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Three novel species of *Russula* Pers.
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Biosphere Reserve in southern China

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Three novel species of *Russula* Pers. subg. *Compactae* (Fr.) Bon from Dinghushan Biosphere Reserve in southern China

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ABSTRACT

Three novel species of *Russula* Pers. subg. *Compactae* (Fr.) Bon from southern China are described with morphological evidence and phylogenetic analyses based on ITS and the concatenated partial LSU, mtSSU, *rpb1*, *rpb2* and *tef1* gene sequences. All three species possess sparse and broad lamellae. *Russula latolamellata* Y.Song & L.H.Qiu, sp. nov., is characterized by its cracking, black-tan pileus, scarlet-turning context on bruising and absence of pileocystidia and caulocystidia. *Russula nigrocarpa* S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov., can be distinguished by its off-white lamellae, small basidiospores, hymenial cystidia and pileocystidia of various forms, often with forked apices and by its gelatinous pileipellis. *Russula ochrobrunnea* S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov., is characterized by its cracking, grayish brown pileus with striate margin, light-brown lamellae whose edges turn dark-brown when mature, small basidiospores and slightly flexuous ormoniliform hymenial cystidia. Their detailed morphological features and phylogenetic positions are discussed and compared among closely related species.

KEY WORDS

Multilocus,
phylogeny,
southern China,
new species.

RÉSUMÉ

Trois nouvelles espèces de Russula Pers. subg. Compactae (Fr.) Bon provenant du sud de la Chine.

Trois nouvelles espèces de *Russula* Pers. subg. *Compactae* (Fr.) Bon provenant du sud de la Chine, toutes possédant des lamelles distantes et larges, sont illustrées et décrites sur la base d'arguments morphologiques et d'analyses phylogénétiques fondées sur l'ITS et les séquences partielles concaténées des gènes LSU, mtSSU, *rpb1*, *rpb2* et *tef1*. *Russula latolamellata* Y.Song & L.H.Qiu, sp. nov., se caractérise par la surface brun noirâtre, finement aréolée de son chapeau, sa chair devenant rouge écarlate à la coupe, la présence fréquente de basides monospores et l'absence de pileo- et caulocystides. *Russula nigrocarpa* S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov., se caractérise par ses lamelles blanc cassé à arête brun foncé à noire à maturité, les cystides hyméniales et pileocystides de forme variable et son pileipellis gélinifé. *Russula ochrobrunnea* S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov., se caractérise par son chapeau gris-brun à marge striée, ses lamelles brun clair à arête brun foncé à maturité, la présence de basides monospores et ses cystides hyméniales légèrement sinueuses ou moniliformes. Leurs caractéristiques morphologiques détaillées et leurs positions phylogénétiques sont discutées et comparées avec les espèces étroitement apparentées.

MOTS CLÉS
Multi-genes,
phylogénie,
sud de la Chine,
espèces nouvelles.

INTRODUCTION

Russula Pers. is one of the most diverse and widely distributed ectomycorrhizal groups of macrofungi (Buyck 1989). The genus has a relatively recent evolutionary age, but has experienced rapid diversification through frequent host switches from conifers to hardwoods with subsequent host expansion (Looney *et al.* 2016). Due to its ectomycorrhizal lifestyle, *Russula* species fulfill important roles in the stabilization of different ecosystems. Usually appearing in the relatively late stage of ecosystem succession (Visser 1995), *Russula* species assist in keeping the balance of multiple soil substances, helping the circulation of C, N and P, and their basidiomata provide food for numerous invertebrates and small mammals (Looney *et al.* 2018). *Russula* species also help the germination of many achlorophyllous hemiparasitic plants and act as host species of different mycoheterotrophic plants within Ericaceae and Orchidaceae (Bidartondo & Bruns 2001; Giralanda *et al.* 2006), enriching the interspecific relationships inside temperate, subtropical to tropical forests.

Buyck *et al.* (2018, 2020) reorganized the infrageneric taxonomic system of *Russula* employing a 5-locus phylogenetic analysis, in which *Russula* subg. *Glutinosae* Buyck & X.H.Wang, *Archaeae* Buyck & V.Hofst. and *Compactae* (Fr.) Bon formed a monophyletic clade with subg. *Glutinosae* being sister to a clade composed of subg. *Compactae* and subg. *Archaeae*. *Russula* subg. *Compactae* mainly consists of species with dull-colored pileus, unpleasant or unremarkable smell and unequal, multiseriate lamellae (Buyck *et al.* 2018). There are about 20 described species in this subgenus with most of them collected from Europe (Romagnesi 1967; Sarnari 1998) and North America (Shaffer 1962; Adamcik & Buyck 2014), but with also a high number of representatives in tropical Africa (Buyck 1993). So far, only four Asian species are described in this subgenus: Indian *R. kangchenjungae* Van de Putte, K.Das & Buyck (Das *et al.* 2010) and *R. schaefferina* Rawla & Sarwal (Rawla & Sarwal 1983), Japanese *R. subnigricans* Hongo (Hongo 1955) and *R. purpureonigra* Petch from Sri Lanka (Petch 1917; Manimohan & Latha 2011).

Recently, in a survey for *Russula* carried out by the authors in Dinghushan Biosphere Reserve (DHSBR, 112°30'39"-112°33'41"E, 23°09'21"-23°11'30"N), a well-known reserve located in Guangdong Province, P. R. of China, 13 new species and one epitype (Das *et al.* 2017; Zhang *et al.* 2017; Song *et al.* 2018a, b; Li *et al.* 2018; Yuan *et al.* 2019), all belonging to *R.* subg. *Heterophyllidiae* Romagn., have been recovered and published. In this study, we report three novel species belonging to *R.* subg. *Compactae* collected from DHSBR during the survey. It is the first time that new species of this subgenus are reported from China.

MATERIAL AND METHODS

SAMPLING AND MORPHOLOGY

Specimens were collected from Dinghushan Biosphere Reserve (DHSBR, 112°30'39"-112°33'41"E, 23°09'21"-23°11'30"N). Specimens were dehydrated at 50-60°C and deposited in the Herbarium of Microbiology Institute of Guangdong (GDGM). Fresh basidiomata 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 basidiomata. 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 Electronic Microscope (JSM-6330F). Basidiospores were examined in Melzer's reagent and measured in side view, excluding ornamentations. Other micromorphological structures (basidia, hymenial cystidia, dermatocystidia, elements of pileipellis and stipitipellis) were observed from hand-sectioned dried tissues rehydrated in 5% or 10% KOH solution and then mounted with 1% aqueous Congo red reagent. All tissues were examined in cresyl blue (Buyck 1989) to verify presence of ortho- or metachromatic reactions. Sulphovanillin

TABLE 1. — Sequences used in phylogenetic analysis based on ITS. Specimens in **bold** correspond to new species.

