**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* widely distributes at low elevations in East Asia. At present, seven taxa have been revealed in the *H. russula* species complex, and a key to these species is provided.

**Keywords** Cryptic species · Ectomycorrhizal fungi · Molecular systematics · Taxonomy

**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; Arnold 1990; Candusso 1997; Lodge et al. 2014). *Hygrophorus* are generally ectomycorrhizal, but a couple is parasitic, such as *H. olivaceoalbus* (Fr.) Fr. and *H. penarius* Fr. (Marino 2008; Agerer 2012). Most form associations with broad-leaved or coniferous trees. This genus appears 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* Murill 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 *Q. serrata*) (Endo et al. 2018).

Lodge et al. (2014) proposed the infrageneric updates of *Hygrophorus* based on molecular phylogeny and revealed, in many cases, that a widely applied name may harbour 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 *Tephroleucii* (Bataille) Singer (Larsson et al. 2018).

*Hygrophorus russula* (Schaeff. ex Fr.) Kauffman was originally described based on material 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 mainly 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 distributing in East Asia.

**Materials and methods**

**Sampling and morphological studies**

Most specimens were collected in central, 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 basal mycelium of stipe were examined with a ZEISS Sigma 300 scanning electron microscope (SEM) at 7.00 kV. The procedures of stipe were examined with a ZEISS Sigma 300 scanning electron microscope. Melzer’s reagent was used to test the amyloidy of basidiospores. Basidiospores and basal mycelium of stipe were examined with a ZEISS Sigma 300 scanning electron microscope (SEM) at 7.00 kV. The procedures of stipe were examined with a ZEISS Sigma 300 scanning electron microscope.

**Chemical reactions**

Seven chemical reagents were applied in this study: 

