

Hygrophorus russula complex (Hygrophoraceae, Agaricales) in China

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Abstract

The Chinese species complex of *Hygrophorus russula* in subsection *Clitocyboides* has been studied. Three new species, *H. orientalis*, *H. qinggangjun* and *H. yunnanensis* were proposed, based on morphology, molecular systematics and chemical reactions. *Hygrophorus qinggangjun* and *H. yunnanensis* occur at high elevations of Yunnan China; *H. orientalis* is widely distributed in East Asia and occurs at low elevations. At present, seven taxa have been revealed in the *H. russula* species complex; a key to these species is provided.

Introduction

The genus *Hygrophorus* Fr. (Hygrophoraceae, Agaricales) contains around 270 species, based on the Index Fungorum (<http://www.indexfungorum.org>). Most species are widespread in the temperate regions of the Northern Hemisphere (Tedersoo et al. 2010) and are characterised by sub-viscid to glutinous pileus, adnate to decurrent lamellae and divergent hymenophoral trama (Hesler and Smith 1963; Bas et al. 1990; Candusso 1997; Lodge et al. 2014). Species of *Hygrophorus* are ectomycorrhizal, excluding a couple of parasitic species, such as *H. olivaceoalbus* (Fr.) Fr. and *H. penarius* Fr. (Marino 2008; Agerer 2012), but possibly behave as facultative ectomycorrhizal species. Most form associations with both broad-leaved and coniferous trees. Many appear to show host preference: for example, *H. alboflavescens* A. Naseer & A.N. Khalid forms ectomycorrhizal associations with *Quercus incana* (Naseer et al. 2019); *H. betulae* K. Bendiksen & E. Larss. is associated with *Betula pubescens* (Larsson and Bendiksen 2020); *H. boyeri* Lebeuf, Bellanger & H. Lambert and *H. meridionalis* Loizides, P.-A. Moreau, Athanassiou & Athanasiades are found in pine forests (Moreau et al. 2018); *H. russuliformis* Murrill occurs with oak in Florida (Hesler and Smith 1963); *H. yadigarii* E. Sesli, Antonín & Contu occurs in hornbeam-spruce-dominated forests (Sesli et al. 2018) and *H. yukishiro* N. Endo, Tokoo & A. Yamada grows on the ground of oak forests (*Quercus acutissima* and *Quercus serrata*) (Endo et al. 2018).

Recently, Lodge et al. (2014) proposed the infrageneric updates of *Hygrophorus*, based on molecular phylogeny and revealed, in many cases, that the same name has been widely applied while presenting several different phylogenetic species. Other studies uncovered hidden diversity in North America and Europe: for example, *H. hypothejus* (Fr.) Fr. complex in section *Aurei* (Bataille) E. Larss. (Moreau et al. 2018) and *H. agathosmus* (Fr.) Fr. group in subsection *Tephroleuci* (Bataille) Singer (Larsson et al. 2018).

Hygrophorus russula (Schaeff. ex Fr.) Kauffman, originally from Europe, (Schaeffer 1774) belongs to subsection *Clitocyboides* (Hesler & A.H. Sm.) E. Larss and is characterised by a pinkish-red to vinaceous-purple pileus. This name has been widely used for some similar species found in Africa (GenBank sequence KU973852), Asia (Hongo 1982; Chen and Li 2013), North America (Hesler and Smith 1963; Siegel and Schwarz 2016) and South America (GenBank sequence KF381523). Recent studies indicate that this name harbours at least a couple of new taxa in Asia, viz. *H. deliciosus* C.Q. Wang & T.H. Li and *H. parvirussula* H.Y. Huang & L.P. Tang, both from south-western China (Huang et al. 2018; Wang and Li 2020). These results indicated that the species diversity within *Hygrophorus* is higher than previous estimates.

In order to explore the genetic diversity, host preference and geographic distribution of the *H. russula* complex, we studied more than 80 well-documented specimens from China. Based on morphological characters, chemical reactions and phylogenetic results, along with sequences of this species complex from GenBank, three species are proposed as new: *H. qinggangjun* and *H. yunnanensis* from south-western China and *H. orientalis* widely distributed in East Asia.

Materials And Methods

Sampling and morphological studies

Most specimens were collected in central (Hubei), northern and south-western China during rainy seasons (July–October). Basidiomes were found in broad-leaved forests, mainly Ericaceae and Fagaceae. Herbarium materials, identified as *H. russula*, were loaned from the University of Alcalá (AH). Other specimens were deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS), the Mycological Herbarium, Institute of Mycology, Chinese Academy of Sciences (HMAS), the Mycological Herbarium of Kunming Medical University (MHKMU) and the Herbarium of the Royal Botanic Garden, Edinburgh (RBGE).

Macro-morphological descriptions were taken from field notes and images of basidiomes, with colour codes following Kornerup and Wanscher (1981). Micro-morphological characters were observed from dried materials after being sectioned and mounted in 5% potassium hydroxide (KOH) and 1% Congo Red solution (w/v) under a Leica DM2500 microscope. Melzer's Reagent was used to test

the amyloidy of basidiospores. Basidiospores and hyphae of the basal mycelium were examined with a ZEISS Sigma 300 scanning electron microscope (SEM) at 7.00 kV. The procedures followed those in Huang et al. (2018, 2020) and are referenced therein. The following abbreviations [n/m/p] indicate n basidiospores measured from m basidiomes of p collections. Dimensions for basidiospores are given using (a) b–c (d), the range of b–c contains a minimum of 90% of the measured values and 'a' and 'd' were the extreme values. Q means the quotient of length and width in the side view of basidiospores and Q_m indicates average Q of all basidiospores \pm sample standard deviation.

Chemical reactions

Seven chemical reagents were applied in this study: C_6H_5OH = 10% (w/v) phenol, $EtOH$ = 95% (w/v) ethanol, $FeCl_3$ = 10% (w/v) ferric chloride, $FeSO_4$ = 10% (w/v) ferrous sulphate, KOH = 5% (w/v) potassium hydroxide, Melzer's = Melzer's Reagent, NH_4OH = 10% (w/v) ammonium hydroxide. A piece of tissue was taken separately from the pilei, context, lamellae and stipes of dried basidiocarps. Colour changes were recorded following the application of reagents.

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from about 10–20 mg of dried basidiome tissue using a modified CTAB method (Doyle 1987). ITS regions and large subunit nuclear ribosomal RNA (nrLSU) region were amplified by a polymerase chain reaction (PCR) using primer pairs ITS5/ITS4 and LROR/LR5 (Vilgalys and Hester 1990; White et al. 1990). PCR reactions were performed in 25 μ l reaction mixtures containing 2.5 μ l of 10 \times amplification buffer (with $MgCl_2$), 0.5 μ l dNTP (200 μ M), 0.2 μ l Taq DNA polymerase (5 U/ μ l), 1 μ l of each primer (10 μ M), 1 μ l DNA template and 18.8 μ l sterile water. PCR conditions followed the programme of Yang et al. (2018). PCR products were checked on 1% agarose gels. Amplified PCR products were sequenced using an ABI 3730 DNA Analyzer (Sangon, Shanghai, China) with the same primers.

Sequence alignment and phylogenetic analyses

Raw sequences were assembled and edited using SeqMan (DNASTAR Lasergene 9) and deposited in GenBank (<http://www.ncbi.nlm.nih.gov>) (see Table 1). DNA sequences of ITS and nrLSU were independently aligned using MUSCLE 3.6 (Edgar 2004) and manually adjusted where necessary in BioEdit 7.0.9 (Hall 1999); the concatenated datasets were manually constructed. The dataset was analysed with Maximum Likelihood (ML) and Bayesian Inference (BI). ML analyses were performed using RAXML 7.0.3 (Stamatakis et al. 2008); GTRGAMMA was set by default as the selected model; statistical support of clades was obtained with 1000 rapid bootstrap replicates. For BI, concatenated sequences were partitioned into ITS1 (1–140), 5.8S (141–309), ITS2 (310–588) and LSU (589–1471). The best-fit model of nucleotide substitution was obtained in PartitionFinder 2 (Lanfear et al. 2016), based on the Akaike Information Criteria (AIC). The selected models were GTR + G for ITS1 and ITS2, K80 for 5.8S and GTR + I + G for nrLSU. Partitioned Bayesian analysis was performed with MrBayes 3.2 (Ronquist et al. 2012) on the CIPRES portal. Four simultaneous Markov chains were run for 6,000,000 generations for ITS and nrLSU, 8,000,000 generations for concatenated sequences and sampled every 1000 generations. At the end of the run, the average deviation of split frequencies was below 0.005. Burn-in values were determined in Tracer 1.7 (Rambaut et al. 2018). The first 25% of generations were discarded as burn-in, when the plot generated by the sump command levelled off and effective sample sizes were well over 200 for all sampled parameters for each run.

Table 1
Sequences used/produced in the present study

Taxon	Voucher	Locality	GenBank Accession No. (ITS)	GenBank Accession No. (LSU)	References
<i>Hygrophorus deliciosus</i>	ZJ0002LS04	China, Sichuan	KU836534	—	Liu et al. unpublished
<i>H. deliciosus</i>	GDGM 79208	China, Yunnan	MT363808	—	Wang and Li 2020
<i>H. deliciosus</i>	HKAS 54703	China, Yunnan	MW290158*	MW290225*	This study
<i>H. deliciosus</i>	HKAS 51000	China, Sichuan	MW290159*	MW290226*	This study
<i>H. deliciosus</i>	HKAS 76246	China, Sichuan	MW290160*	MW290227*	This study
<i>H. deliciosus</i>	HKAS 69670	China, Yunnan	MW290161*	MW290228*	This study
<i>H. deliciosus</i>	HKAS 54510	China, Yunnan	MW290162*	MW290229*	This study
<i>H. deliciosus</i>	MHKMU S.D. Yang 43	China, Yunnan	MW290163*	MW290230*	This study
<i>H. deliciosus</i>	HKAS 55053	China, Yunnan	MW290164*	—	This study
<i>H. deliciosus</i>	HKAS 61276	China, Yunnan	MW290165*	MW290231*	This study
<i>H. deliciosus</i>	HKAS 61315	China, Yunnan	MW290166*	MW290232*	This study
<i>H. deliciosus</i>	HKAS 71624	China, Yunnan	MW290167*	—	This study
<i>H. deliciosus</i>	HMAS 253198	China, Tibet	MW290168*	—	This study
<i>H. deliciosus</i>	HMAS 253233	China, Tibet	MW290169*	MW290233*	This study
<i>H. deliciosus</i>	HMAS 253249	China, Tibet	MW290170*	MW290234*	This study
<i>H. deliciosus</i>	MHKMU H.Y. Huang 725	China, Yunnan	MW290171*	—	This study
<i>H. deliciosus</i>	MHKMU H.Y. Huang 806	China, Yunnan	MW290172*	MW290235*	This study
<i>H. deliciosus</i>	MHKMU H.Y. Huang 807	China, Yunnan	MW290173*	MW290236*	This study
<i>H. deliciosus</i>	MHKMU H.Y. Huang 1006	China, Yunnan	MW290174*	MW290237*	This study
<i>H. deliciosus</i>	MHKMU H.Y. Huang 1008	China, Yunnan	MW290175*	MW290238*	This study
<i>H. orientalis</i> (Holotype)	HKAS 75586	China, Hubei	MW290176*	MW290239*	This study
<i>H. orientalis</i>	HKAS 63417	China, Jilin	MW290177*	MW290240*	This study
<i>H. orientalis</i>	HKAS 71845	China, Jilin	MW290178*	MW290241*	This study
<i>H. orientalis</i>	HKAS 71851	China, Shaanxi	MW290179*	MW290242*	This study
<i>H. orientalis</i>	HKAS 58741	China, Yunnan	MW290180*	MW290243*	This study
<i>H. orientalis</i>	MHKMU H.Y. Huang 472	China, Jilin	MW290181*	—	This study
<i>H. orientalis</i>	CFSZ 20884	China, Inner Mongolia	MW290182*	—	This study
<i>H. parvirussula</i>	HKAS 53605	China, Sichuan	MH160768	MH160772	Huang et al. 2018

* indicates sequences generated in this study

Taxon	Voucher	Locality	GenBank Accession No. (ITS)	GenBank Accession No. (LSU)	References
<i>H. parvirussula</i>	MHKMU L.P. Tang 1691	China, Yunnan	MH160769	MH160773	Huang et al. 2018
<i>H. parvirussula</i>	MHKMU N.K. Zeng 2878	China, Yunnan	MH160770	MH160774	Huang et al. 2018
<i>H. parvirussula</i>	MHKMU S.D. Yang 434	China, Yunnan	MH160771	MH160775	Huang et al. 2018
<i>H. parvirussula</i>	HKAS 56191	China, Yunnan	MW290183*	—	This study
<i>H. parvirussula</i>	HKAS 57447	China, Yunnan	MW290184*	—	This study
<i>H. parvirussula</i>	HKAS 58821	China, Yunnan	MW290185*	—	This study
<i>H. parvirussula</i>	MHKMU S.D. Yang 12	China, Yunnan	MW290186*	MW290244*	This study
<i>H. parvirussula</i>	MHKMU W.H. Zhang 177	China, Yunnan	MW290187*	MW290245*	This study
<i>H. parvirussula</i>	MHKMU W.H. Zhang 178	China, Yunnan	MW290188*	MW290246*	This study
<i>H. parvirussula</i>	MHKMU W.H. Zhang 179	China, Yunnan	MW290189*	MW290247*	This study
<i>H. parvirussula</i>	MHKMU T. Huang 220	China, Yunnan	MW290190*	MW290248*	This study
<i>H. parvirussula</i>	MHKMU H.Y. Huang 501	China, Yunnan	MW290191*	MW290249*	This study
<i>H. parvirussula</i>	MHKMU W.H. Zhang 183	China, Yunnan	MW290192*	MW290250*	This study
<i>H. parvirussula</i>	MHKMU W.H. Zhang 195	China, Yunnan	MW290193*	MW290251*	This study
<i>H. parvirussula</i>	MHKMU H.Y. Huang 534	China, Yunnan	—	MW290252*	This study
<i>H. parvirussula</i>	MHKMU Y.J. Pu 171	China, Yunnan	MW290194*	MW290253*	This study
<i>H. parvirussula</i>	MHKMU H.Y. Huang 911	China, Yunnan	MW290195*	MW290254*	This study
<i>H. parvirussula</i>	MHKMU L.P. Tang 3420	China, Yunnan	MW290196*	MW290255*	This study
<i>H. parvirussula</i>	MHKMU T. Huang 455	China, Yunnan	MW290197*	—	This study
<i>H. parvirussula</i>	MHKMU T. Huang 476	China, Yunnan	—	—	This study
<i>H. parvirussula</i>	MHKMU T. Huang 491	China, Yunnan	MW290198*	—	This study
<i>H. parvirussula</i>	MHKMU M. Mu 770	China, Yunnan	MW290199*	—	This study
<i>H. parvirussula</i>	MHKMU Y.J. Pu 376	China, Yunnan	MW290200*	MW290256*	This study
<i>H. parvirussula</i>	MHKMU Y.J. Pu 399	China, Yunnan	MW290201*	MW290257*	This study

