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Diversity of *Moesziomyces* (Ustilaginales, Ustilaginomycotina) on *Echinochloa* and *Leersia* (Poaceae)

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Abstract

A combined ecological, morphological, and molecular approach was used to examine 26 herbarium specimens and eight strains of *Moesziomyces*. The phylogenetic analysis resolved eight well-supported clades, of which three contained type specimens of known species of *Moesziomyces*. One clade contained two specimens that produced a teleomorph in the flowers of *Echinochloa kimberleyensis* in Australia. The name *Moesziomyces kimberleyensis* is proposed for this smut fungus. Another clade contained specimens that produced sori in the flowers of *Leersia hexandra*. The name *Thecaphora globuligera* (now *Moesziomyces globuligerus*) is available for this species, which is lectotypified. The teleomorph of *Moesziomyces antarcticus*, previously known only from Japan, is found for the first time in China, on *Echinochloa crus-galli*.

Keywords

Ecology, plant pathogens, phylogeny, Ustilaginaceae, Ustilaginomycotina

Introduction

The genus *Moesziomyces* (Ustilaginales, Ustilaginaceae) was established by Vánky (1977) for smut fungi that produce sori in the ovaries of grasses, lack a columella, and have spores with irregular meshes and wings on the surface, bound in firmly

agglutinated spore balls. Vánky (1977) recognized four species, *M. bullatus*, *M. evernius*, *M. globuligerus*, and *M. penicillariae*. Vánky (1986, 2012, 2013) later synonymised these names with the oldest available name, *M. bullatus*, and considered *Moesziomyces* as monotypic. Species of *Moesziomyces* are known to produce both free-living saprobic anamorphs (yeast-like) and plant pathogenic teleomorphs (smuts) (Wang et al. 2015; Kruse et al. 2017). The anamorphs of *Moesziomyces* are readily culturable on artificial media and have been isolated from a range of substrates, while the teleomorphs are formed in ovaries of seven genera of grasses (Poaceae). Wang et al. (2015) recombined four species known only by their anamorphs (*Pseudozyma antarctica*, *P. aphidis*, *P. parantarctica*, and *P. rugulosa*) into *Moesziomyces*, based on a molecular phylogenetic analysis. Subsequently, Tanaka et al. (2019) showed that one of these species, *M. antarcticus*, produced a teleomorph on *Echinochloa crus-galli* in Japan. A further five species, *M. bullatus*, *M. eriocauli*, *M. evernius*, *M. penicillariae*, and *M. verrucosus*, have been characterized from teleomorphs (Vánky 2012; Wang et al. 2015; Kruse et al. 2017). Kruse et al. (2017) recognized six species of *Moesziomyces* based on phylogenetic analysis, and treated *M. aphidis* and *M. rugulosus* as synonyms of *M. bullatus*.

The teleomorphs of Ustilaginaceae are mostly host specific (Stoll et al. 2003, 2005; Skibbe et al. 2010; McTaggart et al. 2012; Li et al. 2017a, 2017b). Given that species of *Moesziomyces* have been reported from seven different genera of grasses (*Echinochloa*, *Leersia*, *Panicum*, *Paspalum*, *Pennisetum*, *Polytrias*, and *Uranthoecium*), it is likely that additional species remain to be discovered. The aim of this study was to build on the work of Kruse et al. (2017) by examining specimens of *Moesziomyces* held in herbaria BRIP (Queensland Plant Pathology Herbarium), HMAS (Herbarium Mycologicum Academiae Sinicae), and HUV (Herbarium Ustilaginales Vánky, now deposited in BRIP), as well as eight yeast strains deposited in LC Culture Collection (personal culture collection held in the laboratory of Dr Lei Cai).

Materials and methods

Specimen examination

Specimens borrowed from several herbaria were examined by light microscopy (Table 1) by mounting the spores in lactic acid (100% v/v). Teliospore measurements were expressed as ranges (min–) mean-standard deviation-mean + standard deviation (–max) (n = 50). Images were captured by using a Nikon DS-Fi1 camera attached to a Nikon Eclipse 80i microscope with Nomarski differential interference contrast. Helicon Focus ver. 4.46.1 (Helicon Soft Ltd) was used to combine images to increase depth of field. Nomenclatural novelties and descriptions were registered in MycoBank (http://www.MycoBank.org).

DNA extraction, PCR amplification and sequencing

Sori were carefully removed from herbarium specimens, up to 149 years old, with a fine needle, sterilized by dipping in 75% ethanol for 30 s, air-dried on sterilized filter paper, and deposited in cell lysis solution (CTAB). Pure yeast colonies grown on yeast extract peptone dextrose (YPD) plates were transferred to cell lysis solution directly. Genomic DNA was extracted following the protocol of Cubero et al. (1999). Fragments of internal transcribed spacer rDNA were amplified by PCR with primers M-ITS1/ITS4 (White et al. 1990; Stoll et al. 2003).

PCR amplifications were carried out in 25 µl reactions containing 1 µl of genomic DNA template, 9.5 µl distilled water, 12.5 µl of 2 X Taq Plus Master Mix (Nanjing Vazyme Biotech Co. Ltd, Nanjing, China) and 1 µl of each primer (10 µM). Amplification reactions were run as follows: initial denaturation of 95 °C for 5 min followed by 35 cycles at 95 °C for 30 s, 45 s at 58 °C (annealing temperature) and 1 min at 72 °C with a final extension of 10 min at 72 °C. PCR products were sent to Tianyihuiyuan (Beijing, China) for sequencing with the forward and reverse primers indicated above. AB1 sequence traces were assembled with Sequencher version 5 (Genecodes, Ann Arbor, USA).

Phylogenetic analyses

The sequences included in this study (Tables 1, 2) were aligned online with MAFFT (https://mafft.cbrc.jp/alignment/server/index.html) using auto strategy, and observed in MEGA 5 (Katoh and Toh 2008). Phylogenetic analyses were based on both maximum likelihood (ML) and Bayesian Inference (BI). RAxML (Stamatakis 2006) and PhyML 3.0 (Guindon et al. 2010) were used for ML analyses. GTRGAMMA was specified as the model of evolution in both programs. The RAxML analyses were run with a rapid Bootstrap analysis (command -f a) using a random starting tree and 1 000 ML bootstrap replicates. The PhyML analyses were implemented using the ATGC bioinformatics platform (available at: http://www.atgcmontpellier.fr/phyml/), with six substitution type and SPR tree improvement, and support obtained from an approximate likelihood ratio test (Anisimova et al. 2011).

For BI, MrBayes was used with a Markov Chain Monte Carlo algorithm incorporating four runs, each consisting of four chains, until the standard deviation of split frequencies was reached. The cold chain was heated at a temperature of 0.25. Substitution model parameters were sampled every 50 generations and trees were saved every 5000 generations. Convergence of the Bayesian analysis was confirmed using AWTY (Nylander et al. 2008) (available at: http://ceb.csit.fsu.edu/awty/). A user-defined tree obtained from the PhyML analyses was used as a starting point for all the Bayesian analyses, which helped to improve convergence of the four runs.

Results

The ITS dataset comprised the newly sequenced *Moesziomyces* specimens and strains (Table 1) together with the reference sequences of *Moesziomyces* from Kruse et al. (2017) and Tanaka et al. (2019) (Table 2) and *Triodiomyces altilis* and *Ustilago echinata* as the outgroup based on the phylogenetic analyses of Wang et al. (2015). The topology of the ML and BI analyses (Fig. 1) were congruent. The phylogenetic

Table 1. Collection details for *Moesziomyces* specimens newly sequenced in this study.

Species	Specimen/strain no.[1]	Host	Source	Location	Year of collection	ITS GenBank accession number[2]
Moesziomyces antarcticus	HMAS 248025	*Echinochloa crus-galli*	Sorus	China	2017	**MK027038**
M. antarcticus	HMAS 248026	*E. crus-galli*	Sorus	China	2017	**MK027039**
M. antarcticus	HMAS 60130	*E. crus-galli*	Sorus	China	1989	**MK027043**
M. bullatus	HMAS 146471	*E. crus-galli*	Sorus	China	2003	**MK027040**
M. bullatus	HMAS 50052	*E. crus-galli*	Sorus	China	1985	**MK027041**
M. bullatus	LC-CLS58-3-2	*Setaria faberii*	Leaf surface	China	2017	**MK024201**
M. bullatus	LC-CLS58-3-21	*S. faberii*	Leaf surface	China	2017	**MK024202**
M. bullatus	LC-CLS58-3-22	*S. faberii*	Leaf surface	China	2017	**MK024203**
M. bullatus	LC-CLS60-2-22	*Pennisetum* sp.	Leaf surface	China	2017	**MK024204**
M. bullatus	LC-CLS60-2-4	*Pennisetum* sp.	Leaf surface	China	2017	**MK024205**
M. bullatus	LC-SY1-2-11	*Digitaria* sp.	Leaf surface	China	2017	**MK024206**
M. bullatus	LC-SY1-2-21	*Digitaria* sp.	Leaf surface	China	2017	**MK024207**
M. bullatus	LC-SY1-2-22	*Digitaria* sp.	Leaf surface	China	2017	**MK024208**
M. bullatus	HMAS 50454	*E. crus-galli*	Sorus	Japan	1985	**MK027042**
M. bullatus	HMAS 70876	*E. crus-galli*	Sorus	China	1991	**MK027045**
M. bullatus	HMAS 73871	*E. crus-galli*	Sorus	China	1996	**MK027046**
M. bullatus	HUV 2442*	*E. crus-galli*	Sorus	Poland	1869	**MK027047**
M. bullatus	HUV 305	*E. crus-galli*	Sorus	Germany	1905	**MK027050**
M. globuligerus	BRIP 27384	*Leersia hexandra*	Sorus	Australia	1998	**MK027025**
M. globuligerus	BRIP 44301	*L. hexandra*	Sorus	Australia	2004	**MK027029**
M. globuligerus	BRIP 44569	*L. hexandra*	Sorus	Australia	2004	**MK027030**
M. globuligerus	BRIP 47767	*L. hexandra*	Sorus	Thailand	2005	**MK027031**
M. globuligerus	BRIP 47768	*L. hexandra*	Sorus	Thailand	2005	**MK027032**
M. globuligerus	BRIP 51872	*L. hexandra*	Sorus	Australia	2008	**MK027035**
M. globuligerus	HMAS 248027	*L. hexandra*	Sorus	China	2017	**MK027037**
M. kimberleyensis	BRIP 51843*	*E. kimberleyensis*	Sorus	Australia	2008	**MK027034**
M. kimberleyensis	BRIP 52498	*E. kimberleyensis*	Sorus	Australia	2009	**MK027036**
M. penicillariae	HUV 2487	*Pe. glaucum*	Sorus	Gambia	1973	**MK027048**
M. penicillariae	HUV 2488	*Pe. glaucum*	Sorus	India	1912	**MK027049**
M. verrucosus	BRIP 39886	*Paspalum distichum*	Sorus	Australia	2003	**MK027026**
M. verrucosus	BRIP 43727	*Pa. distichum*	Sorus	Australia	2004	**MK027027**
M. verrucosus	BRIP 43735	*Pa. distichum*	Sorus	Australia	2004	**MK027028**
M. verrucosus	BRIP 51772	*Pa. distichum*	Sorus	India	1992	**MK027033**
M. verrucosus	HMAS 66437	*Pa. distichum*	Sorus	India	1992	**MK027044**

[1]BRIP: Queensland Plant Pathology Herbarium, Brisbane, Australia; HMAS: Herbarium Mycologicum Academiae Sinicae; HUV: Herbarium Ustilaginales Vánky (located at BRIP). [2]GenBank accessions derived from this study are shown in bold. * Type specimens.

Figure 1. Phylogram obtained from a ML analysis based on the ITS sequence alignment. Values above the branches represent ML bootstrap values (> 70%) from RaxML and PhyML analysis respectively. Thickened branches represent Bayesian posterior probabilities (> 0.95). The scale bar indicates 0.03 expected substitutions per site. * indicates type specimens or type strains.

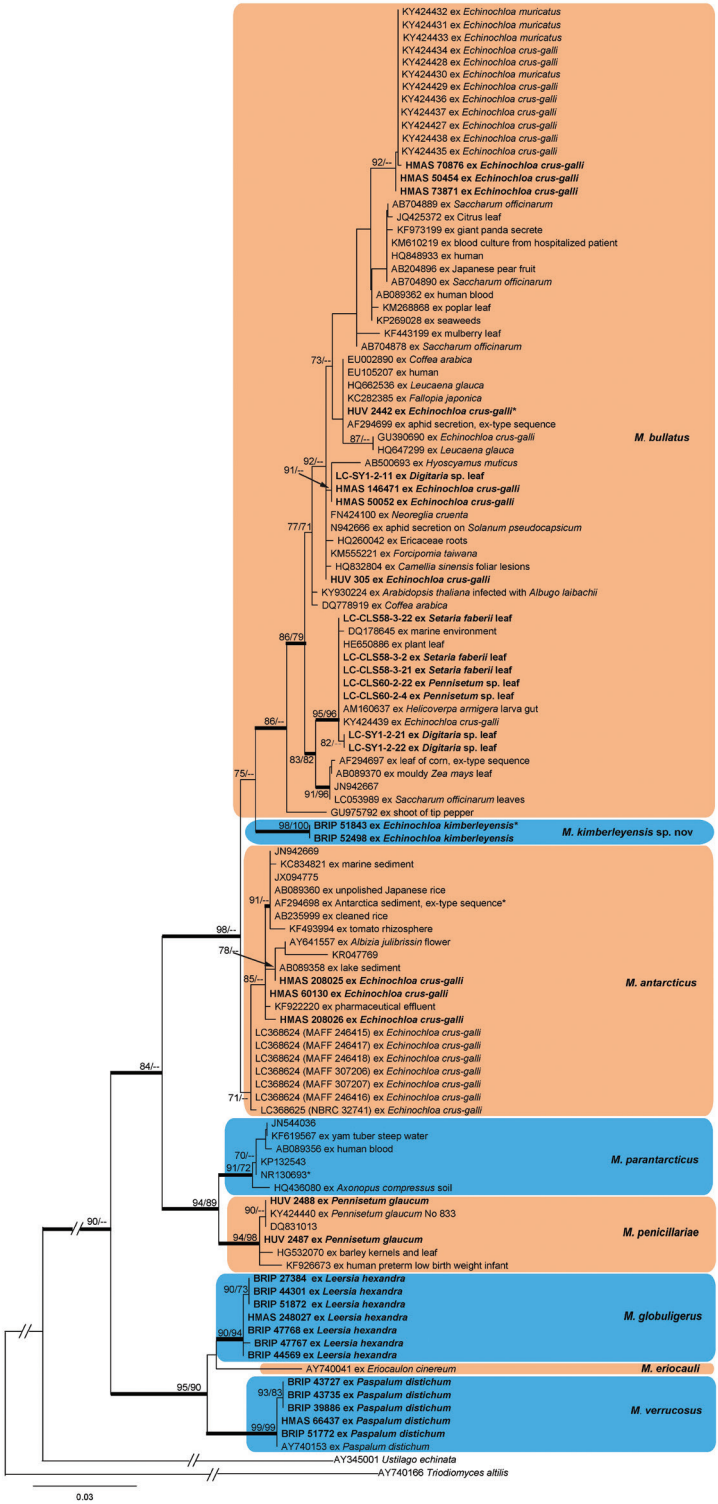
KY424432 ex Echinochloa muricatus
KY424431 ex Echinochloa muricatus
KY424433 ex Echinochloa muricatus
KY424434 ex Echinochloa crus-galli
KY424428 ex Echinochloa crus-galli
KY424430 ex Echinochloa muricatus
KY424429 ex Echinochloa crus-galli
KY424436 ex Echinochloa crus-galli
KY424437 ex Echinochloa crus-galli
KY424427 ex Echinochloa crus-galli
KY424438 ex Echinochloa crus-galli
KY424435 ex Echinochloa crus-galli
92/--
HMAS 70876 ex Echinochloa crus-galli
HMAS 50454 ex Echinochloa crus-galli
HMAS 73871 ex Echinochloa crus-galli
AB704889 ex Saccharum officinarum
JQ425372 ex Citrus leaf
KF973199 ex giant panda secrete
KM610219 ex blood culture from hospitalized patient
HQ848933 ex human
AB204896 ex Japanese pear fruit
AB704890 ex Saccharum officinarum
AB089362 ex human blood
KM268868 ex poplar leaf
KP269028 ex seaweeds
KF443199 ex mulberry leaf
AB704878 ex Saccharum officinarum
73/--
EU002890 ex Coffea arabica
EU105207 ex human
HQ662536 ex Leucaena glauca
KC282385 ex Fallopia japonica
HUV 2442 ex Echinochloa crus-galli*
AF294699 ex aphid secretion, ex-type sequence
87/--
GU390690 ex Echinochloa crus-galli
HQ647299 ex Leucaena glauca
M. bullatus
92/--
91/--
AB500693 ex Hyoscyamus muticus
LC-SY1-2-11 ex Digitaria sp. leaf
HMAS 146471 ex Echinochloa crus-galli
HMAS 50052 ex Echinochloa crus-galli
FN424100 ex Neoreglia cruenta
77/71
N942666 ex aphid secretion on Solanum pseudocapsicum
HQ260042 ex Ericaceae roots
KM555221 ex Forcipomia taiwana
HQ832804 ex Camellia sinensis foliar lesions
HUV 305 ex Echinochloa crus-galli
KY930224 ex Arabidopsis thaliana infected with Albugo laibachii
DQ778919 ex Coffea arabica
LC-CLS58-3-22 ex Setaria faberii leaf
DQ178645 ex marine environment
86/79
HE650886 ex plant leaf
LC-CLS58-3-2 ex Setaria faberii leaf
LC-CLS58-3-21 ex Setaria faberii leaf
LC-CLS60-2-22 ex Pennisetum sp. leaf
LC-CLS60-2-4 ex Pennisetum sp. leaf
95/96
AM160637 ex Helicoverpa armigera larva gut
86/--
KY424439 ex Echinochloa crus-galli
82/
LC-SY1-2-21 ex Digitaria sp. leaf
LC-SY1-2-22 ex Digitaria sp. leaf
83/82
AF294697 ex leaf of corn, ex-type sequence
75/--
AB089370 ex mouldy Zea mays leaf
JN942667
91/96
LC053989 ex Saccharum officinarum leaves
GU975792 ex shoot of tip pepper
98/100
BRIP 51843 ex Echinochloa kimberleyensis*
BRIP 52498 ex Echinochloa kimberleyensis
M. kimberleyensis sp. nov
JN942669
KC834821 ex marine sediment
JX094775
AB089360 ex unpolished Japanese rice
91/--
AF294698 ex Antarctica sediment, ex-type sequence*
AB235999 ex cleaned rice
98/--
KF493994 ex tomato rhizosphere
78/--
AY641557 ex Albizia julibrissin flower
KR047769
AB089358 ex lake sediment
85/--
HMAS 208025 ex Echinochloa crus-galli
HMAS 60130 ex Echinochloa crus-galli
KF922220 ex pharmaceutical effluent
HMAS 208026 ex Echinochloa crus-galli
M. antarcticus
LC368624 (MAFF 246415) ex Echinochloa crus-galli
LC368624 (MAFF 246417) ex Echinochloa crus-galli
LC368624 (MAFF 246418) ex Echinochloa crus-galli
LC368624 (MAFF 307206) ex Echinochloa crus-galli
LC368624 (MAFF 307207) ex Echinochloa crus-galli
84/--
71/--
LC368624 (MAFF 246416) ex Echinochloa crus-galli
LC368625 (NBRC 32741) ex Echinochloa crus-galli
JN544036
KF619567 ex yam tuber steep water
AB089356 ex human blood
70/--
KP132543
91/72
NR130693*
M. parantarcticus
HQ436080 ex Axonopus compressus soil
94/89
HUV 2488 ex Pennisetum glaucum
90/--
KY424440 ex Pennisetum glaucum No 833
DQ831013
94/98
HUV 2487 ex Pennisetum glaucum
M. penicillariae
HG532070 ex barley kernels and leaf
KF926673 ex human preterm low birth weight infant
BRIP 27384 ex Leersia hexandra
90/73
BRIP 44301 ex Leersia hexandra
BRIP 51872 ex Leersia hexandra
HMAS 248027 ex Leersia hexandra
M. globuligerus
90/94
BRIP 47768 ex Leersia hexandra
BRIP 47767 ex Leersia hexandra
BRIP 44569 ex Leersia hexandra
AY740041 ex Eriocaulon cinereum
M. eriocauli
95/90
BRIP 43727 ex Paspalum distichum
93/83
BRIP 43735 ex Paspalum distichum
BRIP 39886 ex Paspalum distichum
HMAS 66437 ex Paspalum distichum
M. verrucosus
99/99
BRIP 51772 ex Paspalum distichum
AY740153 ex Paspalum distichum
AY345001 Ustilago echinata
AY740166 Triodiomyces altiiis
0.03

Table 2. List of *Moesziomyces*, *Triodiomyces*, and *Ustilago* sequences taken from GenBank and used in the phylogenetic analysis.

Species	Source	ITS GenBank accession number	Reference
Moesziomyces antarcticus	–	JX094775	Gujjari et al. (unpubl.)
	–	JN942669	An (unpubl.)
	unpolished Japanese rice	AB089360	Sugita et al. 2003
	Antarctica sediment	AF294698	Avis et al. 2001
	Albizia julibrissin flower	AY641557	Wei et al. 2005
	lake sediment	AB089358	Sugita et al. 2003
	tomato rhizosphere	KF493994	Johnston-Monje et al. (unpubl.)
	Echinochloa crus-galli sorus	LC368624	Tanaka et al. 2019
	Echinochloa crus-galli sorus	LC368624	Tanaka et al. 2019
	Echinochloa crus-galli sorus	LC368624	Tanaka et al. 2019
	Echinochloa crus-galli sorus	LC368624	Tanaka et al. 2019
	Echinochloa crus-galli sorus	LC368624	Tanaka et al. 2019
	Echinochloa crus-galli sorus	LC368624	Tanaka et al. 2019
	Echinochloa crus-galli sorus	LC368625	Tanaka et al. 2019
Moesziomyces bullatus	human preterm low birth weight infant	KF926673	Okolo et al. 2015
	–	DQ831013	Matheny et al. 2006
	Japanese pear fruit	AB204896	Yasuda et al. 2007
	Saccharum officinarum	AB704889	Morita et al. 2012
	Leucaena glauca	HQ662536	Wei et al. 2011
	human	EU105207	Lin et al. 2008
	human blood	AB089362	Sugita et al. 2003
	human	HQ848933	Xie et al. unpubl.
	Fallopia japonica	KC282385	Wang & Liu (unpubl.)
	human blood	KM610219	Bosco-Borgeat & Taverna (unpubl.)
	Leucaena glauca	HQ647299	Wei et al. 2011
	Saccharum officinarum	AB704890	Morita et al. 2012
	poplar leaf	KM268868	Sun & Yan (unpubl.)
	Forcipomyia taiwana	KM555221	Chen (unpubl.)
	seaweed	KP269028	Wang et al. (unpubl.)
	aphid secretion	AF294699	Avis et al. 2001
	Neoreglia cruenta	FN424100	Garcia et al. (unpubl.)
	Saccharum officinarum	AB704878	Morita et al. 2012
	giant panda secretion	KF973199	Li et al. (unpubl.)
	Camellia sinensis leaf lesions	HQ832804	Li et al. (unpubl.)
	Echinochloa crus-galli	GU390690	Hamayun & Ahmad (unpubl.)
	aphid secretion on *Solanum pseudocapsicum*	JN942666	An (unpubl.)
	Citrus leaf	JQ425372	Soliman (unpubl.)
	–	JN942667	An (unpubl.)
	mouldy *Zea mays* leaf	AB089370	Sugita et al. 2003
	plant leaf	HE650886	Han et al. 2012
	ex-leaf of corn	AF294697	Avis et al. 2001
	Hyoscyamus muticus	AB500693	Abdel-Motaal & Itu (unpubl.)
	Coffea arabica	EU002890	Vega et al. (unpubl.)
	Coffea arabica	DQ778919	Vega et al. 2008
	Saccharum officinarum leaf	LC053989	Surussawadee & Limtong (unpubl.)
	marine environment	DQ178645	Chang et al. 2008
	Helicoverpa armigera larva gut	AM160637	Molnar & Prillinger (unpubl.)

Species	Source	ITS GenBank accession number	Reference
Moesziomyces bullatus	marine sediment	KC834821	Qu et al. (unpubl.)
	–	KR047769	Wang et al. (unpubl.)
	pharmaceutical effluent	KF922220	Selvi & Das (unpubl.)
	barley kernels and leaf	HG532070	Korhola et al. 2014
	Ericaceae roots	HQ260042	Walker et al. 2011
	cleaned rice	AB235999	Ikeda et al. 2007
	Arabidopsis thaliana infected with *Albugo laibachii*	KY930224	Kruse et al. 2017
	Echinochloa crus-galli	KY424439	Kruse et al. 2017
	Echinochloa crus-galli	KY424428	Kruse et al. 2017
	Echinochloa crus-galli	KY424429	Kruse et al. 2017
	Echinochloa muricata	KY424430	Kruse et al. 2017
	Echinochloa muricata	KY424431	Kruse et al. 2017
	Echinochloa muricata	KY424432	Kruse et al. 2017
	Echinochloa muricata	KY424433	Kruse et al. 2017
	Echinochloa crus-galli	KY424434	Kruse et al. 2017
	Echinochloa crus-galli	KY424435	Kruse et al. 2017
	Echinochloa crus-galli	KY424436	Kruse et al. 2017
	Echinochloa crus-galli	KY424437	Kruse et al. 2017
	Echinochloa crus-galli	KY424427	Kruse et al. 2017
	Echinochloa crus-galli	KY424438	Kruse et al. 2017
	shoot of tip pepper	GU975792	Sim et al. (unpubl.)
Moesziomyces eriocauli	*Eriocaulon cinereum*	AY740041	Stoll et al. 2005
Moesziomyces parantarcticus	–	KP132543	Irinyi et al. 2015
	human blood	AB089356	Sugita et al. 2003
	–	NR130693	An (unpubl.)
	–	JN544036	Chen (unpubl.)
	yam tuber steep water	KF619567	Babajide et al. 2015
	Axonopus compressus soil	HQ436080	Kee & Chia (unpubl.)
Moesziomyces penicillariae	*Pennisetum glaucum*	KY424440	Kruse et al. 2017
Moesziomyces verrucosus	*Paspalum distichum*	AY740153	Stoll et al. 2005
Triodiomyces altilis	*Triodia pungens*	AY740166	Stoll et al. 2005
Ustilago echinata	*Phalaris arundinacea*	AY345001	Stoll et al. 2003

analyses revealed eight distinct groups with high support values, including six clades consistent with those recovered by Kruse et al. (2017). The largest clade included specimens of *M. bullatus* on *Echinochloa crus-galli* (the host for the type specimen of *M. bullatus*) and *E. muricata* from Europe, related yeast strains as well as strains formerly assigned to the synonymous species names *Pseudozyma aphidis* and *P. rugulosa* (Kruse et al. 2017). Four well-supported clades comprised teleomorphic specimens on *Echinochloa kimberleyensis*, *Leersia hexandra*, *Paspalum distichum*, and *Pennisetum glaucum* (the latter with related yeast strains). One well-supported clade comprised yeast strains assigned to *M. parantarcticus*. One moderately supported clade comprised teleomorphic specimens on *E. crus-galli* from China and Japan and related yeast strains, assigned to *M. antarcticus*. The remaining single-sequence lineage was formed by *Moesziomyces eriocauli* on *Eriocaulon cinereum* (Eriocaulaceae).

Taxonomy

Based on the phylogenetic analysis and the hosts of the teleomorphs, a new species of *Moesziomyces* is described and another species resurrected. Additionally, the teleomorph of *M. antarcticus* is reported for the first time from China.

***Moesziomyces antarcticus* (Goto, Sugiyama & Iizuka) Q.M. Wang, Begerow, F.Y. Bai & Boekhout, Stud. Mycol. 81: 81 (2015)**
Figure 2h–k

Sporobolomyces antarcticus Goto, Sugiyama & Iizuka, Mycologia 61: 759 (1969). [Basionym]
Candida antarctica (Goto, Sugiyama & Iizuka) Kurtzman et al. Yeasts: 86 (1983).
Vanrija antarctica (Goto, Sugiyama & Iizuka) R.T. Moore, Biblthеca Mycol. 108: 167 (1987).
Pseudozyma antarctica (Goto, Sugiyama & Iizuka) Boekhout, J. Gen. Appl. Microbiol. 41: 364 (1995).
Trichosporon oryzae H. Ito, Iizuka & T. Sato, Agric. Biol. Chem. 38: 1599 (1974). (synonymy by Q.M. Wang, Begerow, F.Y. Bai and Boekhout).

Description. Sori in scattered ovaries, sometimes deciduous, globose to ovoid, 2–3 mm in length, covered by a smooth green membrane of host tissue origin that becomes brown and ruptures irregularly to expose a granular, black to dark brown mass of spore balls; columella absent. Spore balls variable in shape and size, globose, subglobose, ovoid, elongate to irregular, 130–200 μm in diameter, dark brown, composed of up to several hundred spores, united firmly by fungal sterile cells and spore meshes and wings. Spore globose, ovoid to irregular, slightly polyhedral, (8–) 8.5–9.5 (–10) × (6–) 7–9 (–10) μm, usually with well-developed meshes and wings, subhyaline to pale yellowish-brown; wall 0.5 μm thick, smooth. Some of the sterile cells empty at maturity, thin-walled, with irregular meshes or wings on the spore surface when the spores separates; other sterile cells, globose, ovoid to irregular, slightly polyhedral, (8–) 8.5–9.5 (–10) × (6–) 7–9 (–10) μm, subhyaline to pale yellowish brown; wall 1–1.5 μm thick, smooth.

Specimens examined. CHINA, Sichuan, Chengdu, on *Echinochloa crus-galli*, 15 Sept. 1989, L. Guo leg., HMAS 60130; Guangxi, on *E. crus-galli*, Oct. 2017, R.G. Shivas, M.D.E. Shivas & Y.-M. Li leg., HMAS 208025; Guangxi, on *E. crus-galli*, Oct. 2017, R.G. Shivas, M.D.E. Shivas & Y.-M. Li leg., HMAS 208026.

Notes. The teleomorph of *Moesziomyces antarcticus* was previously reported from Japan, on *Echinochloa crus-galli* (Tanaka et al. 2019). The current report from China, also on *E. crus-galli*, suggests that this smut fungus may be common in the teleomorphic stage, at least in East Asia.

***Moesziomyces globuligerus* (Berk. & Broome) Vánky, Bot. Not. 130: 135 (1977)**
Figure 2e–g

Thecaphora globuligera Berk. & Broome, Trans. Linn. Soc. London, Bot., Ser. 2, 1: 407 (1880). — Type: AUSTRALIA, Queensland, Brisbane, on *Leersia hexandra*, F.M. Bailey, No. 86 (K(M) 252436, **lectotype designated here**, MBT 385180, not seen; K(M) 252437, syntype). [Basionym]
Tolyposporium globuligerum (Berk. & Broome) Ricker, J. Mycol. 11:112 (1905).
Testicularia leersiae Cornu, Ann. Sci. Nat. Bot., Sér. 6, 15: 275 (1883).

Description. Sori in some of the ovaries, often deciduous, ellipsoidal to oval, 2.5–4 × 1.5–3 mm, green at first, later brown, smooth, ruptures irregularly to reveal a granular, dark brown mass of spore balls; columella absent. Spore balls subglobose, ellipsoidal or irregular, 75–150 µm in diameter, yellowish brown, composed of up to several hundred spores that separate by moderate pressure. Spores subglobose, ovoid to irregularly polyhedral, (8–) 8.5–11 (–13) × (6–) 7–9 (–10) µm ($\bar{x}$ = 9.6 ± 1.2 × 7.9 ± 0.9 µm, n = 50), subhyaline to pale yellowish brown, attached together by multiple narrow cylindrical protuberances about 2 µm wide and 1–2 µm long; wall with irregular meshes and wings, less than 0.5 µm thick, smooth. (Based on specimen BRIP 27384).

Specimens examined. AUSTRALIA, Queensland, Willowbank, on *Leersia hexandra*, 9 Mar. 1998, C. Vánky & K. Vánky leg., BRIP 27384; Queensland, Mareeba, on *L. hexandra*, 1 May 2004, M.D.E. Shivas & R.G. Shivas leg., BRIP 44301; Queensland, Mt Garnet, on *L. hexandra*, 5 May 2005, T.S. Marney & R.G. Shivas leg., BRIP 44569; Northern Territory, Darwin, on *L. hexandra*, 15 Apr. 2008, J. Ray, A.A. Mitchell, A.R. McTaggart & R.G. Shivas leg., BRIP 51872. CHINA, Guangxi province, on *L. hexandra*, Oct. 2017, R.G. Shivas, M.D.E. Shivas, Y.-M. Li, P. Zhao & X.-H. Qi leg., HMAS 248027. THAILAND, Kanchanaburi, on *L. hexandra*, 16 Dec. 2005, R.G. Shivas & M.D.E. Shivas leg., BRIP 47767; Chiang Mai, on *L. hexandra*, 26 Dec. 2005, R.G. Shivas & M.D.E. Shivas leg., BRIP 47768.

Notes. Vánky (1986) considered that *M. globuligerus* was a synonym of *M. bullatus* based on their similar morphologies. Phylogenetic analyses support *M. globuligerus* as a distinct species (Fig. 1), with a teleomorph specific to the pantropical grass *Leersia hexandra* (Berkeley and Broome 1880). The name *Testicularia leersiae* (Cornu 1883), described from infected *Leersia hexandra* in Algeria, is likely a heterotypic synonym of *M. globuligerus*, but this has not been checked by molecular phylogenetic analysis. The type material of *Thecaphora globuligera* was collected circa 1878 from near the Brisbane River, Queensland, Australia by the botanist F. M. Bailey (Berkeley and Broome 1880). Original material of this specimen (F.M. Bailey, No. 86) could not be found in the Australian herbaria BRI and BRIP, where most of F.M. Bailey's specimens are held. Two syntypes were located in K(M), of which K(M) 252436 ex C.E. Broome herbarium (BM) was selected as lectotype of *T. globuligera* (now *M. globuligerus*). The material in the second specimen, K(M) 252437 from the Berkeley herbarium, was scant (Dr Begoña Aguirre-Hudson pers. comm).

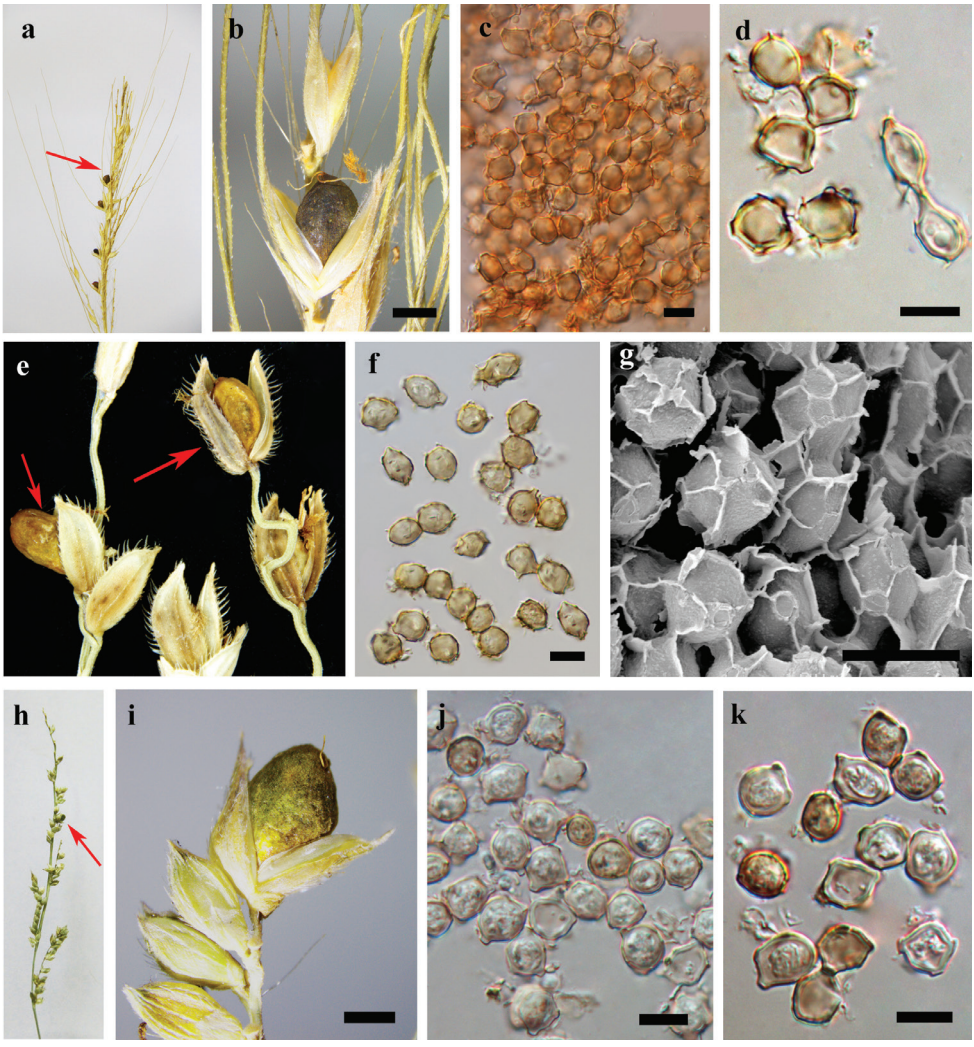

Figure 2. a–d *Moesziomyces kimberleyensis* (holotype BRIP 51843) **e–g** *Moesziomyces globuligerus* (BRIP 27384) **h–k** *Moesziomyces antarcticus* (HMAS 60130). a, b: sori. c, d, f, j, k: spores under LM. g: spores under SEM. Scale bars: 1 mm (**b, i**); 10 μm (**c, d, f, g, j, k**).

Moesziomyces kimberleyensis **Y.M. Li, L. Cai & R.G. Shivas, sp. nov.**
MycoBank: MB827986
Figure 2a–d

Type. AUSTRALIA, Western Australia, Kununurra, Mulligan's Lagoon Road, on *Echinochloa kimberleyensis*, 9 Apr. 2008, A.R. McTaggart, V.L. Challinor, A.D.W. Geering, M.D.E. Shivas & R.G. Shivas leg. (holotype: BRIP 51843).

Etymology. Named after the Kimberley region of northern Western Australia from where it was collected.

Description. Sori in some of the ovaries, often deciduous, globose to ovoid, 3–6 × 2–4 mm, green at first, later brown, smooth, ruptures irregularly to reveal a granular, dark brown mass of spore balls; columella absent. Spore balls subglobose, ovoid, elongate or irregular, 275–100 µm diam, dark brown, composed of up to several hundred spores, separated by moderate pressure. Spore globose, ovoid to irregular, slightly polyhedral, (9–) 9.5–12 (–14.5) × (8–) 8.5–9.5 (–10) µm ($\bar{x}$ = 10.5 ± 1.2 × 8.9 ± 0.7 µm, *n* = 50), subhyaline to yellowish brown, attached together by multiple narrow cylindrical protuberances about 2 µm wide and 1–2 µm long; wall with irregular meshes and wings, 0.5 µm thick, smooth.

Additional specimen examined. AUSTRALIA, Western Australia, Kununurra, Mulligan's Lagoon Road, on *E. kimberleyensis*, 7 May 2009, A.R. McTaggart, M.J. Ryley, M.D.E. Shivas & R.G. Shivas leg. (BRIP 52498).

Notes. *Moesziomyces kimberleyensis* was shown in the phylogenetic analysis to reside in a well-supported clade sister to *M. bullatus*. *Moesziomyces kimberleyensis* is only known from the teleomorph, which forms sori in flowers of *E. kimberleyensis*, and thereby differs from *M. bullatus* by host association. *Moesziomyces kimberleyensis* is only known from one location in Western Australia on *E. kimberleyensis*, which is an endemic grass in the tropical and subtropical woodlands of northern Australia.

Discussion

The phylogenetic analyses in this study supported the host specificity of the teleomorphic stage of six species of *Moesziomyces*, specifically, *M. antarcticus* on *Echinochloa crus-galli*, *M. bullatus* on *E. crus-galli* and *E. muricata*, *M. globuligerus* on *Leersia hexandra*, *M. kimberleyensis* on *E. kimberleyensis*, *M. penicillariae* on *Pennisetum glaucum*, and *M. verrucosus* on *Paspalum distichum*. The teleomorph of *M. eriocauli* may be specific to *Eriocaulon* spp., although this cannot be ascertained from the sequence data of one specimen. Specimens that have been assigned to *M. bullatus* were not well resolved and formed a number of smaller clades with varying degrees of support (Fig. 1). The *M. bullatus* clade contained several anamorphic yeasts isolated from diverse habitats (Wang et al. 2015; Kruse et al. 2017), including leaves of *Digitaria* sp., *Pennisetum* sp., and *Setaria faberii*. This shows that the anamorphs of *Moesziomyces* are widespread in the environment as saprobes.

The anamorphs of *Moesziomyces*, together with most members of the Ustilaginales, have a dimorphic lifecycle comprised of a parasitic dikaryotic phase characterized by teliospores, together with a saprobic yeast-like haploid phase (Brefeld 1883; de Bary 1884; Sampson 1939; Begerow et al. 2014). The teliospores are generally thick-walled and darkened, which protects against desiccation and UV radiation, thereby facilitating survival and long-distance dispersal (Piepenbring et al. 1998). The basidiospores

are usually thin-walled, hyaline, and survive as free-living saprobic yeasts that may occur on a vast diversity of substrates (Wang et al. 2015; Kruse et al. 2017; Tanaka et al. 2019). There is genomic evidence that some saprobic ustilaginalean yeasts, e.g. *M. antarcticus*, *Kalmanozyma brasiliensis* (= *P. brasiliensis*), *Pseudozyma hubeiensis*, and the yeast stage of *M. bullatus* (= *P. aphidis*), have retained the capacity to produce effector proteins, which hints at the possibility that undiscovered plant pathogenic stages may exist for these fungi (Sharma et al. 2018). Indeed, a teleomorph for *M. antarcticus* (=*P. antarctica*) was recently reported for the first time on *Echinochloa crus-galli* (Tanaka et al. 2019). Further collections are needed to resolve the ecological relationships and elucidate the life cycles of the ustilaginalean fungi and their hosts.

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References

Anisimova M, Gil M, Dufayard J-F, Dessimoz C, Gascuel O (2011) Survey of branch support methods demonstrates accuracy, power, and robustness of fast likelihood-based approximation schemes. Systematic Biology 60: 685–699. https://doi.org/10.1093/sysbio/syr041

Ariyawansa HA, Hawksworth DL, Hyde KD, Jones EBG, Maharachchikumbura SSN, Manamgoda DS, Thambugala KM, Udayanga D, Camporesi E, Daranagama A, Jayawardena R, Liu JK, McKenzie EHC, Phookamsak R, Shivas RG (2014) Epitypification and neotypification: guidelines with appropriate and inappropriate examples. Fungal Diversity 69: 57–91. https://doi.org/10.1007/s13225-014-0315-4

Avis TJ, Caron SJ, Boekhout T, Hamelin RC, Bélanger RR (2001) Molecular and physiological analysis of the powdery mildew antagonist *Pseudozyma flocculosa* and related fungi. Phytopathology 91: 249–254. https://doi.org/10.1094/PHYTO.2001.91.3.249

Babajide JM, Maina S, Kiawa B, Skilton R (2015) Identification of fungal isolates from steeped yam (Gbodo): Predominance of *Meyerozyma guilliermondii*. Food Science & Biotechnology 24: 1041–1047. https://doi.org/10.1007/s10068-015-0133-9

Barnett JA, Payne RW, Yarrow D (1983) Yeasts: Characteristics & Identification. Cambridge University Press, New York, 86 pp.

Begerow D, Schafer AM, Kellner R, Yurkov A, Kemler M, Oberwinkler F, Bauer R (2014) Ustilaginomycotina. In: McLaughlin DJ, Spatafora JW (Eds) The Mycota VII: Systematics & Evolution Part A. 2nd edition. Springer-Verlag, Berlin, 295–329.

Berkeley MJ, Broome CE (1880). XXII. List of fungi from Brisbane, Queensland; with descriptions of new species. Transactions of the Linnean Society London, Botany 1: 399–407. https://doi.org/10.1111/j.1095-8339.1879.tb00140.x

Boekhout T (1995) *Pseudozyma* Bandoni emend. Boekhout, a genus for yeast-like anamorphs of Ustilaginales. J Gen Appl Microbiol 41: 359–366. https://doi.org/10.2323/jgam.41.359

Brefeld O (1883) Botanische Untersuchungen uber Hefepilze. 5. Die Brandpilze I (Ustilagineen). A. Felix, Leipzig, Germany, 220 pp.

Chang MH, Kim HJ, Jahng KY, Hong SC (2008) The isolation and characterization of *Pseudozyma* sp. JCC 207, a novel producer of squalene. Applied Microbiology & Biotechnology 78: 963. https://doi.org/10.1007/s00253-008-1395-4

Cornu M (1883) Sur quelques Ustilaginees nouvelles ou peu connues. Ann Sci Nat Bot, Ser 6 15: 269–296.

