

***Russula verrucospora* sp. nov.
and *R. xanthovirens* sp. nov., two novel species
of *Russula* (Russulaceae) from southern China**

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Abstract – Two novel species of *Russula* subgenus *Heterophyllidia*, collected from Guangdong Province, P. R. of China, are described and illustrated based on both morphology and phylogenetic analyses of ITS sequences. *Russula verrucospora* sp. nov. is characterized by a combination of unequal lamellae, verrucose basidiospores, presence of robust caulocystidia, and pleurocystidia changing to reddish-brown in sulphovanillin (SV). *R. xanthovirens* sp. nov. is phylogenetically closely related to *R. aureoviridis* within subsection *Virescentinae*, but it can be separated from the latter by its much thicker pileipellis which is distinctly divided into two layers and orthochromatic in cresyl blue, ellipsoid to globose subterminal cells, bigger basidiospores with higher warts and ridges, and much longer pleurocystidia and cheilocystidia. Both morphological and molecular data consistently verify that *R. verrucospora* and *R. xanthovirens* are new to subg. *Heterophyllidia*. The differences among these two novel species and their related taxa are discussed.

Russulaceae / *Heterophyllidia* / Dinghu Mountain / new taxa / morphology / phylogeny

INTRODUCTION

Russula is a common ectomycorrhizal genus with high species diversity and cosmopolitan distribution, forming ectomycorrhiza with trees in most known host families and playing crucial ecological roles (Buyck *et al.*, 2008, 2015; Buyck & Horak 1999). In recent years, some *Russula* species (Das *et al.*, 2017; Li *et al.*, 2011, 2012, 2013a, 2013b, 2015a, 2015b; Zhang *et al.*, 2017; Zhao *et al.*, 2015) were described from China, but a thorough and systematic study on the genus is still lacking.

The Dinghushan Biosphere Reserve (DHSBR, 112° 31' E, 23° 10' N) is located northwest of Hong Kong, in Zhaoqing City, Guangdong Province, P. R. of China. It is one of three reserves in China selected in 1980 to join the Biosphere Reserve Network of UNESCO due to its rich biological diversity and ecological importance. The region is subjected to a subtropical monsoon climate with the mean

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annual temperature and rainfall of 21°C and 1927 mm, respectively. DHSBR possesses three typical forest formations representing the early, middle and late successional stages of the forest: coniferous forest, coniferous and broad-leaved mixed forest, and broad-leaved forest, including a large area of well preserved monsoon evergreen broadleaved forest of at least 400 years. A thorough and systematic exploration of Russulaceae has been carried out in DHSBR since 2014 by the authors, resulting already in the publication of four new species of *Russula* (Das *et al.*, 2017; Zhang *et al.*, 2017), and three new species and one new variety of *Lactifluus* (Song *et al.*, 2017a, 2017b; Zhang *et al.*, 2016). Two additional novel *Russula* species, *R. verrucospora* sp. nov. and *R. xanthovirens* sp. nov., are proposed in this study, based on the specimens collected during this survey.

MATERIALS AND METHODS

Sampling and morphological studies

Specimens were collected during macrofungal inventories in the Dinghushan Biosphere Reserve (112° 31' E, 23° 10' N), Guangdong Province, P. R. of China from 2014 to 2017. The dominate trees distributed in the sampling area include *Castanopsis chinensis*, *Cryptocarya consinna*, *Aporosa yunnanensis*, *Erythrophleum fordii*, *Machilus chinensis*, *Microdesmis caseariifolia* and *Craibiodendron sleranthum* etc. During the survey, fresh basidiomata were photographed using a digital camera in daylight and detailed field notes of macromorphological features were recorded. Macromorphological descriptions are based on the field notes and photographs taken from fresh basidiomata. A five percent FeSO₄ solution was applied to the stipe trama to note color changes to iron sulfate on fresh specimens. Color designation refers to HTML Color Codes (<http://www.htmlcolorcode.org/>). Specimens were dried at 50-60°C and deposited in the Herbarium of Microbiology Institute of Guangdong (HMIGD).

Dried materials were hand sectioned and observed under a Nikon Eclipse Ni-U Microscope to obtain micromorphological characters. Basidiospores were examined in Melzer's reagent and measured in side view, excluding ornamentations and apiculus. All other microscopic structures (basidia, cystidia and elements of the pileipellis and stipitipellis) were observed from hand-sectioned dried tissues pretreated in 5% KOH solution and then mounted with 1% aqueous Congo red reagent. All tissue were also examined for the presence of ortho- or meta- chromatic contents or incrustations in cresyl blue (Buyck 1989a). Sulphovanillin (SV) was used to test for reactions of cystidia. Scanning electron images of basidiospores were captured with a Field Emission Scanning Electronic Microscope (JSM-6330F). Descriptive terminology followed Vellinga (1988). The abbreviation [x/y/z] indicates that measurements were made on x basidiospores in y basidiomata from z collections. Size of basidiospores is 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. Q indicates length/width ratio of basidiospores, with Q_m the average Q of all basidiospores ± standard deviation.

DNA extraction, PCR, sequencing and phylogenetic analysis.