* indicates sequences generated in this study

Taxon	Voucher	Locality	GenBank Accession No. (ITS)	GenBank Accession No. (LSU)	References
<i>H. parvirussula</i>	MHKMU W.H. Zhang 535	China, Yunnan	MW290202*	MW290258*	This study
<i>H. parvirussula</i>	MHKMU T. Huang 516	China, Yunnan	MW290203*	—	This study
<i>H. parvirussula</i>	MHKMU H.Y. Huang 1007	China, Yunnan	MW290204*	MW290259*	This study
<i>H. penarioides</i>	HGT 000921	Sweden, Gotlands	EF395371	—	Jacobsson and Larsson 2007
<i>H. qinggangjun</i>	Zhao 447	China, Yunnan	MW290147*	MW290218*	This study
<i>H. qinggangjun</i>	HKAS 68397	China, Yunnan	MW290148*	MW290219*	This study
<i>H. qinggangjun</i>	MHKMU S.D. Yang 20	China, Yunnan	MW290149*	MW290220*	This study
<i>H. qinggangjun</i> (Holotype)	MHKMU L.P. Tang 1683	China, Yunnan	MW290150*	MW290221*	This study
<i>H. qinggangjun</i>	HKAS 55496	China, Yunnan	MW290151*	—	This study
<i>H. qinggangjun</i>	HKAS 60481	China, Yunnan	MW290152*	—	This study
<i>H. qinggangjun</i>	HKAS 60530	China, Yunnan	MW290153*	—	This study
<i>H. qinggangjun</i>	HKAS 72665	China, Yunnan	MW290154*	—	This study
<i>H. qinggangjun</i>	MHKMU M. Mu 464	China, Yunnan	MW290155*	MW290222*	This study
<i>H. qinggangjun</i>	MHKMU M. Mu 436	China, Yunnan	MW290156*	MW290223*	This study
<i>H. qinggangjun</i>	MHKMU H.Y. Huang 724	China, Yunnan	MW290157*	MW290224*	This study
<i>H. russula</i>	AH 19677	Spain, Torrelodones	MW290205*	MW290260*	This study
<i>H. russula</i>	AH 37145	Spain, Javierregay	MW290206*	MW290261*	This study
<i>H. russula</i>	LAS 85196	Sweden, —	EF395376	—	Jacobsson and Larsson 2007
<i>H. russula</i>	VAC 8b-09	France, —	JF506764	—	Shahin unpublished
<i>H. russula</i>	11936	Italy, —	JF908065	—	Osmundson et al. 2013
<i>H. russula</i>	992	Italy, —	JF908077	—	Osmundson et al. 2013
<i>H. russula</i>	HE 2787	—	KC505575	—	Sun et al. unpublished
<i>H. russula</i>	CFMR JP-3	Japan, Shiga	KF291216	—	Lodge et al. 2014
<i>H. russula</i>	CLO-4280	Belize, Mountain Pine Ridge	KF381523	—	Lodge et al. 2014
<i>H. russula</i>	GO-2009-116	Mexico, Temascaltepec	KT875017	—	Garibay et al. unpublished
<i>H. russula</i>	4433	Canada, Quebec	KM248883	—	Berube et al. unpublished

* indicates sequences generated in this study

Taxon	Voucher	Locality	GenBank Accession No. (ITS)	GenBank Accession No. (LSU)	References
<i>H. russula</i>	NIFoS 1987	South Korea, —	KX814449	—	Wang et al. 2016
<i>H. russula</i>	NIFoS 2003	South Korea, —	KX814450	—	Wang et al. 2016
<i>H. russula</i>	MB344	USA, Arkansas	KX358034	—	Stephenson et al. 2017
<i>H. russula</i>	EMB 141/86	Italy, —	MF399419	—	Peintner et al. unpublished
<i>H. russula</i>	EMB 710/90	Italy, —	MF399423	—	Peintner et al. unpublished
<i>H. russula</i>	IB19630793	Switzerland, Lucerne	MF399427	—	Peintner et al. unpublished
<i>H. russula</i>	IB19710232	Switzerland, Bern	MF399432	—	Peintner et al. unpublished
<i>H. russula</i>	IB19730073	Switzerland, Bern	MF399433	—	Peintner et al. unpublished
<i>H. russula</i>	IB19740604	France, Provence	MF399434	—	Peintner et al. unpublished
<i>H. russula</i>	iNaturalist 31828832	USA, Arizona	MN498103	—	Clements and Tighe unpublished
<i>H. cf. russula</i>	JLF 7925	USA, Arizona	MT101865	—	Frank unpublished
<i>H. russula</i>	IB 19740604	France, —	MT158400	—	Papetti et al. unpublished
<i>H. russula</i>	EMB 141 86	Italy, —	MT158401	—	Papetti et al. unpublished
<i>H. russula</i>	EMB 710 90	Italy, —	MT158402	—	Papetti et al. unpublished
<i>H. russula</i>	IB19630793	Switzerland, —	MT158403	—	Papetti et al. unpublished
<i>H. russuliformis</i>	MICH 10939	USA, Florida	MF399455	—	Peintner et al. unpublished
<i>H. sordidus</i>	AHSmith 91580	USA, Wisconsin	EF395373	—	Jacobsson and Larsson 2007
<i>H. yunnanensis</i>	MHKMU S.D. Yang 12 – 1	China, Yunnan	—	MW290262*	This study
<i>H. yunnanensis</i>	MHKMU S.D. Yang 12 – 2	China, Yunnan	—	MW290263*	This study
<i>H. yunnanensis</i>	MHKMU J. Zhao 52	China, Yunnan	MW290207*	MW290264*	This study
<i>H. yunnanensis</i>	HKAS 56982	China, Yunnan	MW290208*	MW290265*	This study
<i>H. yunnanensis</i>	HKAS 50442	China, Yunnan	MW290209*	—	This study
<i>H. yunnanensis</i>	HKAS 72912	China, Yunnan	MW290210*	MW290266*	This study
<i>H. yunnanensis</i>	MHKMU L.P. Tang 2751	China, Yunnan	—	MW290267*	This study
<i>H. yunnanensis</i>	MHKMU L.P. Tang 2772	China, Yunnan	MW290211*	MW290268*	This study

* indicates sequences generated in this study

Taxon	Voucher	Locality	GenBank Accession No. (ITS)	GenBank Accession No. (LSU)	References
<i>H. yunnanensis</i>	MHKMU L.P. Tang 2773	China, Yunnan	MW290212*	MW290269*	This study
<i>H. yunnanensis</i>	MHKMU H.Y. Huang 313	China, Yunnan	—	MW290270*	This study
<i>H. yunnanensis</i>	MHKMU H.Y. Huang 316	China, Yunnan	—	MW290271*	This study
<i>H. yunnanensis</i>	MHKMU H.Y. Huang 321	China, Yunnan	MW290213*	MW290272*	This study
<i>H. yunnanensis</i>	MHKMU H.Y. Huang 322	China, Yunnan	MW290214*	MW290273*	This study
(Holotype)					
<i>H. yunnanensis</i>	MHKMU H.Y. Huang 334	China, Yunnan	—	MW290274*	This study
<i>H. yunnanensis</i>	MHKMU H.Y. Huang 336	China, Yunnan	—	MW290275*	This study
<i>H. yunnanensis</i>	0726 07	China, Yunnan	MW290215*	MW290276*	This study
<i>H. yunnanensis</i>	MHKMU M. Mu 439	China, Yunnan	—	MW290277*	This study
<i>H. yunnanensis</i>	MHKMU H.Y. Huang 969	China, Yunnan	—	MW290278*	This study
<i>H. yunnanensis</i>	MHKMU H.Y. Huang 970	China, Yunnan	—	MW290279*	This study
<i>H. yunnanensis</i>	MHKMU H.Y. Huang 971	China, Yunnan	—	MW290280*	This study
* indicates sequences generated in this study					

Results

Phylogenetic analysis

One hundred and forty ITS and LSU sequences were newly generated for the *H. russula* complex in this study. DNA sequences were edited and aligned. The alignment length is 585 characters for the ITS dataset, 888 characters for the LSU dataset and 1471 for the concatenated dataset. Sequences of the *H. russula* complex were selected as the ingroup, *H. sordidus* Peck and *H. penarioides* Jacobsson & E. Larss. in subsection *Clitocyboides* served as outgroups, based on the previous study (Huang et al. 2018). The alignment is available at TreeBASE (Accession 27296 <http://purl.org/phylo/treebase/phylows/study/TB2:S27296>). All sequences acquired from this study are listed in Table 1.

The phylogenetic trees from BI and RAxML were almost identical, while statistical support showed slight differences. The ITS and concatenated trees were almost identical. Twelve phylogenetic species were recovered under the name of “*H. russula*” from Asia, Europe, North America and South America (Figs. 1–4). European “*H. russula*” formed three groups. Sequences of “*H. russula*” from North America represented five phylogenetic species. The Belize sequence of “*H. russula*” represented the South American group forming a single branch, which is the basal group of the *H. russula* species complex in the ITS trees (Fig. 1). The Chinese collections respectively formed five monophyletic clades with strong support, corresponding to five taxa, three new (*H. orientalis*, *H. qinggangjun* and *H. yunnanensis*) and two known taxa (*H. deliciosus* and *H. parvirussula*). These collections of “*H. russula*” from the same continent did not cluster together, although some may have close relationships. For example, *H. deliciosus* exhibited a sister

relationship with *H. qinggangjun* with strong support in the ITS and concatenated trees, but appeared to be sister to *H. yunnanensis* in the LSU tree. *Hygrophorus parvirussula* and *H. russula* were sister species in the ITS, LSU and concatenated trees. *Hygrophorus orientalis* had a close relationship to "*H. russula*" from Europe, probably as its Europe vicariant; the relationships cannot be resolved according to the present data and needs further research.

Chemical reactions studies

FeSO₄, NH₄OH, KOH and Melzer's Reagent were applied to test possible colour changes, which help to delimit sections or distinguish some species in *Hygrophorus* (Helser and Smith 1963; Larsson and Jacobsson 2004; Moreau et al. 2018). In this study, we used seven reagents, based on the previous study (Huang et al. 2018). All species in the *H. russula* complex from China showed a negative reaction to EtOH, FeCl₃, FeSO₄, KOH, NH₄OH and Melzer's Reagent, with the pileus turning pinkish in C₆H₅OH.

Taxonomy

Hygrophorus deliciosus C.Q. Wang & T.H. Li, Phytotaxa 449: 232–242, 2020 (Figs. 5, 6a, 7a, 8a, 9a, 10a)

The following description is mainly taken from Wang and Li (2020), combined with our field notes including macro-morphology, growth habit, distribution, host plants and examination.

Description

Basidiomata sparsely scattered to gregarious on soil, medium-sized, fleshy, rather fragile. *Pileus* 5–14 cm diam., rounded-conical to convex at first, expanding to plane at maturity; dirty white, flesh pink (10A3) to reddish-brown (10C7), covered with pale reddish-brown or reddish-purple (10C7–8) scales. *Context* white to pinkish (10A2), up to 2.4 cm thick. *Lamellae* adnate to decurrent, subdistant, 60–75 pieces of complete lamellae/cap, 0.5–1 cm wide, white to flesh pink (10A2–3), the surface often covered by reddish-purple (10C7–8) spots, short gills or lamellulae numerous and narrowed. *Stipe* 7–12 cm long, 1–2.2 cm diam., central, cylindrical, equal, white, yellowish-white, pinkish (10A2), with red (10A7) to brownish-red (10D7) scales. *Flesh* often pinkish (10A2). *Basal mycelium* white. *Odour* not distinctive. *Taste* mild.

Basidiospores [80/6/6] 6.44–8 (–9) × 4.6–6 µm, Q = 1.25–1.6, Q_m = 1.42 ± 0.12, broad ellipsoid to ellipsoid, smooth under a light microscope and SEM, thin-walled, hyaline, with a distinct hilar appendix, yellowish in Melzer's Reagent. *Basidia* 45–55 × 4–7 µm, mostly 4-spored, occasionally 2-spored, clavate, slender, thin-walled; sterigmata 6–10 µm in length. *Cheilocystidia* 29–43 × 3–4 µm, rare, scattered, subfusiform, narrowly clavate, spathulate, subcylindrical, flexuous, thin-walled, colourless. *Pleurocystidia* 32–50 × 3–6 µm, abundant, scattered, subfusiform, narrowly clavate, spathulate, flexuous, thin-walled, hyaline. *Lamellar trama* divergent, composed of short elements and long hyphae, short elements 10–25 × 3–4 µm, next to hymenium, cylindrical, long ellipsoid, pear-shaped or irregularly-shaped, colourless and hyaline; long hyphae 3–15 µm broad, long ellipsoid, clavate or irregularly-shaped, colourless and hyaline. *Pileipellis* an ixotrichoderm, consisting of narrow hyphae 2–5 µm diam. in gluten, loosely interwoven, branched or non-branched, thin-walled. *Pileal trama* made up of hyphae 6–20 µm diam., thin-walled. *Stipitipellis* a cutis, composed of hyphae 3–5 µm diam., thin-walled, mostly arranged in parallel. *Stipititrampa* made up of hyphae 6–18(–30) µm diam., arranged in parallel, thin-walled. *Mycelial hyphae* 2–4 µm broad, smooth under a light microscope, mostly smooth and occasionally covered with a massive nipple-shaped protuberance under SEM. *Clamp connections* present in all tissues.

Known distribution

South-western China, the subalpine to the alpine belt of Sichuan, Tibet and Yunnan.

Habit and habitat

Scattered or gregarious in broad-leaved forests, mainly Fagaceae (*Quercus aquifolioides*), elev. 1990–3700 m; in summer (July–September).

Additional materials examined: CHINA. Sichuan Province: Litang Prefecture, Junba Town, 30°18.70'N, 100°17.73'E, elev. 3630–3675 m, 26 August 2006, *Z.W. Ge 1414* (HKAS 51000); Muli Prefecture, Liziping Town, elev. 3400–3700 m, 1 August 2012, *T. Guo 554* (HKAS 76246). Tibet Autonomous Region: Linzhi Prefecture, Lulang Town, elev. 3325 m, 11 August 2013, *T.Z. Wei 3592* (HMAS 253198); Milin Prefecture, elev. 2990 m, 12 August 2013, *T.Z. Wei & T.Z. Li & X.Y. Liu & J.Y. Zhuang 3694* (HMAS 253249); Milin Prefecture, elev. 2990

m, 12 August 2013, *T.Z. Wei & T.Z. Li & X.Y. Liu & J.Y. Zhuang 3740* (HMAS 253233). Yunnan Province: Chuxiong City, Nanhua Wild Mushrooms Market, elev. unknown, 5 August 2020, *H.Y. Huang 725* (MHKMU H.Y. Huang 725); Jianchuan Prefecture, Diannan Town, elev. unknown, 10 August 2010, *J. Zhang 5* (HKAS 61315); Jianchuan Prefecture, Shaxi Town, elev. 3018 m, 6 October 2020, *H.Y. Huang 1006* (MHKMU H.Y. Huang 1006), *H.Y. Huang 1008* (MHKMU H.Y. Huang 1008); Jianchuan Prefecture, Shibao Mountain, elev. about 2500 m, 11 August 2010, *X.Y. Zhou 14* (HKAS 61276); the same location, 26°23.73'N, 99°50.41'E, elev. 2540 m, 19 August 2014, *S.D. Yang 43* (MHKMU S.D. Yang 43); Kunming City, the Park of Wild Duck Lake, elev. 1990 m, 17 September 2008, *Z.L. Yang 5214* (HKAS 54510); Lanping Prefecture, elev. 2700 m, 14 August 2011, *J.Y. Hao 515* (HKAS 71624); Lijiang City, Gucheng Area, elev. 2600 m, 18 August 2010, *Q. Zhao 949* (HKAS 69670); Lijiang City, Taian Town, elev. about 3200 m, 19 August 2020, *H.Y. Huang 806* (MHKMU H.Y. Huang 806), *H.Y. Huang 807* (MHKMU H.Y. Huang 807); Yulong Prefecture, Lijiang Observatory, elev. about 3100 m, 20 July 2008, *Q. Zhao 852* (HKAS 55053); Yulong Prefecture, Yulong Snow Mountains, elev. 2940 m, 24 July 2008, *L.P. Tang 472* (HKAS 54703).