Cubero OF, Crespo A, Fatehi J, Bridge PD (1999) DNA extraction and PCR amplification method suitable for fresh, herbarium-stored, lichenized, and other fungi. Plant Systematics & Evolution 216: 243–249. https://doi.org/10.1007/BF01084401

De Bary A (1884) Vergleichende Morphologie und Biologie der Pilze, Mycetozoen und Bacterien. Verlag W. Engelmann, Leipzig, Germany, 558 pp. https://doi.org/10.5962/bhl.title.42380

Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59: 307–321. https://doi.org/10.1093/sysbio/syq010

Goto S, Sugiyama J, Iizuka H (1969) A taxonomic study of Antarctic yeasts. Mycologia 61: 748–774. https://doi.org/10.2307/3757466

Han PJ, Qiu JZ, Wang QM, Bai FY (2012) *Udeniomyces kanasensis* sp. nov., a ballistoconidium-forming yeast species in the Cystofilobasidiales. Antonie van Leeuwenhoek 102: 45–51. https://doi.org/10.1007/s10482-012-9711-5

Ikeda S, Fuji SI, Sato T, Furuya H, Naito H, Ytow N, Ezura H, Minamisawa K, Fujimura T (2007) Microbial diversity in milled rice as revealed by ribosomal intergenic spacer analysis. Microbes & Environments 22: 165–174. https://doi.org/10.1264/jsme2.22.165

Irinyi L, Serena C, Garcia-Hermoso D, Arabatzis M, Desnos-Ollivier M, Vu D, Cardinali G, Arthur I, Normand A-C, Giraldo A, da Cunha KC, Sandoval-Denis M, Hendrickx M, Nishikaku AS, de Azevedo Melo AS, Merseguel KB, Khan1 A, Rocha11 JAP, Sampaio P, da Silva Briones MR, Ferreira RC, de Medeiros Muniz M, Castanon-Olivares LR, Estrada-Barcenas D, Cassagne C, Mary C, Duan SY, Kong FR, Sun AY, Zeng XY, Zhao Z, Gantois N, Bottere F, Robbertse B, Schoch D, Gams W, Ellis D, Halliday C, Chen S, Sorrell TC, Piarroux R, Colombo AL, Pais C, de Hoog S, Zancope-Oliveira RM, Taylor ML, Toriello C, de Almeida Soares CM, Delhaes L, Stubbe D, Dromer F, Ranque S, Guarro J, Cano-Lira JF, Robert V, Velegraki A, Meyer W (2015) International Society of Human & Animal Mycology (ISHAM)-ITS reference DNA barcoding database — the quality controlled standard tool for routine identification of human and animal pathogenic fungi. Medical Mycology 53: 313–317. https://doi.org/10.1093/mmy/myv008

Katoh K, Toh H (2008) Recent developments in the MAFFT multiple sequence alignment program. Briefings in bioinformatics 9 (4): 286–298. https://doi.org/10.1093/bib/bbn013

Korhola M, Hakonen R, Juuti K, Edelmann M, Kariluoto S, Nyström L, Sontag-Strohm T, Piironen V (2014) Production of folate in oat bran fermentation by yeasts isolated from barley and diverse foods. Journal of Applied Microbiology 117: 679–689. https://doi.org/10.1111/jam.12564

Kruse J, Doehlemann G, Kemen E, Thines M (2017) Asexual and sexual morphs of *Moesziomyces* revisited. IMA Fungus 8: 117–129. https://doi.org/10.5598/imafungus.2017.08.01.09

Li Y-M, Shivas RG, Cai L (2017a) Cryptic diversity in *Tranzscheliella* spp. (Ustilaginales) is driven by host switches. Scientific Reports 7: 43549. https://doi.org/10.1038/srep43549

Li Y-M, Shivas RG, McTaggart AR, Zhao P, Cai L (2017b) Ten new species of *Macalpinomyces* on *Eriachne* in northern Australia. Mycologia 109: 408–421. https://doi.org/10.1080/00275514.2017.1330026

Lin SS, Pranikoff T, Smith SF, Brandt ME, Gilbert K, Palavecino EL, Shetty AK (2008) Central venous catheter infection associated with *Pseudozyma aphidis* in a child with short gut syndrome. Journal of Medical Microbiology 57: 516–518. https://doi.org/10.1099/jmm.0.47563-0

Matheny PB, Gossmann JA, Zalar P, Kumar TA, Hibbett DS (2006) Resolving the phylogenetic position of the Wallemiomycetes: an enigmatic major lineage of Basidiomycota. Botany: 84: 1794–1805. https://doi.org/10.1139/b06-128

McTaggart AR, Shivas RG, Geering AD, Callaghan B, Vanky K, Scharaschkin T (2012) Soral synapomorphies are significant for the systematics of the *Ustilago-Sporisorium-Macalpinomyces* complex (Ustilaginaceae). Persoonia 29: 63–77. https://doi.org/10.3767/003158512X660562

Morita T, Fukuoka T, Imura T, Hirose N, Kitamoto D (2012) Isolation and screening of glycolipid biosurfactant producers from sugarcane. Bioscience, Biotechnology, and Biochemistry 76: 1788–1791. https://doi.org/10.1271/bbb.120251

Moore RT (1987) Additions to the genus *Vanrija*. Bibliotheca Mycologica 108: 167–173.

Nylander JA, Wilgenbusch JC, Warren DL, Swofford DL (2008) AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24: 581–583. https://doi.org/10.1093/bioinformatics/btm388

Okolo OM, Van Diepeningen AD, Toma B, Nnadi NE, Ayanbimpe MG, Ikenna KO, Mohammed ZS, Benle EB, Marizeth G, Fabio S, Zanyul DE, Giuseppe C, Orazio R (2015) First report of neonatal sepsis due to *Moesziomyces bullatus* in a preterm low-birth-weight infant. JMM Case Reports 2(2). https://doi.org/10.1099/jmmcr.0.000011

Piepenbring M, Hagedorn G, Oberwinkler F (1998) Spore liberation and dispersal in smut fungi. Botanica Acta 111: 444–460. https://doi.org/10.1111/j.1438-8677.1998.tb00732.x

Sampson K (1939) Life cycles of smut fungi. Transactions of the British Mycological Society 23: 1–23. https://doi.org/10.1016/S0007-1536(39)80012-2

Sharma R, Oekmen B, Doehlemann G, Thines M (2018) *Pseudozyma* saprotrophic yeasts have retained a large effector arsenal, including functional Pep1 orthologs. bioRxiv, 489690. https://doi.org/10.1101/489690

Skibbe DS, Doehlemann G, Fernandes J, Walbot V (2010) Maize tumors caused by *Ustilago maydis* require organ-specific genes in host and pathogen. Science 328: 89–92. https://doi.org/10.1126/science.1185775

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

Stoll M, Begerow D, Oberwinkler F (2005) Molecular phylogeny of *Ustilago*, *Sporisorium*, and related taxa based on combined analyses of rDNA sequences. Mycological Research 109: 342–356. https://doi.org/10.1017/S0953756204002229

Stoll M, Piepenbring M, Begerow D, Oberwinkler F (2003) Molecular phylogeny of *Ustilago* & *Sporisorium* species (Basidiomycota, Ustilaginales) based on internal transcribed spacer (ITS) sequences. Canadian Journal of Botany 81: 976–984. https://doi.org/10.1139/b03-094

Sugita T, Masako T, Natteewan P, Nanthawan M, Kaewjai M, Benjaporn T, Soem P, Pakeenee L, Toshiaki K (2003) The first isolation of ustilaginomycetous anamorphic yeasts, *Pseudozyma* species, from patients' blood and a description of two new species: *P. parantarctica* and *P. thailandica*. Microbiology & Immunology 47: 183–190. https://doi.org/10.1111/j.1348-0421.2003.tb03385.x

Tanaka E, Koitabashi M, Kitamoto H (2019) A teleomorph of the ustilaginalean yeast *Moesziomyces antarcticus* on barnyardgrass in Japan provides bioresources that degrade biodegradable plastics. Antonie van Leeuwenhoek112: 599–561. https://doi.org/10.1007/s10482-018-1190-x

Vánky K (1977) *Moesziomyces*, a new genus of Ustilaginales. Botaniska Notiser 130: 131–135.

Vánky K (1986) The genus *Moesziomyces* (Ustilaginales). Nordic Journal of Botany 6: 67–73. https://doi.org/10.1111/j.1756-1051.1986.tb00860.x

Vánky K (2012) Smut Fungi of the World. St. Paul, Minnesota: APS Press, 418–420.

Vánky K (2013) Illustrated Genera of Smut Fungi, 3rd edition. St Paul, MN, USA, APS Press, 418–420 pp.

Vega FE, Posada F, Aime MC, Peterson SW, Rehner SA (2008) Fungal endophytes in green coffee seeds. Mycosystema 27: 75–84.

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

Walker JF, Aldrich-Wolfe L, Riffel A, Barbare H, Simpson NB, Trowbridge J, Jumpponen A (2011) Diverse Helotiales associated with the roots of three species of Arctic Ericaceae provide no evidence for host specificity. New Phytologist 191: 515–527. https://doi.org/10.1111/j.1469-8137.2011.03703.x

Wang Q-M, Begerow D, Groenewald M, Liu X-Z, Theelen B, Bai F-Y, Boekhout T (2015) Multigene phylogeny and taxonomic revision of yeasts and related fungi in the Ustilaginomycotina. Studies in Mycology 81: 55–83. https://doi.org/10.1016/j.simyco.2015.10.004

Wei YH, Lee F-L, Hsu W-H, Chen S-R, Chen C-C, Wen C-Y, Lin S-J, Chu W-S, Yuan G-F, Liou G-Y (2005) *Pseudozyma antarctica* in Taiwan: a description based on morphologi-

cal, physiological and molecular characteristics. Botanical Bulletin of Academia Sinica 46: 223–229. https://doi.org/10.1016/j.aquabot.2005.02.006

Wei YH, Liou GY, Lee FL (2011) *Pseudozyma aphidis*, a new record of ustilaginomycetous anamorphic fungi in Taiwan. Fungal Science 26: 1–5.

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

Yasuda F, Yamagishi D, Izawa H, Kodama M, Otani H (2007) Fruit stain of Japanese pear caused by basidiomycetous, yeastlike fungi *Meira geulakonigii* & *Pseudozyma aphidis*. Japanese Journal of Phytopathology 73: 166–171. https://doi.org/10.3186/jjphytopath.73.166

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How useful is the current species recognition concept for the determination of true morels? Insights from the Czech Republic

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Abstract

The phylogentic diversity of the genus *Morchella* has only been sporadically studied in Central Europe. In this study, a molecular taxonomic revision of the *Morchella* species of the Czech Republic was performed using available fungarium specimens, fresh collections, and axenic cultures. Molecular phylogenetic analyses based on either ITS or five-locus (ITS, LSU, *RPB1*, *RPB2*, and *EF-1α*) sequencing and the application of principles of the genealogical concordance phylogenetic species recognition (GCPSR) have revealed the occurrence of 11 phylogenetic species in the region, but only six of them could be assigned unequivocally to the previously published phylospecies: Mel-3 (*M. semilibera*), Mel-10 (*M. importuna*), Mel-19 (*M. eohespera*), Mes-4 (*M. americana*), Mes-5 and Mes-8 (*M. esculenta*). One lineage was identified as a new phylospecies and is designated as Mel-39. Four lineages grouped together with two or more previously published phylospecies: Mel-13/26 (*M. deliciosa*), Mel-15/16 (*M. angusticeps* / *M. eximioides*), Mel-20/34 (*M. purpurascens*), and Mel-23/24/31/32 (*M. pulchella*). Our phylogenetic analyses and literature review shed light on the pitfalls of current molecular taxonomy of morels and highlight the ambiguities of present species recognition concepts. The main source of the problems seems to be rooted in the application of different methods (multigene vs single-gene sequencing, phenotypic determination) and approaches (monophyly vs paraphyly, the application or not of GCPSR, degree of differentiation between accepted species, etc.) by various authors for the delimitation of new phylospecies. Therefore, we propose five criteria for distinguishing new phylospecies in the genus *Morchella* based on molecular data, and recommend a more conservative approach in species delimitation.

Keywords
GCPSR, Mel-39, *Morchella*, multigene analysis, phylospecies, species concept

Introduction

True morels (genus *Morchella* Dill. ex Pers.: Fr.) are edible ascomycete fungi characterized by a honeycomb appearance and a spring fruiting (at least in the temperate zone), with the exception of a couple of autumnally occurring species (e.g. Masaphy et al. 2009; Matočec et al. 2014; Taşkın et al. 2015). Morels are amongst the most highly prized fungi worldwide, not only for their taste, but also for their nutritional value and medicinal properties (Tietel and Masaphy 2018). The genus is distributed worldwide. However, recent molecular phylogenetic studies suggest that the individual species exhibit high continental endemism and provincialism in the Northern Hemisphere (O'Donnell et al. 2011), and approximately 20 species have been recorded on more than one continent (Taşkın et al. 2010, 2012, 2015; O'Donnell et al. 2011; Du et al. 2012a; Pildain et al. 2014; Richard et al. 2015; Loizides et al. 2016, 2017; Yatsiuk et al. 2016; Loizides 2017). The highest species diversity of true morels is concentrated in Europe and West Asia, East Asia (mainly China), and North America (Du et al. 2015; Richard et al. 2015). One of the worldwide diversity hotspots is the Mediterranean and adjacent regions, particularly Turkey (with more than 20 species; Taşkın et al. 2010, 2012) and Cyprus (11 species; Loizides et al. 2016).

For taxonomists and field mycologists, true morels are known as a very intricate genus. Three easily distinguishable evolutionary lineages (clades) and three corresponding sections are currently recognized: (i) the basal Rufobrunnea Clade (sect. *Rufobrunnea*, or "white morels"), (ii) the Elata Clade (sect. *Distantes*, or "black morels") and (iii) the Esculenta Clade (sect. *Morchella*, or "yellow morels"). Nevertheless, the lack of discriminatory micromorphological characters and in some cases extreme macromorphological variability and/or plasticity have complicated the delimitation and characterization of species. Therefore, phenotypic characters have often been complemented with the geographic occurrence and/or ecology in recent studies, especially putative associations with particular trees or shrubs, which can sometimes be taxonomically informative (Clowez 2012; Kuo et al. 2012; Clowez et al. 2014; Loizides et al. 2015; Loizides 2017; Baroni et al. 2018). It is supposed that black morels may be either mycorrhizal or saprotrophic, some of them being obligate or facultative pyrophiles (Loizides 2017). Yellow morels are considered to be exclusively mycorrhizal (Li et al. 2013) and, thus, are probably more tightly associated with their autotrophic partners.

Current taxonomic and systematic studies on morels are mostly based on multilocus DNA sequencing (Taşkın et al. 2010, 2012; O'Donnell et al. 2011; Du et al. 2012a; Richard et al. 2015), which allows for species delimitation and phylogeny inference. By employing sequence data from four to five nuclear genomic loci (nuc 28S rDNA [LSU], RNA polymerase largest [*RPB1*] and second largest subunit [*RPB2*], translation elongation factor 1-alpha [*EF-1α*], and for particular groups also nuc rDNA ITS1-5.8S-ITS2

[ITS]) and principles of genealogical concordance phylogenetic species recognition (GCPSR; Taylor et al. 2000), O'Donnell et al. (2011) distinguished 41 phylogenetic species (phylospecies) in three major clades across the globe: 24 in the Elata Clade, 16 in the Esculenta Clade and one species in the Rufobrunnea Clade. In parallel or later, many new phylospecies were distinguished by several authors, who did not always utilize the multigene approach and/or basic phylogenetic principles (such as monophyly), not to speak of GCPSR (Taşkın et al. 2010, 2012; Du et al. 2012a; Elliott et al. 2014; Pildain et al. 2014; Loizides et al. 2016; Voitk et al. 2016a). Because binominal names can be unambiguously assigned to only a part of the phylospecies, they are usually (but not by all authors) denoted by a clade abbreviation followed by an Arabic number (Mel-1 to Mel-38 for the Elata Clade and Mes-1 to Mes-28 for the Esculenta Clade; Taşkın et al. 2010; O'Donnell et al. 2011). In total, 76 distinct (phylo)species have so far been recognized within the genus *Morchella* worldwide, including 25 species recorded in continental Europe (O'Donnell et al. 2011; Du et al. 2012b; Taşkın et al. 2012; Clowez et al. 2014, 2015; Richard et al. 2015; Yatsiuk et al. 2016; Baroni et al. 2018). However, data on the *Morchella* species diversity from Central Europe are lacking.

In the Czech Republic and former Czechoslovakia, studies on Ascomycota have a long tradition and popularity, and several *Morchella* species were even described from the Czech territory (Krombholz 1831–1834; Velenovský 1934; Smotlacha 1947, 1952; Šebek 1973). However, the available literature on morels is rather confusing and far from being clear. With the exception of a few Czech specimens included in the worldwide molecular studies (O'Donnell et al. 2011) and a single study by Ondřej et al. (2011), who employed the sequencing of the 5.8S-ITS2 region and AFLP markers to characterize the diversity of bark mulch morels, all previous studies were limited to phenotypic and ecological species identification. The following species are usually reported as occurring in the Czech Republic: *M. angusticeps* Peck, *M. conica* Pers., *M. crassipes* (Vent.) Pers., *M. elata* Fr., *M. esculenta* (L.) Pers., *M. pragensis* Smotl., *M. semilibera* DC., and *M. vulgaris* (Pers.) Gray (Holec et al. 2012; Mikšík 2015). However, *M. angusticeps* is currently used only for the probably endemic American species, *M. conica* is considered illegitimate (Richard et al. 2015), collections formerly treated as *M. crassipes* were recently determined to be several Esculenta Clade species on the basis of sequencing data (Du et al. 2012b; Richard et al. 2015), the taxonomical status of *M. elata* is still unresolved (Richard et al. 2015), and *M. pragensis* is a rather mysterious species that also remains phylogenetically and taxonomically unresolved (see below).

We therefore performed a detailed molecular taxonomic revision of true morels in the Czech Republic on the basis of recent collections and available fungarium specimens within the framework of phylogenetic species recognition as initiated by O'Donnell et al. (2011) and followed by a number of other authors. However, our analysis has failed to discriminate between several published phylospecies, questioning the accuracy and consistency of currently applied species recognition methods. As a result, a revised phylogenetic species concept is proposed and suggestions regarding the criteria for the recognition of morel species are presented.

Materials and methods

Sampling and culturing

Our sampling aimed at covering the territory of the Czech Republic, and to a lesser extent adjacent parts of Slovakia, using two sources of material. First, for cultivation of axenic cultures and subsequent molecular analysis, 66 fresh specimens of *Morchella* that originated from our own recent collections or were provided by collaborating mycologists in 2008–2018, were used. Fruiting bodies from each micro-locality (unless significantly different in appearance) were considered as a single specimen and a single fruiting body was usually used for cultivation and/or analysis. However, at nine localities two to twelve mulch morels ascomata were analyzed to assess species diversity within the mulch beds (Suppl. material 1, Table S1). Cultures were derived either from the spore prints or from the inner tissues of ascomata transferred into Petri dishes with a malt extract glucose agar medium (MEGA; 10 g/L malt extract, 5 g/L glucose, 15 g/L agar) supplemented with chloramphenicol (100 mg/L). Cultivation was carried out in the dark at 18–20 °C. Axenic cultures of the obtained strains maintained on a rye grain substrate are available as a part of the Collection of Edible & Medicinal Macromycetes (CEMM) maintained within the framework of The Czech National Programme on Conservation & Utilization of Microbial Genetic Resources Important for Agriculture (http://www.vurv.cz/cspp/mikroorganismy/Edible and Medicinal macromycetes.html) at the Crop Research Institute (https://www.vurv.cz). For DNA extraction, the mycelium or sclerotia of individual strains were sampled from jars with the rye grain spawn prepared by the inoculation of pre-soaked and sterilized rye grains with pieces of agar covered with morel mycelium. From 16 specimens (including five samples from Slovakia) in which the derivation of axenic cultures was unsuccessful (marked as n.m.d. in Suppl. material 2, Table S2) DNA was extracted directly from fresh-frozen or dried pieces of ascomata.

Secondly, for the DNA analysis only, 377 morel specimens in total were obtained from the selected Czech public herbaria and one private fungarium, of which 203 were successfully analyzed (abbreviations according to Thiers 2018): BRNM: 73 specimens, CB: 50 specimens, CHOM: 23 specimens, HR: 24 specimens, LIT: 12 specimens, PL: six specimens, PRC: three specimens, and Vavřinec Klener's private fungarium: 12 specimens). The specimens were collected between the years 1950 and 2018. For details see Suppl. material 2, Table S2.

Molecular analysis

Total genomic DNA was extracted from ca < 10 mg of dry fruiting body or an equivalent amount of the fresh mycelium culture or sclerotia by the CTAB method (Doyle and Doyle 1987). The ITS locus was amplified and sequenced in all the studied accessions using the ITS1F (Gardes and Bruns 1993) and ITS4 primers (White et al.

1990) or, in the case of old specimens with fragmented DNA, either with ITS1F and ITS2, or ITS3 and ITS4 (White et al. 1990). Subsequently, at least two representative accessions per detected phylospecies (with respect to the detected variation in ITS) and approximately six accessions within species-rich complexes were selected for further sequencing. *RPB1* was amplified and sequenced with the gRPB1-A and fRPB1-C primers (Matheny et al. 2002), *RPB2* with the fRPB2-7cF (Liu et al. 1999) and RPB2-3053r primers (Reeb et al. 2004), *EF-1α* with the EF-526F and EF1567R primers (Rehner and Buckley 2005), and domains D1 and D2 of 28S rDNA (LSU) with the NL1 and NL4 primers (O'Donnell 1993). All the PCRs were performed in 20-μL reaction mixtures with Kapa polymerase (Kapa Biosystems, Massachusetts, USA) and a touchdown protocol with an annealing temperature of 61–56 °C in the first six cycles and 56 °C in the following 37 cycles. The PCR products were purified by precipitation with polyethylene glycol (10% PEG 6000 and 1.25 M NaCl in the precipitation mixture) and sequenced by the Sanger method at Macrogen Europe (The Netherlands).

Data analysis

Sequences were edited and aligned in Geneious 7.1.7. (Biomatters, New Zealand) using the MAFFT plugin and deposited in NCBI GenBank under the accession numbers MH982584–MH983000. Alleles of the ITS locus were distinguished on the basis of single nucleotide polymorphisms and compared to the publicly available sequences. Bayesian phylogeny inference in MrBayes 3.2.4 (Ronquist et al. 2012) with 10^7 generations, sampling every 1000th tree, in two independent runs, each with six chains, 50% burn-in and a temp parameter of 0.01 was used for the preliminary assignment to the published phylogenetic species. As a reference database, previously published sequence data from one to two accessions per phylospecies were selected in order to cover the total species richness and the widest possible intraspecific variation. Multilocus sequences from the selected accessions were concatenated and ambiguously aligned parts (ITS1 in the Esculenta Clade dataset) and ends of the sequences with many missing data were discarded. Bayesian phylogeny inference for concatenated data was computed in MrBayes with 20 million generations, sampling every 1000th tree, in two independent runs, each with four chains, and the first 10 million generations (50%) were excluded as burn-in. A substitution model for each locus was determined in Partitionfinder 2.1.1 (Lanfear et al. 2017) using the corrected AIC (AICc) and a greedy search, and partitions were subsequently set in MrBayes according to the loci.

Results

Out of a total of 377 fungarium specimens of different ages (mostly < 50 years), we were able to obtain at least a partial informative ITS sequence for 211 specimens,

of which eight specimens from Mel-19 (*M. eohespera* Beug, Voitk & O'Donnell), Mel-20/34 (*M. purpurascens* (Boud.) Jacquet), or Mel-23/24/31/32 (*M. pulchella* Clowez & F. Petit) could not be determined because of the insufficient sequence length and therefore they were excluded from the analyses. The success rate only partly corresponded to the age, as even exsiccata that were several decades old contained relatively well-preserved DNA and many specimens that were one or a few years old had very degraded DNA, particularly if the fruiting body had been attacked by larvae or dried slowly (data not shown). ITS seems to be insufficient for distinguishing between Mel-19 and Mel-20/34, which differ in a single SNP in ITS2 closely adjacent to 5.8S rDNA. This SNP may be uninformative on a wider geographic scale, as the Mel-19 (*M. eohespera*) variant was observed in some published sequences of Mel-20 (*M. purpurascens*). However, because this SNP was stable in our data set, we used it for the determination of specimens analyzed solely by ITS. Though, our method of preliminary identification based on ITS proved to be successful and robust for most fresh and fungarium specimens (Suppl. material 3, Fig. S1).

3766 bp and 3464 bp alignments were constructed from 102 and 39 specimens for the Elata Clade and the Esculenta Clade, respectively, including a representative set of the published sequences (Suppl. material 4, supplementary data). According to our expectations, ITS revealed the highest variability (ca 65% and 67% of variable sites in the Elata and the Esculenta Clade, respectively; see also Suppl. material 5, Table S3) and therefore proved to be the most suitable for screening of the phylogenetic diversity of the whole sample set. The least polymorphic locus was LSU (11% and 7%), *EF-1α* exhibited 27% and 18%, *RPB1* 25% and 12%, and *RPB2* 21% and 12% of polymorphic sites in the Elata and the Esculenta Clade, respectively.

Bayesian analysis of the multilocus data placed all of the Czech specimens into a highly supported branch together with other specimens that were analyzed, but only six of the lineages contained a single published species and could be determined unambiguously: Mel-3 (*M. semilibera*), Mel-10 (*M. importuna* M. Kuo, O'Donnell & T.J. Volk), Mel-19 (*M. eohespera*), Mes-4 (*M. americana* Clowez & Matherly), Mes-5 and Mes-8 (*M. esculenta*) (Figs 1, 2). Mel-19 was separated from Mel-20/34 (*M. purpurascens*) in *EF-1α* and multilocus analysis, yet rather weakly diverged (Suppl. material 5: Table S3), and appeared polyphyletic at *RPB2* (Suppl. material 3, Fig. S1). Four specimens that were used for multigene analysis and six specimens analyzed for ITS only formed a basal lineage to Mel-15 (*M. angusticeps*) and Mel-16 (*M. eximioides* Jacquet.). Although this lineage is highly supported in all analyses (Fig. 1, Suppl. material 3, Fig. S1), its genetic distance from Mel-15 and Mel-16 is only 0–9 SNP's at every locus (Suppl. material 5, Table S3). Six specimens (of which three were included in the multigene analysis) formed a well-separated and highly supported clade sister to Mel-10 (*M. importuna*) in the multigene analysis and all the single-gene analyses (posterior probability 1.0, or 0.98 for ITS; Fig. 1, Suppl. material 3, Fig. S1). The genetic distance of this clade from Mel-10 was 12–21 SNP's at most

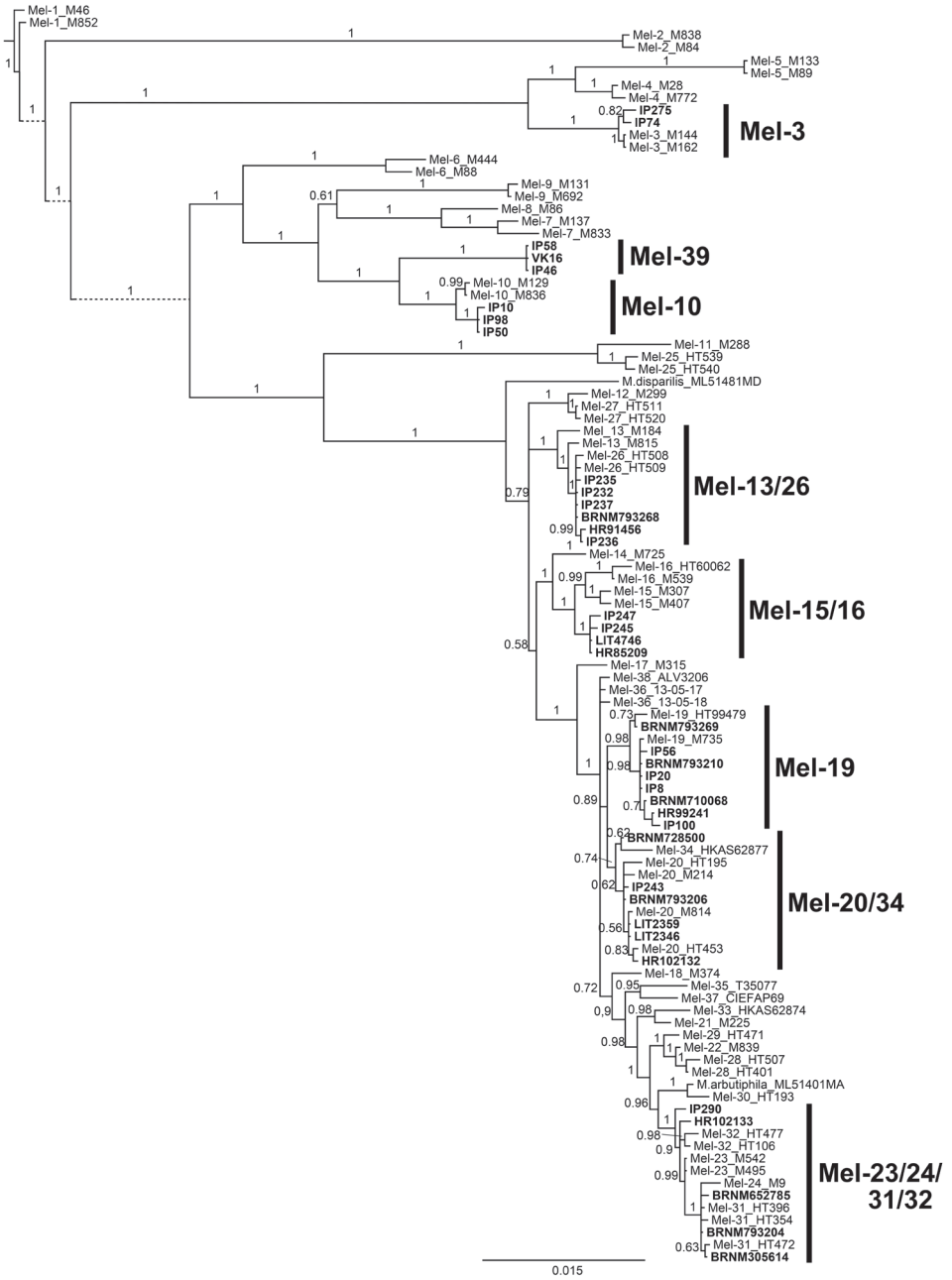

Figure 1. Bayesian phylogeny inference tree based on five-gene concatenated alignment from selected accessions of the Elata Clade. Posterior probabilities (PP) are shown above branches, splits with PP < 50% were collapsed.

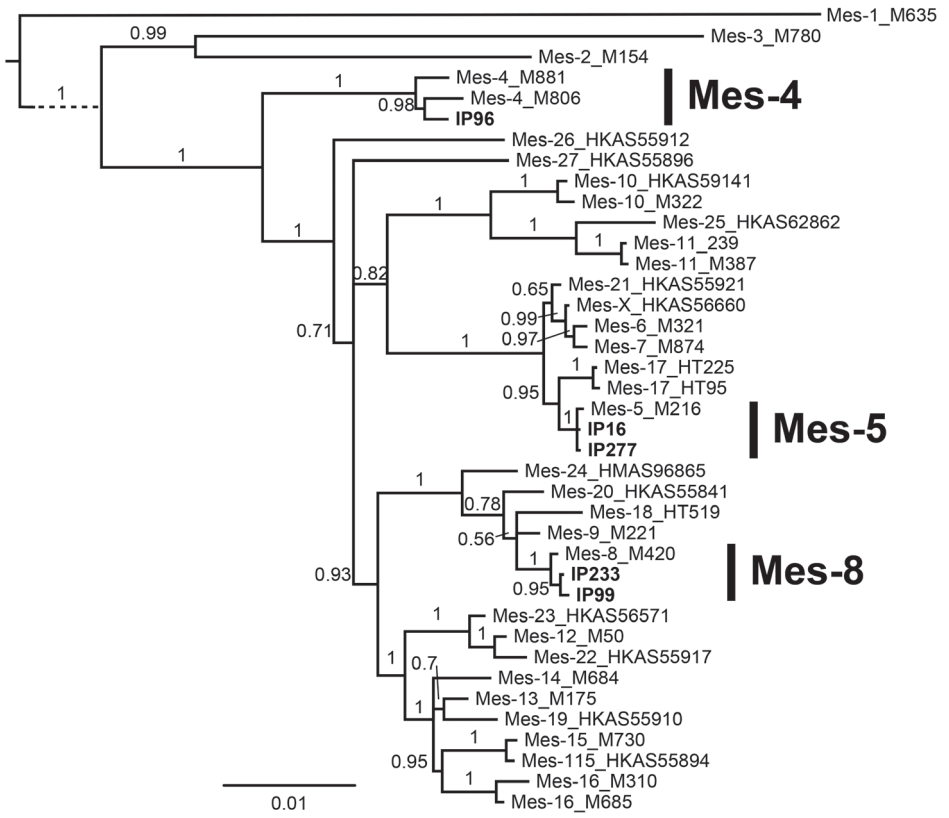

Figure 2. Bayesian phylogeny inference tree based on five-gene concatenated alignment from selected accessions of the Esculenta Clade. Posterior probabilities (PP) are shown above branches, splits with PP < 50% were collapsed.

loci and no variation was detected within the clade (Suppl. material 5, Table S3). These six specimens were designated as a new phylospecies, annotated "Mel-39". Other specimens that were studied were intermixed within the clusters of two or four previously recognized phylospecies and could not be assigned unambiguously to a single one of them because of the high intra-specific variation (autapomorphies) and the lack of shared polymorphism (synapomorphies).

Geographic mapping of the analyzed accessions did not reveal any clear patterns in the phylospecies distribution (Figs 3, 4). Every species was distributed in all the lowland to lower montane areas of the Czech Republic; only Mes-5 was not detected in the southern half of Bohemia and northern parts of Moravia (Fig. 4), and Mel-19 (*M. eohespera*) is underrepresented in the north-western half of Bohemia (Fig. 3), which may, however, only reflect the density of sampling in these regions.

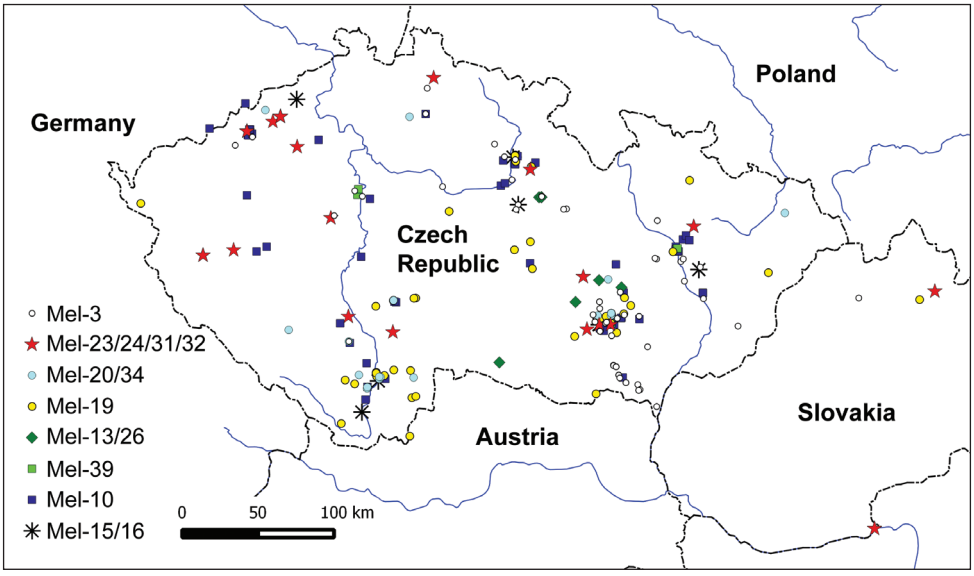

Figure 3. Distribution of the Elata Clade phylospecies in the Czech Republic (and Slovakia) based on identification by ITS or multi-gene sequencing, or phenotypic identification (in the case of Mel-3). For details see Supplementary Table 2.

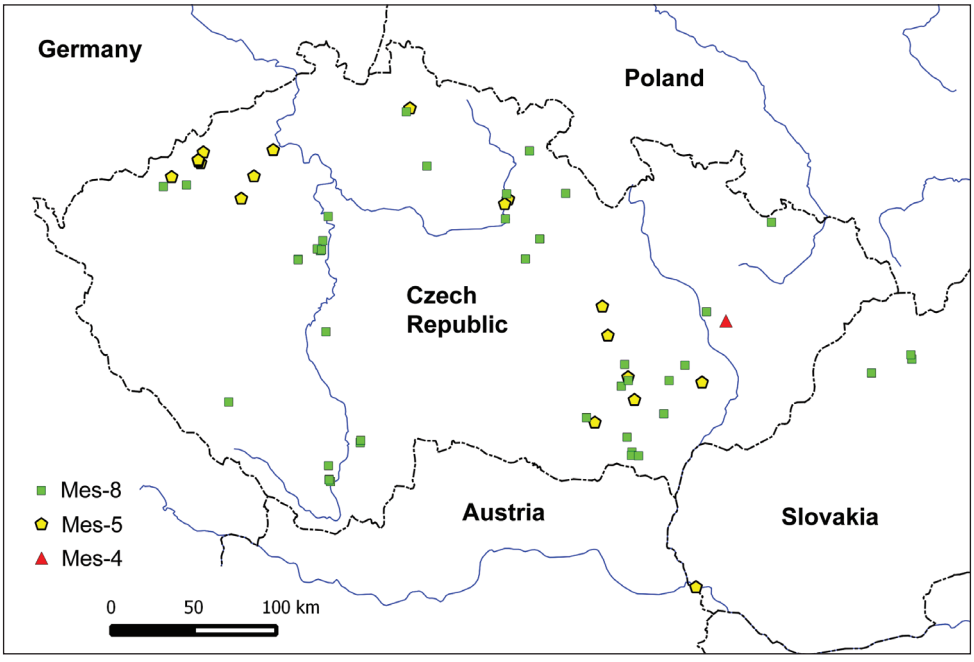

Figure 4. Distribution of the Esculenta Clade phylospecies in the Czech Republic (and Slovakia) based on identification by ITS or multi-gene sequencing. For details see Supplementary Table 2.

Discussion

On the basis of the five-gene sequencing of 41 collections and ITS sequencing of a further 228 collections, we distinguished 11 phylogenetic lineages occurring in the Czech Republic. Only six lineages clustered tightly to a single one of the published phylospecies, whereas four lineages grouped together with two or more previously published species. One lineage was unique, without close affinity to any known phylospecies.

The concept of the phylogenetic species recognition in the genus *Morchella* was developed by O'Donnell et al. (2011) on the basis of multi-gene sequencing of global set of *Morchella* specimens using the principles of genealogical concordance phylogenetic species recognition (GCPSR; Taylor et al. 2000). *Morchella rufobrunnea* Guzmán & F. Tapia, Mel-1 to Mel-24 and Mes-1 to Mes-16 were distinguished first. In parallel, *M. anatolica* Işıloğlu, Spooner, Allı & Solak (the Rufobrunnea Clade), Mes-17, Mes-18 and Mel-25 to Mel-32 were distinguished from Turkey (Işiloğlu et al. 2010; Taşkın et al. 2010, 2012). In China, Du et al. (2012a) recognized eleven new phylospecies (Mel-33 and Mel-34 and Mes-19 to Mes-27). Mel-35 was designated to the Australian species *M. australiana* T.F. Elliott, Bougher, O'Donnell & Trappe (Elliott et al. 2014), Mel-36 (named as *M. laurentiniana* Voitk, Burzynski, O'Donnell) was described from Canada (Voitk et al. 2016a), Mel-37 from Argentina (Pildain et al. 2014), and four new species (Mes-28, Mel-38, *M. disparilis* Loizides & P.-A. Moreau and *M. arbutiphila* Loizides, Bellanger & P.-A. Moreau, both without phylospecies designation) were described from Cyprus (Loizides et al. 2016). From Spain, *M. castaneae* L. Romero & Clowez and *M. palazonii* Clowez & L. Romero (both without a phylospecies designation) were described by Clowez (2012) and Clowez et al. (2015) based on morphology and ITS sequencing. Most recently, *M. kaibabensis* Beug, T.A. Clem. & T.J. Baroni and *M. peruviana* S.A. Cantrell, Lodge, T.J. Baroni & O'Donnell were described from Arizona and Peru (Baroni et al. 2018).

Two independent studies with descriptions of several new species were published in 2012 (Clowez 2012; Kuo et al. 2012; the former not reflecting previous molecular analyses, the latter with the aim of assigning Latin binomials to the unnamed phylospecies). However, the names proposed by Clowez (2012) have priority over those published for the same taxa by Kuo et al. (2012). A unified taxonomy for the known European and North American species was therefore later proposed by Richard et al. (2015), who performed a nomenclatorial revision, typification of some ambiguous names and synonymization of the names published by Clowez (2012) and Kuo et al. (2012). Of the phylospecies originally identified by O'Donnell et al. (2011), binominal names have so far been assigned to 18 Mel- species and nine Mes- species (Richard et al. 2015; Voitk et al. 2016a; Baroni et al 2018). To the phylospecies that were distinguished from Turkey by Taşkın et al. (2010, 2012), scientific names were assigned by Clowez et al. (2014), Richard et al. (2015), and Taşkın et al. (2016). Additionally, Richard et al. (2015) synonymized several different taxa described by Clowez (2012) under the priority name *M. vulgaris*, which corresponds to Mes-17. However,

on the basis of seven nucleotide changes in ITS (mainly ITS1) and morphological and ecological observations, Loizides et al. (2016) separated Mes-17 corresponding to *M. dunensis* (Castañera, J.L. Alonso & G. Moreno) Clowez (incl. *M. andalusiae* Clowez & L. Romero) as a separate sister species to *M. vulgaris*, which was left without a phylospecies designation.

To summarize, 76 (phylo)species have so far been recognized in the genus worldwide. However, taxonomic concepts differ greatly in terms of both methods (multigene sequencing, single-gene sequencing or phenotypic observation) and approaches (monophyly vs paraphyly, GCPSR or not, degree of genetic/phenotypic differentiation between accepted species, minimal number of collections, application of binominals or phylospecies designations, etc.). Together with the relatively high number of recent publications on morels, this conceptual diversity has led to much confusion and many contradictions. Therefore, on the basis of our data and a literature review, we discuss here some of the conceptual problems with *Morchella* species recognition and suggest basic rules that may prevent the introduction of unnecessary new taxa in the future.

Suggestions for a sustainable morel taxonomy

The phylospecies concept of O'Donnell et al. (2011) was based explicitly on two main criteria. First, species were recognized if they were resolved as reciprocally monophyletic in at least one of the individual (i.e., single-locus) phylogenies and in the combined dataset (let us call this the "criterion of monophyly"), and second, if their genealogical exclusivity was not contradicted by analyses of any individual data partition ("genealogical criterion"). Understandably, none of these criteria can be fulfilled in species with a single collection. Therefore, the recognition of such species was based on a third criterion, i.e., genetic divergence from their sisters ("criterion of distinctness"). The three criteria are fully legitimate and intuitive. Nevertheless, whether they are met or not is always dependent on a particular dataset. After the addition of more collections (e.g., from different geographic regions), the criteria may cease to be fulfilled and the species thus become "illegitimate". This seems to be the case of Mel-23 and Mel-24, which appeared to be reciprocally monophyletic and well differentiated originally, but several Czech specimens, as well as specimens previously determined as Mel-31 and Mel-32, share apomorphies with both of the lineages at each of the analyzed loci and, furthermore, exhibit several unique mutations. Their phylogenetic position therefore disrupts the clear distinctness of Mel-23 and Mel-24. At the same time, the Czech specimens do not form a separate lineage(s) and do not fulfill any of the three criteria (Fig. 1, Suppl. material 3, Fig. S1, Suppl. material 5, Table S3) and cannot be distinguished as separate phylospecies.

The criterion of monophyly and the genealogical criterion were not employed in many of the recent studies and were violated either consciously or because only one

specimen was available. This is the case of, e.g., Mel-26 (*M. deliciosa* Fr.), Mel-31 (*M. pulchella*; see below), or Mel-33. Similarly, the criterion of distinctness seems to be considered only rarely and little attention seems to be paid to the genetic differentiation among newly distinguished phylospecies and the related ones in many studies. For instance, Mel-34 was distinguished as a separate lineage sister to Mel-20 (*M. purpurascens*) on the basis of a single specimen. Its genotype was similar or identical to Mel-20 at all the loci except *RPB1*, which provided an almost identical sequence to Mel-23/24/31/32 (*M. pulchella*; Suppl. material 3, Fig. S1, Suppl. material 5, Table S3, see also below). This discrepancy may represent a true biological signal, e.g. incomplete lineage sorting (Leliaert et al. 2014), but divergence between Mel-20 and Mel-23/24/31/32 is substantial (Fig. 1) and such an explanation is therefore questionable. This case illustrates how important it is to justify the distinction of new phylospecies by (i) the number of genetic differences at each locus and (ii) the inclusion of more than one sample in the analyses, which enables the effective exposure of base miscalling, erroneous alignment, PCR mutations, contaminations and other technical and processing errors that pose the high risk of introducing artifacts as new species (Thines et al. 2018). Beside the above-mentioned criteria, we therefore suggest that every newly distinguished phylospecies should be based on several (optimally three or more) different specimens ("criterion of minimal sampling"), and that it should differ from closely related species at most of the highly variable loci (in the case of morels, i.e., *EF-1α*, ITS, *RPB1*, *RPB2*) by at least one, but preferably more SNP's that would be shared by all the individuals that are studied ("criterion of polygenic differentiation"). Although the latter criterion may be rather pragmatic and not fully reflect theoretical evolutionary processes at different loci, our analysis of average genetic differentiation shows that the closely related phylospecies differ at every locus by > 2 (but usually > 10) SNP's (Suppl. matirial 5, Table S3). The criterion is therefore supported by empirical evidence and could also be useful for the potential recognition of new species in the future.

It is important to note that the proposed criteria should not be viewed as definite and insurmountable limits for taxonomy, but rather as a recommendation for cautiousness in introduction of new (phylo)species. Incomplete lineage sorting, hybridization, evolutionary stasis, and other factors may affect phylogenetic signal at each locus, particularly in recently diverged lineages (Mailund et al. 2014). Such closely related lineages may have already achieved reproductive isolation and segregated in distinct ecological or biogeographical compartments, they may even have acquired some diagnostic morphological traits, but DNA phylogenies may fail to assign them to (reciprocally) monophyletic clades (reviewed by Leliaert et al. 2014). Final taxonomic decision may therefore be influenced by stronger lines of evidence than (weak) patterns in DNA sequence variation. Nevertheless, as stressed by Carstens et al. (2013: 4369), taxonomic inferences should be conservative, "for in most contexts it is better to fail to delimit species than it is to falsely delimit entities that do not represent actual evolutionary lineages".

Morchella diversity in the Czech Republic with notes on taxonomy, nomenclature and ecology

According to our analyses, the phylogenetic lineages of morels occurring in the Czech Republic are as follows (arranged by the phylospecies designations):

***Mel-3* (*M. semilibera* DC.; Fig. 5A).** Seventeen *Morchella* cultures or exsiccated specimens (one originating from Slovakia) were proven to be Mel-3, corresponding to *Morchella semilibera* (syn. *Mitrophora semilibera* (DC.) Lév., *Morchella gigas* (Batsch) Pers. or *M. hybrida* Pers.), which is in accordance with the previous phenotypic determination of the specimens. Ascomata were collected from mid-April to mid-May. As morphological features seemed to be highly reliable for the delimitation of this species (there is only the possibility of confusion with *Verpa bohemica* (Krombh.) J. Schröt.), fungarium specimens were mostly not used for DNA analyses. However, collection data for 50 *M. semilibera* fungarium specimens were included on a map (Fig. 3) to demonstrate the species distribution in the Czech Republic. *Morchella semilibera* is a widely distributed Eurasian species that had previously been recorded not only from the Czech Republic, but its occurrence was also confirmed molecularly from France, Germany, Italy, the Netherlands, Spain, Sweden, Turkey, and India (Taşkın et al. 2010, 2012; Kanwal et al. 2011; O'Donnell et al. 2011; Clowez 2012; Du et al. 2012b; Richard et al. 2015).

The Czech collections originated mostly from (semi-)natural habitats such as deciduous or, less frequently, mixed forests and floodplain forests (note that most of what are termed forests in Central Europe are semi-natural or completely artificial), groves, old fruit orchards, shrubs, or rocks. Only rarely was *M. semilibera* found in urban areas, e.g., in gardens, town parks, or also in ornamental beds, but we have no information as to whether there was bark mulch or not. The species appeared most frequently in association with *Fraxinus* spp., *Carpinus betulus*, *Quercus* sp., *Acer* spp., *Prunus* spp. (especially *P. spinosa*), and cherry trees. According to the literature, *M. semilibera* often grows under *Fraxinus excelsior* (Clowez 2012; Richard et al. 2015), and it was also found under *Malus sylvestris*, *Castanea* sp., and *Populus* sp. (Taşkın et al. 2010, 2012; Richard et al. 2015). Judging by our recent collections and the representation of the species in herbaria, *M. semilibera* seems to be one of the most common *Morchella* species and is widespread in lowland areas of the Czech Republic (Fig. 3). However, in the national red list of macromycetes it is treated as Near-Threatened because of the potential overexploitation of natural populations by mushroom gatherers (Antonín 2006).

