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Research article

Phylogenetic and morphological evidence for four new species of *Russula* (Russulaceae, Basidiomycota) from northwestern China

Yu SONG[1,*], Yu-Yu WANG[2], Jin-Ling YU[3], Rui YUAN[4] & Fang LI[5]

[1,2,3,4] School of Biological Science & Engineering,
Shaanxi University of Technology, Hanzhong 723000, China.
[1] Shaanxi Province Key Laboratory of Bio-resources, Hanzhong 723000, China.
[1] Qinba Mountain Area Collaborative Innovation Center of Bioresources Comprehensive Development, Hanzhong 723000, China.
[1] Qinba State Key Laboratory of Biological Resources and Ecological Environment (Incubation), Hanzhong 723000, China.
[5] School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, China.

[*] Corresponding author: songyucanada@foxmail.com
[2] Email: 2472768945@qq.com
[3] Email: 1416349174@qq.com
[4] Email: 1579071050@qq.com
[5] Email: lsslif@mail.sysu.edu.cn

Abstract. Four novel species of subgenus *Russula* crown clade collected from northwestern China are described based on morphological and phylogenetic evidence. Morphologically, *R. griseorosea* Y.Song sp. nov. (subsection *Puellarinae*) is characterized by its brown pileus with a grayish pink tint, basidiospores with warts often connected by fine lines, orthochromatic pileipellis with long terminal cells and septate pileocystidia; *R. micangshanensis* Y.Song sp. nov. (subsection *Olivaceinae*) is diagnosed by its large basidia, hymenial cystidia and basidiospores, and spore ornamentations with unequal crests and often twinned warts, which give the spore distinctive appearance; *R. minirosea* Y.Song sp. nov. (subsection *Laricinae*) has very small basidiocarp with pileus less than 3.3 cm in diameter, basidiospores with fine reticulum, small basidia, and septate pileocystidia; *R. purpureomarginalis* F.Li & Y.Song sp. nov. (subsection *Xerampelinae*) has large basidiospores with often isolated ornamentations, slim basidia and often septate flexuous pileocystidia. Differences between the four novel species and their closely related taxa were analyzed. Phylogenetic analyses based on both ITS and multi-locus (LSU, *rpb2* and *tef1*) were carried out to confirm the distinct taxonomic status of the four novel species.

Keywords. *Russula*, novel species, multi-locus phylogeny, taxonomy, morphology.

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Introduction

Russula Pers. is a hyper diverse ectomycorrhizal genus with a worldwide distribution, playing important ecological roles in the maintenance of different ecosystems (Buyck *et al.* 2015, 2018; Adamčík *et al.* 2019). Many members of this group are edible and medicinal, showing considerable economic value (Wu *et al.* 2019). Based on the newest infrageneric taxonomic system employing a 5-locus phylogenetic analysis, *Russula* was divided into 8 subgenera, in which the subgenus *Russula* was further separated into a core and a crown clade (Buyck *et al.* 2018, 2020).

Species of subg. *Russula* crown clade are mainly characterized by basidiomata ranging from very small to very large, equal gills never forked, spore print color varying from white to deep yellow, spores with an amyloid suprahilar spot, and gloeocystidia or primordial hyphae often with obtuse-rounded apices (Buyck *et al.* 2018). The crown clade shows a high species richness worldwide, at least 67 novel species have been described in this group based on both morphological and molecular data between 2006 and the end of 2023, of which 60 were reported from Asia including 31 from China (Das *et al.* 2006, 2017, 2018; Wang *et al.* 2009, 2019; Adamčík *et al.* 2010, 2015, 2016, 2019; Li *et al.* 2012, 2013a, 2013b, 2015, 2016, 2018a, 2018b; Miller *et al.* 2012; Ariyawansa *et al.* 2015; Paloi *et al.* 2015, 2016, 2018; Ghosh *et al.* 2016, 2017, 2021a, 2021b; Ghosh & Das 2017; Jabeen *et al.* 2017; Jiang *et al.* 2017; Khatua *et al.* 2017; Tibpromma *et al.* 2017; Li & Deng 2018; Caboň *et al.* 2019; Phookamsak *et al.* 2019; Hampe & Manz 2021; Kiran *et al.* 2021; Song *et al.* 2021; Chen *et al.* 2022; Khurshid *et al.* 2022; Zhou *et al.* 2022; Wang *et al.* 2023).

Since 2020, the authors have been investigating *Russula* resources in the Qinling (or Tsinling) Mountains and Xinjiang Uygur Autonomous Region, both located in northwestern China. The Qinling Mountains extend 1500 kilometers east to west, with elevations typically ranging from 1500 to 2500 meters (reaching 4811 meters at peak), with lots of biological reserves and precious plants and animals spreading all over. The mountains divide China into two parts with very different climate and culture, namely northern and southern China. While the species diversity of animals and plants has been well documented due to frequent surveys, an investigation of the macrofungal resources has never been carried out within this century in this important region (Song *et al.* 2022). During the macrofungal survey in northwestern China, the authors found four species of *Russula*, three from Qinling Mountains, and one from Xinjiang Uygur Autonomous Region. Through a combination of morphological examinations and phylogenetic analyses based on ITS and concatenated LSU-*tef1*-*rpb2* sequences, these four species belonging to subg. *Russula* crown clade were identified as novel species. This study presents the first description of new species of *Russula* from the Qinling Mountains.

Material and methods

Morphology

Specimens were collected from evergreen broad-leaf forest dominated by Fagaceae trees in northwestern China. Specimens were dehydrated at 40–50°C and deposited in the Shaanxi Provincial Engineering Research Center (SERC). Fresh basidiocarps were photographed using a Canon EOS 850D camera under daylight in the field, and their macroscopic characteristics were recorded. Macromorphological descriptions are based on the field notes and photographs of the fresh basidiocarps.

Microscopic characters were observed under an Olympus CX33 microscope from hand-sectioned dried material. Scanning electron images of basidiospores were captured with a scanning electron microscope (SU8100). Basidiospores were examined in Melzer's reagent and measured in side view, excluding ornamentations. Other micromorphological structures (basidia, hymenial cystidia, gloeocystidia, elements of pileipellis and stipitipellis) were observed from hand-sectioned dried tissues rehydrated in 5% or 10% KOH solution and then mounted with 1% aqueous Congo red reagent. All tissues were examined in cresyl blue (Buyck 1989) to verify presence of ortho- or metachromatic reactions. Sulphovanillin (SV) was used to test color reactions of cystidia (Sarnari 1998). Descriptive terminology followed Vellinga (1988). Color designation refers to HTML Color Codes (https://htmlcolorcodes.com). The abbreviation [x/y/z] indicates that measurements were made on x basidiospores in y basidiocarp from z collections. Basidiospore size and length / width ratio (Q) are given in the form of (a–)b–m–c(–d). The range b–c contains 95% of the measured values, a and d refer to the extremes of all measurements, and m means average value.

DNA extraction, PCR and sequencing

Genomic DNA was extracted from fresh basidiocarp using a modified CTAB procedure of Doyle & Doyle (1987). ITS was amplified using primer pair ITS1 and ITS4 (White *et al.* 1990). nLSU, *rpb2* and *tef1* were amplified using the primers and protocols described in Buyck *et al.* (2018). The products obtained were purified using E.Z.N.A Gel Extraction Kit (OMEGA) and sequenced on an ABI3730xl DNA Analyzer (IGE, Xian, China). The newly generated sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank).

Phylogenetic analysis

Phylogenetic analyses based on ITS and combined sequences of nLSU-*tef1*-*rpb2* were performed using both maximum likelihood (ML) and Bayesian inference (BI) methods. The datasets were automatically aligned by MAFFT ver. 7.427 (Katoh & Standley 2013), then manually adjusted and trimmed with BioEdit ver. 7.0.9 (Hall 1999). Maximum likelihood analysis was executed in RAxML ver. 7.2.6 (Stamatakis 2006), followed by a rapid bootstrapping (BS) algorithm of 1000 replicates and heuristic ML search for the best tree using the GTR+G substitution model. The best partitioning scheme and evolutionary models for BI analyses were selected using PartitionFinder2 with greedy algorithm and AICc criterion (Lanfear *et al.* 2017). Bayesian inference analyses were performed with MrBayes ver. 3.1.2, two independent runs were computed with four MCMC chains for 10 million generations until the split frequencies fell below 0.01. Trees were sampled every 1000 generations. The first 25% of trees were discarded as burn-in. The remaining 75% of trees were used for computation of Bayesian posterior probabilities (PPs), following a 50% majority-consensus rule. The convergence of runs and burn-in phase were assessed using Tracer ver. 1.7 (Rambaut *et al.* 2018).

Results

Phylogeny

The final ITS alignment contained 98 sequences (Table 1) with 813 bp including gaps, *R. emetica* (Schaeff.) Pers. belonging to subg. *Russula* core clade was chosen as outgroup. For BI analysis of the ITS region, the best substitution model is GTR+G+I. The 3-locus dataset comprised of 50 sequences representing 44 taxa (Table 2) with 2600 bp in length (nLSU 893 bp, *tef1* 932 bp, *rpb2* 775 bp), using *R. glutinosa* Fatto and *R. glutinosoides* Buyck & X.H.Wang as outgroups. For BI analysis of the multi-locus region, the best substitution models for five predefined partitions are GTR+I+G for nLSU, SYM+I+G for *rpb2* exons, HKY+G for *rpb2* introns, SYM+I+G for *tef1* exons, GTR+I+G for *tef1* introns.

Russula griseorosea sp. nov. nested well in subsect. *Puellarinae* Singer. The species clustered with an uncultured *Russula* clone sequence (KY684526) collected from China in the ITS phylogram, with sequence similarity more than 99% (Fig. 1). *Russula griseorosea* was well resolved in the phylogenetic analyses and showed close relationship to *R.* cf. *odorata* and *R. sinoparva* C.L.Hou, H.Zhou & G.Q.Cheng. *Russula micangshanensis* sp. nov. was undoubtedly classified into subsection *Olivaceinae* Singer, and highly supported (BS = 100%, PP = 1) in the ITS phylogram (Fig. 1). *Russula minirosea* sp. nov. was resolved into subsect. *Laricinae* Romagn., and closely related to *R. nauseosa* (Pers.) Fr. and *R. laricina* Velen. In the ITS phylogram, *R. minirosea* clustered with another five unidentified or *R.* cf. *nauseosa* sequences (KX441101, MW554359, GU371293, KX655853 and MT678891) all collected from China, with sequence similarity more than 99% between each other. The species gained high support in multi-locus phylogram (BS = 100%, PP = 1) (Fig. 2). *Russula purpureomarginalis* sp. nov. was nested in subsect. *Xerampelinae* Singer and significantly supported in the ITS phylogram (Fig. 1).

Table 1 (continued on next page). Sequences used in phylogenetic analysis based on ITS. Newly generated sequences are shown in **bold**.

