# ORIGINAL RESEARCH



# The quest for a globally comprehensible *Russula* language

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# Abstract

Since 2007, the quality of *Russula* descriptions has improved and the use of molecular support for species delimitation and the number of published new species has increased. However, the description style is not consistent and has regional or author-specific patterns. Most recent publications still favour descriptions of spores compared to hymenium and pileipellis elements, and usually only the spore size is provided with statistical support. This study proposes standards for descriptions of the microscopic structure of *Russula* species (Russulaceae, Agaricomycetes). We present the description template, the template measurements table, the specific terminology and the essential chemical reagents. The proposed standards were tested by mycologists from 11 countries who met at the Russula Microscopy Workshop in Slovakia. Descriptions of 26 species from 9 countries and four continents were prepared, among them R. amarissima, R. castanopsidis, R. seperina and R. subtilis are re-described and 15 species are introduced as new: R. abietiphila, R. amerorecondita, R. aurantioflava, R. echidna, R. flavobrunnescens, R. fluvialis, R. fortunae, R. garyensis, R. gemmata, R. laevis, R. madrensis, R. olivaceohimalayensis, R. purpureogracilis, R. sancti-pauli and R. wielangtae. Seven descriptions for candidate new species are provided without a formal name assignment. Pairwise comparison of species described in this study with available similar descriptions of related species suggests that microscopic characters from all parts of the basidiomata can be equally important for species recognition and they deserve the same treatment including number of measurements and statistics. The majority of recent studies does not recognise differences between the pileus margin and centre, but more than one-third of the species described in this study show distinct differences between the pileus areas, emphasizing the importance to specify the origin of pileipellis observations. This study proved that there is frequently insufficient difference in the ITS barcode between closely related species and that it is necessary to use more genetic markers combined with ecological and geographical data.

Keywords Morphology standards · New species · Phylogeny · Species delimitation · Taxonomy

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# Introduction

*Russula* Pers. is a large genus of ectomycorrhizal (ECM) fungi distributed worldwide in all ecosystems wherever suitable host plants occur (Buyck et al. 2018; Looney et al. 2018). Looney et al. (2016) analysed available data in public databases and demonstrated that the *Russula* diversity contains more than one thousand molecular operational taxonomical units (MOTUs) in North America, Asia and Europe with some overlap (up to 22%) between continents. Buyck (2007) calculated 419 different *Russula* species described or reported in North America, but he estimated

that the real number for the subcontinent might be three or four times higher. The total number of known tropical African *Russula* species is about 200 taxa (species and varieties), which are all endemic to the continent (Buyck 1993, 1994a, 1997; Sanon et al. 2014).

A lack of knowledge about the distribution of Russula species (incomplete sampling, many underexplored areas) combined with insufficient data for species recognition (missing morphological and molecular data) impedes the description of Russula diversity. Increasing efforts in barcode sequencing have generated a relatively large global dataset of the internal transcribed spacer nrDNA (ITS) region (Looney et al. 2016). A fast-growing barcode data pool is the consequence of progress in molecular techniques, especially the implementation of next generation sequencing approaches (Nilsson et al. 2018). However, the fungal ITS barcode is often insufficient for delimitation in the case of closely related species and sequence identity cut-offs can vary from 98 to 99.5% for species in different infrageneric groups in Russula (Adamčík et al. 2016a; Bazzicalupo et al. 2017). Because there is often an overlap 'in, within and between' species distances (Ryberg 2015), species delimitation should not rely on comparisons of barcode sequences alone. Unfortunately, there is a limited number of alternative marker sequences available. This was demonstrated by the recent comprehensive multi-locus phylogenies of the genus (Looney et al. 2016; Buyck et al. 2018) that are based largely on sequences generated by the authors of these publications and for this purpose. It is clear that besides molecular data, at least in the next decade, morphology will be an essential tool for species recognition in the absence of verified barcode sequences, and also because currently adapted species concepts in up-to-date taxonomy are integrating as many characters as possible (De Crop et al. 2017).

Morphology has not been only relevant to support species concepts but is also important for field research. Although often only the classification into larger infrageneric units is possible, there are also species that might be identified by macro-morphological characters (combined with ecology and particularly host tree association) (Bazzicalupo et al. 2017). Morphology may also help to revive the concept of older species names since the sequencing of old *Russula* types (older than 50 years) has a low success rate (Looney 2015). Even with the prospect of advancing next generation sequencing techniques (Bakker 2017), morphology remains essential to understand species with old names that have no authentic material available and only have an illustration or a description.

Adamčík et al. (2018) presented modern microscopic studies for 28 type specimens of North American *Russula* species described by C.H. Peck (published from 1873 to 1913). Based on this study, eight species changed subgenus

compared to their latest classifications, yet, in several cases the recent study was preceded by several earlier studies of the same types. The need for revised and actual type studies is evident because of (i) previous descriptions with an incomplete set of morphological characters; (ii) various misunderstandings and misinterpretations; (iii) inconsistent terminology; (iv) insufficient statistical support for numerical measurements; (v) outdated techniques; (vi) misuse of chemical reagents; and (vii) inadequate quality of illustrations.

The goal of this study is to unite Russula experts worldwide to agree on using a standard description of micro-morphological characters and to define minimal requirements for an up-to-date description. The aim is to provide description templates in the form of common measurement tables and to test their efficacy by individual investigators on new or interesting Russula species collected in various continents and belonging to different taxonomic groups. We further place these morphological results in a phylogentic context by conducting molecular analyses using ITS and other markers to position these specimens examined for morphological study into lineages consistent with current molecular-based understanding of the genus. We also compare the quality of descriptions published during the last decade and, based on our descriptions, evaluate the importance of individual characters for species delimitation.

# Materials and methods

The "Quest for a globally comprehensible Russula language" started as a ResearchGate (https://www.research gate.net/project/Quest-for-a-globally-comprehensible-Rus sula-language) project initiative aiming to define standards for full descriptions of new species or taxonomic revisions of published species in the genus Russula. To simplify and to boost discussion, the majority of authors of this publication, all working on Russula taxonomy, met at a Russula Microscopy Workshop organised by the Plant Science and Biodiversity Centre of the Slovak Academy of Sciences in Banská Bystrica, Slovakia from 23 to 28 February 2018. Participants prepared full descriptions of new Russula species for at least one species per participant, including line drawings and micro-photo documentation, all using a unified form and methodology for the microscopy. The daily workshop program (http://ibot.sav.sk/dipofungi-en/ project-team/events) consisted of thematic presentations ('technique', 'spore', 'pileipellis', 'hymenium' and 'illustration' days) followed by corresponding microscopic practice.

#### Sampling and preliminary species identifications

The selected *Russula* species originate from Finland, Germany, India, Mexico, Panama, Pakistan, South Korea, Tasmania (Australia), Thailand and the USA. Based on morphological arguments and a BLAST search of ITS sequences, classifications of species were proposed and subjected to new-species hypothesis testing. Among the so far undescribed species, only ones with two or more collections available for morphological study are formally described as new to science.

# **Material sequencing**

DNA extractions were performed using the various protocols of the home laboratories. All sequences generated in this study are listed in the Electronic Supplementary Table S1.

We amplified three DNA regions: (1) the ITS of the ribosomal DNA, (2) partial mitochondrial small subunit ribosomal DNA (mtSSU), and (3) the region between domains six and seven of the nuclear gene encoding the second largest subunit of RNA polymerase II (*rpb2*). The ITS region was amplified using the primers ITS1F–ITS4 (White et al. 1990; Gardes and Bruns 1993). The mtSSU region was amplified using the primer pair MS1 and MS2 (White et al. 1990). For the amplification of *rpb2* we used, depending on the group, forward primers b6f or A-Russ-F and the reverse primer frpb2-7CR (Matheny 2005; Caboň et al. 2017).

# **Phylogenetic analysis**

Sequences were edited in the BioEdit Sequence Alignment Editor version 7.2.5 (Hall 2013) or Geneious version R10 (Kearse et al. 2012). Intra-individual polymorphic sites having more than one signal were marked with NC-IUPAC ambiguity codes. Final datasets were aligned by MAFFT version 7 using the strategy E-INS-i (Katoh and Standley 2013) and further edited in AliView version 1.17.1 (Larsson 2014). All single-locus datasets were concatenated into one multi-loci dataset using SeaView version 4.5.1 (Gouy et al. 2010).

Our multi-locus sequence dataset was aligned to the *Russula* dataset published by Looney et al. (2016) that includes also LSU nrDNA and *rpb1* regions. The *R.* subg. *Russula* was supported by sequences from Caboň et al. (2017). The multi-locus analysis was used to show the position of the described species within the genus.

To show relationships at the lower rank, individual subtrees were prepared based on ITS sequences only. The ITS trees are based on our sequences supplemented by sequences retrieved from GenBank (https://www.ncbi.nlm. nih.gov/genbank) and UNITE (https://unite.ut.ee) databases. The best-matching sequences based on sequence identity were aligned, trimmed and clustered by the Maximum Likelihood method (ML) by Geneious version R10 software using default settings for both Geneious automatic alignment and ML procedure. The resulting alignments were supported by sequences of types or representative samples of morphologically similar and already described species. The representative samples for some described and well-known species were selected by species hypothesis from UNITE. Outgroups were selected based on the position of studied species in our multi-locus analyses. Original identifications are adopted for sequences retrieved from public databases, but if such names are in apparent conflict with known species concept, we used quotation marks.

All alignments have been deposited at TreeBASE (no. 24226). The multi-locus dataset was analysed using two different methods: Bayesian Inference (BI) and Maximum Likelihood (ML). For ML analysis, the concatenated alignment was loaded as a PHYLIP file into the RAxML GUI version 1.2 (Silvestro and Michalak 2012) and analysed as a partitioned dataset under the GAMMA + I + Gmodel with bootstrap iterations set to autoMR. For BI, the multi-locus dataset was divided into partitions: ITS, LSU, mtSSU, and the 1st, 2nd and 3rd codon positions of rpb1 and rpb2. The best substitution model for each partition was computed jointly in PartitionFinder v.1.1.1 (Lanfear et al. 2012). BI was computed in MrBayes version 3.2.6 (Ronquist et al. 2012) with four Markov chain Monte Carlo (MCMC) chains for 10,000,000 iterations until the standard deviation of split frequencies reached below the 0.01 threshold. The convergence of runs was visually assessed. The bootstrap values  $ML \ge 75$  and posterior probability BI > 0.95 are treated as well-supported.

In all trees, samples published in this study are labelled in bold (Electronic Supplementary Table S1) and types are labelled in red. Samples matching the concept of species described in this study are highlighted by dark backgrounds. Names of taxa retrieved from public databases are in their original forms, possible incorrect identifications are in quotation marks. Plant hosts are provided (if available) for environmental (abbreviated as E) samples from ectomycorrhizal roots (as ECM), orchid or mycoheterotrophs (as root) and endophytes (as leaf). Countries are abbreviated by ISO codes, US and Canadian states by postal codes (in parentheses).

#### Morphological descriptions

Field observations were recorded by individual authors before the meeting. Field characters were described using a common template and unified terminology and the information is arranged according to the list of characters in the Electronic Supplementary Table S2.

Most observations and descriptions were prepared during the Russula Microscopy Workshop. Only the authors describing the Pakistani species (Munazza Kiran, Malka Saba and Abdul Nasir Khalid) did not join the workshop and they prepared their descriptions based on communication with other authors. The microscopy room of the workshop had seven Carl Zeiss Axioscope microscopes and two Olympus BX43 and CX41 microscopes, with AxioCamERc5 s or Promicam digital cameras with connection to measuring software AxioVision SE64 or Quick Photo Micro version 3.1. Drawings were prepared using drawings attachments U-DA mounted on Olympus microscopes at a projection scale of  $\times$  2000. Spore illustrations were prepared either as drawings based on printed photos of spores or stacked photos. Spores were measured from spore prints where possible; if no spore print was available, spores were released from dried lamellae by placing lamellae fragments in 0.5 ml of water in 2 ml tubes, mixing for ca. 20 s and gently squashing with a pestle. The stacked photos (Electronic Supplementary Fig. S3) were prepared from spore prints in Melzer's reagent; approximately 20 photos taken in a different focus were stacked using the CombineZ software (https://combinezp.software. informer.com). SEM photos of spores of some species are also provided (Electronic Supplementary Fig. S4).

The Q value is used to indicate the length/width ratio of the spores. Spore measurements exclude ornamentation and apiculus. The spore ornamentation density is estimated following Adamčík and Marhold (2000). Figure 1 gives an example of how the spore ornamentation was measured and described. The hymenial cystidium density estimates follow Buyck (1991), see Fig. 2. After ca. 10 s in aqueous 10% KOH solution to dissolve the gelatinous matrix and facilitate tissue dissociation, the shape and size of elements and contents of hymenial cystidia and pileocystidia were observed and illustrated in ammoniacal Congo Red. The contents of some pileocystidia are indicated schematically (cross-hatched). Tissues were mounted in Cresyl Blue (Buyck 1989), sulfovanillin (Caboň et al. 2017) and treated with carbolfuchsin (Romagnesi 1967) to observe the presence and colour changes of incrustations and cystidium contents. Composition and use of reagents is further explained in Table 1. Hyphal terminations and pileocystidia were observed both near the pileus margin and at the pileus centre.

Statistics for most of the microscopic characteristics were based on 30 measurements per specimen, using three specimens when available, and are expressed as the mean  $\pm$  standard deviation with extreme values in parentheses. Spores were measured to an accuracy of 0.1 µm using the camera and the computer software. All other elements were measured using an eyepiece micrometer with an accuracy of 0.5 µm. Statistics for some elements that were difficult to observe (e.g. cystidia) are based on 20 measurements. The measurements were recorded in the template table (Electronic Supplementary Table S5). Every Russula description consists of the same micro-morphological characters arranged in defined order according to the description template presented in the Table 2. More details about observed characters are in Electronic Supplementary Table S6.

The terminology and scientific terms follow widely used mycological glossaries (Kirk et al. 2008; Vellinga 1988). Because of the inconsistent use of some terms in the



Fig. 1 Example of spore ornamentation counting in a 3  $\mu$ m diam. circle. **a** *Russula flavobrunnescens* sp. nova (TLXM *AK 5024*, holotype)—ornamentation of large, distant to moderately distant amyloid warts or spines, mainly isolated, occasionally fused in pairs or triplets, rarely connected by fine line connections. **b** *Russula madrensis* sp. nova (TLXM *AK 3184*)—ornamentation of relatively small, dense to very dense amyloid warts, subreticulate, rarely

isolated, fused in short or long, branched chains, connected by frequent, short, fine line connections. **c** *Russula fortunae* sp. nova (PMA *Corrales 180*, holotype)—ornamentation of relatively small, moderately distant to dense amyloid warts, reticulated and without isolated elements, fused in usually long chains, connected by short, frequent, fine line connections



Fig. 2 Example of hymenial cystidia counting. a *Russula fortunae* sp. nova (PMA *Corrales 180*, holotype)—hymenial cystidia moderately numerous to numerous. b *Russula garyensis* sp. nova (F PGA17-008, holotype)—hymenial cysdidia dispersed

Table 1 List of reagents and tissue preparations used for micro-morphological observations

Reagent	Composition/manner of use	Purpose of use	References
Carbolfuchsin	5 g phenol + 84 ml $H_2O$ + 1 g fuchsin + 10 ml ethanol/stained with carbolfuchsin, washed and observed in distilled water after incubation for a few seconds in a 10% solution of HCl	Incrustations on primordial hyphae	Romagnesi (1967)
Congo Red	1 ml 25% NH <sub>3</sub> dissolved in filtrated solution of 1.5 g Congo Red and 50 ml H <sub>2</sub> O/used after short treatment in 10% KOH solution	Contrast improvement of elements in hymenium and pileipellis	Heilmann- Clausen et al. (1998)
Cresyl Blue	2 ml Cresyl Blue + 1.3 ml glycerin + 2 ml ethanol + 4.2 ml H <sub>2</sub> O/used dirently	Presence of metachromatic incrustations in pileipellis	Buyck (1989)
Melzeŕs reagent	1.5 g I + 5 g KI + 100 ml $\mathrm{C_2H_3Cl_3O_2}$ + 100 ml H_2O/used directly	Colouring of spore ornamentation	Melzer and Zvára (1927)
Sulfovanillin	1 g of vanillin dissolved in 6 ml $\rm H_2O$ and 5 ml concentrated $\rm H_2SO_4,\!/used$ directly, observed after 5 min and 30 min staining	Colouring of cystidia contents	Caboň et al. (2017)

literature, we list and explain some specific terms used in *Russula* descriptions (Table 3).

# **Character analysis**

The quality of *Russula* microscopic descriptions was estimated based on information included in new *Russula* descriptions from January 2007 to January 2019 (the past 12 years). We compared the presence of microscopic elements of pileus, lamellae and spores, use of statistical support for size (average values and a range estimated statistically), quality of pileipellis illustrations (if terminal elements are distinguishable), use of chemical reagents, measurements taken at pileus margin as well as the pileus centre and use of sequence data (Electronic Supplementary Table S7).

The importance of individual micro-morphological characters was analysed based on comparison of species

described in this study with related and morphologically similar species with available high-quality descriptions. Non-scaled heatmap analysis was plotted under default settings in RStudio version 1.1.463 (RStudio team 2016) using heatmap.2 function implemented in gplots package (Warnes et al. 2009).

# Results

# Phylogeny

Our multi-locus analysis of ITS, LSU, mtSSU, *rpb1* and *rpb2* regions (Fig. 3) with samples representing the whole of the genus *Russula* shows a similar topology to that presented by Looney et al. (2016) and Buyck et al. (2018). The eight major clades recognised by both studies were all well-supported and they correspond to subgenera *Russula* 

 Table 2 The description template used in this study

**Spores** (1) shape and Q value, (2) size; (3) shape of elements in the spore ornamentation, (4) their density [in a 3 μm circle], and (5) prominence (6) general appearance of spore ornamentation, (7) frequency of line connections and fusions [in a 3 μm circle]; (8) size, amyloidy and surface of the suprahilar spot

- **Basidia** (9) size, shape and number of sterigma; (10) estimated size and shape of basidiola. **Hymenial cystidia on lamellae sides** (11) density at 1 mm<sup>2</sup>, (12) size, (13) shape, terminations, presence and length of an appendage, emergence above basidium level, origin and thickness of walls, (14) contents observed in Congo Red and sulfovanillin; **cystidia on lamellae edges** (15) size and (16) relative differences [compared to lamellae sides]. **Lamellae edges** (17) presence and frequency of different cell types; **marginal cells** (18) size, (19) shape, contents and thickness of walls
- **Pileipellis** (20) colour reaction in Cresyl Blue, (21) delimitation from context, (22) depth, (23) distinction and delimitation of supra- and subpellis, (24) gelatinization and presence of extra gelatinous matter; (25) suprapellis depth and arrangement of hyphal terminations; (26) subpellis depth, structure and hyphal width. **Hyphal terminations** near the pileus margin (27) general aspect; (28) terminal cells size, (29) shape and general appearance of the terminal cells; (30) subterminal cells width and relative differences [compared to the terminal cells]. Hyphal terminations near the pileus centre (31) size of terminal cells and (32) relative differences in general aspect, terminal and subterminal cells shape [compared to the pileus margin]
- **Pileocystidia** in suprapellis near the pileus margin (33) number of cells, shape, insertion of basal part, thickness of cell walls and irregularities, (34) size of terminal cells, (35) their shape and terminations, (36) contents observed in Congo Red and sulfovanillin, (37) presence of acid-resistant incrustations and incrustations observed in Congo Red and sulfovanillin. Pileocystidia in suprapellis near the pileus centre (38) size of the terminal cells and (39) relative differences in general aspect and terminal cells shape [compared to the pileus margin]. Cystidioid or oleiferous hyphae (40) presence in subpellis and trama and contents

More details explaining character stages and observation styles are in Electronic Supplementary Table S6, numbering of characters (here in parenthesis) is consistent between both Tables and descriptions in the Taxonomy part

(two well-supported clades), *Malodora* Buyck & V. Hofst., *Compactae* (Fr.) Bon, *Archaea* Buyck & V. Hofst., *Heterophyllidia* Romagn., *Crassotunicata* Buyck & V. Hofst. and *Brevipes* Buyck & V. Hofst. Most studied species are placed in two subgenera, *Russula* and *Heterophyllidia*. Only two species are placed in subgenus *Compactae* and one in subgenus *Brevipes*.

# Subgenus Russula

All 18 studied species of the subgenus Russula are placed in one well-supported clade that corresponds to /russula in Looney et al. (2016). Within this clade, species are placed in several independent lineages. Eleven species are represented by at least two samples and they all received a significant support. The remaining seven species represented by a single sample in the multi-locus phylogeny, show long branch length and their delimitation is further supported by additional ITS-based analysis. Four species belong to the R. xerampelina lineage (that corresponds to Russula subsect. Xerampelinae Singer) and three species belong to the R. seperina lineage. All other species are placed as singles or pairs within different, mainly not directly related (not sister) lineages. For this reason, we present the close relationships of the species described here in 11 individual ITS trees.

# Russula castanopsidis lineage

Russula castanopsidis Hongo and R. purpureogracilis sp. nov. are both placed in a well-supported clade with a

long branch (Fig. 3), but their further position within the subgenus Russula is unresolved. In the ITS tree (Fig. 4), sequences within the 93% identity threshold from the BLAST search are all placed in this clade. Our South Korean collection of R. castanopsidis is clustered together with the Japanese samples and the strong statistical support proved that they represent a single species originally described from Japan. The Indian species R. darjeelingensis Paloi, K. Acharya & K. Das is sister to it. All Asian collections of the lineage form a well-supported clade together with samples from Florida (USA). This clade is sister to another well-supported clade of three species from New Zealand. The R. castanopsidis lineage certainly deserves a taxonomic rank (probably of subsection), and in our current tree at least eight species from three continents are represented, some not described vet.

# Russula seperina lineage

The three blackening species described in this study cluster in a well-supported clade (Fig. 3), but the change of the context colour upon bruising is a polyphyletic character. There are at least two more lineages with this character represented in our tree by *R. decolorans* (Fr.) Fr. (the type of the subsection *Decolorantes* Maire) and *R. claroflava* Grove. Another blackening species, *R. griseocarnosa* X. H. Wang et al. from China, is placed as sister to the *R. seperina* lineage (Fig. 5), but its position is not well-supported and it may represent a third independent *Russula* lineage with blackening context. *Russula* sp. 1 from

Table 3	Explanation	of selected	terms u	used for	descriptions	of	Russula in	1 this	study
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Terms	Explanation					
Acid-resistant incrustations	Incrustations that turn red in carbolfuchsin and retain the colour after being exposed to a weak acid for few seconds					
Acute apical part	Cell walls at the terminal part shaped in a sharp angle (narrowing tip)					
Appendage	Apical constriction of cystidium, resulting in a vermiform, capitulate, fusiform or moniliform 'appendage', which often easily breaks off and allows discharge of cystidial contents					
Chains in spore ornamentation	More than two warts or spines aligned very closely					
Cystidioid hyphae	Hyphae (or portions of hyphae) in subpellis or trama with cystidia-like heteromorphous contents					
Essential number of measurements	At least 20 measurements per specimen, optimum number 3 and more specimens measured per species					
Essential statistics	Length, width and for spores also ratio of length and width (Q value); always provide the average value and the range estimated as the average $\pm$ standard deviation					
Fusions in spore ornamentation	When two warts or spines adhere to each other (twinned elements)					
Heteromorphous contents	Contents of hyphae or pileocystidia, they may have granular, crystalline, banded components or refringent bodies, sometimes they are disconnected or limited to a part of the cystidium					
Hymenial cystidia	Sterile elements in hymenium defined by their contents or shape					
Hyphal terminations	Free terminations of hyphae that end in transition between suprapellis or subpellis; they can be represented by one or multiple cells					
Incrustations	Droplets, crystals or glutinous coatings on the surface of pileocystidia and hyphal terminations					
Line connections	Thin and low, amyloid lines that connect warts or spines of spore ornamentation					
Marginal cells	Sterile cells on lamellae edges that clearly differ from basidiola by their shape and size and have optically empty contents; mostly they are similar to hyphal endings of the pileipellis					
Metachromatic reaction	Colour change to blood red in Cresyl Blue, the negative reaction is orthochromatic					
Mucronate apical part	Having an abruptly projecting point					
Number of cells of pileocystidia	Number of cells that are separated by septa and have heteromorphous contents or a specific shape typical for pileocystidia					
Obtuse apical part	Rounded tips of hymenial elements					
Oleiferous hyphae	Hyphae in subpellis or trama with homogeneous, refractive, oily contents, sometimes pigmented					
Pileipellis	Cuticle on upper surface of pileus delimited from the pileus trama by its specific structure, often also with conspicuous pigmentations and gelatinisation					
Pileocystidia	Hyphal structures in pileipellis with specific contents or shape					
Primordial hyphae	Pileocystidia with acid-resistant incrustations staining red after carbolfuchsin treatment					
Ridges	Linear elements made up of aligned warts or spines that are interconnected by amyloid 'walls' of more than half their height					
Spines on spores	Elements of spore ornamentation with pointed (acute) tips					
Spore shape	Defined by ratio of length and width of spores					
Spore size	Length and width of spores excluding spore ornamentation					
Subpellis	Hyphal structure in pileipellis between trama and suprapellis; many species have a separable pileipellis under the suprapellis					
Subterminal cells	Single cell next to the terminal cell					
Suprahilar spot	Area above the hilum with nearly smooth or smooth surface that is in some species completely or partly amyloid					
Suprapellis	The upper part of pileipellis that is near the surface and is composed of hyphal terminations and usually also pileocystidia					
Terminal cells	Single hyphal cells at the terminal position in suprapellis					
Warts on spores	Elements of spore ornamentation with obtuse tips					
Wings	Same as ridges, but much higher (> 2 $\mu$ m)					

Thailand is sister to *R. olivaceohimalayensis* sp. nov. from India and the European *R. seperina* Dupain is probably not directly related to them. The sequences originating from root samples from China and Japan may represent another undescribed species, while the position of the sample from Czechia might be a result of its low sequence quality. The



**◄ Fig. 3** Phylogenetic ML tree of the genus *Russula* based on ITS, LSU, mtSSU, *rpb1* and *rpb2* regions. Samples in blue are published first in this study (Supplementary Table 1), types are in bold. Branches in bold are supported by ML ≥ 75 or BI ≥ 0.95; black—with full support (ML = 100, BI = 1.00), blue—supported by both ML and BI, grey—supported only by one analysis, asterisk (\*) indicates full support. Countries are abbreviated by ISO codes (https://www.iso.org/iso-3166-country-codes.html), US and Canadian states by postal codes (http://www.icq.eps.harvard.edu/ICQpost. htmlin parentheses)

*Russula seperina* lineage is the second so far unrecognised group that probably deserves a taxonomic rank.

#### Russula xerampelina lineage

The morphological classification of subsection *Xerampelinae* (fishy odour, context turning yellowish brown) proved to be consistent with the monophyletic group that



Fig. 3 continued



**Fig. 4** Phylogenetic tree of the *R. castanopsidis* lineage based on ITS region. Samples in blue are published first in this study (Supplementary Table 1), types are in bold. Samples matching concept of species described in this study are highlighted by shaded backgrounds. Names of taxa retrieved from public databases are in their original forms. Plant hosts are provided (if available) for samples from ectomycorrhizal roots (followed by 'ECM'), orchid or myco-heterothrophs (followed by 'root'). Countries are abbreviated by ISO codes (https://www.iso.org/iso-3166-country-codes.html), in parentheses are US and Canadian states by postal codes (http://www.icq.eps.harvard.edu/ ICQpost.html)

was obtained in the phylogenetic analysis (Fig. 3). The group received strong support and is sister to R. nitida (Pers.) Fr. While all four species described here are supported in the multi-locus phylogeny, the support in the ITS tree is less significant (Fig. 6). The general topology of the ITS tree corresponds to host preferences. Boreal and arctic species are placed at the basal position. A Salix-associated species, R. subrubens (J.E. Lange) Bon forms a clade with samples probably all associated with Quercus. The Fagusassociated R. faginea Romagn. forms a clade with species that are typically associated with conifers. The clustering of conifer-associated samples is consistent with geographical and morphological patterns, but there is low statistical support for most clusters; among the species described in this study only R. flavobrunnescens sp. nov. is well-supported. The Mexican R. madrensis sp. nov. with bright red pilei forms a clade with other samples from Mexico and Arizona (USA) that is sister to the European *R. xerampelina* (Schaeff.) Fr. with similar pileus colour. The clade of yellow species consists of the Mexican *R. flavobrunnescens* associated with *Pinus oocarpa* or *P. montezumae* and *R. katarinae* Adamčík & Buyck from the Appalachian Mts. in eastern USA associated with *P. strobus*. Collections with dull purple colours to the pileus cluster together showing further clustering of *R. sanctipauli* sp. nov. from Mexico and a cluster of western North American collections from California, Oregon, Washington in the USA and British Colombia in Canada. The *Quercus*-associated *R.* sp. 2 from Mexico is close to the European *R. graveolens* Romell. The support values within the *Quercus*-associated species in Europe and North America.

#### Russula cuprea lineage

Russula abietiphila sp. nov. received strong support and is related to R. cuprea Krombh. (Figure 3). The ITS analysis (Fig. 7) shows also strong support for the Korean R. abietiphila as a species associated probably exclusively with Abies, but its relationships within the lineage are not resolved. The European Quercus-associated species R. cuprea and R. juniperina Ubaldi are not closely related to the newly described species and form a well-supported clade. Our ITS tree shows 33 more samples that are very similar to R. abietiphila. Except for two samples from European boreal species, R. olivina Ruots. & Vauras and R. olivobrunnea Ruots. & Vauras, all other samples probably belong to undescribed species. Among the environmental samples, two clades of samples originating from China and Mexico are well-supported, but the clustering and geographical pattern suggest that this clade of closely related species may comprise at least seven species including R. abietiphila. The Russula cuprea lineage represents a well-defined group that may be classified as subsection Cupreinae Bon.

#### Russula globispora lineage

*Russula* sp. 3 is clustered with *R. maculata* Quél. & Roze, *R. globispora* (J. Blum) Bon and *R. dryadicola* Fellner & Landa, members of the well-supported (Fig. 3) subsection *Maculatinae* (Konrad & Josserand) Romagn. defined morphologically by brownish yellow spots on the surface of the basidiomata. Further clustering shows *R.* sp. 3 to be a member of the *R. globispora* lineage defined by large spores. The newly described species from South Korea is sister to *R. heilongjiangensis* G.J. Li & R.L. Zhao from China and it received strong support together with an orchid mycorrhiza sample from South Korea that is probably the same species (Fig. 8). Both Asian species are nested within mainly European samples of the *R.* 



**Fig. 5** Phylogenetic tree of *R. seperina* lineage based on ITS region. Samples in blue are published first in this study (Supplementary Table 1), types are in bold. Samples matching concept of species described in this study are highlighted by shaded backgrounds. Names of taxa retrieved from public databases are in their original forms. Plant hosts are provided (if available) for samples from ectomycorrhizal roots (followed by 'ECM'), orchid or myco-heterothrophs (followed by 'root'). Countries are abbreviated by ISO codes (https://www.iso.org/iso-3166-country-codes.html), in;parentheses are US states by postal codes (http://www.icq.eps.harvard.edu/ICQpost.html)

*globispora* lineage. The ITS tree suggests existence of three species from Europe and two from North America within the *R. globispora* lineage.

#### Russula 'tenuihyphata' lineage

The position of the single sequence of R. sp. 4 in our multilocus tree (Fig. 3) is not resolved and it shows a long branch. Based on clustering of sequences retrieved from GenBank, we identified the third *Russula* clade that has not been recognised so far (Fig. 9). This lineage does not contain a sequence of any described species and we call it *R. 'tenuihyphata'* lineage because of the narrow hyphae of our collection. *Russula* sp. 4 from South Korea is nested between probably two undescribed species represented by a clade of sequences from *Pyrola* roots from Japan and a clade of sequences from basidiomata collected in an *Oreomunnea mexicana* forest in Panama.

#### Russula lilacea lineage

Species with a white spore print and primordial hyphae known in former classifications as subgenus *Incrustatula* Romagn. (Romagnesi 1987) are clustered in a well-



**Fig. 6** Phylogenetic tree of *R. xerampelina* lineage based on ITS region. Samples in blue are published first in this study (Supplementary Table 1), types are in bold. Samples matching concept of species described in this study are highlighted by shaded backgrounds. Names of taxa retrieved from public databases are in their original forms. Plant hosts are provided (if available) for environmental (abbreviated as E) samples from ectomycorrhizal roots (followed by 'ECM'), orchid or myco-heterothrophs (followed by 'root') and endophytes (followed by 'leaf'). Countries are abbreviated by ISO codes (https://www.iso.org/iso-3166-country-codes.html), in parentheses US and Canadian states by postal codes (http://www.icq.eps.harvard.edu/ICQpost.html)

supported clade (Fig. 3). This clade consists of two wellsupported subclades that correspond to morphologically recognised subsections *Lilaceinae* (Melzer & Zvára) Jul. Schäff. and *Roseinae* Sarnari. Two species described in our study based on material from the USA are placed in the first subsection, together with the most common European species *R. lilacea* Quél. The ITS tree (Fig. 10) shows *Russula gemmata* sp. nov. placed in a well-supported clade together with two species from Asia and one probably undescribed species from the USA. *Russula subtilis* Peck is placed in a large clade containing another seven European species represented by sequences chosen based on the UNITE species hypothesis and probably two more North American species, one of which represents the current concept of *R. corallina* Burl. Our sequences from collections of Peck's species match perfectly with four other sequences retrieved from GenBank which suggests a wide distribution of the species along the Appalachian Mts. in North America.