- **C₆H₅OH = 10% (w/v) phenol**
- **EtOH = 95% (w/v) ethanol**
- **FeCl₃ = 10% (w/v) ferric chloride**
- **FeSO₄ = 10% (w/v) ferrous sulphate**
- **KOH = 5% (w/v) potassium hydroxide**
- **NH₄OH = 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). The nuclear ribosomal DNA internal transcribed space regions (ITS), large subunit nuclear ribosomal RNA (LSU), and translation elongation factor 1-α (*TEF1*) were amplified by a polymerase chain reaction (PCR) using primer pairs ITS5/ITS4, LROR/LR5, and EF1-983F/EF1-1567R (Vilgalys and Hester 1990; White et al. 1990). PCR reactions were performed in 25 μl reaction mixtures containing 2.5 μl of 10× amplification buffer (with MgCl₂), 0.5 μl dNTP (200 μM), 0.2 μl Taq DNA polymerase (5 U/μl), 1 μl of each primer (10 μM), 1 μl DNA template, and 18.8 μl 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, LSU, and *TEF1* 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 (1–136), 5.8S (137–300), ITS2 (301–585), LSU (586–1442), and *TEF1* (1443–1981). 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+I for ITS1 and ITS2, SYM+G for 5.8S, GTR+I for LSU, and SYM+G for *TEF1*. 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 LSU, 3,000,000 generations for *TEF1*, and 5,000,000 generations for concatenated sequences and sampled every 1000 generations. At the end of the run, the average
| Taxon              | Voucher | Locality            | GenBank accession no. (ITS) | GenBank accession no. (LSU) | GenBank accession no. (TEF1) | References                      |
|-------------------|---------|---------------------|-----------------------------|-----------------------------|-----------------------------|---------------------------------|
| *Hygrophorus deliciosus* | ZJ0002LS04 | China, Sichuan       | KU836534                    | —                           | —                           | Liu et al. unpublished         |
| *H. deliciosus*   | GDGM 79,208 | China, Yunnan       | MT363808                    | —                           | —                           | Wang and Li 2020                |
| *H. deliciosus*   | HKAS 54,703 | China, Yunnan       | MW290158*                   | MW290225*                   | —                           | This study                      |
| *H. deliciosus*   | HKAS 51,000 | China, Sichuan       | MW290159*                   | MW290226*                   | MW928549*                   | This study                      |
| *H. deliciosus*   | HKAS 76,246 | China, Sichuan       | MW290160*                   | MW290227*                   | MW928550*                   | This study                      |
| *H. deliciosus*   | HKAS 69,670 | China, Yunnan       | MW290161*                   | MW290228*                   | MW928551*                   | This study                      |
| *H. deliciosus*   | HKAS 54,510 | China, Yunnan       | MW290162*                   | MW290229*                   | —                           | This study                      |
| *H. deliciosus*   | MHKMU S.D. Yang 43 | China, Yunnan       | MW290163*                   | MW290230*                   | MW928552*                   | This study                      |
| *H. deliciosus*   | HKAS 55,053 | China, Yunnan       | MW290164*                   | —                           | —                           | This study                      |
| *H. deliciosus*   | HKAS 61,276 | China, Yunnan       | MW290165*                   | MW290231*                   | —                           | This study                      |
| *H. deliciosus*   | HKAS 61,315 | China, Yunnan       | MW290166*                   | MW290232*                   | —                           | This study                      |
| *H. deliciosus*   | HKAS 71,624 | China, Yunnan       | MW290167*                   | —                           | —                           | This study                      |
| *H. deliciosus*   | HMAS 253,198 | China, Tibet        | MW290168*                   | —                           | —                           | This study                      |
| *H. deliciosus*   | HMAS 253,233 | China, Tibet        | MW290169*                   | MW290233*                   | —                           | This study                      |
| *H. deliciosus*   | HMAS 253,249 | China, Tibet        | MW290170*                   | MW290234*                   | —                           | This study                      |
| *H. deliciosus*   | MHKMU H.Y. Huang 725 | China, Yunnan       | MW290171*                   | —                           | —                           | This study                      |
| *H. deliciosus*   | MHKMU H.Y. Huang 806 | China, Yunnan       | MW290172*                   | MW290235*                   | —                           | This study                      |
| *H. deliciosus*   | MHKMU H.Y. Huang 807 | China, Yunnan       | MW290173*                   | MW290236*                   | —                           | This study                      |
| *H. deliciosus*   | MHKMU H.Y. Huang 1006 | China, Yunnan       | MW290174*                   | MW290237*                   | MW928553*                   | This study                      |
| *H. deliciosus*   | MHKMU H.Y. Huang 1008 | China, Yunnan       | MW290175*                   | MW290238*                   | MW928554*                   | This study                      |
| *H. orientalis*   | HKAS 75,586 | China, Hubei        | MW290176*                   | MW290239*                   | MW928555*                   | This study                      |
| *H. orientalis*   | HKAS 63,417 | China, Jilin        | MW290177*                   | MW290240*                   | —                           | This study                      |
| *H. orientalis*   | HKAS 71,845 | China, Jilin        | MW290178*                   | MW290241*                   | —                           | This study                      |
| *H. orientalis*   | HKAS 71,851 | China, Shaanxi      | MW290179*                   | MW290242*                   | —                           | This study                      |
| *H. orientalis*   | HKAS 58,741 | China, Yunnan       | MW290180*                   | MW290243*                   | —                           | This study                      |
| *H. orientalis*   | MHKMU H.Y. Huang 472 | China, Jilin        | MW290181*                   | —                           | —                           | This study                      |
| *H. orientalis*   | CFSZ 20,884 | China, Inner Mongolia | MW290182*              | MW928625*                   | MW928556*                   | This study                      |
| *H. parvirussula* | HKAS 53,605 | China, Sichuan       | MH160768                    | MH160772                    | —                           | Huang et al. 2018               |
| *H. parvirussula* | MHKMU L.P. Tang 1691 | China, Yunnan       | MH160769                    | MH160773                    | —                           | Huang et al. 2018               |
| *H. parvirussula* | MHKMU N.K. Zeng 2878 | China, Yunnan       | MH160770                    | MH160774                    | —                           | Huang et al. 2018               |
| *H. parvirussula* | MHKMU S.D. Yang 434 | China, Yunnan       | MH160771                    | MH160775                    | —                           | Huang et al. 2018               |
| *H. parvirussula* | HKAS 56,191 | China, Yunnan       | MW290183*                   | —                           | —                           | This study                      |
| *H. parvirussula* | HKAS 57,447 | China, Yunnan       | MW290184*                   | —                           | —                           | This study                      |
| *H. parvirussula* | HKAS 58,821 | China, Yunnan       | MW290185*                   | —                           | —                           | This study                      |
| *H. parvirussula* | MHKMU S.D. Yang 12 | China, Yunnan       | MW290186*                   | MW290244*                   | —                           | This study                      |
| *H. parvirussula* | MHKMU W.H. Zhang 177 | China, Yunnan       | MW290187*                   | MW290245*                   | —                           | This study                      |
| Taxon                | Voucher         | Locality          | GenBank accession no. (ITS) | GenBank accession no. (LSU) | GenBank accession no. (TEF) | References                     |
|---------------------|-----------------|-------------------|-----------------------------|-----------------------------|-----------------------------|--------------------------------|
| *H. parvirussula*   | MHKMU W.H. Zhang 178 | China, Yunnan     | MW290188*                   | MW290246*                   | —                           | This study                     |
| *H. parvirussula*   | MHKMU W.H. Zhang 179 | China, Yunnan     | MW290189*                   | MW290247*                   | —                           | This study                     |
| *H. parvirussula*   | MHKMU T. Huang 220 | China, Yunnan     | MW290190*                   | MW290248*                   | MW928557*                   | This study                     |
| *H. parvirussula*   | MHKMU H.Y. Huang 501 | China, Yunnan     | MW290191*                   | MW290249*                   | —                           | This study                     |
| *H. parvirussula*   | MHKMU W.H. Zhang 183 | China, Yunnan     | MW290192*                   | MW290250*                   | —                           | This study                     |
| *H. parvirussula*   | MHKMU W.H. Zhang 195 | China, Yunnan     | MW290193*                   | MW290251*                   | —                           | This study                     |
| *H. parvirussula*   | MHKMU H.Y. Huang 534 | China, Yunnan     | —                           | MW290252*                   | —                           | This study                     |
| *H. parvirussula*   | MHKMU Y.J. Pu 171 | China, Yunnan     | MW290194*                   | MW290253*                   | —                           | This study                     |
| *H. parvirussula*   | MHKMU H.Y. Huang 911 | China, Yunnan     | MW290195*                   | MW290254*                   | MW928558*                   | This study                     |
| *H. parvirussula*   | MHKMU L.P. Tang 3420 | China, Yunnan     | MW290196*                   | MW290255*                   | MW928559*                   | This study                     |
| *H. parvirussula*   | MHKMU T. Huang 455 | China, Yunnan     | MW290197*                   | —                           | —                           | This study                     |
