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New species of *Cylindrocladiella* from plantation soils in South-East Asia

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Abstract

Cylindrocladiella spp. are widely distributed especially in tropical and sub-tropical regions, where they are mainly known as saprobes although some species are plant pathogens. Very little is known about these fungi in South-East Asia. The aim of this study was to identify a collection of *Cylindrocladiella* isolates from soils collected in forest nurseries and plantations in Vietnam and Malaysia. This was achieved using DNA sequence comparisons and morphological observations. The study revealed two previously described species, *Cy. lageniformis* and *Cy. peruviana* as well as five novel taxa, described here as *Cy. arbusta* **sp. nov.**, *Cy. malesiana* **sp. nov.**, *Cy. obpyriformis* **sp. nov.**, *Cy. parvispora* **sp. nov.** and *Cy. solicola* **sp. nov.** A relatively small collection of isolates from a limited geographic sampling revealed an unexpectedly high level of *Cylindrocladiella* diversity suggesting that many more species in this genus await discovery in South-East Asia.

Keywords

multigene phylogeny, plantation forestry, taxonomy

Introduction

Cylindrocladiella (*Hypocreales*, *Nectriaceae*) are soil-borne fungi that have commonly been confused with the asexual morph of the closely related genus *Calonectria* (Crous 2002). Species of *Cylindrocladiella* can be distinguished from *Calonectria* spp. by their aseptate stipe extensions, distinctive conidiophore branching patterns and their small 1-septate conidia. In addition, they have sexual morphs in *Nectricladiella* that are very different to those in *Calonectria* (Boesewinkel 1982, Crous and Wingfield 1993, Schoch et al. 2000, Crous 2002). Multigene phylogenetic inference has led to the description of a relatively large number of novel species and to the delimitation of cryptic species (Schoch et al. 2000, van Coller et al. 2005, Lombard et al. 2012, 2017). Currently, *Cylindrocladiella* accommodates 35 species (Crous 2002, van Coller et al. 2005, Inderbitzin et al. 2012, Lombard et al. 2012, 2017, Crous et al. 2017).

Species of *Cylindrocladiella* are distributed globally, especially in the tropical, sub-tropical and temperate regions of the world (Crous 2002, Lombard et al. 2012). These fungi are not typically considered primary pathogens although their role in causing plant disease is likely underestimated. The fact that they are isolated using baiting with living plant tissue similar to the approach for *Calonectria* spp. (Crous 2002), suggests some level of pathogenicity. Disease symptoms that have been associated with *Cylindrocladiella* include leaf spot (Mohanan and Sharma 1985, Crous et al. 1991, Crous and Wingfield 1993), damping off (Sharma and Mohanan 1982, Scattolin and Montecchio 2007) and shoot die-back (Brielmaier-Liebetanz et al. 2013). *Cylindrocladiella* spp. are, however, most frequently associated with root diseases (Crous et al. 1991, Crous and Wingfield 1993, Crous 2002). They have, for example, been reported causing root rot on *Eucalyptus* spp. (Mohanan and Sharma 1985, Crous and Wingfield 1993) and *Pinus* sp. (Boesewinkel 1982) in forestry nurseries. They have also been associated with root rot of peanut (Crous and Wingfield 1993), tea (Peerally 1974), kiwi fruit (Erper et al. 2013) and black-foot disease of grapevines (Agustí-Brisach and Armengol 2013, Armengol and Gramaje 2016, Carlucci et al. 2017).

Thirteen species of *Cylindrocladiella* have been reported from South-East Asia from Indonesia and Thailand (Crous 2002, Lombard et al. 2012, 2017). Of these, only four species (*Cy. camelliae*, *Cy. infestans*, *Cy. microcylindrica* and *Cy. viticola*), have been isolated from plant tissues, with the other nine species having been isolated from soil (Crous 2002, Lombard et al. 2012, 2017). However, nothing is known regarding their role as plant pathogens in this region.

In order to provide a better understanding about the diversity of *Cylindrocladiella* species in South-East Asia, this study aimed at identifying a collection of *Cylindrocladiella* isolates obtained from soils collected in plantations and nurseries in Malaysia and Vietnam. This was achieved using multigene sequence comparisons and morphological observations.

Materials and methods

Isolates

Soil samples were collected from various plantations and nurseries in Malaysia and Vietnam and baited with germinating alfalfa (*Medicago sativa*) seeds as described by Crous (2002). Direct isolations from fungal structures were made on to malt extract agar (MEA; 2 % w/v; Biolab, Midrand, South Africa). Cultures were incubated for 3–7 d at 25 °C and purified by transferring single hyphal tips from primary isolations to fresh MEA plates. Cultures were deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa with representative isolates in the collection of the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands. Dried specimens were deposited in the National Collection of Fungi (PREM), Pretoria, South Africa.

DNA sequencing and phylogenetic analyses

Seven-day-old fungal cultures grown on MEA at 25 °C were used for DNA extraction using Prepman® Ultra Sample Preparation Reagent (Thermo Fisher Scientific, Waltham, MA, USA) following the protocols provided by the manufacturer. Four loci were amplified and sequenced including the internal transcribed spacer (ITS) region using primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990); partial fragments of the translation elongation factor 1-alpha (*tef1*) gene region using primers EF1-728F (Carbone and Kohn 1999) and EF-2 (O'Donnell et al. 1998); partial fragments of the β-tubulin (*tub2*) gene region using primers T1 (O'Donnell and Cigelnik 1997) and CYLTUB1R (Crous et al. 2004a) and part of the Histone H3 (*his3*) gene region using primers CYLH3F and CYLH3R (Crous et al. 2004a).

The PCR reactions were conducted as described by Pham (2018). Amplified fragments were purified using ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA, USA). The products were sequenced in both directions with the same primers used for amplification, using the BigDye terminator sequencing kit v. 3.1 (Applied Biosystems, USA) on an ABI PRISM 3100 DNA sequencer (Applied Biosystems, USA).

Raw sequences were assembled and edited using Geneious v. 7.0 (Kearse et al. 2012). Sequence data were compared with other closely related *Cylindrocladiella* spp. available on the GenBank database. Sequences were aligned using MAFFT v. 7 (Katoh and Standley 2013), then edited manually in MEGA v. 7 (Kumar et al. 2016).

Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses were performed on data sets for each gene region and the combined data set. For MP, analyses were conducted using PAUP v. 4.0b10 (Swofford 2003) with phylogenic relationships estimated by heuristic searches with 1000 random stepwise addition sequences and tree

bisection and reconstruction (TBR) branch-swapping. Alignment gaps were treated as missing data and all characters were weighted equally. Measures calculated for parsimony included tree length (TL), retention index (RI), consistency index (CI), rescaled consistency index (RC) and homoplasy index (HI). Statistical support for branch nodes in the most parsimonious trees was obtained by performing 1000 bootstrap replicates. For ML, the appropriate substitution model was obtained using the software package jModeltest v. 2.1.5 (Posada 2008). The ML phylogenetic trees were generated using PhyML v. 3.0 (Guindon and Gascuel 2003). Confidence levels for the nodes were determined using 1000 replication bootstrap analyses. For both MP and ML, *Calonectria brachiatica* (CMW 25307) and *Calonectria pauciramosa* (CMW 5638) were used as the outgroup taxa. All resulting trees were viewed using MEGA v. 7 (Kumar et al. 2016).

Taxonomy

Morphological characteristics were assessed using single hyphal tip cultures on synthetic low-nutrient agar (SNA; Nirenburg 1981) and incubated at 25 °C for 3–7 d. In some cases, pieces of carnation leaf were added to the media to induce sporulation. Fungal structures were studied by mounting in 80 % lactic acid on glass sides and examined using a Nikon H550L microscope (Nikon, Japan). Thirty to fifty measurements were made for all taxonomically informative characters depending on their availability. The 95 % confidence levels were determined and extremes of conidial measurements are given in parentheses. For all other fungal structures, only extremes are presented. Colony colour and morphology were assessed using 7-d-old cultures on MEA grown at 25 °C using the colour charts of Rayner (1970). To determine the optimal temperature for growth, cultures were transferred to MEA and incubated at temperatures ranging from 5 to 35 °C at 5 °C intervals. Fungal descriptions and associated metadata were deposited in MycoBank (Crous et al. 2004b).

Results

Isolates

Nineteen isolates in total were obtained from soil baits. Of these, 15 were from Vietnam (nine from Tuyen Quang, four from Nghe An, one from Vinh Phuc and one from Hanoi) and four were from Sabah, Malaysia. The majority (16) of the isolates were from soils collected from *Acacia* plantations (Table 1).

Phylogenetic analyses

Approximately 500–570 bases were obtained for each of the *his3, tef1, tub2* and ITS loci. For the ML analyses of each individual data sets, the TIM2+G model was selected

Table 1. Collection details and GenBank accessions of *Cylindrocladiella* isolates included in the phylogenetic analysis.

Species	Isolate number[1,3]	Substrate	Locality	Genbank accession[2]				References
				tub2	*his3*	*tef1*	ITS	
Cy. arbusta	**CMW 47295[T]; CBS 143546**	soil in *Acacia mangium* plantation	Tan Ky, Nghe An, Vietnam	MH016958	MH016996	MH016977	MH017015	This study
	CMW 47296; CBS 143547	soil in *A. mangium* plantation	Tan Ky, Nghe An, Vietnam	MH016959	MH016997	MH016978	MH017016	This study
Cy. camelliae	CPC 234; PPRI 3990; IMI 346845	*Eucalyptus grandis*	South Africa	AY793471	AY793509	JN099087	AF220952	Boesewinkel 1982
	CPC 237	*E. grandis*	South Africa	JN098749	JN098839	JN099090	JN100573	Boesewinkel 1982
Cy. clavata	CBS 129563; CPC 17591	soil	Australia	JN098751	JN098859	JN098975	JN099096	Lombard et al. 2012
	CBS 129564[T]; CPC 17592	soil	Australia	JN098752	JN098858	JN098974	JN099095	Lombard et al. 2012
Cy. cymbiformis	CBS 129553[T]; CPC 17393	soil	Australia	JN098753	JN098866	JN098988	JN099103	Lombard et al. 2012
Cy. elegans	CBS 338.92[T]; PPRI 4050; IMI 346847	leaf litter	South Africa	AY793474	AY793512	JN099039	AY793444	Crous and Wingfield 1993
	CBS 110801; CPC 525	leaf litter	South Africa	JN098755	JN098916	JN099044	JN100609	Crous and Wingfield 1993
Cy. lageniformis	CBS 340.92[T]; PPRI 4449; UFV 115	*Eucalyptus* sp.	Brazil	AY793481	AY793520	JN099003	AF220959	Crous and Wingfield 1993
	CBS 111060; CPC 1240	*Eucalyptus* sp.	South Africa	JN098770	JN098918	JN099046	JN100611	Crous and Wingfield 1993
	CMW 47419	soil in *E. camaldulensis* plantation	Hoang Mai, Nghe An, Vietnam	MH016972	MH017010	MH016991	MH017029	This study
Cy. lanceolata	CBS 129565; CPC 17566	soil	Australia	JN098788	JN098939	JN099069	JN100632	Lombard et al. 2012
	CBS 129566[T]; CPC 17567	soil	Australia	JN098789	JN098862	JN098978	JN099099	Lombard et al. 2012
Cy. longiphialidica	CBS 129557[T]; CPC 18839	soil	Thailand	JN098790	JN098851	JN098966	JN100585	Lombard et al. 2012
	CBS 129558	soil	Thailand	JN098791	JN098852	JN098967	JN100586	Lombard et al. 2012
Cy. malesiana	**CMW 48276; CBS 143549**	soil in *A. mangium* plantation	Tawau, Sabah, Malaysia	MH016960	MH016998	MH016979	MH017017	This study
	CMW 48277; CBS 143550	soil in *A. mangium* plantation	Tawau, Sabah, Malaysia	MH016961	MH016999	MH016980	MH017018	This study
	CMW 48278[T]; CBS 143548	soil in *A. mangium* plantation	Tawau, Sabah, Malaysia	MH016962	MH017000	MH016981	MH017019	This study

Species	Isolate number[1,3]	Substrate	Locality	Genbank accession[2]				References
				tub2	*his3*	*tef1*	ITS	
Cy. malesiana	**CMW 48279**	soil in *A. mangium* plantation	Tawau, Sabah, Malaysia	MH016963	MH017001	MH016982	MH017020	This study
Cy. microcylindrica	CBS 111794[T]; ATCC 38571; CPC 2375	*Echeveria elegans*	Indonesia	AY793483	AY793523	JN099041	AY793452	Schoch et al. 2000
Cy. natalensis	CBS 110800; CPC 529	soil	South Africa	JN098793	JN098915	JN099043	JN100608	Lombard et al. 2012
	CBS 114943[T]; CPC 456	*Arachis hypogaea*	South Africa	JN098794	JN098895	JN099016	JN100588	Lombard et al. 2012
Cy. nederlandica	CBS 143.95; PD94/1353	*Kalanchoe* sp.	The Netherlands	JN098798	JN098891	JN099013	JN099129	Lombard et al. 2012
	CBS 152.91[T]; PD90/2015	*Pelargonium* sp.	The Netherlands	JN098800	JN098910	JN099033	JN100603	Lombard et al. 2012
Cy. novaezelandica	CBS 486.77[T]; ATCC 44815; CPC 2397	*Rhododendron indicum*	New Zealand	AY793485	AY793525	JN099050	AF220963	Boesewinkel 1982
Cy. obpyriformis	**CMW 47194[T]; CBS 143552**	soil in *Acacia* hybrid plantation	Tuyen Quang, Vietnam	MH016965	MH017003	MH016984	MH017022	This study
	CMW 49940; CBS 143553	soil in *Camellia chrysantha* nursery	Tam Dao, Vinh Phuc, Vietnam	MH016966	MH017004	MH016985	MH017023	This study
Cy. parvispora	**CMW 47193**	soil in *Acacia* hybrid plantation	Tuyen Quang, Vietnam	MH016967	MH017005	MH016986	MH017024	This study
	CMW 47197[T]; CBS 143554	soil in *Acacia* hybrid plantation	Tuyen Quang, Vietnam	MH016968	MH017006	MH016987	MH017025	This study
	CMW 47207; CBS 143555	soil in *Acacia* hybrid plantation	Tuyen Quang, Vietnam	MH016969	MH017007	MH016988	MH017026	This study
	CMW 47208; CBS 143556	soil in *Acacia* hybrid plantation	Tuyen Quang, Vietnam	MH016970	MH017008	MH016989	MH017027	This study
	CMW 47315	soil in *A. mangium* plantation	Son Duong, Tuyen Quang, Vietnam	MH016971	MH017009	MH016990	MH017028	This study
Cy. peruviana	CBS 113022; CPC 4291	*Eucalyptus* sp.	South Africa	JN098801	JN098906	JN099029	JN100599	Boesewinkel 1982
	CPC 2404[T]; IMUR 1843	ants	Peru	AY793500	AY793540	JN098968	AF220966	Boesewinkel 1982
	CMW 47297	soil in *A. mangium* plantation	Tan Ky, Nghe An, Vietnam	MH016973	MH017011	MH016992	MH017030	This study
	CMW 47304	soil in *A. mangium* plantation	Son Duong, Tuyen Quang, Vietnam	MH016974	MH017012	MH016993	MH017031	This study
	CMW 47333	soil in *A. mangium* plantation	Son Duong, Tuyen Quang, Vietnam	MH016975	MH017013	MH016994	MH017032	This study

Species	Isolate number[1,3]	Substrate	Locality	Genbank accession[2]				References
				tub2	*his3*	*tef1*	ITS	
Cy. peruviana	**CMW 47416**	soil	Bac Tu Liem, Hanoi, Vietnam	MH016976	MH017014	MH016995	MH017033	This study
Cy. pseudocamelliae	CBS 129555[T]; CPC 18825	soil	Thailand	JN098814	JN098843	JN098958	JN100577	Lombard et al. 2012
	CBS 129556; CPC 18832	soil	Thailand	JN098815	JN098846	JN098961	JN100580	Lombard et al. 2012
Cy. solicola	**CMW 47198[T]; CBS 143551**	soil in *Acacia* hybrid plantation	Tuyen Quang, Vietnam	MH016964	MH017002	MH016983	MH017021	This study
Cy. variabilis	CBS 375.93; IMI 317057	*Mangifera indica*	India	JN098836	JN098881	JN099000	JN099119	Lombard et al. 2012
	CBS 129561[T]; CPC 17505	soil	Australia	JN098719	JN098950	JN099080	JN100643	Lombard et al. 2012

[1] CBS: Culture collection of Westerdijk Fungal Biodiversity Institute (WI), Utrecht, the Netherlands; CMW: Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; CPC: Pedro Crous working collection housed at WI; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, UK; IMUR: Institute of Mycology, University of Recife, Recife, Brazil; ATCC: American Type Culture Collection, Virginia, U.S.A; PPRI: Plant Protection Research Institute, Agricultural Research Council, Pretoria, South Africa; UFV: Universidade Federal de Viçosa, Viçosa, Brazil.

[2] *tub2* = β-tubulin; *his3* = histone H3; *tef1* = translation elongation factor 1-alpha; ITS = Internal transcribed spacer regions 1 and 2 and the 5.8S gene of the ribosomal RNA.

[T] Ex-type cultures.

[3] Isolates obtained during the survey in this study are indicated in **bold.**

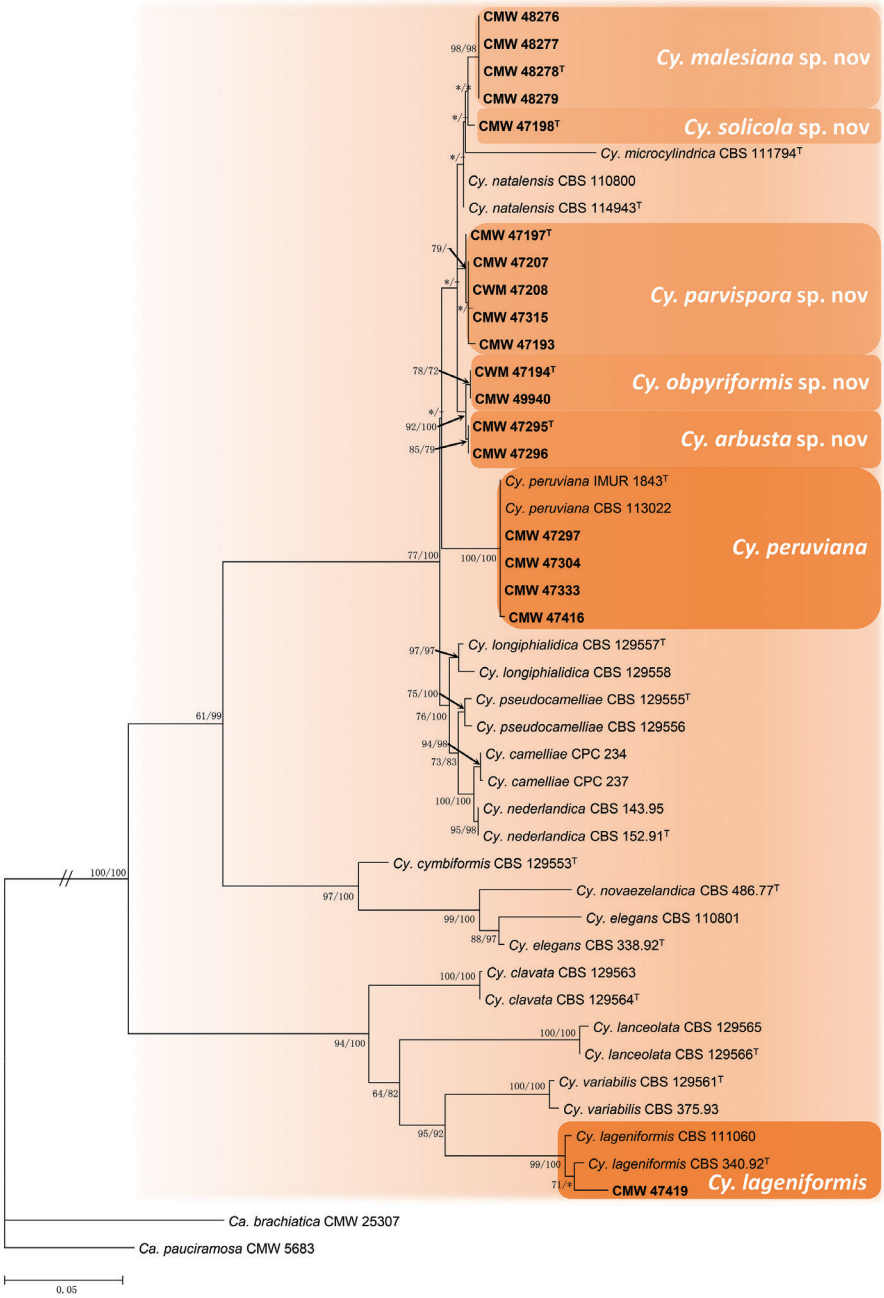

Figure 1. Phylogenetic tree based on maximum likelihood (ML) analysis of a combined data set of *his3, tef1, tub2* and ITS sequence alignments. Bootstrap value ≥ 60 % for maximum parsimony (MP) and ML analyses are indicated at the nodes. Bootstrap values lower than 60 % are marked with "*" and absent are marked with "–". Isolates representing ex–type material are marked with "T" and isolates collected in this study are highlighted in **bold**. *Calonectria brachiatica* (CMW 25307) and *Calonectria pauciramosa* (CMW 5683) represent the outgroups.

for *his3*; GTR+G model for *tef1*; TrN+I+G for *tub2* and the K80+I+G for ITS. The ML tree of each individual gene region with bootstrap support values of both the ML and MP analyses are presented in Suppl. materials 1–4.

The combined data set of *his3, tef1, tub2* and ITS, included 44 ingroup taxa and two outgroup taxa. The data set consisted of 2054 characters, of which 640 were parsimony-informative and 1414 characters were excluded. The MP analysis yielded 1000 trees (TL = 1414; CI = 0.691; RI = 0.880; RC = 0.608; HI = 0.309). The TIM2+I+G model was selected for the combined data set for the ML analyses. The ML tree with bootstrap support values of both the ML and MP analyses is presented in Figure 1.

In the phylogenetic tree (Figure 1), four isolates (CMW 47297, CMW 47304, CMW 47333, CMW 47416) clustered in the clade representing *Cy. peruviana* (ex-type IMUR 1843). *Cylindrocladiella lageniformis* (ex-type CBS 340.92) was represented by CMW 47419. The remaining isolates resided in five distinct clades representing novel taxa, accommodating four isolates (CMW 48276, CMW 48277, CMW 48278, CMW 48279), one isolate (CMW 47198), five isolates (CMW 47193, CMW 47197, CMW 47207, CMW 47208, CMW 47315), two isolates (CMW 47194, CMW 49940) and two isolates (CMW 47295, CMW 47296) respectively.

Taxonomy

Morphological comparisons and phylogenetic inference showed that 19 *Cylindrocladiella* isolates represented five novel species along with two previously described species, *Cy. lageniformis* (CMW 47419) and *Cy. peruviana* (CMW 47297, CMW 47304, CMW 47333, CMW 47416). The novel taxa are provided with names in *Cylindrocladiella* and their important morphological characteristics are compared in Table 2.

Cylindrocladiella arbusta N.Q. Pham, T.Q. Pham & M.J. Wingf., sp. nov.

MycoBank MB824550
Figure 2

Etymology. Name refers to a plantation and the environment where this fungus was isolated.

Type material. VIETNAM. Nghe An Province: Tan Ky, from soil in *Acacia mangium* plantation, Nov. 2013, N.Q. Pham & T.Q. Pham, herbarium specimen of dried culture, PREM 62159 (holotype), CMW 47295 = CBS 143546 (ex-type culture).

Description. *Sexual morph* not observed. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline. *Penicillate conidiophores* comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 116–166.5 × 4–5 µm; stipe extension aseptate, straight, 93–139 µm long, thick-walled with one basal septum, terminating in thin-walled, obpyriform to lanceolate vesicles, 4–5.5 µm wide. *Penicillate conidiogenous*

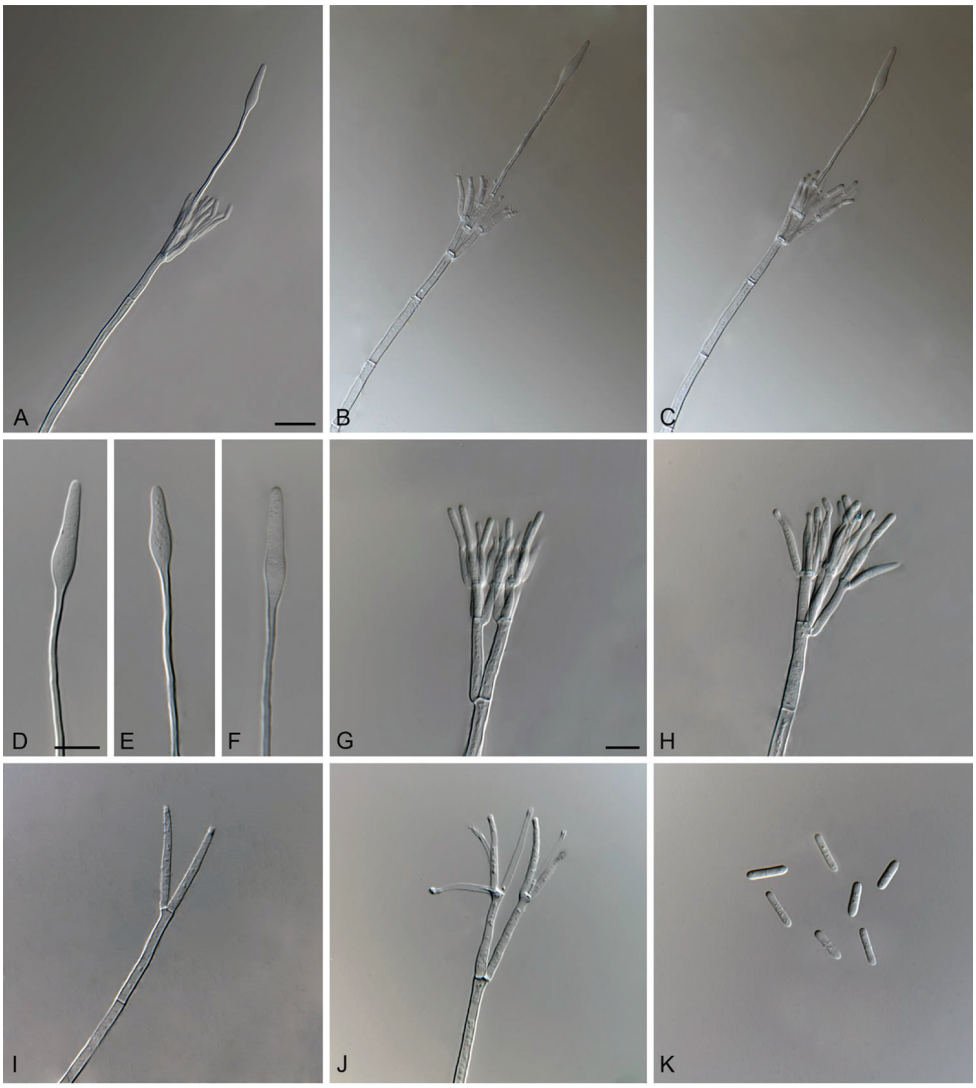

Figure 2. *Cylindrocladiella arbusta* (ex-type CMW 47295). **A–C** Penicillate conidiophores **D–F** Obpyriform to lanceolate vesicles **G–H** Penicillate conidiogenous apparatus **I–J** Subverticillate conidiophores **K** Conidia. Scale bars: **A** = 20 µm (apply to **B–C**); **D** = 10 µm (apply to **E–F**); **G** = 10 µm (apply to **H–K**).

apparatus with primary branches aseptate, 15–28.5 × 2.5–5 µm, secondary branches aseptate, 12–22.5 × 2.5–3.5 µm, each terminal branch producing 2–4 phialides; phialides doliiform to reniform to cymbiform, hyaline, aseptate, 10–18 × 2–3 µm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* in moderate numbers, comprising of a septate stipe and primary branches terminating in 2–4 phialides; primary branches straight, hyaline, 0–1-septate, 25–31 × 2.5–3.5 µm; phialides cymbiform to cylindrical, hyaline, aseptate, 16.5–30.5 × 2–3.5 µm, apex with minute periclinal thickening and collarette. *Conidia* cylindrical, rounded at both

Table 2. Comparisons of morphological characteristics of *Cylindrocladiella* spp. included in this study.

Species	Stipe extension	Vesicle		Macroconidia		Subverticillate conidiophores	References
	Length (µm)	Diam (µm)	Shape	Size (µm)	Average (µm)		
Cy. arbusta	93–139	4–5.5	obpyriform to lanceolate	(8.5–)10–12 (–13.5) × 2–3	11 × 2.5	moderate	This study
Cy. malesiana	114.5–144.5	4.5–6	fusoid to lanceolate	(10–)11–13(–13.5) × (1.5–)2–2.5	12 × 2	abundant	This study
Cy. microcylindrica	70–130	3–4	cylindrical to lanceolate	(10–)12–14 (–15) × 2(–3)	12.5 × 2	abundant	Schoch et al. 2000
Cy. natalensis	82–127	6–8	ellipsoidal to fusoid	(12–)14–16 (–17) × 2–3	15× 3	moderate	Lombard et al. 2012
Cy. obpyriformis	86.5–150	4–7	obpyriform	(9–)11–13(–15) × 2–3(–3.5)	12 × 2.5	abundant	This study
Cy. parvispora	112.5–141	4.5–6.5	fusoid to cylindrical	(8–)10– 12 (–13) × 2–2.5	11 × 2	moderate	This study
Cy. solicola	93.5–170	3.5–6.5	broadly clavate to lanceolate to fusiform	(10.5–)12.5–14.5(–15.5) × 2–3	13.5 × 2.5	abundant	This study

ends, straight, 1-septate, (8.5–)10–12(–13.5) × 2–3 µm (av. = 11 × 2.5 µm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics. Colonies white to buff on the surface and salmon to sienna in reverse on MEA after 7 d; smooth margins; extensive aerial mycelium in the middle and the margins; chlamydospores moderate, arranged in chains. Optimal growth temperature at 25 °C, no growth at 5 °C and 35 °C; after 7 d, colonies at 10 °C, 15 °C, 20 °C, 25 °C and 30 °C reached 3.5 mm, 27.7 mm, 49.2 mm, 67.9 mm and 52.7 mm, respectively.

Additional material examined. VIETNAM, Nghe An Province: Tan Ky, from soil in *Acacia mangium* nursery, Nov. 2013, N.Q. Pham & T.Q. Pham, PREM 62160, culture CMW 47296 = CBS 143547.

Distribution. Nghe An, Vietnam.

Notes. *Cylindrocladiella arbusta* is phylogenetically closely related to *Cy. natalensis*, *Cy. obpyriformis* and *Cy. parvispora*. The stipe extensions of *Cy. arbusta* are longer than those of *Cy. natalensis* and shorter than those of *Cy. obpyriformis* and *Cy. parvispora*. Conidia of *Cy. arbusta* are shorter than those of *Cy. natalensis* and *Cy. obpyriformis* (Table 2).

Cylindrocladiella malesiana **N.Q. Pham & M.J. Wingf., sp. nov.**
MycoBank MB824551
Figure 3

Etymology. Name refers to Malaysia, the country where this species was first collected.

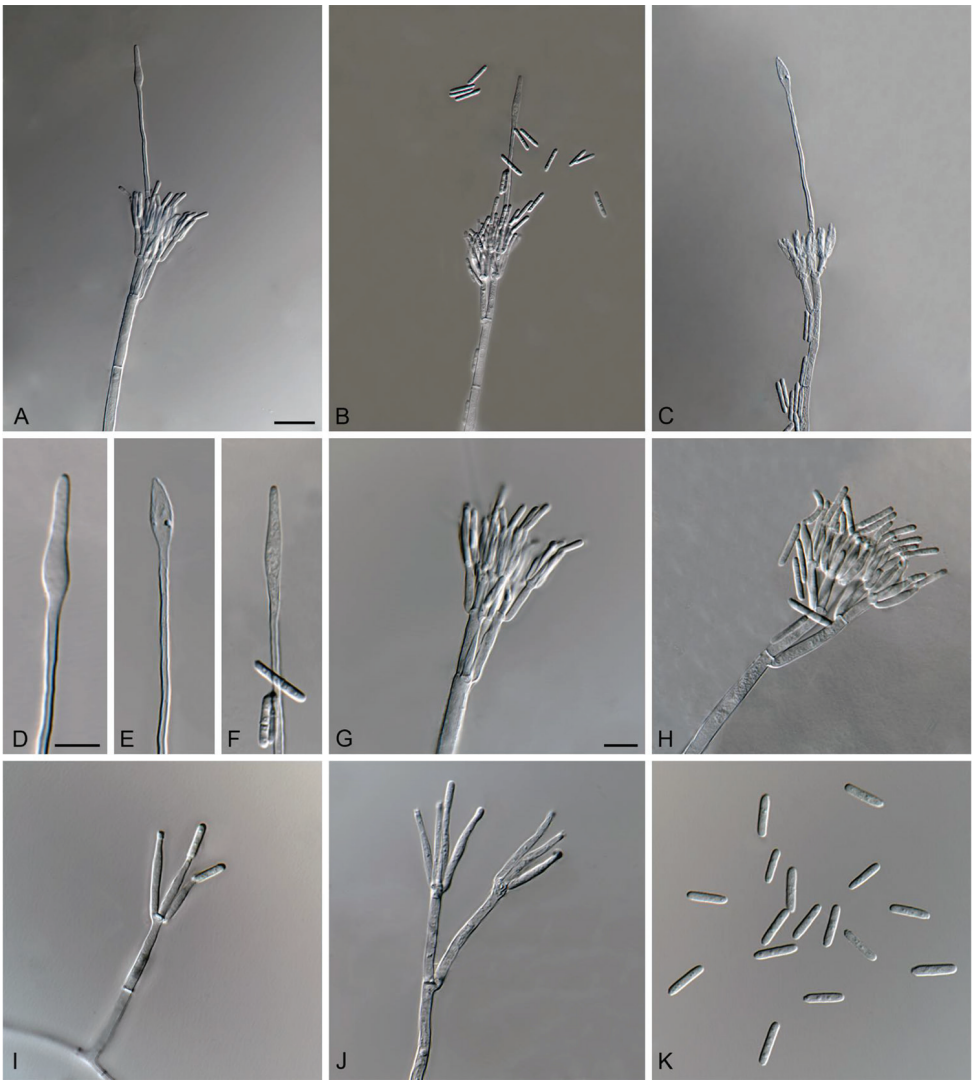

Figure 3. *Cylindrocladiella malesiana* (ex-type CMW 48278). **A–C** Penicillate conidiophores **D–F** Fusoid to lanceolate vesicles **G–H** Penicillate conidiogenous apparatus **I–J** Subverticillate conidiophores **K** Conidia. Scale bars: **A** = 20 µm (apply to **B–C**); **D** = 10 µm (apply to **E–F**); **G** = 10 µm (apply to **H–K**).