**Fig. 7** Phylogenetic tree of *R. cuprea* lineage based on ITS region. Samples in blue are published first in this study (Supplementary Table 1), types is in bold. Samples matching concept of the species described in this study are highlighted by shaded background. Names of taxa retrieved from public databases are in their original forms. Plant hosts are provided (if available) for environmental (abbreviated as E) samples from ectomycorrhizal roots (followed by 'ECM'), orchid or myco-heterothrophs (followed by 'root'). Countries are abbreviated by ISO codes (https://www.iso.org/iso-3166-country-codes.html), in parentheses are US and Canadian states by postal codes (http://www.icq.eps.harvard.edu/ICQpost.html)

#### Russula aurea lineage

The Pakistani collections that are morphologically similar to the European *R. aurea* Pers. because of the yellow tints on the lamellae edges and stipes are grouped in a clade that is sister to the Australian *R. wielangtae* sp. nov. All of the nodes are well-supported (Fig. 3). The clade of European *R. lepida* Fr. and *R. amarissima* Romagn. & E.-J. Gilbert is sister to them, but with weak support. The ITS tree (Fig. 11) places *R. flavida* Peck as more related to the *R. aurata* lineage than to *R. wielangtae*. There is a strong support for grouping *R.* sp. 5 with a collection identified as *R. aurea* from China, but the Chinese collection more



**Fig. 8** Phylogenetic tree of *R. globispora* lineage based on ITS region. Samples in blue are published first in this study (Supplementary Table 1), types are in bold. Samples matching concept of species described in this study are highlighted by shaded background. Names of taxa retrieved from public databases are in their original forms. Plant hosts are provided (if available) for samples from ectomycorrhizal roots (followed by 'ECM'), orchid or myco-heterothrophs (followed by 'root'). Countries are abbreviated by ISO codes (https://www.iso.org/iso-3166-country-codes.html), in parentheses are US states by postal codes (http://www.icq.eps.harvard.edu/ICQpost.html)

likely belongs to the same species as the Pakistani one and not to the European species. Collections of R. aurantioflava sp. nov. from Pakistan form a well-supported clade with European collections of R. aurea. Within this clade, the Pakistani collections and one collection from China (KF002751) received strong support but not with a visible branch length. The ITS tree (Fig. 11) suggests that Asian collections previously named as R. aurea (or as its synonym R. aurata Pers.) are different from European species and belong to at least two species that are described here as new. Three sequences from Mexico and USA represent probably a different, closely related species. We think that the R. aurea lineage together with R. flavida should be taxonomically classified as subsection Auratinae Bon and defined morphologically as having bright yellow tints on the stipe.



**Fig. 9** Phylogenetic tree of *R. 'tenuihyphata'* lineage based on ITS region. The collection of *R.* sp. 4 is in bold and blue. Samples matching concept of species described in this study are highlighted by shaded background. Names of taxa retrieved from public databases are in their original forms. Plant hosts are provided (if available) for samples from orchid or myco-heterothrophs (followed by 'root'). Countries are abbreviated by ISO codes (https://www.iso.org/iso-3166-country-codes.html), in parentheses are US states by postal codes (http://www.icq.eps.harvard.edu/ICQpost.html)

#### Russula wielangtae lineage

Our search of GenBank database yielded 4 other ITS sequences (Fig. 11) that are grouped with strong support with *R. wielangtae*, the species described here based on collections from Tasmania. We think that the collection identified in GenBank as *R. wollumbina* Grgur. represent the same species, and the reason why we have not adopted the latter name is discussed in the note to *R. wielangtae* below. Two collections from New Zealand and the sequestrate *Russula (Cystangium)* from California (USA) are probably another two closely related species that belong to this previously unrecognised lineage.

#### Russula lepida lineage

In our ITS tree (Fig. 11), *R. amarissima* and *R. lepida* Fr. are placed as two independent European species within a strongly supported and widely distributed clade represented also by mainly undescribed species in North America, Asia and Australia. Based on the support and geographical origin of the samples we think that the tree probably contains six non-European species. We confirmed that the *R. lepida* lineage is well-distinguished and previously recognised as *R.* subsection *Lepidinae* (Melzer & Zv.) Singer based on the morphology.

#### Subgenus Compactae

The two Compactae species described in this study are not closely related (Fig. 3). The ITS tree (Fig. 12) places R. fortunae sp. nov. from Panama close to North American R. eccentrica Peck and R. polyphylla Peck, and thus it is probably a member of subsect. Polyphyllinae Singer. Sister to our species is a well-supported Japanese clade labelled as R. subnigricans subgroup B-5, but this clade probably represents a new undescribed species as evidenced by the position of sequence AB291750 of R. subnigricans Hongo in the tree (Fig. 12). The second species from Thailand, Russula sp. 6, is placed in the subsect. Nigricantes Fr. and it is nested in a clade of samples labelled as R. densifolia subgroup A-5, A-6 and A-7 originating from Japan. All these samples are part of a distinct larger clade (ML = 100) that includes also the European R. densifolia Gillet. Placements of other clearly recognised species from Australia (R. ingwa Grgur.) and Europe (R. fuliginosa Sarnari) demonstrate that the Japanese subgroups of R. densifolia probably represent different phylogenetic species, some of them closely related to R. sp. 6.

#### Subgenus Heterophyllidia

All five *Heterophyllidia* species described in this study are placed in a well-supported (ML = 100) clade that corresponds to section *Ingratae* Quél. (Fig. 3). The sequestrate species from Tasmania (Australia), *R. echidna* sp. nov., is at the basal position within the clade and we retrieved only two other sequences from the Southern Hemisphere that are closely related (Fig. 13). The clade that includes *R. echidna* is thus the fifth distinctive lineage of this study recognised for the first time. The agaricoid *R. vinaceocuticulata* McNabb from New Zealand is the closest relative to our species. Very unique in its field appearance (see taxonomic part below) and position within *Ingratae* clade is *R.* sp. 7 from Thailand. This species sits on a long isolated branch not only in the multi-locus but also in the ITS tree, meaning that we were not able to retrieve from



**Fig. 10** Phylogenetic tree of *R. lilacea* lineage based on ITS region. Samples in blue are published first in this study (Supplementary Table 1), types are in bold. Samples matching concept of species described in this study are highlighted by shaded backgrounds. Names of taxa retrieved from public databases are in their original forms. Plant hosts are provided (if available) for samples from ectomycorrhizal roots (followed by 'ECM'), orchid or myco-heterothrophs (followed by 'root'). Countries are abbreviated by ISO codes (https://www.iso.org/iso-3166-country-codes.html), in parentheses are US states by postal codes (http://www.icq.eps.harvard.edu/ICQpost.html)

GenBank anything that has higher than 90% similarity score in a BLAST search. Two species from the USA, *R. amerorecondita* sp. nov. and *R. garyensis* sp. nov., are part of a well-supported clade (Fig. 3: ML = 99, Fig. 13: ML = 96). The multi-locus tree (Fig. 3) shows only the position of European *R. recondita* Melera & Ostellari (collection AT2001049 labelled as *R. pectinatoides* Peck) within this clade, but the ITS tree (Fig. 13) demonstrates that also the European *R. pectinata* Fr. and *R. praetervisa* Sarnari and the Asian *R. ahmadii* S. Jabeen, A. Razaq, A.R. Niazi, I. Ahmad & A.N. Khalid and *R. pseudopectinatoides* G.J. Li & H.A. Wen belong here. Most sequences within this clade are assigned to described species; there is only a small cluster of three sequences closely related to *R.*  garyensis from Western US and Mexico that may represent an undescribed species. *Russula amerorecondita* was apparently mistaken for *R. pectinatoides* Peck in the past, because of morphological similarity, but the latter is placed in a very distant place in both multi-locus and ITS trees as related to several more dark-coloured species. The fifth species, *R. fluvialis* sp. nov. from Finland, belongs to the *R. subfoetens* lineage (Fig. 3). The support for species and higher rank nodes within this lineage is mainly low in the ITS tree (Fig. 13). *Russula fluvialis* is placed in a wellsupported clade (ML = 90) that includes also the type of *R. ombrophila* M.M. Gómez & L.C. Monedero. Although there is little support to distinguish both species, they differ in geographic pattern and climate preference. *Russula* 



**Fig. 11** Phylogenetic tree of *R. aurea*, *R. wielangtae* and *R. lepida* lineages based on ITS region. Samples in blue are published first in this study (Supplementary Table 1), types are in bold. Samples matching concept of species described in this study are highlighted by shaded backgrounds. Names of taxa retrieved from public databases are in their original forms. Plant hosts are provided (if available) for environmental (abbreviated as E) samples from ectomycorrhizal roots (followed by 'ECM'). Countries are abbreviated by ISO codes (https://www.iso.org/iso-3166-country-codes.html), in parentheses US states by postal codes (http://www.icq.eps.harvard.edu/ICQpost.html)

*fluvialis* and another two sequences identified as *R. foetens* Pers. from north-eastern China and British Colombia (Canada) all originate from boreal forest but *R. ombrophila* described from Spain is clustered with two German and one Iranian sequence probably all from temperate or Mediterranean areas.

# Subgenus Brevipes

The single member of this subgenus described in this study is placed in the clade of species with a distinct amyloid suprahilar spot on the spores; the group in Europe is represented by the well-known *R. chloroides* (Krombh.) Bres. and *R. delica* Fr. The BLAST search of the ex-type sequence of *R. laevis* sp. nov. resulted in almost one hundred sequences with 97% and higher identity, many of them originating from environmental samples. The ITS tree (Fig. 14) shows that many of them form, together with the type sequence of *R. laevis*, a large clade with weak support (ML = 42) that is further grouped into a well-supported clade (ML = 74) with a representative sequence of *R. delica* included as the species hypothesis from UNITE. This clade contains sequences originating from different areas of the Northern Hemisphere, some of them identified as *R. delica* or *R. brevipes* Peck. There is also a wellsupported clade (ML = 97) that may represent an undescribed species. Within this clade, most of the European collections are clustered in a terminal clade with weak support (ML = 30). They are all from Scandinavia and most of them appear to be from arctic areas, such as the



Fig. 12 Phylogenetic tree of the subgenus *Compactae* based on ITS region. Samples in blue are published first in this study (Supplementary Table 1), types are in bold. Samples matching concept of species described in this study are highlighted by shaded backgrounds. Names of taxa retrieved from public databases are in their original forms. Countries are abbreviated by ISO codes (https://www.iso.org/iso-3166-country-codes. html), in parentheses are US and Canadian states by postal codes (http://www.icq.eps.harvard.edu/ICQpost.html)

type of *R. laevis*. Three non-European sequences that are very similar might suggest a wide distribution of the species in the Northern Hemisphere, but also might be the result of sequencing errors (especially the one from Thailand). Despite the mess in the ITS tree, we describe *R. laevis* as a new species, because it does not match the UNITE species concept of two already described European species. *Russula laevis* also differs from these others in climate preferences and morphological differences discussed in the taxonomy part below.

#### **Species descriptions**

*Russula abietiphila* Wisitr., H. Lee & Y.W. Lim, sp. nov. *Mycobank no.*: MB 830712, Figs. 15a, 16, 17, 18c *Holotype:* SOUTH KOREA, Gangwon-do, Pyeongchanggun, Mt. Odae, Woljeongsa temple, 37°43′53.86″ N; 128°35′33.21″ E, alt. 666 m, on soil in pure *Abies holophylla* forest, 20 Sep 2006, Soon Ja Seo (HCCN14799; isotype in SFC).



Fig. 13 Phylogenetic tree of the subgenus *Heterophyllidia* based on ITS region. Samples in blue are published first in this study (Supplementary Table 1), types are in bold. Samples matching concept of species described in this study are highlighted by shaded backgrounds. Names of taxa retrieved from public databases are in their original forms. Plant hosts are provided (if available) for environmental (abbreviated as E) samples from ectomycorrhizal roots (followed by 'ECM') and orchid or myco-heterothrophs (followed by 'root'). Countries are abbreviated by ISO codes (https://www.iso.org/ iso-3166-country-codes.html), in parentheses are US and Canadian states by postal codes (http://www.icq.eps.harvard.edu/ICQpost.html)

*Etymology:* 'abietiphila' refers to the host tree of the species.

Short diagnosis: Pileus medium-sized, with yellowish brown to orange-brown colour and with darker brown sports in the centre; stipe and lamellae without yellow– brown spots; spores large, with isolated or occasionally fused, prominent spines; hymenial cystidia clavate or fusiform, ventricose, apically mucronate and appendiculate; pileipellis strongly gelatinized, with narrow and apically unconstricted hyphal terminations and well-defined, conspicuous often 3–4-celled, subcylindrical or clavate, pileocystidia.

**Pileus** medium-sized, 35-50 mm diam., applanate with depressed centre; margin smooth or slightly striate; cuticle dry, peeling to 1/3 of the radius, yellowish brown (5E8), brown (6E8) to copper brown, variegated with dark brown (6F8) and yellowish patches, darker in the centre, brownish orange (7C6-C7) towards the margin, at the margin pale orange. **Lamellae** 4–6 mm deep, pale cream, alternating with relatively frequent shorter lamellulae; edge entire and concolorous. **Stipe** 50–60 × 7–10 mm, cylindrical, longitudinally striate, white; medulla stuffed and becoming partly hollow when mature. **Context** 3 mm thick in a half of the pileus radius, white, when young firm, then compact, unchanging after cutting or bruising. **Spore print** yellowish.

**Spores** (8.4–)9.5–<u>10.3</u>–11.1(–12.1) × (7.1–)7.8–<u>8.6</u>–9.3(– 10.0) µm, broadly ellipsoid, Q = (1.10–)1.16–<u>1.21</u>–1.27(– 1.34); ornamentation of large, moderately distant to dense [4–7(–9) in a 3 µm diam. circle] amyloid spines or warts, which are (0.7–)1.1–1.4(–1.7) µm high, isolated or fused in pairs or short chains [0–3(–4) fusions in the circle]; line connections absent or dispersed; suprahilar spot large, amyloid. **Basidia** (25.5–)33–<u>41.8–50.5(–53.5) × (8–)9.5– <u>11.7–13.5(–16)</u> µm, clavate, 4-spored; basidiola first cylindrical, then clavate, ca. 8.5–14.5 µm wide. **Hymenial cystidia** dispersed to moderately numerous, ca. 300–1100/ mm<sup>2</sup>, (33–)52–<u>67.3–82.5(–100) × (7–)9–11.8–14.5(–16.5)</u> µm, mainly clavate or fusiform, apically acute, mucronate with a 1–9 µm long appendage, originating in subhymenium, thin-walled; contents completely heteromorphous-</u> crystalline, turning grey-brown to black in sulfovanillin; abundant near the lamellae edges, (18.5-)43-58.2-73.5(- $105) \times (6-)8-10.9-13.5(-16) \mu m$ , similar to those on the sides but usually smaller. Lamellae edges fertile; marginal cells  $(13.5-)18-21.3-24.5(-29) \times (3-)5-6.8-9(-10.5) \mu m$ , undifferentiated. Pileipellis orthochromatic in Cresyl Blue, sharply delimited from the underlying context, 250-400 µm deep, with a well-defined, strongly gelatinized, 60-120 µm deep suprapellis composed of ascending to erect hyphal terminations; subpellis 170-330 µm deep, composed of horizontally oriented, dense, intricate and narrow hyphae. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin occasionally branched and narrow, thin-walled; terminal cells (14- $(20.5-29.7-39(-57.5) \times (1.5-)2.5-2.8-3.5(-4) \mu m, cylin$ drical, apically obtuse or slightly constricted; subterminal cells usually equally long and wide, but often also shorter and wider, ca. 2-4 µm wide, branched or not. Hyphal terminations near the pileus centre similar, terminal cells even narrower,  $(10.5-)17.5-24.3-31(-47.5) \times (1.5-)2-$ 2.5-3(-3.5) µm; subterminal cells unbranched or branched and imbedded in intricate hyphae of the subpellis. Pileocystidia near the pileus margin very abundant, typically 3-4-celled, sometimes 1-2-celled, usually clavate, occasionally slightly flexuous, thin-walled, terminal cells variable in length,  $(12.5-)20-36.6-53(-99.5) \times (3-)4-6.6-9(-$ 12.5) µm, mostly subcylindrical or narrowly clavate, apically mainly obtuse, occasionally subacute, contents heteromorphous, usually dense and crystalline-granulose, turning pale yellow-brown or pale grevish brown in sulfovanillin. Pileocystidia near the pileus centre smaller and more closely septate; terminal cells (12.5-)18.5-27.4-36(-62)  $\times$  (3–)4–5.7–7.5(–10) µm, mostly subclavate, cylindrical, ellipsoid or fusiform, apically obtuse but occasionally also subacute to constricted. Cystidioid hyphae in subpellis and context dispersed, with heteromorphousgranulose contents, oleiferous hyphae frequent in the lower part of subpellis and context.

Additional material studied: SOUTH KOREA, Wonju-si, Mt. Chiak, Sangwonsa temple, 37° 20′ 22.15″ N; 128° 03′ 11.19″ E, alt. 671 m, 30 Jul 2009, on soil in pure *Abies holophylla* forest, Soon Ja Seok (HCCN18498, duplicate in SFC); Inje-gun, Mt. Seorak, Baekdamsa temple, 38° 09′ 52.96″ N; 128° 22′ 25.83″ E, alt. 481 m, pure *Abies holophylla* forest or mixed forest dominated by *A. holophylla*, 30 Sep 2009, Soon Ja Seok (HCCN19017, duplicate in SFC).

*Notes*: Among the closely related species (Fig. 7), *Russula abietiphila* is reminiscent of *R. cuprea* or *R. olivobrunnea* with its reddish brown colours, spore size and spore ornamentation. Because European species are insufficiently described, we cannot decide if there are some microscopic differences in hymenium and pileipellis among species



**Fig. 14** Phylogenetic tree of *R. brevipes* lineage based on ITS region. Samples in blue are published first in this study (Supplementary Table 1), types are in bold. Samples matching concept of species described in this study are highlighted by blue background. Names of taxa retrieved from public databases are in their original forms, probably incorrect identifications are in quotation marks. Plant hosts are provided (if available) for environmental (abbreviated as E) samples from ectomycorrhizal roots (followed by 'ECM') and orchid or myco-heterothrophs (followed by 'root'). Countries are abbreviated by ISO codes (https://www.iso.org/iso-3166-country-codes.html), in parentheses are US and Canadian states by postal codes (http://www.icq.eps.harvard.edu/ICQpost.html)

within this group. At the moment, geographical distance and variation in climate and host differences are the only reliable arguments to support the species circumscription. *Russula abietiphila* is associated with *Abies* in the mountains of South Korea, *R. cuprea* with *Quercus* in temperate parts of Europe and *R. olivobrunnea* is only known from the boreal conifer forests of Scandinavia.



**Fig. 15** Spore drawings showing ornamentation in Melzer reagent. **a** *Russula abietiphila* (HCCN14799, holotype). **b** *Russula amarissima* (SAV F-2412, epitype). **c** *Russula amerorecondita* (F PGA17-004, holotype). **d** *Russula aurantioflava* (LAH35410, holotype). **e** *Russula* sp. 7 (GENT FH12-036). **f** *Russula* sp. 6 (GENT FH 12-064). **g** *Russula* sp. 5 (FH00304560). **h** *Russula castanopsidis* (SFC 20140702-14). **i** *Russula echidna* (HO 593336, holotype). Scale bar = 10 µm

*Russula amarissima* Romagn. & E.-J. Gilbert, Bull. Soc. Mycol. France 59: 71. 1943.

Mycobank no.: MB 290597, Figs. 15b, 19, 20, 21d

Lectotype: [France] Romagnesi 22-VIII-43 (PC).

*Epitype* (designated here; identifier no.: MBT 386771): SLOVAKIA, Štiavnické vrchy Mts., Ladzany, N of Buvanec Hill, alt. 450 m, 48° 17′ 14″ N; 18° 52′ 20″ E, on neutral soil in deciduous *Quercus* forest, 26 Jul 2008, S. Adamčík (SAV F-2412).

*Original diagnosis:* Pileo primum globato, dein convexo, durissimo, margine acuta, non sulcata, cute non secernibili, ex atrosanguineo, vel colore cupreo ochraceove infecto,



Fig. 16 Russula abietiphila (HCCN14799, holotype), hymenium. a Basidia. b Basidiola. c Marginal cells on the lamella edges. d Hymenial cystidia near the lamella edges. e Hymenial cystidia on the lamella sides. Cystidia with contents as observed in Congo Red. Scale bar = 10  $\mu$ m

circiter 6 cm lato. Stipite pleno, durissimo scabro, 60 × 20 mm<sup>2</sup>, leviter roseo, paulum flavescente. Carne durissima, crassa, leviter flavescente, odore obsoleto, sapore eximie amaro. Lamellis stipatissimis, antice acutis, angustis, pallidis. Sporis in pulvere non visis, sed verisi militer pallidissimis. Sporis 7,5-8,2 × 6,5-7,5 µm, humillimis verrucis, punctiformibus, frequenter anastomosis. Cystidiis obtusis, longissimis, 115-156 × (6,5)-10-14 µm. Epicute hyphis primordialibus incrustatis. Aestate : sub frondosis arboribus.

**Pileus** large, 120–140 mm diam. when mature, convex, soon expanding to applanate, widely but shallowly depressed in the centre, regular; margin not striate; cuticle dry, matt, velvety smooth or rugulose, hardly peeling, brownish red (10C7, 10D6), madder red (10D7), violet-brown (10F6, 10E7) reddish brown (9D6), in the centre variegated, e.g. cream (4A3), ivory (4B3), flesh colour (6B3). **Lamellae** up to 10 mm deep, moderately distant, adnexed, yellowish white (4A2), pale yellow at the view of edges; lamellulae and furcations absent or rare; edge entire



Fig. 17 Russula abietiphila (HCCN14799, holotype), pileipellis. a Pileocystidia near the pileus centre. b Pileocystidia near the pileus margin. c Hyphal terminations near the pileus centre. d Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red. Scale bar =  $10 \mu m$ 

and red near the margin or along the length. **Stipe**  $40-85 \times 26-35$  mm, cylindrical or narrowly clavate, strongly longitudinally striate, white, often with pinkish flush or pink areas; medulla solid, cortex hardly delimited, ca. 5 mm. **Context** firm in all parts of the basidiomata, white, on damaged places (e.g. by insects) turning brownish yellow; taste mild; odour faint and pleasant. **Spore print** pale cream (Ib–IIa).

**Spores**  $(7.6-)8-\underline{8.4}-8.7(-9.1) \times (7-)7.2-\underline{7.5}-7.9(-8.5)$  µm, subglobose, Q =  $(1.04-)1.08-\underline{1.11}-1.15(-1.18)$ ; ornamentation of medium-sized, dense [6-9(-10) in a 3 µm diam. circle] amyloid spines, 0.5-0.9 µm high, locally reticulate, often fused in long chains (3-6 fusions in the circle), connected with occasional line connections [0-3(-4) line connections in the circle]; suprahillar spot large, amyloid. **Basidia** (45-)47-<u>50.6</u>-54(-56)  $\times$  11-<u>11.7</u>-13(-14) µm, clavate-pedicellate, 4-spored; basidiola first cylindrical, then clavate, ca. 4-12 µm wide. **Hymenial cystidia** dispersed, ca. 450/mm<sup>2</sup>, (69-)82-95.5-109(-

Fig. 18 Basidiomata in the field. **a** Russula sp. 3 (SFC  $\triangleright$  20170821-22a). **b** Russula sp. 4 (SFC 20170712-12). **c** Russula abietiphila (HCCN14799, holotype). **d** Russula sp. 4 (SFC 20170712-12). **e**-**f** Russula olivaceohimalayensis (CAL 1659, holotype). **g** Russula seperina (SAV F-3156, epitype). **h** Russula sp. 1 (GENT FH12-061). Scale bar = 10 mm

 $115) \times (10-)11-13-15(-16)$  µm, fusiform, pedicellate, apically acute and often mucronate, with 4-13(-18) µm long appendage, often with slightly thickened walls; contents strongly heteromorphous (granular or crystalline), reacting weakly (grevish) in sulfovanillin; near the lamellae edges numerous, usually smaller, (36-)51-67.6-84(-89)  $\times$  (7–)8–9.1–10(–11.5) µm, fusiform, clavate or subcylindrical, apically often obtuse and never mucronate, without an appendage, very conspicuous and protruding more than half their length, usually with distinctly thickened walls; contents similar but often distinctly yellow. Lamellae edges sterile; marginal cells (12-)17-21.7-26(-31)  $\times$  (3–)4–4.9–6 µm, conspicuous, mainly narrow, subcylindrical, flexuous, occasionally nodulose. Pileipellis weakly metachromatic in Cresyl Blue, not sharply delimited from the underlying context, 60-90 µm deep, vertically almost homogeneous, composed of irregularly oriented, non-gelatinized hyphae that become denser and more horizontally oriented towards the context; longer hyphal terminations forming conical fascicules near the surface. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin very flexuous, occasionally slightly moniliform, thin-walled, terminal cells (18- $)27-43.4-60(-101) \times 3-3.8-4.5(-5) \mu m$ , cylindrical, often occasionally nodulose, apically obtuse; subterminal cells usually equally long, mainly branched, often embedded in the intricate hyphae of subpellis and difficult to trace, often covered by strong glutinous coating. Hyphal terminations near the pileus centre different in length, longer ones similar to those near the pileus margin and arranged in fascicules, shorter ones more branched and more flexuous, terminal cells  $(10-)15.5-24-32(-45) \times (3-)3.5-4.1-5(-$ 5.5) µm, usually cylindrical, apically constricted or obtuse; subterminal cells very irregular, flexuous-nodulose and often covered with glutinous hyaline coating resembling thick-walled hyphae. Pileocystidia near the pileus margin always 1-celled,  $58-89.7-123 (> 150) \times (3.5-)4.5-5.5-$ 6.5(-7) µm, narrowly clavate to subcylindrical, often flexuous, apically obtuse or acute, contents refringent, yellowish, oleiferous, no reaction in sulfovanillin. Pileocystidia near the pileus centre often narrower and/or  $(13-)24-58.9-93(>150) \times (2.5-)3.5-4.5-5.5($ shorter, 6.5) µm, usually clavate and with similar contents. Oleiferous hyphae frequent in subpellis, dispersed in context, with yellowish refringent contents.





**Fig. 19** *Russula amarissima* (SAV F-2412, epitype), hymenium. **a** Basidia. **b** Basidiola. **c** Marginal cells on the lamellae edges. **d** Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red, some elements with contents indicated schematically by a plus sign (+). Scale bar = 10  $\mu$ m

Additional material studied: GERMANY, Osthausen, forest around Rodberg, associated with *Quercus* and *Carpinus* on calcareous soil, 8 Aug 2010, J. Girwert *FH 2010 BT42* (GENT); ITALY, Province of Imperia, Nava, 19 Aug 2013, R. Jon *1085* (SAV).

*Notes: Russula amarissima* can be easily distinguished by its firm context turning brownish yellow where damaged and bright red colour of pileus descending to lamellae edges. We studied the field aspect of only one collection included in this study, that shows differences from original description in larger pileus and no bitter taste, but this falls within the variation of the species described by Sarnari (2005). We confirmed that both European species, *R. amarissima* and *R. lepida* Fr., are part of a well-distinguished lineage recognised in the literature as *R.* subsect. *Lepidinae* (Melzer & Zv.) Singer, but we did not confirm the presence of the primordial hyphae or the acidresistant incrustations reported by Romagnesi (1967), Sarnari (2005) and our observations rather agree with Tassi



**Fig. 20** *Russula amarissima* (SAV F-2412, epitype), pileipellis. **a** Pileocystidia near the pileus centre. **b** Pileocystidia near the pileus margin. **c** Hyphal terminations near the pileus centre. **d** Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red. Scale bar =  $10 \mu m$ 

(2003). It seems that R. amarissima and R. lepida share similar (sub)reticulate spore ornamentation and very long cystidia that do not change colour in sulfovanillin. The contrast in the width between very slender, one-celled pileocystidia (up to 6.5 µm in this study) and very wide hymenial cystidia (11-15 µm) might be an important distinguishing character for the group (see descriptions of R. *lepida* in the above-mentioned European monographs). According to our experience, the group might be especially well-represented in North America and probably wellknown species (e.g. R. ochroleucoides Kauffman and R. flavisiccans Bills) belong here, but we were not able to find sequences of reliably identified specimens to prove it. Our sampling suggests that R. amarissima occurs in temperate hardwood forests of central, western and southern parts of Europe.

Russula amerorecondita Avis & Barajas, sp. nov. Mycobank no.: MB 830713, Figs. 15c, 22, 23, 24b



Fig. 21 Basidiomata in the field. a Russula aurantioflava (LAH35405). b Russula sp. 5 (FH00304560). c. Russula wielangtae (HO 593331, holotype). d Russula amarissima (SAV F-2412,

*Holotype*: USA, Indiana, Gary, Miller Woods, 13 Jul 2017, P.G. Avis *PGA17-004* (F).

*Etymology*: 'amerorecondita' refers to the fact it is a North American species similar to the European R. *recondita* Melera & Ostellari.

Short diagnosis: Typical medium-sized species of *R. pectinatoides* group; pileus with pale, tan, brown and grey tints and tuberculate-striate margin, developing an unpleasant to bleachy odour; spore print white to pale cream; spores with warts fused in short chains connected by occasional line connections; suprahilar spot not

epitype). e Russula wielangtae (HO 593331, holotype). f-g Russula subtilis (SAV F-3805). h-i Russula gemmata (SAV F-3800). Scale bar = 10 mm

amyloid; terminal cells of hyphal extremities in pileipellis mainly lageniform or subulate, with subterminal cells often shorter, more inflated and unbranched; pileocystidia relatively frequent, single-celled, often long, originating deep in the context, with dispersed granular or banded contents. **Pileus** medium-sized, 40–55 mm diam., convex and sometimes depressed; margin moderately to strongly tuberculate-striate; cuticle viscid to dry, smooth, peeling mostly to <sup>1</sup>/<sub>4</sub> sometimes to <sup>1</sup>/<sub>2</sub> of the radius, in the centre brown to tan, light tan, grey, brown grey, sometimes very faintly orange, tan brown, towards the margin light to dark tan, orange, brown, cream. **Lamellae** 5–7 mm deep, close to subdistant, creamy white, off-white, whitish when not decayed, older with darker hues including orange-brown, sometimes staining slight orange-red, slight pink; lamellulae sometimes present and irregular in length, furcations present especially near the stipe; edge entire, concolorous. **Stipe** 20–40 × 5–15 mm, cylindrical and slightly tapering near the base, smooth, whitish, with some brown, tan or grey at the base; medulla spongy and becoming hollow; cortex ca. 2–3 mm. **Context** ca. 4 mm in a half of the pileus radius, first firm, then spongy, whitish, unchanging when bruised, turning pinkish with FeSO<sub>4</sub>, negative with KOH; taste slightly acrid in lamellae, sometimes almost mild; odour first like parmesan, then unpleasant, bleachy. **Spore print** white to pale cream (Ia–IIa).