Taxon	Voucher specimen	Location	ITS accession numbers
R. abbottabadensis	FH00304589 (holotype)	Pakistan	MG386704
R. adulterina	489RUS25	Europe	AY061651
R. adwanitekae	CAL1821 (holotype)	India	MN263242
R. alutacea	69IJ27	Czech Republic	MG680185
R. aurantioflammans	r3245	Slovakia	KU928167
R. ayubiana	LAH35439 (holotype)	Pakistan	MZ358868
R. brunneopurpurea	LAH35058 (holotype)	Pakistan	KT953613
R. brunneoviolacea	MC01-507	Denmark	AM113956
R. cf. *nauseosa*	LL-31	China	MT678891
R. cf. *nauseosa*	E0866	China	GU371293
R. changbaiensis	HMAS262369 (holotype)	China	KC412162
R. clavatohyphata	CAL1756 (holotype)	India	MG934209
R. coronaspora	GDGM79711 (holotype)	China	MN275689
R. cremeirosea	TENN:069929	USA	KT933983
R. cuprea	FH12250	Germany	KT934010
R. curtipes	FH-12-206 (GENT)	Germany	KT933995
R. dryadicola	TURA 152390	Finland	MG386712
R. emeticicolor	FH12253	Germany	KT934011
R. favrei	SAV:F-2254	Czech Republic	KU205324
R. flavobrunnescens	AK5024	Mexico	MN130082
R. font-queri	FH-12-223 (GENT)	Germany	KT934003
R. gnathangensis	CAL1733 (holotype)	India	MK253441
R. griseorosea **sp. nov.**	**SERC2209 (holotype)**	**China**	**OP828696**
R. griseorosea **sp. nov.**	**SERC2210**	**China**	**OP828697**
R. heilongjiangensis	HMAS255142 (holotype)	China	MG719932
R. juniperina	FH-RUS-11121210 (GENT)	Spain	KU886596
R. kashmiriana	LAH36936 (holotype)	Pakistan	MW882233
R. katarinae	BB03.159 (PC) (holotype)	USA	KP966377
R. khinganensis	HMAS278895 (holotype)	China	MG719928
R. laricina	BB 08.681	Italy	JN944008
R. lilacea	BPL645	Slovakia	KY509453
R. maculata	PC:0084521	France	KU928153
R. madrensis	AK3422 (holotype)	Mexico	MN130093
R. mansehraensis	HUP-SUR180 (holotype)	Pakistan	MG948636
R. mediterraneensis	GK6710 (holotype)	Greece	MK105660
R. messapica var. *messapica*	ALV1991	Spain	MK105669
R. micangshanensis **sp. nov.**	**SERC2201 (holotype)**	**China**	**OP828702**
R. micangshanensis **sp. nov.**	**SERC2202**	**China**	**OP828703**
R. micangshanensis **sp. nov.**	**SERC2303**	**China**	**PP062888**
R. micangshanensis **sp. nov.**	**SERC2304**	**China**	**PP062889**
R. micangshanensis **sp. nov.**	**SERC2305**	**China**	**PP062890**
R. micangshanensis **sp. nov.**	**SERC2306**	**China**	**PP062891**
R. micangshanensis **sp. nov.**	**SERC2307**	**China**	**PP062893**
R. micangshanensis **sp. nov.**	**SERC2308**	**China**	**PP062894**
R. minirosea **sp. nov.**	**SERC2205 (holotype)**	**China**	**OP828699**
R. minirosea **sp. nov.**	**SERC2206**	**China**	**OP828698**
R. minirosea **sp. nov.**	**SERC2207**	**China**	**OP828700**
R. minirosea **sp. nov.**	**SERC2208**	**China**	**OP828701**
R. minirosea **sp. nov.**	**SERC2302**	**China**	**PP062887**
R. minor	GDGM79686 (holotype)	China	MN275666

Table 1 (continued). Sequences used in phylogenetic analysis based on ITS. Newly generated sequences are shown in **bold**.

Taxon	Voucher specimen	Location	ITS accession numbers
R. miyunensis	BJTC Z1355 (holotype)	China	OP133219
R. nauseosa	MYH2013100345	China	KX655853
R. nauseosa	FH-12-173 (GENT)	Germany	KT933985
R. nitida	FH12218	Germany	KT934001
R. nympharum	FH11121505 (holotype)	Spain	KU928157
R. odorata	BB 07.186	Slovakia	JN944010
R. olivacea	SAV F-3943	Slovakia	KY582715
R. paludosa	FH12216	Germany	KT934000
R. paragraveolens	HMAS281158 (holotype)	China	OQ871504
R. plana	BJTC Z1398 (holotype)	China	OP133220
R. pseudochamaeleontina	JMT-17082506 (holotype)	France	MZ567218
R. pseudograveolens	HMAS287384 (holotype)	China	OQ871496
R. puellaris	nl1372 (TUB)	Germany	AF418628
R. puellula	SAV F-3107	Slovakia	KY582704
***R. purpureomarginalis* sp. nov.**	**SERC2211 (holotype)**	**China**	**OP828710**
***R. purpureomarginalis* sp. nov.**	**SERC2212**	**China**	**OP828711**
R. quercus-floribundae	LAH36219 (holotype)	Pakistan	MN053395
R. rubiginosus	HNJAU58933 (holotype)	China	OL828349
R. rubricolor	LAH35071 (holotype)	Pakistan	MK142796
R. rugulosa	BPL654	USA	KY509494
R. sancti-pauli	BB 06.494	Mexico	MN130101
R. sapinea	PA38	Latvia	KR019818
R. shigatseensis	HMAS287389 (holotype)	China	OQ871501
R. sichuanensis	HKAS53792 (holotype)	China	JX391969
R. sinoparva	BJTC Z441 (holotype)	China	OP133221
R. solaris	BB 07.282	Slovakia	JN944007
R. sp.	Q-L-OTU168	China	KY684526
R. sp.	130281MFBPL0206	China	MW554359
R. sp.	HMAS 267755	China	KX441101
R. subrubescens	TENN F18339	USA	NR153232
R. subrutilans	RITF1874 (holotype)	China	KJ868237
R. subsulphurea	TENN:F18743	USA	NR153231
R. subversatilis	BJTC C653 (holotype)	China	MW554190
R. tengii	HMAS262728 (holotype)	China	MG386708
R. uttarakhandia	CAL 1537 (holotype)	India	KY873997
R. versatilis	R55	Czech Republic	MG687329
R. versicolor	BB 07.288	Slovakia	JN944009
R. vidalii	JMV20160517-1 (BCN)	Spain	MK105694
R. vinosobrunneola	HMAS281138 (holotype)	China	MG719927
R. violaceoincarnata	O73136	Netherland	GU234047
R. viridofusca	UBC:F23777	Canada	KC581298
R. xerampelina	UE14.09.2004-3 (UPS)	Sweden	KU205278
R. xerampelinoides	KD 19-001 (holotype)	India	MN922560
R. yadongensis	HMAS287386 (holotype)	China	OQ871498
R. yanheensis	GACP13100308 (holotype)	China	KY195927
R. yanshanensis	BJTC C561 (holotype)	China	MW554232
Outgroup			
R. emetica	lw81 (TUB)	Germany	AF418619
R. emetica	UE05.10.2003-11 (UPS)	Sweden	DQ421997

Table 2 (continued on next page). Sequences used in the multi-locus phylogenetic analysis. Newly generated sequences are shown in **bold**.

Taxon	Voucher specimen	Location	Accession number			Reference
			nucLSU	*rpb2*	*tef1*	
R. aff. *fucosa*	BB 06.596	Canada	KU237457	KU237743	KU237892	Buyck *et al.* 2018
R. aff. *xerampelina*	DM fl07-14	USA	KU237576	KU237862	KU238004	Buyck *et al.* 2018
R. carpini	BB 07.262	Slovakia	KU237543	KU237829	KU237973	Buyck *et al.* 2018
R. cf. *appalachiensis*	BPL250	USA	–	KT933893	–	Looney *et al.* 2016
R. cf. *aurantioflammans*	BB 06.603	Canada	KU237488	KU237774	KU237917	Buyck *et al.* 2018
R. cf. *brunneoviolacea*	BB 06.606	Canada	KU237516	KU237802	KU237946	Buyck *et al.* 2018
R. cf. *decipiens*	BPL266 (TENN)	USA	KT933827	KT933899	–	Looney *et al.* 2016
R. cf. *decipiens*	BB 06.521	Mexico	KU237482	KU237768	KU237911	Buyck *et al.* 2018
R. cf. *katarinae*	BB 06.617	Canada	KU237460	KU237746	KU237895	Buyck *et al.* 2018
R. cf. *odorata*	BB 07.219	Slovakia	KU237517	KU237803	KU237947	Buyck *et al.* 2018
R. cf. *rugulosa*	BPL237 (TENN)	USA	KT933814	KT933885	–	Looney *et al.* 2016
R. cf. *sejuncta*	BB 08.143	Madagascar	KU237547	KU237833	–	Buyck *et al.* 2018
R. cf. *versicolor*	FH12259 (GENT)	Germany	KT933873	KT933944	–	Looney *et al.* 2016
R. cf. *vinosobrunea*	BB 07.231	Slovakia	KU237525	KU237811	KU237955	Buyck *et al.* 2018
R. changbaiensis	HMAS262355	China	KX441304	KX442045	MF893389	–
R. citrinolutea	BB 06.611	Canada	KU237459	KU237745	KU237894	Buyck *et al.* 2018
R. coronaspora	GDGM79711 (holotype)	China	MN839580	MT085657	MT085600	Song *et al.* 2021
R. cremeirosea	BPL289 (TENN)	USA	KT933844	KT933915	–	Looney *et al.* 2016
R. curtipes	FH12206 (GENT)	Germany	KT933856	KT933927	–	Looney *et al.* 2016
R. decipiens	BB 07.178	Slovakia	KU237569	KU237855	KU237997	Buyck *et al.* 2018
R. flavobrunnescens	AK5024 (holotype)	Mexico	–	MN380530	–	Adamčík *et al.* 2019
R. font-queri	FH12223 (GENT)	Germany	KT933864	KT933935	–	Looney *et al.* 2016
R. griseorosea **sp. nov.**	**SERC2209 (holotype)**	**China**	**OP828718**	**OP831169**	**OP857222**	**Present study**
R. laricina	BB 08.681	Italy	KU237560	KU237846	KU237991	Buyck *et al.* 2018
R. madrensis	AK3422 (holotype)	Mexico	–	MN380520	–	Adamčík *et al.* 2019
R. melliolens	BB 07.194	Slovakia	KU237545	KU237831	KU237975	Buyck *et al.* 2018
R. micangshanensis **sp. nov.**	**SERC2201 (holotype)**	**China**	**OP828723**	**OP831174**	**–**	**Present study**
R. minirosea **sp. nov.**	**SERC2205 (holotype)**	**China**	**OP828720**	**OP831171**	**OP857224**	**Present study**
R. minirosea **sp. nov.**	**SERC2206**	**China**	**OP828719**	**OP831170**	**OP857223**	**Present study**
R. minirosea **sp. nov.**	**SERC2207**	**China**	**OP828721**	**OP831172**	**OP857225**	**Present study**
R. minirosea **sp. nov.**	**SERC2208**	**China**	**OP828722**	**OP831173**	**OP857226**	**Present study**
R. nauseosa	BB 07.285	Slovakia	KU237572	KU237858	KU238000	Buyck *et al.* 2018
R. nauseosa	FH12173 (GENT)	Germany	KT933846	KT933917	–	Looney *et al.* 2016
R. nitida	FH12218 (GENT)	Germany	KT933862	KT933933	–	Looney *et al.* 2016
R. obscurosordida	BB 06.564	Canada	KU237575	KU237861	KU238003	Buyck *et al.* 2018
R. odorata	BB 07.186	Slovakia	KU237518	KU237804	KU237948	Buyck *et al.* 2018
R. olivacea	BB 07.223	Slovakia	KU237492	KU237778	KU237921	Buyck *et al.* 2018
R. minor	GDGM79686 (holotype)	China	MK881964	MK880691	MT085599	Song *et al.* 2021
R. minor	GDGM79689	China	MN839576	MT085653	MT085624	Song *et al.* 2021
R. puellaris	BB 07.311	Slovakia	KU237515	KU237801	KU237945	Buyck *et al.* 2018
R. purpureomarginalis **sp. nov.**	**SERC2211 (holotype)**	**China**	**OP828727**	**OP831178**	**OP857230**	**Present study**
R. rugulosa	BPL654	USA	–	KY701373	KY701415	Looney *et al.* 2020

Table 2 (continued). Sequences used in the multi-locus phylogenetic analysis. Newly generated sequences are shown in **bold**.

