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Research article

Species of *Russula* subgenus *Heterophyllidiae* (Russulaceae, Basidiomycota) from Dinghushan Biosphere Reserve

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Abstract. Two new species of subgenus *Heterophyllidiae* subsection *Cyanoxanthinae*, *Russula fusiformata* Y.Song sp. nov. and *R. purpureorosea* Y.Song sp. nov., collected from the Dinghushan Biosphere Reserve (DHSBR), are described based on both morphology and a phylogenetic analysis of the internal transcribed spacer (ITS), further increasing *Heterophyllidiae* species diversity in the area. Differences between the two new species and related taxa are analyzed. The other 17 reported species of *Russula* subgenus *Heterophyllidiae* that have been collected from DHSBR during mushroom explorations since 2014 are also summarized. The dominant species and the ecological distribution of all 19 species are briefly discussed, and most species are presented in macrofungal plates.

Keywords. Morphology, phylogeny, new taxa, species diversity.

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Introduction

Russula Pers. is a hyperdiverse ectomycorrhizal genus with a worldwide distribution from temperate to tropical areas (Buyck 1989; Buyck & Horak 1999; Buyck *et al.* 2015, 2018). Due to its edibility, toxicity, ecological functions and diversity, *Russula* is among the most important lineages of macrofungi (Looney *et al.* 2016; Wu *et al.* 2019). Species of *Russula* are easy to recognize by their brittle context and relatively large and brightly colored surface of the pileus. But the genus displays particular variability in macro- and micromorphological phenotype and chemical reactions, which has led to a complex infrageneric classification that is still artificial (Romagnesi 1967; Singer 1986; Bon 1988; Sarnari 1998). According to the newest classification system based on both multi-locus phylogeny, macrofungal morphology and ectomycorrhizal phenotype, *Russula* is divided into 8 subgenera, namely *Archaeae* Buyck & V.Hofst., *Brevipedum* Buyck & V.Hofst., *Compactae* (Fr.) Bon, emend. Buyck & V.Hofst., *Crassotunicatae* Buyck & V.Hofst., *Glutinosae* Buyck & X.H.Wang, *Heterophyllidiae* Romagnesi, emend. Buyck & V.Hofst., *Malodorae* Buyck & V.Hofst. and *Russula*, emend. Buyck & V.Hofst. (Buyck *et al.* 2018, 2020). Species in subgen. *Heterophyllidiae* mostly share medium to large basidiomata with the pileus coming in almost all possible colors, predominantly equal lamellae, frequently distinct smell, mild to strongly

acrid taste, white or various shades of cream to ochre spore print, spores with inamyloid or partly amyloid suprahilar spot and abundant gloeocystidia (except for subsect. *Amoeninae* (Singer) Buyck).

The Dinghushan Biosphere Reserve (DHSBR, 112°30′39″–112°33′41″ E, 23°9′21″–23°11′30″ N) is located in Zhaoqing City, Guangdong Province, southern China, in the subtropical monsoon climate zone. DHSBR is considered as a region highly diverse in macrofungi (Bi *et al.* 1994), and possesses three typical forest formations representing the early, middle and late succession stages of the forest: coniferous forest, mixed coniferous and broad-leaf forest, and broad-leaf forest. During the macrofungi explorations carried out since 2014, eighteen new species and one epitype of *Russula* (Das *et al.* 2017; Zhang *et al.* 2017; Song *et al.* 2018a, 2018b, 2021; Li *et al.* 2019; Yuan *et al.* 2019; Zhou *et al.* 2020), two new species of *Lactarius* Pers. (Wang *et al.* 2018) and three new species and one new variety of *Lactifluus* (Pers.) Roussel (Zhang *et al.* 2016, 2017; Song *et al.* 2017, 2018c) have been reported from this area. In this study, another two novel species in *Russula* subgen. *Heterophyllidiae* subsection *Cyanoxanthinae* Singer, named as *Russula purpureorosea* Y.Song sp. nov. and *R. fusiformata* Y.Song sp. nov., are proposed based on both morphological and phylogenetic evidence. Until now, a total of 19 *Russula* species in subgen. *Heterophyllidiae* have been reported, 15 of which were first collected and described from DHSBR. All species of *Heterophyllidiae* with voucher specimens and ITS sequences collected from DHSBR since 2014 are summarized (Table 1), species distribution in three different vegetation types is briefly analyzed (Table 2), and most species are presented in macrofungal plates (Appendices 1–12).

Material and methods

Sampling and morphological studies

Fruiting bodies of the specimens were all collected from Dinghushan Biosphere Reserve (DHSBR, 112°33′ E, 23°10′ N), Guangdong Province, China. Specimens were dried at about 40°C and deposited in the Herbarium of Guangdong Institute of Microbiology (GDGM). Macroscopic characteristics of the intact fresh fruit bodies were recognized in daylight in the field and were photographed using a Canon IXUS 220 hs digital camera. HTML Color Codes (https://htmlcolorcodes.com/color-names/) were used to describe the color terminology.

Tissue sections were immersed in 5% KOH and then stained with 1% aqueous Congo red solution to observe the microscopic characters. All tissues were also examined in Cresyl blue to verify the presence of ortho- or metachromatic reactions as explained in Buyck (1989). Sulfovanillin (SV) was used to test for staining reactions of cystidia. Micromorphological features were observed and photographed using a Nikon E200 microscope. Basidiospores were also observed in Melzer's reagent and measured in side view, excluding ornamentation and apiculus which were observed by SEM. The notation (x/y/z) indicates that measurements were made on x basidiospores in y fruit bodies from z specimens. In the notation of basidiospore dimensions '(a–) b–m–c (–d)', b–c is the range including 95% of the measured values for length or width, with 'a' and 'd' corresponding to the extremes of all measurements, and 'm' corresponding to the mean value. Q indicates the length/width ratio of basidiospores.

DNA extraction, PCR, sequencing and phylogenetic analyses

Protocols for DNA extraction followed the method described by Zhou & Liang (2011). The internal transcribed spacer (ITS) regions of nuclear ribosomal DNA were amplified with the primer pair ITS1F/ITS4 (White *et al.* 1990; Gardes & Bruns 1993). The protocol for PCR amplification is as follows: a 5 min activation at 94°C, followed by 32 cycles of 30 s at 94°C, 30 s at 52°C and 1 min at 72°C, and a final 12 min extension at 72°C. PCR products were purified using an E.Z.N.A Gel Extraction Kit (OMEGA) and sequenced on an ABI3730xl DNA Analyzer (IGE, Guangzhou, China) using primers identical to PCR. The newly generated sequences were submitted to GenBank (www.ncbi.nlm.nih.gov) (Table 1).

Table 1 (continued on next three pages). Voucher specimens and ITS accession nos of species of the subgenus *Heterophyllidiae* Romagnesi, emend. Buyck & V.Hofst. collected from Dinghushan Biosphere Reserve.

Taxon	Voucher specimen	ITS accession no.
R. dinghuensis J.B.Zhang & L.H.Qiu (Appendix 1)	GDGM45244 (holotype)	KU863579
	GDGM79644	MN275627
	GDGM79645	MN275628
	GDGM79646	OM021892
	GDGM79647	OM021893
	GDGM79648	OM021894
	GDGM79649	MN275632
	GDGM79650	OM021895
	GDGM79651	MN275634
	GDGM79652	MN275635
	GDGM79653	OM021896
	GDGM79654	MN275637
	GDGM79655	OM021897
	GDGM79656	OM021898
	GDGM79657	OM021899
R. fusiformata Y.Song sp. nov. (Figs 2–3, 6b)	GDGM75333 (holotype)	MK049978
	GDGM75332	MK049979
R. lotus Fang Li (Appendix 2)	GDGM79632	MN275615
	GDGM79633	MN275616
	GDGM79634	MN275617
	GDGM79635	MN275618
R. purpureorosea Y.Song sp. nov. (Figs 4–5, 6a)	GDGM75331 (holotype)	MK049976
	GDGM75334	MK049977
R. subpallidirosea J.B.Zhang & L.H.Qiu (Appendix 3)	GDGM45242 (holotype)	KU863582
	GDGM77425	KU863578
	GDGM77426	OM021900
	GDGM77427	OM021901
	GDGM77428	OM021902
	GDGM77429	OM021903
	GDGM79636	OM021904
	GDGM79637	MN275620
	GDGM79638	MN275621
	GDGM79639	OM021905
	GDGM79640	OM021906
	GDGM79641	MN275624
	GDGM79642	OM021907
	GDGM79643	OM021908
R. bubalina J.W.Li & L.H.Qiu (Appendix 4)	GDGM70728 (holotype)	MG018742
	GDGM70727	MG018741
	GDGM79602	OM021909
	GDGM79603	MN275583
	GDGM79604	OM021910
	GDGM79605	OM021911
	GDGM79606	MN275586
	GDGM79607	OM021912

Table 1 (continued). Voucher specimens and ITS accession nos of species of the subgenus *Heterophyllidiae* collected from Dinghushan Biosphere Reserve.

Taxon	Voucher specimen	ITS accession no.
R. bubalina J.W.Li & L.H.Qiu (Appendix 4)	GDGM79608	MN275588
R. pseudobubalina J.W.Li & L.H.Qiu (Appendix 5)	GDGM70632 (holotype)	MF433036
	GDGM71132	MH422581
	GDGM79611	MN275590
	GDGM79612	MN275591
R. subatropurpurea J.W.Li & L.H.Qiu (Appendix 6)	GDGM70634 (holotype)	MF433038
	GDGM70633	MF433037
	GDGM79609	MN275589
	GDGM79610	MH422579
R. vesca Fr. (Appendix 7)	GDGM79615	OM021913
	GDGM79616	MN275593
	GDGM79617	MN275594
	GDGM79618	MN275595
	GDGM79619	MN275596
	GDGM79620	OM021914
	GDGM79621	OM021915
	GDGM79622	MN275599
	GDGM79623	OM021916
	GDGM79624	MN275601
	GDGM79625	OM021917
	GDGM79626	MN275603
	GDGM79627	OM021918
	GDGM79628	MN603054
R. viridicinnamomea F.Yuan & Y.Song (Appendix 8)	GDGM75339 (holotype)	MK049972
	GDGM75340	MK049973
	GDGM79613	OM021919
	GDGM79614	MN603053
R. albidogrisea J.W.Li & L.H.Qiu (Appendix 9)	GDGM48781 (holotype)	KY767807
	GDGM48782	OM021920
	GDGM48783	OM021921
	GDGM79586	OM021922
	GDGM79587	KY767805
	GDGM79588	OM021923
	GDGM79589	MN275571
	GDGM79590	OM021924
	GDGM79591	OM021925
	GDGM79592	OM021926
	GDGM79593	MN275575
	GDGM79594	OM021927
	GDGM79595	MN275577
	GDGM79596	OM021928
	GDGM79597	OM021929
	GDGM79598	OM021930
	GDGM79599	MN275581
	GDGM79600	MN603051

Table 1 (continued). Voucher specimens and ITS accession nos of species of the subgenus *Heterophyllidiae* collected from Dinghushan Biosphere Reserve.

