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Research article

Two novel species of subgenus *Russula* crown clade (Russulales, Basidiomycota) from China

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Abstract. Two novel species of *Russula* (Russulaceae, Russulales), *R. coronaspora* Y.Song sp. nov. and *R. minor* Y.Song sp. nov. belonging to subgenus *Russula* crown clade, are described based on both morphological and phylogenetic evidence. In morphology, *R. coronaspora* sp. nov. is mainly characterized by its distinct spores ornamented with sparse, cylindrical and isolated spines, which resemble coronavirus, and gelatinized pileipellis with pileocystidia mostly septate and sometimes branched; *R. minor* sp. nov. possesses a very small basidiocarp with pileus less than 2.5 cm in diameter., small basidia, easily peeling and gelatinized pileipellis with slender terminal cells and abundant SV+ pileocystidia. Positions of the two new species in both phylogenetic trees based on ITS and 5-locus sequences (nLSU, mtSSU, *rpb1*, *rpb2* and *tef1*) confirm their distinct taxonomic status.

Keywords. Multi-locus phylogeny, morphology, taxonomy.

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Introduction

Russula Pers. is the type genus of family Russulaceae and the largest genus in the order Russulales. *Russula* can be considered as the second most taxonomically diverse genus of ectomycorrhiza-forming fungi after the genus *Cortinarius* (Pers.) Gray, playing important ecological roles in the maintenance of different ecosystems (Looney *et al.* 2016). At least 78 species of *Russula* are consumed and 30 species are used in traditional medicine for at least 440 years in China (Wu *et al*. 2019).

Buyck *et al.* (2018, 2020) reorganized the infrageneric taxonomic system of *Russula* employing a 5-locus phylogenetic analysis and divided the genus into 8 subgenera, in which subgen. *Russula* was further separated into a core and a crown clade. Species of crown clade mainly characterized by the very small to very large species, equal gills never forked, spore print color from white to deep yellow, spores with an amyloid suprahilar spot, gloeocystidia or primoridial hyphae often with with obtuse-rounded apices (Buyck *et al.* 2018). Subgenus *Russula* crown clade has a very high species richness worldwide, at least 43 novel species have been described in this crown clade based on both morphological characters and molecular data since 2006, of which 34 were reported from Asia and 15 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, 2021; 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).

The Dinghushan Biosphere Reserve (DHSBR) is located in southern China (112°30′39″–112°33′41″ E, 23°09′21″–23°11′30″ N) and subjected to subtropical monsoon climate. The region possesses three typical forest formations: coniferous forest, coniferous and broad-leaf mixed forest, and broad-leaf forest. Recently, during the survey for macrofungi carried out in DHSBR, a well-known reserve for its high biological diversity, another two novel species of subgen. *Russula* crown clade were discovered in a deciduous forest composed mainly of trees of the Fagaceae family. Both new species are here described based on morphological and phylogenetic evidence.

Material and methods

Morphology

Specimens were collected from Dinghushan Biosphere Reserve (DHSBR, 112°30′39″–112°33′41″ E, 23°09′21″–23°11′30″ N) from June to October during the years 2016–2019. Specimens were dehydrated at 40–50 °C and deposited in the Herbarium of Microbiology Institute of Guangdong (GDGM). Fresh basidiocarp were photographed using Canon IXUS 220 hs digital camera or Canon EOS M50 under daylight in the field, and macroscopic characteristics were recorded. Macromorphological descriptions are based on the field notes and photographs taken from fresh basidiocarps. Basidiocarps are considered as "very small" when pileus diameter less than 3 cm.

Microscopic characters were observed under a Nikon Eclipse Ni-U microscope from hand-sectioned dried materials. Scanning electron images of basidiospores were captured with a Field Emission Scanning Electron Microscope (JSM-6330F). 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 the 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 fruiting bodies using a modified CTAB procedure of Doyle & Doyle (1987). ITS was amplified using primer pair ITS1 and ITS4 (White *et al.* 1990). The amplification protocol consists of a 5 min pre-degeneration at 94°C, followed by 32 cycles of 30s at 94°C, 30s at 52°C and 1 min at 72°C, and a final 12 min extension at 72°C. nLSU, mtSSU, *rpb1*, *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, Guangzhou, China). The newly generated sequences were deposited in GenBank (www.ncbi.nlm.nih.gov).

Phylogenetic analysis

Phylogenetic analyses based on both ITS and combined sequences of nLSU-mtSSU-*tef1*-*rpb2*-*rpb1* were performed with Maximum Likelihood method. 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). The final ITS and 5-locus alignments comprised 804 bp and 4137 bp (nLSU 892 bp, mtSSU 548 bp, *tef1* 933 bp, *rpb2* 766 bp, *rpb1* 998 bp), respectively. Incongruence length difference test (ILD test) was performed with PAUP* ver. 4.0b10 to check the homogeneity of different gene locus, the resulting P value is 0.05 (equal to the threshold for concatenated analysis), indicating that the 5-locus alignment can be used for further phylogenetic analysis. Phylogenetic analysis based on ITS consisted of 70 sequences (Table 1), *R. emetica* (Schaeff.) Pers. belonging to core clade was chosen as outgroup; the concatenated 5-locus tree consisted of 39 sequences (Table 2), using *R. glutinosa* Fatto and *R. glutinosoides* Buyck & X.H.Wang as outgroup. Maximum Likelihood (ML) 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 GTRGAMMA model. All parameters in RAxML analysis were kept at default. Bootstrap value (BS) exceeding 70% was considered to be significantly supported.

Results

Phylogeny

The proposed two novel species, *Russula coronaspora* Y.Song sp. nov. and *R. minor* Y.Song sp. nov., are both nested in subgen. *Russula* crown clade in both ITS and 5-locus tree (Fig. 1 and Fig. 2, respectively). *R. coronaspora* formed a significantly supported clade (BS = 93%) sister to *R. brunneoviolacea* Crawshay in the maximum likelihood tree based on ITS, while clustered with *R.* cf. *brunneoviolacea* and *R. melliolens* Quél. in the 5-locus tree with clade highly supported (BS = 95%). *Russula minor* sp. nov. clustered with 5 undescribed sequences collected from North America in ITS tree, but showed evident genetic distance with the latter ones, with sequence similarity less than 95.2%; while formed a distinct clade in the 5-locus tree, showing uniqueness just as its morphological characters reflected. *Russula minor* sp. nov. gained moderate support in the ITS tree with 96% BS value.

Taxonomy

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

Russula coronaspora Y.Song sp. nov.
MycoBank: MB837425
Index Fungorum: IF558819
Figs 3–4, 7A

Diagnosis

Russula coronaspora sp. nov. is mainly characterized by its small basidiocarps with pinkish brown pileus, interveined lamellae with few lamellulae, small spores ornamented with sparse, cylindrical and isolated spines, hymenial and dermatocystidia weakly SV+ or SV-, pileipellis gelatinized and orthochromatic in cresyl blue, pileocystidia mostly septate and sometimes branched.

