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Three new species of *Junghuhnia* (Polyporales, Basidiomycota) from China

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Abstract

In this study, taxonomic and phylogenetic analyses of *Junghuhnia* were performed. Three new species were characterised according to morphological characteristics and molecular phylogenetic analysis using ITS and nLSU sequences. They are *J. austrosinensis* **sp. nov.**, *J. nandinae* **sp. nov.** and *J. subcollabens* **sp. nov.** *Junghuhnia austrosinensis* is characterised by resupinate, thin basidiomata with white to buff-yellow hymenophore, small pores (9–11 per mm), clamped generative hyphae possessing hymenial cystidia, ellipsoid basidiospores (2.5–3 × 1.7–2 μm) and growth on fallen bamboo or angiosperm branch. *Junghuhnia nandinae* is characterised by resupinate basidiomata with pink to salmon pores and a distinct white margin, clamp generative hyphae, interwoven tramal hyphae, ellipsoid basidiospores measuring 2.6–3.2 × 1.8–2 μm and growth on *Nandina domestica*. *Junghuhnia subcollabens* is characterised by resupinate basidiomata with pale salmon to brownish vinaceous hymenophore, small pores (10–12 per mm), generative hyphae with simple septa and clamp connections, interwoven tramal hyphae, lunate basidiospores measuring 2.9–3.4 × 1.6–1.8 μm and thriving on rotten wood of angiosperms.

Keywords

Steccherinaceae, polypore, wood-inhabiting fungi

Introduction

Corda established the genus *Junghuhnia* Corda emend. Ryvarden on the type *Laschia crustacea* Jungh. *Junghuhnia* is characterised by a dimitic hyphal system with clamped generative hyphae and cyanophilous skeletal hyphae, smooth or encrusted skeletocystidia and subglobose or cylindrical basidiospores (Ryvarden and Gilbertson 1993; Núñez and Ryvarden 2001; Yuan and Dai 2008; Yuan et al. 2012). *Junghuhnia* is polyphyletic and has a complicated phylogenetic relationship with *Antrodiella* Ryvarden & I. Johans. and *Steccherinum* Gray (Miettinen et al. 2012; Westphalen et al. 2018; Yuan et al. 2019). These three genera share dimitic hyphal structure with cyanophilous skeletal hyphae and small, smooth, inamyloid, acyanophilous basidiospores (Dai et al. 2004). *Junghuhnia* and *Antrodiella* have poroid hymenophores, while *Steccherinum* have hydnaceous to odontioid hymenophores and *Junghuhnia* differs from *Antrodiella* by having skeletocystidia (Yuan et al. 2012). Previously, more than 30 species were accepted in the genus (Yuan et al. 2012, 2019; Ryvarden 2018, 2019) and 16 species were recorded in China (Yuan and Dai 2008; Miettinen et al. 2012; Yuan et al. 2012, 2019; Wu et al. 2020).

During recent studies on wood-inhabiting fungi in China, samples morphologically belonging to *Junghuhnia* were collected. After microscopic examination and phylogenetic analysis of ITS and nLSU sequences, we identified three new lineages in *Junghuhnia* and they are different from the existing fungal taxa. Therefore, three novel *Junghuhnia* species are characterised.

Materials and methods

Morphology

The samples were evaluated and submitted at the Institute of Microbiology herbaria of BJFC (Beijing Forestry University) and IFP (Institute of Applied Ecology, Chinese Academy of Sciences). The field notes formed the basis of macro-morphological details. Microscopic examination (magnifications ≤ 1000×; Nikon Eclipse 80i microscope) of the sections in phase contrast illumination was undertaken as per the protocols by Dai (2010) and Cui et al. (2019). A drawing tube was used to prepare the drawings. The sections were stained using Melzer's reagent and Cotton Blue to carry out measurements, assess microscopic features and prepare drawings. Sections from the tubes were used to assess the spores. To show the variation in spore sizes, from both ends of the range, 5% of measurements were excluded and are mentioned in parentheses. Abbreviations include KOH, potassium hydroxide (5%); IKI–, Melzer's reagent negative; IKI, Melzer's reagent; CB+, cyanophilous in Cotton blue; Q, the L/W ratio; W, mean spore width and L, mean spore length (both L and W: arithmetic average of all spores); n = number of spores in a specified number of specimens. The terms used for special colour are as per Rayner (1970) and Petersen (1996).

Molecular phylogenetic study

Genomic DNA was isolated from the dried specimens using the CTAB rapid plant genome extraction kit from Aidlab Biotechnologies (Beijing, China), as per provided guidelines with few alterations. The ITS5 and ITS4 primers were used (White et al. 1990) for the amplification of ITS sequences through PCR and the LR0R and LR7 primers were used for nLSU (Vilgalys and Hester 1990). The PCR process for ITS was: 95 °C for 3 min for initial denaturation; 35 cycles for 40 sec at 94 °C, 45 sec at 54 °C, 1 min at 72 °C, 72 °C for 10 min (final extension). The PCR process for nLSU was: 94 °C for 1 min for initial denaturation, 35 cycles for 1 min at 94 °C, 1 min at 50 °C, 1.5 min at 72 °C and 72 °C for 10 min (final extension). After purification of the products from PCR, they were sequenced at Beijing Genomics Institute (China) using the same set of primers.

Phylogenetic analyses were applied to the combined ITS+nLSU dataset. Sequences generated in this study were aligned with additional sequences downloaded from GenBank (Table 1) referred to Miettinen et al. (2012) and Yuan et al. (2019). The alignment of the dataset with *Exidiopsis calcea* (Pers.) K. Wells, as the outgroup following Yuan et al. (2016), was done applying MAFFT 7 with the option of G-INS-i (Katoh and Standley 2013) and the outcome was deposited at TreeBase (submission ID 25589). Construction of the ML (Maximum Likelihood) tree was done applying raxmlGUI 1.2 (Stamatakis 2006; Silvestro and Michalak 2012) with the model GTR + I + G and the option of auto FC (Pattengale 2010) in BS (bootstrap) replicates. The determination of the best-fit evolution model was done using MrModeltest2.3 (Posada and Crandall 1998; Nylander 2004) for the combined dataset for estimating BI (Bayesian Inference), which was estimated using MrBayes3.2.5 (Ronquist et al. 2012). From random starting trees, two runs of four Markov chains were run for the combined datasets for 1 million generations and, every 100 generations, trees were sampled. The initial generations (one-fourth) were rejected as burn-in. Then, for all remaining trees, the majority rule consensus tree was calculated. Branches were considered as significantly supported if they received bootstrap support (BS) for Bayesian posterior probabilities (BPP) and Maximum Likelihood ≥ 0.95 (BPP) and 75% (BS), respectively.

Results

Phylogenetic analysis

The dataset included 54 fungal collections representing 48 species. The best model for the dataset estimated and applied in the BI was GTR+I+G. BI resulted in a similar topology with an average standard deviation of split frequencies = 0.006554 to ML analysis, and thus only the BI tree was provided. Both BPPs (≥ 0.95) and BS values (≥ 50 %) are mentioned at the nodes (Fig. 1). The three new species formed three independent lineages with robust support (BS, 100%; BPP, 1.00).

Table 1. Information for the sequences used in this study.

Species	Specimen no.	Locality	GenBank accession no.	
			ITS	nLSU
Antrodiella americana	HHB 4100-Sp	United States	EU232186	EU232270
Antrodiella faginea	KH Larsson 11977	Sweden	JN710514	JN710514
Antrodiella foliaceodentata	LE 247382	Russia	JN710515	JN710515
Antrodiella onychoides	Miettinen 2312	Finland	JN710517	JN710517
Antrodiella pallescens	Miettinen X1080	Sweden	JN710518	JN710518
Antrodiella romellii	Miettinen 7429	Finland	JN710520	JN710520
Antrodiella semisupina	Labrecque & Labbé 372	Canada	JN710521	JN710521
Ceriporiopsis aneirina	MUAF 888	Czech Republic	EU340895	EU368503
Ceriporiopsis balaenae	Niemelä 2752	Canada	FJ496669	FJ496717
Exidiopsis calcea	MW 331	Canada	AF291280	AF291326
Frantisekia mentschulensis	BRNM 710170	Czech Republic	FJ496670	FJ496728
Frantisekia abieticola	Cui10525	China	KC485534	KC485552
Gloeoporus citrinoalbus	Yuan 9654	China	KU360396	KU360404
Gloeoporus hainanensis	Dai 15253	China	KU360402	KU360408
Hyphodermella poroides	Dai 12045	China	KX008367	KX011852
Irpex oreophilus	Niemelä 7691	Finland	JN710548	JN710548
Junghuhnia austrosinensis	Dai 17540	China	**MN871755**	**MN877768**
Junghuhnia austrosinensis	Dai 17679	China	**MN871756**	**MN877769**
Junghuhnia autumnale	Spirin 2957	Russia	JN710549	JN710549
Junghuhnia collabens	KH Larsson 11848	Sweden	JN710552	JN710552
Junghuhnia crustacea	Miettinen 13852	Indonesia	JN710553	JN710553
Junghuhnia crustacea	Miettinen 2954	Indonesia	JN710554	JN710554
Junghuhnia crustacea	Dai 19138	China	**MN871757**	**MN877770**
Junghuhnia fimbriatella	Miettinen 2091	Russia	JN710555	JN710555
Junghuhnia japonica	Nuñez 1065	Japan	JN710556	JN710556
Junghuhnia lacera	Niemelä 8246	Finland	JN710557	JN710557
Junghuhnia luteoalba	KH Larsson 13238b	Estonia	JN710558	JN710558
Junghuhnia micropora	Spirin 2652	Russia	JN710559	JN710559
Junghuhnia nandinae	Dai 21107	China	**MN833677**	**MN833679**
Junghuhnia nandinae	Dai 21108	China	**MN833678**	**MN833680**
Junghuhnia nitida	KH Larsson 11903	Sweden	JN710560	JN710560
Junghuhnia pseudozilingiana	M Kulju 1004	Finland	JN710561	JN710561
Junghuhnia rhinocephala	Miettinen X460	Australia	JN710562	JN710562
Junghuhnia sp.	Miettinen 10026	China	JN710551	JN710551
Junghuhnia subcollabens	Dai 19344	China	**MN871758**	**MN877771**
Junghuhnia subcollabens	Dai 19345	China	**MN871759**	**MN877772**
Mycoacia cf. columellifera	K Hjortstam 18286	Sweden	JN710572	JN710572
Nigroporus vinosus	B Seitzman 2008-100	USA	JN710575	JN710575
Skeletocutis amorpha	Miettinen 11038	Finland	FN907913	FN907913
Skeletocutis yunnanensis	Dai 15709	China	KU950434	KU950436
Skeletocutis odora	L 13763sp	Canada	KY948830	KY948893
Steccherinum aridum	Bureid 110510	Norway	JN710583	JN710583
Steccherinum bourdotii	Saarenoksa 10195	Finland	JN710584	JN710584
Steccherinum cf. *ciliolatum*	Ryvarden 47033	Estonia	JN710585	JN710585
Steccherinum fimbriatum	KH Larsson 11905	Sweden	JN710530	JN710530
Steccherinum litschaueri	Spirin 2189	Russia	JN710587	JN710587
Steccherinum murashkinskyi	Spirin 2367	Russia	JN710588	JN710588
Steccherinum ochraceum	KH Larsson 11902	Sweden	JN710590	JN710590
Steccherinum robustius	GB 1195	Sweden	JN710591	JN710591
Steccherinum straminellum	KH Larsson 13849	France	JN710597	JN710597
Steccherinum tenue	KH Larsson 12316	United States	JN710598	JN710598
Steccherinum tenuispinum	Miettinen 8065	Finland	JN710599	JN710599
Steccherinum tenuispinum	Spirin 2116	Russia	JN710600	JN710600
Trametopsis brasiliensis	Meijer et al. 3637	Brazil	JN710510	JN710510

New sequences are shown in bold.

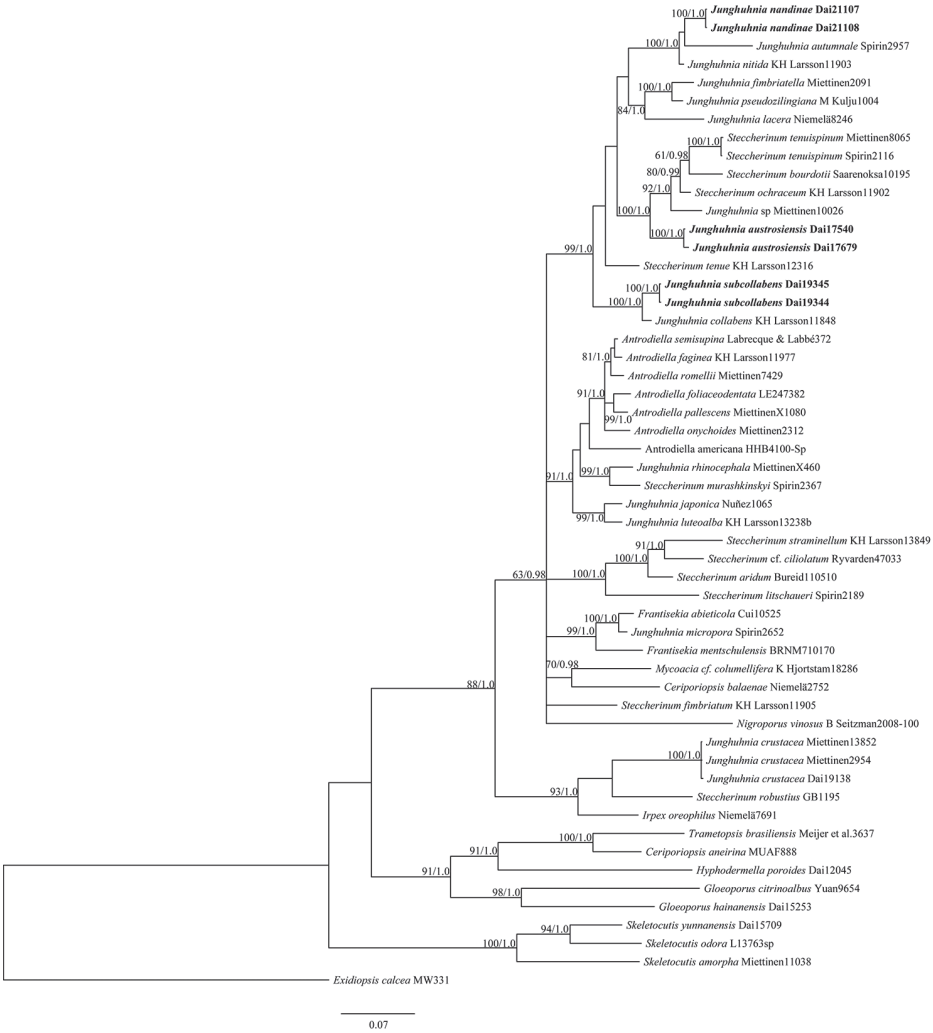

Figure 1. The phylogeny of three new species illustrated by Bayesian Inference tree and other taxa according to the combined ITS+nLSU dataset. Labelling of branches is done with BPP (Bayesian posterior probabilities) = 0.95 and Maximum Likelihood (ML) bootstrap greater than 50% (BS). New species are in bold.

Taxonomy

Junghuhnia austrosinensis **F. Wu, P. Du & X.M. Tian, sp. nov.**
MycoBank No: 834502
Figures 2, 3

Etymology. Refers to the species being collected in the south of China.

Basidiomata. Annual, resupinate, soft corky, without odour or taste when fresh, corky when dried, 7 cm length, 4 cm width and 0.4 mm thick at centre. Pore surface

Figure 2. Basidiomata of *Junghuhnia austrosinensis* (holotype Dai 17540). Scale bar: 10 mm.

white when fresh, cream to buff-yellow when dried; margin distinct, white and nearly 1 mm width; pores round to angular, 9–11 per mm; dissepiments thin, entire. Subiculum cream, paler than tubes, corky when dried, nearly 0.1 mm thick. Tubes concolorous with pore surface, corky, nearly 0.3 mm length.

Hyphal system. Hyphal system dimitic; generative hyphae with clamp connections, skeletal hyphae IKI–, CB+; tissue unchanged in KOH.

Subiculum. Dominated by skeletal hyphae; generative hyphae hyaline, thin- to fairly thick walled, rarely branched, 2–3.5 μm in diam.; skeletal hyphae thick-walled with a wide to narrow lumen, flexuous, unbranched, gelatinised, interwoven, 3–4 μm in diam.

Tubes. Trama dominated by skeletal hyphae; generative hyphae hyaline, thin- to fairly thick walled, rarely branched, 2–3 μm in diam.; skeletal hyphae thick-walled with a wide to narrow lumen, unbranched, more or less straight, subparallel amongst the tube, 2.5–3.8 μm in diam. Skeletocystidia clavate, thick-walled, originated from trama, apex covered with crystals, embedded amongst trama and dissepiments or projecting into hymenium, 30–40 × 6–8 μm; smaller skeletocystidia clavate, thick-walled, 14–18 × 5–6 μm. Basidia barrel-shaped, bearing four sterigmata and a basal clamp connection, 7–8 × 4–4.5 μm; basidioles in shape similar to basidia, but smaller.

Spores. Basidiospores smooth, ellipsoid, thin-walled, hyaline, IKI–, CB–, (2.4–)2.5–3(–3.1) × (1.6–)1.7–2(–2.1) μm, W = 1.83 μm, L = 2.83 μm, Q = 1.51 (n = 30/1).

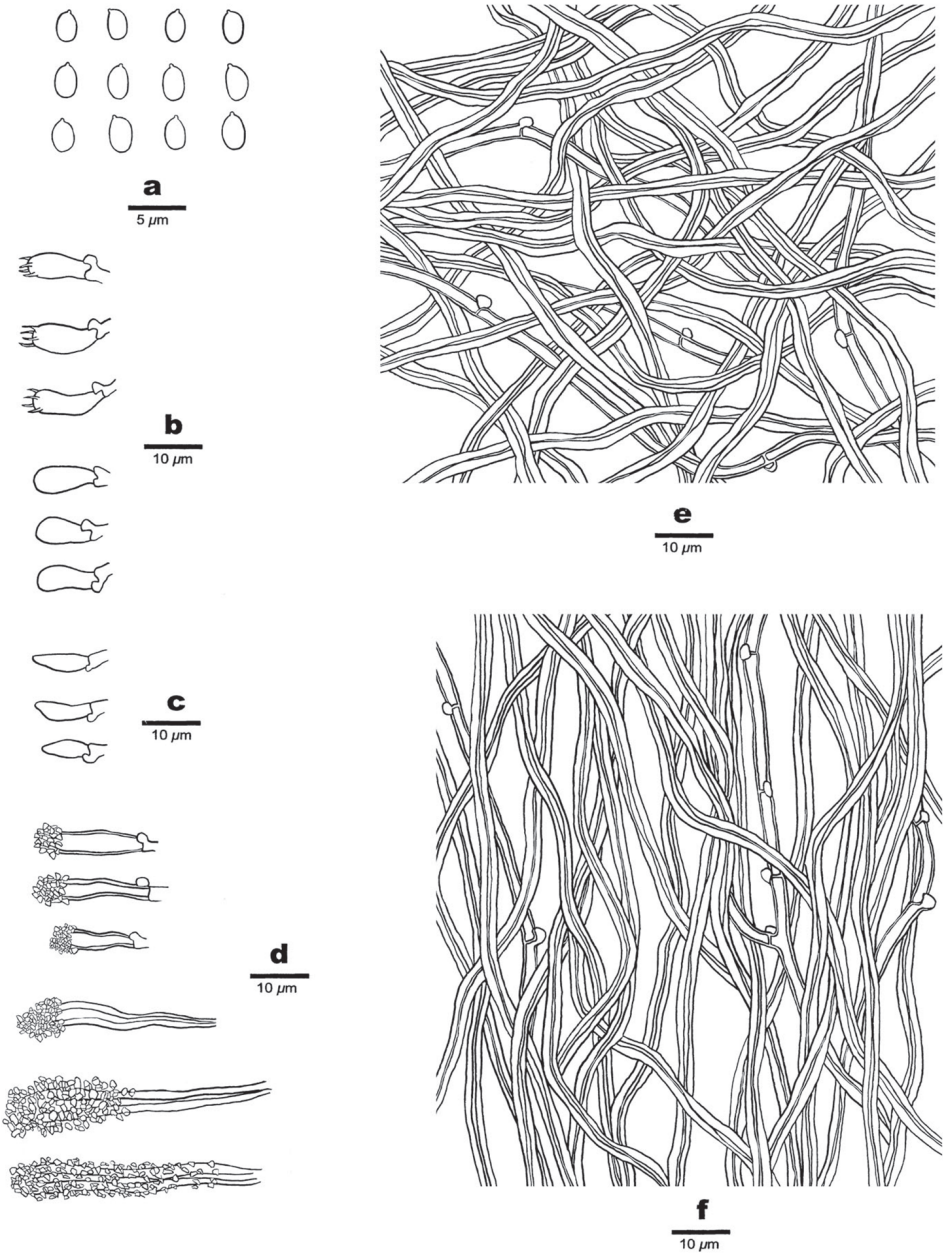

Figure 3. Microscopic assessment of *Junghuhnia austrosinensis* structures (drawn from Dai 17540) **a** basidiospores **b** basidia and basidioles **c** cystidioles **d** two kinds of skeletocystidia **e** hyphae from subiculum **f** hyphae from trama.

Materials examined. China, Yunnan Province, Jinghong, Virgin Forest Park, on fallen bamboo, 17.VI.2017 Dai 17540 (holotype, BJFC025072, isotype in IFP). Hainan Province, Wuzhishan County, Wuzhishan Forest Park, on fallen angiosperm branch, 9.IX.2019 Dai 17679 (paratype, BJFC025211).

Junghuhnia nandinae **F. Wu, P. Du & X.M. Tian, sp. nov.**
MycoBank No: 833784
Figures 4–5

Etymology. Refers to the species growing on *Nandina domestica.*

Basidiomata. Annual, resupinate, coriaceous, without odour or taste when fresh, hard corky when dried, 30 cm length, 3 cm width and 1 mm thick. Pore surface flesh-pink when fresh, pink to salmon when dried; margin distinct, white and nearly 3 mm width; pores round to angular, 6–8 per mm; dissepiments thin, entire. Subiculum buff, paler than tubes, corky when dried, nearly 0.5 mm thick. Tubes concolorous with pore surface, corky, nearly 0.5 mm length.

Hyphal system. Hyphal system dimitic; generative hyphae with clamp connections, skeletal hyphae IKI–, CB+; tissue unchanged in KOH.

Subiculum. Dominated by skeletal hyphae; generative hyphae hyaline, thin-walled, unbranched, 2–3 μm in diam.; skeletal hyphae thick-walled to subsolid, flexuous, unbranched, gelatinised, interwoven, 2.5–4 μm in diam.

Tubes. Trama dominated by skeletal hyphae; generative hyphae hyaline, thin-walled, rarely branched, 2–3 μm in diam.; skeletal hyphae thick-walled to subsolid, unbranched, flexuous, more or less gelatinised, interwoven, 2.5–3.5 μm in diam. Skeletocystidia clavate, thick-walled, originated from trama, apex covered with crystals,

Figure 4. Basidiomata of *Junghuhnia nandinae* (holotype Dai 21107). Scale bar: 8 cm.

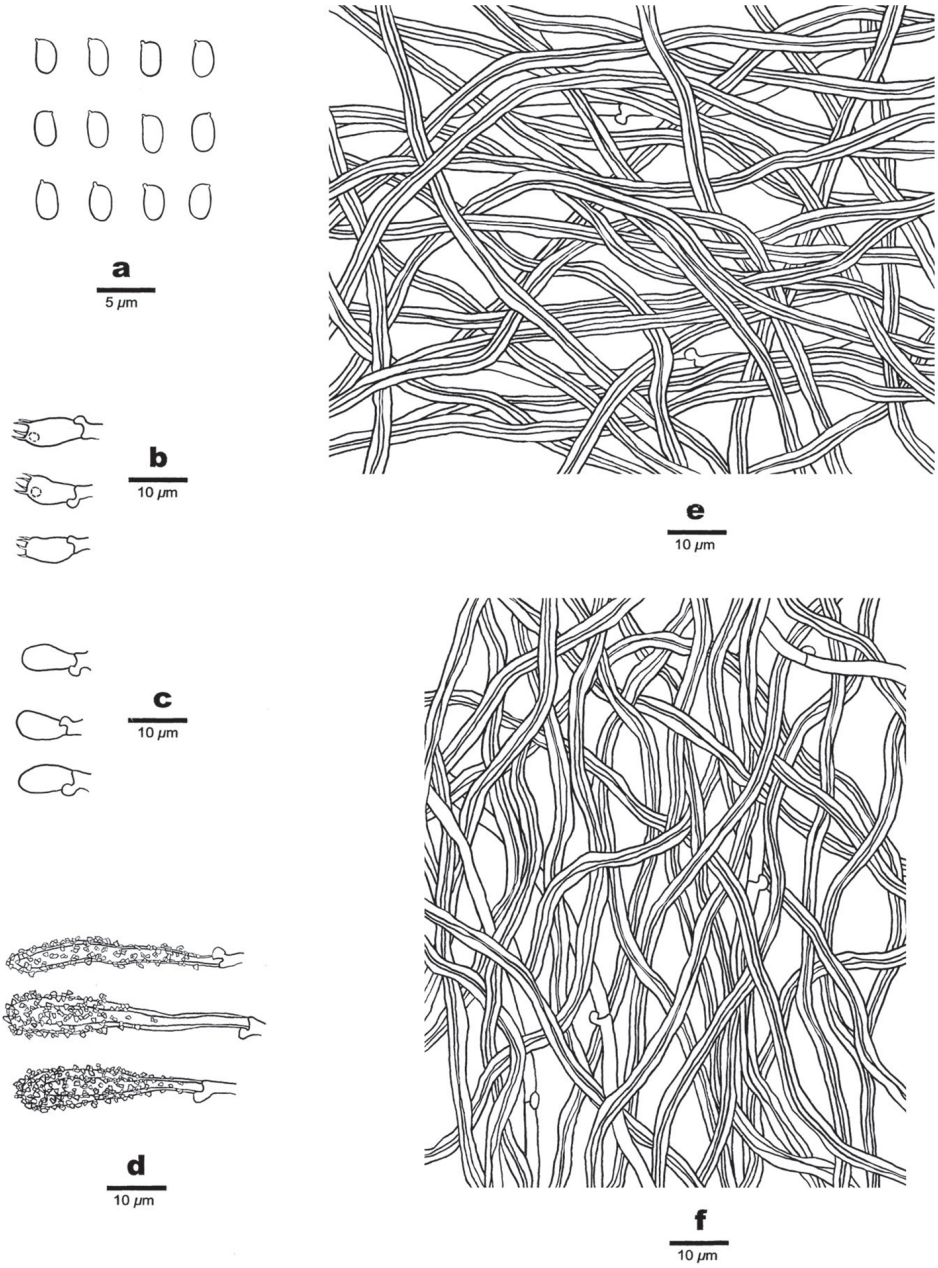

Figure 5. Microscopic assessment of *Junghuhnia nandinae* structures (holotype Dai 21107) **a** basidiospores **b** basidia **c** basidioles **d** skeletocystidia **e** hyphae from subiculum **f** hyphae from trama.

embedded amongst trama and dissepiments or projecting into hymenium, 22–45 × 6–8 µm. Basidia clavate, bearing four sterigmata and a basal clamp connection, 8–11 × 4–4.6 µm; basidioles in shape similar to basidia, but smaller.

Spores. Basidiospores ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, (2.5–)2.6–3.2(–3.3) × (1.6–)1.8–2(–2.1) µm, L = 2.97 µm, W = 1.92 µm, Q = 1.54 (n = 60/2).

Materials examined. China, Chongqing, Nanchuan County, Jinfoshan Forest Park, on dead tree of *Nandina domestica*, 1.XI.2019 Dai 21107 (holotype in BJFC, isotype in IFP) and Dai 21108 (paratype in BJFC).

Junghuhnia subcollabens **F. Wu, P. Du & X.M. Tian, sp. nov.**
MycoBank No: 834505
Figures 6–7

Etymology. Refers to the species similar to *J. collabens*.

Basidiomata. Annual, resupinate, coriaceous, without odour or taste when fresh, hard corky when dried, 8 cm length, 3 cm width and 1.5 mm thick. Pore surface pale salmon when fresh, brownish-vinaceous when dried; margin indistinct to almost lacking; pores round to angular, 10–12 per mm; dissepiments thin to fairly thick, entire. Subiculum vinaceous, darker than pores, hard corky when dried, nearly 0.3 mm thick. Tubes vinaceous, distinctly darker than pore surface, rigid, nearly 1.2 mm length.

Hyphal system. Hyphal system dimitic; generative hyphae with clamp connections and simple septa, skeletal hyphae IKI–, CB+; tissue unchanged in KOH.

Figure 6. Basidiomata of *Junghuhnia subcollabens* (holotype Dai 19345). Bar: 10 mm.

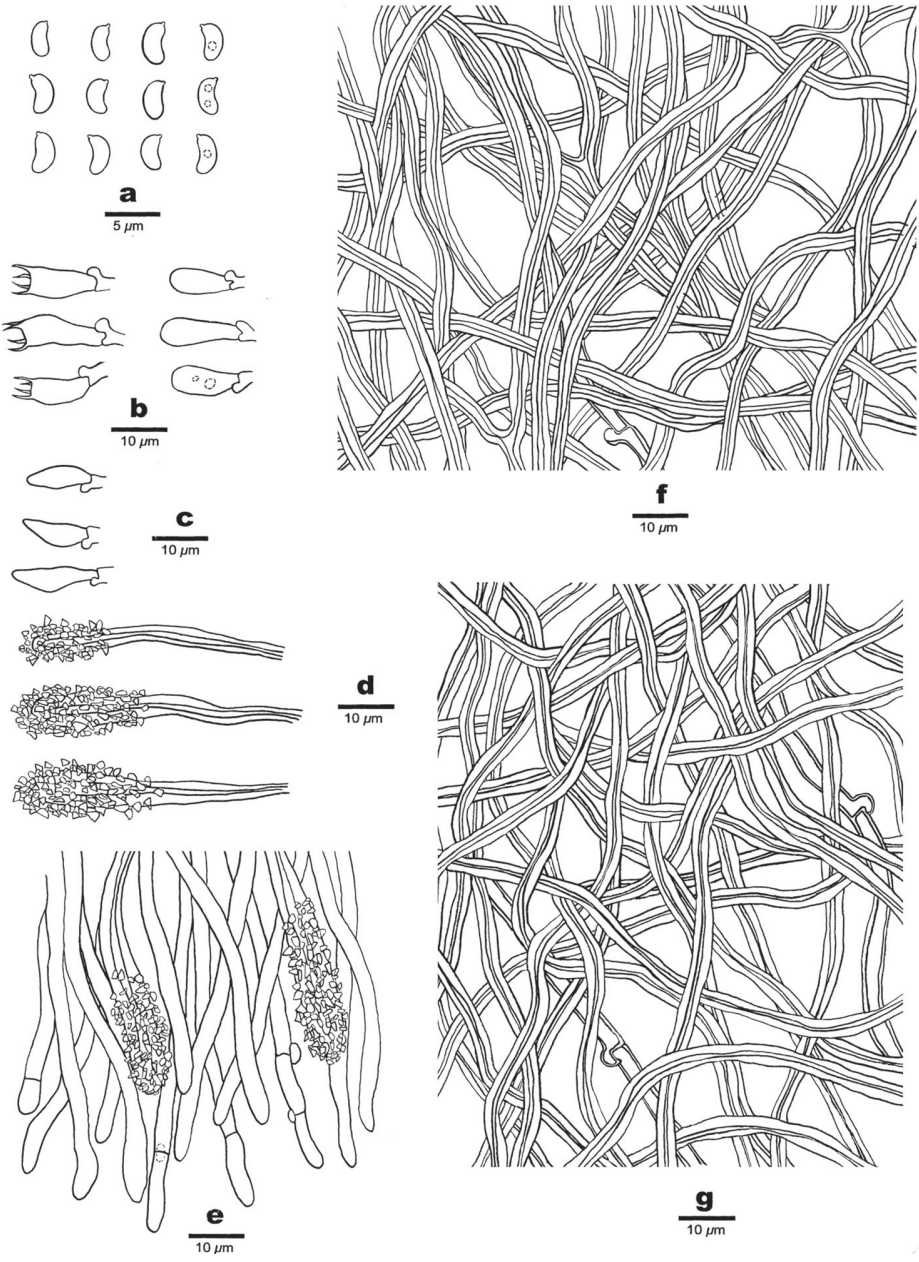

Figure 7. Microscopic structures of *Junghuhnia subcollabens* (holotype Dai 19345) **a** basidiospores **b** basidia and basidioles **c** cystidioles **d** skeletocystidia **e** hyphae and skeletocystidia at dissepiment **f** hyphae from subiculum **g** hyphae from trama.

Subiculum. Dominated by skeletal hyphae; generative hyphae hyaline, thin- to fairly thick-walled, frequently branched, 2.5–3 μm in diam.; skeletal hyphae thick-walled with a wide to narrow lumen, flexuous, occasionally branched, more or less gelatinised, interwoven, 2–4 μm in diam.

Tubes. Trama dominated by skeletal hyphae; generative hyphae hyaline, thin- to fairly thick-walled, frequently branched, with both simple septa and clamp connections, simple septa especially common at dissepiment edge, 2–3.2 μm in diam.; skeletal hyphae thick-walled with a wide to narrow lumen, rarely branched, flexuous, more or less gelatinised, interwoven, 2.5–3.5 μm in diam. Skeletocystidia clavate, thick-walled, originated from trama, apex covered with crystals, embedded amongst trama and dissepiments or projecting into hymenium, 35–50 × 6–9 μm. Fusoid cystidioles present, 8–14 × 3.5–2.5 μm; basidia clavate, bearing four sterigmata and a basal clamp connection, 10–12 × 4–5 μm; basidioles in shape similar to basidia, but smaller.

Spores. Basidiospores mostly lunate, hyaline, thin-walled, smooth, sometimes with one or two small guttules, IKI–, CB–, (2.8–)2.9–3.4(–3.5) × (1.5–)1.6–1.8(–1.9) μm, L = 3.12 μm, W = 1.67 μm, Q = 1.87 (n = 30/1).

Materials examined. China, Yunnan Province, Yongping County, Baitaishan Forest Park, on rotten angiosperm wood, 7.XI.2018 Dai 19345 (holotype, BJFC027813, isotype in IFP) and Dai 19344 (paratype, BJFC027812).

Discussion

Junghuhnia, *Antrodiella* and *Steccherinum* are phylogenetically related and they belong to the family of Steccherinaceae Parmasto in Polyporales (Yuan 2014; Miettinen and Ryvarden 2016; Justo et al. 2017). Our phylogeny also shows similar relationships amongst the species in the three genera (Fig. 1). Morphologically, *Junghuhnia* is distinguished from the other two genera by its poroid hymenophore and skeletocystidia. Based on phylogenetic analyses, several genera of wood-inhabiting fungi include species with lamellate, poroid and hydnaceous hymenophore at the same time (He and Dai 2012; Cui et al. 2019), but we still keep the traditional concepts for the three genera because their limited taxa were analysed according to morphology and phylogeny.

Junghuhnia austrosinensis is related to *Steccherinum bourdotii* Saliba & A. David, *S. ochraceum* (Pers. ex J.F. Gmel.) Gray, *S. tenuispinum* Spirin, Zmitr. & Malysheva and *Junghuhnia* sp. Miettinen 10026 (Fig. 1), but these three *Steccherinum* species have odontioid to hydnoid hymenophore and lack hymenial cystidia (Eriksson et al. 1984; Saliba et al. 1988; Spirin et al. 2007a). *Junghuhnia* sp. Miettinen 10026 was mentioned as *Junghuhnia* cf. *semipileata* (Miettinen et al. 2012), but we did not find the taxon of *Junghuhnia semipileata* (http://www.indexfungorum.org/names/Names.asp; http://www.mycobank.org/Biolomics.aspx?Table=Mycobank&Page=200&ViewMode=Basic). So far, *Skeletocutis semipileata* (Peck) Miettinen & A. Korhonen is the sole taxon with semipileata as epithet, it lacks skeletocystidia and has cylindrical basidiospores 2.8–3.1 × 0.4–0.6 μm (Korhonen et al. 2018).

Junghuhnia minuta I. Lindblad & Ryvarden, *J. neotropica* I. Lindblad & Ryvarden, and *J. austrosinensis* share similar pores (8–12 per mm). However, *J. minuta* has pileate

basidiomata that are roughly subglobose to ellipsoid basidiospores (2–2.5 × 2.5–3 μm, Lindblad and Ryvarden 1999) and *J. neotropica* has smooth cystidia (Lindblad and Ryvarden 1999). *Junghuhnia rhizomorpha* H. S. Yuan & Y. C. Dai resembles *J. austrosinensis* by having resupinate basidiomata and almost the same size pores (8–10 per mm), but the former has rhizomorphs, wider basidiospores and lacks hymenial cystidia (2.7–3 × 1.9–2.1 μm, Yuan and Dai 2008).

Phylogenetically, *Junghuhnia nandinae* is closely related to *J. nitida* (Pers.) Ryvarden and *J. autumnale* Spirin, Zmitr. & Malysheva (Fig. 1), but *J. nitida* has larger basidiospores (4–4.5 × 2.4–2.9 μm, Niemelä 2016) and *J. autumnale* differs from *J. nandinae* by pileate basidiomata, larger pores (5–7 per mm) and larger basidiospores (3.1–4.1 × 2.1–3 μm, Spirin et al. 2007b). Morphologically, *J. nandinae* resembles *J. collabens* (Fr.) Ryvarden in terms of salmon coloured pores, but the latter has cylindrical to suballantoid basidiospores (3.2–3.6 × 1.4–1.7 μm) and grows on gymnosperm wood in temperate and boreal forests (Niemelä 2016), while *J. nandinae* has ellipsoid basidiospores and is so far found in subtropical areas in China. The following names were treated as synonyms of *J. nitida*: *Poria fulgens* Rostk., *Polyporus euporus* P. Karst., *Physisporus vitellinulus* P. Karst. and *Chaetoporus tenuis* P. Karst. (http://www.indexfungorum.org/Names/Names.asp). All these taxa were originally described from Europe and they most probably represent a single species of *J. nitida*.

Junghuhnia subcollabens is phylogenetically closely related to *J. collabens* (Fig. 1) and both species share salmon pore surfaces, but *J. collabens* differs from *J. subcollabens* by larger pores (6–8 per mm), cylindrical to suballantoid basidiospores (3.2–3.6 × 1.4–1.7 μm), lacking simple septa on generative hyphae and growing on gymnosperm wood in temperate and boreal forests (Niemelä 2016), while *J. subcollabens* has smaller pores (10–12 per mm), lunate basidiospores (2.9–3.4 × 1.6–1.8 μm), simple septa on generative hyphae and growing on angiosperm wood in warm temperate forests of southwest China.

Three new species of *Junghuhnia* are described from Southern China in the present paper. Although extensive surveys on wood-decaying fungi in Southern China were carried out, and more than 3000 specimens were collected with 132 new polypore (Dai 2010; Zhao et al. 2015; Chen et al. 2020; Wu et al. 2020), it is expected that more new taxa will be found after additional investigations based on careful morphological examinations and phylogenetic analyses because of the rich woody plant species in subtropical and tropical China.

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References

Cui BK, Li HJ, Ji X, Zhou JL, Song J, Si J, Yang ZL, Dai YC (2019) Species diversity, taxonomy and phylogeny of Polyporaceae (Basidiomycota) in China. Fungal Diversity 97: 137–392. https://doi.org/10.1007/s13225-019-00427-4

Chen Q, Du P, Vlasák J, Wu F, Dai YC (2020) Global diversity and phylogeny of *Fuscoporia* (Hymenochaetales, Basidiomycota). Mycosphere 11: 1477–1513. https://doi.org/10.5943/mycosphere/11/1/10

Dai YC (2010) *Hymenochaetaceae* (Basidiomycota) in China. Fungal Diversity 45: 131–343. https://doi.org/10.1007/s13225-010-0066-9

Eriksson J, Hjortstam K, Ryvarden L (1984) The Corticiaceae of North Europe. Vol. 7, 1282–1449.

He SH, Dai YC (2012) Taxonomy and phylogeny of *Hymenochaete* and allied genera of Hymenochaetaceae (Basidiomycota) in China. Fungal Diversity 56: 77–93. https://doi.org/10.1007/s13225-012-0174-9

Justo A, Miettinen O, Floudas D, Ortiz-Santana B, Sjökvist E, Lindner DL, Nakasone K, Niemelä T, Larsson KH, Ryvarden L, Hibbett DS (2017) A revised family-level classification of the Polyporales (Basidiomycota). Fungal Biology 121: 798–824. https://doi.org/10.1016/j.funbio.2017.05.010

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. https://doi.org/10.1093/molbev/mst010

Korhonen A, Seelan JSS, Miettinen O (2018) Cryptic species diversity in polypores: the *Skeletocutis nivea* species complex. Mycokeys 36: 45–82. https://doi.org/10.3897/mycokeys.36.27002

Lindblad I, Ryvarden L (1999) Studies in neotropical polypores. 3. New and interesting Basidiomycetes (Poriales) from Costa Rica. Mycotaxon 71: 335–359.

Miettinen O, Larsson E, Sjökvist E, Larsson KH (2012) Comprehensive taxon sampling reveals unaccounted diversity and morphological plasticity in a group of dimitic polypores (Polyporales, Basidiomycota). Cladistics 28: 251–270. https://doi.org/10.1111/j.1096-0031.2011.00380.x

Miettinen O, Ryvarden L (2016) Polypore genera *Antella*, *Austeria*, *Butyrea*, *Citripora*, *Metuloidea* and *Trulla* (Steccherinaceae, Polyporales). Annales Botanici Fennici 53: 157–172. https://doi.org/10.5735/085.053.0403

Niemelä T (2016) The polypores of Finland. Norrlinia 31: 1–430.

Núñez M, Ryvarden L (2001) East Asian polypores 2. Polyporaceae *s. lato*. Synopsis Fungorum 14: 170–522.

Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Uppsala University, Uppsala.

Pattengale ND, Alipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A (2010) How many bootstrap replicates are necessary? Journal of Computational Biology 17: 337–354. https://doi.org/10.1089/cmb.2009.0179

Petersen JH (1996) Farvekort. The Danish Mycological Society's colour-chart. Foreningen til Svampekundskabens Fremme, Greve. 6 p.

Posada D, Crandall KA (1998) Modeltest: Testing the model of DNA substitution. Bioinformatics 14: 817–818. https://doi.org/10.1093/bioinformatics/14.9.817

Rayner RW (1970) A Mycological Colour Chart. Commonwealth Mycol. Inst., Kew, 34 pp. [9 pls]

Ronquist F, Teslenko M, van der Mark P, Avres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes3.2: efficient Bayesian phylogenetic inference and model choice, across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Ryvarden L (2018) Studies in African Aphyllophorales 25. New poroid species from East and Central Africa. Synopsis Fungorum 38: 25–32.

Ryvarden L (2019) Studies in African Aphyllophorales 32. Some new African polypores. Synopsis Fungorum 39: 59–71.

Ryvarden L, Gilbertson RL (1993) European polypores 1. Synopsis Fungorum 6: 1–387.

Saliba J, David A (1988) Apports des caractères culturaux et des confrontations dans l'étude des représentants européens du genre *Steccherinum* (Basidiomycètes, Aphyllophorales). Cryptogamie Mycologie 9(2): 93–110.

Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. Organisms Diversity and Evolution 12: 335–337. https://doi.org/10.1007/s13127-011-0056-0

Spirin WA, Zmitrovitch I, Malysheva V (2007a) *Steccherinum tenuispinum* (Polyporales, Basidiomycota), a new species from Russia, and notes on three other species. Annales Botanici Fennici. 44: 298–302.

Spirin WA, Zmitrovitch I, Malysheva V (2007b) New species in *Junghuhnia* (Polyporales, Basidiomycota). Annales Botanici Fennici 44: 303–308.

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238–4246. https://doi.org/10.1128/JB.172.8.4238-4246.1990

Westphalen MC, Rajchenberg M, Tomšovský M, Gugliotta AM (2018) A re-evaluation of Neotropical *Junghuhnia* s.lat. (Polyporales, Basidiomycota) based on morphological and multigene analyses. Persoonia 41: 130–141. https://doi.org/10.3767/persoonia.2018.41.07

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gefand DH, Sninsky JJ, White JT (Eds) PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wu F, Yuan HS, Zhou LW, Yuan Y, Cui BK, Dai YC (2020) Polypore diversity in South China. Mycosystema 39: 653–681.

Yuan HS (2014) Molecular phylogenetic evaluation of *Antrodiella* and morphologically allied genera in China. Mycological Progress 13: 353–364. https://doi.org/10.1007/s11557-013-0921-7

Yuan HS, Dai YC (2008) Two new species of *Junghuhnia* (Basidiomycota, Polyporales), and a key to the species of China. Nordic Journal of Botany 26: 96–100. https://doi.org/10.1111/j.0107-055X.2008.00169.x

Yuan HS, Dai YC, Wu SH (2012) Two new species of *Junghuhnia* (Polyporales) from Taiwan and a key to all species known worldwide of the genus. Sydowia 64: 137–145.

Yuan HS, Lu X, Qin WM (2019) Molecular and morphological analyses separate *Junghuhnia pseudocrustacea* sp. nov. (Basidiomycota) from *Junghuhnia crustacea* complex. Nova Hedwigia 108: 255–264. https://doi.org/10.1127/nova_hedwigia/2018/0497

Yuan Y, Ji XH, Wu F, He SH, Chen JJ (2016) Two new *Gloeoporus* (Polyporales, Basidiomycota) from tropical China. Nova Hedwigia 103: 169–183. https://doi.org/10.1127/nova_hedwigia/2016/0344

Zhao CL, Cui BK, Song J, Dai YC (2015) Fragiliporiaceae, a new family of Polyporales (Basidiomycota). Fungal Diversity 70: 115–126. https://doi.org/10.1007/s13225-014-0299-0

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Phylogeny- and morphology-based recognition of new species in the spider-parasitic genus *Gibellula* (Hypocreales, Cordycipitaceae) from Thailand

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Abstract

Thailand is known to be a part of what is called the Indo-Burma biodiversity hotspot, hosting a vast array of organisms across its diverse ecosystems. This is reflected by the increasing number of new species described over time, especially fungi. However, a very few fungal species from the specialized spider-parasitic genus *Gibellula* have ever been reported from this region. A survey of invertebrate-pathogenic fungi in Thailand over several decades has led to the discovery of a number of fungal specimens with affinities to this genus. Integration of morphological traits into multi-locus phylogenetic analysis uncovered four new species: *G. cebrennini*, *G. fusiformispora*, *G. pigmentosinum*, and *G. scorpioides*. All these appear to be exclusively linked with torrubiella-like sexual morphs with the presence of granulomanus-like asexual morph in *G. pigmentosinum* and *G. cebrennini*. A remarkably high host specificity of these new species towards their spider hosts was revealed, and for the first time, evidence is presented for manipulation of host behavior in *G. scorpioides*.

Keywords

Cordycipitaceae, *Gibellula*, spider specialist fungus, taxonomy

Introduction

To arthropodologists or even arachnologists, it is surprising that fungal pathogens of spiders seem to be generally neglected when the host can be completely overgrown by the pathogens to be unrecognizable as a spider. Nonetheless, this group of fungi has been known and studied for more than two centuries (Evans 2013). Recently, over 80 fungal species, mostly distributed in Cordycipitaceae, have been reported as spider pathogens (Shrestha et al. 2019). Among them, only *Gibellula* and *Hevansia* are obligate parasites of spiders whereas others appear to be natural enemies of different insects and do not show apparent host specificity.

Gibellula is well-known to be a specialized spider-parasitic genus widely distributed worldwide, mostly found in tropical regions (Shrestha et al. 2019). Originally, the type species, *G. pulchra* (Sacc.) Cavara was known as *Corethropsis pulchra* Sacc. collected from Italy, recognized by producing primarily synnematous, aspergillus-like conidiophores with terminal vesicles, each gives rise to phialides produced on metulae (Saccardo 1877; Shrestha et al. 2019). After establishing a genus *Gibellula* Cavara in honor of Prof. Giuseppe Gibelli by Cavara (1894), a number of species in this genus were recorded across the world (Shrestha et al. 2019). Currently, nearly 40 species have been described and listed in the global fungal databases Index Fungorum (www.indexfungorum.org) and MycoBank (www.mycobank.org). According to the review of Shrestha et al. (2019), many of them including *G. arachnophila* (Ditmar) Vuill., *G. arachnophila* f. *arachnophila* (Ditmar) Vuill., *G. arachnophila* f. *macropus* Vuill., *G. aranearum* P. Syd., *G. globosa* Kobayasi & Shimizu, *G. globosostipitata* Kobayasi & Shimizu, *G. haygarthii* Van der Byl, *G. suffulta* Speare and *G. tropicalis* Sawada were synonymized with *G. pulchra* whereas *G. arachnophila* f. *leiopus* Vuill. ex. Maubl., *G. araneae* Sawada and *G. perexigua* (Kobayasi) Koval were synonymized with *G. leiopus* (Vuill. ex Maubl.) Mains. In addition to these species, the identities of several other species reported in this genus still remain doubtful. Petch (1932) expressed uncertainty about the identities of *G. aspergilliformis* (Rostr.) Vuill. and *G. phialobosia* Penz. & Sacc. by pointing out that the narrow metulae and spherical conidia in chains present in *G. aspergilliformis* and the flask-shaped phialides in the latter species were not common features of *Gibellula*. Moreover, description of *G. eximia* Höhn. did not point to the genus. Since *Gibellula* is well-known as an obligate parasite of spiders, Mains (1950) reported that the assignment of *G. elegans* Henn. to this genus might be erroneous, as this species is found occurring on locusts. According to Mains (1950), the description of *G. capillaris* Morgan did not fit the concept of *Gibellula* and re-examination of the type specimen is unfortunately infeasible since it is no longer in a good condition. Tzean et al. (1997) doubted the identity of *G. araneicola* Sawada that produces an isarioid morph instead of *Gibellula*. In the case of *G. petchii* Humber & Rombach, it is still unclear whether the species name should be retained or abandoned. *Gibellula petchii* Humber & Rombach was proposed to accommodate *Cylindrophora aranearum* Petch, which was originally described as the conidial state of *Torrubiella albolanata* Petch and later elevated to generic rank as a new genus, *Granulomanus* de Hoog &

Samson (de Hoog 1978; Humber and Rombach 1987; Petch 1944). From the point of view of Humber and Rombach (1987), *Granulomanus* should be synonymized with *Gibellula* as it almost never occurs in the absence of *Gibellula* and/or its torrubiella-like sexual morph. *Cylindrophora aranearum* (≡*Granulomanus aranearum* (Petch) de Hoog & Samson) was henceforth synonymized with *G. petchii*. On the other hand, Samson and Evans (1992) argued that *Granulomanus* naturally occurs independently on spider hosts either with or without *Gibellula*. Thus, the genus should be retained as an independent asexually typified genus resulting in rejection of *G. petchii.* According to a recent taxonomic revision of the Cordycipitaceae, which was largely based on molecular data, several generic names including *Granulomanus* were suppressed (Kepler et al. 2017). Nevertheless, the taxonomic dilemma of *G. petchii* cannot yet be resolved owing to the lack of its sequence data. Based on these facts, only 17 species have been accepted in *Gibellula* (Shrestha et al. 2019).