Taxon	Voucher specimen	ITS accession no.
R. aureoviridis J.W.Li & L.H.Qiu (Appendix 10)	GDGM48785 (holotype)	KY767809
	GDGM48784	MN275554
	GDGM48786	KY767810
	GDGM48787	OM021931
	GDGM79569	OM021932
	GDGM79570	OM021933
	GDGM79571	OM021934
	GDGM79572	OM021935
	GDGM79573	OM021936
	GDGM79574	MN275555
	GDGM79575	MN275557
	GDGM79576	MN275558
	GDGM79577	OM021937
	GDGM79578	OM021938
	GDGM79579	OM021939
	GDGM79580	OM021940
	GDGM79581	OM021941
	GDGM79582	OM021942
	GDGM79583	OM021943
	GDGM79584	OM021944
R. xanthovirens Y.Song & L.H.Qiu	GDGM71145 (holotype)	MG786055
	GDGM71146	MG786056
R. verrucospora Y.Song & L.H.Qiu (Appendix 11)	GDGM71136 (holotype)	MG786052
	GDGM71137	OM021945
	GDGM71138	OM021946
	GDGM71139	MN275611
	GDGM71140	OM021947
	GDGM71141	OM021948
	GDGM71142	MN275608
	GDGM71143	OM021949
	GDGM71144	MN275610
	GDGM79629	MN275613
R. gelatinosa Y.Song & L.H.Qiu	GDGM71806 (holotype)	MH168575
	GDGM79666	MN275645
	GDGM71808	MH168574
R. insignis Quél.	GDGM79659	MN275641
	GDGM79660	MN275642
R. pseudocatillus F.Yuan & Y.Song	GDGM75338 (holotype)	MK049974
	GDGM79661	MK049975
R. punctipes Singer	GDGM71809	MH168576
	GDGM71810	MH168577
R. rufobasalis Y.Song & L.H.Qiu (Appendix 12)	GDGM71800 (holotype)	MH168570
	GDGM71801	OM021950
	GDGM71802	OM021951
	GDGM71803	MH168571

Table 1 (continued). Voucher specimens and ITS accession nos of species of the subgenus *Heterophyllidiae* collected from Dinghushan Biosphere Reserve.

Taxon	Voucher specimen	ITS accession no.
R. rufobasalis Y.Song & L.H.Qiu (Appendix 12)	GDGM71804	OM021952
	GDGM71805	MH168573
	GDGM79662	OM021953
	GDGM79663	OM021954
	GDGM79664	MN275643
	GDGM79665	OM021955

Table 2. Ecological distribution of species of *Heterophyllidiae* Romagnesi, emend. Buyck & V.Hofst. collected from Dinghushan Biosphere Reserve.

Classification	Taxon	Specimens collected in three vegetation types		
		Broad-leaf forest	Mixed coniferous and broad-leaf forest	Coniferous forest
subsect. *Cyanoxanthinae*	*R. dinghuensis* J.B.Zhang & L.H.Qiu	14	1	–
	R. fusiformata sp. nov.	2	–	–
	R. lotus Fang Li	4	–	–
	R. purpureorosea sp. nov.	–	2	–
	R. subpallidirosea J.B.Zhang & L.H.Qiu	11	3	–
subsect. *Heterophyllinae*	*R. bubalina* J.W.Li & L.H.Qiu	9	–	–
	R. pseudobubalina J.W.Li & L.H.Qiu	4	–	–
	R. subatropurpurea J.W.Li & L.H.Qiu	2	2	–
	R. vesca Fr.	10	4	–
	R. viridicinnamomea F.Yuan & Y.Song	4	–	–
subsect. *Virescentinae*	*R. albidogrisea* J.W.Li & L.H.Qiu	15	3	–
	R. aureoviridis J.W.Li & L.H.Qiu	8	11	1
	R. xanthovirens Y.Song & L.H.Qiu	1	1	–
subsection undefined				
sect. *Heterophyllae*	*R. verrucospora* Y.Song & L.H.Qiu	8	2	–
sect. *Ingratae*	*R. gelatinosa* Y.Song & L.H.Qiu	3	–	–
	R. insignis Quél.	2	–	–
	R. pseudocatillus F.Yuan & Y.Song	2	–	–
	R. punctipes Singer	2	–	–
	R. rufobasalis Y.Song & L.H.Qiu	5	5	–

A BLAST query of the ITS sequences in GenBank indicated that the two novel species should be assigned to *Russula* subgenus *Heterophyllidiae*. A maximum likelihood (ML) analysis was conducted using RAxML ver. 7.2.6 (Stamatakis 2006) for *Russula* subgen. *Heterophyllidiae* based on the rDNA ITS matrix. The ITS dataset was aligned using MAFFT ver. 7 (https://mafft.cbrc.jp/alignment/server/), then manually adjusted and trimmed with BioEdit ver. 7.0.9 (Hall 1999). The final ITS alignment for *R.* subgen. *Heterophyllidiae* comprised 135 sequences and 875 characters including gaps. A rapid bootstrapping (BS) algorithm of 1000 replicates was executed in RAxML, followed by a heuristic ML search for the best tree using the GTRGAMMA model.

Results

Phylogeny

From the phylogram of *Russula* subgen. *Heterophyllidiae* we can see that most of the 19 species collected from DHSBR are significantly supported (Figs 1-1, 1-2, 1-3). The two novel species nested well in subsect. *Cyanoxanthinae*. *Russula fusiformata* sp. nov. forms a distinct clade that, though not highly supported (BS = 53%), is very stable during repetitive phylogenetic analyses. It is relatively close to *R. subpallidirosea* J.B.Zhang & L.H.Qiu and *R. dinghuensis* J.B.Zhang & L.H.Qiu, which were also first reported from DHSBR. *Russula purpureorosea* sp. nov. clustered with its sister species, *R. pallidirosea* Kropp, forming a distinct and highly supported clade (BS = 98%). Both new species are part of a significantly supported clade (BS = 85%) in subsect. *Cyanoxanthinae* that is entirely composed of Asian species.

Taxonomy

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

Russula fusiformata Y.Song sp. nov.
MycoBank: MB828231
Index Fungorum: IF559326
Figs 2–3, 6b

Diagnosis

Russula fusiformata sp. nov. is distinguished by its ITS sequence with a similarity less than 95% compared to other known species. In morphology, it is mainly characterized by the lavender blush to rosy brown pileus with crenate margin, adnate lamellae, metachromatic pileipellis with slender furcated and septate terminal elements, and fusiform cheilocystidia with sharp apices.

Etymology

The name refers to the fusiform cheilocystidia with sharp apices.

Material examined

Holotype

CHINA • Guangdong Province, Zhaoqing City, Dinghushan Biosphere Reserve, on the ground in evergreen broad-leaf forest mainly with Fagaceae trees; 27 May 2015; *Y. Song, K15052703*; GenBank no.: MK049978 (ITS); GDGM75333.

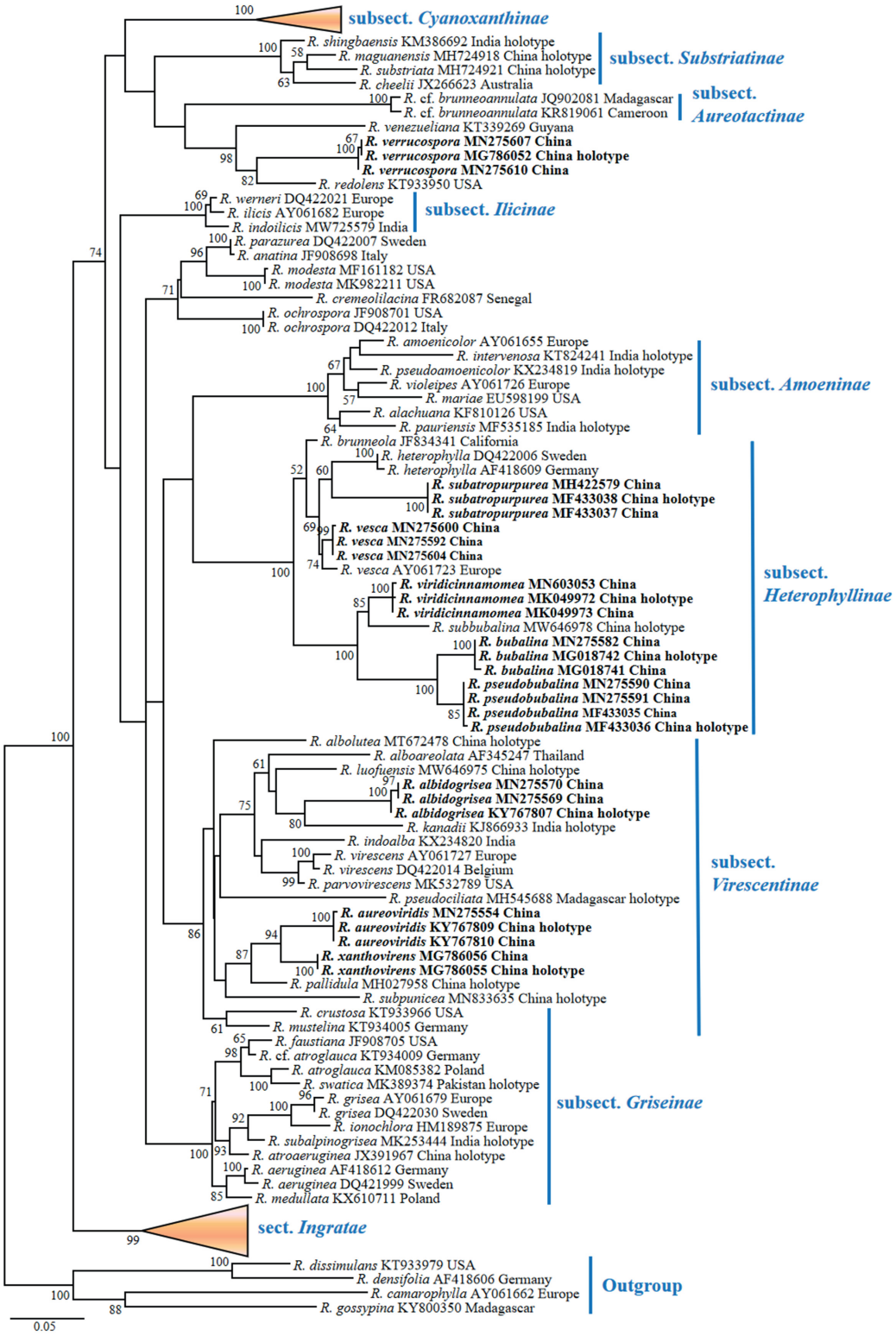

Fig. 1-1. Phylogram of *Russula* Pers. subgen. *Heterophyllidiae* Romagnesi, emend. Buyck & V.Hofst. generated using the maximum likelihood (ML) method based on rDNA ITS sequences performed by RAxML. The clades representing subsect. *Cyanoxanthinae* Singer and sect. *Ingratae* Quel. are compressed and separately displayed in Figs 1-2, 1-3. Bootstrap values higher than 50% are shown above or below the branches. Species collected from Dinghushan Biosphere Reserve are shown in bold.

