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Morphological and phylogenetic characterisations reveal three new species of *Samsoniella* (Cordycipitaceae, Hypocreales) from Guizhou, China

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Abstract

Samsoniella species have been found on lepidopteran larvae or pupae buried in soil or leaf litter. Three new species, *Samsoniella hymenopterorum*, *S. coleopterorum* and *S. lepidopterorum*, parasitic on hymenopteran larvae, coleopteran larvae and lepidopteran pupae, respectively, are reported. Morphological comparisons with extant species and DNA-based phylogenies from analysis of a multigene (ITS, *RPB1*, *RPB2* and *TEF*) dataset supported the establishment of the new species. Unusually, all three new species have mononematous conidiophores. The new species are clearly distinct from other species in *Samsoniella* occurring in separate subclades.

Keywords

Isaria-like, morphology, nutritional preference, phylogeny

Introduction

The genus *Isaria* Pers. was introduced for entomogenous fungi with mononematous or synnematous conidiophores, usually consisting of several verticillate branches, each bearing a dense whorl of phialides characters. The phialides consist of a cylindrical or swollen basal portion, terminating in a thin, often long neck and

produce divergent conidial chains (Samson 1974). However, entomogenous species, morphologically similar to *Isaria*, can be found distributed throughout the Hypocreales (Luangsa-ard et al. 2004).

Kepler et al. (2017) proposed the rejection of *Isaria* in favour of *Cordyceps*, owing to the confusion surrounding the application of *Isaria* and combined 11 species into *Cordyceps*. Mongkolsamrit et al. (2018) described some Isaria-like species and proposed the new genus *Samsoniella* Mongkols., Noisrip., Thanakitp., Spatafora & Luangsa-ard. The typical characteristics of *Samsoniella* are oval to fusiform conidia and bright red-orange teleomorphic stromata and anamorphic synnemata. *Samsoniella* species inhabit lepidopteran larvae and pupae in leaf litter or soil. Currently, *Samsoniella* consists of three species, *S. alboaurantia* (G. Sm.) Mongkols., Noisrip., Thanakitp., Spatafora & Luangsa-ard, *S. aurantia* Mongkols., Noisrip., Thanakitp., Spatafora & Luangsa-ard and *S. inthanonensis* Mongkols., Noisrip., Thanakitp., Spatafora & Luangsa-ard.

Three infected insect specimens were collected during a survey of entomogenous fungi in south-western China. Morphological and molecular phylogenetic analyses suggested that these isolates represented three new species, which are described here as *Samsoniella hymenopterorum* sp. nov., *S. coleopterorum* sp. nov. and *S. lepidopterorum* sp. nov.

Materials and methods

Specimen collection and identification

Three fungus-infected insect specimens were collected from Xishui County (28°29'56.70"N, 106°24'31.04"E) (A1950 and A1952) and Dali, Rongjiang County (26°01'58.70"N, 108°24'48.06"E) (DL1007), Guizhou Province, on 20 July and 1 October 2018, respectively. Isolation of the fungi was done as described by Chen et al. (2019). The surface of the specimens was rinsed with sterile water, followed by surface sterilisation with 75% ethanol for 3–5 sec. A part of the insect body was cut off and inoculated with haemocoel on potato dextrose agar (PDA) and PDA, to which 1% w/v peptone (PDAP) had been added. Fungal colonies emerging from specimens were isolated and cultured at 22 °C for 14 d under 12 h light/12 h dark conditions following protocols described by Zou et al. (2010). Accordingly, strains A19501, A19502, A19521, A19522, DL10071 and DL10072 were obtained. The specimens and the isolated strains were deposited in the Institute of Fungus Resources, Guizhou University (formally Herbarium of Guizhou Agricultural College; code, GZAC), Guiyang City, Guizhou, China.

Macroscopic and microscopic morphological characteristics of the fungi were examined and growth rates determined from PDA cultures incubated at 25 °C for 14 d. Hyphae and conidiogenous structures were mounted in lactophenol cotton blue or 20% lactate solution and observed with an optical microscope (OM, DM4 B, Leica, Germany).

DNA extraction, PCR amplification and nucleotide sequencing

DNA extraction was carried out in accordance with Liang et al. (2011). The extracted DNA was stored at –20 °C. Translation elongation factor 1 alpha (*TEF*) and RNA polymerase II largest subunit 2 (*RPB2*) genes were amplified using 983F/2218R and RPB2-5F/RPB2-7Cr primers, according to van den Brink et al. (2012). The RNA polymerase II largest subunit 1 (*RPB1*) gene was amplified with the primer pair CRPB1 and RPB1-Cr (Castlebury et al. 2004). The internal transcribed spacer (ITS) region was amplified by PCR using ITS4/ITS5, which was described by White et al. (1990). PCR products were purified using the UNIQ-10 column PCR products purification kit (no. SK1141; Sangon Biotech (Shanghai) Co., Shanghai, China) in accordance with the manufacturer's protocol and sequenced at Sangon Biotech (Shanghai) Co. The resulting sequences were submitted to GenBank.

Sequence alignment and phylogenetic analyses

The DNA sequences, generated in this study, were assembled and edited using Lasergene software (version 6.0 DNASTAR). Generated ITS, *RPB1*, *RPB2* and *TEF* sequences were aligned with those published by Mongkolsamrit et al. (2018) and others selected on the basis of BLAST algorithm-based searches in GenBank (Table 1). *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson (isolates CBS 284.36 and CBS 431.87) and *Beauveria bassiana* (Bals.-Criv.) Vuill. (ARSEF 1564) were chosen as outgroup taxa for the analysis of *Samsoniella* in Cordycipitaceae and *Samsoniella* species and closely-related species, respectively. Multiple datasets of ITS, *RPB1*, *RPB2* and *TEF* were aligned using MAFFT v7.037b (Katoh and Standley 2013) and alignments were edited with MEGA6 (Tamura et al. 2013). Sequences were concatenated with SequenceMatrix v.1.7.8 (Vaidya et al. 2011). The partition homogeneity test in PAUP4.0b10 (Swofford 2002) was undertaken by using the command 'hompart'.

Maximum Likelihood (ML) analyses were constructed with RAxMLGUI (Silvestro and Michalak 2012). The GTRGAMMA model was used for all partitions, in accordance with recommendations in the RAxML manual against the use of invariant sites. For Bayesian Inference (BI), a Markov Chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian probabilities using MrBayes v.3.2 (Ronquist et al. 2012) for the combined sequence datasets. The selection of the best-fit nucleotide substitution model for each locus was calculated by the Akaike Information Criterion (AIC) with jModelTest 2 (Darriba et al. 2012). The TIM+I+G model was selected for the concatenated ITS+*RPB1*+*RPB2*+*TEF* sequences. The Bayesian analysis resulted in 20,001 trees after 10,000,000 generations. The first 4,000 trees, representing the burn-in phase of the analyses, were discarded, while the remaining 16,001 trees were used for calculating posterior probabilities in the majority rule consensus tree. After the analysis was finished, each run was examined using the programme Tracer v1.5 (Drummond and Rambaut 2007) to determine burn-in and confirm that both runs had converged. The final alignment is available from TreeBASE under submission ID: 24710 (http://www.treebase.org).

Table 1. Taxa included in the phylogenetic analyses.

Species	Strain No.	GenBank Accession No.			
		ITS	RPB1	RPB2	TEF
Akanthomyces aculeatus	HUA 772	KC519371			KC519366
A. attenuatus	CBS 402.78	AJ292434	EF468888	EF468935	EF468782
A. coccidioperitheciatus	NHJ 6709	JN049865	EU369067	EU369086	
A. farinosa	CBS 541.81	AY624180			JQ425686
A. kanyawimiae	TBRC 7242	MF140751	MF140784	MF140808	MF140838
	TBRC 7243	MF140750	MF140783	MF140807	MF140837
	TBRC 7244	MF140752			MF140836
A. lecanii	CBS 101247	JN049836	DQ522407	DQ522466	DQ522359
A. sulphureus	TBRC 7247	MF140756	MF140785	MF140811	MF140841
	TBRC 7248	MF140758	MF140787	MF140812	MF140843
	TBRC 7249	MF140757	MF140786	MF140734	MF140842
A. thailandicus	TBRC 7245	MF140754		MF140809	MF140839
	TBRC 7246	MF140755		MF140810	MF140840
A. tuberculatus	BCC 16819	GQ250012			GQ250037
	OSC111002	JN049830	DQ522384	DQ522435	DQ522338
A. waltergamsii	TBRC 7250	MF140749			MF140835
	TBRC 7251	MF140747	MF140781	MF140805	MF140833
	TBRC 7252	MF140748	MF140782	MF140806	
Ascopolyporus polychrous	P.C. 546		DQ127236		DQ118745
Beauveria acridophila	HUA 179219		JX003857	JX003841	JQ958613
B. acridophila	QCNE 186726	JQ958605	JX003855		JQ958618
B. bassiana	ARSEF 1564	HQ880761	HQ880833	HQ880905	HQ880974
B. brongniartii	ARSEF 617	HQ880782	HQ880854	HQ880926	HQ880991
	BCC 16585	JN049867	JN049885	JF415991	JF416009
B. caledonica	ARSEF 2567	HQ880817	EF469086	HQ880961	EF469057
B. diapheromeriphila	MCA 1557	JQ958608	JX003851		JQ958612
	QCNE 186272	JQ958599	JX003848		JQ958610
	QCNE 186714	JQ958603	JX003850		JQ958611
B. locustiphila	HUA 179217	JQ958609	JX003847		
	HUA 179218	JQ958606	JX003846	JX003845	JQ958619
B. malawiensis	ARSEF 7760		HQ880897	HQ880969	DQ376246
B. pseudobassiana	ARSEF 3405	AY532022	HQ880864	HQ880936	AY531931
B. scarabaeidicola	ARSEF 5689	JN049827	DQ522380	DQ522431	DQ522335
B. staphylinidicola	ARSEF 5718		EF468881		EF468776
Blackwellomyces cardinalis	OSC 93609		DQ522370	DQ522422	DQ522325
B. cardinalis	OSC 93610	JN049843	EF469088	EF469106	EF469059
B. pseudomilitaris	NBRC 101409	JN943305	JN992482		
	NBRC 101410	JN943307	JN992481		
Cordyceps amoene-rosea	CBS 729.73			MG665235	HM161732
C. amoene-rosea	CBS 107.73	AY624168			
C. bifusispora	EFCC 5690		EF468854	EF468909	
	EFCC 8260		EF468855	EF468910	EF468747
C. blackwelliae	TBRC 7253	MF140739	MF140774	MF140798	MF140825
	TBRC 7254	MF140738	MF140773	MF140797	MF140824
	TBRC 7255	MF140737	MF140772	MF140796	MF140823
	TBRC 7256	MF140736	MF140771	MF140795	
	TBRC 7257	MF140735	MF140770	MF140794	MF140821
C. cateniannulatus	CBS 152.83	AY624172			JQ425687
	TBRC 7258	MF140753	MF140767		MF140850
C. cateniobliqua	CBS 153.83	AY624173		MG665236	JQ425688
C. cf. *farinosa*	OSC 111004		EF468886		EF468780
C. cf. *ochraceostromata*	ARSEF 5691		EF468867	EF468921	EF468759
C. cf. *takaomontana*	NHJ 12623		EF468884	EF468932	EF468778
C. chiangdaoensis	TBRC 7274	KT261393			KT261403

Species	Strain No.	GenBank Accession No.			
		ITS	RPB1	RPB2	TEF
C. coleopterorum	CBS 110.73	AY624177	JN049903	JF416006	JF416028
C. farinosa	CBS 111113	AY624181		GU979973	GQ250022
C. fumosorosea	CBS 107.10	AY624184		MG665237	HM161735
	CBS 244.31	AY624182			JQ425690
	CBS 375.70	AY624183		MG665238	HM161736
	CBS 337.52	EF411219			MG665233
C. javanica	CBS 134.22	AY624186			JQ425683
	TBRC 7259	MF140745	MF140780	MF140804	MF140831
	TBRC 7260	MF140744	MF140779	MF140803	MF140830
	TBRC 7261	MF140743	MF140778	MF140802	MF140829
	TBRC 7262	MF140746			MF140832
C. kintrischica	ARSEF 7218	EU553278			GU734751
	ARSEF 8058	GU734764			GU734750
C. kyusyuensis	EFCC 5886		EF468863	EF468917	
C. lepidopterorum	TBRC 7263	MF140765	MF140768	MF140792	MF140819
	TBRC 7264	MF140766	MF140769	MF140793	MF140820
C. militaris	OSC 93623				
C. morakotii	TBRC 7275	KT261388			KT261398
	TBRC 7276	KT261390			KT261400
C. ninchukispora	EFCC 5197		EF468868		EF468760
	EFCC 5693		EF468869		EF468762
	EGS 38.165		EF468900		EF468795
	EGS 38.166		EF468901		EF468794
	NHJ 10627		EF468870		EF468763
	NHJ 10684		EF468871		EF468761
C. oncoperae	ARSEF 4358		EF468891	EF468936	EF468785
C. piperis	CBS 116719		DQ127240	EU369083	DQ118749
C. pruinosa	ARSEF 5413	JN049826	DQ522397	DQ522451	DQ522351
Cordyceps sp.	CBS 102184		EF468907	EF468948	EF468803
C. takaomontana	BCC 28612	FJ765285			FJ765268
C. tenuipes	ARSEF 5135	AY624196	JN049896	JF416000	JF416020
	OSC 111007		DQ522395	DQ522449	DQ522349
	TBRC 7265	MF140741	MF140776		MF140827
	TBRC 7266	MF140742	MF140777	MF140801	MF140828
	TBRC 7267	MF140740	MF140775	MF140799	MF140826
Engyodontium aranearum	CBS 309.85		DQ522387	DQ522439	DQ522341
Gibellula longispora	NHJ 12014		EU369055	EU369075	EU369017
G. ratticaudata	ARSEF 1915		DQ522408	DQ522467	DQ522360
Gibellula sp.	NHJ 10788		EU369058	EU369078	EU369019
	NHJ 13158		EU369057	EU369077	EU369020
	NHJ 10808		EU369056	EU369076	EU369018
	NHJ 5401		EU369059	EU369079	
	NHJ 7859		EU369064	EU369085	
Hevansia cinerea	NHJ 3510		EU369048	EU369070	EU369009
H. nelumboides	BCC 41864	JN201871			JN201867
H. novoguineensis	NHJ 4314		EU369051	EU369071	EU369012
	NHJ 10469		EU369047		EU369008
	NHJ 11923		EU369052	EU369072	EU369013
	NHJ 13117		EU369049	EU369073	EU369010
	NHJ 13161		EU369050		EU369011
Hyperdermium pulvinatum	P.C. 602		DQ127237		DQ118746
Lecanicillium aranearum	CBS 350.85		DQ522396	DQ522450	DQ522350
L. aranearum	CBS 726.73a		EF468887	EF468934	EF468781
L. fusisporum	CBS 164.70		EF468889		EF468783
L. psalliotae	CBS 101270		EF469095	EF469113	EF469066
	CBS 363.86		EF468890		EF468784
	CBS 532.81		EF469096	EF469112	EF469067

Species	Strain No.	GenBank Accession No.			
		ITS	RPB1	RPB2	TEF
Purpureocillium lilacinum	CBS 284.36	AY624189	EF468792	EF468898	EF468941
P. lilacinum	CBS 431.87	AY624188	EF468897	EF468940	EF468791
Samsoniella alboaurantium	CBS 240.32	AY624178	JN049895	JF415999	JF416019
S. alboaurantium	CBS 262.58	AY624179			JQ425685
S. aurantia	TBRC 7271	MF140764	MF140791	MF140818	MF140846
	TBRC 7272	MF140763		MF140817	MF140845
	TBRC 7273	MF140762		MF140816	MF140844
S. coleopterorum	**A19501**	**MT626376**	**MT642600**	**MN101585**	**MN101586**
	A19502	**MT626625**	**MT642603**	**MN101587**	**MT642602**
S. hymenopterorum	**A19521**	**MN128224**	**MT642601**	**MT642604**	**MN101588**
	A19522	**MN128081**	**MN101589**	**MN101590**	**MN101591**
S. inthanonensis	TBRC 7915	MF140761	MF140790	MF140815	MF140849
	TBRC 7916	MF140760	MF140789	MF140814	MF140848
	TBRC 7270	MF140759	MF140788	MF140813	MF140847
S. lepidopterorum	**DL10071**	**MN128076**	**MN101592**	**MN101593**	**MN101594**
	DL10072	**MN128084**		**MT642605**	**MT642606**
Simplicillium lamellicola	CBS 116.25	AJ292393	DQ522404	DQ522462	DQ522356
S. lanosoniveum	CBS 101267	AJ292395	DQ522405	DQ522463	DQ522357
	CBS 704.86		DQ522406	DQ522464	DQ522358
Torrubiella wallacei	CBS 101237		EF469102	EF469119	EF469073

Results

Phylogenetic analyses

The phylogenetic tree of *Samsoniella* in Cordycipitaceae (Fig. 1) and *Samsoniella* species and closely related species (Fig. 2) were generated from the ML and BI analysis, based on a combined data set of ITS, *RPB1*, *RPB2* and *TEF* sequence data. Statistical support (≥ 50%/0.5) is shown at the nodes for ML bootstrap support/BI posterior probabilities (Figs 1, 2). The strain numbers are noted after each species' name. The concatenated sequences of analysis 1 and analysis 2 included 67 and 17 taxa, and consisted of 2,152 (ITS: 528, *RPB1*: 488, *RPB2*: 442 and *TEF*: 694) and 2,194 (ITS: 477, *RPB1*: 565, *RPB2*: 473 and *TEF*: 679) characters with gaps, respectively.

Analysis 1: *Samsoniella* in Cordycipitaceae. The RAxML analysis of the combined dataset (ITS+*RPB1*+*RPB2*+*TEF*) yielded a best scoring tree (Fig. 1) with a final ML optimisation likelihood value of –28,809.222105. Parameters for the GTR model of the concatenated dataset was as follows: estimated base frequencies; A = 0.234094, C = 0.301291, G = 0.260521, T = 0.204093; substitution rates AC = 1.111784, AG = 3.130020, AT = 0.930972, CG = 0.886915, CT = 6.300092, GT = 1.000000; gamma distribution shape parameter α = 0.390179. In the phylogenetic tree (Fig. 1), *Samsoniella* species were clustered in a clade and resolved into two obvious clades. *Samsoniella* species have a close relationship with *Akanthomyces* species.

Analysis 2: *Samsoniella* species and closely-related species. The RAxML analysis of the combined dataset (ITS+*RPB1*+*RPB2*+*TEF*) yielded a best scoring tree (Fig. 2) with a final ML optimisation likelihood value of –9,722.503130. Parameters for the GTR model of the concatenated data set were as follows: estimated base frequencies;

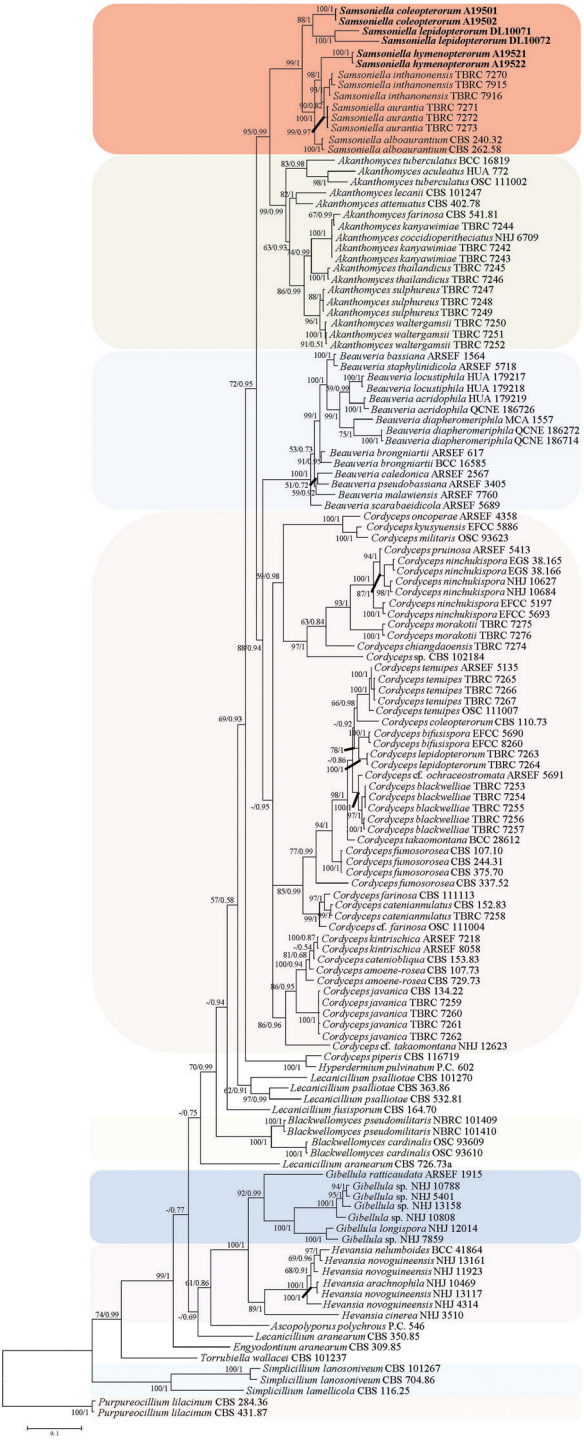

Figure 1. Phylogenetic relationships of the genus *Samsoniella* in Cordycipitaceae, based on multigene dataset (ITS, *RPB1*, *RPB2* and *TEF*). Statistical support values (≥ 50%/0.5) are shown at the nodes for ML bootstrap support/BI posterior probabilities.

Figure 2. Phylogenetic relationships between the genus *Samsoniella* and closely-related species, based on multigene dataset (ITS, *RPB1*, *RPB2* and *TEF*). Statistical support values (≥ 50%/0.5) are shown at the nodes for ML bootstrap support/BI posterior probabilities.

A = 0.233473, C = 0.298686, G = 0.261629, T = 0.206212; substitution rates AC = 1.250081, AG = 2.534760, AT = 0.891128, CG = 0.827805, CT = 5.916085, GT = 1.000000; gamma distribution shape parameter α = 0.674468. In the phylogenetic tree (Fig. 2), *Samsoniella* species were clustered in a clade and easily distinguished with *Akanthomyces* species. *S. coleopterorum* and *S. lepidopterorum* clustered in a clade (Fig. 2) and formed two independent branches. *S. hymenopterorum* was phylogenetically close to *S. inthanonensis* and *S. aurantia*.

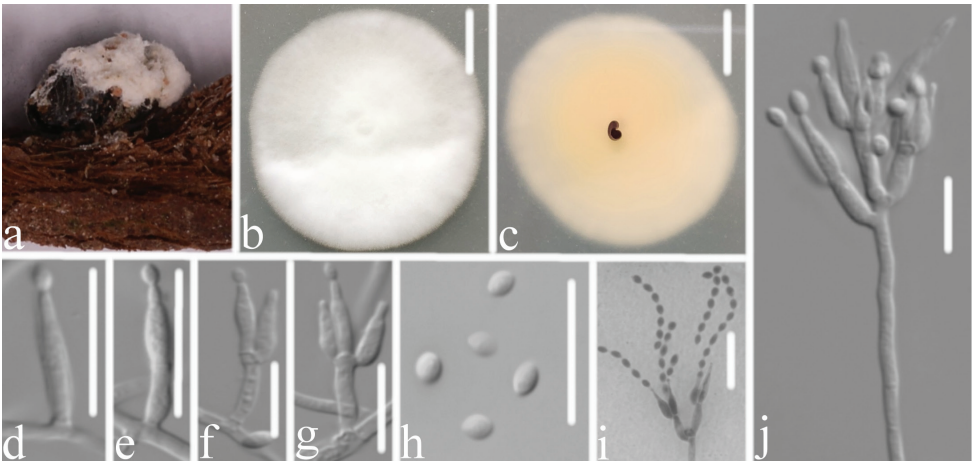

Figure 3. *Samsoniella coleopterorum* **A** infected insect (Coleoptera) **B, C** top (**B**) and underside (**C**) of a colony cultured on PDA medium at 14 d **D, E, F, G, I** phialides and conidia in chains **J** conidiophore and phialides **H** conidia. Scale bars: 10 mm (**B, C**); 10 μm (**D–J**).

Taxonomy

Samsoniella coleopterorum **W.H. Chen, Y.F. Han & Z.Q. Liang, sp. nov.**
MycoBank No: 831735
Fig. 3

Diagnosis. Differs from *Samsoniella aurantia* by having smaller conidia and snout beetle host in the family Curculionidae. Differs from *S. lepidopterorum* by having cylindrical to ellipsoidal phialides, smaller fusiform to ellipsoidal conidia and a different host.

Type. China, Guizhou Province, Xishui County (28°29'56.70"N, 106°24'31.04"E), July 2018, Jiandong Liang, holotype GZAC A1950, ex-type culture GZAC A19501. Sequences from isolated strain A19501 have been deposited in GenBank with accession numbers: ITS = MT626376, *RPB1* = MT642600, *RPB2* = MN101585 and *TEF* = MN101586.

Description. Colonies on PDA, 3.6–4.0 cm diam. in 14 d at 25 °C, white, consisting of a basal felt and cottony, floccose hyphal overgrowth, reverse yellowish. Prostrate hyphae smooth, septate, hyaline, 1.1–1.8 μm diam. Erect conidiophores usually arising from aerial hyphae, Isaria-like with phialides in whorls of two to four. Phialides 5.4–9.7 × 1.2–1.8 μm, with a cylindrical to ellipsoidal basal portion, tapering into a short distinct neck. Conidia in chains, hyaline, fusiform, ellipsoidal or subglobose, one-celled, 1.7–2.5 × 1.2–1.8 μm. Chlamydospores and synnemata not observed. Size and shape of phialides and conidia similar in culture and on natural substratum. Sexual state not observed.

Host. Snout beetle, family Curculionidae.

Distribution. Xishui County, Guizhou Province, China.

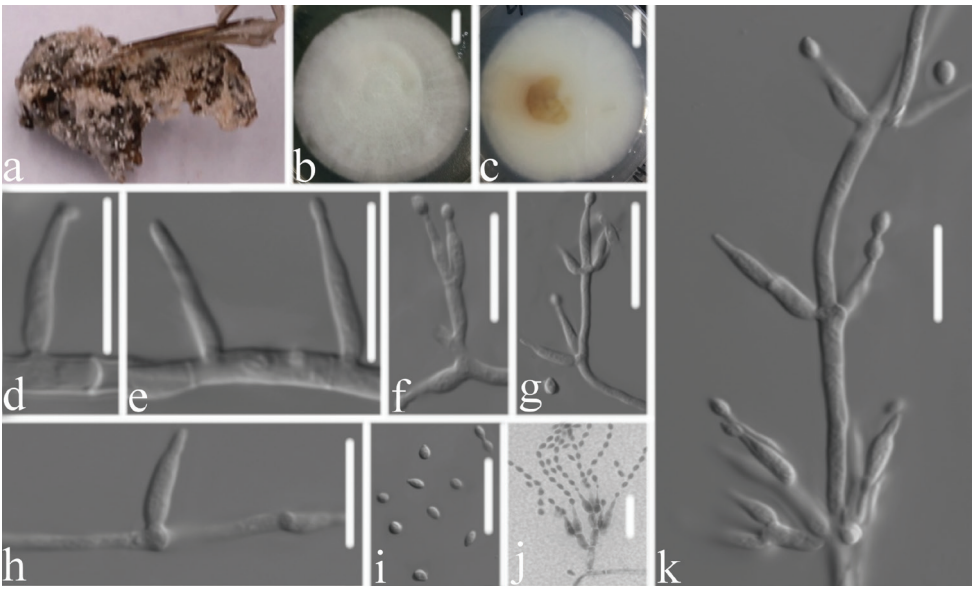

Figure 4. *Samsoniella hymenopterorum* **A** infected insect (Hymenoptera) **B, C** top (**B**) and underside (**C**) of a colony cultured on PDA medium at 14 d **D–H, J** phialides and conidia in chains **K** conidiophore and phialides **I** conidia. Scale bars: 10 mm (**B, C**); 10 μm (**D–K**).

Etymology. Referring to its insect host, order Coleoptera.

Remarks. *Samsoniella coleopterorum* was easily identified as belonging to *Samsoniella* based on the phylogenetic analyses (Fig. 1). Comparing with the typical characteristics of three species (Table 2), *S. coleopterorum* has a close relationship with *S. aurantia* by having cylindrical to ellipsoidal phialides and similar in size. However, it differs from *S. aurantia* by having shorter conidia and snout beetle host in the family Curculionidae. Based on the combined dataset of ITS, *RPB1*, *RPB2* and *TEF* sequences, *S. coleopterorum* has a close relationship with *S. lepidopterorum* (Fig. 2). However, *S. coleopterorum* has cylindrical to ellipsoidal phialides, smaller fusiform to ellipsoidal conidia and a different host.

Samsoniella hymenopterorum **W.H. Chen, Y.F. Han & Z.Q. Liang, sp. nov.**
MycoBank No: 831736
Fig. 4

Diagnosis. Differs from *Samsoniella inthanonensis* and *S. aurantia* by having smaller, fusiform to ovoid conidia and a host in the family Vespidae.

Type. China, Guizhou Province, Xishui County, at 28°29'56.70"N, 106°24'31.04"E, July 2018, Jiandong Liang, holotype GZAC A1952, ex-type culture GZAC A19522. Sequences from isolated strain A19522 have been deposited in GenBank with accession numbers: ITS = MN128224, *RPB1* = MT642603, *RPB2* = MT642604 and *TEF* = MN101588.

Table 2. Morphological comparison of three new species with other *Samsoniella* species.

Species	Morphological characteristics			Reference
	Phialide (µm)	Conidia (µm)	Hosts/substrates	
Samsoniella alboaurantium	5–8 × 2	ovate to lemon-shaped 2.3–2.5(–3) × 1.5–1.8	soil, lepidopterous pupa	Smith 1957
S. aurantia	cylindrical to ellipsoidal (5–)5.5–8.5(–13) × 2–3	fusiform (2–)2.5–3.5(–4) × (1–)1.5(–2)	lepidopterous larvae	Mongkolsamrit et al. 2018
S. inthanonensis	cylindrical (4–)6.5–10(–12) × (1–)1.5–2(–3)	short fusiform (2–)3(–3.5) × 1.5–2	lepidopterous larvae	Mongkolsamrit et al. 2018
S. coleopterorum	cylindrical to ellipsoidal 5.4–9.7 × 1.2–1.8	fusiform, ellipsoidal or subglobose 1.7–2.5 × 1.2–1.8	snout beetle	this study
S. hymenopterorum	cylindrical 6.5–10.6 × 1.2–2.0	fusiform to ovoid 1.9–2.5 × 1.5–2.1	bee	this study
S. lepidopterorum	ellipsoidal 5.2–8.5(–13.1) × 1.1–1.7	fusiform to subglobose 2.0–2.5 × 1.2–2.0	lepidopterous pupa	this study

Description. Colonies on PDA, 6.2–6.4 cm diam. in 14 d at 25 °C, white, consisting of a basal felt and cottony, floccose hyphal overgrowth, reverse yellowish. Prostrate hyphae smooth, septate, hyaline, 1.1–1.6 µm diam. Erect conidiophores usually arising from aerial hyphae, Isaria-like with phialides in whorls of three to four. Phialides 6.5–10.6 × 1.2–2.0 µm, with a cylindrical basal portion, tapering to a distinct neck. Conidia in chains, hyaline, fusiform to ovoid, 1-celled, 1.9–2.5 × 1.5–2.1 µm. Chlamydospores and synnemata not observed. Size and shape of phialides and conidia similar in culture and on natural substratum. Sexual state not observed.

Host. Bee, family Vespidae.

Distribution. Xishui County, Guizhou Province, China.

Etymology. Referring to its insect host, order Hymenoptera.

Remarks. *Samsoniella hymenopterorum* was identified as belonging to *Samsoniella*, based on the phylogenetic analyses (Fig. 1). Comparing with the typical characteristics of the three species (Table 2), *S. hymenopterorum* has a close relationship with *S. inthanonensis* by a having cylindrical basal in phialide and similar in size. However, it is distinguished from *S. inthanonensis* by having smaller, fusiform to ovoid conidia and a host in the family Vespidae. Based on combined dataset of ITS, *RPB1*, *RPB2* and *TEF* sequences, *S. hymenopterorum* is phylogenetically close to *S. aurantia* and *S. inthanonensis* (Fig. 2). However, *S. hymenopterorum* has smaller fusiform to ovoid conidia and a different host.

Samsoniella lepidopterorum **W.H. Chen, Y.F. Han & Z.Q. Liang, sp. nov.**
MycoBank No: 831737
Fig. 5

Diagnosis. Differs from *Samsoniella coleopterorum* by having larger, ellipsoidal phialide conidia and a host in the order Lepidoptera.

Type. China, Guizhou Province, Rongjiang County (26°01'56.13"N, 108°24'48.06"E), October 2018, Wanhao Chen, holotype GZAC DL1007 = RJ1807,

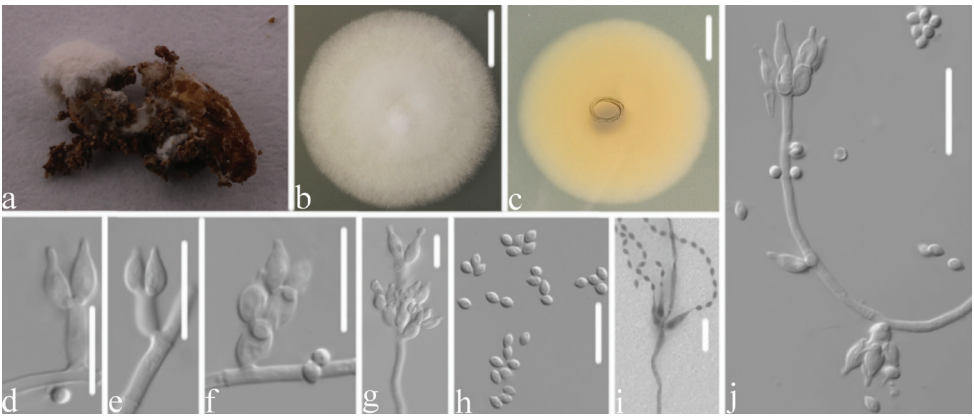

Figure 5. *Samsoniella lepidopterorum* **A** infected insect pupa (Lepidoptera) **B, C** top (**B**) and underside (**C**) of a colony cultured on PDA medium at 14 d **D–G, I** phialides and conidia in chains **H** conidia **J** conidiophore and phialides. Scale bars: 10 mm (**B, C**); 10 μm (**D–J**).

ex-type culture GZAC DL10071 = RJ18071. Sequences from isolated strain DL10071 have been deposited in GenBank with accession numbers: ITS = MN128076, *RPB1* = MN101592, *RPB2* = MN101593 and *TEF* = MN101594.

Description. Colonies on PDA, 3.7–3.8 cm diam. in 14 d at 25 °C, white, consisting of a basal felt and cottony, floccose hyphal overgrowth, reverse yellowish. Prostrate hyphae smooth, septate, hyaline, 1.1–2.2 μm diam. Erect conidiophores usually arising from aerial hyphae, Isaria-like with phialides in whorls of two to four. Phialides 5.2–8.5 (–13.1) × 1.1–1.7 μm, with an ellipsoidal basal portion, tapering into a distinct neck. Conidia in chains, hyaline, fusiform to subglobose, 1-celled, 2.0–2.5 × 1.2–2.0 μm. Chlamydospores and synnemata not observed. Size and shape of phialides and conidia similar in culture and on natural substratum. Sexual state not observed.

Host. Pupa, order Lepidoptera

Distribution. Rongjiang County, Guizhou Province, China

Etymology. Referring to its insect host, order Lepidoptera

Remarks. *Samsoniella lepidopterorum* was easily identified as belonging to *Samsoniella*, based on the phylogenetic analyses (Fig. 1). Based on the combined dataset of ITS, *RPB1*, *RPB2* and *TEF* sequences (Fig. 2) and the typical characteristics of *Samsoniella* species (Table 2), *S. lepidopterorum* has a close relationship with *S. coleopterorum*. However, *S. lepidopterorum* has larger, ellipsoidal phialide conidia and its pupa host is in the order Lepidoptera.