Thailand is one of the most biodiverse countries in Southeast Asia and the BIOTEC culture collection has more than 700 *Gibellula* strains. Despite this number, only very few *Gibellula* species with distinct features could be recognized morphologically (Luangsa-ard et al. 2008, 2010). *Gibellula gamsii* is the most recently described species reported from Thailand (Kuephadungphan et al. 2019).

Our continuous survey of invertebrate-pathogenic fungi in Thailand for over two decades has led to the BIOTEC Bangkok Herbarium (BBH) and the BIOTEC Culture Collection (BCC) owning a very large herbaria, and culture collections, which greatly facilitates the exploration of existing species including *Gibellula*. Here, phylogeny within *Gibellula* species from the ribosomal internal transcribed spacer (ITS) regions analyzed prior to this study enabled recognition of four distinct clades. The morphological and multi-gene phylogenetic data confirm their identities as well as taxonomic placements. Herein, new species are described that are illustrated morphologically and phylogenetically and compared with other species in the same genus.

Materials and methods

Collection of fungal materials and isolation of pure cultures

Spiders parasitized by *Gibellula* spp. firmly attached on the underside of living leaves were collected from various locations throughout Thailand, mostly in the Northeastern region. The leaf bearing the parasitized spider was carefully detached from the tree, placed in a plastic box and transported to the laboratory for immediate isolation of a pure culture according to the protocols described by Kuephadungphan et al. (2014) and Mongkolsamrit et al. (2018). Briefly, the conidia located on the synnemata were gently swiped with small agar plugs of potato dextrose agar (PDA) which were then placed on a PDA plate. The conidia were allowed to germinate at 25 °C for a few days. Thereafter, each agar plug with actively growing mycelia was transferred to a fresh PDA plate where the fungus could readily grow for another 6–8 weeks. For specimens bear-

ing sexual morphs, pure cultures were isolated by enabling ascospores from the perithecia to be discharged onto PDA plates and allowing them to grow at 25 °C for a certain amount of time depending on the growth rate of each individual strain. All cultures were required to be deposited in the BCC, Thailand while the fungal specimens were dried in an electric food dryer (50–55 °C) before being stored at the BBH, Thailand.

Morphological characterization

Microscopic characteristics were studied based on observation of synnemata and perithecia. Each of them was detached from the stroma and mounted on a microscope slide containing a drop of lactophenol cotton blue solution. Shapes and sizes of individual character were determined and measured according to Mongkolsamrit et al. (2018).

Identification of spider hosts

The mummified spiders were identified based on morphological characteristics. To better understand the host-pathogen relationship, posture of spider at attachment on leaf surface (touching or lifting), position of spider on the leaf (under or upper side), and leaf type (monocots or dicots) were herein recorded.

Molecular phylogenetic analyses

DNA extraction, PCR amplification of five DNA regions as well as purification of PCR products were conducted according to the protocols previously described by Kuephadungphan et al. (2019). The PCR amplicons were obtained using primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) for nuc rDNA ITS1-5.8S-ITS2 (ITS barcode), LR5 or LR7 (Vilgalys and Hester 1990) and LROR (Bunyard et al. 1994) for the partial region of nuc 28SrDNA (LSU), 983F and 2218R (Rehner and Buckley 2005) for translation elongation factor 1-alpha (*TEF1*), RPB1-Ac and RPB1-Cr (Murata et al. 2014) for the largest subunit of RNA polymerase II (*RPB1*) and fRPB2-5F and fRPB2-7cR (Liu et al. 1999) for the second largest subunit of RNA polymerase II (*RPB2*).

DNA sequences were checked manually for ambiguous base calls and all sequences were assembled using BioEdit v.7.2.5 (Hall 1999; Hall et al. 2011). Sequence alignment was conducted using MAFFT 7.017 with G-INS as the algorithm and default settings used for gap opening and gap extension penalties (Katoh and Toh 2008). Manual adjustments were subsequently made in BioEdit. Concatenation of multiple loci was performed in GENEIOUS® 7.1.19 (http://www.geneious.com, Kearse et al. 2012).

Phylogenetic relationships were inferred using maximum likelihood (ML) with GTRCAT as the substitution model in RAXML 7.2.8 (Stamatakis 2006) and the rapid

bootstrap analysis algorithm. Relative support for the branches was obtained from bootstrap analysis with 1,000 replicates. Bayesian analysis was performed according to Mongkolsamrit et al. (2019) using MRBAYES v.3.2.7 (Ronquist and Huelsenbeck 2003) on XSEDE via the online CIPRES Science gateway using SYM+G selected by MRMODELTEST 2.2 (Nylander 2004) as the best nucleotide substitution model. Posterior probabilities were performed by Markov Chain Monte Carlo Sampling (MCMC) in which four chains were run for 5,000,000 generations with a tree sampling frequency of 100 and a burn-in of 10% of the total run.

Results

Molecular phylogeny

The combined data set of 43 taxa (Table 1) comprised 4,325 characters including 680, 859, 917, 1,109 and 917 characters derived from ITS, LSU, *TEF1*, *RPB1* and *RPB2*, respectively with *Engyodontium aranearum* as the outgroup. The multigene tree (Fig. 1) comprised seven different genera belonging to the family Cordycipitaceae including *Akanthomyces*, *Beauveria*, *Blackwellomyces*, *Cordyceps*, *Engyodontium*, *Gibellula* and *Hevansia*. The analyses showed the genera segregated corresponding to the recent phylogenetic classification of the Cordycipitaceae (Kepler et al. 2017; Kuephadungphan et al. 2019). The taxa of the new species were distributed in the *Gibellula* clade which was strongly supported (100%) and inferred as a monophyletic group. *Gibellula pigmentosinum* was found to be very close to *Gibellula* cf. *alba* by forming a strong supported clade together. *Gibellula fusiformispora* was inferred as the phylogenetic sister of *G. cebrennini*, whereas *G. scorpioiodes* formed a distinct well-supported sister clade to these species.

Taxonomy

Gibellula cebrennini **Tasanathai, Kuephadungphan & Luangsa-ard, sp. nov.**
MycoBank No: 835113
Figure 2

Typification. Thailand, Nakhon Ratchasima, Khao Yai National Park, Mo Sing To Nature Trail; 14°711'N, 101°421'E; on *Cebrenninus* cf. *magnus* (Thomisidae, Araneae) attached to the underside of unidentified dicot leaf; 20 June 2012; K. Tasanathai, S. Mongkolsamrit, A. Khonsanit, W. Noisripoom, P. Srikitikulchai, K. Sansatchanon, R. Somnuk (Holotype no. BBH 35749, ex-type culture no. BCC 53604, isolated from ascospores and BCC 53605, isolated from conidia). GenBank (BCC 53605): ITS = MT477069, LSU = MT477062, *TEF1* = MT503328, *RPB1* = MT503321, *RPB2* = MT503336.

Etymology. Refers to its spider host.

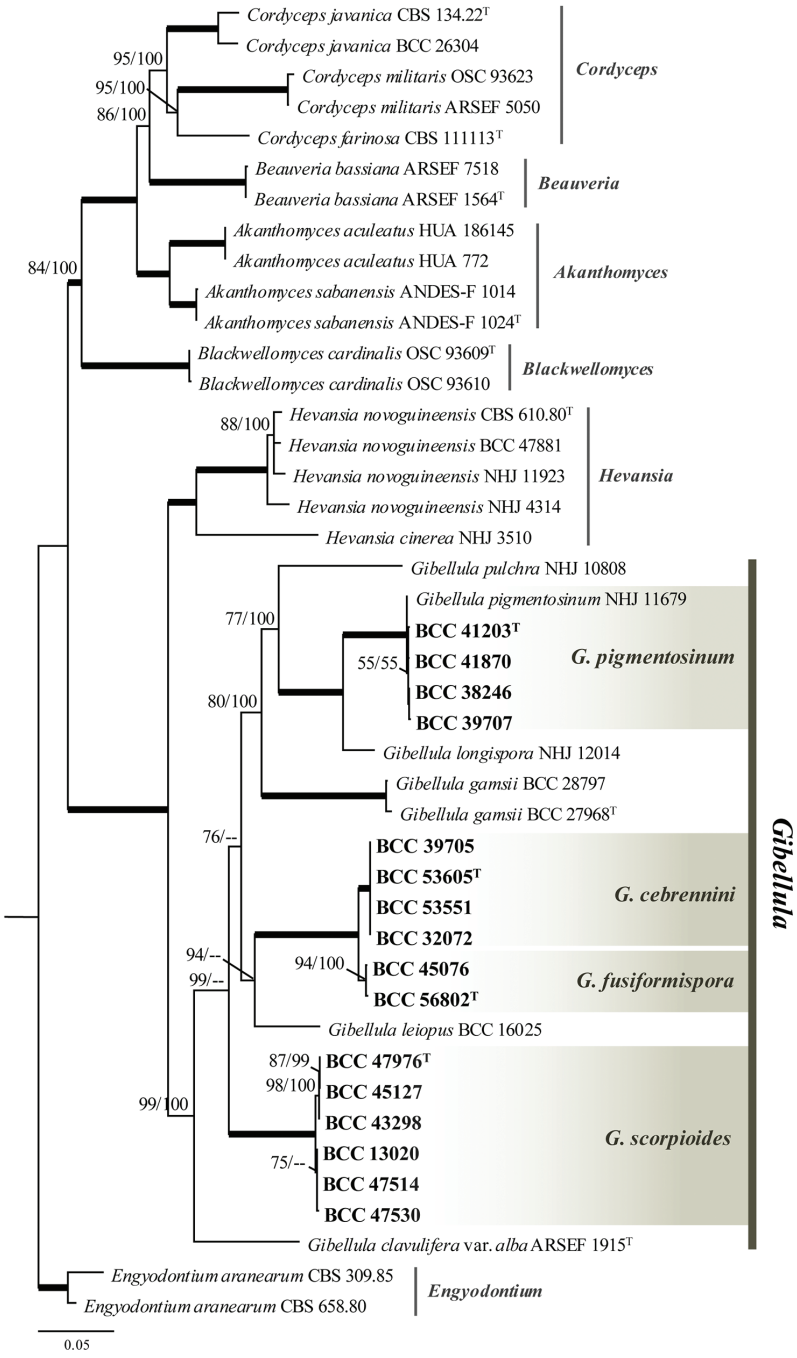

Figure 1. Phylogenetic tree inferred from a RAxML search of a concatenated alignment of ITS, LSU, *TEF1*, *RPB1* and *RPB2* showing the relationship among *Gibellula* and related genera. Bootstrap proportions/ Bayesian posterior probabilities ≥ 50% are provided above corresponding nodes; nodes with 100% support are shown as thick lines. The ex-type strains are marked with a superscript T (T) and the isolates reported in this study are bold.

Table 1. List of taxa included in the phylogenetic analysis and their GenBank accession numbers. The isolates representing four new species and other sequences generated in this study are marked in bold.

Species	Code	GenBank accession numbers					References
		ITS	LSU	*TEF1*	*RPB1*	*RPB2*	
Akanthomyces aculeatus	HUA 772	KC519371	KC519370	KC519366	–	–	Sanjuan et al. 2014
A. aculeatus	HUA 186145	–	MF416520	MF416465	–	–	Kepler et al. 2017
A. sabanensis	ANDES-F 1014	KC633245	KC633248	KC875221	–	–	Chirivi-Salomon et al. 2015
A. sabanensis	ANDES-F 1024^{T}	KC633232	KC875225	KC633266	–	KC633249	Chirivi-Salomon et al. 2015
Beauveria bassiana	ARSEF 7518	HQ880762	–	HQ880975	HQ880834	HQ880906	Rehner et al. 2011
B. bassiana	ARSEF 1564^{T}	NR111594	–	HQ880974	HQ880833	HQ880905	Rehner et al. 2011
Cordyceps militaris	OSC 93623	JN049825	AY184966	DQ522332	DQ522377	AY545732	Kepler et al. 2012; Spatafora et al. 2007; Sung and Spatafora 2004
C. militaris	ARSEF 5050	HQ880829	–	HQ881020	HQ880901	HQ880973	Rehner et al. 2011
C. farinosa	CBS 111113^{T}	AY624181	MF416554	MF416499	MF416656	MF416450	Luangsa-ard et al. 2005; Kepler et al. 2017
C. javanica	CBS 134.22^{T}	NR111172	NG059048	MF416504	MF416661	MF416455	Luangsa-ard et al. 2005; Kepler et al. 2017
C. javanica	BCC 26304	MH532851	**MH394660**	MH521903	**MH521825**	**MH521868**	Helaly et al. 2019, **this study**
Blackwellomyces cardinalis	OSC 93609^{T}	–	AY184962	DQ522325	DQ522370	DQ522422	Sung and Spatafora 2004; Spatafora et al. 2007
B. cardinalis	OSC 93610	JN049843	AY184963	EF469059	EF469088	EF469106	Kepler et al. 2012; Sung and Spatafora 2004; Sung et al. 2007
Engyodontium aranearum	CBS 309.85	JN036556	AF339526	DQ522341	DQ522387	DQ522439	Spatafora et al. 2007; Sung et al. 2001
E. aranearum	CBS 658.80	LC092897	LC092916	–	–	–	Tsang et al. 2016
Hevansia novoguineensis	CBS 610.80^{T}	MH532831	MH394646	MH521885	–	MH521844	Helaly et al. 2019; Mongkolsamrit et al. in press
H. novoguineensis	NHJ 4314	–	–	EU369012	EU369051	EU369071	Johnson et al. 2009
H. novoguineensis	NHJ 11923	–	EU369032	EU369013	EU369052	EU369072	Johnson et al. 2009
H. novoguineensis	BCC 47881	JX192685	**MH394650**	**MH521886**	**MH521807**	**MH521845**	Helaly et al. 2017, **this study**
H. cinerea	NHJ 3510	–	–	EU369009	EU369048	EU369070	Johnson et al. 2009
Gibellula cf. *alba*	NHJ 11679	–	–	EU369016	EU369054	–	Johnson et al. 2009
G. cebrennini	**BCC 32072**	**MT477067**	–	**MT503326**	–	–	**This study**
G. cebrennini	**BCC 39705**	**MH532874**	**MH394673**	**MH521895**	**MH521822**	**MH521859**	**This study**
G. cebrennini	**BCC 53551**	**MT477068**	–	**MT503327**	–	–	**This study**
G. cebrennini	**BCC 53605^{T}**	**MT477069**	**MT477062**	**MT503328**	**MT503321**	**MT503336**	**This study**
G. clavulifera var. *alba*	ARSEF 1915^{T}	–	DQ518777	DQ522360	DQ522408	DQ522467	Spatafora et al. 2007
G. fusiformispora	**BCC 45076**	**MH532882**	**–**	**–**	**MH521823**	**MH521860**	**This study**
G. fusiformispora	**BCC 56802^{T}**	**MT477070**	**MT477063**	**MT503329**	**MT503322**	**MT503337**	**This study**
G. gamsii	BCC 27968^{T}	MH152529	MH152539	MH152560	MH152547	–	Kuephadungphan et al. 2019
G. gamsii	BCC 28797	MH152531	MH152541	MH152562	MH152549	MH152557	Kuephadungphan et al. 2019
G. leiopus	BCC 16025	–	MF416548	MF416492	MF416649	–	Kepler et al. 2017

Species	Code	GenBank accession numbers					References
		ITS	LSU	*TEF1*	*RPB1*	*RPB2*	
G. longispora	NHJ 12014	–	–	EU369017	EU369055	EU369075	Johnson et al. 2009
G. pulchra	NHJ 10808	–	EU369035	EU369018	EU369056	EU369076	Johnson et al. 2009
G. pigmentosinum	**BCC 38246**	MH532872	**MH394672**	MH521893	**MH521800**	**MH521855**	Helaly et al. 2019, **this study**
G. pigmentosinum	**BCC 39707**	MH532875	**MH394674**	MH521894	**MH521801**	**MH521856**	Helaly et al. 2019, **this study**
G. pigmentosinum	**BCC 41203^{T}**	**MT477071**	–	**MT503330**	**MT503323**	–	**This study**
G. pigmentosinum	**BCC 41870**	**MT477072**	**MT477064**	**MT503331**	**MT503324**	–	**This study**
G. scorpioides	**BCC 13020**	**MT477073**	**MH394686**	**MH521901**	**MH521814**	–	**This study**
G. scorpioides	**BCC 43298**	**MT477074**	**MH394677**	**MH521900**	**MH521816**	**MH521858**	**This study**
G. scorpioides	**BCC 45127**	**MT477075**	–	**MT503332**	–	–	**This study**
G. scorpioides	**BCC 47514**	**MT477076**	–	**MT503333**	–	–	**This study**
G. scorpioides	**BCC 47530**	**MT477077**	**MT477065**	**MT503334**	–	**MT503338**	**This study**
G. scorpioides	**BCC 47976^{T}**	**MT477078**	**MT477066**	**MT503335**	**MT503325**	**MT503339**	**This study**

Description. *Synnema* arising from white to cream mycelial mat completely covering the spider host, cylindric, white to cream, slightly enlarged toward the sterile tip, consisting of multiseptate somewhat loosely bound longitudinal hyphae (Fig. 2a, c). *Conidiophores* scattered, arising from a network of hyphae loosely attached to the surface of the synnema, occasionally from a mycelium covering the host, (45–)95–139(–150) × (5–)5.5–7(–8) µm, verrucose, multiseptate, tapering abruptly to a short distinct neck, enlarging into a broadly ellipsoid to globose vesicle, (4.5–)5.5–7.5(–8.5) µm in diam (Fig. 2g). *Metulae* borne on vesicle, broadly obovoid to obovoid, (5–)6–7.5(–9) × (3–)4.5–6(–6.5) µm, bearing a group of narrowly obovoid phialides, thickened towards papillate apices, (4–)5.5–7.5(–9) × 1.5–2.5(–3.5) µm (Fig. 2h). *Vesicle*, metulae and phialides forming spherical heads, (23–)24–29.5(–33.5) µm in diam (Fig. 2h). *Conidia* fusiform, (4–)5.5–7.5(–9) × 1.5–2.5(–3.5) µm (Fig. 2i). *Perithecia* developed on subiculum of the host, arranged sparingly, occasionally crowded, superficial with a loose covering of cream mycelia, reddish yellow, ovoid, (1,150–)1,209–1,400(–1,411) × (375–)427–505(–575) µm (Fig, 2b, d). *Asci* over 600 µm long, (3.5–)4–5(–6) µm wide, ascus cap, (6–)7–8.5(–10) × (3.5–)4–4.5(–5) µm (Fig. 2e). *Ascospores* bacilliform, multiseptate, whole, over 570 µm long, 1–1.5 µm wide (Fig. 2f). Granulomanus-like asexual morph often occurring on the mycelial mat covering the host body. Polyblastic and irregularly shaped phialides developing multiple denticles, each bearing filiform conidium, (6–)7.5–10(–12) × 1–1.5 µm (Fig. 2j).

Culture characteristics. Colonies derived from conidia, on PDA slow-growing, attaining a diam of 1.4±0.05 cm in 4 weeks at 25 °C, white, velvety; reverse cream, becoming light brown with age toward center (Fig. 2k). Sporulation not observed.

Additional specimen examined. Thailand, Nakhon Ratchasima, Khao Yai National Park, Kong Kaeo Waterfall; 14°711'N, 101°421'E; on Araneida, underside of unidentified dicot leaf; 28 November 2006; K. Tasanathai, W. Chaygate, B. Thongnuch (BBH 18890, BCC 23863); Mo Sing To Nature Trail; 14°711'N, 101°421'E; on

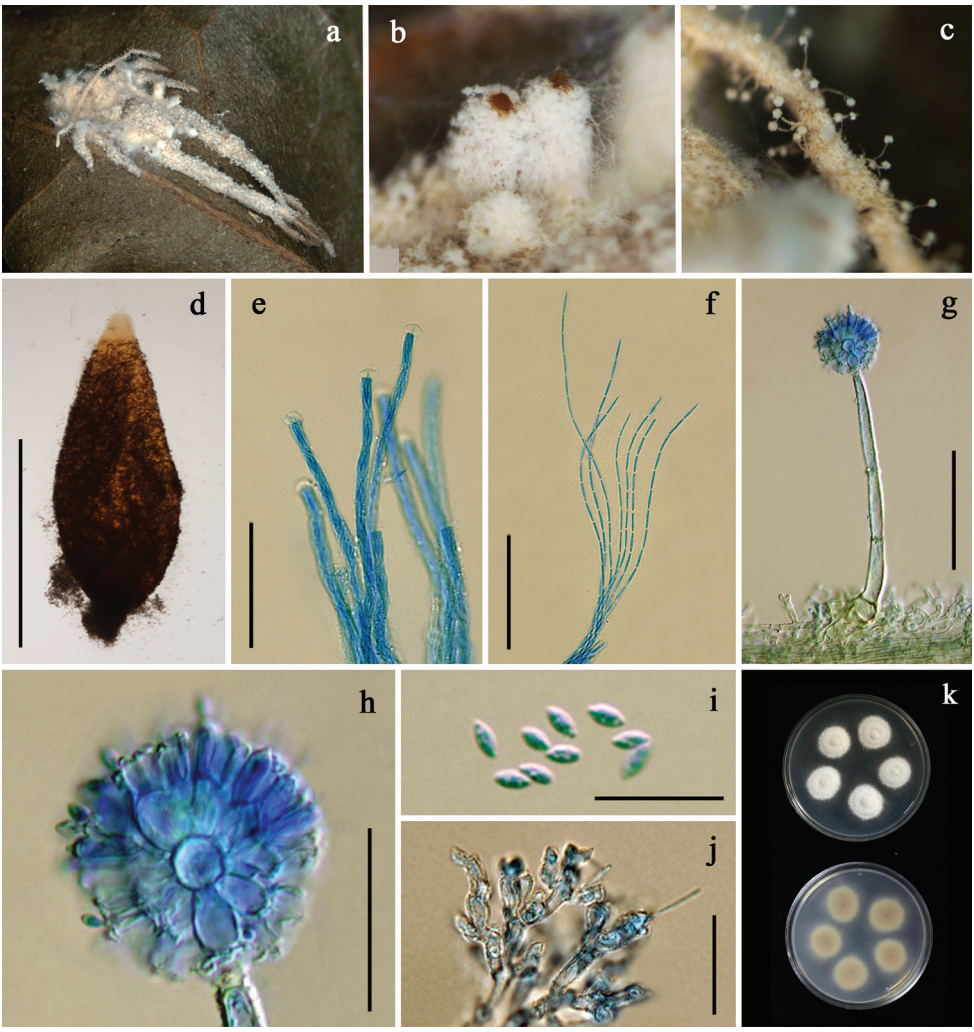

Figure 2. *Gibellula cebrennini* **a** fungus on spider (BBH 35749) **b** perithecia **c** a part of synnema showing conidiophores **d** perithecium **e** asci with apical apparatus **f** ascospores **g** conidiophore **h** conidial head **i** conidia **j** granulomanus-like asexual morph **k** colonies obverse and reverse on PDA at 25 °C after 28 days. Scale bars: 1 mm (**d**); 50 µm (**e–f, g**); 20 µm (**h, j**); 10 µm (**i**).

Cebrenninus cf. *magnus*, underside of unidentified dicot leaf; 18 June 2008; J. Luangsaard, K. Tasanathai, S. Mongkolsamrit, B. Thongnuch, P. Srikitikulchai, R. Ridkaew, W. Chaygate, R. Promharn (BBH 24673, BCC 32072). On *Cebrenninus* cf. *magnus*, underside of unidentified dicot leaf; 11 September 2009; K. Tasanathai, P. Srikitikulchai, S. Mongkolsamrit, T. Chohmee, R. Ridkaew (BBH 32685, BCC 39705 and BCC 39706). On *Cebrenninus* cf. *magnus*, underside of unidentified dicot leaf; 6 June 2012; K. Tasanathai, S. Mongkolsamrit, A. Khonsanit, W. Noisripoom, P. Srikitikulchai (BBH 32589, BCC 53551).

Gibellula fusiformispora **Tasanathai, Kuephadungphan & Luangsa-ard, sp. nov.**
MycoBank No: 835114
Figure 3

Typification. Thailand, Chiang Mai, Chiang Dao District, Ban Huathung; 19°420'N, 98°971'E; on Araneida attached to the underside of unidentified dicot leaf; 5 October 2012; K. Tasanathai, A. Khonsanit, W. Noisripoom, P. Srikitikulchai, R. Promharn (Holotype no. BBH 32918, ex-type culture no. BCC 56802, isolated from conidia) GenBank: ITS = MT477070, LSU = MT477063, *TEF1* = MT503329, *RPB1* = MT503322, *RPB2* = MT503337.

Etymology. Refers to the fusiform part-spores.

Description. Spiders totally covered by the white to cream mycelial mat. A single synnema or synnemata in pairs cream to light brown, often darker than the mycelia covering the host, narrowing toward the apex and terminating in a swollen sterile tip with acute apex (Fig. 3a–c). *Conidiophores* arising laterally from the outer layer of synnemata, crowded, (23–)31–53(–83) × (4–)5.5–6.5(–7.5) µm, mostly verrucose, occasionally slightly roughed for very short conidiophores, abruptly narrowing to a slender apex and forming a globose to subglobose vesicle (Fig. 3c, i). *Vesicles* 6–7(–8) µm in diam, each bearing a number of metulae (Fig. 3j). *Metulae* obovoid to broadly obovoid, (7–)7.5–9(–10) × (4.5–)5–5.5(–6) µm (Fig. 3j). *Phialides* borne on metulae, narrowly obovoid, 7–8.5(–10) × 2–3 µm bearing fusiform to broadly fusiform conidia, (3.5–)4–5(–6) × 1.5–2(–2.5) µm (Fig. 3h, j). *Conidial heads* spherical, (31–)32–34.5(–37) µm in diam (Fig. 3j). *Perithecia* mostly appearing in pairs, ovoid, superficial with a loose covering of white to cream mycelia, reddish yellow, up to 1,000 µm in length, 320–350 µm in width (Fig. 3d–e). *Asci* 600–700 × 7–8 µm (Fig. 3f). *Ascospores* often disarticulating into part-spores. *Part-spores* fusiform, 12–15 × 2–3 µm (Fig. 3g). Granulomanus-like asexual morph absent.

Culture characteristics. Colonies derived from conidia, on PDA slow-growing, attaining a diam of 1.1±0.03 cm in 20 days at 25 °C, white, velvety; reverse cream, becoming light brown with age toward center (Fig. 3k). Sporulation not observed.

Additional specimen examined. Thailand, Chiang Mai, Chiang Dao District, Ban Huathung; 19°420'N, 98°971'E; on Deinopidae (Araneae) attached to the underside of unidentified monocot leaf; 23 September 2010; K. Tasanathai, P. Srikitikulchai, A. Khonsanit, K. Sansatchanon (BBH 38838, BCC 45076 and BCC 45077).

Notes. The sexual morph of *G. fusiformispora* is extremely close to *Torrubiella ellipsoidea* (Kobayasi and Shimizu 1982) in producing slightly curved fusiform part-spores of maximum 3 µm wide, whereas those of *G. fusiformispora* are almost two times wider. Considering the *Gibellula* conidial state, *G. fusiformispora* resembled *G. cebrennini* by forming synnema with sterile swollen tip, aspergillate conidiophores and fusiform conidia. However, *G. fusiformispora* can be easily recognized by having much shorter conidiophores, and the production of more than one synnema on the spider hosts.

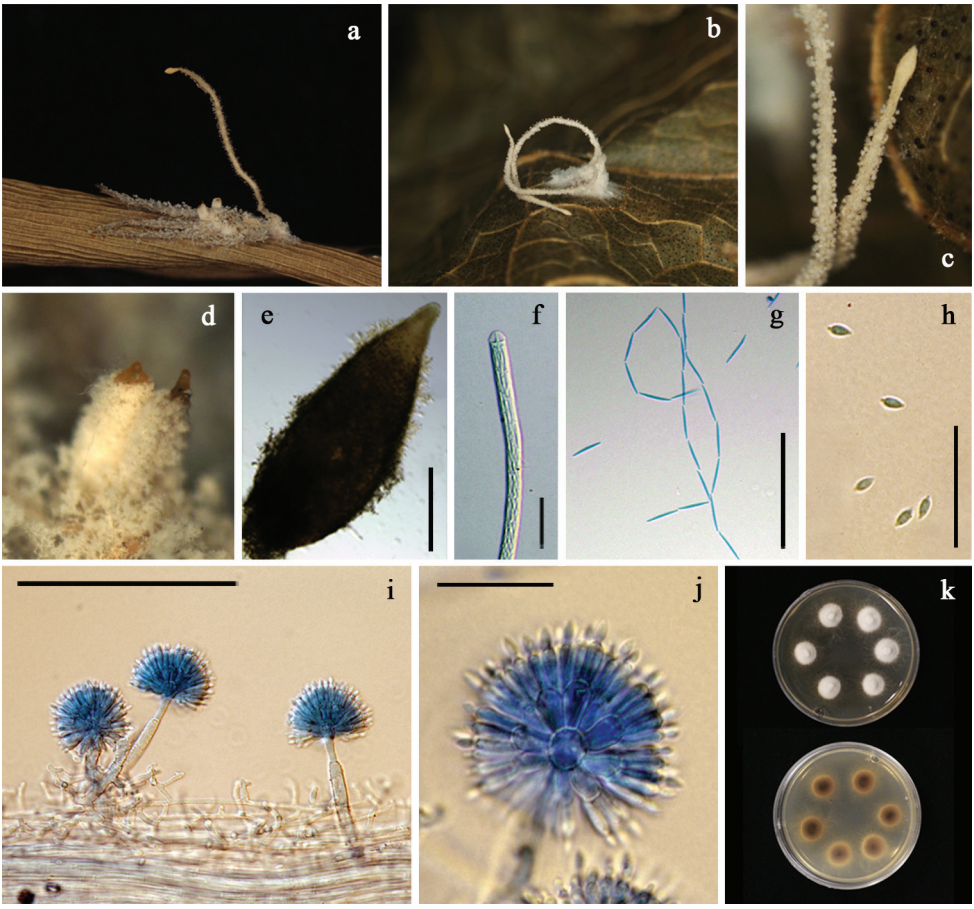

Figure 3. *Gibellula fusiformispora* **a** fungus on a spider (BBH 38838) **b** fungus on a spider (BBH 32918) **c** synnemata (BBH 32918) **d, e** perithecia (BBH 38838) **f** ascus (BBH 38838) **g** ascospores (BBH 38838) **h** conidia (BBH 32918) **i** conidiophores showing conidial heads (BBH 32918) **j** conidial head bearing conidia (BCC 32918) **k** colonies obverse and reverse on PDA at 25 °C after 20 days. Scale bars: 250 µm (**e**); 100 µm (**i**); 50 µm (**g**); 20 µm (**f, h, j**).

Gibellula pigmentosinum **Tasanathai, Kuephadungphan & Luangsa-ard, sp. nov.**
MycoBank No: 835112
Figure 4

Typification. Thailand. Nakhon Ratchasima, Khao Yai National Park, Mo Sing To Nature Trail; 14°711'N, 101°421'E; on *Storenomorpha* sp., attached to underside of unidentified dicot leaf; 10 February 2010; K. Tasanathai, P. Srikitikulchai, S. Mongkolsamrit, T. Chohmee, R. Ridkaew, A. Khonsanit (Holotype no. BBH 28509, ex-type culture no. BCC 41203, isolated from ascospores and BCC 41204, isolated from conidia). GenBank (BCC 41203): ITS = MT477071, *TEF1* = MT503330, *RPB1* = MT503323.

Etymology. Refers to the capability of the fungus to produce pigmentosins.

Description. Spider host completely covered by white to yellowish-white mycelial mat. *Synnemata* solitary or in pairs, cylindrical, white, becoming yellowish-white at the base (Fig. 4a). *Conidiophores* arising along the entire length of the outer hyphae of synnemata and from the mycelia covering the host, crowded, smooth to verrucose, (55–)97.5–170(–226) × (5–)7–10(–12.5) µm, narrowing to a slender apex, and terminating in a swollen vesicle, metulae, phialides bearing conidia, forming a spherical conidial head (Fig. 4a, f). *Conidial heads* (25–)30–39(–45) µm diam (Fig. 4g). *Vesicles* mostly globose, (4.5–)5.5–9(–10) µm diam (Fig. 4g). *Metulae* borne on a vesicle, broadly obovoid, (5.5–)6–8(–10) × (3–)4–6(–7.5) µm (Fig. 4g), bearing phialides. *Phialides* obovoid to clavate, with a distinct short neck, (5–)5.5–8(–9) × 2–3(–4.5) µm (Fig. 4g). *Conidia* produced on a phialide, obovoid with an acute apex, (2.5–)3.5–5(–5.5) × 1–2(–3) µm (Fig. 4h). *Perithecia* produced on the mycelial mat on the head and body of the spider, scattered, superficial with loose mycelia covering only the bottom one-fourth of the perithecium, ovoid, reddish-yellow, (790–)882–1,117(–1,150) × 300–443(–475) µm (Fig. 4a, b). *Asci* cylindrical, 700–750 µm long, (4.5–)5–6(–7) µm wide, ascus cap (4–)5.5–6.5(–7) × 3.5–4(–5.5) µm (Fig. 4c). *Ascospores* filiform, multiseptate, arranged in parallel rows, (666–)670–727(–730) × 2–3 µm, often breaking into 128 part-spores (Fig. 4d). *Part-spores* bacilliform with apices rounded, (3.5–)4–7(–9) × 1–1.5(–3) µm (Fig. 4e). Granulomanus-like asexual morph occasionally present, forming irregularly branched hyphae bearing mono- or polyblastic phialides. *Phialides* irregularly in shape, mostly smooth, with one or more conspicuous denticles. A *conidium* borne on each denticle, long, filiform, (16–)16.5–21.5(–22.5) × 1–1.5 µm (Fig. 4i).

Culture characteristics. Colonies derived from ascospores, on PDA slow-growing, attaining a diam of 1.5±0.2 cm in 4 weeks at 25 °C, white, floccose; reverse light brown, darkening with age toward center (Fig. 4j). Sporulation not observed.

Additional specimens examined. Thailand, Nakhon Ratchasima, Khao Yai National Park, Mo Sing To Nature Trail; 14°711'N, 101°421'E; on *Storenomorpha* sp., underside of unidentified dicot leaf; 13 August 2009; K. Tasanathai, P. Srikitikulchai, S. Mongkolsamrit, T. Chohmee, R. Ridkaew (BBH 26516, BCC 38246 and BCC 38955); on Araneida, underside of unidentified dicot leaf; 11 September 2009; K. Tasanathai, P. Srikitikulchai, S. Mongkolsamrit, T. Chohmee, R. Ridkaew (BBH 27081, BCC 39707 and BCC 39708); on *Storenomorpha* sp., underside of unidentified dicot leaf; 7 April 2010; K. Tasanathai, S. Mongkolsamrit, T. Chohmee, A. Khonsanit, R. Ridkaew (BBH 28533, BCC 41870 and BCC 41871).

Notes. *Gibellula pigmentosinum* shares similarity with *G. pulchra* (Mains 1950) in producing cylindric, yellowish white synnemata bearing aspergillate conidiophores with fusoid-ellipsoid conidia and superficial, reddish brown, ovoid perithecia containing bacilliform part-ascospores. The synnemata in *G. pulchra* are more copious and sometimes more violaceous than in *G. pigmentosinum*. Remarkably, *G. pigmentosinum* distinctly differs from *G. pulchra* in having a granulomanus-like conidial state.

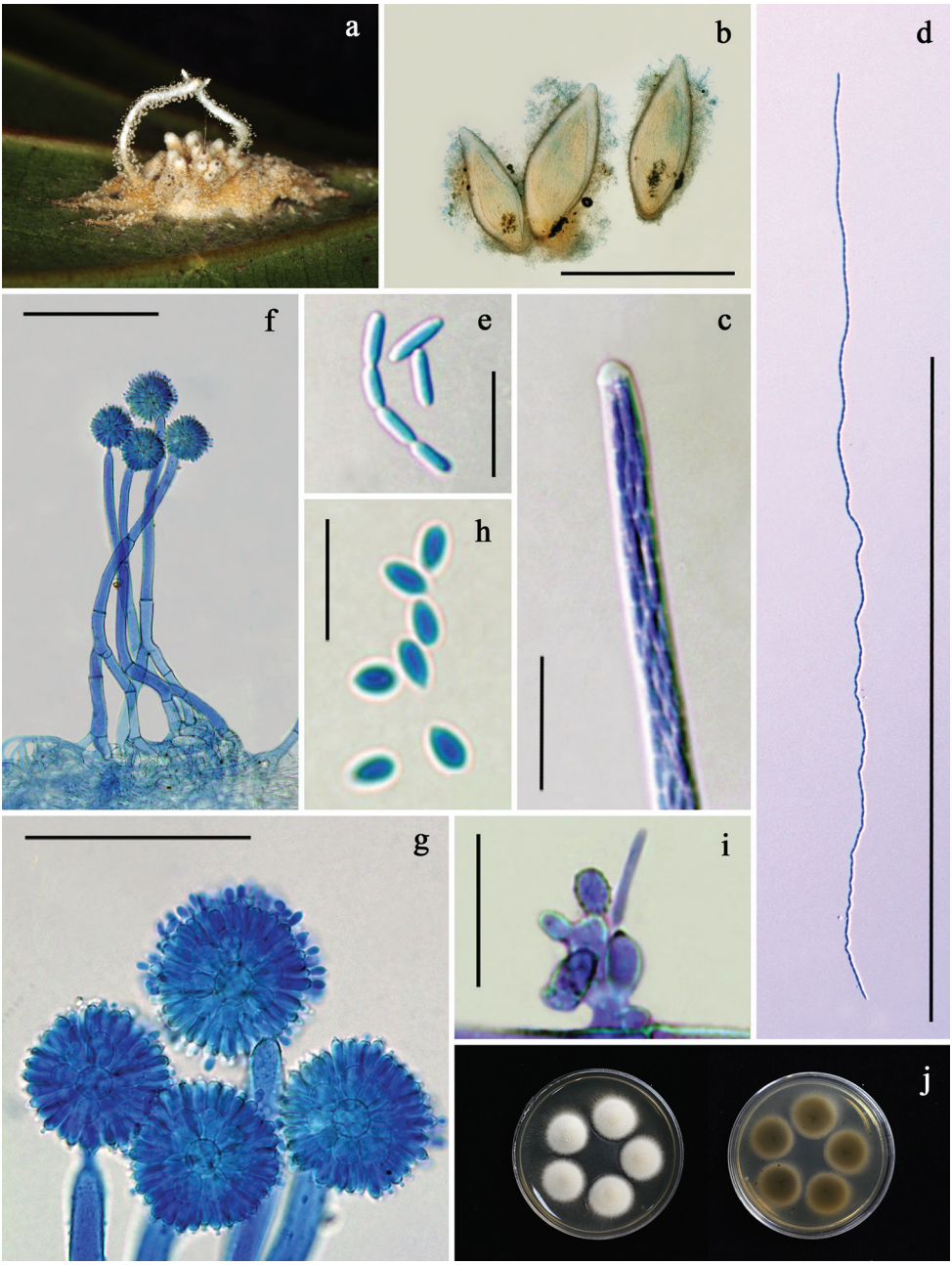

Figure 4. *Gibellula pigmentosinum* **a** fungus on spider (BBH 28509); **b** perithecia; **c** an ascus with an apical apparatus; **d** ascospore; **e** part-spores; **f** conidiophores; **g** conidial heads; **h** conidia; **i** granulomanus-like asexual morph; **j** colonies obverse and reverse on PDA at 25 °C after 28 days. Scale bars: 1 mm (**b**); 500 μm (**d**); 100 μm (**f**); 50 μm (**g**); 20 μm (**c, i**); 10 μm (**e, h**).

Gibellula scorpioides **Tasanathai, Khonsanit, Kuephadungphan & Luangsa-ard, sp. nov.**
MycoBank No: 835115
Figure 5

Typification. Thailand, Nakhon Ratchasima, Khao Yai National Park, Mo Sing To Nature Trail, 14°711'N, 101°421'E; on *Portia* sp. attached to the underside of unidentified dicot leaf; 1 June 2011; K. Tasanathai, P. Srikitikulchai, S. Mongkolsamrit, A. Khonsanit, K. Sansatchanon, W. Noisripoom (Holotype no. BBH 31439, ex-type culture no. BCC 47975, isolated from ascospores and BCC 47976, isolated from conidia) GenBank (BCC 47976): ITS = MT477078, LSU = MT477066, *TEF1* = MT503335, *RPB1* = MT503325, *RPB2* = MT503339.

Etymology. Refers to the outer appearance of the fungus resembling the posture of a scorpion.

Description. White to grayish- or brownish-white mycelial mat velvety, completely covering the spider host, firmly attaching the underside of living leaf by the mycelia covering its legs (Fig. 5a, b). *Synnema* solitary, arising from the posterior of the host abdomen, cylindrical, consisting of a compact bundle of parallel hyphae, 15–20 mm long with blunt tip. *Conidiophores* arising laterally from synnema, stout, smooth, mostly biverticillate, 20–29(–30) × 4 µm (Fig. 5d). *Vesicles* absent or hardly developed, bearing multiple metulae. *Metulae* obovoid, slightly broadening toward the base, (7–)9.5–12.5(–15) × (2–)3–5(–7) µm (Fig. 5e). A number of phialides borne on each metula, broadly cylindrical, abruptly tapering toward the apex, forming thickened distinct short neck, (9–)10–12.5(–14) × (2–)2.5–3.5(–4) µm, each bearing a conidium (Fig. 5e). *Conidia* fusiform, 5–7(–9) × (1.5–)2–3 µm (Fig. 5f). Sexual morph occasionally present. *Perithecia* occurring on the mycelial mat covering the host legs, occasionally on synnema particularly at base, superficial, mostly arranged in groups, ovoid, reddish yellow or light honey-brown, one-third immersed in the loose network of mycelia, 750–836(–870) × 310–361(–380) µm (Fig. 5c). *Asci* over 550 µm in length, (3–)4–5.5(–7) µm in width, ascus tip (4–)4.5–5 × 3–3.5(–4) µm (Fig. 5g, h). *Ascospores* often breaking into part-spores. *Part-spores* bacilliform, (9–)10–15(–22) × 1.5–2 µm (Fig. 5i). Granulomanus-like asexual morph absent.

Culture characteristics. Colonies derived from conidia, on PDA slow-growing, attaining a diam of 1.5±0.1 cm in 4 weeks at 25 °C, floccose, forming irregular margin, white, reverse cream, darkening toward center with age (Fig. 5l). Sporulation occurring after 3–4 months with the absence of synnema, forming a group of conidiophores, grey and scatter. *Conidiophores* biverticillate. *Vesicles* absent or hardly developed. *Metulae* obovoid, (10–)11–14.5(–16) × 3–5.5(–7) µm, each bearing cylindrical *phialides*, (10–)11.5–14(–16) × 3–4 µm. *Conidia* fusiform, 5–6(–7) × 3–3.5(–4) µm.

Additional specimens examined. Thailand, Chumphon, Phato District, Phato Watershed Conservation and Management Unit; 9°784'N, 98°699'E; on *Portia* sp., underside of unidentified dicot leaf; 10 March 2011; K. Tasanathai, P. Srikitikulchai, A. Khonsanit, K. Sansatchanon, D. Thanakitpipattana (BBH 30499, BCC 47530). Nakhon Ratchasima, Khao Yai National Park, Mo Sing To Nature Trail;

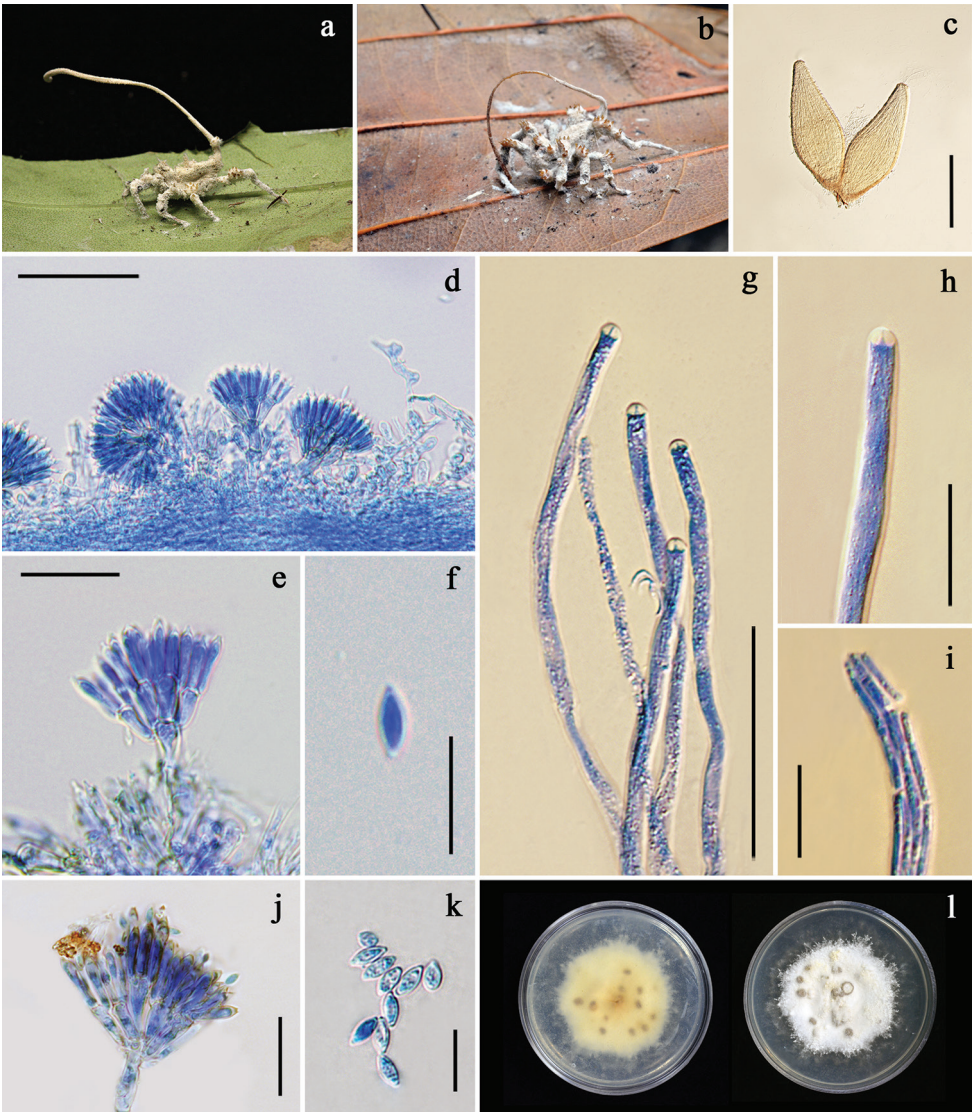

Figure 5. *Gibellula scorpioides* **a** fungus on a spider (BBH 29669) **b** fungus on a spider (BBH 31439) **c** perithecia (BBH 31439) **d** conidiophores arising on synnema (BBH 29669) **e** penicillate conidiophore (BBH 29669) **f** conidia (BBH 29669) **g** asci (BBH 31439) **h** ascus with apical apparatus (BBH 31439) **i** ascospores (BBH 31439) **j** penicillate conidiophore produced on PDA **k** conidia on PDA **l** colonies obverse and reverse on PDA at 25 °C after 4 months. Scale bars: 500 µm (**c**); 50 µm (**d, g**); 20 µm (**e, h–j**); 10 µm (**f, k**).

14°711'N, 101°421'E; on *Portia* sp., underside of unidentified dicot leaf; 1 June 2011; K. Tasanathai, P. Srikitikulchai, S. Mongkolsamrit, A. Khonsanit, K. Sansatchanon, W. Noisripoom (BBH 29669, BCC 43298).