**Spores** (6.5–)7.1–<u>7.6–</u>8.1(–9.5) × (5–)5.6–6.3–6.9(–8) µm, broadly ellipsoid, Q = (1.07–)1.12–<u>1.22</u>–1.31(–1.5); ornamentation of moderately distant to dense [(4–)5–7 in a 3 µm diam. circle] amyloid warts, 0.4–0.6(–0.8) µm high, occasionally to frequently fused in short chains [(0–)1–3 fusions in the circle]; connected by dispersed, short, fine line connections [0–2(–3) in the circle], suprahilar spot not amyloid, but well delimited by low, small warts connected in a chain. **Basidia** (20–)28–<u>34.1–40(–50) × (6–)8–<u>9.6–</u>11(–13) µm, clavate, 4-spored; basidiola clavate, ca.</u>



Fig. 22 Russula amerorecondita (F PGA17-004, holotype), hymenium. a Basidia. b Basidiola. c Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar =  $10 \mu m$ 



Fig. 23 Russula amerorecondita (F PGA17-004, holotype), pileipellis. a Pileocystidia near the pileus centre. b Pileocystidia near the pileus margin. c Hyphal terminations near the pileus centre. d Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red. Scale bar =  $10 \ \mu m$ 

5-7.5 µm wide. Hymenial cystidia moderately numerous, ca.  $1200/\text{mm}^2$ ,  $48-54.8-61.5(-72) \times (6-)7-7.8-8.5(-9)$ µm, mainly fusiform, less frequently clavate, apically acute and often mucronate, usually with a 1-2(-4) µm long appendage, usually originating at approximately the same level as basidia, a few longer originated in the context, thin-walled; contents pale yellowish in Congo Red or in water, with dispersed inconspicuous granulations near the terminal part, staining dark red-brown in sulfovanillin. Lamellae edges fertile and similar to lamellae sides. Pileipellis orthochromatic in Cresyl Blue, not sharply delimited from the underlying context, 80-120 µm deep, two-layered, strongly gelatinized throughout; suprapellis 35–75 µm deep, epithelium-like, composed of large, up to 25-50 µm wide, sphaerical or ellipsoid and near the surface more elongated cells; hyphae often forming pyramidal structures near the pileus margin, gradually passing to the 25-45 µm deep subpellis, composed of horizontally oriented, dense, intricate, 3-8 µm wide hyphae covered by



Fig. 24 Basidiomata in the field. a *Russula fluvialis* (KUO *JR8196*). b *Russula amerorecondita* (F PGA17-004, holotype). c *Russula fluvialis* (KUO *Ruotsalainen 4866*, holotype). d *Russula garyensis* (F

thick, glutinous coating that does not colour in Congo Red. Acid-resistant incrustations absent, but contents of cystidia and cystidiod hyphae acid-resistant (staining red after carbolfuchsin treatment). Hyphal terminations near the pileus margin scarcely branched, occasionally flexuous, thin-walled, terminal cells  $(15-)18-25-31.5(-44) \times (3-)3.5-4.6-5.5(-6.5)$  µm, mainly attenuated or narrowly lageniform, occasionally cylindrical, rarely clavate, lanceolate or fusiform, often irregularly inflated near the base, apically constricted or not; subterminal cells frequently shorter and occasionally inflated, to 8.5 µm wide, rarely

PGA17-008, holotype). **e** *Russula purpureogracilis* (GENT FH12-055, holotype). **f** *Russula castanopsidis* (SFC 20140702-14). Scale bar = 10 mm

branched or with lateral nodules. Terminal cells of hyphae near the pileus centre similar to those near the pileus margin. **Pileocystidia** near the pileus margin relatively numerous, 1-celled, cylindrical, rarely lanceolate, often very long and originating in the context, a few shorter ones originating in the suprapellis,  $(43-)46.5-\underline{89.1}-130(>150) \times 4.5-\underline{5.5}-6(-7)$  µm, apically mainly constricted or attenuated but otherwise obtuse, thin-walled; contents yellow in Congo Red and water, with dispersed granulations or locally heteromorphous-banded, weakly reactine to sulfovanillin (pale yellow–brown or pale red-

brown). Pileocystidia near the pileus centre similar. Oleiferous hyphae frequent in context, with yellowish pigments.

Additional material studied: USA: Indiana, Gary, Miller Woods, 13 Jul 2017, P.G. Avis *PGA17-003*, *PGA17-004*, *PGA17-005*, *PGA17-006* (F); Gary, Marquette Park, research plots 2 and 4, *Quercus velutina* woodland (for plant community data see http://nirmi.org/site.php?pa geNav=133), 13 Jul 2017, P.G. Avis *PGA17-017* (F); Porter, Indiana Dunes National Lakeshore Park, Bog Trail, just 10 m west of Mineral Springs Road, collected during A.H. Smith Mycological Foray in hardwood forest dominated by *Quercus*, 21–24 Aug 2008, P.G. Avis *SMF08080* (F); Shades State Park in Indiana, Waveland, hardwood forest, 28 Aug 2012, S. Russell *SH120828* (F).

*Notes*: This new species is the North American sister species of the recently published *R. recondita*, a recently introduced name for a European species that was in the past commonly referred to as *R. pectinatoides* Peck (Melera et al. 2016). *Russula amerorecondita* appears to be a common species in the United States and was probably also confused with the same *R. pectinatoides* Peck in the past. The microscopic features of the type of *R. pectinatoides* were studied by Adamčík et al. (2013) and the main differences with our new species are the more isolated spore ornamentation and the frequent mucronate pileocystidia in Peck's species.

*Russula amerorecondita* could also be confused with *R. foetentula* Peck because of the nearly identical microscopic features including spores (see Adamčík et al. 2013). However, *R. foetentula* was described as having an almond smell and clearly acrid taste, suggesting it might belong to the *R. laurocerasi* complex, whereas our species clearly has a disagreeable smell and mild taste. Microscopically, the pileocystidia of *R. foetentula* are narrower and apically more constricted, attenuated and often acute. This new species has a very wide distribution covering most of the eastern United States, from Ontario in Canada and Wisconsin all the way down to Florida, including specimens that are involved in associations with mycoheterotrophic orchids (*Corallorhiza*, see Fig. 13).

# *Russula aurantioflava* Kiran & Khalid, sp. nov. *Mycobank no.:* MB 830715, Figs. 15d, 21a, 25, 26

*Holotype:* PAKISTAN, Khyber Pakhtunkhwa province, Dir upper district, Kumrat, alt. 2359 m, in mixed coniferous forest with *Abies pindrow* and *Picea smithiana*, 20 Aug 2016, Munazza Kiran & Abdul Nasir Khalid *KM 133* (LAH35410).

*Etymology:* 'aurantioflava' refers to the bright orange-yellow colour of the cuticle near the pileus margin.

Short diagnosis: Pileus medium-sized, convex, becoming applanate when old, lemon to golden yellow with darker

orange centre; lamellae edges and stipe with vivid yellow hue; spores large, subreticulated, with large warts fused in chains oriented radially from suprahilar spot; hymenial cystidia without well-defined contents more abundant near the lamellae edges, without reaction in sulfovanillin; pileipellis strongly gelatinized, with loose hyphal terminations of two types, apically attenuated with longer terminal cells and apically obtuse with shorter ones, without defined pileocystidia and with hyaline incrustations not colouring in any reagents.

**Pileus** medium-sized, 25–67 mm diam., semiglobose, convex, only when old expanding to plane, slightly depressed in the centre; margin deflexed, smooth and only sometimes striate when mature; cuticle smooth, wet-viscous and shiny but dry-matt and pruinose towards the centre, near the margin golden yellow to bright lemon, in the centre orange, yellow-orange, reddish brown when dry and old. **Lamellae** relatively dense, adnate-emarginate, pale yellow; lamellulae very rare and furcations occasional near the stipe; edge even and vivid yellow. **Stipe**  $25-65 \times 10-30$  mm, obclavate, longitudinally striate, white with vivid yellow hue especially near the lamellae, sometimes almost completely vivid yellow. **Context** white, unchanging, compact. **Spore print** not observed.

**Spores**  $(8-)8.5-9.6-10.7(-11.84) \times (6.2-)7.4-8.5-9.5(-$ 10.6)  $\mu$ m, subglobose to broadly ellipsoid, Q = (1.1–)1.14– 1.17-1.21(-1.23); ornamentation of large, distant to moderately distant (3-6 in a 3 µm diam. circle) amyloid warts or spines, (0.6-)0.8-1.2(-1.4) µm high, subreticulate, often fused in long chains radially oriented from suprahilar spot, often also forming crests or wings [(0-)1-3 fusions in the circle], connected by usually short, fine or thick line connections [(0-)1-3(-4) in the circle]; suprahilar spot large, amyloid, finely warted and with irregular radial amyloid projections. **Basidia**  $(40-)47-55.8-64.5(-74) \times (11.5-$ )13.5–14.7–16(–18.5) µm, broadly clavate, 4-spored; basidiola first cylindrical, then clavate, ca. 5.5-14.5 µm wide. Hymenial cystidia with no well-defined contents and probably moderately numerous, (56.5-)65.5-76.4- $87.5(-89.5) \times (5-)8.5-10.8-13(-15) \mu m$ , mainly fusiform, clavate or lanceolate, apically obtuse or acute, sometimes with round or ellipsoid, 2-6 µm long appendage, thinwalled; contents in almost whole volume optically empty and only apically granulose-crystalline, without reaction in sulfovanillin; near the lamellae edges more numerous, (50- $)69-80.1-91(-103) \times (6-)9-11.4-14(-18.5)$  µm, more distinctly protruding above other elements, similar in size, shape and contents. Lamellae edges fertile; marginal cells undifferentiated. Pileipellis orthochromatic in Cresyl Blue, sharply delimited from the underlying context, 110-145 µm deep, strongly gelatinized throughout, vaguely divided in 65-75 µm deep suprapellis of ascending, loose hyphal terminations, gradually passing to



Fig. 25 *Russula aurantioflava* (LAH35410, holotype), hymenium. a Basidia. b Basidiola. c Hymenial cystidia near the lamellae edges. d Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar =  $10 \ \mu m$ 

35–55 µm deep subpellis of irregularly and near context horizontally oriented, intricate, 2.5-4.5 µm wide hyphae. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin not or only slightly flexuous, often with hyaline incrustations not colouring in any reagent, thin-walled, of two forms: more frequently composed of 1-3 unbranched cells; terminal cells (17-)25-33.7-42.5  $(-50) \times 3.5 - 4.2 - 5(-5.5) \mu m$ , subulate or cylindrical and apically attenuated or constricted to 1-2 µm, less frequently composed of 2-4(-5) unbranched cells; terminal cells often very short,  $(7-)11-16.5-22(-24) \times (2.5-)3-$ 3.8-5(-5.5) µm, cylindrical or lageniform, usually with refringent inclusion or fine granulose pigmentations; subterminal cells usually unbranched, equally large but in case of small terminal cells often longer or more inflated (few 6-9 µm wide). Hyphal terminations near the pileus centre similarly of two forms, the first form with attenuated cells with narrower and shorter but otherwise similar terminal cells,  $(10-)19.5-28.8-38(-53) \times 2.5-4-5(-8)$  µm, the second form resembling pileocystidia in shape, more closeptate, terminal cells (7.5 - )9.5 - 14.9 - 20.5sely  $(-26) \times (2-)2.5-3.8-5(-6)$ often small μm, and



**Fig. 26** *Russula aurantioflava* (LAH35410, holotype), pileipellis. **a** Hyphal terminations near the pileus centre. **b** Hyphal terminations near the pileus margin. Contents of hyphae as observed in Congo Red. Scale bar =  $10 \mu m$ 

cylindrical, usually without heteromorphous contents only with yellowish pigments. Well-defined **pileocystidia** or primordial hyphae absent. Cystidioid or oleiferous hyphae in subpellis or context absent.

Additional material studied: PAKISTAN, Khyber Pakhtunkhwa province, Swat district, Gabin jabba valley, alt. 2483 m, in mixed coniferous forest with *Pinus wallichiana*, *Abies pindrow* and *Picea smithiana*, 4 Sep 2016, Junaid Khan *GJ1640* (LAH35408); ibid., 15 Sep 2016, Junaid Khan *GJ1655* (LAH35409); Khyber Pakhtunkhwa province, Dir upper district, Kalkot, alt. 2044 m, in mixed coniferous forest with *Abies pindrow* and *Picea smithiana*, 20 Aug 2016, Munazza Kiran & Abdul Nasir Khalid *KK* 75, *KK* 83, *KK* 96 (LAH35405, LAH35407, LAH35406). *Notes: Russula aurantioflava* and the European species *R. aurea* differ in four informative positions in ITS (see also Fig. 11) and are very similar macro- and microscopically, although a complete microscopic analysis would be desirable for the European species. Indeed, both Sarnari (2005) and Buyck (2005) illustrated the presence of lateral diverticules on the hyphal extremities in the pileipellis of *R. aurea*, and this, to a lesser degree, was also observed in *R. aurantioflava* (not shown). This therefore suggests that diverticules might be a frequent feature for *R.* subsect. *Auratinae* Bon (as they are for subsect. *Cupreinae*, for example), rather than for individual species. The only other species that has been considered as being very close to *R. aurea* so far, is the North American *R. xantho* Shaffer, which shares the absence of pileocystidia. However, *R. xantho* has much more irregular hyphal extremities in the pileus surface, as well as on the lamellae edge (see Buyck 2005).

# Russula castanopsidis Hongo, Memoirs of Shiga University 23: 42, 1973.

#### Mycobank no.: MB 280394, Figs. 15h, 24f, 27, 28

*Holotype*: [JAPAN] under *Castanopsis cuspidata*, Tanakami-Sekinotsu, Otsu, July 15, 1971 (herbarium T. Hongo no. 4458).

*Original diagnosis*: Pileo 3.5–5.5 cm lato, convexo, dein expanso, medio latius depresso, sicco, e pallide argillaceo isabellino ad marginem pallidiore, cutifracto, margine breviter subsulcata; carne tenui, alba; odore nullo, sapore miti; lamellis attenuato-liberis, confertis, 3–6 mm latis, ventricosis, albis; stipite 4–6.5 cm longo, 6–8 mm crasso, aequali vel ad basim attenuato, albo, rogoso-striato, farcto; sporis in pulvere albis,  $7.5-9(9.5) \times 5.5-7(8)\mu$ ,



Fig. 27 Russula castanopsidis (SFC 20140702-14), hymenium. a Basidia. b Basidiola. c Marginal cells on the lamellae edges. d Hymenial cystidia near the lamellae edges. e Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar = 10  $\mu$ m

subglobosis, echinulatis; cheilocystidiis 48–63  $\times$  7–13  $\mu$ ; pleurocystidiis 58–75  $\times$  11–20 $\mu$ .

**Pileus** medium-sized, 30–60 mm diam., convex when young, later plano-convex and with depressed centre; margin with short and inconspicuous striation, crenulate; cuticle dry and not viscid when wet, typically cracking with age, peeling near the margin, pale greyish brown to pale pinkish buff, near the centre darker, yellowish brown to brown. **Lamellae** 5 mm deep, crowded, adnexed to almost free, white, furcate near the stipe; lamellulae absent; edge smooth and concolorous. **Stipe** 40–70 × 6–11 mm, cylindrical to slightly tapering towards the base, longitudinally striate, white to pale cream, staining brownish with age, hollow. **Context** 2–3 mm thick in a half of the pileus radius, fragile, white, unchanging. **Spore print** pale.

**Spores**  $(7.7-)8.0-8.6-9.2(-9.9) \times (6.1-)6.4-6.8-7.2(-7.6)$  $\mu$ m, broadly ellipsoid to ellipsoid, Q = (1.12–)1.19–1.26– 1.33(-1.38); ornamentation of large, moderately distant to dense [(5-)6-8(-9) in a 3 µm diam. circle] amyloid spines or warts, up to 1.5 µm high, isolated or occasionally fused (0-2 fusions in the circle), line connections dispersed [(0-2))1 in the circle]; suprahilar spot large, amyloid. Basidia  $(31.5-)34.5-37.3-40(-43) \times (9.5-)10-10.8-11.5(-12.5)$ µm, clavate, 4-spored; basidiola mainly clavate, ca. 7-12 µm wide. Hymenial cystidia widely dispersed to dispersed. ca.  $100-600/\text{mm}^2$ , (54-)57-70.0-83(-95.5 × (9.5-)11.5-13.1-15(-16.5) µm, fusiform, lanceolate or clavate, apically acute and often mucronate, usually with 1-9 µm long appendage, thin-walled, contents heteromorphous granulose-crystalline and dense in apical part, in other places dispersed and hardly visible, no reaction in sulfovanillin; near the lamellae edges very narrower, (28.5–)36–<u>46.3</u>–56.5(– abundant, usually  $(67) \times (3.5-)4.5-6.4-8.5(-10)$  µm, narrowly fusiform, lageniform or subulate, usually inflated at base, apically acute and usually with a short appendage. Lamellae edges with occasional basidia; marginal cells (13-)16.5-19.8- $23(-26.5) \times (4.5-)6-7.2-8.5(-9) \mu m$ , usually broadly clavate and shorter than basidiola. Pileipellis orthochromatic in Cresyl Blue, sharply delimited from the underlying context, 80-150 µm deep, not gelatinized throughout, distinctly two-layered; suprapellis 30-55 µm deep, composed of loose, ascending or erect hyphal terminations, abruptly passing to pseudoparenchymatic, 50-100 µm deep subpellis composed of inflated, ellipsoid or globose, 6.5-12.5 µm wide cells. Acid-resistant incrustations absent. Hyphal termination in pileipellis near the pileus margin composed of 2-3 unbranched cells originating in the pseudoparenchymatic subpellis, not flexuous, thinwalled, terminal cells (11.5-)15.5-21.9-28.5(-36.5 × 3.5-4.4-5(-5.5) µm, cylindrical or occasionally narrowly fusiform to narrowly lageniform, apically obtuse occasionally slightly attenuated, contents finely or



Fig. 28 Russula castanopsidis (SFC 20140702-14), pileipellis. a Hyphal terminations near the pileus centre. b Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red. Scale bar =  $10 \mu m$ 

granulose; subterminal cells often shorter and wider, ca. 4.5–7 µm wide, unbranched. Hyphal termination near the pileus centre shorter and usually only composed of 1–2 unbranched cells, terminal cells  $(10.5-)15-20.6-26.5(-29.5) \times 2.5-3.9-5(-6.5)$  µm; subterminal cells often embedded in subpellis, and occasionally branched. **Pileo-cystidia** or primordial hyphae not observed. **Cystidioid hyphae** absent, oleiferous hyphae frequent in the subpellis. *Additional material studied*: SOUTH KOREA, Jeju-do Seogwipo-si, Andeok-myeon, Andeok valley, 33°15′26.44″ N; 126°21′10.39″ E, alt. 106 m, on soil in deciduous forest, 2 Jul 2014, Hyun Lee & Hae Jin Cho (SFC 20140702-14). *Notes*: The collections from Korea are morphologically in complete agreement with the type study by Buyck

(unpublished observations). Our phylogeny does not suggest that the Korean material represents a separate taxon from those collected in Japan from where it was originally described. The Japanese samples suggest further that *R. castanopsidis* is a frequent associate of mycoheterotrophic Monotropoids and orchids (Fig. 4). For the comparison with closely related taxa we refer to the notes under *R. purpureogracilis*.

Russula echidna G.M. Gates, Caboň & Jančovičová, sp. nov.

Mycobank no.: MB 830722, Figs. 15i, 29, 30, 31e-h

*Holotype*: Australia: Tasmania, Lambert Creek Gully, Sandy Bay,  $42^{\circ}$  55' S;  $147^{\circ}$  20' E, on surface of soil in wet gully in dry sclerophyll vegetation, 27 Jul 2015, G. Gates (HO 593336).

*Etymology*: 'echidna' refers to the abundant, erect, subulate pileocystidia that remind one of the spines of the echidna (*Tachyglossus*).

Short diagnosis: Basidiomata hypogeous, sequestrate, 10-32 mm in diameter; cuticle fibrous, dry, orange-pink when young, pale yellow with streaky orange-red mottling to pale brown with red veins when mature; lamellae in form of white gleba with locules of various size and shape; stipe only in form of columella; spores relatively small, with warts fused in long chains and connected by fine line connections; suprahilar spot not amyloid; hilar appendage with amyloid ring; pileipellis composed mainly of abundant lageniform, subulate or fusiform, apically acute-pointed pileocystidia, usually with a  $1-2 \mu m$  long, pearl-like appendage.

**Basidiomata** hypogeous, sequestrate, small, 10–32 mm diam., 12-22 mm deep, irregularly tuberiform; cuticle fibrose, dry, cherry red (10B8) when young, becoming paler when mature, pale yellow with streaky orange-red mottling to pale brown with red veins, with some white pruinosity at the base. Lamellae in form of white gleba with locules of various size and shape. Stipe in form of columella only visible when young, white. Context without odour, taste not observed. Spore print not observed. **Spores**  $(5.7-)6.6-7.2-7.8(-8.6) \times (5.2-)5.7-6.3-6.9(-7.6)$  $\mu$ m, subglobose to broadly ellipsoid, Q = (1.03–)1.1–1.15– 1.19(-1.25); ornamentation of relatively small, moderately distant to dense [(3-)4-7(-8) in a 3 µm diam. circle] amyloid warts, 0.3-0.5 µm high, locally subreticulated, rarely also isolated, fused in long, branched chains [(0-)1-3(-4) fusions in the circle], connected by often short, frequent, fine line connections [(0-)1-4 in the circle]; suprahilar spot covered by lower ornamentation to almost smooth; appendage with strongly amyloid ring around the base. **Basidia**  $(23.5-)28-32.9-37.5(-45) \times (7.5-)8.5-9.6-$ 10.5(-12) µm, clavate, 4-spored; basidiola first cylindrical, then narrowly clavate,  $(10-)16-21.6-27.5(-40) \times (4.5-)6-$ 



Fig. 29 *Russula echidna* (HO 593336, holotype), hymenium. **a** Basidia. **b** Basidiola. **c** Hymenial cystidia. Cystidia with contents as observed in Congo Red. Scale bar =  $10 \ \mu m$ 



Fig. 30 *Russula echidna* (HO 593336, holotype), pileipellis. a Pileocystidia and hyphal terminations near the pileus centre. b Pileocystidia and hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red. Scale bar =  $10 \ \mu m$ 

7.5–9(–11) um. **Hymenial cystidia** widely dispersed to dispersed, 240-560/mm<sup>2</sup>, (20-)29.5-38.3-47(-66)  $\times$  (5-)6.5-7.8-9(-14) µm, lageniform, fusiform or subulate, apically always acute-pointed, mainly with a 1-3(-6) µm long appendage, thin-walled; contents heteromorphous, loose, granular, turning grey-brown in sulfovanillin and orange in Melzer's reagent. Lamellae edges not defined. Pileipellis orthochromatic in Cresvl Blue, sharply delimited from the underlying subhymenium, 350-400 µm deep; pileus context not defined; suprapellis 70-120 µm deep, not gelatinized, composed of ascending to erect, densely arranged hyphae forming a trichoderm, well-delimited from the 250-290 µm deep subpellis of horizontally oriented, loosely arranged, intricate and strongly gelatinized hyphae. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin (near the attachment of basidiomata to the substrate) dispersed and inconspicuous; terminal cells  $9.5-14.6-20(-27) \times (3.5-)4.5-5.3-6(-75)$ µm, conical, subulate, lageniform or cylindrical, apically often gradually narrowing and acute, occasionally also obtuse, thin-walled; subterminal cells usually equal in size, mainly branched. Terminal cells of hyphae near the pileus centre narrower and longer,  $(9-)13-18.6-24(-39) \times (3-)$ )3.5-4.4-5(-6) µm. **Pileocystidia** near the pileus margin very abundant, always 1-celled, lageniform, fusiform or subulate, thin-walled,  $(15-)16.5-20.5-24.5(-31) \times (4.5-$ )5-5.7-6.4(-7.5) µm, apically acute-pointed, usually with a 1-2 µm long, pearl-like appendage; contents yellow, with rather dispersed and sometimes also banded granulations, slightly brownish in sulfovanillin. Pileocystidia near the pileus centre similar, but often longer, (15-)20-26.8- $33.5(-42) \times (4-)4.5-5.5-6.5(-9)$  µm. Oleiferous hyphae in subpellis abundant, with yellow refringent contents, grevish pink in sulfovanillin.

Additional material studied: AUSTRALIA, Tasmania, North West Bay River, 42°57′ S, 147°12′ E, alt. 250 m, at surface of soil in wet sclerophyll forest dominated by *Eucalyptus obliqua*, with *Pomaderris apetala* and *Olearia argophylla* understorey, along a river, 29 Dec 2011, G. Gates (HO 593337); ibid., 2 Feb 2012, G. Gates (HO 593335).

*Notes*: This sequestrate *Russula* collected under eucalypts in Tasmania has no closely related species for which sequences have been deposited in GenBank, nor are we aware of any unsequenced sequestrate Russula species that have been described with similar morphological features. The description of *R. rostraticystidia* T. Lebel from Australia presents somewhat similar morphology, but it differs in a distinct stipe development (*Macowanites* type of basidioma) and lacks pileocystidia (Lebel and Tonkin 2007). It was recently shown to belong in subsect. *Amoeninae* Buyck (see under *R. pseudoamoenicolor* A. Ghosh, Buyck, K. Das, A. Baghela & R.P. Bhatt in



Fig. 31 Basidiomata in the field. a Russula laevis (KUO Taipale 14 Aug 2014). b Russula sp. 6 (GENT FH 12-064). c Russula fortunae (PMA Corrales 180, holotype). d Russula sp. 7 (GENT FH12-036).

Ariyawansa et al. 2015). One of the surprising features in our collections was the presence of a perfect amyloid ring surrounding the very base of the apiculus in many of the spores.

**e-f** *Russula echidna* (HO 593336, holotype). **g-h** *Russula echidna* (HO 593335). Scale bar = 10 mm

Russula flavobrunnescens A. Kong & Buyck, sp. nov. Mycobank no.: MB 830724, Figs. 32a, 33, 34, 35f *Holotype*: MEXICO, Chiapas, Municipality of La Trinitaria, Lagunas de Montebello Park, Laguna Bosque Azul, alt. 1475 m, 16° 07′ 36″ N, 91° 43′ 51″ W, subtropical forest of *Pinus oocarpa*, 6 Oct 2006, A. Kong *5024* (TLXM).

*Etymology*: 'flavobrunnescens' refers to the yellow-brown colours of the pileus and stipe developing during maturation.

Short diagnosis: Basidiomata large; pileus cuticle mainly pale yellowish with some pinkish tints near the margin, a fishy odour and colour change typical for the *R. xerampelina* group; spores relatively large, ornamented with mainly isolated prominent and blunt spines; hymenial cystidia with hardly visible contents; marginal cells well-differentiated, fusiform, often flexuous and nodulose; hyphal terminations near the pileus margin loose, composed of 1–3 unbranched cells, terminal cells mainly cylindrical and apically obtuse, narrower near the pileus centre; pileocystidia 1-celled, relatively narrow, cylindrical or narrowly clavate, with hardly any definable contents.

Pileus large, 75–100 mm diam., convex to plano-convex, depressed in the centre; margin only indistinctly striate and slightly crenulate when old; cuticle slightly viscid and shiny when wet, matt when dry, rugulose towards the margin, in the centre yellowish (3A4-4A4) and also with olive and brownish tints, near the margin paler and often with pink tints. Lamellae 6-12 mm deep, dense, adnexed, vellowish (2A3, 2A4, 3A3); lamellulae absent, furcations frequent near the stipe; edge concolorous, staining yellowish brown. Stipe  $45-100 \times 17-24$  mm, cylidrical or narrowly clavate, distinctly rugoluse-striate, white, staining yellowish brown (5B6) when bruised, with brown spots at the base, solid. Context ca. 4 mm thick in a half of the pileus radius, firm when young, compact and more fragile when old, white, staining yellowish brown when bruised; taste mild; odour weak when fresh, developing to fishy during the drying. Spore print ochre (3A4-4A3).

**Spores**  $(8.1-)8.5-9-9.4(-10.3) \times (6.5-)6.9-7.2-7.6(-7.9)$  $\mu$ m, broadly ellipsoid, Q = (1.16–)1.18–1.24–1.3(–1.45); ornamentation of large, distant to moderately distant (3-6 in a 3 µm diam. circle) amyloid warts or spines, (0.8-)0.9-1.1 µm high, mainly isolated, occasionally fused in pairs or triplets [(0-)1-2(-3)] fusions in the circle], rarely connected by fine line connections [0-1(-2) in the circle]; suprahilar spot large, amyloid. Basidia (34-)37-40.1- $43.5(-46) \times (9.5-)11-11.9-13(-13.5)$  µm, broadly clavate, pedicellate, 4-spored; basidiola ellipsoid or clavate, ca. 7-11 µm wide. Hymenial cystidia widely dispersed,  $250-300/\text{mm}^2$ ,  $(59-)63.5-71.7-80(-90) \times (8$ ca. )9.5-10.9-12.5(-14) µm, fusiform, occasionally also clavate, apically usually acute, rarely obtuse, almost always with a 4-14(-18) µm long appendage, thin-walled; contents optically almost empty, with dispersed, granulose or



Fig. 32 Spore drawings showing ornamentation in Melzer reagent. a Russula flavobrunnescens (TLXM AK5024, holotype). b Russula fluvialis (KUO Ruotsalainen 4866, holotype). c Russula fortunae (PMA Corrales 180, holotype). d Russula garyensis (F PGA17-008, holotype). e Russula gemmata (TENN-F-067624, holotype). f Russula laevis (KUO Ruotsalainen 4016, holotype). g Russula madrensis (TLXM AK3422, holotype). h Russula sp. 1 (GENT FH12-061). i Russula olivaceohimalayensis (CAL 1659, holotype). Scale bar = 10 μm

banded inclusions, no reaction in sulfovanillin; abundant near the lamellae edges,  $(38-)45.5-\underline{59.2}-73(-86) \times (6-)7-\underline{8.4}-10(-13) \mu m$ , mainly clavate or lanceolate, occasionally fusiform, occasionally slightly thick-walled, apically mainly acute, usually with a 5–12(–21)  $\mu$ m long appendage, contents yellowish, usually without any inclusions. Lamellae edges with occasional basidia; **marginal cells** well-differentiated, often resembling small cystidia in shape, (16–)21.5–<u>26.5</u>–31.5(–35) × (3.5–)4.5–<u>5.9</u>– 7.5  $\mu$ m, mainly fusiform, often nodulose and flexuous, apically often constricted or appendiculate, thin-walled.



Fig. 33 Russula flavobrunnescens (TLXM AK5024, holotype), hymenium. a Basidia. b Basidiola. c Marginal cells on the lamellae edges. d Hymenial cystidia near the lamellae edges. e Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar = 10  $\mu$ m

Pileipellis orthochromatic in Cresyl Blue, not sharply delimited from the underlying context, 45-60 µm deep, vaguely divided in 30-40 µm deep, strongly gelatinized suprapellis composed of loose, repent or ascending hyphal terminations and a less gelatinized, 15-25 µm deep subpellis of horizontally oriented, relatively dense, intricate, 2.5–4 µm wide hyphae. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin composed of 1-3(-4) unbranched cells, often with lateral projections at the branching area, usually slightly or distinctly moniliform, thin- or slightly thick-walled; terminal cells  $(22-)29.5-39-48.5(-58) \times (4-)4.5-5.2-6(-7.5)$ um. mainly cylindrical, occasionally narrowly lageniform, rarely clavate, apically usually obtuse but occasionally constricted; subterminal cells often distinctly wider and shorter, usually not branched. Hyphal terminations near the pileus centre narrower, densely arranged, flexuous, moniliform, composed of 1-2 unbranched cells, thin-walled and originating from narrower and thick-walled hypae of subpellis; terminal cells (17-)21-28.7-36.5(-44) × 2.5-3.7-4.5(-6) µm, usually cylindrical or clavate. Pileocystidia near the pileus margin relatively frequent, 1-celled,



**Fig. 34** *Russula flavobrunnescens* (TLXM AK5024, holotype), pileipellis. **a** Pileocystidia near the pileus centre. **b** Pileocystidia near the pileus margin. **c** Hyphal terminations near the pileus centre. **d** Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red. Scale bar =  $10 \mu m$ 

cylindrical or clavate, often flexuous and occasionally slightly moniliform, often slightly thick-walled; terminal cells  $(25-)39-\underline{65.8}-92.5(-114) \times 5.5-\underline{6.8}-8(-11)$  µm, apically obtuse; contents optically empty or with few dispersed inclusions, no reaction in sulfovanillin. Pileocystidia near the pileus centre smaller, 1(-2)-celled, mainly cylindrical, often flexuous, often thick-walled; terminal cells  $(23-)26-\underline{42.8}-59.5(-80) \times (4-)4.5-\underline{5.1}-6(-6.5)$  µm, apically occasionally acute; contents with more abundant inclusions or even conspicuously heteromorphous and usually with yellowish pigments. Cystidioid or oleiferous hyphae not observed.

Additional material studied: MEXICO, Tlaxcala, Municipality of Ixtenco, La Malinche National Park, E slope of La Malintzi Volcano, 0.5 km SW of La Caprina (monitoring house), 19°14'17" N, 97°58'25" W, alt. 3000 m, *Pinus montezumae* forest, 11 Jul 1990, A. Kong *1565* (TLXM). *Notes*: Odour, taste, spore print colour and reactions to chemicals clearly place *R. flavobrunnescens* in *R.* 



Fig. 35 Basidiomata in the field. **a-b** *Russula madrensis* (TLXM AK3422, holotype). **c-d** *Russula sancti-pauli* (PC, *B. Buyck 06.494*, holotype). **e** *Russula* sp. 2 (PC, coll. *Buyck 06.542*). **f** *Russula flavobrunnescens* (TLXM AK5024, holotype). Scale bar = 10 mm

subsect. *Xerampelinae*. The yellow pileus cuticle with only slight pink tints near the margin is an uncommon character within the group but considering the colour variability of its members we have to discuss the species delimitation more generally. Two North American species with yellow pilei, *R. ochrifloridana* Buyck & Adamčík and *R. grundii* Thiers, have fine, low spore ornamentation, different from the prominent spines of the species described here. Two other species associated with conifers in Mexico and described in this study differ not only in the darker red and purple colours of the pileus, but also in the less prominent, low, more numerous and more connected warts of the spore

ornamentation. The closest morphological match is *R. katarinae* Adamčík & Buyck described from eastern US with *Pinus strobus* that is sister to *R. flavobrunnescens* in our tree (Fig. 6) and differs only by 4 informative positions in the ITS sequence. The US species has the same yellow-orange colours of the pileus, similar spore ornamentation and cylindrical hyphal terminations that become more dense and narrower near the pileus centre. Our observations suggest that *R. katarinae* might differ also in smaller spores, narrower terminal cells of hyphae and thinner pileipellis near the pileus margin.