Discussion

Phylogenetic analyses, based on the combined datasets of (ITS+*RPB1*+*RPB2*+*TEF*), suggest that the three new species are members of the Cordycipitaceae and belong to the genus *Samsoniella* (Fig. 1). Mongkolsamrit et al. (2018) noted that the typical

characteristics of *Samsoniella* were oval to fusiform conidia, bright red-orange stromata of the sexual morphs and synnemata of the asexual morphs. The phialides in this genus range from cylindrical to possessing a swollen basal portion. *S. coleopterorum*, *S. hymenopterorum* and *S. lepidopterorum* all have cylindrical phialides and fusiform conidia. However, the three new species had mononematous conidiophores rather than synnemata. Synnematous entomopathogenic fungi (such as *Gibellula* spp.) can be found on abaxial leaf surfaces of shrubbery, forest floors and shallow soil layers (Hywel-Jones 1996). As air flow under the forest canopy is slow and humid, the dispersal of conidia through airflow diffusion may be difficult. Therefore, these entomopathogenic fungi may employ a particular strategy, such as producing synnemata and sticky conidia, to accommodate various arthropod activities and facilitate conidium spread (Abbott 2002). The three new species were located in the more open portion of the forest and this may favour the dispersal of dry conidia. Thus, we could speculate that the mononematous conidiophores of the three new species may be the result of a convergent evolution to adapt to the ecological environment.

The evolutionary dynamics of fungi and their hosts are usually described either by co-evolution or by host shifts. Shifts often occur to new hosts that are evolutionarily distant, but which occupy a common ecological niche (Vega et al. 2009). Nutrient requirements often determine whether host shifts occur (Vega et al. 2009). Relationships between insects and fungi have been described as biotrophy, necrotrophy and hemibiotrophy, *inter alia*. The common ancestor of Hypocreaceae and Clavicipitaceae corresponds to a departure from plant-based nutrition to one that specialises on animals and fungi (Spatafora et al. 2007). Prediction of the characteristics and evolutionary placement of any given member should be based on the correlation between molecular-phylogenetic genealogy and nutritional preferences (Spatafora et al. 2007; Vega et al. 2009). Species of *Samsoniella* were originally found on lepidopteran larvae or pupae buried in soil or leaf litter (Mongkolsamrit et al. 2018). Mongkolsamrit et al. (2018) also reported that the true range of host affiliations of *Samsoniella* in nature may not be currently represented. Here, we report *Samsoniella* spp. from coleopteran, hymenopteran larvae and lepidopteran pupae. The presence of different hosts indicates that the nutrient requirements of *Samsoniella* spp. can change with the environment (Spatafora et al. 2007).

In the present study, a four loci phylogenetic analysis showed that *S. coleopterorum*, *S. lepidopterorum* and *S. hymenopterorum* clustered in separate subclades from other *Samsoniella* species. They represent new taxa, based on morphological characteristics, nutritional preferences and phylogenetic analyses.

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Delimitation, new species and teleomorph-anamorph relationships in *Codinaea*, *Dendrophoma*, *Paragaeumannomyces* and *Striatosphaeria* (Chaetosphaeriaceae)

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Abstract

The Chaetosphaeriaceae are a diverse group of pigmented, predominantly phialidic hyphomycetes comprised of several holomorphic genera including *Chaetosphaeria*, the most prominent genus of the family. Although the morphology of the teleomorphs of the majority of *Chaetosphaeria* is rather uniform, their associated anamorphs primarily exhibit the variability and evolutionary change observed in the genus. An exception from the morphological monotony among *Chaetosphaeria* species is a group characterised by scolecosporous, hyaline to light pink, multiseptate, asymmetrical ascospores and a unique three-layered ascomatal wall. *Paragaeumannomyces sphaerocellularis*, the type species of the genus, exhibits these morphological traits and is compared with similar *Chaetosphaeria* with craspedodidymum- and chloridium-like synanamorphs. Morphological comparison and phylogenetic analyses of the combined ITS-28S sequences of 35 isolates and vouchers with these characteristics revealed a strongly-supported, morphologically well-delimited clade in the Chaetosphaeriaceae containing 16 species. The generic name *Paragaeumannomyces* is applied to this monophyletic clade; eight new combinations and five new species, i.e. *P. abietinus* **sp. nov.**, *P. elegans* **sp. nov.**, *P. granulatus* **sp. nov.**, *P. sabinianus* **sp. nov.** and *P. smokiensis* **sp. nov.**, are proposed. A key to *Paragaeumannomyces* is provided. Using morphology, cultivation studies and phylogenetic analyses of ITS and 28S rDNA, two additional new species from freshwater and

terrestrial habitats, *Codinaea paniculata* **sp. nov.** and *Striatosphaeria castanea* **sp. nov.**, are described in the family. A codinaea-like anamorph of *S. castanea* forms conidia with setulae at each end in axenic culture; this feature expands the known morphology of *Striatosphaeria*. A chaetosphaeria-like teleomorph is experimentally linked to *Dendrophoma cytisporoides*, a sporodochial hyphomycete and type species of *Dendrophoma*, for the first time.

Keywords
molecular phylogeny, phialidic conidiogenesis, scolecosporous, systematics, wood-inhabiting fungi, 15 new taxa

Introduction

The family Chaetosphaeriaceae (Réblová et al. 1999) is a speciose, diverse group of pigmented, predominantly phialidic fungi some of which possess known teleomorphs (sexual and asexual morphs, hereafter teleomorph and anamorph respectively). Members of the family have a world-wide geographical distribution. They are essential components of biodiversity and play a role in decomposition of woody and herbaceous material and leaf litter, occur in soil, and some exhibit an endophytic lifestyle and have been isolated from living herbs and trees (e.g. Gams and Holubová-Jechová 1976; Hughes and Kendrick 1968; Réblová and Gams 1999; Réblová and Seifert 2003; Réblová 2004; Fernández and Huhndorf 2005; Huhndorf and Fernández 2005; Crous et al. 2012; Hashimoto et al. 2015; Yang et al. 2018; Lin et al. 2019; Luo et al. 2019).

Sexually reproducing fungi encompassed in the Chaetosphaeriaceae are perithecial ascomycetes that share several morphological traits such as similar anatomy of the brittle, melanised ascomatal wall, persistent paraphyses, unitunicate, thin-walled asci with a refractive, non-amyloid apical annulus, transversely septate ascospores that germinate by germ tubes and phialidic conidiogenesis. Several species produce both ascospores and conidia, ascomata are often associated with conspicuous conidiophores arranged in the juxtaposition. Most representatives of the family reproduce only asexually and are known as "anamorphic holomorphs" (Seifert et al. 2011). They either permanently lost the ability to sexually reproduce and do not develop the teleomorph, or the latter remains to be discovered.

Most of the sexually reproducing fungi in the family were classified in *Chaetosphaeria* (Tulasne & Tulasne, 1863), a prominent genus of the family. *Chloridium botryoideum* has long been known to be a part of the life cycle of *Ch. innumera*, the generic type (Tulasne and Tulasne 1863; Gams and Holubová-Jechová 1976). Using ITS and 28S DNA sequence data, *Ch. innumera* was resolved as unrelated to other *Chaetosphaeria* and chaetosphaeria-like species associated with morphologically different anamorphs (Réblová and Winka 2000; Fernández et al. 2006; Lin et al. 2019). Following the "one fungus, one name" concept (Hawksworth 2011, 2012; Hawksworth et al. 2011), some of the former *Chaetosphaeria* linked with different anamorphs now belong in the respective anamorphic genera based on priority, for example, *Catenularia* (Berkeley and

Broome 1871; Hughes 1965a; Holubová-Jechová 1982), *Cacumisporium* (Réblová and Gams 1999), *Chloridium* (Gams and Holubová-Jechová 1976; Réblová et al. 2016), *Exserticlava* (Hino 1961; Matsushima 1985; Réblová and Seifert 2003; Fernández and Huhndorf 2005), *Menispora* (Booth 1957, 1958; Holubová-Jechová 1973; Réblová and Seifert 2008), *Sporoschisma* (Müller et al. 1969, Réblová et al. 2016), *Tainosphaeria* (Fernández and Huhndorf 2005) and *Zanclospora* (Hughes and Kendrick 1965b). Other *Chaetosphaeria* that form natural units, characterised primarily by the morphological traits of their anamorphs, will form the basis of generic classification in the family and, thus, need to be re-examined based on phylogenetic studies.

The majority of species accommodated in *Chaetosphaeria* possess ellipsoidal, fusiform to cylindrical-fusiform, 1–5-septate, hyaline, symmetrical ascospores with their length generally ranging from 6 to 40 µm. Ascomata are brown to black, papillate, often glossy with a two-layered ascomatal wall; the outer layer consisting of several rows of brick-like cells with dark brown, opaque walls. The transfer of a scolecosporous *Lasiosphaeria raciborskii* (Carroll and Munk 1964) with a three-layered ascomatal wall to *Chaetosphaeria* by Miller and Huhndorf (2004) expanded the concept of the genus. Huhndorf and Fernández (2005) introduced another four morphologically similar species based on ITS sequence data, i.e. *Ch. ellisii* (= *Ch. longispora*), *Ch. lapaziana*, *Ch. panamensis* and *Ch. rubicunda*, characterised by unique ascomatal wall anatomy, multiseptate scolecosporous ascospores and occurrence on decaying wood. Their ascomatal wall is composed of three layers. The typical chaetosphaeriaceous outer layer is present as the middle layer, while the outer layer consists of thin-walled, mostly globose cells. The ascospores are hyaline, cylindrical-filiform (up to 150 µm long), 7–16-septate, usually asymmetrical with a bluntly rounded apical end and tapering towards the basal end. These species were experimentally linked with a craspedodidymum-like anamorph, and some also form a chloridium-like synanamorph in axenic culture (Huhndorf and Fernández 2005). Atkinson et al. (2007) and Perera et al. (2016) introduced another three *Chaetosphaeria* matching the diagnostic characters of this group. Among the known ascomycetes, the monotypic genus *Paragaeumannomyces* (Matsushima 2003), based on *P. sphaerocellularis*, is remarkably similar to these scolecosporous species of *Chaetosphaeria* in features of ascomata, asci and ascospores and ecology.

Our sampling of saprobic lignicolous fungi in terrestrial biotopes in various localities in Europe, New Zealand and North America revealed several species whose morphological characters best match those of the genus *Paragaeumannomyces* and other scolecosporous *Chaetosphaeria*, i.e. *Ch. albida* (Atkinson et al. 2007), *Ch. longispora* (Barr 1993; Huhndorf and Fernández 2005) and five unknown species. We also collected additional specimens that represent new species, an unknown *Codinaea* (Maire 1937; Hughes and Kendrick 1968) on submerged wood and leaves in France and United Kingdom and an undescribed *Striatosphaeria* (Samuels and Müller 1978) on decaying bark of a woody liana in French Guiana. *Codinaea*, typified by *C. aristata*, comprises fungi forming tufts of fertile or sterile setae accompanied by conidiophores terminating into a phialide and hyaline, aseptate, falcate conidia with setulae at both ends. *Striatosphaeria* is well distinguishable from other members of the family by

brown, 1-septate ascospores with longitudinal ridges and furrows running the entire length of the ascospore and a codinaea-like anamorph with brown, 1-septate conidia.

Dendrophoma (Saccardo 1880; Crous et al. 2012) is characterised by superficial, stromatic, stipitate, cupulate conidiomata, phialidic conidiogenous cells arranged in terminal whorls and naviculate to botuliform, aseptate, hyaline conidia with polar appendages. Using DNA sequence data, Crous et al. (2012) confirmed its systematic placement in the Chaetosphaeriaceae. However, its teleomorph-anamorph relationship remains unknown. A collection of a chaetosphaeria-like species with glabrous, dark, erumpent, aggregated ascomata sometimes in caespitose clusters, stipitate asci with inconspicuous apical annulus and fusiform, hyaline, 1-septate ascospores was encountered in the cracks of the bark of twigs of *Buxus sempervivens* in Germany. The axenic culture derived from ascospores yielded an anamorph similar to *Dendrophoma*. A BLASTn search (Zhang et al. 2000) for possible relatives in GenBank (Sayers et al. 2019) suggested our isolate is similar to *Dendrophoma cytisporoides*, the type species of the genus.

The present study provides new data that improve our understanding of morphological and genetic diversity of the Chaetosphaeriaceae and its pleomorphism. Our longer term goals focus on identification of monophyletic, morphologically well-delimited natural lineages and the life history of species currently assigned to the family. To assess phylogenetic relationships of our isolates, we based the study on morphological and cultivation studies along with the analysis of DNA sequence data from the nuc rDNA internal transcribed spacer region (ITS1-5.8S-ITS2 = ITS) and nuclear large subunit 28S ribosomal DNA gene (28S).

Materials and methods

Herbarium material and fungal strains

Material for this study was collected in north temperate regions of Europe (France, Germany and Ukraine) and North America (North Carolina, Tennessee), south subtropical and temperate climate zones of New Zealand, and in the neotropical regions of the Caribbean (Puerto Rico) and South America (French Guiana). An additional living culture was obtained from BCCM/MUCL Agro-food & Environmental Fungal Collection (MUCL), Université catholique de Louvain, Louvain, Belgium. Representative strains and ex-type strains were deposited at Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, the Netherlands. Holotypes and other herbarium material (as dried voucher specimens) were deposited in the Fungarium of the Illinois Natural History Survey (ILLS), Champaign, Illinois, USA, the New Zealand Fungarium (PDD), Auckland, New Zealand and Herbarium of the Institute of Botany (PRA), Czech Academy of Sciences, Průhonice, Czech Republic. Isolates and specimens, their sources and GenBank accession numbers for ITS and 28S sequences generated in this study, are listed in Table 1.

Table 1. Taxa, isolate information and GenBank accession numbers for new sequences (in bold) determined for this study.

Taxon	Specimen	Status	Country	Host	Substrate	GenBank accessions		Reference
						ITS	28S	
Codinaea paniculata	CBS 145098	T	France	unidentified	submerged decaying wood	**MT118230**	**MT118201**	This study
	CBS 126573		France	*Alnus glutinosa*	submerged decaying wood	**MT118231**	**MT118202**	This study
	CBS 127692		France	*Fraxinus excelsior*	submerged decaying wood	**MT118232**	**MT118203**	This study
	MUCL 34876		United Kingdom	unidentified	submerged dead leaf	**MT118233**	**MT118204**	This study
Dendrophoma cytisporoides	CBS 144107 IMI 506817		Germany	*Buxus sempervivens*	decaying periderm of a twig	**MT118234**	**MT118205**	This study
Paragaeumannomyces abietinus	CBS 145351	T	France	*Abies alba*	decaying wood	**MT118235**	**MT118206**	This study
Paragaeumannomyces albidus	PDD 118738		New Zealand	unidentified	decaying wood	**MT876579**	–	This study
Paragaeumannomyces elegans	PDD 118740	T	New Zealand	unidentified	decaying wood	**MT876580**	–	This study
Paragaeumannomyces granulatus	ICMP 15133	T	New Zealand	unidentified	decaying wood	**MT876575**	**MT876577**	This study
	PDD 118745		New Zealand	unidentified	decaying wood	**MT876576**	**MT876578**	This study
Paragaeumannomyces lapazianus	S.M.H. 2182		Costa Rica	unidentified	decaying wood	AY906945	**MT118207**	Huhndorf and Fernández (2005), this study
	S.M.H. 2900		Puerto Rico	unidentified	decaying wood	AY906946	**MT118208**	Huhndorf and Fernández (2005), this study
	S.M.H. 3043		Puerto Rico	unidentified	decaying wood	AY906947	**MT118209**	Huhndorf and Fernández (2005), this study
Paragaeumannomyces longisporus	A.N.M. 1269		USA, Tennessee	unidentified	decaying wood	**MT118239**	**MT118210**	This study
	ILLS00121385		USA, Tennessee	unidentified	decaying wood	**MT118237**	**MT118211**	This study
	ILLS00121386		USA, Tennessee	unidentified	decaying wood	**MT118238**	**MT118212**	This study
	S.M.H. 2519		USA, Indiana	unidentified	decaying wood	AY906939	**MT118213**	Huhndorf and Fernández (2005), this study
	S.M.H. 2758		USA, North Carolina	unidentified	decaying wood	AY906940	**MT118214**	Huhndorf and Fernández (2005), this study
	S.M.H. 3805		USA, North Carolina	unidentified	decaying wood	**MT118236**	**MT118215**	This study
	S.M.H. 3809		USA, North Carolina	unidentified	decaying wood	AY906942	**MT118216**	Huhndorf and Fernández (2005), this study
	S.M.H. 3860		USA, South Carolina	unidentified	decaying wood	AY906944	**MT118217**	Huhndorf and Fernández (2005), this study
Paragaeumannomyces panamensis	S.M.H. 3596	T	Panama	unidentified	decaying wood	AY906948	**MT118218**	Huhndorf and Fernández (2005), this study
Paragaeumannomyces sp. 1	S.M.H. 2025		Puerto Rico	unidentified	decaying wood	**MT118241**	**MT118219**	This study
	S.M.H. 3014		Puerto Rico	unidentified	decaying wood	AY906952	**MT118222**	Huhndorf and Fernández (2005) (as '*raciborskii*'), this study

Taxon	Specimen	Status	Country	Host	Substrate	GenBank accessions		Reference
						ITS	28S	
Paragaeumannomyces sp. 2	S.M.H. 2036		Puerto Rico	unidentified	decaying wood	AY906950	**MT118220**	Huhndorf and Fernández (2005) (as '*raciborskii*'), this study
	S.M.H. 2132		Puerto Rico	unidentified	decaying wood	AY906951	**MT118221**	Huhndorf and Fernández (2005) (as '*raciborskii*'), this study
Paragaeumannomyces rubicundus	S.M.H. 2881	PT	Puerto Rico	unidentified	decaying wood	AY906954	**MT118223**	Huhndorf and Fernández (2005), this study
	S.M.H. 3221	T	Costa Rica	unidentified	decaying wood	**MT118242**	**MT118224**	This study
Paragaeumannomyces sabinianus	ILLS00121384	T	USA, Tennessee	unidentified	decaying wood	**MT118243**	**MT118225**	This study
	S.M.H. 3807		USA, North Carolina	unidentified	decaying wood	AY906941	**MT118226**	Huhndorf and Fernández (2005), this study
	S.M.H. 3824		USA, North Carolina	unidentified	decaying wood	AY906943	**MT118227**	Huhndorf and Fernández (2005), this study
Paragaeumannomyces smokiensis	ILLS00121398	T	USA, Tennessee	unidentified	decaying wood	**MT118240**	**MT118228**	This study
Striatosphaeria castanea	CBS 145352	T	French Guinea	woody liana	decaying periderm	**MT118244**	**MT118229**	This study
Striatosphaeria codinaeophora	S.M.H. 1524		Puerto Rico	*Nectandra turbacensis*	decaying wood	**MT118245**	AF466088	Huhndorf et al. (2004), this study

Note: T and PT denote ex-type and ex-paratype strains.

Morphological characterisation

Morphological characteristics were obtained from fungi growing on natural substrate and growth media. Descriptions in the key are based on fungi growing on natural substrate. Herbarium material was rehydrated with tap water and examined with an Olympus SZX12 dissecting microscope (Olympus America, Inc., Melville, USA). Hand-sectioned ascomata and centrum material (asci, ascospores and paraphyses), conidiophores and conidia were mounted in 90 % lactic acid, Melzer's reagent, and lactophenol with cotton blue. All measurements were in Melzer's reagent. Means ± standard deviation (SD) based on a minimum of 20–25 measurements are given for dimensions of asci, ascospores and conidia. Micromorphological observations were made using an Olympus BX51 compound microscope with differential interference contrast (DIC) and phase contrast (PC) illumination. Images of microscopic structures were captured with an Olympus DP70 camera operated by Imaging Software Cell^D (Olympus). Macroscopic images of colonies were documented using a Canon EOS 77D digital camera with Canon EF 100mm f/2.8L Macro IS USM objective with daylight spectrum 5500K 16W LED lights (Canon Europe Ltd., Middlesex, United Kingdom). All images were processed with Adobe Photoshop CS6 (Adobe Systems, San Jose, USA).

For comparative purposes, strains were inoculated in triplicate on cornmeal dextrose agar (CMD) [17 g of cornmeal agar (Oxoid Limited, Hampshire, United Kingdom), 2 g of dextrose, 1 L of distilled water, sterilized for 15 min at 121 C], Modified

Leonian's agar (MLA) (Malloch 1981), oat-meal agar (OA) modified after Gooding and Lucas (1959) (30 g of oatmeal cooked in 1 L of distilled water for 15–30 min, filtered through cheesecloth, the filtrate was brought back to volume with distilled water, 15 g of agar, sterilized for 60 min at 121 C) and potato-carrot agar (PCA) (Crous et al. 2019). To induce sporulation, strains were also inoculated on CMA (Crous et al. 2019) with sterile stems of *Urtica dioica*. Descriptions of colonies are based on 4 wk old cultures grown in darkness at 22–23 C.

DNA extraction and amplification

Methods for the DNA extraction and amplification of samples with A.N.M., ILLS and S.M.H. prefixes followed Huhndorf et al. (2004) and Hustad and Miller (2015). Other samples were processed according to the following protocols. Total genomic DNA was extracted from mycelium removed from 3-wk-old cultures grown on MLA using the DNeasy® UltraClean® Microbial Kit (Qiagen GmbH, Germany) following the manufacturer's protocol for filamentous fungi. All PCR amplifications were carried out in 25 µL volume reactions using a Q5 High Fidelity DNA polymerase kit (New England Biolabs Inc., United Kingdom) according to the manufacturer's protocol. Primers used for the amplification included: V9G/LR8 (de Hoog and Gerrits van den Ende 1998; Vilgalys unpublished) for the internal transcribed spacers (ITS) of the nuclear rRNA cistron and D1, D2 and D3 domains (approx. 1900 bp of the 5' end) of the 28S rDNA gene.

PCR was carried out in a BioRad C1000 thermal cycler (Bio-Rad Laboratories Inc., USA) as follows: 98 C for 30 s; 40 cycles of denaturation (98 C for 10 s), annealing (62 C for 30 s) and elongation (72 C for 90 s) and a final extension step at 72 C for 5 min. Amplicons were purified from agarose gels using a NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel GmbH & Co. KG, Germany) following the manufacturer's instructions, with an elution volume of 25 µL. The DNA concentration was assessed fluorimetrically using Quant-iT PicoGreen dsDNA Assay Kit and Qubit fluorometer (Invitrogen / Thermo Fisher Scientific, USA) to assure required sequencing concentrations adjusted for the length of amplicons/ number of reads required.

Each of the amplicons was sequenced in both directions using the PCR primers and nested primers: ITS5, ITS4, JS1, JS7, JS8 and LR7 for ITS-28S (Vilgalys and Hester 1990; White et al. 1990; Landvik 1996; Vilgalys unpublished). Automated sequencing was carried out by Eurofins GATC Biotech Sequencing Service (Cologne, Germany). Raw sequence data were assembled, examined and edited using Sequencher v.5.4.6 (Gene Codes Corp., Ann Arbor, USA).

Alignments and phylogenetic analyses

Two gene markers, ITS and 28S rDNA, were analysed to assess evolutionary relationships of the unknown fungi with members of the Chaetosphaeriaceae. Consensus secondary structure (2D) models for the ITS1 and ITS2 for members of the Chaetosphaeriaceae were built using the Ppfold program v.3.0 (Sukosd et al. 2012).

The obtained 2D consensus models were further improved using the program Mfold (Zuker 2003) and adjusted manually if necessary, based on comparison of homologous positions in the multiple sequence alignment. A predicted 2D model of the 28S of *Saccharomyces cerevisiae* (Gutell et al. 1993) was used to improve the alignment of this gene. The models were highly consistent in all taxa.

ITS and 28S sequences were aligned manually in Bioedit v.7.1.8 (Hall 1999). GenBank accession numbers for ITS and 28S sequences of members of the Chaetosphaeriaceae retrieved from GenBank and published in other studies are listed in Table 2. Single-locus data sets of the Chaetosphaeriaceae (ITS: 89 sequences/602 characters including gaps, 28S: 86/1176) and *Paragaeumannomyces* (ITS: 35/489, 28S: 32/1104) were evaluated using PartitionFinder2 (Lanfear et al. 2016), implemented in the CIPRES Science Gateway v.3.3 (http://www.phylo.org) (Miller et al. 2010), to find the best partitioning scheme for our datasets and to select best-fit models under corrected Akaike information criteria. Conflict-free data sets were concatenated into two alignments (deposited in TreeBASE 25964) that were subjected to subsequent phylogenetic analyses.

Three analyses were employed to estimate phylogenetic relationships. Bayesian Inference (BI) and Maximum Likelihood (ML) analyses were performed through the CIPRES Science Gateway v.3.3. ML analyses were conducted with RAXML-HPC v.8.2.12 (Stamatakis 2014) with a GTRCAT approximation. Nodal support was determined by non-parametric bootstrapping (BS) with 1 000 replicates. BI analyses were performed in a likelihood framework as implemented in MrBayes v.3.2.6 (Huelsenbeck and Ronquist 2001). Two Bayesian searches were performed using default parameters. The B-MCMCMC analyses lasted until the average standard deviation of split frequencies was below 0.01 with trees saved every 1000 generations. The first 25 % of saved trees, representing the burn-in phase of the analysis, were discarded. The remaining trees were used for calculating posterior probabilities (PP) of recovered branches. Maximum Parsimony (MP) analyses were conducted with PAUP 4.0a167 (Swofford 2003). A heuristic search was performed with the stepwise-addition option with 1000 random taxon addition replicates and TBR branch swapping. Because the secondary structures of the ITS and 28S were carefully studied when aligning the sequences, and regions with incomplete sequences were excluded from the analysis, we treated gaps as a fifth character state; they were given equal weight as the other characters. All characters were unordered. Branch support was estimated on the recovered topologies by performing a heuristic search of 1000 bootstrap replicates consisting of ten random-addition replicates for each bootstrap replicate.

Results

Phylogenetic analyses

Employing the predicted 2D structure of the variable ITS region and 28S enabled us to construct a reliable multiple sequence alignment of homologous positions at both

Table 2. Taxa, isolate information and accession numbers for sequences retrieved from GenBank.

Taxon	Strain	Status	Country	Host	Substrate	GenBank accessions		Reference
						ITS	28S	
Adautomilanezia caesalpiniae	CC-LAMIC 102/12	T	Brazil	*Caesalpina echinata*	wood	KX821777	KU170671	Crous et al. (2016)
Anacacumisporium appendiculatum	HMAS 245593	T	China, Hainan	broad-leaved tree	dead stems	KP347129	KT001553	Ma et al. (2016)
Brunneodinemasporium brasiliense	CBS 112007	T	Brazil	unidentified	decaying leaf	JQ889272	JQ889288	Crous et al. (2012)
Brunneodinemasporium jonesii	GZCC 16-0050	T	China	unidentified	decaying wood	KY026058	KY026055	Lu et al. (2016)
Cacumisporium capitulatum	FMR 11339		Spain	unidentified	decaying wood	HF677176	HF677190	Hernández-Restrepo et al. (2017)
Calvolachnella guaviyunis	CBS 134695	T	Uruguay	*Myrcianthes pungens*	bark	KJ834524	KJ834525	Crous et al. (2014a)
Chaetosphaeria chlorotunicata	S.M.H. 1565	T	Puerto Rico	unidentified	decaying wood	–	AF466064	Fernández et al. (2006)
Chaetosphaeria innumera	M.R. 1175		Czech Republic	*Fagus sylvatica*	decaying wood	AF178551	AF178551	Réblová and Winka (2000)
Chaetosphaeria lignomollis	S.M.H. 3015	T	Puerto Rico	unidentified	decaying wood	EU037896	AF466073	Atkinson et al. (2007), Fernández et al. (2006)
Chaetosphaeria myriocarpa	CBS 264.76		The Netherlands	unidentified	decaying wood	AF178552	AF178552	Réblová and Winka (2000)
Chaetosphaeria pygmaea	M.R. 1365		Czech Republic	*Fagus sylvatica*	decaying wood	AF178545	AF178545	Réblová and Winka (2000)
Chloridium caesium	CBS 102339		Austria	*Salix cinerea*	decaying wood	AF178564	AF178564	Réblová and Winka (2000)
Chloridium gonytrichii	CBS 195.60		South Africa	unidentified	unknown	MH857954	MH869503	Vu et al. (2019)
Chloridium virescens	CBS 152.53		France	*Acer* sp.	unknown	MH857142	MH868678	Vu et al. (2019)
Codinaea acaciae	CBS 139907	T	Malaysia, Sarawak	*Acacia mangium*	leaf spot	KR476732	–	Crous et al. (2015b)
Codinaea lambertiae	CBS 143419	T	Australia, N.S. Wales	*Lambertia formosa*	leaves	MG386052	MG386105	Crous et al. (2017)
Codinaea pini	CBS 138866	T	Uganda	*Pinus patula*	dead needles	KP004465	KP004493	Crous et al. (2014b)
Codinaea simplex	CBS 966.69		The Netherlands	*Quercus* sp.	cupule	AF178559	AF178559	Réblová and Winka (2000)
Codinaeopsis gonytrichodes	CBS 593.93		Japan	unidentified	decaying plant material	AF178556	AF178556	Réblová and Winka (2000)
Conicomyces pseudotransvaalensis	HHUF 29956	T	Japan	*Machilus japonica*	dead twig	LC001710	LC001708	Liu et al. (2015)
Cryptophiale udagawae	GZCC 18-0047		China, Guizhou	unidentified	decaying wood	MN104608	MN104619	Lin et al. (2019)
Dendrophoma cytisporoides	CBS 223.95	ET	The Netherlands	*Rhododendron* sp.	branches and twigs	JQ889273	JQ889289	Crous et al. (2012)
Dictyochaeta assamica	CBS 242.66		Guadeloupe	*Musa* sp.	root	MH858788	MH870426	Vu et al. (2019)
Dictyochaeta callimorpha	ICMP 15130		New Zealand	unidentified	decaying wood	MT454483	MT454498	Réblová et al. (2020)
Dictyochaeta cangshanensis	MFLUCC 17-2214	T	China, Yunnan	unidentified	submerged decaying wood	MK828632	MK835832	Luo et al. (2019)
Dictyochaeta ellipsoidea	MFLUCC 18-1574	T	China, Yunnan	unidentified	submerged decaying wood	MK828628	MK835828	Luo et al. (2019)
Dictyochaeta fuegiana	ICMP 15153	T	New Zealand	unidentified	decaying wood	MT454487	EF063574	Réblová and Seifert (2007), Réblová et al. (2020)
Dictyochaeta lignicola	DLUCC 0899	T	China, Yunnan	unidentified	submerged decaying wood	MK828630	MK835830	Luo et al. (2019)

Taxon	Strain	Status	Country	Host	Substrate	GenBank accessions		Reference
						ITS	28S	
Dictyochaeta pandanicola	KUMCC 16-0153	T	China, Yunnan	*Pandanus* sp.	decayng leaf	MH388338	MH376710	Tibpromma et al. (2018)
Dictyochaeta septata	CBS 143386	ET	Chile	*Eucalyptus grandis* × *urophylla*	leaves	MH107889	MH107936	Crous et al. (2018a)
Dictyochaeta siamensis	MFLUCC 15-0614	T	Thailand	unidentified	submerged decaying twig	KX609955	KX609952	Liu et al. (2016)
Dictyochaeta terminalis	GZCC 18-0085	T	China, Guizhou	unidentified	decaying leaves	MN104613	MN104624	Lin et al. (2019)
Dinemasporium americanum	CBS 127127	T	USA, Iowa	n/a	soil of tallgrass prairie	JQ889274	JQ889290	Crous et al. (2012)
Dinemasporium pseudoindicum	CBS 127402	T	USA, Kansas	n/a	soil of tallgrass prairie	JQ889277	JQ889293	Crous et al. (2012)
Ellisembia aurea	CBS 144403	T	France	*Sambucus nigra*	decaying wood	MH836375	MH836376	Hyde et al. (2019)
Ellisembia folliculata	CBS 101317		France	*Salix* sp.	decaying wood	–	AF261071	Réblová and Winka (2001)
Eucalyptostroma eucalypti	CBS 142074	T	Malaysia	*Eucalyptus pellita*	leaf spots	KY173408	KY173500	Crous et al. (2016)
Exserticlava vasiformis	TAMA 450		Japan, Chiba	unidentified	plant debris	–	AB753846	Tsuchiya et al., unpublished
Infundibulomyces cupulatus	BCC 11929	T	Thailand	*Lagerstroemia* sp.	dead leaf	EF113976	EF113979	Plaingam et al. (2003)
Infundibulomyces oblongisporus	BCC 13400	T	Thailand	unidentified, angiosperm	leaf litter	EF113977	EF113980	Somrithipol et al. (2008)
Kionochaeta castaneae	GZCC 18-0025	T	China	*Castanea mollissima*	decaying seed shell	MN104610	MN104621	Lin et al. (2019)
Kionochaeta microspora	GZCC 18-0036	T	China, Guizhou	unidentified	decaying wood	MN104607	MN104618	Lin et al. (2019)
Leptosporella arengae	MFLUCC 15-0330	T	Thailand	*Arenga pinnata*	dead rachis	MG272255	MG272246	Konta et al. (2017)
Leptosporella bambusae	MFLUCC 12-0846	T	Thailand	bamboo	dead culms	KU940134	KU863122	Dai et al. (2016)
Menispora ciliata	CBS 122131	T	Czech Republic	*Acer campestre*	decaying wood	EU488736	–	Réblová and Seifert (2008)
Menispora tortuosa	DAOM 231154		unknown	unidentified	unknown	KT225527	AY544682	Schoch et al. (2009)
Menisporopsis dushanensis	GZCC 18-0084	T	China, Guizhou	unidentified	decaying leaves	MN104615	MN104626	Lin et al. (2019)
Menisporopsis theobromae	MFLUCC 15-0055		Thailand	unidentified	submerged decaying wood	KX609957	KX609954	Liu et al. (2016)
Nawawia filiformis	MFLUCC 17-2394		Thailand	unidentified	decaying wood	MH758196	MH758209	Yang et al. (2018)
Neopseudolachnella acutispora	MAFF 244358	T	Japan, Aomori	*Pleioblastus chino*	dead twigs	AB934065	AB934041	Hashimoto et al. (2015)
Neopseudolachnella magnispora	MAFF 244359	T	Japan, Aomori	*Sasa kurilensis*	dead twigs	AB934066	AB934042	Hashimoto et al. (2015)
Paliphora intermedia	CBS 896.97	IST	Australia, Queensland	unidentified	leaf litter	MH862682	EF204501	Shenoy et al. (2010), Vu et al. (2019)
Paragaeumannomyces albidus	PDD 92537	T	New Zealand	*Nothofagus* sp.	decaying wood	EU037890	EU037898	Atkinson et al. (2007)
P. albidus	PDD 92540		New Zealand	*Nothofagus* sp.	decaying wood	EU037891	–	Atkinson et al. (2007)
Paragaeumannomyces bombycinus	PDD 92538	T	New Zealand	*Nothofagus* sp.	decaying wood	EU037892	–	Atkinson et al. (2007)
Paragaeumannomyces elegans	PDD 92561		New Zealand	unidentified	decaying wood	EU037895	–	Atkinson et al. (2007)
Paragaeumannomyces garethjonesii	MFLUCC 15-1012	T	Thailand	Fabaceae	seed pod	KY212751	KY212759	Perera et al. (2016)

Taxon	Strain	Status	Country	Host	Substrate	GenBank accessions		Reference
						ITS	28S	
Paragaeumannomyces panamensis	MFLUCC 15-1011		Thailand	*Pinus* sp.	decaying wood	KY212752	KY212760	Perera et al. (2016)
Paragaeumannomyces sp. 3	S.M.H. 2017		Puerto Rico	unidentified	decaying wood	AY906949	AF466078	Huhndorf and Fernández (2005) (as '*raciborskii*')
Paragaeumannomyces sp. 4	S.M.H. 3119		Puerto Rico	unidentified	decaying wood	AY906953	AY436402	Huhndorf and Fernández (2005) (as '*raciborskii*')
Phialosporostilbe scutiformis	MFLUCC 17-0227	T	China	unidentified	submerged decaying wood	MH758194	MH758207	Yang et al. (2018)
Polynema podocarpi	CBS 144415	T	New Zealand	*Podocarpus totara*	unknown	MH327797	MH327833	Crous et al. (2018b)
Pseudodinemasporium fabiforme	CBS 140010		Malaysia, Sarawak	*Acacia mangium*	leaf spots	KR611889	KR611906	Crous et al. (2015a)
Pseudolachnea fraxini	CBS 113701	T	Sweden	*Fraxinus excelsior*	unknown	JQ889287	JQ889301	Crous et al. (2012)
Pseudolachnea hispidula	MAFF 244365		Japan, Aomori	*Morus bombycis*	dead twig	AB934072	AB934048	Hashimoto et al. (2015)
Pseudolachnella asymmetrica	MAFF 244366		Japan, Fukuoka	*Phyllostachys nigra* var. *henonis*	dead twig	AB934073	AB934049	Hashimoto et al. (2015)
Pseudolachnella scolecospora	MAFF 244379		Japan, Gifu	Sasa sp.	dead twigs	AB934086	AB934062	Hashimoto et al. (2015)
Pyrigemmula aurantiaca	CBS 126743	T	Hungary	*Vitis vinifera*	bark	HM241692	HM241692	Magyar et al. 2011)
Sporoschisma longicatenatum	MFLUCC 16-0180	T	Thailand	unidentified	submerged decaying wood	KX505871	KX358077	Yang et al. (2016)
Sporoschisma mirabile	FMR 11247		Spain	unidentified	dead wood	HF677174	HF677183	Hernández-Restrepo et al. (2017)
Striatosphaeria castanea	monte6.2		Brazil	*Encyclia ghillanyi*	root	KC928368	–	Almeida et al., unpublished
Striatosphaeria codinaeophora	M.R. 1230		Puerto Rico	*Dacryodes excelsa*	decaying wood	AF178546	AF178546	Réblová and Winka (2000)
Tainosphaeria jonesii	GZCC 16-0065	PT	China, Guangxi	unidentified	submerged decaying wood	KY026060	KY026057	Lu et al. (2016)
Tainosphaeria siamensis	MFLUCC 15-0607	T	Thailand	unidentified	submerged decaying wood	KX609956	KX609953	Liu et al. (2016)
Thozetella nivea	n/a		unknown	unidentified	unknown	EU825201	EU825200	Jeewon et al. (2009)
Thozetella tocklaiensis	CBS 378.58	T	India	*Camellia sinensis*	decaying flower	MH857817	MH869349	Vu et al. (2019)
Tracylla aristata	CBS 141404	ET	Australia, Victoria	*Eucalyptus regnans*	leaf	KX306770	KX306795	Hernández-Restrepo et al. (2016)
Tracylla eucalypti	CBS 144429	T	Colombia	*Eucalyptus urophylla*	spots on living leaves	MH327810	MH327846	Crous et al. (2018b)
Zanclospora iberica	CBS 130426	T	Spain	unidentified	decaying wood	KY853480	KY853544	Hernández-Restrepo et al. (2017)

Note: T, ET, IST and PT denote ex-type, ex-epitype, ex-isotype and ex-paratype strains.

helices and loops, thus eliminating potential ambiguous regions in the alignments. Initially, we compared trees from ML phylogenetic analyses of the two combined data sets (Chaetosphaeriaceae and scolecosporous species of *Chaetosphaeria*) after alignments were improved with the 2D structure, with and without applying Gblocks (Castresana 2000) using default options, to delimit and remove putative ambiguous regions. The

phylogenetic trees based on datasets using Gblocks had lower support for nodes and relationships within and among several clades that could not be resolved (data not shown) compared to trees in which these regions remained. Therefore, the final phylogenies were based on datasets in which Gblocks was not employed.