Notes. The morphology of *G. scorpioides* appeared to be very close to *G. clavulifera* var. *clavulifera* (Samson and Evans 1977), *G. clavulifera* var. *major* (Tzean et al. 1997) and *G. clavulifera* var. *alba* (Humber and Rombach 1987). The penicillate

conidiophores were largely absent from the whip-like stroma in *G. clavulifera* var. *alba* but distinctly present on a synnema of *G. scorpioides*. Based on a comparison of microscopic characteristics among *G. scorpioides*, varieties *clavulifera*, *major* and *alba*, the latter three were found to produce much longer conidiophores (up to 100 μm) than *G. scorpioides* (20–29(–30) × 4 μm) while the other characters such as metulae, phialides as well as conidia were considered to be not significantly different in both shape and size. Considering the presence of the torrubiella-like sexual morph, perithecia of *G. clavulifera* var. *alba* were produced sparingly and separately on the host abdomen while those of *G. scorpioides* distinctly appeared in groups, only on the spider's legs and basally on synnema. Nevertheless, an examination of additional specimens has led us to conclude that the sexual morph is not always present in *G. scorpioides*.

Discussion

A torrubiella-like sexual morph is well-known to be connected with *Gibellula* (Evans 2013; Kepler et al. 2017; Shrestha et al. 2019). Shrestha and colleagues (2019) recently reviewed spider-pathogenic fungi within Hypocreales including *Gibellula* where its sexual morph links are listed. *Torrubiella globosa* Kobayasi & Shimizu, *Torrubiella globosostipitata* Kobayasi & Shimizu, *Torrubiella arachnophila* var. *pulchra* Mains and *Torrubiella gibellulae* Petch were synonymized with *G. pulchra*, species where their conidial and torrubiella-like sexual morphs often concurrently occur on the same substrates. *Gibellula pigmentosinum* appeared to be remarkably close to *G. pulchra* in producing nearly identical microscopic characters in both shapes and sizes. Nonetheless, *G. pigmentosinum* distinctly differs from *G. pulchra* in having a granulomanus-like conidial state. Considering its phylogenetic placement, *G. pigmentosinum* was significantly placed far from the taxon representing *G. pulchra* which supported the morphological differences between them. Noticeably, *G. pigmentosinum* formed a very strongly supported clade together with *Gibellula* cf. *alba*. It is interesting that *Gibellula* cf. *alba* was not proposed as a species and it is unfortunate that the herbarium specimen of NHJ 11679 is no longer in a good condition. According to its placement in the RAxML/Bayesian tree inferred from multiple loci (Fig. 1), *Gibellula* cf. *alba* NHJ 11679 could unambiguously be assigned to *G. pigmentosinum*.

The morphological resemblance between *G. cebrennini* and *G. fusiformispora* as well as a multi-gene phylogenetic analysis indicate a very close relationship among these species. Moreover, they can be distinguished from each other by the length of conidiophores, the shape of ascospores as well as the presence of a granulomanus-like conidial state.

In nature, a torrubiella-like sexual morph may occur on spider hosts without the presence of *Gibellula*. It may be premature to assign the new species to *Gibellula* on the basis of sexual morph, when more than one asexually reproductive genus are known to be linked to a torrubiella-like sexual morph. *Gibellula cebrennini* and *Akanthomyces thailandicus* (Mongkolsamrit et al. 2018) are good examples of such a phenomenon. Based on an investigation of Thai specimens, *G. cebrennini* tended to produce torrubiella-like perithecia on the spider hosts in the absence of *Gibellula* and granulomanus-

like asexual morphs, whereas *A. thailandicus* is an obligate parasite of spiders, of which only its torrubiella-like sexual morph has so far been recorded. These characteristics could lead to misidentification between these two species. The size and shape of part-spores are considered as the only morphological characters that have potential in species discrimination according to the evidence that *G. cebrennini* mostly produces longer bacilliform part-spores than *A. thailandicus*. In *G. cebrennini* and *G. fusiformispora*, it may also be difficult to discriminate them at first glance as the only distinguishing feature is the shape of their part-spores. These similiarities, and the occasional overlap in shape and size of morphological characters, were also demonstrated by Khonsanit et al. (2019) in the *Ophiocordyceps irangiensis* and *O. myrmecophila* species complex, by Mongkolsamrit et al. (2019) in *Paraisaria phuwiangensis* and *P. yodhathaii*, by Luangsaard et al. (2018) in *Ophiocordyceps* spp. with superficial perithecia, and Tasanathai et al. (2019) among termite pathogens in *Ophiocordyceps*. To improve species delimitation among closely related species with such very low morphological differentiation, integrative taxonomy combining a variety of data such as molecular, chemical, biogeographical, ecological characters, etc. is suggested to be very useful (Pante et al. 2015).

It has been over a half century since host specialization was suggested as one of the taxonomic criteria for parasitic fungi (Johnson 1968). In most cases of fungi that have a narrow host range or are restricted to a single host species, host specificity is considered as an important feature that can be used for identification at the species level (Vialle et al. 2013). In the case of invertebrate-pathogenic fungi, host specificity is usually taken into account mostly to evaluate their virulence and potential in terms of using them as biocontrol agents. For taxonomic purposes, Kobmoo and co-workers (2012) proved that host specificity has great potential for reflecting the divergent evolution of the ant-parasitic *Ophiocordyceps unilateralis*. Their success has therefore driven us to put effort for the first time to define the host species of *Gibellula*.

Bishop (1990), Hughes et al. (2016), and Savić et al. (2016) reported spider hosts of *Gibellula* distributed among 11 families consisting of Anyphaenidae, Agelinidae, Araneidae, Corinnidae, Linyphiidae, Pholcidae, Salticidae, Sparassidae, Theridiidae, Thomisidae, Zodariidae which represent approximately 10% of described families worldwide (World Spider Catalog 2020). Herein, three of those including Salticidae, Thomisidae and Zodariidae were found to be the hosts of *G. scorpioides*, *G. cebrennini* and *G. pigmentosinum*, respectively, whereas the family Deinopidae is reported here for the first time as a *Gibellula* host for *G. fusiformispora*.

According to the effort of putting toward species identification of spider hosts (Bristowe1930; Tikader 1965; Jocqué and Bosmans 1989; Deeleman-Reinhold 2001, 2009; Jocqué and Dippenaar-Schoeman 2007; Lehtinen et al. 2008; Benjamin 2011, 2016; Miller et al. 2012; Dhali et al. 2017; Jocqué et al. 2019), *G. pigmentosinum*, *G. cebrennini* as well as *G. scorpioides* were found to be exclusively specific to spider species with the exception of *G. fusiformispora* wherein only one specimen allowed us to identify the spider only to the family rank. Despite *Gibellula* being a well-known spider specialist, only Nentwig (1985), Hughes et al. (2016), and Savić et al. (2016) have ever indicated its hosts at genus or species ranks. In the current study, only the host of *G. cebrennini*

could be identified to the species level as *Cebrenninus* cf. *magnus*, whereas hosts of *G. pigmentosinum* and *G. scorpioides* were assigned to *Storenomorpha* sp. (Zodariidae) and *Portia* sp. (Salticidae), respectively. One common problem is that the fungus tends to cover the host body completely, which can obscure the spider's morphological features making identification infeasible. Tarsal claws and scopulae are important morphological features used to identify spider species (Wolff and Gorb 2012; Wolff et al. 2013; Labarque et al. 2017), and the morphology of spider feet was herein targeted and carefully studied. Since their legs appeared to be the only part that were slightly covered by fungal mycelia, it was thus considered to be the most significant character for distinguishing spider species infected by *Gibellula*. Host identification can be especially challenging for old fungal herbarium specimens that are dry and damaged. We suggest delivering specimens to arachnological taxonomists immediately after field work to allow the identification of spider hosts to species rank. Furthermore, as new species of spider continue to be described (e.g. Miller et al. 2012; Dhali et al. 2017; Jocqué et al. 2019), accurate taxonomy of spider hosts could be important for taxonomy of fungal pathogens on them.

It is notable that all seven host individuals used for identification in this study were found under a leaf. *Portia* sp. has a unique life history. These spiders are not only web invaders or cursorial hunters but are also web builders. Thus, they exist both on and off their own webs. Their webs are used for various activities including trapping, baiting, resting, molting, mating, oviposition, brooding (Jackson and Blest 1982; Jackson and Hallas 1986; Nelson and Jackson 2011a). They are day-active hunters and stay on their own webs at night (Barth 2002; Herland and Jackson 2004; Nelson and Jackson 2011b). Moreover, moribund web-building spiders infected by pathogenic fungi are presumed to stay motionless on their webs (Anotaux et al. 2016). This behaviour may promote growth and reproduction of *G. scorpioides* since spider silks possibly have antifungal properties (Tahir et al. 2015; Phartale et al. 2019). Another distinct feature of the spiders infected by *G. scorpioides* is its firm attachment to the substrate by only the mycelia growing over the tips of their legs allowing the host to sprawl and elevate their bodies upward. Interestingly, the host of *G. scorpioides*, *Portia* sp. is an araneophagic jumping spider that usually assumes such a posture during hunting (Forster and Murphy 1986). Furthermore, spiders are generally found dead with a posture of their legs flexed beneath their body (Pollard et al. 1987; Pizzi 2006; Starr and Taggart 2006). Such dead posture may also support growth and reproduction of *G. scorpioides* by keeping host from predation (Deeleman-Reinhold 2001; Honma et al. 2006), at least at the beginning stage of a fungal growth. From these observations, we believe that the fungus may influence the behaviour of the spider host by forcing it to stay firmly in place and assume an active posture during infection.

Since *G. scorpioides* can be cultured, it is possible to apply fungal spores to *Portia* spiders and study the spider-fungus interaction. It will be particularly interesting to investigate death sites, on or off web and death posture, resting or hunting postures between uninfected control and fungal infected spiders, which may give insight into behavioural manipulation before death by the fungus. Additionally, cultured *G. scorpioides* could be used to test the antifungal properties of *Portia* spider silk.

It is remarkable that our finding has revealed the high possibility to incorporate the host specificity in molecular and morphological criteria for classification and identification of *Gibellula*.

The biggest challenge for molecular phylogeny-based classification of *Gibellula* is the lack of reliable sequence data from type specimens. From sequences available in public databases, identities often appear erroneous, e.g. *G. clavispora* (Chen et al. 2016), *G. curvispora* (Han et al. 2013) and *G. shennongjiaensis* (Zou et al. 2016) appeared to be closer to other ascomycetes than *Gibellula* based on ITS sequence data. In the past, no attempts were made to establish the described species as pure cultures, or the attempts failed, thus making molecular analysis impractical. The lack of sequence data from type strains of *Gibellula* makes it difficult to establish whether query sequences from new specimens represent new or rediscovered taxa.

Despite molecular phylogeny currently being the most powerful approach available in modern fungal classification and taxonomy (Ariyawansa et al. 2014), many attempts to incorporate alternative or polyphasic approaches, such as chemotaxonomy, have been made. This approach provides high-informative data to support morphological and molecular data for identifying fungal species, facilitates solving taxonomic problem as well as unraveling asexual morph-sexual morph links (Frisvad et al. 1990; Stadler et al. 2003; Helaly et al. 2018). As part of our ongoing research on taxonomy and secondary metabolite production of Thai invertebrate-pathogenic fungi, chemotaxonomy has been employed, resulting in the discovery of unprecedented secondary metabolites, including pigmentosin B from *G. pigmentosinum* and gibellamines from *G. gamsii* (Helaly et al. 2019; Kuephadungphan et al. 2019). These compounds were found to be species-specific and could be designated as chemotaxonomic markers for the species. However, it is premature to use such compounds as markers for *Gibellula* since the exploration of their secondary metabolite production is limited to only a few species. Chemotaxonomy must therefore be expanded to other taxa, in particular *G. cebrennini*, *G. fusiformispora* as well as *G. scorpioides*.

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References

Anotaux M, Toscani C, Leborgne R, Chaline N, Pasquet A (2016) Time till death affects spider mobility and web-building behavior during web construction in an orb-web spider. Current Zoology 62: 123–130. https://doi.org/10.1093/cz/zow001

Ariyawansa HA, Hawksworth DL, Hyde KD, Jones EG, Maharachchikumbura SS, Manamgoda DS, Thambugala KM, Udayanga D, Camporesi E, Daranagama A, Jayawardena R (2014) Epitypification and neotypification: guidelines with appropriate and inappropriate examples. Fungal Diversity 69: 5–91. https://doi.org/10.1007/s13225-014-0315-4

Barth FG (2002) Spider's world: senses and behavior. Springer Verlag, Berlin, 394 pp.

Benjamin SP (2011) Phylogenetics and comparative morphology of crab spiders (Araneae: Dionycha, Thomisidae). Zootaxa 3080: 1–108. https://doi.org/10.11646/zootaxa.3080.1.1

Benjamin SP (2016) Revision of *Cebrenninus* Simon, 1887 with description of one new genus and six new species (Araneae: Thomisidae). Revue Suisse de Zoologie 123: 179–200.

Bishop L (1990) Entomophagous fungi as mortality agents of ballooning spiderlings. Journal of Arachnology 18: 237–238.

Bristowe WS (1930) Notes on the biology of spiders. I–III. Annals and Magazine of Natural History 6: 334–353. https://doi.org/10.1080/00222933008673221

Bunyard BA, Nicholson MS, Royse DJ (1994) A systematic assessment of *Morchella* using RFLP analysis of the 28S ribosomal RNA gene. Mycologia 86: 762–772. https://doi.org/10.1080/00275514.1994.12026481

Cavara F (1894) Ulteriore contribuzione alla micologia lombarda. Atti dell'Ist Bot Univ e Lab Crittogamico Pavia 3: 313–350.

Chen WH, Han YF, Liang ZQ, Zou X (2016) Morphological traits, DELTA system, and molecular analysis for *Gibellula clavispora* sp. nov. from China. Mycotaxon 131: 111–121. https://doi.org/10.5248/131.111

Chirivi-Salomon JS, Danies G, Restrepo S, Sanjuan TI (2015) *Lecanicillium sabanense* sp. nov. (Cordycipitaceae) a new fungal entomopathogen of coccids. Phytotaxa 234: 63–74. https://doi.org/10.11646/phytotaxa.234.1.4

de Hoog GS (1978) Notes some fungicolous Hyphomycetes and their relatives. Persoonia 10: 33–81.

Deeleman-Reinhold CL (2001) Forest spiders of South East Asia: with a revision of the sac and ground spiders (Araneae: Clubionidae, Corinnidae, Liocranidae, Gnaphosidae, Prodidomidae and Trochanterriidae [sic]). Brill, Leiden, 591 pp.

Deeleman-Reinhold CL (2009) Spiny theridiids in the Asian tropics. Systematics, notes on behaviour and species richness (Araneae: Theridiidae: Chrysso, Meotipa). Contributions to Natural History 12: 403–436.

Dhali DC, Saha S, Raychaudhuri D (2017) Litter and ground dwelling spiders (Araneae: Arachnida) of reserve forests of Dooars, West Bengal. World Scientific News 63: 1–242.

Evans HC (2013) Fungal pathogens of spiders. In: Nentwig W (Ed.) Spider ecophysiology. Berlin, Germany: Springer, 107–121. https://doi.org/10.1007/978-3-642-33989-9_9

Forster L, Murphy F (1986) Ecology and behaviour in *Portia Schultzii*, with notes on related Species (Araneae, Salticidae). The Journal of Arachnology 14: 29–42.

Frisvad JC, Filtenborg O, Samson RA, Stolk AC (1990) Chemotaxonomy of the genus *Talaromyces*. Antonie van Leeuwenhoek 57: 179–189. https://doi.org/10.1007/BF00403953

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Hall T (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symposium series 41: 95–98.

Hall T, Biosciences I, Carlsbad C (2011) BioEdit: an important software for molecular biology. GERF Bulletin of Bioscience 2: 60–61.

Han YF, Chen WH, Zou X, Liang ZQ (2013) *Gibellula curvispora*, a new species of *Gibellula*. Mycosystema 32: 777–780.

Helaly SE, Kuephadungphan W, Phainuphong P, Ibrahim MA, Tasanathai K, Mongkolsamrit S, Luangsa-ard JJ, Phongpaichit S, Rukachaisirikul V, Stadler M (2019) Pigmentosins from *Gibellula* sp. as antibiofilm agents and a new glycosylated asperfuran from *Cordyceps javanica*. Beilstein Journal of Organic Chemistry 15: 2968–2981. https://doi.org/10.3762/bjoc.15.293

Helaly SE, Kuephadungphan W, Phongpaichit S, Luangsa-ard JJ, Rukachaisirikul V, Stadler M (2017) Five unprecedented secondary metabolites from the spider parasitic fungus *Akanthomyces novoguineensis*. Molecules 22: 991–1001. https://doi.org/10.3390/molecules22060991

Helaly SE, Thongbai B, Stadler M (2018) Diversity of biologically active secondary metabolites from endophytic and saprotrophic fungi of the ascomycete order Xylariales. Natural Products Report 35: 992–1014. https://doi.org/10.1039/C8NP00010G

Herland DP, Jackson RR (2004) Portia perception: the umwelt of an araneophagic jumping spider. In: Prete FR (Ed.) Complex worlds from simpler nervous systems. London, England: MIT Press, 5–40.

Honma A, Oku S, Nishida T (2006) Adaptive significance of death feigning posture as a specialized inducible defence against gape-limited predators. Proceedings of the Royal Society B: Biological Sciences 273: 1631–1636. https://doi.org/10.1098/rspb.2006.3501

Hughes DP, Araújo J, Loreto R, Quevillon L, de Bekker C, Evans HC (2016) From So Simple a Beginning: The Evolution of Behavioral Manipulation by Fungi. In: Lovett B, St. Leger RJ (Eds) Genetics and molecular biology of entomopathogenic fungi (Advances in genetics), vol. 94. Cambridge: Academic Press, 1–33. https://doi.org/10.1016/bs.adgen.2016.01.004

Humber RA, Rombach MC (1987) *Torrubiella ratticaudata* sp. nov. (Pyrenomycetes: Clavicipitales) and other fungi from spiders on the Solomon Islands. Mycologia 79: 375–382. https://doi.org/10.1080/00275514.1987.12025393

Jackson RR, Blest AD (1982) The biology of *Portia fimbriata*, a web-building jumping spider (Araneae, Salticidae) from Queensland: Utilization of webs and predatory versatility. Journal of Zoology 196: 255–293. https://doi.org/10.1111/j.1469-7998.1982.tb03504.x

Jackson RR, Hallas SEA (1986) Comparative biology of *Portia africana*, *P. albimana*, *P. fimbriata*, *P. labiata*, and *P. shultzi*, araneophagic, web-building jumping spiders (Araneae: Salticidae): Utilisation of webs, predatory versatility, and intraspecific interactions. New Zealand Journal of Zoology 13: 423–489. https://doi.org/10.1080/03014223.1986.10422978

Jocqué R, Bosmans R (1989) A revision of the genus *Storenomorpha* Simon (Araneae, Zodariidae). Spixiana 12: 125–134.

Jocqué R, Dippenaar-Schoeman AS (2007) Spider families of the world. Tervuren , Belgium: Royal Museum for Central Africa, 336 pp.

Jocqué R, Jocqué M, Stock W, Rin N, Henrard A (2019) The new Southeast Asian genus *Cambonilla* gen. nov. (Zodariidae, Araneae): 'bisrepetitaplacent'. European Journal of Taxonomy 501: 1–24. https://doi.org/10.5852/ejt.2019.501

Johnson D, Sung GH, Hywel-Jones NL, Luangsa-ard JJ, Bischoff JF, Kepler RM, Spatafora JW (2009) Systematics and evolution of the genus *Torrubiella* (Hypocreales, Ascomycota). Mycological Research 113: 279–289. https://doi.org/10.1016/j.mycres.2008.09.008

Johnson T (1968) Host specialization as a taxonomic criterion. In: Ainsworth GC, Sussman AS (Eds) The fungi advanced treatise vol. 3 the fungal population. New York: Academic Press, 543–554. https://doi.org/10.1016/B978-1-4832-2744-3.50027-4

Katoh K, Toh H (2008) Recent developments in the MAFFT multiple sequence alignment program. Briefings in Bioinformatics 9: 286–298. https://doi.org/10.1093/bib/bbn013

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T (2012) Geneious Basic: an intergrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Kepler RM, Luangsa-ard JJ, Hywel-Jones NL, Quandt C, Sung GH, Rehner SA, Aime MC, Henkel TW, Sanjuan T, Zare R, Chen M, Li Z, Rossman AY, Spatafora JW, Shrestha B (2017) A phylogenetically-based nomenclature for Cordycipitaceae (Hypocreales). IMA Fungus 8: 335–353. https://doi.org/10.5598/imafungus.2017.08.02.08

Kepler RM, Sung GH, Ban S, Nakagiri A, Chen M, Huang B, Li, Z, Spatafora JW (2012) New teleomorph combinations in the entomopathogenic genus *Metacordyceps*. Mycologia 104: 182–197. https://doi.org/10.3852/11-070

Khonsanit A, Luangsa-ard JJ, Thanakitpipattana D, Kobmoo N, Piasai O(2019) Cryptic species within *Ophiocordyceps myrmecophila* complex on formicine ants from Thailand. Mycological progress 18: 147–161. https://doi.org/10.1007/s11557-018-1412-7

Kobayasi Y, Shimizu D (1982) Monograph of the genus *Torrubiella*. Bulletin of the National Science Museum (Tokyo) 8: 43–78.

Kobmoo N, Mongkolsamrit S, Tasanathai K, Thanakitpipattana D, Luangsa-ard JJ (2012) Molecular phylogenies reveal host-specific divergence of *Ophiocordyceps unilateralis* sensu lato following its host ants. Molecular Ecology 21: 3022–3031. https://doi.org/10.1111/j.1365-294X.2012.05574.x

Kuephadungphan W, Macabeo APG, Luangsa-ard JJ, Tasanathai K, Thanakitpipattana D, Phongpaichit S, Yuyama K, Stadler M (2019) Studies on the biologically active secondary metabolites of the new spider parasitic fungus *Gibellula gamsii*. Mycological Progress 18: 135–146. https://doi.org/10.1007/s11557-018-1431-4

Kuephadungphan W, Phongpaichit S, Luangsa-ard JJ, Rukachaisirikul V (2014) Antimicrobial activity of invertebrate-pathogenic fungi in the genera *Akanthomyces* and *Gibellula*. Mycoscience 55: 127–133. https://doi.org/10.1016/j.myc.2013.06.007

Labarque FM, Wolff JO, Michalik P, Griswold CE, Ramírez MJ (2017) The evolution and function of spider feet (Araneae: Arachnida): multiple acquisitions of distal articulations. Zoological Journal of the Linnean Society, 1–34. https://doi.org/10.1093/zoolinnean/zlw030

Lehtinen PT, Marusik YM (2008) A redefinition of *Misumenops* F. O. Pickard- Cambridge, 1900 (Araneae, Thomisidae) and review of the New World species. Bulletin of the British Arachnological Society 14: 173–198. https://doi.org/10.13156/100.014.0406

Liu YL, Whelen S, Hall BD (1999) Phylogenetic relationship among ascomycetes: evidence from an RNA polymerase II subunit. Molecular Biology and Evolution 16: 1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092

Luangsa-ard JJ, Hywel-Jones NL, Manoch L, Samson RA (2005) On the relationships of *Paecilomyces* sect. *Isarioidea* species. Mycological Research 109: 581–589. https://doi.org/10.1017/S0953756205002741

Luangsa-ard JJ, Tasanathai K, Mongkolsamrit S, Hywel-Jones NL (2008) Atlas of invertebrate-pathogenic fungi of Thailand volume 2. National Center of Genetic Engineering and Biotechnology, National Science and Technology Development: Thailand, 88 pp.

Luangsa-ard JJ, Tasanathai K, Mongkolsamrit S, Hywel-Jones NL (2010) Atlas of invertebrate-pathogenic fungi of Thailand volume 3. National Center of Genetic Engineering and Biotechnology, National Science and Technology Development: Thailand, 96 pp.

Luangsa-ard JJ, Tasanathai K, Thanakitpipattana D, Khonsanit A, Stadler M (2018) Novel and interesting *Ophiocordyceps* spp. (Ophiocordycipitaceae, Hypocreales) with superficial perithecia from Thailand. Studies in Mycology 89: 125–142. https://doi.org/10.1016/j.simyco.2018.02.001

Mains EB (1950) The genus *Gibellula* on spiders in North America. Mycological Society of America 42: 306–321. https://doi.org/10.1080/00275514.1950.12017836

Miller JA, Griswold, CE, Scharff N, Rezáč M, Szűts T, Marhabaie M (2012) The velvet spiders: an atlas of the Eresidae (Arachnida, Araneae). Zookeys 195: 1–144. https://doi.org/10.3897/zookeys.195.2342

Mongkolsamrit S, Noisripoom W, Thanakitpipattana D, Wutikhun T, Spatafora JW, Luangsa-ard J (2018) Disentangling cryptic species with isaria-like morphs in Cordycipitaceae. Mycologia 110: 230–257. https://doi.org/10.1080/00275514.2018.1446651

Mongkolsamrit S, Noisripoom W, Arnamnart N, Lamlertthon S, Himaman W, Jangsantear P, Samson RA, Luangsa-ard JJ (2019) Resurrection of *Paraisaria* in the Ophiocordycipitaceae with three new species from Thailand. Mycological Progress 18: 1213–1230. https://doi.org/10.1007/s11557-019-01518-x

Mongkolsamrit S, Noisripoom W, Tasanathai K, Khonsanit A, Thanakitpipattana D, Himaman W, Kobmoo N, Luangsa-ard JJ (in press) Molecular phylogeny and morphology reveal cryptic species in *Blackwellomyces* and *Cordyceps* (Cordycipitaceae) from Thailand. Mycological Progress. https://doi.org/10.1007/s11557-020-01615-2

Murata N, Aoki T, Kusaba M, Tosa Y, Chuma I (2014) Various species of *Pyricularia* constitute a robust clade distinct from *Magnaporthe salvinii* and its relatives in Magnaporthaceae. Journal of General Plant Pathology 80: 66–72. https://doi.org/10.1007/s10327-013-0477-z

Nelson XJ, Jackson RR (2011a) Flexibility in the foraging strategies of spiders. In: Herberstein ME (Ed.) Spider behaviour: flexibility and versatility, Cambridge: Cambridge University Press, Cambridge, 31–56. https://doi.org/10.1017/CBO9780511974496.003

Nelson XJ, Jackson RR (2011b) Flexible use of anti-predator defences. In: Herberstein ME (Ed.) Spider behaviour: flexibility and versatility, Cambridge: Cambridge University Press, 99–126. https://doi.org/10.1017/CBO9780511974496.005

Nentwig W (1985) Parasitic fungi as a mortality factor of spiders. Journal of Arachnology 13: 272–274.

Nylander JAA (2004) MrModeltest v2. Progam distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.

Pante E, Schoelinck C, Puillandre N (2015) From integrative taxonomy to species description: one step beyond. Systematic Biology 64: 152–160. https://doi.org/10.1093/sysbio/syu083

Petch T (1932) *Gibellula*. Annales Mycologici 30: 386–393.

Petch T (1944) Notes on entomogenous fungi. Transactions of the British Mycological Society 27: 81–93. https://doi.org/10.1016/S0007-1536(44)80014-6

Phartale NN, Kadam TA, Bhosale HJ, Karale MA, Garimella G (2019) Exploring the antimicrobial potential of *Pardosa brevivulva* silk. The Journal of Basic and Applied Zoology 80: 31. https://doi.org/10.1186/s41936-019-0102-6

Pizzi R (2006) Spiders. In: Lewbart GA (Ed.) Invertebrate medicine.Ames, Iowa: Blackwell Publishing, 143–168. https://doi.org/10.1002/9780470344606.ch10

Pollard SD, Macnab AM, Jackson RR (1987) Communication with chemicals: pheromones and spiders. In: Nentwig W (Ed.) Ecophysiology of spiders. Heidelberg, German: Springer Verlag, 133–141. https://doi.org/10.1007/978-3-642-71552-5_10

Rehner SA, Buckley E (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97: 84–98. https://doi.org/10.3852/mycologia.97.1.84

Rehner SA, Minnis AM, Sung GH, Luangsa-ard JJ, Devotto L, Humber RA (2011) Phylogeny and systematic of the anamorphic, entomopathogenic genus *Beauveria*. Mycologia 103: 1055–1073. https://doi.org/10.3852/10-302

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1571–1572. https://doi.org/10.1093/bioinformatics/btg180

Saccardo PA (1877) Fungi Italici. Michelia 1: 73–100.

Samson RA, Evans HC (1973) Notes on entomogenous fungi from Ghana I. The genera *Gibellula* and *Pseudogibellula*. Acta Botanica Neerlandica 22: 522–528. https://doi.org/10.1111/j.1438-8677.1973.tb00873.x

Samson RA, Evans HC (1977) Notes on entomogenous fungi from Ghana IV. The genera *Paecilomyces* and *Nomuraea*. Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen: Series C 80: 128–134.

Samson RA, Evans HC (1992) New species of *Gibellula* on spiders (Araneida) from South America. Mycologia 84: 300–314. https://doi.org/10.1080/00275514.1992.12026143

Sanjuan T, Tabima J, Restrepo S, Laessoe T, Spatafora JW, Franco-Malano AE (2014) Entomopathogens of Amazonian stick insects and locusts are members of the *Beauveria* species complex (*Cordyceps* sensu stricto). Mycologia 106: 260–275. https://doi.org/10.3852/13-020

Savić D, Grbić G, Bošković E, Hänggi A (2016) First records of fungi pathogenic on spiders for the Republic of Serbia Arachnologiche Mitteilungen/Arachnology Letters 52: 31–34. https://doi.org/10.5431/aramit5206

Shrestha B, Kubátová A, Tanaka E, Oh J, Yoon DH, Sung JM, Sung GH (2019) Spider-pathogenic fungi within Hypocreales (Ascomycota): their current nomenclature, diversity, and distribution. Mycological Progress 18: 983–1003. https://doi.org/10.1007/s11557-019-01512-3

Spatafora JW, Sung GH, Sung JM, Hywel-Jones NL, White JF (2007) Phylogenetic evidence for an animal pathogen origin of ergot and the grass endophytes. Molecular ecology 16: 1701–1711. https://doi.org/10.1111/j.1365-294X.2007.03225.x

Stadler M, Tichy H-V, Katsiou E, Hellwig V (2003) Chemotaxonomy of *Pochonia* and other conidial fungi with *Verticillium*-like anamorphs. Mycological Progress 2: 95–122. https://doi.org/10.1007/s11557-006-0048-1

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Starr C, Taggart R (2006) Biology: the unity and diversity of life. Belmont, California: Wadsworth Publishing Company, 1056 pp.

Sung GH, Spatafora JW (2004) *Cordyceps cardinalis* sp. nov., a new species of *Cordyceps* with an east Asian-eastern North American distribution. Mycologia 96: 658–666. https://doi.org/10.1080/15572536.2005.11832962

Sung GH, Hywel-Jones NL, Sung JM, Luangsa-ard JJ, Shrestha B, Spatafora JW (2007) Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. Study Mycology 57: 5–59. https://doi.org/10.3114/sim.2007.57.01

Sung GH, Spatafora JW, Zare R, Hodge KT, Gams W (2001)A revision of *Verticillium* sect. Prostrata. II. Phylogenetic analyses of SSU and LSU nuclear rDNA sequences from anamorphs and teleomorphs of the Clavicipitaceae. Nova Hedwigia 72: 311–328.

Tahir HM, Zaheer A, Yaqoob R (2015) Antifungal potential of silk recovered from *Neoscona theisi* (Araneae: Araneidae). Punjab University Journal of Zoology 30: 81–83.

Tasanathai K, Noisripoom W, Chaitika T, Khonsanit A, Hasin S, Luangsa-ard J (2019) Phylogenetic and morphological classification of *Ophiocordyceps* species on termites from Thailand. MycoKeys 56: 101–129. https://doi.org/10.3897/mycokeys.56.37636

Tikader BK (1965) On some new species of spiders of the family Thomisidae from India. Proceedings of the Indian Academy of Science 61: 277–289.

Tsang CC, Chan JF, Pong WM, Chen JH, Ngan AH, Cheung M, Lai CK, Tsang DN, Lau SK, Woo PC (2016) Cutaneous hyalohyphomycosis due to *Parengyodontium album* gen. et. comb. nov. Medical Mycology 54: 699–713. https://doi.org/10.1093/mmy/myw025

Tzean SS, Hsieh LS, Wu WJ (1997) The genus *Gibellula* on spiders from Taiwan. Mycologia 89: 309–318. https://doi.org/10.1080/00275514.1997.12026787

Vialle A, Feau N, Frey P, Bernier L, Hamelin RC (2013) Phylogenetic species recognition reveals host-specific lineages among poplar rust fungi. Molecular Phylogenetics and Evolution 66: 628–644. https://doi.org/10.1016/j.ympev.2012.10.021

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238–4246. https://doi.org/10.1128/JB.172.8.4238-4246.1990

White TJ, Bruns TD, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ

(Eds) PCR protocols: a guide to methods and applications. San Diego: Academic Press, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wolff JO, Gorb SN (2012) Comparative morphology of pretarsal scopulae in eleven spider families. Arthropod structure & development 41: 419–433. https://doi.org/10.1016/j.asd.2012.04.004

Wolff JO, Nentwig W, Gorb SN (2013) Thegreat silk alternative: multiple co-evolution of web loss and sticky hairs in spiders. PLoS ONE 8: e62682. https://doi.org/10.1371/journal.pone.0062682

World Spider Catalog (2020) World spider catalog. Version 21.0 Natural History Museum Bern, available online at http://wsc.nmbe.ch/

Zou X, Chen WH, Han YF, Liang ZQ (2016) A new species of the genus *Gibellula*. Mycosystema 35: 1161–1168.

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Taxonomy of *Verrucaria* species characterised by large spores, perithecia leaving pits in the rock and a pale thin thallus in Finland

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Abstract

Species of *Verrucaria*, characterised by large spores (at least some spores exceeding 25 µm in length), perithecia leaving pits in the rock and a pale thin thallus, form a taxonomically-difficult and poorly-known group. In this study, such species occurring in Finland are revised, based on ITS sequences and morphology. Maximum likelihood analysis of ITS sequence data was used to examine if the species belong to the *Thelidium* group, as suggested by BLAST search. Twelve species are accepted in Finland: *Verrucaria bifurcata* **sp. nov.**, *V. cavernarum* **sp. nov.**, *V. devergens*, *V. difficilis* **sp. nov.**, *V. foveolata*, *V. fuscozonata* **sp. nov.**, *V. karelica*, *V. kuusamoensis* **sp. nov.**, *V. subdevergens* **sp. nov.**, *V. subjunctiva*, *V. subtilis* and *V. vacillans* **sp. nov.** *Verrucaria foveolata* is nested in *V. subjunctiva* in the phylogeny, but due to morphological and ecogeographical differences, the two taxa are treated as separate species pending further studies. Based on the analysis, the study species belong to the *Thelidium* group. The studied species show a rather high infraspecific morphological, but a low genetic variation. Furthermore, they show considerable overlap in their morphology and many specimens cannot be reliably identified, based on morphology only. All species are restricted to calcareous rocks. *Verrucaria alpigena*, *V. cinereorufa* and *V. hochstetteri* are excluded from the lichen flora of Finland. *Verrucaria grossa* is considered a species with unresolved identity. *Verrucaria foveolata* and *V. subtilis* are rather common on calcareous rocks of Finland while *V. devergens* and *V. kuusamoensis* are restricted to northern Finland. *Verrucaria subjunctiva* occurs mainly in northern Finland. *Verrucaria bifurcata* has been found only from southern Finland. *Verrucaria difficilis* has few localities both in SW and NE Finland. *Verrucaria vacillans* is restricted to calcareous rocks (dolomite) on the mountains of the NW corner of Finland. *Verrucaria fuscozonata*, *V. karelica* and

V. subdevergens occur only in the Oulanka area in NE Finland. A lectotype is designated for *V. subjunctiva*. The morphology of the Finnish species was compared with 51 European species of *Verrucaria* presumably belonging to the *Thelidium* group.

Keywords
Ascomycota, calcareous rocks, DNA barcoding, Europe, ITS, lichenised fungi, taxonomic revision

Introduction

Verrucaria Schrader is a notoriously-difficult group of lichens, which has been proven to be highly polyphyletic (Gueidan et al. 2007, 2009). Numerous species have been previously described from Europe. Due to the high number of described species, one would expect to find a published name for each collected specimen. However, recent studies have shown that this is often not the case. During the past twenty years, twenty-four new species of *Verrucaria* have been described from Europe (Orange 2004, 2013a, 2014; Aptroot and Thüs 2011; Breuss and Berger 2012; Thüs et al. 2015, 2018; Pykälä et al. 2017a, b, 2018, 2019).

Species of *Verrucaria* occurring on calcareous rocks and characterised by pale endolithic or thinly epilithic thallus, large spores (at least some spores exceeding 25 µm in length) and perithecia leaving pits in the rock, form a difficult and poorly-known group of species. Numerous species belonging to this morphogroup have been previously described, mainly from Central and Southern Europe (see, for example, Zschacke 1933; Servít 1948, 1950, 1954). The taxonomy of this morphogroup is highly confusing. Many species have not been reported since their original description and many described species have been supposed to be synonyms or treated as dubious names in need of further study. There is no consensus of the species level taxonomy, but different authors accept different species in this group.

The taxonomy of this morphogroup is rather poorly known also in Fennoscandia and authors have treated the species somewhat differently (see, for example, Vainio 1921; Foucard 2001). The recent Fennoscandian checklist (Nordin et al. 2019) accepts 15 species (potentially) belonging to the group: *V. adelminienii* Zschacke, *V. alpigena* Breuss, *V. caesiopsila* Anzi, *V. cinereorufa* Schaer., *V. devergens* Nyl., *V. dolomitica* (A. Massal.) Kremp., *V. foveolata* (Flörke) A. Massal., *V. grossa* Nyl., *V. hochstetteri* Fr., *V. integra* (Nyl.) Nyl., *V. karelica* Vain., *V. mimicrans* Servít, *V. obscura* Th. Fr. (nom. illeg. non (Sm. & Sowerby) Borrer), *V. papillosa* Ach. and *V. subjunctiva* Nyl. All these species, but *V. obscura*, have been reported from Finland. Four of the species (*V. devergens*, *V. grossa*, *V. karelica* and *V. obscura*) have been described from northern Europe. Thus, only very few species of this morphogroup have been described from northern Europe compared to several dozens of described species from Central Europe. Furthermore, the northern European species have been described a century or more ago and based on somewhat limited field sampling. This suggests that several undescribed species may potentially occur in northern Europe.

The phylogenetic position of large-spored (at least some spores exceeding 25 µm in length) species of *Verrucaria* leaving deep pits in the rock is mainly not known because species of this group are poorly represented in the phylogenetic studies of Verrucariaceae. However, based on the phylogeny of Gueidan et al. (2009), *V. hochstetteri* belongs to the so-called *Thelidium* group. This suggests that species of this morphogroup may belong to the *Thelidium* group.

In this paper, we revise the Finnish species of *Verrucaria* characterised by large spores, thin, predominantly endolithic, thallus and perithecia leaving pits in the rock, using morphology and ITS sequences. We compare the Finnish species with 51 previously-described European species which may presumably belong to the *Thelidium* group, based on their morphology. We also describe seven new species of *Verrucaria* belonging to this group.

Materials and methods

Verrucaria specimens were collected during the large-scale field study of lichens of calcareous rocks and lime quarries in Finland (see Pykälä and Myllys 2016; Pykälä et al. 2017a, b). Type material of 47 relevant species of *Verrucaria* from herbaria B, G, H, H-NYL, M, PRM, S, TUR-V, UPS, VER and W were studied for comparison. Furthermore, the material was compared with four species for which type material could not be located.

Morphology

Perithecia and thalli were hand-sectioned with razor blades. The sections were examined and measured in tap water. Asci and ascospores were also studied in squash preparations of perithecia mounted in water. Sections and squash preparations of old herbarium specimens were studied using potassium hydroxide (KOH, 10% solution). Additionally, involucrellum characters and exciple colour and diameter were studied by cutting perithecia into two pieces and studying the pieces using a binocular microscope.

The range of ascospore size is indicated as arithmetic mean and standard deviation. Minimum and maximum values are given in parentheses. The size of the perithecia (in diameter) is given in surface view. The colour of the wall of the exciple is the colour of the base of the exciple.

DNA extraction and sequencing

Total genomic DNA was extracted from perithecia (1–3) of two- to six-year-old herbarium specimens. Most samples were placed in 96-well microplates and sent to the

Canadian Centre for DNA Barcoding (**CCDB**). CCDB's standard protocols (documentation available at http://ccdb.ca/resources.php) were used for extraction, PCR and sequencing. Primers ITS1-LM (Myllys et al. 1999) and ITS4 (White et al. 1990) were used both for PCR and sequencing of the nuclear ribosomal ITS region. The barcode sequences, their trace files along with all relevant collection data and photographs of the voucher specimens were uploaded to the Barcode of Life Data Systems (BOLD, http://www.boldsystems.org) database. The sequences are available in GenBank (see Table 1 for accession numbers).

The DNA of 25 specimens (26865, 29589, 31528, 32606, 33120, 34601, 35326, 35361, 35857, 35920, 35922, 35930, 35933, 35965, 36222, 36244, 36245, 36254, 36294, 36304, 36308, 36335, 36371, 37331, 39475) was extracted using DNeasy Blood & Tissue kit by Qiagen following the protocol described in Myllys et al. (2011). PCR reactions were prepared using PuReTaq Ready-To-Go PCR beads (GE Healthcare). The 25 µl reaction volume contained 19 µl dH2O, 0.4 µM of each primer and 4 µl extracted DNA. PCR was run under the following conditions: initial denaturation for 5 min at 95 °C followed by five cycles of 30 s at 95 °C (denaturation), 30 s at 58 °C (annealing), and 1 min at 72 °C (extension); in the remaining 35 cycles, the annealing temperature was decreased to 56 °C; the PCR schedule ended with a final extension for 7 min at 72 °C. PCR products were cleaned and sequenced by Macrogen Inc., South Korea (www.macrogen.fi). Primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) were used both for PCR amplification and the sequencing of the ITS regions.

Phylogenetic analyses

The BLAST search facility in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to find the closest relatives for our material. Based on this search, the studied species are most closely related to *Thelidium umbilicatum* Th. Fr. (95% sequence similarity), *Verrucaria deversa* Vain. (94% sequence similarity) and *Polyblastia abscondita* (Nyl.) Arnold (94% sequence similarity). These species belong to the so-called *Thelidium* group which is morphologically variable with regard to thallus structure, perithecium anatomy, spore pigmentation and spore septation (Gueidan et al. 2007, 2009). Consequently, we included 15 species from this group in our phylogeny (Table 1). *Polyblastia albida* Arnold and *P. fuscoargillacea* Anzi from the *Polyblastia* group were used as outgroup because they are closely related to the *Thelidium* group, based on the phylogeny of Gueidan et al. (2009).

A total of 138 ITS sequences were aligned with MUSCLE v.3.8.31 (Edgar 2004) using EMBL-EBI's web service (http://www.ebi.ac.uk/Tools/msa/muscle/). The aligned dataset was subjected to Maximum Likelihood analysis (ML). The analysis was performed with RAxML v.8.1.3 (Stamatakis 2014) located at CSC – IT Center for Science (http://www.csc.fi/english). The ITS region was partitioned into ITS1, 5.8S and ITS2. The GTRGAMMA model was used for all partitions. Node support was estimated with 1000 bootstrap replications using the rapid bootstrap algorithm.

Results

We obtained 119 new nuITS sequences in this study (Table 1). The topology of the ML tree obtained from the ITS dataset is shown in Fig. 1. The Finnish specimens were divided into eleven strongly-supported lineages of which seven are here described as new species: *V. bifurcata*, *V. cavernarum*, *V. difficilis*, *V. fuscozonata* (represented by only one specimen), *V. subdevergens*, *V. kuusamoensis* and *V. vacillans* (see also Fig. 2). In addition to our new species, *V. subtilis*, *V. devergens* and *V. karelica* are monophyletic, whereas the monophyly of either *V. foveolata* or *V. subjunctiva* could not be recovered. Instead, the two species together form a strongly-supported group.

The monophyly of the ingroup was strongly supported, which suggests that all Finnish species in our study are members of the *Thelidium* group *sensu* Gueidan et al. (2009). However, the ITS phylogeny was otherwise poorly resolved. The relationships between the Finnish species remained mostly unclear and only one strongly-supported group was detected: *V. karelica*, *V. subdevergens* and *V. devergens* form a clade. *Verrucaria bifurcata*, *V. difficilis*, *V. cavernarum* and *V. subtilis* also group together, but without any support. *V. calkinsiana* collected in Canada also belongs in this latter clade.

All the studied species had relatively-low infraspecific genetic variation in their ITS sequences, but there seems to be species-specific variation (Table 2). The highest variation was detected in *V. foveolata* with 98.5% sequence similarity. In *V. difficilis* (n = 4) and *V. subdevergens* (n = 3), the sequences were completely identical between the specimens. For comparison, the maximum sequence similarity between closely related *V. devergens* and *V. subdevergens* was 98.7%, but the two species can be separated by the size of the involucrellum (see below for Taxonomy).

Infraspecific morphological variation usually appeared to be rather high. For instance, in most study species, more than one major involucrellum type was detected and infraspecific variation of other perithecium characters was also considerable (Fig. 3, Table 3).

Discussion

Based on the Maximum Likelihood analysis, all the studied species in the morphogroup with large spores, perithecia leaving pits in the rock and a pale thin thallus belong to the *Thelidium* group, but they do not form a monophyletic group. Instead, they are widely distributed within the *Thelidium* group.

Molecular data show that the number of the Finnish species in the morphogroup is higher than previously expected. Similar results have often been obtained from other molecular studies in *Verrucaria* (Orange 2013a; Pykälä et al. 2019), as well as in many other lichen groups (e.g. Kraichak et al. 2015; Jüriado et al. 2017; Launis et al. 2019). We could find previously-published names for only four of the species, even though the type material of 47 previously-described European species, potentially belonging to the *Thelidium* group, was studied. This suggests that Fennoscandian and Central European *Verrucaria* mycobiota largely differ from each other. Similar results have been obtained amongst other previously-studied *Verrucaria* taxa (Pykälä et al. 2017a, b, 2019).

Table 1. Specimens used in the phylogenetic analyses. New sequences are in bold.

