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Research article

Two new species of *Russula* subgenus *Heterophyllidia* (Russulaceae, Russulales) from Yanshan Mountains, North China

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Abstract. Two new species, *Russula pseudopunicea* C.L.Hou, G.Q.Cheng & H.Zhou sp. nov. and *R. wulingshanensis* C.L.Hou, G.Q.Cheng & H.Zhou sp. nov., from Yanshan mountains in North China are described herein based on morphological and phylogenetic analyses of nrITS, and nrLSU-*rpb2*-mtSSU gene regions. Morphologically, *R. pseudopunicea* sp. nov. is characterised by a reddish brown, light brown to brownish orange pileus with a greyish yellow margin, subglobose to broadly ellipsoid basidiospores with warts forming a partial reticulum and pleurocystidia turning grey to purplish red in sulfovanillin. *Russula wulingshanensis* sp. nov. is characterised by a purple pinkish pileus with a grey-white to grey-purple margin, subglobose to broadly ellipsoid basidiospores with isolated warts, and pileocystidia turning black in sulfovanillin. Phylogenetic and morphological analyses resolved the two species in *Russula* subg. *Heterophyllidia*. *Russula pseudopunicea* sp. nov. and *R. wulingshanensis* sp. nov. were placed in the lineages of subsect. *Virescentinae* and subsect. *Griseinae*, respectively.

Keywords. Basidiomycota, phylogenetic analysis, new taxon, taxonomy.

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Introduction

Russula Pers., the largest genus of Russulaceae Lotsy, was established by Persoon (1796), and its species are widely distributed globally (Looney *et al.* 2016). Through multi-locus phylogenetic analyses and morphological evidence, Buyck *et al.* (2018, 2020) recently resolved species of *Russula* into eight subgenera. Among them, *Russula* subgenus *Heterophyllidia* Romagn. is characterised by a pileus of variable colours, ranging from white to purple, most species having a mild taste, white- to cream-coloured (rarely ochre) spore prints, and pileipellis lacking primordial hyphae (Romagnesi 1987). China is rich in *Russula* species (Song *et al.* 2007; Li *et al.* 2013; Li 2014; Wang 2020). In the last 10 years, 59 new species

of *Russula* have been described worldwide. Of them, 31 species belong to the subgenus *Heterophyllidia* (Li *et al.* 2013, 2018, 2020, 2021a, 2021b; Hyde *et al.* 2019; Deng *et al.* 2020; Song *et al.* 2020, 2021, 2022; Zhou *et al.* 2020; Chen *et al.* 2021a, 2021b, 2021c, 2022).

The Yanshan Mountains (115°–119°47' E, 39°40'–41°20' N), located in North China, have a warm temperate continental monsoon climate. This region is known for its high plant diversity, with the main vegetation types being deciduous broad-leaved forest and mixed coniferous and broad-leaved forest. Dominant ectomycorrhizal trees in this region include *Quercus mongolica* Fisch. ex Ledeb., *Betula platyphylla* Sukaczew., *Abies nephrolepis* (Trautv.) Maxim., *Populus tomentosa* Carrière and *Pinus tabuliformis* Carrière (Wang *et al.* 2021). This region has an annual precipitation of approximately 350–700 mm, and the altitude ranges from 200 to 2200 m (Zhou *et al.* 2022a). Information about *Russula* from Yanshan Mountains is as yet limited (Lu *et al.* 2015; Wu *et al.* 2017; Yang *et al.* 2021; Zhou *et al.* 2022b).

In this study, we describe two new species of the *Russula* subgenus *Heterophyllidia* from Yanshan Mountains based on morphological and molecular phylogenetic analyses. Detailed macro- and micromorphological descriptions and illustrations are provided. A phylogenetic tree based on nrITS and nrLSU-*rpb2*-mtSSU datasets was also constructed.

Materials and methods

Sampling and morphological analyses

Specimens were collected during 2019 and 2021. The collected fresh specimens were photographed in the field, and their characters such as colour, odour, and viscosity were noted. Colour codes were from the reference website colorhexa (<https://www.colorhexa.com>). Specimens were dried using a Dorrex dryer at 50°C, and deposited in the Herbarium of the College of Life Science, Capital Normal University, Beijing, China (BJTC).

Thin sections of the dried material mounted in 3% KOH or sterilized water were used to observe microscopic characters. Congo red (1%) was used to improve the visibility of structures. Melzer's reagent was used to test the amyloid reaction of basidiospores. Using Cresyl blue, all tissues were examined to verify the presence of ortho- or metachromatic reactions as explained in Buyck (1989). Cystidia contents were examined in a sulfovanillin (SV) solution (Caboň *et al.* 2017). Microscopic structures (e.g., basidiospores, basidia, and cystidia) were observed and measured under a light microscope (Olympus DP71, Tokyo, Japan), and basidiospore ornamentations were observed under a field emission scanning electron microscope (SEM, Hitachi S-4800, Tokyo, Japan). Basidiospore measurements are presented as (Min–)AV–SD–AV–AV+SD(–Max). Min, Max, AV, and SD represent the minimum value, maximum value, average value, and standard deviation, respectively, and Q represents the length/width ratio of the basidiospores (Song *et al.* 2022).

DNA extraction and sequencing

DNA was extracted using the M5 Plant Genomic DNA Kit (Mei5 Biotechnology, Co., Ltd, China). PCR amplifications were performed in a Bio-Rad S1000 TM Thermal Cycler (Bio-Rad Laboratories, Inc, USA). Primers nrITS1/nrITS4 (White *et al.* 1990) were used to amplify the rDNA ITS region, primers sets LR5/LR0R (Vilgalys & Hester 1990) to amplify the large subunit nuclear ribosomal DNA (nrLSU rDNA) region, primers sets *rpb2*-6f/*rpb2*-7r (Matheny 2005) to amplify the second largest subunit of RNA polymerase II (*rpb2*) region, and primers sets MS1/MS2 (White *et al.* 1990) to amplify the ribosomal mitochondrial small subunit (mtSSU) region. PCRs were performed in a 25 µL volume consisting of 12.5 µL of 2 × Master Mix (Mei5 Biotechnology Co., Ltd, China), 1 µL of each primer (10 µmol/L), 8.5 µL ddH₂O, and 2 µL template sequence (215 µg/µL). DNA sequences were obtained through Sanger dideoxy sequencing (Zhongkexilin Biotechnology, Co., Ltd, Beijing, China). The newly obtained sequences were submitted to GenBank (<https://www.ncbi.nlm.nih.gov>) under accession numbers MW554231, MW554431, MW554144, OP133163–OP133166, OP133202–OP133207, OP143935–143940, and OP156851–156856 (Fig. 2 and Table 1).

Table 1 (continued on next page). Information on sequences of *Russula* Pers. used in the phylogenetic analysis in this study. The newly generated sequences are in **bold**; “–” show no sequence.