Taxon	**Voucher specimen**	**Location**	**Accession number**			**Reference**
			nucLSU	*rpb2*	*tef1*	
R. sinoparva	BJTC C540	China	OP133234	OP156829	OP156839	Zhou *et al.* 2022
R. solaris	BB 07.282	Slovakia	KU237549	KU237835	KU237978	Buyck *et al.* 2018
R. tlaxcalae	BB 06.542	Mexico	KU237463	KU237749	KU237897	Buyck *et al.* 2018
R. subversatilis	BJTC C653 (holotype)	China	OP133238	OP156832	OP156844	Zhou *et al.* 2022
R. versicolor	BB 07.288	Slovakia	KU237573	KU237859	KU238001	Buyck *et al.* 2018
R. yanshanensis	BJTC Z1305	China	OP133243	OP156834	OP156848	Zhou *et al.* 2022
Outgroup						
R. glutinosa	Roody WRWV 04.1154 (holotype)	USA	MN315511	MN326798	MN326799	Buyck *et al.* 2020
R. glutinosoides	HKAS106678 (holotype)	China	MN428827	–	–	Buyck *et al.* 2020

Taxonomy

Order Russulales Kreisel ex P.M.Kirk, P.F.Cannon & J.C.David
Family Russulaceae Lotsy
Genus *Russula* Pers.

Russula griseorosea Y.Song sp. nov.

MycoBank: MB847717
Figs 1–4, 11E–F

Diagnosis

Russula griseorosea sp. nov. differs from the phylogenetically closely related *R. odorata* Romagn. by its grayish pink pileus and irregularly bifurcated lamellae; while the equally close *R. sinoparva* has very small fruiting bodies (pileus 1.8–3.3 cm in diam.), a pink to deep red pileus and pileocystidia that turn light ash black in SV.

Etymology

The name refers to the mostly grayish pink color of the pileipellis.

Type material

Holotype

CHINA • Shaanxi Province, Hanzhong City, Lianchen Mountain; 33°12′25″ N, 106°57′39″ E; 593 m a.s.l.; 3 Sep. 2021; *Y. Song*, *K21090308*; gregarious on the ground in mixed coniferous and broad-leaf forest, mainly with *Quercus variabilis* Bl., *Q. aliena* Bl., *Q. acutissima* Carruth. and *Pinus massoniana* Lamb.; GenBank nos: OP828696 (ITS), OP828718 (LSU), OP831169 (*rpb2*), OP857222 (*tef1*); SERC [SERC2209].

Additional material examined

CHINA • Shaanxi Province, Hanzhong City, Lianchen Mountain; 33°12′25″ N, 106°57′39″ E; 593 m a.s.l.; 13 Sep. 2021; *Y. Song*, *K21091306*; gregarious on the ground in mixed coniferous and broad-leaf forest, mainly with *Quercus variabilis* Bl., *Q. aliena* Bl., *Q. acutissima* Carruth. and *Pinus massoniana* Lamb.; GenBank no: OP828697 (ITS); SERC [SERC2210].

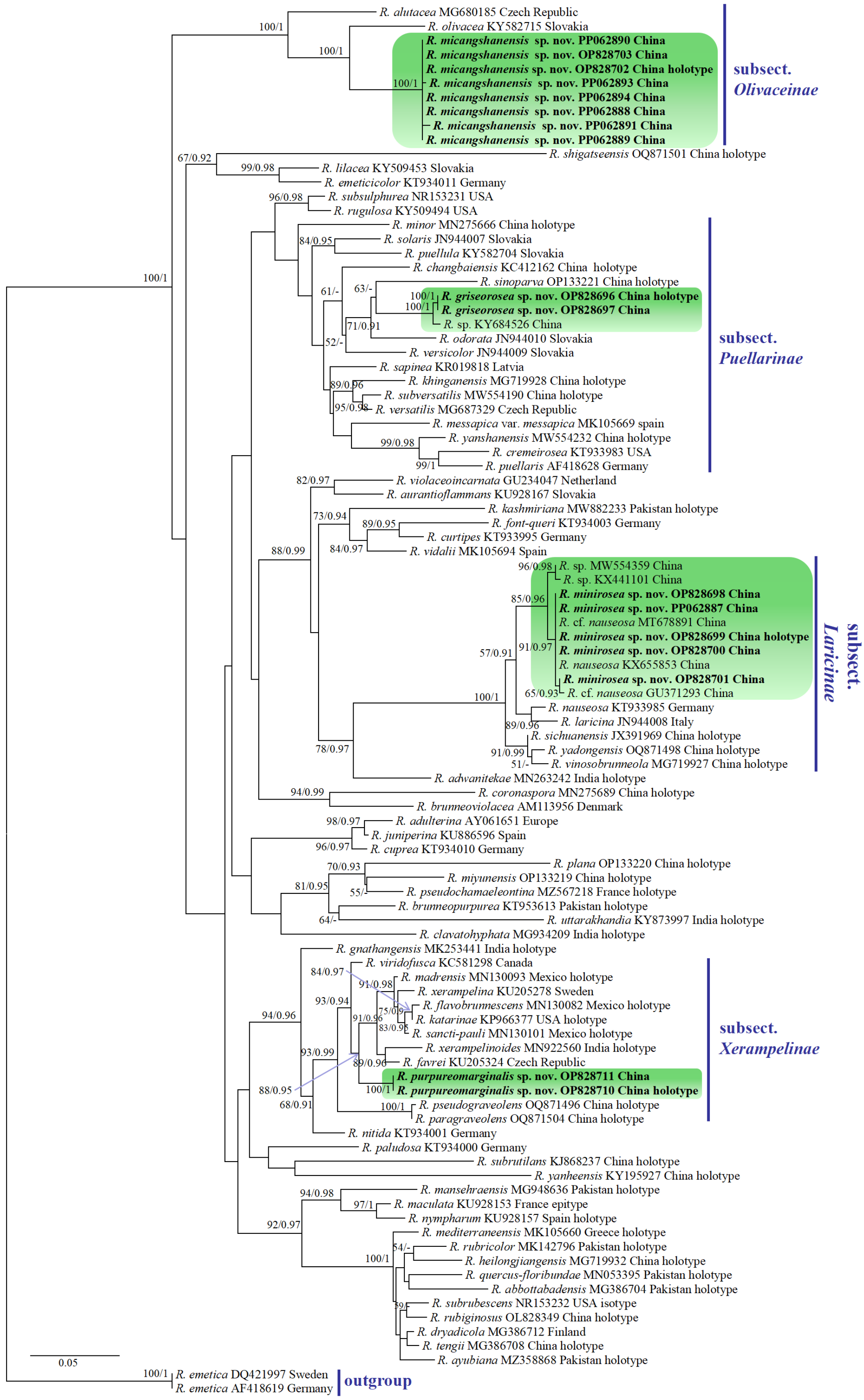

Fig. 1. Maximum likelihood tree of subg. *Russula* crown clade based on ITS sequences, bootstrap (BS) values higher than 50% and posterior probability (PP) values higher than 0.9 are displayed around nodes. Newly generated sequences are shown in bold and clades with four new species are highlighted in green.

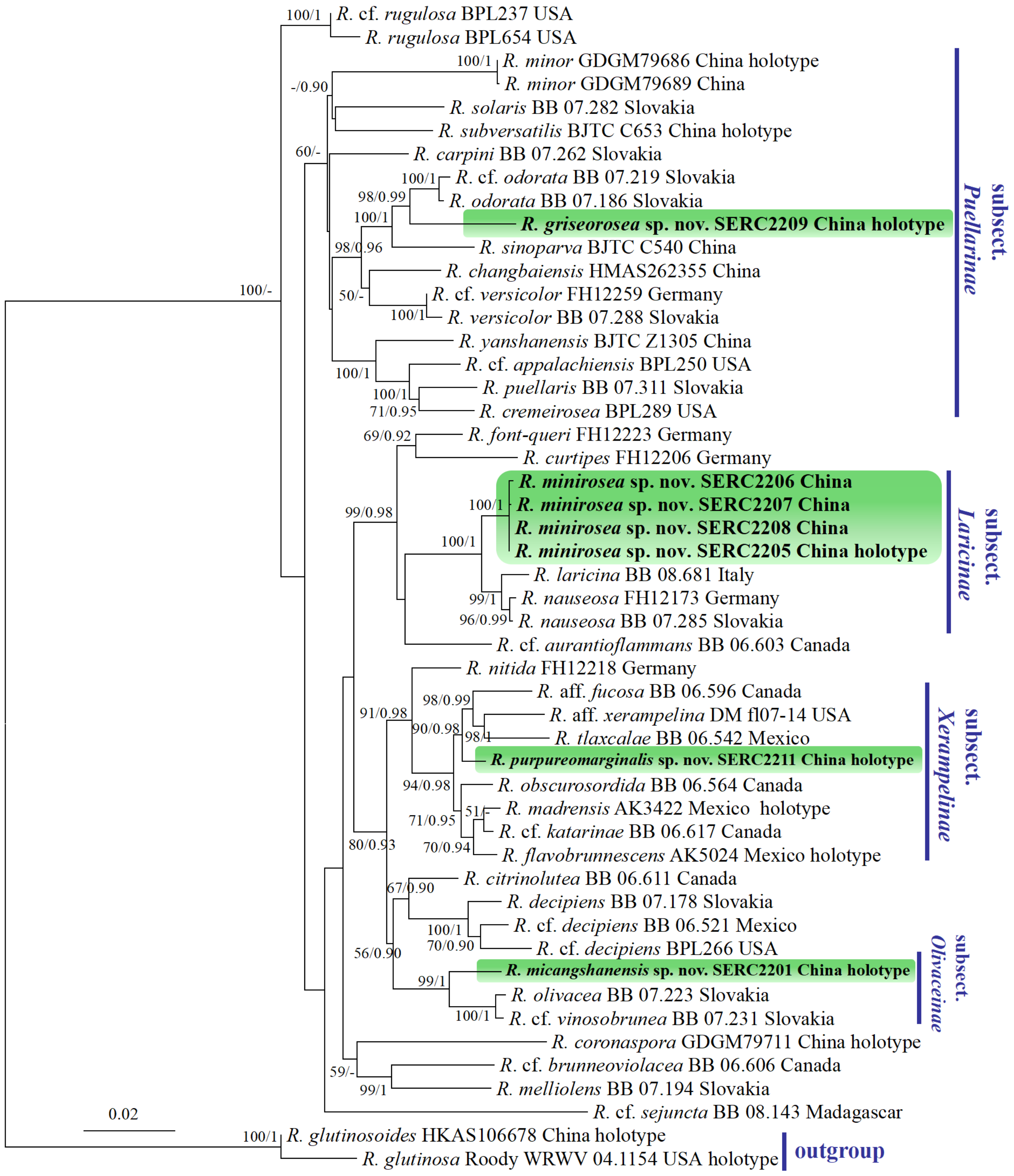

Fig. 2. Maximum likelihood tree of subgen. *Russula* crown clade based on 3-locus (nLSU-*tef1*-*rpb2*) combined sequences, bootstrap (BS) values higher than 50% and posterior probability (PP) values higher than 0.9 are presented around nodes. Newly generated sequences are shown in bold and four new species are highlighted in green.