Species	Country	Voucher	GenBank accession numbers
Polyblastia abscondita	Sweden	Tibell 23641 (UPS)	EU553507
P. albida	Sweden	Savić 3021 (UPS)	EU553492
P. clandestina	Sweden	Nordin 5466 (UPS)	EU559740
P. fuscoargillacea	Sweden	Palice 7666 (hb. Palice)	EU553498
P. lutosa	Sweden	Savić 3163 (UPS)	EU559734
P. moravica	Sweden	Savić 3154 (UPS)	EU553522
P. nidulans	Sweden	Savić 3015 (UPS)	EU553491
Staurothele rupifraga	Sweden	Savić 3003 (UPS)	EU553490
Thelidium decipiens	Sweden	Tibell 23959 (UPS)	EU553511
T. papulare	UK	Orange 16318 (NMW)	FJ645268
T. pyrenophorum	Sweden	Tibell 23649 (UPS)	EU553500
T. umbilicatum	Sweden	Tibell 23525 (UPS)	EU559737
Verrucaria aethiobola	UK	Orange 16278 (NMW)	FJ664863
V. aethiobola	UK	Orange 16309 (NMW)	FJ664864
V. anziana	UK	Orange 15898 (NMW)	FJ664829
V. anziana	UK	Orange 16103 (NMW)	FJ664830
V. anziana	Sweden	Orange 16377 (NMW)	FJ664831
V. bifurcata	**Finland**	**Pykälä 33120 (H)**	**MT229719**
V. bifurcata	**Finland**	**Pykälä 36722 (H)**	**MT229720**
V. bifurcata	**Finland**	**Pykälä 37228 (H)**	**MT229721**
V. bifurcata	**Finland**	**Pykälä 45762 (H)**	**MT229722**
V. calkinsiana	Canada	McMullin (OAC)	KT695332
V. cavernarum	**Finland**	**Pykälä 34527 (H)**	**MT229723**
V. cavernarum	**Finland**	**Pykälä 37975 (H)**	**MT229724**
V. cavernarum	**Finland**	**Pykälä 41568 (H)**	**MT229725**
V. deversa	Sweden	Savić 3063 (UPS)	EU553496
V. devergens	**Finland**	**Pykälä 35922 (H)**	**MT229726**
V. devergens	**Finland**	**Pykälä 35933 (H)**	**MT229727**
V. devergens	**Finland**	**Pykälä 36220 (H)**	**MT229728**
V. devergens	**Finland**	**Pykälä 36234 (H)**	**MT229729**
V. devergens	**Finland**	**Pykälä 36244 (H)**	**MT229730**
V. devergens	**Finland**	**Pykälä 36245 (H)**	**MT229731**
V. devergens	**Finland**	**Pykälä 36271 (H)**	**MT229732**
V. devergens	**Finland**	**Pykälä 36304 (H)**	**MT229733**
V. devergens	**Finland**	**Pykälä 36344 (H)**	**MT229734**
V. devergens	**Finland**	**Pykälä 39898 (H)**	**MT229735**
V. devergens	**Finland**	**Pykälä 39901 (H)**	**MT229736**
V. devergens	**Finland**	**Pykälä 43421 (H)**	**MT229737**
V. devergens	**Finland**	**Pykälä 44042 (H)**	**MT229738**
V. devergens	**Finland**	**Pykälä 44914 (H)**	**MT229739**
V. devergens	**Finland**	**Pykälä 45090 (H)**	**MT229740**
V. devergens	**Finland**	**Pykälä 45367 (H)**	**MT229741**
V. difficilis	**Finland**	**Pykälä 32687 (H)**	**MT229742**
V. difficilis	**Finland**	**Pykälä 39060 (H)**	**MT229743**
V. difficilis	**Finland**	**Pykälä 41859 (H)**	**MT229744**
V. difficilis	**Finland**	**Pykälä 44811 (H)**	**MT229745**
V. foveolata	**Finland**	**Pykälä 31528 (H)**	**MT229746**
V. foveolata	**Finland**	**Pykälä 34953 (H)**	**MT229747**
V. foveolata	**Finland**	**Pykälä 35395 (H)**	**MT229748**
V. foveolata	**Finland**	**Pykälä 35965 (H)**	**MT229749**
V. foveolata	**Finland**	**Pykälä 37728 (H)**	**MT229750**
V. foveolata	**Finland**	**Pykälä 38119 (H)**	**MT229751**

Species	Country	Voucher	GenBank accession numbers
V. foveolata	**Finland**	**Pykälä 38719 (H)**	**MT229752**
V. foveolata	**Finland**	**Pykälä 39028 (H)**	**MT229753**
V. foveolata	**Finland**	**Pykälä 39294 (H)**	**MT229754**
V. foveolata	**Finland**	**Pykälä 40195 (H)**	**MT229755**
V. foveolata	**Finland**	**Pykälä 44553 (H)**	**MT229756**
V. foveolata	**Finland**	**Pykälä 44952 (H)**	**MT229757**
V. fuscozonata	**Finland**	**Pykälä 36222 (H)**	**MT229758**
V. karelica	**Finland**	**Pykälä 39625 (H)**	**MT229759**
V. karelica	**Finland**	**Pykälä 39991 (H)**	**MT229760**
V. karelica	**Finland**	**Pykälä 40235 (H)**	**MT229761**
V. karelica	**Finland**	**Pykälä 40325 (H)**	**MT229762**
V. kuusamoensis	**Finland**	**Pykälä 35710 (H)**	**MT229763**
V. kuusamoensis	**Finland**	**Pykälä 35857 (H)**	**MT229764**
V. kuusamoensis	**Finland**	**Pykälä 35920 (H)**	**MT229765**
V. kuusamoensis	**Finland**	**Pykälä 36254 (H)**	**MT229766**
V. kuusamoensis	**Finland**	**Pykälä 36294 (H)**	**MT229767**
V. kuusamoensis	**Finland**	**Pykälä 36335 (H)**	**MT229768**
V. kuusamoensis	**Finland**	**Pykälä 39052 (H)**	**MT229769**
V. kuusamoensis	**Finland**	**Pykälä 39900 (H)**	**MT229770**
V. kuusamoensis	**Finland**	**Pykälä 40219 (H)**	**MT229771**
V. kuusamoensis	**Finland**	**Pykälä 44563 (H)**	**MT229772**
V. kuusamoensis	**Finland**	**Pykälä 44570 (H)**	**MT229773**
V. kuusamoensis	**Finland**	**Pykälä 44694 (H)**	**MT229774**
V. kuusamoensis	**Finland**	**Pykälä 44696 (H)**	**MT229775**
V. kuusamoensis	**Finland**	**Pykälä 44703 (H)**	**MT229776**
V. kuusamoensis	**Finland**	**Pykälä 44744 (H)**	**MT229777**
V. kuusamoensis	**Finland**	**Pykälä 44980 (H)**	**MT229778**
V. kuusamoensis	**Finland**	**Pykälä 45231 (H)**	**MT229779**
V. kuusamoensis	**Finland**	**Pykälä 45330 (H)**	**MT229780**
V. latebrosa	Switzerland	Thues W1135	EU249473
V. latebrosa	Switzerland	Thues W1097	EU249474
V. subdevergens	**Finland**	**Pykälä 39128 (H)**	**MT229781**
V. subdevergens	**Finland**	**Pykälä 44550 (H)**	**MT229782**
V. subdevergens	**Finland**	**Pykälä 45109 (H)**	**MT229783**
V. subjunctiva	**Finland**	**Pykälä 35326 (H)**	**MT229784**
V. subjunctiva	**Finland**	**Pykälä 35361 (H)**	**MT229785**
V. subjunctiva	**Finland**	**Pykälä 35930 (H)**	**MT229786**
V. subjunctiva	**Finland**	**Pykälä 36308 (H)**	**MT229787**
V. subjunctiva	**Finland**	**Pykälä 36371 (H)**	**MT229788**
V. subjunctiva	**Finland**	**Pykälä 37746 (H)**	**MT229789**
V. subjunctiva	**Finland**	**Pykälä 39475 (H)**	**MT229790**
V. subjunctiva	**Finland**	**Pykälä 39478 (H)**	**MT229791**
V. subjunctiva	**Finland**	**Pykälä 39491 (H)**	**MT229792**
V. subjunctiva	**Finland**	**Pykälä 39803 (H)**	**MT229793**
V. subjunctiva	**Finland**	**Pykälä 40284 (H)**	**MT229794**
V. subjunctiva	**Finland**	**Pykälä 42392 (H)**	**MT229795**
V. subjunctiva	**Finland**	**Pykälä 42406 (H)**	**MT229796**
V. subjunctiva	**Finland**	**Pykälä 42419 (H)**	**MT229797**
V. subjunctiva	**Finland**	**Pykälä 42510 (H)**	**MT229798**
V. subjunctiva	**Finland**	**Pykälä 44671 (H)**	**MT229799**
V. subjunctiva	**Finland**	**Pykälä 44734 (H)**	**MT229800**
V. subjunctiva	**Finland**	**Pykälä 44881 (H)**	**MT229801**
V. subtilis	**Finland**	**Pykälä 26865 (H)**	**MT229802**
V. subtilis	**Finland**	**Pykälä 29589 (H)**	**MT229803**

Species	Country	Voucher	GenBank accession numbers
V. subtilis	Finland	Pykälä 32606 (H)	MT229804
V. subtilis	Finland	Pykälä 32749 (H)	MT229805
V. subtilis	Finland	Pykälä 34601 (H)	MT229806
V. subtilis	Finland	Pykälä 35093 (H)	MT229807
V. subtilis	Finland	Pykälä 36819 (H)	MT229808
V. subtilis	Finland	Pykälä 37102 (H)	MT229809
V. subtilis	Finland	Pykälä 37329 (H)	MT229810
V. subtilis	Finland	Pykälä 37331 (H)	MT229811
V. subtilis	Finland	Pykälä 37794 (H)	MT229812
V. subtilis	Finland	Pykälä 38140 (H)	MT229813
V. subtilis	Finland	Pykälä 39870 (H)	MT229814
V. subtilis	Finland	Pykälä 40280 (H)	MT229815
V. subtilis	Finland	Pykälä 40596 (H)	MT229816
V. subtilis	Finland	Pykälä 40833 (H)	MT229817
V. subtilis	Finland	Pykälä 40859 (H)	MT229818
V. subtilis	Finland	Pykälä 40874 (H)	MT229819
V. subtilis	Finland	Pykälä 41857 (H)	MT229820
V. subtilis	Finland	Pykälä 42225 (H)	MT229821
V. subtilis	Finland	Pykälä 42540 (H)	MT229822
V. subtilis	Finland	Pykälä 44843 (H)	MT229823
V. subtilis	Finland	Pykälä 44844 (H)	MT229824
V. subtilis	Finland	Pykälä 45794 (H)	MT229825
V. subtilis	Finland	Pykälä 45817 (H)	MT229826
V. subtilis	Finland	Pykälä 45847 (H)	MT229827
V. vacillans	Finland	Pykälä 43058 (H)	MT229828
V. vacillans	Finland	Pykälä 43118 H)	MT229829
V. vacillans	Finland	Pykälä 43232 (H)	MT229830
V. vacillans	Finland	Pykälä 43272 (H)	MT229831
V. vacillans	Finland	Pykälä 43296 (H)	MT229832
V. vacillans	Finland	Pykälä 43302 (H)	MT229833
V. vacillans	Finland	Pykälä 43384 (H)	MT229834
V. vacillans	Finland	Pykälä 44075 (H)	MT229835
V. vacillans	Finland	Pykälä 44081 (H)	MT229836
V. vacillans	Finland	Pykälä 44081b (H)	MT229837

Of the new species, *Verrucaria bifurcata*, *V. cavernarum*, *V. difficilis* and *V. subtilis* form a weakly-supported group of closely-related species (*V. subtilis* complex). Similarly, *V. devergens*, *V. karelica* and *V. subdevergens* are closely related and belong to the so-called *V. devergens* complex. The species in both complexes can be seen as examples of cryptic species: while they are genetically distinct, there are no clear morphological differences that can used to separate between different lineages (see Crespo and Lumbsch 2010). However, there seems to be some ecogeographical differences between the species (as discussed in the Taxonomy section). Furthermore, there are differences in the infraspecific morphological variation between the species.

Verrucaria foveolata and *V. subjunctiva* do not differ in their ITS sequences, even if the species are usually identifiable, based on morphology. Furthermore, the two species have ecological and geographical differences in Finland, as discussed below. Thus, we prefer to treat them as different species pending further study using other molecular markers. It is generally acknowledged that ITS sometimes fails to separate closely-

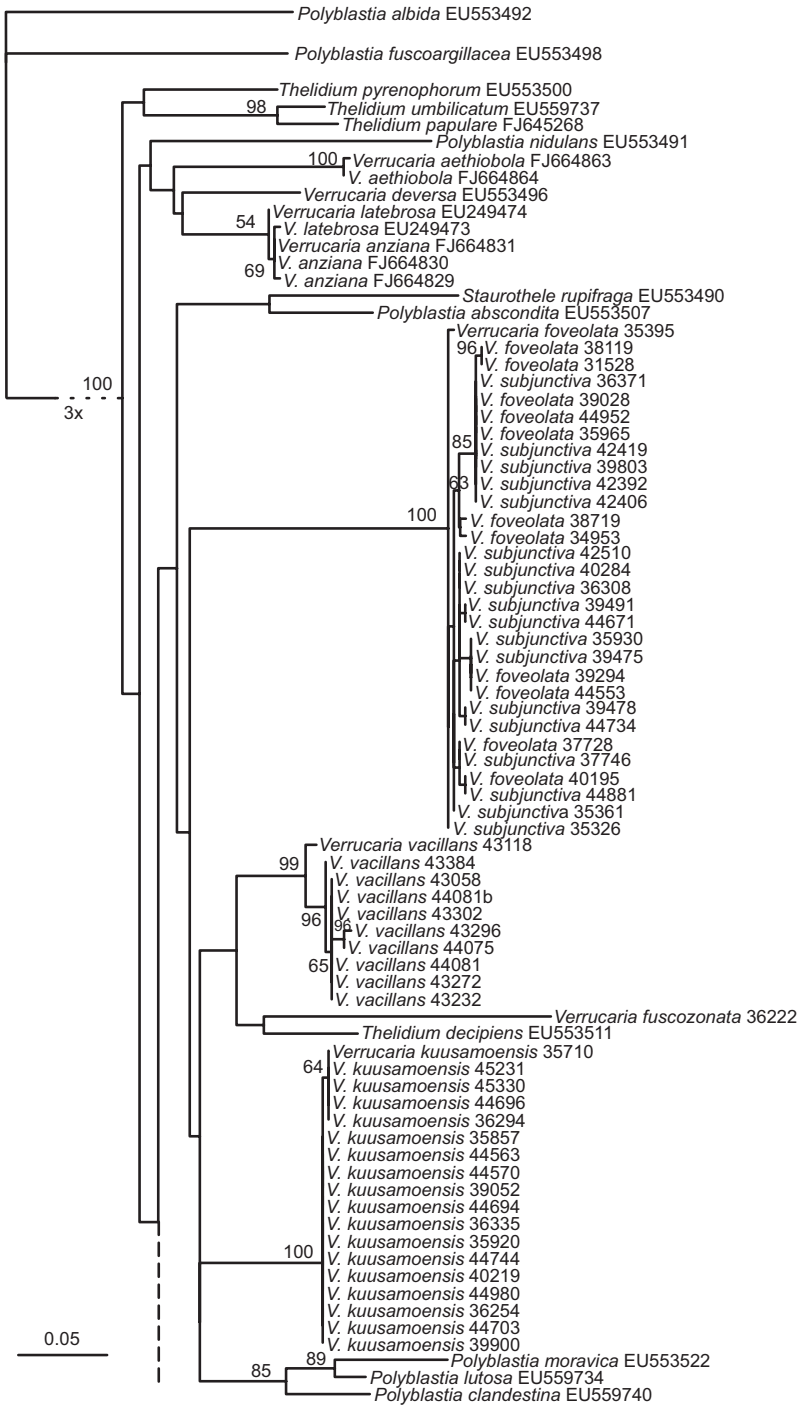

Figure 1. Phylogenetic relationships of *Verrucaria* with large spores, perithecia leaving pits in the rock and pale thin thallus belonging to the *Thelidium* group. A Maximum Likelihood phylogram obtained from the RAxML analysis is based on the ITS dataset. Bootstrap values (> 50%) are shown at nodes. The node leading to the ingroup is shortened and is in reality three times longer.

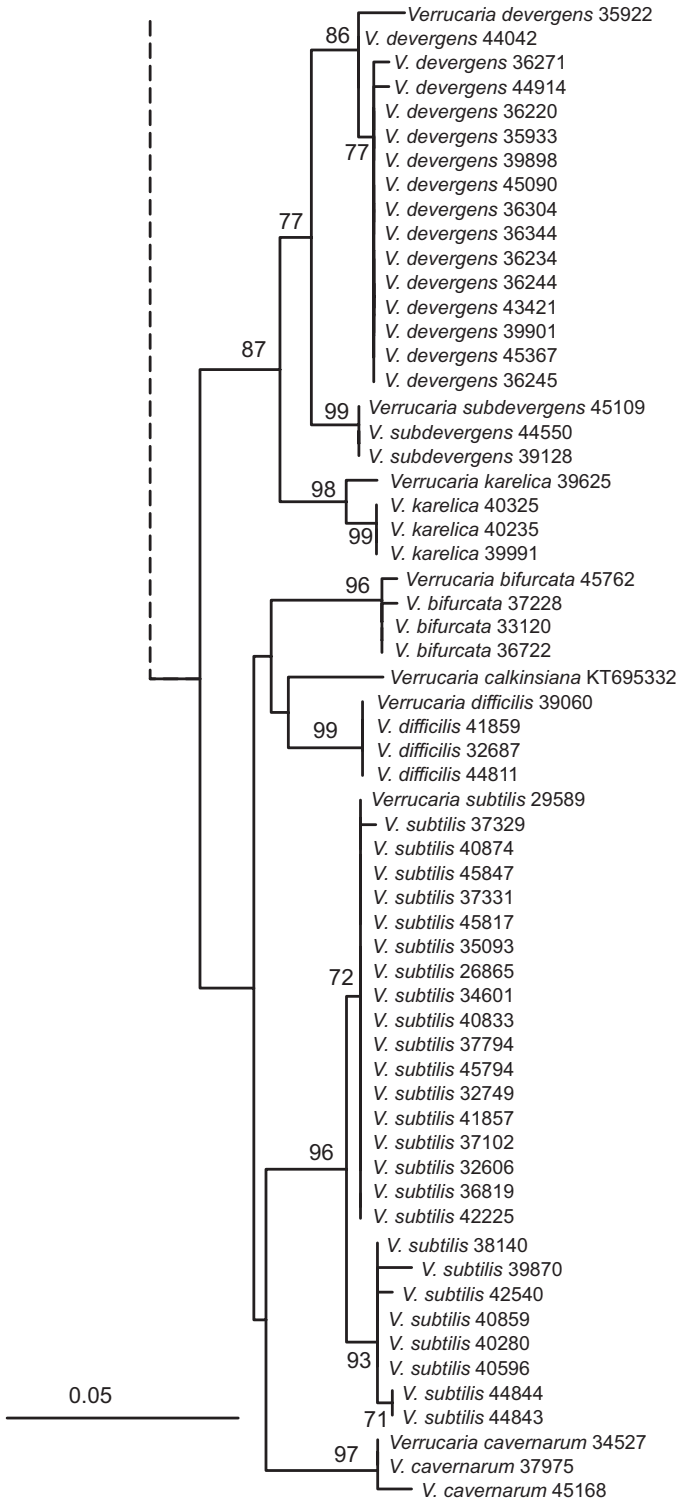

Figure 1. Continued.

Table 2. Minimum infraspecific sequence similarity of the ITS region of the species. n = number of studied specimens.

	n	Minimum sequence similarity
V. bifurcata	4	99.6%
V. cavernarum	3	99.5%
V. devergens	16	98.9%
V. difficilis	4	100%
V. foveolata	12	98.5%
V. karelica	4	99.1%
V. kuusamoensis	18	99.8%
V. subdevergens	3	100%
V. subjunctiva	18	98.7%
V. subtilis	26	98.7%
V. vacillans	10	98.6%

Table 3. The main perithecium characters of the study species. Per = Perithecia size (mm), Inv = Involucrellum: ab = absent, ap = apical, ce = covering half of the exciple, bl = to the exciple base level, ee = enveloping the exciple, Invthick = Involucrellum thickness (mm), Exc = Exciple size in diameter (mm), Spores = Ascospore size (mm), minimum, mean and maximum values.

Species	Per	Inv	Invthick	Exc	Spores
V. bifurcata	0.13–0.26	ab, ap, bl, ee	0–60	0.18–0.27	21–26–30 × 9–11–13
V. cavernarum	0.15–0.28	ap, ce	30–60	0.16–0.32	23–28–34 × 10–12–14
V. devergens	0.13–0.40	ab, ap, ce	0–80	0.20–0.35	20–27–35 × 10–13–16
V. difficilis	0.18–0.36	ce, bl	40–70	0.16–0.28	23–27–34 × 10–11–13
V. foveolata	0.11–0.42	ab, ap, ce	0–60	0.19–0.42	24–30–37 × 10–13–17
V. fuscozonata	0.11–0.26	bl	50–60	0.18–0.25	21–26–29 × 10–12–13
V. karelica	0.07–0.37	ap, ce	50–70	0.21–0.28	23–28–31 × 10–12–14
V. kuusamoensis	0.17–0.45	ce, bl, ee	30–80	0.19–0.29	21–28–34 × 9–12–14
V. subdevergens	0.21–0.42	ce, bl, ee	30–80	0.21–0.34	23–28–35 × 11–13–15
V. subjunctiva	0.16–0.45	ce, bl, ee	40–100	0.20–0.36	23–30–40 × 12–14–17
V. subtilis	0.15–0.44	ap, ce	30–80	0.16–0.33	20–25–31 × 8–10–13
V. vacillans	0.15–0.47	ap, ce, bl	30–90	0.15–0.26	18–25–32 × 8–12–15

related species of lichens (see, for instance, Leavitt et al. 2013; Pino-Bodas et al. 2013; Magain and Sérusiaux 2015).

We included multiple specimens per species in our study to examine genetic and morphological infraspecific variation. Interestingly, in most of the species, we found one or a few specimens that differed morphologically from the other specimens and could not be reliably identified at species level. This suggests that a rather high number of specimens needs to be sequenced to cover the infraspecific morphological variation of the species. Even if the studied species are characterised by a high infraspecific morphological variation and even overlap in morphology, the infraspecific variation in the ITS sequence is rather low. This result is similar to the recently analysed *Verrucaria kalenskyi* – *V. xyloxena* complex (Pykälä et al. 2019). The results suggest that reliable identification of the studied species, based on morphology, is often not possible, especially if a specimen lacks well-developed spores. Particularly, specimens with unusually small or large spores or with involucrellum deviating from normal are easily misidentified.

The studied species show, on average, differences in several morphological characters, but there is a considerable overlap in all these characters between different species. Such semi-cryptic species may be common in *Verrucaria* and related genera (Orange 2012, 2013a, 2014; Thüs et al. 2015; Pykälä et al. 2017b, 2018, 2019).

For example, the occurrence of dark lines between contiguous conspecific thalli varies between the species. Such lines are common in *V. vacillans*, fairly common in the *V. devergens* complex, infrequent in *V. kuusamoensis* and absent in *V. foveolata*, *V. subjunctiva* and in the *V. subtilis* complex.

The study group is characterised by a predominance of a dark exciple wall. In most species, pale exciple walls have not been seen. Pale exciples are rather common only in *V. subtilis*, although most specimens have only dark exciples. In *V. kuusamoensis*, over 95% of the specimens have only dark exciples, but very few specimens (two confirmed by ITS) include only or also pale exciples.

The occurrence of a halonate perispore has been confirmed for all studied Finnish species, but *V. karelica*. However, many specimens were studied when a few years (3–6 years) old. Then the occurrence of the halonate perispore was often not confirmed. In specimens that are a few years old, a halonate perispore was seen only in few spores or it was not found. It remains to be studied whether a halonate perispore can always be detected in fresh material.

Most study species have a northern distribution in Finland. The result was unexpected, because most previously-described species in the morphogroup are from Central Europe. This result suggests that most Finnish species may be restricted to the boreal and arctic zones or be at least rare south of the boreal zone. Dolomite rocks in the Oulanka area in the biogeographical Province of Koillismaa have the highest species richness of large-spored *Verrucaria* leaving pits in the rock. *Verrucaria subtilis* and *V. bifurcata* may be the only species in the group which seem to be more common in southern than in northern Finland and the latter species may possibly occur only in southern Finland.

Taxonomy

Species descriptions are based on the Finnish sequenced specimens.

Verrucaria bifurcata **Pykälä, Kantelinen & Myllys, sp. nov.**
MycoBank No: 835669
Fig. 2A

Diagnosis. Species characterised by pale, usually endolithic thallus, perithecia leaving shallow to deep pits in the rock, very variable involucrellum appressed to the exciple and ascospores (21–)24–28(–30) × (9–)10–12(–13) mm, morphologically rather similar to the other Finnish species of the *V. subtilis* complex, but the ITS sequence divergence between the species is 1.7–3.9%.

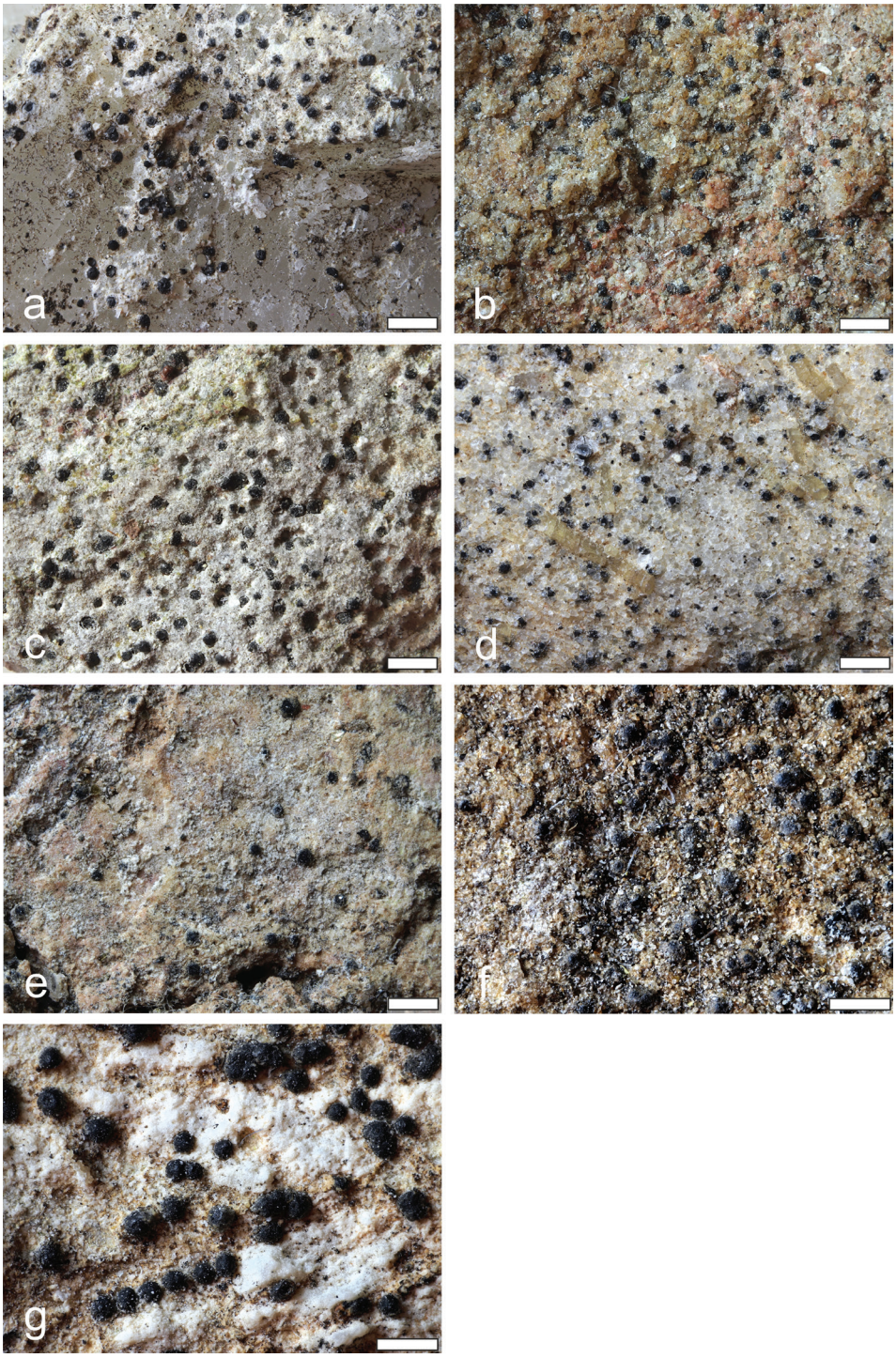

Figure 2. Habitus of the new *Verrucaria* species **A** *V. bifurcata* (holotype) **B** *V. cavernarum* (holotype) **C** *V. difficilis* (holotype) **D** *V. fuscozonata* (holotype) **E** *V. kuusamoensis* (holotype) **F** *V. subdevergens* (holotype) **G** *V. vacillans* (holotype). Scale bars: 1 mm (**A–D**), 0.5 mm (**E–G**).

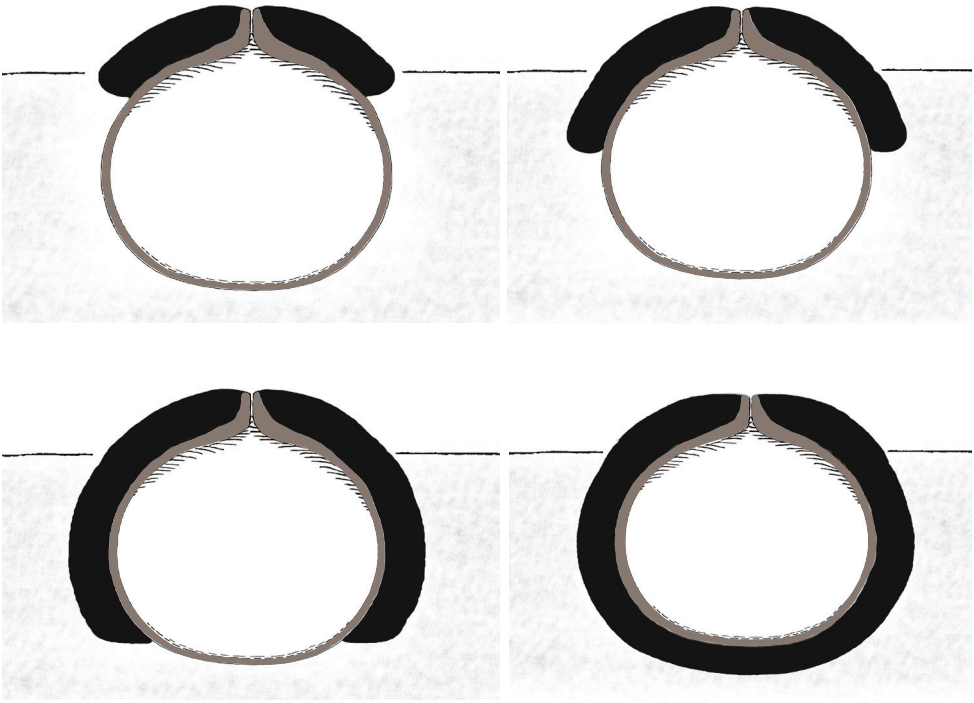

Figure 3. Schematic drawings of sections of perithecia of the study species **A** involucrellum apical **B** involucrellum covering half of the exciple **C** involucrellum reaching the exciple base level **D** involucrellum enveloping the exciple.

Holotype. Finland. Varsinais-Suomi, Länsi-Turunmaa (Parainen), Ersby, 150 m SE of Stormossen, abandoned lime quarry, quarry waste hill, S-slope, on pebbles, 27 m alt., 60°17'N, 22°15'E, 3 Sept 2009 J. Pykälä 36722 (H9205739, GenBank accession number: MT229720).

Description. Prothallus absent. Thallus white, grey or pale greyish-brown, endolithic or thinly epilithic, continuous or small patches surrounding perithecia, ca. 20–60 mm thick, algal cells (4–)5–8 mm. Perithecia 0.13–0.26 mm in diam., (1/2–)3/4(–1)–immersed, leaving shallow to deep pits in the rock, sometimes thinly thalline covered; 80–140 perithecia/cm^2. Ostiole inconspicuous, tiny, pale or dark, plane or depressed, ca. 20–30 mm wide. Involucrellum absent, apical, to the exciple base level or enveloping the exciple, 20–60 mm thick, appressed to the exciple. Exciple 0.18–0.27 mm in diam., wall dark brown or black, ca. 20–30 mm thick. Periphysoids ca. 25–35 × 1.5–2.5 mm. Asci 60–104 × 22–33 mm, 8-spored. Ascospores 0-septate, (20.6–)23.8–25.8–27.8(–30.3) × (8.7–)10.0–11.0–12.0(–12.9) mm (n = 117), perispore 1–1.5 mm thick.

Habitat and distribution. All finds are from lime quarries or road cuttings of calcareous rocks. The species seems to prefer pebbles and stones in lime quarries. It occurs

both in sun-exposed and rather shady habitats. The specimens are from SW and SE Finland. This suggests that *V. bifurcata* has a southern distribution in Finland.

Etylomogy. The epithet refers to the dualistic nature of the involucrellum of the species: absent or short vs. long or enveloping the exciple.

Other specimens examined. Finland. Varsinais-Suomi, Särkisalo, Förby, E of Vähämaankaula, abandoned lime quarry, beneath NW-facing wall, on stone, 7 m alt., 60°05'N, 22°52'E, 23 July 2008, J. Pykälä 33120 (H); Länsi-Turunmaa (Parainen), Simonby, Gropen, abandoned lime quarry, road cutting of calciferous rock, on pebbles, 15 m alt., 60°16'N, 22°13'E, 16 Sept 2009, J. Pykälä 37228 (H); Etelä-Savo, Kerimäki, Ruokojärvi, Pitkäniemi, abandoned lime quarry, on NE-facing wall, 90 m alt., 61°56'N, 29°00'E, 15 Sept 2011, J. Pykälä 45762 (H).

Notes. *Verrucaria bifurcata* is a somewhat puzzling species as it has a very variable involucrellum. Two specimens are characterised by an absent or small involucrellum and two by a deep reaching involucrellum. In the former case, the involucrellum varies within a specimen from absent to apical. In the latter case, the involucrellum extends to the exciple base level or envelopes the exciple. *Verrucaria bifurcata* cannot be identified with certainty without ITS sequencing. Nevertheless, it shows morphological variation differing from the other species in the *V. subtilis* complex. *Verrucaria bifurcata* is the only species in the *V. subtilis* complex in which involucrellum may be absent or enveloping the exciple. In *V. bifurcata*, the involucrellum is always tightly appressed to the exciple and sometimes it is difficult to find out whether the involucrellum is absent or enveloping the exciple. The specimen 45762 was originally identified as *V. adelminienii* Zschacke (Pykälä 2013). However, the type of *V. adelminienii* is not identifiable (Pykälä 2016). Furthermore, the spore size in the original description (Zschacke 1933) is smaller than the spore size in the Finnish specimen.

***Verrucaria cavernarum* Pykälä & Myllys, sp. nov.**
MycoBank No: 835670
Fig. 2B

Diagnosis. Species morphologically somewhat similar to *V. subtilis*, ascospores slightly larger: (23–)25–30(–34) × (10–)11–13(–14) mm and the ITS sequence divergence between the species is 2.8–3.4%.

Holotype. Finland. Koillismaa, Kuusamo, Oulanka National Park, Mataraniemi, shore of Oulankajoki river, treeless stony river shore, on dolomite stones, 145 m alt., 66°22'N, 29°20'E, 26 Aug 2011, J. Pykälä 45168 (H9205102, GenBank accession number: MT229725).

Description. Prothallus absent. Thallus grey to pale greyish-brown, endolithic or thinly epilithic, continuous, 20–80 mm thick, algal cells 5–8 mm. Perithecia 0.15–0.28 mm in diam., 1/2–1-immersed, leaving shallow to deep pits in the rock, often surrounded by thallus collar, few perithecia thinly thalline covered; 80–160 perithecia/ cm^2. Ostiole inconspicuous, dark, plane or depressed. Involucrellum apical to covering half of the exciple, in one specimen also few longer involucrella almost reaching the exciple

base level present, 30–60 mm thick, appressed to the exciple or slightly diverging from the exciple. Exciple 0.16–0.32 mm, wall dark brown or black, ca. 15–25 mm thick. Periphysoids (25–)30–40(–50) × 1.5–2.5 mm, branching. Asci 8-spored. Ascospores 0-septate, two 1-septate spores seen in one specimen, (23.1–)25.1–27.5–29.8(–34.1) × (9.8–)10.7–11.6–12.6(–13.7) mm (n = 111), perispore 1 mm thick.

Habitat and distribution. Two specimens of the species are from SW Finland and one specimen from NE Finland. The three sequenced specimens are from different kinds of habitats: dolomite stone on river shore (apparently periodically submerged), calcareous rock on seashore (perhaps not submerged) and in a lime quarry on pebbles. The species may prefer more humid (but preferably sun-exposed?) habitats than the other species in the *V. subtilis* complex.

Etymology. The perithecia of the species leave shallow to deep pits in the rock when decayed.

Other specimens examined. Finland. Varsinais-Suomi, Raasepori (Karjaa), Knapsby, Mustio lime quarry, deciduous forest on lime quarry waste, on pebbles, 45 m alt., 60°10'N, 23°49'E, 2 July 2009, J. Pykälä 34527 (H); Länsi-Turunmaa (Iniö), Söderby, Biskopsö island, calcareous rock outcrop on shore of the Baltic Sea, on N-slope, scarce, 7 m alt., 60°20'N, 21°28'E, 9 June 2010, J. Pykälä 37975 (H).

Notes. The species cannot be morphologically separated with certainty from the other species of the *V. subtilis* complex. It is most difficult to separate from *V. subtilis*. On average, *V. cavernarum* has slightly longer (mean 2.3 mm longer than in *V. subtilis*) and broader (mean 1.1 mm broader than in *V. subtilis*) spores and pale exciples have not been found.

Verrucaria devergens Nyl., Flora 55: 362, 1872 (as *V. divergens* Nyl., a typographic error)

Type. [Russia,] Suojärvi, ad saxa calcarea Pöpönsaari, 1870, Norrlin (H!, H-NYL 3036a!, syntypes).

Description. Prothallus absent. Thallus white, grey or pale brown, endolithic, rarely epilithic (two sequenced specimens), thin, continuous, algal cells 5–8 mm, occasionally (three sequenced specimens) contiguous conspecific thalli separated by dark brown lines, 0.13–0.22 mm wide. Perithecia 0.13–0.40 mm in diam., (1/4–)1/2–1-immersed, leaving shallow to deep pits in the rock, few perithecia occasionally not leaving pits, often surrounded by a thalline collar, sometimes thinly thalline covered; 50–140 perithecia/cm^2. Ostiole usually inconspicuous, pale or dark, plane or depressed, ca. 20–50 mm wide. Involucrellum absent or apical, short, rarely covering half of the exciple (two sequenced specimens), (40–)50–80 mm thick, appressed to the exciple or diverging from the exciple. Exciple 0.20–0.35 mm in diam., wall dark brown or black, ca. 27–40 mm thick, apex thickened to ca. 50–100 mm thick if the involucrellum is absent. Periphysoids ca. 30–50(–60) × 1–2.5 mm, branching or branched-anastomosing. Ascospores 0-septate, (20.2–)24.6–27.4–30.2(–34.8) × (10.2–)11.7–12.6–13.5(–15.7) mm (n = 281), perispore 1 mm thick.

Habitat and distribution. The species is a strict calcicole occurring on calcareous rocks. It may prefer fairly humid habitats. *Verrucaria devergens* seems to be able to tolerate moderate flooding and it also grows on subaquatic calcareous rocks on river shores in the Oulanka area. It is not rare on dolomite rocks in the Oulanka and Kilpisjärvi areas in northern Finland, but seems to be absent from southern Finland.

Other specimens examined. Finland. Koillismaa, Kuusamo, Oulanka National Park, Pikkukönkäänkuru, *Pinus sylvestris*-dominated forest, SW-slope, on dolomite stones, 178 m alt., 66°21'N, 29°19'E, 8 Aug 2009, J. Pykälä 35922 (H); Kuusamo, Oulanka National Park, Pikkukönkäänkuru, dolomite rock crop, on overhanging SW-facing wall, 173 m alt., 66°21'N, 29°19'E, 8 Aug 2009, J. Pykälä 35933 (H); Kuusamo, Oulanka National Park, Pikkuköngäs, N shore of river Oulankajoki, dolomite rock outcrop, on SW-facing wall, 160 m alt., 66°22'N, 29°19'E, 12 Aug 2009, J. Pykälä 36220 (H), 36244 (H), 36245 (H); Kuusamo, Oulanka National Park, Pikkuköngäs, N shore of river Oulankajoki, dolomite rock outcrop, stony shore, on stones, 160 m alt., 66°22'N, 29°19'E, 12 Aug 2009, J. Pykälä 36234 (H); Kuusamo, Oulanka National Park, Pikkuköngäs, N shore of river Oulankajoki, dolomite rock outcrop, on 1 m high SW-facing wall, 160 m alt. 66°22'N, 29°19'E, 12.VIII.2009, J. Pykälä 36271 (H); Kuusamo, Oulanka National Park, Kiutaköngäs, N shore of river Oulankajoki, dolomite rock outcrop, on SE-slope, 150 m alt., 66°22'N, 29°20'E, 12 Aug 2009, J. Pykälä 36304 (H); Kuusamo, Oulanka National Park, Kiutaköngäs, by the rapids, S shore of Oulankajoki river, calciferous (dolomite) schistose rock outcrop, NE-slope, on E-facing wall, 152 m alt., 66°22'N, 29°19'E, 13 Aug 2010, J. Pykälä 39898 (H); Kuusamo, Oulanka National Park, Kiutaköngäs, by the rapids, S shore of Oulankajoki river, calciferous (dolomite) schistose rock outcrop, on gentle NE-slope, 152 m alt., 66°22'N, 29°19'E, 13 Aug 2010, J. Pykälä 39901 (H); Kuusamo, Oulanka National Park, Taivalköngäs, shore of Oulankajoki river, stony river shore, on dolomite stone, 170 m alt., 66°24'N, 29°11'E, 25 Aug 2011, J. Pykälä 45090 (H); Kuusamo, Oulanka National Park, Mataraniemi W, shore of Oulankajoki river, small dolomite rock outcrop, on 40 cm high SE-facing wall, 145 m alt., 66°22'N, 29°20'E, 28 Aug 2011, J. Pykälä 45367 (H); Salla, Oulanka National Park, 400 m N of Savilampi, shore of river Savinajoki, cliff, dolomite rock outcrop, on overhanging NE-facing wall, 177 m alt. 66°25'N, 29°10'E, 13 Aug 2009, J. Pykälä 36344 (H); Salla, Oulanka National Park, Savilampi 1.4 km NE, shore of Savinajoki river, dolomite rock outcrop, SE-slope, on dolomite boulder, 184 m alt., 66°26'N, 29°11'E, 23 Aug 2011, J. Pykälä 44914 (H); Enontekiön Lappi, Enontekiö, Porojärvet, Toskalharji, Toskaljärvi N, fell, gentle SW-slope, dolomite scree, on dolomite boulders , 710 m alt., 69°11'N, 21°26'E, 3 Aug 2011, J. Pykälä 43421 (H); Enontekiö, Kilpisjärvi, Saana, nature reserve, E-part, fell, dolomite rock outcrop, on SW-facing wall, 880 m alt., 69°02'N, 20°50'E, 10 Aug 2011, J. Pykälä 44042 (H).

Notes. Based on the ITS phylogeny, *V. devergens*, *V. karelica* and *V. subdevergens* are very closely related. They are here considered as distinct species, based on the ITS phylogeny and because of a barcoding gap between the species. *Verrucaria devergens* is morphologically more variable than previously known (Pykälä 2007).

Usually, the species has no involucrellum, but the apex of the exciple is thickened. However, specimens with an apical involucrellum, as well as two specimens in which the involucrellum covers half of the exciple, have an identical ITS sequence compared to the typical *V. devergens*. Typically, *V. devergens* has perithecia varying from half-immersed to immersed in the same specimen, but in some specimens, the perithecia are 3/4–1-immersed, while in a few others, they are 1/4–1/2-immersed.

Verrucaria devergens is difficult to separate from *V. foveolata*, *V. karelica* and *V. subdevergens*. *V. devergens* and *V. foveolata* show similar variation in the involucrellum, i.e. absent or apical. *Verrucaria foveolata* has larger spores, but there is a wide overlap in the spore size. *Verrucaria foveolata* usually has immersed perithecia, while *V. devergens* has 1/2–1-immersed perithecia. However, some specimens of *V. devergens* are similar to *V. foveolata* in having 3/4–1-immersed perithecia. No consistent morphological differences were found between *V. devergens* and *V. karelica*, although all specimens of *V. karelica* have an involucrellum. *Verrucaria karelica* may have more often an epilithic thallus and dark lines between contiguous conspecific thalli. *Verrucaria subdevergens* has a longer involucrellum than *V. devergens* in all studied specimens predominantly exceeding half of the exciple.

Specimens of *V. devergens* with untypically deep reaching involucrellum may be difficult to separate from *V. kuusamoensis* and *V. subtilis*. *Verrucaria kuusamoensis* tend to have a smaller exciple and shorter periphysoids, the thallus is usually epilithic and the involucrellum usually exceeds half of the exciple. *Verrucaria subtilis* has thinner and smaller exciple and, on average, smaller spores. In some specimens of *V. subtilis*, pale exciples are present, while they have never been found from *V. devergens*.

Verrucaria difficilis **Pykälä & Myllys, sp. nov.**
MycoBank No: 835671
Fig. 2C

Diagnosis. Species characterised by perithecia 1/4–3/4-immersed, leaving usually shallow pits, involucrellum covering half of the exciple or almost to the exciple base, ascospores (23–)25–29(–34) × (10–)11–12(–13) mm, morphologically rather similar to the other Finnish species of the *V. subtilis* complex, but the sequence divergence in ITS 1.7–2.6%.

Holotype. Finland, Varsinais-Suomi, Karkkila, Haavisto, 100 m S of E-part of Iitalammi, S-slope, clear cut herb-rich forest, on calcareous stone, 60°31'N, 24°23'E, 123 m alt., 7 June 2008 J. Pykälä 32687 (H9205096, GenBank accession number: MT229742).

Description. Prothallus absent. Thallus white or grey, inconspicuous, endolithic to thinly epilithic, continuous to irregularly rimose, ca. 20–80 mm thick, algal cells 5–7(–8) mm. Perithecia 0.18–0.36 mm in diam., 1/4–3/4(–1)–immersed, leaving shallow to more rarely deep pits in the rock, often thinly thalline covered except apex; 60–160 perithecia/cm^2. Ostiole inconspicuous, tiny, pale to usually dark, plane or

depressed, ca. 20–30 mm wide. Involucrellum covering half of the exciple or almost to the exciple base, 40–70 mm thick, appressed to the exciple or slightly or moderately diverging from it. Exciple 0.16–0.28 mm in diam., wall dark brown, ca. 20–25 mm thick. Periphysoids (20–)25–35(–40) × 1.5–2.5 mm, some branching. Asci 77–101 × 23–28 mm, 8-spored. Ascospores (22.7–)25.1–27.0–28.9(–33.6) × (9.6–)10.6–11.4–12.3(–13.3) (n = 78), perispore 1 mm thick.

Habitat and distribution. Four sequenced specimens occur: two from SW Finland and two from NE Finland. The species grows on calcareous rocks and in lime quarries, on walls, boulders, stones and pebbles. *Verrucaria difficilis* may prefer half-shady habitats. The species is rare, but may also have been overlooked due to its morphological similarity to several other species.

Etymology. The species may be mixed up with several other species of *Verrucaria*.

Other specimens examined. Finland, Koillismaa, Kuusamo, Oulanka National Park, Kiutaköngäs 400 m N, *Pinus sylvestris*-dominated forest, small dolomite rock outcrop, SW-slope, on pebbles, 165 m alt., 66°22'N, 29°19'E, 2 Aug 2010, J. Pykälä 39060 (H); Kuusamo, Juuma, Niskakoski, calciferous (dolomite) schistose rock outcrop, on calciferous boulder, 225 m alt., 66°13'N, 29°24'E, 22 Aug 2011, J. Pykälä 44811 (H); Uusimaa, Vantaa, Sotunki, Bisa, 300 m E-NE, herb-rich forest, abandoned lime quarry, on SW-facing wall, 35 m alt., 60°17'N, 25°08'E, 7 June 2011, J. Pykälä 41859 (H).

Notes. Based on the ITS phylogeny, *V. difficilis* belongs to the *V. subtilis* complex with *V. bifurcata*, *V. cavernarum* and *V. subtilis*. The involucrellum is usually longer than in *V. cavernarum* and *V. subtilis*. Furthermore, the perithecia of *V. difficilis* are, on average, less immersed, often only 1/4–1/2-immersed in rock. *Verrucaria bifurcata* differs in more immersed perithecia with the involucrellum appressed to the exciple. Nevertheless, *V. difficilis* may not be identified with certainty without sequencing. *Verrucaria difficilis* is also difficult to separate from *V. kuusamoensis*. This species has slightly longer spores and the perithecia commonly leave deep pits in the rock.

A Genbank sequence *Verrucaria calkinsiana* Servít (KT695332) has 98% similarity to *V. difficilis* and it remains to be studied whether it is a closely-related species or possibly conspecific. Based on the morphology of the type specimen (PRM-857016!), *V. calkinsiana* does not belong to the *V. subtilis* complex and the sequenced specimen is apparently misidentified.

Verrucaria foveolata (Flörke) A. Massal., Ric. auton. lich. crost.: 346, 1852

= *Verrucaria latzeliana* Servít, Stud. Bot. Čech. 9: 89, 1948. Type. Ragusa: Gartenmauer am 3. Aquidotto, ca. 200 m, Kalk, 28.7.1907, A. Latzel (PRM-859178!, holotype)
Verrucaria schraderi Sommerf. var. *foveolata* Flörke, Deutschl. Lich. 6, 1815. Basionym.

Type. Not seen. Protologue: “auf Kalksteinen bei Rüdersdorf”.

Description. Prothallus absent. Thallus white, grey or pale brown, endolithic, often inconspicuous, rarely thinly epilithic, algal cells 5–9 mm. Perithecia 0.11–0.42 mm, (1/2–)3/4–1-immersed in rock, leaving deep pits in the rock, commonly surrounded by a thallus collar, sometimes covered by a thin thalline layer except for the apex; (30–)60–120 perithecia/cm^2. Ostiole usually inconspicuous, tiny, pale or dark, plane or depressed, ca. 20–40(–50) mm wide, wider ostiolar depression rarely present up to 80 mm wide. Involucrellum absent or apical, rarely covering half of the exciple, 40–60 mm thick. Exciple 0.19–0.42 mm in diam., usually round, but sometimes pear-shaped or at least longer than broad, medium brown (rarely), dark brown or black, ca. (20–)25–43(–60) mm thick, apex sometimes thickened to ca. 40–60 mm thick if the involucrellum is absent. Periphysoids ca. (30–)40–65 × 1–2(–3) mm, branching. Asci 78–102 × 27–39 mm, 8-spored. Ascospores 0-septate, rarely solitary spores 1-septate, (23.6–)27.4–30.5–33.7(–37.3) × (10.4–)12.1–13.4–14.6(–17.1) mm (n = 197), perispore 1–1.5 mm thick.

Habitat and distribution. The species grows on calcareous rocks and in lime quarries, both on sun-exposed and shady rocks, both in southern and in northern Finland.

Other specimens examined. Finland. Varsinais-Suomi, Lohja, Torhola, 400 m E of Torhola cave, S-slope, calcareous rock outcrop, 40 m alt., 60°15'N, 23°52'E, 20 July 2007, J. Pykälä 31528 (H); Salo (Kisko), Leilä, Kalkuuni, *Pinus sylvestris*-dominated forest, SW-slope, on calcareous rock wall, 60 m alt., 60°12'N, 23°35'E, 14 July 2009, J. Pykälä 34953 (H); Länsi-Turunmaa (Korppoo), Åfvensår, Kilamo, abandoned lime quarry, on SW-facing wall, 13 m alt., 60°17'N, 21°32'E, 28 July 2009, J. Pykälä 35395 (H); Salo (Kisko), Haapaniemi, Plantmaannokka, calcareous rock outcrop on shore of Lake Määrjärvi, on NE-facing wall, 42 m alt. , 60°12'N, 23°31'E, 4 June 2010, J. Pykälä 37728 (H); Salo (Kisko), Jyly, 200 m NE of Purslammi, calcareous rock outcrop, on NW-facing wall, 67 m alt., 60°14'N, 23°36'E, 17 June 2010, J. Pykälä 38119 (H); Kemiönsaari (Dragsfjärd), Olmos, Kolaskär island, calcareous rock outcrop on shore of the Baltic Sea, beneath SE-facing wall, on pebbles, 2 m alt., 60°03'N, 22°19'E, 12 July 2010, J. Pykälä 38719 (H); Koillismaa, Kuusamo, Oulanka, Putaanoja, 500 m W-NW of Hautala, *Pinus sylvestris*-dominated semi-open forest, dolomite rock outcrop, on N-slope, 230 m alt., 66°22'N, 29°25'E, 9 Aug 2009, J. Pykälä 35965 (H); Kuusamo, Kallunki, Merenvaara, *Pinus sylvestris*-dominated forest, NW-slope, small dolomite rock outcrop, on W-facing wall, 225 m alt., 66°20'N, 29°20'E, 2 Aug 2010, J. Pykälä 39028 (H); Kuusamo, Oulanka National Park, Kiutaköngäs 400 m N, SE-slope, *Pinus sylvestris*-dominated forest, small dolomite rock outcrop, on SW-facing wall, 170 m alt., 66°22'N, 29°19'E, 5 Aug 2010, J. Pykälä 39294 (H); Salla, Oulanka National Park, W of Savikoski, cliff, dolomite rock outcrop, NE-slope, on dolomite boulder, 185 m alt., 66°25'N, 29°10'E, 17 Aug 2010, J. Pykälä 40195 (H); Kuusamo, Oulanka National Park, Taivalköngäs, shore of Oulankajoki river, *Picea abies*-dominated herb-rich forest, dolomite rock outcrop, NE-slope, on dolomite boulder, 174 m alt., 66°24'N, 29°11'E, 20 Aug 2011, J. Pykälä 44553 (H); Salla, Hautajärvi, Kurtinniittykuru, dolomite rock outcrop, on flat surface, 195 m alt., 66°26'N, 29°09'E, 24 Aug 2011, J. Pykälä 44952 (H).