<i>Taxon</i>	<i>Voucher</i>	<i>Location</i>	<i>nrLSU</i>	<i>rpb2</i>	<i>mtSSU</i>	<i>Reference</i>
<i>R. aeruginea</i>	AT2003017	Europe	DQ421999	DQ421946	–	Buyck <i>et al.</i> 2008
<i>R. albidogrisea</i>	RITF1871	China	MW397128	–	MW403841	–
<i>R. albolutea</i>	RITF2653	China	MW397120	MW411340	MW403833	Chen <i>et al.</i> 2021c
<i>R. albolutea</i>	RITF4460	China	MW397121	MW411341	MW403834	Chen <i>et al.</i> 2021c
<i>R. albonigra</i>	544/BB 07.291	Slovakia	KU237536	KU237822	KU237382	Buyck <i>et al.</i> 2018
<i>R. amoenolens</i>	BB 08.675	Italy	KU237562	KU237848	KU237410	Buyck <i>et al.</i> 2018
<i>R. cf. amoenicolor</i>	SAV F–20302	Greece	–	MT417196	MT417188	Wisitrassameewong <i>et al.</i> 2020
<i>R. atroglauca</i>	SAV F-3066	Sweden	MT738252	MT732160	–	–
<i>R. aureoviridis</i>	RITF4709	China	MW646992	–	MW647003	Chen <i>et al.</i> 2021a
<i>R. bella</i>	SFC20121010-06	South Korea	KF361858	KF361758	–	Park <i>et al.</i> 2013
<i>R. aff. brunneoannulata</i>	BB 06.029	Madagascar	KU237452	KU237738	KU237295	Buyck <i>et al.</i> 2018
<i>R. columbicolor</i>	2010BT108A	Germany	JN389003	JN375606	–	Van de Putte <i>et al.</i> 2012
<i>R. crustosa</i>	BPL265	USA	KT933826	KT933898	–	Looney <i>et al.</i> 2016
<i>R. cyanoxantha</i>	UE29.09.2002-2	Sweden	DQ422033	DQ421970	–	Buyck <i>et al.</i> 2008
<i>R. elastica</i>	BB 06.009	Madagascar	KU237451	KU237737	KU237294	Buyck <i>et al.</i> 2018
<i>R. faustiana</i>	FH 2011 BT314	Germany	MT738253	MT732163	–	–
<i>R. floriformis</i>	Corrales943	Colombia	MT023729	MT021752	MT039861	Vera <i>et al.</i> 2021
<i>R. floriformis</i> subsp. <i>floriformis</i>	Corrales591	Panama	MT023730	MT021753	MT039862	Vera <i>et al.</i> 2021
<i>R. floriformis</i> subsp. <i>symphoniae</i>						
<i>R. galochroa</i>	FH 2010 BT137	Germany	MT738255	MT732165	–	–
<i>R. heterophylla</i>	UE20.08.2004-2	Sweden	DQ422006	DQ421951	–	Buyck <i>et al.</i> 2008
<i>R. ilicis</i>	1174/MF 00.300	Italy	KU237595	KU237880	KU237443	Buyck <i>et al.</i> 2018
<i>R. ionochlora</i>	FH 2009 BT01	Germany	MT738264	MT732172	–	–
<i>R. ionochlora</i>	448/BB 07.338	Slovakia	KU237508	KU237794	KU237354	Buyck <i>et al.</i> 2018
<i>R. lakhanpalii</i>	RITF2600	China	MW646995	–	MW647006	Chen <i>et al.</i> 2021b
<i>R. langei</i>	450/BB 07.792	France	KU237510	KU237796	KU237356	Buyck <i>et al.</i> 2018
<i>R. luofuensis</i>	RITF4707	China	MW646986	–	MW646997	Chen <i>et al.</i> 2021b
<i>R. luofuensis</i>	RITF4708	China	MW646987	–	MW646998	Chen <i>et al.</i> 2021b
<i>R. mariae</i>	546/BB 07.038	USA	KU237538	KU237824	KU237384	Buyck <i>et al.</i> 2018
<i>R. medullata</i>	555/BB 07.252	Slovakia	KU237546	KU237832	KU237392	Buyck <i>et al.</i> 2018
<i>R. mustelina</i>	FH12226	Germany	KT933866	KT933937	–	Looney <i>et al.</i> 2016
<i>R. mustelina</i>	1176/S. Adamcik 09.88	Germany	KU237596	KU237881	KU237444	Buyck <i>et al.</i> 2018
<i>R. nigricans</i>	429/BB 07.342	Slovakia	KU237495	KU237781	KU237339	Buyck <i>et al.</i> 2018
<i>R. oleifera</i>	BB 98.024	Burundi	KU237490	KU237776	KU237334	Buyck <i>et al.</i> 2018
<i>R. orientipurpurea</i>	HCCN19111	South Korea	KF361812	KF361712	–	Park <i>et al.</i> 2013
<i>R. orientipurpurea</i>	SFC20170725–37	South Korea	–	MT199639	MT196927	Wisitrassameewong <i>et al.</i> 2020
<i>R. ornaticeps</i>	46/BB 06.530	Mexico	KU237466	KU237752	KU237310	Buyck <i>et al.</i> 2018
<i>R. pallidula</i>	RITF3331	China	MH027960	MH091698	MW403845	Chen <i>et al.</i> 2021c
<i>R. pseudopunicea</i> sp. nov.	BJTC C335	China	OP133202	OP156851	OP143935	This work
<i>R. pseudopunicea</i> sp. nov.	BJTC ZH1389	China	OP133203	OP156852	OP143936	This work
<i>R. pseudopunicea</i> sp. nov.	BJTC ZH1392	China	OP133204	OP156853	OP143937	This work
<i>R. pulverulenta</i>	BB 05.160	USA	KU237563	KU237849	KU237411	Buyck <i>et al.</i> 2018

Table 1 (continued). Information on sequences of *Russula* Pers. used in the phylogenetic analysis in this study. The newly generated sequences are in **bold**; “–” show no sequence.

<i>Taxon</i>	<i>Voucher</i>	<i>Location</i>	<i>nrLSU</i>	<i>rpb2</i>	<i>mtSSU</i>	<i>Reference</i>
<i>R. shawarensis</i>	LAH36425	Pakistan	MT738267	MT732176	–	–
<i>R. shawarensis</i>	LAH36426	Pakistan	MT738268	MT732177	–	–
<i>R. subbubalina</i>	RITF4710	China	MW646990	–	MW647001	Chen <i>et al.</i> 2021b
<i>R. subpunicea</i>	RITF3715	China	MW397124	MW411344	MW403837	Chen <i>et al.</i> 2021c
<i>R. subpunicea</i>	RITF1435	China	MW397126	MW411346	MW403839	Chen <i>et al.</i> 2021c
<i>R. subpunicea</i>	RITF2648	China	MW397125	MW411345	MW403838	Chen <i>et al.</i> 2021c
<i>R. subpunicea</i>	RITF2615	China	MW397127	MW411347	MW403840	Chen <i>et al.</i> 2021c
<i>R. substriata</i>	XHW4766	China	MH714540	MH939992	–	Wang <i>et al.</i> 2019
<i>R. cf. vesca</i>	45/BB 06.525	Mexico	KU237465	KU237751	KU237309	Buyck <i>et al.</i> 2018
<i>R. violeipes</i>	542/BB 07.273	Slovakia	KU237534	KU237820	KU237380	Buyck <i>et al.</i> 2018
<i>R. virescens</i>	HJB9989	Sweden	DQ422014	DQ421955	–	Buyck <i>et al.</i> 2008
<i>R. viridicin-namomea</i>	RITF3324	China	MW397130	MW411348	MW403847	–
<i>R. wlingshanensis</i> <i>sp. nov.</i>	BJTC C399	China	OP133205	OP156854	OP143938	This work
<i>R. wlingshanensis</i> <i>sp. nov.</i>	BJTC C403	China	OP133206	OP156855	OP143939	This work
<i>R. wlingshanensis</i> <i>sp. nov.</i>	BJTC L278	China	OP133207	OP156856	OP143940	This work

Molecular phylogenetic analyses

The nrLSU-*rpb2*-mtSSU multi-locus dataset included 54 ingroup samples, and these samples were used to determine the subsection in which the specimens were located. The nrITS dataset included 77 ingroup samples, and these samples were used to analyse whether the specimen was a new species. Based on previous studies and the GenBank or UNITE database (<https://unite.ut.ee>), all reference sequences of subg. *Heterophyllidia* of the dataset were selected for phylogenetic analyses (Buyck *et al.* 2008, 2018; Chen *et al.* 2021a, 2021b, 2021c) (Table 1). *Russula albonigra* (Krombh.) Fr. and *Russula nigricans* Fr. were used as outgroups following Ghosh *et al.* (2020).