Genomic DNA was extracted from the context of fresh specimen with CTAB procedure described by Doyle & Doyle (1987). ITS region 1 and 2, and the 5.8S rDNA were amplified using primer pair ITS1 and ITS4 (White *et al.*, 1990). The DNA fragments were amplified on a MyGene L Series Peltier thermal cycler. A hot start of 5 min at 94°C was followed by 35 cycles consisting of 30s at 94°C, 30s at 52°C, 1 min at 72°C, and a final extension step of 10 min at 72°C. PCR products were checked on 1% agarose gel stained with GelStain T155 dye (TransGen Biotech, China). PCR products were purified using E.Z.N.A Gel Extraction Kit (OMEGA) and were subjected to automated DNA sequencing on an ABI3730xl DNA Analyzer (IGE, Guangzhou, China) using primers identical to PCR. The newly generated sequences were deposited in GenBank (www.ncbi.nlm.nih.gov).

Blast query in GenBank with ITS sequences showed that the examined specimens were assigned to *Russula*. To exactly verify their taxonomic positions, Maximum Likelihood (ML) analysis based on rDNA ITS matrix was performed using RAxML 7.2.6 (Stamatakis 2006). A dataset containing 41 sequences was automatically aligned by ClustalW, then manually adjusted and trimmed with BioEdit v7.0.9 (Hall 1999). A rapid bootstrapping (BS) algorithm of 1000 replicates was executed in RAxML, followed by a heuristic ML search for the best tree using the GTRGAMMAI model. All parameters in RAxML analysis were kept at default, bootstrap value (BV) exceeding 70% was considered significantly supported.

RESULTS

Molecular phylogeny

A dataset composed of 41 sequences (listed in Table 1) involving 31 taxa representing four subgenera was used in the phylogenetic analysis, including five newly generated sequences and 36 sequences retrieved from GenBank or UNITE. *Russula archaea* and *R. camarophylla* in subg. *Archaea*, as well as *R. densifolia* and *R. nigricans* in subg. *Compactae* were chosen as outgroups, referring to Das *et al.* (2017). The final alignment comprised 682 characters including gaps. The resulting ML phylogram is shown in Fig. 1. In the phylogram, species of subg. *Heterophyllidia* formed a monophyletic clade with strong support (BS 100%), and 5 subclades that represent five recognized subsections of subg. *Heterophyllidia* are also significantly supported with high or moderate bootstrap values. The two novel species to be described both belong in subg. *Heterophyllidia* but each occupied a distinct phylogenetic position in different clades. *Russula verrucospora* sp. nov., is monophyletic with *R. shingbaensis* (Das *et al.*, 2014) with high bootstrap support (BS = 84%). Both species formed together a sister clade to subsect. *Cyanoxanthinae* with significant support (BS 75%). For the moment, the clade that is composed by these two species has not yet been named.

R. xanthovirens sp. nov., is part of a strongly supported subsect. *Virescentinae* clade, together with *R. aureoviridis*.

Table 1. Specimens and GenBank accession numbers of ITS sequences used in molecular analysis. Newly generated sequences are indicated in bold

<i>Species</i>	<i>Voucher</i>	<i>Location</i>	<i>ITS Accession No.</i>
subgenus <i>Heterophyllidia</i>			
<i>Russula aeruginea</i>	NI1292	Germany	UDB000341
<i>R. albidogrisea</i>	GDGM 48783	China	KY767807
<i>R. alboareolata</i>	SUT-1	Thailand	AF345247
<i>R. atroaeruginea</i>	HKAS53626	China	JX391967
<i>R. aureoviridis</i>	GDGM 48786	China	KY767809
<i>R. crustosa</i>	BB2004-214, PC	USA	EU598193
<i>R. cyanoxantha</i>	HKAS78376	China	KF002766
<i>R. cyanoxantha</i>	RUS24	Europe	AY061669
<i>R. dinghuensis</i>	K15052704-3	China	KU863581
<i>R. grisea</i>	Watling27098, E	Europe	AY061679
<i>R. heterophylla</i>	Hue103 (TUB)	Germany	AF418609
<i>R. heterophylla</i>	Buyck99.803, PC	Europe	AY061681
<i>R. ilicis</i>	Sarnari10/18/99	Europe	AY061682
<i>R. indoalba</i>	AG15-628	India	KX234820
<i>R. ionochlora</i>	BB72-407	Europe	HM189875
<i>R. kanadii</i>	CAL1162	India	KJ866933
<i>R. nigrovirens</i>	HKAS55222	China	KP171173
<i>R. pallidivirens</i>	UTC00274382	USA	KR831283
<i>R. shingbaensis</i>	KD11-094	India	KM386692
<i>R. subpallidivirens</i>	GDGM 45242	China	KU863582
<i>R. variata</i>	JMP0078	USA	EU819436
<i>R. verrucospora</i>	GDGM 71136 (holotype)	China	MG786052
<i>R. verrucospora</i>	GDGM 71140	China	MG786053
<i>R. verrucospora</i>	GDGM 71137	China	MG786054
<i>R. vesca</i>	Buyck99.802, PC	Europe	AY061723
<i>R. vesca</i>	AT2002091, UPS	Europe	DQ422018
<i>R. virescens</i>	Buyck99.808, PC	Europe	AY061727
<i>R. werneri</i>	IB1997/0786	Europe	DQ422021
<i>R. xanthovirens</i>	GDGM 71145 (holotype)	China	MG786055
<i>R. xanthovirens</i>	GDGM 71146	China	MG786056
subg. <i>Ingratula</i>			
<i>R. granulata</i>	BB2004-226, PC	USA	EU598192
<i>R. granulata</i>	BB2004-228, PC	USA	EU598189
<i>R. pectinatoides</i>	MICH52692	USA	KF245518
<i>R. pectinatoides</i>	NAMA499	USA	EU819493
<i>R. praetervisa</i>	IB1997/0812	Italy	UDB019331
<i>R. praetervisa</i>	UE2006-11-12-01	Italy	UDB019333
outgroup			
<i>R. archaea</i>	IS79	Europe	AY061737
<i>R. camarophylla</i>	IS68	Europe	AY061662
<i>R. densifolia</i>	Ue116 (TUB)	Europe	AF418606
<i>R. nigricans</i>	Fo46792 (TUB)	Europe	AF418607
<i>R. nigricans</i>	MC01-511	Europe	AM113962