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

Taxon	**Voucher specimen**	**Location**	**ITS accession number**
R. abbottabadensis	FH00304589 (holotype)	Pakistan	MG386704
R. abietiphila	HCCN14799 (holotype)	South Korea	MN130060
R. aurantioflammans	r3245	Slovakia	KU928167
R. brunneopurpurea	LAH35058 (holotype)	Pakistan	KT953613
R. brunneovinacea	RITF 2242 (holotype)	China	KY114148
R. brunneoviolacea	MC01-507	Denmark	AM113956
R. brunneoviolacea	PRM 922557	Canada	MG687327
R. buyckii	CUHAM277 (holotype)	India	KT962833
R. changbaiensis	HMAS262369 (holotype)	China	KC412162
R. clavatohyphata	CAL1756 (holotype)	India	MG934209
R. corallina	BB 06.324	USA	JN944006
***R. coronaspora* sp. nov.**	**GDGM79711 (holotype)**	**China**	**MN275689**
***R. coronaspora* sp. nov.**	**GDGM79712**	**China**	**MN275690**
R. cremeirosea	TENN:069929	USA	KT933983
R. curtipes	FH-12-206 (GENT)	Germany	KT933995
R. dhakuriana	CUHAM343	India	MK414576
R. flavobrunnescens	AK5024	Mexico	MN130082
R. font-queri	FH-12-223 (GENT)	Germany	KT934003
R. gemmata	BPL666 (holotype)	USA	KY509511
R. gnathangensis	CAL1733 (holotype)	India	MK253441
R. griseocarnosa	KUN F51839 (holotype)	China	EF627042
R. guangxiensis	HMAS267867 (holotype)	China	KT286852
R. hakkae	HMAS267765 (holotype)	China	KT286848
R. heilongjiangensis	HMAS255142 (holotype)	China	MG719932
R. hookeri	CUHAM275 (holotype)	India	KP713777
R. jilinensis	HMAS194253 (holotype)	China	GU966632
R. katarinae	BB03.159 (PC) (holotype)	USA	KP966377
R. kewzingensis	CAL1636 (holotype)	India	MG674302
R. khinganensis	HMAS278895 (holotype)	China	MG719928
R. laricina	BB 08.681	Italy	JN944008
R. madrensis	AK3422 (holotype)	Mexico	MN130093
R. magica	FH 12-061 (GENT)	Thailand	MN130096
R. messapica var. *messapica*	ALV1991	Spain	MK105669
R. messapica var. *messapicoides*	JL1493	Spain	MK105674
***R. minor* sp. nov.**	**GDGM79686 (holotype)**	**China**	**MN275666**
***R. minor* sp. nov.**	**GDGM79687**	**China**	**MN275665**
***R. minor* sp. nov.**	**GDGM79688**	**China**	**MN275667**
***R. minor* sp. nov.**	**GDGM79689**	**China**	**MN275668**
R. nauseosa	FH-12-173 (GENT)	Germany	KT933985
R. nympharum	FH11121505 (holotype)	Spain	KU928157
R. odorata	BB 07.186	Slovakia	JN944010
R. olivaceohimalayensis	CAL1659 (holotype)	India	MN130097
R. puellaris	nl1372 (TUB)	Germany	AF418628

Table 1. Newly generated sequences used in phylogenetic analysis based on ITS. Newly generated sequences are shown in bold.

Taxon	**Voucher specimen**	**Location**	**ITS accession number**
R. puellula	SAVF 3107	Slovakia	KY582704
R. purpureoverrucosa	GDGM32902 (holotype)	China	MG214692
R. rosea	BB 07.780	France	JN944003
R. rugulosa	BPL654	USA	KY848516
R. sancti-pauli	BB 06.494	Mexico	MN130101
R. sapinea	PA38	Latvia	KR019818
R. seperina	SAVF 3156 (epitype)	Slovakia	MN130108
R. sichuanensis	HKAS53792 (holotype)	China	JX391969
R. solaris	BB 07.282	Slovakia	JN944007
R. subrutilans	RITF1874 (holotype)	China	KJ868237
R. subsulphurea	F18743	Europe	KF810135
R. subsulphurea	TENN:F18743	USA	NR153231
R. subtilis	SAVF 3805 (epitype)	USA	KY509504
R. sp. 1	SR48-10MX	Mexico	KT697966
R. sp. 2	FLAS-F-61609	USA	MH211995
R. sp. 2	FLAS-F-61146	USA	MH211767
R. sp. 3	S.D. Russell 439	USA	MK397035
R. sp. 3	S.D. Russell 7799	USA	MK532803
R. tengii	HMAS262728 (holotype)	China	MG386708
R. uttarakhandia	CAL 1537 (holotype)	India	KY873997
R. versatilis	PRM 922558	Czech Republic	MG687329
R. versicolor	BB 07.288	Slovakia	JN944009
R. vinosobrunneola	HMAS281138 (holotype)	China	MG719927
R. violaceoincarnata	O73136	Netherland	GU234047
R. zvarae	BB 08.639	Italy	JN944004
Outgroup			
R. emetica	lw81 (TUB)	Germany	AF418619
R. emetica	UE05.10.2003-11 (UPS)	Sweden	DQ421997

Etymology

Named after its basidiospores which resemble a coronavirus.

Type material

Holotype

CHINA • Guangdong Province, Zhaoqing City, Dinghushan Biosphere Reserve, on the ground in evergreen broad-leaf forest; 22 Apr. 2019; *Y. Song*, *K19042201*; GenBank nos: MN275689 (ITS), MN839580 (nLSU), MN839630 (mtSSU), MT085562 (*rpb1*), MT085657 (*rpb2*), MT085600 (*tef1*); GDGM79711.

Additional material examined

CHINA • Guangdong Province, Zhaoqing City, Dinghushan Biosphere Reserve, on the ground in evergreen broad-leaf forest; 6 Apr. 2016; *Y. Song, K16040650*; GenBank no: MN275690 (ITS); GDGM79712.

Table 2. Sequences used in phylogenetic analysis based on 5-locus data. Newly generated sequences are shown in bold.