Taxon	Voucher specimen	Location	ITS accession number
<i>R. acrifolia</i> Romagn.	UE12.09.2003-3 (UPS)	Sweden	DQ421998
<i>R. adusta</i> (Pers.) Fr.	Buyck 6020 (PC)	Europe	AY061652
<i>R. aff. earlei</i> Peck	Ex1186/BB 07.108 (PC)	Europe	KY800363
<i>R. albonigra</i> (Krombh.) Fr.	AT2002064 (UPS)	Sweden	DQ422029
<i>R. anthracina</i> Romagn.	LAH-EM2-2013	Pakistan	KR011881
<i>R. archaea</i> R.Heim	BB2004-255 (PC)	United States	EU598165
<i>R. archaeosuberis</i> Sarnari	1118/BB 12.085	Italy	KY930355
<i>R. butyroindica</i> K.Das & Buyck	CAL1511 (holotype)	India	KY674859
<i>R. camarophylla</i> Romagn.	PAM01081108 (PC)	France	DQ421982
<i>R. camarophylla</i> Romagn.	Sarnari 8/8/94 (PC)	Europe	AY061662
<i>R. cantharellicola</i> Romagn.	RG1	United States	KF306037
<i>R. cortinarioides</i> Buyck, Adamík, D.P.Lewis & V.Hofst.	567/BB 07.103	United States	KP033480
<i>R. cf. eccentrica</i> Peck	431/BB 07.132	United States	KP033478
<i>R. densifolia</i> Secr. ex Gillet	ue116 (TUB)	Germany	AF418606
<i>R. dissimulans</i> Shaffer	BPL285 (TENN)	United States	KT933979
<i>R. earlei</i> Peck	WRW00-412 (PC)	United States	DQ422025
<i>R. earlei</i> Peck	BPL245 (TENN)	United States	KT933961
<i>R. eccentrica</i> Peck	BB2004-248 (PC)	United States	EU598197
<i>R. fortunae</i> Corrales	TUC (Corrales 180) (holotype)	Panama	KM594806
<i>R. fuliginosa</i> Sarnari	IB 1992/0454 (holotype)	Italy	HG798529
<i>R. glutinosa</i> Fatto	Roody WRWV 04.1154 (holotype)	United States	MN315541
<i>R. glutinosoides</i> Buyck & X.H.Wang	HKAS106678 (holotype)	China	MN434187
<i>R. gossypina</i> Buyck	2/BB 06.002	Madagascar	KY800350
<i>R. khanchanjungae</i> Van de Putte, K.Das & Buyck	AV-KD-KVP09-106 (GENT)	India	KR364129
<i>R. lateritcola</i> (R.Heim) Singer	7/BB 06.031	Madagascar	KP033476
<i>R. latolamellata</i> Y.Song & L.H.Qiu, sp. nov.	GDGM 79561 (holotype)	China	MN275543
<i>R. latolamellata</i> Y.Song & L.H.Qiu, sp. nov.	GDGM 79562	China	MN275544
<i>R. latolamellata</i> Y.Song & L.H.Qiu, sp. nov.	GDGM 79563	China	MN275545
<i>R. nigricans</i> Fr.	UE20.09.2004-07 (UPS)	Sweden	DQ422010
<i>R. nigrocarpa</i> S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov.	GDGM 79720 (holotype)	China	MN688794
<i>R. nigrocarpa</i> S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov.	GDGM 79721	China	MN688795
<i>R. ochrobrunnea</i> S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov.	GDGM 79718 (holotype)	China	MN688792
<i>R. ochrobrunnea</i> S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov.	GDGM 79719	China	MN688793
<i>R. polyphylla</i> Peck	568/BB 07.023	United States	KP033481
<i>R. polyphylla</i> Peck	1194/BB 07.134	United States	KP033486
<i>R. pseudoaurantiophylla</i> Buyck & V.Hofst.	BB 09.223 (PC) (holotype)	New Caledonia	KY800354
<i>R. subnigricans</i> Hongjo	OSA-MY-4267	Japan	AB291750

(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 basidiomata 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, SEQUENCING AND PHYLOGENETIC ANALYSES

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 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. LSU, mtSSU, *rpb1*, *rpb2* and *tefl* were amplified using the primers and protocols described in Buyck *et al.* (2018). The products obtained were purified using E.Z.N.A Gel Extraction Kit (OMEGA) and sequenced on an ABI3730xl DNA Analyzer (IGE, Guangzhou, China).

The newly generated sequences were deposited in GenBank (www.ncbi.nlm.nih.gov).

Blast query in GenBank with newly generated ITS sequences showed that three proposed novel species were assigned to *Russula* subg. *Compactae*. Phylogenetic analyses based on both ITS and combined sequences of LSU-mtSSU-*tefl-rpb2-rpb1* were performed with Maximum Likelihood method. The datasets were automatically aligned by MAFFT v 7.427 (Katoh & Standley 2013), then manually adjusted and trimmed with BioEdit v7.0.9 (Hall 1999). Ambiguous sites at the beginning, end and middle parts of the datasets were removed. The final ITS and 5-locus alignments comprised 759 bp and 4163 bp (LSU 904 bp, mtSSU 551 bp, *tefl* 924 bp, *rpb2* 779 bp, *rpb1* 1005 bp), respectively. The ITS and the concatenated 5-locus datasets used in the analyses consisted of 37 (Table 1) and 33 (Table 2) sequences, respectively, and both using *R. glutinosa* Fatto and *R. glutinosoides* Buyck & X. H. Wang as outgroups. A rapid bootstrapping (BS) algorithm of 1000 replicates was executed in RAxML 7.2.6 (Stamatakis 2006), followed by a heuristic ML search for the best tree using the GTRGAMMA model. All parameters in RAxML analysis were kept at default. Bootstrap value (BS) exceeding 70% was considered to be significantly supported.

TABLE 2. — Sequences used in phylogenetic analysis based on 5-locus data (LSU-mtSSU-*tef1-rpb2-rpb1*). Specimens in **bold** correspond to new species.