Description

Basidiomata relatively small sized. Pileus 3–6 cm in diam., hemispherical to convex when young, turning applanate with depressed center at maturity; surface viscid, easy to peel off ⅔ radius when mature, grayish pink (#F8F3DD) when young, then grayish (#FAF9F5) at margin, reddish brown (#E5AB70) at center; margin smooth and entire at first, turning striate or sulcate with age. *Lamellae* adnate, interveined, white (#FEFEFE), often stained with rust brown (#F0BF8E) when bruised; edge concolorous, smooth. *Stipe* central, fleshy, fragile, smooth, cylindrical, often tapering upwards, solid at first, turning hollow with age, 2.5–7 × 0.7–1.5 cm, chalky white to white (#FCF3CF, #FBFCFC), often stained with grayish brown (#E5B280) when bruised. *Context* white (#FCF3CF), unchanging when bruised. *Taste* mild. *Odor* indistinct. *Spore print* not observed.

Basidiospores subglobose to broad ellipsoid, (40/2/2) (6.5–)6.7–7.2–7.5(–8.0) × (5.4–)5.6–5.9–6.3(–6.6) µm, [Q = (1.10–)1.15–1.21–1.24(–1.29)], hyaline in 5% KOH; ornamentation amyloid, conical to cylindircal warts less than 1.5 µm in height, moderately distant to dense ((4–)6–7 in a 3 µm diam. circle), often fused into short crests or chains (1–3(–5) in the circle), connected by fine lines, line connections dispersed (1–2 in the circle), forming partial reticulum; suprahilar spot amyloid. *Basidia* (30–)31.5–35–39(–42) × (8.5–)10–11.5–12.5 µm, clavate, 2- or 4-spored, thin-walled, with oil droplets; *sterigmata* 3.4–5.5–8.5 × 1.4–2.0–2.6 µm. *Pleurocystidia* subcylindrical to subclavate, (37.5–)40.5–52.5–67(–92) × 8–10–13(–14.5) µm, with obtuse, papillate or mucronate apices, thin-walled, with irregular refractive contents, projecting up to 24 µm, unchanging in SV. *Cheilocystidia* similar to pleurocystidia in shape, but relatively smaller, measuring (29–)35.5–46–53(–60) × 7–10–12 µm. *Lamellar trama* composed of numerous sphaerocytes surrounded by connective hyphae, sphaerocytes measuring 8–43 × 5–36 µm. *Subhymenium* pseudoparenchymatous. *Pileipellis* orthochromatic in cresyl blue, thin, 80–150 µm thick, gelatinized, divided into two layers: suprapellis 30–60 µm thick, composed of ascending to erect hyphae and pileocystidia; subpellis thin, 40–110 µm deep, composed of more horizontal hyphae and pileocystidia; hyphae 2.5–6 µm in diam. *Acidoresistant incrustations* not observed. *Hyphal terminations* near the pileus margin thin-walled, occasionally narrow; terminal cells measuring 9–17.5–29(–50) × 2.5–3.5–5 µm, subcylindrical, with obtuse or sharp apices; subterminal cells usually equally wide. *Hyphal terminations* near the pileus centre of similar structure; terminal cells measuring (11–)12.5–18–25.5(–33) × 2–3–4 µm, cylindrical or slightly narrowed towards apex, apically obtuse-rounded; subterminal cells usually equally wide. *Pileocystidia* near the pileus margin (29–)31–58–121(–135.5) × 4–6–8 µm, abundant, 1- to 5-celled, thin-walled, cylindrical to subclavate, flexuous, with obtuse apices, with refractive contents, unchanging in SV. *Pileocystidia* near the pileus center of similar shape, 1- to 5-celled, abundant, measuring (28–)31–47.5–97(–103) × 3–6–9 µm, with refractive contents, unchanging in SV. *Cystidioid hyphae* in subpellis and context with granulose or crystalline contents. *Oleiferous hyphae* in the subpellis. *Clamp connections* absent in all tissues.

Remarks

Russula griseorosea sp. nov. belongs to the subsect. *Puellarinae*, a group mainly comprised of small russulas. *Russula sinoparva*, described from China (Zhou *et al.* 2022), is also a member of the subsect. *Puellarinae* and closely related to *R. griseorosea* in the phylogeny. Both species are somewhat similar in spore and basidia size and have septate pileocystidia, but *R. sinoparva* has a very small fruiting body (pileus 1.8–3.3 cm in diam.), a pink to deep red pileus and pileocystidia turning light ash black in SV, which differs from *R. griseorosea*. *Russula yanshanensis* C.L.Hou, H.Zhou & G.Q.Cheng, *R. subversatilis* C.L.Hou, H.Zhou & G.Q.Cheng and *R. khinganensis* G.J.Li & R.L.Zhao reported from China also belong to subsect. *Puellarinae*. *Russula yanshanensis* is diagnosed by a light pink to pink pileus, basidiospores ornamented with short warts (less than 0.6 µm in height); *R. subversatilis* has a light gray-red to deep red pileus, and pileocystidia becoming light red in SV (Zhou *et al.* 2022); *R. khinganensis* is characterized by a blackish red pileus and slender hymenial cystidia (6–9 µm in diam.) becoming blackish in SV (Li

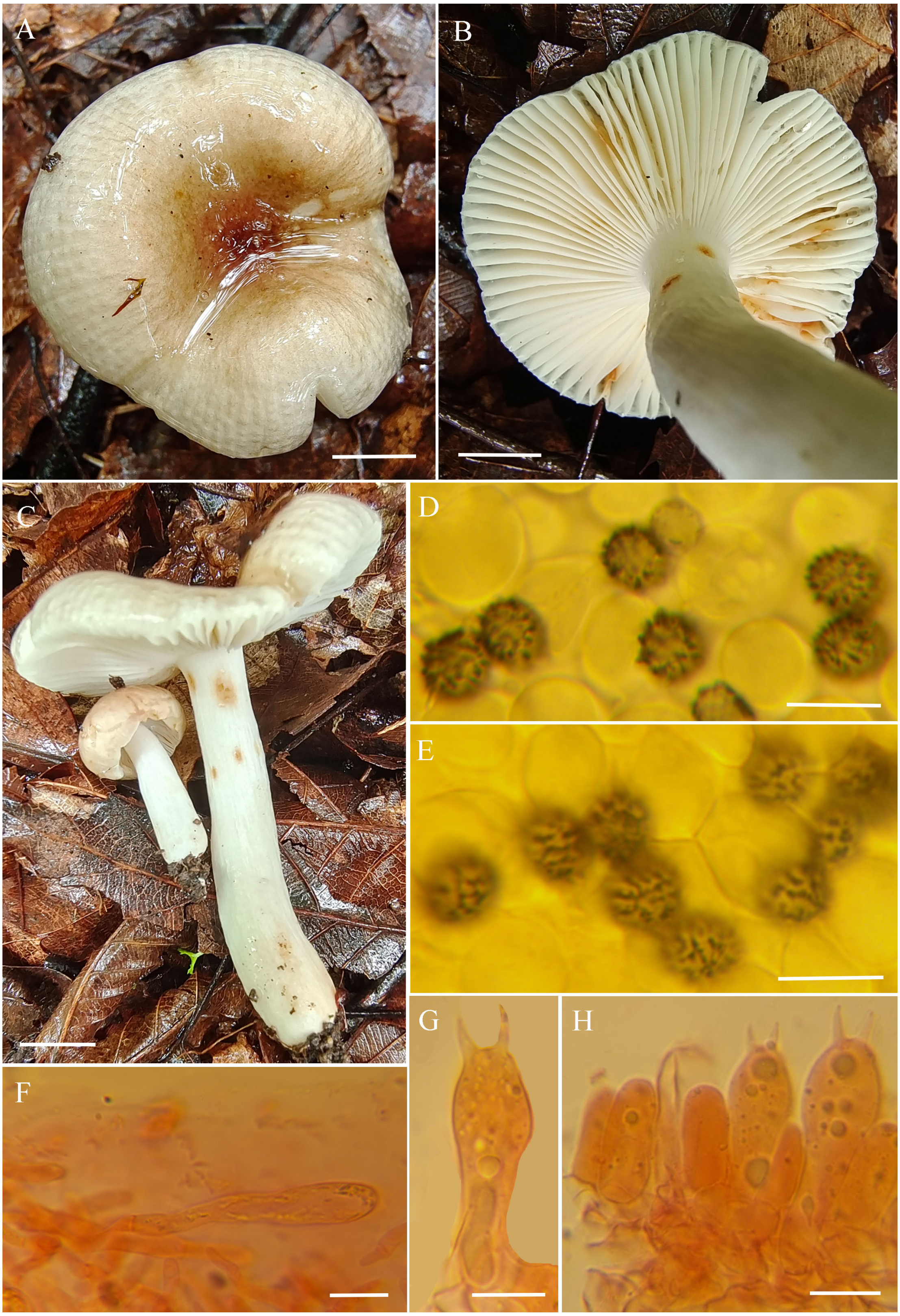

Fig. 3. *Russula griseorosea* Y.Song sp. nov., holotype (SERC2209). **A–C**. Fruiting bodies. **D–E**. Basidiospores in Melzer's reagent showing the amyloid ornamentations. **F**. Pileocystidia near pileus margin. **G–H**. Basidia and basidiole in the hymenium. Scale bars: A–C = 1 cm; D–H = 10 µm.