Evolutionary relationships of studied fungi were evaluated in the phylogenetic analysis based on the combined ITS and 28S sequences of 87 representative species of the Chaetosphaeriaceae. *Leptosporella arengae*, *L. bambusae* (Leptosporellaceae), and *Tracylla eucalypti* and *T. aristata* (Tracyllaceae) were used to root the tree. 76 nucleotides (nt) at the 5'-end and 606 nt at the 3'-end of 28S were excluded from the alignment because of missing data in the majority of sequences. The alignment had 1778 characters including gaps and 882 unique character sites (RAxML). In the MP analysis, 1021 characters were constant (proportion = 57.42 %), 134 variable characters were parsimony-uninformative, 623 characters were parsimony-informative (included); two most parsimonious trees were produced (length = 5066 steps, consistency index = 0.0.298, homoplasy index = 0.702, retention index = 0.631). For the BI analysis, GTR+I+G model was selected for ITS and 28S partitions. The ML tree (RAxML) is shown in Fig. 1. There were no conflicts among the trees generated by the three different phylogenetic analyses. The Chaetosphaeriaceae were resolved as a strongly supported clade; some of the nodes of the backbone tree, which obtained support in the ML and/or BI analyses, were not statistically supported in MP analysis. The 39 identified terminal clades corresponded to individual genera or natural groups of species. *Codinaea* was resolved as polyphyletic in three subclades. The unknown species was grouped in a clade (92 % ML BS/1.0 PP/92 % MP BS) containing seven *Codinaea* or *Dictyochaeta* species, three of which possess the typical *Codinaea* phenotype, while other morphologically similar species with setulate conidia clustered in the other two subclades, *C. lambertiae*, *C. pini* and *C. simplex* (100/1.0/100) and *Dictyochaeta septata* and *D. cangshanensis* (96/1.0/100). The new species *Codinaea paniculata*, based on four strains, was resolved as a monophyletic clade in all three analyses, although the statistical support varied. In ML and MP analyses the clade obtained 97 % and 100 % support, respectively, in the BI analysis it was weakly supported with 0.77 PP. The intraspecific variability of *C. paniculata*, based on ITS sequences, varied slightly. Three strains (CBS 145098 ex-type, CBS 126573, MUCL 34876) had identical ITS sequences, strain CBS 127692 differed from them by one base pair. The 28S sequence similarity of all strains of *C. paniculata* was 100 %. The undescribed *Striatosphaeria* was nested in the monophyletic *Striatosphaeria* (100/1.0/100) clade as sister to *S. codinaeophora*. It clustered in a subclade (93/0.98/99) with an endophytic isolate *Striatosphaeria* sp. monte6.2; their ITS exhibited 98.5 % sequence similarity. The five unknown chaetosphaeria-like species with scolecosporous ascospores were nested in a strongly-supported monophyletic clade (99/1.0/97). This clade contained eight additional morphologically similar species with scolecosporous ascospores and three-layered ascomatal wall. The clade is introduced as *Paragaeumannomyces* in this study. A chaetosphaeria-like species grouped with the ex-type strain of *Dendrophoma cytisporoides* CBS 223.95

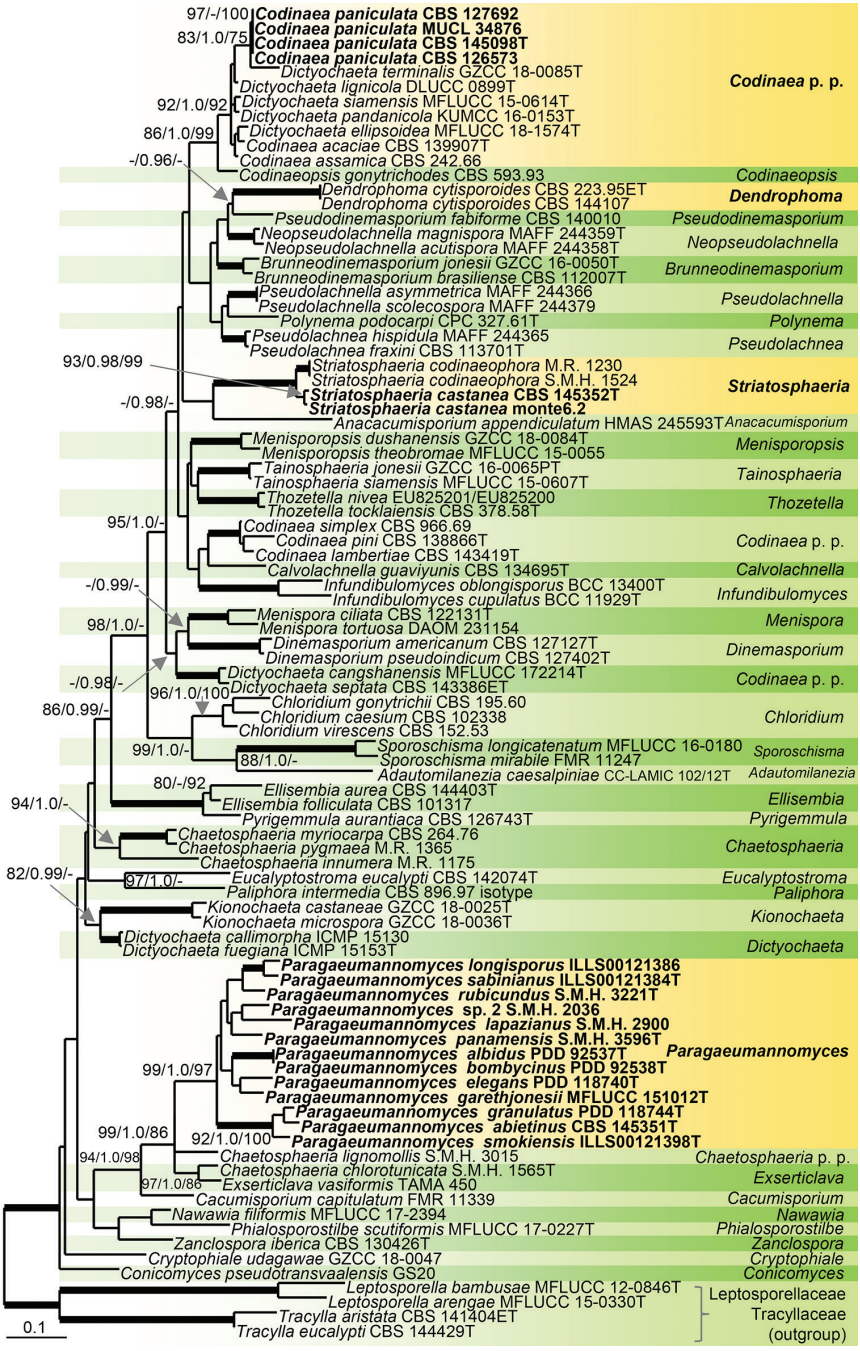

Figure 1. Combined phylogeny using ITS and 28S of selected members of the Chaetosphaeriaceae. Species names given in bold are taxonomic novelties; T, ET, IST and PT indicate ex-type, ex-epitype, ex-isotype and ex-paratype strains. Thickened branches indicate branch support with ML BS = 100%, PP values = 1.0 and MP = 100 %. Branch support of nodes ≥ 75 % ML and MP BS, and ≥ 0.95 PP is indicated above branches.

in a monophyletic clade (100/1.0/100). *Dendrophoma* was resolved as a member of a large, statistically weakly-supported grouping containing six other genera characterised by sporodochial conidiomata.

Phylogenetic relationships within the genus *Paragaeumannomyces* were assessed in the second analysis of the combined ITS-28S loci. *Chaetosphaeria fusiformis* and *Ch. lignomollis* (Chaetosphaeriaceae) were used to root the tree, and thus served as outgroup. The analysis included 35 sequences belonging to 16 species. 28 nt at the 5'-end and 714 nt at the 3'-end of 28S were excluded from the alignment due to missing data in the majority of sequences. The alignment had 1593 characters including gaps and 410 unique character sites (RAxML). In the MP analysis, 1247 characters were constant (proportion = 78.28 %), 78 variable characters were parsimony-uninformative, and 268 characters were parsimony-informative (included); 286 most parsimonious trees were produced (length = 832 steps, consistency index = 0.6118, homoplasy index = 0.3882, retention index = 0.8082). For the BI analysis, SYM+G and GTR+I+G models were selected for ITS and 28S partitions, respectively. The ML tree is shown in Fig. 2. There were no conflicts among the trees generated by the three different phylogenetic analyses. In the MP strict consensus tree, branches collapsed within the *P. longisporus*, *P. sabinianus* and *P. albidus-bombycinus* clades. The unknown species from wood of *Abies alba* clustered in a subclade (100/1.0/100) with two other unknown species from New Zealand and USA. They were introduced as new species sharing similar ascoma morphology, i.e. *P. abietinus*, *P. granulatus* and *P. smokiensis*. Another unknown species from New Zealand with densely setose, brownish-grey ascomata was grouped as a sister to *P. garethjonesii* and is introduced as *P. elegans*. The subclade (100/1.0/100) identified initially as *Chaetosphaeria ellisii fide* Huhndorf and Fernández (2005) [= *Chaetosphaeria longispora fide* Kirk (2014)] was segregated into two well-supported subclades distinguished by ascospore morphology. These subclades represent two species, *P. longisporus* (99/1.0/100) and the new species, *P. sabinianus* (100/1.0/100). *Paragaeumannomyces raciborskii fide* Huhndorf and Fernández (2005) was resolved as polyphyletic forming four subclades accompanied by different anamorph morphology. Because none of these subclades could be designated '*raciborskii* s. str.', they were labelled *Paragaeumannomyces* sp. 1–4.

Taxonomy

Codinaea paniculata **Réblová & J. Fourn., sp. nov.**
MycoBank No: 836526
Figure 3

Typification. France – Ariège • Pyrénées Mts., Rimont, La Maille brook; alt. 550 m; 28 May 2018 (incubated in moist chamber for 1 wk); on submerged decaying wood; J. Fournier leg.; M.R. 3950 (**holotype**: PRA-16319!, ex-type culture CBS 145098).

Etymology. *Panicula* (Latin) tuft, referring to the dense groups of setae and conidiophores on the natural substrate.

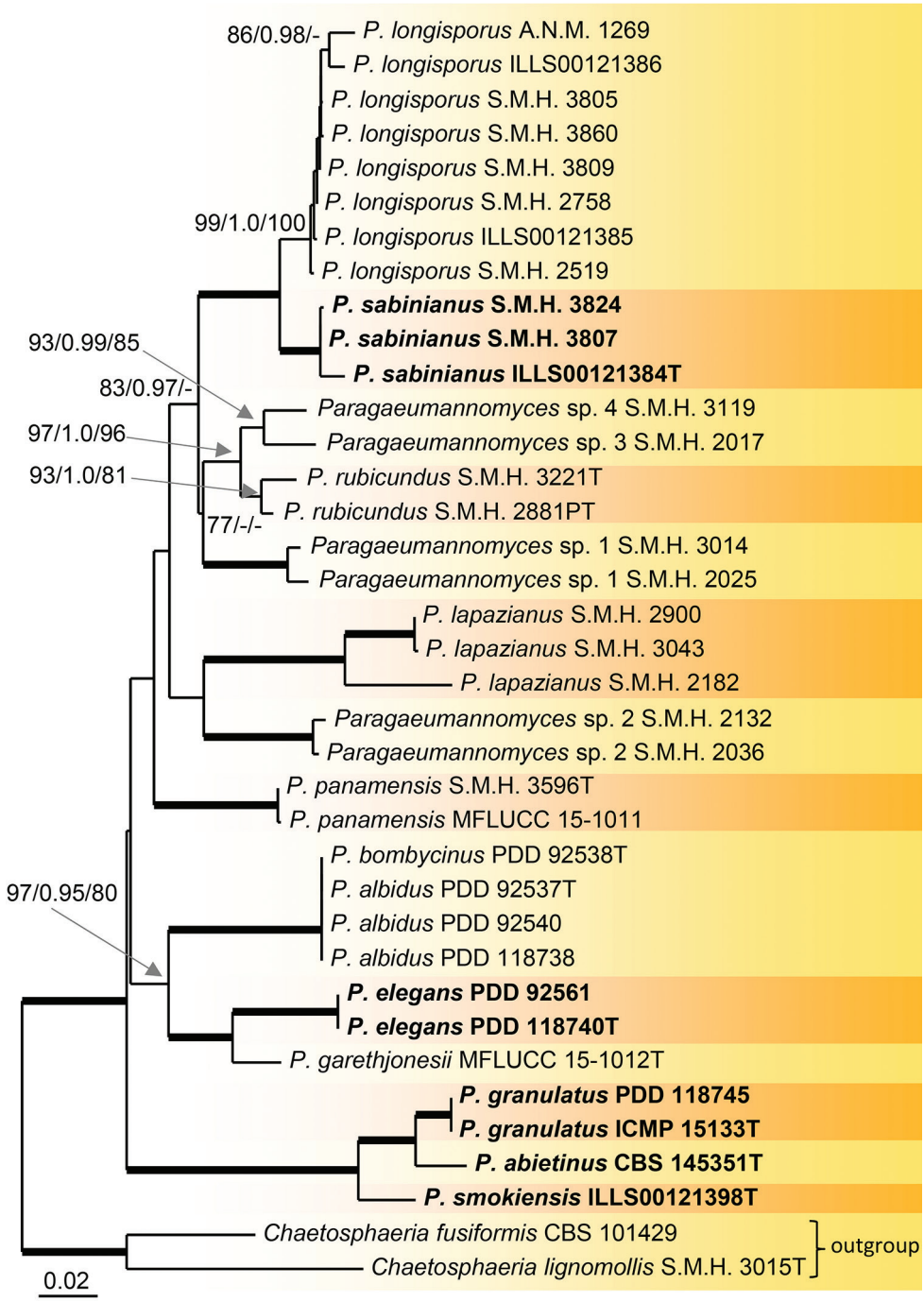

Figure 2. Combined phylogeny using ITS and 28S of 35 members of *Paragaeumannomyces*. Species names given in bold are new species; T and PT indicate ex-type and ex-paratype strains. Thickened branches indicate branch support with ML BS = 100%, PP values = 1.0 and MP = 100 %. Branch support of nodes ≥ 75 % ML and MP BS, and ≥ 0.95 PP is indicated above branches.

Description on the natural substrate. Colonies on the nature substrate effuse, hairy, greyish-brown. Setae erect, straight or slightly flexuous, smooth-walled, dark brown and thick-walled, becoming pale brown to subhyaline and thin-walled towards the apex, 230–290 µm long, 6–7.5 µm wide above the base, tapering gradually towards the apex which almost always develops into a monophialide. Conidiophores macronematous, mononematous, 62–127 × 3.5–4.5 µm, septate, erect, straight or flexuous, arising singly or in groups of 4–6 from hyphal cells associated with the bases of setae, septate, mid-brown to pale brown becoming gradually paler towards the apex. Conidiogenous cells 16.5–30(–38) × 3.5–5 µm, tapering to 1.5–2 µm just below the collarette, integrated, terminal, monophialidic, cylindrical to cylindrical-lageniform, subhyaline or pale brown at the base becoming hyaline to subhyaline towards the apex, smooth-walled; collarettes funnel-shaped, 3.5–4.5 µm wide, 1.5–2.5 µm deep. Conidia in slimy droplets, hyaline in mass, (11.5–)12–17 × (2–)2.5–3(–3.5) µm (mean ± SD = 14.7 ± 1.5 × 2.5 ± 0.3 µm), of two types, narrower and longer, 13.5–17(–17.5) × 2.5–3.5 µm (mean ± SD = 15.2 ± 1.0 × 2.8 ± 0.3 µm), and shorter and usually wider, 11.5–13.5(–14) × 3–3.5(–4) µm, falcate, asymmetrical, rounded at the apical end, with an inconspicuous scar at the basal end, hyaline, aseptate, smooth-walled, with simple, straight or gently curved setulae at both ends, 5–8 µm long; setulae inserted on the concave sides of the conidia.

Description on MLA. Vegetative hyphae hyaline to pale brown. Setae absent. Conidiophores 95–150(–195) µm long, 3.5–4.5 µm wide, conidiogenous cells 25–35 × 3.5–4(–4.5) µm, tapering to 1.5 µm just below the collarette, integrated, terminal, polyphialidic, usually cylindrical, pale brown to subhyaline, smooth-walled; collarette funnel-shaped, 3.5–4(–4.5) µm wide, 1.5–2 µm deep. Conidia in slimy droplets, of two types, narrower and longer (13–)13.5–15.5(–17) × 2.5–3 µm (mean ± SD = 14.4 ± 0.9 × 2.7 ± 0.2 µm), usually slightly wider and shorter 11–13 × 2.5–3.5 µm (mean ± SD = 12.0 ± 0.7 × 3.1 ± 0.3 µm), falcate, asymmetrical, hyaline, with simple setulae 3.5–5.5(–7.5) µm long at both ends.

Culture characteristics. On CMD colonies 80–85 mm diam, circular, flat, margin fimbriate, aerial mycelium restricted mainly to the centre and margin of the colony, sparsely lanose, floccose centrally becoming mucoid towards the margin, cobwebby at the margin, colony centre whitish, pale brown to creamy towards the margin, pale brown pigment diffusing from the centre of the colony to the agar; reverse creamy. On MLA colonies 65–70 mm diam, circular, slightly raised, margin filiform, lanose, floccose, colony centre whitish becoming brown-grey towards the margin with a brown outer zone of submerged growth, pale brown pigment diffusing to the agar; reverse dark brown. On OA colonies 89–95 mm diam, circular, raised, margin filiform, aerial mycelium occasionally reduced or absent, colonies similar to those on MLA, lanose, floccose, locally mucoid and smooth or cobwebby, whitish becoming dark grey at the margin, a dark brown to burgundy brown pigment diffusing to the agar; reverse dark grey. On PCA colonies 78–89 mm diam, circular, flat to slightly raised, margin entire to weakly filiform, lanose, floccose, occasionally locally mucoid

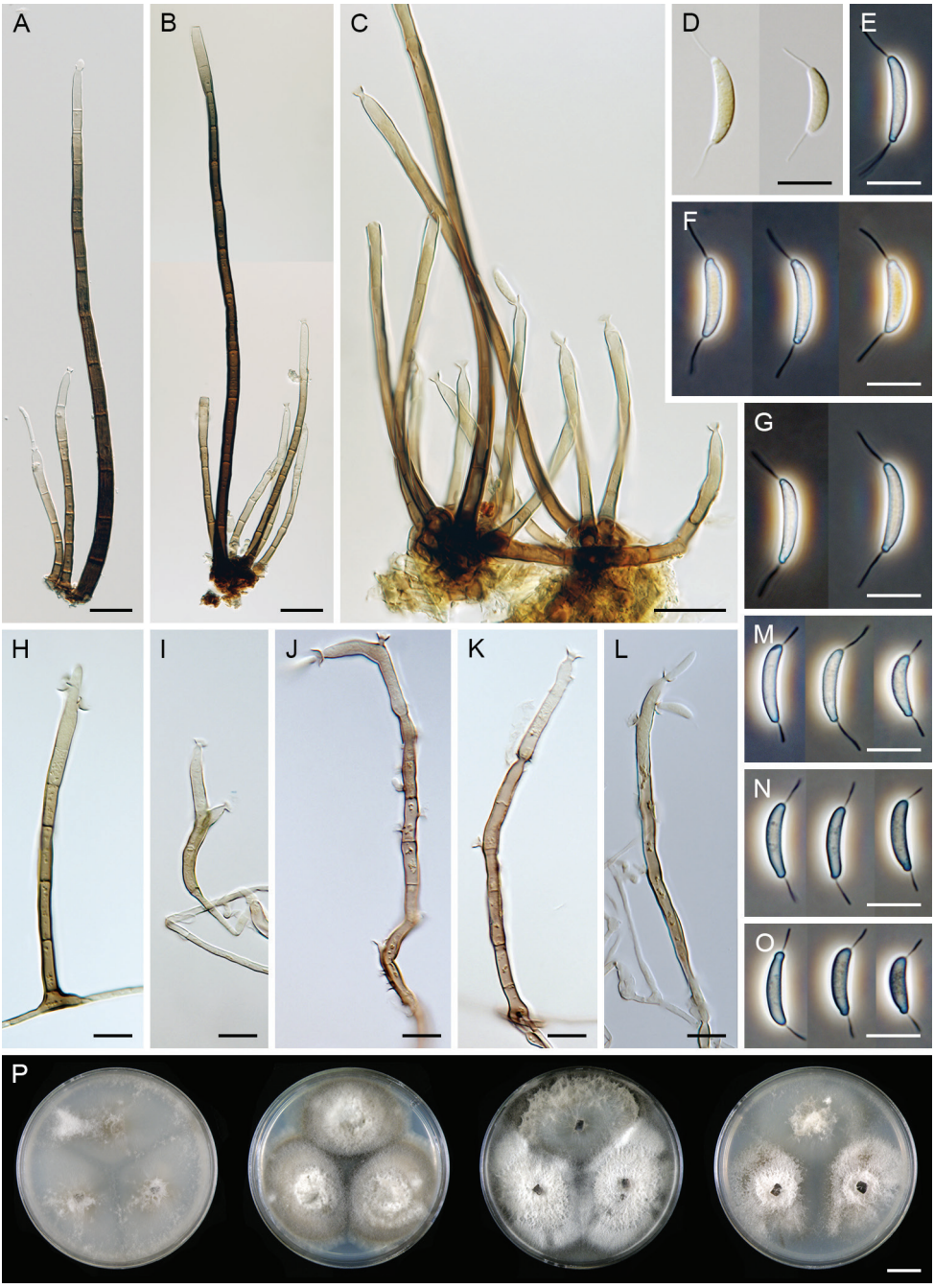

Figure 3. *Codinaea paniculata*. **A–C** setae and conidiophores on nature substrate **D–G** conidia on nature substrate **H–L** conidiophores in MLA culture (6 wk) **M–O** conidia in MLA culture (6 wk) **P** colonies on CMD, MLA, OA and PCA after 4 wk (from left to right). Images: CBS 145098 (**A, B, G–O**); CBS 126573 (**C**); CBS 127692 (**D–F**). Scale bars: 20 µm (**A–C**); 10 µm (**D–O**); 1 cm (**P**).

and smooth or with sparse decumbent aerial hyphae, cobwebby at the margin, whitish becoming brown towards the margin; reverse olivaceous brown. Sporulation on MLA, OA, CMD after 8 wk.

Other specimen examined. France – Ariège • Pyrénées Mts., Rimont, Le Baup stream, ca. 1.5 km from the village along D18 road; alt. 550 m; 12 Jun. 2009; on submerged wood of *Fraxinus excelsior*; J. Fournier leg.; J.F. 09153 (PRA-16320, culture CBS 127692) • *Ibid*.; 23 May 2008; on submerged wood of *Alnus glutinosa*; J. Fournier & M. Delpont leg.; J.F. 08124 (PRA-16321, culture CBS 126573). United Kingdom • Liverpool, University Campus Liverpool; 1992; on submerged dead leaf in a pool; G.L. Hennebert leg.; (culture MUCL 34876).

Habitat and distribution. All four isolates analysed in this study originated from the freshwater environment and occurred on decaying wood or leaves of *Alnus glutinosa*, *Fraxinus excelsior* and other unidentified hosts. Based on the BLASTn search of the ITS sequence of *C. paniculata* in GenBank, two isolates from roots of *Elymus mollis* (ITS: KU838460, KU839605, David et al. 2016), a native beach grass on the USA Pacific Northwest coast, and one environmental soil sample from ancient woodland enclosing a conifer plantation in the United Kingdom (ITS: KM374380, Johnson et al. 2014) showed 100 % sequence similarity. Based on these records, *C. paniculata* is known from the north temperate region in Europe in France and United Kingdom and North America in USA, Oregon.

Notes. Among known *Codinaea* species, *C. assamica* is similar to *C. paniculata*, but differs by slightly longer (14.6–16.8 × 2.6–2.8 µm) conidia with longer (9.6–12.8 µm) setulae (Hughes and Kendrick 1968) and formation of polyphialides in vivo. *Dictyochaeta terminalis* (Lin et al. 2019) matches *C. paniculata* in monophialidic conidiogenous cells formed in vivo and aseptate conidia, which are slightly longer and wider (14.7–20.7 × 2.9–4.2 µm). The ITS sequences of examined strains of *C. paniculata* exhibit 99.94–100 % similarity; their comparison with ITS sequences of the closely related *C. assamica* CBS 242.66 (MH858788) and *D. terminalis* GZCC 18-0085 (MN104613) showed 89.7 % and 89.85 % similarity, respectively.

Paragaeumannomyces Matsush., Matsush. Mycol. Mem. 10: 156. (2003) [2001]. Emend. Réblová & A. N. Miller.

Type species. *Paragaeumannomyces sphaerocellularis* Matsush., Mycol. Mem. 10: 156. (2003) [2001].

Description. Teleomorph: Ascomata perithecial, non-stromatic, superficial, subglobose to conical, solitary, in small groups or aggregated, sometimes collapsing laterally upon drying, ranging from white, yellow-white, light fawn-grey, ginger-brown, reddish-brown, russet to dark brown, papillate, glabrous or setose, setae dark brown, acute, opaque, scattered over entire ascoma and/or clustered around the ostiole, centrum sometimes pink to pale red. Ostiole periphysate. Ascomatal wall three-layered;

outer layer composed of thin-walled, globose, subglobose to polyhedral cells, sometimes containing pale purple pigment when fresh; middle layer composed of brick-like, dark brown cells with opaque walls; inner layer of flattened, thin-walled, subhyaline cells. Paraphyses persistent, branching, tapering. Asci unitunicate, 8-spored, cylindrical-fusiform, stipitate, apex with a non-amyloid apical annulus. Ascospores asymmetrical, cylindrical-filiform, slightly tapering towards the basal end, multiseptate, hyaline, occasionally light pink, with negative or positive dextrinoid reaction in Melzer's reagent. Synanamorphs: Craspedodidymum-like. Conidiophores mononematous, semi-macronematous to micronematous, brown, septate, unbranched or reduced to single conidiogenous cells. Conidiogenous cells phialidic, obclavate or broadly lageniform, brown, with an apical opening; collarettes flared or cup-shaped. Conidia globose, subglobose, subangular to triangular, unicellular, hyaline, with setulae. Chloridium-like. Conidiophores mononematous, macronematous, brown, septate, unbranched. Conidiogenous cells phialidic, cylindrical, subhyaline, elongating percurrently, with an apical opening; collarette indistinct or flared. Conidia globose, ovoid to clavate, unicellular, hyaline, non-setulate, accumulating in slimy droplets. [Characteristics of the synanamorphs adopted from Huhndorf and Fernández (2005)].

Notes. The holotype of *P. sphaerocellularis* (Japan, Schimizu-cho, Wakayama Pref., on decaying twig of unknown broadleaf tree, Apr. 2000, MFC-21077), the type species of *Paragaeumannomyces* (Matsushima 2003), was not available to us. A comparison of its protologue with our specimens and descriptions of other scolecosporous species of *Chaetosphaeria* (Carroll and Munk 1964; Huhndorf and Fernández 2005; Atkinson et al. 2007; Perera et al. 2016), combined with phylogenetic analysis of the ITS-28S sequences of 35 isolates, provided sufficient evidence to consider them congeneric. *Paragaeumannomyces* is proposed as the correct name for this morphologically and phylogenetically well-delimited group of chaetosphaeriaceous fungi. The width of the ascus is sometimes variable even within a single collection depending on the arrangement of ascospores in the sporiferous part, whether they are 2–3-seriate, 4-seriate end-to-end or in a fascicle.

Members of *Paragaeumannomyces* display a wide geographical distribution pattern; they have a predominantly pantropical distribution in Central America and Asia but were also encountered in the subtropical and temperate climate zones of Europe, Japan, New Zealand and North America.

Key to *Paragaeumannomyces* species

1 Ascomata almost white, yellowish-white or light fawn-grey when fresh, dark yellow, buff, tawny to dark ginger-brown or yellow-grey when dried, purple pigment absent in cells of the outer ascomatal layer, setae absent **2**

– Ascomata reddish, reddish-brown, russet or brown, sometimes with red surface crystals, globose cells in the outer ascomatal layer may contain pale purple pigment when fresh, setae present or absent **3**

2 Ascomata almost white to yellowish-white, translucent, areolate when fresh, with a distinct black papilla, asci 270–295 × 18.5–20.5 µm, ascospores (3–)5–11-septate, (55–)69–86 × 5–6.5(–7) µm ..... ***P. albidus***

– Ascomata light fawn-grey, not areolate when fresh, papilla indistinct or absent, asci 215–270 × 11–14 µm, ascospores (7–)11(–13)-septate, 62–88 × 4.5–6 µm ..... ***P. bombycinus***

3 Ascomatal setae present only at the apex or absent **4**

– Ascomatal setae present at the apex and also scattered over entire surface of the ascoma, or setae only scattered over ascoma, occasionally absent **8**

4 Ascomata with red surface crystals, ascospores 7-septate, 80–100 × 3.5–4.2 µm, anamorph craspedodidymum-like, conidia globose to subglobose in a vertical position, with setulae ..... ***P. rubicundus***

– Ascomata without red surface crystals, ascospores with seven or more septa **5**

5 Ascomata more than 500 µm diam, setae occasionally absent, ascospores 7-septate, 50–100 × 4.5–6 µm, anamorph craspedodidymum-like, conidia oblate to horizontally oblong with a short abscission scar or frill, without setulae, tropical distribution ..... ***P. lapazianus***

– Ascomata less than 500 µm diam, setae present at the apex, ascospores with seven or more septa, temperate and subtropical distribution **6**

6 Ascospores (7–)11–13-septate, (90–)95–123.5 × 4–5(–5.5) µm, asci 210–295 × (16.5–)17–24.5 µm ..... ***P. granulatus***

– Ascospores with up to 11 septa, 87 µm and shorter **7**

7 Ascospores 9–11-septate, (58–)60.5–80.5 × (3–)3.5–4.5(–5) µm, asci (134–)140–174(–189) × 11–13(–14) µm ..... ***P. smokiensis***

– Ascospores (5–)7–9(–11)-septate, (62–)65–87 × (3.5–)4–5.5 µm, asci (185–)195–240 × 12–14.5(–15.5) µm ..... ***P. abietinus***

8 Setae present at the apex and also scattered over entire surface of ascoma, ascospores 7-septate **9**

– Setae scattered over entire surface of ascoma, occasionally absent, ascospores with seven or more septa **10**

9 Ascospores (50.4–)52.5–68 × 3.5–4.5 µm, craspedodidymum- and chloridium-like synanamorphs, conidia without setulae ..... ***P. longisporus***

– Ascospores (64.5–)68.5–86.5(–88.5) × (3–)3.5–4.5 µm, anamorph unknown ..... ***P. sabinianus***

10 Ascospores 7-septate **11**

– Ascospores with more than seven septa **14**

11 Asci up to 152 µm long **12**

– Asci 150 µm and longer **13**

12 Ascomatal setae 60–75 µm long, ascospores 65–75 × 3–4 µm, asci 123–140 × 10–11 µm, craspedodidymum-like anamorph, conidia without setulae, purple-pigmented aleuriospore-like cells present in culture .... ***P. panamensis***

– Ascomatal setae 38–47 µm long, ascospores 63.3–75 × 2.3–3.7 µm, asci 120–152 × 10.7–13.3 µm, anamorph unknown ..... ***P. garethjonesii***

13 Ascospores (50–)60–100(–150) × 3–3.75(–4.5) µm, asci (150–)180–250(–350) × 10–20(–27) µm, craspedodidymum-like anamorph, conidia with or without setulae, some strains also with a chloridium-like synanamorph .... ***P. raciborskii s. lat.*** (*fide* Huhndorf and Fernández 2005; as *Paragaeumannomyces* sp. 1–4 in the phylogeny, Fig. 2)

– Ascospores (57.5–)60–73(–75) × (3.5–)4–4.5(–5) µm, asci (152–)174–221(–227) × 10.5–15(–20) µm, anamorph unknown ..... ***P. elegans***

14 Ascospores 5–10-septate, 65–90 × 3–4 µm, asci 105–125 × 10–12.5 µm .... ***P. sphaerocellularis***

– Ascospores 13–16-septate, 50–65 × 2–4 µm, asci 70–100 × 10–13 µm ..... ***P. raciborskii s. str.*** (*fide* Penzig and Saccardo 1897; Carroll and Munk 1964)

Paragaeumannomyces abietinus **Réblová, J. Fourn. & A.N. Mill., sp. nov.**
MycoBank No: 836527
Figure 4

Typification. France – Ariège • Pyrénées Mts., Ustou, Cirque de Cagateille, path up to the La Hillette lake, mixed *Abies* forest; alt. 1550 m; 18 Jul. 2018; on decaying wood of a trunk of *Abies alba*; J. Fournier leg.; J.F. 18057 (**holotype**: PRA-16323!, ex-type culture CBS 145351).

