Biology Department
Research Group Mycology

A BIG WHITE MESS: DELIMITING SPECIES WITHIN *RUSSULA* SUBGENUS *BREVIPEDUM*

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Contents

1. Corona preambule

Under normal circumstances spore drawings are done using a Zeiss Axioscop 2 microscope and camera lucida with an enhancement of 6000x. And there would have been the possibility of having SEM (Scanning electronic microscopy) photos taken of the spores.
Another 3 extra markers would have been used for the fruitbody samples, namely LSU, rpb2 and Tef1-α.

2. Introduction

2.1. Taxonomy

Taxonomy is the science dealing with delimitation, naming and describing of species, and categorize them. In these days of modern taxonomy and advancing insight it seems that the nomenclatural changes are never ending. The introduction of modern molecular techniques made it possible to untangle and clarify a lot of relationships between taxa. Fungi were traditionally grouped based upon macroscopic (shape of the fruiting body, hymenophore type, spore print colour etc.) and microscopic features (spore, basidia and cystidia size, chemical reactions etc.). The Morphological Species Concept was used, species were diagnosed and grouped by morphological characters. Nowadays the Biological and Phylogenetic Species Concept are mainly used and DNA sequences are used to make phylogenies (Taylor *et al.*, 2000). Since 2010 the use of molecular techniques has significantly increased the number of new species described (Hawksworth & Lücking, 2017). New fungal species are mostly found through inventory in poorly studied areas or habitats or through environmental sequencing. Another source of unknown fungal species is discovered when known taxa are revised using molecular techniques (Hawksworth & Lücking, 2017). By using multiple molecular markers, we are now able to make more correct phylogenies and find hidden correlations or untangle species complexes. Nowadays even whole genomes can be sequenced, the future of taxonomy probably lies in using these full genome sequences to make phylogenies (Wu *et al.*, 2019).

Environmental sequencing is a technique which allows to detect multiple organisms in environmental samples, like soil and water samples. This technique can detect trace amounts of DNA of the whole community present in the sample. For (ecto)mycorrhizal fungi this technique can give us insights in the diversity present in the soil in contrast with the above ground fungal diversity which is visible through the presence of fruitbodies. As well as provide us with information about the mycorrhizal fungi-host connection by analysing DNA found in root tips covered in mycorrhiza.

The family of the Russulaceae (Taylor & Bruns 1999) was early differentiated from the other Agaricomycetes, mainly because sphaerocytes in their trama make their fruitbodies structure brittle unlike other fungi. *Russula* is the largest ectomycorrhizal genus within the Russulaceae (over 4500 species estimated), with over 3000 estimated species (He *et al.*, 2019). *Russula* differentiates from the other ectomycorrhizal Russulaceae by its species that never produce latex. Some *Multifurca* Buyck & V. Hofst. species produce latex, this genus contains species that were previously placed within either *Lactarius* or *Russula*. *Lactifluus* (Pers.) Roussel and *Lactarius* Pers. species always produce latex.

2.2. Russula

Russula is a genus of ectomycorrhizal (ECM) fungi with a cosmopolitan distribution given that their host plants occur (Buyck *et al*., 2018). In many ecosystems worldwide it is one of the dominant ECM genera. There is a high diversity in macroscopic, microscopic and even chemical features that can be found within the *Russula* fruitbodies (e.g. Sarnari 1998; Singer 1986; Romagnesi 1967; Bon 1988). Despite the extensive research that has been done within this genus there is still an unknown diversity. There are several reasons contributing to this unknown diversity. One being unequal sampling, most mycologist focussing on *Russula* are based within Europe and this has

caused an undersampling and restricted knowledge of tropical species and species in North America (Buyck & Adamcik, 2013; Buyck *et al.*, 2018). Another reason is the existence of species complexes, cryptic and pseudo-cryptic species. Species complexes are often composed by species with high resemblance despite showing genomic differences. The high resemblance makes it impossible to differentiate these species in the field, and even microscopically. Cryptic species only showcase molecular differences. Pseudo-cryptic species have high resemblance however there are microscopic differences that can be found. Furthermore some *Russula* species have host specificity on top of having a habitat preference and vegetation successional stage (Bigg, 2000; Geml *et al.*, 2010). The knowledge about host connections is very limited, these connections are made underground and the occurring fruitbodies can be situated quite a distance from their host plant.

In this work, we will mainly focus on unravelling species complexes of pseudo-cryptic species and identification of the host tree connection. All selected species belong to *Russula* subg. *Brevipedum* (Buyck & V. Hofst., 2015). *R.* subg. *Brevipedum* was described in 2015 as *R*. subg. *Brevipes* after the American type species *R.* brevipes, but this was an invalid name and is changed into *Brevipedum* in 2020 (Buyck *et al.*, 2020). The species within *R.* subg. *Brevipedum* were formerly classified within *R.* subg. *Compactae (Fr.) Bon*. A short overview of how the taxonomy of the groups of interest for this work have evolved over the years is given here.

As mentioned before, there have been several changes within the Russulaceae, likewise within *Russula* and its subgenus *Compactae* (Fr.) Bon. This subgenus is a basal group within *Russula* and is mainly characterized by the presence of lamellulae, firm, compact and large fruitbodies which lack the colour diversity that is so typical for the genus, instead they mainly have black and white pigments.

Fries grouped species in R. subg. *Compactae* as primitive species closely related to the "*Lactaria",* the milkcaps. *R*. subg. *Compactae* is a basal group within *Russula*, the species within this group portray ancestral characteristics like pale spores and pale or brownish cap colour. Subsequent the split between *Russula* and the Lactaria (*Lactarius*, *Lactifluus* and *Multifurca*)*, R*. subg*. Compactae* is assumed to be the second group to diversify within *Russula* after *R*. sect*. Heterophyllae* (Looney *et al.*, 2016)*.* R. subg. *Compactae* is characterised by the abundant presence of lamellulae, the lamellae are white, cream or yellow. The fruitbody is fleshy, firm at least in the juvenile phase, whitish in the beginning, later stained with ochre, brown, blackish colours. The cap has an acute margin, is rigid and never furrowed, little differentiated and has a smooth surface. The spores barely have an amyloid spot. The basidia are remarkably narrow. The epicutis often has poorly characterized dermatocystidia, which are little to not septate. The epicutis has late-setting brown vacuolar pigment, particularly striking in black-discolouring forms. There is never a veil.