Comments

Hygrophorus deliciosus is quite common in SW China as a wild commercial fungus known from the local name “Qinggangjun” or “Mitangjun”. “Mitang” is a kind of soup made from rice. “Mitangjun” means that this mushroom soup is thick and looks like “Mitang”. This species grows scattered to gregarious at varied elevations from 1900 to 3700 m. It is distinguished by its medium-sized basidiomata, thick context (up to 2.4 cm), broad ellipsoid basidiospores and probably associated with *Quercus aquifolioides*.

This species is most likely to be confused with *H. qinggangjun*; both are sold as edible mushrooms in Yunnan Province markets and has been identified from the samples of Nanhua Wild Mushrooms Market. These two species have a very weak or mild taste when fresh and a little bitter after cooking. *Hygrophorus qinggangjun* has a narrow distribution (Yunnan Province) and elevation (2000–2780 m), wavy and uplifted margin, thin context (0.4–0.9 cm), broad basidia (43–49 × 6–9 µm) and varied basidiospores (broad ellipsoid, ellipsoid and oblong). This taxon prefers to grow in a solitary manner or scattered in mixed forests, probably associated with *Quercus serrata*.

According to our phylogenetic analyses, *H. deliciosus* exhibits a sister relationship with *H. qinggangjun* with strong support in the ITS and concatenated trees, but appears to be sister to *H. yunnanensis* in the LSU tree (Figs. 1–3). However, *H. yunnanensis* has small basidiomata (pileus 5–15 cm), thin context (0.3–0.7 cm), narrow basidiospores [(7–) 7.5–9.5 × 4–5 µm], is so far restricted to Yunnan Province and prefers to grow alone or sparsely scattered in low elevations (2100–2600 m). This taxon is probably associated with *Lyonia ovalifolia* and *Ternstroemia gymnanthera*.

Hygrophorus orientalis H.Y. Huang & L.P. Tang, sp. nov. (Figs. 6b, 7b, 8b, 9b, 10b, 11)

MycoBank: MB 838040.

Diagnosis

Hygrophorus orientalis is different from other species of the *H. russula* complex in having large basidiomata, dense lamellae, small basidiospores and an extensive distribution.

Etymology

Latin “orientalis” means the species is widely distributed in East Asia.

Holotype: CHINA. Hubei Province: Shennongjia Forestry District, Muyu Town, in forests dominated by Fagaceae, 31°40.83'N, 110°26.45'E, elev. 1900 m, 18 July 2012, *Q. Cai 852* (HKAS 75586).

Description

Basidiomata solitary to gregarious on soil, large. *Pileus* 9–25 cm diam., often with a depressed centre and an in-rolled margin; pink (10A2–3), purplish-pink (14A4) to rose red (14A8), darker at the centre, covered with reddish-brown (10C7–8) scales. *Context* white (–A1), up to 3 cm thick. *Lamellae* adnate to slightly decurrent, crowded, 120–145 pieces of complete lamellae/cap, 0.5–1 cm wide, white to yellowish (1A2), rather fragile, concolorous (14A4) with the pileus when damaged, some gills branched. *Stipe* 7–15 cm long, 2.5–5 cm diam., solid, fibrillose, central to slightly eccentric, cylindrical, equal, more or less concolorous (10A2–3) with the pileus, covered with reddish-brown (10C7–8) scales, surface cracking and upward curved with age; flesh pink (10A2–3); *basal mycelium* white. *Odour* and *taste* not recorded.

Basidiospores [80/5/5] 5.5–7 (–8) × 3.5–4.8 (–5) µm, Q = (1.3–) 1.33–1.79, Q_m = 1.57 ± 0.15, ellipsoid, smooth under a light microscope and SEM, thin-walled, hyaline, with a distinct hilar appendix, yellowish in Melzer's Reagent. *Basidia* 30–40 × 4–7 µm, 4-spored, clavate, thin-walled; sterigmata 4–7 µm in length. *Cheilocystidia* 30–40 × 3–5 µm, rare, scattered, subfusiform, narrowly clavate, spathulate, subcylindrical, flexuous, thin-walled, colourless. *Pleurocystidia* 28–35 × 3–5 µm, rare, scattered, subfusiform, spathulate, flexuous, thin-walled, hyaline. *Lamellar trama* divergent, composed of short elements and long hyphae, short elements 5–15 × 4–7 µm, next to hymenium, subcylindrical, broadly ellipsoid, pear-shaped or irregularly-shaped, colourless and hyaline; long hyphae 4–18 µm broad, long ellipsoid, clavate or irregularly-shaped, colourless and hyaline. *Pileipellis* an ixotrichoderm, consisting of narrow hyphae (2.5–4.5 µm diam.) in gluten, loosely interwoven, branched or non-branched, thin-walled. *Pileal trama* made up of hyphae 4–22 µm diam., thin-walled. *Stipitipellis* a cutis, composed of hyphae 3–6 µm diam., thin-walled, mostly arranged in parallel. *Stipititrampa* made up of hyphae 6–20 µm diam., arranged in parallel, thin-walled. *Mycelial hyphae* 2–5 µm broad, smooth under a light microscope, mostly smooth and occasionally covered with a massive nipple-shaped protuberance under SEM. *Clamp connections* present in all tissues.

Known distribution: East Asia: including China, Japan (GenBank accession KF291216), South Korea (GenBank accession KX814449–814450).

Habit and habitat

Scattered to gregarious, at times in arcs or fairy rings, in broad-leaved forests, mainly Fagaceae, probably associated with *Cyclobalanopsis* sp. or *Quercus mongolica*, elev. 750–2340 m; in summer (July–September).

Additional materials examined: CHINA. Inner Mongolia: Ningcheng Prefecture, Heilihe National Nature Reserve, in broad-leaved forests, mainly *Quercus mongolica*, mixed with *Corylus heterophylla* and *Corylus mandshurica*, elev. ca. 770–1840 m, 20 August 2019, T. Z. Liu 19–520 (CFSZ 20884). Jilin Province: Antu Prefecture, the Changbai Mountain Scenic Area, in broad-leaved forests, mainly Fagaceae, elev. 750 m, 7 August 2010, X.F. Shi 450 (HKAS 63417, HKAS 71845); the same location, in broad-leaved forests, mainly Fagaceae (*Q. mongolica*), mixed with a few Betulaceae (e.g. *Betula platyphylla* and *C. heterophylla*), Pinaceae (*Pinus koraiensis*), 42°24.27'N, 128°6.10'E, elev. 760 m, 2 September 2019, H.Y. Huang 472 (MHKMU H.Y. Huang 472). Shanxi Province: Mei Prefecture, Yingtou Town, in broad-leaved forests, mainly *Cyclobalanopsis* sp. and Fagaceae, elev. 1280 m, 4 September 2010, X.F. Shi 659 (HKAS 71851). Yunnan Province: Yongping Prefecture, Longmen Town, elev. 2340 m, 1 August 2009, Q. Cai 74 (HKAS 58741).

Comments

Hygrophorus orientalis can be recognised from other species of the *H. russula* complex by its large basidiomata, dense lamellae, small basidiospores and scattered to gregarious (at times in arcs or fairy rings) growth habit, probably associated with *Cyclobalanopsis* sp. or *Quercus mongolica*. This taxon has a wide distribution in East Asia (including China, Japan and South Korea), usually occurring below elev. 2400 m. It is known as an edible mushroom in NE China, where locals prefer it raw rather than cooked.

Phylogenetically, this species is related to "*H. russula*" from Europe, probably as its Europe vicariant; the sister relationship of *H. orientalis* cannot be resolved according to the present data and needs further research.

Hygrophorus qinggangjun H.Y. Huang & L.P. Tang, sp. nov. (Figs. 6d, 7c, 8c, 9c, 10c, 12)

MycoBank: MB 838039.

Diagnosis

Hygrophorus qinggangjun is different from other species of the *H. russula* complex in its medium-sized basidiomata, uplifted and wavy margin, thinner context, variable basidiospores (broad ellipsoid, ellipsoid and oblong) and so far, only being known from Yunnan.

Etymology

Latin "*qinggangjun*" means the host plant of this mushroom, "qinggang" trees referring to oak trees in SW China.

Holotype: CHINA. Yunnan Province: Jianchuan Prefecture, Shibao Mountain, in a mixed forest with Ericaceae, Fagaceae and a few *Pinus yunnanensis*, 26°23.73'N, 99°50.41'E, elev. 2500 m, 18 August 2014, L.P. Tang 1683 (MHKMU L.P. Tang 1683).

Description

Basidiomata solitary to sparsely scattered on soil, medium-sized to large, fleshy, rather fragile. *Pileus* 8–14 cm diam., convex with an incurved margin when young, becoming plane with a depressed centre, margin often wavy and uplifted in age, reddish-white to pink (10A2–3), pale reddish-purple (12A5), with reddish-brown (10C7–8) scales, viscid to slimy when wet. *Context* white to pink-white (10A2), becoming darker (10A3) on exposure, 0.4–0.9 cm thick. *Lamellae* decurrent, subdistant, 60–80 pieces of complete lamellae/cap, 0.9–1.2 cm wide, pinkish (10A2–3) to reddish (10A5), short gills or lamellulae narrowed, rather fragile. *Stipe* 7–10 cm long, 1–1.7 cm diam., central to slightly eccentric, cylindrical, equal, surface pinkish (10A2–3) to reddish-purple (14A2), with dark reddish-violet (10C7–8) scales. *Flesh* firm, pinkish-white to pinkish (10A2), pale reddish-purple (10A3), becoming darker when cut or on exposure. *Basal mycelium* white. *Odour* not distinctive. *Taste* very weak.

Basidiospores [90/4/4] 6.44–9 (–9.5) × (4–) 4.4–6 (–6.5) μm, $Q = (1.15–) 1.24–1.64 (–1.89)$, $Q_m = 1.46 \pm 0.14$, broad ellipsoid, ellipsoid, oblong, smooth under a light microscope and SEM, thin-walled, hyaline, with a distinct hilar appendix, yellowish in Melzer's Reagent. *Basidia* 43–49 × 6–9 μm, mostly 4-spored, clavate, thin-walled; sterigmata 5–10 μm in length. *Cheilocystidia* 25–40 × 3–7 μm, rare, scattered, subfusiform, narrowly clavate, spathulate, irregularly-shaped, flexuous, thin-walled, colourless. *Pleurocystidia* 25–40 × 4–6 μm, scattered, narrowly clavate, spathulate, flexuous, thin-walled, hyaline. *Lamellar trama* divergent, composed of short elements and long hyphae, short elements 6–20 × 3–5 μm, next to hymenium, cylindrical, long ellipsoid, pear-shaped or irregularly-shaped, colourless and hyaline; long hyphae 5–20 (–28) μm broad, long ellipsoid, clavate or irregularly-shaped, colourless and hyaline. *Pileipellis* an ixotrichoderm, consisting of narrow hyphae (2–6 μm diam.) in gluten, loosely interwoven, branched or non-branched, thin-walled. *Pileal trama* made up of hyphae 7–20 μm diam., thin-walled. *Stipitipellis* a cutis, composed of hyphae 3–6 μm diam., thin-walled, mostly arranged in parallel. *Stipititrampa* made up of hyphae 6–18 μm diam., arranged in parallel, thin-walled. *Mycelial hyphae* 2–5 μm broad, smooth under a light microscope, mostly smooth and occasionally covered with a massive nipple-shaped protuberance under SEM. *Clamp connections* present in all tissues.

Known distribution

Yunnan Province.

Habit and habitat

Solitary or scattered in mixed forests, mainly including Ericaceae and Fagaceae, probably associated with *Quercus serrata*; elev. 2000–2780 m; in late summer and autumn (August–October).

Additional materials examined: CHINA. Yunnan Province: Chuxiong City, Nanhua Wild Mushrooms Market, elev. unknown, 5 August 2020, *H.Y. Huang 724* (MHKMU H.Y. Huang 724); Jianchuan Prefecture, Shibao Mountain, in a mixed forest with Ericaceae, Fagaceae and Pinaceae, 26°23.73'N, 99°50.41'E, elev. 2500 m, 18 August 2014, *S.D. Yang 20* (MHKMU S.D. Yang 20); the same location, occurred under *Quercus* sp., probably *Q. serrata*, 26°23.78'N, 99°50.32'E, elev. 2530 m, 13 September 2019, *M. Mu 436* (MHKMU M. Mu 436); the same location, in a mixed forest with Ericaceae, *Quercus* sp. and a few *Pinus yunnanensis*, 26°23.67'N, 99°50.18'E, elev. 2520 m, 14 September 2019, *M. Mu 464* (MHKMU M. Mu 464); Kunming City, Shuanglong Town, elev. about 2000 m, 17 September 2008, *X.H. Du 39* (HKAS 55496); Lijiang City, Qihe Town, elev. 2780 m, 19 August 2010, *X.T. Zhu 221* (HKAS 68397); Weixi Prefecture, Qizong Town, elev. unknown, 19 September 2010, *X.H. Wang 44* (HKAS 60530); Weixi Prefecture, Tacheng Town, elev. unknown, 20 September 2010, *X.H. Wang 121* (HKAS 60481); the same location, elev. unknown, 15 October 2011, *X.H. Wang 127* (HKAS 72665); location unknown, elev. unknown, 1 August 2009, *Q. Zhao 447*.

Comments

In SW China, *H. qinggangjun* is a wild edible mushroom, also known locally as “Mitangjun” or “Qinggangjun”. Thus, there are at least two species under this common name based on our research, viz. *H. deliciosus* and *H. qinggangjun*. Currently, *H. qinggangjun* is known only from Yunnan Province, occurring at high elevation regions (2000–2780 m). This mushroom is distinguished by its medium-sized basidiomata, wavy and uplifted margin, thin context (0.4–0.9 cm), broad basidia (43–49 × 6–9 μm) and varied basidiospores (broad ellipsoid, ellipsoid and oblong) and is solitary or scattered in mixed forests, probably in association with *Quercus serrata*.

The morphological and phylogenetic analyses (except the LSU tree) show that *H. qinggangjun* is closely related to *H. deliciosus*; the comparison between *H. deliciosus* and *H. qinggangjun* can be found in our treatment of *H. deliciosus*.

Hygrophorus russula (Schaeff. ex Fr.) Kauffman, Publications Michigan Geology Biology Survey, Biology Series 5 26: 185, 1918 (Fig. 6e)

The following description is mainly taken from Bas et al. (1990) and Candusso (1997), combined with our field notes including macro-morphology, growth habit, distribution, host plants and examination.

Description

Basidiomata solitary to sub-gregarious on soil, medium-sized, fleshy, rather fragile. *Pileus* 4–10 (–15) cm diam., hemispherical, convex, applanate, firstly almost white to pale pink with scattered pink to wine-red spots or appressed, fibrillose scales, gradually darker, becoming pinkish-red to wine-red at the centre, finally dark purplish-red, with small to larger concolorous spots elsewhere, surface dry to slight viscid. *Context* white, becoming pale pink to pinkish-purple when damaged, up to 2.5 cm thick. *Lamellae* adnate to short-decurrent, rather crowded to subdistant, 70–150 pieces of complete lamellae/cap, narrow, up to 0.5 cm wide, at first white to very pale pink, then spotted wine-red, brown-red or purplish-red, finally entirely dark reddish. *Stipe* 6–10 (–15) cm long, 0.8–2.5 (–4) cm diam., cylindrical, solid, dry, equal or slightly tapering towards base, white with some wine-red to purplish-red spots to almost completely brownish-purple. *Odour* absent to rather weakly unpleasant, sweetish. *Taste* mild.

