FACULTY OF SCIENCES

A search for the perfect match: host specificity as a character in Russula phylogeny

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Contents

1. Introduction

1.1 Genus *Russula*

Russula is among the most important genera of ectomycorrhiza (ECM) -forming fungi. It is one of the largest and extensive monographed genera of the ECM Basidiomycota, but also one of the best recognizable (Sarnari, 1998). The genus *Russula* can be recognized by their mostly colourful fruit bodies and the presence of sphaerocytes in the context, which give them a chalk-like structure. The fruit bodies of *Russula* exhibit a high diversity of macroscopic, microscopic and chemical features, which resulted in very complex and highly structured, multilevel classifications (Buyck et al., 2018). However, the diversity of *Russula* has been highly underestimated. Incomplete sampling and many underexplored areas cause a lack of knowledge about the distribution of *Russula* species (Adamčík et al., 2019). Most research on *Russula* diversity is centred around Europe, hereby underestimating and ignoring the *Russula* diversity of other continents (Buyck & Adamcik, 2013). Data for species recognition is also deemed to be insufficient, due to missing morphological and molecular data (Adamčík et al., 2019). The genus *Russula* has many cryptic species and species with phenotypic plasticity, making it difficult to differentiate species based on morphological features alone. Differentiation based on ecological features, like host specificity, can be a solution.

The genus *Russula* is divided in multiple subgenera and sections. The classification has changed over the years. In the classification of Singer (1987), *Russula* was divided in 7 sections. Romagnesi (1987) divided *Russula* in 9 subgenera. In the classification of Sarnari (1998), only 6 subgenera remained. Some of them were transferred to the subgenus *R.* Romagn. emend. as sections within that subgenus. The most recent classification follows Buyck et al. (2018) and divides *Russula* in 7 sebgenera.

1.2 Subgenus *Compactae*

The *Russula* subgenus *Compactae* (Fr.) Bon[1] was seen as a distinct morphological group, characterised by the presence of numerous lamellulae and firm, compact fruit bodies with a yellow, white or brown cap. The classification of *R*. subgenus *Compactae* (Fr.) Bon has changed over the years.

Classification of Singer (1986, 'The Agaricales in modern taxonomy'): In one of the earliest classifications, Singer put *Russula* species with unequal gills (polydymous gills) in the section *Compacta* Fr. No further subdivisions were made.

Classification of Romagnesi (1987, 'Les Russules') and Bon (1988)**:** Romagnesi divided *Russula* in different subgenera. Species with a compact fruit body and lamellulae of different lengths were put in *Russula* subgenus *Compactae* (Fr.) Bon. (classificatie van Bon zoeken) The subgenus was subdivided in 3 sections: *R*ussula section *Archaeinae* Heim, *R*ussula section *Nigricantinae* Bataille (blackening context), *R*ussula section *Plorantinae* Bataille. In Bon (1988) *R*ussula section *Nigricantinae* was renamed to *R*ussula section *Compactae*, and *R*ussula section *Plorantinae* to *R*. sect. *Plorantes*.

[1] Former *Russula* subgenus *Compactae* (Fr.) Bon will be referred to as *Russula* subgenus *Compactae* s.l. The current *Russula* subgenus *Compactae* (Fr.) Bon, emend. Buyck & V. Hofst. will be referred to as *Russula* subgenus *Compactae* s.s.

Classification of Sarnari (1998, 'Monografia illustrata del genere Russula in Europa'): *Russula Compactae* was again subdivided in 3 different sections: *R*ussula section *Archaeinae* R. Heim ex Buyck & Sarnari, *R*ussula section *Compactae* Fr. and *R*ussula section *Lactarioides* (Bataille) Konr. & Joss. The *R*. sect. *Lactarioides* was previously named *R*ussula section *Plorantinae* in the classification of Romagnesi

Buyck et al. 2018 and Buyck et al. 2020: Recent molecular analysis scattered *Russula* subgenus *Compactae* (fr.) Bon in 5 different *Russula* clades: *Russula* subgenus *Archaeae* Buyck & V. Hofst. (Hongsanan et al., 2015), *Russula* subgenus *Compactae* (Fr.) Bon, emend. Buyck & V. Hofst. (Hongsanan et al., 2015), *Russula* subgenus *Malodorae* Buyck & V. Hofst., *Russula* subgenus *Brevipedum* Buyck & V. Hofst. (formerly known as *Brevipes)* (Buyck et al., 2020) and, most recently, *Russula* subgenus *Glutinosae* Buyck & X.H. Wang (Buyck et al., 2020). Only the first 4 clades will be discussed in this research, as *R*. subg. *Glutinosae* only consists of 2 species until now.

Below is an overview of the most important microscopic and macroscopic characteristics of the 4 subgenera (Buyck et al., 2018):

Russula subgenus *Archaeae* Buyck & V. Hofst.: Moderately large to small species, compact to very thin-fleshed. Cap dull coloured, yellowish, brownish or gray. Gills irregularly unequal, with lamellulae either more or less abundant than normal gills. Context yellowing, browning, greying or reddening. Spore print white. Spores very small, with inamyloid suprahilar spot. Gloeocystidia mucronate to obtuse. Ectomycorrhizal mantle with a plectenchymatic outer layer, producing abundant, emergent, hyphal extremities. Gloeocystidia inconspicuous, terminal, one-celled, minutely capitate with mostly one terminal knob.

Russula subgenus *Compactae* (Fr.) Bon, emend. Buyck & V. Hofst.: Fruiting bodies very large to very small, thick-fleshed. Cap dull-coloured, white, brown, grey to black. Gills regularly unequal. Context reddening, greying, blackening, rarely browning, with or without distinct, mostly disagreeable smell. Spore print white. Spores with inamyloid suprahilar spot. Gloeocystidia present in all tissues or not, sometimes restricted to the hymenium only and there mostly minutely capitate with one central knob, elsewhere often with two excentrical knobs (the "Mickey Mouse type"), more rarely obtuse rounded. Ectomycorrhizal mantle covered with emergent, one-celled, flask-shaped gloeocystidia that are mostly mucronate with one central knob or, more frequently, two excentrical knobs.

Russula subgenus *Malodorae* Buyck & V. Hofst: Medium to large species, often firm and compact, never extremely thin-fleshed. Cap dull coloured, yellow brown, grey to almost black or whitish. Gills regularly unequal to frequently and almost regularly forking. Context greying or browning, mostly developing rapidly a strongly disagreeable smell and taste. White spore print. Spores with inamyloid suprahilar spot. Gloeocystidia moderately numerous to numerous on gill surface, mucronate and inconspicuous to absent elsewhere from the fruiting body. Hyphal extremities of cap surface typically with inflated, often voluminous cells, and strongly septate. Ectomycorrhizal mantle with dispersed to rare gloeocystidia that are emergent, one-celled, flask-shaped, minutely capitate with one or rarely two knobs, sometimes accompanied by emergent, apically tapering to cylindrical, thick-walled hyphal extremities.

Russula subgenus *Brevipedum* Buyck & V. Hofst.: Mostly medium to very large species that are very thick-fleshed, only exceptionally also small and thin-fleshed. Cap whitish, often rapidly developing

yellowish brown to reddish brown stains. Gills regularly unequal. Context turning yellowish to rusty brown, mostly with distinct smell, acrid to strongly acrid, (rarely mild?). Spore print whitish to yellow. Spores with inamyloid or amyloid suprahilar spot. Gloeocystidia mucronate to obtuse-rounded, in all parts of the fruiting body. Ectomycorrhizal mantle covered with emergent, one-celled to secondarily septate, short, flask-shaped, mostly thick-walled gloeocystidia that are generally minutely capitate with one or rarely two, central knobs.

1.3 Species descriptions

The descriptions of species within the genus *Russula* are often incomplete. Microscopic descriptions in many publications mostly favour spores, more specifically spore size, over hymenium and pileipellis (Adamčík et al., 2019). Also, the significance of below-ground characteristics as meaningful descriptive features is often ignored (Buyck et al., 2018). Additionally, the description style between authors and regions is not always consistent. In Adamčík et al. (2019), a more consistent species description style for all *Russula* is suggested and encouraged, which we will also follow in our descriptions of some species.

Different species concepts can be used for delimiting species. For Fungi, the most used species concept in the past was the Morphological Species Concept (MSC), which delimits species based on morphological features, both microscopic and macroscopic (Aldhebiani, 2018). The MSC has both its strengths and weaknesses. One of its strengths is that it is widely used for Fungi: an easy comparison can be made between different taxa as well as between new and existing taxa (Taylor et al., 2000). However, with the rise of a new species concept, the Phylogenetic Species Concept (PSC), it became clear that morphological features are not always sufficient to reflect the real phylogeny behind different fungal clades. The MSC ignores the existence of so-called cryptic species, which are two or more species who are morphologically similar, but are genetically distinct (Taylor et al., 2000). In the genus *Russula*, phenotypic plasticity and obscure morphological and anatomical discontinuities have been observed, which also makes it difficult to differentiate individual species (Miller & Buyck, 2002) based on morphological characteristics. Overall, the morphology-based taxonomy has led to an underestimation of the fungal diversity and an inadequate classification (Agapow et al., 2004).

The description of *Russula* diversity is however impeded by missing data for species recognition (both morphological and molecular). The fungal ITS barcode is often proven to be insufficient for delimitation between closely related species (Adamčík et al., 2016). 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. Therefore, more elaborate and consistent morphologic descriptions can be an important tool for species delimitation and to support species concepts.

Besides species delimitation, better species descriptions also benefit field research and revive older species names, as sequencing of old *Russula* specimens from (e.g. type specimens) often have a low success rate (Looney, 2015).

1.4 Host specificity

The Morphological Species Concept (MSC) and the Phylogenetic Species Concept (PSC) were already mentioned in the previous section. However, another species concept is overlooked for delimiting species of Fungi, namely the Ecological Species Concept (ESC). The ESC is based on ecological competition and states that the more similar two individuals are, the more their needs are likely to

overlap. Therefore, they are expected to contest, and consequently, the more similar their ecological features, the more likely that they are of the same species (Aldhebiani, 2018). An interesting ecological feature for species delimitation can be the host plants of ectomycorrhizal (ECM)-forming fungi.

ECM -forming fungi form symbiotic relationships with plant hosts without penetrating the cells of the host. The ECM root consists of three structural components: a sheath or mantle from fungal tissue which encloses the root of the host, an inward growth of hyphae between host cells called the Hartig net, and the external mycelium which grows outwards and forms connections with the soil and the fruiting body (Smith & Read, 1997). The fungus gets carbohydrates from the host, while the fungus helps the host to take up minerals and water more efficiently (Smith & Read, 1997). ECMs are important in ecosystem-functioning of forests.

The different host plants of ECM-forming fungi can be an important ecological feature for species recognition and delimitation. Caution is needed, as many possible host species can co-occur in the field, making it difficult to determine which of them is the host. Another problem is the possibility of specialized cryptic species within generalist species (Bruns et al., 2002). Most species of the genus *Russula*, for example *Russula brevipes*, appear to have a wide range of hosts and are considered generalists. Other species of *Russula* however are highly host specific (Kernaghan et al., 2003; Geml et al., 2010), e.g., *R. decolorans* and *R. nauseosa* are considered to be specific to species of Pinaceae (Molina et al., 1992). In this paper, the host specificity is investigated in *Russula* subgenus *Compactae* s.l., which has many morphologically similar species.

2. Objectives

A first objective is to find a link between the species of *Russula* subgenus *Compactae* s.l. and the different hosts they connect to. Hereby, we will use phylogenetic analyses of *R*. subg. *Archaeae*, *R*. subg. *Compactae* s.s., *R*. subg. *Malodorae* and *R*. subg. *Brevipedum* and evaluate the host specificity. We will try to find a link between the species trees of these groups and their host species.

A second objective focusses more on *R*. subg. *Brevipedum*. Some species of this subgenus will be described microscopically for the first time. These taxa were based on phylogenetic analyses with the ITS, LSU, RBP2 and EF1 markers and descriptions were based on the guidelines in Adamčík et al. (2019). Also, an additional species delimitation analysis will be done based on 4 different markers: ITS, LSU, RBP2 and EF1.

3. Material and methods

3.1 Sampling

The specimens for the descriptions were collected by Ruben De Lange, Felix Hampe, Helga Marxmüller and Jean Michel Trendel. The *R. delica* Fr. var. *delica* paratype/topotype was collected by Henri Romagnesi and was collected in the same area as the holotype. Specimens originate from Italy, France and Belgium. Collections from Ruben De Lange and Felix Hampe were stored in the Herbarium Universitatis Gandavensis (GENT). Supplementary collections were requested from the personal collection of Jean Michel Trendel, Helga Marxmüller (deposited in the State Museum of Natural History Karlsruhe, KR). The collection of Henri Romagnesi was stored in Muséum National d'Histoire Naturelle in Paris, France.

From the collections of Ruben De Lange, Felix Hampe and Jean Michel Trendel, parts of the fruiting bodies were collected and preserved in small tubes with CTAB (Cetyl trimethyl-ammonium bromide).

3.2 Data host species

Databases used for obtaining ecological information and sequences of species from *R*. subg. *Archaeae*, *R*. subg. *Compactae* s.s., *R*. subg. *Malodorae* and *R*. subg. *Brevipedum* were the database from our research group, UNITE (Kõljalg et al., 2005) and GlobalFungi (Větrovský et al., 2020). For each subgenus, Maximum Likelihood (ML) phylogenetic trees based on the ITS (internal transcribed spacer) sequence were composed by Ruben De Lange. From these ML trees, samples from BioloMICS were selected, based on the different species in the trees. The ITS sequences of these specimens were used to search the databases UNITE and GlobalFungi for the corresponding species hypotheses (SH) per specimen. Each sample has multiple SHs with different thresholds, expressed in percentage. A threshold of 1% means that all the members of that SH have a difference in their ITS sequence of maximum 1%, meaning that samples with more differences belong to another SH. Per sample, three SH's were selected with thresholds of 3%, 1,5% and 0,5% or 0,0%. In these SH's, data about host plants, habitat, location and corresponding ITS sequence of different samples were exported from UNITE and GlobalFungi and collected into a dataset.

3.3 Morphologic analysis

3.3.1 Macroscopy

The macroscopic descriptions are based on observations from fresh material. The colour codes used follow the Methuen book of colours (Kornerup & Wanscher, 1978), guaiac reactions refer to Chalange (2014), and spore print colour codes follow the scale of Romagnesi (1967)

3.3.2 Microscopy

Microscopic descriptions follow Adamcik et al (2019). Microscopic characters were studied from dried specimens. Spores were observed in Melzer's reagent. Spore measurements were done using a Nikon Eclipse Ni-U microscope with crosshair eyepiece with a magnification of 1000×. Line drawings of spores were made based on stacked photographs taken with a Nikon DsFi3 camera ((Nikon Eclipse Ni-U microscope, stacking software: Extended Depth of Field, Nikon Nis Elements module) at original magnification of 5000×.

Characters of the hymenium and pileipellis were observed in Congo Red. The elements of the hymenium were measured and observed on both the lamellae side and lamellae edge. For the lamellae side, the basidia and the cystidia were measured and observed and for the lamellae edge, the cystidia and marginal cells. Pileocystidia and hyphal terminations were measured and observed at both the pileipellis margin and centre. The vertical structure of the pileipellis was measured and observed at mid-radius. Different chemical reactions were tested on the hymenium and pileipellis. The metachromatic reaction with Cresyl blue and the reaction of the cystidia to sulfovanillin on the cystidia were tested on both the pileipellis and hymenium. Carbolfuchsin was used to test for the presence of acid-resistant incrustations of primordial hyphae and was only tested on the pileipellis. Drawings of the pileipellis and hymenial elements were made with an Olympus CX31 microscope with a drawing tube at original magnification of 1500×.

For all described species except *R.* sp. 1, multiple collections were used. Statistics for all microscopic characteristics were based on at least 10 measurements per collection. Measurements are given as (minimum –) average minus standard deviation (SD) – average – average plus SD (– maximum). For the spores, a Q-value was also calculated, which indicates the length/width ratio. The density of the spore ornamentation is computed following Adamcik & Marhold, (2000).

3.4 Molecular analysis

3.4.1 Phylogenetic trees

Performed by Ruben De Lange

In Ghent University, DNA was extracted from the samples using the CTAB extraction method from Nuytinck and Verbeken (2003). DNA from dried material was extracted using a modified CTAB protocol (Tel-Zur et al., 1999; modified by Meise Botanic Garden and Research Group Mycology of Ghent University). Phylogenetic trees were made for *R*. subg. *Archaeae*, *R*. subg. *Compactae* s.s., *R*. subg. *Malodorae* and *R*. subg. *Brevipedum.* The molecular marker that was amplified for these phylogenetic trees is the internal transcribed spacer (ITS), which is the spacer DNA between the small-subunit and the large-subunit ribosomal DNA (rDNA). Specifically, the ITS1 and ITS2 regions and the ribosomal gene 5.8S were amplified, using the ITS1-F and ITS4 primers (White et al., 1990; Gardes & Bruns, 1993). For the concatenated tree, the following 4 markers were used: (1) the internal transcribed spacer region of ribosomal DNA (ITS), (2) a part of the ribosomal large subunit 28S region (LSU), using primers LR0R and LR5 (Moncalvo et al., 2000) (3) the region between the conserved domains 6 and 7 of the second largest subunit of the RNA polymerase II (RPB2), using primers bRPB2-6F and fRPB2-7cR or bRPB2-7.1R (Liu et al., 1999; Matheny, 2005), (4) the translation elongation factor 1-alpha (TEF1α), using primer pairs EF1-1018F and EF1-1620R or tef1F and tef1R (Morehouse et al., 2003; Stielow et al., 2015).Protocols for PCR amplification follow Le et al. (2007). PCR products from Ghent University were sequenced using an automated ABI 3730 XL capillary sequencer at Macrogen. Forward and reverse sequences were assembled into contigs and edited where needed with BioloMICS.

Performed by me:

To assemble the phylogenetic trees of the four subgenera, both the ITS sequences from BioloMICS and our own dataset, with ITS sequences from UNITE, were used. For the alignment of the sequences, the online version of the multiple sequence alignment program MAFFT v 7 was used, performing the E-INS-I strategy, which is an iterative refinement method (Katoh et al., 2019). Partitioning of the aligned ITS sequences and exclusion the trailing ends was done in MEGA-X (Tamura et al., 2018). Therefore, the sequences were partitioned in 18S rRNA, ITS1, 5.8S rRNA, ITS2 and 28S rRNA, from which the borders of each segment were found via the partitioning of GenBank sequences. Maximum likelihood analysis was performed with RAxML-HPC BlackBox, which uses rapid bootstrapping (1000 replicates) (Stamatakis, 2014), via the online CIPRES science gateway (Miller et al., 2010).

Performed by Ruben De Lange:

For the alignment of the 4 markers ITS, LSU, RBP2 and EF1, the online version of the multiple sequence alignment program MAFFT v 7 was used, performing the E-INS-I strategy. The alignments

were partitioned into following partitions: ITS-LSU-alignment: partial 18S, ITS1, 5.8S, ITS2, LSU; RPB2-alignment: the RPB2 intron and the first, second and third codon positions of the exon; TEF1α-alignment: the first and second intron and the first, second and third codon positions. Maximum likelihood (ML) analyses were conducted with IQ-Tree (Nguyen et al., 2014; Chernomor et al., 2016). using standard bootstrapping analysis (1000 replicates). Convergence and Effective Sample Size (ESS) statistics of the runs were also examined with Tracer v1.7.1 (Rambaut et al., 2018). A burn-in sample of 20% was excluded before constructing the majority rule consensus tree. Analyses were first performed on each alignment separately and visually checked for incongruence. Significant incongruence was assumed if two different relationships (one monophyletic and the other non-monophyletic) for any set of taxa were supported with bootstrap values (BS) ≥ 70 or posterior probabilities (PP) ≥ 90. The resulting gene tree did not show any supported conflicts, therefore all alignments could be concatenated. The concatenated alignment was used for the multi-locus phylogenetic analyses.

3.4.2 Coalescent species delimitation

Potential species units were specified based on 4 ML for each marker: ITS, LSU, RBP2 and EF1, made by Ruben De Lange, with a total of 33 as the full model. Each of species unit was assigned a letter code. For species delimitation, we used Bayesian Phylogenetics and Phylogeography, BP&P v4.3.8 (Yang, 2015). Analysis A11 (Yang & Rannala, 2014) was performed, for unguided species delimitation using reversible-jump Markov chain Monte Carlo (rjMCMC) algorithm 0 (Yang & Rannala, 2010). We used ε=2 for the fine-tune parameter, assigned equal probabilities to the rooted species trees as a species model prior, and $\theta \sim IG(3,0.002)$ and $\tau \sim IG(3,0.002)$ as priors on the ancestral population size and root age. The BP&P v4.3.8 program was run 3 times with the same input to test consistency between runs.

4. Results

4.1 Host specificity

4.1.1 *R.* subg. *Malodorae* (Appendix A)

We found hosts for most of the species of *Russula* subg. *Malodorae*, except for *R. griseobrunnea* McNabb*, R. capillaris* Buyck and several unnamed species.

Angiosperms are most common among the hosts, but gymnosperms are also present, though far less common. We found hosts in 8 different families, 6 of them containing tree hosts: Fagaceae (*Quercus Castanopsis*, *Lithocarpus*), Dipterocarpaceae (*Marquesia*) Nyctaginaceae (*Neea*), Salicaceae (*Populus*), Phyllanthaceae (*Uapaca*), Caesalpiniaceae (sometimes named as subfamily Caesalpinioideae within the family Fabaceae) and Pinaceae (*Pinus*, *Abies*). The 2 other families Orchidaceae (*Cymbidium*, *Lecanorchis*, *Cypripedium*) and Ericaceae (*Monotropa*, *Monotropastrum*) contain herbs. The genus *Uapaca* is seen as a host for 7 different species of this subgenus, therefore being the most abundant host genus.

4.1.2 *R.* subg. *Archaea* (Appendix B)

For *Russula* subg. *Archaea*, information about the host species in the databases was limited, as we only found host species for 6 species: *Russula camarophylla* Romagn., *Russula butyroindica* K.Das & Buyck, *Russula earlei* Peck, *Russula archaea* R.Heim and two unnamed species. For *R. camarophylla*, *R. archaea* and the 2 unnamed species, only one host was found, while *R. botyrundica* and *R. aerlei* have multiple hosts.

Most hosts are angiosperms, but gymnosperms are also represented as host for 2 species. The hosts are mostly tree species, which are members of only 3 families: Fagaceae (*Castanopsis*, *Fagus*, *Quercus*), Phyllanthaceae (*Uapaca*) and Pinaceae (*Picea*, *Pinus*, *Tsuga*), but an herb of the family Ericaceae (*Pyrola*) was also mentioned. The genus *Quercus* is the most abundant host in this subgenus, being the host of 3 different species.

4.1.3 *R.* subg. *Compactae* s.s. (Appendix C)

Hosts were found for most species of *R.* subg. *Compactae* s.s. (Fig. 3, see addendum) Most of the species only had one host species, but sometimes, mainly if the group contains more specimens, more host species could be found.