In the classification of Romagnesi (1967, amendment 1985, 1987) *R. subg. Compactae* was then divided in the sections *Nigricantinae* (Bataille), *Plorantinae* (Bataille) and *Archaeinae* (Heim ex Bataille). *R*. sect. *Nigricantinae* is characterized by reddening and/or blackening of the flesh of the fruitbody when damaged or by old age. R. sect. *Plorantinae* is characterized by white flesh, that slowly (multiple hours) discolours brown but not red or black, and some have a green or blue coloration of the lamellae or at the top of the stipe.

Later, in the classification of Bon (1988), *R.* subg. *Compactae* was seen as a subgenus with sections *Compactae*, the *Plorantes* Bataille & Singer and the section *Archaeinae* Heim. *Russula* sect. *Plorantes* was divided in the subsections *Delicinae* Bataille and *Pallidosporinae* Bon. In fact, no new groups were created, but they were renamed. *Russula* sect. *Compactae* is the former section *Nigricantinae*, and *R*. sect. *Plorantes* is the former section *Plorantinae.*

Mauro Sarnari's classification used in 'Monografia illustrate del Genere *Russula* in Europa' (1998) divides the subgenus *Compactae* in 3 sections; *Compactae* Fries, *Archaeinae* Heim ex Buyck & Sarnari and *Lactarioides* Bataille, Konrad & Josserand. Another name change has occurred here, this time *R.* sect. *Plorantes* is renamed as *R.* sect. *Lactarioides*.

Recent DNA analysis with multiple markers has changed the phylogeny of *Russula* overall and *R.* subg. *Compactae* (Fr.) Bon in specific (Miller & Buyck, 2002; Looney & Matheny, 2016; Buyck *et al.*, 2018). The species of the former *R.* subg. *Compactae* (Fr.) Bon are now divided over 5 subgenera; *R.* subg. *Glutinosae* Buyck & X.H. Wang (Buyck *et al.*, 2020), *R.* subg. *Archaeae* Buyck & V. Hofst. (Hongsanan *et al.*, 2015)*,R.* subg. *Compactae*[1] (Fr.) Bon, emend. Buyck & V. Hofst. (Hongsanan *et al.*, 2015)*,R.* subg. *Malodorae* Buyck & V. Hofst. (Hongsanan *et al.*, 2015) and *R.* subg. *Brevipedum* Buyck & V. Hofst. (Hongsanan *et al.*, 2015; Buyck *et al.*, 2020). *R.* subg. *Brevipedum* contains the species that were previously placed *within R*. sect. *Lactarioideae* and this is the group of interest off this research.

2.3. *Russula* subgenus *Brevipedum* and species delineation

The species within *Russula* subgenus *Brevipedum* have a white cap, that can have yellow to red-brown stains. This is unlike most *Russula*, this genus is famous for the broad variety of cap colours. The flesh of these fungi is white but turns slowly yellow to rusty brown when exposed to the air. The spore print is white to yellow, the lamellae or the top of the stipe can have a blue or green hue. At the moment only 7 species within the subgenus *Brevipedum* are described in Europe: *R. chloroides* Krombholz*, R. delica* Fries*, R. flavispora* (Blum in Romagn.) Romagn.*, R. pallidospora* (Blum in Romagn.) Romagn.*, R. littoralis, R. pseudodelica* Lange (nec J. Schaef.) sec. Blum and *R. laevis* Kälviäinen, Ruotsalainen & Taipale (Adamčík *et al.*, 2019). Within these 7 species, varieties are known in the species *R. chloroides* and the species *R. delica* and these varieties can differ between the different authors. However, when phylogenetic trees based on ITS (internal transcribed spacer) sequences are made, there seem to be at least 31 species within *R.* subg. *Brevipedum* (figure 1). This finding implicates that a lot of research is still needed within this subgenus to sort out the species complexes and describe the new species. To be able to differentiate these species without using DNA, macroscopic or microscopic differences need to be found and described.

[1] From this point onwards if *R.* subg. *Compactae* is mentioned it is referring to this group.