Notes. Fennoscandian specimens of *Verrucaria* with large spores, perithecia leaving deep pits in the rock and immersed in rock, lacking an involucrellum and with endolithic pale thallus have been predominantly treated as *V. foveolata* (e.g. Foucard 2001). It remains uncertain whether *V. foveolata* is the correct name for this common species, as the type material was not located. The absence of involucrellum has been used as the main character to separate *V. foveolata* from morphologically-similar species with apical involucrella, such as *V. dolomitica* (A. Massal.) Kremp. (Breuss 2004). However, the sequenced Finnish specimens with an apical involucrellum do not differ from specimens without an involucrellum.

Based on the ITS phylogeny, *Verrucaria foveolata* and *V. subjunctiva* are not monophyletic, but together form a strongly-supported group. However, the two taxa are, for the time being, treated as separate species pending further study. Most specimens can be identified by their morphology, although we found some intermediate specimens having morphological characters pointing to both species. However, overlap in the morphology is not larger than compared to several, not closely related species of *Verrucaria*. *Verrucaria foveolata* is more difficult to be separated from *V. devergens* (see the species) than from *V. subjunctiva*. Furthermore, some ecological and biogeographical differences seem to occur between *V. foveolata* and *V. subjunctiva. Verrucaria subjunctiva* has not been found from lime quarries, whereas several populations of *V. foveolata* occur in lime quarries. *Verrucaria foveolata* is fairly common on calcareous rocks both in southern and northern Finland, whereas *V. subjunctiva* is rare in southern Finland.

Verrucaria fuscozonata **Pykälä, Kantelinen & Myllys, sp. nov.**
MycoBank No: 835672
Fig. 2D

Diagnosis. Species characterised by dark lines between contiguous conspecific thalli, pale endolithic thallus, small perithecia leaving shallow to deep pits in the rock, involucrellum reaching the exciple base level and appressed to the exciple, ascospores measuring (21–)24–28(–29) × (10–)11–12(–13) mm.

Holotype. Finland. Koillismaa, Kuusamo, Oulanka National Park, Pikkuköngäs, N shore of river Oulankajoki, dolomite rock outcrop, on SW-facing wall, 160 m alt., 66°22'N, 29°19'E, 12 Aug 2009, J. Pykälä 36222 (H, GenBank accession number: MT229758).

Description. Prothallus not seen. Thallus pale grey, endolithic, dark lines between contiguous conspecific thalli, 0.21–0.35 mm wide. Perithecia 0.11–0.26 mm in diam., (1/2–)3/4–immersed, leaving shallow to deep pits in the rock, surrounded by a thallus collar; 120–140 perithecia/cm^2. Ostiole inconspicuous, tiny, pale to dark, plane or depressed, ca. 30 mm wide. Involucrellum reaching the exciple base, 50–60 mm thick, appressed to the exciple. Exciple 0.18–0.25 mm in diam., wall dark brown to black. Periphysoids ca. 25–35 × 2–2.5 mm. Asci 8-spored. Ascospores 0-septate, (21.2–)24.5–26.5–28.4(–29.4) × (10.0–)10.9–11.7–12.5(–13.2) mm (n = 36), perispore 1 mm thick.

Habitat and distribution. The only known specimen is from a dolomite rock on a river shore in north-eastern Finland, in Kuusamo.

Etymology. The only specimen available is characterised by dark lines between contiguous conspecific thalli.

Notes. *Verrucaria fuscozonata* did not group with any of the examined species in the ITS phylogeny. However, it is morphologically rather similar to *V. bifurcata*, *V. kuusamoensis* and *V. subdevergens*. In *V. bifurcata*, dark lines between contiguous conspecific thalli are absent and the involucrellum usually thinner. In *V. kuusamoensis* and *V. subdevergens*, the spores are larger and the perithecia occur less densely. More material is needed to find out whether *V. fuscozonata* can be unambiguously identified, based on morphology only.

Verrucaria karelica Vain., Acta Soc. Fauna Flora Fenn. 49(2): 46, 1921

Type. Russia, Karelia Onegensis, Mundjärvi, supra saxa dolomitica cinerea, J. P. Norrlin (H-NYL 3146!, H!, syntypes).

Description. Prothallus absent. Thallus white, grey or pale greyish-brown, endolithic or thinly epilithic, farinose, algal cells 5–8 mm, contiguous conspecific thalli often separated by dark lines, 0.13–0.22 mm wide. Perithecia 0.07–0.37 mm, (1/2–)3/4–1-immersed, leaving shallow to usually deep pits in the rock, surrounded by a thalline collar; 40–80 perithecia/cm^2. Ostiole pale or dark, plane or depressed ca. 20–40(–60) mm wide. Involucrellum apical or covering half of the exciple, 50–70 mm thick, appressed to the exciple or diverging from the exciple. Exciple 0.21–0.28 mm in diam., wall dark brown to black, ca. 20–31 mm thick. Periphysoids ca. 30–50 × 2–2.5(–3) mm. Asci ca. 66–84 × 26–33 mm, 8-spored. Ascospores 0-septate, (23.2–)26.2–27.9–29.5(–31.3) × (10.3–)11.7–12.3–13.0(–14.1) mm (n = 63), perispore not seen, but may have vanished during storage.

Habitat and distribution. This species is known from Finland only from the Oulanka area in the biogeographical province of Koillismaa in NE Finland where it grows on dolomite rocks. It seems to occur in fairly shady habitats.

Other specimens examined. Finland. Koillismaa, Salla, Oulanka National Park, Savikoski 300 m W, *Pinus sylvestris*-forest, steep N-slope, dolomite rock outcrop, on N-facing wall, 180 m alt., 66°25'N, 29°10'E, 10 Aug 2010, J. Pykälä 39625 (H); Kuusamo, Oulanka, Putaanoja, 500 m W-NW of Hautala, NE-slope, dolomite rock outcrop, on 50 cm high SW-facing wall, scarce, 232 m alt., 66°22'N, 29°25'E, 15 Aug 2010, J. Pykälä 39991 (H); Kuusamo, Oulanka National Park, Kiutaköngäs N, steep S-slope, *Pinus sylvestris*-dominated forest, dolomite rock outcrop, on SW-facing wall, 182 m alt., 66°22'N, 29°19'E, 19 Aug 2010, J. Pykälä 40325 (H); Salla, Oulanka National Park, W of Savikoski, cliff, dolomite rock outcrop, on NE-facing wall, scarce, 185 m alt., 66°25'N, 29°10'E, 17 Aug 2010, J. Pykälä 40235 (H).

Notes. The type specimens of *V. karelica* have epilithic thalli and contiguous conspecific thalli are separated by dark lines. None of the Finnish specimens has both

epilithic thalli and dark lines. However, one of the sequenced specimens has epilithic thalli and another specimen has dark lines. Thus, based on morphology, this entity probably belongs to *V. karelica*. The type locality of *V. karelica* (Vainio 1921) is situated rather close to the Oulanka area, suggesting that the species would probably occur in the Oulanka area. The species is closely related to *V. devergens* and *V. subdevergens. V. devergens* and *V. karelica* may not be unambiguously separated by morphology only. *Verrucaria devergens* usually has endolithic thalli and several specimens lack an involucrellum. *Verrucaria karelica* may be absent from subaquatic habitats unlike *V. devergens* which often grows on river shores. *Verrucaria subdevergens* has an involucrellum usually exceeding half of the exciple height. The species is also difficult to be separated from several other species of *Verrucaria* belonging to the *Thelidium* group. *Verrucaria cavernarum*, *V. difficilis* and *V. subtilis* always lack dark lines between contiguous conspecific thalli and the spores are smaller. *Verrucaria kuusamoensis* usually has an involucrellum exceeding half of the exciple.

Verrucaria kuusamoensis **Pykälä, Kantelinen & Myllys, sp. nov.**
MycoBank No: 835673
Fig. 2E

Diagnosis. Species characterised by pale, usually thinly epilithic thallus, rather large perithecia leaving shallow to deep pits in the rock, involucrellum usually covering more than half of the exciple, ascospores (21–)26–30(–34) × (9–)11–13(–14) mm, morphologically difficult to separate from *V. subdevergens*, but the sequence divergence in ITS 6.8–7.4%.

Type. Finland. Koillismaa, Kuusamo, Juuma, Oulanka National Park, Hautaniitynvuoma, gorge, dolomite rock outcrop, on high NE-facing wall, 190 m alt., 66°15'N, 29°26'E, 21 Aug 2011, J. Pykälä 44703 (H9205113 – holotype, UPS – isotype, GenBank accession number: MT229776).

Description. Prothallus absent. Thallus white, grey or more rarely pale brown, endolithic or usually thinly epilithic, continuous or rimose, often farinose, up to 0.2 mm thick, algal cells (4–)5–7 mm, contiguous conspecific thalli sometimes separated by a dark line, 0.12–0.35 mm wide, present in only few specimens. Perithecia 0.17–0.45 mm in diam., (1/4–)1/2–3/4(–1)-immersed, leaving shallow to deep pits in the rock, rarely few perithecia not leaving pits, often thinly thalline covered except apex; (30–)40–120 perithecia/cm^2. Ostiole tiny, pale or dark, plane or depressed, ca. 20–40(–60) mm wide, occasionally wider ostiolar depression up to 110 mm wide. Involucrellum covering half of the exciple or to the exciple base level, rarely in few perithecia enveloping the exciple, (30–)40–70(–80) mm thick, appressed to the exciple or slightly or moderately diverging from it. Exciple 0.19–0.29 mm in diam., wall dark brown or black, rarely pale, ca. 20–42 mm thick. Periphysoids ca. (20–)25–40 × (1.5–)2–2.5(–3) mm. Asci 68–102 × 25–34 mm, 8-spored. Ascospores 0-septate, (21.4–)25.5–27.9–30.3(–34.5) × (9.3–)11.3–12.2–13.1(–14.2) mm (n = 312), perispore 1 mm thick.

Habitat and distribution. *Verrucaria kuusamoensis* is rather common on dolomite rocks in the Oulanka area in the municipalities of Kuusamo and Salla in the biogeographical Province of Koillismaa (Ks). It seems not to occur in southern Finland.

Etymology. Most specimens of the species originate from the Kuusamo area.

Other specimens examined. Finland. Koillismaa, Kuusamo, Paljakka, E shore of Kuusinkijoki river, Kiukaankorva, dolomite rock outcrop, on overhanging NW-facing wall, scarce, 213 m alt., 66°11'N, 29°38'E, 5 Aug 2009, J. Pykälä 35710 (H); Kuusamo, Oulanka National Park, Pikkukönkäänkuru, herb-rich heath forest, small dolomite rock outcrop, on W-facing wall, 165 m alt., 66°21'N, 29°19'E, 6 Aug 2009, J. Pykälä 35857 (H); Kuusamo, Oulanka National Park, Pikkukönkäänkuru, dolomite rock outcrop, on SW-facing wall, 175 m alt., 66°21'N, 29°19'E, 8 Aug 2009, J. Pykälä 35920 (H); Kuusamo, Oulanka National Park, Pikkuköngäs, N shore of river Oulankajoki, dolomite rock outcrop, on SW-facing wall, 160 m alt., 66°22'N, 29°19'E, 12 Aug 2009, J. Pykälä 36254 (H); Kuusamo, Oulanka National Park, Kiutaköngäs, *Pinus sylvestris*-dominated forest, steep SE-slope, dolomite rock outcrop, on SE-facing wall, rather scarce, 175 m alt., 66°22'N, 29°19'E, 12 Aug 2009, J. Pykälä 36294 (H); Salla, Oulanka National Park, 400 m N of Savilampi, shore of river Savinajoki, dolomite rock outcrop, on NE-slope, scarce, 178 m alt., 66°25'N, 29°10'E, 13 Aug 2009, J. Pykälä 36335 (H); Kuusamo, Oulanka National Park, Kiutaköngäs 400 m N, *Pinus sylvestris*-dominated forest, small dolomite rock outcrop, on SW-slope, 165 m alt., 66°22'N, 29°19'E, 2 Aug 2010, J. Pykälä 39052 (H); Kuusamo, Oulanka National Park, Kiutaköngäs, by the rapids, S shore of Oulankajoki river, calciferous (dolomite) schistose rock outcrop, NE-slope, on E-facing wall, rather scarce, 152 m alt., 66°22'N, 29°20'E, 13 Aug 2010, J. Pykälä 39900 (H); Salla, Oulanka National Park, W of Savikoski, cliff, dolomite rock outcrop, on NW-facing wall, very scarce, 185 m alt., 66°25'N, 29°10'E, 17 Aug 2010, J. Pykälä 40219 (H); Kuusamo, Oulanka National Park, Taivalköngäs, shore of Oulankajoki river, *Picea abies*-dominated herb-rich forest, dolomite rock outcrop, on NE-facing wall, 175 m alt., 66°24'N, 29°11'E, 20 Aug 2011, J. Pykälä 44563 (H); Kuusamo, Oulanka National Park, Taivalköngäs, shore of Oulankajoki river, *Picea abies*-dominated herb-rich forest, dolomite rock outcrop, on E-facing wall, 175 m alt., 66°24'N, 29°11'E, 20 Aug 2011, J. Pykälä 44570 (H); Kuusamo, Juuma, Oulanka National Park, Hautaniitynvuoma, gorge, dolomite rock outcrop, on high NE-facing wall, 190 m alt., 66°15'N, 29°26'E, 21 Aug 2011, J. Pykälä 44694 (H), 44696 (H); Kuusamo, Juuma, Oulanka National Park, Hautaniitynvuoma, gorge, stony NW-slope with sparse stunted birches, close to bottom, on dolomite stone, 182 m alt., 66°15'N, 29°26'E, 21 Aug 2011, J. Pykälä 44744 (H); Salla, Hautajärvi, Kurtinniittykuru, cliff, dolomite rock outcrop, on SE-facing wall, scarce, 195 m alt., 66°26'N, 29°09'E, 24 Aug 2011, J. Pykälä 44980 (H); Kuusamo, Oulanka National Park, Halosenkuru gorge, NW-slope, *Picea abies*-dominated forest, dolomite rock outcrop, on NW-facing wall, 215 m alt., 66°21'N, 29°26'E, 27 Aug 2011, J. Pykälä 45231 (H); Kuusamo, Oulanka National Park, Halosenkuru, gorge, dolomite rock outcrop, on SE-facing wall, scarce, 235 m alt., 66°21'N, 29°26'E, 28 Aug 2011, J. Pykälä 45330 (H).

Notes. The exciple wall of *V. kuusamoensis* is usually dark brown or black. However, one specimen with a pale exciple wall and one specimen with both pale and dark exciple walls have similar ITS sequences compared to the specimens with dark exciple walls. This species was erroneously reported as *V. subjunctiva* by Pykälä (2010a), while *V. subjunctiva* has larger spores and longer periphysoids. *Verrucaria devergens* has a shorter involucrellum. *Verrucaria kuusamoensis* may be most difficult to separate from *V. subdevergens* and *V. difficilis*. These species show wide overlap in their morphology. *Verrucaria subdevergens* more often has pale brownish thallus and slightly longer periphysoids. *Verrucaria difficilis* has, on average, less-developed thallus and the perithecia and the spores slightly smaller.

Verrucaria subdevergens **Pykälä & Myllys, sp. nov.**
MycoBank No: 835674
Fig. 2F

Diagnosis. Differing from *V. devergens* by longer involucrellum, morphologically difficult to separate from *V. kuusamoensis*, but the sequence divergence in ITS 5.4–6.0%.

Holotype. Finland, Koillismaa, Kuusamo, Oulanka National Park, Taivalköngäs, shore of Oulankajoki river, dolomite rock outcrop, on gentle NE-slope, 165 m alt., 66°24'N, 29°11'E, 25 Aug 2011, J. Pykälä 45109 (holotype: H9205097, GenBank accession number: MT229783).

Description. Prothallus absent. Thallus white, grey, ochraceous or pale greyish-brown, endolithic to thinly epilithic, continuous to irregularly rimose, in one specimen contiguous conspecific thalli separated by a dark line. Perithecia 0.21–0.42 mm, 1/2–3/4-immersed, leaving shallow to deep pits in the rock, often surrounded by a thallus collar, in one specimen, thalline covered except apex, thalline cover 8–20 mm thick; 80–120 perithecia/cm^2. Ostiole inconspicuous, tiny, pale to dark, plane or depressed, ca. 20–40 mm wide. Involucrellum covering half of the exciple or to the exciple base, in few perithecia may envelope the exciple, 30–80 mm thick, in one specimen, often apically thickened to 50–70 mm thick, appressed to the exciple. Exciple 0.21–0.34 mm in diam., wall blackish-brown, ca. 15–25 mm thick. Periphysoids ca. 25–50 × 1.5–2 mm. Asci 82–94 × 27–33 mm, 8-spored. Ascospores 0-septate, (23.0–)25.4–28.2–31.0(–34.9) × (11.2–)12.0–13.0–13.9(–15.2) mm (n = 83), perispore 1–1.5 mm thick.

Habitat and distribution. All three finds are from the Oulanka area in NE Finland where the species grows on dolomite rock outcrops and on a dolomite boulder.

Etymology. The species is close to *V. devergens*.

Other specimens examined. Finland. Koillismaa, Kuusamo, Oulanka National Park, Kiutaköngäs 400 m N, *Pinus sylvestris*-herb-rich forest, small dolomite rock outcrop, on small S-facing wall, 165 m alt., 66°22'N, 29°19'E, 3 Aug 2010, J. Pykälä 39128 (H); Kuusamo, Oulanka National Park, Taivalköngäs, shore of Oulankajoki

river, *Picea abies*-dominated herb-rich forest, dolomite rock outcrop, NE-slope, on dolomite boulder, 174 m alt., 66°24'N, 29°11'E, 20 Aug 2011, J. Pykälä 44550 (H).

Notes. This species is close to *V. devergens* and *V. karelica*, based on the ITS phylogeny. It differs from these species in a longer involucrellum mainly exceeding half of the exciple. Morphologically, *V. subdevergens* is most difficult to separate from *V. kuusamoensis*, which tends to have shorter periphysoids and the thallus is more often white.

Verrucaria subjunctiva Nyl., Flora 67: 218, 1884

MycoBank No: 392473

= ?*Verrucaria lacerata* Servít, Stud. Bot. Čech. 11: 115, 1950. Type. Slovakia, Tatry Bielské, rup, calc. pr. Tatranská kotlina, 800 m alt., 1925 Suza (PRM-859169!, syntype)

Type. [Russia,] Sibiria Septentrionalis: Si nus Konyam ad fretum Bering, 64°50' lat. bor., 173° long. occid. (Greenw.) 28–30.7.1879 E. Almquist (S-L46!, lectotype, designated here); Fretum Behring, Kongar Bay, E. Almquist (H-NYL 3512!, isolectotype).

Description. Prothallus absent. Thallus white or grey, rarely pale ochraceous, endolithic or thinly epilithic, continuous or rimose, up to 0.1 mm thick, algal cells 5–8 mm. Perithecia (0.16–)0.23–0.45 mm in diam., (1/4–)1/2–3/4(–1)-immersed, not leaving pits or usually leaving shallow or deep pits in the rock, sometimes covered by a thin thalline layer except for the apex, often surrounded by a thalline collar; ca. (10–)30–100(–120) perithecia/cm^2. Ostiole tiny, pale or dark, plane or depressed, ca. 20–40(–50) mm wide, ostiolar depression rarely wide, up to 130 mm wide. Involucrellum exceeding half of the exciple or reaching the exciple base level, rarely enveloping the exciple, (40–)50–100 mm thick, appressed to the exciple or slightly to moderately diverging from the exciple. Exciple 0.20–0.36 mm in diam., wall dark brown or black, ca. 22–45 mm thick. Periphysoids ca. 30–60 × (1–)1.5–2.5 mm, branching. Asci 84–109 × 32–40 mm, 8-spored. Ascospores 0-septate, rarely very few spores 1-septate, (23.4–)27.0–30.4–33.8(–40.1) × (11.7–)12.6–13.8–15.0(–17.4) mm (n = 242), perispore 1–2 mm thick.

Habitat and distribution. The species occurs on calcareous rocks in both sun-exposed and shady sites. Most sequenced specimens are from the biogeographical province of Koillismaa. Three sequenced specimens (two localities) originate from eastern Finland (biogeographical Province of Pohjois-Karjala) and three (two localities) from southern Finland (biogeographical Province of Varsinais-Suomi). In southern Finland, the species seems to be very rare. *Verrucaria subjunctiva* has not been collected in Finland from lime quarries.

Other specimens examined. Finland. Varsinais-Suomi, Länsi-Turunmaa (Korppoo), Åfvensår, Kilamo, calcareous rock outcrop, on flat rock, scarce, 17 m. alt., 60°17'N, 21°32'E, 28 July 2009, J. Pykälä 35326 (H); Länsi-Turunmaa (Korppoo), Åfvensår, Kilamo, calcareous rock outcrop, on flat rock, on pebbles, 17 m alt., 60°17'N, 21°32'E, 28 July 2009, J. Pykälä 35361 (H); Salo (Kisko), Haapaniemi, Plantmaan-

nokka, calcareous rock outcrop on shore of Lake Määrjärvi, on calcareous boulder, 43 m alt., 60°12'N, 23°31'E, 4 June 2010, J. Pykälä 37746 (H); Koillismaa, Kuusamo, Oulanka National Park, Pikkukönkäänkuru, dolomite rock outcrop, on SW-facing wall, 173 m alt., 66°21'N, 29°19'E, 8 Aug 2009, J. Pykälä 35930 (H); Kuusamo, Oulanka National Park, Kiutaköngäs, N-shore of river Oulankajoki, dolomite rock outcrop, on SE-slope, 150 m alt., 66°22'N, 29°20'E, 12 Aug 2009, J. Pykälä 36308 (H); Salla, Oulanka National Park, 400 m N of Savilampi, shore of river Savinajoki, cliff, dolomite rock outcrop, on NW-facing wall, 177 m alt., 66°25'N, 29°10'E, 13 Aug 2009, J. Pykälä 36371 (H); Kuusamo, Juuma, Lammasvuoma, gorge, calciferous (dolomite) schistose rock outcrop, on NE-facing wall, 225 m alt., 66°16'N, 29°26'E, 8 Aug 2010, J. Pykälä 39475 (H), 39478 (H), 39491 (H); Salla, Oulanka National Park, Savilamminniemi, shore of lake Savilampi, cliff, dolomite rock outcrop, on E-facing wall, scarce, 185 m alt., 66°25'N, 29°10'E, 12 Aug 2010, J. Pykälä 39803 (H); Kuusamo, Oulanka National Park, Kiutaköngäs, by the rapids, shore of Oulankajoki river, calciferous (dolomite) schistose rock outcrop, N-slope, on boulder, 152 m alt., 66°22'N, 29°20'E, 18 Aug 2010, J. Pykälä 40284 (H); Kuusamo, Juuma, Oulanka National Park, Hautaniitynvuoma, gorge, dolomite rock outcrop, on high NE-facing wall, very scarce, 190 m alt., 66°15'N, 29°26'E, 21 Aug 2011, J. Pykälä 44671 (H); Kuusamo, Juuma, Oulanka National Park, Hautaniitynvuoma, gorge, stony NW-slope with sparse stunted birches, close to bottom, on dolomite boulder, 181 m alt., 66°15'N, 29°26'E, 21 Aug 2011, J. Pykälä 44734 (H); Salla, Oulanka National Park, Savilampi 1.2 km NE, steep E-slope, open area in forest, on small dolomite rock, 190 m alt., 66°26'N, 29°11'E, 23 Aug 2011, J. Pykälä 44881 (H); Pohjois-Karjala, Juuka, Polvela, Valkealampi, close by E-shore, *Pinus sylvestris*-dominated forest, calcareous rock outcrop, on W-slope, 176 m alt., 63°10'N, 29°07'E, 11 July 2011, J. Pykälä 42392 (H), 42419 (H); Juuka, Polvela, Valkealampi, close by E-shore, *Pinus sylvestris*-dominated forest, calcareous rock outcrop, W-slope, directly on rock, rather scarce, 175 m alt., 63°10'N, 29°07'E, 11 July 2011, J. Pykälä 42406 (H); Juuka, Petrovaara, Riihilahti S, shore of lake Polvijärvi, calcareous rock outcrop, on W-facing wall, 171 m alt., 63°09'N, 28°58'E, 13 July 2011, J. Pykälä 42510 (H).

Notes. This species has usually been treated as *V. papillosa* Ach. and was also reported from Finland as *V. papillosa* (Pykälä 2010a). However, Orange (2004b) showed that the type specimen of *V. papillosa* belongs to *V. viridula* (Schrad.) Ach. The type specimen of *V. lacerata* is small, but it fits rather well with the Finnish material. However, ITS sequences from Central Europe are needed to confirm the identity of *V. lacerata*. According to Breuss (2008b), the exciple size in *V. lacerata* is 0.4–0.6 mm, i.e. exceeding the size of *V. subjunctiva*. The ITS phylogeny does not separate *V. subjunctiva* from *V. foveolata*. These two taxa are here kept separated pending further study (see *V. foveolata*). *Verrucaria subjunctiva* and *V. foveolata* have larger spores than the other studied species. However, there is much overlap in the spore size of *V. devergens* and *V. kuusamoensis* and specimens with suboptimally-developed spores are easily misidentified. *Verrucaria subjunctiva* has larger perithecia and longer periphysoids than *V. kuusamoensis*.

***Verrucaria subtilis* Müll. Arg., Flora 57: 536, 1874**

= *Verrucaria hypophaea* (J. Steiner & Zahlbr.) Servít, Stud. Bot. Cechoslov. 11(3): 114, 1950

Verrucaria rupestris var. *hypophaea* J. Steiner & Zahlbr., Ann. K. K. naturh. Hofmus. Wien 22: 107, 1908.Basionym. Type. [Croatia] Hungaria; ad saxa dolomitica prope pagum Pulac supra Fiume, ca. 250 m a.s.m, leg. J. Schuler, Kryptogamie exsiccatae 1521 (M-0164001!, PRM-789449!, syntypes).

=? *Verrucaria infidula* Zschacke, Rabenh. Krypt.-Fl. 9(1)1: 135, 1933. Type. [Poland,] Eitner, Sammlung H. Zschacke 4708 (B-600194849!, syntype?) (see Pykälä 2016)

Type. [Switzerland] Bagnes-Thal, nördl. vom Hotel Monvoisin gegen den Plaine an Dolomitfelsen 16.9.1873 (G-00295028!, syntype); … Monvoisin & Bonat Mepa in Bagnes-Thal 1874 (G-00260361!, syntype?).

Description. Prothallus absent. Thallus white, grey or pale brown, endolithic, or thinly epilithic, continuous to rimose, up to 0.1 mm thick. Perithecia 0.15–0.34(–0.44) mm in diam., (1/2–)3/4(–1)-immersed, leaving shallow to deep pits in the rock, few perithecia occasionally not leaving pits, sometimes covered by a thin thalline layer except for the ostiolar region; 40–160 perithecia/cm^2. Ostiole inconspicuous, tiny, pale or dark, plane or depressed, in two specimens, several ostioles slightly projecting, ca. 20–40(–70) mm wide. Involucrellum apical or covering half of the exciple, rarely in few perithecia exceeding half of the exciple, 30–70(–80) mm thick, appressed to the exciple to clearly diverging from the exciple. Exciple 0.16–0.33 mm in diam., wall pale or pale brown (rather rare), usually dark brown or black, 18–30 mm thick. Periphysoids ca. 20–40(–50) × (1–)1.5–2.5(–3) mm, branching. Asci 58–84 × 22–28 mm, 8-spored. Ascospores 0-septate, (19.8–)22.9–25.2–27.4(–30.7) × (8.3–)9.6–10.5–11.4(–12.8) mm (n = 400), perispore 1 mm thick.

Habitat and distribution. The species grows on various calcareous rocks and in lime quarries. It occurs both in sun-exposed and shady habitats. It is amongst the most common species of *Verrucaria* on calcareous rocks of southern Finland. It may occur in the whole country, but the northernmost sequenced specimens are from the biogeographical province of Koillismaa. In Finland, *V. subtilis* is the most common species of *Verrucaria* belonging to the *Thelidium* group and having perithecia leaving pits in the rock.

Other specimens examined. Finland. Varsinais-Suomi, Lohja, Paavola, N of Rautaniemi, stony SE-slope, young *Pinus sylvestris*-plantation, on calcareous stone, 50 m alt., 60°13'N, 23°54'E, 21 May 2005, J. Pykälä 26865 (H); Pohja, Kuovila, 150 m NW of Valkjärvi, small rather flat calcareous rock outcrop, 50 m alt., 60°08'N, 23°23'E, 9 October 2006, J. Pykälä 29589 (H); Karkkila, Haavisto, 200 m N of Saaressuo, on calcareous rock outcrop, 132 m alt., 60°31'N, 24°22'E, 24 May 2008, J. Pykälä 32606 (H); Suomusjärvi, Sallittu, Huuttavanmäki, S-slope, on calciferous boulder, 110 m alt., 60°18'N, 23°37'E, 28 June 2008, J. Pykälä 32749 (H); Salo (Kiikala), Saari, Kalkkimäki, abandoned lime quarry, on NW-facing wall, 105 m alt., 60°25'N, 23°40'E, 4 July 2009, J. Pykälä 34601 (H); Salo (Kisko), Haapaniemi,

Multsilta, calcareous rock outcrop, on shady N-facing wall, 65 m alt., 60°13'N, 23°29'E, 17 July 2009, J. Pykälä 35093 (H); Kemiönsaari (Västanfjärd), Billböle, Svinberget, calcareous rock outcrop, on W-slope, st pc, 25 m alt., 60°03'N, 22°43'E, 4 Sept 2009, J. Pykälä 36819 (H); Länsi-Turunmaa (Parainen), Hyvilemp, Hyvilemp, abandoned lime quarry, on SW-facing wall, scarce, 15 m alt., 60°17'N, 22°12'E, 14 Sept 2009, J. Pykälä 37102 (H); Karjalohja, Pyöli, E of Innoonlampi, rocky forest, on calcareous boulder, 46 m alt., 60°13'N, 23°49'E, 28 Sept 2009, J. Pykälä 37329 (H), 37331 (H); Salo (Kisko), Haapaniemi, Sorronniemi, abandoned lime quarry, on SE-facing wall, scarce, 65 m alt., 60°13'N, 23°30'E, 4 June 2010, J. Pykälä 37794 (H);

Salo (Kisko), Jyly, 200 m NE of Purslammi, calcareous rock outcrop, on NW-facing wall, 68 m alt., 60°14'N, 23°36'E, 17 June 2010, J. Pykälä 38140 (H); Salo (Särkisalo), Kaukosalo, Pyölinmäki, abandoned lime quarry, quarry spoil heap, NW-slope, on calcareous pebbles, 15 m alt., 60°07'N, 22°58'E, 17 June 2011, J. Pykälä 42225 (H); Koillismaa, Salla, Oulanka National Park, Pikkuköngäs, shore of river Oulankajoki, high cliff, dolomite rock outcrop, on SW-slope, 180 m alt., 66°25'N, 29°08'E, 13 Aug 2010, J. Pykälä 39870 (H); Kuusamo, Liikasenvaara, Iso Sirkkalampi 200 m E, SW-slope, young *Larix*-plantation, on dolomite boulder, rather scarce, 295 m alt., 66°21'N, 29°35'E, 18 Aug 2010, J. Pykälä 40280 (H); Salla, Oulanka National Park, Savilampi 850 m N, shore of Savinajoki river, river shore, on dolomite boulder, on S-facing wall, 182 m alt., 66°26'N, 29°10'E, 23 Aug 2011, J. Pykälä 44843 (H), 44844 (H); Keski-Pohjanmaa, Vimpeli, Vimpeli, Ryytimaa, lime quarry, quarry spoil heap, young deciduous forest, on calcareous boulders, rather scarce, 135 m alt., 63°09'N, 24°01'E, 31 Aug 2010, J. Pykälä 40596 (H);

Vimpeli, Vimpeli, Ryytimaa, lime quarry, S-slope, on pebbles, 125 m alt., 63°09'N, 24°01'E, 2 Sept 2010, J. Pykälä 40833 (H); Vimpeli, Möksy, Kotakangas, abandoned lime quarry, small quarry spoil heap, on pebbles, 122 m alt., 63°07'N, 23°58'E, 2 Sept 2010, J. Pykälä 40859 (H); Vimpeli, Möksy, Kotakangas, by abandoned lime quarry, quarry spoil heap, W-slope, on boulders, 120 m alt., 63°07'N, 23°58'E, 2 Sept 2010, J. Pykälä 40874 (H); Uusimaa, Vantaa, Sotunki, Bisa, 300 m E-NE, herb-rich forest, abandoned lime quarry, on SW-facing wall, 35 m alt., 60°17'N, 25°09'E, 7 June 2011, J. Pykälä 41857 (H); Pohjois-Karjala, Juuka, Nunnanlahti, Mustanvaara, dolomite rock outcrop, on SE-slope, 140 m alt., 63°09'N, 29°09'E, 14 July 2011, J. Pykälä 42540 (H); Etelä-Savo, Kerimäki, Ruokojärvi, Pitkäniemi, abandoned lime quarry, gravelly field, on calcareous pebbles, 85 m alt., 61°56'N, 29°00'E, 15 Sept 2011, J. Pykälä 45794 (H), 45817 (H), 45847 (H).

Notes. *Verrucaria subtilis* may be confused with several other species treated in this paper (see descriptions of these species). *Verrucaria cavernarum* and *V. difficilis* may differ by often longer involucrellum and slightly larger spores. The species may also be mixed up with *Verrucaria epilithea* Vain. and *Verrucaria muralis* Ach. These species have shorter spores (17–26 mm long) and the perithecia are not leaving pits in the rock or the pits are shallow. The first specimens of *V. subtilis* from Finland were identified as *Verrucaria mimicrans* Servít and *V. transfugiens* Zschacke (Pykälä and Breuss 2008). The type material of *V. mimicrans* has not been located and the identity of this spe-

cies remains to be studied. *Verrucaria transfugiens* (see Pykälä 2016) differs in smaller spores and the presence of dark lines between contiguous conspecific thalli. *Verrucaria hypophaea* has usually been considered to belong to *V. muralis* or to *V. schindleri* Servít, which is said to differ from *V. muralis* by a dark exciple (Breuss 2007). However, *V. hypophaea* clearly differs from *V. muralis* by larger spores and the perithecia commonly leaving deep pits in the rock. The characters of *V. hypophaea* fit well with *V. subtilis*.

Verrucaria vacillans **Pykälä & Myllys, sp. nov.**
MycoBank No: 835675
Fig. 2G

Diagnosis. Species characterised by dark lines between contiguous conspecific thalli, pale usually endolithic thallus, perithecia leaving shallow to deep pits in the rock, very variable involucrellum, ascospores (18–)23–28(–32) × (8–)11–13(–15) mm, morphologically rather similar to the Finnish species of the *V. subtilis* complex, but the sequence divergence in ITS 4.5–6.8%.

Holotype. Finland. Enontekiön Lappi, Enontekiö, Porojärvet, Toskalharji, Toskalpahta, fell, SW-slope, scree, on dolomite boulder, 795 m alt., 69°11'N, 21°29'E, 1 Aug 2011, J. Pykälä 43118 (H9205851, GenBank accession number: MT229829).

Description. Prothallus absent. Thallus white, whitish grey or pale brownish, mainly endolithic to thinly epilithic, 20–170 mm thick, algal cells 5–10 mm, contiguous conspecific thalli separated by dark lines, 0.21–0.41 mm wide. Perithecia 0.15–0.47 mm in diam., 1/4–3/4-immersed, usually leaving shallow to fairly deep pits in the rock, rarely few perithecia not leaving pits, often surrounded by a thalline collar, 60–160(–200) perithecia/cm^2. Ostiole tiny or conspicuous, pale to dark, plane or depressed, ca. 20–40(–60) mm wide, wider ostiolar depression occasionally present, up to 160 mm wide. Involucrellum apical, covering half of the exciple, exceeding half of the exciple or rarely to the exciple base, 30–70(–90) mm thick, appressed to the exciple, moderately diverging from the exciple, strongly diverging from the exciple or even spreading outwards away from the exciple. Exciple 0.15–0.26 mm, wall dark brown or black, 17–35 mm thick. Periphysoids ca. 25–40(–50) × 1.5–2.5 mm, branching. Asci 67–84 × 27–28 mm, 8-spored. Ascospores 0-septate, (18.1–)22.7–25.3–28.0(–31.7) × (8.3–)10.8–11.9–13.1(–15.2) mm (n = 228), perispore 1–1.5 mm thick.

Habitat and distribution. The species is restricted in Finland to the calcareous mountains (Scandes) in NW Finland above the tree level. It always grows on dolomite. It grows on rock outcrops, boulders, stones and pebbles.

Etymology. The specific epithet refers to the high morphological variation in the involucrellum from apical to (rarely) reaching the exciple base level, from being appressed to the exciple to spreading outwards away from the exciple and from fairly thin to thick.

Other specimens examined. Finland. Enontekiön Lappi, Enontekiö, Porojärvet, Toskalharji, Toskalpahta, fell, SW-slope, scree, on dolomite pebbles, 785 m alt.,

69°11'N, 21°29'E, 1 Aug 2011, J. Pykälä 43058 (H); Enontekiö, Porojärvet, Toskalharji, Toskaljärvi N, fell, gentle SE-slope, dolomite rock outcrop, on dolomite stones, with *V. foveolata*, 730 m alt., 69°12'N, 21°26'E, 2 Aug 2011, J. Pykälä 43232 (H); Enontekiö, Porojärvet, Toskalharji, Toskaljärvi N, fell, dolomite rock, gentle S-slope, on dolomite stone, 720 m alt., 69°12'N, 21°26'E, 2 Aug 2011, J. Pykälä 43272 (H); Enontekiö, Porojärvet, Toskalharji, Toskaljärvi N, fell, dolomite rock, on SE-facing wall, 720 m alt., 69°12'N, 21°26'E, 2 Aug 2011, J. Pykälä 43296 (H); Enontekiö, Porojärvet, Toskalharji, Toskaljärvi N, fell, dolomite rock, gentle SE-slope, on dolomite pebbles, 730 m alt., 69°12'N, 21°26'E, 2 Aug 2011, J. Pykälä 43302 (H); Enontekiö, Porojärvet, Toskalharji, Toskaljärvi N, fell, dolomite scree, on dolomite boulder, rather abundant, 710 m alt., 69°11'N, 21°26'E, 2 Aug 2011, J. Pykälä 43384 (H); Enontekiö, Kilpisjärvi, Saana, nature reserve, E-part, fell, dolomite rock, on SW-facing wall, 880 m alt., 69°02'N, 20°51'E, 10 Aug 2011, J. Pykälä 44075, 44081b (H); Enontekiö, Kilpisjärvi, Saana, fell, steep NE-slope, dolomite rock, on NE-facing wall, 820 m alt., 69°02'N, 20°51'E, 11 Aug 2011, J. Pykälä 44142, 44162 (H); Enontekiö, Kilpisjärvi, Saana, nature reserve, E-part, fell, steep SW-slope, dolomite rock, on SW-facing wall, 730 m alt., 69°02'N, 20°51'E, 12 Aug 2011, J. Pykälä 44255 (H).

Notes. Based on ITS sequences, *V. vacillans* is genetically well distinct from other *Verrucaria* species. However, it may be confused with several other species. *Verrucaria vacillans* is most difficult to separate from *V. cavernarum*, *V. difficilis* and *V. subtilis*. In these three species, dark lines between contiguous conspecific thalli are never present. *Verrucaria cavernarum* and *V. subtilis* have an involucrellum seldom exceeding half of the exciple (and then only in a minority of perithecia). The exciple of *V. subtilis* is sometimes pale (although usually dark). The spores tend to be slightly broader in *V. vacillans* than in *V. subtilis*. However, specimens of *V. vacillans* without dark lines and with a short involucrellum may not be possible to separate from *V. cavernarum* and *V. subtilis* by morphology. Specimens of *V. vacillans* with a deep reaching involucrellum may not be separable from *V. difficilis* if dark lines are absent. *Verrucaria vacillans* may also be confused with *V. devergens*, *V. kuusamoensis*, *V. epilithea* and *V. muralis*. *Verrucaria kuusamoensis* has an involucrellum usually exceeding half of the exciple, larger spores and dark lines are rather rare. *Verrucaria devergens* has larger spores and the involucrellum is usually absent or sometimes apical. *Verrucaria epilithea* and *V. muralis* have perithecia not leaving pits or the pits are shallow, the spores do not exceed 26 mm in length and dark lines are absent.

Names considered inapplicable to the species treated above

***Verrucaria adelminienii* Zschacke, Rabenh. Krypt.-Fl. 9(1) 1: 160, 1933**

Type. France, Cantal: Auf hartem Kalk bei St. Santin, 1886, F. Adelminien (B600191351!, syntype).

Notes. The specimen in B is tiny with ca. 10 perithecia, of which all but two are covered by glue. The specimen is not identifiable and the species is better to be treated as a species with unresolved status (Pykälä 2016).

Verrucaria aljazevi Servít, Stud. Bot. Čech. 9: 71, 1948

Type. [Slovenia] Carniola, Mojakrana, Aljazev dom, 1100 m, 1931, Servít (PRM-858477!, holotype?).

Description. Prothallus not seen. Thallus white, endolithic. Perithecia 0.11–0.36 mm, immersed, leaving deep pits in the rock. Involucrellum absent. Exciple ca. 0.25 mm in diam., wall dark. Periphysoids ca. 25–35 × 2–3 mm, sparsely branching, *Bagliettoa*-like. Ascospores 0-septate (only few seen), 15–18 × 6–8 mm.

Notes. According to the protologue (Servít 1948), the spore size is 20–28 × 7–8 mm. The species may be related to *Bagliettoa calciseda* (DC.) Gueidan & Cl. Roux.

Verrucaria alpigena Breuss, nom. nov., Sauteria 15: 122, 2008

Type. Austria, Niederösterreich, Voralpen, Bez. Lilienfeld, Gem. Kleinzell, SE von Salzerbad, Weg von Reintal zum Kruckensattel, 550–650 m alt., 29.3.2002, O. Breuss (8060) 19.990 (LI-01763881!, holotype).

Description. Prothallus rather weakly developed, medium brown, weakly fimbriate. Thallus pale greyish-brown with frequent medium brown flecks, rimose, ca. 0.05–0.15 mm thick. Perithecia 0.22–0.38 mm, 1/2–3/4-immersed, not leaving pits to leaving shallow pits in the rock, thinly thalline covered except apex; ca. 80–100 perithecia cm^2. Ostiole pale brown, plane, ca. 20–60 mm wide. Involucrellum to the exciple base level, occasionally enveloping the exciple, ca. 40–60 mm thick, appressed to the exciple. Exciple 0.21–0.24 mm in diam., wall pale to dark brown. Ascospores 0-septate, (22.7–)26.1–28.1– 30.9(–33.6) × (12.1–)12.4–13.5–14.5(–15.8) mm (n = 20).

Notes. This species was erroneously reported from Finland by Pykälä (2011), but based on the ITS phylogeny, the specimen belongs to *V. subjunctiva*. It differs from the other Finnish specimens of *V. subjunctiva* by the pale exciple wall. Originally, *V. alpigena* was described as a species related to *V. muralis*, but differing by larger spores (Breuss 2008). Studying the type specimen of *V. alpigena* revealed that the species may not be related to *V. muralis* nor to the *Thelidium* group. It has a superficial morphological similarity to *Verrucaria ahtii* Pykälä, Launis & Myllys (Pykälä et al. 2017), but the spores are larger. *Verrucaria alpigena* may belong to the so-called Endocarpon group such as *V. ahtii*.

Verrucaria bavarica Servít, Stud. Bot. Čech. 9: 73, 1948

Type. [Germany,] Alg. Alpen, Britzelmeyer (PRM-858488!, holotype?).

Description. Prothallus not seen. Thallus whitish grey, endolithic. Perithecia 0.22–0.36 mm, 3/4–1-immersed, leaving deep pits in the rock. Involucrellum apical, ca. 60 mm thick, appressed to the exciple. Exciple ca. 0.24 mm in diam., wall dark. Ascospores 0-septate, 23–31 × 11–13 mm.

Notes. The specimen is small and only one perithecium was dissected. Our spore measurements match well with the original description (26–32 × 10–12(–14) mm, according to Servít 1948), as well as the values given by Breuss (26–32 × 10–13 mm, according to Breuss 2016). According to Servít (1948), the size of the exciple is 0.4 mm. *Verrucaria bavarica* is morphologically close to *V. cavernarum* and *V. subtilis*, but may differ in having a larger exciple (which was not confirmed due to the very small size of the specimen) and slightly larger spores.

Verrucaria caesiopsila Anzi, Comm. Soc. Critt. Ital. 2(1): 23, 1864

Type. [Switzerland] ad saxa dolomitica in alpe Camsciano sopra Poschiavo, Anzi nro. 364 (S-L140!, syntype).

Description. Prothallus not seen. Thallus inconspicuous, endolithic. Perithecia 0.15–0.25 mm, 3/4–1-immersed, leaving deep pits in the rock. Involucrellum absent. Exciple ca. 0.25–0.3 mm in diam., wall black. Periphysoids ca. 30–40 × 2 mm. Ascospores 0-septate, 17–23 × 11–12(–14) mm.

Notes. The species differs from *V. devergens*, *V. foveolata* and other species treated in the Taxonomy section in smaller spores.

Verrucaria carnea (Arnold) Servít, Stud. Bot. Čechoslov. 9: 77, 1948

Verrucaria leightonii var. *carnea* Arnold in Zwackh, Flora 47: 87, 1864. Basionym.

Type. [Germany] an einer Sandsteinmauer in den Weinbergen bei Neuenheim, Febr. 1863, W. von Zwackh (M-0023494!, syntype?).

Description. Prothallus not seen. Thallus pale grey, rimose to areolate, areoles 0.3–0.7 mm. Perithecia 0.22–0.26 mm, immersed in thallus. Involucrellum absent. Exciple wall pale. Periphysoids ca. 50–80 × 2.5–3 mm, branching. Ascospores 0-septate (only few seen), 20–28 × 13–14 mm.

Notes. Krzewicka (2012) treated *V. carnea* as a pigment-deficient mutant of *V. hochstetteri* and Gilbert (1996) as an albino form of *V. macrostoma* Dufour ex DC. However, it differs in several morphological characters from these species. Oïhénart et al. (2018) accepted *V. carnea* as a distinct species.

Verrucaria cinereorufa Schaer., Lich. Helv. Spicil. 7, 338, 1836

Type. [France,] Saléve (H-NYL3038!, UPS!, probably syntypes).

Description. Prothallus not seen. Thallus pale greyish-brown, thinly epilithic, continuous. Perithecia 0.38–0.61 mm, 1/2–3/4-immersed, leaving fairly deep to deep pits in the rock; ca. 30–60 perithecia/cm^2. Ostiole plane to depressed, ca. 20–60 mm wide. Involucrellum covering half of the exciple, ca. 70–180 mm thick. Exciple 0.23–0.38 mm in diam., wall black. Periphysoids long, ca. 1.5–3 mm thick. Ascospores 0-septate, 30–38 × 12–15 mm.

Notes. The species may be related to *Verrucaria depressula* Servít, but has larger perithecia and thicker involucrellum. The species was erroneously reported by Pykälä (2010a) from Finland.

Verrucaria clauzadei de Lesd., Bull. Bot. Soc. France 97: 171, 1950

Type. [France] Calcaire argileux enposé au N, á 100 m au NE du pas du Bourreau Allaunch, 7.7.1951, Clauzade (PRM-858628!, syntype?).

Description. Prothallus not seen (but, according to the protologue, "linea nigra marginatus"). Thallus grey with tiny brown flecks, thinly epilithic, continuous. Perithecia 0.25–0.45 mm, 3/4–1-immersed, leaving deep pits in the rock; ca. 70–80 perithecia/cm^2. Involucrellum covering half of the exciple, ca. 60–80 mm thick. Exciple ca. 0.25 mm in diam., wall black. Periphysoids ca. 35–50 × 2–2.5 mm. Ascospores 0-septate, 28–34(–38) × 12–13 mm.

Notes. The studied specimen is tiny and better material is needed to solve the identity of the species. The specimen matches in most respects with *V. subjunctiva*. The spores seen were narrower, but the spore size given in the protologue (Bouly de Lesdain 1950) 25–33 × 13–16 mm is similar to *V. subjunctiva*.

Verrucaria cryptica (Arnold) J. Steiner, nom. inval., Verh. zool.-bot. Ges. Wien 61: 41, 1911

Amphoridium crypticum Arnold, nom. inval., Lich. Frank. Jura 257, 1885. Basionym.