All sequences were aligned using MAFFT ver. 6 (Katoh & Toh 2010), and manually trimmed using MEGA ver. 6 (Tamura *et al.* 2013). Phylogenetic analyses were performed using MrBayes ver. 3.1.2 for Bayesian inference (BI) (Ronquist & Huelsenbeck 2003) and maximum likelihood (ML) gene-trees were estimated using RAxML ver. 7.4.2 (Stamatakis 2006; Stamatakis *et al.* 2008).

The Bayesian analysis was performed using the best substitution model GTR+I+G, as determined through MrModeltest ver. 2.3 (Nylander 2004). Two Markov chain Monte Carlo (MCMC) chains were run from random trees for 10 000 000 generations and stopped when the average standard deviation of split frequencies was <0.01. Trees were saved every 1000th generation (Rannala & Yang 1996). After the first 25% of the trees were discarded as the burn-in phase in each analysis, branches with significant Bayesian posterior probabilities (BPP) were estimated in the remaining trees (Posada & Crandall 1998; Zhou *et al.* 2022a). Maximum likelihood (ML) analysis was performed under a GTR site substitution model (Guindon *et al.* 2010). Branch support was calculated using a bootstrapping (BS) method of 1000 replicates (Hillis & Bull 1993). Clades with a bootstrap support (BS) of $\geq 70\%$ and Bayesian posterior probability (PP) of ≥ 0.95 were considered as well supported (Hillis & Bull 1993).

Results

Phylogenetic analyses

The nrLSU-*rpb2*-mtSSU and nrITS datasets were analysed through maximum likelihood (ML) and Bayesian inference (BI) analyses. The topologies of ML and BI phylogenetic trees were almost identical, and only RAxML trees are shown in Figs 1–2, with an indication of the ML bootstrap support and BI posterior probabilities. The multi-locus phylogenetic analyses (Fig. 1) resolved the species of *Russula* subgenus *Heterophyllidia* as a monophyletic lineage with strong support, and 11 clades were further recognized, which corresponded to the subsections of this subgenus. Our collected specimens formed two distinct and strongly supported branches, which were nested in the subsections *Heterophyllidia* and *Virescentinae*, respectively.

The nrITS phylogenetic analysis and the multi-locus phylogenetic tree exhibited similar topologies, and our collected specimens also formed two strongly supported terminal branches (BS = 100%, BPP = 1.00) (Fig. 2). These results confirm that our specimens represent two new species, which are described here.

Russula pseudopunicea sp. nov. formed a sister clade to *R. subpunicea* B.Chen & J.F.Liang with a strong support in the ITS tree (Fig. 2) and multi-locus tree (Fig. 1). *Russula wulingshanensis* sp. nov. clustered with *R. grisea* Fr. and *R. ionochlora* Romagn. with high support values in the ITS tree (Fig. 2) and multi-locus tree (Fig. 1).

Taxonomic treatments

Phylum Basidiomycota R.T.Moore
Class Agaricomycetes Doweld
Order Russulales Kreisel ex P.M.Kirk, P.F.Cannon & J.C.David
Family Russulaceae Lotsy
Genus *Russula* Pers.

Russula pseudopunicea C.L.Hou, H.Zhou, & G.Q.Cheng sp. nov.
Mycobank: MB844536
Figs 3–4

Diagnosis

Russula pseudopunicea sp. nov. differs from other species by the reddish brown, light brown to brownish orange pileus disc with a greyish yellow margin, small basidiospores ($5.9\text{--}6.4\text{--}6.9 \times 5.2\text{--}5.5\text{--}5.8 \mu\text{m}$), short basidia ($35\text{--}40\text{--}45 \times 9.1\text{--}11\text{--}12 \mu\text{m}$) and distinct position in the nrITS phylogenetic tree.

Etymology

The epithet '*pseudopunicea*' refers to its phylogenetic position relative to *R. subpunicea*.

Material examined

Holotype

CHINA • Beijing, Miyun District, Sileng Mountains; 40°28'23" N, 117°6'17" E; elev. 713 m; in broadleaf forest dominated by *Betula costata* Trautv.; 30 Aug. 2021; C.L. Hou, G.Q. Cheng & H. Zhao; BJTC[BJTC ZH1392].

Additional material

CHINA • Beijing, Miyun District, Sileng Mountains; 40°28'23" N, 117°6'18" E; elev. 710 m; in broadleaf forest dominated by *Betula costata*; 30 Aug. 2021; C.L. Hou, G.Q. Cheng & H. Zhao; BJTC[BJTC ZH1389]

• Hebei Province, Chengde City, Xinglong County, Badaziling; 40°18'36.6" N, 117°35'6.0" E; elev. 879 m; in broadleaf forest dominated by *Populus davidiana* Dode; 22 Aug. 2020; C.L. Hou & G.Q. Cheng; BJTC[BJTC C335] • Beijing, Huairou District, Sunzhazi Village; 40°56'33.9" N, 116°30'25.4" E; elev.

