RESEARCH ARTICLE



Two new species of Hymenochaetaceae on Dracaena cambodiana from tropical China

Ping Du¹, Tian-Xu Cao¹, Ying-Da Wu², Meng Zhou³, Zhan-Bo Liu³

College of Life Science and Technology, Yangtze Normal University, Chongqing 408100, China 2 China Fire and Rescue Institute, Beijing 102202, China 3 School of Ecology and Nature Conservation, Beijing Forestry University, Beijing 100083, China

Corresponding author: Zhan-Bo Liu (zhanboliu@bjfu.edu.cn)

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Abstract

Two new wood-rotting fungi in the family Hymenochaetaceae, *Fulvifomes dracaenicola* **sp. nov.** and *Hymenochaete dracaenicola* **sp. nov.**, are described and illustrated from tropical China based on morphological characteristics and molecular data. It is worth to mention that both of them grow on *Dracaena cambodiana* which is a kind of angiosperm tree distributed in tropical regions. *F. dracaenicola* is characterised by perennial, pileate, triquetrous basidioma with yellowish brown fresh pores which becoming honey yellow with silk sheening upon drying, a dimitic hyphal system in trama and monomitic in context, and subglobose basidiospores measuring $4.8-5 \times 4-4.1 \mu m$. *H. dracaenicola* is characterised by annual, resupinate basidioma with a clay buff hymenophore, a dimitic hyphal system, absence of tomentum and cortex, presence of subulate setae, absence of cystidia, presence of cystidioles and simple hyphidia, and oblong ellipsoid basidiospores measuring $5.2-5.8 \times 2.5-2.8 \mu m$. The phylogenetic analyses based on ITS + nLSU rDNA sequences confirm the placement of two new species respectively in *Fulvifomes* and *Hymenochaete*. Phylogenetically closely related species to the two new species are discussed.

Keywords

Phylogenetic analysis, taxonomy, wood-rotting fungi

Introduction

Fulvifomes Murrill (Hymenochaetaceae, Hymenochaetales) was erected in 1914 and typified by *F. robiniae* (Murrill) Murrill (Murrill 1914). Wagner and Fischer (2002) thought that *Fulvifomes* comprises species with a dimitic hyphal system, absence of

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setae, and yellowish, thick-walled basidiospores. Hattori et al. (2014) provided a key to worldwide species of *Fulvifomes* and other species possibly belonging to *Fulvifomes*. Zhou (2014) treated *Aurificaria* D.A. Reid as a taxonomic synonym of *Fulvifomes* and transferred *Aurificaria indica* (Massee) D.A. Reid to *Fulvifomes*. However, *Fulvifomes indicus* (Massee) L.W. Zhou has a monomitic hyphal system, but he thought that the hyphal system might be not a stable character at the generic level within Hymenochaetaceae. Salvador-Montoya et al. (2018) redefined *Fulvifomes* and thought *Fulvifomes* should encompass species with a monomitic hyphal system in the context, a dimitic hyphal system in the trama. We agree with Zhou and Salvador-Montoya et al., and consider the genus *Fulvifomes* has a monomitic or dimitic hyphal system.

Hymenochaete Lév. (Hymenochaetaceae, Hymenochaetales) was erected in 1846 and typified by *H. rubiginosa* (Dicks.) Lév. (Léveillé 1846). Léger (1998) wrote a world monograph of *Hymenochaete* and provided a key of the genus. The genus comprises more than 120 species around the world (He and Dai 2012). *Hymenochaete* is characterised by annual to perennial, resupinate, effused-reflexed to pileate basidioma with smooth, tuberculate, lamellate, poroid or hydnoid hymenophores; a monomitic or dimitic hyphal system; presence of setae, and hyaline, thin-walled, narrowly cylindrical to globose basidiospores (Léger 1998; Parmasto 2001; He and Dai 2012).

During investigations on the diversity of wood-rotting fungi from China, five unknown specimens were collected from Hainan Province, and their morphology corresponds to the concepts of *Fulvifomes* and *Hymenochaete*. To confirm their affinity, phylogenetic analyses based on the ITS and nLSU rDNA sequences were carried out. Both morphological characteristics and molecular evidence demonstrated these five specimens represent two new species of Hymenochaetaceae, which we describe in the present paper.

Materials and methods

Morphological studies

Macro-morphological descriptions were based on field notes and dry herbarium specimens. Microscopic measurements and drawings were made from slide preparations of dried tissues stained with Cotton Blue and Melzer's reagent following Dai (2010). Pores were measured by subjectively choosing as straight a line of pores as possible and measuring how many fit per mm. The following abbreviations are used: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = neither amyloid nor dextrinoid, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between specimens studied, and n (a/b) = number of spores (a) measured from given number of specimens (b). In presenting spore size variation, 5% of measurements were excluded from each end of the range and this value is given in parentheses. Special color terms follow Anonymous (1969) and Petersen (1996). Herbarium abbreviations follow Thiers (2018). The studied specimens were deposited at the herbarium of the Institute of Microbiology, Beijing Forestry University (**BJFC**).

Molecular studies and phylogenetic analysis

A CTAB rapid plant genome extraction kit (Aidlab Biotechnologies Co., Ltd., Beijing, China) was used to extract total genomic DNA from dried specimens following the manufacturer's instructions with some modifications (Cui et al. 2019; Shen et al. 2019). ITS regions were amplified with primers ITS4 and ITS5 (White et al. 1990), and the nLSU with primers LR0R and LR7. The polymerase chain reaction (PCR) procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 58 °C for 45 s, and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94 °C for 1 min, and 72 °C for 1.5 min, and a final extension of 72 °C for 10 min (Chen et al. 2015). The PCR products were purified and sequenced in the Beijing Genomics Institute, China, with the same primers used in the PCR reactions.

Phylogenetic trees were constructed using ITS and nLSU rDNA sequences, and phylogenetic analyses were computed with maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI) methods. Sequences of Fulvifomes were adopted mainly from ITS + nLSU tree topologies established by Liu et al. (2020). Sequences of Hymenochaete were adopted mainly from ITS + nLSU tree topologies established by He et al. (2017) and Rossi et al. (2020). New sequences generated in this study, along with reference sequences retrieved from GenBank (Table 1 and Table 2), were aligned by MAFFT 6 (Katoh and Toh 2008; http://mafft.cbrc.jp/alignment/server/) using the "G-INS-i" strategy and manually adjusted in BioEdit (Hall 1999). The data matrix was edited in Mesquite v3.04 software (Maddison and Maddison 2010). The sequence alignment was deposited at TreeBase (Fulvifomes, http://purl.org/phylo/treebase/phylows/study/TB2:S27995; submission ID 27995) and (Hymenochaete, http://purl.org/phylo/treebase/phylows/study/TB2:S27696; submission ID 27696). Sequences of Phellinus laevigatus (P. Karst.) Bourdot & Galzin and P. populicola Niemelä obtained from GenBank were used as outgroups of *Fulvifomes* to root trees following Ji et al. (2017) in the ITS + nLSU analysis. Sequences of *Hydnoporia tabacina* (Sowerby) Spirin, Miettinen & K.H. Larss. obtained from GenBank were used as outgroups of Hymenochaete to root trees following He et al. (2017) in the ITS + nLSU analysis.

Maximum parsimony analysis was applied to the ITS + nLSU dataset sequences. Approaches to phylogenetic analysis followed Song et al. (2016), and the tree construction procedure was computed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with tree bisection and reconnection (TBR) branch swapping and 1000 random sequence additions maxtrees were set to 5000, branches of zero length were collapsed, and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), Consistency Index (CI), Retention Index (RI), Rescaled Consistency index (RC), and Homoplasy Index (HI) were calculated for each maximum parsimonious tree (MPT) generated. Sequences were also analyzed using maximum likelihood (ML) with RAxML-HPC through the CIPRES Science Gateway (Miller et al. 2009; http://www.phylo.org). Branch support for ML analysis was determined by 1000 bootstrap replicates.

Taxa	Voucher	ITS	LSU
Fomitiporella caryophylli	CBS 448.76	AY558611	AY059021
Fulvifomes centroamericanus	JV 0611/III	KX960763	KX960764
F. centroamericanus	JV 0611/8P	KX960757	_
F. dracaenicola	Dai 22093	MW559799	MW559804
F. dracaenicola	Dai 22097	MW559800	MW559805
F. fastuosus	LWZ 20140731-13	KR905674	KR905668
F. fastuosus	LWZ 20140718-29	KR905673	_
F. fastuosus	Dai 18292	MH390411	MH390381
F. grenadensis	JV 1212/2J	KX960756	_
F. grenadensis	JV 1607/66	KX960758	_
F. hainanensis	Dai 11573	KC879263	JX866779
F. halophilus	XG 4	JX104705	JX104752
F. halophilus	JV 1502/4	MH390427	MH390392
F. imbricatus	LWZ 20140728-16	KR905677	KR905670
F. imbricatus	LWZ 20140729-25	KR905678	_
F. imbricatus	LWZ 20140729-26	KR905679	KR905671
F. indicus	Yuan 5932	KC879261	JX866777
F. indicus	O 25034	KC879262	KC879259
F. krugiodendri	JV 0904/1	KX960762	KX960765
F. krugiodendri	JV 0312/24.10J	KX960760	KX960766
F. krugiodendri	JV 1008/21	KX960761	KX960767
F. merrillii		JX484013	—
F. nilgheriensis	URM 3028	MH390431	MH390384
F. nilgheriensis	PPT152	MH048095	MH048085
F. rimosus	M 2392655	MH628255	MH628017
F. robiniae	CBS 211.36	AY558646	AF411825
F. robiniae	—	EF088656	—
F. siamensis	XG 2	JX104709	JX104756
F. siamensis	Dai 18309	MH390434	MH390389
E sp.	PM 950703-1	EU035311	—
F. squamosus	C\$385	MF479268	MF479265
F. squamosus	CS444	MF479269	MF479264
F. submerrillii	Dai 17911	MH390405	MH390371
F. submerrillii	Dai 17917	MH390406	MH390372
F. thailandicus	LWZ 20140731-1	KR905672	KR905665
F. xylocarpicola	MU 8	JX104676	JX104723
Inocutis rheades	—	AF237731	—
Inonotus hispidus	CBS 388.61	AY558602	—
I. lloydii	Dai 10809	MH390428	MH390378
I. lloydii	Dai 9642	MH390429	MH390379
I. lloydii	Dai 11978	MH390430	MH390380
I. porrectus	CBS 296.56	AY558603	AY059051
I. rigidus	Dai 17496	MH390432	MH390398
I. rigidus	Dai 17507	MH390433	MH390399
Phellinotus neoaridus	URM 80362	KM211294	KM211286
P. piptadeniae	URM 80766	KM211293	KM211285
Phellinus laevigatus	CBS 122.40	MH856059	MH867554
P. populicola	CBS 638.75	MH860960	MH872729
Phylloporia crataegi	Dai 18133	MH151191	MH165865
P. ribis	CBS 579.50	MH856765	MN240818

Table 1. A list of species, specimens and GenBank accession numbers of sequences used in the phylogenetic analysis of *Fulviformes*.

New species is shown in bold.

Taxa	Voucher	ITS	LSU
Hydnoporia tabacina	He 390	JQ279610	JQ279625
Hymenochaete acerosa	He 338	JQ279543	JQ279657
H. adusta	He 207	JQ279523	KU975497
H. angustispora	Dai 17045	MF370592	MF370598
H. angustispora	Dai 17049	MF370593	MF370599
H. anomala	He 592	JQ279566	JQ279650
H. asetosa	Dai 10756	JQ279559	JQ279642
H. attenuata	He 28	JQ279526	JQ279633
H. bambusicola	He 4116	KY425674	KY425681
H. berteroi	He 1488	KU975459	KU975498
H. biformisetosa	He 1445	KF908247	KU975499
H. boddingii	MEH 66068	MN030343	MN030345
H. boddingii	MEH 69996	MN030341	MN030347
H. boddingii	MEH 66150	MN030344	MN030344
H. borbonica	CBS 731.86	MH862026	MH873716
H. cana	He 1305	KF438169	KF438172
H. cinnamomea	He 755	IQ279548	IQ279658
H. colliculosa	Dai 16427	MF370595	MF370602
H. colliculosa	Dai 16428	MF370596	MF370603
H. colliculosa	Dai 16429	MF370597	MF370604
H. conchata	MEH 70144	MF373838	
H. contiformis	He 1166	KU975461	KU975501
H. cruenta	He 766	IQ279595	IQ279681
H. cyclolamellata	Cui 7393	IQ279513	IQ279629
H. damicornis	URM 84261	KC348466	_
H. damicornis	URM 84263	KC348467	_
H. denticulata	He 1271	KF438171	KF438174
H. dracaenicola	Dai 22090	MW559797	MW559802
H. dracaenicola	Dai 22096	MW559798	MW559803
H. duportii	AFTOL ID666	DO404386	AY635770
H. epichlora	He 525	IO279549	IO279659
H. floridea	He 536	IO279597	IO279683
H. fuliginosa	He 1188	KU975465	KU975506
H. fulva	He 640	IO279565	IO279648
H. globispora	He 911	_	KU975508
H. huangshanensis	He 432	IQ279533	IQ279671
H. hvdnoides	He 245	IO279590	IO279680
H. innexa	He 555	IO279584	IO279674
H. legeri	He 960	KU975469	KU975511
H. longispora	He 217	IQ279537	KU975514
H. luteobadia	He 8	IQ279569	KU975515
H. macrochloae	ARAN-Fungi 7079	MF990738	MF990743
H. megaspora	He 302	IQ279553	IQ279660
H. minor	He 933	IQ279555	IO279654
H. minuscula	He 253	IO279546	KU975516
H. murina	He 569	IO716406	IO716412
H. muroiana	He 405	IO279542	KU975517
H. nanospora	He 475	IO279531	IO279672
H. ochromarginata	He 47	IO279579	10279666
H. odontoides	Dai 11635	IO279563	IO279647
H. orientalis	He 4601	KY425677	KY425685
H. parmastoi	He 867	IQ780063	KU975518
H. paucisetigera	Cui 7845	IQ279560	IQ279644
H. quercicola	He 373	KU975474	KU975521
H. rhabarbarina	He 280	IO279574	KY425688
H. rheicolor	Cui 8317	IO279529	
H. rhododendricola	He 389	JQ279577	JQ279653

Table 2. A list of species, specimens and GenBank accession numbers of sequences used in the phylogenetic analysis of *Hymenochaete*.

Taxa	Voucher	ITS	LSU
H. rubiginosa	He 1049	JQ716407	JQ279667
H. rufomarginata	He 1489	KU975477	KU975524
H. separabilis	He 460	JQ279572	JQ279655
H. setipora	Cui 6301	JQ279515	JQ279639
H. sharmae	CAL 1535	KY929017	KY929018
H. sharmae	66088	MK588753	MK588836
H. spathulata	He 685	JQ279591	KU975529
H. sphaericola	He 303	JQ279599	JQ279684
H. sphaerospora	He 715	JQ279594	KU975531
H. subferruginea	Cui 8122	JQ279521	_
H. subferruginea	He 1598	KU975481	—
H. tasmanica	He 449	JQ279582	JQ279663
H. tongbiguanensis	He 1552	KF908248	KU975532
H. tenuis	He 779	JQ279538	JQ279641
H. tropica	He 574	JQ279587	JQ279675
H. ulmicola	He 864	JQ780065	KU975534
H. unicolor	He 468a	JQ279551	JQ279662
H. verruculosa	Dai 17047	_	MF370600
H. verruculosa	Dai 17052	MF370594	MF370601
H. villosa	He 537	JQ279528	JQ279634
H. xerantica	Cui 9209	JQ279519	JQ279635
H. yunnanensis	He 1447	KU975486	KU975538

New species is shown in bold.

MrModeltest 2.3 (Posada and Crandall 1998; Nylander 2004) was used to determine the best-fit evolution model for the combined dataset for Bayesian Inference (BI). BI was performed using MrBayes v. 3.2.7a (Ronquist and Huelsenbeck 2003) with four simultaneous independent chains for two datasets, performing 3 million generations (*Fulvifomes*) and 5 million generations (*Hymenochaete*) until the split deviation frequency value < 0.01, and sampled every 1000th generation. The first 25% sampled trees were discarded as burn-in, while the remaining ones were used to calculate Bayesian posterior probabilities (BPP) of the clades.

Branches that received bootstrap support for maximum likelihood (BS), maximum parsimony (BP), and Bayesian posterior probabilities (BPP) greater than 70% (BS), 50% (BP) and 0.95 (BPP) were considered as significantly supported, respectively. FigTree v1.4.2 (Rambaut 2012) was used to visualize the resulting tree.

Results

Phylogeny results

Fulvifomes

The combined ITS + nLSU dataset included sequences from 50 specimens representing 31 species (Table 1). The dataset had an aligned length of 1693 characters, of which 1013 (60%) were constant, 186 (11%) were variable but parsimony-uninformative, and 494 (29%) were parsimony-informative. MP analysis yielded two equally parsimonious trees (TL = 1841, CI = 0.546, RI = 0.712, RC = 0.389, HI = 0.454). The best model-fi for the ITS + nLSU dataset estimated and applied in the Bayesian analysis was GTR+I+G. Bayesian analysis and ML analysis resulted in a similar topology to the MP analysis, with an average standard deviation of split frequencies of 0.004578 (BI).

The phylogeny (Fig. 1) inferred from the ITS and nLSU sequences demonstrated that the new species *Fulvifomes dracaenicola* nested in the *Fulvifomes* clade. Moreover, two specimens of *F. dracaenicola* form a lineage with strong support (100% BP, 100% BS, 1.00 BPP, Fig. 1).



Figure 1. Phylogeny of *Fulvifomes* and related species by MP analysis based on combined ITS and nLSU rDNA sequences. Branches are labelled with maximum likelihood bootstrap > 70%, parsimony bootstrap proportions > 50%, and Bayesian posterior probabilities > 0.95, respectively. New species is in bold.

Hymenochaete

The combined ITS + nLSU dataset included sequences from 79 specimens representing 69 species (Table 2). The dataset had an aligned length of 2249 characters, of which 1486 (66%) were constant, 248 (11%) were variable but parsimony-uninformative, and 515 (23%) were parsimony-informative. MP analysis yielded 48 equally parsimonious trees (TL = 3261, CI = 0.365, RI = 0.619, RC = 0.226, HI = 0.635). The best model for the ITS + nLSU dataset estimated and applied in the Bayesian analysis was GTR+I+G. Bayesian analysis and MP analysis resulted in a similar topology to the ML analysis, with an average standard deviation of split frequencies of 0.009996 (BI).

The phylogeny (Fig. 2) inferred from the ITS and nLSU sequences demonstrated that the new species *Hymenochaete dracaenicola* clustered in the *Hymenochaete* clade and two specimens of *H. dracaenicola* form a lineage with strong support (100% BS, 100% BP, 1.00 BPP, Fig. 2).



Figure 2. Phylogeny of *Hymenochaete* and related species by ML analysis based on combined ITS and nLSU rDNA sequences. Branches are labelled with maximum likelihood bootstrap > 70%, parsimony bootstrap proportions > 50%, and Bayesian posterior probabilities > 0.95, respectively. New species is in bold.

Taxonomy

Fulvifomes dracaenicola Z.B. Liu & Y.C. Dai, sp. nov.

MycoBank No: 838682 Figs 3, 4

Diagnosis. *Fulvifomes dracaenicola* is characterised by perennial, pileate, triquetrous basidioma with yellowish brown fresh pores which becoming honey yellow with silk sheening upon drying, a dimitic hyphal system in trama and monomitic in context, subglobose basidiospores measuring $4.8-5 \times 4-4.1 \mu m$.

Holotype. China. Hainan Province, Sanya, Daxiaodongtian Park, N18.299, E109.172, on living tree of *Dracaena cambodiana*, 15.XI.2020, Dai 22097 (BJFC 035989).

Etymology. *Dracaenicola* (Lat.): referring to the species growing on *Dracaena cambodiana*.

Fruiting body. Basidioma perennial, pileate, without odor or taste and woody hard when fresh, light in weight when dry. Pilei triquetrous, projecting up to 2.5 cm, 2.3 cm wide and 2.6 cm thick at base. Pileal surface yellowish brown to grayish brown when fresh, vinaceous brown when dry, encrusted, glabrous, zonate, uncracked, mar-



Figure 3. A basidiocarp of *Fulvifomes dracaenicola* (Holotype, Dai 22097). Scale bar: 1.0 cm. Photo by: Yu-Cheng Dai.



Figure 4. Microscopic structures of *Fulvifomes dracaenicola* (Holotype, Dai 22097) **a** basidiospores **b** hyphae of context **c** hyphae of the tubes. Drawings by: Meng Zhou.

gin olivaceous brown. Pore surface yellowish brown when fresh, honey yellow with silk sheening when dry; sterile margin indistinct; pores circular, 5–7 per mm; dissepiments thin, entire. Context cinnamon buff to fawn, corky, often darker near the pileus surface, up to 1.4 cm thick, with a distinct crust (black line) near pileus surface at the basal area, partly with additional crust (black line) within context or above tubes. Tubes cinnamon buff to cinnamon, woody hard, up to 1.2 cm thick, tube layers distinctly stratified, individual tube layer up to 0.5 cm long.

Hyphal structure. Hyphal system dimitic in trama, monomitic in context; generative hyphae simple septate; tissues darkening but otherwise unchanged in KOH.

Context. Generative hyphae apricot-orange to brownish-orange, thick-walled with a wide lumen, simple septate, unbranched, regularly arranged, $4.5-6 \mu m$ in diam.

Trama of the tubes. Generative hyphae hyaline, thick-walled, simple septate, occasionally branched, 2–2.5 mm in diam; skeletal hyphae apricot-orange to brownish-orange, thick-walled to subsolid, unbranched, loosely interwoven, 3.5–4 mm in diam. Setae or setal hyphae absent; hymenium collapsed in the studied material, basidia and basidioles not seen.

Spores. Basidiospores subglobose with an apiculus, yellowish brown, thick-walled, smooth, IKI–, CB–, occasionally collapsed when mature, $4.8-5(-5.5) \times 4-4.1 \mu m$, L = 5.02 μm , W = 4.04 μm , Q = 1.22–1.25 (n = 90/3).

Additional specimens (paratypes) examined. China. Hainan Province, Sanya, Daxiaodongtian Park, N18.299, E109.172, on rotten wood of living *Dracaena cambodiana*, 15.XI.2020, Dai 22093 (BJFC 035986), Dai 22095 (BJFC 035987).

Hymenochaete dracaenicola Z.B. Liu & Y.C. Dai, sp. nov.

MycoBank No: 838683 Figs 5, 6

Diagnosis. *Hymenochaete dracaenicola* is characterised by annual, resupinate basidioma with a clay buff hymenophore, a dimitic hyphal system, absence of tomentum and cortex, subulate setae present in hyphal layer, absence of cystidia, presence of cystidioles and simple hyphidia, and oblong ellipsoid basidiospores measuring $5.2-5.8 \times 2.5-2.8 \mu m$.

Holotype. China. Hainan Province, Sanya, Daxiaodongtian Park, N18.299, E109.172, on dead tree of *Dracaena cambodiana*, 15.XI.2020, Dai 22090 (BJFC 035983).

Etymology. Dracaenicola (Lat.): referring to the species s growing on Dracaena cambodiana.

Fruiting body. Basidioma annual, resupinate, adnate, not separable from substrate, hard corky, up to 7.5 cm long, 2 cm wide, and less than 0.1 mm thick at center. Hymenophore surface smooth or locally verruculose, clay buff, with some scattered crevices; margin cinnamon buff, up to 0.4 mm.



Figure 5. A basidiocarp of *Hymenochaete dracaenicola* (Holotype, Dai 22090). Scale bar: 1.0 cm. Photo by: Zhan-Bo Liu.



Figure 6. Microscopic structures of *Hymenochaete dracaenicola* (Holotype, Dai 22090) a basidiospores
b basidia and basidioles c cystidioles d hyphidia e setae f Hyphae from hyphal layer. Drawings by: Meng Zhou.

Hyphal structure. Hyphal system dimitic; generative hyphae infrequent, simple septate; skeletal hyphae dominant; tissues darkening but otherwise unchanged in KOH.

Subiculum. Tomentum and cortex absent; hyphal layer present. Generative hyphae infrequent, hyaline, thick-walled, simple septate, often branched, $1-2 \mu m$ in diam. Skeletal hyphae cinnamon to orange brown, thick-walled to subsolid, rarely branched, interwoven, $1.5-2.5 \mu m$ in diam.

Hymenium. Hyphae similar to those in hyphal layer. Setal layer present, thickening with age, with one to several rows of overlapping setae. Setae numerous, subulate with blunt to acute tips, orange brown to reddish brown, smooth, occasionally with a hyphal sheath, distinctly thick-walled, $30-57 \times 6-10 \mu m$, embedded or projecting up to 35 µm beyond the hymenium. Cystidia absent; cystidioles present, fusoid, hyaline, thin-walled, basally swollen, with a sharp or often hyphoid neck, $10-17 \times 2.5-4 \mu m$; Simple hyphidia present, scattered, thick-walled, $15-36 \times 2-3.5 \mu m$. Basidia subclavate to subcylindrical, with walls thickening toward the base, with four sterigmata and a basal simple septum, $17-23(-25) \times 3.5-5 \mu m$; basidioles similar to basidia but smaller.

Spores. Basidiospores oblong ellipsoid with an apiculus, hyaline, thin-walled, smooth, IKI–, CB–, occasionally bearing a guttule, $(5–)5.2–5.8(-6.1) \times 2.5–2.8 \mu m$, L = 5.6 μm , W = 2.68 μm , Q = 2.03–2.15 (n = 60/2).

Additional specimen (paratype) examined. China. China. Hainan Province, Sanya, Daxiaodongtian Park, N18.299, E109.172, on fallen branch of *Dracaena cambodiana*, 15.XI.2020, Dai 22096 (BJFC 035988).

Discussion

Fulvifomes dracaenicola and *Hymenochaete dracaenicola* were found in tropical regions of China. It is interesting that both species growing on *Dracaena cambodiana*.

Morphologically, *Fulvifomes dracaenicola* (5–7 per mm) shares similar pores with *F. kawakamii* (M.J. Larsen, Lombard & Hodges) T. Wagner & M. Fisch. (5–7 per mm, Larsen et al. 1985), *F. robiniae* (5–6 per mm, Salvador-Montoya et al. 2018), *F. swieteniae* Murrill (5–7 per mm, Hattori et al. 2014) and *F. thailandicus* L.W. Zhou (6–7 per mm, Zhou 2015). *F. dracaenicola* and *F. kawakamii* share perennial, pileate basidioma, but basidioma of *F. dracaenicola* is solitary and glabrous, while basidioma of *F. kawakamii* is imbricate and nodulose. In addition, basidioma of *F. dracaenicola* are bigger than that of *F. dracaenicola* and *F. kawakamii* (4.8–5 × 4–4.1 µm vs. 4.5 × 3.5 µm, Larsen et al. 1985). *F. dracaenicola* and *F. robiniae* share perennial, triquetrous and solitary basidioma, a monomitic hyphal system in context and dimitic in trama, however, *F. robiniae* can be distinguished from *F. dracaenicola* by its bigger basidiospores (5.5–6 × 4–5.5 µm vs. 4.8–5 × 4–4.1 µm). In addition,

F. dracaenicola has a distinct crust (black line) on the pileal surface, but the crust (black line) is absent in *F. robiniae* (Gilbertson and Ryvarden 1987). *F. dracaenicola* resembles *F. swieteniae* by perennial, glabrous basidioma, a dimitic hyphal system in trama, however, basidioma of *F. swieteniae* is ungulate and pileal surface of *F. swieteniae* is azonate, while basidioma of *F. dracaenicola* is triquetrous and its pileal surface is zonate. And *F. dracaenicola* can be also distinguished from *F. swieteniae* by its wider basidiospores (4–4.1 µm vs. 3–4 µm, Salvador-Montoya et al. 2018). *F. dracaenicola* is similar to *F. thailandicus* by sharing perennial, solitary basidioma, but *F. thailandicus* can be distinguished from *F. system* in context, and its bigger basidiospores (5–5.8 × 4.1–4.8 µm vs. 4.8–5 × 4–4.1 µm, Zhou 2015).

Two specimens of *Fulvifomes dracaenicola* form a lineage with strong support (100% BP, 100% BS, 1.00 BPP, Fig. 1) in our phylogeny. *E dracaenicola* is closely related to *E siamensis* T. Hatt. et al. (98% BP, 99% BS, 1.00 BPP, Fig. 1) and both species share perennial, pileate basidioma, a dimitic hyphal system in trama and monomitic in context, and occurring in tropical Asia. Morphologically they can be easily differentiated by the presence of crust on the pileus surface. *E dracaenicola* has a distinct crust (black line) on the pileus surface and partly with additional crust (black line) within context or above tubes, but the crust (black line) is absent in. *E siamensis* (Hattori et al. 2014). Besides, *E siamensis* has applanate pilei pores 7–8 per mm, thin- to thick-walled contextual generative hyphae 2–8 μ m wide, and basidiospore 4–5 μ m wide (Hattori et al. 2014), while *E dracaenicola* has triquetrous pilei, pores 5–7 per mm, thick-walled contextual generative hyphae 4.5–6 μ m wide, and basidiospore 4–4.1 μ m wide. In addition, *E siamensis* grows on *Xylocarpus granatum* in mangrove while *E dracaenicola* grows on *Dracaena cambodiana* in terrestrial ecosystem.

Morphologically, to avoid redescribing the existed species, we review the monograph by Léger (1998) and compare Hymenochaete dracaenicola with all the species in the monograph. H. dracaenicola belongs to the section "FULTOCHAETE" and is similar to H. epichlora (Berk. & M.A. Curtis) Cooke. Both species share resupinate and adnate basidioma, absence of tomentum and cortex, similar setae $(30-57 \times 6-10 \ \mu m \ vs. \ 30-60$ × 5.5-9 µm in H. epichlora, Cooke 1880), but H. dracaenicola has a dimitic hyphal system, while H. epichlora has a monomitic or subdimitic hyphal system and smaller basidiospores (5.2–5.8 × 2.5–2.8 μm vs. 3.5–5 × 1.8–2.5 μm, Cooke 1880). Besides, H. dracaenicola has simple hyphidia in hymenium, but hyphidia are absent in H. epichlora (Cooke 1880). Phylogenetically, two specimens of Hymenochaete dracaenicola form a lineage with strong support (100% BP, 100% BS, 1.00 BPP, Fig. 2). H. dracaenicola clusters together with H. borbonica J.C. Léger & Lanq., H. angustispora S.H. He & Y.C. Dai and H. tenuis Peck with strong support (100% BS, 98% BP, 1.00 BPP, Fig. 2). Morphologically, setae in *H. dracaenicola* are shorter than in *H. borbonica* (30-57 µm vs. 60-70 µm in *H. borbonica*). In addition, basidiospores of *H. dracaenicola* are oblong ellipsoid while they are suballantoid in H. borbonica (5-6 × 2 µm, Léger and Lanquetin 1987). H. angustispora is different from H. dracaenicola by a monomitic hyphal system and narrowly cylindrical to allantoid basidiospores (5–7 × 1.8–2.2 μ m, He et al. 2017). *H. tenuis* can be distinguished from *H. dracaenicola* through its smaller basidiospores $(4.5-5.5 \times 2-2.5)$ μ m vs. 5.2–5.8 × 2.5–2.8 μ m) and a monomitic hyphal system (Peck 1887).

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RESEARCH ARTICLE



Brahmaculus gen. nov. (Leotiomycetes, Chlorociboriaceae)

Peter R. Johnston¹, Duckchul Park¹, Matthew E. Smith², Alija B. Mujic², Tom W. May³

Manaaki Whenua–Landcare Research, Private Bag 92170, Auckland 1142, (Aotearoa) New Zealand
 University of Florida, Department of Plant Pathology, Gainesville FL 32611, USA 3 Royal Botanic Gardens Victoria, Melbourne, Victoria 3004, Australia

Corresponding author: Peter R. Johnston (johnstonp@landcareresearch.co.nz)

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Abstract

A second genus in Chlorociboriaceae is described here as *Brahmaculus* gen. nov. Macroscopically distinctive, all species have bright yellow apothecia with several apothecial cups held on short branches at the tip of a long stipe. The genus is widely distributed across the Southern Hemisphere; the four new species described here include two from Chile (*B. magellanicus* sp. nov., *B. osornoensis* sp. nov.) and one each from New Zealand (*B. moonlighticus* sp. nov.) and Australia (*B. packhamiae* sp. nov.). They differ from species referred to *Chlorociboria*, the only other genus in Chlorociboriaceae, in their terrestrial habitat and ascomata that are noticeably more hairy than the known *Chlorociboria* species, most of which have apothecia with short, macroscopically indistinct hair-like elements. Based on our analyses, *Chlorociboria* as accepted here is paraphyletic. Additional study is needed to clarify where alternative, monophyletic generic limits should be drawn and how these genera may be recognised morphologically. Also described here are three new *Chlorociboria* spp. from New Zealand (*C. metrosideri* sp. nov., *C. solandri* sp. nov., *C. subtilis* sp. nov.), distinctive in developing on dead leaves rather than wood and in two of them not forming the green pigmentation characteristic of most *Chlorociboria* species. New Zealand specimens previously incorrectly identified as *Chlorociboria argentinensis* are provided with a new name, *C. novae-zelandiae* sp. nov.

Keywords

Chlorociboria, Cyttariaceae, fungi, molecular phylogeny, systematics, 9 new taxa

Introduction

The modern-day distribution of Nothofagaceae forests of the Southern Hemisphere and their associated fungi are often explained in terms of vicariance in relation to the breakup of Gondwana (e.g. Horak 1983). This explanation has been challenged in recent years (May 2017), with their distribution now thought to be due to a complex mix of ancient vicariant and geologically more recent long distance dispersal events, with evidence from Nothofagaceae phylogeny (e.g. Knapp et al. 2005) along with the phylogeny of some of their specialised fungal associates (e.g. Peterson et al. 2010b). The importance of these forests to the vegetation of southern South America and New Zealand has meant they have been amongst the most intensively studied mycologically in these regions (McKenzie et al. 2000; Johnston et al. 2012, Gamundí et al. 2017; Romano et al. 2017a). Despite this, much of the fungal diversity in these forests remains undiscovered (e.g. Johnston et al. 2012; Romano et al. 2017b).

An example of this undiscovered diversity comes from recent collections of a beautiful, small terrestrial fungus from Nothofagaceae forests in South America, New Zealand and Australia that could not be matched to any known genus. Microscopically they had a clear affinity to Leotiomycetes. The unique apothecia are morphologically complex with a branched stipe and each branch ending in one or more cups, the hymenial surface in these cups forming a complex pattern comprising separate regions with asci and paraphyses, and with hair-like elements. Preliminary sequencing of ribosomal genes of both Australasian and South American specimens showed that these fungi are phylogenetically closely related and that they are also related to the Leotiomycetes genus *Chlorociboria*.

