

Two novel and a forgotten *Russula* species in sect. *Ingratae* (Russulales) from Dinghushan Biosphere Reserve in southern China

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Abstract – Two novel species of *Russula* section *Ingratae*, collected in the Dinghushan Biosphere Reserve, southern China, are described and illustrated based on both morphology and phylogenetic analyses of rDNA ITS sequences. *Russula gelatinosa* sp. nov. is characterized by the gelatinized pileus with tuberculate striate margin, irregularly forked and inter-veined lamellae, winged basidiospores (ornamentations up to 3.5 µm), a suprapellis entirely composed of short erect chains of inflated terminal elements, and abundant gloeocystidia (hymenial cystidia and dermatocystidia) changing to reddish brown in SV. *Russula rufobasalis* sp. nov. is mainly recognized by the reddish stipe base, inter-veined lamellae and frequent lamellulae, cystidia blackened in SV, thick pileipellis with slender, but often thick-walled terminal elements, stipe trama with scattered cystidioid hyphae becoming blackish brown in SV. The differences and similarities with related species are discussed. *Russula punctipes* is redescribed here for the first time since it was originally published nearly a century ago, on the basis of new collections from the same area. Our analyses placed it sister to the Japanese *R. senecis*.

ITS sequence data / new taxa / morphology / phylogeny / *Russula punctipes*

INTRODUCTION

The genus *Russula* is one of the most highly diverse groups of macrofungi and has a cosmopolitan distribution from tropical to polar ecosystems (Buyck 1989; Buyck *et al.*, 1996; Miller & Buyck 2002; Park *et al.*, 2013). Species of *Russula* can form ectomycorrhizae with diverse plants, playing important ecological roles in sustaining forest biodiversity and as nutrient sources for some animals (Yamashita & Hijii 2007; Fogel & Trappe 1978). The genus is characterized by conspicuous basidiomata with colorful pileus, brittle context with abundant sphaerocytes, amyloid spore ornamentations, absence of lactifers and clamp connections, presence of

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gloeocystidia becoming brownish-gray over violaceous to black-tinged in sulphovanillin (SV) (Romagnesi 1967; Singer 1986; Buyck 1989; Sarnari 1998). *Russula* sect. *Ingratae*, commonly distributed in Europe and North America, is mainly recognized by their strong odors (either spermatic, waxy, fetid or of bitter almonds), pale to dark yellowish brown to dark greyish brown colors, acute to subacute lamellae, tuberculate striate pileus margin, inamyloid supraphilar spot and short-celled and branched hyphal extremities at the pileus surface (Shaffer 1972; Romagnesi 1985; Sarnari 1998).

The Dinghushan Biosphere Reserve (DHSBR, 112° 30'39"-112° 33'41" E, 23° 9'21"-23° 11'30" N) is located in Guangdong Province, southern China. As one of three Chinese reserves selected in 1980 to join the Biosphere Reserve Network of UNESCO, DHSBR has rich biological diversity and ecological importance. The region is subjected to subtropical monsoon climate with high Russulaceae species richness (Bi *et al.*, 1986; Zheng *et al.*, 1985). During the thorough exploration of Russulaceae carried out in DHSBR since 2014 by the authors, six new species of *Russula* (Das *et al.*, 2017; Zhang *et al.*, 2017; Song *et al.*, 2018), two new species of *Lactarius* (Wang *et al.*, 2018) and three new species and one new variety of *Lactifluus* (Song *et al.*, 2018, 2017; Zhang *et al.*, 2016) have been published. In this study, *R. gelatinosa* and *R. rufobasalis*, two additional novel species of *Russula* sect. *Ingratae*) are proposed based on the specimens collected during the survey. The successive reports of new taxa in Russulaceae from DHSBR point to the rich undiscovered species diversity in this subtropical area. In addition, *R. punctipes* is redescribed here based on the specimen collected from the same Biosphere Reserve, which is the first report of the collection of this species that was originally published nearly a century ago from Hunan Province (Singer 1935).

MATERIALS AND METHODS

Sampling and morphological studies

Specimens were collected during macrofungal survey in the Dinghushan Biosphere Reserve (DHSBR, 112° 30'39"-112° 33'41" E, 23° 9'21"-23° 11'30" N), Guangdong Province, southern China. Ectomycorrhiza-forming plants, distributed in the area, include trees from such diverse families as Fabaceae, Phyllanthaceae, Fagaceae, Pinaceae, Cupressaceae, Juglandaceae, Tiliaceae, etc. Detailed field notes of macroscopic features were recorded and fresh basidiomata were photographed using a digital camera. 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 test color changes to iron sulfate on fresh specimens. Specimens were air dried at 50-60°C in an oven and deposited in the Herbarium of Microbiology Institute of Guangdong (GDGM).

Microscopic characters were observed under a Nikon Eclipse Ni-U microscope from hand-sectioned dried materials. Basidiospores were examined in Melzer's reagent and measured in side view, excluding ornamentations. The other micromorphological structures (basidia, hymenial cystidia, dermatocystidia, elements of pileipellis and stiptipellis) were observed from hand-sectioned dried tissues rehydrated in 5% KOH solution and then mounted with 1% aqueous Congo red reagent. All tissues were also examined for the presence of ortho- or meta- chromatic

contents or incrustations in cresyl blue (Buyck 1989). Sulphovanillin (SV) was used to test color reactions of cystidia. Scanning electron images of basidiospores were captured with a Field Emission Scanning Electronic Microscope (JSM-6330F). Descriptive terminology followed Vellinga (1988). Color designation refers to HTML Color Codes (<http://www.htmlcolorcode.org/>). 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. The infrageneric classification follows the latest phylogeny published for the genus (Buyck *et al.* 2018), thus abandoning *Ingratula* as a separate subgenus.

DNA extraction, PCR, sequencing and phylogenetic analysis

Genomic DNA was extracted from fresh basidiomata with CTAB method described by Doyle & Doyle (1987). ITS region was amplified using primer pair ITS1 and ITS4 (White *et al.*, 1990) by a MyGene L Series Peltier thermal cycler, with the following program: a hot start of 5 min at 94 °C 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 tested in 1% agarose gel stained with GelStain T155 dye (TransGen Biotech, China), purified using E.Z.N.A Gel Extraction Kit (OMEGA), and then sequenced on an ABI3730xl DNA Analyzer (IGE, Guangzhou, China) using primers same to PCR. The newly generated sequences were deposited in GenBank (www.ncbi.nlm.nih.gov).