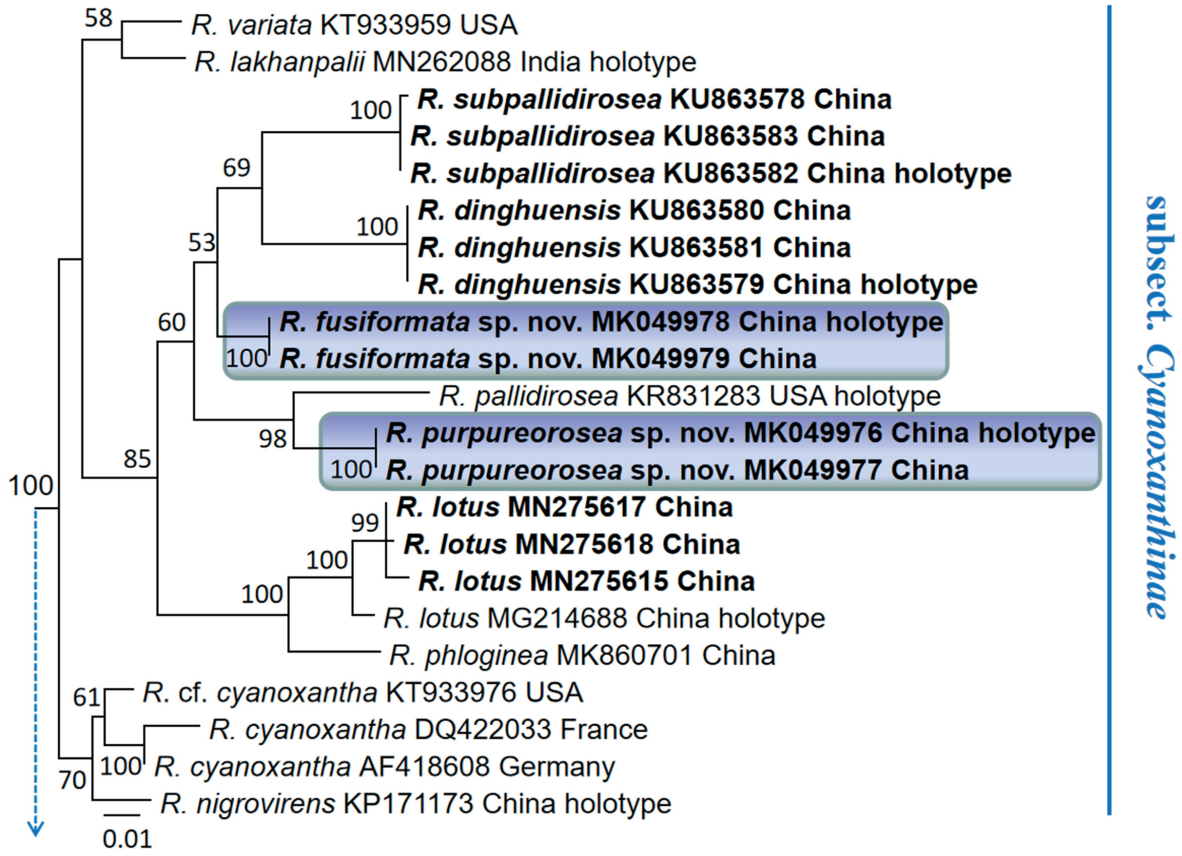

Fig. 1-2. Phylogram of subsect. *Cyanoxanthinae* Singer. Bootstrap values higher than 50% are shown above or below the branches. Species collected from Dinghushan Biosphere Reserve are shown in bold, the two novel species are highlighted with a blue background.

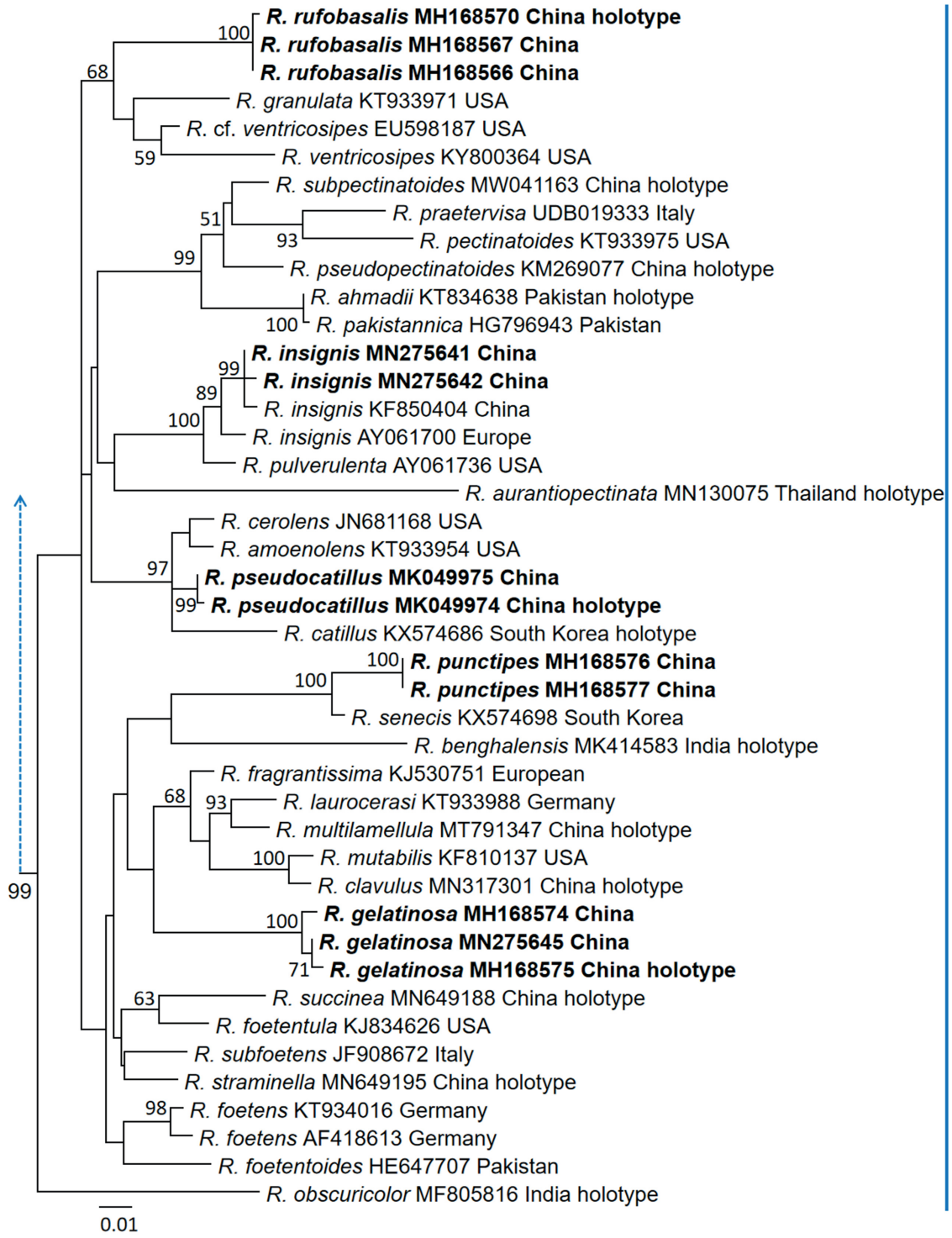

Fig. 1-3. Phylogram of sect. *Ingratae* Quel. Bootstrap values higher than 50% are shown above or below the branches. Species collected from Dinghushan Biosphere Reserve are shown in bold.

Additional material

CHINA • Guangdong Province, Zhaoqing City, Dinghushan Biosphere Reserve, on the ground in evergreen broad-leaf forest mainly with Fagaceae trees; 12 Jun. 2015; *Y. Song*; GenBank no.: MK049979 (ITS); GDGM75332.

Description

Basidiomata medium-sized. Pileus 6–9 cm in diam., applanate to plano-concave with a depressed center, lavender blush (#FFF0F5) with thistle tint (#D8BFD8), often tan (#D2B48C) to rosy brown (#BC8F8F) at center, rarely with a pale yellow tinge; margin entire or waved, striate. Lamellae adnate, ivory (#FFFFF0), cream yellow when dried, unchanging after bruising, equal, not or rarely forking, with even, concolorous edges. Stipe 6–8 × 1.5–2 cm, white (#FFFFFF), cylindrical, smooth, dry, central, solid, often longitudinally rugulose. Context 6–8 mm thick, whitish, unchanging after bruising or with $FeSO_4$, cream when dry. Taste mild. Odor indistinct. Spore print whitish.

Basidiospores subglobose to broadly ellipsoid, (40/2/2) (4.8–)5.6–6.5–7.4(–7.6) × (4.5–)5.1–5.7–6.3(–6.7) μm, Q = (1–)1.04–1.15–1.28(–1.3), hyaline in 5% KOH; ornamentation amyloid, composed of conical to subcylindrical warts, not exceeding 0.7 μm in height, mostly isolated, but a few connected with short lines, but not forming a reticulum; suprahilar spot distinct, not amyloid. Basidia 26.5–42–49.5 × 8–12–14 μm, 4-spored, rarely 2-spored, narrowly cylindrical to subcylindrical or broadly clavate; sterigmata not exceeding 5.7 μm in length. Lamellar trama mainly composed of nested spherocytes (13–55 × 7.5–42 μm) surrounded by connective hyphae. Pleurocystidia 43–50–53 × 7–8–9 μm, slender, cylindrical to subfusiform, apex obtuse, bluntly acuminate or mucronate, with abundant granular contents; negative in SV. Cheilocystidia 36.5–52–78 × 5–9–11 μm, clavate to fusoid, often with bluntly acuminate to mucronate apices, thin-walled, with refractive contents, unchanged in SV. Marginal cells not differentiated. Pileipellis metachromatic in cresyl blue, 67–140.5 μm thick, composed of suprapellis and subpellis; suprapellis a trichoderm composed of ascending to erect hyphae and subpellis composed of horizontally oriented hyphae 1.8–5 μm in diam.; terminal elements at center 5.5–14 × 3–8 μm, cylindrical, apices obtuse; terminal cells at margin slender cylindrical with obtuse apices. Pileocystidia 15.5–64 × 2–10 μm, always one-celled, slender, apex mucronate or subterminally constricted, cylindrical to clavate. Stipitipellis a cutis, composed of thin-walled, often ramifying, septate hyphae 1.5–3.6 μm in diam. Caulocystidia 19–31 × 3–10.5 μm, subcylindrical to fusoid, bluntly acuminate or mucronate, with refractive contents. Clamp connections absent.

Remarks

Our phylogenetic analysis based on ITS shows that *Russula fusiformata* sp. nov. is well nested in subsect. *Cyanoxanthinae* Singer and relatively close to *R. dinghuensis* J.B.Zhang & L.H.Qiu and *R. subpallidirosea* J.B.Zhang & L.H.Qiu. The three species have a common habitat and distribution, namely all are gregarious in monsoon evergreen broad-leaf forest and mixed pine-broad-leaf forest in DHSBR. *Russula dinghuensis* (Appendix 1) is characterized by the olive green pileus with acute and incurved margin, white and rarely forked lamellae, thick metachromatic pileipellis. *Russula subpallidirosea* (Appendix 3) can be recognized by the pale pink to pale grayish-pink pileus, white and forked lamellae, metachromatic pileipellis with short terminal elements. *Russula fusiformata* can be differentiated from related species by its lavender blush to rosy brown pileus and fusiform cheilocystidia with sharp apices. In addition, the margin of *R. dinghuensis* and *R. subpallidirosea* is even and incurved while in *R. fusiformata* it is crenate. The lamellae of *R. dinghuensis* and *R. fusiformata* are rarely forked, but those of *R. subpallidirosea* are often forking. Moreover, the chemical reactions of cystidia and pileipellis of the three species are totally different. Pleurocystidia in *R. fusiformata* are SV negative, but positive in *R. dinghuensis* and *R. subpallidirosea.*