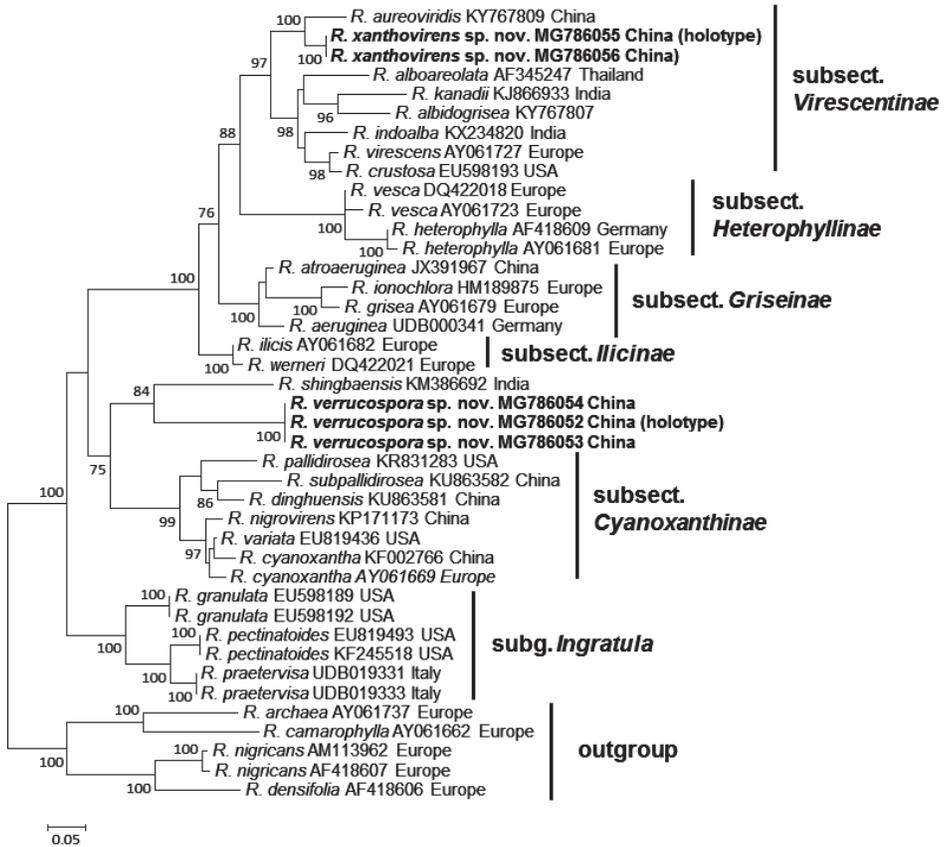


Fig. 1. Phylogram generated from Maximum Likelihood (ML) method based on rDNA ITS sequences performed by RAxML. One thousand bootstrap replicates followed by a heuristic ML tree search was analyzed. Bootstrap values higher than 70% are shown above or below the branch. Two novel species are shown in bold.

Taxonomy

***Russula verrucospora* Y. Song & L. H. Qiu sp. nov.**

Figs 2 a-c; 3

Mycobank: MB823961

Systematic position: Basidiomycota, Agaricomycetes, Russulales, Russulaceae.

Etymology: Referring to its verrucose spore ornamentation composed of totally isolated warts in different sizes.

Diagnosis: *Russula verrucospora* sp. nov. is characterized by a combination of unequal lamellae with lamellulae, verrucose basidiospores (ornamentations composed of completely isolated warts in different sizes), presence of robust caulocystidia, and pleurocystidia changing to reddish-brown in sulphovanillin (SV).

Holotype: CHINA. Guangdong Province, Zhaoqing City, Dinghushan Biosphere Reserve, on the ground in broad-leaf forest, 25 September 2017, J. W. Li K17092512 (GDGM 71136, **holotype**).