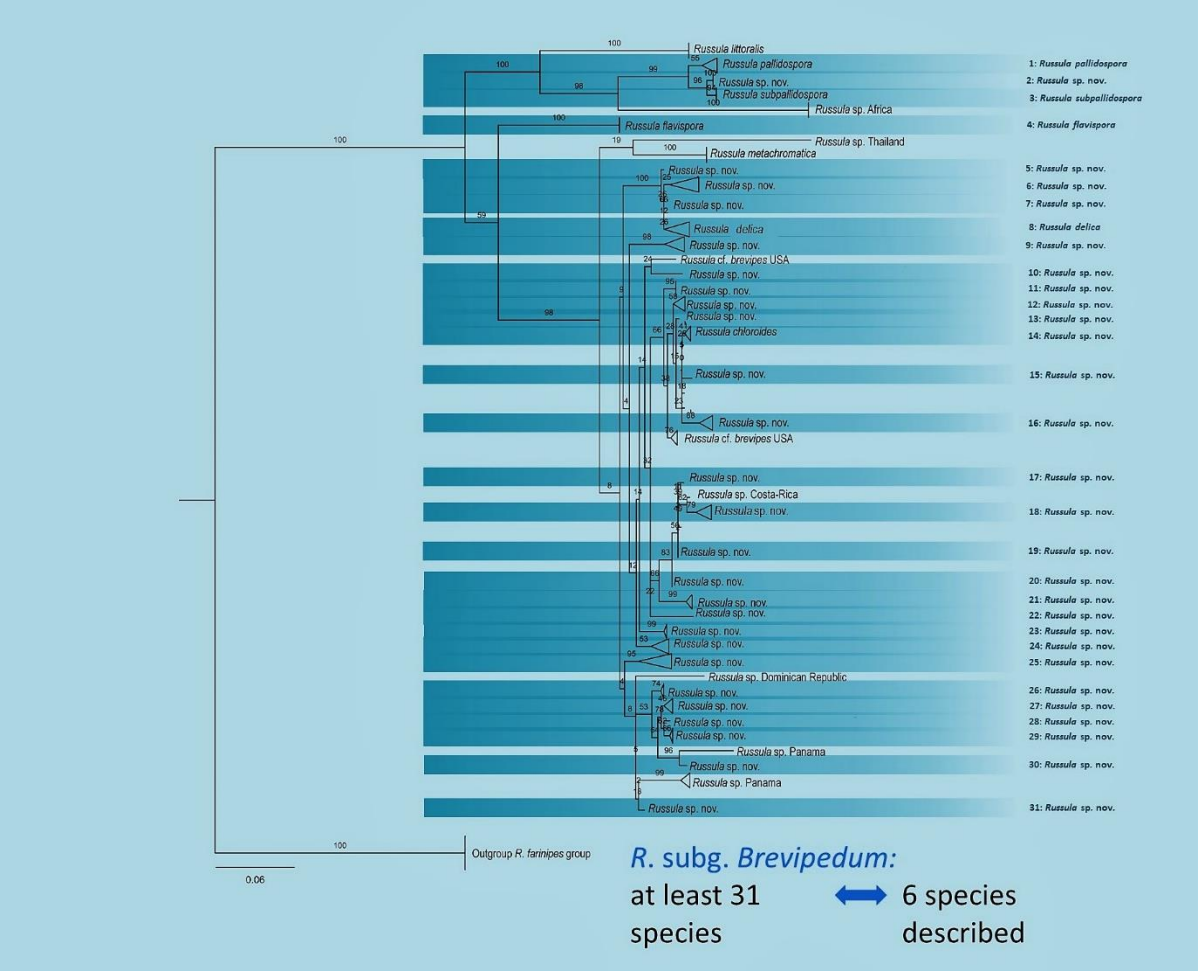


Figure 1. Phylogenetic tree of European species within *Russula* subgenus *Brevipedum* by Ruben De Lange (unpublished)

In North and South America 11 species are known within *R.* subg. *Brevipedum*.(Singer, 1952, 1963; Shaffer, 1964; Buyck & Ovrebo, 2002; Kong, Montoya, & Estrada-Torres, 2002; Buyck & Adamčík, 2013). The four species described into detail by Buyck and Adamcik (2013) are *R. brevipes* Peck, Ann. Rep. N.Y. St. Mus. Nat. Hist. 43: 20. 1890*, R. inopina* Shaffer (Shaffer, 1964)*, R. romagnesiana* Shaffer (Shaffer, 1964) and *R. vesicatoria* Burl. (Burlingham, 1944). The other species are *R. littoralis, R. fuegiana* Singer, NA Sing., Rev. Mycol. Paris 15:125. 1950 *, R. cascadensis* Shaffer (Shaffer, 1964)*, R. delicula* Romag., Bull. Soc. Mycol. Fr. 61: 30 1946*, R. idroboi* Singer (Singer, 1963)*, R. austrodelica, R. herrerae* Kong, Montoya et Estrada (Kong *et al.*, 2002) and *R. aucarum* Singer (Singer, 1975)*. R. herrerae* is characterised by the presence of a marginal veil and this character differentiates this species from all other Brevipedum species. *R. aucarum* is a species of the section *Delicoarchaeae* found in Panama. The distinction between *R.* sect. *Delicoarchaeae* and the former *R.* sect. *Lactariodeae* are unclear, some suggest *R.* sect. *Delicoarchaeae* is a synomym of *R.* sect. *Lactariodeae* (Buyck & Ovrebo, 2002)*.*

In his monograph 'Les Russules d'Europe et d'Afrique du Nord', Romagnesi (1967) already mentioned that *R.* section *Plorantinae* (now R. subg. *Brevipedum*) is a tricky group, since the

characteristics which are used to distinguish and differentiate species in other *Russula* groups have little to no use in this group. The descriptions of *R. delica* even differs between Fries, Singer and Kühner & Romagnesi (Shaffer, 1964).

2.4. Species descriptions

Most of the descriptions of *Russulales* species are not complete, most have information about spores, basidia and cystidia sizes and ornamentation, but not of density of these structures and differences between the pileipellis margin and centre or between lamellae sides and edges (Adamčík *et al.*, 2019). Information about the mycorrhizal structures, their accompanied host plant is missing in most descriptions. Besides being incomplete, the descriptions are not consistent between different continents, and are often author specific. The combination of these factors makes comparing descriptions difficult to even impossible. With their paper, Adamčík et al. (2019) are now encouraging others to make a consistent description of *Russula* species. They created a standard template, with a manual and examples, which is universally applicable.

The below-ground features of fungi, the mycorrhizae, are often not, or in restricted amounts, examined. There is still a lot to discover about ectomycorrhizae and about the correlation between above and below-ground parts of the fungi (Buyck *et al.*, 2018). *Russula* species have contact exploration type ectomycorrhizae, this type of ectomycorrhizae have a smooth mantle with a small number of emanating hyphae (Agerer, 2001). *Russula* subg. Brevipedum has ectomycorrhizae whose cystidia have exclusively russuloid forms (Agerer, 2006).

The key to unravel species complexes of pseudo-cryptic species could lay in more detailed description of macroscopic and microscopic characteristics, including those of the mycorrhizae. Differences between lamellae sides and edges and between pileus margin and centre, are often not described while this could potentially be a discriminating factor. Cystidal density is another characteristic that is absent in descriptions found in Romagnesi (1967) and Sarnari (1998), while these are still the principal works for the European species.

3. Objectives

A first objective is to make a complete description of possible new taxa within the *Russula* subgenus *Brevipedum*. Those taxa are suggested by the molecular phylogeny based on ITS markers and were chosen based upon availability of specimens. These taxa were initially recognised to be other species within this subgenus, but molecular analysis shows they are different species (De Lange et al., unpublished).

The second objective is to compare these descriptions carefully to find microscopic differences to delimitate species within species complexes of pseudo-cryptic species. This master thesis frames in the research project done by Ruben De Lange on the former *R.* subg. *Compactae* (Fr.) Bon which is now known not to be a monophyletic group. The aim of his project is to delimit species within the subgenera *Archaeae*, *Compactae, Malodorae*, *Glutinosae* and *Brevipedum* based on morphological, molecular and ecological characters.