Type. [Italy] An Kalksteinen einer Schutthalde unterhalb der Kalkwände an der Südseite des Latemar –Gebirges oberhalb Predazzo, Südtirol, 21. Aug. 1883, Arnold (H-NYL 7009!, H!, UPS-L-169663!, isotypes).

Description. Prothallus absent. Thallus endolithic, grey. Perithecia 0.15–0.39 mm, (3/4–)1-immersed in rock, leaving deep pits in the rock. Involucrellum absent or possibly in some perithecia, small apical involucrellum. Exciple ca. 0.25–0.40 mm in diam., apex thickened, wall black, ca. 30 mm thick. Periphysoids ca. 50–70 × 2 mm, branched-anastomosing. Ascospores 0-septate, 25–30(–32) × (12–)13–16(–17) mm, perispore 1(–1.5) mm thick, persistent.

Notes. The species is rather similar to *V. foveolata*, but the halonate perispore seems to be persistent. This species seems not to have been validly published as the species description is missing from Arnold (1885) and Steiner (1911).

Verrucaria depressula Servít, nom. nov., Stud. Bot. Čechoslov. 9: 80, 1948

= *Verrucaria depressa* Stenhammar, nom. illeg. non Meyen & Flot., Öfvers. K. Svensk. Vetensk.-Akad. Förhandl. 14: 120, 1857. Type. Sweden, Gotland, Lojsta, Lojsta, in collybus calcareis, 1846–55, C. Stenhammar (H!, two syntypes)
= *Verrucaria obscura* Th. Fr., nom. illeg. non (Sm. & Sowerby) Borrer 1836, Öfvers. K. Svensk. Vetensk.-Akad. Förhandl. 21: 276, 1865. Type. Sweden, Resmo, C. Stenhammar (UPS!, syntype)

Description. Prothallus not seen. Thallus grey, pale brown, medium brown or rarely dark brown, with a violet tinge, continuous, rimose or areolate, thallus colour may be variable within specimen, 0.05–0.2(–0.3) mm thick, contiguous conspecific thalli separated by dark lines. Perithecia 0.26–0.52 mm, (1/2–)3/4-immersed, leaving shallow to deep pits in the rock; ca. 60–120 perithecia/cm^2. Involucrellum apical, strongly diverging from the exciple (mainly spreading outwards away from the exciple), (40–)50–90 µm thick. Exciple 0.25–0.4 mm in diameter, pale or dark. Periphysoids ca. 40–60 × (1–)1.5–2 µm. Ascospores 0-septate, (24–)27–35(–45) × 10–18(–20) µm, few spores 1-septate.

Notes. Based on morphology, *V. depressula* may belong to the *Thelidium* group or perhaps more probably be related to *V. viridula*. The type locality is in Sweden and rather close to Finland, but nevertheless, no specimens fitting with *V. depressula* have been found from Finland.

Verrucaria dermatoidea (A. Massal.) Servít, Stud. Bot. Čechoslov. 9: 81, 1948

Verrucaria veronensis f. *dermatoidea* A. Massal., Anzi, Lich. exs. minus rari Italiae superioris 377. Basionym.

Type. [Italy] ad saxa calcarea prope Veronam Mass., Anzi, Lich. Exs. minus rari Italiae superioris 377 (UPS!, syntype).

Description. Prothallus not seen. Thallus grey, rimose to areolate, 0.2–0.4 mm thick. Perithecia 0.18–0.23 mm, immersed in thallus. Involucrellum apical, ca. 30–40 mm thick. Exciple ca. 0.4–0.45 mm in diam., pear-shaped, wall black. Ascospores 0-septate, 27–32 × 13–15 mm.

Notes. The studied specimen is conspecific with *V. viridula*.

Verrucaria dolomitica (A. Massal.) Kremp., Denkschrft. K. Bayer. Bot. Gesellsch. 4(2): 238, 1861

Amphoridium dolomiticum A. Massal., Symmict. Lich. 80, 1855. Basionym.

Type. [Italy,] in op. Giazza ad saxa dolomitica, 1853, A. Massalongo (VER!, syntype); Ad saxa dolomitica in oppido Giazza Prov. Veron. Massal., Massalongo Lichens Ital. Exsiccatae 250 (VER!, syntype).

Description. Prothallus not seen. Thallus pale greyish-cream, endolithic to thinly epilithic surrounding perithecia, slightly rimose, thalli bordered by a blackish-brown line. Perithecia 0.26–0.53 mm, (1/2–)3/4-immersed, leaving deep pits in the rock; 70–100 perithecia/cm^2. Ostiole, pale, plane or depressed, ca. 40–150 mm wide. Involucrellum apical, 50–80 mm thick. Exciple 0.24–0.42 mm in diam., wall medium brown to blackish-brown, pale in one studied perithecium. Periphysoids ca. 40–50 × 2 mm. Ascospores 0-septate, 26–37 × 11–18 mm.

Notes. *Verrucaria dolomitica* and *V. foveolata* have been treated as separate taxa because of the presence (in the former) or absence (in the latter) of an apical involucrellum (Breuss 2004). *Verrucaria dolomitica* was reported as new to Finland by Pykälä (2010 b). However, the Finnish specimens with and without an apical involucrellum have identical ITS sequences or the sequences differ only by a few bases. The Finnish specimens originally identified as *V. dolomitica* are conspecific with *V. foveolata* and treated as such in Stenroos et al. (2016). The Finnish specimens identified as *V. dolomitica* usually have endolithic thalli and no dark lines have been observed between thalli. The syntypes of *V. dolomitica* studied have a partly epilithic thin thallus bordered by a dark line. Furthermore, the perithecia are larger than in the Finnish specimens. Thus, *V. dolomitica* is apparently distinct from *V. foveolata*, but *V. dolomitica* does not occur in Finland.

Verrucaria epipolaea Ach., Lichenogr. Univers. p. 285, 1810

Type. [Switzerland] Helvetia (H-ACH 686!, holotype?, piece on the upper left).

Description. Prothallus not seen. Thallus pale grey, thin, continuous, up to 0.1 mm thick. Perithecia 0.26–0.41 mm, 1/4–1/2-immersed in thallus, sometimes with thin irregular thalline cover; ca. 70–100 perithecia/cm^2. Ostiole inconspicuous, dark, plane or depressed, ca. 20–70 mm wide. Involucrellum slightly exceeding half of the exciple or reaching the exciple base level, 50–70 mm thick, appressed to the exciple or slightly diverging from it. Exciple 0.25–0.32 mm in diam., wall pale. Periphysoids ca. 30–40 × 1.5–2 mm, branching. Asci 75–117 × 25–37 mm, 8-spored. Ascospores 0-septate, (25.3–)26.8–29.6–32.3(–34.4) × (10.4–)11.8–12.5–13.2(–13.4) mm (n = 37).

Notes. *Verrucaria epipolaea* is reminiscent of rare morphs of *V. kuusamoensis* with a pale exciple. It differs in less immersed perithecia by not leaving pits and in the consistently hyaline exciple wall.

Verrucaria euboensis Servít, Stud. Bot. Čech. 9: 83, 1948

Type. [Greece,] Euboea: Berg Xerowuni, ca. 1100 m alt., 1931, Rechinger (PRM-858655!, isotype).

Description. Prothallus not seen. Thallus white to whitish-grey, endolithic. Perithecia 0.2–0.35 mm, 3/4–1-immersed, leaving deep pits in the rock. Involucrellum covering half of the exciple, ca. 100 mm thick, appressed to the exciple. Exciple ca. 0.35–0.45 mm in diam., wall black. Periphysoids ca. 40–50 × 2–2.5 mm. Ascospores 0-septate, 25–30 × 12–16 mm.

Notes. The species may be conspecific with *V. viridula*, but the thallus is endolithic.

Verrucaria grossa Nyl., in Nylander & Saelan, Herb. Mus. Fennici 111, 1859

Type. [Russia,] Lapponia Rossica, 1843, F. Nylander (H!, holotype or syntype).

Description. Prothallus not seen. Thallus grey, rimose. Perithecia ca. 0.4–0.6 mm, 1/2-immersed, leaving deep pits in the rock. Involucrellum enveloping the exciple, ca. 50–100 mm thick, thicker at apex. Exciple ca. 0.3–0.4 mm in diam., wall black. Ascospores in very poor condition, 0-septate, ca. 22–25 × 10 mm.

Notes. The specimen in H is small and in a very poor condition. Vainio (1921) reported the spore size of 15–24 × 10–18 mm. Pykälä (2010b) reported the species new to Finland. The Finnish specimen (the sequencing failed) may be rather similar to the one sectioned perithecium of the type of *V. grossa*, which, however, had spores in poor condition. *Verrucaria grossa* should be treated as a species with unresolved identity unless better type material can be located.

Verrucaria hercegensis Servít, Stud. Bot. Čech. 9: 85, 1948

Type. [Montenegro] Dalmatia mer., Herceg Novi, 80 m, 1929, M. Servít (PRM-760604!, holotype).

Description. Prothallus not seen. Thallus white to grey, endolithic. Perithecia 0.12–0.26 mm, immersed, leaving deep pits in the rock; ca. 30–40 perithecia/cm^2. Involucrellum absent. Exciple ca. 0.4 mm in diam., wall black. Periphysoids ca. 35–50 × 1.5–2 mm, branched-anastomosing. Ascospores 0-septate (only few seen), 20–23 × 10–11 mm.

Notes. The spore size given in the protologue (Servít 1948) is 21–25(–32) × 12–13(–15) mm. The species differs from *V. devergens* and *V. foveolata* in smaller spores. *Verrucaria devergens* has smaller exciple (up to 0.35 mm). *Verrucaria caesiopsila* may differ in a smaller exciple and possibly in smaller spores.

Verrucaria hochstetteri Fr., Lichenogr. Europ. Reform. 435, 1831

Type. Germania: Regni Würtemberg, Blabyrae, ad rupes calcareas, Hochstetter (UPS-L-708716!, holotype).

Description. Prothallus not seen. Thallus light grey, endolithic, dark lines between contiguous conspecific thalli present. Perithecia immersed, leaving deep pits in the rock. Involucrellum absent. Exciple 0.32–0.4 mm in diam., longer than wide, wall black, rather thin. Asci ca. 110–125 × 30–38 mm. Ascospores 0-septate, (25–)26–30(–35) × 16–20 mm.

Notes. The specimen is small and the description above is based on only one perithecium dissected. *Verrucaria hochstetteri* was reported from Finland by Pykälä (2007). However, all the Finnish specimens have narrower spores (max. 18 mm broad) than in the type specimen of *V. hochstetteri* and no dark lines between contiguous conspecific thalli. Thus, they apparently do not belong to *V. hochstetteri*, but to *V. foveolata*.

Verrucaria integra (Nyl.) Nyl., Notiser ur Sällskapets pro Fauna et Flora Fennica Förhandlingar, 5: 276, 1861

Verrucaria rupestris var. *integra* Nyl., Actes Soc. Linn. Bordeaux, 21: 183, 1856. Basionym.

Type. Not in H-NYL, protologue: "in Gallia passim (Ejus statum ochraceo-tinctum, E Cebennis inferioribus in hb. Mougeot vidi)".

Notes. The type material has not been located (possibly in Paris). Nylander had a very wide circumscription for *V. integra*. Specimens identified by Nylander as *V. integra* in H-NYL represent several species of *Verrucaria*. Thus, the identity of *V. integra* cannot be solved without studying the type material. Two old records of this species have been reported from Finland (Vainio 1921), but these specimens belong to *V. viridula*. Based on Vainio's interpretation of *V. integra*, the species may be conspecific with *V. viridula*.

Verrucaria integrella (Nyl.) Nyl., Lich. Pyrenaeorum Orient. Obs. Nov.: 21, 1891

Verrucaria integra (Nyl.) Nyl. f. *integrella* Nyl, Flora 64: 457, 1881. Basionym.

Type. [Switzerland] ad dolomit supra Poschiavo, Anzi (H-NYL 3384!, syntype).

Description. Prothallus absent. Thallus inconspicuous, endolithic. Perithecia 0.18–0.23 mm, 3/4–1-immersed, leaving deep pits in the rock; ca. 100–110 perithecia/cm^2. Ostiole depressed, ca. 20–50 mm wide. Involucrellum absent (?). Exciple ca. 0.2 mm in diam., wall dark. Ascospores 0-septate, ca. 17–21 × 11–12 mm.

Notes. The studied specimen may be a tiny syntype. Nylander has annotated to the specimen: spores 18–24 × 11–14 mm. *Verrucaria integrella* may be synonymous with *V. caesiopsila* as often stated in literature (e.g. Clauzade and Roux 1985; Santesson et al. 2004).

Verrucaria koerberi Hepp, Flechten Eur. 692, 1860

Type. [Germany] an Dolomitfelsen in Laubwäldern bei Eichstätt (Baiern), F. Arnold, Hepp, Flechten Eur. 692 (UPS-L-069713!, syntype).

Description. Prothallus not seen. Thallus white, endolithic to thinly epilithic. Perithecia 0.2–0.3 mm, 3/4–1-immersed, leaving deep pits in the rock, surrounded by a thalline collar, ca. 50–120 perithecia/cm^2. Ostiole inconspicuous, dark, depressed, ostiolar depression up to 100 mm wide. Involucrellum apical, 40–70 mm thick, appressed to the exciple. Exciple 0.17–0.25 mm in diam., wall dark. Periphysoids ca. 30 × 1.5 mm. Ascospores 0-septate, (17–)18–21 × (7–)8 mm.

Notes. This species differs from *V. subtilis* in smaller spores. The specimen H-NYL 7012 does not belong to the type material because it has too large spores (25–33 × 12–16 mm).

Verrucaria mastoidea (A. Massal.) Trevis., Conspect. Verruc. p. 8, 1860

Amphoridium mastoideum A. Massal., Symmict. Lich. 82, 1855. Basionym.

Type. [Italy,] in op. Tregnago – Viacara (VER!, syntype).

Description. Prothallus not seen. Thallus pale brownish-grey, continuous to rimose. Perithecia 0.12–0.21 mm, 3/4–immersed in thallus. Involucrellum apical, ca. 40–50 mm thick, appressed to the exciple. Exciple 0.27–0.33 mm in diam., wall black. Periphysoids ca. 40–45 × 2 mm. Ascospores 0-septate, 28–31 × 12–15 mm.

Notes. The syntype specimen studied is probably conspecific with *V. viridula*.

Verrucaria mimicrans Servít, Stud. Bot. Čech. 11: 116, 1950

Type. ?, protologue: "Jugoslavia, Pulac pr. Rijeka (Fiume), 250 m, dolom., Schuler (P)".

Notes. The type material was not located. *Verrucaria mimicrans* was reported from Finland by Pykälä and Breuss (2008), but the specimen belongs to *V. subtilis*. In the original description, the spore size of *V. mimicrans* is 25–31 × 12–15 mm (Servít 1950) which exceeds the values of *V. subtilis*. Thus, *V. mimicrans* may be distinct from *V. subtilis*, but not present in Finland.

Verrucaria montenegrina Servít, Stud. Bot. Čech. 9: 94, 1948

Type. [Montenegro,] Lovcen, Veterni mlin, 1400 m, 1929, M. Servít (PRM-859152!, holotype).

Description. Prothallus not seen. Thallus grey with frequent tiny brown flecks, endolithic. Perithecia 0.18–0.26 mm, 3/4(–1)-immersed, leaving deep pits in the rock; ca. 60–80 perithecia/cm^2. Involucrellum reaching the exciple base or enveloping the exciple, in the latter case diffusely pigmented under the exciple, ca. 70–110 mm thick, appressed to the exciple. Exciple ca. 0.20–0.22 mm in diam., wall dark. Periphysoids ca. 20–25 × 2.5–3 mm. Ascospores 0-septate (only few seen), 20–25 × 11–14 mm.

Notes. The species differs from the species of the *V. subtilis* complex by thicker involucrellum and shorter spores. *Verrucaria samosensis* Servít has thinner involucrellum and shorter spores.

Verrucaria moravica Servít, Stud. Bot. Čech. 9: 95, 1948

Type. [Czech Republic,] Moravia, Kopřivnice, Piskovnice, 490 m alt., 1922, Suza (PRM-760594!, syntype).

Description. Prothallus not seen. Thallus whitish-grey with abundant medium greenish-brown punctae, endolithic, a dark line between contiguous conspecific thalli. Perithecia 0.23–0.35 mm, 3/4–1-immersed, leaving deep pits in the rock, surrounded by a thalline collar; ca. 40–60 perithecia/cm^2. Involucrellum apical. Exciple ca. 0.26 mm in diam., wall brown. Ascospores 0-septate, 20–25 × 9–12 mm.

Notes. Perithecia are mostly over-mature. One perithecium was sectioned. *V. moravica* may be rather similar to *V. subtilis*, but differs in the presence of dark lines between contiguous conspecific thalli. In the original description, the spore length was reported to be more variable: 18–28 × 9–12 (Servít 1948). In *V. transfugiens* Zschacke, the involucrellum is absent.

Verrucaria muelleriana Servít, Věstn. Krá. České Společ. Nauk 10: 14 (1948) [1947]

Type. [France] Salève, J. Müller (M-0193432!, holotype).

Description. Prothallus not seen. Thallus pale brown with a violet tinge, continuous, hemi-endolithic, contiguous conspecific thalli separated by dark lines. Perithecia 0.38–0.46 mm, 3/4-immersed, leaving deep pits in the rock; ca. 30–50 perithecia/cm^2. Involucrellum apical, ca. 50–60 mm thick, appressed to the exciple. Exciple ca. 0.34–0.35 mm in diam., wall black, ca. 25 mm thick. Periphysoids ca. 50–80 × 1–1.5 mm. Ascospores 0-septate, 32–41 × 12–15 mm.

Notes. The species is morphologically close to *V. depressula* or may even be conspecific.

Verrucaria nylanderiana Servít, Stud. Bot. Čech. 9: 96, 1948

Type. [France] Gallia, Sevres (M-0193237!, holotype).

Description. Prothallus not seen. Thallus greenish-grey with green flecks, continuous, ca. 0.1–0.3 mm thick. Perithecia 0.12–0.32 mm, immersed, leaving deep pits in the rock; ca. 80–100 perithecia/cm^2. Involucrellum absent. Exciple ca. 0.27–0.41 mm in diam., higher than broad, often pear-shaped, wall dark brown. Periphysoids ca. 40–60 × 2–2.5 mm, branched-anastomosing. Asci 85–106 × 25–29 mm, 8-spored. Ascospores 0-septate, 18–23 × 12–14 mm.

Notes. The species differs from *V. viridula* by shorter spores and absence of an involucrellum.

Verrucaria oligocarpa Servít, Stud. Bot. Čech. 9: 97, 1948

Type. [Germany] Eichstätt, ober dem Tiefenthale, 2. 1887, Boll (M-0204594!, holotype).

Description. Prothallus not seen. Thallus grey, endolithic. Perithecia 0.08–0.21 mm, immersed, leaving deep pits in the rock; ca. 60–100 perithecia/cm^2. Involucrellum absent. Exciple ca. 0.19–0.24 mm in diam., wall medium brown to dark brown, apex often thickened. Periphysoids ca. 15–30 × 2–2.5 mm. Asci ca. 61–69 × 20–21 mm, 8-spored. Ascospores 0-septate, 18–23 × 8–11 mm.

Notes. The species may differ from *V. caesiopsila* by narrower spores and shorter periphysoids. *Verrucaria koerberi* has an apical involucrellum and narrower spores.

Verrucaria pallidocarpa Servít, Stud. Bot. Čech. 9: 98, 1948

Type. Jugoslavia, Lovčen, Sanatorium, 1240 m, 1929, M. Servít (PRM-858454!, holotype?).

Description. Prothallus not seen. Thallus grey with brown punctae, endolithic, contiguous conspecific thalli separated by dark lines. Perithecia 0.15–0.2 mm, 3/4(–1)-immersed, leaving deep pits in the rock; ca. 80–240 perithecia/cm^2. Involucrellum absent. Exciple ca. 0.21–0.24 mm in diam., wall pale brown to medium brown, apex thickened to ca. 40–50 mm thick. Ascospores 0-septate 16–24 × 10–13(–14) mm.

Notes. The species is rather similar to *V. transfugiens*, but has a paler exciple wall and slightly larger spores.

Verrucaria paradolomitica Servít, Stud. Bot. Čech. 9: 99, 1948

Type. [Austria,] Dolomit … Grosser Rettenstein bei Kizbühel im Tirol, 1869, Arnold (PRM-858456!, isotype).

Description. Prothallus not seen. Thallus pale brown, epilithic, thin, continuous. Perithecia 0.15–0.23 mm, (3/4–)1-immersed, leaving deep pits in the rock. Involucrellum absent or apical, ca. 70–90 mm thick. Exciple ca. 0.22–0.25 mm in diam., wall blackish-brown, the apex is strongly thickened when the involucrellum is absent. Ascospores 0-septate, 27–37 × 12–15 mm.

Notes. The species may fall within the variation of *V. foveolata*, although it has an epilithic pale brown thallus.

Verrucaria periphysata Zahlbr., Österr. Bot. Zeitschrift 68: 67, 1919

Type. [Croatia] Dalmatien: Schlossruine Vrlika a.d. … Granuga, an Kalk… c. 550 m, 5.7.1911, J. Baumgartner 4250 (W-4250!).

Description. Prothallus not seen. Thallus endolithic, grey. Perithecia 0.15–0.34 mm, immersed, leaving deep pits in the rock; ca. 100–130 perithecia/cm^2. Involucrellum absent. Exciple ca. 0.35–0.5 mm in diam., longer than wide, often pear-shaped, apex thickened, wall black. Periphysoids ca. 50–80 × 2 mm. Ascospores 0-septate, 26–35 × 12–14 mm.

Notes. Material similar to *V. periphysata* has not been observed in Finland. The exciple of the species is larger than in *V. foveolata* (0.2–0.4 mm in diam.). The periphysoids may also be longer.

Verrucaria praecellens (Arnold) Servít, Stud. Bot. Čech. 9: 100, 1948

Amphoridium praecellens Arnold, Verh. Zool. Bot. Ges. 19: 651, 1869. Basionym.

Type. [Italy] 87. Dolomitfelsen in der Schlernklamm ober … in Süd Tirol, 7.1867, Arnold (H-NYL 3208!, H-NYL 3209!, syntypes).

Description. Prothallus absent. Thallus endolithic, grey with a violet tinge, a dark line between contiguous conspecific thalli present, 0.15–0.22 mm wide. Perithecia 0.21–0.44 mm, immersed in rock, leaving deep pits in the rock. Ostiolar depression large. Involucrellum absent or possibly in some perithecia, small apical involucrellum. Exciple ca. 0.4 mm in diam., apex thickened to ca. 60–80 mm, wall black. Periphysoids ca. 50–60 × 2 mm. Ascospores 0-septate, ca. 26–34 × 16–20 mm, perispore ca. 1–1.5 mm thick.

Notes. The perithecia of the syntypes in H-NYL are mainly over-mature. The spore size annotated by Nylander to the specimen is larger (40–48 × 23–26 mm) than the few spores measured by us. Servít (1948) reported high variation in the spore size: 20–45 × 18–26 mm. *Verrucaria praecellens* seems to be characterised by broad spores and a persistent perispore. *Verrucaria cryptica* has narrower spores. *V. praecellens* has been synonymised with *V. hochstetteri* (Krzewicka 2012), but it may differ by persistent perispores and larger spores.

***Verrucaria pustulifera* Servít, Stud. Bot. Čech. 11(3): 120, 1950**

Type. Slovakia, in valle fl. Hnilec, pr. R. Ztratená, 800 m alt., calc., 1933, Suza (PRM 858074!, syntype).

Description. Prothallus not seen. Thallus grey, endolithic to semi-endolithic. Perithecia 0.25–0.33 mm, 3/4-immersed, leaving deep pits in the rock, usually surrounded by a thalline collar or is covered by a thin thalline layer except for the apex. Ostiole pale, plane, ca. 20–50 mm wide. Involucrellum covering half of the exciple, ca. 50–70 mm thick, diverging from the exciple. Exciple ca. 0.3–0.33 mm in diam., wall pale brown. Periphysoids ca. 25–30 × 2–2.5 mm. Ascospores 0-septate, 27–38 × 12–15 mm.

Notes. *Verrucaria pustulifera* differs from *V. subjunctiva* in a pale brown exciple and shorter periphysoids. The involucrellum is also smaller than usually in *V. subjunctiva*. *Verrucaria kuusamoensis* has smaller spores.

***Verrucaria reculetensis* Servít, Stud. Bot. Čech. 11(3): 103, 1950**

Type. [France] Reculet, Jan. 1855, J. Müller (M-0220250!, holotype).

Description. Prothallus not seen. Thallus pale brown, epilithic, thin, continuous. Perithecia 0.38–0.55 mm, 1/2–3/4-immersed, leaving deep pits in the rock; ca. 30–40 perithecia/cm^2. Ostiole tiny, inconspicuous, dark, plane, often surrounded by a projecting neck up to ca. 150 mm wide. Involucrellum absent. Exciple ca. 0.38–0.45 mm in diam., wall dark, ca. 30–40 mm thick, apex thickened to 70–100 mm thick. Periphysoids ca. 50–60 × 1.5–2 mm, branched-anastomosing. Ascospores 0-septate, 25–30 × 13–16 mm.

Notes. The species is rather similar to *V. foveolata*, but may differ by slightly larger perithecia and an epilithic pale brown thallus.

***Verrucaria samosensis* Servít, Stud. Bot. Čech. 9: 105, 1948**

Type. [Greece,] Samos, Vathy, Rechinger (PRM-858434!, holotype).

Description. Prothallus not seen. Thallus whitish-grey, endolithic to thinly epilithic, occasionally irregularly rimose around perithecia. Perithecia 0.22–0.28 mm, (1/2–)3/4-immersed, leaving shallow to deep pits in the rock; ca. 70–80 perithecia/cm^2. Involucrellum enveloping the exciple, 40–50 mm thick. Exciple 0.19–0.28 mm in diam., wall black. Periphysoids ca. 50–60 × 2–2.5 mm. Ascospores 0-septate, ca. 21–25 × 11–13 mm.

Notes. According to the protologue, the spores may be larger: 20–29 × 9–15 mm (Servít 1948). The species differs from *V. bifurcata* by longer periphysoids and possibly by slightly shorter, but broader spores.

Verrucaria saprophila (A. Massal.) Trevis., Conspect. Verruc.: 8, 1860

Amphoridium saprophilum A. Massal., Symmicta Lich. 79, 1855. Basionym.

Type. [Italy,] avi in op. Avesa ([Monte] Ongarine) ad saxa putrida eocenica (VER!, syntype).

Description. Prothallus not seen. Thallus whitish-grey, endolithic to semi-endolithic, a black line between thalli present. Perithecia 0.16–0.23 mm, immersed, leaving deep pits in the rock. Involucrellum absent. Exciple ca. 0.26 mm in diam., wall brown. Ascospores 0-septate, 24–33 × 12–18 mm.

Notes. Krzewicka (2012) treated *V. saprophila* as a synonym of *V. hochstetteri*. However, *V. hochstetteri* has a larger exciple and broader spores. The species differs from *V. foveolata* with the presence of a dark line. *Verrucaria dolomitica* has larger perithecia and an apical involucrellum.

Verrucaria sbarbaronis de Lesd., Bull. Soc. Bot. Fr. 94: 199, 1948

Type. Not seen. Protologue: "Italia, in Valle Bisagno prope Genuam, loco Prato, supra rupem calcaream colore fuscorufo tinctam. leg. Sbarbaro, 1946".

Notes. The type material of *V. sbarbaronis* has not been located. Breuss (2008a) treated *V. sbarbaronis* as a species rather similar to *V. lacerata* (which is here considered conspecific with *V. subjunctiva*), but with clearly smaller spores (20–26 × 11–15 mm). Such specimens have not been found from Finland.

Verrucaria serlosensis Servít, Stud. Bot. Čech. 9: 106, 1948

Type. [Austria], Kalksteine des Serlosgipfels 8200' Matrei-Tirol, 7. 1869, Arnold (M-0193173!, holotype).

Description. Prothallus not seen. Thallus grey to pale brown, endolithic, somewhat inconspicuous. Perithecia 0.12–0.22 mm, immersed, leaving deep pits in the rock; ca. 60–100 perithecia/cm^2. Involucrellum absent. Exciple ca. 0.2 mm in diam., wall pale to pale brown, apex dark, thickened. Ascospores 0-septate, (23.2–)23.9–24.7–25.4(–25.5) × (12.7–)12.8–13.7–14.6(–15.1) mm (n = 15).

Notes. The specimen is rather poor. The species differs from *V. foveolata* by a pale exciple wall, shorter spores and perhaps by a smaller exciple. *Verrucaria caesiopsila* has smaller spores and a dark exciple wall.

Verrucaria slovaca Servít, Stud. Bot. Čech. 11: 125, 1950

Type. Slovakia, Liptovskě hole, Zuberec, Osobita, 1650–1680 m, 1935, Suza (PRM-765231!, syntype).

Description. Prothallus not seen. Thallus white or grey, endolithic. Perithecia 0.25–0.35 mm, 1/2–3/4(–1)-immersed, leaving shallow to deep pits in the rock. Involucrellum reaching the exciple base, ca. 70–90 mm thick, appressed to the exciple or slightly diverging from the exciple. Exciple ca. 0.15–0.25 mm in diam., wall pale. Periphysoids ca. 25 × 2–2.5 mm. Ascospores 0-septate, few spores 1-septate, ca. 20–27 × 9–10 mm, not well developed.

Notes. *Verrucaria slovaca* may possibly belong to the *V. subtilis* complex, but similar specimens have not been found in Finland. The spore size given by Servít (1950) is 24–30 × 9–11 mm. Breuss (2016) apparently found better-developed spores than in this study: (22–)24–27(–28) × (7.5–)9–11–(12) mm.

Verrucaria strasseri Servít, Stud. Bot. Čech. 9: 107, 1948

Type. [Italy], Auf Kalkconglommerat in Villa Lagarina bei Roveredo in Südtirol, 1.5.1883, P. Strasser (M-02039301!, holotype).

Description. Prothallus not seen. Thallus whitish-grey, endolithic. Perithecia 0.15–0.38 mm, (3/4)–1-immersed, leaving deep pits in the rock. Involucrellum apical, ca. 50–90 mm thick, appressed to the exciple. Exciple ca. 0.22–0.26 mm in diam., wall pale brown to dark brown. Periphysoids ca. 30–50 × 1.5–2.5 mm, branching. Asci ca. 88–95 × 26–28 mm, 8-spored. Ascospores 0-septate, (23.6–)24.5–26.6–28.8(–30.2) × (10.1–)10.4–11.4–12.4(–13.7) mm (n = 17).

Notes. The species may differ from the *V. devergens* and *V. subtilis* complexes by mostly fully immersed perithecia and from the *V. subtilis* complex by slightly larger perithecia and a thicker involucrellum.

Verrucaria transfugiens Zschacke, Rabenh. Krypt.-Fl. 9, 1(1): 85, 1933

Type. Deutschland. Thüringen, Jonastal bei Arnstadt, alt. 350–400 m, co-ord. 10°55'E, 50°49'N. An Muschelkalkplättchen, 7.7.1907, G. Lettau (B-600025730!); Deutschland.Sachsen-Anhalt: Vorland des Nord-Ost-Harzes, Steinbruch am Hackel. co-ord. 11°19'E, 51°53'N, 1910, H. Zschacke 4664 (B-600194785!); Deutschland.Sachsen-Anhalt: Harz-Vorland, Ostseite des Hackels. co-ord. 11°19'E, 51°53'N, 1.2.1906, H. Zschacke 4664 (B-600194786!); Deutschland. Thüringen: Dosdorfer Haart, unweit Arnstadt, alt. 450 m, an Muschel Kalk-Felsbänken, accomp. *Tichothecium erraticum*, *Caloplaca lactea* 11.9.1907, G. Lettau 614 (B-600194783!); Deutschland. Thüringen: Dosdorfer Haart, unweit Arnstadt, alt. 450 m, an Muschel Kalk-Felsbänken, 1907?, G. Lettau (B-600194781!). Syntypes.

For the description of the species, see Pykälä (2016). *V. transfugiens* has been reported from Finland by Pykälä and Breuss (2008), but the specimens belong to *V. subtilis* (Stenroos et al. 2016).

***Verrucaria veronensis* A. Massal., Ric. Auton. Lich. Crost. 173, 1852**

Type. [Italy,] S. Leonardo, L. Tonini (VER!, syntype); ad saxa eocenica circa urbem Veronam (S. Leonardo), leg. Tonini, Massalongo Lichenes Ital. Exsiccatae 8 (VER!, syntype); Massalongo, Lich. Ital. exs. 8 (UPS!, syntype).

Description. Prothallus absent. Thallus greenish-grey or grey with some brown pigmentation, epilithic, rimose, ca. 0.2–0.3(–0.4) mm thick. Perithecia 0.12–0.32 mm, 3/4–1-immersed in thallus. Involucrellum apical, ca. 60–70 mm thick. Exciple ca. (0.2–)0.3–0.5 mm in diam., often longer than broad, wall dark. Ascospores 0-septate, 27–35 × 11–15 mm.

Notes. The type material of the species is morphologically similar to *V. viridula* and, based on the morphological similarity, the species is likely to be conspecific with *V. viridula*.

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References

Aptroot A, Thüs H (2011) *Verrucaria rhizicola*. In: Lumbsch HT, et al. (Eds) One hundred species of lichenized fungi. A signature of undiscovered global diversity. Phytotaxa 18: 112–114.

Arnold F (1885) Die Lichenen des fränkischen Jura. Neubauer, Regensburg.

Bouly de Lesdain M (1950) Notes lichenologiques No. XXXV. Bulletin de la Société botanique de France 97: 169–171. https://doi.org/10.1080/00378941.1950.10834796

Breuss O (2004) Neue Felchtenfunde, vorviegend pyrenocarper Arten, aus Oberösterreich. Österreichische Zeitschrift für Pilzkunde 13: 267–275.

Breuss O (2007) *Verrucaria*. In: Nash TH III, Gries C, Bungartz F (Eds) Lichen flora of the Greater Sonoran Desert Region. Volume III. Lichens Unlimited, Tempe, 335–377.

Breuss O (2008a) Bemerkungen zu einigen Arten der Flechtengattung *Verrucaria*. Sauteria 15: 121–138.

Breuss O (2008b) Neue Funde pyrenocarper Flechten aus den julischen Alpen (Slowenien und Italien). Herzogia 21: 85–92.

Breuss O (2016) Über einige von Miroslav Servít beschriebene *Verrucaria*-Arten (lichenisierte Ascomycota, Verrucariaceae). Herzogia 29(2): 374–382. https://doi.org/10.13158/heia.29.2.2016.374

Breuss O, Berger F (2010) Die *Verrucaria*-Arten mit braunem Lager in den österreichischen Kalkalpen. Eine vorläufige Übersicht mit Bestimmungschlüssel. Bibliotheca Lichenologica 104: 77–116.

Breuss O, Berger F (2012) Die Validierung von *Verrucaria finitima* und Bemerkungen über den Formenkreis von *Verrucaria tristis* (lichenisierte Ascomyceten, Verrucariaceae). Österreichische Zeitschrift für Pilzkunde 21: 117–126.

Clauzade G, Roux C (1985) Likenoj de Okcidenta Europo. Ilustrita Determinlibro. Bulletin de la Societe Botanique du Centre-Ouest, Nouvelle Serie, Numero Special 7. Royan, 893 pp.

Crespo A, Lumbsch TH (2010) Cryptic species in lichen-forming fungi. IMA Fungus 1(2): 167–170. https://doi.org/10.5598/imafungus.2010.01.02.09

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1792–1797. https://doi.org/10.1093/nar/gkh340

Foucard T (2001) Svenska skorplavar och svampar som växer på dem. Interpublishing, Stockholm.

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology 2(2): 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Gueidan C, Roux C, Lutzoni F (2007) Using a multigene phylogenetic analysis to assess generic delineation and character evolution in Verrucariaceae (Verrucariales, Ascomycota). Mycological Research 111: 1147–1168. https://doi.org/10.1016/j.mycres.2007.08.010

Gueidan C, Savić S, Thüs H, Roux C, Keller C, Tibell L, Prieto M, Heiðmarsson S, Breuss O, Orange A, Fröberg L, Amtoft Wynns A, Navarro-Rosinés P, Krzewicka B, Pykälä J, Grube M, Lutzoni F (2009) Generic classification of the Verrucariaceae (Ascomycota) based on molecular and morphological evidence: recent progress and remaining challenges. Taxon 58: 184–208. https://doi.org/10.1002/tax.581019

Jüriado I, Kaasalainen, U, Rikkinen J (2017) Specialist taxa restricted to threatened habitats contribute significantly to the regional diversity of *Peltigera* (Lecanoromycetes, Ascomycota) in Estonia. Fungal Ecology 30: 76–87. https://doi.org/10.1016/j.funeco.2017.08.004

Kraichak E, Lücking R, Aptroot A, Beck A, Dornes P, John V, Lendemer JC, Nelsen MP, Neuwirth G, Nutakki A, Parnmen S, Sohrabi M, Tønsberg T, Lumbsch HT (2015) Hidden diversity in the morphologically variable script lichen (*Graphis scripta*) complex (Ascomycota, Ostropales, Graphidaceae). Organisms Diversity & Evolution 15: 447–458. https://doi.org/10.1007/s13127-015-0219-5

Krzewicka B (2012) A revision of *Verrucaria* s. l. (Verrucariaceae) in Poland. Polish Botanical Studies 27: 1–143.

Launis A, Pykälä J, van den Boom PPG, Serusiaux E, Myllys L (2019) Four new epiphytic species in the *Micarea prasina* group from Europe. Lichenologist 51(1): 7–25. https://doi.org/10.1017/S0024282918000555

Leavitt SD, Lumbsch HT, Stenroos S, St Clair LL (2013) Pleistocene speciation in North American lichenized fungi and the impact of alternative species circumscriptions and rates of molecular evolution on divergence estimates. PLoS ONE 8(12): e85240. https://doi.org/10.1371/journal.pone.0085240

Magain N, Sérusiaux E (2015) Dismantling the treasured flagship lichen *Sticta fuliginosa* (Peltigerales) into four species in Western Europe. Mycological Progress 14: 1–97. https://doi.org/10.1007/s11557-015-1109-0

Myllys L, Lohtander K, Källersjö M, Tehler A (1999) Sequence insertions and ITS data provide congruent information on *Roccella canariensis* and *R. tuberculata* (Arthoniales, Euascomycetes) phylogeny. Molecular Phylogenetics and Evolution 12(3): 295–309. https://doi.org/10.1006/mpev.1999.0620

Myllys L, Velmala S, Holien H, Halonen P, Wang LS, Goward T (2011) Phylogeny of the genus *Bryoria*. Lichenologist 43(6): 617–638. https://doi.org/10.1017/S0024282911000132

Nordin A, Moberg R, Tønsberg T, Vitikainen O, Dalsätt Å, Myrdal M, Snitting D, Ekman S (2019) Santesson's checklist of Fennoscandian lichen-forming and lichenicolous fungi. http://www.evolutionsmuseet.uu.se/databaser/santesson.html [downloaded 2.11.2019]

Oïhénart M, Clerc P, Breuss O (2018) New and interesting species of the lichen genus *Verrucaria* (Verrucariaceae, Ascomycota) for Switzerland and France. Herzogia 31(1): 209–218. https://doi.org/10.13158/099.031.0117

Orange A (2004a) A remarkable new freshwater *Verrucaria* from Europe. Lichenologist 36(6): 349–354. https://doi.org/10.1017/S002428290401446X

Orange A (2004b) *Verrucaria papillosa* is a synonym of *Verrucaria viridula*. Lichenologist 36(6): 445–447. https://doi.org/10.1017/S0024282904014471

Orange A (2012) Semi-cryptic marine species of *Hydropunctaria* (Verrucariaceae, lichenized Ascomycota) from north-west Europe. Lichenologist 44(3): 299–320. https://doi.org/10.1017/S0024282911000867

Orange A (2013a) Four new species of *Verrucaria* (Verrucariaceae, lichenized Ascomycota) from freshwater habitats in Europe. Lichenologist 45(3): 305–322. https://doi.org/10.1017/S0024282912000898

Orange A (2013b) British and Other Pyrenocarpous Lichens. Department of Biodiversity and Systematic Biology, National Museum of Wales, 250 pp. https://www.museumwales/media/Orange-A-2013-British-and-other-pyrenocarpous-lichens.pdf

Orange A (2014) Two new or misunderstood species related to *Verrucaria praetermissa* (Verrucariaceae, lichenized Ascomycota). Lichenologist 46(5): 605–615. https://doi.org/10.1017/S0024282914000176

Pino-Bodas R, Martin AP, Burgaz AR, Lumbsch HT (2013) Species delimitation in *Cladonia* (Ascomycota): a challenge to the DNA barcoding philosophy. Molecular Ecology Resources 13(6): 1058–1068. https://doi.org/10.1111/1755-0998.12086

Pykälä J (2007) Additions to the lichen flora of Finland. II. Calcareous rocks and associated soils in Lohja. Graphis Scripta 19(1): 17–32.

Pykälä J (2010a) Notes on the lichen flora of Saana and Malla fells in northern Finland. Memoranda Societatis pro Fauna et Flora Fennica 86: 34–42.

Pykälä J (2010b) Additions to the lichen flora of Finland. V. Graphis Scripta 22(2): 54–62.

Pykälä J (2011) Additions to the lichen flora of Finland. VI. Graphis Scripta 23(2): 47–55.

Pykälä J (2013) Additions to the lichen flora of Finland. VII. Graphis Scripta 25(1): 21–29.

Pykälä J (2016) Examination of types of twenty-two species of *Verrucaria* described by Hermann Zschacke. Herzogia 29(2): 721–729. https://doi.org/10.13158/heia.29.2.2016.721

Pykälä J, Breuss O (2008) Eleven *Verrucaria* species new to Finland. Österreichische Zeitschrift für Pilzkunde 17: 35–40.

Pykälä J, Breuss O (2011) Notes on some rare *Verrucaria* species (lichenized Ascomycotina, Verrucariales). Österreichische Zeitschrift für Pilzkunde 20: 29–34.

Pykälä J, Launis A, Myllys L (2017a) Four new species of *Verrucaria* from calcareous rocks in Finland Lichenologist 49(1): 27–37. https://doi.org/10.1017/S0024282916000542

Pykälä J, Launis A, Myllys L (2017b) *Verrucaria ahtii*, *V. oulankaensis* and *V. vitikainenii*, three new species from the Endocarpon group (Verrucariaceae, lichenized Ascomycota). Lichenologist 49(2): 107–116. https://doi.org/10.1017/S0024282916000694

Pykälä J, Launis A, Myllys L (2018) *Verrucaria tenebrosa* (Verrucariaceae), a new lichen species from Finland and Norway, and notes on the taxonomy of epiphytic taxa belonging to the *V. hydrophila* complex. Phytotaxa 361(2): 211–221. https://doi.org/10.11646/phytotaxa.361.2.6

Pykälä J, Launis A, Myllys L (2019) Taxonomy of the *Verrucaria kalenskyi* – *V. xyloxena* species complex in Finland. Nova Hedwigia 109(3–4): 489–511. https://doi.org/10.1127/nova_hedwigia/2019/0553

Santesson R, Moberg R, Nordin A, Tønsberg T, Vitikainen O (2004) Lichen-Forming and Lichenicolous Fungi of Fennoscandia. Museum of Evolution, Uppsala University, Uppsala, 359 pp.

Servít M (1948) The new lichens of the Pyrenocarpae-group. II. Studia Botanica Čechoslovaca 9: 67–115.

Servít M (1950) The new lichens of the Pyrenocarpae-Group – IV. Studia Botanica Čechoslovaca 11: 101–144.

Servít M (1954) Československé Lišejníky Čeledi Verrucariaceae. Lichenes familiae Verrucariacearum. Nakladatelství Československé Akademie Věd, Praha.

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Steiner J (1911) Flechten aus dem italienisch-französischen Grenzgebiete und aus Mittelitalien. Verhandlungen der Kaiserlich-Königlichen Zoologisch-Botanischen Gesellschaft in Wien 1911: 29–64.

Stenroos S, Velmala S, Pykälä J, Ahti T (2016) Lichens of Finland. Norrlinia 30: 1–896.

Thüs H, Killmann D, Leh B, Fischer E (2018) *Verrucaria hunsrueckensis* (Verrucariaceae, lichenized Ascomycota), a new rare species with exceptionally slender ascospores from Germany. Phytotaxa 345(1): 26–34. https://doi.org/10.11646/phytotaxa.345.1.3

Thüs H, Orange A, Gueidan C, Pykälä J, Ruberti C, Lo Schiavo F, Nascimbene J (2015) Revision of the *Verrucaria elaeomelaena* species complex and morphologically similar

freshwater lichens (Verrucariaceae, Ascomycota). Phytotaxa 197(3): 161–185. https://doi.org/10.11646/phytotaxa.197.3.1

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal DNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR Protocols: a guide to methods and applications. Academic Press, San Diego, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zschacke H (1933) Epigloeaceae, Verrucariaceae und Dermatocarpaceae. In: Zahlbruckner A (Ed.) Dr. L. Rabenhorst's Kryptogamen-Flora von Deutschland, Österreich und der Schweiz, 2 Auflage 9 (1/1): 44–480, Akademische Verlagsanstalt, Leipzig.

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Two new species of *Microdochium* from *Indocalamus longiauritus* in south-western China

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Abstract

Microdochium species have often been reported as plant pathogens and saprophytes and are commonly isolated from some diseased plant hosts. The primary aim of the present study was to describe and illustrate two new *Microdochium* species isolated from the leaf spot of *Indocalamus longiauritus* in Yunnan Province, China, namely *Microdochium yunnanense* and *M. indocalami*, spp. nov., based on their morphology and multilocus phylogenetic analyses of the combined ITS, LSU, TUB2, and RPB2. DNA sequence data indicate that six strains represent three independent groups from related and similar species in *Microdochium*. *Microdochium indocalami* **sp. nov.** clustered with *M. fisheri*, *M. lycopodinum*, *M. rhopalostylidis*, and *M. phragmitis*. *Microdochium yunnanense* **sp. nov.** grouped with *M. bolleyi*. In addition, the strain SAUCC1017 is recorded as an unidentified species in *Microdochium*. Descriptions and illustrations of the new species in the genus and *Microdochium* sp. indet. are provided.

Keywords

Microdochiaceae, multigene phylogeny, new species, taxonomy, Xylariales

Introduction

Microdochium is a genus in Microdochiaceae (Xylariales), which has been well-studied in recent years by Hernández-Restrepo et al. (2016), Zhang et al. (2017), Crous et al. (2018, 2019), and Marin-Felix et al. (2019) by incorporating morphological and molecular data with appropriate genes to resolve species limitations in the genus.

Microdochium phragmitis, the type of the genus, was introduced by Sydow (1924) for an ascomycetous fungal plant pathogen found on leaves of *Phragmites australis* (= *Phragmites communis*) in Germany in 1919, which has globose, erumpent stromata of minute, hyaline cells, small papillate conoid conidiogenous cells, and solitary, fusiform to subfalcate hyaline conidia. Currently, about 48 species of this genus are listed in Index Fungorum (http://www.indexfungorum.org/; accessed 1 May 2020), but only about two-fifths of them are well known and have been studied in pure culture (Crous et al. 2018, 2019; Marin-Felix et al. 2019).

Monographella was described by Petrak (1924) and was considered the sexual morph of *Microdochium* for many years (Hernández-Restrepo et al. 2016). Nevertheless, *Microdochium* has more species, is more commonly encountered, and the name is more frequently used in the literature. With the implementation of "one fungus one name" nomenclature, *Microdochium* has been retained as a genus name (Hernández-Restrepo et al. 2016).

Microdochium included important plant pathogens, particularly on grasses and cereals. Kwasna et al. (2007) newly described *M. triticicola* which was isolated from roots of wheat in the United Kingdom. Zhang et al. (2015) identified *M. paspali*, which caused leaf blight of seashore paspalum (*Paspalum vaginatum*), a turfgrass widely used in tropical and subtropical golf courses. Crous et al. (2018) described *M. musae* isolated from leaves of *Musa* sp. *Microdochium rhopalostylidis*, found on the leaves of *Rhopalostylis sapida* (Arecaceae) in New Zealand, was identified and described by Crous et al. (2019). From turf leaves (Poaceae) in New Zealand, *M. novae-zelandiae* was isolated by Marin-Felix et al. (2019) and described as a new species.

Many taxonomic problems have occurred in *Microdochium*, and the genus was shown to be polyphyletic (Hernández-Restrepo et al. 2016). However, some species have been reclassified based on molecular analyses (Glynn et al. 2005; Jewell and Hsiang 2013; Hernández-Restrepo et al. 2016). For example, phylogenetic analysis of the translation elongation factor 1-alpha gene (TEF1) showed that the isolates previously described as varieties of *Microdochium nivale* shown a distinct heterogeneity between isolates and these isolates were generated as two separate species, *M. majus* and *M. nivale* (Glynn et al. 2005). The study of multigene differences between *M. nivale* and *M. majus* by Jewell and Hsiang (2013) supported the reclassification of *M. nivale* and *M. majus* as sister species rather than varieties. Three species of *Microdochium* were revised by Hernández-Restrepo et al. (2016), which were initially recognised as *M. gracile* (CBS 493.70), *M. tripsaci* (CBS 857.72), and *M. fusarioides* (CBS 740.83, CBS 741.83, and CBS 742.83), and were renamed *Paramicrodochium gracile* (Sordariomycetes *incertae sedis*), *Ephelis tripsaci* (Clavicipitaceae, Hypocreales), and *Microdochiella fusarioides* (Orbiliales).

In this study, we introduce two novel species, *M. yunnanense* and *M. indocalami* spp. nov., which were isolated from the leaves of *Indocalamus longiauritus* in China. These two species are introduced based on both morphological features and molecular sequence data.