Basidiomata small sized. *Pileus* 2-4 cm in diam., plano-convex when young, becoming applanate with a depressed centre, then sub-infundibuliform with age; surface glabrous, dry, green-tinged ochre (#C9B39C) when young, often with reddish brown center, becoming olivaceous (#ACA16B) with brown center, purplish

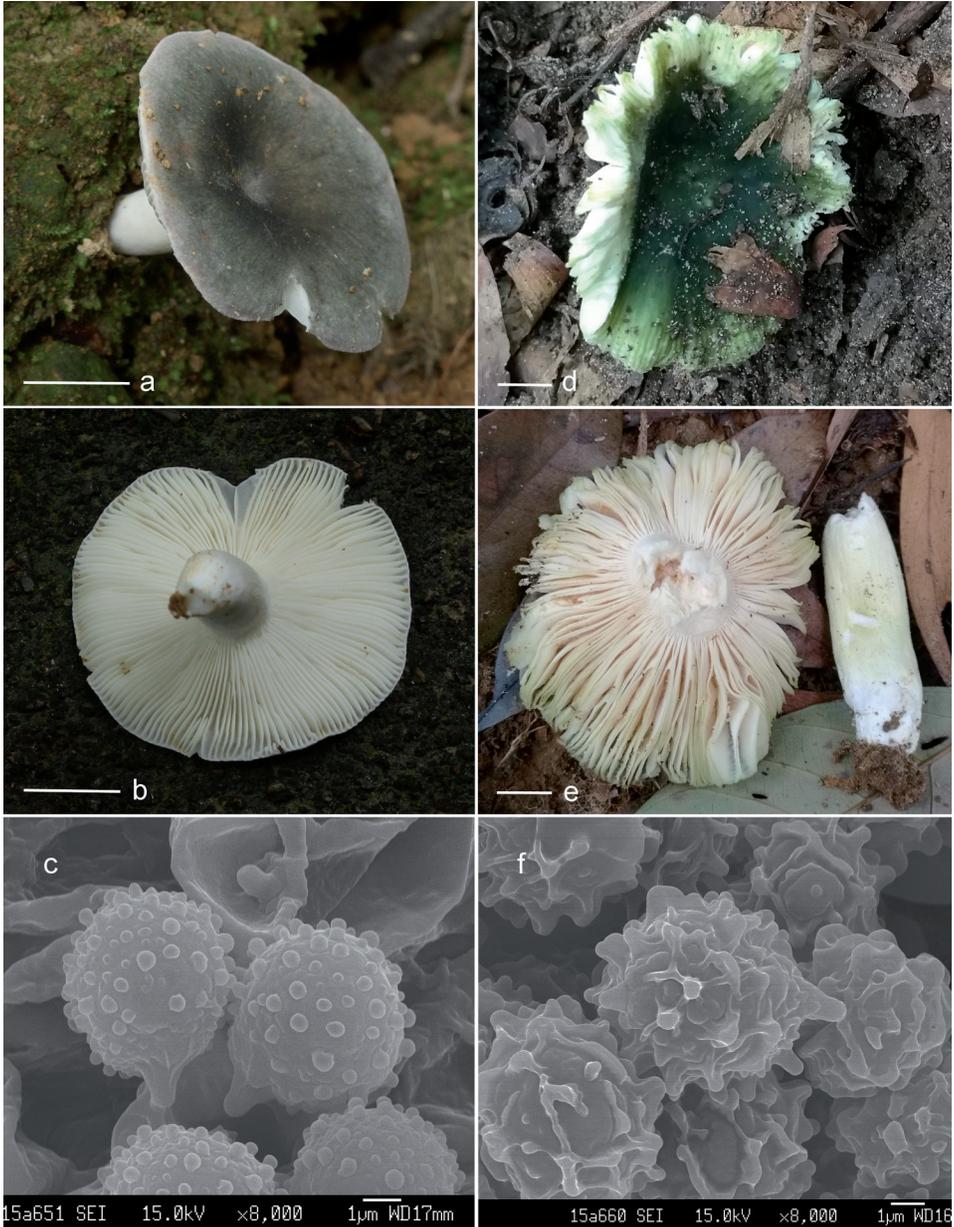


Fig. 2. **a-c.** *Russula verrucospora* (GDGM 71136, holotype); **d-f.** *Russula xanthovirens* (GDGM 71145, Holotype). Scale bars: **a-b, d-e.** (photographs of basidiocarps) = 1 cm; **c, f.** (Scanning Electronic Micrographs of basidiospores) = 1 µm.

grey (#646A5E) with darker center or livid vinaceous (#8C8280) at maturity, pale ochre (#C1C2BA) or brown with age; margin entire or slightly wavy, smooth or slightly striate when young, striate and cracked with age, readily peeling to 1/3 radius. *Lamellae* adnate to subdecurrent, whitish, 1-3 mm wide, unchanging after bruising, unequal, not or rarely forking, rarely inter-veined, with even, concolorous edges; lamellulae frequent but not regularly polytomous. *Stipe* 22-40 × 3.5-7.5 mm, cylindrical, often slightly tapering towards base, central, solid; surface whitish, dry, longitudinally rugulose. *Context* 1-3 mm thick, whitish, unchanging after bruising or with FeSO₄. *Taste* mild. *Odour* indistinct. *Spore print* whitish.