Etymology. Referring to the host *Abies alba*.

Description on the natural substrate. Teleomorph: Ascomata perithecial, non-stromatic, superficial, solitary or in small groups, 350–450 µm diam, 360–500 µm high, broadly conical, collapsing laterally upon drying, finely roughened, dark reddish-brown, glabrous except for the black conical papilla, with dark brown, stiff, acute setae, 32–40 × 3–4 µm, densely clustered around the ostiole; centrum pink. Ostiole periphysate. Ascomatal wall leathery, three-layered. Outer layer of textura angularis, 33–58 µm thick, consisting of thin-walled, globose, subglobose to polyhedral, dark ginger-brown to reddish-brown cells, 6.5–11 µm diam, grading into smaller cells towards the exterior. Middle layer of textura prismatica, 18–25 µm thick, composed of thick-walled, polyhedral, dark brown, melanised cells. Inner layer of textura prismatica, 10–15 µm thick, composed of thin-walled, flattened and elongated hyaline cells. Paraphyses abundant, hyaline, sparsely branched, septate, 4.5–7 µm wide, tapering to 2–2.5 µm, longer than the asci. Asci (185–)195–240 × 12–14.5(–15.5) µm (mean ± SD = 209.2 ± 12.0 × 14.1 ± 0.8 µm), (145–)155–205 µm (mean ± SD = 172.6 ± 14.3 µm) long in the sporiferous part, cylindrical-fusiform, stipitate, apically rounded, ascal apex non-amyloid with a distinct apical annulus 3–3.5 µm wide, 2–3 µm high. Ascospores (62–)65–87 × (3.5–)4–5.5 µm, filiform to cylindrical, straight or slightly curved to sigmoid, hyaline, light pink in mass, with dextrinoid reaction in Melzer's reagent turning reddish-brown except for the end cells which remain hyaline, (5–)7–9(–11)-septate, septa often unevenly distributed, not constricted or slightly constricted at the septa, especially at the septa above and below the middle, asymmetrical, rounded at the apical end, tapering

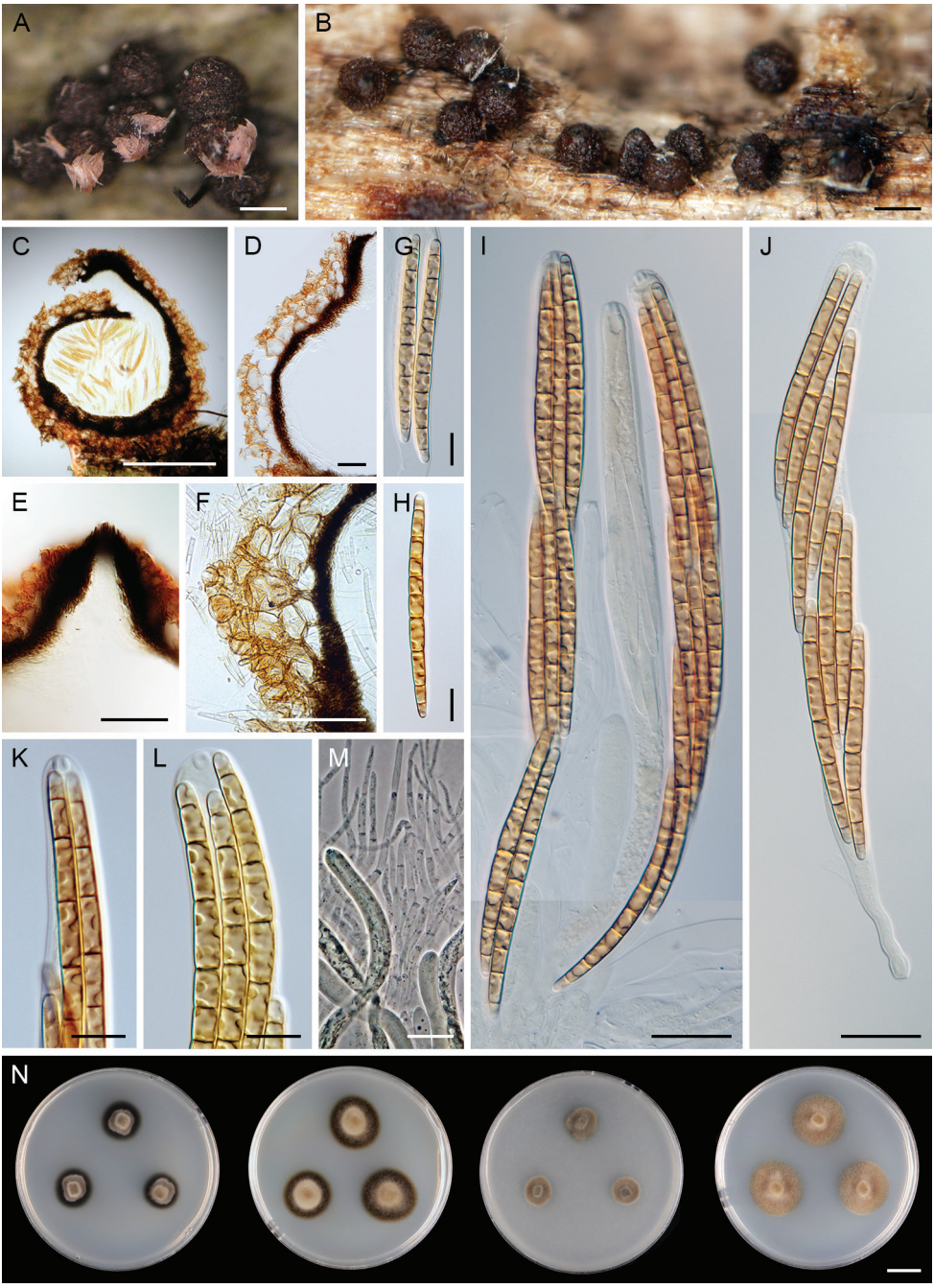

Figure 4. *Paragaeumannomyces abietinus*. **A, B** ascomata **C, D, F** vertical section of ascomal wall **E** vertical section of the ascomal wall and papilla with apical setae **G, H** ascospores **I, J** asci **K, L** ascal apex with apical ring **M** paraphyses **N** colonies on CMD, MLA, OA and PCA after 4 wk (from left to right). Images: PRA-16327 (**A**); CBS 145351 (**B, F–L, N**); PRA-16324 (**C**); PRA-16325 (**D, E, M**). Scale bars: 250 µm (**A, B**); 200 µm (**C**); 50 µm (**D–F**); 20 µm (**I, J, M**); 10 µm (**G, H, K, L**); 1 cm (**N**).

towards the basal end, with one or two guttules in each cell, 2–3-seriate or 4-seriate and partially overlapping or 4-seriate forming two fascicles end to end. Anamorph: Unknown.

Culture characteristics. On CMD colonies 10–11 mm diam, circular, slightly convex, margin entire to weakly fimbriate, lanose, beige-brown with a dark brown outer zone of submerged growth, dark brown pigment diffusing from the colony margin to agar; reverse dark brown to black. On MLA colonies 12–15 mm diam, circular, slightly convex, margin entire, lanose, floccose, cobwebby at the margin, beige-brown with a dark brown outer zone of submerged growth, brown pigment diffusing from the colony margin to agar; reverse dark brown. On OA colonies 8–9 mm diam, circular, convex, margin entire, lanose, beige-brown, with a paler outer ring; reverse brown. On PCA colonies 14–15 mm diam, circular, convex, margin entire, lanose, floccose, cobwebby towards the margin, beige, pale brown towards the margin; reverse brown. Sporulation absent on all media, even after prolonged incubation (> 3 mo).

Other specimen examined. Ukraine • Carpathian Mts., Kvasi, Bliznica near Rachiv, right bank of the upper flow of the Tisa river; alt. 1000 m; 28 Jun. 1997; on decaying wood of *Abies alba*; M. Réblová leg.; M.R. 946 (PRA-16324). • *Ibid.*; M.R. 947 (PRA-16325). • *Ibid.*; M.R. 959 (PRA-16326). Ukraine • Carpathian Mts., Massif Boržava, Guklivij; 21 Jul. 1998; on decaying wood of *Abies alba*; M. Réblová leg.; M.R. 1309 (PRA-16327).

Habitat and distribution. All specimens of *P. abietinus* occur on decaying wood of *Abies alba*. The species has been collected in mountain areas and is known in Europe in France and Ukraine.

Notes. Attempts to cultivate this species were unsuccessful for the Ukrainian specimens; the ascospores germinated over five days with long inflated germ tubes from both ends but did not grow after isolation on agar medium. The axenic culture derived from the ascospore isolate of the French material yielded sterile mycelium only.

Paragaeumannomyces abietinus is similar to *P. rubicundus* and *P. lapazianus* in reddish-brown ascomata, the arrangement of setae around the ostiole and distribution in the north temperate region. *Paragaeumannomyces rubicundus* (Huhndorf and Fernández 2005) can be distinguished from the present species in having 7-septate, longer (80–100 × 3.5–4.2 µm) ascospores and red surface crystals; *P. lapazianus* has 7-septate ascospores and a broader range of ascospore lengths including shorter and broader ascospores [(45–)50–100(–120) × (3–)4.5–6(–7) µm] and larger ascomata [(400–)500–950 µm diam, 525–825(–1025) µm high]. In the ITS-28S phylogenetic tree (Fig. 2), *P. abietinus* was clustered with *P. granulatus* (New Zealand) and *P. smokiensis* (USA). These species are morphologically highly similar; they share glabrous, dark brown to reddish-brown ascomata except for the black papilla containing short, appressed setae, and ascospores exhibiting a dextrinoid reaction in Melzer's reagent. *Paragaeumannomyces granulatus* differs from *P. abietinus* in longer [(90–)95–123.5 µm], (7–)11–13-septate ascospores, while *P. smokiensis* is distinguished from the latter species by shorter and slightly narrower asci [(134–)140–174(–189) × 11–13(–14) µm] and ascospores with more septa (9–11-septate).

Paragaeumannomyces albidus **(T.J. Atk., A.N. Mill. & Huhndorf) Réblová & A.N. Mill., comb. nov.**
MycoBank No: 836528
Figure 5

Basionym. *Chaetosphaeria albida* T.J. Atk., A.N. Mill. & Huhndorf, New Zealand J. Bot. 45: 688. 2007.

Specimens examined. New Zealand – Tasman • Tasman District, Abel Tasman National Park, Pigeon Saddle point, unpaved road between Tata Beach and Totaranui ca. 10 km NW of Totaranui; 24 Feb. 2003; on decaying wood of *Nothofagus* sp. buried in soil; M. Réblová leg.; M.R. 2605/NZ 76 (PDD 118737). – West Coast • Westland District, Arthur's Pass National Park, Kelly Shelter ca. 5 km W of Otira, Cockayane Nature Walk, a podocarp-broadleaf forest; 16 Mar. 2003; on decaying wood of a trunk; M. Réblová leg.; M.R. 2840/NZ 351 (PDD 118738). • *Ibid.*; 16 Mar. 2003; on decaying wood and bark of a branch; M. Réblová leg.; M.R. 2843/NZ 355 (PDD 118739).

Habitat and distribution. *Paragaeumannomyces albidus* has been collected on *Metrosideros robusta*, *Metrosideros* sp., *Nothofagus* sp. and other unidentified hosts and is known from New Zealand (Atkinson et al. 2007; this study).

Notes. For additional illustrations and description, see Atkinson et al. (2007).

Paragaeumannomyces albidus is the only species of the genus characterised by a wide range of ascoma colours that change when ascomata are young and fresh or mature and dried. Different colours were used by Atkinson et al. (2007) to distinguish *P. albidus* from closely related *P. bombycinus*. *Paragaeumannomyces albidus* differs from the latter species in having distinctly papillate ascomata, which are almost white, yellowish-white, areolate and translucent when young except for the black papilla (Fig. 5A, B). In older specimens and after drying, ascomata often become laterally pinched, dark yellow, buff, tawny to dark ginger-brown (Fig. 5C–E). The ascomatal wall of *P. albidus* is thicker than that of *P. bombycinus*, with an outer layer containing an external melanised section. In our material, asci were longer than those in the original description, 270–295 × 18.5–20.5 µm long and 155–225 µm long in the sporiferous part vs 220–260 × 16–20 µm *fide* Atkinson et al. (2007). The size and septation of ascospores matched those given in the protologue, though being slightly longer in the upper range: (3–)5–11-septate, (55–)69–86 × 5–6.5(–7) µm vs (5–)7(–12)-septate, (47–)60–80 × 5–7 µm *fide* Atkinson et al. (2007). The ascospores exhibited a dextrinoid reaction in Melzer's reagent turning reddish-brown except for the tips of the end cells that remain hyaline.

Attempts to isolate our specimens of *P. albidus* in living culture were not successful. Therefore, the DNA was extracted from herbarium material of all three collections, but only ITS1 of PDD 118738 could be amplified and sequenced. Comparison of the ITS1 sequences of our specimen and the holotype of *P. albidus* revealed 100 % similarity (Fig. 2).

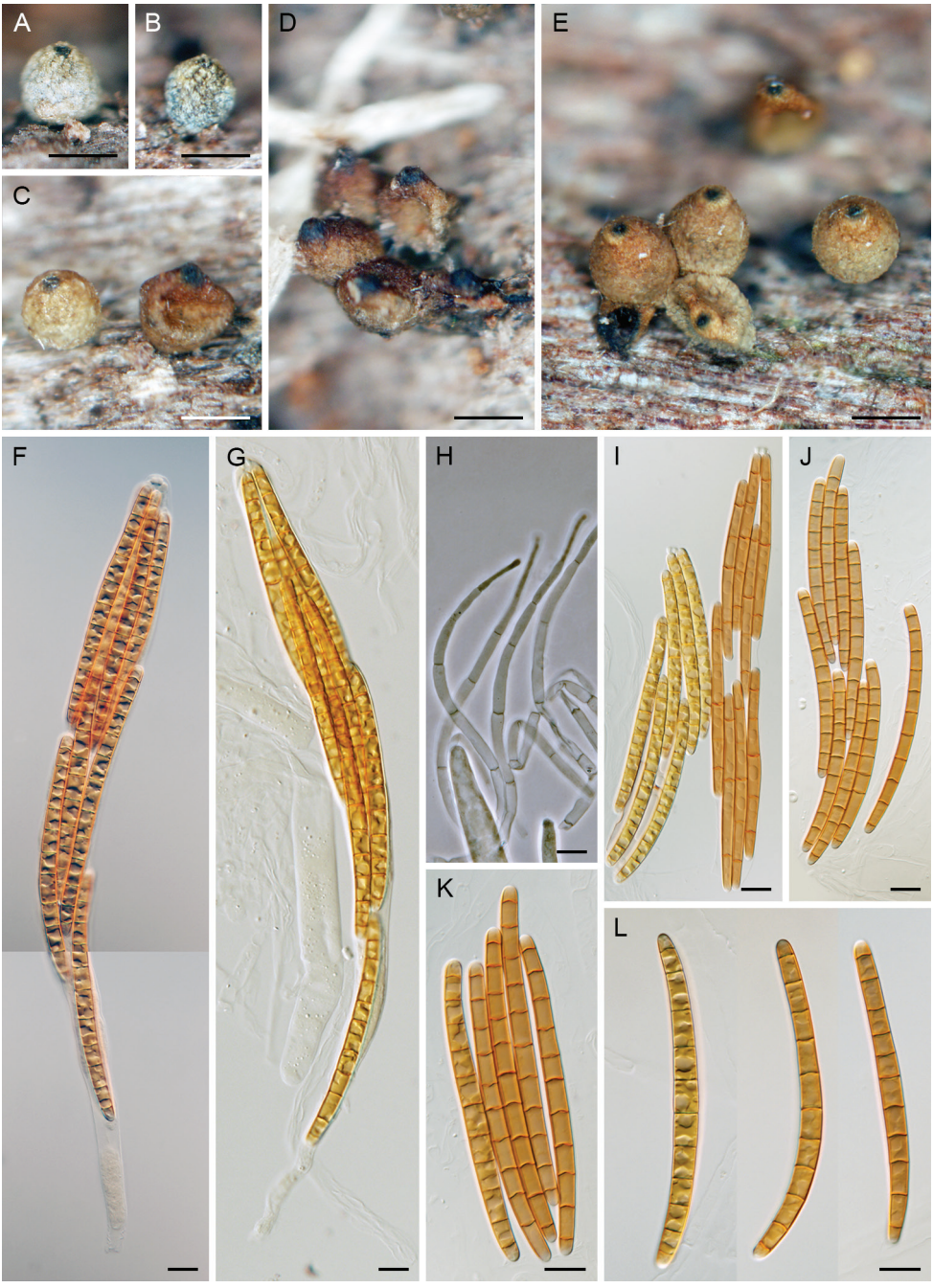

Figure 5. *Paragaeumannomyces albidus* (PDD 118738). **A, B** young ascomata **C–E** mature ascomata **F, G** asci **H** paraphyses **I, J** sporiferous parts of the asci **K, L** ascospores. Scale bars: 250 μm (**A–E**); 10 μm (**F–L**).

***Paragaeumannomyces bombycinus* (T.J. Atk., A.N. Mill. & Huhndorf) Réblová & A.N. Mill., comb. nov.**
MycoBank No: 836529

Basionym. *Chaetosphaeria bombycina* T.J. Atk., A.N. Mill. & Huhndorf, New Zealand J. Bot. 45: 691. 2007.

Habitat and distribution. The species has been collected on decaying wood of *Nothofagus* sp. and is known from New Zealand (Atkinson et al. 2007).

Notes. For description, illustration and holotype information, see Atkinson et al. (2007). Although *P. albidus* and *P. bombycinus* share identical ITS sequences and the size of their ascomata, asci and ascospores considerably overlap, the latter species was distinguished by characters in the ascomatal wall, ascoma appearance and ascospore septation. The ascomata of *P. bombycinus* are light fawn-grey and non-areolate when fresh, the ascomatal wall lacks the external melanised layer or the melanisation is only weakly present, and the black papilla is lacking or indistinct being covered by the outer layer. The ascospores of *P. bombycinus* are (7–)11(–13)-septate compared to (5–)7(–12)-septate ascospores of *P. albidus*. Atkinson et al. (2007) considered the ITS sequence identity uninformative in the light of distinct morphologies between the two species. Another explanation to this peculiar case could be that *P. bombycinus* is a described anomaly within *P. albidus* based on a single collection. More specimens of the "*bombycinus*" phenotype need to be examined, and their ITS and possibly other loci analysed.

***Paragaeumannomyces elegans* Réblová & A.N. Mill., sp. nov.**
MycoBank No: 836530
Figure 6

Typification. New Zealand – West Coast • Westland District, Mount Aspiring National Park, Haast, Roaring Billy track; 22 Mar. 2005; on decaying wood; M. Réblová leg.; M.R. 3295/NZ 566A (**holotype**: PDD 118740!).

Etymology. *Elegans* (L) elegant, referring to elegant and lovely ascomata adorned with setae.

Description on the natural substrate. Teleomorph: Ascomata perithecial, nonstromatic, superficial, in small groups, often gregarious, 290–350 µm diam, 280–350 µm high, subglobose to slightly conical, dully glossy, brown with a light grey tinge except for the tiny black papilla composed of thick-walled, dark brown cells, ascomata densely setose, setae 28–60 × 4.5–6 µm, stiff, acute, dark brown, thick-walled, opaque. Ostiole periphysate. Ascomatal wall leathery, three-layered. Outer layer of textura angularis, 33–41 µm thick, consisting of thin-walled, globose to subglobose to polyhedral, reddish-brown cells ca. 5–12 µm diam. Middle layer of textura prismatica, 9.5–18 µm thick, composed of thick-walled, polyhedral, elongated, dark brown, melanised cells. Inner layer of textura prismatica, 5–8 µm thick, composed of thin-walled, flattened and elongated hyaline cells. Paraphyses abundant, hyaline,

Figure 6. *Paragaeumannomyces elegans*. **A, B** ascomata **C** vertical section of ascomal wall **D** ascal apex with apical annulus **E–G** asci **H** ascospores **I** paraphyses. Images: PDD 118740 (**A, B, D, H, I**); PDD 118741 (**C, E–G**). Scale bars: 250 µm (**A, B**); 20 µm (**C**); 10 µm (**D–I**).

sparsely branched, septate, 3.5–5 µm wide, tapering to ca. 2 µm, longer than the asci. Asci (152–)174–221(–227) × 10.5–15(–20) µm (mean ± SD = 204.8 ± 13.7 × 12.3 ± 1.5 µm), (129–)141–197(–204) µm (mean ± SD = 168.2 ± 17.2 µm) long in

the sporiferous part, cylindrical-fusiform, stipitate, apically rounded, ascal apex non-amyloid with a distinct apical annulus 2.5–3 µm wide, 2–2.5 µm high. Ascospores (57.5–)60–73(–75) × (3.5–)4–4.5(–5) µm (mean ± SD = 65.5 ± 3.2 × 4.1 ± 0.2 µm), filiform to cylindrical, straight or slightly curved to sigmoid, hyaline, with negative or very weak dextrinoid reaction in Melzer's reagent, 7–septate, septa usually obscured by large guttules, not constricted at the septa, asymmetrical, rounded at the apical end, slightly tapering towards the basal end, with one or two guttules in each cell, 2–3-seriate or 3–4-seriate, partially overlapping. Anamorph: Unknown.

Other specimen examined. New Zealand – Otago • Clutha District, The Catlins, Catlins Coastal Rain Forest Park, MacLennan Range, Lake Wilkie Walk; 17 Mar. 2005; on decaying wood of a branch; M. Réblová leg.; M.R. 3289/NZ 549 (PDD 118742). – West Coast • Westland District, Ship Creek Point, Kahikatea Swamp Forest walk; 8 Mar. 2003; on decaying wood; M. Réblová leg.; M.R. 2819/NZ 329 (PDD 118741). – West Coast • Westland District, Ross, Totara Valley Road, 12 Apr. 2005; on decaying wood; M. Réblová leg.; M.R. 3486/NZ 775 (PDD 118743).

Habitat and distribution. The present species is a saprobe on decaying wood of *Nothofagus* sp. and other unidentified hosts, known from New Zealand (Atkinson et al. 2007; this study).

Notes. *Paragaeumannomyces elegans* is distinguishable from other members of the genus by densely setose, dull glistening brown ascomata with a light grey tinge, which gives them an almost grey appearance when dried. The new species resembles *P. garethjonesii* (Perera et al. 2016) and *P. panamensis* (Huhndorf and Fernández 2005) in 7-septate ascospores and setose ascomata with acute, stiff, opaque setae scattered over the entire surface, but differs from them in larger ascomata, asci and wider ascospores (for a detailed comparison see the key).

Comparison of the ITS sequence of the holotype of *P. elegans* with available *Paragaeumannomyces* sequences revealed 100 % sequence similarity with a specimen PDD 92561 (New Zealand, Taupo, Ohakune, ITS: EUO37895) tentatively identified as *P. raciborskii* (Atkinson et al. 2007) (Fig. 2).

Paragaeumannomyces garethjonesii **(R.H. Perera, Maharachch. & K.D. Hyde) Réblová & A.N. Mill., comb. nov.**
MycoBank No: 836531

Basionym. *Chaetosphaeria garethjonesii* R.H. Perera, Maharachch. & K.D. Hyde, Mycosphere 7: 1308. 2016.

Habitat and distribution. *Paragaeumannomyces garethjonesii* was collected on a Fabaceae seed pod and is known from Asia in Thailand (Perera et al. 2016).

Notes. For description, illustration and holotype information see Perera et al. (2016). *Paragaeumannomyces garethjonesii* resembles *P. panamensis* (Huhndorf and Fernández 2005) in size of ascomata, which are the smallest (up to 250 µm diam and 270 µm high) within the genus, setae scattered over the entire ascoma, overlapping length of their asci and 7-septate ascospores, but it differs by shorter (38–47 µm) setae,

slightly wider (10.7–13.3 µm) asci and the absence of aleuriospore-like cells in culture (for a detailed comparison see the key).

Paragaeumannomyces granulatus **Réblová & A.N. Mill., sp. nov.**
MycoBank No: 836532
Figure 7

Typification. New Zealand – West Coast • Westland District, Hokitika, Mananui Point, Lake Mahinapua, Swimmers Beach walks; 5 Mar. 2003; on decaying wood; M. Réblová leg.; M.R. 2715/NZ 216 (**holotype**: PDD 118744!, ex-type culture ICMP 15133).

Etymology. *Granulum* (L), granule, small grain, diminutive of *granum*, referring to the roughened surface of the ascomatal wall composed of globose cells, which appears granulose in the surface view.

Description on the natural substrate. Teleomorph: Ascomata perithecial, nonstromatic, superficial, solitary or in small groups, 380–495 µm diam, 415–530 µm high, subglobose to conical, finely roughened, dark brown to dark reddish-brown, sometimes with irregular reddish colour except for the black papilla; papilla composed of dark brown, thick-walled, cylindrical to subulate, apically narrowly rounded soft setae; centrum pink. Ostiole periphysate. Ascomatal wall leathery, three-layered. Outer layer of textura angularis, 95–115 µm thick, consisting of thin-walled, globose to subglobose ginger-brown cells ca. 27–33 µm diam, grading into smaller cells 8–16 µm diam. Middle layer of textura prismatica, 14–21 µm thick, composed of thick-walled, polyhedral, elongated, dark brown, melanised cells. Inner layer of textura prismatica, 7–12 µm thick, composed of thin-walled, flattened and elongated hyaline cells. Paraphyses abundant, hyaline, sparsely branched, septate, 3.5–5 µm wide, tapering to 2–2.5 µm, longer than the asci. Asci 210–295 × (16.5–)17–24.5 µm (mean ± SD = 239.7 ± 15.5 × 20.3 ± 2.1 µm), 165–200(–250) µm (mean ± SD = 184.7 ± 10.3 µm) long in the sporiferous part, cylindrical-fusiform, stipitate, apically rounded, ascal apex non-amyloid with a distinct apical annulus 3.5–4 µm wide, 2.5–3(–3.5) µm high. Ascospores (90–)95–123.5 × 4–5(–5.5) µm (mean ± SD = 101.4 ± 10.2 × 4.8 ± 0.4 µm), filiform to cylindrical, straight or slightly curved to sigmoid, hyaline to very light pink, light pink-brown in mass, with dextrinoid reaction in Melzer's reagent turning reddish-brown except for the end cells which remain hyaline, (7–)11–13-septate, septa often unevenly distributed, not constricted or slightly constricted at the septa, asymmetrical, broadly rounded at the apical end, tapering and narrowly rounded at the basal end, with one or two guttules in each cell, 2–3-seriate or 4-seriate and partially overlapping. Anamorph: Unknown.

Culture characteristics. On CMD colonies 14–16 mm diam, circular, convex, margin fimbriate, lanose, grey-brown, reverse dark brown to almost black. On MLA colonies 19–20 mm diam, circular, raised, margin entire to weakly fimbriate, lanose, beige-brown, with a dark brown outer zone, reverse dark brown to almost black. On OA colonies 13–16mm diam, circular, raised, margin weakly fimbriate, lanose, beige-grey becoming grey towards the periphery, reverse dark brown to almost black. On

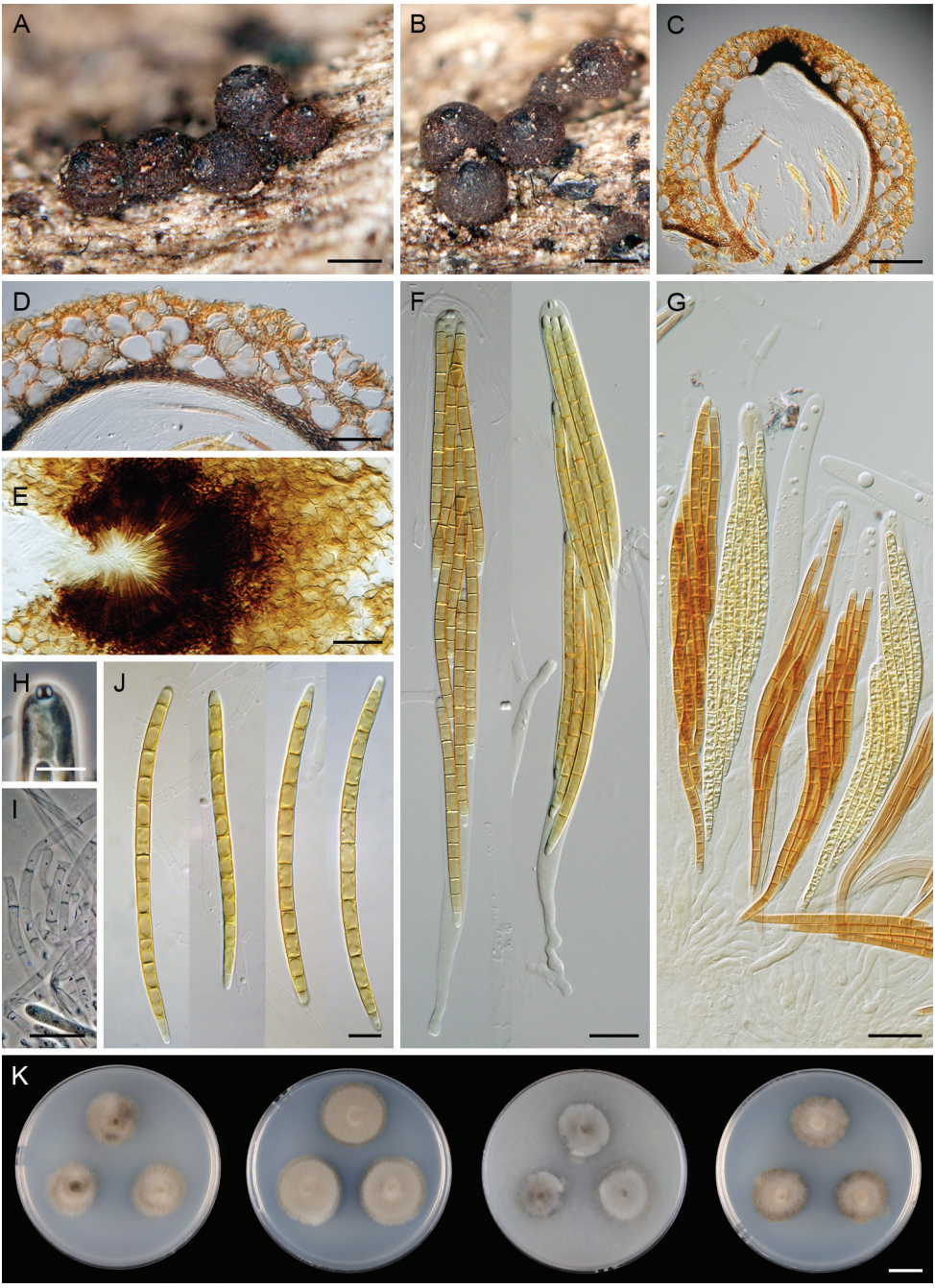

Figure 7. *Paragaeumannomyces granulatus* (PDD 118744). **A, B** ascomata **C, D** vertical section of ascomal wall **E** papilla and the upper part of ascomal wall in surface view **F, G** asci **H** ascal apex with apical annulus **I** paraphyses **J** ascospores **K** colonies on CMD, MLA, OA and PCA after 4 wk (from left to right). Scale bars: 250 µm (**A, B**); 100 µm (**C**); 25 µm (**G**); 20 µm (**D–F, I**); 10 µm (**H, J**); 1 cm (**K**).

PCA colonies 15–17 mm diam, circular, slightly convex, margin weakly fimbriate, lanose, beige, pale brown at the margin, reverse black. Sporulation absent on all media.

Other specimen examined. New Zealand – Auckland • Auckland district, Waitakere Ranges Nature Reserve, Anawhata Road; 24 Apr. 2005; on decaying wood; M. Réblová leg.; M.R. 3543/NZ 838 (PDD 118745).

Habitat and distribution. A saprobe on decaying wood, known from New Zealand.

Notes. *Paragaeumannomyces granulatus* most closely resembles *P. abietinus* in the ascoma appearance, pink content of the ascoma centrum, ascospores with usually more than seven septa and positive dextrinoid reaction in Melzer's reagent but both species are separated by size of asci and ascospores. The ascospores of *P. abietinus* are (5–)7–9(–11)-septate and shorter [(62–)65–87 µm] and asci are shorter and narrower [(185–)195–240 × 12–14.5(–15.5) µm].

***Paragaeumannomyces lapazianus* (G.C. Carroll & Munk) Réblová & A.N. Mill., comb. nov.**
MycoBank No: 836533

≡ *Chaetosphaeria lapaziana* (G.C. Carroll & Munk) F.A. Fernández & Huhndorf, Fung. Diver. 18: 49. 2005.

Basionym. *Lasiosphaeria lapaziana* G.C. Carroll & Munk, Mycologia 56: 90. 1964.

Habitat and distribution. *Paragaeumannomyces lapazianus* is common on decaying wood in the neotropics and is known from the Caribbean in Puerto Rico and Jamaica, from Central America in Costa Rica, and from South America in French Guiana (Carroll and Munk 1964; Huhndorf and Fernández 2005).

Notes. For description, illustration and holotype information see Carroll and Munk (1964) and Huhndorf and Fernández (2005). *Paragaeumannomyces lapazianus* has 7-septate ascospores and the largest ascomata in the genus, (400–)500–950 µm diam and 525–825(–1025) µm high *fide* Huhndorf and Fernández (2005), and forms a craspedodidymum-like anamorph in vitro. The anamorph is characterised by inflated, pigmented conidiogenous cells with a flared collarette and oblate to horizontally oblong conidia with a short abscission scar or frill and without setulae.

***Paragaeumannomyces longisporus* (Sacc.) Réblová & A.N. Mill., comb. nov.**
MycoBank No: 836534
Figure 8

≡ *Sphaeria longispora* Ellis, Bull. Torrey Bot. Club 6: 135. 1877 non Currey 1859 nec Karsten 1873. (Nom. illegit., Art. 53.1)
≡ *Ceratostomella longispora* (Sacc.) Cooke, Grevillea 17: 50. 1889.

≡ *Chaetosphaeria longispora* (Sacc.) P.M. Kirk, Index Fung. 120: 1. 2014.
= *Lasiosphaeria ellisii* M.E. Barr, Mycotaxon 46: 48. 1993.
≡ *Chaetosphaeria ellisii* (M.E. Barr) Huhndorf & F.A. Fernández, Fung. Diver. 19: 27. 2005.

Basionym. *Ophioceras longisporum* Sacc., Syll. fung. 2: 360. 1883.

Specimens examined. USA – Tennessee • Cocke Co., Great Smoky Mountains National Park, Cosby, Cosby Nature Trail; alt. 716 m; 23 Mar. 2007; on decaying wood; A.N. Miller, P. Chaudhary & H.A. Raja leg.; A.N.M. 1134 (ILLS00121385). • *Ibid.*; 19 Jul. 2007; T.J. Atkinson & P. Chaudhary leg.; A.N.M. 1250 (ILLS00121386).

Habitat and distribution. The species occurs on decaying wood and is known from the north temperate region in the USA (Indiana, New Jersey, North Carolina, South Carolina, Tennessee) (Barr 1993; Huhndorf and Fernández 2005; this study).

Notes. For description and illustration, refer to Barr (1993), and Huhndorf and Fernández (2005). Our specimens of *P. longisporus* match well the fungus described and illustrated by Barr (1993) based on the holotype of *Sphaeria longispora* (Ellis 1877), only the asci are longer and agree with the measurements given by Huhndorf and Fernández (2005). Based on examination of our material (Fig. 8), ascomata are reddish-brown, subglobose to globose, with three-layered wall, setose, setae dark brown, acute, scattered over entire ascomatal surface and also aggregated around the ostiole, asci (140.5–)165–183 × 10.5–12.5 µm and (114–)133–157.5 µm long in the sporiferous part, ascospores (50.5–)52.5–68 × 3.5–4.5 µm, 7-septate, asymmetrical, tapering towards the basal end, with a negative or very weak dextrinoid reaction in Melzer's reagent.