| *H. parvirussula*   | MHKMU T. Huang 476 | China, Yunnan     | —                           | —                           | —                           | This study                     |
| *H. parvirussula*   | MHKMU T. Huang 491 | China, Yunnan     | MW290198*                   | —                           | —                           | This study                     |
| *H. parvirussula*   | MHKMU M. Mu 770  | China, Yunnan     | MW290199*                   | —                           | —                           | This study                     |
| *H. parvirussula*   | MHKMU Y.J. Pu 376 | China, Yunnan     | MW290200*                   | MW290256*                   | —                           | This study                     |
| *H. parvirussula*   | MHKMU Y.J. Pu 399 | China, Yunnan     | MW290201*                   | MW290257*                   | —                           | This study                     |
| *H. parvirussula*   | MHKMU W.H. Zhang 535 | China, Yunnan     | MW290202*                   | MW290258*                   | MW928560*                   | This study                     |
| *H. parvirussula*   | MHKMU T. Huang 516 | China, Yunnan     | MW290203*                   | —                           | —                           | This study                     |
| *H. parvirussula*   | MHKMU H.Y. Huang 1007 | China, Yunnan     | MW290204*                   | MW290259*                   | —                           | This study                     |
| *H. penarioides*    | SJ94067         | Sweden, Gotlands  | EF395370                    | —                           | —                           | Jacobsson and Larsson 2007    |
| *H. penarioides*    | G0490           | Hungary, —        | —                           | MK278196                    | —                           | —                              |
| *H. pudorinus*      | PBM2721         | USA, Colorado     | —                           | —                           | GU187710                     | —                              |
| *H. qinggangjun*    | Zhao 447        | China, Yunnan     | MW290147*                   | MW290218*                   | —                           | —                              |
| *H. qinggangjun*    | HKAS 68,397     | China, Yunnan     | MW290148*                   | MW290219*                   | MW928544*                   | —                              |
| *H. qinggangjun*    | MHKMU S.D. Yang 20 | China, Yunnan     | MW290149*                   | MW290220*                   | MW928545*                   | —                              |
| *H. qinggangjun*    | MHKMU L.P. Tang 1683 | China, Yunnan     | MW290150*                   | MW290221*                   | —                           | This study                     |
| *H. qinggangjun*    | HKAS 55,496     | China, Yunnan     | MW290151*                   | —                           | —                           | This study                     |
| *H. qinggangjun*    | HKAS 60,481     | China, Yunnan     | MW290152*                   | —                           | —                           | This study                     |
| *H. qinggangjun*    | HKAS 60,530     | China, Yunnan     | MW290153*                   | —                           | —                           | This study                     |
| *H. qinggangjun*    | HKAS 72,665     | China, Yunnan     | MW290154*                   | —                           | —                           | This study                     |
| *H. qinggangjun*    | MHKMU M. Mu 464 | China, Yunnan     | MW290155*                   | MW290222*                   | MW928546*                   | This study                     |
| *H. qinggangjun*    | MHKMU M. Mu 436 | China, Yunnan     | MW290156*                   | MW290223*                   | MW928547*                   | This study                     |
| Taxon          | Voucher     | Locality                        | GenBank accession no. (ITS) | GenBank accession no. (LSU) | GenBank accession no. (TEF1) | References                        |
|---------------|-------------|---------------------------------|-----------------------------|-----------------------------|--------------------------------|-----------------------------------|
| *H. qinggangjun* | MHKMU H.Y. Huang 724 | China, Yunnan                  | MW290157*                   | MW290224*                   | MW928548*                      | This study                        |
| *H. russula*   | AH 19,677   | Spain, Torrelodones             | MW290205*                   | MW290260*                   | —                              | This study                        |
| *H. russula*   | AH 37,145   | Spain, Javierregay              | MW290206*                   | MW290261*                   | —                              | This study                        |
| *H. russula*   | LAS 85,196  | Sweden, —                       | EF395376                    | —                           | —                              | Jacobson and Larsson 2007         |
| *H. russula*   | VAC 8b-09   | France, —                       | JF506764                    | —                           | —                              | Shahin unpublished                |
| *H. russula*   | 11,936      | Italy, —                        | JF908065                    | —                           | —                              | Osmundson et al. 2013             |
| *H. russula*   | 992         | Italy, —                        | JF908077                    | —                           | —                              | Osmundson et al. 2013             |
| *H. russula*   | HE 2787     | —                               | KC505575                    | —                           | —                              | Sun et al. unpublished            |
| *H. russula*   | CFMR JP-3   | Japan, Shiga                    | KF291216                    | KF291217                    | —                              | Lodge et al. 2014                 |
| *H. russula*   | CLO-4280    | Belize, Mountain Pine Ridge     | KF381523                    | —                           | —                              | Lodge et al. 2014                 |
| *H. russula*   | GO-2009–116 | Mexico, Temascaltepec           | KT875017                    | —                           | —                              | Garibay et al. unpublished        |
| *H. russula*   | 4433        | Canada, Quebec                  | KM248883                    | —                           | —                              | Berube et al. unpublished         |
| *H. russula*   | NIFoS 1987  | South Korea, —                  | KX814449                    | —                           | —                              | Wang et al. 2016                  |
| *H. russula*   | NIFoS 2003  | South Korea, —                  | KX814450                    | —                           | —                              | Wang et al. 2016                  |
| *H. russula*   | MB344       | USA, Arkansas                   | KX358034                    | —                           | —                              | Stephenson et al. 2017            |
| *H. russula*   | EMB 141/86  | Italy, —                        | MF399419                    | —                           | —                              | Peintner et al. unpublished       |
| *H. russula*   | EMB 710/90  | Italy, —                        | MF399423                    | —                           | —                              | Peintner et al. unpublished       |
| *H. russula*   | IB19630793  | Switzerland, Lucerne            | MF399427                    | —                           | —                              | Peintner et al. unpublished       |
| *H. russula*   | IB19710232  | Switzerland, Bern               | MF399432                    | —                           | —                              | Peintner et al. unpublished       |
| *H. russula*   | IB19730073  | Switzerland, Bern               | MF399433                    | —                           | —                              | Peintner et al. unpublished       |
| *H. russula*   | IB19740604  | France, Provence                | MF399434                    | —                           | —                              | Peintner et al. unpublished       |
| *H. russula*   | 420,526MF0429 | China, —                  | MG712352                    | —                           | —                              | Wang et al. unpublished            |
| *H. russula*   | iNaturalist 31,828,832 | USA, Arizona | MN498103 | — | — | Clements and Tighe unpublished |
| *H. cf. russula* | JLF 7925   | USA, Arizona                    | MT101865                    | —                           | —                              | Frank unpublished                 |
| *H. russula*   | IB 19,740,604 | France, —                  | MT158400                    | —                           | —                              | Papetti et al. unpublished        |
| *H. russula*   | EMB 141 86  | Italy, —                        | MT158401                    | —                           | —                              | Papetti et al. unpublished        |
| *H. russula*   | EMB 710 90  | Italy, —                        | MT158402                    | —                           | —                              | Papetti et al. unpublished        |
| *H. russula*   | IB19630793  | Switzerland, —                 | MT158403                    | —                           | —                              | Papetti et al. unpublished        |
| *H. russula*   | GDGM25,922  | China, Jilin                     | MT363801                    | —                           | —                              | Wang and Li 2020                  |
| *H. russula*   | GDGM42,113  | China, Jilin                     | MT363802                    | —                           | —                              | Wang and Li 2020                  |
| *H. russula*   | GDGM41,951  | China, Jilin                     | MT363803                    | —                           | —                              | Wang and Li 2020                  |
| *H. russula*   | CFSZ18,156  | China, Inner Mongolia           | MT363804                    | —                           | —                              | Wang and Li 2020                  |
| *H. russula*   | CFSZ19,893  | China, Inner Mongolia           | MT363805                    | —                           | —                              | Wang and Li 2020                  |
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.

