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Cryptic species of *Curvularia* in the culture collection of the Queensland Plant Pathology Herbarium

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Abstract

Several unidentified specimens of *Curvularia* deposited in the Queensland Plant Pathology Herbarium were re-examined. Phylogenetic analyses based on sequence data of the internal transcribed spacer region, partial fragments of the glyceraldehyde-3-phosphate dehydrogenase and the translation elongation factor 1-α genes, supported the introduction of 13 novel *Curvularia* species. Eight of the species described, namely, *C. beasleyi* **sp. nov.**, *C. beerburrumensis* **sp. nov.**, *C. eragrosticola* **sp. nov.**, *C. kenpeggii* **sp. nov.**, *C. mebaldsii* **sp. nov.**, *C. petersonii* **sp. nov.**, *C. platzii* **sp. nov.** and *C. warraberensis* **sp. nov.**, were isolated from grasses (Poaceae) exotic to Australia. Only two species, *C. lamingtonensis* **sp. nov.** and *C. sporobolicola* **sp. nov.**, were described from native Australian grasses. Two species were described from hosts in other families, namely, *C. coatesiae* **sp. nov.** from *Litchi chinensis* (Sapindaceae) and *C. colbranii* **sp. nov.** from *Crinum zeylanicum* (Amaryllidaceae). *Curvularia reesii* **sp. nov.** was described from an isolate obtained from an air sample. Furthermore, DNA sequences from ex-type cultures supported the generic placement of *C. neoindica* and the transfer of *Drechslera boeremae* to *Curvularia*.

Keywords

Dothideomycetes, multigene phylogeny, taxonomy, 13 new species

Introduction

Curvularia is a species-rich genus of pathogens and saprobes associated with plant, human and animals worldwide (Sivanesan 1987, Hyde et al. 2014, Madrid et al. 2014, Manamgoda et al. 2015, Marin-Felix et al. 2017a, b). *Curvularia* species have also been reported from substrates such as air (Almaguer et al. 2012, Hargreaves et al. 2013), aquatic environments (Verma et al. 2013, Su et al. 2015, Sharma et al. 2016) and soil (Manamgoda et al. 2011, Marin-Felix et al. 2017a).

Species delimitation within *Curvularia* based solely on morphology is difficult as many species share similar characters and have overlapping conidial dimensions. Currently, there are 131 species of *Curvularia* (excluding varieties) listed in *Index Fungorum* (accessed on 4 January 2018). Phylogenetic studies based on multilocus sequence analyses of ex-type or reference cultures have recently delimited many cryptic species (Deng et al. 2014, Manamgoda et al. 2014, Tan et al. 2014, Manamgoda et al. 2015, Marin-Felix et al. 2017a, 2017b). Presently, there are 81 accepted species for which taxonomic placement has been established by DNA barcodes to allow accurate identification and comparison (Marin-Felix et al. 2017a, b).

In Australia, 64 species of *Curvularia* have been reported (DAF Biological Collections 2018, Farr and Rossman 2018). Of these, 17 species were described from Australia, namely *C. australiensis*, *C. australis*, *C. bothriochloae*, *C. crustacea*, *C. dactyloctenii*, *C. graminicola*, *C. harveyi*, *C. heteropogonis*, *C. micrairae*, *C. ovariicola*, *C. perotidis*, *C. queenslandica*, *C. ravenelii*, *C. richardiae*, *C. ryleyi*, *C. sorghina* and *C. tripogonis*. Eight of the Australian *Curvularia* species were originally placed in the closely related genus, *Bipolaris*, before transfer to *Curvularia* based on molecular studies (Manamgoda et al. 2012, 2014, Tan et al. 2014).

In this study, 17 unidentified isolates of *Curvularia* maintained in the culture collection held in the Queensland Plant Pathology Herbarium (BRIP) were compared with ex-type and reference isolates. Thirteen new species of *Curvularia* were revealed based on multilocus phylogenetic analyses and are formally described here. In addition, phylogenetic analyses of ex-type cultures have confirmed the placement of a *Curvularia* species, as well as the introduction of a new combination.

Materials and methods

Isolates and morphology

Unidentified isolates of *Curvularia* were obtained from BRIP (Table 1), which retains cultures in a metabolically inactive state at -80 °C in a sterile solution of 15% v/v glycerol. In order to observe conidia and conidiophores, living cultures were grown on sterilised leaf pieces of *Zea mays* on modified Sachs agar and on sterilised wheat straws on water agar, incubated at room temperature (approx. 25 °C) for seven days and exposed to near ultraviolet light on a 12 h light/dark diurnal cycle (Sivanesan 1987). Conidia

Table 1. *Curvularia* isolates examined.

Species	**Isolate no.**[1]	***Host***	**Location**	**GenBank accession numbers**[2]		
				ITS	***gapdh***	***tef1a***
Bipolaris maydis	CBS 136.29 [T]	*Zea mays*	USA	AF071325	KM034846	KM093794
Curvularia aeria	CBS 294.61 [T]	air	Brazil	HF934910	HG779148	–
C. affinis	CBS 154.34 [T]	unknown	Indonesia	KJ909780	KM230401	KM196566
C. akaii	CBS 317.86	unknown	Japan	KJ909782	KM230402	KM196569
C. akaiiensis	BRIP 16080 [T]	unknown	India	KJ415539	KJ415407	KJ415453
C. alcornii	MFLUCC 10-0703 [T]	*Zea mays*	Thailand	JX256420	JX276433	JX266589
C. americana	UTHSC 08-3414 [T]	*Homo sapiens*	USA	HE861833	HF565488	–
C. asiatica	MFLUCC 10-0711 [T]	*Panicum* sp.	Thailand	JX256424	JX276436	JX266593
C. australiensis	BRIP 12044 [T]	*Oryza sativa*	Australia	KJ415540	KJ415406	KJ415452
C. australis	BRIP 12521 [T]	*Sporobolus caroli*	Australia	KJ415541	KJ415405	KJ415451
C. bannonii	BRIP 16732 [T]	*Jacquemontia tamnifolia*	USA	KJ415542	KJ415404	KJ415450
***C. beasleyi* sp. nov.**	BRIP 10972 [T]	*Chloris gayana*	Australia	**MH414892**	**MH433638**	**MH433654**
	BRIP 15854	*Leersia hexandra*	Australia	**MH414893**	**MH433639**	**MH433655**
***C. beerburrumensis* sp. nov.**	BRIP 12942 [T]	*Eragrostis bahiensis*	Australia	**MH414894**	**MH433634**	**MH433657**
	BRIP 12555	*Eragrostis sororia*	Australia	**MH414895**	**MH433640**	**MH433656**
***C. boeremae* comb. nov.**	IMI 164633 [T]	*Portulaca oleracea*	India	**MH414911**	**MH433641**	–
C. borreriae	MFLUCC 11-0422	unknown Poaceae	Thailand	KP400638	KP419987	KM196571
C. bothriochloae	BRIP 12522 [T]	*Bothriochloa bladhii*	Australia	KJ415543	KJ415403	KJ415449
C. brachyspora	CBS 186.50	Soil	India	KJ922372	KM061784	KM230405
C. buchloës	CBS 246.49 [T]	*Buchloë dactyloides*	USA	KJ909765	KM061789	KM196588
C. carica-papayae	CBS 135941 [T]	*Carica papaya*	India	HG778984	HG779146	–
C. chiangmaiensis	CPC 28829 [T]	*Zea mays*	Thailand	MF490814	MF490836	MF490857
C. chlamydospora	UTHSC 07-2764 [T]	*Homo sapiens*	USA	HG779021	HG779151	–
***C. coatesiae* sp. nov.**	BRIP 24170	air	Australia	**MH414896**	**MH433635**	**MH433658**
	BRIP 24261 [T]	*Litchi chinensis*	Australia	**MH414897**	**MH433636**	**MH433659**
C. clavata	BRIP 61680b	*Oryza rufipogon*	Australia	KU552205	KU552167	KU552159
C. coicis	CBS 192.29 [T]	*Coix lacryma-jobi*	Japan	AF081447	AF081410	JN601006
***C. colbranii* sp. nov.**	BRIP 13066 [T]	*Crinum zeylanicum*	Australia	**MH414898**	MH433642	MH433660
C. crustacea	BRIP 13524 [T]	*Sporobolus* sp.	Indonesia	KJ415544	KJ415402	KJ415448
C. cymbopogonis	CBS 419.78	*Yucca* sp.	Netherlands	HG778985	HG779129	–
C. dactyloctenicola	CPC 28810 [T]	*Dactyloctenium aegyptium*	Thailand	MF490815	MF490837	MF490858
C. dactyloctenii	BRIP 12846 [T]	*Dactyloctenium radulans*	Australia	KJ415545	KJ415401	KJ415447
C. ellisii	CBS 193.62 [T]	air	Pakistan	JN192375	JN600963	JN601007
C. eragrostidis	CBS 189.48	*Sorghum* sp.	Indonesia	HG778986	HG779154	–
***C. eragrosticola* sp. nov.**	BRIP 12538 [T]	*Eragrostis pilosa*	Australia	**MH414899**	**MH433643**	**MH433661**
C. geniculata	CBS 187.50	*Andropogon sorghum*	Indonesia	KJ909781	KM083609	KM230410
C. gladioli	CBS 210.79	*Gladiolus* sp.	Romania	HG778987	HG779123	–
C. graminicola	BRIP 23186 [T]	*Aristida ingrata*	Australia	JN192376	JN600964	JN601008
C. harveyi	BRIP 57412 [T]	*Triticum aestivum*	Australia	KJ415546	KJ415400	KJ415446
C. hawaiiensis	BRIP 11987 [T]	*Oryza sativa*	USA	KJ415547	KJ415399	KJ415445
C. heteropogonicola	BRIP 14579 [T]	*Heteropogon contortus*	India	KJ415548	KJ415398	KJ415444

Species	Isolate no.[1]	Host	Location	GenBank accession numbers[2]		
				ITS	*gapdh*	*tef1a*
C. heteropogonis	CBS 284.91 T	*Heteropogon contortus*	Australia	KJ415549	JN600969	JN601013
C. hominis	CBS 136985 T	*Homo sapiens*	USA	HG779011	HG779106	
C. homomorpha	CBS 156.60 T	air	USA	JN192380	JN600970	JN601014
C. inaequalis	CBS 102.42 T	soil	France	KJ922375	KM061787	KM196574
C. intermedia	CBS 334.64	*Avena versicolor*	USA	HG778991	HG779155	–
C. ischaemi	CBS 630.82 T	*Ischaemum indicum*	Solomon Islands	JX256428	JX276440	–
***C. kenpeggii* sp. nov.**	BRIP 14530 T	*Triticum aestivum*	Australia	**MH414900**	**MH433644**	**MH433662**
C. kusanoi	CBS 137.29	*Eragrostis major*	Japan	JN192381	–	JN601016
***C. lamingtonensis* sp. nov.**	BRIP 12259 T	*Microlaena stipoides*	Australia	**MH414901**	**MH433645**	**MH433663**
C. lunata	CBS 730.96 T	*Homo sapiens*	USA	JX256429	JX276441	JX266596
C. malina	CBS 131274 T	*Zoysia matrella*	USA	JF812154	KP153179	KR493095
***C. mebaldsii* sp. nov.**	BRIP 12900 T	*Cynodon transvaalensis*	Australia	**MH414902**	**MH433647**	**MH433664**
	BRIP 13983	*Cynodon dactylon x transvaalensis*	Australia	**MH414903**	**MH433646**	**MH433665**
C. miyakei	CBS 197.29 T	*Eragrostis pilosa*	Japan	KJ909770	KM083611	KM196568
C. muehlenbeckiae	CBS 144.63 T	*Sorghum* sp.	USA	KP400647	KP419996	KM196578
C. neergaardii	BRIP 12919 T	*Oryza sativa*	Ghana	KJ415550	KJ415397	KJ415443
C. neoindica	IMI 129790 T	*Brassica nigra*	India	**MH414910**	**MH433649**	**MH433667**
C. nicotiae	BRIP 11983 T	soil	Algeria	KJ415551	KJ415396	KJ415442
C. nodosa	CPC 28800 T	*Digitaria ciliaris*	Thailand	MF490816	MF490838	MF490859
C. nodulosa	CBS 160.58	*Eleusine indica*	USA	JN601033	JN600975	JN601019
C. oryzae	CBS 169.53 T	*Oryza sativa*	Vietnam	KP400650	KP645344	KM196590
C. ovariicola	CBS 470.90 T	*Eragrostis interrupta*	Australia	JN192384	JN600976	JN601020
C. pallescens	CBS 156.35 T	air	Indonesia	KJ922380	KM083606	KM196570
C. papendorfii	CBS 308.67 T	*Acacia karroo*	South Africa	KJ415552	KJ415395	KJ415441
***C. petersonii* sp. nov.**	BRIP 14642 T	*Dactyloctenium aegyptium*	Australia	**MH414905**	**MH433667**	**MH433668**
C. perotidis	CBS 350.90 T	*Perotis rara*	Australia	JN192385	KJ415394	JN601021
C. pisi	CBS 190.48 T	*Pisum sativum*	Canada	KY905678	KY905690	KY905697
***C. platzii* sp. nov.**	BRIP 27703b T	*Cenchrus clandestinus*	Australia	**MH414906**	**MH433651**	**MH433669**
C. portulacae	BRIP 14541 T	*Portulaca oleracea*	USA	KJ415553	KJ415393	KJ415440
C. prasadii	CBS 143.64 T	*Jasminum sambac*	India	KJ922373	KM061785	KM230408
C. protuberata	CBS 376.65 T	*Deschampsia flexuosa*	UK	KJ922376	KM083605	KM196576
C. pseudobrachyspora	CPC 28808 T	*Eleusine indica*	Thailand	MF490819	MF490841	MF490862
C. pseudolunata	UTHSC 09-2092 T	*Homo sapiens*	USA	HE861842	HE861842	–
C. pseudorobusta	UTHSC 08-3458	*Homo sapiens*	USA	HE861838	HF565476	–
C. ravenelii	BRIP 13165 T	*Sporobolus fertilis*	Australia	JN192386	JN600978	JN601024
***C. reesii* sp. nov.**	BRIP 4358 T	air	Australia	**MH414907**	**MH433637**	**MH433670**
C. richardiae	BRIP 4371 T	*Richardia brasiliensis*	Australia	KJ415555	KJ415391	KJ415438
C. robusta	CBS 624.68 T	*Dichanthium annulatum*	USA	KJ909783	KM083613	KM196577
C. ryleyi	BRIP 12554 T	*Sporobolus creber*	Australia	KJ415556	KJ415390	KJ415437

Species	**Isolate no.**[1]	***Host***	**Location**	**GenBank accession numbers**[2]		
				ITS	***gapdh***	***tef1a***
C. senegalensis	CBS 149.71	unknown	Nigeria	HG779001	HG779128	–
C. soli	CBS 222.96[T]	soil	Papua New Guinea	KY905679	KY905691	KY905698
C. sorghina	BRIP 15900[T]	*Sorghum bicolor*	Australia	KJ415558	KJ415388	KJ415435
C. spicifera	CBS 274.52	soil	Spain	JN192387	JN600979	JN601023
***C. sporobolicola* sp. nov.**	BRIP 23040b[T]	*Sporobolus australasicus*	Australia	**MH414908**	**MH433652**	**MH433671**
C. subpapendorfii	CBS 656.74[T]	soil	Egypt	KJ909777	KM061791	KM196585
C. trifolii	CBS 173.55	*Trifolium repens*	USA	HG779023	HG779124	–
C. tripogonis	BRIP 12375[T]	*Tripogon loliiformis*	Australia	JN192388	JN600980	JN601025
C. tropicalis	BRIP 14834[T]	*Coffea arabica*	India	KJ415559	KJ415387	KJ415434
C. tsudae	ATCC 44764[T]	*Chloris gayana*	Japan	KC424596	KC747745	KC503940
C. tuberculata	CBS 146.63[T]	*Zea mays*	India	JX256433	JX276445	JX266599
C. uncinata	CBS 221.52[T]	*Oryza sativa*	Vietnam	HG779024	HG779134	–
C. variabilis	CPC 28815[T]	*Chloris barbata*	Thailand	MF490822	MF490844	MF490865
C. verruciformis	CBS 537.75	*Vanellus miles*	New Zealand	HG779026	HG779133	–
C. verruculosa	CBS 150.63	*Punica granatum*	India	KP400652	KP645346	KP735695
***C. warraberensis* sp. nov.**	BRIP 14817[T]	*Dactyloctenium aegyptium*	Australia	**MH414909**	**MH433653**	**MH433672**
Curvularia sp.	BRIP 17068b	*Micraira subulifolia*	Australia	**MH414904**	**MH433648**	**MH433666**
	BRIP 17439	*Trianthema portulacastrum*	Australia	AF081449	AF081406	**MH445455**

[1]ATCC: American Type Culture Collection, Manassas, Virginia, USA; BRIP: Queensland Plant Pathology Herbarium, Brisbane, Australia; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CPC: cultures of Pedro Crous, housed at Westerdijk Fungal Biodiversity Institute; ICMP: International Collection of Microorganisms for Plants, Auckland, New Zealand; IMI: International Mycological Institute, CABI-Bioscience, Egham, United Kingdom; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; UTHSC: Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, Texas, USA.

[T]Ex-type isolates.

GenBank accessions derived from this study are shown in **bold**.

and conidiophores were mounted on glass slides in lactic acid (100% v/v). Images were captured with a Leica DFC 500 camera attached to a Leica DM5500B compound microscope with Nomarski differential interference contrast illumination. Conidial widths were measured at the widest part of each conidium. Means and standard deviations (SD) were calculated from at least 20 measurements. Ranges were expressed as (minimum value–) mean-SD – mean+SD (–maximum value) with values rounded to 0.5 µm.

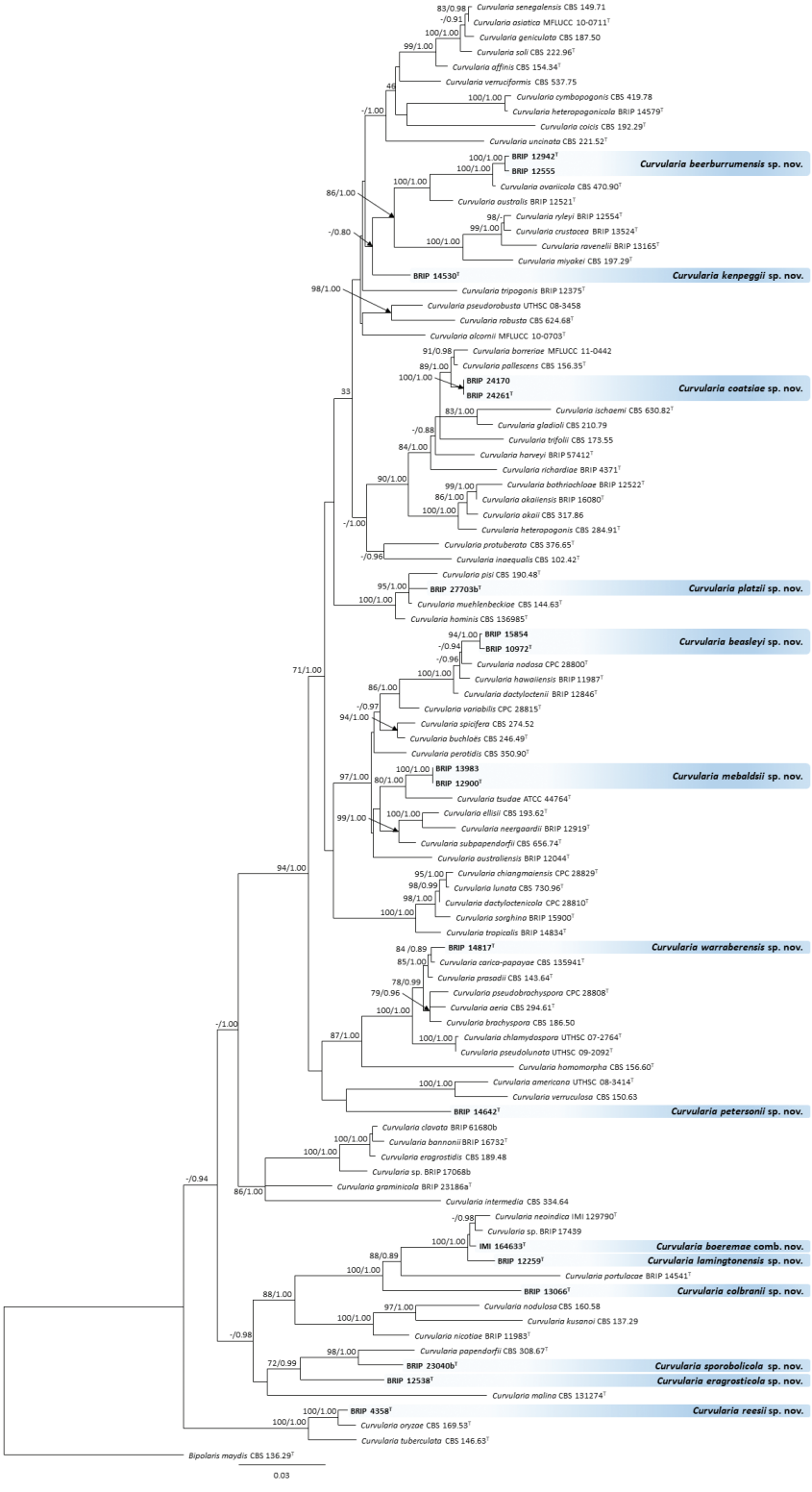
Colonies were described from 7-d-old cultures grown on potato dextrose agar (PDA) (Becton Dickinson), incubated at room temperature (approx. 25 °C) and exposed to near-ultraviolet light on a diurnal cycle. Images of the colonies and herbarium specimens were captured by an Epson Perfection V700 scanner at a 300 dpi resolution. Colour of the colonies was rated according to Rayner (1970). Taxonomic novelties were deposited in MycoBank (http://www.MycoBank.org; Crous et al. 2004).

DNA isolation, amplification, and phylogenetic analyses

Isolates were grown on PDA for 7 d at room temperature (approx. 25 °C). Mycelium was scraped off the PDA cultures and macerated with 0.5 mm glass beads (Daintree Scientific) in a Tissue Lyser (Qiagen). Genomic DNA was extracted with the Gentra Puregene DNA Extraction Kit (Qiagen) according to the manufacturer's instructions. Amplification and sequencing of the internal transcribed spacer (ITS) region, glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) and the translation elongation factor 1-alpha (*tef1a*) loci followed the methods by Tan et al. (2014). All sequences generated were assembled using Geneious v. 9.1.8 (Biomatters Ltd) and deposited in GenBank (Table 1, in bold). Sequences were aligned with selected sequences of *Curvularia* species obtained from GenBank (Table 1) using the MAFFT alignment algorithm (Katoh et al. 2009) in Geneious. *Bipolaris maydis* (CBS 136.29) was included as the outgroup. The sequences of each locus were aligned separately and manually adjusted where necessary. The alignment included sequences from ex-type cultures of 63 species of *Curvularia* and from the reference cultures of 16 species. The Maximum-Likelihood (ML) and Bayesian Inference (BI) methods were used in phylogenetic analyses as described by Tan et al. (2016). Briefly, the ML analysis was run using RAxML v.7.2.8 (Stamatakis and Alachiotis 2010) in Geneious and started from a random tree topology. The nucleotide substitution model used was GTR with a gamma-distributed rate variation. The Markov chain Monte Carlo (MCMC) algorithm was used to create a phylogenetic tree based on Bayesian probabilities using MrBayes v.3.2.1 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003) in Geneious. To remove the need for *a priori* model testing, the MCMC analysis was set to sample across the entire general time-reversible (GTR) model space with a gamma-distributed rate variation across the sites. Ten million random trees were generated using the MCMC procedure with four chains. The sample frequency was set at 100 and the temperature of the heated chain was 0.1. Burn-in was set at 25%, after which the likelihood values were stationary. The concatenated alignment was deposited in TreeBASE (S22563).

Unique fixed nucleotide positions were used to characterise and describe two cryptic species (see applicable species notes). For each of the cryptic species that was described, the closest phylogenetic neighbour was selected (Fig. 1) and this focused dataset was subjected to single nucleotide polymorphism (SNP) analysis. These SNPs were determined for each aligned locus using the Find Variation/SNPs feature in Geneious. The SNPs were determined based on a minimum variant frequency of 0.2.

Figure 1. Phylogenetic tree based on maximum likelihood analysis of the combined multilocus alignment. RAxML bootstrap values (bs) greater than 70% and Bayesian posterior probabilities (pp) greater than 0.7 are given at the nodes (bs/pp). Novel species names are highlighted in blue. Ex-type isolates are marked with a T. The outgroup is *Bipolaris maydis* ex-type strain CBS 136.29.


Curvularia senegalensis CBS 149.71
Curvularia asiatica MFLUCC 10-0711T
Curvularia geniculata CBS 187.50
Curvularia soli CBS 222.96T
Curvularia affinis CBS 154.34T
Curvularia verruciformis CBS 537.75
Curvularia cymbopogonis CBS 419.78
Curvularia heteropogonicola BRIP 14579T
Curvularia coicis CBS 192.29T
Curvularia uncinata CBS 221.52T
BRIP 12942T
BRIP 12555
Curvularia beerburrumensis sp. nov.
Curvularia avaricola CBS 470.90T
Curvularia australis BRIP 12521T
Curvularia ryleyi BRIP 12554T
Curvularia crustacea BRIP 13524T
Curvularia ravenelii BRIP 13165T
Curvularia miyakei CBS 197.29T
BRIP 14530T
Curvularia kenpeggii sp. nov.
Curvularia tripogonis BRIP 12375T
Curvularia pseudorobusta UTHSC 08-3458
Curvularia robusta CBS 624.68T
Curvularia alcornii MFLUCC 10-0703T
Curvularia borreriae MFLUCC 11-0442
Curvularia pallescens CBS 156.35T
BRIP 24170
BRIP 24261T
Curvularia coatsiae sp. nov.
Curvularia ischaemi CBS 630.82T
Curvularia gladioli CBS 210.79
Curvularia trifolii CBS 173.55
Curvularia harveyi BRIP 57412T
Curvularia richardiae BRIP 4371T
Curvularia bothriochloae BRIP 12522T
Curvularia akaiiensis BRIP 16080T
Curvularia akaii CBS 317.86
Curvularia heteropogonis CBS 284.91T
Curvularia protuberata CBS 376.65T
Curvularia inaequalis CBS 102.42T
Curvularia pisi CBS 190.48T
BRIP 27703bT
Curvularia platzii sp. nov.
Curvularia muehlenbeckiae CBS 144.63T
Curvularia hominis CBS 136985T
BRIP 15854
BRIP 10972T
Curvularia beasleyi sp. nov.
Curvularia nodosa CPC 28800T
Curvularia hawaiiensis BRIP 11987T
Curvularia dactyloctenii BRIP 12846T
Curvularia variabilis CPC 28815T
Curvularia spicifera CBS 274.52
Curvularia buchloës CBS 246.49T
Curvularia perotidis CBS 350.90T
BRIP 13983
BRIP 12900T
Curvularia mebaldsii sp. nov.
Curvularia tsudae ATCC 44764T
Curvularia ellisii CBS 193.62T
Curvularia neergaardii BRIP 12919T
Curvularia subpapendorfii CBS 656.74T
Curvularia australiensis BRIP 12044T
Curvularia chiangmaiensis CPC 28829T
Curvularia lunata CBS 730.96T
Curvularia dactyloctenicola CPC 28810T
Curvularia sorghina BRIP 15900T
Curvularia tropicalis BRIP 14834T
BRIP 14817T
Curvularia warraberensis sp. nov.
Curvularia carica-papayae CBS 135941T
Curvularia prasadii CBS 143.64T
Curvularia pseudobrachyspora CPC 28808T
Curvularia aeria CBS 294.61T
Curvularia brachyspora CBS 186.50
Curvularia chlamydospora UTHSC 07-2764T
Curvularia pseudolunata UTHSC 09-2092T
Curvularia homomorpha CBS 156.60T
Curvularia americana UTHSC 08-3414T
Curvularia verruculosa CBS 150.63
BRIP 14642T
Curvularia petersonii sp. nov.
Curvularia clavata BRIP 61680b
Curvularia bannonii BRIP 16732T
Curvularia eragrostidis CBS 189.48
Curvularia sp. BRIP 17068b
Curvularia graminicola BRIP 23186aT
Curvularia intermedia CBS 334.64
Curvularia neoindica IMI 129790T
Curvularia sp. BRIP 17439
IMI 164633T
Curvularia boeremae comb. nov.
BRIP 12259T
Curvularia lamingtonensis sp. nov.
Curvularia portulacae BRIP 14541T
BRIP 13066T
Curvularia colbranii sp. nov.
Curvularia nodulosa CBS 160.58
Curvularia kusanoi CBS 137.29
Curvularia nicotiae BRIP 11983T
Curvularia papendorfii CBS 308.67T
BRIP 23040bT
Curvularia sporobolicola sp. nov.
BRIP 12538T
Curvularia eragrosticola sp. nov.
Curvularia molina CBS 131274T
BRIP 4358T
Curvularia reesii sp. nov.
Curvularia oryzae CBS 169.53T
Curvularia tuberculata CBS 146.63T
Bipolaris maydis CBS 136.29T
0.03

Results

Molecular phylogeny

Approximately 800 bp of the ITS region, 598 bp of the partial region of the *gapdh* gene and 969 bp of the partial region of the *tef1a* gene were sequenced from the BRIP isolates. After removing ambiguously aligned regions, the ITS, *gapdh* and *tef1a* alignments were trimmed to 474 bp, 544 bp and 867 bp, respectively. The ITS phylogeny was able to resolve 53 of 79 *Curvularia* species, including 10 of the new species (data not shown). The *gapdh* phylogeny inferred 12 new species and the *tef1a* phylogeny resolved all 13 of the new species (data not shown). As the topologies of the single locus phylogenies for the tree datasets did not show any conflicts, they were analysed in a concatenated alignment. The phylogenetic tree based on the concatenated alignment resolved the 17 BRIP isolates into 13 well-supported and unique clades (Fig. 1), which are described in this study as novel species.

Taxonomy

Curvularia beasleyi **Y.P. Tan & R.G. Shivas, sp. nov.**
MycoBank MB825449
Fig. 2A–D

Type. AUSTRALIA, Queensland, Beaudesert, from leaf spot on *Chloris gayana*, 9 Jan. 1974, *J.L. Alcorn* (holotype BRIP 10972, includes ex-type culture).

Description. *Colonies* on PDA approx. 4 cm diam. after 7 d at 25 °C, surface funiculose, margin fimbriate, olivaceous black. *Hyphae* subhyaline, smooth to branched, septate, up to 3 µm in width. *Conidiophores* branched, erect, straight to flexuous, geniculate towards apex, brown, paler towards apex, smooth, septate, up to 110 µm long, 4 µm wide; basal cell swollen and darker than the other cells, up to 6 µm diam. *Conidiogenous cells* integrated, terminal or intercalary, sympodial, pale brown, smooth, with darkened scars. *Conidia* fusiform, straight to slightly curved, rounded at the apex, (14–) 26–29 (–34) × (5–) 6.5–7.5 (–9) µm, brown to dark brown, 3–7 (mostly 5)-distoseptate; hila conspicuous, slightly protuberant, thickened and darkened, 1–1.5 µm wide.

Etymology. In recognition of Dr Dean R. Beasley, an Australian plant pathologist, for his dedication and numerous innovative contributions to the curation and promotion of the Queensland Plant Pathology Herbarium (BRIP).

Additional material examined. AUSTRALIA, Queensland, Atherton, from leaf spot on *Leersia hexandra*, 1 May 1987, *J.L. Alcorn*, BRIP 15854 (includes culture).

Notes. *Curvularia beasleyi* is placed in the same clade as *C. dactyloctenii*, *C. hawaiiensis* and *C. nodosa* (Fig. 1). *Curvularia dactyloctenii* and *C. hawaiiensis* have been recorded in Australia (Sivanesan 1987, Tan et al. 2014), but the recently described *C. nodosa* has only been reported from Thailand (Marin-Felix et al. 2017b). *Curvularia beasleyi* is dis-

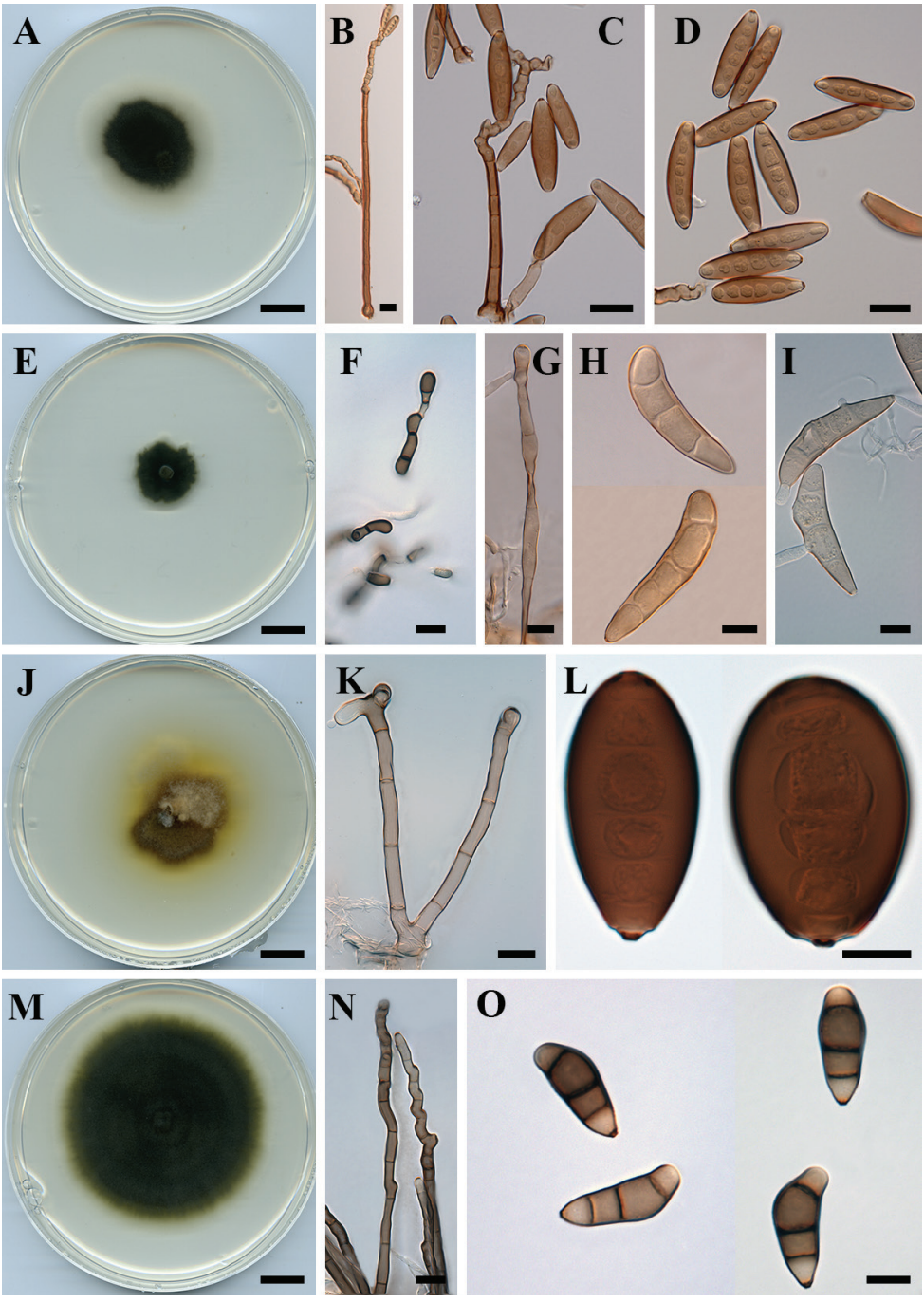


Figure 2. *Curvularia beasleyi* (BRIP 10972): **A** colony on PDA **B–C** conidiophores and conidia **D** conidia. *Curvularia beerburrumensis* (BRIP 12942) **E** colony on PDA **F** chlamydospores **G** conidiophore **H–I** conidia. *Curvularia boeremae* (IMI 164633) **J** colony on PDA **K c**onidiophores **L** conidia. *Curvularia coatesiae* (BRIP 24261) **M** colony on PDA **N** conidiophores **O** conidia. Scale bars: 1 cm (**A, E, J, M**); all others – 10 µm.

tinguished in two loci from the ex-type cultures of *C. dactyloctenii* (99% in *gapdh* and 99% in *tef1a*), *C. hawaiiensis* (98% in *gapdh* and 99% in *tef1a*) and *C. nodosa* (99% in *gapdh* and 99% in *tef1a*). The conidia of *C. beasleyi* are longer than those of *C. nodosa* (12–25 µm, Marin-Felix et al. 2017b) and shorter than those of *C. dactyloctenii* (32–55 µm, Sivanesan 1987). *Curvularia beasleyi* is morphologically similar to *C. hawaiiensis*, however the later species has never been recorded on *Leersia* (Farr and Rossman 2018).