			Accession number				
Taxon	**Voucher specimen**	**Location**	***nucLSU***	***mtSSU***	*rpb1*	*rpb2*	*tef1*
R. amara	BB 07.782	France	KU237524	KU237370	KU237674	KU237810	KU237954
R. amara	FH-12-213 (GENT)	Germany	KT933859	–	KT957370	KT933930	–
R. aurea	BB 07.211	Slovakia	KU237539	KU237385	KU237688	KU237825	KU237969
R. azurea	BB 08.668	Italy	KU237529	KU237375	KU237679	KU237815	KU237959
R. badia	BB 07.324	Slovakia	KU237571	KU237419	KU237715	KU237857	KU237999
R. burlinghamiae	BB 05.108	USA	KU237540	KU237386	KU237689	KU237826	KU237970
R. carpini	BB 07.262	Slovakia	KU237543	KU237389	KU237691	KU237829	KU237973
R. cf. *brunneoviolacea*	BB 06.606	Canada	KU237516	KU237362	KU237666	KU237802	KU237946
R. changbaiensis	HMAS262355	China	KX441304	KX441551	KX441798	KX442045	MF893389
R. corallina	BB 06.324	USA	KU237481	KU237325	KU237630	KU237767	KU237910
***R. coronaspora* sp. nov.**	**GDGM79711 (holotype)**	**China**	**MN839580**	**MN839630**	**MT085562**	**MT085657**	**MT085600**
R. cuprea	BB 07.233	Slovakia	KU237555	KU237401	KU237699	KU237841	KU237984
R. decipiens	BB 07.178	Slovakia	KU237569	KU237417	KU237713	KU237855	KU237997
R. decolorans	BB 07.322	Slovakia	KU237541	KU237387	KU237735	KU237827	KU237971
R. flavisiccans	BB 06.336	Mexico	KU237485	KU237329	KU237634	KU237771	KU237914
R. gemmata	BB 05.107	USA	KU237528	KU237374	KU237678	KU237814	KU237958
R. globispora	BB 07.243	Slovakia	KU237499	KU237344	KU237648	KU237785	KU237929
R. laricina	BB 08.681	Italy	KU237560	KU237408	KU237704	KU237846	KU237991
R. lepida	BB 07.189	Slovakia	KU237500	KU237345	KU237649	KU237786	KU237930
R. lilacea	BB 07.213	Slovakia	KU237498	KU237343	KU237647	KU237784	KU237928
R. melliolens	BB 07.194	Slovakia	KU237545	KU237391	KU237692	KU237831	KU237975
R. minutula	BB 08.636	Italy	KU237531	KU237377	–	KU237817	KU237961
***R. minor* sp. nov.**	**GDGM79686 (holotype)**	**China**	**MK881964**	**MK882091**	**MT085496**	**MK880691**	**MT085599**
R. nauseosa	BB 07.285	Slovakia	KU237572	KU237420	KU237716	KU237858	KU238000
R. obscurosordida	BB 06.564 (holotype)	Canada	KU237575	KU237423	KU237719	KU237861	KU238003
R. odorata	BB 07.186	Slovakia	KU237518	KU237364	KU237668	KU237804	KU237948
R. olivascens	BB 08.663	Italy	KU237522	KU237368	KU237672	KU237808	KU237952
R. pelargonia	BB 07.169	Slovakia	KU237570	KU237418	KU237714	KU237856	KU237998
R. puellaris	BB 07.311	Slovakia	KU237515	KU237361	KU237665	KU237801	KU237945
R. romellii	BB 07.202	Slovakia	KU237493	KU237337	KU237641	KU237779	KU237922
R. romellii	FH-12-177 (GENT)	Germany	KT933848	–	KT957359	KT933919	–
R. rosea	BB 07.780	France	KU237496	KU237340	KU237644	KU237782	KU237925
R. roseinae	BB 09.172	New Caledonia	KU237588	KU237436	KU237728	KU237873	KU238015
R. solaris	BB 07.282	Slovakia	KU237549	KU237395	KU237695	KU237835	KU237978
R. turci	BB 07.328	Slovakia	KU237520	KU237366	KU237670	KU237806	KU237950
R. versicolor	BB 07.288	Slovakia	KU237573	KU237421	KU237717	KU237859	KU238001
R. zvarae	BB 08.639	Italy	KU237530	KU237376	KU237680	KU237816	KU237960
Outgroup							
R. glutinosa	Roody WRWV 04.1154 (holotype)	USA	MN315511	MN315532	–	MN326798	MN326799
R. glutinosoides	HKAS106678 (holotype)	China	MN428827	MN460313	–	–	–