Here we describe four species in the newly erected genus *Brahmaculus* based on a combination of unique morphological and molecular characters. We incorporate *Brahmaculus* DNA sequences into a broad multigene Leotiomycetes phylogeny to show that these fungi represent a second genus in Chlorociboriaceae. Including *Brahmaculus* in the phylogeny makes *Chlorociboria* paraphyletic but the morphological and ecological differences between *Chlorociboria* and *Brahmaculus* species means that it is not sensible to treat them as a single genus. More intensive genetic sampling of additional *Chlorociboria* species will be needed to better resolve phylogenetic relationships within Chlorociboriaceae and to clearly define the phylogenetic and morphological limits of the genus *Chlorociboria*.

It is surprising that specimens of the morphologically spectacular *Brahmaculus* have not been collected more often in the Nothofagaceae forests of the Southern Hemisphere. Although clearly widespread geographically, these fungi presumably fruit rarely.

Methods

Samples

Specimens were collected during surveys of fungal diversity in Southern Hemisphere forests. Brief notes on macroscopic appearance were prepared and then the specimens

dried and stored in the New Zealand Fungarium (**PDD**), National Herbarium of Victoria (**MEL**), Museo Nacional de Historia Natural (**SGO**) and the Florida Museum of Natural History (**FLAS**).

Morphology and culturing

Microscopic examinations were made from dried material routinely rehydrated and in 3% KOH and mounted in Melzer's Reagent, or where indicated, rehydrated and mounted in water. Vertical sections about 10 μ m thick were prepared from apothecia rehydrated in 3% KOH using a freezing microtome and mounted in lactic acid. Where available, living cultures were grown from germinated ascospores and are stored in the ICMP culture collection, Manaaki Whenua–Landcare Research, Auckland.

DNA extraction and PCR amplification

DNA was extracted from apothecia that had been placed in buffer when fresh, from dried apothecia, or from mycelium from living cultures, using a QIAamp DNA mini kit (QIAGEN, US) on the QIAcube nucleic acid extraction robot (QIAGEN, US). Amplification primers used for each of the genes were: SSU – NS1 and NS4 (White et al. 1990); ITS – ITS-1F and ITS4 (White et al. 1990; Gardes and Bruns 1993); LSU – LROR and LR5 (Bunyard et al. 1994; Vilgalys and Hester 1990); MCM7 – mcm7-709for and mcm7-1348rev (Schmitt et al. 2009); RPB1 – RPB1-Af and RPB1-Cr (Stiller and Hall 1997; Matheny et al. 2002); and RPB2 – RPB2-5f2 and fRPB2-7cR (Liu et al. 1999; Sung et al. 2007).

Phylogenetic analyses

Two phylogenetic analyses were carried out. In the first, LSU, ITS, MCM7, RPB1 and RPB2 sequences from *Brahmaculus* specimens from South America and New Zealand, together with a set of *Chlorociboria* and *Cyttaria* specimens with multi-gene data available (Table 1), were incorporated into the alignments from Johnston et al. (2019 – data available from https://doi.org/10.7931/T5YV-BE95). *Cyttaria* was added because the analysis presented by Peterson and Pfister (2010a) suggested a relationship to Chlorociboriaceae and additional genes had recently become available for *Cyttaria nigra*. The expanded dataset was reanalysed using the same methods as Johnston et al. (2019). Briefly, genes were aligned using MAFFT (Katoh and Standley 2013), a maximum likelihood (ML) analysis of the concatenated alignments was run using IQ-TREE (Nguyen et al. 2015; Chernomor et al. 2016), using models selected by ModelFinder (Kalyaanamoorthy et al. 2017) for each partitioned gene, and ultrafast bootstrap (BS) analysis with 1000 replicates estimated branch support in the ML tree (Hoang et al. 2018). *Xylaria hypoxylon* and *Neurospora crassa* were used as outgroups.

The second analysis used ITS sequences only, treating all four *Brahmaculus* species, together with all *Chlorociboria* species with ITS sequences available, using Cenangiaceae

1 numbers for DNA sequences of Brahmaculus, Chlorociboria and Cyttaria specimens used for phylogeny in Fig. 1, and for newly gener-	geny in Fig. 2. Sequences generated as part of this project in bold. Data for other taxa included in the Fig. 1 phylogeny from Johnston	ore/10.7931/T5YV-BE95.
ble I. GenBank accession numbers for DNA	d sequences used in phylogeny in Fig. 2. Sequ	d. (2019), see https://doi.org/10.7931/T5YV

Species	Voucher (T = type specimen)	SSU	STI	ISU	MCM7	RPB1	RPB2	TEF	mtSSU	β—tubulin
Brahmaculus magellanicus	PDD 116650 (T)	MW364563	MW364557	MW364560	MW350087	1	MW350085	1	1	1
B. moonlighticus	PDD 112225 (T)	MK248054	MK248036	MK248011	MK241483	MK241482	MK241484	I	I	I
B. osornoensis	FLAS-F-65492 (T)	I	MW575608	I	I	I	I	I		I
B. packhamiae	PDD 117311 (T)	I	MW364556	I	I	I	I	I	I	I
Chlorociboria aeuruginascens	TNS-F-36241	LC434588	LC425045	LC429376	I	LC431689	LC431723	I	I	I
C. aeuruginascens	DSM 107184 (isolate	PRJNA382475								
	IHIA39, genome)									
C. aeuruginascens ssp. australis	ICMP 15642 (T)	JN939873	NR_119520	JN939932	JN993274	JN985222	JN985532	I	I	I
C. aeruginosa	AFTOL-ID 151	AY544713	DQ491501	AY544669	I	DQ471125	DQ470886	I	I	I
C. aeruginosa	TNS-F-13596	LC434578	LC425047	LC429383	I	LC431687	I	I	I	I
C. aeruginella	TAAM 198514	KX090875	MH752067	I	I	KX090769	KX090722	I	I	I
C. argentinensis	ICMP 16995	JN939876	EF520123	JN939930	JN993275	JN985197	JN985515	I	I	I
C. novae-zelandiae	ICMP 18766 (T)	JN939875	JN943456	JN939940	JN993286	JN985223	JN985514	I	I	I
C. awakinoana	ICMP 15631	JN939870	JN943461	JN939921	JN993273	JN985219	JN985504	I	I	I
C. clavula	ICMP 15634	JN939866	JN943465	JN939924	JN993299	JN985215	JN985519	I	I	I
C. duriligna	ICMP 18763 (T)	JN939863	JN943468	JN939934	JN993279	JN985212	JN985500	I	I	I
C. glauca	TAAM 198458	KX090872	I	KX090821	I	KX090766	I	I	I	I
C. halonata	ICMP 18764	JN939860	JN943471	JN939935	JN993296	JN985209	JN985502	I	I	I
C. metrosideri	ICMP 23410 (T)	I	MW364558	I	I	I	I	I	I	I
C. poutoensis	ICMP 15618	I	AY755352	I	MH700576	MH682247	I	I	I	I
C. solandri	ICMP 23686 (T)	I	MW364559	I	I	I	I	I	I	I
C. spathulata	ICMP 18760	JN939868	JN943463	JN939923	JN993272	JN985217	JN985530	I	I	I
C. subtilis	PDD 112247	I	MH921854	I	I	I	I	I	I	I
Cyttaria darwinii	FH (Peterson and Pfister 2010. isolare 40. 45)	EU107180	I	EU107209	I	I	I	EU107250	EU107236	I
Cuttania haniatii	EH (Peterson and Pfister	FI1107194	I	FI1107217	I	I	I	FII107251	FI1107245	I
Operation 13a1 10 mil	2010, isolate 44)	1/1/0107		17/0107				1/7/0107	17/0107	
Cyttaria nigra	PDD 117571	MW364564	I	MW364561	MW350086	MW363493	MW350084	MW350088	MW364562	MW350089

as the outgroup. The methods were the same as those used for the multi-gene analysis, except with the TIM2+F+I+G4 model, selected using ModelFinder.

Alignments and partitions for each of the analyses are provided through the Landcare Research – Manaaki Whenua Datastore, https://doi.org/10.7931/2xet-fc88.

Results

Phylogenetic analyses

Helotiales form a strongly supported monophyletic clade, and most families accepted within this order also form strongly supported clades (Fig. 1). The family-level clades of those families clustered in collapsed clades in Fig. 1 have 100% bootstrap support. Chlorociboriaceae and Cyttariaceae are strongly supported as monophyletic but their relationship to each other, and to other basal family-level clades within the Helotiales, is poorly resolved. A fully expanded version of Fig. 1 is available as a nexus file from the Landcare Research – Manaaki Whenua Datastore, https://doi.org/10.7931/2xet-fc88.

In both the multi-gene and ITS analyses, *Brahmaculus* forms a monophyletic clade within Chlorociboriaceae, but *Chlorociboria* as accepted here is paraphyletic (Figs 1, 2). The *Brahmaculus* species form a well-supported clade sister to a well-supported clade comprised of *Chlorociboria aeruginella* and *C. halonata* (from Northern Europe and New Zealand, respectively). The clade comprised of *Brahmaculus* plus these two species of *Chlorociboria* is sister to a clade containing the bulk of sequenced species of *Chlorociboria*, including the type species of the genus, *C. aeruginosa*.

At the species level, the ITS analysis supports the molecular phylogenetic distinctiveness of the novel species of *Brahmaculus* and *Chlorociboria* accepted here (Fig. 2).

Taxonomy

Brahmaculus P.R.Johnst. gen. nov.

MycoBank No: 838724

Type species. Brahmaculus moonlighticus P.R.Johnst.

Etymology. From Hindu mythology, named after Brahma, the four-headed creator god, reflecting the multiple heads of the apothecia, and the masculine diminutive -culus.

Diagnosis. Phylogenetically Chlorociboriaceae, distinguished from *Chlorociboria* by its terrestrial habitat, and apothecium with stipe branched near apex, each branch with an apothecial cup.

Description. Apothecia stipitate, yellow rhizomorphs at base of stipe, the stipe branched apically several times, each branch holding an apothecial cup. Receptacle and stipe densely covered with short hairs. Hairs more or less straight, cylindric, thin walled, with a few septa, pale brown intracellular pigment, externally densely encrusted with yellowish material, encrusting material dissolving in KOH + Melzer's reagent. The hymenium



Figure 1. ML tree based on a multi-gene alignment, placing *Brahmaculus* within Chlorociboriaceae and both Chlorociboriaceae and Cyttariaceae in Helotiales. Taxa newly named in this paper in bold. Bootstrap values where >90%. See Methods and Table 1.

within each apothecial cup is typically divided into smaller segments, with areas comprising asci and paraphyses separated by clumps of hair-like elements. Excipulum comprises cylindric cells arranged more or less parallel to the surface, cells mostly long-cylindric, but



Figure 2. ML tree based on an ITS alignment, treating all *Chlorociboria* and *Brahmaculus* species with ITS sequences available. Taxa newly named in this paper in bold. Bootstrap values where >90%.

sometimes with outermost 1–2 layers of cells short and broad-cylindric, cell walls slightly thickened, hyaline, cells near base of hairs with pale brown vacuolar pigment. Asci with wall thickened at apex, amyloid pore extending through the wall, flaring near the inside and especially toward outside of the wall, 8-spored, with croziers. Paraphyses simple or tapering to apex, of similar length as asci. Ascospores oblong-elliptic, 0-septate, hyaline.

Notes. The four species described below are phylogenetically distinct but remarkably similar morphologically. There appear to be small differences in size and colour of the apothecia and shape of the paraphyses and hairs but having only a single specimen available for each species makes the significance of these differences difficult to assess. The rhizomorphs at the base of the stipe appear to be associated with tree roots. Based on the collecting sites, in South America and New Zealand the roots are likely to be Nothofagaceae, in Australia they may also be Nothofagaceae but *Eucalyptus* species were also growing in the vicinity. Observations from the South American specimens showed a loose weft of mycelium around the Nothofagaceae roots but there was no clear evidence of a mantle or ectomycorrhizal association. It is possible that these fungi are root endophytes, or perhaps parasites of Nothofagaceae-associated ectomycorrhizal fungi.

Brahmaculus magellanicus M.E.Sm. & P.R.Johnst. sp. nov.

MycoBank No: 838730 Figure 3

Typification. CHILE – **Magallanes** • Puente San Pedro, south of Punta Arenas, stream near the end of the road, *Nothofagus betuloides* forest; -53.6993, 70.9695; Alija Mujic (MES2454) leg.; 5 Apr 2017; SGO – *Holotype*; FLAS-F-65086 – *isotype*; PDD 116650 – *isotype*.

Etymology. Refers to the Magellanic forests of the type locality.

Diagnosis. Phylogenetically distinct from other known *Brahmaculus* spp., apothecia $3-8 \times 1-2.5$ mm, paraphyses undifferentiated to rounded apex, ascospores $5.5-9 \times 1.5-2 \mu m$ (average $7.3 \times 1.7 \mu m$).

Description. Apothecia 3-8 mm high, stipe 0.4-0.6 mm wide, cap 1-2.5 mm wide, the more or less globose cap comprising several closely packed apothecial cups, these arising from short, branches at the top of the stipe, hymenium pale yellow, hymenial areas broken into smaller segments by groups of bright yellow, hair-like elements amongst the fertile parts of the hymenium. Receptacle densely covered with stiff, bright yellow hairs, stipe with shorter hairs. Hairs $45-70 \times 2.5-4.5 \mu m$, straight, cylindric, tapering gradually in apical half toward small, rounded apex, thin-walled, sparsely septate, pale brown vacuolar pigment, densely encrusted with coarse, bright yellow crystals that dissolve in KOH + Melzers. Ectal excipulum comprising narrow-cylindric cells $8-20 \times 2.5-3 \mu m$ oriented at low angle to receptacle surface, wall slightly thickened, mostly hyaline except cells at the base of hairs have pale brown vacuolar pigment. Medullary excipulum similar in structure but cells wider, 4.5–8 µm diam. Paraphyses 1.5-2.5 µm diam., undifferentiated at rounded apex, about same length as asci. Asci $40-55 \times 4-5 \mu m$, cylindric, apex rounded, wall thickened, amyloid pore extends through the wall, diffuse and flaring slightly towards the outside of the wall, crozier at base, 8-spored. Ascospores $5.5-9 \times 1.5-2 \ \mu m$ (average 7.3 \times 1.7 µm, n = 12), oblong elliptic, ends rounded, flattened on one side, straight to slightly curved, 0-septate, hyaline.



Figure 3. *Brahmaculus magellanicus* (PDD 116650) **A, B** fresh apothecia **C** dried apothecia **D** ascoma in vertical section, showing multiple apothecial cups on short branches **E** ascoma in vertical section showing excipular tissue and hairs, and a clump of hairs within the hymenium **F** squash mount showing hairs in KOH **G** asci **H** paraphyses **I** ascospores. Scale bars: 1 mm (**A–C**); 100 μ m (**D**); 10 μ m (**E–I**).

Notes. The two Chilean species differ macroscopically, *B. magellanicus* having noticeably thinner stipes than *B. osornoensis*. The only known collection of *B. magellanicus* is from Magellanic subpolar forest in Patagonia that is dominated by *Nothofagus betuloides*. It is possible that this *Brahmaculus* species is restricted to these sub Antarctic cold southern forests but more specimens are needed to determine the range of the species.

Brahmaculus moonlighticus P.R.Johnst. sp. nov.

MycoBank No: 838733 Figure 4

Typification. New ZEALAND – **Buller** • Moonlight Creek; -42.2713, 171.4587; on soil under Nothofagaceae; A. Chinn leg.; 10 May 2018; PDD 112225 – *bolotype*.

Etymology. From the type locality. Historically important as a gold mining area (where T.H. Chinn, the great-great grandfather of the collector of the type specimen, prospected for gold in the 1880's), the name also reflects the deep golden colour of the apothecia of this fungus.

Diagnosis. Phylogenetically distinct from other known *Brahmaculus* spp., apothecia $1.5-3 \times 1-1.8$ mm, paraphyses taper slightly to rounded apex, ascospores $6.5-8.5 \times 1.5-2(-3)$ µm (average 7.7×1.9 µm).

Description. Apothecia 1.5-3 mm high, stipe 0.25-0.5 mm wide, cap 1-1.8 mm wide, bright golden-yellow when fresh, consistently with four short branches arising from top of stipe, each branch with its own apothecial cup, hymenium pale yellow, divided into a complex pattern with hymenial areas separated by narrow groups of golden yellow hair-like elements. Receptacle densely covered with stiff, bright yellow hairs. Hairs $40-60 \times 3-4 \mu m$, straight, cylindric, tapering slightly towards rounded apex, pale brown vacuolar pigment, wall smooth, encrusted with coarse yellow-brown crystals that dissolve in KOH + Melzer's, few-septate. Ectal excipulum comprising long-cylindric cells $15-25 \times 3-5 \mu m$, but with the outermost 1-2 layers of cells short and broad-cylindric, 6-8 µm diam., cell walls slightly thickened, hyaline, cells near base of hairs with pale brown vacuolar pigment. Medullary excipulum comprising partly tangled hyphae 3-4 µm diam. with walls thin, hyaline. Paraphyses 2-3 µm diam., tapering slightly towards rounded apex, about same length as asci. Asci 45-55 × 5.5-6 µm, cylindric, tapering slightly to broad subtruncate apex, wall uniformly thickened across apex, amyloid pore extending through wall, flaring slightly towards both inside and outside of wall. Ascospores 6.5–8.5 \times 1.5–2 (–3) µm (average 7.7 \times 1.9 μ m, n = 50), oblong-elliptic to subfusoid, sometimes tapering suddenly to a narrower lower half, ends rounded, flat one side in side view, sometimes slightly curved or sigmoid, 0-septate, hyaline.

Notes. *Brahmaculus moonlighticus* has a stipe that consistently has 4 distinct branches near the apex. The other species have several separate hymenial cups, but these are held on very short branches arising from across the apex of the stipe, the margins of these cups superficially forming a more or less continuous layer.



Figure 4. *Brahmaculus moonlighticus* (PDD 112225) **A** fresh apothecia (dried apothecium inset) **B** detail, fresh apothecia **C** ascoma in vertical section, showing multiple apothecial cups **D** ascoma in vertical section showing excipular tissue and hairs **E** hairs in squash mount in KOH **F** asci and ascospores **G** asci and paraphyses **H**, **I** ascospores. Scale bars: 1 mm (**A**, **B**); 100 μm (**C**); 10 μm (**D–I**).

Brahmaculus osornoensis M.E.Sm. & P.R.Johnst. sp. nov.

MycoBank No: 838734 Figure 5

Typification. CHILE • Parque Nacional Vicente Perez Rosales, Volcan Osorno, on the road to the ski area just above Mirador el Bosque, *Nothofagus dombeyi* forest; -41.1382, 72.5370; Matthew Smith and Alija Mujic (MES2942) leg.; 17 April 2017; SGO – *holotype*; FLAS-F-65492 – *isotype*; PDD 116649 – *isotype*.

Etymology. Refers to the type locality, Volcan Osorno.

Diagnosis. Phylogenetically distinct from other known *Brahmaculus* spp., apothecia $3-6 \times 1-2.5$ mm, paraphyses taper slightly to rounded apex, ascospores $6.5-10(-11) \times 1.5-2$ µm (average 8.3×2 µm).

Description. Apothecia 3–6 mm high, stipe 0.5–1 mm wide, cap 1–2.5 mm wide, the more or less globose cap comprising several closely packed apothecial cups, these arising from short, branches at the top of the stipe, hymenium pale yellow, hymenial areas broken into smaller segments by groups of bright yellow, hair-like elements amongst the fertile parts of the hymenium. Receptacle densely covered with stiff, bright yellow hairs, stipe with shorter hairs. Hairs 50–85 × 2.5–4 µm, straight, with a broad basal cell then cylindric, apically tapering suddenly to the narrow-rounded apex, thin-walled, sparsely septate, densely encrusted with coarse, bright yellow crystals that dissolve in KOH + Melzers. In squash mount, excipular cells broad-cylindric, about 15–30 × 8–12 µm, wall slightly thickened, hyaline. Paraphyses 2–2.5 µm, tapering slightly to rounded apex, about same length as asci. Asci 40–50 × 4–4.5 µm, cylindric, apex rounded, wall thickened, amyloid pore extends through the wall, flaring slightly towards the outside. Ascospores 6.5–10(–11) × 1.5–2.5 µm (average 8.3 × 2.0 µm, n = 50), oblong elliptic, ends rounded, one side flat in side view, sometimes slightly curved, 0-septate, hyaline.

Notes. The two Chilean species differ macroscopically, *Brahmaculus osornoensis* having noticeably broader stipes than *B. magellanicus* and slightly longer ascospores. *B. osornoensis* is known only from *Nothofagus dombeyi* forest in northern Patagonia on Volcan Osorno in the Vicente Perez Rosales National Park. It is possible that this *Brahmaculus* species is restricted to the wetter and warmer forests in northern Patagonia, but more specimens are needed to determine the range of the species.

Brahmaculus packhamiae T.W.May & P.R.Johnst. sp. nov.

MycoBank No: 838729 Figure 6

Typification. AUSTRALIA – **Tasmania** • Geeveston District, Hermons Rd; -43.2652, 146.8613; J.M. Packham (6/R6/26) leg.; 5 June 1995; MEL 2363173 – *holotype*; PDD 117311 – *isotype*.



Figure 5. *Brahmaculus osornoensis* (PDD 116649) **A** fresh apothecia (dried apothecia inset) **B** squash mount, excipulum and hairs in 3% KOH + Melzer's reagent **C** squash mount, hairs in water showing encrusting crystals **D** paraphyses and asci **E** ascospores. Scale bars: 1 mm (**A**); 10 μ m (**B–E**).



Figure 6. *Brahmaculus packhamiae* (PDD 117311) **A, B** dried apothecia **C** detail of head of dried apothecium **D** hymenial surface of rehydrated apothecium, showing multiple separate apothecial cups **E** squash mount showing excipular cells and hairs in KOH + Melzer's reagent **F** ascoma in vertical section **G** paraphyses, asci, and ascospores **H** ascospores. Scale bars: 1 mm (**A–C**); 0.1 mm (**D**); 10 μm (**E–H**).

Etymology. Named after the late Jillian ("Jill") Mary Packham whose assiduous collecting activities detected the type collection.

Diagnosis. Phylogenetically distinct from other known *Brahmaculus* spp., apothecia up to 11×2.5 mm, paraphyses undifferentiated to rounded apex, ascospores $5.5-8.5 \times 1.5-2.5 \mu m$ (average $7.2 \times 1.8 \mu m$).

Description. Apothecia up to 11 mm high, stipe up to 0.8 mm wide, cap up to 2.5 mm wide, the cap comprising several closely packed apothecial cups, these arising from short branches at the top of the stipe, hymenium white when fresh. Receptacle densely covered with stiff, bright yellow hairs, stipe with shorter hairs, yellow rhizomorphs at base. Hairs $40-60 \times 2.5-3.5 \mu m$, straight, narrow flask-shaped, broad near base then tapering suddenly to narrow-cylindric apical part, apex rounded, thinwalled, 1–2 septate near the base, densely encrusted with coarse, bright yellow crystals, that dissolve in KOH + Melzers. Ectal excipulum comprising cylindric cells 8-15 × 3-5 µm, oriented at a low angle to the receptacle surface, walls slightly thickened, hyaline. Medullary excipulum comprises partly tangled hyphae 3-5 µm diam. with walls thin, hyaline. Paraphyses 2–2.5 µm diam., undifferentiated at the rounded apex, about the same length as the asci. Asci $35-45 \times 4.5-5.5 \mu m$, cylindric, tapering slightly to broad, subtruncate apex, wall thickened across apex, amyloid pore extending through wall, flaring toward outside of wall. Ascospores $5.5-8.5 \times 1.5-2.5 \mu m$ (average 7.2 × 1.8 μ m, n = 20), oblong-elliptic, tapering slightly to rounded ends, one side flat in side view, sometimes slightly curved, 0-septate, hyaline.

Notes. *Brahmaculus packhamiae* is macroscopically and microscopically similar to the Chilean *B. magellanicus*, both species having relatively long and narrow stipes. Notes with the specimen, indicate that when fresh the ascomata "seem to be attached to roots".

Chlorociboria metrosideri P.R.Johnst., sp. nov.

MycoBank No: 838735 Figure 7

Typification. NEW ZEALAND – **Bay of Plenty** • vic. Rotorua, Tarawera Falls (-38.1573, 176.5193); on fallen leaves *Metrosideros excelsa*; P.R. Johnston (D2565) leg.; 16 May 2019; PDD 116740 – *bolotype*; ICMP 23410 – ex type culture.

Etymology. Refers to the host substrate of the known specimens.

Diagnosis. Phylogenetically a *Chlorociboria*, differs in developing on dead leaves rather than wood and in the asci being 4-spored when mature.

Description. Apothecia developing on partly decomposed fallen leaves, not associated with pigmentation of substrate. Apothecia less than 1 mm diam., sessile, with short, matted hairs around the margin, hymenium yellow. Hairs 20–45 × 4 μ m, cy-lindric, walls thin, roughened. Apothecium in vertical section with ectal excipulum 30–40 μ m wide, comprising short, broad-cylindric cells 5–7.5 μ m diam., with walls hyaline, slightly thickened, rows of cells arranged at a high angle to the receptacle



Figure 7. *Chlorociboria metrosideri* (PDD 116740) **A** fresh apothecia **B** margin of receptacle in vertical section **C** surface of receptacle in squash mount showing rough-walled hairs **D** apex of asci and paraphyses **E** immature ascus with 8 spores, and mature asci with 4 spores. Scale bars: 0.1 mm (**A**); 20 μ m (**B**, **C**); 10 μ m (**D**, **E**).

surface near the base of the cup, more parallel to the surface near edge of cup; medullary excipulum of narrow-cylindric cells with thin walls. Paraphyses 2–3 μ m diam., taper slightly and gradually to rounded apex, extending 5–10 μ m beyond asci. Asci $40-55 \times 5.5-7 \mu m$, cylindric, tapering slightly to broad, subtruncate apex, wall thickened at apex with amyloid pore extending as two narrow, parallel bands extending through the wall, initially with 8 spores, 4 spores aborting and 4–spored at maturity, crozier present. Ascospores 7.5–9.5 × 2.5–3.5 µm (average 8.3 × 3.1 µm, n = 20), oblong-elliptic, tapering to rounded ends, one side flat in side view, widest point towards one end, 0–septate, hyaline.

Additional specimen examined. NEW ZEALAND – Auckland • Rangitoto Island, Kidney Fern Glen; -36.805544, 174.860064; on fallen, partly rotten *Metrosideros excelsa* leaves; P.R. Johnston (D2329) leg.; 23 Apr 2012; PDD 102723.

Notes. The substrate in both specimens was partly rotted leaves. It is possible that this fungus has a broader host range as most host-specialised, leaf-inhabiting Leotiomycetes are found on recently fallen leaves of their preferred host. Cultures are slow growing (on PDA, 9 mm after 8 weeks) with sparse mycelium and pale brownish pigmentation, remaining sterile.

Chlorociboria novae-zelandiae P.R.Johnst. sp. nov.

MycoBank No: 838736

Typification. NEW ZEALAND – **Fiordland** • Kepler Track, control gates; -45.4396, 167.6822; on Nothofagaceae sp. dead wood; P.R. Johnston (D1484), R.E. Beever, S.R. Pennycook, R. Leschen, T. Lebel leg.; 10 May 2000; PDD 77447 – *holotype*; ICMP 18766 – ex type culture.

Etymology. Refers to the country of origin, in contrast to Argentina and the morphologically similar *C. argentinensis*, with which *C. novae-zelandiae* was previously confused.

Diagnosis. Similar to *Chlorociboria argentinensis* in having small, allantoid ascospores and lacking tomentum hyphae, but phylogenetically distinct and with smaller ascospores and narrower asci.

Additional specimens examined. *C. novae-zelandiae*: NEW ZEALAND – Fiordland. • Borland Lodge nature trail; on Nothofagaceae sp. dead wood; P.R. Johnston (D1471.2) leg.; 9 May 2000; PDD 77446 – North Canterbury. • Mt Thomas Forest, Richardson Track; on Nothofagaceae sp. dead wood; P.R. Johnston (D679) leg.; 15 Mar 1991; PDD 58574, ICMP 15616 – Nelson. • Arthur Range, Graham Valley Rd, track from Flora car park to Mt Arthur Hut; P.R. Johnston (D993) leg.; 6 May 1994; PDD 77444.

C. argentinensis: ARGENTINA – Tierra del Fuego • Lago Fagnano, vic. Kosobo, road to hot springs; on *Nothofagus pumilio* fallen wood; P.R. Johnston (SA86), L. Lorenzo leg.; 22 Mar 1996; PDD 92026; ICMP 16994 – Patagonia. • Rio Negro, Nahuel Huapai National Park, path from Puerto Blest to Los Cantaros; on *Nothofagus dombeyi* fallen wood; P.R. Johnston (SA 188), I. Gamundí, C. Brion leg.; 2 Apr 1996; PDD 92027; ICMP 16995.

Notes. Johnston and Park (2005: 690-693) provided a description of *C. novae-zelandiae*, from New Zealand specimens reported under the name *C. argentinensis*.

Subsequent DNA sequencing of specimens from Argentina identified as *C. argentinensis*, showed that the New Zealand species is phylogenetically distinct. Morphologically, the two species are similar, both with an excipulum comprising highly gelatinous *textura intricata*, the apothecia lacking hair-like tomentum hyphae, and with small, allantoid ascospores. The New Zealand species has somewhat smaller ascospores (average $7.0 \times 1.5 \ \mu m$ versus $9.9 \times 1.9 \ \mu m$) and narrower asci ($3.5-4.5 \ \mu m$ versus $4-5.5 \ \mu m$) compared with the Argentinian specimens recognised here as *C. argentinensis*. The Argentinian specimens match closely the description of Dixon (1975).

Chlorociboria solandri P.R.Johnst., sp. nov.

MycoBank No: 838737 Figure 8

Typification. NEW ZEALAND – **Fiordland** • Fiordland National park, Kepler Track, Rainbow Reach; -45.4429, 167.6802; on *Fuscopora solandri* fallen leaves; P.R. Johnston (D686) leg.; 17 Mar 1991; PDD 58580 – *holotype*; ICMP 23686 – ex-type culture.

Etymology. Refers to the host substrate of the holotype.

Diagnosis. Phylogenetically a *Chlorociboria*, developing on fallen leaves rather than wood, differs from *Chlorociboria metrosideri* in having flexuous, coiled hairs and lanceolate paraphyses.

Description. Apothecia developing on fallen leaves, not associated with any pigmentation of substrate. Apothecia less than 1 mm diam., short-stipitate, receptacle densely covered with short, white hairs, hymenium pale yellow. Hairs 30-40 × 3-4 µm, short-cylindric, undifferentiated to apex, septate, thin-walled, roughened all over, flexuous, coiled and tangled. Apothecia in vertical section with ectal excipulum 45 µm wide, comprising short-cylindric to subglobose cells 5-8 µm diam. oriented at high angle to receptacle surface, with walls hyaline, thickened, agglutinated, amyloid in some specimens. Medullary excipulum non-gelatinous, comprising narrow-cylindric hyphae with thin walls. Tissue at base of stipe of gelatinous textura intricata. Paraphyses up to 5 µm diam., lanceolate, tapering to narrow rounded apex, extending 20-30 µm beyond asci, wall distinctively thickened at base, amyloid in some specimens. Asci 40-55 × 4.5-5.5 µm, cylindric, tapering gradually to small, subtruncate apex, wall thickened at apex, amyloid pore extending through wall, flaring towards outside of wall, crozier present, 8-spored. Ascospores $8-11.5 \times 1.5-2 \mu m$ (average $10.0 \times 1.7 \mu m$, n = 20), oblong-elliptic to subfusoid, widest point slightly towards the upper end, taper to narrow-rounded ends, 0-septate, hyaline.

Additional specimens examined. NEW ZEALAND – Taupo • Kaimanawa Forest Park, Tree Trunk Gorge; on *Fuscopora solandri* fallen leaves; P.R. Johnston (D877), I. Gamundí leg.; 1 Feb 1993; PDD 61833 – Mid Canterbury • Craigieburn, Cave Stream; on *Fuscopora solandi* fallen leaves; E. Horak leg.; 31 Mar 1983; PDD 92925.


Figure 8. *Chlorociboria solandri* **A** dried apothecium **B** fresh apothecium **C** margin of receptacle in vertical section **D** ascospores **E** paraphyses **F** amyloid paraphysis **G** base of paraphysis with thick wall **H** details of coiling, rough-walled excipular hairs. Images: PDD 58580 (**A**, **C**, **D**, **F–H**); PDD 61833 (**B**, **E**). Scale bars: 0.1 mm (**A**, **B**); 20 µm (**C**); 10 µm (**D–H**).

Notes. *Chlorociboria solandri* is micromorphologically distinctive in having scattered, large, lanceolate paraphyses, short-cylindric to more or less globose, thick-walled excipular cells, excipular tissue reacting either blue or red to Melzer's reagent, and coiling, rough-walled hairs. Known from two specimens from *Fuscopora solandri* leaves. A third specimen in poor condition, PDD 92925, could be the same species; it is morphologically similar but has longer hairs than the other two specimens. Cultures on agar are very slow growing (10 cm after 4 weeks), have little aerial mycelium and pale yellow brown pigments, remaining sterile.

Chlorociboria subtilis P.R.Johnst., sp. nov.

MycoBank No: 838738 Figure 9

Typification. New ZEALAND – **Westland** • Haast Pass Summit, Lookout Track; -44.1063, 169.3519; on fallen leaves *Dracophyllum* sp.; P.R. Johnston (D2515), M. Padamsee leg.; 16 May 2018; PDD 112247 – *bolotype*.

Etymology. From subtilis (delicate) referring to the stature of the apothecia.

Diagnosis. Blue-green apothecia on blue-green stained fallen, partly decomposed leaves, hairs on receptacle rough-walled, somewhat flexuous, ascospores filiform, $45-55 \times 1 \mu m$.



Figure 9. *Chlorociboria subtilus* (PDD 112247) **A** dried apothecium **B** apothecium in vertical section **C** detail of margin of receptacle in vertical section **D** asci, ascospores and paraphyses **E** detail of apex of paraphyses and asci **F** excipular hairs (squash mount). Scale bars: 0.1 mm (**A**); 100 μ m (**B**); 10 μ m (**C–F**).

Description. Apothecia erumpent from blue-green stained leaf tissue. Apothecia less than 1 mm diam., cupulate with short, broad stipe, receptacle pale blue-green with tangled, white hairs, especially near the edge of the cup. Hairs $55-75 \times 3-4 \mu m$, somewhat flexuous, wall roughened. Apothecia in vertical section with ectal excipulum up to 30 µm wide, cells 6–10 µm diam., short-cylindric to square, walls thick, cells arranged in rows with a high angle to the receptacle surface; medullary excipulum poorly developed, two or three rows of narrow-cylindric cells, walls encrusted with blue-green material; stipe with thick-walled *textura intricata*. Paraphyses 1.5–2 µm diam., slightly wider towards the apex, often branched in the upper 20–30 µm, extending 15 µm beyond asci. Asci 85–105 µm × 5.5–6.5 µm cylindric, tapering gradually to small, truncate apex, wall thickened at apex, amyloid pore in inner half of wall, reaction most intense on inner edge of wall, pore appears more or less U-shaped, sloping outwards slightly through the wall, 8-spored, spores confined to the upper 60–100 µm of ascus, crozier present. Ascospores $45-55 \times 1 \mu m$, filiform, straight, 0–septate, hyaline.