### Results

#### Phylogenetic analysis

One hundred fifty-six ITS, LSU, and TEF1 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, 874 characters for the LSU...
dataset, 592 characters for the TEF1 dataset, and 1981 for the concatenated dataset. Sequences of the H. russula complex were selected as the in-group, H. sordidus Peck and H. penaroides Jacobsson & E. Larss. in subsection Clitocyboides served as outgroups for ITS and LSU dataset based on the previous study (Huang et al. 2018); Hygrophorus pudorinus (Fr.) Fr. in subsection Pudorini served as outgroups for the TEF1 dataset. The alignment is available at TreeBASE (Accession 27,296). 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. The phylogenetic analysis recovered 13 supported clades from Asia, Europe, North America, and South America, corresponding to unique lineages within the sequence data of H. russula (Figs. 1, 2, and 3, Appendix 1–2). European “H. russula” formed two 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 tree (Fig. 1). The Chinese collections respectively formed five monophyletic clades with strong support, corresponding to five taxa, three new species (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 species have close relationships. For example, H. deliciosus exhibited a sister relationship with H. qinggangjun with strong support in the ITS and concatenated trees, while appeared to be sister to H. yunnanensis in the LSU tree. Hygrophorus parvirussula and H. russula were sister species in the ITS and LSU, while appeared to be sister to H. yunnanensis in the TEF1 tree. 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 research (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, while the pileus turning pinkish in C₆H₅OH.

Taxonomy

Hygrophorus deliciosus C.Q. Wang & T.H. Li, Phytotaxa 449: 232–242, 2020 (Figs. 4a–c, 5a, 6a–b, 7a, 8a).

The following description is mainly taken from Wang and Li (2020), combined with our field notes including macro-morphology, 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, 1.2–2.2 cm diam., central, cylindrical, equal, white, yellowish-white, pinkish (10A2), with red (10A7) to brownish-red (10D7) scales; flesh often pinkish (10A2); basal mycelium (bundles of hyphae attached to the base of stipe) white. Odour not distinctive. Taste mild.

Basidiospores [80/6/16] 6.44–8 (−9) × 4.6–6 μm, Q = 1.25–1.6, Qm = 1.42 ± 0.12, broad ellipsoid to ellipsoidoid, smooth under a light microscope and SEM, thin-walled, hyaline, non-amyloid, with a distinct hilar appendix. 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 diam., long ellipsoid, clavate or irregularly shaped, colourless and hyaline. Pileipellis an ixorichoderm, 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. Stipititrama made up of hyphae 6–18 (~30) μm diam., arranged in parallel, thin-walled. Mycelial hyphae 2–4 μm diam., 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).

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 51,000); Muli Prefecture, Liziping Town, elev. 3400–3700 m, 1 August 2012, T. Guo 554 (HKAS 76,246). Tibet Autonomous Region: Linzhi Prefecture, Lulang Town, elev. 3325 m, 11 August 2013, T.Z. Wei 3592 (HAMAS 253,198); Milin Prefecture, elev. 2990 m, 12 August 2013, T.Z. Wei & T.Z. Li & X.Y. Liu & J.Y. Zhuang 3740 (MHKMU H.Y. Huang 806). 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 61,315); Lijiang 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 61,276); 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 54,510); Laping Prefecture, elev. 2700 m, 14 August 2011, Y.J. Hao 515 (HKAS 71,624); Lijiang City, Gucheng Area, elev. 2600 m, 18 August 2010, Q. Zhao 949 (HKAS 69,670); 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 55,053); Yulong Prefecture, Yulong Snow Mountains, elev. 2940 m, 24 July 2008, L.P. Tang 472 (HKAS 54,703).