Taxon	Voucher specimen	Location	Accession number				
			nuLSU	mtSSU	<i>rpb1</i>	<i>rpb2</i>	<i>tef1</i>
<i>R. acrifolia</i> Romagn.	543/BB 08.662	Italy	KU237535	KU237381	KU237684	KU237821	KU237965
<i>R. adusta</i> (Pers.) Fr.	223/BB 06.562	Canada	KU237476	KU237320	KU237625	KU237762	KU237907
<i>R. aff. areolata</i> Buyck	79/BB 06.090	Madagascar	KU237471	KU237315	–	KU237757	KU237903
<i>R. aff. earlei</i> Peck	1181/MT s.n.	Costa Rica	KU237598	KU237446	–	KU237883	–
<i>R. albonigra</i> (Krombh.) Fr.	544/BB 07.291	Slovakia	KU237536	KU237382	KU237685	KU237822	KU237966
<i>R. albonigra</i> (Krombh.) Fr.	AT2002064 (UPS)	Sweden	DQ422029	–	–	DQ421966	–
<i>R. archaeosuberis</i> Sarnari	1118/BB 12.085	Italy	KU237593	KU237441	KU237732	KU237878	KU238019
<i>R. camarophylla</i> Romagn.	PAM01081108 (PC)	France	DQ421982	–	–	DQ421938	–
<i>R. cf. camarophylla</i> Romagn.	639/MPG11-7-09	Spain	KP033579	KU237427	KU237722	KU237865	KU238006
<i>R. cf. eccentrica</i> Peck	431/BB 07.132	United States	KP033489	KU237341	KU237645	KP033500	KU237926
<i>R. cf. subfistulosa</i> Buyck	550/BB 08.176	Madagascar	KU237542	KU237388	KU237690	KU237828	KU237972
<i>R. cortinarioides</i> Buyck, Adamčík, D.P.Lewis & V.Hofst.	567/BB 07.103	United States	KP033491	KU237402	–	KP033502	KU237985
<i>R. densifolia</i> Secr. ex Gillet	439/BB 07.344	Slovakia	KU237502	KU237347	KU237651	KU237788	KU237932
<i>R. dissimulans</i> Shaffer	BPL285 (TENN)	United States	KT933840	–	–	KT933911	–
<i>R. earlei</i> Peck	BPL245 (TENN)	United States	KT933820	–	KT957331	KT933891	–
<i>R. eccentrica</i> Peck	447/BB 07.044	United States	KP033490	KU237353	KU237657	KP033501	KU237937
<i>R. fistulosinae</i>	535/BB 08.105	Madagascar	KU237527	KU237373	KU237677	KU237813	KU237957
<i>R. fuliginosa</i> Sarnari	1178/MF s.n.	Italy	KU237597	KU237445	–	KU237882	–
<i>R. glutinosa</i> Fatto	Roody WRWV 04.1154 (holotype)	United States	MN315511	MN315532	–	MN326798	MN326799
<i>R. glutinosoides</i> Buyck & X.H.Wang	HKAS106678 (holotype)	China	MN428827	MN460313	–	–	–
<i>R. gossypina</i> Buyck	2/BB 06.002	Madagascar	KU237450	KU237293	–	KU237736	KU237886
<i>R. inornata</i>	571/BB 08.194	Madagascar	KU237558	KU237406	–	KU237844	KU237989
<i>R. khanchanjungae</i> Van de Putte, K.Das & Buyck	AV-KD-KVP09-106 (GENT)	India	JN389004	–	JN389201	JN375607	–
<i>R. lateritcola</i> (R.Heim) Singer	7/BB 06.031	Madagascar	KP033487	KU237297	KU237604	KP033498	KU237888
<i>R. latolamellata</i> Y.Song & L.H.Qiu, sp. nov.	GDGM 79561 (holotype)	China	MK881933	MK882061	MT085500	MK880660	MT085574
<i>R. latolamellata</i> Y.Song & L.H.Qiu, sp. nov.	GDGM 79563 (holotype)	China	MN839557	MN839605	MT085525	MT085632	MT085604
<i>R. nigricans</i> Fr.	429/BB 07.342	Slovakia	KU237495	KU237339	KU237643	KU237781	KU237924
<i>R. nigricans</i> Fr.	UE20.09.2004-07 (UPS)	Sweden	DQ422010	–	–	DQ421952	–
<i>R. nigrocarpa</i> S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov.	GDGM 79720 (holotype)	China	MN839585	MN839635	MT085531	MT085662	MT085630
<i>R. ochrobrunnea</i> S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov.	GDGM 79718 (holotype)	China	MN839584	MN839634	–	MT085661	MT085629
<i>R. polyphyllinae</i>	737/BB 09.215	New Caledonia	KU237590	KU237438	–	KU237875	–
<i>R. polyphylla</i> Peck	568/BB 07.023	United States	KP033492	KU237403	KU237700	KP033503	KU237986
<i>R. pseudoaurantiophylla</i> Buyck & V.Hofst.	740/BB 09.219	New Caledonia	KU237591	KU237439	KU237730	KU237876	KU238017

RESULTS

PHYLOGENETIC ANALYSES

The final alignments of the ITS and 5-locus datasets used in Maximum Likelihood analyses were 759 bp and 4179 bp, and the resulting trees are shown in Figures 1 and 2, respectively.

Subgenera *Archaeae* and *Compactae* were fully resolved in both phylogenetic analyses (BS = 100%) with all three newly proposed species nested in subg. *Compactae*. *Russula ochrobrunnea* S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov., was highly supported as member of a clade corresponding to sect. *Polyphyllae* Buyck & V.Hofst. (BS = 100%) in both analyses and placed sister with full support (BS = 100%) to *R. eccentrica* Peck. The other two novel species were placed with significant support (BS = 82%) by our multilocus analysis (Fig. 2) in a clade that corresponds to sect. *Nigricantinae* Bataille, but could not be placed with significant support to any particular species in the ITS analysis (Fig. 1) whereas, in our multilocus analysis, *R. latolamellata* Y.Song &

L.H.Qiu, sp. nov., maintains an isolated position and *R. nigrocarpa* S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov., is grouped with *R. acrifolia* Romagn. with full support (BS = 100%).

TAXONOMY

Phylum BASIDIOMYCOTA R.T.Moore
 Class AGARICOMYCETES Doweld
 Order RUSSULALES Kreisel ex P.M.Kirk,
 P.F.Cannon & J.C.David
 Family RUSSULACEAE Lotsy
 Genus *Russula* Pers.
 Subgenus *Compactae* (Fr.) Bon

***Russula latolamellata* Y.Song & L.H.Qiu, sp. nov.**
 (Figs 3; 4)

MYCOBANK NUMBER. — MB 835726.

HOLOTYPE. — China. Guangdong Province, Zhaoqing City, Dinghushan Biosphere Reserve, on the ground in broadleaf forest, 6.IV.2015, J. B. Zhang K15060604 (GDGM 79561).

ETYMOLOGY. — Named after its broad lamellae.

DIAGNOSIS. — Mainly characterized by its distinctly cracking and black-tan pileus, broad and sparse lamellae, stipe and context becoming scarlet when bruised, basidiospores with completely reticulate ornamentations, common presence of 1-spored basidia, encrusted hyphae with brown pigments in pileipellis and absence of both pileocystidia and caulocystidia.

HABITAT AND DISTRIBUTION. — Solitary or gregarious in broadleaf forest.

ADDITIONAL SPECIMENS EXAMINED. — China. Guangdong Province, Zhaoqing City, Dinghushan Biosphere Reserve, on the ground in broadleaf forest, 13. IX. 2016, J. W. Li, Y. Song K16091311 (GDGM 79562); 13.VII.2015, J. W. Li, Y. Song K17071307 (GDGM 79563).

DESCRIPTION

Basidiomata

Medium to large sized, agaricoid.

Pileus

6-12 cm in diameter, hemispherical when young, becoming appanate with a slightly depressed center when mature; surface dry, cracking, tan, grayish to blackish brown, not easy to peel; margin entire or undulate.

Lamellae

Adnate, sparse, up to 8 mm broad, irregularly unequal, sometimes forked near stipe or at pileus margin, sometimes interveined; surface smooth, white, off-white to cream, tinged with reddish brown; lamella edge concolorous.

Stipe

4-8 cm long, central, cylindrical, sometimes tapering upwards or downwards, longitudinally rugulose, white to off-white, becoming grayish white when old, turning reddish-brown to scarlet when bruised, solid or stuffed.

Context

White, becoming reddish-brown when bruised, turning yellowish-brown in reaction with 5% FeSO₄.

Odour

Slightly unpleasant.

Taste

Mild.

Spore print

White.

Basidiospores

Subglobose to ellipsoid, [100/5/3] (5.9-) 6-6.8-7.4 (-7.5) × (4.9-) 5.1-5.8-6.5 (-6.9) μm, Q = (1.04-) 1.06-1.17-1.32 (-1.37); ornamentations amyloid, composed of low ridges, forming a complete reticulum; suprahilar spot inamyloid.

Basidia

41-60 × 6-12 μm, clavate to narrowly clavate, 1-, 2-, 3- or 4-spored, hyaline or containing oil droplets; sterigmata 4.8-8.3 × 1.4-2.2 μm.

Lamellar trama

Composed of nested sphaerocytes measuring 18.5-55.5 × 17-43 μm and surrounded by connective hyphae.

Pleurocystidia

(35.5-) 38-62-83 (-91) × (4.5-) 6-7.5-10 μm, protruding up to 30 μm, narrowly clavate to narrowly cylindrical with obtuse or mucronate apices, thin-walled, with refractive contents, unchanging in SV.

Cheilocystidia

(34-)39-43-47(-51) × 4.5-5-6.5 μm, resembling pleurocystidia in shape but smaller in size, thin-walled, with refractive contents, unchanging in SV.

Marginal cells

Not differentiated.