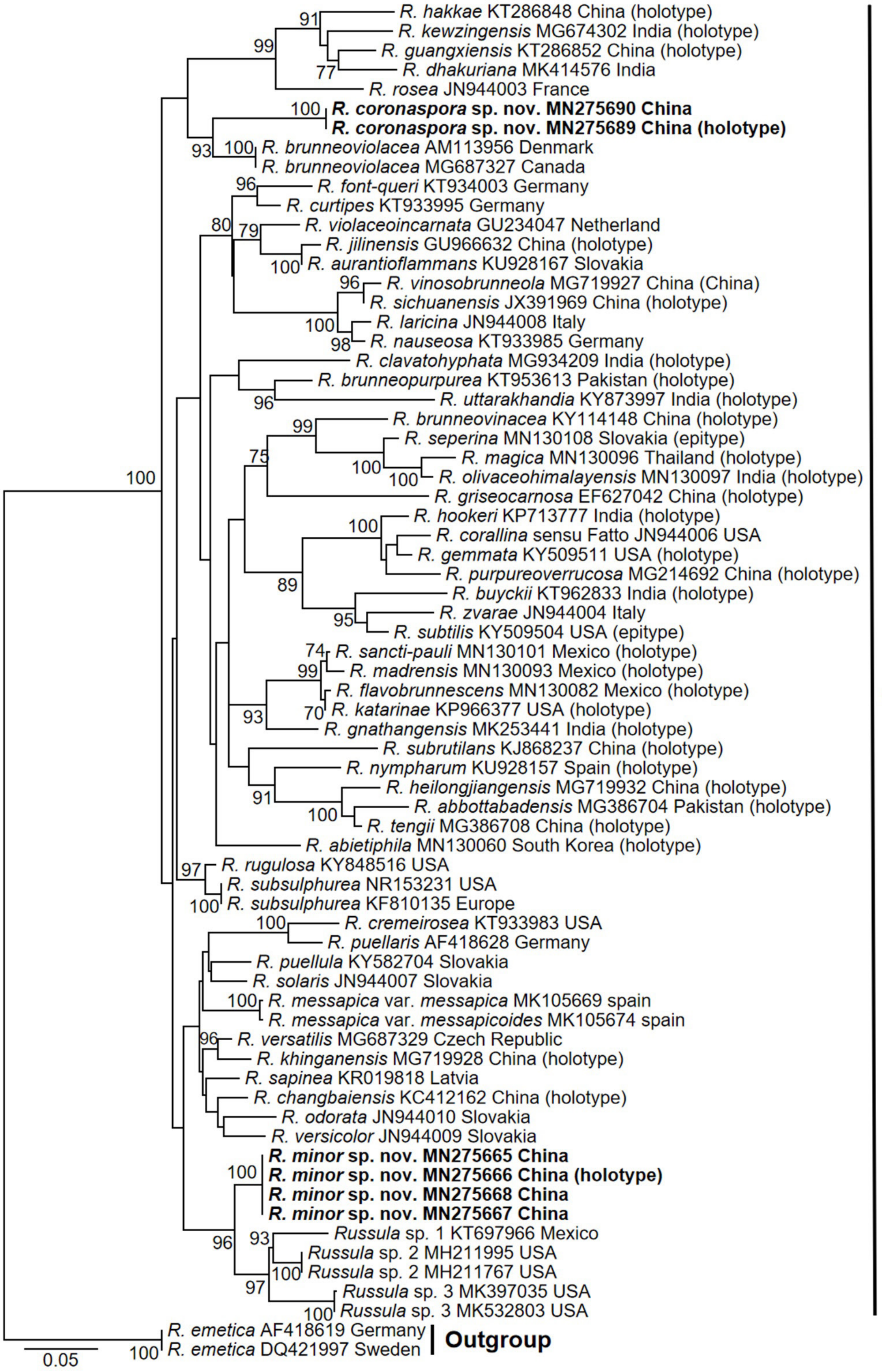

Fig. 1. Maximum Likelihood tree of subgen. *Russula* crown clade based on ITS sequences, bootstrap values higher than 70% were displayed around nodes. Accession numbers of the two novel species are shown in bold.

Description

Basidiocarp small sized. Pileus 2.5–4 cm in diam., hemispherical to convex at first, turning applanate with depressed center at maturity; surface glabrous, dry, viscid when wet, pinkish (#FDF2E9) or brownish (#FCF3CF), sometime white (#FFFFF0) at margin; margin entire, sometimes cracked, striate when young. Lamellae white (#FFFFF0), interveined, mixed with few, dispersed shorter lamellulae; edge entire, concolorous, smooth. Stipe central, 2–3.5 × 0.5–1 cm, cylindrical, sometimes tapering upwards, solid at first, turning spongy to hollow with age, fleshy, fragile, white (#FBFCFC). Context white, not changing when bruised; taste mild; odor indistinct. Spore print pale cream (#FBFBEF).

Basidiospores subglobose to ellipsoid, rarely globose, very small, (60/3/2) (4.5–)4.7–5.1–5.6 (–6.2) × (3.6–) 3.8–4.2–4.6(–4.8) µm, [Q = 1.12–1.20–1.32(–1.38)], hyaline in 5% KOH; ornamentation amyloid, spines cylindrical to subclavate, sometimes tapering upwards or downwards, less than 2 µm in height, sparsely distributed [2–(4–)5 in a 3 µm diam. circle], isolated, long warts scattered, never connected; suprahilar spot indistinct, amyloid. Basidia clavate, 2- or 4-spored, thin-walled, often with irregular contents or droplets, (19.5–)21.5–25–30(–35) × 7–9–10.5(–11) µm; sterigmata 2.4–5.1 × 1.2–1.5 µm. Hymenial gloeocystidia on gill sides subcylindrical to fusiform, with papillate, mucronate or branched apices, thin-walled, with irregular refractive contents, weakly SV+ or SV-, (21–)23.5–33–49(–51) × 4.5–5.5–7.5 µm; on gill edges not abundant, 33–40–46(–50) × 4.5–5–6 µm. Subhymenium pseudoparenchymatous.

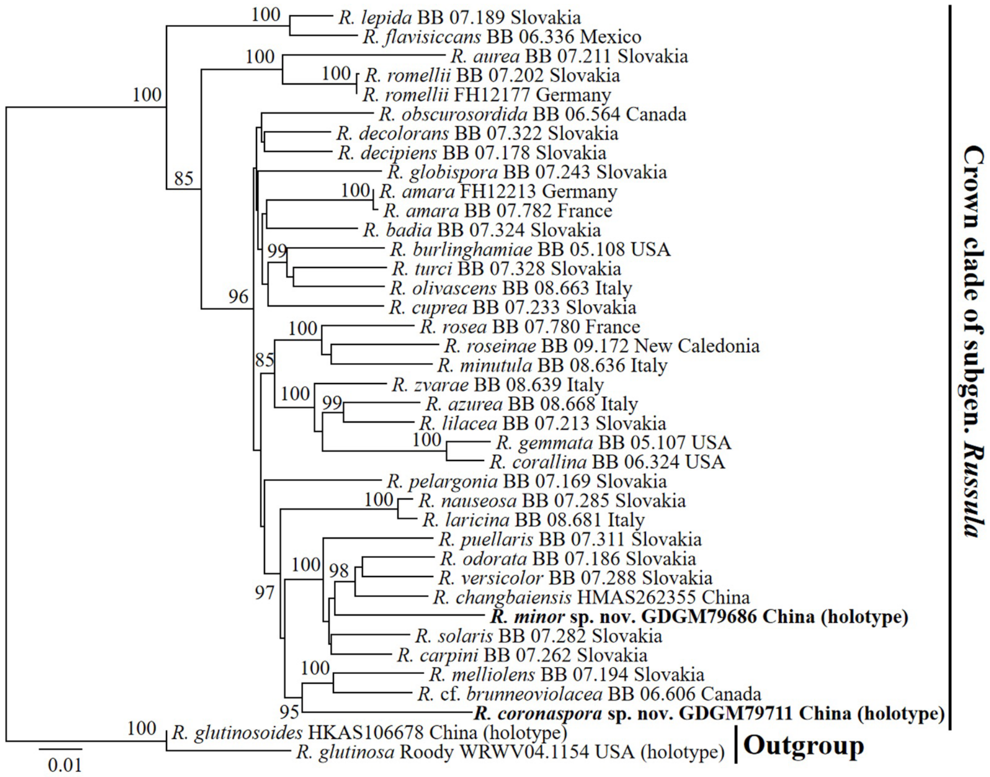

Fig. 2. Maximum Likelihood tree of subgen. *Russula* crown clade based on 5-locus (nLSU-mtSSU-*tef1*-*rpb2*-*rpb1*) combined sequences, bootstrap values higher than 70% were displayed around nodes. Collections of the two novel species are shown in bold.