The hosts of *R.* subg. *Compactae* s.s. are mostly angiosperms, but gymnosperms also occur abundantly as hosts. The biggest part of the hosts are trees and are represented by 12 different families, from which 10 families contain trees: Fagaceae (*Quercus, Lithocarpus, Castanopsis, Fagus*), Fabaceae (*Acacia, Gilbertiodendron, Microberlinia, Intsia*), Juglandaceae (*Oreomunnea*), Betulaceae (*Betula, Carpinus*), Salicaceae (*Populus*), Eucalypteae (*Eucalyptus*), Phyllanthaceae (*Uapaca*), Asteropeiaceae (*Asteropeia*), Dipterocarpaceae (*Dipterocarpus*), Caesalpiniaceae and Pinaceae (*Pinus, Abies, Picea, Pseudotsuga, Larix*). Herbs are represented by 2 families: Ericaceae (*Monotropa, Pyrola*) and Orchidaceae (*Aphyllorchis, Cymbidium, Epipactis*). The genera *Pinus* and *Quercus* represent the largest part of the hosts.

4.1.4 *R.* subg. *Brevipedum* (Appendix D)

Again, the large majority of the hosts are angiosperms, but gymnosperms are also present as host trees. *Russula* subg. *Brevipedum* has the most diversity in host families of all the subgenera mentioned (Fig. 4, see addendum). The number of families counts 14, with the majority -around 10- containing trees species: Nothofagaceae (*Nothofagus*), Fabaceae (*Dicymbe*), Fagaceae (*Quercus, Fagus*), Betulaceae (*Betula*), Phyllanthaceae (*Uapaca*), Malvaceae (*Tilia*), Salicaceae (*Populus, Salix*), Nyctaginaceae (*Pisonia*), Burseraceae (*Bursera*) and Polygonaceae (*Coccoloba*). Additionally, *R*. subg. *Brevipedum* has some herb hosts from the families Cistaceae (*Cistus*) and Orchidaceae (*Limodorum, Cymbidium, Monotropa, Hexalectris*) and shrubs from the family Rosaceae (*Dryas*). Remarkably, the genus *Kobresia*, which is a grass from the family Cyperaceae, was also mentioned as a host in two cases.

Similar as in *Russula* subg. *Compactae* s.s., the genera *Pinus* and *Quercus* are most abundant as hosts in *R*. subg. *Brevipedum.*

4.2 Species delimitation (Appendix E)

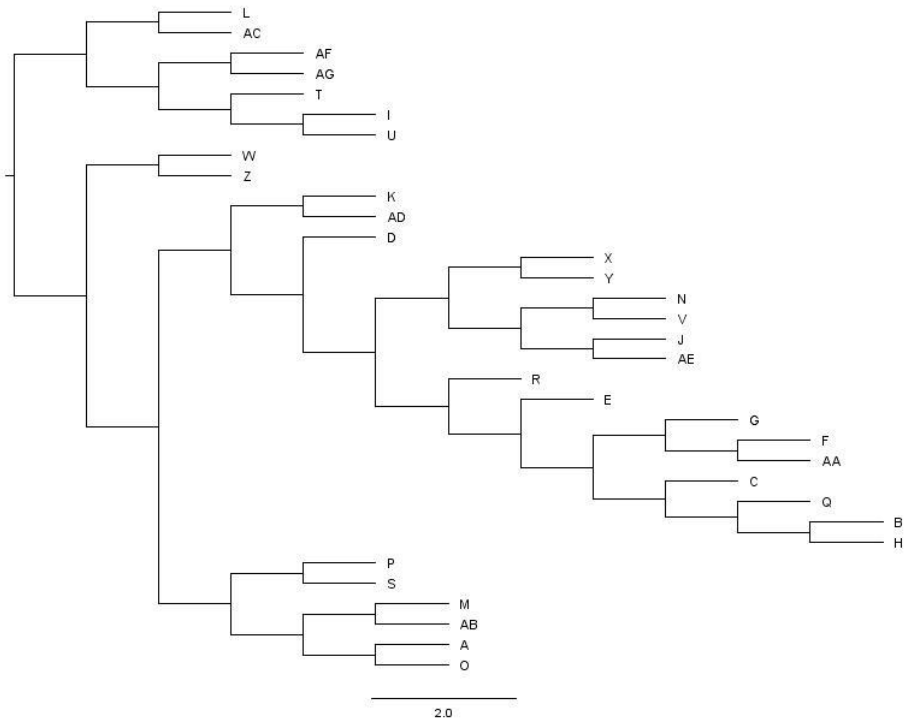

Figure 1. Best species tree model after the first run. The letter codes follow those of appendix E.

In total, 33 species were specified based on the ML trees of the 4 markers (ITS, LSU, RBP2, EF1) (Appendix E). The program was run three times, with each run a different output.

After the first run, the full set of 33 proposed species was recovered as the highest supported species model in the BP&P analysis, with a posterior probability of 0.45 (Fig. 1). The posterior probabilities of the delimited species in figure 1 range from 1.00 (A, B, L, O, T, W, Z) to 0.64 (K, AD)

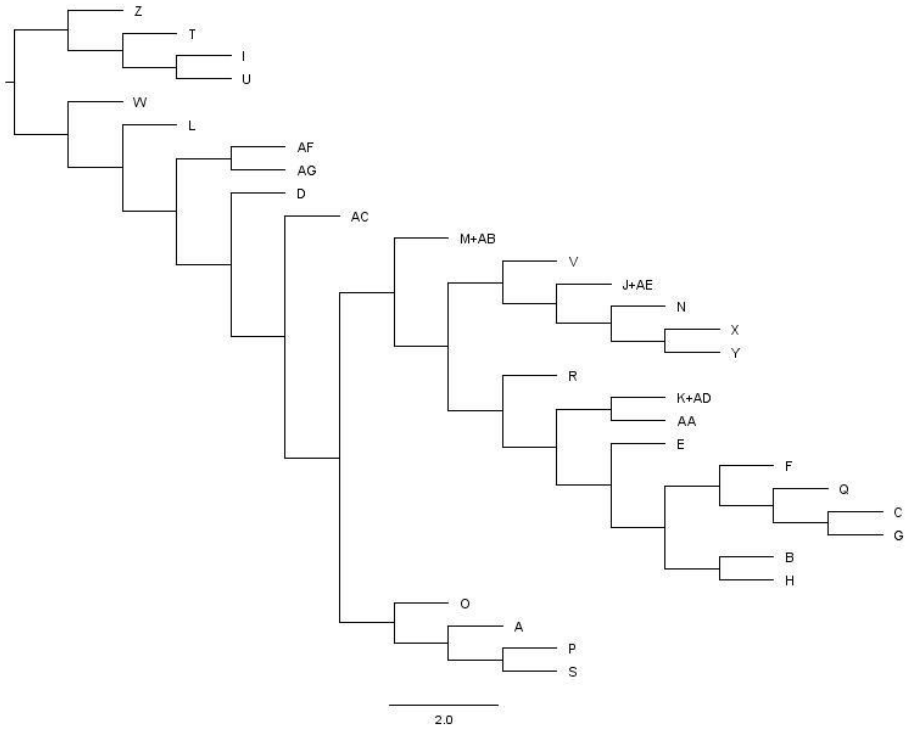

Figure 2. Best species tree model after the second run. The letter codes follow those of appendix E.

After the second run, a set of 30 species, from the 33 species we proposed, was recovered as the highest supported species model in the BP&P analysis (Fig. 2). This model had a posterior probability of 0.56. The posterior probabilities of the delimited species in figure 2 range from 1.00 (A, B, D, L, O, R, T, W, Z, AC) to 0.79 (J+AE).

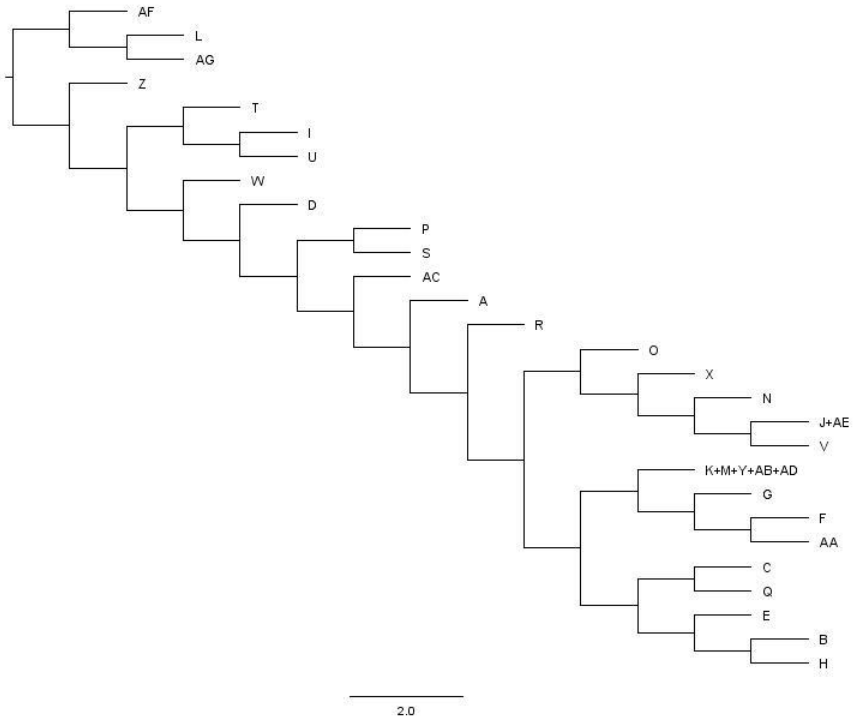

Figure 3. Best species tree model after the third run. The letter codes follow those of appendix E.

After the third run, a set of 28 species was retrieved as the highest reported species model in the BP&P analysis (Fig. 3). This model had the highest posterior probability of the three runs, with a value of 0.58. The posterior probabilities of the delimited species in figure 3 range from 1.00 (A, B, O, T, W) to 0.69 (J+AE).

4.3 Species description

***Russula* sp. 1 (Fig. 4)**

Description: Pileus medium-sized, 65 mm diam. **Lamellae** yellow. **Context** taste mild; odour fruity; reactions not observed.

Hymenial cystidia (49–)57.0–66.3–75.5 (–80) × (5–)6.4–7.4–8.3 (– 9) µm, cylindrical to narrowly clavate, apically obtuse, thin-walled; contents refringent, oily, reacting weakly (greyish) in sulfovanillin; near the lamellae edges, (50–)55.6–66.0–76.3(–82) × (6–)6.9–7.8–8.6(–9) µm, cylindrical to narrowly clavate, apically obtuse, thin-walled; content same as on lamellae sides. Lamellae edges sterile; **marginal cells** (8 –)12.7–21.8–30.8(–40) × (3–)3.9–4.8–5.6(–6) µm, undifferentiated, cylindrical to narrowly clavate. **Pileipellis** orthochromatic in Cresyl Blue, 425-575 µm deep, not sharply delimited from trama, gradually passing; subpellis not delimited from suprapellis; hyphae 2-4 µm wide near trama densely arranged, irregularly oriented, more horizontally oriented towards the trama, with no distinct gelatinous coating. **Acid-resistant incrustations** absent. **Hyphal terminations** near the pileus margin long, sometimes flexuose with multiple septa, thin-walled; terminal cells (20–)30.6–39.8–49.0(–60) × (2–)2.3–3.0–3.6(–4) µm, cylindrical, apically obtuse; subterminal cells and the cells below irregular, sometimes branched. Hyphal terminations near the pileus centre sometimes flexuose, with multiple septa, thin walled, terminal cells slightly smaller (25–)29.0–35.7–42.3(–49) × (2–)2.6–3.0–3.3(–4) µm, cylindrical, apically obtuse; subterminal cells and the cells below very irregular, sometimes branched. **Pileocystidia** a near the pileus margin, 1-3 celled, terminal cells (37–)42.4–50.9–59.4(–64) × (4–)4.0–4.8–5.6(–7) µm, cylindrical to narrowly fusiform, often flexuose, apically obtuse, contents refringent, slight reaction with sulfovanillin (greyish). Pileocystidia near the pileus centre slightly less broad, terminal cells (24–)30.7–45.0–59.3(–75) × (3–)3.5–4.2–4.9(–5) µm, similar in shape and content.

Ecology: No data.

Distribution: Known from Italy.

Specimen examined: Italy, 11 november 2016, R. De Lange (RDL 16-059).

Notes: In specimen RDL 16-059, no basidia and spores were observed.

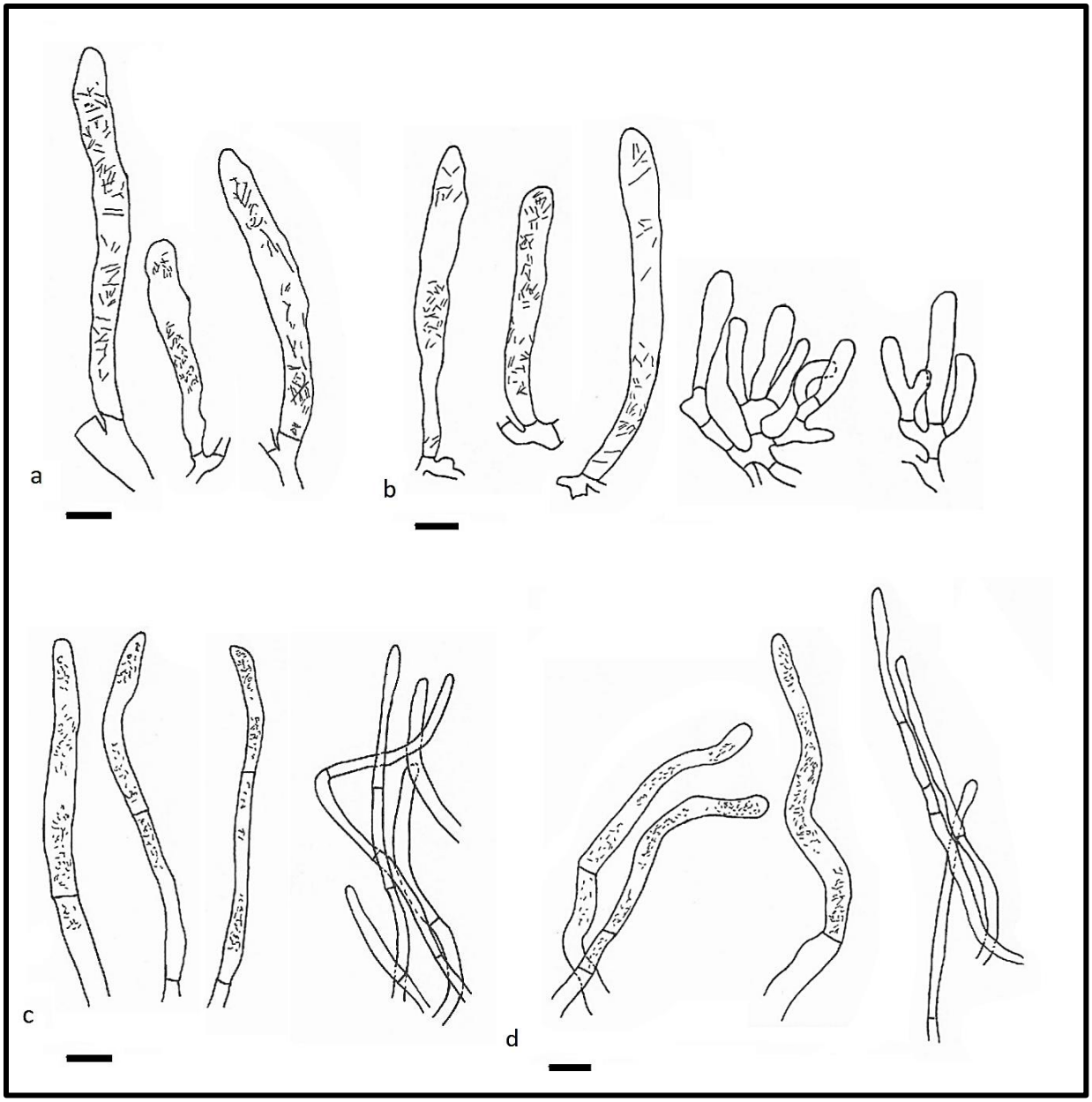

Figure 4. Russula sp.1; a) Cystidia of the hymenial side; b) Cystidia and marginal cells of the hymenial edge; c) Cystidia and hyphal terminations of the pileipellis centre; d) Cystidia and hyphal terminations of the pileipellis margin.

***Russula subpallidospora* Marxm. nom. prov. (Fig. 5-6)**

Figure 5. Russula subpallidospora (Marxmüller, 2014)

Etymology: Look-alike of the closely related Russula pallidospora J. Blum ex Romagnesi.

Suggested holotype: EUROPE, France, Drôme (department), Commune de Gigors et Lozeron, Le Savelat, 13 September 1994, H. Marxmüller (MxM R-9422).

Description: Pileus white in centre (4A1), more golden yellow (5B7) to the margin; infundibuliform with a depression in the centre. **Lamellae** golden yellow (5A3, 5B7). **Stipe** white (4A1); medulla solid. **Context** taste mild; odour fruity, but not strong; turning light pink with $FeSO_4$; slow reaction with guaiac; KOH not observed. **Spore print** pale cream (IIb).

Basidiospores (6.8–)7.6–8.4–9.3(–11.0) × (5.4–)6.1–6.6–7.1(–7.8) µm, broadly ellipsoid to ellipsoid, Q= (1.13–)1.19–1.28–1.36(–1.51); ornamentation low to normal, moderately distant to dense [(4–)5–7(–8) in a 3 µm diam. circle], amyloid warts, 0.4-0.8 µm high, subreticulate, occasionally fused in chains [0–3 fusions in a 3 µm diam. circle], connected with dispersed to occasional line connections [0–2(–4) in a 3 µm diam. circle], suprahilar spot large, not amyloid. **Basidia** (35–)57.8–70.8–83.9(–87) × (6–)8.6–10.1–11.6(–13) µm, narrowly clavate, 4-spored. **Hymenial cystidia** (62–)72.8–87.4–102.1(–131) × (7–)7.4–8.4–9.3(– 11) µm, narrowly fusiform, apically obtuse to mucronate or with 1-2 appendages or with double constriction, thin-walled; contents refringent, oily, slight reaction with sulfovanillin (greyish); near the lamellae edges smaller, (50–)57.7–68.8–79.9(–111) × 7–8.1–9.2(–10) µm, fusiform to narrowly clavate, with or without appendage, thin walled; content same as on lamellae sides. Lamellae edges sterile; **marginal cells** (11–)18.1–24.3–30.5(–35) × (3–)3.9–5.0–6.1(–7) µm, poorly differentiated, cylindrical. **Pileipellis** orthochromatic in Cresyl Blue, 285-525 µm deep, sharply delimited from trama; subpellis not delimited from suprapellis; hyphae 2-4 µm wide near trama, densely arranged, irregularly oriented, more horizontally oriented towards the trama, with no distinct gelatinous coating. **Acid-resistant incrustations** absent. **Hyphal terminations** near the pileus margin long, with multiple septa, flexuose; terminal cells (19–)26.1–37.0–47.9 (–70) × (2–)2.1–3.7–4.9(–6) µm, cylindrical; subterminal cells and the cells below similar in length or slightly shorter, similar in width, sometimes branched. Hyphal terminations near the pileus centre similar, terminal cells similar in length (22–)27.3–39.7–52.0(–73) × (2–)2.4–3.1–3.7(–5) µm, cylindrical; subterminal cells and cells below sometimes branching. **Pileocystidia** near the pileus margin dispersed, 1 -3 celled, long, terminal cells (32–)43.7–73.3–103.0(–184) × (4–)4.7–5.6–6.5(–7) µm, cylindrical to narrowly fusiform, flexuose, obtuse or mucronate, with granulose content, reacting weakly (greyish) in sulfovanillin; near the pileus centre rare, 1-2 celled, (41–)59.4–74.8–90.1(–104) × (5–)5.3–6.1–7.0(–7) µm, cylindrical, narrowly fusiform or narrowly clavate, flexuose, obtuse, contents same as near pileus margin.

Ecology: Associated with *Pinus sylvestis*

Distribution: Known from France.

Specimens examined: France, Drôme, Gigors et Lozeron, Gigors St. Pancrace, terrain marneux, with *Pinus sylvestis*, 22 September 2004, H. Marxmüller (MxM R-4048); H. Marxmüller (MxM R-1508)

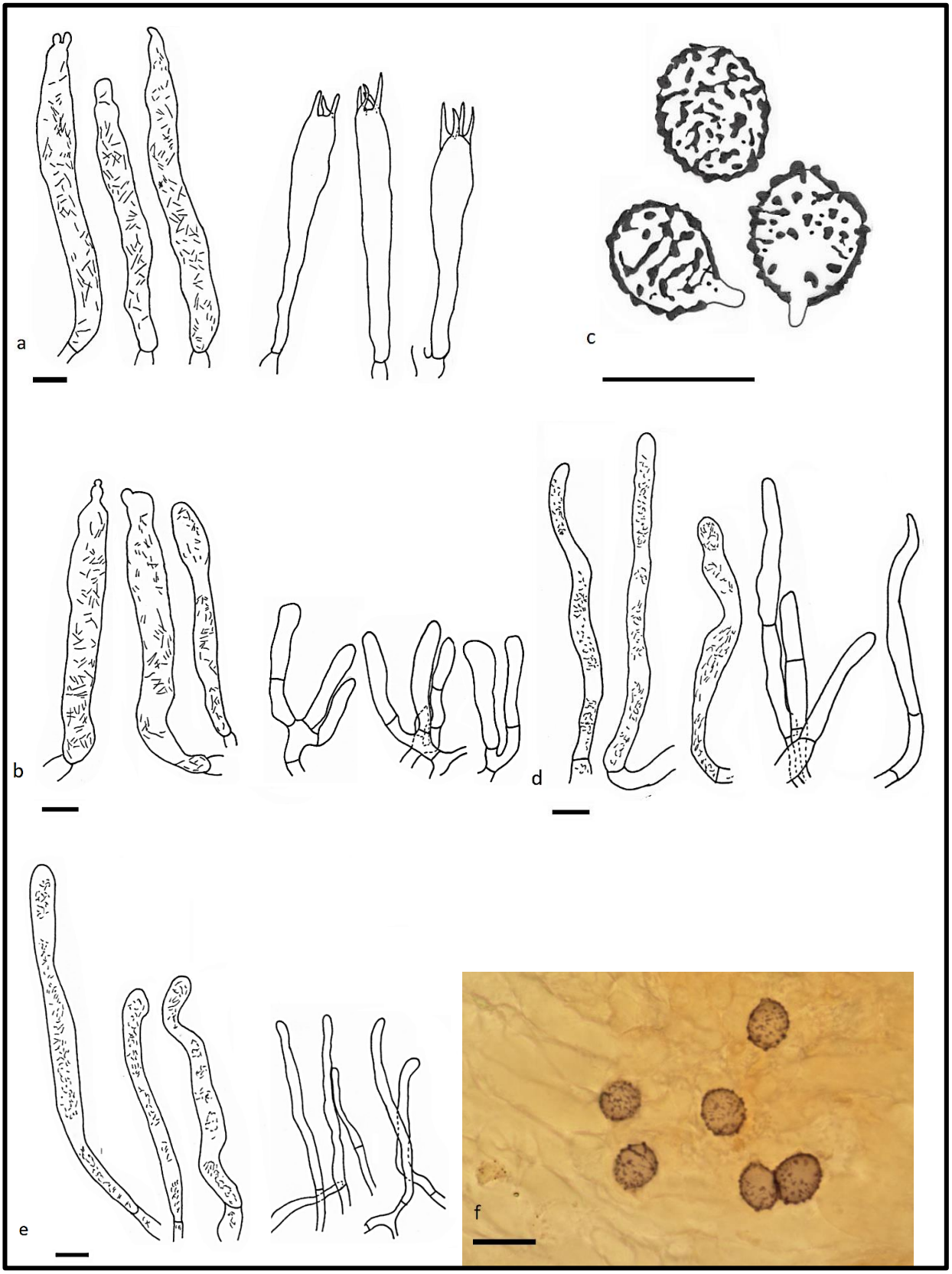

Figure 6. Russula subpallidospora; a) Cystidia of the hymenial side; b) Cystidia and marginal cells of the hymenial edge; c and f) spores; d) Cystidia and hyphal terminations of the pileipellis margin; e) Cystidia and hyphal terminations of the pileipellis centre.