Basidiospores [20/2/2] $8-10 \times 5-6.5 \mu\text{m}$, $Q = 1.33-1.72$, $Q_m = 1.55 \pm 0.12$, broad ellipsoid to ellipsoid, smooth under a light microscope, thin-walled, hyaline, with a distinct hilar appendix, yellowish in Melzer's Reagent. *Basidia* $55-68 (-80) \times 7-9 (-10) \mu\text{m}$, 4-spored, clavate, slender, thin-walled; sterigmata $4-7 \mu\text{m}$ in length. *Cheilocystidia* $35-50 \times 3-5 \mu\text{m}$, rare, scattered, irregularly-shaped, narrowly clavate, flexuous, thin-walled, colourless. *Pleurocystidia* $40-55 \times 3-9 \mu\text{m}$, scattered, irregularly-shaped, narrowly clavate, flexuous, thin-walled, hyaline. *Lamellar trama* divergent, composed of short elements and long hyphae, short elements $15-26 \times 3-6 \mu\text{m}$, next to hymenium, cylindrical, long ellipsoid, pear-shaped or irregularly-shaped, colourless and hyaline; long hyphae $3-10 (-25) \mu\text{m}$ broad, up to $70 \mu\text{m}$ long, long ellipsoid, clavate or irregularly-shaped, colourless and hyaline. *Pileipellis* an ixotrichoderm, consisting of narrow hyphae ($2-4 \mu\text{m}$ diam.) in gluten, loosely interwoven, branched or non-branched, thin-walled. *Pileal trama* made up of hyphae $4-25 \mu\text{m}$ diam., thin-walled. *Stipitipellis* a cutis, composed of hyphae $2.5-4.5 \mu\text{m}$ diam., thin-walled, mostly arranged in parallel. *Clamp connections* present in all tissues.

Known distribution

Europe.

Habit and habitat

Solitary to sub-gregarious in mixed forests, associated with *Quercus* spp., mainly *Q. ilex*, elev. below 1100 m; in autumn to winter (September–December).

Additional materials examined

SPAIN. Huesca city, Javierregay, on humus under oak and *Quercus* sp., elev. 680 m, 5 December 2009, A. González & F. Prieto s. n. (AH 37145); Madrid City, Torrelodones, in mixed forests, including *Pinus pinaster* and *Quercus ilex* subsp. *ballota*, elev. 845 m, 7 December 2000, F. Prieto s. n. (AH 19676); the same location, in mixed forests, including *P. pinaster* and *Q. ilex* subsp. *ballota*, elev. 845 m, 1 December 2001, F. Prieto s. n. (AH 19677).

Comments

Hygrophorus russula was originally described from Germany as *Agaricus russula* Schaeff. in 1774 (Schaeffer 1774, Fries 1821), but with few macroscopic details and without microscopic details in the original description. Subsequently, it was placed in the genus *Tricholoma* in 1878 (Gillet 1878). It was then placed in the genus *Hygrophorus*, i.e. *H. russula* (Schaeff. ex Fr.) Kauffman in 1918 (Kauffman 1918). Kauffman CH firstly described this mushroom in detail, based on American collections. *Hygrophorus russula* was long assumed to have a widespread distribution and has been reported from several countries and continents (Hesler and Smith 1963; Hongo 1982; Bas et al. 1990; Candusso 1997; Chen and Li 2013; Siegel and Schwarz 2016). Spanish material in this study matches the description based on European collections (Bas et al. 1990; Candusso 1997). According to those references and our study, European *H. russula* is distinguished by its solitary to sub-gregarious habit at low elevations (below 1100 m), medium-sized basidiomata, narrow

lamellae and large basidiospores, occurring under *Quercus* spp., principally *Q. ilex*, more rarely *Q. cerris*, *Q. pubescens*, *Q. pyrenaica* and *Q. suber*, mainly on basic soil. We confirm that *H. russula* is a strictly European species, based on this study.

The phylogenetic analyses show that this species is a sister species of *H. parvirussula*. However, *H. parvirussula* differs from *H. russula* by its solitary to sparsely scattered habit at high elevations (2500–3300 m), associated with *Pieris formosa* and *Rhododendron concinnum*, smaller basidiomata and narrower basidiospores ($6.9\text{--}9.0 \times 4.6\text{--}6.9 \mu\text{m}$, $Q = 1.13\text{--}1.83$, $Q_m = 1.48 \pm 0.23$).

Hygrophorus yunnanensis H.Y. Huang & L.P. Tang, sp. nov. (Figs. 6f, 7d, 8d, 9d, 10d, 13)

MycoBank: MB 838041.

Diagnosis

Hygrophorus yunnanensis is different from other species of the *H. russula* complex by its small basidiomata, thin context, narrow basidiospores and, so far, is only known from Yunnan.

Etymology

Latin “*yunnanensis*” means the species from Yunnan Province.

Holotype: CHINA. Yunnan Province: Qujing City, Shizong Town, in broad-leaved forests mainly dominated by *Lyonia ovalifolia*, mixed with a few *Lithocarpus* sp., *Pinus yunnanensis*, *Quercus* sp. and *Rhododendron decorum*, 24°38.03'N, 104°8.98'E, elev. 2330 m, 12 August 2019, *H.Y. Huang 322* (MHKMU H.Y. Huang 322).

Description

Basidiomata solitary to sparsely scattered on soil, small to medium-sized, firm, fleshy. *Pileus* 4–8 cm diam., convex to hemispherical with an in-rolled margin at first, expanding to plane at maturity; pale reddish-purple (12A5), dark red to reddish-brown (10C7–8), with a dark centre covered with scales. *Context* pinkish-white (10A2), becoming darker (10A3) on exposure, 0.3–0.7 cm thick. *Lamellae* adnate to slightly decurrent, subdistant, 70–95 pieces of complete lamellae/cap, 0.3–0.8 cm wide, pale pinkish (10A2), pinkish (10A3), dirty pink (10B2), staining reddish-purple (12B5) when damaged and occasionally showing reddish-purple (12B5) spots, short gills or lamellulae narrowed. *Stipe* 4–8 (–12) cm long, 0.5–1.5 cm diam., central, cylindrical, pinkish-white (10A2), pinkish-purple (12B5), staining reddish-violet (11B5), reddish-brown (10C7–8) when rubbed or with age. *Flesh* initially firm, becoming soft with age, whitish to pinkish-white (10A2), becoming darker (10A3) when cut or damaged. *Basal mycelium* white. *Odour* not distinctive. *Taste* mild.

Basidiospores [80/3/3] (7–) $7.5\text{--}9.5 \times 4\text{--}5 \mu\text{m}$, $Q = 1.6\text{--}2.13$, $Q_m = 1.86 \pm 0.14$, ellipsoid to oblong, ovoid, smooth under a light microscope and SEM, thin-walled, hyaline, with a distinct hilar appendix, yellowish in Melzer's Reagent. *Basidia* 38–45 (–50) $\times 5\text{--}10 \mu\text{m}$, mostly 4-spored, clavate, slender, thin-walled; sterigmata 4–9 μm in length. *Cheilocystidia* 30–40 $\times 3\text{--}6 \mu\text{m}$, rare, scattered, subfusiform, narrowly clavate, spathulate, subcylindrical, flexuous, thin-walled, colourless. *Pleurocystidia* 43–50 $\times 3\text{--}7 \mu\text{m}$, rare, scattered, subfusiform, narrowly clavate, spathulate, flexuous, thin-walled, hyaline. *Lamellar trama* divergent, composed of short elements and long hyphae, short elements 5–12 $\times 3\text{--}6 \mu\text{m}$, next to hymenium, cylindrical, long ellipsoid, pear-shaped or irregularly-shaped, colourless and hyaline; long hyphae 6–18 μm broad, long ellipsoid, clavate or irregularly-shaped, colourless and hyaline. *Pileipellis* an ixotrichoderm, consisting of hyphae (3–5 μm diam.) in gluten, loosely interwoven, branched or non-branched, thin-walled. *Pileal trama* made up of thin-walled hyphae 4–20 μm diam. *Stipitipellis* a cutis, composed of hyphae 3–5 μm diam., thin-walled, mostly arranged in parallel. *Stipititrampa* made up of hyphae 5–18 μm diam., arranged in parallel, thin-walled. *Mycelial hyphae* 2–5 μm broad, smooth under a light microscope, mostly smooth and occasionally covered with a massive nipple-shaped protuberance under SEM. *Clamp connections* present in all tissues.

Known distribution

Yunnan Province.

Habit and habitat

Solitary to sparsely scattered in broad-leaved forests probably associated with *Lyonia ovalifolia* and *Ternstroemia gymnanthera*, elev. 2100–2600 m; in late summer and autumn (August–October).

Additional materials examined: CHINA. Yunnan Province: Kunming City, Miaogao Temple, elev. 2100 m, 6 August 2006, *Y.C. Li 688* (HKAS 50442); Jianchuan Prefecture, Shibao Mountain, 26°23.73'N, 99°50.41'E, elev. 2500 m, 17 August 2014, *S.D. Yang 12* (MHKMU S.D. Yang 12); the same location, in broad-leaved forests, mainly Ericaceae and Fagaceae, elev. 2590 m, 20 August 2014, *J. Zhao 52* (MHKMU J. Zhao 52); the same location, in mixed forests with Ericaceae, *Quercus* sp. and a few *Pinus yunnanensis*, 26°23.78'N, 99°50.32'E, elev. 2530 m, 13 September 2019, *M. Mu 439* (MHKMU M. Mu 439); the same location, occurred under *Ternstroemia gymnanthera*, mixed with Ericaceae (*Pieris formosa* and *Rhododendron decorum*), Fagaceae and a few *P. yunnanensis*, elev. 2530 m, 5 October 2020, *H.Y. Huang 969* (MHKMU H.Y. Huang 969), *H.Y. Huang 970* (MHKMU H.Y. Huang 970), *H.Y. Huang 971* (MHKMU H.Y. Huang 971); Qujing City, Junzi Mountain, in broad-leaved forests mainly dominated by *Lyonia ovalifolia*, mixed with a few *Lithocarpus* sp., *P. yunnanensis*, *Quercus* sp. and *R. decorum*, 24°38.03'N, 104°8.98'E, elev. 2330 m, 11 August 2019, *H.Y. Huang 313* (MHKMU H.Y. Huang 313), *H.Y. Huang 316* (MHKMU H.Y. Huang 316), *H.Y. Huang 321* (MHKMU H.Y. Huang 321), *T. Huang 59* (MHKMU T. Huang 59), *L.P. Tang 2751* (MHKMU L.P. Tang 2751); the same location, 24°38.15'N, 104°9.13'E, elev. 2350 m, 12 August 2019, *H.Y. Huang 330* (MHKMU H.Y. Huang 330), *H.Y. Huang 334* (MHKMU H.Y. Huang 334), *H.Y. Huang 335* (MHKMU H.Y. Huang 335), *H.Y. Huang 336* (MHKMU H.Y. Huang 336), *L.P. Tang 2772* (MHKMU L.P. Tang 2772), *L.P. Tang 2773* (MHKMU L.P. Tang 2773); Yongping Prefecture, National Highway 320, 25°29.71'N, 99°39.40'E, elev. 2200 m, 30 July 2009, *L.P. Tang 1025* (HKAS 56982); Yunlong Prefecture, Nuodeng Village, elev. unknown, 26 August 2011, *R. Wang 95* (HKAS 72912).

Comments

Hygrophorus yunnanensis is distinctive by its small basidiomata, thin context, narrow basidiospores, so far restricted to Yunnan Province and occurring at high elevations (2100–2600 m). This taxon is common under shrub wood, mainly *Lyonia ovalifolia* in Junzi Mountain and occasionally occurring under *Ternstroemia gymnanthera*.

Morphologically, *H. yunnanensis* is quite similar to *H. parvirussula*. However, *H. parvirussula* occurs at high elevations (2500–3300 m) associated with *Pieris formosa* and *Rhododendron concinnum* and has wide basidiospores ($6.9\text{--}9.0 \times 4.6\text{--}6.9 \mu\text{m}$, $Q = 1.13\text{--}1.83$, $Q_m = 1.48 \pm 0.23$).

The LSU phylogenetic analyses showed that *H. yunnanensis* was sister to *H. deliciosus*, the comparison between *H. deliciosus* and *H. yunnanensis* being found in our treatment of *H. deliciosus*.

Taxonomic Key to Species of *Hygrophorus russula* Complex

- 1 Strictly North American distribution, at low elevations < 500 m; context unchanged on exposure; basidiospores < 3.2 μm in width.....*H. russuliformis*
- 1* Asia and Europe distribution, at high elevations > 500 m; context changed on exposure; basidiospores > 3.2 μm in width.....2
- 2 East Asia distribution; pileus > 15 cm diam.; basidiospores small, $5.5\text{--}7\text{--}(8) \times 3.5\text{--}4.8\text{--}(5) \mu\text{m}$*H. orientalis*
- 2* Narrow distribution; pileus < 15 cm diam.; basidiospores slightly large.....3
- 3 Europe distribution, at low elevations < 1500 m; basidiospores large, $8\text{--}10 \times 5\text{--}6.5 \mu\text{m}$*H. russula*
- 3* SW China distribution, at high elevations > 1500 m; basidiospores small.....4
- 4 Pileus > 10 cm diam.; associated with Fagaceae.....5
- 4* Pileus < 10 cm diam.; associated with Ericaceae or Theaceae.....6
- 5 Scattered to gregarious at elevations 1900–3700 m; pileus applanate, context thick (up to 2.4 cm); basidiospores broad ellipsoid to ellipsoid, $6.44\text{--}8\text{--}(9) \times 4.6\text{--}6 \mu\text{m}$, $Q_m = 1.42 \pm 0.12$*H. deliciosus*
- 5* Solitary or scattered at elevations 2000–2800 m; pileus wavy and uplifted, context thin (0.4–0.9 cm); varied basidiospores, $6.44\text{--}9\text{--}(9.5) \times (4\text{--}) 4.4\text{--}6\text{--}(6.5) \mu\text{m}$, $Q_m = 1.46 \pm 0.14$*H. qinggangjun*

- 6 Occurring at elevations 2500–3300 m; basidiospores broad ellipsoid, surface bacillate ornamentation, $6.9\text{--}9.0 \times 4.6\text{--}6.6$ (-6.9) μm , $Q_m = 1.48 \pm 0.23$*H. parvirussula*
- 6* Occurring at elevations 2100–2600 m; basidiospores narrow ellipsoid, surface smooth, (7--) $7.5\text{--}9.5 \times 4\text{--}5$ μm , $Q_m = 1.86 \pm 0.14$*H. yunnanensis*

Discussion

Species delimitations of *Hygrophorus russula* complex

Due to its recognisable characteristics in the field, consisting of a pale flesh pink to reddish-purple pileus, *H. russula* was once considered a widely-distributed species in the Northern Hemisphere (Hongo 1982; Bas et al. 1990; Hesler and Smith 1963; Chen and Li 2013). With the application of molecular methods, seven molecularly-distinct species have been revealed to be hidden under this name, supported by diagnosable criteria, including morphological characters, ecological traits and geographical distribution (see Table 2). Three species, *H. orientalis*, *H. qinggangjun* and *H. yunnanensis* from China, are newly described in this study.

