinae, aseptatae, fusiformes, lunatae vel falcatae, vagina gelatinosa

praesens vel absens. Anamorphe *Custingophora*; conidiophora monoverticillata vel penicillata, fusca, conidiis aseptatis modo phialidico orientibus, in massa mucida aggregatis.

Typus: *Gondwanamyces* G.J. Marais & M.J. Wingf., Mycologia 90: 139. 1998.

Stromata absent. *Ascomata* perithecioid, black, necks relatively long, tapered toward apex; ostiolar hyphae present. *Perithecial wall* fragile, thin-walled. *Interascal tissue* absent. *Asci* evanescent. *Ascospores* hyaline, aseptate, fusiform to lunate to falcate, with or without a gelatinous sheath. Anamorph: *Custingophora*; conidiophores monoverticillate or penicillate, brown, conidiogenesis phialidic, conidia aseptate, slimy.

Chadefaudiellaceae

Chadefaudiellaceae was described and validly published by Benny & Kimbrough (1980) for the coprophilous genus *Chadefaudiella*. Cannon & Kirk (2007) added a second genus to the family, *Faurelina* (Locquin-Linard 1975). Locquin-Linard (1973) and Parguey-Leduc (1977) placed the *Chadefaudiella* in the *Microascales* because of its perithecial acomata, catenate asci, and characteristic centrum structures, *i.e.* asci arising from a fertile layer lining the bottom of the cavity, ascogenous hyphae ramifying upwards, asci differentiated without croziers and liberated by basal dissolution to float free in the centrum (Benny & Kimbrough 1980). Ascospores are 1-celled, nondextrinoid, striate, and lack germ pores. No anamorph has been reported. *Faurelina* was described for coprophilous, cleistothecial fungi, otherwise reminiscent of *Chadefaudiella*, but differing by dextrinoid ascospores, and the absence of apical anastomosing setae on its ascomata. The ascomatal wall of *Faurelina* is cephalothecoid and the asci are catenate, irregularly disposed in the centrum at maturity, characters reminiscent of *Chadefaudiella* (Udagawa & Furuya 1973, Furuya 1978, von Arx *et al*. 1981). Von Arx (1978) and von Arx *et al*. (1981) regarded the anamorph of *Faurelina* as similar to the *Arthrographis* anamorph of *Pithoascus langeronii*, producing arthroconidia and secondary small blastoconidia in axenic culture (CBS 126.78).

The classification of *Faurelina* has been problematic. Despite the similarities with *Chadefaudiella* noted by Locquin-Linard (1975), Parguey-Leduc & Locquin-Linard (1976) concluded that *Faurelina* should be placed in the *Loculoascomycetes*. *Faurelina* was later transferred by von Arx (1978) to the *Microascaceae* because of its dextrinoid ascospores, which lack germ pores. He speculated on a relationship with *Neurospora* in the *Sordariaceae* (*Sordariomycetes*), which is characterised by elongate, striate ascospores with apical germ pores, and an anamorph with 1-celled, inflated arthroconidia or perhaps even with the *Testudinaceae* (*Dothideomycetes*). Benny & Kimbrough (1980) accepted *Faurelina* in the *Pithoascaceae* (= *Microascaceae fide* Kirk *et al*. 2008), a family erected for members of the *Microascales* with arthroconidial anamorphs and narrowly fusoid or naviculate ascospores. Recently both genera were placed in the *Chadefaudiellaceae, Microascales* (Cannon & Kirk 2007). This was in part based on the conclusions of Tang *et al.* (2007), who sequenced a single strain of *Faurelina indica* (CBS 126.78) and obtained ncLSU, ncSSU, and RPB2 sequences identical to those of *Ceratocystis fimbriata*, the type species of *Ceratocystis.*

We studied two authentic strains of *Faurelina indica,* the ex-type strain CBS 126.78 and CBS 301.78. They both grew slowly and mature ascomata did not develop on OA after 2 mo, but an arthroconidial anamorph with 0–1-septate conidia was observed similar to that illustrated by von Arx *et al.* (1981). No structures resembling phialides or *Ceratocystis*-type ascomata were produced. We generated new ITS and ncLSU sequences (ITS: GU291802; ncLSU: GU180653, GU180654) for these two strains. Phylogenetic analysis of ncLSU sequences (Fig. 5) suggests a relationship with the *Didymellaceae* (*Pleosporales, Dothideomycetes*). ITS sequences (phylogeny not shown) were similar to those of *Eremomyces* and *Arthrographis* species (90–91 % overall similarity), which also have arthroconidial anamorphs. We are confident that our sequences represent the fungus described by von Arx *et al*. (1981); those reported by Tang *et al*. (2007) were based on a different fungus. Our morphological and molecular studies fail to support the phylogenetic relationship of *Faurelina* with *Ceratocystis* suggested by Tang *et al*. (2007).

Based on these results, we confirm the hypothesis originally proposed by Parguey-Leduc & Locquin-Linard (1976) that *Faurelina* originated in the group of fungi with ascolocular development. Based on ncLSU sequences, we cannot confirm a close relationship of *Faurelina* with the *Testudinaceae* (von Arx 1978) or the *Eremomycetaceae*; the latter includes the morphologically similar *Arthrographis* (Fig. 5).

This phylogenetic reevaluation eliminates the *Chadefaudiellaceae* as an appropriate family name for the *Ceratocystis* clade. *Chadefaudiella* is morphologically slightly different from *Faurelina.* A further molecular analysis may lead to a re-establishment of the *Chadefaudiellaceae* in the *Microascales,* but with the exclusion of *Faurelina* from the family and distinct from the *Ceratocystidaceae*.

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