Basidiospores subglobose to broadly ellipsoid, small, [100/5/3] (4.8) 5.0-5.7-6.7 (7.5) × (4.0) 4.3-5.0-5.9 (6.1) μm, Q = 1.11-1.13-1.18(1.22), hyaline in 5% KOH; ornamentation amyloid, not exceeding 0.7 μm in height but very variable in size, completely composed of isolated warts; warts pustulose to hemispherical, almost cylindrical when higher, never strongly attenuated and sharp toward the tip (not spinose); suprahilar spot distinct but not amyloid. *Basidia* (27) 28-42.5 (46) × (7) 8-11 (11.5) μm, mostly 4-spored, rarely 2-spored, clavate to subcylindrical; sterigmata 1.7-7.4 × 1.2-2.6 μm. *Subhymenium* pseudoparenchymatous. *Lamellar trama* composed of numerous sphaerocytes surrounded by connective hyphae; sphaerocytes measuring 14-32.5 × 7.5-22.5 μm. *Pleurocystidia* 33-74 × 7-10.5 μm, clavate to subcylindrical, with mucronate or appendiculate apices, abundant, thin-walled, mostly with refractive heteromorphous contents that are restricted to the upper part of the cystidia, reddish-brown in sulphovanillin (SV). *Cheilocystidia* 31.5-72.5 × 11-18.5 μm, fusoid to subcylindrical, often with mucronate or moniliform apices, thin-walled, with distinct refractive contents. *Marginal cells* not differentiated. *Pileipellis* orthochromatic in cresyl blue, composed of subparallel to erect hyphae (up to 8 μm broad) and cystidia; hyphal extremities having terminal cells measuring 11-24 × 4.5-6 μm, cylindrical to subconical with rounded apices. *Pileocystidia* abundant, one-celled, 28-133.5 × 5.5-13 μm, subulate to cylindrical, with mucronate or capitate apices, with distinct, dense content, becoming slightly reddish brown in SV, also present in subpellis and in lower pileus context. *Stipitipellis* a cutis, composed of parallel hyphae (up to 6 μm broad), orthochromatic in cresyl blue; caulocystidia 32.5-82 × 4.5-13.5 μm, fusoid to lanceolate, with mucronate to moniliform apices. *Stipe trama* composed of connective hyphae and nested sphaerocytes measuring 18-45 × 11.5-32 μm. *Clamp connections* absent in all tissues.

Habitat and distribution: Solitary or gregarious on the ground in monsoon evergreen broad-leaf forest and pine-broadleaf mixed forest.

Additional specimens examined: CHINA, Guangdong Province, Zhaoqing City, Dinghu Mountain, on the ground in mixed pine-broadleaf forest, 14 September 2016, J. W. Li H16091404 (GDGM 71144); CHINA, Guangdong Province, Zhaoqing City, Dinghu Mountain, on the ground in broad-leaf forest, 28 May 2015, J. B. Zhang K15052817 (GDGM 71143); 7 June 2015, J. B. Zhang K15060704 (GDGM 71142); 12 September 2016, J.W. Li K16091205 (GDGM 71140); 12 July 2017, J. W. Li K17071212 (GDGM 71139); 25 September 2017, J. W. Li K17092506 (GDGM 71137).

***Russula xanthovirens* Y. Song & L. H. Qiu sp. nov.**

Figs 2 d-f; 4

Mycobank: MB823964

Systematic position: Basidiomycota, Agaricomycetes, Russulales, Russulaceae.

Etymology: Referring to its yellowish green pileus.