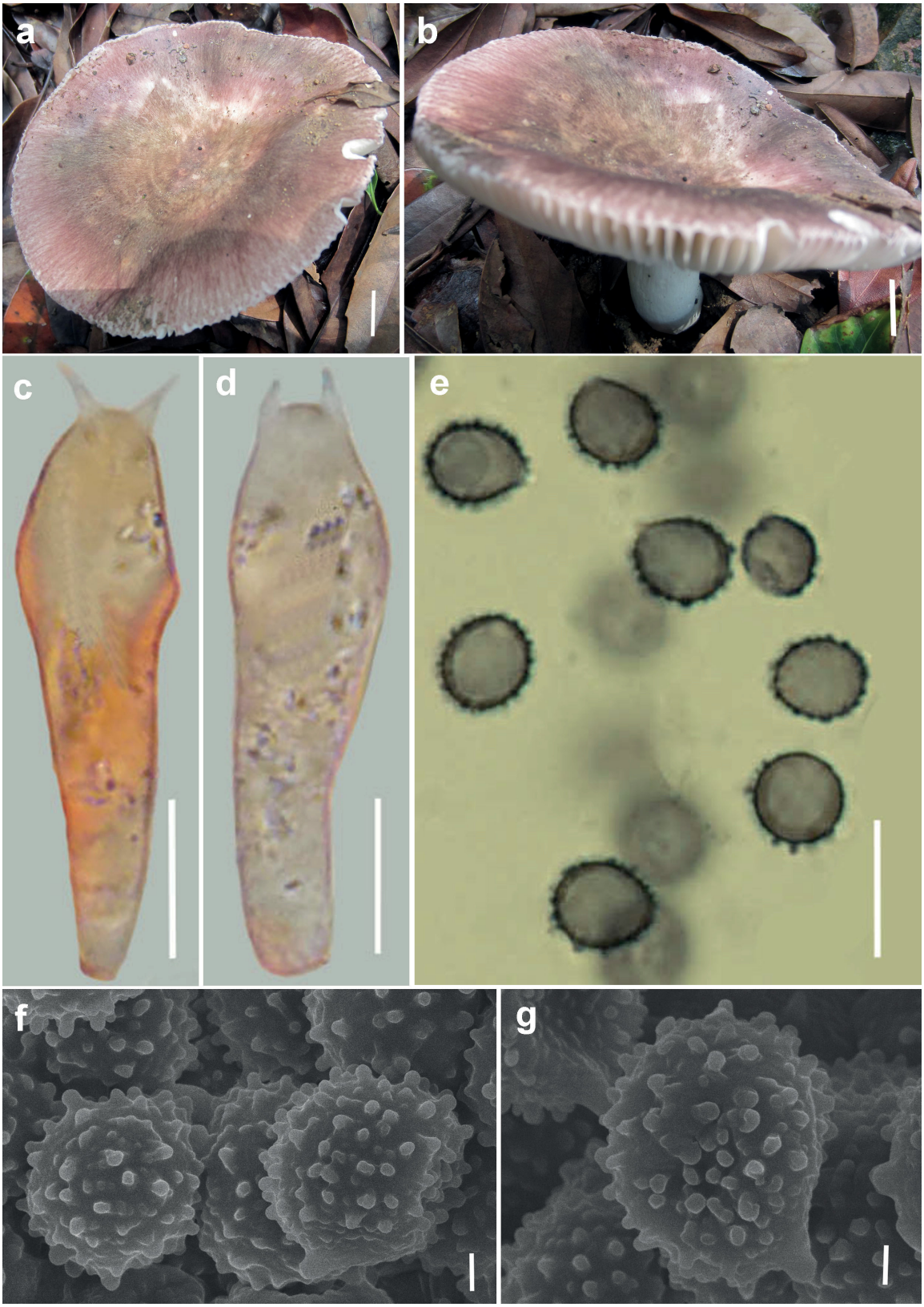

Fig. 2. *Russula fusiformata* Y.Song sp. nov., holotype (GDGM75333). **a–b**. Fruiting bodies. **c–d**. Basidia. **e**. Basidiospores in Melzer's reagent. **f–g**. Basidiospores under scanning electron microscope. Scale bars: a–b = 1 cm; c–e = 10 μm; f–g = 1 μm.

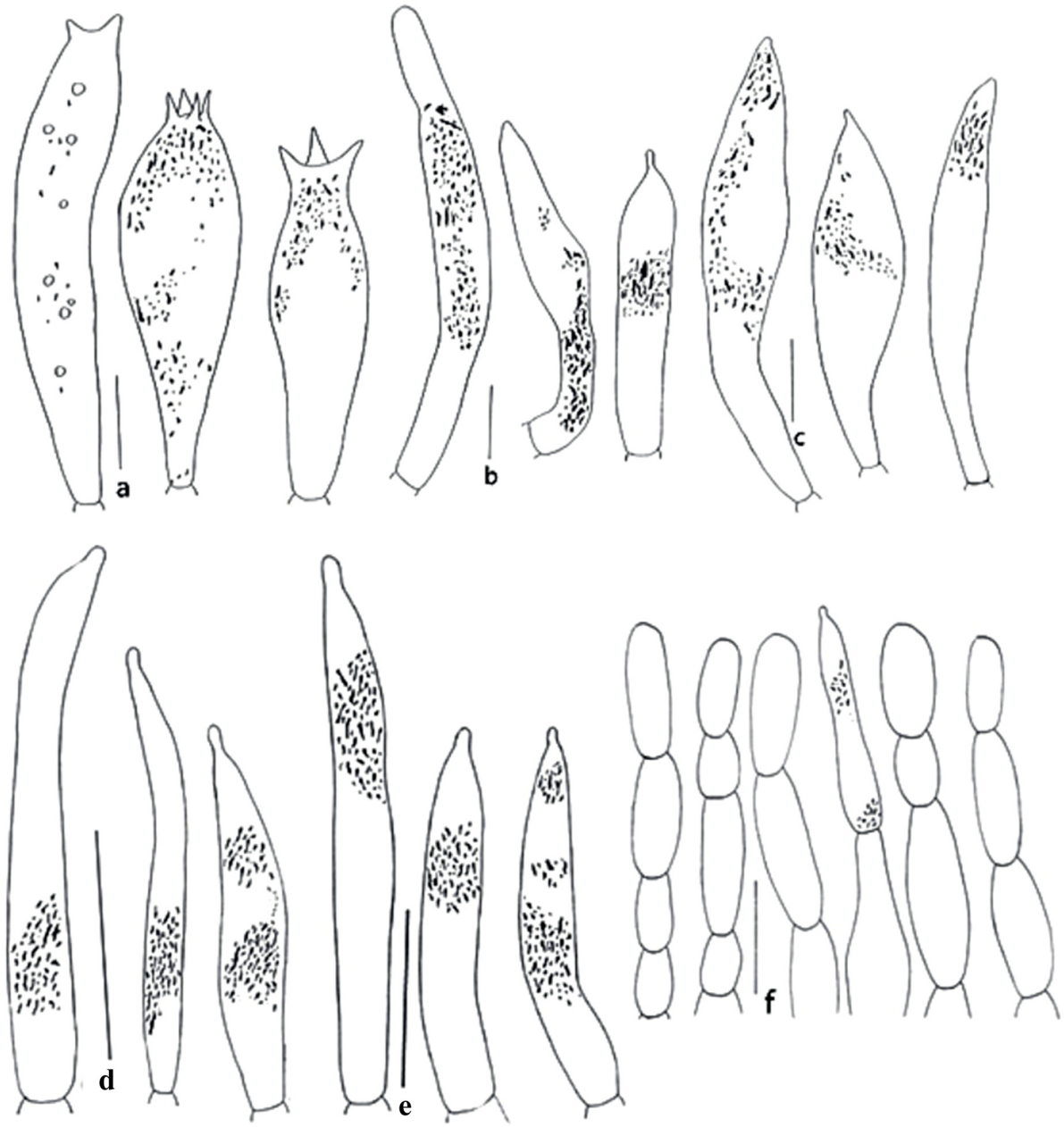

Fig. 3. *Russula fusiformata* Y.Song sp. nov., holotype (GDGM75333). **a**. Basidia. **b**. Pleurocystidia. **c**. Cheilocystidia. **d**. Pileocystidia. **e**. Caulocystidia. **f**. Pileipellis. Scale bars = 10 μm.

Russula purpureorosea Y.Song sp. nov.
MycoBank: MB828229
Index Fungorum: IF559327
Figs 4–5, 6a

Diagnosis

Russula purpureorosea sp. nov. can be separated from other known species by its ITS sequence with a similarity less than 95.5% when aligned. The species is morphologically characterized by the pale pinkish purple pileus with even or incurved margin, adnate and crowded lamellae with lamellula, thick metachromatic pileipellis, terminal elements of pileipellis with some inflated terminal and subterminal cells.

Etymology

The name refers to pale pinkish purple pileus.

Material examined

Holotype

CHINA • Guangdong Province, Zhaoqing City, Dinghushan Biosphere Reserve, on the ground in mixed coniferous and broad-leaf forest; 5 May 2017; *Y. Song, H17050506*; GenBank no.: MK049976 (ITS); GDGM75331.

Additional material

CHINA • Guangdong Province, Zhaoqing City, Dinghushan Biosphere Reserve, on the ground in broad-leaf forest mainly with Fagaceae trees; 18 May 2018; *Y. Song*; GenBank no.: MK049977 (ITS); GDGM75334.

Description

Basidiomata small to medium-sized. Pileus 3.5–6 cm in diam., applanate or with a slightly depressed center; surface glabrous, dry, pale pinkish purple (#D8BFD8), often with rosy brown (#BC8F8F) center; margin even or slightly incurved, smooth to slightly striate when young, sulcate or cracked with age. Lamellae adnate, ivory (#FFFFF0), yellowish when dried, unchanging after bruising, not forking, interveined, with even, concolorous edges; lamellulae frequent when young, but not regularly polydymous. Stipe 25–40 × 8–12 mm, cylindrical, central, solid; surface white (#FFFFFF), glabrous, dry, slightly longitudinally rugulose. Context 3–5 mm thick, whitish (#FFFFFF), unchanging after bruising or with $FeSO_4$. Taste mild. Odor indistinct. Spore print whitish.

Basidiospores subglobose to broadly ellipsoid, (40/2/2) (6.1–)6.4–7.0–7.7(–7.8) × (5.3–)5.4–5.8–6.3(–6.6) µm, Q = (1.06–)1.18–1.20–1.28(–1.36); ornamentation amyloid, composed of conical to subcylindrical warts, not exceeding 0.6 µm in height, mostly isolated; hyaline in 5% KOH. Basidia 27–41–46 × 8.5–12–14.5 µm, mostly 4-spored, rarely 2-spored, clavate to subcylindrical; sterigmata up to 6 µm in height. Lamellar trama mainly composed of nested spherocytes (18–69 × 7–42 µm) surrounded by connective hyphae. Pleurocystidia 32–55–85 × 3.5–7–9 µm, subcylindrical to subfusiform, apex obtuse or mucronate, often with refractive heteromorphous contents that are mostly restricted to upper part of cystidia, negative in SV. Cheilocystidia 54–60–95 × 7–8.5–10 µm, slender, clavate to subfusiform, apex obtuse, with abundant refractive heteromorphous contents. Marginal cells not differentiated. Pileipellis metachromatic in cresyl blue, 57–112 µm thick, composed of suprapellis and subpellis; suprapellis trichoderm to palisade, composed of ascending to erect hyphae, some with chains of inflated subterminal cells; subpellis composed of horizontally oriented hyphae, 2–9 µm in diam.; terminal cells at center 6.5–15.5 × 2–5.5 µm, cylindrical to clavate, some inflated, apices obtuse, rarely attenuate; terminal cells at margin similar in shape, but more or less slender, rarely inflated. Pileocystidia 17–53 × 4.5–9 µm, clavate to fusiform, one-celled, thin-walled, some subterminally constricted, with mucronate or capitate apices, with distinct contents, unchanging in SV, also present in subpellis. Stipitipellis a cutis, composed of