Pileipellis

Composed of ascending to erect hyphae, orthochromatic in cresyl blue; hyphae 2-6 μm in width, cylindrical, septate, some with incrustation and brown dispersive pigments; terminal cells (8-)11-23-36(-38.5) × 2.5-4-6(-7.5) μm, usually cylindrical with obtuse apices and brown pigments, lageniform, clavate to cylindrical, some with short rostrate apices or granular contents.

Pileocystidia

None found.

Stipitipellis

Composed of erect hyphae, 40-90 μm wide; hyphae 1.5-4 μm in width, narrowly cylindrical, septate; terminal cells (6.5-)13-22-31(-39) × 2-4-6(-7.5) μm, thin-walled, usually hyaline, cylindrical to clavate with obtuse apices.

Caulocystidia

None found.

Clamp connections

Absent.

NOTES

Russula latolamellata Y.Song & L.H.Qiu, sp. nov., morphologically resembles *R. schaefferina* Rawla & Sarwal, including pileus color and features of pileipellis, size and ornamentations of basidiospores and absence of both pileocystidia and caulocystidia, etc. However, it differs substantially from our species in its finely pruinose pileus with uncracking surface, thin and crowded lamellae, and further also by shape and size of pleurocystidia (85-98 × 10-14 μm), and cheilocystidia (35-50 × 9-13 μm), and the

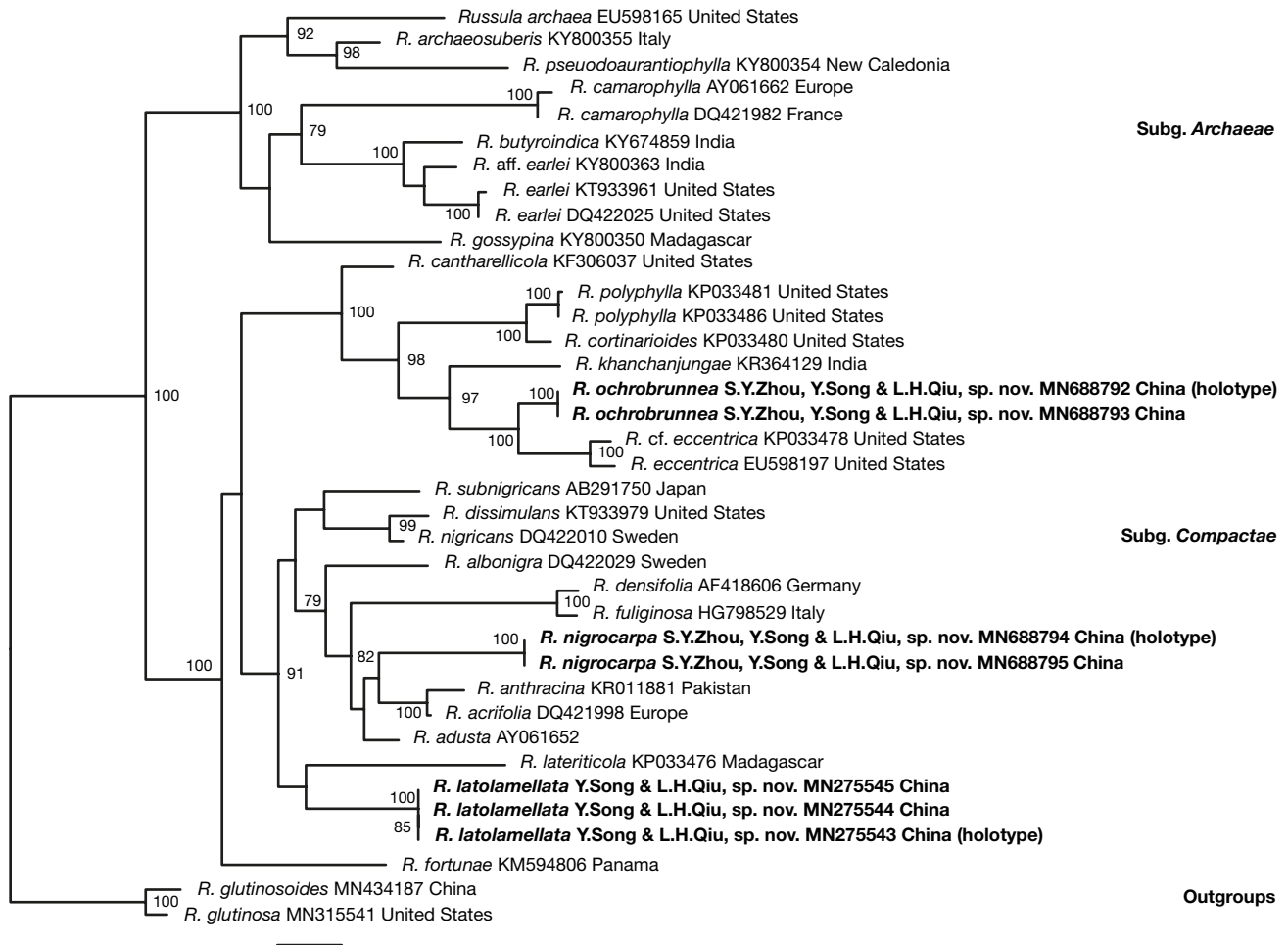


Fig. 1. — Maximum Likelihood tree of *Russula* Pers. subg. *Archaeae* Buyck & V.Hofst. and *Compactae* (Fr.) Bon based on ITS sequences, bootstrap values higher than 70% were displayed around nodes. Scale bar: 0.03 substitutions.

hollow stipe covered with ochraceous or brown depressed fibrillose scales (Rawla & Sarwal 1983). When bruised, context of *R. latolamellata* Y.Song & L.H.Qiu, sp. nov., directly becomes reddish brown without turning black first.

Although all three specimens of *R. latolamellata* Y.Song & L.H.Qiu, sp. nov., were collected from the broadleaf forest of DHSBR, several ITS sequences that have a sequence similarity of about 99% to that of *R. latolamellata* Y.Song & L.H.Qiu, sp. nov., retrieved from soil in the pine and broadleaf mixed forest of DHSBR are available in GenBank, implying that the species may be common in DHSBR.

Russula nigrocarpa S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov.
(Figs 5; 6)

MYCOBANK NUMBER. — MB 835727.

HOLOTYPE. — **China**. Guangdong Province, Zhaoqing City, Dinghushan Biosphere Reserve, on the ground in broadleaf forest, 16.VII.2019, S. Y. Zhou K19071603 (GDGM 79720).

ETYMOLOGY. — Named after its dark black pileus.

DIAGNOSIS. — Mainly characterized by its dry, cracking, dark brown to dark black pileus, off-white lamellae, basidiospores with complete reticulate ornamentations, hymenial cystidia and pileocystidia of various forms with often forked apices, strongly glutinous pileipellis composed of hyphae usually with dark brown vacuolar pigments.

HABITAT AND DISTRIBUTION. — Solitary or gregarious in evergreen broadleaf forest.

ADDITIONAL SPECIMENS EXAMINED. — **China**. Guangdong Province, Zhaoqing City, Dinghushan Biosphere Reserve, on the ground in broadleaf forest, 5.V.2018, J. W. Li, Y. Song K18050529 (GDGM 79721).

DESCRIPTION

Basidiomata

Medium to large sized, agaricoid.

Pileus

6-10 cm in diameter, applanate to concave when mature; surface dry, dark brown to dark black; margin entire, turning slightly upward.