Blast query of newly generated ITS sequences in GenBank showed that the examined specimens were all assigned to *Russula*. Both Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses were performed based on ITS matrix. Maximum parsimony analysis was performed using PAUP* 4.0b10 (Swofford 2002). Parsimony trees were inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions, with all characters equally weighted and all gaps treated as missing data. Tree length [TL], consistency index [CI], retention index [RI], rescaled consistency index [RC] and homoplasy index [HI] were calculated. The robustness of the most parsimonious trees was evaluated by 1,000 bootstrap replications. Maximum Likelihood (ML) analysis was performed using RAxML 7.2.6 (Stamatakis 2006). ITS matrix was aligned by MAFFT v 7 (Katoh & Standley 2013) with the default settings, 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 GTRGAMMA model. All parameters in RAxML analysis were kept at default, bootstrap value (BS) exceeding or equal to 70% in ML analysis and 50% in MP were considered significantly supported.

RESULTS AND DISCUSSION

Molecular phylogeny

A dataset composed of 44 ITS sequences of *Russula* was used in MP phylogenetic analysis. *Russula densifolia*, *R. nigricans* in subg. *Compactae*, *R. archaea*, *R. camarophylla* in subg. *Archaea* and *R. emetica*, *R. lepida* in subg. *Russula* were chosen as outgroups. The final alignment comprised 763 characters including gaps. The resulting MP phylogram is shown in Fig. 1. The ITS alignment used in ML analysis (Fig. 2.) consisted of 54 sequences with 730 characters including gaps. *R. cyanoxantha*, *R. verrucospora*, *R. shingbaensis* in subg. *Heterophyllidia* sect. *Heterophyllae* and *R. crassotunicata* in subg. *Crassotunicata* were chosen as

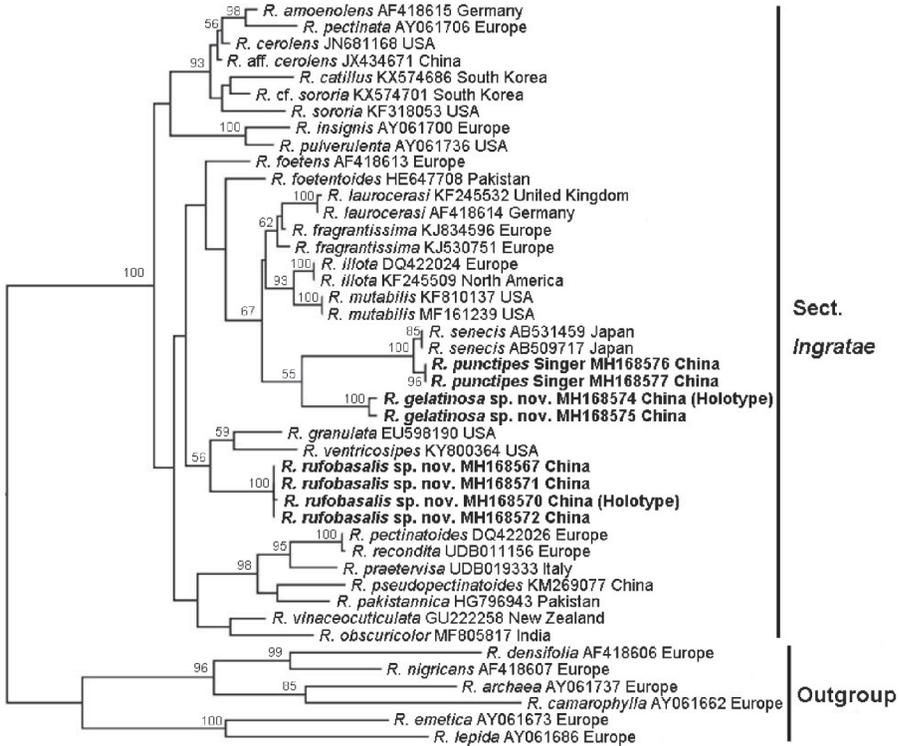


Fig. 1. Maximum parsimony (MP) phylogram of *Russula* sect. *Ingratae* generated from ITS sequences with PAUP. Data were analyzed with random addition sequence, unweighted parsimony and treating gaps as missing data. Six *Russula* species from other subgenera were used as outgroups. Bootstrap values higher than 50% are shown.

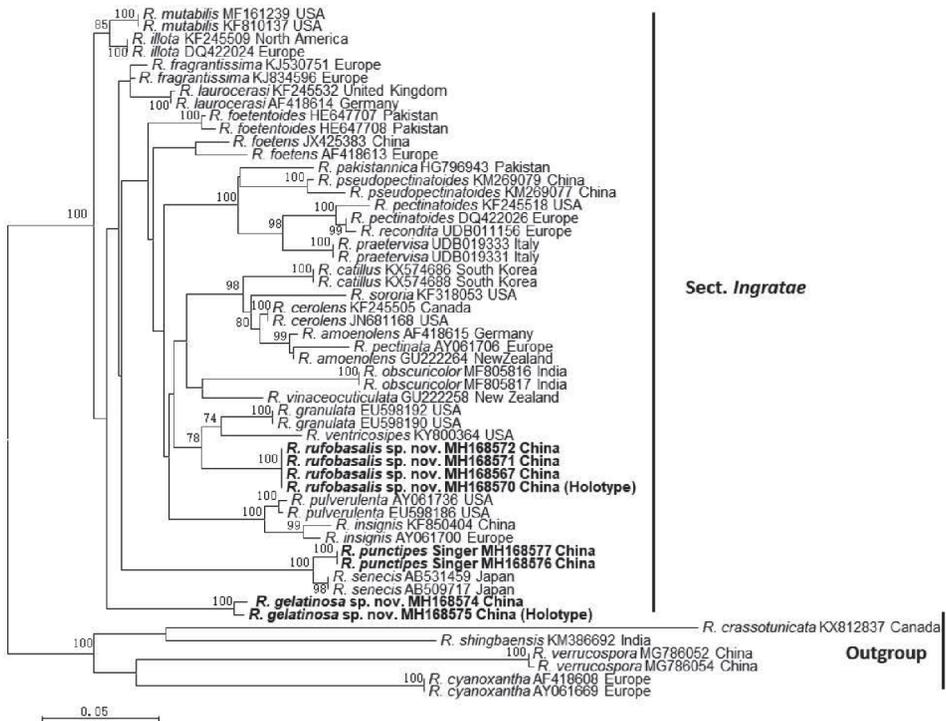


Fig. 2. Phylogram generated from Maximum Likelihood (ML) method based on rDNA ITS sequences performed by RAxML. One thousand bootstrap replicates followed by a heuristic ML tree search was analyzed. Bootstrap values higher than 70% are shown above or below the branch. The three discussed species are shown in bold.

outgroups. MP analysis of rDNA ITS sequences places *R. gelatinosa* in a significantly supported clade (MP BS = 67%) together with the Asian *R. senecis*, *R. punctipes* and four European species: *R. fragrantissima*, *R. mutabilis*, *R. illota* and *R. laurocerasi*. *R. rufobasalis* sp. nov. forms a distinct clade (MP BS = 56%) together with the North American *R. granulata* and *R. ventricosipes*, which is also confirmed in ML phylogram (ML BS = 78%).