Type material. MALAYSIA. Sabah State: Tawau, Brumas, from soil in *Acacia mangium* plantation, Mar. 2013, M.J. Wingfield, herbarium specimen of dried culture, PREM 62161 (holotype), CMW 48278 = CBS 143548 (ex-type culture).

Description. *Sexual morph* not observed. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline. *Penicillate conidiophores* comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 76.5–126 × 3.5–5 µm; stipe extension aseptate, straight, 114.5–144.5 µm long, thick-walled with one basal septum, terminating

in thin-walled, fusoid to lanceolate vesicles, 4.5–6 µm wide. *Penicillate conidiogenous apparatus* with primary branches aseptate, 16.5–24 × 3–4.5 µm, secondary branches aseptate, 10.5–15 × 2–3.5 µm, each terminal branch producing 2–4 phialides; phialides cymbiform to cylindrical, hyaline, aseptate, 9–15.5 × 2–3.5 µm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* abundant, comprising of a septate stipe and primary branches terminating in 2–4 phialides; primary branches straight, hyaline, 0–1-septate, 13.5–35 × 2.5–4 µm; phialides cymbiform to cylindrical, hyaline, aseptate, 14.5–27 × 2–3.5 µm, apex with minute periclinal thickening and collarette. *Conidia* cylindrical, rounded at both ends, straight, 1-septate, (10–)11–13(–13.5) × (1.5–)2–2.5 µm (av. = 12 × 2 µm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics. Colonies buff to hazel on the surface and dark brick to brown vinaceous in reverse on MEA after 7 d; smooth to undulate margins; moderate aerial mycelium; chlamydospores moderate, arranged in chains. Optimal growth temperature at 25 °C, no growth at 5 °C and 35 °C; after 7 d, colonies at 10 °C, 15 °C, 20 °C, 25 °C and 30 °C reached 3.8 mm, 24.3 mm, 45.2 mm, 74.4 mm and 48.8 mm, respectively.

Distribution. Sabah, Malaysia

Additional material examined. MALAYSIA. Sabah state: Tawau, Brumas, from soil in *Acacia mangium* plantation, Mar. 2013, M.J. Wingfield, PREM 62162, culture CMW 48276 = CBS 143549; *ibid*., PREM 62163, culture CMW 48277 = CBS 143550.

Notes. *Cylindrocladiella malesiana* is phylogenetically closely related to *Cy. microcylindrica*, *Cy. natalensis* and *Cy. solicola*. Conidia of *Cy. malesiana* are shorter than those of *Cy. microcylindrica*, *Cy. natalensis* and *Cy. solicola* (Table 2).

Cylindrocladiella obpyriformis **N.Q. Pham, T.Q. Pham & M.J. Wingf., sp. nov.**
MycoBank MB824552
Figure 4

Etymology. Name refers to the obpyriform terminating vesicles in this species.

Type material. VIETNAM. Tuyen Quang Province, from soil in *Acacia* hybrid plantation, Nov. 2013, N.Q. Pham & T.Q. Pham, herbarium specimen of dried culture, PREM 62165 (holotype), CMW 47194 = CBS 143552 (ex-type culture).

Description. *Sexual morph* not observed. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline. *Penicillate conidiophores* comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 58.5–148 × 4–6 µm; stipe extension aseptate, straight, 86.5–150 µm long, thick-walled with one basal septum, terminating in thin-walled, obpyriform vesicles, 4–7 µm wide. *Penicillate conidiogenous apparatus* with primary branches aseptate, 17.5–31.5 × 3–5 µm, secondary branches aseptate, 10–19 × 2–4 µm, each terminal branch producing 2–4 phialides; phialides cymbiform to cy-

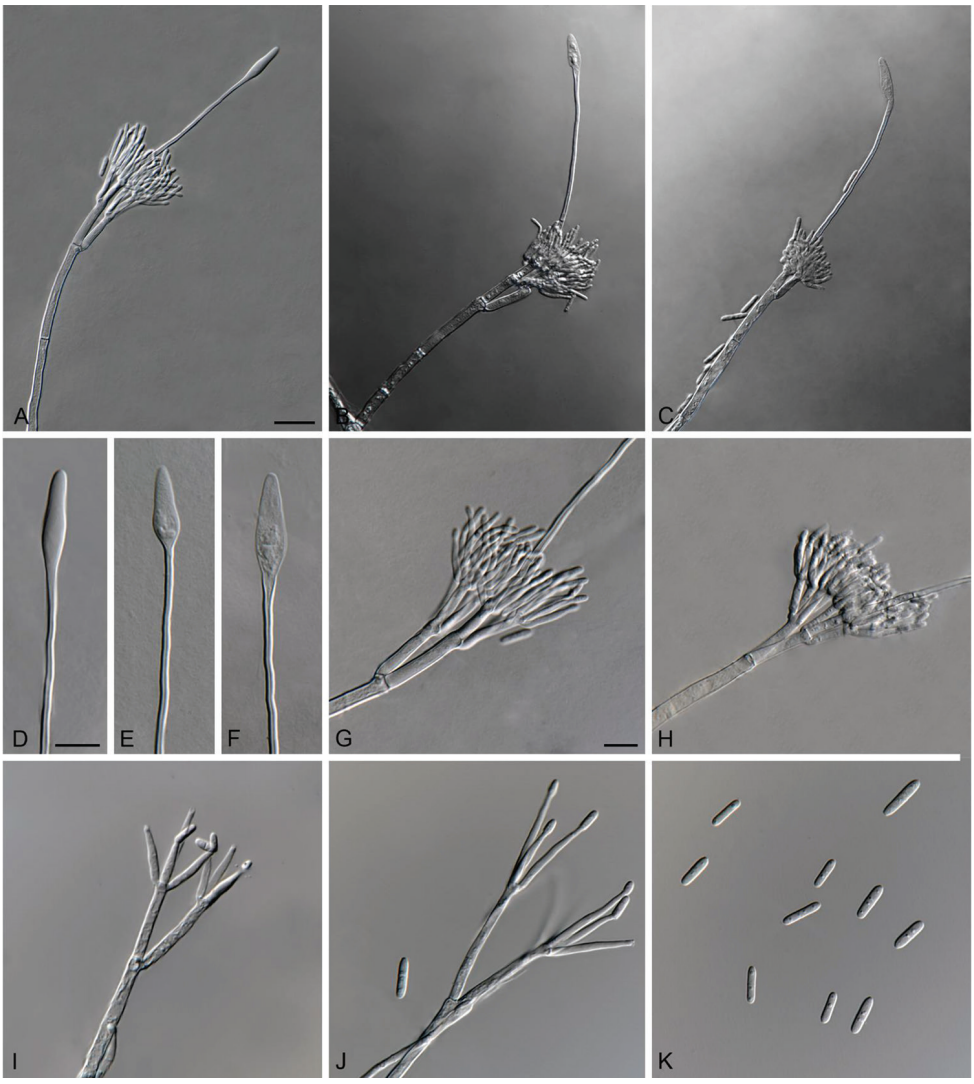

Figure 4. *Cylindrocladiella obpyriformis* (ex-type CMW 47194). **A–C** Penicillate conidiophores **D–F** Obpyriform vesicles **G–H** Penicillate conidiogenous apparatus **I–J** Subverticillate conidiophores **K** Conidia. Scale bars: **A** = 20 µm (apply to **B–C**); **D** = 10 µm (apply to **E–F**); **G** = 10 µm (apply to **H–K**).

lindrical, hyaline, aseptate, 10.5–18 × 2–3 µm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* abundant, comprising of a septate stipe and primary branches terminating in 2–4 phialides; primary branches straight, hyaline, 0–1-septate, 15–38.5 × 2–4 µm; phialides cymbiform to cylindrical, hyaline, aseptate, 13–30.5 × 2–3 µm, apex with minute periclinal thickening and collarette. *Conidia* cylindrical, rounded at both ends, straight, 1-septate, (9–)11–13(–15) × 2–3(–3.5) µm (av. = 12 × 2.5 µm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics. Colonies buff to isabelline on the surface and dark brick to sepia in reverse on MEA after 7 d; smooth to undulate margins; extensive aerial mycelium especially in the middle; chlamydospores moderate, arranged in chains. Optimal growth temperature at 25 °C, no growth at 5 °C and 35 °C; after 7 d, colonies at 10 °C, 15 °C, 20 °C, 25 °C and 30 °C reached 5.4 mm, 25.5 mm, 47.2 mm, 74.0 mm and 50.8 mm, respectively.

Distribution. Tuyen Quang & Vinh Phuc, Vietnam

Additional material examined. VIETNAM. Vinh Phuc Province: Tam Dao, from soil in *Camellia chrysantha* nursery, Sept. 2013, N.Q. Pham, Q.N. Dang & T.Q. Pham, PREM 62166, culture CMW 49940 = CBS 143553.

Notes. *Cylindrocladiella obpyriformis* is phylogenetically closely related to *Cy. arbusta*, *Cy. natalensis* and *Cy. parvispora*. The stipe extensions of *Cy. obpyriformis* are longer than those of *Cy. arbusta*, *Cy. natalensis* and *Cy. parvispora* (Table 2).

Cylindrocladiella parvispora **N.Q. Pham, T.Q. Pham & M.J. Wingfield, sp. nov.**
MycoBank MB824553
Figure 5

Etymology. Name refers to the small conidia produced by this species.

Type material. VIETNAM. Tuyen Quang Province, from soil in *Acacia* hybrid plantation, Nov. 2013, N.Q. Pham & T.Q. Pham, herbarium specimen of dried culture, PREM 62167 (holotype), CMW 47197 = CBS 143554 (ex-type culture).

Description. *Sexual morph* not observed. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline. *Penicillate conidiophores* comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 67–107 × 3–6.5 µm; stipe extension aseptate, straight, 112.5–141 µm long, thick-walled with one basal septum, terminating in thin-walled, fusoid to cylindrical vesicles, 4.5–6.5 µm wide. *Penicillate conidiogenous apparatus* with primary branches aseptate, 10.5–25 × 2–4 µm, secondary branches aseptate, 7.5–17 × 2–3 µm, each terminal branch producing 2–4 phialides; phialides doliiform to reniform to cymbiform, hyaline, aseptate, 7.5–13 × 2–3 µm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* in moderate numbers, comprising of a septate stipe and primary branches terminating in 2–4 phialides; primary branches straight, hyaline, 0–1-septate, 15.5–27 × 2.5–4 µm; phialides cymbiform to cylindrical, hyaline, aseptate, 13.5–41 × 2.5–6 µm, apex with minute periclinal thickening and collarette. *Conidia* cylindrical, rounded at both ends, straight, 1-septate, (8–)10– 12(–13) × 2–2.5 µm (av. = 11 × 2 µm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics. Colonies buff to honey to isabelline on the surface and umber to sepia in reverse on MEA after 7 d; smooth to undulate margin; abundant aerial mycelium especially in the middle; chlamydospores moderate, arranged in chains.

Figure 5. *Cylindrocladiella parvispora* (ex-type CMW 47197). **A–C** Penicillate conidiophores **D–F** Fusoid to cylindrical vesicles **G–H** Penicillate conidiogenous apparatus **I–J** Subverticillate conidiophores **K** Conidia. Scale bars: **A** = 20 μm (apply to **B–C**); **D** = 10 μm (apply to **E–F**); **G** = 10 μm (apply to **H–K**).

Optimal growth temperature at 25 °C, no growth at 5 °C and 35 °C; after 7 d, colonies at 10 °C, 15 °C, 20 °C, 25 °C and 30°C reached 5.5 mm, 23.4 mm, 43.8 mm, 63.6 mm and 49.2 mm, respectively.

Distribution. Tuyen Quang, Vietnam

Additional material examined. VIETNAM. Tuyen Quang Province, from soil in *Acacia* hybrid plantation, Nov. 2013, N.Q. Pham & T.Q. Pham, PREM 62168,

culture CMW 47207 = CBS 143555; *ibid.*, PREM 62169, culture CMW 47208 = CBS 143556.

Notes. *Cylindrocladiella parvispora* is phylogenetically closely related to *Cy. arbusta*, *Cy. natalensis* and *Cy. obpyriformis*. Conidia of *Cy. parvispora* are slightly smaller than those of *Cy. arbusta*, *Cy. natalensis* and *Cy. obpyriformis* (Table 2).

Cylindrocladiella solicola **N.Q. Pham, T.Q. Pham & M.J. Wingf., sp. nov.**
MycoBank MB824554
Figure 6

Etymology. Name refers to soil, the substrate from which this fungus was first isolated.

Type material. VIETNAM. Tuyen Quang Province, from soil in *Acacia* hybrid plantation, Nov. 2013, N.Q. Pham & T.Q. Pham, herbarium specimen of dried culture, PREM 62164 (holotype), CMW 47198 = CBS 143551 (ex-type culture).

Description. *Sexual morph* not observed. *Conidiophores* dimorphic, penicillate and subverticillate, mononematous and hyaline. *Penicillate conidiophores* comprising a stipe, a penicillate arrangement of fertile branches, a stipe extension and a terminal vesicle; stipe septate, hyaline, smooth, 58.5–120 × 2.5–5 µm; stipe extension aseptate, straight, 93.5–170 µm long, thick-walled with one basal septum, terminating in thin-walled, broadly clavate to lanceolate to fusiform vesicles, 3.5–6.5 µm wide. *Penicillate conidiogenous apparatus* with primary branches aseptate, 16–36.5 × 3–4.5 µm, secondary branches aseptate, 10–16 × 2.5–3.5 µm, each terminal branch producing 2–4 phialides; phialides cymbiform to cylindrical, hyaline, aseptate, 9–15.5 × 2–3 µm, apex with minute periclinal thickening and collarette. *Subverticillate conidiophores* abundant, comprising of a septate stipe and primary branches terminating in 2–4 phialides; primary branches straight, hyaline, 0–1-septate, 16.5–25 × 2.5–5 µm; phialides cymbiform to cylindrical, hyaline, aseptate, 12–28 × 2.5–4 µm, apex with minute periclinal thickening and collarette. *Conidia* cylindrical, rounded at both ends, straight, 1-septate, (10.5–)12.5–14.5(–15.5) × 2–2.5(–3) µm (av. = 13.5 × 2.5 µm), frequently slightly flattened at the base, held in asymmetrical clusters by colourless slime.

Culture characteristics. Colonies honey to isabelline on the surface and sepia to brown vinaceous in reverse on MEA after 7 d; undulate margins; extensive aerial mycelium especially in the middle; chlamydospores moderate, arranged in chains. Optimal growth temperature at 25 °C, no growth at 5 °C and 35 °C; after 7 d, colonies at 10 °C, 15 °C, 20 °C, 25 °C and 30 °C reached 5.2 mm, 20.4 mm, 37.8 mm, 61.2 mm and 37.1 mm, respectively.

Distribution. Tuyen Quang, Vietnam

Notes. *Cylindrocladiella solicola* is phylogenetically closely related to *Cy. malesiana*, *Cy. microcylindrica* and *Cy. natalensis*. The stipe extensions of *Cy. solicola* are longer than those of *Cy. malesiana*, *Cy. microcylindrica* and *Cy. natalensis* (Table 2).

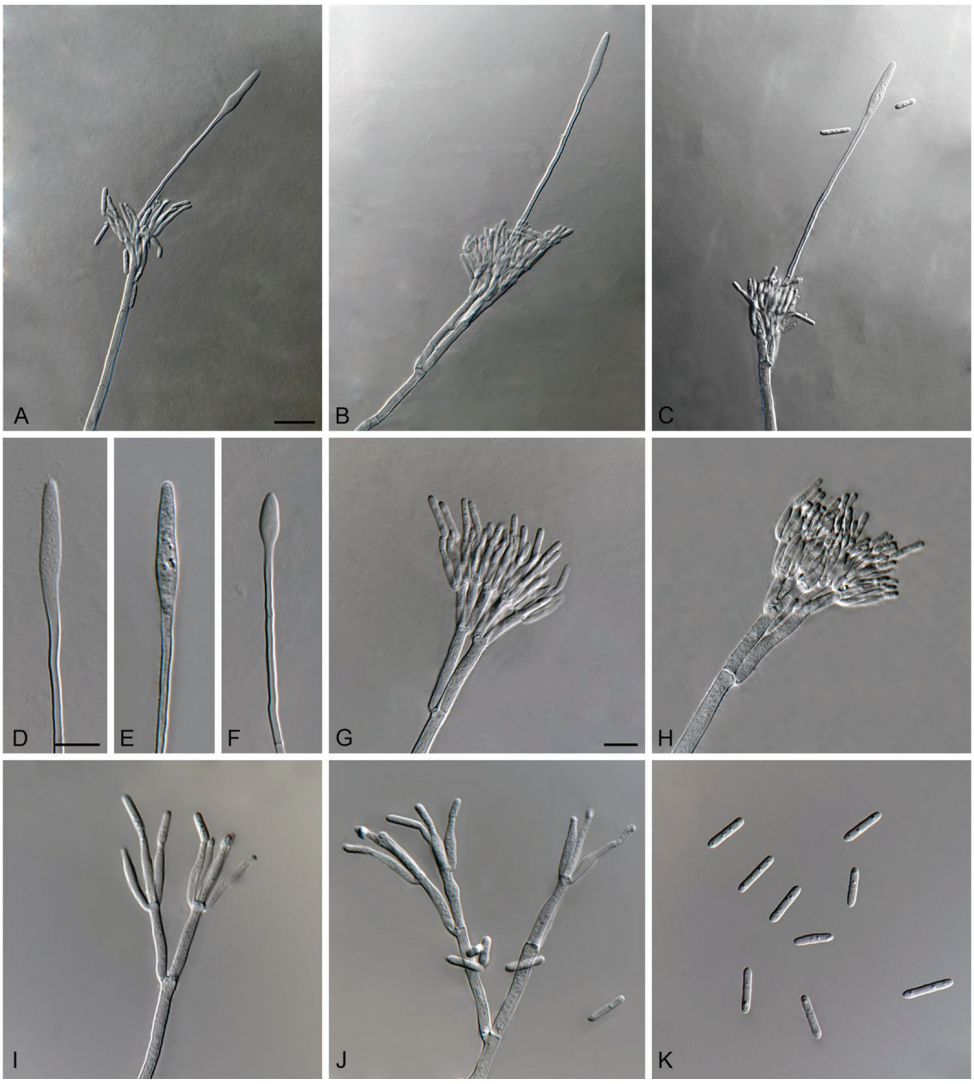

Figure 6. *Cylindrocladiella solicola* (ex-type CMW 47198). **A–C** Penicillate conidiophores **D–F** Broadly clavate to lanceolate to fusiform vesicles **G–H** Penicillate conidiogenous apparatus **I–J** Subverticillate conidiophores **K** Conidia. Scale bars: **A** = 20 µm (apply to **B–C**); **D** = 10 µm (apply to **E–F**); **G** = 10 µm (apply to **H–K**).

Discussion

Application of multigene phylogenetic inference made it possible to identify five novel and two known species of *Cylindrocladiella* in this study. The seven species found bring the number of *Cylindrocladiella* known from South-East Asia to 20 (Crous 2002, Lombard et al. 2012, 2017), thus suggesting that this geographical region could be a possible centre of diversity for the genus *Cylindrocladiella*. A relatively small collection of isolates was shown to represent a high diversity of *Cylindrocladiella* spp. This indicates that more *Cylindrocladiella* spp. remain to be discovered in South-East Asia.

The *his3* gene region provided the best resolution for species delineation amongst the four gene regions applied. This was the only gene region that could distinguish between all five novel species in the study. The ITS could not resolve any single lineage and the *tef1* gene region failed to distinguish between *Cy. arbusta* and *Cy. parvispora*. The phylogenetic relationship between *Cy. arbusta*, *Cy. malesiana* and *Cy. obpyriformis* could not be resolved using the *tub2* gene region (Suppl. materials 1–4). In the most recent study of species of *Cylindrocladiella* (Lombard et al. 2017), the *his3* gene region was not used in the analyses because it provided limited information compared with *tef1* and *tub2* gene sequences that were more informative. However, the results of the present study suggest that *his3* sequence data should be included in future studies as they provide valuable additional information on the relationships amongst some groups of species.

Five novel species, described as *Cy. arbusta*, *Cy. malesiana*, *Cy. obpyriformis, Cy. parvispora* and *Cy. solicola*, were all isolated from soil samples associated with *Acacia* plantations across Malaysia and Vietnam. In comparison with a previous study on *Calonectria* spp. from South-East Asia (Pham 2018), even though they share similar ecological niches, *Cylindrocladiella* spp. seemed to have a relatively narrow distribution and host association. This suggests that there is some substrate specialisation for these species of *Cylindrocladiella.* It is possible that they are mild pathogens of roots but no evidence of disease was observed.

This study includes the first report of *Cy. lageniformis* and *Cy. peruviana* in Vietnam. These two species have been reported as causal agents of black-foot disease, one of the most economically important fungal disease and a major constraint to wine and grape production (van Coller et al. 2005, Koike et al. 2016). The detection of these species from plantations soils in Vietnam might suggest that they infect the roots of *Acacia* spp. but this would require further investigation. These species have also been reported to cause leaf spots as well as root and cutting rot of *Eucalyptus* in Brazil (Crous et al. 1991, Crous and Wingfield 1993) and they clearly deserve further study in South-East Asia.

Acknowledgements

We acknowledge financial support from the Tree Protection and Cooperation Programme (TPCP) and the DST/NRF Centre of Excellence in Tree Health Biotechnology (CTHB), South Africa. We thank the members of the Forest Protection Research Centre (FPRC), Vietnam, especially Quynh N. Dang for the valuable assistance with the cultures.

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Supplementary material 1

Figure S1. Phylogenetic tree based on maximum likelihood (ML) analysis of *his3* sequence alignments

Authors: Nam Q. Pham, Irene Barnes, ShuaiFei Chen, Thu Q. Pham, Lorenzo Lombard, Pedro W. Crous, Michael J. Wingfield

Data type: molecular data

Explanation note: Bootstrap value ≥ 60 % for maximum parsimony (MP) and ML analyses are indicated at the nodes. Bootstrap values lower than 60 % are marked with "*" and absent are marked with "–". Isolates representing ex–type material are marked with "T" and isolates collected in this study are highlighted in **bold**. *Calonectria brachiatica* (CMW 25307) and *Calonectria pauciramosa* (CMW 5683) represent the outgroups.

Link: https://doi.org/10.3897/mycokeys.32.23754.suppl1

Supplementary material 2

Figure S2. Phylogenetic tree based on maximum likelihood (ML) analysis of *tef*1 sequence alignments

Authors: Nam Q. Pham, Irene Barnes, ShuaiFei Chen, Thu Q. Pham, Lorenzo Lombard, Pedro W. Crous, Michael J. Wingfield

Data type: molecular data

Explanation note: Phylogenetic tree based on maximum likelihood (ML) analysis of *tef1* sequence alignments. Bootstrap value ≥ 60 % for maximum parsimony (MP) and ML analyses are indicated at the nodes. Bootstrap values lower than 60% are marked with "*" and absent are marked with "–". Isolates representing ex–type material are marked with "T" and isolates collected in this study are highlighted in **bold**. *Calonectria brachiatica* (CMW 25307) and *Calonectria pauciramosa* (CMW 5683) represent the outgroups.

Link: https://doi.org/10.3897/mycokeys.32.23754.suppl2

Supplementary material 3

Figure S3. Phylogenetic tree based on maximum likelihood (ML) analysis of *tub*2 sequence alignments

Authors: Nam Q. Pham, Irene Barnes, ShuaiFei Chen, Thu Q. Pham, Lorenzo Lombard, Pedro W. Crous, Michael J. Wingfield

Data type: molecular data

Explanation note: Bootstrap value ≥ 60 % for maximum parsimony (MP) and ML analyses are indicated at the nodes. Bootstrap values lower than 60 % are marked with "*" and absent are marked with "–". Isolates representing ex–type material are marked with "T" and isolates collected in this study are highlighted in **bold**. *Calonectria brachiatica* (CMW 25307) and *Calonectria pauciramosa* (CMW 5683) represent the outgroups.

Link: https://doi.org/10.3897/mycokeys.32.23754.suppl3

Supplementary material 4

Figure S4. Phylogenetic tree based on maximum likelihood (ML) analysis of ITS sequence alignments

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Data type: molecular data

Explanation note: Bootstrap value ≥ 60 % for maximum parsimony (MP) and ML analyses are indicated at the nodes. Bootstrap values lower than 60 % are marked with "*" and absent are marked with "–". Isolates representing ex–type material are marked with "T" and isolates collected in this study are highlighted in **bold**. *Calonectria brachiatica* (CMW 25307) and *Calonectria pauciramosa* (CMW 5683) represent the outgroups.

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Taxonomy and phylogeny of *Lopharia* s.s., *Dendrodontia*, *Dentocorticium* and *Fuscocerrena* (Basidiomycota, Polyporales)

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Abstract

Eleven taxa of *Lopharia* s.s., *Dendrodontia*, *Dentocorticium* and *Fuscocerrena* in Polyporales are included in the phylogenetic analyses of nuc rDNA ITS1-5.8S-ITS2 (ITS), D1-D2 domains of nuc 28S rDNA (28S) and RNA polymerase II second-largest subunit (*rpb2*) sequences. New species *Lopharia resupinata* and *L. sinensis* are described and illustrated. *Lopharia resupinata*, from south-eastern China, is closely related to *L. ayresii*, and *L. sinensis*, from northern China, is related to *L. cinerascens* and *L. mirabilis*. *Lopharia mirabilis* specimens from temperate to tropical areas with varied hymenophore configurations all cluster together in a fully supported clade. *Dendrodontia* and *Fuscocerrena* are shown to be synonyms of *Dentocorticium*, which is phylogenetically related to *Lopharia*. Four new combinations, *Dentocorticium bicolor*, *D. hyphopaxillosum*, *D. portoricense* and *D. taiwanianum*, are proposed. Revised generic descriptions of *Lopharia* and *Dentocorticium* are provided with keys to the six accepted species in each genus. A list of all names in *Lopharia* and *Dentocorticium* are presented with their current taxonomic status. Type specimens of *Dentocorticium brasiliense* and *D. irregulare* were examined and determined to be later synonyms of *Punctularia subhepatica* and *Diplomitoporus daedaleiformis*, respectively.

Keywords

Corticioid fungi, dendrohyphidia, species complex, wood-inhabiting fungi

Introduction

The genus *Lopharia* s.s., typified by *L. lirellosa* Kalchbr. & MacOwan (= *Radulum mirabile* Berk. & Broome), is characterised by a dimitic hyphal system with clamped generative hyphae, large basidia and basidiospores and large, encrusted, hyaline, thick-walled cystidia (Hjortstam and Ryvarden 1990, Boidin and Gilles 2002, Bernicchia and Gorjón 2010). Of 35 taxa placed in *Lopharia*, Hjortstam and Ryvarden (1990) accepted only *L. cinerascens* (Schwein.) G. Cunn. and *L. mirabilis* (Berk. & Broome) Pat. and Boidin and Gilles (2002) additionally accepted *L. pseudocinerascens* Boidin & Gilles. Welden (1975, 2010) adopted a broad interpretation of *Lopharia* that included species of *Porostereum* Pilát. A few phylogenetic studies that have included *Lopharia* s.s. and *Porostereum spadiceum* (Pers.) Hjortstam & Ryvarden (generic type) showed that they are distantly related (Ko et al. 2001, Yoon et al. 2003, Wu et al. 2007, Jang et al. 2016). Both genera are included in the Polyporales with *Lopharia* in the Polyporaceae and *Porostereum* in the Phanerochaetaceae (Justo et al. 2017).

Dentocorticium (Parmasto) M.J. Larsen & Gilb. was segregated from *Laeticorticium* Donk to accommodate *L. ussuricum* Parmasto (generic type) and *Hydnum sulphurellum* Peck (Larsen and Gilbertson 1974) because they lack probasidia. Subsequently, nine species were described or transferred to the genus (Larsen and Gilbertson 1977, Ryvarden 1978, Domański 1988, Boidin et al. 1996, Boidin and Gilles 1998, Duhem and Michel 2009).

Dendrodontia Hjortstam & Ryvarden (generic type *Grandinia bicolor* P.H.B. Talbot) is similar to *Dentocorticium* in possessing tuberculate to odontoid hymenophore, dendrohyphidia and thin-walled smooth basidiospores, but differs by its dimitic hyphal system with brown skeletal hyphae (Hjortstam and Ryvarden 1980, Boidin and Gilles 1998). The monotypic genus *Fuscocerrena* Ryvarden was erected for *Polyporus portoricensis* Spreng. ex Fr. This taxon is characterised by dark brown, effused, effused-reflexed to pileate basidiocarps with a poroid to spinose hymenophore, a dimitic hyphal system with brown skeletal hyphae and dendrohyphidia (Ryvarden 1982). Except for the variable hymenophore configuration and greenish-yellow hymenial surface, *F. portoricensis* (Spreng. ex Fr.) Ryvarden is similar to many species of *Dendrodontia* and *Dentocorticium* at the microscopic level.

Morphologically, *Lopharia* s.s. is distinct from *Dentocorticium* and *Dendrodontia* but are phylogenetically closely related as shown in phylogenetic studies based on two to six taxa (Yoon et al. 2003, Wu et al. 2007, Justo and Hibbett 2011, 2017, Jang et al. 2016). In this study, eleven taxa of *Lopharia* s.s., *Dentocorticium*, *Dendrodontia* and *Fuscocerrena* from North America and East Asia were included in phylogenetic analyses of a concatenated 3-gene dataset of ITS, 28S and *rpb2* sequences.

Materials and methods

Morphological studies. Voucher specimens are deposited in the herbarium of Beijing Forestry University (BJFC), the National Museum of Natural Science in Taiwan (TNM)

and the Centre for Forest Mycology Research (CFMR). Samples for microscopic examination were mounted in 0.2 % cotton blue in lactic acid, 1 % phloxine and Melzer's reagent. The following abbreviations are used: L = mean spore length, W = mean spore width, Q = L/W ratio, n (a/b) = number of spores (a) measured from given number of specimens (b). Colour codes and names follow Kornerup and Wanscher (1978).

DNA extraction and sequencing. A CTAB plant genome rapid extraction kit-DN14 (Aidlab Biotechnologies Co. Ltd, Beijing) was employed for DNA extraction and PCR amplification from dried specimens. The ITS, 28S and *rpb2* gene regions were amplified with the primer pairs ITS5 and ITS4 (White et al. 1990), LR0R and LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm) and *rpb2*-f5F and *rpb2*-7.1R (Liu et al. 1999, Matheny et al. 2007), respectively. The PCR procedures for ITS and 28S followed Liu et al. (2017), while the procedure for *rpb2* was the same as Justo and Hibbett (2011). DNA sequencing was performed at Beijing Genomics Institute and the sequences are deposited in GenBank (Table 1).

Phylogenetic analyses. The molecular phylogeny used a combined dataset of ITS, 28S and *rpb2* sequences. Justo and Hibbett (2011) was consulted for taxon sampling and outgroup selection. The sequences were aligned using the MAFFT v.6 (Katoh and Toh 2008, http://mafft.cbrc.jp/alignment/server/). Alignments were optimised manually in BioEdit 7.0.5.3 (Hall 1999) and deposited at TreeBase (http://treebase.org/treebase-web/home.html, submission ID: 21717).

Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian Inference (BI) analyses were performed by using RAxML 7.2.6 (Stamatakis 2006), PAUP* 4.0b10 (Swofford 2002) and MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), respectively. In ML analysis, statistical support values were obtained from rapid bootstrapping of 1000 replicates using default settings for other parameters. In MP analysis, gaps in the alignments were treated as missing data. Trees were generated using 100 replicates of random stepwise addition of sequence and tree-bisection reconnection (TBR) branch-swapping algorithm with all characters given equal weight. Branch supports for all parsimony analyses were estimated by performing 1000 bootstrap replicates (Felsenstein 1985) with a heuristic search of 10 random-addition replicates for each bootstrap replicate. For BI, best models of evolution were estimated by using MrModeltest 2.2 (Nylander 2004) and the Bayesian posterior probabilities (BPP) were determined by Markov Chain Monte Carlo sampling in MrBayes 3.1.2. Four simultaneous Markov chains were run for two million generations and trees were sampled every 100th generation. The first quarter of the trees, which represented the burn-in phase of the analyses, were discarded and the remaining trees were used to calculate posterior probabilities in the majority rule consensus tree.

Phylogeny results

The ITS-28S-*rpb2* sequences dataset contained 54 ITS, 55 nuc 28S and 40 *rpb2* sequences from 56 samples representing 38 ingroup and 2 outgroup taxa (Table 1). Twenty-three

Table 1. Species and sequences used in the phylogenetic analyses. Newly generated sequences are set in bold.