***Russula* sp. 2 (Fig. 7-8)**

Description: Pileus large sized, 90-135 mm diam.; infundibuliform with depression in the centre; white to orange-yellow (4A1, 4A6) and on some areas yellowish brown (4D5). **Lamellae** dense, 5-8L/cm; white to light yellow (4A1, 4A3, 4A4); anastomosing near stipe, with light yellow reflex (4A4). **Stipe** 40-60 × 22-25 mm; white to yellowish white (4A1, 4A2); turning yellowish brown (5D8) on damaged places. **Context** mild taste in stipe, somewhat acrid in cap; odour fruity and like coriander leaves; turning orange with $FeSO_4$; strong reaction with guaiac. **Spore print** white (Ib). Turning yellow in stipe and context and yellow-orange in lamellae with KOH.

Figure 7. Russula sp. 2

Basidiospores (7.9–)8.9–9.5–10.1(–10.9) × (6.3–)7.1–7.5–8.0(–8.6) µm, broadly ellipsoid to ellipsoid, Q= (1.01–)1.18–1.26–1.34(–1.47); ornamentation of low to normal, moderately distant to dense [(3–)4–7(–8) in a 3 µm diam. circle], amyloid warts, 0.3-1.2 µm high, branched, occasionally to frequently fused in chains [(0–)1–3 fusions in a 3 µm diam. circle], connected with dispersed to frequent line connections [1–3(–4) in a 3 µm diam. circle], suprahilar spot large, amyloid. **Basidia** (62–)68.6–76.7–84.9(–95) × (6–)8.6–10.1–11.6(–13) µm, narrowly clavate, 4-spored. **Hymenial cystidia** (68–)79.3–95.3–111.4(–139) × (7–)8.3–9.4–10.5(– 12) µm, cylindrical to narrowly fusiform, apically obtuse to rostrate or with small appendage or with double constriction, thin-walled; contents granulose, slight reaction with sulfovanillin (greyish); near the lamellae edges smaller, (45–)53.3–70.3–87.4(–114) × (5–)7.0–8.3–9.6(–11) µm, narrowly clavate to narrowly fusiform, apically obtuse to rostrate, thin-walled; content same as on lamellae sides. Lamellae edges sterile; **marginal cells** (11–)16.1–22.4–28.8(–39) × (4–)4.1–5.3–6.4(–8) µm, poorly differentiated, cylindrical to narrowly clavate, flexuose, sometimes bifurcating, thin-walled. **Pileipellis** orthochromatic in Cresyl Blue, 125-520 µm deep, not sharply delimited from trama, gradually passing; subpellis not delimited from suprapellis; hyphae 3–8 µm wide near trama, densely arranged, irregularly oriented, more horizontally oriented near trama, with no distinct gelatinous coating. **Acid-resistant incrustations** absent. **Hyphal terminations** near the pileus margin long and wide, with multiple septa, flexuose; terminal cells (14–)26.4–40.6–54.7(–84) × (2–)2.9–4.2–5.4(–8) µm, cylindrical, flexuose; subterminal cells and the cells below similar in length or shorter, similar in width, subterminal cells and the cells below sometimes branched. Hyphal terminations near the pileus centre long, with multiple septa, terminal cells usually less broad (24–)31.3–43.3–55.3 (–77) × (2–)2.7–3.3–3.9(–5) µm, subterminal cells and cells below sometimes branched. **Pileocystidia** hard to find; near the pileus margin mostly 1-celled, but up to 3-celled, long, terminal cells (39–)63.8–94.7–126.0(–165) × (3–)4.1–5.2–6.2(–8) µm, cylindrical to narrowly clavate, flexuose, apically obtuse or with slight constriction, contents granulose, reacting weakly (greyish) in sulfovanillin; near the pileus centre rare, 1-2 celled, generally shorter but still long, terminal cells (33–

)56.0–83.1–110.0(–145) × (3–)4.1–5.2–6.2(–8) µm, cylindrical, flexuose, apically obtuse or with double constriction, contents same as near pileus margin.

Ecology: Associated with *Pinus halepensis, Quercus ilex, Cistus monspeliensis*.

Distribution: Known from France, Italy, Belgium, Spain.

Specimens examined: Jean-Marie Trendel (JMT-17080703); France, Drome, Gigors et Lozeron, Gigors le Savel, Gigors village, La Charousse, with *Pinus*, 01 September 1991, H. Marxmüller (MxM R-9125); Italy, 05 November 2016, R. De Lange (RDL 16-021); Belgium, Luxemburg (province), R. De Lange (RDL 17-026).

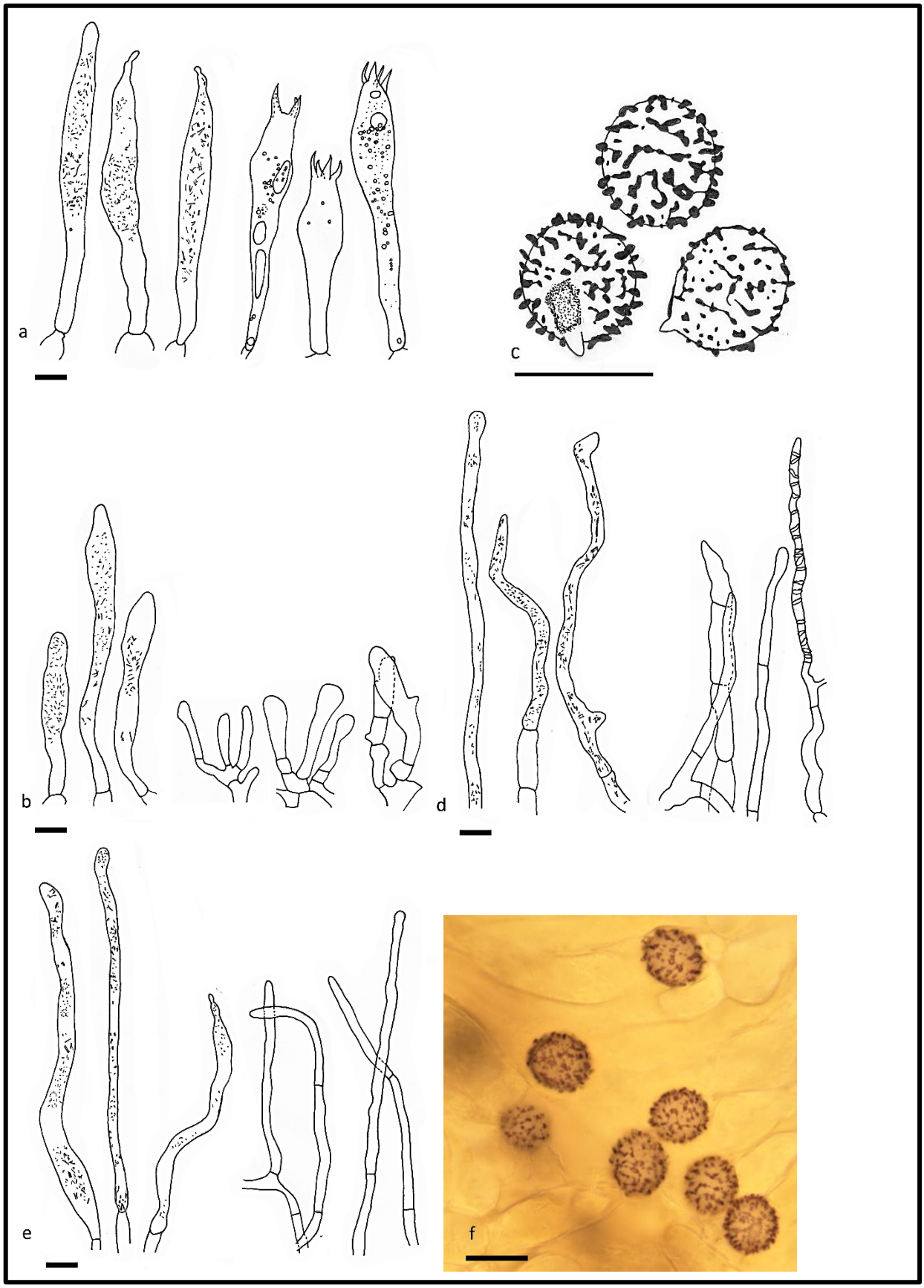

Figure 8. Russula sp.2; a) Cystidia of the hymenial side; b) Cystidia and marginal cells of the hymenial edge; c and f) spores; d) Cystidia and hyphal terminations of the pileipellis margin; e) Cystidia and hyphal terminations of the pileipellis centre.

***Russula* sp.3 (Fig. 9-10)**

Pileus medium sized, 70-90 mm diam.; infundibuliform with a deep depression in the centre; white to pale orange (5A1, 5A3) with light brown (6D8) zone in centre. **Lamellae** crowded, L = 115; slightly decurrent; with weak blue-greenish hues (24A1-2). **Stipe** 20-25 × 15-20 mm; white (4A1); turning light brown (6D8) when damaged; medulla solid. **Context** mild taste; odour fruity or like paint; turning slightly orange with $FeSO_4$; strong reaction with guaiac. **Spore print** not observed. Reaction with KOH not observed.

Figure 9. *Russula* sp. 3

Spores (7.2–)7.6–8.2–8.7(–9.5) × (6.3–)7.1–7.5–8.0(–8.6) µm, broadly ellipsoid Q= (1.13–)1.17–1.22–1.27(–1.38); ornamentation of low to normal, moderately distant [4–6(–7) in a 3 µm diam. circle] amyloid warts, 0.4–0.8 µm high, subreticulate, dispersedly to frequently fused in chains [(0–)1–3(–4) fusions in a 3 µm diam. circle], connected with dispersed to frequent line connections [(0–)1–3(–5) in a 3 µm diam. circle], suprahilar spot large, amyloid. **Basidia** (41–)54.7–61.6–68.6(–74) × (9–)10.6–11.9–13.2(–14) µm, narrowly clavate, 4-spored. **Hymenial cystidia** (51–)65.4–81.3–97.2(–134) × (6–)7.2–8.3–9.3(– 11) µm, cylindrical to narrowly fusiform, apically obtuse to rostrate or with 1 appendage or double constriction, thin-walled; contents granulose, no reaction with sulfovanillin; near the lamellae edges smaller, (47–)50.7–63.5–76.3(–122) × (6–)7.0–7.9–8.8(– 10) µm, cylindrical to narrowly fusiform, apically obtuse to mucronate or with 1 appendage or double constriction, thin-walled; content same as on lamellae sides. Lamellae edges sterile; **marginal cells** (8–)14.3–20.0–25.8(–35) × (3–)4.0–5.3–6.6(–8) µm, poorly differentiated, cylindrical to narrowly clavate, sometimes flexuose, thin-walled. **Pileipellis** orthochromatic in Cresyl Blue, 170-530 µm deep, not sharply delimited from trama, gradually passing; subpellis not delimited from suprapellis; hyphae 4–9 µm wide near trama, densely arranged, irregularly oriented, with no distinct gelatinous coating. **Acid-resistant incrustations** present. **Hyphal terminations** near the pileus margin long and wide, with multiple septa, flexuose; terminal cells (19–)30.9–45.7–60.4(–84) × (3–)4.2–5.9–7.6(–9) µm, cylindrical to clavate, flexuose; subterminal cells and the cells below similar in length or shorter, similar in width, subterminal cells and the cells below sometimes branched. Hyphal terminations near the pileus centre long, with multiple septa, terminal cells usually less broad (21–)32.0–46.3–60.7(–81) × (3–)3.0–3.7–4.4(–5) µm, sometimes bifurcating, subterminal cells and cells below sometimes branched. **Pileocystidia** near the pileus margin mostly 1-celled, but up to 4-celled, terminal cells (26–)40.3–60.1–79.8(–107) × (4–)4.4–5.8–7.3(–10) µm, cylindrical to narrowly clavate, flexuose, apically obtuse or with slight constriction, sometimes bifurcating, contents granulose, reacting weakly (greyish) in sulfovanillin, near the pileus centre 1-3 celled, terminal cells (30–)35.6–

56.6–77.6(–125) × (4–)4.1–5.2–6.3(–8) µm, cylindrical to narrowly clavate, flexuose, apically obtuse or with slight constriction, contents same as near pileus margin.

Ecology: Associated with *Quercus ilex, Pinus halepensis*.

Distribution: Known from France, Italy, Spain.

Specimens examined: France, Drôme, Val des Nymphes, with *Quercus ilex*, H. Marxmüller (MxM R-010120); Italy, 03 November 2016, R. De Lange (RDL 16-017); 15 August 2016, F. Hampe (FH RUS 16081503).

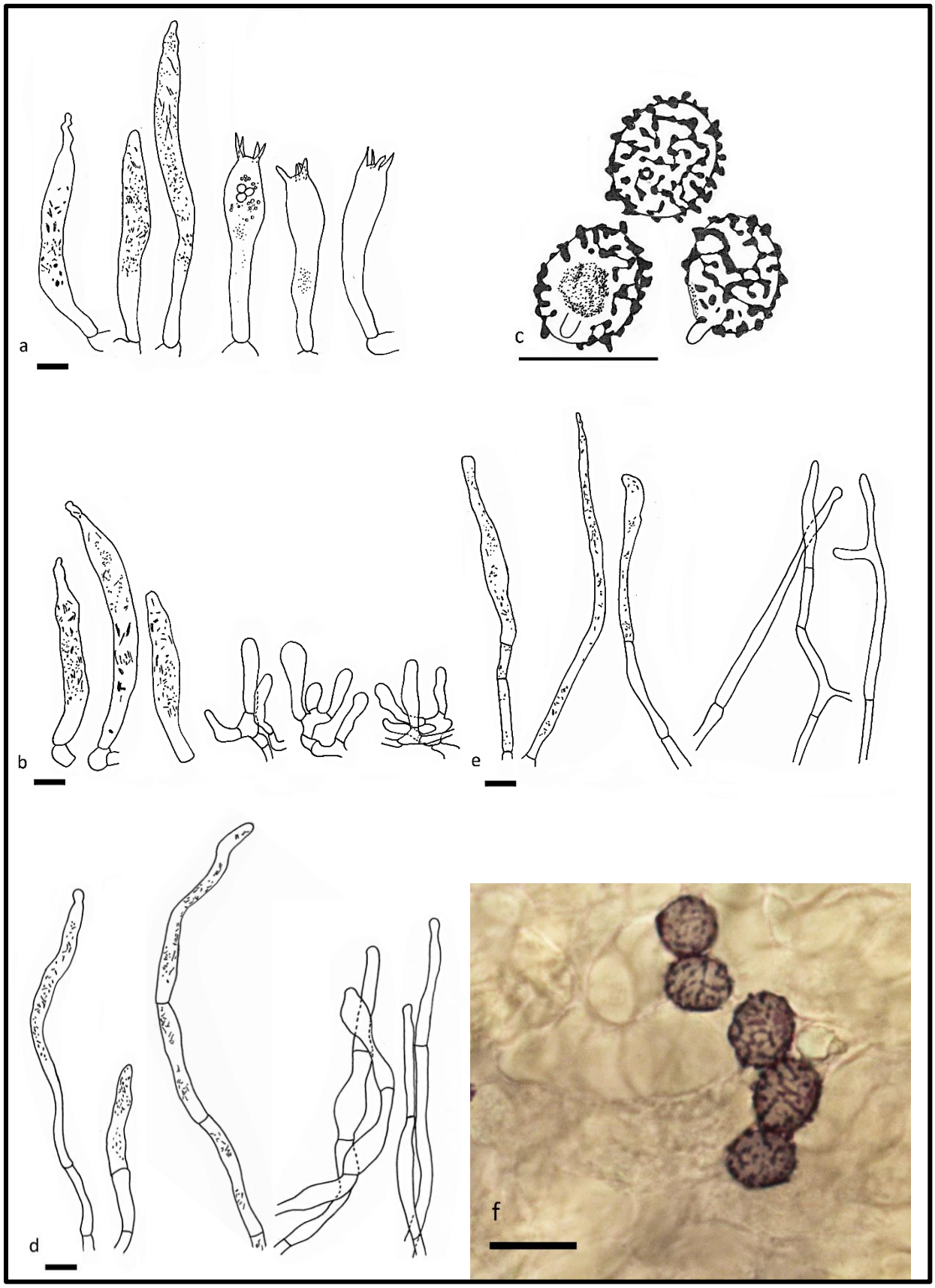

Figure 10. Russula sp.3; a) Cystidia of the hymenial side; b) Cystidia and marginal cells of the hymenial edge; c and f) spores; d) Cystidia and hyphal terminations of the pileipellis margin; e) Cystidia and hyphal terminations of the pileipellis centre.

***Russula delica* Fr. var*. delica* paratype/topotype (Fig. 11)**

Specimen: France, Bellefontaine, H. Romagnesi (Rom 1viii 62).

Microscopical description: Basidiospores (8.6–)8.9–9.6–10.4(–10.9) × (6.9–)7.0–7.7–8.3(–9.2) µm, broadly ellipsoid to ellipsoid Q= (1.17–)1.19–1.26–1.33(–1.38); ornamentation of low to normal, moderately distant [(3–)4–6(–8) in a 3 µm diam. circle] amyloid warts, 0.5–0.8 µm high, subreticulate, dispersedly to frequently fused in chains [(0–)0.5–3.1(–4) fusions in a 3 µm diam. circle], connected with dispersed to frequent line connections [(0–)0.4–2.8(–4) in a 3 µm diam. circle], suprahilar spot large, amyloid. **Basidia** (49–)54.5–60.3–66.0(–72) × (10–)12.2–13.7–15.2(–16) µm, narrowly clavate, 4-spored. **Hymenial cystidia** (67–)71.0–82.3–93.5(–106) × (7–)8.4–9.3– 10 µm, cylindrical to narrowly fusiform or narrowly clavate, apically obtuse or with 1 appendage or double constriction, thin-walled; contents granulose, slight reaction with sulfovanillin (greyish). Lamellae edges not present. **Pileipellis orthochromatic** in Cresyl Blue, 125-510 µm deep, not sharply delimited from trama, gradually passing; subpellis not delimited from suprapellis; hyphae 4–10 µm wide near trama, densely arranged, irregularly oriented, with no distinct gelatinous coating. **Acid-resistant incrustations** absent. **Hyphal terminations** near the pileus margin long and wide, with multiple septa, flexuose; terminal cells (19–)27.1–41.1–55.1(–77) × (3–)3.8–6.4–8.9(–11) µm, cylindrical to clavate, flexuose; subterminal cells and the cells below similar in length or shorter, similar in width or wider, subterminal cells and the cells below sometimes branched; pileus centre not present. **Pileocystidia** near the pileus margin 1-3 celled; terminal cells (49–)62.1–105.0–148.0(–170) × (4–)4.6–5.6–6.6(–7) µm, cylindrical to clavate, flexuose, apically obtuse or mucronate, contents granulose, reacting weakly (greyish) in sulfovanillin.

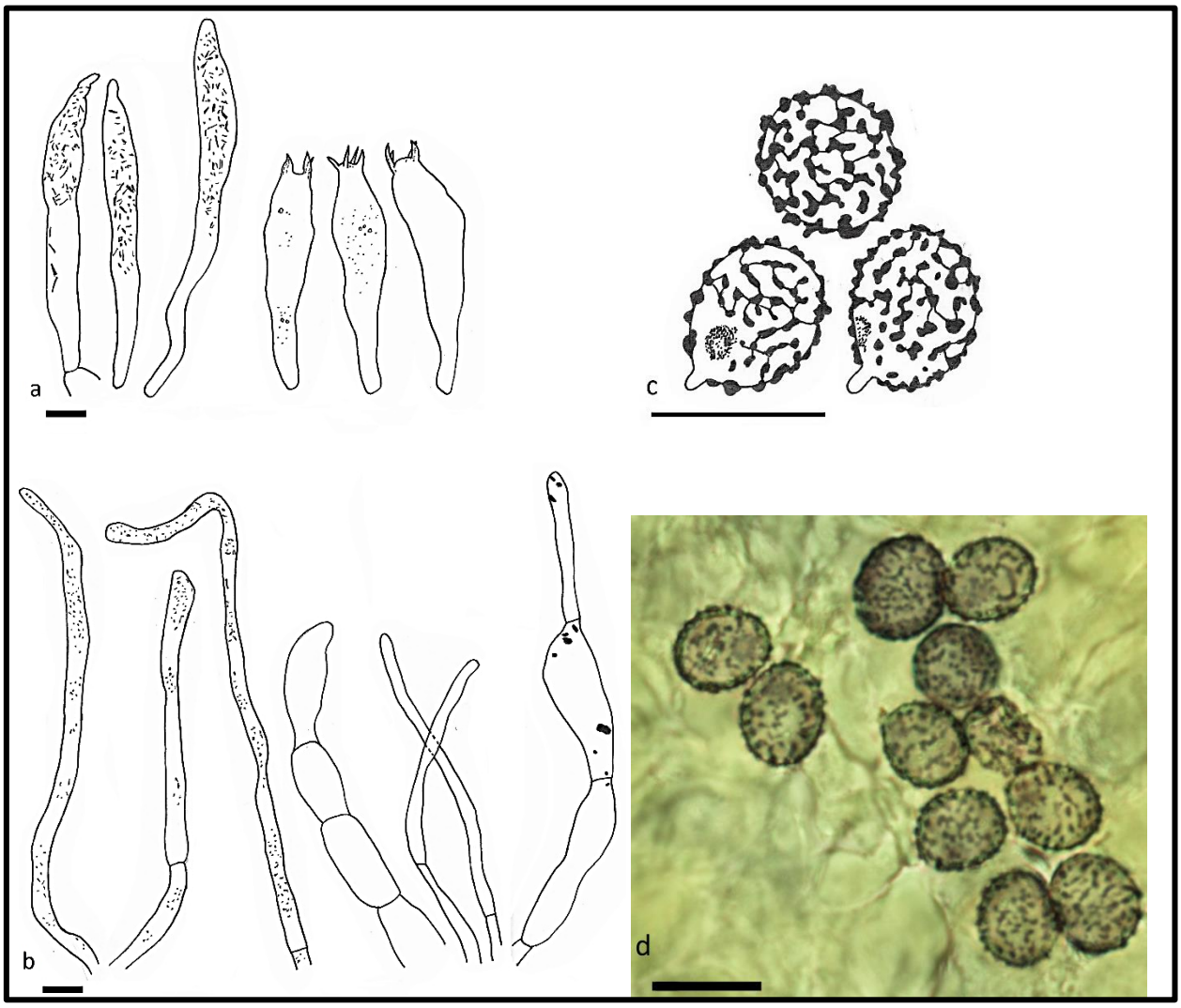

Figure 11. Russula delica var. *delica paratype/topotype*; a) Cystidia of the hymenial side; b) Cystidia and hyphal terminations of the pileipellis margin; c and d) spores.