***Mel-10* (*M. importuna* M. Kuo, O'Donnell & T.J. Volk; Fig. 5B).** Mel-10 is a newly recognized species for the Czech Republic, although our results show that it has already been a part of the Czech mycobiota for decades. In total, 70 *Morchella* cultures or exsiccated specimens (the oldest one was collected in 1950) previously morphologically identified mostly as *M. pragensis* (18 specimens), *M. conica* (12 specimens), or

Figure 5. Examples of the fruiting bodies of *Morchella* phylospecies in the Czech Republic. **A1–2** Mel-3 (*M. semilibera*; A1. accession number VK13, A2. IP229) **B** Mel-10 (*M. importuna*; IP26) **C** Mel-13/26 (HR86151) **D** Mel-15/16 (IP245 and IP247) **E** Mel-19 (*M. eohespera*; HR99241) **F** Mel-20/34 (HR102132) **G** Mel-23/24/31/32 (HR102133) **H** Mel-39 (VK17) **I** Mes-4 (*M. americana*; IP297) **J** Mes-5 (IP350, IP351) **K** Mes-8 (*M. esculenta*; IP341) Photographers: Vavřinec Klener (**A1, H, K**); Irena Petrželová (**A2, B, D, I, J**); Jan Kramoliš (**F, G**); Dušan Bureš (**C, E**).

M. elata (nine specimens), or designated just as "black mulch morel" (18 specimens) were determined as Mel-10. The ascomata of the Czech collections were extremely variable in shape. The specimens that were examined were collected from mid-April

to mid-May. It is noteworthy that *M. importuna* is probably a later synonym for several validly published names, e.g., *M. elata*, *M. hortensis* Boud. or *M. vaporaria* Brond. However, the interpretation of these names is unresolved and the name *M. importuna* was therefore provisionally retained for Mel-10 by Richard et al. (2015). Among Czech mycologists and morel hunters, the species is often treated as *M. pragensis*. This name was published twice (Smotlacha 1947, 1952) on the basis of collections from the surroundings of Prague, firstly without the Latin diagnosis, secondly without the holotype being indicated (which, nevertheless, was not necessary before 1958) and as two forms without the nominate form being specified. The nomenclatural errors were later corrected and the neotype was assigned by Moravec (1970). However, sequencing of the neotype has not been successful yet; its identity needs to be determined in future studies. Despite the formal errors, *M. pragensis* became widely known among the public and the name has been commonly used for various collections from anthropogenic habitats, particularly ruderal places such as waste dumps and debris after demolition, but also gardens, yards, ornamental beds with bark mulch, etc.

Morchella importuna was described from the USA in 2012 (Kuo et al. 2012) and it was hypothesized as originating in western North America, from where it has spread in association with horticulture and silviculture (Taşkın et al. 2010), but it has also been reported from Germany, Poland, Finland, France, Switzerland, Spain, Turkey, Cyprus, Israel, Canada, and China (Taşkın et al. 2010, 2012; Du et al. 2012a, 2012b; Richard et al. 2015; Loizides et al. 2016). The species appears to be a saprotroph (Mann and Mann 2014), and therefore it can be cultivated artificially (Du et al. 2015). *Morchella importuna* is also known as a facultative post-fire species (Clowez 2012; Du et al. 2012a; Loizides 2017), and recent research has shown that it can even be grown on fire-treated fields (Li et al. 2017). A morphological description of *M. importuna* was given by Clowez (2012; under the name *M. vaporaria*) and Kuo et al. (2012). It may be difficult to distinguish *M. importuna* from other species in the Elata Clade morphologically. However, the best clue for its identification may be its occurrence in urban habitats in combination with regularly laddered, vertically oriented pits and ridges on ascomata (Kuo et al. 2012; Mann and Mann 2014). Both in its presumed native distribution area and in the Czech Republic it occurs in various urban habitats, particularly woodchip or mulch beds (Kuo et al. 2012); therefore, it is sometimes called the "mulch morel" (Mann and Mann 2014). But it is also frequently found in the yards of houses, in masonry, dumps of rubble, sand, wood or bark, and one Czech specimen was collected in an old fire pit. Only occasionally was this species found in semi-natural habitats such as forests, along forest paths, or in meadows or town parks.

***Mel-13/26* (*M. deliciosa* Fr.; Fig. 5C).** Eight *Morchella* cultures or fungarium specimens phenotypically identified mostly as *M. conica* (four specimens) clustered with both Mel-13 (no Latin binomial) and Mel-26 (*M. deliciosa*). While the former species was distinguished by O'Donnell et al. (2011), the latter one was delimited by Taşkın et al. (2010), but without Mel-13 being used in their analysis. It was only later that

Taşkın et al. (2012) analyzed both species together and revealed the paraphyly of Mel-13 because of the exclusion of Mel-26 from the clade. The separation of Mel-26 can therefore be considered inappropriate and both species were combined by Du et al. (2015) as Mel-13/26 (*M. deliciosa* being the only known Latin binomial). Our results confirm this treatment unambiguously. Mel-13 has so far only been reported from Asia (China, India, and Turkey; O'Donnell et al. 2011; Du et al. 2012a; Taşkın et al. 2012; Richard et al. 2015), while Mel-26 has, in addition to Turkey (Taşkın et al. 2010, 2012), also been reported from some European countries (France, Poland, and Sweden; Clowez 2012, as several varieties of *M. conica*; Taşkın et al. 2012; Richard et al. 2015; Baran and Boroń 2017). The Czech specimens that were examined were collected in mid-April in mixed forests, mainly under *Fraxinus excelsior*, *Picea* sp., and *Pinus* sp. Other nearby trees or shrubs were *Quercus* sp., *Larix decidua*, *Fagus sylvatica*, and *Sambucus nigra*. Other authors (Taşkın et al. 2010, 2012; Clowez 2012) mainly reported associations with conifers (*L. decidua*, *Picea abies*, *Pinus* sp.), while Baran and Boroń (2017) also observed *Abies alba*, *Tilia platyphyllos*, *Acer pseudoplatanus*, and *Euonymus verrucosa* at the localities where *M. deliciosa* was collected.

***Mel-15/16* (*M. angusticeps* Peck / *M. eximioides* Jacquet.; Fig. 5D).** Nine *Morchella* cultures or fungarium specimens previously morphologically recognized mostly as *M. conica* (six specimens) clustered with Mel-15 (*M. angusticeps*) or Mel-16 (*M. eximioides*). Both species were originally delimited by O'Donnell et al. (2011) on the basis of a sample set of seven eastern North American (Mel-15) and four Scandinavian specimens (Mel-16), which exhibited stable polymorphism (i.e., synapomorphies) at *EF-1α* and *RPB2*, but not at LSU and *RPB1*. Nevertheless, the Czech samples share synapomorphies with both of the species and form a basal lineage to them (Fig. 1). The Czech Mel-15/16 specimens fulfill the criterion of monophyly and the genealogical criterion (Suppl. material 3, Fig. S1) and thus could be distinguished as a separate phylospecies according to O'Donnell et al. (2011). However, the three lineages are distinguishable only by *EF-1α* and *RPB2* (Suppl. material 3, Fig. S1, Suppl. material 5, Table S3), and the total detected genetic distance of the Czech specimens from Mel-15 and Mel-16 is eight and 15 SNP's, respectively. Moreover, variation within branches is higher than that among branches at some loci and the split into three lineages therefore may be caused, hypothetically, by geographic variation and limited sampling. Consequently, we prefer not to assign the Czech lineage as a new phylospecies before additional (e.g., phenotypic) data prove its distinctness.

It is generally supposed that Mel-15 is endemic to eastern North America (O'Donnell et al. 2011; Kuo et al. 2012; Richard et al. 2015), while Mel-16 has been reported from Northern Europe and China (O'Donnell et al. 2011; Du et al. 2012a). This early fruiting species (recent collections were made in approximately mid-April) appeared in both (semi-)natural and anthropogenic habitats. Five specimens were found in deciduous forests, a town park or an old orchard. Two other specimens were collected in the vicinity of paper mills on paper or wood waste. Two specimens (collected in different years) were found by a sedimentation basin of a heating plant. The

species grew together with various deciduous trees and shrubs, including *Prunus* spp., *Fraxinus* sp., *Acer* sp., *Populus* sp., *Crataegus* sp., *Cornus sanguinea*, *Betula* sp., or *Salix* sp. Du et al. (2012a) also reported the association of Mel-16 with *Picea* sp.

***Mel-19* (*M. eohespera* Beug, Voitk & O'Donnell; Fig. 5E).** Forty-three *Morchella* cultures or fungarium specimens morphologically identified mostly as *M. conica* (24 specimens), *M. elata* (seven specimens), or *M. pragensis* (six specimens) were determined as Mel-19 (*M. eohespera*; an older name is possibly *M. norvegiensis* Jacquet.; see Voitk et al. 2016b). Although Mel-19 was previously recorded from the Netherlands, Sweden, Switzerland, China, and the USA (O'Donnell et al. 2011; Du et al. 2012a; Taşkın et al. 2012; Beug and O'Donnell 2014; Richard et al. 2015), it was only after the collections from Canada that the Latin binominal was given to this phylogenetic species (Beug and O'Donnell 2014; Voitk et al. 2016a). A morphological description of this species is available in Voitk et al. (2016a). In the Czech Republic, *M. eohespera* appeared from mid-April to mid-May, but the most recent collections were mostly made around mid-April. The specimens that were examined were collected in a variety of habitats including different types of forests (where they often occurred along roads, on deposits of wood on wood waste, on hillsides, and in river or creek valleys), in gardens, old yards, rubble sites, railway stations, and other urban habitats, sandstone quarries, a brick factory, a meadow, and also on bark mulch. Voitk et al. (2016a) also reported the occurrence of this species both in natural habitats and at sites significantly affected by human activities. The Czech collections of *M. eohespera* were frequently found together with *Populus* spp. (mostly *P. tremula*), *Betula pendula*, *Picea abies*, or *Pinus sylvestris*, while other nearby trees were *Salix* spp., *Fagus sylvatica*, *Fraxinus excelsior*, *Quercus* sp., and *Malus domestica*. The species can also be found in association with other trees such as *Alnus* sp., *Corylus* sp., or *Abies* sp. (Du et al. 2012a ; Voitk et al. 2016a).

***Mel-20/34* (*M. purpurascens* (Boud.) Jacquet; Fig. 5F).** One culture and 21 fungarium specimens previously morphologically identified mostly as *M. conica* (11 specimens) or *M. elata* (six specimens) were determined as Mel-20, corresponding to *M. purpurascens*, or as Mel-34, which lacks a Latin binominal. Mel-20 was originally distinguished by O'Donnell et al. (2011) as a sister lineage to Mel-19 (*M. eohespera*) with a very low bootstrap support at most of the loci (< 50%) and 93% support at *EF-1α*, which contains almost all of the few apomorphies that distinguish the two lineages. Later, Du et al. (2012a) distinguished Mel-34 on the basis of a single specimen from China that is almost identical to Mel-20 at all loci except *RPB1*, which provided an almost identical sequence to Mel-23/24/31/32 (*M. pulchella*; Suppl. material 3, Fig. S1, Suppl. material 5, Table S3). Therefore, we propose merging Mel-34 with Mel-20 (*M. purpurascens* being the only known binomial) provisionally until more collections are made and this extraordinary pattern is confirmed. The distinctness of Mel-19 and Mel-20 is still clear after the inclusion of the Czech samples, although the difference is very small, the latter branch is poorly supported

(posterior probability 0.74) and distinction of the two lineages may thus be an artifact of anagenesis in the *EF-1α* gene. Not surprisingly, distinguishing between these species on the sole basis of ITS is tricky, as they differ in a single SNP. Moreover, this polymorphism is not stable across the whole ranges of these species, but it was stable for all of the Czech specimens that were studied. We therefore considered it as diagnostic in ITS-based determinations.

Mel-20 (*M. purpurascens*) is known from France, Scandinavia, Turkey, China, and Taiwan (Taşkın et al. 2010, 2012; O'Donnell et al. 2011; Clowez 2012, as a variety of *M. conica*; Du et al. 2012a; Richard et al. 2015). The ascomata of the Czech specimens were collected between late April and mid-May, both in anthropogenic habitats (railways, roadsides, gardens, and also in a junkyard, places with deposits of various materials such as rubble, sand, wood, or pure brick clay, between stones or even on concrete) and in forests, and co-occurred with both conifers (often growing under *Picea* sp. or *Pinus* sp.) and deciduous trees and shrubs (*Quercus* sp., *Prunus domestica*, *Betula* sp., *Crataegus* sp., *Populus* sp., *Salix* sp.). Other authors also reported the frequent co-occurrence of Mel-20 with conifers such as *Abies* sp., *Pinus* sp., or *Cedrus* sp. and also with *Populus* sp. or *Quercus* sp. (Taşkın et al. 2010, 2012 ; Clowez 2012 ; Du et al. 2012a).

***Mel-23/24/31/32* (*M. pulchella* Clowez & F. Petit; Fig. 5G).** Twenty-five specimens (including two samples from Slovakia) originally determined mostly as *M. conica* (10 specimens), *M. elata* (four specimens), or *M. pragensis* (five specimens), grouped together with Mel-23, Mel-24, Mel-31, and Mel-32. Mel-23 (no Latin binominal) and Mel-24 (*M. septentrionalis* M. Kuo, J.D. Moore & Zordani) were originally distinguished by O'Donnell et al. (2011) on the basis of three Scandinavian specimens and one specimen (plus five additional that were not shown) from the eastern USA and Canada, respectively. Mel-31 (*M. pulchella*) was delimited in parallel by Taşkın et al. (2010) without the inclusion of Mel-23 and Mel-24 in the analyses and has so far been reported from China, Pakistan, Turkey, and France (Taşkın et al. 2010, 2012; Du et al. 2012a; Richard et al. 2015; Badshah et al. 2018). Mel-32 (*M. conifericola* Taşkın, Büyükalaca & H.H. Doğan) was later distinguished by Taşkın et al. (2012) in Turkey, although the clade was poorly supported (BS = 59 for concatenated data and < 50% for individual loci). Moreover, the latter study revealed the paraphyly of Mel-31 as a result of the exclusion of Mel-24, and also very low support (if any) for each of the four species. These facts were confirmed by Richard et al. (2015) and our own data (Fig. 1). Therefore, we suggest combining the four formerly delimited species and to treat them as one, with the oldest known Latin binomial being *M. pulchella*, and *M. septentrionalis* and *M. conifericola* being later synonyms. Two specimens corresponding to the Mel-23/24/31/32 lineage were also reported from India (Du et al. 2012b). The degree of endemism, therefore, appears to be overestimated and the recorded variation may be attributed to phylogenetically young mutations and sometimes to intraspecific geographic variability. The Czech specimens were collected from mid-April to early May, mostly in forests (often along roads or forest edges), but also along railways or

in sandstone quarries. A special collection site was a surface coal mine where a stable population of morels was visited in several successive years in the 1970s. Ascomata were mostly found under deciduous trees such as *Populus tremula*, *Carpinus betulus*, *Betula pendula*, or *Fraxinus* sp., while other nearby trees were *Tilia* sp., *Salix caprea*, *Quercus* sp., and occasionally conifers (*Pinus* sp., *Picea* sp.).

Mel-39 **(newly designated phylogenetic species; Fig. 5H).** Two cultures and four exsiccated specimens formed a well-separated and well-supported lineage sister to Mel-10 (*M. importuna*). Although closely related to Mel-10, this lineage differs at each of the loci that were studied by three (LSU) to 18 (*EF-1α*) synapomorphic SNP's (59 SNP's in total; Suppl. material 5, Table S3). This lineage is identical to the New-2 clade sensu Du et al. (2012b), which was reported from China and Germany based on ITS only; however, it has not been definitely distinguished as a phylospecies until now. Beside the significant genetic differences and genealogical concordance, Mel-10 and Mel-39 differ in several phenotypic traits, particularly on sclerotia under experimental cultivation. Whereas the sclerotia of Mel-39 are very tiny (mostly not bigger than 1 mm), spherical, not coalescing, dark, red-brown, mature sclerotia of *M. importuna* are of a light color varying from that of a walnut shell to somewhat orange, and coalesce into big hardened bodies of an irregular shape that are up to several centimeters long (Petrželová unpublished data). Nevertheless, formal taxonomic treatment needs to be based on extensive phenotypic analyses and the study of the type material of related taxa, and cannot be performed at this stage. The species was mostly collected on bark mulch, mostly in late April or early May, i.e., similarly to or slightly earlier than Mel-10.

Mes-4 **(*M. americana* Clowez & Matherly; Fig. 5I).** One *Morchella* strain maintained as an axenic culture and previously morphologically determined as *M. esculenta* was identified as Mes-4 (Fig. 2; Suppl. material 2, Table S2). This species was described under several binominals (Clowez 2012; Kuo et al. 2012), and *M. americana* was selected by Richard et al. (2015) as the most appropriate among the priority names. However, Clowez (2012) used the name *M. rigida* (Krombh.) Boud. for the French specimens that Richard et al. (2015) found as conspecific with Mes-4. If this name of Krombholz (a 19th-century Prague mycologist) was used correctly, *M. rigida* (basionym *M. conica* var. *rigida*; Krombholz 1831–1834) is probably the oldest name for the species. Nevertheless, we follow the latest treatment of Richard et al. (2015) for now.

Morchella americana appears to be native to North America, where it is the most widely distributed Esculenta clade species (O'Donnell et al. 2011; Du et al. 2012b; Kuo et al. 2012; Richard et al. 2015). To date, it has also been reported from France, Spain, Turkey, and China (Taşkın et al. 2010, 2012; Du et al. 2012b; Richard et al. 2015), and this is the first record of the species for the Czech Republic (not considering Krombholz's collections). In North America it mostly co-occurs with *Fraxinus* spp., *Ulmus americana*, *Populus* spp., *Platanus occidentalis*, *Acer* sp., or *Quercus* spp., but it can also be found in old apple orchards and occasionally

together with conifers (Kuo et al. 2012; Richard et al. 2015). Its association with *Buxus sempervirens* has also been reported (Clowez 2012; Richard et al. 2015). It has been suggested that *M. americana* has only recently been introduced to Europe, as most records come from sites with a strong anthropogenic influence, especially from hybrid poplar plantations (Richard et al. 2015). The Czech specimen, nevertheless, occurred in the Žebračka National Nature Reserve, i.e., a site with one of the most valuable natural alluvial forests in the Czech Republic. Moreover, if Krombholz's specimens originating from Czech Republic were really identical with Mes-4, human-mediated introduction from North America would be rather unlikely. The studied specimen was found under young *Populus tremula* trees at the beginning of May.

***Mes-5* (Fig. 5J).** Nineteen *Morchella* cultures or exsiccated specimens (including two samples from Slovakia) that had previously been phenotypically determined differently, mostly as *M. vulgaris* (eight specimens) or *M. esculenta* (three specimens), but also as a variety of species of black morels, were identified as Mes-5 (no Latin binominal). Although the multi-gene approach was only used for two specimens, no clear polymorphism was found at ITS among our Mes-5 accessions, whereas two SNP's were observed between Mes-5 and the sister Mes-17 (*M. dunensis*). Therefore, identification based on ITS should be sufficient. Mes-5 has so far been found in Denmark, France, and Norway (O'Donnell et al. 2011). The Czech specimens were mostly collected from mid-April to early May, often in gardens (also on bark mulch) or in forests, but some collections were also made in a park, an orchard, a meadow and a waste dump near a summer cottage. Ascomata of this species were often found under fruit trees (*Malus domestica*, *Pyrus* sp., *Prunus persica*), and Rosaceae shrubs (e.g., *Crataegus* sp.); other nearby trees that were recorded were *Fraxinus excelsior*, *Acer pseudoplatanus*, *Populus* sp., *Robinia pseudoaccacia*, and *Picea abies*, and in Slovakia also *Swida* sp. and *Pinus nigra*.

***Mes-8* (*M. esculenta* (L.) Pers.; Fig. 5K).** Forty-seven *Morchella* cultures or exsiccated specimens, (including three samples from Slovakia) previously morphologically determined mostly as *M. esculenta* (29 specimens) or less frequently as *M. crassipes* (eight specimens) or *M. vulgaris* (six specimens), were identified as Mes-8 (corresponding to *M. esculenta*). *Morchella esculenta* is the common and widely distributed European morel species recorded from the Czech Republic, Poland, Germany, Switzerland, France, Spain, Belgium, the Netherlands, Norway, Sweden, and Turkey (Taşkın et al. 2010, 2012; O'Donnell et al. 2011; Clowez 2012; Du et al. 2012b; Richard et al. 2015; Baran and Boroń 2017) but also from China (Du et al. 2012a). The Czech collections were mostly obtained from mid-April to early May, occasionally up to mid-May, mostly in (semi-)natural habitats in deciduous (including floodplain) forests and shrubs, less often in limestone quarries, old orchards, parks, or gardens (here also on bark mulch). A special collection site was the edge of a reed bed. Uncommonly, *M. esculenta* was found in ruderal or urban habitats such as the yards of buildings, a rubble site or even the concrete floor of a woodshed.

M. esculenta has been found in association with a variety of deciduous wood species. The Czech collections were more frequently collected under *Fraxinus* sp. (often *F. excelsior*), *Crataegus* sp., *Prunus* spp. (especially *P. spinosa*, *P. domestica*, and *P. avium*), *Quercus* spp., and *Acer* spp. Other nearby trees were *Alnus* sp., *Carpinus betulus*, *Populus tremula*, *Betula* sp., *Salix caprea*, *Malus domestica*, *Aesculus hippocastanum*, *Robinia pseudoaccacia*, and occasionally conifers such as *Picea* sp., *Larix* sp., *Pinus sylvestris*, or *Thuja* sp. According to other authors, *M. esculenta* can also grow under *Ulmus minor, Malus sylvestris, Cydonia oblonga, Mahonia* sp., or, rarely, Cupressaceae species (Clowez 2012) or *Abies* sp. (Taşkın et al. 2012).

Mulch morels

What are known as the "mulch morels" represent a specific ecological group of morels that occur massively in newly created ornamental beds with bark mulch, mostly in gardens or around construction zones and newly built houses. On the basis of our observations, the macromorphological variation both within and among populations of mulch morels is remarkable, sometimes to such an extent that it brings to mind the variation among species. Therefore, we aimed at an estimation of the number of species within and among neighboring localities. However, with the only exception of one site with Mel-10 (*M. importuna*) and Mel-39, all the samples from the same ornamental bed belonged to the same species (Mel-10; Suppl. material 1, Table S1). Nevertheless, among the total of 48 specimens that originated from different localities with bark mulch, five *Morchella* species were recognized. A total of 36 specimens were determined as Mel-10 (*M. importuna*), five as Mel-39, four as Mes-5, two as Mel-19 (*M. eohespera*), and one as Mes-8 (*M. esculenta*; Suppl. material 2, Table S2).

Conclusions

Morchella taxonomy may give the impression of being opaque for many field mycologists. Much of the confusion appears to stem from the excessive or inappropriate over-splitting of some phylogenetic clades into smaller and poorly supported subclades and from the apparent lack of consensus on taxonomical principles. Therefore, we propose five criteria for distinguishing the new phylospecies in *Morchella*: the criterion of monophyly, the genealogical criterion, the criterion of distinctness, the criterion of minimal sampling, and the criterion of polygenic differentiation. Surely, none of them absolutely reflects natural processes related to speciation and DNA sequence evolution (Leliaert et al. 2014) and each of them can be modified in specific cases. Nevertheless, we believe that the application of these five criteria in distinguishing new phylospecies could prevent further confusion in the molecular taxonomy of morels, although some phylospecies may remain overlooked and undetected. Our approach, therefore, is conservative and pragmatic, aiming at the practical usage of

taxonomy, rather than at identification of all possibly existing small evolutionary units. It is stressed that in this study we rely on molecular phylogenetics only. The most straightforward method for recognition of the species would be one based on phenotypic traits, which should also serve as a support for the delimitation of species. Considering that phenotypic traits are often highly influenced by plasticity (i.e. environmental conditions) and/or intraspecific variability, identification of discriminating macro-and microscopic characters that correspond to the phylogenetic species will be the greatest challenge. This is, nevertheless, necessary in order to link the various phylospecies to the appropriate binomials, especially the old names whose type material is not available for molecular analyses. Integrative studies combining both phenotypic and molecular methods will, hopefully, result in a clearer, phylogeny-based and sustainable *Morchella* taxonomy.

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References

Antonín V (2006) *Morchella semilibera* DC.: Fr. In: Holec J, Beran M (Eds) Červený seznam hub (makromycetů) České republiky, Příroda 24, 56. [in Czech]

Badshah H, Ali B, Shah SA, Alam MM, Aly HI, Mumtaz AS (2018) First record of *Morchella pulchella* from Pakistan. Mycotaxon 133: 201–207. https://doi.org/10.5248/133.201

Baran J, Boroń P (2017) Two species of true morels (the genus *Morchella, Ascomycota*) recorded in the Ojców National Park (south Poland). Acta Mycologica 52: 1094. https://doi.org/10.5586/am.1094

Baroni TJ, Beug MW, Cantrell SA, Clements TA, Iturriaga T, Læssøe T, Holgado Rojas ME, Aguilar FM, Quispe MO, Lodge DJ, O'Donnell K (2018) Four new species of *Morchella* from the Americas. Mycologia. https://doi.org/10.1080/00275514.2018.1533772

Beug M, O'Donnell K (2014) Morel species Mel-19 – preliminary report. Omphalina 5: 13–14. http://www.mykoweb.com/misc/Omphalina/O-V-2.pdf

Carstens BC, Pelletier TA, Reid NM, Satler JD (2013) How to fail at species delimitation. Molecular Ecology 22: 4369–4383. https://doi.org/10.1111/mec.12413

Clowez P (2012) Les morilles. Une nouvelle approche mondiale du genre *Morchella*. Bulletin de la Société Mycologique de France 126: 199–376.

Clowez P, Alvarado P, Becerra M, Bilbao T, Moreau PA (2014) *Morchella fluvialis* sp. nov. (Ascomycota, Pezizales): a new but widespread morel in Spain. Boletín de la Sociedad Micológica de Madrid 38: 253–262. http://www.ascofrance.com/uploads/document/20-Morchella-fluvialis-BSMM-0001.pdf

Clowez P, Bellanger JM, Romero de la Osa L, Moreau PA (2015) *Morchella palazonii* sp. nov. (Ascomycota, Pezizales): une nouvelle morille méditerranéenne. Clé des *Morchella* sect. *Morchella* en Europe. Documents mycologiques XXXVI: 71–84. http://www.ascofrance.com/uploads/forum_file/DM36-reprint-Clowez-Morchella-palazonii-0001.pdf

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Du XH, Zhao Q, O'Donnell K, Rooney AP, Yang ZL (2012a) Multigene molecular phylogenetics reveals true morels (*Morchella*) are especially species-rich in China. Fungal Genetics & Biology 49: 455–469. https://doi.org/10.1016/j.fgb.2012.03.006

Du XH, Zhao Q, Yang ZL (2015) A review on research advances, issues, and perspectives of morels. Mycology 6: 78–85. https://doi.org/10.1080/21501203.2015.1016561

Du XH, Zhao Q, Yang ZL, Hansen K, Taşkın H, Büyükalaca S, Dewsburry D, Moncalvo JM, Douhan GW, Robert VARG, Crous PW, Rehner SA, Rooney AP, Sink S, O'Donnell K (2012b) How well do ITS rDNA sequences differentiate species of true morels (*Morchella*)? Mycologia 104: 1351–1368. https://doi.org/10.3852/12-056

Elliott TF, Bougher NL, O'Donnell K, Trappe JM (2014) *Morchella australiana* sp. nov., an apparent Australian endemic from New South Wales and Victoria. Mycologia 106: 113–118. https://doi.org/10.3852/13-065

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

Holec J, Bielich A, Beran M (2012) Přehled hub střední Evropy. Academia, Praha, 1–624 [in Czech]

Işiloğlu M, Alli H, Spooner BM, Solak MH (2010) *Morchella anatolica* (Ascomycota), a new species from southwestern Anatolia, Turkey. Mycologia 102: 455–458. https://doi.org/10.3852/09-186

Kanwal HK, Acharya K, Ramesh G, Reddy MS (2011) Molecular characterization of *Morchella* species from the Western Himalayan region of India. Current Microbiology 62: 1245–1252. https://doi.org/10.1007/s00284-010-9849-1

Krombholz JV (1831–1834) Naturgetreue Abbildungen und Beschreibungen der essbaren, schädlichen and verdächtigen Schwämme. J.G. Calve, Praha, 1–152.

Kuo M, Dewsburry DR, O'Donnell K, Carter MC, Rehner SA, Moore JD, Moncalvo JM, Canfield SA, Methven AS, Volk TJ (2012) Taxonomic revision of true morels (*Morchella*) in Canada and the United States. Mycologia 104: 1159–1177. https://doi.org/10.3852/11-375

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2017) PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology & Evolution 34: 772–773. https://doi.org/10.1093/molbev/msw260

Leliaert F, Verbruggen H, Vanormelingen P, Steen F, López-Bautista JM, Zuccarello GC, Clerck O De (2014) DNA-based species delimitation in algae. Europaean Journal of Phycology 49: 179–196. https://doi.org/10.1080/09670262.2014.904524

Li QL, Ding C, Fan L (2013) Trophic manner of morels analyzed by using stable carbon isotopes. Mycosystema 32: 213–223. http://manu40.magtech.com.cn/Jwxb/CN/abstract/abstract1126.shtml

Li Q, Xiong C, Huang W, Li X (2017) Controlled surface fire for improving yields of *Morchella importuna*. Mycological Progress 16: 1057–1063. https://doi.org/10.1007/s11557-017-1350-9

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

Loizides M (2017) Morels: the story so far. Field Mycology 18: 42–53. https://doi.org/10.1016/j.fldmyc.2017.04.004

Loizides M, Bellanger JM, Clowez P, Richard F, Moreau PA (2016) Combined phylogenetic and morphological studies of true morels (*Pezizales*, *Ascomycota*) in Cyprus reveal significant diversity, including *Morchella arbutiphila* and *M. disparilis* spp. nov. Mycological Progress 15: 39. https://doi.org/10.1007/s11557-016-1180-1

Mailund T, Munch K, Schierup MH (2014) Lineage sorting in apes. Annual Reviews in Genetics 48: 519–535. https://doi.org/10.1146/annurev-genet-120213-092532

Mann H, Mann P (2014) *Morchella importuna*. The Pasadena mulch morel. Omphalina 5: 11–12. http://www.mykoweb.com/misc/Omphalina/O-V-2.pdf

Masaphy S., Zabari L., Goldberg D (2009) New long-season ecotype of *Morchella rufobrunnea* from Northern Israel. Micologia Aplicada International 21: 45–55. https://www.redalyc.org/html/685/68511349005/

Matheny PB, Liu YJ, Ammirati JF, Hall BD (2002) Using RPB1 sequences to improve phylogenetic inference among mushrooms (*Inocybe*, Agaricales). American Journal of Botany 89: 688–698. https://doi.org/10.3732/ajb.89.4.688

Matočec N., Kušan I., Mrvoš D., Raguzin E (2014) The autumnal occurrence of the vernal genus *Morchella* (Ascomycota, Fungi). Natura Croatica 23: 163-178. http://fulir.irb.hr/1997/1/Matocec_etal(2014)Autumnal_occurrence_Morchella.pdf

Mikšík M (2015) 1000 českých a slovenských hub. Svojtka & Co., Praha, 1–1024. (in Czech)

Moravec J (1970) *Morchella pragensis* Smotlacha 1952, species male nota bohemica. Czech Mycology 24: 32–39. [in Czech] http://www.czechmycology.org/_cm/CM241.pdf

O'Donnell K (1993) *Fusarium* and its near relatives. In: Reynolds DR, Taylor JW (Eds) The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics. CAB International, Wallingford, 225–233.

O'Donnell K, Rooney AP, Mills GL, Kuo M, Weber NS, Rehner SA (2011) Phylogeny and historical biogeography of true morels (*Morchella*) reveals an early Cretaceous origin and high continental endemism and provincialism in the Holarctic. Fungal Genetics & Biology 48: 252–265. https://doi.org/10.1016/j.fgb.2010.09.006

Ondřej V, Havránek P, Kitner M, Němcová P (2011) Molecular identification and characterization of the edible and medicinal Morchellaceae germplasm collection of "mulch morels". International Journal of Medicinal Mushrooms 13: 369–375. https://doi.org/10.1615/IntJMedMushr.v13.i4.70

Pildain MB, Visnovsky SB, Barroetaveña C (2014) Phylogenetic diversity of true morels (*Morchella*), the main edible non-timber product from native Patagonian forests of Argentina. Fungal Biology 118: 755–763. https://doi.org/10.1016/j.funbio.2014.03.008

Reeb V, Lutzoni F, Roux C (2004) Contribution of *RPB2* to multilocus phylogenetic studies of the euascomycetes (Pezizomycotina, Fungi) with special emphasis on the lichen-forming Acarosporaceae and evolution of polyspory. Molecular Phylogenetics & Evolution 32: 1036–1060. https://doi.org/10.1016/j.ympev.2004.04.012

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

Richard F, Bellanger JM, Clowez P, Hansen K, O'Donnell K, Urban A, Sauve M, Courtecuisse M, Moreau PA (2015) True morels (*Morchella*, Pezizales) of Europe and North America: evolutionary relationships inferred from multilocus data and a unified taxonomy. Mycologia 107: 259–382. https://doi.org/10.3852/14-166

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

Smotlacha F (1947) Atlas hub jedlých a nejedlých, Ed. 1. Melantrich, Praha, 1–297. (in Czech)

Smotlacha F (1952) Smrž pražská, a) podivuhodná, b) věžitá. *Morchella Pragensis*: a) *mirabilis*, b) *turriformis* Smotlacha. Časopis českých houbařů 29: 33–37. [in Czech]

Šebek S (1973) Naše chřapáčovité a smržovité houby. Oblastní muzeum v Poděbradech, Poděbrady, 1–40. [in Czech]

Taşkın H, Büyükalaca S, Doğan HH, Rehner AA, O'Donnell K (2010) A multigene molecular phylogenetic assessment of true morels (*Morchella*) in Turkey. Fungal Genetics & Biology 47: 672–682. https://doi.org/10.1016/j.fgb.2010.05.004

Taşkın H, Büyükalaca S, Hansen K, O'Donnell K (2012) Multilocus phylogenetic analysis of true morels (*Morchella*) reveals high levels of endemics in Turkey relative to other regions of Europe. Mycologia 104: 446–461. https://doi.org/10.3852/11-180

Taşkın H, Doğan HH, Büyükalaca S (2015) *Morchella galilaea*, an autumn species from Turkey. Mycotaxon 130: 215–221. https://doi.org/10.5248/130.215

Taşkın H, Doğan HH, Büyükalaca S, Clowez P, Moreau PA, O'Donell K (2016) Four new morel (*Morchella*) species in the elata subclade (*M.* sect. *Distantes*) from Turkey. Mycotaxon 131: 467–782. https://10.5248/131.467

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC (2000) Phylogenetic species recognition and species concepts in fungi. Fungal Genetics & Biology 31: 21–32. https://doi.org/10.1006/fgbi.2000.1228

Thiers B (2018) Index Herbariorum: a global directory of public herbaria and associated staff. New York botanical Garden's virtual herbarium. http://sweetgum.nybg.org/ih/

Thines M, Crous PW, Aime MC, Aoki T, Cai L, Hyde KD, Miller AN, Zhang N, Stadler M. (2018) Ten reasons why a sequence-based nomenclature is not useful for fungi anytime soon. IMA Fungus 9: 177–183. https://doi.org/10.5598/imafungus.2018.09.01.11

Tietel Z, Masaphy S (2018) True morels (*Morchella*) – nutritional and phytochemical composition, health benefits and flavor: a review. Critical Reviews in Food Science & Nutrition 58: 1888–1901. https://doi.org/10.1080/10408398.2017.1285269

Velenovský J (1934) Monographia Discomycetum Bohemiae. Velenovský, Praha, 1–436.

Voitk A, Beug MW, O'Donnell K, Burzynski M (2016a) Two new species of true morels from Newfoundland and Labrador: cosmopolitan *Morchella eohespera* and parochial *M. laurentiana*. Mycologia 108: 31–37. https://doi.org/10.3852/15-149

Voitk A, O'Donnell K, Beug M, Burzynski M, Mann H (2016b) Our morels are named! Omphalina 7(2): 3–10. http://www.mykoweb.com/misc/Omphalina/O-VII-2.pdf

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

Yatsiuk I, Saar I, Kalamees K, Sulaymonov S, Gafforov Y, O'Donnell K (2016) Epitypification of *Morchella steppicola* (Morchellaceae, Pezizales), a morphologically, phylogenetically and biogeographically distinct member of the Esculenta Clade from central Eurasia. Phytotaxa 284 (1): 31–40. https://doi.org/10.11646/phytotaxa.284.1.3

Supplementary material 1

List of localities of mulch morels from which more than one ascoma was analyzed

Authors: Irena Petrželová, Michal Sochor

Data type: occurrence

Link: https://doi.org/10.3897/mycokeys.52.32335.suppl1

Supplementary material 2

List of analysed *Morchella* specimens with collection data, determination and Genbank accession numbers

Authors: Irena Petrželová, Michal Sochor

Data type: species data

Link: https://doi.org/10.3897/mycokeys.52.32335.suppl2

Supplementary material 3

Single-gene bayesian trees; posterior probabilites shown above branches
Authors: Irena Petrželová, Michal Sochor
Data type: phylogenetic data

Link: https://doi.org/10.3897/mycokeys.52.32335.suppl3

Supplementary material 4

Supplementary data
Authors: Irena Petrželová, Michal Sochor
Data type: measurement

Link: https://doi.org/10.3897/mycokeys.52.32335.suppl4

Supplementary material 5

Matrices of genetic distances detected at each locus for selected accessions. A. ITS, B. LSU and EF-1a, C. RPB1 and RPB2
Authors: Irena Petrželová, Michal Sochor
Data type: molecular data

Link: https://doi.org/10.3897/mycokeys.52.32335.suppl5

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RESEARCH ARTICLE

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Looking for *Lepiota psalion* Huijser & Vellinga (Agaricales, Agaricaceae)

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Abstract

Lepiota psalion is fully described based on a recent collection from Sardinia (Italy) and the holotype. NrITS- and nrLSU-based phylogeny demonstrates that sequences deposited in GenBank as "*L. psalion*" and generated from two Dutch and one Chinese collections are not conspecific with the holotype and represent two distinct, undescribed species. These species are here proposed as *Lepiota recondita* sp. nov. and *Lepiota sinorecondita* ad int.

Keywords

Agaricomycetes, Basidiomycota, cryptic species, hymeniform pileus covering, taxonomy

Introduction

Recent molecular analyses have indicated that the genus *Lepiota* (Pers.) Gray is a paraphyletic assemblage that is monophyletic only if it is considered together with species of *Cystolepiota* Singer, *Echinoderma* (Locq. ex Bon) Bon, *Melanophyllum* Velen.,

and *Pulverolepiota* Bon (Johnson 1999; Vellinga 2003, 2004; Vellinga et al. 2011). Consequently, according to the modern concept of Vellinga (2003, 2004), the genus *Lepiota* s.l. includes the pale-spored members of the Agaricaceae Chevall., which are circumscribed by having non-metachromatic, dextrinoid, and usually binucleate spores, cheilocystidia usually present, pleurocystidia absent, a regular hymenophoral trama, and clamp-connections usually present. The structure of the pileus covering has been shown to be a key character to divide the genus into operative, morphology-based sections (Vellinga and Huijser 1999; Vellinga 2001, 2003, 2010).

Species of *Lepiota* with a hymeniform pileus covering were distributed by Bon (1993) over three different sections, *Cristatae* (Kühner ex Wasser) Bon, *Integrellae* (Kühner ex Bon) Bon and *Lilaceae* Bon, based mainly on different spore shapes (either ellipsoid or spurred) and spore nuclear number (mononucleate vs binucleate); all species were included by Vellinga and Huijser (1999) and Vellinga (2001) in an emended large section *Lilaceae*.

According to recent molecular analyses, the species with a hymeniform pileus covering do not form a monophyletic lineage (Vellinga 2003, 2004, 2010; Vizzini et al. 2014a, b; Justo et al. 2015; Qasim et al. 2015; Hosen et al. 2016), even though most of them (with different spore shapes and nuclear number) fall in a clade (named clade 3 by Vellinga 2003) which also includes taxa as *L. albogranulosa* T. Qasim & A.N. Khalid, *L. cystophoroides* Joss. & Riousset, *L. luteophylla* Sundb., and *L. scaberula* Vellinga with a hymeniderm giving rise to loose globose elements (a transition between hymeniderm and epithelium, Vellinga 1988).

During a 3-year survey of macrofungi in the Botanical Garden of Cagliari (Sardinia, Italy), a collection of a *Lepiota* with a hymeniform pileus covering was recorded. It showed striking morphological affinities with *L. psalion* Huijser & Vellinga. The present paper fully describes this collection using morphological features and molecular data, and infers, through sequencing of the holotype, the phylogenetic placement of *L. psalion*. Additionally, two morphologically allied taxa, *Lepiota recondita* sp. nov. and *L. sinorecondita* ad int. are described.

Materials and methods

Morphology

Macroscopic description was based on detailed field notes of fresh basidiomes. Colour terms in capital letters (e.g., Pale Cinnamon-Pink, Plate XXIX) are those of Ridgway (1912). HTML alphanumeric colour codes (https://html-color-codes.info/) were obtained using GIMP (GNU Image Manipulation Program, https://www.gimp.org/) with the "Color Picker" tool on photographs taken in natural light of fresh basidiomes. Micromorphological features were observed on dried material; sections were rehydrated in water or 5% KOH and mounted separately in ammoniacal Congo Red, Cotton

Blue, Cresyl Blue, and Melzer's reagent. Measurements of the microscopic features of *Lepiota psalion* and *L. recondita* were made by photographing all the elements occurring in the visual field of an Optika B-383 PLi light microscope. Measurements were performed using the Piximètre 5.9 R 1530 software (http://ach.log.free.fr/Piximetre/) at 1000× magnification. The microphotographs were taken by an Optikam B5, 5 MP× camera.

When possible, dimensions of the microscopic elements are given as: (minimum–) average minus standard deviation – average plus standard deviation (–maximum) of length × (minimum–) average minus standard deviation – average plus standard deviation (–maximum) of width. Spore dimensions do not include the hilar appendix. The width of each basidium was measured at the widest part, and the length was measured from the apex (sterigmata excluded) to the basal septum. The DNA fluorescent dye 4′,6-diamidino-2-phenyl-indoldihydrochloride (DAPI) was used to stain nuclei in spores following Horton (2006). The number of nuclei in spores were then determined using a Leica TCS-SP2 confocal microscope. Samples were excited with 405 nm light and fluorescence was recorded at 440–500 nm. The following abbreviations are used: l = number of lamellulae between each pair of lamellae reaching the stipe; the notation [X, Y, Z] indicates that measurements were made on X randomly selected spores (taken from spore-prints), in Y samples from Z collections; Q = the spore quotient (length/width ratio); Qav = the average spore quotient. Terminology for descriptive terms is according to Vellinga (1988, 2001). Herbarium abbreviations follow Thiers (2019, continuously updated). Author citations follow the Index Fungorum – Authors of Fungal Names (http://www.indexfungorum.org/authorsoffungalnames.htm).

DNA extraction, PCR amplification and DNA sequencing

Total DNA was extracted from seven dry basidiomes (Tab. 1): two basidiomes (labelled as "a" and "b") from the same *L. psalion* CAG P.11_9/7.68 collection, one basidiome from the *L. psalion* holotype (WU 5152), two basidiomes from two collections of the new species *L. recondita*, and two basidiomes from two collections of *L. sanguineofracta* Vizzini (TO-HG2916, holotype and TO-HG2917). DNA extraction and PCR amplifications were performed as described by Alvarado et al. (2015). Primers ITS1F and ITS4 (White et al. 1990; Gardes and Bruns 1993) were used for the nrITS region; primers LR0R and LR5 (Vilgalys and Hester 1990) were used for the nrLSU (28S) rDNA, and finally EF1-983F and EF1-1567R (Rehner and Buckley 2005) for the translation elongation factor 1-α (*tef1-α*) gene. Chromatograms were checked searching for putative reading errors, and these were corrected. The PCR products were purified with the Wizard SV Gel and PCR Clean-UP System (Promega) following manufacturer's instructions and sequenced forward and reverse by MACROGEN Inc. (Seoul, Republic of Korea). Sequences were checked and assembled using Geneious v. 5.3 (Drummond et al. 2010) and submitted to GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Accession numbers are reported in Table 1.

Table 1. Taxa, vouchers and GenBank accession numbers used in the molecular analyses. Newly sequenced collections are in bold.

