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 *Corinarius* (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 primordial hyphae often with with obtuse-rounded apices...
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; Caboń 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 Dimgushan 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 basidiocarps 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
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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 GTR+gamma 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       | Pakistan         | MG386704             |
| R. abietiphila                       | HCCN14799        | South Korea      | MN130060             |
| R. aurantioflamans                   | r3245            | Slovakia         | KU928167             |
| R. brunneporpurea                     | LAH35058         | Pakistan         | KT953613             |
| R. brunneporvica                     | RITF 2242        | China            | KY114148             |
| R. brunneporvica                     | MC01-507         | Denmark          | AM113956             |
| R. brunneporvica                     | PRM 922557       | Canada           | MG687327             |
| R. bayckii                           | CUHAM277         | India            | KT962833             |
| R. changbaiensis                     | HMAS262369       | China            | KC412162             |
| R. clavatothorata                    | CAL1756          | India            | MG934209             |
| R. corallina                         | BB 06.324        | USA              | JN944006             |
| R. coronaspora sp. nov.             | GDGM79711        | China            | MN275689             |
| R. coronaspora sp. nov.             | GDGM79712        | China            | MN275690             |
| R. cremerioides                      | TENN:069929      | USA              | KT933983             |
| R. curtipes                          | FH-12-206        | Germany          | KT933995             |
| R. dhakariana                        | CUHAM343         | India            | MK414576             |
| R. flavohyphata                      | AK5024           | Mexico           | MN130082             |
| R. font-queri                        | FH-12-223        | Germany          | KT934003             |
| R. gemmata                           | BPL666           | USA              | KY509511             |
| R. gnathangensis                     | CAL1733          | India            | MK253441             |
| R. grisescarnosa                     | KUN F51839       | China            | EF627042             |
| R. guangxienensis                    | HMAS267867       | China            | KT286852             |
| R. hakkii                            | HMAS267765       | China            | KT286848             |
| R. heilongjiangensis                 | HMAS255142       | China            | MG719932             |
| R. hookeri                           | CUHAM275         | India            | KP713777             |
| R. jilinensis                        | HMAS194253       | China            | GU966632             |
| R. katarinae                         | BB03.159         | USA              | KF966377             |
| R. kewzingensis                      | CAL1636          | India            | MG674302             |
| R. kheinganensis                     | HMAS278895       | China            | MG719928             |
| R. laricina                          | BB 08.681        | Italy             | JN944008             |
| R. madrensis                         | AK3422           | Mexico           | MN130093             |
| R. magicia                           | FH 12-061        | Thailand         | MN130096             |
| R. messapica var. messapica          | ALV1991          | Spain            | MK105669             |
| R. messapica var. messapicoides      | JL1493           | Spain            | MK105674             |
| R. minor sp. nov.                    | GDGM796686       | 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        | Germany          | KT933985             |
| R. nymphaema                          | FH11121505       | Spain            | KU928157             |
| R. odorata                            | BB 07.186        | Slovakia         | JN944010             |
| R. olivaceaohimalayensis             | CAL1659          | India            | MN130097             |
| R. puellaris                         | nli372 (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. 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

|          |                | Location                  |                  |
|----------|----------------|---------------------------|------------------|
| *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 (tefl); 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.

| Taxon                        | Voucher specimen | Location | Accession number          |
|------------------------------|------------------|----------|---------------------------|
|                             |                  |          | **nucLSU** | **mtSSU** | **rpb1** | **rpb2** | **tef1** |
| **R. amara**                 | BB 07.782        | France   | KU237524 | KU237370 | KU237674 | KU237810 | KU237954 |
| **R. amara**                 | FH-12-213 (GENT) | Germany  | KT335859 | —         | 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 | KU237387 | KU237735 | KU237827 | KU237971 |
| **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. coronaspera**           | GDGM79711 (holotype) | China | MN839500 | MN839360 | 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. flavissicccans**        | 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. larcica**               | BB 08.681        | Italy    | KU237560 | KU237406 | 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. meliliolens**           | 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. obscuroscordida**       | 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. pellargonia**           | 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  | KT33845  | —         | KT957359 | KT933919 | —         |
| **R. rosea**                 | BB 07.780        | France   | KU237496 | KU237340 | KU237644 | KU237782 | KU237925 |
| **R. roseinae**              | BB 09.172        | New Caledonia | KU237588 | KU237436 | KU237728 | 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 planate with depressed center at maturity; surface glabrous, dry, viscid when wet, pinkish (#FDF2E9) or brownish (#FCF3CF), sometime white (#FFFFFF) at margin; margin entire, sometimes cracked, striate when young. Lamellae white (#FFFFFF), interwoven, 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 (#FBFBF). 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-tefl1-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 $21 \times 19 \mu m$. Pilepellis orthochromatic in cresyl blue, gelatinized, composed of ascending to erect hyphae, 33–80 $\mu m$ thick; terminal cells cylindrical, with obtuse or tapering apices, thin-walled, hyaline, $(7.5–)8.5–14–19(–24) \times 1.5–2–3 \mu m$. Pileocystidia abundant, cylindrical to fusiform, with obtuse or tapering apices, mostly 1- or 2-septa, sometimes branched, with refractive contents, weakly SV+ or SV-, $(29–)30.5–62–94.5(–98.5) \times 3–4.5–6(8.5) \mu m$. Stipitipellis a cutis, composed of septate and hyaline hyphae measuring 1–3 $\mu m$ wide; terminal cells cylindrical with obtuse apices, thin-walled, hyaline. Caulocystidia frequent, cylindrical, obtuse or papillate, mostly septate, with refractive contents, $(30.5–)33–39.5–49(–57.5) \times 2–3–4 \mu m$. 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., pilepellis 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 pilepellis 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 apllanate 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. meliolens (subsect. Melliolentinae Singer) and a R. ‘cf brunneoviolacea’ (the European species is part of Romagnesi’s subsect. Spagnoophilinae 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. meliolens 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], pilepellis 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 Kullerm. 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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