Table 2
Comparison of the diagnostic characteristics between *Hygrophorus russula* complex

	<i>H. deliciosus</i>	<i>H. orientalis</i>	<i>H. parvirussula</i>	<i>H. qinggangjun</i>	<i>H. russula</i>	<i>H. russuliformis</i>	<i>H. yunnanensis</i>
Pileus	5–14 cm, applanate	9–25 cm, with an in-rolled margin	6–8 (–10) cm, applanate	8–14 cm, margin wavy and uplifted	4–10 (–15) cm, applanate	5–8 cm, convex	4–8 cm, applanate
Lamellae	60–75 pieces 0.5–1 cm deep	120–145 pieces 0.5–1 cm deep	60–80 pieces 0.4–0.9 cm deep	60–80 pieces 0.9–1.2 cm deep	70–150 pieces up to 0.5 cm deep	Crowded, unknown Narrow, unknown	70–95 pieces 0.3–0.8 cm deep
Stipe	7–12 \times 1–2.2	7–15 \times 2.5–5	6.5–13 \times 0.5–1.5	7–10 \times 1–1.7	6–10 (–15) \times 0.8–2.5 (–4)	1–4 \times 1.4–2	4–8 (–12) \times 0.5–1.5
Context (cm)	Up to 2.4	Up to 3	0.4–1	0.4–0.9	Up to 2.5	Unknown	0.3–0.7
Spores (μm)	6.44–8 (–9) \times 4.6–6	5.5–7 (–8) \times 3.5–4.8 (–5)	6.9–9.0 \times 4.6–6.6 (–6.9)	6.44–9 (–9.5) \times (4–) 4.4–6 (–6.5)	8–10 \times 5–6.5	8–10 (–12) \times 2–3.2	(7–) 7.5–9.5 \times 4–5
Q_m	1.42 \pm 0.12	1.57 \pm 0.15	1.48 \pm 0.23	1.46 \pm 0.14	1.55 \pm 0.12	Unknown	1.86 \pm 0.14
Basidia (μm)	45–55 \times 4–7	30–40 \times 4–7	30–55 \times 5–10	43–49 \times 6–9	55–68 (–80) \times 7–9	38–57 \times 5–7	38–45 (–50) \times 5–10
Habit	Scattered to gregarious	scattered to gregarious, at times in arcs or fairy rings	Solitary or scattered	Solitary or scattered	Solitary to subgregarious	Unknown	Solitary to sparsely scattered
Hosts	<i>Quercus aquifolioides</i>	<i>Cyclobalanopsis</i> sp. <i>Q. mongolica</i>	<i>Pieris formosa</i> <i>Rhododendron concinnum</i>	<i>Quercus</i> sp. (maybe <i>Q. serrata</i>)	<i>Q. ilex</i>	oak	<i>Lyonia ovalifolia</i> <i>Ternstroemia gymnanthera</i>

Morphologically, *H. russula* complex has similar colours (pale reddish-purple, dark red, reddish-brown, brownish-vinaceous), which are hard to identify in the field. Still, they are divergent in geographical distribution, host information, the size of basidiomata and microscopic characters.

Three species, *H. parvirussula*, *H. russuliformis* and *H. yunnanensis*, have a similar pileus less than 10 cm diam. However, *H. russuliformis*, so far restricted to North America, occurs under oak trees at low elevations (less than 500 m) of Florida, the context is

unchanged on exposure and has narrower basidiospores measuring $8-10 (-12) \times 2-3.2 \mu\text{m}$ (Hesler and Smith 1963). *Hygrophorus parvirussula* and *H. yunnanensis* were both found in south-western China and the context became darker on exposure.

Regarding *H. deliciosus*, *H. qinggangjun* and *H. russula*, these species are medium-sized with pileus 5–15 cm diam. However, *H. russula* occurs at low elevations (< 1100 m), has narrow lamellae (up to 0.5 cm wide) and large basidiospores ($8-10 \times 5-6.5 \mu\text{m}$) (Bas et al. 1990; Candusso 1997). Both *Hygrophorus deliciosus* and *H. qinggangjun* were found at high elevations (> 2500 m), have shorter basidia ($43-49 \times 6-9 \mu\text{m}$; $45-55 \times 4-7 \mu\text{m}$) and broader basidiospores [$6.44-9 (-9.5) \times (4-)$ $4.4-6 (-6.5) \mu\text{m}$, $Q = (1.15-)$ $1.24-1.64 (-1.89)$, $Q_m = 1.46 \pm 0.14$; $6.44-8 (-9) \times 4.6-6 \mu\text{m}$, $Q = 1.25-1.6$, $Q_m = 1.42 \pm 0.12$].

Hygrophorus orientalis differs from other species in the *H. russula* complex by its extensive distribution, wide range of elevation (750–2340 m), scattered to gregarious, at times in arcs or fairy rings, probably associated with *Cyclobalanopsis* sp. or *Quercus mongolica*, larger basidiomata (pileus up to 25 cm diam., stipe up to 15 cm long, 5 cm diam.), more dense lamellae (120–145 pieces/cap), shorter basidia ($30-40 \times 4-7 \mu\text{m}$) and smaller basidiospores [$5.5-7 (-8) \times 3.5-4.8 (-5) \mu\text{m}$].

Ecological traits of species in the *Hygrophorus russula* complex

Our data indicate that ecological traits, including its range of elevation, distribution and host preference, are useful for separating morphologically-similar species in the *H. russula* complex. European *Hygrophorus russula* was probably associated with *Quercus ilex* (Bas et al. 1990; Candusso 1997). *Hygrophorus russuliformis* from North America occurs under oak trees in Florida (Hesler and Smith 1963). To date, there are five taxa of the *H. russula* complex from Yunnan, China. Their distributions show a distinct pattern, varying with the elevation gradient. *Hygrophorus orientalis* is widely distributed, but in low-elevation environments. *Hygrophorus qinggangjun* and *H. yunnanensis* seem to be restricted to high elevations between 2000–3000 m. *H. deliciosus* and *H. parvirussula* can be found only in the subalpine belt (above 3000 m). This distribution usually correlates with the host plant. Our field records indicate that *H. yunnanensis* is associated with *Lyonia ovalifolia* and *Temstroemia gymnanthera*, which grows in forests below 2800 m; *H. deliciosus* is connected to *Quercus aquifolioides* and *H. parvirussula* is associated with *Pieris formosa* and *Rhododendron concinnum*, both their host plants being distributed at high elevations above 3000 m (see Fig. 14).

The taxonomic importance of comprehensive data in the *Hygrophorus russula* complex

Due to the similar characteristics, it is quite difficult to identify the species complex. Thus, the application of comprehensive evidence is very important for the accurate identification of this species complex. In our study, SEM characteristics and chemical reactions are helpful to distinguish the complex species of *H. russula*. *Hygrophorus parvirussula* is characterised by bacillate ornamentation of basidiospores under SEM; the other four taxa in this complex are smooth (see Fig. 7). The characteristics of basal hyphae are not very common in fungal taxonomy. Huang et al. (2020) firstly reported abundant morphological characters of the basal hyphae of seven species from *Clavariadelphus* as taxonomical evidence. In *H. russula* complex, the basal hyphae also vary from smooth to a massive nipple-shaped protuberance under SEM (see Fig. 10). It indicates the basal hyphae are valuable in the classification of *Hygrophorus*. In terms of chemical reactions, we found that $\text{C}_6\text{H}_5\text{OH}$ can be used to distinguish the *Hygrophorus* species.

Molecular methods effectively detect hidden species in fungi (Tang et al. 2014, 2017; Huang et al. 2018; Yang et al. 2018). In addition, disentangling the species complex of *H. russula* needs many different types of information, including a phylogenetic, morphological and taxonomical investigation. SEM characteristics and chemical reactions help distinguish and separate species.

Declarations

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Authors' contributions

All authors contributed to the study conception and design. Material preparation, sampling, collecting, DNA isolation and preparation for sequencing were performed by Hong-Yan Huang, Li-Ping Tang, Moreno Gabriel, Tie-Zhi Liu, Ting Huang and Wen-Hao Zhang. Data

analysis were performed by Hong-Yan Huang. The first draft of the manuscript was written by Hong-Yan Huang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

The datasets generated during and/or analysed during the current study are available in the GenBank, <http://www.ncbi.nlm.nih.gov>.

Ethics declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Consent for publication

Not applicable

Ethical approval and Consent to participate

Not applicable

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Figures

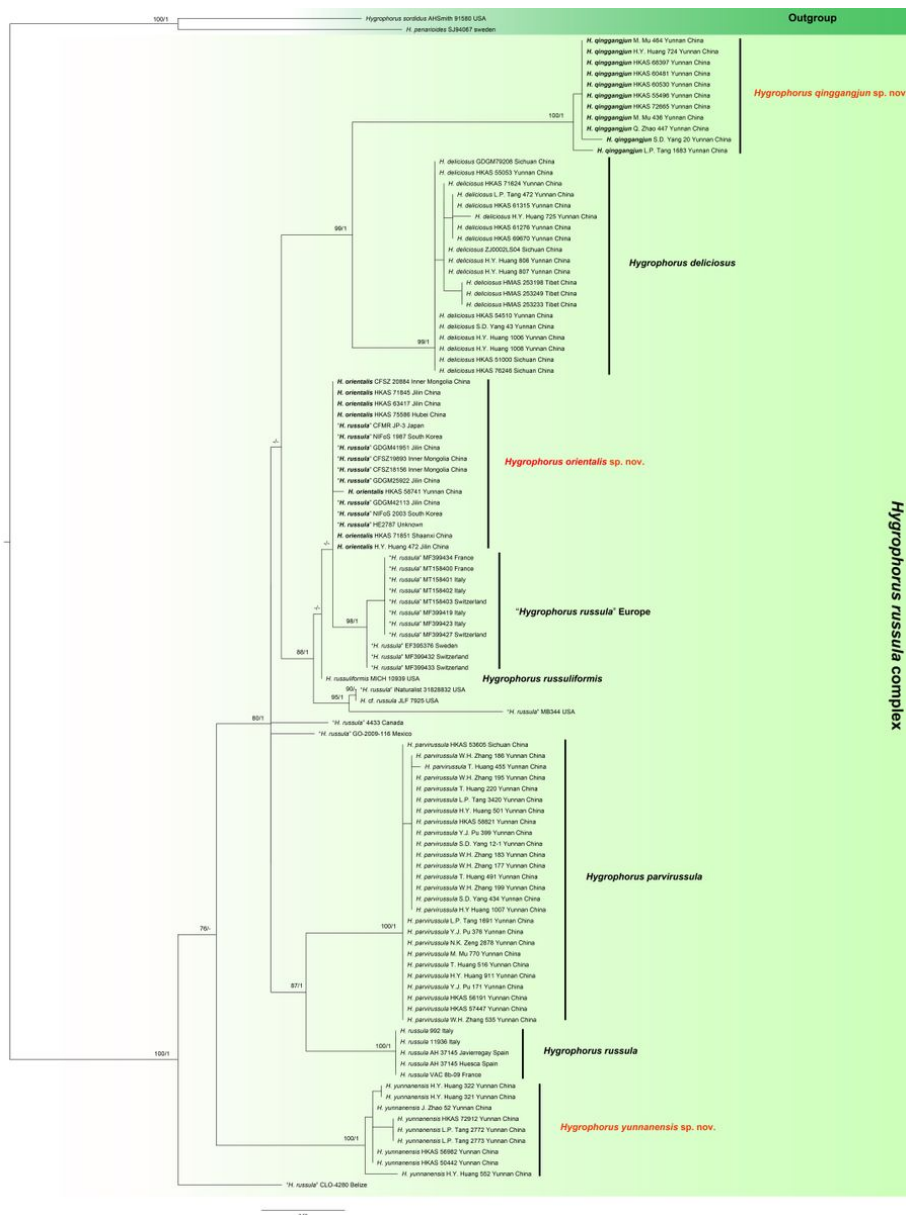


Figure 1

Phylogenetic tree of *Hygrophorus russula* complex, based on ITS sequence data. RAxML BP values ($\geq 70\%$) are shown above branches, Bayesian posterior probabilities (≥ 0.90) are shown above branches

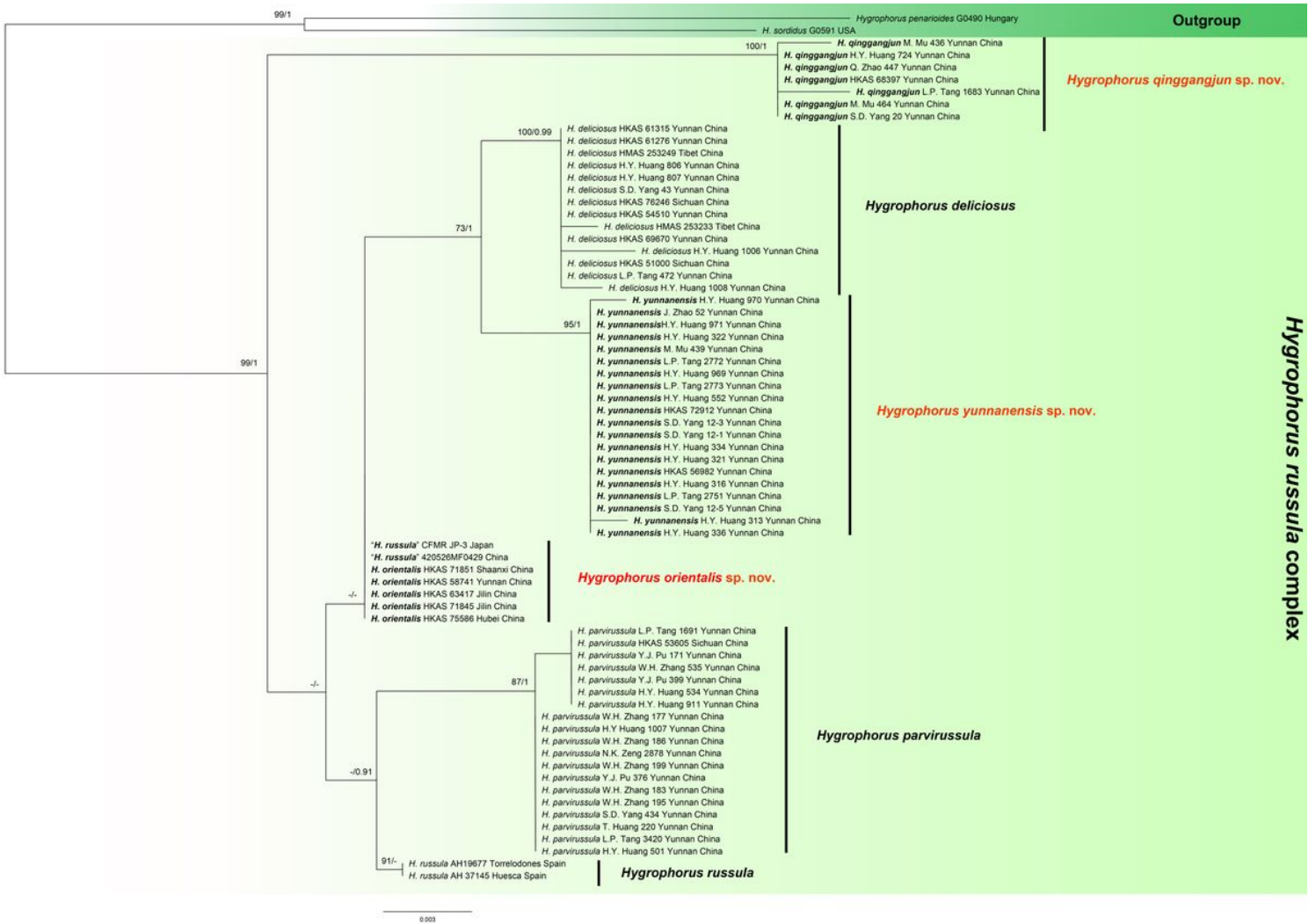


Figure 2

Phylogenetic tree of *Hygrophorus russula* complex, based on LSU sequence data. RAxML BP values ($\geq 70\%$) are shown above branches, Bayesian posterior probabilities (≥ 0.90) are shown above branches