Curvularia beasleyi is only known from Queensland on two unrelated grasses, the introduced host *Chloris gayana* and the native *Leersia hexandra*. There are many *Curvularia* species reported as associated with *Chloris* spp. (*C. australiensis*, *C. australis, C. hawaiiensis*, *C. lunata*, *C. nodosa*, *C. pallescens*, *C. tsudae*, *C. variabilis*, C. *verruculosa*) (Sivanesan 1987, Deng et al. 2014, Manamgoda et al. 2014, Marin-Felix et al. 2017b) and *Leersia* spp. (*C. australiensis*, *C. geniculata*, and *C. heteropogonicola*) (DAF Biological Collections 2018, Farr and Rossman 2018, Herbarium Catalogue 2018), although not all of the reports have been verified by molecular phylogenetic analyses.

Curvularia beerburrumensis **Y.P. Tan & R.G. Shivas, sp. nov.**
MycoBank MB825450
Fig. 2E–I

Type. AUSTRALIA, Queensland, Beerburrum, from blackened inflorescence of *Eragrostis bahiensis*, 24 May 1979, *J.L. Alcorn* (holotype BRIP 12942, includes ex-type culture).

Description. *Colonies* on PDA approx. 2 cm diam. after 7 d at 25 °C, surface funiculose, margin fimbrillate, olivaceous black. *Hyphae* subhyaline, smooth to asperulate, branched, septate, 3–4 µm in width; chlamydospores intercalary in chains, 4–9 µm, smooth, thick-walled. *Conidiophores* erect, straight to flexuous, geniculate towards apex, subhyaline to pale brown, smooth, septate, up to 500 µm long, 5–6 µm wide. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic, with darkened scars. *Conidia* fusiform to subcylindrical or clavate, straight to slightly curved, rounded at the apex, (40–) 51–56 (–71) × (10–) 12–13 (–14) µm, subhyaline to pale yellowish-brown, 2–4 (mostly 3)-distoseptate; hila mostly inconspicuous or minutely thickened and darkened.

Etymology. Named after the town Beerburrum, where the holotype was collected.

Additional material examined. AUSTRALIA, Queensland, Beerburrum, New South Wales, Yetman, blackened inflorescence of *Eragrostis sororia*, 12 May 1977, *J.L. Alcorn*, BRIP 12555 (includes culture).

Notes. *Curvularia beerburrumensis* is phylogenetically sister to *C. australis* and *C. ovariicola* (Fig. 1), which have both been recorded in Australia on *Eragrostis* (Sivanesan 1987, Tan et al. 2014). *Curvularia beerburrumensis* is distinguished from the ex-type culture of *C. australis* in three loci (98% in ITS, 96% in *gapdh* and 98% in *tef1a*). Furthermore, *C. beerburrumensis* has larger conidia than *C. australis* (25–48 × 9.0–12.5 µm, Sivanesan 1987). *Curvularia beerburrumensis* differs from the ex-type culture of

C. ovariicola in three loci (99% in ITS, 99% in *gapdh* and 99% in *tef1a*). *Curvularia beerburrumensis* has longer conidiophores than *C. ovariicola* (up to 325 µm, Sivanesan 1987). *Curvularia beerburrumensis* also produced chlamydospores in culture, which are not known for *C. australis* and *C. ovariicola*.

Curvularia beerburrumensis is only known from inflorescences of the invasive South American grass *Eragrostis bahiensis*, as well as the Australian native *E. sororia* (Simon and Alfonso 2011). Other *Curvularia* associated with *Eragrostis* include *C. australis*, *C. clavata*, *C. crustacea*, *C. ellisii*, *C. eragrostidis*, *C. geniculata*, *C. kusanoi*, *C. lunata*, *C. miyakei*, *C. nodulosa*, *C. ovariicola*, *C. perotidis*, *C. protuberata*, *C. ravenelii* and *C. verrucosa*, (Sivanesan 1987, Farr and Rossman 2018, Herbarium Catalogue 2018), although many of these reports are yet to be verified by molecular phylogenetic analyses.

Curvularia boeremae **(A.S. Patil & V.G. Rao) Y.P. Tan & R.G. Shivas, comb. nov.**
MycoBank MB825451
Fig. 2J–L

Basionym. *Drechslera boeremae* A.S. Patil & V.G. Rao, *Antonie van Leeuwenhoek* 42: 129 (1976).

Description. *Colonies* on PDA approx. 3 cm diam. after 7 d at 25 °C, surface funiculose, margin fimbriate, olivaceous green to citrine, velutinous with aerial mycelium. *Hyphae* subhyaline, smooth to asperulate, branched, septate, 2–3 µm in width. *Conidiophores* straight to flexuous, slightly geniculate towards apex, uniformly subhyaline to pale brown, smooth, septate, up to 110 µm long, 4 µm wide. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic, with darkened scars. *Conidia* broadly ellipsoidal to oval, brown to dark brown, smooth, (42–) 46–52 (–55) × (17–) 20–23 (–25) µm, brown to dark brown, 4–6-distoseptate, hila protuberant, thickened and darkened, 2–3 µm wide.

Type. INDIA, Poona, from leaves of *Portulaca oleracea*, 28 Apr. 1970, *A.S. Patil* (holotype IMI 164633, includes ex-type culture), (isotype BRIP 13934, includes ex-type culture).

Notes. Multilocus phylogenetic analyses placed the ex-type culture of *D. boeremae* within the clade that includes *C. lunata*, the type species of the genus (Fig. 1). *Curvularia boeremae* differs from *C. neoindica* in one locus (98% identities in *gapdh*). Furthermore, *C. boeremae* has shorter conidia than *C. neoindica* (27–65 µm, Manamgoda et al. 2014). Sivanesan's (1987) synonymy of *Drechslera boeremae* with *Bipolaris indica* was based on similar conidial morphology and is not supported by the phylogenetic analyses in this study.

Curvularia boeremae is only known from the type specimen on *P. oleraceae* and has not been recorded in Australia. *Curvularia portulacae* is the only other species recorded on *P. oleraceae* (Farr and Rossman 2018). *Curvularia boeremae* is morphologically distinct from *C. portulacae*, which has comparatively long, cylindrical conidia (average 110 × 13 µm, Rader 1948).

Curvularia coatesiae **Y.P. Tan & R.G. Shivas, sp. nov.**
MycoBank MB825452
Fig. 2M–O

Type. AUSTRALIA, Queensland, Eudlo, from rotted fruit of *Litchi chinensis*, 28 Jan. 1992, *L.M. Coates* (holotype BRIP 24261, includes ex-type culture).

Description. *Colonies* on PDA 6–7 cm diam. after 7 d at 25 °C, surface funiculose, floccose, olivaceous black at the centre, olivaceous to grey olivaceous towards the edge, margin fimbriate. *Hyphae* subhyaline, smooth to asperulate, septate, up to 3 µm in width. *Conidiophores* erect, flexuous, geniculate in the top half, uniformly brown, sometimes pale towards apex, septate, up to 190 µm long, 4 µm wide; basal cell sometimes swollen, up to 8 µm diam. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown, mono- or polytretic, with darkened nodes. *Conidia* ellipsoidal to obovoid, asymmetrical, sometimes the third cell from base is unequally enlarged, intermediate cells dark brown and usually verruculose, end cells paler and less ornamented than central cells, (20–) 23–26 (–30) × (7–) 8–9 (–10) µm, 3-distoseptate; hila protuberant, thickened and darkened, 1–2 µm wide.

Etymology. Named after Dr Lindel (Lindy) M. Coates, an Australian plant pathologist in recognition of her contributions to the study of post-harvest fruit pathology.

Additional material examined. AUSTRALIA, New South Wales, Alstonville, isolated from the air in a mango orchard, 11 Mar. 1991, *G.I. Johnson*, BRIP 24170 (includes culture).

Notes. *Curvularia coatesiae* is morphologically similar and phylogenetically related to a reference culture of *C. borreriae* and the ex-type culture of *C. pallescens* (Fig. 1). *Curvularia coatesiae* differs from the ex-type culture of *C. pallescens* in three loci: ITS position 439 (T); *gapdh* positions 219 (C), 287 (C); *tef1a* positions 43 (C), 257 (C), 259 (C). Although *C. borreriae* and *C. pallescens* have been recorded in Australia, these have not been verified by molecular phylogenetic analyses and there have been no additional records beyond the 1980s (Sivanesan 1987, Shivas 1989). Other species recorded from *L. chinensis* are *C. geniculata*, *C. hawaiiensis*, *C. lunata* and *C. pallescens* (DAF Biological Collections 2018, Herbarium Catalogue 2018), although not all the reports have been verified by molecular phylogenetic analyses.

Curvularia colbranii **Y.P. Tan & R.G. Shivas, sp. nov.**
MycoBank MB825453
Fig. 3A–D

Type. AUSTRALIA, Queensland, Brisbane, from leaf spot on *Crinum zeylanicum*, 11 Oct. 1976, *R.C. Colbran* (holotype BRIP 13066, includes ex-type culture).

Description. *Colonies* on PDA approx. 5 cm diam. after 7 d at 25 °C, surface funiculose, margin fimbriate, olivaceous black, aerial mycelium white. *Hyphae* subhyaline, smooth, septate, up to 3 µm in width. *Conidiophores* erect, flexuous, geniculate,

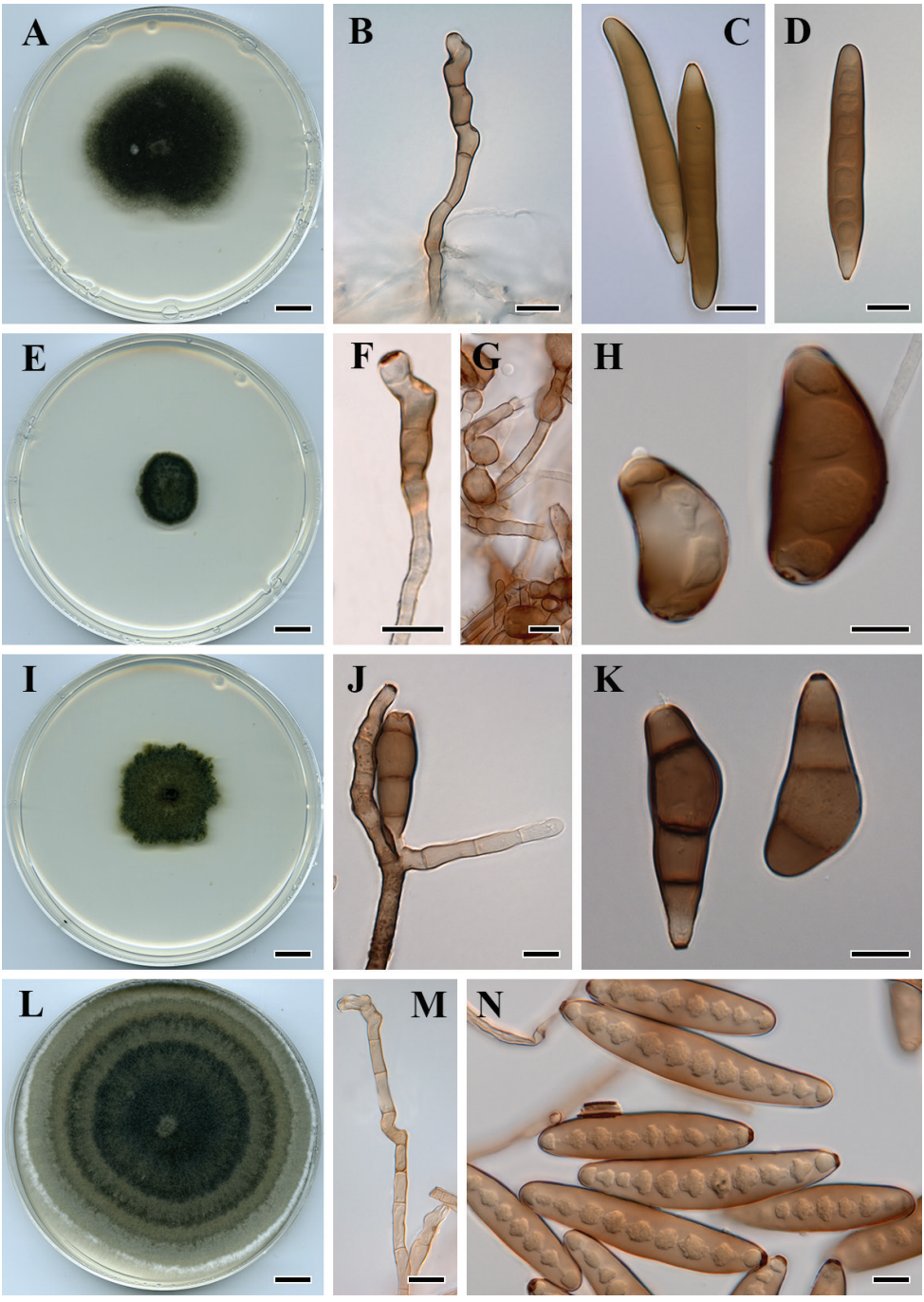


Figure 3. *Curvularia colbranii* (BRIP 13066): **A** colony on PDA **B** conidiophore **C–D** conidia. *Curvularia eragrosticola* (BRIP 12538) **E** colony on PDA **F** conidiophore **G** chlamydosphores **H** conidia. *Curvularia kenpeggii* (BRIP 14530) **I** colony on PDA **J** conidiophores and conidium **K** conidia. *Curvularia lamingtonensis* (BRIP 12259) **L** colony on PDA **M** conidiophore **O** conidia. Scale bars: 1 cm (**A, E, I, L**); all others – 10 µm.

uniformly pale brown to brown, smooth, septate, up to 145 µm long, 4–6 µm wide, basal cell sometimes swollen, up to 8 µm diam. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic, with darkened scars. *Conidia* fusiform to subcylindrical with rounded apex and obconical at the base, brown, end cells pale, (54–) 83–92 (–110) × (13–) 14–16 (–17) µm, brown to dark brown, 6–9-distoseptate; hila slightly protuberant, thickened and darkened, 1–2 µm wide.

Etymology. Named after Dr Robert (Bob) Chester Colbran (1926–2010), an Australian nematologist and Director of the Plant Pathology Branch, Queensland Department of Primary Industries, in recognition of his significant contributions to Australian plant pathology.

Notes. *Curvularia colbranii* is sister to *C. boeremae*, *C. lamingtonensis* (see this paper), *C. neoindica* and *C. portulacae*, although separated by a considerable genetic distance (Fig. 1). *Curvularia colbranii* has fusiform to subcylindrical conidia that are distinct from the ellipsoidal to oval conidia of *C. boeremae* (42–55 × 17–25 µm, this study) and *C. neoindica* (27–65 × 17–27 µm, Manamgoda et al. 2014) and longer than those of *C. lamingtonensis* (45–76 × 11–14 µm, this study). *Curvularia colbranii* has conidia that are 6–9-distoseptate, while *C. portulacae* has conidia reported as 3–15 celled (Rader 1948).

Only one other species, *C. trifolii*, has been reported on *Crinum* sp. (Shaw 1984), but this record has not been verified by phylogenetic analyses. *Curvularia colbranii* is morphologically distinct from *C. trifolii*, which has curved conidia.

Curvularia eragrosticola **Y.P. Tan & R.G. Shivas, sp. nov.**
MycoBank MB825454
Fig. 3E–H

Type. AUSTRALIA, New South Wales, Yetman, from inflorescence on *Eragrostis pilosa*, 12 May 1977, *J.L. Alcorn* (holotype BRIP 12538, includes ex-type culture).

Description. *Colonies* on PDA approx. 2 cm diam. after 7 d at 25 °C, surface funiculose, margin fimbriate, dark olive with white patches, velutinous with some aerial mycelium. *Hyphae* subhyaline, smooth, branched, septate, 4–5 µm wide; chlamydospores abundant, subglobose to ellipsoidal or irregular, terminal and intercalary, 5–20 µm diam. *Conidiophores* erect, straight to flexuous, slightly geniculate, pale brown to brown, paler towards apex smooth, septate, up to 145 µm long, 4–5 µm wide. *Conidiogenous cells* integrated, terminal or intercalary, sympodial, pale brown to brown, smooth, with darkened scars. *Conidia* hemi-ellipsoidal, curved, asymmetrical, brown to dark brown, end cells slightly paler, (25–) 26–30 (–34) × (9–) 13–15 (–19) µm, 3-distoseptate with a faint narrow median septum; hila non-protuberant, minutely thickened and darkened.

Etymology. Named after *Eragrostis*, the grass genus from which this fungus was isolated.

Notes. *Curvularia eragrosticola* is phylogenetically close to *C. papendorfii* and *C. sporobolicola* (see this paper) (Fig. 1). *Curvularia eragrosticola* is distinguished in three loci from the ex-type culture of *C. papendorfii* (97% in ITS, 92% in *gapdh* and 98% in *tef1a*) and *C. sporobolicola* (98% in ITS, 92% in *gapdh* and 98% in *tef1a*). *Curvularia eragrosticola* has conidia that are smaller than *C. papendorfii* (30–50 × 17–30 µm, Sivanesan 1987) and *C. sporobolicola* (34–45 × 14–23 µm, this study).

Curvularia eragrosticola is only known from the type specimen on *Eragrostis pilosa*, which is native to Eurasia and Africa and is considered a troublesome weed in Australia (Simon and Alfonso 2011). Neither *C. papendorfii* nor *C. sporobolicola* have been reported on *Eragrostis*. Other *Curvularia* spp. associated with *Eragrostis* are listed in the notes for *C. beerburrumensis*.

***Curvularia kenpeggii* Y.P. Tan & R.G. Shivas, sp. nov.**
MycoBank MB825455
Fig. 3H–J

Type. AUSTRALIA, Queensland, from mouldy grain of *Triticum aestivum*, 26 Oct. 1984, *J.L. Alcorn* (holotype BRIP 14530, includes ex-type culture), (isotype IMI 290719).

Description. *Colonies* on PDA 3–4 cm diam. after 7 d at 25 °C, surface funiculose, margin fimbriate, floccose and olivaceous black at the centre with white patches, velutinous with some aerial mycelium. *Hyphae* hyaline, asperulate, branched, septate, 4–5 µm in width. *Conidiophores* erect, straight to flexuous, slightly geniculate in the upper part, pale brown to brown, sometimes paler towards the apex, verrucose, septate, up to 360 µm long, 4–5 µm wide, basal cell sometimes swollen, up to 8 µm. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic, with darkened scars. *Conidia* ellipsoidal to clavate to obovoid, asymmetrical, third cell from the base is unequally enlarged, brown, end cells paler, verruculose, (31–) 35–39 (–42) × (10–) 13–14 (–15) µm, 3-distoseptate, hila protuberant, thickened and darkened, 1–2 µm wide.

Etymology. Named after Dr Kenneth G. Pegg AM (member of the Order of Australia), in celebration of his 60 years of dedication to plant pathology in Australia and to thank him for his generous mentorship.

Notes. *Curvularia kenpeggii* is only known from the holotype specimen and is genetically distinct from all other *Curvularia* species (Fig. 1). *Curvularia kenpeggii* is basal to a clade comprised of *C. australis*, *C. beerburrumensis*, *C. crustacea*, *C. miyakei*, *C. ovariicola*, *C. ravenelii* and *C. ryleyi*. These species are mostly reported as pathogens of *Eragrostis* and *Sporobolus* spp. and not known to be associated with wheat (*Triticum aestivum*). *Curvularia* species associated with *T. aestivum* in Australia are *C. brachyspora*, *C. harveyi*, *C. hawaiiensis*, *C. lunata*, *C. perotidis*, *C. ramosa* and *C. spicifera*, (Shivas 1989, Farr and Rossman 2018), although not all the reports have been verified by molecular phylogenetic analyses.

Curvularia lamingtonensis **Y.P. Tan & R.G. Shivas, sp. nov.**
MycoBank MB825456
Fig. 3L–N

Type. AUSTRALIA, Queensland, Lamington National Park, from *Microlaena stipoides*, 09 May 1977, *J.L. Alcorn* (holotype BRIP 12259, includes ex-type culture).

Description. *Colonies* on PDA cover the whole plate after 7 d at 25 °C, surface funiculose, margin fimbriate, olivaceous green, velutinous with some aerial mycelium. *Hyphae* hyaline, branched, septate, 4 µm in width. *Conidiophores* erect, straight to flexuous, geniculate towards apex, pale brown to dark brown on wheat straw agar, septate, up to 160 µm long, 3–4 µm wide. *Conidiogenous cells* integrated, terminal or intercalary, sympodial, pale brown to brown, smooth, with darkened scars. *Conidia* ellipsoidal to fusiform, straight, pale brown, (45–) 59–66 (–76) × (11–) 11.5–13 (–14) µm, 4–11-distoseptate with inconspicuous transverse septa, hila protuberant, thickened and darkened, 1–2 µm wide.

Etymology. Named after the locality, Lamington National Park, where the holotype was collected.

Notes. *Curvularia lamingtonensis* is phylogenetically closely related to *C. boeremae* and *C. neoindica*. *Curvularia lamingtonensis* is distinguished from the ex-type culture of *C. boeremae* in two loci (96% in ITS and 98% in *gapdh*) and from the ex-type culture of *C. neoindica* in three loci (95% in ITS, 98% in *gapdh* and 99% in *tef1a*). *Curvularia lamingtonensis* has longer and straighter conidia than *C. boeremae* and *C. neoindica*, both of which have broad, ellipsoidal conidia (42–55 × 20–23 µm, and 27–65 × 17–27 µm, respectively). *Curvularia lamingtonensis* is only known from the type specimen on *Microlaena stipoides*. This is the first record of a *Curvularia* species associated with *Microlaena*.

Curvularia mebaldsii **Y.P. Tan & R.G. Shivas, sp. nov.**
MycoBank MB825457
Fig. 4A–C

Type. AUSTRALIA, Victoria, Hopetoun, from *Cynodon transvaalensis*, Apr. 1979, *M. Mebalds* (holotype BRIP 12900, includes ex-type culture).

Description. *Colonies* on PDA approx. 5 cm diam. after 7 d at 25 °C, surface funiculose, margin fimbriate, olivaceous black with white patches, velutinous with some aerial mycelium. *Hyphae* hyaline to subhyaline, smooth to asperulate, septate, 3–4 µm wide. *Conidiophores* erect, straight to flexuous, sometimes slightly geniculate towards apex, branched, uniformly brown, paler at apex, smooth to asperulate, septate, up to 180 µm long, 4–5 µm wide. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, subhyaline to pale brown, smooth, mono- or polytretic, with darkened scars. *Conidia* ellipsoidal to obovoid, sometimes straight to slightly curved, rounded at the apex, (22–) 25–28 (–30) × (7–) 8–9 (–10) µm, pale brown to brown, 3-distoseptate, hila protuberant, thickened and darkened, 1–2 µm wide.

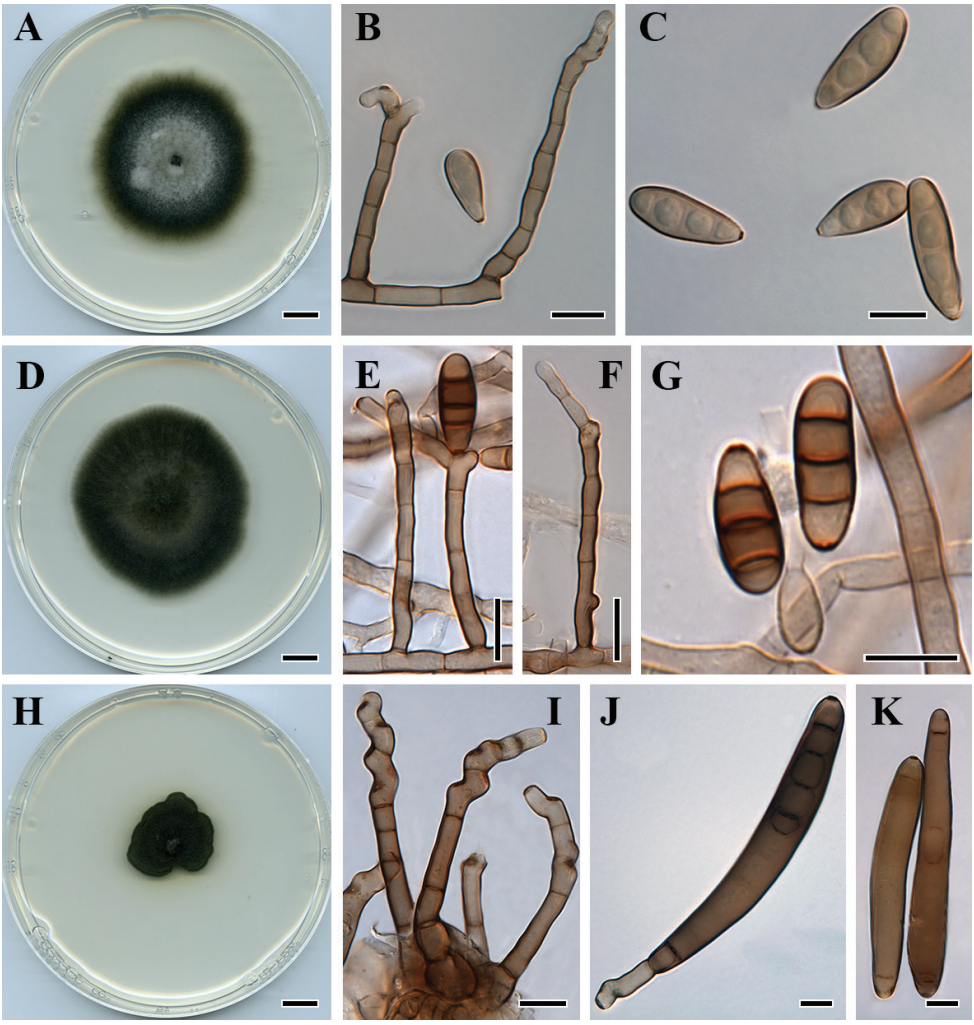


Figure 4. *Curvularia mebaldsii* (BRIP 12900): **A** colony on PDA **B** conidiophores and conidium **C** conidia. *Curvularia petersonii* (BRIP 14642) **D** colony on PDA **E–F** conidiophores and conidium **G** conidia. *Curvularia platzii* (BRIP 27703b) **H** colony on PDA **I** conidiophores **J–K** conidia. Scale bars: 1 cm (**A, D, H**); all others – 10 μm.

Etymology. Named after the collector, Martin Mebalds, in recognition of his contributions to Australian plant pathology and biosecurity.

Additional material examined. AUSTRALIA, New South Wales, Tweed Heads, from necrotic leaf on *Cynodon dactylon* × *transvaalensis*, 10 Jun. 1983, *G. Thomas*, BRIP 13983 (includes culture).

Notes. The multilocus phylogenetic analyses showed that *C. mebaldsii* was sister to *C. tsudae*, although separated by a considerable genetic distance (Fig. 1). *Curvularia mebaldsii* is distinguished from the ex-type culture of *C. tsudae* in three loci (98% in

ITS, 97% in *gapdh* and 99% in *tef1a*). Morphologically, *C. mebaldsii* cannot be reliably separated from *C. tsudae*.

Curvularia mebaldsii is known from two specimens on *Cynodon* spp. Several *Curvularia* species have been associated with *Cynodon*, including *C. aeria*, *C. australiensis*, *C. brachyspora*, *C. clavata*, *C. fallax*, *C. geniculata*, *C. hawaiiensis*, *C. inaequalis*, *C. lunata*, *C. pallescens*, *C. ramosa*, *C. senegalensis*, *C. spicata*, *C. spicifera* and *C. verruculosa* (DAF Biological Collections 2018, Farr and Rossman 2018, Herbarium Catalogue 2018), although these records have not been verified by phylogenetic analyses.

Curvularia petersonii **Y.P. Tan & R.G. Shivas, sp. nov.**
MycoBank MB825458
Fig. 4D–G

Type. AUSTRALIA, Northern Territory, Daly Waters, from leaf spot on *Dactyloctenium aegyptium*, 20 Mar. 1985, *R.A. Peterson* (holotype BRIP 14642, includes ex-type culture).

Description. *Colonies* on PDA approx. 5 cm diam. after 7 d at 25 °C, surface funiculose, olivaceous black, velutinous with some aerial mycelium, margin fimbriate. *Hyphae* subhyaline, smooth to asperulate, septate, up to 3 µm in width. *Conidiophores* erect, straight to flexuous, rarely branched, slightly geniculate, uniformly brown, sometimes pale brown at apex, smooth, septate, up to 110 µm long, 4 µm wide. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic, with darkened scars. *Conidia* obovoid to ellipsoidal, straight to slightly curved, (15–) 17–19 (–21) × (5–) 5.5–6 (–7) µm, brown, end cells pale, 3-distoseptate, hila non-protuberant, thickened and darkened.

Etymology. Named after Ron A. Peterson, an Australian plant pathologist, in recognition of his contributions to tropical plant pathology.

Notes. The multilocus phylogenetic analyses placed *C. petersonii* sister to *C. americana* and *C. verruculosa*, although separated by a considerable genetic distance (Fig. 1). Both *C. americana* and *C. verruculosa* have been found in Australia (DAF Biological Collections 2018, Herbarium Catalogue 2018). *Curvularia petersonii* is distinguished from the ex-type culture of *C. americana* in two loci (94% in ITS and 92% in *gapdh*) and from a reference culture of *C. verruculosa* in three loci (92% in ITS, 92% in *gapdh* and 98% in *tef1a*). *Curvularia petersonii* has smaller conidia than *C. americana* (13–28 × 7–15 µm, Madrid et al. 2014) and *C. verruculosa* (20–40 × 12–17 µm, Sivanesan 1987).

Curvularia petersonii is only known from a single specimen on *Dactyloctenium aegyptium* in the Northern Territory. Many *Curvularia* species have been associated with *Dactyloctenium*, including *C. clavata*, *C. dactyloctenicola*, *C. dactyloctenii*, *C. eragrostidis*, *C. lunata*, *C. neergaardii*, *C. pallescens* and *C. verruculosa* (Sivanesan 1987, Manamgoda et al. 2014, Farr and Rossman 2018, Herbarium Catalogue 2018, Marin-Felix et al. 2017b), although these records have not been verified by phylogenetic analyses.

Curvularia platzii **Y.P. Tan & R.G. Shivas, sp. nov.**
MycoBank MB825459
Fig. 4H–I

Type. AUSTRALIA, Queensland, Warwick, from leaf spot on *Cenchrus clandestinus*, 24 Jan. 2001, *G.J. Platz* (holotype BRIP 27703b, includes ex-type culture).

Description. *Colonies* on PDA approx. 2 cm diam. after 7 d at 25 °C, surface dark olivaceous green. *Hyphae* subhyaline, smooth, septate, up to 3 µm wide. *Conidiophores* erect, straight to flexuous, geniculate towards apex, uniformly brown, sometimes pale brown towards apex, septate, up to 75 µm long, 5–6 µm wide, swollen at base, 8–10 µm. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic, with darkened scars. *Conidia* fusiform to narrowly clavate, brown, end cells sometimes paler, (65–) 94–105 (–115) × (11–) 12.5–13.5 (–14) µm, 9–13-distoseptate; hila non-protuberant, thickened and darkened.

Etymology. Named after Gregory (Greg) J. Platz, in recognition of his contributions to Australian cereal plant pathology for the past 30 years, as well as his prowess as an international and Queensland rugby league footballer.

Notes. The multilocus phylogenetic analyses indicated *C. platzii* was sister to *C. hominis*, *C. meuhlenbeckiae* and *C. pisi* (Fig. 1). *Curvularia platzii* is distinguished in one locus from the ex-type culture of *C. hominis* (97% in *tef1a*) and in two loci from the reference culture of *C. meuhlenbeckiae* (99% in *gapdh* and 99% in *tef1a*) and the ex-type culture of *C. pisi* (98% in *gapdh* and 99% in *tef1a*). *Curvularia platzii* differs from *C. hominis*, *C. meuhlenbeckiae* and *C. pisi*, which have much shorter asymmetrical conidia with fewer septa (Madrid et al. 2014, Marin-Felix et al. 2017a).

Curvularia platzii is only known from the holotype. The host, *Cenchrus clandestinus* (syn. *Pennisetum clandestinus*), is a perennial grass with a worldwide distribution (Simon and Alfonso 2011). Other *Curvularia* species associated with *C. clandestinus* are *C. lunata*, *C. nodulosa* and *C. trifolii* (Farr and Rossman 2018, Herbarium Catalogue 2017), although these records have not been verified by phylogenetic analyses.

Curvularia reesii **Y.P. Tan & R.G. Shivas, sp. nov.**
MycoBank MB825460
Fig. 5A–C

Type. AUSTRALIA, Queensland, Brisbane, isolated from air, 22 Jun. 1963, *R.G. Rees* (holotype BRIP 4358, includes ex-type culture).

Description. *Colonies* on PDA approx. 6–7 cm diam. after 7 d at 25 °C, surface funiculose, greenish-grey, velutinous with some aerial mycelium, margin fimbriate. *Hyphae* hyaline, branched, septate, 3–4 µm in width. *Conidiophores* erect, straight to flexuous, slightly geniculate towards apex, pale brown to brown, sometimes paler towards the apex, septate, up to 200 µm long, 4–5 µm wide. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic, with darkened scars. *Conidia* ellipsoidal to obclavate, straight,

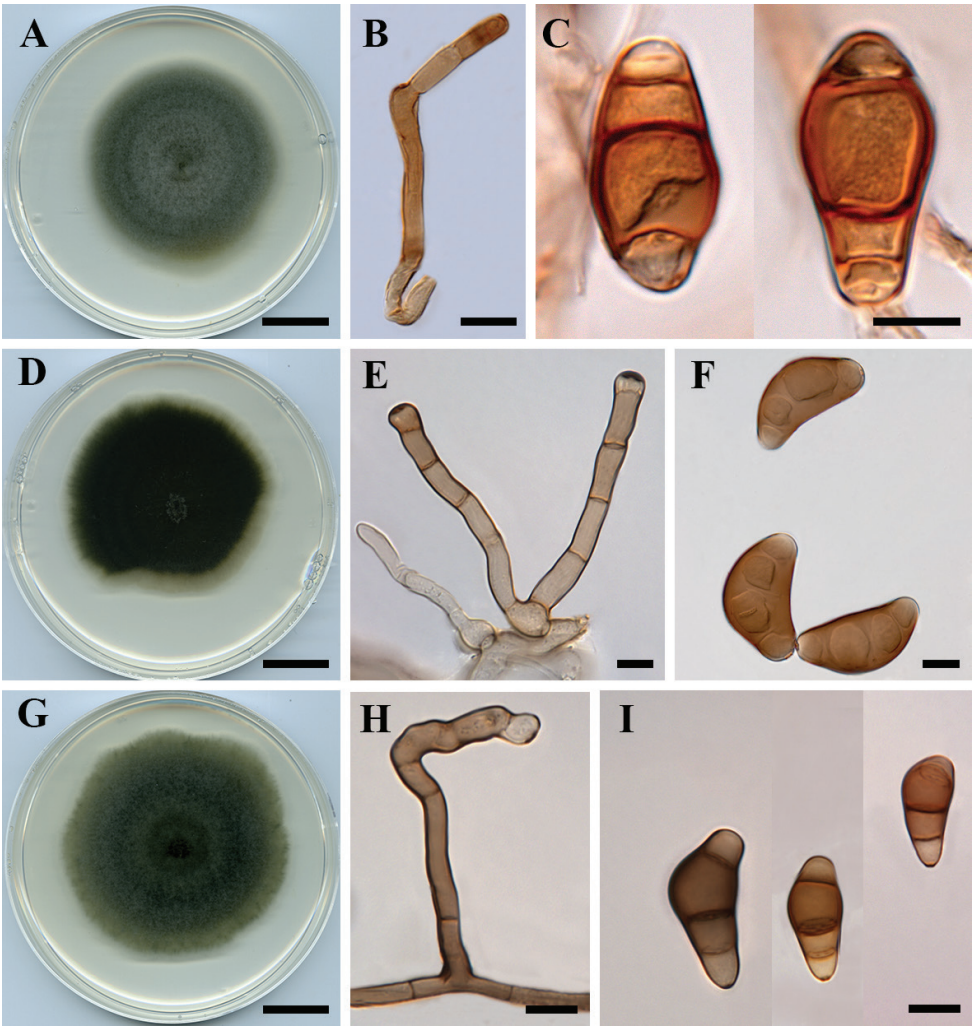


Figure 5. *Curvularia reesii* (BRIP 4358): **A** colony on PDA **B** conidiophore **C** conidia. *Curvularia sporobolicola* (BRIP 23040b) **D** colony on PDA **E** conidiophores **F** conidia. *Curvularia warraberensis* (BRIP 14817) **G** colony on PDA **H** conidiophore **I** conidia. Scale bars: 1 cm (**A, D, G**); all others – 10 µm.

third cell from pore swollen, brown, end cells paler, smooth, (28–) 31–35 (–39) × (10–) 12–13 (–14) µm, mostly 3 septate; hila inconspicuous, sometimes darkened.