Sphaeria longispora (Ellis 1877) is a later homonym of *S. longispora* (Currey 1859) and *S. longispora* (Karsten 1873). Barr (1993) revised the holotype of *S. longispora* Ellis (USA, New Jersey, Newfield, on fallen branch of *Kalmia latifolia*, 20 Jul 1874, J.B. Ellis, NY) and concluded that the fungus is better placed in *Lasiosphaeria* due to filiform, septate ascospores and setose ascomata and proposed a replacement name, *Lasiosphaeria ellisii* as a nomen novum. This species was later transferred to *Chaetosphaeria* by Huhndorf and Fernández (2005) as *Ch. ellisii*. Kirk (2014) considered the first combination of *S. longispora* in *Ophioceras* by Saccardo (1883) as the earliest legitimate name of the taxon in the same rank (Art. 41.3) to replace *Sphaeria longispora* Ellis. *Ophioceras longisporum* Sacc. is, therefore, a basionym for all future combinations. Kirk (2014) proposed a new combination *Chaetosphaeria longispora* but erroneously cited *S. longispora* as the basionym, which does not affect the valid publication of the new combination (Art. 41.8c).

***Paragaeumannomyces panamensis* (Huhndorf & F.A. Fernández) Réblová & A.N. Mill., comb. nov.**
MycoBank No: 836535

Basionym. *Chaetosphaeria panamensis* Huhndorf & F.A. Fernández, Fung. Diver. 19:33. 2005.

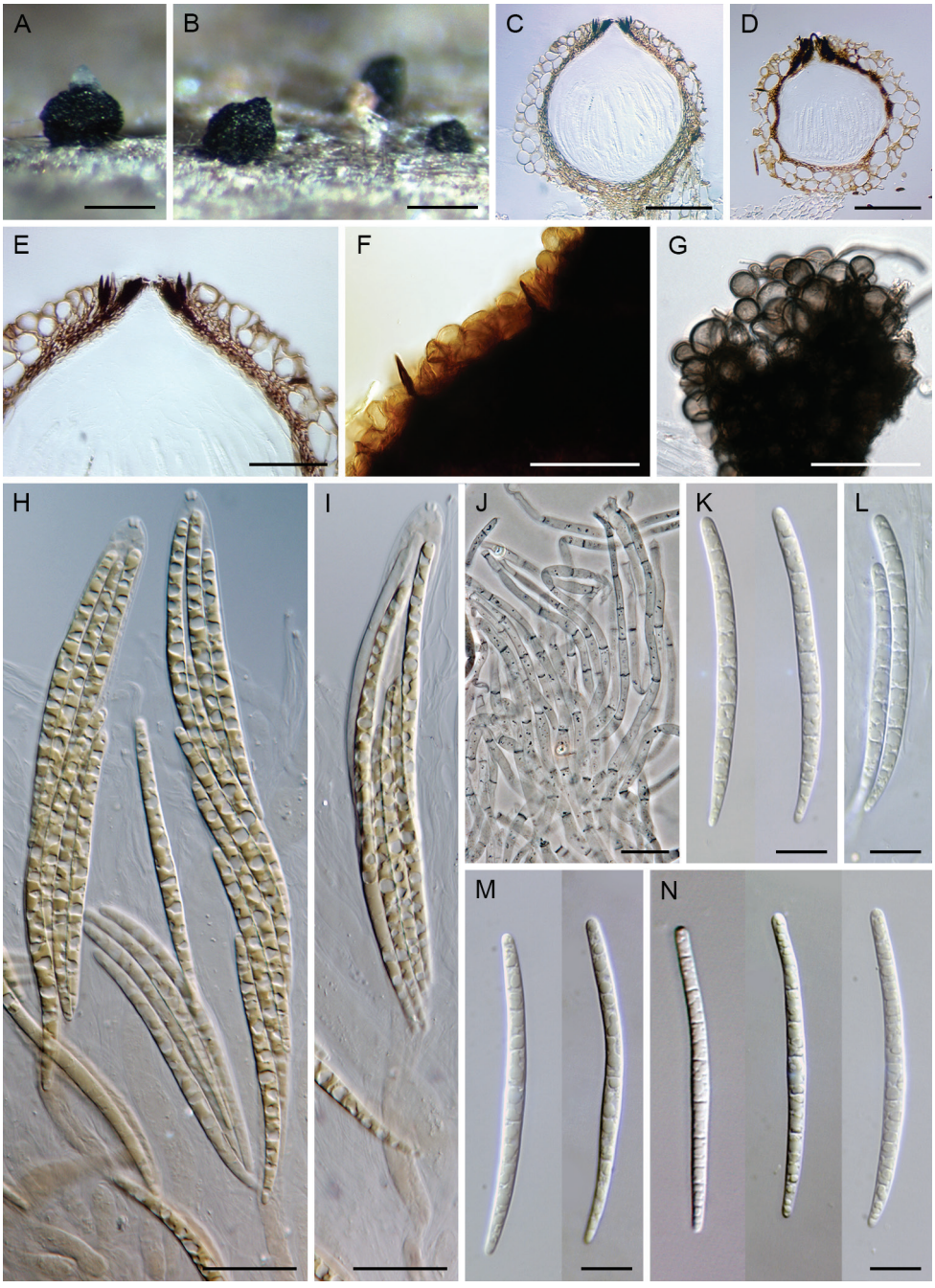

Figure 8. *Paragaeumannomyces longisporus*. **A, B** ascomata **C, D** vertical section of ascomal wall **E** vertical section of ascomal wall and papilla with apical of setae **F** ascomal wall with setae **G** globose cells of the outer layer of the ascomal wall **H, I** asci **J** paraphyses **K–N** ascospores. Images: ILLS00121385 (**A, B, G**); S.M.H. 3860 (**C, E, M**); S.M.H. 2519 (**D**); ILLS00121386 (**F, H–J, K**); S.M.H. 2758 (**L**); S.M.H. 3809 (**N**). Scale bars: 250 µm (**A–D**); 50 µm (**E–G**); 20 µm (**H–J**); 10 µm (**K–N**).

Habitat and distribution. *Paragaeumannomyces panamensis* has been collected on decaying wood of *Pinus* sp. and an unidentified host, and it is known from Asia in Thailand and Central America in Panama (Huhndorf and Fernández 2005; Perera et al. 2016).

Notes. For description, illustrations and holotype information see Huhndorf and Fernández (2005) and Perera et al. (2016). *Paragaeumannomyces panamensis* is similar to *P. sphaerocellularis* in ascoma and is more or less comparable in size of asci, but differs by shorter, always 7-septate ascospores and occurrence in the tropics. For detailed comparison, see notes to *P. sphaerocellularis*.

Paragaeumannomyces raciborskii **(Penz. & Sacc.) Réblová & A.N. Mill., comb. nov.**
MycoBank No: 836536

≡ *Lasiosphaeria raciborskii* (Penz. & Sacc.) G.C. Carroll & Munk, Mycologia 56: 91. 1964.
≡ *Chaetosphaeria raciborskii* (Penz. & Sacc.) F.A. Fernández & Huhndorf, Mycol. Res. 108: 29. 2004.

Basionym. *Ophiochaeta raciborskii* Penz. & Sacc., Malpighia 11: 406. 1897.

Habitat and distribution. *Paragaeumannomyces raciborskii* has been collected on culms of *Chusquea* bamboo and other unidentified bamboo species, on palm wood and fruit, and decaying wood of unknown trees. The species has a pantropical geographical distribution and is probably the most commonly encountered species of the genus; it is known from Indonesia in Java and Central America in Costa Rica (Penzig and Saccardo 1897; Carroll and Munk 1964). Other collections published under this name, which may represent different species, originate from Asia in Thailand, the Caribbean in Cuba, Jamaica and Puerto Rico, Central America in Costa Rica and Panama, and South America in Ecuador, French Guiana and Venezuela (Huhndorf and Fernández 2005).

Notes. For descriptions and illustrations, see Carroll and Munk (1964). The holotype of *P. raciborskii* (Penzig and Saccardo 1897) originates from decaying wood in Java. In the protologue, the species was described with black, setose ascomata 250 µm diam, short-stipitate asci 130–150 × 9–10 µm and hyaline, multiseptate ascospores 60–70 × 3 µm. Carroll and Munk (1964) redescribed the species based on the holotype and an additional collection from Costa Rica as having setose, reddish-brown ascomata 250–300 µm diam, 13–16-septate ascospores 50–65 × 2–4 µm, and asci 70–100 × 10–13 µm. Huhndorf and Fernández (2005) introduced a broader species concept of *P. raciborskii*, which was based on numerous specimens of a tropical geographical distribution originating mainly from Central and South America. The species was characterised by setose, reddish, russet or brown ascomata (150–)200–450 µm diam with stiff, dark setae scattered over the entire ascoma or absent in some specimens, 7-septate ascospores (50–)60–100(–150) × 3–3.75(–4.5) µm, and short-stipitate asci (150–)180–250(–350) × 10–20(–27) µm.

Paragaeumannomyces raciborskii fide Huhndorf and Fernández (2005) shows a high degree of ITS sequence variability accompanied by a low phenotypic plasticity, which resulted in the species being polyphyletic and segregated into four lineages labelled *Paragaeumannomyces* sp. 1–4 (Fig. 2). Two anamorphic craspedodidymum-like morphotypes with and without setulae and a chloridium-like synanamorph have been experimentally linked to several strains of *P.* '*raciborskii*' by Huhndorf and Fernández (2005). Although the in vitro anamorphic characters seem promising in becoming another important set of diagnostic features to distinguish species of *Paragaeumannomyces*, isolated strains often form sterile mycelium in vitro or they are difficult to isolate into living culture.

Paragaeumannomyces rubicundus **(Huhndorf & F.A. Fernández) Réblová & A.N. Mill., comb. nov.**
MycoBank No: 836537

Basionym. *Chaetosphaeria rubicunda* Huhndorf & F.A. Fernández, Fung. Diver. 19: 39. 2005.

Habitat and distribution. *Paragaeumannomyces rubicundus* occurs on decaying wood and is known from the Caribbean in Puerto Rico and from Central America in Costa Rica (Huhndorf and Fernández 2005).

Notes. For description, illustration and holotype information see Huhndorf and Fernández (2005). *Paragaeumannomyces rubicundus* is distinguished from other species of the genus by ascomata with red surface crystals not dissolving in water, 3 % KOH or lactophenol. Similar to *Paragaeumannomyces* sp. 4 (S.M.H. 3119), the craspedodidymum-like anamorph forms conidia with three setulae.

Paragaeumannomyces sabinianus **Réblová & A.N. Mill., sp. nov.**
MycoBank No: 836538
Figure 9

Typification. USA – Tennessee • Sevier Co., Great Smoky Mountains National Park, Twin Creeks, Twin Creeks Nature Trail, near ATBI plot; alt. 549 m; 11 Oct. 2006; on decaying wood; A.N. Miller & P. Chaudhary leg.; A.N.M. 1011 (**holotype**: ILLS00121384!).

Etymology. The species epithet is proposed in honour of Sabine M. Huhndorf for her contribution to mycology and studies in *Chaetosphaeria*.

Description on the natural substrate. Teleomorph: Ascomata perithecial, nonstromatic, superficial, usually solitary or in small groups, 250–300(–400) μm diam, 280–320 μm high, subglobose to broadly conical, rarely collapsing laterally upon drying, finely roughened, dark reddish-brown except for a black indistinct papilla, setose, setae 30–41.5 × 4–5 μm, dark brown, stiff, acute, scattered over entire ascoma, shorter

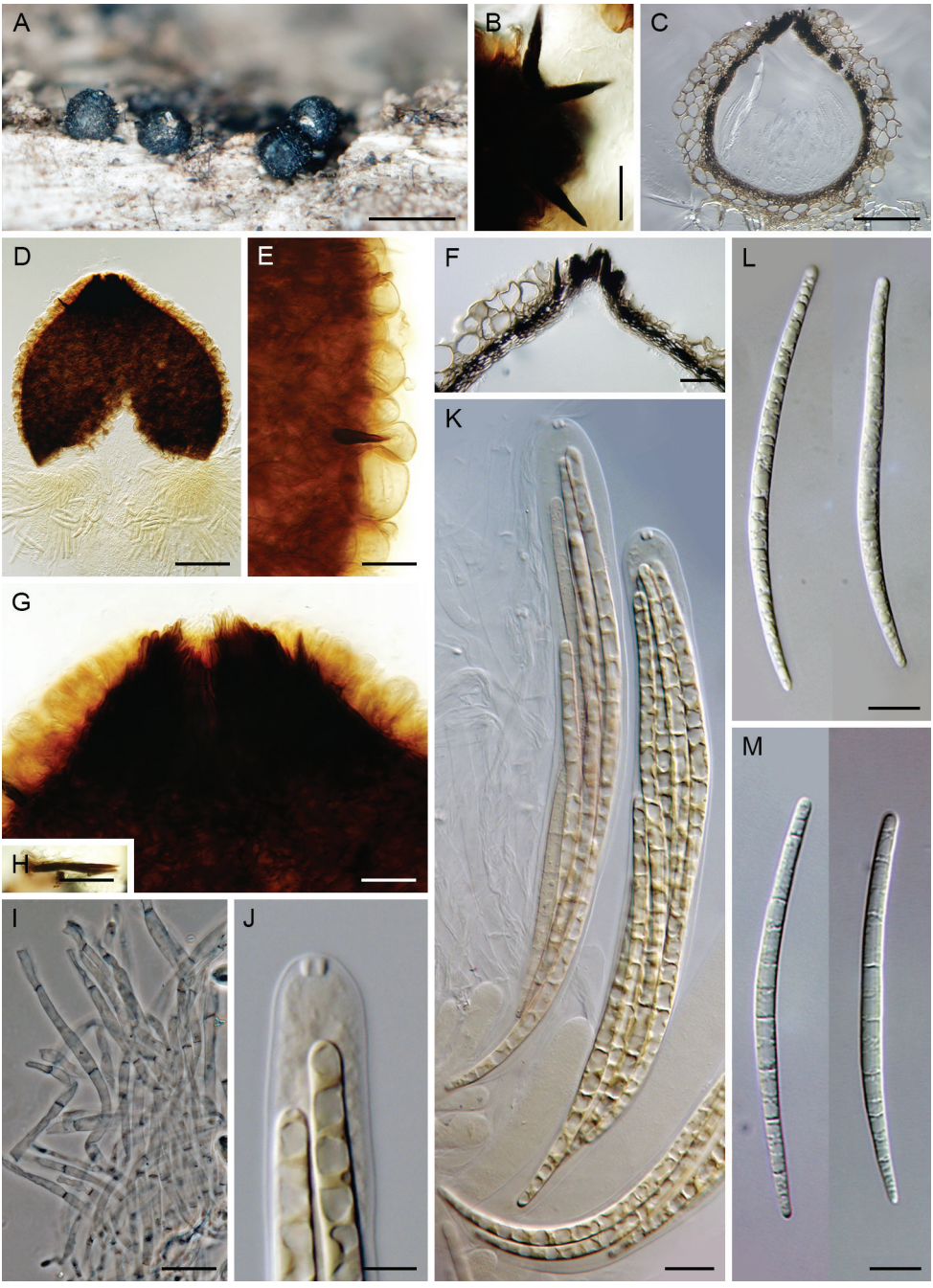

Figure 9. *Paragaeumannomyces sabinianus*. **A** ascomata. **B** ascomatal setae **C** vertical section of ascomal wall **D, E** ascomal wall **F, G** upper part of the ascoma with ostiole surrounded by setae **H** setae from the ostiolar region **I** paraphyses **J** ascal apex with apical ring **K** asci **L, M** ascospores. Images: ILLS00121384 (**A, B, D, E, G–K**); S.M.H. 3824 (**C, F, L**); S.M.H. 3807 (**M**). Scale bars: 500 μm (**A**); 20 μm (**B, E, G, H**); 100 μm (**C, D**); 25 μm (**F**); 5 μm (**J**); 10 μm (**I, K–M**).

and narrower setae 16.5–35 × 2.5–3 µm densely aggregated around the ostiole. Ostiole periphysate. Ascomatal wall leathery, three-layered. Outer layer of textura angularis, 32–53 µm thick, consisting of thin-walled, globose to subglobose, dark orange-brown to reddish-brown cells, 11.5–28 µm diam. Middle layer of textura prismatica, 12–22 µm thick, composed of thick-walled, polyhedral, dark brown, melanised cells. Inner layer of textura prismatica, 3–5 µm thick, composed of thin-walled, flattened and elongated hyaline to subhyaline cells. Paraphyses abundant, hyaline, sparsely branched, septate, 3.5–4.5(–6) µm wide, tapering to 2–2.5 µm, longer than the asci. Asci (154–)161–189 × (11–)12.5–14.5(–15.5) µm (mean ± SD = 174.2 ± 8.7 × 13.0 ± 0.8 µm), (130–)144–165 µm (mean ± SD = 155.2 ± 8.3 µm) long in the sporiferous part, cylindrical-fusiform, stipitate, apically broadly rounded to obtuse, ascal apex non-amyloid with a distinct apical annulus 2.5–3 µm wide, 1.5–2 µm high. Ascospores (64.5–)68.5–86.5(–88.5) × (3–)3.5–4.5 µm, (mean ± SD = 79.1 ± 5.3 × 4.0 ± 0.3 µm), filiform to cylindrical, straight or slightly curved to sigmoid, hyaline, 7-septate, septa often unevenly distributed, not constricted at the septa, asymmetrical, broadly rounded at the apical end and tapering towards the narrowly rounded basal end, with one or two guttules in each cell, 2–3-seriate, rarely 4-seriate, partially overlapping, with a negative or weak dextrinoid reaction in Melzer's reagent. Anamorph: Unknown.

Other specimen examined. USA – North Carolina • Macon Co., Coweeta Hydrological Laboratory; 27 Jun. 1998, on decorticated wood; F.A. Fernández leg.; S.M.H. 3807. • *Ibid.*, Horse Cove Drive & Bull Pen Road, alt. 1000 m; 27 Jun. 1998; on decorticated wood; F.A. Fernández leg.; S.M.H. 3824.

Habitat and distribution. A saprobe on decaying wood, so far known from North America in the USA (North Carolina, Tennessee) (Ellis 1887; Huhndorf and Fernández 2005).

Notes. Huhndorf and Fernández (2005) reported *P. longisporus* (as *Ch. ellisii*) from numerous collections from North America; the phylogenetic analysis of ITS sequences of six specimens resolved this species as a statistically unsupported clade with two strongly supported subclades. Although Huhndorf and Fernández (2005) described *P. longisporus* with setae scattered over the entire ascoma, in discussion, they admitted the presence of setae also around the ostiole: "In *C. ellisii*, *C. raciborskii* and *C. panamensis* the setae tend to be scattered over the entire surface of the ascomata, however some specimens of *C. ellisii* may have setae concentrated only at the apex."

Barr (1993) described the ostiole of the holotype of *S. longispora* surrounded by a crown of dark brown, stiff setae. We examined three collections tentatively identified as *P. longisporus* from North America (ILLS00121384, ILLS00121385, ILLS00121386) and in each the ostiole was delimited by densely aggregated acute setae. Apart from the ostiolar setae, additional setae were scattered over the entire ascoma, but they differed by their density among collections. The ascomata and asci of these three collections are comparable in size; the main difference lies in the ascospore length. The specimen ILLS00121384 has longer [(64.5–)68.5–86.5(–88.5) µm] ascospores compared to ILLS00121385 and ILLS00121386, which have shorter [(50.5–)52.5–68 µm] ascospores corresponding to the size given by Barr (1993) for the *S. longispora* holotype.

In the description of *P. longisporus fide* Huhndorf and Fernández (2005), a wide range of ascospore lengths [(40–)50–75(–80) µm] is given, the upper limit matching the ascospore size of ILLS00121384.

In our ITS-28S phylogeny, *P. longisporus fide* Huhndorf and Fernández (2005) was resolved as a strongly supported clade (100/1.0/100) with two subclades. The first subclade (100/1.0/100) was introduced as a new species *P. sabinianus*, including ILLS00121384 (Fig. 9), S.M.H. 3807 (Huhndorf and Fernández 2005: fig. 13) and S.M.H. 3824 (Huhndorf and Fernández 2005: fig. 15) with longer ascospores, distinguished from the second subclade *P. longisporus* (99/1.0/100) with shorter ascospores (Fig. 8). The anamorph of *P. sabinianus* is unknown; our specimen was not isolated in axenic culture and the strains S.M.H. 3807 and S.M.H. 3824 formed only sterile mycelium in vitro (Huhndorf and Fernández 2005).

Paragaeumannomyces smokiensis **Réblová & A.N. Mill., sp. nov.**
MycoBank No: 836539
Figure 10

Typification. USA – Tennessee • Sevier Co., Great Smoky Mountains National Park, Greenbrier, alternative side trail to Whaley Cemetery; alt. 549 m; 10 Jul. 2005; on decaying wood; A.N. Miller & A.M. Stchigel leg.; A.N.M. 466 (**holotype**: ILLS00121398!).

Etymology. Named after the Great Smoky Mountains National Park from where it was collected.

Description on the natural substrate. Teleomorph: Ascomata perithecial, nonstromatic, superficial, solitary or in small groups, 270–390 µm diam, 320–400 µm high, subglobose to broadly conical, finely roughened, dark reddish-brown, glabrous except for the black papilla with dark brown, stiff, acute setae, 9.5–13.5 × 2–2.5 µm, densely aggregated around the ostiole. Ostiole periphysate. Ascomatal wall leathery, three-layered; outer layer of textura angularis consisting of globose to subglobose, reddish-brown cells; middle layer composed of brick-like, brown cells; inner layer of flattened, thin-walled, subhyaline cells. Paraphyses abundant, hyaline, longer than the asci, tapering. Asci (134–)140–174(–189) × 11–13(–14) µm (mean ± SD = 158.2 ± 19.4 × 12.3 ± 0.9 µm), cylindrical-fusiform, stipitate, apically narrowly rounded, ascal apex non-amyloid with a distinct apical annulus 2–2.5 µm wide, 1–1.5 µm high. Ascospores (58–)60.5–80.5 × (3–)3.5–4.5(–5) µm (mean ± SD = 69.8 ± 6.3 × 4.0 ± 0.5 µm), filiform to cylindrical, straight or slightly curved, hyaline, 9–11-septate, septa often unevenly distributed, not constricted at the septa, asymmetrical, rounded at the apical end, tapering towards the basal end, with one or two guttules in each cell, 2–3-seriate or 4-seriate and partially overlapping, seldom in a single fascicle. Anamorph: Unknown.

Habitat and distribution. A saprobe on decaying wood, known only from the USA.

Notes. The present species is most similar to *P. abietinus*, the only member of the genus known from Europe. They share dark reddish-brown, glabrous ascomata with

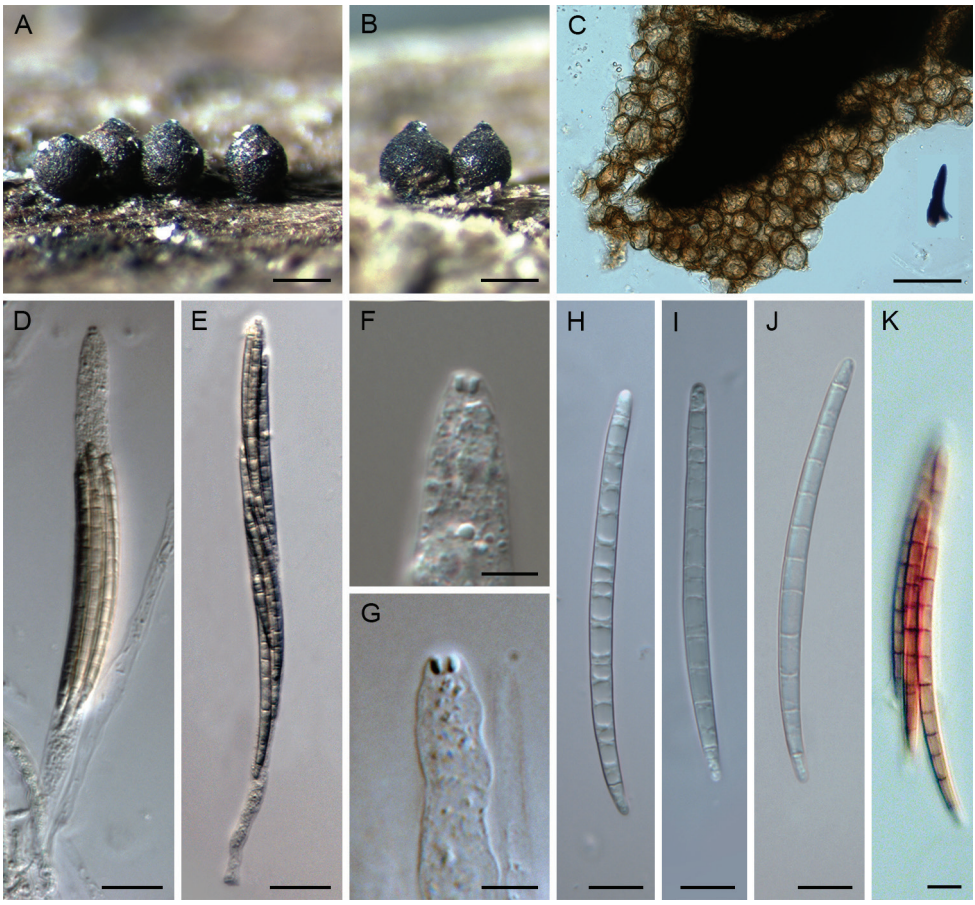

Figure 10. *Paragaeumannomyces smokiensis* (ILLS00121398). **A, B** ascomata **C** globose cells of the outer layer of the ascomal wall and an ostiolar seta **D, E** asci **F, G** ascal apex with apical annulus **H–K** ascospores. Scale bars: 250 µm (**A, B**); 50 µm (**C**); 20 µm (**D, E**); 10 µm (**F–K**).

short setae surrounding the ostiole. Although the size of ascospores of both species overlap, *P. abietinus* differs from *P. smokiensis* by longer [(185–)195–240 × 12–14.5(–15.5) µm] asci and slightly longer and wider ascospores [(62–)65–87 × (3.5–)4–5.5 µm] with usually less septa [(5–)7–9(–11)].

Paragaeumannomyces sphaerocellularis **Matsush., Matsush. Mycol. Mem. 10: 156. (2003) [2001].**

Habitat and distribution. The species was described from dead twigs of an unknown broadleaf tree and is so far known only from the subtropical climate zone of the northern hemisphere in Japan, Wakayama Prefecture (Matsushima 2003).

Notes. *Paragaeumannomyces sphaerocellularis* is similar to *P. panamensis* (Huhndorf and Fernández 2005; Perera et al. 2016) in the morphology of reddish-brown, setose ascomata with acute, dark, opaque setae scattered over the entire surface and hyaline ascospores, but differs from it in larger ascomata 200–350 × 300–425 µm vs 185–235 × 190–270 µm, slightly shorter asci 105–125 µm vs 123–140 µm, and 5–10-septate ascospores longer in their upper range 65–90 × 3–4 µm vs always 7-septate, shorter ascospores 65–75 × 3–4 of *P. panamensis*. Although the size of the asci may vary, often dependent on the arrangement of ascospores, shorter ascospores with the constant occurrence of seven septa of *P. panamensis* is considered an important character. In other *Paragaeumannomyces* species with 7-septate ascospores, such as *P. elegans, P. lapazianus* and *P. rubicundus*, the number of seven septa remains constant and is considered a diagnostic feature. While *P. sphaerocellularis* was collected only once in Japan, two collections of *P. panamensis* originating from Panama and Thailand suggest that this species has a pantropical distribution. Although the two species are remarkably similar, without molecular evidence we prefer to consider them as separate. For a detailed comparison, see the key.

Striatosphaeria castanea **Réblová & J. Fourn., sp. nov.**
MycoBank No: 836540
Figure 11

Typification. French Guiana • Maripasoula, Saül, sentier des gros arbres, disturbed secondary rainforest; alt. 200 m; 25 Aug. 2018; on the bark of decaying woody liana on the ground associated with *Xylaria papillatoides*; C. Lechat leg.; GY.J.F. 18140-1 (**holotype**: PRA-16328!, ex-type culture CBS 145352).

Etymology. *Castanea* (Latin) chestnut-coloured, referring to the colour of conidia.

Description on the natural substrate. Teleomorph: Ascomata perithecial, nonstromatic or formed on rudimentary basal stroma, superficial, solitary or in small groups or dense clusters, 160–200 µm diam, 170–220 µm high, subglobose to broadly conical, dark brown, glabrous, papillate. Ostiole periphysate. Ascomatal wall fragile, carbonaceous, 20–27 µm thick, two-layered. Outer layer of textura prismatica, consisting of brown, polyhedral cells with opaque walls. Inner layer of textura prismatica, consisting of several rows of thin-walled, hyaline, flattened cells. Paraphyses sparse, partially disintegrating at maturity, septate, 3–5 µm wide, tapering to ca. 2.5 µm, longer than the asci. Asci (75–)78–97(–102) × (10.5–)11–14.5 µm (mean ± SD = 87.3 ± 5.0 × 12.5 ± 1.1 µm), (56.5–)65–77(–82.5) µm (mean ± SD = 70.0 ± 4.9 µm) long in the sporiferous part, cylindrical-fusiform, stipitate, apically obtuse, ascal apex with a shallow, non-amyloid apical annulus 3.5–4.5 µm wide, 1–1.5 µm high. Ascospores (10.5–)11–13.5(–14.5) × (5.5–)6–7.5 µm (mean ± SD = 12.2 ± 0.5 × 6.7 ± 0.5 µm), ellipsoidal-fusiform, dark brown to chestnut brown, 1-septate; septum median, dark brown, with a central pore, not constricted or slightly constricted at the septum, with longitudinally arranged darker

Figure 11. *Striatosphaeria castanea* (CBS 145352). **A, B** ascomata **C** asci **D** ascospores **E** ascal apex with apical annulus **F** colonies on CMA with an *Urtica dioica* stem after 8 wk **G–J** conidia **K–Q** conidiophores **R** colonies on CMD, MLA, OA and PCA after 4 wk (from left to right). Scale bars: 500 µm (**A, B**); 20 µm (**C, E**); 10 µm (**D, G–Q**); 1 cm (**F, R**).

brown ridges alternating with lighter brown furrows, ascospores uniseriate or obliquely uniseriate in the ascus. Anamorph: Codinaea-like, not present on the nature substrate.

Description on CMA with sterile stems of *Urtica dioica*. Colonies effuse, vegetative hyphae hyaline, branched, 2.5–3.5 µm wide. Conidiophores macronematous, mononematous, 22–66 µm long, 3.5–5 µm wide near the base, erect, straight, cylindrical, several-septate, brown, paler towards the apex, unbranched, smooth-walled, or reduced to single conidiogenous cells. Conidiogenous cells (9.5–)12.5–25(–33) × 4–4.5 µm, tapering to 2.5–3.5 µm just below the collarette, monophialidic, integrated, terminal, cylindrical to cylindrical-lageniform, pale brown, subhyaline towards the apex, smooth-walled; collarettes funnel-shaped, 4–6.5 µm wide, (1.5–)2–2.5 µm deep. Conidia (10–)11–13.5 × 4–5.5 µm (mean ± SD = 11.9 ± 0.8 × 4.8 ± 0.4 µm), reniform to ellipsoidal, straight or slightly curved, asymmetrical, narrowly rounded at the apical end, truncate at the basal end, brown, 1-septate, not constricted or slightly constricted at the septum, with 1–2.5 µm long, hyaline setulae at each end, smooth-walled, in slimy droplets, dark brown in mass.

Culture characteristics. On CMD colonies 23–25 mm diam, circular, flat, margin entire, lanose, floccose, funiculose at the centre, cobwebby towards the periphery, whitish with irregular pale brown spots due to pigmented funiculose mycelium, with an isabelline outer zone of submerged growth; reverse beige. On MLA colonies 22–25 mm diam, circular, raised, margin entire, lanose, floccose, zonate, with grey, brown and white zones, with an isabelline outer zone of submerged growth; reverse dark grey. On OA colonies 31–33 mm diam, circular, flat, margin entire, sparsely lanose, floccose, cobwebby at the margin, zonate, whitish, colony centre with irregular dark brown spots due to pigmented submerged mycelium, pale brown towards the margin, with an olivaceous outer zone of submerged growth, dark brown pigment diffusing to agar at the colony centre; reverse olivaceous grey. On PCA colonies 17–19 mm diam, circular, slightly convex centrally, margin entire, lanose, floccose becoming cobwebby towards the periphery, isabelline to light beige with irregular brown spots due to pigmented mycelium; reverse light beige. Sporulation abundant on CMD, CMA with *Urtica* stems and PCA, sparse on MLA and OA.

Other specimen. Brazil • Bahia; isolated from roots of *Encyclia ghillanyi*; isolate monte6.2; GenBank (ITS): KC928368, unpublished. (Specimen not available).

Habitat and distribution. *Striatosphaeria castanea* occurs on the bark of woody liana and as an endophyte of *Encyclia ghillanyi*. It is known from South America in Brazil and French Guiana.

Notes. *Striatosphaeria codinaeophora* closely resembles *S. castanea*, but differs in having larger [(130–)140–160(–170) × 25–35(– 40) µm] asci, [(17–)19–23(–26) × (6–)7–9(–10) µm] ascospores and (15–20 × 4.5–6 µm) conidia (Samuels and Müller 1978). Based on the present phylogeny (Fig. 1) and comparison of ITS sequences, *S. castanea* has also been recorded as an endophyte, isolated from roots of *Encyclia ghillanyi*, a rupiculous orchid inhabiting rock surfaces in semiarid areas in the Bahia state of northern Brazil (strain monte6.2, ITS: KC928368, Almeida et al. unpublished).

***Dendrophoma cytisporoides* Sacc., Michelia 2: 4. 1880.**
Figure 12

≡ *Phoma cytisporoides* Sacc., Michelia 1: 522. 1879.
≡ *Phoma cytisporoides* subsp. *punicina* Sacc., Michelia 2: 273. 1881.
≡ *Dendrophoma cytisporoides* var. *punicina* (Sacc.) Sacc., Syll. fung. 3: 180. 1884.
≡ *Dendrophoma cytisporoides* var. *pruni-virginianae* Sacc., Riv. Accad. di Padova 33: 169. 1917.
= *Dendrophoma punicina* (Sacc.) Sacc., Rabenh. Krypt.-Fl., Edn. 2, 1(6): 409. 1901.

Description on the natural substrate. Teleomorph: Ascomata perithecial, non-stromatic, immersed becoming erumpent, in small groups or dense caespitose clusters on the bark of the host, 120–150 µm diam, 180–200 µm high, subglobose to broadly conical, dark brown, glabrous, papillate. Ostiole periphysate. Ascomatal wall fragile, carbonaceous, 22–28 µm thick, two-layered. Outer layer of textura prismatica, consisting of brown, polyhedral cells with opaque walls. Inner layer of textura prismatica, consisting of several rows of thin-walled, hyaline, flattened cells. Paraphyses sparse, persistent, septate, anastomosing, 2–2.5 µm wide, tapering to ca. 1–1.5 µm, longer than the asci. Asci (66–)69–88.5(–92) × 6.5–8 µm (mean ± SD = 77.5 ± 5.9 × 7.3 ± 0.5 µm), (35.5–)43.5–63(–69) µm (mean ± SD = 53.9 ± 5.7 µm) long in the sporiferous part, unitunicate, arising from densely branched, short ascogenous hyphae, 8-spored, cylindrical to cylindrical-clavate, stipitate, apically broadly rounded, ascal apex with an indistinct, non-amyloid apical annulus visible only with the PC illumination, ca. 1.5 µm wide, 1.5–2 µm high. Ascospores 8–10.5(–11) × 2.5–3.5 µm (mean ± SD = 9.5 ± 0.6 × 3.0 ± 0.3 µm), fusiform, hyaline, 1-septate, not constricted at the median septum, uniseriate, obliquely uniseriate or partially biseriate in the ascus. Anamorph: Not observed.