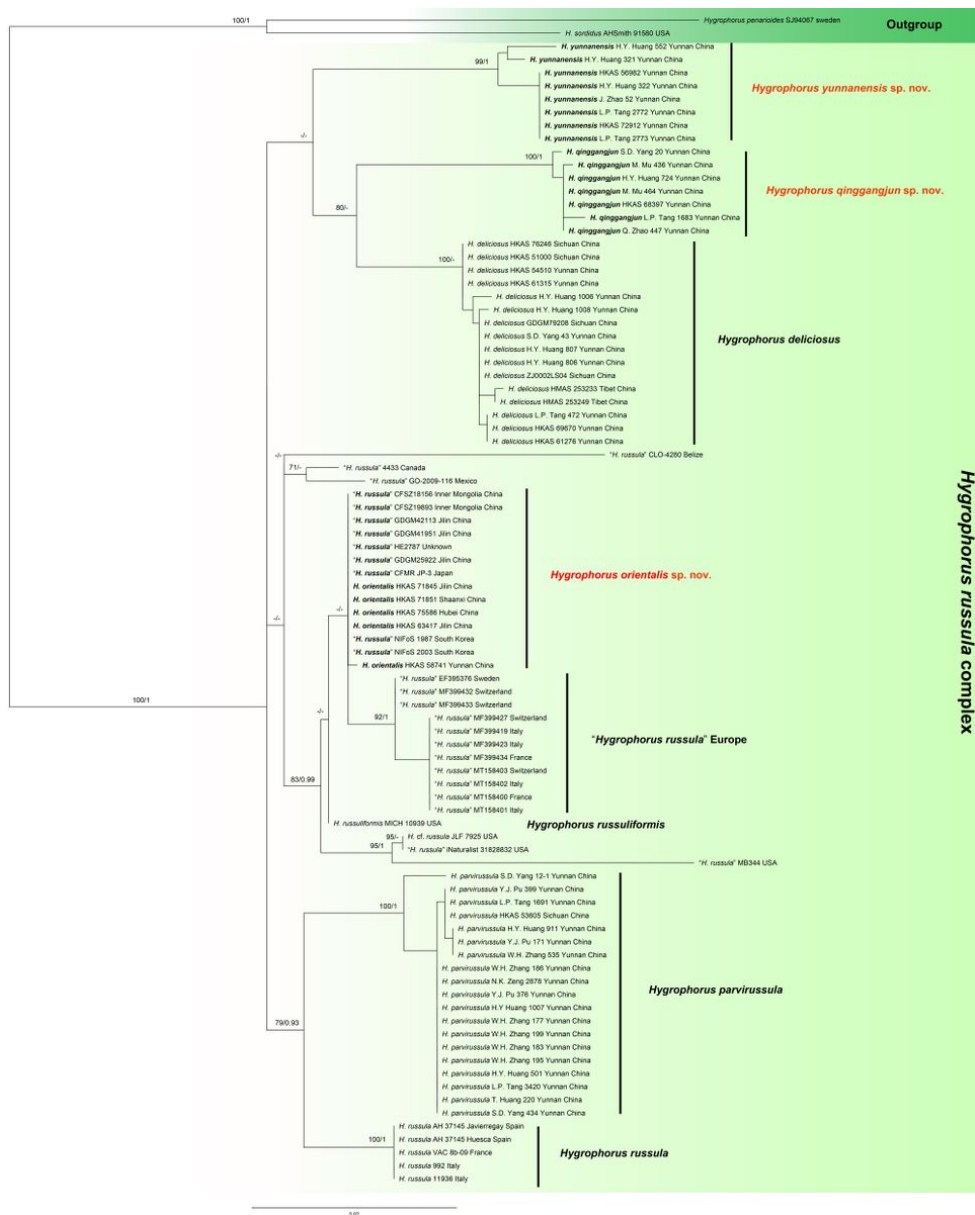


Figure 3

Phylogenetic tree of *Hygrophorus russula* complex, based on ITS and LSU sequence data. RAxML BP values ($\geq 70\%$) are shown above branches, Bayesian posterior probabilities (≥ 0.90) are shown above branches

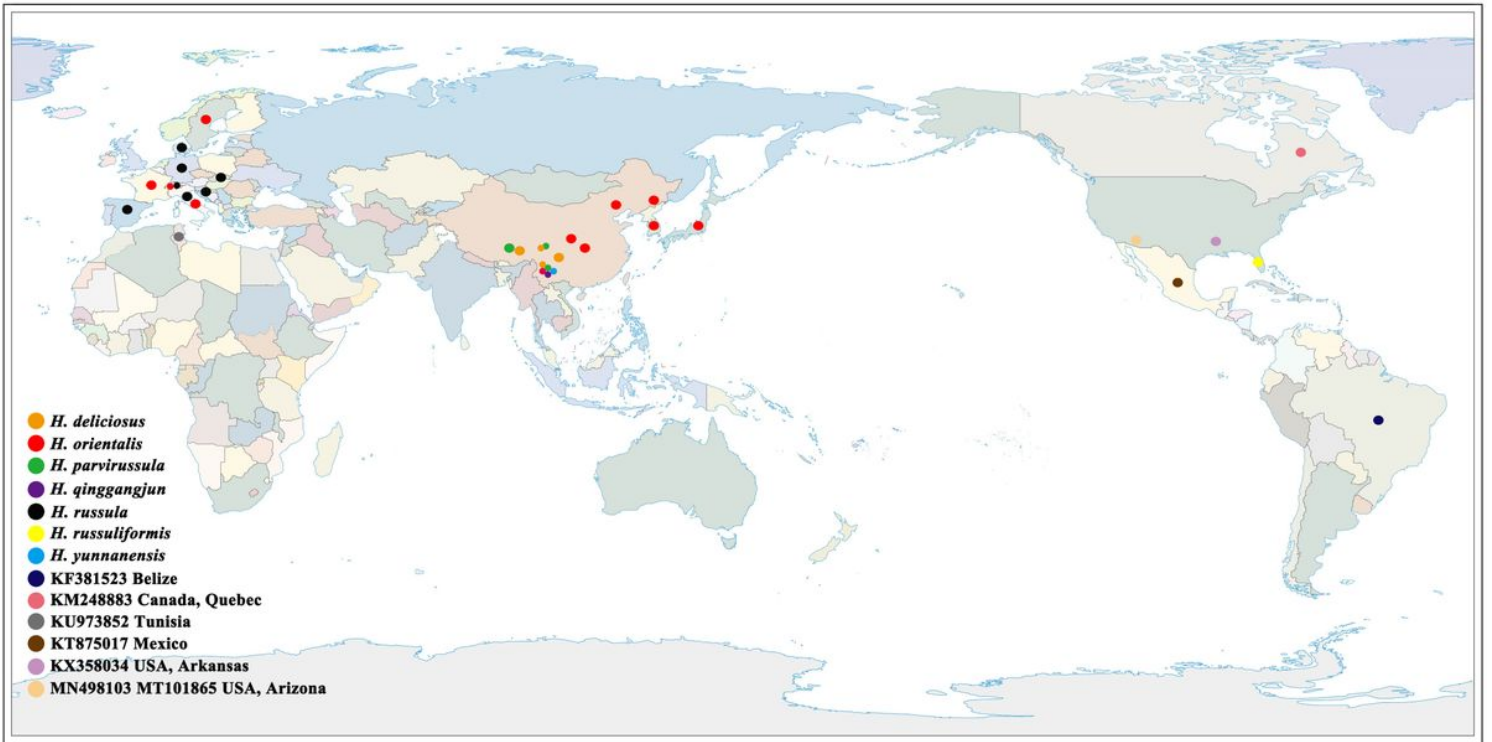


Figure 4

Distribution of *Hygrophorus russula* complex. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

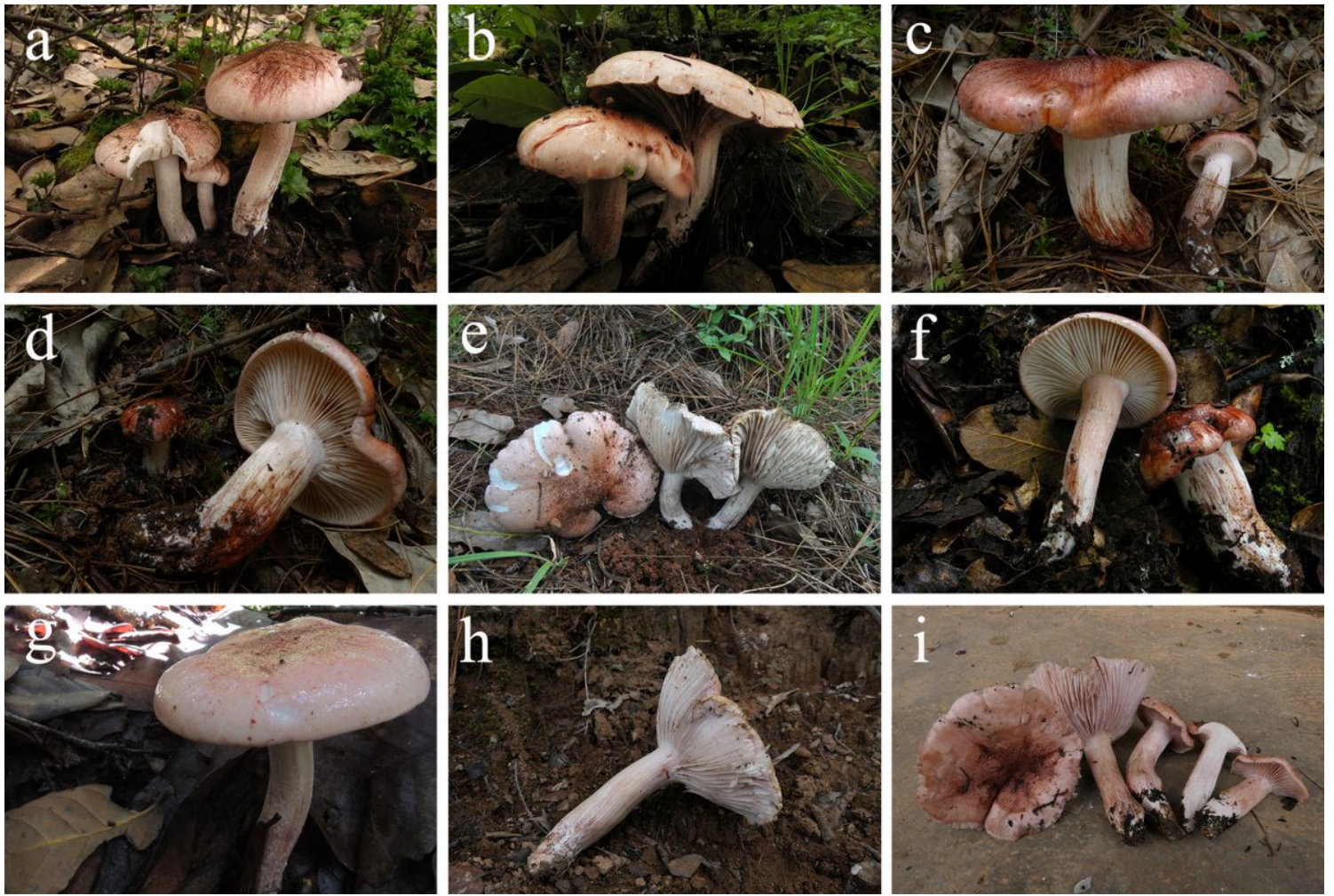


Figure 5

Basidiomata of *Hygrophorus deliciosus*. a. from HKAS 54510; b. from HKAS 54703; c, d. from HKAS 71624; e. from HKAS 69670; f. from HKAS 76246; g. from MHKMU S.D. Yang 43; h. from MHKMU H.Y. Huang 1006; i. from MHKMU H.Y. Huang 806 (a photo by Z.L. Yang; b photo by L.P. Tang; c, d photos by J.Y. Hao; e photo by Q. Zhao; f photo by T. Guo; g photo by S.D. Yang; h–i photos by H.Y. Huang)

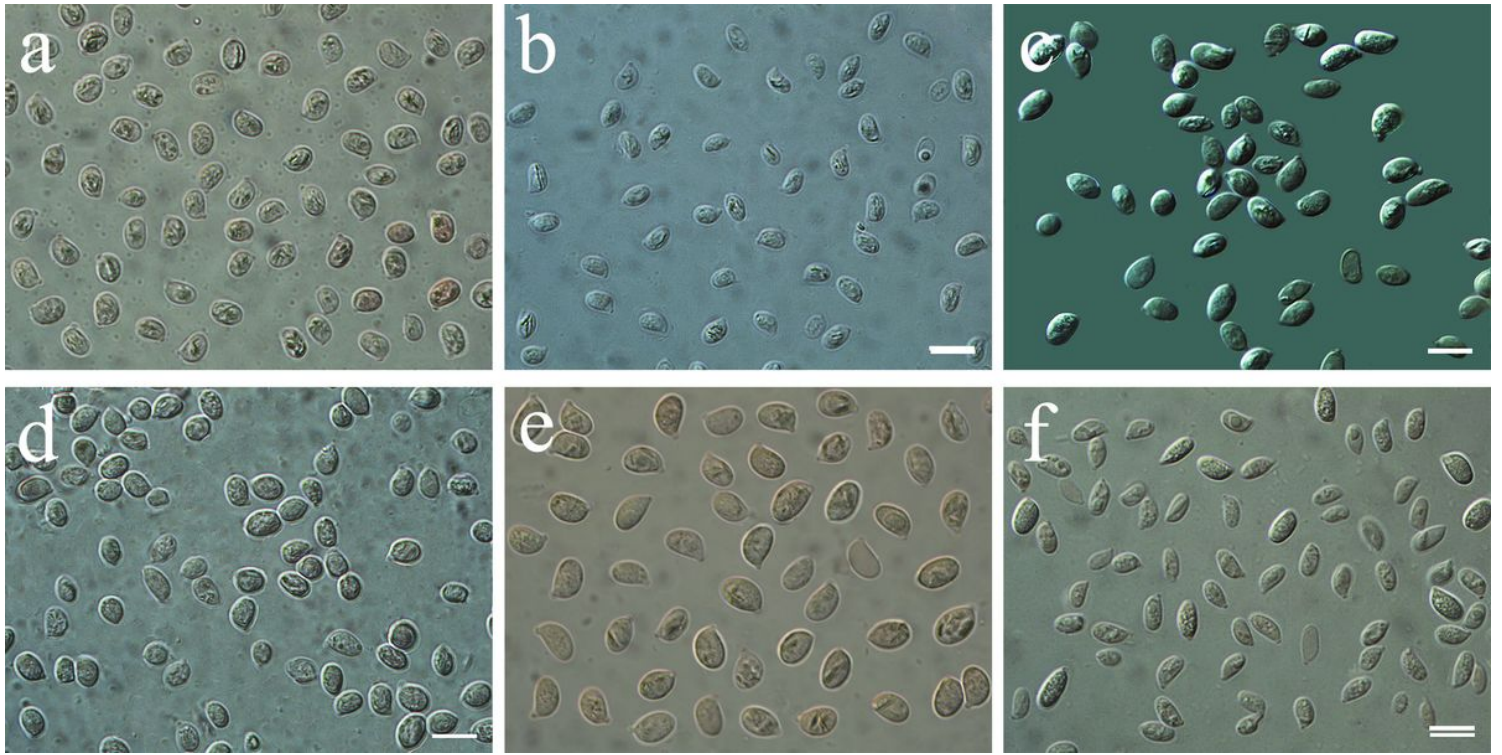


Figure 6

Basidiospores of *Hygrophorus russula* complex under a light microscope. a. *H. deliciosus* (HKAS 61315); b. *H. orientalis* (MHKMU H.Y. Huang 472); c. *H. parvirussula* (MHKMU N.K. Zeng 2878); d. *H. qinggangjun* (MHKMU L.P. Tang 1683); e. *H. russula* (AH 19677); f. *H. yunnanensis* (MHKMU S.D. Yang 12). Bars = 10 μm