Etymology. Named after Dr Robert (Bob) G. Rees, an Australian plant pathologist, in recognition of his extensive contributions to cereal pathology.

Notes. The multilocus phylogenetic analyses indicated *C. reesii* was sister to *C. oryzae* and *C. tuberculata*. *Curvularia reesii* is distinguished in two loci from the ex-type cultures of *C. oryzae* (98% in *gapdh* and 99% in *tef1a*) and *C. tuberculata* (96% in *gapdh* and 99% in *tef1a*). Morphologically, *C. reesii* has conidia similar in size to *C. oryzae* (24–40 × 12–22 µm, Sivanesan 1987) and *C. tuberculata* (23–52 × 13–20 µm, Sivanesan 1987). The isolate of *C. reesii* examined in this study had become sterile.

Curvularia sporobolicola **Y.P. Tan & R.G. Shivas, sp. nov.**
MycoBank MB825461
Fig. 5C–E

Type. AUSTRALIA, Queensland, Musselbrook Reserve, leaf of *Sporobolus australasicus*, 2 May 1995, *J.L. Alcorn* (holotype BRIP 23040b, includes ex-type culture).

Description. *Colonies* on PDA approx. 6 cm diam. after 7 d at 25 °C, surface funiculose, margin fimbriate, olivaceous black, velutinous. *Hyphae* subhyaline, smooth, branched, septate, 3 µm wide. *Conidiophores* erect, straight to flexuous, geniculate, pale yellowish-brown, septate, up to 55 µm long, 4–5 µm wide, basal cell swollen, 6–10 µm diam. *Conidiogenous cells* cylindrical, slightly flared at the apex, integrated, sympodial, pale brown, smooth, with darkened and thickened scars. *Conidia* hemi-ellipsoidal, curved, 4-distoseptate with a faint narrow median septum, (34–) 37–41 (–45) × (14–) 17–20 (–23) µm, brown to dark brown, end cells rounded and paler, hila non-protuberant, sometimes darkened.

Etymology. Named after *Sporobolus*, the grass genus from which it was isolated.

Notes. Based on multilocus phylogenetic analyses, *C. sporobolicola* clustered sister to *C. papendorfii*, which are both sister to *C. eragrosticola* (Fig. 1). *Curvularia sporobolicola* is distinguished in three loci from the ex-type cultures of *C. papendorfii* (99% in ITS, 96% in *gapdh* and 98% in *tef1a*) and *C. eragrosticola* (98% in ITS, 92% in *gapdh* and 98% in *tef1a*). These three species are similar in having dark brown, hemi-ellipsoidal, curved, conidia, which makes identification by morphology difficult. The conidia of *C. sporobolicola* tend to be wider than those of C. *eragrosticola* (25–35 × 9–19 µm, this study) and *C. papendorfii* (30–50 × 9–19 µm, Sivanesan 1987).

Curvularia sporobolicola is only known from the type specimen on *S. australasicus*, which is a native Australian grass with a broad distribution in the tropics and subtropics (Simon and Alfonso 2011). Other *Curvularia* species associated with *Sporobolus* include *C. australis*, *C. crustacea*, *C. eragrostidis*, *C. geniculata*, *C. lunata*, *C. ovariicola*, *C. pallescens*, *C. ravenelii* and *C. ryleyi* (Sivanesan 1987, Farr and Rossman 2018), although this is the first *Curvularia* species associated with *S. australasicus*.

Curvularia warraberensis **Y.P. Tan & R.G. Shivas, sp. nov.**
MycoBank MB825462
Fig. 5F–H

Type. AUSTRALIA, Queensland, Torres Strait, Warraber Island, from leaf spot on *Dactyloctenium aegyptium*, 2 Jun. 1985, *R.A. Peterson* (holotype BRIP 14817, includes ex-type culture).

Description. *Colonies* on PDA 6–7 mm diam. after 7 d at 25 °C, surface funiculose, margin fimbriate, olivaceous green, velutinous with some aerial mycelium. *Hyphae* subhyaline, smooth, septate, up to 3 µm wide. *Conidiophores* erect, flexuous, geniculate towards apex, uniformly pale brown to brown, septate, up to 360 µm long, 4–5 µm wide, basal cell sometimes swollen, 6–8 µm diam. *Conidiogenous cells* inte-

grated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic, with darkened scars. *Conidia* ellipsoidal, curved, the third cell from base swollen, end cells paler, smooth, (20–) 23–26 (–28) × (8–) 9.5–11 µm, pale brown to brown, 3-distoseptate, hila conspicuous, sometimes slightly protuberant, thickened and darkened.

Etymology. Named after the locality, Warraber Island in the Torres Straits, where the specimen was collected.

Notes. Multilocus phylogenetic analyses placed *C. warraberensis* sister to *C. caricae-papayae* and *C. prasadii* (Fig. 1). *Curvularia warraberensis* differs from the ex-type culture of *C. caricae-papayae* in *gapdh* positions 40 (C), 102 (C), 230 (A), 233 (C) and 321 (A) and from the ex-type culture of *C. prasadii* in two loci, *gapdh* positions 102 (C), 131 (C), 230 (A), 233 (C), 321 (A) and *tef1a* positions 214 (C), 337 (C), 542 (A), 543 (C), 685 (C). These three species belong to the *lunata*-clade sensu Madrid et al. (2014), which also includes *C. aeria*, *C. brachyspora*, *C. chlamydospora, C. lunata* and *C. pseudolunata*. All the species in the *lunata*-clade sensu Madrid et al. (2014) have 4-celled conidia in which the third cell from the base is often swollen (unequally sided and larger) and darker than the other cells. *Curvularia warraberensis* has longer conidiophores than *C. caricae-papayae* (up to 100 µm long, Srivastava and Bilgrami 1963) and longer conidia than *C. caricae-papayae* (12.8–18.0 × 6–8 µm) and *C. prasadii* (12.8–18.0 × 6–8 µm, Mathur and Mathur 1959).

Curvularia warraberensis is only known from the holotype. *Curvularia* species associated with *Dactyloctenium* are listed in the notes for *C. petersonii*.

Discussion

Although the ITS locus is the universal barcode marker for fungi (Schoch et al. 2012), secondary loci are often essential for the accurate identification of many helminthosporioid species (Manamgoda et al. 2012, 2015, Madrid et al. 2014, Tan et al. 2014, 2016, Stielow et al. 2015. Hernández-Restrepo et al. 2018). The protein-coding loci of *gapdh*, *tef1a* and RNA polymerase II second largest subunit (*rpb2*) have been reported as phylogenetically informative in the phylogenetic analyses of sequence data from species of *Curvularia* (Hernández-Restrepo et al. 2018, Manamgoda et al. 2014, Marin-Felix et al. 2017a, 2017b). In this study, sequences of three loci (ITS, *gapdh* and *tef1a*) from 17 cultures in BRIP were compared with those from ex-type cultures as well as published reference cultures for species of *Bipolaris* and *Curvularia*. The phylogenetic analyses of the concatenated three-locus dataset resolved the 17 BRIP isolates into 13 novel *Curvularia* species.

Eight *Curvularia* species are described here from grasses (Poaceae) exotic to Australia, namely, *C. beasleyi* on *Chloris gayana*, *C. beerburrumensis* on *Eragrostis bahiensis*, *C. eragrosticola* on *E. pilosa*, *C. kenpeggii* on *Triticum aestivum*, *C. mebaldsii* on *Cynodon dactylon* × *transvaalensis*, *C. petersonii* and *C. warraberensis* on *Dactyloctenium aegyptium* and *C. platzii* on *Cenchrus clandestinus*. Only two species were described

from native Australian grasses, *C. lamingtonensis* on *Microlaena stipoides* and *C. sporobolicola* on *Sporobolus australasicus*. Two species were described from other hosts, *C. coatesiae* from *Litchi chinensis* (Sapindaceae) and *C. colbranii* from *Crinum zeylanicum* (Amaryllidaceae). One species, *C. reesii*, was described from an isolate obtained from an air sample. Furthermore, DNA sequences derived from ex-type cultures have supported the generic placement of *C. neoindica* and the transfer of *Drechslera boeremae* to *Curvularia*.

It is not known whether the species described here are pathogens, endophytes or saprobes. It is also unclear as to whether these species are native or introduced. *Curvularia beasleyi* and *C. beerburrumensis* were both isolated from a native Australian grass species, as well as an exotic host. Some grass species have been reported to be associated with multiple *Curvularia* species, such as *Chloris* and *Cynodon*, with nine and 15 species, respectively. Many of the published records on *Chloris* and *Cynodon* have not been verified by molecular analyses. The number of new species described from non-Australian grasses indicates a need for a molecular-based reassessment of previous host-species records. The description of these species provides a foundation upon which additional sampling and accumulation of molecular data will improve knowledge of the host ranges and ecological roles of helminthosporioid fungi in Australia and overseas.

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RESEARCH ARTICLE

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Molecular and morphological evidence reveal a new genus and species in Auriculariales from tropical China

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Abstract

Grammatus labyrinthinus **gen. et sp. nov.** is proposed based on DNA sequences data and morphological characteristics. It is known so far from southern, tropical China. The new species is characterised by an annual, resupinate basidiocarp with a shallow, subporoid hymenophore, a hymenium restricted to the bottom of the tubes, a dimitic hyphal system, presence of encrusted skeletocystidia and dendrohyphidia, longitudinally septate basidia and smooth, oblong-ellipsoid to cylindrical, acyanophilous basidiospores. Phylogenetic analyses based on ITS + nLSU DNA sequences data indicate that *G. labyrinthinus* belongs to Auriculariaceae in which it has an isolated position. Phylogenetic inferences show *G. labyrinthinus* to be related to *Heteroradulum*. However, the ITS sequences similarity between *G. labyrinthinus* and *H. kmetii*, the type species of *Heteroradulum*, were 89.84% and support the establishment of the new genus. Inversely, *Heteroradulum semis* clustered with *G. labyrinthinus* with strong support and it is transferred to *Grammatus*.

Keywords

Grammatus labyrinthinus, ITS and nLSU, lignicolous fungi, phylogeny, taxonomy

Introduction

Auriculariales was established by Schroeter (1889) and originally accommodated species which, with transversely septate basidia, is also known as auricularioid basidia. Based on the micromorphology and ultra-structure of the septal pore and the spindle

pole body, Bandoni (1984) redefined Auriculariales, in order to accommodate all heterobasidiomycetes with continuous parenthesomes, transversely or longitudinally septate basidia and hyphal haploid stages. Currently, one family, Auriculariaceae is accepted and 198 species are distributed into 32 genera of Auriculariales (Kirk et al. 2008).

Auriculariaceae are diverse as long as their basidiocarp consistency (flesh gelatinous, wax-like and corky) and hymenophore structures (smooth, plicate, hydnoid and poroid) are concerned. According to the Dictionary of the fungi (10th edition), the family includes 7 genera: *Auricularia* Bull., *Eichleriella* Bres., *Elmerina* Bres., *Exidia* Fr., *Exidiopsis* (Bref.) Möller, *Fibulosebacea* K. Wells & Raitv., *Heterochaete* Pat. and 112 species (Kirk et al. 2008). Two other genera related to *Elmerina*, *viz*. *Aporpium* Bondartsev & Singer and *Protodaedalea* Imazeki, were also described. However, the phylogenetic position of the type species of *Elmerina* is still unclear (Sotome et al. 2014). The latest study of Auriculariales using ITS + nLSU DNA sequences data introduced or revalidated several new genera, *viz*. *Amphistereum* Spirin & V. Malysheva, *Sclerotrema* Spirin & V. Malysheva, *Heteroradulum* Lloyd and allowed the inclusion of *Exidia glandulosa* (Bull.) Fr., *Hirneolina hirneoloides* (Pat.) Pat., *Tremellochaete japonica* (Lloyd) Raitviir in Auriculariaceae (Malysheva and Spirin 2017).

Molecular phylogeny had been widely used to investigate phylogenetic relationships amongst the genera and species in Auriculariales (Swann and Taylor 1993, Berres et al. 1995, Weiß and Oberwinkler 2001, Kirschner and Chen 2004, Wells et al. 2004, Kirschner et al. 2010, 2012, Zhou and Dai 2013, Sotome et al. 2014, Malysheva and Spirin 2017). These phylogenetic contributions provided a general overview of Auriculariales and show strong support at the species level, but so far, not all the deeper nodes received high support from molecular evidence.

China is very rich in wood-decaying fungi and extensive studies on species diversity, taxonomy, ecology and phylogeny of wood-decaying fungi have been carried out recently (Yuan and Dai 2008; Dai 2010, 2011, 2012; Yuan et al. 2015). During a continuous survey of wood-decaying Basidiomycetes in the Yunnan Province, tropical China, a species of Auriculariaceae was collected but could not be confidently identified to any known species. The morphology characteristics suggested they represent an undescribed genus in Auriculariaceae. The aim of this paper is to clarify the taxonomic status of the genus and to describe new taxa.

Materials and methods

Morphological studies. Specimens are deposited at the herbarium of Institute of Applied Ecology, Chinese Academy of Sciences (IFP). Microscopic procedures follow Yuan and Qin (2018). The microscopic studies were made from sections mounted in Cotton Blue (CB): 0.1 mg aniline blue dissolved in 60 g pure lactic acid; CB+/– = cyanophilous / acyanophilous. Amyloid and dextrinoid reactions were tested in Melzer's reagent (IKI): 1.5 g KI (potassium iodide), 0.5 g I (crystalline iodine), 22 g chloral hydrate, aq. dest. 20 ml; IKI– = neither amyloid nor dextrinoid reaction. KOH (5%) was used as a

mounting reagent. Sections were studied at magnifications up to 1000× using a Nikon Eclipse E600 microscope and phase contrast illumination and dimensions were estimated subjectively with an accuracy of 0.1 μm. For spore measurements, the apiculus was excluded. In presenting the variation of spore size, 5% of the measurements at each end of the range are given in parentheses. The following abbreviations are used in the text: L = mean spore length, W = mean spore width, Q = range of length/width ratios for studied specimens and n = total number of spores measured from a given number of specimens. Special colour terms are from Petersen (1996).

Molecular procedures and phylogenetic analyses. The fungal taxa and strains used in this study are listed in Table 1. Phire Plant Direct PCR Kit (Finnzymes, Finland) procedure was used to extract total genomic DNA from the fruiting body and for the polymerase chain reaction (PCR). PCR amplification was confirmed on 1% agarose electrophoresis gels stained with ethidium bromide (Stöger et al. 2006). DNA sequencing was performed at Beijing Genomics Institute (BGI). All newly generated sequences have been submitted to GenBank and are listed in Table 1.

Nuclear ribosomal RNA genes were used to determine the phylogenetic position of the new species. The internal transcribed spacer (ITS) regions were amplified with the primers ITS4 and ITS5 and the partial nLSU regions were amplified with primers LR7 and LR0R (White et al. 1990). The most similar sequences were searched for in GenBank NCBI (http://www.ncbi.nlm.gov) using the BLAST option and downloaded (Table 1). Sequences were aligned using ClustalX (Thompson et al. 1997) and the alignment was deposited in TreeBASE (http://treebase.org/treebase-web/) (submission ID: 22496). Identity/similarity between two sequences was calculated using the BioEdit v. 7.2.6 (Hall 2005). Maximum parsimony (MP), Maximum likelihood (ML) and Bayesian inference were applied to the ITS + LSU dataset. All characters were weighted and gaps were treated as missing data. Maximum parsimony analysis (PAUP* version 4.0b10) was used (Swofford 2002). Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000 and no-increase, branches of zero length were collapsed and all parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Maximum likelihood (ML) analysis was performed in RAxML v8.2.4 with GTR + I + G model (Stamatakis 2014). The best tree was obtained by executing 100 rapid bootstrap inferences and, thereafter, a thorough search for the most likely tree using one distinct model/data partition with joint branch length optimisation (Stamatakis et al. 2008). Bayesian analysis with the latest version of MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003) implementing the Markov Chain Monte Carlo (MCMC) technique. The best-fit models (GTR + I + G) were selected by hLRT in MrModeltest 2.3 (Nylander 2004). Four simultaneous Markov chains were run starting from random trees and keeping one tree every 100th generation until the average standard deviation of split frequencies was below 0.01. The value of burn-in was set to discard 25% of trees when calculating the posterior probabilities. Bayesian posterior probabilities were obtained from the 50% majority rule consensus of the trees kept.

Table 1. DNA sequences used in the present study.

Species	Collector/herbarium number	ITS GenBank#	LSU GenBank#	Source
Amphistereum leveilleanum	Lentz FP-106715 (CFMR)	KX262119	KX262168	Malysheva and Spirin 2017
A. schrenkii	Burdsall 8476 (CFMR)	KX262130	KX262178	Malysheva and Spirin 2017
Aporpium hexagonoides	ML297 (TFM)	AB871754	AB871735	Sotome et al. 2014
Auricularia cornea	AU110	KF297960	KF297995	unpublished
A. fuscosuccinea	MW530	AB615231	AF291291	Weiß and Oberwinkler 2001
A. mesenterica	FO 25132	AF291271	AF291292	Weiß and Oberwinkler 2001
A. mesenterica	TUFC12805	AB915192	AB915191	Sotome et al. 2014
A. polytricha	TUFC12920	AB871752	AB871733	Sotome et al. 2014
Basidiodendron caesiocinereum	MW 320	–	AF291293	Weiß and Oberwinkler 2001
B. eyrei	MW 529	–	AF291296	Weiß and Oberwinkler 2001
B. eyrei	TUFC14484	AB871753	AB871734	Sotome et al. 2014
Bourdotia galzinii	FO 2278	–	AF291301	Weiß and Oberwinkler 2001
Ductifera pululahuana	KW 1733	–	AF291315	Weiß and Oberwinkler 2001
Eichleriella alliciens	Burdsall 7194 (CFMR)	KX262120	KX262169	Malysheva and Spirin 2017
E. bactriana	I. Parmasto (TAAM 96698)	KX262123	KX262172	Malysheva and Spirin 2017
E. bactriana	E. Parmasto (TAAM 104431)	KX262138	KX262186	Malysheva and Spirin 2017
E. crocata	E. Parmasto (TAAM 101077)	KX262100	KX262147	Malysheva and Spirin 2017
E. crocata	E. Parmasto (TAAM 125909)	KX262118	KX262167	Malysheva and Spirin 2017
E. desertorum	Ryvarden 49350 (O)	KX262142	KX262190	Malysheva and Spirin 2017
E. flavida	Ryvarden 49412 (H)	KX262137	KX262185	Malysheva and Spirin 2017
E. leucophaea	Barsukova (LE 303261)	KX262111	KX262161	Malysheva and Spirin 2017
E. leucophaea	Larsson 15299 (O)	KX262136	KX262184	Malysheva and Spirin 2017
E. shearii	USJ 54609	AF291284	AF291335	Weiß and Oberwinkler 2001
E. sicca	Miettinen 17349 (H)	KX262143	KX262191	Malysheva and Spirin 2017
E. tenuicula	Ryvarden 17599 (O)	KX262141	KX262189	Malysheva and Spirin 2017
Elmerina caryae	WD2207	AB871751	AB871730	Sotome et al. 2014
E. caryae	Dai 4549	JQ764652	JQ764631	Zhou and Dai 2013
E.cladophora	Wei 5621	JQ764659	JQ764634	Zhou and Dai 2013
E. dimidiata	O18238	JQ764663	JQ764640	Zhou and Dai 2013
E. dimidiata	O18261	JQ764664	JQ764641	Zhou and Dai 2013
E. efibulata	Dai 9322	JQ764669	JQ764647	Zhou and Dai 2013
E. foliacea	Yuan 5691	JQ764666	JQ764644	Zhou and Dai 2013
E. hispida	WD548 (TFM)	AB871768	AB871749	Sotome et al. 2014
E. hispida	E701	AB871767	AB871748	Sotome et al. 2014
E. hispida	Wei 5584	JQ764667	JQ764645	Zhou and Dai 2013
Exidia glandulosa	TUFC 34008	AB871761	AB871742	Sotome et al. 2014
E. glandulosa	MW 355	AF291273	AF291319	Weiß and Oberwinkler 2001
E. pithya	MW 313	AF291275	AF291321	Weiß and Oberwinkler 2001
E. uvapsassa	AFTOL-ID 461	DQ241776	AY645056	unpublished
Exidiopsis calcea	MW 331	AF291280	AF291326	Weiß and Oberwinkler 2001
E. effusa	Miettinen 19136 (H)	KX262145	KX262193	Malysheva and Spirin 2017

Species	Collector/herbarium number	ITS GenBank#	LSU GenBank#	Source
E. grisea	RK 162	AF291281	AF291328	Weiß and Oberwinkler 2001
*E. gris*ea	TUFC100049	AB871765	AB871746	Sotome et al. 2014
E. sp.	TUFC34333	AB871764	AB871745	Sotome et al. 2014
E. sp.	FO 46291	AF291282	AF291329	Weiß and Oberwinkler 2001
Grammatus labyrinthinus	Yuan 1759	KM379137	KM379138	This study
G. labyrinthinus	Yuan 1600	KM379139	KM379140	This study
Heterochaete andina	Lagerheim (FH, lectotype)	–	KX262187	Malysheva and Spirin 2017
H. delicata	TUFC33717	AB871766	AB871747	Sotome et al. 2014
Heterochaetella brachyspora	RK 96	–	AF291337	Weiß and Oberwinkler 2001
Heteroradulum adnatum	Ryvarden 23453 (O)	KX262116	KX262165	Malysheva and Spirin 2017
H. deglubens	LE 38182	KX262112	KX262162	Malysheva and Spirin 2017
H. deglubens	TAAM 064782	KX262101	KX262148	Malysheva and Spirin 2017
H. kmetii	Kmet (H, lectotype)	KX262124	KX262173	Malysheva and Spirin 2017
H. kmetii	Spirin 6466 (H)	KX262104	KX262152	Malysheva and Spirin 2017
H. semis	Miettinen 10618.1 (H)	KX262146	KX262194	Malysheva and Spirin 2017
Myxarium grilletii	RK 218	–	AF291349	Weiß and Oberwinkler 2001
M. nucleatum	ZP TRE2M	–	AF291351	Weiß and Oberwinkler 2001
Protodontia subgelatinosa	USJ 54661	–	AF291357	Weiß and Oberwinkler 2001
Protomerulius africanus	Ryvarden 9800 (O)	–	AF291358	Weiß and Oberwinkler 2001
Pseudohydnum gelatinosum	MW 298	–	AF291360	Weiß and Oberwinkler 2001
Sclerotrema griseobrunneum	Niemelä 2722 (H)	KX262144	KX262192	Malysheva and Spirin 2017
Sistotrema brinkmannii	Isolate 236	JX535169	JX535170	GenBank
Tremellochaete japonica	LE 303446	KX262110	KX262160	Malysheva and Spirin 2017
Tremellodendropsis sp.	USJ 54427	–	AF291375	Weiß and Oberwinkler 2001
Tremiscus helvelloides	MW 337	–	AF291377	Weiß and Oberwinkler 2001

Results

Phylogenetic analyses

The combined ITS + nLSU sequence dataset includes the new species and other related species in Auriculariales. *Sistotrema brinkmannii* was used as outgroup (Malysheva and Spirin 2017). The data matrix comprised 1413 base pairs with 818 constant characters, 206 parsimony-uninformative variable characters and 389 parsimony informative positions. Maximum parsimony analysis was performed and a strict consensus tree was obtained from the 2 equally most parsimonious trees. The same dataset and alignment was analysed using RAxML v8.2.4 and MrBayes 3.2.6 with the best-fit model (GTR + I + G) selected by MrModeltest 2.3 and a similar topology was generated and the maximum likelihood tree is shown in Fig. 1. Bayesian analysis ran 4 million generations and resulted in average standard deviation of split frequencies = 0.008219. In the phylogenetic tree, two sampled specimens of *Grammatus labyrinthinus* group together with full support and form a monophyletic lineage with *Heteroradulum semis* with strong support (97 % in ML, 100 % in MP and 1.00 BPP). The new taxon belongs to Auriculariales in which it has an isolated position.

Taxonomy

Grammatus **H.S. Yuan & C. Decock, gen. nov.**
MycoBank no.: MB825392

Notes. Basidiocarps annual, resupinate; hymenophoral surface hydnoid, irregularly poroid to labyrinthine, hymenium restricted to the area surround the spines or the bottom of the tubes; Hyphal system dimitic; skeletocystidia heavily encrusted in trama; dendrohyphidia thin- to slightly thick-walled; basidia longitudinally septate; basidiospores thin-walled, smooth, oblong-ellipsoid to cylindrical.

Type species. *Grammatus labyrinthinus* H.S. Yuan & C. Decock.

Etymology. *grammatus*: referring to the hymenophore striped with raised lines.

Basidiocarps annual, resupinate, coriaceous; hymenophoral surface cream to pale buff, covered by evenly distributed blunt-pointed spines or irregularly irpicoid to subporoid, then developing into labyrinthiform to sinuous pores; hymenium restricted to the area surrounding the spines or the bottom of the tubes. Subiculum very thin. Spine or tubes corky, concolorous with hymenophoral surface, shallow. Hyphal system dimitic; generative hyphae bearing clamp connections; skeletal hyphae IKI–, CB+; tissue unchanged in KOH. Skeletocystidia clavate, upper part heavily encrusted. Dendrohyphidia present. Basidia subglobose, longitudinally septate. Basidiospores oblong-ellipsoid to cylindrical, hyaline, thin-walled, smooth, IKI–, CB–.

Grammatus labyrinthinus **H.S. Yuan & C. Decock, sp. nov.**
MycoBank no.: MB825393
Figures 3–4

Diagnoses. Basidiocarps annual, resupinate; hymenium restricted to the base of the tubes. Hymenophoral surface irregularly irpicoid to subporoid, then labyrinthine to sinuous. Subiculum very thin. Tubes shallow. Hyphal system dimitic; generative hyphae bearing clamp connections; skeletal hyphae IKI–, CB+. Skeletocystidia clavate, the upper part heavily encrusted. Dendrohyphidia present, thin- to slightly thick-walled. Basidia subglobose, longitudinally septate. Basidiospores oblong-ellipsoid to cylindrical, hyaline, thin-walled, smooth, IKI–, CB–.

Type. China. Yunnan Province, Xishuangbanna, Jinghong County, Nabanhe Nat. Res., fallen angiosperm branch, 17.VIII.2005 *Yuan 1759* (holotype: IFP 019121).

Etymology. *labyrinthinus* (Lat.): refers to labyrinthine hymenophore.

Basidiocarps annual, resupinate, coriaceous, without special odour or taste when fresh, corky when dry, up to 15 cm long, 3 cm wide and 0.2 mm thick. Hymenophoral surface cream to pale buff when fresh, cinnamon-buff to yellowish-brown upon drying, firstly irregularly irpicoid to subporoid, the separate plates grow laterally and then develop into labyrinthine to sinuous pores, mostly 4–5 per mm, dissepiments thin; sterile margin up to 0.2 mm wide, pale yellow. Subiculum very thin (ca. 0.1

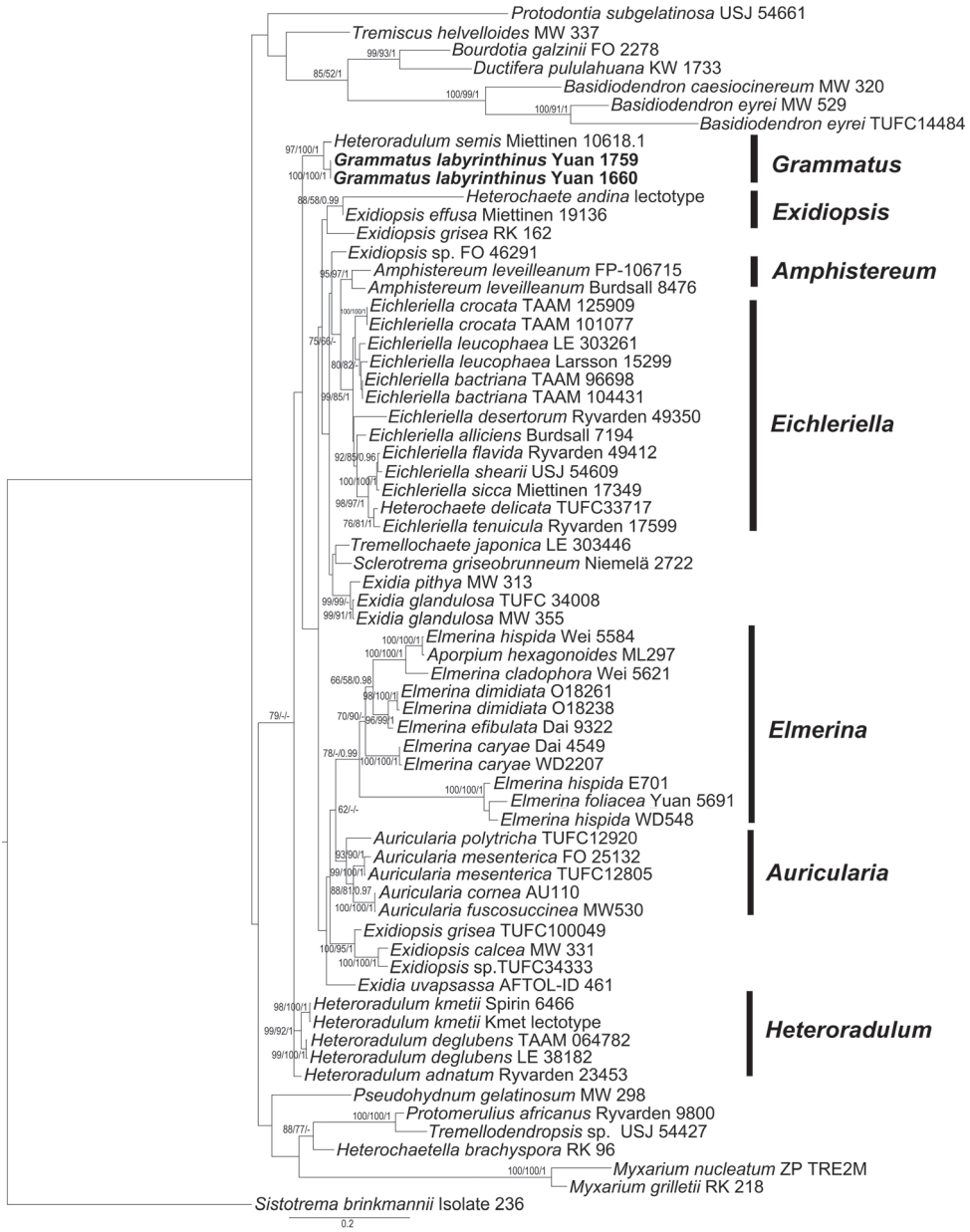

Figure 1. Maximum likelihood tree illustrating the phylogeny of *Grammatus labyrinthinus* and related taxa in Auriculariales, based on the combined ITS + nLSU sequence dataset. Branches are labelled with maximum likelihood bootstrap higher than 50%, parsimony bootstrap proportions higher than 50% and Bayesian posterior probabilities more than 0.95.

mm thick), cream to pale buff. Tubes corky, concolorous with pore surface, shallow, up to 130 µm deep, tube walls 120–200 µm thick. Hymenium restricted to the base of the tubes.

Figure 2. Basidiocarps of *Grammatus labyrinthinus* (*Yuan 1734*).

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

Subiculum. Dominated by skeletal hyphae; generative hyphae hyaline, thin-walled, rarely branched, 1.5–2.8 µm diam; skeletal hyphae hyaline, thick-walled to subsolid, straight to flexuous, covered by fine crystals, occasionally branched, interwoven, 1.8–3 µm diam.

Tubes. Generative hyphae infrequent, hyaline, thin-walled, rarely branched, 1.5–2.5 µm diam; skeletal hyphae dominant, hyaline, thick-walled to subsolid, moderately branched, interwoven, 1.8–2.8 µm diam. Skeletocystidia numerous, clavate, thick-walled, originating from and tightly embedded in trama, upper part heavily encrusted, 10–30 × 4–8 µm (with encrustation). Dendrohyphidia present, especially along the dissepiments, arising from generative hyphae, thin- to slightly thick-walled, apically moderately to strongly branched. Basidia subglobose, longitudinally septate, already septate as probasidia, 18–25 × 10–13 µm, epibasidia divided into four parts up to 20 µm long, bearing four sterigmata and without clamp connection at the base, sterigmata up to 20 µm long.

Basidiospores. Oblong-ellipsoid to cylindrical, hyaline, thin-walled, smooth, IKI–, CB–, (13–)13.3–15.7(–16) × (6–)6.4–7.4(–7.7) µm, L = 14.4 µm, W = 6.94 µm, Q = 2.07–2.1 (n = 60/2).

Type of rot. White rot.

Additional specimens examined – **China.** Yunnan Province, Xishuangbanna, Jinghong County, Elephant Valley Forest Park, fallen angiosperm branch, 14.VIII.2005

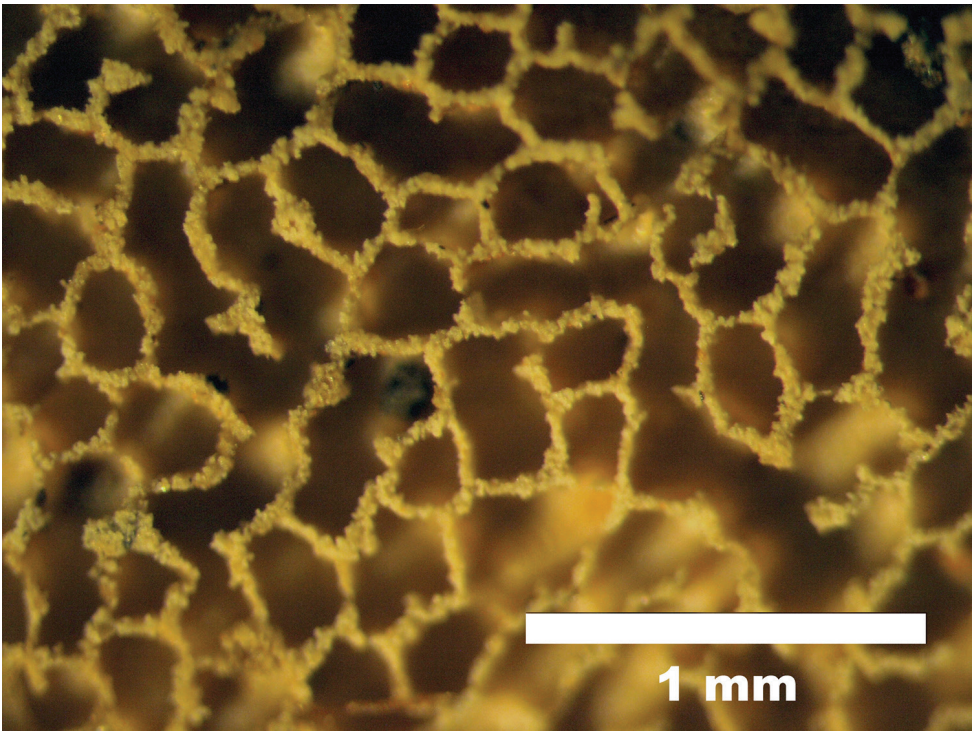

Figure 3. Hymenophoral surface of *Grammatus labyrinthinus* under ×8 lens (holotype).