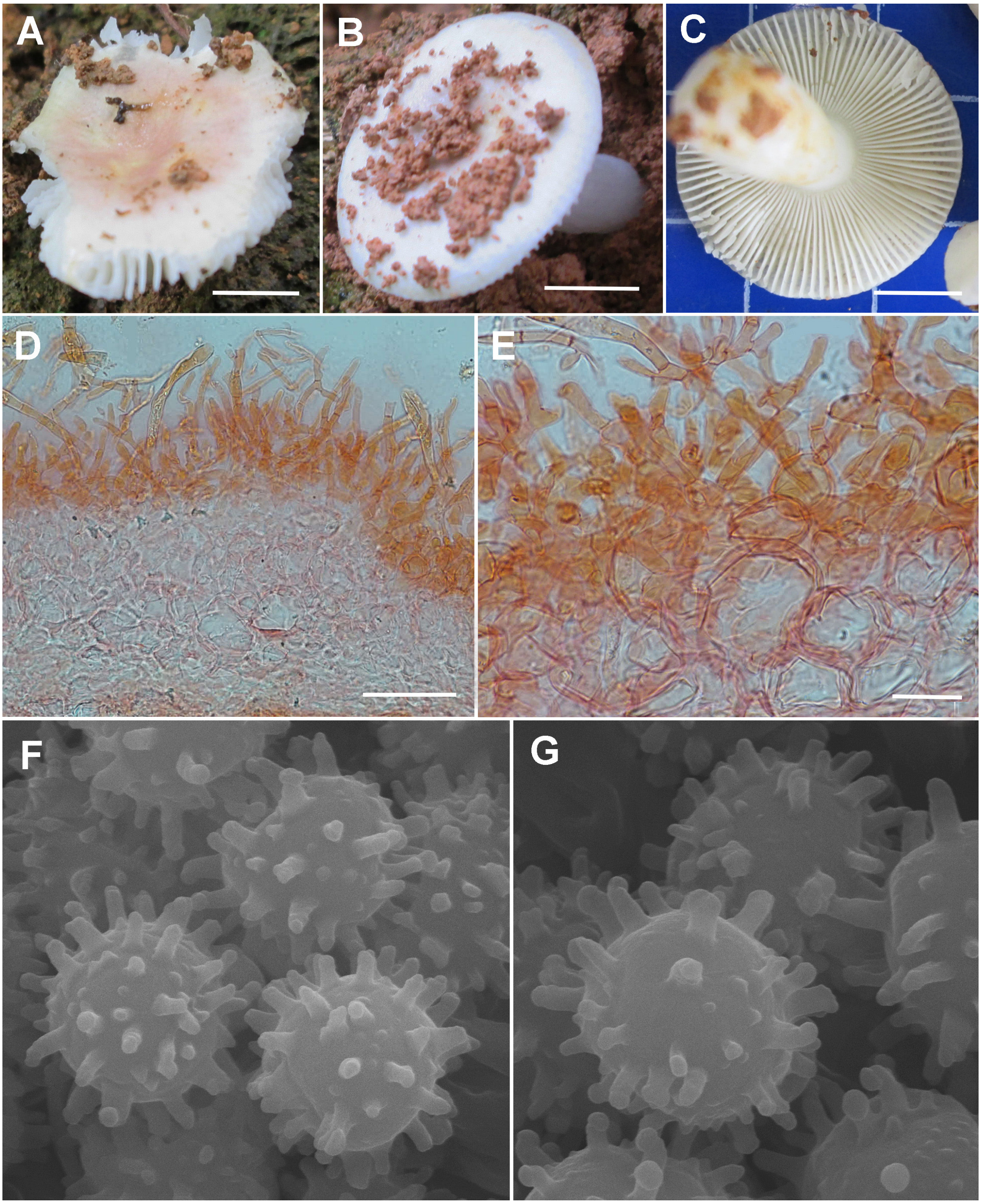

Fig. 3. *Russula coronaspora* Y.Song sp. nov. (GDGM79711, holotype). **A–C**. Fruiting bodies. **D–E**. Pileipellis. **F–G**. Basidiospores under scanning electron microscope. Scale bars: A–C = 1 cm; D = 25 µm; E = 10 µm; F–G = 2 µm.

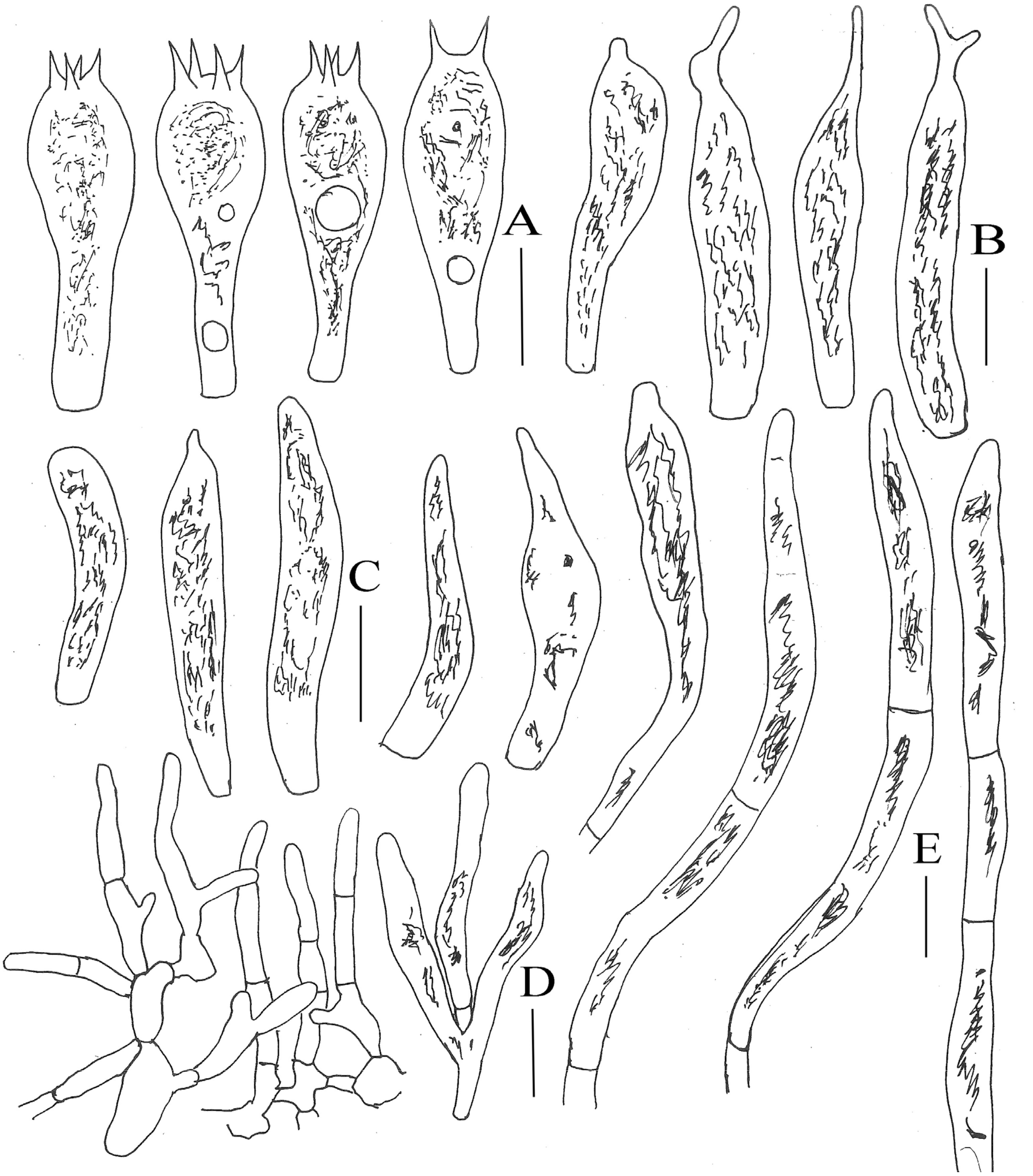

Fig. 4. *Russula coronaspora* Y.Song sp. nov. (GDGM79711, holotype). **A**. Basidia. **B**. Pleurocystidia. **C**. Cheilocystidia. **D**. Terminal elements in pileipellis. **E**. Pileocystidia. Scale bars = 10 µm. Drawing by Yu Song.