Species	Collection No.	Origin	GenBank accession No.	
			nrITS	nrLSU
Chamaemyces fracidus	Th.W. Kuyper 960 (L)	Belgium	AY176343	AY176344
Cystolepiota cystophora	MCVE 56163	Italy	GQ141550	–
Cystolepiota seminuda	4-X-1989, H.A. Huijser s.n. (herb. Huijser)	The Netherlands	AY176350	–
	MCVE 9247	Italy	JF907983	–
Lepiota aff. *grangei*	TENN 064380, ECV4063	USA	–	MF797685
Lepiota acutesquamosa	DUKE-JJ177	USA	–	U85293
Lepiota albogranulosa	LAH. NO. 10152012, Holotype	Pakistan	LK932284	–
	LAH. NO. 9992012	Pakistan	LK932285	–
Lepiota apatelia	26-IX-1990, H.A. Huijser (herb. Huijser)	The Netherlands	AY176462	–
	04-X-1991, H.A. Huijser (herb. Huijser)	The Netherlands	GQ203819	–
Lepiota aspera	E.C. Vellinga 2233 (L)	The Netherlands	AY176354	–
	GLM 45944	Germany	–	AY207219
Lepiota bengalensis	Iqbal 825 GDGM 45684 Holotype	Bangladesh	KU563148	KU563150
	Iqbal 860 Paratype	Bangladesh	KU563149	–
Lepiota brunneoincarnata	DB4157	Hungary	–	MK278258
	NL-5409	Hungary	–	MK278260
Lepiota castanea	TENN 064371, ECV4016	USA	–	MF797675
	NL-2980	Hungary	–	MK278259
Lepiota castaneidisca	E.C. Vellinga 2594 (UC)	USA	AF391055	–
	E.C. Vellinga 2410 (UC)	USA	AF391064	–
	E.C. Vellinga 2805 (UC)	USA	GQ203808	–
	E.C. Vellinga 2756 (UC)	USA	GQ203816	–
Lepiota cf. *aspera*	MFLU 09-0061	Thailand	–	HM488788
Lepiota cf. *cristata*	E.C. Vellinga 2515 (UC)	USA	AF391052	–
	E.C. Vellinga 2677 (UCB)	USA	AY176466	–
	E.C. Vellinga 2714 (UC)	USA	GQ203807	–
Lepiota clypeolaria	E.C. Vellinga 1683 (L)	Germany	AY176361	–
	TENN 064372, ECV4003	USA	–	MF797684
	VPI-OKM22029	South Korea	–	U85291
	CBS 146.42	Sweden	–	MH867601
Lepiota coloratipes	9-X-1991, H.A. Huijser (herb. Huijser)	The Netherlands	AF391066	–
	MCVE 16888	Italy	FJ998406	–
	Zhu L. Yang 4790	China	KC819621	–
	Zhu L. Yang 4951	China	KC819622	–
	SAV F-3212	Spain	KC900376	–
	SAV F-3213, Holotype	Spain	KC900377	–
	NL-5353	Hungary	–	MK278270
Lepiota cortinarius	NL-1602	Hungary	–	MK278262
Lepiota cristata	22-IX-1993, H.A. Huijser (herb. Huijser)	The Netherlands	AF391042	–
	20-IX-1989, H.A. Huijser (L)	The Netherlands	AF391043	–
	9-VII-1998, Z.L. Yang 2238 (HKAS)	China	AF391044	–
	8-XII-2000, E.C. Vellinga 2611 (UC)	USA	AF391045	–
	30-I-1993, D.E. Desjardin 5658 (SFSU)	USA	AF391050	–
	24-IX-2000, S. Clark (coll. P.B. Matheny 1958) (WTU)	USA	AF391051	–
	AFTOL-ID 1625, ECV 2449 (UC)	USA	–	DQ457685
	E.C. Vellinga 2780 (UC)	USA	GQ203806	–
	E.C. Vellinga 2750 (UC)	USA	GQ203815	–
	DUKE1582	USA	–	U85292
	420526MF0542	China	–	MH141343
	420526MF0550	China	–	MG712361
Lepiota cristatoides	5-IX-1996, H.A. Huijser s.n. (herb. Huijser)	The Netherlands	AY176363	–
Lepiota cystophoroides	E.C. Vellinga 2142 (L)	France	AF391031	–
Lepiota erminea	NL-3095	Hungary	–	MK278263

Species	Collection No.	Origin	GenBank accession No.	
			nrITS	nrLSU
Lepiota felina	VPI-OKM20596	USA	U85330	U85295
	NL-4207	Slovakia	–	MK278264
Lepiota geogenia	MEL 2358504	Australia	–	JX179270
	MEL:2358503	Australia	–	JX179271
Lepiota griseovirens	MCVE 13747	Italy	FJ998403	–
Lepiota hymenoderma	E.C. Vellinga 2017 (L)	The Netherlands	AF391083	–
Lepiota laevigata	FP2012-11-02	Hungary	–	MK278266
Lepiota lilacea	E.C. Vellinga 2451 (UCB)	USA	AY176379	–
	E. Brown (coll. E.C. Vellinga 1873) (L)	United Kingdom	GQ203820	–
Lepiota luteophylla	H.V. Smith 284 (MICH)	USA	AY176475	–
Lepiota maculans	TENN 064381	USA	–	HQ832458
Lepiota mandarina	HKAS 50028	China	–	KM214816
Lepiota neophana	E.C. Vellinga 2602 (UCB)	USA	AY176492	–
	E.C. Vellinga 3947 (UC)	USA	GQ203812	–
	rh24 08/27/07 (ISC)	USA	GQ375546	–
	rh39 08/11/07 (ISC)	USA	GQ375547	–
	E.C. Vellinga ecv3955 (UC)	USA	–	HM488785
Lepiota ochraceofulva	E.C. Vellinga 2267) (L)	The Netherlands	AF391032	–
	E.C. Vellinga 2273 (L)	The Netherlands	AY176386	–
Lepiota ochraceofulva	NL-2973	Hungary	–	MK278267
Lepiota ochraceoumbonata	Murhula Cizungu 39	Gabon	–	MK278268
Lepiota oreadiformis	FO 46679	Germany	–	AF291344
Lepiota phaeoderma	E.C. Vellinga 3000 (UC)	USA	GQ203810	–
Lepiota psalion	**WU 5152 Holotype**	**AUSTRIA**	**MG581687**	**MG581699**
Lepiota psalion basidiome **a**	**CAG P.11_9/7.68**	**Italy**	**MG581688**	–
Lepiota psalion basidiome **b**	**CAG P.11_9/7.68**	**Italy**	**MG581689**	**MG581700**
Lepiota psalion (*L. recondita*)	15-IX-1999, H.A. Huijser (herb. Huijser) hah6153	The Netherlands	AY176390	–
	3-VIII-1999, H.A. Huijser s.n. (herb. Huijser)	The Netherlands	–	AY176391
	H.A. Huijser (herb. Huijser) hah6177	The Netherlands	GQ203823	–
Lepiota psalion (*L. sinorecondita ad interim*)	HMJAU3799	China	GU199362	GU199355
Lepiota pseudohelveola	GLM 45945	Germany	–	AY207220
Lepiota pyrochroa	E.C. Vellinga 2006 (L)	The Netherlands	AY176477	–
Lepiota recondita	**TR gmb 01481, paratype**	**The Netherlands**	**MK508899**	**MK508901**
	TR gmb 01482, holotype	**The Netherlands**	**MK508900**	**MK508902**
Lepiota rhodophylla	E.C. Vellinga 2610 (UCB)	USA	AY176480	–
Lepiota sanguineofracta	**TO-HG2916, Holotype**	**Italy**	KF879620	**MG581701**
	TO-HG2917	**Italy**	KF879621	**MG581702**
Lepiota scaberula	E.C. Vellinga 2307 (UC)	USA	AF391029	–
	E.C. Vellinga 2595 (holotype) (UC)	USA	AF391030	–
	UC1999143	USA	–	MK278271
Lepiota subcastanea	HKAS 45633	China	–	KM214817
Lepiota subgranulosa	ANGE253 (JBSD, duplicate in MEXU)	The Dominican Republic	KR022007	–
Lepiota subalba	E.C. Vellinga 2242 (L)	The Netherlands	AY176489	–
Lepiota subincarnata	E.C. Vellinga 2234 (L)	The Netherlands	AY176491	–
	VPI-OKM22153	South Korea	–	U85294
	NL-2022	Hungary	–	MK278273
Lepiota thiersii	E.C. Vellinga 2590 (UCB)	USA	AY176485	–
	E.C. Vellinga 2589 (UC)	USA	GQ203817	–
Lepiota xanthophylla	TUB 011553	Germany	–	DQ071712
Uncultured Basidiomycota	Environmental sample, man22_soil_G02	USA	GU328508	–

Sequence alignment, dataset assembly and phylogenetic analysis

Sequences obtained in this study were compared to those available in the GenBank (http://www.ncbi.nlm.nih.gov/) and UNITE (http://unite.ut.ee/) databases by using the Blastn algorithm (Altschul et al. 1990).

Based on the BLASTn results (sequences were selected based on the greatest similarity) and outcomes of recent phylogenetic studies incorporating *Lepiota* sequences (Vellinga 2003, 2004, 2010; Vizzini et al. 2014a, b; Justo et al. 2015; Qasim et al. 2015; Hosen et al. 2016) sequences were retrieved from GenBank for the comparative phylogenetic analysis. The nrITS and nrLSU datasets were analysed separately. The combined nrITS/nrLSU phylogeny was not inferred as most *Lepiota* collections in GenBank are not provided with both molecular markers (Table 1). Although *tef1-α* sequences were generated for *L. psalion*, they were not included in phylogenetic analyses because comparable sequences for most *Lepiota* taxa are currently unavailable in public databases, and, in this case, only the Blastn results were provided in the Results. In the nrITS dataset, besides *Lepiota* species with a hymeniform pileus covering, eight species (indicated by an asterisk in Fig. 1) representative of the major clades in *Lepiota* as delimited by Vellinga (2003) were chosen for comparison. The nrLSU dataset consists of all the *Lepiota* s.l. collections determined at species level present in GenBank. Alignments were generated for each nrITS and nrLSU dataset using MAFFT (Katoh et al. 2002) with default conditions for gap openings and gap extension penalties. The two alignments were imported into MEGA v. 6.0 (Tamura et al. 2013) for manual adjustment. The best-fit substitution model for each single alignment was estimated by the Bayesian information criterion (BIC) with jModelTest 2 (Darriba et al. 2012). The GTR + G model was chosen for the nrITS alignment and the TrN+I+G for the nrLSU alignment. The nrITS dataset was partitioned into ITS1, 5.8S and ITS2 subsets. *Chamaemyces fracidus* (AY176343 and AY176344) was used as an outgroup taxon in both the nrITS and nrLSU analyses because it is basal in the Agaricaceae (Vellinga 2004, 2010).

Phylogenetic hypotheses were constructed with Bayesian inference (BI) and Maximum likelihood (ML) criteria. The BI was performed with MrBayes v. 3.2.6 (Ronquist et al. 2012) with one cold and three incrementally heated simultaneous Monte Carlo Markov chains (MCMC) run for 10 million generations, under the selected evolutionary model. Two simultaneous runs were performed independently. Trees were sampled every 1,000 generations, resulting in overall sampling of 10,001 trees per single run; the first 2,500 trees (25%) were discarded as burn-in. For the remaining trees of the two independent runs, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian posterior probabilities (BPP).

ML estimation was performed with RAxML v. 7.3.2 (Stamatakis 2006), with 1,000 bootstrap replicates (Felsenstein 1985) using the GTRGAMMA algorithm to perform a tree inference and search for a good topology. Support values from bootstrapping runs (MLB) were mapped on the globally best tree using the "-f a" option of RAxML and "-x 12345" as a random seed to invoke the novel rapid

bootstrapping algorithm. BI and ML analyses were run on the CIPRES Science Gateway web server (Miller et al. 2010). Only BPP and MLB values over 0.70 and 50%, respectively, are reported in the resulting trees (Figs 1, 2). Pairwise % identity values (P%IV) of the sequences were calculated using MEGA v. 6.0 (Tamura et al. 2013). Alignments and phylogenetic trees are available at TreeBASE (www.treebase.org) under ID S22021.

Results

Molecular analysis

The PCR product was 476–729 bp (nrITS) and 894–1128 bp (nrLSU). The nrITS data matrix comprised 68 sequences (including 63 from GenBank). This dataset was 814 bp long and contained 545 (66.9 %) variable sites. The nrLSU data matrix comprised 45 sequences (including 39 from GenBank). This dataset was 953 bp long and contained 335 (35.2%) variable sites.

As both Bayesian and Maximum likelihood analyses produced a consistent topology, only the Bayesian trees with both BPP and MLB values are shown (Figs 1, 2).

In both the nrITS and nrLSU analyses (Figs 1, 2), the sequences of the holotype of *L. psalion* and of the Sardinian collection clustered together in a strongly supported clade (BPP = 1.00, MLB = 100% and BPP = 1.00, MLB = 99%, respectively). The sequences of this clade show a P%IV of 98.9% for the nrITS and of 99.6% for the nrLSU. According to the nrITS analysis, which is based on a larger taxon sampling (Fig. 1), *L. psalion* is sister (BPP = 1.00; MLB = 85%) to *L. coloratipes* Vizzini, J.F. Liang, Jančovičová & Zhu L. Yang. The Blastn results of the *tef1-α* sequences obtained from the two Sardinian specimens of CAG P.11_9/7.68 (MG597229 and MG597230) show an identity value of 83% with *Lepiota phaeoderma* Vellinga (GQ375549), 81% with *Coniolepiota spongodes* (Berk. & Broome) Vellinga (HM488881, HM488883 and HM488884) and with *Lepiota neophana* Morgan (GQ375550 and GQ375551).

Both the nrITS and nrLSU analyses (Figs 1, 2) highlight the presence of sequences in GenBank from Dutch [GQ203823, AY176390 (nrITS), the Netherlands, Limburg province, Valkenburg, Schaelsberg, H.A. Huijser (herb. Huijser), 15-IX-1999, and AY176391 (nrLSU), ibidem, H.A. Huijser (herb. Huijser), 23-VIII-1999] and Chinese collections [GU199362 (nrITS) and GU199355 (nrLSU), China: Jilin province, Changchun, Jinyuetan Park, herb. HMJAU3799] which are named as "*Lepiota psalion*", but are clearly distinct from the holotype and the Sardinian collection of *L. psalion*. The Dutch "*Lepiota psalion*" sequences form a strongly supported clade (BPP = 1.00 and MLB = 100% in the nrITS analysis; BPP = 1.00 and MLB = 99% in the nrLSU analysis) with sequences from the two collections of *L. recondita* (recondita clade). The sequences of this clade show a P%IV of 99.3% for both the nrITS and the nrLSU. The Chinese "*Lepiota psalion*" is sister (BPP = 1.00 and MLB = 98% in the nrITS analysis; BPP = 1.00 and MLB = 94% in the nrLSU analysis) to the recondita clade.

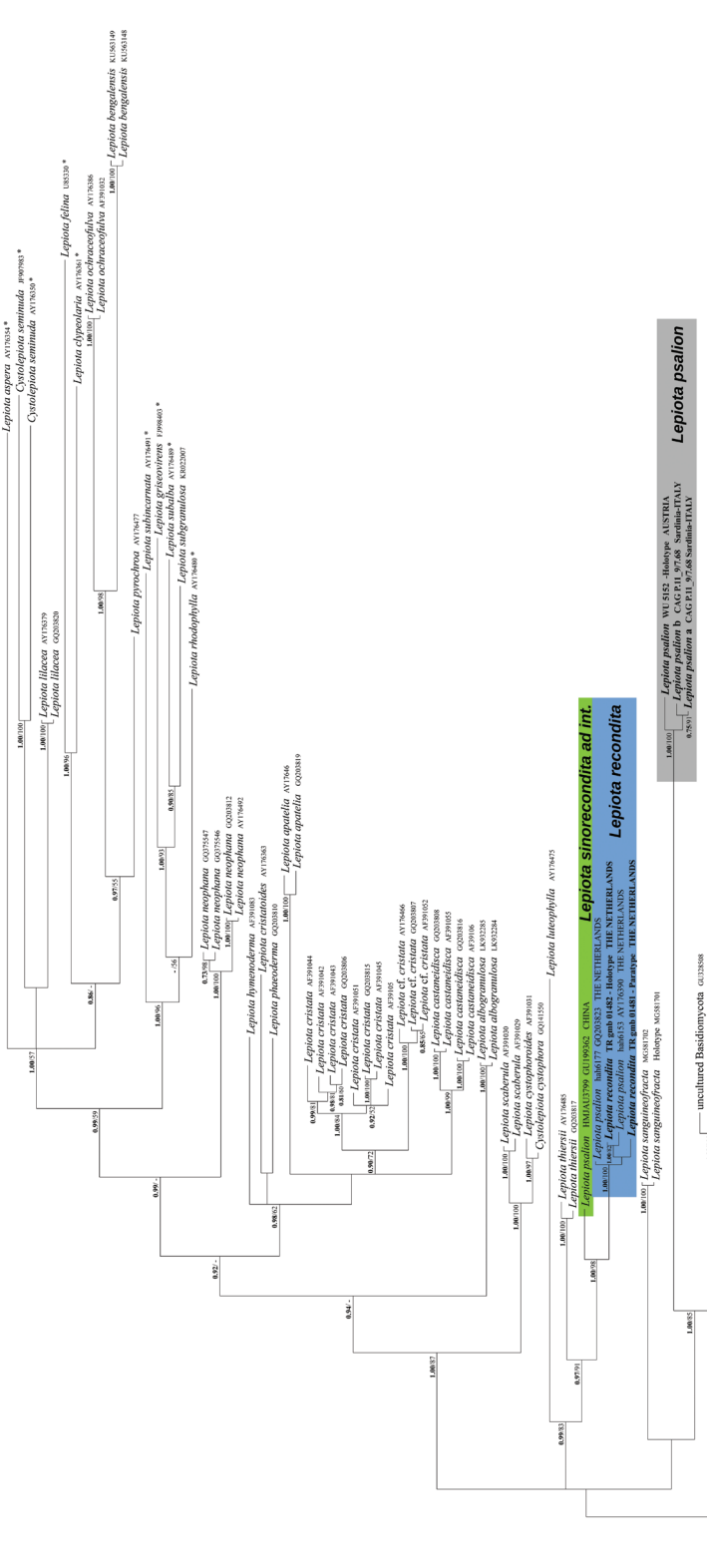

Figure 1. Bayesian phylogram obtained from the general nrITS sequence alignment of *Lepiota* spp. Here there are included *Lepiota* species with a hymeniform pileus covering, eight species representative of the major clades in *Lepiota* (indicated by *), and *Chamaemyces fracidus* as an outgroup taxon. Support values in either the Bayesian (Posterior Probabilities values [BPP]) or Maximum likelihood (ML Bootstrap percentage [MLB]) analyses are indicated. Only BPP values over 0.70 (in bold) and MLB values over 50% are given above clade branches. Newly sequenced collections are in bold.

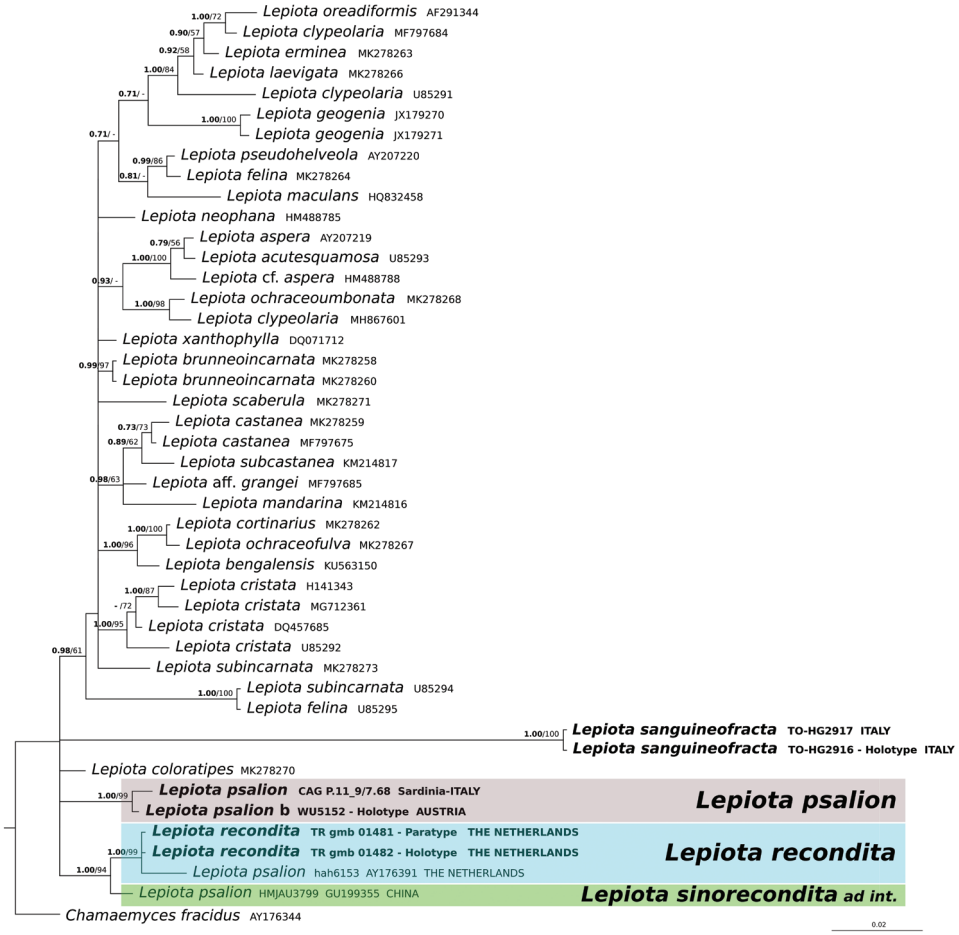

Figure 2. Bayesian phylogram obtained from the general nrLSU sequence alignment of *Lepiota* spp. *Chamaemyces fracidus* was used as an outgroup taxon. Support values in either the Bayesian (Posterior Probabilities values [BPP]) or Maximum likelihood (ML Bootstrap percentage [MLB]) analyses are indicated. Only BPP values over 0.70 (in bold) and MLB values over 50% are given above clade branches. Newly sequenced collections are in bold.

Taxonomy

Lepiota psalion **Huijser & Vellinga, in Vellinga & Huijser, Belg. J. Bot. 131(2): 203 (1999) [1998]**
Figs 3–6

Description. Macrocharacters (Fig. 3). *Pileus* 8–36 mm wide, at first slightly obtusely campanulate, hemispherical-trapezoid or broadly conical, later plano-convex to applanate-expanded, subumbonate, with a shallow umbo; not hygrophanous; margin

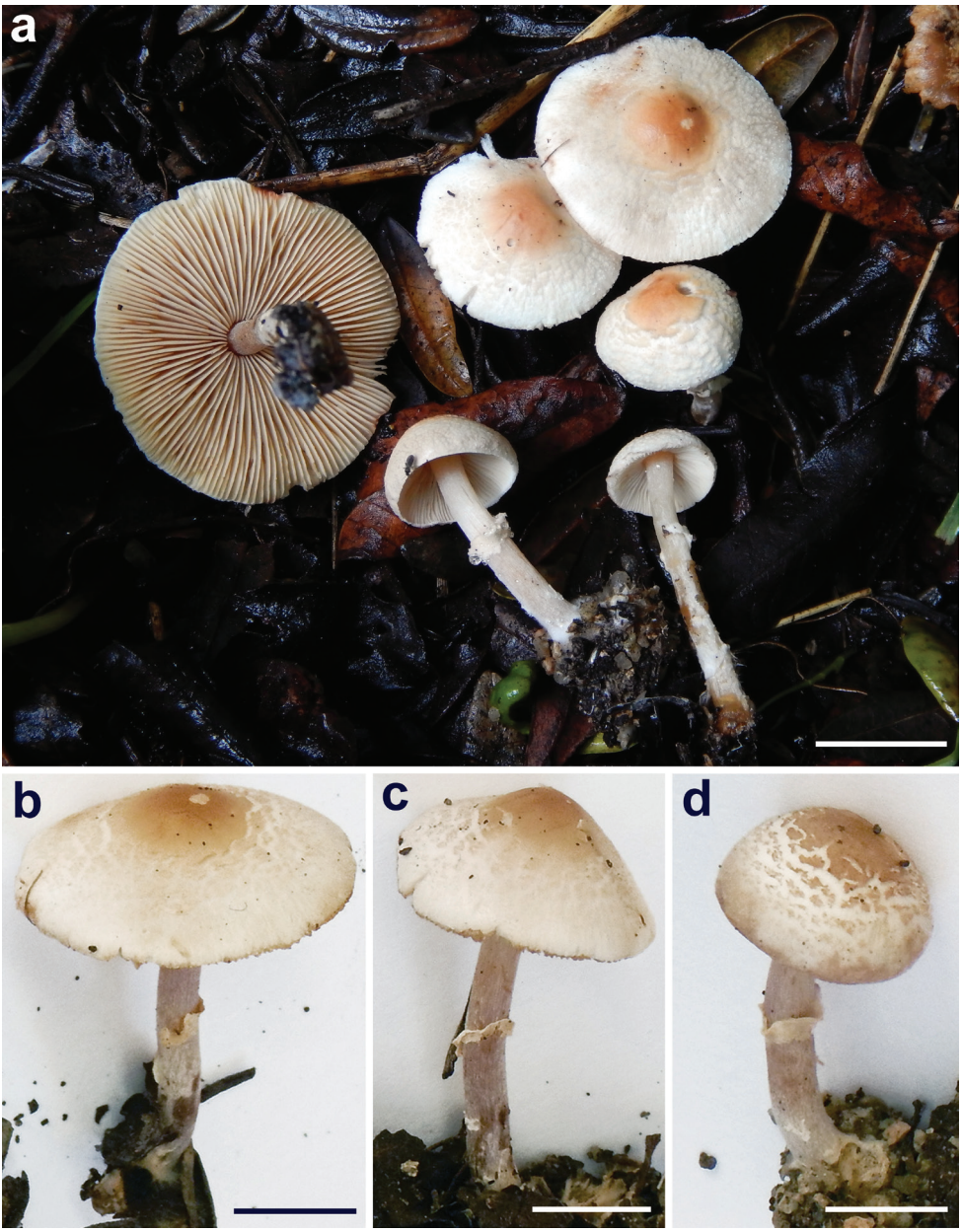

Figure 3. *Lepiota psalion*. Fresh basidiomes (CAG P.11_9/7.68) **a** Basidiomes in situ **b–d** Details of pileus surface, stipe and annulus. Scale bars: 10 mm (**a**); 5 mm (**b–d**). Photographs by A. Tatti.

not striated, slightly exceeding the lamellae when young, sinuous-undulate, entire or slightly fringed with age, with minute adhering remnants of partial veil when young; surface dry, at first smooth, later irregularly cracking around centre into concentric non-uplifted squamules; cream to pinkish-light brown at centre [*Vinaceous-buff

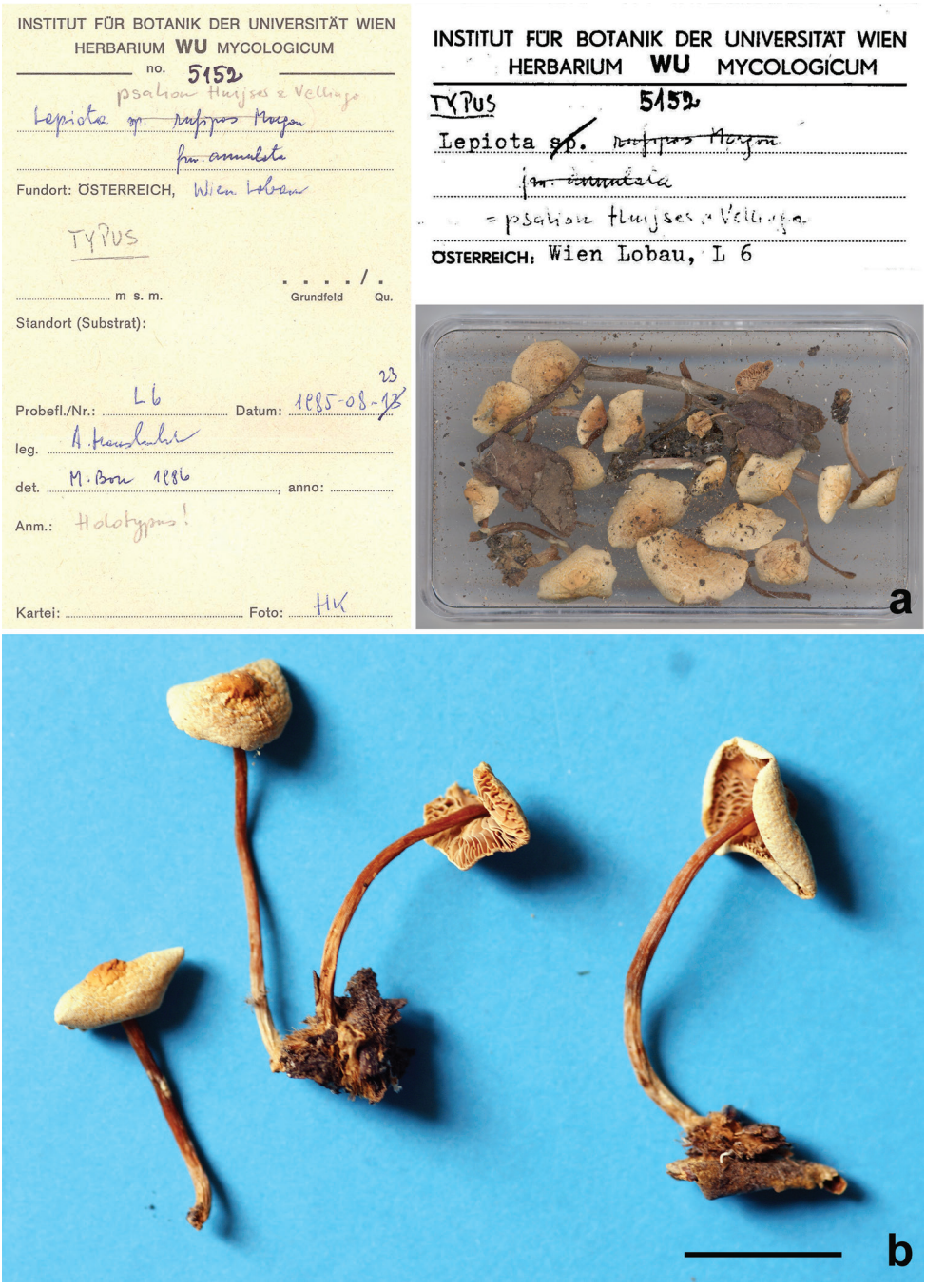

Figure 4. *Lepiota psalion*. Holotype (WU 5152) **a** Labels and collection **b** Four basidiomes from the collection. Scale bar: 10 mm. Photographs: **a** by W. Till; **b** by A. Vizzini.

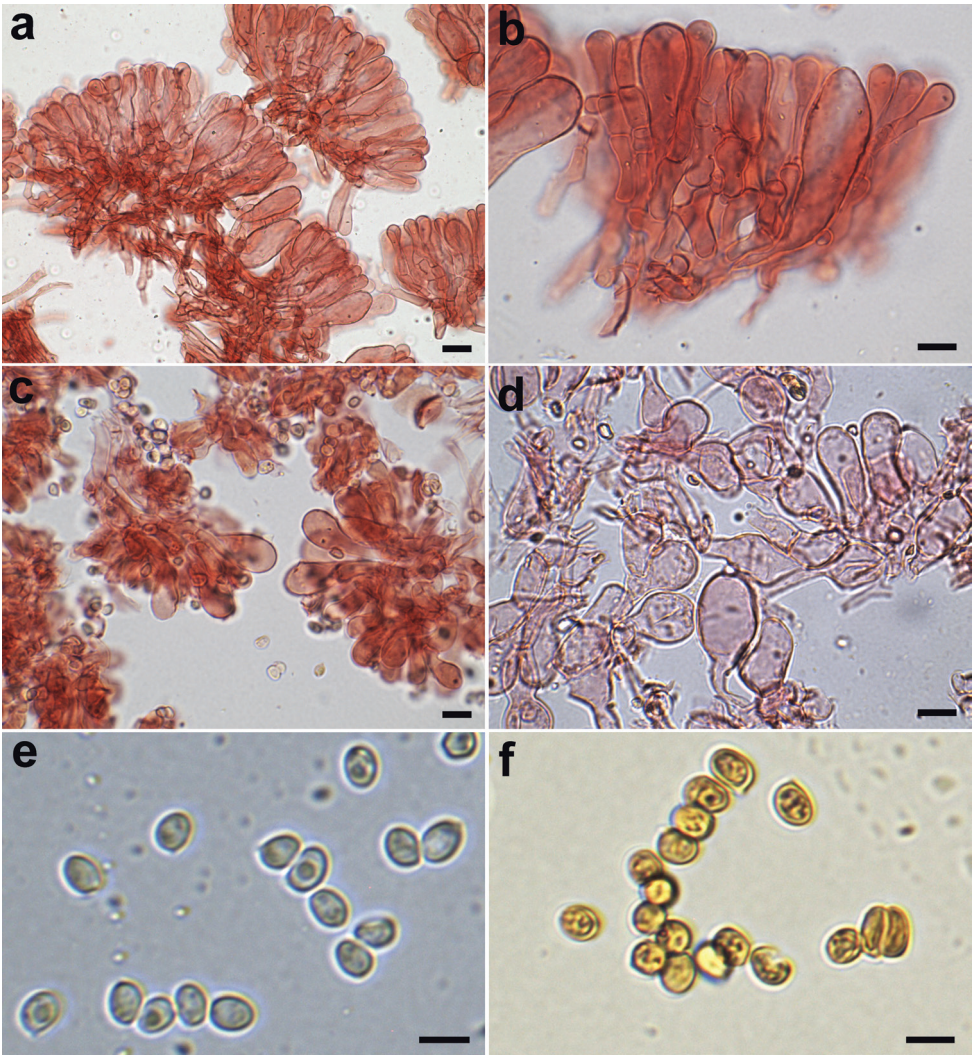

Figure 5. *Lepiota psalion*. Microscopic features (CAG P.11_9/7.68) **a–b** Elements of the pileus covering **c** Cheilocystidia **d** Elements of the annulus **e–f** Spores. **a–d** in ammoniacal Congo red **e** in 5% KOH **f** in Melzer's reagent. Scale bars: 10 µm (**a–d**); 5 µm (**e–f**). Photographs by A. Tatti.

(Plate XL 17"".c-y./d) HTML d3b094 to Orange-Cinnamon (Plate XXIX 13".ou-o.) or Ochraceous-Tawny (Plate XV 15'.y-o./i) HTML bc7e4d], paler towards the margin [Pale Cinnamon-Pink (Plate XXIX - 13".oy-o./f) HTML e5d6c3 to Pale Smoke-Gray (Plate XLVI 21"".o-y./d) HTML cdc9c6]. *Stipe* 22–33 × 1.5–2 mm, central, cylindrical, usually regular, but sometimes also slightly flexuous, hollow; shiny, at first white, soon becoming pink-brown [Tilleul-Buff (Plate XL - 17"".c-y./f), HTML c3b092 to *Drab Gray (Plate XLVI 17"".o-y./d) HTML bda599] starting from the base and pro-

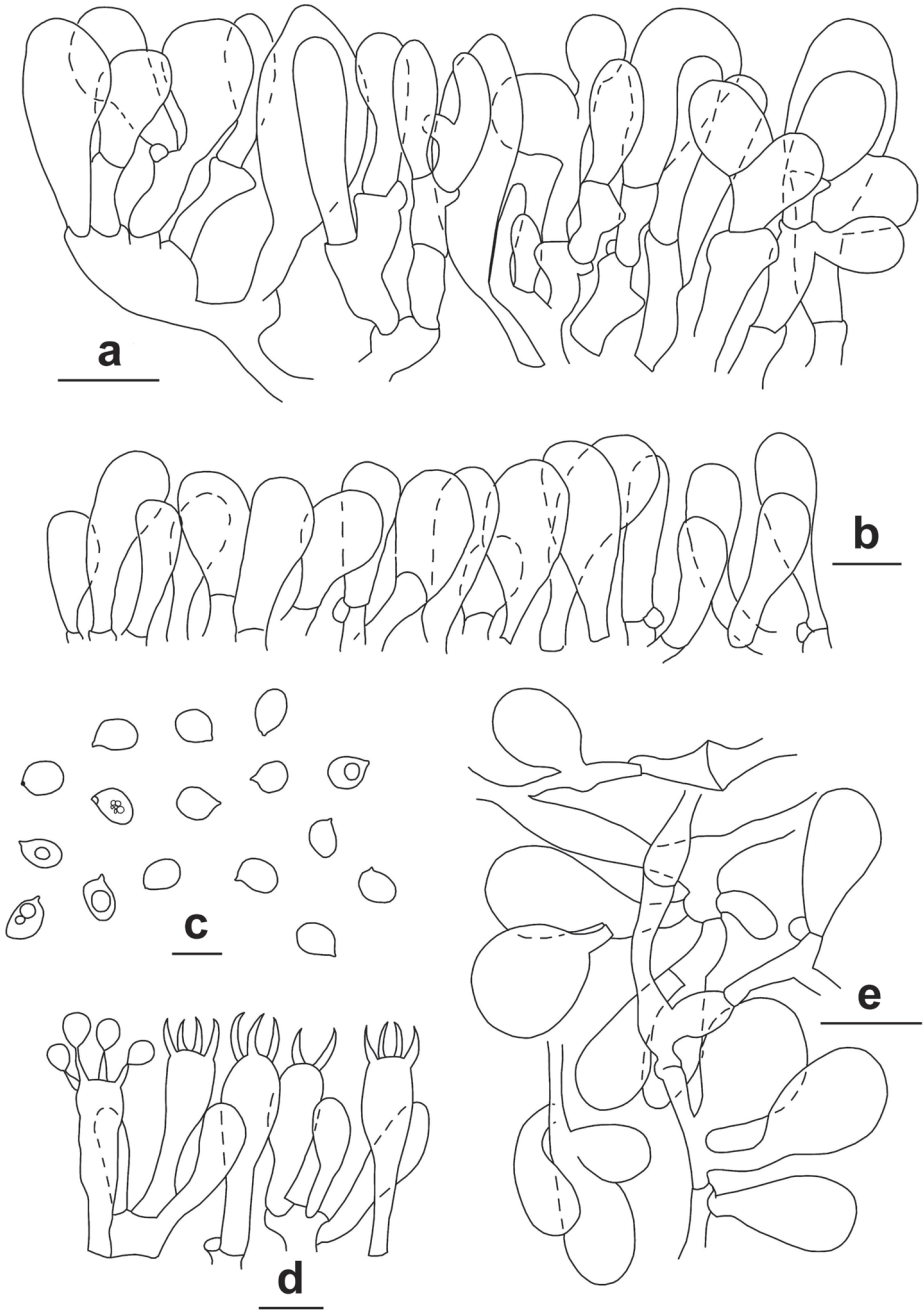

Figure 6. *Lepiota psalion*. Microscopic features (CAG P.11_9/7.68) **a** Elements of the pileus covering **b** Cheilocystidia **c** Spores **d** Basidia **e** Elements of the annulus. Scale bars: 20 μm (**a, e**); 10 μm (**b, d**); 5 μm (**c**). Drawings by A. Tatti.

gressing upward; minutely silky fibrillose along all length; with whitish [Pale pinkish buff (Plate XXIX 17".o-y./f) HTML ede2d4], ascending and often incomplete annulus on the upper part of the stipe, sometimes disappearing in age; often with minute white rhizomorphs. *Lamellae* 2–3(4) mm wide, l = 1–3(4), free, crowded, at first white, soon with evident pinkish tints [Cream-Buff (Plate XXX 19" .yo-y /d) HTML dfc38c to Clay-Color Plate (XXIX 17" .o-y.) HTML ce9b44]; edge finely granulose. *Context* elastic, whitish, pink-brown towards the stipe base; without specific smell and taste. *Spore-print* pale cream.

Microcharacters (Figs 5, 6). *Spores* [700, 6, 2] (2.7–)3.5–4.3(–4.9) × (2.0–)2.6–3.2(–3.9) µm, on average 3.9 × 2.9 µm, Q = (1.03–)1.23–1.49(–1.78), Qav = 1.36, from broadly ellipsoid to ellipsoid, hyaline, thin-walled, smooth, not verruculose in Melzer's reagent, binucleate, not metachromatic in Cresyl Blue, nonamyloid, non-dextrinoid, cyanophilic in Cotton Blue (Figs 5e, f, 6c). *Basidia* mainly 4-spored, (15.5–)17.1–21(–22.0) × (4.2–)4.7–5.8 (–6.0) µm (*n* = 54), rarely 1- or 2-spored, clavate, hyaline, thin-walled; sterigmata (2.6–) 3.0–4.2 (–4.9) × (0.5–)0.6–1.1(–1.2) µm (*n* = 67) (Fig. 6d). *Lamella edge* sterile. *Cheilocystidia* (10.0–)13.7–21.1 (–26.3) × (4.6–)6.2–8.7(–10.0) µm (*n* = 84), numerous and crowded, hyaline, thin-walled, various in shape, mostly clavate to subutriform, occasionally subfusiform, subcapitulate (Figs 5c, 6b). *Pleurocystidia* absent. *Pileus covering* a (140.7–)153.7–179.1(–201.1) µm (*n* = 16) thick hymeniderm with transition to an epithelium (Figs 5a,b, 6a), with up to 2(or 3) colourless elements on top of each other; terminal elements not tightly packed, (10.4–)18.0–53.6(–62.3) × (3.9–)7.7–19.3(–24.0) µm (*n* = 62), vesiculose, sphaeropedunculate to clavate-pyriform, utriform; slightly thick-walled (walls ca 0.5 µm), with walls embedded in a thin gelatinous matrix; subpellis composed of densely arranged and branching cylindrical hyphae, (21.3–)49.0–108.5(–136.8) × (3.8–)4.5–8.8(–9.7) µm (*n* = 38). *Pileitrama* of cylindrical hyphae, (33.1–)42.1–93.2(–111.8) × (2.7–)4.3–9.8(–14.4) µm (*n* = 45). *Hymenophoral trama* subregular, consisting of cylindrical hyphae (33.8–)36.5–64.4(–83.1) × (6.0–)7.6–15.8(–17.3) µm (*n* = 61). *Stipe covering* consisting of cylindrical hyphae, (23.8–)80.1–214.4(–370.8) × (2.6–)5.4–12.1(–15.4) µm (*n* = 58). *Stipe trama* consisting of cylindrical hyphae, (21.8–)58.5–178.9(–302.7) × (2.5–) 3.3–11.6(–12.5) µm (*n* = 32). *Caulocystidia* absent. *Partial veil* (annulus) composed of cylindrical elements, (21.1–)27.5–52.7(–94.7) × (2.2–)2.9–4.8(–8.5) µm (*n* = 36) with terminal clavate elements, (12.4–)17.9–34.0(–40.3) × (8.4–)10.6–17.7(–19.8) µm (n = 60) (Figs. 5d, 6e). *Clamp-connections* present and abundant everywhere.

Ecology and distribution. Gregarious on bare soil, in gardens and parks; so far known only from the type locality (Austria) and Sardinia (Italy).

Collections examined. Italy, Sardinia, Cagliari, Botanical Garden, 6 basidiomes growing among the *Searsia*/*Rhus* sp. litter, calcareous soil, 17 January 2017, Alessia Tatti and Giacomo Calvia (CAG P.11_9/7.68). Austria, Wien-Lobau, N. Uferhaus, 23 August 1985, Anton Hausknecht (WU 5152, holotype) (Fig. 4).

***Lepiota recondita* Tatti, Huijser & Vizzini, sp. nov.**
MycoBank No: MB 829963
Figs 7–9

Holotype. The Netherlands, prov. Limburg, Valkenburg, Schaelsberg, 02 September 2004, Henk A. Huijser (TR gmb 01482).

Etymology. From the Latin "reconditus", meaning hidden, forgotten, which refers to its resemblance with *L. psalion* with which it was confused.

Diagnosis. It is distinguished from *Lepiota psalion* by larger spores (3.7–)4.4–5.4(–5.9) × (2.4–)2.9–3.6(–4.3) µm, versiform cheilocystidia and different nrITS and nrLSU sequences.

Description. Macrocharacters (Fig. 7). *Pileus* 9–26 mm wide, at first slightly obtusely campanulate, hemispherical-trapezoid or broadly conical, later plano-convex to applanate-expanded, subumbonate, with a shallow umbo; not hygrophanous; margin not striated, slightly exceeding the lamellae when young, sinuous-undulate, entire or slightly fringed with age, with minute adhering remnants of partial veil when young; surface dry, at first smooth, later irregularly cracking around centre into concentric non-uplifted squamules; pinkish-light brown at centre from [Light Pinkish Cinnamon (Plate XXIX, 15".Y-O./d) HTML f19b5f] to [Mikado brown (Plate XXIX 13".OY-O./i), HTML 9f5425] or [Sayal Brown (Plate XXIX, 15".Y-O./i) HTML bc662d], paler towards the margin: [Capucine Bluff (Plate III, 13.OY-O./f) HTML fee6cc] or [Orange Pink (Plate II, 11.ORANGE/f) HTML ecc8a3]. *Stipe* 26–47 × 1.5–3 mm, central, cylindrical, at first white, becoming pink-brown with manipulation [Pinkish Cinnamon (Plate XXIX, 15".Y-O./b) HTML e1934f]; minutely silky fibrillose along all length; with whitish, ascending and often incomplete annulus on the upper part of the stipe, sometimes disappearing in age; often with minute white rhizomorphs. *Lamellae* free, crowded, l = 1–3, at first white, soon with evident yellowish tints [Catrige Buff (Plate XXX 19" .yo-y /f) HTML cdaf68] becoming [Honey Yellow (Plate XXX 19".YO-Y) HTML de9e42] when dry. *Context* elastic, whitish, smell weak, *Lepiota cristata*-like, taste not recorded. *Spore-print* whitish.

Microcharacters (Figs 8, 9). *Spores* [350, 6, 2] (3.7–)4.4–5.4(–5.9) × (2.4–)2.9–3.6(–4.3) µm, on average 4.8 × 3.3 µm, Q = (1.1–)1.3–1.7(–2.0), Qav = 1.5, from subglobose to oblong, mainly ellipsoid, hyaline, thin-walled, smooth, not verruculose in Melzer's reagent, binucleate, not metachromatic in Cresyl Blue, nonamyloid, non-dextrinoid, cyanophilic in Cotton Blue (Figs 8f, 9c). *Basidia* mainly 4-spored, (15.8–)17.4–25.4(–28.6) × (5.7–)6–7.3(–8.8) µm (*n* = 60), sometimes 1–2-spored, clavate, hyaline, thin-walled (Fig. 9d); sterigmata (1.9–)2.4–4.2(–4.8) × (0.4–)0.6–1.2(–1.5) µm (*n* = 70). *Lamella edge* sterile. *Cheilocystidia* (20.1–)25.4–44(–50.0) × (3.2–)7.2–10.4(–12.0) µm (*n* = 66), numerous and crowded, hyaline, thin-walled, various in shape, mostly clavate, cylindrical-clavate, sphaeropedunculate to submoniliform, occasionally pyriform, cylindrical (Figs 8b–d, 9b). *Pleurocystidia* absent.

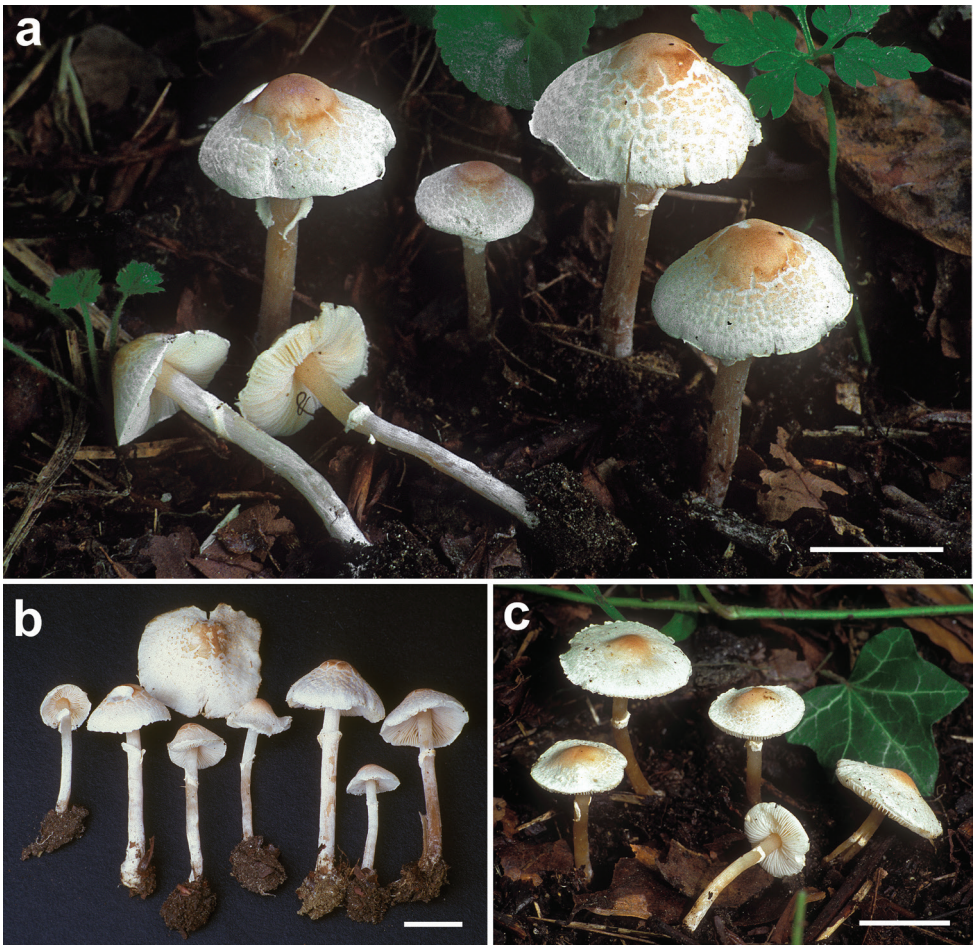

Figure 7. *Lepiota recondita*. Fresh basidiomes **a–b** (TR gmb 01482, holotype) **c** (TR gmb 01481, paratype). Scale bars= 10 mm. Photographs by H.A. Huijser.