Taxa	Voucher	Locality	ITS	28S	rpb2
Amauroderma rugosum	ML 56	Japan	AB509712	AB368061	AB368119
Boletopsis leucomelaena	AFTOL 1527	USA	DQ484064	DQ154112	GU187820
Climacodon septentrionalis	AFTOL 767	USA	AY854082	AY684165	AY780941
Coriolopsis gallica	RLG-7630-Sp	USA	JN165013	JN164814	JN164821
Coriolopsis trogii	RLG-4826-Sp	USA	JN164993	JN164808	JN164867
Daedaleopsis confragosa	WD 747	Japan	GU731549	AB368062	AB368120
Datronia mollis	RLG-6304-Sp	USA	JN165002	JN164791	JN164872
Datronia scutellata	RLG-9584-T	USA	JN165004	JN164792	JN164873
Dendrocorticium bicolor	**He 2772**	**China**	**MF626354**	**MF626378**	**–**
Dendrocorticium bicolor	**He 2757**	**China**	**MF626355**	**MF626379**	**–**
Dendrocorticium portoricense	**He 2161**	**USA**	**MF626356**	**MF626380**	**MF626397**
Dendrocorticium portoricense	**He 2202**	**USA**	**MF626357**	**MF626381**	**–**
Dendrocorticium taiwanianum	**He 3383**	**China**	**MF626361**	**MF626385**	**–**
Dendrocorticium taiwanianum	**He 4615**	**China**	**MF626362**	**MF626386**	**–**
Dendrocorticium taiwanianum	**He 3777**	**China**	**–**	**MF626388**	**–**
Dendrocorticium taiwanianum	**Wu 9907-1 (type)**	**China**	**MF626363**	**MF626387**	**–**
Dendrocorticium ussuricum	**He 3322**	**China**	**MF626360**	**MF626384**	**MF626399**
Dendrocorticium ussuricum	**He 3278**	**China**	**MF626358**	**MF626382**	**–**
Dendrocorticium ussuricum	**He 3294**	**China**	**MF626359**	**MF626383**	**MF626398**
Dentocorticium sulphurellum	T 609	Canada	JN165015	JN164815	JN164875
Earliella scabrosa	PR 1209	Puerto Rico	JN165009	JN164793	JN164866
Fomitopsis pinicola	AFTOL 770	USA	AY854083	AY684164	AY786056
Ganoderma lucidum	WD 565	Japan	EU021460	AB368068	AB368126
Ganoderma tsugae	AFTOL 771	USA	DQ206985	AY684163	DQ408116
Grifola sordulenta	AFTOL 562	USA	AY854085	AY645050	AY786058
Hydnellum geogenium	AFTOL 680	USA	DQ218304	AY631900	DQ408133
Irpex lacteus	TM 03-480	Japan	AB079264	EU522839	DQ408117
Lentinus squarrosulus	WD 1729	Japan	GU001951	AB368071	AB368129
Lentinus tigrinus	MUCL 22821	Japan	AF516520	AB368072	AB368130
Lenzites betulinus	AJ 150	USA	JN164915	–	–
Lopharia ayresii	**He 20120724-4**	**China**	**MF626352**	**MF626375**	**–**
Lopharia ayresii	**He 2778**	**China**	**MF626353**	**MF626376**	**–**
Lopharia cinerascens	**He 2188**	**USA**	**MF626350**	**MF626373**	**MF626395**
Lopharia cinerascens	**He 2228**	**USA**	**MF626351**	**MF626374**	**–**
Lopharia resupinata	**He 4401 (type)**	**China**	**–**	**MF626377**	**MF626396**
Lopharia mirabilis	**Dai 5147**	**China**	**MF626342**	**MF626365**	**MF626389**
Lopharia mirabilis	**Yuan 2532**	**China**	**MF626343**	**MF626366**	**MF626390**
Lopharia mirabilis	**Dai 5598**	**China**	**MF626341**	**MF626364**	**–**
Lopharia mirabilis	**He 4558**	**China**	**MF626344**	**MF626367**	**–**
Lopharia mirabilis	**Dai 14978**	**China**	**MF626345**	**MF626368**	**MF626391**
Lopharia mirabilis	**Dai 13722**	**China**	**MF626346**	**MF626369**	**MF626392**
Lopharia sinensis	**He 2428 (type)**	**China**	**MF626347**	**MF626370**	**MF626393**
Lopharia sinensis	**He 2510**	**China**	**MF626348**	**MF626371**	**MF626394**

Taxa	Voucher	Locality	ITS	28S	rpb2
Lopharia sinensis	**He 2424**	**China**	**MF626349**	**MF626372**	**–**
Lopharia sp.	FP-105043	USA	JN165019	JN164813	JN164874
Phanerochaete chrysosporium	FPL 5175	USA	AF854086	AF287883	–
Phlebia radiata	FPL 6140	USA	AY854087	AF287885	AY218502
Polyporus squamosus	AFTOL 704	USA	DQ267123	AY629320	DQ408120
Polyporus umbellatus	WD 719	Japan	EU442276	AB368109	AB368166
Pseudofavolus cucullatus	WD 2157	Japan	AF516601	AB368114	AB368170
Pycnoporus sanguineus	PR-SC-95	Puerto Rico	JN164982	JN164795	JN164858
Pycnoporus cinnabarinus	ZW 02-30	China	DQ411525	AY684160	DQ408121
Trametes ectypa	FP-106037-T	USA	JN164929	JN164803	JN164848
Trametes hirsuta	RLG-5133-T	USA	JN164941	JN164801	JN164854
Trametes versicolor	FP-135156-Sp	USA	JN164919	JN164809	JN164850
Trametopsis cervina	TJV-93-216-Sp	USA	JN165020	JN164796	JN164877

ITS, 25 nuc 28S and 11 *rpb2* sequences were generated for this study (Table 1). The dataset had an aligned length of 2806 characters, of which 836 were parsimony informative. MP analysis yielded four equally parsimonious trees (TL = 5240, CI = 0.323, RI = 0.594, RC = 0.192, HI = 0.677). The best model estimated and applied in the Bayesian analysis was GTR+I+G. MP and BI analyses resulted in almost the same tree topologies as that of ML analysis, which is similar to that of Justo and Hibbett (2011). Only the ML tree is shown in Fig. 1 with maximum likelihood and maximum parsimony bootstrap values ≥ 50 % and BPP ≥ 0.95 labelled along the branches. In the tree, the Dentocorticium clade sensu Justo and Hibbett (2011) was recovered and strongly supported. The five species of *Lopharia* s.s. and FP-105043 (as *Lopharia* sp.) are in a strongly supported lineage with two subclades – (1) *Lopharia sinensis*, *L. mirabilis* and *L. cinerascens* and (2) *L. resupinata* and *L. ayresii*. The *Dentocorticium* species are in a clade with five distinct and well-supported lineages representing the species *D. ussuricum*, *D. sulphurellum*, *D. bicolor*, *D. taiwanianum* and *D. portoricense*.

Taxonomy of *Lopharia* species

Lopharia resupinata **S.H. He, S.L. Liu & Y.C. Dai, sp. nov.**
MycoBank: MB823071
Figs 2A–B, 3

Diagnosis. Distinguished from other *Lopharia* species by its resupinate basidiocarps, a densely compact texture, a monomitic hyphal system and small basidiospores 7–9(–10) × 4–5 µm.

Holotype. CHINA. Jiangxi Province: Anyuan County, Sanbaishan Forest Park, on fallen angiosperm branch, 15 Aug. 2016, He 4401 (holotype, BJFC 023842!).

Etymology. "*resupinata*" (Lat.) refers to the resupinate basidiocarps.

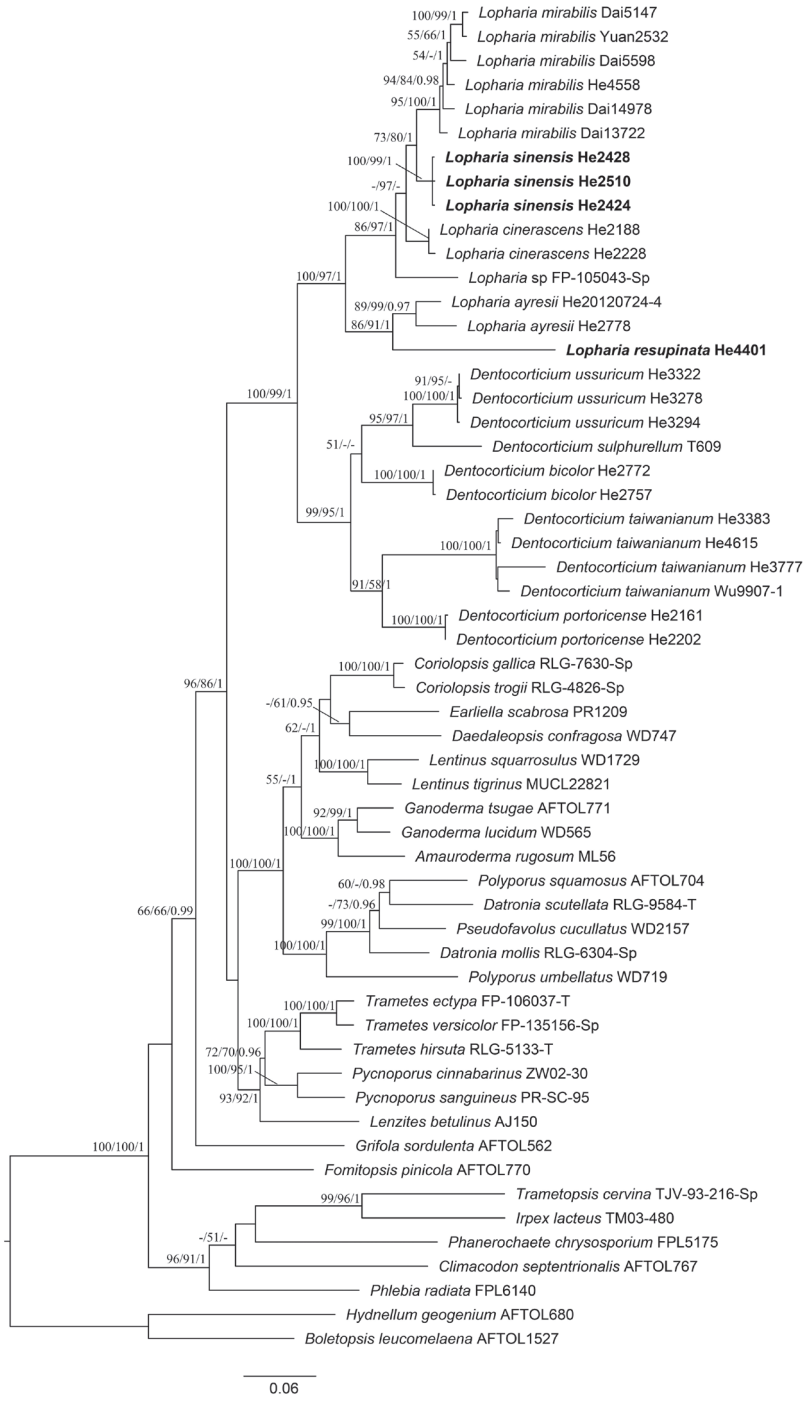

Figure 1. Phylogenetic tree inferred from maximum likelihood analysis of the combined ITS, 28S and *rpb2* sequences of taxa in Polyporales. Branches are labelled with maximum likelihood and maximum parsimony bootstrap values ≥ 50 % and Bayesian posterior probabilities ≥ 0.95.

Figure 2. Basidiocarps of *Lopharia* species. **A–B** *L. resupinata* (holotype, He 4401) **C–D** *L. sinensis* (**C** holotype, He 2428 **D** He 2510) **E** *L. ayresii* (He 3884) **F** *L. cinerascens* (He 2228). Scale bars: 1 cm.

Fruiting body. Annual, resupinate, adnate, ceraceous, hygrophanous, not separable from the substrate when fresh, becoming crustaceous, brittle and easily detached from substrate upon drying, first as small patches, later confluent up to 20 cm long, 2.5 cm wide, up to 400 μm thick. Hymenophore smooth, under a lens pilose from projecting cystidia, pale orange (6A3), orange grey (6B2) to greyish-orange (6B3) when fresh, becoming brownish-orange [6C(2–4)] to light brown [6D(4–5)] upon drying, uncracked; margin abrupt, concolorous when fresh, reflexed and incurved upon drying, abhymenial surface white (6A1).

Microscopic structures. Hyphal system monomitic, generative hyphae with clamp connections. Subiculum thin, with numerous small crystals; hyphae hyaline, thin- to slightly thick-walled, moderately septate and branched, interwoven, 2–3.5 μm

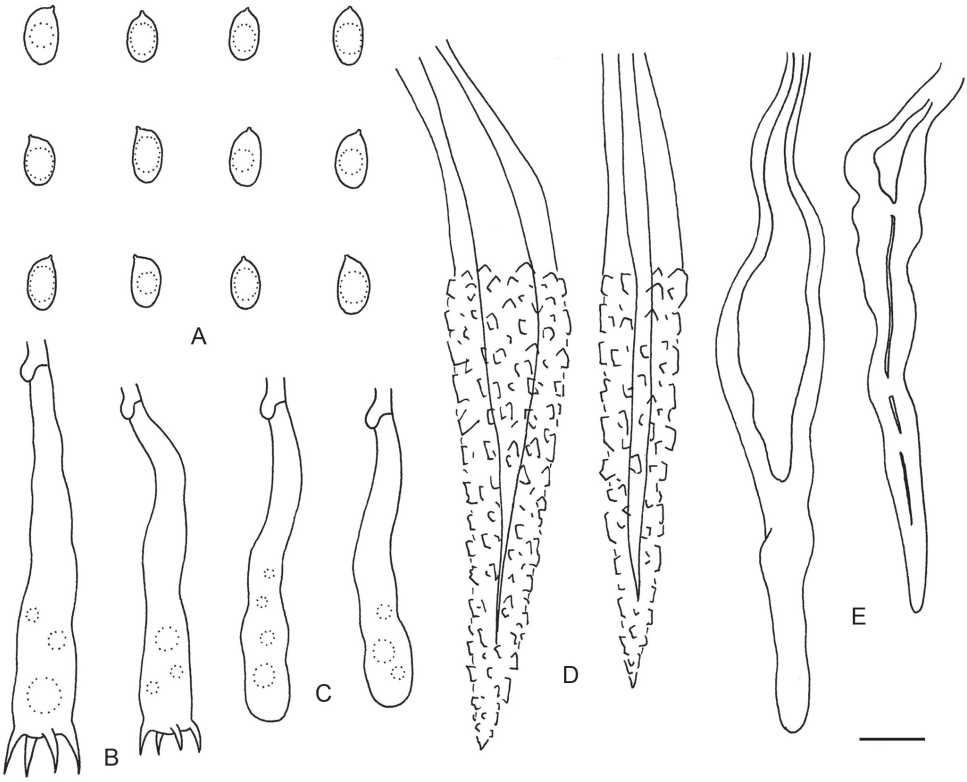

Figure 3. Microscopic structures of *Lopharia resupinata* (drawn from the holotype). **A** Basidiospores **B** Basidia **C** Basidioles **D–E** Lamprocystidia (**D** in cotton blue **E** in KOH).

in diam. Subhymenium thickening, up to 300 μm thick; hyphae hyaline, slightly thick-walled, vertically arranged, densely agglutinated, 2–4 μm in diam. Lamprocystidia abundant, arising from subhymenium, subulate, heavily encrusted with crystals, distinctly thick-walled, embedded in subhymenium or exerted, 80–150 × 10–20 μm. Basidia clavate, with a basal clamp connection and four sterigmata, 50–65 × 8–10 μm; basidioles dominating in hymenium, similar to basidia but smaller. Basidiospores ellipsoid, hyaline, thin-walled, smooth, containing a large guttule, IKI–, CB–, 7–9(–10) × 4–5 μm, L = 7.9 μm, W = 4.4 μm, Q = 1.81 (n = 30/1).

Remarks. *Lopharia resupinata*, like *L. ayresii*, has a resupinate habit, a monomitic hyphal system and a densely compact texture. *Lopharia ayresii* (Fig. 2E), however, has larger basidiospores (11.2 ± 0.7 × 6.4 ± 0.4 μm, from type, Boidin and Gilles 1991). In Fig. 1, *L. resupinata* and *L. ayresii* cluster together. *Lopharia cinerascens* and *L. mirabilis* differ from *L. resupinata* by having effused-reflexed to pileate basidiocarps, a dimitic hyphal system and larger basidiospores (Hjortstam and Ryvarden 1990, Boidin and Gilles 2002). *Lopharia resupinata* has a thickening subhymenium with embedded lamprocystidia, characters that are also found in species of *Phlebiopsis* Jülich.

***Lopharia sinensis* S.H. He, S.L. Liu & Y.C. Dai, sp. nov.**
MycoBank: MB823072
Figs 2C–D, 4

Diagnosis. Differs from *L. cinerascens* by its ellipsoid basidiospores and long, projecting cystidia. Known only from northern China.

Holotype. CHINA. Ningxia Autonomous Region: Jingyuan County, Liupanshan Forest Park, on dead angiosperm branch, 4 Aug. 2015, He 2428 (holotype, BJFC 020881!).

Etymology. "*sinensis*" (Lat.) refers to the type locality in China.

Fruiting body. Annual, effused to effused-reflexed, adnate, coriaceous, first as small patches, later confluent, effused part up to 8 cm long, 2.5 cm wide, up to 1 mm thick, pilei projecting up to 1 cm, 3 cm wide. Abhymenial surface tomentose to glabrous, greyish-orange (6B3) to brownish-grey [6D(2–4)]. Hymenophore smooth, greyish-orange (6B3), greyish-brown (6D3) to light brown [6D(4–6)], uncracked; margin thinning out, lighter than hymenophore surface, up to 1.5 mm wide, becoming indistinct and concolorous with age.

Microscopic structures. Hyphal system dimitic, generative hyphae with clamp connections. Cortex and tomentum present. Subiculum well developed, hyphae more or less regularly arranged, interwoven. Skeletal hyphae dominant, thick-walled, pale yellow, unbranched and septate, flexuous, 3–6 μm in diam. Generative hyphae hyaline, thin- to slightly thick-walled, rarely branched and septate, 2–4 μm in diam. Lamprocystidia abundant, large, subulate, distinctly thick-walled, arising from subhymenium, 100–280 × 8–20 μm, projecting up to 200 μm beyond hymenium. Basidia clavate, with a basal clamp and four sterigmata, 45–70 × 9–13 μm; basidioles dominating in hymenium, in shape similar to basidia, but smaller. Basidiospores ellipsoid, hyaline, thin-walled, smooth, containing a large guttule, IKI–, CB–, 11–14 × (6–)6.5–8 μm, L = 12.6 μm, W = 7.1 μm, Q = 1.75–1.79 (n = 60/2).

Additional specimens examined. CHINA. Gansu Province: Pingliang County, Kongtongshan Forest park, on fallen trunk of *Euonymus maackii*, 3 Aug 2015, He 2401 (BJFC 020855); on dead angiosperm branch, 3 Aug 2015, He 2408 (BJFC 020862); Tianshui County, Dangchuan Forest Farm, on construction wood, 8 Aug 2015, He 2510 (BJFC 020963). Hebei Province: Xinglong County, Wulingshan Nature Reserve, on fallen angiosperm branch, 2 Sep 2017, He 5005 (BJFC). Ningxia Autonomous Region: Jingyuan County, Liupanshan Forest Park, on dead angiosperm trunk, 4 Aug 2015, He 2424 (BJFC 020877) & He 2438 (BJFC 020891).

Remarks. *Lopharia sinensis* belongs to the *L. cinerascens* clade (Fig. 1). It differs from *L. mirabilis* by its smooth hymenophore surface and north temperate distribution and from *L. cinerascens* by its ellipsoid basidiospores and long, projecting cystidia (Hjortstam and Ryvarden 1990, Dai 2002). *Lopharia pseudocinerascens* from Africa also belongs to the *L. cinerascens* group and can be distinguished from *L. sinensis* by narrower basidiospores (8–14 × 4.5–6.5 μm, Boidin and Gilles 2002).

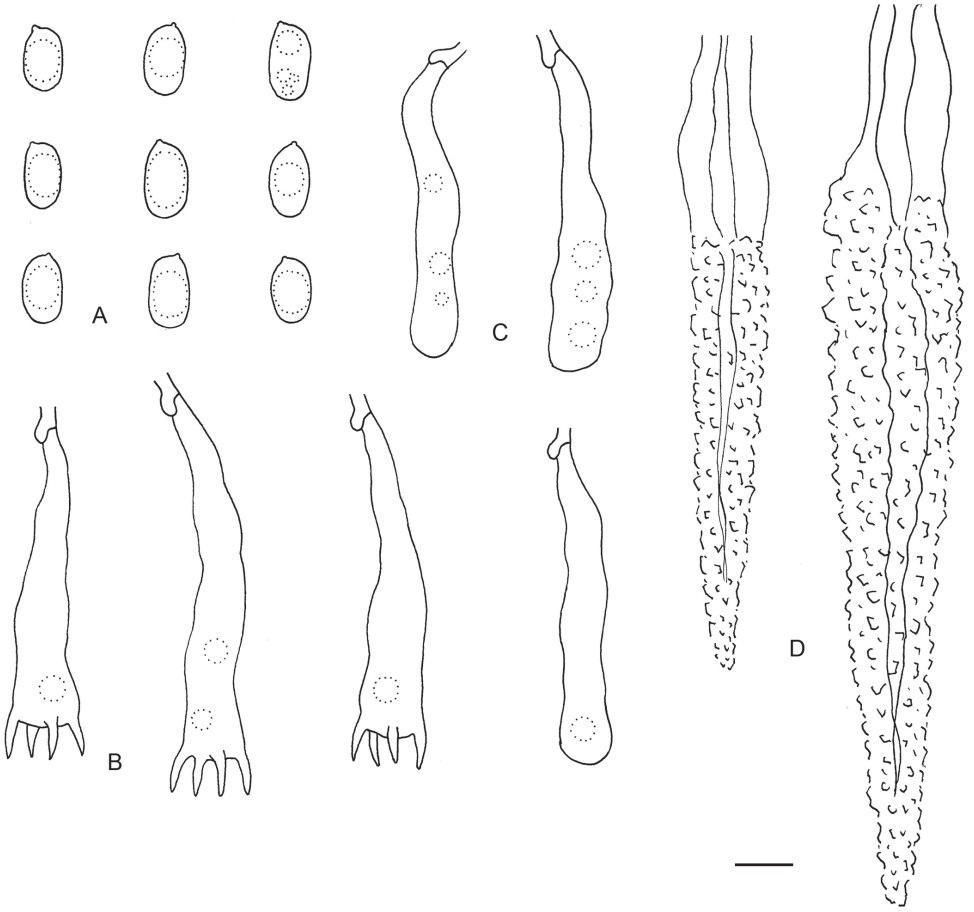

Figure 4. Microscopic structures of *Lopharia sinensis* (drawn from holotype). **A** Basidiospores **B** Basidia **C** Basidioles **D** Lamprocystidia.

Six species of *Lopharia*, *L. ayresii*, *L. cinerascens*, *L. resupinata*, *L. mirabilis*, *L. sinensis* and *Lopharia* sp. (FP-105043) are included in a fully supported monophyletic clade (Fig. 1). They all develop the large encrusted cystidia, the large basidia (> 50 µm long) and the relatively large basidiospores (> 8 µm long and 4 µm wide) that characterise the genus. *Lopharia mirabilis*, the generic type, is a tropical species possessing a tuberculate, odontoid, irpicoid to semiporoid hymenophore (Hjortstam and Ryvarden 1990, Dai 2002). The authors' phylogenetic analyses show that collections from temperate to tropical areas in China, with smooth to semiporoid hymenophores, cluster together, thus extending the geographical range and hymenophore variability for *L. mirabilis* (Figs 1, 5). Thus, specimens from Taiwan, previously identified as *L. cinerascens* (Boidin and Gilles 2002, Wu 2010) because of their smooth hymenophore, are in fact *L. mirabilis*.

Figure 5. Basidiocarps of *Lopharia mirabilis*. **A** He 4558 **B** Dai 15094 **C** Dai 14978 **D** He 20120923-7 **E** He 1657 **F** Cui 9330.

Lopharia cinerascens is a cosmopolitan species in temperate to subtropical areas (Hjortstam and Ryvarden 1990, Boidin and Gilles 2002). These phylogenetic analyses suggest that it is a species complex (Fig. 1). Two specimens (He 2188 and He 2228, Fig. 2F) from Wisconsin in northern United States are probably *L. cinerascens* s.s. for it is near the type locality of Pennsylvania. They are phylogenetically distinct from FP-105043 (listed as *L. cinerascens* in Justo and Hibbett, 2011) which was collected in Mississippi, southern United States.

Lopharia ayresii nests within the *Lopharia* clade and forms with *L. resupinata* a strongly supported lineage sister to the *L. mirabilis* group (Fig. 1). These two species have resupinate basidiocarps, a monomitic hyphal system, a thin to indistinct subiculum and a thickened subhymenium. Otherwise, they fit well with other *Lopharia* spe-

cies in developing large basidia and basidiospores and encrusted cystidia. The addition of these species requires that the genus description of *Lopharia* be modified to include monomitic taxa.

It is still premature to make a conclusion about the distribution of *Lopharia* species with present data. Three species, *L. pseudocinerascens*, *L. sinensis* and *L. resupinata*, have been found from the type localities only (Boidin and Gilles 2002, present study). *Lopharia mirabilis* is reported from tropical Africa to temperate to tropical East Asia (Hjortstam and Ryvarden 1990, present study). *Lopharia ayresii* seems to be pantropical and is reported from Mauritius, Réunion (Boidin and Gilles 1991), southern China (Wu 2008), Taiwan (Wu 2010), Okinawa (Maekawa et al. 2003) and South America (Hjortstam et al. 2005, Hjortstam and Ryvarden 2008).

Lopharia Kalchbr. & MacOwan, Grevillea 10: 58, 1881, emended

Note. Basidiocarps annual, effused, effused-reflexed or pileate, crustaceous, coriaceous or corky. Pilei tomentose to glabrous. Hymenophore surface smooth, tuberculate, odontoid, irpicoid to semiporoid, cream, greyish-brown to light brown. Hyphal system monomitic or dimitic; generative hyphae with clamp connections. Lamprocystidia metuloid, large, subulate, hyaline, distinctly thick-walled. Dendrohyphidia absent, simple hyphidia hyphoid, thin-walled, hyaline. Basidia clavate with 4 sterigmata, large (> 50 µm long). Basidiospores ellipsoid to cylindrical, hyaline, thin-walled, smooth, negative in Melzer's reagent, acyanophilous.

Type species. *Lopharia mirabilis* (Berk. & Broome) Pat., *Bulletin de la Société Mycologique de France* 11: 14, 1895.

Key to species of *Lopharia* s.s.

1	Hymenophore tuberculate, odontoid, irpicoid to subporoid	***L. mirabilis***
–	Hymenophore smooth or slightly tuberculate	**2**
2	Basidiocarps effused-reflexed to pileate; hyphal system dimitic	**3**
–	Basidiocarps resupinate; hyphal system monomitic	**6**
3	Basidiospores 4.5–6.5 µm wide; reported from Africa	***L. pseudocinerascens***
–	Basidiospores 6.5–8 µm wide	**4**
4	From Taiwan	***L. mirabilis***
–	From elsewhere	**5**
5	Cystidia projecting up to 70 µm; basidiospores Q value > 1.9; from northern United States	***L. cinerascens***
–	Cystidia projecting up to 200 µm; basidiospores Q value < 1.9; from northern China	***L. sinensis***
6	Basidiospores > 10 µm long	***L. ayresii***
–	Basidiospores < 10 µm long	***L. resupinata***

List of names in *Lopharia* and their current taxonomic status

The list by species epithet is obtained from Index Fungorum (http://www.indexfungorum.org, 25 Sep. 2017). If a name is accepted, a direct statement is made with supporting evidence cited. Note that Miettinen et al. (2017: 26) consider *Hjortstamia* Boidin & Gilles to be a synonym of *Phlebiopsis* based on molecular and morphological criteria. Hjortstam and Ryvarden (1990) compiled the first nomenclature of *Lopharia* species.

abietina (Pers.) Z.S. Bi & G.Y. Zheng, [Macrofungus flora of the mountainous district of North Guangdong]: 62 (1990). Accepted as ***Veluticeps abietina*** (Pers.) Hjortstam & Tellería. Supported by ITS (Yang et al. 2016) and multi-gene phylogenetic analyses (Garcia-Sandoval et al. 2011).

albida Rick, *Brotéria, Ci. Nat.* 7: 13 (1938). An unidentifiable species of ***Hyphodontia*** as reported by Hjortstam and Ryvarden (1990: 59) and Baltazar et al. (2016: 119) for the type is sterile.

americana Rick, *Egatea* 13: 435 (1928). Hjortstam and Ryvarden (1990: 59) reported that the type is lost.

amethystea (Hjortstam & Ryvarden) A.L. Welden, *Flora Neotropica Monograph* 106: 70 (2010). = ***Hjortstamia amethystea*** (Hjortstam & Ryvarden) Boidin & Gilles. Hjortstam and Ryvarden (1990: 29) observed that the species is close to *Porostereum* (*Phlebiopsis*) *crassum* (Lév.) Hjortstam & Ryvarden.

areolata G. Cunn., Bull. *New Zealand Dept. Sci. Industr. Res.* 145: 331 (1963). = ***Phanerochaete areolata*** (G.H. Cunn.) Hjortstam & Ryvarden. Welden (1975: 547) noted that the type was related to the genus *Phanerochaete*. Hjortstam and Ryvarden (1990: 59) also examined the type and pointed out similarities to *Phanerochaete hiulca* (Burt) Welden.

ayresii (Berk. ex Cooke) Hjortstam, *Mycotaxon* 54: 188 (1995). Accepted in ***Lopharia*** and supported by phylogenetic analyses (fig. 1 herein). The type (Kew 35450, Mauritius, P.B. Ayres) was examined.

bambusae Rick, *Iheringia* 7: 199 (1960). Accepted as a synonym of ***Fomitiporia bambusarum*** (Rick) Campos-Santana & Decock. Hjortstam and Ryvarden (1990: 59) and Baltazar et al. (2016: 119) examined the type and agreed that it belongs to the *Phellinus* (*Fomitiporia*) *punctatus* species complex.

cheesmanii (Wakef.) G. Cunn., Bull. *New Zealand Dept. Sci. Industr. Res.* 145: 195 (1963). Accepted as a synonym of ***Laurilia sulcata*** (Burt) Pouzar as proposed by Hjortstam and Ryvarden (1990: 59) who examined the type at Kew. In addition, Boidin (1969: 190) observed finely echinulate, amyloid basidiospores in the type specimen.

cinerascens (Schwein.) G. Cunn., *Trans. Roy. Soc. New Zealand* 83: 622 (1956). Accepted in ***Lopharia*** and supported by phylogenetic analyses (fig. 1 herein).

crassa (Lév.) Boidin, *Bull. Trimestriel Soc. Mycol. France* 74: 479 (1959). Accepted as ***Phlebiopsis crassa*** (Lév.) Floudas & Hibbett and supported by multi-gene phylogenetic analyses; see (Floudas and Hibbett 2015: figs 1, 3) and (Miettinen et al. 2016: fig. 2 part 2).

cystidiosa (Rehill & B.K. Bakshi) Boidin, *Rev. Mycol.* (*Paris*) 34: 191 (1969). = ***Porostereum cystidiosum*** (Rehill & B.K. Bakshi) Hjortstam & Ryvarden.

dregeana (Berk.) P.H.B Talbot, *Bothalia* 6: 57 (1951). = ***Australohydnum dregeanum*** (Berk.) Hjortstam & Ryvarden.

fulva (Lév.) Boidin, *Bull. Mens. Soc. Linn. Lyon* 28: 213 (1959). Accepted as ***Porostereum fulvum*** (Lév.) Boidin & Gilles. Although considered a synonym of *P. spadiceum* by Hjortstam and Ryvarden (1990: 61), Boidin and Gilles (2002: 109) showed by crossing experiments and differences in basidiospore shape and size that *P. fulvum* was distinct from *P. spadiceum*. Welden (1975) also noted basidiospore size differences. In addition, they have distinct distributions — *P. fulvum* is reported from Africa, Reunion, India, Pakistan, Nepal, Philippines, Australia, New Zealand and Siberia, whereas *P. spadiceum* is known from Europe, Armenia and Morocco (Boidin and Gilles 2002, Talbot 1954, Welden 1975).

heterospora (Burt) D.A. Reid, *Rev. Mycol.* (*Paris*) 33: 251 (1969). Accepted as a synonym of ***Dendrophora albobadia*** (Schwein.) Chamuris. Welden (1975: 547), Boidin and Lanquetin (1977: 120) and Chamuris (1987) examined the type specimen, Matthews 27 and agreed that it is conspecific with ***D. albobadia***.

involuta (Klotzsch) G. Cunn., *Bull. New Zealand Dept. Sci. Industr. Res.* 145: 194 (1963). = ***Podoscyha involuta*** (Klotzsch) Imazeki. In a phylogenetic study of stipitate stereoid fungi, Sjökvist et al. (2012) showed that *Podoscypha* was paraphyletic with *P. involuta* and two other species in a lineage separate from the larger group of *Podoscypha* species.

javanica Henn. & E. Nyman, *Monsunia* 1: 144 (1900) [1899]. A possible synonym of *L. mirabilis* (Talbot 1954: 342; Boidin 1959: 207) or *L. cinerascens* (Welden 1975: 536). A portion of the type may be at NY (no. 00775916).

lilacina (Berk. & Broome) A.L. Welden, *Flora Neotropica Monograph* 106: 71 (2010). = ***Porostereum lilacinum*** (Berk. & Broome) Hjortstam & Ryvarden.

lirellosa Kalchbr. & MacOwan, in Kalchbrenner, *Grevillea* 10 (54): 58 (1881). Accepted as a synonym of ***L. mirabilis*** as proposed by Talbot (1951: 56; 1954: 340). Hjortstam and Ryvarden (1990: 62) and Boidin and Gilles (2002: 94) follow Talbot's synonymy.

mexicana A.L. Welden, *Tulane Stud. Zool. Bot.* 17: 19 (1971). = ***Hjortstamia mexicana*** (A.L. Welden) Boidin & Gilles.

mirabilis (Berk. & Broome) Pat., *Bull. Soc. Mycol. France* 11: 14 (1895). Type species of ***Lopharia***.

novae-granata A.L. Welden, *Mycologia* 67: 540 (1975). = ***Hjortstamia novae-granata*** (Welden) Hjortstam & Ryvarden.

ochracea G. Cunn., *Bull. New Zealand Dept. Sci. Industr. Res.* 145: 196 (1963). Accepted as ***Amylostereum areolatum*** (Fr.) Boidin based on basidiospore size (Thomsen, 1998) and its occurrence in New Zealand (Talbot 1964, Gaut 1969). Boidin and Lanquetin (1984) identified two paratype specimens as a species of *Amylostereum*. Hjortstam and Ryvarden (1990: 62) reported that the type specimen was morphologically indistinguishable from *A. chailletii* (Fr.) Boidin.

papyracea (Bres.) D.A. Reid, *Kew Bull.* 12: 131 (1957). Accepted as ***Phlebiopsis friesii*** (Lév.) Spirin & Miettinen. Originally published as *L. papyracea* (Jungh.) D.A. Reid.