Lamellar trama composed of numerous sphaerocytes surrounded by connective hyphae, sphaerocytes up to 21 × 19 µm. Pileipellis orthochromatic in cresyl blue, gelatinized, composed of ascending to erect hyphae, 33–80 µm thick; terminal cells cylindrical, with obtuse or tapering apices, thin-walled, hyaline, (7.5–)8.5–14–19(–24) × 1.5–2–3 µm. Pileocystidia abundant, cylindrical to fusiform, with obtuse or tapering apices, mostly 1- or 2-septa, sometimes branched, with refractive contents, weakly SV+ or SV-, (29–)30.5–62–94.5(–98.5) × 3–4.5–6(8.5) µm. Stipitipellis a cutis, composed of septate and hyaline hyphae measuring 1–3 µm wide; terminal cells cylindrical with obtuse apices, thin-walled, hyaline. Caulocystidia frequent, cylindrical, obtuse or papillate, mostly septate, with refractive contents, (30.5–)33–39.5–49(–57.5) × 2–3–4 µm. Clamp connections absent in all tissues.

Russula minor Y.Song sp. nov.

MycoBank : MB837427
Index Fungorum: IF558489
Figs 5–6, 7B

Diagnosis

Russula minor sp. nov. is mainly characterized by its very small basidiocarp with thin context and pink to rosy pileus less than 2.5 cm in diam., pileipellis very easy to peel off, white to cream lamellae with few lamellulae, often curved and hollow to multi-chambered stipe, spores with conical to cylindrical warts never connected, very small basidia and gelatinized pileipellis with slender terminal cells and abundant pileocystidia, and pleurocystidia weakly SV+ (slightly becoming brownish) or SV-, while pileocystidia becoming brown in SV.

Etymology

Referring to its very small basidiocarp.

Type material

Holotype

CHINA • Guangdong Province, Zhaoqing City, Dinghushan Biosphere Reserve, on the ground in evergreen broad-leaf forest mainly with plants in Fagaceae; 12 Sep. 2016; *Y. Song*, *K18043001*; GenBank nos.: MN275666 (ITS), MK881964 (nLSU), MK882091 (mtSSU), MT085496 (*rpb1*), MK880691(*rpb2*), MT085599 (*tef1*); GDGM79686.

Additional material examined

CHINA • Guangdong Province, Zhaoqing City, Dinghushan Biosphere Reserve, on the ground in evergreen broad-leaf forest; 8 Sep. 2018; *F. Yuan and Y. Song, K18090802*; GenBank no: MN275665 (ITS); GDGM79687 • same data as for preceding; *F. Yuan and Y. Song, K18090827*; GenBank no: MN275667; GDGM79688 • same data as for preceding; 10 Sep. 2018; *F. Yuan and Y. Song, K18091022*; GenBank no: MN275668; GDGM79689.

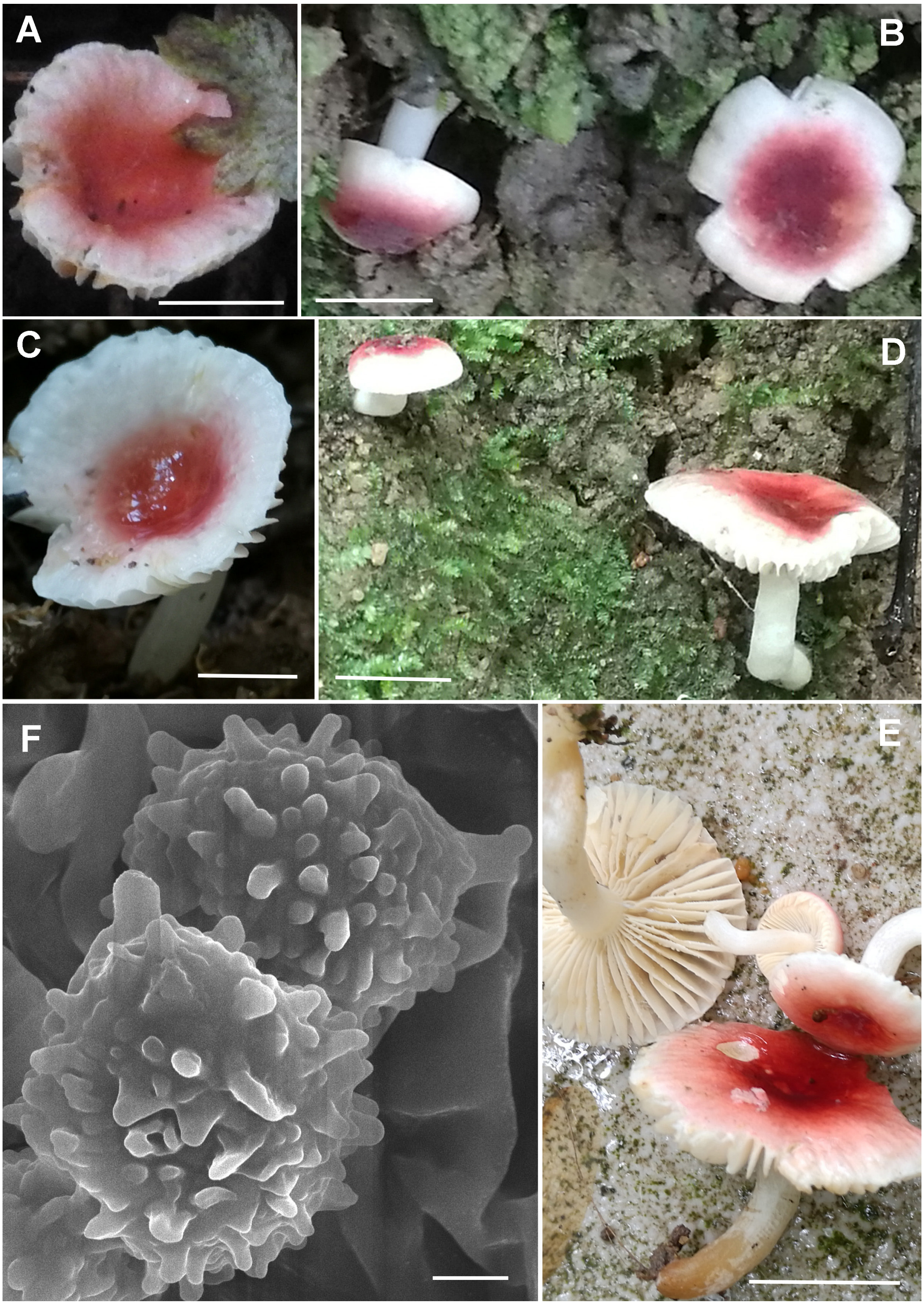

Fig. 5. *Russula minor* Y.Song sp. nov. **A**, **E–F**. Holotype, GDGM79686. **B**. GDGM79689. **C**. GDGM79687. **D**. GDGM79688. **A–E**. Fruiting bodies. **F**. Basidiospores under scanning electron microscope. Scale bars: A–E = 1 cm; F = 1 µm.