Yuan 1600 (IFP 019118); Nabanhe Nat. Res., fallen angiosperm branch, 15.VIII.2005 *Yuan 1683* (IFP 019119); fallen angiosperm branch, 17.VIII.2005 *Yuan 1734* (IFP 019120).

***Grammatus semis* (Spirin & Malysheva) H.S. Yuan & C. Decock, comb. nov.**
MycoBank MB825394

Basionym. *Heteroradulum semis* Spirin & Malysheva, in Malysheva & Spirin, Fungal Biology 121: 712. 2017.

Discussion

Anatomically, the longitudinally septate basidia of *Grammatus labyrinthinus* point toward affinities with Auriculariales, which is confirmed by molecular data. The new taxa are phylogenetically closely related to *Heteroradulum*. *Heteroradulum kmetii*, type of the genus, has perennial, effused-reflexed and pinkish or reddish basidiocarps with hymenial surface first smooth then with irregularly arranged, sharpened outgrowths (Malysheva and Spirin 2017), in which feature, it differs from *G. labyrinthinus*. The

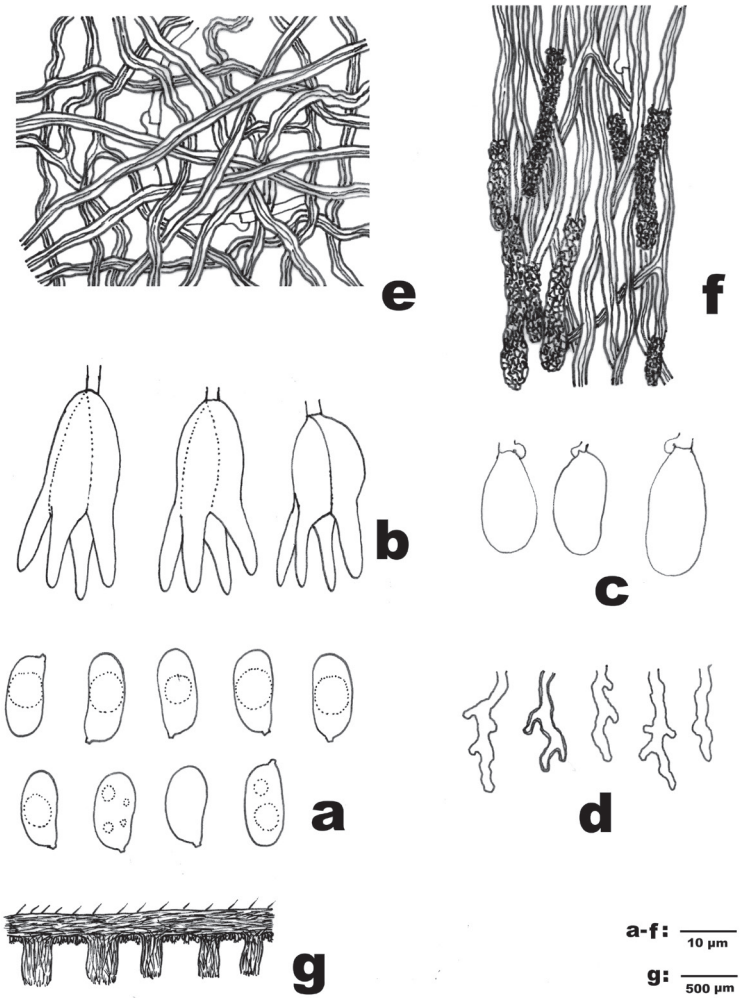

Figure 4. Microscopic structures of *Grammatus labyrinthinus* (drawn from the holotype). **a** Basidiospores **b** Probasidia **c** Probasidia transection **d** Epibasidia **e** Dendrohyphidia **f** Hyphae from subiculum **g** Hyphae from trama **h** Basidiocarp transection.

similarity between the ITS sequences of *G. labyrinthinus* and *H. kmetii* are of 89.84%. The unique morphological characteristics and molecular sequence analyses both support the establishment of the new genus.

Heteroradulum semis was originally found and described from high elevation temperate north-eastern China. It is characterised by resupinate, leathery basidiocarps covered by blunt-pointed spines, a dimitic hyphal structure with clamped generative hyphae, encrusted tramal skeletocystidia, simple or sparsely branched dendrohyphidia, longitudinally septate basidia and broadly cylindrical to narrowly obovate basidiospores (Malysheva and Spirin 2017). The sterile and blunt-pointed outgrowths on the hymenial surface of *H. semis* identify the irregularly irpicoid and separate plates

of *G. labyrinthinus* when young, but the irregularly irpicoid and separate plates of *G. labyrinthinus* would form the labyrinthine or subporoid structure when old and can be distinguished from the former species. Phylogenetic analyses confirm that *H. semis* clustered with *G. labyrinthinus* with strong support. So it is transferred to *Grammatus* and a new combination, *G. semis* is proposed.

Aporpium, *Elmerina* and *Protomerulius* Möller all have a poroid hymenophore (Ryvarden and Johansen 1980, Núñez and Ryvarden 2001, Sotome et al. 2014). However, they are all distant from *Grammatus labyrinthinus* in phylogenetic inferences (Fig. 1).

There are 216 genera and more than 1800 species of wood-inhabiting fungi in Polyporales (Kirk et al 2008) with high diversity of hymenophore structure from smooth, hydnoid, lamellate and poroid. Amongst the poroid taxa, *Grammothele* Berk. & M.A. Curtis, *Hymenogramme* Mont. & Berk., *Porogramme* (Pat.) Pat. and *Thelepo-rus* Fr. are characterised by a hymenium restricted to the bottom of the pores, which differentiate them from the other typical polypores. These genera are characterised by non-septate basidia and are members of Polyporales. In comparison, Auriculariales are relatively poor in genera and species, but still, their hymenophore structures are diverse. *Grammatus labyrinthinus* is the representative of poroid species with a hymenium restricted to the bottom in Auriculariales. It is another instance of morphological convergent evolution across the order.

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Short-spored *Subulicystidium* (Trechisporales, Basidiomycota): high morphological diversity and only partly clear species boundaries

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Abstract

Diversity of corticioid fungi (resupinate Basidiomycota), especially outside the northern temperate climatic zone, remains poorly explored. Furthermore, most of the known species are delimited by morphological concepts only and, not rarely, these concepts are too broad and need to be tested by molecular tools. For many decades, the delimitation of species in the genus *Subulicystidium* (Hydnodontaceae, Trechisporales) was a challenge for mycologists. The presence of numerous transitional forms as to basidiospore size and shape hindered species delimitation and almost no data on molecular diversity have been available. In this study, an extensive set of 144 *Subulicystidium* specimens from Paleo- and Neotropics was examined. Forty-nine sequences of ITS nuclear ribosomal DNA region and 51 sequences of 28S nuclear ribosomal DNA region from fruit bodies of *Subulicystidium* were obtained and analysed within the barcoding gap framework and with phylogenetic Bayesian and Maximum likelihood approaches. Eleven new species of *Subulicystidium* are described based on morphology and molecular analyses: *Subulicystidium boidinii*, *S. fusisporum*, *S. grandisporum*, *S. harpagum*, *S. inornatum*, *S. oberwinkleri*, *S. parvisporum*, *S. rarocrystallinum*, *S. robustius*, *S. ryvardenii* and *S. tedersooi*. Morphological and DNA-evidenced borders were revised for the five previously known species: *S. naviculatum, S. nikau*, *S. obtusisporum*, *S. brachysporum* and *S. meridense*. Species-level variation in basidiospore size and shape was estimated based on systematic measurements of 2840 spores from 67 sequenced specimens. An updated identification key to all known species of *Subulicystidium* is provided.

Keywords
basidiospores, biodiversity, biometry, crystals, cystidia, DNA barcode, encrustation, genetic distance, internal transcribed spacer, large subunit, species delimitation, taxonomy

Introduction

The genus *Subulicystidium* was created by Parmasto (1968) to accommodate corticioid fungi with long subulate or sword-like cystidia with a unique morphology. The smooth thick crystalline sheath of cystidia is covered with two chains of the bow-tie-shaped crystals, which are seen in the light microscope as four chains of rectangular crystals along the cystidium body (Jülich 1975, Keller 1985). Other morphological characters of the genus are resupinate arachnoid fruit-bodies, loosely interwoven hyphae with constant clamps and suburniform basidia (Oberwinkler 1977, Duhem and Michel 2001). Repetobasidia were also noted by some authors (Jülich 1968, Liberta 1980). The genus belongs to the order Trechisporales K.H.Larss., though its relationship with the other genera within the family Hydnodontaceae Jülich remains unclear (Larsson 2007, Telleria et al. 2013). Fruit-bodies of *Subulicystidium* are found on decayed wood or other plant debris at the forest floor but exact nutrition mode of the genus is not known (Hibbett et al. 2014).

Currently, nine species are recognised based on morphological features (Index Fungorum 2018). The generitype *S. longisporum* (Pat.) Parmasto is often reported and mapped in mycodiversity surveys worldwide (e.g. see Subulicystidium 2017). In contrast, other species are still known either from the type locality only (*S. curvisporum* Gorjón, Gresl. & Rajchenb.) or from a few localities: *S. brachysporum* (P.H.B. Talbot & V.C. Green) Jülich, *S. cochleum* Punugu, *S. meridense* Oberw., *S. naviculatum* Oberw., *S. nikau* (G. Cunn.) Jülich and *S. obtusisporum* Duhem & H. Michel (Punugu et al. 1980, Gorjón et al. 2012). Some species records represent more than one continent but in all cases these reports are based on a morphological species concept (Boidin and Gilles 1988, Duhem and Michel 2001, Volobuev 2016).

Species delimitation in *Subulicystidium* has remained challenging. Basidiospore size and shape were traditionally used as the main discriminating characters, while other microscopic structures of fruit-bodies were considered as generally invariable (Oberwinkler 1977, Boidin and Gilles 1988, Duhem and Michel 2001). However, overlap of spore size between species is reported, as well as high morphological variability of the spores within single collections (Liberta 1980, Hjortstam and Ryvarden 1986). This has led to doubts on the identity of some taxa. For example, Liberta (1980) regarded *S. longisporum* as a highly variable "species complex" and that *S. brachysporum* (P.H.B. Talbot & V.C. Green) Jülich and *S. meridense* Oberw. should not be accepted until additional data for species limit evaluation became available (Oberwinkler 1977, Liberta 1980).

Despite the general progress in molecular identification of fungi during the last three decades (Kõljalg et al. 2013), almost no data on the genetic diversity within

Subulicystidium have been published and the genus remains poorly represented in all kinds of molecular studies. Currently available public sequences are usually identified to genus level only or even just named "Trechisporales". Public sequences from fungal fruit-bodies annotated to the species level are few (Volobuev 2016).

During recent decades, extensive collections of *Subulicystidium* were made by us in Paleo- and Neotropics. In this paper, 11 new species of *Subulicystidium* are reported based on morphological and molecular evidence (similarities and phylogenies based on rDNA ITS and 28S sequences). The concepts of five previously known species are clarified and the possibility of species presence on several continents is verified. In the current study, we focus on rich material with relatively short basidiospores, i.e. non-acicular and often less than 10 μm long, thus leaving out *S. longisporum*-like material for a future study.

Materials and methods

Assembling dataset

In this study, we examined 144 herbarium specimens of the genus *Subulicystidium*, which were collected in several regions of Paleotropics (Réunion Island, Madagascar, Africa, South-East Asia) and Neotropics (Caribbean region, various countries of South America). This material was collected during the last six decades, with the oldest collection (PDD13816) from 1954 and the most recent ones from 2015 (e.g. KAS:L 1860). Collections are preserved in the following herbaria: O (Natural History Museum, Oslo University, Norway), GB (Gothenburg University, Sweden), MG (Museu Paraense Emílio Goeldi, Belém, Brasil), SP (Instituto de Botânica, São Paulo, Brasil), KAS (University of Kassel, Germany), FR (Senckenberg Research Institute and Natural History Museum, Frankfurt am Main, Germany) and LY (University of Lyon, France). We examined also holotype specimens of *Subulicystidium meridense* Oberw. (TUB, Tübingen University, Germany), *S. nikau* (G. Cunn.) Jülich (PDD, New Zealand Fungal Herbarium, Landcare Research, Auckland) and the collection of *S. allantosporum* Boidin and Gilles ad interim (Boidin and Gilles 1988) from LY. Attempts to obtain the type specimen of *S. brachysporum* from PREM (Plant Protection Research Institute, Queenswood, South Africa) were not successful.

For a better biodiversity data availability and reusability, in Suppl. material 1, the table with detailed information on all 144 specimens examined is provided. If missing on the original specimen labels, data on higher-and lower rank administrative units were mined and added to the corresponding columns in Suppl. material 1. The table also includes geographic coordinates for each specimen in decimal degrees format (DD) with minus signs used to indicate southern and western hemispheres. Originally, geographic coordinates were available for 65 specimens. For the other 69 specimens, an attempt to estimate the coordinates from a map was made using resources Google Maps (via http://www.gpskoordinaten.de/), OpenStreetMap (https://www.openstreet-

map.org) and the georeferencing calculator of Wieczorek and Wieczorek (2015). In ten specimens, the locality was not precisely indicated to estimate the coordinates. The manner, in which coordinates were obtained, was specified for each specimen in the Suppl. material 1.

Field data and photos of recent collections from Réunion Island (stored in FR and KAS) are accessible via PlutoF workbench (Abarenkov et al. 2010) under the project "Ordynets_Fungi of Reunion Island" and as a part of GBIF occurrence dataset of the Senckenberg herbarium FR (Senckenberg 2018).

Analysing microscopic traits

Sections from dried herbarium specimens were examined in 3% aqueous solution of potassium hydroxide (KOH) mixed with 1% aqueous solution of Phloxine, using 100× immersion oil lens of a Leica DM500 light microscope. Images were captured with a built-in ICC 50 HD Camera using Leica Application Suite EZ V.3.2.1 software (Leica Microsystems Ltd., Switzerland). Measurements were done with the software "Makroaufmaßprogramm" from Jens Rüdigs (https://ruedig.de/tmp/messprogramm.htm) and analysed with the software "Smaff" version 3.2 (Wilk 2012). At least 30 basidiospores per specimen were measured where possible for the sequenced specimens or otherwise important collections. When referring to the basidiospore measurement results, abbreviation L was used for the spore length, W for the spore width, Q for the length to width ratio and N x/y for the x number of spores measured from y specimens.

The raw spore measurements were undertaken as follows. First, for each collection, automated search for size outliers was performed with the "Smaff" software (Wilk 2012). To account for the outliers in both spore length and width simultaneously, the parameters of (i) length multiplied by width and (ii) volume were calculated for each spore by the programme. These values, (i) followed by (ii), were checked to represent the outliers in the sample on a 95% probability level, using simultaneously the tests of Verma and Quiroz-Ruiz (2006), David et al. (1954) and Grubbs (1950). Upon their detection, outliers were excluded from the sample as recommended by Wilk (2012). This procedure usually resulted in a better fit of the spore measurements to the normal distribution. The spore measurements after excluding outliers are provided in the Suppl. material 2.

These filtered spore measurements were used to calculate the spore size range of the species. The main range was presented as the interval into which 90% of non-outlier measurements fall, while 5% of the smallest and 5% of the largest non-outlier measurements were included in parentheses. For the species with more than one specimen available, the filtered spore measurements were pooled together and the main range (90% of the data) with 5% of the smallest and 5% of the largest values were defined for this pooled sample. Calculations were done in R version 3.3.3 (R Core Team 2017) and script is available from Ordynets and Denecke (2018). Additionally, for species with at least three sequenced specimens, hypothetical intervals were calculated within

which 90% of all existing individuals' specimen mean values lie. This way of representing basidiospore size variability in species was highly recommended by Parmasto and Parmasto (1987) and Raitviir (1972). These 90% tolerance intervals were calculated for the 90% probability level, with the method of Howe (1969) implemented in the "normtol.int" function of the "tolerance" R package version 1.3.0 (Young 2010). R script for these calculations is available in Ordynets (2018b).

At least 10 basidia and cystidia were measured per specimen and their size variation was presented simply as the range between minimum and maximum values for the pooled measurements of all collections belonging to one species. When basally swollen cystidia was a regular feature, both the largest diameter at the place of swelling and the diameter next to the swelling were noted. The protruding bow-tie crystals were included in the measurements of cystidium diameter. The shape of the cystidium apex followed terminology for sterile hymenial elements of Yurchenko and Wu (2016) and included the following options: tapering, acute and acuminate. Cystidial ornamentation was described as seen under the light microscope.

DNA extraction, amplification and sequencing

Sequences of two nuclear ribosomal DNA regions were considered in our study: internal transcribed spacer (ITS) and ribosomal large subunit-coding DNA (28S). Sequences were obtained from dried herbarium specimens. Total DNA was isolated according to the protocol of Izumitsu et al. (2012). For that, 1-2 mg of fungal fruit body tissue were suspended in 100 µl TE buffer in a 1.5 ml tube. The tubes were microwaved (700 Watt) for 1 min two times, with a 30 seconds pause while keeping the tubes at room temperature. Tubes were cooled at -20°C for 20 min and centrifuged at 10000 rpm for 5 min. The supernatants were 10 or 100 times diluted and in this form used in PCR.

Primer pairs used to amplify the complete ITS region were ITS1F/ITS4, ITS1/ITS4 and ITS1/ALR0 (White et al. 1990, Gardes and Bruns 1993, Collopy et al. 2001). The D1–D2 domains at the 5' end of 28S were amplified with primer pairs NL1/NL4 (O'Donnell 1992) and less frequently with LR0R/LR5 (Hopple and Vilgalys 1999). PCRs of the collections from Réunion Island (FR and KAS herbaria) were performed as explained in Ordynets et al. (2015). The PCR for the remaining material was performed on 53 µl solution containing 5 µl of extracted DNA and 48 µl Master Mix (BIOLINE GmbH, Luckenwalde, Germany). One Master mix portion contained 30.2 µl H_2O, 10 µl reaction buffer (30 mM $MgCl_2$) coloured with red and orange dyes, 2 µl $MgCl_2$ (50 mM), 2 µl dNTPs (6 mM), 2 µl Bovine Serum Albumin (20 µg/µl), 0.8 µl of each forward and reverse primers (25 pM) and 0.2 µl Mango-Taq DNA Polymerase (5 units/µl).

Amplifications were performed in 96-well TGradient Thermocycler (Biometra, Göttingen, Germany). PCR with primer pairs ITS1F/ITS4, ITS1/ALRO and NL1/NL4 was set as initial denaturation at 94°C for 3 min followed by 29 cycles of dena-

turation 94°C for 30 s, annealing 55°C for 45 s and extension 72°C for 60 s; final elongation was done at 72°C for 7 min. PCR with primer pair LR0R/LR5 differed only in having the annealing temperature as 48°C.

PCR products were checked on 1% agarose gel stained with GelRed fluorescence dye (BIOTIUM, Hayward, CA, USA) in the Transilluminator Biometra Ti5 equipped with BioDocAnalyze software (Biometra GmbH, Göttingen, Germany). PCR products were cleaned with QIAquick PCR Purification Kit according to manufacturer's instructions (QIAGEN GmbH, Hilden, Germany). Sanger sequencing of purified products was performed in the facilities of the Senckenberg Research Institute and Natural History Museum (Frankfurt am Main, Germany) and by the company GATC Biotech AG (Constance, Germany). The primers used for sequencing were identical to those used for amplification.

The oldest specimen we succeeded to sequence, with regard to both ITS and 28S regions, was from the year 1978 (LR 15483 in O:F 918488). Attempts of DNA amplification from the type specimens of *S. meridense*, *S. nikau* and *S. allantosporum* Boidin ad interim (Boidin and Gilles 1988) were not successful, as well as an attempt to sequence the type specimen of the new species *Subulicystidium ryvardenii* Ordynets, Langer & K.H. Larss. sp. nov. We did not succeed in amplifying two protein-coding genes from any of the specimens: a partial segment (511 bp) of the translation elongation factor 1α (TEF1α) with EF-595f and EF-1160r primer pair (Kauserud and Schumacher 2001), as well as the largest subunit of RNA polymerase II gene (RPB1) with RPB1-Af and RPB1-Cr primer pair (Matheny et al. 2002).

DNA sequence-based analyses

All sequences obtained in this study went through the standard quality assessment steps outlined by Nilsson et al. (2012). Raw sequence data were processed with Geneious version 5.6.7 (http://www.geneious.com, Kearse et al. 2012). For various sequence format conversions and alignment viewing, Mesquite version 3.40 (Maddison and Maddison 2018), AliView version 1.19 (Larsson 2014) and Seaview version 4 (Gouy et al. 2010) were used.

In this study, 49 sequences of ITS rDNA region and 51 sequences of 28S rDNA region of *Subulicystidium* were generated and submitted to GenBank (Benson et al. 2013). They are available as accessions MH041511-MH041559 for ITS and MH041560-MH041610 for 28S region. Additional ten ITS and six 28S sequences of *Subulicystidium*, earlier available in GenBank and UNITE database (Kõljalg et al. 2013), were downloaded and used in our analyses. Finally, we included sequences of *Brevicellicium exile* (H.S. Jacks.) K.H. Larss. & Hjortstam and *B. olivascens* (Bres.) K.H. Larss. & Hjortstam to serve as an outgroup in our sequence-based analyses. All sequences used in the study are listed with brief metadata in Table 1.

Sequences from each locus, ITS and 28S, were pre-aligned in Geneious version 5.6.7 (Kearse et al. 2012) with MUSCLE algorithm (eight iterations) (Edgar 2004).

Table 1. Ribosomal DNA sequences used in this study with information on voucher specimens. Most sequences are newly generated for this study and ITS and 28S region were sequenced separately. For specimens GB:KHL 14229 and 16100 and TU 124388, single accession number in each case refers to a sequence containing both ITS and 28S regions. Sequences retrieved from other studies are marked with an asterisk. Abbreviation "na" means sequence is not available. In the species *S. brachysporum,* "B" means morphological species concept following Boidin and Gilles (1988), while "T" means the species as described by Talbot (1958).

Species	Locality	Voucher specimen	Collector(s)	GenBank/UNITE accession numbers	
				ITS	28S
Subulicystidium boidinii	Costa Rica: Puntarenas	GB:KHL 12830	K.-H. Larsson	MH041537	MH041570
S. boidinii (holotype)	Reunion: Saint-Benoit	KAS:L 1584a	M. Striegel	MH041527	na
S. brachysporum B	Argentina: Misiones	O:F: 506782	L. Ryvarden	MH041518	MH041572
S. brachysporum B	Brazil: Paraiba	O:F: KHL 16100	K.-H. Larsson	MH000599*	MH000599*
S. brachysporum B	Brazil: Rondonia	O:F:KHL 15352	K.-H. Larsson	MH041553	MH041576
S. brachysporum B	Brazil: Sao Paulo	GB:Hjm 16573	K. Hjortstam	MH041545	MH041596
S. brachysporum B	Colombia: Magdalena	O:F: 918493	L. Ryvarden	MH041522	MH041605
S. brachysporum B	Costa Rica: Alajuela	GB:KHL 11216	K.-H. Larsson	MH041517	MH041580
S. brachysporum B	Jamaica: Cornwall	GB:KHL 10763	K.-H. Larsson	MH041546	MH041598
S. brachysporum B	Jamaica: Middlesex	GB:KHL 10566	K.-H. Larsson	na	MH041599
S. brachysporum B	Madagascar: Anosy	O:F:KHL 14537	K.-H. Larsson	MH041552	MH041573
S. brachysporum B	Puerto Rico: Isabela	GB:KHL 9544	K.-H. Larsson	MH041555	MH041560
S. brachysporum B	Puerto Rico: Luquillo	GB:KHL 10406	K.-H. Larsson	MH041543	MH041600
S. brachysporum B	Puerto Rico: Luquillo	GB:KHL 10411	K.-H. Larsson	MH041549	MH041601
S. brachysporum B	Reunion: Saint Pierre	KAS:L 0134	E. Langer	MH041541	MH041593
S. brachysporum B	Reunion: Saint-Benoit	KAS:L 1584b	M. Striegel	MH041544	MH041610
S. brachysporum B	Reunion: Saint-Pierre	KAS:L 1147	J. Riebesehl; M. Schroth	MH041542	MH041594
S. brachysporum B	Reunion: Saint-Pierre	KAS:L 1498	M. Striegel	MH041526	na
S. brachysporum B	Reunion: Saint-Pierre	KAS:L 1795	M. Striegel	MH041525	MH041579
S. brachysporum B	Reunion: Saint-Pierre	LY 12293	G. Gilles	MH041550	MH041571
S. brachysporum B	Reunion: Saint-Pierre	LY 12772	G. Gilles	na	MH041595
S. brachysporum T	Brazil: Rondonia	O:F:KHL 15318	K.-H. Larsson	MH041557	MH041577
S. brachysporum T	Brazil: Rondonia	O:F:KHL 15327	K.-H. Larsson	MH041539	MH041603
S. brachysporum T	Brazil: Sao Paulo	O:F:LR 24170	D. Pegler; K. Hjortstam; L. Ryvarden	MH041556	na
S. brachysporum T	Reunion: Saint-Paul	LY 11378	J. Boidin	na	MH041574
S. fusisporum	Costa Rica: Puntarenas	GB:KHL 12761	K.-H. Larsson	MH041536	MH041568
S. fusisporum	Puerto Rico: Rio Grande	GB:KHL 9093	K.-H. Larsson	MH041534	na
S. fusisporum (holotype)	Puerto Rico: Rio Grande	GB:KHL 10360	K.-H. Larsson	MH041535	MH041567
S. grandisporum (holotype)	Costa Rica: Cartago	O:F: 506781	L. Ryvarden	MH041547	MH041592

Species	Locality	Voucher specimen	Collector(s)	GenBank/UNITE accession numbers	
				ITS	28S
S. harpagum	Colombia: Magdalena	O:F:LR 15736	L. Ryvarden	MH041531	MH041586
S. harpagum	Jamaica: Cornwall	GB:KHL 10733	K.-H. Larsson	MH041520	MH041563
S. harpagum	Reunion: Saint-Benoit	KAS:L 0244	E. Langer	MH041533	MH041609
S. harpagum (holotype)	Reunion: Saint-Pierre	KAS:L 1726a	M. Striegel	MH041532	MH041588
S. inornatum (holotype)	Puerto Rico: Rio Grande	GB:KHL 10444	K.-H. Larsson	MH041558	MH041569
S. longisporum	Italy: Sicily	TU 124391	A. Saitta	UDB028356*	UDB028356*
S. longisporum	Russia: Orel	LE 292121	S. Volobuev	KP268491*	na
S. longisporum	Sweden: Skåne	GB:KHL 14229	K.-H. Larsson	MH000601*	MH000601*
S. meridense	Brazil: Rondonia	O:F:KHL 15322	K.-H. Larsson	MH041540	MH041602
S. meridense	Brazil: Sao Paulo	GB:Hjm 16400	D. Pegler; K. Hjortstam; L. Ryvarden	MH041538	MH041604
S. meridense	Costa Rica: Guanacaste	GB:KHL 11355	K.-H. Larsson	na	MH041583
S. meridense	Costa Rica: Guanacaste	GB:KHL 11365	K.-H. Larsson	MH041523	MH041584
S. meridense	Reunion: Saint-Benoit	LY 12816	G. Gilles	na	MH041597
S. meridense	Taiwan: Nantou	KAS:GEL 3520	E. Langer; G. Langer; C.-J. Chen	MH041548	na
S. aff. *meridense*	Argentina: Misiones	O:F:LR 19581	L. Ryvarden	MH041551	MH041578
S. aff. *meridense*	Brazil: Rondonia	O:F:KHL 15325	K.-H. Larsson	na	MH041585
S. aff. *meridense*	Colombia: Magdalena	O:F: 918846	L. Ryvarden	MH041554	MH041575
S. aff. *meridense*	Puerto Rico: Cerro Alto	GB:KHL 9561	K.-H. Larsson	MH041524	MH041581
S. aff. *meridense*	Puerto Rico: Luquillo	GB:KHL 10397	K.-H. Larsson	MH041519	MH041582
S. nikau	Reunion: Saint-Pierre	KAS:L 1296	J. Riebesehl; M. Schroth	MH041513	MH041565
S. oberwinkleri	Venezuela: Aragua	GB:KHL 11042	K.-H. Larsson	na	MH041561
S. oberwinkleri (holotype)	Reunion: Saint-Pierre	KAS:L 1860	J. Riebesehl	MH041511	MH041562
S. obtusisporum	Germany: Hesse	FR: Piepenbrink & Lotz-Winter W213-3-I	O. Koukol	MH041521	MH041566
S. obtusisporum	Jamaica: Cornwall	GB:KHL 10622	K.-H. Larsson	MH041559	MH041606
S. parvisporum	Reunion: Saint-Benoit	KAS:L 1226	J. Riebesehl	MH041528	MH041587
S. parvisporum	Reunion: Saint-Pierre	KAS:GEL 5032	E. Langer; E. Hennen	MH041530	MH041591
S. parvisporum	Reunion: Saint-Pierre	LY 12750	G. Gilles	na	MH041589
S. parvisporum (holotype)	Reunion: Saint-Pierre	KAS:L 0140	E. Langer	MH041529	MH041590
S. perlongisporum	Italy: Sicily	TU124388	A.Saitta	UDB028355*	UDB028355*
S. perlongisporum	Russia: Kaluga	LE 302156	S. Volobuev	KP268489*	na
S. rarocrystallinum (holotype)	Colombia: Cundinamarcha	O:F: 918488	L. Ryvarden	MH041512	MH041564

Species	Locality	Voucher specimen	Collector(s)	GenBank/UNITE accession numbers	
				ITS	28S
S. robustius	Jamaica: Cornwall	GB:KHL 10780	K.-H. Larsson	AY463468*	AY586714*
S. robustius	Puerto Rico: Luquillo	GB:KHL 10039	K.-H. Larsson	MH041515	na
S. robustius	Puerto Rico: Rio Grande	GB:KHL 10272	K.-H. Larsson	MH041516	MH041607
S. robustius (holotype)	Jamaica: Cornwall	GB:KHL 10813	K.-H. Larsson	MH041514	MH041608
S. tedersooi	Vietnam: Ninh Bình	TU 110895	L. Tedersoo	UDB014162*	na
S. tedersooi (holotype)	Vietnam: Ninh Bình	TU 110894	L. Tedersoo	UDB014161*	na
outgroup: *Brevicellicium exile*	Spain: Huesca	MA:F 26554	M. Dueñas,	HE963777*	HE963778*
outgroup: *Brevicellicium olivascens*	Sweden: Bohuslän	GB:KHL 8571	K.-H. Larsson	HE963792*	HE963793*

Final alignments of each locus were produced in the online mode of MAFFT version 7 (Katoh et al. 2017), with L-INS-i algorithm and other settings as default.

The small fragments of 18S rDNA and 28S rDNA were automatically trimmed from the target ITS region with the ITSx software (Bengtsson-Palme et al. 2013) implemented in the PlutoF workbench (Abarenkov et al. 2010). The same tool was used to partition ITS into ITS1, 5.8 and ITS2 regions prior to phylogenetic analyses of ITS alignment, to estimate the evolutionary model parameters for each partition separately. The 28S alignment was trimmed manually to produce the sequences of the same lengths and with fewer (if any) gaps at both ends and was not partitioned. Key properties of the final alignments were explored and described using RAxML terminology (Stamatakis 2016).

Morphologically outlined species were compared in terms of genetic distances estimated separately for the trimmed ITS and 28S alignments. For this, raw (also called uncorrected) pairwise dissimilarities of sequences in each alignment were calculated, defined as the percentage of sites that differ between each two full-length sequences including gap positions (Schoch et al. 2012, Kõljalg et al. 2013). This procedure was done with the "dist.dna" function of "ape" R package (Paradis et al. 2004) with option pairwise.deletion=FALSE (i.e. without deleting the sites with missing data in a pairwise way). Results were visualised with the ggplot2 R graphics (Wickham 2009) and R script can be viewed in Ordynets (2018a). Pairwise sequence dissimilarities were further analysed on the intraspecific versus interspecific level. The two levels of sequence variability were segregated with the "sppDist" function of the "spider" R package (Brown et al. 2012) and plotted simultaneously as histograms in a search for the barcoding gap (Meyer et al. 2005). As this classical approach provides only a general overview of sequence variability, i.e. for the pooled dataset, we applied also the recommended alternative which considers the species identity. For each sequence, the maximum intraspecific distance was contrasted with the minimum interspecific

distance as recommended by Collins and Cruickshank (2012). Both types of distance for each sequence were estimated, respectively, with the functions "maxInDist" and "nonConDist" of the "spider" R package (Brown et al. 2012) and visualised as a scatterplot. R script for these procedures is available in Ordynets (2018c).

All phylogenetic analyses were performed using the GTR+G evolutionary model. We performed separate analyses of the ITS alignment (partitioned into ITS1, 5.8 and ITS2 regions), unpartitioned 28S alignment and concatenated ITS+28S alignment partitioned into four regions (ITS1, 5.8, ITS2 and 28S). For Bayesian inference of phylogeny, MrBayes 3.2.3 (Ronquist et al. 2012) was used. Two independent MCMC processes, each in 4 chains, were run. Five million trees were generated, sample frequency was set to 1000 and burnin fraction to 0.2. The acceptability of selected settings and mixing sampled trees, were confirmed by the standard deviation of split frequencies, by the potential scale reduction factor, by the sum of average effective sample size in two runs and by tracing likelihood scores of generated trees with Tracer 1.6 (Ronquist et al. 2011, Rambaut et al. 2014). For 8002 sampled trees per analysis (burn-in fraction excluded), a majority rule consensus tree was computed with branch supports representing the relative frequencies of bipartitions (posterior probabilities). Maximum likelihood analyses were performed in RAxML 8.2.10 (Stamatakis 2014). The search for the best-scoring maximum likelihood tree and bootstrap analysis (1000 replicates) were performed in a single run. Both RAxML and MrBayes were run on CIPRES Science Gateway V 3.3 (Miller et al. 2010; http://www.phylo.org). Resulting phylogenetic trees were first viewed in FigTree v. 1.4.2 (Rambaut 2014). Further visualisation and annotation of the phylogenetic trees were done in R version 3.3.3 (R Core Team 2017) and R script is available in Ordynets (2018d). The multiple sequence alignments, details of phylogenetic analyses and trees generated in the study were deposited in TreeBASE: http://purl.org/phylo/treebase/phylows/study/TB2:S22473.

Results

Descriptions of new species

Subulicystidium boidinii **Ordynets, M.M.Striegel & Langer, sp. nov.**
Index Fungorum No: IF554263
Figs 5e–g; 10q

Diagnosis. Species with broader allantoid spores (2.8–3.5 μm) and less heavily encrusted cystidia than in *Subulicystidium meridense* Oberw.

Type. RÉUNION. Saint-Benoît: Salazie, Hell Bourg, ca 1000 m, -21.0642, 55.5269, on dead woody branch, 23 Mar 2015, M.Striegel (L 1584a in FR; isotype in KAS).

Etymology. *boidinii*, in honour of Jacques Boidin, a great explorer of fungi of Réunion Island, who collected this species and suggested an independent status for it.

Description. *Basidiomata* annual, effused, resupinate, soft and fragile, arachnoid, thin, loosely adnate. Hymenophore smooth, finely velutinous due to numerous protruding cystidia, whitish. Margin thinning out, pruinose, adnate.

Hyphal system monomitic. All septa with clamps. Subiculum thin, with loosely interwoven richly branched hyphae 1.5–2.5 µm wide, thin-walled, hyaline and smooth. Subhymenium thin, with hyphae similar to those in subiculum but occasionally bearing slight amorphous hyaline encrustation. *Cystidia* subulate, rather narrow, 45–65 × 2.5–3.5 µm including encrustation, projecting up to 30 µm, without basal swelling, terminal or pleural, with thin hyaline cell wall and outer hyaline crystalline sheath covering the whole cystidia except the tapering, thin-walled, acuminate apex. Crystal protrusions on cystidium are small and clearly rectangular and arranged in longitudinal rows.