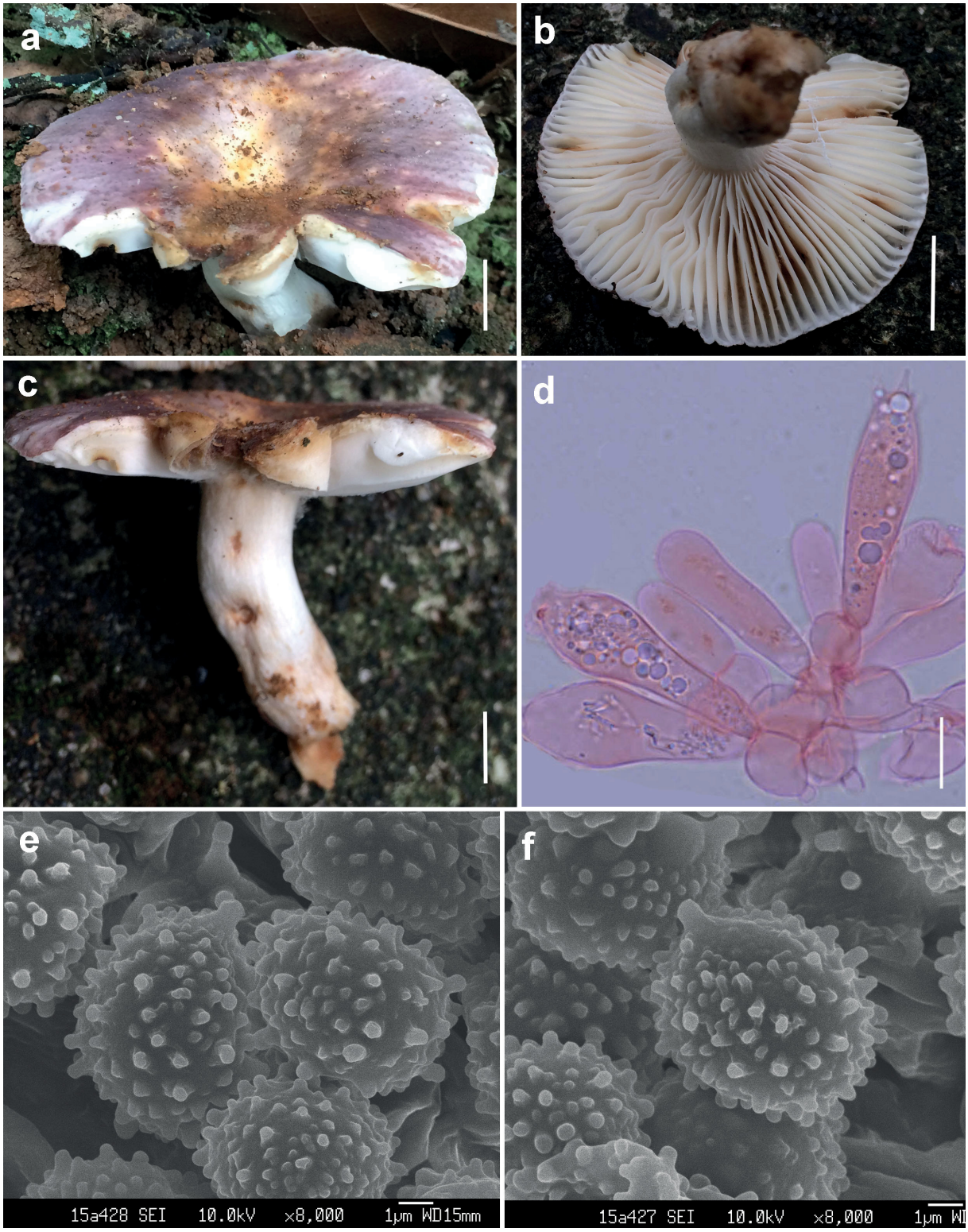

Fig. 4. *Russula purpureorosea* Y.Song sp. nov., holotype (GDGM75331). **a–c**. Fruiting bodies. **d**. Basidia. **e–f**. Basidiospores under scanning electron microscope. Scale bars: a–c = 1 cm; d = 10 μm; e–f = 1 μm.

Fig. 5. *Russula purpureorosea* Y.Song sp. nov., holotype (GDGM75331). **a**. Basidia. **b**. Pleurocystidia. **c**. Cheilocystidia. **d**. Pileocystidia. **e**. Caulocystidia. **f**. Pileipellis. Scale bars = 10 μm.

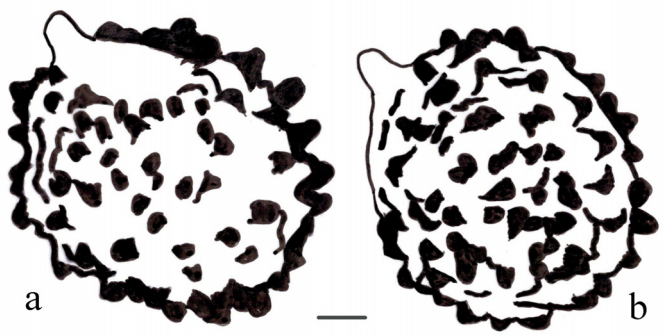

Fig. 6. Line drawings of basidiospores. **a**. *Russula purpureorosea* Y.Song sp. nov., holotype (GDGM75331). **b**. *Russula fusiformata* Y.Song sp. nov., holotype (GDGM 75333). Scale bar = 1 μm.

thin-walled, interwoven, septate, cylindrical hyphae 1.5–3.7 μm in diam. Caulocystidia 9–38 × 2.3–7 μm, rare, subcylindrical to narrow clavate. Clamp connections absent.

Remarks

Russula purpureorosea sp. nov. has equal gills, lamellulae not frequent, context unchanging, spore print whitish, spores with inamyloid suprahilar spot, primordial hyphae absent, gloeocystidia abundant, terminal elements inflated; all these characters indicate that *R. purpureorosea* belongs to subgen. *Heterophyllidiae* subsect. *Cyanoxanthinae* in morphology. Our phylogenetic analysis based on ITS shows that *R. purpureorosea* is closely related to *R. pallidirosea* Kropp within subsect. *Cyanoxanthinae*. *Russula purpureorosea* can be distinguished from related species by its pinkish purple to rosy brown pileus with lamellulae. Compared with *R. purpureorosea*, *R. pallidirosea* has smaller pinkish pileus (cap 1.5–2.5 cm in diam.) with margins inrolled at first, becoming uplifted with age, lamellae occasionally forked, lamellula present and stipe tapering toward the base. Basidiospores of *R. pallidirosea* have low unconnected amyloid ornamentations which are generally shorter than in *R. purpureorosea.*

Discussion

The phylogenetic analyses based on ITS showed that *R. purpureorosea* sp. nov. clusters with its sister species *R. pallidirosea* (Kropp 2016) forming a distinct clade, while *R. fusiformata* sp. nov. forms a distinct clade parallel to the clade comprising *R. dinghuensis* and *R. subpallidirosea.* A detailed morphological comparison of the two novel species and their related taxa is given in Table 3. Both morphological and phylogenetic analyses demonstrate that *R. purpureorosea* and *R. pallidirosea* are two novel species in *R.* subgen. *Heterophyllidiae* subsect. *Cyanoxanthinae*.

The Dinghushan Biosphere Reserve is located in southern China. It is one of three reserves in China selected in 1980 to join the Biosphere Reserve Network of UNESCO for their rich biological diversity and ecological importance. The region is subjected to a subtropical monsoon climate and possesses three vegetation types: coniferous forest, mixed coniferous and broad-leaf forest, and broad-leaf forest. The distinct climate and ecological environment allow for a diversity of species of *Russula*. Until now, up to 141 specimens representing 19 species (including the two novel species proposed in this study) of *Russula* subgen. *Heterophyllidiae* have been collected, of which 15 species were first described from DHSBR. All the collected species of *Heterophyllidiae* with voucher specimens and distribution are listed in Table 1 and Table 2, respectively. Of all the 141 specimens, 106 samples representing 18 species were collected from broad-leaf forest, 34 samples representing 10 species from mixed coniferous and broad-leaf forest, and only one sample from coniferous forest. The species richness and diversity of *Russula* in broad-leaf forest is higher compared to the other two vegetation types. *Russula aureoviridis* J.W.Li & L.H.Qiu, distributed in all three types of forest and collected every year with the most specimens, seems to be the dominant species among the native Russulas in DHSBR.

Of all the 19 species of subgen. *Heterophyllidiae* collected from DHSBR, 12 species are supplemented with more voucher specimens and macro-morphological photographs in different growth stages or environments in this study (Appendices 1–12). For most species, macrofungal features of newly collected specimens are consistent with original descriptions. For a few other species, such as *R. verrucospora* Y.Song & L.H.Qiu and *R. viridicinnamomea* F.Yuan & Y.Song, there appears to exist a significant variation in morphological characters.

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Table 3. Detailed morphological comparison of two new taxa with their phylogenetically related species. Morphological characters that can be used to separate species from their relatives are given in bold.

Character	*R. dinghuensis*	*R. subpallidirosea*	*R. pallidirosea*	*R. lotus*	*R. purpureorosea* **sp. nov.**	*R. fusiformata* **sp. nov.**
Pileus size (cm)	4–8	3–7	**1.5–2.5**	3.5–9.5	3–5	6–9
Pileus color	**olive green with rusty tone**	pale pink with yellowish brown spot	pinkish	pinkish white to purplish pink	pale pinkish purple	lavender blush to rosy brown
Pileus margin	even or incurved	even or incurved	**faintly sulcate**	**slightly striate**	even or incurved	**crenate**
Lamellae	rarely forked	**often forking**	**occasionally forked**	**occasionally forked**	rarely forked	rarely forked
Pileipellis in cresyl blue	metachromatic	metachromatic	–	–	metachromatic	metachromatic
Stipe (mm)	3–6.5 × 0.8–1.2	3.1–6.5 × 0.8–1.3	**1.2–2.2 × 0.3–0.5**	3–8 × 0.8–1.8	2.5–4 × 0.5–1.8	**6–8 × 1.5–2**
Spore size (µm)	(5.5–)6–6.9–8.0 (–8.5) × 5.0–6.3–7.0	(5.5–)6–6.7–8.0 (–9.0) × 5.0–5.9–7.0(–8.0)	6.0–8.5 × 5.0–7.0	(6.5–)7–8(–10) × (5–)6–7(–8)	(6.1–)6.4–7.0-7.7 (–7.8) × (5.3–)5.4–5.8–6.3(–6.6)	(4.8–)5.6–6.5–7.4 (–7.6) × (4.5–)5.1–5.7–6.3(–6.7)
Spore ornamentation	isolated or not forming a reticulum	mostly not forming a reticulum	–	disconnected	mostly isolated and not forming a reticulum	mostly isolated and not forming a reticulum
Pleurocystidia (µm)	44–67 × 6–10	35–50 × 5–8	40–55 × 5–7	**52–70 × 10–16**	32–55–85 × 3.5–7–9	43–50–53 × 7–8–9
Pleurocystidia in SV	**dark grey**	**grey**	–	–	negative	negative
Cheilocystidia (µm)	45–52 × 4–6	55–63 × 6–10	40–55 × 5–7	52–70 × 10–16	54–60–95 × 7–8.5–10	36.5–52–78 × 5–9–11
Caulocystidia (µm)	**43–76 × 5–6.3**	**50–83 × 4–6**	17–50 × 2–4	–	9–38 × 2.3–7	19–31 × 3–10.5
Pileocystidia (µm)	32–53 × 2.5–5	27–38 × 3–5	17–50 × 2–4	15–60 × 4–8	17–53 × 2.5–9	15.5–64 × 2–10