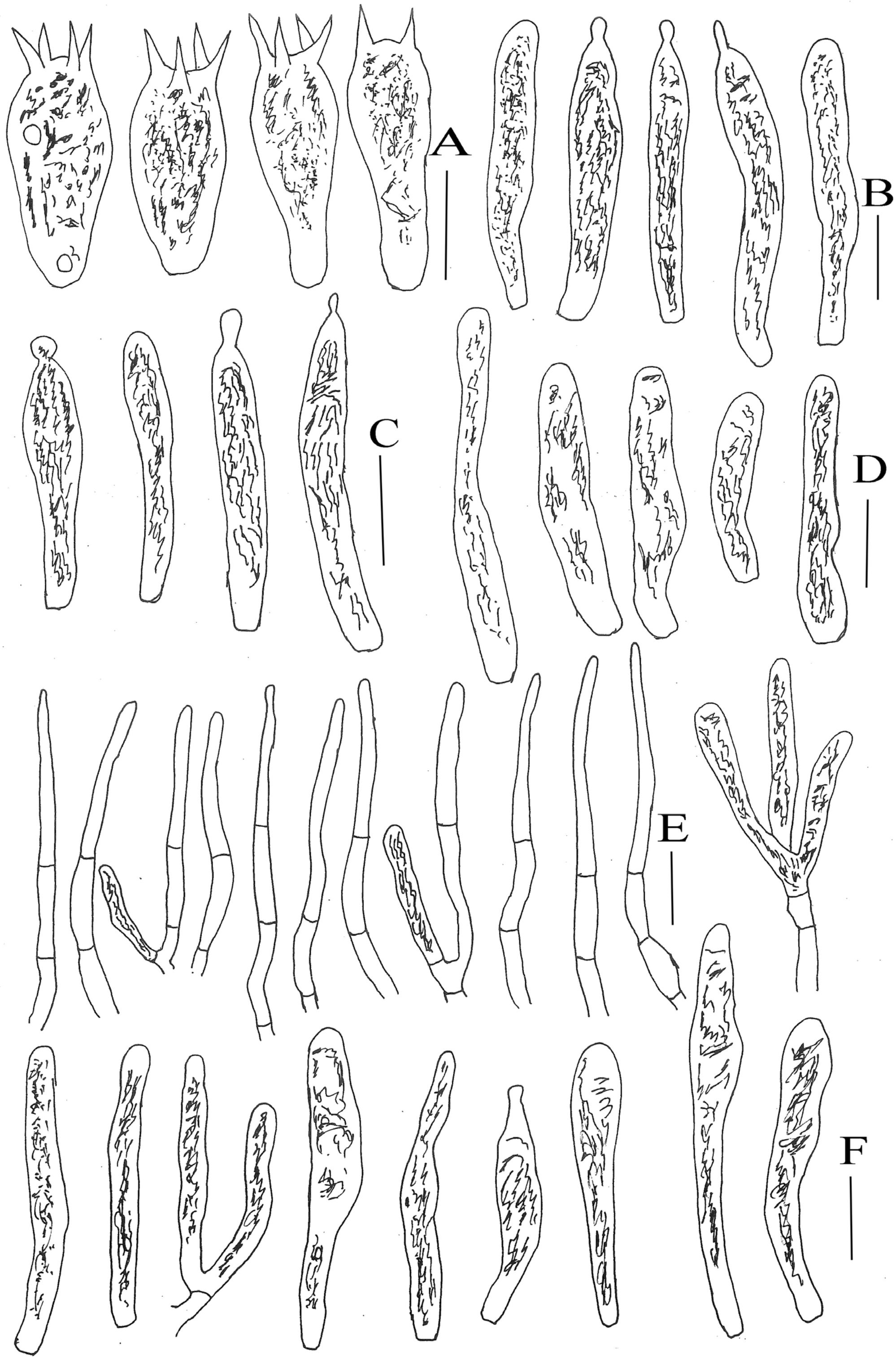

Fig. 6. *Russula minor* Y.Song sp. nov., holotype (GDGM79686). **A**. Basidia. **B**. Pleurocystidia. **C**. Cheilocystidia. **D**. Caulocystidia. **E**. Terminal elements of pileipellis. **F**. Pileocystidia. Scale bars = 10 µm. Drawing by Yu Song.

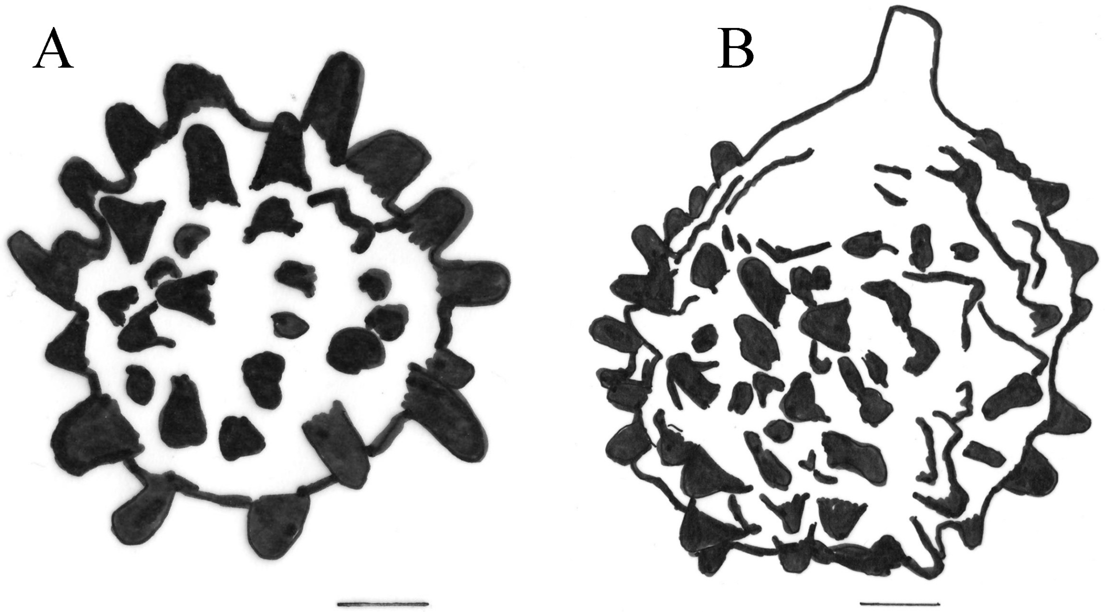

Fig. 7. Line drawings of basidiospores. **A**. *Russula coronaspora* Y.Song sp. nov. (GDGM79711) **B**. *R. minor* Y.Song sp. nov., holotype (GDGM79686). Scale bars = 1 µm. Drawing by Yu Song.