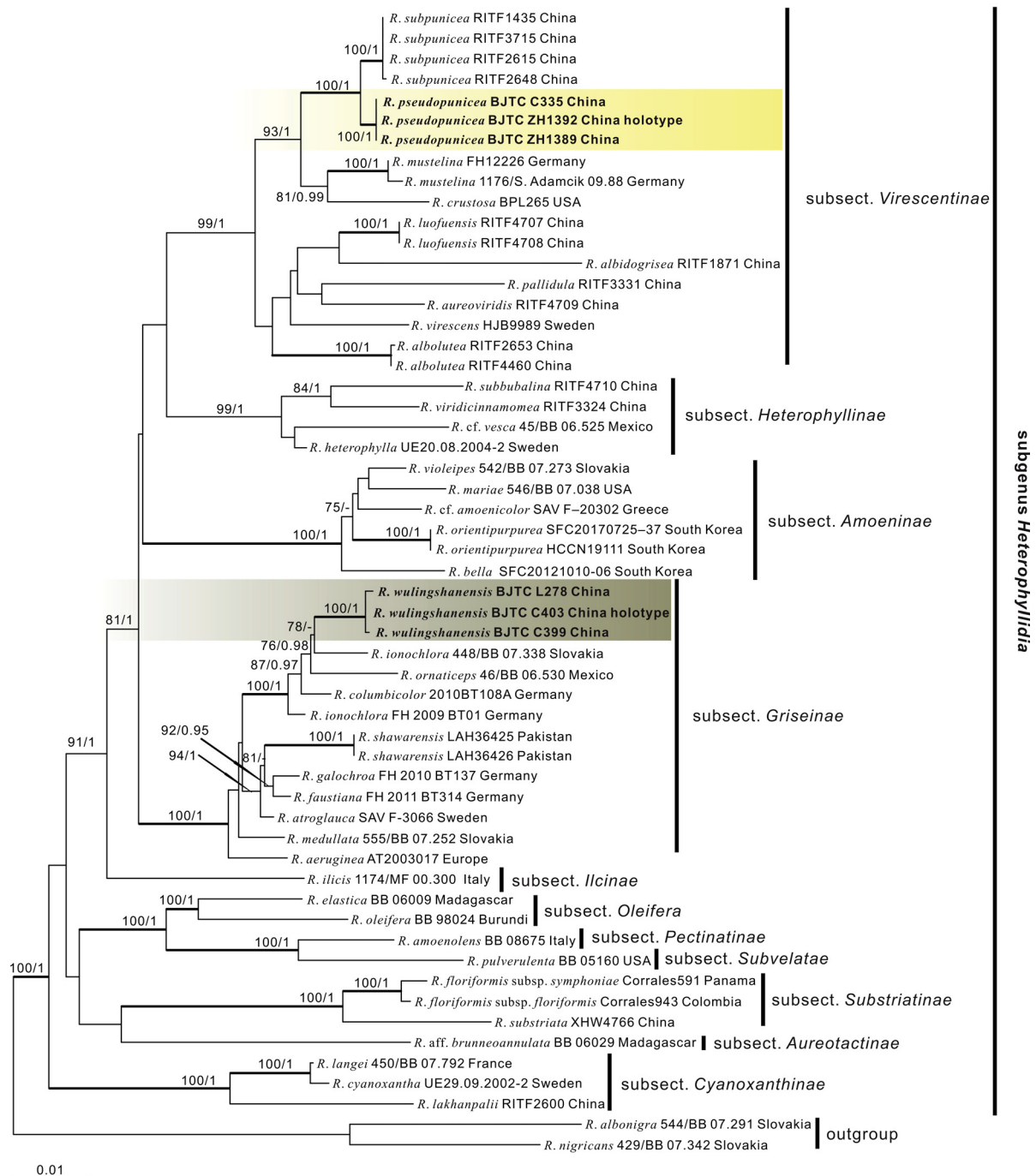


Fig. 1. Maximum likelihood (ML) phylogenetic tree inferred from nrLSU-*rpb2*-mtSSU. The two new species described in this paper are indicated in **bold**. Values next to nodes indicate ML bootstrap values (left) and Bayesian posterior probabilities (right). Only ML bootstrap values $\geq 70\%$ and Bayesian posterior probabilities ≥ 0.95 are shown.

791 m; in coniferous and broad-leaved forest dominated by *Populus davidiana* and *Pinus tabuliformis* Carr.; 22 Aug. 2020; C.L. Hou & G.Q. Cheng; BJTC[BJTC C565].

Description

Basidiomata medium to large-sized. Pileus 35–113 mm in diam., hemispheric when young, applanate with a depressed centre at maturity; margin smooth or with indistinct striae, 3–12 mm, surface viscid when

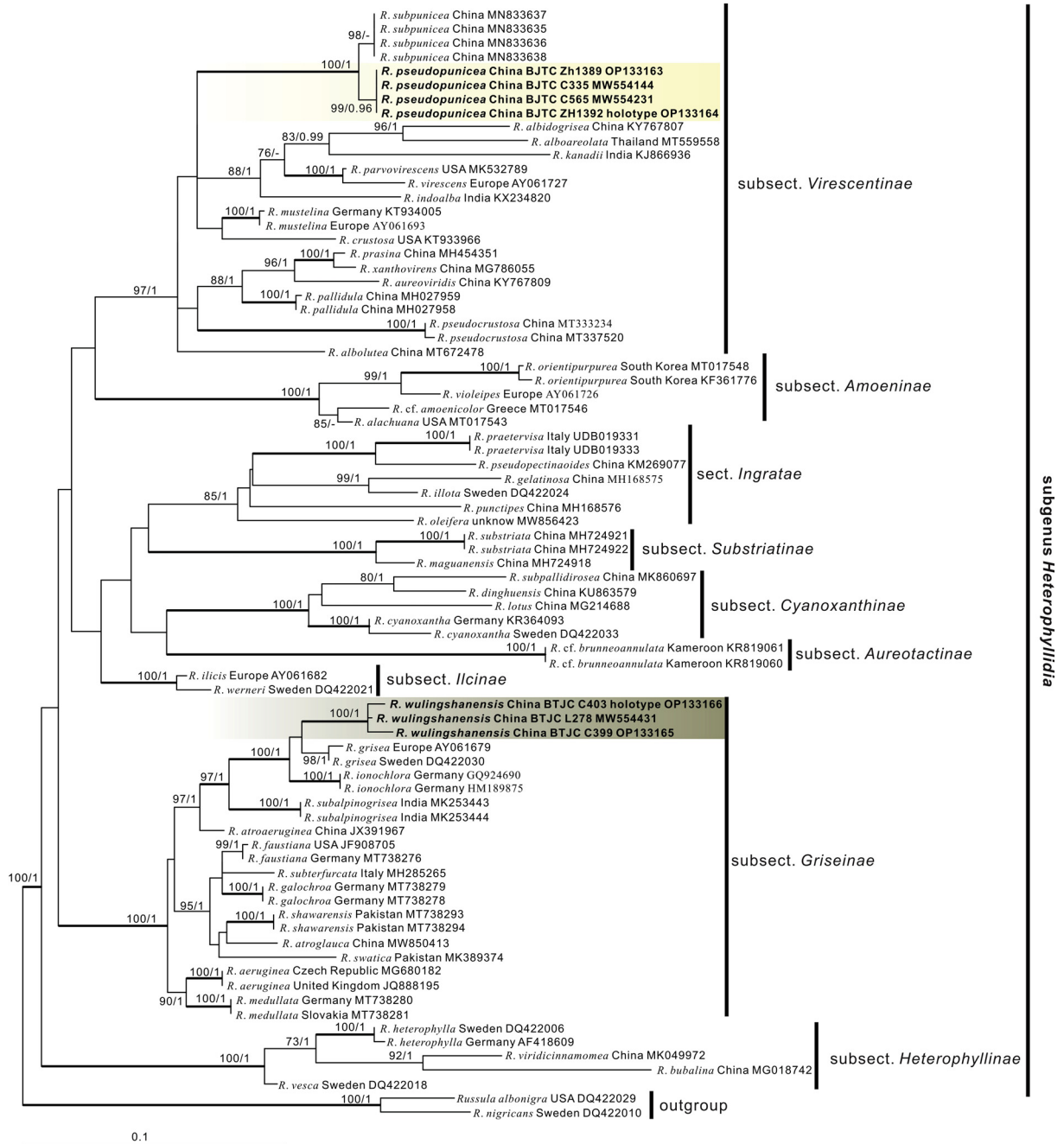


Fig. 2. Maximum likelihood (ML) phylogenetic tree inferred from nrITS. The two new species described in this paper are indicated in **bold**. Values next to nodes indicate ML bootstrap values (left) and Bayesian posterior probabilities (right). Only ML bootstrap values $\geq 70\%$ and Bayesian posterior probabilities ≥ 0.95 are shown.

wet, cuticle peeling approximately to one-third of the pileus radius; reddish brown (#9a603e), light brown (#8e4d1e) to brownish orange (#c5994b), greyish brown (#a79e9a), margin greyish yellow (#ceb98f), pale yellow (#feed4) to light grey-brown (#fddaa2). Lamellae cream (#ffffff) to pale yellow (#ffe7a3), adnate to adnexed, approximately 11–14 pieces/cm near the pileus margin, not forked, lamellulae absent. Stipe 41–94 × 21–38 mm, white (#ffffff), grey-white (#d3cbba) to cream-white (#d3cfba), cylindrical, solid. Context 5–15 mm at the pileus centre, white (#ffffff), unchanging when bruised, odour not obvious, taste mild. Spore print not observed.