Fig. 36 *Russula fluvialis* (KUO *Ruotsalainen 4866*, holotype), hymenium. **a** Basidia. **b** Basidiola. **c** Marginal cells on the lamellae edges. **d** Hymenial cystidia near the lamellae edges. **e** Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar =  $10 \mu m$ 

*Russula fluvialis* Taipale, Ruotsalainen & Kälviäinen, sp. nov.

Mycobank no.: MB 830725, Figs. 24a, c, 32b, 36, 37

*Holotype*: FINLAND, Northern Savonia, Nilsiä Huutavanholma, primary calcium rich forest, dominated by *Picea*, also mixed with some *Betula* and *Pinus*, 23 Aug 2012, J. Ruotsalainen *JR8666* (KUO).

*Etymology*: 'fluvialis' refers to the fact that most of the collections were collected close to a stream.

Short diagnosis: Basidiomata strongly reminiscent of *R. subfoetens*, from which it differs by the thin-fleshed pileus with nearly transparent margin; spores with large obtuse and often winged warts connected by occasional lines and fusions; hymenial cystidia fusiform or lanceolate, apically appendiculate, with yellowish contents and sometimes with a few dispersed granulations; hyphal terminations of pileipellis intricate, flexuous, moniliform, cylindrical and apically often constricted; pileocystidia 1-celled, similar to hymenial cystidia, apically with a small, pearl-like appendage.

**Pileus** medium-sized, 40–73 mm diam., hemispherical when young, convex to applanate when mature, in the centre often somewhat depressed; margin tuberculate-



**Fig. 37** *Russula fluvialis* (KUO *Ruotsalainen 4866*, holotype), pileipellis. **a** Pileocystidia near the pileus centre. **b** Pileocystidia near the pileus margin. **c** Hyphal terminations near the pileus centre. **d** Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red. Scale bar =  $10 \mu m$ 

striate up to the 1/3 of the radius; cuticle viscid, shiny when wet, towards the centre rough, ochraceous yellow to ochraceous brown. Lamellae moderately distant, adnexed, cream; lamellulae of various length, occasional; furcations occasional, especially near the stipe; edge even and concolorous. Stipe  $40-60 \times 9-15$  mm, cylindrical or sometimes narrowed towards the base, near the base often curved due to growth on stream bank, finely longitudinally striate, light cream with some brownish spots when mature, cavernate, cortex 2-3 mm thick. Context 3 mm thick in half of the radius of average-sized indivuduals, near the margin very thin, membranaceous and transparent, compact, cream, with age or with drying brownish; taste not observed; odour inconspicuous. Spore print not observed. **Spores**  $(7.1-)7.4-7.7-8.1(-8.7) \times (6.1-)6.3-6.6-6.9(-7.4)$  $\mu$ m, subglobose to broadly ellipsoid, Q = (1.03–)1.13– 1.18-1.22(-1.26); ornamentation of large, distant to moderately distant [(2–)3–5(–6) in a 3  $\mu$ m diam. circle] amyloid, prominent, obtuse warts or wings, (0.6-)0.8-1.2(-1.5)

µm high, locally subreticulate, rarely isolated, fused in pairs or short chains [0-2(-3)] fusions in the circle], connected by occasional, fine, long line connections [0-2(-3)]in the circle]; suprahilar spot not or partly amyloid, covered bv lower ornamentation. **Basidia** (35–)41–46–51(– 52) × (9.5–)10–10.8–11.5(–12.5)  $\mu$ m, clavate, 4-spored; basidiola first cylindrical, then narrowly clavate, ca. 4-9 µm wide. Hymenial cystidia moderately numerous, ca.  $850-1100/\text{mm}^2$ ,  $(51-)61-74-87(-104) \times (6.5-)7-8-$ 9 µm, fusiform, lanceolate, rarely clavate, apically mainly acute, with a 2-7(-9) µm long appendage, often originating deep in lamellae context, thin-walled; contents yellowish, optically empty or with a few dispersed granulations near the apical part, turning black in sulfovanillin; near the lamellae edges smaller,  $(36-)43-50.6-58(-71) \times (4.5-)6-$ 6.9-8(-8.5) µm, mainly clavate, occasionally fusiform or lanceolate, contents usually yellow and optically empty, rarely with few granulose inclusions. Lamellae edges with dispersed basidia; marginal cells (16-)18-21.8-25.5(-32)  $\times$  (4–)4.5–5.8–7(–10) µm, clavate or cylindrical, obtuse. Pileipellis orthochromatic in Cresyl Blue, sharply delimited from the underlying context, 200-270 µm deep, strongly gelatinized, vaguely divided into a 60-115 µm deep suprapellis of loose, irregularly oriented and often repent hyphal terminations forming sometimes mammillar structures, and 160-210 µm deep subpellis of horizontally oriented, loose, but near the context dense, 2.5-6 µm wide hyphae. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin intricate, very flexuous or sinuous, often moniliform, thin-walled; terminal cells (21- $)27-37.6-48.5(-62) \times (2.5-)3-3.7-4.5(-5) \mu m$ , cylindrical, apically usually attenuated and constricted to at least 2.5 µm, occasionally nodulose or diverticulate; subterminal cells branched or not, often with lateral projections or nodules, less flexuous, often slightly wider. Hyphal terminations near the pileus centre more densely arranged, less flexuous, with shorter terminal cells (16-)20-28.9-38(-53) × (2–)2.5–3.1–3.5(–4)  $\mu$ m, usually cylindrical, apically obtuse and usually not distinctly constricted. Pileocystidia near the pileus margin frequent, always 1-celled, cylindrical, narrowly fusiform or lanceolate, rarely clavate, entire or curved but not flexuous, thin-walled, (28-)38- $56.3-74.5(-85) \times (3-)3.5-3.9-4.5(-5) \mu m$ , apically acute, always with one pearl-like, 1-3 µm long appendage; contents with dispersed granulations or partly banded, often optically empty, often with yellowish intracellular pigments, weakly greying in sulfovanillin. Pileocystidia near the pileus centre even more frequent, similar in shape and contents, but in average shorter (28-)32-45.5-58(-71)  $\times$  (3–)3.5–3.9–4.5(–5) µm, apically rarely also with two appendages. Cystidioid hyphae abundant in subpellis and context, with dispersed granulose contents.

Additional material studied: FINLAND, Northern Savonia, Nilsiä Haluna, along the stream from Kaakonlampi-lake, with *Picea*; coord. 63° 06′ 40.14″ N, 27° 59′ 51.55″ E, 15 Sep 2010, M.-L. Aho & J. Ruotsalainen *JR8313* (KUO); Ostrobottnia Kajanensis biogeographical province, Paltamo Tololanmäki, stream of Kylmäpuro, nature reserve, nutrition rich swamp with *Betula*, *Picea*, *Alnus incana* and some *Pinus*, 60° 20′ 26.99″ N, 28° 02′ 42.75″ E, 20 Aug 2010, J. Ruosalainen, J. Vauras, & A.M. Moron *JR8196* (KUO).

Notes: Russula fluvialis is possibly a common boreal species in Picea-dominated forests. Most of the collections are found along small streams in nutrition-rich places. The species is very similar to a common European species R. subfoetens, with which it has been probably previously confused, because it has very similar field appearance and may grow in similar places. Russula fluvialis differs from R. subfoetens by the negative or weak, yellowish reaction of the flesh or stipe surface to KOH. A similar species common in boreal areas of Northern Europe is R. foetens but that has usually much more robust basidiomata and ellipsoid, larger spores with less prominent warts. The Mediterranean species R. ombrophila and R. inamoena have similar spore ornamentation as R. fluvialis. The former species is even clustered with the newly described species and their delimitation is not resolved in our ITS tree (Fig. 13). Despite low support in the phylogeny, not only the ecology and the climate are different, but also the spores of R. ombrophilla have less crested spines that are rarely connected by line connections and the pileocystidia are wider than 5 µm (Gómez and Monedero 2011). The second Mediterranean species, R. inamoena, seems to be morphologically very similar to R. fluvialis and differs not only by its habitat but also in the hyphal terminations in the pileipellis that are attenuated and less flexuous. The type study of R. foetentula Peck, the species described from temperate climate of Long Island, New York (USA) more than one hundred years before 2018 (Adamčík et al. 2018) shows surprisingly similar morphology to our species. However, the recent collection of the North American species is not clustered with R. fluvialis or R. ombrophila, and it has distinctly narrower hyphal terminations in the pileipellis near the pileus centre and they are more conspiculously flexuous near the pileus margin. We are aware of the probably wide geographical distribution of R. fluvialis with little morphological and ITS differences, but taking into account all the differences mentioned above, we think that it is a good species of a complex that needs further multi-source studies.

Russula fortunae Corrales, sp. nov. Mycobank no.: MB 830726, Figs. 31c, 32c, 38, 39



Fig. 38 *Russula fortunae* (PMA *Corrales 180*, holotype), hymenium. a Basidia. b Basidiola. c Marginal cells on the lamellae edges. d Hymenial cystidia near the lamellae edges. e Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar = 10  $\mu$ m

*Holotype*: PANAMA, province of Chiriquí, Fortuna Forest Reserve, Bocas del Toro road, 2.4 km S of Valle de la Mina, Alto Frio, 08° 39' 24.96" N, 82°12' 55.50" W, alt. 1000 m, montane forest, on soil in a monodominant *Oreomunnea mexicana* forest, 22 May 2012, A. Corrales & C. Velásquez, *Corrales 180* (holotype, PMA; isotype, ARIZ). *Etymology*: 'fortunae' refers to the Fortuna forest reserve, where the type specimen was collected.

Short diagnosis: basidiomata very pale; pileus mediumsized, becoming distinctly striate-crenulate toward the margin; pileus surface areolate-warted; lamellae distant; spores with reticulated ornamentation; suprahilar spot not amyloid; hymenial cystidia numerous, mainly cylindrical or narrowly clavate and near the lamellae edges typically lageniform or fusiform; marginal cells well-differentiated and of very variable shapes; pileipellis with pseudoparenchymatic suprapellis, composed of nodulose and frequently branched hyphal terminations with terminal



Fig. 39 *Russula fortunae* (PMA *Corrales 180*, holotype), pileipellis. a Pileocystidia near the pileus centre. b Hyphal terminations near the pileus centre. c Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red. Scale bar =  $10 \mu m$ 

cells of very variable shape; pileocystidia present only near the pileus centre and very dispersed and inconspicuous.

Pileus medium-sized, 60-66 mm diam., convex to planoconvex, slightly depressed in the centre; margin distinctly striate to 1/3 of the pileus radius, deflexed also when mature, crenulated; cuticle dry, matt, areolate with irregular warts usually radially arranged, pale brownish to beige. Lamellae 4-9 mm deep, very distant, only ca. 16-22 reaching the stipe, broadly adnate and decurrent with a short tooth, pale brownish with a faint pinkish shade when mature; lamellulae 1-3(-7) between each pair of the lamellae, occasionally furcate or anastomosed; edge finely fimbriate or serrulate, segmentiform and concolorous. Stipe  $21-45 \times 7-14$  mm, central or excentric, cylindrical, usually narrowed at the base, longitudinally finely areolate and striate, beige-orange, solid. Context only ca. 1 mm thick in a half of the pileus radius, pale brownish, unchanging, young firm, compact and more fragile when old. Spore print not observed.

**Spores**  $(6.7-)7.2-7.5-7.8(-8.2) \times (6.1-)6.3-6.6-6.9(-7.3)$  $\mu$ m, subglobose to broadly ellipsoid, Q = (1.08–)1.1–1.14– 1.18(-1.23); ornamentation of relatively small, moderately distant to dense [(5-)6-7(-8) in a 3 µm diam. circle], amyloid warts, 0.3-0.5 µm high, reticulated and without isolated elements; warts fused in usually long chains [(1-)2-5(-6) fusions in the circle], connected by short, frequent, fine line connections [(0-)1-4 in the circle]; suprahilar spot large, distinct but smooth, not amyloid. **Basidia**  $(41.5-)48.5-55-62(-65) \times (6-)7.5-8.6-9.5(-10)$ µm, narrowly clavate, 4-spored; basidiola cylindrical or narrowly clavate, ca. 3-7.5 µm wide. Hymenial cystidia moderately numerous to numerous, 1350-2800/mm<sup>2</sup>, (52- $58.5-70-81.5(-88) \times (5-6.5-7.6-8.5(-9) \mu m$ , cylindrical or narrowly clavate, rarely fusiform, often slightly flexuous and moniliform, apically usually obtuse, rarely acute, rarely with a small appendage, originating deeper in context, not or slightly protruding above basidia, thinwalled; contents heteromorphous, mainly loose and banded, yellowish in Congo Red, weakly greying in sulfovanillin; near the lamellae edges dispersed, (52-)56.5- $68.7-81(-97) \times (5.5-)6.5-7.8-8.5(-10)$  µm, frequently narrowly lageniform or fusiform, apically often distinctly moniliform, obtuse and not appendiculate. Lamellae edges sterile; marginal cells  $(12-)22.5-31.4-40(-45) \times 5-7.4-$ 9.5(-11.5) µm, very variable in shape, lageniform, clavate, fusiform, subulate, subcylindrical, apically obtuse or acute, thin-walled, often with short inflated ellipsoid subterminal cell. Pileipellis orthochromatic in Cresyl Blue, sharply delimited from the underlying context, 175-285 µm deep, strongly gelatinized throughout and often embedded in up to 30 µm thick gelatinous matter, vaguely divided in pseudoparenchymatic, 50-170 µm deep suprapellis, composed of large fascicules of ascending to erect hyphae forming mammillar or pyramidal, 30-50 µm high structures, depressions in between with repent hyphae, gradually passing to 90-135 µm deep subpellis of horizontally oriented, relatively dense, intricate, 2-4 µm wide hyphae. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin usually branched and intricate, flexuous and nodulose, usually constricted on septa, thinwalled; terminal cells (6-)19-27.8-36.5(-46)  $\times$  (5-)5.5-8-10(-13.5) µm, variable in shape, cylindrical, clavate, fusiform, lageniform, often nodulose or lobate, occasionally with projections, apically usually obtuse; subterminal cells mainly distinctly wider, inflated, ellipsoid or pyriform, often branched, below usually with some more inflated cells forming short or long chains. Hyphal terminations near the pileus centre usually of pairs of terminal and subterminal cells forming an epithelium, more densely arranged, less flexuous and nodulose, terminal cells (18- $)23-32.6-42(-58) \times (5-)5.5-8.4-11(-16) \mu m$ , often thickwalled (walls up to 1 µm thick). Pileocystidia near the



Fig. 40 *Russula garyensis* (F PGA17-008, holotype), hymenium. a Basidia. b Basidiola. c Hymenial cystidia near the lamellae edges. d Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar =  $10 \ \mu m$ 



**Fig. 41** *Russula garyensis* (F PGA17-008, holotype), pileipellis. Pileocystidia and hyphal terminations near the pileus centre. Cystidial contents as observed in Congo Red. Scale bar =  $10 \mu m$ 

pileus margin not observed, near the pileus centre very dispersed, inconspicuous, usually 1-celled, cylindrical, entire, ca.  $33-75-100(-120) \times 4-5.5-8 \mu m$ , apically indistinctly moniliform and sometimes with a well-

delimited appendage, thin-walled, often with glutinous incrustation or coating that is not reactive to any reagent; contents optically empty except for a few dispersed inclusions or crystals in the apical part, turning weakly to reddish grey in sulfovanillin, hardly visible. **Cystidioid** hyphae in the subpellis present, absent in the context, greyish in sulfovanillin.

Additional material studied: PANAMA, province of Chiriquí, Fortuna Forest Reserve, Trail to Cerro Hornito from Bocas del Toro Road, near "Mirador". 8° 40.440′ N, 82° 13.038′ W, montane forest, on soil in a monodominant *Oreomunnea mexicana* forest, 1 Jun 2015, C.L. Ovrebo 5504 (CSU).

*Notes*: This entirely pale brownish to off-white species with widely spaced unequal lamellae, is strongly reminiscent of other tropical species in *R*. subg. *Archaea* and *R*. subg. *Compactae*. It differs from all species in the former subgenus in the 'normal' (i.e. not exceptionally small) size of its spores. In *R*. subg. *Compactae*, species with similarly spaced lamellae are only found so far in *R*. subsect. *Polyphyllinae* (viz. in the *R. eccentrica* complex) and *R*. subsect. *Nigricantinae* (viz. in the *R. nigricans* complex). Our phylogeny (Fig. 12) clearly places *R. fortunae* in *R.* subsect. *Polyphyllinae* where it is sister to one of the Japanese clades identified as '*R. subnigricans*', but certainly not closely related to *R. eccentrica*.

## Russula garyensis Avis & Barajas, sp. nov.

Mycobank no.: MB 830727, Figs. 24d, 32d, 40, 41

*Holotype*: USA, Indiana, Gary, Marquette Park, research plots 2 and 4, *Quercus velutina* woodland (for plant community data see http://nirmi.org/site.php?pageNav=133), 13 Jul 2017, P.G. Avis *PGA17-008* (F).

*Etymology*: 'garyensis' refers to the city of Gary, which is approximately the center of the currently known distribution in eastern North America of this species.

Short diagnosis: basidiomata medium-sized to large, similar to *R. pectinatoides*, with pileus having a strongly tuberculate-striate margin, and with tan, amber, golden, orange, and greyish tints especially towards the pileus centre; context with unpleasant, bleachy odour; white to pale cream spore print; spore ornamentation subreticulate with warts fused in short chains connected by occasional line connections, and an inamyloid suprahilar spot; terminal cells of hyphal extremities in pileipellis mainly lageniform or subulate and subterminal cells often shorter, more inflated and unbranched; pileocystidia relatively frequent, single-celled, often long and originating deep in the context, with dispersed granular or banded contents.

**Pileus** medium-sized to large, 28–90 mm diam., convex, depressed expanding to applanate, then infundibuliform when old; margin strongly tuberculate-striate; cuticle sometimes lubricous, sticky to slightly viscid, rough and

near centre often rugulose, peeling usually to  $\frac{1}{4}$ , rarely even up to 2/3 of the radius, in the centre brown, tan to light tan, sometimes slightly grey, towards margin amber, golden, orange, brown, tan, cream or whitish sometimes also slightly grey. Lamellae 4-12 mm deep, subdistant to distant, off-white, tannish white, cream, ivory, creamy tan, creamy orange, sometimes staining rusty orange, amber orange, brown, red, or orange; lamellulae often present, usually irregular in length, often anastomosing with the lamellae; furcations present especially near the stipe; edge entire, concolorous. Stipe  $20-67 \times 10-18(-25)$  mm, cylindrical and slightly tapering near the base, smooth, but sometimes longitudinally striate or pitted near the base, towards apex whitish mostly or creamy grey, towards or at base often with distinct red or rusty red colour, sometimes also brown and/or greyish, cortex ca. 2-4 mm. Context ca. 4-5 mm in a half of the pileus radius, firm, whitish, sometimes with orange-brown, cream, tan or slightly yellow, unchanging when bruised, turning pinkish or orange with FeSO<sub>4</sub>, negative with KOH; taste slightly acrid and somewhat unpleasant in lamellae; odour first like parmesan, then unpleasant, bleachy, later also slightly fishy. Spore print cream to pale ochre (IIb-IIIb).

 $(6-)6.6-7.1-7.5(-7.88) \times (5-)5.4-5.9-6.4(-6.5)$ Spores  $\mu$ m, broadly ellipsoid, Q = (1.09–)1.13–1.21–1.32(–1.41); ornamentation of large, moderately distant [4-6(-7)] in a 3 µm diam. circle] amyloid warts or pustules, (0.6-)0.8-1(-1.4) µm high, rarely isolated, frequently fused in crests or chains [(0-)1-3(-4)] fusions in the circle], connected by occasional, fine line connections [0-2(-3)] in the circle], suprahilar spot not amyloid, but marginated by a chain of low warts. Basidia (27-)31-35.2-39.5(- $45) \times (7.5-8.5-9.8-11(-12)) \mu m$ , clavate, 4-spored; basidiola cylindrical, ellipsoid or clavate, ca. 5-7.5 µm wide. Hymenial cystidia dispersed, ca. 450–600/mm<sup>2</sup>,  $(62-)71.5-81.4-91(-103) \times 7-8.1-9(-10)$  µm, mainly fusiform or lanceolate, apically always acute, mucronate or pointed, with one or two, 1-8(-13) µm long, usually narrow appendages, base usually curved and originating deep (30-70 µm) in the subhymenium or context, thin-walled; contents yellowish, poorly defined, finely granulose, staining dark red-brown in sulfovanillin; near the lamellae edges similar but often distinctly shorter, (49-)55.5-68.3- $81(-98) \times (7-)7.5-8.3-9(-10) \mu m$ . Lamellae edges fertile; marginal cells undifferentiated. Pileipellis orthochromatic in Cresyl Blue, sharply delimited from the underlying context, 40-50 µm deep, one-layered, cutis-like, strongly gelatinized, composed of dense, horizontally oriented,  $3-5 \mu m$  wide hyphae, with some loose, repent terminations near the surface. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin dispersed, 3-4.5 µm wide, scarcely branched, embedded in a thick, gelatinous matter that does not dissolve in potassium



Fig. 42 Russula gemmata (TENN-F-067624, holotype), hymenium. a Basidia. b Basidiola. c Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar =  $10 \mu m$ 



Fig. 43 *Russula gemmata* (TENN-F-067624, holotype), pileipellis. a Primordial hyphae near the pileus centre. b Primordial hyphae near the pileus margin. c Hyphal terminations near the pileus centre. d Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red. Scale bar =  $10 \mu m$ 

hydroxide and makes the hyphal terminations difficult to trace; terminal cells ca.  $20-50 \mu m \log$ , cylindrical, entire, usually not distinctly narrowed or flexuous, apically obtuse; subterminal cells in size more or less equal, rarely branched. Hyphal terminations near the pileus centre more

distinctive, more densely arranged, cylindrical, often branched, not or slightly flexuous; terminal cells (13– )16.5–<u>21.5</u>–26.5(–35) × 2.5–<u>3.1</u>–3.5(–4) µm; subterminal cells often shorter and branched or nodulose. **Pileocystidia** near the pileus margin not observed, near the pileus centre more frequent than hyphal terminations, 1-celled, narrowly fusiform to subcylindrical, some longer descending to the subpellis or deeper to the context, 25–<u>39.7</u>–56.5(– 82) × 3.5–<u>4.2</u>–5(–5.5) µm, apically acute and often pointed, usually with a 1–2 µm large, pearl-like appendage, thin-walled; contents yellowish, dispersed, heteromorphous-banded, hardly reacting to sulfovanillin (brownish yellow). **Oleiferous hyphae** abundant in pileipellis near the context and dispersed in the context, contents yellowish and acid-resistant.

Additional material studied: USA, Indiana, Gary, Miller Woods, 13 Jul 2017, P.G. Avis PGA17-002 (F); Marquette Park, Quercus velutina woodland, 13 Jul 2017, P.G. Avis PGA17-007. PGA17-010. PGA17-011. PGA17-012. PGA17-014, PGA17-015 (F); Indiana Dunes National Lakeshore Park, section west of Grand Boulevard in the Miller Section of Gary, in grassy yard of 6417 Ash Avenue, less than 10 m from Quercus velutina woodland, Jun 2010, P.G. Avis PGA10-001 (F); Indiana Dunes National Lakeshore Park, just west of Michigan City, along the Mt. Baldy Trail from the parking lot to top of Mt. Baldy, under closed canopy hardwood forest dominated by Quercus, 10 Sep 2015, P.G. Avis PGA15-910 (F); Minnesota, along Dakota Valley Trail, on ground under Quercus macrocarpa, 29 Aug 2007, D.J. McLaughlin MCBS157 (MIN 896153); Anoka, Cedar Creek Natural History Area, Field D, Control, N addition expt. plot 5, point 4, coord. 45°24'46.20" N, 93°11'44.22" W, 10 Aug 2002, P.G. Avis & B.T.M. Dentinger PA275 (MIN877051); West Virginia, Randolph County, the Lower Glady Fork of Monongahela National Forest, on ground in Tsuga canadensis and mixed broadleaf moist ravine, 7 Aug 2005, D.M. Mitchell (DEWV05-898).

*Notes*: This species differs from the closely related *R*. *amerorecondita* in the more yellowish pileus, the frequently shorter and clearly mucronate pileocystidia with pearl-like appendage, as well as in the slightly acrid taste. They both have a wide distribution in the east and central part of the USA and are occasionally associatiated with mycoheterotrophic orchids in the genus *Corallorhiza*.

## Russula gemmata Looney, sp. nov.

Mycobank no.: MB 830728, Figs. 21h-i, 32e, 42, 43

*Holotype*: USA, North Carolina, Haywood Co., Great Smoky Mountains National Park, Big Creek, Chestnut Branch trail, 43° 38' 19.39" N; 116° 14' 28.86" W, mixed cove forest with *Quercus, Pinus, Fagus grandifolia*, and

*Tsuga canadensis*, 7 Jul 2015, B.P. Looney *BPL666* (TENN-F-067624).

*Etymology*: 'gemmate' refers to the granulose encrustations present on the stipe and pileus surface that resemble mineralized crystals.

Short diagnosis: Pileus small to medium-sized; cuticle pruinose or encrusted with granulose tufts over entire surface, dull red and more vivid and dark red towards the centre; stipe mainly pastel red; spore print cream; spores with moderately prominent, dense, amyloid warts connected to subreticulate ornamentation; suprahilar spot amyloid; hymenial cystidia clavate, apically obtuse and heteromorphous only in the central part; hyphal terminations in pileipellis frequently branched, with clavate and apically obtuse terminal cells; primordial hyphae usually 2–3-celled, cylindrical, flexuous, with acid-resistant incrustations.

Pileus small to medium-sized, 25-43 mm diam., campanulate, applanate to plano-convex, often pinched or shaldepressed in the centre, becoming nearly lowly infundibuliform with age; margin even to faintly tuberculate or crenate with lamellae edges sometimes protruding; cuticle when wet viscid near the centre, radially rugulosewrinkled, smooth, matt, peeling to 1/2-3/4 of the radius, near the margin dull red (9B4, 10B4), greyish red (10B6-10C5) to brownish red (10C5-6), towards the centre cherry red (10B8) to blood red (10C8), near the centre darker and more brown, violet brown (10E5, 10E8, 10F8), brownish red (10C6), often discolouring to greyish red (9B5, 11C6), pale red (10A3) to pinkish white (10A2), minutely pruinose or encrusted with granulose tufts over entire surface which are darker than the background. Lamellae narrow, thin, close to crowded, 9-14 at 1 cm near the pileus margin, adnate to subdecurrent, pale cream (2A2), some forking near the stipe; edges even and concolorous. Stipe  $19-48 \times 5-8$  mm, cylindrical or slightly clavate, with most of the surface covered by pastel red (10A4) dotted hue, white at apex and base, smooth, pruinose near the apex; medulla stuffed. Context white, soft, turning brownish when damaged; guaiac negative but weakly positive on the lamellae surface, PDAB negative, FeSO<sub>4</sub> pinkish; taste mild, nutty or onion; odour sweet like baked apples. Spore print cream (IIb).

**Spores** (5.5–)5.8–<u>6.2</u>–6.5(–7.0) × (4.3–)4.8–<u>5.1–</u>5.4(–5.7) µm, broadly ellipsoid, Q = (1.12–)1.14–<u>1.21</u>–1.28(–1.38); ornamentation subreticulate, of moderately large, dense [(5–)6–9(–11) in a 3 µm diam. circle] amyloid warts, up to (0.4–)0.5–0.7(–0.8) µm high, fused in short or long, occasionally branched chains [(0–)1–3(-5) fusion in the circle], connected by occasional line connections [(0–)1–3(-5) in the circle]; suprahilar spot large, not descending on hilum, amyloid. **Basidia** (17–)19–<u>22.8</u>–26.5(–29) × (7–)8–<u>8.6</u>–9.5(–11) µm, narrowly clavate or fusiform, 4-spored;

basidiola cylindrical or clavate, ca. 3-9 µm wide. Hymenial cystidia moderately numerous to numerous, ca.  $1000-1600/\text{mm}^2$ ,  $(35-)38-43.5-49(-55) \times (8-)9.5-11.1-$ 12.5(-13.5) µm, clavate to broadly clavate, apically obtuse, without appendages, originating in subhymenium and somewhat protruding over basidia, thin-walled; contents in Congo Red oleiferous or granulose, heteromorphous in the central part, hardly reacting to sulfovanillin; near the lamellae edges dispersed, smaller, (19-)26.5-34.9-43.5(-56)  $\times$  (5.5–)7–7.8–9(–9.5) µm, cylindrical to clavate. Lamellae edges fertile; marginal cells undifferentiated. Pileipellis orthochromatic in Cresyl Blue, sharply delimited from the underlying context, 80-100 µm deep, vaguely divided in a 20-40 µm deep suprapellis of loosely arranged, weakly gelatinized, ascending hyphal terminations, gradually passing to a 40-80 µm deep subpellis of strongly gelatinized, irregularly oriented, dense hyphae that are slightly wider than those near the pileus surface. Acidresistant incrustations present on primordial hyphae. Hyphal terminations near the pileus margin frequently branched, moniliform, constricted at septa, thin-walled; terminal cells (12-)17.5-27.7-38(-57) × (2.5-)3.5-4.6-6(-9) µm, mainly clavate, occasionally cylindrical, rarely narrowly fusiform, apically obtuse; subterminal cells often wider (more than 6 µm) and longer. Hyphal terminations near the pileus centre of shorter cells; terminal cells (10- $(13.5-21.1-29(-38) \times (3-)3.5-4.6-5.5(-6.5) \mu m$ , mainly cylindrical and with more frequently branched subterminal cells. **Primordial hyphae** near the pileus margin typically 2- to 3-celled, rarely 1-celled, flexuous, occasionally slightly moniliform, thin-walled; terminal cells (11-)16.5- $30.3-44(-72) \times (3-)3.5-3.9-4.5(-5) \mu m$ , mainly cylindrical, sometimes lanceolate or narrowly lageniform, with refringent or oily contents usually only in terminal cell, occasionally also in subterminal cells; acid-resistant incrustations abundant especially on the subterminal cells. Primordial hyphae near the pileus centre more often 1-celled, shorter, terminal cells  $15-20.8-27(-37) \times (2.5-$ )3-3.8-4.5(-5) µm. Cystidioid or oleiferous hyphae not observed.

Additional material studied: USA, North Carolina. Great Smoky Mountains National Park, Swain Co., Big Creek, Chestnut Branch trail, coord. 35°45′57″ N; 83°06′33″ W, mixed cove forest with *Quercus*, *Pinus*, *Fagus grandifolia*, and *Tsuga canadensis*, 9 Aug. 2012, S. Trudell *BPL275* (TENN-F-067624); ibid., Haywood Co., Baxter creek trail near the campground, mixed forest with *Fagus grandifolia*, *Quercus rubra*, *Tsuga canadensis*, coord. 35°45′05″N; 83°06′35″W, alt. ca. 600 m, 9 Aug 2012, P. Marstad (SAV F-3800); ibid., Haywood Co., Chestnut Branch Trail, Big Creek, coord. 35° 45′ 33″ N; 83° 06′ 22″ W, mixed cove forest with *Quercus*, *Pinus*, *Fagus grandifolia*, and *Tsuga canadensis*, 7 Jul 2015, B.P. Looney *BPL665* (TENN-F-



Fig. 44 *Russula laevis* (KUO *Ruotsalainen 4016*, holotype), hymenium. a Basidia. b Basidiola. c Marginal cells on the lamellae edges. d Hymenial cystidia near the lamellae edges. e Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar = 10  $\mu$ m

070376); Tennessee. White Co., Sparta, Virgin Falls Natural Area, Virgin Falls trail, coord.  $35^{\circ} 51' 13'' \text{ N } 85^{\circ} 18' 21'' \text{ W}$ , 30 Jun 2013, R.H. Petersen *BPL317* (TENN-F-069937).

Notes: Recent collections putatively named Russula subtilis represent a striking species of Russula in eastern North America and are recognized here as R. gemmata sp. nov. Three species of Russula subsect. Lilaceinae (Melzer & Zvára) Jul. Schäff. are described and widely reported from the eastern United States: R. subtilis Burl., Russula corallina Burl., and Russula uncialis Peck. Burlingham (1924) differentiates R. subtilis from R. corallina by having more fragile basidiomata, more distant lamellae that remain nearly white after drying, and a slate violet color with salmon-lilac hues compared to the pale rosy pink of R. corallina. Given the described pileus colours for R. subtilis and dark pigment of some of the pilei of the dried holotype exsiccati, it seems improbable our collections of R. gemmata are R. subtilis as a dark violet colour was never observed on any of the recent specimens collected. The pileus colour more closely matches that described for R.