Lamellae

Adnate to slightly decurrent, sparse, 6 pieces of lamellae and lamellulae/cm at the margin of pileus, broad, unequal; surface

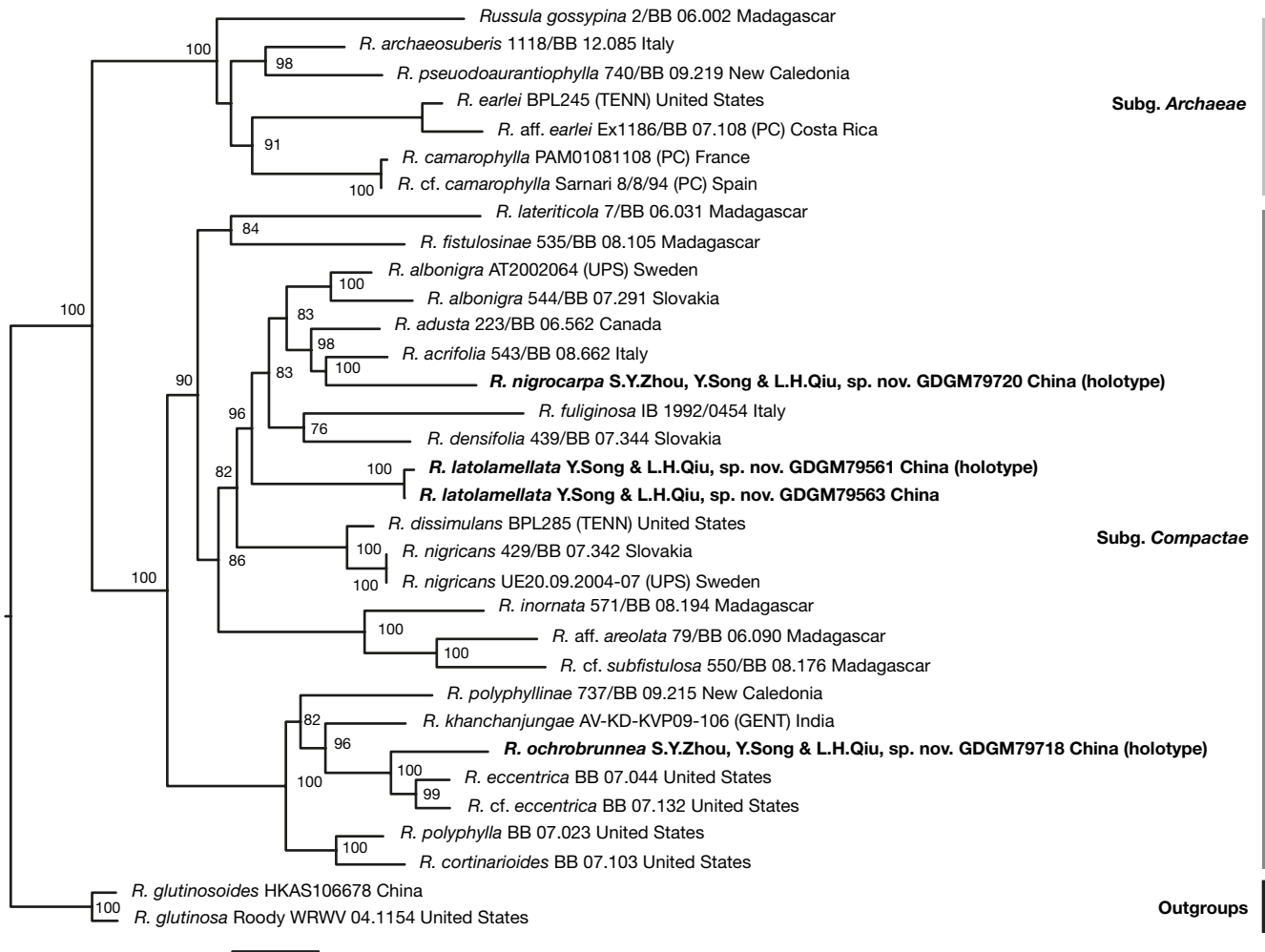


FIG. 2. — Maximum Likelihood tree of *Russula* Pers. subg. *Archaeae* Buyck & V.Hofst. and *Compactae* (Fr.) Bon based on LSU-mtSSU-*tef1-rpb2-rpb1* combined sequences, bootstrap values higher than 70% were displayed around nodes. Scale bar: 0.02 substitutions.

off-white to cream or yellowish, becoming dark brown when bruised; gill edge concolorous, becoming dark brown to black when old (probably from drying out).

Stipe

3-5 × 2.5-4 cm, central, cylindrical, sometimes tapering downwards, solid, off-white, becoming grayish white when mature, turning dark brown when bruised.

Context

White, becoming directly black when bruised without reddening first, 6-8 mm thick near stipe.

Odour

Unpleasant.

Taste

Not taken.

Spore print

White to cream.

Basidiospores

Subglobose to ellipsoid, [40/2/2] 4.3-4.8-5.4 (-5.8) × (3.4-) 3.7-4-4.4 (-4.6) μm, Q = 1.10-1.19-1.28 (-1.35); ornamentations amyloid, composed of low ridges forming a complete reticulum; suprahilar spot inamyloid.

Basidia

(22-) 26.5-31-35 (-46.5) × 5-6.5-8 μm, clavate to cylindrical, 1-, 2-, 3- or 4-spored, with refractive contents; sterigmata 1.8-6 μm long.

Lamellar trama

Composed of nested sphaerocytes surrounded by connective hyphae.

Pleurocystidia

(31-) 35-41-52 (-58) × 3.5-5-6.5 μm, of various forms, narrowly cylindrical to slightly flexuose with obtuse, mucronate, moniliform, inflorescence-like or forked apices, thin-walled, with refractive contents, unchanging in SV.