Comments: *Hygrophorus deliciosus* is quite common in SW China as a wild commercial fungus known from the local name “Qinggangjun” or “Mitangjun” (Fig. 1). “Mitang” is a kind of soup made from rice. “Mitangjun” means that this mushroom soup is thick and looks like “Mitang”. It is distinguished by its medium-sized basidiomata, thick context (up to 2.4 cm), broad ellipsoid basidiospores, probably associated with *Quercus aquifolioides*, and scattered to gregarious at varied elevations from 1900 to 3700 m.

This species is likely to be confused with *Hygrophorus qinggangjun*, both are sold as edible mushrooms in Yunnan Province markets and have 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 wavy and uplifted margin, thin context (0.4–0.9 cm), broad basidiole (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*, and with narrow distribution (Yunnan Province) and elevation (2000–2780 m).

According to our phylogenetic analyses, *H. deliciosus* exhibits a sister relationship with *H. qinggangjun* with strong support in the ITS and concatenated trees (Figs. 1 and 2), while appears to be sister to *H. yunnanensis* in the LSU tree (Appendix 1). Differently, *H. yunnanensis* has small basidiomata (pileus 4–8 cm), thin context (0.3–0.7 cm), narrow basidiospores (5.5–7.5 × 4–5 μm), probably associated with *Lyonia ovalifolia* and *Ternstroemia gymnanthera*, is so far restricted to Yunnan Province and prefers to grow alone or sparsely scattered in low elevations (2100–2600 m).

### Hygrophorus orientalis

H.Y. Huang & L.P. Tang, sp. nov. (Figs. 4d–f, 5b, 6c–d, 7b, 8b). MycoBank: MB 838040.

**Diagnosis:** *Hygrophorus orientalis* is different from other species of the *H. russula* complex in having large basidiomata (pileus > 15 cm diam.), dense lamellae (> 100 pieces of complete lamellae/cap), small basidiospores (5.5–7.5 × 3.5–4.8 μm), and extensive distribution.

**Etymology:** Latin “orientalis” means the species widely distributing in East Asia.

**Holotype:** CHINA. Hubei Province: Shennongjia Forestry District, Miyu Town, 31° 40.83′ N, 110° 26.45′ E, elev. 1900 m, 18 July 2012, Q. Cai 852 (HKAS 75,586), ITS GenBank Accession No. MW290176.

**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 white to yellowish (1A2), rather fragile, concolorous (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 (bundles of hyphae attached to the base of stipe) 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, Qm = 1.57 ± 0.15, ellipsoid, smooth under a light microscope and SEM, thin-walled.
hyaline, non-amyloid, with a distinct hilar appendix. 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, 

Fig. 2 Phylogenetic tree of *Hygrophorus russula* complex, based on the concatenated dataset of ITS, LSU and *TEF1*. RAxML BP values (≥ 70%) and Bayesian posterior probabilities (≥ 0.90) are shown above branches.

Fig. 3 Distribution of *Hygrophorus russula* complex.
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 diam., 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. Stipititrama made up of hyphae 6–20 μm diam., arranged in parallel, thin-walled. Mycelial hyphae 2–5 μm diam., 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 KX814449–814,450).
Fig. 5 Basidiospores of *Hygrophorus russula* complex under a light microscope. a *H.* deliciosus (HKAS 61,315). 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 19,677). f *H.* yunnanensis (MHKMU S.D. Yang 12). Bars = 10 μm

Fig. 6 Basidia and cystidia of *Hygrophorus russula* complex. a, b *H.* deliciosus. c, d *H.* orientalis. e, f *H.* qinggangjun. g, h *H.* yunnanensis. i–l *H.* russula. a, c, e, g, i, j basidia and cheilocystidia. b, d, f, h, k, l basidia and pleurocystidia. Bars = 10 μm
**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 Fagaceae, elev. 750–2340 m; in summer (July–September).

- **a** *H. deliciosus* (HKAS 54,510).
- **b** *H. orientalis* (HKAS 71,845).
- **c** *H. qinggangjun* (MHKMU L.P. Tang 1683).
- **d** *H. yunnanensis* (MHKMU H.Y. Huang 322).

Bars = 10 μm

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Fig. 7 Hymenium (including basidia, pleurocystidia, and lamellar trama) of *Hygrophorus russula* complex. **a** *H. deliciosus* (HKAS 54,510). **b** *H. orientalis* (HKAS 71,845). **c** *H. qinggangjun* (MHKMU L.P. Tang 1683). **d** *H. yunnanensis* (MHKMU H.Y. Huang 322). Bars = 10 μm.
1280 m, 4 September 2010, X.F. Shi 659 (HKAS 71,851). Yunnan Province: Yongping Prefecture, Longmen Town, elev. 2340 m, 1 August 2009, Q. Cai 74 (HKAS 58,741).

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) 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.

*Hygrophorus qinggangjun* H.Y. Huang & L.P. Tang, sp. nov. (Figs. 4g–i, 5d, 6e–f, 7c, 8c).

MycoBank: MB 838039.