Basidiospores (5.2–)5.9–6.4–6.9(–7.5) × (4.8–)5.2–5.5–5.8(–6.2) μm, Q = 1.16 ± 0.16, globose to ellipsoid, ornamented with small and amyloid warts of height 0.2–0.6 μm, which are usually connected by the lower line and sometimes form a partial reticulum; hilar appendix usually conspicuous, imamyloid.

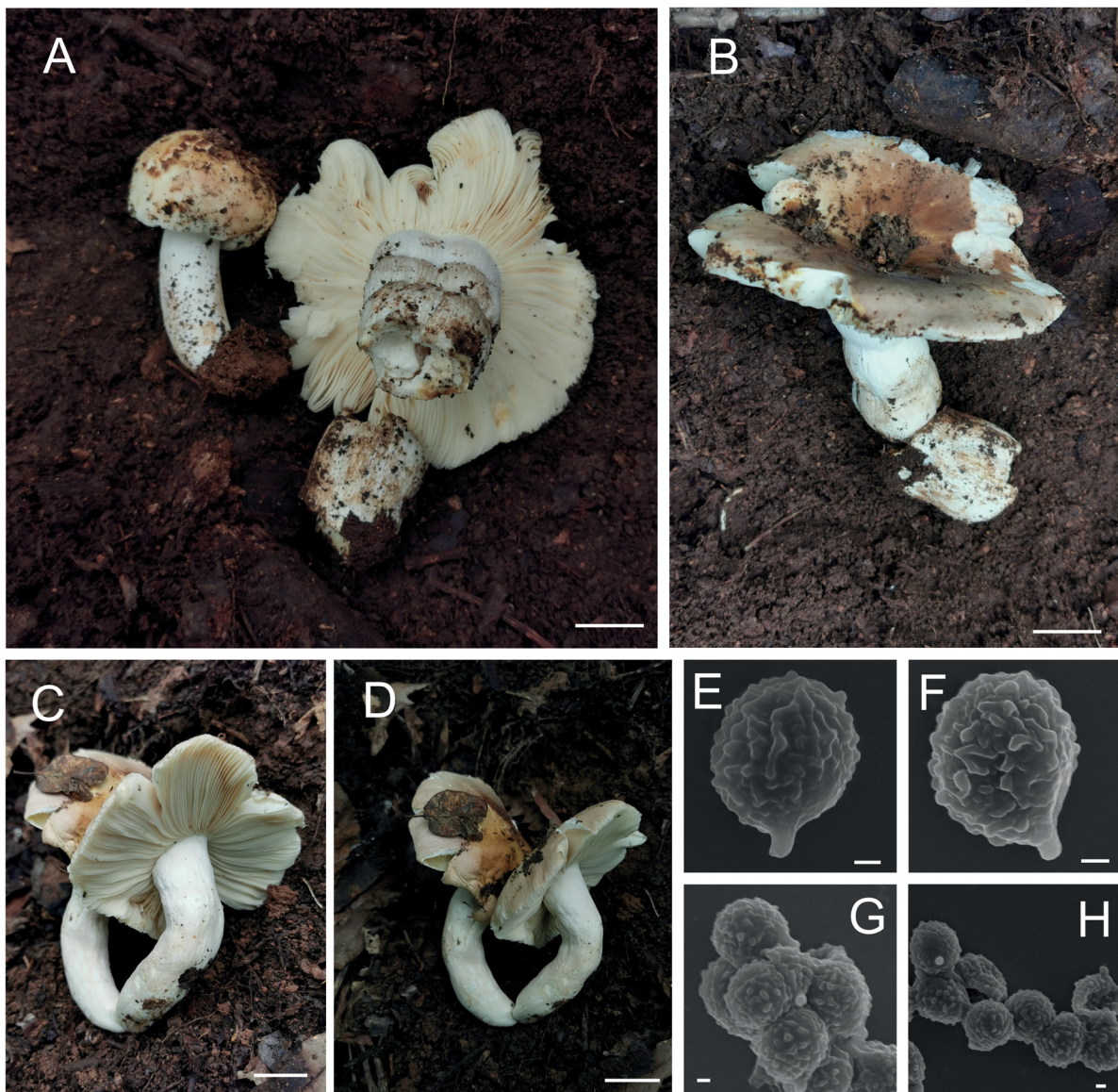


Fig. 3. Basidiomata and basidiospores of *Russula pseudopunicea* C.L.Hou, G.Q.Cheng & H.Zhou sp. nov. A–D. Basidiomata (A–B = BJTC ZH1392, holotype; C–D = BJTC ZH1389). E–H. SEM micrograph of basidiospores (BJTC ZH1392, holotype). Scale bars: A–D = 10 mm, E–H = 1 μm.