5. Discussion

5.1 Host specificity

5.1.1 *R.* subg. *Malodorae*

In appendix A, we see that the *Russula* subgenus *Malodorae* divides early on in 3 clades.

The first clade is rather small, with only one unnamed *Russula* species from Brazil and Ecuador (1). For this clade, only one host has been observed, namely a tree species of the genus *Neea*, which is a tree that grows in South- and Central- America (Rossetto et al., 2019). The genus may be a good delimitation feature for this species, as the genus is only found for this *Russula* species.

The second clade makes up the largest part of *R.* subg. *Malodorae* and includes *R. pseudocompacta* A.Ghosh, K.Das, R.P.Bhatt & B.Buyck (2), *R. compactoides* K.Das, A.Ghosh & B.Buyck (7), and multiple species that are named *R. compacta* Frost (4, 5, 6, 8, 9) or that are unnamed (1, 3). This clade has also the largest diversity in plant hosts. It has hosts from the families Fagaceae (*Quercus Castanopsis*, *Lithocarpus*), Salicaceae (*Populus*), Pinaceae (*Pinus*, *Abies*), Orchidaceae (*Cymbidium*, *Lecanorchis*, *Cypripedium*) and Ericaceae (*Monotropa*, *Monotropastrum*) and thus, has a mix from gymnosperms and angiosperms, and trees and herbs. All these families, except the Pinaceae, are not found in the other two clades of *R.* subg. *Malodorae*, meaning that they may be a distinctive factor between this clade and the others. The different families and genera are scattered completely over this clade and do not follow a pattern for more closely related species. If we look on species level, the different *Russula* species seem to have a different set of host species. *Russula compacta* Japan/Thailand (4), one of the species named *R. compacta* United States (9) and *R. compactoides* (7) each only have one host species (resp. *Cymbidium macrorhizon*, *Monotropa uniflora* and *Abies densa*), but each of these host species was only mentioned once in the databases. We are not certain if these host species do not interact with other *Russula* species. *Russula* sp. Japan (3), *R. compacta* Thailand (4) and *R. pseudocompacta* (2) were part of different species hypotheses in UNITE, but seem to be one species in the ML tree (namely *R. pseudocompacta*). We see that the genus *Quercus* (*Quercus* sp., *Quercus miyagii, Quercus multinervis*) and the family Orchidaceae (*Cymbidium macrorhizon*, *Lecanorchis trachycaula*) recurring as hosts of *R. pseudocompacta* (2, 3, 4). The species of this clade which had multiple host species each have a different set of hosts. Different species with specimens labelled as *R. compacta* had all different host species. Except for *Monotropa uniflora* (with 5 and 9), there was no host species that had interactions with multiple species labelled *R. compacta*, which means that host specificity can be a feature to delimit between these different species.

The third clade (with *R. blennia* Buyck (12)*, R. capillaris* Buyck, *R. edulis* Buyck (10) and many unnamed species(11, 13, 14, 15, 16,17)) are all species from African countries and the largest part of the hosts are members of the genus *Uapaca*, but also some species of the genera *Pinus* and *Marquesia*, and the family *Caesalpiniaceae* were mentioned*. Uapaca* is an endemic tree genus of Africa and Madagascar (Breteler, 2013; McPherson, 2011). The fact that this host genus, or even the species *Uapaca guineensis,* is so abundantly found in this clade, means that it may be a good determining factor for this clade of *R.* subg. *Malodorae*. However, this clade is the only clade of *R.* subg. *Malodorae* that has African species. The geographic distribution alone is thus a good determining factor for this clade. Finding good delimiting hosts like *Uapaca* is a logical consequence of the geographic distribution of this clade.

Overall, the host species seem to follow a pattern for the largest clades in *R.* subg. *Malodorae*. However, on species level, it is difficult to determine if the host species are a delimiting feature between the *Russula* species, as data is often limited. However, the data that is available suggests that hosts can be good indicators for the *Russula* species.

5.1.2 *R.* subg. *Archaea*

In appendix B, we see that *R*. subg. *Archaea* had the least data of the 4 subgenera, making it difficult to draw some conclusion for the host specificity within this subgenus. Overall, the *Russula* species for which some host species were found, had mostly a different set of hosts, except *Russula sp* Panama (3) and *R.* aff. *archaea* Panama (6), which had both one host from the genus *Quercus*. Only *R. butyroindica* K.Das & Buyck (4) had also a herb (*Pyrola japonica*) as a host, the rest of the host species were trees. No hosts were found in the databases for *R. archaeosuberis* Sarnari. However, in Sarnari (1998), the habitat of *R. archaeosuberis* is described as a forest only consisting of cork oak (*Quercus suber*), which is probably a host for this species. In Romagnesi (1967), the holotype of *R. camarophylla* Romagn. (2) was found in a forest of (*Quercus, Betula, Fagus* and *Corylus*). The only host found in the databases was *Picea abies. Russula camarophylla* has probably more host species than mentioned in the databases.

Overall, the available data suggests that host specificity is a good indicator for the different species of *R*. subg. *Archaea*.

5.1.3 *R.* subg. *Compactae* s.s.

In appendix C, we see that *Russula* subgenus *Compactae* s.s. divides early on in three clades.

The first clade only has 2 species, *R. subnigricans* Hongo and *R. fortunae* Corrales (1) that originate from Japan and Panama respectively. Only for *R. fortunae* (1), a host species was found. *Oreomunnea Mexicana* is a tree species from the family Juglandaceae and is found in Central- and South- America. *Oreomunnea Mexicana* is found for other species *R.* subg. *Compactae* s.s. (*Russula* sp. Panama (5), *Russula* aff. *nigricans* Panama (16) and *Russula* sp. (19)) and seems to be a typical host tree for species of Panama.

The second clade is larger than the first and consists of *R. griseobrunnea* (2), *R. cantharellicola* D.Arora & N.H.Nguyen (3), *R. khanchanjungae* Van de Putte, K. Das & Buyck, 2 species with the name *R. polyphylla* Peck (4), *R. ochrobrunnea* Y.Song & L.H.Qiu, *R. cortinarioides* Buyck, Adamčík, D.P.Lewis & V.Hofst. (7), different species labelled as *R.eccentrica* Peck (6) and some unnamed species (8). The species originate from Australia, the United States, Panama and different Asian countries (Thailand, Korea, India, China). This clade has hosts of the families Fabaceae, Juglandaceae, Fagaceae and Pinaceae, which are also found in the other clades of this subgenus. The hosts of *R. griseobrunnea* (2) and *Russula* sp. Cameroon (8) (resp. *Acacia auriculiformis* and *Gilbertiodendron dewevrei*) are only found for these two species. *Acacia* and *Gilbertiodendron* are both from the family Fagaceae. *Russula cantharellicola* (3) and *Russula* sp Panama (5) also have only one host species, respectively *Quercus agrifolia* and *Oreomunnea Mexicana*. *Quercus agrifolia* is only found once as a host in *R.* subg. *Compactae* s.s. and is observed multiple times to be a host of *R. cantharellicola* (3), meaning that this host species can be a determining factor for this *Russula* species. *Quercus oleoides* is the only host found for *R. polyphylla* Costa Rica and is not observed to be a host for another species of *R.* subg. *Compactae* s.s. It could be a good feature for *R. polyphylla* Costa Rica (4). *Pinus densiflora* is the host

of *R. eccentrica* (6) and *R. cortinarioides* (7), but is also observed to be a host for a species of the next clade.

The third clade is the largest clade. Early on, it divides into two branches. The smallest subclade has species which originate from Africa and only one species that originates from Australia: two species named *R. fistulosa* R.Heim (11)*, R. subfistulosa* Buyck (10)*, R. ingwa* Grgur.*, R. lateriticola* R. Heim (12) and some unnamed species (9). As these are the only African species in our tree of *R.* subg. *Compactae* s.s., it seems logical that some of the hosts are not found for other species of this subgenus. Host species are from the families Fabaceae (*Acacia, Microberlinia, Intsia*), Phyllanthaceae (*Uapaca)*, Asteropeiaceae (*Asteropeia*) and Pinaceae (*Picea abies*) and are all tree species. *Asteropeia mcphersoni* is endemic to Madagascar (Henry et al., 2015) and is only found as a host tree for two species of *R.* subg. *Compactae* s.s. (*R. subfistulosa* (10) and *R. lateriticola* (12)). These species can both be found in Madagascar but are not clustered together and thus not particularly closely related. But the fact that *Asteropeia mcphersoni* is not found for any other *Russula* species of our four subgenera, only in this branch of *R.* subg. *Compactae* s.s., may mean that this host species is a good delimiting host for this branch. All the other host species of this branch (*Intsia bijuga, Uapaca littoralis, Uapaca guineensis, Microberlinia bisulcata* and the family Caesalpiniaceae), except *Picea abies,* are also only found in this branch of *R.* subg. *Compactae* s.s.. Moreover, *Intsia bijuga*, *Uapaca littoralis* and *Microberlinia bisulcata* cannot be found in the other subgenera, but *Uapaca guineensis* is observed in all subgenera. *Intsia bijuga* and *Uapaca littoralis* could be specific and determining for *R. subfistulosa* (10) and *Microberlinia bisulcata* for *Russula* sp Cameroon (9). It is remarkable that, just like in *R.* subg. *Archaea*, all African species seem to cluster together in one clade.

The second, much larger subclade contains species from almost all continents, except Africa and contains the largest part of the species tree. It contains host species from the families Fagaceae (*Quercus, Lithocarpus, Castanopsis, Fagus*), Pinaceae (*Pinus, Abies, Picea, Pseudotsuga, Larix*), Juglandaceae (*Oreomunnea*), Betulaceae (*Betula, Carpinus*), Salicaceae (*Populus*), Ericaceae (*Monotropa, Pyrola*), Eucalypteae (*Eucalyptus*), Dipterocarpaceae (*Dipterocarpus*) and Orchidaceae (*Aphyllorchis, Cymbidium, Epipactis*). The genera *Quercus* and *Pinus* are found throughout the whole branch. This clade has many species which are morphological very similar, which is evident by the names assigned to the specimens. These names were assigned to the specimens based on first macromorphological observations in the field, but after further microscopic and molecular delimitation, we see that these specimens are part of different species. We will compare species which are morphological similar, based on these specimen names.

Species units 13, 23, 24, 25, 26 and 27*:* *Russula albonigra* (Krombh.) Fr. was seen as one species in the past, but recently, it was divided in 5 species: *R. sardoa* De Lange sp. nov. (23), *R. albonigra* (Krombh.) Fr. (24), *R. nigrifacta* De Lange & Adamčík, sp. nov. (25), *R. ustulata* De Lange & Verbeken, sp. nov. (26) and *R. ambusta* De Lange, Adamčík & F. Hampe, sp. nov. (27). All species originate from Europe, except *R. albonigra* (24), which has also specimens from the United States and Russia. This large distribution range implies that *R. albonigra* (24) still consists of multiple species. One species, *Russula* sp. Belgium (13), has one specimen which was also labelled *R. albonigra*, but is not closely related to the other species. *Russula* sp. Belgium (13) has only one host, *Quercus robur*, which is also a possible host for *R. nigrifacta* (25). The species which were part of the former *R. albonigra* each have a different set of host species which are alle members of the Fagales (*Quercus, Fagus, Carpinus, Betula*) or Pinaceae (*Abies, Picea, Pinus*), but there is some overlap. *Quercus suber*, *Quercus robur,*

Pinus sylvestris, and the genera *Carpinus* and *Picea* are possible hosts for multiple species. *Abies alba* and *Fagus sylvatica* only interact with *R. albonigra* (24). *Quercus ilex* is only a possible host of *R. nigrifacta* (25). *Betula pendula* is only found for *R. ambusta* (27). Species *Pinus koraiensis* and *Pinus pinaster* only interact with *R. albonigra* (24). *Pinus koraiensis* is a species native to Eastern Asia and Russia (Thomas & Farjon, 2013) and was found interacting with a Russian specimen.

Species unit 14 contains specimens labelled as *Russula subnigricans* Hongo. In the first clade, *R. subnigricans* was also mentioned, but no hosts were found for this species unit. For species unit 14, only one host species was found, namely *Quercus serrata*. All specimens labelled as *R. subnigricans* were found in Japan, which makes it also hard to delimit between these species.

Species units 15, 16, 17, 18, 22, 39 have many specimens which were labelled as *Russula nigricans* Fr.. Species units 15, 16, 17 and 18 are closely related to each other, but are probably different species, based on their distributions range (resp. Europe, Panama, Europe and North- America). The hosts are very different between species with a different distribution. Species unit 16 only has one host species, *Oreomunnea mexicana*, which is also a host species for other species from Panama within *R.* subg. *Compactae* s.s.. Species unit 18 has *Pseudotsuga menziesii* as its only host species. There is some overlap in hosts between the European species units 15 and 17, as they share *Fagus sylvatica* as a common host species. However, *Abies alba, Picea abies* and *Quercus* sp. are observed to be host species of species unit 17, but not of species unit 15. Species unit 22 is not closely related to the other species units and originates from Europe. One of its specimens is labelled *R. anthracina* var. *insipida* Romagn. Its only host species, *Quercus robur*, is not observed to be a host of the other species units mentioned here. Last, species unit 39 is also not closely related to the other species units mentioned here. The specimens originate from Belize, the Dominican Republic and Panama. This species unit can be found with *Pinus caribaea* and *Quercus* sp. *Quercus* sp. is found in other species units, but *Pinus caribaea* can be a good delimiting feature. However, its geographic range does not overlap with the other species unit, thus there should be no confusion with the other species units.

Species units 20 and 21 both have specimens labelled *Russula dissimulans*. Species unit 20 is the real *Russula dissimulans* Shaffer, as this species is from North- America, while species unit 21 has specimens from Japan. These species will not be confused with each other, as their distributions do not overlap. Their hosts are also different. Species unit 20 only has one host species, namely *Pinus taeda*, while species unit 21 only has tree species from the genus *Quercus* (*Quercus glauca, Quercus serrata*).

Russula ingwa (28) is the only Australian species from *R.* subgenus *Compactae* s.s. Another specimen labelled with the same name was clustered together with the previous clade of African species, but no host species were found. Only one host species was found for *R. ingwa* (28), namely *Eucalyptus regrans*, which is only found in Australia (Fensham et al., 2019)

Species units 30, 35, 36, 43 have many specimens labelled as *Russula densifolia* Secr., but are not particularly closely related. The distribution of species unit 30 (Japan and China) and species unit 36 (Japan) overlaps. Only one host was found for species unit 30, namely *Pinus massoniana*, which is native to Taiwan and China (Farjon, 2013c). Species unit 36 has a larger variety in host species. It not only contains tree hosts (*Quercus serrata, Fagus japonica*), but also some herb hosts from the families Ericaceae (*Pyrola japonica*) and Orchidaceae (*Aphyllorchis caudata, Cymbidium lancifolium*),

which are not found in the other two species units. Species units 35 and 43 both originate from Europe. There is some overlap in tree hosts, as both species units have host species from the genus *Pinus*. However, species unit 35 has a larger variety of tree hosts from the family Pinaceae (*Picea abies, Pinus pinaster, Pinus sylvestris*) but also hosts of the genera *Quercus* (*Quercus robur, Quercus suber*) and *Populus* (*Populus alba, Populus tremula*) and *Fagus sylvatica*. *Fagus sylvatica, Picea abies, Populus alba, Populus tremula, Quercus robur* and *Quercus suber* were not found with the other species units.

Russula atramentosa Sarnari (31) has a wide distribution range (Europe, North- America), thus is probably not just one species. Only one host species is found, namely *Fagus grandifolia*, which is also a host of species unit 32, a closely related species, which makes it hard to delimit between the two based on host specificity.

Russula densissima Romagn.(33) is found in Germany and Switzerland and has a variety of host trees from the Fagales (*Fagus sylvatica, Quercus petraea*) and Pinaceae (*Picea abies, Pinus sylvestris*). All these species are not exclusively found for this species unit.

Species units 42, 45, 46 all contain specimens labelled as *Russula acrifolia* Romagn. and are all closely related. Alle three specie units originate from Europe, but species unit 42 has also specimens from the United States, Panama and Iran and species unit 45 has a specimen from Japan. All three species units have a different set of host species that do not overlap. Specimen 42 has *Picea abies,* native to North- America (Farjon, 2017b) and Fagales as hosts. The wide distribution range implies that this species unit contains multiple species. Species unit 45 has a wide variety of host species: both herbs from the families Orchidaceae (*Epipactis helleborine*) and Ericaceae (*Pyrola rotundifolia*) and trees from the families Pinaceae (*Picea jezoensis*) and Fagaceae (*Quercus petraea, Quercus ilex*). *Picea jezoensis* is an Asian species (Thomas et al., 2013) and was a host from the Japanese specimen. This Japanese specimen is possibly part of a different species. Species unit 46 has only host species of the genus *Populus*.

Russula roseonigra Pidlich-Aigner (44) is closely related to the species units 42, 43, 45 and 46. The specimens of this species unit originate from Europe. Only on host was found, namely *Abies alba*, which was not found in the closely related species units.

Species units 47 and 48 both have specimens labelled as *Russula adusta* (Pers.) Fr., but originate respectively from North- America and Europe, which reduces possible confusion between the two. Both are associated with tree species from the family Pinaceae, but there is no overlap between the host species. Species unit 47 has hosts from the genus *Pinus* (*Pinus banksiana, Pinus contorta*). Species unit 48 has a wider variety of hosts: *Larix decidua, Picea abies, Pinus cembra, Pinus sylvestris*.

In conclusion, most unrelated species which were falsely labelled with the same species name based on macromorphology, can be delimited in most cases by looking at the host trees and the distribution range.

5.1.4 *R.* subg. *Brevipedum*

In appendix D, we see that *Russula* subgenus *Brevipedum* divides early on in 2 large clades.

The first large clade consists of a subclade with species *R. fuegiana* Singer (1)*, R. metachromatica* Singer (2) and *R. metachromatica* subsp. *tarumaensis* Singer (3) which are all South- American

species. *Russula fuegiana* (1) only has one host tree: *Nothofagus dombeyi*, which is native to Chile (Barstow et al., 2017). *Nothofagus dombeyi* has not been observed to make an interaction with other species, thus can be a good delimiting feature for *R. fuegiana* (1). *Russula metachromatica* (2) and *R. metachromatica* subsp. *Tarumaensis* (3) both have the same host species, namely *Dicymbe corymbosa*, which makes sense, as these two are related. *Dicymbe corymbosa* is not found to be a host for other species. A second subclade consists of *R. littoralis* Romagn (AG)., *R. cascadensis* Shaffer (4), *R. subpallidospora* Marx. (U), *Russula* sp.1 (I), *R. pallidospora* J.Blum ex Romagn. (T) and an unnamed *Russula* sp. Cameroon (5). The host species of this subclade are members of different families. *Russula littoralis* (AG) is only found in Europe. It has two herb hosts from the family Cistaceae (*Cistus salviifolius, Halimium halimifolium*) and one tree host, namely *Pinus pinaster*. No other species of *R*. subg. *Brevipedum* have hosts from the Cistaceae, so this family may be a good indicator for *R. littoralis* (AG). *Russula cascadensis* (4) has specimens from Uruguay, North- America and Japan. All of the host species are members of Pinaceae (*Pinus parviflora, Pinus ponderosa, Pseudotsuga menziesii*). The host species are native to North- America (Farjon, 2013e; Farjon, 2013g), except *Pinus parviflora,* which is native to Japan (Farjon, 2013d). *Russula cascadensis* (4) seems to have a wide distribution range, but most specimens are from North- America. Specimens from Uruguay and Japan are probably members of another species, but more research is needed. *Russula subpallidospora* (U) has specimens from the United States, Europe and Iran. Its host species are *Quercus kelloggii* and *Betula* sp. *Quercus kelloggii* has no interaction with other species of *R.* subg. *Brevipedum*, and could be a good indicator species. The specimen of *R. pallidospora* are found only in Europe. *Quercus robur, Fagus* sp. are its host trees. The hosts of *R. pallidospora* (T) and *R. subpallidospora* (U) are all members of the order Fagales. Strangely, in the literature, *R. subpallidospora* (U) was observed to be associating with the genus *Pinus* (Marxmüller, 2014), but this genus was not mentioned in the databases. *Russula* sp. Cameroon (5) has only one host species, *Uapaca guineensis*, which seems to be a common host for African species of *Russula* subgenus *Compactae* s.l.

The second clade of *Russula* subg. *Brevipedum* is quite complicated, due to many specimens which were wrongfully named *Russula delica* Fr. and *Russula chloroides* Romagn. due to their macromorphological.

Species unit X can only be found in Europe. It has two different host species: one tree species, *Tilia cordata* and one orchid species, *Limodorum abortivum*. *Limodorum abortivum* is also a host for other species of *R.* subg. *Brevipedum*. *Tilia cordata* is only found for this species unit and could be a good delimiting feature.

Species unit Y has one host species, *Picea abies*, and its specimens originate from Norther Europe. *Picea abies* is also a host for species unit J, which is relatively closely related to species unit Y.

Species unit N has specimens from South- and Central- America. Its host species are all members of the genus *Quercus* (*Quercus oleoides, Quercus seemanii, Quercus salicifolia*) and are all native to Central- America (Gallagher, 2018; Govaerts & Frodin, 1998; Jerome, 2018). There is no other species of *R.* subg. *Brevipedum Quercus oleoides* which interacts with *Quercus oleoides*. *Quercus seemanii* and *Quercus salicifolia* are also host of species unit M, which is not closely related to N, but also originates from Central- America.

Species unit J: UNITE assigned different species hypotheses to different parts of species unit J. Therefore, J was divided in 5 different parts which were assigned their hosts species. It is indeed unlikely that J is just one species (see section 5.2) as the geographical range is too wide: we have specimens from North- America, China, Northern Europe and Thailand. There is however some overlap between the host species, e.g. *Dryas octopetala, Picea abies*, and the genera *Populus* and *Betula* are host species for multiple subdivisions of species unit J. Other hosts are more exclusive, e.g. *Larix chinensis* and the genus *Salix*. More research is probably needed for species unit J.

Species unit V is only found in North- America. Only one host species has been found for this species unit, namely *Pseudotsuga menziesii*. *Pseudotsuga menziesii* is native to America (Farjon, 2013g), and interacts with many other species of *R.* subg. *Compactae* s.l.

Species unit M contains specimens from Mexico and Panama. It has two species from the genus *Quercus* (*Quercus salicifoliaii, Quercus seemanii*) as host species. Both *Quercus salicifoliaii* and *Quercus seemanii* are native to South- and Central- America (Govaerts & Frodin, 1998; Jerome, 2018) and are also hosts of the species unit N.

Species unit R has specimens from Europe, Costa Rica and North- America and is probably composed of multiple species. Specimens of North- America have the trees *Fagus grandifolia, Pseudotsuga menziesii* and the herb *Monotropa uniflora* from the Ericaceae as host species. Specimens from Europe have *Quercus petraea* and *Fagus sylvatica* as host species.