Additional specimens examined. NEW ZEALAND – Nelson • Arthur Range, Graham River Valley Rd, track from Flora car park to Mt Arthur Hut; on *Dracophyllum pyrimidale* fallen leaves; P.R. Johnston (D990) leg.; 6 May 1994; PDD 105292 – Central Otago •vic. Dunedin, Great Moss Swamp; on *Dracophyllum uniflorum* fallen leaves; P.R. Johnston (D82) leg.; 12 May 1984; PDD 105293 – Mid Canterbury • Craigieburn, Cave Stream; on *Dracophyllum uniflorum* fallen leaves; P.R. Johnston (D248) leg.; 23 Feb 1988; PDD 105294 – Taupo • Tongariro National Park, Ohakune Mountain Road, Blyth Track; on *Fuscopora cliffortioides* fallen leaves; P.R. Johnston (D353) leg.; 20 May 1989; PDD 55523 • Rangitoto Station, Ranginui Summit; on *Dracophyllum pyrimidale* fallen leaves; P.R. Johnston (D1622), S.R. Whitton leg.; 6 May 2001; PDD 117584.

Notes. Most specimens are on fallen leaves of *Dracophyllum* spp., but the host range may be more extensive. A specimen on *Fuscopora cliffortioides* (PDD 55523) is morphologically similar, but perhaps with longer ascospores.

Discussion

The *Brahmaculus* species described here are so morphologically and ecologically divergent from *Chlorociboria* that they must be placed in their own genus. All four new species are members of a well-supported monophyletic lineage within Chlorociboriaceae (Fig. 2). However, in both the multigene and ITS analyses (Figs 1, 2) the *Brahmaculus* clade makes *Chlorociboria*, as currently understood in a morphological sense, paraphyletic. If alternative generic limits were to be drawn to recognise only monophyletic genera within Chlorociboriaceae, it is unclear how these genera could be distinguished morphologically. The type of *Chlorociboria* (*C. aeruginosa*) sits within the main *Chlorociboria* clade, and hence the name *Chlorociboria* will remain attached to the bulk of the species so far described in the genus. However, further sampling of *Chlorociboria*, including of species lacking green pigments (see below) is required before redrawing generic limits, especially in regard to the distinguishing morphological characters of the main *Chlorociboria* clade in relation to the phylogenetically differentiated species *C. halonata* and *C. aeruginella*. The multi-gene phylogeny places Chlorociboriaceae in an isolated position near the base of Helotiales. Earlier analyses had suggested a relationship between Chlorociboriaceae and Cyttariaceae (Peterson and Pfister (2010a). The multiple genes newly available from a *Cyttaria nigra* specimen (PDD 117571) allowed Cyttariaceae to be treated in the multi-gene analysis. This showed that although Cyttariaceae was similar to Chlorociboriaceae in having an isolated position near the base of Helotiales, no particular phylogenetic relationship was found between the families. Cyttariales is treated here as a synonym of Helotiales.

Direct observations of the mycelium at the base of the stipes of several of the *Brahmaculus* spp. suggests a biotrophic relationship with either the roots of Nothofagaceae (possibly as root endophytes), or the mycorrhizal fungi associated with those roots (possibly as parasites). Johnston and Park (2005) noted a possible ecological relationship between wood rotting basidiomycetes and some of the wood-inhabiting *Chlorociboria* spp.

Not all of the specimens accepted here as *Chlorociboria* develop green pigment on their substrate. These include *C. glauca* and two of the newly described species from New Zealand (*C. metrosideri* and *C. solandri*). Both of these newly described species develop on fallen leaves, they have whitish rather than green apothecia, form no green pigment on their substrate, but have an excipular structure and the short, rough-walled, hair-like elements typical of several of the New Zealand representatives of the genus. The third newly named species from New Zealand, *Chlorociboria subtilis*, also develops on fallen leaves, but both the apothecia and the adjacent parts of the leaf have a blue-green pigment. The apothecial hairs of this species are better developed than those of most *Chlorociboria* species. Fungi morphologically similar to *C. subtilis* occur on fallen leaves in both eastern Australia (e.g. PDD 117581) and southern South America (unpubl. data) but they are not named here as only small specimens, and no DNA sequences, are available for these fungi.

Most known *Chlorociboria* species develop on green-stained, fallen wood. It is likely that there are other unrecognised *Chlorociboria* species, placed in other genera because they lack green pigment and have substrates apart from wood, the visually obvious features historically regarded as characteristic of *Chlorociboria*. Their true phylogenetic relationship may be revealed only when DNA sequence data becomes available for them, unless an alternative set of morphological features is discovered that is found to be characteristic of the Chlorociboriaceae clade. Huhtinen et al. (2010) discuss other seemingly ecologically or morphologically atypical *Chlorociboria* spp. from Europe. If these are shown to be *Chlorociboria* phylogenetically, they may be key to discovering phylogenetically informative morphological characters for the genus and family.

Conclusions

The phylogenetic breadth of Chlorociboriaceae is becoming better understood with the identification of *Brahmaculus* as a distinct lineage. For *Chlorociboria*, recognis-

ing that not all species form apothecia on green-stained wood is an important step in characterising the genus and family both morphologically and phylogenetically, and in resolving more accurately its geographic distribution globally.

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Multi-gene phylogenetic evidence indicates that Pleurodesmospora belongs in Cordycipitaceae (Hypocreales, Hypocreomycetidae) and Pleurodesmospora lepidopterorum sp. nov. on pupa from China

Wan-Hao Chen¹, Yan-Feng Han², Jian-Dong Liang¹, Wei-Yi Tian¹, Zong-Qi Liang²

 Basic Medical School, Guizhou University of Traditional Chinese Medicine, Guiyang 550025, Guizhou, China 2 Institute of Fungus Resources, Department of Ecology, College of Life Sciences, Guizhou University, Guiyang 550025, Guizhou, China

Corresponding author: Yan-Feng Han (swallow1128@126.com)

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Abstract

A new species, *Pleurodesmospora lepidopterorum*, isolated from a pupa, is introduced. Morphological comparisons and phylogenetic analyses based on multigene datasets (ITS+RPB1+RPB2+TEF) support the establishment of the new species. *Pleurodesmospora lepidopterorum* is distinguished from *P. coccorum* by its longer conidiogenous pegs located in the terminal or lateral conidiophores, and smaller subglobose or ellipsoidal conidia. A combined dataset of RPB1, RPB2, and TEF confirmed the taxonomic placement of *Pleurodesmospora* in Cordycipitaceae for the first time.

Keywords

Insect, morphological characteristic, new species, phylogenetic analysis, taxonomic placement

Introduction

The genus *Pleurodesmospora* was established for the type species *P. coccorum* (Petch) Samson, W. Gams & H.C. Evans (Samson et al. 1980). The typical characteristic of *Pleurodesmospora* is its erect or procumbent conidiophores, which bear numerous min-

ute phialidic conidiogenous pegs in the terminal or mostly intercalary position, often in whorls below the septa. Conidiogenous pegs are short-cylindrical and give rise to short chains of conidia. Conidia are ellipsoid to dacryoid with a slightly truncate base (Samson et al. 1980).

Pleurodesmospora species have diverse ecological characteristics, and have been found on scale insects, whitefly, aphids, leaf-hoppers, spider and scavenger mites (Petch 1931; Samson and McCoy 1982; Samson et al. 1980). Li et al. (1991) reported *Pleurodesmospora* as a newly recorded genus in China and confirmed for the first time that *P. coccorum* has strong pathogenicity to black whitefly. According to Index Fungorum, the taxonomic status of *Pleurodesmospora* is incertae sedis.

During a survey of entomopathogenic fungi from Southwest China, a new insect-associated species was found. The morphological characteristics of the new species resembled *Pleurodesmospora*. In our phylogenetic analyses of combined RPB1, RPB2 and TEF sequences, *Pleurodesmospora* clustered in Cordycipitaceae (Hypocreales, Hypocreomycetidae) with strong statistical support and was closely related to *Beauveria* Vuill. and *Akanthomyces* Lebert. Thus, we propose that *Pleurodesmospora* belongs to family Cordycipitaceae and introduce *Pleurodesmospora lepidopterorum* sp. nov. as a new insect-associated species on the basis of morphological comparison and molecular phylogenetic analyses.

Materials and methods

Specimen collection and identification

An infected pupa of Lepidoptera specimen (DY1050) was collected from Duyun City (26°21'24.71"N, 107°22'48.22"E), Qiannan Buyi and Miao Autonomous Prefecture, Guizhou Province, on 1 October 2019. Isolation of strains was conducted as described by Chen et al. (2019). Fungal colonies emerging from specimens were isolated and cultured at 25 °C for 14 days under 12 h light/12 h dark conditions following protocols described by Zou et al. (2010). Specimens and the isolated strains were deposited in the Institute of Fungus Resources, Guizhou University (formally Herbarium of Guizhou Agricultural College; code, GZAC), Guiyang City, Guizhou, China.

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

DNA extraction, polymerase chain reaction amplification and nucleotide sequencing

DNA extraction was carried out by Fungal genomic DNA Extraction Kit (DP2033, BioTeke Corporation) in accordance with Liang et al. (2011). The extracted DNA was stored at -20 °C. The internal transcribed spacer (ITS) region, RNA polymerase II

largest subunit 1 (RPB1), RNA polymerase II largest subunit 2 (RPB2) and translation elongation factor 1 alpha (TEF) were amplified by PCR as described by White et al. (1990), Castlebury et al. (2004) and van den Brink et al. (2004), respectively. PCR products were purified and sequenced at Sangon Biotech (Shanghai) Co. The resulting sequences were submitted to GenBank.

Sequence alignment and phylogenetic analyses

Lasergene software (version 6.0, DNASTAR) was applied for the assembling and editing of DNA sequence. The ITS, RPB1, RPB2 and TEF sequences were downloaded from GenBank, based on Mongkolsamrit et al. (2018, 2020) and others selected on the basis of BLAST algorithm-based searches in GenBank (Table 1). The multiple datasets of ITS, RPB1, RPB2 and TEF were aligned and edited by MAFFT v7.037b (Katoh and Standley 2013) and MEGA6 (Tamura et al. 2013). Assembling of the combined datasets (RPB1+RPB2+TEF and ITS+RPB1+RPB2+TEF) was performed by SequenceMatrix v.1.7.8 (Vaidya et al. 2011). The model was selected for Bayesian analysis by ModelFinder (Kalyaanamoorthy et al. 2017) in the software PhyloSuite (Zhang et al. 2020).

The datasets (RPB1+RPB2+TEF and ITS+RPB1+RPB2+TEF) were analyzed by Bayesian inference (BI) and maximum likelihood (ML) methods to determine the relationship among *Pleurodesmospora* and related genera in the order Hypocreales (analysis 1) and the relationship among *Pleurodesmospora* and related genera in the family Cordycipitaceae (analysis 2), respectively. For BI, a Markov chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian probabilities using MrBayes v.3.2 (Ronquist et al. 2012) for the combined sequence datasets. The Bayesian analysis resulted in 20,001 trees after 10,000,000 generations. The first 4,000 trees, representing the burn-in phase of the analyses, were discarded, while the remaining 16,001 trees were used for calculating posterior probabilities in the majority rule consensus tree. After the analysis was finished, each run was examined using the program Tracer v1.5 (Drummond and Rambaut 2007) to determine burnin and confirm that both runs had converged. ML analyses were constructed with RAxMLGUI (Silvestro et al. 2012). The GTRGAMMA model was used for all partitions, in accordance with recommendations in the RAxML manual against the use of invariant sites.

Results

Phylogenetic analyses

Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams isolates (AFTOL ID.187 and GJS 90227) were used as the outgroup in analysis 1 (Fig. 1), and *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson isolates (CBS 284.36 and CBS 431.87) were used as the outgroup in analysis 2 (Fig. 2).

Species	Strain No.	GenBank accession No.			
	-	ITS	RPB1	RPB2	TEF
Akanthomyces aculeatus	HUA 186145	-	_	_	MF416465
	HUA 772	KC519371	-	-	KC519366
Akanthomyces attenuates	CBS 402.78	-	EF468888	EF468935	EF468782
Akanthomyces lecanii	CBS 101247	-	DQ522407	DQ522466	DQ522359
Akanthomyces waltergamsii	TBRC 7251	-	MF140781	MF140805	MF140833
	TBRC 7252		MF140782	MF140806	MF140834
Ascopolyporus polychrous	P.C. 546	_	DQ127236	-	DQ118745
Ascopolyporus villosus	ARSEF 6355	AY886544	DQ127241	-	DQ118750
Beauveria bassiana	ARSEF 1564	HQ880761	HQ880833	HQ880905	HQ880974
	ARSEF 7518	HQ880762	HQ880834	HQ880906	HQ880975
Beauveria brongniartii	ARSEF 617	-	HQ880854	HQ880926	HQ880991
Beauveria caledonica	ARSEF 2567	_	HQ880889	HQ880961	EF469057
Blackwellomyces cardinalis	OSC 93609	_	DQ522370	DQ522370	DQ522325
	OSC 93610	JN049843	EF469088	EF469106	EF469059
Claviceps purpurea	S.A. cp11	_	EF469087	EF469105	EF469058
Clonostachys rosea	AFTOL ID.187	-	-	DQ862029	-
	GJS 90227	_	-	-	AY489611
Conoideocrella luteorostrata	NHJ 11343	_	EF468906	-	EF468801
	NHJ 12516	_	EF468905	EF468946	EF468800
Cordyceps kyusyuensis	EFCC 5886	_	EF468863	-	EF468754
Cordyceps militaris	OSC 93623	JN049825	DQ522377	-	DQ522332
Cordyceps ninchukispora	E.G.S.38.165	_	EF468900	-	EF468795
	E.G.S.38.166	_	EF468901	-	EF468794
Cordyceps piperis	CBS 116719	_	DQ127240	EU369083	DQ118749
Gibellula gamsii	BCC 25798	MH152532	EU369056	EU369076	EU369018
-	BCC 27968	MH152529	MH152547	-	MH152560
Hevansia novoguineensis	CBS 610.80	MH532831	-	MH521844	MH521885
	NHJ 11923	_	EU369052	EU369072	EU369013
Hyperdermium pulvinatum	P.C. 602	_	DQ127237	-	DQ118746
Lecanicillium antillanum	CBS 350.85	MH861888	DQ522396	DQ522450	DQ522350
Lecanicillium psalliotae	CBS 101270	_	EF469096	EF469112	EF469067
-	CBS 532.81	_	EF469095	EF469113	EF469066
Lecanicllium tenuipes	CBS 309.85	_	DQ522387	DQ522439	DQ522341
Metarhizium anisopliae	ARSEF 7487	_	DQ468355	DQ468370	DQ463996
	CBS 130.71	_	MT078861	MT078918	MT078845
Metarhizium flavoviride	CBS 125.65	-	MT078862	MT078919	MT078846
	CBS 700.74	_	MT078863	MT078920	MT078847
Neotorrubiella chinghridicola	BCC 39684	_	MK632071	MK632181	MK632148
_	BCC 80733	_	MK632072	MK632176	MK632149
Ophiocordyceps gracilis	EFCC 8572	_	EF468859	EF468912	EF468751
Ophiocordyceps sinensis	EFCC 7287	-	EF468874	EF468924	EF468767
Orbiocrella petchii	NHJ 6209	-	EU369061	EU369081	EU369023
Pleurodesmospora coccorum	CBS 458.73	MH860741	_	_	_
-	CBS 459.73	MH860742	_	_	_
	CBS 460.73	MH860743	-	-	-
Pleurodesmospora	DY10501	MW826576	MW834315	MW834316	MW834317
lepidopterorum	DY10502	MW826577	-	MW834318	MW834319
Polycephalomyces formosus	ARSEF 1424	_	DQ127245	KF049671	DQ118754
Polycephalomyces paracuboideus	NBRC 101742	_	KF049647	KF049669	KF049685
Purpureocillium lilacinum	ARSEF 2181	_	EF468896	_	EF468790
	CBS 431.87	_	EF468897	EF468940	EF468791

Table 1. Taxa included in the phylogenetic analyses.

Species	Strain No.	GenBank accession No.			
		ITS	RPB1	RPB2	TEF
Purpureocillium lilacinum	CBS 284.36	MH855800	EF468898	EF468941	EF468792
Samsoniella aurantia	TBRC 7271	_	MF140791	-	MF140846
	TBRC 7272	MF140763	_	MF140817	MF140845
Simplicillium lanosoniveum	CBS 101267	_	DQ522405	DQ522463	DQ522357
	CBS 704.86	AJ292396	DQ522406	DQ522464	DQ522358
Yosiokobayasia kusanagiensis	TNS-F18494	-	JN049890	_	JF416014



Figure 1. Phylogenetic relationships among *Pleurodesmospora* and related genera in the order Hypocreales based on a multigene dataset (RPB1, RPB2, and TEF). Statistical support values (\geq 50%/0.5) are shown at the nodes for maximum likelihood bootstrap support/ Bayesian inference posterior probabilities.



Figure 2. Phylogenetic relationships among *Pleurodesmospora* and related genera in the family Cordycipitaceae based on a multigene dataset (ITS, RPB1, RPB2 and TEF). Statistical support values (\geq 50%/0.5) are shown at the nodes for maximum likelihood bootstrap support/Bayesian inference posterior probabilities.

The concatenated sequences of analysis 1 and 2 included 23 and 21 taxa, respectively, and consisted of 2,262 (RPB1, 561; RPB2, 821; and TEF, 880) and 2,711 (ITS, 597; RPB1, 508; RPB2, 852; and TEF, 754) characters with gaps, respectively.

Analysis 1: The final value of the highest scoring tree was -18,860.236896, which was obtained from the ML analysis of the dataset (RPB1+RPB2+TEF). The parameters of GTR model to analysis of the dataset were estimated base frequencies; A = 0.240138,

C = 0.290732, G = 0.262224, T = 0.206905; substitution rates AC = 1.004710, AG = 3.103423, AT = 0.837508, CG = 0.886482, CT = 5.821155, GT = 1.000000; gamma distribution shape parameter α = 0.309925. The selected model for BI analysis were K2P+G4 (RPB2) and GTR+F+I+G4 (RPB1+TEF). In the order-level phylogenetic tree (Fig. 1), the maximum likelihood and Bayesian inference trees were generally congruent, and most branches were strongly supported. The new strains clustered with the genera *Cordyceps, Akanthomyces*, and *Beauveria*, and belonged to family Cordycipitaceae.

Analysis 2: The final value of the highest scoring tree was -19,321.404482, which was obtained from the ML analysis of the dataset (ITS+RPB1+RPB2+TEF). The parameters of GTR model to analysis of the dataset were estimated base frequencies; A = 0.238334, C = 0.298168, G = 0.261443, T = 0.202055; substitution rates AC = 0.963749, AG = 2.807654, AT = 0.822463, CG = 0.766574, CT = 5.738062, GT = 1.000000; gamma distribution shape parameter α = 0.339059. The selected model for BI analysis were HKY+F+G4 (ITS) and GTR+F+I+G4 (RPB1+RPB2+TEF). In the family-level phylogenetic tree (Fig. 2), the maximum likelihood and Bayesian inference trees were generally congruent, and most branches were strongly supported. The new strains formed an independent branch but clustered with *Pleurodesmospora coccorum*; therefore, these strains represent a new species described as *P. lepidopterorum*.

Taxonomy

Pleurodesmospora lepidopterorum W.H. Chen, Y.F. Han & Z.Q. Liang, sp. nov. MycoBank No: 839148 Figure 3

Diagnosis. Differs from *P. coccorum* by having longer conidiogenous pegs located in the terminal or lateral conidiophores, and smaller subglobose or ellipsoidal conidia.

Type. CHINA, Guizhou Province, Qiannan Buyi and Miao Autonomous Prefecture, Duyun City (26°21'24.71"N, 107°22'48.22"E), 1 October 2019, Wanhao Chen, holotype GZAC DY1050, ex-type culture GZAC DY10501. Sequences from isolated strain DY10501 have been deposited in GenBank with accession numbers: ITS = MW826576, RPB1 = MW834315, RPB2 = MW834316 and TEF = MW834317.

Description. Colonies on PDA, 3.9–4.1 cm diam. in 14 d at 25 °C, white, consisting of a basal felt and cottony, floccose hyphal overgrowth, reverse pale yellowish. Prostrate hyphae smooth, septate, hyaline, 1.3–1.9 μ m diam. Erect or procumbent conidiophores usually arising from aerial hyphae, barely differentiated from vegetative hyphae, usually branched. Conidiogenous cells polyphialidic, terminal and intercalary, bearing numerous short-cylindrical, 1.8–3.5 μ m long and 0.7–1.3 μ m wide conidiogenous cells cylindrical, 5.9–12.0 × 1.8–2.2 μ m. Conidia in chains, hyaline, smooth-walled, subglobose or ellipsoidal, one-celled, 2.3–3.6 × 1.7–3.3 μ m. Chlamydospores and synnemata not observed. Size and shape of phialides and conidia similar in culture on PDA, OA agar and on natural substrate. Sexual state not observed.

Host. Pupa, order Lepidoptera.



Figure 3. *Pleurodesmospora lepidopterorum* **A** infected pupa (Lepidoptera) **B**, **C** top (**B**) and underside (**C**) of a colony cultured on PDA medium at 14 d **D–J** conidiogenous pegs and conidia **K** conidia in chains. Scale bars: 10 mm (**B**, **C**) 10 µm (**D–K**).

Distribution. Duyun City, Qiannan Buyi and Miao Autonomous Prefecture, Guizhou Province, China.

Etymology. Referring to its insect host, which belongs to order Lepidoptera.

Remarks. *Pleurodesmospora lepidopterorum* was readily identified as belonging to *Pleurodesmospora* in the family-level phylogenetic tree (Fig. 2). When compared with the typical characteristics of *P. coccorum*, *P. lepidopterorum* was easily distinguished by its longer conidiogenous pegs located in the terminal or lateral conidiophores, and smaller subglobose or ellipsoidal conidia.

Discussion

BLAST results of ITS, RPB1, RPB2, and TEF sequence data revealed that the strain DY10501 was similar to several taxa in GenBank: ITS, 98.62% similar to *Lecanicillium* sp. (isolate ICMP:20146); RPB1, 88.55% similar to *Beauveria caledonica* Bissett & Widden (isolate ARSEF 7117); RPB2, 86.53% similar to *Cordyceps* sp. (isolate

A12116); TEF, 95.33% similar to *Beauveria bassiana* (Bals.-Criv.) Vuill. (isolate CHE-CNRCB 82). In the family-level phylogenetic tree, strains DY10501 and DY10502 formed an independent branch and clustered with *P. coccorum* in a subclade.

Samson et al. (1980) introduced the genus *Pleurodesmospora* with *P. coccorum*, but the taxonomic status of the genus was unclear. Unfortunately, *P. coccorum* lacked RPB1, RPB2, and TEF sequences in GenBank. Therefore, *P. lepidopterorum* was used for multigene analysis of *Pleurodesmospora* and related genera in the order Hypocreales. In the order-level phylogenetic tree, *P. lepidopterorum* clustered into Cordycipitaceae (Hypocreales, Hypocreomycetidae, Sordariomycetes). Thus, the combined dataset of RPB1, RPB2, and TEF confirmed the taxonomic placement of *Pleurodesmospora* in Cordycipitaceae for the first time.

Acknowledgements

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Supplementary material I

Dataset for Figure 1

Authors: Wan-Hao Chen, Yan-Feng Han, Jian-Dong Liang, Wei-Yi Tian, Zong-Qi Liang Data type: molecular data

Explanation note: A dataset of RPB1, RPB2 and TEF for Figure 1.

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Link: https://doi.org/10.3897/mycokeys.80.66794.suppl1

Supplementary material 2

Dataset for Figure 2

Authors: Wan-Hao Chen, Yan-Feng Han, Jian-Dong Liang, Wei-Yi Tian, Zong-Qi Liang Data type: molecular data

Explanation note: A dataset of ITS, RPB1, RPB2 and TEF for Figure 2

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Supplementary material 3

Table S1. Taxa included in the phylogenetic analyses

Authors: Wan-Hao Chen, Yan-Feng Han, Jian-Dong Liang, Wei-Yi Tian, Zong-Qi Liang Data type: molecular data

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RESEARCH ARTICLE



Diversity of Plectosphaerella within aquatic plants from southwest China, with P. endophytica and P. sichuanensis spp. nov.

Xiao Qian Yang^{1*}, Shi Yun Ma^{1*}, Ze Xiang Peng², Zhong Qiao Wang², Min Qiao¹, Zefen Yu¹

I Laboratory for Conservation and Utilization of Bio-resources, Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University, Kunming, Yunnan, 650091, China 2 Tianma development office of Yiliang county, Yunnan Province, China

Corresponding author: Zefen Yu (zfyuqm@hotmail.com)

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Abstract

Members of *Plectosphaerella* inhabit different substrates, including plants, soil and insects, and most species are pathogens causing large losses in agriculture. During a survey of endophytic fungi in aquatic plants in southwest China, 112 strains of *Plectosphaerella* were isolated, representing two new species, *P. endophytica* **sp. nov.** and *P. sichuanensis* **sp. nov.**, as well as two known species, *P. cucumerina* and *P. pauciseptata*. The novel taxa are described and illustrated here using combined morphological and multi-locus phylogenetic (LSU-ITS-TEF-1 α -TUB2) analyses. Our result revealed *Plectosphaerella* species inhabiting within aquatic plants in southwest China, and the separation frequency of each species was presented.

Keywords

Endophytic fungi, multi-locus phylogeny, new species, Plectosphaerellaceae, Sordariomycetes

^{*} Contributed equally as the first authors.

Introduction

The genus *Plectosphaerella* Kleb. was established to accommodate *P. cucumeris* Kleb. from young cucumber plants (Klebahn 1929). Previously, it was always placed in Hypocreaceae (Sordariomycetes, Hypocreales) and Sordariaceae (Sordariomycetes, Sordariales) (Domsch and Gams 1972; Uecker 1993), until Zare et al. (2007) established Plectosphaerellaceae W. Gams, Summerb. & Zare (Glomerellales) to accommodate it (Réblová et al. 2011). The genus *Plectosporium* Palm, Gams & Nirenberg was described as the asexual morph of *Plectosphaerella* (Palm et al. 1995). Given its priority, *Plectosphaerella* was recommended as the accepted generic name (Réblová et al. 2016), and all species of *Plectosporium* were transferred into *Plectosphaerella* (Carlucci et al. 2012). At present, there are 23 species records for *Plectosphaerella* as listed in Index Fungorum (2021), two species, *P. himantia* (Pers.) Kirschst. and *P. melaena* (Fr.) Kirschst. are not recognized for *Plectosphaerella*.

Species of *Plectosphaerella* are distributed in a variety of habitats and have wide geographic distribution (Carlucci et al. 2012; Zhang et al. 2019). Some species are pathogens on the fruit, root or collar in Cucurbitaceae plant, causing large losses of melon, pumpkin and zucchini crops (Alfaro-García et al. 1996; Toyozo et al. 2005; Raimondo and Carlucci 2018). Several species harm other plants such as tomato, pepper, bamboo, and asparagus. And the symptoms of hosts are decomposition, breakdown and death (Antignani et al. 2008; Carlucci et al. 2012; Arzanlou et al. 2013). Besides diseased plants, *P. sinensis* Lei Su & Y.C. Niu was reported as endophytic fungus without causing obvious symptoms (Su et al. 2017). In addition, most species causing plant diseases were also isolated from soil, except for *P. oratosquillae* (P.M. Duc et al.) A.J.L. Phillips et al. originating from arthropod *Oratosquilla oratoria* (Duc et al. 2009).

Plectosphaerella cucumerina is the most widely distributed species, thriving on more than nine plant genera: *Arabidopsis, Cucumis, Galium, Hydrilla, Nicotiana, Pyrus, Solanum, Viola* and *Austropotamobius* etc., and also occurring in soil and paper (Alderman and Polglase 1985; Palm et al. 1995; Smith-Kopperl et al. 1999; Domsch et al. 2007; Giraldo and Crous 2019). Meanwhile, this species has a wide geographic distribution, and it has been reported from England, Italy, Canada, the Netherlands, Egypt etc. (Raimondo and Carlucci 2018, Giraldo et al. 2019). The second species with wide geographic distribution, *P. plurivora* A.J.L. Phillips et al., was found in Australia, the Netherlands, Germany, USA etc. Its hosts include *Lolium, Solanum, Nicotiana, Asparagus* and it also occurs in soil (Carlucci et al. 2012; Giraldo and Crous 2019). However, other species did not show obvious habitats diversity and wide geographic distribution.

During our investigation of endophytic fungal diversity of aquatic plants in southwest China, among 1697 acquired strains, 112 strains belonging to *Plectosphaerella* were isolated. Based on morphological characteristics and phylogenetic analysis, two known species, *P. cucumerina* and *P. pauciseptata*, were described, and two new species, *P. endophytica* and *P. sichuanensis*, were proposed and illustrated. The geographic distribution and habitat diversity of *Plectosphaerella* in this study were also discussed.

Materials and methods

Isolates and Morphology

Samples were collected from Yunnan, Guizhou, Sichuan provinces, Chongqing and Tibet from 2014 to 2017. The dominant hosts are Myriophyllum, Potamogeton, Hydrilla and Hippuris. Samples were placed in plastic bags, labeled and transported to the laboratory. Each leaf and stem was cut into segments 30-40 mm in length and washed thoroughly with tap water, then a surface-disinfection was carried out according to Su et al. (2016). The segments were cut into smaller sections of about 5×5 mm under the aseptic operating table. Firstly, washed 30 s in sterile water and soaked 2 min in 0.5% hypochlorite solution, then 30 s in sterile water and 2 min in 75% ethanol, finally 30 s in sterile water. Each ten sections were randomly isolated on rose bengal agar (RBA, Guangdong Huankai Microbial Sci and Tech), the antibiotics chloramphenicol (0.1 g 1⁻¹) was added to restrain bacterial growth. When a fungus grew up from the segments, some hyphae were picked up and transferred to potato dextrose agar (PDA, 200 g potato, 20 g dextrose, 18 g agar, 1000 ml distilled water) plates for incubation at 28 °C. After 10 days, colonies were transferred to different plates, including corn meal agar (CMA, 20 g cornmeal, 18 g agar, 1000 ml distilled water) and oatmeal agar (OA, 30 g filtered oat flakes, 20 g agar, 1000 ml distilled water). Colony characteristics, growth speed and other macrostructure from PDA plates were observed after 10 days. Microscopic characteristics such as mycelium, conidiophores and conidia were examined and measured after 3 days on CMA using BX51 microscope (Olympus); the sterile water was used as a mounting medium.

Pure cultures were deposited in the Herbarium of the Laboratory for Conservation and Utilization of Bio resources, Yunnan University, Kunming, Yunnan, P.R. China (YMF, formerly Key Laboratory of Industrial Microbiology and Fermentation Technology of Yunnan) and at the China Center for Type Culture Collection (**CCTCC**).

DNA extraction, PCR amplification and sequencing

Actively growing mycelium was scraped off from the surface of the culture and transferred to 2 ml Eppendorf micro-centrifuge tubes. Total genomic DNA was extracted follow the protocol of Guo et al. (2000). The internal transcribed spacer (ITS) and the 28S large subunit nuclear ribosomal RNA (LSU rRNA) were amplified using the primer pairs ITS1/ITS4 (White et al. 1990) and LROR/LR5 (Vilgalys and Hester 1990), respectively. Translation elongation factor 1-alpha (TEF-1 α) and partial β -tubulin (TUB2) were amplified using the primer pairs EF-1251R/EF-688F (Alves et al. 2008) and Bt2a/Bt2b (Glass and Donaldson 1995), respectively. The PCR amplifications were conducted in 25 µl final volumes which consisted of 1.0 µl DNA template, 1.0 µl of each forward and reverse primers, 12.5 µl 2 × Master Mix and 9.5 µl ddH₂O. The PCR reaction cycles were as follows: initial denaturation at 94 °C for 3 min; followed by 35 cycles of denaturation at 94 °C for 40 sec; the annealing extension dependent on the amplified loci (48 °C for LSU, 54 °C for ITS, 55 °C for TEF-1 α and 58 °C for TUB2) for 1 min and extension at 72 °C for 2 min; a final extension at 72 °C for 10 min. PCR products were sequenced by TSINGKE Biological Technology in Kunming, China. The sequences are deposited in GenBank database and the accession numbers are listed in Table 1.

Phylogenetic analyses

The ITS sequences generated in this study were used as a query to search similar DNA sequences using BLASTn. All published DNA sequences were obtained from the Gen-Bank from relevant studies (Su et al. 2017; Phookamsak et al. 2019; Zhang et al. 2019), *Monilochaetes infuscans* Harter (Australiascaceae) was selected as an outgroup. The generated sequences were manually aligned with CLUSTAL_X v. 1.83 (Thompson et al. 1997) with default parameters. Aligned sequences of multiple loci were concatenated and manually adjusted through BioEdit version v. 7.0.4.1 (Hall 1999), and ambiguously aligned regions were excluded. The combined sequence was converted to a NEXUS file using MEGA6 (Tamura 2013). The alignment was deposited at Tree-Base http://purl.org/phylo/treebase/phylows/study/TB2:S27363.

Maximum-likelihood (ML) analysis was conducted by using RAxML (Stamatakis 2006) with the PHY files generated with CLUSTAL X v. 1.83 (Thompson et al. 1997), using the GTR+GAMMA model. ML bootstrap proportions (MLBPs) were computed with 1000 replicates. Bayesian inference (BI) analysis was executed with MrBayes v. 3.2.2 (Ronquist and Huelsenbeck 2003). The Akaike information criterion (AIC) implemented in jModelTest version 2.0 was used to select the best fit models after likelihood score calculations (Posada 2008). HKY+I+G was estimated as the best-fit model under the output strategy of AIC, Lsetnst=6, rates=gamma. A Markov Chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian probabilities. Two runs were executed simultaneously for 6,000,000 generations and sampled every 500th generations, four chains containing one cold and three heated were run until the average standard deviation of the split frequencies dropped below 0.01, the stationarity of the analyses was confirmed in line with standards described by Sun and Guo (2010). The initial 25% of the generations of MCMC sampling were discarded as burn-in. The refinement of the phylogenetic tree was used for estimating BI posterior probability (BIPP) values. The tree was viewed in FigTree version 1.4 (Rambaut 2012).

Results

Phylogenetic analyses

A total of 112 strains were identified as members of *Plectosphaerella* according to the BLASTn search results using the ITS sequences. At first, we carried out individual

Table 1. Plectosphaerella species used in phylogenetic analyses.