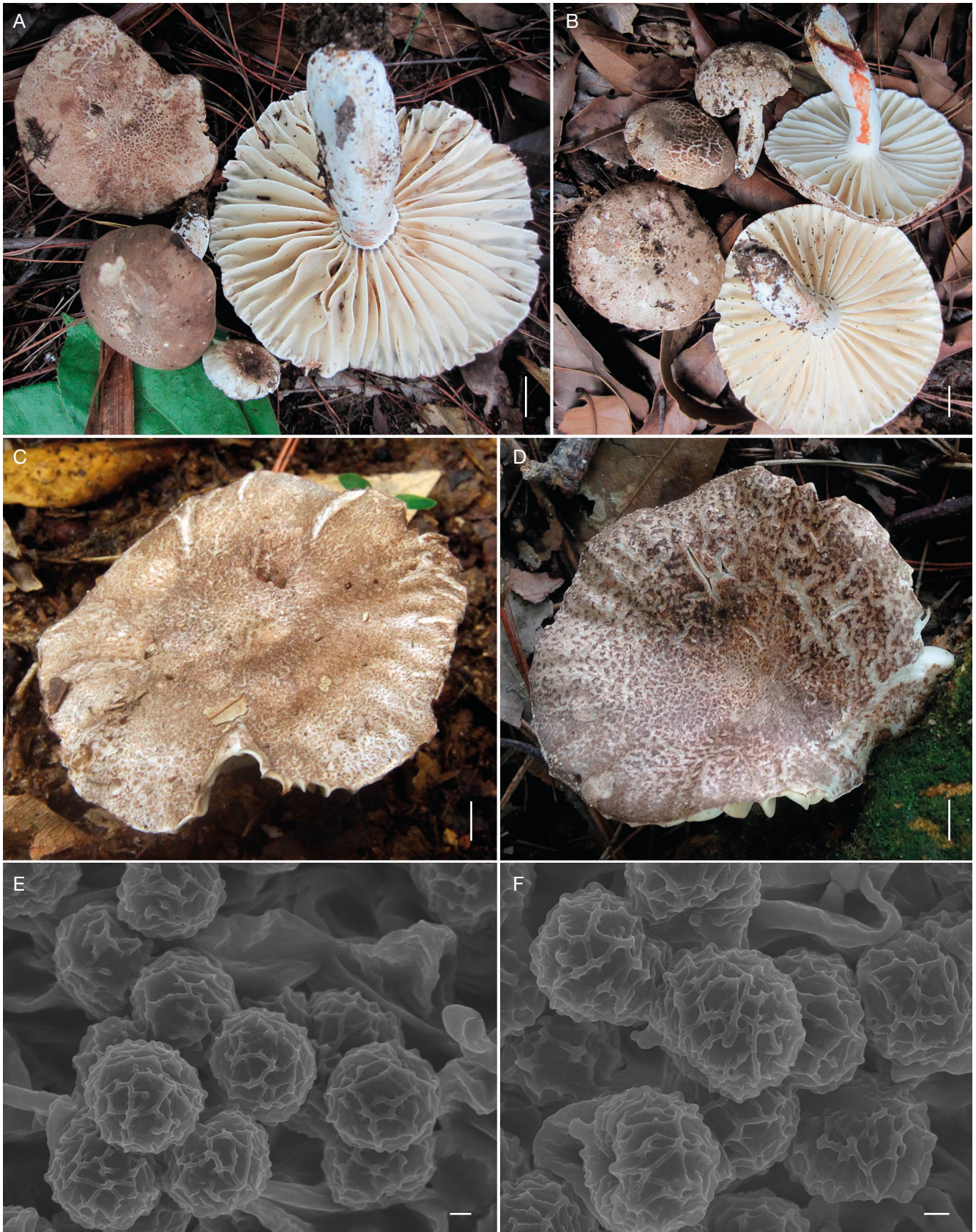


FIG. 3. — *Russula latolamellata* Y.Song & L.H.Qiu, sp. nov. (GDGM 79561, holotype): **A-D**, fruiting bodies; **E, F**, scanning electronic micrographs of basidiospores. Scale bars: A-D, 1 cm; E, F, 1 μ m.

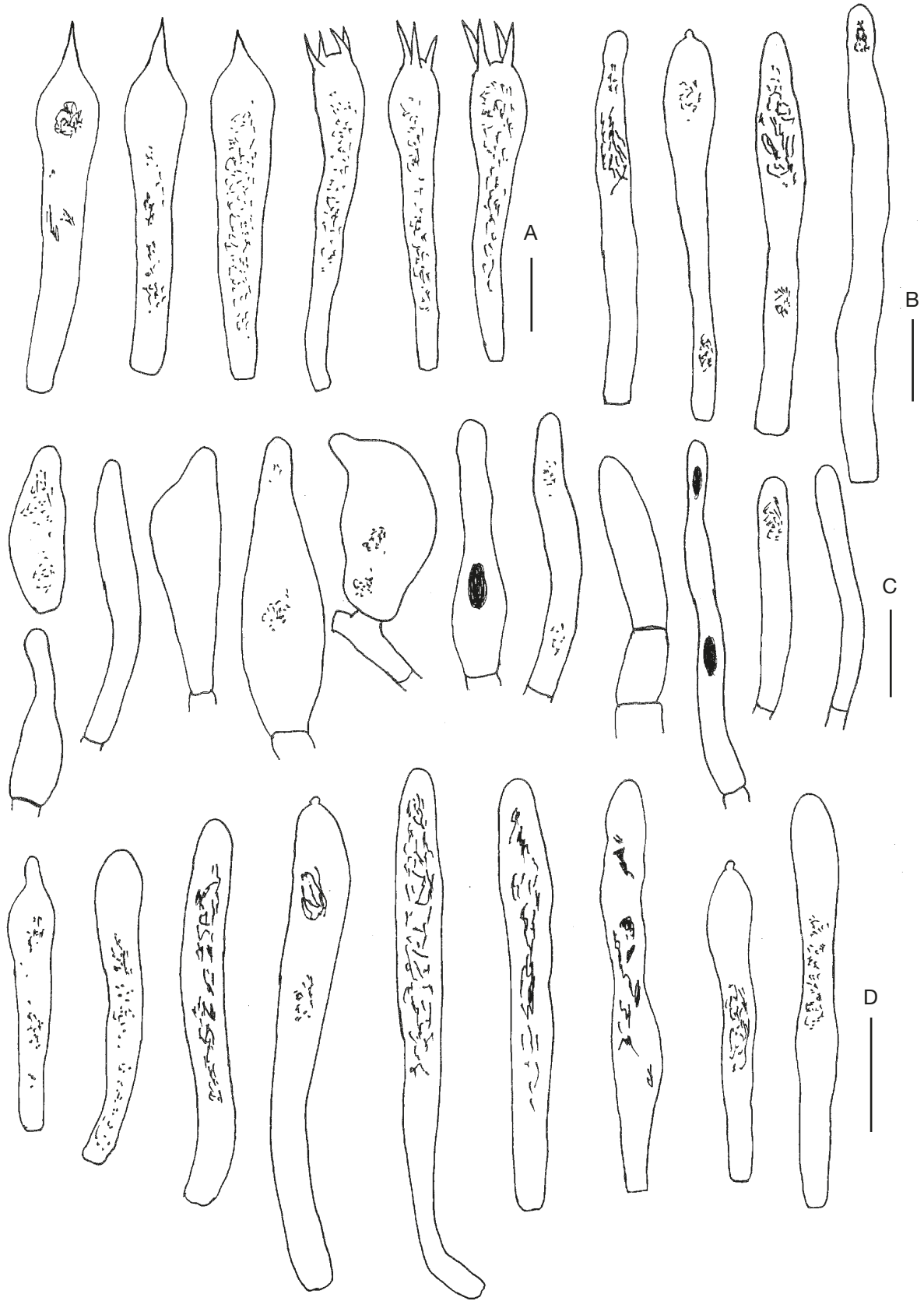


FIG. 4. — *Russula latolamellata* Y.Song & L.H.Qiu, sp. nov. (GDGM 79561, holotype): A, basidia; B, cheilocystidia; C, terminal elements of pileipellis; D, pleurocystidia. Scale bars: A-D, 10 μ m.



FIG. 5. — *Russula nigrocarpa* S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov. (GDGM 79720, holotype): **A, B**, fruiting bodies; **C-E**, pleurocystidia; **F-H**, pileocystidia; **I, J**, scanning electronic micrographs of basidiospores. Scale bars: A, B, 1 cm; C-H, 10 μ m; I, J, 1 μ m.