Description on OA. Colonies effuse, vegetative hyphae hyaline, 2–3 µm wide. Conidiomata stromatic, globose becoming cupulate, up to 350 µm diam. Setae absent. Conidiophores macronematous, septate, branched or unbranched, up to 60 µm long, hyaline. Conidiogenous cells 7–13 × 1.5 µm, monophialidic, integrated and terminal or discrete and lateral, subcylindrical, single or in terminal whorls, hyaline, tapering to ca. 1 µm; collarettes indistinct. Conidia 2.5–3.5 × 1–1.5 (mean ± SD = 3.1 ± 0.3 × 1.2 ± 0.1 µm), naviculate to botuliform, with 0.5–1 µm long setulae at each end, aseptate, smooth-walled, in slimy droplets, hyaline in mass.

Culture characteristics. On CMD colonies 18–20 mm diam, circular, flat, margin entire, velvety-lanose, mucoid at the margin, white, isabelline towards the periphery, reverse white. On MLA colonies 18–21 mm diam, slightly convex, circular, margin entire to fimbriate, lanose, floccose, cobwebby at the margin, white, with an isabelline outer zone of submerged growth, reverse isabelline. On OA colonies 19–21 mm diam, circular, flat, raised margin, margin entire, velvety becoming lanose towards the margin, mucoid at the margin, zonate, colony centre grey to whitish-grey

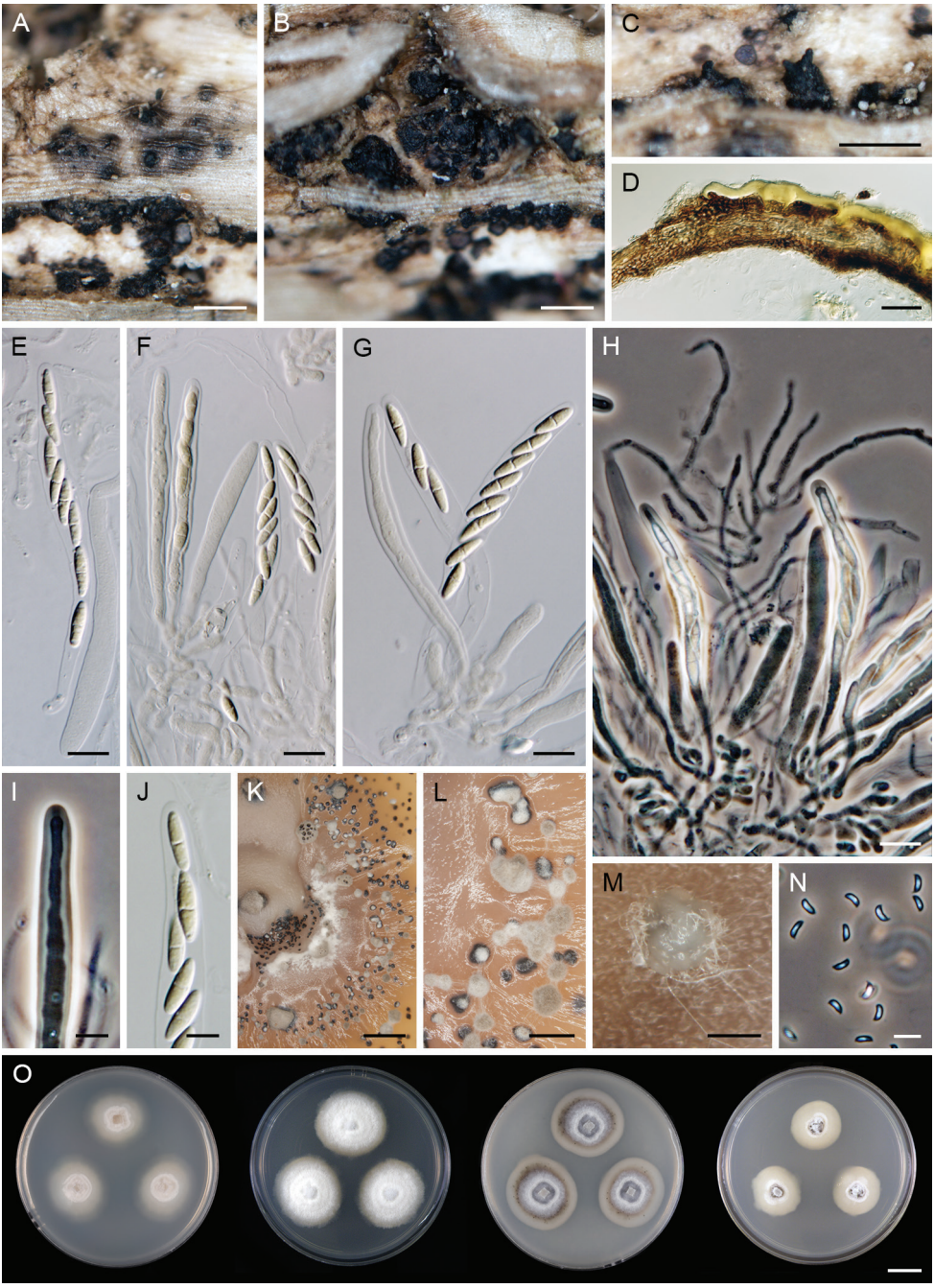

Figure 12. *Dendrophoma cytisporoides* (CBS 144107). **A–C** ascomata **D** vertical section of the ascomal wall with remnants of the periderm **E–G** asci **H** paraphyses, asci and ascogenous hyphae **I, J** ascal apex **K, L** sterile primordia of sporodochia on MLA after 6 mo **M** sporodochium on OA after 10 wk **N** conidia **O** colonies on CMD, MLA, OA and PCA after 4 wk (from left to right). Scale bars: 300 µm (**A–C, K**); 20 µm (**D**); 10 µm (**E–H**); 5 µm (**I, J, N**); 100 µm (**L, M**); 1 cm (**O**).

with intermediate white zone becoming brown, beige at the margin, reverse grey. On PCA colonies 13–15 mm diam, circular, flat, slightly convex centrally, margin entire, velvety-lanose, floccose becoming mucoid towards the periphery, white, pale brown centrally, with an isabelline outer zone of submerged growth, reverse isabelline. Sporulation absent on all media after 4 wk; abundant on OA and PCA after 10 wk or 4 mo, respectively, on MLA sterile primordia of conidiomata were formed.

Specimen examined. Germany • 16 Jan. 2012; on bark and wood of a dead twig of *Buxus sempervivens*; R. Schumacher leg.; (PRA-16322, culture CBS 144107 = IMI 506817).

Habitat and distribution. Saprobic on decaying wood and bark of *Buxus sempervivens*, *Deutzia scabra*, *Rhododendron* sp., and *Ulmus* sp. The species is known from Europe in France, Germany and the Netherlands (Saccardo 1879; Sutton 1965; Crous et al. 2012; this study).

Notes. Our strain sporulated in vitro only after prolonged incubation. On OA it formed globose to pulvinate fertile conidiomata, while on PCA the conidiomata often became confluent. The comparison of ITS and 28S sequences of our strain with those of the epitype strain of *D. cytisporoides* CBS 223.95 (Crous et al. 2012) confirmed they are conspecific; the two strains share 100 % sequence similarity.

Discussion

Based on morphology, cultivation studies and phylogenetic analysis of the combined ITS-28S loci, seven new species and eight new combinations are introduced in the Chaetosphaeriaceae. *Paragaeumannomyces* (Matsushima 2003) is proposed for the monophyletic, strongly supported clade of former *Chaetosphaeria* species (Figs 1, 2) characterised by scolecosporous, multiseptate, asymmetrical, hyaline to light pink ascospores and unique three-layered ascomatal wall. The unusual wall was first brought to attention by Carroll and Munk (1964). The colour of the outer wall ranges from white, whitish-yellow, ginger-brown to reddish-brown, russet to dark brown and is composed of thin-walled cells of textura angularis, which may contain pale purple pigment when fresh, occasionally with red surface crystals. Setae, if present, are scattered over the entire ascoma or only surround the ostiole. They are stiff, acute, dark brown with opaque walls, arise from the middle, carbonaceous layer and penetrate the outer layer of globose cells. Members of the genus occur on decayed plant material, especially on strongly decayed decorticated wood.

Examination of our material of *Paragaeumannomyces* revealed that ascospores of four species exhibit a strong dextrinoid reaction in Melzer's reagent, namely *P. abietinus*, *P. albidus*, *P. granulatus* and *P. smokiensis*. The ascospores turned reddish-brown except for the end cells, which remained partially hyaline, especially at the tips. Interestingly, these species share glabrous, non-setose ascomata or only minute setae are arranged around the ostiole. The chemical reaction is visible in ascospores without guttules, which otherwise fill the cells and obscure the colour. The ascospores of

P. elegans, *P. longisporus* and *P. sabinianus* exhibit a negative or weak dextrinoid reaction; some mature ascospores turned light pink-brown. These species share setose ascomata, sometimes with ostioles surrounded by minute setae. Although more species need to be examined to evaluate this character, we hope it is not premature to argue that the dextrinoid reaction of ascospores is species-specific and has the potential to become another diagnostic feature facilitating species identification. Because we did not examine all known species of *Paragaeumannomyces*, this character has not been used in the key, but it is mentioned in the species descriptions, if known.

Paragaeumannomyces, typified by *P. sphaerocellularis*, encompasses 18 species. The present phylogenetic tree (Fig. 2) contains 12 of them and four subclades labelled *Paragaeumannomyces* sp. 1–4, which represent separate, yet undescribed taxa at the species rank. The molecular data of *P. raciborskii* and *P. sphaerocellularis* are unavailable. The closest relatives to *Paragaeumannomyces* are species of *Exserticlava* and *Chaetosphaeria lignomollis* with a kylindria-like anamorph, characterised by septate, versicolorous or hyaline ascospores, respectively (Fig. 1).

Members of *Paragaeumannomyces* are not easily cultivated and only seldom sporulate in vitro. Huhndorf and Fernández (2005) noted that even isolates from the same specimen varied in their ability to produce the anamorph in culture. The craspedodidymum-like anamorph with usually semi-macronematous to micronematous conidiophores, inflated phialides, deeply flared, cup-shaped collarettes and hyaline non-septate conidia, with or without setulae, was reported for *P. lapazianus*, *P. longisporus*, *P. panamensis*, *P. rubicundus*, and *Paragaeumannomyces* sp. 1–4, while the chloridium-like synanamorph is known only in *P. longisporus* and *Paragaeumannomyces* sp. 2 (Huhndorf and Fernández 2005; Perera et al. 2016). The systematic placement of *Craspedodidymum* (Holubová-Jechová 1972), typified by *C. elatum*, is unknown. The genus was erected for a hyphomycete forming effuse colonies on an old petiole of *Phoenix canariensis* in a green house in the Czech Republic. To date, 15 binomials were introduced in the genus (Index Fungorum). *Craspedodidymum elatum* differs from *Paragaeumannomyces* anamorphs by macronematous, dichotomously branched conidiophores and non-septate, dark brown conidia with a basal hilum. In hyaline, unicellular, globose or triangular conidia with setulae, the *Paragaeumannomyces* anamorphs also resemble *Bahusutrabeeja* (Subramanian and Bhat 1977) and *Nawawia* (Marvanová1980), respectively. *Bahusutrabeeja* and *Nawawia* are similar to each other but differ in shape of conidia. *Bahusutrabeeja*, typified with *B. dwaya*, forms globose conidia on solitary conidiophores, while *Nawawia*, based on *N. filiformis*, have conidia triangular, round-tetrahedral or obpyramidal-shaped on conidiophores arising from small stromata. Their molecular data (Yang et al. 2016; Vu et al. 2019) suggest a distant relationship to *Paragaeumannomyces*.

The original *P. longisporus* clade with two strongly supported subclades was recognized as two species, the short-spored *P. longisporus* and the long-spored *P. sabinianus*. In general, a high degree of ITS sequence variability and more or less uniform teleomorphic phenotype pose special problems in species identification, especially in

P. raciborskii, which was resolved as polyphyletic (Huhndorf and Fernández 2005; this study). Morphology of this species was studied by Huhndorf and Fernández (2005) based on more than 100 species, mostly from the neotropics. The broad species concept of *P. raciborskii fide* Huhndorf and Fernández (2005) includes specimens with wider ascomata, longer ascospores with less septa and longer asci than reported in the protologue (Penzig and Saccardo 1897) and re-description of this species prepared by Carroll and Munk (1964); for details see above. Although no significant variability among ascomata, asci and ascospores was encountered, Huhndorf and Fernández (2005) reported intraspecific variability regarding setae, which were lacking in some specimens. On the other hand, certain variability at the anamorphic level, typical of many natural groups of the Chaetosphaeriaceae, also occurs in *P. raciborskii fide* Huhndorf and Fernández (2005) and to some extent delimits the four subclades. One isolate (S.M.H. 3119) produced a craspedodidymum-like anamorph with triangular conidia with setulae, while others with the morphologically similar anamorph produced globose, non-setulate conidia and can be further distinguished by formation of the chloridium-like synanamorph (S.M.H. 2036, S.M.H. 2132), or its absence but are separated by dark, purplish-brown phialides (S.M.H. 2017) or light brown phialides (S.M.H. 3014) (Huhndorf and Fernández 2005). A close comparison of the descriptions of the holotype of *P. raciborskii* (Penzig and Saccardo 1897; Carroll and Munk 1964) with collections gathered by Huhndorf and Fernández (2005) and identified as *P. raciborskii* confirms that none of the four subclades inferred in the ITS-28S ML tree (Fig. 2) could be delimited as *P. raciborskii* s. str. From a biogeographical perspective it is more likely that they belong to different species entirely. Therefore, the name *P. raciborskii* for these strains was rejected in our phylogeny; instead, the four subclades were designated *Paragaeumannomyces* sp. 1–4.

Codinaea (Maire 1937) is one of the largest genera of the Chaetosphaeriaceae with a turbulent taxonomic history. Based on a cluster analysis of phialidic dematiaceous hyphomycetes, Arambarri and Cabello (1989) considered *Codinaea*, with usually falcate, septate or non-septate conidia bearing setulae at both end, and *Dictyochaeta* (Spegazzini 1923) with non-setulate, non-septate, cylindrical and asymmetrical conidia, congeneric. Since then, *Dictyochaeta* (syn. *Codinaea*) became a broadly circumscribed genus with more than 100 species and the new morphological concept was followed by many mycologists. Réblová and Seifert (2007) confirmed with DNA sequence data that *Dictyochaeta fuegiana*, the type species of the genus, is a member of the Chaetosphaeriaceae. Based on the ITS-28S phylogeny (Fig. 1), *D. fuegiana* is unrelated to morphologically similar species with setulate conidia, classified in *Codinaea* or *Dictyochaeta*, and resolved as polyphyletic, which is in agreement with Lin et al. (2019) and Réblová et al. (2020). However, in the absence of molecular DNA data of *Codinaea aristata*, the generic type, it is difficult to delimit *Codinaea* phylogenetically. The morphological traits delimiting the new species *C. paniculata*, i.e. presence of setae, unbranched and shorter conidiophores growing at the base of the setae with monophialidic conidiogenous cells in vivo and falcate, non-septate conidia with setulae at both ends, best match

those of *Codinaea*. In the subclade where *C. paniculata* was clustered, several species sharing the same *Codinaea* morphotype were present, namely *C. assamica* (Hughes and Kendrick 1968), *D. siamensis* (Liu et al. 2016) and *D. terminalis* (Lin et al. 2019).

Based on published records, *Striatosphaeria* is an uncommon lignicolous genus with a known distribution in the neotropics. It was introduced by Samuels and Müller (1978) based on two Brazilian collections of *S. codinaeophora*. Additional specimens of *S. codinaeophora* known to us were collected on decaying wood of *Dacryodes excelsa* and *Nectandra turbacensis* and unidentified hosts in Costa Rica, French Guiana and Puerto Rico (Réblová and Winka 2000; Fernández et al. 2006; S.M. Huhndorf pers. data). The codinaea-like anamorph develops only in axenic culture. The conidia are asymmetrical, brown, 1-septate with minute, hyaline setulae at each end. Although *S. codinaeophora* was described with non-setulate conidia (Samuels and Müller 1978), a photograph of conidia with setulae accompanied the *S. codinaeophora* lineage on a phylogenetic tree (Fernández et al. 2006: fig. 1, 6g). The setulate conidia were also present in the new species, *S. castanea*. It is probable that setulae are formed later, after conidia detach from the conidiogenous cells. The conidia with setulae at both ends are formally introduced in *Striatosphaeria* for the first time in this study.

The genus *Dendrophoma* with a single species, *D. cytisporoides*, was proposed by Saccardo (1880) for fungi with phoma-like fruit bodies, botuliform hyaline conidia and conidiogenous cells arranged in a verticillate fashion. Sutton (1965) lectotypified *Phoma cytisporoides* (Saccardo 1879) and reported additional characters not mentioned by Saccardo in the protologue, i.e. dark brown, acute setae accompanying conidiomata and minute, unbranched setulae at both ends of conidia. Sutton (1965) compared *D. cytisporoides* with *Dinemasporium graminum*, the type species of the genus, and reduced *Dendrophoma* to synonymy with *Dinemasporium* (Léveillé 1846). Using nuclear ribosomal loci, Crous et al. (2012) re-established *Dendrophoma* and placed the genus in the Chaetosphaeriaceae, where it emerged as a separate lineage from *Dinemasporium*. The anamorph-teleomorph relationship of *Dendrophoma* has been established for the first time in this study. The teleomorph is morphologically similar to *Chaetosphaeria* (Tulasne and Tulasne 1863), but differs in having immersed to erumpent ascomata, densely branched ascogenous hyphae (Fig. 12H), the ascal apex lacking a visible discharge mechanism, which can only be seen as a minute apical ring with PC illumination (Fig. 12I) and a morphologically distinct anamorph forming stromatic, stipitate, cupulate sporodochial conidiomata.

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RESEARCH ARTICLE

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Mrakia panshiensis sp. nov. a new member of the Cystofilobasidiales from soil in China, and description of the teleomorphic-stage of *M. arctica*

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Abstract

In a study on the fungal diversity in Northeast China, twelve yeast isolates were obtained from soils collected in three provinces, Helongjiang, Jilin and Liaoning. Morphological assessment and phylogenetic analyses of the nuc rDNA internal transcribed spacer (ITS) region and the D1/D2 domains of the nuc 28S rDNA (nuc 28S) gene of the 12 cultures placed them in the genus *Mrakia*, namely *Mrakia aquatica*, *Mrakia arctica*, *Mrakia frigida*, *Mrakia gelida* and *Mrakia robertii*. A total of three isolates represented a hitherto undescribed species, which is described here as *M. panshiensis* **sp. nov.** (MB 834813). The species *M. panshiensis* **sp. nov.** shares several morphological characters with *M. niccombsii*, *M. aquatica*, *M. fibulata* and *M. hoshinonis*. These species can be distinguished based on physiological traits and pairwise rDNA sequence similarities. The study also describes for the first time the formation of teliospores by previously described *M. arctica*.

Keywords

Cystofilobasidiales, *Mrakiaceae*, one new species, soil-inhabiting yeasts, taxonomy

Introduction

The first *Mrakia* species was described as a new species *Candida curiosa* from a frozen food (Komagata and Nakase 1965). Subsequently, three new *Candida* species, *Candida frigida*, *C. gelida* and *C. nivalis*, were obtained from soil samples in Antarctic and

Greenland (Di Menna 1966). Fell et al. (1969) observed that these *Candida* species exhibit a heterobasidiomycetous lifecycle, and hence reclassified them in the basidiomycetous genus *Leucosporidium*. Yamada and Komagata (1987) reclassified these taxa to a new genus once again, *Mrakia* (Mrakiaceae, Cystofilobasidiales), as *M. frigida*, *M. gelida*, *M. nivalis* (now considered a synonym of *M. frigida*), and *M. stokesii* (now considered a synonym of *M. gelida*), since these isolates have a CoQ8 system while other *Leucosporidium* species have CoQ9 or CoQ10 (Tsuji et al. 2019). Since then, seven further species have been described, namely *M. curviuscula* (now classified in the genus *Krasilnikovozyma*) (Bab'eva et al. 2002; Liu et al. 2015), *M. psychrophila* (Xin and Zhou 2007), *M. robertii*, *M. blollopis* (Thomas-Hall et al. 2010), *M. arctica* (Tsuji et al. 2018), *M. hoshinonis* (Tsuji et al. 2019), and *M. fibulata* (Yurkov et al. 2020). In addition to sexual species classified in the genus *Mrakia*, the order Cystofilobasidiales (Fell et al., 1999) included anamorphic species that were originally classified in the genus *Cryptococcus* and later in the anamorphic genus *Mrakiella* (Margesin and Fell 2008). With the end of dual naming of pleomorphic fungi, asexual species of the genus *Mrakiella* (*M. aquatica*, *M. cryoconiti*, and *M. niccombsii*) were transferred in the genus *Mrakia*, which was emended to include asexual states (Liu et al. 2015). In the same study, *M. curviuscula* was excluded from the emended genus *Mrakia* because this species built a distinct clade together with the anamorph species *Krasilnikovozyma* (formerly *Cryptococcus*) *huempii* and a few other closely related species (Liu et al. 2015). Recently, based on molecular and morphological analyses, two new taxa, namely *M. montana* and *M. stelviica*, were described in *Mrakia* (Turchetti et al. 2020). Thus, twelve species are presently accepted in the genus *Mrakia*.

The genus *Mrakia* has been demonstrated to be a monophyletic group based on phylogenetic analyses of sequences of D1/D2 domains of nuc 28S rDNA and ITS region (Fell et al. 2000; Scorzetti et al. 2002; Margesin and Fell 2008), and the multigene phylogenetic analysis that limited to its current circumscription, the *Mrakia* clade recognised in Liu et al. (2015). This genus is characterized by the formation of true hyphae, pseudohyphae, lack of basidiocarps, the ability to utilize nitrate and nitrite, and have coenzyme Q-8, Q-9 or Q-10 system (Liu et al. 2015). Asexual reproduction is by polar budding; sexual reproduction is by formation of teliospores that occur terminally and intercalarly (Fell and Margesin 2011; Fell 2011). *Mrakia* species share a remarkable adaptation to low temperatures, in some case even below freezing point (Buzzini et al. 2018; Yurkov et al. 2020), by producing diverse extracellular cold-active enzymes such as lipases, amylases proteases, pectinases, cellulases, chitinases, and ligninolytic enzymes (Tasselli et al. 2017; Tsuji et al. 2018, 2019). These abilities have been applied to wastewater treatment and bioethanol production and low temperatures fermentation (Tsuji et al. 2013a, b; De Francesco et al. 2018).

During a study on the fungal diversity in Northeast China, in the provinces of Helongjiang, Jilin and Liaoning, several soil-inhabiting yeasts were cultured. Based on the identification sequences of the D1/D2 domains of the nuc 28S rDNA (nuc 28S) gene, these isolates were assigned to the genus *Mrakia*. After a detailed phylogenetic

analysis of a concatenated alignment of sequences of the complete ITS region and D1/D2 domains as well as a micro-morphological study, the yeasts were identified as six *Mrakia* species, including one potential novel species. This novel species is described here as *Mrakia panshiensis* sp. nov.. Additionally, using a culture isolated in the present study, we describe the formation of teliospores by previously described *M. arctica*.

Materials and methods

Sample collection, morphological studies and isolation

Biodiversity assessment of soil yeasts was performed in three provinces of China, Helongjiang, Jilin and Liaoning. Forty-five soil samples were collected from the Lianhuashan National Forest Park (approximate GPS coordinates: 43°92'N, 125°71'E) in Panshi city, Jilin Province. The climate of this area is temperate, with an annual precipitation between 650 to 720 mm, and an average temperature ranging from –3 to 10 °C. Thirty-five soil samples were collected from the Maoershan National Forest Park (approximate GPS coordinates: 45°28'N, 127°58'E) in Yanji city, Heilongjiang Province. The forest park is characterized by temperate and humid climates, with annual precipitation between 400 to 650 mm, and an average temperature from –2 to 6 °C. Forty soil samples were collected from the Tianhuashan National Forest Park (approximate GPS coordinates: 41°09'N, 124°62'E) in Dandong city, Liaoning Province. The climate of this area is temperate and humid, with annual precipitation between 800 to 1100 mm, and an average temperature from 5 to 7 °C. Three studied locations are situated in Northeast China. The sampling sites were forested areas in the mountain cold broad-leaved and mixed forest biomes; they have a long and very cold winter.

Soil samples were collected in September 2018 as following: samples were taken from the top 15 cm layer, avoiding stones and organic materials as much as possible, and transferred into two 20 ml plastic tubes with screw-lids. Within 1 h after the sampling, samples were cooled and stored at 4 °C until analysis. Yeast strains were isolated following the method described by Groenewald et al. (2018). Specifically, an aliquot of each sample (1 g) was subjected to a serial dilution in sterile distilled water and mixed by vortex for 30 s. Subsequently, 0.1 ml of soil suspension ranging from 10^{-1}–10^{-4} (w/v) were spread on four plates containing yeast extract-malt extract (YM) agar (1% glucose, 0.5% peptone, 0.3% yeast extract, 0.3% malt extract and 2% plain agar; pH 5.4) supplemented with 0.02% chloramphenicol. The plates were incubated at 10 °C for up to three weeks and examined every three days. Colonies were differentiated into macro-morphological types using dissection microscopy by colony morphology and color, counted and two representatives of each colony morphotype per sample were purified by streaking at least twice, and then stored in 15% glycerol at –80 °C.

The morphological observations and metabolic tests were performed according to the standard procedures described by Kurtzman et al. (2011). Assimilation tests of

carbon and nitrogen sources were performed in liquid media at 15 °C. Starved inocula were used in nitrogen and vitamin assimilation tests, and the results were read after 5 and 21 days of incubation. Induction of the sexual stage was tested by incubating cultures singly or crossed pair-wise on cornmeal (CM) agar, YM agar or 5% malt extract (5% ME) agar at 10 °C for 2 months (Yurkov et al. 2020). Photomicrographs were taken with a Leica DM5000B microscope (Leica). Culture growth was examined at 1–30 °C in YM liquid culture and on YM agar plates.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from the cultures using the Ezup Column Yeast Genomic DNA Purification Kit, according to the manufacturer's protocol (Sangon Biotech, Shanghai, China). The nuc rDNA internal transcribed spacer (ITS) region was amplified using the primer pairs ITS1/ITS4 (White et al. 1990). The D1/D2 domain of the nuc 28S rDNA gene was amplified using the primer pair NL1/NL4 (Kurtzman and Robnett 1998). Amplification of ITS and nuc 28S rDNA gene was accomplished by an initial step of 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 51 °C (for ITS and nuc 28S rDNA, respectively) and 40 s at 72 °C, with a final extension of 10 min at 72 °C (Liu et al. 2016). PCR products were directly purified and sequenced by Sangon Biotech Inc. (Shanghai, China). We determined the identity and accuracy of newly-obtained sequences by comparing them to sequences of isolates of the genus *Mrakia* in GenBank. Sequences were assembled using BioEdit V7.0.9.0 (Hall 1999). Newly-obtained sequences were submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/) under accession numbers provided in Table 2.

Phylogenetic analysis

The ITS region, the nuc 28S rDNA gene and the concatenated alignment of ITS and nuc 28S rDNA sequences were employed to delimit species of the genus *Mrakia*. The selection of strains and species within the genus *Mrakia* followed most recent descriptions of species in the genus, Yurkov et al. (2020), Tsuji et al. (2017, 2019). The ITS region and the nuc 28S rDNA gene sequences were aligned with the MAFFT program ver. 7.273 (Katoh and Standley 2013) using the G-INS-I algorithm. The alignments were deposited in TreeBASE (Submission ID: S26876 and S26877; www.treebase.org), respectively. Major insertions and ambiguous regions were identified and eliminated with Gblocks version 0.91b (Castresana 2000) using a relaxed selection (minimum number of sequences for a conserved position = 15, minimum number of sequences for a flank position = 15, maximum number of contiguous non-conserved positions = 8, minimum length of a block = 5 and allowed gap positions = 'with half'), following Talavera and Castresana (2007). The edited alignment was deposited at TreeBase (submission ID S26878 and S26879; www.treebase.org). Maximum likelihood (ML) with an HKY+G+I model was performed using MEGA 7 (Kumar et al. 2016). Bayesian inference (BI) was constructed using MrBayes 3.2.5 (Ronquist et al. 2012) with a GTR+I+G model and

5,000,000 generations, two independent runs, and four chains. The other parameters were set as the default values. We discarded 25% of these trees, and remaining trees were used to compute a 50% majority rule consensus tree to estimate posterior probabilities. A bootstrap analysis with 1000 replicates was performed to estimate the confidence of the tree nodes and a bootstrap percentage (BP) of 50% or Bayesian posterior probability (BPP) of 0.9 was considered supportive in all constructed trees in this study.

Results

Phylogenetic analyses

A total of 135 yeast isolates were isolated from the 120 soil samples. Twelve isolates from 12 different soil samples built a rather uniform group based on a careful examination of morphological characteristics. Based on pair-wise sequence similarity comparisons and phylogenetic analyses of the ITS region and the nuc 28S rDNA, these 12 strains were identified as members of the genus *Mrakia*, *Mrakia gelida* (three isolates), *Mrakia robertii* (two isolates), *Mrakia frigida* (one isolate), *Mrakia aquatica* (two isolates) and one isolates was classified as *Mrakia arctica* (Table 1). Three strains represented a potential new species, which is described in detail in the present study. The remaining more than 100 isolates from soils require further identification and characterization.

The three isolates (NYNU 18562, NYNU 1941, NYNU 18410) shared identical sequences of both nuc 28S rDNA and ITS region. In terms of pairwise sequence similarity, the novel species showed a sequence divergence of 1.5% (9 substitutions and 0 gap over 602 bases) in nuc 28S rDNA from the closest relative *M. niccombsii*. The novel species also differed from other members of the *M. aquatica* sub-clade, *M. aquatica*, *M. fibulata* and *M. hoshinonis*, by sequence divergences ranging from 1.7% to 2% (10 to 12 substitutions and 0 to 1 gap over 602 bases) and from 3.3% to 2.5% (11 to 14 substitutions and 4 to 6 gaps over 603 bases), in nuc 28S rDNA and ITS, respectively. The phylogram, based on concatenated alignments of sequences of the the ITS region

Table 1. Strains isolated in this work and correspondent isolation sources.

Species	Strain	Source	Location
Mrakia aquatica	NYNU 18538	Soil and lichen	The Lianhuashan National Forest Park, Jilin Province, China
	NYNU 19451	Soil	The Lianhuashan National Forest Park, Jilin Province, China
Mrakia arctica	NYNU 18469	Soil	The Tianhuashan National Forest Park Liaoning Province, China
Mrakia frigida	NYNU 1846	Soil	The Tianhuashan National Forest Park Liaoning Province, China
Mrakia gelida	NYNU 18513	Soil	The Tianhuashan National Forest Park Liaoning Province, China
	NYNU 1834	Soil and lichen	The Tianhuashan National Forest Park Liaoning Province, China
	NYNU 18473	Soil	The Tianhuashan National Forest Park Liaoning Province, China
Mrakia panshiensis	NYNU 18562	Soil	The Lianhuashan National Forest Park, Jilin Province China
	NYNU 18410	Soil	The Maoershan National Forest Park Heilongjiang Province, China
	NYNU 1941	Soil	The Maoershan National Forest Park Heilongjiang Province, China
Mrakia robertii	NYNU 18415	Soil	The Lianhuashan National Forest Park, Jilin Province, China
	NYNU 184159	Soil and lichen	The Lianhuashan National Forest Park, Jilin Province, China

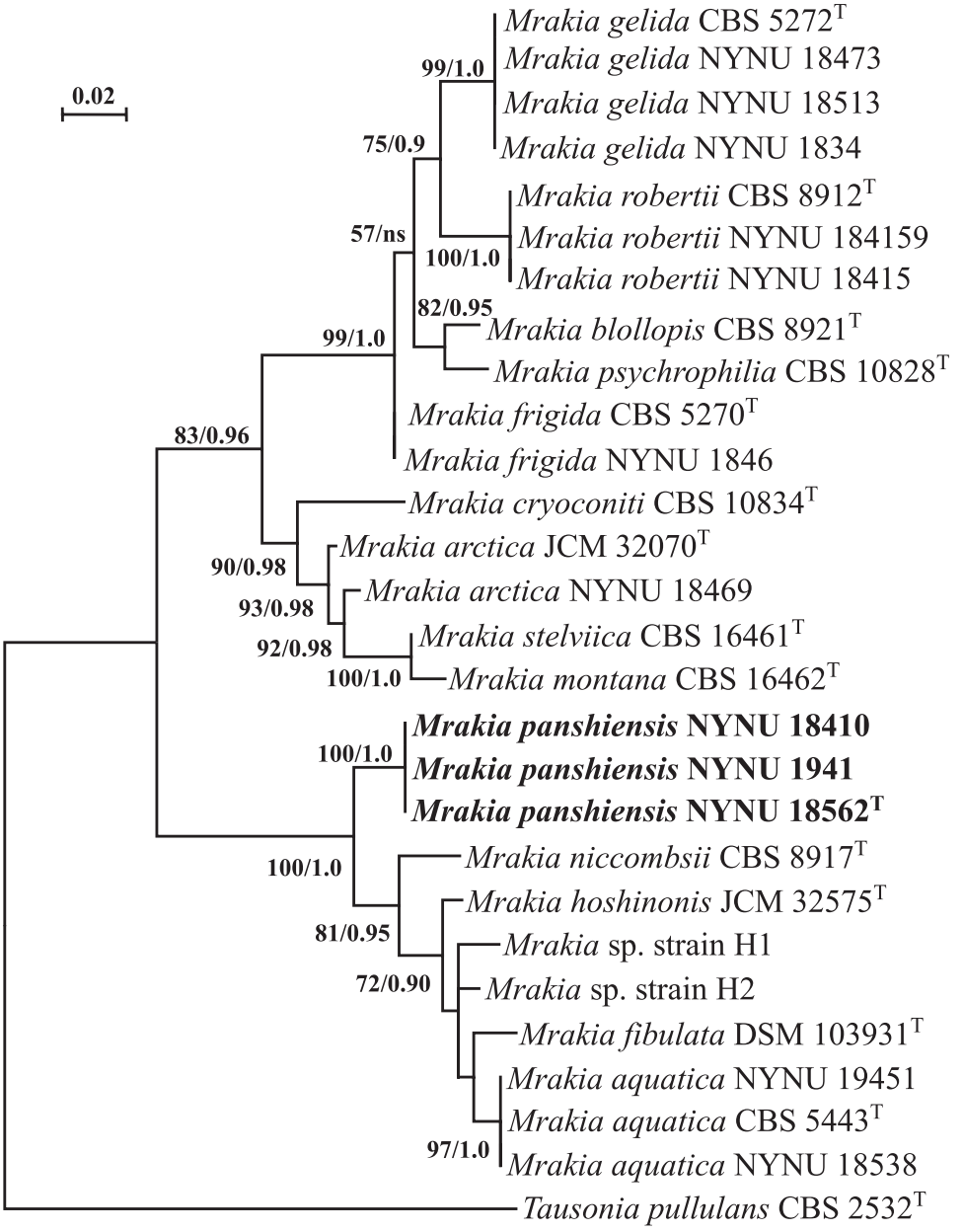

Figure 1. Phylogram inferred from Maximum likelihood analysis of the concatenated ITS and nuc 28S rDNA dataset of taxa in *Mrakia* s.s. *Mrakia panshiensis* strains investigated in this study are highlighted in bold font. *Tausonia pullulans* CBS 2532^{T} was designated as the outgroup. The tree backbone was constructed by maximum likelihood analysis with MEGA7. Bootstrap percentages of maximum likelihood over 50% from 1000 bootstrap replicates and posterior probabilities of Bayesian inference above 0.9 are shown from left on the branches. The scale bar represents 0.02 substitutions per nucleotide.

Table 2. Sequences used in molecular phylogentic analysis. Entries in bold are newly generated for this study.