Materials and methods

Isolation and morphological studies

The samples were collected from Yunnan Province, China. The strains of *Microdochium* were isolated from diseased or healthy leaves of *Indocalamus longiauritus* using single spore and tissue isolation methods (Chomnunti et al. 2014). Single spore isolation following the protocol of Choi et al. (1999) and Zhang et al. (2013) was adopted for collection with visible foliar sporulation. The spore suspension was obtained and spread onto potato dextrose agar (PDA) and incubated overnight under normal conditions. The germinated spores were then transferred to a new PDA plate to obtain a pure culture. Besides, the surface-sterilised plant tissue isolation was also used to obtain sterile isolates from plant host. Fungi were isolated by cutting eight fragments (5 × 5 mm) per leaf from the margin of leaf lesions and surface-sterilized by consecutively immersing in 75% ethanol solution for 1 min, 5% sodium hypochlorite solution for 30 s, and then rinsing in sterile distilled water for 1 min (Gao et al. 2014; Liu et al. 2015). The samples were dried with sterilized paper towels and placed on potato dextrose agar (PDA) (Cai et al. 2009). All the plates were incubated at biochemical incubator at 25 °C for 3–4 days, then hyphae were picked out of the periphery of the colonies and inoculated onto new PDA plates.

Following 2–3 weeks of incubation, morphological characters were recorded as by Hernández-Restrepo et al. (2016). Photographs of the colonies were taken at 7 days and 15 days using a Powershot G7X mark II digital camera. Micromorphological characters were observed using an Olympus SZX10 stereomicroscope and an Olympus BX53 microscope, both fitted with Olympus DP80 high definition color digital cameras to photo-document fungal structures. All fungal strains were stored in 10% sterilized glycerin at 4 °C for further studies. Voucher specimens were deposited in the Herbarium of the Department of Plant Pathology, Shandong Agricultural University (HSAUP). Living cultures were deposited in the Shandong Agricultural University Culture Collection (SAUCC). Taxonomic information of the new taxa was submitted to MycoBank (http://www.mycobank.org).

DNA extraction and amplification

Genomic DNA was extracted from fungal mycelia grown on PDA, using a modified cetyltrimethylammonium bromide (CTAB) protocol as described in Guo et al. (2000). Four pairs of primers were adopted to amplify gene sequences (Hernández-Restrepo et al. 2016). The partial large subunit (LSU) rDNA, the internal transcribed spacer region with intervening 5.8S nrRNA gene (ITS), part of the beta-tubulin gene region (TUB2), and partial RNA polymerase II second largest subunit (RPB2) region were amplified and sequenced using primers pairs LR0R/LR5 (Vilgalys and Hester 1990),

ITS4/ITS5 (White et al. 1990), Btub526F and Btub1332R (Jewell and Hsiang 2013), and RPB2-5F2/fRPB2-7cR (Liu et al. 1999; Sung et al. 2007), respectively.

PCR was performed using an Eppendorf Master Thermocycler (Hamburg, Germany). Amplification reactions were performed in a 25 μL reaction volume, which contained 12.5 μL Green Taq Mix (vazyme, Nanjing, China), 1 μL of each forward and reverse primer (10 μM) (Biosune, Shanghai, China), and 1 μL template genomic DNA in amplifier, and were adjusted with distilled deionized water to a total volume of 25 μL.

PCR parameters were as follows: 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at a suitable temperature for 30 s, extension at 72 °C for 1 min and a final elongation step at 72 °C for 10 min. Annealing temperature for each gene were 55 °C for ITS, 51 °C for LSU, 56 °C for RPB2 and 53 °C for TUB2. The PCR products were visualised on 1% agarose electrophoresis gel. Sequencing was done bi-directionally, conducted by the Biosune Company Limited (Shanghai, China). Consensus sequences were obtained using MEGA 7.0 (Kumar et al. 2016). All sequences generated in this study were deposited in GenBank (Table 1).

Phylogenetic analyses

Novel sequences generated from the six strains in this study, and all reference available sequences of *Microdochium* species downloaded from GenBank (mostly used in Hernández-Restrepo et al. 2016; Zhang et al. 2017; Marin-Felix et al. 2019; Crous et al. 2018, 2019) were used for phylogenetic analyses. Alignments of the individual locus were determined using MAFFT v. 7.110 by default settings (Katoh et al. 2017) and manually corrected where necessary. To establish the identity of the isolates at species level, phylogenetic analyses were conducted first individually for each locus and then as combined analyses of four loci (ITS, LSU, TUB2, and RPB2 regions). Phylogenetic analyses were based on maximum likelihood (ML) and Bayesian inference (BI) for the multi-locus analyses. For BI, the best evolutionary model for each partition was determined using MrModeltest v. 2.3 (Nylander 2004) and incorporated into the analyses. ML and BI were run on the CIPRES Science Gateway portal (https://www.phylo.org/) (Miller et al. 2012) using RaxML-HPC2 on XSEDE (8.2.12) (Stamatakis 2014) and MrBayes on XSEDE (3.2.7a) (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003; Ronquist et al. 2012), respectively. For ML analyses the default parameters were used and BI was carried out using the rapid bootstrapping algorithm with the automatic halt option. Bayesian analyses included four parallel runs of 5,000,000 generations, with the stop rule option and a sampling frequency of 500 generations. The burn-in fraction was set to 0.25 and posterior probabilities (PP) were determined from the remaining trees. The resulting trees were plotted using FigTree v. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree) and edited with Adobe Illustrator CS5.1. New sequences generated in this study were deposited at GenBank (https://www.ncbi.nlm.nih.gov; Table 1), the alignments and trees were deposited in TreeBASE (http://treebase.org/treebase-web/home.html).

Table 1. Specimens and GenBank accession numbers of DNA sequences used in this study.

Species	Voucher	Host/Substrate	Country	GeneBank accession numbers			
				LSU	ITS	TUB2	RPB2
Idriella lunata	CBS 204.56*	Root of *Fragaria chiloensis*	USA	KP858981	KP859044	–	–
Microdochium albescens	CBS 291.79	On *Oryza sativa*	Ivory Coast	KP858932	KP858996	KP859059	KP859105
	CBS 243.83	Seed *Oryza sativa*	Unknown country	KP858930	KP858994	KP859057	KP859103
M. bolleyi	CBS 540.92	Root of *Hordeum vulgare*	Syria	KP858946	KP859010	KP859073	KP859119
M. citrinidiscum	CBS 109067*	Leaf of *Eichhornia crassipes*	Peru	KP858939	KP859003	KP859066	KP859112
M. chrysanthemoides	LC5363 = CGMCC3.17929*	Unnamed Karst Cave	China	KU746736	KU746690	KU746781	–
	LC5466 = CGMCC3.17930	Unnamed Karst Cave	China	KU746735	KU746689	KU746782	–
M. colombiense	CBS 624.94*	On *Musa sapientum*	Colombia	KP858935	KP858999	KP859062	KP859108
M. fisheri	CBS 242.90*	Stem of *Oryza sativa*	UK	KP858951	KP859015	KP859079	KP859124
M. indocalami	SAUCC1016*	Leaves of *Indocalamus longiauritus*	China	MT199878	MT199884	MT435653	MT510550
M. lycopodinum	CBS 146.68	Air sample	The Netherlands	KP858929	KP858993	KP859056	KP859102
	CBS 109397	On *Phragmites australis*	Germany	KP858940	KP859004	KP859067	KP859113
	CBS 109398	On *Phragmites australis*	Germany	KP858941	KP859005	KP859068	KP859114
M. majus	CBS 741.79	On *Triticum aestivum*	Germany	KP858937	KP859001	KP859064	KP859110
M. musae	CBS 111018 = CPC 5380	*Musa* cv. Cavendish	Costa Rica	–	AY293061	–	–
	CBS 143499 = CPC 32809	Leaves of *Musa* sp.	Malaysia	MH107941	MH107894	MH108040	–
	CBS 143500* = CPC 32689	Leaves of *Musa* sp.	Malaysia	MH107942	MH107895	MH108041	MH108003
	CPC 11234	Leaves of *Musa* sp.	Mauritius	MH107943	MH107896	MH108042	–
	CPC 11240	Leaves of *Musa* sp.	Mauritius	MH107944	MH107897	MH108043	–
	CPC 16258	Leaves of *Musa* sp.	Mexico	MH107945	MH107898	MH108044	–
	CPC 32681	Leaves of *Musa* sp.	Malaysia	MH107946	MH107899	–	–
M. neoqueenslandicum	CBS 445.95	On *Juncus effusus*	The Netherlands	KP858933	KP858997	KP859060	KP859106
	CBS 108926*	On *Agrostis* sp.	New Zealand	KP858938	KP859002	KP859065	KP859111
M. nivale	CBS 116205*	Roots of *Triticum aestivum*	UK	KP858944	KP859008	KP859071	KP859117
M. nivale var. *nivale*	CBS 288.50	Unknown	Unknown country	MH868135	MH856626	–	–
M. novae-zelandiae	CBS 143847	From turf leaves (Poaceae)	New Zealand	–	LT990655	LT990608	LT990641
	CPC 29693	From turf leaves (Poaceae)	New Zealand	–	LT990656	LT990609	LT990642
M. paspali	HK-ML-1371	*Paspalum vaginatum*	China	–	KJ569509	KJ569514	–
	QH-BA-48	*Paspalum vaginatum*	China	–	KJ569510	KJ569515	–
	SY-LQG66	*Paspalum vaginatum*	China	–	KJ569511	KJ569516	–
	WC-WC-85	*Paspalum vaginatum*	China	–	KJ569512	KJ569517	–
	WN-BD-452	*Paspalum vaginatum*	China	–	KJ569513	KJ569518	–
M. phragmitis	CBS 285.71*	On *Phragmites australis*	Poland	KP858949	KP859013	KP859077	KP859122
	CBS 423.78	On *Phragmites communis*	Germany	KP858948	KP859012	KP859076	KP859121
M. rhopalostylidis	CPC 34449 = CBS 145125*	*Rhopalostylis sapida*	New Zealand	MK442532	MK442592	MK442735	MK442667
M. seminicola	KAS3576 = CBS 139951*	Maize kernels	Switzerland	KP858974	KP859038	KP859101	KP859147
	KAS1516 = CPC 26001	On grain	Canada	KP858961	KP859025	KP859088	KP859134
	KAS3574 = DAOM 250155	Maize kernels	Switzerland	KP858973	KP859037	KP859100	KP859146
	KAS3158 = DAOM 250161	On *Triticum aestivum*	Canada	KP858970	KP859034	KP859097	KP859143
	KAS1527 = DAOM 250165	On grain	Canada	KP858966	KP859030	KP859093	KP859139
	KAS1473 = DAOM 250176	On *Triticum aestivum*	Canada	KP858955	KP859019	KP859082	KP859128

Species	Voucher	Host/Substrate	Country	GeneBank accession numbers			
				LSU	ITS	TUB2	RPB2
M. sorghi	CBS 691.96	Living *Sorghum halepense*	Cuba	KP858936	KP859000	KP859063	KP859109
***M.* sp. indet.**	SAUCC1017	Leaves of *Indocalamus longiauritus*	China	MT199879	MT199885	MT435654	–
M. tainanense	CBS 269.76*	Root of *Saccharum officinarum*	China, Taiwan	KP858945	KP859009	KP859072	KP859118
	CBS 270.76	Root of *Saccharum officinarum*	China, Taiwan	KP858931	KP858995	KP859058	KP859104
M. trichocladiopsis	CBS 623.77*	Rhizosphere of *Triticum aestivum*	Unknown country	KP858934	KP858998	KP859061	KP859107
M. yunnanense	SAUCC1011*	Leaves of *Indocalamus longiauritus*	China	MT199875	MT199881	MT435650	MT510547
	SAUCC1012	Leaves of *Indocalamus longiauritus*	China	MT199876	MT199882	MT543651	MT510548
	SAUCC1015	Leaves of *Indocalamus longiauritus*	China	MT199877	MT199883	MT435652	MT510549
	SAUCC1018	Leaves of *Indocalamus longiauritus*	China	MT199880	MT199886	MT435655	–

Isolates marked with "*" are ex-type or ex-epitype strain.

Results

Phylogenetic analyses

Six *Microdochium* strains isolated from plant hosts were sequenced. *Microdochium* was analysed by using multilocus data (ITS, LSU, TUB2 and RPB2) composed of 50 isolates of *Microdochium* and *Idriella lunata* (CBS 204.56) as the outgroup taxon. A total of 3257 characters including gaps were obtained in the phylogenetic analysis, viz. ITS: 1–572, LSU: 573–1429, TUB2: 1430–2395, RPB2: 2396–3257. Of these characters, 2019 were constant, 219 were variable and parsimony-uninformative, and 1019 were parsimony-informative. For the BI and ML analyses, GTR+I+G for LSU and RPB2, SYM+I+G for ITS, and GTR+G for TUB2 were selected and incorporated into the analyses. The ML tree topology confirmed the tree topologies obtained from the BI analyses, and therefore, only the ML tree is presented (Fig. 1).

ML bootstrap support values (≥ 75%) and Bayesian posterior probability (≥ 0.95) are shown as first and second position above nodes, respectively. The 50 strains were assigned to 23 species clades based on the four gene loci phylogeny (Fig. 1). The six strains studied here represented two novel species. The new species of *Microdochium yunnanense* showed a close relationship to *M. bolleyi* (CBS 540.92) with good support (ML-BS: 98% and BYPP: 1.00). *Microdochium indocalami* (SAUCC1016) appeared most closely related to *M. fisheri* (CBS 242.90), *M. lycopodinum* (CBS 146.68), *M. rhopalostylidis* (CBS 145125), and *M. phragmitis* (CBS 285.71) with high support by the multi-locus phylogeny. From the tree (Fig. 1), strain SAUCC1017 formed a conspicuous branch independent from other *Microdochium* species, thus supporting the introduction of SAUCC1017 as an indeterminate species.

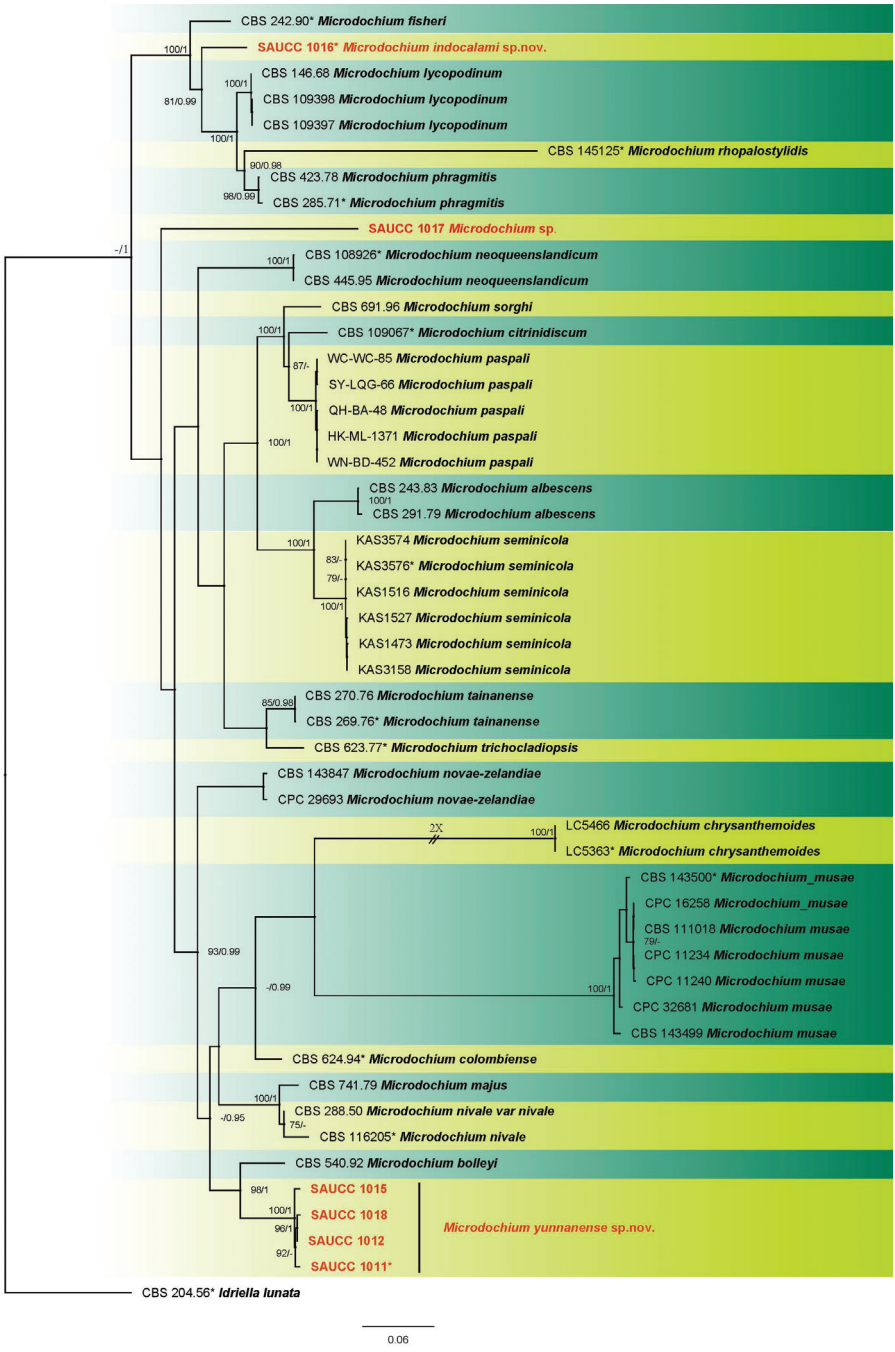

Figure 1. Phylogram of *Microdochium* based on combined ITS, LSU, TUB2 and RPB2 genes. The ML and BI bootstrap support values above 75% and 0.95 BYPP are shown at the first and second position, respectively. Strains marked with "*" are ex-type or ex-epitype. Strains from the current study are in red. Some branches were shortened to fit them to the page – these are indicated by two diagonal lines with the number of times a branch was shortened indicated next to the lines.

Taxonomy

Microdochium indocalami **S.T. Huang, J.W. Xia, X.G. Zhang, W.X. Sun & Z. Li, sp. nov.**

MycoBank No: 835766

Figure 2

Etymology. Name refers to the genus of the host plant *Indocalamus longiauritus*.

Diagnosis. Characterised by the size of conidia and the number of septa of conidia.

Type. China, Yunnan Province: Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, on diseased leaves of *Indocalamus longiauritus*. 16 April 2019, S.T. Huang, HSAUP1016, holotype, ex-type living culture SAUCC1016.

Description. Colonies on PDA attaining 46.1–51.2 mm in diameter after 7 days, formed a conspicuous concentric circle, periphery of aerial mycelium cottony, centre with scarce aerial mycelium, white initially, then becoming greyish sepia after 25 days. Some aerial hyphae aggregated and form a sporodochium within 15 days or longer. Mycelium composed of hyaline, immersed and superficial, smooth, branched, septate, 2.0–3.0 μm wide hyphae. Due to the soluble pigment secreted, reverse white to salmon. Conidiophores straight or slightly curved, aseptate, aggregated in the aerial mycelium, often reduced to conidiogenous cells borne directly from the hyphae. Conidiogenous cells terminal or intercalary, mono-or polyblastic, denticulate, smooth, hyaline, cylindrical, straight or bent, 11.0–28.3 × 1.5–2.9 μm. Conidia cylindrical, clavate to obovoid, 1–3-septate, 13.0–15.5 × 3.5–5.5 μm, base usually flattened 0.5–1.0 μm. Sometimes borne directly from the mycelial hyphae. Sexual morph: unknown.

Culture characteristics. Colonies on OA 62.0–64.0 mm in diameter after 7 days, centre with aerial mycelium cottony, periphery with scarce aerial mycelium. Mycelium mostly immersed, hyphae hyaline, septate, smooth, exudate and soluble pigment produced, reverse white initially, then becoming pale mouse-grey in periphery and mouse-grey in center. Sporodochia formed on agar surface. Colonies on MEA 50.8–52.7 mm in diameter after 7 days, aerial mycelium abundant, with concentric rings, white to pale pink, periphery with cottony aerial mycelium, centre with scarce aerial mycelium, exudate absent. Reverse white to pale pink with age.

Habitat and distribution. Isolated from leaves of *Indocalamus longiauritus* in China.

Notes. *Microdochium* and allied genera were revised by Hernández-Restrepo et al. (2016). Phylogenetic analysis of a combined four gene showed that *M. indocalami* (strain SAUCC1016) formed a separated branch as the clade of *M. fisheri*, *M. lycopodinum*, *M. phragmites*, and *M. rhopalostylidis* with good support (ML-BS: 100% and BYPP: 1.00) (Fig. 1). Additionally, conidiogenous cells of *M. indocalami* are terminal or intercalary, denticulate, cylindrical which are similar to the species in this clade. The size of conidia and the number of septa of conidia reported for *M. fisheri* (7.0–12.0 × 3.0–4.0 μm, 0–1-septate), *M. lycopodinum* (8.0–15.5 × 2.5–4.0 μm, 0–1-septate), *M. phragmites* (10.0–14.5 × 2.0–3.0 μm, 0–1-septate), and *M. rhopalostylidis* ((13.0–) 16.0–20.0 (–23.0) × (2.5–) 3.0 (–4.0) μm, 1–3-septate) (Hernández-Restrepo et al. 2016; Crous et al. 2019) were different to *M. indocalami* (13.0–15.5 × 3.5–5.5 μm, 1–3-septate).

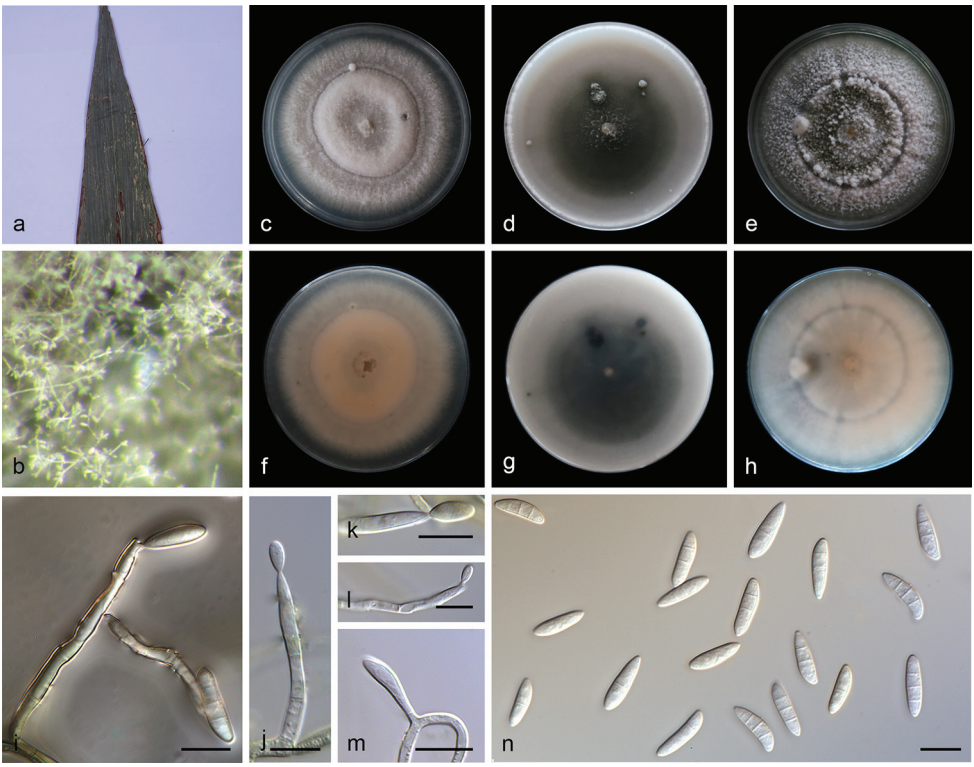

Figure 2. *Microdochium indocalami* (SAUCC1016) **a** leaves of host plant **b** colony overview **c–e** surface of colony after 15 days on PDA (**c**), OA (**d**), MEA (**e**) **f–h** reverse of colony after 15 days on PDA (**f**) OA (**g**) MEA (**h**) **i–m** conidiophores and conidiogenous cells **n** conidia. Scale bars: 10μm (**i–n**).

Microdochium yunnanense **S.T. Huang, J.W. Xia, X.G. Zhang, W.X. Sun & Z. Li, sp. nov.**
MycoBank No: 835765
Figure 3

Etymology. Named after Yunnan Province, where the fungus was collected.

Diagnosis. Characterised by conidiomata sporodochium-like and the size of conidia.

Type. China, Yunnan Province: Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, on diseased leaves of *Indocalamus longiauritus*. 16 April 2019, S.T. Huang, HSAUP1011 holotype, ex-type living culture SAUCC1011.

Description. Colonies on PDA attaining 48.0–61.5 mm in diameter after 15 days, felty, compact, erose or dentate, initially white, then becoming yellowish with age. Mycelium superficial, consisting of hyaline, smooth, branched, septate, 1.0–2.5 μm wide hyphae. Conidiomata sporodochium-like, appeared within 15 days or longer, formed in aerial mycelium or on agar surface, slimy, hyaline or orange, semi-submerged. Exudate occasionally appeared on old sporodochia. Reverse colorless to yellowish, due to the soluble pigment secreted. Conidiophores formed terminal or lateral with sympo-

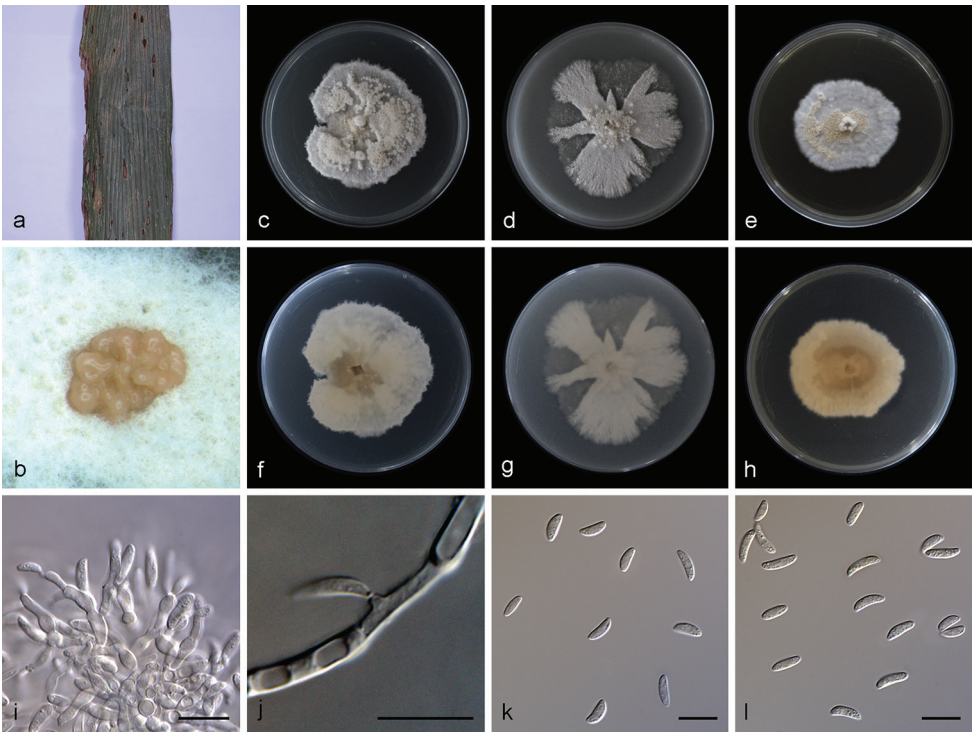

Figure 3. *Microdochium yunnanense* (SAUCC1011) **a** leaves of host plant **b** sporodochia on media surface **c–e** surface of colony after 15 days on PDA (**c**), OA (**d**), MEA (**e**) **f–h** reverse of colony after 15 days on PDA (**f**), OA (**g**), MEA (**h**) **i** sporodochial conidiophores and conidiogenous cells with conidia **j** conidiogenous cells **k–l** conidia. Scale bars: 10μm (**i–l**).

dial proliferation, solitary or aggregated. Most conidiophores tightly aggregated in a sporodochium, inconspicuous flat-tipped loci, irregularly branched, or borne directly on mycelial hyphae, straight or slightly curved, aseptate, guttulate, smooth-walled, apex subobtuse, base truncate. Conidiogenous cells of two types: some polyblastic, ampulliform, lageniform, with percurrent proliferations, 6.5–10.0 × 2.5–3.4 μm, neck up to 4.5 μm long, 1.0–1.5 μm wide, others straight or bent, smooth, cylindrical up to 10.0–11.5 μm long, 1.0–2.0 μm wide. Conidia aseptate, mostly lunate, a few ellipsoid and cylindrical, hyaline, straight or curved, obtuse, 0–2 guttulate in mature conidia, 6.8–10.0 × 2.4–3.5 μm. Chlamydospores was not observed. Sexual morph: unknown.

Culture characteristics. Colonies on OA 58.1–61.5 mm in diameter after 15 days, entire, flat, white, lobate and radially margin, aerial mycelia cottony or sparse. Less exudate. Reverse white. Vegetative hyphae hyaline, abundant, branched, septate, thin-walled. Colonies on MEA 39.5–48.2 mm in diameter after 15 days, dense, initially white, becoming pale yellow, the centre of aerial mycelium cottony, periphery with scarce aerial mycelium, aerial mycelium formed a protuberance at center of colony.

Habitat and distribution. Isolated from leaves of *Indocalamus longiauritus* in China.

Additional specimens examined. China, Yunnan Province: Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, on diseased leaves of *Indocalamus longiauritus*. 16 April 2019, S.T. Huang, HSAUP1012, HSAUP1015, and HSAUP1018 paratype; living culture SAUCC1012, SAUCC1015, and SAUCC1018.

Notes. Strains SAUCC1011, SAUCC1012, SAUCC1015, and SAUCC1018 belong to a single species as they have similar morphological features including culture characteristics, sporodochium, and conidia, the nearly identical sequence data, and cluster in a separate branch with a good support (ML-BS: 100% and BYPP: 1.00). The species is most phylogenetically close to *M. bolleyi*, and their branch lengths are slightly different. Nevertheless, the morphology of *M. yunnanense* and *M. bolleyi* (Hong et al. 2008) are different in having sporodochium-like conidiomata, conidiogenous cells, and conidia. *Microdochium yunnanense* produced some sporodochium-like conidiomata, slimy, hyaline or orange, semi-submerged on the agar surface, with most conidial droplets formed on it and a few formed laterally along with hyaline hyphal cells. However, the conidia of *M. bolleyi* only formed laterally along with hyaline hyphal cells. They all produced two types of conidiogenous cells, cylindrical and ampulliform, but the size of *M. yunnanense* (10.0–11.5 × 1.0–2.0 μm (average 10.7 × 1.6 μm) and 6.5–10.0 × 2.5–3.4 μm (average 8.4 × 2.9 μm)), *M. bolleyi* (1.5–2.7 × 0.8–1.4 μm (average 2.1 × 1.0 μm) and 3.1–6.4 × 2.5–3.8 μm (average 4.9 × 3.2 μm)) were clearly different. Conidial shape was differed little, but the conidial size of *M. yunnanense* (6.8–10.0 × 2.4–3.5μm (average 8.3 × 3.1 μm)) has much larger than *M. bolleyi* (5.0–8.7 × 1.6–2.3 μm (average 6.4 × 1.9 μm)).

***Microdochium* sp. indet.**
Figure 4

Description. Colonies on PDA attaining 73.9–80.4 mm in diameter after 15 days, felty to cottony, flat, margin entire or dentate, white, aerial mycelium abundant. Mycelium superficial, hyphae hyaline, septate, branched, smooth-walled. Reverse white to pale yellow, with yellow pigment produced with aging. Aerial hyphae aggregated to form numerous chlamydospores on agar surface. Chlamydospores thick-walled, terminal or intercalary, more frequently arranged in chains than clusters. Conidiophores not observed. Colonies on OA attaining 79.9–81.7 mm in diameter after 15 days, fluffy, margin entire, white. Reverse white. Colonies on MEA attaining 73.2–78.4 mm in diameter after 15 days, flat, with pale pink inconspicuous concentric circle near the centre, margin entire and white, aerial mycelium abundant.

Material examined. China, Yunnan Province: Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, on diseased leaves of *Indocalamus longiauritus*. 16 April 2019, S.T. Huang, HSAUP1017, living culture SAUCC1017.

Note. Strain SAUCC1017 failed to produce conidia and lacks a complete morphological description. It formed a conspicuous independent lineage from other *Microdochium* species in the tree. ITS sequence BLASTn search of SAUCC1017 showed

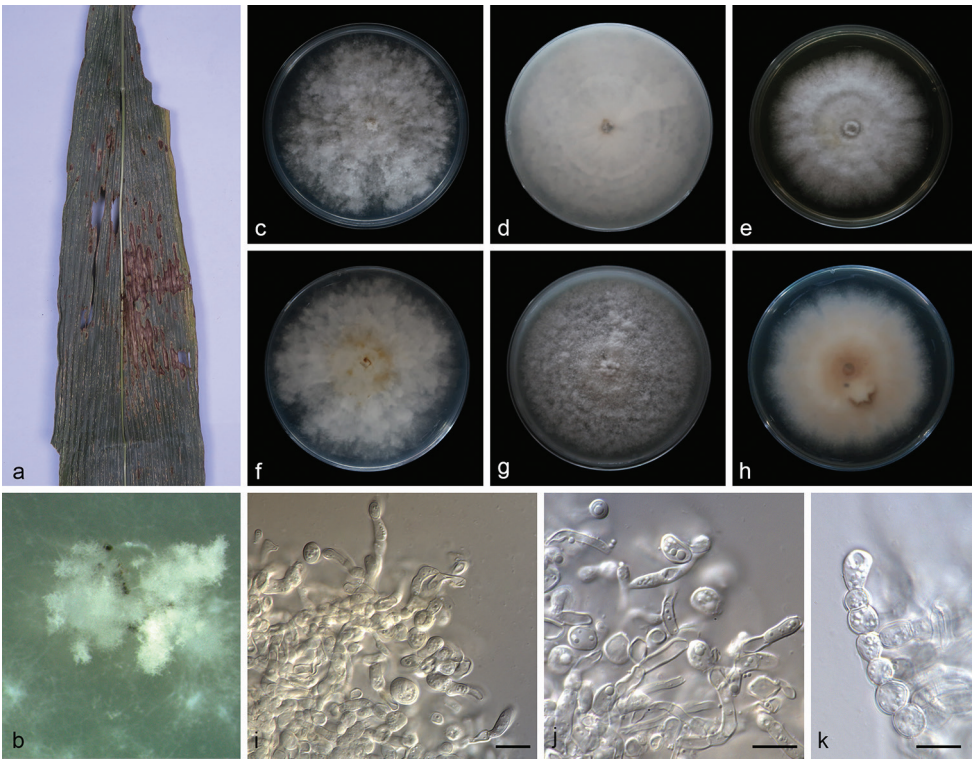

Figure 4. *Microdochium* sp. (SAUCC1017) **a** leaves of host plant **b** colony overview **c–e** surface of colony after 15 days on PDA (**c**) OA (**d**) MEA (**e**) **f–h** reverse of colony after 15 days on PDA (**f**) OA (**g**) MEA (**h**) **i–k** chlamydospores. Scale bars: 10μm (**i–k**).

many different species with 97% identity. BLASTn searches with LSU (GenBank MH869857) sequences result in 99% identity with *M. bolleyi* (CBS 172.63) and TUB2 (GenBank AB625368) sequences result in 99% identity with *Xylaria cubensis* (strain BCC 18758). Thus, here we listed it as an unidentified species.

Discussion

Previous studies placed *Microdochium* in Amphisphaeriaceae (Parkinson et al. 1981; Samuels and Hallet 1983; von Arx 1984; Jaklitsch and Voglmayr 2012), which is a large heterogeneous family possessing pestalotiopsis-like asexual morphs characterised by holoblastic conidiogenous cells that produce septate, brown or hyaline conidia with appendages at both ends (Tanaka et al. 2011; Maharachchikumbura et al. 2014). Nevertheless, based on the results of phylogenetic analyses, *Microdochium*, *Idriella*, and *Selenodriella* were incorporated to a new family introduced as Microdochiaceae by Hernández-Restrepo et al. (2016), which is characterised by asexual morphs that

produce polyblastic, sympodial or annellidic conidiogenous cells with hyaline conidia without appendages and sexual morphs that are monographella-like. In *Microdochium*, the color of conidiogenous cells is hyaline, and the shape of conidia seem to be taxonomic important feature. The conidial shape of *Microdochium* is more variable from cylindrical, fusoid or oblong, to lunate, straight or curved, with truncate bases and apices mainly rounded.

Three sections were widely accepted in *Microdochium* based on the type of conidiogenous cells and conidia by Braun (1995) and Hernández-Restrepo et al. (2016). Type I: *Microdochium* sect. *Gerlachia* forming annellidic conidiogenous cells with percurrent proliferations; Type II: *Microdochium* forming sympodial, often subdenticulate conidiogenous cells, and relatively more or less fusiform, straight to somewhat curved or falcate, 0–3-septate or even pluriseptate conidia; and Type III: *Gloeocercospora* forming sympodial conidiogenous cells, and very long, scolecosporous and pluriseptate conidia.

From the previous molecular studies of *Microdochium* (Jaklitsch and Voglmayr 2012; Jewell and Hsiang 2013; Zhang et al. 2015), the four gene regions (ITS, LSU, RPB2, TUB2) were chosen in this study. The LSU is informative enough for generic placement of *Microdochium*. The individual gene regions of ITS, TUB2, and RPB2 proved to be able to resolve species in *Microdochium* (results not shown). However, TUB2 was the more informative than other gene regions and showed longer distances between species and higher support values. This results in our study agree with previous studies in other xylariaceous genera (Hsieh et al. 2005; Læssøe et al. 2013; Hernández-Restrepo et al. 2016). By combining phylogenetic analysis and morphology, two species of *Microdochium* were delimited as new species, namely *M. yunnanense* sp. nov. and *M. indocalami* sp. nov. In order to support the validity of these new species, we followed the guidelines of Hernández-Restrepo et al. (2016).

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References

von Arx JA (1984) Notes on *Monographella* and *Microdochium*. Transactions of The British Mycological Society 83(2): 373–374. https://doi.org/10.1016/S0007-1536(84)80168-0

Braun U (1995) A Monograph of *Cercosporella*, *Ramularia* and allied genera (phytopathogenic *Hyphomycetes*) (Vol. 1). IHW-Verlag, Eching, 494 pp.

Cai L, Hyde KD, Taylor PWJ, Weir B, Waller J, Abang MM, Zhang ZJ, Yang YL, Phoulivong S, Liu ZY, Prihastuti H, Shivas RG, McKenzie EHC, Johnston PR (2009) A polyphasic approach for studying *Colletotrichum*. Fungal Diversity 39: 183–204.

Choi YW, Hyde KD, WH Ho (1999) Single spore isolation of fungi. Fungal Diversity 3: 29–38.

Chomnunti P, Hongsanan S, Aguirre-Hudson B, Tian Q, Peršoh D, Dhami MK, Alias AS, Xu JC, Liu XZ, Stadler M (2014) The sooty moulds. Fungal Diversity 66(1): 1–36. https://doi.org/10.1007/s13225-014-0278-5

Crous PW, Schumacher RK, Akulov A, Thangavel R, Hernández-Restrepo M, Carnegie AJ, Cheewangkoon R, Wingfield MJ, Summerell BA, Quaedvlieg W (2019) New and interesting fungi 2. Fungal Systematics and Evolution 3 (1): 57–134. https://doi.org/10.3114/fuse.2019.03.06

Crous PW, Schumacher RK, Wingfield MJ, Akulov A, Denman S, Roux J, Braun U, Burgess TI, Carnegie AJ, Váczy KZ (2018) New and interesting fungi 1. Fungal Systematics and Evolution 1(1): 169–215. https://doi.org/10.3114/fuse.2018.01.08

Gao YH, Sun W, Su YY, Cai L (2014) Three new species of *Phomopsis* in Gutianshan Nature Reserve in China. Mycological Progress 13(1): 111–121. https://doi.org/10.1007/s11557-013-0898-2

Glynn NC, Hare MC, Parry DW, Edwards SG (2005) Phylogenetic analysis of EF-1 alpha gene sequences from isolates of *Microdochium Nivale* leads to elevation of varieties *Majus* and *Nivale* to species status. Fungal Biology 109(8): 872–880. https://doi.org/10.1017/S0953756205003370

Guo LD, Hyde KD, Liew ECY (2000) Identification of endophytic fungi from *Livistona chinensis* based on morphology and rDNA sequences. New Phytologist 147(3): 617–630. https://doi.org/10.1046/j.1469-8137.2000.00716.x

Hernández-Restrepo M, Groenewald JZ, Crous PW (2016) Taxonomic and phylogenetic re-evaluation of *Microdochium*, *Monographella* and *Idriella*. Persoonia 36(1): 57–82. https://doi.org/10.3767/003158516X688676

Hong SK, Kim WG, Choi HW, Lee SY (2008) Identification of *Microdochium bolleyi* associated with basal rot of creeping bent grass in Korea. Mycobiology 36(2): 77–80. https://doi.org/10.4489/MYCO.2008.36.2.077

Hsieh HM, Ju YM, Rogers JD (2005) Molecular phylogeny of *Hypoxylon* and closely related genera. Mycologia 97(4): 844–865. https://doi.org/10.1080/15572536.2006.11832776

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17(17): 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Jaklitsch WM, Voglmayr H (2012) Phylogenetic relationships of five genera of Xylariales and *Rosasphaeria* gen. nov. (Hypocreales). Fungal Diversity 52(1): 75–98. https://doi.org/10.1007/s13225-011-0104-2

Jewell LE, Hsiang T (2013) Multigene differences between *Microdochium Nivale* and *Microdochium majus*. Botany 91(2): 99–106. https://doi.org/10.1139/cjb-2012-0178

Katoh K, Rozewicki J, Yamada KD (2017) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4): 1160–1166. https://doi.org/10.1093/bib/bbx108

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870–1874. https://doi.org/10.1093/molbev/msw054

Kwasna H, Bateman GL (2007) *Microdochium Triticicola* sp. nov. from roots of *Triticum aestivum* in the United Kingdom. Mycologia 99(5): 765–776. https://doi.org/10.3852/mycologia.99.5.765

Læssøe T, Srikitikulchai P, Luangsa-ard JJD, Stadler M (2013) *Theissenia* reconsidered, including molecular phylogeny of the type species *T. Pyrenocrata* and a new genus *Durotheca* (Xylariaceae, Ascomycota). IMA Fungus 4(1): 57–69. https://doi.org/10.5598/imafungus.2013.04.01.07

Liu FF, Weir BS, Damm U, Crous PW, Wang Y, Liu B, Wang M, Zhang MM, Cai L (2015) Unravelling *Colletotrichum* species associated with *Camellia*: employing ApMat and GS loci to resolve species in the *C. Gloeosporioides* complex. Persoonia 35: 63–86. https://doi.org/10.3767/003158515X687597

Liu YJ, Whelen S, Hall BD (1999) Phylogenetic Relationships among Ascomycetes: evidence from an RNA polymerse II subunit. Molecular Biology and Evolution 16(12): 1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092

Maharachchikumbura SSN, Hyde KD, Groenewald JZ (2014) Pestalotiopsis revisited. Studies in Mycology 79: 121–186. https://doi.org/10.1016/j.simyco.2014.09.005

Marin-Felix Y, Hernández-Restrepo M, Wingfield MJ, Akulov A, Carnegie AJ, Cheewangkoon R, Gramaje D, Groenewald JZ, Guarnaccia V, Halleen F (2019) Genera of phytopathogenic fungi: GOPHY 2. Studies in Mycology 92: 47–133. https://doi.org/10.1016/j.simyco.2018.04.002

Miller MA, Pfeiffer W, Schwartz T (2012) The CIPRES science gateway: enabling high-impact science for phylogenetics researchers with limited resources. Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment. Bridging from the extreme to the campus and beyond. Association for Computing Machinery, USA, 8 pp. https://doi.org/10.1145/2335755.2335836

Nylander JAA (2004) MrModelTest v. 2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Parkinson VO, Sivanesan A, Booth C (1981) The perfect state of the rice leaf scald fungus and the taxonomy of both the perfect and imperfect states. Transactions of The British Mycological Society 76(1): 59–69. https://doi.org/10.1016/S0007-1536(81)80009-5

Petrak F (1924) Mykologische Notizen. VII (No. 144–145). Annales Mycologici 22(1–2):1–182.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian Phylogenetic Inference under Mixed Models. Bioinformatics 19(12): 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Samuels GJ, Hallett IC (1983) *Microdochium stoveri* and *Monographella stoveri*, new combinations for *Fusarium stoveri* and *Micronectriella stoveri*. Transactions of The British Mycological Society 81(3): 473–483. https://doi.org/10.1016/S0007-1536(83)80115-6

Stamatakis A (2014) RAxML Version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Sung GH, Sung JM, Hywel-Jones NL, Spatafora JW (2007) A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): identification of localized incongruence using a combinational bootstrap approach. Molecular Phylogenetics and Evolution 44(3): 1204–1223. https://doi.org/10.1016/j.ympev.2007.03.011

Sydow H (1924) Mycotheca germanic. Fasc. XLII–XLV (No. 2051–2250). Annales Mycologici 22: 257–268.

Tanaka K, Endo M, Hirayama K, Okane I, Hosoya T, Sato T (2011) Phylogeny of *Discosia* and *Seimatosporium*, and introduction of *Adisciso* and *Immersidiscosia* genera nova. Persoonia 26(1): 85–98. https://doi.org/10.3767/003158511X576666

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172(8): 4238–4246. https://doi.org/10.1128/JB.172.8.4238-4246.1990

White TJ, Bruns T, Lee S, Taylor FJRM, Lee SH, Taylor L, Shawe-Taylor J (1990) Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics. Innis MA, Gelfand DH, Sninsky JJ (Eds) PCR Protocols: a Guide to Methods and Applications. Academic Press Inc., New York, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zhang K, Su YY, Cai L (2013) An optimized protocol of single spore isolation for fungi. Cryptogamie Mycologie 34(4): 349–356. https://doi.org/10.7872/crym.v34.iss4.2013.349

Zhang W, Nan ZB, Tian P, Hu MJ, Gao ZY, Li M, Liu GD (2015) *Microdochium Paspali*, a new species causing seashore paspalum disease in southern China. Mycologia 107(1): 80–89. https://doi.org/10.3852/14-119

Zhang ZF, Liu F, Zhou X, Liu XZ, Liu SJ, Cai L (2017) Culturable Mycobiota from karst caves in China, with descriptions of 20 new species. Persoonia 39(1): 1–31. https://doi.org/10.3767/persoonia.2017.39.01

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RESEARCH ARTICLE

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When mycologists describe new species, not all relevant information is provided (clearly enough)

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Abstract

Taxonomic mycology struggles with what seems to be a perpetual shortage of resources. Logically, fungal taxonomists should therefore leverage every opportunity to highlight and visualize the importance of taxonomic work, the usefulness of taxonomic data far beyond taxonomy, and the integrative and collaborative nature of modern taxonomy at large. Is mycology really doing that, though? In this study, we went through ten years' worth (2009–2018) of species descriptions of extant fungal taxa – 1,097 studies describing at most ten new species – in five major mycological journals plus one plant journal. We estimated the frequency at which a range of key words, illustrations, and concepts related to ecology, geography, taxonomy, molecular data, and data availability were provided with the descriptions. We also considered a range of science-demographical aspects such as gender bias and the rejuvenation of taxonomy and taxonomists as well as public availability of the results. Our results show that the target audience of fungal species descriptions appears to be other fungal taxonomists, because many aspects of the new species were presented only implicitly, if at all. Although many of the parameters we estimated show a gradual, and in some cases marked, change for the better over time, they still paint a somewhat bleak picture of mycological taxonomy as a male-dominated field where the wants and needs of an extended target audience are often not understood or even considered. This study hopes to leave a mark on the way fungal species are described by putting the focus on ways in which fungal taxonomy can better anticipate the end users of species descriptions – be they mycologists, other researchers, the public at large, or even algorithms. In the end, fungal taxonomy, too, is likely to benefit from such measures.

Keywords
collaboration, gender equality, metadata, reproducibility, species description, taxonomy

Introduction

Taxonomy is the science that discovers, identifies, classifies, and describes organisms. Like in any scientific field, the knowledge gained in taxonomy has a value in itself, but it also caters to the needs of other research areas. Almost all studies in biology, and many other sciences, are performed on a taxon (often a species), derivatives from samples of a specific taxon (e.g., a protein), or pertain to the diversity of taxa. This view of the fundamental nature of taxonomy is certain to be shared by scientists and decision makers alike, but surprisingly this is not enough to guarantee a steady long-term supply of resources to taxonomy (Drew 2011). In fact, the funding for taxonomy is at a record low. In what has become known as the "taxonomy crisis" and the "taxonomic impediment" (Wheeler 2004; de Carvalho 2007), taxonomists are finding themselves at nearly the same risk of extinction as the very species they are supposed to study. Various mechanisms have been put forward to visualize and highlight the importance of taxonomy and to give credit to taxonomic work, such as citing authors of species names – and the underlying publications – when using those species names in publications (Wägele et al. 2011). However, the extent to which these suggestions seem to have taken effect appears to be limited, as taxonomy remains locked in a state of crisis (Magoga et al. 2016).

Since the "taxonomy crisis" has been acting out gradually during at least the last 20 years, it is reasonable to think that few biologists are unaware of it. Taxonomists, in particular, are certain to be all too familiar with it, often reporting feeling marginalized in comparison to ecological or molecular initiatives in the context of, e.g., grant writing and scientific funding (Giangrande 2003; Coleman 2015). In this context it is important to focus on the values of taxonomy and how they can be communicated to a wide audience. In this way of thinking, every new species description is a potential outlet for information that is useful not only in taxonomy but also in ecology, conservation biology, agriculture, and so on. The potential of each and every such outlet should be maximized in the interest of taxonomy. But are taxonomic papers written accordingly?