Taxonomy

Russula gelatinosa Y. Song & L. H. Qiu sp. nov.

Figs. 3-4

Mycobank: MB 824893

Systematic position: Basidiomycota, Agaricomycetes, Russulales, Russulaceae, *Russula* subg. *Heterophyllidia*, sect. *Ingratae*.

Etymology: Referring to the gelatinized pileus.

Diagnosis: resembles *R. punctipes* in terms of general stature and basidiospores ornamented with up to 3.5 μ m high wings and crests, but differs in the suprapellis structure, being more palisadic rather than pseudoparenchymatic, and

composed of short, more or less parallel chains of inflated to globular subterminal and terminal elements.

Holotype: CHINA. Guangdong Province, Zhaoqing City, Dinghu Mountain, on the ground in broad-leaf forest, 26 May 2015, J. W. Li K15052626 (GDGM 71806, *holotype*).

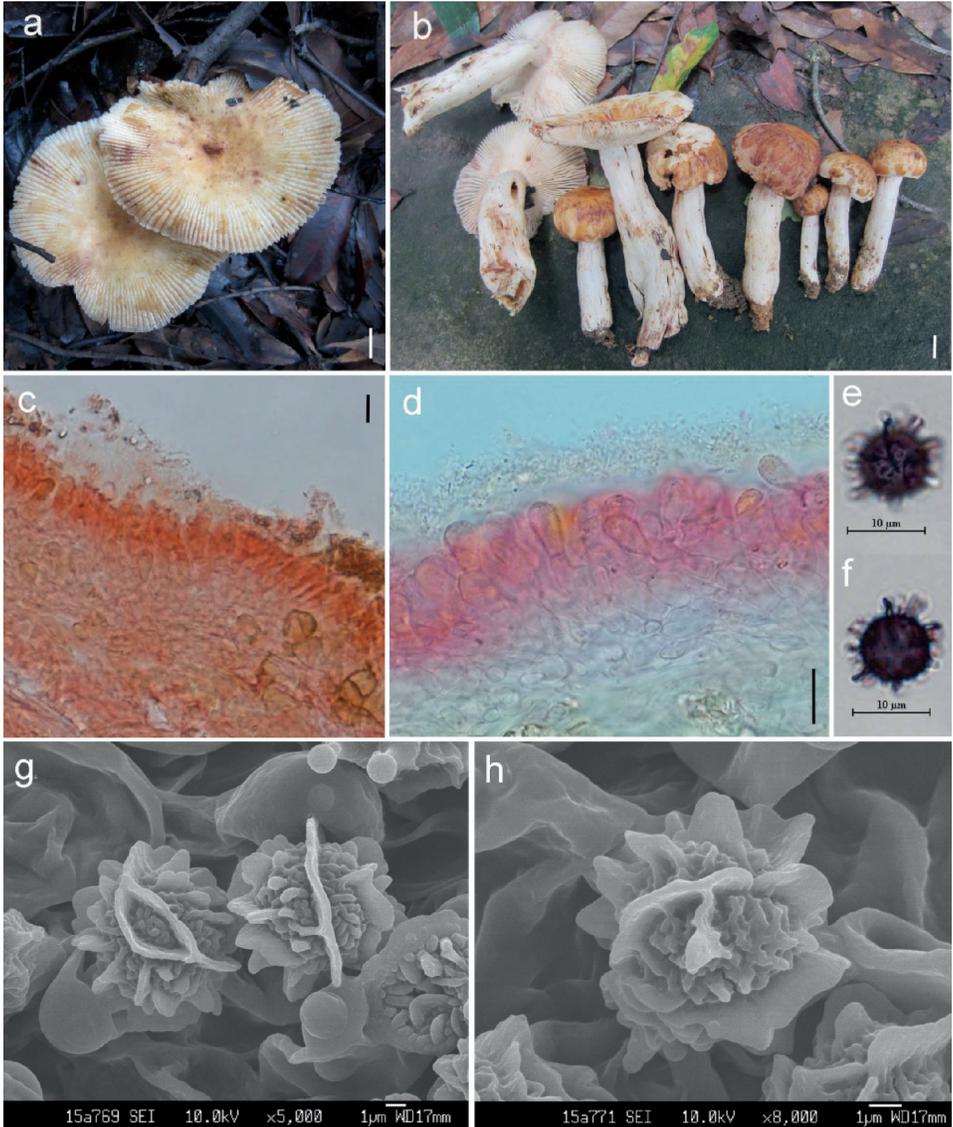


Fig. 3. *Russula gelatinosa* (GDGM 71806, holotype). **a-b**. Photos of basidiomata; **c-d**. Pileipellis and terminal elements in Congo Red reagent; **e-f**. Basidiospores in Melzer's reagent; **g-h**. Scanning Electron Micrographs of basidiospores. Scale bars: a-b = 1 cm; c-f = 10 μm; g-h = 1 μm.

Basidiomata medium sized. *Pileus* 4-8 cm in diam., hemispherical when young, convex to applanate with a depressed center when mature, slightly cracked with age; surface gelatinized, reddish brown (#BC8463) at first, ochre to brown tinged (#BAB3A3) when mature, dark brown (#AE95AB) when old, glabrous, dry, not viscid when wet; margin acute, tuberculate-striate, inward when young, becoming cracked with age. *Lamellae* adnate, even, smooth, rarely forked, interveined, 3-4 mm in height, whitish with reddish hue (#D0C6C4), unchanging when bruised; edge entire, darker than lamellae surface or slightly tinged purplish (#B28C89); lamellulae rare. *Stipe* 7-9.5 × 1.2-1.7 cm, cylindrical, slightly attenuating upwards when mature, central, solid at first, becoming hollow with age; surface dry, white with reddish brown tinge (#C9ABA1), longitudinally rugulose. *Context* whitish, 3-5 mm thick, not changing when bruised. *Taste* mild. *Odour* indistinct. *Spore print* pale cream.