4. Materials and Methods

4.1. Specimens

The specimens were collected fresh by Ruben De Lange (*R. macrostigma*, 2 and 3), Jesko Kleine (*R. macrostigma*), Ronny Boeykens (*Russula boeykensii*), Felix Hampe and Cathrin Manz (*Russula hampei*). Three species were collected within Europe and the other species was collected in Panama, Central America, by Felix Hampe and Cathrin Manz. These collections were dried and stored in the Herbarium Universitatis Gandavensis (GENT). A small fragment of each specimen was deposited in a strong detergent, 2*CTAB buffer (2% cetyltrimethylammonium bromide). For all four species, all available specimens at the GENT Herbarium were microscopically examined.
At the finding location of each specimen (only *R. macrostigma* & 2) roots were collected by carefully removing the upper soil layers and uncovering plant roots. These plant roots are then collected and preserved in aluminium foil together with some surrounding soil, to prevent desiccation. Later these were soaked in water and studied under a binocular microscope with small magnification to determine whether ectomycorrhiza was present on these root tips. The root tips without ectomycorrhiza were discarded. For each collection of root tips, a sample is preserved in 70% ethanol and another sample is preserved in 2*CTAB buffer.

4.2. Morphology

4.2.1. Macroscopy

While collecting, short macromorphological descriptions were made and photographs were taken of the specimens by the collectors. The colour codes used are from the Methuen book of colours (Kornerup & Wanscher, 1978). Spore deposits were available for the specimens collected by Ronny Boeykens (*Russula boeykensii*).

4.2.2. Microscopy

Microscopy was done on dried specimens. Spores were observed, measured and photographed in Melzer's reagent, elements of the hymenium and pileipellis were observed and measured in Congo-Red. The hymenial elements were observed and measured both at the lamellae edge and the lamellae sides. Hyphal terminations and pileocystidia examined and measured near the pileus margin and the pileus centre. Spore measurements were done using a Zeiss Axioscop 2 microscope and pictures were taken with a Nikon Eclipse Ni-U microscope at 1000x magnification and a Bresser MikroCamII Full HD HSP camera. Bresser MikroCamLabII software was used to make stacking images, these images were used to create spore drawings. Basidia measurements are without the sterigmata. Drawings of the pileipellis and hymenial elements were made with an Olympus CX21 microscope with a drawing tube at 1000x magnification. Chemical reactions in Cresyl Blue (Buyk, 1989), carbolfuchsin (Romagnesi, 1967) and sulfovanillin (Caboň *et al.*, 2017) were examined to respectively observe the presence of metachromatic incrustations in the pileipellis, incrustations on primordial hyphae and colouring of cystidia contents.
Per described species there were at least 2 specimens (maximum 4). Statistics for all microscopic characteristics, except for spores, were based on average on 10 measurements per specimens. Per specimen 20 spores were measured in side view excluding ornamentation. Measurements are given as (minimum –) average minus standard deviation (SD) – average – average plus SD (– maximum). Q indicates the length/width ratio of the spores. The spore ornamentation density is

computed following (Adamcik & Marhold, 2000).The density of hymenial cystidia is computed following (Buyck, 1991).

4.3. Molecular work

This part was performed by Ruben De Lange.

Little fragments of fresh material of the fruitbodies and root tips were preserved in small tubes with CTAB (Cetyl trimethyl-ammonium bromide). Afterwards DNA was extracted from these samples using the CTAB extraction method described in Nuytinck and Verbeken (2003). For collections of which no fresh fragments were preserved in CTAB, a modified CTAB protocol (Tel-Zur et al. 1999; mod. by Agentschap Plantentuin Meise) was used.

4.3.1. Fruitbodies

The marker that was amplified for the fruitbody samples is the internal transcribed spacer region of ribosomal DNA (ITS), specifically the ITS1 and ITS2 spacer regions and the ribosomal gene 5.8S, using primers ITS-1F and ITS4 (White *et al.*, 1990; Gardes & Bruns, 1993) and protocols for PCR amplification follow Le et al. (2007). An automated ABI 3730 XL capillary sequencer at Macrogen was used to sequence the PCR products. Assembly of the forward and reverse sequences into contigs and where needed edited with BioloMICS (BioAware SA NV).

4.3.2. Root tips

The internal transcribed spacer region of ribosomal DNA (ITS) was amplified, more specifically ITS1, both for plant and fungal DNA, and ITS2 spacer, solely for fungal DNA, regions. The forward primers ITS1-F and fITS7 and reverse primers ITS2 and ITS4 were used respectively for the fungal ITS1 and ITS2 markers (White *et al.*, 1990; Gardes & Bruns, 1993; Tedersoo *et al.*, 2013). The forward primer ITS-p5 and reverse primer ITS-u2 were used for the plant ITS1 marker (Cheng *et al.*, 2016). Amplification was done using a two-step PCR process. In the first step of PCR, the above mentioned primers prolonged with Nextera™ tails (Illumina) were used with the setting following the description of (Le *et al.*, 2007). Subsequent a DNA quantity and quality check, the PCR product was polished with the NucleoMag NGS Clean-up and Size Select kit (Machery-Nagel). In the second PCR step, a Nextera™ XT label (Illumina) was added to the amplicon under the following quantities: 3 µL of template DNA, 1 µL of each primer (10 pmol/µL), and 15 µL of Master Mix for a final volume of 20 µL. Amplification conditions were: 95 °C for 10 min, 8 cycles of 30 s at 95 °C, 60 s at 55 °C and 30 s at 72 °C, followed by 7 min at 72 °C. Subsequent quantification and clean-up, the sample was sent to BaseClear (Leiden, the Netherlands) for paired-end sequencing using the Illumina MiSeq technology (2 × 300 bp) amongst a batch of other amplicons with different Nextera™ labels.