Species unit G only has specimens from Italy and two host species: the oak *Quercus ilex* and the orchid *Limodorum abortivum*. *Quercus ilex* is also a host of species unit H. *Limodorum abortivum* is a common host for *R.* subg. *Brevipedum.*

Species unit C has specimens from Europe. There are only three host species: two of the genus *Populus* (*Populus alba, Populus canescens* (*Populus alba* × *tremula*)) and one of the genus *Tilia*.

Species unit Q seems to be one species in the concatenated ML tree and our coalescent species delimitation method seems to support this species unit (posterior probability > 0.99, see section 5.2). However, it seems possible that species unit Q is not just one species, as the geographic range is large. In our ML tree based on the ITS marker, the species unit Q is divided in 3 different parts with different host species. All host species are members of the family Pinaceae. Q1 has specimens from Japan and the United States. The host species are *Pinus pumila*, native to Asia (Farjon, 2013f), and *Pseudotsuga menziesii*, native to North- America (Farjon, 2013g). Q2 has specimens from Europe and the Canary Islands (Spain). Only one host species was found, namely *Pinus canariensis*, which is native to the Canary Islands (Thomas, 2017). Q3 has specimens from Canada and Italy. Three host species were found, namely *Abies alba, Abies balsamea* and *Picea mariana*. *Abies alba* is a European species (Farjon, 2017a), while both *Abies balsamea* and *Picea mariana* are native to North- America (Farjon, 2013a; Farjon, 2013b).

Species unit B has specimens from Europe and has quite a large variety of host species. Host species and genera are Tila sp., members of the family Pinaceae (*Abies alba*, *Picea* sp., *Pinus* sp.) and the order Fagales (*Betula* sp., *Populus* sp.). All these host species and genera are common among the species of *R.* subg. *Brevipedum*.

Species unit F has specimens originating from Europe. The host species are trees from the order Fagales (*Fagus sylvatica, Quercus petraea, Quercus rotundifolia*) and 2 orchid species from the genus *Limodorum* (*Limodorum abortivum, Limodorum brulloi*). *Limodorum brulloi* and *Quercus rotundifolia* are not observed to form interactions with other species of *R.* subg. *Brevipedum* and could be good indicator species.

Species unit O has only two hosts: *Pinus pinaster*, which is also a host of *R. littoralis* (AG), and *Limodorum abortivum*, which is a common host for the species of *R.* subg. *Brevipedum*.

Species unit A (*Russula* sp.2): The specimens of species unit A originate from West- and North-Europe. The variety of host species is quite high, as species unit A has two species from the genus *Pinus* (*Pinus halepensis, Pinus sylvestris*) *Quercus* sp., *Tilia* sp. and the orchid *Limodorum abortivum* as its host. *Pinus halepensis* is an exclusive host of species unit A. *Limodorum abortivum* is a host for different species of *R.* subg. *Brevipedum*, but not for the other subgenera. *Pinus sylvestris* is a common host for the species of *R.* subg. *Compactae* s.l., but only interacts with this species unit within *R.* subg. *Brevipedum*.

Species unit H has a variety of host species and a large geographic range (Europe and Canada). Host species are members of the order Fagales (*Betula pendula, Populus tremula, Quercus ilex*) and the family Pinaceae (*Picea* sp., *Pinus* sp.). It is possible that species unit H also consists of more species, as it has a wide host variety and geographic range.

Species unit S (*Russula* sp.3) has specimens originating from Europe. It has only two hosts from the orchid genus *Limodorum* (*Limodorum abortivum, Limodorum trabutianum*). *Limodorum abortivum* is a common host for the species of *R.* subg. *Brevipedum*, while *Limodorum trabutianum* is exclusively found as a host for this species unit.

Species unit W (*R. metachromatica*) is only found in Martinique. This species unit has 4 host species, which are not observed to interact with other species of *R.* subg. *Compactae* s.l. *Coccoloba pubescens, Coccoloba swartzii* and *Pisonia fragrans* are tree species native to the Caribbean region (Hassler, 2021a; Hassler, 2021b; Hassler, 2021c). *R. metachromatica* was also mentioned as a species in the first clade (2, 3), but had only one host species *Dicymbe corymbosa*, which was not found for species unit W. There is no overlap between host species, so these species can easily be delimited by host specificity.

There were also some unnamed species with host species data who were not assigned to a species unit. Remarkably, two related species units (10, 11) had the grass *Kobresia* from the family Cyperaceae as host. This is the only grass host we found. *Kobresia* is the only sedge known to form ECM associations and associates with *Cenoccocum, Sebacina, Tomentella* etc (Mühlmann & Peintner, 2008). Interactions with *Russula* have also been reported (Schadt, 2002).

Overall, we see many differences in host species between morphological similar species.

5.1.5 Intermediate conclusion and problems

The different species of the different subgenera seem to be host specific. Many species interact with hosts from one or two different families or with hosts that form no interactions with other species of *R*. subg. *Compactae* s.l. Even closely related species sometimes have hosts from the same species, genus or family, thus reflecting their relatedness. Similar species or different specimen falsely

labelled under the same name have in most cases a different variety of host species, which makes it easy to differentiate between them based on their surrounding hosts.

However, some caution is needed when we make conclusions for the host specificity, as some problems arise. Specimens retrieved from the UNITE and GlobalFungi database often reflect another and often wider geographic range than the specimens of our research group database. It is difficult to line the species hypotheses up to species in our tree, as specimens of the same species hypotheses can sometimes be placed at a whole different branch compared to the other specimens of the same species hypotheses. The ITS marker may also not be sufficient to differentiate between these different species complexes. Another problem is that relatively new species and species which are rare have less data available, meaning that they may have a larger variety of hosts, which overlap with hosts of morphological similar species. Species with a large variety of hosts, often have a large geographic range, which makes it more likely that these species can be divided in more different species. More molecular research, preferably with different markers, is needed. We also find more data about hosts for European species compared to the rest of the world, probably because most *Russula* research is centred around Europe (Buyck & Adamcik, 2013). Species from other parts of the world can have a wider host variety than we described.

5.2 Species delimitation

From the three runs, the third had the highest posterior probability for its best species model (0.58), with a set of 28 species. However, the posterior probability is rather low. The posterior probabilities of the delimited species are high for 27 species (0.93 to 1.00), but one species (J+AE) has a low posterior probability (0.69). The posterior probabilities of the best species models after the other two runs were lower (0.45 after run 1; 0.56 after run 2), and they had a higher set of species (resp. 33 and 30). After the second run, species J+AE had also the lowest probability (0.79), which implies it is unlikely that J+AE is one species. After the first run, species units K and AD had the lowest probability (both 0.64). K and AD are clustered together in the second run (K+AD) and third run (K+M+Y+A+B+AD) with high probabilities (both around 0.95), implying that these initial species units are probably clustered together in one species. Overall, our coalescent species delimitation approach does not seem to suffice to delimit the species of *Russula* subgenus *Brevipedum*. The fact that the outcomes of the three runs change so drastically, implies that the species models are unstable.

Maybe if we divided our initial set of species units in more, smaller species units, the posterior probability would be higher. Another way we could refine our species delimitation method is by adding more markers. We could also try to use another method for species delimitation to compare with our results.

5.3 Species comparisons

The new descriptions of *R*. subg. *Brevipedum* species will be compared to closely related and similar species, described in Romagnesi (1967) and Sarnari (1998). We will compare *Russula* sp.1 and *Russula subpallidospora* with each other and with *Russula pallidospora* Romagn. Further, *Russula* sp.2 and *Russula* sp.3 will be compared to each other and with our description of the *Russula delica* var*. delica* paratype/topotype. We will also compare *R.* sp.2 and *R.* sp.3 with the other variations of *R. delica* (*R. delica* var. *puta*, *R. delica* var. *trachyspora*) and to *Russula chloroides*.

5.3.1 *Russula* sp.1 VS. *Russula subpallidospora*

The hymenial cystidia of *Russula* sp.1 ((49–)57.0–66.3–75.5 (–80) × (5–)6.4–7.4–8.3 (– 9) µm) are smaller compared to the cystidia of *Russula subpallidospora* ((62–)72.8–87.4–102.1(–131) × (7–)7.4–8.4–9.3(– 11) µm). The hymenial cystidia of *R.* sp.1 are always apically obtuse, while those of *R. subpallidospora* can be apically mucronate or can have appendages. The marginal cells of *R.* sp.1 are similar in size ((8 –)12.7–21.8–30.8(–40) × (3–)3.9–4.8–5.6(–6) µm) compared to *R. subpallidospora* ((11–)18.1–24.3–30.5(–35) × (3–)3.9–5.0–6.1(–7) µm). The hyphal terminal cells in the pileus margin are similar in size (*R.* sp.1: (20–)30.6–39.8–49.0(–60) × (2–)2.3–3.0–3.6(–4) µm; *R. subpallidospora*: (19–)26.1–37.0–47.9 (–70) × (2–)2.1–3.7–4.9(–6) µm), but hyphal terminal cells are smaller in the pileus centre of *R.* sp.1 ((25–)29.0–35.7–42.3(–49) × (2–)2.6–3.0–3.3(–4) µm) compared to *R. subpallidospora* ((22–)27.3–39.7–52.0(–73) × (2–)2.4–3.1–3.7(–5) µm). Terminal cells of the pileocystidia of *R.* sp.1 are smaller ((37–)42.4–50.9–59.4(–64) × (4–)4.0–4.8–5.6(–7) µm) compared those of *R. subpallidospora* ((32–)43.7–73.3–103.0(–184) × (4–)4.7–5.6–6.5(–7) µm). The pileocystidia are 1-3 celled in both species. In both species, there is a slight reaction with sulfovanillin in the cystidia. Acid-resistant incrustations are not observed in both species.

Intermediate conclusion

The most notable differences between *Russula* sp.1 and *Russula subpallidospora* are the size difference of the hymenial cystidia and terminal cells of the pileocystidia and the difference in apical shape of the hymenial cystidia.

5.3.2 *Russula* sp.1

***Russula* sp.1 VS. *Russula pallidospora* Romagn.**

1. Romagnesi
   Microscopic: Hymenial cystidia are larger in *R. pallidospora* Romagn. (65-165 × 6,5-10 µm) than in *R.* sp.1 ((49–)57.0–66.3–75.5 (–80) × (5–)6.4–7.4–8.3 (– 9) µm). Apical appendages are present on the hymenial cystidia of *R. pallidospora* Romagn., but are not observed on the hymenial cystidia of *R.* sp.1, which are apically obtuse.
2. Sarnari
   Microscopic: Hymenial cystidia of *R. pallidospora* Romagn. are slightly wider (7-10.5 µm) compared to *R.* sp.1 ((5–)6.4–7.4–8.3 (– 9) µm).

Intermediate conclusion

Because only one collection of *Russula* sp.1 was available for the description, some parts of the description are missing (basidia and spores). This makes it difficult to draw a complete conclusion for this species. We see some differences in the hymenial cystidia compared to *R. pallidospora*: the hymenial cystidia of *Russula* sp.1 are smaller and lack apical appendages.

5.3.3 *Russula subpallidospora*

***Russula subpallidospora* VS. *Russula pallidospora* Romagn.**

1. Romagnesi
   Macroscopic: The spore print of *R. pallidospora* Romagn. is dark (IId), while the spore print of *R. subpallidospora* is pale cream (IIb).
   Microscopic: Spores of *R. pallidospora* Romagn. are smaller (7,5-8,75 × 6-7 µm) than the spores of *R.* sub*pallidospora* ((6.8–)7.6–8.4–9.3(–11.0) × (5.4–)6.1–6.6–7.1(–7.8) µm). Spores of *R. pallidospora* Romagn. have a weak amyloid, suprahilar spot, while the spores of *R. subpallidospora* have a large, suprahilar spot that is not amyloid. Basidia of *R. pallidospora* Romagn. (52-60 × 9-11 µm) are smaller than the basidia of *R. subpallidospora* ((35–)57.8–70.8–83.9(–87) × (6–)8.6–10.1–11.6(–13) µm). The hymenial cystidia of *R. pallidospora* Romagn. are larger (65-165 × 6,5-10 µm) compared to the cystidia of *R. subpallidospora* ((62–)72.8–87.4–102.1(–131) × (7–)7.4–8.4–9.3(– 11) µm).
2. Sarnari
   Macroscopic: *R. pallidospora* Romagn. has a slightly darker spore print (IIc-d) compared to *R. subpallidospora* (IIb).
   Microscopic: *R. pallidospora* Romagn. has slightly smaller spores (7.2-9 × 5.9-6.8 µm) compared to *R. subpallidospora* ((6.8–)7.6–8.4–9.3(–11.0) × (5.4–)6.1–6.6–7.1(–7.8) µm). The suprahilar spot of *R. pallidospora* Romagn. is small and amyloid, while that of *R. subpallidospora* is large and not amyloid. *Russula pallidospora* Romagn. has smaller basidia (48-62 × 8-11 µm) compared to *R. subpallidospora* (35–)57.8–70.8–83.9(–87) × (6–)8.6–10.1–11.6(–13) µm. Hymenial cystidia are similar in width. The hyphal terminations are less broad in *R. pallidospora* Romagn. (1.3-2.5 µm) compared to *R. subpallidospora* ((2–)2.1–3.7–4.9(–6) µm).

Intermediate conclusion

The most notable differences between *R. subpallidospora* and *R. pallidospora* are the suprahilar spot, which is not amyloid on the spores of *R. subpallidospora*, and the larger basidia of *R. subpallidospora*.

5.3.4 The real *Russula delica* var. *delica*

We made two descriptions based on specimens of two different clades which were both closely related to *Russula delica* (labelled here as *Russula* sp.2 and *R.* sp.3) and which contained both a paratype of *R. delica* var. *delica*. We will compare the descriptions of these two clades with the description of the *Russula delica* var. *delica* paratype/topotype, found in the same area as the holotype, to find out which of the two is more closely related.

***Russula* sp.2 VS. *Russula delica Fr.* var. *delica* paratype/topotype**

Microscopic: The spores of the paratype/topotype have a similar size ((8.6–)8.9–9.6–10.4(–10.9) × (6.9–)7.0–7.7–8.3(–9.2) µm) compared to *R.* sp.2 ((7.9–)8.9–9.5–10.1(–10.9) × (6.3–)7.1–7.5–8.0(–8.6) µm). Ornamentation of the spores of the *R. delica* paratype is slightly less high (0.5–0.8 µm) compared to *R.* sp.2 (0.3-1.2 µm). The basidia of the paratype/topotype are smaller, but wider ((49–)54.5–60.3–66.0(–72) × (10–)12.2–13.7–15.2(–16) µm) than *R.* sp.2 ((62–)68.6–76.7–84.9(–95) × (6–)8.6–10.1–11.6(–13) µm). Hymenial cystidia are also smaller, but slightly less broad for the paratype/topotype ((67–)71.0–82.3–93.5(–106) × (7–)8.4–9.3– 10 µm) compared to *R.* sp.2 ((68–)79.3–95.3–111.4(–139) × (7–)8.3–9.4–10.5(– 12) µm). The hymenial cystidia of both *R.* sp.2 and the paratype/topotype react slightly to sulfovanillin. The hyphal terminal cells of the pileipellis are slightly wider in the paratype/topotype ((19–)27.1–41.1–55.1(–77) × (3–)3.8–6.4–8.9(–11) µm) than *R.* sp.2 ((14–)26.4–40.6–54.7(–84) × (2–)2.9–4.2–5.4(–8) µm), but are similar in length. Terminal cells of the pileocystidia of the paratype/topotype are larger ((49–)62.1–105.0–148.0(–170) × (4–)4.6–5.6–6.6(–7) µm) compared to *R.* sp.2 (39–)63.8–94.7–126.0(–165) × (3–)4.1–5.2–6.2(–8) µm and are sometimes apically mucronate for the paratype/topotype, which is not observed for *R.* sp.2.

***Russula* sp.3 VS. *Russula delica Fr.* var. *delica* paratype/topotype**

Microscopic: Spores of the *R. delica* var. *delica* paratype/topotype are larger ((8.6–)8.9–9.6–10.4(–10.9) × (6.9–)7.0–7.7–8.3(–9.2) µm) than the spores of *R.* sp.3 ((7.2–)7.6–8.2–8.7(–9.5) × (6.3–)7.1–7.5–8.0(–8.6) µm). Basidia of the paratype/topotype are slightly smaller, but wider ((49–)54.5–60.3–66.0(–72) × (10–)12.2–13.7–15.2(–16) µm) compared to *R.* sp.3 (41–)54.7–61.6–68.6(–74) × (9–)10.6–11.9–13.2(–14) µm. The hymenial cystidia are never observed to be apically rostrate in the paratype/topotype, while this is sometimes observed in *R.* sp.3. Also, no reaction with sulfovanillin was observed in the hymenial cystidia of *R.* sp.3, while we did see this reaction in the cystidia of the paratype/topotype. Acid-resistant incrustations are absent in the paratype/topotype, while these are present in *R.* sp.3. The terminal cells of the pileipellis hyphae are slightly smaller in the paratype/topotype ((19–)27.1–41.1–55.1(–77) × (3–)3.8–6.4–8.9(–11) µm) compared to *R.* sp.3 ((19–)30.9–45.7–60.4(–84) × (3–)4.2–5.9–7.6(–9) µm). The pileocystidia of the paratype/topotype counted 1-3 cells, while these of *R.* sp.3 were up to 4 cells. The pileocystidia of the paratype/topotype are also much larger ((49–)62.1–105.0–148.0(–170) × (4–)4.6–5.6–6.6(–7) µm) than the pileocystidia of *R.* sp.3 ((26–)40.3–60.1–79.8(–107) × (4–)4.4–5.8–7.3(–10)) and were never observed to be bifurcating in the paratype/topotype, in contrary to the pileocystidia of *R.* sp.3.

Intermediate conclusion

Our description of *R.* sp.2 matches the description of the *R. delica* var. *delica* paratype/topotype the most. The measurements of the spores, basidia, cystidia and terminal cells are slightly different, but lie closer to the measurement of *R.* sp.2 than *R.* sp.3. Also, there is no reaction seen with sulfovanillin

in the cystidia of *R.* sp.3, but the reaction with sulfovanillin is observed in both *R.* sp.2 and the paratype/topotype. Acid-resistant incrustations were present in *R.* sp.3, but absent in both our *R.* sp.2 and the paratype/topotype.

5.3.5 *Russula* sp.2 VS. *Russula* sp.3

The spores of *Russula* sp.2 are slightly larger ((7.9–)8.9–9.5–10.1(–10.9) × (6.3–)7.1–7.5–8.0(–8.6) µm) compared to the spores of *Russula* sp.3 ((7.2–)7.6–8.2–8.7(–9.5) × (6.3–)7.1–7.5–8.0(–8.6) µm). The hymenial cystidia of *R.* sp.2 ((68–)79.3–95.3–111.4(–139) × (7–)8.3–9.4–10.5(– 12)) are larger compared to the cystidia of *R.* sp.3 ((51–)65.4–81.3–97.2(–134) × (6–)7.2–8.3–9.3(– 11) µm), but have a similar shape. The basidia of *R.* sp.2 are larger ((62–)68.6–76.7–84.9(–95) × (6–)8.6–10.1–11.6(–13) µm) compared to those of *R.* sp.3 ((41–)54.7–61.6–68.6(–74) × (9–)10.6–11.9–13.2(–14) µm). The marginal cells of *R.* sp.2 are slightly larger ((11–)16.1–22.4–28.8(–39) × (4–)4.1–5.3–6.4(–8) µm) compared to *R.* sp.3 ((8–)14.3–20.0–25.8(–35) × (3–)4.0–5.3–6.6(–8) µm). The hyphal terminal cells of *R.* sp.2 are similar in size (pileus margin: (14–)26.4–40.6–54.7(–84) × (2–)2.9–4.2–5.4(–8) µm; pileus centre: (24–)31.3–43.3–55.3 (–77) × (2–)2.7–3.3–3.9(–5)) compared to *R.* sp.3 (pileus margin:(19–)30.9–45.7–60.4(–84) × (3–)4.2–5.9–7.6(–9) µm; pileus centre: (21–)32.0–46.3–60.7(–81) × (3–)3.0–3.7–4.4(–5)). Terminal cells of the pileocystidia of *R.* sp.2 are larger ((39–)63.8–94.7–126.0(–165) × (3–)4.1–5.2–6.2(–8) µm) compared those of *R.* sp.3 ((26–)40.3–60.1–79.8(–107) × (4–)4.4–5.8–7.3(–10) µm). The pileocystidia are 1-3 celled in *R.* sp.2, but 1-4 celled in *R.* sp.3. The hymenial cystidia of *R.* sp.2 react slightly with sulfovanillin, while there is no reaction in the hymenial cystidia of *R.* sp.3 with sulfovanillin. *R.* sp.3 Acid-resistant incrustations are present in *R.* sp.3, but absent in *R.* sp.2.

Intermediate conclusion

The most notable differences between *Russula* sp.2 and *Russula* sp.3 are the difference in size of the basidia and terminal cells of the pileocystidia, no reaction of sulfovanillin in the cystidia of *R.* sp.3 and the presence of acid-resistant incrustations in *R.* sp.3, which are absent in *R.* sp.2.

5.3.6 *Russula* sp.2

***Russula* sp.2 VS. *Russula delica* var. *puta* Romagn.**

1. Romagnesi
   Macroscopic: Pileus size is larger for our description of *R.* sp.2 (90-135 mm) compared to the pileus of *R. delica* var. *puta* (55-120 mm). Stipe has a similar size in both descriptions. The flavour is mild for both descriptions. *Russula delica* var. *puta* has a bright reddish pink reaction to $FeSO_4$, while *R.* sp.2 has an orange reaction. Spore print is similar in colour (Ib)
   Microscopic: The spore size is similar for both *R. delica* var. *puta* (7.7-9.2-(9.7) × 6.5-7-(8) µm) and *R.* sp.2 ((7.9–)8.9–9.5–10.1(–10.9) × (6.3–)7.1–7.5–8.0(–8.6) µm). Basidia of *R. delica* var. *puta* are slightly smaller ((47)-62-75 × 12-13 µm) compared to those of *R.* sp.2 ((62–)68.6–76.7–84.9(–95) × (6–)8.6–10.1–11.6(–13) µm). Hymenial cystidia of *R. delica* var. *puta* are slightly larger (65-150 × (6.5)-7.2- 11.5-(13,5) µm) compared to *R.* sp.2 ((68–)79.3–95.3–111.4(–139) × (7–)8.3–9.4–10.5(– 12) µm).
2. Sarnari
   Macroscopic: The pileus size of *R. delica* var. *puta* is smaller (90-110 mm) than the pileus size of *R.* sp.2 (90-135 mm). The lamellae density is higher for *R. delica* var. *puta* (10L/cm) compared to *R.* sp.2 (5-8L/cm). The stipe of *R. delica* var. *puta* is smaller (20-30 × 15-20 mm) than *R.* sp.2 (40-60 × 22-25 mm). *Russula delica* var. *puta* has a salty odour, while *R.* sp.2 smells fruity and like coriander leaves. Both have a reaction to $FeSO_4$ and guaiac. The spore print has a similar colour in both descriptions (Ib)
   Microscopic: Spore size is similar for *R. delica* var. *puta* (8-9.6 × 6.7-8 µm) and *R.* sp.2 ((7.9–)8.9–9.5–10.1(–10.9) × (6.3–)7.1–7.5–8.0(–8.6) µm). The basidia of *R. delica* var. *puta* are smaller (40-68 × 10-12 µm) compared to the basidia of *R.* sp.2 ((62–)68.6–76.7–84.9(–95) × (6–)8.6–10.1–11.6(–13) µm), but similar in shape. Hymenial cystidia have a similar shape.