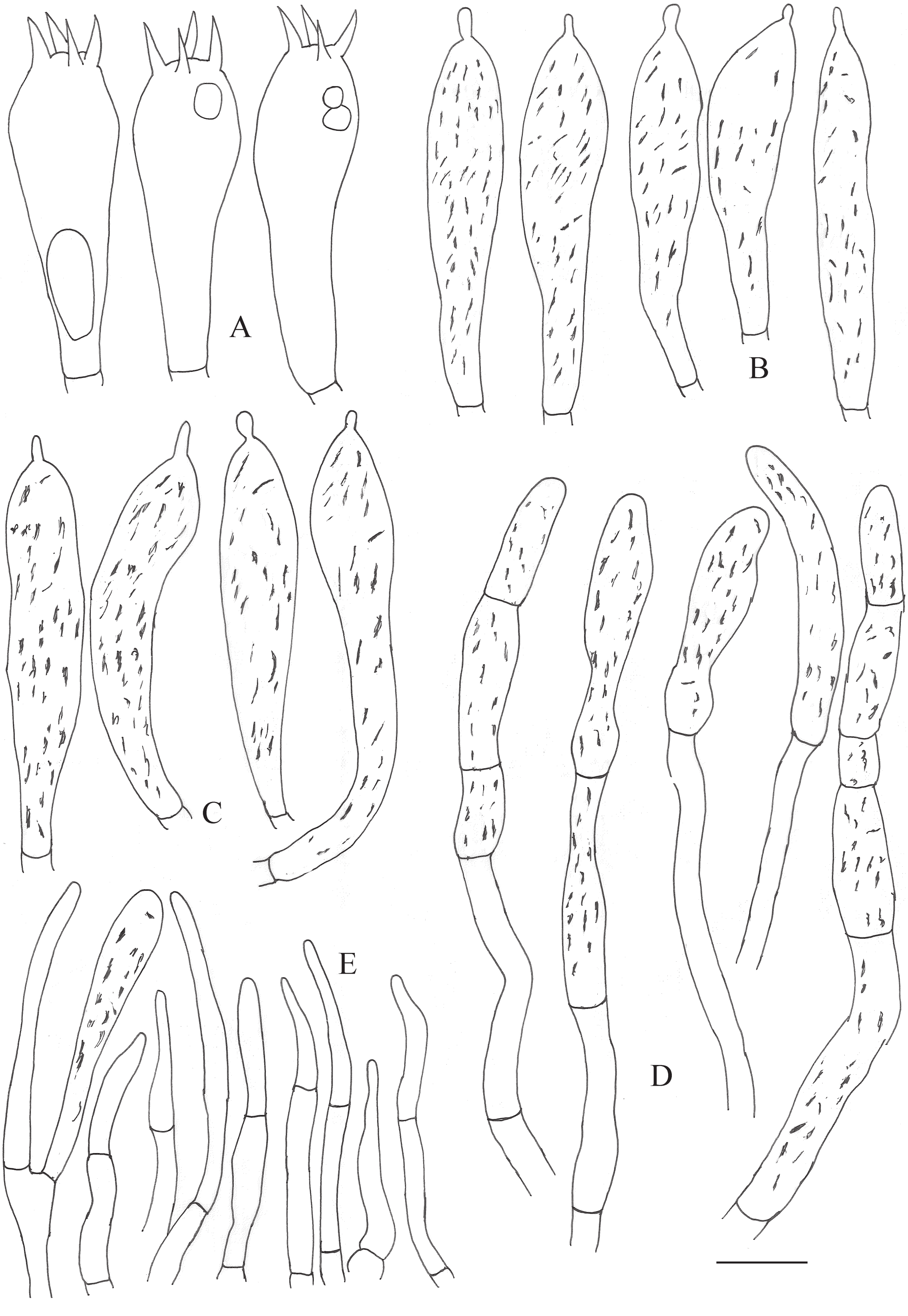

Fig. 4. Line drawings of *Russula griseorosea* Y.Song sp. nov., holotype (SERC2209). **A**. Basidia. **B**. Cheilocystidia. **C**. Pleurocystidia. **D**. Pileocystidia near pileus margin. **E**. Hyphal terminations near pileus margin. Scale bar = 10 µm.

et al. 2018b), all differ from *R. griseorosea*. The European *R. odorata*, another phylogenetically related species, can be distinguished from *R. griseorosea* by its robust fruiting bodies, a central brown or olive pileus without a pinkish tint and irregularly bifurcated lamellae.

Russula micangshanensis Y.Song sp. nov.

MycoBank: MB847656

Figs 1–2, 5–6, 11A–B

Diagnosis

Russula micangshanensis sp. nov. differs from other species of the subsection *Olivaceinae* by a unique combination of characteristics: spore ornamentation composed of unequal crests and often twinned warts, large basidia (43.5–70.5 × 12.5–17.5 µm], hymenial cystidia (62–157 × 7.5–17 µm) and basidiospores (8.1–10 × 6.5–8.8 µm), and sequence data.

Etymology

The name refers to the locality of the type specimen.

Type material

Holotype

CHINA • Shaanxi Province, Hanzhong City, Xi-xiang County, Micangshan Biosphere Reserve; 32°37′66″ N, 107°25′49″ E; 1010 m a.s.l.; 2 Aug. 2021; *Y. Song*, *K21080208*; gregarious on the ground in mixed coniferous and broad-leaf forest, mainly with *Quercus variabilis* Bl., *Q. aliena* Bl., *Q. acutissima* Carruth. and *Pinus massoniana* Lamb.; GenBank nos: OP828702 (ITS), OP828723 (LSU), OP831174 (*rpb2*); SERC [SERC2201].

Additional material examined

CHINA • Shaanxi Province, Hanzhong City, Xi-xiang County, Micangshan Biosphere Reserve; 32°37′66″ N, 107°25′49″ E; 1010 m a.s.l.; 2 Oct. 2021; *Y. Song*, *K21100211*; gregarious on the ground in mixed coniferous and broad-leaf forest, mainly with *Quercus variabilis* Bl., *Q. aliena* Bl., *Q. acutissima* Carruth. and *Pinus massoniana* Lamb.; GenBank no: OP828703 (ITS); SERC [SERC2202] • Hanzhong City, Mian County; 11 Aug. 2023; *Y. Song*, *Y.Y. Wang*, *23081122*; gregarious on the ground in mixed coniferous and broad-leaf forest; GenBank no: PP062888 (ITS); SERC [SERC2303] • Hanzhong City, Ning-qiang County; 21 Aug. 2023; *Y. Song*, *R. Yuan*, *23082119*; gregarious on the ground in mixed coniferous and broad-leaf forest, mainly with *Quercus variabilis* Bl., *Q. aliena* Bl., *Q. acutissima* Carruth. and *Pinus massoniana* Lamb.; GenBank no: PP062889 (ITS); SERC [SERC2304] • same data as for preceding; *Y. Song*, *23082126*; GenBank no: PP062890 (ITS); SERC [SERC2305] • same data as for preceding; *Y. Song*, *23082128*; GenBank no: PP062891 (ITS); SERC [SERC2306] • Hanzhong City, Nan-zheng District; 21 Aug. 2023; *Y. Song*, *23082139*; gregarious on the ground in mixed coniferous and broad-leaf forest; GenBank no: PP062893 (ITS); SERC [SERC2307] • same data as for preceding; *Y. Song*, *23082142*; GenBank no: PP062894 (ITS); SERC [SERC2308].

Description

Basidiomata medium to relatively large sized. Pileus 5.5–8 cm in diam., hemispherical to convex at first, turning applanate with depressed center at maturity; surface glabrous, dry, smooth, sometimes wrinkled concentrically near margin, bright crimson (#FA8072) to light tangerine (#F08080), darker at center, red (#CD5C5C) to light burgundy (#ED6755) when old, sometimes brown (#F9D278) at center; margin entire, often cracked when old. *Lamellae* adnate, cream (#FFFACD), becoming yellow (#F7D03A) with age, interveined, often forked; edge entire, concolorous, smooth. *Stipe* central, 4–8 × 1.5–3 cm, cylindrical,

Fig. 5. *Russula micangshanensis* Y.Song sp. nov. **A–H**. Fruiting bodies. **A, H**. Holotype (SERC2201). **B–C**. SERC2202. **D**. SERC2303. **E**. SERC2304. **F**. SERC2305. **G**. SERC2307. **I**. Basidiospores in Melzer's reagent showing the amyloid ornamentations (SERC2201). Scale bars: A–H = 1 cm; I = 10 µm.

sometimes tapering or inflating upwards, solid, fleshy, pinkish (#FFD6BE). *Context* white, not changing when bruised. *Taste* mild. *Odor* indistinct. *Spore print* cream (#FFFAD1).

Basidiospores subglobose to broadly ellipsoid, (80/4/4) (8.1–)8.3–9.1–9.8(–10) × (6.5–)6.8–7.8–8.7(–8.8) µm, [Q = (1.06–)1.09–1.15–1.27], hyaline in 5% KOH; ornamentation amyloid, composed

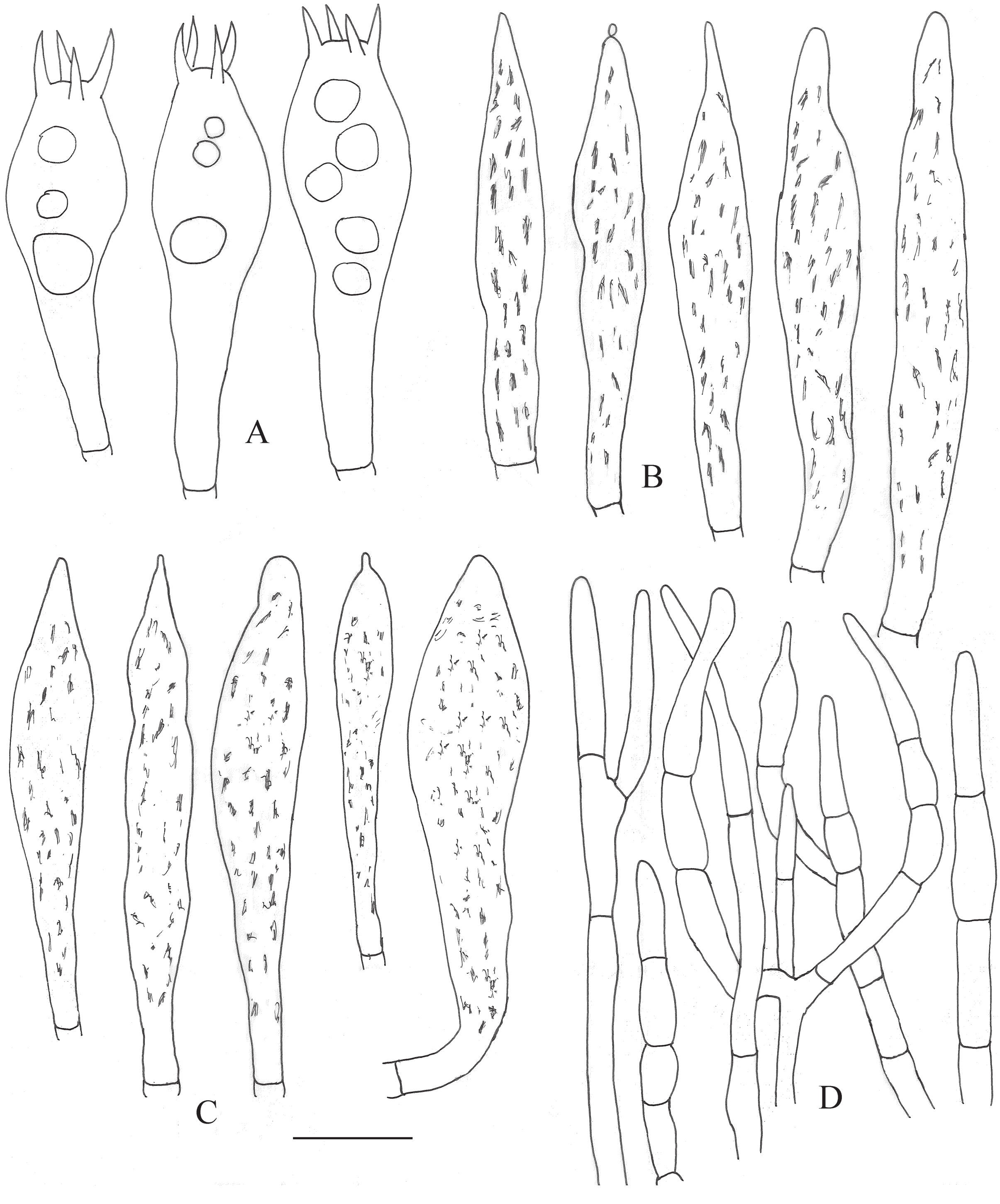

Fig. 6. Line drawings of *Russula micangshanensis* Y.Song sp. nov., holotype (SERC2201). **A**. Basidia. **B**. Cheilocystidia. **C**. Pleurocystidia. **D**. Hyphal terminations near pileus margin. Scale bar = 10 µm.