4.3.3. Dataprocessing

Performed by me and Ruben De Lange

The Naturalis Galaxy v.19.01 instance was used to process the Illumina sequence reads. The reads for each specific specimen were isolated by demultiplexing the reads based upon their unique tags. Merging of the R1 and R2 reads from the paired-end sequencing was done using FLASH (Magoc

& SL, 2011) with the minimum overlap size set at 100 bp. We discarded the reads shorter than 250 bp or with more than 8 consecutive N's or a Phred score lower than 28 and trimmed the primers utilizing Cutadapt (Martin, 2011). The sequences were dereplicated subsequent quality control with PRINSEQ (Schmieder & Edwards, 2011). The dereplicated sequences were arranged by size and clustered in zero-radius OTU's with the UNOISE algorithm (Edgar & Flyvbjerg, 2015; Edgar, 2016) to denoise the amplicon reads. The VSEARCH UCHIME algorithm (Edgar *et al.*, 2011)was used to discard the chimera sequences. A BLASTN search (Altschul *et al.*, 1997) against the UNITE and GenBank databases was used to create an OTU abundance and taxonomic assignment table. The online MAFFT v7 program (Katoh & Toh, 2008) was used to align the sequences, using the E-INS-I strategy. Trimming of the trailing ends and manual edits of the alignment where necessary were done using Mega 6 (Tamura *et al.*, 2013). The ITS alignment was divided into partial 18S, ITS1, 5.8S , ITS2 and partial 28S. RAxML v8.0.24 (Stamatakis & A, 2014) was used to perform maximum likelihood (ML) analyses. These were then combined with the Rapid Bootstrapping algorithm with 1000 replicates under the GTRCAT option (Stamatakis, Hoover, & Rougemont, 2008).
All analyses were conducted on the CIPRES Science Gateway (Miller, Pfeiffer, & Schwartz, 2010). The fungal and plant DNA from the root tip samples is combined to find the most probable mycorrhizal fungi-host connection based on the OTU-abundance table. Only the samples which contain compactoid *Russula* are used.

5. Results

5.1. Species description

5.1.1. *Russula macrostigma*

Russula macrostigma Beel & De Lange **nom. prov.**
Holotype: EUROPE**,** Italy**,** Tuscany, 8 Nov 2016, R. De Lange (RDL 16-032/1)
Etymology: 'macrostigma' refers to the large and very amyloid suprahilar spot.

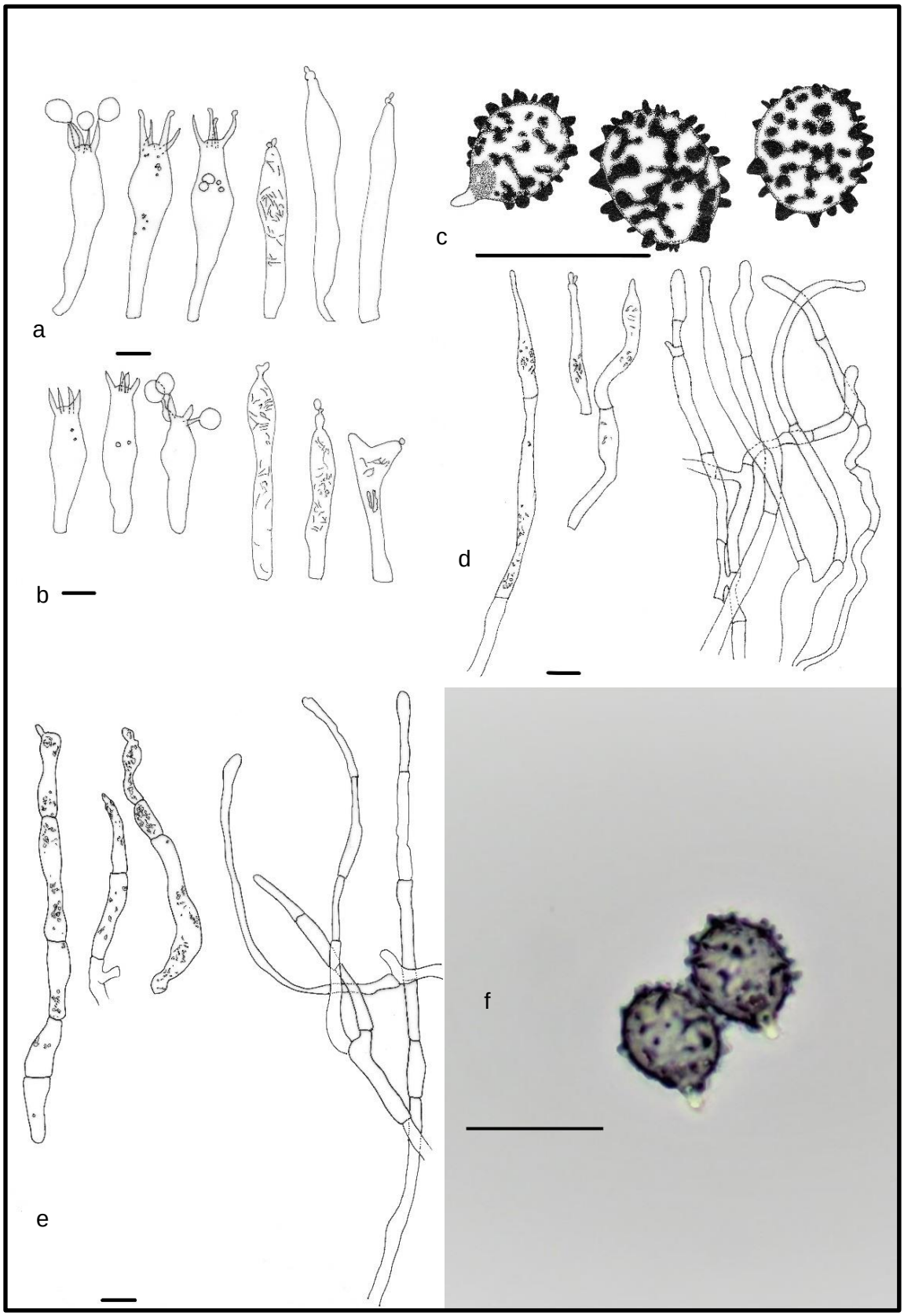
Pileus medium to large sized,77–127 mm diam., when mature infundibuliform with a deep depression in the centre; margin smooth, not striate, cuticle smooth, matt, usually retaining some debris; white/yellow-white (3A2, 4A2) to light yellow (4A3, 4A4, 4A5), brownish orange (5C6) with spots of light brown (6D8), orange brown (5C6) and dark yellow (4A8). **Lamellae** up to 3 mm deep, dense (6–9L + 3–5l/cm), with decurrent tooth, white to yellow-white (3A2, 4A2); lamellulae general, of different lengths, furcation's absent or rare. **Stipe** 25–26 x 25–29 mm, cylindrical or narrowly clavate, white to yellow-white (3A2, 4A2) at the top and darker at the base, brownish orange (5C6); medulla solid. **Context** firm in all parts of the basidiomata, white-pale cream, on damaged places turning brownish orange; taste mild, afterwards slightly acrid; odour fruity. **Spore print** white to pale cream (Ib–IIa). FeSO4 brown-orange. Guaiac stipe, lamellae and context all strong and fast reaction. KOH stipe and lamellae yellowish, context yellow to slightly reddening.