Species	Strain no.	GenBank accession no.			
•	-	LSU	ITS	TEF-1a	TUB2
Monilochaetes infuscans	CBS 379.77	GU180645	LR026764	-	-
Plectosphaerella alismatis	CBS 113362 ^T	KY662261	KY399815	KY421328	KY421304
P. citrullae	CBS 131740	KY662254	KY399821	KY421319	KY421307
	CBS 131741 ^T	KY662255	KY399822	KY421320	KY421308
P. cucumerina	3F17	KY399831	KY401602	KY421331	KY421300
	3F24	KY399832	KY401603	KY421332	KY421301
	3F42	KY399829	KY401604	KY421330	KY421299
	3F43	KY399830	KY401605	KY421334	KY421302
	CBS 131739	KY662258	KY399816	_	_
	CBS 137.37	KY662256	KY399827	-	-
	YMF 1.04692	MW326671	MW024075	MW441776	MW441782
P. delsorboi	CBS 116708 ^T	EF543843	KY499237	KY499238	KY499240
P. endophytica	YMF 1.04701	MW024052	MW024054	MW029607	MW029608
P. guizhouensis	CGMCC 3.19658 ^T	MK880431	MK880441	_	_
	CGMCC 3.19659	MK880432	MK880442	_	_
	CGMCC 3.19660	MK880433	MK880443	-	-
P. hanneae	CBS 144925	LR590378	LR590201	-	-
P. humicola	CBS 423.66 ^T	LR025949	LR026811	_	_
P. kunmingensis	KUMCC 18-0181	MK993015	MK993014	-	-
P. melonis	CBS 489.96 ^T	KY662260	KY399813	KY421329	KY421295
	CBS 409.95	KY662259	KY399814	_	KY421296
P. nauculaspora	CGMCC 3.19656 ^T	MK880424	MK880439	-	-
	CGMCC 3.19657	MK880425	MK880440	-	-
P. oligotrophica	CGMCC 3.15078 ^T	JX508806	KY399817	KY421315	KY421309
	CGMCC 3.15079	JX508807	KY399818	KY421316	KY421310
P. oratosquillae	NJM 0662 ^T	-	AB425974	-	-
	NJM 0665	-	AB425975	-	-
P. pauciseptata	CBS 131744	KY662251	KY399824	KY421322	-
	CBS 131745 ^T	KY662250	KY399823	KY421321	-
	YMF 1.05088	MW326672	MW024087	MW441777	-
	YMF 1.04679	MW326673	MW024105	MW441778	MW441783
	YMF 1.04725	MW466575	MW024104	MW441779	MW441784
P. populi	CBS139623 ^T	KY662257	KY399828	KY421336	KY421311
	CBS 139624	KR476784	KR476751	-	-
P. plurivora	CBS131742 ^T	KY662248	KY399826	KY421324	KY421298
P. ramiseptata	CBS 131743	KY662252	KY399819	KY421318	KY421306
	CBS 131861 ^T	KY662253	KY399820	KY421317	KY421305
P. sichuanensis	YMF 1.05081	MW027535	MW027539	MW309880	MW441780
	YMF 1.05082	MW027536	MW027534	MW441775	MW441781
P. sinensis	ACCC 39144	KX527892	KX527889	KY421326	KY421313
	ACCC39145 ^T	KX527891	KX527888	KY421325	KY421312
	ACCC 39146	KX527893	KX527890	KY421327	KY421314
P. verschoorii	CBS 144924 ^T	LR590476	LR590240	-	-
	JW 13006	LR590415	LR590241	-	-
	JW 62006	LR590426	LR590252	-	-

Strains and sequences generated in this study are emphasized in bold face. ^Tex-type cultures.

phylogenetic analyses with ITS sequences to resolve the taxonomic position of our strains using the sequences of the accepted species into *Plectosphaerella*. This tree is shown in Figure 1. The result indicated that there are 77 strains clustered together with *P. pauciseptata* A.J.L. Phillips et al. with 0.92 Bayesian posterior probability, 27 strains clustered together with *P. cucumerina* with 0.96 Bayesian posterior probability, whereas



Figure 1. Phylogenetic tree inferred from a Bayesian analysis based on ITS sequences of 112 *Plectosphaerella* strains obtained in this study. BIPP over 80% are shown on the respective branches. The scale bar shows the expected changes per site. Two new species are given in boldface. *Monilochaetes infuscans* CBS 379.77 serves as an outgroup.



Figure 1. Continued.

other strains formed two individual groups. Therefore, three representative strains of *P. pauciseptata*, a representative strain of *P. cucumerina*, and three representative strains of two novel taxa were chosen among the 112 strains for single-gene and combined phylogenetic analysis.

The four Bayesian trees derived from the single-gene sequence alignments (LSU, ITS, TEF-1 α , TUB2) confirmed that the novel taxa were distant from other known species in *Plectosphaerella*. The Bayesian trees are available in the Suppl. material 1. The resulting combined sequence matrix included 2136 nucleotide positions (804 from LSU, 545 from ITS, 413 from TEF-1 α , 374 from TUB2), with *M. infuscans* CBS 379.77 as the outgroup. The tree topology is shown in Figure 2, with the Bayesian posterior probabilities over 80% and ML bootstrap support over 50% indicated for respective clades. In this phylogenetic tree, *P. endophytica* represented by strain YMF 1.04701 was close to *P. oratosquillae* (NJM 0662 and NJM 0665) and formed a single clade with 0.97 Bayesian posterior probability and 57% ML bootstrap proportions. Similarly, *P. sichuanensis* represented by strains YMF 1.05081 and YMF 1.05082 were close to *P. populi* Ullah et al. (CBS 139623) with 1.00 Bayesian posterior probability and 100% ML bootstrap proportions. Considering distinct morphological differences, we propose to describe our isolates as two new species in *Plectosphaerella*.



Figure 2. Phylogenetic tree of *Plectosphaerella* based on Bayesian analyses and Maximum Likelihood analyses of the combined sequences dataset of LSU, ITS, TEF-1 α and TUB2. The numbers above branches represent BIPP (left) and MLBPs (right). BIPP over 80% and MLBPs over 50% are shown on the respective branches. The scale bar shows the expected changes per site. Two new species are given in boldface. *Monilochaetes infuscans* CBS 379.77 serves as an outgroup.

Taxonomy

Plectosphaerella endophytica Z.F. Yu & X.Q. Yang, sp. nov.

MycoBank No: 838656 Figure 3

Etymology. Latin, endophytica meaning endophytic, growing within plant tissue.

Description. Colony on CMA after 3 d, hyphae hyaline, smooth, septate, thinwalled, branched, 1.9–3.3 μ m ($\bar{x} = 2.6 \mu$ m, n = 10) wide. Conidiophores macronematous, mononematous, erect, straight or flexuous, smooth-walled, hyaline, unbranched or occasionally irregular branched, sometimes 1–2-septate. Conidiogenous cells phialides, subulate, integrated, terminal, determinate, hyaline, smooth-walled. Conidia solitary, acrogenous, broadly navicular to broadly fusiform, suboblong or ellipsoidal, 0–1-septate, usually constricted at septum, bi-guttulate, hyaline, smooth-walled, aseptate conidia abundant, 5–9.1 × 2.5–3.5 μ m ($\bar{x} = 7.8 \times 3.1 \mu$ m, n = 30); septate conidia scarce, 8.8–10.1 × 3.7–4.6 μ m ($\bar{x} = 9.4 \times 4.1 \mu$ m, n = 30), forming hyaline to white mucilaginous masses. Sexual morph and chlamydospores absent.

Culture characteristics. Colonies on OA reaching 52 mm diameter, on PDA reaching 48 mm diameter and on CMA reaching 43 mm diameter in 14 d at 25 °C. On PDA, colonies white, dense, fluffy hyphae growth in the medium surface, outermost mycelia formed an annule, margin smooth and entire, sporulation abundant, reverse pale yellow to white.

Typification. CHINA, Yunnan Province, Kunming, The Dian Lake, 24°96'N, 102°66'E, 1886 m alt., isolated from *Hydrilla verticillata* (L.f.) Royle as an endophyte, 20 Jul. 2014, Z.F. Yu, YMF 1.04701 (Holotype), ex-type CCTCC AF 2021053.

Notes. Although the phylogenetic analyses showed that our isolate *Plectosphaerella endophytica* is close to *P. oratosquillae*, the conidia of *P. oratosquillae* are aseptate, multiguttulate (Duc et al. 2009). Furthermore, *P. endophytica* is most similar to *P. verschoorii* Hern.-Restr. & Giraldo López in the septa of conidia; both species produce 0–1-septate conidia, and septate conidia are larger than aseptate conidia (*P. verschoorii*: 1-septate conidia, 8–11.5 × 2–3 µm; aseptate conidia, 3–8.5 × 2–3 µm), but there are obvious difference in the shape of conidia, *P. endophytica* was deeply constricted at septa. Besides, the phialides of *P. verschoorii* are shorter (up to 14 µm) (Giraldo et al. 2019).

Plectosphaerella sichuanensis Z.F. Yu & X.Q. Yang, sp. nov.

MycoBank No: 838657 Figure 4

Etymology. Latin, *sichuanensis*, referred to Sichuan Province, the locality where the fungus was found.

Description. Colony on CMA after 3 d, vegetative hyphae hyaline, septate, commonly branched, smooth, thin-walled, mostly 2.5–3.5 μ m ($\bar{x} = 2.9 \mu$ m, n = 10) wide. Conidiophores macronematous, mononematous, erect, straight or flexuous to sinu-



Figure 3. *Plectosphaerella endophytica* (YMF 1.04701, holotype) **A–C** colony on OA, PDA and CMA after 14 d **D–F** conidiophores and Phialides **G** conidia. Scale bars: 1.35 cm (**A–C**), 10 μm (**D–G**).



Figure 4. *Plectosphaerella sichuanensis* (YMF 1.05081, holotype) **A–C** colony on OA, PDA and CMA after 14 d **D–H** conidiophores and Phialides I conidia. Scale bars: 1.35 cm (**A–C**), 10 μm (**D–I**).

ate, hyaline, smooth, unbranched or rarely branched, aseptate. Conidiogenous cells phialides, integrated, terminal, determinate, subulate, hyaline, smooth. Conidia acrogenous, ellipsoidal, unicellular, smooth-walled, hyaline, 1–3 guttulate, 4.2–6.8 × 2.5–3.7 μ m ($\bar{x} = 5.2 \times 3.3 \mu$ m, n = 30), forming hyaline to white mucilaginous masses. Sexual morph and chlamydospores absent.

Culture characteristics. Colonies on OA reaching 50 mm diameter, on PDA reaching 47 mm diameter and on CMA reaching 42 mm diameter in 14 d at 25 °C. On PDA, colonies pale brown to white, flat, repressed, plicated, partly immersed, a few white aerial hyphae grew in the middle of the medium, margin regular, frontier distinct, reverse pale brown to white.

Typification. CHINA, Sichuan Province, Daofu, 30°98'N, 101°13'E, 2960 m alt., isolated from *Potamogeton pectinatus* as an endophyte, 20 Jul. 2015, Z.F. Yu, YMF 1.05081 (Holotype), ex-type CCTCC AF 2021054, another strain checked: YMF 1.05082.

Notes. In the phylogenetic tree, the closest species to *Plectosphaerella sichuanensis* is *P. populi*, but *P. populi* can be distinguished from *P. sichuanensis* by its smaller aseptate conidia (Crous et al. 2015). The size and shape of conidia of *P. sichuanensis* is more similar to *P. cucumerina*, expect that *P. cucumerina* presents longer phialides (up to 69 µm) (Carlucci et al. 2012). In addition, *P. sichuanensis* resembles *P. citrullae* Carlucci et al., *P. pauciseptata* and *P. oratosquillae* in lacking septate conidia. However, it can be distinguished from *P. citrullae* by polyphialides conidiogenous cells; from *P. pauciseptata* and *P. oratosquillae* by bi-guttulate conidia (Duc et al. 2009; Carlucci et al. 2012).

Plectosphaerella cucumerina (Lindf.) W. Gams, in Domsch & Gams Figure 5

Description. Colony on CMA after 3 d, hyphae hyaline, septate, smooth, thin-walled, branched, 2.5–3.5 μ m ($\bar{x} = 3.2 \mu$ m, n = 10) wide. Conidiophores macronematous, mononematous, erect or flexuous to sinuate, hyaline, smooth, branched, occasionally forming hyphal coils. Conidiogenous cells phialides, terminal, determinate, subulate. Conidia acrogenous, hyaline, unicellular, smooth-walled, oblong-ellipsoidal, 1–2 guttulate, 6.6–10.7 × 2.3–3.6 μ m ($\bar{x} = 8.7 \times 3.1 \mu$ m, n = 30), forming hyaline to white mucilaginous masses. Sexual morph and chlamydospores absent.

Culture characteristics. Colonies on OA reaching 55 mm diameter, on PDA reaching 48 mm diameter and on CMA reaching 44 mm diameter in 14 d at 25 °C. On PDA, colonies pale brown, repressed, flat, partly immersed, some aerial hyphae grew in the middle and margin of the medium, margin regular, reverse pale brown.

Strain examined. CHINA, Sichuan Province, Baiyu, 31°00'N, 99°41'E, 4013 m alt., isolated from *Myriophyllum spicatum* as an endophyte, 20 Aug. 2015, Z.F. Yu, YMF 1.04692.



Figure 5. *Plectosphaerella cucumerina* (YMF 1.04692) **A–C** colony on OA, PDA and CMA after 14 d **D** hyphal coils **E**, **F** conidiophores and Phialides **G** conidia. Scale bars: 1.35 cm (**A–C**), 10 µm (**D–G**).



Figure 6. *Plectosphaerella pauciseptata* (YMF 1.05088) **A–C** colony on OA, PDA and CMA after 14 d **D–F** conidiophores and Phialides **G** conidia. Scale bars: 1.35 cm (**A–C**), 10 μm (**D–G**).

Plectosphaerella pauciseptata A.J.L. Phillips, A. Carlucci & M.L. Raimondo Figure 6

Description. Colony on CMA after 3 d, hyphae hyaline, septate, commonly branched, thin-walled, smooth, 2.5–3.0 μ m (\bar{x} = 2.6 μ m, n = 10) wide. Conidiophores macronematous, mononematous, erect, straight or flexuous, hyaline, smooth, aseptate, occasionally branched. Conidiogenous cells phialides, terminal, determinate, subulate, hyaline, smooth, thin-walled. Conidia acrogenous, hyaline, oblong-ellipsoidal, unicellular, smooth-walled, multi-guttulate, 5.5–12.5 × 2.5–3.5 μ m (\bar{x} = 9.7 × 3.3 μ m, n = 30), forming hyaline to white mucilaginous masses. Sexual morph and chlamydospores absent.

Culture characteristics. Colonies on OA reaching 55 mm diameter, on PDA reaching 49 mm diameter and on CMA reaching 45 mm diameter in 14 d at 25 °C. On PDA, colonies white, dense, raised, aerial hyphae growth in the medium surface, margin regular, frontier distinct, reverse pale brown to white.

Strain examined. CHINA, Yunnan Province, Erhai, 25°43'N, 100°11'E, 1964 m alt., isolated from *Myriophyllum spicatum* as an endophyte, 31 Jul. 2014, Z.F. Yu, YMF 1.05088, YMF 1.04679, YMF 1.04725.

Discussion

Sexual reproduction of *Plectosphaerella* has only been reported for three species (Giraldo and Crous 2019; Phookamsak et al. 2019). Most strains, by contrast, show asexual morphs in substrate, so the main distinguishing characteristics of this genus are based on the ratio of septate conidia, conidial dimensions and shape, absence or presence of chlamydospores (Carlucci et al. 2012; Arzanlou et al. 2013). These simple features and similar lifestyle make identification more difficult, so the classical way is no longer a valid and reliable marker (Palm et al. 1995; Zare et al. 2007; Carlucci et al. 2012). The ITS and LSU regions were considered to be a necessary condition for species identification of *Plectosphaerella* (Duc et al. 2009; Carlucci et al. 2012; Liu et al. 2013; Crous et al. 2015). However, *Plectosphaerella* species exhibit a relatively low degree of ITS or LSU molecular diversity, showing more molecular markers are needed to distinguish species (Carlucci et al. 2012). Recently, fragments of several protein-coding genes were selected to efficiently elucidate the taxonomy of this genus, including CaM, TEF-1 α , TUB2 and RPB2 (Su et al. 2017; Giraldo et al. 2019; Giraldo and Crous 2019; Phookamsak et al. 2019; Zhang et al. 2019). In this study, a multi-locus analysis improved the diagnostic level of the whole genus.

In this study, among 129 sample sites in Yunnan and Sichuan province, *Plectosphaere-lla* species were isolated from 13 sampling sites. Regions are arranged in order of isolation frequency: Eehai (39.29%), Dian Lake (20.54%), Fuxian Lake (13.39%), Daofu (8.04%) and other places were below 5%. It seems that distribution of *Plectosphaerella* is impacted by human activities, because there are more human activities around the first three sites. In addition, *Plectosphaerella* species only occur in six aquatic plants including *Batrachium, Halerpestes, Hippuris, Hydrilla, Myriophyllum*, and *Potamogeton*, although

we investigated 30 aquatic plants. So *Plectosphaerella* species exhibit medium host diversity and relatively narrow geographic distribution when aquatic plants serve as hosts.

Secondly, the separation frequency of *Plectosphaerella pauciseptata* was the highest at 68.78%, followed by *P. cucumerina* at 24.11%, and two new species were the lowest at 3.57%. According to this data and previous studies, *P. cucumerina* and *P. pauciseptata* are still the most widely distributed species either on land or within aquatic plants, although *P. pauciseptata* is more common in aquatic plants. On the other hand, only four species were identified among 112 strains. One reason may be that species diversity of *Plectosphaerella* is low within aquatic plants. Other reasons may be *Plectosphaerella* species exhibit a relatively low degree of ITS molecular diversity. Based on this, perhaps we treat some different species as two known species, which reduce the diversity of *Plectosphaerella*. Regrettably, we did not preserve all strains except for representative strains. Or else, we should sequence other loci of all strains, especially for two species, *P. pauciseptata*, *P. cucumerina*, more loci are needed to distinguish them.

This study revealed *Plectosphaerella* species within aquatic plants from the southwest of China, although only 112 strains and four species were reported, which also enrich the ecology and diversity of the genus. However, our survey was limited, hence it is necessary to know if our result is concordant with those of other regions. So in-depth work is required to obtain a more integrated knowledge of their biodiversity and distribution in water bodies.

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Supplementary material I

Figures S1–S4

Authors: Xiao Qian Yang, Shi Yun Ma, Ze Xiang Peng, Zhong Qiao Wang, Min Qiao, Zefen Yu

Data type: phylogenetic tree

- Explanation note: **Figure S1.** Phylogenetic tree generated by Bayesian inference based on sequences of the ITS. Monilochaetes infuscans CBS 379.77 serves as an outgroup. Bayesian posterior probability over 80% are shown at the nodes. Two new species are given in boldface. **Figure S2.** Phylogenetic tree generated by Bayesian inference based on sequences of the LSU. Monilochaetes infuscans CBS 379.77 serves as an outgroup. Bayesian posterior probability over 80% are shown at the nodes. Two new species are given in boldface. **Figure S3.** Phylogenetic tree generated by Bayesian inference based on sequences of the TEF-1α. Bayesian posterior probability over 80% are shown at the nodes. Two new species are given in boldface. **Figure S4.** Phylogenetic tree generated by Bayesian inference based on sequences of the TUB2. Bayesian posterior probability over 80% are shown at the nodes. Two new species are given in boldface.
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Characterization of Diaporthe species associated with peach constriction canker, with two novel species from China

Xianhong Wang¹, Yashuang Guo¹, Yamin Du¹, Ziling Yang¹, Xinzhong Huang³, Ni Hong^{1,2}, Wenxing Xu^{1,2}, Guoping Wang^{1,2}

I Key Lab of Plant Pathology of Hubei Province, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, 430070, China 2 Key Laboratory of Horticultural Crop (Fruit Trees) Biology and Germplasm Creation of the Ministry of Agriculture, Wuhan, Hubei, 430070, China 3 Research Institute of Pomology, Fujian Academy of Agricultural Sciences, Fuzhou, Fujian, 350013, China

Corresponding author: Guoping Wang (gpwang@mail.hzau.edu.cn)

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Abstract

Species of *Diaporthe* infect a wide range of plants and live *in vivo* as endophytes, saprobes or pathogens. However, those in peach plants are poorly characterized. In this study, 52 *Diaporthe* strains were isolated from peach branches with buds, showing constriction canker symptoms. Phylogenetic analyses were conducted using five gene regions: internal transcribed spacer of the ribosomal DNA (ITS), translation elongation factor $1-\alpha$ (*TEF*), β -tubulin (*TUB*), histone (*HIS*), and calmodulin (*CAL*). These results coupled with morphology revealed seven species of *Diaporthe*, including five known species (*D. caryae*, *D. cercidis*, *D. eres*, *D. hongkongensis*, and *D. unshiuensis*). In addition, two novel species *D. jinxiu* and *D. zaofenghuang* are introduced. Except for the previously reported *D. eres*, this study represents the first characterization of *Diaporthe* species associated with peach constriction canker in China, and contributes useful data for practicable disease management.

Keywords

Constriction canker, Diaporthe, multi-gene phylogeny, Prunus persica, taxonomy, two new taxa

Introduction

The genus Diaporthe (asexual morph Phomopsis) was established by Nitschke in 1870 and predates its sexual morph established in 1905, thus Diaporthe is used for this genus (Rossman et al. 2015). The sexual morph of Diaporthe is characterized by black spherical ascomata with single or multiple tapering perithecial necks. Their asci are unitunicate, 8-spored, sessile, and elongate to clavate. The ascospores are hyaline, two-celled, often biguttulate, and elliptical to fusiform (Udayanga et al. 2015; Guo et al. 2020). The asexual morph is characterized by black or dark brown conidiomata, with cylindrical phialides producing three types of conidia (Udayanga et al. 2011; Gomes et al. 2013; Guo et al. 2020). In early studies, species of Diaporthe were identified based on host association, morphology and cultural characteristics (Uecker 1988). Recently, studies have shown that many species of *Diaporthe* are not host-specific i.e., one species may infect more than one host species, e.g., D. eres can infect blackberry (Vrandecic et al. 2011), pear (Bai et al. 2015), and jujube (Zhang et al. 2018). Moreover, a single species is prone to morphological changes depending on the incubation conditions (Gomes et al. 2013). Therefore, molecular data have been adopted to resolve the circumscription of species of *Diaporthe*, initially relying on the internal transcribed spacer (ITS) of the ribosomal DNA region (Santos et al. 2009; Thompson et al. 2011), and recently on multiple loci including ITS, translation elongation factor 1- α (*TEF*), β -tubulin (*TUB*), histone (*HIS*), and calmodulin (CAL) gene regions (Santos et al. 2017). At present, the five-locus dataset (ITS-TEF-CAL-HIS-TUB) has been optimally adopted for the species delimitation by recent authors (Gao et al. 2017; Yang et al. 2018, 2020; Crous et al. 2020; Guo et al. 2020; Hyde et al. 2020; Sun et al. 2021).

Peach (Prunus persica L.) originated from China, where it has been cultivated for more than 3,000 years (Faust and Timon 2010). In the past ten years, the national annual production of peach and nectarine was 10-15 million tons, accounting for 50% of global production (http://www.fao.org/faostat/en/#data/QC). In recent years, peach constriction canker has been frequently observed in peach orchards in Fujian province, one of the important peach-cultivation areas in China. This disease can cause flower bud necrosis, no flowering, and even kill the shoots, resulting in a severe economic loss for growers. Peach constriction canker was firstly observed in 1934 in New Jersey, USA (Daines et al. 1958), and usually infects peach buds leading to the formation of reddish-brown elongate cankers around twig nodes, which can girdle and kill buds the following summer (Lalancette and Robison 2001). In a previous study, P. amygdali was identified as the cause of peach constriction canker based on morphology in Spain (Tuset et al. 1989). However, morphology alone is not adequate for determining a species in *Diaporthe*. Moreover, *Diaporthe* spp. associated with peach plants have not been well documented. To understand the etiology of peach constriction canker in China, diseased samples were collected and isolates obtained from them. The characterization of these isolates revealed five known and two novel *Diaporthe* species associated with the disease.

Materials and methods

Sampling and isolation

The infected peach branches with buds showing constriction canker symptoms were collected in Fujian province of China in 2017–2018. The collected samples were subjected to fungal isolation following the protocol described by Bai et al. (2015). Diseased tissues (4–5 mm²) were excised from infected bud scales after they were surface-sterilized with 75% ethanol for 45 s and 1% NaOCl for 45 s, rinsed twice with sterilized water, and air-dried. The excised tissues were placed on potato dextrose agar (PDA, 20% diced potatoes, 2% dextrose and 1.5% agar) plates and incubated at 25 °C in the dark for 3–5 d. After colonies grow, their mycelium was transferred to a new PDA plate and each colony was designated as a specific isolate. Each isolate was further purified by culturing from a single conidium (Choi et al. 1999). The obtained isolates were stored in 25% glycerol at -80 °C for further usage. Specimens of new species were deposited in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (**HMAS**). Ex-type living cultures were deposited in the China General Microbiological Culture Collection Centre (**CGMCC**), Beijing, China. Sequences of the novel species were submitted to MycoBank (http://www.mycobank.org).

Morphological analyses

Fungal morphology was determined by culturing a 5-d-old mycelial disc (5 mm in diameter) on a petri dish containing PDA and oatmeal agar (OA) (Udayanga et al. 2014), respectively. Cultures were incubated at 25 °C with a 14/10 h fluorescent light/ dark cycle. Growth rate (mm/d) was determined by measuring the colony diameters of each isolate on PDA daily for 3 days. The colony morphologies were recorded after 14 d. Moreover, the shape, color, and size of conidiomata, conidia and conidiophore were observed using an optical microscope (Olympus BX63 or Olympus SZX16, Japan), and 50 conidia of each isolate were measured to determine their size.

DNA extraction and determination of the taxonomic region sequences

Genomic DNA was extracted from pure culture using modified cetyltrimethyl-ammonium bromide (CTAB) protocol (Udayanga et al. 2012), and subjected to PCR amplification of the partial regions of the five loci comprised ITS, *TUB*, *TEF*, *CAL*, and *HIS* using corresponding primer pairs: ITS5/ITS4 (White et al. 1990), Bt2a/Bt2b (Glass and Donaldson 1995), EF1-728F/EF1-986R (Carbone and Kohn 1999), CAL-228F/CAL-737R (Carbone and Kohn 1999), and CYLH3F/H3-1b (Glass and Donaldson 1995, Crous et al. 2006), respectively. PCR programs were initiated with 95 °C for 5 min, followed by 34 cycles of denaturation at 95 °C for 30 s, annealing at a suitable temperature for 30 sec (56 °C for ITS, 52 °C for *TEF*, 54 °C for *CAL*, 57 °C for *HIS* and 60°C for *TUB*), and extension at 72 °C for 30 sec, and terminated with a final elongation step at 72 °C for 10 min. The PCR amplicons were purified and sequenced at the Sangon Biotech (Shanghai, China) Company. Consensus sequences were obtained using DNAMAN (v. 9.0, Lynnon Biosoft), and deposited in GenBank (Suppl. material 1: Table S1).

Phylogenetic analyses

Sequences generated in this study were blasted against the NCBI GenBank nucleotide database to determine the closest relatives. Alignment of different gene regions of isolates obtained in this study, their relatives and the ones of the type species (Suppl. material 2: Table S2) was initially performed using the MAFFT v. 7 online servers (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh and Standley 2013) with default settings, and the alignment was manually adjusted in MEGA v. 7 (Kumar et al. 2016).

Phylogenetic analyses were conducted based on the concatenated five loci. Bayesian inference (BI) was used to construct phylogenies using MrBayes v. 3.1.2 (Ronquist and Huelsenbeck 2003). The best-fit model of nucleotide substitution for each partition was determined using MrModeltest v. 2.3 (Nylander 2004) and incorporated into the analyses. Two analyses of four Markov Chain Monte Carlo (MCMC) chains were conducted from random trees with 1.8×10^8 generations. The analyses were sampled every 1,000 generations, which were stopped once the average standard deviation of split frequencies was below 0.01. The first 25% of the trees were discarded as the burnin phase of each analysis, and the remaining trees were summarized to calculate the posterior probabilities (PP) of each monophyletic clade.

Additionally, maximum parsimony analyses (MP) were performed on the multi-locus alignment using PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford 2002). Phylogenetic trees were generated using the heuristic search option with Tree Bisection Reconnection (TBR) branch swapping and 1,000 random sequence additions. Max trees were set up to 5,000, zero-length branches were collapsed, and all multiple parsimonious trees were saved. Clade stability was assessed using a bootstrap analysis with 1,000 replicates. Tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated. Furthermore, maximum likelihood (ML) analysis was performed using IQtree v. 1.6.8. The analysis was performed with a GTR site substitution model. Branch support was evaluated with a bootstrapping (BS) method of 1,000 replicates (Hillis and Bull 1993). Phylogenetic trees were visualized in FigTree v. 1.4.2 (Rambaut 2014). The alignments and phylogenetic trees were deposited in TreeBASE (submission ID: 28014).

Results

Collection of Diaporthe strains

In the surveyed orchards, this disease caused flower-bud necrosis, little or no flowering (Fig. 1A), black-brown lesions around the buds (Fig. 1B), petrified blossom buds



Figure 1. Representative symptoms of peach constriction canker in the field **A** blossom bud necrosis, few or no flowers on peach trees in spring **B** black-brown lesions around buds **C** petrified blossom buds **D** rigid leaf buds.

(Fig. 1C), and rigid leaf buds (Fig. 1D). A total of 52 *Diaporthe* strains were obtained from 27 infected bud samples of *Prunus persica* cultivars (cvs.) Baifeng, Jinxiu, Jinyuan, and Zaofenghuang by fungal isolation and single spore culturing. All strains were used in morphological observation and phylogenetic analysis.

Phylogenetic analyses

The strains mentioned above together with 49 reference isolates of previously described species (Suppl. material 2: Table S2) were subjected to multi-locus phylogenetic analyses with concatenated ITS, *TEF*, *CAL*, *HIS*, and *TUB* sequences. *Diaporthella corylina* (CBS 121124) was selected as the outgroup. A total of 2,514 characters of nucleotides and gaps (ITS: 1–578, *TEF*: 579–960, *CAL*: 961–1,469, *HIS*: 1,470–1,989, *TUB*: 1,990–2,514) were included in the phylogenetic analysis. The maximum-likelihood (ML) tree was generated with the GTR model. The best nucleotide substitution models were recommended by MrModeltest and used in the Bayesian analysis: SYM+I+G for ITS, GTR+G for *TEF*, HKY+I+G for *TUB*, and GTR+I+G for both *CAL* and *HIS*. The heuristic search using MP generated 1,000 parsimonious trees (TL = 3,182, CI = 0.52, RI = 0.87, RC = 0.45), and branches of zero length were collapsed and all multiple parsimonious trees were saved. MP and ML bootstrap support values above 50% are shown as second and third position



above the nodes, respectively. In the phylogenetic tree, 48 of the 52 isolates obtained in this study were assigned to five known species, *D. caryae* (one strain), *D. cercidis* (one), *D. eres* (nine), *D. hongkongensis* (26), and *D. unshiuensis* (11). Four strains formed two distinct clades, and were identified as two novel species, described herein as *D. zaofenghuang* (two strains, closely related to *D. penetriteum*), and *D. jinxiu* (two strains, close to *D. rhoina*), respectively (Fig. 2).

Taxonomy

Diaporthe jinxiu X.H. Wang & G.P. Wang, sp. nov.

MycoBank No: 838502 Fig. 3

Etymology. Named for the host variety (*Prunus persica* cv. Jinxiu), from which the species was isolated.

Description. Sexual morph: not observed. Asexual morph on alfalfa stems after 15 days. *Pycnidial conidiomata* small, covered by pale yellow discharged conidial masses at maturity, 385–810 µm diam. *Conidiophore* hyaline, cylindrical, smooth, phialidic, unbranched, straight or slightly curved, $16-21 \times 2-2.5$ µm. *Conidiogenous cells* phialidic, cylindrical. Alpha *conidia* hyaline, aseptate, ellipsoidal, biguttulate, rounded at each end, $5.8-7.1 \times 2.7-4.0$ µm (mean = $6.4 \pm 0.4 \times 3.5 \pm 0.3$ µm, n = 50). *Beta* and *gamma conidia* not observed.

Culture characteristics. Cultures incubated on PDA at 25 °C in cycle of light/ darkness, growth rate 11.5 mm per day. On PDA medium, colonies were sparse and villous, flourishing at edge of colony. On OA medium, colonies dense with neat edges, with yellow pigment in the center.

Materials examined. China, Fujian Province, Sanming City, on buds of *Prunus persica* cv. Zaofenghuang, 23 March 2017, Y. S. Guo (holotype HMAS 249837, culture ex-holotype culture CGMCC3.20269 = TZFH20); ibid., ex-isotype culture TZFH25.

Notes. In the phylogenetic, multi-locus tree, *D. jinxiu* forms a distinct clade with maximum support (1/100/99) and is most closely related to *D. rhoina*, but with smaller pycnidial conidiomata than the later (*D. jinxiu* = 386–807 µm vs *D. rhoina* = 500–2500 µm) (Feltgen 1901). Moreover, the sequence differences were significant, and all five regions were able to distinguish them (28/578 in ITS, 38/382 in *TEF*, 21/509 in *CAL*, 28/520 in *HIS*, and 11/525 in *TUB*).

Figure 2. A phylogenetic tree generated by Bayesian analysis based on combined ITS, *TEF*, *CAL*, *HIS*, and *TUB* sequence. *Diaporthella corylina* (CBS121124) was selected as the outgroup. Bayesian posterior probability ($PP \ge 0.90$), MP bootstrap support values ($MP \ge 50\%$) and RAxML bootstrap support values ($ML \ge 50\%$) are shown at the nodes (PP/ML/MP). The branches of the new *Diaporthe* species are marked with red stars **B**, **C** are partial phylogenetic taxa highlighting *D. zaofenghuang* and *D. jinxiu* together with their closely related species, respectively.



Figure 3. *Diaporthe jinxiu*. Front and back views of colonies on PDA (**A**, **B**) and OA (**C**, **D**), respectively **E** conidiomata on alfalfa stems **F** conidioma **G** conidiophores **H** *alpha* conidia **I–J** section view of conidioma. Scale bars: 2 mm (**E**); 200 μm (**F**); 20 μm (**I**); 10 μm (**G**, **H**, **J**).

Diaporthe zaofenghuang X.H. Wang & G.P. Wang, sp. nov.

MycoBank No: 838501 Fig. 4

Etymology. Named after the host species (*Prunus persica* cv. Zaofenghuang) from which the species was isolated.