**Fig. 45** *Russula laevis* (KUO *Ruotsalainen 4016*, holotype), pileipellis. **a** Pileocystidia near the pileus centre. **b** Pileocystidia near the pileus margin. **c** Hyphal terminations near the pileus centre. **d** Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red. Scale bar =  $10 \mu m$ 

corallina. Another differentiating feature is the smaller spores of our species (average length of 6.2–6.7 µm) compared to the type of R. subtilis (average length of 7.2 µm). Kibby and Fatto (1990) described R. subtilis as having smaller spores  $(6-7.7 \times 4.5-6.3 \,\mu\text{m})$  than R.  $(7-9 \times 5.5-7 \ \mu m)$ uncialis and *R*. corallina  $(6.5-9 \times 5.5-6.5 \ \mu m)$ . Examinations of recent material of other North American species of subsection Lilaceinae show larger spores in other species compared to R. gemmata. A final discordance between these recent collections and the type of R. subtilis is the density of lamellar cystidia, which is much higher in recent collections  $(1000-1600/\text{mm}^2)$  than the type of *R. subtilis* (400-600/ mm<sup>2</sup>). Given this body of evidence, we believe that this taxon represents an undescribed species in this complex and should therefore be described as a new taxon. The species likely has a restricted distribution, with reports matching this description coming from Mississippi, North Carolina, Tennessee, and West Virginia.

Russula laevis Kälviäinen, Ruotsalainen & Taipale, sp. nov.

Mycobank no.: MB 830729, Figs. 31a, 32f, 44, 45

*Holotype*: FINLAND, Lapponia Enontekionensis, Kilpisjärvi, Biological station, inside the fenced area, on calcareous soil among herbal vegatation, associated with *Betula pubescens, B. czerepanovii, B. nana, Salix* spp., 21 Aug 1995, J. Ruotsalainen 4016 (KUO).

*Etymology*: 'laevis' refers to the smooth, shiny pileus cuticle of the species.

Short diagnosis: Pileus medium-sized, infundibuliform; cuticle smooth, shiny, cream to pale brownish; hymenium with abundant lamellulae; stipe short, taste acrid after a while; spore print cream; spores subreticulate, with relatively prominent spines or warts connected by frequent lines; suprahilar spot amyloid; pileipellis an ixocutis, hyphal terminations relatively wide and apically obtuse, subterminal cells often branched; pileocystidia long, 1-celled, often with one or two central knobs.

Pileus medium-sized, 40-75 mm diam., when mature infundibuliform and deeply depressed in the centre, near the margin smooth and remaining involute for a long time; cuticle completely smooth, shiny and somewhat slimy when wet, usually remaining clean from debris, sometimes peeling near margin, ochraceous cream to pale brownish. Lamellae to 5 mm deep, in average 9 per 10 mm near the pileus margin including short lamellulae, adnexed, white, turning brownish when bruised, without a bluish tint; furcations absent, unequal with (0-)1-3(-7) lamellulae between two lamellae reaching the stipe; lamellae edge entire and concolorous. Stipe  $16-25(-53) \times 12-20$  mm, cylindrical, smooth, cream, turning brownish with age or when bruised, medulla solid. Context ca. 6 mm thick at mid-radius, pale cream, spotted ochraceous to pale brown when bruised; taste slowly acrid; odour indistinct. Spore print creme (IIb-d).

**Spores**  $(9.2-)9.5-10-10.5(-11.3) \times (7.6-)8-8.5-8.9(-9.6)$  $\mu$ m, subglobose to broadly ellipsoid, Q = (1.14–)1.16– 1.18–1.21(–1.26); ornamentation of large, moderately distant [(3-)4-5(-6) in a 3 µm diam. circle] amyloid spines or warts, 0.8-1.1(-1.3) µm high, occasionally reticulate, not isolated, fused in short or long, branched chains [1-3 fusions in the circle], connected by frequent, fine line connections [(0-)1-3 in the circle]; suprahilar spot small and irregular, amyloid or partly amyloid. Basidia (41-)49- $54.2-59.5(-65) \times 11-12.1-13(-15.5)$ um. clavate, 4-spored; basidiola cylindrical, then narrowly clavate, ca. 4.5–10.5 μm wide. Hymenial cystidia moderately  $850-900/\text{mm}^2$ , (72-)79.5-86.8-94(numerous, ca. 98)  $\times$  (7–)7.5–8–8.5(–9.5) µm, narrowly fusiform, subulate, rarely lanceolate, apically always acute-pointed, mainly with a 2-4 µm long appendage, thin-walled; contents heteromorphous, granulose-banded, turning dark grey-brown in sulfovanillin; cystidia near the lamellae edges smaller,  $(43-)49-58.8-68.5(-76) \times (5-)6.5-7.3-8(-$ 9) µm, fusiform, lanceolate or clavate, contents dispersed, usually heteromorphous only near the apical part, granulose-banded. Lamellae edges with dispersed basidia; marginal cells  $(10.5-)16.5-20.7-24.5(-27) \times (3-)4-6-$ 8(-10.5) µm, clavate or cylindrical, obtuse. Pileipellis orthochromatic in Cresvl Blue but with slightly metachromatic pileocystidia, sharply delimited from the underlying context, 110-130 µm deep, not stratified, strongly gelatinized, composed of horizontally oriented, intricate, relatively loose, but near the context denser, 3.5-7 µm wide hyphae. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin usually composed of 1-2 unbranched cells, often slightly flexuous, thin-walled. terminal cells (20-)36.5-53.6-71.5(-116)  $\times$  (4–)5–6–7(–9) µm, mainly clavate, occasionally cylindrical, apically usually obtuse and not constricted; subterminal cells mainly branched, often intricate and covered by a glutinous coating not colouring in Congo Red, usually equally wide. Hyphal terminations near the pileus centre more closely septate and more densely arranged,



**Fig. 46** *Russula madrensis* (TLXM AK3422, holotype), hymenium. **a** Basidia. **b** Basidiola. **c** Marginal cells on the lamellae edges. **d** Hymenial cystidia near the lamellae edges. **e** Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red, some elements with contents indicated schematically by a plus sign (+). Scale bar = 10  $\mu$ m



**Fig. 47** *Russula madrensis* (TLXM AK3422, holotype), pileipellis. **a** Pileocystidia near the pileus centre. **b** Pileocystidia near the pileus margin. **c** Hyphal terminations near the pileus centre. **d** Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red, some elements with contents indicated schematically by a plus sign (+). Scale bar = 10  $\mu$ m

with shorter terminal cells (20-)23.5-31.9-40.5(-56) × (3.5–)4–5–6(–7)  $\mu$ m, usually cylindrical, less frequently clavate or fusiform, apically obtuse, often with the similar glutinous coating. Pileocystidia near the pileus margin very widely dispersed, always 1-celled, lanceolate, fusiform or clavate, flexuous, thin-walled, (40-)46.5-79.8-113(> 200)  $\times$  4.5–5.9–7(–8)  $\mu m,$  apically mainly acute, frequently with one or two  $2-4 \mu m$  long appendages; contents dispersed, heteromorphous-granulose, sometimes occasionally partly crystalline, hardly reactive in sulfovanillin. Pileocystidia near the pileus centre more frequent to numerous, more conspicuous, (44-)60.5-91.1-129(-144) × (3.5–)4.5–5.7–7(–8)  $\mu$ m, apically occasionally attenuated, with dispersed but more abundant heteromorphous contents. Cystidioid or oleiferous hyphae in context not observed.

*Additional material studied*: FINLAND, Kuusamo, Iivaara southern slope, 18 Aug 1997, J. Ruotsalainen *4376* (KUO); NORWAY: Salloaivi, near the Finnish border, alt. 700 m, 14 Aug 2014, T. Taipale & V. Kälviäinen (KUO).

*Notes*: This medium-sized new species in *R*. subg. *Brevipes* is apparently a typical and common inhabitant of arctic areas as evidenced by the many environmental sequences deposited for it in GenBank (see Fig. 14).

It strongly resembles the American *R. romagnesiana* Shaffer in the composition of the pileipellis (see Buyck and Adamčík 2013a), including the slender pileocystidia with one or two central knobs at the tip. However, *R. romagnesiana* was described from a different habitat, *Quercus-Carya* woodland in Michigan, and it has much smaller spores (mean values:  $6.3 \times 5.4 \mu m$ , Q = 1.16). The UNITE species hypothesis based on collections from the temperate belt of Europe excludes similarity of our species with *R. delica* and *R. chloroides*. From a morphological point of view, however, *R. brevipes* and *R. chloroides* share more inflated, frequently ellipsoid terminal cells in the pileipellis (see Buyck and Adamčík 2013a; Sarnari 1998), which is quite different from *R. laevis* where hyphal extremities are much slenderer.

The species delimitation of *R. laevis* is uncertain, and our ITS tree shows unresolved clustering of either various geographical variants or closely related species. One surprising element in our analysis (Fig. 14) is the presence of a sequence that was apparently generated from material collected in Thailand and is clustered within all the other collections of *R. laevis* from boreal areas. We have no explanation for this.

## Russula madrensis A. Kong & Buyck, sp. nov.

Mycobank no.: MB 830730, Figs. 32g, 35a-b, 46, 47

*Holotype*: MEXICO, Chihuahua, Municipality of Bocoyna, El Pinabetal, coord. 27° 45′ 41″ N, 107° 41′ 40″ W, alt. 2350 m, *Picea chihuahuana-Pinus arizonica* forest, 30 Jul 2001, A. Kong *3422* (TXLM)

*Etymology*: 'madrensis' refers to the Sierra Madre Occidental (or Madrean Region), a mountain range in northwestern Mexico.

Short diagnosis: Basidiomata reminiscent of *Russula xer-ampelina*, with context typically developing brownish colour and fishy smell with maturing or drying; spore ornamentation of dense to very dense warts forming a subreticulate structure; hyphal terminations in pileipellis near the pileus margin composed of long attenuated terminal cells followed by 1–2 shorter and often wider sub-terminal cells; pileocystidia present, coarsely granular, hardly reactive in sulfovanillin.

**Basidiomata** in colour very similar to the European *Rus*sula xerampelina. **Pileus** medium-sized to large, first convex and soon expanding to plane; margin deflexed, smooth and only weakly striate when old; cuticle smooth and shiny near the margin, rough to rugulose and matt towards the centre, bright red (cherry red 10B8, blood red 10C8 to strawbery red 10D8), centre discoloured to brick or carrot red and with ochraceous spotts. **Lamellae** moderately distant, yellowish white (4A2), ivory (4A3); lamellulae absent; furcations frequent near the stipe; edges bright red near the pileus margin. **Stipe** cylindrical; surface strongly longitudinaly striate, white and flushed reddish white (7A2), often almost completely pink. **Context** white, turning **y**ellowish brown when bruised, mild; odour turning fishy. **Spore print** ochre (IIIc).

**Spores**  $(8-)8.8-9.4-10.1(-11.3) \times (6-)6.9-7.3-7.7(-8.2)$  $\mu$ m, broadly ellipsoid to ellipsoid, Q = (1.11–)1.22–1.3– 1.38(-1.45); ornamentation of relatively small, dense to very dense [(7-)8-11(-12) in a 3 µm diam. circle] amyloid warts, 0.3-0.5 µm high, subreticulate, rarely isolated, fused in short or long, branched chains [(1-)3-6(-7)] fusions in the circle], connected by frequent, short, fine line connections [(0-)1-4(-6) in the circle]; suprahilar spot large, amyloid. Basidia (40-)42-45.1-48(-53) × 11-12-13(-14.5) µm, clavate-pedicellate, 4-spored; basidiola first narrowly clavate or fusiform, then clavate, to 8 µm wide. Hymenial cystidia dispersed, less than 700/mm<sup>2</sup>, (73–)79–  $83.7-88.5(-94) \times (9.5-)10.5-11.5-12.5(-13)$  µm, fusiform or clavate, apically usually acute, occasionally with a small, 1-3 µm long appendage, thin-walled; contents granular or crystalline-banded, hardly greying in sulfovanillin; near the lamellae edges dispersed, smaller, (35- $)44-54.7-65.5(-73) \times (7-)7.5-8.6-10(-11) \ \mu m. \ Lamellar$ edges with dispersed basidia; marginal cells (14.5-)16.5- $21.9-27(-34) \times 4-5.8-7.5(-8.5)$  µm, mainly clavate, some also narrowly moniliform and occasionally nodulose. Pileipellis orthochromatic in Cresyl Blue, sharply delimited from the underlying context, 85-110 µm deep, twolayered; suprapellis 55-80 µm deep, strongly gelatinized, of loose, ascending, but near the surface often repent hyphae; subpellis 20-40 µm deep, less gelatinized, composed of horizontally oriented, dense, intricate, 2-5 µm wide hyphae. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin of similar structure as in some groups of the R. subg. Heterophyllidia (e.g. R. grisea), with attenuated terminal cells followed by 1-2(-3)unbranched, shorter and often wider cells; terminal cells  $(9-)14.5-25.4-36.5(-55) \times (3-)4-4.8-6(-9) \ \mu m$ , usually subulate, lageniform or subcylindrical, few shorter fusiform or ellipsoid, not monilliform or flexuous, usually inflated near the septum, apically obtuse, thin-walled or sometimes with slightly thickened walls; subterminal cells shorter and usually wider (ca 5-6.5 µm), unbranched. Hyphal termination near the pileus centre usually with smaller terminal cells  $(9-)12.5-19.9-27(-43) \times (2.5)3-$ 4.3-5.5(-8.5) µm, often inflated, fusiform or ellipsoid,



**Fig. 48** *Russula olivaceohimalayensis* (CAL 1659, holotype), hymenium. **a** Basidia. **b** Hymenial cystidia near the lamellae edges. **c** Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red, some elements with contents indicated schematically by a plus sign (+). Scale bar =  $10 \mu m$ 

frequently also cylindrical or attenuated; subterminal cells usually not shorter. **Pileocystidia** near the pileus margin relatively numerous, 1-2-celled, narrowly clavate or subcylindrical, thin-walled, terminal cells  $(16-)21.5-37.4-53(-92) \times (3.5-)4.5-5.8-7(-9) \mu m$ , apically obtuse, rarely subacute, contents hardly visible, minutely to coarsely granular, hardly reacting in sulfovanillin. Pileocystidia near the pileus centre similar in size, terminal cells  $(16-)21.5-33.4-45(-55) \times 4.5-5.4-6(-6.5) \mu m$ , cylindrical or fusiform, apically obtuse or subacute; contents even less conspicuous. **Cystidioid hyphae** in subpellis and context absent, oleiferous hyphae with yellowish, refringent contents present in subpellis.

Additional material studied: MEXICO, Chihuahua, Municipality of Bocoyna, Batuyvo, 27° 44′ 49″ N, 107° 42′ 07″ W, alt. 2375 m, coniferous forest of *Picea chihuahuana* and *Pinus arizonica*, 26 Jul 2001, A. Kong *3184*, BB 06.663 (TXLM); ibid., El Pinabetal, coord. 27° 45′ 41″ N, 107° 41′ 40″ W, alt. 2350 m, *Picea chihuahuana Pinus arizonica* forest, 30 Jul 2001, leg. A. Kong *3421* (TXLM).

*Notes*: Previous collections of *R. madrensis* have probably been mistaken for the European species *R. xerampelina* (Schaeff.) Fr., and the two samples represented in our tree (Fig. 6) confirm that. Together with another Mexican species described here, *R. sancti-pauli*, both are typical members of *Xerampelinae* with a purple or red pileus and



**Fig. 49** *Russula olivaceohimalayensis* (CAL 1659, holotype), pileipellis. **a** Pileocystidia near the pileus centre. **b** Pileocystidia near the pileus margin. **c** Hyphal terminations near the pileus centre. **d** Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red, some elements with contents indicated schematically by a plus sign (+). Scale bar =  $10 \mu m$ 

are probably strictly associated with conifers. Both Mexican species share a similar spore character: densely arranged warts forming an incomplete reticulum that distinguishes them from the European *R. xerampelina* with more prominent spines. *Russula madrensis* can easily be distinguished from both related species by the structure of the pileipellis near the pileus margin, formed by subulate or attenuated longer terminal cells contrasting to cylindrical or ellipsoid, shorter and wider subterminal cells. The related species *R. xerampelina* and *R. sancti-pauli* do not have ellipsoid short subterminal cells. Terminal cells near the pileus centre are often composed of short ellipsoid, often inflated cells in *R. madrensis*.

# *Russula olivaceohimalayensis* A. Ghosh, K. Das & R.P. Bhatt, sp. nov.

Mycobank no.: MB 830743, Figs. 18e-f, 32i, 48, 49

*Holotype*: INDIA, Pauri distrinct, Phedkhal, N 30° 09' 40.74" N; 78° 51' 14.22" E, alt. 1899 m, 11 Jul 2017, under

*Quercus* sp. in mixed forests dominated by *Quercus*, *Rhododendron* and *Cupressus*, A. Ghosh *AG* 17–1447 (CAL 1659).

*Etymology*: 'olivaceohimalayensis' refers to the olivaceous colour of the pileus and origin of the type collection from the Himalaya Mountains.

Short diagnosis: pileus medium-sized, greyish green to olive colour and greyish yellow in the centre; surface of basidiomata and context turning red and then black when bruised; taste mild; spores with usually dense, narrow, and mainly isolated spines; hymenial cystidia numerous, with abundant, heteromophous contents, fusiform, appendiculate and acute, near the lamellae edges mainly obtuse; pileipellis thick, hyphal terminations in suprapellis composed of 1–2 unbranched cells originating in branched cells below; terminal cells apically attenuated, often longer and narrower than subterminal cells; pileocystidia near the pileus margin 2–6–celled, cylindrical, with heteromorphous contents that turn grey to black in sulfovanillin.

Pileus medium-sized, 41-86 mm diam., hemispherical when young, then convex, plano-convex to applanate, broadly but shallowly depressed in the centre when mature; margin first involute to inflexed, entire when mature, obscurely tuberculate-striate; cuticle dry, viscid when wet, peeling to 1/4 of the radius, greyish green (30B4-6) to olive (2D5-7), sometimes paler towards the margin, in the centre greyish yellow (2B6-8). Lamellae 2-9 mm deep, narrowing towards the margin, relatively dense, 9-14 at 1 cm near the pileus margin, adnate to adnexed, yellowish white to pale vellow (1A2-3), often forked; lamellulae frequent, in two series; edge entire and concolorous. Stipe  $43-94 \times 11-22$  mm, cylindrical to obclavate, broader at the base, dry, smooth, longitudinally striate, chalky white (1-2A1); medulla solid. Context 3-6 mm thick in the half of the pileus radius, chalky white (1-2A1), when cut or bruised turning first red, then black, compact; with guaiac turning brown (8D5-8), with phenol pale red (10A3) and with FeSO<sub>4</sub> dark green (27E5–7); taste mild; odour indistinctive. Spore print not observed.

**Spores**  $(6.4-)7.3-7.9-8.4(-9.3) \times (5.6-)6.1-6.6-7.2(-7.8)$  $\mu$ m, subglobose to broadly ellipsoid, Q = (1.04–)1.12– 1.19–1.26(–1.36); ornamentation of relatively small, dense [6-10(-13) in a 3 µm diam. circle] amyloid spines, 0.5-0.8 µm high, mainly isolated, occasionally fused in pairs or triplets (1–3 fusions in the circle), line connections absent; suprahilar spot relatively small, amyloid. Basidia  $(30.5-)31-37-40(-45.5) \times (10-)10.5-11.3-12 \ \mu m$ clavate, 2- or 4-spored; basidiola first cylindrical then clavate, to 11 µm wide. Hymenial cystidia numerous, ca. 1800–2000/mm<sup>2</sup>,  $(34-)37.5-50.3-63(-77) \times (8-)8.5-$ 10.2-12(-14) µm, cylindrical, subclavate to clavate, apically acute, often mucronate and with an up to 8 µm long appendage, emerging up to 30 µm above basidium level, thin-walled; contents abundant, heteromorphous and partly also with crystalline components, staining dark grey-black in sulfovanillin; near the lamellae edges (33.5-)36-45.5- $55(-62) \times (9-)9.5-11.1-12.5(-13.5) \mu m$ , narrowly clavate to clavate, apically mainly obtuse-rounded, usually without an appendage. Lamellae edges fertile; marginal cells undifferentiated. Pileipellis orthochromatic in Cresyl Blue, delimited from the underlying sharply context. 125-160 µm deep, two-layered; suprapellis 60-95 µm deep, strongly gelatinized, composed of erect or ascending and near the surface loose hyphal terminations; subpellis 60-90 µm deep, less gelatinized, composed of intricate and more or less horizontally oriented hyphae. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin usually composed of 1-2 cells originating from the branched cells below, often slightly flexuous, occasionally nodulose or with lateral projections, thin-walled; terminal cells (22–)28–39.6–51(–61.5) × (2.5–)3–4.1–5(–6.5)  $\mu$ m, mainly subulate, often narrowly lageniform or fusiform,



Fig. 50 Spore drawings showing ornamentation in Melzer reagent. a Russula purpureogracilis (GENT FH12-055, holotype). b Russula sancti-pauli (PC, B. Buyck 06.494, holotype). c Russula seperina (SAV F-3156, epitype). d Russula sp. 3 (SFC 20170821-22a). e Russula subtilis (NY00760993, lectotype). f Russula sp. 4 (SFC 20170712-12). g Russula sp. 2 (PC, coll. Buyck 06.542). h Russula wielangtae (HO 593331, holotype). Scale bar = 10 μm



Fig. 51 *Russula purpureogracilis* (GENT FH12-055, holotype), hymenium. **a** Basidia. **b** Basidiola. **c** Hymenial cystidia near the lamellae edges. **d** Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar =  $10 \mu m$ 

apically acute and distinctly attenuated; subterminal cells often shorter and wider, mainly unbranched. Hyphal terminations near the pileus centre more frequently with lateral projections, sometimes forked; terminal cells often shorter and occasionally ventricose, (15-)21-32.3-43.5(-58.5 × (2–)2.5–4.1–5.5(–9), subulate or lageniform; subterminal cells more frequently branched. Pileocystidia near the pileus margin 2-6-celled, cylindrical and often constricted near septa, usually originating deep in the subpellis, thin-walled; terminal cells (13-)24-36.3-48.5(-(4.5-)5-5.9-6.5(-7) µm, ellipsoid or cylindrical, obtuse, without any incrustations, contents heteromorphous-crystalline, staining grey to black in sulfovanillin. Pileocystidia near the pileus centre with often more distant septa; terminal cells  $(18-)29.5-50.6-71.5(-112) \times (4-)5-$ 5.5–6(–6.5)  $\mu$ m. Cystidioid and oleiferous hyphae in the context absent.

Additional material studied: INDIA, Uttarakhand, Pauri district, Phedkhal, 30° 09' 42.66" N; 78° 51' 12" E, alt. 1903 m, 12 Aug 2015, A. Ghosh *AG 15–910* (CAL 1664, paratype).

*Notes*: As mentioned bellow under R. sp. 1 from Thailand, this Indian species shares near-identical microscopic features, and is part of the same well-supported small clade to which belongs also the European R. *seperina*. The latter differs from both Asian species in its more reticulated spore ornamentation and the mainly single-celled pileocystidia that are more frequently inflated in their upper portion. The blackening species of this clade are apparently



**Fig. 52** *Russula purpureogracilis* (GENT FH12-055, holotype), pileipellis. **a** Primordial hyphae near the pileus centre. **b** Primordial hyphae near the pileus margin. **c** Hyphal terminations near the pileus centre. **d** Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red, some elements with acidoresistant incrustation stained by karbolfuchsin. Scale bar = 10  $\mu$ m

unrelated to other blackening species in *R*. subg. *Polychromidia* such as to the *R. vinosa-R. claroflava* group with their primordial hyphae and incrustations turning pink in sulfovanillin (Caboň et al. 2017), or to *R. griseocarnosa* which lacks pileocystidia (Wang et al. 2009), not even to *R. decolorans* (Fr.) Fr. or *R. subdensifolia* Murrill which do not have incrusted pileocystidia (Adamčík and Buyck 2011a; Adamčík et al. 2015). All three blackening species studied here have dense spore ornamentation and it is possible that this character defines the group, but more observations on other blackening species are needed to decide this.

*Russula purpureogracilis* F. Hampe, Looney & Manz, sp. nov.

Mycobank no.: MB 830744, Figs. 24e, 50a, 51, 52

Holotype: THAILAND, Mae Taeng district, province Chiang Mai, around temple near Mushroom Research

Center (MRC), 19° 06′ 38″ N; 98° 44′ 32″ E, alt. 1055 m, on red clay soil with *Dipterocarpus* sp., 25 Jun 2012, F. Hampe & B. Looney *BPL186* (GENT FH12-055).

Etymology: 'purpureogracilis' refers to the colour of the pileus and thin-fleshed basidiomata with long narrow stipe. Short diagnosis: pileus small; cuticle finely granulose and towards the margin finely areolate, grey or brownish violet; stipe longer than pileus diameter and covered by pastel red granulations; spore print cream; spore ornamentation locally subreticulate; suprahilar spot large, amyloid; hymenial cystidia fusiform, relatively wide, with large refractive body in apical part; hyphal terminations in pileipellis near the pileus centre forming a dense hymeniderm, near the pileus margin locally loosely arranged, densely septate, originating in pseudoparenchymatic subpellis of inflated hyphae; primordial hyphae inconspicuous, small, scattered, with acid-resistant incrustations, near the pileus margin 2-4-celled, near the pileus centre 1-2-celled. **Pileus** small, 10–35 mm diam., convex to applanate, in the centre slightly depressed with low broad umbo; margin usually finely striate; cuticle dry, matt, finely granulose, towards the margin finely areolate, hardly peeling, in the centre violet-brown (11F4, 11F5), dark ruby (12F4) to dark violet (16F4), sometimes greyish ruby (12D4) when cracked with age, sometimes towards the margin paler brownish violet (11D6), near the margin greyish ruby (12D6), greyish Magenta (13E4, 14D6) or greyish red (11C5, 11C6). Lamellae narrow, 1-3 mm deep, dense, adnate, cream becoming dark cream with age; lamellulae and furcations absent; edge even and concolorous. Stipe  $30-60 \times 3-9$  mm, cylindrical, narrowed towards the base, sometimes slightly compressed, white, covered with fine pastel red (10A4 to 10A5) granulations, stuffed to hollow. Context white, unchanging, fragile in pileus, flexible in stipe; reaction with guaiac after 5 s negative at the stipe and light blue (++) on lamellae, with FeSO<sub>4</sub> almost negative (pale orange), and with sulfovanillin purple red; taste first mild then bitter; odour inconspicuous. Spore print cream (IIa).

**Spores** (6.9–)7.2–<u>7.5</u>–7.9(–8.2) × (5.8–)6.2–<u>6.5</u>–6.8(–7.5) µm, subglobose to broadly ellipsoid, Q = (1.07–)1.11– <u>1.15–1.2</u>(–1.26); ornamentation of relatively large, moderately distant to dense [(3–)4–7(–8) spines in a 3 µm circle] amyloid spines, 0.8–1(–1.2) µm high, locally subreticulated, rarely isolated, occasionally fused in pairs, triplet or rarely short chains [0–3(–4) fusions in the circle], connected by occasional, short or long, fine to thick line connections [(0–)1–3(–4) in the circle]; suprahilar spot moderately large, amyloid. **Basidia** (25–)28–<u>32.2</u>–36(– 40) × (9–)10–<u>10.8</u>–11.5(–12.5) µm, clavate to obpyriform, 4-spored; basidiola mainly clavate, ca. 4.5–11 µm wide. **Hymenial cystidia** widely dispersed, ca. 240/mm<sup>2</sup>, (32.5–)42–52–61(–79) × (9.5–)11–13–15(–16.5) µm, mainly fusiform, occasionally clavate, apically mainly obtuse, with a small, 1-3 µm long appendage, thin-walled or occasionally with slightly thickened walls (to 1 µm thick); contents with a large refractive body in the apical part, otherwise optically empty or with dispersed inclusions, no reaction in sulfovanillin; near the lamellae edges numerous,  $(31-)40-46-52(-55) \times (5-)5.5-7-8(-10)$  µm, narrowly fusiform, cylindrical, lageniform, apically usually obtuse and rarely with a small appendage, usually with slightly (ca. 0.5 µm) thickened wall, contents optically empty or with few, dispersed refringent inclusions. Lamellae edges sterile, mainly composed of cysditia; marginal cells undifferentiated. Pileipellis orthochromatic in Cresyl Blue, sharply delimited from the underlying context, 65-85 µm deep, but near the pileus centre 90-110 µm deep, vaguely divided into a 25-40 deep suprapellis of ascending to erect, strongly gelatinized hyphal terminations, gradually passing to a pseudoparenchymatic, 40-60 µm deep subpellis of mainly inflated, densely arranged, ellipsoid to globose, 4-12 µm wide cells. Acid-resistant incrustations present. Hyphal terminations near the pileus margin variable, some erect and densely arranged, others loose and irregularly oriented, densely septate and composed of 1-3 cells, thin-walled, terminal cells  $(7.5-)8.5-13-17.5(-26) \times (3.5-)4-4.9-$ 5.5(-7) µm, mainly cylindrical or ellipsoid, occasionally lageniform, pyriform or clavate, apically obtuse and often with glutinous coating not colouring in any reagent; subterminal cells and cells below occasionally branched, few inflated, ellipsoid or cylindrical, equal in size, originating from longer and narrower cells of subpellis, 3-9 µm wide. Hyphal terminations near the pileus centre forming a dense hymeniderm, always erect and originating from 3-5 ranks of inflated ellipsoid or subglobose cells of pseudoparenchymatic subpellis often thick walled, terminal cells  $(5-)8-11-14(-16.5) \times (3.5-)4.5-5.4-6.5(-7) \ \mu m$ , similar in shape, maximum two originating from a single subterminal cell. Primordial hyphae near the pileus margin inconspicuous, dispersed but also in small fascicules, (1-)2-4-celled, cylindrical, terminal cells (11.5-)12-19.1- $26(-37) \times (3-)3.5-4.4-5(-6) \mu m$ , apically obtuse, only occasionally slightly constricted, contents with few dispersed granulations or refringent patches only in the terminal cells, no reaction in sulfovanillin, with few large droplets of acid-resistant incrustations, in sulfovanillin incrusted with small, red droplets at the terminal cells and more strongly incrusted in the subpellis. Primordial hyphae near the pileus centre dispersed, narrow, cylindrical and usually protruding over other hyphal terminations, 1-2celled, terminal cells  $(7-)9.5-14.3-19(-25) \times (2.5-)3-$ 3.9-5(-6) µm, cylindrical, apically obtuse, contents dispersed, granulose. Cystidioid or oleiferous hyphae in the context absent.

Additional material studied: THAILAND, Mae Taeng district, province Chiang Mai, around temple near Mushroom Research Center (MRC), 19° 06′ 38″ N; 98° 44′ 32″ E, alt. 1055 m, on red clay soil with *Dipterocarpus* sp., 25 Jun 2012, F. Hampe & B. Looney *BPL188* (GENT FH-12-057)

*Notes*: This tropical Asian species is part of a well-supported clade consisting of *R. castanopsidis* Hongo, *R. darjeelingensis* S. Paloi, K. Acharya & K. Das and some samples from Florida. Our analysis suggests that the American samples represent the sister clade for the three Asian species, thereby conforming to a generally observed phenomenon that North American and Asian species seem to be often more closely related than either group from these two continents are with European species. Both *R. purpureogracilis* and *R. castanopsidis* appear to have a wide distribution in Asia, although different geographically restricted species or infraspecific taxa might be involved but an answer to this question would need a wider sampling.

When comparing the morphology of the three Asian species, one is struck by the many shared similarities: all have a slender, hollowing stipe that is distinctly longer than the pileus diameter, all have a pseudoparenchymatic structure of the pileipellis resulting in a pileus surface that is dull, dry and hardly peeling, but easily rupturing or fissuring, all are lacking well-differentiated pileocystidia, but have encrusted extremities that surpass the general level of the pseudoparenchyma, all possess rather short but wide basidia that produce spores with a near identical ornamentation of isolated spines with few line connections, all produce white or near-white spore prints and have a context that is mild and unchanging when cut. The main difference between these species is the overall colour, which is white for R. darjeelingensis, yellowish greyish brown for R. castanopsidis and brownish violet for our new Thai species. Russula castanopsidis was never subjected to a phylogenetic analysis, while R. darjeelingensis was suggested to belong in R. subg. Polychromidia subsect. Paraintegrinae. Our phylogeny suggests that this lineage deserves it own taxonomic group probably at the rank of subsection.

Russula sancti-pauli A. Kong & Buyck, sp. nov.

Mycobank no.: MB 830745, Figs. 35c-d, 50b, 53, 54

Holotype: MEXICO, La Malinche Volcano, Cañada Grande, in *Pinus hartwegii* woodland, 26 Jul 2006, B. Buyck & A. Kong *B. Buyck 06.494* (PC)

*Etymology*: 'sancti-pauli' refers to the popular name of this edible fungus 'San Pablero', a name derived from the Spanish 'San Pablo' or Saint Paul.