Lloydella papyracea Bres. 1910 is the replacement name for *Thelephora papyracea* Jungh. which is a later homonym of *T. papyracea* Schrader ex J.F. Gmelin 1792.

papyrina (Mont.) Boidin, *Bull. Mens. Soc. Linn. Lyon* 28: 210 (1959). Accepted as ***Phlebiopsis papyrina*** (Mont.) Miettinen & Spirin.

perplexa D.A. Reid, *Kew Bull.* 17: 297 (1963). = ***Hjortstamia perplexum*** (D.A. Reid) Boidin & Gilles.

phellodendri (Pilát) Boidin, *Bull. Mens. Soc. Linn. Lyon* 28: 207 (1959). = ***Porostereum phellodendri*** Pilát, type of *Porostereum*. A possible synonym of *P. fulva* (Boidin and Gilles, 2002: 108) or *P. spadiceum* (Hjortstam & Ryvarden, 1990: 62). See discussion under *L. fulva*.

pilosiuscula (Hjortstam & Ryvarden) A.L. Welden, *Fl. Neotrop. Monogr.* 106: 73 (2010). Placement is uncertain for it is not typical of *Porostereum* (Hjortstam and Ryvarden 1990: 49) nor of *Lopharia* s.s. (Welden 2010: 73).

pseudocinerascens Boidin & Gilles, *Bull. Trimestriel Soc. Mycol. France* 118: 96 (2002). Accepted in ***Lopharia***.

rhodocarpa (Rehill & B.K. Bakshi) S.S. Rattan, *Biblioth. Mycol.* 60: 172 (1977). Accepted as ***Peniophora rhodocarpa*** Rehill & B.K. Bakshi. The authors follow Hjortstam & Ryvarden (1990: 62) who examined the isotype at Kew.

rimosissima Rick in Rambo, Iheringia, *Ser. Bot.* 7: 199 (1960). The protologue does not provide enough information to identify this species but it may be a *Xylodon* species. A line after the protologue states that it appears to be identical to *Odontia rimosissima* Peck [= *Xylodon rimosissimus* (Peck) Hjortstam & Ryvarden].

rimosissima (Berk. & M.A. Curtis) A.L. Welden, *Mycologia* 67: 544 (1975). = ***Hjortstamia rimosissima*** Boidin & Gilles. Known only from the type from Nicaragua collected on dead cane. Although the type lacks basidiospores, it is otherwise similar to *P. crassa* (Burt 1925: 342; Welden 1975: 544, 2010: 73).

rugulosa (Berk. & M.A. Curtis) Hjortstam, *Mycotaxon* 54: 188. 1995. Of uncertain generic disposition because of conflicting observations of the type specimen (Ginns 1971: 230, Hjortstam 1990: 420, Ryvarden 2010: 115).

sharpiana A.L. Welden, *Tulane Stud. Zool. Bot.* 17: 18 (1971). = ***Porostereum sharpianum*** (A.L. Welden) Hjortstam & Ryvarden. Hjortstam and Ryvarden (1990: 51) made the transfer after examining the type specimen. Welden (2010: 74), however, believed it is better placed in *Lopharia* s.s.

spadicea (Pers.) Boidin, *Bull. Mens. Soc. Linn. Lyon* 28: 211 (1959). Accepted as ***Porostereum spadiceum*** (Pers.) Hjortstam & Ryvarden. See *L. fulva* for additional information.

umbrinoalutacea (Wakef.) A.L. Welden, *Mycologia* 67: 546 (1975). Accepted as ***Porostereum umbrinoalutacea*** (Wakef.) Hjortstam & Ryvarden. Hjortstam and Ryvarden (1990: 63) made the transfer to *Porostereum* after examining the type specimen. Welden (1975: 539) noted that *P. umbrinoalutacea* was closely related to *P. fulvum* and *P. spadiceum*.

vinosa (Berk.) G. Cunn., *Trans. Roy. Soc. New Zealand* 83: 625 (1956). Accepted as a synonym of ***Phlebiopsis crassa***. Lentz (1955: 20), (Cunningham 1956: 624, fig. 2)

Figure 6. Basidiocarps of *Dentocorticium* species. **A** *D. bicolor* (He 2757) **B** *D. portoricense* (He 2161) **C–D** *D. taiwanianum* (**C** He 3383 **D** He 4635) **E–F** *D. ussuricum* (**E** He 3278 **F** He 3294). Scale bars: 1 cm.

and Hjortstam and Ryvarden (1990: 63) examined the type of *Corticium vinosum* Berk. They all agree that *C. vinosum* is conspecific with *Thelephora crassa* Lév. Note that some authors have mistakenly used *Thelephora vinosa* Berk. instead of *Corticium vinosum* Berk. as the proper basionym; see May et al. (2003: 295) for a summary.

Taxonomy of *Dentocorticium*, *Dendrodontia* and *Fuscocerrena* species

Dendrodontia bicolor (generic type, Fig. 6A), *Fuscocerrena portoricensis* (generic type, Fig. 6B), *Dentocorticium sulphurellum*, *Dentocorticium taiwanianum* (Fig. 6C–D) and *Dentocorticium ussuricum* (Parmasto) M.J. Larsen & Gilb. (generic type, Fig. 6E–F) cluster in a strongly supported clade (Fig. 1). The phylogenetic analyses demonstrate

that the three genera are closely related and support merging the genera together. Amongst the three generic names, *Dentocorticium* (1974) has priority over *Dendrodontia* (1980) and *Fuscocerrena* (1982). Thus, the latter two genera are treated as synonyms of *Dentocorticium* and four new combinations are proposed. An expanded and more inclusive generic circumscription of *Dentocorticium* is presented below.

Dentocorticium **(Parmasto) M.J. Larsen & Gilb., Norwegian Journal of Botany 21: 225, 1974, emended**

Laeticorticium sect. *Dentocorticium* Parmasto, Conspectus Systematis Corticiacearum: 151, 1968; *Dendrodontia* Hjortstam & Ryvarden, *Mycotaxon* 10: 273, 1980; *Fuscocerrena* Ryvarden, *Transactions of the British Mycological Society* 79: 279, 1982.

Note. Basidiocarps annual, effused, effused-reflexed or pileate, membranous, coriaceous or soft corky. Hymenophore surface odontoid, tuberculate, spinose, poroid, daedaleoid, sometimes developing irregular ridges or hyphal pegs. Hyphal system dimitic or trimitic; generative hyphae with clamp connections, brown skeletal hyphae in subiculum, spine trama and hyphal pegs, microbinding hyphae may be present in subiculum or substrate. Dendrohyphidia present. Cylindrical to subfusiform cystidia may be present. Basidia clavate with 4 sterigmata. Basidiospores ellipsoid to cylindrical, hyaline, thin-walled, smooth, negative in Melzer's reagent, acyanophilous.

Type species. *Laeticorticium ussuricum* Parmasto, *Eesti NSV Teaduste Akadeemia Toimetised* 14: 229, 1965.

Key to species of *Dentocorticium*

1 With hyphal peg **2**
– Without hyphal peg **3**
2 Sterile margin distinct and brown; hyphal pegs 4–5 per mm; subiculum brown ..... ***D. taiwanianum***
– Sterile margin indistinct; hyphal pegs > 5 per mm; subiculum grey..... ***D. hyphopaxillosum***
3 Hymenophore poroid or with ridges, hydnoid to spinose, from North and South America ..... ***D. portoricense***
– Hymenophore smooth, tuberculate, odontoid, rarely spinose **4**
4 Hymenial surface white to yellow, basidiospores 7–9.5 × 2.5–3 µm long, reported from North America ..... ***D. sulphurellum***
– Hymenial surface cream, brown to violaceous, basidiospores 5–7 × 2.2–2.5 µm long, reported from East Asia ..... ***D. ussuricum***
– Hymenial surface cream, yellow or brown, basidiospores 8–9 × 3–4 µm long, reported from southern Africa, Australia, East Asia, North and South America ..... ***D. bicolor***

Dentocorticium bicolor **(P.H.B. Talbot) Nakasone & S.H. He, comb. nov.**
MycoBank: MB823073
Fig. 6A

Dendrodontia bicolor (P.H.B. Talbot) Hjortstam & Ryvarden, *Mycotaxon* 10: 273, 1980.

Basionym. *Grandinia bicolor* P.H.B. Talbot, *Bothalia* 4: 947, 1948.

Type specimen examined. South Africa: Natal Province: Pietermaritzburg District, Town bush valley, on dead wood, Aug. 1934, W.G. Rump 100, UDA Herb. No. 27756 [K, K(M)15722, holotype].

Other specimens examined. China. Anhui Province: Qimen County, Guniujiang Nature Reserve, on fallen angiosperm branch, 8 Aug 2013, He 1722 (BJFC 016189, CFMR). Yunnan Province: Yongde County, Daxueshan Nature Reserve, on dead *Juglans* branch, 28 Aug 2015 He 2757 (BJFC 021195, CFMR) & He 2772 (BJFC 021210, CFMR). Zhejiang Province: Lin'an County, Tianmushan Nature Reserve, on dead angiosperm branch, 6 Aug 2013, He 1691 (BJFC 016158, CFMR). South Africa, Natal Province, Pietermaritzburg District, Town bush, on (corticated) indigenous wood, Oct 1934, W.G. Rump 215, herb. no. 28291, W.G. Rump 217, herb no. 28292, W.G. Rump 270 herb. No. 28502 (PREM).

Remarks. See Hjortstam and Ryvarden (1980) for a description and illustration of this species. The authors were unable to obtain sequences of *Dentocorticium bicolor* from the type locality in South Africa. Maekawa (1994) reported *D. sulphurellum* from Japan; however, the Japanese specimens may be *D. bicolor*, for *D. sulphurellum* appears to be restricted to North America.

Dentocorticium hyphopaxillosum **(M.J. Li & H.S. Yuan) Nakasone & S.H. He, comb. nov.**
MycoBank: MB823080

Basionym. *Dendrodontia hyphopaxillosa* M.J. Li & H.S. Yuan, *Phytotaxa* 156: 183, 2014.

Type specimen examined. China. Guangxi Autonomous Region: Shangsi County, Shiwandashan Forest Park, on fallen angiosperm branch, 24 Jul 2012, Yuan 6269 (CFMR, isotype).

Remarks. Although not included in phylogenetic analyses, this combination is made based on morphological evidence. See Li and Yuan (2014) for description and illustration.

Dentocorticium portoricense **(Spreng. ex Fr.) Nakasone & S.H. He, comb. nov.**
MycoBank: MB823074
Fig. 6B

Fuscocerrena portoricensis (Spreng. ex Fr.) Ryvarden, *Transactions of the British Mycological Society* 79: 280, 1982.

Basionym. *Polyporus portoricensis* Spreng. ex Fr., *Elenchus Fungorum* 1: 115, 1828.

Specimens examined. Costa Rica. San José Province: Jardin, on hardwood, 9 Aug 1963, J.L. Lowe 13402 (CFMR). Uruguay. Depto. Tacuarembó, Ext. Paso Baltasar, on *Eucalyptus globulus*, 11 Nov 2001, L. Bettucci and S. Lupo, MVHC 5038 (CFMR). USA. Florida: Alachua County, Devil's Millhopper, on *Magnolia* sp., 18 July 1972, H.H. Burdsall, Jr., HHB 19632 (CFMR). Tennessee: Cocke County, Cosby Nature Trail, on *Liriodendron tulipifera* log, 2 Aug 2010, H.H. Burdsall, Jr., HHB 6651 (CFMR). Wisconsin: Dane County, Madison, Picnic Point, on dead angiosperm tree, 7 Oct 2014, He 2161 (BJFC 018806, CFMR); 11 Oct 2014, He 2202 (BJFC 018832, CFMR).

Remarks. *Dentocorticium portoricense* is easily recognised by its poroid, hydnoid to spinose, dark brown hymenophore and greenish-yellow hymenial surface. Phylogenetically, it is closely related to *D. taiwanianum* (Fig. 1). See Ryvarden (1982) for description and drawing of this species with synonymy.

***Dentocorticium taiwanianum* (H.C. Wang & Sheng H. Wu) Nakasone & S.H. He, comb. nov.**
MycoBank: MB823075
Fig. 6C–D

Basionym. *Dendrodontia taiwaniana* H.C. Wang & Sheng H. Wu, *Mycologia* 102: 1153, 2010.

Type specimen examined. Taiwan: Nantou County, Hsitou, alt. 1000 m, on (corticate) branch of angiosperm, 3 Jul. 1999, *S.H. Wu 9907-1*, F10258 (TNM, holotype).

Other specimens examined. China. Guizhou Province: Libo County, Maolan Nature Reserve, on dead angiosperm branch, 14 Jun 2016, He 3777 (BJFC 022276). Hainan Province: Wuzhishan County, Wuzhishan Nature Reserve, on dead angiosperm branch, 10 Jun 2016, He 3927 (BJFC 022429). Taiwan: Nantou County, Nandongyan Mountains, on fallen angiosperm trunk, 7 Dec 2016, He 4615 (BJFC 024057); Xitou, on dead angiosperm branch, 11 Dec 2016, He 4635 (BJFC 024078) & He 4639 (BJFC 024082). Yunnan Province: Baoshan County, Baihualing, on fallen angiosperm branch, 30 Nov 2015, He 3383 (BJFC 021778).

Remarks. This is a common species in tropical China. See Wang et al. (2010) for a description and illustration of this species.

List of names in *Dentocorticium* and their current taxonomic status

The list by species epithet is obtained from Index Fungorum (http://www.indexfungorum.org, 25 Sep. 2017). If a name is accepted, a direct statement is made with supporting evidence cited.

blastanos Boidin & Gilles, *Cryptog. Mycol.* 19: 193 (1998). Accepted as ***Neocampanella blastanos*** (Boidin & Gilles) Nakasone, Hibbett & Goranova and supported by molecular data (Nakasone et al. 2009: fig. 1).

brasiliense M.J. Larsen & Gilb., *Norweg. J. Bot.* 24: 117 (1977). Accepted as ***Punctularia subhepatica*** (Berk.) Hjortstam. The isotype at CFMR (Brazil, Rio Grande du Sol, ad ligna angiosperma, 1936, Rick) was examined. It has rare basidiospores (6.5–8.7 × 3.2–3.7 μm) and characteristic knobby dendrohyphidia that are brown in the upper portion and hyaline at the base. The holotype at FH is apparently lost.

expallens (Bres.) Domański, Mala Flora Grzybów. Tom I: Basidiomycetes (Podstawczaki), Aphyllophorales (Bezblaszkowce). Corticiaeae, *Acanthobasidium – Irpicodon* 5: 248 (1988). = ***Crustomyces expallens*** (Bres.) Hjortstam. In addition to *Corticium*, *Dentocorticium*, and *Crustomyces*, this species has been transferred to *Phlebia* and *Laeticorticium*, but none of these generic placements is satisfactory.

irregulare Ryvarden, *Bull. Jardin Bot. Natl. Belg.* 48: 84 (1978). Accepted as a synonym of ***Diplomitoporus daedaleiformis*** (Henn.) Ryvarden. The holotype of *D. irregulare* (JR 4316, GENT) and isotype of *Poria daedaleiformis* (US0239243, BPI) were examined. Basidiospores of *D. irregulare* were narrower [(2.8–) 3–3.5 μm] than reported by Ryvarden (1978) and similar to those of *D. daedaleiformis* (Ryvarden 2012: 16). Also in *D. irregulare,* skeletal hyphae were observed in the ridges and spines and obclavate, subfusiform cystidioles (11.5–21 × 4–5.5 μm) in the hymenium; these were not described earlier. Cystidioles were also observed in the isotype of *P. daedaleiformis* but no basidiospores. Both species develop elongated pores and ridges, clamped generative, dendrohyphidia and occur in the same geographical area in Africa.

nephrolepidis Boidin & Gilles, *Cryptog. Mycol.* 19: 193 (1998). Accepted as a synonym of ***L. cyathae*** (S. Ito & S. Imai) Hjortstam & Ryvarden as determined by Nakasone (2005) who examined the holotype.

pilatii (Parmasto) Duhem & H. Michel, *Cryptog. Mycol.* 30: 165 (2009). Accepted as ***Phlebiopsis pilatii*** (Parmasto) Spirin & Miettinen based on ITS and 28S sequences analyses (Miettinen et al. 2016: fig. 2 part 2). However, ***P. pilatii*** differs from other ***Phlebiopsis*** species in the absence of lamprocystidia and presence of dendrohyphidia and microbinding hyphae (Duhem and Michel 2009: figs 7–17).

sasae (Boidin, Cand. & Gilles) Boidin, Lanq. & Duhem, *Bulletin de la Société Mycologique de France* 112: 116 (1996). Accepted as ***Leptocorticium sasae*** (Boidin, Cand. & Gilles) Nakasone based on morphological criteria (Nakasone 2005).

sinapicolor Boidin, Gilles & Duhem, *Cryptog. Mycol.* 19: 194 (1998). A poorly studied species. Duhem and Michel (2009: 171) cite morphological similarities between this species and ***P. pilatii***.

sulphurellum (Peck) M.J. Larsen & Gilb., *Norweg. J. Bot.* 21: 226 (1974). Accepted in ***Dentocorticium*** as inferred from multi-gene sequences (Fig. 1 herein) and morphology.

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RESEARCH ARTICLE

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A new species of *Stamnaria* (Leotiomycetes, Helotiales) from Western Siberia

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Abstract

A new species of *Stamnaria* is described based on morphology and molecular data from a collection made in West Siberia. *Stamnaria yugrana* is differentiated by lanceolate, strongly protruding paraphyses and comparatively narrow, fusoid-clavate ascospores. The apothecia are urn-shaped due to a prominent and even collar as in *S. persoonii*. The species grows on fallen side branches of *Equisetum sylvaticum*, a rarely recorded host for *Stamnaria*. The authors formally describe the new species and provide colour illustrations. In addition, the literature is reviewed on previously described species of *Stamnaria*. Phylogenetic reconstruction of the *Stamnaria* lineage, based on the ITS ribosomal DNA, strongly supports the three currently recognised species: *S. americana*, *S. persoonii* and *S. yugrana*.

Keywords

Ascomycota, ecology, *Equisetum*, ITS rDNA, *Stamnaria*, taxonomy

Introduction

During ongoing studies of the Helotiales (see Baral and Haelewaters 2015, Baral et al. 2015), material of a species of *Stamnaria* Fuckel, which had been collected and morphologically documented by one of the authors (NVF), was investigated by molecular

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methods. Collections were made during surveys in the vicinities of Shapsha field station of Yugra State University in Western Siberia, Russia. After the first collection of the species in June 2008, the same locality was visited in June 2012, February 2014 and May 2015. During all these visits, abundant apothecia were observed. The species was always found growing on *Equisetum sylvaticum*. This plant species had rarely been mentioned before as a host for the genus *Stamnaria* (Sydow 1898: 432, *S. equiseti*; Jaap 1922: 15, *S. persoonii*). Moreover, the microscopic features of the collections differed markedly from all earlier taxa described in the genus *Stamnaria*. Therefore, it was decided to formally describe this fungus as a new species based on morphological and molecular characteristics.

Members of the genus *Stamnaria* (Ascomycota, Leotiomycetes, Helotiales) share the following characteristics: 1) the presence of a thick hyaline gelatinised layer of textura oblita outside the ectal excipulum of textura prismatica, 2) cells of ectal excipulum and paraphyses containing yellow-orange carotenoids, 3) apothecia erumpent through epidermis, 4) all species growing on members of *Equisetum* and 5) with asexual state in the genus *Titaeospora* Bubák (von Höhnel 1928, Gruber 2006, Baral in Jaklitsch et al. 2016). According to Johnston et al. (2014), the older name *Stamnaria* is recommended to be used for the holomorph, instead of *Titaeospora*. Based on the currently available sequence data, the genus forms its own clade within the Helotiales (Baral and Haelewaters 2015). Its isolated position was emphasised by Baral in Jaklitsch et al. (2016) by the informal recognition as "*Stamnaria* lineage."

Thus far, seven species have been referred to as *Stamnaria* in the literature: *S. americana* Massee & Morgan, *S. herjedalensis* (Rehm) Bubák, *S. hyalopus* P. Karst., *S. equiseti* (Hoffm.) Sacc., *S. persoonii* (Moug.) Fuckel, *S. pusio* (Berk. & M.A. Curtis) Massee and *S. thujae* Seaver, according to the Index Fungorum (2018). However, *S. hyalopus*, *S. pusio* and *S. thujae* do not belong in the genus *Stamnaria* because they grow on hosts other than *Equisetum*, amongst other reasons (Gruber 2006). *Stamnaria hyalopus* occurs on decaying leaves of *Carex vesicaria* and *S. thujae* on *Thuja occidentalis*. *Stamnaria pusio* grows on rotting debris in the soil and was placed in tentative synonymy with *Sarcoscypha occidentalis* (Schwein.) Sacc. by Harrington (1990). *Stamnaria herjedalensis* appears to be a synonym of *Roseodiscus equisetinus* (Velen.) Baral (O. Eriksson pers. comm.). *Stamnaria equiseti* is considered a synonym of *S. persoonii* by accepting the older name *equiseti* (Saccardo 1889) but *S. equiseti* is of uncertain identity whereas that of *S. persoonii* could be clarified based on the lectotype (Gruber 2006). Taken together, only two species are currently accepted in the genus *Stamnaria*: *S. americana* and *S. persoonii*.

Material and methods

Study site ecology

The study site is located in the middle taiga sub-zone of Western Siberia, in Russia. The area is characterised by a subarctic climate with average yearly temperatures of -1.1 °C,

ranging from averages of -20 °C in January to 18 °C in July. The total annual precipitation is 553 mm. The period without snow cover usually lasts from May until October (Bulatov et al. 2007).

The collections were made in a mixed coniferous-deciduous forest close to a stream and a forest path 1.2 km SSE of Shapsha village, 61.07929"N, 69.46925"E. The tree canopy was dominated by *Pinus sibirica*, *Picea obovata* and *Abies sibirica* with admixture of *Betula pubescens*, *Populus tremula*, *Sorbus sibirica* and *Salix* spp. The plant layer was made up of *Equisetum sylvaticum*, *Rubus arcticus*, *Milium effusum* and *Luzula pilosa*.

Several years ago, a fire took place in this forest, resulting in the dominance of *E. sylvaticum*. Ground fires generally result in completely new successional trajectories and one dominant component of early post-fire vegetation communities is *E. sylvaticum*. Its rhizomes are buried deep in the soil and thus are resistant to fire (Viereck 1983). Interestingly, the new *Stamnaria* species was only collected from these post-fire mass populations of *E. sylvaticum*. Elsewhere in coniferous forests in Khanty-Mansi Autonomous Okrug – Yugra, *E. sylvaticum* is common but occurs in sparse densities and no growth of *Stamnaria* has been observed.

Morphological studies

The species was discovered after examining the litter of the host plant *in situ* by the naked eye. Time of collection was always at the beginning of summer (about three weeks after snow melt). The substrate (*Equisetum sylvaticum* side branches) extracted from under the snow in February 2014 also gave abundant fruiting after three weeks of incubation in a moist chamber.

The litter was collected and brought to the laboratory where it was studied and documented the same day. Hereafter, the material (side branches with attached fruiting bodies) was dried at room temperature and stored as dry collection at Yugra State University Biological Collection (YSU). Voucher specimens are also preserved at Farlow Herbarium of Harvard University, Cambridge, Massachusetts (FH) and at V.L. Komarov Botanical Institute, Saint-Petersburg (LE).

The morphological features of the species were studied using a Zeiss Stemi 2000-C stereomicroscope, with magnification from 6 to 50× and a Zeiss Axiostar transmitted light microscope (with Achromat 10/0.25, 40/0.65 dry and 100/1.25 oil immersion objectives). Microstructures were studied and measured from living material in tap water and later compared to dead material from dried specimens. The iodine reaction was tested with Lugol's solution and Congo Red in water was used to stain the sections and the structure of the excipulum.

Macro- and micro-photographs were obtained using a Canon EOS 50D digital camera and Axiocam ERc 5s digital camera. Abbreviations used: * = living state, † = dead state, CR = Congo Red, VB = vacuolar body.

DNA extraction, PCR and sequencing

DNA was extracted from dry apothecia with the help of the Extract-N-Amp Plant PCR Kit (Sigma-Aldrich, St Louis, MO), DNeasy Plant Mini Kit (Qiagen, Valencia, CA) and the QIAamp DNA Micro Kit (Qiagen). Per extraction, 1 to 4 apothecia were used. Apothecia were crushed in 1.5 mL tubes using a 1.5 mL pellet pestle (Kimble, Rockwood, TN, #749521-1500) or cut in half using a sterile no. 10 surgical blade on a disposable Bard-Parker handle (Aspen Surgical, Caledonia, MI). Undiluted DNA was used for PCR amplification of the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA). The ITS was amplified using the forward primer ITS1f (5'–CTTGGTCATTTAGAGGAAGTAA–3') in combination with either ITS4 (5'–TCCTCCGCTTATTGATATGC–3') or the Ascomycota-specific primer ITS4A (5'–CGCCGTTACTGGGGCAATCCCTG–3') (White et al. 1990, Gardes and Bruns 1993, Larena et al. 1999). All PCR reactions were done in a Mastercycler ep gradient thermocycler (Eppendorf, Hauppauge, NY) using Sigma-Aldrich's REDTaq DNA Polymerase enzyme. PCR conditions were as follows: an initial denaturation step at 94 °C for 3 min, then 35 cycles of 94 °C for 1 min, 50 °C for 45 s and 72 °C for 90 s and a final extension step at 72 °C for 10 min (ITS).

Products with clear bands on agarose gel were cleaned with the Qiaquick PCR Purification Kit (Qiagen) and subsequently sequenced with the same primers (3 µl of purified PCR product per 10 µl sequencing reaction). Sequencing reactions were performed using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Life Technologies, Carlsbad, CA). Sequences were trimmed, edited and assembled in Sequencher v. 4.10.1. All generated sequences have been deposited in GenBank (Table 1). BLAST searches for similar sequences were undertaken at: http://ncbi.nlm.nih.gov/blast/Blast.cgi.

Sequence alignment, nucleotide divergence and phylogenetic analyses

An ITS dataset was constructed to investigate the phylogenetic structure within the genus *Stamnaria*. Sequences were aligned using Muscle v3.7 (Edgar 2004), available on the Cipres Science Gateway version 3.3 (Miller et al. 2010). Pairwise evolutionary distances, the amounts of genetic variation between two species, were calculated using Paup on XSEDE, also on the Cipres Science Gateway. The p-distance is calculated as the proportion of sites in a pairwise alignment at which the compared sequences are different. This is the number of nucleotide differences divided by the number of nucleotides compared. In addition, Jukes-Cantor (JC69) and Kimura 2-parameter (K2P) distance metrics (Jukes and Cantor 1969, Kimura 1980) were calculated. Neighbour joining (NJ) analyses (Saitou and Nei 1987) were performed to cluster taxa based on the respective distance matrices. The three resulting NJ trees were statistically compared with the SH test (Shimodaira and Hasegawa 1999).

Maximum parsimony (MP) and maximum likelihood (ML) analyses were run using Paup on XSEDE. MP was estimated with heuristic searches consisting of 500 step-

Table 1. Isolates used in phylogenetic analyses, with voucher information and GenBank accession numbers. Accession numbers of sequences generated during this study are in bold. *This sequence was retrieved from the Biological Resource Center of the National Institute of Technology and Evaluation, Japan (NBRC).

Species	Isolate	Voucher	GenBank accession number
Geoglossum nigritum	AFTOL-ID 56	OSC 100009	DQ491490
Geoglossum umbratile	ANM Acc377	ILLS:61040	JQ256422
Hymenoscyphus epiphyllus	–	H.B. 7054	DQ431180
Leotia lubrica	ZW-Geo59-Clark	–	AY789360
Microglossum rufum	–	–	AY144533
Rommelaarsia flavovirens	E5	H.B. 9684	KT958772
Roseodiscus rhodoleucus	DH257	H.B. 8488a	KT972704
Sarcoleotia globosa	–	OSC 63633	AY789410
Stamnaria americana	DH258a	H.B. 7261	KT972707
	DH941c	Gruber 152/226	**MG662188**
	DH941d	Gruber 152/226	**MG662189**
	FC-2732	TNS-F-39244	NBRC 108774*
Stamnaria persoonii	DH671a	Gruber 119/183	**MG662201**
	NLU003b	Gruber 118/182	**MG662202**
Stamnaria yugrana sp. nov.	DH603a	FH 01146308	**MG662203**
	DH603b	FH 01146308	**MG662204**

wise-addition trees obtained using random sequence addition replicates followed by tree bisection-reconnection (TBR) branch swapping (MulTrees in effect) and saving all equally most parsimonious trees. Robustness of branches was estimated by maximum parsimony bootstrap proportions (BP) using 1000 bootstrap replicates, with heuristic searches consisting of 10 stepwise-addition trees obtained using random sequence addition replicates followed by TBR branch swapping, with MaxTrees set at 100. ML inference was run under a TIM2ef+I+G model of nucleotide substitution, as selected by jModeltest 2.1 (Darriba et al. 2012) following the Akaike Information Criterion. Rapid bootstrapping was implemented with 500 replicates.

Bayesian analyses were done with a Markov chain Monte Carlo (MCMC) coalescent approach implemented in Beast 1.8.4 (Drummond et al. 2012), with a strict clock with uniform rates of evolution across branches. A Yule speciation (Yule 1925) tree prior was selected with the TIM2ef+G nucleotide substitution model (considering the Bayesian Information Criterion from the earlier jModelTest 2.1 analysis). Four independent runs were performed from a random starting tree for 40 million generations, with a sampling frequency of 4000. Tracer 1.6 (Rambaut et al. 2014) was used to check trace plots and effective sample sizes (ESS). Burnin values were 5 % for all runs. Logged parameters were checked to have combined ESS values of at least 200. TreeAnnotator 1.8.4 was used to generate consensus trees with 0 % burnin and to infer the Maximum Clade Credibility (MCC) tree, with the highest product of individual clade posterior probabilities. The different trees with bootstrap values (BS) and posterior probabilities (pp) were visualised in FigTree 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

Taxonomy

Stamnaria yugrana **Filippova, Haelew. & Baral, sp. nov.**
MycoBank: MB823742

Diagnosis. Characterised by the presence of both lanceolate and cylindrical paraphyses, fusoid-clavate ascospores with a length/width ratio of predominantly >4 and free-ending hyphae at the inner excipulum of the tube-shaped, even collar. Saprophytic on dead branches of *E. sylvaticum*.

Types. Holotype: Russia, Western Siberia, Khanty-Mansi Autonomous Okrug – Yugra, 25 km ENE of Khanty-Mansiysk town, 1.2 km SSE of Shapsha village, 61.07929"N, 69.46925"E, alt. 40 m, 9 Jun 2012, *leg*. N.V. Filippova, on fallen side branches of *Equisetum sylvaticum* L. lying amongst other forest litter in a mixed coniferous-deciduous forest; Biological Collection of Yugra State University (YSU-F-03519). Isotypes: LE-295215; FH 01146308. Paratypes: *ibid.*, 16 Jun 2008 (YSU-F-00097, material lost; LE-295060); *ibid.*, 22 Feb 2014, substrate collected from under snow and grown in a moist chamber (YSU-F-04933); *ibid.*, 25 Feb 2015 (YSU-F-06579; LE-296061).

Description. ***Apothecia*** urn-shaped, stipitate, 0.25–0.6 mm in diameter when mature, 0.5–1 mm high, varying depending on light conditions, being stouter with shorter stipe when substrate exposed to sunlight; receptacle light yellow(-ochraceous) when fresh, with even, whitish collar ~80–120 µm high, stipe pale yellowish-translucent,

Figure 1. Study site of *Stamnaria yugrana* growing on litter of *Equisetum sylvaticum* in Western Siberia near the Khanty-Mansiysk town.