Description. Sexual morph not observed. Asexual morph on alfalfa stems. *Pycnidial conidiomata* conical, yellowish translucent conidial drops exuded from ostioles, 650–1430 μ m diam. *Conidiophores* fasciculate, hyaline, long cylindrical, straight or slightly curved, apex pointed, 13.7–20.9 × 1.8–2.7 μ m. *Conidiogenous cells* phialidic, cylindrical. *Alpha conidia* hyaline, aseptate, ellipsoidal, biguttulate, rounded at one



Figure 4. *Diaporthe zaofenghuang*. Front and back views of colonies on PDA (**A**, **B**) and OA (**C**, **D**), respectively **E** conidiomata on alfalfa stems **F** conidioma **G** conidiophores **H** *alpha* conidia **I–J** section view of conidioma. Scale bars: 2 mm (**E**); 200 μm (**F**); 20 μm (**G**, **I**); 10 μm (**H**, **J**).

end, slightly apex at another end, $5.3-7.5 \times 2.9-3.7 \mu m$ (mean = $6.0 \pm 0.6 \times 3.1 \pm 0.3 \mu m$, n = 50). *Beta* and *gamma conidia* not observed.

Culture characteristics. Cultures incubated on PDA at 25 °C in cycle of light/ darkness, growth rate 8.5 mm per day. Colonies initially white on surface, producing black pigment from center of medium and expanding outwardly after 5–7 d. On PDA, edge of colony petal-like, irregular; on OA, edge relatively flat.

Materials examined. China, Fujian Province, Sanming City, on buds of *Prunus persica* cv. Zaofenghuang, 23 March 2017, Y. S. Guo (holotype HMAS 249835, culture ex-holotype CGMCC3.20271 = TZFH1); ibid., culture TZFH3.

Notes. Two isolates representing *D. zaofenghuang* form a well-supported clade (1/100/100) and appear to be most closely related to *D. penetriteum*. *Diaporthe zaofen-ghuang* can be distinguished from *D. penetriteum* based on ITS, *HIS*, and *TUB* loci (10/578 in ITS, 45/520 in *HIS*, and 7/525 in *TUB*). Morphologically, *D. zaofeng-huang* differs from *D. penetriteum* in having larger conidiomata (*D. zaofenghuang* = 653–1433 µm vs *D. penetriteum* = 180–490 µm) and *alpha* conidia (*D. zaofenghuang* = 6.0 \pm 0.6×3.1 \pm 0.3 µm vs *D. penetriteum* = 5.0 \pm 0.3 × 2.2 \pm 0.2 µm). Additionally, *D. penetriteum* produces two types of conidia, but *D. zaofenghuang* produces only *alpha* conidia (Gao et al. 2016).

Discussion

In this study, phylogenetic analyses based on the five combined loci (ITS, *TEF*, *CAL*, *HIS*, and *TUB*) coupled with morphology revealed seven *Diaporthe* species (viz. *D. caryae*, *D. cercidis*, *D. eres*, *D. hongkongensis*, *D. jinxiu*, *D. unshiuensis*, and *D. zaofenghuang*) associated with peach constriction canker. Of these species, two novel species *D. jinxiu* and *D. zaofenghuang* in distinct clades were described. *Diapothe jinxiu* has smaller conidiomata as compared to its closest relative *D. rhoina*. However, it is not possible to have more morphological comparisons at this stage because limited biological information is available for this species (Feltgen 1901). Moreover, *D. jinxiu* clearly differs from other phylogenetically related species, e.g., *D. psoraleae-pinnatae*, by having shorter and wider *alpha* conidia (*D. jinxiu* = 5.8–7.1 × 2.7–4.0 µm vs *D. psoraleae-pinnatae* = 7.0–12.0 × 2.5–3.0 µm) (Crous et al. 2013), and yellow pigments (Fig. 3A–D). *Diaporthe zaofenghuang* showed different morphologies compared with *D. penetriteum*, e.g., larger *alpha* conidia, larger conidiomata, and no *beta* conidia (Gao et al. 2016). Therefore, both novel species described here are clearly separated from known ones in the phylogeny and morphology.

Based on molecular data, several *Diaporthe* species associated with peach diseases in other countries have been identified and characterized, including *D. amygdali*, which is responsible for apical dead shoot, twig and shoot blight in peach and nectarine in Uruguay (Sessa et al. 2017), and *D. eres* for stem canker on peach in Italy and Greece (Thomidis et al. 2009; Prencipe et al. 2017). *Diaporthe amygdali* was not found in the present study but Dai et al. (2012) recorded it as *Phomopsis amygdali* related to twig canker of peach. Previously, six *Diaporthe* spp. infecting peach trees have been characterized in China. The reported *D. hongkongensis* caused fruit rot (Zhang et al. 2021), *D. eres, D. momicola, D. pescicola*, and *D. taoicola* were related to tree dieback (Dissanayake 2017), and *D. amygdali* was related to twig canker (Dai et al. 2012). To our knowledge, besides *D. eres*, this is the first report of *D. caryae*, *D. cercidis*, *D. hongkongensis*, *D. jinxiu*, *D. unshiuensis*, and *D. zaofenghuang* as the cause of peach constriction canker. This study contributes useful information for practicable disease management.

Previous studies have revealed that species of *Diaporthe* are highly divergent and closely linked to sampling areas, such as 19 species of *Diaporthe* infecting pears cultivated in 15 provinces of China (Guo et al. 2020). Further studies are required to

include an extensive collection of *Diaporthe* isolates from other peach-cultivated regions in China. For effective disease management, more knowledge is required about *Diaporthe* species related to peach constriction canker in China.

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Supplementary material I

GenBank accession numbers of isolates included in this study

Author: Xianhong Wang

Data type: GenBank accession numbers

Explanation note: GenBank accession numbers of sequences obtained from this study. Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

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Supplementary material 2

List of *Diaporthe* species used to phylogenetic analysis in this study, with details about host, country, and GenBank accession numbers

Author: Xianhong Wang

Data type: GenBank accession numbers

Explanation note: GenBank accession numbers of sequences downloaded from GenBank. Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

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Two new species and a new record of yellow Cantharellus from tropical Quercus forests in eastern Mexico with the proposal of a new name for the replacement of Craterellus confluens

Leticia Montoya¹, Mariana Herrera¹, Victor M. Bandala¹, Antero Ramos¹

Red Biodiversidad y Sistemática, Instituto de Ecología A.C., P.O. Box 63, Xalapa, Veracruz, 91000, México

Corresponding author: Victor M. Bandala (victor.bandala@inecol.mx)

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Abstract

Two new species of yellow *Cantharellus* and a new record of *Cantharellus tabernensis* associated with tropical species of *Quercus* are presented, based on the taxonomic study of fresh specimens and in a phylogenetic analysis of transcription elongation factor 1-alpha (*tef*-1 α) and the large subunit of the ribosome (nLSU) sequences. One of the new species proposed here, corresponds to a choice edible mushroom, which, in our molecular phylogeny, resulted in it being related to the group of species around *C. lateritius* and sister with *Craterellus confluens* type specimen. This latter is here formally transferred to *Cantharellus confluens* (Schwein.) Schwein. 1834 a later synonym of *Byssomerulius corium*. Detailed macroscopic and microscopic descriptions accompanied with illustrations and a taxonomic discussion are presented for each species.

Keywords

American *Cantharellus*, *Craterellus*, ectomycorrhizal mushrooms, Neotropical *Cantharellus* or chanterelles, oak, wild edible mushrooms

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Introduction

In the American continent, especially from USA, new species of *Cantharellus* had been proposed, several of them look-alikes of the commonly cited *C. cibarius* Fr., *C. cinnabarinus* Fr. and *C. lateritius* (Berk.) Singer (Arora and Dunham 2008; Buyck et al. 2010, 2011, 2016a, b; Buyck and Hofstetter 2011; Foltz et al. 2013; Leacock et al. 2016; Thorn et al. 2017). Further explorations in tropical America are achieving also the discovery of undescribed species of the genus (e.g. Wartchow et al. 2012; Henkel et al. 2014; Nascimento et al. 2014; Buyck et al. 2016b; Herrera et al. 2018) as occurred also with *Craterellus* (Wilson et al. 2012).

Species delimitation in *Cantharellus* is often said to be hard to address, especially because of the overlap of phenotypic variation, including scarce microscopic morphoanatomic taxonomically informative features. In such a sense, Buyck et al. (2014) explicitly defined that basidiomes of *Cantharellus* species "...under the microscope ... exhibit a discouraging monotony...". Studying *Cantharellus* specimens from Mexico, we have noted that the difficulty in revising early records is exacerbated by frequent incomplete data accompanying herbaria specimens. For or instance, there is poor or no information on features like hymenophore and color variations of basidiomes along their development or even by weathering effects. It is of primary importance then, to be able to count on accurate observations of specimens in fresh that lead to the characterization of their phenotypes and establish robust concepts for pertinent taxonomic conclusions. It is important even to count on data on the spatial/temporal distribution, and associated tree species.

In Cantharellus violaceovinosus (Herrera et al. 2018), for example, it was possible to document wide macromorphological and color information through a register of samples collected over more than five years, even in weekly explorations along three years sampling. Such a record allowed us to recognize its phenology between July-October in pure stands of Quercus oleoides, and found it less frequent in association with Q. glaucescens and Q. sapotifolia. In fact, such a record together with molecular information facilitated the distinction of C. violaceovinosus from other phenotypically similar species. Olariaga et al. (2015) informed about the identity of some taxa previously described solely based on colored or unpigmented variants, i.e., while in C. amethysteus (Quel.) Sacc., C. cibarius Fr., C. ferruginascens P.D. Orton, C. pallens Pilát and C. romagnesianus Eyssart. & Buyck, white specimens may occasionally occur, in C. cibarius and C. pallens orange forms can be found. Among other conclusions, these authors demonstrated that white forms of C. cibarius already described as varieties (var. inodorus Velen, f. pallidus R. Schulz) corresponded molecularly indeed to a single taxon, and C. gallaecicus (Blanco-Dios) Olariaga, lacking yellow-orange tones, is in fact the same as the orange-yellow to ochre-yellow *C. romagnesianus* (Olariaga et al. 2017).

Yellow chantherelles, such as *Cantharellus cibarius* Fr., *C. lateritius* (Berk.) Singer, *C. odoratus* (Schwein.) Fr. and *Craterellus confluens* Berk. & M.A. Curtis have been reported from different regions of Mexico (Berkeley 1867; Guzmán and Sampieri 1984; Guzmán 1985; Guevara et al. 2004; Pérez-Moreno et al. 2008; Garibay-Orijel et al.

2009; Kong et al. 2018; Corona-González 2019). *Craterellus confluens* was described by Berkeley (1867) from Mexico (Orizaba at central Veracruz state), in a locality relatively close to one of the current study sites in the Municipality of Zentla, Veracruz. Particularly from this latter region, an edible yellow chantherelle species common in the surrounding *Quercus* forest and even sold in popular markets, was previously reported under *C. odoratus*, considering it contaxic with *Cr. confluens* (Guzmán and Sampieri 1984; Guzmán 1985).

During a systematic multiyear sampling of basidiomes, as part of a project focused to study ectomycorrhizal fungi in tropical *Quercus* forests in eastern Mexico (Montoya et al. 2019a, b), we found coexisting three species of yellow *Cantharellus*. Two of these taxa are distinctive by having short-sized basidiomes with veined to gill-like folded hymenophore, while a third one, is distinctive by its medium-sized, moderately robust basidiomes, with smooth or at times rugulose hymenophore, this latter apparently corresponding to what was earlier reported as "*C. odoratus*".

We report here the results of both, a morphological study of fresh specimens and a phylogenetic analysis of the transcription elongation factor 1-alpha (*tef*-1 α) and the large subunit of the ribosome (nLSU) sequences obtained from our recent collections and those available in GeneBank. Three well-supported clades inferred in the phylogenetic tree, allowed us to recognize two new species and the record in Mexico of *C. tabernensis*, described from Southern Mississippi in USA (Feibelman et al. 1996). One of the new species here proposed, corresponds to the yellow *Cantharellus* with smooth hymenophore, which interestingly, in our phylogenetic analysis appears independent of *Craterellus confluens* (holotype), *Cantharellus lateritius* (holotype) and *C. flavolateritius* Buyck & V. Hofst. (paratype) sequences. Both macromorphological and color variation mentioned in the descriptions were recovered from fresh basidiomes through seven years of sampling. The monitoring of monodominant stands of three different species of tropical *Quercus*, allowed registering also, the putative ectomycorrhizal interaction of the studied species of *Cantharellus*.

Materials and methods

Sampling and morphological study

Cantharellus basidiomes were collected through a weekly sampling during June-October 2015–2018, with sporadic collections among 2009–2014, in tropical oak forests from Municipalities of both Zentla (837–850 m a.s.l.) and Alto Lucero (400–500 m a.s.l.) in central Veracruz (eastern Mexico). In these oak forests, *Quercus oleoides* is dominant, and even forming pure stands. In the Zentla locality, *Q. glaucescens* and *Q. sapotifolia* are also present, and form monodominant small stands. Descriptions are derived from recording the morpho-anatomical features of fresh samples, the records of color follow Kornerup and Wanscher (1978) (e.g. 4A4–8) and Munsell (1994) (e.g. 2.5Y 7/8–8/8). Basidiomes were dried in a hot air dehydrator (45 °C) for their preser-

vation. Microscopic features were examined from desiccated specimens, measured in 3% KOH and stained with 1% Congo red or analyzed in Melzer's solution. Thirty-five basidiospores per collection were measured in lateral view following Montoya et al. (2019b). In the descriptions \overline{X} denotes an interval of mean values of basidiospores length and width per collection in *n* collections, and \overline{Q} refers to the range of coef. Q (where Q is the average of the ratio of basidiospore length/basidiospore width in each collection). Line drawings were made with the aid of a drawing tube. Collections form 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

Genomic DNA was isolated from fresh material according to Cesar et al. (2018). We amplified the transcription elongation factor 1-alpha (*tef*-1 α) using the pairs of primers *tef*-1F/*tef*-1R (Morehouse et al. 2003) and *tef*-1Fcanth/*tef*-1Rcanth (Buyck et al. 2014). We amplified the large subunit of ribosome (nLSU) using combinations of the pair of primers LR0R/LR7 (Vilgalys and Hesler 1990) and the pair of primers designed LRCA1(5'-GTTGCACTGTCCGAGTTGTA-3')/LRCA2(5'-AGACTGATG-GCGAGGTATGA-3'). PCR was performed according to Herrera et al. (2018). A capillary sequencer, Genetic Analyzer 3730XL (Applied Biosystems), was used to obtain the sequences of the amplified PCR products. These sequences were assembled, edited, and deposited at GenBank database (Benson et al. 2017), the accession numbers are indicated in Table 1.

Phylogenetic analysis

We constructed a concatenated dataset, using PhyDE v.0.9971 (Müller et al. 2010), with 19 sequences obtained here (nLSU and *tef*-1 α) (Table 1), together with sequences of related taxonomic groups, and additionally taking as reference works on chantarelles by An et al. (2017), Buyck et al. (2014, 2016a, b, c, d), Herrera et al. (2018) and Olariaga et al. (2017). The dataset was aligned with MAFFT online service (Katoh et al. 2019), and the inconsistencies were corrected manually. Phylogenetic trees were generated according to Montoya et al. (2019a). The evolutionary model was calculated using the IQ-Tree 2.0-rc1 (Minh et al. 2020; Kalyaanamoorthy et al. 2017) and the best-fit model selected using the Bayesian Information Criterion (BIC), the Akaike Information Criterion (AIC) and corrected AIC. This later was used to generate a phylogenetic tree with the Maximum Likelihood (ML) method, with a Nearest Neighbour Interchange (NNI) heuristic, with the TIMe+I+G4 evolutionary model. A consensus tree was also generated calculating the Robinson-Foulds distance between the ML tree and the consensus tree, the branches being tested by means of Ultrafast Approach Bootstrap (UFBoot), SH-like approximate Likelihood Ratio Test (SH-aLRT), Approximate Bayes test (aBayes) and Bootstrap Standard (BS). Another phylogenetic tree (not

Taxa	Voucher	Locality	LSU	tef-1a
Cantharellus addaiensis	BB 98.033 TYPE	Tanzania	KF294667	IX192992
Cantharellus afrocibarius	BB 96.235 TYPE	Zambia	KF294668	JX192993
Cantharellus albidolutescens	BB 08.070 TYPE	Madagascar	KF294646	JX192982
Cantharellus alborufescens	BB 12.075	Italy	KX929161	KX907243
5	BB 12.076	Italy	KX907222	KX907244
Cantharellus altipes	BB 07.019 TYPE	USA	KF294627	GQ914939
Cantharellus ambohitantelyensis	BB 08.336 TYPE	Madagascar	KF294656	IX192989
Cantharellus amethysteus	AH44796 TYPE	Spain	KR677550	KX828819
Cantharellus appalachiensis	BB 07.123	USA	KF294635	GQ914979
Cantharellus camphoratus	TENN:F-38025 TYPE	Canada	KX896788	_
Cantharellus cerinoalbus	AV 06.051 TYPE	Malaysia	KF294663	-
Cantharellus cibarius	BIO10986 TYPE	Sweden	KR677539	KX828823
Cantharellus cinnabarinus	BB 07.001 TYPE	USA	KF294624	GQ914985
Cantharellus coccolobae	1065/RC 11.25 TYPE	Guadeloupe	KX857089	KX857021
Cantharellus congolensis	BB 98.039	Tanzania	KF294609	JX193015
	BB 98.058	Tanzania	KF294673	JX192996
Cantharellus corallinus	JJ MO-Canth-2 TYPE	USA	KX896776	KX857031
Cantharellus decolorans	BB 08.278 TYPE	Madagascar	KF294654	GQ914968
Cantharellus enelensis	13.08.21.av02 TYPE	Canada	KX592712	-
Cantharellus ferruginascens	BB 07.283	Slovakia	KF294638	GQ914952
Cantharellus fistulosus	DT 43 TYPE	Tanzania	KF294674	JX192992
Cantharellus flavolateritius	Halling 6252	USA	MT371334	-
	JJ/NC-CANT-2	USA	KX896783	KX857027
Cantharellus flavus	C066WI TYPE	USA	JX030437	-
Cantharellus formosus	SAR220712	Canada	KR677553	KX828830
Cantharellus friesii	AH44798	Spain	KR677522	KX828831
Cantharellus garnierii	RF32 PC TYPE	New Caledonia	AY392767	-
Cantharellus gracilis	BB 98.234 TYPE	Tanzania	KF294612	JX192970
Cantharellus guyanensis	1501/MRG07	Guyane	KX857094	KX857060
	1517/MR	Guyane	KX857095	KX857061
Cantharellus hainanensis	N.K. Zeng2289 TYPE	China	KY407524	KY407536
Cantharellus heinemannianus	BB 96.307 TYPE	Zambia	KF294665	-
Cantharellus humidicolus	BB 98.036 TYPE	Tanzania	KF294666	JX193005
Cantharellus ibityensis	BB 08.196 TYPE	Madagascar	KF294650	GQ914980
Cantharellus isabellinus var. parvisporus	BB 98.020 TYPE	Tanzania	KF294614	JX192972
Cantharellus iuventateviridis	BP Looney 523 TYPE	USA	NG_060428	KX857047
Cantharellus lateritius	TJ Baroni 8059F	USA	MT371335	-
	TJ Baroni 8117L	USA	MT371336	-
	BB 07.025 TYPE	USA	KF294633	GQ914959
Cantharellus lewisii	BB 07.003 TYPE	USA	JN940597	GQ914962
Cantharellus lilacinopruinatus	BB 07.221	Slovakia	KF294637	GQ914951
Cantharellus miniatescens	1683/TH9870	Cameroon	KX857108	KX857079
Cantharellus minor	BB 07.002	USA	KF294625	JX192978
	BB 07.057	USA	KF294632	JX192979
Cantharellus miomboensis	BB 98.021 TYPE	Tanzania	KF294613	JX192971
Cantharellus pallens	BB 09.441	Italy	KX907218	KX907240
	BB 12.082	Italy	KX857092	KX857036
Cantharellus parvoflavus	Montoya 5423 TYPE	Mexico	MT371337	MT449706
	Herrera 204	Mexico	MT371338	MT449707
	Herrera 229	Mexico	MT371339	MT449708
Cantharellus paucifurcatus	BB 08.320 TYPE	Madagascar	KF294655	JX192988
Cantharellus phasmatis	C073WITYPE	USA	JX030426	-
Cantnarellus platyphyllus	BB 98.126 I YPE	Ianzania	KF294620	JX1929/5
Cantnarellus platyphyllus subsp. bojeriensis	BB 08.160	Madagascar	KF294648	JX192984
Cantnarellus pseudominimus	JV 00.663	France	KF294657	JX192991

Table 1. *Cantharellus* taxa: Fungal names, specimen vouchers, locations and GenBank accession numbers (for 28S and tef- 1α). Newly sequenced collections in bold.

Taxa	Voucher	Locality	LSU	tef—1a.
Cantharellus quercophilus	BB 07.097 TYPE	USA	KF294644	JX192981
Cantharellus romagnesianus	AH44218	Spain	KX828807	KX828836
Cantharellus roseofagetorum	AH44789 TYPE	Georgia	NG_058962	KX828839
Cantharellus sebosus	BB 08.234 TYPE	Madagascar	KF294652	JX192986
Cantharellus spectaculus	C081WI TYPE	USA	JX030421	JX030414
Cantharellus splendens	BB 96.306 TYPE	Zambia	KF294670	_
Cantharellus subalbidus	BB 13.014B	USA	KX896782	KX857038
Cantharellus subamethysteus	DS 06.218 TYPE	Malaysia	KF294664	_
Cantharellus subcyanoxanthus	BB 00.1137 TYPE	Madagascar	-	JX192990
Cantharellus subincarnatus subsp.	BB 06.080 TYPE	Madagascar	KF294601	JX192962
rubrosalmoneus				
Cantharellus symoensii	BB 98.113 TYPE	Tanzania	KF294619	JX192974
Cantharellus tabernensis	Herrera 120	Mexico	MT371340	MT449709
	Herrera 121	Mexico	MT371341	MT449710
	BB 07.056 TYPE	USA	KF294631	GQ914974
Cantharellus tanzanicus	BB 98.040 TYPE	Tanzania	KF294622	JX192977
Cantharellus tenuithrix	BB 07.125 TYPE	USA	JN940600	GQ914947
Cantharellus texensis	BB 07.018 TYPE	USA	KF294626	GQ914988
Cantharellus tomentosus	BB 98.060 TYPE	Tanzania	KF294672	JX192995
Cantharellus veraecrucis	Herrera 142	Mexico	MT371342	-
	Herrera 58	Mexico	MT371343	MT449711
	Bandala 4505 TYPE	Mexico	MT371344	MT449712
Cantharellus violaceovinosus	Corona 648 TYPE	Mexico	NG_064465	MF616521
Craterellus confluens	Botteri 6 TYPE	Mexico	MT371345	-
Craterellus tubaeformis	BB 07.293	Slovakia	KF294640	GQ914989
Hydnum repandum	BB 07.341	Slovakia	KF294643	JX192980

shown) was also generated by Bayesian Inference (BI), using Mr Bayes v. 3.2.7 (Ronquist et al. 2012) according to Montoya et al. (2019a), with the previously calculated evolutionary model. The phylogenies from ML and BI analyses were displayed using FigTree v1.4.4 (Rambaut 2018). Only bootstrap values (BS) of \geq 70% and Bayesian posterior probabilities (BPP) of \geq 0.90 were considered and indicated on the tree branches (BS/BPP) of Fig. 1.

Results

We studied 78 specimens in the field (not all conserved) of *Cantharellus* species, each with basidiomes in different growth stages, most of them showing an annual fruiting pattern between August-October. We generated 19 new DNA sequences from eight fresh specimens and four from desiccated herbarium collections, twelve from nLSU and seven from *tef*-1 α (Table 1). The built dataset includes a total of 80 sequences, using *Craterellus tubaeformis* and *Hydnum repandum* sequences as the outgroup (Table 1); the alignment is deposited in TreeBASE as 26146. In the inferred molecular phylogeny two groups of the produced sequences clustered in isolated position. One of them, the *Cantharellus lateritius* and *C. flavolateritius*, and the other group appeared close to *C. minor* and *C. romagnesianus* (Fig. 1). Based on the distinctive morphological fea-



Figure 1. Phylogenetic relationships within *Cantharellus* species inferred from the combined nLSU (large subunit of the ribosome) and *tef*-1 α (transcription elongation factor 1-alpha) sequences, by maximum likelihood method and Bayesian inference. The new species are indicated in bold letters. Bootstrap scores (only values \geq 70) and Bayesian Posterior Probabilities (only values \geq 0.90) are indicated above branches.

tures and color variation of specimens of two clades, as well as their isolated position in the phylogeny obtained, we concluded that these Mexican specimens represent two distinct species, which are proposed here as new to science (described below). A third group of sequences clustered with strong support together with sequences of *C. tabernensis* type specimen (Fig. 1). Although Mexican samples, in contrast with the morphological description by Feibelman et al. (1996) that shows some minor differences (below discussed), all share the taxonomic distinctive characters to interpret them as being conspecific. In the classification proposed by Buyck et al. (2014) the *Cantharellus* with smooth hymenophore, clustered within subgenus *Cantharellus* and the other new species proposed here, together with *C. tabernensis*, within subgenus *Parvocantharellus*.

Taxonomy

Description of the new species

Cantharellus veraecrucis Bandala, Montoya & M. Herrera, sp. nov.

MycoBank No: 838105 Figs 2a, b, 3

Holotype. MEXICO. Veracruz: Municipality of Zentla, around town of Zentla, 850 m a.s.l., gregarious on ground, under *Quercus oleoides* Schltdl. & Cham., 5 July 2012, Bandala 4505 (XAL).

Diagnosis. Differing from other related yellow *Cantharellus* species (subgenus *Cantharellus*) by the smooth hymenophore, often rugulose or with low, close, fine, irregular veins, pinkish-yellow, ellipsoid basidiospores 7–9 (–10.5) × (4.5–) 5–6.5 μ m [\bar{Q} = 1.36–1.65], basidia (43–) 49–96 (–104) × 5–12 μ m, pileipellis terminal hyphae 22–60 (–73) × 4–5.5 μ m, subcylindrical, rarely subventricose, straight to moderately flexuous, wall ≤ 1 μ m thick.

Gene sequences ex-holotype. nLSU MT371344; *tef*-1a MT449712.

Etymology. Referring to the locality of origin, in the State of Veracruz, Mexico.

Description. *Pileus* 20–80 (–100) mm diam, convex to plane convex, then more or less applanate and centrally depressed, becoming concave and finally broadly infundibuliform; involute margin when young, later incurved and becoming recurved or plane or uplifted in old specimens, not striate, at first entire, becoming variably lobed and undulate; surface dry, when young with appressed fibrils forming a moderately fine, squamulose surface especially at the center, smooth to glabrescent with age, yellow, light yellow (2.5Y 8/3, 7/12, 10YR 4–5/2), pale orange to bright yellow-orange (3A7–8, 4A4–8, 5A4–8, 2.5Y 7/8–8/8, 10YR 6/8, 7/6–8, 8/8) and even brownish-orange (5B7), at times light gray (10YR 7/1–2, 7.5YR 7/1, 4B2) at the center, orange-buff (5B5), salmon-orange to dirty peach-orange (6A6, 6B3, 6B5) or even brown (6E5). *Hymenophore* decurrent, smooth overall, often rugulose or with low, close, simple or forked, fine, irregular veins; paler than the pileus, light rose (10YR 8/2–3;7.5YR 7/3–4, 8/4, 5A2–4) when young although with age still preserv-



Figure 2. Basidiomes of *Cantharellus* species **a**, **b** *C. veraecrucis* (**a** Bandala 4505, holotype **b** Herrera 142) **c**, **d** *C. parvoflavus* (**c** Montoya 5423, holotype **d** Herrera 229) **e**, **f** *C. tabernensis* (**e** Herrera 120 **f** Herrera 131). Scale bars: 10 mm.

ing pinkish tints on a pale yellow (4A2–3), light yellow (10YR 8/3–4, 8/6, 2.5Y 8/4), light orange (6A3–4), or even egg yellow (4A8) ground. *Stipe* 10–75 × 6–21 mm, equal, tapering gradually downwards, somewhat sinuous or curved, central, occasionally somewhat eccentric, solid, glabrous to subtomentose, at times with age the surface becomes detached in scattered fibrils concolorous with hymenophore, whitish with yellow tinges (4A3–4), pale to bright yellow (4A6–8), orange (5A4), to orange-brown tinges (4A8, 4B7–8, 5B7) especially towards the base, often staining ochraceous or rusty orange color when handle; base in some specimens villous to finely villous under lens. *Context* fleshy, fibrous in stipe, concolorous with pileus or paler, yellowish-buff, odor agreeable fruity, faintly to peach or somewhat recalling butter; taste mild, fruity agreeable, finally somewhat bitter. KOH 3% negative, only somewhat orange on pileus, NH₄OH 10% negative.

Basidiospores 7–9 (–10.5) × (4.5–) 5–6.5 µm [\overline{X} = 7.8–8.9 × 5.3–6.1 µm, \overline{Q} = 1.36–1.65 (n = 12)], ellipsoid, smooth, thin-walled, hyaline, inamyloid. **Basidia** (43–) 49–96 (–104) × 5–12 µm, narrowly clavate to subcylindrical, with 2–5 sterigmata, thin-walled, hyaline, subhymenium composed of cylindrical hyphae 3–5 µm diam. **Cystidia** absent. **Pileipellis** a cutis composed of cylindrical hyphae 4–6 µm diam., intermingled in a compact arrangement, hyaline, yellowish colored in group; terminal hyphae 22–60 (–73) × 4–5.5 µm, subcylindrical, rarely subventricose, scattered, straight to moderately flexuous, smooth, hyaline, inamyloid, thick-walled (<1 µm thick). **Pileus trama** composed of cylindrical hyphae, 4–5 µm diam, slightly thickwalled (<1 µm thick), hyaline, some with weakly refringent contents. **Hymenophoral trama** composed of hyphae 4–5 µm diam, thin-walled, some with weakly refringent contents. Clamp connections present in all tissues.

Habitat. Solitary to gregarious, on soil, in tropical oak forest, in the studied sites it is recorded frequently in monodominant stands of *Quercus oleoides*, being less frequent in monodominant stands of *Q. glaucescens* Bonpl. or *Q. sapotifolia* Liebm.; fruiting in June-October at the coastal plain of central Veracruz State, east coast of Mexico.

Specimens examined. MEXICO. Veracruz, Municipality of Zentla, Road Puentecilla-La Piña, 837 m a.s.l., 2 Jul 2009, Ramos 192, 193, 194; 21 Jun 2012, Herrera 23, 24, 28; 5 Jul 2012, Corona 649, 650, 653; 31 Jul 2012, Montoya 4887; 6 Nov 2013, Herrera 68. Around town of Zentla, 850 m a.s.l., 26 Jun 2013, Herrera 58, 59; 15 Jun 2016, Herrera 153, 154; 23 Jun 2016, Herrera 156; 6 Jul 2016, Herrera 175, 181, 183; 12 Jul 2016, Caro 71, Herrera 185, 186, 188, 190; 10 Aug 2016, Herrera 193; 5 Oct 2016, Melecio 16; 23 Oct 2018, Herrera 159; 12 Jul 2017, Montoya 5347; 21 Sep 2017, Garay 394, Garrido 88, 89; 20 Jun 2018, Herrera 232. Municipality of Alto Lucero, NE Mesa de Venticuatro, 450–500 m a.s.l., 17 Sep 2015, Herrera 140; 4 Sep 2018, Herrera 244. Jaguarundi Park, Coatzacoalcos 29 Sep 2015, Herrera 142, 143, 144, 145 (all at XAL).

Remarks. *Cantharellus veraecrucis* is distinguished by the basidiome colors, hymenophore smooth (or at times discontinuously rugulose) with pinkish tinges, and pileus surface with appressed fibrils. In some stage of development, it superficially might look like *C. flavolateritius*; this latter, however, according to Buyck et al. (2016a) exhibits bright yellow colors on pileus, the hymenophore is composed of radially oriented, low anastomosing veins,"... locally almost smooth...", paler stipe (yellow to off-white), narrowly ellipsoid, somewhat phaseoliform basidiospores (7.1–) 7.2–7.88–8.5 (–10.0) × (4.0–) 4.2– 4.71–5.2 (–5.8) µm, Q = (1.4–) 1.5–1.69–1.8 (–2.1) and pileipellis terminal hyphae often rather short, clavulate or apically slightly inflated, rarely ellipsoid, mostly 20–50 (–70) µm long, sometimes more or less wavy-undulate in outline.

In our phylogenetic analysis, *C. veraecrucis* is related also with *C. lateritius*. This latter species exhibits pale to deep yellow or even apricot orange (Buyck et al. 2011) or bright orange or slightly pinkish orange colors (Petersen 1979). Buyck et al. (2011) with their field experience also cited that *C. lateritius* "... has an often excentrical,



Figure 3. *Cantharellus veraecrucis* (Bandala 4505, holotype) **a** basidiospores **b** terminal elements of the pileipellis **c** basidia **d** longitudinal section of pileipellis. Scale bars: $5 \mu m$ (**a**); $10 \mu m$ (**b**, **c**); $25 \mu m$ (**d**).

sometimes laterally compressed, short to long, more or less yellow stipe that can remain white at the base but is concolorous with the cap higher up, and it has an almost smooth to clearly veined often slightly pinkish tinted hymenophore (the senior author has never seen absolutely smooth specimens)...". Based on our revision of the epitype of *C. lateritius* (Buyck 07.025 kept at PC, designated by Buyck and Hofstetter 2011), it microscopically differs from *C. veraecrucis* by the basidiospores shape (ellipsoid to slightly phaseoliform) and the terminal hyphae of the pileipellis, which are (19–) 21– 60 (–70) × 5–11 μ m, cylindrical to subclavate, tending to be wider than those of *C. veraecrucis* (Fig. 7).

The Asian *Cantharellus hiananensis* N.K. Zeng, Zhi Q. Liang & S. Jiang, appears related also to *C. veraecrucis*, but according to data by An et al. (2017), it differs from the Mexican species by its smaller basidiome size (pileus 25–55 mm diam., stipe 30–55 × 8–10 mm), paler hymenophore (cream to yellowish white), stipe usually hollow covered with tiny, yellow to pale yellowish brown scales, smaller, subcylindrical basidiospores [6–7.09–8 (–9) × (4–) 4.5–4.84–5 (–5.5) µm], and smaller basidia (50–70 × 7–10 µm), (4–) 5 (–6) -spored and pileipellis terminal hyphae 23–82 × 3–8 mm, narrowly clavate or subcylindrical, sometimes subfusiform, with obtuse apex.

Cantharellus veraecrucis represents a wild edible mushroom that is harvested for consumption and commercialization during the rainy season, in the study site and surroundings; it is known as "Oak mushroom". After our systematic multiyear sampling of basidiomes in the forests studied, we could observe that *C. veraecrucis* is a frequent chanterelle, and shares the same habit preferences as *C. violaceovinosus*, recently described from the same region (Herrera et al. 2018).