Short diagnosis: pileus medium-sized to large; cuticle continuous, smooth in maturity, developing predominantly



Fig. 53 Russula sancti-pauli (PC, B. Buyck 06.494, holotype), hymenium. a Basidia. b Basidiola. c Marginal cells on the lamellae edges. d Hymenial cystidia near the lamellae edges. e Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar = 10  $\mu$ m

red-purple colour and more variegated in the centre; surface of basidiomata and context turning yellow-brown after bruising; odour weak fishy, becoming strong when drying; spore print dark ochre, spores subreticulate, with dense, small warts; suprahilar spot large, amyloid; hyphal terminations in pileipellis loose, composed of 1-3 unbranched cells arising from dense and branched cells below; hyphal terminations variable in shape and apically obtuse, occasionally appendiculate; pileocystidia mainly 1-2-celled, narrowly clavate and with dispersed granular contents. Pileus medium-sized to large, (35-)65-75(-125) mm diam., convex, expanding to plane; centre depressed when mature; margin smooth, regular or undulate near the margin; cuticle not disrupted in smaller fragments but continuous and smooth, glabrous, young with pruinose aspect towards the margin, dull but slightly viscid when wet, peeling to ca. 1/3 of the radius, very young specimens predominantly lemon yellow, with red-purple tints developing gradually, sometimes near the margin with orange, olive or reddish and in the centre with yellowish



**Fig. 54** *Russula sancti-pauli* (PC, *B. Buyck 06.494*, holotype), pileipellis. **a** Pileocystidia near the pileus centre. **b** Pileocystidia near the pileus margin. **c** Hyphal terminations near the pileus centre. **d** Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red, some elements with contents indicated schematically by a plus sign (+). Scale bar =  $10 \mu m$ 

(ochraceous orange) and reddish areas, others dark purplish red with blackish purple tints in the centre. Lamellae 6-9 mm deep, moderately distant, 10 at 1 cm near the pileus margin, adnexed, rounded near pileus margin, cream but with distinct ochre flush when mature; lamellulae absent; furcations rare or sometimes more frequent near the stipe attachment, with radial anastomosing veins in dorsal part; edges even and concolorous. Stipe  $45-55 \times 21-23$  mm, usually slightly narrowing upwards, shorter than pileus diameter, longitudinally wrinkled, not pruinose, chalk white when young, often tinged with pink, quickly becoming yellow-brown from handling, stout; medulla without cavities, stuffed. Context 7 mm thick above the lamellae attachment, white, quickly becoming grey-brown inside stipe, compact and fragile in lamellae; reaction with FeSO<sub>4</sub> greenish grey on the stipe surface, less

so on the stipe interior; taste mild; odour weak fishy when fresh. **Spore print** dark ochre (IIId).

**Spores** (7.6–)8.4–9.1–9.8(–10.6)  $\times$  (6.2–)6.9–7.5–8.2(–9.3)  $\mu$ m, subglobose to broadly ellipsoid, Q = (1.03-)1.14-1.21-1.28(-1.39); ornamentation of relatively small, moderately distant to dense [(5-)6-8(-10) in a 3 µm diam. circle] amyloid warts, 0.3-0.5(-0.7) µm high, subreticulate, rarely isolated, fused in short to long, irregularly oriented chains or crests [(0-)1-4(-5)] fusions in the circle, connected by occasional, short, fine line connections [(0-)1-3] in the circle]; suprahilar spot large, amyloid. Basidia (42-)44.5- $50-55.5(-60) \times (11-)11.5-12.4-13.5(-15)$  µm, clavatepedicellate, 4-spored; basidiola first cylindrical, utriform or ellipsoid, then clavate, ca. 6-12 µm wide. Hymenial cystidia moderately numerous, 650-1200/mm<sup>2</sup>, (60-)77- $87.6-98(-108) \times (8.5-)9-10.5-12(-13)$  µm, mainly fusiform, apically acute, often with a 1-3.5(-10.5) µm long appendage, thin- to slightly thick-walled ( $< 0.5 \mu m$ ); contents with only dispersed granulations and sometimes also partly crystalline, weakly greying in sulfovanillin; near the lamellae edges narrower, (58-)66.5-79.9-93.5(- $102) \times (7.5-8.5-9.5-10.5 \ \mu\text{m}$ , similar in shape but apically often obtuse, contents dispersed, occasionally optically empty. Lamellar edges with dispersed basidia; marginal cells  $(12.5-)16-24.1-32(-38) \times 3-4.2-5(-6)$ µm, subcylindrical or fusiform, often nodulose or diverticulate, sometimes apically acute or constricted and resembling small cystidia. Pileipellis orthochromatic in Cresyl Blue, not well-delimited from the underlying context, ca. 200-270 µm deep, not distinctly stratified, strongly gelatinized throughout except near the pileus surface and the context, suprapellis 100-150 µm deep, of loose and irregularly oriented hyphae, gradually passing to 150-170 µm deep subpellis of intricate, horizontally oriented, 3-12 µm wide hyphae. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin composed of 1-3 unbranched cells arising from densely arranged and branched cells below, thin-walled, terminal cells  $(11.5-)20.5-32.5-45(-68.5) \times (3-)3.5-4.7-6(-8)$ μm, very variable in shape and size, mainly subcylindrical, often also fusiform or clavate, with obtuse or constricted apices, often also appendiculate, often distinctly moniliform, sometimes flexuous or nodulose; subterminal cells branched or not, equally wide and usually also equally long. Hyphal terminations near the pileus centre terminal cells  $(11-)14.5-21.6-28(-43.5) \times (3.5)4-5.1-6(10) \mu m$ , less irregular in shape, mainly cylindrical, clavate or fusiform-pedicellate, apically obtuse and not constricted, usually not distinctly moniliform but often with one central constriction, thin-walled or occasionally with slightly thickened walls. Pileocystidia near the pileus margin relatively frequent, 1-2(-3)-celled, narrowly clavate or subcylindrical, thin-walled, few with short apical or lateral

projections (appendages or diverticules), terminal cells  $(23-)29-\underline{47.3}-66(-92) \times (4-)4.5-\underline{5.7}-7(-7.5) \ \mu\text{m}$ , apically mainly obtuse, contents finely granular, often dispersed or nearly absent, hardly reacting in sulfovanillin, often with yellowish pigments. Pileocystidia near the pileus centre similar, terminal cells  $(24-)33.5-\underline{49.8}-66(-76) \times (4.5-)5.5-\underline{6.3}-7.5(-8.5) \ \mu\text{m}$ . Oleiferous hyphae very numerous in the subpellis just above the context, contents refringent, yellow.

Additional material studied: MEXICO, Tlaxcala, La Malinche volcano, Cañada Grande, in *Pinus hartwegii* woodland, 26 Jul 2006, B. Buyck & A. Kong (PC, coll. B. Buyck 06.499, 06.502, 06.504); Tlaxcala, La Malinche National Park, SE slope of La Malinche volcano, Miunicipality of Trinidad Sánchez Santos, coord. 19° 11' 48" N, 97° 58' 38" W, alt. 3000 m, *Pinus montezumae* forest, 31 Aug 1989, leg. A. Montoya *AK* 487 (TLXM); ibid., E slope of La Malinche Volcano, 0.5 km SW of La Caprina (monitoring house), coord. 19° 14' 27" N, 98° 00' 37" W, alt. 3000 m, 10 Jun 1992, A. Kong *AK2272* (TLXM); ibid., Municipality of Huamantla, Cañada Grande, E slope of La Malinche Volcano, coord. 19° 14' 27" N, 98° 00' 37" W, alt. 3450 m, *Abies religiosa-Pinus hartwegii* forest, 28 Aug 1992, leg. A. Kong *AK 2451* (TLXM).

Notes: This is a well-known, edible species traded in the state of Tlaxcala. The dark purple pilei are reminiscent of the European R. xerampelina that is indeed a related species together with another species described here based on collections from Mexico (see the discussion on R. madrensis). Russula sancti-pauli differs from any known members of R. xerampelina lineage by cylindrical and obtuse hyphal terminations near the pileus margin in combination with dense warts forming subreticulate spore ornamentation. Bazzicalupo et al. (2017) recognised among collections of Benjamin Woo from the Pacific Northwest of North America the operational unit "Woo sp. 61" which corresponds to the European R. xerampelina according to the authors. However, all ITS2 sequences of those collections clustered together and form a clade related rather more to the Mexican R. sancti-pauli than to the European species.

Russula seperina Dupain, Bull. Soc. Mycol. France 29: 181. 1913.

Mycobank no.: MB 209782, Figs. 18g, 50c, 55, 56

*Lectotype*: plate VII in Dupain, Bull. Soc. Mycol. France 29: 185. 1913.

*Epitype* (designated here; identifier no.: MBT 386774): SLOVAKIA, Tríbeč Mts., Žibrica, SE slopes of Vápeník hill, alt. ca. 400 m, 48° 21′ 39″ N; 18° 09′ 43″ E, on calcareous but also nitrogen-rich soil in a deciduous forest of *Quercus* and *Tilia*, 1 Sep 2010, S. Adamčík (SAV F-3156).



Fig. 55 *Russula seperina* (SAV F-3156, epitype), hymenium. **a** Basidia. **b** Basidiola. **c** Marginal cells on the lamellae edges. **d** Hymenial cystidia near the lamellae edges. **e** Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar = 10  $\mu$ m

Original description: Medius, magnitudine 5 à 7 cent. latus. Pileus sphaericus, expansus dein depressus, primum roseo-luteolus dein rubro-purpureus, tandem nigricans, innatis nigris fibrillis notatus; tenui separabili pelliculâ a humiditate viscosâ et siccitate nitidâ tectus. Margo primum laevis demum sulcata tuberculosaque. Stipes farctus, tum cylindricus, tum apice paulo attenuatus, primum firmus, dein spongiosus corticatusque, striatus et apice pruinosus, albus dein cinerascens, demum praecipue deorsum nigricans. Lamellae maxime fragiles, primum albae cremore tinctae, dein flavo citrinae, confertae, crassae, adnatae, latae, venis unitae antice latiores, tactu rubentes dein nigricantes. Caro in primâ aetate firma, dein grumosa lacunosaque, mitis avellanae sapore, alba, tactu rubens dein nigricans. Sporae citrino-flavae, reticulato-cristulatae, paulo ellipsoidae, ocellam ostendentes.

**Pileus** medium-sized, 62–72 mm diam., convex, soon expanding to plane, widely but shallowly depressed in the centre, often lobate, not striate or only indistinctly up to



**Fig. 56** *Russula seperina* (SAV F-3156, epitype), pileipellis. **a** Pileocystidia near the pileus centre. **b** Pileocystidia near the pileus margin. **c.** Hyphal terminations near the pileus centre. **d** Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red. Scale bar =  $10 \mu m$ 

5 mm from margin when old; cuticle shiny and viscid when wet, dry, matt and smooth near the margin, rough near the centre, peeling to 1/3 of the pileus radius, pastel red (10A5), red (10B6), dull red (10B3), brownish red (10C7), towards the centre greyish red (10D4, 10D5), violet-brown (10E6); centre more brown and variegated with grey-brown to yellowish grey colours, e.g. nutria brown 5E3, greyish orange 5B3 or Madeira 8E5, Lamellae to 7 mm deep, moderately distant, ca. 7 at 1 cm near the pileus margin, adnexed, light yellow (4A4); lamellulae absent; furcations sometimes frequent, especially near the stipe; edge entire and concolorous. Stipe  $40-55 \times 16-22$  mm, cylindrical or tapering towards the base, moderately strongly longitudinally striate, first white, later becoming pale grey, blackish grey to black locally, stuffed. Context compact; when bruised turning first red, later grey to completely black; taste mild; odour indistinct and somewhat unpleasant when old. Spore print yellow (IVc).

**Spores**  $(8.2-)8.5-9-9.5(-9.9) \times (6.4-)6.8-7.2-7.6(-8.1)$  $\mu$ m, broadly ellipsoid, Q = (1.17–)1.21–1.25–1.29(–1.35); ornamentation of moderately large, moderately distant to dense [6-8(-9) in a 3 µm diam. circle] amyloid warts, 0.6-0.9 µm high, locally reticulate, rarely isolated, frequently fused in short or long, often branched chains [2-5(-6) fusions in the circle], connected by occasional line connections [0-2(-3)] in the circle]; suprahilar spot large, amyloid. **Basidia** (34–)40–46.3–52.5(–57) × (11– )12-12.8-14 µm, clavate-pedicellate, rarely fusiform, 4-spored; basidiola first cylindrical, then clavate, often flexuous-nodulose and sometimes with lateral outgrowths, ca. 4-11 µm wide. Hymenial cystidia numerous, ca.  $1700-2100/\text{mm}^2$ , (66-)73.5-83.1-92.5(-102) × (8-)9.5-10.3–11(–12) µm, clavate or fusiform, pedicellate, apically usually acute and mucronate, with a (4-)7-14(-17) µm long appendage, often with slightly thickened walls; contents slightly heteromorphous or with only dispersed inclusions, sometimes optically empty, turning dark greybrown in sulfovanillin; near the lamellae edges numerous, usually smaller,  $(31-)45-57-68.5(-73) \times (6.5-)7.5-8.8-$ 10(-11.5) µm, 1-celled but rarely also 2-celled, fusiform of clavate, apically often obtuse, occasionally with 1-5 µm long appendage; contents more often optically empty. Lamellae edges sterile, marginal cells (19-)22-27.6-33(- $39) \times (4-)4.5-5.8-7(-7.5)$  µm, narrowly clavate, subcylindrical or fusiform, sometimes in shape a transition to cystidia, often flexuous-nodulose. Pileipellis weakly metachromatic in Cresyl Blue, not sharply delimited from the underlying context, 65-100 µm deep, vaguely divided into a strongly gelatinized, 45-65 µm deep suprapellis composed of ascending and loose hyphal terminations and gradually passing into a 25-40 µm deep subpellis of very dense, less gelatinized, horizontally oriented, ca. 2-5 µm wide hyphae. Acid-resistant incrustation absent. Hyphal terminations near the pileus margin narrow and branched, thin-walled; terminal cells (18-)23.5-33.5-43.5(-63)  $\times$  2.5–3.1–3.5 µm, mostly narrowly cylindrical, usually entire, apically obtuse or constricted to 1-2 µm; subterminal cells usually equally long and wide, mainly branched, flexuous or nodulose and often embedded into the intricate hyphae of subpellis. Hyphal terminations near the pileus centre narrower, denser and more erect; terminal cells (14–)25–32.2–39(–49)  $\times$  2–3.6–5(–9.5) µm, subulate or cylindrical, rarely lageniform or pyriform, mainly inflated near the base. Pileocystidia near the pileus margin mainly 1-celled, less frequently 2-celled, clavate-pedicellate, often with strongly constricted and attenuated basal part, originating upper or deeper in the subpellis, thinwalled; terminal cells (25.5-)33.5-49.1-64.5(-84) × (4- $)5-\underline{6.9}-8.5(-10.5)$  µm; contents heteromophous-banded, turning dark grey to black in sulfovanillin. Pileocystidia near the pileus centre narrower; terminal cells (18-)35<u>51.9</u>–69(–84) × (3.5–)4–<u>5</u>–6(–7.5)  $\mu$ m, usually with very long, attenuated and flexuous basal part. **Cystidioid hyphae** in subpellis frequent, dispersed in context.

Additional material studied: ITALY, Sardinia, Tempio Pausania, Baldo, in Mediterranean maquis (scrubby) vegetation associated with *Quercus suber*, 4 Nov 2000, A. Verbeken 2000-135 (GENT).

Notes: R. seperina is one of three closely related species described in this study in which the context turns first red and then black (see notes to Asian species R. sp. 1 and R. olicaveohimalayensis). The European R. seperina differs from the two Asian species in subreticulate spore ornamentation, narrower hyphal terminations and 1-2-celled pileocystidia. Russula seperina might be easily recognised also by the purple pilei, but paler coloured variants of the species have been reported (Sarnari 2005) and these are in colour reminiscent of the Asian species. Both Romagnesi (1967) and Sarnari (2005) reported acid-resistant incrustations that were not observed in this study neither for R. seperina or any other species of this clade. Our study proved that this species has a wide distribution in Europe and is associated with Quercus spp. on calcareous soil in thermophilous areas.

## Russula sp. 1

Figures 18h, 32h, 57, 58



Fig. 57 *Russula* sp. 1 (GENT FH12-061), hymenium. **a** Basidia. **b** Basidiola. **c** Hymenial cystidia near the lamellae edges. **d** Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar =  $10 \mu m$ 



Fig. 58 *Russula* sp. 1 (GENT FH12-061), pileipellis. a Pileocystidia near the pileus centre. b Pileocystidia near the pileus margin. c Hyphal terminations near the pileus centre. d Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red. Scale bar =  $10 \mu m$ 

Short diagnosis: pileus small to medium-sized, white and towards centre dull yellow and grey; surface of basidiomata and context turning red and then black when bruised; taste mild; spore print ochre; spores with usually dense, narrow, and mainly isolated spines; hymenial cystidia with poorly defined contents, fusiform, appendiculate and acute, near the lamellae edges obtuse; hyphal terminations in the pileipellis branched usually at the subterminal cell or the cell just below, with cylindrical obtuse or apically attenuated terminal cells; pileocystidia near the pileus margin often 3–6-celled, constricted at septa and with well-defined contents that hardly react in sulfovanillin.

**Pileus** small to medium-sized, 20–65 mm diam., planoconvex to applanate, depressed in the centre; margin even and not distinctly striate even when mature; cuticle dry, matt, smooth and with minuscule squamules towards the margin, hardly peeling to 1/3 of the radius, first completely white, later from the centre towards the margin become a dull yellow (3B3) and grey (3F3) to coal grey (3F1). **Lamellae** 4–4.5 mm deep, moderately distant, adnateemarginate, white, later dull yellow (3B3), after handling staining reddish brown (8D6), brownish grey (8C2) to black, without lamellulae; edge even and concolorous. **Stipe**  $30-40 \times 8-11$  mm, obclavate; surface dry, matt, rugulose, white, staining reddish brown (8D6) then black-ish on handling; medulla stuffed. **Context** firm, lamellae fragile, white, turning red and then black when bruised; taste mild; odour indistinct; reaction with guaiac after 5 s strong and intensively blue, with FeSO<sub>4</sub> greenish with a pink outline. **Spore print** ochre (IIIc).

**Spores**  $(6.8-)6.9-7.4-7.8(-8.5) \times (5.3-)5.7-6.0-6.4(-7.1)$  $\mu$ m, broadly ellipsoid, Q = (1.06–)1.16–1.22–1.29(–1.45); ornamentation of relatively small, moderately distant to dense [5-9(-10) in a 3 µm diam. circle] amyloid spines, 0.5-0.9(-1.2) µm high, mainly isolated, occasionally fused in pairs or small groups [0-3(-4)] fusions in the circle], line connections dispersed or absent; suprahilar spot large, amyloid. Basidia (26-)30-33-36(-38) × (10.5-)11-12.9-14.5(-16.5) µm, clavate or fusiform, 4-spored; basidiola cylindrical or clavate, ca. 6-10 µm wide. Hymenial cystidia probably widely dispersed, (30.5-)41-55.3-69.5(-80)  $\times$  (8.5–)11–12.8–14.5(–16) µm, mainly clavate, less frequently fusiform, sometimes almost cylindrical or also centrally constricted, apically usually acute or mucronate, with a 2-5(-10) µm long appendage, thin-walled; contents mainly heteromorphous-crystalline, occasionally optically empty or with only few refringent inclusions, slightly yellow-brown in KOH, hardly reacting in sulfovanillin; near the lamellae edges dispersed, (36-)39-47.3-55.5(- $(68) \times (7.5-)9-11.1-13.5(-14.5) \ \mu m$ , slender, cylindrical to subclavate, sometimes slightly flexuous or constricted, apically obtuse, contents less heteromorphous. Lamellae edges fertile; marginal cells undifferentiated. Pileipellis orthochromatic in Cresyl Blue, gradually passing to the underlying context, ca. 150 µm deep, not gelatinized, vaguely divided into a suprapellis composed of ascending hyphal terminations, and a subpellis of irregularly oriented, moderately dense, 3-5 µm wide hyphae. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin usually branched at the subterminal cells or the cells just below, occasionally slightly flexuous, thin-walled; terminal cells  $(18.5-)25-36.1-47(-70) \times (2-)3-4-5$ (-5.5) µm, cylindrical, obtuse but occasionally apically constricted or attenuated and wider near the base; subterminal cells usually equal in size, rarely with lateral projections, ca. 2.5–7 µm wide. Hyphal terminations near the pileus centre densely septate, with shorter terminal cells  $18-25.6-33.5(-45) \times (2-)2.5-3.4-4(-5) \mu m$ ; subterminal cells occasionally wider to ventricose. Pileocystidia near abundant, mainly the pileus margin 3–6-celled,

occasionally 1-2-celled, cylindrical to narrowly clavate, sometimes slightly flexuous or moniliform, near septa often constricted, often originating deep in the subpellis, thinwalled; terminal cells  $(12.5-)15-22.9-31(-47) \times (4-)4.5-$ 5.6-6.5(-8.5) µm, cylindrical, rarely lageniform, apically obtuse or rarely somewhat constricted; contents heteromorphous, banded, hardly reacting in sulfovanillin. Pileocvstidia near the pileus centre mainly 1(-3)-celled and often originating from branched subterminal cells; terminal  $(18-)20-25.9-32(-39) \times (3-)4-4.7-5.5(-6)$ cells μm. Cystidioid hyphae only present in the subpellis and scattered, contents often banded, not observed in the context. Material studied: THAILAND, Mae Taeng district, province Chiang Mai, around temple near Mushroom Research Center (MRC), 19° 06' 38" N; 98° 44' 32" E, alt. 1055 m, scattered on soil under Lithocarpus sp., Dipterocarpus sp. and Castanopsis sp., 27 Jun 2012, F. Hampe & B.P. Looney BPL201 (GENT FH12-061).

*Notes*: This reddening and blackening *Russula* from the tropical forests in Thailand forms a well-supported clade with the Indian *R. olivaceohimalayensis* (described in this study) and the European *R. seperina* (Fig. 5). Although the pileus colour might be much more variable in this Thai species, particularly because *R. seperina* is reputed for its high variability of overall colour, one of a few morphological differences between *R.* sp. 1 and the Indian *R.* 



**Fig. 59** *Russula* sp. 2 (PC, coll. *Buyck 06.542*), hymenium. **a** Basidia. **b** Basidiola. **c** Marginal cells on the lamellae edges. **d** Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red, some elements with contents indicated schematically by a plus sign (+). Scale bar = 10  $\mu$ m



**Fig. 60** *Russula* sp. 2 (PC, coll. *Buyck 06.542*), pileipellis. **a** Pileocystidia near the pileus centre. **b** Pileocystidia near the pileus margin. **c** Hyphal terminations near the pileus centre. **d** Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red, some elements with contents indicated schematically by a plus sign (+). Scale bar = 10  $\mu$ m

*olivaceohimalayensis* remains for the moment the pileus colour. The main microscopic difference is the hyphal terminations near the pileus margin, which are cylindrical and obtuse or apically contricted in the Thai species and subulate, narrowly lageniform or fusiform and apically attenuated and acute in the Indian species.

#### Russula sp. 2

#### Figures 35e, 50g, 59, 60

*Short diagnosis*: Pileus medium-sized; cuticle smooth, slightly viscid, dark ruby and later dark brown in the centre; basidiomata surface and context turning yellow or brown after bruising; odour slightly fishy when fresh; spore print yellow; ornamentations of warts forming long, sometimes branched chains; suprahilar spot large, amyloid; marginal cells well defined, moniliform, cylindrical or fusiform; pileipellis strongly gelatinized; terminal cells near the pileus margin ascending, narrow, long, attenuated,

near the pileus centre comparatively shorter; pileocystidia 1–2-celled, clavate or cylindrical, with inconspicuous, dispersed granular contents that hardly react in sulfovanillin.

Pileus medium-sized, 55–70 mm diam., applanate, depressed in the centre, regular; margin smooth, then shortly striate up to 5-7 mm from the margin; cuticle smooth, peeling to 1/3 of the pileus radius, glabrous, matt, slightly viscid and shiny, first dark ruby (12E8) to reddish brown (8D6, 9E7), later dark ruby (12F8-7) at the margin and dark brown (7F5-6) in the centre. Lamellae 5-9 mm deep, moderately distant, ca. 10 at 1 cm near the pileus margin, shortly adnate to adnexed, cream, no lamellulae, rarely anastomosing or forking; edge even and concolorous. Stipe  $40-80 \times 8-17$  mm, subcylindrical, usually longer than pileus diameter, smooth, whitish but becoming yellowish white (3A2), pale yellow (3A4-B4) or honey vellow (5D6) from handling; medulla stuffed. Context 7 mm thick above lamellae attachment, white, yellowbrown with age, quickly becoming grey-brown inside stipe; taste mild, odour not strong, fishy, turning greenish grey with FeSO<sub>4</sub> on the stipe surface. Spore print yellow (IVc). **Spores**  $(7.6-)8-8.4-8.9(-9.5) \times (6.6-)6.9-7.3-7.6(-8)$  $\mu$ m, subglobose to broadly ellipsoid, Q = (1.09–)1.12– 1.16–1.21(–1.23); ornamentation of moderately large, moderately distant [(3-)4-6(-7) in a 3 µm circle] amyloid warts, 0.7-1 µm high, rarely isolated, mainly fused in long and sometimes branched chains [1-3(-5)] fusions in the circle], connected by short, occasional, fine line connections [(0-)1-3(-4) in the circle]; suprahilar spot large, amyloid. Basidia 35–41.2–48  $\times$  10.5–12.5–14.5 µm, clavate or fusiform, 4-spored; basidiola cylindrical, ellipsoid or clavate, ca. 3.5-10 µm wide. Hymenial cystidia moderately numerous, ca. 1000/mm<sup>2</sup>, (51-)59-67-75(-78)  $\times$  (9–)10.5–12–13.5(–14.5) µm, mainly clavate, less frequently fusiform, apically obtuse or acute, occasionally mucronate or with a small, 1-2(-6) µm long appendage, thin-walled; contents poorly defined, some turning strongly vellow-brown in warm potassium solution, no reaction in sulfovanillin; cystidia near the lamellae edges not observed. Lamellae edges sterile; marginal cells (16-)23- $34.8-46.5(-61) \times 4-5.4-6.5(-8) \ \mu m$ , cylindrical or fusiform, apically constricted, moniliform, thin-walled. Pileipellis orthochromatic in Cresyl Blue, sharply delimited from the underlying context, 130-170 µm deep, strongly gelatinized throughout, two-layered, a suprapellis 110–130 µm deep, often embedded in up to 30 µm thick gelatinous matter, composed of narrow, ascending hyphal terminations, and a subpellis 35-50 µm deep, composed of horizontally oriented, dense, intricate, 2.5-6 µm wide hyphae. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin flexuous and downwards also nodulose, thin-walled, terminal cells often very long,

 $(33-)49-72.6-96.5(-126) \times (2-)3-4.2-5.5(-6.5)$ μm. mainly attenuated, rarely narrowly cylindrical or subulate, near the base often irregularly inflated, apically constricted to at least 2.5 µm and often with refringent vellowish contents; subterminal cells much shorter, often irregularly branched and more inflated, intricate and embedded in the subpellis. Hyphal terminations near the pileus centre slenderer and with shorter terminal cells (19.5-)24-36- $48(-82.5) \times 3-3.3-4 \mu m$ . **Pileocystidia** near the pileus margin dispersed, inconspicuous, usually 1-2-celled, clavate or cylindrical, rarely fusiform, often laterally diverticulate, often with slightly thickened walls (up to 0.5 µm), terminal cells (20-)32.5-51.4-70.5(-86) × 6-7.2-8.5(-10) µm, cylindrical, clavate, apically mainly obtuse-rounded, sometimes slightly constricted, contents rather dispersed, granular to crystalline, hardly reacting in sulfovanillin. Pileocystidia near the pileus centre similar in shape and size but more frequent. Cystidioid hyphae in the context absent, oleiferous hyphae frequent in the subpellis just above the context.

*Material studied*: MEXICO, Tlaxcala, Municipality of Panotla, E of San Francisco Temezontla, 19° 20' 52" N 98° 16' 26" W, alt. 2600 m, monodominant *Quercus* forest, 29 Jul 2006, B. Buyck & A. Kong *Buyck 06.542* (PC).

Additional material studied: MEXICO, Tlaxcala, Municipality of Panotla, 1 km E of San Francisco Temezontla, alt. 2600 m, deciduous *Quercus* forest, 13 Sep 1994, A. Kong *AK2786* (TXLM); ibid, deciduous *Quercus* forest, 2640 m alt, 19° 20′ 46″ N, 98° 16′ 24″ W, 23 Jul 1996, A. Kong *AK2895* (TXLM); ibid, Quercus forest, 2640 m alt, 19° 20′ 46″ N, 98° 16′ 24″ W, 23 Jul 1996 A. Kong *AK2895* (TXLM).

*Notes*: Our ITS phylogeny (Fig. 6) places *R*. sp. 2 in a group of *R*. graveolens and other members of Xerampelinae that are all probably associated with Quercus or other less frequently occurring ectomycorrhizal alternative host trees. *Russula* sp. 2 can be morphologically recognised by the very long terminal cells of the hyphae in the pileipellis near the pileus margin usually originating directly from the intricate hyphae of the subpellis and spores with warts forming long chains.

## Russula sp. 3

#### Figures 18a, 50d, 61, 62

Short diagnosis: Pileus medium-sized; cuticle cream with yellowish brown spots and pale pinkish shade near the margin; context firm; spores large with prominent ornamentation composed of spines fused in short chains or ridges; hymenial cystidia numerous, large, fusiform to lanceolate; hyphal termination in pileipellis near the pileus margin cylindrical with attenuated tips; pileocystidia numerous, 2–4-celled, cylindrical to lanceolate, apically



Fig. 61 *Russula* sp. 3 (SFC 20170821-22a), hymenium. **a** Basidia. **b** Basidiola. **c** Marginal cells on the lamellae edges. **d** Hymenial cystidia near the lamellae edges. **e** Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar =  $10 \mu m$ 

obtuse,  $4.4-8.8 \ \mu m$  wide, with crystalline-granular contents.

**Pileus** medium-sized, 60–75 mm diam., convex to applanate, depressed in the centre; margin inconspicuously striate; cuticle smooth, peeling to 1/4-1/3 of the pileus radius, cream near the centre, pale pinkish to pink near the margin, with yellowish brown spots. **Lamellae** 5–6.5 mm deep, fragile, adnate to slightly decurrent, first cream, then yellowish, with yellowish brown spots with age, furcate often near stipe. **Stipe** 60–90 × 10–15 mm, cylindrical, sometimes broader towards the base, longitudinally striate, white, turning yellowish brown with age or when bruised. **Context** firm, white; taste and odour not recorded. **Spore print** not obtained.

**Spores** (10.9–)11.6–<u>12.1</u>–12.6(–12.8) × (9.5–)–9.9–<u>10.4–</u> 11.0(–11.8) µm, subglobose to broadly ellipsoid,  $Q = (1.08–)1.11-\underline{1.16}-1.21(-1.24)$ ; ornamentation of large, moderately distant to dense [(6–8(–9) in a 3 µm diam. circle] amyloid spines, 1.1–1.9(–2.3) µm high, rarely isolated, frequently fused in short to long chains or ridges (0–6 fusions in the circle), connected by occasional, fine



Fig. 62 *Russula* sp. 3 (SFC 20170821-22a), pileipellis. **a** Pileocystidia near the pileus centre. **b** Pileocystidia near the pileus margin. **c** Hyphal terminations near the pileus centre. **d** Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red. Scale bar = 10  $\mu$ m

line connections (0–2 in the circle); suprahilar spot large, amyloid. **Basidia**  $(27.5-)31.5-39.3-47(-52.5) \times (10-$ )11.5-14-16.5(-18) µm, clavate, 4-spored; basidiola clavate, ca. 6.5-16.5 µm wide. Hymenial cystidia widely dispersed to dispersed, ca. 300-700/mm<sup>2</sup>, (88-)96-113.5- $131(-155.5) \times (10.5-)12-13.8-15.5(-18)$ μm, mainly fusiform or lanceolate, apically acute and often mucronate, mainly with an up to 3 µm long appendage, thin-walled; contents heteromorphous, crystalline, turning dark grey in sulfovanillin; abundant near the lamellae edges, (59.5-)65- $75.9-87(-100) \times (8-)8.5-10.6-12.5(-14.5)$  µm, mainly fusiform or lanceolate, apically often obtuse and often without appendage. Lamellae edges fertile; marginal cells  $(16.5-)20-23.4-27(-30.5) \times (4-)5-6.5-8(-9) \mu m$ , clavate. Pileipellis orthochromatic in Cresyl Blue, sharply delimited from the underlying context, 200-400 µm deep, composed of a well-defined, 55-110 µm deep suprapellis of ascending to erect, loosely arranged hyphal terminations, and a 95-310 µm deep subpellis of horizontally oriented, intricate, dense and narrow hyphae. Acidresistant incrustations absent. Hyphal terminations in pileipellis near the pileus margin, scarcely branched, often slightly flexuous, thin-walled; terminal cells (24.5-)35- $45.5-56(-63.5) \times 2-2.5-3 \mu m$ , cylindrical, apically usually slightly attenuated; subterminal cells usually equally wide and long, mainly not branched. Hyphal termination near the pileus centre similar, terminal cells (24.5-)31- $39.7-48.5(-56.5) \times 2-2.5-3 \mu m$ , more frequently without an apical constriction. Pileocystidia near the pileus margin very abundant, mainly 2-3-celled, occasionally 1-celled, narrowly clavate, thin-walled, terminal cells (20.5-)26- $55.4-85(-118.5) \times (3.5-)5-5.9-7 \ \mu m$ , mainly cylindrical, occasionally fusiform or clavate, apically mainly obtuse, contents heteromorphous, crystalline-granulose, in sulfovanillin turning black. Pileocystidia near the pileus centre similar, occasionally with lateral nodes or projections near the base, terminal cells  $(23.5-)33-65.1-97(-134) \times (4-$ )4.5-5.4-6.5(-7) µm. Cystidioid hyphae in subpellis and context dispersed, contents heteromorphous-granulose, oleiferous hyphae frequent in the subpellis near the context. Material studied: SOUTH KOREA, Kyeongsangbuk-do, Ulleung-gun, Ulleungdo, Nari Basin, on soil in a forest with Quercus and Acer trees, 37°31′2.84″ N: 130°52'11.44" E, alt. 395 m, 21 Aug 2017, Nam Kyu Kim & Jae Young Park (SFC 20170821-22a).