Diagnosis: *Hygrophorus qinggangjun* is different from other species of the *H. russula* complex in its medium-sized basidiomata (pileus 8–14 cm diam.), uplifted and wavy margin, thinner context, variable basidiospores (broad ellipsoid, ellipsoid and oblong), and so far, only 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), ITS GenBank Accession No. MW290150.

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), 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 (bundles of hyphae attached to the base of stipe) 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), Qm = 1.46 ± 0.14, broad ellipsoid, ellipsoid, oblong, smooth under a light microscope and SEM, thin-walled, hyaline, non-amyloid, with a distinct hilar appendix. *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 diam., 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. Stipititrama made up of hyphae 6–18 μm diam., arranged in parallel, thin-walled. Mycelial hyphae 2–5 μm diam., 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, Shibo 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 fewPinus 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 2019, X.H. Du 39 (HKAS 55,496); Lijiang City, Qihe Town, elev. 2780 m, 19 August 2010, X.T. Zhu 221 (HKAS 68,397); Weixi Prefecture, Qizong Town, elev. unknown, 19 September 2010, X.H. Wang 44 (HKAS 60,530); Weixi Prefecture, Tacheng Town, elev. unknown, 20 September 2010, X.H. Wang 127 (HKAS 60,481); the same location, elev. unknown, 15 October 2011, X.H. Wang 127 (HKAS 72,665); 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*. 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), varied basidiospores (broad ellipsoid, ellipsoid and oblong), solitary or scattered in mixed forests, and probably in association with *Quercus serrata*. Currently, *H. qinggangjun* is known only from Yunnan Province, occurring at high elevation regions (2000–2780 m).

The morphological and phylogenetic analyses (ITS and concatenated trees) 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*.

*Hygrohirus russula* (Schaeff. ex Fr.) Kauffman, Publications Michigan Geology Biology Survey, Biology Series 5 26: 185, 1918 (Figs. 5e and 6i–l).

The following description is mainly taken from Arnolds (1990) and Candusso (1997), combined with the field notes including macro-morphology, 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 large concolorous spots elsewhere, surface dry to slightly 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 × 5–6.5 μm, Q = 1.33–1.72, Qm = 1.55 ± 0.12, broad ellipsoid to ellipsoid, smooth under a light microscope, thin-walled, hyaline, non-amyloid, with a distinct hilar appendix. *Basidium* 55–68 (~80) × 7–9 (~10) μm, 4-spored, clavate, slender, thin-walled; sterigma 4–7 μm in length. *Cheilocystidia* 35–50 × 3–5 μm, rare, scattered, irregularly shaped, narrowly clavate, flexuous, thin-walled, colourless. *Pleurocystidia* 40–55 × 3–9 μm, scattered, irregularly shaped, narrowly clavate, flexuous, thin-walled, hyaline. Lamellar trama divergent, composed of short elements and long hyphae, short elements 15–26 × 3–6 μm, next to hymenium, cylindrical, long ellipsoid, pear-shaped or irregularly shaped, colourless and hyaline; long hyphae 3–10 (~25) μm diam., up to 70 μm long, long ellipsoid, clavate or irregularly shaped, colourless and hyaline. *Pileipellis* an ixotrichoderm, consisting of narrow hyphae 2–4 μm diam. in gluten, loosely interwoven, branched or non-branched, thin-walled. *Pileal trama* made up of hyphae 4–25 μm diam., thin-walled. Stipitipellis a cutis, composed of hyphae 2.5–4.5 μm 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., elev. below 1100 m; in autumn to winter (September–December).

**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 37,145); 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 19,676); 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 19,677).

**Comments:** *Hygrophorus russula* was originally described from Germany as *Agaricus russula* Schaeff. in 1774 (Schaeffer 1774; Fries 1821), but with few macrospores (4–5 μm wide), so far, and only known from Yunnan.

**Materials examined:** 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), ITS GenBank Accession No. MW290214.

**Description:** Basidiomata solitary to sparsely scattered on soil, small to medium-sized, firm, fleshy. *Pileus* 4–8 cm diam., convex to hemispherical with an inrolled 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, 0.3–0.5 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 or with age; flesh initially firm, becoming soft with age, whitish to pinkish-white (10A2), becoming darker (10A3) when cut or damaged; basal mycelium (bundles of hyphae attached to the base of stipe) white. *Odour* not distinctive. *Taste* mild.

**Basidiospores** [80/3/3] (7–) 7.5–9.5 × 4–5 μm, Q = 1.6–2.13, Qm = 1.86 ± 0.14, ellipsoid to oblong, ovoid, smooth under a light microscope and SEM, thin-walled, hyaline, non-amyloid, with a distinct hilar appendix. *Basidia* 38–45 (–50) × 5–10 μm, mostly 4-spored, clavate, slender, thin-walled; sterigmata 4–9 μm in length. *Cheilocystidia* 30–40 × 3–6 μm, rare, scattered, subfusciform, narrowly clavate, spathulate, subcylindrical, flexuous, thin-walled, colourless. *Pleurocystidia* 43–50 × 3–7 μm, rare, scattered, subfusciform, narrowly clavate, spathulate, flexuous, thin-walled, hyaline. *Lamellar trama* divergent, composed of short elements and long hyphae, short elements 5–12 × 3–6 μm, next to hymenium, cylindrical, long ellipsoid, pear-shaped or irregularly shaped, colourless and hyaline; long hyphae 6–18 μm diam., 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. *Stipititrama* made up of hyphae 5–18 μm diam., arranged in parallel, thin-walled. *Pleural hyphae* 2–5 μm diam., smooth under a light microscope, occasionally covered with a massive nipple-shaped

**Hygrophorus yunnanensis** H.Y. Huang & L.P. Tang, sp. nov. (Figs. 4j–l, 5f, 6g–h, 7d, 8d).

**MycoBank:** MB 838041.

**Diagnosis:** *Hygrophorus yunnanensis* is different from other species of the *H. russula* complex by its small basidiomata (*pileus* 4–8 cm diam.), thin context (≤0.7 cm), narrow basidiospores (4–5 μm wide), so far, and 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), ITS GenBank Accession No. MW290214.