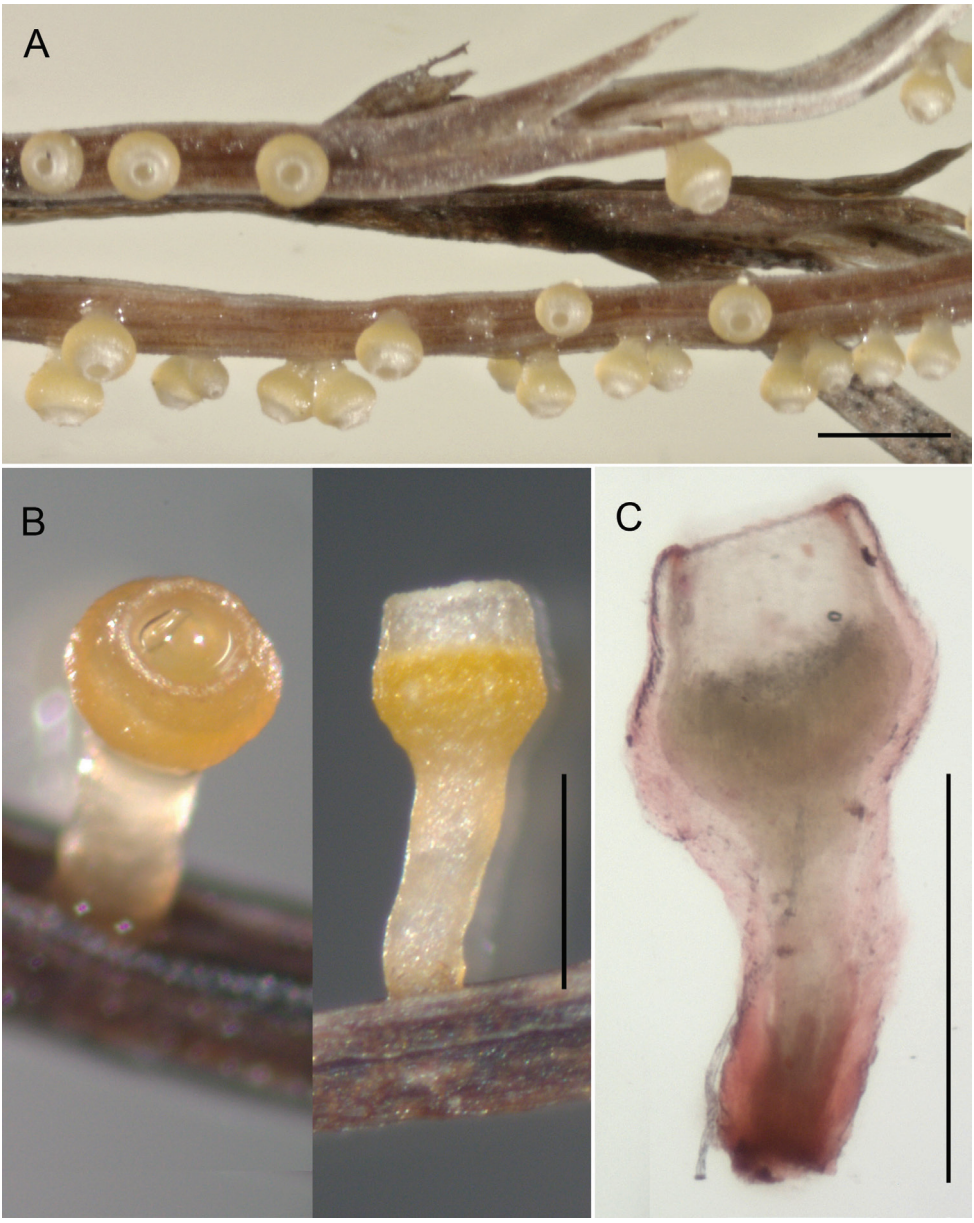

Figure 2. Apothecia of *Stamnaria yugrana* on side branches of *Equisetum sylvaticum*: **A** Apothecia grown *in situ* under well-lit conditions **B** Apothecia grown in shady conditions after incubation in a moist chamber **C** Median section through an apothecium after incubation in a moist chamber (dead, in CR). A from YSU-F-03519, B from YSU-F-04933, C from YSU-F00097. Scale bars: **A** 1.0 mm, **B, C** 0.5 mm.

100–380 × 130–300 µm, receptacle becoming light brown on drying; scattered to moderately gregarious, often abundant on the branches. ***Ectal excipulum*** outer layer *40–45 µm thick at middle flanks and margin, made up of strongly gelatinous tissue of

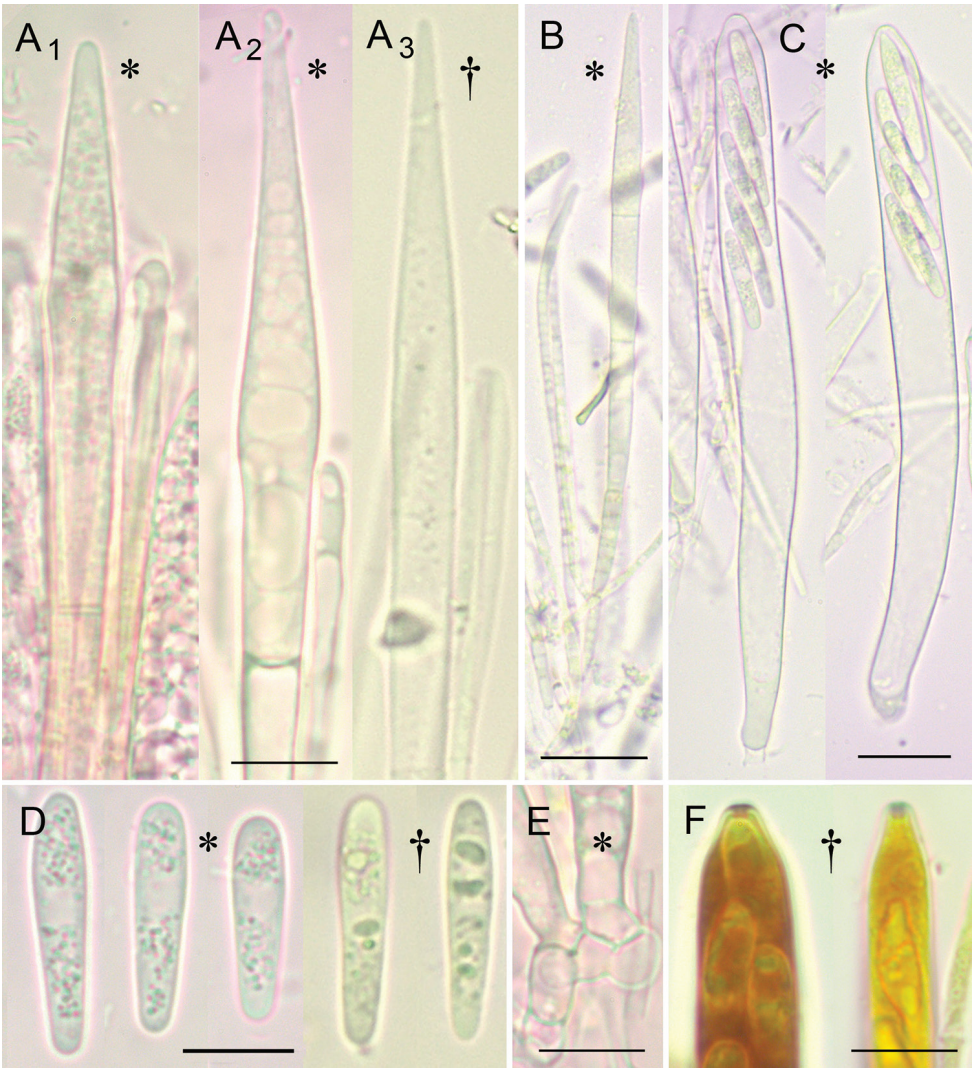

Figure 3. **A_1** Young living paraphysis with multiguttulate, medium refractive vacuolar content (VBs), **A_2** Mature living paraphysis with large non-refractive vacuoles **A_3** Dead paraphysis with small oil drops (LBs), vacuoles disappeared **B** Paraphyses of cylindrical and lanceolate type **C** Mature living asci **D** Mature ejected ascospores with granular lipid content of minute LBs (confluent in dead state) **E** Ascus bases with croziers **F** Apices of mature and immature ascus (stained in IKI). All * and F from YSU-F-04933, all other † from YSU-F-00097. Scale bars: **A, D, E, F** 10 µm, **B, C** 20 µm.

loose, parallel to wavy hyphae 2–3 µm broad, septate, embedded in abundant gelatinous matrix (textura oblita); inner layer *~45 µm thick at middle flanks, made up of textura prismatica-porrecta running parallel to outside, cells at middle flanks *17–44 × 7–12 µm, slightly narrower in the collar region, much narrower in stipe (*20–45 × 3–5 µm); the

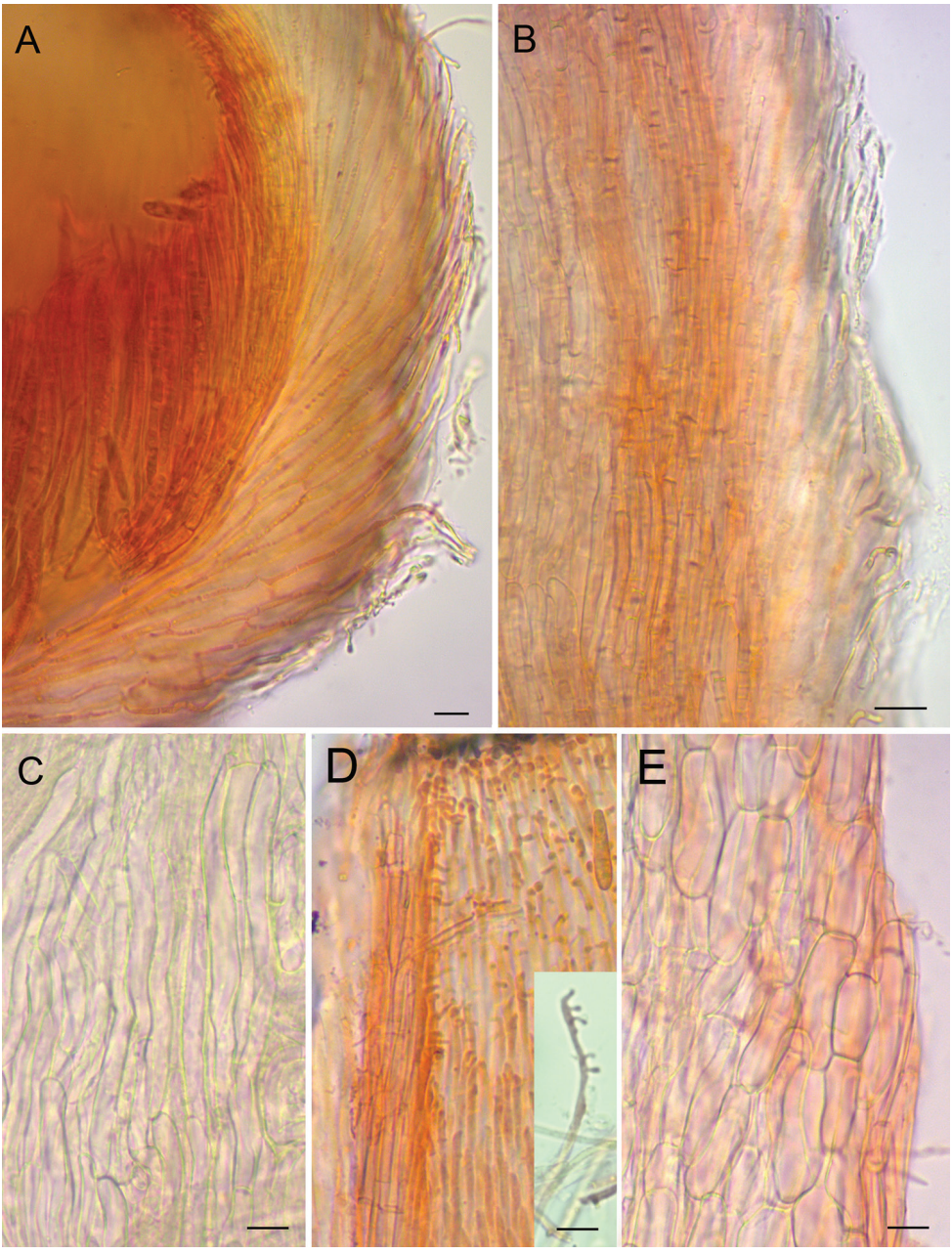

Figure 4.A Median section through receptacle, showing gelatinised outer layer of ectal excipulum **B** Median section through stipe, showing outer gelatinised layer, inner layer of textura porrecta (orange) and transition to medullary excipulum **C** Medullary excipulum in squash mount **D** Inner part of collar in squash mount showing hyphae forming outgrowths (single hyphae shown in insert) **E** Ectal excipulum of textura prismatica at receptacle flank in squash mount (outer layer absent). All stained by CR in water (cells partly in vital state), from YSU-F-04933. Scale bars: 10 μm.

inner layer of the collar composed of narrow hyphae, free upper part of these hyphae internally covered by lateral cellular outgrowths *2–5 × 1.5–2 µm. ***Medullary excipulum*** well developed, of dense, parallel, septate hyphae (textura porrecta) without gel, cells *65–90 × 2.5–6 (–7.7) µm; subhymenium well developed (*20–30 thick), of intricate hyphae *2 µm broad. ***Asci*** cylindrical, developing from croziers which are difficult to see in mature asci, with apical thickening enclosing a hemiamyloid ring of *Calycina*-type (rb: dirty red at high, blue at low concentration), *146 × 12.5 [123–159 (–206) × 11.7–13.5] µm, †98 × 9 (90–110 × 8–10.5) µm, 8-spored, spores *obliquely biseriate. ***Paraphyses*** of two types: (1) lanceolate, exceeding asci for *12–20 µm when young and *30–40 µm when fully developed, septate in lower part, non-septate in broad upper part, with quite acute tip, in young paraphyses with granular (multiguttulate) vacuolar content of moderate refractivity (VBs), later replaced by larger non-refractive vacuoles, *5–7 (†3–6) µm broad in upper part; (2) cylindrical, more abundant, not exceeding the asci, *2.3–3 µm broad above, septate, with obtuse tip, rarely branched below and scarcely enlarged in upper segment, without VBs, with pale yellow-orange pigment in middle and lower part. ***Ascospores*** fusoid-clavate, slightly to distinctly heteropolar, with rounded to obtuse ends, usually without any gel around, filled with granular oil content in both halves, leaving a central zone for the single nucleus, variable in length, *19.8 × 4.8 (16.5–24.5 × 4.2–5.6), n=18, Q=4.1 (YSU-F-04933); †20.5 × 4.0 (17.2–24.2 × 3.6–4.6) µm, n=37, Q=5.1 (YSU-F-03519, YSU-F-04933).

Etymology. Referring to Yugra, the historical name of the region (currently "Khanty-Mansi Autonomous Okrug – Yugra").

Distribution. Known only from the type locality.

Nucleotide alignment dataset and phylogenetic inferences

The ITS rDNA dataset consists of 16 isolates and 671 characters, of which 387 are constant and 187 are parsimony-informative. Taxonomical sampling covers the genera *Hymenoscyphus* Gray (1 isolate), *Leotia* Pers. (1), *Microglossum* Gillet (1), *Rommelaarsia* Baral & Haelew. (1), *Roseodiscus* Baral (1) and *Stamnaria* (8); *Geoglossum* Pers. (2) and *Sarcoleotia* S. Ito & S. Imai (1) served as outgroup taxa (Geoglossomycetes, Geoglossales, *sensu* Schoch et al. 2006). This dataset includes the three currently recognised species in the genus *Stamnaria*.

Intra-specific divergence in the ITS region ranges from 0.0 to 0.9 % while inter-specific divergences range between 8.0 and 13.7 % (p-distances) or between 8.4–8.5 and 15.1 % (JC69, K2P). The p-distances are 8.0 % between *S. americana* and *S. persoonii*, 10.6 % between *S. persoonii* and *S. yugrana* and 13.7 % between *S. americana* and *S. yugrana*. JC69 and K2P distances are almost identical, higher but equivalent to the p-distances (Table 2). The NJ phenogram constructed from the Jukes–Cantor distance matrix has a higher likelihood score (-lnL = 791.63111) compared to the analyses based on p-distances and Kimura 2-parameter distances, but the differences are insignificant.

Table 2. Intra- and interspecific distances for and between *S. americana*, *S. persoonii* and *S. yugrana*. Distances are given as percentages (%).

	p-distance	JC69	K2P
S. americana, intraspecific	0.2	0.2	0.2
S. persoonii, intraspecific	0.9	0.9	0.9
S. yugrana, intraspecific	0.0	0.0	0.0
S. americana–S. persoonii	8.0	8.4	8.5
S. americana–S. yugrana	13.7	15.1	15.1
S. persoonii–S. yugrana	10.6	11.4	11.4

The genus *Stamnaria* is retrieved as a monophyletic clade in all three phylogenetic reconstructions (MP BS = 74, ML BS = 98, pp = 1.0). All morphologically delineated species of *Stamnaria* have maximum support. The position of the new species within the genus is unresolved. Under MP and ML inference, *S. yugrana* is sister to *S. persoonii* but this sister relationship is only supported by ML BS = 81. In the Bayesian analysis, on the other hand, *S. yugrana* is sister to (*S. americana*, *S. persoonii*), with moderate support for the sister relationship between the two latter species (pp = 0.8).

Discussion

The morphological and ecological features of *S. yugrana* (Table 3) are considerably different compared to the two previously recognised species, the most pronounced features being paraphyses of two types, including strongly protruding, broadly lanceolate ones and heteropolar, comparatively narrow ascospores. No previous reports of a *Stamnaria* species with ascertained identity are known from *E. sylvaticum*, when applying the revised species concept as reviewed in the introduction. Jaap (1922) presented a report of *S. persoonii* on *E. sylvaticum* but did not provide a morphological description. It is impossible to assess whether this report represents *S. persoonii* or *S. yugrana* because the collection does not seem to have been preserved (no voucher number cited).

Stamnaria yugrana differs from *S. americana* by solitary, never fasciculate, distinctly stalked apothecia, presence of a pronounced raised collar with free-ending hyphae at its inner excipulum, presence of two types of paraphyses (cylindrical and lanceolate) and shorter and especially narrower, fusoid-clavate ascospores (Table 3). The ecology of these two species is also quite different: the apothecia of *S. americana* occur on living stems of *Equisetum hyemale*, while those of *S. yugrana* are found on dead fallen side branches of *E. sylvaticum*. Both *S. yugrana* and *S. persoonii* have stipitate apothecia with a distinct collar. However, *S. yugrana* differs by the presence of paraphyses of two types and much narrower, heteropolar spores with higher length/width ratio (Table 3). Apothecia of *S. persoonii* also grow on dead stems, but on another host plant, *E. fluviatile* (Gruber 2006).

Many *Stamnaria* collections have been misidentified and/or reported under misidentified host plants. Currently, the *Stamnaria* lineage has no taxonomic assignment at the family level. In their ITS+LSU rDNA phylogeny, Baral et al. (2015) retrieved it as

Table 3. Comparison of the ecological and morphological characteristics between described *Stamnaria* species.

Species	*S. americana*	*S. persoonii*	*S. yugrana*
Host association	Parasitic on *E. hyemale*	Saprophytic on *E. fluviatile*, rarely on *E. arvense*	Saprophytic on *E. sylvaticum*
Apothecia, diameter	0.3–0.7 (–1.0) mm	0.4–1.0 mm	0.25–0.6 mm
Apothecia, margin	Without collar	With even collar	With even collar
Asci, measurements	*(110–) 140–190 (–210) × 13.5–14.6 µm, †120–157 × (11.5–) 12–14 (–15) µm	*190–230 × 14–16 µm, †130 × 12 µm	*123–159 (–206) × 11.7–13.5 µm, †90–110 × 8.0–10.5 µm
Asci, apex	Inamyloid, thin-walled	With thick amyloid ring	With thick amyloid ring
Ascospores, measurements	*(22–) 24–28 (–34) × (6–) 6.5–7.5 (–8.4) µm, †20–29 × 5.5–7.0 µm	*16–23 × 7.5–9.5 µm, †15–18 × 5.0–8.0 µm	*16.5–24.5 × 4.2–5.6 µm, †16.5–23.2 × 4.0–4.8 µm
Ascospores, shape	Fusoid, not or only slightly heteropolar	Broadly ellipsoid with rounded ends	Fusoid-clavate
Paraphyses	Cylindrical, with apical part enlarged to *4.0–6.7 µm	Cylindrical, with apical part enlarged to *2.5–4.5 µm	Lanceolate, strongly exceeding, *5–7 (†3–6) µm broad; and cylindrical, not exceeding, *2.3–3.0 µm broad above
Reference	Künkele et al. (2005)	Dennis (1968), H.-O. Baral (pers. obs.)	This paper

a sister clade to Erysiphales, but without support for this sister relationship. Baral and Haelewaters (2015) found a sister relationship of *Stamnaria* and *Roseodiscus*, although only moderately supported by Bayesian analysis (pp = 0.83). Hosoya et al. (2013) suggested a transfer of the genus *Stamnaria* from Helotiaceae to the small family Leotiaceae. This was based on the strongly supported sister relationship with *Leotia lubrica* (Scop.) Pers. obtained in the maximum parsimony multigene analysis using rDNA, *EF1* and *RPB* genes, with bootstrap values of 100 from neighbour-joining and 99 from maximum parsimony. Morphologically, Hosoya et al. (2013) mentioned "a gelatinized layer in the external part of apothecia" as a synapomorphic character and suggested to include *Stamnaria* in Leotiaceae in a restricted sense, which traces back to a concept introduced by Korf & Lizoň (2001). However, recent molecular research indicated that the family Leotiaceae includes solely soil-inhabiting species with large ascomata with convex, pileate or clavate fertile part. Amongst the genera in this family, *Leotia* Pers. is the only one showing a gelatinised external layer outside the ectal excipulum (Baral in Jaklitsch et al. 2016). In addition, the ascospores of Leotiaceae consistently contain large oil drops (Baral in Jaklitsch et al. 2016), unlike *Stamnaria*.

Detailed morphological studies in the genus *Stamnaria* by Gruber (2006) revealed several other species, including collections that had been misidentified as *S. americana* or *S. persoonii* (*sensu* Magnes and Hafellner 1991, Brunelli 1992, Künkele et al. 2005).

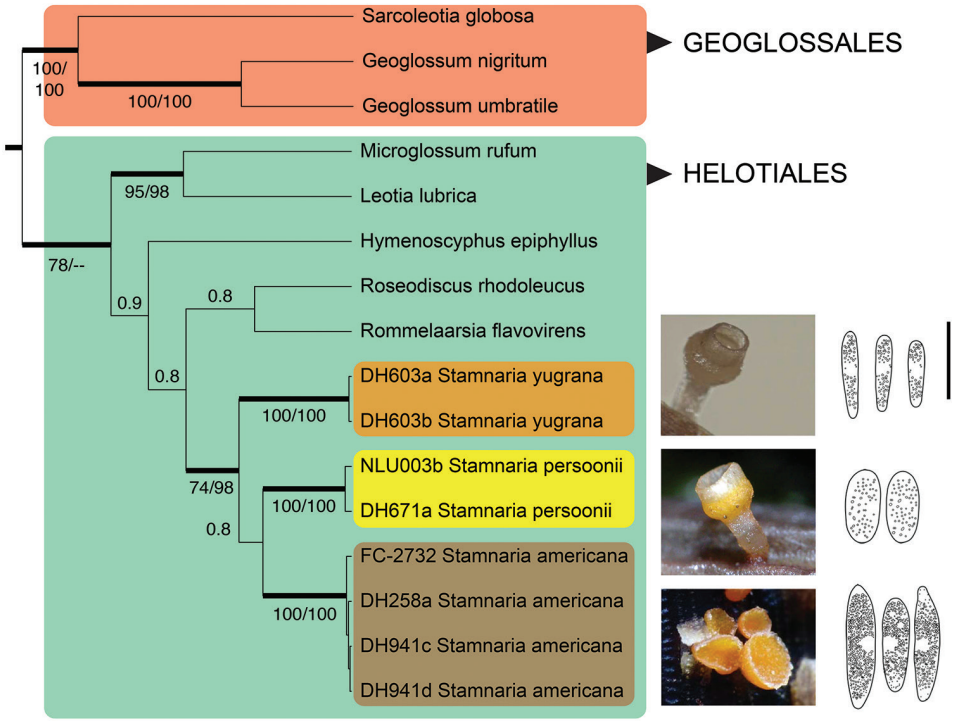

Figure 5. Bayesian MCC tree, with node values indicating posterior probabilities (above) and MP/ML bootstrap values (below). Thick branches indicate maximum Bayesian support (pp = 1.0). Photos of apothecia (left) and drawings of ascospores (right) from top to bottom: *S. yugrana*, holotype, YSU-F-03519, Russia, 9 Jun 2012; *S. persoonii*, Gilbert Moyne, H.B. 8889, France, 24.VI.2008; *S. americana*, Claude Page, France, 7 Apr 2016 (first French report in Moingeon and Page 2003). Scale bar: 20 µm.

The author proposed several new names for species that he considered undescribed, but they have not been validly published. Formal descriptions of these new species along with molecular phylogenetic analyses are planned in the near future. It is clear that the genus *Stamnaria* is in need of thorough revision. The discovery and detailed description of *S. yugrana* will help in the further delimitation of the genus.

Acknowledgements

The authors are very thankful to Erwin Gruber (Karl-Franzens-Universität, Graz, Austria) for providing collections that were used in molecular analyses, to Ove Eriksson (Umeå University, Sweden) for valuable information during the course of this study and to Kanchi Gandhi (Harvard University Herbaria) for nomenclatural advice. Nadia L. Urrea (Harvard College) is acknowledged for providing assistance in the molecular lab. This study was partially supported by two Russian Foundation of Basic Research grants (Nos. 18-34-00291 & 18-44-860017).

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A multi-gene phylogeny of *Chlorophyllum* (*Agaricaceae*, *Basidiomycota*): new species, new combination and infrageneric classification

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Abstract

Taxonomic and phylogenetic studies of *Chlorophyllum* were carried out on the basis of morphological differences and molecular phylogenetic analyses. Based on the phylogeny inferred from the internal transcribed spacer (ITS), the partial large subunit nuclear ribosomal DNA (nrLSU), the second largest subunit of RNA polymerase II (*rpb2*) and translation elongation factor 1-α (*tef1*) sequences, six well-supported clades and 17 phylogenetic species are recognised. Within this phylogenetic framework and considering the diagnostic morphological characters, two new species, *C. africanum* and *C. palaeotropicum*, are described. In addition, a new infrageneric classification of *Chlorophyllum* is proposed, in which the genus is divided into six sections. One new combination is also made. This study provides a robust basis for a more detailed investigation of diversity and biogeography of *Chlorophyllum*.

Keywords

Agaricales, *Lepiota*, *Macrolepiota*, multigene phylogeny, new taxa

Introduction

The genus *Chlorophyllum* Massee, 1898 (*Agaricaceae*, *Agaricales*) is typified by *Chlorophyllum molybdites* (G. Mey.) Massee. This genus currently accommodates ca. 16 species (Kirk et al. 2011) and 30 records can be found in Index Fungorum (http://www.indexfungorum.org/Names/NAMES.ASP). Traditionally, this genus was monotypic, only containing the green-spored species, *C. molybdites*. Based on similarities in morphology and/or molecular evidence, a few species previously placed in *Macrolepiota* Singer or *Lepiota* (Pers.) Gray, were transferred into it (Vellinga 2002). Similarly, *Endoptychum agaricoides* was also transferred to this genus based on molecular evidence and the proposal to conserve *Chlorophyllum* (hereafter abbreviated as *C.*) against *Endoptychum* was submitted to retain the genus name for the well-known toxic species *C. molybdites* (Vellinga and De Kok 2002) and accepted (Gams 2005). Members of this genus are characterised by the following unique combination of morphological characters: the pileus covering is hymenidermal, the stipe (if present) is smooth and basidiospores lack a germ pore or have a germ pore caused by a depression in the episporium without a hyaline covering. The basidiospores are white, green, brownish or brown in deposit and the habit varies from agaricoid, secotioid to gasteroid (Crous et al. 2015a; Ge and Yang 2006; Vellinga 2003a; 2004b; Vellinga et al. 2003). Species within this genus are saprotrophic and distributed worldwide, often growing in urban and ruderal habitats, with a preference for tropical and subtropical regions (Vellinga 2004a).

Recently, three species, *C. lusitanicum* G. Moreno, Mohedano, Manjón, Carlavilla & Altés, *C. pseudoglobosum* J. Sarkar, A.K. Dutta & Acharya and *C. sphaerosporum* Z.W. Ge & Zhu L. Yang were described from Spain, India and China, respectively (Crous et al. 2015a; Crous et al. 2015b; Ge and Yang 2006). These studies provided a better understanding of the species diversity within the genus, but are confined to certain specific regions and samples from other poorly explored areas such as Africa have seldom been included. Such studies have been focused on new species descriptions, but an infrageneric classification for the genus is still lacking because infrageneric relationships are poorly known.

Phylogenetic studies have shown that *Chlorophyllum* is nested within *Agaricaceae* (Ge and Yang 2017; Vellinga 2004b; Vellinga et al. 2003; Vellinga et al. 2011). However, due to limited taxon sampling and /or use of the ribosomal RNA genes only (ITS and /or nrLSU), limited information on infrageneric relationships could be gleaned. Further sampling of more species and phylogenetic analyses based on protein coding genes are needed to clarify relationships within *Chlorophyllum*.

Based on investigations of lepiotoid fungi in China, Dominican Republic, Germany, Italy, South Africa, Thailand, United Kingdom and the United States of America, detailed morphological and molecular studies were carried out in this study. The aims were to:

1. elucidate species diversity within *Chlorophyllum* based on both morphological characters and phylogenetic analysis, describe novel species and provide more information on poorly known species;

2. use a combined multi-gene dataset (ITS, nrLSU, *rpb2* and *tef1*) to provide a robust hypothesis for relationships amongst *Chlorophyllum* species;
3. examine diagnostic characters for recognised clades and establish an infrageneric classification that best reflects the evolutionary history of the genus.

Materials and methods

Taxon sampling and morphological studies

Fifty-nine collections were newly sampled from China, Dominican Republic, Germany, Italy, South Africa, Thailand, the United Kingdom and United States of America and deposited in HMAS, PREM, HKAS (Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences) and MFLU. Twelve out of the 16 recognised species, plus two recently described species, as well as two putative new species and a new combination were represented in this study. Morphological characters were studied from field notes, colour images of the material and complemented with literature data. Colour names and codes are from Kornerup and Wanscher (1978). Microscopic character observations were conducted under a light microscope using thin handmade sections rehydrated in 5% aqueous potassium hydroxide (KOH) (w/v). Melzer's reagent was used to test the amyloidity of basidiospores and cresyl blue was used to study the metachromatic reaction (Largent et al. 1977). In the descriptions of basidiospores, the abbreviation [*n*/*m*/*p*] indicates *n* basidiospores measured from *m* basidiocarps of *p* collections; (a)b–c(d) stands for the dimensions of the basidiospores, with b–c containing a minimum of 90 % of the measured values and (a) and (d) extreme values. Q is used to mean "length/width ratio" of a basidiospore and Qav represents average of Q of all basidiospores studied.

DNA extraction, primers, PCR and sequencing

A small piece of dried basidiocarp was excised from a specimen and ground in an Eppendorf tube. Genomic DNA was isolated using the CTAB method (Doyle and Doyle 1987). Optimal dilutions of the DNAs were used to amplify the following regions: internal transcribed spacer (ITS), the large subunit nuclear ribosomal DNA (nrLSU), the second largest subunit of RNA polymerase II (*rpb2*) and the translation elongation factor 1-α gene (*tef1*). PCR amplifications used the previously described primers: ITS1F/ITS4 for ITS, LR0R/LR5 for nrLSU (Gardes and Bruns 1993; White et al. 1990), bRPB2-6F /bRPB2-7.1R for *rpb2* (Matheny 2005) and 983F/1567R for *tef1* (Rehner and Buckley 2005). PCR conditions were as recommended by the Taq polymerase manufacturer (Bioteke, Beijing, China), using an ABI 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA). PCR products were cleaned and sequenced by Sangon Biotech (Shanghai) Co. Ltd. (Shanghai, China) and Kunming Shuoqing Biotech Ltd (Kunming, China).

Phylogenetic analyses

In this study, 144 sequences were produced using standard methods and deposited in GenBank (MG741961–MG742106), viz., 59 ITS, 29 nrLSU, 28 *rpb2* and 28 *tef1* (Figure 1 and Table 1) sequences. To obtain an estimate of *Chlorophyllum* genetic diversity, 96 ITS sequences were also retrieved from GenBank and included in the phylogenetic analyses (GenBank nos. included in Figure 1).

The ITS data set included 155 *Chlorophyllum* sequences. From these, a subset of 29 collections was chosen to represent the full range of phylogenetic diversity sampled for a four-locus dataset comprising portions of the ITS, nrLSU, *rpb2* and *tef1* (Table 1). To test the monophyly of *Chlorophyllum* within *Agaricaceae*, ML analysis of the *rpb2* dataset, which has much fewer ambiguous aligned sections in the matrix compared to the ITS dataset, was conducted with representative genera of the *Agaricaceae* (Ge and Yang 2017; Ge et al. 2015; Vellinga et al. 2011). As *Chlorophyllum* is confirmed as a monophyletic group close to *Agaricus* L. and allied genera in the present study (Suppl. material 1) and recent studies (Ge and Yang 2017; Ge et al. 2015; Vellinga et al. 2011), representative species of these genera were included as outgroups for rooting purposes for analyses; these are *Agaricus campestris* L., *Clarkeinda trachodes* (Berk.) Singer, *Coniolepiota spongodes* (Berk. & Broome) Vellinga, *Eriocybe chionea* Vellinga, *Heinemannomyces splendidissimus* Watling and *Pseudolepiota zangmui* Z.W. Ge.

Sequences were aligned using MAFFT 6.8 (Katoh et al. 2009) and further optimised visually. The best-fit evolutionary model for each dataset was determined using MRMODELTEST (Nylander 2004). Maximum Likelihood (ML) analyses were conducted in RAxML 7.2.6-WIN with default settings using a GTRGAMMA model (Stamatakis et al. 2007). Clade robustness was assessed using a bootstrap analysis with 1000 replicates (Felsenstein 1985).

The ITS-nrLSU, *rpb2* and *tef1* datasets were analysed separately before concatenation. As no significant (bootstrap support above 70 %) incongruence was detected, the resulting three alignments (ITS-nrLSU, *rpb2* and *tef1*) were combined for further multigene analyses. Unavailable sequences of loci from a few species were treated as missing data in the phylogenetic analyses. Final alignments have been deposited in TREEBASE (http://www.treebase.org) with accession number (S22068).

Results

The ITS alignment contained 787 sites, all of which were included in the analyses. The ML tree is shown in Figure 1 with the final ML optimisation likelihood at -5625.939348. According to the ML tree, 17 phylogenetic species were recovered. The new species were nested within *Ellipsoidospororum* clade (*C. africanum*) and *Chlorophyllum* clade (*C. palaeotropicum*).

The combined data set included subsamples of the 17 species recovered in the ITS tree. This alignment contained 2896 nucleotide sites (including gaps), consisting of 785, 851,

Figure 1. ML tree inferred from ITS data. Bootstrap values >50 % are indicated at internodes. Names of new taxa are in bold.