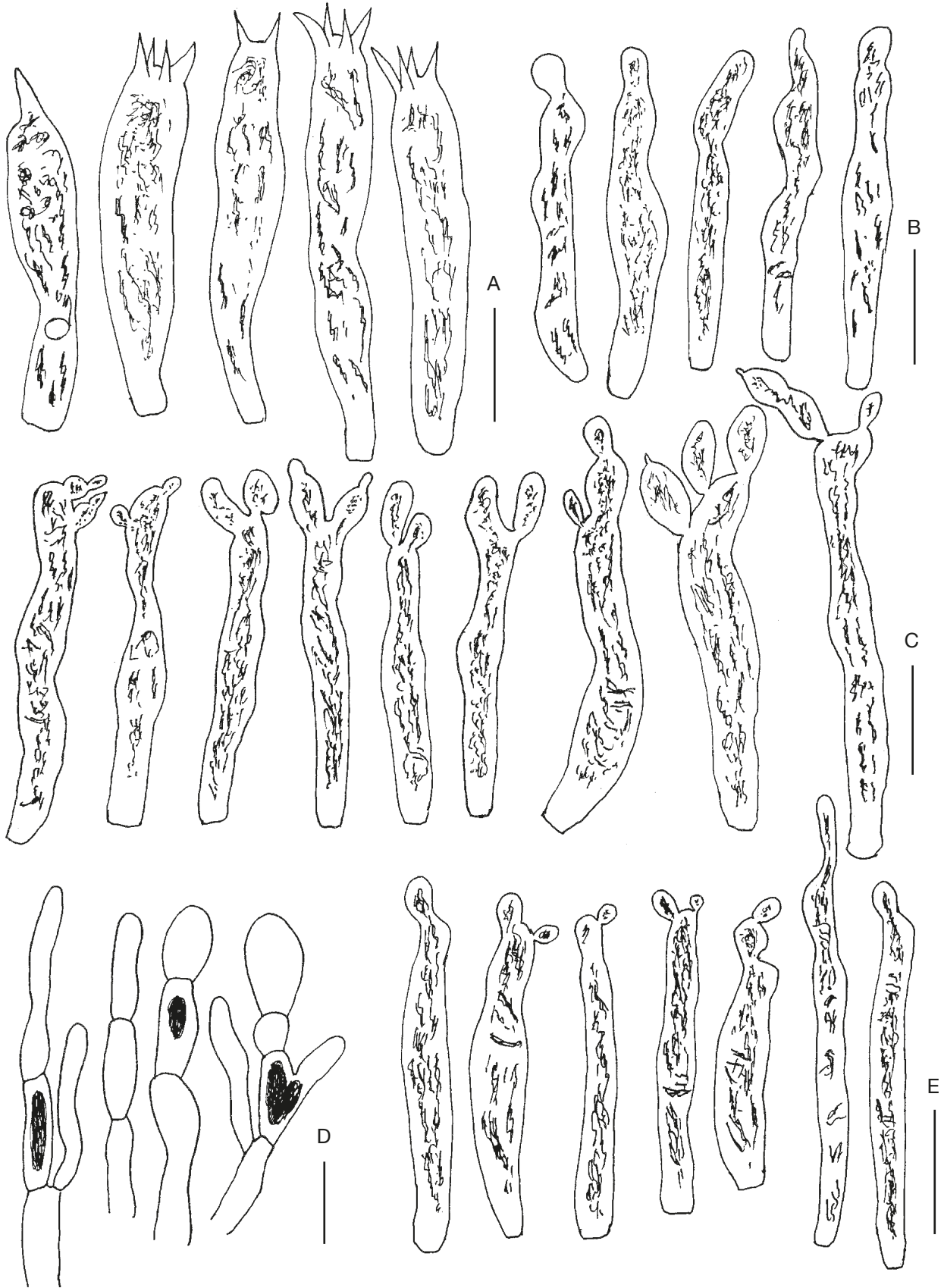


FIG. 6. — *Russula nigrocarpa* S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov. (GDGM 79720, holotype): A, basidia; B, cheilocystidia; C, pleurocystidia; D, terminal elements of pileipellis; E, pileocystidia. Scale bars: A-E, 10 μ m.



FIG. 7. — *Russula ochrobrunnea* S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov. (GDGM 79718, holotype): **A-D**, fruiting bodies; **E, F**, scanning electronic micrographs of basidiospores. Scale bars: A-D, 1 cm; E, F, 1 μ m.

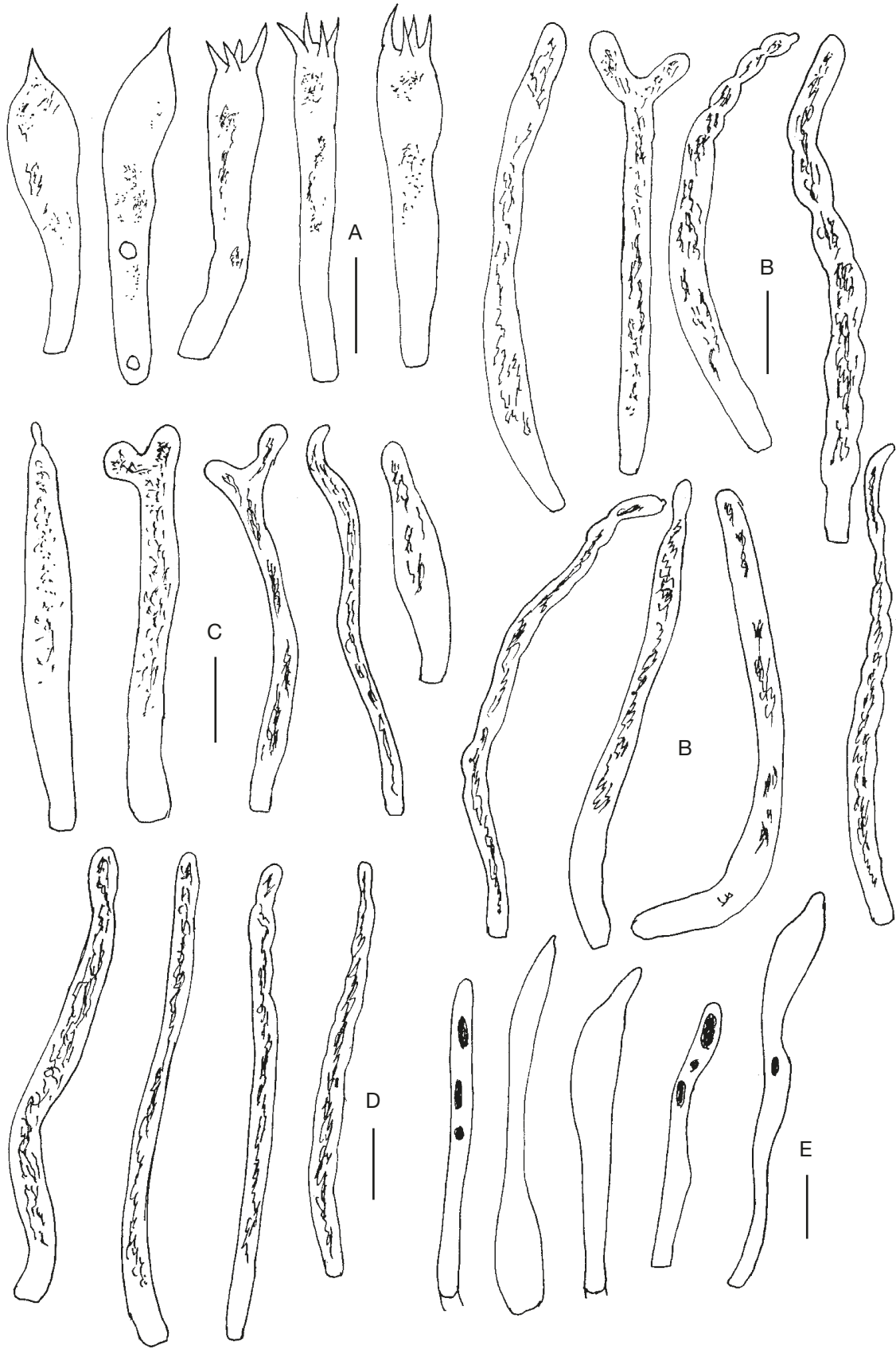


FIG. 8. — *Russula ochrobrunnea* S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov. (GDGM 79718, holotype): **A**, basidia; **B**, pleurocystidia; **C**, cheilocystidia; **D**, caulocystidia; **E**, terminal elements of pileipellis. Scale bars: A-D, 10 μ m.