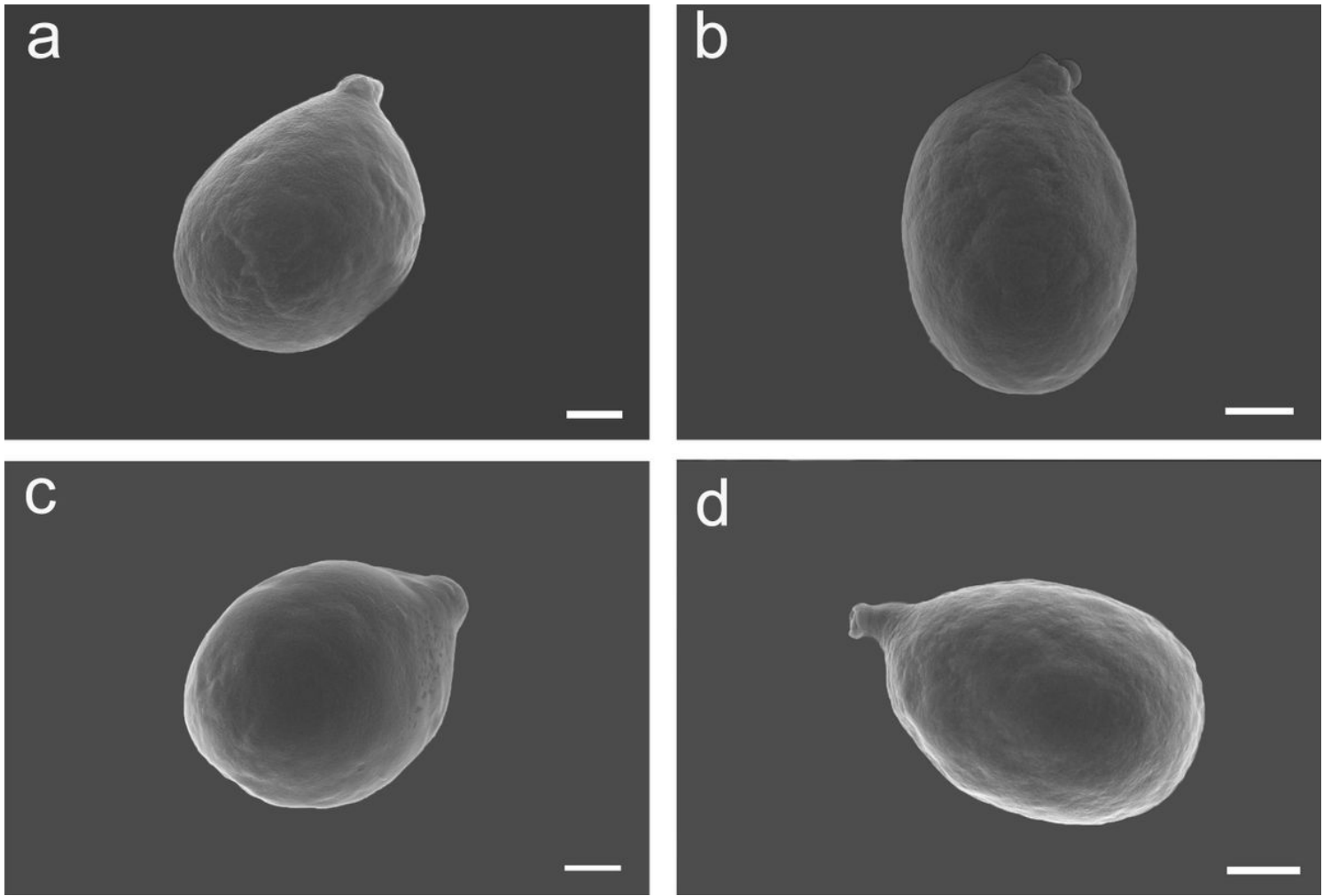


Figure 7

Basidiospores of *Hygrophorus russula* complex under SEM. a. *H. deliciosus* (HKAS 54510); b. *H. orientalis* (HKAS 75586); c. *H. qinggangjun* (HKAS 60530); d. *H. yunnanensis* (MHKMU S.D. Yang 12). Bars = 1 µm

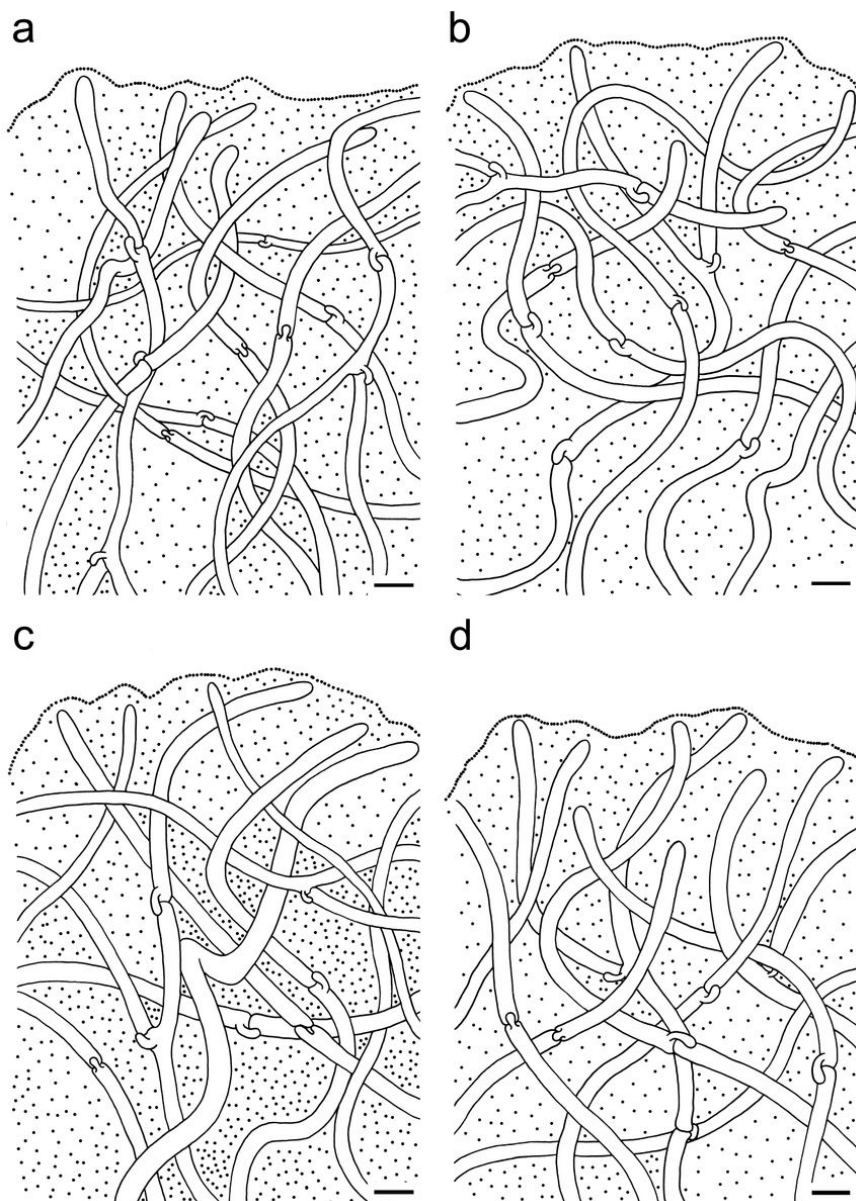


Figure 8

Pileipellis of *Hygrophorus russula* complex. a. *H. deliciosus* (HKAS 54510); b. *H. orientalis* (HKAS 63417); c. *H. qinggangjun* (MHKMU L.P. Tang 1683); d. *H. yunnanensis* (HKAS 50442). Bars = 10 μ m

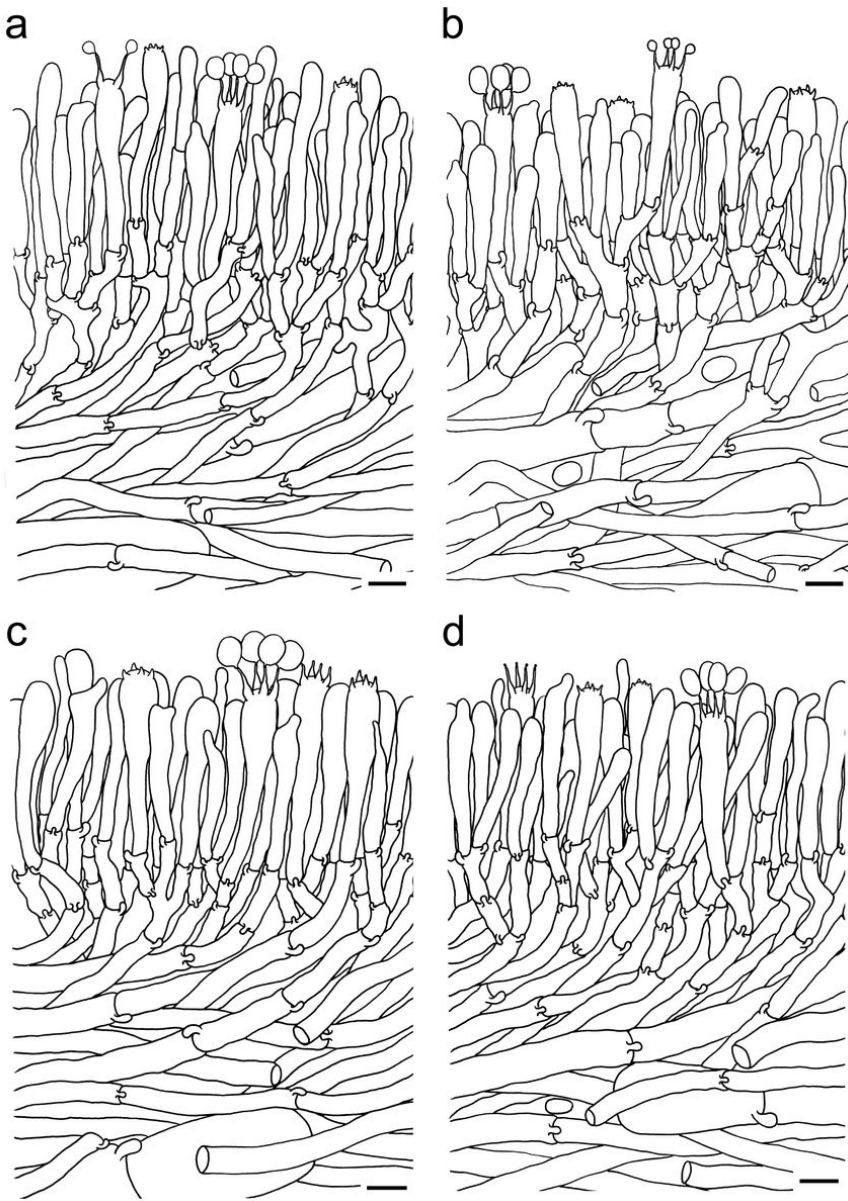


Figure 9

Hymenium (including basidia, pleurocystidia and lamellar trama) of *Hygrophorus russula* complex. a. *H. deliciosus* (HKAS 54510); b. *H. orientalis* (HKAS 71845); c. *H. qinggangjun* (MHKMU L.P. Tang 1683); d. *H. yunnanensis* (MHKMU H.Y. Huang 322). Bars = 10 μ m

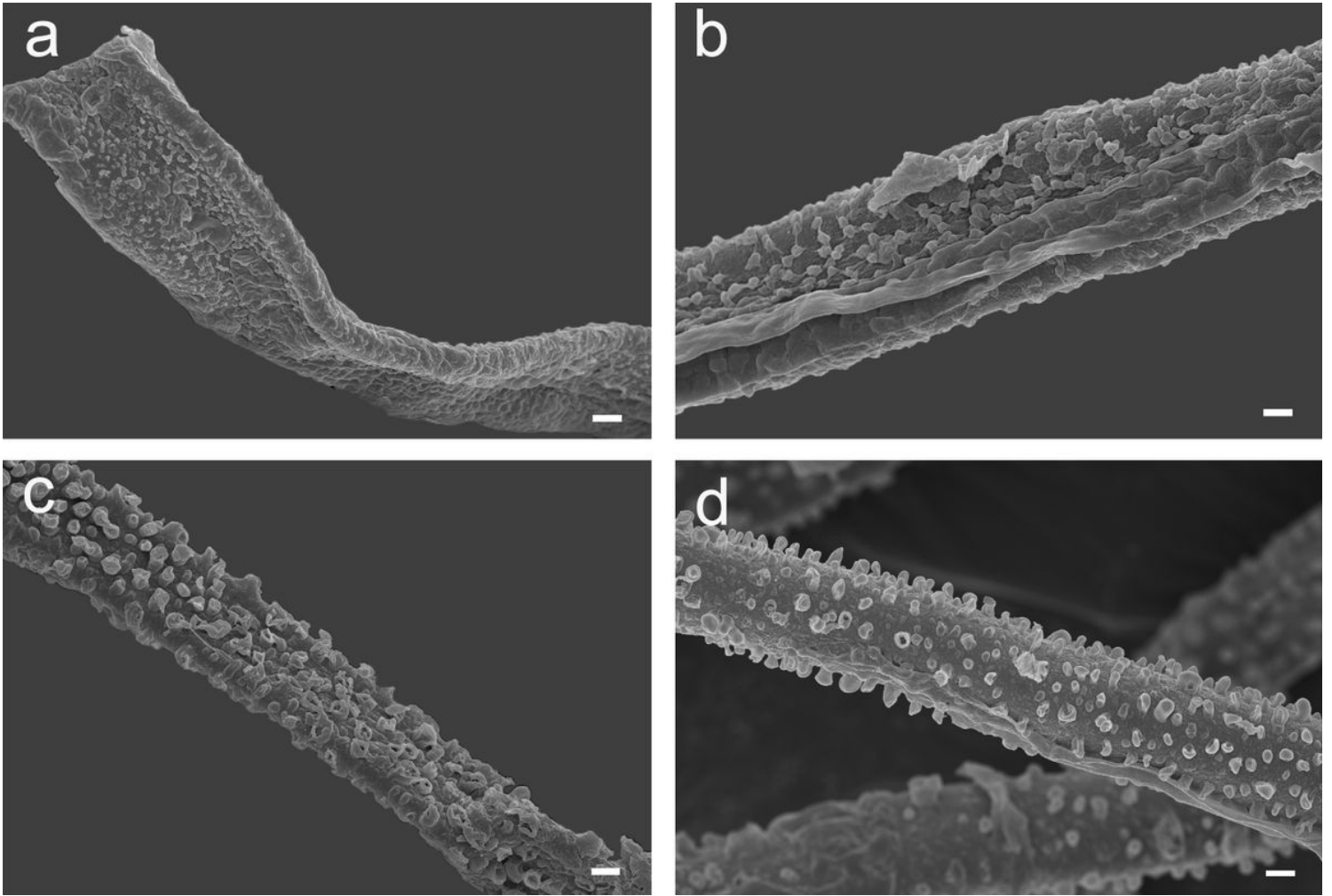


Figure 10

Basal mycelial hyphae of *Hygrophorus russula* complex under SEM. a. *H. deliciosus* (MHKMU S.D. Yang 70); b. *H. orientalis* (HKAS 75586); c. *H. qinggangjun* (MHKMU L.P. Tang 1683); d. *H. yunnanensis* (MHKMU S.D. Yang 12). Bars = 1 μ m