699 and 561 sites (including gaps) for ITS, nrLSU, *rpb2* and *tef1*, respectively. The analyses identified six distinct well-supported clades within *Chlorophyllum*, each representing one to four species (Figure 2). These clades are: *Chlorophyllum* clade, *Ellipsoidospororum* clade,

Table 1. Taxa, vouchers, geographic origin, and GenBank accession numbers of DNA sequences of *Chlorophyllum* and outgroups used in this study. New sequences generated by this work are in bold.

Taxon	Collection	Origin	nrITS	nrLSU	*rpb2*	*tef1*
Chlorophyllum africanum	PREM 62140	South Africa	**MG741961**	**MG742041**	**MG742070**	**MG742098**
C. africanum	PREM 62141	South Africa	**MG741963**	**MG742042**	**MG742071**	**MG742099**
C. agaricoides	HKAS 101312	Russia	**MG742003**	**MG742020**	**MG742050**	**MG742078**
C. agaricoides	HMAS 71678	China: Neimenggu	**MG742004**	**MG742021**	**MG742051**	**MG742079**
C. arizonicum	AH31724	Mexico	KR233490	KR233499	N/A	N/A
C. arizonicum	Trappe 11481 (AZ80)	USA	HQ020416	HQ020419	N/A	N/A
C. brunneum	HKAS 101315	Italy	**MG742013**	**MG742022**	**MG742052**	**MG742080**
C. brunneum			AY083206	AF482886	HM488804	HM488886
C. demangei	Z. W. Ge 3112	China: Yunnan	**MG741965**	**MG742027**	**MG742056**	**MG742084**
C. demangei	Z. W. Ge 3574	China: Yunnan	**MG741964**	**MG742025**	**MG742055**	**MG742083**
C. globosum	Z. W. Ge 2006-1	China: Yunnan	**MG741995**	**MG742023**	N/A	N/A
C. globosum	PREM 62147	South Africa	**MG742002**	**MG742024**	**MG742053**	**MG742081**
C. hortense	HKAS 101317	China: Hainan	**MG741967**	**MG742026**	**MG742054**	**MG742082**
C. hortense	Z. W. Ge 3115	China: Yunnan	**MG741968**	**MG742028**	**MG742057**	**MG742085**
C. hortense	HKAS 90470	China: Yunnan	**MG741971**	**MG742029**	**MG742058**	**MG742086**
C. lusitanicum	AH45540	Spain	KR233482	KR233491	N/A	N/A
C. lusitanicum	AH43927	Spain	KR233483	KR233492	N/A	N/A
C. molybdites	HKAS 45051	China: Hunan	**MG741985**	**MG742030**	**MG742059**	**MG742087**
C. molybdites	Z. W. Ge 3381	USA: Florida	**MG741993**	**MG742034**	**MG742063**	**MG742091**
C. molybdites	Z. W. Ge 3146	China: Yunnan	**MG741987**	**MG742031**	**MG742060**	**MG742088**
C. molybdites	HKAS 101322	Italy	**MG741988**	**MG742032**	**MG742061**	**MG742089**
C. molybdites	Z. W. Ge 3377	USA: Florida	**MG741992**	**MG742033**	**MG742062**	**MG742090**
C. neomastoideum	HKAS 83208	China: Zhejiang	**MG741976**	**MG742035**	**MG742064**	**MG742092**
C. olivieri	HKAS 31587	Germany: Marburg	**MG742016**	**MG742036**	**MG742065**	**MG742093**
C. olivieri	HKAS 53466	Germany: Marburg	**MG742017**	**MG742037**	**MG742066**	**MG742094**
C. palaeotropicum	PREM 62142	South Africa	**MG741978**	**MG742038**	**MG742067**	**MG742095**
C. palaeotropicum	PREM 62145	South Africa	**MG741982**	**MG742039**	**MG742068**	**MG742096**
C. palaeotropicum	HKAS 93747	Benin: Okpara	**MG741983**	**MG742040**	**MG742069**	**MG742097**
C. pseudoglobosum	AM155	India: West Bengal	KP642506	KR080484	N/A	N/A
C. rhacodes			AF482849	AY176345	N/A	HM488885
C. rhacodes			U85312	U85277	HM488803	KC884736
C. sphaerosporum	HMAS 66153	China: Neimenggu	**MG742011**	**MG742043**	**MG742072**	**MG742100**
C. sphaerosporum	HMAS 71683	China: Neimenggu	**MG742012**	**MG742044**	**MG742073**	**MG742101**
C. subrhacodes	Z. W. Ge 3411	USA: Florida	**MG741975**	**MG742045**	**MG742074**	**MG742102**
C. subrhacodes	Z. W. Ge 3232	USA: Florida	**MG741973**	**MG742046**	**MG742075**	**MG742103**
C. subrhacodes	Z. W. Ge 3385	USA: Florida	**MG741972**	**MG742048**	**MG742077**	**MG742105**
C. subrhacodes	Z. W. Ge 3242	USA: Florida	**MG741974**	**MG742047**	**MG742076**	**MG742104**
Outgroups						
Agaricus campestris			KM657927	KR006607	KT951556	KR006636
Clarkeinda trachodes			HM488751	KY418837	HM488802	N/A
Coniolepiota spongodes	png012	Thailand	HM488756	HM488774	HM488796	HM488883
Eriocybe chionea			HM488753	HM488772	HM488800	N/A
Heinemannomyces splendidissimus			HM488760	HM488769	HM488793	KT951657
Pseudolepiota zangmui	Z. W. Ge 2175	China: Yunnan	KY768928	**MG742049**	KY768929	**MG742106**

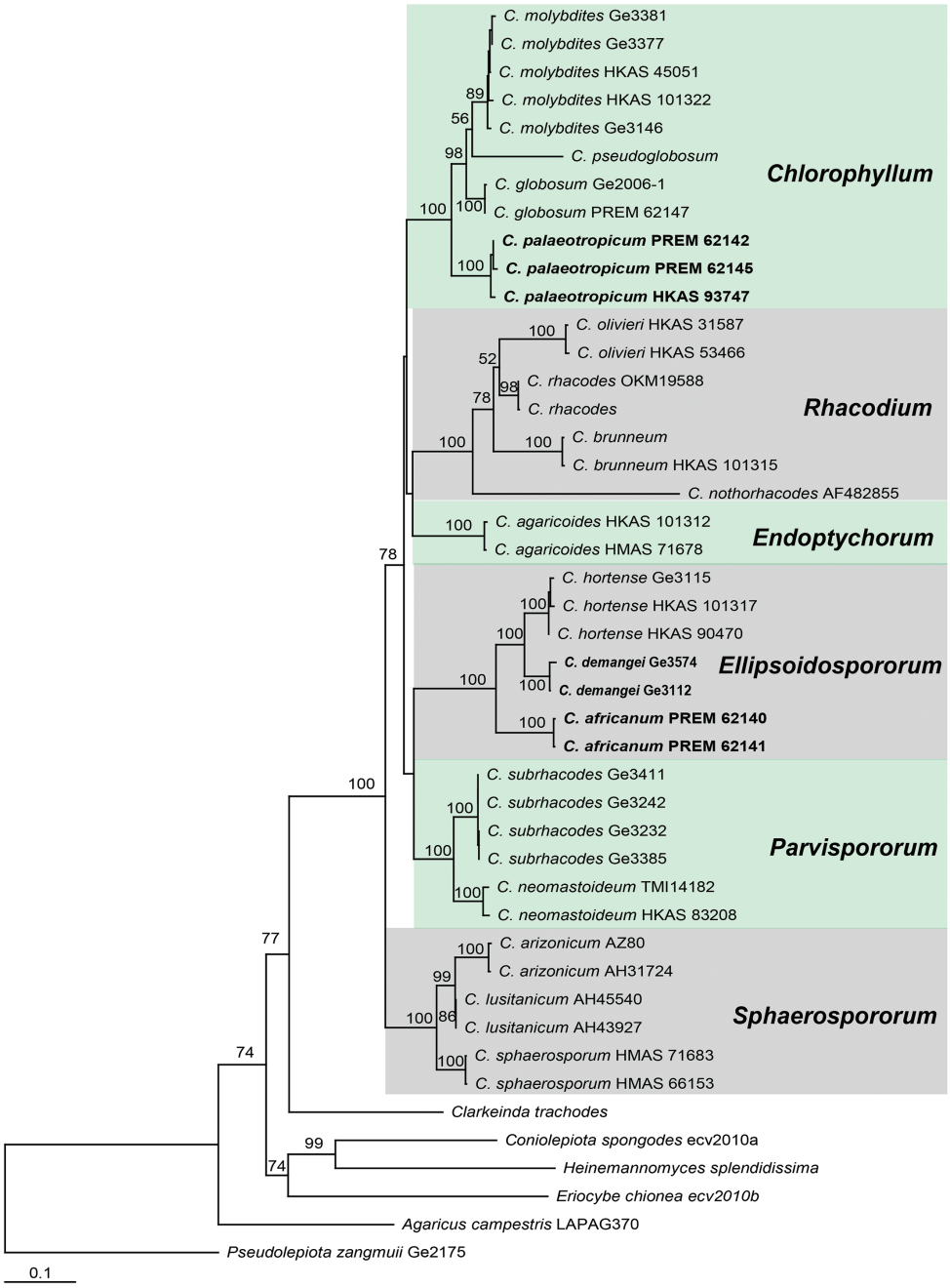

Figure 2. ML tree inferred from the combined alignment based on ITS, nrLSU, *rpb2* and *tef1*. Bootstrap values >50 % are indicated at internodes. Names of new taxa are shown in bold.

Endoptychorum clade, *Rhacodium* clade, *Parvispororum* clade and *Sphaerospororum* clade. Each of these clades received 100 percent maximum boot strap (ML-BP) support in the combined data set and strong support (≥87 % boot strap support) in the individual data sets (ITS-nrLSU, *rpb2* and *tef1* respectively). Species relationships within these six clades are largely resolved, but relationships amongst all clades were not resolved with confidence.

Emphasising both molecular data and morphological characters, a new infrageneric classification for *Chlorophyllum* and two new distinct species, *C. africanum* and *C. palaeotropicum* are proposed.

Taxonomy

Infrageneric classification of Chlorophyllum

The genus *Chlorophyllum* is divided into six sections: sect. *Chlorophyllum*, Sect. *Ellipsoidospororum*, sect. *Rhacodium*, sect. *Parvispororum*, sect. *Endoptychorum* and sect. *Sphaerospororum*.

Chlorophyllum sect. *Chlorophyllum* Massee

Type. *Chlorophyllum molybdites* (G. Mey.) Massee. Bull. Misc. Inf., Kew: 136. 1898.

≡ *Agaricus molybdites* G. Mey., Prim. fl. esseq.: 300. 1818.

Description. Basidiocarps medium to large sized, stout, agaricoid, with obvious plate-like squamules. Basidiospores olive to greenish-white, thick-walled, ellipsoid to amygdaliform with a truncate apex, except for *C. palaeotropicum*, which produce subglobose basidiospores without germ pore. Cheilocystidia broadly clavate to sphaeropedunculate. Pileipellis is a palisade of hyphae with terminal elements clavate to subfusiform.

Discussion. This section contains the type species of this genus, *C. molybdites* and also *C. globosum*, *C. pseudoglobosum*, as well as the novel taxon *C. palaeotropicum*.

Chlorophyllum sect. *Ellipsoidospororum* Z.W. Ge, sect. nov.

MycoBank MB 823853

Diagnosis. Differs from other sections by the slender basidiocarps with furfuraceous squamules on the pileus, the non-pored, ellipsoid basidiospores and subcylindric to slightly fusiform cheilocystidia.

Type. *Chlorophyllum hortense* (Murrill) Vellinga, Mycotaxon 83: 416. 2002.

≡ *Lepiota hortensis* Murrill, N. Amer. Fl. (New York) 10 (1): 59. 1917.

Description. Basidiocarps agaricoid, small to medium sized, with furfuraceous squamules. Basidiospores ellipsoid to ovoid without germ pore. Cheilocystidia narrowly clavate to subcylindrical. Pileipellis a loose hymeniderm made up of clavate to subfusiform hyphae.

Discussion. This section is represented by *C. hortense*, *C. demangei* and the new taxon *C. africanum*. *Chlorophyllum alborubescens* (Hongo) Vellinga, *C. humei* (Murrill) Vellinga, *C. mammillatum* (Murrill) Vellinga, *C. subfulvidiscum* (Murrill) Vellinga, *Leucoagaricus bisporus* Heinem., which were treated as synonyms of *C. hortense* (Murrill) Vellinga (Vellinga 2003; Akers and Sundberg 1997) also belong here (Figure 1).

***Chlorophyllum* sect. *Endoptychorum* (Czernajew) Z.W. Ge, comb. & stat. nov.**
MycoBank MB 823854

Basionym. *Endoptychum* Czern., Bull. Soc. Imp. nat. Moscou 18(2, III): 146 1845.

Type. *Chlorophyllum agaricoides* (Czern.) Vellinga, Mycotaxon 83: 416. 2002.

≡ *Endoptychum agaricoides* Czern., Bull. Soc. Imp. nat. Moscou 18(2, III): 148. 1845.

Description. Basidiocarps secotioid, with inconspicuous squamules. Basidiospores thick-walled, without germ pore.

Discussion. This section currently contains only one species (*C. agaricoides*), known from America, Asia and Europe.

***Chlorophyllum* sect. *Parvisporum* Z.W. Ge, sect. nov.**
MycoBank MB 823859

Diagnosis. Differs from other sections by the relatively smaller, porous basidiospores (less than 10 μm long) and a pileipellis composed of a palisade of hyphae with cylindrical terminal elements.

Type. *Chlorophyllum subrhacodes* (Murrill) Vellinga, Mycotaxon 83: 416. 2002.

≡ *Lepiota subrhacodes* Murrill, Lloydia 6: 223. 1943.

Description. Basidiocarps small to medium-sized, agaricoid, covered with large squamules contrasting in colour with the background. Basidiospores relatively small (less than 10 μm long), with germ pore, forming a truncated apex. Cheilocystidia clavate to mucronate clavate. Pileipellis a palisade of hyphae with cylindrical terminal elements. Hyphae without clamp connections.

Discussion. This section contains the species from south-eastern North America (*C. subrhacodes*) and east Asia (*C. neomastoideum*) displaying an America-Asia disjunct distribution.

***Chlorophyllum* sect. *Rhacodium* Z.W. Ge, sect. nov.**
MycoBank MB 823865

Diagnosis. Differs from other sections by the stout basidiocarps with plate- like squamules on the pileus and white lamellae, basidiospores with wide germ pore and pileipellis composed of a tightly packed hymeniderm of cylindrical and flexuous, or narrowly clavate or narrowly lageniform elements.

Type. *Chlorophyllum rhacodes* (Vittad.) Vellinga, Mycotaxon 83: 416. 2002.
≡ *Agaricus rhacodes* Vittad. [as 'rachodes'], Descr. fung. mang. Italia: 158. 1835.

Description. Basidiocarps medium to large sized, stout, agaricoid, with plate like squamules, basidiospores with wide germ pore, forming a truncated apex. Cheilocystidia clavate to sphaeropedunculate. Pileipellis a tightly packed hymeniderm of cylindrical and flexuous, or narrowly clavate or narrowly lageniform elements.

Discussion. This section contains *C. nothorhacodes*, *C. brunneum*, *C. rhacodes*, *C. olivieri* and *C. venenatum* (Bon) C. Lange & Vellinga. There was controversy over the spelling of the species epithet '*rhacodes*' (originally published as '*rachodes*'). The Nomenclature Committee for Fungi debated the issue for years and the General Committee made the final decision that the epithet of *Agaricus rhacodes* Vittad. (Descr. Fung. Mang.: 158. 1833) is to be so spelled, even though it was originally spelled '*rachodes*', which was approved by the International Botanical Congress in Shenzhen, China (Wilson 2017).

***Chlorophyllum* sect. *Sphaerosporum* Z.W. Ge, sect. nov.**
MycoBank MB 823860

Diagnosis. Differs from other sections by the nonporous, globose to subglobose basidiospores and gasteroid basidiocarps or globose to subglobose basidiospores and a hymenodermal pileipellis made up of loosely arranged clavate to broadly clavate elements when the basidiocarps are agaricoid.

Type. *Chlorophyllum sphaerosporum* Z.W. Ge & Zhu L. Yang, Mycotaxon 96: 187. 2006.

Description. Basidiocarps agaricoid or gasteroid, covered with inconspicuous squamules. Basidiospores subglobose to globose without germ pore (with rounded apex). Cheilocystidia (if present) clavate to broadly clavate. Pileipellis a hymeniderm made up of loosely arranged clavate to broadly clavate elements.

Discussion. This section contains the agaricoid *C. sphaerosporum* and two hypogeous taxa, *C. arizonicum* and *C. lusitanicum*. It is so far the only clade containing gasteroid species.

Recognition of two new species and the transfer of *Lepiota demangei* from *Lepiota* to *Chlorophyllum*

***Chlorophyllum africanum* Z.W. Ge & A. Jacobs, sp. nov.**
MycoBank MB 823861
Figs 3A, 4

Diagnosis. This species is distinguished from other *Chlorophyllum* species by relatively small basidiocarps with yellow grey to grey orange (5B4) furfuraceous squamules, the

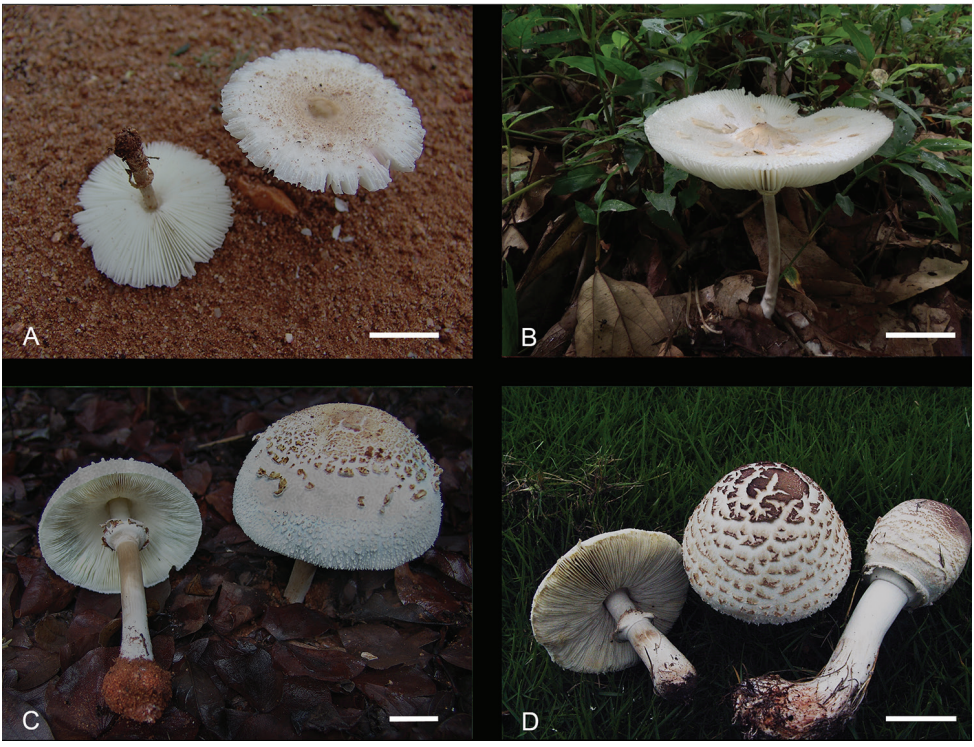

Figure 3. Basidiocarps of representative species of *Chlorophyllum*. **A** *Chlorophyllum africanum* (PREM 62141) **B** *Chlorophyllum demangei* (HKAS 89157) **C** *Chlorophyllum globosum* (PREM 62152) **D** *Chlorophyllum palaeotropicum* (HKAS 60195).

squamules composed of a hymenidermal layer made up of greyish-yellow to dull yellow, narrowly clavate to clavate elements, the hyaline ellipsoid basidiospores without a germ pore and the hyaline, cylindric to slightly fusiform cheilocystidia.

Type. SOUTH AFRICA. 2229 BB Beit Bridge, Farm Matolege 133 MS (–22°14.91'S, 29°47.29'E), alt. ca. 560 m, growing in disturbed area with large volume of animal droppings, 9 February 2014, Van Der Walt, R 885 (holotype: PREM 62143!; isotype: HKAS!). ITS barcoding sequence: MG741962.

Description. Pileus 30–50 mm broad, hemispherical to convex when young, expanding to broadly convex or applanate with age, sometimes with a prominent umbo; margin sulcate striate; surface covered with thin, yellow grey (4B2–4B3), orange grey (6B2) to grey orange (5B4) furfuraceous squamules, these remaining intact on the disc but elsewhere diffracted-scaly with expansion and receding from pileus margin. Lamellae free and remote from stipe, white to off white, crowded, narrow, up to 6 mm deep, with 1–2 series of lamellulae; edges entire, white. Stipe 35–60 × 3–6 mm, subcylindric, slightly enlarged at base, glabrous, white to light brown, hollow, nearly stuffed, with a simple annulus about 10–15 mm from top of the stipe. Context white, 1–2 mm thick in pileus, discolouring brown to red where bruised or handled, with strong mushroom odour, taste mild. Spore print white to cream.

Basidiospores [100,5,2] (7.5)8.0–9.0 × (5.5) 6.0–6.5(7.0) µm (mean 8.2 ± 0.4 × 6.2 ± 0.3 µm), Q = (1.2)1.3–1.4 (1.5), Qav = 1.3 ± 0.05, ellipsoid, occasionally ovoid in side view or in frontal view, with rounded apex, smooth, hyaline, congophilous, dextrinoid, with one guttule in most cases, without germ pore, slightly thick walled, becoming purplish-red (14A6–14A7) in cresyl blue. Basidia 29–33 × 10.0–11.0 µm, clavate, hyaline, 4-spored, rarely 2-spored. Cheilocystidia 28–50 × 6.0–10.0 µm, cylindric to slightly fusiform, hyaline. Pleurocystidia not observed. Lamella trama regular to slightly interwoven, made up of subcylindrical hyaline hyphae, 7.0–12.0 µm diam. Pileipellis a hymenidermal layer made up of greyish-yellow (1B4, 2B3) to dull yellow (3B3), clavate elements of 21–50 × 9.0–14.0(16.0) µm, slightly thick walled, with greyish-yellow vacuolar pigments; wall greyish-yellow; terminal elements mostly narrowly clavate. Clamp connections not observed.

Distribution. So far, only known from South Africa.

Ecology. Saprotrophic, solitary to scattered, terrestrial.

Etymology. (L.) in reference to Africa where it is collected.

Additional specimens examined. SOUTH AFRICA. 2229 BB Beit Bridge, Farm Matolege 133MS, –22°14.66'S, 29°46.75'E, 574 m, on soil. 10 January 2014, Van Der Walt, R787 (PREM 62140). 2229 BD Kamkusi, Farm Ludwigslust 163 MS (Farm Yard), –22°16.64'S, 29°48.22'E, alt. ca. 610 m, 9 March 2014, Van Der Walt, R935 (PREM 62141). Scattered in sandy soil of semi-shade to full sun, cleared area.

Discussion. *Chlorophyllum africanum* is morphologically very similar to *C. bharatense* Sathe & S.M. Kulk. Both species have a small-sized convex to applanate pileus covered with pale olivaceous brown squamules, clavate cheilocystidia and broadly ellipsoid basidiospores. However, *C. bharatense* differs from *C. africanum* by the umbonate pileus, lamellae that become reddish- brown on drying, basidiospores with an indistinct or absent germpore and squamules composed of a trichodermal layer (Sathe et al. 1981 ('1980')).

Chlorophyllum africanum is also similar to *C. hortense* on account of the small-sized basidiocarps, ellipsoid basidiospores and subcylindrical cheilocystidia. However, *C. hortense* differs from *C. africanum* by 2-spored basidia and the whitish context of the stipe becoming reddish where bruised (Akers and Sundberg 1997; Vellinga 2003b).

Chlorophyllum demangei (see below) is characterised by the frequent and obviously umbonate pileus and large basidiospores measuring (7.5) 8.0–10.5 (12.5) × (5.0) 5.5–7.0 (7.5) µm. Molecular phylogenetic results clearly support the recognition of the two as separate species.

Leucocoprinus zeylanicus (Berk.) Boedijn, described from Sri Lanka, is also similar to *C. africanum* due to the small-sized pileus with a distinct umbo, the subcylindric cheilocystidia and the short ellipsoid basidiospores (Pegler 1986). However, the finely radially silky-striate pileus of *Lc. zeylanicus* beset with sparse, minute, blackish-brown repent squamules and the basidiospores with a small germ pore (Pegler 1986), differentiate this species from *C. africanum*.

Lepiota zeyheri (Berk.) Sacc., a species also found in South Africa, is somewhat similar to *C. africanum* on account of the whitish pileus with a clay brown umbo that is elsewhere covered with cream or brown squamules and the ellipsoid basidiospores.

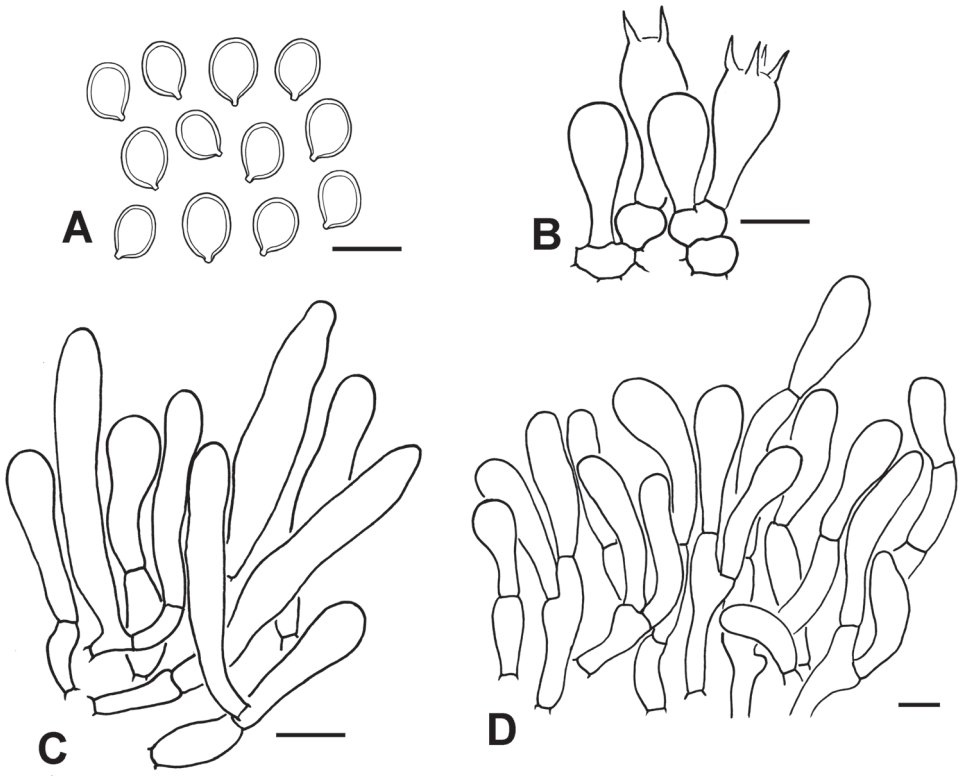

Figure 4. Micro-morphological features of *C. africanum* (PREM 62143, type). **A** Basidiospores **B** Basidia **C** Cheilocystidia **D** Elements of squamules on pileus. Scale bars: 10 μm (**A, D**); 20 μm (**B, C**).

However, *L. zeyheri* has much larger basidiocarps measuring 10–22 cm or larger, a pale pink spore deposit, larger broadly ellipsoid basidiospores (15.0–17.0 × 10.0–12.0 μm) with a germ pore and clavate cheilocystidia (Pearson 1950).

***Chlorophyllum palaeotropicum* Z.W. Ge & A. Jacobs, sp. nov.**
MycoBank MB 823862
Figs 3D, 5

Diagnosis. This species is distinguished from other *Chlorophyllum* species by medium-sized basidiocarps with distinct brownish squamules composed of a trichodermal layer of subcylindrical brownish hyphae and slightly enlarged terminal elements, the greenish subglobose basidiospores without a germ pore and the clavate to narrowly clavate cheilocystidia with brownish to fuscous brown vacuolar pigments.

Type. SOUTH AFRICA. 2229 BD Kamkusi, Farm Ludwigslust 163MS (-22°15.39'S, 29°47.48'E), alt. 582 m, near open area along dirt road, growing in loam soil, compost-rich – mopane (*Colophospermum mopane*) leaf layer, 30 November

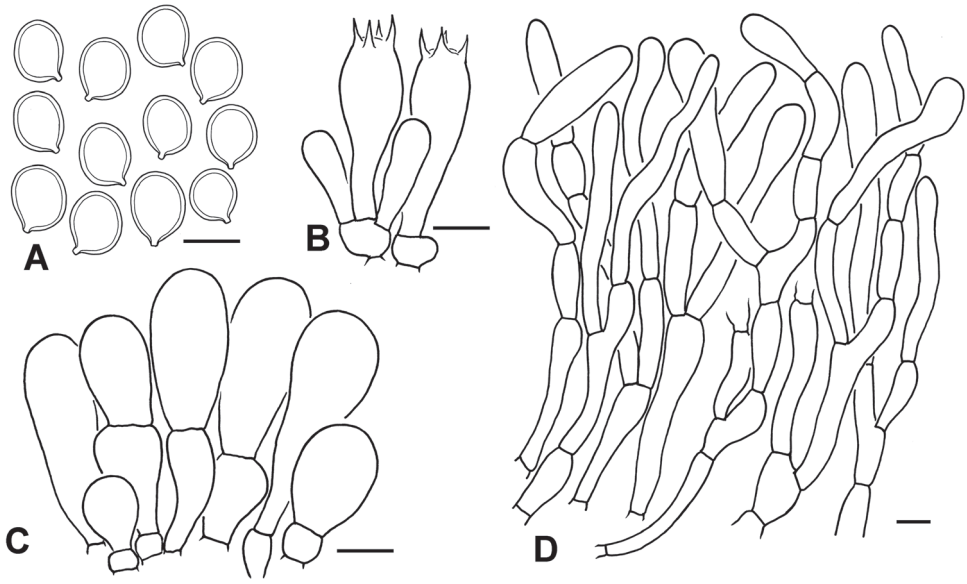

Figure 5. Micro-morphological features of *C. palaeotropicum* (PREM 62142, type). **A** Basidiospores **B** Basidia **C** Cheilocystidia **D** Elements of squamules on pileus. Scale bars: 10 μm (**A, D**); 20 μm (**B, C**).

2013, Van Der Walt, R 715 (Holotype: PREM 62142!; isotype: HKAS!). ITS barcoding sequence: MG741978.

Description. Pileus 50–100 mm broad, hemispherical to convex at first, expanding to convex to broadly convex with age; surface covered with fibrillose, tufted, reddish-white (7A2) to brownish-orange (6C3) squamules at the margin and brownish-grey (6C2), orange grey (6B2), to greyish-brown (7D3) plate-like squamules at the centre. Lamellae free and remote from stipe; white to off-white when young, whitish to greenish-white (26A2) when mature, crowded, 6–11 mm deep, with 1–2 series of lamellulae. Stipe 16–90 × 3.5–8 mm, subcylindrical, with slightly enlarged base, straight or curved, white; hollow, nearly stuffed, with an annulus at the middle part of the stipe. Context white, 4–6 mm thick in pileus, white in pileus and stipe, discolouring pastel pink (7A4) when drying, with a distinct mushroom smell, taste mild. Spore print greyish-green (30B3–30B4).

Basidiospores [100,5,3] (8.0)8.5–11.0(12.0) × (6.0)7.0–9.0(10.0) μm (mean 9.8 ± 0.9 × 8.0 ± 0.8 μm), Q = 1.0–1.4, Qav = 1.2 ± 0.05, ellipsoid, oblong in side view or in frontal view, with rounded apex, smooth, hyaline when young, greenish-white (27A2), olive to brownish (in KOH) when mature, congophilous, dextrinoid, without germ pore, slightly thick-walled; mature basidiospores staining purplish-red (14A6–14A7) in cresyl blue; immature basidiospores staining bluish-violet (18B7) in cresyl blue. Basidia 29–33 × 10.0–12.0(15.0) μm, clavate, hyaline, 4-spored. Cheilocystidia (13)20–55(63) × 10.0–15.0(20.0) μm, clavate, rarely broadly clavate or narrowly clavate, brownish to fuscous brown, sometimes septate. Pleurocystidia absent. Lamella trama slightly interwoven, made up of subcylindrical hyaline hyphae, 8–14 μm diam. Pileipellis a trichoderm made up of filamentous or cylindrical hyphae, slightly inter-

woven, interspersed with brown to tea brown hyphae, 8–14 µm in diam., thick-walled, with brownish vacuolar pigments; wall brownish-yellow; terminal elements mostly slightly enlarged to narrowly clavate, rarely cylindrical. Clamp connections present on basal septa of young basidia and tissue of annulus, but not common.

Distribution. Known from Benin and South Africa in Africa and from China in Asia.

Ecology. Saprotrophic, solitary to scattered, terrestrial.

Etymology. (L.) with reference to distribution of this species in the Old World tropics.

Additional specimens examined. BENIN. Okpara: countryside of North-eastern Parakou, 15 km from Parakou, alt. ca 330 m, 18 June, 2015, G. Wu 1370 (HKAS 93747). CHINA. Hainan Province: Sanya, Yalongwan, on man-made lawn near seaside, 29 June 2010, Z.W. Ge 2519 (HKAS 60195). SOUTH AFRICA. 2229 BB Beit Bridge, Farm Wimpsh 139 MS, 12 February 2014, Van Der Walt, R891 (PREM 62144), growing in cleared area, near water hole, among *Bulbostylis hispidula*; 2229 BD Kamkusi, Farm Ludwigslust 163 MS (farm yard), –22°16.64'S, 29°48.22'E, alt. 606 m, growing in loam soil in cleared area, in semi-shade to full sun, 9 March 2014, Van Der Walt, R 938 (PREM 62145); 2229 BD Kamkusi, Farm Ludwigslust 163MS (–22°16.64'S, 29°48.22'E), alt. ca. 610 m, growing in loam soil in cleared area, 14 February 2014, Van Der Walt, R 905 (PREM 62146).