of blunt, cylindrical to subcylindrical warts less than 1.5 µm in height, moderately distant to dense ((4–)6–8 in a 3 µm diam. circle), often fused into short and unequal crests varying in height, warts often twinned, connected by fine lines forming a partial reticulum; suprahilar spot indistinct, amyloid. *Basidia* (43.5–)46–60–68(–70.5) × (12.5–)14–15.5–17.5 µm, long clavate, 4-spored, thin-walled, often with oil droplets; *sterigmata* (6.2–)6.4–7.1–8.7 × 1.8–2.2–2.7(–2.9) µm. *Pleurocystidia* (62–) 65–94–125 (–157) × (7.5–)11–14–17 µm, subcylindrical to fusiform, with papillate, mucronate apices, thin-walled, with irregular refractive contents, projecting up to 53 µm, unchanging in SV. *Cheilocystidia* 66.5–86.5–96 × 10–13–15 µm, fusiform, with papillate, mucronate apices, thin-walled, with irregular refractive contents, unchanging in SV. *Subhymenium* pseudoparenchymatous. *Marginal cells* not observed. *Lamellar trama* composed of numerous sphaerocytes surrounded by connective hyphae. *Pileipellis* orthochromatic in cresyl blue, vaguely divided into two layers: 60–120 µm deep suprapellis of ascending to erect hyphae and 30–60 µm deep subpellis composed of more horizontal and dense hyphae; hyphae 3–7 µm in diam., thin-walled, hyaline. *Acidoresistant incrustations* not observed. *Hyphal terminations* near the pileus margin flexuous, thin-walled, often narrowed; terminal cells measuring (12.5–)14–23–38.5(–40.5) × 3–4.5–6 µm, subcylindrical, with obtuse or tapering apices, often with slightly wider subterminal cells (up to 8 µm). *Hyphal terminations* near the pileus centre of similar structure; terminal cells measuring (12–)13–24–41.5(–5) × 3–4.5–6 µm, cylindrical or slightly narrowed towards apex, apically obtuse-rounded or acute; subterminal cells usually equally long but sometimes slightly wider (up to 8 µm). *Oleiferous hyphae* in the subpellis. *Pileocystidia* and *primordial hyphae* absent. *Clamp connections* absent in all tissues.

Remarks

The pileus surface typically wrinkled concentrically near the margin, the absence of dermatocystidia and primordial hyphae in both pileipellis and stipitipellis, and the orthochromatic pileipellis, all confirm the taxonomic placement of *R. micangshanensis* sp. nov. within the subsection *Olivaceinae*. This small group comprises few species, namely *R. olivacea* Pers., *R. alutacea* (Fr.) Fr. and *R. vinosobrunnea* (Bres.) Romagn. The cap color of *R. olivacea* is variable, initially olive (the reason for taking the species as a greenish *Russula*), later often tinged with red, purple, brown and even gray (Roberts 2007; Siegel & Schwarz 2016). But all eight specimens of *R. micangshanensis* mainly appear red and never show any olive tint. While *R. olivacea* resembles *R. micangshanensis* in its pinkish-vinaceous flushed stipe, cream-yellow gills and relatively large spores (8.5–10.5 × 7.5–9.0 µm), its non-reticulate spores differ from the latter species' spores, which are ornamented with unequal crests and often twinned warts forming a partial reticulum. Phylogenetically, the ITS sequence similarity between *R. micangshanensis* and *R. olivacea* is 96.69%, and they show a clear genetic distance in the multi-locus phylogram. The pileus color of *R. alutacea* often appears brown and dark red, while *R. vinosobrunnea* shows dark brown to dark purple, both differing from *R. micangshanensis* with its bright red cap. Additionally, the former two species have a fruity odor and sweet taste, while *R. micangshanensis* has an indistinct odor and mild taste. *Russula micangshanensis* has a wide distribution and a high yield in the Qinling mountains and is popularly consumed as an edible mushroom.

Russula minirosea Y.Song sp. nov.

MycoBank: MB847690
Figs 1–2, 7–8, 11C–D

Diagnosis

Russula minirosea sp. nov. can be separated from other morphologically and phylogenetically related species by a unique combination of characteristics: very small basidiocarps with thin context, peach pink to rosy pileus less than 3.3 cm in diam., basidiospores with low ornamentation and fine reticulum, septate pileocystidia and sequence data.

Fig. 7. *Russula minirosea* Y.Song sp. nov. **A–H**. Fruiting bodies. **A, D–E**. Holotype (SERC2205) **B**. SERC2206. **C**. SERC2207. **F–G**. SERC2208. **H**. SERC2302. **I**. Basidiospores in Melzer's reagent showing the amyloid ornamentations, holotype (SERC2205). Scale bars: A–H = 1 cm; I = 10 µm.

Etymology

The species name refers to its small basidiocarp often with rosy pileus.

Type material

Holotype

CHINA • Shaanxi Province, Hanzhong City, Micangshan Biosphere Reserve; 32°37′66″ N, 107°25′49″ E; 1010 m a.s.l.; 21 Sep. 2022; *Y. Song*, *K22092145*; gregarious on the ground in mixed coniferous and broad-leaf forest, mainly with *Quercus variabilis* Bl., *Q. aliena* Bl., *Q. acutissima* Carruth. and *Pinus massoniana* Lamb.; GenBank nos: OP828699 (ITS), OP828720 (LSU), OP831171 (*rpb2*), OP857224 (*tef1*); SERC [SERC2205].

Additional material examined

CHINA • Shaanxi Province, Hanzhong City, Liancheng Mountain; 33°12′25″ N, 106°57′39″ E; 593 m a.s.l. ; 6 Sep. 2021; *Y. Song, K21090617*; gregarious on the ground in mixed coniferous and broad-leaf forest; GenBank nos: OP828698 (ITS), OP828719 (LSU), OP831170 (*rpb2*), OP857223 (*tef1*); SERC [SERC2206] • Shaanxi Province, Hanzhong City, Han Mountain; 5 Oct. 2022; *Y. Song, K22100508*; gregarious on the ground in mixed coniferous and broad-leaf forest; GenBank nos: OP828700 (ITS), OP828721 (LSU), OP831172 (*rpb2*), OP857225 (*tef1*); SERC [SERC2207] • same data as for preceding;

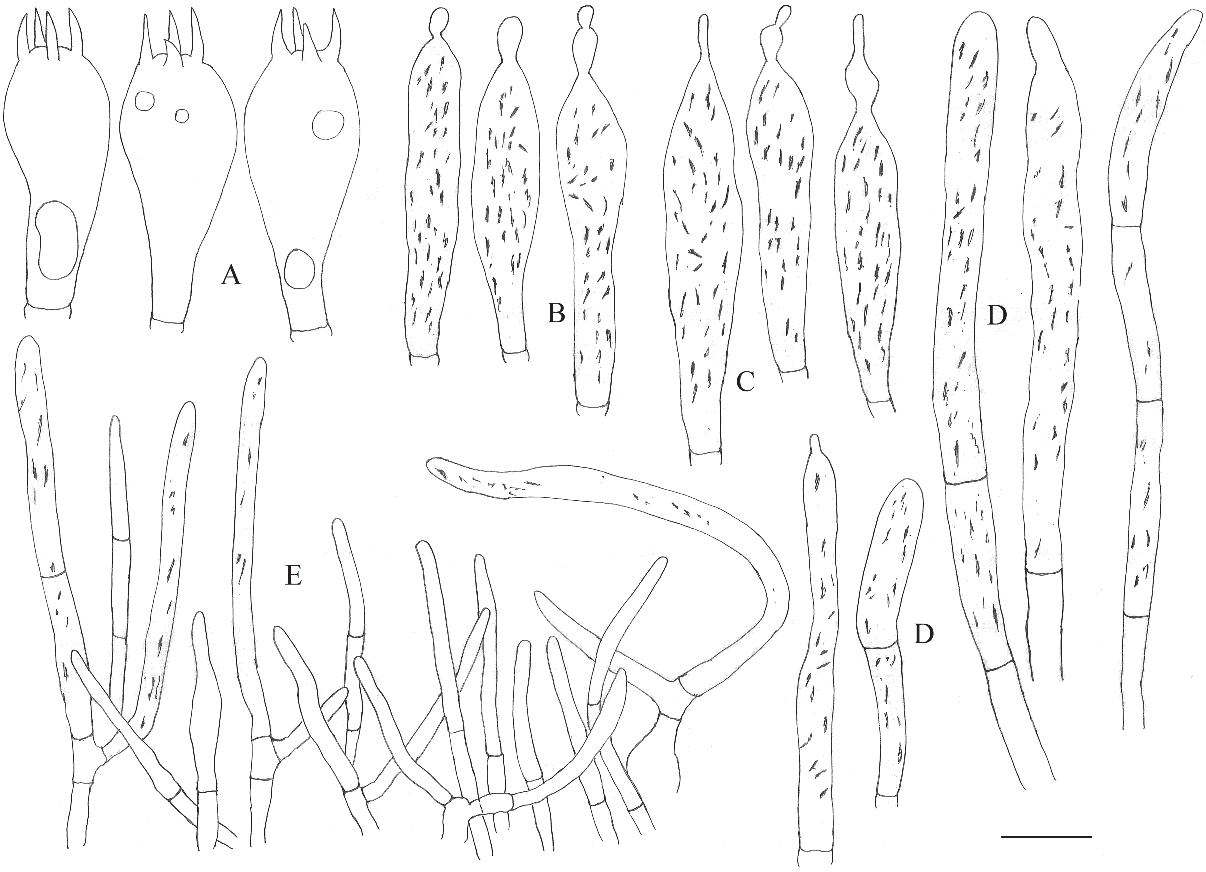

Fig. 8. Line drawings of *Russula minirosea* Y.Song sp. nov., holotype (SERC2205). **A**. Basidia. **B**. Cheilocystidia. **C**. Pleurocystidia. **D**. Pileocystidia near pileus margin. **E**. Hyphal terminations near pileus margin. Scale bar = 10 µm.

5 Oct. 2022; *Y. Song, K22100509*; GenBank nos: OP828701 (ITS), OP828722 (LSU), OP831173 (*rpb2*), OP857226 (*tef1*); SERC [SERC2208] • Hanzhong City, Nan-zheng District, Qing-shu Town; 30 Sep. 2023; *Y. Song*, *J.L. Yu*, *23093003*; gregarious on the ground in mixed coniferous and broad-leaf forest; GenBank no.: PP062887 (ITS); SERC [SERC2302].

Description

Basidiomata very small sized. Pileus 1.8–3.3 cm in diam., hemispherical to convex when young, turning applanate with depressed center at maturity; surface viscid, easy to peel off ⅔ radius when mature, dark blood red (#DE3C37) to deep rosea (#EC7063) when young, becoming peach pink (#F97D8E) at margin, fuchsia pink to rosy (#C85868, #DF828F) at center; margin smooth and entire at first, turning striate with age. *Lamellae* adnate, interveined, sometimes forked near stipe, white when young, turning yellowish cream (#FCF3CF, #FEF9E7) at maturity; edge concolorous, smooth; lamellulae rare, irregularly dispersed. *Stipe* central, fleshy, fragile, smooth, cylindrical, often tapering upwards, solid at first, turning hollow to multi-chambered with age, 2.5–3.9 × 0.5–1.3 cm, chalky white to white (#FCF3CF, #FBFCFC). *Context* white (#FCF3CF), unchanging when bruised. *Taste* mild. *Odor* indistinct. *Spore print* not observed.