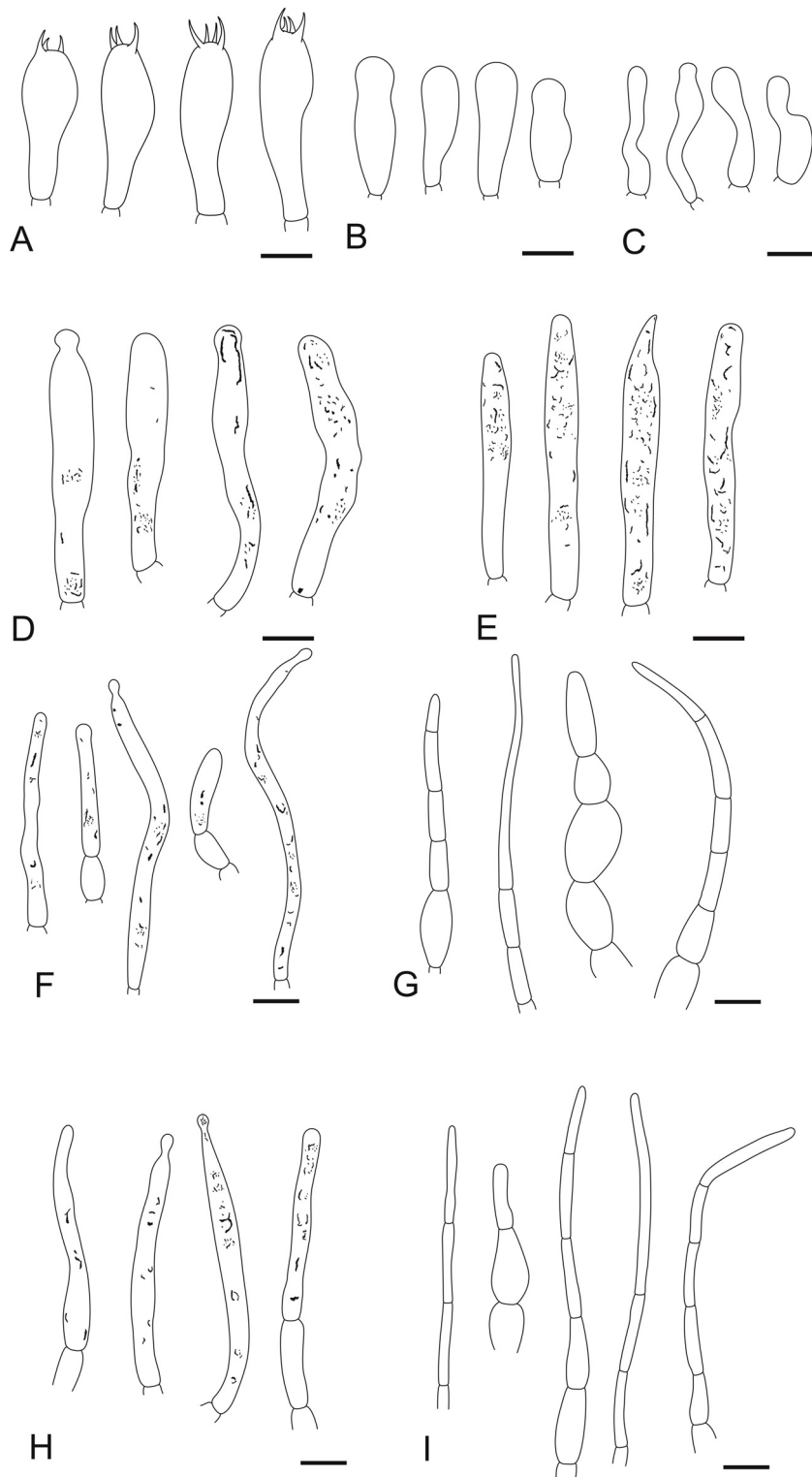


Fig. 4. Microscopic structures of lamellae of *Russula pseudopunicea* C.L.Hou, G.Q.Cheng & H.Zhou sp. nov., holotype (BJTC ZH1392). **A.** Basidia. **B.** Basidiola. **C.** Marginal cells. **D.** Pleurocystidia. **E.** Cheilocystidia. **F.** Pileocystidia near pileus margin. **G.** Hyphal terminations near pileus margin. **H.** Pileocystidia near pileus centre. **I.** Hyphal terminations near pileus centre. Scale bars = 10 μ m.

Basidia (32–)35–40–44(–50) × (7.4–)9.1–10–11(–12) μm, clavate, 2-, 4-spored, thin-walled, with guttate or granular contents; basidiola clavate or subcylindrical, ca 5–10 μm wide. Pleurocystidia dispersed to moderately numerous, (54–)57–68–76(–84) × (7.1–)7.4–8.4–9.4(–10.2) μm, cylindrical, clavate or subfusiform, apically mainly obtuse, presence of heteromorphous-crystalline, occasionally banded contents, turning grey to purplish red (#694f89) in sulfovanillin. Cheilocystidia (50–)52–60–67(–76) × (5.8–)6.3–7.1–7.9(–9.2) μm, clavate, occasionally cylindrical or subfusiform, with crystalline or granulose, few banded contents. Marginal cells (15–)17–21–25(–27) × (4.0–)4.2–5.8–7.4(–11) μm, subcylindrical, often flexuous. Pileipellis orthochromatic in cresyl blue, sharply delimited from the underlying context, 210–410 μm thick, two-layered, suprapellis 60–190 μm thick, not gelatinized, composed of erect, repent or ascending and near the surface loose hyphal terminations, inflated at base and attenuated towards terminal cells; subpellis 150–270 μm thick, less gelatinized, composed of interwoven hyphae of width 2.5–5.5 μm. Hyphal terminations near the pileus margin not branched, occasionally flexuous, thin-walled; terminal cells (8.1–)9.2–22–36(–55) × (2.0–)2.4–3–3.6(–4.1) μm, subcylindrical to cylindrical, sometimes apically attenuated, subterminal cells often short or inflated, 2–8 μm wide, forked. Hyphal terminations near the pileus centre similar to those near the pileus margin, terminal cells (18–)20–29–38(–47) × (2.0–)2.4–2.8–3.2(–3.6) μm, mostly subcylindrical. Pileocystidia near the pileus margin (34–)38–53–67(–74) × (3.0–)3.4–4.2–5(–5.4) μm, thin-walled, cylindrical, subcylindrical or narrowly fusiform, sometimes with a 3–5 μm long appendage, contents heteromorphous granulose or banded, becoming purple (#800080) in sulfovanillin; pileocystidia near the pileus centre (22–)25–39–53(–71) × (3.0–)3.2–4–4.8(–5.1) μm, thin-walled, cylindrical, subcylindrical or narrowly fusiform, apically mainly obtuse, sometimes with a 2–4 μm long appendage, contents heteromorphous-granulose. Cystidioid hyphae absent.

Russula wulingshanensis C.L.Hou, H.Zhou, & G.Q.Cheng sp. nov.

Mycobank: MB844537

Figs 5–6

Diagnosis

Russula wulingshanensis sp. nov. differs from other species by the purple pinkish, pale brown or greyish white pileus disc with grey-white to grey-purple margin at maturity, small basidiospores (5.8–6.3–6.8 × 4.9–5.2–5.5), pleurocystidia turning pink in sulfovanillin and a separate phylogenetic position.

Etymology

The epithet ‘*wulingshanensis*’ refers to the locality where the type specimen was collected.

Material examined

Holotype

CHINA • Hebei Province, Xinglong County, Wulingshan Mountain National Nature Reserve; 40°33′40.7″ N, 117°28′44.9″ E; elev. 1248 m; in broadleaf forest dominated by *Betula dahurica* Pall.; 23 Aug. 2020; C.L. Hou & G.Q. Cheng; BJTC[BJTC C403].

Additional material

CHINA • Hebei Province, Xinglong County, Wulingshan Mountain National Nature Reserve; 40°33′39.7″ N, 117°28′44.7″ E; elev. 1248 m; in broadleaf forest dominated by *Betula dahurica*; 19 Aug. 2019; H. Zhao & J.Q. Li; BJTC[BJTC L278] • *ibid.*; 40°33′40.1″ N, 117°28′44.7″ E; elev. 1256 m; in broadleaf forest dominated by *B. dahurica*; 23 Aug. 2020; C.L. Hou, G.Q. Cheng & R.T. Zhang; BJTC[BJTC C399].

Description

Basidiomata small to medium-sized. Pileus 14–63 mm in diam., hemispheric at first, appanate with a depressed centre to infundibuliform at maturity, margin smooth when young, often fine striate at maturity, cuticle peeling often one-fifth of the pileus radius; purple pinkish (#ff989b), pale brown (#a95b4c) or

greyish white (#e9e9e6), margin grey-white (#e9e9e6), grey-purple (#aaaad5). Lamellae white (#ffff) to pale yellow (#ffffcc), adnate, fragile, up to 6 mm wide, approximately 10–12 pieces per cm near the pileus margin, lamellulae absent, usually forked near the stipe. Stipe 72–105 × 12–27 mm, solid, subcylindrical to slightly inflated at the base, sometimes light brown to brownish orange (#ae6020) when bruised. Context unchanging when bruised, odour unpleasant, taste mild. Spore print not observed.