Spores (7.1–)8.3–*9.1*–9.8(–10.8) × (5.3–)6.7–*7.2*–7.8(–8.4) µm, broadly ellipsoid to ellipsoid, Q = (1.15–)1.19–*1.25*–1.31(–1.43), n= 60; ornamentation small sized, moderately distant to dense [3–8 in a 3 µm diam. circle] amyloid warts, 0.2–0.5 µm high, locally subreticulate, sometimes fused in chains (3–5 fusions in a 3 µm diam. circle), connected with occasional line connections [0–3(–7) line connections in a 3 µm diam. circle] suprahilar spot large, amyloid. **Basidia** (38–) 45.2–*54.3*–63.4 (–80) × (5–) 8.9–*11.4*–13.9 (–19) µm, narrowly clavate to clavate, 4–spored; basidiola first cylindrical, then clavate, ca. 4–12 µm wide. **Hymenial cystidia** numerous to abundant, ca. 38000/mm², (43–) 56.5–*72.4*–88.3 (–110) × (4–) 6.1–*7.7*–9.3 (–11) µm, narrowly cylindrical to narrowly clavate or narrowly fusiform, often slightly moniliform and flexuous, apically acute or obtuse, with or without appendage 2–6.63(–10) µm long; contents strongly heteromorphous (refringent), reacting weakly (greyish) in sulfovanillin; near the lamellae edges numerous, usually smaller, (38–)*11.1*–45.12–(–80) × (5–)6.1–*8.0*–9.9(–15) µm, fusiform, clavate or subcylindrical, apically often obtuse, mostly with an appendage 3–4.5(–5); contents similar. Lamellae edges fertile; **marginal cells** not distinctive. **Pileipellis** orthochromatic in Cresyl Blue, not sharply delimited from the underlying context, 140–250 µm deep, vertically almost homogeneous, composed of irregularly oriented, non-gelatinized hyphae that become denser and more horizontally oriented towards the

context; longer hyphal terminations forming conical fascicules near the surface. **Acid-resistant incrustations** absent. Hyphal terminations near the pileus margin very flexuous, thin-walled, terminal cells (12–) 20–*28*–36(–42) × 3–*5.2*–7.4(–11) µm, cylindrical or clavate, apically obtuse; subterminal cells very irregular, sometimes branched, often covered by strong glutinous coating. Hyphal terminations near the pileus centre different in length, terminal cells (10–)16.1–*27.2*–38.3(–60) × (2–)2.9–*3.9*–4.9(–7) µm, usually cylindrical, apically obtuse; subterminal cells very irregular, flexuous and often covered with glutinous hyaline coating. **Pileocystidia** near the pileus margin 1–to–5 celled, (14–)34.8–*58.5*– 82.3(–131) × (2–)3.5–*5.2*–6.9(–10) µm, narrowly clavate to subcylindrical, often flexuous, apically obtuse or acute, with or without appendage, contents refringent, yellowish, no reaction in sulfovanillin. Pileocystidia near the pileus centre often longer, (20–)39.7–*65.9*–92.1(135) × 3–*5.2*–7.3(–11) µm, usually clavate and with similar contents.

Habitat: Specimens found in Italy by evergreen oaks, specifically *Quercus ilex* and *Quercus suber*.

Additional material studied: EUROPE**,** Italy**,** Tuscany, 7 Nov 2016, R. De Lange (RDL 16-026/2); ibid., 7 Nov 2016, J. Kleine (RUS 16110702).



R. macrostigma; a) basidia and cystidia of the lamellae sides; b) basidia and cystidia of the lamellae edges; c) & f) spores; d) pileocystidia and hairs of the pileipellis centre; e) pileocystidia and hairs of the pileipellis centre. The scale is 10µm.

5.1.2. *Russula zebrihyphis*

Russula zebrihyphis Beel & De Lange **nom. prov.**
Holotype: EUROPE, Sweden, Medelpad (Province), Borgsjö parish, Sodra Sillre, 62°34'22.4"N 15°47'46.7"E, 31 Aug 2018, R. De Lange (RDL_18_043).
Etymology: 'zebrihyphis' refers to the zebroid incrustations of the pileipellis hyphae.