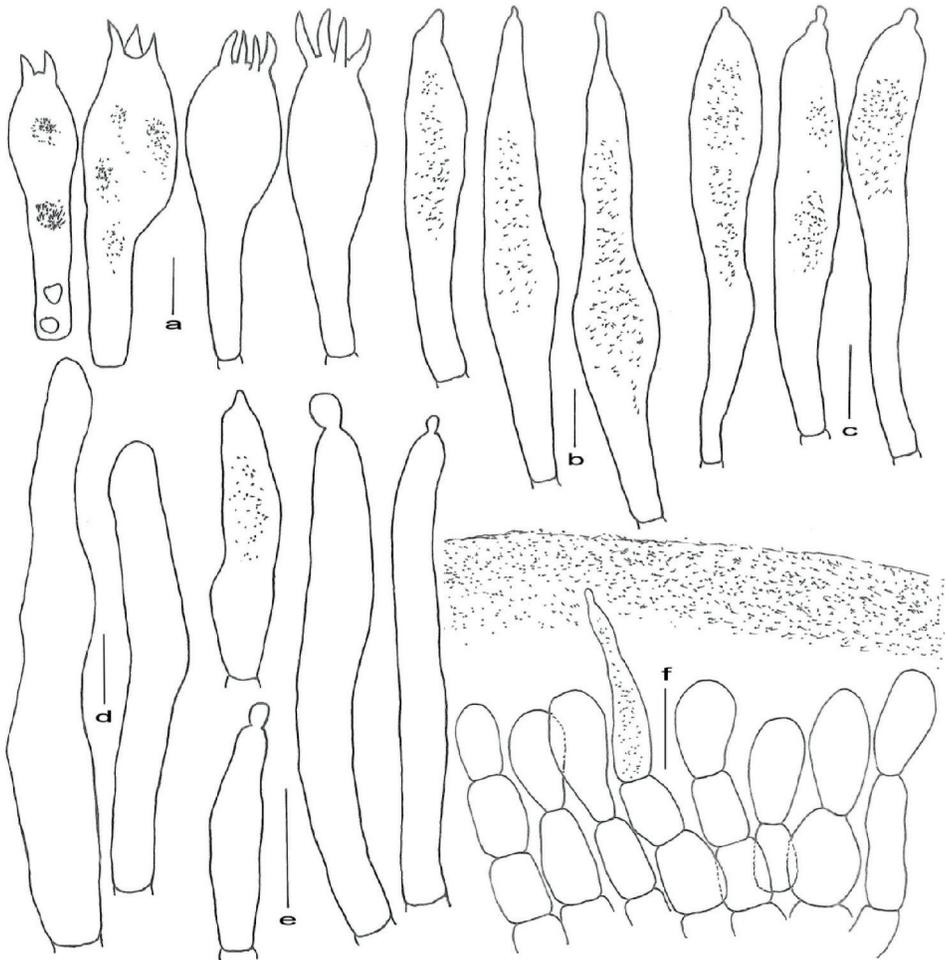


Fig. 4. *Russula gelatinosa* (GDGM 71806, holotype). a. Basidia; b. Pleurocystidia; c. Cheilocystidia; d. Caulocystidia; e. Pileocystidia; f. Pileipellis. Scale bars = 10 μ m.

Basidiospores globose to subglobose and quite large, [40/2/2] (7.6) 8.2-9.0-9.8 × (7.0) 7.5-8.4-9.1 (9.5) μm, Q = (1.03) 1.05-1.06-1.12 (1.14), hyaline in 5% KOH; ornamentation strongly amyloid, composed of up to 3.5 μm high wings running over more or less long distances on the spore surface or even nearly encircling the spores, mixed with shorter and often less high, laterally flattened, conical ornamentations (reminiscent of the bony plates on the back of Stegosaurus dinosaurs) and, in between these, the spore surface is covered with a dense, low network of short, blunt ridges and warts forming an incomplete network, intermixed with crowded, isolated warts and large spines, some spines partly connected; suprahilar spot inamyloid and indistinct. *Basidia* clavate, (44) 48-67.5 (69) × (10) 12-19 (20.5) μm, mostly 2- and 4-spored, rarely 3-spored, hyaline, some containing oil droplets when young; sterigmata 2.5-10.5 × 2-3.5 μm. *Subhymenium* pseudoparenchymatous. *Lamellar trama* mainly composed of sphaerocytes measuring up to 20 μm in diam. *Pleurocystidia* 60-75 × 13-20.5 μm, emergent up to 30 μm, fusoid to subclavate, with mucronate or papillate apices, thin-walled, rarely with refractive granules, becoming reddish brown in SV. *Cheilocystidia* 41.5-78 × 8-16 μm, clavate to subcylindrical, mostly with papillate or moniliform apices, thin-walled, some with refractive, granular contents. *Marginal cells* not differentiated. *Pileipellis* orthochromatic, divided into two layers; suprapellis an ixo-palisade (gelatin up to 12 μm), 80-100 μm thick, composed of ascending to erect, short-celled hyphal terminations, often with inflated subterminal cells; subpellis 100-130 μm deep, composed of horizontally oriented hyphae up to 5 μm wide, septate, thin-walled, hyaline or with rare granular contents; terminal cells clavate to cylindrical, mostly with rounded apices, 8.5-26 × 4-7 μm, some slightly refractive; subterminal cells subcylindrical to subglobose, measuring 7-17 × 4-8 μm. *Incrustations* absent. *Pileocystidia* abundant, changing to reddish brown in SV, fusoid to cylindrical in suprapellis, mostly 22.5-45.5 × 4-8.5 μm, one-celled, with mucronate or appendiculate apices, some with granular contents; cylindrical in subpellis, 5-10.5 μm wide, long, non-septate, with distinctly refractive, non-crystalline granules. *Stiptipellis* a cutis, orthochromatic in cresyl blue, composed of repent, thin-walled, septate hyphae up to 4 μm broad; terminal cells subcylindrical, rarely subulate. *Caulocystidia* frequent, fusoid to cylindrical, mostly 39.5-84.5 × 6.5-12 μm, with appendiculate or rounded apex, non-septate, with refractive contents, reddish brown in SV. *Stipe trama* composed of connective hyphae and nested sphaerocytes up to 30 μm in diam. *Clamp connections* absent in all tissues.

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

Additional specimens examined: CHINA, Guangdong Province, Zhaoqing City, Dinghu Mountain, on the ground in broad-leaf forest, 31 May 2016, J. W. Li K16053118 (GDGM 71807); J. W. Li K16053119 (GDGM 71808).