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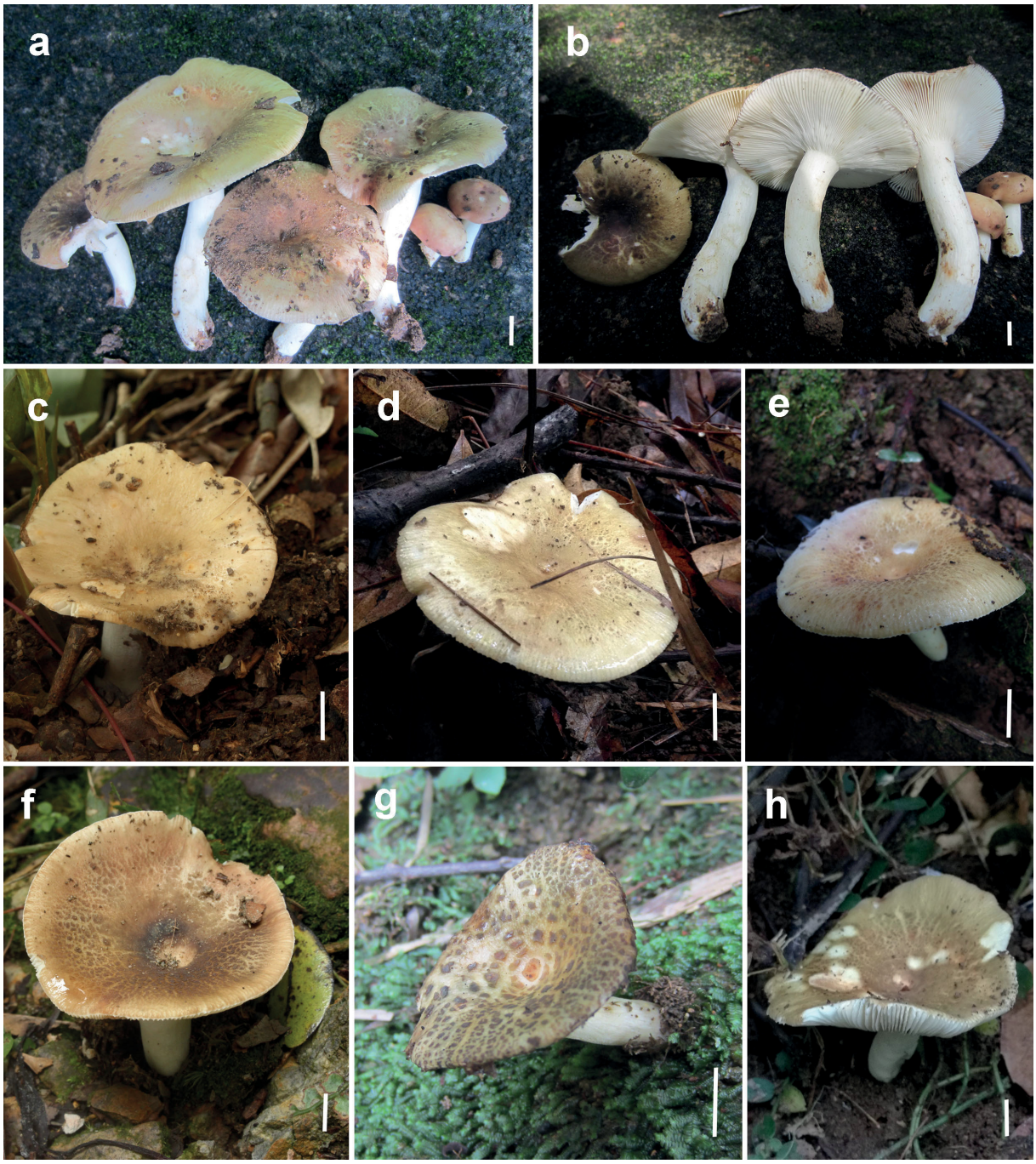
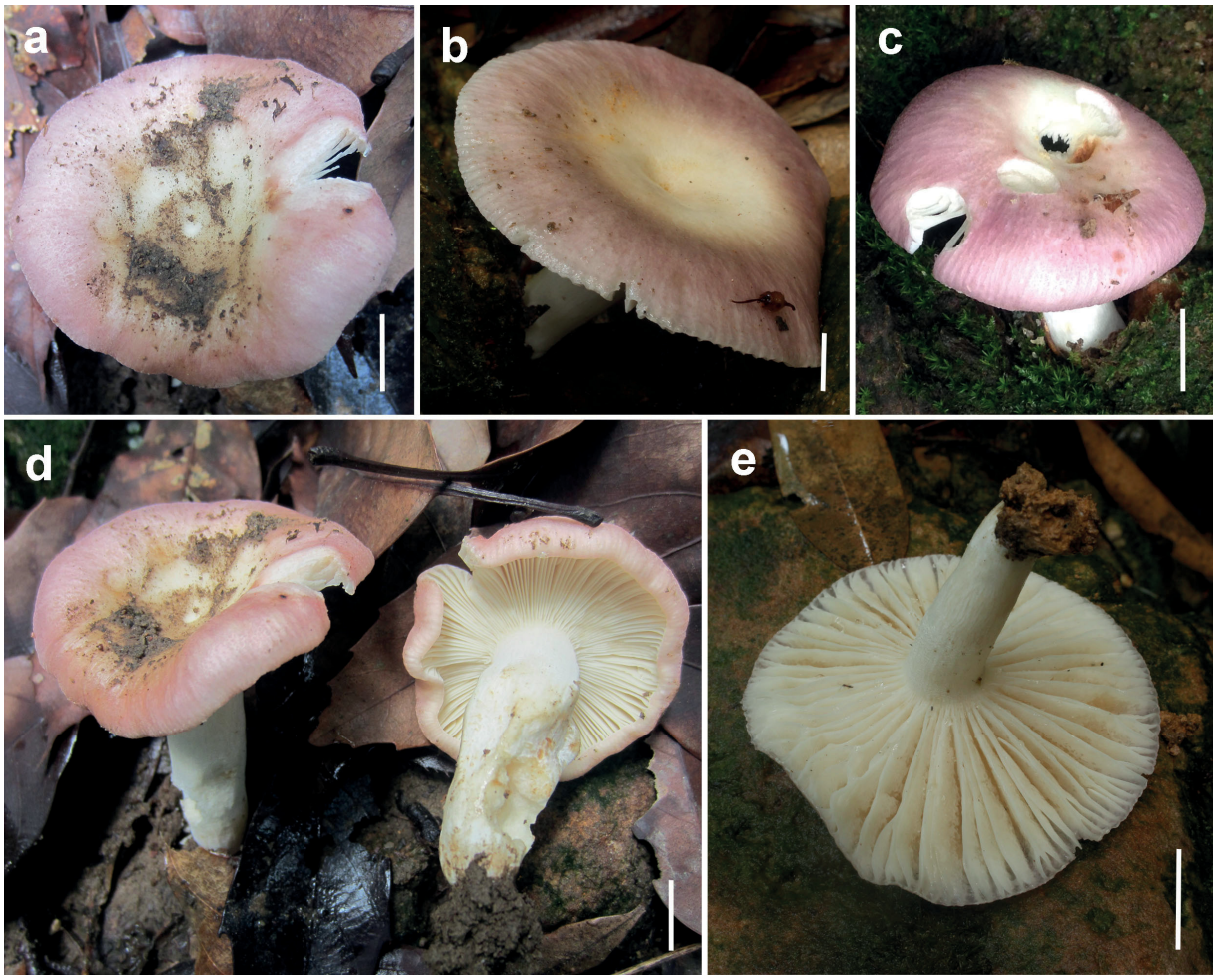
Appendix 1

Russula dinghuensis J.B.Zhang & L.H.Qiu. **a–b**. GDGM45244 (holotype). **c**. GDGM79644. **d**. GDGM79646. **e**. GDGM79650. **f**. GDGM79645. **g**. GDGM79655. **h**. GDGM79652. Scale bars = 1cm.



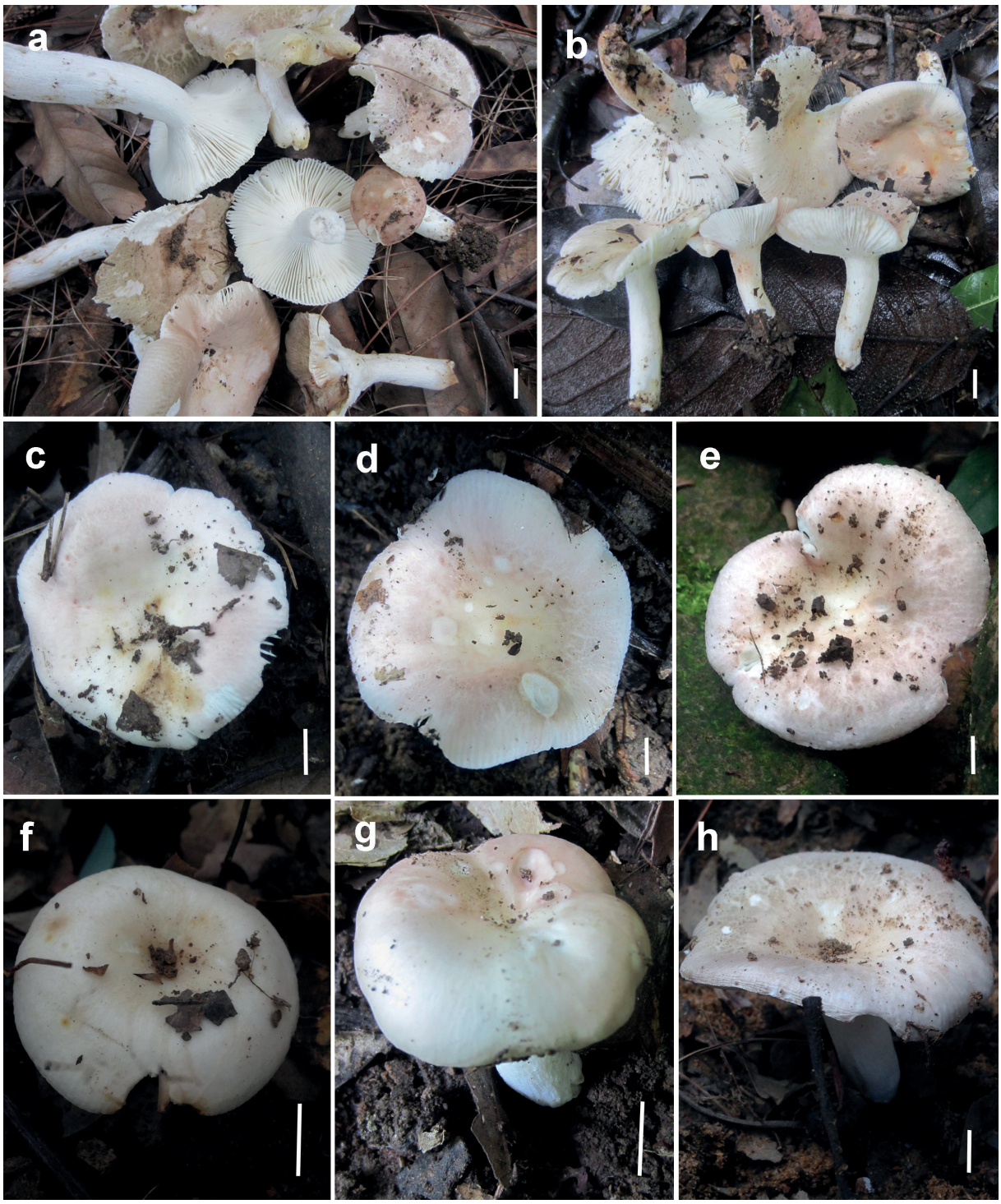
Appendix 2

Russula lotus FangLi. **a, d**. GDGM79632. **b, e**. GDGM79634. **c**. GDGM79635. Scale bars = 1cm.