Pileus covering hymenidermic: terminal elements not tightly packed, (17–)24.7–51.1(–59.6) × (8.1–)10–14(–27.3) µm (*n* = 70), vesiculose, sphaeropedunculate to clavate-pyriform (Figs 8a, 9a); slightly thick-walled (walls ca 0.5 µm), with walls embedded in a thin gelatinous matrix; subpellis composed of densely arranged and branching cylindrical hyphae, (40.6–)47.0–118.3(–156.2) × (5.8–)7.6–16.2(–17.1) µm (*n* = 20) and containing scattered ramified oleiferous hyphae, (1.5–)1.8–5.3(–8.0) µm wide (*n* = 30). *Hymenophoral trama* subregular, consisting of ovate hyphae (20.9–)21.1–40.3(–42) × (7–)9.6–13(–14.5) µm (*n* = 12). *Stipe covering* and *trama* indistinguishable, consisting of cylindrical hyphae, (55.3–) 67.0–165.7 (–213.0) × (5.5–)7.6–15.0(–21.0) µm. *Caulocystidia* absent. *Partial veil* (annulus) composed of cylindrical elements, (7.2–)22.3–59(–70.0) × (2.0–)2.5–4.2(–4.7) µm (*n* = 20) with terminal clavate elements, (10.1–)12.4–26.7(–38.1) × (7.0–)9.5–16.7(–28.4) µm (*n* = 40) (Figs 8e, 9e). *Clamp-connections* present and abundant everywhere.

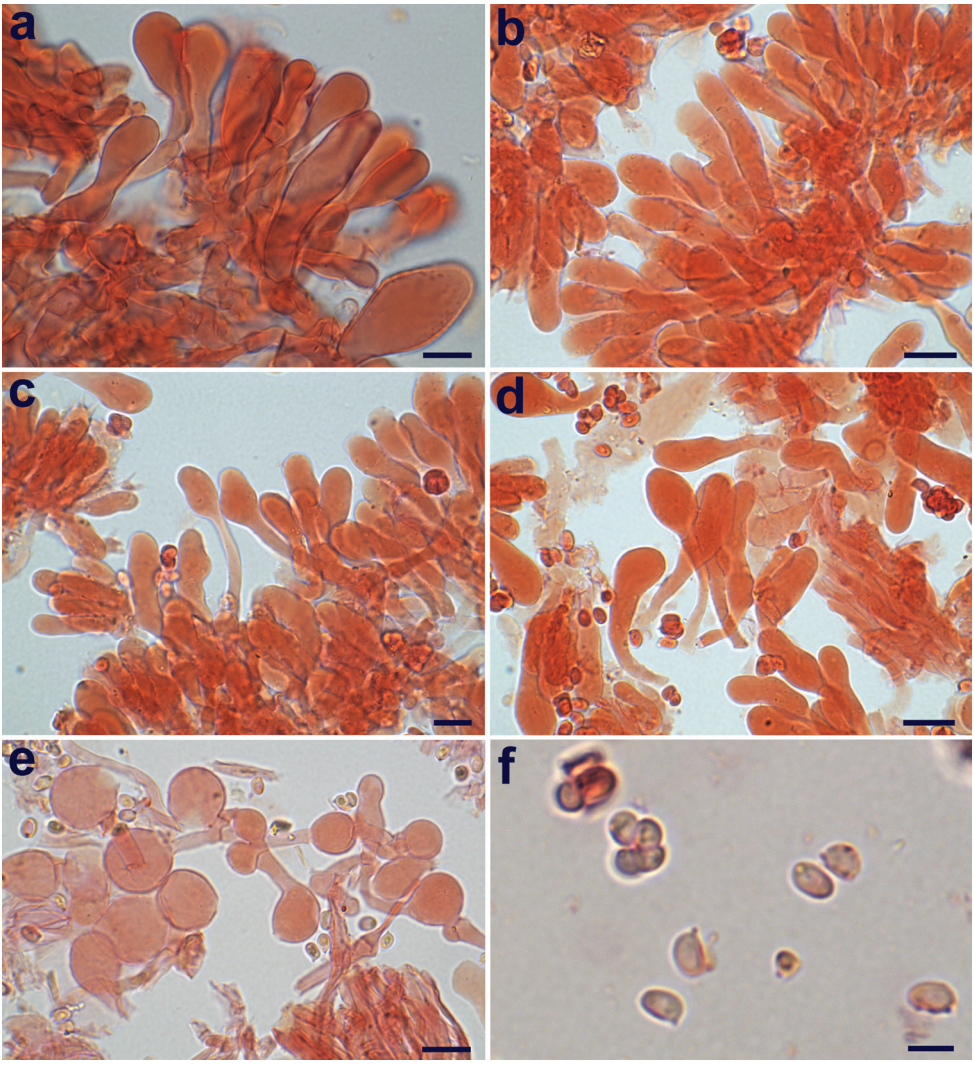

Figure 8. *Lepiota recondita*. Microscopic features (in ammoniacal Congo red, TR gmb 01482, holotype) **a** Elements of the pileus covering **b–d** Cheilocystidia **e** Elements of the annulus **f** Spores. Scale bars: 10 µm (**a–e**); 5 µm (**f**). Photographs by A. Tatti.

Ecology and distribution. Gregarious on rich in nutrients and lime (marl) bare soil, in a mixed deciduous forest; so far known only from the type locality.

Collections examined. The Netherlands, Limburg province, Valkenburg, Schaelsberg, man-made (anthropized) hilly grove with mainly deciduous trees (*Quercus*, *Fagus*, *Corylus*, *Fraxinus*, *Robinia*, *Prunus*, *Sambucus*), together with *Lepiota tomentella*, *L. poliochloodes*, *Melanophyllum eyrei*, and *Limacella ochraceolutea*, 22 September 2001, Henk A. Huijser (TR gmb 01481, paratype); *ibidem*, 02 September 2004, Henk A. Huijser (TR gmb 01482, holotype).

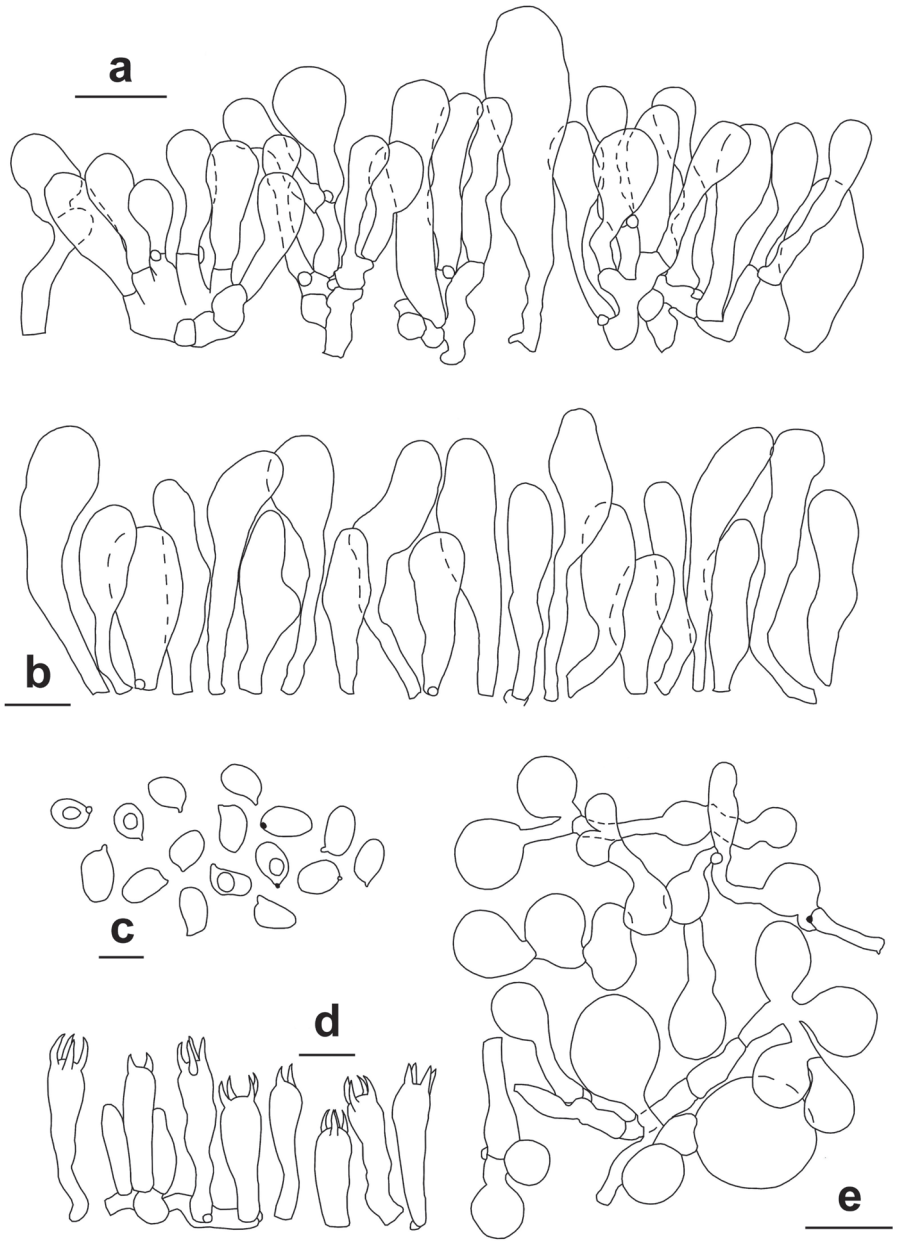

Figure 9. *Lepiota recondita*. Microscopic features (TR gmb 01482, holotype) **a** Elements of the pileus covering **b** Cheilocystidia **c** Spores **d** Basidia **e** Elements of the annulus. Scale bars: 20 μm (**a, e**); 10 μm (**b, d**); 5 μm (**c**). Drawings by A. Tatti.

***Lepiota sinorecondita* ad interim**
Fig. 10

Description. The specific epithet is a combination of Medieval Latin "sino" (which means Chinese) and "recondita", referring to the strong affinity of the Chinese taxon to the European *L. recondita*.

Basidiomata small (Fig. 10a). *Pileus* 9–17 mm wide, expanding to convex with obtuse umbo; at centre on umbo smooth, dark yellowish brown to dark brown, around umbo split up into pale brown concentrically arranged patches on dirty white to cream background, paler and smaller towards margin. *Stipe* 35–37 × 1–4 mm, subcylindrical or attenuate, slightly inflated at base; hollow, dirty white and glabrous at the apical part, surface whitish, covered white, tomentose at lower part, with white mycelial cords at base; annulus membranous, superior, whitish on upper surface, with small yellowish brown to brownish squamules on lower whitish surface. *Lamellae* free, cream, yellow to brown when dry, crowded with lamellulae, edge wavy.

Spores [60,3,1] (4.0–)4.5–5.5 × 2.5–3.0(–3.5) µm, Q = 1.50–1.80(–1.83), Qav = 1.64 (Fig. 10b), ellipsoid to oblong in side and front view, without suprahilar depression, sometimes with straight adaxial side; hyaline, smooth, non-dextrinoid, congophilous but very weakly, slightly reddish purple in Cresyl Blue. Basidia 17–22 × 5–6

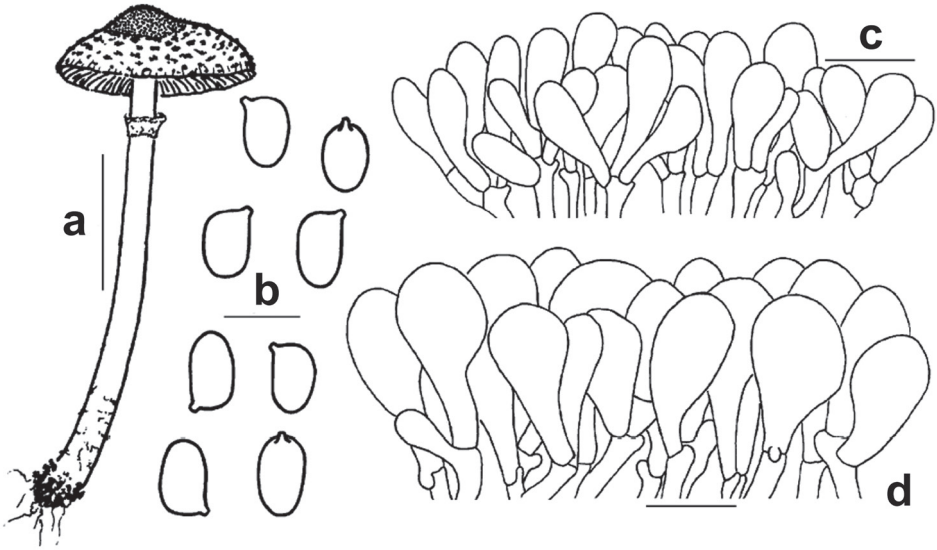

Figure 10. *Lepiota sinorecondita* (HMJAU 3799) **a** Basidiome **b** Spores **c** Cheilocystidia **d** Elements of the pileus covering. Scale bars: 10 mm (**a**); 5 µm (**b**); 20 µm (**c–d**). Drawings by J.F. Liang.

μm, narrowly clavate or subcylindrical, 4-spored. *Lamella edge* sterile. *Cheilocystidia* 21–40 × 6–13 μm, clavate to narrowly clavate, rarely broadly clavate, colourless, hyaline, thin-walled (Fig. 10c). *Pleurocystidia* absent. *Pileus covering* a hymeniderm made up of broadly clavate, clavate to obpyriform terminal elements, 18–50 × 10–20 μm, with pale yellowish brown intracellular pigment (Fig. 10d). *Clamp-connections* present in all tissues.

Collection examined. China, Jilin Province, Changchun City, Jinyuetan Park, 7 July 2005, Wang Jianrui (HMJAU 3799).

Ecology and distribution. Solitary, terrestrial, on the ground in a larch forest in summer and autumn. So far known only from China.

Discussion

Distinguishing characters of *L. psalion* and allied species

The morphological differences among the *Lepiota* species with hymeniform pileus covering are often subtle (Vellinga and Huijser 1999; Vellinga 2010), but nrlTS sequence data support the morphologically recognized species (Vellinga 2010; Vizzini et al. 2014a, b; Justo et al. 2015; Qasim et al. 2015; Hosen et al. 2016).

Lepiota psalion is distinguished by having a non-smooth pileus with concentric non-uplifted squamules, a distinct annulus, and mostly clavate cheilocystidia (Vellinga and Huijser 1999; Vellinga 2001; our observations). The annulus is quite evanescent (Fig. 3) mainly because it is predominantly composed of inflated elements (Figs 5d, 6e).

Lepiota "cf. *rufipes* f. *phaeophylla*" sensu Winterhoff and Bon (1994) and *L. rufipes* sensu Babos (1974), Wasser (1980), and Krieglsteiner (1991), all with a distinct annulus, are probably referable to *L. psalion* (Vellinga and Huijser 1999; Vellinga 2001), but see below.

The phylogenetically closest species are *L. coloratipes* (= *L. rufipes* ss. Auct. europ. non ss. orig.) and *L. sanguineofracta* (Fig. 1). *Lepiota coloratipes* differs from *L. psalion* in having a usually smooth pileus surface, a very evanescent partial veil not forming an annulus but leaving fibrillose remnants on stipe surface, a stipe with reddish tinges at base, the presence of oil droplets in all tissues (including spore surface), the hymeniform pileus covering consisting of very tightly arranged clavate to sphaeropedunculate elements, the presence of uninucleate spores which are often verruculose in Melzer's reagent, versiform cheilocystidia (mostly lageniform or lecythiform), and the presence of caulocystidia (Bon 1981, 1993; Candusso and Lanzoni 1990; Vellinga and Huijser 1999; Vellinga 2001; Vizzini et al. 2014b). *Lepiota sanguineofracta*, recently described from Italy, is characterized by a micaceous but not squamulose pileus surface with distinct green tinges when mature, a fugacious partial veil not forming an annulus, a stipe with reddish tinges towards the base, the context smelling of dried rose petals, basidiome surfaces and context strongly reddening on handling, binucleate spores, and versiform cheilocystidia (clavate to subutriform, subfusiform) (Vizzini et al. 2014a).

The other morphologically allied species of *Lepiota* with a hymeniform pileus covering, ellipsoid spores, and a well-formed annulus, phylogenetically far from *L. psalion* (Figs 1, 2), show distinctive morphological traits: *L. apatelia* Vellinga & Huijser, *L. cristatoides* Einhell. (both from Europe), and *L. thiersii* Sundb. (from western North America) have no cheilocystidia (Einhellinger 1973; Sundberg 1989; Vellinga and Huijser 1999; Vellinga 2001, 2010; Hausknecht and Pidlich-Aigener 2005; Kosakyan et al. 2008; Mertens 2010; Gierczyk et al. 2011). *Lepiota neophana* (including var. *europaea* Bizio & Migl. and f. *papillata* Migl. & L. Perrone) shows a smooth pileus surface with a buff to dark-brown and umbonate centre, very rare clamp-connections in the pileus trama and no cheilocystidia (Anonymous 1992; Bizio et al. 1993; Vellinga and Huijser 1999; Vellinga 2010). Finally, pale collections of *L. lilacea* Bres. are distinguished by whitish lamellae, an annulus with lilac-brown tinges on the lower part and margin, and metachromatic (in Cresyl Blue) up to 6 µm long spores (Bon 1981, 1993; Migliozzi and Clericuzio 1989; Candusso and Lanzoni 1990; Vellinga 2001).

The *Lepiota psalion* complex

Lepiota psalion was established by Vellinga and Huijser (1999) based on an Austrian collection made by A. Hausknecht on 23 August 1985 (WU 5152) and determined by M. Bon as *L. rufipes* fo. *annulata* ined. (Fig. 4a). The extended description they provided is heterogeneous: the macromorphology was taken from Krieglsteiner (1991) who described a German collection as *L. rufipes*, collection considered by Vellinga and Huijser as *L. psalion*, while the micromorphology was based on the analysis of the holotype made by the same Dutch mycologists. NrITS and nrLSU sequences later deposited in GenBank as *L. psalion* were generated by Vellinga (2004, 2010) not from the holotype, but from three Dutch collections (vouchers 23-VIII-1999, 15-IX-1999, and hah6177, H.A. Huijser, herb. Huijser).

When the Sardinian specimens were collected, they were morphologically attributed to *L. psalion*, but when they were sequenced to obtain molecular evidence, they did not cluster either with the Dutch collections or with a collection named *L. psalion* from China (herb. HMJAU3799; Liang et al. 2011) (tree not shown). Consequently, we decided to request the holotype collection from WU and sequenced it. Phylogenetic analyses highlighted that Sardinian collection and the holotype are conspecific (Figs 1, 2) and sister to *L. coloratipes* (Fig. 1). Molecular data so confirm *L. psalion* as independent species in the genus *Lepiota*; Dutch and Chinese collections are two distinct and yet undescribed new species, phylogenetically close (BPP = 0.97; MLB = 91%) to *L. thiersii* (Fig. 1). Unfortunately, the collections of the Dutch taxon whose sequences are deposited in GenBank were subsequently lost (Vellinga, pers. comm.) but, based on two newly sequenced additional collections from the same original area of the Dutch taxon, the new species *L. recondita* is here described. As only one collection (consisting of three basidiomes) is available for the Chinese taxon, it was decided to propose it only as an *ad interim* species. Further collections will be necessary to describe it as a new species.

Lepiota psalion, *L. recondita*, *L.* "*sinorecondita*", *L. apatelia*, and *L. thiersii* constitute a homogeneous morphology-based but not monophyletic group, here named the "*L. psalion* complex", which is circumscribed by a set of shared characters: a pileus surface breaking into small squamules, well-formed white partial veil (usually forming an annulus, but see *L. apatelia*), hymeniform pileus covering, and ellipsoid spores.

An identification key for the taxa belonging to this complex is proposed below.

Key to the species of the *Lepiota psalion* complex

1 Cheilocystidia absent **2**
– Cheilocystidia present **3**
2 Smell farinaceous, annulus often adhering to pileus margin (as velar remnants), spores weakly dextrinoid ..... ***L. apatelia*** **(Europe)**
– Smell *L. cristata*-like, annulus usually ascending on stipe, spores non-dextrinoid ..... ***L. thiersii*** **(North America)**
3 Spores ellipsoid, on average = 3.9 µm long, Qav = 1.36 .... ***L. psalion*** **(Europe)**
– Spores ellipsoid to oblong, on average > 4.0 µm long, Qav > 1.4 ..... **4**
4 Cheilocystidia versiform, spores ellipsoid, Qav = 1.5, annulus entirely smooth ..... ***L. recondita*** **(Europe)**
– Cheilocystidia mainly clavate, spores oblong, Qav = 1.64, annulus covered by minute yellowish brown squamules on lower surface..... ..... ***L. sinorecondita ad int.*** **(China)**

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References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. Journal of Molecular Biology 215: 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Alvarado P, Moreno G, Vizzini A, Consiglio Manjón JL, Setti L (2015) *Atractosporocybe*, *Leucocybe* and *Rhizocybe*, three new clitocyboid genera in the Tricholomatoid clade (Agaricales) and notes on some whitish species of *Clitocybe* and *Lepista*. Mycologia 107(1): 123–136. https://doi.org/10.3852/13-369

Anonymous (1992) Nova taxa in *Lepiota* s.l. Bollettino dell' Associazione Micologica ed Ecologica Romana 9(27): 44–45.

Babos M (1974) Studies on Hungarian *Lepiota* s.l. species, IV. Annales Historico-Naturales Musei Nationali Hungarici 66: 65–75.

Bizio E, Migliozzi V, Zecchin G (1993) La sezione *Integrellae* (Kühner ex M. Bon) M. Bon del genere *Lepiota* (Persoon) Gray. Rivista di Micologia 36: 223–244.

Bon M (1981) Clé monographique des Lépiotes d'Europe (Agaricaceae, tribus Lepioteae et Leucocoprineae). Documents Mycologiques 11(43): 1–77.

Bon M (1991) Les genres *Echinoderma* (Locq. ex Bon) st. nov. et *Rugosomyces* Raithelhuber ss lato. Documents Mycologiques 21(82): 61–66.

Bon M (1993) Flore mycologique d'Europe, 3. Les lépiotes. Lepiotaceae Roze. Documents Mycologiques Mémoire hors série no. 3. L'Association d'Ecologie et Mycologie, Lille.

Candusso M, Lanzoni G (1990) *Lepiota* s.1. Fungi Europaei 4. G. Biella, Saronno.

Darriba D, Taboada GL, Doallo R, Posada D (2012) "jModelTest 2: more models, new heuristics and parallel computing". Nature Methods 9(8): 772. https://doi.org/10.1038/nmeth.2109

Drummond AJ, Ashton B, Cheung M, Heled J, Kearse M, Moir R, Stones-Havas S, Thierer T, Wilson A (2010) Geneious v. 5.3. http://www.geneious.com [2017-4-12]

Einhellinger A (1973) Die Pilze der Pflanzengesellschaften des Auwaldgebietes der Isar zwischen München und Grüneck. Berichte der Bayerischen Botanischen Gesellschaft 44: 5–100.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

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

Gierczyk B, Kujawa A, Szczepkowski A, Chachuła P (2011) Rare species of *Lepiota* and related genera. Acta Mycologica 46: 137–178. https://doi.org/10.5586/am.2011.010

Hausknecht A, Pidlich-Aigener H (2005) Lepiotaceae (Schirmlinge) in Österreich 2. Die Gattung *Lepiota*. Österreichische Zeitschrift für Pilzkunde 14: 41–78.

Horton TR (2006) The number of nuclei in basidiospores of 63 species of ectomycorrhizal Homobasidiomycetes. Mycologia 98: 233–238. https://doi.org/10.1080/15572536.2006.11832695

Hosen MI, Li TH, Ge ZW, Vellinga EC (2016) *Lepiota bengalensis*, a new species of *Lepiota* section *Lilaceae* from Bangladesh. Sydowia 68: 187–192. https://doi.org/10.12905/0380.sydowia68-2016-0187

Johnson J (1999) Phylogenetic relationships within *Lepiota* sensu lato based on morphological and molecular data. Mycologia 91: 443–458. https://doi.org/10.2307/3761345

Justo A, Angelini C, Bizzi A (2015) Two new species and a new record of *Lepiota* (Basidiomycota, Agaricales) from the Dominican Republic. Mycological Progress 14: 56. https://doi.org/10.1007/s11557-015-1080-9

Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30: 3059–3066. https://doi.org/10.1093/nar/gkf436

Kosakyan A, Ur Y, Wasser SP, Nevo E (2008) Rare and noteworthy lepiotaceous species (Basidiomycota, Agaricales, Agaricaceae) from Israel. Mycotaxon 103: 59–74.

Krieglsteiner GJ (1991) Über neue, seltene, kritische Makromyzeten in Westdeutschland (ehemalige BR Deutschland, Mitteleuropa). XII. Röhrlinge und Blätterpilze. Beiträge zur Kenntnis der Pilze Mitteleuropas 7: 61–79.

Liang JF, Yang ZL, Xu DP (2011) A new species of *Lepiota* from China. Mycologia 103(4): 820–830. https://doi.org/10.3852/10-216

Mertens C (2010) Deux taxons nouveaux pour la Belgique: *Marasmius favrei* var. *sorbi* et *Lepiota apatelia*. Revue du Cercle de Mycologie de Bruxelles 10: 43–48.

Migliozzi V, Clericuzio M (1989) Alcune lepiotee nell'area mediterranea: *Leucoagaricus macrorhizus* var. *pinguipes*, *Lepiota lilacea* f. *pallida*, *Lepiota ignicolor*. Micologia e Vegetazione Mediterranea 4(1): 29–40.

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, LA, 1–8. https://doi.org/10.1109/GCE.2010.5676129

Qasim T, Khalid AN, Vellinga EC, Razaq A (2015) *Lepiota albogranulosa* sp. nov. (Agaricales, Agaricaceae) from Lahore, Pakistan. Mycological Progress 14(5/24): 1–6. https://doi.org/10.1007/s11557-015-1037-z

Rehner SA, Buckley E (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97: 84–98. https://doi.org/10.1080/15572536.2006.11832842

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

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

Sundberg WJ (1989) *Lepiota* sensu lato in California. III. Species with a hymeniform pileipellis. Mycotaxon 34: 239–248.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA 6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30(12): 2725–2729. https://doi.org/10.1093/molbev/mst197

Thiers B (2019) [continuously updated] Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/science/ih [2019-2-11]

Vellinga EC, Huijser HA (1999) Studies in *Lepiota* I. Species with a hymeniform pileus covering. Belgian Journal of Botany 131[1998]: 191–210.

Vellinga EC (1988) Glossary. In: Bas C, Kuyper ThW, Noordeloos ME, Vellinga EC (Eds) Flora Agaricina Neerlandica. Vol. 1. A.A. Balkema, Rotterdam, 54–64.

Vellinga EC (2001) *Lepiota* (Pers.: Fr.) S.F. Gray. In: Noordeloos ME, Kuyper TW, Vellinga EC (Eds) Flora Agaricina Neerlandica. Vol. 5. A.A. Balkema Publishers, Lisse, 109–151.

Vellinga EC (2003) Phylogeny of *Lepiota* (Agaricaceae) – evidence from nrITS and nrLSU sequences. Mycological Progress 2: 305–322. https://doi.org/10.1007/s11557-006-0068-x

Vellinga EC (2004) Genera in the family Agaricaceae: evidence from nrITS and nrLSU sequences. Mycological Research 108: 354–377. https://doi.org/10.1017/S0953756204009700

Vellinga EC (2010) *Lepiota* in California: species with a hymeniform pileus covering. Mycologia 102: 664–674. https://doi.org/10.3852/09-180

Vellinga EC, Sysouphanthong S, Hyde KD (2011) The family Agaricaceae: phylogenies and two new white-spored genera. Mycologia 103: 494–509. https://doi.org/10.3852/10-204

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

Vizzini A, Ercole E, Voyron S (2014a) *Lepiota sanguineofracta* (Basidiomycota, Agaricales), a new species with a hymeniform pileus covering from Italy. Mycological Progress 13: 683–690. https://doi.org/10.1007/s11557-013-0950-2

Vizzini A, Liang JF, Jančovičová S, Adamčík S, Ercole E, Contu M, Yang ZL, Vellinga EC (2014b) *Lepiota coloratipes*, a new species for *Lepiota rufipes* ss. auct. europ. non ss. orig. Mycological Progress 13: 171–179. https://doi.org/10.1007/s11557-013-0905-7

Wasser SP (1980) Flora gribov Ukrainy, Agarikovye griby. [Fungal Flora of the Ukraine: Agaricoid Fungi]. Naukova Dumka, Kiev, 327 pp.

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

Winterhoff W, Bon M (1994) Zum Vorkommen seltener Schirmlinge (*Lepiota* s.l.) im nördlichen Oberrheingebiet. Carolinea 52: 5–10.

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Four new East Asian species of *Aleurodiscus* with echinulate basidiospores

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Abstract

Four new species of *Aleurodiscus* sensu lato with echinulate basidiospores are described from East Asia: *A. alpinus*, *A. pinicola*, *A. senticosus*, and *A. sichuanensis*. *Aleurodiscus alpinus* is from northwest Yunnan of China where it occurs on *Rhododendron* in montane habitats. *Aleurodiscus pinicola* occurs on *Pinus* in montane settings in Taiwan and northwest Yunnan. *Aleurodiscus senticosus* is from subtropical Taiwan, where it occurs on angiosperms. *Aleurodiscus sichuanensis* is reported from southwest China on angiosperms in montane environments. Phylogenetic relationships of these four new species were inferred from analyses of a combined dataset consisting of three genetic markers, viz. 28S, nuc rDNA ITS1-5.8S-ITS2 (ITS), and a portion of the translation elongation factor 1-alpha gene, *TEF1*.

Keywords

China, corticioid fungi, Taiwan, taxonomy, wood-decaying fungi

Introduction

The genus *Aleurodiscus* Rabenh. ex J. Schröt. belongs to the Stereaceae Pilát of the Russulales Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David. However, whether to keep *Aleurodiscus* in a broad or a narrow sense has long been a puzzling issue in the taxonomy of Basidiomycota (Boidin et al 1985; Núñez and Ryvarden 1997; Wu et al. 2001; Larsson and Larsson 2003; Miller et al. 2006; Larsson 2007; Wu et al. 2010), because diagnostic characters are highly variable among species. *Aleurodiscus* s.l. is characterized by cupulate,

effused or effused-reflexed basidiocarps, a monomitic or dimitic hyphal system with simple-septate or clamped generative hyphae, smooth or ornamented amyloid basidiospores, and sterile organs such as acanthophyses, gloeocystidia, hyphidia, and dendrohyphidia (Núñez and Ryvarden 1997). The characteristics used for separating segregate genera within *Aleurodiscus* s.l. (*Acanthobasidium* Oberw., *Acanthofungus* Sheng H. Wu et al, *Acanthophysellum* Parmasto, *Aleurobotrys* Boidin, *Aleurodiscus* s.s., *Aleurocystidiellum* P.A. Lemke, *Gloeosoma* Bres., and *Neoaleurodiscus* Sheng H. Wu) as well as *Stereum* Hill ex Pers. and *Xylobolus* P. Karst. were provided by Wu et al. (2001, table 1) and Wu et al. (2010, table 1). Currently, 169 names are recorded under *Aleurodiscus*, of which about 85 taxa are generally accepted worldwide (http://www.indexfungorum.org/). Since the year 2000, new species of *Aleurodiscus* s.l. have been proposed by Simpson and Grgurinovic (2003), Hjortstam et al. (2009), Ryvarden et al. (2012), Gorjón et al. (2013), Maninder et al. (2014), Dai and He (2016), Dai et al. (2017a, b), Ghobad-Nejhad and Langer (2018), and Tian et al. (2018). Since the phylogenetic relationships of the taxa in *Aleurodiscus* s.l., as well as in the Stereaceae at large, are not resolved, we adopt a broad and inclusive generic concept of *Aleurodiscus* for the new taxa presented in this study.

During a two-decade long, ongoing survey of corticioid fungi from mainland China and Taiwan, we have found four new species of *Aleurodiscus* with echinulate basidiospores based on morphological characters. In addition, phylogenetic analyses of a nuclear rDNA 28S D1–D2 domains (28S) dataset and analyses of a second dataset consisting of three genetic markers – nuc rDNA 28S D1–D2 domains (28S), nuc rDNA ITS1-5.8S-ITS2 (ITS), and translation elongation factor 1-alpha (*TEF1*) – are performed to complement our morphological observations and place the newly described species in a molecular phylogenetic framework.

Materials and methods

Morphological and cultural studies

Macroscopic and microscopic studies were based on dried specimens. Color names from Rayner (1970) are capitalized. Thin free-hand sections of basidiocarps were prepared for microscopic study. For observations and measurements of microscopic characters, sections were mounted in 5% KOH to ensure rehydration. A blue-black color change with Melzer's reagent (IKI) indicates an amyloid reaction. Cotton blue (CB) was used as mounting medium to determine cyanophily. Sulphoaldehyde (SA) was used to detect a sulphuric reaction of gloeocystidia; a bluish black color change with SA indicates a positive reaction. The following abbreviations are used for basidiospore measurements: L = mean spore length with standard deviation, W = mean spore width with standard deviation, Q = variation in L/W ratio, and n = number of spores measured from each specimen. Apiculi and ornamentation were excluded in spore measurements. Living mycelia were isolated from the woody substratum beneath the basidiocarps, and were cultured on 1.5% malt extract agar (MEA). Fungal specimens and living cultures used in this study are deposited in the herbaria of the National Mu-

seum of Natural Science of ROC (TNM; Taichung City, Taiwan) and Beijing Forestry University (BJFC; Beijing, China).

DNA extraction, polymerase chain reaction (PCR), and sequencing

Dried specimens or the mycelial colonies cultured on MEA were used for DNA extraction, carried out with a Plant Genomic DNA Extraction Miniprep System (Viogene-Biotek Corp., New Taipei City, Taiwan). Liquid N and Tissue Lyser II (Qiagen, Hilden, Germany) were used to disrupt and homogenize the fungal tissues before DNA extraction process. The primer pairs ITS1/ITS4 or ITS1F/LR22 were used for the ITS region (White et al. 1990, Gardes and Bruns 1993), and LR0R/LR3 and LR0R/LR5 were used for the 28S region (Vilgalys and Hester 1990). Efdf/1953R and 983F/2218R were used to amplify a portion of the *TEF1* gene (Rehner & Buckley 2005; Matheny et al. 2007). PCR products were purified and directly sequenced by MB Mission Biotech Company (Taipei City, Taiwan). We examined the technical quality of the newly obtained sequences by comparison to entries in GenBank. Sequences were assembled using BioEdit v7.2.5 (Hall 1999). Newly obtained sequences (Supplementary Table 1) were submitted to either GenBank through the National Center for Biotechnology Information (NCBI) or DNA Data Bank of Japan (DDBJ) (Mashima et al. 2016, Benson et al. 2018).

Alignment and phylogenetic analyses

The newly generated sequences were added to the DNA sequence dataset employed by Dai and He (2016), so far the most inclusive alignments for analyzing *Aleurodiscus* s.l. based on three genetic markers. To achieve a comprehensive analysis, we also added some related taxa of the genera *Boidinia* Stalpers & Hjortstam, *Conferticium* Hallenb., *Gloeocystidiellum* Donk and *Megalocystidium* Jülich to the ingroup. We tried to include the type species of the genera as far as possible (Table 1). The phylogenetic tree of the 28S+ITS+ *TEF1* dataset was inferred through Maximum likelihood (ML) and Bayesian inference (BI) methods using RAxML v. 8.2.4 (Stamatakis 2014) and MrBayes v. 3.2.6 (Ronquist et al. 2012), respectively (Ronquist et al. 2012, Stamatakis 2014). The alignments were inferred in MAFFT v. 7 using the FFT-N-i strategy for 28S and *TEF1*, and Q-INS-i strategy for ITS. For the BI analysis, the best-fit model for each alignment partition was estimated by jModelTest 2 (Darriba et al. 2012) using the Akaike information criterion (AIC). For ML bootstrapping, the extended majority-rule consensus tree criterion was specified under a GTRGAMMA model with 1000 replicates. In the BI analysis, four MCMC chains were run simultaneously from a random starting tree for ten million generations. Trees were sampled every 1000 generations resulting in 10000 trees in the posterior distribution; the first 25% trees were discarded as the burn-in. Posterior probabilities (PP) were calculated based on the post-burn-in trees. ML bootstrap values (BS) and BI posterior probability (PP) values ≥ 50% and ≥ 0.7 are indicated at the nodes of the ML tree. The final sequence alignments and the phylogenetic trees are available at TreeBASE (S23581; www.treebase.org).

Table 1. List of species, specimens and sequences used in this study. Sequences generated in this study are shown in boldface.

Fungal species	Specimen or strain no.	DDBJ/GenBank/EMBL accession no.		
		ITS	28S	*TEF1*
Acanthobasidium bambusicola	He2357	KU559343	KU574833	–
Acanthofungus rimosus#	Wu9601-1	MF043521	AY039333	–
Acanthophysellum cerussatum	He20120920-3	KU559339	KU574830	KU992716
Aleurobotrys botryosus#	He2712	KX306877	KY450788	–
Aleurocystidiellum disciforme	He3159	KU559340	KU574831	KU992721
Aleurocystidiellum subcruentatum#	He2886	KU559341	KU574847	KU992720
Aleurodiscus alpinus	**Wu1407-59**	**MF043522**	**MF043527**	**–**
Aleurodiscus alpinus	**Wu1407-55***	**–**	**MF043526**	**LC269190**
Aleurodiscus alpinus	**Wu1407-61**	**MF043523**	**MF043528**	**–**
Aleurodiscus amorphus#	Ghobad-Nejhad-2464	KU559342	KU574832	KU992717
Aleurodiscus amorphus#	KHL4240	AF506397	AF506397	–
Aleurodiscus bambusinus	He 4261	KY706207	KY706219	**LC430911**
Aleurodiscus canadensis	Wu 1207-90	KY706203	KY706225	–
Aleurodiscus dextrinoideocerussatus	EL25-97	AF506401	AF506401	–
Aleurodiscus dextrinoideophyses	He 4105	MH109050	KY450784	–
Aleurodiscus effusus	He2261	KU559344	KU574834	KU992719
Aleurodiscus gigasporus	Wu 0108-15	KY706205	KY706213	–
Aleurodiscus grantii	HHB-14417	KU559363	KU574821	KU992708
Aleurodiscus grantii	HHB-14418	KU559364	KU574822	–
Aleurodiscus isabellinus	He 5283	MH109052	MH109046	**LC430912**
Aleurodiscus mesaverdense	FP-120155	KU559359	KU574817	–
Aleurodiscus mirabilis	Dai13281	KU559350	KU574839	KU992711
Aleurodiscus oakesii	He2243	KU559352	KU574840	–
Aleurodiscus oakesii	HHB11890-A-sp	KU559365	KU574823	–
Aleurodiscus pinicola	**Wu1106-16**	**MF043524**	**MF043529**	**–**
Aleurodiscus pinicola	**Wu1308-54***	**MF043525**	**MF043530**	**LC269191**
Aleurodiscus senticosus	**Wu1209-7***	**MH596849**	**MF043531**	**LC271169**
Aleurodiscus senticosus	**Wu1209-9**	**MH596850**	**MF043533**	**LC269192**
Aleurodiscus senticosus	**Wu9610-1**	**MH596851**	**MF043532**	**LC269193**
Aleurodiscus sichuanensis	**Wu0010-18***	**MH596852**	**MF043534**	**LC269194**
Aleurodiscus sichuanensis	He 4935	**LC430904**	**LC430907**	–
Aleurodiscus subroseus	He 4807	MH109054	MH109048	–
Aleurodiscus subroseus	He 4895	**LC430903**	**LC430910**	**LC430913**
Aleurodiscus tenuissimus	He3575	KX306880	KX842529	–
Aleurodiscus thailandicus	He 4099	KY450781	KY450782	–
Aleurodiscus tropicus	He3830	KX553875	KX578720	LC269195
Aleurodiscus verrucosporus	He 4491	KY450786	KY450790	–
Aleurodiscus wakefieldiae	He2580	KU559353	KU574841	KU992710
Boidinia macrospora	Wu9202-21	AF506377	AF506377	–
Conferticium heimii	CBS321.66	AF506381	AF506381	–
Conferticium ravum	NH13291	AF506382	AF506382	–
Gloeocystidiellum aspellum	LIN625	AF506432	AF506432	–
Gloeocystidiellum porosum#	**Wu 1608-176**	**LC430905**	**LC430908**	**–**
Gloeocystidiopsis cryptacanthus	KHL10334	AF506442	AF506442	–
Gloeocystidiopsis flammea#	CBS324.66	AF506437	AF506437	–
Heterobasidion parviporum	91605	KJ651503	KJ651561	KU985089
Megalocystidium chelidonium	LodgeSJ110.1	AF506441	AF506441	–
Megalocystidium leucoxanthum#	HK9808	AF506420	AF506420	–
Megalocystidium wakullum	Oslo-930107	AF506443	AF506443	–
Neoaleurodiscus fujii#	He2921	KU559357	KU574845	KU992709
Stereum complicatum	He2234	KU559368	KU574828	KU992706
Stereum hirsutum#	Wu 1109-127	**LC430906**	**LC430909**	–
Stereum ostrea	SHe2067	KU559366	KU574826	KU992703
Stereum sanguinolentum	He2111	KU559367	KU574827	KU992705
Xylobolus frustulatus	He2231	KU881905	KU574825	KU992704

* Holotype, # Generic type

Phylogeny results

The three-marker dataset was composed of 55 taxa and 2502 sites including gaps (of which 29% were parsimony-informative): 953 characters for 28S, 949 characters for ITS and 600 characters for *TEF1*. Missing sequences were treated as missing data (Table 1). After the ML search, 1000 rapid bootstrap inferences were executed. For the BI analysis, the GTR+I+G model was chosen as the best model for the 28S and *TEF1* alignments, and GTR+G was chosen for the ITS alignment. After 2.79 million

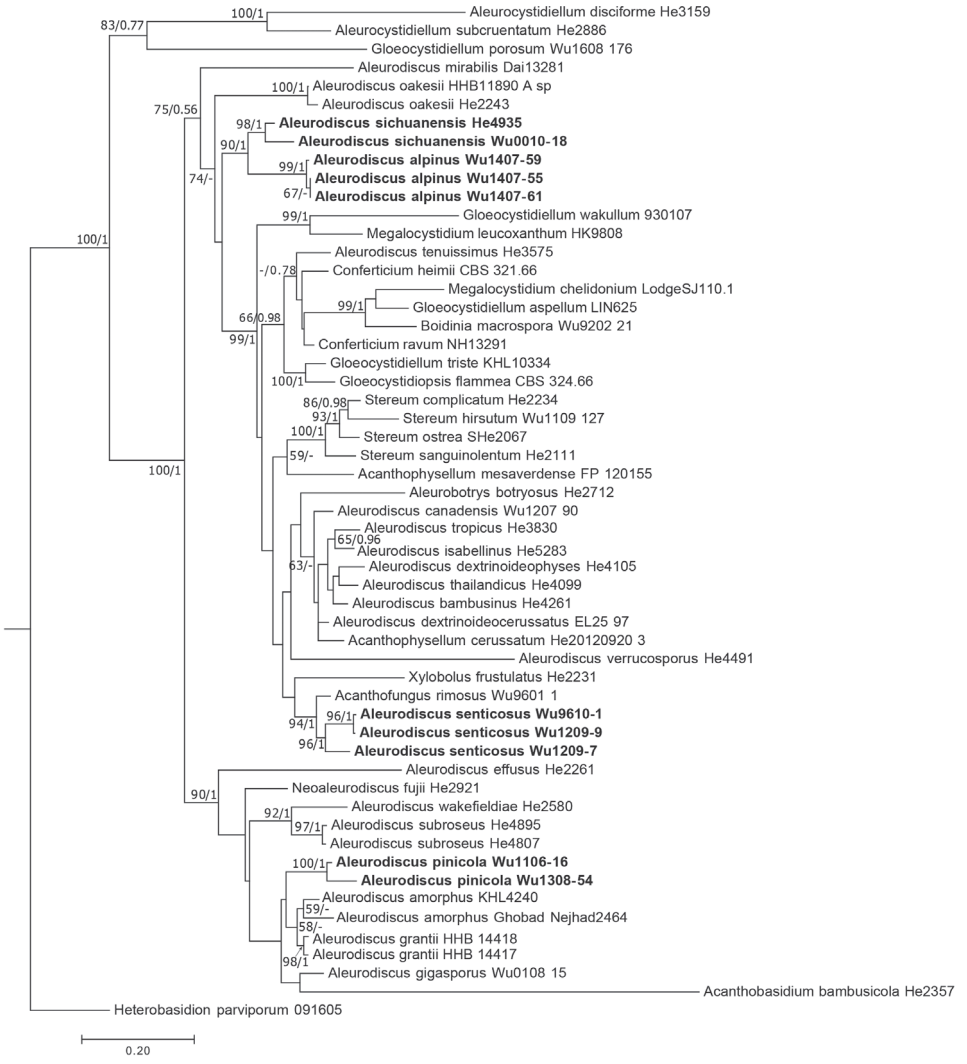

Figure 1. ML tree of *Aleurodiscus* and related genera of Stereaceae inferred from the 28S-ITS-*TEF1* markers. ML bootstrap values ≥ 50% and PP ≥ 0.7 from the Bayesian analysis are indicated at internodes. The presented new species are shown in boldface type.

generations, average standard deviation of split frequencies fell to 0.0099. Only the ML tree is shown given that the ML and BI analyses yielded similar topologies. The ML tree of the combined 28S, ITS and *TEF1-α* dataset (Fig. 1) showed that strains of *Aleurodiscus alpinus*, *A. pinicola*, *A. sichuanensis*, and *A. senticosus* formed separate clades in distinct lineages with high statistical support (BS = 96–100%, PP = 1). The strain of *A. sichuanensis* was sister to *A. alpinus* with significant support, BS: 90% and PP: 1.

Taxonomy

Aleurodiscus alpinus **Sheng H. Wu, sp. nov.**
MycoBank MB823178
Figs 2A, 3

Typification. CHINA. YUNNAN PROVINCE: Shangrila County, Pudacuo National Park, Bita Lake, 27°43'N, 99°58'E, 3640 m, on branch of *Rhododendron* sp., 10 Jul 2014, *S.H. Wu*, *Wu 1407-55* (holotype TNM F27976). GenBank 28S = MF043526, *TEF1* = LC269190.

Etymology. *alpinus* (L.), referring to the occurrence at high elevations.

Diagnosis. Resembles *Aleurodiscus cupulatus* Núñez & Ryvarden in having discoid basidiomes, clamped hyphae, similar gloeocystidia, absence of acanthophyses, branched or unbranched hyphidia, and echinulate basidiospores. *Aleurodiscus cupulatus* features much wider basidiospores than *A. alpinus*. It differs from its closest phylogenetic relative, *A. sichuanensis*, by having clamped hyphae, but lacks acanthophyses.

Description. Basidiomes cupuloid or discoid, solitary, occasionally fused, adnate, 350–750 μm thick in section. Hymenial surface Buff, Pale Luteous or Luteous, subceraceous, covered with crystal masses, not cracked; margin concolorous or paler, incurved, filamentous.