Commentary: *R. gelatinosa* was in all of our analyses suggested to be most closely related to a clade that is sometimes referred to (Das *et al.* 2009) as the “*R. laurocerasi* complex”. The latter clade is composed of species with nearly (sub) globose and relatively large spores that have (except for *R. mutabilis* Murrill) a well-developed spore ornamentation made up of more or less high ridges and crests (winged spores) with some typically running over long distances on the spore surface, exactly as in our new species. In Europe this group is principally represented by *R. laurocerasi* Melzer, *R. fragrantissima* Romagn. and *R. illota* Romagn., while in North America the same European names are used for still undescribed relatives, in addition to *R. mutabilis*. Apart from the misapplication of European names in America, there are also nomenclatural issues to be solved in this complex as there

is a growing cleavage between mycologists who use now *R. grata* Britzelm. as the current name for *R. laurocerasi*; even Mycobank and Species Fungorum have opposite views on the question. However, all these European and American species develop a distinct smell of bitter almonds, which is absent from *R. gelatinosa*. Our new species resembles in its general habit particularly three other Asian species: *R. punctipes* Singer (Singer 1936) described from Hunan prov. in China, *R. senecis* (either a later name for *R. punctipes* Singer or a valid name if the Japanese collections are sufficiently different) originally described from Japan (Imai 1938) and *R. tsokae* K. Das, Van de Putte & Buyck from the Indian Himalaya. All these Asian species lack the smell of bitter almonds, but all have the typically winged spores characteristic for most of the laurocerasi-clade. Deposited sequences in GenBank suggest that the *R. punctipes-senecis* complex is largely distributed in Asia (Khatua *et al.* 2015) and might represent a species complex or that there are at least distinct geographical populations (ITS sequence similarity variation range is 3%). *Russula tsokae* is possibly a close relative of our new species and shares the mild taste with it (both *R. senecis* and *R. punctipes* are strongly acrid), but it differs in the intense yellow color of the entire stipe surface and gill edges without darker punctuations. There are no ITS sequences available for *R. tsokae*, but preliminary multigene analyses of available protein coding genes for the type collection of *R. tsokae* suggest that it is unrelated to the acrid *R. punctipes* and *R. senecis* (not shown). A more in-depth study of the *R. punctipes-senecis* complex is necessary to establish the extent of variation for the various macro- and micromorphological features, such as, for example, the interstitial space between the high wings on the spores which is nearly smooth in *R. tsokae*, but strongly ornamented in *R. senecis* and *R. punctipes*, as well as in *R. gelatinosa*.

Russula punctipes Singer, *Annales Mycologici* 33 (5-6): 312 (1935) **Figs. 5-6**

Systematic position: Basidiomycota, Agaricomycetes, Russulales, Russulaceae, *Russula* subg. *Heterophyllidia*, sect. *Ingratae*.

Basidiomata small sized. *Pileus* 3-5 cm in diam., semispherical at first, becoming convex, applanate to plano-concave with a depressed center when mature; surface glabrous, viscid, ochraceous brown (#C47E64) when young, becoming brown (#ECB66E) with darker center (#B47645); margin acute, tuberculate striate, cracked with age. *Lamellae* whitish or buff (#BC8C68), adnate, 2-3 mm broad, unchanging after bruising, unequal, inter-veined; edge darker than lamellae surface, ochraceous brown, with warts, slightly waved; lamellulae frequent but not regularly polytomous. *Stipe* 35-55 × 6-12 mm, cylindrical, central, hollow, slightly curved; surface dry, slightly longitudinally rugulose, ceamy buff at first, becoming dull yellow to brownish (#E1C6AB) with age, with ochraceous brown to dark brown (#96674B) punctuations. *Context* 2-4 mm thick near stipe, whitish or buff, unchanging after bruising, brownish with FeSO₄. *Taste* acrid. *Odour* strongly fetid. *Spore print* whitish.

Basidiospores globose to broad ellipsoid, big, [60/3/2] (7.1) 7.9-9.3-10.3 (11) × (6.0) 7.2-8.7-10.1 (10.3) μm, Q = 1.00-1.06-1.17 (1.24), hyaline in 5% KOH; ornamentation strongly amyloid, composed of up to 3 μm high wings running over more or less long distances on the spore surface or even nearly encircling the spores, mixed with shorter and often less high, laterally flattened, conical ornamentations (reminiscent of the bony plates on the back of Stegosaurus dinosaurs) and, in between these, the spore surface is covered with a dense, low network of short, blunt ridges and warts forming an incomplete network, intermixed with crowded, isolated warts and large spines, some spines partly connected; suprahilar spot inamyloid and



Fig. 5. *Russula punctipes* (GDGM 71809, epitype). **a-c.** Photos of basidiomata; **d-f.** Pileipellis and inflated globular terminal elements in Congo Red reagent; **g-h.** Basidiospores in Melzer's reagent; **i-j.** Scanning Electron Micrographs of basidiospores. Scale bars: a-c = 1 cm; d-h = 10 μm; i-j = 1 μm.

indistinct. *Basidia* (32) $33.5\text{--}48.5$ (54) \times (10.5) $11\text{--}18.5$ μm , mostly 4-spored, rarely 2-spored, clavate, thin-walled; sterigmata $4\text{--}8 \times 1.5\text{--}3.5$ μm . *Subhymenium* pseudoparenchymatous. *Lamellar trama* mainly composed of sphaerocytes measuring up to 30 μm in diam. *Pleurocystidia* $39.5\text{--}76.5 \times 7\text{--}11$ μm , subcylindrical, with mucronate to moniliform apices, thin-walled, mostly with refractive granular contents that are restricted to the upper part of the cystidia, blackening in sulphovanillin (SV). *Cheilocystidia* $30.5\text{--}62.5 \times 9\text{--}18.5$ μm , fusoid to subcylindrical, often with rounded apices, thin-walled, some with rare refractive granules. *Marginal cells* not differentiated. *Pileipellis* orthochromatic, $140\text{--}200$ μm thick, divided into two layers: suprapellis pseudoparenchyma, mainly composed of erect hyphae with chains of inflated subterminal cells, and a subpellis composed of subparallel hyphae up to 5 μm in diam.; terminal cells clavate to subglobose, $9\text{--}23 \times 4.5\text{--}10$ μm ; subterminal cells mostly inflated to subglobose measuring $8.5\text{--}33 \times 5.5\text{--}30$ μm , rarely cylindrical. *Incrustations* absent. *Pileocystidia* abundant, blackening in SV, fusoid to cylindrical