Cantharellus parvoflavus M. Herrera, Bandala & Montoya, sp. nov.

MycoBank No: 838106 Figs 2c, d, 4

Holotype. MEXICO. Veracruz: Municipality of Alto Lucero, NE Mesa de Venticuatro, 450–500 m a.s.l. gregarious, on ground, under *Quercus oleoides* Schltdl. & Cham., 2 Oct 2017, Montoya 5423 (XAL).

Diagnosis. Differing from other related *Cantharellus* species (subgenus *Parvocan-tharellus*) by the pileus surface with appressed fibrils at center, broadly ellipsoid basidiospores $6-9 (-9.5) \times 4.5-5 \mu m$ [\overline{Q} = 1.52–1.57 (n=3)], pileipellis terminal hyphae (23–) 25–75 (–80) $\mu m \times (3.5-)$ 4–8 μm , mostly cylindrical, often subclaviform, subventricose or somewhat narrowly utriform.

Gene sequences ex-holotype. nLSU MT371337; tef-1a MT449706.

Etymology. Referring to a small, yellow chanterelle; from *parvus* (Lat.): small and *flavus* (Lat.): yellow

Description. *Pileus* 6–26 mm diam, subhemispheric in young, becoming convex to plane convex and centrally depressed, some finally irregularly infundibuliform; margin incurved when young, becoming inflexed to somewhat straight, undulate or irregular or more or less crenate, not or obscurely translucid striate; surface dry, with appressed fibrils at center when young, glabrous at remaining areas, with waxy appearance, bright yellow-orange (5A5–A8) with tiny white to light yellow scales in the center when young, paler at edge when young. *Hymenophore* decurrent or shortly decurrent, with gill-like folds up to 2 mm deep, subdistant to more frequently distant,



Figure 4. *Cantharellus parvoflavus* (Montoya 5423, holotype) **a** basidiospores **b** Terminal elements of the pileipellis **c** basidia **d** longitudinal section of pileipellis. Scale bars: $5 \mu m$ (**a**); $10 \mu m$ (**b**, **c**); $25 \mu m$ (**d**).

at times forked, moderately thick with margin entire or often irregular or eroded, frequently intervenose, some specimens (especially towards the stipe) with irregular low and sinuous veins, often with lower irregular anastomosis among the folds, in some specimens the anastomosis occur practically in the whole hymenophore, while in others only at some areas, especially at pileus margin, with some short lamellulae-like folds, concolorous with the pileus. *Stipe* (10–) $15-42 \times 2-6$ mm, broadened towards the apex, somewhat fused, compressed at times or furrowed, solid but soon fistulous to hollow, glabrous, concolorous with pileus. *Context* fleshy, concolorous with pileus or somewhat paler, with waxy appearance, odor mild, agreeable; taste mild, agreeable.

Basidiospores 6–9 (–9.5) × 4.5–5 µm [\overline{X} = 7.6–7.8 × 4.9–5 µm, \overline{Q} = 1.52–1.57 (n = 3)], broadly ellipsoid, smooth, thin-walled, hyaline, inamyloid, with granular contents or refractive droplets. **Basidia** 50–83 (–89) × (6–) 7–10 µm, narrowly clavate to subcylindrical, with 2–5 sterigmata, thin-walled, hyaline; subhymenium composed of cylindrical hyphae 4–6 µm diam. **Cystidia** absent. **Pileipellis** composed of intermingled hyphae of 4–7 µm diam, cylindrical, hyaline, yellowish in group, terminal hyphae (23–) 25–75 (–80) × (3.5–) 4–8 µm, mostly cylindrical, often subclaviform, subventricose or somewhat narrowly utriform, moderately straight to flexuous, inamyloid, thick-walled (<1 µm thick), smooth, hyaline. **Pileus trama** composed of cylindrical to inflated hyphae, 4–7 µm diam, slightly thick-walled (<1 µm thick), hyaline, some with weakly refringent contents. **Hymenophoral trama** composed of hyphae 4–5 µm diam, thin-walled, some with weakly refringent contents. Clamp connections present in all tissues.

Habitat. Solitary to gregarious, rare in the study area, on soil, in tropical oak forest, under *Quercus oleoides*, September-October, known in the coastal plain of central Veracruz State, east coast of Mexico.

Specimens examined. MEXICO. Veracruz, Municipality of Alto Lucero, NE Mesa de Venticuatro, 392–433 m a.s.l., 27 Sep 2016, Herrera 204; 20 Oct 2017, Herrera 229 (all at XAL).

Remarks. The phylogenetic analysis supports (with high values of bootstrap and Bayesian posterior probabilities 100/1) the distinction of *Cantharellus parvoflavus* as a new species, sister to *C. appalachiensis* from USA. This latter species, besides their basidiomes being somewhat larger [pileus 10–50 mm/stipe 15–75 × 3–10 (–13) mm], are not distinctly yellow, only dingy yellow, usually dull brown, pale or yellowish-brown at margin, darker to brown on disc (Petersen and Ryvarden 1971; Bigelow 1978). Moreover, *C. appalachiensis* has wider broadly-ellipsoid basidiospores [(6.6–) 7.4–8.2 (–8.9) × (4.4–) 4.8–5.6 (–5.9) µm or (6–) 7.5–9 (–10.5) × (4–) 4.5–5.5 (–6) µm] and wider pileipellis hyphae (3–14.5 µm diam. or 9–14 µm diam.) (Petersen and Ryvarden 1971; Bigelow 1978).

Cantharellus parvoflavus is similar to yellow forms of *C. minor*, because they have a hygrophoroid appearance, but this latter is usually bright yellow orange to orange, fading to pale orange-buff or pale orange, with glabrous pileus surface, bigger, ellipsoid, slightly phaseoliform basidiospores (6–) 7.5–10 (–11.5) × (4–) 4.5–6 (–6.5) µm and pileipellis terminal elements subcylindrical to subventricose (Bigelow 1978; Buyck et al. 2010). *Cantharellus romagnesianus* is close to *C. parvoflavus* but it develops greybrown colors in the pileus, its hymenophore has forked veins, often spaced, larger basidiospores [(8–) 9–11.5 (–12.5) × 4–6 (–6.5), Q = 1.71–2.28] and with different shape (Olariaga et al. 2017).

New record of Cantharellus tabernensis in Mexico

Cantharellus tabernensis Feib. & Cibula, Mycologia 88: 299 (1996) Figs 2e, f, 5

Description. *Pileus* 10–30 mm diam, hemispheric to convex, becoming broadly conical to plane convex and faintly depressed in the disc, margin incurved when young, somewhat inflexed to straight with age or somewhat reflexed, not strate, not or faintly undulate or crenulate; hygrophanous, with dull appearance, some with greyish appressed fibrils at center and smooth at the margin when young, smooth to glabrescent with age; light yellow (2.5Y 8/6–8/8, 4A5). *Hymenophore* decurrent or shortly decurrent, with gills up to 3 mm deep, subdistant to more frequently distant, continuous, or forked at different levels, moderately thick; margin entire, at times with irregular anastomosis among folds, with short lamellulae-like folds; yellow to egg yellow (10YR 8/8) brighter than the pileus. *Stipe* (15–) 19–40 × 2–6 mm, central or at times slightly eccentric, equal, occasionally somewhat applanate, at times slightly fused or broader at base, solid to hollow, often furrowed especially below, hygrophanous, surface smooth, concolorous with the pileus; mycelium whitish to pale yellowish. *Context* 1–3 mm thick cream color to yellowish, odor mild, agreeable; taste mild, agreeable.

Basidiospores 6.5–8.5 × 4.5–5 µm [\overline{X} = 7.32–7.34 × 4.8–4.9 µm, \overline{X} = 1.49–1.52, (n = 2)], ellipsoid, smooth, thin-walled, hyaline, inamyloid, with granular contents or refractive droplets. **Basidia** (53–) 56–87 (–99) × 6–10 µm, narrowly clavate to subcylindrical, with 2–4 sterigmata, thin-walled, hyaline; subhymenium composed of cylindrical hyphae 3–5 µm diam. **Cystidia** absent. **Pileipellis** a cutis composed of hyphae 5–8 µm diam, intermingled in a compact arrangement, cylindrical, hyaline, inamyloid, with terminal hyphae cylindrical to somewhat subclavate, 62–75 × 6–10 µm, slightly thick-walled (<1 µm thick), smooth, hyaline, inamyloid, usually abundant. **Pileus trama** composed of cylindrical hyphae, 3–8 µm diam, slightly thick-walled (<1 µm thick), hyaline. **Hymenophoral trama** composed of hyphae 3–6 µm diam, thin-walled. Clamp connections present in all tissues.

Habitat. Solitary to gregarious, rare in the study area, on soil, in tropical oak forest, under *Quercus oleoides* and *Q. sapotifolia*, fruiting in June at the coastal plain of central Veracruz State, east coast of Mexico.

Specimens examined. MEXICO. Veracruz, Municipality of Zentla, Road Puentecilla-La Piña, 837 m a.s.l.,11 Jun 2015, Herrera 120, 121;10 Sep 2015, Herrera 131 (all at XAL).

Remarks. In our phylogeny Mexican sequences of specimens Herrera 120 and 121 clustered (Fig. 1) with high values of Bootstrap and Bayesian posterior probabilities (96/0.99) with a sequence of the type specimen of *Cantharellus tabernensis* from U.S.A., produced by Buyck et al. (2014). The morphological description provided above includes both mentioned specimens, and in fact, in the most relevant characters, those specimens agree with the species. It should be mentioned, however, that the



Figure 5. *Cantharellus tabernensis* (Herrera 131) **a** basidiospores **b** basidia **c** Terminal elements of the pileipellis **d** longitudinal section of pileipellis. Scale bass: $5 \mu m$ (**a**); $10 \mu m$ (**b**, **c**); $25 \mu m$ (**d**).

following features recorded in the description provided by Feibelman et al. (1996) were not observed in the Mexican material: pileus mat felted overall, often umbilicate, sometimes perforated, basidia 4–5–6 -spored and dark plasmatic pigment confined to clavate terminal cells of the surface hyphae at disc.

The record presented here of *C. tabernensis*, in its turn provides additional information on the species distribution. It is known from the mixed pine and hardwood forests, usually near *Pinus elliotii* Engelm., at the Gulf coastal plain in Texas, Mississippi, and Louisiana states in USA (Feibelman et al. 1996), and now *C. tabernensis* is known also in the tropical *Quercus* forest from Veracruz, in the coastal plain of Veracruz state in the Gulf of Mexico.

Proposal of a new name for the replacement of Craterellus confluens

Derived from the fact that *Craterellus confluens* was described by Berkeley (1867) from the Orizaba region in Veracruz (Mexico), later records of yellow chantherelles occurring in the Zentla region (north of Orizaba) were referred to in the past by Guzmán and Sampieri (1984) as "Cantharellus odoratus" following Corner (1966). This latter author introduced that C. lateritius and Cr. confluens were the same as Schweinitz (1822) described as Merulius odoratus. Burt (1914) mentioned particularly the macroscopic resemblance among the Cr. confluens isotype specimen in Schweinitz herbarium and the specimens of C. odoratus that he studied, thus he synonymized the former and pointed out that "...the type of *Cr. confluens* has the hymenium rugose-wrinkled, as is often the case in specimens of *C. odoratus*; its habit, dimensions, structure, coloration, and spores are quite those of *C. odoratus...*". In the molecular phylogeny here generated (Fig. 1) Cr. confluens holotype specimen (Botteri 6, kept at K) is supported with high values of bootstrap and Bayesian posterior probabilities sister to C. veraecrucis here described (above), and both are closely related with C. lateritius (including a sequence of the type) and C. flavolateritius. Petersen (1979) after type studies considered indeed, separately C. lateritius, Cr. odoratus (Schwein.) Fr. and Cr. confluens, being a combination of characters such as clamps (present or not), basidiome colors and the leathery, funnel-shaped basidiocarps (with a hollow stipe), among other features, considered in the distinction of such taxa. Molecular studies have also shown that Schweinitz's species belongs to Craterellus (i.e. Cr. odoratus) (Feibelman et al. 1997; Dahlman et al. 2000) and now, our analysis confirms (Fig. 1) that Cr. confluens holotype specimen belongs to Cantharellus, among the group of yellow species around C. lateritius. Buyck and Hofstetter (2011) suggested "...to refrain from using the name C. confluens any longer...", but rather a new specific name in Cantharellus is required for such taxon because in *Cantharellus* the specific name is preoccupied by *C. confluens* (Schwein.) Schwein. 1834, i.e. Merulius confluens Schwein. 1822, a meruliod species (Burt 1917) member of Byssomerulius (Ginns 1975; Zmitrovich et al. 2006). Possibly Cr. confluens exhibits a rare occurrence in the site that we explored in the Zentla region or it has a more restricted occurrence in some other ecosystem, near or around the city of Orizaba, Veracruz. Considering the features of the fruitbodies ("...stem divided...") mentioned by Berkeley (1867) in the diagnosis, we propose to replace the name as follows:



Figure 6. *Cantharellus furcatus* (Botteri 6, holotype of *Craterellus confluens*) **a** basidiospores **b** terminal elements of the pileipellis. Scale bars: $5 \mu m$ (**a**); $10 \mu m$ (**b**).

Cantharellus furcatus Bandala, Montoya & Ramos, nom. nov.

Mycobank No: 838107

Bas. Craterellus confluens Berk. & M.A. Curtis, J. Linn. Soc., Bot. 9: 423 (1867).

- Syn. *Cantharellus confluens* (Berk. & M.A. Curtis) R.H. Petersen, Sydowia 32: 201 (1979) nom. illeg.
- Non *Cantharellus confluens* (Schwein.) Schwein., Trans. Am. Phil. Soc., New Series 4: 153 (1834).
- = Merulius confluens Schwein., Schr. Nat. Ges. Leipzig 1: 92 (66 in reprint) (1822).
- = Byssomerulius corium (Pers.: Fr.) Parmasto, Eesti NSV Tead. Akad. Toim., ser Biol. 16: 383 (1967).

Holotype. MEXICO. Veracruz, Orizaba. Botteri 6 [ex herb. M.J. Berkeley] KM 173247 (K). Gene sequences ex-holotype. nLSU MT371345.

Etymology. From *furcatus* (Lat.): forked, referring to a bifurcation developed in the basidiome.

Remarks. Presumably having been separated from the entire collection, the holotype specimen consists of a single unipileate basidiome but the diagnostic feature mentioned by Berkeley (1867) "... stem divided above into numerous pilei...", a feature practically not observed in close related species (*C. flavolateritius*, *C. lateritius*, *C. veraecrucis*) is present, as noted and depicted by Burt (1914), in the isotype collection at Farlow Herbarium (https://huh.harvard.edu/pages/farlow-herbarium-fh), and it is


Figure 7. Terminal elements of the pileipellis of *Cantharellus* species **a**, **b** *C. lateritius* (**a** Buyck 05.058 **b** Buyck 07.025 epitype) **c** *C. veraecrucis* (Bandala 4505, holotype). Scale bar: 10 μm.

well-depicted and described for collections from SE USA studied by Petersen (1979). The particularity of producing multipileate basidiomes and/or with fused stipes, in combination with the smooth pileus surface, pileus and hymenophore predominantly orange colored (*aurantiacus* in the diagnosis) hymenophore rugulose, irregularly forking and anastomosing, rarely smooth, with yellow stipe and lacking pinkish shades (Petersen 1979), are the distinctive macroscopic features of *C. furcatus*.

The holotype specimen Botteri 6 (at K) of *Cr. confluens* was preserved in such a poor condition that it does not allow a proper rehydration of the tissues. The microscopic features recovered were: basidiospores of 7.5–8.5 × 5–6 µm (\overline{X} = 7.8 × 5.3 µm), \overline{Q} = 1.46, broadly ellipsoid to ellipsoid, some subglobose, somewhat flattened adaxially, smooth, hyaline, thin-walled, inamyloid. Pileipellis a cutis composed of cylindrical hyphae 5–7 µm diam, compactly arranged, hyaline, yellowish colored in group; terminal hyphae 36–57 × 8–12 µm, clavate to broadly clavate, scattered, smooth, hyaline, inamyloid, thin to thick-walled (<1 µm thick). Clamp connections present (Fig. 6). In the holotype Petersen (1979) registered basidiospores of 6.7–8.9 × 4.8–5.9 µm, \overline{Q} = 1.29–1.54 and of 7–10 × 5–6.3 µm, while in the isotype collection there is an annotation made in 1980 by Dr. H.E. Bigelow, describing basidiospores: 8–10 × 5.5–6.5 µm, ellipsoid or broadly ellipsoid or subglobose, smooth, inamyloid, basidia mostly collapsed, ± 41–52 × 6–7.5 µm, pileus with hyphae 4–10 µm diam, clamped, pigment apparently intracellular (https://huh.harvard.edu/pages/farlow-herbarium-fh).

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RESEARCH ARTICLE



A new genus and four new species in the /Psathyrella s.l. clade from China

Tolgor Bau¹, Jun-Qing Yan²

I Key Laboratory of Edible Fungal Resources and Utilization (North), Ministry of Agriculture and Rural Affairs, Jilin Agricultural University, Changchun 130118, China 2 Jiangxi Key Laboratory for Conservation and Utilization of Fungal Resources, Jiangxi Agricultural University, Nanchang, Jiangxi 330045, China

Corresponding authors: Tolgor Bau (junwusuo@126.com); Jun-Qing Yan (yanjunqing1990@126.com)

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Abstract

Based on traditional morphological and phylogenetic analyses (ITS, LSU, *tef-1a* and β -tub) of psathyrelloid specimens collected from China, four new species are here described: *Heteropsathyrella macrocystidia*, *Psathyrella amygdalinospora*, *P. piluliformoides*, and *P. truncatisporoides*. *H. macrocystidia* forms a distinct lineage and groups together with *Cystoagaricus*, *Kauffmania*, and *Typhrasa* in the /Psathyrella s.l. clade, based on the Maximum Likelihood and Bayesian analyses. Thus, the monospecific genus *Heteropsathyrella* gen. nov. is introduced for the single species. Detailed descriptions, colour photos, and illustrations are presented in this paper.

Keywords

Agaricales, Basidiomycete, four new taxa, Psathyrellaceae, taxonomy

Introduction

Psathyrella (Fr.) Quél. is characterized by usually fragile basidiomata, a hygrophanous pileus, brown to black-brown spore prints, always present cheilocystidia and basidiospores fading to greyish in concentrated sulphuric acid (H_2SO_4) (Kits van Waveren 1985; Örstadius et al. 2015). There are records of more than 1000 names, including synonyms and subspecies, since Fries established the tribe *Psathyrella* under *Agaricus* L. (Fries 1838; Smith 1972; Kits van Waveren 1985; Örstadius and Knudsen

2012). This group has been classified in the Coprinaceae Roze ex Overeem subfamily Psathyrelloideae (Hawksworth et al. 1983; Hawksworth et al. 1995; Kirk et al. 2001) and then incorporated into Psathyrellaceae Vilgalys, Moncalvo & Redhead, based on the study of Redhead et al. (2001). Further studies found that *Psathyrella* is polyphyletic and /Psathyrella s.l. was limited by Örstadius et al. (2015). /Psathyrella s.l. consists of five major supported clades: /*Coprinellus*, /*Cystoagaricus*, /*Kauffmania*, /*Psathyrella* s.s., and /*Typhrasa*. Each major clade represents a genus. Hence, *Kauffmania* Örstadius & E. Larss. and *Typhrasa* Örstadius & E. Larss. were established, Cystoagaricus Singer emend. Örstadius & E. Larss. was redefined (Örstadius et al. 2015). Most species in *Cystoagaricus*, *Kauffmania*, and *Typhrasa* were incorporated from *Psathyrella*. Species with cap surface breaking up into dark fibrils or scales were classified into *Cystoagaricus*. Species having rostrate hymenial cystidia with oily drops were classified into *Typhrasa*. *P. larga* (Kauffman) A.H. Sm., which has large basidiomata, scanty veil, and pale spores, was classified into *Kauffmania*.

As a part of the study of Chinese psathyrelloid species, four new species were discovered, during our investigations in temperate and subtropical regions of China from 2016–2019. Among them was a new species morphologically similar to *Psathyrella* but phylogenetically distinguished from it, and which formed a separate lineage. We recognize this new taxon as a new genus based on traditional morphological and phylogenetic analyses. In this paper, detailed information on the new taxa is presented.

Materials and methods

Morphological studies

Macroscopic characteristics of fresh specimens were recorded. Colour codes followed Kornerup and Wanscher (1978). Thirty basidiospores, cystidia, and basidia were measured under a microscope in water and 5% aqueous KOH for each specimen. The measurements and Q values are given as (a)b–c(d), in which "a" is the lowest value, "b–c" covers a minimum of 90% of the values and "d" is the highest value. "Q" stands for the ratio of length to width of a spore (Bas 1969; Yu et al. 2020). Photographs of some microscopic characteristics are shown in Suppl. material 1: Figure S1. Specimens were deposited in the Herbarium of Mycology, Jilin Agricultural University (**HMJAU**).

DNA extraction and sequencing

DNA was extracted from dried specimens with the NuClean Plant Genomic DNA kit (CWBIO, China). Four regions (ITS, LSU, *tef-1a* and β -*tub*) were amplified for the study, which using ITS1/ITS4 (White et al. 1990), LR0R/LR7 (Hopple and Vilgalys 1999), EF983F/EF2218R (Örstadius et al. 2015), and B36f/B12r (Nagy et al. 2011), respectively. PCR was performed using a touchdown program for all regions as follows: 5 min at 95 °C; 1 min at 95 °C; 30 s at 65 °C (add -1 °C per cycle); and 1 min at 72 °C

for a cycle of 15 times; 1 min at 95 °C; 30 s at 50 °C; and 1 min at 72 °C for a cycle of 20 times; and 10 min at 72 °C (Yan and Bau 2018b). DNA sequencing was performed by Qing Ke Biotechnology Co., Ltd. (Wuhan City, China), using primers listed above.

Data analyses

ITS1+5.8S+ITS2 sequences of four new species were tested with BLAST in GenBank, species sharing over 95% similarity are selected. Based on the BLAST results, morphological similarities and then compared to the research of Örstadius et al. (2015) and Yan and Bau (2018a). Totally, 176 sequences of 46 taxa, including 4 regions (ITS, LSU, *tef-1a*, and β -*tub*) which divided into 7 partitions (ITS, LSU, Tef 1st, Tef 2nd, Tef 3rd, Tub 1st, and Tub 2nd) were downloaded for phylogenetic analyses. The details are presented in Table 1. Sequences were aligned by the online version of the multiple sequence alignment program MAFFT v7 (Katoh and Standley 2013) and were manually adjusted in BIOEDIT v7.1.3.0 (Hall 1999). Phylogenetic analyses were conducted using Bayesian inference (BI) in MRBAYES v3.2.6 (Ronquist and Huelsenbeck 2003) and maximum likelihood (ML) in IQTREE v1.5.6 (Nguyen et al. 2014). For BI analyses, the best model was selected by AIC in MRMODELTEST 2.3, and gaps were treated as missing data (Nylander 2004; Örstadius et al. 2015). Four Markov chains (MCMCs) were run for two million generations, with sampling every 100th generation. The first 25% of trees were discarded (Ronquist and Huelsenbeck 2003). ML analyses were executed by applying the ultrafast bootstrap approximation with 1000 replicates. The sequence alignment was deposited in TreeBASE (http://purl.org/phylo/ treebase/phylows/study/TB2:S27605?x-access-code=ad75ae6bd4198cfa6d444a89586 3bc1b&format=html).

Results

Phylogenetic results

The aligned complete dataset consisted of 54 taxa and 2606 characters (ITS 711 bp, LSU 829 bp, Tef 1st 69 bp, Tef 2nd 136 bp, Tef 3rd 497 bp, Tub 1st 125 bp, and Tub 2nd 239 bp). Due to the different number of models supported by Mrbayes and IQtree, the best models are calculated separately, and the results are as follows: the best models for Bayesian analysis were GTR+I+G for the ITS, LSU, Tef 3rd, and Tub 2nd, HKY+I for Tef 1st, SYM+G for Tef 2nd, and SYM+I+G for Tub 1st; the best models for ML analysis were TIM2+F+I+G4 for the ITS and LSU, TNe+FQ+I for Tef 1st and Tef 2nd, TIM2+F+G4 for Tef 3rd, TIMe+FQ+G4 for Tub 1st, and HKY+F+G4 for Tub 2nd.

For Bayes analysis, the average standard deviation of split frequencies less than 0.01 after 610 thousand generations. The Bayesian inference (BI) and ML bootstrap proportions are shown in the Bayesian tree (Fig. 1). In addition, the ML tree is shown in Suppl. material 2: Figure S2. The phylogenetic tree analyses recovered 8 major supported clades

Taxon	Voucher	Locality	ITS	LSU	tef-1a	β-Tub
Coprinellus	LO141-08 type	Sweden	KC992944	KC992944	KJ732823	-
christianopolitanus			E) (050015	EL (OE (OE (EN Jaco (ana
C. disseminatus	SZMC-NL-233/		FM8/801/	FM8/62/4	_	FN396282
C. silvaticus	LO172-08	Sweden	KC992943	KC992943	KJ732822	KJ664911
C. truncorum	SZMC-NL-1101	Sweden	FM878006	FM876262	FM897225	FN396328
Cystoagaricus	Ramsholm800927	Finland	KC992945	KC992945	-	-
hirtosquamulosus						
C. olivaceogriseus	WK 8/15/63-5 (MICH) Type	USA	KC992948	KC992948	-	-
C. sylvestris	LO191-92	Sweden	KC992949	KC992949	-	-
C. squarrosiceps	Laessoe44835	Ecuador	KC992950	-	-	-
C. strobilomyces	E. Nagasawa 9740		AY176347	AY176348	-	-
Heteropsathyrella macrocystidia	HMJAU37802 Type	China:Jilin	MW405102	MW413359	MW411004	MW410997
H. macrocvstidia	HMIAU37803	China:Iilin	MW405101	MW413358	MW411003	_
H. macrocystidia	HMIAU37912	China:Jilin	MW405103	MW413360	MW411005	_
Kauffmania larga	I A\$97-054	Sweden	DO389695	DO389695	_	_
K lavaa	LÖ223.90	Sweden	DQ389694	DQ389694	K1732824	K1664912
R. urgu Deselementes shistingta	Smith 59672 (MICH) Trans	TICA	VC002801	VC002801	KJ/ 32824	KJ004912
	LIMIAU27052 T	CLINEL	KC992891	KC992891	-	-
r. amygaainospora	HMJA05/952 Type		MW405104	MW415501	MW410999	MW410991
P. amygaaiinospora	HMJAU3/044	China:Sichuan	MW405105	-	-	-
P. conferta	GE02.00/ (PC) Type	France	KC992890	KC992890	-	-
P. echinata	Z1120/3	NewZealand	KC992925	KC992925	-	KJ664900
P. fagetophila	LO210-85 (M) Type	Sweden	KC992902	KC992902	_	KJ6648/9
P. fennoscandica	HMJAU37918	China:Heilongjiang	MG734723	MW413365	MW411000	MW410993
P. fennoscandica	LO484-05 Type	Sweden	KC992903	KC992903	KJ732790	KJ664881
P. fennoscandica	LO95-96	Sweden	KC992904	KC992904	KJ732791	KJ664882
P. fusca	LÖ287-04	Sweden	KC992892	KC992892	KJ732779	-
P. mucrocystis	LÖ103-98	Sweden	DQ389700	-	KJ732810	KJ664901
P. noli-tangere	LÖ83-03 Neotype	Sweden	DQ389713	DQ389713	-	KJ664890
P. oboensis	HMJAU37936	China:Yunnan	MT429164	MW413366	-	MW410996
P. oboensis	DED 8234 Type	SãoTomé	NR148107	-	-	-
P. olympiana	LÖ32-02	Sweden	DQ389722	DQ389722	KJ732817	KJ664906
P. panaeoloides	LÖ44-03	Sweden	DQ389719	DQ389719	KJ732782	KJ664873
P. panaeoloides	HMJAU23696	China:Jilin	MG734733	MH155958	-	MH161165
P. pertinax	HMJAU6830	China:Jilin	MG734735	_	_	MW410995
P. pertinax	LO259-91 Neotype	Sweden	DQ389701	DQ389701	KJ732809	_
P. piluliformis	HMJAU37922	China:Heilongjiang	MG734716	MW413364	MW411001	MW410994
P. piluliformis	LÖ162-02	Germany	DQ389699	DQ389699	KJ732808	KJ664899
P. piluliformoides	HMIAU37923 Type	China:Yunnan	MW405106	MW413362	MW411002	_
P. twomaea	LÖ97-04	Sweden	DO389718	DO389718	KI732811	KI664902
P twomaea	HMIAU37850	China Iilin	MG734744	MH155959	MH161170	MH161166
P ryheraji	I Ö373-06 Type	Sweden	KC992893	KC992893	KI732781	KI664872
P sabonacea	HMILIA 37935	China:Shanyi	MH155965	MH155960	-	MH161167
P sabonacea	LÖ204-96	Sweden	DO389717		KI732780	KI664871
D cominada	Smith 3/091 (MICH) True	LISA	KC992907	- KC992907	KJ/ 52/00	10040/1
D		ChinadanaManaalia	MC72/722	RC))2)0/	_	MW/410002
r. senex	LÖ115 02	China:Innerwongona	MG/34/32	-	_	WW410992
T. senex	LU113-02	Germany	DQ389/12	DQ389/12	-	KJ004880
P. truncatisporoides	HMJAU3/94/ Type	China:Zhejiang	MW40510/	MW413363	MW410998	MW410990
P. truncatisporoides	HMJAU5/045	China:Zhejiang	MW405108	-	-	-
1'. warrenensis	Smith/0162 (MICH) Type	USA	KC992906	KC992906	-	-
Iyphrasa gossypina	Schumacher024	Germany	KC992946	KC992946	KJ732825	-
T. nanispora	Barta980706 Type	Austria	KC992947	KC992947	-	-
Outgroup						
Coprinopsis cineraria	CBM-FB-24142 Type	Japan	KC992962	-	-	-
C. musae	JV06-179 Type	Denmark	KC992965	KC992965	-	KJ664920
C. submicrospora	AH27055 Type	Spain	KC992959	KC992959	-	KJ664918
C. udicola	AM1240 Type	Germany	KC992967	KC992967	KJ732831	KJ664922
C. uliginicola	Smith34903 (MICH) Type	USA	KC992960	KC992960	_	_

Table 1. Sequences used in this study. Newly generated sequences are in bold.



Figure 1. Phylogram generated by Bayesian inference (BI) analysis based on sequences of a concatenated data set from four nuclear genes (ITS, LSU, *tef-1a* and β -*tub*), rooted with *Coprinopsis* spp. Bayesian inference (BI-PP) \geq 0.95 and ML bootstrap proportions (ML-BP) \geq 75 are shown as BI-PP/ML-BP. • indicates newly described taxa.

(6 genura), with a high statistical support value (BPP ≥ 0.95 , bootstrap ≥ 75). They are *Psathyrella* (including 3 clades), *Coprinellus, Kauffmania, Cystoagaricus, Typhrasa*, and the new genus – *Heteropsathyrella. P. amygdalinospora*, *P. piluliformoides*, *P. truncatisporoides* were separated into individual lineages and are independent from the close taxa in *Psathyrella. P. amygdalinospora* forms a distinct lineage in the /pygmaea clade, *P. piluliformoides* belongs to /piluliformis and groups together with *P. oboensis* Desjardin & B.A. Perry, and *P. truncatisporoides* belongs to /noli-tangere and groups together with

P. rybergii Örstadius & E. Larss. *H. macrocystidia* forms a distinct lineage and groups together with the lineage consisting of *Cystoagaricus*, *Kauffmania*, and *Typhrasa*.

Taxonomy

Heteropsathyrella T. Bau & J.Q. Yan, gen. nov.

MycoBank No: 838372

Remarks. Pileus hygrophanous, tawny to brown, non-deliquescent. Veil present. Lamellae adnexed. Stipe central, hollow. Basidiospores ellipsoid to subellipsoid, smooth, brown in 5% KOH, pale mouse grey in H_2SO_4 . Hymenium hyaline. Basidia monomorphic. Pseudoparaphyses abundant and regularly distributed. Pleurocystidia and cheilocystidia present. Pileipellis composed of saccate to subglobose cells covered by a 1 cell deep layer of periclinal hyphae which are covered by scattered and irregular deposits dissolving in 5% KOH.

Etymology. *Heteropsathyrella*, referring to its morphological similarity to *Psathyrella*. **Type species.** *Heteropsathyrella macrocystidia* T. Bau & J.Q. Yan, *sp. nov.*

Heteropsathyrella macrocystidia T. Bau & J.Q. Yan, sp. nov.

MycoBank No: 838373 Figs 2a–c, 3

Etymology. *macrocystidia*, referring to its large pleurocystidia, which are up to 83 μm long. **Type.** CHINA. Changbai Mountain, Antu County, Yanbian Korean Autonomous Prefecture, Jun-Qing Yan, Herbarium of Mycology, Jilin Agricultural University (HM-JAU37802).

Diagnosis. Differs from *Psathyrella epimyces* by saprophytic and abundant pseudoparaphyses.

Pileus 35–70 mm broad, obtusely conic when young, expanding to plane, with a small obtuse umbo, hygrophanous, tawny to brown (7C6–7D7), darker at center (7E7), striate up to 2/3 from margin, becoming dirty white as pileus dries (7A1–7B2). Veil scattered, small, white (7A1), fibrillose, evanescent. Context hygrophanous, thin and fragile, approximately 1.0–1.5 mm at the centre. Lamellae 3.0–6.0 mm broad, crowded, adnexed, dirty white (7A1–7B2), becoming pale brown to brown (7E7–7F7) as spores mature, edge white (7A1) and even. Stipe 35–100 mm long, 5.0–15 mm thick, white (7A1), cylindrical, gradually thickening towards base, fragile, hollow, but context thick, surface uneven, with small grainy bulb, covered with small, white, evanescent fibrils. Odour and taste indistinctive. Spore print grey brown (7E3–7E4).

Spores 7.8–9.2 × 4.9–5.4 µm, Q = 1.6–1.8, elongated-ellipsoid in face view, in profile flattened on one side, pale brown in water, darker brown in 5% KOH, smooth, with or without 1–2 guttules, germ pore indistinct, approximately 1.0 µm in diam. Basidia 26–34 × 7.3–9.8 µm, clavate, hyaline, 4- or 2-spored. Pseudoparaphyses abundant and regular distribution. Pleurocystidia 59–83 × 12–20 µm, abundant, utriform



Figure 2. Basidiomata **a–c** *Heteropsathyrella macrocystidia* (HMJAU37802) **d–f** *Psathyrella amygdalinospora* (HMJAU37952) **g, h** *P. piluliformoides* (HMJAU37923) **i** *P. truncatisporoides* (HMJAU37947). Scale bars: 10 mm (**a–i**).

with broadly obtuse apex, slightly thick-walled, glabrous or covered by irregular deposits, base tapering to a long stipe. Cheilocystidia $37-56 \times 9.8-17 \mu m$, utriform to fusoid with obtuse apex, base tapering to a short stipe. Caulocystidia $29-61 \times 12-22 \mu m$, caespitose, various, utriform, fusoid or utriform with abrupt narrow neck terminating in a capitellum, base tapering to a long or short stipe. Trama of gills irregular. Pileipellis a 1–2-cell-deep layer of vesiculose cells, up to 61 μm long, covered by a 1-cell-deep layer of periclinal hyphae which are approximately 3.6 μm in diam and covered by scattered and irregular deposition dissolving in 5% KOH. Clamps present.