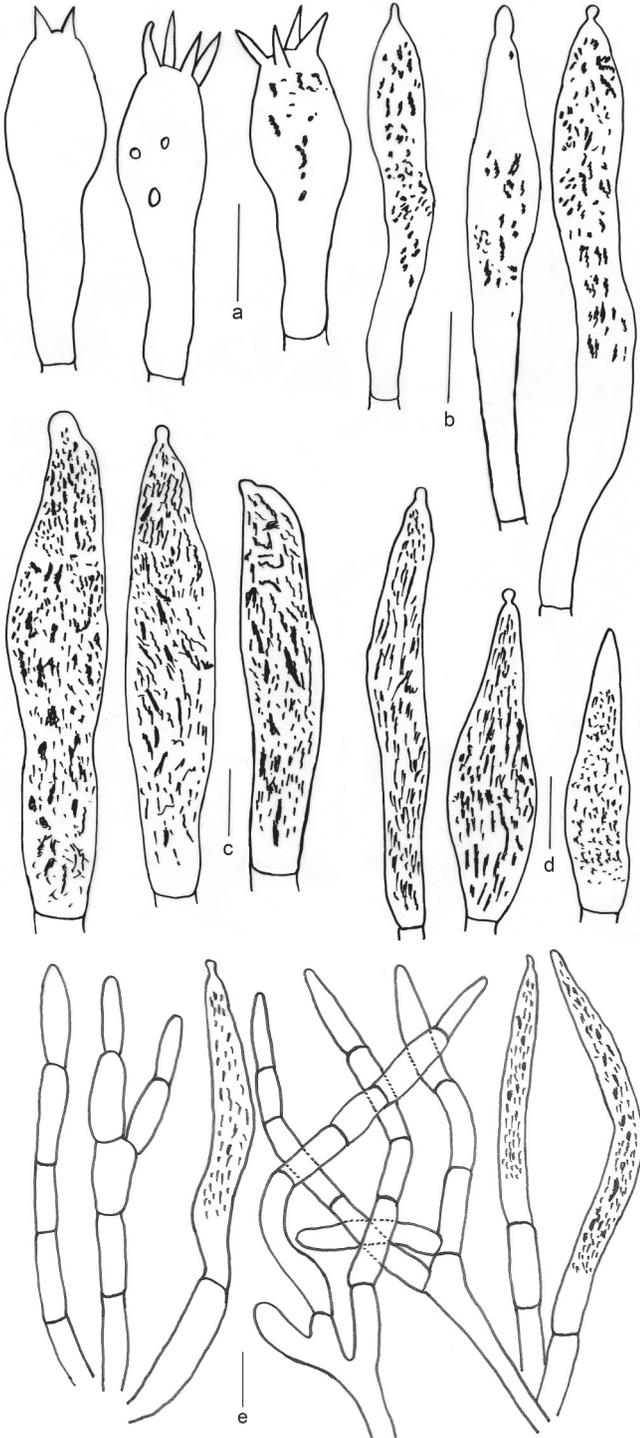


Fig. 3. *Russula verrucospora* (GDGM 71136, holotype). a. Basidia; b. Pleurocystidia; c. Cheilocystidia; d. Caulocystidia; e. Pileipellis. Scale bars = 10 μ m.

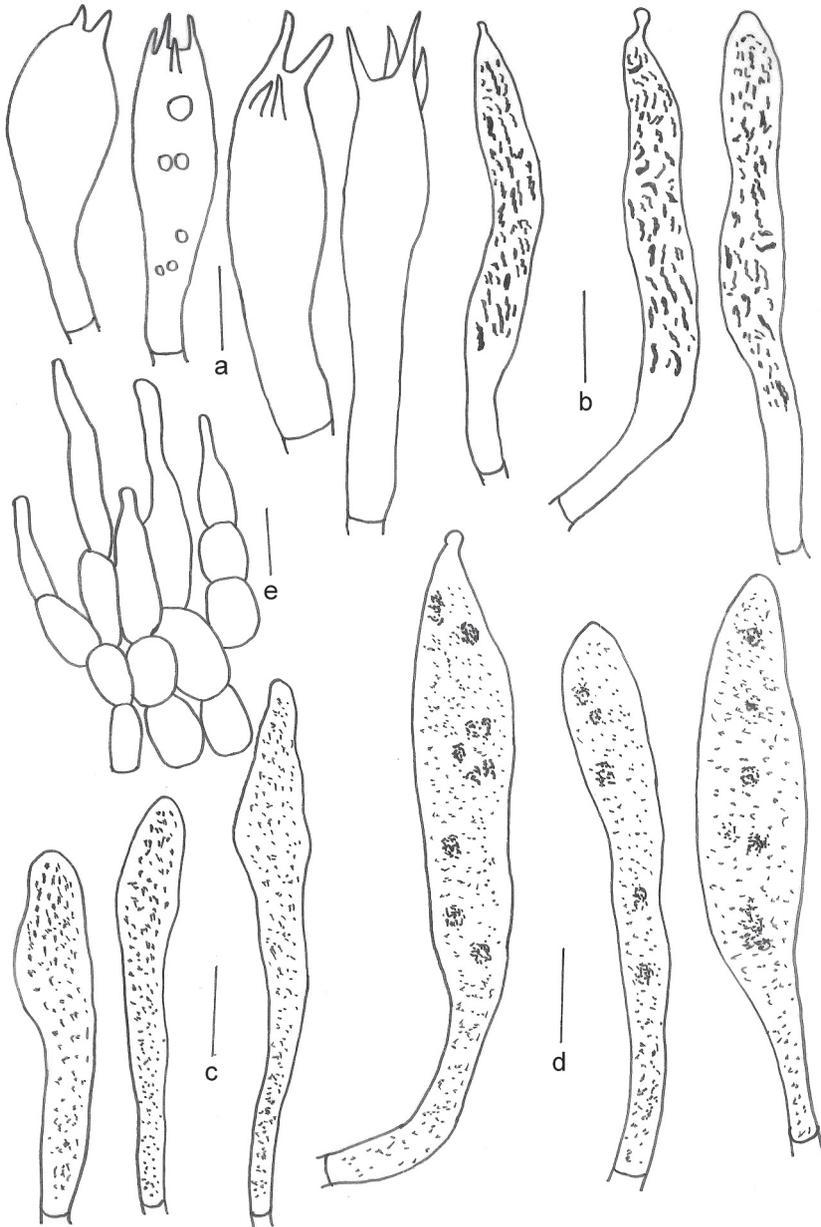


Fig. 4. *Russula xanthovirens* (GDGM 71145, holotype). **a.** Basidia; **b.** Pleurocystidia; **c.** Pileocystidia; **d.** Cheilocystidia; **e.** Terminal and subterminal cells of Pileipellis. Scale bars = 10 μ m.