**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 or with age; flesh initially firm, becoming soft with age, whitish to pinkish-white (10A2), becoming darker (10A3) when cut or damaged; basal mycelium (bundles of hyphae attached to the base of stipe) white. *Odour* not distinctive. *Taste* mild.

**Basidiospores** [80/3/3] (7–) 7.5–9.5 × 4–5 μm, Q = 1.6–2.13, Qm = 1.86 ± 0.14, ellipsoid to oblong, ovoid, smooth under a light microscope and SEM, thin-walled, hyaline, non-amyloid, with a distinct hilar appendix. *Basidia* 38–45 (–50) × 5–10 μm, mostly 4-spored, clavate, slender, thin-walled; sterigmata 4–9 μm in length. *Cheilocystidia* 30–40 × 3–6 μm, rare, scattered, subfusciform, narrowly clavate, spathulate, subcylindrical, flexuous, thin-walled, colourless. *Pleurocystidia* 43–50 × 3–7 μm, rare, scattered, subfusciform, narrowly clavate, spathulate, flexuous, thin-walled, hyaline. *Lamellar trama* divergent, composed of short elements and long hyphae, short elements 5–12 × 3–6 μm, next to hymenium, cylindrical, long ellipsoid, pear-shaped or irregularly shaped, colourless and hyaline; long hyphae 6–18 μm diam., 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. *Stipititrama* made up of hyphae 5–18 μm diam., arranged in parallel, thin-walled. *Pleural hyphae* 2–5 μm diam., smooth under a light microscope, 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, Miaoao Temple, elev. 2100 m, 6 August 2006, Y.C. Li 688 (HKAS 50,442); Jianchuan Prefecture, Shibao Mountain, 26° 23.73′N, 99° 50.41′E, elev. 2500 m, 17 August 2014, S.D. Yang J2 (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. yunnanensis* 970 (MHKMU H.Y. Huang 970), *H. 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. Huang 313* (MHKMU H.Y. Huang 313), *H. Huang 316* (MHKMU H.Y. Huang 316), *H. 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. Huang 330* (MHKMU H.Y. Huang 330), *H. Huang 334* (MHKMU H.Y. Huang 334), *H. Huang 335* (MHKMU H.Y. Huang 335), *H. Huang 336* (MHKMU H.Y. Huang 336), *T. Huang 59* (MHKMU T. Huang 59), *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 56,982); Yunlong Prefecture, Nuding Village, elev. unknown, 26 August 2011, *R. Wang 95* (HKAS 72,912).

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

The morphological and phylogenetic analyses (TEF1 tree), *H. yunnanensis* is quite similar to *H. parvirussula*. However, *H. parvirussula* has wide basidiospores (6.9–9.0×4.6–6.9 μm, Q = 1.13–1.83, Q_m = 1.48 ± 0.23), occurring at high elevations (2500–3300 m), and probably associated with *Pieris formosa* and *Rhododendron concinnum*.

The LSU phylogenetic analyses showed that *H. yunnanensis* was sister to *H. deliciosus*. The comparison between *H. deliciosus* and *H. yunnanensis* sees 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 European distribution, at high elevations >500 m; context changed on exposure; basidiospores >3.2 μm in width.................................................................2

2 East Asian distribution; pileus >15 cm diam.; basidiospores small, 5.5–7×3.5–4.8 μm............................ *H. orientalis* 2* Narrow distribution; pileus <15 cm diam.; basidiospores slightly large..............................3

3 European distribution, at low elevations <1500 m; basidiospores large, 8–10×5–6.5 μm............................ *H. russula* 3* Narrow distribution; pileus <15 cm diam.; basidiospores slightly large..............................4

4 Pileus <10 cm diam.; associated with Fagaceae........................................................................5

4* Pileus <10 cm diam.; probably 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, 6.44–8×4.6–6 μm, Q_m = 1.42 ± 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, 6.44–9×4.4–6 μm, Q_m = 1.46 ± 0.14.............................................................................. *H. qinggangjun* 6 Occurring at elevations 2500–3300 m; basidiospores broad ellipsoid to ellipsoid, surface basillate ornamentation, 6.9–9.0×4.6–6.6 μm, Q_m = 1.48 ± 0.23.................................................. *H. parvirussula* 6* Occurring at elevations 2100–2600 m; basidiospores narrow ellipsoid, surface smooth, 7.5–9.5×4.5 μm, Q_m = 1.86 ± 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
Table 2 Comparison of the diagnostic characteristics between Hygrophorus russula complex