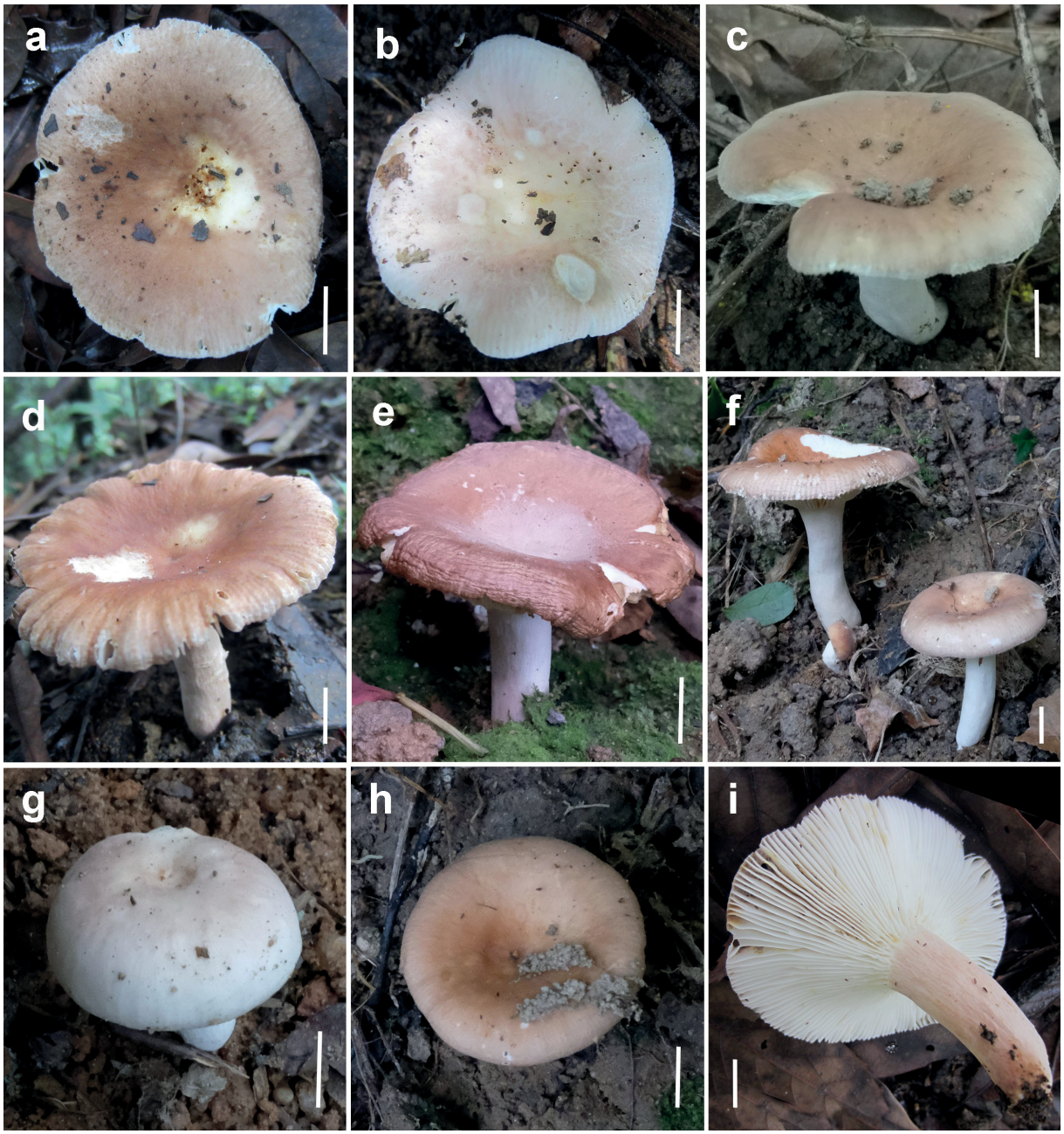
Appendix 3

Russula subpallidirosea J.B.Zhang & L.H.Qiu. **a–b**. GDGM45242 (holotype). **c**. GDGM79641. **d**. GDGM79638. **e**. GDGM79642. **f**. GDGM79640. **g**. GDGM77428. **h**. GDGM79643. Scale bars = 1cm.



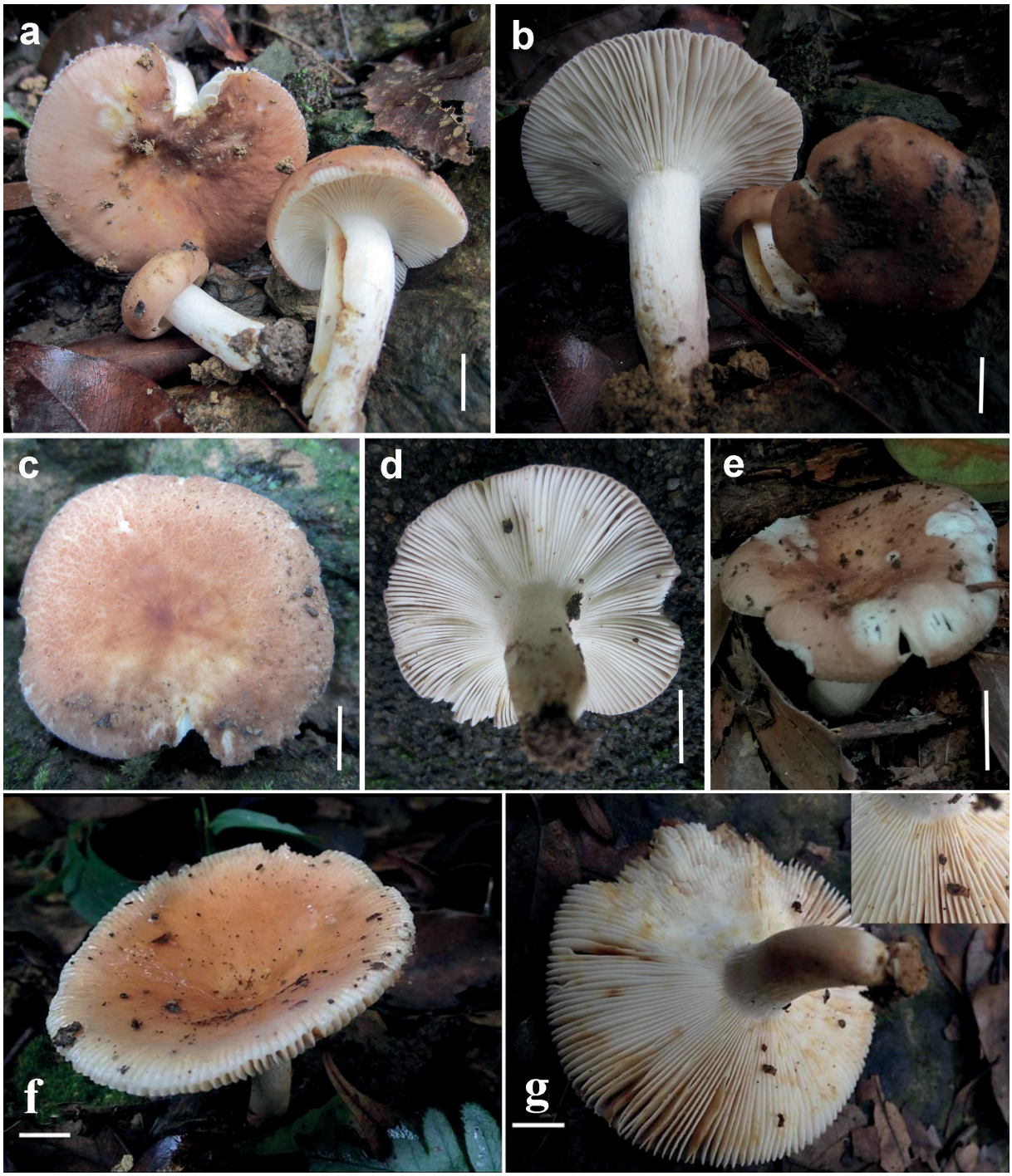
Appendix 4

Russula bubalina J.W.Li & L.H.Qiu. **a**. GDGM70727. **b**. GDGM79603. **c**. GDGM79602. **d, i**. GDGM70728 (holotype). **e**. GDGM79604. **f**. GDGM79606. **g**. GDGM79608. **h**. GDGM79607. Scale bars = 1cm.



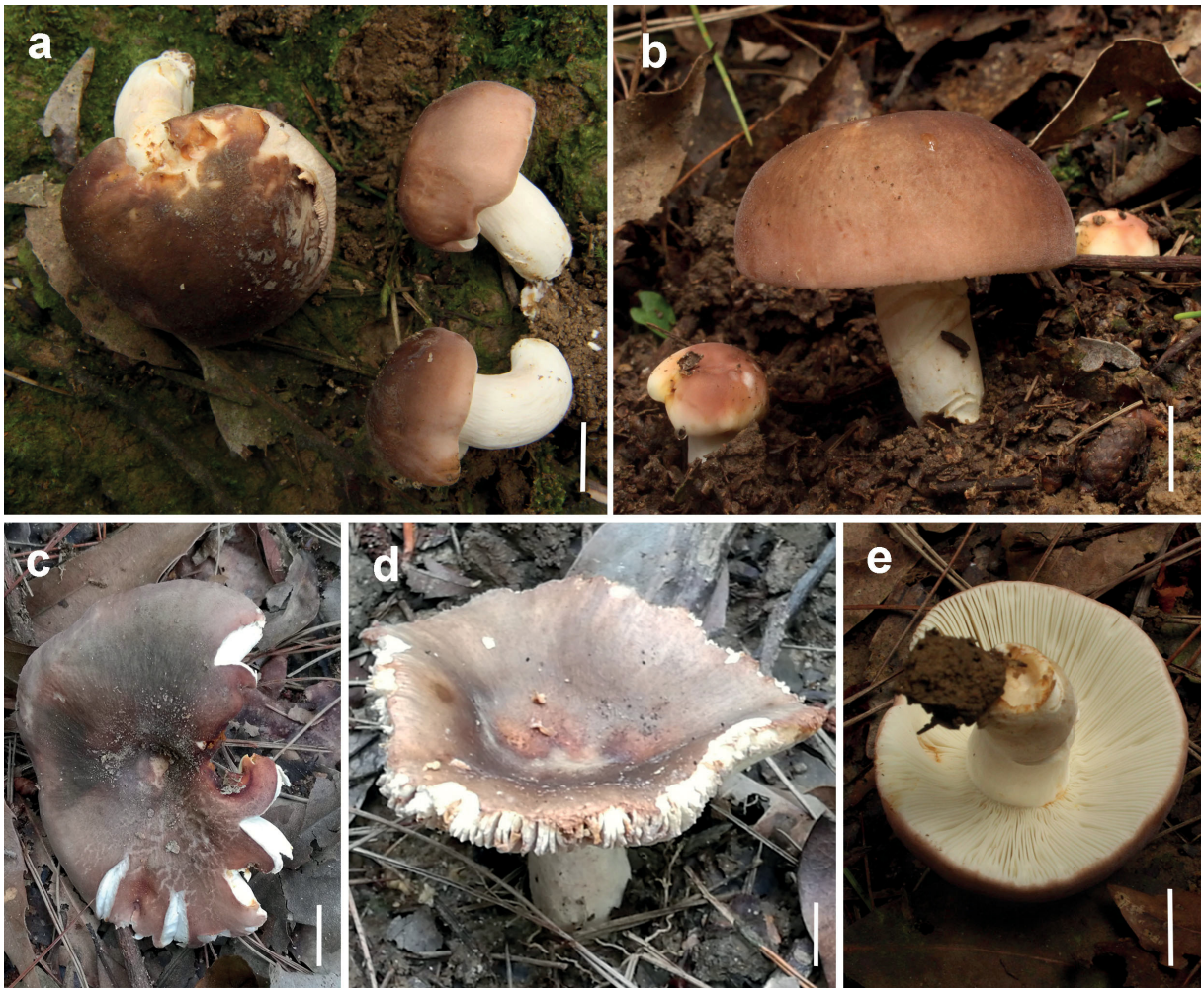
Appendix 5

Russula pseudobubalina J.W.Li & L.H.Qiu. **a–b**. GDGM70632 (holotype). **c–d**. GDGM79611. **e**. GDGM79612. **f–g**. GDGM71132. Scale bars = 1cm.