Diagnosis: *R. xanthovirens* sp. nov. is characterized by its thick pileipellis which is distinctly divided into two layers and orthochromatic in cresyl blue, ellipsoid to globose subterminal cells, basidiospores composed of warts and ridges forming incomplete reticulum, and context unchanged when bruising.

Holotype: CHINA. Guangdong Province, Guangzhou City, Baiyun Mountain, on the ground in broad-leaf forest, 16 September 2017, L. H. Qiu B17091630 (GDGM 71145, **holotype**).

Basidiomata medium sized. *Pileus* 5-7.5 cm in diam., applanate to plano-concave with a slightly depressed center, often subinfunduliform and cracked with age; margin entire, striate, becoming cracked and sulcate with age; surface dry, glabrous, yellowish green (#AAC381), deep green (#182720) with cracked yellowish margin when aged, readily peeling to 1/4 radius. *Lamellae* adnate to subdecurrent, regular, smooth, compact, frequently forked, 3-4 mm deep, whitish with reddish hue, unchanging when bruised; edge even, concolorous, becoming slightly green-tinged when old. *Stipe* 4-6 × 0.8-1.2 cm, cylindrical, slightly attenuating towards base, central, solid; surface dry, white with green tinge, longitudinally rugulose. *Context* whitish, 3-6 mm thick, not changing when bruised. *Taste* mild. *Odour* indistinct. *Spore print* cream.

Basidiospores subglobose to broadly ellipsoid, small, [60/3/2] (5.2) 5.6-6.4-7.6 (8.3) × (4.5) 4.6-5.4-6.2 (6.4) μm, Q = (1.16) 1.17-1.19-1.23 (1.30), hyaline in 5% KOH; ornamentation amyloid, composed of conical to subcylindrical warts and ridges forming incomplete reticulum, warts measuring 0.4-0.8 μm high, ridges up to 0.5 μm high; suprahilar spot distinct, but not amyloid. *Basidia* (29) 30-51.5 (60) × (9.5) 10-14.5 (15) μm, mostly 4-spored, rarely 2-spored, clavate to subcylindrical, some containing oil droplets when young; sterigmata 1.2-8.6 × 1-3.5 μm. *Subhymenium* pseudoparenchymatous. *Lamellar trama* mainly composed of sphaerocytes measuring up to 60 μm diam. *Pleurocystidia* 49-79 × 6-11.5 μm, emergent up to 20 μm, subcylindrical, with mucronate or appendiculate apices, thin-walled, mostly with refractive heteromorphous contents that are restricted to the central or upper part of the cystidia, negative with sulphovanillin (SV). *Cheilocystidia* 38-58.5 × 6.5-13.5 μm, clavate to fusoid, often with papillate to mucronate apices, thin-walled, content blank or with rare refractive granules, unchanged in SV. *Marginal cells* not differentiated. *Pileipellis* orthochromatic in cresyl blue, 230-320 μm thick, distinctly divided into two layers: suprapellis (140-200 μm thick) composed of ascending to erect hyphae mostly with chains of inflated subterminal cells and a dense subpellis (80-170 μm deep) composed of horizontally oriented hyphae; terminal cells 11-49 × 5-7.5 μm, subcylindrical to subulate; subterminal cells 5.5-18.5 × 5-13.5 μm, ellipsoid to globose or cylindrical. *Incrustations* absent. *Pileocystidia* 40-69.5 × 4-9 μm, narrowly clavate to fusiform, only present in suprapellis, one-celled, thin-walled, some subterminally constricted, with rounded apices, rarely mucronate, unchanging in SV; cystioid hyphae absent. *Stipitipellis* a cutis, orthochromatic in cresyl blue, composed of repent to interwoven hyphae up to 6 μm broad with subulate terminal cells, measuring 17-30 × 4-8 μm. *Caulocystidia* absent. *Stipe trama* composed of connective hyphae and nested sphaerocytes up to 50 μm diam. *Clamp connections* absent in all tissues.

Habitat and distribution: Gregarious on the ground in monsoon evergreen broad-leaf forest and pine-broadleaf mixed forest.

Additional specimens examined: CHINA, Guangdong Province, Zhaoqing City, Dinghu Mountain, on the ground in pine-broadleaf mixed forest, 6 June 2015, J. B. Zhang H15060611 (GDGM 71146).

DISCUSSION

Russula verrucospora sp. nov. and *R. xanthovirens* sp. nov. are proposed based on morphological and phylogenetic evidence. In terms of the phylogenetic analysis based on rDNA ITS sequences, *R. verrucospora* and its sister species *R. shingbaensis* (Das *et al.*, 2014) form a distinct clade in *Russula* subgenus *Heterophyllidia*, sister to subsect. *Cyanoxanthinae* Singer. Both species share the verrucose basidiospores with inamyloid suprahilar spot, mucronate dermatocystidia and short-celled hyphal extremities, but *R. verrucospora* can be distinguished by its unforked lamellae mixed with frequent lamellulae, uninflated terminal cells in pileipellis, smaller basidiospores ($7.0\text{-}7.9\text{-}8.9 \times 6.1\text{-}6.7\text{-}7.6 \mu\text{m}$ in *R. shingbaensis*), larger cheilocystidia and pileocystidia, presence of caulocystidia, and SV-positive (changing to reddish-brown) pleurocystidia. In addition, the tuberculate-striate pileus margin and multichambered stipe context of *R. shingbaensis* are totally different from the almost smooth pileus margin and stuffed stipe of *R. verrucospora*.