Species	Strain	ITS	D1/D2
Mrakia aquatica	CBS 5443^{T}	AF410469	NG_042348
	NYNU 18538	**MT126040**	**MT126039**
	NYNU 19451	**MT140347**	**MT140348**
Mrakia arctica	JCM32070^{T}	LC222845	LC222845
	NYNU 18469	**MK682823**	**MK682821**
Mrakia blollopis	CBS 8921^{T}	AY038826	NG_057710
Mrakia cryoconiti	CBS 10834^{T}	AJ866976	KY108575
Mrakia fibulata	DSM 103931^{T}	MK372216	MK372216
Mrakia frigida	CBS 5270^{T}	AF144483	NG_042346
	NYNU 1846	**MT126288**	**MT133538**
Mrakia gelida	CBS 5272^{T}	AF144485	AF189831
	NYNU 1834	**MT126029**	**MT126028**
	NYNU 18473	**MT133535**	**MT133534**
	NYNU 18513	**MT133539**	**MT133537**
Mrakia hoshinonis	JCM 32575^{T}	LC335798	LC335798
Mrakia niccombsii	CBS 8917^{T}	AY029346	NG_060242
Mrakia panshiensis	**NYNU 18410**	**MT133553**	**MT133536**
	NYNU 18562^{T}	**MK682818**	**MK682815**
	NYNU 1941	**MT133552**	**MT133554**
Mrakia psychrophilia	CBS 10828^{T}	EU224267	KY108586
Mrakia robertii	CBS 8912^{T}	AY038829	AY038811
	NYNU 18415	**MT125967**	**MT125965**
	NYNU 184159	**MT133533**	**MT133532**
Mrakia sp.	strain H2	AY052488	AY052480
	strain H1	AY052487	AY052479
Mrakia stelviica	CBS 16461	MT347764	MT347768
Mrakia montana	CBS 16462	MT347765	MT347769
Tausonia pullulans	CBS 2532^{T}	AF444417	NG_042352

Abbreviations: **CBS**: CBS-KNAW Collections, Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; **JCM**: RIKEN BioResource Research Center-Japan Collection of Microorganisms, Takao, Japan; **DSM**: the German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany; **NYNU**: Microbiology Lab, Nanyang Normal University, Henan, China; **T**: type strain.

and nuc 28S rDNA showed that the three isolates represent a novel species in the *Mrakia aquatica* sub-clade, close to *M. niccombsii*, *M. aquatica*, *M. fibulata* and *M. hoshinonis* (Fig. 1). Topologies of phylogenetic trees that were made using alignments of either ITS region or nuc 28S rDNA and inferred from BI and ML algorithms were visually similar and, thus, only ML trees were shown (Suppl. material 1: Figs S1, S2). The combined ITS and nuc 28S rDNA dataset included 28 yeast collections representing 12 species of the genus *Mrakia* with *Tausonia pullulans* CBS 2532^{T} as the outgroup (Fig. 1). The alignment had a total of 1243 characters, of which 1031 characters were constant, 210 were variable and parsimony-uninformative and 114 were parsimony-informative. ML and BI analyses yielded trees which were topologically congruent in terms of species clusters (examined visually). Three strains of the new species formed a well-supported branch basal to other members of the *M. aquatica* sub-clade with high statistical support of 100% BP and 1.0 BPP in ML and BI analyses, respectively. The strain NYNU 18469 clustered with high support with the type strain of *M. arctica* showing 99.8% and 99.3% sequence identities in the nuc 28S rDNA and ITS region, respectively.

Taxonomy

Mrakia panshiensis **R.R. Jia & F.L. Hui, sp. nov.**
MycoBank No: 834813
Fig. 2

Etymology. The species name *panshiensis* (N.L. fem. adj.) refers to the geographical origin of the type strain of this species.

Description. The physiological profiles of the three strains of the novel species were almost identical. In YM broth after 3 days at 15 °C, the cells are ovoid to elongated (3.5–7 × 3.5–5 μm) and proliferate by polar budding (Fig. 2A). Streak culture for 1 week at 15 °C on YM agar produces colonies that are white to yellowish-cream, round, convex and smooth with an entire margin. After 2 weeks in Dalmau plate culture on CM agar at 15 °C, pseudohyphae and true hyphae are formed. Teliospores were observed after 2 months at 10 °C terminally and intercalarily on the hyphae on CM agar (Fig. 2B). Teliospores are spherical, 8–13 μm in diameter, single and in short chains of 2–4 spores (Fig. 2C). Teliospores may germinate by a bud-like projection (Fig. 2D). The fermentation of sugars is absent. Glucose, galactose, L-sorbose, D-ribose, D-xylose, L-arabinose, L-rhamnose, sucrose, maltose, trehalose, methyl α-D-glucoside, cellobiose, salicin, arbutin, melibiose, lactose, raffinose, melezitose, inulin, glycerol, erythritol, ribitol, xylitol, L-arabinitol, D-glucitol, D-mannitol, galactitol, 2-keto-D-gluconate, D-gluconate, D-glucuronate, succinate, citrate and ethanol are assimilated. No growth occurs in D-glucosamine, D-arabinose, *myo*-inositol, D-glucono-1, 5-lactone, 5-keto-D-gluconate, DL-lactate or methanol. For the assimilation of nitrogen compounds, growth on nitrate, nitrite, L-lysine, glucosamine and D-tryptophan is positive, whereas on ethylamine, cadaverine, creatine, creatinine or imidazole, it is negative. There is no growth in the presence of 5% glucose medium with 10% NaCl and 0.01% cycloheximide. Diazonium blue B test and urease activity are positive. The maximum growth temperature is 18 °C and optimal growth temperature is 15 °C.

Molecular characteristics (holotype). nucleotide sequences of ITS and nuc 28S rDNA gene sequences are deposited in NCBI GenBank under the accession numbers are MK682818 and MK682815, respectively.

Deposits. ***holotype***, NYNU 18562 culture was isolated from the soil of the Lianhuashan National Forest Park, Jilin Province, China, in September 2018. The holotype culture is preserved in a metabolically inactive state at Microbiology Lab, Nanyang Normal University, Henan, China. Ex-type cultures are deposited at the China Centre of Industrial Culture Collection (CICC), Beijing, China, as CICC 33355, and at the CBS Yeast Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands, as CBS 15868.

Strains studied. NYNU 18562; paratypes: NYNU 1941 and NYNU 18410 from two different soil samples in the Maoershan National Forest Park in Yanji city, Heilongjiang Province, China.

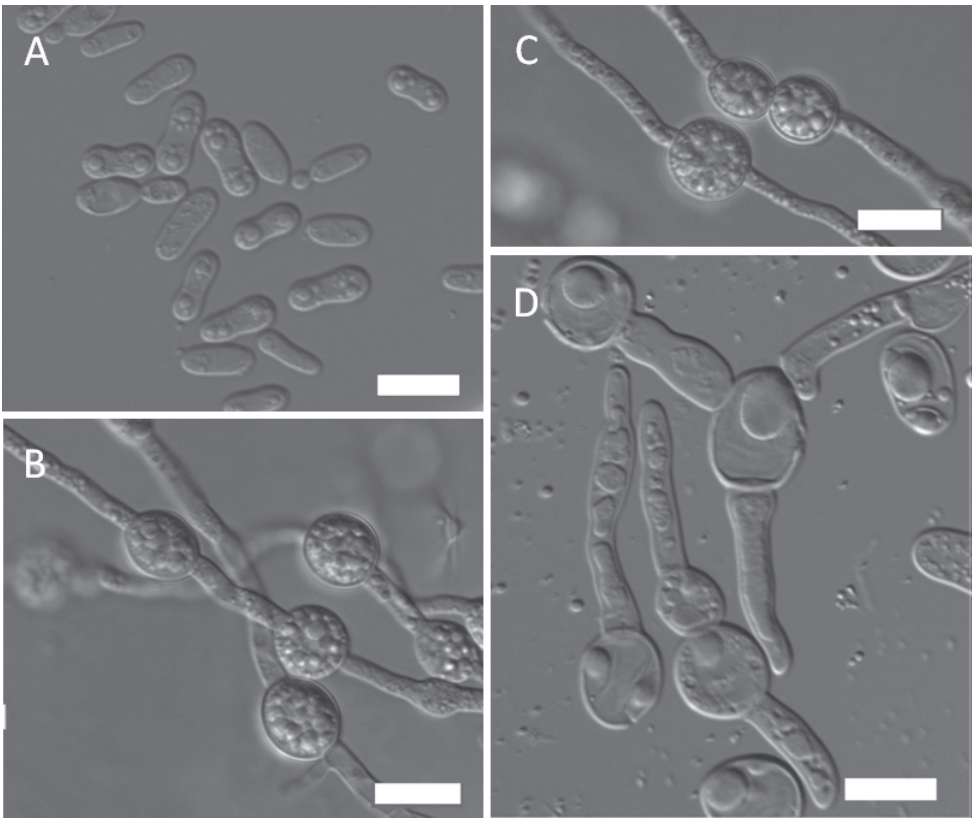

Figure 2. Morphology of *Mrakia panshiensis* (NYNU 18562[T]) **A** budding cells **B** true hyphae with clamp connections and teliospores **C** teliospores in pairs **D** teliospores produced with a bud-like projection. Scale bars: 10 μm.

Mrakia arctica M. Tsuji emend. R.R. Jia & F.L. Hui

Description. The yeast *Mrakia arctica* was described as an asexual species (Tsuji et al. 2018). Among soil yeasts isolated in the present study, strain NYNU 18469 collected from Liaoning provinces, formed hyphae and teliospores on CM agar (Fig. 3). In spite of the observation of sexual behavior in that species, the description of *Mrakia arctica* M. Tsuji, Mycoscience 59 (1): 57. 2018 (MB 821502) is emended below.

True hyphae with terminally and intercalarily teliospores are developed after 2 months at 10 °C on CM agar (Fig. 3A). Culture NYNU 18469 demonstrated similar morphological characteristics with those of the previously reported yeast-stage morphs, except for the yeast-forming teliospores. Teliospores are spherical, 6–12 μm in diameter, single and in short chains of 2–4 spores. Teliospores germinate by a bud-like projection (Fig. 3B, C, D). Physiologically, both asexual and sexual morphs of *M .arctica* were identical, except for a few reactions on standard growth. The asexual morph of

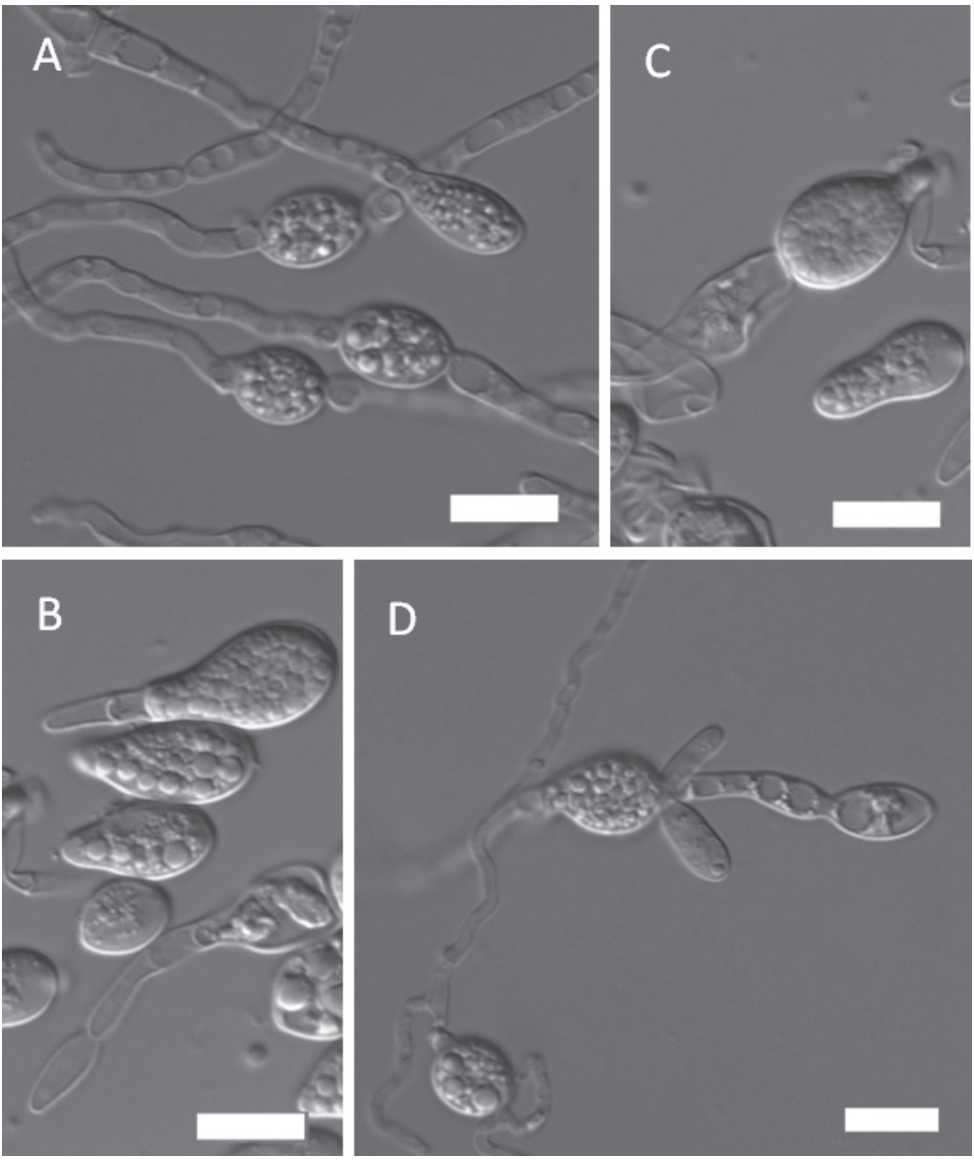

Figure 3. The teliospore-stage morph of *Mrakia arctica* (NYNU 18469) **A.** True hyphae with teliospores terminally and intercalary; **B/C/D.** Teliospores produced with a bud-like projection. Scale bars: 10 μm.

M .arctica can assimilate L-sorbose and grows in the presence of 0.01% cycloheximide, and grows at 20 °C, but not for the sexual morph.

Deposits. culture NYNU 18469 was isolated from the soil of the Tianhuashan National Forest Park, Liaoning Province, China, in September 2018. It is preserved in a metabolically inactive state at Microbiology Lab, Nanyang Normal University, Henan, China. Ex-type cultures are deposited at the China Centre of Industrial Culture Collection (CICC), Beijing, China, as CICC 33354, and at the CBS Yeast Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands, as CBS 10867.

Discussion

Mrakia is currently placed in the monotypic family Mrakiaceae (Liu et al. 2015). In this study, we identified 12 isolates of *Mrakia* species from soil samples in Northeast China and characterized them using molecular sequence analyses and microscopy. As a result, a novel species was discovered among these isolates, and characterized and described as *M. panshiensis* sp. nov. Physiologically, *M. panshiensis* is different from the closely related *M. niccombsii* (Fell and Margesin 2011) in the ability to assimilate ribitol and the inability to assimilate D-glucosamine, D-arabinose and *myo*-inositol. The novel species differs from the other four members of the *M. aquatica* sub-clade in its inability to grow in 5% glucose medium with 10% NaCl and at 20 °C. Additionally, we obtained four reported *Mrakia* species, *M. aquatica*, *M. gelida*, *M. frigida* and *M. robertii*, have been reported from cold environments, such as the Alps, Alaska and Antarctica (Thomas-Hall et al. 2010; Fell 2011; Fell and Margesin 2011). These four species can be distinguished by both morphological and molecular approaches. A careful examination of isolates also resulted in the observation of the teleomorphic stage of *M. arctica*. Considering the newly described *M. panshiensis*, the genus *Mrakia* includes thirteen species. Amongst them, *M. aquatica*, *M. hoshinonis*, *M. cryoconiti* and *M. niccombsii* were described as asexual morphs. Sexual reproduction with hyphae and teliospores was observed in seven species, viz. *M. blollopis*, *M. fibulata*, *M. frigida*, *M. gelida*, *M. psychrophila*, *M. robertii*, *M. montana*, *M. stelviica* and the newly-described *M. panshiensis* sp. nov. (Fell 2011; Tsuji et al. 2018, 2019; Yurkov et al. 2020; Turchetti et al. 2020). The question of whether or not the teleomorphs of soil yeasts depend on their hosts as well as the degree of the host specificity may shed new light on the ecology of below ground microorganisms.

Most species of the genus *Mrakia* have an optimal temperature for growth of approximately 12–15 °C and are not able to grow at temperatures above 20 °C (Fell 2011; Fell and Margesin 2011). Thus these yeasts can be defined as obligate psychrophilic yeasts (Watson 1987; Raspor and Zupan 2006). Psychrophilic yeasts are noted for their ability to grow at low temperatures and even below freezing point (Panikov and Sizova 2007). Twelve *Mrakia* isolates characterized in the present study were obtained from three regions in Northeast China. The three regions are characterized by average temperatures between –2 and 10 °C. The region represents a seasonally cold environment in Northeast China. Isolation of *Mrakia* species from seasonal soils is in line with previous observations of these yeasts from cold habitats and suggests an ecological role of *Mrakia* species in extreme environments characterized by low temperatures. Frequent sources of isolation include a variety of low temperature environments, such as ice, glacier sediment, snow and meltwater (Fell 2011; Fell and Margesin 2011; Tsuji et al. 2018, 2019). However, biodiversity and distribution of psychrophilic yeasts outside cold habitats are not well known (Yurkov et al. 2020). Sylvester et al. (2015) supposed that, although *Mrakia* seems to have a competitive advantage at 10 °C, it can grow at warmer temperatures. *M. frigida* was isolated from an oak bark in different sites across the northern United States, using an enrichment protocol and incubation at 10 °C (Sylvester et al. 2015). Several species of *Mrakia* were also obtained from

xylem sap of *Betula pendula* and *Carpinus betulus* in Germany, including *M. fibulata* which exhibited maximum growth temperature at 25 °C (Yurkov et al. 2020). *Mrakia aquatica*, *M. blollopis* and *M. robertii* were isolated in summer periods from urban soils in Moscow, but not from adjacent low-managed soils (Tepeeva et al. 2018). Isolation of *M. aquatica*, *M. arctica*, *M. blollopis*, *M. gelida*, *M. fibulata*, *M. frigida*, *M. robertii M. stelviica*, *M. montana* and the newly-described *M. panshiensis* sp. nov. from these temperate climates suggests that these yeasts might be common inhabitants of diverse environments in boreal and temperate climates.

Soils were regarded as a mere reservoir for yeasts that reside in habitats above it. Our knowledge of soil yeasts is further biased towards temperate and boreal forests. The distribution of soil yeasts is determined by plant, insect and fungal hosts and vectors (Yurkov 2018). Di Menna (1966) found *Mrakia* to be the dominant yeast genus in Antarctic soils, representing 24% of the yeast species isolated in that study. In the present study, we identified 12 isolates of *Mrakia* species from forest soils collected in Northeast China, which is corresponding to 10% of the total number of isolated yeast cultures. The sampling sites were located in temperate deciduous and broadleaf forests that are commonly found in areas with warm, moist summers and mild winters. Typical soils of temperate forests are Alfisols and Spodosols along with some Histosols. Yeast communities respond to forest properties rather than to basic soil parameters even though the mechanisms underlying these effects remain unknown (Yurkov 2017). Forest management is known to influence substrate-dependent taxa, such as bryophytes and sporocarp (fruiting body) forming fungi (Ódor et al. 2006). In view of the rapid decline of many natural habitats, studies of soil yeasts in undisturbed or low managed biotopes became extremely valuable. To date, the isolation of *Mrakia* yeasts from temperate soils is limited to a few studies from Europe (Mašínová et al. 2017; Yurkov et al. 2016; Tepeeva et al. 2018). The present study demonstrated that yeasts of the genus *Mrakia* are present in temperate forest soils in Asia.

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Supplementary material 1

Figures S1 and S2

Authors: Kai-Hong Zhang, Cheng-Feng Shi, Chun-Yue Chai, Feng-Li Hui

Data type: phylogenetic trees

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RESEARCH ARTICLE

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Cortinarius pakistanicus and *C. pseudotorvus*: two new species in oak forests in the Pakistan Himalayas

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Abstract

The genus of basidiomycetous fungi *Cortinarius* occurs worldwide, from subtropical to boreal latitudes. Although molecular systematics has triggered the study of these fungi in the Americas and Europe in the last two decades, there is still limited research on its diversity in large portions of the planet, such as the high mountain ranges of Asia. Several collections of *Cortinarius* were made during mycological field trips conducted between 2014 and 2018 in pure oak forests in the Pakistan Himalayas. An integrative framework combining morphological and phylogenetic data was employed for their study. As a result, the two species *C. pakistanicus* and *C. pseudotorvus* are here described as new to science. Detailed macro- and micro-morphological descriptions, including SEM images of spores, and a molecular phylogenetic reconstruction based on nrITS sequence data are provided and used to discriminate the new species from morphologically and phylogenetically close taxa. Whereas our phylogenetic tree inference gave unequivocal support for the inclusion of *C. pseudotorvus* within *C.* sect. *Telamonia*, the assignment of *C. pakistanicus* to any known sections remained elusive. These species likely establish ectomycorrhizal associations with trees in the genus *Quercus*, making this type of forest in the Pakistan Himalayas a promising focus for future research on the diversity of *Cortinarius*.

Keywords

Biodiversity, ectomycorrhizal fungi, ITS, phylogeny, systematics, taxonomy

Introduction

Cortinarius (Pers.) Gray (*Cortinariaceae*) is a relatively well known mushroom-forming genus of basidiomycetous fungi characterized by the fugacious veil forming a fine cobweb ("cortina") between the stipe and pileus margin and by the production of ornamented, cinnamon brown to fulvous basidiospores (Kirk et al. 2008; Niskanen 2008). It is one of the most species rich, abundant and widespread ectomycorrhizal genera in *Agaricales* (Geml et al. 2012; Nouhra et al. 2013; Nuske et al. 2019), encompassing ca. 3000 species worldwide (Niskanen et al. 2018). Whereas the distributional ranges for many species are known to be rather restricted (e.g. Ballarà et al. 2017; Soop et al. 2019), other species occur across several regions and continents (Dima et al. 2014; Harrower et al. 2015; Liimatainen et al. 2017). Furthermore, evidence gained in recent years due to the use of high-throughput sequencing techniques has shown that *Cortinarius* establishes ectomycorrhizal associations with a myriad of plant hosts, including not only members in the important families *Fagaceae*, *Betulaceae*, *Pinaceae*, *Salicaceae* and *Cistaceae*, but also some herbaceous plants in the *Cyperaceae* and *Polygonaceae* (Geml et al. 2012; Horton et al. 2017; Pérez-Izquierdo et al. 2017; Nuske et al. 2019). However, the vast majority of systematic studies on *Cortinarius* have been conducted in Europe and the Americas, and more recently in Oceania (Australia, Tasmania and New Zealand), and therefore little is known about its diversity and range of plant hosts in other areas of the planet. In particular, we know virtually nothing about the species of *Cortinarius* growing in forests in the high mountain ranges north of Pakistan, which belong to the Himalayas.

Taxonomic studies on *Cortinarius* throughout the whole of Pakistan are in fact scant. Ahmad et al. (1997) reported *C. bulliardii* (Pers.) Fr., *C. cinnamomeus* (L.) Gray, *C. hinnuleus* Fr., and *C. phoeniceus* (Vent.) Maire from moist temperate forests of *Abies*, *Cedrus*, *Picea* and *Pinus* in Khanspur, Shogran, Dungagali and adjoining areas of the Pakistan Himalayas. After more than 20 years, Saba et al. (2017) described *C. longistipitatus* Saba, S. Jabeen, Khalid & Dima as a new species in *C.* subgenus *Telamonia* sect. *Cinnabarini* based on phylogenetic data. This species was collected in mixed *Cedrus deodara* and *Pinus wallichiana* forests in the Hazara and Malakand divisions. Recently, *C. brunneocarpus* Razaq & Khalid has been described from Khanspur growing in a mixed *P. wallichiana* and *Abies pindrow* forest, and the phylogenetic analysis found it in *C.* section *Hinnulei* (Song et al. 2019). In the same work, an additional species, *C. lilacinoarmillatus* Semwal & Dima, was described from the Indian Himalayas. All in all, further research on the diversity of this genus in the whole region is required. The aim of the present work is to provide new insights about the diversity of *Cortinarius* species associated with oaks in the Pakistan Himalayas.

Several collections of *Cortinarius* were sampled and preliminarily assigned to *C.* subgenus *Telamonia* (Fr.) Trog. during a 2014–2018 exploration campaign to oak forests from the Khyber Pakhtunkhwa province (Swat District, Pakistan, Fig. 1). This subgenus initially comprised a very large number of species, but thanks to the advent of molecular techniques, we now know that it is polyphyletic (Høiland and Holst-Jensen 2000; Niskanen et al. 2013a; Soop et al. 2019). Here, we combine data of the nuclear ribosomal internal transcribed spacer (nrITS) region as well as morphology

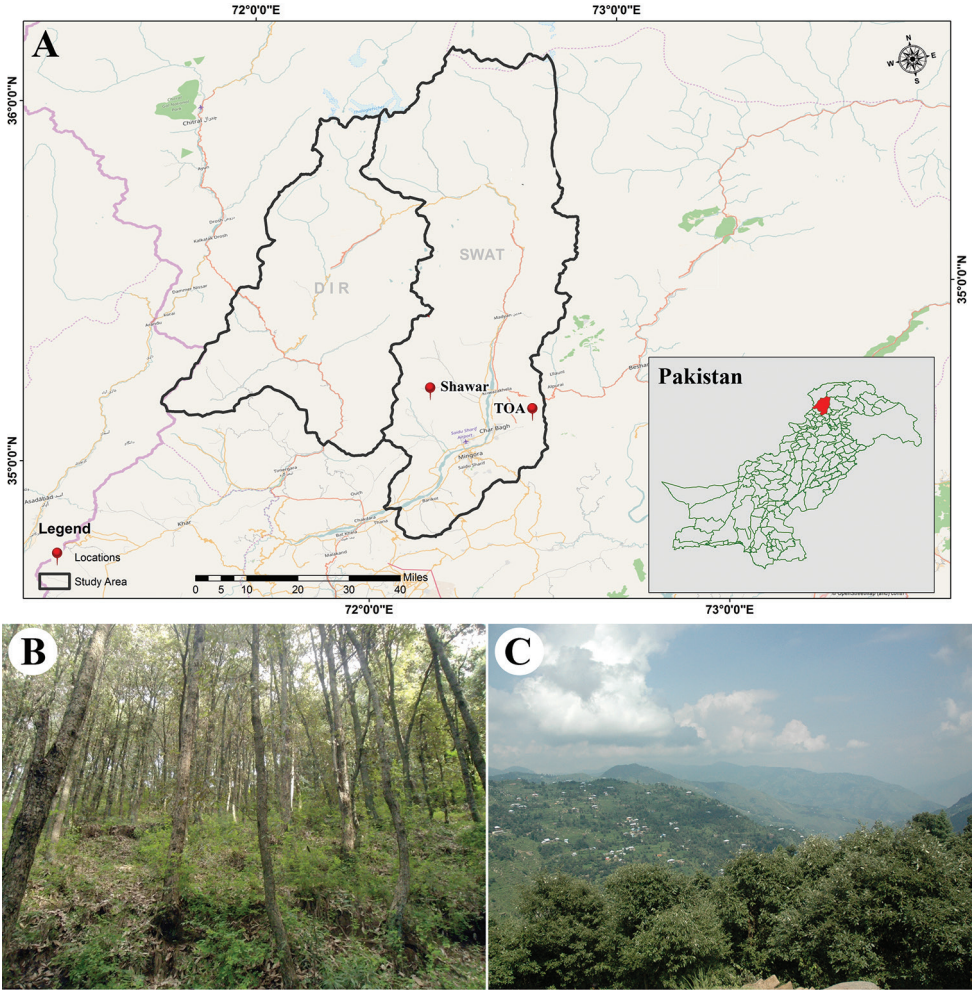

Figure 1. A Map of the two surveyed localities in the Swat District (Pakistan) **B** forest of *Quercus oblongata* in Shawar Valley **C** *Quercus oblongata* trees in the Alpuri forests (Toa). Photo: A. Naseer.

and habitat to propose two novel *Cortinarius* species for science and study their affinities to any known sections within this genus.

Methods

Morphological and anatomical studies

Basidiomata were collected following Lodge et al. (2014) and photographed in their natural habitats using a Nikon D70S camera. Morphological characters were recorded from fresh specimens. Colors were designated with reference to mColorMeter application (Yan-

mei He, Mac App Store). Collections of the newly described species were deposited in the Herbarium of the Department of Botany, University of the Punjab, Lahore, Pakistan (acronym LAH). Microscopic characters are based on freehand sections from fresh and dried specimens mounted in 5% (w/v) aqueous Potassium Hydroxide (KOH) solution. Tissues from lamellae and pileipellis were mounted in phloxine (1%) for increasing contrast, and examined using a Meiji Techno MX4300H compound microscope. A total of 30 basidiospores, basidia, cystidia and hyphae from pilei were measured from each collection. For basidiospores, the abbreviation "*n/m/p*" indicates *n* basidiospores measured from *m* fruit bodies of *p* collections. Dimensions for basidiospores are given using length × width (L × W), and extreme values are given in parentheses. The range contains a minimum of 90% of the values. Measurements include the arithmetic mean of spore length and width.

Laboratory procedures and sequence alignment and phylogenetic analyses

Genomic DNA was extracted from portions of lamellae following a modified CTAB extraction method (Bruns 1995). Primers used for amplification of the nrITS marker were ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). Polymerase chain reactions (PCR) were performed in a total volume of 25 µL and consisted of an initial 4 minutes denaturation step at 94 °C, 40 cycles of 1 minute at 94 °C, 1 min at 55 °C, 1 min at 72 °C, and a final extension step of 10 minutes at 72 °C. Visualization of PCR products on a 1.5% agarose electrophoretic gel was done staining with SYBR Green. Successful amplicons were purified by enzymatic purification using Exonuclease I and Shrimp Alkaline Phosphatase enzymes (Werle et al. 1994). Bidirectional sequencing of purified products was done by Macrogen (Republic of Korea). Chromatograms were checked and assembled using SeqmanII v.5.07 (Dnastar Inc.).

The online tool BLAST (Altschul et al. 1997) and the databases GenBank (http://www.ncbi.nlm.nih.gov/) and UNITE (Nilsson et al. 2018) were used to check for possible PCR-product contamination and to identify and retrieve available, highly similar *Cortinarius* nrITS sequences to the four newly produced sequences (Table 1). Eighty-one sequences were downloaded, of which 52 corresponded to the sequence of the species' type material. To provide a detailed view of *Cortinarius* phylodiversity, representative species of several sections were included: *C.* sect. *Anomali* (*Ano*, 2), *C.* sect. *Biveli* (*Biv*, 2), *C.* sect. *Boulderenses* (*Bou*, 2), *C.* sect. *Bovini* (*Bov*, 3), *C.* sect *Brunnei* (*Bru*, 2), *C.* sect. *Castanei* (*Cas*, 2), *C.* sect. *Cinnabarini* (*Cin*, 2), *C.* sect. *Colymbadini* (*Col*, 2), *C.* sect. *Crassispori* (*Cra*, 2), *C.* sect. *Disjungendi* (*Dis*, 2), *C.* sect. *Duracini* (*Dur*, 2), *C.* sect. *Firmiores* (*Fir*, 7), *C.* sect. *Hinnulei* (*Hin*, 4), *C.* sect. *Hydrocybe* (*Hyd*, 4), *C.* sect. *Illumini* (*Ill*, 2), *C.* sect. *Incrustati* (*Inc*, 1), *C.* sect. *Infracti* (*Inf*, 2), *C.* sect. *Laeti* (*Lae*, 2), *C.* sect. *Malachii* (*Mal*, 2), *C.* sect. *Obtusi* (*Obt*, 2), *C.* sect. *Paleacei* (*Pal*, 2), *C.* sect. *Parvuli* (*Par*, 2), the "*C. puellaris* group" (*Pue*, 3), *C.* sect. *Safranopedes* (*Saf*, 2), *C.* sect. *Saniosi* (*San*, 1), *C.* sect. *Saturnini* (*Sat*, 2), *C.* sect. *Sciophylli* (*Sci*, 2), *C.* sect. *Telamonia* s. str. (*Tel*, 8 spp.), *C.* sect. *Uracei* (*Ura*, 2), and *C.* sect. *Urbici* (*Urb*, 2). Two taxa within *C.* sect. *Callochroi* (*Cal*) were selected as outgroup: *C. calochrous* (Pers.) Gray and *C. barbarorum* Bidaud, Moënne-Locc. & Reumaux.

MAFFT v.7.308 (Katoh et al. 2002; Katoh and Standley 2013) was used to build a multiple sequence alignment (MSA). The following parameter options were selected: the FFT-NS-I x1000 algorithm, the 200PAM / k = 2 scoring matrix, a gap open penalty of 1.5 and an offset value of 0.123. Manual editing of the resulting alignment was carried out in Geneious v.11.0.5 and consisted of trimming alignment ends of longer sequences that included part of the 18S–28S ribosomal subunits, and replacing gaps at the ends of shorter sequences with N (IUPAC bases representing any base). To assess the effect of keeping ambiguously aligned regions in the dataset, two analyses were conducted: one using the original MSA as input data, and the other using an MSA processed in GBlocks v.0.91b (Castresana 2000). This software allows for automatically removing these conflicting regions in the alignment and, to do this, the least stringent parameters were selected but allowing gaps in 50% of the sequences. Phylogenetic analyses were conducted under a Maximum Likelihood (ML) and Bayesian Inference (BI) perspectives, and used either the original MSA as well as the one trimmed with GBlocks. The ML phylogeny was inferred with the online version of RAxML-HPC2 hosted at the CIPRES Science Gateway (Miller et al. 2010; Stamatakis 2006; Stamatakis et al. 2008). Nodal support was evaluated with 1000 bootstrap pseudoreplicates. Then, the MrBayes analyses were conducted with two parallel, simultaneous four-chain runs executed over 5×10^7 generations starting with a random tree, and sampling after every 500th step. The first 25% of data were discarded as burn-in, and the 50% majority-rule consensus tree and corresponding posterior probabilities were calculated from the remaining trees. Optimal substitution models for the two partitions within the nrITS (ITS1+2, 5.8S) used in the above analyses were inferred with PartitionFinder v.1.1.1 (Lanfear et al. 2012) considering a model with linked branch lengths and the Bayesian Information Criterion (BIC). This analysis favored the GTR+Γ model for the two nrITS partitions in the RAxML analyses, whereas in the MrBayes analyses, the GTR+Γ and K80 models were selected for ITS1+2 and 5.8S, respectively. Average standard deviation of split frequencies (ASDSF) values below 0.005 and potential scale reduction factor (PSRF) values approaching 1.00 were considered as indicators of chain convergence in the Bayesian analyses. As for tree nodal support, nodes showing Bootstrap support (BS) values equal or higher than 70% (RAxML analyses) and Bayesian posterior probabilities (PP) equal or higher than 0.95 (MrBayes analyses) were regarded as significantly supported. Phylogenetic trees were visualized in FigTree v.1.4 (available at http://tree.bio.ed.ac.uk/software/tracer/) and Adobe Illustrator CS5 was used for artwork.

Results

Molecular phylogenetic analyses

The original MSA produced with MAFFT was 701 base pairs in length (347 variable and 105 singleton sites), whereas the GBlocks-trimmed MSA comprised 494 positions (70% of the original length with 216 variable and 60 singleton sites) distributed in 45 blocks (Table 2). The ML analysis in RAxML generated a phylogeny with Ln = -6706.607367 (original MSA) and Ln = -5097.173288 (GBlocks-trimmed). The

Table 1. Specimens included in phylogenetic analyses. Sequences produced in this study are highlighted in bold. Country codes follow www.country-code.cl/es/.