Description

Basidiocarp very small (pileus diameter less than 3 cm). Pileus 0.8–2.5 cm in diam., hemispherical to convex when young, turning applanate with depressed center or infundibuliform at maturity; surface dry, viscid when wet, sometimes slightly villose at center, easy to peel to 2/3 radius, white (#FFFAFA) to pinkish (#FAEBD7) at margin, pink, fuchsia pink to rosy (#F97D8E, #C85868, #DF828F) at center; margin smooth and entire at first, turning striate with age, sometimes cracked. Lamellae adnate, dense at first, becoming scattered with age, interveined, white when young, turning yellowish cream (#FCF3CF, #FEF9E7) at maturity; edge concolorous, smooth; lamellulae irregularly dispersed. Stipe central, fleshy, fragile, cylindrical, often curved, solid at first, turning hollow to multi-chambered with age, 0.7–1.2 × 0.2–0.4 cm, white to yellowish cream (#FCF3CF, #F8F9C2), sometimes slightly villose. Context white, sometimes yellowing at lower part of the stipe; taste mild; odor indistinct. Spore print pale cream (#F3E2A9).

Basidiospores subglobose to ellipsoid, rarely globose, (40/2/2) (5.0–)5.3–5.8–6.1(–6.6) × (4.1–) 4.3–4.6–4.9(–5.2) µm, [Q = (1.11–)1.15–1.22–1.30], hyaline in 5% KOH; ornamentation amyloid, conical to cylindrical warts less than 1.5 µm in height, mostly isolated, rarely fused into short crest, but not reticulate; suprahilar spot amyloid. Basidia clavate, 2- or 4-spored, thin-walled, with irregular contents or dorplets, small, (17–)17.5–21–27(–29.5) × 7–8.5–9.5(–10) µm; sterigmata 2.2–4.8 × 0.9–1.7 µm. Pleurocystidia subcylindrical to fusiform, with obtuse, papillate or mucronate apices, thin-walled, with irregular refractive contents, weakly SV+ or SV-, 26.5–36–48(–52.5) × 4–6–7 µm. Cheilocystidia similar to pleurocystidia, but shorter, 20–31–40(–47) in length. Subhymenium pseudoparenchymatous. Lamellar trama composed of numerous sphaerocytes surrounded by connective hyphae, sphaerocytes measuring 21–28 µm in diam. Pileipellis 60–120 µm thick, gelatinized, composed of ascending to erect hyphae; hyphae cylindrical, thin-walled, hyaline, sometimes branched, 1.5–4 µm wide; terminal cells cylindrical, slender, with obtuse or tapering apices, thin-walled, hyaline, (7–)8–18–31(–44) × 2–2.5–3.5

µm. Pileocystidia one-celled, abundant, subcylindrical to subclavate, obtuse or papillate, sometimes branched, with refractive contents, becoming brown in SV, (15.5–)16–46–106(–130) × 3–4.5–6.5 µm. Stipitipellis a cutis, composed of cylindrical, septate, thin-walled, hyaline hyphae measuring 1-3 µm in diam.; terminal cells cylindrical with obtuse apices, hyaline, 9–13.5–16(–18) × 2.5–3–4 µm. Caulocystidia subcylindrical, with obtuse apices, with irregular refractive contents, becoming brown in SV, (16.5–)21–35–62(–85.5) × 3–5–6.5 µm. Clamp connections absent in all tissues.

Discussion

Our multigene phylogeny (Fig. 2) places *Russula coronaspora* sp. nov. and *R. minor* sp. nov. in a significantly supported subclade (BS = 97%) within the crown calde of subgen. *Russula*. This subclade corresponds largely to subgen. *Tenellula* Romagn. as it is principally composed of species of the subsections *Puellarinae* Singer and *Laricinae* Romagn., both harboring typically small species with abundant dermatocystidia and short basidia and various colors of spore print. *Russula coronaspora* sp. nov. is here placed outside of *Puellarinae* and *Laricinae* but in a strongly supported subclade (95%) together with *R. melliolens* (subsect. *Melliolentinae* Singer) and a *R.* ‘cf *brunneoviolacea*’(the European species is part of Romagnesi’s subsect. *Sphagnophilinae* Romagn., equally placed in subgen. *Tenellula*, but this specimen was collected in Canada and is likely a still undescribed American taxon). In the phylogenetic tree based on ITS, *R. coronaspora* is closely related to *R. brunneoviolacea*, while *R. minor* sp. nov. clustered with several undescribed taxa from North America.

The basidiospores of *Russula coronaspora* sp. nov. which are exceptionally small, yet ornamented with large, very sparse, cylindrical and completely isolated warts or spines, resembling coronavirus, make *R. coronaspora* very special compared with its phylogenetically related species. *Russula brunneoviolacea* Crawshay and *R. melliolens* Quél. are phylogenetically related to *R. coronaspora* sp. nov., but the former two species all have a much darker pileus and spores with ornamentations more or less connected (Schaeffer *et al*. 1949; Kaur *et al.* 2011).

Russula minutula var. *minor* Z.S.Bi was previously described from Dinghushan Biosphere Reserve (Bi & Li 1986), and it resembles *R. minor* sp. nov. in the field because of small size and similar color, but their size of spores, basidia and hymenial cystidia (also form of cystidia on gill sides) proved to be different.

Russula rugulosa Peck also has small fruiting bodies and small reddish pileus (2–4 cm in diam.) with epidermis easy to peel off, which are similar to those of *R. minor* sp. nov., but the former species has tuberculately striate pileus, dense lamellae, reticulate and much bigger spores [(6.9–)7.1–7.4–7.7(–8.7) × 6.2–6.4–6.6(–7.2) µm], pileipellis orthochromatic in cresyl blue (Adamčík *et al.* 2018), which distinguish from *R. minor* sp. nov. *Russula zhejiangensis* G.J.Li & H.A.Wen and *R. nana* Killerm. all share the small basidiocarps of *R. minor* sp. nov., but the former species have obviously bigger spores, basidia and hymenial cystidia (Li *et al*. 2011), while *R. nana* has dark claret-red pileus and arctic alpine habitat. Based on their morphology, both species can be separated from *R. minor* sp. nov.

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