The limited sampling used in Das *et al.* (2014) did not allow for a precise placement of *R. shingbaensis* within subg. *Heterophyllidia*, unlike our analysis which positions *R. verrucospora* sister to subsection *Cyanoxanthinae* with significant support. Our species shares with *Cyanoxanthinae* the insensitivity to FeSO_4 , which is typical for most *Cyanoxanthinae* (pale salmon reaction in *R. shingbaensis*). However, our species differs from most known *Cyanoxanthinae* in its orthochromatic pileipellis in cresyl blue (metachromatic in most *Cyanoxanthinae*), spore ornamentation composed of isolated, large warts that have a circular base (not laterally prolonged warts or subreticulate spores as in most *Cyanoxanthinae*). *Cyanoxanthinae* have predominantly been described from the temperate zone of the northern hemisphere, but were also reported as quite common in Central Africa (Buyck 1989b, 1996). The systematic placement of these tropical African species was until now exclusively based on their similar morphology, but multigene sequence data have now confirmed this (Buyck *et al.* 2018) and have additionally suggested that this subsection is sister to another species group that has so far an exclusive tropical African distribution: *Aureotactinae* Heim ex Buyck. Species of the latter subsection share with the subtropical *R. verrucospora* a very similar spore ornamentation as well as similar dermatocystidia, but they differ in their strongly yellowing, often very acrid taste, as well as in their frequently very thin context and lacunose stipe (in both latter features thus resembling more *R. shingbaensis*). Finally, the frequent lamellulae of this new species are unique within the context of subg. *Heterophyllidia*. It is interesting to note that Das *et al.* (2017) recently described *R. aureorubra* from India in subsect. *Emeticinae* based on sequence data as the sole representative of this subsection to possess many lamellulae amongst a group of species that all have equal gills.

Our second species, *R. xanthovirens* sp. nov. fits both morphologically and molecularly in subg. *Heterophyllidia* subsect. *Virescentinae*, where it is part of a fully supported subclade composed of two Chinese species. Our phylogeny places it as sister to *R. aureoviridis* which has an ITS sequence similarity of only 95.1%. *R. xanthovirens* can be separated from the latter species by its much thicker pileipellis which is conspicuously divided into two layers, the ellipsoid to globose subterminal cells, bigger basidiospores with higher warts and ridges, and much longer pleurocystidia and cheilocystidia (see Table 2).

Table 2. Detailed comparison of morphological characters for two new taxa with their phylogenetically closely related species

<i>Character</i>	<i>R. verrucospora</i>	<i>R. shingbaensis</i>	<i>R. xanthovirens</i>	<i>R. aureoviridis</i>
Pileus size (cm)	small sized, 2-4	medium sized, 4.1-6.2	medium sized, 5-7.5	medium sized, 4.5-8.2
Pileus color	mainly olivaceous to purplish grey	<u>colorful, mainly greyish green</u>	yellowish green to deep green	yellowish green to golden green
Pileus margin	smooth to slightly striate	<u>tuberculate striate-sulcate</u>	striate, sulcate and cracked with age	smooth, striate with age
Lamellae	adnate, unforked or rarely forked	<u>adnexed, forked near stipe</u>	adnate to subdecurent, forked	adnate, forked near stipe
Lamellulae	frequent	<u>absent</u>	absent	absent
Stipe (mm)	solid	<u>multichambered</u>	solid	–
Context bruised or in FeSO ₄	unchanging	unchanging	unchanging when bruised	turning brown when bruised, salmon red with FeSO₄
Spore size (µm)	(4.8) 5.0-5.7-6.7 (7.5) × (4.0) 4.3-5.0-5.9 (6.1)	<u>7.0-7.9-8.9 × 6.1-6.7-7.6</u>	(5.2) 5.6-6.4-7.6 (8.3) × (4.5) 4.6-5.4-6.2 (6.4)	(4.9) 5.3-5.7-6.1 (6.7) × (4.4) 4.8-5.1-5.5 (6.0)
Spore ornamentation	isolated warts	isolated warts	conical warts and ridges forming incomplete reticulum	ridges forming incomplete reticulum
Pleurocystidia	33-74 × 7-10.5	58-76 × 7-9	49-79 × 6-11.5	38-50 × 7-12
Pleurocystidia in SV	changing to reddish brown	–	unchanging	unchanging
Cheilocystidia	31.5-72.5 × 11-18.5	<u>33-41 × 7-10.5</u>	38-58.5 × 6.5-13.5	27-40 × 6-10
Pileipellis	cutis to trichoderm	<u>ixocutis to ixotrichoderm</u>	thick, distinctly divided into supra- and subpellis	composed of supra- and subpellis
Subterminal cells	uninflated	<u>globose to elongated inflated cells</u>	ellipsoid to globose	slightly inflated
Caulocystidia	present	<u>absent</u>	absent	absent

Characters underlined are those differ from *R. verrucospora*; those in bold fonts differ from *R. xanthovirens*.

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