Habit and habitat. Scattered on mossy rotten wood in mixed forests of larch and birch.

Other specimens examined. CHINA. Changbai Mountain, Antu County, Yanbian Korean Autonomous Prefecture, Jun-Qing Yan, 16 July 2016, HMJAU37803; 28 July 2017, HMJAU37912.

Psathyrella amygdalinospora **T. Bau & J.Q. Yan, sp. nov.** MycoBank No: 838374 Figs 2d–f, 4

Etymology. Referring to the spore shape.



Figure 3. *Heteropsathyrella macrocystidia* (HMJAU37802) **a** basidiomata **b** basidiospores **c** basidia **d** pileipellis **e** pleurocystidia **f** cheilocystidia **g** caulocystidia. Scale basis = 10 mm (**a**); 10 µm (**b–g**).

Type. CHINA. Scenic Spot of Kangding Love Song (Mugecuo), Kangding City, Tibetan Autonomous Prefecture of Garzê, Sichuan Province, 30°08'49.19"N, 101°51'39.18"E, 3790 m, 21 August 2017, Jun-Qing Yan, Herbarium of Mycology, Jilin Agricultural University (HMJAU37952).

Diagnosis. Differs from *P. obtusata* by its spores, ovoid in front view, amygdaliform in profile and dark brown and gradually becoming black-brown in 5% KOH.

Pileus 15–25 mm broad, paraboloid, hygrophanous, chestnut (8F6–8F7), becoming dirty white (8A1–8B1) as pileus dries. Veil not observed. Context approximately 2.0 mm at the centre, fragile, concolorous with pileus. Lamellae 4.0 mm, light brown (8D3–8D5), edges white (8A1), even. Stipes 45–60 mm long, 2.5–3.0 mm thick, fragile, hollow, cylindrical, equal or slightly expanded at base, dirty white (8A1–8B1). Odour and taste indistinctive.



Figure 4. *Psathyrella amygdalinospora* (HMJAU37952) **a** basidiomata **b** basidiospores **c** basidia **d** pileipellis **e** pleurocystidia **f** cheilocystidia. Scale bars: 10 mm (**a**); 10 µm (**b–f**).

Spores 8.8–9.7 × 4.9–5.8 μ m, Q = 1.5–1.9, ovoid in front view, amygdaliform in profile, reddish brown in water, dark brown and gradually becoming black-brown in 5% KOH, inamyloid, smooth, germ pore absent. Basidia 17–20 × 7.3–9.8 μ m, clavate, hyaline, 4-spored. Pleurocystidia abundant, 44–68 × 9.8–13 μ m, fusiform to narrowly utriform, thin-walled, apex obtuse to subacute, rarely subcapitate. Pleurocystidioid cheilocystidia abundant, 22–32 × 7.3–12 μ m, fusiform to utriform, short mucronate or obtuse at apex, rarely mixed with pyriform cells. Trama of gills irregular. Pileipellis consisting of a 1–2-cell-deep layer of subglobose cells that were 30–40 μ m broad. Clamps present.

Habit and habitat. Scattered on mosses in mixed forests of *Cunninghamia* spp., *Pinus* spp. and *Quercus semecarpifolia*.

Other specimens examined. CHINA. Scenic Spot of Kangding Love Song (Mugecuo), Kangding City, Tibetan Autonomous Prefecture of Garzê, Sichuan Province, 22 August 2017, Jun-Qing Yan, HMJAU57044.

Psathyrella piluliformoides T. Bau & J.Q. Yan, sp. nov.

MycoBank No: 838375 Figs 2g, h, 5

Etymology. Reference to its characteristics similar to *Psathyrella piluliformis*.

Type. CHINA. Kunming Institute of Botany, Kunming City, Yunnan Province, 9 September 2017, Herbarium of Mycology, Jilin Agricultural University (HM-JAU37923).

Diagnosis. Differs from *Psathyrella piluliformis* by having ring and yellow amorphous incrustation at the apex of pleurocystidia.

Pileus 50–60 mm broad, plane, hygrophanous, brown (7C7–7D7) at centre, pale (6B6–6B7) at margin, smooth, striations indistinct at margin. Context thin and fragile, approximately 2.0 mm at the centre, same colour as pileus. Lamellae approximately 4.0 mm, very closed, pale coffee (6C5–6D5), edges paler and even (6B4). Stipe 5.5 mm long, 5.0 mm thick, fragile, cylindrical, hollow, slightly thickened towards base, white (6A1) at apex, base slightly brown, with white evanescent squama. Ring present at 1/3 from stipe apex.

Spores 5.6–6.3 × 3.1–4.4 μ m, Q = 1.3–1.9, ellipsoid to oblong-ellipsoid, in profile flattened on one side, pale brown in water, dirty brown in 5% KOH, inamyloid, smooth, germ pore distinct, truncate, 1.1–1.9 μ m broad. Basidia 15–18 × 4.9–6.1 μ m, clavate, hyaline, 4- or 2-spored. Pleurocystidia 39–54 × 11–15 μ m, abundant, utriform to narrowly utriform, or lageniform, rarely fusiform, thick-walled or thin-walled, apex obtuse or broadly obtuse, covered by yellow amorphous incrustation, dissolving in 5% KOH. Cheilocystidia scattered, 24–37 × 9.8–15 μ m, utriform, thick-walled or thin-walled, apex obtuse or broadly obtuse, mixed with subglobose to spheropedunculate cells, cells 11–16 × 9.8–14 μ m, slightly thick-walled or not. Trama of gills irregular. Pileipellis consisting of a 2–3-cell-deep layer of subglobose cells 34–40 μ m broad. Clamps present.

Habit and habitat. Solitary on moss.

Psathyrella truncatisporoides T. Bau & J.Q. Yan, sp. nov.

MycoBank No: 838378 Figs 2i, 6

Etymology. Referring to the truncate spore.



Figure 5. *Psathyrella piluliformoides* (HMJAU37923) **a** basidiomata **b** basidiospores **c** basidia **d** pileipellis **e** pleurocystidia **f** cheilocystidia. Scale bars: 10 mm (**a**); 10 µm (**b–f**).

Type. CHINA. Wulingken, Baishanzhu, Qingyuan County, Lishui City, Zhejiang Province, Tolgor Bau, Jun-Qing Yan, 16 August 2015, Herbarium of Mycology, Jilin Agricultural University (HMJAU37947).

Diagnosis. Differs from *P. rybergii* by its shorter spores (6.8–7.8 µm).

Pileus 8.0–13 mm broad, spreading broadly conical to plane, hygrophanous, pale brown (7C6–7D7), white (7A1–7B1) at margin, striate up to 2/3 from margin. Veil of a thin coating of white to dirty white (7A1–7B1) fibrils, evanescent. Context thin and very fragile, same colour as pileus, approximately 1.0 mm at centre. Lamellae approximately



Figure 6. *Psathyrella truncatisporoides* (HMJAU37947) **a** basidiomata **b** basidiospores **c** basidia **d** pileipellis **e** pleurocystidia **f** cheilocystidia. Scale bars: 10 mm (**a**); 10 μm (**b–f**).

1.5 mm broad, pale brown (7B4–7C4), close, adnate, margin even. Stipes 10–25 mm long, approximately 1.5 mm thick, white (7A1), fragile, hollow, smooth but irregularly lumpy, with the base slightly expanding or not. Odour and taste indistinctive.

Spores (5.8)6.8–7.8(8.3) × 4.4–4.9 μ m, Q=1.2–1.8, broadly ellipsoid to ellipsoid, in profile flattened on one side, inamyloid, smooth, apex obviously truncate, germ pore distinct, 1.5–2.4 μ m broad. Basidia 13–17 × 6.1–7.3 μ m, clavate, 4-spored. Pleurocystidia 37–49 × 12–16 μ m, utriform to broadly utriform, with obtuse to broad apex, base tapering to a long or short stipe, thin-walled. Cheilocystidia 19–31 × 7.3–12 μ m, abundant, similar to pleurocystidia, rarely spheropedunculate, rarely with crystals. Trama of gills irregular. Pileipellis a hymeniderm with 29–39 μ m broad cells. Clamps present.

Habit and habitat. Scattered, terrestrial, in bamboo forest.

Other specimens examined. CHINA. Wulingken, Baishanzhu, Qingyuan County, Lishui City, Zhejiang Province, Tolgor Bau, Jun-Qing Yan, 18 August 2015, HM-JAU57045.

Discussion

The species in the family Psathyrellaceae can be roughly divided into two types by macromorphology: psathyrelloid and coprinoid. Heteropsathyrella is macromorphologically similar to *Psathyrella* s.s. but phylogenetically and micromorphologically distinguished from it, differing in the special pileipellis which composed of utriform to subglobose cells covered by a 1 cell deep layer of periclinal hyphae and abundant pseudoparaphyses. There are no other genera in this family, like Heteropsathyrella, that match the characteristics of psathyrelloid basidiomata, lamellae adnexed, basidia monomorphic, pseudoparaphyses abundant and pileipellis composed of a cellular subpellis below a hyphal suprapellis covered by scattered and irregular deposits, which dissolve in 5% KOH. Based on the study of this family (Smith 1972; Kits van Waveren 1985; Nagy et al. 2013; Örstadius et al. 2015), a detailed feature comparison between Heteropsathyrella and related genera are shown in Table 2. The type species, H. macrocystidia, is characterized by stout basidiomata, large pleurocystidia up to 80 µm long, and the generic characters above cited. Thus, this taxon is obviously unique and distinguished from all known species. In the case of not comparing the pileipellis and pseudoparaphyses, few species have the aspect of H. macrocystidia: P. epimyces (Peck) A.H. Sm. has stout basidiomata, and large pleurocystidia up to 70 µm long, but parasitic on Coprinus- or Coprinopsis- species (Smith 1972); P. parvifibrillosa A.H. Sm. and P. lauricola A.H. Sm. & Hesler has large pleurocystidia up to 70 µm long, but basidiomata small, and the shape of pleurocystidia are fusoid and utriform without long stipe at the base, respectively (Smith 1972).

For several of the already formally described and circumscribed clades within *Psathyrella*, phylogenetic analyses suggest that they include a morphologically heterogeneous assemblage of species, and morphological characterization is difficult (Örstadius et al. 2015). The boundaries between species in the /noli-tangere clade are difficult to characterize; these taxa share the characteristics of spores less than 10 µm long, and utriform, fusiform, lageniform or transition-type pleurocystidia present at the same time. The new species *P. amygdalinospora* forms an independent lineage and differs from the others in spores being ovoid in front view, amygdaliform in profile, and germ pore being absent. Macromorphologically, this species is similar to *P. obtusata* (Pers.) A.H. Sm. and *P. fulvescens* (Romagn.) M.M. Moser ex A.H. Sm, but the spores of *P. obtusata* are ellipsoid to oblong-ellipsoid and pale yellow in 5% KOH (Örstadius and Knudsen 2012), while *P. fulvescens* has an obvious germ pore (Smith 1972).

P. amygdalinospora can be classified into section *Pennatae* in Kits van Waveren's classification system (Kits van Waveren 1985) and in subsection *Limicolae* in Smith's

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Table 2. A sun	nmary of morpl	hological chara	acteristics used	to discriminate	the ten genera	·				
	Coprinellus	Coprinopsis	Cystoagaricus	Heterops at by rella	Homophron	Kauffmania	Lacrymaria	Parasola	Psathyrella	Typhrasa
Veil	subglobose cells,	hyphae,	hyphae	hyphae	absent	hyphae	hyphae	absent	hyphae, rarely	hyphae
	hyphae, or absent	subglobose cells,							subglobose cells	
		or mixtures								
Cap or lamellae	fully, partilly, or	deliquescent,	non-deliquescent	non-deliquescent	non-deliquescent	non-deliquescent	non-deliquescent	non-deliquescent,	non-deliquescent	non-deliquescent
	non-deliquescent	rarely non- deliquescent						or collapsing		
Spore surface	smooth, rarely	smooth, rarely	smooth	smooth	smooth	smooth	often warty	smooth	smooth, rarely	smooth
	warty	warty or with							granulose or with	
		myxosporium							myxosporium	
Basidia	mono-, di-, tri-, or	Dimorphic	monomorphic	monomorphic	monomorphic	monomorphic	mono- to	di- to trimorphic	monomorphic	monomorphic
	tetramorphic						dimorphic	1		I
Pseudoparaphyses	present	present, rarely	absent	present	absent	absent	absent	present	rarely present	absent
		absent								
Pileipellis	hymeniderm to	Cutis	paraderm	hymeniderm	hymeniderm to	hymeniderm to	hymeniderm	hymeniderm	hymeniderm,	hymeniderm to
	paraderm			to paraderm,	paraderm	paraderm			paraderm, rarely	paraderm
				covered by a 1					cutis	
				cells deep layer of						
				periclinal hyphae						
Pileocystidia	often present	Absent	absent	abundant	simple hairs	absent	absent	absent	very rarely present	absent
				and regular	sometimes					
				distribution	present					
Sclerocystidia	sometimes present	Absent	absent	absent	absent	absent	absent	absent	absent	absent

classification system (Smith 1972). The closest related species can be separated as follows: the pleurocystidia of *P. pennata* (Fr.) A. Pearson & Dennis are fusoid-ventricose with an acute apex and thickened wall (Kits van Waveren 1985); the spores of *P. borealis* A.H. Sm. are ellipsoid and have an obvious germ pore (Smith 1972).

The species in the /pygmaea clade share abundant cheilocystidia and utriform pleurocystidia. The new species *P. truncatisporoides* forms a distinct lineage and groups together with *P. rybergii* Örstadius & E. Larss. in this clade. The closely related *P. rybergii* differs in having spore lengths of 8.5–9.5 µm. Macromorphologically, there are hardly any other species that match the characteristics of *P. truncatisporoides* and they can be separated as follows: *P. rubiginosa* A. H. Sm. has subdistant lamellae and a very inconspicuous germ pores (Smith 1972); the pleurocystidia of *P. noli-tangere* (Fr.) A. Pearson & Dennis are narrowly utriform to lageniform and rarely forked (Kits van Waveren 1985); the spores of *P. elliptispora* A.H. Sm. are 8.0–11.0 µm long (Smith 1972).

The morphological boundary of the /piluliformis clade is basically the same as that of section *Hydrophilae* delineated by Kits van Waveren (1985). The new species *P. piluliformoides* forms a distinct lineage in this clade and can be separated by having an obvious ring. The closely related *P. oboensis* also exhibits very closed lamellae but differs in absence of a ring and clavate-mucronate pleurocystidia. Few species have been described resembling *P. piluliformoides* and they can be separated as follows: *P. piluliformis* (Bull.) P.D. Orton has no ring and without yellow amorphous incrustation at the apex of pleurocystidia (Örstadius and Knudsen 2012); *P. laevissima* (Romagn.) Singer has mucronate pleurocystidia (Kits van Waveren 1985).

Acknowledgements

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Supplementary material I

Figure S1. Photographs under the microscope

Authors: Tolgor Bau, Jun-Qing Yan

Data type: images

- Explanation note: *Heteropsathyrella macrocystidia*: a. Basidiospores, Basidia, Pseudoparaphyses, and Pleurocystidia; b. Marginal cell; c. Pileipellis; *Psathyrella amygdalinospora*: d. Basidiospores; e. Pleurocystidia; f. Marginal cell; *P. piluliformoides*: g. Basidiospores; h1. Apex of pleurocystidia covered by yellow amorphous incrustation in water; h2. Pleurocystidia; i. Marginal cell; *P. truncatisporoides*: j. Basidiospores; k. Pleurocystidia; l: Marginal cell. Observed under 5% aqueous KOH. Congo Red was used as a stain when necessary. Scale bars: 10 µm.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/mycokeys.80.65123.suppl1

Supplementary material 2

Figure S2. Phylogram generated by maximum likelihood (ML) analysis

Authors: Tolgor Bau, Jun-Qing Yan

Data type: phylogenetic

- Explanation note: Phylogram generated by maximum likelihood (ML) analysis based on sequences of a concatenated data set from four nuclear genes (ITS, LSU, *Tef-1a* and β -*tub*) rooted with *Coprinopsis* spp. ML bootstrap proportion (ML-BP) ³ 75% is shown • indicates newly described species..
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
- Link: https://doi.org/10.3897/mycokeys.80.65123.suppl2

RESEARCH ARTICLE



Three new Melanoleuca species (Agaricales, Basidiomycota) from north-eastern China, supported by morphological and molecular data

Ying Pei^{1*}, Hong-Bo Guo^{2*}, Tie-Zhi Liu³, Wei-Qiang Qin⁴, Di Zhao¹, Xiao-Jian Qi¹, Xiao-Dan Yu¹

College of Biological Science and Technology, Shenyang Agricultural University, Shenyang 110866, China
College of Life Engineering, Shenyang Institute of Technology, Fushun 113122, China 3 College of Chemistry and Life Sciences, Chifeng University, Chifeng 024000, China 4 Jishou University, Zhangjiajie 42700, China

Corresponding author: Xiao-Dan Yu (yuxd126@126.com)

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Abstract

Three new *Melanoleuca* species, *M. chifengense*, *M. griseoflava* and *M. pallidorosea*, were discovered in the northeast of China. *Melanoleuca chifengense* is morphologically characterised by its grey to yellowish-grey pileus, decurrent lamellae, grey to yellowish-brown stipe, yellowish-grey context, ellipsoid basidiospores with irregular warts and lack of hymenial cystidia. *Melanoleuca griseoflava* is mainly characterised by its greyish-brown pileus, adnexed to adnate lamellae, greyish-yellow context, fusiform cystidia and almost reticulate basidiospores. *Melanoleuca pallidorosea* is characterised by its pinkish-white pileus, white and decurrent lamellae, ellipsoid basidiospores with round and scattered warts and lack of hymenial cystidia. The phylogenetic relationship of the three species was determined by the analyses of the ITS region and the combined data matrix (ITS-nrLSU-RPB2), respectively. The results showed that the three species formed three independent lineages. Based on the combination of both morphological and molecular data, *M. chifengense, M. griseoflava* and *M. pallidorosea* were confirmed to be new species. The morphological similarities of the three new species is also discussed.

Keywords

Agaricales, morphology, phylogenetic analysis, Pluteoid clade, taxonomy

^{*} Contributed equally as the first authors.

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Introduction

Melaleuca Pat. was erected by Patouillard in 1887. As the name '*Melaleuca*' was found to be the same as that of a plant species, Patouillard (1897) changed it to the current name *Melanoleuca* Pat. The genus was traditionally included in the family Tricholo-mataceae, subtribus Leucopaxillaceae Singer mainly because the species present a regular hymenophoral trama, amyloid basidiospores and a white spore print (Singer 1948; Singer 1986). However, molecular data showed that the genus *Melanoleuca* is close to the species of Pluteaceae and Amanitaceae (Moncalvo et al. 2002; Bodensteiner et al. 2004; Matheny et al. 2006; Garnica et al. 2007; Justo et al. 2011; Vizzini et al. 2011; Binder et al. 2014; Yu et al. 2014). Therefore, *Melanoleuca* was assumed to belong to the Pluteoid clade by Matheny et al. (2006) and Sánchez-García et al. (2014).

The species of *Melanoleuca* are often characterised by having a convex to slightly depressed pileus, mostly hymenial cystidia, amyloid ornamented basidiospores and all hyphae without clamp connections (Singer 1986; Boekhout 1988; Vizzini et al. 2011). The genus *Melanoleuca* always grows directly on humus-rich soil, in meadows, in and outside of woods and is distributed in temperate and frigid zones of both hemispheres (Singer 1986). In recent years, many new species of *Melanoleuca* have been reported around the world (Vizzini et al. 2010, 2011; Sánchez-García et al. 2013; Antonín et al. 2014, 2017; Yu et al. 2014; Nawaz et al. 2017; Xu et al. 2019; Antonín et al. 2021). Up to now, there are 221 validly published names reported in the world (Index Fungorum 2021).

Although *Melanoleuca* has been proved to be a monophyletic group, the classification system within the genus remains controversial. Based on the colour of the pileus and the size of the carpophore, Singer (1986) divided the genus into four sections, i.e. sect. Alboflavidae Singer, sect. Humiles Singer, sect. Oreinae Singer and sect. Melanoleuca Pat. As Boekhout (1988) believed that the cystidia should play an important role in the classification system of *Melanoleuca*, the genus was, therefore, divided into three subgenera, based on the types of cystidia, i.e. subgen. Macrocystis Boekhout, subgen. Melanoleuca Pat. and subgen. Urticocystis Boekhout. Subgen. Macrocystis and subgen. Urticocystis are characterised by the presence of fusiform to lageniform cystidia and urticiform cystidia, respectively while subgen. Melanoleuca is characterised by the absence of cystidia. However, these morphological classification systems are not supported by molecular data. The result of ITS region analysis supported the fact that Melanoleuca included two subgenera, i.e. subgen. Urticocystis and subgen. Melanoleuca (Vizzini et al. 2011). The species of subgen. Melanoleuca are characterised by basidiomata with non-septate macrocystidia. Subgen. Urticocystis was composed of the taxa mainly with urticocystidia, but also without any cystidia and with macrocystidia and brightly coloured pilei (Vizzini et al. 2011).

In this paper, the authors studied three *Melanoleuca* species collected in north-eastern China from 2017 to 2019. Morphological observation and phylogenetic analyses confirmed that they are novel taxa in the genus *Melanoleuca*.

Materials and methods

Morphological studies

All of the fungal specimens were described and photographed in the field. Specimens were dried in an electric drier and deposited in the Fungal Herbarium of Shenyang Agricultural University (SYAU-FUNGI) and Fungal Herbarium of Chifeng University (CFSZ). Tissue blocks were removed from the inner part of the fresh basidiomata for DNA analyses. Macroscopic characters of the basidiomata described here were based on observations of fresh specimens. The names of colours were based on Kornerup and Wanscher (1963). Methods used for morphological descriptions followed those of Li et al. (2017). For the microscopic study, dried materials were observed in 5% potassium hydroxide (KOH) solution. Melzer's reagent was used for testing colour reactions of the tissues and basidiospores. The notation "(n/m/p)" of basidiospores indicates that the measurements were conducted for n basidiospores from m basidiomata of p collections. The Q value (length:breadth ratio) for each spore was calculated and the mean values are presented in the descriptions. For observation of the surface of the spores, the gills were covered with a thin gold film by using an Ion Sputter Coater (MC1000, Hitachi, Japan) before imaging by a scanning electron microscope (REGLUS 8100, HITACHI, Japan). Line drawings were prepared by freehand.

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from fresh blocks of tissue, dried with silica gel using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1987). Primer pairs ITS5/ITS4 (White et al. 1990), LR0R/LR5 (Michot et al. 1984) and b6F/b7.1R (Matheny et al. 2007) were used to amplify the internal transcribed spacer (ITS) region, the large subunit nuclear ribosomal RNA (nrLSU) region and the second largest subunit of the nuclear RNA polymerase enzyme II (RPB2), respectively. PCR protocol and sequencing were conducted as described by Wang et al. (2019).

Phylogenetic analyses

High-quality and representative sequences of *Melanoleuca* in previous studies (Sánchez-García et al. 2013; Yu et al. 2014; Antonín et al. 2014, 2015, 2017; Nawaz et al. 2017; Xu et al. 2019; Antonín et al. 2021) were downloaded from GenBank and aligned with the sequences obtained from this study by Bioedit v7.0.9 (Hall 1999) and MAFFT v7.313 (Katoh and Standley 2013). *Pluteus romellii* (AY854065 for ITS; AY634279 for nrLSU; AY786063 for RPB2) was used as the outgroup in this study. Data partition homogeneity tests (Farris et al. 1995) were implemented in PAUP 4.0b4a (Swofford 2003). This test detected no conflicts among ITS, nrLSU and RPB2 regions (P-value = 0.33), suggesting that sequences of the three genes can be combined for phyloge-

netic analysis. The final ITS data matrices consisted of 125 samples of 669 characters, whereas the combined data set (ITS-nrLSU-RPB2) consisted of 67 samples of 2204 characters. Maximum likelihood (ML) analysis was performed with RAxML-8.2.10-WIN using a GTR-GAMMA model of evolution (Stamatakis 2014). Nodal bootstrap support (BS) was assessed with nonparametric bootstrapping using 1000 replicates. Bayesian Inference (BI) analysis was conducted with MrBayes v.3.2.6 (Ronquist et al. 2012). ModelFinder (Kalyaanamoorthy et al. 2017) and PartitionFinder 2 (Lanfear et al. 2016) were used for the selection of the best-fitting model of sequence evolution for ITS dataset (GTR+I+G+F) and the combined dataset (GTR+I+G for ITS and nrLSU, SYM+I+G for RPB2), respectively. Both of the two data sets were run for 5 000 000 generations, with four chains, and trees sampled every 500 generations. The average split frequencies were checked to determine optimal convergence of the chains below 0.01. The first 25% of the sample trees was designated as burn-in, and the remaining samples were retained for further analyses. The topologies were used to generate a 50% majority-rule consensus tree for posterior probabilities (PP). The best tree was viewed in FIGTREE v1.4.4 (Rambaut 2018) and was compiled in Adobe Illustrator CC. Both of the final alignments were submitted to TreeBASE (Submission ID 28200).

Results

Molecular phylogenetic results

The GenBank accession numbers of the sequences, determined in this study, are from MW258676 to MW258689 and MW281543 to MW281548 (Table 1). The BI and ML analyses produced similar topologies for the ITS and combined regions datasets. The BI trees were selected for display (Figures 1, 2). The results showed that the species in the genus *Melanoleuca* formed a monophyletic group in both ITS regions and combined regions analyses (PP=1.00, BS=100, Figures 1, 2), which is consistent with the previous results (Yu et al. 2014; Vizzini et al. 2011). A total of five clades (A to E) can be recognized within *Melanoleuca* (Figures 1, 2). Based on the analyses of the two datasets, the collections named *M. grisoflava* (SYAU-FUNGI-061 to SYAU-FUNGI-064) formed an independent lineage with strong statistical support (PP =

Species	Voucher collection	Origin	GenBank accession No.		n No.
			ITS	nrLSU	RPB2
Melanoleuca pallidorosea	SYAU-FUNGI-058	Xilingole League, Inner Mongolia, China	MW258676	MW258684	MW281543
M. pallidorosea	SYAU-FUNGI-065	Xilingole League, Inner Mongolia, China	MW258677	MW258687	MW281545
M. griseoflava	SYAU-FUNGI-061	Fuxin City, Liaoning Province, China	MW258680	MW258685	MW281544
M. griseoflava	SYAU-FUNGI-062	Shenyang City, Liaoning Province, China	MW258681	_	_
M. griseoflava	SYAU-FUNGI-063	Shenyang City, Liaoning Province, China	MW258682	_	_
M. griseoflava	SYAU-FUNGI-064	Chifeng City, Inner Mongolia, China	MW258683	MW258686	MW281548
M. chifengense	SYAU-FUNGI-059	Chifeng City, Inner Mongolia, China	MW258678	MW258688	MW281546
M. chifengense	SYAU-FUNGI-060	Chifeng City, Inner Mongolia, China	MW258679	MW258689	MW281547

Table 1. Collections of *Melanoleuca* used for DNA sequence analyses.



Figure 1. Phylogenetic placements of the three new *Melanoleuca*, inferred from the ITS region using MrBayes. The lineages with new species were shown in boxes. PP ≥ 0.95 and BS $\ge 75\%$ were indicated around the branches. Accession numbers of ITS in GenBank follow the fungal names.



Figure 2. Phylogenetic placements of the three new *Melanoleuca*, inferred from the combined regions (ITS-nrLSU-RPB2) using MrBayes. The lineages with new species were shown in boxes. PP \ge 0.95 and BS \ge 75% were indicated around the branches. Accession numbers in GenBank (ITS, nrLSU, RPB2) follow the fungal names.

1.00, BS \geq 97), located within clade A, and sister to a clade containing sequences of *M. arcuata* (Bull.) Singer, *M. heterocystidiosa* (Beller & Bon) Bon, *M. robusta* (Bres.) Fontenla, Gottardi & Para and *M. subpulverulenta* (Pers.) Singer. In clade E, *Melanoleuca chifengense* consist of two collections (SYAU-FUNGI-059 and SYAU-FUNGI-060) that form an independent lineage with high support (PP \geq 0.98, BP \geq 99) and close to

M. humilis (Pers.) Pat. and *M. malenconii* Bon. The collections (SYAU-FUNGI-058 and SYAU-FUNGI-065) named *M. pallidorosea* group together in clade E with well support ($PP \ge 0.99$, $BP \ge 94$).

Taxonomy

Melanoleuca chifengense X.D. Yu & H.B. Guo, sp. nov.

MycoBank No: 838026 Figs 3, 6a–c

Etymology. The epithet refers to the species found in Chifeng City in north-eastern China.

Diagnosis. The new species is distinguished from *M. exscissa* in having yellowish tinct pileus and without any type of cystidia.

Type. CHINA. Inner Mongolia: Chifeng City, Linxi County, Xinlin Town, Dauran Village, alt. 1200 m, 44.00°N, 118.07°E, 21 Aug 2017, H.B. Guo (SYAU-FUN-GI-059).

Description. Pileus 30–60 mm diam., flat at first, becoming depressed at disc when mature, margin sometimes cracking, surface glabrous, grey to yellowish-grey (4B1 to 4B2), greyish-brown (4B4 to 4B6) at centre, often darker at margin. Lamellae crowded, adnate to decurrent, white to yellowish-white (4A2), 2.5–3.0 mm broad, with lamellulae, edge entire. Stipe cylindrical, 20–35 mm long \times 2–5 mm diam., central, broadened at base, solid, surface grey to yellowish-grey at first (4B1 to 4B2), becoming yellowish-brown (5D8, 5E8) with age or after touching, striate, often with whitish basal tomentum. Pileus context up to 10 mm thick near stipe attachment, thin at margin, yellowish-grey (4B2), grayish brown to yellowish brown (5D3 to 5E5) in stipe cortex, up to brown (6E7) in stipe base. Odour none, taste mild. Spore print white.

Basidiospores (90/6/2) 7.0–8.5 (9.0) × 4.0–6.2(6.5) μ m, av. 7.5 × 5.2 μ m, Q = 1.40–1.45(1.50), ellipsoid, hyaline, amyloid, ornamentation vertuculose, with irregular warts, sometimes with ridges. Basidia (20) 23–29 (30) × (7.0) 7.5–9.0 (10.0) μ m,



Figure 3. *Melanoleuca chifengense* (holotype, SYAU-FUNGI-059) **A** macroscopic habit **B** surface of basidiospores. Scale bars: 1 cm (**A**); 5 μ m (**B**).

av. 26 × 8.5 μ m, clavate, 4-spored, sometimes 2-spored, subhyaline. Hymenial cystidia absent, lamella edge sterile. Hymenophoral trama 42–85 μ m broad, regular with thin-walled hyphae 5.5–16.5 μ m diam., hyphae not pigmented. Subhymenium poorly developed. Pileipellis a cutis of numerous repent branched hyphae, 5.5–7.5 μ m wide, thin-walled. Stipitipellis hyphae 3.5–8.0 μ m diam., thin-walled, hyaline. Caulocystidia absent. Clamp connections absent.

Habit, ecology and distribution. On soil or meadow outside of a forest, often on the roadside near a forest. Known from north-eastern China.

Additional specimens examined. CHINA. Inner Mongolia: Chifeng City, Linxi County, Xinlin Town, Dauran Village, alt. 1201 m, 44.07°N, 118.08°E, 22 Aug 2017, H.B. Guo (SYAU-FUNGI-060).

Melanoleuca griseoflava X.D. Yu & H.B. Guo, sp. nov.

MycoBank No: 838027 Figs 4, 6d–g

Etymology. The epithet refers to the colour of the pileus which is greyish-brown.

Diagnosis. The new species is distinguished from *M. exscissa* in having adnexed to adnate lamellae and fusiform cheilocystidia.

Type. CHINA. Liaoning Province: Shenyang City, Tianzhu Mountain, on the soil in woods, 31 Aug 2019, X.D. Yu (holotype: SYAU-FUNGI-062).

Description. Pileus 35–60 mm diam., flat at first, then gradually depressed, margin slightly inflexed when mature, surface fibrillose, greyish-brown (4B3 to 4B5), becoming deep yellow (4C6 to 4C8) at centre. Lamellae crowded, adnexed to adnate, white, 2.5–3.0 mm broad, with lamellulae, edge entire. Stipe cylindrical, 30–50 mm long \times 3–5 mm diam., central, somewhat broadened at the base, fibrous, expanded at base, solid, surface yellowish-grey to greyish at first (4B2 to 4C2), becoming yellowishbrown (5E7 to 5E8) with age, striate, with whitish basal tomentum. Pileus context up to 10 mm thick near stipe attachment, thin at margin, greyish-yellow to yellowish-grey (4B4 to 4B2), yellowish-grey (4B2) in stipe cortex, whitish in stipe base. Odour none, taste mild. Spore deposit white.

Basidiospores (234/10/8) (5.0) 6.0–7.2 (8.0) × 4.0–5.0 (6.0) μ m, av. 6.5 × 4.5 μ m, Q = (1.30)1.45–1.55 (1.60), ellipsoid, hyaline, amyloid, ornamentation verruculose, warts with ridges, almost reticulate. Basidia (18) 20–25 (28) × (4.0) 5.0–6.5 (7.0) μ m, av. 22 × 6.0 μ m, clavate, 4-spored, occasionally 2-spored, hyaline. Cheilocystidia (40) 45–55 (60) × (6.0) 8.0–12.0 (15.0) μ m, fusiform, thinwalled, with encrusted crystals at apex, abundance. Pleurocystidia scattered, similar to cheilocystidia. Hymenophoral trama 90–150 μ m broad, regular with thin-walled hyphae 3.0–14.0 μ m diam., hyphae not pigmented, lamella edge sterile, Subhymenium poorly developed. Pileipellis a cutis of numerous repent branched hyphae 7.5–10.5 μ m wide, thin-walled, pigmented with light violet. Stipitipellis hyphae 3–10.0 μ m diam., smooth, thin-walled, pigmented. Caulocystidia of two types of cells, (1) 40–90 × 6.0–10.0 μ m, fusiform, thin-walled, some with encrusted crystals



Figure 4. *Melanoleuca griseoflava* (holotype, SYAU-FUNGI-062) **A** macroscopic habit **B**, **C** surface of basidiospores **D** hymenial cystidia with encrusted crystals at apex. Scale bars: 1 cm (**A**); 5 μ m (**B**, **C**); 10 μ m (**D**).

at apex, similar to cheilocystidia; (2) $30-40 \times 7.0-10.0 \mu m$, clavate, thin-walled, without crystals. Clamp connections absent.