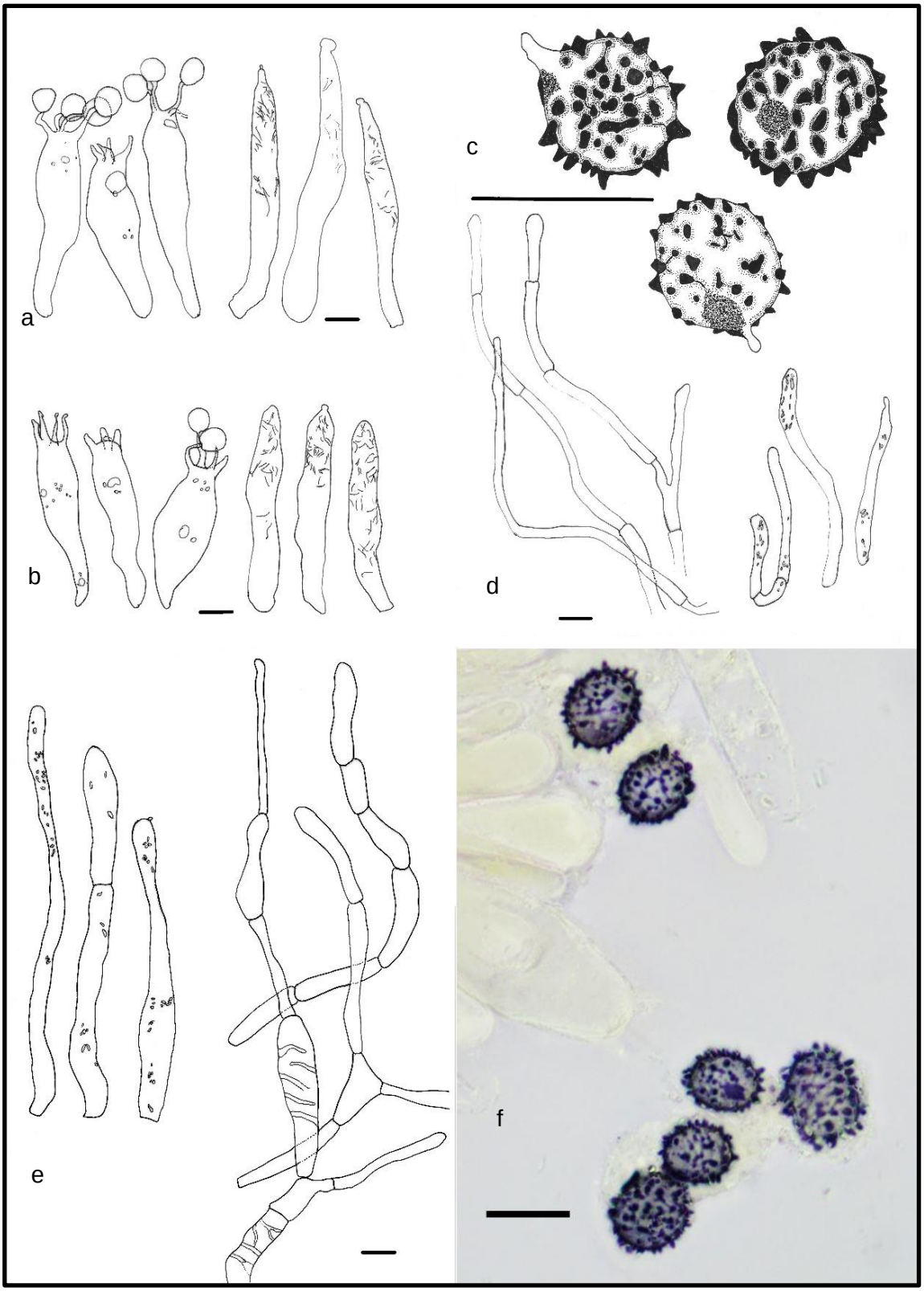
Pileus medium to large sized, 85–160 mm diam. when mature, infundibuliform with a deep depression in the centre; margin smooth, not striate; yellow–white (4A2, 4A3, 4A4), with spots of brownish orange (5D8) and yellow (4A6). **Lamellae** up to 5 mm deep, dense (5–8L + 1–2l/cm), with decurrent tooth, white to yellow-white (3A2, 4A2); lamellulae general, of different lengths; furcation's absent or rare. **Stipe** 20–40 x 25–55 mm, cylindrical or narrowly clavate, white to yellow-white (3A2, 4A2), turning brownish orange (5D8) and yellow (4A6) on damaged places; medulla solid. **Context** firm in all parts of the basidiomata; taste mild, sometimes very light sharp tinge; odour fruity, flowery, like *R*. *pectinatoides* (fishy when old). **Spore print** white to pale cream (Ib–IIa). $FeSO_4$ no reaction. Guaiac stipe and lamellae strong and fast reaction. KOH stipe, lamellae and context no reaction.

Spores (7.7–)8.5–*9.4*–10.2(–11.6) × (6.6–)7.2–*7.9*–8.6(–10.2) µm, subglobose to broadly ellipsoid, Q = (1.1–)1.12–*1.19*–1.25(–1.33) n= 80; ornamentation normal to high, moderately distant to very dense [4–12 in a 3 µm diam. circle] amyloid warts, 0.9–1.6 µm high, locally subreticulate, sometimes fused in chains (0–9 fusions in a 3 µm diam. circle), connected with occasional to frequent line connections [0–2(–6) line connections in a 3 µm diam. circle] suprahilar spot large, amyloid. **Basidia** (37–) 43.9–*52.4*–60.8 (–69) × (10–) 11.1–*13.4*–15.9 (–19) µm, narrowly clavate to clavate, 4–spored; basidiola first cylindrical, then clavate, ca. 4–12 µm wide. **Hymenial cystidia** abundant, 5000–20000/mm², (45–) 50.9–*65.9*–80.8 (–108) × (7–) 7.9–*9.1*–10.1 (–11) µm, narrowly cylindrical to narrowly clavate or narrowly fusiform, often slightly flexuose, apically acute or obtuse, mostly with appendage 1–4.1(–9) µm long; contents strongly heteromorphous, refringent, reacting strong (blackening) in sulfovanillin; near the lamellae edges numerous, usually smaller, (36–)47.8–*56.4*–65.0(–71) × (5–)7.0–*8.8*–10.5(–12) µm, fusiform, clavate or subcylindrical, apically often obtuse, without or with an appendage 2–*3*(–5); contents similar. Lamellae edges fertile; **marginal cells** not distinctive. **Pileipellis** orthochromatic in Cresyl Blue, not sharply delimited from the underlying context, 150–540 µm deep, vertically almost homogeneous, composed of irregularly oriented, non-gelatinized hyphae that become denser and more horizontally oriented towards the context. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin very flexuous, thin-walled, terminal cells (10–) 18.4–*37.3*–56.3(–118) × (2–)3.4–*6.5*–9.6(–13) µm, cylindrical or clavate, apically obtuse; subterminal cells very irregular, sometimes branched. Hyphal terminations near the pileus centre, terminal cells 18–*41.8*–65.6(–122) × 2.9–*5.1*–7.3(–12) µm, usually cylindrical, apically obtuse; subterminal cells very irregular, flexuous and often covered with

glutinous hyaline coating, sometimes with zebroid incrustation (see figure e). **Pileocystidia** near the pileus margin 1–to–4 celled, (28–)45.5–*91.4*–137(–237) × (3–)3.8–*7.8*–11.7(–23) µm, narrowly clavate to subcylindrical, often flexuous, apically obtuse or acute, rarely with appendage, contents refringent, yellowish, no reaction in sulfovanillin. Pileocystidia near the pileus centre often shorter and less broad, (27–)30.7–*47*–63.3(–84) × (3–) 3.5–*5.6*–7.7(–11) µm, usually clavate and with similar contents.

Habitat: Specimens found in Sweden in proximity to *Picea*, *Betula*, *Populus*, (*Alnus* and *Pinus*).

Additional material studied: EUROPE**,** Sweden**,** Medelpad (Province), Borgsjö parish, Sodra Sillre, 62°34'22.4"N 15°47'46.7"E, 31 Aug 2018, R. De Lange (RDL 18-041, RDL 18-042); ibid., 62°31'20.6"N 15°57'04.7"E, 31 Aug 2018, R. De Lange (RDL 18-038).