***Russula* sp.2 VS. *Russula delica* var. *trachyspora* Romagn.**

1. Romagnesi
   Macroscopic: The pileus size of *R. delica* var. *trachyspora* is slightly smaller (80-110 mm) than the pileus size of *R.* sp.2 (90-135 mm). The lamellae density is higher for *R. delica* var. *trachyspora* (6-9L/cm) compared to *R.* sp.2 (5-8L/cm). The stipe of *R. delica* var. *trachyspora* is smaller (20-50 × 15-24 mm) than *R.* sp.2 (40-60 × 22-25 mm). Both have a mild taste. *Russula delica* var. *trachyspora* turns light orange-pink with the reaction of $FeSO_4$, while *R.* sp.2 turns orange. The spore print is light for both (Ib), but sometimes a little darker (IIa) for *R. delica* var. *trachyspora*
   Microscopic: Spore size is similar in *R. delica* var. *trachyspora* (8.5-10.7 × 7-8.5 µm) and our *R. delica* ((7.9–)8.9–9.5–10.1(–10.9) × (6.3–)7.1–7.5–8.0(–8.6) µm). The basidia of *R. delica* var. *trachyspora* are smaller (43-57 × 11-12.5) compared to the basidia of *R.* sp.2 ((62–)68.6–76.7–84.9(–95) × (6–)8.6–10.1–11.6(–13) µm). The hymenial cystidia are similar in size *R. delica* var. *trachyspora* (78-135 × 6-11.5 µm) compared to *R.* sp.2 (68–)79.3–95.3–111.4(–139) × (7–)8.3–9.4–10.5(– 12) µm).

***Russula* sp.2 VS. *Russula chloroides* (Krombh.) Bres.**

1. Romagnesi
   Macroscopic: The pileus of *R. chloroides* reaches smaller sizes (var. *chloroides*: 45-130 mm; var. *parvispora*: 45-100 mm) than *R.* sp.2 (90-135 mm). The stipe of *R. chloroides* is similar or smaller (var. *chloroides*: (15)-30-50-(90) × 10-30 mm; var. *parvispora*: 25-40 × 10-18-(23) mm) than *R.* sp.2 (40-60 × 22-25 mm). Taste is mild of both *R. chloroides* and *R.* sp.2. The odour of *R. chloroides* is first unpleasant, then strongly fruity (var. *chloroides*) or the other way around (var. *parvispora*), while the odour of *R.* sp.2 is fruity with the smell of coriander leaves. *Russula chloroides* var. *chloroides* turns reddish and *R. chloroides* var. *parvispora* turns pink-orange with the reaction of $FeSO_4$, while *R.* sp.2 turns orange. *Russula chloroides* and *R.* sp.2 react strongly to guaiac. The spore print is similar for both (Ib) or slightly darker (Ib-IIa for *R. chloroides* var. *chloroides*)
   Microscopic: The spores of *R. chloroides* var. *chloroides* are similar in size (7-10-11 × 6-8.7 µm) to *R.* sp.2 ((7.9–)8.9–9.5–10.1(–10.9) × (6.3–)7.1–7.5–8.0(–8.6) µm), while the spores of *R. chloroides* var. *parvispora* are smaller (6.5-8 × 6-6.7 µm). Ornamentation of the spores reaches slightly bigger lengths for *R. chloroides* var. *chloroides* (max. 1.5 µm) compared to *R.* sp.2 (0.3-1.2 µm). The basidia of *R. chloroides* are smaller (var. *chloroides*: 50-67 × (9)-10-12,5-(15) µm; var. *parvispora*: 45-62 × 6,7-10 µm) compared to the basidia of *R.* sp.2 ((62–)68.6–76.7–84.9(–95) × (6–)8.6–10.1–11.6(–13) µm). The hymenial cystidia of *R. chloroides* have a similar size (var. *chloroides*: 50-130 × 6,5 -11 µm; var. *parvispora*: (57)-65-115 × 6,5-9 µm) compared to the cystidia of *R.* sp.2 ((68–)79.3–95.3–111.4(–139) × (7–)8.3–9.4–10.5(–12) µm).
2. Sarnari
   Macroscopic: The pileus of *R. chloroides* reaches larger (var. *chloroides*: 65-150 mm) or similar (var. *trachyspora*: 100-130 mm) sizes compared to *R.* sp.2 (90-135 mm). The stipe of *R. chloroides* is mostly smaller (var. *chloroides*: 25-45 (58) × 20-30 mm; var. *trachyspora*: 30-45 × 18-22 nm) than *R.* sp.2 (40-60 × 22-25 mm). The taste *R. chloroides* is spicy, while *R.* sp.2 has a mild taste. The odour of *R. chloroides* is unpleasant, like saltwater, while *R.* sp.2 smells fruity and like coriander leaves. The spore print is the same for *R. chloroides* var. *chloroides* and our *R.* sp.2 (Ib), but a bit darker for *R. chloroides* var. *trachyspora* (IIa)
   Microscopic: The spores of *R. chloroides* var. *chloroides* (8- 11.2 × 7.2-8.8 µm) and *R. chloroides* var. *trachyspora* (9,5-11.4 × 8-10.5 µm) are larger than *R.* sp.2 ((7.9–)8.9–9.5–10.1(–10.9) × (6.3–)7.1–7.5–8.0(–8.6) µm), while *R. chloroides* var. *parvispora* (6.4-8 × 6-7 µm) has smaller spores. Ornamentation of the spores is higher for *R. chloroides* var. *chloroides* (1.3-1.6 µm) and *R. chloroides* var. *trachyspora* (max. 1.5 µm), but similar for *R. chloroides* var. *parvispora* (max. 1 µm) compared to *R.* sp.2 (0.3-1.2 µm). The basidia of *R. chloroides* (var. *chloroides*: 48-70 × 10-15 µm; var. *trachyspora*: 40-68 × 11-13 µm; var. *parvispora*: 40-69 × 8-11 µm) are smaller than the basidia of *R.* sp.2 ((62–)68.6–76.7–84.9(–95) × (6–)8.6–10.1–11.6(–13) µm). The hymenial cystidia of *R. chloroides* have a similar width (var. *chloroides*: larger than 7- 13 µm; var. *trachyspora*: 11 µm; var. *parvispora*: 10 µm) compared to the cystidia of *R.* sp.2 ((7–)8.3–9.4–10.5(– 12) µm).

Intermediate conclusion

The basidia of *Russula* sp.2 are larger compared to all the *R. delica* varieties and *R. chloroides*. The smell of coriander leaves in the context of *R.* sp.2 is not observed in the *R. delica* varieties and *R. chloroides***.**

5.3.7 *Russula* sp.3

***Russula* sp.3 *VS Russula delica* var. *puta* Romagn.**

1. Romagnesi
   Macroscopic: Pileus size is smaller for our description of *R.* sp.3 (70-90 mm) compared to the pileus of *R. delica* var. *puta* (55-120 mm). The stipe of *R. delica* var. *puta* is larger (35-65 × 13-17 mm) than the stipe of *R.* sp.3 (20-25 × 15-20 mm). The flavour is mild for both descriptions. *Russula delica* var. *puta* has a bright reddish pink reaction to $FeSO_4$, while *R.* sp.3 has slightly orange reaction.
   Microscopic: The spore size is similar for both *R. delica* var. *puta* (7.7-9.2-(9.7) × 6.5-7-(8) µm) and *R.* sp.3 ((7.2–)7.6–8.2–8.7(–9.5) × (6.3–)7.1–7.5–8.0(–8.6) µm). Basidia of *R. delica* var. *puta* are similar in size ((47)-62-75 × 12-13 µm) compared to those of *R.* sp.3 ((41–)54.7–61.6–68.6(–74) × (9–)10.6–11.9–13.2(–14) µm). Hymenial cystidia of *R. delica* var. *puta* reach larger sizes (65-150 × (6.5)-7.2- 11.5- (13,5) µm) than *R.* sp.3 ((51–)65.4–81.3–97.2(–134) × (6–)7.2–8.3–9.3(– 11) µm).
2. Sarnari
   Macroscopic: The pileus size of *R. delica* var. *puta* is larger (90-110 mm) than the pileus size of *R.* sp.3 (70-90 mm). The stipe of *R. delica* var. *puta* is similar in size (20-30 × 15-20 mm) compared to *R.* sp.3 (20-25 × 15-20 mm). *Russula delica* var. *puta* has a salty odour, while *R.* sp.3 smells fruity and like paint. Both have a reaction to $FeSO_4$ and guaiac.
   Microscopic: Spore size is similar for *R. delica* var. *puta* (8-9.6 × 6.7-8 µm) and *R.* sp.3 ((7.2–)7.6–8.2–8.7(–9.5) × (6.3–)7.1–7.5–8.0(–8.6) µm). The basidia of *R. delica* var. *puta* are similar in size (40-68 × 10-12 µm) compared to the basidia of *R.* sp.3 ((41–)54.7–61.6–68.6(–74) × (9–)10.6–11.9–13.2(–14) µm) and have a similar shape. Hymenial cystidia have a similar shape.

***Russula* sp.3 *VS Russula delica* var. *trachyspora* Romagn.**

1. Romagnesi
   Macroscopic: The pileus size of *R. delica* var. *trachyspora* reaches a larger size (80-110 mm) than the pileus size of *R.* sp.3 (70-90 mm). The stipe of *R. delica* var. *trachyspora* reaches larger sizes (20-50 × 15-24 mm) than *R.* sp.3 (20-25 × 15-20 mm). Both have a mild taste. *Russula delica* var. *trachyspora* turns light orange-pink with the reaction of $FeSO_4$, while *R.* sp.3 turns slightly orange.
   Microscopic: The spores of *R. delica* var. *trachyspora* (8.5-10.7 × 7-8.5 µm) are slightly larger than the spores of *R.* sp.3 ((7.2–)7.6–8.2–8.7(–9.5) × (6.3–)7.1–7.5–8.0(–8.6) µm). The basidia of *R. delica* var. *trachyspora* are smaller (43-57 × 11-12.5) compared to the basidia of *R.* sp.3 ((41–)54.7–61.6–68.6(–74) × (9–)10.6–11.9–13.2(–14) µm). The hymenial cystidia of *R. delica* var. *trachyspora* are similar in size (78-135 × 6-11.5 µm) compared to the cystidia of *R.* sp.3 ((51–)65.4–81.3–97.2(–134) × (6–)7.2–8.3–9.3(– 11) µm).

***Russula* sp.3 *VS Russula chloroides* (Krombh.) Bres.**

1. Romagnesi
   Macroscopic: The pileus of *R. chloroides* reaches slightly larger sizes (var. *chloroides*: 45-130 mm; var. *parvispora*: 45-100 mm) than *R.* sp.3 (70-90 mm). The stipe of *R. chloroides* is mostly larger (var. *chloroides*: (15)-30-50-(90) × 10-30 mm; var. *parvispora*: 25-40 × 10-18-(23) mm) than *R.* sp.3 (20-25 × 15-20 mm). Taste is mild of both *R. chloroides* and *R.* sp.3. The

odour of *R. chloroides* is first unpleasant, then strongly fruity (var. *chloroides*) or the other way around (var. *parvispora*), while the odour of *R.* sp.3 is fruity and smells a bit like paint. *Russula chloroides* var. *chloroides* turns reddish and *R. chloroides* var. *parvispora* turns pink-orange with the reaction of $FeSO_4$, while *R.* sp.3 turns slightly orange. Both *R. chloroides* and *R.* sp.3 react strongly to guaiac.

Microscopic: The spores of *R. chloroides* var. *chloroides* reach slightly larger sizes (7-10-11 × 6-8.7 µm) compared to *R.* sp.3 ((7.2–)7.6–8.2–8.7(–9.5) × (6.3–)7.1–7.5–8.0(–8.6) µm), while the spores of *R. chloroides* var. *parvispora* are smaller (6.5-8 × 6-6.7 µm). Ornamentation of the spores reaches bigger lengths for *R. chloroides* var. *chloroides* (max. 1.5 µm) compared to *R.* sp.3 (0.4–0.8 µm). The basidia of *R. chloroides* are similar in size (var. *chloroides*: 50-67 × (9)-10-12,5-(15) µm; var. *parvispora*: 45-62 × 6,7-10 µm) compared to the basidia of *R.* sp.3 ((41–)54.7–61.6–68.6(–74) × (9–)10.6–11.9–13.2(–14) µm). The hymenial cystidia of *R. chloroides* have a similar size (var. *chloroides*: 50-130 and larger × 6,5 -11 µm; var. *parvispora*: (57)-65-115 × 6,5-9 µm) compared to the cystidia of *R.* sp.3 ((51–)65.4–81.3–97.2(–134) × (6–)7.2–8.3–9.3(– 11) µm).

2. Sarnari

   Macroscopic: The pileus of *R. chloroides* reaches larger sizes (var. *chloroides*: 65-150 mm; var. *trachyspora*: 100-130 mm) compared to *R.* sp.3 (70-90 mm). The stipe of *R. chloroides* is mostly smaller (var. *chloroides*: 25-45 (58) × 20-30 mm; var. *trachyspora*: 30-45 × 18-22 nm) than *R.* sp.3 (20-25 × 15-20 mm). The taste *R. chloroides* is spicy, while *R.* sp.3 has a mild taste. The odour of *R. chloroides* is unpleasant, like saltwater, while *R.* sp.3 smells fruity and like paint.

   Microscopic: The spores of *R. chloroides* var. *chloroides* (8- 11.2 × 7.2-8.8 µm) and *R. chloroides* var. *trachyspora* (9,5-11.4 × 8-10.5 µm) are larger than *R.* sp.3 ((7.2–)7.6–8.2–8.7(–9.5) × (6.3–)7.1–7.5–8.0(–8.6) µm), while *R. chloroides* var. *parvispora* (6.4-8 × 6-7 µm) has slightly smaller spores. Ornamentation of the spores is higher for *R. chloroides* (var. *chloroides*: 1.3-1.6 µm; var. *trachyspora:* max. 1.5 µm; var. *parvispora*: max. 1 µm) compared to *R.* sp.3 (0.4–0.8 µm). The basidia of *R. chloroides* (var. *chloroides*: 48-70 × 10-15 µm; var. *trachyspora*: 40-68 × 11-13 µm; var. *parvispora*: 40-69 × 8-11 µm) are similar in size compared to the basidia of *R.* sp.3 ((41–)54.7–61.6–68.6(–74) × (9–)10.6–11.9–13.2(–14) µm). The hymenial cystidia of *R. chloroides* have a similar or slightly larger width (var. *chloroides*: larger than 7- 13 µm; var. *trachyspora*: 11 µm; var. *parvispora*: 10 µm) compared to the cystidia of *R.* sp.3 ((6–)7.2–8.3–9.3(– 11) µm).

Intermediate conclusion

The spores of *Russula* sp.3 are larger compared to *Russula chloroides* and *Russula delica* var. *trachyspora*. The smell of paint in the context of *R.* sp.3 is not observed in the other varieties of *Russula delica* and *Russula chloroides*.

6. Conclusion

Species delimitation of *Russula* subgenus *Compactae* s.l. remains challenging to this day. Incomplete morphological descriptions and the insufficiency of the ITS marker to delimit closely related species cause an underestimation in the species diversity.

Our four subgenera *Russula* subgenus *Archaeae*, *Russula* subgenus *Compactae* s.s., *Russula* subgenus *Malodorae* and *Russula* subgenus *Brevipedum* contain both generalists and specialists for host interactions. The host specificity reflects in most cases the diversity or relatedness of the species of *R*. subg. *Compactae* s.l. However, some scepticism is needed when making conclusions, as the databases we used are probably partly incomplete, because data is focused around abundant and European species. Species hypotheses of the online databases are often not in line with the Maximum Likelihood trees, based on the ITS marker, of the different subgenera. Overall, we think that host specificity is a good feature to delimit between morphological similar species, but more research is needed.

The coalescent species delimitation method used in this paper does not suffice for the species delimitation of *R.* subg. *Brevipedum*. The third run had a set of 28 species as the highest supported species model, with a posterior probability of 0.58. This number was the highest of the three runs, but still rather low. The three runs had different posterior probabilities and species models, implying that the species model was unstable. More initial species units and/or more different markers could benefit our approach. The use of another species delimitation method could further verify our species model.

Russula sp.1 and *Russula subpallidospora* are closest related to each other and to *Russula pallidospora*. *Russula* sp.1 and *Russula subpallidospora* differ in size of the hymenial cystidia and terminal cells of the pileocystidia. Another notable difference is the apical shape of the hymenial cystidia, which is always obtuse in the case of *Russula* sp.1, but can be obtuse to mucronate or with appendages in the case of *Russula subpallidospora*. The most notable difference between *Russula* sp.1 and *Russula pallidospora* are the hymenial cystida, which are smaller and lack apical appendages in the case of *Russula* sp.1. *Russula subpallidospora* and *Russula pallidospora* differ in basidia and the suprahilar spot of the spores, which are respectively larger and not amyloid in the case of *Russula subpallidospora*. *Russula* sp.2 has more similarities with the *Russula delica* var. *delica* paratype/topotype than *Russula* sp.3 and is probably the real *Russula delica.* The most notable differences between *Russula* sp.2 and *Russula* sp.3 are the larger size of the basidia and terminal cells of the pileocystidia in *Russula* sp.2, no reaction of sulfovanillin in the cystidia of *Russula* sp.3 and the presence of acid-resistant incrustations in *Russula* sp.3, which are absent in *Russula* sp.2.

7. Summary

Russula is an important ectomycorrhiza (ECM)-forming genus, which is recognizable by its colourful fruit bodies and chalk-like context. The real diversity of *Russula* is underestimated due to lack of complete species descriptions, missing molecular data and sampling being almost restricted to Europe. The classification of the genus *Russula* has also changed over the years. For example, the *Russula* subgenus *Compactae* (Fr.) Bon s.l. -characterised by the presence of numerous lamellulae and firm, compact fruit bodies with a yellow, white or brown cap- is in recent classifications scattered in five subgenera: *Russula* subgenus *Archaeae* Buyck & V. Hofst., *Russula* subgenus *Compactae* (Fr.) Bon s.s., emend. Buyck & V. Hofst., *Russula* subgenus *Malodorae* Buyck & V. Hofst., *Russula* subgenus *Brevipedum* Buyck & V. Hofst. and, most recently, *Russula* subgenus *Glutinosae* Buyck & X.H. Wang.

In the past, species delimitation of Fungi was based on morphological features (Morphological Species Concept, MSC). However, with the rise of the Phylogenetic Species Concept (PSC), it became clear the morphological descriptions of *Russula* species did not suffice to describe the real diversity of *Russula*. The *Russula* diversity was underestimated, due to the existence of cryptic species and phenotypic plasticity within species. Thus came the rise of the Ecological Species Concept (ESP): which states that we can delimit species based on their different ecological features. For *Russula* subgenus *Compactae* (Fr.) Bon s.l., we chose host specificity as a feature to delimit the different species.

In this study, we focus on 4 different subgenera: *Russula* subgenus *Archaeae*, *Russula* subgenus *Compactae* s.s., *Russula* subgenus *Brevipedum* and *Russula* subgenus *Malodorae*. Data about host plants species was collected for each species of the subgenera from different Fungi databases. Maximum Likelihood (ML) trees based on the ITS marker were compiled for all 4 subgenera. Also, a coalescent species delimitation method with different markers (ITS, LSU, RBP2 and ETF1) was tested to delimit the species of *Russula* subgenus *Brevipedum.* A model of 33 different species units was proposed for this species delimitation method. Last, four new species of *Russula* subgenus *Brevipedum* were morphologically described, based on the description style from Adamčík et al. (2019).

The host specificity seems to be a good feature for species delimitation between morphological similar species, and in some cases, it even reflects relatedness between *Russula* species. However, the databases were mainly focused on European species and thus were probably incomplete. The species hypotheses of the databases did not follow the species units of our ML trees in many cases. Also, there is more data available for abundant species than species that are rarer. In conclusion, host specificity can be a good delimiting feature, but more research is needed.

The best species model of our coalescent species delimitation method assigns 28 different species to *Russula* subgenus *Brevipedum*. However, the posterior probability is rather low (0.58). Maybe, if more initial species units were assigned or if more different markers were used, the species delimitation method could have a more satisfying result. Also, other species delimitation methods could be used to verify the species model.

Descriptions of four new species were made. *Russula* sp.1 and *Russula subpallidospora* are closest related to each other *Russula pallidospora* Romagn. *Russula* sp.1 has smaller hymenial cystidia compared to *Russula pallidospora.* The hymenial cystidia of *Russula* sp.1 also do not have apical

appendages, while the hymenial cystidia of *Russula pallidospora* do. *Russula subpallidospora* has larger hymenial cystidia and basidia than *Russula pallidospora*. The spores of *Russula subpallidospora* have an inamyloid suprahilar spot, while the suprahilar spot of *Russula pallidospora* spores is amyloid. The most notable differences between *Russula* sp.1 and *Russula subpallidospora* are the larger sizes of the hymenial cystidia and terminal cells of the pileocystidia in *Russula subpallidospora* and the difference in apical shape of the hymenial cystidia. The hymenial cystidia are always apically obtuse in the case of *Russula* sp.1, but can be apically obtuse to mucronate or with appendages in the case of *Russula subpallidospora.*

Russula sp.2 and *Russula* sp.3 were compared to different subspecies of *Russula delica* Fries, *Russula chloroides* (Krombh.) Bres. and to the *Russula delica* Fr. var*. delica* paratype/topotype. The latter was found in the same area as the *Russula delica* Fries holotype and is the closest we could go to the real holotype. *Russula* sp.2 has more similarities to the *R. delica* Fr. var*. delica* paratype/topotype than *Russula* sp. 3 does, thus is probably the real *Russula delica.* The most notable differences between *Russula* sp.2 and *Russula* sp.3 are the larger size of the basidia and terminal cells of the pileocystidia in *Russula* sp.2, no reaction of sulfovanillin in the cystidia of *Russula* sp.3 and the presence of acid-resistant incrustations in *Russula* sp.3, which are absent in *Russula* sp.2.

8. Samenvatting

Russula is een belangrijk ectomycorrhiza (ECM)-vormend genus, herkenbaar aan de kleurrijke vruchtlichamen en krijtachtige context. De echte diversiteit van *Russula* wordt onderschat, omdat complete soortbeschrijvingen en moleculaire gegevens vaak ontbreken en het verzamelen van stalen vaak beperkt blijft tot Europa. Ook de classificatie van *Russula* is in de loop der jaren veranderd. *Russula* subgenus *Compactae* (Fr.) Bon s.l. -gekenmerkt door de aanwezigheid van talrijke lamellulae en stevige, compacte vruchtlichamen met een gele, witte of bruine hoed- werd in recente classificaties verspreid over vijf subgenera: *Russula* subgenus *Archaeae* Buyck & V. Hofst., *Russula* subgenus *Compactae* (Fr.) Bon s.s., emend. Buyck & V. Hofst., *Russula* subgenus *Malodorae* Buyck & V. Hofst., *Russula* subgenus *Brevipedum* Buyck & V. Hofst. en, meest recentelijk, *Russula* subgenus *Glutinosae* Buyck & X.H. Wang.