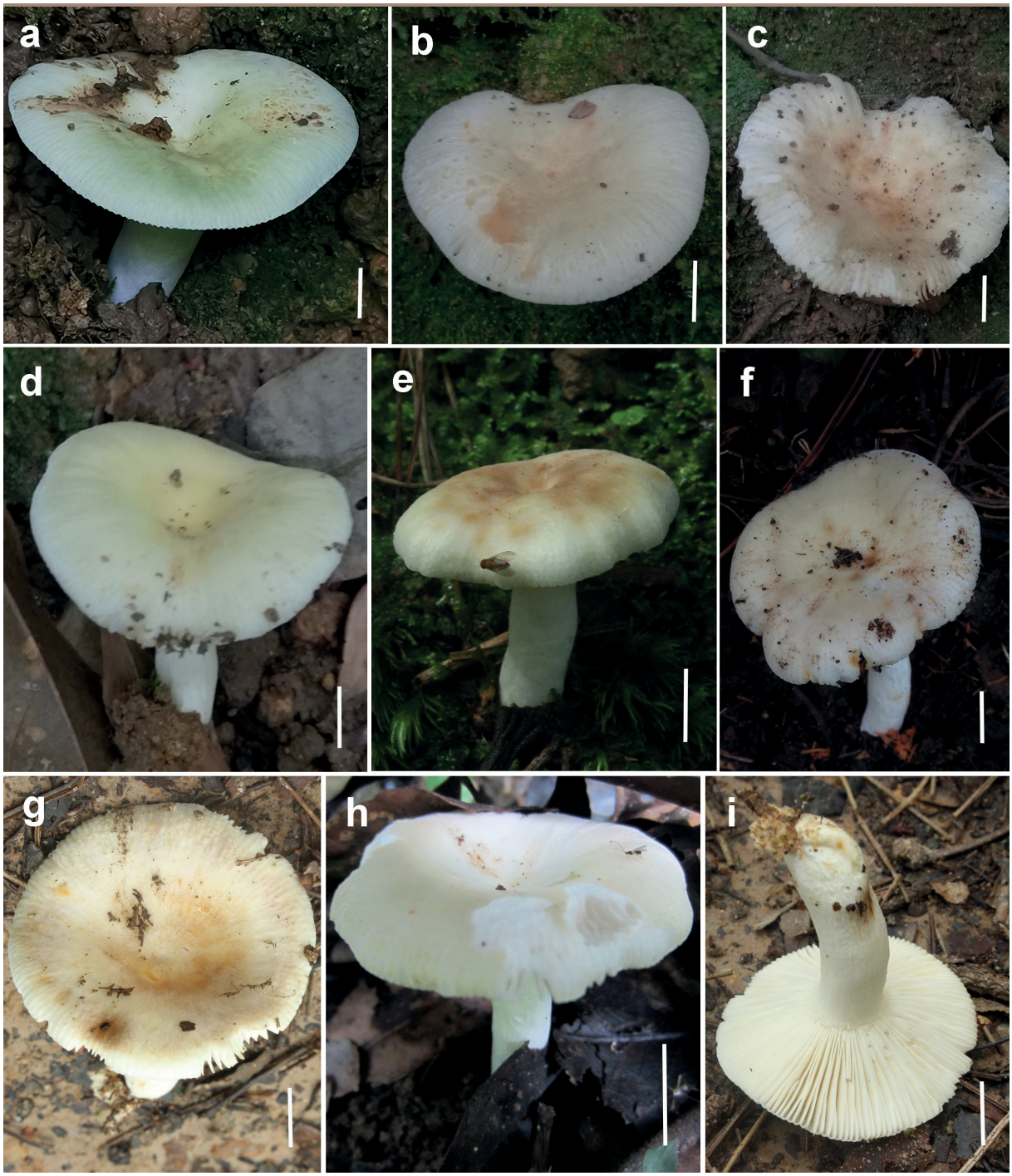
Appendix 6

Russula subatropurpurea J.W.Li & L.H.Qiu. **a**. GDGM79609. **b, e**. GDGM60633. **c–d**. GDGM70634 (holotype). Scale bars = 1cm.



Appendix 7

Russula vesca Fr. **a**. GDGM79628. **b**. GDGM79624. **c**. GDGM79615. **d**. GDGM79621. **e**. GDGM79622. **f**. GDGM79618. **g**. GDGM79620. **h–i**. GDGM79627. Scale bars = 1cm.

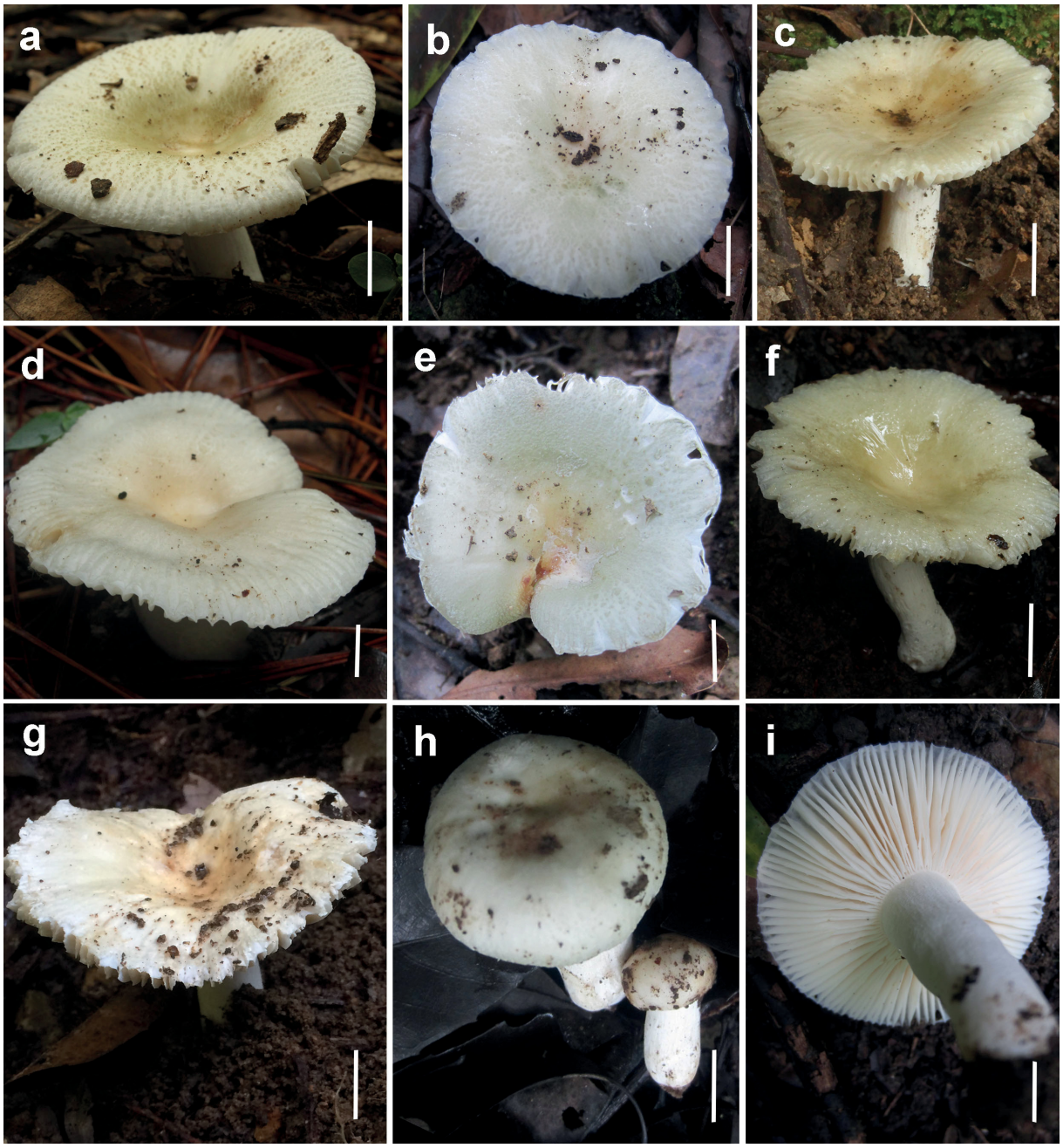


Appendix 8

Russula viridicinnamomea F.Yuan & Y.Song. **a–b**. GDGM79613. **c–d**. GDGM79614. **e–f**. GDGM75339 (holotype). Scale bars = 1cm.

Appendix 9

Russula albidogrisea J.W.Li & L.H.Qiu. **a**. GDGM79589. **b**. GDGM48781 (holotype). **c**. GDGM79591. **d**. GDGM79598. **e**. GDGM79596. **f**. GDGM79600. **g**. GDGM79592. **h**. GDGM79593. **i**. GDGM79586. Scale bars = 1cm.

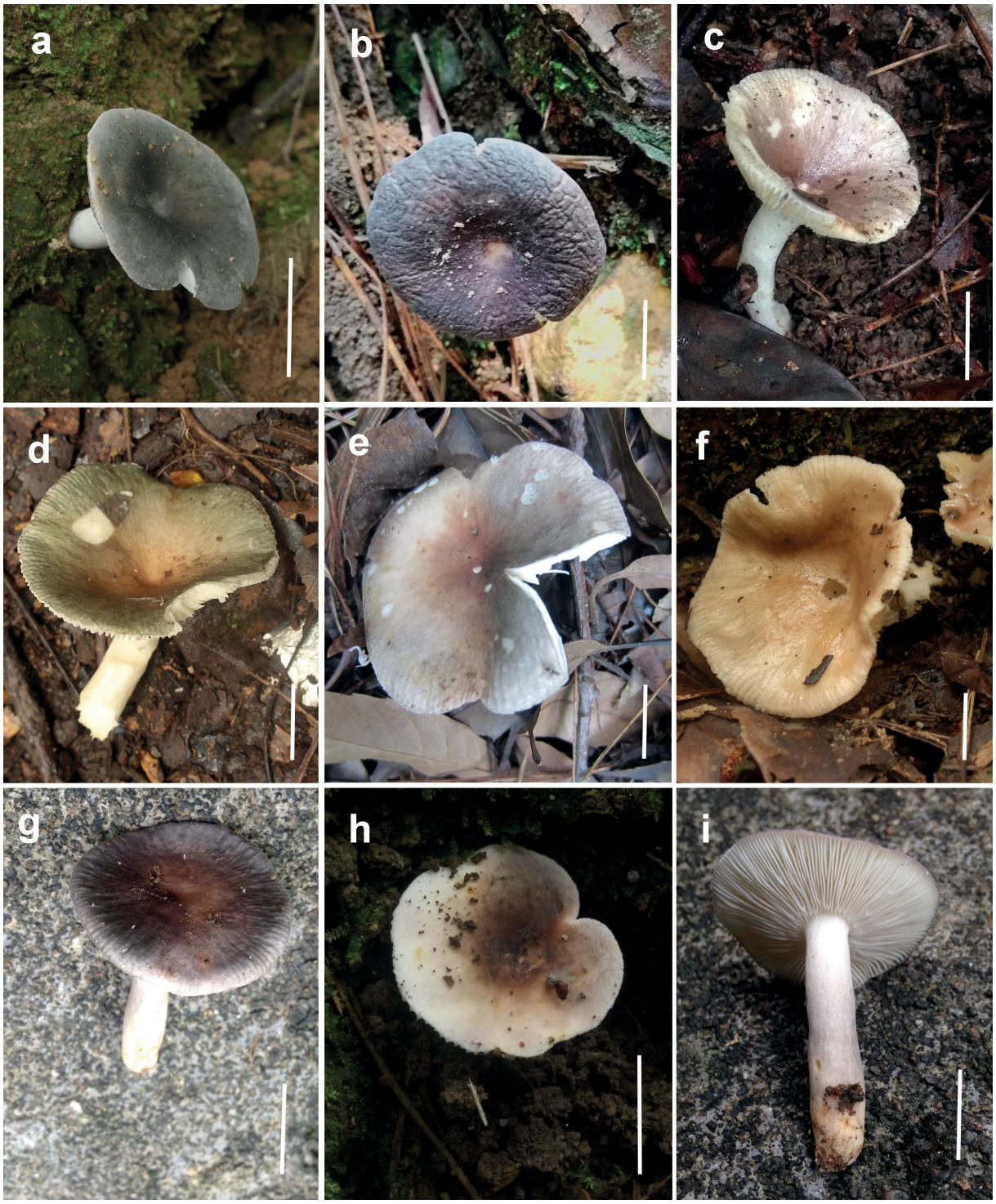


Appendix 10

Russula aureoviridis J.W.Li & L.H.Qiu. **a**. GDGM48785 (holotype). **b**. GDGM48786. **c**. GDGM79578. **d**. GDGM79574. **e**. GDGM48787. **f**. GDGM79575. **g**. GDGM79576. **h–i**. GDGM79581. Scale bars = 1cm.

Appendix 11

Russula verrucospora Y.Song & L.H.Qiu. **a**. GDGM71136 (holotype). **b**. GDGM71144. **c**. GDGM71140. **d**. GDGM71138. **e**. GDGM71141. **f**. GDGM71139. **g, i**. GDGM79629. **h**. GDGM71137. Scale bars = 1cm.



Appendix 12

Russula rufobasalis Y.Song & L.H.Qiu. **a–c**. GDGM71800 (holotype). **d**. GDGM71803. **e**. GDGM71805. **f–g**. GDGM79664. **h**. GDGM71801. **i**. GDGM71804. Scale bars = 1cm.