Species	Voucher	Country	GenBank and UNITE accessions (*nrITS*)
Cortinarius acutus	OS576	NO	KC842420
C. alboviolaceus	1734	IT	JF907875
C. anomalus	S: CFP1154 (Type)	SE	KX302224
C. barbarorum	TF2004-030	SE	DQ663237
C. biformis	SMIA42	CA	FJ039574
C. bivelus	IK-00518	PL	KX355542
C. bovinus	H: IK04-038 (Type)	FI	NR_120189
C. boulderensis	MICH: 10323 (Type)	US	NR_121207
C. brunneocarpus	LAH 240810 (Type)	PK	NR_166355
C. brunneovernus	WTU: JF Ammirati 13331 (Type)	US	NR_131826
C. brunneus	CFP587 (Type)	SE	DQ117927
C. calochrous	TF2001-113	SE	DQ663250
C. caninus	S: CFP627 (Type)	SE	KX302250
C. chrysomallus (= *C. saniosus*)	LY69_217 (Type)	FR	DQ102670
C. cinnabarinus	S: F248436 (Type)	SE	NR_120163
C. coccineus	GK: 435745 (Type)	FR	JX114945
C. colymbadinus	S: F248443 (Type)	SE	NR_131819
C. confirmatus	PC: R Henry 3195 (Type)	FR	KX964438
C. crassisporus	H: IK95-1085 (Type)	SE	NR_131882
C. decipiens	PML 366 (Type)	FR	FN428988
C. decipiens var. *hoffmannii* (= *C. casimiri*)	PML 559 (Type)	FR	FN429015
C. denigrates	WTU: M Beug 02MWB043014 (Type)	US	NR_153056
C. disjungendus	H: PA Karsten 4370 (Type)	FI	KP013190
C. duboisensis	WTU: J.F. Ammirati 13311 (Type)	US	NR_153057
C. duracinus	G: PML 349 (Type)	FR	KX964582
C. flexipes	MC01-551	DK	AJ889971
C. fragantissimus	WTU: M Beug 10MWB111913 (Type)	US	NR_153058
C. fructuodorus	H: 7001104 (Type)	US	NR_131827
C. fulvescens	TN04-935 (Type)	FI	NR_153077
C. fuscescens	H: 6001898 (Type)	FI	NR_131879
C. gallurae	CONS 00076 (Type)	IT	FN428979
C. helobius	HLCFP542	n/a	DQ102686
C. hinnuleoarmillatus	G: 00052098 (Type)	FR	NR_131790
C. hinnuleus	TUB 011512	DE	AY669665
C. illuminus	S: F44877 (Type)	SE	KP866156
C. impennoides	O: TE Brandrud TEB 281-09	NO	KT591607
C. infractus	TUB 011441	DE	AY174781
C. infractiflavus	SMI286	CA	FJ039612
C. intempestivus	PC: PML 1157 (Type)	FR	KX831120
C. iunii	J Ballarà JB-6989/10 (Type)	ES	MF000335
C. laetus	F15817	CA	FJ157034
C. lilacinoarmillatus	CAL: KCS2428 (Type)	IN	NR_166356
C. malachius	IK98-1298	FI	JX407332
C. microglobisporus	IB 20110123 (Type)	IT	NR_153027
C. millaresensis	J Ballarà JB-7369/11 (Type)	ES	KU953933
C. minusculus	H: TN12-032 (Type)	FI	MK211177
C. aff. *multicolor*	UBC: F17146 OC74	CA	GQ159889
C. murinascens	H: IK 08-958 (Type)	FI	KP165570
C. neofallax	PC: PML 1158 (Type)	FR	KF048129
C. neofurvolaesus	S: CFP1438 (Type)	SE	NR131789

Species	Voucher	Country	GenBank and UNITE accessions (*nrITS*)
C. niveotraganus	TN04-014a	FI	KM273104
C. nolaneiformis	PRM: J Velenovsky 857042 (Type)	CZ	NR_131833
C. obtusus	OS577	NO	KC842421
C. olididisjungendus	H: TN07-191 (Type)	CA	KM273091
C. ortovernus	JB-6048-08 (Type)	ES	KX964566
C. oxytoneus	PC: R Henry 931 (Type)	FR	KX964567
C. pakistanicus	**AST332, LAH36366 (Type)**	**PK**	**MN864283**
C. pakistanicus	**ASSW58, LAH35227**	**PK**	**MN864282**
C. persimilis	PC: GE 16.029 (Type)	FR	MH485205
C. pseudofallax	PC: 0124963 (Type)	FR	NR_131831
C. pseudotorvus	**AST20, LAH35257 (Type)**	**PK**	**MN864285**
C. pseudotorvus	**MJ-15103, LAH36368**	**PK**	**MN864286**
C. puellaris	O: TE Brandrud TEB 431-14 (Type)	NO	KT591581
C. quarciticus	H: CFP765 (Type)	SE	UDB000748
C. roseocastaneus	H:_6001997 (Type)	FI	NR_131866
C. rubrovioleipes	H: IK04-031 (Type)	FI	DQ497191
C. saturninoides	X Carteret 2013-144	FR	KX964573
C. saturninus	S: H Lindström CFP514 (Type)	SE	KX964584
C. sciophylloides	PC: A Bidaud 99-10-254 (Type)	FR	KX964576
C. suberi	IK95-349	FI	HQ845172
C. subpuellaris	O: TE Brandrud TEB562-15 (Type)	NO	KX831129
C. subscotoides	H: TN12-010 (Type)	FI	MK211175
C. subserratissimus	H: IK11-017 (Type)	SE	KP165552
C. subturibulosus	MES 3779	ES	FN428987
C. torvus	TUB 011515	DE	AY669668
C. torvus	TF01-035	DK	AJ889977
C. torvus	MIN: DJM1489	US	KY964778
C. turgidoides	PML35	FR	MH784724
C. turgidus	AB01-09-69	FR	MH784708
C. umbrinobellus	H: 7018158 (Type)	SE	NR_131874
C. uraceisporus	H: 6001791 (Type)	FI	NR_131878
C. uraceonemoralis	H: TN04-1116 (Type)	IT	KJ206515
C. uraceus	H: TN04-872 (Type)	FI	KJ206522
C. urbicus	H Lindström CFP486	SE	UDB000743
C. venustus	F16350	CA	FJ039571

MrBayes analyses reached an average standard deviation of split frequencies of 0.005 after 2.636×10^7 (original MSA) and 1.2295×10^7 (GBlocks-trimmed) generations. Average Estimated Sample Sizes (EESs) were well above 200 in both Bayesian analyses. Because the four inferred phylogenies (two ML, and two BI) were largely coherent, with no supported conflicts, the topology inferred with MrBayes using the GBlocks-trimmed MSA is presented in Fig. 2.

In general, analyses retrieved BS > 70% and PP > 0.95 for clades that represented several *Cortinarius* sections (Fig. 2). All analyses supported a clade containing *C.* sections *Hinnulei*, *Hydrocybe*, *Parvuli*, *Paleacei*, *Safranopedes*, *Incrustati*, *Castanei*, *Saniosi*, *Boulderenses*, *Crassispori*, *Cinnabarini*, *Colymbadini*, *Brunnei*, *Uracei*, *Bovini*, *Disjungendi*, *Telamonia*, *Duracini*, *Firmiores*, *Saturnini*, *Urbici*, *Biveli*, *Sciophylli*, *Malachii* and the "*C. puellaris* group". Among these, evolutionary relationships remained mostly opaque, as is often the case in phylogenetic analysis including only one molecular marker, and

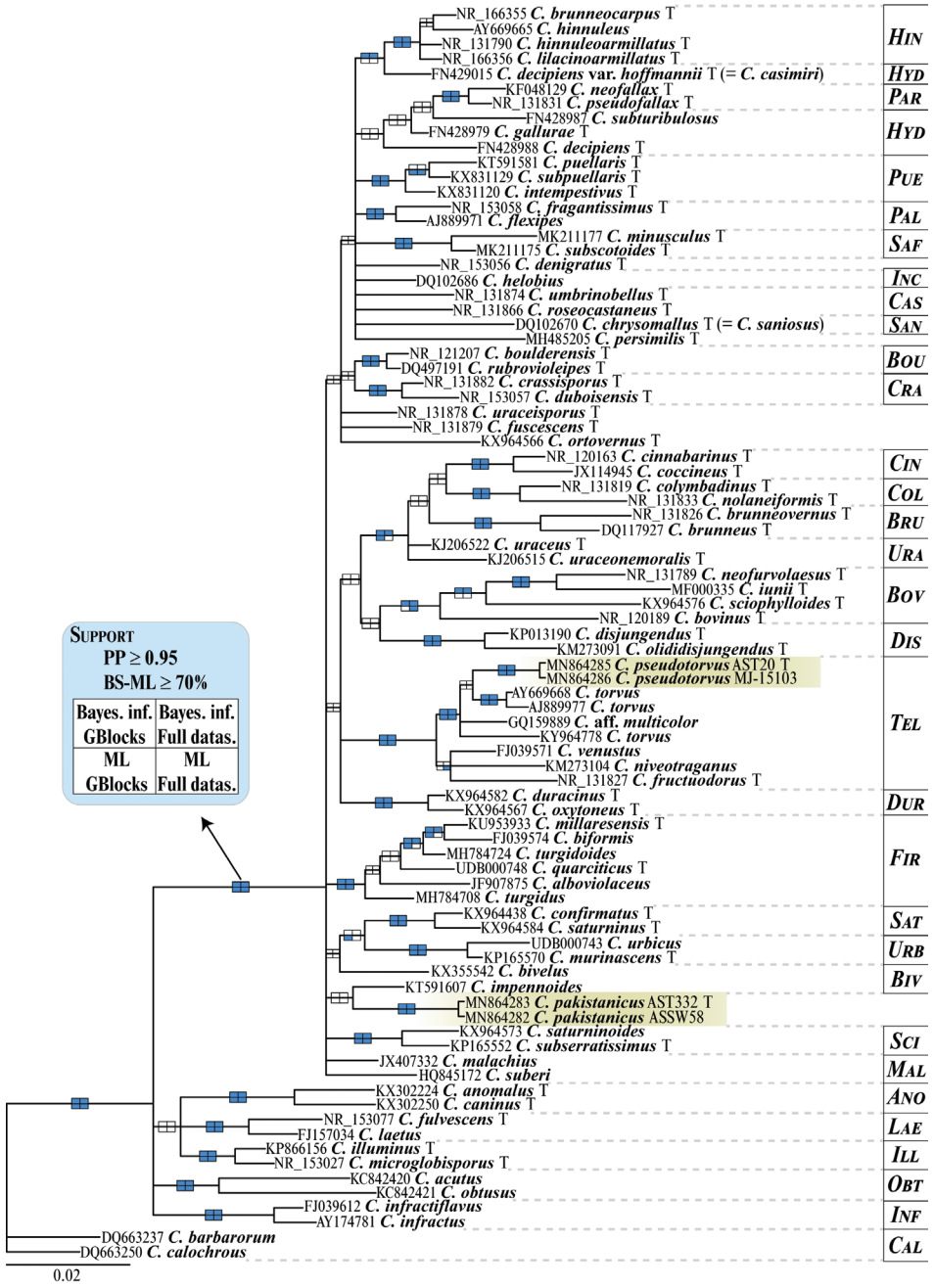

Figure 2. Phylogram depicting the evolutionary relationships of the new *Cortinarius* species and their relatives. The represented topology was obtained under a Bayesian framework using MrBayes and the GBlocks-trimmed dataset. For each terminal, the GenBank nrITS accession number, species name and an indication when they are type material (T) are given. Posterior Probabilities (PP, Bayesian analyses) and Bootstrap support (BS, RAxML analyses) are represented on branches leading to nodes. Blue-filled rectangles indicate nodal support for any of the four analyses performed in this study. *Cortinarius* sections in which the considered species belong are showed in the right column (see abbreviations list in section Material and methods).

Table 2. Polymorphism statistics for the two versions of the nrITS dataset. In both cases, the number of species included was 52.

	Full *nrITS* dataset	**GBlocks-trimmed *nrITS* dataset**
Number of aligned sites (bp)	701	494
Partition ranges (bp)	*ITS1+ITS2*: 1–543 *5.8S*: 544–701	*ITS1+ITS2*: 1–336 *5.8S*: 337–494
Singleton sites	105	60
Parsimony informative sites	235	156
Number of polymorphic sites	347	216
Conserved sites	328	278

very divergent sequences among taxa (e.g. Brandrud et al. 2018; Garrido-Benavent et al. 2019). The two Pakistani collections AST20 and MJ-15103 were genetically identical. The closest relatives were two GenBank samples (AY669668, AJ889977) labelled as *C. torvus* (Fr.) Fr. that were collected in Europe (Germany and Denmark) although this relationship was not statistically supported. The number of divergent nucleotides between AST20, MJ-15103 and these two *C. torvus* samples were nine plus 5 indels. A third *C. torvus* sample (KY964778) and a sample labelled as *C.* aff. *multicolor* (GQ159889), both collected in North America, showed a higher number of nucleotide differences. Along with *C. venustus* P. Karst., *C. fructuodorus* Niskanen, Liimat. & Ammirati and *C. niveotraganus* Kytöv., Niskanen & Liimat., these samples formed a well-supported clade corresponding with *C.* sect. *Telamonia*. Therefore, these results demonstrate that this clade of cortinarii is widely distributed across several landmasses in the Northern Hemisphere.

The two Pakistani collections AST332 and SW58 were genetically identical as well, but showed no close relationship to any other *Cortinarius* sample found in current databases. The closest relatives in the phylogenetic reconstructions obtained in this study were members in *C.* sections *Biveli*, *Sciophylly*, *Urbici*, *Saturnini*, and *Firmiores* although no statistical support was found for the inferred relationships (Fig. 2). Sequences in these clades diverged from AST332 and SW58 by at least 20 nucleotides and a number of indel positions. Given the evidence collected in these phylogenetic analyses and morphological comparisons with non-sequenced telamonioid taxa found in literature, the two lineages of *Cortinarius* sampled in the Pakistan Himalayas are considered to represent new species for science and hence are described below.

Taxonomy

***Cortinarius pakistanicus* A. Naseer & A. N. Khalid, sp. nov.**
MycoBank No: 833818
Figure 3

Diagnosis. *Cortinarius pakistanicus* is an oak-associated species that forms small basidiomata, with campanulate to obtusely umbonate, slightly hygrophanous, dark reddish to brownish pilei with margins first incurved and with whitish veil traces; lamellae are

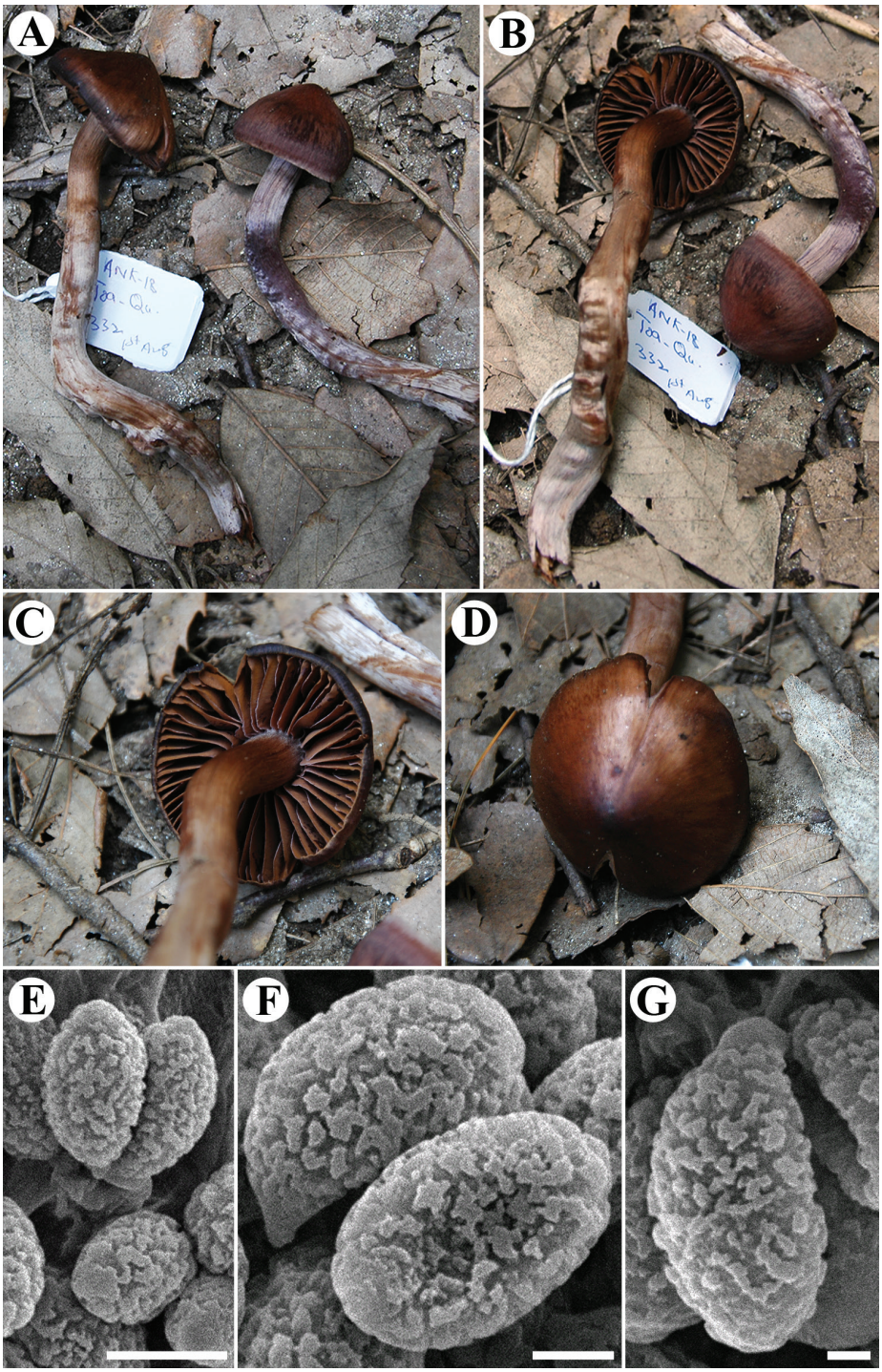

Figure 3. The new species *Cortinarius pakistanicus*, AST332 (LAH36366, holotype). **A–D** Basidiomata **E–G** Basidiospores observed with the SEM technique. Photo: A. Naseer (**A–D**).Scale bars: 5 µm (**E**), 2 µm (**F**), 1 µm (**G**).

fairly distant, first pale brown and later dark brown, with edges lighter and fimbriate; stipes slender, cylindrical to slightly fusiform, hollow, with a barely fibrillose surface, lilaceous towards the apex and whitish-tomentose towards the base, the remaining brown to dark brown with age and without annular traces; it produces amygdaliform, profusely and coarsely verrucose basidiospores measuring 8.6 × 5.5 µm, and shows cylindrical to narrowly utriform marginal cells.

Type. Pakistan, Khyber Pakhtunkhwa province, Swat, Toa, Alpuri forests, 34°51'51.2"N, 72°39'48.0"E, 2800 m a.s.l., on soil under *Quercus oblongata*, leg. Arooj Naseer & Abdul Nasir Khalid, 1 August 2018, AST332 (LAH36366).

Etymology. The epithet "*pakistanicus*" refers to Pakistan, where the species was collected.

Description. *Basidiomata* small sized. ***Pilei*** campanulate to umbonate, 2 to 3.3 cm in diameter, with margins first incurved, and then involute when mature, slightly hygrophanous; cuticle dark reddish to brownish (7.6 YR 3.6/5.8) with lighter brown tinges (1.6Y 6.6/3) towards the margin, fibrillose, and margins fimbriate. ***Lamellae*** medium spaced to fairly distant, emarginate, serrate to undulate, broad, first pale brown and later dark brown (4.7 YR 1.8/2.7). ***Stipe*** clavate to cylindrical, straight to curved, 5–9 cm long, 0.5–1 cm thick, slightly tapering towards the apex, which is about 0.3–0.7 cm, hollow; surface longitudinally striate, lilaceous towards the apex, and brown (1.8Y 6.6/2.7) to dark brown (7.6 YR 3.6/5.8) with age, base whitish (7.2GY 8.4/0.7). ***Context*** of pileus and stipe the same color as the cuticle. ***Smell*** distinct, earth-like and ***taste*** not recorded.

Basidiospores thin-walled, ellipsoid, [90/6/3] (7.8–) 7.9–9.4 (–9.8) × (4.7–) 4.9–6.3 (–6.2) µm in size, avl × avw = 8.62 × 5.56 µm, non-amyloid. ***Basidia*** clavate, 27.38 × 7.81 µm, 4-spored, clamped at the base, hyaline in 5% KOH. Scant cellular elements in lamellar pleura cylindrical to narrowly utriform 30–35 × 5–8 µm, clamped at the base. ***Pileipellis*** duplex; epicutis composed of individual hyphae 3–4 µm in diameter, clamped at septa, and with clavate to cylindrical terminal ends.

Ecology. Gregarious; growing in mountainous pure oak forests of *Quercus oblongata* at an altitude greater than 2000 m a.s.l. The soil pH ranged between 6–8.4.

Additional material examined. Pakistan, Khyber Pakhtunkhwa province, Swat, Toa, Alpuri forests, 34°51'51.2"N, 72°39'48.0"E, 2800 m a.s.l., on soil under *Quercus oblongata*, leg. Arooj Naseer & Abdul Nasir Khalid, 6 August 2016, AST132 (LAH36367); Swat, Matta, Shawar Valley, 34°58'59.8"N, 72°19'36.5"E, 2100 m a.s.l., solitary on ground under *Quercus oblongata*, 14 July 2015, Arooj Naseer & Abdul Nasir Khalid, ASSW58 (LAH35227).

Cortinarius pseudotorvus **A. Naseer, J. Khan & A. N. Khalid, sp. nov.**
MycoBank No: 833817
Figure 4

Diagnosis. *Cortinarius pseudotorvus* is an oak-associated species that differs from *C. torvus* by the smaller and slender basidiomata, and by the slightly more felty surface of pilei; it has broadly ellipsoid to sub-amygdaliform basidiospores (10.9 × 7.1 µm in average).

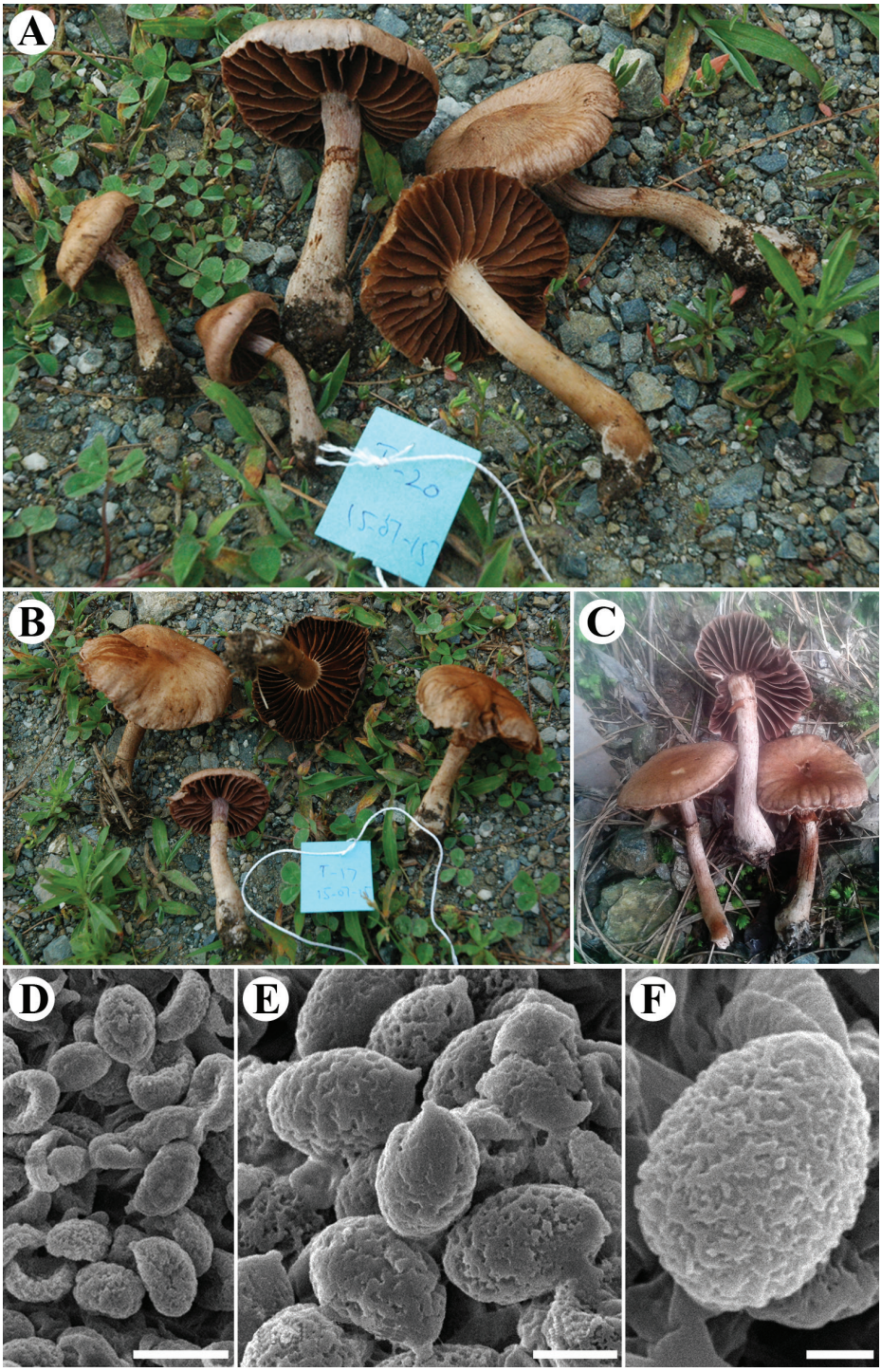

Figure 4. The new species *Cortinarius pseudotorvus*, AST20 (LAH35257, holotype). **A–C** Basidiomata **A, B** AST20 (holotype) **C** MJ-15103 (LAH36368) **D–F** Basidiospores of AST20 (holotype) observed with the SEM technique. Photo: A. Naseer (**A, B**), J. Khan (**C**). Scale bars: 10 µm (**D**), 5 µm (**E**), 2 µm (**F**).

Type. Pakistan, Khyber Pakhtunkhwa province, Swat, Toa, Alpuri forests, 34°51'51.2"N, 72°39'48.0"E, 2800 m a.s.l., on soil under *Quercus oblongata*, Arooj Naseer & Abdul Nasir Khalid, 15 July 2015, AST20 (holotype: LAH35257).

Etymology. The epithet "*pseudotorvus*" indicates its morphological resemblance and close phylogenetic position to *Cortinarius torvus.*

Description. *Basidiomata* small sized and slender. ***Pileus*** campanulate when young, becoming subumbonate and sometimes flat to plano-convex in mature stages, 15–30 mm in diameter, with margins deflexed, undulate, sometimes moderately to strongly striate; cuticle light brown (8.3YR 6.7/2.5), smooth to finely or even coarsely fibrillose (felty), with dark brown (4.7YR 2.6/5.5) fibrils radiating from the center. ***Lamellae*** adnate, broad, distant and relatively thick, with an evenly smooth margin, dark brown with age (4.1 YR 1.8/3.1); *lamellulae* present, regular. ***Stipe*** cylindrical, up to 56 mm long, 4–9 mm at apex and 1.1–1.3 cm thick at base, which is slightly bulbous, solid; surface brown (7.8YR 4.4/4.7) becoming whitish (3Y 7.2/1.5) in upper half and the base, finely fibrillose, the ¾ of stipe covered with universal veil remnants when young, and partial veil present, whitish in young specimens and brownish when mature, forming in general a persistent annulus. ***Context*** of pileus and stipe of the same color as the cuticle. ***Smell*** indistinct and ***taste*** not recorded.

Basidiospores broadly ellipsoid to sub-amygdaliform, [90/6/3] (9.2–) 9.9–11.6 (–12.5) × (6.1–) 6.7–7.7 (–8.1) µm, avl × avw= 10.9 × 7.1 µm, light yellowish brown to dark brown in 5% KOH, reddish brown in Melzer's reagent, densely ornamented. ***Basidia*** clavate, 25–35 × 7–8 µm, 4-spored, clamped at the base, hyaline in 5% KOH, darker when stained in Congo red. Scant cellular elements in lamellar pleura cylindrical to narrowly utriform, 30–35 × 5–8 µm, and clamped at the base. ***Pileipellis*** duplex; epicutis composed of individual hyphae 3–4 µm in diameter, clamped at septa and with clavate to cylindrical terminal elements.

Ecology. Gregarious; growing in either pure oak forests (*Quercus oblongata*) or mixed forests with oaks and pines (*Pinus wallichiana*) at an altitude greater than 2000 m a.s.l. The soil pH was around 8.4.

Additional material examined. Pakistan, Khyber Pakhtunkhwa, District Swat, Malam Jabba valley, 34°50'57.6"N 72°33'15.7"E, on ground in mixed forests of oak and pines, Junaid Khan, 10 August 2018, MJ-15103 (LAH36368); Toa, Alpuri forests, 34°51'51.2"N 72°39'48.0"E, 2800 m a.s.l., on soil under *Quercus oblongata*, Arooj Naseer & Abdul Nasir Khalid, 15 July 2015, AST17 (LAH35256).

Discussion

In the present study, *Cortinarius pakistanicus* and *C. pseudotorvus* are described as new to science based on an integrative taxonomic approach. The brownish color with occasional lilaceous tinges displayed by basidiomata of these species, which are both of rather small size, suggested their inclusion within the complicated *C.* subgenus *Telamonia* s. lat. Although recent phylogenetic surveys have helped to better circumscribe it, telamonioid cortinarii are still poorly known and constitute one of the most taxo-

nomically challenging groups within the genus (Niskanen et al. 2013b; Brandrud et al. 2015, Liimatainen et al. 2015; Garrido-Benavent et al. 2019).

Our phylogenetic inference showed that *C. pakistanicus* is not in any known sections. The closest taxa to this species were *C. bivelus* (Fr.) Fr. and *C. impennoides* Bidaud, Moënne-Locc. & Reumaux in *C.* sect. *Biveli*, which produce larger basidiomata that become pale ochraceous when dry, their spores are shorter and they usually grow in subalpine or boreal regions (Brandrud et al. 2015). In any case, phylogenetic relationships among these species were not supported by any of the four different phylogenetic analyses implemented in the present study. Furthermore, other species in *C.* sect. *Biveli* usually have abundant whitish veil remnants and lack any traces of lilaceous tinges in basidiomata which are otherwise shown by *C. pakistanicus* at least on the stipe surface. Young specimens of *C. pakistanicus* may resemble several species in *C.* sect. *Hydrocybe* due to overall morphology, pigmentation and the presence of traces of veil in the surface of pilei (Suárez-Santiago et al. 2009), although our reconstructed phylogenetic tree showed that members of this section are phylogenetically unrelated to the new species. The type species of this section is *C. decipiens* Fr., which usually shows grayish lamellae and stipe cortex when young, traits that were not noticed on our specimens. Besides, *C. decipiens* has had various interpretations in the literature and it probably forms a species complex in need of detailed morphological and phylogenetic study (e.g. Esteve-Raventós et al. 2014; Brandrud et al. 2015). *Cortinarius gallurae* D. Antonini, M. Antonini & Cons. can be larger in size, with flattened pilei ca. 60 mm showing abundant veil traces, and has shorter spores than the new species and associates with thermophilous *Quercus* spp. in the Mediterranean region (Consiglio et al. 2005). *Cortinarius casimiri* (Velen.) Huijsman displays a warmer pigmentation than *C. pakistanicus* and its spores are larger, ca. 12 × 7 µm. *Cortinarius subturibulosus* Kizlik & Trescol has a distinct smell like orange flower and associates with thermophilous *Quercus* spp. (Ortega and Mahiques 1995). On the other hand, mature and dry *C. pakistanicus* specimens with an umbonate pileus may bear a slight resemblance to species in *C.* sect. *Hinnulei*, but these normally lack lilaceous tinges, show more evident veil remnants on the stipes and their spores are more coarsely ornamented. *Cortinarius saniosus* (Fr.) Fr. in *C.* sect. *Saniosi* produces basidiomata with a similar size but these display warmer colors and a striking yellowish veil. Members in other sections like *C.* sect. *Brunnei* and *Uracei* commonly associate with coniferous trees and are characterized by producing slender or large basidiomata, with darker pigmentation than *C. pakistanicus*, sometimes necropigmented, and highly hygrophanous pilei.

On the other hand, the inferred nrITS phylogeny was unequivocal in including *C. pseudotorvus* within *C.* sect. *Telamonia*, together with several representatives of the section's type species *C. torvus*. Both species share the habitat under deciduous trees, the fibrillose to felty and matte pileus cuticle, which is slightly more felty in the new species, and the relatively thick and distant lamellae (Brandrud et al. 1992: B13). In *C. torvus* lamellae are initially grayish, a character that was not noticed in our collections, and then they turn brownish and even darker with age as happens in *C. pseudotorvus*. Spore dimensions are similar in both taxa, despite verrucae in the new

species' spores seeming to be less prominent. Nevertheless, the main distinguishing character between *C. torvus* and *C. pseudotorvus* is that the former produces stouter and larger basidiomata. This is particularly true for *C. torvus* stipes, whose morphology is also more variable, from cylindrical (as in *C. pseudotorvus*) to fusiform and especially clavate. A further character that should be tested with new collections of *C. pseudotorvus* is the smell, which in *C. torvus* is described as persistently sweet and pleasant. There are other species within *C.* sect. *Telamonia* displaying such smell and with abundant veil remnants: *C. venustus* P. Karst., whose basidiomata are more vividly pigmented, with lilaceous to orangish tinges (Brandrud et al. 1994: C50); and the very hygrophanous *C. agathosmus*, with dark pilei when moist and with longer stipes (Brandrud et al. 1990: A05). In contrast to *C. pseudotorvus*, both species fructify in acidophilous, subalpine coniferous forests. Finally, *C. tigrinipes* Bergeron displays conspicuous bands or girdles on the stipe, which is not thickened at the base (as in *C. pseudotorvus*), and the spores are smaller (7.5) 8–10.5 × 5–6.5 µm (Bidaud et al. 1999: pl. 250, f. 410).

With the new data provided in the present study, the number of *Cortinarius* species for all of Pakistan increases to eight. In neighboring countries, such as India, the number of recorded species for this genus seems to be lower than 20 as well (Verma et al. 2019). All in all, these data suggest that our knowledge of the diversity of *Cortinarius* in high mountain areas in Asia and the Himalayan forests of Pakistan is still in its infancy. By combining morphological and molecular analyses, which has proven to be a straightforward approach to disentangle the diversity of other fungal groups in the region (Saba et al. 2015; Khan et al. 2017; Sarwar et al. 2018; Naseer et al. 2019), we expect to further improve our knowledge of the mycobiota in this area of the planet.

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Recently, in a revision of the phylogeny and generic classification of fruticose Ramalinaceae (Spjut et al. 2020), two new combinations and a lectotypification were invalidly published by omission of the identifier. This note validates the names and the lectotypification.

Namibialina melanothrix **(Laurer) Spjut & Sérus., comb. nov.**
MycoBank No: 837518

= *Ramalina melanothrix* Laurer, Syn. Meth. Lich. 1(2): 290, tab. VIII, fig. 26, 1860, MB 403773
= *Trichoramalina melanothrix* (Laurer) Rundel & Bowler, Bryologist 77: 194, 1974, MB 343799
= *Niebla melanothrix* (Laurer) Kistenich, Timdal, Bendiksby, S. Ekman, Taxon 67: 893, 2018, MB 824407

Vermilacinia granulans **(Sipman) Spjut & Sérus., comb. nov.**
MycoBank No: 837536

= *Niebla granulans* Sipman, Bibliotheca Lichenologica 106: 300, 2011, MB 569717

Vermilacinia procera **(Rundel & Bowler) Spjut, Sida, Bot. Misc. 14: 168, 199.**
MycoBank No: 416069

= *Niebla procera* Rundel & Bowler, Phytologia 77: 34, 1994, MB 363506.

Type. USA, California, San Luis Obispo Co., found on rock outcrops in high marsh at Morro Bay State Park, Riefner 87–100, left hand specimen (ASU – lectotype, herewith selected).

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Reference

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