*Notes*: The field appearance of this species matches perfectly *R. globispora*: the pileus cuticle is pale pink near the margin and cream towards the centre and the whole of the basidioma has yellowish brown spots. Accordingly, several microscopic characters agree well with *R. globispora* (Adamčík and Jančovičová 2013): the pileocystidia are mainly 2–3-celled, hyphal terminations in pileipellis are



**Fig. 63** *Russula* sp. 4 (SFC 20170712-12), hymenium. **a** Basidia. **b** Basidiola. **c** Marginal cells on the lamellae edges. **d** Hymenial cystidia near the lamellae edges. **e** Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar = 10  $\mu$ m



**Fig. 64** *Russula* sp. 4 (SFC 20170712-12), pileipellis. **a** Pileocystidia near the pileus centre. **b** Pileocystidia near the pileus margin. **c** Hyphal terminations near the pileus centre. **d** Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red. Scale bar = 10  $\mu$ m

very narrow and spores are very large and subglobose to broadly ellipsoid. It differs from both known European species of the group (*R. globispora* and *R. dryadicola*) in the spore ornamentation that consists of spines that are frequently fused in long chains or crests. The most closely related published species in our phylogeny (Fig. 8) is *R. heilongjiangensis* G.J. Li & R.L. Zhao described from China (Li et al. 2018) and this species has very similar spore ornamentation. The Chinese species is considerably smaller, has a bright red pileus cuticle, smaller spores (up to 10 µm long), smaller basidia, narrower pleurocystidia, 1-celled pileocystidia, and wider (3–6 µm) hyphal terminations in the pileipellis.

#### Russula sp. 4

#### Figures 18b, d, 50f, 63, 64

Short diagnosis: Pileus medium-sized, with pastel red to pinkish colour, yellow-brown to dark brown spots present on all parts of basidiomata; stipe longer than pileus diameter; context fragile, turning brown after bruising; taste moderately acrid; spore print ochre; hymenial cystidia relatively small, acute at lamellae sides and obtuse near the edges; spores relatively small, with isolate and low warts; hyphal terminations in pileipellis densely septate, narrow and apically contricted; pileocystidia abundant, well-defined, narrow, mainly 2–4-celled.

Pileus medium-sized, 50 mm diam., soon expanding and infundibuliform when mature, margin striate to ca. 5 mm of pileus radius, crenulate, cuticle smooth, slightly viscid, peeling to 1/2 of the pileus radius, pastel red to pinkish, variegated with orange-brown and yellow-brown spots, turning pale vellowish orange with KOH. Lamellae 5.5–6 mm deep, moderately distant, ca. 9 at 1 cm near the pileus margin, adnate to slightly decurrent, fragile, whitish to pale cream; lamellulae frequent; furcations and anastomoses present; edge entire and concolorous. Stipe relatively long, ca.  $84 \times 11-15$  mm, longitudinally striate, white, often with yellow-brown spots, staining orangebrown when bruised, stuffed and partly hollow when mature. Context 3 mm thick in a half of the pileus radius, white, turning brown after cutting, soft in the pileus and fragile in the stipe; taste moderately acrid; odour indistinct; not changing with FeSO<sub>4</sub> and PDAB, turning pale orange with 10% KOH. Spore print ochre.

**Spores** (6.0–)6.1–6.5–6.8(–7.5) × (4.8–)5.2–5.5–5.8(–6.0)  $\mu$ m, subglobose to broadly ellipsoid, Q = (1.07–)1.11– 1.19-1.26(-1.31); ornamentation of relatively small, moderately distant to dense (5-10 in a 3 µm diam. circle) amyloid warts or spines, 0.5-0.8(-1.1) µm high, mainly isolated, occasionally fused (0-2 fusions in the circle); line connections absent or very rare; suprahilar spot large, amyloid. Basidia (25.5-)27-30.3-33.5 × 8.5-9.1-9.5(-10) µm, clavate, 4-spored; basidiola clavate, ca. 7-9 µm wide. Hymenial cystidia dispersed to moderately numerous, ca. 500–900/mm<sup>2</sup>,  $(33-)43.5-52.4-61.5(-65) \times (7-$ )7.5–8.6–9.5(–10.5) µm, clavate or fusiform, apically acute and often mucronate, with a 2-11 µm long appendage, thin-walled, contents heteromorphous, crystalline-granulose, turning grey in sulfovanillin; abundant near the lamellae edges,  $(38-)38.5-47.2-56(-67) \times 7.5-8.9-10.5(-$ 12.5) µm, clavate and apically obtuse. Lamellae edges fertile; marginal cells  $(13.5-)17-20.6-24(-27) \times (6-)6.5-$ 7.6-8.5 µm, clavate to cylindrical, apically obtuse. Pileipellis orthochromatic in Cresyl Blue, sharply delimited from the underlying context, 150-400 µm deep, distinctly two-layered; a suprapellis 90-260 µm deep, strongly gelatinized, composed of ascending to erect hyphae; subpellis 70-150 µm deep, composed of horizontally oriented, dense, intricate, narrow hyphae. Acid-resistant incrustrations absent. Hyphal terminations near the pileus margin narrow, scarcely branched, often slightly moniliform or flexuous, thin-walled; terminal cells (15- $(19.5-25.1-31(-35) \times (2-)2.5-2.9-3.5(-4))$ μm,

cylindrical or narrowly fusiform, apically mainly constricted and subacute, occasionally obtuse; subterminal cells branched or not, equally wide (ca. 3-5 µm). Hyphal terminations near the pileus centre more closely septate and shorter, usually with shorter terminal cells (11-)17-20.6- $24(-27) \times (2-)2.5-2.9-3.5(-4) \ \mu m$ . **Pileocystidia** near the pileus margin abundant, usually (1-)2-4-celled, rarely 5-celled, cylindrical and often slightly flexuous, thin-walled, terminal cells  $(12.5-)15.5-28.1-40.5(-59.5) \times (3-$ )3.5-3.9-4.5(-5) µm, mainly cylindrical, apically obtuse, contents heteromorphous, with dispersed small crystalline particles, in sulfovanillin turning to dark brown to almost black. Pileocystidia near the pileus centre similar, terminal cells  $(12.5-)18.5-27.2-36(-45) \times 3-3.7-4.5(-5.5)$  µm. Cystidioid hyphae in the subpellis and the context dispersed, with heteromorphous-granulose contents, oleiferous hyphae frequent in the subpellis close to the context. Material studied: SOUTH KOREA, Jeollanam-do, Goheung-gun, Yeongnam-myeon, Paryeongsan Natural Recreation Forest, 34° 37' 25.94" N; 127° 26' 9.04" E, alt. 417 m, on soil among leaf litter in deciduous forest dominated by Quercus acutissima and Castanea crenata, 12 Jul 2017, K. Wisitrassameewong & J.Y. Park (SFC 20170712-12).

*Notes*: With its context turning brown and moderately acrid taste, *R*. sp. 4 is similar to *R. globispora*. However, the microscopic stuructures of these two species are very



Fig. 65 *Russula* sp. 5 (FH00304560), hymenium. **a** Basidia. **b** Basidiola. **c** Hymenial cystidia near the lamellae edges. **d** Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar =  $10 \ \mu m$ 



Fig. 66 *Russula* sp. 5 (FH00304560), pileipellis. **a** Hyphal terminations near the pileus centre. **b** Hyphal terminations near the pileus margin. Contents of hyphae as observed as observed in Congo Red. Scale bar =  $10 \mu m$ 

different, our new species has much smaller spores, hyphae and other elements. The microscopic structures of *R*. sp. 4 are unusually small for subgenus *Russula*.

#### Russula sp. 5

#### Figures 15g, 21b, 65, 66

Short diagnosis: Pileus small, convex and soon expanding to applanate, colour at the margin vivid orange, towards the centre paler; edges of lamellae concolorous with sides; stipe with vivid yellow hue; spores moderately large, with moderately prominent warts forming long and often parallel chains or crests; hymenial cystidia without well-defined contents, more abundant near the lamellae edges, no reaction in sulfovanillin; pileipellis strongly gelatinized, composed of loose, hyphae with obtuse, cylindrical or clavate terminal cells, without pileocystidia or incrustations, gradually denser towards the context.

**Pileus** small, 16–37 mm diam., convex, soon expanding to applanate, when mature often slightly depressed in the centre; margin first deflexed, when mature inflexed and

striate; cuticle smooth, when wet viscous, when dry matt and covered with adherent soil and debris particles, at margin vivid orange, towards the centre paler orange yellow to egg yellow. **Lamellae** moderately distant, adnexed to adnate, pale yellow; lamellulae and furcations rare or absent; edge even and concolorous. **Stipe** 26–38 × 8–13, obclavate, longitudinally striate, white, partly with vivid yellow hue especially near the lamellae; medulla stuffed. **Context** white, unchanging, compact, later fragile. **Spore** print not observed.

**Spores**  $(6.8-)7.2-7.5-7.8(-8.1) \times (5.6-)6-6.3-6.6(-6.9)$  $\mu$ m, subglobose to broadly ellipsoid, O = (1.1-)1.15-1.18-1.22(-1.23); ornamentation of medium-sized to large, moderately distant to dense [(4-)5-7(-8) in a 3 µm diam. circle] amyloid warts, 0.4-0.6(-0.7) µm high, rarely isolated, fused in very long parallel chains with some oriented transversally to them, often forming crests [1-6(-6)] fusions in the circle], connected by usually short, fine or thick line connections [(0-)1-3 in the circle]; suprahilar spot relatively small, amyloid. **Basidia**  $(27-)30-33-36(-39) \times (9-)$ )10-10.8-11.5(-12) µm, clavate, 4-spored; basidiola first ellipsoid or cylindrical, then clavate, ca. 3.5-10 µm wide. Hymenial cystidia probably widely dispersed, (46–)51.5–  $60-68.5(-77) \times (8-)8.5-10.3-12(-14)$  µm, mainly fusiform, apically obtuse or acute and often pointed, usually with a 2-4 µm long appendage, thin-walled, contents poorly defined, with loose, heteromorphous-granulose inclusions near the apical part, no reaction in sulfovanillin; more numerous near the lamellae edges, (35-)40-47.5- $55(-60) \times (8-)8.5-10-11.5(-11.5)$  µm, fusiform, lanceolate, clavate or subulate, apically mainly acute, but often also obtuse, occasionally with a short appendage, optically empty or with a few dispersed, refringent inclusions. Lamellae edges sterile, or with a few dispersed basidia; marginal cells undifferentiated. Pileipellis orthochromatic in Cresyl Blue, sharply delimited from the underlying context, 70-110 µm deep, not distinctly stratified, strongly gelatinized throughout; hyphae near the surface relatively loose, ascending and gradually pass to horizontally oriented, intricate, dense and  $2-3 \mu m$  wide hyphae near the context. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin composed of 1-3 unbranched cells originating in a layer of intricate hyphae underneath, often slightly flexuous, thin-walled, terminal cells (18–)21.5–29.8–38.5(–64)  $\times$  (2.5–)3.5–3.9–4.5  $\mu$ m, mainly cylindrical, often also clavate, sometimes narrowly fusiform, apically obtuse or rarely slightly constricted; subterminal cells usually equally wide and long, mainly not branched and entire. Terminal cells of hyphae near the pileus centre similar in shape, occasionally with some shorter terminal cells,  $(13-)18-24.6-31(-39) \times (2.5-)3-$ 3.7-4(-5) µm. Pileocystidia absent. Cystidioid and oleiferous hyphae absent.



Fig. 67 *Russula* sp. 6 (GENT FH 12-064), hymenium. **a** Basidia. **b** Basidiola. **c** Hymenial cystidia near the lamellae edges. **d** Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar =  $10 \mu m$ 

*Material studied:* PAKISTAN, Pakhtunkhwa, Mansehra, Batrasi, associated with *Pinus roxburghii*, 3 Aug 2013, Malka Saba *MSM#0076* (FH00304560); *ibid.*, associated with *Pinus roxburghii*, 12 Sep 2017, Malka Saba *MSM#00186*.

*Notes*: This is yet another new species in *R*. subsect. *Auratinae*. It differs from all other known species of the group by the almost regular cylindrical terminal cells in the pileipellis. All other species have spores that are approximately 2  $\mu$ m longer, and both *R*. *aurantioflava* and *R*. *aurea* have distinctly tapering terminal cells in the pileipellis, while *R*. *xantho* has very irregular, nodulose-tortuous terminal cells in the pileipellis.

#### Russula sp. 6

## Figures 15f, 31b, 67, 68

Short diagnosis: Pileus medium-sized, regular in outline, fleshy, with smooth margin; cuticle smooth, glabrous, with grey-brown colours, paler in the center; lamellae narrow, crowded, unequal with numerous lamellulae of different lengths and frequent furcations at different distances from the stipe; stipe shorter than pileus diameter, solid and not hollowing; context firm, blackening without first turning red, mild; ornamentation subreticulate, of moderately large warts fused and connected by frequent line connections; suprahilar spot not amyloid; hymenial cystidia filled with brown, irregular, refractive bodies; hyphal terminations at pileus surface long, branched at bases, gradually narrowing upwards, with multiple septa and filled with brown,



Fig. 68 *Russula* sp. 6 (GENT FH 12-064), pileipellis. a Hyphal terminations near the pileus centre. b Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red. Scale bar =  $10 \ \mu m$ 

refractive bodies; pileocystidia and gloeoplerous elements absent, also from the underlying context; primordial incrusted hyphae absent.

**Pileus** medium-sized, 34–72 mm diam., planoconvex to applanate, centrally depressed to umbilicate, with smooth margin; pileus surface smooth, shiny when wet, velvety when dry, yellowish brown to greyish brown (5D3, 5E4, 5F3), paler in the centre. **Lamellae** narrow, 2–4 mm deep, crowded, adnate, cream, with numerous lamellulae of different lengths and frequent furcations at different distances from the stipe, blackening with age; edges concolorous and even. **Stipe** 11–25 × 5–10 mm, cylindrical, firm and fleshy, smooth, white, becoming mustard brown (5E6) to black, solid inside. **Context** ca. 3 mm thick at mid-radius, white, firm, upon bruising directly blackening without turning red; first turning pink with FeSO<sub>4</sub>, immediately dark blue with guaiac (strong reaction); taste mild; odour indistinct. **Spore print** white (Ia).

**Spores** (5.2–)5.5–<u>5.9</u>–6.3(–6.8) × (4.5–)4.8–<u>5.1</u>–5.3(–5.6)  $\mu$ m, subglobose to broadly ellipsoid, Q = (1.05–)1.10–

1.16-1.23(-1.35); ornamentation of moderately large. dense [(4-)6-9(-12) in a 3 µm diam. circle] amyloid warts, 0.4-0.7 µm high, subreticulate, occasionally fused in chains [(0-)1-3 fusions in the circle], connected by abundant, short, fine line connections [(1-)3-8(-11)] in the circle]; suprahilar spot small, not amyloid. Basidia (31-)32- $35-38(-43) \times (7-)8-8.9-9.5(-11) \mu m$ , narrowly clavate to clavate, 4-spored; basidiola cylindrical to clavate, ca. 5-8 µm wide. Hymenial cystidia probably widely dispersed,  $(49-)54.5-64.6-75(-85) \times (6-)7-8.4-10(-11) \ \mu m$ , cylindrical to flexuous, apically mainly acute, with a 2-5 µm long appendage, thin-walled; contents with irregular, brown refractive bodies, without reaction in sulfovanillin; abundant near the lamellae edges, (27-)36-44.9- $54(-59) \times (6-)6.5-8.3-10(-11)$  µm, subulate, apically obtuse, with a 2-6 µm long appendage, thin-walled, completely filled with refractive brown pigments. Lamellae edges sterile; marginal cells (12-)12.5-15.1-18(-20)  $\times$  (4–)4.5–5.6–7(–8) µm, cylindrical or clavate, thinwalled. Pileipellis orthochromatic in Cresyl Blue, sharply delimited from the underlying context, 180-250 µm deep, two-layered; suprapellis 100-200 µm deep, composed of narrow, ascending hyphal terminations; subpellis 25-75 µm deep, composed of horizontally oriented, dense, 4-8 µm wide hyphae. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin narrow, long, with multiple septa, scarcely branched at the bases, flexuous, thin-walled, filled with irregular refractive bodies containing brown pigments; terminal cells (20-)25-31.1- $37(-40) \times (3-)3.5-4.2-5(-6)$  µm, narrowly cylindrical to subulate, apically mainly constricted to at least 2.5 µm; subterminal cells and the cells below often gradually wider, usually shorter, subterminal cells never branched. Hyphal terminations near the pileus centre less branched and apically more attenuated, terminal cells (20-)27.5-34.8-42(-55)  $\times$  (3–)3.5–4.3–5(–6) µm. **Pileocystidia** not observed. Oleiferous hyphae containing brown pigments present in the context of both pileus and lamellae.

*Material studied*: Thailand: Chiang Mai Province, Mae Taeng District, around temple near Mushroom Research Centre (MRC), 19° 06' 38" N, 98° 44' 32" E, alt. 1055 m, scattered in soil under *Lithocarpus*, *Castanopsis* and *Dipterocarpus*, 27 Jun 2012, F. Hampe & B.P. Looney *BPL 204* (GENT FH 12-064).

*Notes*: This species is strongly reminiscent of *R. albonigra* because of the strong and rapid blackening of the basidiomata which is not preceded by any reddening of the tissues. Our phylogenetic analysis (Fig. 12) shows that there are apparently still many undescribed taxa among similar species with close to crowded lamellae. As no new species have been published in *R.* subg. *Compactae* from Asia in recent years, this is one of the first endemic Asian species in the subgenus.



Fig. 69 *Russula* sp. 7 (GENT FH12-036), hymenium. **a** Basidia. **b** Basidiola. **c** Hymenial cystidia near the lamellae edges. **d** Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar =  $10 \ \mu m$ 

# Russula sp. 7

## Figures 15e, 31d, 69, 70

Short diagnosis: Pileus small to medium-sized, first hemispherical, when mature expanding to applanate with depressed centre; margin tuberculate-striate when mature; granulose-pubescent at the surface and cracking into a starlike pattern near the margin, predominantly orange; stipe tapering towards the base, with orange granules on pale yellow background; spores relatively small, subreticulate and ornamented with low warts fused in pairs or in short chains and connected by occasional to frequent line connections; suprahilar spot not amyloid; hymenial cystidia widely dispersed on lamellae sides, without heteromorphous contents but with yellow pigments; pileipellis with well-defined suprapellis made up of fascicles of long, densely septate and multi-celled, yellow-incrusted hyphal terminations originating from a dense layer of more inflated cells on top of the horizontally oriented, densely arranged hyphae of the subpellis; well-differentiated pileocystidia not observed.

**Pileus** small to medium-sized, 5–40 mm diam., first hemispherical, later convex, when mature applanate with depressed centre; margin when young smooth, soon tuberculate-striate; pileus surface dry, matt, finely granular when young, later becoming coarsely granular to pubescent, and towards the margin cracking into a star-like or areolate pattern, when young orange to orange-red bordered with a yellow line near the pileus margin, later turning to reddish golden (6C7), brownish orange (6C8),



Fig. 70 *Russula* sp. 7 (GENT FH12-036), pileipellis. **a** Hyphal terminations near the pileus centre. **b** Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red. Scale bar =  $10 \mu m$ 

carrot red (6B7), mandarin orange (6B8), dark orange (5A8), orange (5A7, 6A6), Persian orange (6A7) or light brown (6D7, 6D8), not peeling. Lamellae moderately distant, 40-60 in total reaching the stipe, white to cream, equal, with a few lamellulae and occasional furcations; edge entire and concolorous Stipe  $40-50 \times 2.5-4$  mm, cylindrical, tapering toward the base, matt, finely granulose, fragile, near the lamellae first almost white, later pale yellow (4A3) to light yellow (4A5), most of the surface with orange-red granulations on light yellow (4A6) to sunflower yellow (4A7) background. Context in the pileus thin (ca. 1 mm at mid-radius), white, unchanging, fragile; taste mild; odour inconspicuous; turning blue after 5 s with guaiac, pale pink with FeSO<sub>4</sub>. Spore print not observed.  $(5.9-)6.3-6.6-6.8(-7.1) \times (5-)5.4-5.7-6(-6.6)$ Spores  $\mu$ m, subglobose to broadly ellipsoid, Q = (1.05–)1.11– 1.15-1.19(-1.23); ornamentation of small to mediumsized, moderately distant to dense [(3-)5-7(-8) in a 3  $\mu$ m diam. circle] amyloid warts, 0.4-0.6 µm high, subreticulate, occasionally fused in pairs or short chains [(0-)1-2(-3) fusions in the circle], occasionally connected by line

connections [(0-)1-3 in the circle]; suprahilar spot medium-sized, not amyloid, smooth. Basidia (20-)22.5-27.8- $33(-37) \times (8-)8.5-9.8-11(12.5)$  µm, clavate, 4-spored; basidiola cylindrical, clavate or ellipsoid, ca. 5.5-10 µm wide. Hymenial cystidia widely dispersed, ca. 50-100/ mm<sup>2</sup>,  $(44-)52.5-58.8-65 \times (6-)7-7.5-8(-9.5)$  µm, fusiform or lanceolate, apically acute and often moniliform, with a small, 1-3(-4) µm long appendage, thin-walled; contents not heteromorphous, yellow, turning pale brown in sulfovanillin; near the lamellae edges more frequent and narrower,  $(30-)45-51.3-64(-70.5) \times 5.5-6.3-7(-8)$  µm. Lamella edges fertile; marginal cells undifferentiated. Pileipellis orthochromatic in Cresyl Blue, not sharply delimited from the underlying context, 85-110 µm deep, distinctly two-layered; suprapellis 65-90 µm deep, composed of non-gelatinized, ascending or erect, loose but downwards gradually denser hyphae, abruptly delimited from 18-26 µm deep subpellis of horizontally oriented, dense, interwoven, undulate, 2.5-6 µm wide hyphae with thick (up to 1.5 µm) glutinous coating. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin flexuous, very variable in length, some very long, apically attenuated and fasciculated, others shorter and constricted on septa, near transition to the subpellis occasionally branched and forming a pseudoparenchymatic layer of ellipsoid or ventricose cells, long hyphal terminations composed of 5-10 unbranched, downwards gradually wider cells, often with yellow-orange granular incrustations and also with yellow intracellular pigments of variable intensity, thin-walled, terminal cells (10.8-)12.5- $30.2-48(-85) \times (3-)3.5-4.5-5.5(-6.5)$  µm, mainly cylindrical, occasionally attenuated or ellipsoid, apically usually not distinctly constricted and obtuse; subterminal cells equally long but often wider, usually unbranched. Hyphal terminations near the pileus centre similar, but with less distinct incrustations, terminal cells (6.5-)14.5-25.7-37(-52)  $\times$  (2.5–)3–4.4–5.5(–8) µm. Pileocystidia with welldifferentiated contents not observed, some dispersed fusiform elements, similar in shape to hymenial cystidia (apically appendiculate) without reaction in sulfovanillin observed near the pileus centre. Cystidioid hyphae in the context absent, oleiferous hyphae dispersed, with yellowish contents not reacting in sulfovanillin.

*Material studied*: THAILAND, Mae Taeng district, province Chiang Mai, Bua Tong Waterfalls and Rainbow spring, 19°04′14.28″ N; 99°04′47.95″ E, alt. 513 m, on stony and clayey soil with *Dipterocarpus tuberculatus*, 23 Jun 2012, F. Hampe (GENT FH12-036)

*Notes*: This beautiful species is strongly reminiscent of several similarly colored African species, with or without a ring, but all of these had small but distinct, mucronate pileocystidia (Buyck 1993, 1994a). In our species, we were unable to find pileocystidia with typical contents.



Fig. 71 *Russula subtilis* (NY00760993, lectotype), hymenium. **a** Basidia. **b** Basidiola. **c** Marginal cells on the lamellae edges. **d** Hymenial cystidia near the lamellae edges. **e** Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar =  $10 \mu m$ 

*Russula subtilis* Burl., Mycologia 16(1): 20. 1924. *Mycobank no.*: MB 214963, Figs. 21f-g, 50e, 71, 72

*Lectotype* (designated here; identifier no.: MBT 386775): [USA] Vermont, Newfane Hill, 7 Aug 1915, G.S. Burlingham (NY00760993).

*Epitype* (designated here; identifier no.: MBT 386776): USA, North Carolina, Geat Smoky Mountains National Park, Haywood Co., Baxter creek trail near the campground, coord. 35° 45′ 5″ N; 83° 6′ 35″ W, alt. ca. 650 m, associated with *Fagus grandifolia*, *Carpinus caroliniana*, *Rhododendron maximum*, 9 Aug 2012, S. Adamčík (SAV F-3805).

*Original diagnosis*: Pileus broadly convex, becoming plane to centrally depressed, up to 4 cm. broad; surface slateviolet or with a faint wash of salmon-lilac, darker toward the center, pruinose, granulose toward the margin when viewed with a lens, viscid when wet, cuticle separable, margin even; context mild, then imparting a stinging sensation to the throat; lamellae white, equal, rounded at the outer and narrowed at the inner ends, subdistant, broad; stipe washed with Corinthian-red, equal or tapering downward, pruinose, especially at the apex, which is whitestuffed, 3 cm. by 1.5 cm.; spores white, somewhat ovoid to



**Fig. 72** *Russula subtilis* (NY00760993, lectotype), pileipellis. **a** Primordial hyphae near the pileus centre. **b** Primordial hyphae near the pileus margin. **c** Hyphal terminations near the pileus centre. **d** Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red. Scale bar =  $10 \mu m$ 

broadly ellipsoid, apiculate, unsymmetrical in one view, minutely echinulate,  $7.5 \times 10 \mu$ , including the appiculus, which is  $1 \mu$  long.

**Pileus** (macromorphological characters described based on recent collection SAV F-3805) small, 28 mm diam., convex, soon expanding to plane, depressed in the centre, not lobate; margin strongly striate to ca. 1/2 of the radius; cuticle dry, glabrous, near the margin finely granulose, near the centre pruinose, colour at the margin dull red (10C4), centre reddish brown (oxblood red, 9E7). **Lamellae** 4 mm deep, distant, 8 at 1 cm near the pileus margin, adnexed, white; lamellulae and furcations rare, anastomosed at the connection to the pileus; edge entire and near the pileus margin of the same colour as pileus cuticle. **Stipe**  $38 \times 7$  mm, narrowly clavate, finely longitudinally striate, near lamellae white, below pinkish white (10A2) to pale red (10A3), medulla stuffed, cortex 1 mm thick. **Context** fragile, white, unchanging; taste mild; odour none; reaction with guaiac slow and weak, turning to green-blue. **Spore print** pale white (Ia).

**Spores**  $(6.2-)6.8-7.2-7.6(-7.8) \times (4.8-)5.4-5.8-6.1(-6.4)$  $\mu$ m, broadly ellipsoid, O = (1.15-)1.2-1.24-1.29(-1.34);ornamentation of moderately large, moderately distant to dense (4-7 in a 3 µm diam. circle) amyloid warts, 0.3-0.6(-0.7) µm high, rarely isolated, fused in usually short, often branched chains (0-3 fusions in the circle), connected by occasional, fine line connections [0-3 (-4) inthe circle]; suprahillar spot moderately large, amyloid. **Basidia** (29–)30.5–33.4–36(–38)  $\times$  9–10.3–11.5(–12) µm, broadly clavate, pedicellate, 4-spored, rarely also 1-2 spored; basidiola first cylindrical or ellipsoid, then clavate, ca. a 3-11 µm wide. Hymenial cystidia dispersed, ca.  $400-600/\text{mm}^2$ ,  $(48-)54.5-61-67.5(-77) \times (6-)7-8.4-$ 9.5(-12)  $\mu$ m, fusiform, rarely clavate, pedicellate, apically mainly acute, always with 3-9(-11) µm long appendage, thin-walled, contents partly heteromorphous, oleiferous, granulose or sometimes crystalline, slowly turning dark brown in sulfovanillin; near the lamellae edges dispersed, very variable in size, usually smaller, (31-)37-44.4-52(-59)  $\times$  (6–)7–8.2–9.5 µm, clavate or fusiform, apically mainly obtuse, with or without a 3-9(-17) µm long appendage, contents dispersed, finely granulose. Lamellae edges with occasional basidia; marginal cells (10-)12- $15.8-19.5(-22) \times (6-)7-8.6-10.5 \ \mu m$ , broadly clavate or obpyriform, obtuse. Pileipellis orthochromatic in Cresyl Blue, sharply delimited from the underlying context, 75-85 µm deep, vaguely divided into a 25-40 µm deep, non-gelatinized suprapellis of ascending or repent, near the surface loose, downwards gradually denser hyphae, and a pseudoparenchymatic, 45-60 µm deep subpellis of more or less horizontally oriented, intricate, irregularly inflated, 3-12 µm wide hyphae. Acid-resistant incrustations present, but usually distinct only on subterminal cells of primordial hyphae. Hyphal terminations near the pileus margin branched, often flexuous, sinuous or nodulose, thinwalled; terminal cells  $(18-)21-32.2-43.5(-66) \times (3-)3.5-$ 5.2–6.5(–8)  $\mu$ m, very variable in shape, mainly subulate, often ventricose, lageniform or fusiform, apically constricted or attenuated, rarely cylindrical or clavate and obtuse, apically often moniliform; subterminal cells mainly branched, usually embedded in the subpellis, variable in length, often wider. Hyphal terminations near the pileus centre composed of 1-3 cells, narrow, scarcely branched, arising from a pseudoparenchymatic subpellis of inflated (5–13 µm wide) cells, often lobate or nodulose, terminal cells  $(13-)16-24.5-33(-42) \times 3.5-4.6-6(-7)$  µm, cylindrical, narrowly fusiform or narrowly lageniform, apically mainly obtuse, occasionally slightly constricted. Primordial hyphae near the pileus margin inconspicuous, 1-3(-4)-celled, subcylindrical and less flexuous than other hyphae; terminal cells (18-)25.5-35.4-45(-57) × 3-3.54 μm, cylindrical, apically mainly acute and constricted, contents optically empty or with a few inclusions in terminal or subterminal cells, no reaction in sulfovanillin. Primordial hyphae near the pileus centre even less conspicuous and apically more attenuated. Cystidioid or oleiferous hyphae absent in the subpellis and the context. *Additional material studied*: USA: Mississippi, George, Wiggins, Wiggins, Pascagoula River Wildlife Management Area, coord. 30° 51′ 5″ N; 88° 44′ 44″ W, 12 Jul 2014, collected by participants of Gulf States Mycological Society Foray *BPL533* (TENN-F-070208).

Notes: Russula subtilis and R. corralina are two commonly used names for members of subsection Lilaceinae in the eastern USA. However, the first was probably widely used for another species described here as R. gemmata (see the note to the species above). Our recent collection of R. subtilis is morphologically a perfect match with our type study. The pileus cuticle has dull dark red colours that correspond to the original description, the spores are larger than those of R. gemmata and the terminal cells of the hyphae in the pileipellis near the pileus margin are apically constricted to attenuate and not obtuse. According to the type study (Adamčík et al. 2013), R. uncialis Peck described from USA has a very similar pileipellis structure but the pileus has bright pink and red colours and the spores have low spore ornamentation and are narrow (Q in average 1.4). Another US member of Lilaceinae, R. corallina, has pale pink and red colours, lower spore ornamentation and, based on similarities described by Fatto (1998), is a possible synonym of R. uncialis. Sequences of collections identified by R. Fatto as R. corralina are clustered as a separate clade close to R. subtilis. The specimen labelled as the possible syntype of R. subtilis in the herbarium of the New York Botanical Garden is undoubtedly part of the original material and we are designating it as a lectotype here. We propose our recent collection with sequence data as the epitype of the species.

*Russula wielangtae* G.M. Gates, Caboň & Jančovičová, sp. nov.

Mycobank no.: MB 830749, Figs. 21c, e, 50h, 73, 74

*Holotype*: AUSTRALIA, Tasmania, Wielangta, 42° 42′ S; 147° 51′ E, alt. 200 m, wet sclerophyll forest, *Eucalyptus* dominated with understorey of *Pomaderris apetala* and *Olearia argophylla*, 17 Jan 2015, G.M. Gates (HO 593331).

*Etymology*: 'wielangtae' refers to the township of Wielangta where the type was collected.