Basidia suburniform to almost clavate, 10–12 × 4–5.5 µm, thin-walled, with 4 sterigmata and a basal clamp, without or with slight amorphous hyaline encrustation at the base. *Basidiospores* allantoid, L=(5.7–)5.9–7.2(–7.9) µm, W=(2.6–)2.8–3.3(–3.5) µm, Q=(1.8–)2.0–2.4(–2.5), N=116/2, with minute apiculus, smooth, thin-walled, hyaline, often with two oil drops (one at each pole), negative in Melzer's reagent.

Additional specimens examined. COSTA RICA. Puntarenas: Coto Brus, Sabalito, Zona Protectora Las Tablas, Finca Cafrosa, El Tajo, 1560 m, 8.9225, -82.7956, on stem of angiosperm tree, 6 Nov 2004, K.-H.Larsson (KHL 12830 in GB). RÉUNION. Saint Pierre: Cilaos, A_Cilaos X, Forêt de la Mare-a-Joseph, kiosque au milieus des *Cryptomeria* D.Don, alt. 1400 m, on strongly decayed wood of *Cryptomeria japonica* D.Don, 20 Apr 1985, J.Boidin (LY 11247).

Remarks on species. Boidin and Gilles (1988) in their survey of *Subulicystidium* from Réunion Island reported "*Subulicystidium allantosporum* ad interim" and referred to their specimens LY 11247 and LY 12750. We show in the current study that these two collections represent different species and only the former may be assigned to *S. boidinii*. We were not able to sequence LY 11247, but description and illustration (Fig. 38A) provided by Boidin and Gilles (1988) and our re-measuring of basidiospores in their specimen (Supplementary files 2 and 3) agree well with our concept of *S. boidinii*.

***Subulicystidium fusisporum* Ordynets & K.H.Larss., sp. nov.**
Index Fungorum No: IF554264
Figs 4a–c; 10e

Diagnosis. Differs from *Subulicystidium longisporum* (Pat.) Parmasto by fusiform basidiospores which are ca. 10–13 µm long and 2.5–3.5 µm broad.

Type. PUERTO RICO. Municipio Rio Grande, Luquillo Mts, El Verde Research Area, between Field Station and 16-hectare grid, 320–380 m, 18.3233, -65.8172, on fallen tree log, 9 Jun 1998, K.-H.Larsson (KHL 10360 in GB).

Etymology. *fusisporum* (Lat.), having fusiform basidiospores.

Description. *Basidiomata* annual, effused, resupinate, soft and fragile, arachnoid, thin, loosely adnate. Hymenophore smooth, finely velutinous due to numerous protruding cystidia, whitish. Margin thinning out, adnate.

Hyphal system monomitic. All septa with clamps. Subiculum thin, with loosely interwoven richly branched hyphae 2.5–3.5 μm wide, usually thin-walled, hyaline and smooth. Subhymenium thin, with hyphae slightly broader than in subiculum, 2.7–4 μm wide, compactly arranged, often slightly thick-walled and covered with hyaline crystalline sheath. *Cystidia* subulate, 65–90 × 3.5–5 μm including encrustation, projecting up to 40 μm, without or occasionally with basal swelling (up to 6 μm wide), terminal, with thick hyaline cell wall and outer hyaline crystalline sheath covering the whole cystidium except the thin-walled, acuminate apex. Crystal protrusions on cystidium are small to moderately large and clearly rectangular and arranged in longitudinal rows.

Basidia suburniform, 12–14 × 4.5–6 μm, thin-walled, with 4 sterigmata and a basal clamp, often with a hyaline crystal collar at the base. *Basidiospores* fusiform, L=(9.7–)10.7–12.8(–13.3) μm, W=(2.1–)2.4–3.4(–3.7) μm, Q= (3.0–)3.3–4.9(–5.9), N=127/3, with minute apiculus, smooth, thin-walled, hyaline, occasionally with oil drops, negative in Melzer's reagent. Tolerance limits for basidiospore length, width and length to width ratio in *S. fusisporum* based on 3 sequenced specimens are provided in the Table 2.

Additional specimens examined. COSTA RICA. Puntarenas: Coto Brus, Sabalito, Zona Protectora Las Tablas, La Neblina, 8.9149, -82.7719, on stem of angiosperm tree, 5 Nov 2004, K.-H.Larsson (KHL 12761 in GB). CÔTE D'IVOIRE. Abidjan: Foret du Banco, 5.3932, -4.0525, on dead wood, 6 Jul 1974, G.Gilles (LY 7375). JAMAICA. Cornwall County: Trelawny parish, N of Crowlands, trail/road into park area, 18.2611, -77.6511, on stem of angiosperm tree, 10 Jun 1999, K.-H. Larsson (KHL 10612 in GB). PUERTO RICO. Municipio Rio Grande, Luquillo Mts, El Verde Research Area, between Field Station and 16-hectare grid, 320-380 m, 18.3233, -65.8172, on strongly decayed stem of angiosperm tree, 19 Jun 1996, K.-H. Larsson (KHL 9093 in GB), on uprooted angiosperm tree, 19 Jun 1996, K.-H.Larsson (KHL 9061 in GB).

Remarks on species. Amongst the species considered in this study, *S. fusisporum* is the most probable to be confused with *S. longisporum*. However, careful measurement of basidiospores (length below 13 μm, see Fig. 10e vs. 10g) and rDNA sequence identity clearly point to the species of its own. The regular rectangular shape of crystal protrusion as well as their dense arrangement in longitudinal rows on cystidia in *S. fusisporum* is also prominent.

Subulicystidium grandisporum Ordynets & K.H.Larss. sp. nov.

Index Fungorum No: IF554265
Figs 6a–c; 10i

Diagnosis. Species with the largest cylindrical basidiospores ever observed in the genus (10.5–14.5 × 3.3–3.9 μm) and relatively large cystidia with prominent regular encrustation.

Table 2. 90% tolerance limits defined for the 90% probability level for the mean basidiospore length, width and length to width ratio for *Subulicystidium* species with at least 3 sequenced specimens. The following specimens were used to estimate tolerance limits for species: *Subulicystidium fusisporum*: GB:KHL 9093, 10360 and 12761; *S. harpagum*: GB:KHL 10733, O:F:LR 15736, KAS:L 0244 and 1726a; *S. parvisporum*: KAS:GEL 5032, KAS:L 0140 and 1226 and LY 12750; *S. robustius*: GB:KHL 10039, 10272, 10780 and 10813.

Measurement type	Estimate	Species			
		Subulicystidium fusisporum	*Subulicystidium harpagum*	*Subulicystidium parvisporum*	*Subulicystidium robustius*
Spore length, µm	Sample mean	11.78	6.74	5.61	9.78
	Lower limit of 90% tolerance interval	9.64	4.34	4.78	7.81
	Upper limit of 90% tolerance interval	13.92	9.13	6.43	11.75
Spore width, µm	Sample mean	2.92	2.6	2.51	3.00
	Lower limit of 90% tolerance interval	1.65	1.62	2.06	2.44
	Upper limit of 90% tolerance interval	4.19	3.58	2.95	3.57
Spore length/width ratio	Sample mean	4.09	2.63	2.25	3.27
	Lower limit of 90% tolerance interval	1.89	0.83	1.91	2.52
	Upper limit of 90% tolerance interval	6.28	4.42	2.59	4.02

Type. COSTA RICA. Cartago: Faldas del volcano Irazu, 1800 m, on decayed twig, 28 May 1991, L.Ryvarden (LR 29162 in O:F 506781).

Etymology. *grandisporum* (Lat.), having large basidiospores.

Description. *Basidiomata* annual, effused, resupinate, soft and fragile, arachnoid, thin, loosely adnate. Hymenophore smooth, finely velutinous due to numerous protruding cystidia, whitish. Margin thinning out, pruinose, adnate.

Hyphal system monomitic. All septa with clamps. Subiculum thin, with loosely interwoven richly branched hyphae 3–4 µm wide, thin-walled, hyaline and smooth. Subhymenium thin, compact, with richly branched hyphae 3–3.5 µm wide, often covered with thin hyaline crystalline sheath. *Cystidia* subulate, 70–90 × 5–7 µm including encrustation, projecting up to 60 µm, without basal swelling, terminal or pleural, with thick hyaline cell wall and outer hyaline crystalline sheath covering the whole cystidium except the thin-walled, tapering apex. Crystal protrusions on cystidium are large, clearly rectangular to rounded, rather sparsely arranged in longitudinal rows.

Basidia suburniform, 13–19 × 5.5–7 µm, thin-walled, with 4 sterigmata and a basal clamp, often with hyaline crystalline collar at the base. *Basidiospores* cylindric, adaxial side slightly concave, L=(10–)10.6–14.5(–15.3) µm, W=(3.2–)3.3–3.9(–4.2) µm, Q= (2.9–)3.0–4.0, N=48/1, with minute apiculus, smooth, thin-walled, hyaline, negative in Melzer's reagent.

Remarks on species. Until now, it is the only known *Subulicystidium* species with such large cylindrical basidiospores. Additionally, large cystidia with regular large protrusions, together with large basidia, make the species remarkable.

Subulicystidium harpagum **Ordynets, M.M.Striegel & K.H.Larss., sp. nov.**
Index Fungorum No: IF554266
Photo of fresh collection in PlutoF: link 1
Figs 7a, b; 10r

Diagnosis. Differs from other *Subulicystidium* species by the cystidia which resemble a harpoon due to protruded backward pointing individual crystals and moderately large cylindric to allantoid basidiospores (5.7–8.2 × 2.2–3.0 μm).

Type. RÉUNION. Saint-Pierre: Saint-Philippe, Forêt de Mare Longue, 495 m, -21.3438, 55.7410, on dead tree branch, 28 Mar 2015, M.Striegel (L 1726a in FR, isotype in KAS).

Etymology. *harpagum*, from the Latin "harpaga", English "harpoon", a spear with barbs and serrated edges used in fishing. Epithet refers to the cystidium encrustation pattern.

Description. *Basidiomata* annual, effused, resupinate, soft and fragile, arachnoid, loosely adnate and easily separable. Hymenophore smooth, velutinous due to numerous protruding large cystidia, whitish. Margin not differentiated.

Hyphal system monomitic. All septa with clamps. Subiculum thin, with interwoven richly branched hyphae 2-3 μm wide, thin-walled to very slightly thick-walled, hyaline, often with rough surface because of slight encrustation. In the older fruitbody parts, encrustation represents an up to 1 μm thick sheath over the hypha. Subhymenium thin, with hyphae identical to those in subiculum. *Cystidia* subulate, 35–62 × 2.5–3.5 μm including encrustation, projecting up to 30 μm, without basal swelling, terminal or pleural, with thin to slightly thickened hyaline cell wall and outer hyaline crystal sheath covering the whole cystidium except the thin-walled, acuminate and particularly narrow, apex. Crystal protrusions on cystidium are formed like short rods that project backwards under acute angle, thus making cystidia resembling a harpoon.

Basidia suburniform, 9–12 × 4.2–5.7 μm, thin-walled, with 4 sterigmata and a basal clamp, basally slightly encrusted. *Basidiospores* weakly allantoid, adaxial side concave, L=(4.5–)5.7–8.2(–8.7) μm, W=(2.0–)2.2–3.0(–3.3) μm, Q=(1.7–)2.1–3.4(–3.8), N=178/4, with minute apiculus, smooth, thin-walled, hyaline, often with two oil drops (one at each pole), negative in Melzer's reagent. Tolerance limits for basidiospore length, width and length to width ratio in *S. harpagum* based on 4 sequenced specimens are provided in Table 2.

Additional specimens examined. RÉUNION. Saint-Benoît: Sainte-Rose, Forêt de Bois Blanc, 640 m, -21.2081, 55.7981, on strongly decayed wood, 21 Mar 2013, E.Langer (L 0244 in FR and KAS). JAMAICA. Cornwall County: Trelawny parish, Windsor Cave, along trail to Troy, 18.3564, -77.6472, on twig of angiosperm tree, 12 Jun 1999, K.-H.Larsson (KHL 10733 in GB). COLOMBIA. Magdalena: Parque Nacional Tayrona, Estacion de Gairaca, 0-30 m, 11.3170, -74.1063, on dead twig, 12 Jun 1978, L.Ryvarden (LR 15736 in O:F).

Remarks on species. The holotype specimen contains also a small piece of *S. perlongisporum*, now kept in a separate clearly labelled envelope within the voucher.

Despite being mixed, the specimen was still selected as type because of the hymenium and subhymenium are better preserved and the ITS and 28S sequences retrieved are of higher quality.

***Subulicystidium inornatum* Ordynets & K.H.Larss. sp. nov.**
Index Fungorum No: IF554267
Figs 4d–f; 10d

Diagnosis. The species has cystidia that do not possess individual crystal protrusions but are instead smooth or only slightly rough and basidiospores that are fusiform and moderately large, 8.1–10.9 × 2.7–3.3 µm.

Type. PUERTO RICO. Municipio Rio Grande, Luquillo Mts, El Yunque, Mount Britton Trail, between upper road and trail head, 760-880 m, 18.3003, -65.7917, on wet dead wood, 11 Jun 1998, K.-H.Larsson (KHL 10444 in GB).

Etymology. *inornatum* (Lat.), without ornament, referring to the almost smooth cystidia.

Description. *Basidiomata* annual, effused, resupinate, soft and fragile, arachnoid, thin, loosely adnate. Hymenophore smooth, finely velutinous due to numerous protruding cystidia, whitish. Margin thinning out, adnate.

Hyphal system monomitic. All septa with clamps. Subiculum thin, with loosely interwoven richly branched hyphae 3–4 µm wide, hyaline, thin-walled to slightly thick-walled, covered by a thin hyaline crystal sheath giving them a slightly rough appearance. Subhymenial hyphae similar to those in subiculum, but more compactly arranged and slightly agglutinated. *Cystidia* subulate, 45–60 × 4–5.5 µm including encrustation, projecting up to 45 µm, occasionally with slight basal swelling (up to 6 µm), terminal, thick-walled and with an outer hyaline crystal sheath covering the whole cystidium except the thin-walled acuminate apex. Surface of the crystal sheath slightly rough, crystal protrusions lacking.

Basidia suburniform to almost clavate, 10–14 × 4.5–6 µm, thin-walled, with 4 sterigmata and a basal clamp, often with hyaline crystalline collar at the base. *Basidiospores* fusiform, L=(7.2)8.1–10.9(–11.0) µm, W=(2.5–)2.7–3.3(–3.5) µm, Q=(2.4–)2.7–3.8(–4.1), N=97/1, with minute apiculus, smooth, thin-walled, hyaline, negative in Melzer's reagent.

Additional specimens examined. COSTA RICA. Puntarenas: Carrara Biologica Reserva, ca. 50 m, 9.7472, -84.6278, on dead fruit-bodies of *Coriolopsis rigida* (Berk. & Mont.) Murill, 14 Jun 1991, L.Ryvarden (LR 29823 in O:F 506780). PUERTO RICO. Municipio Cayey, Bosque Estatal Carite, Guavate Picnic area, 18.1264, -66.0764, on dead wood, 23 Jun 1996, K.-H.Larsson (KHL 9289 and 9337 in GB).

Remarks on species. This is the only species in which cystidia and hyphae have a similar surface, which is smooth or slightly rough due to a thin layer of crystalline matter.

***Subulicystidium oberwinkleri* Ordynets, Riebesehl & K.H.Larss., sp. nov.**
Index Fungorum No: IF554268
Figs 5a, b; 10t

Diagnosis. differs from *Subulicystidium nikau* (G. Cunn.) Jülich by having plate-like to irregular crystals on cystidium and longer basidiospores (7.8–10.8 µm long).

Type. RÉUNION. Saint-Pierre: Saint-Philippe, Forêt de Mare Longue, 495 m, -21.3438, 55.7410, on dead woody branch, 28 Mar 2015, J.Riebesehl (L 1860 in FR; isotype in KAS).

Etymology. *oberwinkleri*, named after Franz Oberwinkler, a German mycologist who provided a perceptive view into the species concepts in *Subulicystidium* and was an early collector of the species in South America.

Description. *Basidiomata* annual, effused, resupinate, soft and fragile, arachnoid, loosely adnate and easily separable. Hymenophore smooth, velutinous due to numerous protruding large cystidia, porulose, whitish to yellow. Margin abrupt, not differentiated.

Hyphal system monomitic. All septa with clamps. Subiculum with interwoven and richly branched hyphae 3–4 µm wide, occasionally swollen up to 6 µm, slightly to moderately thick-walled, hyaline. Subhymenium thin and loose. Subhymenial hyphae richly branched, intricate, regular or occasionally slightly inflated, 3–4 µm wide, thin-walled. *Cystidia* tubular, 80–150 × 5.5–10 µm including encrustation, projecting up to 70 µm, without basal swelling, with septa having or devoid of clamps, with thin or only slightly thickened hyaline cell wall and outer hyaline crystalline sheath (up to 3.5 µm thick) covering at least the lower half and, at a maximum, almost the whole cystidium except the short, 2–3 µm wide, hyphoid, cylindrical or tapering apex. The crystal protrusions on cystidium are large, plate-like, slightly rhomboid or irregular in outline, somewhat imbricately arranged. Similar encrustation pattern is found also on the subicular and especially subhymenial hyphae and sometimes on the bases of basidia.

Basidia suburniform to urniform, 12–18 × 6–8 µm, thin-walled, with 4 sterigmata and a basal clamp, terminal or sometimes pleural. *Basidiospores* broad cylindric to reniform, adaxial side slightly concave, L=(7.4–)7.8–10.8(–11.6) µm, W=(3.7–)4.0–5.5(–5.8) µm, Q=(1.6–)1.6–2.3(–2.4), N=99/3, with a prominent apiculus, smooth, thin-walled, hyaline, negative in Melzer's reagent.

Additional specimens examined. RÉUNION. Saint-Benoit: Saint-Benoit, Forêt de Bébour, Bebour-I-87, *Cryptomeria* forest, 1200 m, on dead wood of *Cryptomeria japonica*, 24 May 1987, J.Boidin (LY 12488). VENEZUELA. Estado Aragua: Maracay, National Park Henri Pittier, Rancho Grande, 10.3800, -67.6190, on dead wood, 30 Aug 1999, K.-H.Larsson (KHL 11042 in GB). Estado Merida: La Carbonera, Road Merida-La Azulita, 2000–2200 m, on dead wood, 19 Jan 1969, F.Oberwinkler (FO 14338 in TUB).

Remarks on species. Specimens of *S. oberwinkleri* were noticed for the peculiar cystidia previously by Oberwinkler (1977) and later by Maekawa (1998). Neither author was prepared to assign them to a separate species and instead labelled them as *S. nikau* (characterised by regularly ornamented cystidia). Our examination of the specimens TUB:FO 14338 from Venezuela (Oberwinkler 1977, fig. 31) and LY 12488 from Réunion (Boidin and Gilles 1988, fig. 39A) showed that both represent *S. oberwinkleri*.

***Subulicystidium parvisporum* Ordynets & Langer, sp. nov.**
Index Fungorum No: IF554270
Photo of fresh collection (holotype) in PlutoF: link 1
Figs 7c, d; 10s

Diagnosis. The species with the smallest basidiospores known in the genus, 5.0–6.2 × 2.2–2.8 µm and allantoid, combined with rather small cystidia with regular delicate encrustation.

Holotype. RÉUNION. Saint-Pierre: Cilaos, Cirque de Cilaos, Roche Merveilleux, Sentiere botanique, 1300 m, -21.1232, 55.4920, on strongly decayed wood, 15 Mar 2013, E.Langer (L 0140 in FR; isotype in KAS).

Etymology. *parvisporum* (Lat.), having small basidiospores.

Description. *Basidiomata* annual, effused, resupinate, soft and fragile, arachnoid, thin, loosely adnate. Hymenophore smooth, finely velutinous due to numerous protruding cystidia, whitish. Margin thinning out, pruinose, adnate.

Hyphal system monomitic. All septa with clamps. Subiculum thin, with loosely interwoven richly branched hyphae 1.8–3 µm wide, thin-walled, hyaline and smooth. Subhymenium thin, with hyphae similar to those in subiculum but occasionally bearing slight amorphous hyaline encrustation. *Cystidia* subulate, 45–65 × 2.5–3 µm including encrustation, projecting up to 30 µm, without basal swelling, terminal or pleural, with thin hyaline cell wall and outer hyaline crystalline sheath covering the whole cystidium except the thin-walled, narrow, acuminate apex. Crystal protrusions on cystidium are low but clearly rectangular and arranged in longitudinal rows.

Basidia suburniform to almost clavate, 10–15 × 4–5 µm, thin-walled, with 4 sterigmata and a basal clamp, occasionally with slight amorphous hyaline encrustation at the base. *Basidiospores* allantoid, often with a slight constriction in the middle part, L= (4.3)5.0–6.2(–6.8) µm, W=(1.8–)2.2–2.8(–3.0) µm, Q=(1.8–)1.9–2.6(–3.1), N=151/4, with minute apiculus, smooth, thin-walled, hyaline, occasionally with one or two oil drops, negative in Melzer's reagent. Tolerance limits for basidiospore length, width and length to width ratio in *S. parvisporum,* based on 4 sequenced specimens, are provided in the Table 2.

Additional specimens examined. RÉUNION. Saint-Benoit: Saint-Benoit, Forêt Margarithe, ca. 450 m, -21.1031, 55.6926, on dead wood, 24 Mar 2015, J.Riebesehl (L 1226 in FR and KAS). Saint-Pierre: Cilaos, Cilaos XII-87, forêt de la Mare à Joseph, au-dessus du hameau de Bras Sec, 1400 m, -21.1239, 55.4957, on dead wood, 4 Apr 1987, G.Gilles (LY 12750); le Tampon, Notre dame de la Paix, Forêt de la Riviere des Remparts, Sentier Botanique, -21.2559, 55.5987, on dead wood, 23 Mar 1998, E.Langer & E.Hennen (GEL 5032 in KAS).

Remarks on species. Boidin and Gilles (1988) mentioned one collection with such small spores for his *S. allantosporum* ad interim (LY12750). After examining and sequencing the specimen, we conclude that it clearly represents our new species *S. parvisporum*. Both ours and specimens of Boidin and Gilles originate exclusively from Réunion.

Subulicystidium rarocrystallinum **Ordynets & K.H.Larss. sp. nov.**
Index Fungorum No: IF554269
Figs 6f–h; 10k

Diagnosis. Differs from all other *Subulicystidium* species by cystidia which bear few spaced and irregularly located crystals and have a thick cell wall.

Type. COLOMBIA. Cundinamarcha: 23rd kilometre of a highway from Medellin (direction SE) to Tenjo, alt 2600 m, 6.0605, -75.4095, on dead twig, 4 Jun 1978, L.Ryvarden (LR 15483 in O:F 918488).

Etymology. *rarocrystallinum* (Lat.), having few spaced crystals on cystidium.

Description. *Basidiomata* annual, effused, resupinate, fragile, porulose, thin, adnate. Hymenophore smooth, finely velutinous due to numerous protruding cystidia, whitish. Margin thinning out, adnate.

Hyphal system monomitic. All septa with clamps. Subiculum thin, compact, with richly branched hyphae 3–3.5 µm wide, thin-walled to slightly thick-walled, hyaline and smooth. Subhymenium thin, compact, with richly branched hyphae 3–3.5 µm wide, thin-walled, smooth. *Cystidia* subulate, 45–65(–80) × 3.7–5 µm including encrustation, projecting up to 50 µm, with especially thick-walled, occasionally slightly swollen (up to 5.5 µm), basal part, with outer hyaline crystalline sheath covering the whole cystidium except the tapering, thin-walled apex. Crystal protrusions on cystidium are moderately large, rectangular to rounded, rather sparse and allocated rather irregularly and mostly in the medial part.

Basidia suburniform, 11–15 × 4.5–5.5 µm, thin-walled, with 4 sterigmata and a basal clamp, without encrustation. *Basidiospores* cylindric, adaxial side slightly concave, L=(7.5–)8.0–10.5(–10.8) µm, W=(2.8)2.9–3.7(–3.8) µm, Q=(2.2–)2.5–3.2(–3.7), N=72/1, with minute apiculus, smooth, thin-walled, hyaline, negative in Melzer's reagent.

Remarks on species. The few spaced far from each other crystals on cystidium and thick cell wall of cystidium are peculiar. Furthermore, in the single collection studied, cystidia were relatively infrequent and subhymenium was more compact than in other species. Species can be distinguished from *Subulicystidium brachysporum* also by larger cylindric basidiospores.

Subulicystidium robustius **K.H.Larss. & Ordynets, sp. nov.**
Index Fungorum No: IF554271
Figs 3f–h; 10c

Diagnosis. The species is characterised by numerous large and most prominently ornamented cystidia with regular ornamentation and by moderately broad fusiform basidiospores 10.5–12.5 × 2.5–3.5 µm.

Type. JAMAICA. Cornwall County: Trelawny parish, Windsor Cave, along trail to Troy, 18.3564, -77.6472, on trunk of angiosperm tree, 13 Jun 1999, K.-H.Larsson (KHL 10813 in GB).

Etymology. *robustius* (Lat.), having large cystidia with large crystal protrusions.

Description. *Basidiomata* annual, effused, resupinate, soft and fragile, arachnoid, loosely adnate and easily separable. Hymenophore smooth, hirsute due to numerous protruding large cystidia, yellowish. Margin thinning out, pruinose, adnate.

Hyphal system monomitic. All septa with clamps. Subiculum thick, with interwoven richly branched hyphae 2–3 µm wide, thin-walled to slightly thick-walled, hyaline to yellowish, smooth or with sparse granulose encrustation. Subhymenium rather thick, up to 60 µm. Subhymenial hyphae richly branched, intricate, regular or occasionally slightly inflated, 2–4 µm wide, thin-walled, occasionally weakly encrusted by yellowish crystalline material. *Cystidia* subulate, 80–105 × 4.5–6 µm including encrustation, projecting up to 65 µm, without basal swelling, terminal or pleural, with thick yellowish wall and outer hyaline crystalline sheath covering the whole cystidium except the small tapering or acuminate apex. Crystal protrusions on cystidium are large and clearly rectangular, arranged in longitudinal rows.

Basidia clavate to suburniform, 13–20 × 4–6 µm, thin-walled, with 4 sterigmata and a basal clamp, without encrustation or rarely with a slight crystalline crust at the base. *Basidiospores* fusiform, adaxial side convex, L= (8.1–)8.5–10.9(–11.7) µm, W=(2.5–)2.7–3.5(–3.7) µm, Q=(2.4–)2.6–3.6(–4.0), N=197/4, with minute apiculus, smooth, thin-walled, hyaline, negative in Melzer's reagent. Tolerance limits for basidiospore length, width and length to width ratio in *S. robustius*, based on 4 sequenced specimens, are provided in Table 2.

Additional specimens examined. BRAZIL. Sao Paulo: Cananeia, Ilha do Cardoso, -25.1336, -47.9617, on dead wood, 2-5 Feb 1987, D.Pegler, K.Hjortstam & L.Ryvarden (LR 24792 in O:F). COLOMBIA. Magdalena: Parque Nacional Tayrona, Estacion de Gairaca, 0-30 m, 11.3170, -74.1063, on dead wood, 12 Jun 1978, L.Ryvarden (LR 15791 in O:F 918494). COSTA RICA. Alajuela: Bijagua, Albergue Heliconias, Sendero Heliconias, 770 m, 10.7181, -85.0453, on log of angiosperm tree, 12 Jul 2001, K.-H.Larsson (KHL 11245 and 11259 in GB); San Ramon, Reserva Forestal Colonia Palmarena, 850 m., 10.2500, -84.5667, on dead wood, 14 Mar 1991, L.Horovitz (FO 42968 in TUB). ECUADOR. Orellana: Yasuni National Park, Yasuni Scientific Research Station, -0.6859, -76.3953, on dead wood, 9-12 Mar 2002, L.Ryvarden (LR 44667 in O:F 505981 and LR 44688 in O:F 505799). JAMAICA. Cornwall County: Trelawny parish, N of Crowlands, trail/road into park area, 18.2611, -77.6511, on stem of angiosperm tree, 10 Jun 1999, K.-H.Larsson (KHL 10661 in GB); Windsor Cave, along trail to Troy, 18.3564, -77.6472, on trunk of angiosperm tree, 13 Jun 1999, K.-H.Larsson (KHL 10780 and 10814 in GB). Surrey County: Portland parish, between reach and Ecclesdown hillside to the east, alt 500 m, 18.0433, -76.3108, on of angiosperm trunk, 16 Jun 1999, K.-H.Larsson (KHL 10895 in GB). PUERTO RICO. Municipio Juana Diaz, Bosque Estatal Toro Negro, near DNR office, downstream from Road 143, 18.1539, -66.5356, on dead wood, 24 Jun 1996, K.-H.Larsson (KHL 9381 in GB). Municipio Luquillo, Luquillo Mts, Bisley Experimental Watersheds, along the logging road, 215 m a.s.l., 18.3161, -65.7467, on log of angiosperm tree, 6 Jun 1997, K.-H.Larsson (KHL 10039 in GB); Sabana, above Chicken Farm & Rio Sabana, 70 m a.s.l., 18.3500, -65.7344, on log, 10 Jun 1998,

K.-H.Larsson (KHL 10423 in GB). Municipio Maricao, Reserva Forestal Maricao, near Fish Hatchery, 18.1922, -66.9933, on decaying log of angiosperm tree, 25 Jun 1996, K.-H.Larsson (KHL 9454 in GB). Municipio Rio Grande, Luquillo Mts, El Verde Research Area, between Field Station and 16-hectare grid, 320-380 m, 18.3233, -65.8172, on log, 7 Jun 1998, K.-H.Larsson (KHL 10272 in GB); El Verde Research Area, lower part of 16-hectare grid, 345-360 m, 18.3239, -65.8172, on dead wood, 28 Jun 1996, K.-H.Larsson (KHL 9574 in GB). VENEZUELA. Estado Amazonas: Manapiare, Yutajé, 5.6142, -66.1236, on dead wood of angiosperm tree, 12-19 Apr 1998, L.Ryvarden (LR 40545 in O:F). Estado Aragua: Maracay, National Park Henri Pittier, Rancho Grande, 10.3800, -67.6190, on dead wood of angiosperm tree, 25 Apr 1998, L.Ryvarden (LR 40767 in O:F).

Remarks on species. Our data shows that the species is widespread in the Caribbean region and in South America. We were able to examine the specimen mentioned and illustrated from Costa Rica by Kisimova-Horovitz et al. (1997) under the name *S. naviculatum* and re-identified it as *S. robustius*.

Subulicystidium ryvardenii **Ordynets & K.H.Larss. sp. nov.**
Index Fungorum No: IF554272
Figs 3c–e; 10b

Diagnosis. Species with fusiform basidiospores with the width range 3.5–4.2 µm, halfway between the width ranges of *Subulicystidium robustius* K.H. Larss. & Ordynets and *S. naviculatum* Oberw.

Type. ETHIOPIA. Arussi: Munessa Forest east of Lake Lagano, 7.5833, 38.9167, on dead wood, 10 Jan 1973, L.Ryvarden (LR 8860/b in O:F 909583).

Etymology. *ryvardenii*, named after Leif Ryvarden, a Norwegian mycologist, enthusiastic explorer of the tropical fungal diversity and collector of the type specimen.

Description. *Basidiomata* annual, effused, resupinate, soft and fragile, arachnoid, thin, loosely adnate. Hymenophore smooth, hirsute due to numerous large protruding cystidia, yellowish. Margin thinning out, adnate.

Hyphal system monomitic. All septa with clamps. Subiculum thin, with loosely interwoven richly branched hyphae 3–4 µm wide, thin-walled to slightly thick-walled, hyaline and smooth. Subhymenium weakly developed, with hyphae 3–4 µm wide, loosely arranged, slightly thick-walled and often covered with a hyaline crystal sheath. *Cystidia* subulate, 65–115 × 4.5–6 µm including encrustation, projecting up to 50 µm, with or without a slight basal swelling (up to 6.5 µm diam.), terminal, with thick hyaline cell wall and an outer hyaline crystal sheath covering the whole cystidium except the tapering, thin-walled apex. Crystal protrusions on cystidium are large and mostly rounded and sparsely arranged in longitudinal rows.

Basidia subclavate to suburniform, 15–20 × 4–5 µm, thin-walled, with 4 sterigmata and a basal clamp, often with a hyaline crystal collar at the base. *Basidiospores* broadly fusiform, L=(8.5–)8.7–11.2(–11.6) µm, W= 3.5–4.2(–4.4) µm, Q=(2.3–)2.4–3.0(–

3.2), N=31/1, with minute apiculus, smooth, thin-walled, hyaline, occasionally with oil drops, negative in Melzer's reagent.

Remarks on species. With its hirsute hymenium which has numerous large cystidia, the species is similar to *S. robustius*, but differs by broader basidiospores and more rounded single crystals on cystidia.

***Subulicystidium tedersooi* Ordynets, Scherf & Langer, sp. nov.**
Index Fungorum No: IF554273
Figs 4g, h; 10f

Diagnosis. Species with particularly narrow fusiform basidiospores, 8.5–11.5 × 2–2.5 µm and long, 85–125 µm, regularly encrusted cystidia.

Type. VIETNAM. Ninh Bình Province: Cuc Phuong National Park, sampling area G2906, 20.3500, 105.6026, on fallen decayed twig, 15 Oct 2012, L.Tedersoo (TU 110894).

Etymology. *tedersooi*, named after Leho Tedersoo, an Estonian mycologist, the vigorous explorer of the global soil fungal diversity and collector of the type specimen.

Description. *Basidiomata* annual, effused, resupinate, soft and fragile, arachnoid, thin, loosely adnate. Hymenophore smooth, finely velutinous due to numerous protruding cystidia, whitish. Margin thinning out, adnate.

Hyphal system monomitic. All septa with clamps. Subicular and subhymenial layer weakly differentiated, consisting of richly branched hyphae 2–3 µm wide, thin-walled, with rough surface due to a subinvisible hyaline crystal sheath. *Cystidia* subulate, 85–125 × 4.5–5 µm, usually without basal swelling, terminal, with thick hyaline cell wall and an outer hyaline crystal sheath covering the whole cystidium except the acuminate apex. Crystal protrusions on cystidium are rectangular, moderately large, regularly arranged in longitudinal rows.

Basidia suburniform to cylindrical, 9–13 × 4.5–5, thin-walled, with 4 sterigmata and a basal clamp, occasionally with a thin hyaline crystal collar at the base. *Basidiospores* narrowly fusiform, L=(7.9–)8.4–11.5(–11.8) µm, W=(1.9–)2.1–2.6(–2.8) µm, Q=(3.4–)3.5–5.0(–5.7), N=81/2, with straight to slightly curved base, thin-walled, often with two large or many smaller oil drops, negative in Melzer's reagent.

Additional specimens examined. VIETNAM. Ninh Bình Province: Cuc Phuong National Park, sampling area G2906, 20.3500, 105.6026, on fallen decayed twig, 15 Oct 2012, L.Tedersoo (TU 110895).

Remarks on species. The narrow spores of *S. tedersooi* are comparable in width only with *S. perlongisporum* (see Fig. 10f vs. 10j). However, the spore length of two species drastically differs: 8.4–11.5 µm in *S. tedersooi* vs. 17–25 µm in *S. perlongisporum* (Boidin and Gilles 1988). *S. tedersooi* also has shorter basidiospores and longer cystidia than its sister species *S. fusisporum* (see Figs 4, 10 and 11 for spore and cystidia comparisons and Figs 12–14 for phylogenetic inference).

Sequence dissimilarities and barcoding gap

The aligned ITS dataset included 59 *Subulicystidium* sequences and two outgroup sequences of *Brevicellicium*. The dataset consisted of 671 characters (gaps included) and contained 477 distinct alignment patterns, namely 238 in ITS1, 28 in 5.8S and 211 in ITS2 region. The proportion of gaps and completely undetermined characters in this alignment was 20.02%.

Aligned ITS sequences fell into several dissimilarity categories. All the *Subulicystidium* sequences were at least 10% different from two *Brevicellicium* sequences (outgroup), as well as from single sequence of *Subulicystidium oberwinkleri* (Fig. 1a). The sequences of *S. robustius* were at least 3% and maximum 10% different from the rest of the genus. The sequences of *S. harpagum* and *S. parvisporum* were most distant from *S. robustius* (7–10%) and 3-7% distant from the rest of the genus. Sequences mostly belonging to morphospecies *S. meridense* and *S. brachysporum* formed four groups within which they were all 0-3% (in many cases only up to 1%) dissimilar. One of these groups included also sequences of *S. fusisporum* and *S. tedersooi*, another group—*S. longisporum* and *S. grandisporum* and the third—single sequence of *S. obtusisporum*. Therefore, both easier and harder distinguishable species, in terms of ITS region identity, were found in the dataset.