Basidiospores subglobose to ellipsoid, (80/4/4) (6.5–)7–7.5–8(–8.3) × (5.5–)5.7–6.2–6.8 µm, [Q = 1.14–1.21–1.30(–1.45)], hyaline in 5% KOH; ornamentation amyloid, less than 0.8 µm in height, moderately distant to dense ((5–7(–8) in a 3 µm circle), forming a complete reticulum; suprahilar spot amyloid. *Basidia* (26.5–)28–33–39(–41) × 10–12–13.5(–15) µm, clavate, 4-spored, thin-walled, with oil dorplets; *sterigmata* 3.7–5.9–8.5 × 1.4–2.0–2.6 µm. *Pleurocystidia* subcylindrical to fusiform, (38.5–)41–49–60.5 (–66) × 7.5–9.5–11(–13.5) µm, with obtuse, papillate or mucronate apices, thin-walled, with irregular refractive contents, projecting up to 30 µm, unchanging in SV. *Cheilocystidia* similar to pleurocystidia in shape, but relatively smaller, measuring (31–)38–40–46(–56) × 6–8–12 µm. *Lamellar trama* composed of numerous sphaerocytes surrounded by connective hyphae, sphaerocytes measuring 30–62 × 23–42 µm. *Subhymenium* pseudoparenchymatous. *Pileipellis* orthochromatic in cresyl blue, thin, 100–150 µm thick, gelatinized, vaguely divided into two layers: suprapellis 80–120 µm thick, composed of ascending to erect hyphae and pileocystidia; subpellis thin, 20–50 µm deep, composed of more horizontal hyphae and pileocystidia; hyphae 2.5–5 µm in diam. *Acidoresistant incrustations* not observed. *Hyphal terminations* near the pileus margin often branched, thin-walled, sometimes narrowed; terminal cells measuring (16–)18.5–27–43 × 2.5–3.5–5 µm, subcylindrical, with obtuse apices; subterminal cells usually equally wide. *Hyphal terminations* near the pileus centre of similar structure; terminal cells measuring (13.5–)16–21–27 × 2–3–5 µm, cylindrical or slightly narrowed towards apex, apically obtuse-rounded; subterminal cells usually equally wide. *Pileocystidia* near the pileus margin (31–)42–68–119(–138) × 4–6–8 µm, abundant, 1- to 4-celled, thin-walled, cylindrical to subclavate, flexuous, with obtuse apices, with refractive contents, weakly becoming pinkish in SV. *Pileocystidia* near the pileus center of similar shape, 1- to 4-celled, abundant, measuring (26–)37–54–97(–112) × 3–5–7.5 µm, with refractive contents, weakly SV+. *Cystidioid hyphae* in subpellis and context with heteromorphous granulose contents. *Oleiferous hyphae* in the subpellis. *Clamp connections* absent in all tissues.

Remarks

Russula minirosea sp. nov. is classified into subsect. *Laricinae*, which typically harbors small species of *Russula* with abundant dermatocystidia, short basidia and various colors of spore print. There are few species with reticulate-crested spores in *Laricinae*, most having isolated warts. *Russula yadongensis* S.H.Wang, R.L.Zhao & B.Cao, also a member of subsect. *Laricinae* reported from northwestern China, can be distinguished from *R. minirosea* by its medium-sized pileus (3.2–4.6 mm in diam.) with a purple coloration and spores with isolated ornamentations (Wang *et al.* 2023). Two other members of subsect. *Laricinae* reported from China differ from *R. minirosea*: *R. sichuanensis* G.J.Li & H.A.Wen is characterized by its dirty white to pale tinged pileus and larger basidiospores [(8.8–)9.4–14.1(–15.5) × (7.6–)7.9–12.8(–13.1) µm] with cristulate to subreticulate ornamentations (Li *et al.* 2013b), while

R. vinosobrunneola G.J.Li & R.L.Zhao has a brown pileus and larger basidiospores [7.7–9.6 (–10.1) × 6.4–8 (–8.6) µm] (Li *et al.* 2018a).

Russula minirosea sp. nov. shows a close relationship to the European species *R. nauseosa* and *R. laricina*. *Russula nauseosa* is variable in pileus color (reddish, purplish, brownish and whitish, but mostly with purplish tint) and size. Basidiospores of *R. nauseosa* are larger (7.8–10 × 6.6–7.8 µm) and often ornamented with isolated warts or spines never forming a reticulum (Miller *et al.* 2012), distinguishing it from *R. minirosea*. *Russula laricina* is easily differentiated from *R. minirosea* by its purple pileus and reddish-tinged stipe.

In terms of very small basidiocarps and rosy pileus color, *R. minirosea* sp. nov. resembles *R. minor* Y.Song which belongs to subsect. *Puellarinae*. But the latter species has a pileus often with a white margin, much smaller basidiospores [(5.0–)5.3–5.8–6.1(–6.6) × (4.1–) 4.3–4.6–4.9(–5.2) µm] with isolated warts, very small basidia [(17–)17.5–21–27(–29.5) × 7–8.5–9.5(–10) µm] and non-septate pileocystidia (Song *et al.* 2021).

In the phylogenetic analysis based on ITS, two unidentified sequences (KX441101, MW554359) and three sequences identified as *R.* cf. *nauseosa* (GU371293, KX655853, MT678891), all collected from China, clustered with *R. minirosea* sp. nov. (Fig. 1), with sequence similarity more than 99% between each other. Consequently, these five sequences should also be reclassified as *R. minirosea*, which also indicates the relative abundance of the species in China.

Russula purpureomarginalis F.Li & Y.Song sp. nov.

MycoBank: MB847718

Figs 1–2, 9–10, 11G–H

Diagnosis

Russula purpureomarginalis sp. nov. differs from the very similar *R. xerampelinoides* K.Das, I.Bera, A.Ghosh & Buyck from India in several aspects: it is not associated with *Abies densa* Griff., it has larger basidia, unchanging pileocystidia in SV, and it has distinct ITS sequence data.

Etymology

The species name refers to the reddish purple margin of its pileus.

Type material

Holotype

CHINA • Xinjiang Uygur Autonomous Region, Burqin County, Hemu Village; 2 Aug. 2022; *F. Li*, *Hm23*; solitary on the ground in mixed coniferous and broad-leaf forest; GenBank nos: OP828710 (ITS), OP828727 (LSU), OP831178 (*rpb2*), OP857230 (*tef1*); SERC [SERC2211].

Additional material examined

CHINA • Shaanxi Province, Hanzhong City, Micangshan Biosphere Reserve; 32°37′66″ N, 107°25′49″ E; 9 Se. 2021; *Y. Song*, *K21090918*; solitary on the ground in mixed coniferous and broad-leaf forest; GenBank no: OP828711 (ITS); SERC [SERC2212].

Description

Basidiomata medium to relatively large sized. Pileus 5–9 cm in diam., hemispherical when young, turning convex to applanate at maturity; surface dry, viscid when wet, not easy to peel off, pale pinkish purple (#D8BFD8) to reddish purple (#FDE9FB) at margin, yellow to brown (#FDFEE6) at center; margin

Fig. 9. *Russula purpureomarginalis* F.Li & Y.Song sp. nov., holotype (SERC2211). **A**–**C**. Fruiting bodies. **D**–**E**. Basidia. **F**. Pileocystidia near pileus margin. **G**. Basidiospores in Melzer's reagent showing the amyloid ornamentations. Scale bars: A–C = 1 cm; D–G = 10 µm.

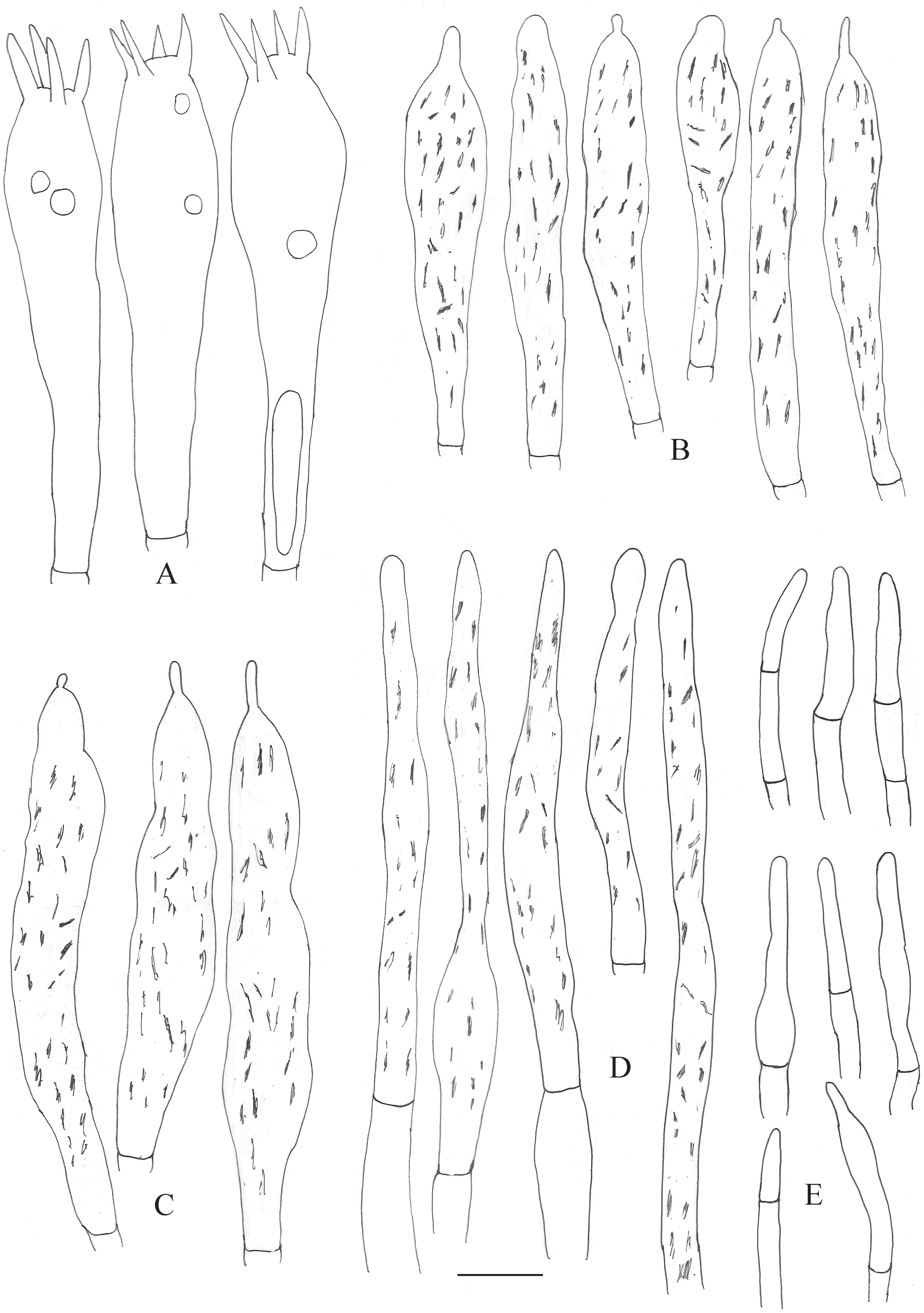

Fig. 10. Line drawings of *Russula purpureomarginalis* F.Li & Y.Song sp. nov., holotype (SERC2211). **A**. Basidia. **B**. Cheilocystidia. **C**. Pleurocystidia. **D**. Pileocystidia near pileus margin. **E**. Hyphal terminations near pileus margin. Scale bar = 10 µm.

smooth and entire at first, slightly striate with age. *Lamellae* adnate, interveined, white (#FEFEFE) to pale cream (#FFF8D1), up to 8 mm in diam.; edge concolorous, smooth. *Stipe* central, fleshy, cylindrical, often slightly tapering upwards, solid, 3.5–7 × 1–1.6 cm, white (#FCF3CF, #FBFCFC) with pinkish tinge (#FFF3FC). *Context* white (#FCF3CF), 9 mm thick in pileus, unchanging when bruised. *Taste* mild. *Odor* indistinct. *Spore print* not observed.