Short diagnosis: Pileus medium-sized to large, dry and velvety, reddish; taste mild; spore print ochre; spores subreticulate to reticulate with prominent, interconnected spines and amyloid suprahilar spot; hymenial cystidia widely dispersed, mainly fusiform, with sparse granular



Fig. 73 *Russula wielangtae* (HO 593331, holotype), hymenium. a Basidia. b Basidiola. c Marginal cells on the lamellae edges. d Hymenial cystidia near the lamellae edges. e Hymenial cystidia on the lamellae sides. Cystidia with contents as observed in Congo Red. Scale bar = 10  $\mu$ m

contents; marginal cells well-differentiated, variable in shape and apically narrowing; Hyphal terminations near the pileus margin with terminal cells often basally inflated and with smaller subterminal cells, but near the pileus centre apically less constricted and often nodulose or branched; pileocystidia doubtful.

**Pileus** medium-sized to large; 43–125 mm diam., when young plano-convex, becoming shallowly depressed in the center with age; near the margin smooth, rarely slightly striate, dry, velvety, radially fissuring near the margin upon expansion, peeling to 1/3 of the radius, pale orange, deep red (9B7) or greyish red (9B5) near the margin, towards the centre more brownish orange (7C6-C7), to darker, yellowish brown (5E8), brown (6E8) or copper brown, variegated with darker brown (6F8) and yellowish patches. **Lamellae** to 9 mm deep, dense, adnate or sometimes decurrent with a small tooth, subventricose, white to cream when young, ochre (5B5–C5) when mature, becoming very thick with age, furcate, equal; edges even, fimbriate toward



**Fig. 74** *Russula wielangtae* (HO 593331, holotype), pileipellis. **a** Pileocystidia near the pileus centre. **b** Pileocystidia near the pileus margin. **c** Hyphal terminations near the pileus centre. **d** Hyphal terminations near the pileus margin. Cystidial contents as observed in Congo Red. Scale bar =  $10 \mu m$ 

the pileus margin, concolorous. Stipe  $25-87 \times 13-35$  mm, cylindrical, longitudinally fibrillose, chalky, smooth, white with pinkish hue near the base, stuffed inner. Context white, unchanging, compact, reaction with FeSO<sub>4</sub> brown on the stipe surface; taste mild, odour weakly of pelargonium. Spore print ochre.

**Spores**  $(7.7-)8.2-\underline{8.6}-9.1(-9.7) \times (6.6-)7-\underline{7.5}-8(-8.8)$  µm, subglobose to broadly ellipsoid, Q =  $(1.06-)1.11-\underline{1.15}-1.2(-1.28)$ ; ornamentation of large, moderately distant [(3-)4-6 in a 3 µm diam. circle] amyloid spines, 0.8-1.4 µm high, reticulated or subreticulated, frequently fused in short to long chains [(0-)1-3(-4) fusions in the circle], connected by occasional, fine line connections [1-3(-4) in the circle]; suprahilar spot large, amyloid. **Basidia** (29-)35-<u>38.8</u>-43(-53) × (11-)11.5-<u>12.6</u>-13.5(-16) µm, clavate, 4-spored; basidiola cylindrical or ellipsoid, then clavate to broadly clavate, (13-)19-<u>25.6</u>-32(-44) × (6-)8.5-10.4-12.5(-14) µm. **Hymenial cystidia** 

widely dispersed, ca. 300/mm<sup>2</sup>, (40-)58-70-81.5(-105)  $\times$  (8–)9–10.4–12(–13) µm, fusiform or rarely clavate, apically usually acute, rarely mucronate, often with a 3-10(-13) µm long appendage, originating in the subhymenium or occasionally deeper in context, distinctly protruding above basidia, thin-walled or with 0.5-1 µm thick walls; heteromorphous contents mainly loose, granulose and banded, not reracting to sulfovanilin; near the lamellae edges dispersed,  $(46-)58.5-69.6-81(-104) \times (6.5-)8.5-$ 10.7–12.5(–16) µm, fusiform, narrowly clavate or narrowly lageniform, occasionally slightly moniliform, apically obtuse or rarely acute, without an appendage. Lamellae edges sterile; marginal cells (16-)22.5-35.1-48(- $75) \times (5-)6.5-9.8-13(-19.5)$  µm, variable in shape, lageniform, clavate, fusiform, or lanceolate, occasionally nodulose, apically mainly acute, sometimes also with an appendage, thin-walled. Pileipellis orthochromatic in Cresyl Blue, not sharply delimited from the underlying context, 70-100 µm deep, not conspicuously stratified, strongly gelatinized near the surface and not gelatinized near the context; suprapellis 25-35 µm deep, composed of ascending to erect, loose but towards the pileus centre denser hyphal terminations; gradually passing into a 45-70 µm deep subpellis of horizontally oriented, towards the context more intricate, dense and irregularly arranged, 3–7 µm wide hyphae. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin composed of 1-3 unbranched cells originating in subpellis, often flexuous and occasionally also nodulose, thin-walled; terminal cells  $(8-)13.5-22.7-31.5(-46) \times (3-)4.5-6-8(-10.5) \mu m$ , attenuated, cylindrical, lageniform or fusiform, often inflated near the base, apically often constricted to 3 µm or more, and occasionally with yellowish pigments; subterminal cells distinctly shorter, cylindrical, mainly not inflated, usually not branched. Hyphal terminations near the pileus centre more nodulose and branched, often also with lateral branches, more densely septate and composed of more frequently inflated cells forming an epithelium; terminal cells  $(5.5-)10-16.1-22(-32) \times (3-)4.5-6.4-8(-$ 10) µm, ellipsoid, cylindrical, pyriform, lageniform, clavate or fusiform. Pileocystidia near the pileus margin dispersed but often 2-5 clustered in fascicules, (2-)3-4(-5)-celled, cylindrical and often slightly flexuous, exceeding other hyphal terminations and repent near the surface of pileipellis, thin-walled; terminal cells (11.5-)16-26.3-37(-44)  $\times$  (5–)5.5–6.8–8(–10) µm, clavate or cylindrical, apically obtuse; without defined contents and incrustations, optically empty; contents do not react in sulfovanillin. Pileocystidia near the pileus centre similar. Cystidioid or oleiferous hyphae in the subpellis and the context not observed.

Additional material studied: AUSTRALIA, Tasmania, Pipeline Track, Mt Wellington, 42° 55′ S, 147° 15′ E, alt.

500 m, at edge of a fire trail in wet sclerophyll forest dominated by *Eucalyptus obliqua* with an understory of mainly *Pomaderris apetala* and *Olearia argophylla*, 2 Feb 2012, G. Gates (HO 593332); ibid, Nerinna Creek Track, Margate,  $43^{\circ}$  02' S;  $147^{\circ}$  15' E, 7 Jan 2014, G. Gates (HO 593333); ibid., North West Bay River,  $42^{\circ}$  57' S;  $147^{\circ}$  12' E, 20 Jan 2014, G. Gates (HO 593334).

*Notes*: This species, which is apparently associated with *Eucalyptus* spp., is closely related to *R. atroviridis* Buyck, a replacement name for *R. atrovirens* McNabb, 1973 (the latter homonym of *R.* atrovirens Beeli, 1928). The latter species was described from *Nothofagus* vegetation in New Zealand and shares with our species a very similar structure and composition of the pileipellis, equally lacking well-differentiated pileocystidia, and having a similar spore ornamentation, but differs principally in the more greyish green–brown colours.

Its relation to *R. wollumbina* Grug., a new name for another *Eucalyptus* associated *Russula* in southern Australia that was originally identified by Cleland as *R. xerampelina*, is problematic. Indeed, *R. wollumbina* is very similar to our species but seems to differ essentially in its more isolated and less prominent spore ornamentation (Grgurinovic 1997). The only available sequence for *R. wollumbina* was provided by Lebel and Tonkin (2007) but we think that this sequence might actually correspond to our species as there is only a 4 base pair difference (< 99% similarity) between both. Our observations on both the type and the sequenced specimen correspond to this, the spores of the type have low and scarcely isolated warts, while the spore ornamentation of the specimen from which the



**Fig. 75** Chart showing global data on the number of *Russula* species described per year (blue), description quality estimated based on presence and quality of 23 selected micro-morphological characters (green) and percentage of sequenced types (red)

sequence originated corresponds to the species described here.

# Analyses of micromorphological characters

## Recent trends in quality of Russula descriptions

During the past 12 years (January 2007 till January 2019), 160 Russula species have been described worldwide: 11 from Africa and Madagascar, 23 from America, 67 from Asia, 11 from Australia and Oceania, and 48 from Europe. Since 2007, 2-32 Russula species have been described annually with increasing trend (Fig. 75). Molecular support for the delimitation of the new taxa and description quality are also increasing. From 2007 to 2014, only 25 of 82 newly described Russula species (27%) were provided with a sequence of the type specimen, compared with 73 of 76 Russula species (95.1%) newly described from 2015 to 2018. The negative contribution to the general quality of descriptions and molecular support is largely due to European amateur mycologists publishing in local journals and basing their new species solely on morphology and ecology. Since 2007, the estimated average quality of European Russula descriptions was only 61.6% compared to the 83.9% quality of the descriptions from the rest of the world (Electronic Supplementary Table S7). Even more noticeable is the difference in molecular support since 2007. Only 5 of 47 (11%) Russula species described from Europe were provided with sequence data, compared to 82 of 101 (81%) species described from other parts of the world. Several new European species are the result of misunderstanding of the phylogenetic signals of some morphological characters combined with low statistical support and lack of sequence data, and according to our knowledge (e.g. Adamčík et al. 2016a) and our experience (incl. personal communication with U. Eberhardt), sequences of these recently described species usually match previously described and well-known Russula species. Another weakness in European Russula taxonomy is the lack of type studies and comprehensive phylogenetic studies confirming the concept of older species names. To correct the situation, good descriptions of representative collections provided by statistically supported morphological observations and molecular support are needed, in fact we need a modern scientific monograph. Furthermore, progressive studies on Russula diversity require phylogenetic studies with well-represented lineages of closely related species and a comprehensive sampling for an estimation of distribution areas and history of species evolution.

Almost all recent *Russula* protologues (with one exception) describe spores and at least a part of pileipellis structures, 91% some hymenial elements and 51%

Fig. 76 Non-scaled heat map plotted in ggplots showing presence of information about microscopic elements, statistical support, use of chemical reagents and molecular support in 160 descriptions of *Russula* species published in 2007–2018. Information about individual species is in rows, the presence is indicated by pale and absence by red bars. Species are clustered horizontally based on similar pattern in their descriptions. Vertical arrangement (in columns) is based on similarities among individual analysed parts of descriptions. The full dataset is in Electronic Supplementary Table S7

stipitipellis (Fig. 76, Electronic Supplementary Table S7). Most attention is paid to spores and the structure of the spore ornamentation is the only character that is present in nearly all descriptions. More than three quarters of the protologues also give the ratio of spore length and width (81%), prominence of spore ornamentation (91%), and characters of the suprahilar spot (74%). For the hymenium, the majority of protologues describe the basidia (91%) and hymenial cystidia on lamella sides (90%, often called 'pleurocystidia'), but only a few describe cystidia near the lamella edges (43%, usually called 'cheilocystidia') and marginal cells (28%). Nearly all protologues describe hyphal terminations in the pileipellis (95%) and pileocystidia (93% including also primordial hyphae) but descriptions of the vertical structure of the pileipellis are less consistent (65%). In general, pileipellis descriptions are often incomplete, i.e. descriptions of the hyphal terminations often miss details about the terminal cells (29%) and pileocystidia appear to be often overlooked (treated as absent or not observed). The majority of species descriptions (93%) do not provide observations from both areas of the pileus surface and do not mention from which part of the pileus their observations originate. Russula experts pay most attention to the spores; they provide most of the spore dimensions with a range and average values (75%), but only 12% give average values of hymenial elements (basidia, cystidia) and only 9% of pileipellis elements (Fig. 76). Moreover, the majority of descriptions (54%) provide only an estimated width of cystidia and hyphae in pileipellis. All protologues have illustrations of spores (of very variable quality), but less consistent are illustrations of hymenial elements (79%) and pileipellis (93%). Some pileipellis drawings (20%) do not show enough detail to recognise terminal elements. The use of chemical reagents is very inconsistent: 91% of spores are observed in Melzer's reagent (spores are often illustrated by SEM photos), 51% of cystidia in sulfovanillin, 20% species are provided with observations of incrustations in the pileipellis using carbolfuchsin and 23% of publications used Cresyl Blue.

The clustering of European *Russula* descriptions at the bottom of the heat map (Fig. 76) and Asian ones mainly at the upper half of the chart suggest regional preferences of the character selection to describe new species. In addition, we labelled clusters published by five different authors



suggesting that even individual people or teams use their own templates.

# Importance of individual characters for species identification

Table 4 shows the results of a pairwise comparison of selected micro-morphological characters between species described in this study and morphologically similar related species with available reliable description (Adamčík 2002; Adamčík and Buyck 2011a, b, 2014a; Adamčík and Jan-čovičová 2013; Adamčík et al. 2013, 2015, 2016a, b, 2018; Buyck 2005; Buyck and Adamčík 2013; Ghosh et al. 2016; Song et al. 2018). Non-overlapping values within the range estimated as average minus and average plus standard deviation are treated as different. Differences in presence/ absence of non-numerical characters were also scored.

A minimum of two differences was scored between *R. madrensis* and *R. xerampelina* and between *R. fluvialis* and *R. foetentula*. Six species show differences in 50% or more of the analysed characters, among them also *R. olivaceo-himalayensis* and *R. seperina* that are closely related species of the well-defined lineage (Fig. 5). Morphological difference is not statistically correlated with pairwaise sequence identity (see last two columns of the Table 4).

Considering different parts of the basidiomata, the highest average rate of differences (39.2%) concerns pileipellis structures; spore characters are 32.3% different in average (excluding suprahilar spot) and hymenial elements 31.9%. There are three species pairs showing no difference in spores, four showing no difference in hymenial elements but not one with a completely similar pileipellis. Characters showing the most frequent differences between species pairs were pileocystidia size (both near the margin and near the centre) and spore size. This does not mean that any one of the characters showing low difference rate in the table is not important. For example, the lowest rate for species resolution accounts for the suprahilar spot, but this character is important for higher rank classification. Moreover, R. echidna is the only Russula species known so far to have an amyloid ring on the hilar appendage, which makes it as an excellent distinguishing character.

Our study confirmed the importance to distinguish between the margin and centre of the pileus when it comes to describing the elements. Nine of the total 26 species described here show distinct differences in pileipellis structure between the two areas of the pileus. Accordingly, species comparison revealed five species pairs that differ in the terminal cell size at the pileus centre, but not near the margin (Table 4).

# Discussion

# Estimation of global Russula diversity

Tedersoo et al. (2014) estimated that the highest richness of ECM fungi peaked at mid-latitudes, especially in temperate forests and the Mediterranean biomes of the Northern Hemisphere. Our analysis of the data used in this publication shows only small differences of Russula MOTU (molecular operational taxonomic unit) diversity among the tropical, the temperate, the boreal and the arctic environments (Fig. 77) and the most diverse biome seems to be savannah. The diversity pattern of individual genera of ECM fungi is not known yet and individual genera might have different patterns, e.g. boreal forests are usually dominated rather by Cortinarius or Tomentella than by Russula (Morgado et al. 2015). However, Corrales et al. (2016) discovered that *Russula* is the most dominant and diverse genus of ECM communities in neotropical montane forests of Panama.

Biogeographical factors strongly influence tropical ECM symbioses (Corrales et al. 2018) and these observations are also consistent with our ITS analyses of individual phylogenetic lineages originating from temperate or subtropical areas. Riviere et al. (2007) compared ECM basidiomycete diversity in two tropical rainforests of Africa and India and did not report any overlap in species diversity of both Indian and African forests (altogether 40 species identified). Kleine et al. (2013) compared ITS and atp6 regions of three Russula species reported in association with Uapaca from both continental Africa and Madagascar and they revealed significant sequence differences suggesting that long geographical disjunction across sea might have contributed to speciation. Our study demonstrated the distinct geographical pattern of closely related North American Xerampelinae species associated with conifers that are different among central Mexico and eastern and western North America (Fig. 6). Boreal species, represented in our dataset by R. fluvialis and R. laevis, may contribute to the overlap of species diversity between continents suggested by Looney et al. (2016) and Bazzicalupo et al. (2017). The studies by Geml et al. (2012) and Caboň et al. (2019) also confirmed the intercontinental conspecificity of boreal species.

Looney et al. (2016) recognised 1064 *Russula* molecular MOTUs worldwide based on available ITS data. They estimated the number of continent-specific *Russula* MOTUs known from well-researched areas of Europe to be 226 and the total number of species for the continent to 295. In our study, we recognised geographical elements of species distribution among regions of Central and western and eastern North America (*R. xerampelina* lineage),
among Europe, India and Thailand (R. seperina lineage), and Australian Russula species may form their own lineages (R. wielangtae lineage). Based on the published data (Kleine et al. 2013; Trierveiler-Pereira et al. 2015), Africa, Madagascar and South America are other Russula diversity pools. Most of the above mentioned 10 regions have as much or even more variable biomes than Europe and also equal or even higher ECM host diversity. While in Europe the number of Russula names probably significantly exceed the number of biological species (Buyck and Adamčík 2013b), in North America there are still many undescribed species (Avis 2012; Looney et al. 2016; Adamčík et al. 2018). In Asia, describing the local diversity is rapidly progressing but it has only just started (Li et al. 2016; Ghosh and Das 2017), while the *Russula* diversity in South and Latin America and Australia is very fragmentary (https://biogeodb.stri.si.edu/fungi; Grgurinovic 1997: https://www.funnz.org.nz). In Africa, the knowledge is very local and the number of species described during the last decade is very low, compared to the high rate of diversity continent unexplored in the (Buyck 1993, 1994a, b, 1997). Taking into account the 10 possible Russula-specific biogeographical areas, and the minimum of 200 species for each of them as an average, we estimate that there are at least 2000 species of Russula worldwide. This is probably only a minimum number considering a number of local disjunctions (climatic, orographic or by a sea) not discussed above.

#### Human factor

Human imagination, opinions, views and personal skills have sometimes contributed to more differences in species descriptions than nature itself (e.g. see results of type studies of Peck's Russula species by Adamčík et al. 2018). Furthermore, subjective observations used to describe new taxa can lead to divergent taxonomic opinions. Because of different opinions and skills, the text below is mainly prepared from the perspective of the first and the last authors. This study started with the ResearchGate discussion (https://www.researchgate.net/project/Quest-for-aglobally-comprehensible-Russula-language) that resulted in a common will to meet, discuss and test description standards in order to reduce differences caused by the human factor. All authors of this study had at least field experience with Russula before the meeting at Russula Microscopy Workshop in Slovakia. The genus attracted their attention not only because of the striking field appearance, but also because of its important mycorrhizal role in ecosystems and local economies (e.g. Xerampelinae in Mexico) as an important and traded food resource. Undiscovered diversity in some areas of the world motivates mycologists to study the genus, also because new



**Fig. 77** Chart showing number of Russulaceae OTUs in different biomes based on data retrieved from Tedersoo et al. (2014). Red line connects average values and blue horizontal line shows the trend. Trend lines use the method of least squares to find a line that best fits the points

species descriptions are believed to be a relatively easy source of scientific publications. At least some workshop participants are not focused on the taxonomy and systematics of a single genus or a limited fungal group but are interested in achieving skills applicable for fungi generally. Many of the workshop participants and authors are researchers starting their careers and several of them came from laboratories with no experience with Russula or agaric taxonomy at all. During the initial conversations, we were frustrated at not being able to compare descriptions because they were in very different styles. This caused difficulty in describing new taxa or to assigning existing collections to already described species because everybody experienced an inability to identify their collections with certainty. As a result, the outcomes expected from the workshop were that, by the end, we could speak one 'Russula language', meaning that we could compare our descriptions without missing some important characters or dealing with different terminologies or techniques.

#### **Field descriptions**

Macro-morphological descriptions were prepared by the workshop participants before the *Russula* Microscopy

Workshop in Slovakia and they were unified in style (Electronic Supplementary Table S2). However, several field characters are missing in some descriptions, among them often important characters like taste, odour and colour change of the context and colour of spore print. We observed a pattern consistent with the literature that the pileus colour as a 'character of interest' is present in all descriptions. The description of macro-morphological characters is an important part of species description and delimitation. It often allows collections to be classified or even to identify them reasonably well without any other observations. For the purpose of field work, the efficient way to obtain experience is to participate at meetings such as the Russulales workshop that has been held every 2 years since 2010 (Fraiture 2013). Examples of description forms for field work are presented by Bazzicalupo et al. (2017) or at the Russulales news webpage (http://www2. muse.it/russulales-news/). It is important to realize that in Russula, some characters, such as spore print colour and taste of the context, that are usually not taken into account in other genera, have an important taxonomic and phylogenetic signal.

### Individual performances

Is there a subjective input to the description, if all selected characters are clearly defined and they are described by defined terminology? What makes descriptions of the same specimen by various persons different? First, the variation largely depends on the quality of preparation of the material for microscopy. If the material is thick, dirty or too dense with hyphae, it is difficult or impossible to recognise microscopic elements. If the material is prepared with too much pressure or damage, the microscopic structures may rupture. Lastly, experience is needed for recognising the quality of a preparation and to interpret elements such as the contents, incrustation or damage of the microscopic structures. Individual performance can thus influence the perception of the quality of the spore ornamentation, contents of the cystidia, recognising of septa, etc.

For a suitable microscopic preparation, well-dried, mature specimens and a carefully prepared thin microscopic section is needed. For most elements, fresh 10% KOH solution helps to dissociate tissues, before colouring them with Congo Red for examination. Spore prints are a very convenient and efficient way to observe spores and provide a better chance of getting mature spores. Preparing spores from the lamellae is more difficult and may lead to damaged ornamentation, so that the elements of the ornamentation look more irregular, more connected and more numerous. Microscopic structures that are particularly difficult to recognize and interpret are cystidia. Some species, for example *R. aurantioflava* and *R.* sp. 5, have

very inconspicuous hymenial cystidia because they are almost optically invisible. The primordial hyphae of R. purpureogracilis are very inconspicuous and the presence of pileocystidia in R. sp. 7 is questionable. Several participants of the Russula workshop had initial difficulties interpreting the results of Cresyl Blue and carbolfuchsin staining. We recommend 'calibrating' Cresyl Blue using species with known reactions as controls, for example R. cyanoxantha (Schaeff.) Fr. as a positive and R. virescens (Schaeff.) Fr. as a negative control. The staining of acidresistant incrustations is sometimes also not convincing and may be sensitive to washing in HCl solution and water, for example, we observed very abundant large glutinous droplets on hyphae in the pileipellis of R. castanopsidis and R. purpureogracilis, but only inconspicuous incrustation of primordial hyphae of the latter species that were acid-resistant, and the red staining disappeared towards the margin of the material. For counting hymenial cystidia, sulfovanillin reaction is particularly convenient, in several cases we observed that darkening of the contents in sulfovanillin appears after several minutes and was strong enough only after at least a half an hour.

The template measurement tables (Electronic Supplementary Table S5) contain some characters that were used to describe the terminal cells of hyphae in pileipellis near the pileus margin: thickness near tips, difference between thickness at widest part and near tips and the presence of base-inflated, cylindrical and top-inflated cells. When used properly, these characters are useful in distinguishing between some species. For example, we used them to distinguish between *R*. sp. 1 which has less than 40% of the terminal cells base-inflated (subulate, attenuated, lageniform) and often obtuse and not inflated apically while the terminal cells of *R. olivaceohimalayensis* are mostly base-inflated (> 90%) and are apically usually constricted to  $1-2 \ \mu m$ .

Drawings of microscopic elements are very important to explain their overall arrangement and shape. The best option for spores might be SEM photos, but those are not always available, and they do not show spore ornamentation as seen in Melzer's reagent (http://www2.muse.it/rus sulales-news/tc\_spores.asp). We also tried to stack photos digitally, but this was very difficult and time consuming and the resulting figures show rather low quality and gridded views, despite looking very realistic (Electronic Supplementary Fig. S3). Consequently and to be more consistent with traditional literature, we prepared pencil line drawings (Figs. 15, 32, 50). For drawings of other elements, using line drawings made by standard optical drawing attachment (or alternatively in a data projector view) is the most realistic and efficient. The drawing tube approach is better than tracing photos from microscopemounted cameras as these can suffer from overlapping

Table 4	Analysis of reliability	y of characters based of	on comparison of our s	pecies in this study	y with available descrip	ptions of similar sp	pecies



Characters showing differences between species pairs are labelled by number one (1) in red field, zero (0) in blue indicates similarities, white fields data not available

elements (difficulties to trace individual hyphae and their cells) and unarranged hyphal orientations. Editing of digital photos from microscope also might take more time and produce a less informative result. Although line drawings are the preferred approach, they are (together with object preparation) the most difficult part of *Russula* descriptions. The main disadvantage is different personal style, as can be seen by comparing the species *R. olivaceohimalayensis* prepared by A. Ghosh, of *R.* sp. 1 by C. Manz and F. Hampe and *R. seperina* by S. Jančovičová and S. Adamčík in this study.

#### Character selection and their importance

The first effort to describe in depth the terminology and variety of microscopic characters of the genus *Russula* was by Buyck (1991) who focused on spores and hymenial elements. He pointed out the importance of some neglected characters in previous *Russula* descriptions, for example, the density of hymenial cystidia on lamella sides. In this study we do not want to introduce new characters, but rather use those that have been tested and recognized as important for *Russula* taxonomy.

In their study of four type specimens of Maculatinae, Adamčík and Jančovičová (2013) listed many important micromorphology differences that were previously not recognised in the literature. Most of these differences were in the pileipellis structure. Bazzicalupo et al. (2017) concluded that only 48.5% of specimens were correctly assigned to their species in a canonical variate analysis of combined field and spore characters. In some cases, characters of pileocystidia and hyphal terminations in pileipellis are the only available distinguishing characters (Adamčík et al. 2016a, b). These publications clearly show the major problems, viz. the general underuse of characters describing hyphal extremities and hyphal structures and that excessive attention is paid only to macro-morphological characters and spores (see also discussion on recent trends above and Electronic Supplementary Table S7). Accordingly, our character analysis (Table 4) showed that pileocystidia deserve to be described in detail and with the same statistical tests as for spores.

The differences between descriptions of the same species by different authors may be based on low number of observations that does not cover species variability. However, measuring all characters proposed in this study (Electronic Supplementary Table S5) is time consuming when assessing the variability from multiple specimens. To recognize species within a lineage of closely related species, Adamčík et al. (2016a) proposed to use complete descriptions of single representative collections per species by selecting characters that show differences between species. Such reduced selection of characters measured on an additional couple of collections per species allows elimination of even more characters showing overlapping values. The character reduction facilitates observations of multiple collections and increases efficiency by focusing only on informative characters.

Several studies have demonstrated the importance of recognising differences between the pileipellis near the pileus margin and the pileipellis near the centre for various Russula groups (Adamčík and Buyck 2011b; Buyck and Adamčík 2011). However, most of recent studies do not recognize or use the difference between the pileus margin and centre (Fig. 76) and the authors explained (in personal communication) that their observations originate from the intermediate zone or the pileus margin. Some Russula groups, particularly R. subg. Heterophyllidia, have welldeveloped and abundant pileocystidia only near the pileus centre. On the other hand, members of R. subg. Russula often have more attenuated terminal cells in the pileipellis near the margin than in the centre. In any case if the origin of pileipellis observations is not specified, not only is there a loss of information, but the comparison between species may result in wrong conclusions.

### Molecular and phylogenetic part

Most of the species described in this study originate from areas with few published taxonomic studies on Russula. In this case, a very valuable source for species delimitation is sequences originating from environmental samples rather than from basidiomata. As shown in Avis (2012), sequences from basidiomata of uncertain identity can be compared (e.g. by BLAST and other common and simple phylogenetic methods) to build a robust understanding of the sampled Russula species and diversity in a given region. Of course, this approach is limited by sequencing bias and the sequence length, and thus can result in decreased support for species clades. We think, that the combination of missing multi-locus data with low sequence quality and uncertain sample identification of publicly available ITS sequences are reasons why recent studies dealing with Russula use environmental samples sparingly, for example compare this study with Foetentinae in Song et al. (2018) and Maculatinae in Li et al. (2018). On the other hand, sequences of environmental origin helped us to improve our knowledge about (i) host range, (ii)

distribution, (iii) clustering and close relationships and (iv) possible infraspecific sequence variability.

To improve the selection of a closely related or ingroup species we selected type sequences or representative sequences from the UNITE species hypothesis (https:// unite.ut.ee). If the sequences were not available, it was important to check the identification of available sequences to their source or publication and to avoid ones with origin or ecology incongruent with the original publication of the species.

Molecular techniques have revolutionized the study of fungal taxonomy. In the past, species delimitation was mainly based on analyses of ITS region due to various reasons. Some examples in this study prove that the ITS is often insufficient for delimitation of closely related species (Hofstetter et al. 2019). We think that in addition to the ITS, more DNA regions should be provided to facilitate species delimitation and to recognize closely related *Russula* species. We recommend using of ITS, LSU, mtSSU, *rpb2* and *tef1* $\alpha$  regions.

Although we generated multi-locus data for most species described in this study, there are no such data available for related species to improve the support in our phylogenetic trees. In several cases, we used ecological, geographical and especially morphological arguments to support insufficiently supported ITS clades in our phylogeny. For *R*. subsect. *Xerampelinae*, the comparison of support at species level and at the higher rank between our multi-locus tree (Fig. 3) and ITS tree (Fig. 6) suggests that the ITS region is, in some early diversified lineages, an insufficient marker for species delimitation and more loci may support the recognition of phylogenetic species and elucidate their relationships.

## Conclusion

In this study, we estimate that at least 2000 Russula species occur worldwide. Most of them are regional in distribution, and probably only boreal-arctic species of the Northern Hemisphere contribute to overlap in the diversity between continents. We recognised 10 regions with a specific Russula diversity, but only Europe and eastern North America are well-researched, meaning that probably more than half of the existing Russula species are still undescribed. In the period 2007-2019, 160 Russula species were described, showing not only progress in the number of described species per year, but also in the quality of descriptions and molecular support for the species delimitation. The majority of Russula descriptions describe only spores in sufficient detail and with statistical support for the size, while descriptions of the hymenial and pileipellis structures are non-existent, brief or imprecise. Moreover, recent descriptions of new *Russula* species showed regional or author specific patterns. Given these issues, we propose *Russula* description standards that can be widely accepted and followed not only by *Russula*-centric investigators, but also by the wider mycological community. In so doing, we provide here a description template, template measurements table and specified terminology to facilitate descriptions of new *Russula* species.

In this study we deal with the question of which micromorphological characters are necessary for the description of new species; and to what extent (i.e. how many observations) these characters should be measured. Also, we deal with the question of whether spore characters are more reliable than other microscopic characters. For this reason, we analysed pairwise comparisons of species described in this study with most similar available descriptions of related species. Characters of spore, hymenium and pileipellis appear to be almost equally informative. Because of this, we argue that not only spores, but all elements deserve a similar approach that includes size range and average value estimated based on multiple measurements (in this study 30 or 20 replicated on 3 collections per species).

Different species pairs analysed in our study combined various sets of diagnostic characters. For this reason we recommend the following in order to describe new Russula species: conduct a full descriptive profile of the species based on select, single collections including measurements of all characters recommended in the template of this study; but, for the study of subsequent collections of the species of interest, limit observations of other collections to those characters which show significant differences to related and previously described species. This approach is feasible for well-represented lineages of closely related species that are well sampled by collections from different areas and habitats. For under-explored and unstudied Russula lineages, which diagnostic characters will be useful remain unknown, thus reducing the number of collections studied or the morphological characters and their measurements at the initial stage may trigger a future need of further studies and additional observations. Given this, for new lineages, we recommend the measurement of all characters provided in this study on multiple collections that are available. Several of our pairwise comparisons were based on a single (type) specimen per species; increased sampling will undoubtedly extend the knowledge of the variability of the known species and improve species delimitation. The descriptions of new species based on a single collection are only welcome in case of areas with difficult access (because of economic or political reasons) or originating from endangered habitats but they should be described in detail, using the characters, terminology and templates recommended in this study.

Species descriptions of this study use most of the characters traditionally used in the literature but not all. For instance, we did not use some characters such as the surface of the stipe, subhymenium and sphaerocytes of the context, because we do not know of any such study as truly diagnostic. In our study, we propose and show that measuring spore ornamentation, hymenial cystidia density and pileipellis characteristics are especially relevant and informative. Furthermore, we think that the proposed terminology and template will facilitate assessment of best diagnostic characters. We found it particularly important to recognize between the pileus margin and centre, because one-third of species described here show distinct differences between these pileus areas. Despite that, the majority of recent Russula descriptions do not provide information about the pileal origin of their observations. Comparison of observations from different parts of the pileus may lead to the wrong conclusion nd observing only one area of the pileus may lead to loss of important characters.

In this study we combined knowledge about geography, ecology, ITS and morphological data to support our species hypotheses. Most current *Russula* studies avoid using sequences from environmental samples, because they are usually not identified to species, but our study showed that they can be very useful to support the sampling. For instance, if sequences originate from ECM root tips and match sequences of well-studied and described basid-iomata, they can provide precise data about the host plant. Because of possible sequencing biases and short reads, the sequences of environmental origin sequenced by next generation amplicon sequencing technology should be combined by ones produced by Sanger sequencing in order to maximize the information gained from both the study of basidiomata and the environment.

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