Habit, habitat and distribution. Solitary, saprotrophic on the soil, on the grass, on roadsides, in woods. Known from north-eastern China.

Additional specimens examined. CHINA. Liaoning Province: Shenyang City, Tianzhu Mountain, on the soil in woods, 31 Aug 2019, X.D. Yu (SYAU-FUNGI-063); Fuxin City, Haitang Mountain, on roadsides, 20 Jul 2019, H.B. Guo (SYAU-FUN-GI-061). Inner Mongolia: Chifeng City, Linxi County, Xinlin Town, Dauran Village, alt. 1286 m, 43.08°N, 118.07°E, 22 Aug 2017, X.D. Yu (SYAU-FUNGI-064). Chifeng City, Karakqin Banner, Maanshan, 2 Sep 2019, T.Z. Liu & Y.M. Gao (CFSZ 21439).

Melanoleuca pallidorosea X.D. Yu & H.B. Guo, sp. nov.

MycoBank No: 838028 Figs 5, 6h, i

Etymology. The epithet refers to the species which has a pallid rose pileus.

Diagnosis. The new species is distinguished from *M. grammopodia* and *M. leucopoda* in having a pinkish-white pileus.



Figure 5. *Melanoleuca pallidorosea* (holotype, SYAU-FUNGI-058) **A** macroscopic habit **B** surface of basidiospores. Scale bars: 1 cm (**A**); 2 μm (**B**).

Type. CHINA. Inner Mongolia: Xilingole League, Xiwuzhumuqin Banner, on the grass in woods, 1051 m alt., 44.48°N, 117.86°E, 22 Aug 2017, X.D. Yu (holotype: SYAU-FUNGI-058).

Description. Pileus 30–65 mm diam., flat, with depressed centre, margin slightly undulating, expanding to uplifted, sometimes slightly lacerate when mature, surface glabrous, camel (9E8 to 10E8) at centre, pinkish-white (10A3 to 10A4) towards the margin. Lamellae rather distant, adnate to decurrent, white, 3.0–4.5 mm broad, with lamellulae of two lengths, but not intervening, edge entire. Stipe cylindrical, 20–50 mm long × 5–8 mm diam., in upper part of stipe apricot (6C8 to 6D8), becoming yellowish-brown (5E8) towards base, with whitish flocculose apex, longitudinally striate, with whitish basal tomentum. Context up to 2–5 mm thick at the pileus base, whitish to creamy, whitish in stipe cortex and base. Smell fungoid smell, taste mild. Spore print white.

Basidiospores (130/7/4) (6.5) 7.0–8.5 (9.0) × 5.0–6.0 (6.5) μ m, av. 7.4 × 5.5 μ m, Q = (1.28)1.31–1.40(1.44), ellipsoid, hyaline, ornamentation vertuculose, warts mainly round and scattered, amyloid. Basidia (20) 25–33 (35) × (6.0) 6.5–9.5 (10.5) μ m, av. 28 × 8.5 μ m, clavate, 4-spored, occasionally 2-spored, subhyaline. Hymenial cystidia absent. Lamella edge sterile. Hymenophoral trama 95–159 μ m wide, regular, with thin-walled hyphae, 5.0–10.0 μ m diam., hyphae not pigmented. Subhymenium poorly developed. Pileipellis a cutis of numerous repent branched hyphae, 4.0–10.0 μ m wide, inflated cell to 21.0 μ m, thin-walled. Stipitipellis hyphae 7.0–10.0 μ m, thin-walled, hyaline. Caulocystidia absent. Clamp connections absent.

Habit, ecology and distribution. Solitary or in small group, saprotrophic in grass. Known from north-eastern China.

Additional specimens examined. CHINA. Inner Mongolia: Xilingole League, Xiwuzhumuqin Banner, on the grass in woods, 1051 m alt., 44.48°N, 117.86°E, 22 Aug 2017, X.D. Yu (SYAU-FUNGI-065). Chifeng City, Bahrain Banner Saihanwula, 10 Sep 2016, T.Z. Liu & Z.L. Song (CFSZ 12136); 12 Sep 2016, T.Z. Liu & Z.L. Song (CFSZ 12253).



Figure 6. Line drawings of the three new *Melanoleuca* species A–C *Melanoleuca chifengense* (holotype, SYAU-FUNGI-059) A basidiospores B, C basidia and basidioles D–G *Melanoleuca griseoflava* (holotype, SYAU-FUNGI-062) D basidia and basidioles E caulocystidia F basidiospores G cheilocystidia H, I *Melanoleuca pallidorosea* (holotype, SYAU-FUNGI-058) H basidiospores I basidia and basidioles. Scale bars: 10 µm.

Discussion

Morphologically, the most distinctive features of *M. pallidorosea* are a pinkish-white pileus, a yellowish stipe, white and decurrent lamellae, lack of hymenial cystidia, ellipsoid basidiospores with round and scattered warts, $7.0-8.5 \times 5.0-6.0 \mu$ m. According to the classification system of Singer (1986), *Melanoleuca pallidorosea* should belong to sect. *Alboflavidae* because of the pinkish-white pileus. Four species with a whitish pileus in the section were similar to *M. pallidorosea*, i.e. *M. balansae* (Speg.) Singer (Spegazzini 1883), *M. candida* Singer (Singer 1943), *M. kavinae* (Pilát & Veselý) Singer (Pilát and Veselý 1932) and *M. strictipes* (P. Karst.) Jul. Schäff. (Ďuriška et al. 2017). The latter three species mainly differ on account of their large pileus size (up to 12 cm diam.). Moreover, all of them have macrocystidia which differs from *M. pallidorosea*. Considering the size of the pileus (up to 6 cm diam.), *M. balansae* (Speg.) Singer is similar to *M. pallidorosea* to some extent. However, *M. balansae*, originally reported from Paraguay, differs on account of its white stipe and smaller basidiospores (7–7.5 × 4–5 µm).

Melanoleuca chifengense is easily recognised by its grey to yellowish-grey pileus, decurrent lamellae, grey to yellowish-brown stipe and yellowish-grey context, and lack of hymenial cystidia. *Melanoleuca griseoflava* is characterised by a greyish-brown pileus, adnexed to adnate lamellae, yellowish-grey stipe, greyish-yellow context and fusiform cystidia. The two species have similar-sized basidiomata and grey pileus, Melanoleuca griseoflava differs from M. chifengense by the adnexed to adnate gills and having abundant fusiform cystidia. According to Singer (1986), both M. chifengense and M. griseoflava belonged to sect. Oreinae, based on their grey pileus, narrow lamellae and nearly pallid stipe. Amongst the section Oreinae, the two new species differ from the other species by their small-size basidiomata, including M. catalaunica Singer, M. graminicola (Velen.) Kühner & Maire, M. microcephala (P. Karst.) Singer and M. oreina (Fr.) Kühner & Maire (Singer 1943). Some species in sect. Oreinae have the urticoid hymenial cystidia, making them easily distinguishable from *M. chifengense* and *M. griseoflava*, such as M. paedida (Fr.) Kühner & Maire (Vizzini et al. 2011), M. exscissa (Fr.) Singer (Antonín et al. 2017), M. humilis (Pers.) Pat. (Antonín et al. 2015), and M. rasilis (Fr.) Singer (Antonín et al. 2017). Melanoleuca griseoflava can be distinguished from the above species by its fusiform hymenial cystidia. In addition, *M. chifengense* differs from them by its lack of any form of cystidia; Melanoleuca subcinereiforme Murrill, originally reported in Oregon, differs on account of its finely pruinose, smoky pileus and white stipe (Murrill 1914); Melanoleuca deserticola (Speg.) Singer mainly differs on account of its spotted-pileus, short and solid stipe and larger basidiospores (9–11 \times 4–6 μ m) (Spegazzini 1900); *M. strictipes* differs by its larger basidiomata (55–115 mm broad), leathery yellow pileus and a distinct bulb stipe (Duriška et al. 2017).

In the present study, both phylogenetic analyses, based on a single region (ITS) and three regions (ITS-nrLSU-RPB2), showed that there were nine clades in the genus *Melanoleuca* (Figures 1, 2). According to the phylogram, *M. griseoflava* is sister to the other four species in clade A, i.e. *M. arcuata*, *M. heterocystidiosa*, *M. robusta* and *M. sub*-
pulverulenta. Melanoleuca arcuata differs by its brick-red pileus and decurrent lamellae (Fries 1821). The other two species, *M. heterocystidiosa* and *M. subpulverulenta*, can also be easily separated from *M. griseoflava*, based on their small basidiomata (Singer 1939; Bon 1984); *M. robusta* differs on account of its grey-brown pileus, grey lamellae, brown context and caespitose growth (Vizzini et al. 2011). In clade E, *M. chifengense* is closely related to *M. humilis* and *M. malenconii* with high support. However, both the two species differ from *M. chifengense* in their dark brown pileus (Fries 1821; Bon 1990). In the analysis of both ITS region and three regions (ITS-nrLSU-RPB2), *Melanoleuca pallidorosea* form an individual clade (clade I) and far away from the other species of *Melanoleuca*.

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RESEARCH ARTICLE



Two new rare species of Candolleomyces with pale spores from China

Tolgor Bau¹, Jun-Qing Yan²

I Key Laboratory of Edible Fungal Resources and Utilization (North), Ministry of Agriculture and Rural Affairs, Jilin Agricultural University, Changchun 130118, China 2 Jiangxi Key Laboratory for Conservation and Utilization of Fungal Resource, Jiangxi Agricultural University, Nanchang, Jiangxi 330045, China

Corresponding authors: Tolgor Bau (junwusuo@126.com); Jun-Qing Yan (yanjunqing1990@126.com)

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Abstract

Most species of *Candolleomyces* have brown or dark brown spores. Although pale-spored members are rare in the genus, we frequently collected two such species from many Provinces during our investigations in subtropical China from 2016–2020. As revealed by morphological characterisation and multigene phylogenetic analyses (ITS, LSU, β -tub and tef-1a), these species, which we have named *C. subcacao* and *C. subminutisporus*, are unique and distinct from known taxa. In addition, a new combination, *C. cladiimarisci*, is proposed on the basis of ITS sequence analysis of the type specimen. Detailed descriptions, colour photos, illustrations and a key to related species are presented.

Keywords

Basidiomycete, new taxon, Psathyrellaceae, phylogenetic analysis, taxonomy

Introduction

On the basis of extensive comparisons of gene sequences and phylogenetic analyses, the historical genus *Psathyrella* (Fr.) Quél. has been split into several genera. One of these genera is *Candolleomyces* D. Wächt. & A. Melzer, which differs from *Psathyrella* s.s. in lacking pleurocystidia (Örstadius et al. 2015; Wächter and Melzer 2020). Approximately 100 taxa (including synonyms and subspecies) without pleurocystidia have been previously described in *Psathyrella* s.l. (Fries 1838; Smith 1972; Kits van Waveren 1985; Örstadius and Kundsen 2012; Battistin et al. 2014); however, many of these taxa

have been treated as synonyms, as unique features, based on conventional methods, are scarce (Galland et al. 1979; Kits van Waveren 1980; Knudsen and Vesterholt 2012). Currently, 26 species have been assigned to *Candolleomyces* (Wächter & Melzer, 2020).

According to the research of Wächter and Melzer (2020), *Candolleomyces* may be more speciose than previously thought and better delimitation of species boundaries is needed. Although controversies still exist regarding some species boundaries, the number of new taxa is steadily increasing (Melzer et al. 2018; Sicoli et al. 2019a; Büttner et al. 2020). This continuous discovery of new taxa with clear boundaries deepens understanding of the species in this genus.

Approximately eight taxa in the genus *Candolleomyces* have previously been reported from China (Yan 2018). During our investigations in subtropical China from 2016–2020, we frequently collected two unknown *Candolleomyces* species with pale spores from many Provinces. Spores that are pale or almost colourless in water and 5% potassium hydroxide (KOH) are very rare in this genus. On the basis of our morphological and phylogenetic analyses, the specimens are described as new species in this paper.

Materials and methods

Morphological studies

Specimens were deposited in the Herbarium of Mycology, Jilin Agricultural University (**HMJAU**) and the Herbarium of Fungi, Jiangxi Agricultural University (**HFJAU**). Macromorphological characters and habitat details were recorded from fresh basidiomata. Colour codes were based on the Methuen Handbook of Colour (Kornerup and Wanscher 1978). More than 30 spores, cystidia and basidia in water and 5% aqueous KOH were measured under a microscope. In subsequent descriptions, measurements are shown as (a)b-c(d), where *a* is the lowest value, b-c encompasses at least 90% of values and *d* is the highest value, while *Q* is the length–width ratio of a spore (Bas 1969; Yu et al. 2020).

DNA extraction and sequencing

DNA was extracted from dried specimens using a NuClean Plant Genomic DNA kit (CWBIO, China). Four DNA regions (ITS, LSU, *Tef-1a* and β -tub) were selected for analysis (Örstadius et al. 2015) and were respectively amplified using the primer pairs ITS1/ITS4 (White et al. 1990), LR0R/LR7 (Hopple and Vilgalys 1999), EF983F/EF2218R (Örstadius et al. 2015) and B36f/B12r (Nagy et al. 2011). PCR amplifications were performed using the following touchdown programme: 5 min at 95 °C, followed by 15 rounds of 1 min at 95 °C, 30 s at 65 °C (lowered by 1 °C per cycle) and 1 min at 72 °C, followed by 20 rounds of 1 min at 72 °C (Yan and Bau 2018b). Sequencing was carried out by Qing Ke Biotechnology Co. (Wuhan, China).

Taxa	Voucher	Locality	ITS	LSU	β-Tub	tef-1a
Candolleomyces albipes	DED8340	Sao Tome	KX017209	_	_	_
C. aberdarensis	GLM-F116094	Kenya	MH880928	_	_	_
C. badhyzensis	79478 (TAA) Type	Turkmenistan	KC992883	KC992883	_	_
C. badiophyllus	SZMC-NL-2347	-	FN430699	FM876268	FN396261	FM897252
C. cacao	SFSU DED 8339	Sao Tome	NR148106	_	_	_
	FP1R4	USA	KU847452	_	_	_
	MP2R2	USA	KU847436	_	_	_
C. candolleanus	LAS73030 Neotype	Sweden	KM030175	KM030175	_	_
C. efflorescens	Pegler2133 (K)	Sri Lanka	KC992941	_	_	_
C. eurysporus	GLM-F126263 Type	Germany	MT651560	MT651560	_	_
C. leucotephrus	LÖ138-01 (UPS)	Sweden	KC992885	KC992885	KJ664865	KJ732775
C. luteopallidus	Sharp20863 (MICH) Type	USA	KC992884	KC992884	_	_
	HMJAU5148	China: Jilin	MG734736	MW301084	MW314056	MW314073
C. secotioides	UES2918 Type	Mexico	KR003281	KR003282	_	KR003283
C. singeri	HMJUA37867	China: Jilin	MG734718	MW301088	MW314059	MW314077
	HMJAU37877	China: Chongqing	MW301073	MW301091	MW314062	MW314080
Candolleomyces sp.	BAB-4773	India	KP686450	_	_	_
	BAB-5172	India	KR349656	_	_	_
	BAB-4748	India	KR154977	_	_	_
	BAB-4747	India	KR154976	_	_	_
	BAB-5202	India	KT188611	_	_	_
C. subcacao	HMJAU37807 Type	China: Henan	MW301064	MW301092	MW314063	MW314081
	HMJAU37808	China: Henan	MW301065	MW301093	MW314064	MW314082
	HFJAU1014	China: Jiangxi	MW559218	_	_	_
	HFJAU1274	China: Jiangxi	MW559219	-	-	-
	HFJAU1488	China:Anhui	MW559220	_	_	_
C. subminutisporus	HMJAU37801 Type	China: Hubei	MW301066	MW301094	MW314065	MW314083
	HMJAU37916	China: Henan	MW301067	MW301095	MW314066	MW314084
C. subsingeri	HMJAU37811 Type	China: Jilin	MG734715	MW301097	MW314067	MW314085
	HMJAU37913	China: Jilin	MG734725	MW301098	MW314068	MW314086
C. sulcatotuberculosus	GB:LO55-12	-	KJ138422	KJ138422	_	_
	HFJAU1515	China: Fujian	MW375696	_	MW382967	MW382965
	Chiarello 07-10-2013	-	KJ138423	_	_	_
C. trinitatensis	TL9035 (C)	Ecuador	KC992882	KC992882	KJ664863	_
	ADK4162 (BR)	Togo	KC992886	KC992886	-	-
Psathyrella cladii-marisci	CLUF302 Type	Italy	MK080112			
Outgroup						
Psathyrella multipedata	LÖ237-04	Sweden	KC992888	KC992888	KJ664867	KJ732777

Table 1. Sequences used in this study.

Note: Newly-generated sequences are in bold.

Data analyses

Taking into consideration the results of BLAST searching against GenBank and the research of Büttner et al. (2020) and Wächter and Melzer (2020), we analysed ITS, LSU, *tef-1a* (Tef 1st, Tef 2nd and Tef 3rd) and β -*tub* (Tub 1st and Tub 2nd) sequences from 37 taxa. Details are presented in Table 1. Sequences were aligned using the online version of the multiple sequence alignment programme MAFFT v.7 (Katoh and Standley 2013), followed by manual adjustment in BIOEDIT v.7.1.3.0 (Hall 1999). Phylogenetic analyses were conducted using Bayesian Inference (BI) in MrBayes v.3.2.6 (Ronquist et al. 2012) and by Maximum Likelihood (ML) in IQTREE v.1.5.6 (Nguyen et al. 2014). For the BI analyses, four Monte Carlo Markov chains were run

for 10 million generations, with sampling every 100th generation and with the first 25% of trees discarded as burn-in (Ronquist et al. 2012). ML analyses were undertaken by applying the ultrafast bootstrap approximation with 1000 replicates. The sequence alignment has been deposited in TreeBASE (S28074).

Results

According to a BLAST analysis, the ITS sequence of C. subcacao is 98% similar (eight different loci) to that of C. cacao (Desjardin & B.A. Perry) D. Wächt. & A. Melzer and approximately 97% similar (19 different loci) to five ITS sequences from two unnamed species (KP686450 for BAB-4773, KR349656 for BAB-5172, KR154977 for BAB-4748, KR154976 for BAB-4747 and KT188611 for BAB-5202) isolated from Oeceoclades maculata (Lindley) Lindley (Bayman et al. 2016). The ITS sequence of C. subminutisporus shares 97% similarity (22 different loci) with that of C. sulcatotuberculosus (J. Favre) D. Wächt. & A. Melzer. The generated BI and ML trees are shown in Fig. 1 and Suppl. material 1, respectively. In both trees, sequences of the two new species comprise strongly supported clades that are distinct from closely-related taxa. The C. subcacao clade groups together with C. cacao and two unnamed species with high statistical support, while the C. subminutisporus clade clusters with C. singeri (A.H. Sm.) D. Wächt. & A. Melzer and C. sulcatotuberculosus. The type sequence of Psathyrella cladii-marisci Sicoli, N.G. Passal., De Giuseppe, Palermo & Pellegrino is clearly nested within Candolleomyces, where it groups most closely, although with only weak to moderate support, with C. badhyzensis (Kalamees) D. Wächt. & A. Melzer, C. badiophyllus (Romagn.) D. Wächt. & A. Melzer and C. candolleanus (Fr.) D. Wächt. & A. Melzer.

Taxonomy

Candolleomyces subcacao T. Bau & J.Q. Yan, sp. nov.

MycoBank No: 839231 Fig. 2

Holotype. CHINA. Henan Province: Bird Island, Nanwan Lake, Xinyang City, 32°06'43.32"N, 113°06'03.06"E, 124 m elevation, 17 July 2016, Tolgor Bau, Jun-Qing Yan, HMJAU37807 (holotype!)

Etymology. Referring to its morphological similarity to C. cacao.

Diagnosis. Differs from C. cacao in having a distinct spore germ pore.

Description. Pileus 11–35 mm, spreading hemispherically to planar, hygrophanous, brown (7E7–7E8), striate up to halfway from the margin or indistinct, becoming slightly dirty white (7B1–7B2) upon drying. Veil pale brown (7A5–7B6), thin, fibrillose, falling off easily. Context thin and very fragile, dirty white (7B1–7B2), approximately 1.0 mm thick at the centre. Lamellae 3.0–4.0 mm wide, moderately



Figure 1. Phylogenetic tree of *Candolleomyces*. The tree was generated by Bayesian analysis of a concatenated dataset of sequences from four nuclear regions (ITS, LSU, *tef-1a* and β -*tub*). *Psathyrella multipedata* (Peck) A.H. Sm. was used as an outgroup. Bayesian posterior probabilities (BI-PP) ³ 0.95 and Maximum Likelihood bootstrap support values (ML) \geq 75% are shown above nodes as BI-PP/ML. • indicates newly-described species.

close, adnate to slightly adnexed, pale brown (C3–C4) to dark brown (7D6–7E6), saw-toothed under $20 \times$ magnification. Stipe 40–50 mm long, approximately 2.0 mm thick, white (7A1–7B1), hollow, equal, smooth, with white fibrils (7A1–7B1) at the base. Odour and taste indistinct.

Spores 6.8–8.0(8.8) × 3.9–4.9 µm, Q = 1.4–1.8, ellipsoid to oblong-ellipsoid, profile slightly flattened on one side, rarely phaseoliform, inamyloid, smooth, pale yellow-brown, darkening in 5% KOH, pale brown, germ pores distinct, but small, approximately 1.0 µm wide. Basidia 17–22 × 6.1–7.3 µm, clavate, hyaline, 4-spored. Pleurocystidia absent. Cheilocystidia 22–36 × 9.8–14 µm, scattered to moderately numerous, various, utriform to fusiform, with an obtuse to broadly obtuse apex, rarely subcapitate or clavate, ovoid, thin-walled. Trama of gills irregular. Pileipellis consisting of 2–3 cells in the deep layer of the subglobose cell, 20–37 µm wide.

Habit and habitat. Solitary to scattered on rotten wood in oak forest.

Other specimens examined. CHINA. Henan Province: Bird Island, Nanwan Lake, Xinyang City, 17 July 2016, Tolgor Bau and Jun-Qing Yan, HMJAU37808, HMJAU37809; Borden Forest Park, Xinyang City, 17 July 2017, Jun-Qing Yan,



Figure 2. Basidiomata and microscopic features of *Candolleomyces subcacao* **a–c** Basidiomata **d** spores **e** basidia **f** pileipellis **g** cheilocystidia. Scale bars: 10 mm (**a–c**); 10 µm (**d–g**).

HMJAU37898, HMJAU37899, HMJAU37900, HMJAU37948, HMJAU44554; Jiangxi Province: Jiangxi Agricultural University, Nanchang City, 3 June 2019, Jun-Qing Yan, HFJAU0716, 9 June 2019, Jun-Qing Yan, HFJAU1274; Yun Bi Feng National Forest Park, Shangrao City, 5 July 2019, HFJAU1014.

Candolleomyces subminutisporus T. Bau & J.Q. Yan, sp. nov.

MycoBank No: 839232 Fig. 3

Etymology. Referring to the small spores.

Holotype. CHINA. Henan Province: Boerdeng National Forest Park, Xinyang City, 16 July 2017, Tolgor Bau and Jun-Qing Yan, HMJAU37801 (holotype!).

Diagnosis. Differs from *C. sulcatotuberculosus* in having smaller spores $(5.8-6.8 \ \mu m \ long)$.

Description. Pileus 8.0–22 mm, spreading hemispherically to broadly conical convex, hygrophanous, pale yellow-brown (6C7–6C8) at the centre, pale at the margin (6A2–6A4), striate from margin to centre, becoming pale brown (6B6–6B7) when dry. Veil present in early stages, thin, white (6A1), fibrillose, evanescent. Context thin and very fragile, 1.0–1.5 mm thick at the centre, same colour as the pileus. Lamellae 2.5–3.0 mm wide, adnate, moderately close, white (6B1) to pale coffee (6B2–6B3), edges saw-toothed under 20× magnification. Stipes 15–40 mm long, 1.0–2.0 mm thick, cylindrical, hollow, white (6B1), sometimes subhyaline or slightly yellow-brown (6A2–6B2) at the base, apex pruinose, evanescent, slightly expanded at the base. Odour and taste indistinct.

Spores 5.8–6.8(7.8) × 3.8–4.9 µm, Q = 1.4–1.8, ovoid, ellipsoid to oblongellipsoid, in profile flattened on one side, rarely phaseoliform, inamyloid, smooth, very pale, nearly hyaline in water and 5% KOH, germ pore absent. Basidia 14–20 × 7.3–7.8 µm, 4-spored, clavate, hyaline. Pleurocystidia absent. Cheilocystidia 20–32 × 11–17 µm, utriform, with obtuse apex, bottom side tapering to the long or short stipe. Caulocystidia 27–42 × 6.1–9.8 µm, present at the apex, mostly solitary, various, similar to cheilocystidia or clavate and subcapitate or not. Trama of gills irregular. Pileipellis consists of 1–2 cells in a deep layer of the subglobose cell, up to 36 µm broad.

Habit and habitat. Scattered on rotten wood or humus in *Pinus massoniana* and oak forests.

Other specimens examined. CHINA. Anhui Province: Huangshan City, 3 July 2018, Jun-Qing Yan, HFJAU1253, HFJAU1361; Guangxi Zhuang Autonomous Region: Qingxiushan National Forest Park, Nanning City, 12 Aug 2016, HMJAU37930; Phoenix Valley Forest Park, Nanning City, 17 Aug 2016, Jun-Qing Yan, HMJAU37950; Henan Province: Boerdeng National Forest Park, Xinyang City, 16 July 2017, Jun-Qing Yan, HMJAU37916, HMJAU37958; 17 July 2017, Jun-Qing Yan, HMJAU37950, HMJAU37960, HMJAU37961: Hubei Province: Dagui Temple National Forest Park,



Figure 3. Basidiomata and microscopic features of *Candolleomyces subminutisporus* **a–c** Basidiomata **d** spores **e** basidia **f** pileipellis **g** cheilocystidia **h** caulocystidia. Scale bars: 10 mm (**a–c**); 10 µm (**d–h**).

Suizhou City, 16 July 2016, Tolgor Bau and Jun-Qing Yan, HMJAU37800; Jiangxi Province: Lushan Mountain, Jiujiang City, 30 June 2020, Jun-Qing Yan, HFJAU0921; Yunnan Province: Kunming Botanical Garden, Kunming City, 6 Aug 2016, Jun-Qing Yan, HMJAU37929.

Candolleomyces cladii-marisci (G. Sicoli, N.G. Passalacqua, A.B. De Giuseppe, A.M. Palermo & G. Pellegrino) J.Q. Yan, comb. nov. MycoBank No: 839233

Psathyrella cladii-marisci Sicoli, N.G. Passal., De Giuseppe, Palermo & Pellegrino, MycoKeys 52: 99, 2019. Basionym.

Note. According to the ITS phylogenetic analysis including the type specimen, *P. cladii-marisci* belongs to *Candolleomyces* and has a close phylogenetic relationship with *C. candolleanus, C. badiophyllus* and *C. trinitatensis.* In addition, the morphological characteristics of this species correspond to *Candolleomyces*, which lack pleurocystidia.

For detailed descriptions and line drawings of this species, see Sicoli et al. (2019a; b).

Discussion

Most species of *Candolleomyces* have dark brown or brown spores, whereas species with pale spores are rare. *Candolleomyces subcacao* is very easily confused with *C. cacao* in the field because of their similar macroscopic characteristics. In addition, these two species have highly similar ITS regions (98%). Nevertheless, some members of *Candolleomyces* with high ITS similarity are still treated as separate species on the basis of morphological characters (Sicoli et al. 2019a; Büttner et al. 2020; Wächter and Melzer 2020). *Candolleomyces subcacao* and *C. cacao* group together, but comprise independent lineages, in the phylogenetic tree (Fig. 1). Moreover, *C. cacao* has ventricose to broad lageniform cheilocystidia, an indistinct germ pore in 5% KOH and a tropical distribution (Desjardin 2016).

On the basis of morphology, *C. subcacao* has been classified into *Psathyrella* sect. *Spintrigerae* using the classification system of Kits van Waveren (1985; 1987) and *Psathyrella* sect. *Subatratae*, based on the system of Smith (1972). Some species in these sections lack pleurocystidia and may thus actually belong to *Candolleomyces*, but molecular analyses of type materials are needed prior to their possible reassignment. In this paper, we have, therefore, only compared these species and the new ones with respect to morphology (see the key below). In particular, two species in these sections possess the combined characteristics of small basidiomata, a pale brown and evanescent veil and pale yellow-brown spores with a distinct germ pore: *P. lacuum* Huijsman, which can be distinguished from *C. subcacao* by the presence of a veil with dispersed white arachnoid fibrils or flocci, abundant pyriform cells at the marginal of the lamellae and very rare utriform cheilocystidia (Kits van Waveren 1985; Battistin et al. 2014) and *P. cordobaensis* A.H. Sm., which differs mainly in having a 10 mm wide pileus, an indistinct germ pore and saccate to ellipsoid cheilocystidia (Smith 1972; Desjardin 2016).

Candolleomyces subminutisporus is characterised by the presence of small basidiomata, a pileus that is striate from the margin up to the centre and very pale to nearly hyaline spores that are mainly less than 7.0 μ m long. *Candolleomyces sulcatotuberculo*- sus and *C. subminutisporus* are morphologically very similar and are phylogenetically closely related (Fig. 1); however, the former has a sulcate-tuberculose pileus surface and much larger spores, which measure $(7.6)7.9-8.5(9) \times (4.5)4.6-5.0(5.2) \mu m$ (Einhellinger 1976). Although Kits van Waveren (1985) and Battistini (2014) detected some smaller spores in these species, which measured $(6.2)6.9-7.8(8.9) \times (3.6)4.1-4.7(5.0) \mu m$, most spores of *C. sulcatotuberculosus* are clearly longer than 7.0 μm .

Candolleomyces singeri (A.H. Sm.) D. Wächt. & A. Melzer, *C. eurysporus* A. Karich, E. Büttner & R. Ullrich and *C. aberdarensis* (A. Melzer, Kimani & R. Ullrich) D. Wächt. & A. Melzer group together with *C. subminutisporus* in the phylogenetic tree (Fig. 1). These species can be separated as follows: *C. singeri* has larger spores, mostly 6.8–7.8 µm long (Smith 1972, pers. obs. of HMJUA37867 by JQ Yan), whereas *C. eurysporus* can be separated on the basis of its broader spores, a *Q*-value of 1.2-1.6(-1.7) and brown lamellae at maturity (Büttner et al. 2020) and *C. aberdarensis* is distinguished by having larger spores [7.5–8(–8.8) µm long] (Melzer et al. 2018). In addition, two species are morphologically similar to *C. subminutisporus* in having more-or-less pale spores, germ pores that are indistinct or lacking and no pleurocystidia. These species can be separated from *C. subminutisporus* as follows: *C. halophilus* (Esteve-Rav. & Enderle) D. Wächt. & A. Melzer has larger spores, which are $8.6-11 \times 4.8-6.2$ µm (Esteve-Raventós and Enderle 1992; Battistin et al. 2014) and *C. subsingeri* (T. Bau & J.Q. Yan) D. Wächt. & A. Melzer is easily distinguished on the basis of its stout basidiomata (Yan and Bau 2018a).

Finally, *P. cladii-marisci* was described by Sicoli et. al. (2019) and is characterised by the absence of pleurocystidia and the presence of large spores up to 11 µm long (Sicoli et al. 2019a; b). According to our phylogenetic analysis, this species is relatively closely related to *C. candolleanus*, *C. badiophyllus* and *C. trinitatensis* and should be moved to *Candolleomyces*. A new combination is thus proposed.

Key to related species

1	Spores very pale, nearly hyaline in 5% KOH	2
_	Spores pale yellow-brown, greyish-brown or darker	7
2	Spores mostly less than 7.0 µm	3
_	Spores up to 8.0 µm	í
3	Spores broader, $Q = 1.2-1.6$, lamellae brown at maturity <i>C. eurysporu</i>	s
_	Spores slenderer, $Q = 1.4-1.8$, lamellae pale coffee at maturity	•
	C. subminutisporu	s
4	Surface of pileus is sulcate-tuberculose, up to two-thirds of the radius	•
		s
_	Not as above	5
5	Pileus less than 10 mm wide, lamellae brown	5
_	Not as above	5
6	Basidiomata stout, spores up to 5.5 μm broad C. singer	i
_	Basidiomata slender, spores up to 4.5 µm broad C. subsinger	i
7	Spores up to 11 µm, growing on plant debris in brackish water C. halophilu	s
_	Not as above	3

8	Germ pore distinct
_	Germ pore indistinct
9	Margin of lamellae with abundant pyriform cells, utriform cheilocystidia very
	rare
_	Not as above
10	Cheilocystidia ventricose to broadly lageniform
_	Cheilocystidia saccate to ellipsoid

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Supplementary material I

Phylogram generated by Maximum Likelihood (ML) analysis

Authors: Tolgor Bau, Jun-Qing Yan

Data type: phylogenetic tree

- Explanation note: Phylogram generated by Maximum Likelihood (ML) analysis of *Candolleomyces* based on sequences of a concatenated data set from four nuclear regions (ITS, LSU, *Tef-1a* and β*-tub*), rooted with *Psathyrella multipedata* (Peck) A.H. Sm. (/multipedata clade). ML bootstrap proportion (ML-BP)≥ 75% are shown.
 - indicates newly described species.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/mycokeys.80.67166.suppl1

CORRIGENDA



Corrigendum: Pykälä J, Kantelinen A, Myllys L (2020) Taxonomy of Verrucaria species characterised by large spores, perithecia leaving pits in the rock and a pale thin thallus in Finland. MycoKeys 72: 43–92. https://doi.org/10.3897/mycokeys.72.56223

Juha Pykälä¹, Annina Kantelinen², Leena Myllys²

l Biodiversity Centre, Finnish Environment Institute, Latokartanonkaari 11, 00790, Helsinki, Finland **2** Botanical Museum, Finnish Museum of Natural History, P.O. Box 7, FI-00014, University of Helsinki, Finland

Corresponding author: Juha Pykälä ((juha.pykala@syke.fi)

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Almost all micrometres (μ m) have been erroneously changed into millimetres (mm). All measurements of algal cells, ostiole, involucrellum thickness, exciple wall thickness, periphysoids, asci, ascospores and perispores should be in micrometres (μ m). Furthermore, thallus thickness should be in micrometres in *Verrucaria bifurcata*, *V. cavernarum*, *V. difficilis* and *V. vacillans*.

In the lectotypification of *Verrucaria subjunctiva* registration number MBT392473, MBT is missing from the MycoBank number. This note validates the lectotypification.

Verrucaria subjunctiva Nyl., Flora 67: 218, 1884

MycoBank No: 392473

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

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