Discussion. *Chlorophyllum palaeotropicum* is very similar morphologically to *C. shimogaense* Sathe & S.M. Kulk. Both species have medium-sized, hemispherical to convex pilei covered with reddish-white to brownish-orange squamules composed of a trichodermal layer. Both species also possess clavate cheilocystidia and subglobose basidiospores. However, *C. shimogaense* possesses an umbonate pileus, basidiospores with an indistinct or absent germ pore, much smaller basidia (13–24 × 6–8.5 µm) and no clamp connections (Sathe et al. 1981 ('1980')).

Chlorophyllum palaeotropicum is also similar to *C. molybdites*, *C. globosum* and *C. pseudoglobosum* in general appearance due to the brownish to reddish discolourations where bruised, but *C. palaeotropicum* differs from these three species in having subglobose to globose basidiospores without a germ pore (apex rounded), while *C. molybdites*, *C. globosum* and *C. pseudoglobosum* have amygdaliform basidiospores with large germ pores (apex truncate).

Chlorophyllum palaeotropicum also resembles *C. sphaerosporum* on account of the basidiocarps bearing similar subglobose basidiospores without a germ pore. However, the squamules of *C. sphaerosporum* are made up of a hymenidermal layer composed of clavate to broadly clavate terminal elements. Furthermore, the context of *C. sphaerosporum* does not change colour when bruised. So far, *C. sphaerosporum* has only been recorded from temperate regions in northern China. These two species also belong to two different sections (Figure 1).

Chlorophyllum palaeotropicum is somewhat similar to *L. zeyheri* on account of the overall appearance, the broadly ellipsoid basidiospores and clavate cheilocystidia. However, *L. zeyheri* has much larger basidiocarps measuring 10–22 cm or larger and pale pink spore prints (Pearson 1950). Furthermore, *L. zeyheri* has larger basidiospores (15.0–17.0 × 10.0–12.0 µm) with a germ pore (Pearson 1950).

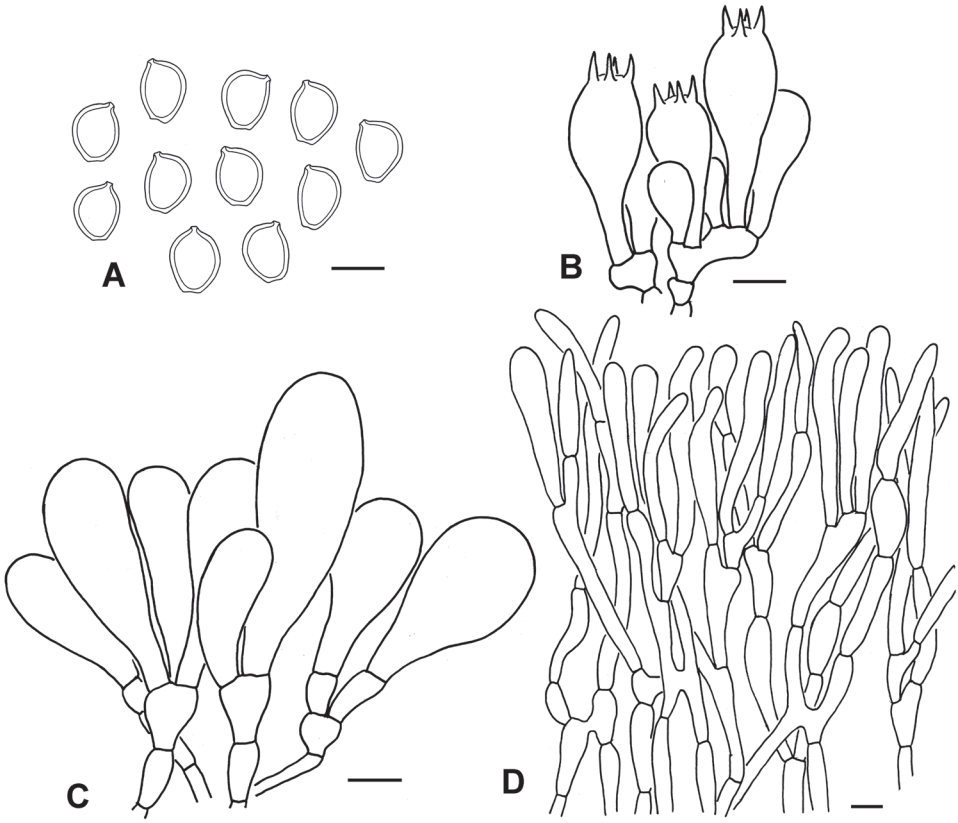

Figure 6. Micro-morphological features of *C. globosum* (HKAS 52741). **A** Basidiospores **B** Basidia **C** Cheilocystidia **D** Elements of squamules on pileus. Scale bars: 10 μm (**A, D**); 20 μm (**B, C**).

Chlorophyllum demangei **(Pat.) Z.W. Ge & Zhu L. Yang, comb. nov.**
MycoBank MB 823863

Basionym. *Lepiota demangei* Pat., Bull. trimest. Soc. mycol. Fr. 23(2): 78. 1907.

Type. VIETNAM. Hanoi: Tonkin, M. Demange 236 (Herb. Patouillard, FH 4244–holotype!).

Description. Pileus small to medium-sized, 2.5–8.5 cm in diam. (Figure 3B), umbonate, white to cream coloured, covered with concentrically arranged, ochraceous to yellowish-brown squamules; margin finely striate. Lamellae free, white to cream-coloured, 5–7 mm in height. Stipe 5–6 × 0.2–0.5 cm, whitish, becoming yellowish to brownish on bruising, slender, annulus 1–1.5 cm below apex of the stipe, persistent. Context of pileus and stipe white, becoming reddish, pinkish or orange red when cut, thin in pileus. Spore print white.

Basidiospores [45/2/1] (7.5) 8.0–10.0 (12.5) × (5.0) 5.5–7.0 (7.5) μm, 8.7 ± 0.4 × 6.3 ± 0.3 μm, Q= (1.3)1.4–1.7 (1.8), Qav =1.5 ± 0.09; ellipsoid, hyaline, strongly dextrinoid, slightly thick-walled, apex lacking germ pore but somewhat thinner than other

areas. Basidia 25–30 × 7–9 μm, clavate, 4-spored. Squamules on pileus (pileus disc of the smaller slice of the holotype) composed of clavate to narrowly clavate cells, 45–66 × 11–15 μm, hyaline to very pale brownish in KOH. Clamp connections not observed.

Distribution. Known from China and Vietnam in Asia.

Ecology. Saprotrophic, solitary to scattered, terrestrial.

Additional specimens examined. CHINA. Yunnan Province: Xishuanbangna Prefecture, Mengla County, Mengyuan, alt. ca. 770 m, July 2, 2014, Z.W. Ge 3574 (HKAS 84412); same locality, K. Zhao 494 (HKAS 89157); Honghe Prefecture, Gejiu City, Manghao town, September 25, 2011, Z.W. Ge 3112 (HKAS 70616).

Discussion. The distinctive characters of *Chlorophyllum demangei* are the discolouration of basidiocarps when bruised, the ellipsoid basidiospores without a germ pore and the pileal squamules composed of clavate to narrowly clavate elements. From the examination of specimens newly collected from southern Yunnan in China, not far away from the locality where the type of *Lepiota demangei* was collected, the distinctive characters were found that fit the description of *Lepiota demangei* (Yang 2000) very well. Thus, *Lepiota demangei* is transferred from *Lepiota* to *Chlorophyllum*.

Chlorophyllum globosum (Mossebo) Vellinga

Type. CAMEROON. Yaoundé, alt. 780 m, growing on humus in shade under tree, 1 November, 1996, D. C. Mossebo, D. C. Mossebo 98-1 kept in the Herbarium of University of Yaoundé I (*non vide*).

Description. Basidiocarps medium to large-sized (Figure 3C). Pileus 5.0–20.0 cm broad, ovoid to subglobose when young, expanding to parabolic, convex to broadly convex with age; margin inflexed, with short, fine striations; surface covered with yellowish-white (3A2) to yellowish-grey (4A2), greyish-yellow (4B3–4B4), brownish-orange (6C6) to greyish-brown (5D3) squamules. The squamules remain intact at disc, but elsewhere diffract with expansion and recede from pileus margin, displaying the white to yellowish-white (2A2, 3A2, 4A2) felted or fibrillose background which turned pastel red to red (9A4–6) when touched. Lamellae free and remote from stipe with obvious gutter, white to orange-white (5A1–2) when young, turning pastel red to red (9A4–6) when touched, pastel green to greyish-green (29A4, 29B4) when fully mature, crowded, ventricose and narrow near pileal margin, crowded, up to 8 mm wide, with 1–2 series of lamellulae; edge finely fimbriate, white to yellowish-grey (4A2). Stipe 8.5–28.0 × 1.0–3.1 cm, subcylindric, tapering to apex, with bulb-like, 3.0–3.4 cm wide; glabrous, white to brownish-orange (6C3–6), hollow, nearly stuffed, with an annulus about 1/3 away from the stipe apex (Figure 3C); sometimes with distant white fibrillose at apex zone, turning pastel red to red (9A4–6) when touched, with white rhizomorph connected to substrate. Context thick, white in pileus and stipe, brownish-orange (6C3–6) at apex zone, paler to middle zone and white downward base, discolouring pastel red to red (9A4–6) in both pileus and stipe context when bruised, with mushroom odour. Taste mild. Spore print yellowish-white (2A2) to pale yellow (2A3) to greyish-green (29D3–5, 29D5–6).

Basidiospores [40,2,2] (10.5)11.5–12.0 (12.5) × (8.0) 8.5–9.0 μm (mean 11.8 ± 0.4 × 8.7 ± 0.3 μm), Q = 1.3–1.4 (1.5), Qav = 1.4 ± 0.05, broadly amygdaliform in side view, ovoid in frontal view, with truncate apex, smooth, greenish-white (28A2), congophilous, dextrinoid, thick-walled (Figure 6A), becoming purplish-red (14A6–14A7) in cresyl blue. Basidia 29–38 × 12.0–14.0 μm, clavate, hyaline, 4-spored, sometimes 2-spored, rarely 1-spored. Cheilocystidia 42–65 × (15.0)18.0–29.0 μm, clavate occasionally with slightly long stalk, hyaline, sometimes with greyish-yellow vacuolar pigments. Pleurocystidia absent. Pileipellis a hymenidermal layer made up of subcylindrical hyphae (5.0–11.0 μm in diam.), slightly thick walled, with dull yellow (3B3) vacuolar pigments; terminal elements with rounded or attenuate apex, mostly narrowly clavate. *Clamp connections* not observed.

Distribution. Known from Cameroon, Nigeria and South Africa in Africa and from China, India and Thailand in Asia.

Ecology. Saprotrophic, solitary to scattered, terrestrial.

Specimens examined. CHINA. Yunnan Province: between Yuanmou and Yongren, 28 June 2006, Z.W. Ge 2006-1 (HKAS 52741). SOUTH AFRICA. 2229 BD Kamkusi, Farm Ludwigslust 163 MS, –22°16.27'S, 29°49.02'E, alt. ca. 580 m, 12 March 2014, Van Der Walt, R 957 (PREM 62147), growing in sandy soil under Sickle bush (*Dichrostachys cinerea*) and Umbrella thorn trees (*Vachellia tortilis*, formerly *Acacia tortilis*); 2229 BD Kamkusi, Farm Ludwigslust 163 MS, alt. 584m, growing in sandy soil under Sickle bush and Umbrella thorn trees, 7 February 2014, Van Der Walt, R 869 (PREM 62148); same locality, 9 March 2014, Van Der Walt, R 936 (PREM 62149); 2229 BB Beit Bridge, Farm Matolege 133 MS, alt. ca. 580m, shady area under blue thorn (*Senegalia erubescens*, formerly *Acacia erubescens*), compost-rich, adjacent to lawn in hunting camp, 12 February 2014, Van Der Walt, R 892 (PREM 62150); 2229 BB Beit Bridge, Farm Wimpsh 139 MS, alt. ca. 604 m, loam soil, amongst grass – adjacent to seasonally waterlogged pan, 12 January 2014, Van Der Walt, R 821 (PREM 62151); same locality, 14 February 2014, Van Der Walt, R 900 (PREM 62152). THAILAND. Chiang Mai Province: Mae Taeng District, Pongduad Village, 16°06'N, 99°43'E, 780–810 m, 16 June 2010, P. Sysouphanthong, P37 (MFLU100555); Chiang Rai Province: Muang District, Ratjabhat University campus, 30 August 2012, P. Sysouphanthong, 2012-21 (MFLU121815).

Discussion. *Chlorophyllum globosum* was originally described from Cameroon in the genus *Macrolepiota*. It was said to differ from *Macrolepiota odorata* "by the globose pileus and the ochraceous spore print" (Mossebo et al. 2000). In fact, the pileus does not stay globose during maturation but becomes broadly convex (Figure 3C). Based on the morphological characters such as the truncate basidiospores and its phylogenetic position, Vellinga (2002) transferred it to *Chlorophyllum*. The authors' molecular phylogeny confirms that *C. globosum* nests in *Chlorophyllum* close to *C. molybdites*, but it differs from the latter in having a pale yellow spore print and clavate cheilocystidia. This species was first described from Africa, but its presence in several Asian countries is confirmed.

Key to the 17 species of *Chlorophyllum* included in the present study

1 Basidiocarps sequestrate (either secotioid or gasteroid), basidiospores statismosporic **2**
– Basidiocarps agaricoid, basidiospores ballistosporic **4**
2 Basidiocarps secotioid, the margin of the pileus does not break free from the stipe, hymenophore (gleba) labyrinthiform to sub-lamellate... ***C. agaricoides***
– Basidiocarps gasteroid, stipe absent or rudimentary with a thick whitish mycelial cord, gleba crossed by a columella and capillitium **3**
3 Columella not fully developed; basidiospores 7–12 µm in diam... ***C. arizonicum***
– Columella well-developed, reaching halfway up the basidiocarp; basidiospores 10–14(–15) × 10–13(–14) µm ..... ***C. lusitanicum***
4 Basidiocarps overall white; basidiospores without germ pore, with rounded apex **5**
– Basidiocarps with distinct dark brown patches and squamules; basidiospores with germ pore; apex truncated **9**
5 Basidiospores subglobose to globose; cheilocystidia clavate to broadly clavate.... **6**
– Basidiospores ellipsoid; cheilocystidia subcylindric to slightly fusiform **7**
6 Spore print greyish-green, known from the palaeotropical regions ..... ***C. palaeotropicum***
– Spore print white, known from temperate region in Northern China ..... ***C. sphaerosporum***
7 Basidia 2-spored; widely distributed in Africa, America and Asia ..... ***C. hortense***
– Basidia 4-spored; known from palaeotropics **8**
8 Pileus with obvious umbo, basidiospores measuring (7.5) 8.0–10.5 (12.5) × (5.0) 5.5–7.0 (7.5), known from Southeast Asia ..... ***C. demangei***
– Pileus without obvious umbo, basidiospores (7.5) 8.0–9.0 × (5.5) 6.0–6.5(7.0) µm), known from South Africa ..... ***C. africanum***
9 Basidiospores less than 10 µm long; terminal elements of pileipellis cylindrical, basidiocarps grown in bamboo forest in east Asia or under oaks of Florida in south-eastern U.S.A **10**
– Basidiospores longer than 10 µm; terminal elements of pileipellis clavate to narrowly clavate; basidiocarps in various habitats (meadows, pastures, lawns, greenhouse, natural forests) **11**
10 Cheilocystidia clavate, without apical excrescences; clamp connections present at base of basidia and cheilocystidia; distributed in Asia ..... ***C. neomastoideum***
– Cheilocystidia clavate, some mucronate or with apical excrescences; clamp connections absent at base of basidia and cheilocystidia; distributed in North America ..... ***C. subrhacodes***
11 Spore print green; lamellae completely greenish with age ..... ***C. molybdites***
– Spore print white or off-white; lamellae whitish or brownish with age, never totally green; sometimes a bluish-green shade is present near the stipe **12**

12 Cheilocystidia sphaeropedunculate to broadly clavate, often catenate.....**13**
– Cheilocystidia narrowly clavate to clavate.....**14**
13 Pileus squamules of similar colour as background, olivaceous brown to greyish-brown .....***C. olivieri***
– Pileus squamules brown (different shades) on white to cream background, which is distinctly paler than squamules ..... ***C. rhacodes***
14 Clamp connections absent at base of basidia and cheilocystidia, cheilocystidia clavate to fusiform; annulus relatively simple ..... ***C. nothorhacodes***
– Clamp connections present at base of basidia and cheilocystidia, cheilocystidia narrowly clavate to fusiform; annulus relatively simple or complex.....**15**
15 Basidiocarps with abruptly to marginately bulbous stipe base; annulus relatively simple, without a double crown, but with a tough brown patch on the underside..... ***C. brunneum***
– Basidiocarps with widened base of stipe, but not abruptly so; annulus complex, with double crown.....**16**
16 Spore print yellowish-white to pale yellow to greyish-green, basidiospores greenish-white, 8–11 × 5–6 (7) μm..... ***C. globosum***
– Spore print white, basidiospores hyaline, 10–10.7 (11) × (7) 8–8.5 (9.5) μm.....
.....***C. pseudoglobosum***

Discussion

Monophyly and infrageneric classification of Chlorophyllum

In the present study, based on the extensive dataset comprising 75 % of all known species, four gene regions were used to clarify the evolutionary relationships of *Chlorophyllum*, in separate and multi-locus analyses. Based on molecular data, the genus *Chlorophyllum* is monophyletic and the genus *Clarkeinda* appeared to be the likely sister clade to *Chlorophyllum* (Figure 2). A six-section infrageneric classification of *Chlorophyllum* was proposed based on the demarked morphological characters of well-supported clades. This phylogeny also elucidated the systematic positions of previously not included taxa, such as *C. demangei*, *C. sphaerosporum* and *C. subrhacodes* (Figure 2). In addition, two new species, *C. africanum* and *C. palaeotropicum* have been added.

Useful morphological characters in delimitation sections and species within *Chlorophyllum*

The morphological diversity within *Chlorophyllum* is mainly reflected in the general appearance of basidiocarp, colour reaction of context when bruised, the structure of pileus squamules, colour, shape and size of basidiospores and presence / absence of germ pore, shape of cheilocystidia and presence /absence of clamp connections. Based on the morphological characters chiefly used for species delimitation, morphological features

in *Chlorophyllum* appear to be fast evolving and prone to shifts and no synapomorphic characteristics have been found to consistently separate the sections. This is probably due to the fact that major evolutionary radiations might have occurred in a relatively short time as it can be seen that most of the deep branches in the phylogenies are short. Nevertheless, several character combinations are phylogenetically informative thus are useful for delineating sections and species.

1. **General habitus of basidiocarps.** The sequestrate (secotioid / gasteroid) form of basidiocarps is considered to be the result of selective pressures (Bruns et al. 1989) and the loss of forcible spore discharge has been found in several predominantly agaricoid genera within the family *Agaricaceae* (Gube and Dörfelt 2012), e.g. *Agaricus* (Vellinga 2004), *Macrolepiota* (Lebel and Syme 2012) and *Lepiota* (Ge and Smith 2013; Vidal et al. 2015). The transition from agaricoid to secotioid or gasteroid is thought to be irreversible (Hibbett 2004). The majority of *Chlorophyllum* species is agaricoid and the secotioid / gasteroid habit is an apomorphy for the genus *Chlorophyllum* and consequently can be used together with other characteristics to distinguish clades. These phylogenetic analyses demonstrate that the secotioid *C. agaricoides* forms an independent clade, while the gasteroid *C. arizonicum* and *C. lusitanicum* jointly form a clade that is sister to the agaricoid *C. sphaerosporum* (Figure 2). These results suggest that the gasteroid *C. arizonicum* and *C. lusitanicum* derived from the same ancestor as *C. sphaerosporum*, while the secotioid *C. agaricoides* may have evolved independently from a different agaricoid ancestor in the genus.
2. **Colour reaction of the context when bruised.** The context of *Chlorophyllum* species shows reddening changes when exposed to the air, from light sienna, pinkish, pinkish cinnamon, reddish, dull brownish-orange to orange red (Crous et al. 2015a; Crous et al. 2015b; Ge and Yang 2006; Vellinga 2003a, 2003b). These changes are difficult to quantify and have not been uniformly recorded according to the same criteria and thus, cannot be used to distinguish sections. Nevertheless, this character can be used in combination with other characters in delimitation of species as this character varies amongst species: some species have a strong reddening reaction, some only weakly change pinkish, in others the context becomes reddish first, then changes to brown.
3. **Structure of pileus squamules.** The squamules in *Chlorophyllum* are considered to be a hymeniform layer in general, but can be further divided into three different types: i. a palisade of hyphae with terminal clavate to subfusiform elements; ii. a tightly packed hymeniderm of cylindrical and flexuous, narrowly clavate or narrowly lageniform elements; and iii. a hymeniderm of loosely arranged clavate to subfusiform hyphae. These different types of structure of squamules can be used to delimit sections in combination with other characters. For instance, the squamules of species in section *Chlorophyllum* are of type i, those of section *Rhacodium* are of type ii, while those of section *Ellipsoidosporum* and section *Sphaerosporum* are of type iii.
4. **Colour, shape and size of basidiospores and presence/absence of germ pore.** The basidiospores of *Chlorophyllum* vary from subglobose to globose without germ pore,

ellipsoid without germ pore and amygdaliform to ellipsoid with large germ pore that causes the spore apex to be obviously truncated. The "ellipsoid without germ pore" shape is a conspicuous synapomorphy for the *Ellipsoidospororum* clade. Similarly, "subglobose to globose basidiospores without a germ pore" is characteristic of the *Sphaerospororum* clade, while all species in section *Parvispororum* have relatively small (less than 10 μm long) basidiospores with a truncate apex. Basidiospores can be hyaline or olive to greenish and this character can be used to separate certain clades: species within the *Chlorophyllum* clade and *Endoptychorum* clade may have olive to greenish basidiospores, while the remaining clades have hyaline basidiospores.

5. **Shape of cheilocystidia.** Shape of cheilocystidia within *Chlorophyllum* ranges from subcylindrical, slightly fusiform, narrowly clavate, clavate, mucronate clavate, broadly clavate to sphaeropedunculate. These changes are informative in recognising certain, but not all sections. For example, the cheilocystidia of species in section *Ellipsoidospororum* are narrowly clavate to subcylindrical, while in other sections, the cheilocystidia are clavate to sphaeropedunculate.
6. **Presence/absence clamp connections.** Amongst the species studied in the present study, most species have clamp connections with the exception of the following five species: *C. agaricoides*, *C. africanum*, *C. demangei*, *C. nothorhacodes* and *C. subrhacodes*. Since clamp connections occur in five different sections, this character is not informative at section level.

Conclusions and future directions

This study constitutes the first multigene molecular phylogenetic analysis of the genus *Chlorophyllum*. Previous analyses included only a limited number of ITS/nrLSU sequences. This study significantly increased the molecular sampling for this group and included a wider array of taxa from a broader geographic range. Several previously unsampled species were included (i.e. *C. africanum*, *C. demangei*, *C. palaeotropicum*, *C. sphaerosporum* and *C. subrhacodes*). Based on these results, the genus *Chlorophyllum* is monophyletic and composed of six well-supported monophyletic groups that were classified as sections (Figure 2). Each section is also characterised by several morphological features. Although the relationships amongst all sections are not yet fully resolved, relationships amongst species within sections are. The majority of *Chlorophyllum* species occurs in disturbed or arid habitats in subtropical to tropical regions and many species have a wide distribution over more than one continent. The role of humans in some of these distribution patterns should be investigated.

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Supplementary material 1

Figure S1. Maximum Likelihood tree showing the monophyly of Chlorophyllum inferred from the rpb2 data set

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Data type: molecular data

Explanation note: Bootstrap values (>50) are indicated along nodes. The clade where *Chlorophyllum* species are nested is highlighted in grey.

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RESEARCH ARTICLE

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Cantharellus violaceovinosus, a new species from tropical *Quercus* forests in eastern Mexico

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Abstract

During explorations of tropical oak forests in central Veracruz (eastern Mexico), the authors discovered a *Cantharellus* species that produces basidiomes with strikingly violet pileus and a hymenium with yellow, raised gill-like folds. It is harvested locally and valued as a prized edible wild mushroom. Systematic multiyear sampling of basidiomes allowed the recording of the morphological variation exhibited by fresh fruit bodies in different growth stages, which supports the recognition of this *Cantharellus* species from others in the genus. Two molecular phylogenetic analyses based on a set of sequences of species of all major clades in *Cantharellus*, one including sequences of the transcription elongation factor 1-alpha (tef-1α) and a combined tef-1α and nLSU region (the large subunit of the ribosome), confirm the isolated position of the new species in a clade close to *C. lewisii* from USA, in the subgenus *Cantharellus*. Detailed macroscopic and microscopic descriptions, accompanied by illustrations and a taxonomic discussion are presented.

Keywords

Cantharellales, ectomycorrhizal fungi, Neotropical fungi, oak forest, wild edible mushrooms

Introduction

The diversity of species in *Cantharellus* Adans.: Fr., in combination with their ectomycorrhizal nature (mycobionts of several plant lineages), as well as their highly prized value as edible wild mushrooms, have attracted the attention of specialists from different fields worldwide (Smith and Morse 1947, Trappe 1962, Chandra 1989, Molina et al. 1992, Homola 1993, Thoen 1993, Watling 1997, Danell 1999, Pilz et al.

2002, Lee et al. 2003,Yun and Hall 2004, Boa 2005, Agerer 2006, Arora and Dunham 2008, Kumari et al. 2011, Wilson et al. 2012).

Cantharellus encompasses fungi with long-lived, gymnocarpic, fleshy, variedly coloured, trumpet-shaped basidiomes with nearly smooth, veined, gill-like folded to distinctly lamellate hymenophore, pileipellis poorly differentiated, cystidia lacking, smooth and thin-walled spores, with or without clamps (Wilson et al. 2012, Buyck et al. 2014). In many cases, basidiomes of members of closely related species or inclusive, unrelated look-alike species are difficult to identify in a strict sense, especially if there is not an accurate record of the variation of morpho-anatomical characters and colours in fresh condition. Few microscopic features in the genus had been considered discriminative, especially clamps (presence or absence), wall thickness of the terminal elements of the pileipellis hyphae and the basidiospore features (size and form).

The two former features are considered amongst the most taxonomically informative at subgeneric level and the latter used to distinguish species (Eyssartier and Buyck 2001, Buyck et al. 2014). Additionally, it has been hypothesized that there are cryptic species still undefined taxonomically, even amongst the best known *Cantharellus* species, especially from tropical regions but also from temperate regions (Smith and Morse 1947, Corner 1966, Heinemann 1958, 1966, Smith 1968, Petersen and Ryvarden 1971, Petersen 1976, Bigelow 1978, Petersen and Mueller 1992, Buyck and Hofstetter 2011, Buyck et al. 2012, Eyssartier et al. 2003, De Kesel et al. 2011, Tian et al. 2012, Wartchow et al. 2012, Buyck and Randrianjohany 2013, Foltz et al. 2013, Buyck et al. 2014, 2016a, c, Leacock et al. 2016).

Taxonomic research on *Cantharellus* has increased substantially in the last decade, especially by combining DNA and morphological information to support the definition of early recognised species and others recently discovered (Buyck and Hofstetter 2011, Buyck et al. 2011, Tibuhwa et al. 2012, Wilson et al. 2012, Buyck et al. 2012, 2013, 2014, 2015, 2016a, 2016b, Foltz et al. 2013, Shao et al. 2014, Shao et al. 2016, Leacock et al. 2016).

The earliest description of *Cantharellus* in Mexico dates from Fries (1855), who proposed *C. mexicanus* based on a specimen with "… *pileo carnoso turbinato-infundibuliformi glabro griseofusco*… *lamellis augustissimis longe decurrentibus*". It was collected by F.M. Liebmann at El Mirador, Veracruz and, years later, considered by Corner (1966) as "… *incert. sed.* (? *Gomphus*)…" no longer recorded in the literature. From the same region (Orizaba, relatively near to the current study site), *Craterellus confluens* Berk. & M.A. Curtis was described by Berkeley (1867). This species is characterised by its yellow basidiomes, it is closely related to *Cantharellus lateritius* (Berk.) Singer, with which it has been confused or even with other yellow chanterelles, such as *C. cibarius* Fr. and *Craterellus odoratus* (Schwein.) Fr. (Heim 1954, Corner 1966, Petersen 1979, Guzmán and Sampieri 1984, Buyck and Hofstetter 2011). After such descriptions of new *Cantharellus* species from Mexico, only some additional records of about ten species described from other latitudes have been mentioned to occur in different forest ecosystems in the country, including *Quercus* forests (Guzmán and Sampieri 1984, Guzmán 1985, Guevara et al. 2004, Perez-Moreno et al. 2008, Garibay-Orijel et al. 2009). The identity of these records, however, has not been confirmed with molecular evidence. Recently, *C. coccolobae* Buyck,

Moreau & Courtecuisse was described from the Caribbean (Guadeloupe), including two collections from Yucatán, Mexico (Buyck et al. 2016c).

During the authors' long term explorations in tropical oak forests in central Veracruz, a *Cantharellus* species was found with a striking habit, distinctive when compared to the previous records from Mexico. In fact, this fungus is unique because the fresh basidiomes in different growth stages possess a strikingly violet pileus and yellow, raised gill-like folded hymenophore, in combination with ellipsoid basidiopores and terminal elements of pileipellis slightly thick-walled. The macro- and micromorphological features depicted in this fungus, as well as its distinct position in two phylogenetic analyses, one of tef-1α and other of a combined tef-1α+nLSU sequences datasets, allowed its recognition as a new species. This *Cantharellus* species is locally considered a prized edible mushroom.

Materials and methods

Sampling and morphological study

Cantharellus basidiomes were collected during June-October, through six consecutive years (2012–2017) including some collections in 2009 and 2011, in tropical oak forests from Zentla (837–850 m a.s.l.) and Alto Lucero (400–500 m a.s.l.) counties in central Veracruz (eastern Mexico). In these oak forests, *Quercus oleoides* is dominant and even forms pure stands. In the Zentla locality, however, *Q. glaucescens* and *Q. sapotifolia* are also present and, at times, also form monodominant small patches. Descriptions of morpho-anatomical features were achieved based on fresh samples and following Largent (1973). The colour notations indicated in the descriptions follow Kornerup and Wanscher (1978) and Munsell colour chart (1994). Basidiomes were dried in a hot air dehydrator (45 °C). Microscopic features were observed and measured after tissues were rehydrated in 3 % potassium hydroxide (KOH) and stained with 1 % Congo red or analysed in Melzer´s solution. At least thirty-five basidiospores per collection were measured in length and width. Mean ranges denoted as $\bar{X}m$ and the length/width ratio ($\bar{Q}$) of basidiospores, in side view, are given as an interval of mean values per collection (n=15 collections). The form of the basidiospores was interpreted after calculating the Q values, following Bas (1969). Line drawings were made with the aid of a drawing tube. Collections are part of XAL Herbarium (Thiers B. [continuously updated] Index Herbariorum: a global directory of public herbaria and associate staff. New York Botanical Garden`s Virtual Herbarium. http://sweetgum.nybg.org/science/ih/).

DNA extraction, PCR and sequencing

DNA was isolated from fresh material using DNAeasy Plant Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer´s recommendations. The transcription elongation factor 1-alpha (tef-1α) was amplified using the primers tef1F and tef1R

(Morehouse et al. 2003) and the large subunit of ribosome (nLSU) using the primers LR0R and LR7 (Vilgalys and Hesler 1990). PCR conditions were performed with an initial denaturation at 94 °C for 3 min; 35 cycles of 1 min 94 °C, 1 min at 55 °C and 2 min at 72 °C; and final elongation at 72 °C for 7 min. Amplified PCR products were purified with the DNA Clean & Concentrator Kit (Zymo Research, USA) following the manufacturer's instructions. Cycle sequencing reactions were made using BigDye Terminator 3.1 Cycle Sequencing kit (Applied Biosystems, USA); reactions were purified with ZR DNA Sequencing Clean-up Kit (Zymo Research, USA) and run in a sequencer, ABIPrism 310 Genetic Analyzer (Applied Biosystems). Sequences obtained were assembled and edited in BioEdit (Hall 1999) and deposited at GenBank database (Benson et al. 2017) (Table 1).

Table 1. *Cantharellus* species included in this study: samples, location and accession number for tef-1α and nLSU sequences.