In het verleden was de soortafbakening van Fungi gebaseerd op morfologische kenmerken (Morfologisch Soort Concept, MSC). Met de opkomst van het Phylogenetische Soort Concept (PSC), werd het echter duidelijk dat de morfologische beschrijvingen van *Russula* soorten niet volstonden om de echte diversiteit van *Russula* te omvatten. De diversiteit werd onderschat door het bestaan van cryptische soorten en fenotypische plasticiteit binnen soorten. Het Ecologische Soort Concept (ESP) is misschien de oplossing. Het ESP stelt dat soorten kunnen worden afgebakend op basis van ecologische kenmerken. Voor *Russula* subgenus *Compactae* (Fr.) Bon s.l. onderzochten we of we soorten kunnen afbakenen op basis van gastheerspecificiteit.

In deze studie richten we ons op 4 verschillende subgenera: *Russula* subgenus *Archaeae*, *Russula* subgenus *Compactae* s.s., *Russula* subgenus *Brevipedum* en *Russula* subgenus *Malodorae*. Gegevens over gastheerplanten werden verzameld voor elke soort aan de hand van verschillende databases met gegevens over Fungi. Maximum Likelihood (ML) bomen werden opgesteld voor alle 4 subgenera. Ook werd een 'coalescent species delimitation method' met vier verschillende markers (ITS, LSU, RBP2 en ETF1) getest om de soorten van *Russula* subgenus *Brevipedum* af te bakenen. Voor deze methode werd een initieel model van 33 verschillende soorteenheden voorgesteld. Als laatste werden vier nieuwe soorten van *Russula* subgenus *Brevipedum* morfologisch beschreven, gebaseerd op de beschrijvingen van Adamčík et al. (2019).

De gastheerspecificiteit leek een goede eigenschap te zijn voor het afbakenen van morfologische gelijkaardige soorten en in sommige gevallen weerspiegelde het zelfs verwantschappen. De databases waren echter gericht op Europese soorten en waren dus mogelijk onvolledig. De soort hypotheses van de databases volgden vaak niet de soorteenheden van onze ML bomen. Het is mogelijk dat er fouten werden gemaakt als stalen in de database werden toegewezen aan soorten, maar dat is moeilijk na te gaan. In conclusie is er meer onderzoek nodig naar gastheerspecificiteit als kenmerk om soorten af te bakenen.

Het beste model dat uit onze 'coalescent species delimitation method' voortkwam, verdeelde *Russula* subgenus *Brevipedum* in 28 verschillende soorten. De posterior probability was echter vrij laag (0.58). Misschien was dit resultaat beter als er meer initiële soorteenheden werden onderscheiden of als er meer verschillende markers werden gebruikt. Om ons soortmodel te verifiëren, kon misschien een andere methode gebruikt worden.

Er werden beschrijvingen gemaakt van vier nieuwe soorten. *Russula* sp.1 en *Russula subpallidospora* zijn het meest verwant aan *Russula pallidospora* Romagn. *Russula* sp.1 heeft kleinere cystidia in het hymenium in vergelijking met *Russula pallidospora*. Deze hebben ook geen apicale aanhangsels bij *Russula* sp.1, terwijl de cystidia van *Russula pallidospora* dat wel hebben. *Russula subpallidospora* heeft grotere cystidia in het hymenium en basidia dan *Russula pallidospora*. De sporen van *Russula subpallidospora* hebben een inamyloïde suprahilaire vlek, terwijl de suprahilaire vlek van *Russula pallidospora* amyloïde is. Het meest opvallende verschil tussen *Russula* sp.1 en *Russula subpallidospora* zijn de gotere cystidia in het hymenium van *Russula subpallidospora* en de grotere terminale cellen van de pileocystidia van *Russula subpallidospora.* Nog een groot verschil is dat de cystidia van het hymenium van *Russula* sp.1 altijd stomp zijn aan de apex, terwijl deze bij *Russula subpallidospora* ook mucronaat kunnen zijn of aanhangsels kunnen hebben.

Russula sp.2 en *Russula* sp.3 werden vergeleken met elkaar, verschillende ondersoorten van *Russula delica* Fries, *Russula chloroides* (Krombh.) Bres. en met het *Russula delica* Fr. var. *delica* paratype/topotype. Die laatste werd gevonden in hetzelfde gebied als het holotype van *Russula delica* Fries en komt, van wat we beschikbaar hadden, het dichtst in de buurt van het echte holotype. De opvallendste verschillen tussen *Russula* sp.2 en *Russula* sp.3 zijn de grotere basidia en terminale cellen van de pileocystidia in *Russula* sp.2, geen reactie met sulfovanillin in de cystidia van *Russula* sp.3 en de aanwezigheid van zuurbestendige incrustaties in *Russula* sp.3, die zijn niet aanwezig in *Russula* sp.2.

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W., Lewis, C. T., Hambleton, S., Moussa, T. A. A., Al-Zahrani, H. S., Almaghrabi, O. A., Louis-Seize, G., Assabgui, R., McCormick, W., Omer, G., Dukik, K., Cardinali, G., Eberhardt, U., de Vries, M., Robert V. (2015). One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia* 35:242-263.

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11. Appendices

Legend of appendices

Appendix A: Maximum Likelihood tree of *Russula* subgenus *Malodorae* based on ITS. ML bootstrap values are shown. In red are numbers of the species units. Host species in bold were found for multiple specimens of the respective *Russula* species.

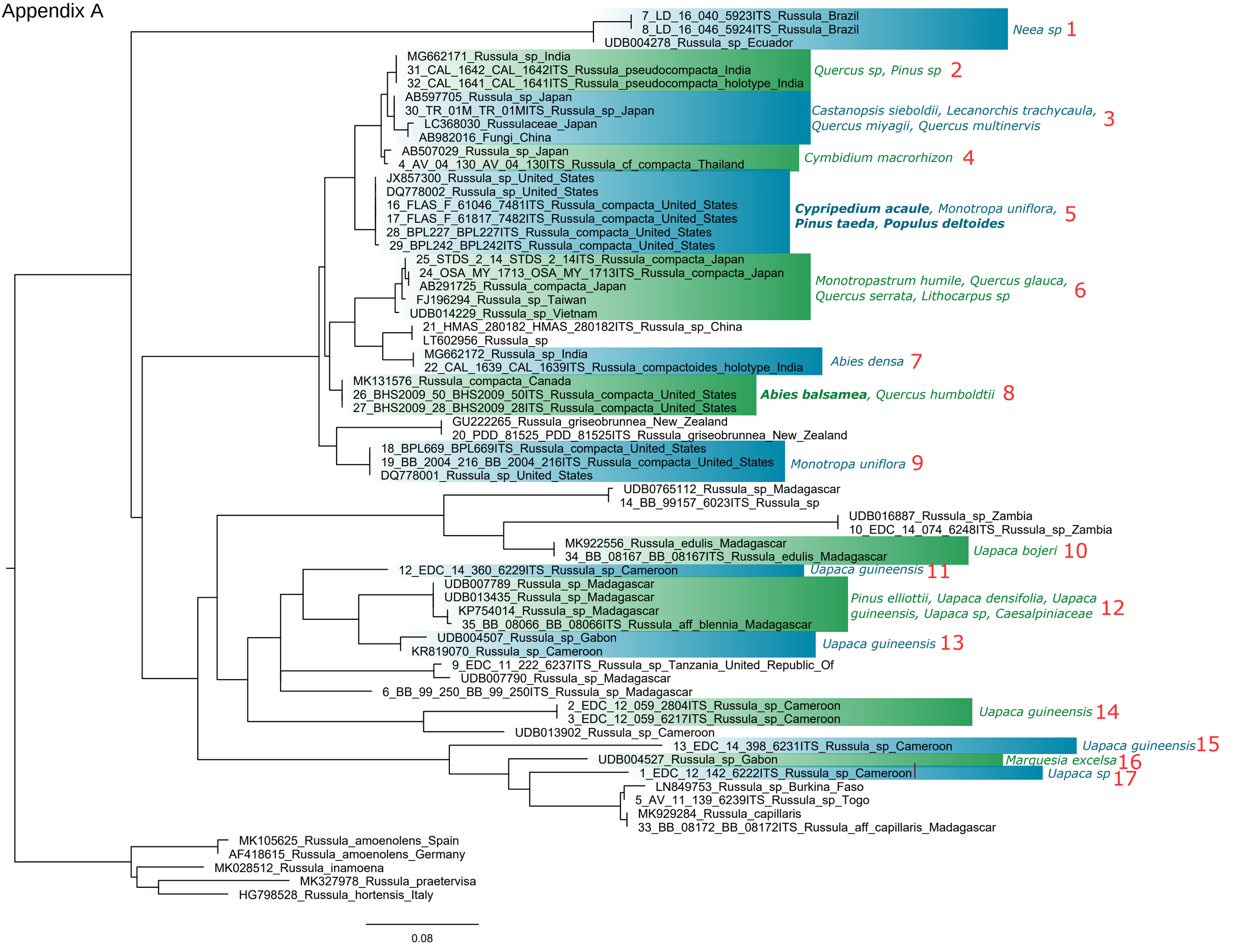
Appendix B: Maximum Likelihood tree of *Russula* subgenus *Archaea* based on ITS. ML bootstrap values are shown. Host species represented in colour per *Russula* species. In red are numbers of the species units.

Appendix C: Maximum Likelihood tree of *Russula* subgenus *Compactae* s.s. based on ITS. ML bootstrap values are shown. Host species represented in colour per *Russula* species. In red are numbers of the species units. Host species in bold were found for multiple specimens of the respective species. Species with symbol '*' were not mentioned in the databases, but are possible host species retrieved from De Lange et al. (2021).

Appendix D: Maximum Likelihood tree of *Russula* subgenus *Brevipedum* based on ITS. ML bootstrap values are shown. Host species represented in colours green and blue per Russula species. Species units are in red. Letters follow species units of appendix E, numbers are additional species units. Host species in bold were found for multiple specimens of the respective Russula species.

Appendix E: Maximum Likelihood tree of *Russula* subgenus *Brevipedum* based on concatenated ITS, LSU, RPB2 and EF1. In green and blue are the specified species used for the species delimitation with their respective letter code. ML bootstraps are shown. In red the are the species describe in 4.3.

Appendix A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8_LD_16_046_5924ITS_Russula_Brazil
UDB004278_Russula_sp_Ecuador
Neea sp 1
MG662171_Russula_sp_India
31_CAL_1642_CAL_1642ITS_Russula_pseudocompacta_India
32_CAL_1641_CAL_1641ITS_Russula_pseudocompacta_holotype_India
Quercus sp, Pinus sp 2
AB597705_Russula_sp_Japan
30_TR_01M_TR_01MITS_Russula_sp_Japan
LC368030_Russulaceae_Japan
AB982016_Fungi_China
Castanopsis sieboldii, Lecanorchis trachycaula, Quercus miyagii, Quercus multinervis 3
AB507029_Russula_sp_Japan
4_AV_04_130_AV_04_130ITS_Russula_cf_compacta_Thailand
Cymbidium macrorhizon 4
JX857300_Russula_sp_United_States
DQ778002_Russula_sp_United_States
16_FLAS_F_61046_7481ITS_Russula_compacta_United_States
17_FLAS_F_61817_7482ITS_Russula_compacta_United_States
28_BPL227_BPL227ITS_Russula_compacta_United_States
29_BPL242_BPL242ITS_Russula_compacta_United_States
Cypripedium acaule, Monotropa uniflora, Pinus taeda, Populus deltoides 5
25_STDS_2_14_STDS_2_14ITS_Russula_compacta_Japan
24_OSA_MY_1713_OSA_MY_1713ITS_Russula_compacta_Japan
AB291725_Russula_compacta_Japan
FJ196294_Russula_sp_Taiwan
UDB014229_Russula_sp_Vietnam
Monotropastrum humile, Quercus glauca, Quercus serrata, Lithocarpus sp 6
21_HMAS_280182_HMAS_280182ITS_Russula_sp_China
LT602956_Russula_sp
MG662172_Russula_sp_India
22_CAL_1639_CAL_1639ITS_Russula_compactoides_holotype_India
Abies densa 7
MK131576_Russula_compacta_Canada
26_BHS2009_50_BHS2009_50ITS_Russula_compacta_United_States
27_BHS2009_28_BHS2009_28ITS_Russula_compacta_United_States
Abies balsamea, Quercus humboldtii 8
GU222265_Russula_griseobrunnea_New_Zealand
20_PDD_81525_PDD_81525ITS_Russula_griseobrunnea_New_Zealand
18_BPL669_BPL669ITS_Russula_compacta_United_States
19_BB_2004_216_BB_2004_216ITS_Russula_compacta_United_States
DQ778001_Russula_sp_United_States
Monotropa uniflora 9
UDB0765112_Russula_sp_Madagascar
14_BB_99157_6023ITS_Russula_sp
UDB016887_Russula_sp_Zambia
10_EDC_14_074_6248ITS_Russula_sp_Zambia
MK922556_Russula_edulis_Madagascar
34_BB_08167_BB_08167ITS_Russula_edulis_Madagascar
Uapaca bojeri 10
12_EDC_14_360_6229ITS_Russula_sp_Cameroon
Uapaca guineensis 11
UDB007789_Russula_sp_Madagascar
UDB013435_Russula_sp_Madagascar
KP754014_Russula_sp_Madagascar
35_BB_08066_BB_08066ITS_Russula_aff_blennia_Madagascar
Pinus elliottii, Uapaca densifolia, Uapaca guineensis, Uapaca sp, Caesalpiniaceae 12
UDB004507_Russula_sp_Gabon
KR819070_Russula_sp_Cameroon
Uapaca guineensis 13
9_EDC_11_222_6237ITS_Russula_sp_Tanzania_United_Republic_Of
UDB007790_Russula_sp_Madagascar
6_BB_99_250_BB_99_250ITS_Russula_sp_Madagascar
2_EDC_12_059_2804ITS_Russula_sp_Cameroon
3_EDC_12_059_6217ITS_Russula_sp_Cameroon
Uapaca guineensis 14
UDB013902_Russula_sp_Cameroon
13_EDC_14_398_6231ITS_Russula_sp_Cameroon
Uapaca guineensis 15
UDB004527_Russula_sp_Gabon
Marquesia excelsa 16
1_EDC_12_142_6222ITS_Russula_sp_Cameroon
Uapaca sp 17
LN849753_Russula_sp_Burkina_Faso
5_AV_11_139_6239ITS_Russula_sp_Togo
MK929284_Russula_capillaris
33_BB_08172_BB_08172ITS_Russula_aff_capillaris_Madagascar
MK105625_Russula_amoenolens_Spain
AF418615_Russula_amoenolens_Germany
MK028512_Russula_inamoena
MK327978_Russula_praetervisa
HG798528_Russula_hortensis_Italy
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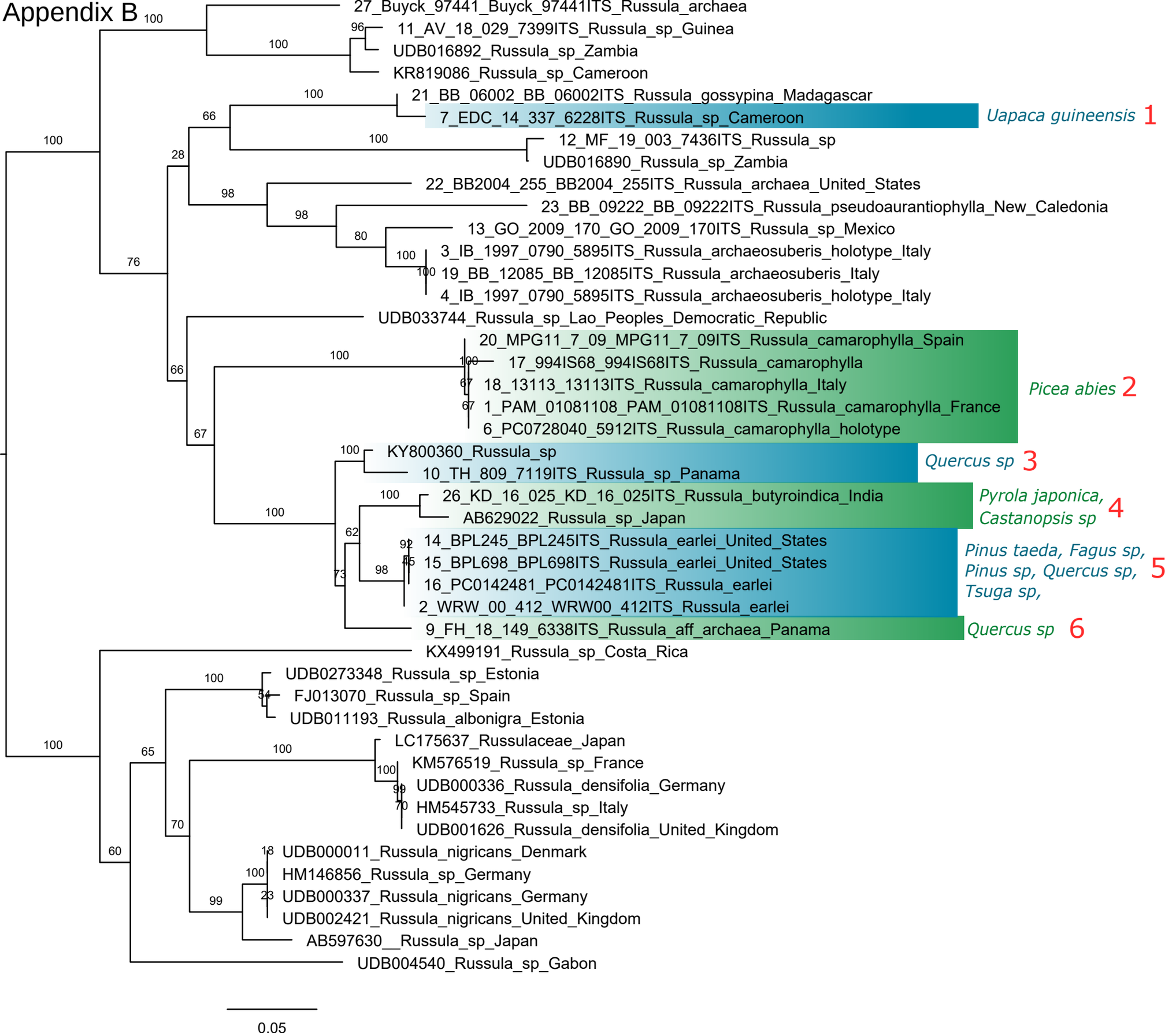
Appendix B
27_Buyck_97441_Buyck_97441ITS_Russula_archaea
11_AV_18_029_7399ITS_Russula_sp_Guinea
UDB016892_Russula_sp_Zambia
KR819086_Russula_sp_Cameroon
21_BB_06002_BB_06002ITS_Russula_gossypina_Madagascar
7_EDC_14_337_6228ITS_Russula_sp_Cameroon
Uapaca guineensis 1
12_MF_19_003_7436ITS_Russula_sp
UDB016890_Russula_sp_Zambia
22_BB2004_255_BB2004_255ITS_Russula_archaea_United_States
23_BB_09222_BB_09222ITS_Russula_pseudoaurantiophylla_New_Caledonia
13_GO_2009_170_GO_2009_170ITS_Russula_sp_Mexico
3_IB_1997_0790_5895ITS_Russula_archaeosuberis_holotype_Italy
19_BB_12085_BB_12085ITS_Russula_archaeosuberis_Italy
4_IB_1997_0790_5895ITS_Russula_archaeosuberis_holotype_Italy
UDB033744_Russula_sp_Lao_Peoples_Democratic_Republic
20_MPG11_7_09_MPG11_7_09ITS_Russula_camarophylla_Spain
17_994IS68_994IS68ITS_Russula_camarophylla
18_13113_13113ITS_Russula_camarophylla_Italy
1_PAM_01081108_PAM_01081108ITS_Russula_camarophylla_France
6_PC0728040_5912ITS_Russula_camarophylla_holotype
Picea abies 2
KY800360_Russula_sp
10_TH_809_7119ITS_Russula_sp_Panama
Quercus sp 3
26_KD_16_025_KD_16_025ITS_Russula_butyroindica_India
AB629022_Russula_sp_Japan
Pyrola japonica,
Castanopsis sp 4
14_BPL245_BPL245ITS_Russula_earlei_United_States
15_BPL698_BPL698ITS_Russula_earlei_United_States
16_PC0142481_PC0142481ITS_Russula_earlei
2_WRW_00_412_WRW00_412ITS_Russula_earlei
Pinus taeda, Fagus sp,
Pinus sp, Quercus sp,
Tsuga sp, 5
9_FH_18_149_6338ITS_Russula_aff_archaea_Panama
Quercus sp 6
KX499191_Russula_sp_Costa_Rica
UDB0273348_Russula_sp_Estonia
FJ013070_Russula_sp_Spain
UDB011193_Russula_albonigra_Estonia
LC175637_Russulaceae_Japan
KM576519_Russula_sp_France
UDB000336_Russula_densifolia_Germany
HM545733_Russula_sp_Italy
UDB001626_Russula_densifolia_United_Kingdom
UDB000011_Russula_nigricans_Denmark
HM146856_Russula_sp_Germany
UDB000337_Russula_nigricans_Germany
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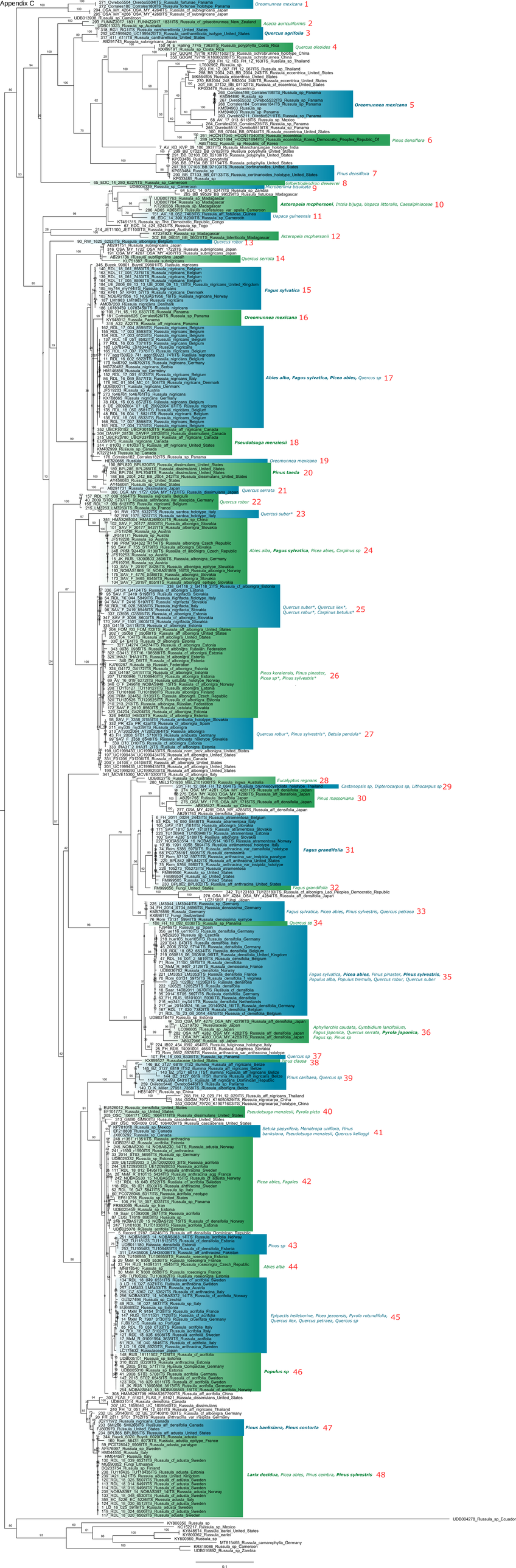
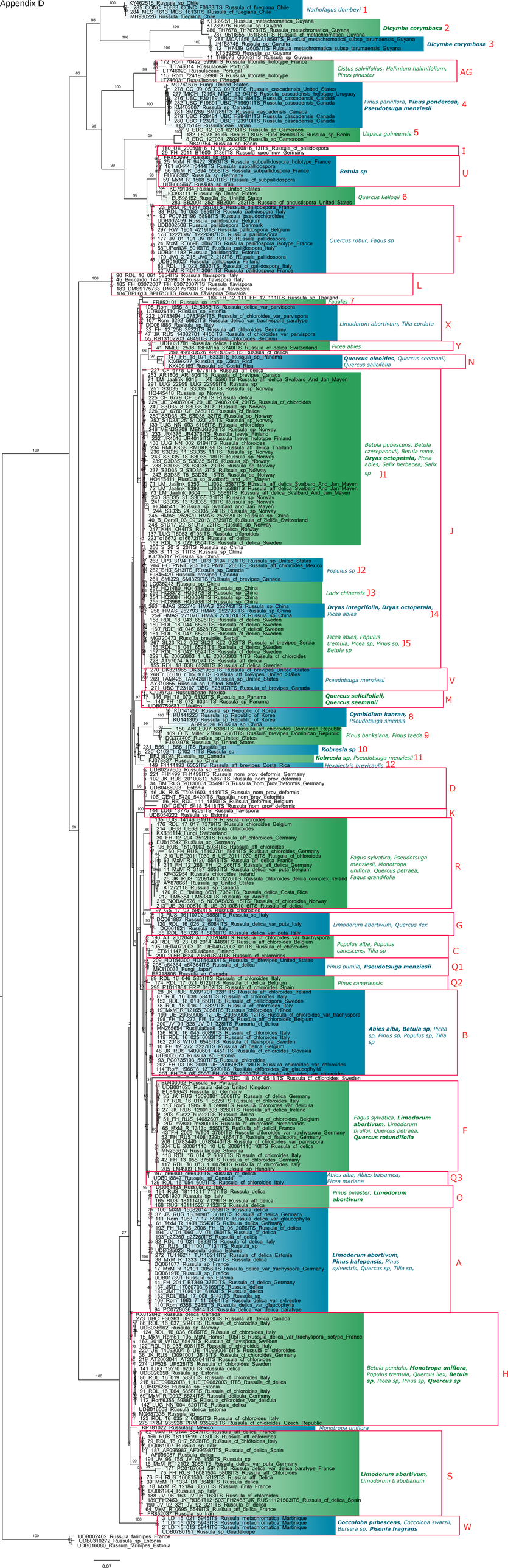
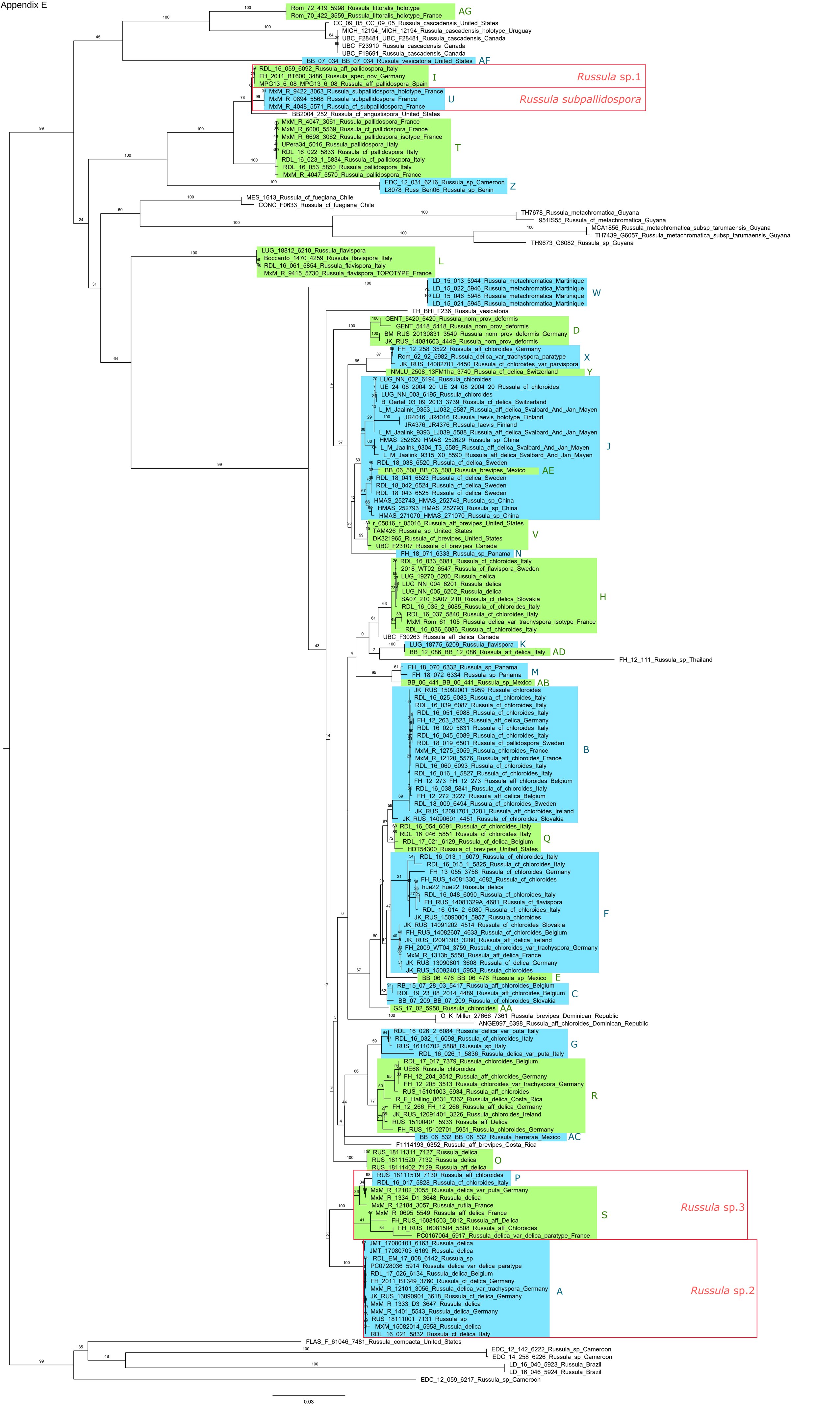