Cheilocystidia

25-29-35 × 3-4-5 µm, narrowly cylindrical to slightly flexuous with obtuse or rostrate apices, with refractive contents, thin-walled, unchanging in SV.

Marginal cells

Not differentiated.

Pileipellis

Composed of ascending to erect hyphae, strongly gelatinized, 150-220 µm thick, orthochromatic in cresyl blue; hyphae 2-6 µm wide, narrowly cylindrical, septate, often with dark brown pigments; terminal cells 12.5-17-22 (-25) × 2.5-4-6 (-7) µm, cylindrical to narrowly clavate with obtuse or slightly acute apices, some with dark brown pigments.

Pileocystidia

16.5-25-33.5 × 2.5-4-5.5 µm, subclavate to cylindrical, apices mucronate to inflorescence-like, with refractive contents, thin-walled, unchanging in SV.

Stipitipellis

Composed of ascending to erect hyphae, 60-100 µm thick, gelatinous; hyphae 1.5-4 µm wide, narrowly cylindrical, septate; terminal cells (8-) 10-19-31 (-34) × 2.5-5-9 (-10) µm, cylindrical to narrowly clavate with obtuse apices, sometimes containing brown pigments.

Caulocystidia

(16.5-) 21-34-52 (-56) × 3.5-4.5-6 µm, thin-walled, cylindrical with obtuse or short rostrate apices.

Clamp connections

Absent.

NOTES

R. nigrocarpa S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov., is closely related to *R. acrifolia* in our multilocus phylogeny (Figs 1; 2). The latter species differs from *R. nigrocarpa* S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov., in its viscid, grayish brown pileus, context that turns red then gray to black when bruised, much crowded lamellae and grayish brown stipe but, above all, in the much larger size (6.0-9.5 × 5.5-7.5 µm) of its basidiospores (Çolak & Işiloğlu 2016). The exceptionally small size of the spores of *R. nigrocarpa* S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov., comparable to those of species in subg. *Archaeae*, was hitherto undocumented within subg. *Compactae*, making it impossible to confuse our species with any of the other Asian or northern hemisphere species in the same subgenus.

Russula ochrobrunnea S.Y.Zhou, Y.Song &
L.H.Qiu, sp. nov.
(Figs 7; 8)

MYCOBANK NUMBER. — MB 835743.

HOLOTYPE. — **China**. Guangdong Province, Zhaoqing City, Dinghushan Biosphere Reserve, on the ground in broadleaf forest, 15.VII.2019, S. Y. Zhou K19071502 (GDGM 79718).

ETYMOLOGY. — Named after its light brown lamellae with ochre margin when mature.

DIAGNOSIS. — Characterized by its grayish-brown pileus with striate to slightly cracking margin, sparse and light brown lamellae with ochre margin when mature, small basidiospores, presence of 1-spored basidia, flexuous to cylindrical hymenial cystidia often with papillate or branched apices and hyphae usually with brown pigments in pileipellis.

HABITAT AND DISTRIBUTION. — Solitary or gregarious in evergreen broadleaf forest.

ADDITIONAL SPECIMENS EXAMINED. — **China**. Guangdong Province, Zhaoqing City, Dinghushan Biosphere Reserve, on the ground in broadleaf forest, 2.VI.2018, S. Y. Zhou K18060208 (GDGM 79719).

DESCRIPTION

Basidiomata

Medium to large sized, agaricoid.

Pileus

7-9 cm in diameter, applanate with a depressed center to concave when mature; surface dry, not viscid, grayish-brown to tan, cracking into reticulum; margin slightly undulate or upward, striate.

Lamellae

Adnate to decurrent, distant (3-4 pieces of lamellae and lamellulae/cm at the margin of pileus), thick, firm, irregularly unequal; light brown to ochre; gill edge concolorous, but becoming tan to dark brown from drying out when old.

Stipe

4-6 × 2.4-3 cm, central to eccentric, cylindrical, mostly tapering downwards or slightly curving, solid, off-white.

Context

White, unchanging, 3-5 mm thick near stipe.

Odour

Unpleasant.

Taste

Not taken.

Spore print

White.

Basidiospores

Subglobose to ellipsoid, [40/2/2] (3.9-) 4.1-4.4-4.7 (-5.1) × (3.4-) 3.5-3.7-4.0 (-4.3) µm, Q = (1.09-) 1.10-1.18-1.29 (-1.30); ornamentations amyloid, composed of dense warts, some fused into ridges, forming a partial reticulum; suprahilar spot inamyloid.

Basidia

(24-) 26.5-32-39 (-42.5) × 4.5-6-7.5 µm, clavate to cylindrical, 1-, 2-, 3- or 4-spored, hyaline or containing granular contents.

Lamellar trama

Composed of nested sphaerocytes surrounded by connective hyphae.

Pleurocystidia

(60.5-) 71-98-136 (-146.5) × 3.5-4.5-6µm, narrowly cylindrical or flexuous with obtuse, mucronate, moniliform or sometimes forked apices, thin-walled, filled with refractive contents, unchanging in SV.

Cheilocystidia

Resembling pleurocystidia.

Marginal cells

Not differentiated.

Pileipellis

A cutis, 70-110 µm thick, strongly gelatinized, orthochromatic in cresyl blue; hyphae 2-6 µm wide, narrowly cylindrical, septate, often with brown pigments; terminal cells (22-) 26-35.5-58 (-63) × 3-5-6 (-7) µm, cylindrical to narrowly clavate with obtuse or slightly acute apices, sometimes with brown pigments.

Pileocystidia

(53.5-) 55-62.5-76 (-90.5) × 3-4.5-7.5 µm, cylindrical to fusiform with obtuse, mucronate or forked apices, some filled with refractive contents, negative in SV.

Stipitipellis

A cutis; hyphae 1.5-4 µm wide, narrowly cylindrical, septate, many with brown pigments; terminal cells cylindrical or lageniform with obtuse apices.

Caulocystidia

Cylindrical to narrowly clavate with obtuse or slightly acute apices, up to 7 µm wide, thin-walled, filled with refractive contents.

Clamp connections

Absent.

NOTES

Both our phylogenetic analyses place our species firmly in sect. *Polyphyllae* Buyck & V.Hofst., being closely related to the North American *R. eccentrica* and already separated by a much longer branch from the Indian *R. khanchanjungae* Van de Putte, K. Das & Buyck (Fig. 2). BLAST results of its ITS sequence in GenBank show top scores that are all <94% similar to our species, in this case for specimens collected in Japan and Korea (Park *et al.* 2014). *Russula ochrobrunnea* S.Y.Zhou, Y.Song & L.H.Qiu, sp. nov., resembles *R. eccentrica* in overall morphology, but the latter has pink

lamellae and much larger basidiospores (6-7.8 × 5-6 µm) and basidia (52-69 × 6.5-9.5 µm) (Adamčík *et al.* 2018). *Russula cartaginis* Buyck & Halling, described from Costa Rica (Buyck & Halling 2004) differs from *R. eccentrica* and our species in the presence of a brownish gill edge resulting from the presence of colored, branching marginal cells. Typical for most *Polyphyllae* is the often distinctly inflated lower portion of hymenial cystidia.

The Indian species *R. khanchanjungae* differs particularly by its crowded and forked lamellae becoming brown when bruised, and by the viscid and brown stipe with surface finely cracking exactly as the pileus surface; it also has much larger basidiospores (7.3-9.4 × 6.3-7.8 µm) and basidia (49-61 × 8-12 µm), as well as wider pleurocystidia (8-10 µm in width) (Das *et al.* 2010). Also *R. purpureonigra* differs principally in its larger spores and crowded lamellae (Manimohan & Latha 2011).

Acknowledgements

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