Hyphal system monomitic; hyphae nodose-septate. Pileus hyphae subcolorless to brownish, straight, thick-walled, walls usually thinner towards apices, usually with excreted material near apices. Subiculum uniform, with dense to compact texture, 150–500 μm thick; hyphae near substrate more or less vertical, moderately ramified, colorless, 3.5–8 μm diam, with 0.7–1.5 μm thick walls, occasionally guttulate; hyphae near hymenial layer more or less vertical, moderately ramified, colorless, fairly straight, 2.5–5 μm diam, thin- or slightly thick-walled, anastomoses occasional. Hymenial layer thickening, subhymenium differentiated from subiculum, 200–250 μm thick, with dense texture; hyphae fairly vertical, colorless, guttulate, 2–4 μm diam, thin-walled. Crystals sparsely scattered throughout section. Gloeocystidia numerous, immersed or slightly projecting, tubular, sometimes with adventitious septa near basal parts, colorless, (50–)70–200 × 4.5–12.5 μm, thin-walled, guttulate, SA+. Hyphidia numerous, sometimes branched, 40–130 × 2–6.5 μm. Basidia narrowly clavate, occasionally with one or two small protuberances, 85–165 × 16–20 μm, slightly thick-walled (ca. 0.5 μm thick), 4-sterigmate. Basidiospores ellipsoid to narrowly ellipsoid, adaxially concave, finely aculeate, thin-walled, homogenous or guttulate, amyloid, CB–, mostly 22–26 × 11–14 μm.

(22–)22.2–26(–27.8) × (11–)11.8–13.5(–14.8) μm, L = 24.2±1.7 μm, W = 12.6±2.2 μm, Q = 1.95 (n = 30) (holotype, *Wu 1407-55*); (22–)23–24.5(–26) × (10.2–)10.8–13(–14) μm, L = 23.8±1.0 μm, W = 11.8±1.0 μm, Q = 2.02 (n = 30) (*Wu 1407-59*).

Ecology and distribution. On dead branches of *Rhododendron* and other angiosperms at very high elevations, China, Jul.

Additional specimens examined. CHINA. YUNNAN PROVINCE: Shangrila County, Pudacuo National Park, Bita Lake, 27°43'N, 99°58'E, 3640 m, on branch of *Rhododendron* sp., 10 Jul 2014, *S.H. Wu*, *Wu 1407-59* (TNM F27979), *Wu 1407-61* (TNM F27981); Pudacuo National Park, 3600 m, on dead branch of *Rhododendron* sp., 28 Jul 2017, *S.H. He*, *He 4924* (BJFC), *He 4942* (BJFC); Jianchuan County, Laochunshan, 26°38'N, 99°47'E, 3400 m, on angiosperm branch, 26 Jul 2001, *S.H. Wu* & *S.Z. Chen*, *Wu 0107-22* (TNM F13507), *Wu 0107-25* (TNM F13510).

***Aleurodiscus pinicola* Sheng H. Wu, sp. nov.**
MycoBank MB823179
Figs 2B, 4

Typification. CHINA. YUNNAN PROVINCE: Lichiang, High Mountain Workstation of Kunning Botanical Garden, 27°00'N, 100°11'E, 3250 m, on branch of *Pinus densata*, 30 Aug 2013, *S.H. Wu*, *Wu 1308-54* (holotype TNM F27182). GenBank: ITS = MF043525, 28S = MF043530, *TEF1* = LC269191.

Etymology. *pinicola* (L.), dwelling on *Pinus*, in reference to the substrate.

Diagnosis. *Aleurodiscus pinicola* and *Acanthobasidium penicillatus* Burt share the features of moniliform gloeocystidia, acanthophyses with apical spines, dendrohyphidia, basidia with lateral protuberances, and aculeate basidiospores; the latter, however, has clamped hyphae and narrower basidiospores 18–27 × 12–14 (–17) μm. *Aleurodiscus pinicola* also resembles *A. oakesii* (Berk. & M.A. Curtis) Pat., however, the latter occurs on deciduous trees and has smaller basidiospores (15–20 × 13–17 μm).

Basidiomes discoid, each one up to 3.5 × 3 mm, adnate, membranaceous-subceraceous, 180–400 μm thick in section. Hymenial surface Buff or Pale Luteous, smooth, occasionally cracked; margin whitish, incurved, filamentous.

Hyphal system monomitic; most hyphae simple-septate, a few hyphal septa in junction of hymenium and subiculum with clamp connections. Subiculum uniform, with fairly dense dense texture, 60–160 μm thick; hyphae more or less vertical at resupinate parts, ± horizontal at marginal curved parts, moderately ramified, more or less interwoven, colorless, 2.5–6 μm diam, slightly thick-walled or containing thick walls up to 2 μm thick, sometimes with small oily drops, anastomoses occasional, some basal hyphae brownish yellow, with thicker walls than those elsewhere. Hymenial layer thickening, subhymenium more or less differentiated from subiculum, with dense texture, 100–270 μm thick; hyphae more or less vertical, colorless, sometimes with a short branch, usually containing minute oily drops, 2.5–5.5 μm diam, thin-walled. Crystal masses scattered throughout hymenial layer. Gloeocystidia numerous, mostly immersed or slightly projecting, cylindrical, usually strongly moniliform toward apices, usually forked, with

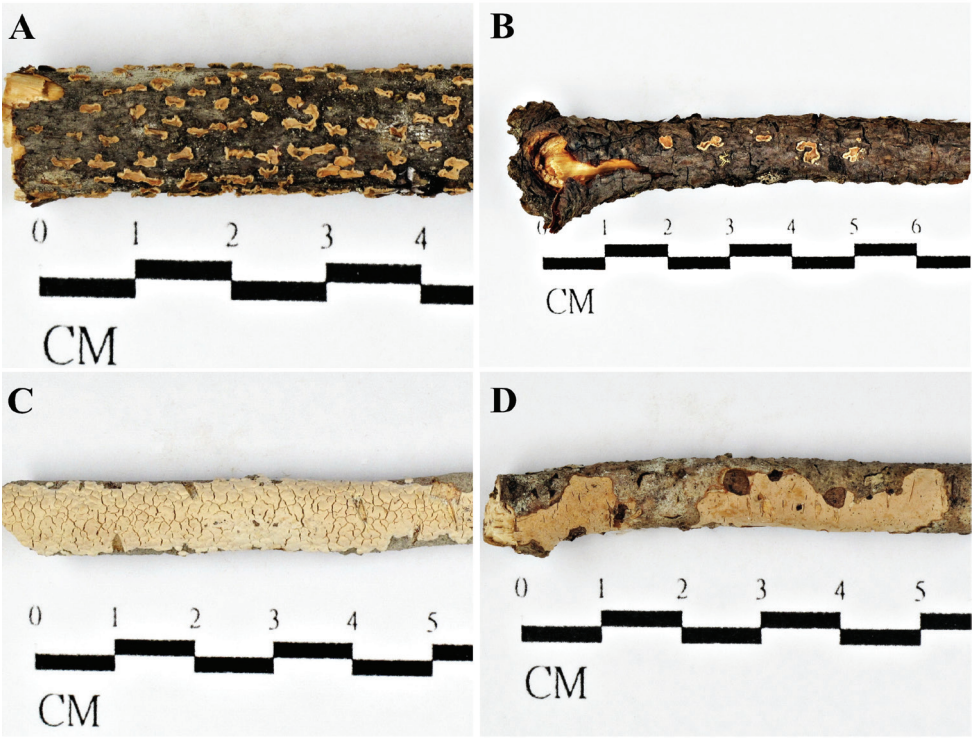

Figure 2. Basidiocarps **A** *Aleurodiscus alpinus* (holotype, *Wu 1407-55*) **B** *A. pinicola* (holotype, *Wu 1308-54*) **C** *A. senticosus* (holotype, *Wu 1308-54*) **D** *A. sichuanensis* (holotype, *Wu 0010-18*).

numerous minute oily drops, colorless, 65–200 × 8.2–15.5 µm, thin- to slightly thick-walled, SA–. Acanthophyses numerous, clavate to broadly clavate, fusiform, stalked, colorless, apical parts with numerous protuberances, 50–100 × 5–30 µm, up to 1.2 µm thick walls, aculei 1–7 × 1–2 µm. Dendrohyphidia numerous, 37–90 × 3–4.8 µm. Hyphidia numerous, 35–80 × 2.4–4.2 µm. Basidia clavate, middle parts usually with several protuberances, 65–130 × 20–32 µm, up to 1.2 µm thick walls, 4-sterigmate. Basidiospores broadly ellipsoid to subglobose, adaxially flattened, aculeate, thin- to thick-walled, up to 3 µm thick walls, with a distinct apiculus, homogenous or with several oil-drops, amyloid, CB–, mostly 22.5–27.5 × 19–24 µm. (22.5–)23.5–27.2(–29) × (18.2–)19.2–22.8(–24) µm, L = 25.4±1.3 µm, W = 20.7±1.6 µm, Q = 1.23 (n = 30) (holotype, *Wu 1308-54*); (22.2–)23–26.5(–28) × (18.2–)20–22.5(–25.5) µm, L = 24.8±1.3 µm, W = 21.2±1.5 µm, Q = 1.17 (n = 30) (*Wu 1106-14*).

Ecology and distribution. On *Pinus* branches at high elevations, China and Taiwan, Jun to Aug.

Additional specimens examined. TAIWAN. Taichung, Siaosyueshan, Tienchih, 24°17'N, 121°01'E, 2580 m, on branch of *Pinus armandii*, 8 Jun 2011, *S.H. Wu*, *Wu 1106-14* (TNM F25532); *ibid. Wu 1106-16* (TNM F25534).

***Aleurodiscus senticosus* Sheng H. Wu, sp. nov.**
MycoBank MB823180
Figs 2C, 5

Typification. TAIWAN. New Taipei City, Wulai, 24°51'N, 121°33'E, 448 m, on branch of angiosperm, 10 Sep 2012, *S.H. Wu*, *Wu 1209-7* (holotype TNM F26702). GenBank: ITS = MH596849, 28S = MF043531, *TEF1* = LC271169.

Etymology. *senticosus* (L.) = full of thorns, referring to the surface of basidia and cystidia.

Diagnosis. Macroscopically featured in having a more or less cracked hymenophore, resulting from the fusion of numerous basidiome patches. Microscopically its basidia are diagnostic in having large lateral echinulate bladder-like swollen structure. Morphologically it resembles *Xylobolus* spp., although the latter cause a white-pocket rot in wood and have smooth basidiospores.

Description. Basidiomes resupinate, beginning as small orbicular patches, gradually extending and fusing together then becoming effused, adnate, membranaceous, 250–600 μm thick in section. Hymenial surface Buff or Light Buff, slightly tuberculate, with a more or less cracked hymenophore; margin paler, usually determinate, occasionally thinning and byssoid.

Hyphal system monomitic; hyphae simple-septate, colorless. Subiculum with dense texture, 200–350 μm thick; hyphae next to substrate more or less horizontal, slightly interwoven, colorless, moderately ramified, at the junction of basidiocarp patches more or less vertical, 2–4(–5) μm diam, walls up to 1.5 μm thick. Hymenial layer thickening, with dense texture, 150–250 μm thick, not clearly differentiated from the subiculum; hyphae mainly vertical, colorless, 2–4 μm diam, thin- to slightly thick-walled. Gloeocystidia numerous, immersed or slightly projecting, cylindrical or tubular, with stalked bases, apically sometimes forked, sometimes with one or more constrictions near apices or slightly moniliform, colorless, 45–135 × 5–12 μm, with walls up to 1.5 μm thick, SA–. Acanthophyses numerous, subclavate or clavate, basal parts thin-walled, thick-walled toward apices, colorless, median to apical parts echinulate, 25–65 × 4–13 μm (spines excluded). Hyphidia numerous, 35–65 × 2–4 μm. Basidia clavate, 60–82 × 10–15 μm, with walls up to 2 μm thick, 4-sterigmate, usually with large lateral echinulate bladder-like swollen structure. Basidiospores broadly ellipsoid to subglobose, adaxially flattened, aculeate, with 1–3 μm thick walls, homogeneous or sometimes with several oily drops, amyloid, CB–, mostly 13.5–16.5 × 11–13 μm. (13–)13.5–15.8(–17) × (10–)11.2–12.5(–13) μm, L = 14.8±1.00 μm, W = 11.8±0.6 μm, Q = 1.25 (n = 30) (holotype, *Wu 1209-7*); (13–)14–16(–17.2) × (10–)11.2–13(–15) μm, L = 15.1±1.0 μm, W = 11.9±1.0 μm, Q = 1.26 (n = 30) (*GC 1604-46*).

Ecology and distribution. On angiosperm branches, Taiwan, Apr to Sep.

Additional specimens examined. Taiwan, New Taipei City, Wulai, 24°51'N, 121°33'E, 448 m, on angiosperm branch, 10 Sep 2012, *S.H. Wu*, *Wu 1209-9* (TNM F26704); Nantou, Lienhuachih. 23°56'N, 120°53'E, 700 m, on angiosperm branch, 08 Oct 1996, *S.H. Wu*, *Wu 9610-1* (TNM F5344); on angiosperm branch, 09 Apr 2016, *G.C. Chen*, *GC 1604-46* (TNM F30771).

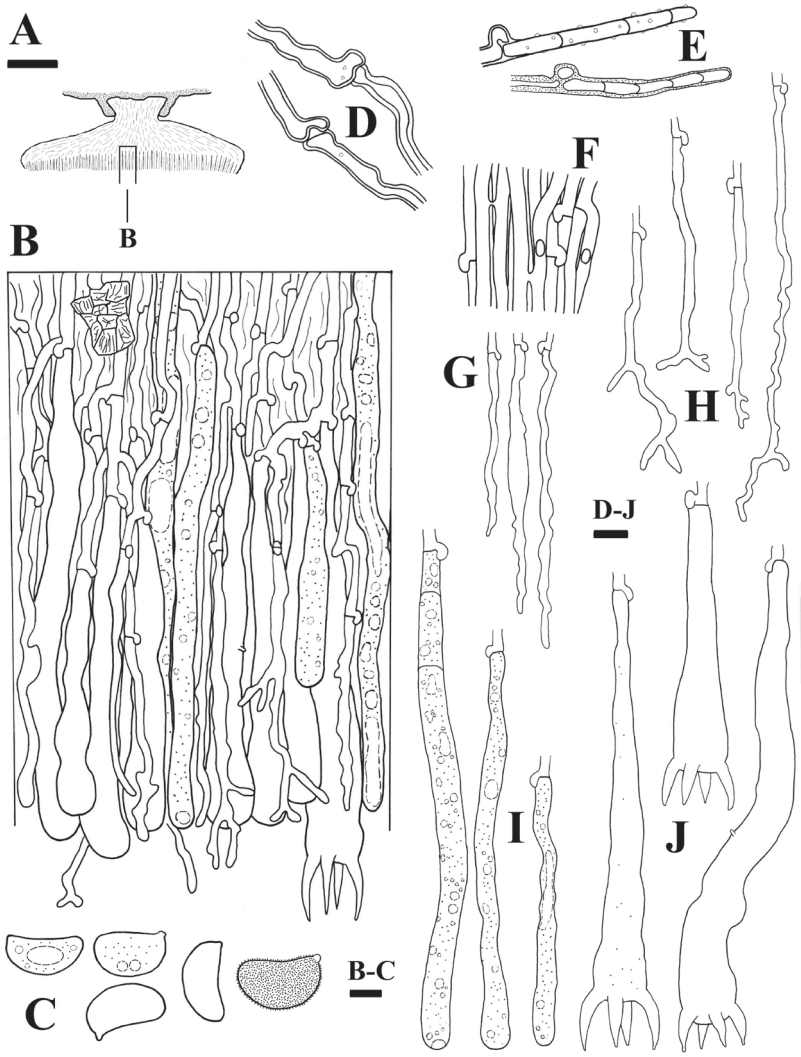

Figure 3. Microscopic structures of *Aleurodiscus alpinus* (holotype, *Wu 1407-55*) **A** profile of basidiocarp section **B** subhymenial and hymenial section **C** basidiospores (far right: in IKI) **D** subicular hyphae near substrate **E** pileus hyphae **F** subhymenial hyphae **G** hyphidia **H** branched hyphidia **I** gloeocystidia **J** basidia. Bars: 300 μm (**A**); 10 μm (**B–J**).

Aleurodiscus sichuanensis **Sheng H. Wu, sp. nov.**
MycoBank MB823181
Figs 2D, 6

Typification. CHINA. SICHUAN PROVINCE: Wolungshan, Tengsheng, 2700 m, under bark of angiosperm, 11 Oct 2000, *S.H. Wu & S.C. Wu*, *Wu 0010-18* (holotype TNM F12097). GenBank: ITS = MH596852, 28S = MF043534, *TEF1* = LC269194.

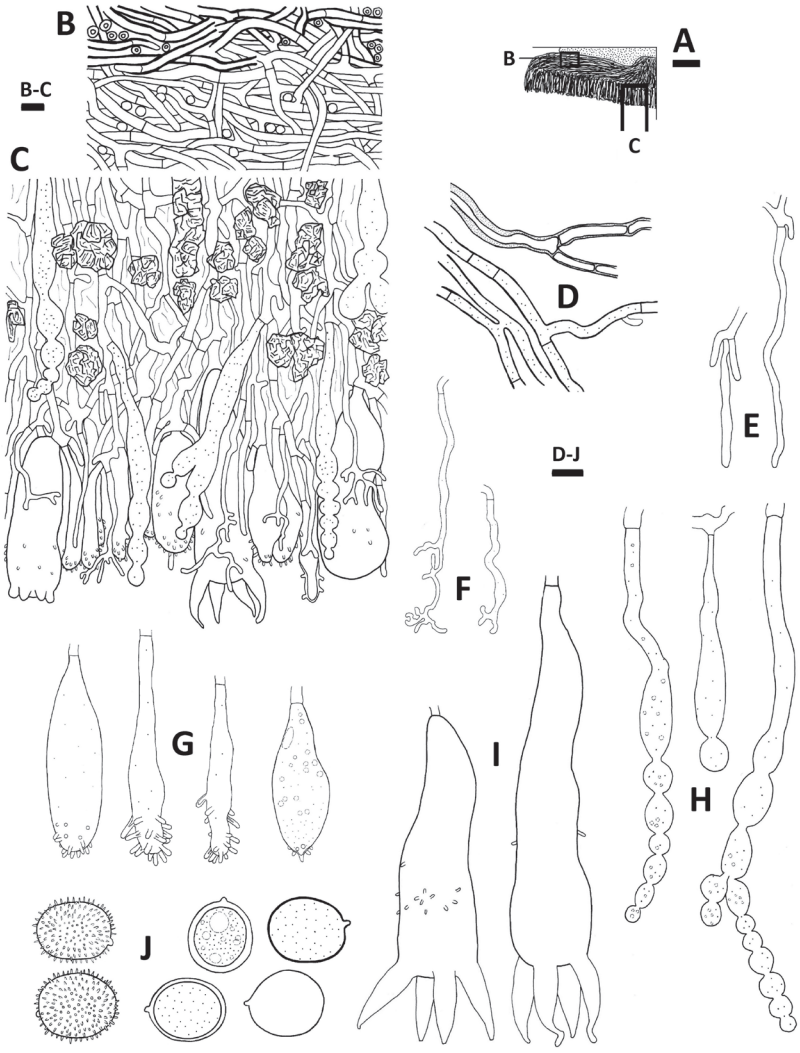

Figure 4. Microscopic structures of *Aleurodiscus pinicola* (holotype, *Wu 1308-54*) **A** profile of basidiocarp section **B** subicular hyphae of basidiocarp section **C** subhymenial and hymenial section **D** generative hyphae **E** hyphidia **F** dendrohyphidia **G** acanthophyses **H** gloeocystidia **I** basidia **J** basidiospores (left: in IKI, right: in KOH). Scale bars: 200 μm (**A**); 10 μm (**B–J**).

Etymology. *sichuanensis* (L.), referring to Sichuan Province, the type locality.

Diagnosis. *Aleurodiscus sichuanensis* resembles *A. oakesii* in having acanthophyses, simple-septate generative hyphae, and gloeocystidia occasionally with protuberances. However, clamped hyphae are rarely present in *A. oakesii*. Protuberances of acanthophyses of *A. oakesii* are antler-like, while aculei of acanthophyses in *A. sichuanensis* are fairly small. Basidiospores of *A. sichuanensis* are D-shaped or broadly ellipsoid, while those of *A. oakesii* are ovoid-ellipsoid and slightly smaller (18–27 ×12–14(–17) μm).

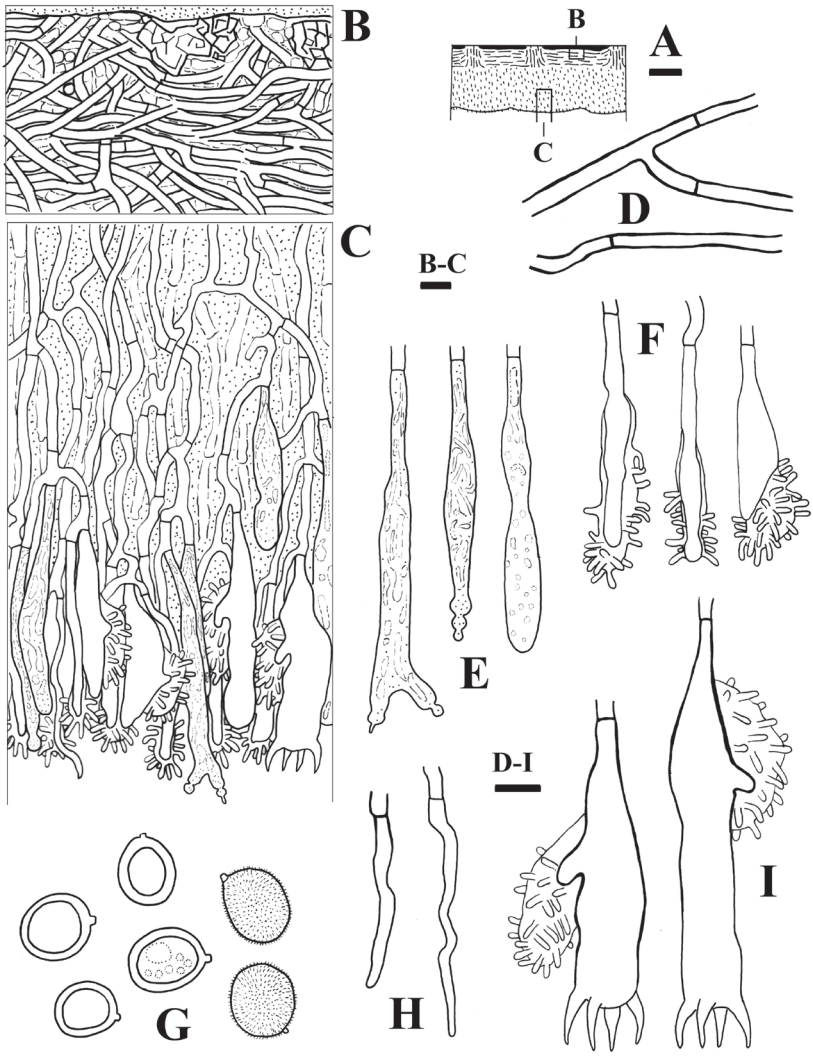

Figure 5. Microscopic structures of *Aleurodiscus senticosus* (holotype, *Wu 1209-7*) **A** profile of basidiocarp section **B** basal of basidiocarp section **C** section of hymenium **D** subicular hyphae **E** gloeocystidia **F** acanthophyses **G** basidiospores (left: in KOH, right: in IKI) **H** hyphidia **I** basidia. Scale bars: 200 μm (**A**); 10 μm (**B–I**).

Aleurodiscus sichuanensis, however, is most closely related to *A. alpinus* and differs from it by having acanthophyses and simple-septate hyphae.

Basidiomes resupinate, effused, adnate, membranaceous-subceraceous, 150–350 μm thick in section. Hymenial surface smooth, Buff or Buff Yellow, occasionally cracked; margin concolorous, determinate.

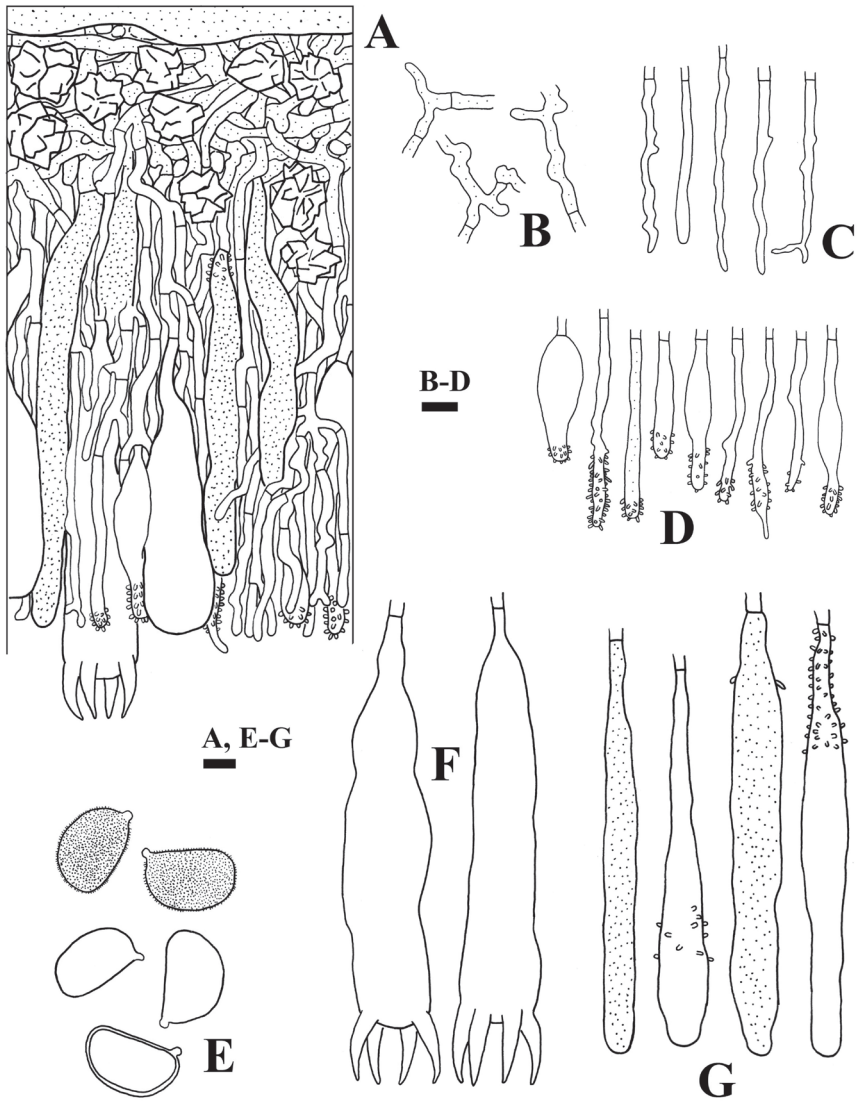

Figure 6. Microscopic structures of *Aleurodiscus sichuanensis* (holotype, *Wu 0010-18*) **A** basidiocarp section **B** subicular hyphae **C** hyphidia **D** acanthophyses **E** basidiospores (upper: in IKI, lower: in KOH) **F** basidia **G** gloeocysitidia. Scale bars: 10 μm.

Hyphal system monomitic; hyphae simple-septate. Subiculum uniform, with dense texture, thin or up to 150 μm thick; hyphae interwoven, colorless, richly ramified, tortuous, usually full of small oily drops, 2.5–5.5 μm diam, thin-walled. Hymenial layer with dense texture, 100–200 μm thick; hyphae vertical, colorless, ± straight, 2.5–4.5 μm diam, thin-walled. Crystal masses scattered in subiculum, yellowish. Gloeocystidia

numerous, immersed or projecting, yellowish or pale brownish yellow, cylindrical, narrowly clavate or tubular, with oily contents or homogeneous, SA+, basal or median portion occasionally with small aculei, 70–135 × 7–14 μm, with 0.5–1 μm thick walls. Acanthophyses numerous, irregularly cylindrical or narrowly clavate, sometimes subfusiform, colorless, apical parts with numerous aculei, 30–70 × 3–8(–12) μm (aculei excluded), thin-walled. Hyphidia numerous, occasionally branched, 35–85 × 2.5–4.5 μm. Basidia clavate, 4-sterigmate, 100–130 × 20–25 μm, with 0.8–1.2 μm thick walls. Basidiospores D-shaped or broadly ellipsoid, adaxially flattened, finely aculeate, thin-walled or 1–2 μm thick, sometimes with oily contents, amyloid, CB–, mostly 25.5–28.5 × 15–18 μm. (25–)26–28.2(–29) × (14.5–)15.2–17(–19) μm, L = 27.1±1.0 μm, W = 15.9±1.1 μm, Q = 1.71 (n = 30) (holotype, *Wu 0010-18*).

Ecology and distribution. On dead branches of *Quercus* and other angiosperms at high elevations, China, Jul to Oct.

Additional specimens examined. CHINA. SICHUAN PROVINCE: Wolungshan, Tengsheng, 2700 m, on angiosperm branch, 11 Oct 2000, *S.H. Wu & S.C. Wu*, *Wu 0010-42* (TNM F12118). YUNNAN PROVINCE: Shangrila County, Pudacuo National Park, 3600 m, on dead branch of *Quercus apuifolloides*, 28 Jul 2017, *S.H. He*, *He 4923*, *He 4926*, *He 4930*, *He 4935* (BJFC).

Discussion

A number of phylogenetic studies of *Aleurodiscus* s.l. have been conducted in the past twenty years (Wu et al. 2001; Larsson and Larsson 2003; Miller et al. 2006; Larsson 2007; Dai and He 2016; Dai et al. 2017). Miller et al. (2006) and Larsson (2007) tried to establish a family level classification for *Aleurodiscus* s.l., as well as related taxa of the Russulales. However, a fully resolved and robust phylogeny of *Aleurodiscus* s.l. and related taxa was not achievable with ribosomal genes alone. Dai and He (2016) and our study have addressed this by including *TEF1* for phylogenetic analyses. From our phylogenetic analyses of three DNA genetic markers (Fig. 1) we can conclude the following about evolutionary relationships in the Stereaceae: (i) *Aleurodiscus* s.l. is highly polyphyletic; (ii) *Acanthophysellum* is polyphyletic; (iii) *Gloeocystidiellum* is polyphyletic; (iv) *Megalocystidium* is polyphyletic; and (v) *Conferticium* is paraphyletic.

Aleurodiscus alpinus is reminiscent of *Aleurodiscus* s.s. (*A. amorphus* (Pers.) J. Schröt. and *A. grantii* Lloyd) due to the discoid basidiocarp and echinulate basidiospores, as well as the absence of acanthophyses. However, the gloeocystidia of *Aleurodiscus* s.s. are paraphysis-like, narrow and moniliform, while those of *A. alpinus* are much wider and not moniliform. In addition, *A. alpinus* has unbranched or branched hyphidia, which are lacking in *Aleurodiscus* s.s. *Aleurodiscus alpinus* formed a clade with *A. sichuanensis* (Fig. 1), however, the latter has simple-septate hyphae and acanthophyses. *Aleurodiscus alpinus* and *A. cupulatus* share most morphological features, except the latter has much wider basidiospores. *Aleurodiscus alpinus* grows on *Rhododendron* sp. in Yunnan of

China, while *A. cupulatus* occurs on *Pseudotsuga menziesii* in Idaho of USA. No DNA sequence of the latter has been obtained to examine their relationship.

Aleurodiscus pinicola presents protuberances in the basidia and this is reminiscent of *Acanthobasidium*. However, this feature is not limited to *Acanthobasidium* spp. For example, basidia of *Aleurodiscus mirabilis* (Berk. & M.A. Curtis) Höhn. and *A. wakefieldiae* Boidin & Beller occasionally possess protuberances, but they and *A. pinicola* do not belong to *Acanthobasidium* (Fig. 1).

Aleurodiscus senticosus is macroscopically distinct in having more or less cracked hymenophore from the fusion of smaller basidiocarp patches; microscopically, its basidia bear a large, spiny, bladder-like structure that is unique among *Aleurodiscus* s.l. The present phylogenetic analyses (Fig. 1) indicated that *A. senticosus* formed a clade with *Xylobolus* and *Acanthofungus*, but without strong support. However, these two genera differ from *A. senticosus* by causing a white-pocket rot in wood and by bearing smooth basidiospores.

Aleurodiscus sichuanensis cannot be accommodated in any segregate genus of *Aleurodiscus* s.l., according to the combined features of effused basidiocarp, simple-septate hyphae, acanthophyses, gloeocystidia with aculei, and echinulate basidiospores.

In conclusion, the status of each segregate genus of *Aleurodiscus* s.l. should be further examined by multi-gene analysis of more species to evaluate which ones can be recognized and which cannot. Although the four new species we introduce cannot be accommodated in any segregate genus of *Aleurodiscus* s.l. according to the present combined morphological and phylogenetic studies, they are still placed under the broad sense of *Aleurodiscus* at the present time.

Acknowledgments

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References

Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Ostell J, Pruitt KD, Sayers EW (2018) GenBank. Nucleic Acids Research 46 (D1): D41–D46. https://doi.org/10.1093/nar/gkx1094

Boidin J, Lanquetin P, Gilles G, Candoussau F, Hugueney R (1985) Contribution à la connaissance des Aleurodiscoideae à spores amyloides (Basidiomycotina, Corticiaceae). Bulletin de la Société Mycologique de France 101: 333–367.

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772–772. https://doi.org/10.1038/nmeth.2109

Dai LD, He SH (2016) New species and new records of *Aleurodiscus* s.l. (Basidiomycota) in China. Mycological Progress 15: 717–730. https://doi.org/10.1007/s11557-016-1202-z

Dai LD, Wu SH, Nakasone KK, Burdsall HH, He SH (2017a) Two new species of *Aleurodiscus* s.l. (Russulales, Basidiomycota) on bamboo from tropics. Mycoscience 58: 213–220. https://doi.org/10.1016/j.myc.2017.02.001

Dai LD, Zhao Y, He SH (2017b) Three new species of *Aleurodiscus* s.l. (Russulales, Basidiomycota) on bamboos from East Asia. Cryptogamie, Mycologie 38: 227–239. https://doi.org/10.7872/crym/v38.iss2.2017.227

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

Ghobad-Nejhad M, Langer E (2018) A new species in *Aleurodiscus* s.l. (Stereaceae, Russulales) from Iran. Phytotaxa 351: 264–272. https://doi.org/10.11646/phytotaxa.351.4.2

Gorjón SP, Greslebin AG, Rajchenberg M (2013) The genus *Aleurodiscus* s.l. (Stereaceae, Russulales) in the Patagonian Andes. Mycological Progress 12: 91–108. https://doi.org/10.1007/s11557-012-0820-3

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hjortstam K, Roberts PJ, Spooner BM (2009) Corticioid fungi from the Kimberley region, Western Australia. Kew Bull 64: 353–368. https://doi.org/10.1007/s12225-009-9104-8

Larsson KH (2007) Re-thinking the classification of corticioid fungi. Mycological Research 111: 1040–1063. https://doi.org/10.1016/j.mycres.2007.08.001

Larsson E, Larsson KH (2003) Phylogenetic relationships of russuloid basidiomycetes with emphasis on aphyllophoralean taxa. Mycologia 95: 1037–1065. https://doi.org/10.2307/3761912

Maninder K, Avneet PS, Dhingra GS, Ryvarden L (2014) *Aleurodiscus himalaicus* (Agaricomycetes) sp. nov. from India. Synopsis Fungorum 32: 5–7.

Matheny BP, Wang Z, Binder M, Curtis JM, Lim YW, Nilsson RH, Hughes KW, Hofstetter V, Ammirati JF, Schoch CL, Langer E, Langer G, McLaughlin DJ, Wilson AW, Frøslev T, Ge Z-W, Kerrigan RW, Slot JC, Yang ZL, Baroni TJ, Fischer M, Hosaka K, Matsuura K, Seidl MT, Vauras J, Hibbett DS (2007) Contributions of rpb2 and tef1 to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). Molecular Phylogenetics and Evolution 43: 430–451. https://doi.org/10.1016/j.ympev.2006.08.024

Mashima J, Kodama Y, Kosuge T, Fujisawa T, Katayama T, Nagasaki H, Okuda Y, Kaminuma E, Ogasawara O, Okubo K, Nakamura Y, Takagi T (2016) DNA data bank of Japan (DDBJ) progress report. Nucleic Acids Research 44: D51–D57. https://doi.org/10.1093/nar/gkv1105

Miller SL, Larsson E, Larsson K-H, Verbeken A, Nuytinck J (2006) Perspectives in the new Russulales. Mycologia 986: 960–970. https://doi.org/10.1080/15572536.2006.11832625

Núñez M, Ryvarden L (1997) The genus *Aleurodiscus* (Basidiomycotina). Synopsis Fungorum 12: 1–164.

Rayner RW (1970) A Mycological Colour Chart. Commonwealth Mycological Institute, Kew 34 pp.

Rehner SA, Buckley E (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97: 84–98. https://doi.org/10.1080/15572536.2006.11832842

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

Ryvarden L, Sanyal SK, Dhingra GS (2012) *Aleurodiscus indicus* (Agaricomycetes) sp. nov. from India. Synopsis Fungorum 30: 14–16.

Simpson JA, Grgurinovic CA (2003) A new species of *Aleurodiscus* (Stereaceae) from Mt Kosciuszko, Australia. Australasian Mycologist 22: 15–19.

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

Tian Y, Ghobad-Nejhad M, He SH, Dai YC (2018) Three new species of *Aleurodiscus* s.l. (Russulales, Basidiomycota) from southern China. MycoKeys 37: 93–107. https://doi.org/10.3897/mycokeys.37.25901

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

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

Wu SH, Hibbett DS, Binder M (2001) Phylogenetic analyses of *Aleurodiscus* s.l. and allied genera. Mycologia 93: 720–731. https://doi.org/10.2307/3761826

Wu SH, Wang DM, Yu SY (2010) *Neoaleurodiscus fujii*, a new genus and new species found at the timberline in Japan. Mycologia 102: 217–223. https://doi.org/10.3852/09-052

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RESEARCH ARTICLE

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A new species of *Psathyrella* (Psathyrellaceae, Agaricales) from Italy

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Abstract

Sporophores of a new *Psathyrella* species have been reported for the first time as growing at the base of *Cladium mariscus* culms in the Botanical Garden of the University of Calabria, Rende, Cosenza, southern Italy. The fungus was initially identified as *P. thujina* (= *P. almerensis*) by means of both ecology and macro- and microscopic characteristics of the basidiomes, then referred to *P. cladii-marisci* sp. nov. after extraction, amplification, purification and analysis of the rDNA ITS region. We came to this conclusion after comparing our specimen with the descriptions of the taxa available in the literature for the genus *Psathyrella*.

Keywords

Agaricomycetes, Basidiomycota, Fen-sedge, Marshes, southern Italy, Taxonomy

Introduction

Within the cosmopolitan fungal genus *Psathyrella* (Fr.) Quél. (*Agaricales*, *Psathyrellaceae*), about one hundred species have traditionally been recognised in Europe, almost all saprotrophs and found in many and diverse environments. Either terrestrial or lignicolous, they grow mainly on organic debris from various origins, such as dung, post-fire locations and dead stems of larger herbaceous plants (Vesterholt and Knudsen 1992). *Psathyrella* basidiomes are pileate, stipitated and exannulate or, at most, with a fugacious ring and the hymenophore is gilled, pale pink when young, turning brown with age due to a dark

spore print. Moreover, they have, as the etymology indicates, a very fragile and ephemeral consistency. Despite these common macroscopic characters of the basidiomes, a recent phylogenetic analysis revealed the extremely complex origin of this genus, recognising species as belonging to a *Psathyrella sensu stricto* group or to *P. sensu lato* complex, the former including 19 clades and the latter involving eight genera (*Coprinellus*, *Kauffmania*, *Cystoagaricus*, *Typhrasa*, *Lacrymaria*, *Homophron*, *Coprinopsis*, *Parasola*), thus consistently widening the list of such "psathyrelloid" basidiomycetes (Örstadius et al. 2015).

During an investigation on the mycoflora of the Botanical Garden at the University of Calabria (Rende, Cosenza, Italy), basidiomes of an apparently "psathyrelloid" fungus were detected at the base of a fen-sedge [*Cladium mariscus* (L.) Pohl (*Cyperaceae*)], a cosmopolitan-distributed plant species (Lansdown et al. 2018) occurring in marshy places of most Italian regions (Bartolucci et al. 2018), but rarely in southern Italy.

Based on records reported by Örstadius et al. (2015), nine clades of *Psathyrella s.s.* include species associated with moist soils and marshy places: "*spadiceogrisea*" (four species), "*fibrillosa*", "*noli-tangere*" and "*prona*" (two species each), "*candolleana*", "*cystopsathyra*", "*lutensis*", "*obtusata*" and "*pygmaea*" (one species each). Nevertheless, only three species have been found to be growing on sticks or on remnants of hygrophilous plants: *P. lutensis* (Romagn.) Bon, as a monospecific "*lutensis*" clade, *P. thujina* A.H. Sm. (=*P. almerensis* Kits van Wav.) in the "*spadiceogrisea*" clade and *P. typhae* (Kalchbr.) A. Pearson & Dennis in the "*candolleana*" clade.

The aim of this work was therefore to identify our basidiomes by using both morpho-ecological and biomolecular tools. This was highly encouraged by the habitat peculiarity and the close relationship with a plant species with which no species of *Psathyrellaceae* had ever been found associated.

Materials and methods

Eight basidiomes of the above "psathyrelloid" fungus were observed and collected on 10 April 2018, as gregarious all around and at the base of *Cladium mariscus* cut culms (Fig. 1). In 2012, that plant had been removed, together with the whole clump of mud attached to its roots, from a natural marsh named Lago dell'Aquila (Laureana di Borrello, Reggio Calabria, southern Italy) and transplanted to the Botanical Garden at the corner of a 90 × 37 cm-wide and 30 cm-deep concrete tank, which had permanently been kept full to the brim with water. Since then, some leaves of water lily (*Nymphaea alba* L.) have been introduced to float on the water surface inside the tank and the mud mass has been increasing, while the *C. mariscus* plant has been expanding and producing new culms that are cut every year.

Morphology

The basidiomes were first macroscopically examined for features, colours, sizes, hymenophore shape, pileus and stipe ornamentations, smell and taste. Then, the structures of the basidiome

Figure 1. A tuft of *Cladium mariscus* planted in a tank at the Botanical Garden of the University of Calabria, southern Italy (**A**), and first-sight features of *Psathyrella* basidiomes at the base and in-between of remnants of excised culms of the plant (**B**).

were microscopically inspected for cheilo- and pleurocystidia occurrence and features, presence of clamp connections, basidia and spore features. These observations were carried out under a light microscope (Axioplan 2 Imaging Microscope, Carl Zeiss, Germany) at 400 and 1,000 magnifications on fragments of *pileipellis* and gills placed on slides in 10%

NH_4OH. The results were compared with those published in the morphological keys for the *Psathyrella* species and, more specifically, with those species reported as the closest, according to morphology and ecological site conditions, i.e. *P. thujina*, *P. typhae* and *P. lutensis* (Kits van Waveren 1985, Vesterholt and Knudsen 1992, Christan et al. 2017, Henrici 2017).

DNA Extraction, Amplification and Sequencing

One of the basidiomes was dehydrated at room temperature and destroyed for molecular analysis: DNA extraction, amplification, purification and sequencing of the nuc rDNA internal transcribed spacer region (ITS). DNA extraction was implemented by using CTAB protocol (Doyle and Doyle 1987) and the ITS region was amplified using the primer combination ITS1F/ITS4 (White et al. 1990). The polymerase chain reaction (PCR) was performed in a 25-µl reaction volume containing 1.0 µl DNA, 2.5 µl 10 × 5-Prime–MasterMix Buffer (Thermo Fischer Scientific, Waltham, Massachusetts, USA) and 1.25 µl of each primer (10 µM/µl). The PCR was carried out according to the following amplification programme: 3 min initial denaturation at 94 °C, 35 cycles (30 s denaturation at 94 °C, 1 min annealing at 55 °C, 45 s extension at 72 °C) and a 10 min final extension at 72 °C. This programme was carried out in a T1000 Thermocycler (Biometra, Goettingen, Germany). The PCR products were purified using a QIAquick PCR purification kit (Qiagen Inc., Valencia, California, USA). Sequencing was performed by means of a Bigdye terminator cycle sequencing kit (Applied Biosystems, Foster City, California, USA). The sequencing reaction was run by BMR Genomics (Padua, Italy) on a 96-capillaries ABI 3730XL DNA Sequencer.

Forward and reverse DNA fragment electropherograms were checked by means of the CHROMAS 2.6.5 software (technelysium.com.au) for a complete reconstruction of the ITS1, ITS2 and 5.8 gene fragments. Ambiguous regions at the start and the end of the alignment were deleted and gaps were manually adjusted to optimise the alignment. The sequence generated for this study is deposited in GenBank with the code MK080112.

Alignment and Phylogenetic Analysis

Consensus sequences were generated from both forward and reverse primer reads in the BioEdit sequence alignment editor, version 7.2.5 (Hall 1999), then homology searches were performed at the National Centre for Biotechnology Information (NCBI) Web site using BLAST. This sequence was then compared with those of the *Psathyrella* species deposited in GenBank on which the phylogenetic analysis had recently been performed (Padamsee et al. 2008, Battistin et al. 2014, Örstadius et al. 2015, Yan and Bau 2018). A total of 45 ITS sequences, including three *Coprinellus* spp. (Table 2) were aligned using MAFFT with the L-INS-i option (Katoh et al. 2017). The aligned ITS dataset consisted of 702 nucleotide sites (including gaps). FASTA alignments from MAFFT were loaded in

IQ-TREE 1.5.6 (Nguyen et al. 2014) to perform Maximum Likelihood Analysis. Clade robustness was assessed using a bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Phylogenetic trees were visualised using the FigTree v1.3.1 (Rambaut 2009).

Results

Morphology

The macro- and micro-morphological features of the basidiomes collected at the base of the fen-sedge plant in the Botanical Garden are shown in Figures 2, 3. At first sight, by observing the macro-level characters, i.e. the small-medium size, the extreme fragility at handling and the brown-blackish spore print, the basidiomes were easily assigned to the *Psathyrella* genus (Vesterholt and Knudsen 1992). Secondly, the occurrence of sphaeropedunculate and clavate cells along the gill edge and the utriform shape of some cheilo and pleurocystidia seemed to direct them to the Section *Spadiceogriseae* Kits van Wav., subsection *Spadiceogriseae* (Romagn.) ex Kits van Wav. (Kits van Waveren 1985).

If we compare the morphological features of our specimens with those belonging to the closest *Psathyrella* species, a number of differences emerge (Table 1). Our specimens appeared to be more similar to *P. thujina* (Henrici, 2017), previously described as *P. almerensis* (Kits van Waveren 1985, Vesterholt and Knudsen 1992), except for

Table 1. Main differences between our *Psathyrella* sp. and the closest species, according to the morphological characteristics of basidiomes and mycelium, and ecology. (Differences from our specimen are in bold characters).