Appendix C
Oreomunnea mexicana 1
Acacia auriculiformis 2
Quercus agrifolia 3
Quercus oleoides 4
Oreomunnea mexicana 5
Pinus densiflora 6
Pinus densiflora 7
Gilbertiodendron dewevrei 8
Microberlinia bisulcata 9
Asteropeia mcphersonii, Intsia bijuga, Uapaca littoralis, Caesalpiniaceae 10
Uapaca guineensis 11
Asteropeia mcphersonii 12
Quercus robur 13
Quercus serrata 14
Fagus sylvatica 15
Oreomunnea mexicana 16
Abies alba, Fagus sylvatica, Picea abies, Quercus sp 17
Pseudotsuga menziesii 18
Oreomunnea mexicana 19
Pinus taeda 20
Quercus serrata 21
Quercus robur 22
Quercus suber* 23
Abies alba, Fagus sylvatica, Picea abies, Carpinus sp 24
Quercus suber*, Quercus ilex*, Quercus robur*, Carpinus betulus* 25
Pinus koraiensis, Pinus pinaster, Picea sp*, Pinus sylvestris* 26
Fagus grandifolia 31
Fagus grandifolia 32
Fagus sylvatica, Picea abies, Pinus sylvestris, Quercus petraea 33
Quercus sp 34
Eucalyptus regnans 28
Castanopsis sp, Dipterocarpus sp, Lithocarpus sp 29
Pinus massoniana 30
Fagus sylvatica, Picea abies, Pinus pinaster, Pinus sylvestris, Populus alba, Populus tremula, Quercus robur, Quercus suber 35
Aphyllorchis caudata, Cymbidium lancifolium, Fagus japonica, Quercus serrata, Pyrola japonica, Pinus sp 36
Quercus sp 37
Cinus clausa 38
Pinus caribaea, Quercus sp 39
Pseudotsuga menziesii, Pyrola picta 40
Betula papyrifera, Monotropa uniflora, Pinus banksiana, Pseudotsuga menziesii, Quercus kelloggi 41
Picea abies, Fagales 42
Pinus sp 43
Abies alba 44
Epipactis helleborine, Picea jezoensis, Pyrola rotundifolia, Quercus ilex, Quercus petraea, Quercus sp 45
Populus sp 46
Pinus banksiana, Pinus contorta 47
Larix decidua, Picea abies, Pinus cembra, Pinus sylvestris 48
UDB004278_Russula_sp_Ecuador
0.1


Appendix D
Nothofagus dombeyi 1
Dicymbe corymbosa 2
Dicymbe corymbosa 3
Cistus salviifolius, Halimium halimifolium, Pinus pinaster
AG
Pinus parviflora, Pinus ponderosa, 4
Pseudotsuga menziesii
Uapaca guineensis 5
I
U
Betula sp
Quercus kelloglii 6
Quercus robur, Fagus sp
T
Fagales 7
Limodorum abortivum, Tilia cordata
X
Picea abies
Y
Quercus oleoides, Quercus seemanii,
N
Quercus salicifolia
Betula pubescens, Betula
czerepanovii, Betula nana,
Dryas octopetala, Picea
abies, Salix herbacea, Salix
sp J1
J
Populus sp J2
Larix chinensis J3
Dryas integrifolia, Dryas octopetala,
Picea abies J4
Picea abies, Populus
tremula, Picea sp, Pinus sp, J5
Betula sp
Pseudotsuga menziesii
V
Quercus salicifoliaii,
Quercus seemanii
M
Cymbidium kanran, 8
Pseudotsuga sinensis
Pinus banksiana, Pinus taeda 9
Kobresia sp 10
Kobresia sp, Pseudotsuga menziesii 11
Hexalectris brevicaulis 12
D
K
Fagus sylvatica, Pseudotsuga
menziesii, Monotropa
uniflora, Quercus petraea,
Fagus grandifolia
R
Limodorum abortivum, Quercus ilex
G
Populus alba, Populus
canescens, Tilia sp
C
Pinus pumila, Pseudotsuga menziesii Q1
Pinus canariensis Q2
Abies alba, Betula sp, Picea
sp, Pinus sp, Populus sp, Tilia
sp
B
Fagus sylvatica, Limodorum
abortivum, Limodorum
brulloi, Quercus petraea,
Quercus rotundifolia
F
Abies alba, Abies balsamea,
Picea mariana Q3
Pinus pinaster, Limodorum
abortivum
O
Limodorum abortivum,
Pinus halepensis, Pinus
sylvestris, Quercus sp, Tilia sp,
A
Betula pendula, Monotropa uniflora,
Populus tremula, Quercus ilex, Betula
sp, Picea sp, Pinus sp, Quercus sp
H
Monotropa uniflora
Limodorum abortivum,
Limodorum trabutianum
S
Coccoloba pubescens, Coccoloba swarzii,
Bursera sp, Pisonia fragrans
W
0.07

Appendix E


Rom_72_419_5998_Russula_littoralis_holotype
Rom_70_422_3559_Russula_littoralis_holotype_France
AG
CC_09_05_CC_09_05_Russula_cascadensis_United_States
MICH_12194_MICH_12194_Russula_cascadensis_holotype_Uruguay
UBC_F28481_UBC_F28481_Russula_cascadensis_Canada
UBC_F23910_Russula_cascadensis_Canada
UBC_F19691_Russula_cascadensis_Canada
BB_07_034_BB_07_034_Russula_vesicatoria_United_States
AF
RDL_16_059_6092_Russula_aff_pallidospora_Italy
FH_2011_BT600_3486_Russula_spec_nov_Germany
MPG13_6_08_MPG13_6_08_Russula_aff_pallidospora_Spain
I
Russula sp.1
MxM_R_9422_3063_Russula_subpallidospora_holotype_France
MxM_R_0894_5568_Russula_subpallidospora_France
MxM_R_4048_5571_Russula_cf_subpallidospora_France
U
Russula subpallidospora
BB2004_252_Russula_cf_angustispora_United_States
MxM_R_4047_3061_Russula_pallidospora_France
MxM_R_6000_5569_Russula_cf_pallidospora_France
MxM_R_6698_3062_Russula_pallidospora_isotype_France
UPera34_5016_Russula_pallidospora_Italy
RDL_16_022_5833_Russula_cf_pallidospora_Italy
RDL_16_023_1_5834_Russula_cf_pallidospora_Italy
RDL_16_053_5850_Russula_pallidospora_Italy
MxM_R_4047_5570_Russula_pallidospora_France
T
EDC_12_031_6216_Russula_sp_Cameroon
L8078_Russ_Ben06_Russula_sp_Benin
Z
MES_1613_Russula_cf_fuegiana_Chile
CONC_F0633_Russula_cf_fuegiana_Chile
TH7678_Russula_metachromatica_Guyana
951IS55_Russula_cf_metachromatica_Guyana
MCA1856_Russula_metachromatica_subsp_tarumaensis_Guyana
TH7439_G6057_Russula_metachromatica_subsp_tarumaensis_Guyana
TH9673_G6082_Russula_sp_Guyana
LUG_18812_6210_Russula_flavispora
Boccardo_1470_4259_Russula_flavispora_Italy
RDL_16_061_5854_Russula_flavispora_Italy
MxM_R_9415_5730_Russula_flavispora_TOPOTYPE_France
L
LD_15_013_5944_Russula_metachromatica_Martinique
LD_15_022_5946_Russula_metachromatica_Martinique
LD_15_046_5948_Russula_metachromatica_Martinique
LD_15_021_5945_Russula_metachromatica_Martinique
W
FH_BHI_F236_Russula_vesicatoria
GENT_5420_5420_Russula_nom_prov_deformis
GENT_5418_5418_Russula_nom_prov_deformis
BM_RUS_20130831_3549_Russula_nom_prov_deformis_Germany
JK_RUS_14081603_4449_Russula_nom_prov_deformis
D
FH_12_258_3522_Russula_aff_chloroides_Germany
Rom_62_92_5982_Russula_delica_var_trachyspora_paratype
JK_RUS_14082701_4450_Russula_cf_chloroides_var_parvispora
X
NMLU_2508_13FM1ha_3740_Russula_cf_delica_Switzerland
Y
LUG_NN_002_6194_Russula_chloroides
UE_24_08_2004_20_UE_24_08_2004_20_Russula_cf_chloroides
LUG_NN_003_6195_Russula_chloroides
B_Oertel_03_09_2013_3739_Russula_cf_delica_Switzerland
L_M_Jaalink_9353_LJ032_5587_Russula_aff_delica_Svalbard_And_Jan_Mayen
JR4016_JR4016_Russula_laevis_holotype_Finland
JR4376_JR4376_Russula_laevis_Finland
L_M_Jaalink_9393_LJ039_5588_Russula_aff_delica_Svalbard_And_Jan_Mayen
HMAS_252629_HMAS_252629_Russula_sp_China
L_M_Jaalink_9304_T3_5589_Russula_aff_delica_Svalbard_And_Jan_Mayen
L_M_Jaalink_9315_X0_5590_Russula_aff_delica_Svalbard_And_Jan_Mayen
RDL_18_038_6520_Russula_cf_delica_Sweden
BB_06_508_BB_06_508_Russula_brevipes_Mexico
AE
RDL_18_041_6523_Russula_cf_delica_Sweden
RDL_18_042_6524_Russula_cf_delica_Sweden
RDL_18_043_6525_Russula_cf_delica_Sweden
HMAS_252743_HMAS_252743_Russula_sp_China
HMAS_252793_HMAS_252793_Russula_sp_China
HMAS_271070_HMAS_271070_Russula_sp_China
J
r_05016_r_05016_Russula_aff_brevipes_United_States
TAM426_Russula_sp_United_States
DK321965_Russula_cf_brevipes_United_States
UBC_F23107_Russula_cf_brevipes_Canada
V
FH_18_071_6333_Russula_sp_Panama
N
RDL_16_033_6081_Russula_cf_chloroides_Italy
2018_WT02_6547_Russula_cf_flavispora_Sweden
LUG_19270_6200_Russula_delica
LUG_NN_004_6201_Russula_delica
LUG_NN_005_6202_Russula_delica
SA07_210_SA07_210_Russula_cf_delica_Slovakia
RDL_16_035_2_6085_Russula_cf_chloroides_Italy
RDL_16_037_5840_Russula_cf_chloroides_Italy
MxM_Rom_61_105_Russula_delica_var_trachyspora_isotype_France
RDL_16_036_6086_Russula_cf_chloroides_Italy
H
UBC_F30263_Russula_aff_delica_Canada
LUG_18775_6209_Russula_flavispora
K
BB_12_086_BB_12_086_Russula_aff_delica_Italy
AD
FH_12_111_Russula_sp_Thailand
FH_18_070_6332_Russula_sp_Panama
FH_18_072_6334_Russula_sp_Panama
M
BB_06_441_BB_06_441_Russula_sp_Mexico
AB
JK_RUS_15092001_5959_Russula_chloroides
RDL_16_025_6083_Russula_cf_chloroides_Italy
RDL_16_039_6087_Russula_cf_chloroides_Italy
RDL_16_051_6088_Russula_cf_chloroides_Italy
FH_12_263_3523_Russula_aff_delica_Germany
RDL_16_020_5831_Russula_cf_chloroides_Italy
RDL_16_045_6089_Russula_cf_chloroides_Italy
RDL_18_019_6501_Russula_cf_pallidospora_Sweden
MxM_R_1275_3059_Russula_chloroides_France
MxM_R_12120_5576_Russula_aff_chloroides_France
RDL_16_060_6093_Russula_cf_chloroides_Italy
RDL_16_016_1_5827_Russula_cf_chloroides_Italy
FH_12_273_FH_12_273_Russula_aff_chloroides_Belgium
RDL_16_038_5841_Russula_cf_chloroides_Italy
FH_12_272_3227_Russula_aff_delica_Belgium
RDL_18_009_6494_Russula_cf_chloroides_Sweden
JK_RUS_12091701_3281_Russula_aff_chloroides_Ireland
JK_RUS_14090601_4451_Russula_cf_chloroides_Slovakia
B
RDL_16_054_6091_Russula_cf_chloroides_Italy
RDL_16_046_5851_Russula_cf_chloroides_Italy
RDL_17_021_6129_Russula_cf_delica_Belgium
HDT54300_Russula_cf_brevipes_United_States
Q
RDL_16_013_1_6079_Russula_cf_chloroides_Italy
RDL_16_015_1_5825_Russula_cf_chloroides_Italy
FH_13_055_3758_Russula_cf_chloroides_Germany
FH_RUS_14081330_4682_Russula_cf_chloroides
hue22_hue22_Russula_delica
RDL_16_048_6090_Russula_cf_chloroides_Italy
FH_RUS_14081329A_4681_Russula_cf_flavispora
RDL_16_014_2_6080_Russula_cf_chloroides_Italy
JK_RUS_15090801_5957_Russula_chloroides
JK_RUS_14091202_4514_Russula_cf_chloroides_Slovakia
FH_RUS_14082607_4633_Russula_cf_chloroides_Belgium
JK_RUS_12091303_3280_Russula_aff_delica_Ireland
FH_2009_WT04_3759_Russula_chloroides_var_trachyspora_Germany
MxM_R_1313b_5550_Russula_aff_delica_France
JK_RUS_13090801_3608_Russula_cf_delica_Germany
JK_RUS_15092401_5953_Russula_chloroides
F
BB_06_476_BB_06_476_Russula_sp_Mexico
E
RB_15_07_28_03_5417_Russula_aff_chloroides_Belgium
RDL_19_23_08_2014_4489_Russula_aff_chloroides_Belgium
BB_07_209_BB_07_209_Russula_cf_chloroides_Slovakia
C
GS_17_02_5950_Russula_chloroides
AA
O_K_Miller_27666_7361_Russula_brevipes_Dominican_Republic
ANGE997_6398_Russula_aff_chloroides_Dominican_Republic
RDL_16_026_2_6084_Russula_delica_var_puta_Italy
RDL_16_032_1_6098_Russula_cf_chloroides_Italy
RUS_16110702_5888_Russula_sp_Italy
RDL_16_026_1_5836_Russula_delica_var_puta_Italy
G
RDL_17_017_7379_Russula_chloroides_Belgium
UE68_Russula_chloroides
FH_12_204_3512_Russula_aff_chloroides_Germany
FH_12_205_3513_Russula_chloroides_var_trachyspora_Germany
RUS_15101003_5934_Russula_aff_chloroides
R_E_Halling_8631_7362_Russula_delica_Costa_Rica
FH_12_266_FH_12_266_Russula_aff_delica_Germany
JK_RUS_12091401_3226_Russula_chloroides_Ireland
RUS_15100401_5933_Russula_aff_Delica
FH_RUS_15102701_5951_Russula_chloroides_Germany
R
BB_06_532_BB_06_532_Russula_herrerae_Mexico
AC
F1114193_6352_Russula_aff_brevipes_Costa_Rica
RUS_18111311_7127_Russula_delica
RUS_18111520_7132_Russula_delica
RUS_18111402_7129_Russula_aff_delica
O
RUS_18111519_7130_Russula_aff_chloroides
RDL_16_017_5828_Russula_cf_chloroides_Italy
P
MxM_R_12102_3055_Russula_delica_var_puta_Germany
MxM_R_1334_D1_3648_Russula_delica
MxM_R_12184_3057_Russula_rutila_France
MxM_R_0695_5549_Russula_aff_delica_France
FH_RUS_16081503_5812_Russula_aff_Delica
FH_RUS_16081504_5808_Russula_aff_Chloroides
PC0167064_5917_Russula_delica_var_delica_paratype_France
S
Russula sp.3
JMT_17080101_6163_Russula_delica
JMT_17080703_6169_Russula_delica
RDL_EM_17_008_6142_Russula_sp
PC0728036_5914_Russula_delica_var_delica_paratype
RDL_17_026_6134_Russula_delica_Belgium
FH_2011_BT349_3760_Russula_cf_delica_Germany
MxM_R_12101_3056_Russula_delica_var_trachyspora_Germany
JK_RUS_13090901_3618_Russula_cf_delica_Germany
MxM_R_1333_D3_3647_Russula_delica
MxM_R_1401_5543_Russula_delica_Germany
RUS_18111001_7131_Russula_sp
MXM_15082014_5958_Russula_delica
RDL_16_021_5832_Russula_cf_delica_Italy
A
Russula sp.2
FLAS_F_61046_7481_Russula_compacta_United_States
EDC_12_142_6222_Russula_sp_Cameroon
EDC_14_258_6226_Russula_sp_Cameroon
LD_16_040_5923_Russula_Brazil
LD_16_046_5924_Russula_Brazil
EDC_12_059_6217_Russula_sp_Cameroon
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