The pattern seen through a visual inspection of the ITS sequence dissimilarity matrix was confirmed by the barcoding gap analysis. Throughout the dataset, intraspecific and interspecific distances strongly overlap and no universal for the genus *Subulicystidium* barcoding gap could be detected (Fig. 2b). Mean and maximal intraspecific distances were 2.87 and 7.73%, while mean and minimal interspecific distances were 5.06 and 0%, respectively. At the level of individual species, a barcode gap existed for *S. fusisporum*, *S. parvisporum*, *S. robustius* and *S. tedersooi* (Fig. 2a).

The aligned 28S dataset included 57 *Subulicystidium* sequences and two outgroup sequences of *Brevicellicium*. The dataset consisted of 617 characters (gaps included) and contained 246 alignment patterns, while the proportion of gaps and completely undetermined characters was 7.54%.

Pairwise 28S sequence dissimilarities were structured differently compared to the ITS dataset (Fig. 1b). The most distinct species in terms of 28S identity was *S. oberwinkleri.* The dissimilarity of its two sequences from the rest of *Subulicystidium* and two *Brevicellicium* sequences was 10–20%. The next most distinct group was formed by the sequences of *S. harpagum* and *S. parvisporum* which were 7–10% dissimilar from the rest of the genus except one group containing *S. meridense* and *S. brachysporum* sequences (2–3%). The majority of dissimilarities lay in the range 1–5% and were clearly grouped (Fig. 1b).

In a whole 28S dataset, intraspecific and interspecific distances strongly overlapped and thus showed no universal for the genus *Subulicystidium* barcode gap (Fig 2d). Mean and maximal intraspecific distances were 2.52 and 12.5%, while mean and minimal interspecific distances were 5.58 and 0%, respectively. At the level of individual species, the barcode gap was evident for *S. oberwinkleri*, *S. fusisporum,* and *S. robustius* (Fig 2c).

Phylogenetic analyses

Bayesian analysis (BA) of the ITS alignment was finished with the standard deviation of split frequencies of 0.008 (equals average) and was characterised by the average potential scale reduction factor 1.00 (maximal 1.002) and pooled effective sample size from two MCMC runs 4151.3494. Maximum likelihood analysis (ML) resulted in a tree with a final optimisation log likelihood of -7019.372. BA produced a tree with a partly similar topology to ML tree but contained large polytomy at one of the basal nodes. Hereinafter we present and discuss the topology of the BA tree plotted with both posterior probabilities (pp) from BA and bootstrap supports (bs) from ML.

The phylogenetic tree, generated for the ITS dataset, contains monophyletic and polyphyletic taxa as well as several species represented by a single sequence (Fig. 12). *Subulicystidium oberwinkleri* is the most basal member of the ingroup. Other singletons descending from the basal nodes are *S. nikau*, the most deviating sequence of the morphospecies *S. brachysporum* (TU 110416) (see Discussion for the explanation) and *S. rarocrystallinum*. The clade dominated by Reunionese collections (pp=1, bs=87%) contains the new species *S. parvisporum* (pp=1, bs=99%) and *S. harpagum* (pp=1, bs=98%). The latter includes also L. Ryvarden's collection from Colombia (LR 15736 in O:F: 918487). Two sequences of *S. perlongisporum* are also placed in the basal part of the tree but do not form a separate clade and, moreover, one sequence (from LE 302156) forms a clade with *S. boidinii* (KAS:L 1584a). *S. robustius* is recovered as a distinct clade (pp=1, bs=100%) of four sequences from Neotropics subtended by a long branch. *S. fusisporum,* represented by three sequences from the Caribbean region (pp=1, bs=98%), is a sister species to *S. tedersooi* represented by two sequences from Vietnam (branch support pp=1, bs=98%).

The remaining three clades each contain a mixture of sequences belonging to the morphospecies *S. brachysporum* and *S. meridense* with their likes. One clade contains also single sequences of *S. obtusisporum* from Germany (FR: W213-3-I) and *S. harpagum* from Jamaica (GB:KHL 10733). This clade is joined by three sequences of *S. brachysporum*: first by two sequences from Réunion (KAS:L 1498 and 1795) and at the next ancestor node with one sequence from Costa Rica (GB:KHL 11216). Another large clade is roughly equally rich in sequences of *S. brachysporum* and *S. meridense* (pp=1, bs=100%) and joined by a single sequence of *S. inornatum* (pp=0.88, bs=41%). One large clade (pp=1, bs=99%) included more collections of *S. brachysporum*, mostly sensu Boidin and Gilles (1988) and less of *S. meridense*, but also a single sequence of *S. grandisporum* (LR 29162 in O:F 506781). One more sequence of *S. brachysporum* (GB:KHL 10411) formed a weakly supported clade with the three sequences of *S. longisporum* from Europe (pp=0.84, bs<50%).

Bayesian analysis (BA) of the 28S alignment was finished with the standard deviation of split frequencies of 0.004 (equals average) and was characterised by the average potential scale reduction factor 1.00 (maximal 1.005) and pooled effective sample size from two MCMC runs 4673.55. Maximum likelihood analysis (ML) resulted in a tree with a final optimisation log likelihood of -2209.83. BA produced the tree with the topology highly similar to that of the ML tree. Hereinafter we present and discuss

the topology of the BA tree plotted with both posterior probabilities (pp) from BA and bootstrap supports (bs) from ML, mostly focusing on differences from the results obtained for the ITS dataset.

The most basal ingroup members on the 28S tree were *S. oberwinkleri* (clade with two sequences, pp=1, bs=100%), *S. rarocrystallinum* and *S. harpagum* from Jamaica (GB:KHL 10733) (Fig. 13). The species *S. harpagum* and *S. parvisporum* remained in the single clade (pp=1, bs=92%) but were recovered as polyphyletic due to the placement of sequence from the KAS:L 1226 amongst the sequences of *S. harpagum*. They were joined by the clade containing sequences from *S. obtusisporum* (GB: KHL 10622) and *S. brachysporum* (TU 110416). The remaining sequences, mostly belonging to *S. brachysporum* and *S. meridense*, occupy upper nodes of the tree without clear grouping by morphospecies. 28S dataset was importantly enriched by the sequences from specimens, for which the ITS region could not be sequenced: GB:KHL 10566, O:F 506782 and LY 12772 (*S. brachysporum* sensu Boidin and Gilles), LY 11378 (*S. brachysporum* sensu Talbot), GB:KHL 11355 and LY 12816 (*S. meridense*), KHL 15325 (*S.* aff. *meridense*) and LY 12750 (*S. parvisporum*).

Bayesian and Maximum likelihood phylogenetic analyses of the concatenated alignment (ITS+28S) resulted in a tree topology which was contributed by both ITS and 28S regions (Fig. 14). In line with the ITS-based tree, *S. boidinii* and *S. harpagum* were recovered as polyphyletic but *S. parvisporum* as monophyletic (cf. Fig. 12). Furthermore, the German sequence of *S. obtusisporum* was found in the brachysporum-meridense clade and not between the sequences of *S. longisporum* (cf. Fig. 13). On the other hand, similarly to 28S-based tree, *S. fusisporum* and *S. tedersooi* intruded the brachysporum-meridense clades. Additionally, the 28S portion of concatenated alignment contributed to resolving a polytomy at one of the basal node containing sequences of *S. nikau* and *S. rarocrystallinum*.

Spore-based species comparisons

We measured in total 2840 basidiospores from 67 specimens of *Subulicystidium*. We defined three groups of species according to the principal basidiospore shape: species with fusiform, cylindric and allantoid basidiospores. We found that some of the species could be delimited based on the basidiospore morphology solely, while, for other species, this was not possible and additional morphological characters had to be considered.

The species with fusiform basidiospores are barely distinguishable according to the basidiospore length. It varied generally from 8 to 11 µm, while the mean value did not exceed 10 µm (Fig. 11a). The only exception was *S. fusisporum* which had spores 10.7–12.3 µm long (main range, i.e. 5–95% quantiles of measurements data) and 11.5 µm long in average. Three species, viz. *S. robustius*, *S. inornatum* and *S. fusisporum* were indistinguishable in the spore width which varied for three of them between 2.5 and 3.5 µm. In contrast, *S. ryvardenii* had broader basidiospores with the main range 3.5–4.2 µm and mean value 3.8 µm. *S. naviculatum* was distinguished by the broadest

fusiform basidiospores (main range 4.3–5.0 µm, mean value 4.6 µm), while *S. tedersooi* by the narrowest fusiform basidiospores (main range 2.1–2.6 µm, mean value 2.4 µm). The spore length to width ratio was as useful as spore width to discriminate the species and was remarkably the lowest in *S. naviculatum* (2.0–2.5, mean 2.2) and the highest in *S. tedersooi* (3.5–5.0, mean 4.2).

Under allantoid basidiospores, we considered those with adaxial side clearly concave and having length to width ratio around 2, thus looking rather as reniform or phaseoliform. Amongst the species with such spores, *S. oberwinkleri* had distinctly the longest and the broadest spores: mean length and width were 9.2 and 4.7 µm, respectively (Fig. 11b). *S. nikau* could be distinguished from *S. boidinii* by broader spores. The spore width in *S. nikau* was 3.9–4.6 µm (mean 4.2 µm) and length-width ratio is 1.6–2.0 (mean=1.8), while *S. boidinii* has spores 2.8–3.3 µm broad (mean 3.1 µm) and length-width ratio 1.9–2.4 (mean=2.1). Comparing our own data with the Cunningham's specimen of *S. nikau* (holotype) and Boidin's specimen of *S. boidinii* showed that we have the same understanding of the respective species as the mentioned authors. *S. harpagum* and *S. parvisporum* had rather overlapping spore width and length to width ratio but differed in the spore length: 5.6–8.3 µm (mean 6.7 µm) in the former versus 5.0–6.2 µm (mean 5.6 µm) in the latter.

Species with cylindric basidiospores (Fig. 11c) were characterised by the average length to width ratio of at least 2.8 and were well distinguished by the mean spore length: 12.7 µm in *S. grandisporum*, 10.7 µm in *S. obtusisporum* and 9.2 µm in *S. cylindrosporum*. The collections of the meridense-brachysporum morphogroup were also characterised by cylindrical basidiospores but of the smaller length and were less clearly distinguishable. Examples of single collections representing each morphogroup (Fig. 11d) showed that *S. brachysporum* sensu Boidin and Gilles (1988) had on average slightly longer basidiospores than *S. brachysporum* sensu Talbot (1958), viz. 7.9 µm versus 7.3 µm. Curved spores of the classical *S. meridense* were on average shorter than straight spores of *S.* aff. *meridense*, viz. 6.9 µm versus 8.2 µm. Spores in the type specimen of *S. meridense* were of the intermediate average length compared to the two former examples (7.4 µm). The spore width and length to width ratio were very much overlapping in the material of meridense-brachysporum morphogroup.

With reference to the newly obtained data, in the next section we present the key to the genus *Subulicystidium*. We used the successful key of Gorjon et al. (2011) as the basis and a source of the information on the long-spored taxa.

Morphological key to the genus *Subulicystidium*

1 Basidiospores acicular, Q>4.5 ..... **2**
– Basidiospores cylindrical, fusiform, allantoid to reniform, Q<4.5..... **5**
2 Basidiospores 12–16 × 2–3 µm, Q=4.5–7..... ***longisporum***
– Basidiospores longer, Q>7..... **3**
3 Basidiospores spirally curved, 27–35 µm long..... ***curvisporum***
– Basidiospores straight or only slightly curved, shorter **4**

4 Basidiospores 20–27 × 2–3 µm, cystidial crystalline sheath ends with a bundle of needle-like crystals ..... ***cochleum***
– Basidiospores 16–25 × 1.5–2.5 µm, cystidia with regular ornamentation (rows of rectangular crystals) ..... ***perlongisporum***
5 Basidiospores fusiform **6**
– Basidiospores cylindric to broad cylindric (straight or curved) **11**
6 Basidiospores 4–5 µm wide ..... ***naviculatum***
– Basidiospores narrower **7**
7 Cystidia almost smooth, without regular rectangular crystalline protrusions ..... ***inornatum***
– Cystidia with regular ornamentation (rows of rectangular crystals) **8**
8 Cystidia with large crystalline protrusions **9**
– Cystidia with small to moderately large crystalline protrusions **10**
9 Basidiospores 2.5–3.5 µm wide ..... ***robustius***
– Basidiospores broader than 3.5 µm ..... ***ryvardenii***
10 Basidiospores 8.5–11.5 × 2–2.5 µm wide ..... ***tedersooi***
– Basidiospores 10.5–12.5 × 2.5–3.5 µm wide ..... ***fusisporum***
11 Basidiospores broad cylindric, Q=1.5–2.5 ..... **12**
– Basidiospores cylindric, Q=2.5–4.5 ..... **14**
12 Cystidia covered with irregularly shaped large crystalline plates, 80–150 × 5.5–10 µm, basidiospores 8–11 × 4.0–5.5 µm ..... ***oberwinkleri***
– Cystidia with regular ornamentation (rows of rectangular crystals) and smaller, basidiospores also smaller **13**
13 Basidiospores 7–9 × 3.5–4.5 µm ..... ***nikau***
– Basidiospores 6–8 × 2.8–3.5 µm ..... ***boidinii***
14 Basidiospores 3–4 µm wide **15**
– Basidiospores 2–3 µm wide **17**
15 Basidiospores 10–15 µm long ..... ***grandisporum***
– Basidiospores shorter **16**
16 Basidiospores 9–13 µm long, cystidia with regular rows of rectangular crystals ..... ***obtusisporum***
– Basidiospores 8–10.5 µm long, cystidia bear rectangular to rounded, rather sparse and irregularly arranged crystals ..... ***rarocrystallinum***
17 Basidiospores 5.0–6.2 µm long ..... ***parvisporum***
– Basidiospores longer **18**
18 Basidiospores 7–10.5 µm long ... ***brachysporum*** **sensu Boidin and Gilles (1988)**
– Basidiospores 6–8 µm long **19**
19 Crystal protrusions on cystidia are short rods that project backwards under acute angle, giving cystidia the resemblance of a harpoon ..... ***harpagum***
– Cystidia with regular ornamentation (rows of rectangular crystals) **20**
20 Basidiospores elliptic with attenuated base, usually straight ..... ***brachysporum*** **sensu Talbot (1958)**
– Basidiospores cylindric, straight to regularly curved ..... ***meridense***

Discussion

General remarks

In this study, we describe 11 new species of *Subulicystidium* based on morphological evidence and rDNA ITS and 28S sequence analyses. Ten of these species are characterised by a unique combination of basidiospore and cystidium morphology and rDNA sequence identity. One species (*S. ryvardenii*) could not be sequenced but the morphological evidence itself was sufficient for describing it as a new species. With our contribution, the number of the known species in the genus *Subulicystidium* now totals 20. The provided morphological key to all known species should facilitate identification of specimens previously treated as highly variable *S. longisporum* or left without species name. Such literature is urgently needed to assist in tropical fungal inventories.

We revised also the morphological and genetic borders of the five previously known species. One of them, *S. naviculatum,* could not be sequenced, while for *S. nikau*, only one specimen with amplifiable DNA was available. For the two morphospecies, *S. brachysporum* and *S. meridense*, numerous sequences from different localities were obtained. Our data show that, despite differences in the protologues, the species are hard to separate morphologically and molecularly. They share, to a large extent, basidiospore size and shape as well as highly similar ITS and 28S sequences, leading to strongly intermixed clades in phylogenetic trees. Therefore, *S. brachysporum* and *S. meridense,* in current understanding, are highly polyphyletic.

Species distributions

Our study is based on examination of a large set of specimens from numerous localities in Paleo- and Neotropics. Upon this, we could show that the diversity of the short-spored *Subulicystidium* species is much higher than previously known. The newly described *S. robustius* is in fact a frequently occurring species in the Caribbean region and in South America. Furthermore, we could report a multicontinental distribution for several species, verified by DNA sequence data. *S. brachysporum*, *S. boidinii*, *S. harpagum* and *S. oberwinkleri* are typified by material from Paleotropics (South Africa in the first species and Réunion in three others), but were found by us also in South America. The morphospecies *S. meridense*, described from Venezuela (Oberwinkler 1977) and later found in Costa Rica (Kisimova-Horovitz et al. 1997), was also found on Réunion Island by Boidin and Gilles (1988). In addition to sequenced collections from a few more countries in South America, we confirmed the species presence on Réunion by sequencing collections of Boidin and Gilles (1988). In this study, we also report *S. meridense* for the first time from South-East Asia (Taiwan). The new species *S. fusisporum* was first considered by us as a Caribbean endemic. Re-identification as *S. fusisporum* of the specimen collected by G. Gilles in Côte D'ivoire (LY 7375, originally labelled as *S. longisporum,* DNA could not be amplified) may suggest that the species is present in West Africa as well.

It was surprising for us to find the species occurring on more than one continent or on islands separated by thousands of kilometres. For fungi with spores carried by wind, dispersal limitation was shown to act strongly even at small spatial scales (Peay et al. 2010, Norros et al. 2012). Given the architecture and location of *Subulicystidium* fruit-bodies (next to the ground, not rarely underside of the logs), one would expect prevailing spore dispersal distance smaller than 1 m (Galante et al. 2011). However, in macrofungi, there remains a probability of spore travel on a distance of kilometres (Nordén and Larsson 2000, Peay et al. 2012, Norros et al. 2014) and also overseas (Geml et al. 2012). Spore morphology traits have been recently discussed in a connection with dispersal and arrival success of a species (Norros et al. 2014, Calhim et al. 2018). In this regard, the genus *Subulicystidium*, with a high diversity of spore size and shape between species, is an interesting object to correlate spore traits and biogeography in future studies.

Remarks on the previously known species

***Subulicystidium brachysporum* (P.H.B. Talbot & V.C. Green) Jülich**
Photos of fresh collections in PlutoF: link 1, link 2
Figs 8; 10l, m

Notes. Unfortunately, we were not able to study the type specimen of *Peniophora longispora* var. *brachyspora* (Talbot's No. 40683 in PREM, Mycology Division, ARC-Plant Protection Research Institute, Queensland, South Africa). However, reviewing taxonomic literature on *Subulicystidium* brought us to delineating two morphogroups in the species *S. brachysporum*, according to the views of the earlier authors. Talbot (1958), while describing *Peniophora longispora* var. *brachyspora*, characterised its basidiospores as "elliptic-fusoid, 6.4–8 × 2.2–3.2 µm … sometimes with a faint band about the middle". Boidin and Gilles (1988) described the basidiospores of *S. brachysporum* from Réunion as elliptic in frontal face and bananiform (cylindric with slightly attenuated apex, slightly curved) in lateral face, 7.5–10 × 2–2.5(–3) µm. Therefore, we differentiated groups of (i) *S. brachysporum* sensu Talbot, i.e. sensu typi, with straight oblong-elliptic basidiospores having long attenuated base, with the mean length below 7.5 µm and mean length to width ratio hardly reaching 3 (see Fig. 10m); and (ii) *S. brachysporum* sensu Boidin and Gilles with cylindric and slightly curved basidiospores with the mean length over 7.5 µm and length to width ratio between 3 and 4 (Fig. 10l). In our dataset, *S. brachysporum* sensu Boidin and Gilles was represented by far more specimens than *S. brachysporum* sensu Talbot.

The description and illustration of *S. brachysporum* by Duhem and Michel (2001) (specimen Bourdot No. 7986) and by Oberwinkler (1977, fig. 25, "authentical material") correspond to the protologue and drawing of *S. brachysporum* by Talbot (1958). On the other hand, our examination of Venezuelan collection (FO15970 in TUB, see Oberwinkler 1977, fig. 29) let us assign it to *S. brachysporum* sensu Boidin and Gilles

(1988). Descriptions by Liberta (1980), Maekawa (1998), Martini (2016) and illustrations by Eriksson et al. (1984, Fig. 764 f, g – for single specimens from Canada and USA), match *S. brachysporum* sensu Boidin and Gilles (1988) as well.

DNA sequence similarity analyses and phylogenetic reconstructions did not support the presence of two morphogroups of *S. brachysporum*. Sequences of both morphotypes occured in the same clade and, moreover, shared the clade with sequences from the morphospecies *S. meridense*. For the moment, we prefer to retain two morphogroups of *S. brachysporum*, thus promoting further exploration of species limits and examination of the type specimen.

The most comprehensive overview of global occurrence of *S. brachysporum* was provided by Martini (2016). We can add that the species is present also in South America, Caribbean region and Madagascar.

Specimens examined: *Subulicystidium brachysporum* sensu Talbot (1958). ARGENTINA. Misiones: Iguazu National Park, Cataratas de Iguazu, -25.6748, -54.4532, on dead wood of angiosperm tree, 1-5 Mar 1982, L.Ryvarden (LR 19687 in O:F 506779). BRAZIL. Rondonia: Porto Velho, Rua Rio Madeira 7014, Nova Esperanca, -8.7160, -63.8785, on dead wood of angiosperm tree, 11 Mar 2012, K.-H. Larsson (KHL 15318 and 15327 in O:F). Sao Paulo: Santos, Ubatuba, Ilha Anchieta, -23.5500, -45.0667, on dead wood, 17-18 Jan 1987, D.Pegler, K.Hjortstam & L.Ryvarden (LR 24170 and LR 24203 in O:F). COLOMBIA. Magdalena: Parque Nacional Tayrona, Estacion de Gairaca, 0-30 m, 11.3170, -74.1063, on dead wood, 12 Jun 1978, L.Ryvarden (LR 15755 in O:F 918492). RÉUNION. Saint-Paul: Saint-Paul, St-Gilles-II, Ravine de St. Gilles, abandoned orchard, 200 m, on dead wood, 26 Apr 1985, J.Boidin (LY 11378).

Specimens examined: *Subulicystidium brachysporum* sensu Boidin and Gilles (1988). ARGENTINA. Misiones: Iguazu National Park, Cataratas de Iguazu, -25.6748, -54.4532, on dead wood of angiosperm tree, 1-5 Mar 1982, L.Ryvarden (LR 19533 in O:F 506782). BRAZIL. Pará: Belem, Museo Goeldii Scientific Centre, -1.4525, -48.4764, on dead wood, 25 Nov 2013, K.-H.Larsson (KHL 16461 in O:F). Paraiba: Areia, Reserva Estadual Mata do Pau-Fero, -6.9642, -35.7496, on dead wood of angiosperm tree, 28 Apr 2013, K.-H.Larsson (KHL 16100 in O:F). Rondonia: Porto Velho, Rua Rio Madeira 7014, Nova Esperanca, -8.7160, -63.8785, on dead wood of angiosperm tree, 11 Mar 2012, K.-H.Larsson (KHL 15330 in O:F); Rubber plantation park, -8.7324, -63.9008, on dead wood of angiosperm tree, 11 Mar 2012, K.-H.Larsson (KHL 15352 in O:F). Sao Paulo: Sao Paulo, Instituto de Botanica, -23.6450, -46.6261, on dead wood, 24 Jan 1987, K.Hjortstam (Hjm 16573 in GB). COLOMBIA. Magdalena: Parque Nacional Tayrona, Estacion de Gairaca, 0-30 m, 11.3170, -74.1063, on dead wood, 12 Jun 1978, L.Ryvarden (LR 15784 in O:F 918490; LR 15744B in O:F 918491; LR 15784 in O:F 918493). COSTA RICA. Alajuela: Bijagua, Albergue Heliconias, Sendero Heliconias, 770 m a.s.l, 10.7181, -85.0453, on branch of angiosperm tree, 12 Jul 2001, K.-H.Larsson (KHL 11216 in GB); La Fortuna de San Carlos, Parque Nacional Volcán Arenal, Sendero Pilón, 650 m, 10.4589, -84.7644, on dead wood of angiosperm tree, 15 Jul 2001, K.-H.Larsson

(KHL 11419 in GB). Puntarenas: Coto Brus, Sabalito, Zona Protectora Las Tablas, La Neblina, 8.9149, -82.7719, on stem of angiosperm tree, 5 Nov 2004, K.-H.Larsson (KHL 12764 in GB). DOMINICAN REPUBLIC. Provincia La Altagracia: Parque Nacional del Este, Playa Guaraguao, 18.3269, -68.8092, on dead wood, 3 Jun 1997, K.-H.Larsson (KHL 9920 in GB). ETHIOPIA. Oromia: West Shewa Zone, Ginchu, Chilomo Forest, 9.0900, 38.1700, on dead wood, 10 Jul 1990, L.Ryvarden (LR 28047 in O:F 909592). JAMAICA. Cornwall County: Trelawny parish, Cockpit Country, Ramgoat Cave, 350 m a.s.l., 18.3378, -77.5568, on twigs of angiosperm tree, 11 Jun 1999, K.-H.Larsson (KHL 10686 in GB); N of Crowlands, trail/road into park area, 18.2611, -77.6511, on branch of angiosperm tree, 10 Jun 1999, K.-H.Larsson (KHL 10633 in GB); Windsor Cave, along trail to Troy, 18.3564, -77.6472, on branch of angiosperm tree, 13 Jun 1999, K.-H.Larsson (KHL 10763 in GB). Middlesex County: Manchester parish, Marshalls Pen, Sutton Farm, 18.0589, -77.5308, on branches of angiosperm tree, 8 Jun 1999, K.-H.Larsson (KHL 10505 in GB), on stem of angiosperm tree, 8 Jun 1999, K.-H.Larsson (KHL 10566 in GB). Surrey County: Portland parish, between reach and Ecclesdown hillside to the east, 500 m, 18.0433, -76.3108, on branch of angiosperm tree, 16 Jun 1999, K.-H.Larsson (KHL 10855 in GB). MADAGASCAR. Anosy: Tolagnaro, Mandena Conservation Zone, -24.9529, 47.0028, on dead wood of angiosperm trunk, 10 Mar 2010, K.-H.Larsson (KHL 14359 and 14365 in O:F); Nahampoana, -24.9667, 46.9667, on dead wood of angiosperm trunk, 12 Mar 2010, K.-H.Larsson (KHL 14390 in O:F); Petriky Conservation Zone, -25.0667, 46.8500, on dead wood of angiosperm trunk, 14 Mar 2010, K.-H. Larsson (KHL 14486, 14505 and 14516 in O:F); Sainte Luce Conservation Zone, -24.8028, 47.1636, on dead wood of angiosperm trunk, 15 Mar 2010, K.-H.Larsson (KHL 14537 in O:F). Ihorombe: Isalo National Park, Namaza, along track from entrance to waterfall, -22.5583, 45.4000, on dead wood of angiosperm trunk, 7 Mar 2010, K.-H.Larsson (KHL 14293 in O:F). PAPUA NEW GUINEA. Morobe: Bawituc, Lae 12-Mile, Uphill of Bawituc, -6.6390, 146.9089, on dead twig, 3 Jul 1905, L.Tedersoo (TU 110416). PUERTO RICO. Municipio Isabela, Moñtanas Aymamón, Bosque Estatal de Guajataca, Verada Nueva Trail, 230 m, 18.4242, -66.9678, on dead fruitbodies of polypore, 26 Jun 1996, K.-H.Larsson (KHL 9544 in GB). Municipio Luquillo, Luquillo Mts, Bisley Experimental Watersheds, along the logging road, 215 m, 18.3161, -65.7467, on log, 6 Jun 1998, K.-H.Larsson (KHL 10270 in GB); Sabana, above Chicken Farm & Rio Sabana, 70 m, 18.3500, -65.7344, 10 Jun 1998, on woody branch, K.-H.Larsson (KHL 10406 in GB) and on wet log, K.-H.Larsson (KHL 10411 in GB), on wood of angiosperm tree, 7 Jun 1997, K.-H.Larsson (KHL 10088, 10095 and 10097 in GB). RÉUNION. Saint-Benoît: La Plaine-des-Palmistes, Palmistes III-87, en descendant vers St Benoit, alt 800 m, on dead wood, 23 Mar 1987, G.Gilles (LY 12456); Salazie, Hell Bourg, ca 1000 m, -21.0642, 55.5269, on dead branch, 23 Mar 2015, M.Striegel (L 1584b in FR and KAS). Saint Pierre: Cilaos, Cirque de Cilaos, Roche Merveilleux, Sentiere botanique, 1300 m, -21.1232, 55.4920, on strongly decayed wood, 15 Mar 2013, E.Langer (L 0134 in FR and KAS); L'Étang-Salé, Étang-Salé-87: forêt domaniale, alt 60-80 m, on dead wood of Fabaceae

tree, 14 Mar 1987, G.Gilles (LY 12293); Saint-Philippe, Baril-II-87, depart du sentier botanique, 100-150 m, on dead branch, 6 Apr 1987, G.Gilles (LY 12772), Forêt de Mare Longue, 495 m, -21.3438, 55.7410, on dead branch, 28 Mar 2015, M.Striegel (L 1795 in FR and KAS); Saint-Pierre, Piton de Mont Vert, ca 560 m, -21.3279, 55.5413, on dead wood, 18 Mar 2015, M.Striegel (L 1498 in FR and KAS), Piton de Mont Vert, hiking path, ca 560 m, -21.3279, 55.5413, on dead branch, 18 Mar 2015, J.Riebesehl & M.Schroth (L 1147 in FR and KAS). VENEZUELA. Estado Amazonas: Manapiare, Yutajé, 110 m, 5.6142, -66.1236, on dead wood of angiosperm tree, 12-19 Jun 1997, L.Ryvarden (LR 40650A in O:F 909582). Estado Merida: Merida, 8.6249, -71.1395, on dead wood, 1969, F.Oberwinkler (FO 15970 in TUB).

Subulicystidium longisporum (Pat.) Parmasto

Note. The following specimen was used to illustrate the species on Fig. 10g:

UKRAINE. Zakarpatska: Carpathian Biosphere Reserve, vicinities of Mala Uholka village, 670 m, 48.2632, 23.6175, on decayed deciduous wood, 11 Sep 2013, A.Ordynets (CWU 6737).

Subulicystidium meridense Oberw.

Figs 9; 10n–p

Notes. According to Oberwinkler (1977), the basidiospore size of the type collection (FO13761 in TUB) is 6–8 × 2.5–3 µm, suggesting the mean basidiospore length, width and their ratio are 6.5 µm, 2.75 µm and 2.36, respectively. Our re-measuring revealed longer basidiospores, namely 6.6–8.1 × 2.4–2.8 µm, with the length to width ratio 2.6–3.2 (2.9) (see Supplementary files 2-4).

When describing *S. meridense*, Oberwinkler (1977) stressed the importance of allantoid, i.e. clearly curved, basidiospores. We adhered to this concept assigning our collections to *S. meridense* (see Figs 10 n, o). We named those with similar spore size but with straight cylindric spores "*Subulicystidium* aff. *meridense*" (Fig. 10p). However, molecular level sequence similarity analyses and phylogenetic reconstructions did not support the presence of two distinct groups.

In addition to the South American and Reunionese specimens, we examined also specimens of *S. meridense* from India and Taiwan. Boidin and Gilles (1988) reported LY 12456 and LY 12772 from Réunion as *S. meridense* with the note that the spores are larger and more elongated than in the Venezuelan type material. Our morphological examination (and DNA sequence data for LY 12772) showed that the Reunionese collection represent *S. brachysporum* sensu Boidin and Gilles. However, we concur with Boidin and Gilles that LY 12816 (sequenced) from Réunion and LY 9144 from Gabon should be named *S. meridense*. The description of the specimen TMI 25520 from Vanuatu (Maekawa 2002) corresponds to our concept of *Subulicystidium* aff. *meridense*.

Specimens examined: ***Subulicystidium*** **aff.** ***meridense***. ARGENTINA. Misiones: Iguazu National Park, Cataratas de Iguazu, -25.6748, -54.4532, on wood of angiosperm tree, 1-5 Mar 1982, L.Ryvarden (LR 19581 in O:F). BRAZIL. Rondonia: Porto Velho, Rubber plantation park, -8.7324, -63.9008, on dead wood of angiosperm tree, 11 Mar 2012, K.-H.Larsson (KHL 15325 in O:F). Sao Paulo: Santos, Ubatuba, Ilha Anchieta, -23.5500, -45.0667, on dead wood, 17-18 Jan 1987, D.Pegler, K.Hjortstam & L.Ryvarden (LR 24201 in O:F). COLOMBIA. Magdalena: Parque Nacional Tayrona, Estacion de Gairaca, 0-30 m, 11.3170, -74.1063, on dead wood, 12 Jun 1978, L.Ryvarden (LR 15812 in O:F 918846). PUERTO RICO: Municipio de Cerro Alto, Montanas Aymamon, limestone magote near Parador Guajataca, 60 m, 18.4828, -66.9583, on strongly decayed log of angiosperm tree, 27 Jun 1996, K.-H.Larsson (KHL 9561 in GB). Municipio Luquillo, Luquillo Mts, Sabana, above Chicken Farm & Rio Sabana, 70 m, 18.3500, -65.7344, on log, 10 Jun 1998, K.-H. Larsson (KHL 10397 in GB).

Specimens examined: ***Subulicystidium meridense*****.** ARGENTINA. Misiones: Iguazu National Park, Cataratas de Iguazu, -25.6748, -54.4532, dead wood of angiosperm tree, 1-5 Mar 1982, L.Ryvarden (LR 19688 in O:F 506784). BRAZIL. Rondonia: Porto Velho, Rua Rio Madeira 7014, Nova Esperanca, -8.7160, -63.8785, on dead wood of angiosperm tree, 11 Mar 2012, K.-H.Larsson (KHL 15322 in O:F). Sao Paulo: Santos, Ubatuba, Ilha Anchieta, -23.5500, -45.0667, on decayed wood, 17-18 Jan 1987, D.Pegler, K.Hjortstam & L.Ryvarden (Hjm 16400 in GB). CENTRAL AFRICAN REPUBLIC. Lobaye: Nola, Boukoko, 3.8929, 17.9153, on dead tree trunk, 18 May 1965, J.Boidin (in LY 5476). COSTA RICA. Guanacaste: Area cons. Tempisque, Reserva Biologica Lomas Barbudal, near the entrance, 20-40 m, 10.5103, -85.3744, on dead wood of angiosperm tree, 14 Jul 2001, K.-H.Larsson (KHL 11355, 11365 and 11368 in GB). Puntarenas: Coto Brus, Sabalito, Zona Protectora Las Tablas, Progreso, Camino a Cotoncito, 1560 m, 8.9306, -82.8031, on wood of angiosperm tree, 3 Nov 2004, K.-H.Larsson (KHL 12557 in GB), La Neblina, 1350 m, 8.9149, -82.7719, on wood of angiosperm tree, 5 Nov 2004, K.-H.Larsson (KHL 12732 in GB). San José: Dota, San Gerardo, around Hotel Savegre, ca 2000 m, 9.5643, -83.8016, on branch of angiosperm tree, 9 Nov 2004, K.-H.Larsson (KHL 12969 in GB). GABON. Estuaire: Libreville, Bush littoral, km 13 N Libreville, 0.5338, 9.4673, on bark of dead wood, 2 Feb 1979, G.Gilles (LY 9144). INDIA. Darjeeling: Sukna, About 4 km from Sukna towards RongTong, 26.8246, 88.3625, on bark of dead/decaying branch of angiosperm, 9 Aug 1980, G.S.Dhingra (19201 in O:F 909586). RÉUNION. Saint-Benoit: Saint-Benoit, Route forestière 3 de Takamaka, -21.1038, 55.5724, on dead wood, 8 Apr 1987, G.Gilles (LY 12816). TAIWAN. Nantou: Huisun Recreation Area, path to the “stone frog”, 24.0912, 121.0337, on dead wood, 26 Apr 1996, G.Langer, E.Langer & C.-J.Chen (GEL 3520 and 3530 in KAS). VENEZUELA. Estado Aragua: Maracay, National Park Henri Pittier, Rancho Grande, 10.3800, -67.6190, on hardwood, 22 Jun 1995, L.Ryvarden (LR 35544/C in O:F). Estado Merida: Merida, Vicinities of Instituto Forestal Latino-Americano, 8.6249, -71.1395, on dead twigs, 27 Nov 1968, F.Oberwinkler (FO 13761 in TUB, holotype).