Morpho-ecological characteristics	***Psathyrella* sp.**	***P. thujina***	***P. typhae***	***P. lutensis***
Pileus diameter (cm)	3.5	**2.5**	**2.5**	**4.0**
Pileus colour	Hazelnut brown, then beige brown	Warm brown, then beige brown	**Pinkish-ochre brown, then pale flesh brown**	**Dark reddish brown, then very pale brown**
Stem colour	White with a pruinose apex	White with a pruinose apex	**Whitish to pale brown**	**White with a pruinose apex, brownish base**
Spore size (µm)	7.2–11.8 x 4.3–6.0	**9.0–11.5 x 4.5–6.5**	**7.5–11.5(12.0) x 5.5–8.0**	**9.0–10.0 x 4.5–5.5**
Cheilocystidia	Versiform, chiefly utriform	**Utriform**	Versiform, chiefly utriform	Versiform, chiefly utriform
Pleurocystidia	Utriform	Utriform	**Absent**	**Versiform, chiefly utriform to ventricose**
Mucoid deposits on cystidia	NO	NO	NO	**YES**
Habitat	Marshes, on cut culms of *Cladium*	Marshes, on cut culms of *Typha*, *Phragmites*, *Cirsium*, *Epilobium*	Marshes, on cut culms of *Typha*, *Epilobium*, *Scirpus*, *Phragmites*, *Rumex*, *Iris*	**Deciduous forests, on sticks in mud**
Seasonal occurrence	Spring	**Autumn to winter**	**Summer**	**Summer to autumn**

Figure 2. Macro-morphological characteristics of the *Psathyrella* basidiomes: scales of velar origin on pilei tops and margins, and beige-coloured gills (**A**); cylindrical, white and exannulate stems under a lateral profile (**B**); colour-shading of a cap hygrophany and fibrillose details of velar-originated scales (**C**); gills turning brown-purplish with spore maturation and a fibrillose surface of a stem base (**D**); a pruinose stem apex bearing a mature hymenophore with white gill edge lines (**E**).

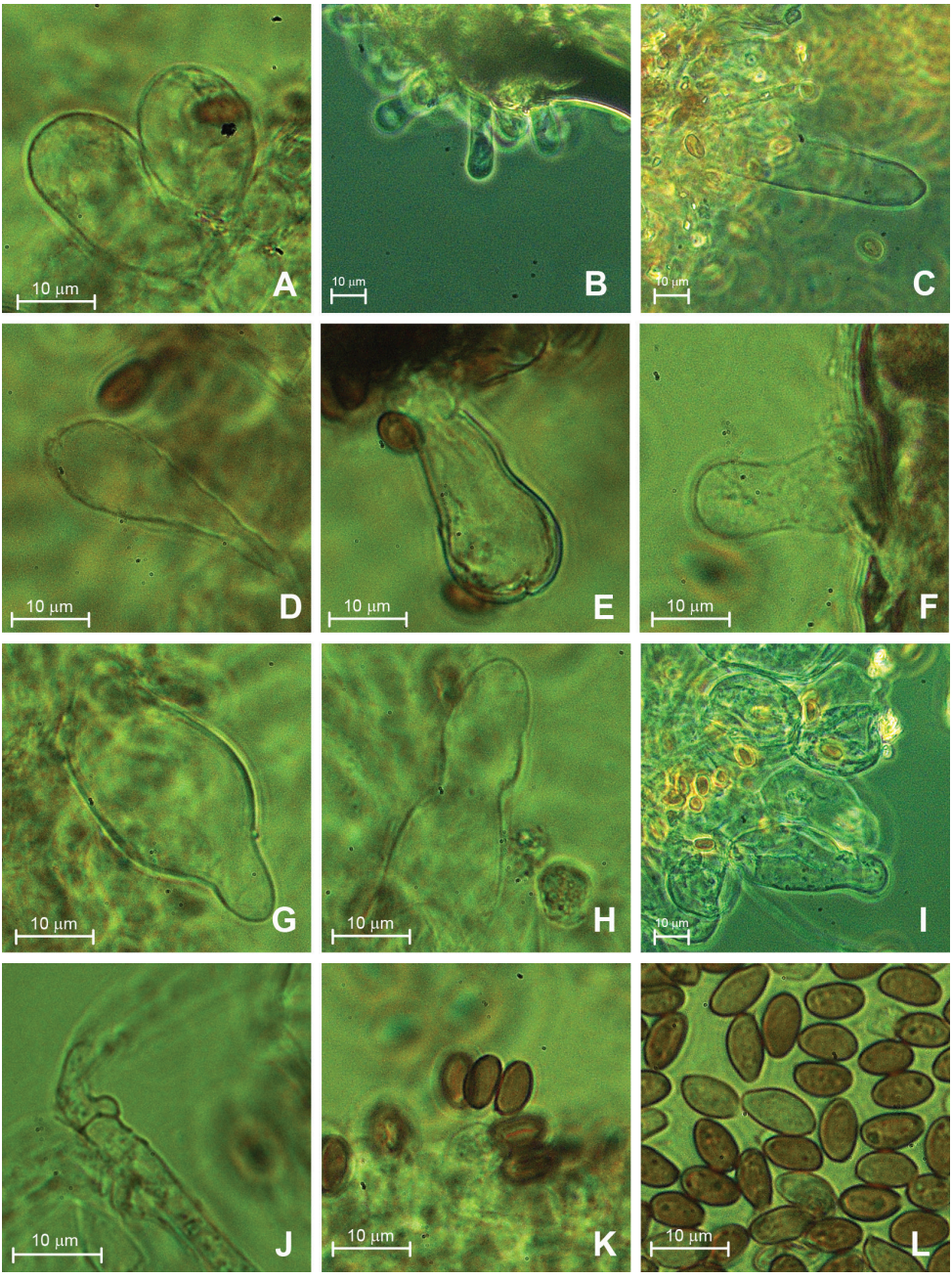

Figure 3. Micro-morphological characteristics of the *Psathyrella* mycelium: clavate and sphaeropedunculate (**A**), and cylindric (**B, C**) cells at a gill edge; differently clavate (**D, E**) and utriform (**F**) cheilocystidia; variously utriform-shaped pleurocystidia (**G, H, I**); a fibulate hypha (**J**); a 4-spored basidium (**K**); basidiospores (**L**).

the pileus diameter reaching 3.5 cm in our specimens, but never exceeding 2.5 cm in this species. Furthermore, our *Psathyrella* revealed versiform-shaped cheilocystidia, while those reported for *P. thujina* are only utriform. *P. typhae* was also divergent for the pileus diameter, not exceeding 2.5 cm, but even for pileus and stipe colours and for lacking pleurocystidia. On the other hand, the mucoid deposits, characterising the pleurocystidioid cheilocystidia of *P. lutensis*, were absent in our specimens. In addition, the spore length range was wider in our specimens than in *P. thujina* and *P. lutensis* and all the closest three species, which showed larger spores on average.

As for ecology, the plant genus *Cladium* Browne has never been reported as a substrate to any other *Psathyrella*, although *P. thujina* and *P. typhae* are commonly found on the remnants of ecologically similar plants (Kits van Waveren 1985, Vesterholt and Knudsen 1992, Örstadius et al. 2015, Henrici 2017). Furthermore, the genus *Cladium* was not mentioned in the unique Italian report of *P. thujina*, which was found "in open sites, close to any hygrophilous plants" (Voto 2016), in accordance with Henrici (2017) who refers this species to reed-beds and generic damp marshy habitats. Finally, our specimen was collected in the spring, whereas the above three other *Psathyrella* species seem to occur in other seasons.

DNA Analysis

The obtained nrDNA sequence was 702 bp long. By comparing it with those published in GenBank, we obtained a data matrix composed of 44 taxa and 710 characters, 276 gap-free sites and 240 conserved sites. The highest homology (99%) was observed with *P. candolleana* (Fr.) Maire, which was confirmed by the phylogenetic analysis (Fig. 4). Indeed, the phylogenetic tree shows that our specimen falls into the "*candolleana*" clade, such a heterogeneous group, including taxa from different morphology, ecology and geographic provenance and, amongst them, the above-cited *P. typhae* (Battistin et al. 2014, Örstadius et al. 2015, Yan and Bau 2018).

Discussion and conclusions

Based on results from both morphological and molecular analysis, our collection cannot be assigned to a known species. According to morphology, our *Psathyrella* should be closer to *P. thujina* (Section *Spadiceogriseae*). By contrast, the DNA ITS sequence would undoubtedly include it in the "*candolleana*" clade, where each species showed up to a 99% ITS sequence similarity with our sample. The most widespread and known species in this clade, *P. candolleana* and *P. leucotephra* (Berk. & Broome) P.D. Orton, both commonly occurring in Europe, too, are however morphologically very different from our specimen, by forming large pilei (diameter up to 8.0 cm) and lacking pleurocystidia; furthermore, the latter frequently even shows a torn annulus in the upper part of the stem, which we did not observe in our *Psathyrella* (Kits van Waveren 1985,

Table 2. Species used for the phylogenetic analyses including GenBank Accession Numbers and published references.

Species	GenBank accession No.	Reference
Psathyrella abieticola	KC992891	Örstadius et al. 2015
P. almerensis	KC992874	Örstadius et al. 2015
P. almerensis	KC992873	Örstadius et al. 2015
P. ammophila	KC992872	Örstadius et al. 2015
P. candolleana	AB306311	Ogura-Tsujita and Yukawa 2008
P. candolleana	DQ389720	Larsson and Örstadius 2008
P. candolleana	MG734719	Yan and Bau 2018
P. candolleana	MG734720	Yan and Bau 2018
P. cladii-marisci	MK080112	This study
P. conferta	KC992890	Örstadius et al. 2015
P. conica	MG734713	Yan and Bau 2018
P. flexispora	MF966494	Heykoop and Moreno 2002
P. fusca	MF966503	Heykoop and Moreno 2002
P. impexa	KC992900	Örstadius et al. 2015
P. kellermanii	KC992920	Örstadius et al. 2015
P. luteopallida	MG734736	Yan and Bau 2018
P. lutensis	MG734748	Yan and Bau 2018
P. lutensis	DQ389685	Larsson and Örstadius 2008
P. lutulenta	KC992875	Örstadius et al. 2015
P. madida	KC992932	Örstadius et al. 2015
P. parva	KC992912	Örstadius et al. 2015
P. prona	KJ939634	Larsson and Örstadius 2008
P. pseudogracilis	KC992853	Örstadius et al. 2015
P. purpureobadia	NR_119670	Larsson and Örstadius 2008
P. romagnesii	DQ389716	Larsson and Örstadius 2008
P. saponacea	MH155965	Yan and Bau 2018
P. senex	MG734732	Yan and Bau 2018
P. singeri	MG734718	Yan and Bau 2018
P. squamosa	KC992939	Örstadius et al. 2015
P. squamosa	MG367206	Yan and Bau 2018
P. subsingeri	MG734714	Yan and Bau 2018
P. sulcatotuberculosa	KJ138423	Battistin et al. 2014
P. tenera	FJ899635	Frank et al. 2010
P. tenuicula	DQ389706	Larsson and Örstadius 2008
P. thujina	KC992873	Örstadius et al. 2015
P. thujina	KC992874	Örstadius et al. 2015
P. thujina	KY680791	Örstadius et al. 2015
P. thujina	KY680792	Örstadius et al. 2015
P. trinitatensis	KC992882	Örstadius et al. 2015
P. tuberculata	MH497604	Yan and Bau 2018
P. typhae	DQ389721	Larsson and Örstadius 2008
Coprinellus heterothrix	FM878018	Nagy et al. 2011
C. impatiens	FM163177	Nagy et al. 2011
C. silvaticus	KC992943	Örstadius et al. 2015

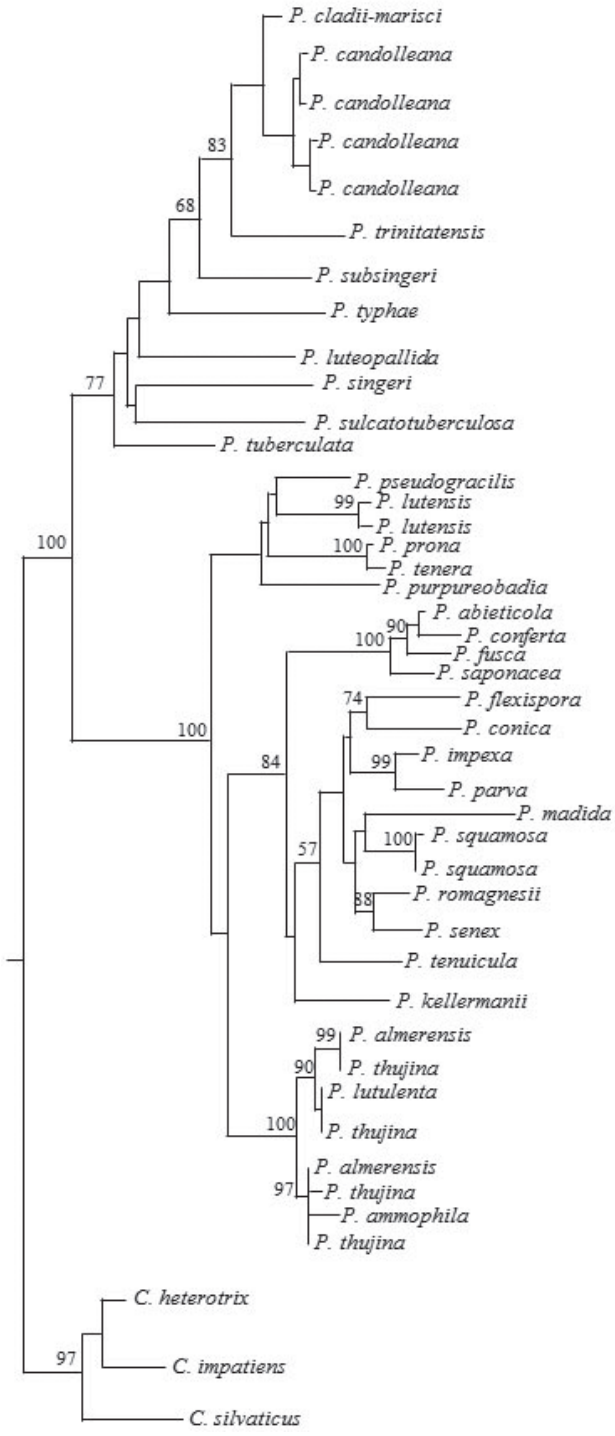

Figure 4. One of the most parsimonius trees from the phylogenetic analysis of *Psathyrella* spp. based on nrDNA sequence data. Bootstrap values are shown above branches based on 1,000 replicates (values below 50 are not shown).

Vesterholt and Knudsen 1992, Consiglio 2005). The "*candolleana*" clade encompasses two more European species according to two recent phylogenetic analyses (Nagy et al. 2011, Battistin et al. 2014): *P. sulcatotuberculosa* (J. Favre) Einhell., previously regarded as a variety of *P. typhae* (Kits van Waveren 1985), which mainly differs from our *Psathyrella* and from *P. typhae* itself with a partially-sulcate and -tuberculate pileus surface, and *P. badiophylla* (Romagn.) Bon which forms spores normally exceeding 10–11 µm in length (Kits van Waveren 1985, Vesterholt and Knudsen 1992); in addition, both also lack pleurocystidia, which was considered to be such a morphologically relevant character to induce the establishment of the Section *Spintrigerae* within the subgenus *Psathyra* (Fr.) Sing. ex Kits van Wav. (Kits van Waveren 1985). Moreover, except for *P. typhae*, which is the only *Psathyrella* ecologically comparable to our collection, all the above species are reported to grow in diverse site conditions, i.e. close to stumps of trees or on branches, on moist ground, in grass, on mossy woods or on various other vegetable matter (Kits van Waveren 1985, Vesterholt and Knudsen 1992). Finally, as far as we know, other species in the "*candolleana*" clade are even geographically more distant, each colonising a different kind of organic debris (Padamsee et al. 2008, Örstadius et al. 2015, Yan and Bau 2018).

Therefore, within this framework, the placement of our fungus into the "*candolleana*" clade, together with other species showing strong differences for geographic and ecologic reasons, should not prevent the recognition of a new *Psathyrella* species.

Anyhow, more and more scientific contributions are remarking that the genetic analysis of a fungus aiming at taxonomic purposes can alone generate artefacts, i.e. "false positive" or "chimeras", especially when such analysis is implemented by using a unique gene (Thines et al. 2018, Lücking et al. 2018). A polyphasic approach, i.e. based on the combination and integration of all the available informative data (Colwell 1970), is becoming more and more desirable for taxonomic research in mycology, whereas the ITS rDNA region is still considered as the universal genetic marker for fungi (Schoch et al. 2012).

On the basis of the outcomes deriving from the morphologic, ecologic and biomolecular characteristics which we have identified in this note, we are therefore inclined to establish a new species of *Psathyrella*.

Taxonomy

Psathyrella cladii-marisci **Sicoli, NG Passal., De Giuseppe, Palermo & Pellegrino, sp. nov.**
Figs 1–3

Etymology. The specific epithet derives from *Cladium mariscus*, the name of the plant where it was first detected.

Diagnosis. Similar to *P. thujina* from which it differs by showing a larger pileus (about 40% larger), a wider range of spore length, versiform cheilocystidia and basidiomes occurring in spring.

Holotype. Italy. Calabria, Cosenza, Rende, Orto Botanico Università della Calabria. 39°21'25.05"N, 16°13'44.57"E, 220 m a.s.l., marsh at the base of cut culms of a *Cladium mariscus* (L.) Pohl plant, transplanted from Lago dell'Aquila (Laureana di Borrello, Reggio Calabria, southern Italy) at the corner of a concrete tank maintained full of water, 10 April 2018, Antonio Biagio De Giuseppe & Giovanni Sicoli (CLU F302).

Description. *Habit* psathyrelloid. *Pileus* up to 3.5 cm diam., conical-convex when young, hemispheric to applanate at maturity, with a deeply striate margin, hazelnut in colour, turning to pale beige when dry. *Pileipellis* with evident concentric arachnoid fibrils of velar origin, whitish and easily removable, often exceeding the cuticle margin. *Lamellae* distant, ventricose, adnate, intermingled with numerous lamellulae, initially pale pink, then intensely brown-purplish. *Lamella edge* whitish with numerous sphaeropedunculate cells. *Stipe*, very fragile, cylindrical, white, exannulate with a diffuse fibrillosity especially on the basal surface, apical surface pruinose. *Basidiospores* 7.2–11.8 × 4.3–6.0 µm (n = 100), ellipsoid to ovoid-ellipsoid, with a thick and smooth wall, adaxially flattened with a central 2µm-wide germ pore and a distinct hilar appendix. *Spore-print* dark brown. *Basidia* clavate, 4-spored. *Cheilocystidia* versiform, often utriform, seldom cylindrical to clavate. *Pleurocystidia* utriform-shaped. *Mycelium* septate and clamped. *Context* with apparently no smell, taste mild.

Habit, habitat and distribution. In small groups (gregarious), on the culm remnants of *Cladium mariscus*. So far, known only from the type locality.

Conclusions

This probably rare and, apparently, never before detected species could occur more commonly if further surveys confirmed a sort of preference for *C. mariscus* as a growing substrate for the fungus. This plant was observed all over Italy (Bartolucci et al. 2018), although becoming more and more scattered due to the progressive surface reduction of its natural growing environment, i.e. marshes and wet sites quite close to the sea at mid-low altitudes. These sites have been long subjected to draining and other forms of anthropogenic land uses. Since human activities have been causing a deep influence and restriction on density and distribution of the spontaneous flora, including *C. mariscus*, the gradual depletion of plant biodiversity in such sites could also result in negative effects on fungal diversity, thus rendering even more scarce the occurrence of basidiomes of such taxa as *P. cladii-marisci* in Italy.

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References

Bartolucci F, Peruzzi L, Galasso G, Albano A, Alessandrini A, et al. (2018) An updated checklist of the vascular flora native to Italy. Plant Biosystems – An International Journal Dealing with all Aspects of Plant Biology 152(2): 179–303. https://doi.org/10.1080/11263504.2017.1419996

Battistin E, Chiarello O, Vizzini A, Örstadius L, Larsson E (2014) Morphological characterisation and phylogenetic placement of the very rare species *Psathyrella sulcatotuberculosa*. Sydowia 66(2): 171–181. http://hdl.handle.net/2318/152677

Christan J, Hussong A, Dondl M (2017) Beiträge zur Familie Psathyrellaceae: *Psathyrella spintrigeroides*, *Psathyrella supernula*, *Psathyrella typhae*. Mycologia Bavarica 18: 35–58.

Colwell RR (1970) Polyphasic Taxonomy of the Genus Vibrio: Numerical Taxonomy of Vibrio cholerae, Vibrio parahaemolyticus, and Related Vibrio Species. Journal of Bacteriology 104(1): 410–433. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC248227/

Consiglio G (2005) Contributo alla conoscenza dei Macromiceti dell'Emilia-Romagna. XXIII. Famiglia Coprinaceae - Parte terza. Bollettino del Gruppo Micologico G. Bresadola – Nuova Serie BGMB 48(2): 7–22.

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12: 13–15.

Felsestein J (1985) Phylogenies and comparative method. The American Naturalist 125(1): 1–15. https://doi.org/10.1086/284325

Frank JL, Coffan RA, Southworth D (2010) Aquatic gilled mushrooms: *Psathyrella* fruiting in the Rogue River in southern Oregon. Mycologia 102: 93–10. https://doi.org/10.3852/07-190

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium, Series 41: 95–98.

Henrici A (2017) *Psathyrella*: the state of play – including *P. thujina* new to Britain. Field Mycology 18(3): 87–91. https://doi.org/10.1016/j.fl dmyc.2017.07.007

Heykoop M, Moreno G (2002) Studies in the genus *Psathyrella* in Spain. IV. *Psathyrella submicrospora sp. nov.* and *P. microsporoides nom. nov.* Mycotaxon 83: 425–433.

Katoh K, Rozewicki J, Yamada KD (2017) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics: 1–7. https://doi.org/10.1093/bib/bbx108

Kits van Waveren E (1985) The Dutch, French and British species of *Psathyrella*. Persoonia, Suppl. Vol. 2: 1–300.

Larsson E, Örstadius L (2008) Fourteen coprophilous species of *Psathyrella* identified in the Nordic countries using morphology and nuclear rDNA sequence data. Mycological Research 112: 1165–1185. https://doi.org/10.1016/j.mycres.2008.04.003

Lücking R, Kirk PM, Hawksworth DL (2018) Sequence-based nomenclature: a reply to Thines et al. and Zamora et al. and provisions for an amended proposal "from the floor" to allow DNA sequences as types of names. IMA Fungus 9(1): 185–198. https://doi.org/10.5598/imafungus.2018.09.01.12

Nagy LG, Walther G, Házi J, Vágvölgyi C, Papp T (2011) Understanding the evolutionary processes of fungal fruiting bodies: correlated evolution and divergence times in the Psathyrellaceae. Systematic Biology 60: 303–317. https://doi.org/10.1093/sysbio/syr005

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2014) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Ogura-Tsujita Y, Yukawa T (2008) High mycorrhizal specificity in a widespread mycoheterotrophic plant, *Eulophia zollingeri* (Orchidaceae). American Journal of Botany 95: 93–97. https://doi.org/10.3732/ajb.95.1.93

Örstadius, L, Ryberg M, Larsson E (2015) Molecular phylogenetics and taxonomy in Psathyrellaceae (Agaricales) with focus on psathyrelloid species: introduction of three new genera and 18 new species. Mycological Progress 14:25. https://doi.org/10.1007/s11557-015-1047-x

Padamsee M, Matheny B, Dentinger BTM, McLaughlin DJ (2008) The mushroom family Psathyrellaceae: Evidence for large-scale polyphyly of the genus *Psathyrella*. Molecular Phylogenetics and Evolution 46: 415–429. https://doi.org/10.1016/j.ympev.2007.11.004

Rambaut A (2009) FigTree version 1.3.1 [computer program] http://tree.bio.ed.ac.uk

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

Thines M, Crous PW, Aime MC, Aoki T, Cai L, Hyde KD, Miller AN, Zhang N, Stadler M (2018) Ten reasons why a sequence-based nomenclature is not useful for fungi anytime soon. IMA Fungus 9(1): 177–183. https://doi.org/10.5598/imafungus.2018.09.01.11

Vesterholt J, Knudsen H (1992) *Psathyrella* (Fr.) Quél. In: Nordic Macromycetes (Vol. 2), Hansen N and Knudsen H (Eds) Nordsvamp, Copenhagen, 236–252.

Voto P (2016) Rare Agaricales in Polesine I: *Psathyrella*, *Conocybe*, *Lepista*. Rivista di Micologia 59(2): 163–174.

Yan JQ, Bau T (2018) The Northeast Chinese species of *Psathyrella* (Agaricales Psathyrellaceae). MycoKeys 33: 85–102. https://mycokeys.pensoft.net/article/24704/

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

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RESEARCH ARTICLE

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Recognition of *Mycena* sect. *Amparoina* sect. nov. (Mycenaceae, Agaricales), including four new species and revision of the limits of sect. *Sacchariferae*

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Abstract

Phylogenetic reconstruction revealed that *Mycena* stirps *Amparoina*, which is traditionally classified in sect. *Sacchariferae*, should be treated at section level. Section *Amparoina* is characterised by the presence or absence of cherocytes, the presence of acanthocysts and spinulose caulocystidia. Eight species referred to *Mycena* sect. *Amparoina* **sect. nov.** are recognised in China. Of these taxa, four new species classified in the new section are formally described: *M. bicystidiata* **sp. nov.**, *M. griseotincta* **sp. nov.**, *M. hygrophoroides* **sp. nov.** and *M. miscanthi* **sp. nov.** The new species are characterised by the absence of both cherocytes and a basal disc, along with the presence of acanthocysts on the pileus, spinulose cheilocystidia and caulocystidia. Descriptions of the new species, accompanied by illustrations of morphological characters and comparisons with closely related taxa, are provided. A multi-locus analysis utilising the ITS + nLSU + SSU regions was carried out using maximum likelihood and Bayesian Inference. A key to the 12 species of sect. *Amparoina* **sect. nov.** and sect. *Sacchariferae* that are found in China is provided.

Keywords

Agarics, new taxon, systematics, taxonomy

Introduction

The genus *Mycena* (Pers.) Roussel is characterised by small basidiomata, thin and convex pileus with sulcate margin, non-deliquescent lamellae and hollow stipe (Persoon 1797). The genus comprises more than 500 species and is distributed worldwide (Kirk

et al. 2008). *Mycena* sect. *Sacchariferae* Kühner ex Singer, which is one of the largest sections in the genus, was first published as a *nomen nudum* by Kühner (1938), who defined the section to include members that possess a granulose or "sugar coated" pileus. In 1958, Singer erected the monotypic genus *Amparoina* Singer to house *Marasmius spinosissimus* Singer based on the collections from Argentina (Singer 1958). Later, Singer (1976) established Amparoinaceae with *A. spinosissima* (Singer) Singer as type species and introduced another species in *Amparoina*, *A. heteracantha* Singer. Meanwhile he suggested that *Amparoina* was similar to sect. *Sacchariferae*, but maintained the autonomy of the former due to inamyloid basidiospores and revised sect. *Sacchariferae* to be characterised by a pileipellis with acanthocysts, which remain as terminal cells overlaid by a universal veil (Singer 1976). The pileus of cherocytes and acanthocysts distinguish taxa of sect. *Sacchariferae* from all other *Mycena* species. Section *Sacchariferae* was subdivided by Desjardin (1995) into stirps *Amparoina* Desjardin, stirps *Alphitophora* Desjardin and stirps *Adscendens* Desjardin, with 55 epithets classified into 27 taxa, based on presence or absence of a basal disc, cherocytes, and diverse caulocystidia. Maas Geesteranus and de Meijer (1997) established a fourth stirps, named stirps *Fuscinea* Maas Geest. & de Meijer, in which the acanthocysts possess brown contents, a character similar to that of stirps *Amparoina*. Only two species have been classified in stirps *Fuscinea*, namely *M. fuscinea* Maas Geest. & de Meijer and *M. fuliginea* Maas Geest. & de Meijer (Maas Geesteranus and de Meijer 1998). The morphology-based infrasectional classification of *Mycena* sect. *Sacchariferae*, proposed by Desjardin (1995), has been widely adopted. However, no phylogenetic reconstruction of relationships in sect. *Sacchariferae* has been published to assess the validity of the infrasectional classification.

Previous studies of sect. *Sacchariferae* have focused on species distributed in Europe and North and South America, with more than 60 species studied in the past 30 years (Maas Geesteranus 1983, 1992a, 1992b; Lodge 1988; Takahashi 1999; Perry 2002; Grgurinovic 2003; Robich 2003, 2016; Tanaka and Hongo 2003; Nealel 2009; Robich and Hausknecht 2009; Zamora and Català 2013; Cortéspérez et al. 2015; Aronsen and Læssøe 2016). In contrast, studies of Asian taxa have been scanty until recent years. Aravindakshan and Manimohan (2015) described ten taxa, including six new species in sect. *Sacchariferae* from India. Only three species, *M. anoectochili* L. Fan & S.X. Guo, *M. alphitophora* (Berk.) Sacc. and *M. cornephora* Maas Geest., were formerly reported from China (Guo et al. 1997; Li et al. 2015). However, recently, three new taxa of sect. *Sacchariferae* were described, namely *M. castaneicola* T. Bau & Q. Na, *M. hyalinostipitata* T. Bau & Q. Na and *M. substylobates* T. Bau & Q. Na, from subtropical regions of China (Na and Bau 2019).

A phylogenetic reconstruction of *Mycena* was incongruous with the traditional classification of stirps *Amparoina* within sect. *Sacchariferae* and indicated that the taxonomic classification of the section should be reconsidered. During our ongoing research on *Mycena*, four new taxa without a basal disc and cherocytes, belonging to the new section, were found in southern China in Chongqing City, Guangdong Province,

Henan Province, Hubei Province, Tibet Autonomous Region, Yunnan Province and Zhejiang Province. These species are described here. Based on the phylogenetic analyses, an identification key to the 12 species of sect. *Sacchariferae* and sect. *Amparoina* currently known from China is provided.

Materials and methods

Morphological study

Macroscopic characters were described from fresh specimens following conventional taxonomic methods. Colour terms and notations refer to those of Kornerup and Wanscher (1978). Microscopic characters were observed from dried specimens rehydrated in 5% potassium hydroxide (KOH) and stained with Congo red, using a Nikon 80i light microscope. Melzer's reagent was used for testing amyloid and dextrinoid reactions of all tissues (Horak 2005). The spore shape quotient (spore length divided by spore width; $Q = L/B$) was calculated from 40 mature basidiospores; 90% of the numerical range is indicated outside parentheses and the 10% extreme values are enclosed in parentheses. Author abbreviations are based on those used in Index Fungorum (https://www.indexfungorum.org). Voucher specimens have been deposited in the Herbarium Mycology of Jilin Agricultural University (HMJAU).

DNA extraction, PCR amplification and DNA sequencing

Material for DNA isolation was taken from dried specimens. Genomic DNA was extracted from samples using the NuClean Plant Genomic DNA Kit (Kangwei Century Biotechnology Company Limited, Beijing, China). The internal transcribed spacer (ITS) region was amplified with the primer pair ITS1 and ITS4 (White et al. 1990). The nLSU and SSU regions were amplified using the primers LROR/LR7 and MS1/MS2, respectively (Ward et al. 1992; Hopple and Vilgalys 1999). The PCR cycling schedule for the ITS, nLSU and SSU region used a touchdown programme (Na and Bau 2018). All newly generated sequences were deposited in GenBank (Table 1).

Sequence alignment and phylogenetic analysis

A dataset, comprising sequences for the ITS + nLSU + SSU region from 96 accessions with taxonomic coverage of Europe, North America, Australia, Africa and Asia, was compiled and analysed. Sequences for 32 accessions were downloaded from GenBank and 64 newly generated sequences obtained in this study were aligned and adjusted manually using BioEdit 7.0.4.1 and Clustal X (Thompson et al. 1997;

Table 1. Sequenced specimens used in phylogenetic analysis.

Taxa	Voucher	Locality	GenBank accession no.		
			ITS	nLSU	SSU
Infundibulicybe gibba (Pers.) Harmaja	AFTOL-ID 1508	USA	DQ490635	DQ457682	–
I. gibba	FLAS-F-60947	Unpublished	MH016906	–	–
Mycena abramsii (Murrill) Murrill	HMJAU 43282	Jilin: Jingyuetan National Scenic Area, Changchun City	MH396626	MK629348	MK629326
M. abramsii	HMJAU 43468	Jilin: Jingyuetan National Scenic Area, Changchun City	MH396627	–	MK629328
	HMJAU 43523	Jilin: Songjiang Town, Jiaohe City	MH396628	MK629350	MK629330
	HMJAU 43606	Inner Mongolia Autonomous Region: Mangui Town, Hulunbeier City	MH396629	MK629355	MK629336
M. adscendens Maas Geest.	Orstadius329-05	Norway: Strengsdal Village, Vestfold	KT900141	–	–
M. adscendens	Aronsen061119	Norway: Strengsdal Village, Vestfold	KT900142	–	–
	Aronsen120826	Norway: Strengsdal Village, Vestfold	KT900143	–	–
M. alphitophora	HMJAU 43498	Jilin: Shenglihe forest farms, Jiaohe City	MH136830	–	MK629329
	HMJAU 43686	Yunnan: Zixi Mountain National Nature Reserve, Chuxiong City	MH136831	–	MK629343
M. arcangeliana Bres.	252b	Italy: Venice Museum of Natural History, Venice	JF908401	–	–
M. arcangeliana	252f	Italy: Venice Museum of Natural History, Venice	JF908402	–	–
M. bicystidiata T.Bau & Q.Na	HMJAU 43589	Hubei: Yandongwan, Lichuan County	MK309774	–	–
M. bicystidiata	HMJAU 43593	Hubei: Xingdou Mountain National Nature Reserves	MK309775	MK629354	–
	HMJAU 43648, **Type**	Chongqing: Dafengbao Scenic Regions, Huangshui Town	MK309773	MK629359	MK629341
	HMJAU 43744	Zhejiang: Tianmu Mountain National Nature Reserves, Hangzhou City	MK309776	–	–
M. castaneicola T.Bau & Q.Na	HMJAU 43578, **Type**	Henan: Jigong Mountain National Nature, Xinyang City	MH136826	–	MK629334
M. castaneicola	HMJAU 43581	Henan: Bolden National Forest Park, Xinyang City	MH136827	–	–
M. citrinomarginata Gillet	HMJAU 43563	Shanxi: Wutai Mountain National Nature, Xinzhou City	MG654739	MK629351	MK629331
M. citrinomarginata	317h	Italy: Venice Museum of Natural History, Venice	JF908416	–	–
	AD4TN	Tunisia: Aïn Draham	KU973883	–	–
M. corynephora Maas Geest.	HMJAU 43574	Henan: Xinyang City	MH136832	–	MK629332
M. corynephora	HMJAU 43576	Henan: Xinyang City	MH136833	–	MK629333
M. diosma Krieglst.&Schwöbel	320f	Italy: Venice Museum of Natural History, Venice	JF908417	–	–
M. griseotincta T.Bau & Q.Na	HMJAU 43800, **Type**	Yunnan: Shangri-La Pudacuo National Park	MK309783	MK629363	MK629346
M. griseotincta	HMJAU 43805	Yunnan: Shangri-La Pudacuo National Park	MK309782	–	–
	HMJAU 43819	Tibet: Zhuqudeng Village, Nyingchi City	MK309784	–	–
M. heteracantha (Singer) Desjardin	HMJAU 43709,	Hunan: Yuelu Mountain, Changsha City	MK309785	MK629362	MK629345
M. heteracantha	HMJAU 43711	Hunan: Xiaoxi National Nature Reserves	MK309786	–	–
	HMJAU 43716	Hunan: Gaowangjie National Nature Reserves	MK309787	–	–
M. hyalinostipitata T.Bau&Q. Na	HMJAU 43693, **Type**	Yunnan: Yeyahu Scenic Spot, Kunming City	MH136828	MK629361	MK629344

Taxa	Voucher	Locality	GenBank accession no.		
			ITS	nLSU	SSU
M. hyalinostipitata	HMJAU 43701	Yunnan: Yeyahu Scenic Spot, Kunming City	MH136829	–	–
M. hygrophoroides	HMJAU 43417, **Type**	Guangdong: Chebaling National Nature Reserve, Shaoguan City	MK309780	MK629349	MK629327
	HMJAU 43421	Guangdong: Shangxie Village, Shaoguan City	MK309781	–	–
M. meliigena (Berk.&Cooke) Sacc.	39	Italy: Venice Museum of Natural History, Venice	JF908423	–	–
M. meliigena	39d	Italy: Venice Museum of Natural History, Venice	JF908429	–	–
M. miscanthi T.Bau & Q.Na	HMJAU 43573	Henan: Jinniu Mountain, Xinyang City	MK309777	MK629352	–
M. miscanthi	HMJAU 43582	Henan: Bolden National Forest Park, Xinyang City	MK309778	–	–
	HMJAU 43584, **Type**	Henan: Jigong Mountain National Nature, Xinyang City	MK309779	MK629353	MK629335
M. pearsoniana Dennis ex Singer	FCME25817	USA: Great Smoky Mountains National Park, Tennessee	JN182198	–	–
M. pearsoniana	TENN61544	USA: Great Smoky Mountains National Park, Tennessee	JN182199	–	–
	TENN61384	USA: Great Smoky Mountains National Park, Tennessee	JN182200	–	–
M. pelianthina (Fr.) Quél.	108b	Italy: Venice Museum of Natural History, Venice	JF908379	–	–
M. pelianthina	108f	Italy: Venice Museum of Natural History, Venice	JF908380	–	–
	CBH164	Denmark: Jutland, Paderup Mose	FN394548	–	–
M. pseudocorticola Kühner	124a	Italy: Venice Museum of Natural History, Venice	JF908386	–	–
M. pura (Pers.) P. Kumm.	HMJAU 43121	Liaoning: Ant Ridge, Dandong City	MK309793	–	–
M. pura	HMJAU 43179	Heilongjiang: Shengshan National Nature Reserve	MK309794	–	–
	TENN65043	USA: Great Smoky Mountains National Park, Tennessee	JN182202	–	–
M. rosea Gramberg	CBH409	Germany: Baden-Württemberg, Schwarzwald	FN394551	–	–
M. rosea	TL12409	Denmark: Jutland, Skivum Nørrekrat	FN394557	–	–
M. rosella (Fr.) P. Kumm.	938a	Italy: Venice Museum of Natural History, Venice	JF908488	–	–
M. rosella	Champ-21	JGI MycoCosm database	KX449424	–	–
M. seminau A.L.C.Chew&Desjardin	ACL136	Malaysia: Ulu Gombak, Selangor	KF537250	–	–
M. seminau	ACL308	Malaysia: Ulu Gombak, Selangor	KF537252	–	–
M. silvae-nigrae Maas Geest.&Schwöbel	515	Italy: Venice Museum of Natural History, Venice	JF908452	–	–
M. silvae-nigrae	CC 13-12	USA: Great Smoky Mountains National Park	KF359604	–	–
M. substylobates T.Bau & Q.Na	HMJAU 43418, **Type**	Guangdong: Chebaling National Nature Reserve, Shaoguan City	MH216189	–	–
M. substylobates	HMJAU 43444	Guangxi Zhuang Autonomous Region: Nonggang National Nature Reserve, Chongzuo City	MH216190	–	–
M. supina (Fr.) P. Kumm.	128a	Italy: Venice Museum of Natural History, Venice	JF908388	–	–
M. tenerrima Maas Geest.	HMJAU 43646	Chongqing: Huangshui Town	MK309795	–	MK629340
M. tenerrima	HMJAU 43816	Tibet: Bomi County, Nyingchi City	MK309796	MK629364	–
M. zephirus (Fr.) P. Kumm.	CBS 270.48	Netherlands: Microbial Biological Resource Centres	MH856339	–	–
M. zephirus	CBS 273.48	Netherlands: Microbial Biological Resource Centres	MH856341	–	–

Hall 1999). The alignment was deposited with TreeBase (submission ID, 24326; study accession URL: http://purl.org/phylo/treebase/phylows/study/TB2:S24326). *Infundibulicybe gibba* were chosen as the outgroup. The aligned dataset consisted of 817 ITS, 1530 nLSU and 620 SSU nucleotide sites (including gaps). The best-fit evolutionary model was identified using Modeltest 2.3 for each of the ITS, nLSU and SSU data partitions for Bayesian Inference (BI), which was implemented with MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003; Nylander 2004). Markov chain Monte Carlo (MCMC) chains were run for one million generations, sampling every 100th generation until the critical value for the topological convergence diagnostic was less than 0.01 (Ronquist and Huelsenbeck 2003). Maximum Likelihood (ML) analysis was performed in raxmlGUI 1.5b1, with a rapid bootstrapping algorithm involving 1,000 replicates (Stamatakis et al. 2004). Topology support values greater than 75% bootstrap support (ML) 0.95 and Bayesian posterior probabilities (BPP) are shown at each branch node.

Results

Phylogeny

Sect. *Amparoina* (Clade 5) formed a distinct clade separated from sect. *Sacchariferae* (Clade 4), sect. *Calodontes* (Clade 3), sect. *Supinaae* (Clade 2) and sect. *Fragilipedes* (Clade 1), as a sister group to all other clades within the ingroup with high statistical support (ML ≥ 75%, BPP ≥ 1.00) and should be elevated to section level.

Phylogenetic reconstructions obtained using BI and ML showed similar topologies. The best-scoring Maximum Likelihood (ML) tree was selected as a representative phylogeny (Fig. 1). The optimal evolutionary model for the 5.8S and nLSU partition were lset nst = 6, rates = invgamma and prset statefreqpr = dirichlet (1,1,1,1) and SSU was lset nst = 6, rates = gamma and prset statefreqpr = dirichlet (1,1,1,1). The phylogenetic tree contained six clades, five including species of *Mycena*. The latter clade was nested within the clades of *Mycena* species. Each of the five clades of *Mycena* species corresponded with a taxonomic section, circumscribed from morphological characters, with high statistical support (ML ≥ 75%, BPP ≥ 0.95).

Samples of the four new species were placed in separate monophyletic lineages, each with high statistical support (*M. bicystidiatum*, ML = 99%, BPP = 1.00; *M. griseotincta*, ML = 99%, BPP = 1.00; *M. hygrophoroides*, ML = 98%, BPP = 0.99; *M. miscanthi*, ML = 100%, BPP = 1.00; Fig. 1). The phylogenetic tree resolved a strongly supported stirps *Alphitophora* comprising these species along with *M. alphitophora* (Berk.) Sacc., *M. corynephora* Maas Geest. in Clade 5 with ML = 100%, BPP = 1.00. Then stirps *Amparoina*, also located in Clade 5 as sister group with stirps *Alphitophora*, formed a monophyletic lineage with high statistical support in accordance with a basal disc in morphology. The distinction of the new taxa from the closely related species, *M. alphitophora* and *M. corynephora*, was also supported.

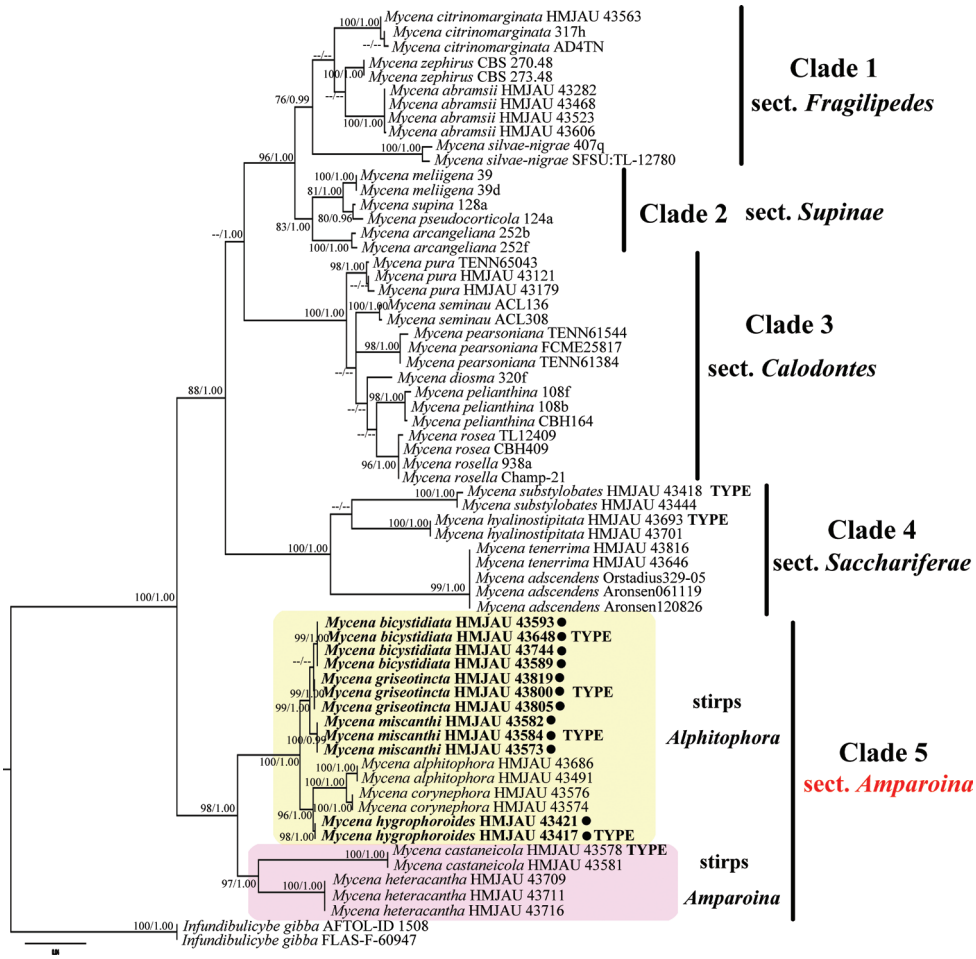

Figure 1. Maximum Likelihood and Bayesian tree concatenated ITS+nLSU+SSU dataset (ML ≥ 75%, BPP ≥ 0.95 are indicated). The tree is rooted with *Infundibulicybe gibba*. The new species are marked by ●.

Taxonomy

Key to species of sect. *Amparoina* and sect. *Saccharifera* in China

1 Basal disc present, cherocytes absent, acanthocysts present, caulocystidia smooth or with few spines ..... **(sect. *Saccharifera*) 2**

– Basal disc present or absent, cherocytes present or absent, acanthocysts present, caulocystidia spinulose..... **(sect. *Amparoina*) 5**

2 Pileus grey-black ..... ***M. anoectochila***

– Pileus white **3**

3 Caulocystidia irregularly shaped..... ***M. substylobates***

– Caulocystidia fusiform **4**

4 Cheilocystidia fusiform with spines in the middle part..... ***M. tenerrima***
– Cheilocystidia sphaeropedunculate with spines overall..... ***M. hyalinostipitata***
5 Basal disc and cherocytes present ..... (**stirps *Amparoina*)6**
– Basal disc and cherocytes absent..... (**stirps *Alphitophora*)7**
6 Habitat on fruits of *Castanea*, pileus slightly pubescent..... ***M. castaneicola***
– Habitat on dead wood or humus layer, pileus with bran-like covering .... ***M. heteracantha***
7 Lamellae distant, L < 10, I < 3 ..... ***M. hygrophoroides***
– Lamellae normal, L > 15, I > 6..... **8**
8 Basidiomata typically grey..... ***M. griseotincta***
– Basidiomata white..... **9**
9 Caulocystidia of two types, sphaeropedunculate or clavate..... ***M. bicystidiata***
– Caulocystidia clavate..... **10**
10 Basidiospores globose..... ***M. corynephora***
– Basidiospores ellipsoid ***11***
11 Acanthocysts of one type, sphaeropedunculate..... ***M. miscanthi***
– Acanthocysts of two types, globose or long-clavate..... ***M. alphitophora***

Section *Amparoina* T.Bau & Q.Na, sect. nov.
MycoBank: MB829096

Diagnosis. Pileus densely pubescent to furfuraceous. Stipe arising from a well-developed basal disc or base swollen without a basal disc. Cheilocystidia with spines. Cherocystes present or absent. Acanthocysts present and overlying universal veil. Caulocystidia densely spinulose overall, never smooth.

Type species. *Mycena spinosissima* (Singer) Desjardin

Etymology. Name refers to the name of stirps *Amparoina*.