Basidiospores subglobose to ellipsoid, (40/2/2) (7.6–)7.9–9.0–9.6(–10) × (5.8–)6.3–7.0–7.7(–8.0) µm, [Q = (1.14–)1.21–1.28–1.40(–1.42)], hyaline in 5% KOH; ornamentation amyloid, conical to cylindircal warts less than 1.5 µm in height, moderately distant to dense (5–7(–8) in a 3 µm circle), often isolated, occasionally connected by line, line connections dispersed (0–1 in the circle); suprahilar spot amyloid. *Basidia* (46.5–)48–59–70(–74) × (8.5–)10.5–12.5–14 µm, long clavate, 4-spored, thin-walled, with irregular contents and oil dorplets; *sterigmata* 5.6–6.9–8.7 × 1.8–2.2–2.8 µm. *Pleurocystidia* cylindrical to fusiform, (48.5–)57–67.5.5–83(–87) × 6.5–10.5–14 µm, with obtuse, papillate or mucronate apices, thin-walled, with irregular refractive contents, projecting up to 20 µm, unchanging in SV. *Cheilocystidia* similar to pleurocystidia in shape, relatively smaller, measuring 46.5–57–66.5(–81) × 6.5–8.9–11 µm. *Subhymenium* pseudoparenchymatous. *Lamellar trama* composed of numerous sphaerocytes surrounded by connective hyphae, sphaerocytes measuring 14–31 × 11–27 µm. *Pileipellis* orthochromatic in cresyl blue, 120–200 µm thick, gelatinized, divided into two layers: suprapellis 60–90 µm thick, composed of ascending to erect hyphae; subpellis thin, 50–110 µm deep, composed of more horizontal hyphae; hyphae 2.5–6 µm in diam., some wider and strongly pigmented, up to 10 µm in diam. *Acidoresistant incrustations* rare. *Hyphal terminations* near the pileus margin thin-walled, often narrowed; terminal cells measuring 14–25.5–33(–40) × 2.5–4–5 µm, subcylindrical, with obtuse or sharp apices, rarely inflated, coracoid, up to 11 µm wide; subterminal cells usually equally wide. *Hyphal terminations* near the pileus centre of similar structure. *Pileocystidia* near the pileus margin (31–)53–82–108(–113) × 4–6–8.5 µm, abundant, 1- to 3-celled, thin-walled, cylindrical to subclavate, flexuous, with obtuse apices, with refractive contents, unchanging in SV. *Pileocystidia* near the pileus center of similar shape, 1- to 3-celled, abundant, measuring (36.5–)42–67.5–96.5(–106) × 3–6–7(8.5) µm, with refractive contents, unchanging in SV. *Cystidioid hyphae* in subpellis and context with granulose or crystalline contents. *Oleiferous hyphae* in the subpellis. *Clamp connections* absent in all tissues.

Remarks

Russula purpureomarginalis sp. nov. is classified into subsect. *Xerampelinae*, forming a distinct clade in both ITS and multi-locus phylogenetic trees. *Russula paragraveolens* S.H.Wang, G.J.Li, R.L.Zhao & B.Cao and *R. pseudograveolens* S.H.Wang, G.J.Li, R.L.Zhao & B.Cao reported from China also belong to this subsection (Wang *et al.* 2023). *Russula paragraveolens* has a bright red pileus, much smaller basidiospores [(5.0–)5.5– 5.9–6.3(–6.6) × (4.0–)4.6–5.0–5.4(–5.6) µm] and basidia [(31–)31–36–41(–50) × (10–)10–11–12(–14) µm]; *R. pseudograveolens* is diagnosed by its violet brown to brownish red pileus, much smaller basidiospores [(5.5–)6.0–6.3–6.6(–6.9) × (4.7–)5.0–5.2–5.5(–5.7) µm] and basidia [(27–)28–30–32(–35) × (8.5–)9–10(–11) µm], all can be distinguished. *Russula xerampelinoides* reported from India resembles *R. purpureomarginalis* in spore size and ornamentations, but the former species has smaller basidia (40–44–48(–50) × 9–10–11(–12) µm) and grey-black pileocystidia in SV. Moreover, *R. xerampelinoides* is distributed in subalpine forest and associated with *Abies densa* (Buyck *et al.* 2021), while *R. purpureomarginalis* inhabits mixed coniferous and broad-leaf forest mainly with *Quercus* and *Pinus* trees.

The European and American members of subsect. *Xerampelinae*, such as *R. madrensis* A.Kong & Buyck, *R. sancti-pauli* A.Kong & Buyck and *R. xerampelina* (Schaeff.) Fr., show a bright red to dark red pileus color (Adamčík *et al.* 2019). *Russula flavobrunnescens* A.Kong & Buyck and *R. katarinae* Adamčík & Buyck have a yellowish or brownish pileus, but not stained with purplish tint (Adamčík *et al.* 2015). *Russula purpureomarginalis* sp. nov., characterized by its distinctly reddish purple tinged pileus, can be easily distinguished from these related species.

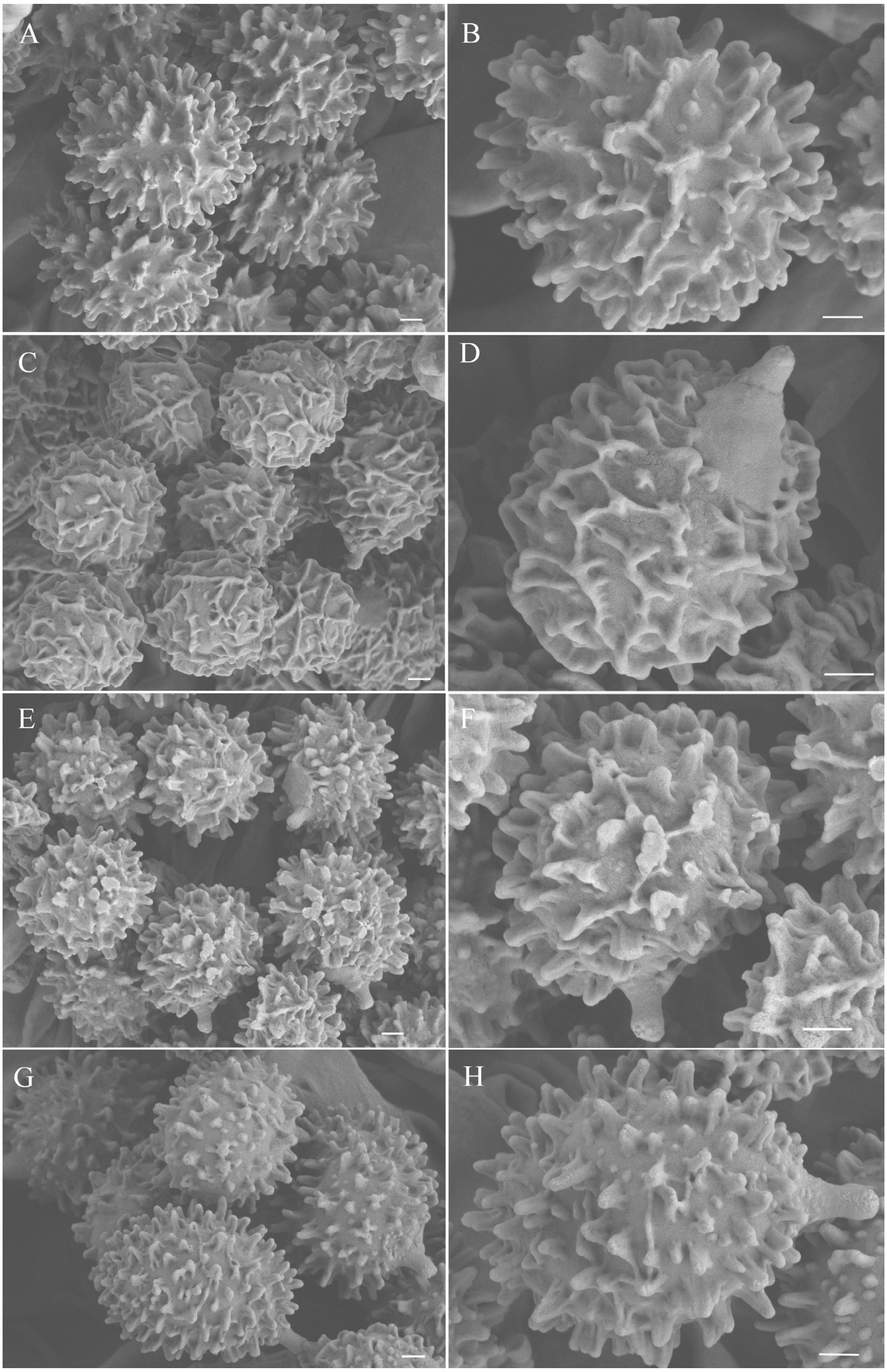

Fig. 11. Photograph of basidiospores under scanning electron microscope. **A–B**. *Russula micangshanensis* Y.Song sp. nov., holotype (SERC2201). **C–D**. *R. minirosea* Y.Song sp. nov., holotype (SERC2201). **E–F**. *R. griseorosea* Y.Song sp. nov., holotype (SERC2209). **G–H**. *R. purpureomarginalis* F.Li & Y.Song sp. nov., holotype (SERC2211). Scale bars = 1 µm.

Discussion

Both morphological and phylogenetic analyses based on ITS and multi-locus datasets support the distinct positions of the four novel species in subg. *Russula* crown clade. *Russula minirosea* sp. nov. is an additional member of subsect. *Laricinae* Romagn., *R. griseorosea* sp. nov. belongs to subsect. *Puellarinae* Singer; these two subsections typically harbor small species with abundant dermatocystidia, short basidia and various colors of spore print. The proposal of *R. micangshanensis* sp. nov. increased the species diversity of subsect. *Olivaceinae*. *Russula purpureomarginalis* sp. nov. forms a distinct clade in subsect. *Xerampelinae* Singer, a group mainly composed of species from Europe and North America.

China has a vast territory with diverse ecosystems, breeding abundant *Russula* resources. There have been plenty of novel *Russula* species reported from southwestern, northeastern, southern, eastern and northern China (Li *et al.* 2011, 2013a, 2013b; Song *et al.* 2018; Zhou *et al.* 2022), but the northwestern part has been largely overlooked. Wang *et al.* (2023) first reported two new species of *Russula* from Xizang Autonomous Region in northwestern China. The descriptions of our four novel species in *Russula* further unraveled the species diversity of the group in northwestern China, highlighting the need for more intensive studies on *Russula* in this region.

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