Basidiospores (5.5–)5.8–6.3–6.8(–7.3) × (4.7–)4.9–5.2–5.5(–5.6) μm, Q=(1.02–)1.10–1.21–1.32(–1.45), subglobose to ellipsoid, ornamented with small and amyloid warts of height 0.2–0.9 μm; hilar appendix small, amyloid or weakly amyloid. Basidia (31–)35–40–45(–48) × (8.5–)9.4–11–12(–15) μm, 2- and 4-spored, clavate and slightly inflated in the upper half, thin-walled, with guttate or granular contents, sterigmata 4.3–5.8 × 0.9–1.2 μm; basidiola clavate or subcylindrical, 7–11 μm wide. Pleurocystidia dispersed, (48–)58–71–84(–95) × (8.6–)9.7–11–12(–14) μm, clavate, fusiform, subcylindrical sometimes lanceolate, apically always obtuse, few mucronate, sometimes with a 2–7 μm long appendage, thin-walled, contents granulose, heteromorphous-crystalline, pink in sulfovanillin. Cheilocystidia (33–)40–51–62(–67) × (6.2–)8.1–9.1–10(–11) μm, clavate and subfusiform, apically always obtuse, sometimes with 3–12 μm long appendage; contents granulose, heteromorphous-crystalline, often dispersed in the

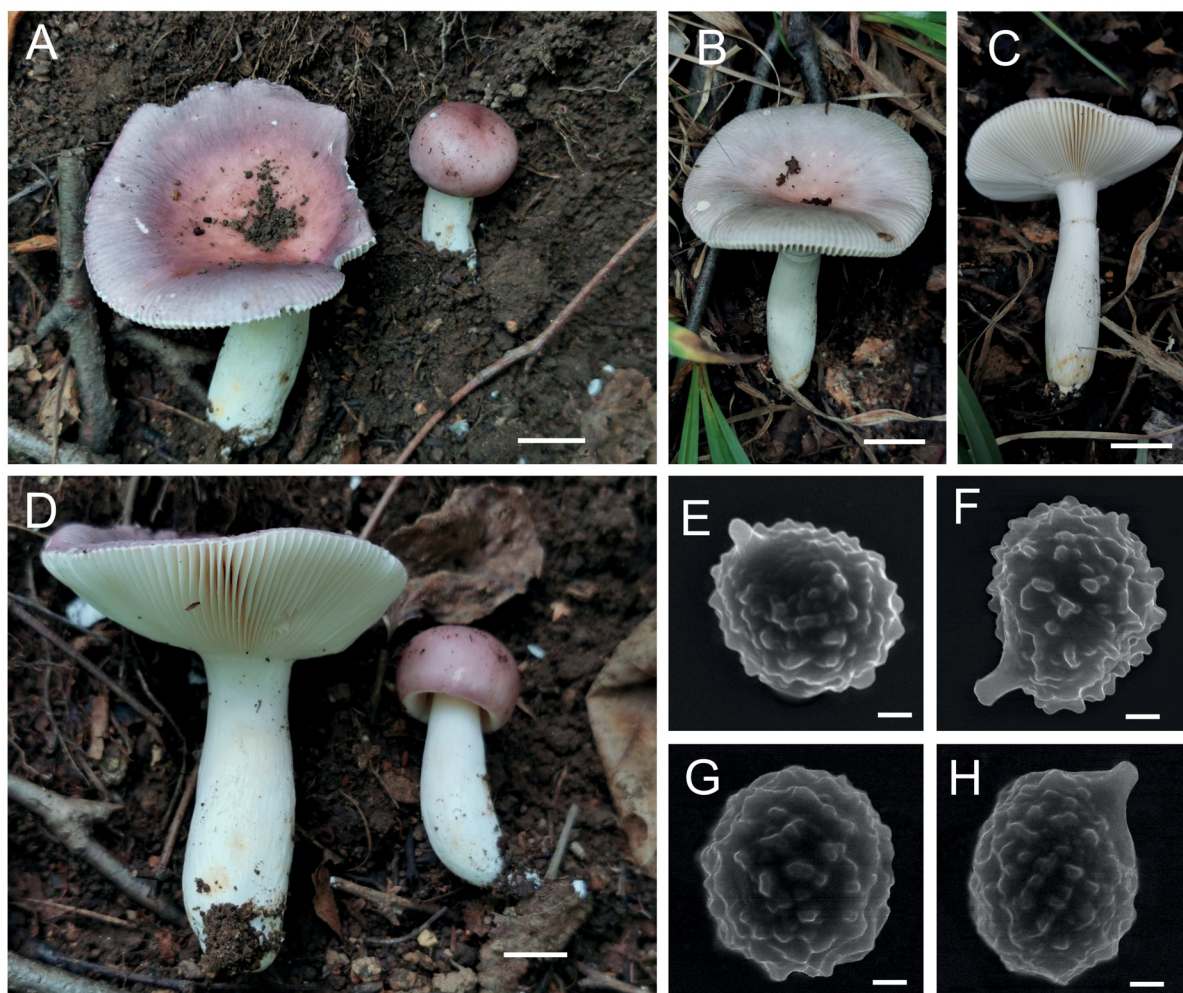


Fig. 5. Basidiomata and basidiospores of *Russula wulingshanensis* C.L.Hou, G.Q.Cheng & H.Zhou sp. nov. A–D. Basidiomata (A–D = BJTC C403, holotype; B–C = BJTC C399). E–H. SEM micrograph of basidiospores (BJTC C403, holotype). Scale bars: A–D = 10 mm, E–H = 1 μm.