***Mycena bicystidiata* T.Bau & Q.Na, sp. nov.**
MycoBank: MB829097
Figs 2c–d, 3

Diagnosis. Pileus furfuraceous to pruinose. Stipe without basal disc. Basidiospores small, 6.1–7.9 × 3.7–4.6 μm. Cheilocystidia clustered, sphaero-pedunculate to utriform with numerous sharp excrescences. Cherocytes absent. Acanthocysts pyriform to vesicular. Caulocystidia of two types, sphaero-pedunculate or clavate covered with conic spines. Clamps present.

Holotype. CHINA. Chongqing City, Dafengbao Scenic Regions, 15 Aug 2017, Qin Na, HMJAU 43648.

Etymology. Name refers to its two types of caulocystidia.

Description. Pileus 2.8–5.2 mm in diam., conical when young, becoming nearly hemispherical with age, pure white all over, sulcate, translucent-striate, pruinose,

Figure 2. Basidiomata of sect. *Amparoina* species. stirps *Alphitophora*: **a–b** *Mycena alphitophora* (Berk.) Sacc. **c–d** *Mycena bicystidiata* T.Bau & Q.Na **e** *Mycena corynephora* Maas Geest. **f–g** *Mycena griseotincta* T.Bau & Q.Na **h** *Mycena hygroporoides* T.Bau & Q.Na **i** *Mycena miscanthi* T.Bau & Q.Na; stirps *Amparoina*: **j** *Mycena castaneicola* T.Bau & Q.Na **k–m** *Mycena heteracantha* (Singer) Desjardin. Basidiomata of sect. *Saccariferae* species **n–o** *Mycena hyalinostipitata* T.Bau & Q.Na **p–q** *Mycena substylobates* T.Bau & Q.Na **r** *Mycena tenerrima* (Berk.) Quél. (=*Mycena adscendens* Maas Geest.) Scale bars: 10 mm (**a–g, i–m, r**), 5 mm (**h, n–q**). Photographs **a–r** by Qin Na.

furfur-like scattered, margin entire first, then nearly plane and finally fissile. Context very thin and fragile, pure white. Lamellae 0.5 mm thick, narrowly adnate, off-white, concolorous with the sides. Stipe slender, 15–28 × 0.5–1.0 mm, cylindrical, hollow, fragile, pure white, densely pruinose on the whole surface, base swollen and not forming a basal disc, hirsute. Odour and taste inconspicuous.

Basidiospores (5.6-)6.1–7.9(-8.3) × (3.5)3.7–4.6(4.9) μm, Q=1.6–2.0, ellipsoid to oblong-ellipsoid, hyaline, with drops, thin walled, amyloid. Basidia 20–26 × 6–9 μm, clavate, hyaline, 4- or 2-spored. Cheilocystidia 19–32 × 12–18 μm, clustered, sphaeropedunculate to utriform with numerous sharp spines, thin-walled and hyaline, inamyloid. Pleurocystidia absent. Pileipellis hyphae 4–7 μm wide, weakly dextrinoid; cherocytes absent; a cutis overlaid by elements of universal veil, not in chains; acanthocysts of one type, numerous, pyriform to vesicular, 29–62 × 24–51 μm, inamyloid. Hyphae of the stipitipellis 3–14 μm wide, smooth, dextrinoid; caulocystidia abundant, of two types, utriform, sphaero-pedunculate, 21–85 × 14–66 μm or clavate, long-elliptic, 21–85 × 11–26 μm, densely and evenly spinulose overall, hyaline, thin-walled, inamyloid. Clamps present in all tissues.

Habit and habitat. Solitary to scattered on rotten wood in mixed forests, Bamboos, *Cunninghamia*, *Ginkgo* and *Platycladus* forests.

Other specimens examined. CHINA. Hubei Province, Enshi Tujia and Miao Autonomous Prefecture, Lichuan County, Yandongwan, 19 Jul 2017, Qin Na, HMJAU 43589; Xingdou Mountain National Nature Reserves, 20 Jul 2017, Qin Na, HMJAU 43593; Zhejiang Province, Hangzhou City, Tianmu Mountain National Nature Reserves, 4 Jul 2018, Qin Na and Tolgor Bau, HMJAU 43774.

Remarks. *Mycena bicystidiata* is unique in sect. *Amparoina* stirps *Alphitophora* because of the two types of caulocystidia covered with conic spines. *Mycena alphitophora*, which is the most widely distributed species of sect. *Amparoina*, shows the most morphological similarities to *M. bicystidiatum*; however, the former differs in forming cylindric spores (7.5–10 × 4.5–5.5 μm), sphaero-pedunculate cheilocystidia and caulocystidia that are only clavate in shape (Desjardin 1995). *Mycena depilata* Singer is easily mistaken for *M. bicystidiata* by the stipe without a basal disc and the similar shape and size of spores and cheilocystidia, but *M. depilata* is distinguished from *M. bicystidiata* by its small basidiomata (pileus < 0.3 mm), larger spores (8.5–10 × 4.5–5.2 μm), and long-cylindrical and larger caulocystidia (30–120 × 5–20 μm) (Desjardin 1995). In contrast to *M. bicystidiata*, basidiospores of *M. corynephora*, *M. distincta* (Manim. & Leelav.) Aravind. & Manim., *M. globispora* (Manim. & Leelav.) Aravind. & Manim. and *M. yalensis* Singer are globose or broadly ellipsoid (Desjardin 1995; Aravindakshan and Manimohan 2015). The bright or dark colour on the pileus distinguishes *M. brunneospinosa* Desjardin, *M. incarnativelum* Desjardin and *M. roseotincta* Aravind. & Manim. from *M. bicystidiata* (Desjardin 1995; Aravindakshan and Manimohan 2015). In addition, *M. hemitrichialis* Singer produces caulocystidia that are only partially spinulose (Singer 1989).

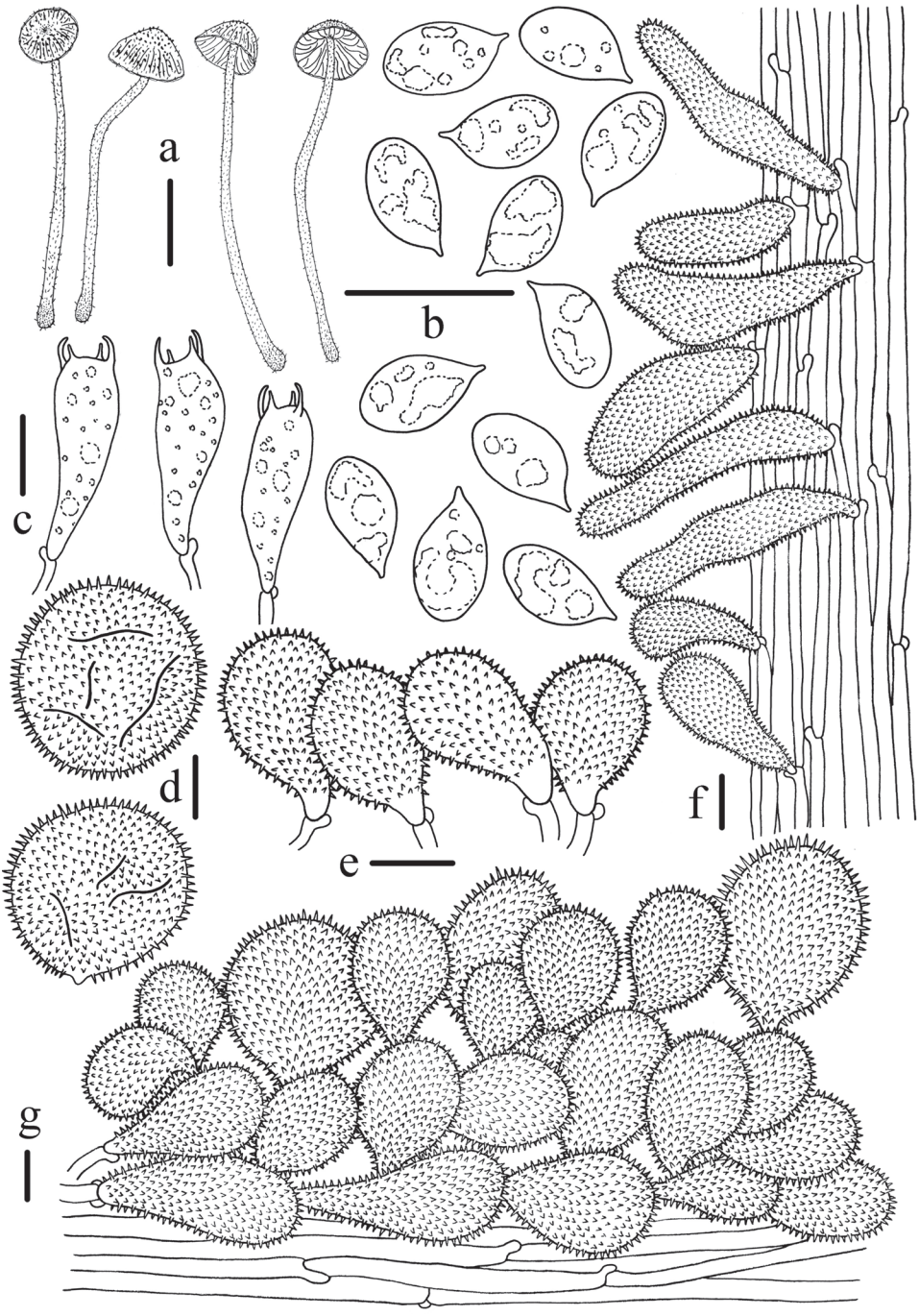

Figure 3. Microscopic features of *Mycena bicystidiata* (HMJAU 43648, holotype) **a** Basidiomata **b** Basidiospores **c** Basidia **d** Universal veil acanthocysts **e** Cheilocystidia **f** Caulocystidia **g** Pileipellis. Scale bars: 5 mm (**a**); 10 μm (**b–g**). Drawing by Qin Na.

Mycena griseotincta **T.Bau & Q.Na, sp. nov.**
MycoBank: MB829098
Figs 2f–g, 4

Diagnosis. Pileus, lamellae and stipe with greyish tint, especially when old. Stipe base swollen. Basidiospores pip-shaped. Pileipellis with two types of acanthocysts. Caulocystidia up to 200 μm long with spines.

Holotype. CHINA. Yunnan Province, Diqing Tibetan Autonomous Prefecture, Shangri-La Pudacuo National Park, 14 August 2018, Qin Na, HMJAU 43800.

Etymology. Name refers to the grey-tinted basidiomata.

Description. Pileus 1.5–12.8 mm in diam., conical when young, campanulate with age, obtusely umbonate in the centre, translucent-striate, white, greyish-white when old (4B1), floccose, pubescent, pruinose, with crenate margin when young, then becoming nearly plane and finely torn. Context pure white, thin, fragile. Lamellae 0.2–0.5 mm thick, narrowly adnate or adnexed, pure white to slightly pale grey (4B1); edges finely torn, concolorous with the sides. Stipe 13–64 × 0.5–1.0 mm, central, terete, almost equal or slightly tapering to apex, hollow, greyish-white (5B1), pubescent or puberulous, with white, fine hairs, base swollen. Odourless, taste mild.

Basidiospores (5.6-)6.3–8.2(-8.5) × (3.5-)4.2–4.6(-5.2) μm, Q=1.5–1.9, Qav=1.7, pip-shaped, hyaline, guttulate, thin walled, amyloid. Basidia 19–23 × 7–9 μm, hyaline, clavate, 4-spored. Cheilocystidia 17–28 × 11–19 μm, oblong or clavate, with short and sharp spines, hyaline, inamyloid. Pleurocystidia absent. Pileipellis hyphae 6–10 μm wide, strongly dextrinoid; cherocytes absent; acanthocysts of two types, pyriform to vesicular, 8–22 × 7–18 μm or clavate to cylindric, 17–51 × 8–13 μm; universal veil composed of acanthocysts, globose, subglobose or sphaero-pedunculate, 28–67 × 26–58 μm, hyaline, covered with long, cylindrical excrescences or long and flexuous spinules, not in chains. Hyphae of the stipitipellis 2–7 μm wide, dextrinoid; caulocystidia abundant, clavate or long cylindrical, 77–216 × 9–11 μm, covered with densely conic spines, inamyloid. Clamps not seen.

Habit and habitat. Scattered to gregarious on litter layer in *Quercus*, *Picea*, *Abies*, *Pinus* mixed forests.

Other specimens examined. Yunnan Province, Diqing Tibetan Autonomous Prefecture, Shangri-La Pudacuo National Park, 15 August 2018, Qin Na, HMJAU 43805; Tibet Autonomous Region, Nyingchi City, Zhuqudeng Village, 20 August 2018, Qin Na, HMJAU 43819.

Remarks. *Mycena griseotincta* is considered a new species in sect. *Amparoina* stirps *Alphitophora* on account of the absence of both a basal disc and cherocytes on the pileal surface (Desjardin 1995). Five species have ellipsoid basidiospores, caulocystidia covered with excrescences and a universal veil composed of acanthocysts: *M. alphitophora*, *M. brunneospinosa*, *M. depilata*, *M. hemitrichialis* and *M. incarnativelum*. *Mycena alphitophora* most resembles *M. griseotincta*, but the former differs in having pure white lamellae, a white and shorter stipe (< 50 mm), sphaero-pedunculate or obovoid cheilocystidia and larger spores (8.1–9.7 × 4.5–5.5 μm), as reported in the original de-

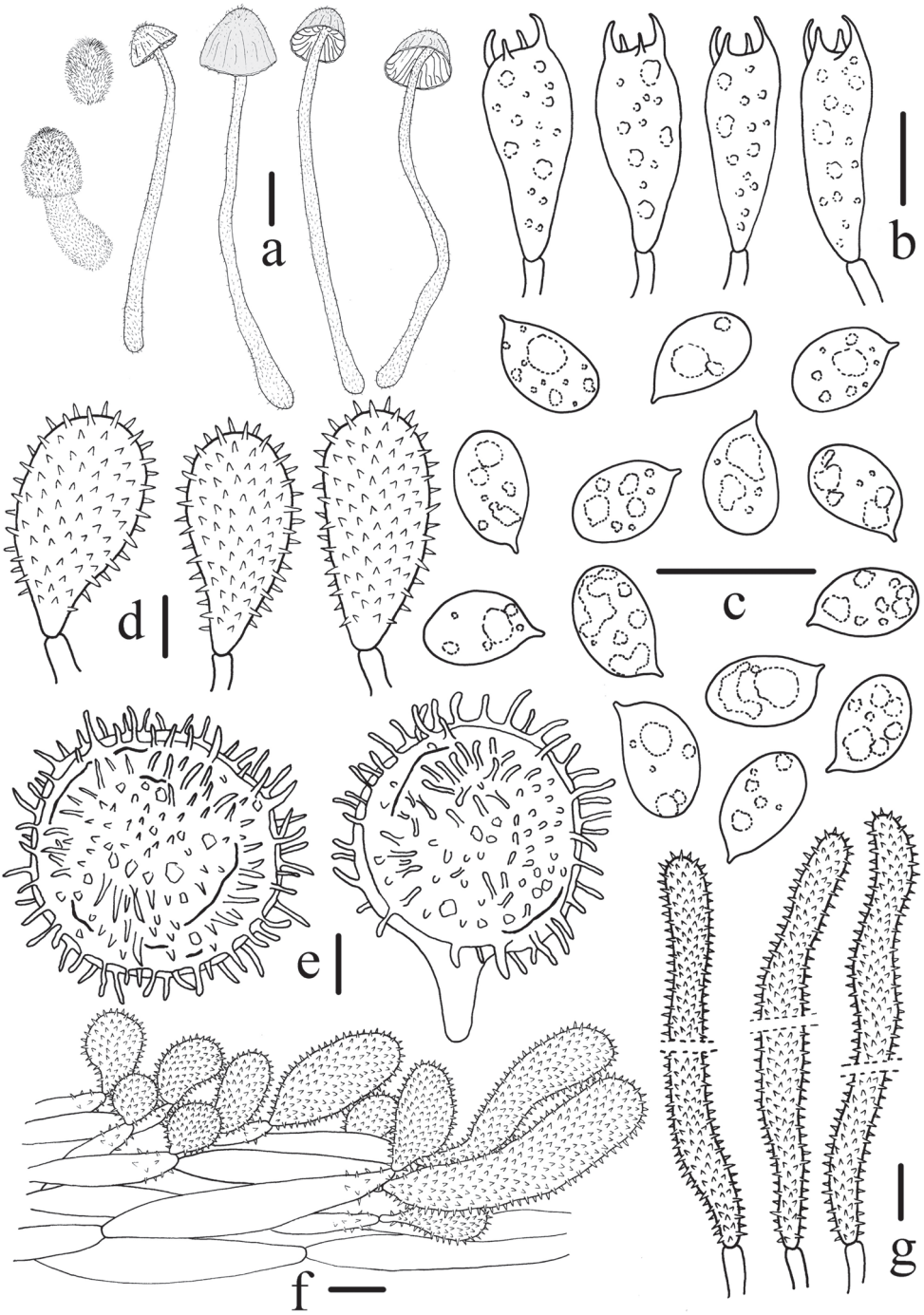

Figure 4. Microscopic features of *Mycena griseotincta* (HMJAU 43800, holotype). **a** Basidiomata **b** Basidia **c** Basidiospores **d** Cheilocystidia **e** Universal veil acanthocysts **f** Pileipellis **g** Caulocystidia. Scale bars: 10 mm (**a**); 10 μm (**b–g**). Drawing by Qin Na.

scription (Maas Geesteranus 1980, 1992b). *Mycena brunneospinosa*, a taxon named by Desjardin (1995), is readily identified by its dull brown or purplish-brown pileus, globose acanthocysts forming chains and broadly ellipsoid spores. *Mycena incarnativelum* is a unique species in sect. *Sacchariferae*, distinguished by the absence of cheilocystidia and deep pink basidiomata when young (Desjardin 1995). *Mycena depilata* is closely allied to *M. griseotincta*, but differs in the convex pileus less than 1 mm in diameter and short and broadly clavate caulocystidia (Singer 1989). *Mycena hemitrichialis* can be mistaken for *M. griseotincta* on account of its grey or pallid pileus and ellipsoid spores, but is distinguished from *M. griseotincta* by its white stipe, free lamellae and pilose stipe forming a flattened ring of mycelium (Desjardin 1995). *Mycena corynephora* is widely distributed worldwide and is recognised by its tiny basidiomata (pileus < 2.4 mm), absence of a basal bulb or basal disc and large globose to subglobose basidiospores, typical of stirps *Alphitophora* (Desjardin 1995; Robich 2003; Aronsen and Læssøe 2016). The same spore shape occurs in *M. yalensis* of which the holotype was collected from Argentina (Singer 1973). Aravindakshan and Manimohan (2015) reported one new species and two others newly combined in *Mycena*, collected from India. The new taxon, *M. roseotincta*, differs from *M. griseotincta* in its pink pileus and universal veil, subcylindrical spores and smaller caulocystidia (Aravindakshan and Manimohan 2015). *Mycena globispora* and *M. distincta* are mainly distinguished in macromorphology from *M. griseotincta* by their white basidiomata and, in micromorphology, by the globose spores and subcylindrical spores, respectively (Aravindakshan and Manimohan 2015).

Mycena hygrophoroides **T.Bau & Q.Na, sp. nov.**
MycoBank: MB829099
Figs 2h, 5

Diagnosis. Pileus concave with slight pruinose. Lamellae distant. Stipe with dense white fibrils and swollen base. Acanthocysts forming two types. Caulocystidia long-elliptic with conical excrescences, up to 120 μm long.

Holotype. CHINA. Guangdong Province, Shaoguan City, Chebaling National Nature Reserve, 8 May 2017, Qin Na, HMJAU 43417.

Etymology. Name refers to its sparse lamellae.

Description. Pileus 1.5–2.5 mm in diam., campanulate to hemispherical, applanate or slightly concave at centre, white with greyish shade (6B1), shallowly sulcate, translucent-striate, slightly pruinose, pubescent. Context white, thin and very fragile. Lamellae distant, sparse, white, concolorous with the sides. Stipe 4.5–8.2 × 0.5–0.8 mm, cylindrical, hollow, fragile, pure white (5A1) with a greyish (5B1) base, covered with dense white fibrils, base swollen and not forming basal disc, hirsute. Odour and taste indistinctive.

Basidiospores (6.9-)7.2-8.9(-9.3) × (5.3-)6.4-6.7(-7.1) μm, Q=1.2–1.5, Qav=1.31, broadly-ellipsoid, hyaline in water and 5% KOH, amyloid, smooth. Basidia 15–21 × 7–9 μm, 4- or 2-spored, clavate, hyaline. Cheilocystidia 23–37 × 19–28 μm, subglo-

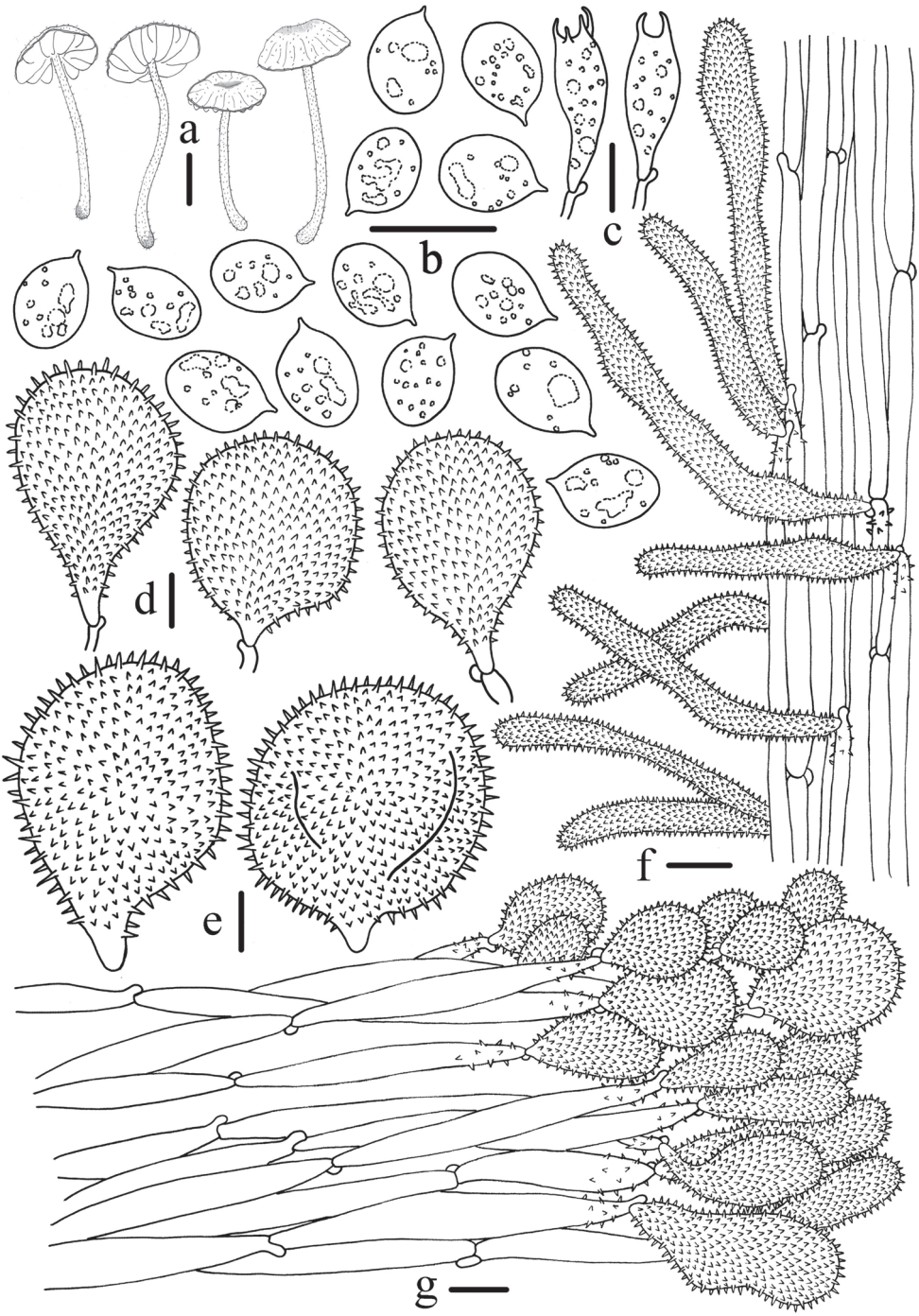

Figure 5. Microscopic features of *Mycena hygrophoroides* (HMJAU 43417, holotype) **a** Basidiomata **b** Basidia **c** Basidiospores **d** Cheilocystidia **e** Universal veil acanthocysts **f** Caulocystidia **g** Pileipellis. Scale bars: 2 mm (**a**); 10 μm (**b–g**). Drawing by Qin Na.

bose, sphaero-pedunculate to utriform with numerous sharp spines, thin-walled and hyaline, inamyloid. Pleurocystidia absent. Pileipellis hyphae 3–9 μm wide, dextrinoid; cherocytes absent; a cutis overlaid by elements of universal veil, not in chains; acanthocysts forming two types, pyriform to vesicular, 13–29 × 11–24 μm, clavate to ovoid or obovoid, 29–42 × 14–20 μm, inamyloid. Hyphae of the stipitipellis 3–7 μm wide, smooth, dextrinoid; caulocystidia abundant, clavate, long-elliptic, 32–122 × 8–11 μm, with numbers of conical spines, inamyloid. Clamps present in all tissues.

Habit and habitat. Scattered on rotten wood of coniferous trees, ex. *Cunninghamia*.

Other specimens examined. Guangdong Province, Shaoguan City, Liangjiang Town, Shangxie Village, 7 May 2017, Qin Na, HMJAU 43421.

Remarks. *Mycena hygrophoroides* could be considered to be a member of *Hemimycena* Singer owing to the tiny basidiomata and sparse lamellae, but the absence of a basal disc, amyloid spores and spinulose cheilocystidia, acanthocysts and caulocystidia are diagnostic characters for *M. hygrophoroides*, which should be placed in *Mycena* sect. *Amparoina* stirps *Alphitophora*. *Mycena acanthophila* J.C.Zamora&Català, of which the holotype was collected from Spain growing on dead branches of Leguminosae, most resembles *M. hygrophoroides*, but differs in having a yellow pileus, smaller cheilocystidia (13.5–22 × 8.5–12 μm) and diverse caulocystidia (Zamora and Català 2012). *Mycena depilata*, a species of stirps *Alphitophora*, shows some morphological similarities to *M. hygrophoroides* in possessing white and tiny basidiomata, distant lamellae (L = 7–9) and globose-pedicellate acanthocysts with hyaline contents. However, *M. depilata* differs in producing ellipsoid spores (Q = 1.64 ± 0.11), broadly clavate cheilocystidia and shorter caulocystidia (16–50 × 5–16 μm; Singer 1989). *Mycena hemitrichialis* is difficult to distinguish from *M. hygrophoroides*, but *M. hemitrichialis* has free to subfree lamellae, longer caulocystidia (100–300 × 5–15 μm) and ellipsoid spores (Singer 1989). In comparison with *M. hygrophoroides*, *M. alphitophora* and *M. distincta* have larger basidiomata and longer caulocystidia of more than 400 μm and 300 μm, respectively (Desjardin 1995; Aravindakshan and Manimohan 2015). Their noticeably pigmented pileus enables discrimination of *M. brunneospinosa*, *M. incarnativelum* and *M. roseotincta* from *M. hygrophoroides* (Desjardin 1995; Aravindakshan and Manimohan 2015). The significantly larger basidiomata and globose spores can be used to distinguish *M. corynephora*, *M. globispora* and *M. yalensis* from *M. hygrophoroides*.

***Mycena miscanthi* T.Bau & Q.Na, sp. nov.**
MycoBank: MB829100
Figs 2i, 6

Diagnosis. Growing on dead stem of *Miscanthus*. Pileus sparsely pruinose. Basidiospores cylindric. Cherocytes absent. Acanthocysts forming two types. Caulocystidia sphaero-pedunculate covered with spines. Clamps present.

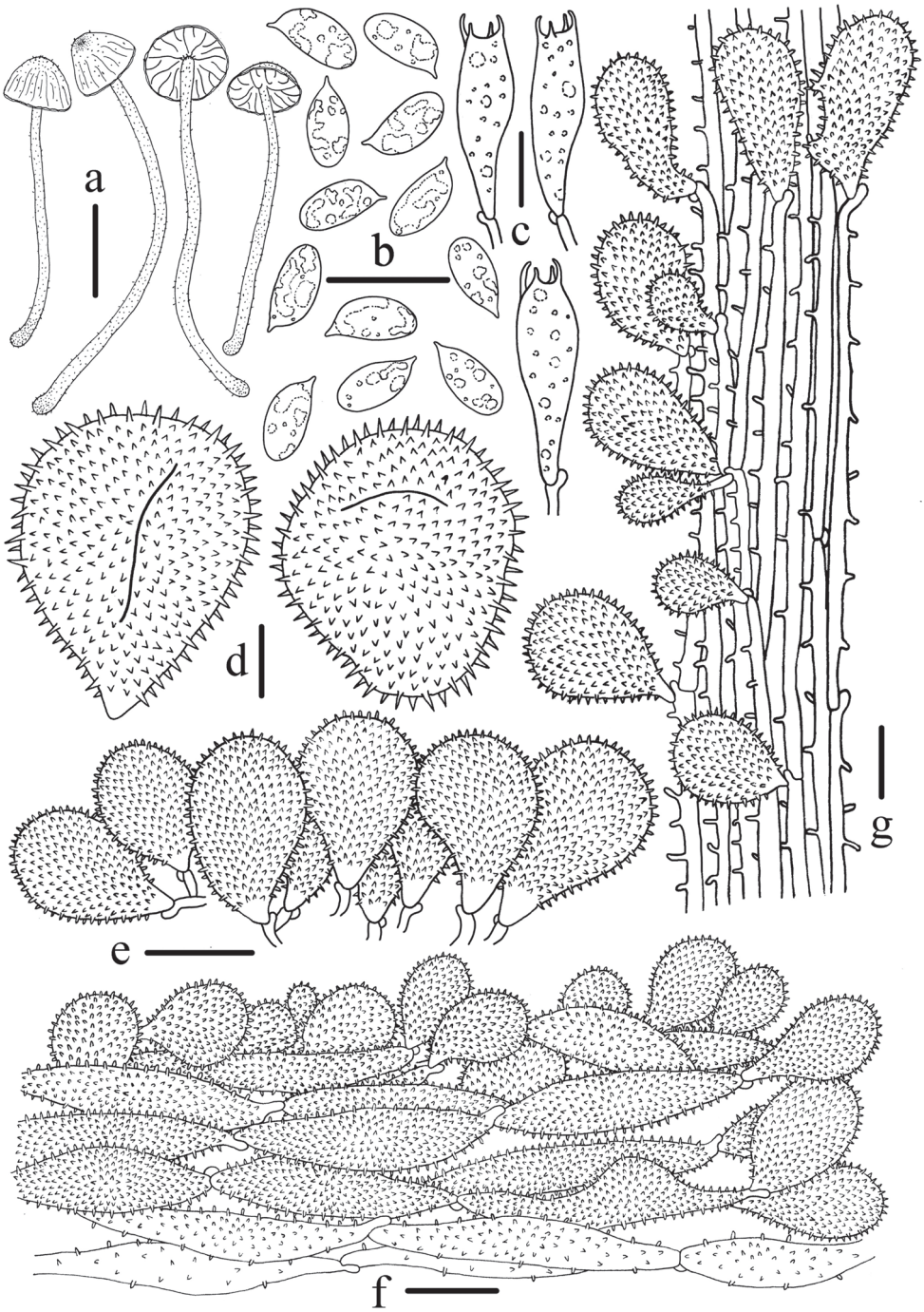

Figure 6. Microscopic features of *Mycena miscanthi* (HMJAU 43584, holotype) **a** Basidiomata **b** Basidiospores **c** Basidia **d** Universal veil acanthocysts **e** Cheilocystidia **f** Pileipellis **g** Caulocystidia. Scale bars: 10 mm (**a**); 10 μm (**b–g**). Drawing by Qin Na.

Holotype. CHINA. Henan Province: Xinyang City, Jigong Mountain, 16 Jul 2017, Qin Na and Tolgor Bau, HMJAU 43584.

Etymology. Name refers to the substratum where the new species was found.

Description. Pileus 3.5–7.8 mm in diam., hemispherical, broadly conical to convex, occasionally ± centrally depressed when young, sulcate, translucent-striate, pure white, pubescent to inconspicuously puberulous, margin nearly plane, undulate. Context white, thin, very fragile, about 1.0 mm thick at centre. Lamellae narrowly adnate or adnexed, off-white, concolorous with the sides. Stipe 26–38 × 0.5–1.0 mm, pure white, central, terete, hollow, equal, surface covered with slight white pubescent, base swollen but not discoid, pruinose. Odour and taste not distinctive.

Basidiospores (6.2-)6.7–8.6(-9.1) × (3.1)3.3–4.2(4.5) μm, Q=1.8–2.3, Qav=2.07, cylindric to narrow-ellipsoid, hyaline, guttulate, thin walled, amyloid. Basidia 18–24 × 6–9 μm, clavate, hyaline, 4-spored. Cheilocystidia 13–26 × 9–14 μm, abundant, lageniform, utriform or sphaero-pedunculate, with short and conical spines. Pleurocystidia absent. Pileipellis hyphae 3–8 μm wide, strongly dextrinoid; cherocytes absent; universal veil composed of acanthocysts, forming two types, pyriform, vesicular or clavate, 12–32 × 10–17 μm, inamyloid. Hyphae of the stipitipellis 2–8 μm wide, with coarse excrescences, 0.9–2.8 × 0.5–0.9 μm, strongly dextrinoid; caulocystidia abundant, elliptic, utriform, sphaero-pedunculate, 15–37 × 7–15 μm, with conical or cylindrical spines inamyloid. Clamps present in all tissues.

Habit and habitat. Solitary to scattered on dead stem of *Miscanthus*.

Other specimens examined. Henan Province, Xinyang City, Jinniu Mountain, 14 Jul 2017, HMJAU 43573; Xinyang City, Bolden National Forest Park, 17 July 2017, Qin Na and Tolgor Bau, HMJAU 43582.

Remarks. The distinctive features of *Mycena miscanthi* include a white, granulose pileus, a pubescent stipe without forming a basal disc, narrow-ellipsoid spores, two types of acanthocysts and growth on dead stems of *Miscanthus* species. In combination, these features support the placement of *M. miscanthi* in sect. *Amparoina* stirps *Alphitophora*. Similar to *M. miscanthi*, *M. alphitophora* and *M. depilata* produce pure white basidiomata, cylindric spores and sphaero-pedunculate and spinulose cheilocystidia (Desjardin 1995; Aravindakshan and Manimohan 2015). However, the two types of acanthocysts and longer caulocystidia can be used to distinguish *M. alphitophora* and *M. depilata* from *M. miscanthi* (Desjardin 1995). *Mycena hemitrichialis* is closely allied to *M. miscanthi*, but differs in producing caulocystidia up to 400 μm in length that lack spinulae or with a few spinulae in the upper half (Singer 1989). *Mycena distincta*, which was originally described as *M. alphitophora* var. *distincta*, was elevated to species level by Manimohan and Leelavathy (1989). It differs from *M. miscanthi* in producing broadly ellipsoid spores and caulocystidia up to 300 μm in length (Aravindakshan and Manimohan 2015). The pigmented pileus present in *M. brunneospinosa*, *M. incarnativelum* and *M. roseotincta* readily distinguishes these species from *M. miscanthi* (Desjardin 1995; Aravindakshan and Manimohan 2015). *Mycena corynephora*, *M. globispora* and *M. yalensis* of stirps *Alphitophora* are characterised by globose to subglobose spores (Maas Geesteranus 1980; Robich 2003; Aravindakshan and Manimohan 2015; Aronsen and Læssøe 2016).

Discussion

The present phylogenetic analysis showed that sect. *Amparoina* formed a distinct clade independent from sect. *Sacchariferae* with high BPP and BS support. This finding suggests that the presence of caulocystidia with dense spines is the most important character to separate sect. *Amparoina* from sect. *Sacchariferae*. However, in the presence of a basal disc, the species of sect. *Sacchariferae* are similar to stirps *Amparoina* and, in the acanthocysts on the pileus sect. *Amparoina* stirps, *Amparoina* resembles sect. *Sacchariferae*. It can be concluded that the difference in caulocystidia can be used to distinguish sect. *Amparoina* and sect. *Sacchariferae* and the basal disc and cherocytes are the basis of an infrasectional classification of sect. *Amparoina*. Thus, the circumscription of sect. *Sacchariferae* should be revised, for which the diagnostic characters are a well-developed basal disc, cherocytes absent, pileipellis a cutis not overlaid by elements of a universal veil composed of acanthocysts and caulocystidia smooth overall.

In morphology, sect. *Amparoina* and sect. *Sacchariferae* are closely allied with sect. *Polyadelphiae* Singer ex Maas Geest. and sect. *Basipedes* (Fr.) Quél (Desjardin et al. 2003). Species of sect. *Polyadelphiae* lack both ornamented pileipellis elements and a stipe with a basal disc and thus differ from species classified in sect. *Amparoina* and sect. *Sacchariferae*. Section *Basipedes* shares the same habitat and a stipe forming a developed basal disc, but the cheilocystidia are covered with rounded and few excrescences. Morphological characters distinguish sect. *Polyadelphiae* and sect. *Basipedes* from sect. *Amparoina* and sect. *Sacchariferae* and only one ITS sequence for *M. stylobates* (Pers.) P. Kumm. (JF908439) is currently deposited in GenBank.

Morphological characters and molecular evidence support the classification of the four new *Mycena* species as members of sect. *Amparoina* stirps *Alphitophora*. The four species share the same furfuraceous or farinose pileus, swollen stipe base without a basal disc, universal veil composed of acanthocysts and absence of both cherocytes and spinose caulocystidia. *Mycena bicystidiatum* is distinguished from *M. griseotincta*, *M. hygrophoroides* and *M. miscanthi* by producing two types of caulocystidia covered with conic spines. *Mycena griseotincta* is readily discriminated from *M. bicystidiatum*, *M. hygrophoroides* and *M. miscanthi* based on the greyish basidiomata and acanthocysts forming a universal veil with long, cylindrical excrescences. Compared with *M. bicystidiatum*, *M. griseotincta*, and *M. miscanthi*, *M. hygrophoroides* is distinct on account of the sparse lamellae and broadly ellipsoid basidiospores. *Mycena miscanthi* differ from *M. bicystidiatum*, *M. griseotincta* and *M. hygrophoroides* in growing on stems of *Miscanthus* and, in addition, the basidiospores are narrow ellipsoid.

It is worth mentioning that the placement of *M. echinocephala* (G.F. Atk.) Desjardin and *M. cylindrospora* A.H. Sm. remains unclear. The species are tentatively placed in stirps *Alphitophora* because of the lack of a basal disc on the stipe, but their caulocystidia are extraordinary in being smooth, terminated by a spinulose apex or smooth with an amorphous apex (Atkinson 1902; Smith 1947; Desjardin 1993). Both species show obvious differences to the four newly described taxa. Furthermore, *M. cryptomeriicola* Imazeki & Toki is distinctive in producing inamyloid spores and a basal disc, which is unusual for specimens of sect. *Sacchariferae* from Japan (Imazeki and Toki

1995). An additional unusual species, *M. minya* Grgur., which lacks caulocystidia, was reported from Australia (Grgurinovic 2003). No species similar in morphology to *M. cryptomeriicola* and *M. minya* are classified in sect. *Sacchariferae*, so the two species are tentatively accepted in sect. *Sacchariferae*.

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References

Aravindakshan DM, Manimohan P (2015) Mycenas of Kerala. SporePrint Books, Calicut, India. https://doi.org/10.13140/RG.2.1.2116.4003

Aronsen A, Læssøe T (2016) The Genus *Mycena* s.l. Fungi of Northern Europe Vol. 5. Narayana Press, Gylling, Denmark.

Atkinson GF (1902) Three new genera of higher fungi. Botanical Gazette 34: 36–43. https://doi.org/10.1086/328258

Cortéspérez A, Ramírezguillén F, Guzmán G (2015) Nuevos registros de *Mycena* sección *Sacchariferae* (Basidiomycota) para México. Revista Mexicana de Micologia 41: 79–87

Desjardin DE (1993) Notes on *Mycena cylindrospora* and *Eomycenella echinocephala*. Mycologia, 85(3): 509–513. https://doi.org/10.2307/3760711

Desjardin DE (1995) A preliminary accounting of the worldwide members of *Mycena* sect. *Sacchariferae*. Bibliotheca Mycologica 159: 1–89.

Desjardin DE, Boonpratuang T, Hywel-Jones N (2003) New spinose species of *Mycena* in sections *Basipedes* and *Polyadelphia* from Thailand. Fungal Diversity 12: 7–17.

Grgurinovic CA (2003) The genus *Mycena* in south-eastern Australia. Fungal Diversity Press, Canberra, Australia.

Guo SX, Fan L, Cao WQ, Xu JT, Xiao PG (1997) *Mycena anoectochila* sp. nov. isolated from mycorrhizal roots of *Anoectochilus roxburghii* from Xishuangbanna, China. Mycologia 89: 952–954. https://doi.org/10.2307/3761116

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hopple JS, Vilgalys R (1999) Phylogenetic relationships in the mushroom genus *Coprinus* and dark-spored allies based on sequence data from the nuclear gene coding for the large ribosomal subunit RNA: divergent domains, outgroups, and monophyly. Molecular Phylogenetics & Evolution 13(1): 1–19. https://doi.org/10.1006/mpev.1999.0634

Horak E (2005) Röhrlinge und Blätterpilze in Europa: Bestimmungsschlüssel für Polyporales (pp), Boletales, Agaricales, Russulales. Elsevier, Spektrum Akad Verlag.

Imazeki R, Toki S (1955) Contributions to the knowledge of Japanese Agaricales. Bulletin of the Government Forest Experimental Station Meguro, 79: 1–14.

Kirk PM, Cannon PE, Minter DW, Stalpers JA (2008) Dictionary of the Fungi (10 edition). Wallingford: CABI International.

Kornerup A, Wanscher JHK (1978) The Methuen Handbook of Colour. Eyre Methuen, London.

Kühner R (1938) Le genre *Mycena* (Fries). Encyclopédie Mycologique X. P. Lechevalier 10: 1–710.

Li Y, Li TH, Yang ZL, Bau T, Dai YC (2015) Atlas of Chinese Macrofungal Resources. Central Chinese Farmer Press, Zhengzhou, China.

Lodge DJ (1988) Three new *Mycena* species (Basidiomycota: Tricholomataceae) from Puerto Rico. Transactions of the British Mycological Society 91(1): 109–116. https://doi.org/10.1016/s0007-1536(88)80011-1

Maas Geesteranus RA (1980) Studies in Mycenas-15. Persoonia 11: 93–120.

Maas Geesteranus RA (1983) Conspectus of the Mycenas of the Northern Hemisphere-1, Sections *Sacchariferae*, *Basipedes*, *Bulbosae*, *Clavulares*, *Exiguae*, and *Longisetae*. Proceedings van de Koninklijke Nederlandse Akademie van Wetenschappen (Ser C), Amsterdam, North-Holland 86: 401–421.

Maas Geesteranus RA (1992a) Mycenas of the Northern Hemisphere I. Studies in Mycenas and other papers. Proceedings van de Koninklijke Nederlandse Akademie van Wetenschappen, Amsterdam, North-Holland.

Maas Geesteranus RA (1992b) Mycenas of the Northern Hemisphere II. Studies in Mycenas and other papers. Proceedings van de Koninklijke Nederlandse Akademie van Wetenschappen, Amsterdam, North-Holland.

Maas Geesteranus RA, de Meijer AAR (1997) Mycenae Paranaenses. Proc K Ned Akad Wet, Amsterdam, North-Holland.

Maas Geesteranus RA, de Meijer AAR (1998) Further Mycenas from the state of Paraná, Brazil. Persoonia 17(1): 29–46.

Na Q, Bau T (2018) New species of *Mycena* (Mycenaceae, Agaricales) with colored lamellae and three new species records from China. Phytotaxa 361(3): 266–278. https://doi.org/10.11646/phytotaxa.361.3.2

Na Q, Bau T (2019) *Mycena* section *Sacchariferae*: three new species with basal discs from China. Mycological Progress 18: 483–493. https://doi.org/10.1007/s11557-018-1456-8

Nealel B (2009) Two intimately co-occurring species of *Mycena* section *Sacchariferae* in southwest Australia. Mycotaxon 108(4): 159–174. https://doi.org/10.5248/108.159

Nylander J (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala.

Perry BA (2002) A taxonomic investigation of *Mycena* in California. Doctoral dissertation, San Francisco State University, California, USA.

Persoon CH (1797) Tentamen dispositionis methodicae fungorum in classes ordines, genera et familias. Lipsiae. https://doi.org/10.5962/bhl.title.42674

Robich G (2003) *Mycena* d'Europa. Associazione Micologica Bresadola, Trento, Italy.

Robich G, Hausknecht A (2009) *Mycena bhuglooi*, a new species of section *Sacchariferae* (Agaricales, Tricholomataceae) from Mauritius (Africa). Österr Z Pilzk 18: 7–14.

Robich G (2016) *Mycena* d'Europa Volume 2. Associazione Micologica Bresadola, Trento, Italy.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https://doi.org/10.1093/bioinformatics/btg180

Singer R (1958) New genera of fungi VIII. Notes concerning the sections of the genus *Marasmius* Fr. Mycologia 50: 103–110. https://doi.org/10.1080/00275514.1958.12024714

Singer R (1973) Diagnose fungorum novorum Agaricalium III. Sydowia 15: 45–83.

Singer R (1976) Amparoinaceae and Montagneaceae. Revue de Mycologie 40: 57–64.

Singer R (1989) New taxa and new combinations of Agaricales (Diagnose fungorum novorum Agaricalium IV). Fieldiana 21: 1–133. https://doi.org/10.5962/bhl.title.2537

Smith AH (1947) North American species of *Mycena*. University Michigan Press, Ann Arbor, Michigan.

Stamatakis A, Ludwig T, Meier H (2004) RAxML-III: a fast program for maximum likelyhood-based inference of large phylogenetic trees. Bioinformatics 21(4): 456–463. https://doi.org/10.1093/bioinformatics/bti191

Takahashi H (1999) *Mycena auricoma*, a new species of *Mycena*, section *Radiatae*, from Japan, and *Mycena spinosissima*, a new record in Japan. Mycoscience 40(1): 73–80. https://doi.org/10.1007/bf02465677

Tanaka I, Hongo T (2003) Two new records of *Mycena* sect. *Sacchariferae* from Japan and type study of *Mycena cryptomeriicola* (sect. *Sacchariferae*). Mycoscience 44(6): 421–424. https://doi.org/10.1007/s10267-003-0134-z

Thompson JD, Gibson TJ, Plewniak F (1997) The Clustal-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 63: 215–228. https://doi.org/10.1093/nar/25.24.4876

Ward E, Gray RM (2010) Generation of a ribosomal DNA probe by PCR and its use in identification of fungi within the Gaeumannomyces-*Phialophora* complex. Plant Pathology 41(6): 730–736. https://doi.org/10.1111/j.1365-3059.1992.tb02556.x

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR protocols: a guide to methods and applications. Academic, San Diego, 315–322. https://doi.org/10.1016/b978-0-12-372180-8.50042-1

Zamora JC, Català S (2013) A new species of *Mycena* sect. *Sacchariferae* from the Iberian cushion-shaped Genisteae. Mycotaxon 122(4): 361–368. https://doi.org/10.5248/122.361