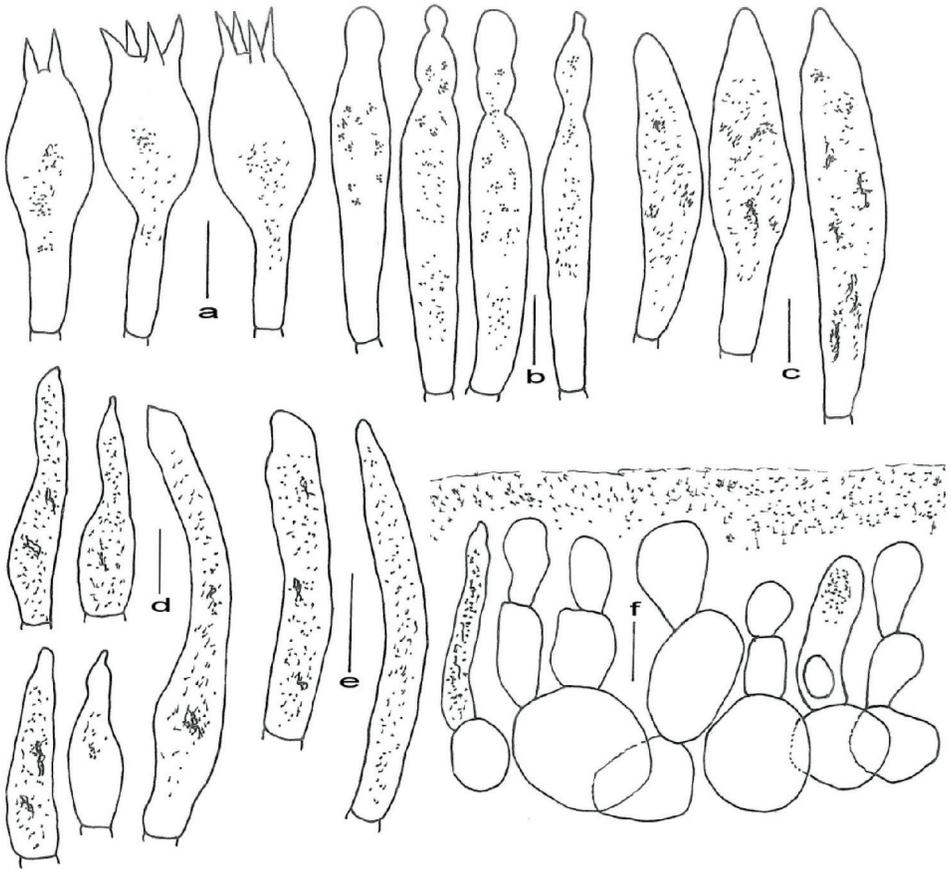


Fig. 6. *Russula punctipes* (GDGM 71809, epitype). a. Basidia; b. Pleurocystidia; c. Cheilocystidia; d. Pileocystidia; e. Caulocystidia; f. Pileipellis. Scale bars = 10 μm .

in suprapellis, mostly $28\text{--}113.5 \times 5.5\text{--}11 \mu\text{m}$, one-celled, with mucronate or appendiculate apices, some with heteromorphous refractive contents and oil droplets; cylindrical in subpellis, $4\text{--}12.5 \mu\text{m}$ wide, long, nonseptate, with distinct refractive granules. *Stipitipellis* a cutis, orthochromatic in cresyl blue, composed of repent to interwoven hyphae up to $4 \mu\text{m}$ in diam.; terminal cells subcylindrical, with rounded apices, measuring $10.5\text{--}33 \times 2\text{--}4 \mu\text{m}$. *Caulocystidia* frequent, fusoid to cylindrical, mostly $17.5\text{--}59.5 \times 4.5\text{--}7 \mu\text{m}$, with appendiculate or rounded apex, some septate, with heteromorphous refractive contents, becoming blackish in SV. *Stipe trama* composed of connective hyphae and nested sphaerocytes up to $35 \mu\text{m}$ in diam. *Clamp connections* absent in all tissues.

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

Specimens examined: CHINA, Guangdong Province, Zhaoqing City, Dinghu Mountain, on the ground in broad-leaf forest, 10 May 2016, J. W. Li K16051001 (GDGM 81124); *ibid.*, on the ground in mixed pine-broadleaf forest, 23 May 2017, J. W. Li H17052318 (GDGM 81123).

Commentary: The rediscovery of Singer's *R. punctipes* will solve part of the existing ambiguity and confusion among Asian Russulas. We assume the species has hitherto been identified as *R. senecis* in China, which appears indeed to be its sister-taxon from Japan (see Fig. 1). *Russula punctipes* principally differs from *R. gelatinosa* in its well-developed pseudoparenchyma on the cap surface, which is comparable to that of some *Virescentinae* of the *R. crustosa* Peck group. However, whereas *R. crustosa* has usually tapering terminal cells (Adamčík *et al.* 2018), Singer's species has mostly clavate terminal cells. The punctuation on the stipe surface may probably be quite variable as suggested by our collections, but our specimen (GDGM 71809) clearly shows this strong punctuation which Singer (1936) compared to the one observed on some *Hygrophorus*.

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

Figs. 7-8

Mycobank: MB 824892

Systematic position: Basidiomycota, Agaricomycetes, Russulales, Russulaceae, *Russula* subg. *Heterophyllidia*, sect. *Ingratae*.

Etymology: Referring to the reddish stipe base.

Diagnosis: *R. rufobasalis* is mainly characterized by its reddish stipe base, interveined lamellae and frequent lamellulae, cystidia (hymenial cystidia and dermatocystidia) blackening in SV, thick pileipellis composed of slender, tapering and often thick-walled, frequently undulate-sinuous or irregularly constricted terminal cells, stipe trama with scattered cystidioid hyphae becoming blackish brown in SV.

Holotype: CHINA. Guangdong Province, Zhaoqing City, Dinghushan Biosphere Reserve, on the ground in mixed pine-broadleaf forest, 22 May 2017, J. W. Li H17052204 (GDGM 71800, **holotype**)

Basidiomata small to medium sized. *Pileus* 3-6 cm in diam., semispherical when young, becoming applanate with a depressed center, then sub-infundibuliform with age; surface glabrous, dry, reddish brown (#AD713D) when young, becoming ochre (#BF956B) with darker center (#7A4E31); margin acute, waved, smooth when young, striate and cracked with age. *Lamellae* whitish or rusty tinged (#7F6A59), adnate to subdecurrent, 2-4 mm wide, unchanging after bruising, unequal with frequent, but irregularly inserted lamellulae, rarely forking near stipe, interveined, with even, concolorous edges. *Stipe* 22-35 \times 6-15 mm, cylindrical, central, solid at first, becoming spongy when mature; surface dry, longitudinally rugulose, whitish,

often with reddish brown tinge (#CFB4A1), reddish (#8B504A) at base. *Context* 2-4 mm thick near stipe, whitish, unchanging after bruising or with FeSO_4 . *Taste* mild. *Odour* indistinct. *Spore print* cream.