***Subulicystidium naviculatum* Oberw.**
Figs 3a, b; 10a

Notes. We examined a single collection from Costa Rica (KHL 11566 in GB) which had broad fusiform basidiospores (8.6–)8.8–11.2(–11.6) × (4.0–)4.3–5.0(–5.3) µm, i.e. slightly shorter than in the holotype specimen FO 12778 (TUB) from Venezuela: 10–12 × 4.5–5 µm (Oberwinkler 1977). Furthermore, the cystidia in our specimen were covered with rows of rectangular crystals while the ornamentation pattern of cystidia in the holotype resembled that of *S. harpagum*, i.e. short rod-like protrusions that project backwards under an acute angle, giving cystidia the resemblance of a harpoon (see fig. 32 in Oberwinkler 1977). Unfortunately, the holotype in TUB could not be located.

Kisimova-Horovitz et al. (1997) reported a collection from Costa Rica (207a-I, =FO 42968 in TUB) as *S. naviculatum.* However, we re-identified the collection as *S. robustius*.

Specimens examined. COSTA RICA. San José: Reserva Los Santos, Cerro de la Muerte, 1.5 km from Interamerican Highway along road to San Gerardo de Dota, 2850 m, 9.5964, -83.7986, on stem of angiosperm tree, 18 Jul 2001, K.-H.Larsson (KHL 11566 in GB).

***Subulicystidium nikau* (G. Cunn.) Jülich**
Figs 5c,d; 10u

Notes. The species was described by Cunningham (1955) as *Peniophora sororia* based on material from the midribs of the dead leaves of nikau palm (*Rhopalostylis sapida*) which is endemic to New Zealand. Later Cunningham (1963) noticed that the name was occupied (Bourdot and Galzin 1912; p. 386) and provided the legitimate name *Peniophora nikau*.

The holotype of *S. nikau* (PDD 13816) has basidiospores 7–9 × 4–5 µm as reported by (Cunningham 1955). We measured the holotype basidiospores as slightly narrower, viz. (6.8–)6.9–8.6(–9.0) × 3.3–4.1(–4.4) µm (see Supplementary files 2–4), confirming the measurements by Oberwinkler (1977) and Liberta (1980).

As the basidiospores are similar, *S. nikau* has been confused with *S. oberwinkleri*. The former has cystidia with regular ornamentation (rows of rectangular crystals) typical for the genus *Subulicystidium* and the generitype *S. longisporum*. In contrast, *S. oberwinkleri* has larger cystidia with large, irregularly shaped crystalline plates. Cunningham (1955) illustrated cystidia of *S. nikau* correctly, while the characterisation by Stalpers and Buchanan (1991) as “covered with plate-like crystals” is misleading. Oberwinkler (1977) and Maekawa (1998) realised the discrepancies in cystidial ornamentation but did not provide a solution. We re-identified the record of *S. nikau* from Reunion, LY12488 (Boidin and Gilles 1988) as *S. oberwinkleri*. We also collected and sequenced a Reunionese specimen with regularly ornamented cystidia

(KAS: L1296). Though sampled on dead wood and far from locus classicus, we keep it under the name *S. nikau* for the time being. Réunion is thus the second known locality of the species after New Zealand. The record from Venezuela reported by Liberta (1980) has not been studied and is hard to interpret because no illustration of cystidia was provided.

Specimens examined. RÉUNION. Saint-Pierre: Saint-Philippe, Sentier de Takamaka, ca 840 m, -21.0913, 55.6199, on dead wood, 26 Mar 2015, J.Riebesehl & M.Schroth (L 1296 in FR and KAS). NEW ZEALAND. Auckland: Cascades, Waitakere Ranges, on dead leaf midribs of palm *Rhopalostylis sapida*, 3 Apr 1954, S.D.Baker (PDD 13816, holotype).

Subulicystidium obtusisporum Duhem & H. Michel

Figs 6d, e; 10h

Notes. Duhem and Michel (2001) identified a collection from Venezuela (Oberwinkler 1977, Fig. 29, FO15970 in TUB) as *S. obtusisporum*, while we regard the same specimen as *S. brachysporum* sensu Boidin and Gilles (1988). Maekawa (2002) reported *S. obtusisporum* from Vanuatu and Ghobad-Nejhad et al. (2009) from Russian Caucasus. Here we report the species from East Asia and Caribbean region.

The first sequenced material of *S. obtusisporum* is our collection from Frankfurt am Main, central Germany (FR:W213-3-I). Fruit-body morphology, as well as microhabitat (exposed dead wood) agree with the data for the type specimen and related collections from southern France (Duhem and Michel 2001). Another sequenced specimen GB:KHL 10622 from Jamaica was very distant from the German specimen in terms of ITS and 28S sequence identity and position on the phylogenetic tree, which means *S. obtusisporum* is polyphyletic. Sequencing additional specimens, not least from Asia, is needed to clarify the taxonomy of this morphospecies.

Specimens examined. CHINA. Jilin: Chang Bai Shan Forest Reserve, Hangcong hou, 750 m, on dead wood of *Acer sp.*, 11-17 Sept 1983, L.Ryvarden (LR 21774 in O:F 909590). COSTA RICA. Guanacaste: Area cons. Tempisque, Reserva Biologica Lomas Barbudal, near the entrance, 20-40 m, 10.5103, -85.3744, on deadwood of angiosperm tree, 14 Jul 2001, K.-H.Larsson (KHL 11373 in GB). GERMANY. Hesse: Frankfurt, Science Park at the Campus Riedberg of Frankfurt University, 50.1701, 8.6300, on decayed trunk, 31 Mar 2016, O.Koukol (W213-3-I in FR). ITALY. Italy. Latina: Circeo Natural park, Selva de Circeo, 41.3429, 13.0534, on wood of *Quercus sp.*, 22-25 Oct 1984, K.Hjortstam, K.-H.Larsson & L.Ryvarden (LR 22458 in O:F 505520). JAMAICA. Cornwall County: Trelawny parish, Crown Lands, trail/road into park area, 18.2611, -77.6511, on branches of angiosperm tree, 10 Jun 1999, K.-H.Larsson (KHL 10622 in GB). PUERTO RICO. Municipio Luquillo, Luquillo Mts, Bisley Experimental Watersheds, along track from parking place, 215 m, 18.3161, -65.7467, on branch of angiosperm tree, 6 Jun 1997, K.-H.Larsson (KHL 9955 in GB). TAIWAN. Chiayi: Shi Ding, road No. 18 in direction to Alishan at

km 60, ca 1500 m alt, 23.4801, 120.4491, on dead wood, 1 May 1996, G.Langer, E.Langer & C.-J.Chen (GEL 3677 in KAS). Miaoli: Sheipa National Park, Kuanwu, forest ca 500 m in direction of Le Shan, trail on the left side of the road, ca 2100 m, 24.3624, 121.1252, on dead wood, 19 Apr 1996, G.Langer, E.Langer & C.-J.Chen (GEL 3409 in KAS).

***Subulicystidium perlongisporum* Boidin & Gilles**

Notes. The following specimen was used to illustrate the species on Fig. 10j: GERMANY. Hesse: Biedenkopf, little valley of Martinsbach creek, mixed forest 280 m, 50.5410, 8.3037, on dead wood, 23 Apr 2016, A.Ordynets & M.Theiss (Ordynets 00158 in KAS).

Which morphological characters are useful for the species delimitation in *Subulicystidium*?

In general, spore size and shape are of crucial importance for the taxonomy of fungi (Parmasto et al. 1987). In our study, however, we found that basidiospore morphology itself may be insufficient for species-rank identifications in *Subulicystidium*. In this regard, the usefulness of other morphological characters is worth discussing.

Jülich (1975) studied cystidia of *S. brachysporum* and *S. longisporum* under a scanning electron microscope and concluded the identity of their ornamentation pattern. Jülich (1975) hypothesised, after observing cystidia of *S. nikau* under a light microscope, that this ornamentation pattern was universal at the genus level. Regarding the shape of cystidia, Jülich (1975) noticed the less prominent basal swelling in *S. brachysporum* compared to *S. longisporum*. Oberwinkler (1977) characterised cystidia of *Subulicystidium* as uniform, but his remarks and especially illustrations displayed several deviations from the common pattern regarding both size and ornamentation. We further developed the idea of the importance of cystidial morphology and showed the presence of interspecific size differences as well as species-specific types of cystidial ornamentation (*S. oberwinkleri*, *S. harpagum*, *S. robustius*, *S. rarocrystallinum*). The important finding of Jülich (1975) is that the shape of single crystals can vary within a collection. The sharpness of the crystals is reduced with age, resulting in rounded instead of rectangular crystals as observed in a light microscope.

Beside cystidia, also hyphae and hymenial elements can have encrustation. Oberwinkler (1977) illustrated crystalline collars on the bases of basidia in the holotype of *S. meridense* and in the specimen of *S. brachysporum* sensu Boidin and Gilles (Figs 30 and 29. respectively in Oberwinkler 1977) but not in other species. Kisimova-Horovitz et al. (1997) noticed a nearly ubiquitous presence of hymenial encrustation in *Subulicystidium*. We share this opinion after examining our collections. Jülich (1968) and Liberta (1980) observed repetobasidia and considered them to be a criterion of

the genus *Subulicystidium*. On the contrary, Eriksson et al. (1984) did not observe any repetobasidia in the North European collections of *S. longisporum.* In our large set of tropical specimens we did not find any repetobasidia. Thus we suppose that repetobasidia observed by Jülich and Liberta are simply basidia with a well-developed crystalline collar.

Oberwinkler (1977) illustrated slightly thick-walled subicular hyphae in *S. meridense* but thin-walled in the rest of the species. We confirm this pattern for *S. meridense* but also observed similar deviating subicular hyphae in several other species, viz. *S. brachysporum*, *S. robustius*, *S. ryvardenii* and *S. oberwinkleri*. However, in all these cases, we found that the hyphal surface is rough and the wall is highly light-refractive, which means they are covered by crystalline material. In *S. harpagum,* this crystalline sheath around hyphae can reach a thickness of one and in *S. oberwinkleri* several micrometres. Under a light microscope, it is not possible to decide to what extent thickness depends on the cell wall or is due to the deposition of crystalline material.

Bourdot and Galzin (1912) reported a series of collections deviating from typical *S. longisporum* in fruit-body thickness and colouration and cystidial uniformity. The several varieties proposed by the French authors were not accepted by Jülich (1975) who considered them to represent normal variation and different developmental stages of *S. longisporum*. In line with Jülich (1975), after examining our specimens, we conclude that fruit-body thickness and density is variable within the species and thus of little value for the species-level identifications. Nevertheless, an experienced eye can differentiate the hirsute hymenial surface of the species with more robust cystidia (*S. robustius*, *S. ryvardenii*) from the velutinous hymenial surface of the rest of the genus. In a single species, *S. robustius*, we consistently observed slightly yellowish fruiting bodies, which was due to a yellowish hue of the hyphal and cystidial walls.

Morphology complements molecular data for species delimitation

Partitioning sequence dissimilarity of both ITS and 28S into interspecific and intraspecific components revealed a clear barcode gap for some of the species but problems to delimit others. Therefore, both cases when morphology and available molecular information are congruent and cases when they are in conflict were found. This points to the importance of careful morphological examination and the need to combine morphology, rDNA barcode data and other DNA markers when defining species in *Subulicystidium*.

Figures

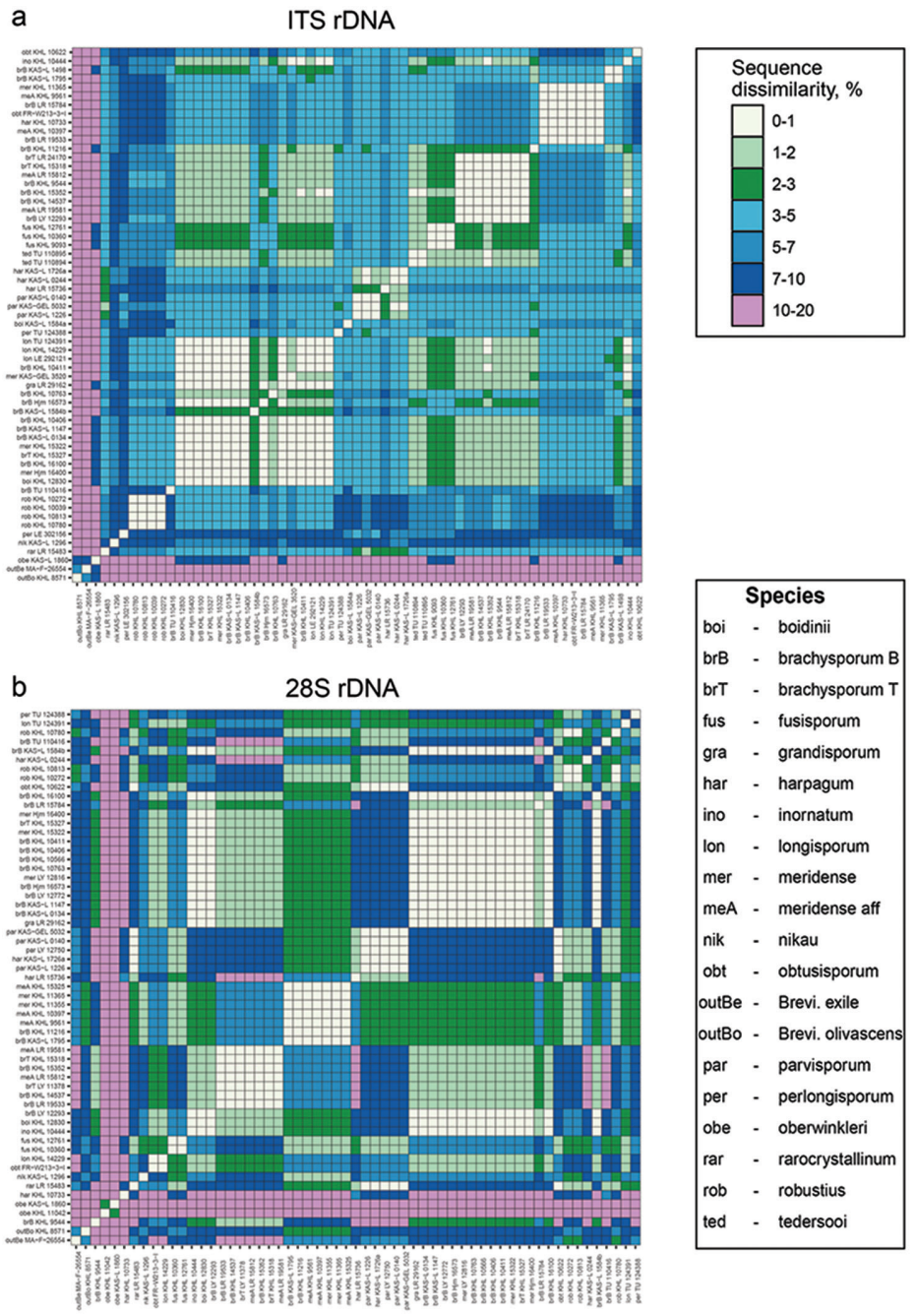

Figure 1. Raw pairwise dissimilarities (proportion of the differing sites, %) between *Subulicystidium* sequences of (**a**) ITS and (**b**) 28S region. Three-letter code before each specimen's number corresponds to a species epithet as explained in the legend.

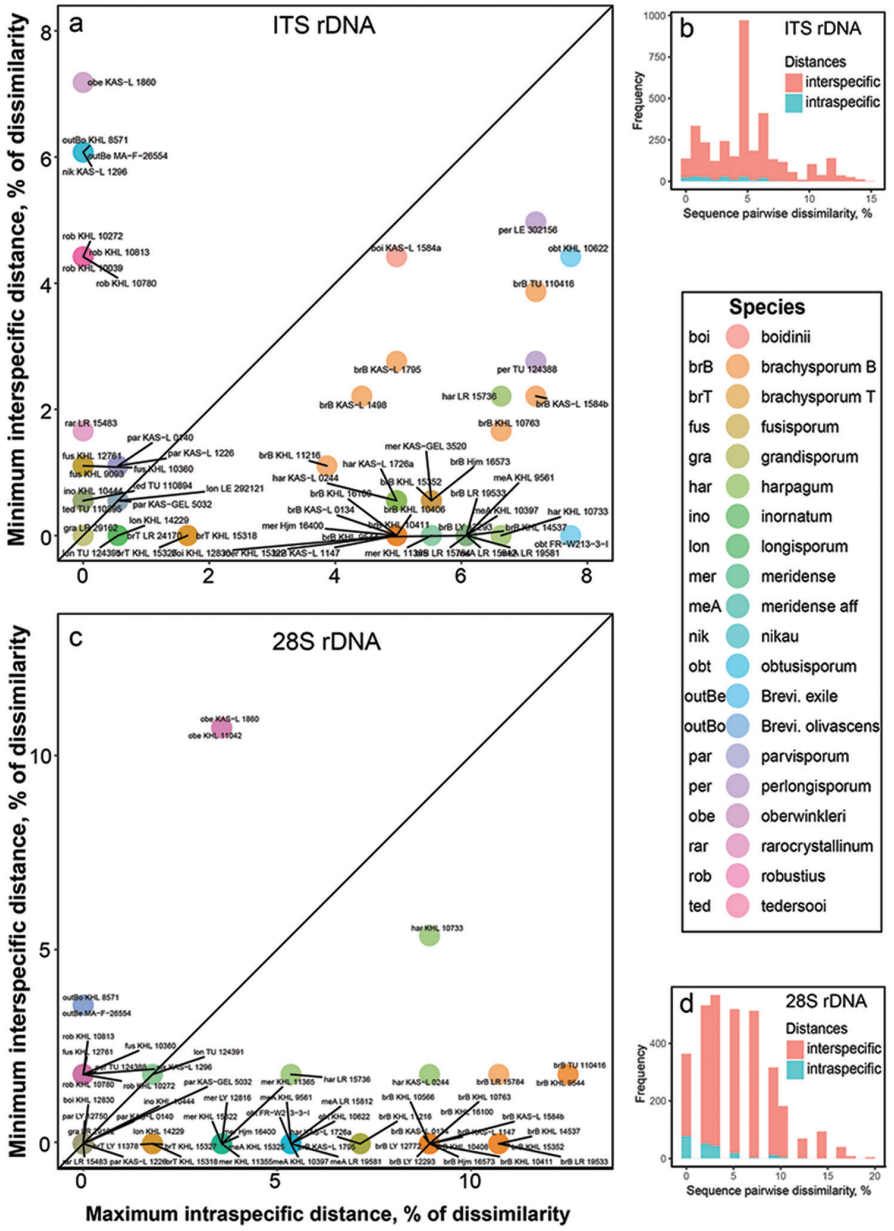

Figure 2. Verifying the presence of the barcode gap in *Subulicystidium* rDNA sequences of ITS (**a, b**) and 28S (**c, d**) regions. **a, c** Maximal intraspecific divergence compared with minimal interspecific distances between the aligned rDNA sequences in ITS (**a**) and 28S (**c**) datasets. Specimens falling above 1:1 line indicate the presence of the barcoding gap (molecular distinctness of the species) **b, d** Frequency distributions of intra- and interspecific distances without referring to particular species in ITS (**b**) and 28S (**d**) datasets. In the legend, the capital "B" following epithet in *S. brachysporum* means morphological species concept following Boidin and Gilles (1988), while "T" means the species as described by Talbot (1958). Three-letter code before each specimen's number corresponds to a species epithet as explained in the legend

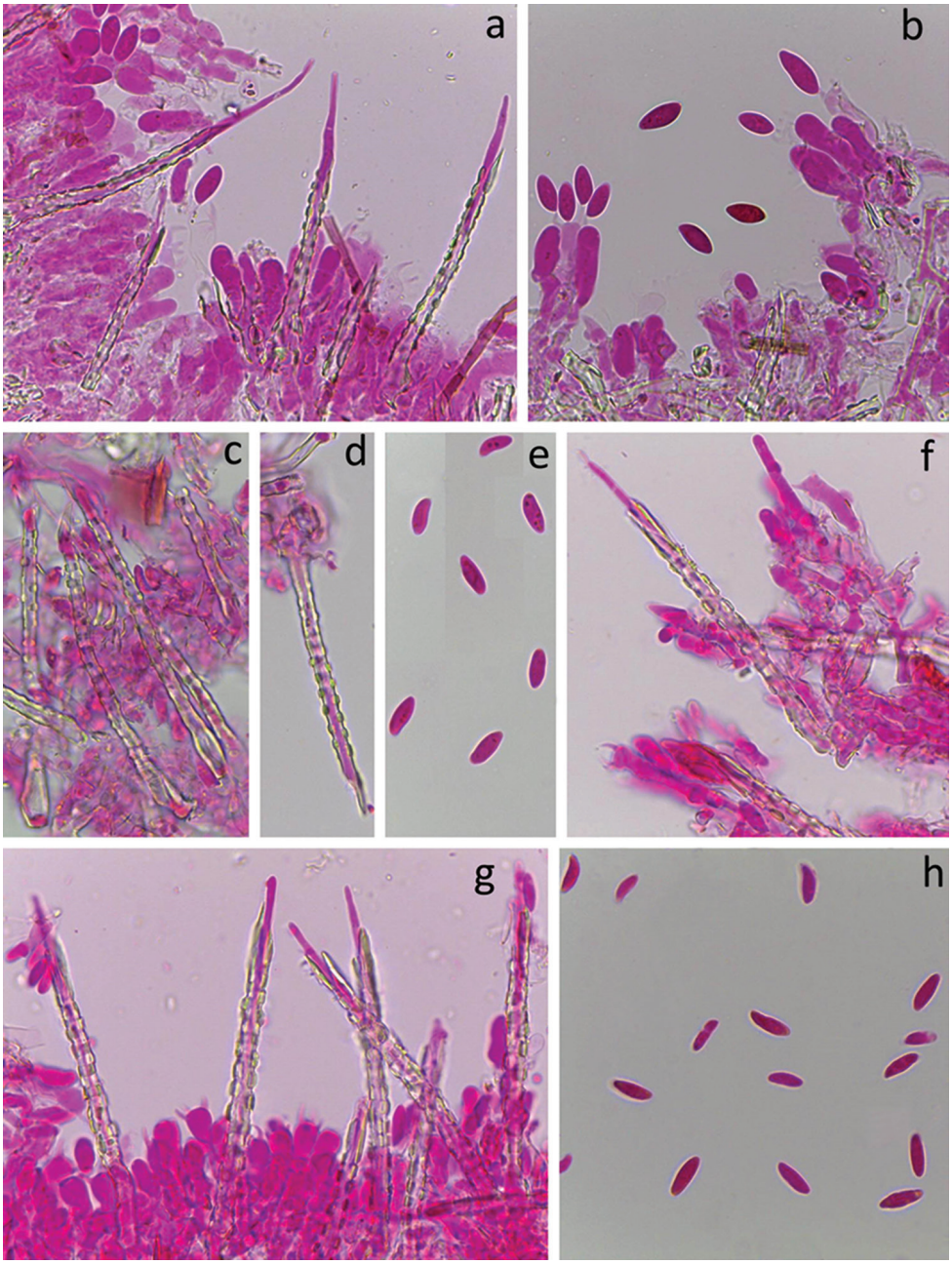

Figure 3. Species of *Subulicystidium* with broad fusiform basidiospores. *Subulicystidium naviculatum* (GB:KHL 11566): **a, b** hymenium and basidiospores. *Subulicystidium ryvardenii* (LR 8860/b in O:F 909583, holotype): **c, d** cystidia **e** basidiospores. *Subulicystidium robustius* (GB:KHL 10813, holotype): **f, g** cystidia in hymenium **h** basidiospores. All preparations done in 3% aqueous solution of potassium hydroxide (KOH) mixed with 1% aqueous solution of Phloxine. All scale bars equal 10 μm.

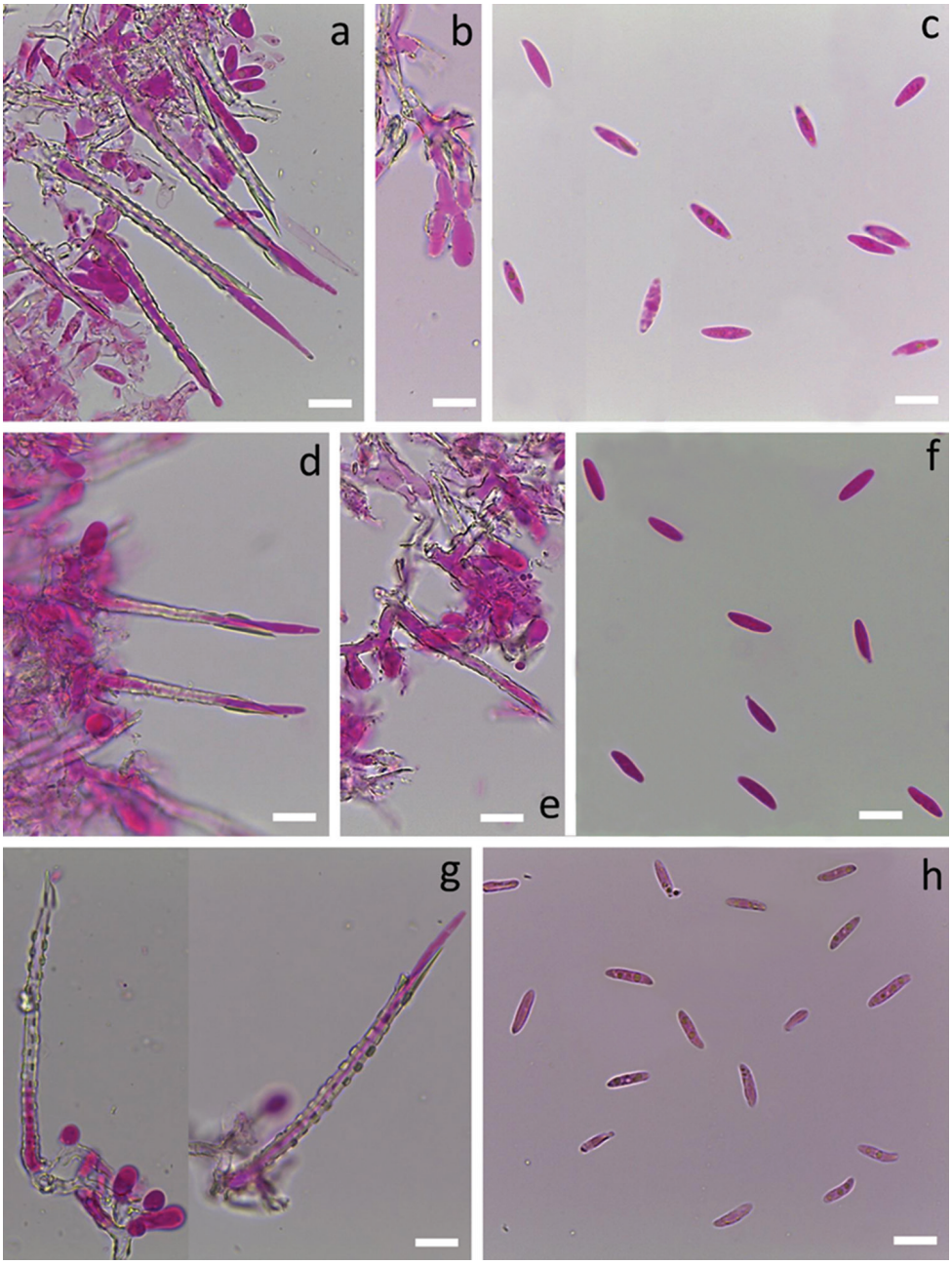

Figure 4. Species of *Subulicystidium* with narrow fusiform basidiospores. *Subulicystidium fusisporum* (GB:KHL 10360, holotype): **a** cystidia **b** crystalline encrustation of hymenium **c** basidiospores. *Subulicystidium inornatum* (GB:KHL 10444, holotype): **d** cystidia **e** young hymenium with slight overall encrustation **f** basidiospores. *Subulicystidium tedersooi* (TU 110894, holotype): **g** cystidia, h basidiospores. All preparations done in 3% aqueous solution of potassium hydroxide (KOH) mixed with 1% aqueous solution of Phloxine. All scale bars equal 10 μm.

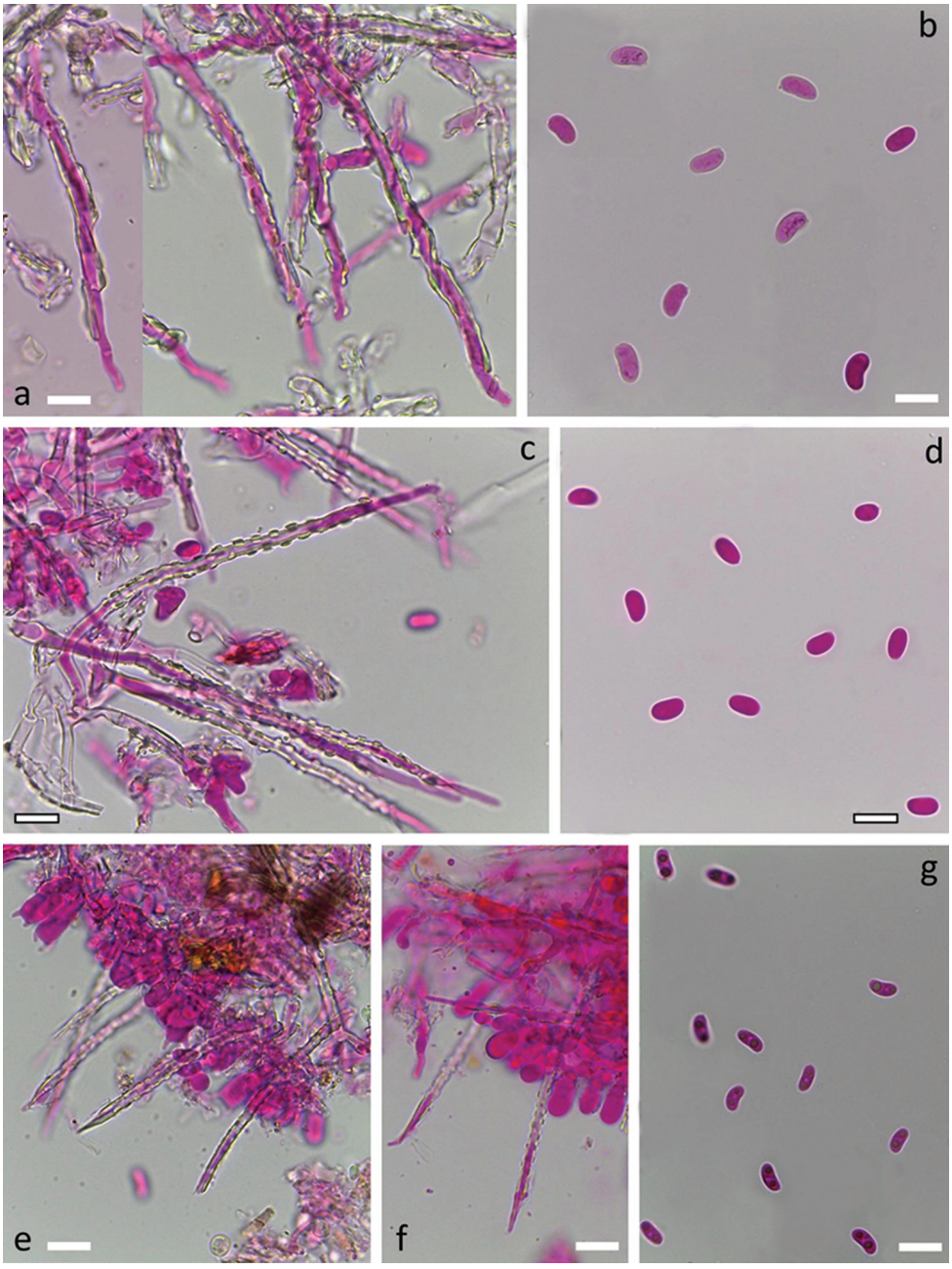

Figure 5. Species of *Subulicystidium* with broad cylindric basidiospores. *Subulicystidium oberwinkleri* (KAS:L 1860, holotype): **a** cystidia **b** basidiospores. *Subulicystidium nikau* (KAS:L 1296): **c** cystidia **d** basidiospores. *Subulicystidium boidinii* (KAS:L 1584a, holotype): **e** mature hymenium **f** young hymenium **g** basidiospores. All preparations done in 3% aqueous solution of potassium hydroxide (KOH) mixed with 1% aqueous solution of Phloxine. All scale bars equal 10 μm.

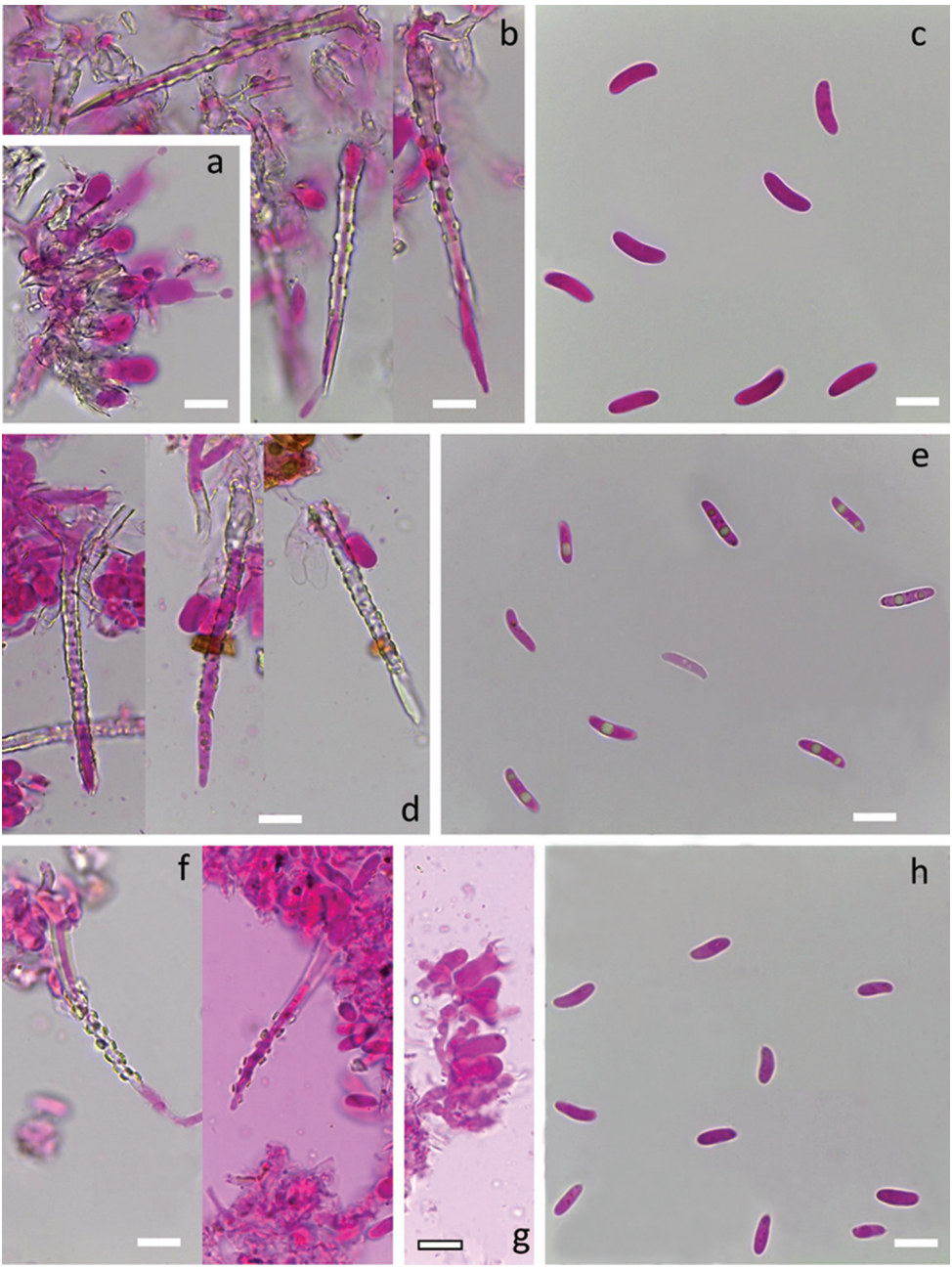

Figure 6. Species of *Subulicystidium* with long cylindric basidiospores. *Subulicystidium grandisporum* (LR 29162 in O:F 506781): **a** hymenium with rich crystalline encrustation **b** cystidia **c** basidiospores. *Subulicystidium obtusisporum* (FR: W213-3-I): **d** cystidia **e** basidiospores. *Subulicystidium rarocrystallinum* (LR 15483 in O:F 918488, holotype): **f** cystidia **g** hymenium **h** basidiospores. All preparations done in 3% aqueous solution of potassium hydroxide (KOH) mixed with 1% aqueous solution of Phloxine. All scale bars equal 10 μm.