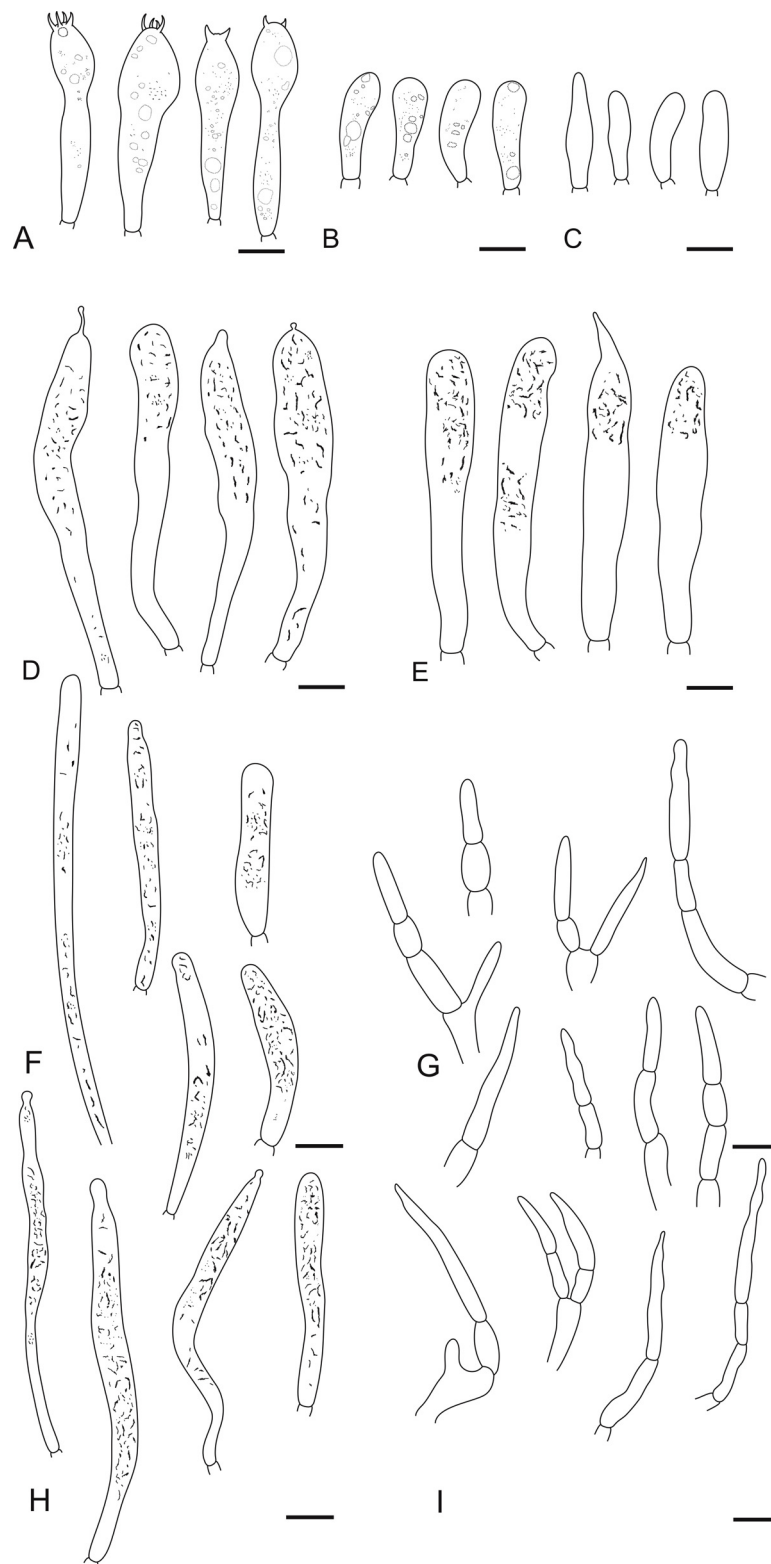


Fig. 6. Microscopic structures of lamellae of *Russula wulingshanensis* C.L.Hou, G.Q.Cheng & H.Zhou sp. nov., holotype (BJTC C403). **A.** Basidia. **B.** Basidiola. **C.** Marginal cells. **D.** Pleurocystidia. **E.** Cheilocystidia. **F.** Pileocystidia near the pileus margin. **G.** Pileocystidia near the pileus centre. **H.** Hyphal terminations near the pileus margin. **I.** Hyphal terminations near the pileus centre. Scale bars = 10 μm .

upper and middle layers. Marginal cells (20–)22–24–26(–29) × (4.9–)5.6–6.7–7.8(–9.4) μm, clavate or subfusiform, sometimes fusiform. Pileipellis orthochromatic in cresyl blue, sharply delimited from the underlying context, 120–180 μm thick, two-layered, strongly gelatinized, suprapellis 50–80 μm thick, composed of ascending to erect hyphal terminations; subpellis 70–100 μm thick, composed of horizontally oriented, interwoven hyphae. Hyphal terminations near the pileus margin intricate and sometimes branched, occasionally flexuous, thin-walled; terminal cells (11–)15–21–27(–31) × (3.0–)3.3–4.1–4.9(–5.9) μm, mostly subcylindrical, occasionally subfusiform or lageniform, apically usually obtuse, sometimes attenuated or constricted, subterminal cells frequently shorter and slightly inflated, ca 4–7 μm wide, rarely branched. Hyphal terminations near the pileus centre similar to those near the pileus margin; terminal cells (12–)18–26–35(–44) × (2.1–)3.5–4.4–5.3(–6.2) μm, subcylindrical, subfusiform or lageniform, apically usually obtuse, sometimes attenuated or constricted, subterminal cells frequently short, 2–6 μm wide, rarely branched. Pileocystidia near the pileus margin (35–)39–50–62(–68) × (5.0–)5.2–5.8–6.4(–7.5) μm, thin-walled, cylindrical, clavate or subfusiform, apically usually obtuse, sometimes with a 2–5 μm long appendage, contents heteromorphous-granulose, black in sulfovanillin. Pileocystidia near the pileus centre (32–)41–50–59(–71) × (4.3–)4.7–5.8–6.9(–8.9) μm, thin-walled, apically usually obtuse, sometimes with 1–4 μm long appendage, contents with granulose or heteromorphous-crystalline, black in sulfovanillin. Cystidioid hyphae dispersed in subpellis and context, with heteromorphous-granulose contents, oleiferous hyphae frequent in subpellis and context.

Discussion

Our phylogenetic analyses revealed *R. pseudopunicea* sp. nov. as a distinct and well-supported species, closely related to *R. subpunicea* (Figs 1–2). Morphologically, *R. pseudopunicea* sp. nov. differs from *R. subpunicea* in pileus colour, i.e., the pileus of *R. subpunicea* is pinkish or greyish-orange, while that of *R. pseudopunicea* sp. nov. is reddish brown, light brown to brownish orange. Moreover, *R. subpunicea* has pileocystidia turning reddish in sulfovanillin, thinner pileipellis (120–180 μm thick), short and wide hyphal terminations near the pileus centre (9.5–16.6–20.5 × 2.8–4.6–6 μm), and wider pileocystidia (4.5–6–7.3 μm in the pileus centre) (Chen *et al.* 2021b). Both *R. mustelina* Fr. and *R. pseudocrustosa* G.J.Li & C.Y.Deng are similar to *R. pseudopunicea* sp. nov. in having a light brown to brownish orange pileus colour; however, *R. mustelina* is distinguished by the pileus with a reddish-brown tone, large basidiospores (6–9 × 5–8 μm), long basidia (35–46 × 7–12 μm) and large pleurocystidia (39–80 × 9–11 μm) (Li 2014). *Russula pseudocrustosa* differs from *R. mustelina* by the lamellae adnate to subfree, wider basidiospores (5–7.5 × 4.5–6.0 μm), and basidiospore ornamentation not forming complete network (Deng *et al.* 2020).

Russula wulingshanensis sp. nov. is a well-supported species in our phylogenetic analyses (Figs 1–2), closely related to *R. grisea* and *R. ionochlora*. *Russula grisea* can be distinguished from our new species by its pileus with blue-purple or blue-green tints (Sarnari 1998), and *R. ionochlora* can be distinguished from our new species by its pileus with a green tint disc and pinkish discolouration when bruised (Romagnesi 1952; Sikora & Luszczynski 2011). The other three species similar to *R. wulingshanensis* sp. nov. with purple tints of pileus and solid stipe, white to light, inflated at the stipe base are *R. columbicolor* Jurkeit & Herches, *R. orientipurpurea* Wisitr., H.Lee & Y.W.Lim and *R. ornaticeps* Burl. *Russula columbicolor* invariably grows in *Fagus*, *Quercus*, *Carpinus* and *Tilia* forests (Jurkeit & Herches 2007), and differs from our new species by its yellow pileus disc, purple staining stipe, partly reticulate ornamentation of basidiospores, and usually a conspicuous hilar appendix. *Russula orientipurpurea* invariably grows in a mixed forest with *Quercus* and *Pinus* trees (Wisitrassameewong *et al.* 2020), and differs from our new species by its pale or darker purple pileus, stipe sometimes staining red flush, large basidiospores (6.9–7.3–7.8 × 6–6.4–6.9 μm), absence of a hilar appendix on basidiospores, negative in sulfovanillin, and absence of pileocystidia. *Russula ornaticeps* invariably grows with *Carpinus* trees and differs from our new species by its lilac-mauve pileus disc (Burlingham 1921).

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Authors' contributions

Conceptualization: H. Zhou, G.Q. Cheng, X.B. Huang & C.L. Hou; data curation: H. Zhou, G.Q. Cheng and X.B. Huang; formal analysis: H. Zhou & X.B. Huang; funding acquisition: C.L. Hou; methodology: H. Zhou, G.Q. Cheng and C.L. Hou; project administration: C.L. Hou; resources: H. Zhou, G.Q. Cheng, X.B. Huang and C.L. Hou; supervision: C.L. Hou; writing-original draft: H. Zhou, G.Q. Cheng and X.B. Huang; writing-review and editing: H. Zhou, G.Q. Cheng and C.L. Hou. All authors have read and agreed to the published version of the manuscript.

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