Basidiospores subglobose to broadly ellipsoid, small, [60/3/3] (5.7) 5.9-6.6-7.5 (7.7) \times (4.3) 4.8-5.4-6.0 (6.2) μm , $Q = 1.11$ -1.16-1.23 (1.25), hyaline in 5% KOH; ornamentation amyloid, composed of verrucose to subcylindrical warts 0.3-

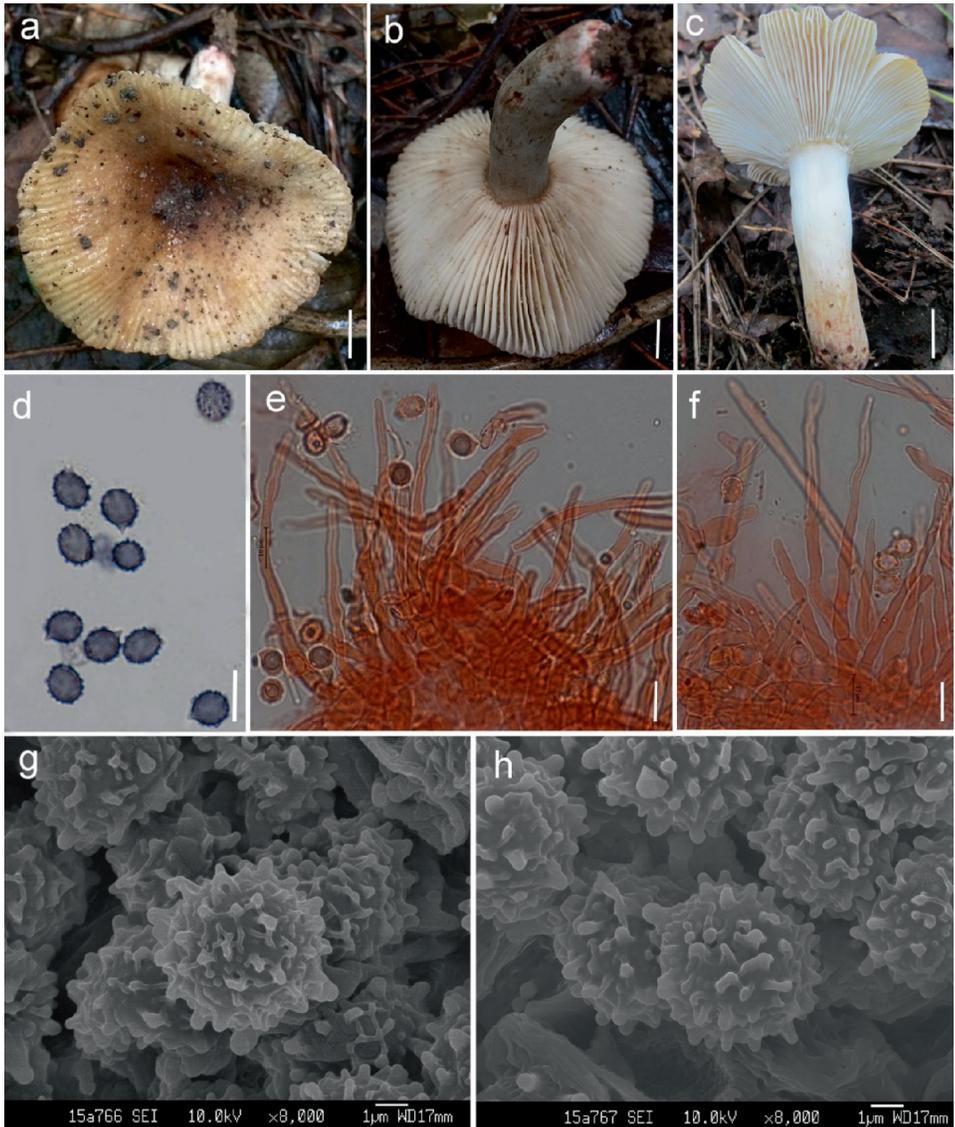


Fig. 7. *Russula rufobasalis* (GDGM 71800, holotype). **a-c.** Photos of basidiomata; **d.** Basidiospores in Melzer's reagent; **e-f.** Terminal elements of suprapellis in Congo Red reagent; **g-h.** Scanning Electron Micrographs of basidiospores. Scale bars: a-c = 1 cm; d-f = 10 μm ; g-h = 1 μm .

0.8 μm in height, often linked as small crests and ridges forming incomplete reticulum, intermixed with isolated warts; suprahilar spot distinct but not amyloid. *Basidia* (32.5) 35-48 \times 8-10.5 (11) μm , mostly 4-spored, rarely 2- and 3-spored, clavate to subcylindrical; sterigmata 3.4-7 \times 1.4-2.2 μm . *Subhymenium* pseudoparenchymatous. *Lamellar trama* composed of numerous sphaerocytes surrounded by connective hyphae; sphaerocytes up to 30 μm in diam. *Pleurocystidia* 39-74 \times 6.5-11.5 μm , fusoid to subcylindrical, with mucronate or appendiculate apices, thin-walled, mostly with refractive heteromorphous contents, blackening in sulphovanillin (SV). *Cheilocystidia* 38.5-67 \times 6.5-9.5 μm , clavate to subcylindrical,