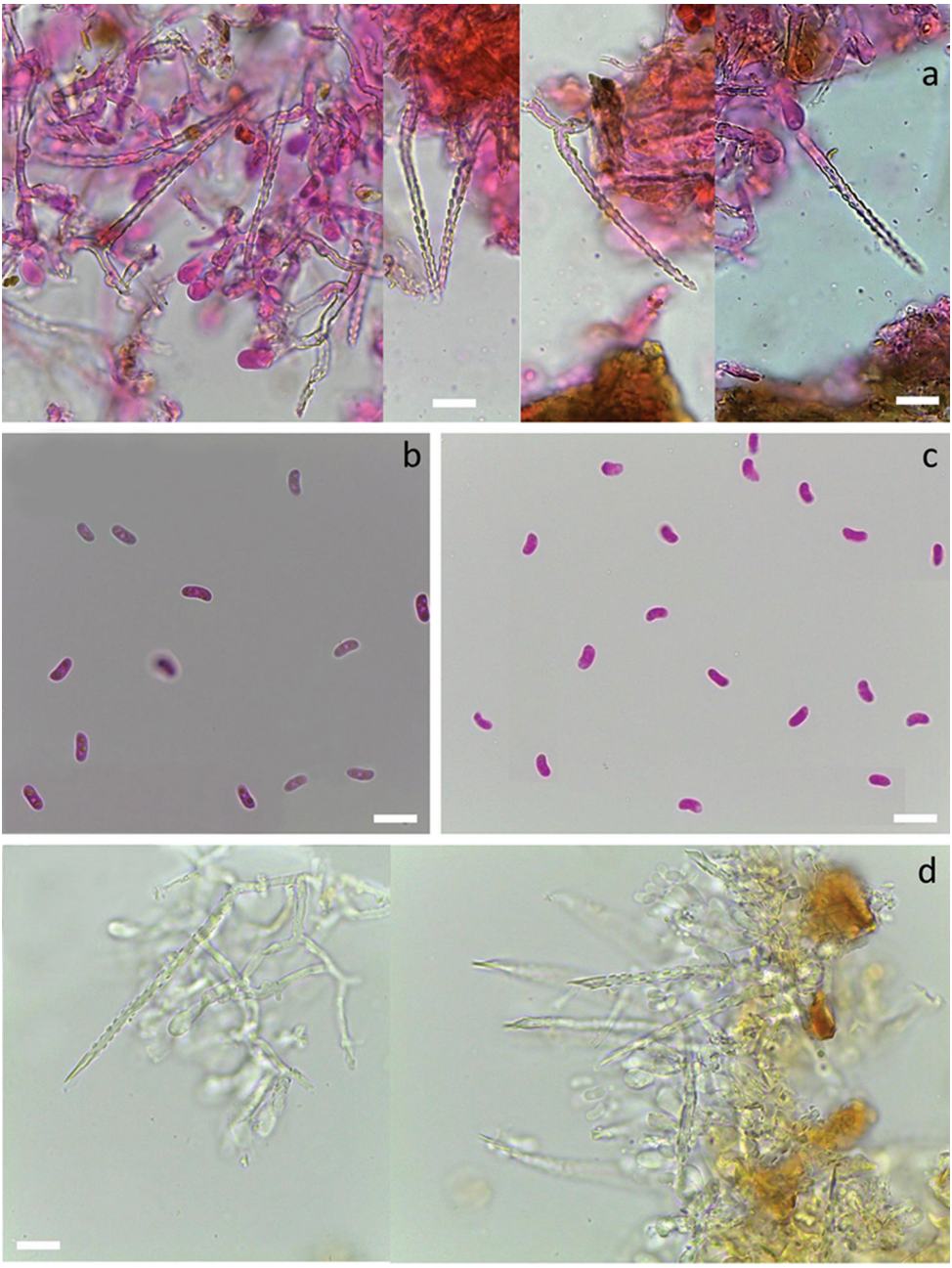

Figure 7. Species of *Subulicystidium* with smallest cylindric basidiospores. *Subulicystidium harpagum* (KAS:L 1726a, holotype): **a** cystidia **b** basidiospores. *Subulicystidium parvisporum* (KAS:L 0140, holotype): **c** basidiospores **d** cross sections through fruit-body. Preparations **a, b, c** done in 3% aqueous solution of potassium hydroxide (KOH) mixed with 1% aqueous solution of Phloxine, preparation **d** simply in KOH. All scale bars equal 10 μm.

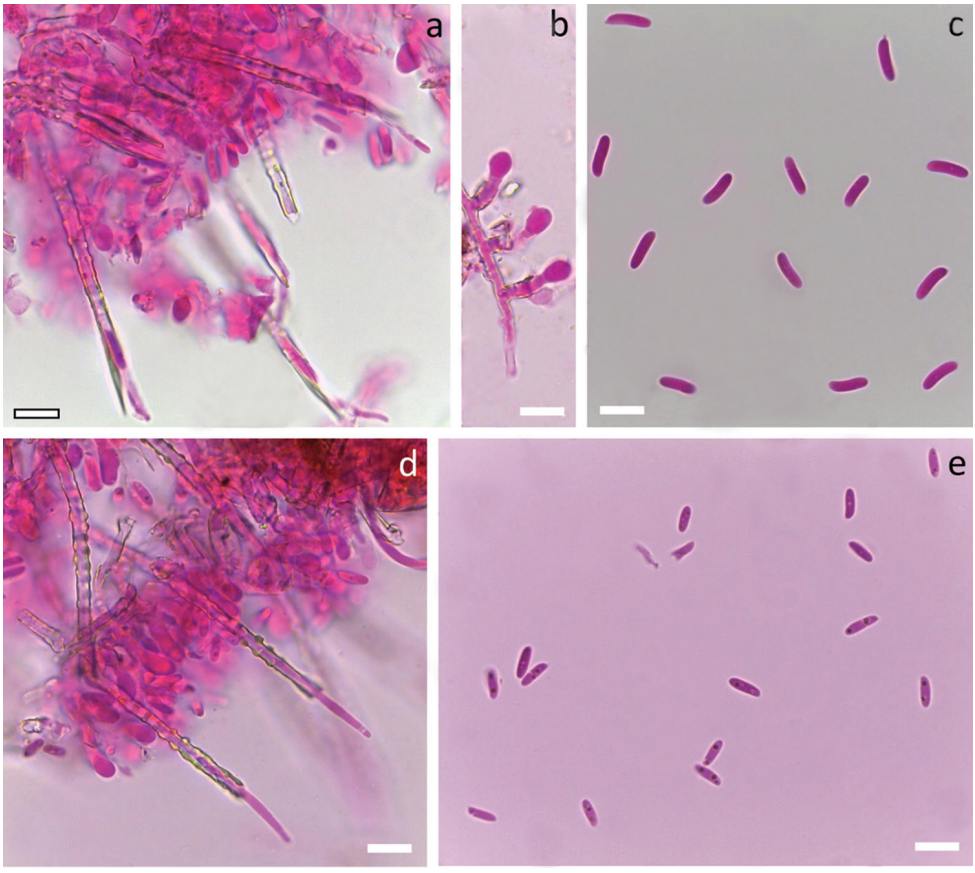

Figure 8. Species of *Subulicystidium brachysporum* morphotype. *Subulicystidium brachysporum* sensu Boidin and Gilles (LR 15784 in O:F 918493): **a** cystidia in hymenium **b** crystalline collars on basidioles and slightly encrustated subhymenial hyphae **c** basidiospores. *Subulicystidium brachysporum* sensu Talbot (LR 24170 in O:F): **d** cystidia in hymenium **e** basidiospores. All preparations done in 3% aqueous solution of potassium hydroxide (KOH) mixed with 1% aqueous solution of Phloxine. All scale bars equal 10 μm.

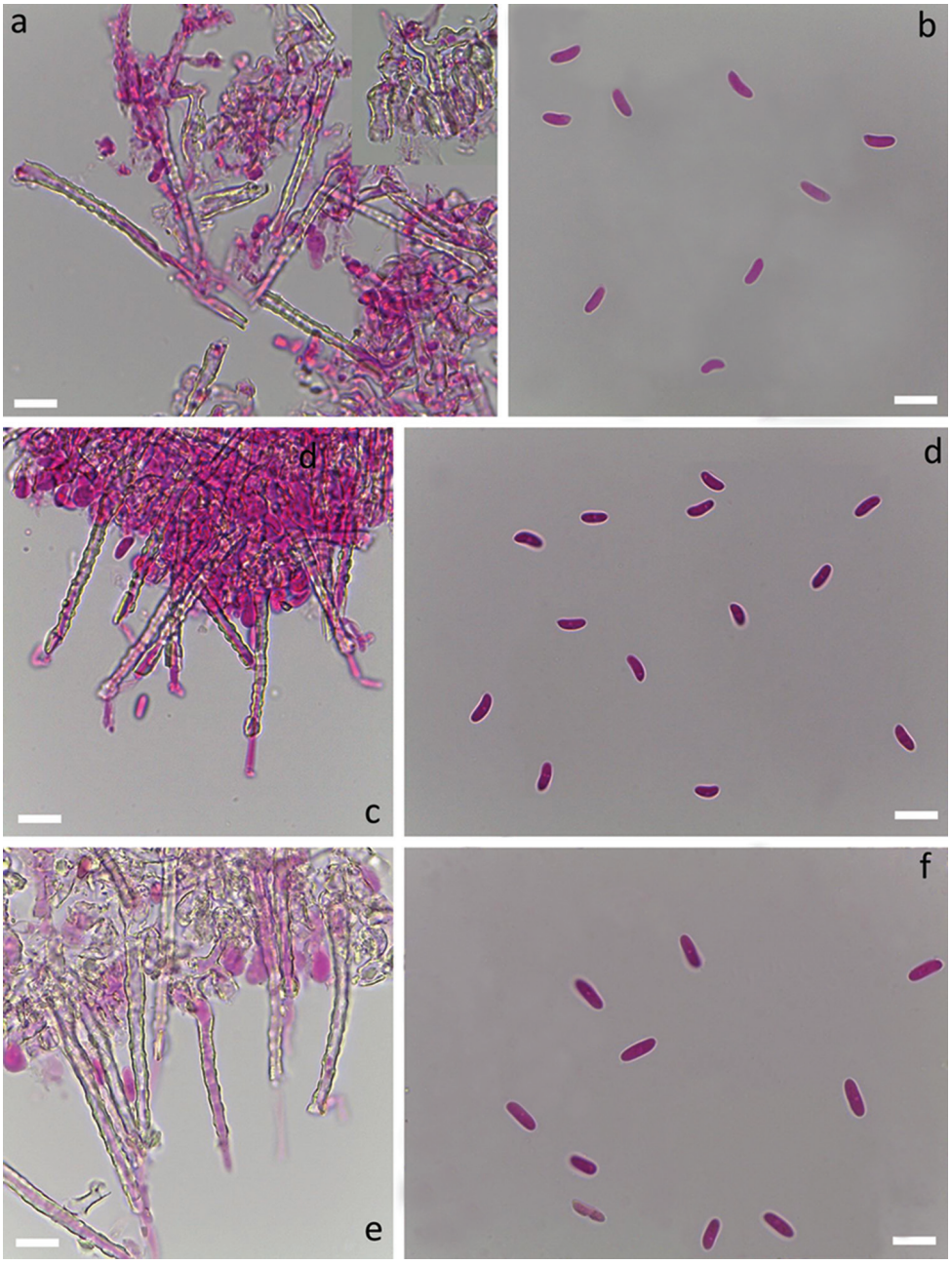

Figure 9. Species of *Subulicystidium meridense* morphotype. *Subulicystidium meridense*, holotype (TUB:FO 13761): **a** hymenium with crystalline encrustation and cystidia **b** basidiospores. *S. meridense* from own study (GB:KHL 11365): **c** cystidia in hymenium **d** basidiospores. *Subulicystidium* aff. *meridense* (GB:KHL 9561): **e** cystidia in hymenium **f** basidiospores. All preparations done in 3% aqueous solution of potassium hydroxide (KOH) mixed with 1% aqueous solution of Phloxine. All scale bars equal 10 μm.

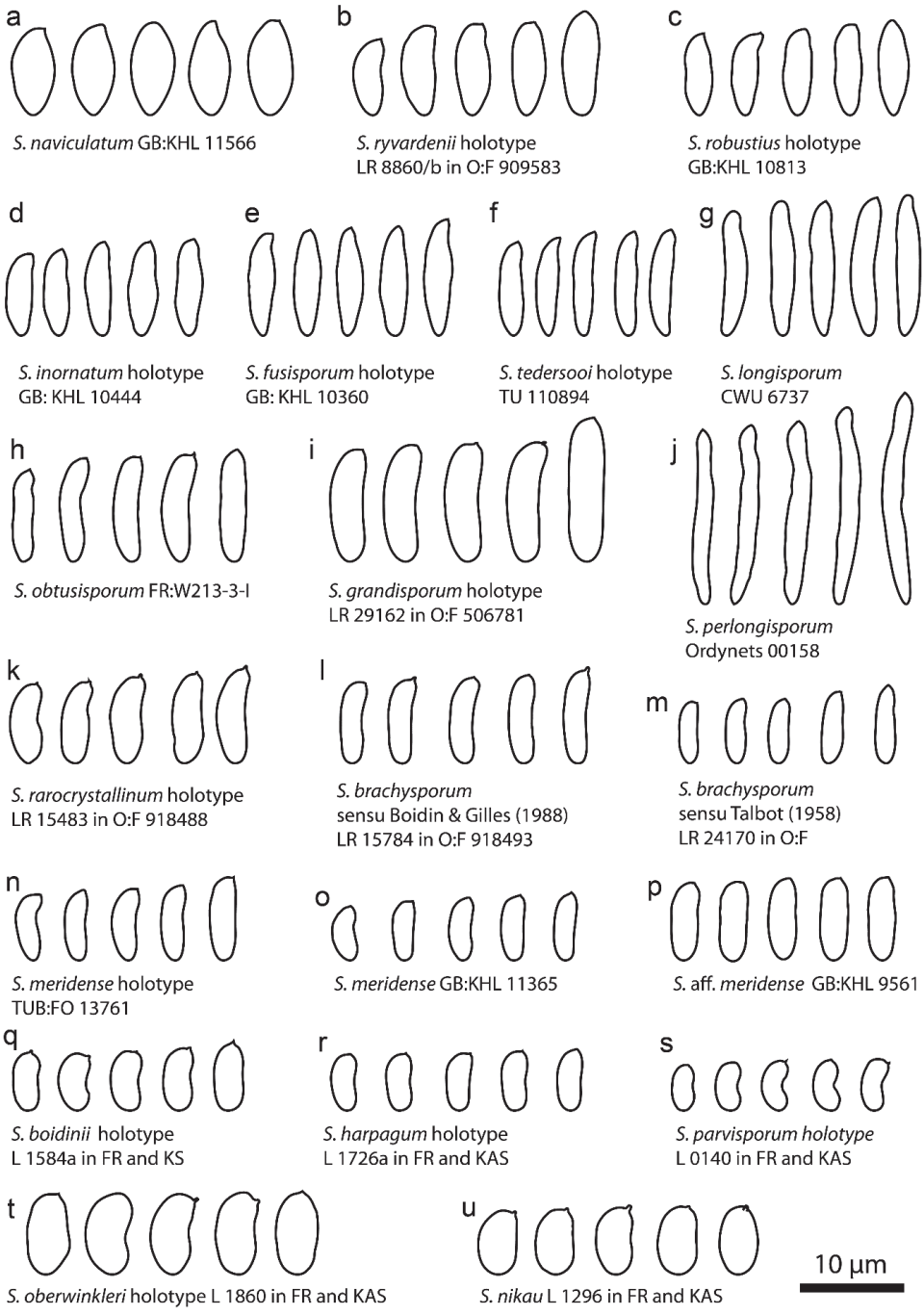

Figure 10. Basidiospore shape and size in all studied species of *Subulicystidium*. Each species is illustrated by a single specimen and herbarium codes are indicated on the figure.

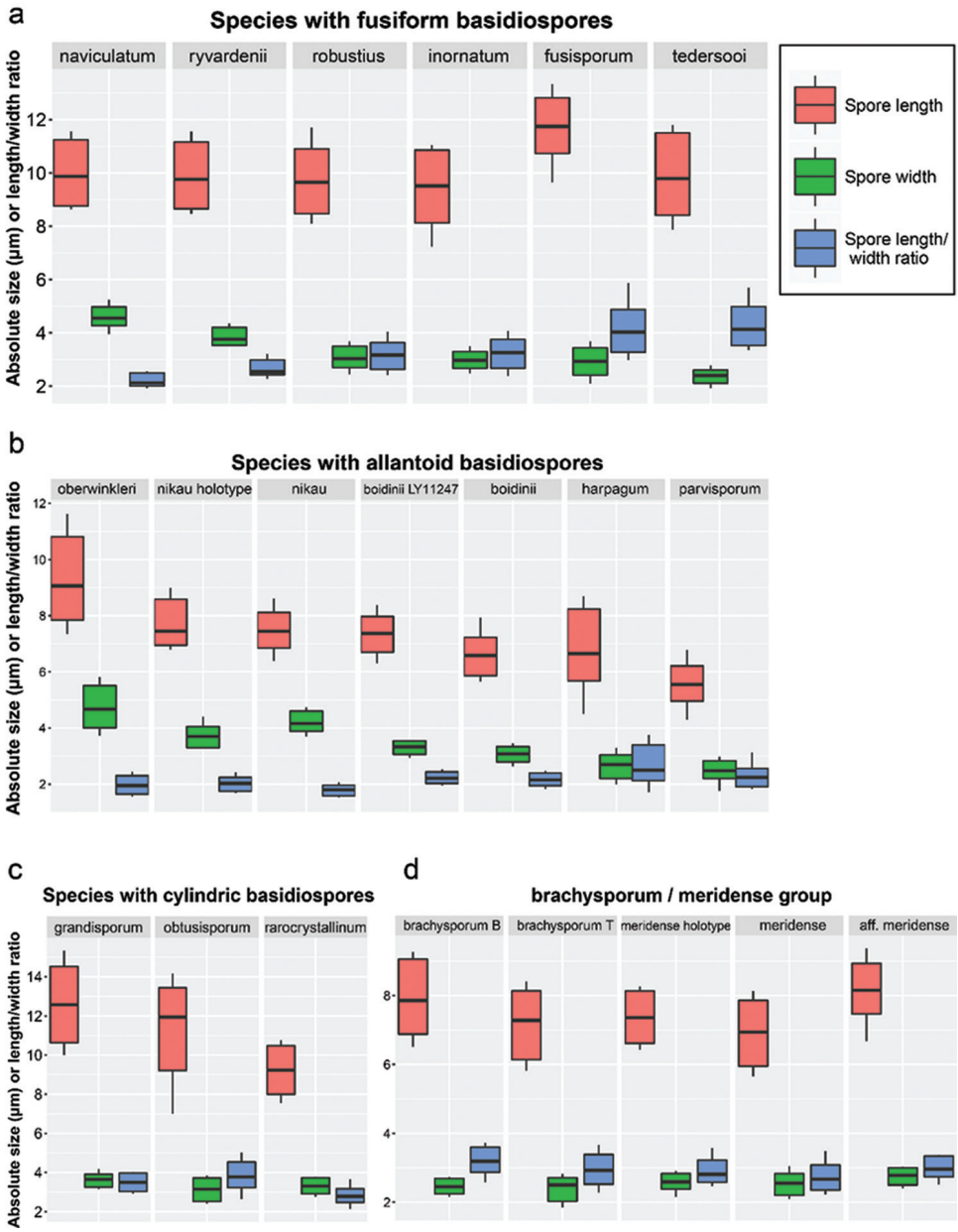

Figure 11. Basidiospore size range in the short-spored species of *Subulicystidium*. Only measurements from sequenced or important historical collections were included in calculations (in total 67 specimens, 2840 basidiospores). Boxes (with median inside) delimit the range between 5% and 95% data quantiles, while the whiskers show minimum and maximum values without considering outliers (see Materials and Methods for details on excluding outliers). If more than one sequenced specimen was available for species, raw measurements without outliers were pooled to calculate basidiospore size range of the species. In *S. brachysporum*, the capital "B" following epithet means morphological species concept following Boidin and Gilles (1988), while "T" means the species as described by Talbot (1958).

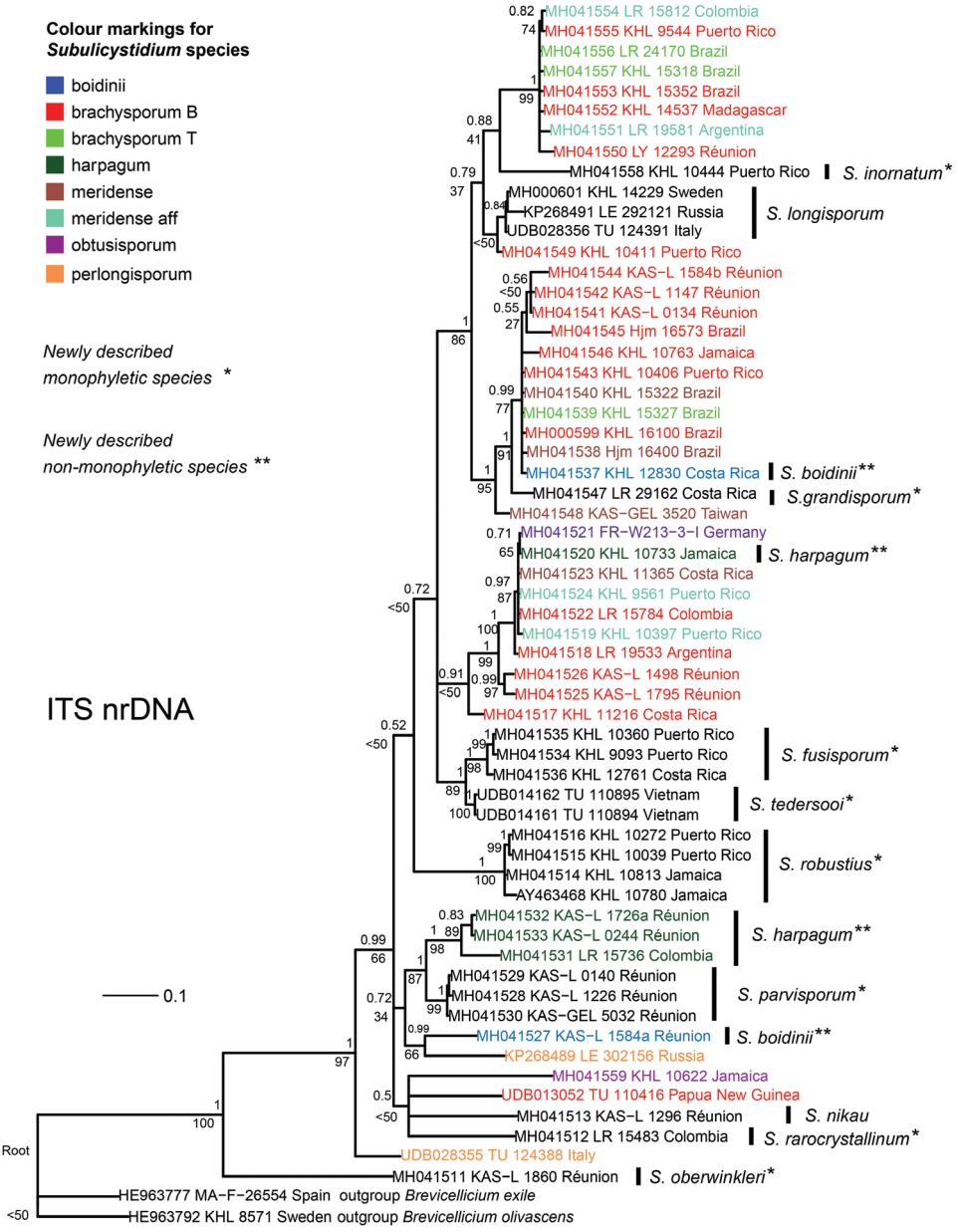

Figure 12. Phylogenetic relationship of *Subulicystidium* based on ITS nrDNA sequences. 50% majority-rule consensus tree from Bayesian analysis is shown, with posterior probabilities above the branches and bootstrap support values from the maximum likelihood estimation below the branches. Tips of the tree are annotated according to morphological identification and marked with colours in non-monophyletic taxa (see legend). In the legend, the capital "B" following epithet in *S. brachysporum* means morphological species concept following Boidin and Gilles (1988), while "T" means the species as described by Talbot (1958).

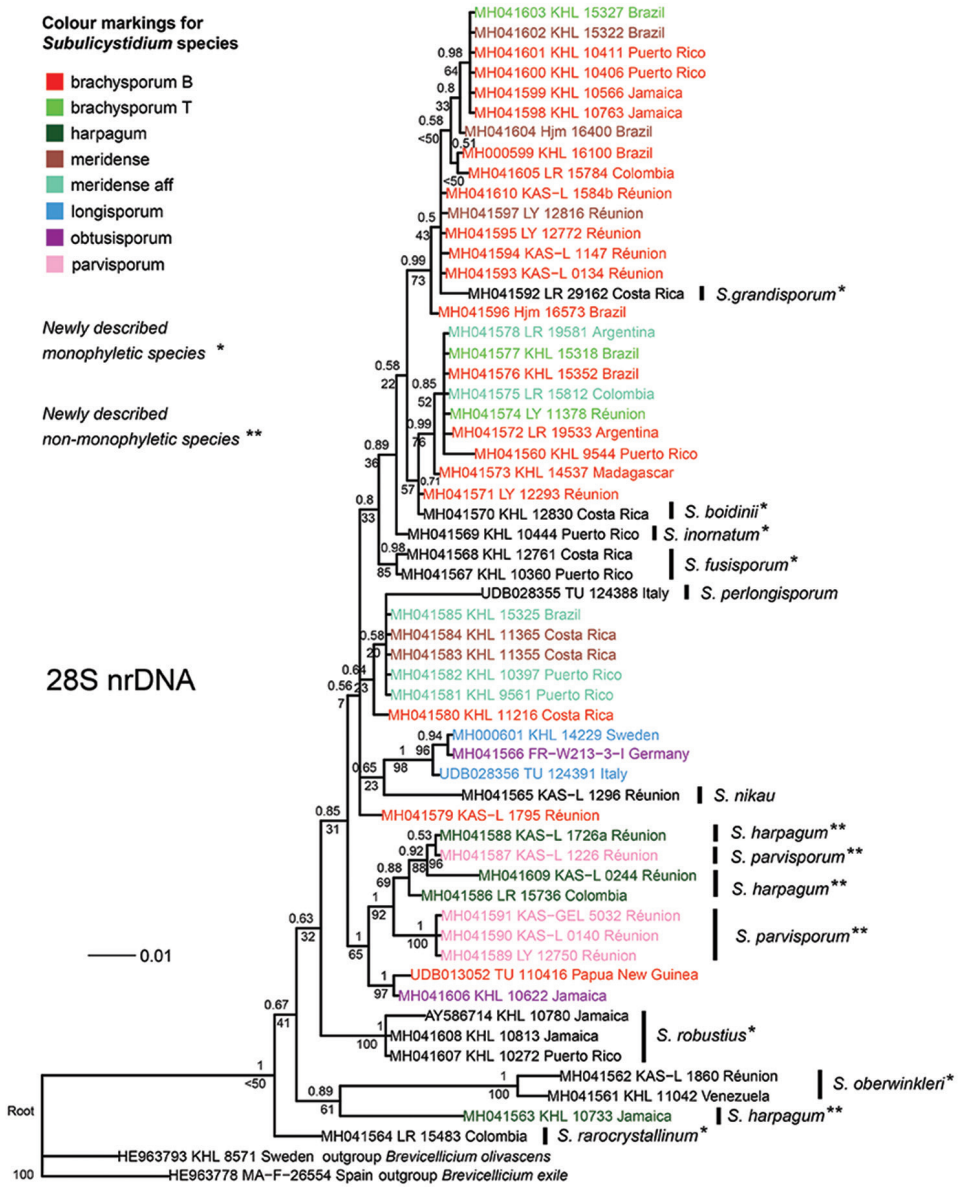

Figure 13. Phylogenetic relationship of *Subulicystidium* based on 28S nrDNA sequences. 50% majority-rule consensus tree from Bayesian analysis is shown, with posterior probabilities above the branches and bootstrap support values from the maximum likelihood estimation below the branches. Tips of the tree are annotated according to morphological identification and marked with colours in non-monophyletic taxa (see legend). In the legend, the capital "B" following epithet in *S. brachysporum* means morphological species concept following Boidin and Gilles (1988), while "T" means the species as described by Talbot (1958).

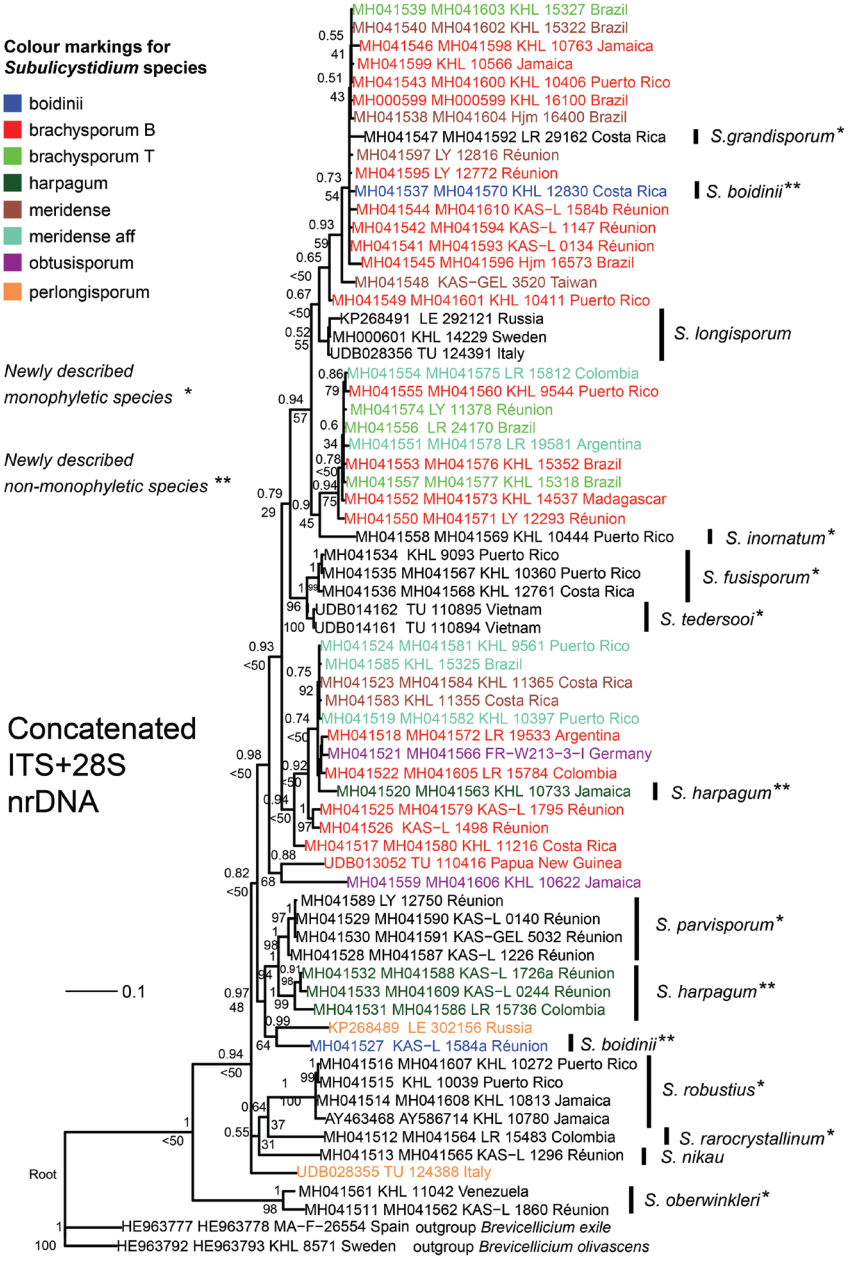

Figure 14. Phylogenetic relationship of *Subulicystidium* based on concatenated ITS+28S nrDNA alignment. 50% majority-rule consensus tree from Bayesian analysis is shown, with posterior probabilities above the branches and bootstrap support values from the maximum likelihood estimation below the branches. Tips of the tree include GenBank/UNITE accession numbers of ITS followed by 28S region. Tips are annotated according to morphological identification and marked with colours in non-monophyletic taxa (see legend). In the legend, the capital "B" following epithet in *S. brachysporum* means morphological species concept following Boidin and Gilles (1988), while "T" means the species as described by Talbot (1958).

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Supplementary material 1

Detailed data on 144 specimens of *Subulicystidium* used in the study

Authors: Alexander Ordynets, David Scherf, Felix Pansegrau, Jonathan Denecke, Ludmila Lysenko, Karl-Henrik Larsson, Ewald Langer

Data type: occurences

Explanation note: Specimens for which coordinates were available on herbarium labels or from field notes contain "gps" in the column "COORD_source". Specimens for which coordinates were estimated from the map web sources (see Materials and Methods) are marked with the word "map".

Link: https://doi.org/10.3897/mycokeys.35.25678.suppl1

Supplementary material 2

Measurements of 2840 basidiospores from 67 *Subulicystidium* specimens which were sequenced or represent important historical collections

Authors: Alexander Ordynets, David Scherf, Felix Pansegrau, Jonathan Denecke, Ludmila Lysenko, Karl-Henrik Larsson, Ewald Langer

Data type: morphological

Explanation note: Each row contains data on length (L_sp), width (W_sp) and length to width ratio (Q_sp) of a single basidiospore and information on specimen and species from which the basidiospore was measured is provided in separate columns. Dataset does not contain outliers which were found for some specimens in raw data (see Materials and methods for details of excluding outliers). In the case of *S. brachysporum*, the capital "B" following epithet means morphological species concept following Boidin and Gilles (1988), while "T" means the species as described by Talbot (1958).

Link: https://doi.org/10.3897/mycokeys.35.25678.suppl2

Supplementary material 3

Basidiospore size ranges of 67 *Subulicystidium* specimens which were sequenced or represent important historical collections

Authors: Alexander Ordynets, David Scherf, Felix Pansegrau, Jonathan Denecke, Ludmila Lysenko, Karl-Henrik Larsson, Ewald Langer

Data type: morphological

Explanation note: For the parameters of basidiospore length (L), width (W) and length to width ratio (Q), the following values are presented: minimal value, 5% data quantile, mean, 95% data quantile and maximum. Minimum and maximum do not consider outliers which were found for some specimens in raw data (see Materials and methods for details of excluding outliers).

Link: https://doi.org/10.3897/mycokeys.35.25678.suppl3

Supplementary material 4

Basidiospore size ranges of *Subulicystidium* species included in the study

Authors: Alexander Ordynets, David Scherf, Felix Pansegrau, Jonathan Denecke, Ludmila Lysenko, Karl-Henrik Larsson, Ewald Langer

Data type: morphological

Explanation note: Calculations are based on a set of 67 specimens which were sequenced or represent important historical collections. For the parameters of basidiospore length (L), width (W) and length to width ratio (Q), the following values are presented: minimal value, 5% data quantile, mean, 95% data quantile and maximum. Minimum and maximum do not consider outliers which were found for some specimens in raw data (see Materials and methods for details of excluding outliers). Calculations were provided separately for the type specimens (of *S. meridense* and *S. nikau*), as well as for the collection as *S. boidinii* LY 11247 which was used to propose "*Subulicystidium allantosporum* ad interim" (Boidin and Gilles 1988). In *S. brachysporum*, the capital "B" following epithet means morphological species concept following Boidin and Gilles (1988), while "T" means the species as described by Talbot (1958).

Link: https://doi.org/10.3897/mycokeys.35.25678.suppl4