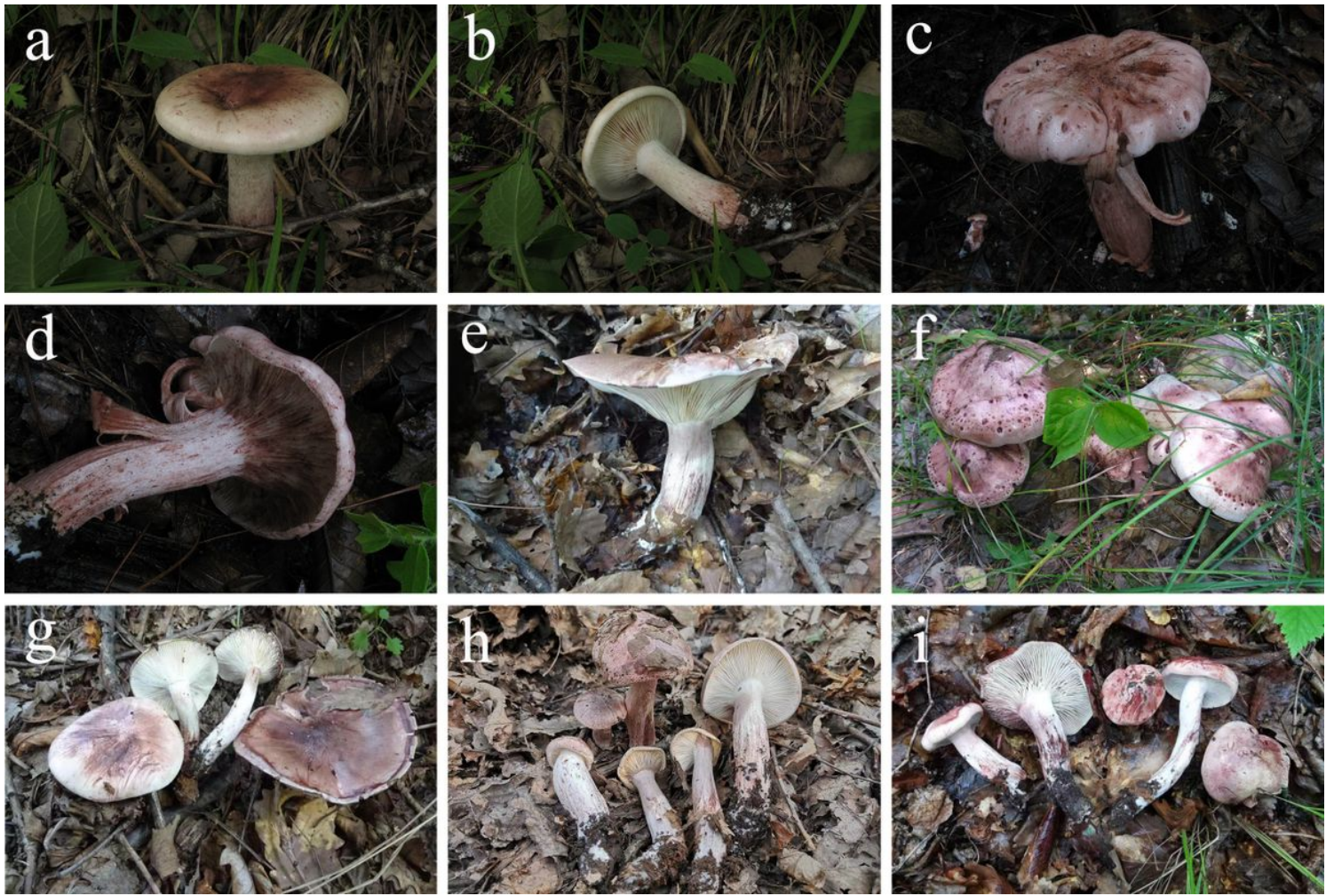


Figure 11

Basidiomata of *Hygrophorus orientalis*. a, b. from HKAS 75586; c–d. from HKAS 58741; e, g–h. from CFSZ 20884; f. from MHKMU H.Y. Huang 472 (a–d photos by Q. Cai; e, g–h photos by T.Z Liu; f photo by B. Wang)

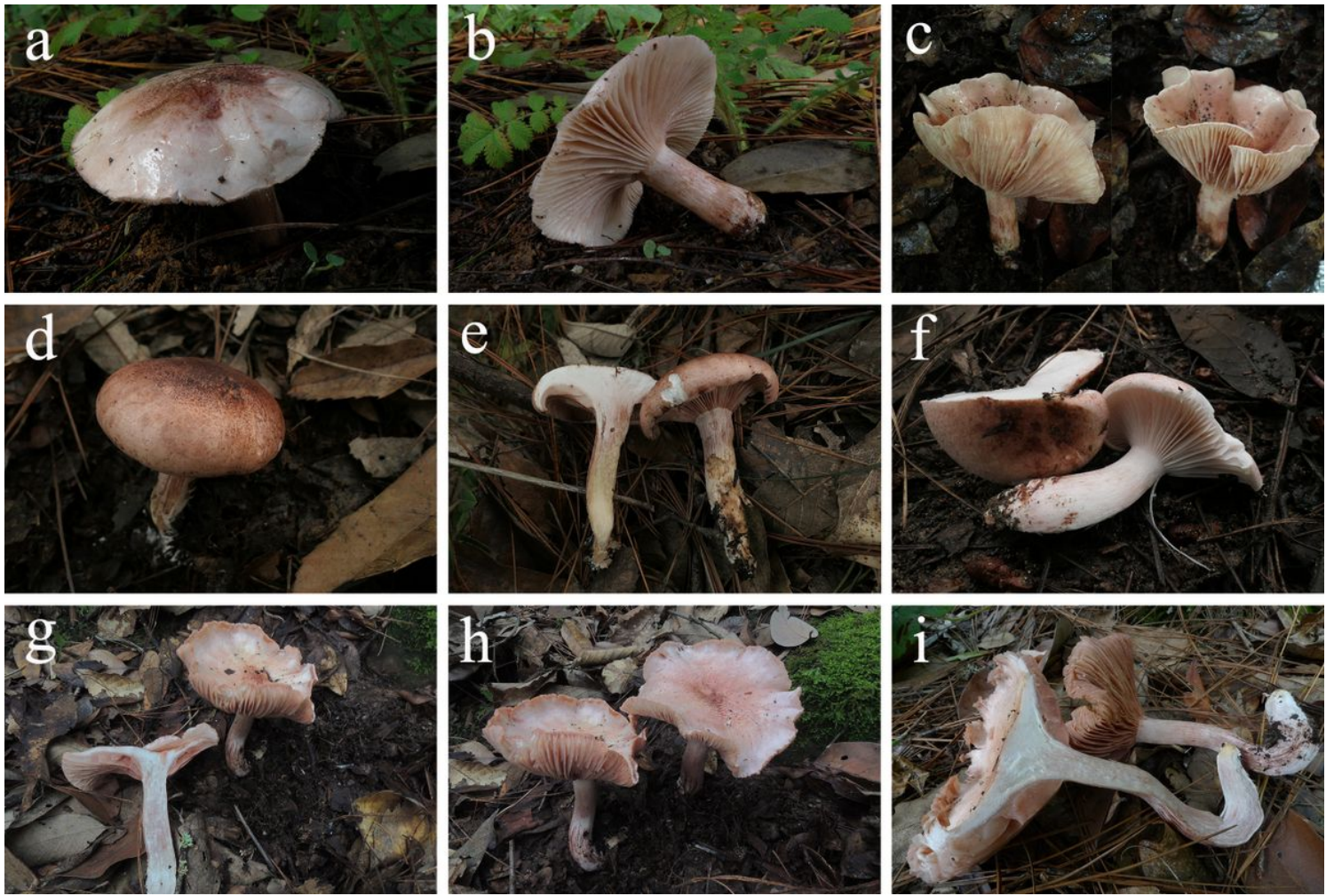


Figure 12

Basidiomata of *Hygrophorus qinggangjun*. a, b. from HKAS 68397; c. from Q. Zhao 447; d–e. from MHKMU M. Mu 436; f. from MHKMU M. Mu 464; g–h. from MHKMU S.D. Yang 20; i. from MHKMU L.P. Tang 1683 (a, b photos by X.T. Zhu; c photo by Q. Zhao; d–f photos by M. Mu; g–h photos by S.D. Yang; i photo by L.P. Tang)

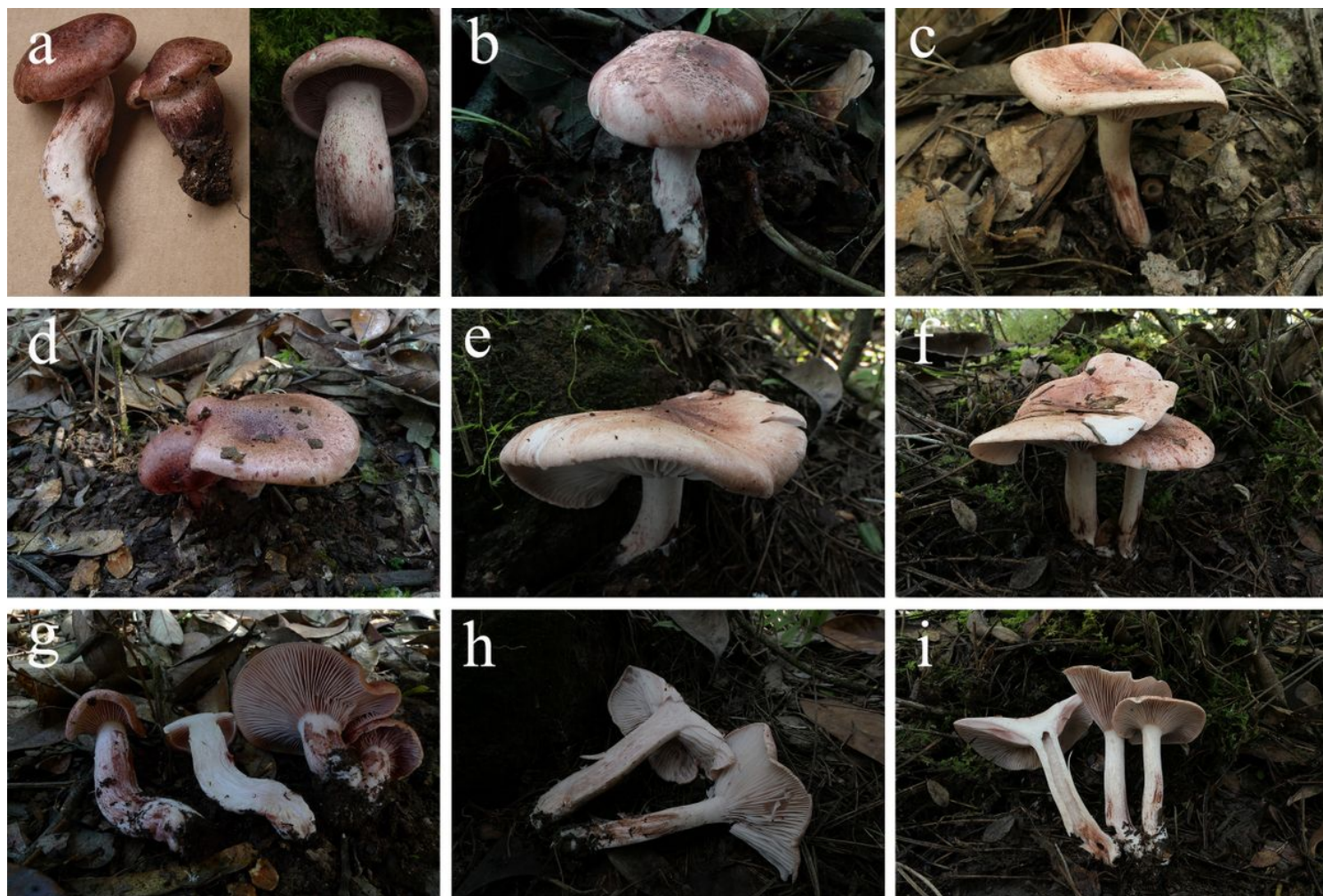


Figure 13

Basidiomata of *Hygrophorus yunnanensis*. a. left from HKAS 50442, right from MHKMU J. Zhao 52; b. from MHKMU L.P. Tang 2751; c. from MHKMU H.Y. Huang 971; d, g. from MHKMU L.P. Tang 2772; e, h. from MHKMU H.Y. Huang 316; f, i. from MHKMU H.Y. Huang 322 (a left photo by Y.C. Li, right photo by J. Zhao; b, d, g photos by L.P. Tang; c, e, f, h, i photos by H.Y. Huang)

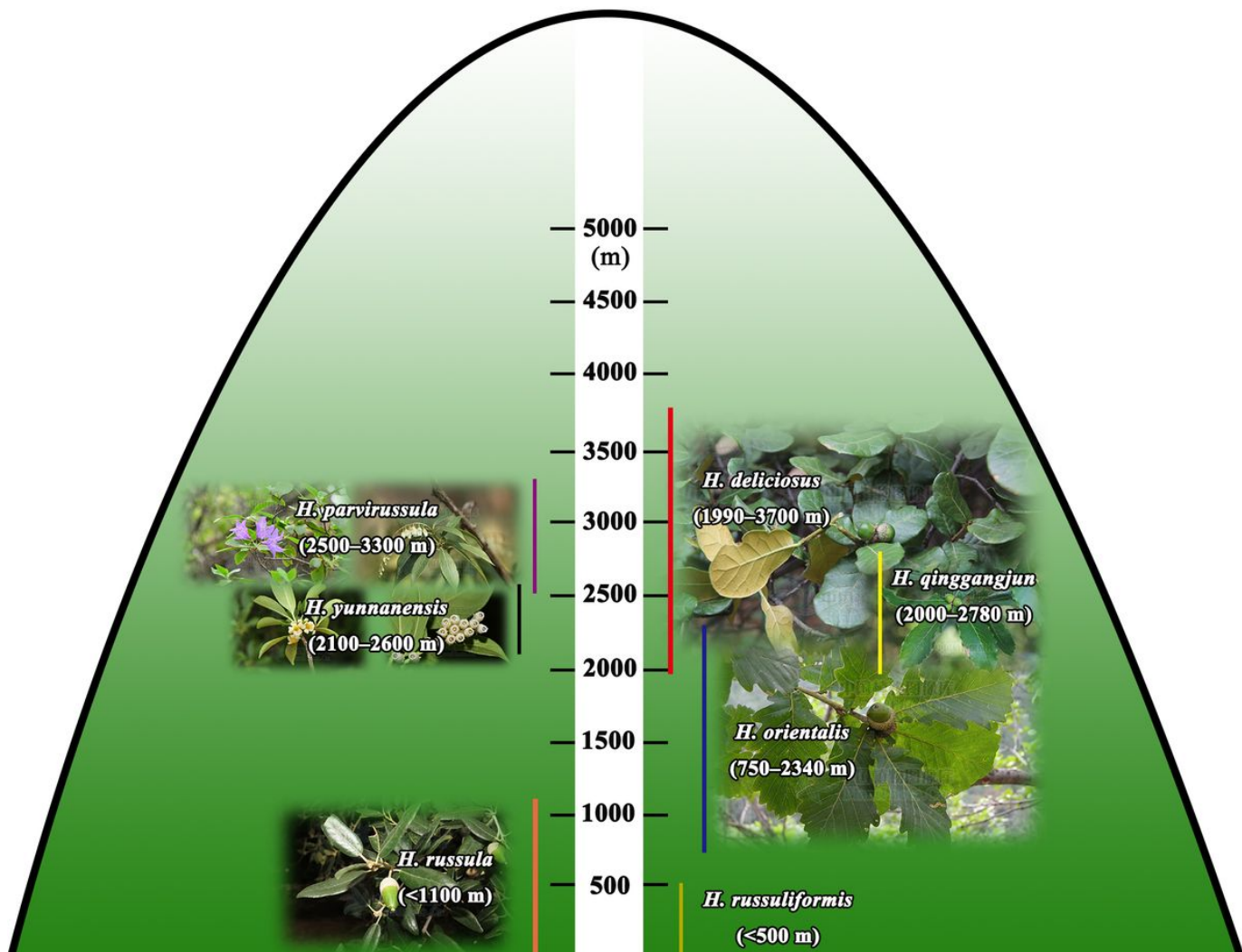


Figure 14

Distribution elevation and host plants of *Hygrophorus russula* complex. [The host plants' photos from the website: Plant Photo Bank of China (PPBC)]

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [H.russulacomplexITSLSU.nxs](#)
- [H.russulacomplexITS.nxs](#)
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