Taxon	Voucher Specimen	Location	GenBank	
			tef-1α	**nLSU**
C. addaiensis	BB 98.033	Tanzania	JX192992	KF294667
C. afrocibarius	BB 96.236	Zambia	JX192993	KF294668
C. albidolutescens	BB 08.057	Madagascar	KF294752	KF294645
C. albidolutescens	BB 08.080	Madagascar	JX192982	–
C. ambohitantelyensis	BB 08.336	Madagascar	JX192989	–
C. amethysteus	BB 07.284	Slovakia	GQ914953	KF294639
C. amethysteus	BB 07.309	Slovakia	GQ914954	KF294642
C. appalachiensis	BB 07.123	USA	GQ914979	KF294565
C. cascadensis	BB 13.251	USA	KX857044	–
C. chicagoensis	JJ/MO-CANT1	USA	KX857025	–
C. cibarius	BB 07.300	Slovakia	GQ914950	KF294641
C. cibarius	GE 07.025	France	GQ914949	–
C. cinnabarinus	BB 07.053	USA	GQ914984	KF294630
C. cinnabarinus	BB 07.001	USA	GQ914985	KF294624
C. congolensis	BB 98.039	Tanzania	JX193015	KF294609
C. congolensis	BB 98.058	Tanzania	JX192996	KF294673
C. corallinus	JJ/MO-CANT2	USA	KX857031	–
C. corallinus	JJ/MO-CANT5	USA	KX857034	–
C. deceptivus	JJ/NC-CANT5	USA	KX857029	–
C. decolorans	BB 08.278	Madagascar	GQ914968	–
C. decolorans	BB 08.243	Madagascar	JX192987	–
C. densifolius	BB 98.013	Tanzania	JX193014	KF294616
C. ferruginascens	BB 07.283	Slovakia	GQ914952	KF294638
C. fistulosus	DT 43	Tanzania	JX192997	KF294674
C. flavolateritius	VH 1076	USA	KX857027	–
C. flavolteritius	VH1078	USA	KX857029	–
C. gracilis	BB 98.234	Tanzania	JX192970	–
C. humidicolus	BB 98.036	Tanzania	JX193005	KF294666
C. ibityensis	BB 08.203	Madagascar	JX192985	KF294651
C. isabellinus var. parvisporus	BB 98.020	Tanzania	JX192972	KF294614

Taxon	Voucher Specimen	Location	GenBank	
			tef-1α	nLSU
C. iuventateviridis	SH13/7/2012	USA	KX857063	–
C. iuventateviridis	SH14/7/2012	USA	KX857064	–
C. lateritius	BB 07.025	USA	GQ914957	KF294628
C. lateritius	BB 07.058	USA	GQ914959	KF294633
C. lewisii	BB 02.197	USA	GQ914961	KF294623
C. lewisii	BB 07.003	USA	GQ914962	–
C. lilacinopruinatus	BB 07.221	Slovakia	GQ914951	KF294637
C. minor	BB 07.002	USA	JX192978	KF294625
C. minor	BB 07.057	USA	JX192979	KF294632
C. pallens	BB 09.441	Italy	KX857013	–
C. pallens	BB 12.082	Italy	KX857035	–
C. paucifurcatus	BB 08.320	Madagascar	KF294655	JK192988
C. persicinus	MH 15.001	USA	KX857080	–
C. phasmasis	CO57	USA	JX030417	–
C. phasmasis	CO74	USA	JX030418	–
C. platyphyllus	BB 98.012	Tanzania	GQ914969	KF294617
C. platyphyllus subsp. *bojeriensis*	BB 08.160	Madagascar	JX192984	KF294648
C. pseudominimus	JV 00.663	Portugal	JX192991	KF294657
C. quercophilus	BB 07.097	USA	JX192981	KF294644
C. sebosus	BB 08.234	Madagascar	JX192986	KF294652
C. spectaculus	C081	USA	JX030414	–
C. cf subamethysteus	AV 12.003	Thailand	KX857062	–
C. subcyanoxanthus	BB 00.1137	Madagascar	JX192990	–
C. subincarnatus subsp. *rubrosalmoneus*	BB 06.080	Madagascar	JX192962	KF294601
C. subincarnatus subsp. *rubrosalmoneus*	BB 06.096	Madagascar	JX192963	KF294602
C. symoensii	BB 98.011	Tanzania	GQ914970	KF294618
C. symoensii	BB 98.113	Tanzania	JX192974	KF294619
C. tabernensis	BB 07.119	USA	GQ914976	KF294634
C. tabernensis	BB 07.020	USA	GQ914971	–
C. tanzanicus	BB 98.040	Tanzania	JX192977	KF294622
C. tenuithrix	BB 14.008	USA	KX857045	–
C. tenuithrix	BB 14.009	USA	KX857045	–
C. tomentosus	BB 98.038	Tanzania	GQ914965	KF294610
C. vellutinus	VH 1583	USA	KX857070	–
C. vellutinus	WR WV 07.074	USA	KX857068	–
C. versicolor	Tian 160	China	KM893857	–
C. versicolor	Yu 24	China	KM893856	–
*C. violaceovinosus**	Bandala 4513	Mexico	MF616520	MF616524
*C. violaceovinosus**	Corona 648	Mexico	MF616521	MF616525
*C. violaceovinosus**	Herrera125	Mexico	MF616522	MF616526
Craterellus tubaeformis	BB 07.293	Slovakia	GQ914989	KF294640
Hydnum repandum	BB 07.341	Slovakia	JX192980	KF294643

*samples and sequences obtained here

Phylogenetic analysis

Six tef-1α and nLSU sequences obtained in this study, together with 113 sequences of *Cantharellus* species from all major clades across the genus (after Buyck et al. 2014) and with the highest similarity scores from the results of BLAST (Altschul et al. 1997) were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/) and used to construct two datasets. One dataset consisted of tef-1α and other combined tef-1α+nLSU sequences. *Craterellus tubaeformis* and *Hydnum repandum* were included as outgroup taxa (Table 1 and alignment in TreeBASE S21920). Both datasets were assembled in the data editor PhyDE v.0.995 programme (Müller et al. 2010). They were aligned using Muscle (Edgar 2004) with inconsistencies corrected manually. A phylogeny of each dataset was constructed under maximum likelihood (ML) and Bayesian Inference (BI) methods. The best evolutionary model for both datasets was calculated with Mega 6.06 (Tamura et al. 2013). ML analyses were also performed using Mega 6.06 with 500 replicates of bootstrap. BI analyses were implemented with MrBayes on XSEDE (3.2.6) on CIPRES portal (Miller et al. 2010) with settings as described in Montoya et al. (2014). The phylogenies from ML and BI analyses were displayed using Mega 6.06 and FigTree v 1.3.1 (Rambaut 2009), respectively.

Results

Sixty fresh collections were obtained of the violet *Cantharellus* species, including basidiomes in different growth stages, most of them detected between August-October, in both localities explored. Six new tef-1α and nLSU sequences from three collections were generated in this study (Table 1). In the inferred molecular phylogenies (from tef-1α and tef-1α+nLSU sequences datasets) (Figs 1–2), the generated sequences from the Mexican specimens, clustered in a terminal clade, strongly supported only bootstrap values ≥70 and posterior probabilities ≥0.90 were considered and indicated (BS/BPP) on the branches of each tree. Both trees were congruent and the sequences of the Mexican *Cantharellus* cluster in a sister clade to *C. lewisii* from USA, in the subgenus *Cantharellus* (Buyck et al. 2014). Based on the distinctive morphological features and colour variation of the studied *Cantharellus* specimens, as well as the isolated position of the samples in the phylogenies obtained, it was concluded that this should be proposed as a new *Cantharellus* species, which inhabits the tropical *Quercus* forests in eastern Mexico.

Description of the new species

Cantharellus violaceovinosus **M. Herrera, Bandala & Montoya, sp. nov.**
MycoBank: MB823600
Figs 3–5

Holotype. MEXICO. Veracruz: Municipality of Zentla, around town of Zentla, 850 m a.s.l., gregarious in soil, under *Quercus oleoides* Schltdl. & Cham., 5 July 2012, Corona 648 (XAL).

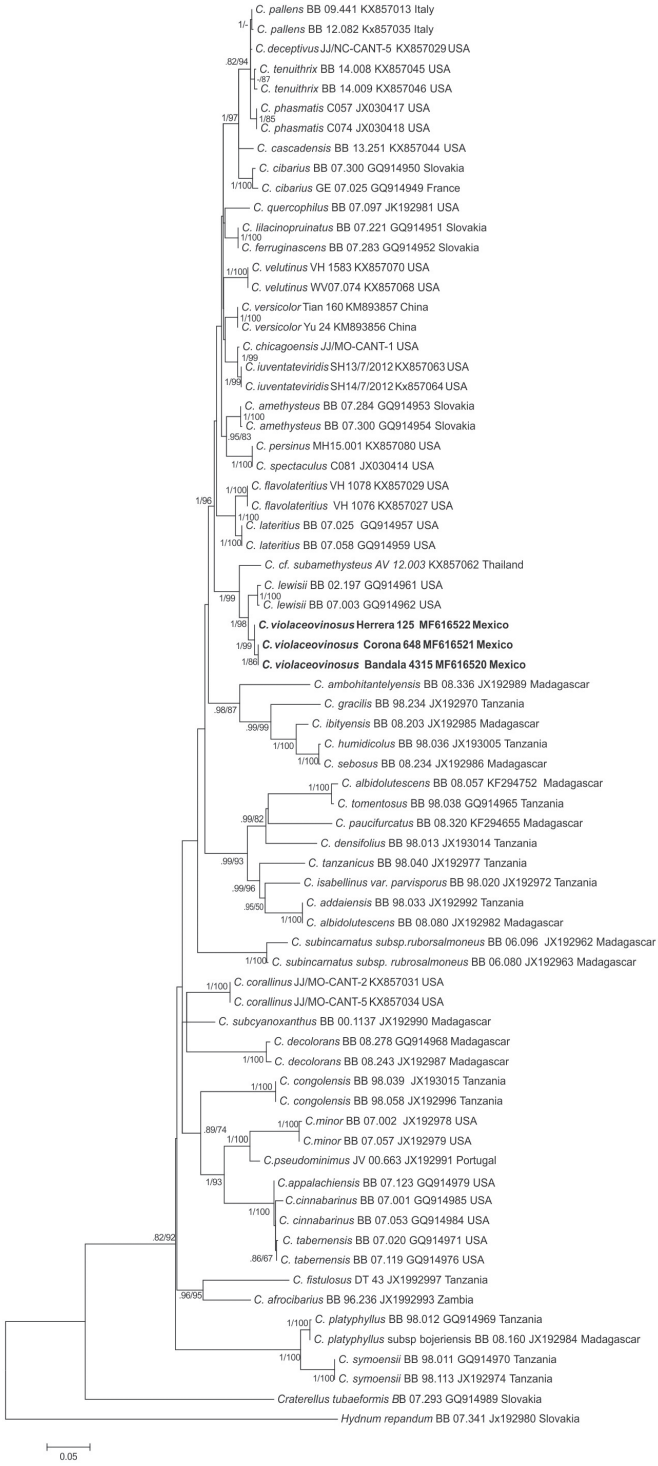

Figure 1. Molecular phylogenetic analysis by maximum likelihood of tef-1α sequences dataset of *Cantharellus* species. Posterior probabilities and Bootstrap values (BPP/BS) are indicated on the tree branches.

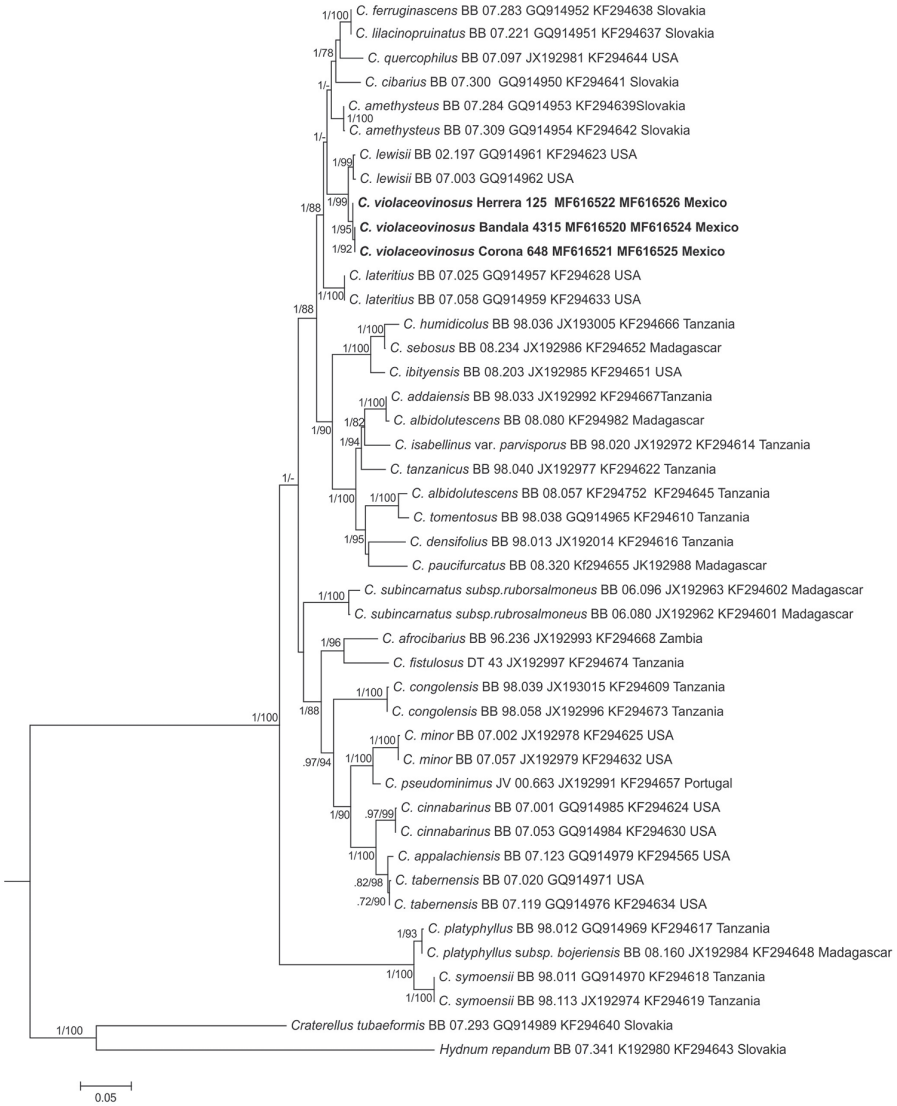

Figure 2. Molecular phylogenetic analysis by maximum likelihood of tef-1α+nLSU sequences dataset of *Cantharellus* species. Posterior probabilities and Bootstrap values (BPP/BS) are indicated on the tree branches.

Diagnosis. Differing from other *Cantharellus* species by: uniformly dark violet, violet-grey to violet-wine or violet-reddish pileus; yellow, gill-like folded hymenophore and ellipsoid basidiospores 7–10 (–11) × (4.5–) 5–6.5 (–7) µm. $\bar{X}m$ = 7.8–9× 5.1–6.3 µm, $\bar{Q}$= 1.31–1.66, basidia (40–) 45–114 (–125) × (6–) 7–11 (–12) µm, with (1–) 2–5 sterigmata, and terminal elements of the pileipellis 4–6 µm diam, slightly thick-walled.

Gene sequences ex-holotype. MF616521 (tef-1α), MF616525 (nLSU).

Etymology. Referring to the dark violaceous, becoming wine to reddish pileus.

Figure 3. Basidiomes of *Cantharellus violaceovinosus:* **a** Corona 648 (holotype) **b** Bandala 4550 **c** Del Moral 427 **d** Bandala 4490. Scale bars: 20 mm.

Pileus (15–) 25–113 mm diam, convex to broadly-convex with margin incurved when young, expanding to plane or subplane, often shallowly depressed or finally broadly infundibuliform, surface dry, not hygrophanous, dull, smooth, glabrescent, surface at times breaking in faintly tesselate-rimose-like pattern, then appearing appressed fibrillose with age and not forming scales; surface uniformly dark violet (15D4, 15F2–7, 16D3–4, 16D6, 16F4–5) to pale violet with age (15DE5–7) or violet-grey (16D3–4, 16D6), lilac or greyish-lilac (15A3, 15C3–4, 16C2–3), becoming violet-wine or violet-reddish (14E5–8, 14EF4–5), wine (12D4), fading with age and sun exposure, developing pinkish, lilac and reddish tints, especially towards the margin (13A3–4, 13D3–4, 15A4–5), naked parts showing the yellow context (4A2–3); margin incurved or straight, entire or slightly crenate, undulate or irregular, often incised, rarely lobed, not striated. *Hymenophore* with well-defined gill-like folds, up to 3 mm deep, decurrent, subdistant, in some specimens almost straight and inclusive thin, in other materials with faintly sinuous or irregular thicker folds, frequently forking at different levels or only towards the pileus margin, with lower irregular anastomosis amongst the folds, in some specimens the anastomosis occur practically in the whole hymenophore, while in others only at some areas, especially at pileus margin, some specimens (specially towards the stipe) with irregular low veins amongst the folds or the folds become as low and sinuous vein-like; butter-yellow or yellow (2.5Y 8/4,10YR

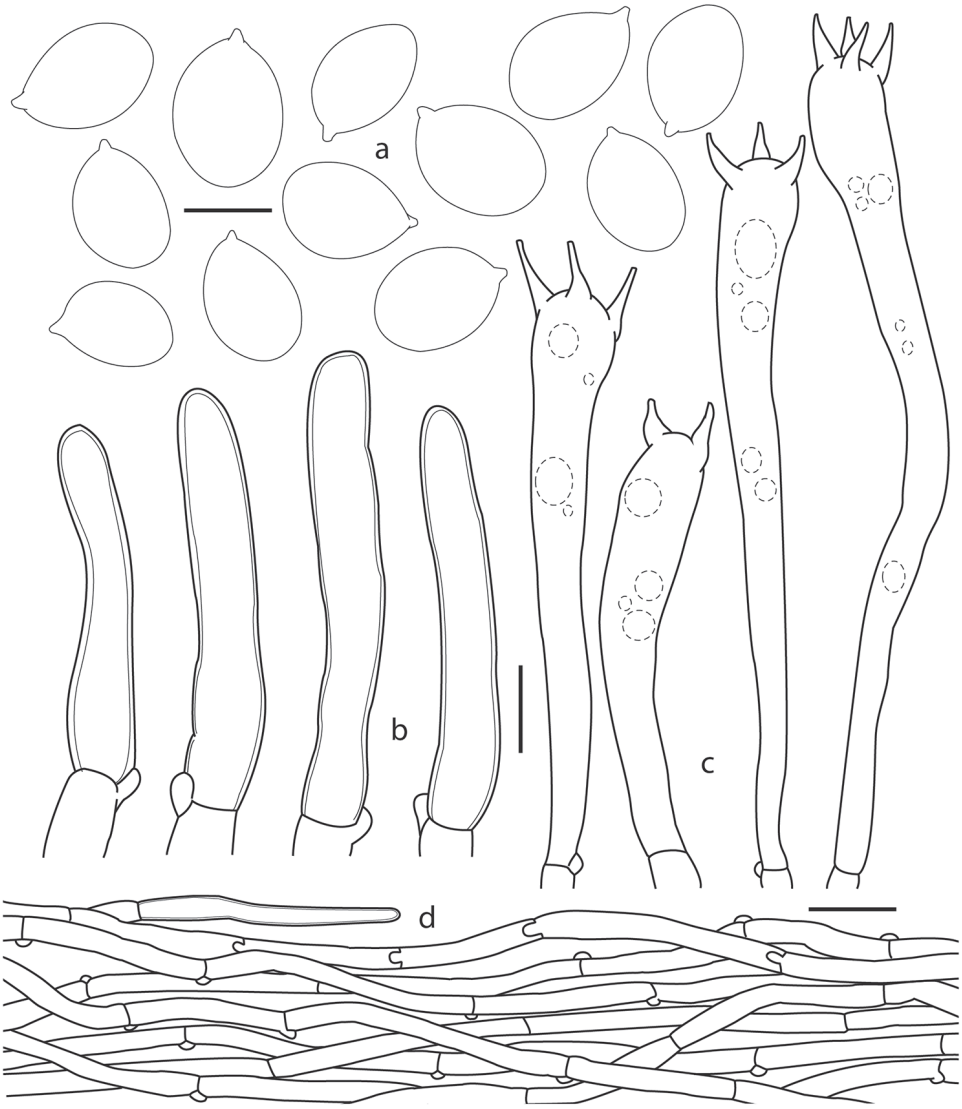

Figure 4. *Cantharellus violaceovinosus* (Corona 648, holotype): **a** basidiospores **b** terminal elements of the pileipellis **c** basidia **d** pileipellis. Scale bars: 5 μm (**a**); 10 μm (**b, c**); 25 μm (**d**).

8/6; 4A3–4). *Stipe* (20–) 25–75 × 5–18 mm, equal and only slightly swollen at base or widening above and tapering gradually downwards, solid, surface glabrous, concolorous with hymenophore, often staining ochraceous or rusty orange colour when handled, occasionally with whitish, small rhizomorphs at base. *Context* whitish to yellow (4A2–3), at times wax-like, odour mild, agreeable, at time fruity somewhat to apricot; taste mild, agreeable.

Basidiospores 7–10 (–11) × (4.5–) 5– 6.5 (–7) μm, [$\overline{X}m$ = 7.8–9 × 5.1–6.3 μm, $\overline{Q}$= 1.31–1.66, (n=13)], ellipsoid, smooth, thin-walled, hyaline, inamyloid, devoid of gran-

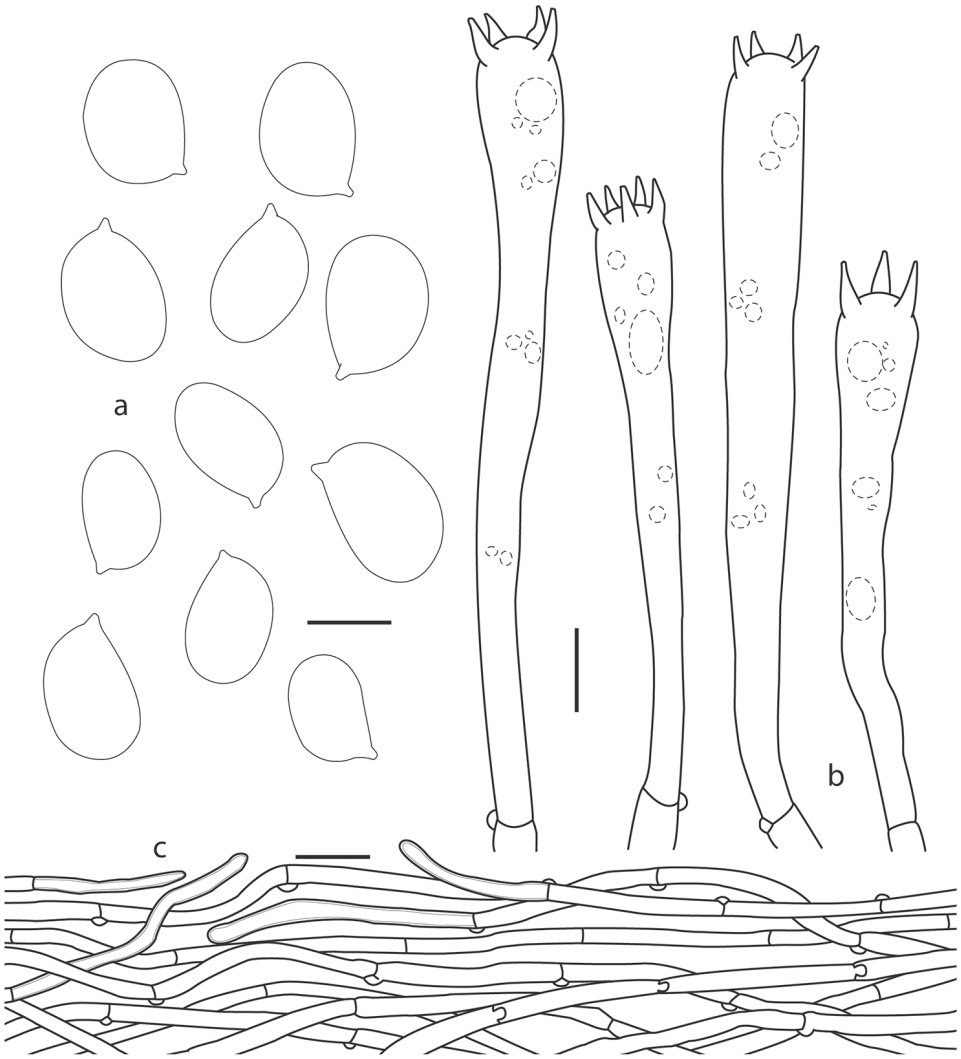

Figure 5. *Cantharellus violaceovinosus:* **a** basidiospores **b** basidia (Gutiérrez 23) **c** pileipellis (Herrera 61). Scale bars: 5 µm (**a**); 10 µm (**b**); 25 µm (**c**).

ular contents or refractive droplets. *Basidia* (40–) 45–114 (–125) × (6–) 7–11(–12) µm, narrowly clavate to subcylindrical, with (1–) 2–5 sterigmata 8–10 µm long, thin-walled, hyaline; subhymenium composed of cylindrical hyphae 4–5 µm diam. *Cystidia* absent. *Pileipellis* a cutis composed of hyphae 4–6 µm diam, intermingled in a compact arrangement, cylindrical, hyaline to yellowish, inamyloid, often some of them with pale brownish contents, these decidedly brown coloured in group; distinctive terminal elements 4–6 µm broad, slightly thick-walled (<1 µm thick), smooth, hyaline, some pale brownish, scattered on the surface. *Pileus trama* composed of cylindrical to inflated hyphae, 3–12 µm diam, slightly thick-walled (<1 µm thick), hyaline, yellowish in mass, some

of the hyphal segments completely filled with darker contents. *Hymenophoral trama* composed of hyphae 3–5 µm diam, thin-walled, some with weakly refringent contents. *Clamp connections* present on hyphae in all tissues.

Habitat. Solitary to gregarious, in soil, at tropical oak forest, under *Quercus oleoides*, less frequently also under both *Q. glaucescens* Bonpl. and *Q. sapotifolia* Liebm. June-October, known in the coastal plain of central Veracruz State, east coast of Mexico.

Specimen examined. MEXICO. Veracruz, Zentla Co., Road Puentecilla-La Piña, 837 m a.s.l., 2 Jul 2009, Del Moral 427, Ramos 216; 27 Oct 2009, García 20, García 22; 16 Jun 2011, Bandala 4490; 21 Jul 2012, Herrera 25; 31 Jul 2012, Bandala 4513; 20 Sep 2012, Bandala 4550, Corona 743; 4 Oct 2012, Bandala 4569, 4573; 4 Jul 2013, Gutiérrez 23; 12 Jul 2013, Bandala 4671; 20 Sep 2013, Herrera 67; 15 Sep 2015, Herrera 135. Around town of Zentla, 850 m a.s.l., 5 Jul 2012, Corona 648; 25 Jun 2013, Herrera 60, 61; 15 Sep 2015, Herrera 137, Santillan 16; 1 Oct 2015, Herrera 151; 30 Jun 2016, Herrera 172; 6 Jul 2016, Herrera 184; 12 Jul 2016, Herrera 187; 22 Sep 2016, Herrera 200, 201, 202, 203; 5 Oct 2016, De la Cruz 14,15; 13 Oct 2016, De la Cruz 42; 27 Oct 2016, Herrera 210, 211; 6 Jul 2017, Garay 350; 3 Aug 2017, Garay 364; 31 Aug 17, Garrido 79; 7 Sep 2017, Herrera 214, 215, 216; 15 Sep 17, Montoya 5403; 21 Sep 17, Corona 1420; 5 Oct 17, Mateo 5. Alto Lucero Co., NE Mesa de Venticuatro, 450–500 m a.s.l., 2 Jul 15, Herrera 125, Herrera 126; 17 Sep 2015, Herrera 138; 2 Aug 2016, Herrera 191; 10 Aug 2016, Herrera 192; 20 Sep 2016, Herrera 195, 196, 197, 198; 27 Sep 2016, Herrera 205, 206, 207; 4 Oct 2016, Herrera 208, 209; 22 Aug 17, Herrera 214; 12 Sep 2017, Garay 375; 19 Sep 2017, Garay 392; 2 Oct 17, Mateo 1 (all at XAL).

Discussion

Distinctive features of this species include the medium to large size basidiomes, with pileus practically homogeneously violet pigmented (only fading with age), smooth, with surface free of scales, at times with disrupted pileus surfaces due to age, hymenophore bearing yellow gill-like folds, ellipsoid, medium-sized basidiospores [7–10 (–11) × (4.5–) 5– 6.5 (–7) µm], medium to large basidia [(40–) 45–114 (–125) × (6–) 7–11(–12) µm] and terminal elements of pileipellis 4–6 µm diam, slightly thick-walled (<1 µm thick). Molecular phylogenetic analyses support that the species is genetically distinct from other *Cantharellus* taxa, in both analyses, *C. violaceovinosus* was nested in an isolated and well-supported clade (95–99/1) (Figs 1–2).

Cantharellus species with basidiomata having violet pileus are rare but occur in various regions worldwide (Eyssartier et al. 2009; Buyck et al. 2012). Amongst about 45 species of the genus known from USA, Mexico, Central and South America (Guzmán and Sampieri 1984, Guzmán 1985, Eyssartier et al. 2003, Guevara et al. 2004, Henkel et al. 2006, Wartchow et al. 2012, Wilson et al. 2012, Pinheiro and Wartchow 2013, Wartchow et al. 2013, Nascimento et al. 2014, Buyck et al. 2016c), *C. lewissi* Buyck & V. Hofst., *C. atrolilacinus* Eyssart., Buyck & Halling and the new

C. violaceovinosus are, up to now, the species known to produce basidiomes with violet tints in the Americas.

Cantharellus lewisii grows in floodplain hardwoods, in Water Oak plots next to a *Taxodium* swamp, in beech-magnolia-loblolly pine forests and also under beech-white oak-loblolly pine-magnolia forests in the south of USA (Buyck and Hofstetter 2011). In the inferred phylogeny, it appears as sister of *C. violaceovinosus*, but differs because its pileus is pale yellow, dull to greyish-yellow or ochre to pale brownish-orange, sometimes reddish-brown near the margin, with a surface covered with dark purplish-lilac appressed fibrils (in young stages, *C. lewisii* is often entirely dark lilac-purple) and with terminal elements of pileipellis conspicuously thick-walled (mostly 1–1.5 µm thick) (Buyck and Hofstetter 2011). According to the original description, *C. lewisii* also differs by its ellipsoid or often somewhat reniform and narrower basidiospores [(7.08–) 7.16–7.62–8.07 (–8.96) × (4.17–) 4.24–4.58–4.93 (–5.21) µm; Q= (1.42–) 1.45–1.57–1.70 (–1.80)] and by 5–6-spored and shorter basidia (60–75 × 7–8 µm) (Buyck and Hofstetter 2011). Two Texan collections of *C. lewisii* (holotype BB 07.003 and BB 02.197, both at PC) were studied. Based on observations, it was confirmed that this later species differs from the Mexican *C. violaceovinosus*, because of its markedly reniform, narrower basidiospores, then tending to be "more ellipsoid" [BB 07.003, holotype: 7.5–9.5 × (4–) 4.5–5.5 µm, $\bar{X}m$ = 8.4 × 5 µm, $\bar{Q}$= 1.68; BB 02.197: 7.5–10 (–11) × 4–5.5 µm, $\bar{X}m$ = 9 × 4.8 µm, $\bar{Q}$= 1.87).

Cantharellus atrolilacinus was described from Costa Rica, growing under *Quercus corrugata* Hook.) and *Q.* sp. (Eyssartier et al. 2003). According to the data on this species (R. Halling, www.nybg.org/bsci/res/hall/canlilac.html; Eyssartier et al. 2003), it differs from *C. violaceovinosus* because its pileus colours tend to be darker, even blackish, dark lilac-grey or brown-lilac, with tomentose surface at the disc, with strong radial, adnate fibrils at the margin, and the stipe whitish with lilac tints. Microscopically, *C. atrolilacinus* has basidiospores (7–) 7.5–8–8.5 (–9) × 4.5–5–5.5 (–6) µm, tending to be more ellipsoid (Eyssartier et al. 2003, fig. 1:2) and having wider pileipellis hyphae [(4–) 5–10 (–15) µm] with a very thick wall ("..très nettement épaissies..").

Although *Cantharellus amethysteus* (Quél.) Sacc. (subg. *Cantharellus*) from Europe, may appear superficially similar to some forms of *C. violaceovinosus*, the former however, especially has a pileus surface covered with vinous or lilac, small scales. The authors studied two specimens of *C. amethysteus* from France (BB 07.284 and BB 07.309 at PC) displaying elongate basidiospores, 9.5–12 (–12.5) × 5–7 µm ($\bar{X}m$ = 11–11.2 × 5.9–6.4 µm; $\bar{Q}$= 1.76–1.86), as Eyssartier and Buyck (2000) reported [(9–) 9.5–10.37–11.5 (–12.5) × 6–6.5–7 µm], resulting in being larger and more elongate than in the Mexican species. Also, it is interesting that one sequence of tef-1α of a specimen from Thailand (GB coded KX857062, Table 1) identified as "*C.* cf. *subamethysteus*", appeared close to *C. violaceovinosus* (Fig. 1). *Cantharellus subamethysteus* indeed is phylogenetically related to *C. lewisii* (Buyck et al. 2014) and differs from *C. violaceovinosus* in the shorter basidiomes (pileus 20–65 mm; stipe 42–57 × 5–11 mm), deep and bright yellow pileus surface, covered with squamules even with rather brown to dark brown tinges, shorter basidiospores [7–8 (8.75) × (4.75) 5–6 µm] and

wider pileipellis elements (8–15 µm width) (Eyssartier et al. 2009). Additionally, the hymenophore of this species is rugose to faintly veined (as depicted in the picture accompanying the description).

Cantharellus goossensiae (Beeli) Heinem., *C. cyanoxanthus* R. Heim ex Heinem., *C. subcyanoxanthus* Buyck, Randrianjohany & Eyssart. and *C. longisporus* Heinem. represent African species with basidiomes displaying violaceous tinges (Buyck et al. 2012) therefore, at some stages their basidiomes could resemble those of *C. violaceovinosus.* However, the three former species have the pileipellis with thin-walled hyphal extremities, thus differing from members of subgenus *Cantharellus*, including the new species here described. Moreover, the four African taxa have basidiospores distinctly narrowly ellipsoid to elongate (Q>1.70) and often slightly reniform, curved or even somewhat peanut-shaped (Beeli 1928, Heinemann 1958, 1959, Buyck et al. 2012).

Cantarellus violaceovinosus was recorded as a common fleshy mushroom, during the multiyear sampling developed in the tropical *Quercus* forests studied. It was found in ectomycorrhizal association with native trees of *Quercus* species. This mushroom was very often recorded in pure stands of *Q. oleoides* and less frequently in *Q. glaucescens* and *Q. sapotifolia* patches. This violet pigmented chanterelle shares the same habit preferences as *C. lateritius*, also found in the study sites. A similar co-ocurrence has been reported between *C. lewissi* (the sister relative of *C. violaceovinosus*) and *C. lateritius* in the State of Texas in the USA (Buyck et al. 2011). Basidiomes of *C. violaceovinosus* and *C. lateritius* are abundant in the local oak forests studied and both are considered choice wild mushrooms although the latter is more highly prized. They are even more appreciated than species of *Amanita* or *Lactarius*, representing an income source for wild mushroom collectors. Benefits from mushrooms harvesting, as well as other ecosystemic services, are motivating some owners to conserve relicts of the tropical *Quercus* forest of the region.

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