| Species          | Pileus (cm) | Lamellae | Spores (μm) | Basidia (μm) | Sapor Qm | Hosts | Comments |
|------------------|-------------|----------|-------------|--------------|----------|-------|----------|
| H. deliciosus    | 5–14        | 60–75    | 6.5–12      | 45–55        | 1.42     | Quercus spp. | Medium-sized with pileus 5–15 cm diam. |
| H. qinggangjun   | 9–18        | 60–90    | 5.5–7      | 1.25–1.6     | 1.46     | Quercus sp. | Broadly similar to H. deliciosus |
| H. parvirussula  | 6–8         | 60–80    | 6.5–9      | 1.48–1.6    | 1.57     | Cyclobalanopsis | Difference in basidiospores and basidia |
| H. orientalis    | 6–8         | 60–90    | 6.5–9      | 1.48–1.6    | 1.57     | Quercus mongolica | Medium-sized with pileus 5–15 cm diam. |
| H. yunnanensis   | 5–14        | 60–80    | 6.5–9      | 1.48–1.6    | 1.57     | Quercus sp. | Broadly similar to H. deliciosus |
| H. yunnanensis   | 5–14        | 60–80    | 6.5–9      | 1.48–1.6    | 1.57     | Quercus sp. | Broadly similar to H. deliciosus |

Considered a species with a wide distribution in the Northern Hemisphere (Hong 1982; Arnolds 1990; Hesler and Smith 1963; Chen and Li 2013) and South Hemisphere according to the data from GenBank. With the application of molecular methods, seven molecularly distinct species have been revealed under this name, and 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.

Morphologically, H. russula complex has similar colours (pale redish-purple, dark red, reddish-brown, to brownish-vinaceous), which are hard to identify in the field. Still, they are divergent in geographical distribution, host preferences, 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 8–10×2–3.2 μm (Hesler and Smith 1963). Hygrophorus parvirussula and H. yunnanensis were both found in south-western China; the context became darker on exposure, and probably associated with Ericaceae.

Regarding H. deliciosus, H. qinggangjun, and H. russula, these species are medium-sized with pileus 5–15 cm diam. However, H. russula has narrow lamellae (up to 0.5 cm wide), slightly long basidia 55–68×7–9 μm, large basidiospores (8–10×5–6.5 μm), and occurring at low elevations (<1100 m) (Arnolds 1990; Candusso 1997). Both H. deliciosus and H. qinggangjun have shorter basidia (45–55×4–7 μm; 43–49×6–9 μm), and broader basidiospores (6.44–8×4.6–6 μm, Q = 1.25–1.6, Qm = 1.42 ± 0.12; 6.44–9×4.4–6 μm, Q = 1.24–1.64, Qm = 1.46 ± 0.14), and fruiting at high elevations (>2500 m).

Hygrophorus orientalis differs from other species in the H. russula complex by its 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 (30–40×4–7 μm), smaller basidiospores (5.5–7×3.5–4.8 μm); scattered to gregarious, at times in arcs or fairy rings, probably associated with Cyclobalanopsis sp. or Quercus mongolica, wide range of elevation (750–2340 m), and extensive distribution.

Ecological traits of species in the Hygrophorus russula complex

Our data indicate that ecological traits, including its host preference, range of elevation, and distribution, are useful for separating morphologically similar species in the H. russula complex. European H. russula was probably associated with Quercus spp., likely Q. ilex in the south and Q. robur in the northern part (Arnolds 1990; Candusso 1997).
Hygrophorus russuliformis from North America occurs with 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 has a wide distribution, but in low-elevation environments. Hygrophorus qinggangjun and H. yunnanensis seem to be restricted to high elevations between 2000 and 3000 m. Hygrophorus 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 might be associated with Lyonia ovalifolia and Ternstroemia gymnantha, which grow in forests below 2800 m, while H. deliciosus is connected to Quercus aquifolioides and H. parvirussula is likely associated with Pieris formosa and Rhododendron concinnum, both their host plants distributing at high elevations above 3000 m.

The taxonomic importance of comprehensive data in the Hygrophorus russula complex

Due to similar characteristics, it is quite difficult to identify the species complex. Thus, the application of comprehensive evidence is important for the accurate identification of this species complex. In our study, SEM characteristics 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. In fungal taxonomy, the application of characteristics of basal hyphae is rarely seen. Huang et al. (2020) firstly reported abundant morphological characters of the basal hyphae of seven species from Clavariadelphus as taxonomical evidence. In the H. russula complex, the basal hyphae also vary from smooth to a massive nipple-shaped protuberance under SEM (see Fig. 8). It indicates the basal hyphae are valuable in the classification of Hygrophorus. In terms of chemical reactions, we found that phenol can be used to distinguish the H. russula complex and H. lucorum Kalchbr. within Hygrophorus, while most species in Hygrophorus sect. Aurei (Bataille) E. Larss. yielded a negative reaction (unpublished data).

Phylogenetic tree of Hygrophorus russula complex based on TEF1 sequence data. RAXML BP values (≥ 70%) and Bayesian posterior probabilities (≥ 0.90) are shown above branches (JPG 2.55 MB)Supplementary File 3 (NXS 103 KB)Supplementary File 4 (NXS 102 KB)Supplementary File 5 (NXS 21.3 KB)Supplementary File 6 (NXS 64.0 KB)Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11557-021-01715-7.

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Author contribution All authors contributed to the study conception and design. Material preparation was performed by Hong-Yan Huang, Li-Ping Tang, Moreno Gabriel, Tie-Zhi Liu, Ting Huang, and Wen-Hao Zhang. DNA isolation, preparation for sequencing, and data analyses were performed by Hong-Yan Huang. The draft of the manuscript was written by Hong-Yan Huang, polished by Li-Ping Tang, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability The datasets generated and downloaded during the current study are available in the GenBank, http://www.ncbi.nlm.nih.gov.

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