Several of the present authors have spent significant time going through published species descriptions for key data on, e.g., taxonomy, ecology, and geography for compilation into community-driven efforts such as UNITE (Nilsson et al. 2019), PlutoF (Abarenkov et al. 2010), FUNGuild (Nguyen et al. 2016), and Wikipedia (https://www.wikipedia.org/). We have found that scrutinizing species descriptions for key information can be surprisingly frustrating and time-consuming, largely owing to the heterogeneous or implicit ways in which information is sometimes provided (or omitted altogether) in species descriptions. Our experience is that straightforward questions such as "What does the new species do for a living?" are often not addressed at all, or are treated only very indirectly by statements such as "on dead branch of *Quercus*" (perhaps implying a saprotrophic

ecology). Another highly relevant question – "Where in the fungal tree of life does the new species belong?" – is similarly often hard to make out from the paper, often requiring a genus name query in NCBI Taxonomy (Federhen 2011) or some similar database. Questions on, e.g., the geographical distribution of the new species are likewise often hard to answer. This lack of information is most unfortunate – certainly, species descriptions should be more or less self-sustained, such that they should not expect significant mycological experience or Google searches on the part of the reader. Our initial observations would indicate that species descriptions are written either for narrow intradisciplinary communication or are disconnected from the wants and needs of many readers. This lowers their impact considerably and is hardly in the interest of taxonomy or mycology at large. Similarly, the field of fungal taxonomy would do good to show that it is, in fact, a vibrant, modern, and collaborative discipline – a discipline that cares little for country borders, where both genders take an active part, and where knowledge is readily shared with, and passed on to, aspiring researchers as well as the public at large. But is that really happening?

To assess whether fungal species descriptions are attuned to both the wants and needs of a target audience beyond taxonomists and the sign of the times, we explored 10 years' worth of fungal species descriptions of extant mycological taxa in five major mycological journals (plus one botany journal for reference) for a range of factors pertaining to inter- and intra-scientific terms and concepts, science-demographical aspects, and illustrations and visualisations (Tables 1, 2; Suppl. materials 1, 2). We processed the underlying PDF files using a text mining approach where specific keywords were used to simulate a non-taxonomic reader. We also scored each paper manually for a number of features deemed relevant to the overall reader experience – such as the presence of a color photo (or illustration) of the organism being described, whether a map or a habitus photo was provided, and whether we could access the paper from a computer not connected to a university network (Table 2). Our results show that there is much that can be improved in taxonomic descriptions to increase their availability, appeal, and usefulness to a wider scientific and public community and thus the impact of the work and of taxonomy itself. Similarly, fungal taxonomists should adapt their output to an increasing number of automated readers, including data aggregators and search engines. Fortunately, many of these improvements can be implemented in manuscripts in a matter of minutes and at zero cost. Other aspects of our results should make mycologists rethink who should be invited to our studies, and how we would like taxonomic expertise to be passed on to younger researchers. The present study seeks to

Table 1. The journals targeted for species descriptions 2009–2018.

Journal name	Journal field	Continent
Mycologia	Mycology	North America
Fungal Biology (Mycological Research)	Mycology	Europe
Mycoscience	Mycology	Asia
Mycological Progress	Mycology	Europe
Studies in Mycology	Mycology	Europe
Plant Systematics and Evolution	Botany	Europe

Table 2. Estimates obtained by the automated and manual parsing of the PDF files, broken down to three individual years (columns 2–4) as well as overall (column 5). Column 6 indicates our interpretation of the mycological repercussions of the trend in the data. Suppl. material 2 breaks down these estimates onto the individual 1,097 mycological papers, and Suppl. material 1 shows the full syntax used to query the individual papers for each parameter group in column 1. The following estimates are given in per cent. Altitude – altitude of sampling. Biodiversity – whether the term "biodiversity" was mentioned. Climate – whether the word "climate" was used. Climate zone – whether reference to climatic zone was used. Collection – whether reference to "herbarium" etc. was used. Distribution – whether reference to geographical distribution was used. Ecological association – whether any ecological association was indicated. Ecological mode – whether the ecological mode was indicated. Ecology, the term – whether variations of the word "ecology" was used. Family (classification) – whether a family name was provided. GIS/GPS – whether GIS/GPS co-ordinates were provided. Index Fungorum – whether this resource was mentioned. Locality, the term – whether variations of "locality" were mentioned. Molecular availability (TreeBase/Dryad) – whether reference to TreeBase or Dryad was made. Molecular search (BLAST) – whether reference to BLAST was made. Molecular database (e.g., GenBank) – whether reference to, e.g., GenBank was made. Molecular data used – whether DNA data was used. Mycobank – whether Mycobank was mentioned. Order (classification) – whether order was provided. Phylum (classification) – whether phylum was provided. Societal implications – whether societal implications were alluded to. Supplementary data – whether supplementary data were bundled with the paper. Threatened (endangered) – whether the taxon was highlighted as threatened or endangered. Color photo/illustration – whether a depiction of more or less the entire fungus was provided, as opposed to only micromorphological details. Determination key provided – whether a determination key was provided. Discussion section present – whether a dedicated Discussion section was provided. Electron microscopy used – whether electron microscopy was used. Fungal culture shown – whether a photo of a fungal culture was shown. Lead author male – whether the lead author was male. Macro-photo indicates size – whether macroscopic images used scale bars/fiducial markers. Manual micromorphology illustration – papers illustrating micromorphological features using manual illustrations. Map used – whether a map was provided. Paper available – whether the paper was found to be openly accessible. Phylogenetic tree shown – whether a phylogenetic tree was provided (molecular or otherwise). Photo showing biological context – whether a photo or illustration indicating the biological context of the species was provided. Photo of micromorphology – whether microscopic details were illustrated by photos. Spore print provided – whether a spore print was provided. The following estimates are provided as averages. Academic age, last author – the academic age of the last author as assessed through Google Scholar profiles. Academic age, lead author – the academic age of the lead author. Co-authors – the number of co-authors. Co-author continents – the number of continents in the list of co-authors. Co-author countries – the number of countries in the list of co-authors. Co-author departments – the number of departments in the list of co-authors. Female co-authors – the number of female co-authors. Pages – the number of pages. Statistical figures – the number of statistical figures/data visualizations.

Parameter group (automated search)	2009	2013	2018	All years	Trend interpretation
Altitude	26.76	13.64	19.30	16.86	Unclear
Biodiversity	12.68	22.73	27.19	23.25	Positive
Climate	7.04	10.91	18.42	13.67	Positive
Climate zone	88.73	83.64	92.98	89.15	Positive
Collection (specimen/culture repository)	76.06	85.45	92.11	84.59	Positive
Distribution (geography)	74.65	78.18	92.11	82.77	Positive
Ecological association	92.96	75.45	93.86	88.15	Unclear
Ecological mode	77.46	69.09	82.46	75.39	Positive
Ecology, the term	29.58	45.45	56.14	42.02	Positive
Family (classification)	64.79	74.55	85.96	75.39	Positive
GIS/GPS	8.45	2.73	4.39	4.47	Negative

Parameter group (automated search)	2009	2013	2018	All years	Trend interpretation
Index Fungorum	4.23	6.36	14.91	11.12	Positive
Locality, the term	29.58	42.73	37.72	35.64	Unclear
Molecular availability (TreeBase/Dryad)	33.80	50.91	69.30	53.78	Positive
Molecular search (BLAST)	9.86	34.55	46.49	35.46	Positive
Molecular database (e.g., GenBank)	57.75	84.55	94.74	85.69	Positive
Molecular data used	71.83	90.91	97.37	90.79	Positive
Mycobank	53.52	94.55	92.11	88.06	Positive
Order (classification)	57.75	64.55	71.93	64.81	Positive
Phylum (classification)	21.13	34.55	50.88	31.91	Positive
Societal implications	50.70	48.18	64.91	53.60	Positive
Supplementary data	5.63	19.09	37.72	24.43	Positive
Threatened (endangered)	0.00	2.73	3.51	2.37	Positive
Parameter group (manual search)					
Colour photo/illustration	30.99	70.91	88.60	73.02	Positive
Determination key provided	29.58	27.27	18.42	24.52	Negative
Discussion section present	71.83	75.45	79.82	77.58	Positive
Electron microscopy used	23.94	26.36	22.81	24.89	No change
Fungal culture shown	22.54	21.82	34.21	25.16	Unclear
Lead author male	72.73	69.33	60.26	68.73	Positive
Macro-photo indicates size	60.98	58.02	60.64	63.34	No change
Manual micromorphology illustration	40.85	53.64	35.09	42.66	Unclear
Map used	9.86	10.00	7.02	6.38	Negative
Paper available	71.83	74.55	72.81	77.85	No change
Phylogenetic tree shown	61.97	84.55	93.86	84.59	Positive
Photo showing biological context	52.11	59.09	71.93	62.26	Positive
Photo of micromorphology	81.69	68.18	72.81	74.20	Unclear
Spore print provided	0.00	0.00	0.00	0.09	No change
Averages (manual search)					
Academic age, last author	29.47	30.66	28.00	27.99	Unclear
Academic age, lead author	23.11	20.65	12.30	18.11	Positive
Co-authors	3.66	4.18	4.97	4.40	Positive
Co-author continents	1.38	1.41	1.55	1.45	Positive
Co-author countries	1.52	1.77	2.06	1.85	Positive
Co-author departments	2.38	2.95	3.33	2.98	Positive
Female co-authors	0.89	1.06	1.71	1.21	Positive
Pages	9.00	9.75	12.87	10.88	Positive
Data visualizations	0.17	0.13	0.09	0.17	Negative

leave a mark on the way fungal species are described, but we also hope to provide food for thought for editors, reviewers, and members of scientific boards.

Materials and methods

Assembly of species descriptions

We went through each issue (2009–2018) of five major, well-reputed mycological journals known to publish new species regularly (Table 1). The journals come from three different continents and are known for their high standards, such that the species

descriptions we examined are likely to represent international mycology at its finest. For reference we also included a botanical journal, where we included the fungal descriptions in the fungal description corpus and the plant descriptions in the plant description corpus. All articles whose title made it clear that one or more new species were being described were examined more closely. We retained articles describing at most 10 new species. Efforts such as Fungal Planet (e.g., Crous et al. 2018) – where 100+ species are described in a single paper by 50+ co-authors – were deemed to be too heterogeneous to score meaningfully in a semi-automated context, as we were interested in singular research efforts by coherent groups of taxonomy-oriented co-authors. Descriptions of fossil taxa were excluded. We retained all descriptions of non-fungal taxa (e.g., myxomycetes and oomycetes), but studies where existing species were simply recombined into other genera were excluded. All individual papers that met our criteria were downloaded as PDF files (Suppl. material 2).

Automated and manual examination of the PDF files

The resulting 1,097 PDF files were converted to text using pdftotext version 2.1.4 (https://pypi.org/project/pdftotext/). The text files were mined using a Python script (Suppl. material 1) which searched for the presence of key words and terms related to ecology, geography, taxonomy, molecular data, and data availability (Table 2). In this process, the article titles, author names and affiliations, abstracts, acknowledgements, and literature cited were excluded from the search to reduce the risk of false-positive matches. Out of the 1,097 papers that were scored automatically, we went through 10% manually to verify that the automatic parsing produced reliable results. A number of features relevant to the description of species were not amenable to straightforward algorithmic interpretation – such as the presence of a full-color photo or illustration showing the whole organism being described rather than just micro-morphological details – and these aspects were scored manually by going through each PDF file in Adobe Illustrator CC 2017 (www.adobe.com).

Assessment of demographic parameters

The number of co-authors, distinct co-author departments, countries of origin and continents of origin (using the seven-continent system) of the co-authors were counted manually to quantify the extent to which taxonomy is practised as a collaborative pursuit. We sought to establish the gender of all co-authors by brief queries in Google, Google Scholar, and ORCID (https://orcid.org/). Only articles where we could determine the gender of all co-authors were used to infer the proportion of female co-authors and lead male authors. In an attempt at quantifying recruitment of aspiring researchers into taxonomy, we made the admittedly coarse assumptions that the last author was the supervisor, mentor, or taxonomic expert, and that the first author was a

student or a nascent taxonomist. Google Scholar was used to determine the academic age of an author: year-of-the-oldest-publication minus year-of-the-most-recent-publication, in a way that dismissed obvious homonyms and ambiguous entries. Unresolved cases were left out from the comparison.

Results and discussion

For convenience we group our results and discussion under the headings Ecology and geography, Systematics and taxonomy, Metadata and data availability, Visualisation, and Demographical aspects. The overall automated and manual estimates are found in Table 2, whereas the full set of results broken down to each individual paper is found in Suppl. material 2.

Ecology and geography

Most biologists would probably agree that taxonomy should be pursued in light of as many data sources as possible, including molecular, morphological, and ecological information. The output of taxonomic work should similarly be rich and many-faceted. However, the fact that the word "ecology" (and its variations) was used in only 42.0% of the examined studies somehow speaks against this assertion. On a more positive note, explicit reference to host, substrate, habitat, or partner was made in 88.1% of the cases, and a reference to the nutritional mode of the new species was made in 75.4% of the cases. The word "ecology" and any of the 19 other ecology-related keywords (Suppl. material 1) we used were completely absent in 3.2% of the studies (Suppl. material 2), suggesting that only a very small number of species descriptions are nucleated in what seems to be either complete disregard or lack of ecological data, or in total ignorance of the wants and needs of the scientific community.

We acknowledge that when a new species is described, there may be no or limited occurrence data beyond the type locality. Still, variations of the words "distribution" and "geography" were mentioned in a strong 82.8% of the studies. Although explicit reference to variations of "climate" was found in only 13.7% of the studies, a full 89.2% of the studies featured climate-related words such as "temperate" or "tropical". GIS/GPS co-ordinates were provided in a much more modest 4.5% of the studies, and 6.4% of the studies provided a map. 62 (5.9%) of the studies that did not provide GIS/GPS co-ordinates provided a map instead. A total of 89.9% of the studies provided neither GIS coordinates nor a map, and 64.4% lacked any relevant variation of the word "locality". This does little to facilitate recollection of the species at the type locality. Altitude/elevation was mentioned explicitly in 16.9% of the studies. It strains credibility that more than 80% of all fungi described during 2009–2018 were collected at sea level, suggesting that the absence of altitude information should not be taken to mean sea level.

62.3% of the studies featured at least one photo or illustration that gave at least some sort of feeling for the biological context of the new species, typically by showing the collection site, the collection spot, or the substrate of collection. We feel that there is room for improvement here, particularly if taxonomy indeed seeks to produce results of relevance and interest that extend beyond the field.

Systematics and taxonomy

It is surprisingly common to describe a new fungal species without mentioning where in the fungal tree of life it belongs: a phylum-level name was found in 31.9% of the studies; order, 64.8%; and family, 75.4%. The intersection of these estimates was 20.8%. In a few cases, some of this information may be truly unknown for the species being described (e.g., Torres-Cruz et al. 2017), but we argue that phylum, order, and perhaps family assignment is known for the vast majority of fungi being described. By knowing where the new species belongs, but not writing it out explicitly, the underlying authors rely on the reader to fill in the mycological gaps. This strikes us as unfortunate, because non-mycologists as well as automated data extraction tools such as data aggregators and search engine indexation software may lack this expertise. In fact, even many mycologists will probably have to look up where, e.g., the family Sphinctrinaceae belongs. The situation is not alleviated by the fact that Index Fungorum, MycoBank, and NCBI Taxonomy regularly disagree on family- and order-level placement of genera (owing to, e.g., differential updates and taxonomic opinion). Thus, even readers who actively go looking for this information may be left clueless or misinformed. Although the fungal family level is in a state of flux in many parts of the fungal tree of life, we can't think of any good reason not to mention at least the phylum and order level affiliations of new species. It is thus not surprising that Catalogue of Life (https://www.catalogueoflife.org/) and similar efforts often fall short of providing the precise taxonomic placement of individual species in the fungal tree of life, even when this information was known to the mycologists who described the species in the first place. Clearly, mycology does not stand to benefit from the presence of incomplete taxonomic information in online repositories.

Although taxonomy represents a core aspect of biodiversity, variations of the word "biodiversity" are not commonly used in papers describing new species of fungi – only 23.2% of the studies used it. This comes across as a missed opportunity to place the new species in a richer context – and to have the underlying paper indexed properly in search engines and automated classifiers of scientific papers. Highlighting the importance or relevance of the new species to society (e.g., agriculture, forestry, or biotechnology) – if motivated – would similarly lead to a wider readership and better article indexation. However, a moderate 53.6% of the studies featured such keywords. A much lower number of studies – 2.4% – made a reference to the threatened or endangered nature of the new species or its habitat, although this may be difficult to know at the time of description.

Where is the underlying specimen or culture deposited? We found it quite common (15.4% of the cases) to provide this information in a way that does not employ any variations of the words "herbarium", "fungarium", "museum", or "culture collection" – an example would be "deposited in H". The reader would then have to know – or find out – that H is a herbarium at the University of Helsinki, Finland. This poses no challenge to a seasoned taxonomist (through recourse to, e.g., Index Herbariorum at http://sweetgum.nybg.org/science/ih/ or GRSciColl at https://www.gbif.org/grscicoll), but we imagine that other readers would struggle with this, as would data mining efforts to extract information from scientific papers. Improving clarity by writing, say, "deposited in herbarium H" – and why not write out the name of the herbarium in full? – should be easy enough. 11.1% of the papers mention "Index Fungorum" (http://www.indexfungorum.org/names/names.asp) and 88.1% "MycoBank" (Robert et al. 2013) explicitly, showing that writing out names in full is common in other contexts.

Identification keys help define what exactly differentiates the new species from others, and 24.5% of the papers we examined featured an identification key. There can be many reasons why a key would be premature or impossible to construct for various fungal taxa, such that whether 24.5% is a comforting estimate or not is hard to say. Determination keys are, however, becoming rarer over time (Table 2).

Metadata and data availability

The proportion of species descriptions making use of DNA sequence data – as deduced from the use of variations of keywords such as "PCR", "DNA", and "sequencing" – is on the rise, from 71.8% in 2009 to 97.4% in 2018 (Fig. 1). Across all years, at least one DNA sequence-related keyword was found in 90.8% of the studies, an estimate that blends well with the 94% reported for fungi by Miralles et al. (2020), who examined papers from 2002, 2010, and 2018. The proportion of DNA-based studies depositing their multiple sequence alignments or phylogenetic trees in TreeBase (Vos et al. 2012) or Dryad (https://datadryad.org/) is similarly on the rise: overall, 53.8% of the studies mention TreeBase/Dryad, up from 33.8% in 2009 but down from the 69.3% of 2018. The problem of poor data availability in systematic mycology has been made apparent before (Nilsson et al. 2011; Drew et al. 2013), so we were happy to note a gradual – albeit slow – improvement over time. If taxonomy is to be a modern, reproducible, integrative, and standards-compliant field (see, e.g., Dimitrova et al. 2019 and the MIAPA standard, Leebens-Mack et al. 2006), then there is simply no excuse for not sharing the underlying phylogenetic – and other – data in standardized ways (Grandcolas 2019; Miralles et al. 2020). Increased data deposition is furthermore likely to improve the visibility of the underlying article and thus taxonomy at large.

To simulate whether the general reader could access the underlying PDF publications by Google searches, we queried Google by pasting the name of the paper in quotation marks and then scrutinizing the first two pages of hits manually (February 2020). We did this from computers not connected to any university network. We ac-

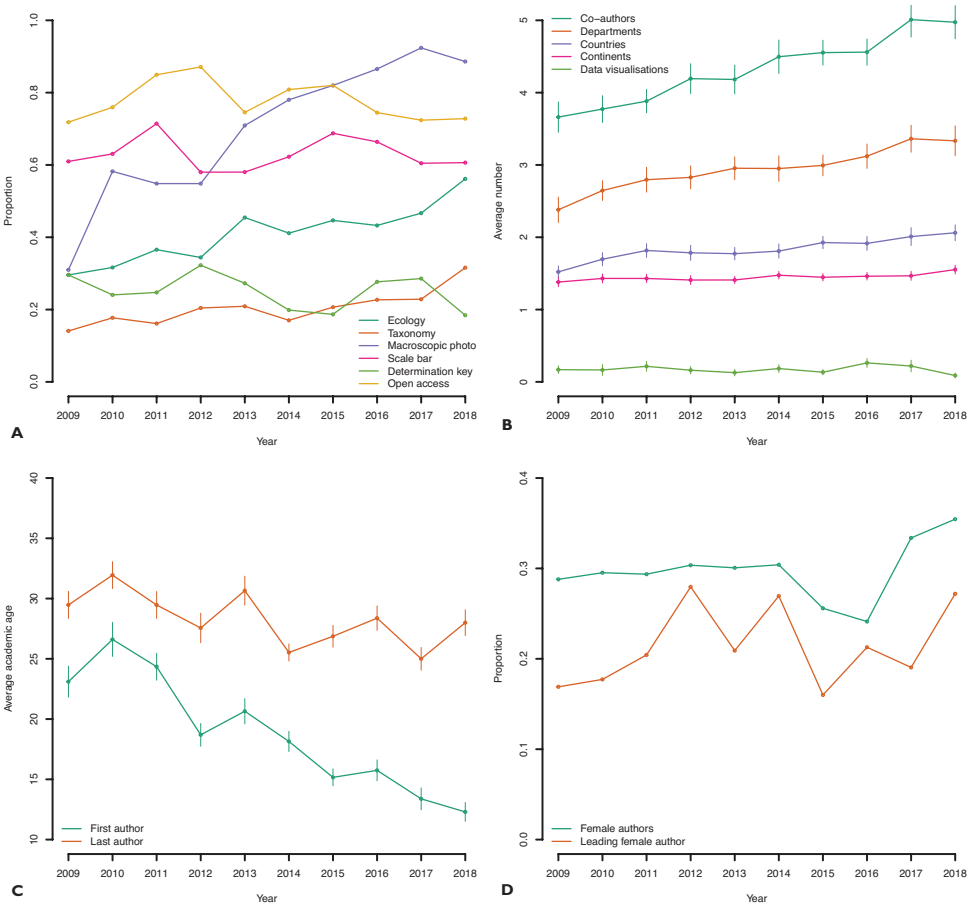

Figure 1. A Data and metadata in the description of fungal species 2009–2018. The x axis depicts year and the y axis depicts proportion of studies (from 0 to 1) fulfilling a specific criterion. Dark green – proportion of studies mentioning the word "ecology" or its variations; brown – proportion of studies giving a complete account of the taxonomic affiliation of the new species (family, order, and phylum); purple – proportion of studies with a macroscopic colour photo/illustration of the new species; pink – proportion of studies with macroscopic photos, that also indicate the size of the depicted object through a scale bar or a fiducial marker; light green – proportion of studies with an identification key; yellow – proportion of openly available papers for each year as assessed in 2020 **B** demographical and publication trends showing the average number of co-authors (dark green), departments (brown), countries (purple), continents (pink), and number of data visualizations (light green) over time. The bars indicate the yearly standard error **C** the average academic age of the first (green) and last (brown) co-author over time. The bars indicate the yearly standard error **D** the proportion of female co-authors (green) over time plus the proportion of female lead authors (brown).

cepted hits to PDF files and full-text papers in the HTML format of both the final, published paper and to any preprints in, e.g, bioRxiv (https://www.biorxiv.org/), and we accepted both legal as well as juridically more dubious sources of PDF files. If any sort of registration was needed to access the PDF file, we scored it as "not avail-

able". We found that 77.8% of the studies could be accessed from outside university networks. The observation that more than 20% of the taxonomic output of the mycological community cannot be readily accessed by the general public comes across as unfortunate. However, all of the journals we targeted allow the submission of preprints to online repositories. Thus, submitting a vetted preprint at least post-publication (in order not to confuse effective publication dates of names) is a way around this inaccessibility (cf. Kwon 2020). It is, evidently, a solution that is not being explored to the extent it could have been by the mycological community.

Many cases of taxonomic mistakes, redundant species descriptions, and laboratory contaminations would have been avoided if the authors had subjected the newly generated DNA sequences to a simple BLAST search in, e.g., GenBank (Nilsson et al. 2012). Sadly, only 35.5% of the studies mention BLAST, although the trend is positive (Table 2). A clear majority (85.7%) of the examined studies make explicit reference to one of the INSDC repositories (GenBank, EMBL, and DDBJ), again in a positive trend. Bundling supplementary material with species descriptions is a good way to increase reproducibility and provide additional, helpful information with respect to the new species without consuming valuable page space in print journals. Here we envisage additional photos or drawings of fungal specimens or the collection site, or perhaps extended maps, field notes, or laboratory details. However, a somewhat disappointing 24.4% of the studies saw fit to include at least one supplementary item.

Visualization

The average study was 10.9 pages long, although we did not correct for the number of described species in each paper. The studies grew more voluminous over time (Table 2), possibly as a consequence of the inclusion of more analyses based on molecular data. A high 73.0% featured at least one color photo or illustration showing more or less the whole fungus being described (in the sense of a full fruiting body rather than only microscopic details or a spore print). Somewhat disappointingly, only 63.3% of the macroscopic photos/illustrations featured a scale bar or a fiducial marker, leaving assessment of size problematical for more than a third. 25.2% of all studies contained at least one photo of a fungal culture, and a spore print was presented in 0.1% of the studies. 98.5% of the studies featured at least one visualization of a micromorphological detail or structure. In total, micro-morphological details were illustrated by line drawings in 42.7% of the studies; photos (74.2%); and electron microscopy (24.9%). 19.2% of the studies featured both a micromorphological photo and a line drawing; a total of 6.2% of the studies commendably used all three techniques. A phylogenetic tree was displayed in 84.6% of the studies. Since 90.8% of the studies used molecular data, this means that a few studies used molecular data without presenting a phylogenetic tree. A cursory look at a few of these indicated that techniques such as RFLP had been used to generate a fingerprint of the new species. The average study featured 0.17 data visualizations (e.g., a graph or a chart other than a phylogenetic tree).

Demographical aspects

The average number of co-authors was 4.4, which was higher than we expected given that taxonomy is sometimes touted as a solitary discipline. The average number of departments, countries, and continents were 3.0, 1.9, and 1.5 – again higher than we had expected. Plotting the number of co-authors and countries over time (Fig. 1) suggests that fungal taxonomy is slowly becoming an increasingly collaborative and international discipline. It is, however, a discipline dominated by males: out of the 549 papers for which we were able to establish the gender of all co-authors, 30.4% were male-only papers and 3.6% were female-only papers. These 549 papers comprised a total of 2,224 co-authors, of which 662 (29.8%) were female. Males were lead authors in 68.7% of the 758 papers for which we were able to determine the gender of the lead author. Our gender-related estimates certainly leave room for improvement of inclusivity and career opportunities in mycology (cf. Salerno et al. 2019), although they slowly improve over time (Table 2). Our admittedly crude attempts at quantifying the extent to which recruitment of aspiring researchers and the passing on of knowledge is going on in fungal taxonomy showed that the average academic age of the last author (28.0 years) is higher than that of the first author (18.1 years), perhaps hinting that knowledge does seem to be passed on to younger generations (or at least a younger generation) albeit somewhat slowly. One hundred non-taxonomical mycological papers from the same journals produced similar figures – 27 and 16 years, respectively – hinting that this issue may not be specific to taxonomy. In fact, the high academic age of the authors may be part of explaining the gender bias, as gender equality tends to decrease upwards in the academic hierarchy (Potvin et al. 2018).

Comparison with the botanical species descriptions

Although the key terms and concepts to look for in a mycological species description will be somewhat different from those of a botanical counterpart, we did find some notable differences between the description of fungi and plants. It should be kept in mind that our botanical reference corpus was limited to a single journal and 40 papers, and the extent to which our results can be extrapolated to botany at large remains unknown. Nevertheless, botany comes out on top of mycology when it comes to specifying the type locality through either a map or GIS/GPS co-ordinates: 65% of the botanical studies did this, as compared to only 10.4% of the mycological. On the other hand, the use of molecular data is more widespread in mycological species descriptions (90.8%) than in botanical (60.0%). 59.2% of the mycological studies that relied on molecular data made these available in TreeBase/Dryad, compared to 8.3% of the corresponding botanical papers. Full-color macro-illustrations of the species being described were more common in mycology (73.0%) than in botany (55.0%). The number of co-authors on botanical species descriptions is lower (3.6) than in mycology (4.4), and so is the average number of female co-authors (0.94 vs. 1.2). Mycology comes across as a somewhat more collaborative discipline in that the average number of co-authors from different depart-

ments, countries, and continents are all higher in mycology, but botany struggles somewhat less with recruitment of aspiring taxonomists (Suppl. material 2). These figures should be interpreted with the very limited size of the reference corpus in mind, but they do seem to suggest that botany and mycology can learn from each other when it comes to describing new species in the 21st century. Joint meetings such as the 2009 meeting of the Mycological Society of America, American Bryological and Lichenological Society, American Fern Society, American Society of Plant Taxonomists, and Botanical Society of America (http://2009.botanyconference.org/) are commendable in this respect.

Biases and shortcomings of our approach

The semi-automated nature of our approach is not without potential shortcomings, and we are likely to have both under- and over-estimated some of our parameter values. As an example of an overestimation, a study could mention "DNA" or perhaps "PCR" without actually making use of sequence data in the description of the new species. This would have led us to the incorrect conclusion that DNA sequence data was used in the description of the species. As an example of an underestimation, a study could conceivably provide information on the ecology or nutritional mode of the new species without using any of the ~20 terms we looked for, leading us to the erroneous conclusion that nothing was said about the ecology of the new species. Since we processed nearly 1,100 mycological papers, such outlier cases will not have contributed much to our estimates. Our manual verification of 10% of the papers did not reveal significant cause for concern with respect to over- or under-estimations.

A potentially larger bias lies in our choice of journals. We purposely selected five major international mycological journals with significant impact factors, stringent editorial and review processes, and very detailed author instructions. The journals are not solely focused on taxonomy but cover a wide spectrum of mycological subdisciplines, and the papers published therein can therefore be expected to be geared towards a more general mycological audience. However, fungal species are described also in other outlets. For instance, there are 29 mycological journals with a formal Web of Science impact factor for 2019. Indeed, Schoch et al. (2012) found that the fungal ribosomal nuclear internal transcribed spacer (ITS) sequences in GenBank stemmed from over 500 different scientific journals. Yet other journals would not even be represented in GenBank because they have yet to publish their first species using sequence data (focusing instead on morphology-based descriptions). We speculate that at least some of these other journals may have less stringent editorial and review processes (in fact, several journals that publish new species of fungi do not use formal peer-review at all). Along the same line, many of these journals are not available digitally, and some are printed in black and white. Thus, our estimates pertain to the state-of-the-art species descriptions of fungi rather than the full spectrum of fungal species descriptions. While not all of our estimates are flattering for fungal taxonomy (e.g., Table 2), it is likely that they still paint an overly optimistic picture of fungal species descriptions at large.

Take-home messages for mycology

The International Code of Nomenclature for algae, fungi, and plants (Turland 2018) stipulates the minimal requirements for publication of new names (species). Notions of, for example, ecology or geographical distributions, or inclusion of illustrations, are not part of those requirements (Seifert and Rossman 2010; Hawksworth 2011). But rather than asking what the minimal requirements are, mycologists should strive to showcase taxonomy as a vibrant, exciting field where species are described in the richest possible way, where all data and metadata elements are machine readable with persistent identifiers, and where ample auxiliary data are posted to online repositories and community initiatives such as Zenodo (https://zenodo.org/), FigShare (https://figshare.com/), Wikimedia Commons (https://commons.wikimedia.org/), and Open Tree of Life (https://tree.opentreeoflife.org/) in standards-compliant formats and data structures (Mons et al. 2017). To some extent, our results question whether this is how fungal species are described at present. The fact that a full 58.0% of the studies did not mention any variations of the word "ecology", or that 76.8% of the studies lack the word "biodiversity", is certainly not encouraging, for instance. We argue that fungal species should be described in the richest possible way; in fact, a moderate 77.6% of the studies featured a formal "Discussion" section, which for the remaining studies seems like missed opportunities to anchor the new species in a richer mycological and biological context. With an average of 4.4 co-authors on the 1,097 papers, there would clearly be room to invite one or more additional co-authors to add, e.g., ecological aspects to the description. Similarly, many opportunities to bundle helpful supplementary data are waiting to be filled (75.6% of the studies did not bundle any supplementary material). We do realize that there are situations where the, e.g., ecology or geographical distribution (or even origin) of a new species is entirely unknown; thinly annotated herbarium specimens come to mind. We are not against species descriptions based on legacy specimens, but a random selection of 100 studies in Suppl. material 2 did not present a single such case. Our dataset presumably still contains examples of this and other special cases, but the vast majority of the studies covered represented relatively recently collected material. This makes the lack of data on ecology and geography harder to swallow.

We were happy to note that the proportion of species descriptions using sequence data is on the rise (Fig. 1). DNA data are important for taxonomic identification in many studies. Failure to provide characters for taxonomic identification using DNA sequencing (typically in the form of sequences deposited in a public repository such as GenBank) is therefore limiting the value of a description. Put negatively, 9.2% of the studies in Suppl. material 2 were done without DNA data. We ask the fungal taxonomists of the world to always bundle at least an ITS sequence (and preferably also an nLSU sequence) with all new species (and genomes, for that matter), even if those sequences were not analyzed or used in the study in question. We recognize that not all mycologists have access to DNA sequencing equipment, and we hope that more well-equipped mycological laboratories will be able to assist nearby, less well-equipped mycologists with the generation of such data. The cost of generating a Sanger sequence is low today, and all of mycology would stand to benefit from such generous acts (cf. Womack 2015).

Adopting a species description to be meaningful also to a non-taxonomic reader may be challenging enough, but we argue that mycological taxonomy needs to go one step further. In a world where information is increasingly culled through automatic means, mycologists should no longer assume that all readers of species descriptions are human to begin with. This means that all data and metadata items should be machine-readable and available online, come with globally unique and persistent identifiers (including ORCIDs for humans, accession numbers/DOIs for sequence data/datasets, DOIs for cited publications, and Open Tree of Life identifiers for phylogenies). The notion of automated readers also brings about changes in the way manuscripts should be written in that it becomes particularly important to provide clear and precise information, almost to the point of tabularization. We argue that standardized terms should be used even when they cannot be parameterized; "Ecology: unknown" is incomparably more helpful to human and automated readers alike than simply leaving out the word "ecology" altogether. Along the same lines, and although brevity may suffer somewhat, "in herbarium GB (University of Gothenburg)" is immeasurably more helpful than "in GB". No automated reader, and few non-taxonomists, will be able to contextualize the acronym "GB" in a meaningful way. To assume that the reader will be able to extrapolate the position of the species in the fungal tree of life, or the GPS co-ordinates of the collection spot, and so on, should similarly be avoided.

Our demographical estimates suffered from various potential shortcomings and biases: online information can be hard to find (particularly when it comes to authors who have not registered on ORCID), Google Scholar profiles are not necessarily complete or correct in terms of their publication lists, the last author does not have to be a supervisor or a mentor figure, and so on. Getting around these shortcomings in a study of the present kind is next to impossible, and we feel that our demographical results should be seen merely as rough estimates of trends. But a surprisingly strong signal still came out of them: taxonomy is no longer – and perhaps never really was – an entirely solitary discipline, but instead comes across as a reasonably collaborative, international discipline where knowledge seems to be passed on to younger researchers, at least to some extent. This offers hope for the future – taxonomy may actually be on its way to shake some of the misconceptions surrounding it (Coleman 2015). Still, taxonomy needs to be wary of gender aspects as evinced by our estimate that a total of 29.8% of the co-authors in our dataset were female, indicating that a lot of potential competence and perspectives are neglected by the field. There may also be other inequalities in the discipline, not estimated here.

Our data generally, but not exclusively, indicate what we feel is a gradual improvement in the richness of species descriptions and in the demographical aspects of fungal taxonomy over time (Table 2). The improvements come across as slow but at least steady. There is little in our data to invite complacency, though. On the contrary, we feel that our data highlight the need for fungal taxonomy to make a sincere effort to be aware and to improve. It is not enough that mycological species descriptions perhaps come across as a bit more robust and reproducible than plant descriptions in our somewhat compromised comparison. We should set the bar really high – this-and-that is what we want species descriptions to look like from the scientific, demographic, and popular science points of

view, so this-and-that is indeed what we will deliver. We feel that there are several measures that fungal taxonomists can and should take to improve the impact and outreach of their work. Our suggestions – with the exception of generating and bundling a DNA sequence with each species description – do not come with any direct costs and do not call for acquisition of any machines or utensils. That said, we don't want our suggestions to be used as excuses for cutting down other parts of species descriptions – we don't feel that the inclusion of a macroscopic color photo justifies shortening the corresponding text-based description of the macromorphology, for instance. Our suggestions will add somewhat to the time it takes to describe a new species – or serve as the editor or reviewer of a manuscript in which a new species is described – but we argue that it is a price worth paying (de Carvalho et al. 2008). Indeed, we feel, it is a price that mycology simply must pay to make it a reproducible field at the heart of systematics and biodiversity at large.

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References

Abarenkov K, Tedersoo L, Nilsson RH, Vellak K, Saar I, Veldre V, Parmasto E, Prous M, Aan A, Ots M, Kurina O, Ostonen I, Jõgeva J, Halapuu S, Põldmaa K, Toots M, Truu J, Larsson K-H, Kõljalg U (2010) PlutoF – a web-based workbench for ecological and taxonomical research, with an online implementation for fungal ITS sequences. Evolutionary Bioinformatics 6: 189–196. https://doi.org/10.4137/EBO.S6271

Coleman CO (2015) Taxonomy in times of the taxonomic impediment – examples from the community of experts on amphipod crustaceans. Journal of Crustacean Biology 35(6): 729–740. https://doi.org/10.1163/1937240X-00002381

Crous PW, Luangsa-Ard JJ, Wingfield MJ, Carnegie AJ, Hernández-Restrepo M, Lombard L, Roux J, Barreto RW, Baseia IG, Cano-Lira JF, Martín MP, Morozova OV, Stchigel AM, Summerell BA, Brandrud TE, Dima B, García D, Giraldo A, Guarro J, Gusmão LFP, Khamsuntorn P, Noordeloos ME, Nuankaew S, Pinruan U, Rodríguez-Andrade E, Souza-Motta CM, Thangavel R, van Iperen AL, Abreu VP, Accioly T, Alves JL, Andrade JP, Bahram M, Baral H-O, Barbier E, Barnes CW, Bendiksen E, Bernard E, Bezerra JDP, Bezerra JL, Bizio E, Blair JE, Bulyonkova TM, Cabral TS, Caiafa MV, Cantillo T, Colmán AA, Conceição LB, Cruz S, Cunha AOB, Darveaux BA, da Silva AL, da Silva GA, da Silva GM, da Silva RMF, de Oliveira RJV, Oliveira RL, De Souza JT, Dueñas M, Evans HC, Epifani F, Felipe MTC, Fernández-López J, Ferreira BW, Figueiredo CN, Filippova NV, Flores JA, Gené J, Ghorbani G, Gibertoni TB, Glushakova AM, Healy R, Huhndorf SM, Iturrieta-González I, Javan-

Nikkhah M, Juciano RF, Jurjević Ž, Kachalkin AV, Keochanpheng K, Krisai-Greilhuber I, Li Y-C, Lima AA, Machado AR, Madrid H, Magalhães OMC, Marbach PAS, Melanda GCS, Miller AN, Mongkolsamrit S, Nascimento RP, Oliveira TGL, Ordoñez ME, Orzes R, Palma MA, Pearce CJ, Pereira OL, Perrone G, Peterson SW, Pham THG, Piontelli E, Pordel A, Quijada L, Raja HA, Rosas de Paz E, Ryvarden L, Saitta A, Salcedo SS, Sandoval-Denis M, Santos TAB, Seifert KA, Silva BDB, Smith ME, Soares AM, Sommai S, Sousa JO, Suetrong S, Susca A, Tedersoo L, Telleria MT, Thanakitpipattana D, Valenzuela-Lopez N, Visagie CM, Zapata M, Groenewald JZ (2018) Fungal Planet description sheets: 785–867. Persoonia 41: 238–417. https://doi.org/10.3767/persoonia.2018.41.12

de Carvalho MR, Bockmann FA, Amorim DS, Brandao CRF (2008) Systematics must embrace comparative biology and evolution, not speed and automation. Evolutionary Biology 35(2): 150–157. https://doi.org/10.1007/s11692-008-9018-7

de Carvalho MR, Bockmann FA, Amorim DS, Brandão CRF, de Vivo M, de Figueiredo JL, Britski HA, de Pinna MCC, Menezes NA, Marques FPL, Papavero N, Cancello EM, Crisci JV, McEachran JD, Schelly RC, Lundberg JG, Gill AC, Britz R, Wheeler QD, Stiassny MLJ, Parenti LR, Page LM, Wheeler WC, Faivovich J, Vari RP, Grande L, Humphries CJ, DeSalle R, Ebach MC, Nelson GJ (2007) Taxonomic impediment or impediment to taxonomy? A commentary on systematics and the cybertaxonomic-automation paradigm. Evolutionary Biology 34(3–4): 140–143. https://doi.org/10.1007/s11692-007-9011-6

Dimitrova M, Senderov V, Georgiev T, Zhelezov G, Penev L (2019) OpenBiodiv: Linking type materials, institutions, locations and taxonomic names extracted from scholarly literature. Biodiversity Information Science and Standards 3: e35089. https://doi.org/10.3897/biss.3.35089

Drew LW (2011) Are we losing the science of taxonomy? As need grows, numbers and training are failing to keep up. BioScience 61(12): 942–946. https://doi.org/10.1525/bio.2011.61.12.4

Drew BT, Gazis R, Cabezas P, Swithers KS, Deng J, Rodriguez R, Katz LA, Crandall KA, Hibbett DS, Soltis DE (2013) Lost branches on the tree of life. PLoS Biology 11(9): e1001636. https://doi.org/10.1371/journal.pbio.1001636

Federhen S (2011) The NCBI taxonomy database. Nucleic Acids Research 40(D1): D136–D143. https://doi.org/10.1093/nar/gkr1178

Giangrande A (2003) Biodiversity, conservation, and the 'Taxonomic impediment'. Aquatic Conservation: Marine and Freshwater Ecosystems 13(5): 451–459. https://doi.org/10.1002/aqc.584

Grandcolas P (2019) The rise of "Digital Biology": We need not only open, FAIR but also sustainable data! Biodiversity Information Science and Standards 3: e37508. https://doi.org/10.3897/biss.3.37508

Hawksworth L (2011) A new dawn for the naming of fungi: impacts of decisions made in Melbourne in July 2011 on the future publication and regulation of fungal names. MycoKeys 1: 7–20. https://doi.org/10.3897/mycokeys.1.2062

Kwon D (2020) How swamped preprint servers are blocking bad coronavirus research. Nature 581: 130–131. https://doi.org/10.1038/d41586-020-01394-6

Leebens-Mack J, Vision T, Brenner E, Bowers JE, Cannon S, Clement MJ, Cunningham CW, DePamphilis C, DeSalle R, Doyle JJ, Eisen JA, Gu X, Harshman J, Jansen RK, Kellogg EA, Koonin EV, Mishler BD, Philippe H, Pires JC, Qiu Y-L, Rhee SY, Sjölander K, Soltis DE, Soltis PS, Stevenson DW, Wall K, Warnow T, Zmasek C (2006) Taking the first steps

towards a standard for reporting on phylogenies: Minimum Information About a Phylogenetic Analysis (MIAPA). Omics: a Journal of Integrative Biology 10(2): 231–237. https://doi.org/10.1089/omi.2006.10.231

Magoga G, Sassi D, Daccordi M, Leonardi C, Mirzaei M, Regalin R, Lozzia G, Montagna M (2016) Barcoding Chrysomelidae: a resource for taxonomy and biodiversity conservation in the Mediterranean Region. ZooKeys (597): 1–27. https://doi.org/10.3897/zookeys.597.7241

Miralles A, Bruy T, Wolcott K, Scherz MD, Begerow D, Beszteri B, Bonkowski M, Felden J, Gemeinholzer B, Glaw F, Glöckner FO, Hawlitschek O, Kostadinov I, Nattkemper TW, Printzen C, Renz J, Rybalka N, Stadler M, Weibulat T, Wilke T, Renner SS, Vences M (2020) Repositories for taxonomic data: where we are and what is missing. Systematic Biology (in press). https://doi.org/10.1093/sysbio/syaa026

Mons B, Neylon C, Velterop J, Dumontier M, da Silva Santos LOB, Wilkinson MD (2017) Cloudy, increasingly FAIR; revisiting the FAIR Data guiding principles for the European Open Science Cloud. Information Services & Use 37(1): 49–56. https://doi.org/10.3233/ISU-170824

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG (2016) FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecology 20: 241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Nilsson RH, Larsson K-H, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, Kennedy P, Picard K, Glöckner FO, Tedersoo L, Saar I, Kõljalg U, Abarenkov K (2019) The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Research 47: D259–D264. https://doi.org/10.1093/nar/gky1022

Nilsson RH, Ryberg M, Sjökvist E, Abarenkov K (2011) Rethinking taxon sampling in the light of environmental sequencing. Cladistics 27(2): 197–203. https://doi.org/10.1111/j.1096-0031.2010.00336.x

Nilsson RH, Tedersoo L, Abarenkov K, Ryberg M, Kristiansson E, Hartmann M, Schoch CL, Nylander JAA, Bergsten J, Porter TM, Jumpponen A, Vaishampayan P, Ovaskainen O, Hallenberg N, Bengtsson-Palme J, Eriksson KM, Larsson K-H, Larsson E, Kõljalg U (2012) Five simple guidelines for establishing basic authenticity and reliability of newly generated fungal ITS sequences. MycoKeys 4: 1–37. https://doi.org/10.3897/mycokeys.4.3606

Potvin DA, Burdfield-Steel E, Potvin JM, Heap SM (2018) Diversity begets diversity: A global perspective on gender equality in scientific society leadership. PLoS One 13(5): e0197280. https://doi.org/10.1371/journal.pone.0197280

Robert V, Vu D, Amor ABH, van de Wiele N, Brouwer C, Jabas B, Szoke S, Dridi A, Triki M, ben Daoud S, Chouchen O, Vaas L, de Cock A, Stalpers JA, Stalpers D, Verkley GJM, Groenewald M, dos Santos FB, Stegehuis G, Li W, Wu L, Zhang R, Ma J, Zhou M, Gorjón SP, Eurwilaichitr L, Ingsriswang S, Hansen K, Schoch C, Robbertse B, Irinyi L, Meyer W, Cardinali G, Hawksworth DL, Taylor JW, Crous PW (2013) MycoBank gearing up for new horizons. IMA Fungus 4(2): 1–371. https://doi.org/10.5598/imafungus.2013.04.02.16

Salerno PE, Páez-Vacas M, Guayasamin JM, Stynoski JL (2019) Male principal investigators (almost) don't publish with women in ecology and zoology. PloS ONE 14(6): e0218598. https://doi.org/10.1371/journal.pone.0218598

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences 109(16): 6241–6246. https://doi.org/10.1073/pnas.1117018109

Seifert KA, Rossman AY (2010) How to describe a new fungal species. IMA Fungus 1(2): 109–116. https://doi.org/10.5598/imafungus.2010.01.02.02

Torres-Cruz TJ, Billingsley Tobias TL, Almatruk M, Hesse CN, Kuske CR, Desirò A, Benucci GMN, Bonito G, Stajich JE, Dunlap C, Arnold AE, Porras-Alfaro A (2017) *Bifiguratus adelaidae*, gen. et sp. nov., a new member of Mucoromycotina in endophytic and soil-dwelling habitats. Mycologia 109(3): 363–378. https://doi.org/10.1080/00275514.2017.1364958

Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth D, Herendeen P, Knapp S, Kusber W-H, Li D-Z, Marhold K, May TW, Mcneill J, Monro AM, Prado J, Price MJ, Smith GF (2018) International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Glashütten: Koeltz Botanical Books. https://doi.org/10.12705/Code.2018

Vos RA, Balhoff JP, Caravas JA, Holder MT, Lapp H, Maddison WP, Midford PE, Priyam A, Sukumaran J, Xia X, Stoltzfus A (2012) NeXML: rich, extensible, and verifiable representation of comparative data and metadata. Systematic Biology 61(4): 675–689. https://doi.org/10.1093/sysbio/sys025

Wägele H, Klussmann-Kolb A, Kuhlmann M, Haszprunar G, Lindberg D, Koch A, Wägele JW (2011) The taxonomist – an endangered race. A practical proposal for its survival. Frontiers in Zoology 8(1): 1–25. https://doi.org/10.1186/1742-9994-8-25

Wheeler QD (2004) Taxonomic triage and the poverty of phylogeny. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 359(1444): 571–583. https://doi.org/10.1098/rstb.2003.1452

Womack RP (2015) Research data in core journals in biology, chemistry, mathematics, and physics. PloS ONE 10(12): e0143460. https://doi.org/10.1371/journal.pone.0143460

Supplementary material 1

Python code responsible for the PDF parsing

Authors: Louisa Durkin, Tobias Jansson, Marisol Sanchez, Maryia Khomich, Martin Ryberg, Erik Kristiansson, R. Henrik Nilsson

Data type: Source code (PDF)

Explanation note: The Python code responsible for the PDF parsing, including the full details on how searches were made for each of the entries in Table 2.

Link: https://doi.org/10.3897/mycokeys.72.56691.suppl1

Supplementary material 2

The automatic and manual estimates for the targeted mycological papers

Authors: Louisa Durkin, Tobias Jansson, Marisol Sanchez, Maryia Khomich, Martin Ryberg, Erik Kristiansson, R. Henrik Nilsson

Data type: Raw data (statistics)

Explanation note: The automatic and manual estimates for each of the 1,097 mycological papers targeted. The corresponding plant data are available on the second sheet.

Link: https://doi.org/10.3897/mycokeys.72.56691.suppl2