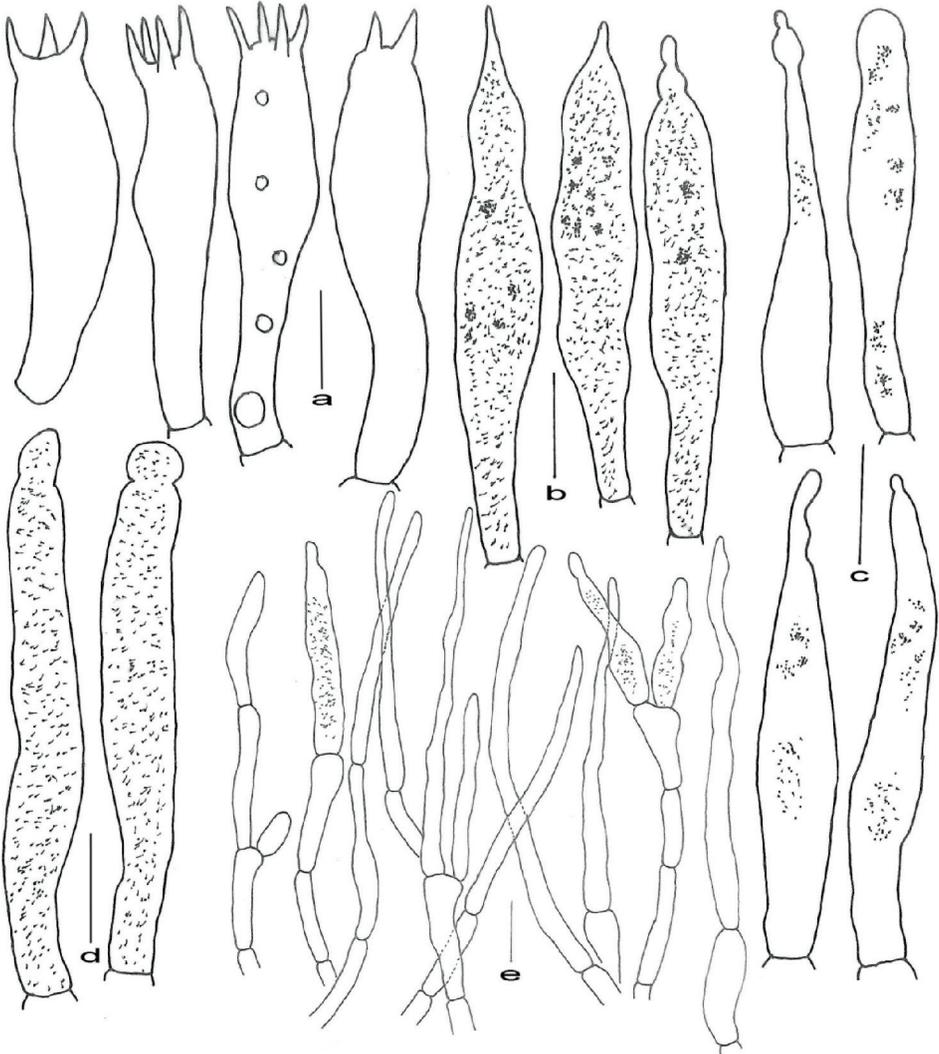


Fig. 8. *Russula rufobasalis* (GDGM 71800, holotype). a. Basidia; b. Pleurocystidia; c. Pileocystidia; d. Cheilocystidia; e. Pileipellis. Scale bars = 10 μm .

often with rounded apices, thin-walled, with distinct refractive contents. *Marginal cells* not differentiated. *Pileipellis* orthochromatic in cresyl blue, thick, 360-500 μm broad, two-layered: suprapellis a gelatinized trichoderm, 100-160 μm thick, mostly composed of ascending to erect hyphae; terminal cells 18-60 \times 2-5 μm , narrowly subcylindrical or tapering upward, often thick-walled, flexuous or irregularly constricted; subterminal cells usually shorter and wider than terminal cells; subpellis a loose cutis, 250-350 μm thick, composed of long, septate, equally wide hyphae up to 7 μm in diam. *Pileocystidia* one-celled, 27-48 \times 4-7 μm , subulate to cylindrical, with mucronate or capitate apices, with distinct, dense content, becoming blackish brown in SV, rarely present in subpellis and in lower pileus context. *Stipitipellis* a cutis, orthochromatic in cresyl blue, thin, 30-70 μm thick, composed of thin-walled, septate, cylindrical, hyaline hyphae up to 5 μm broad; terminal cells of stipitipellis hyphae subulate to broadcylindrical, with obtuse to tapered apex; caulocystidia rare, cylindrical to subclavate, 4-6 μm wide, contents blackish brown in SV. *Stipe trama* composed of sphaerocytes measuring 25-50 μm in diam., and scattered cystidioid hyphae up to 6 μm wide, becoming blackish brown in SV. *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, 6 June 2015, J. B. Zhang H15060622 (GDGM 71801); 20 June 2016, J. W. Li H16062035 (GDGM 71802); CHINA, Guangdong Province, Zhaoqing City, Dinghu Mountain, on the ground in broad-leaf forest, 12 September 2015, J. B. Zhang K15091235 (GDGM 8112871803); 14 September 2015, J. B. Zhang K15091420 (GDGM 71804); 8 May 2016, J.W. Li K16050813 (GDGM 71805).

Commentary: Our second new species, *R. rufobasalis*, received sufficient support to relate it to the American *R. ventricosipes* Peck and *R. granulata* Peck. The types of both these American species have recently been re-examined, and at least *R. ventricosipes* has very different spores (Adamčík *et al.* 2013); differences for *R. granulata* include the clear fragmentation of the suprapellis in appressed squamae, but otherwise both species are quite similar (Adamčík *et al.* 2018). However, *R. rufobasalis* differs from all other species with a reddish stipe base in its frequently thick-walled and irregularly constricted terminal cells in the pileipellis.

The red pigment near the stipe base of our new species might be indicative of relationships with other species groups in sect. *Ingratae*. At least *R. ventricosipes* may occasionally turn strongly red, even in other parts of the fruiting body, when exuding red droplets from the hymenium for example. A red stipe base, however, is also typical of some species in subsect. *Pectinatinae* Bon, some of which share with *R. rufobasalis* a similar overall coloration (see the European *R. praetervisa* Sarnari). In Asia, one of the most similar species probably includes *R. obscuricolor* K.Das, A. Ghosh & Buyck (in Das *et al.* 2017) which is morphologically extremely similar, sharing the same red stipe base and identical size and ornamentation of spores and even near-identical other microscopic features (except for the thick-walled terminal cells), leaving essentially its pungent-bitterish taste and salmon pink reaction to FeSO_4 as sole other differences apart from the darker pileus color. However, the general color in our species is very variable between different collections, although we never found it so dark as in *R. obscuricolor*. The phylogenetic analysis in Das *et al.* (2017) even suggests a very similar phylogenetic position for *R. obscuricolor*, i.e. close to *R. ventricosipes* Peck and *R. granulata* Peck. Our analyses place the same ITS sequences of *R. obscuricolor* closer to *R. vinaceocuticulata* from New

Zealand (another species with a vinaceous stipe base) but a reliable placement of all these species will need multigene approaches. We refer to Das *et al.* (2017) for a discussion of other, more or less similar Asian relatives.

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