R. zebrihyphis; a) basidia and cystidia of the lamellae sides; b) basidia and cystidia of the lamellae edges; c) & f) spores; d) pileocystidia and hairs of the pileipellis centre; e) pileocystidia and hairs of the pileipellis centre. The scale is 10µm.

5.1.3. *Russula boeykensii*

Russula boeykensii Beel & De Lange **nom. prov.**
Holotype: EUROPE**,** Belgium**,** Limburg, Vliermaalroot (Kortessem), Jongenbos, 50°52'45.5"N 5°26'21.1"E, R. Boeykens (RB 15 08 17 08).
Etymology: 'boeykensii' refers to the name one of the collectors of this species: Ronny Boeykens.

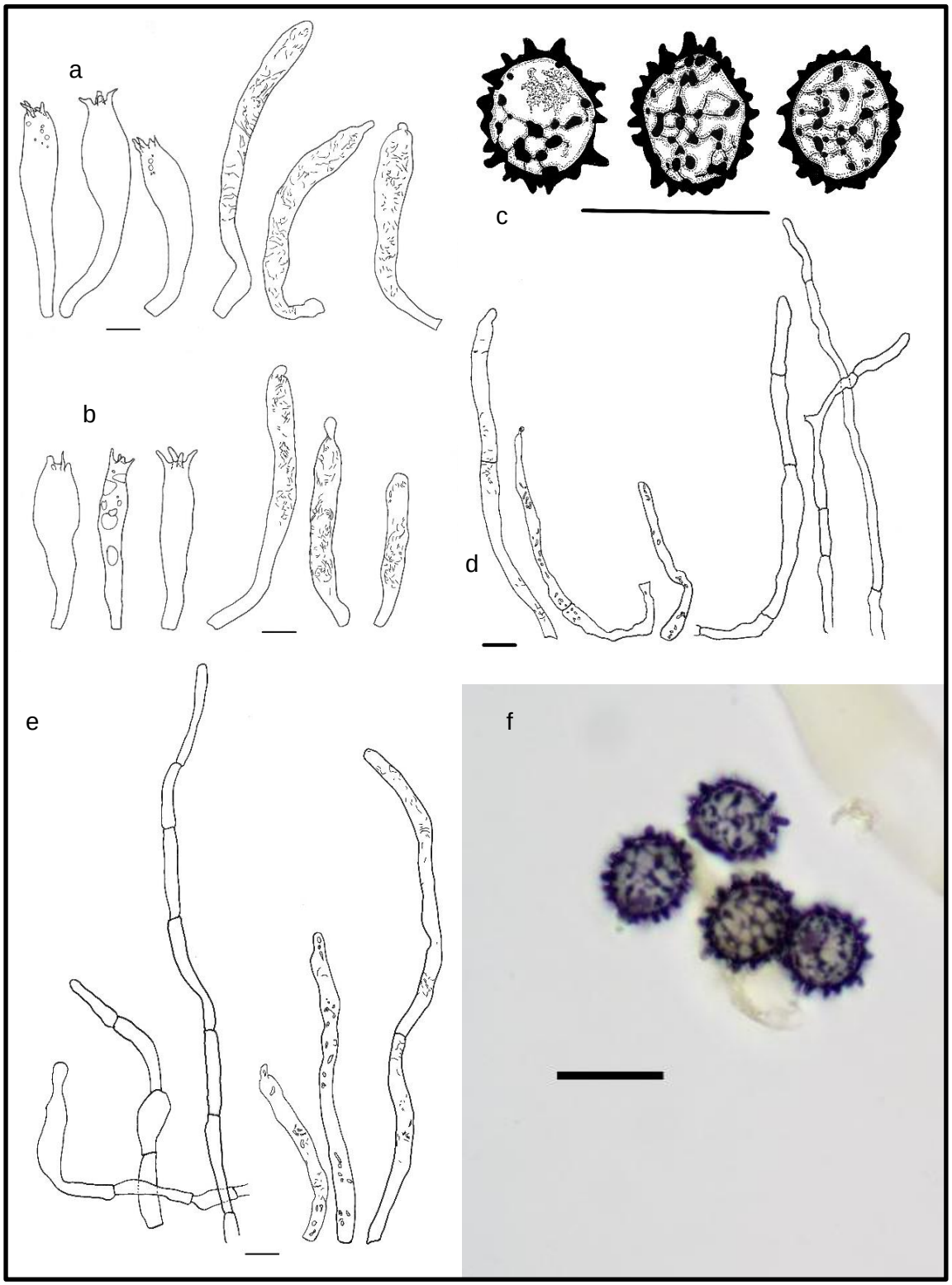
Pileus when mature, infundibuliform with a deep depression in the centre; white/yellow with brown spots (4A8: dark yellow; 5D8: yellowish brown; 5DF: umber). **Lamellae** yellowish white; lamellulae general, of different lengths; furcation's absent or rare. **Stipe** white to yellowish-white (34A, 4A2). **Context** not observed; taste acrid; odour fruity. **Spore print** whitish to pale cream (Ia–IIb). $FeSO_4$ orange or pink and afterwards grey; Guaiac stipe and lamellae strong and fast reaction; KOH not observed.

Spores (7.2–)7.7–*8.2*–8.6(–9.0) × (6.0–)6.4–*6.8*–7.2(–7.5) µm, subglobose to broadly ellipsoid, Q = (1.14–)1.16–*1.20*–1.24(–1.29), n= 40; ornamentation normal to high, distant to very dense [2–12 in a 3 µm diam. circle] amyloid warts, 0.6–2.1 µm high, locally subreticulate, sometimes fused in chains (0–6 fusions in a 3 µm diam. circle), connected with occasional to frequent line connections [0–2(–6) line connections in a 3 µm diam. circle] suprahilar spot irregular, faintly amyloid to amyloid. **Basidia** (40–) 52.5–*58.9*–65.2 (–72) × (9–) 10.7–*12.1*–13.5 (–14) µm, narrowly clavate to clavate, 4–spored; basidiola first cylindrical, then clavate, ca. 5–12 µm wide; near the lamellae edges usually smaller. **Hymenial cystidia** abundant, 120000–220000/mm², (57–) 62.2–*71.9*–81.5 (–94) × (7–) 7.2–*8.2*–9.2 (–10) µm, narrowly cylindrical to narrowly clavate or narrowly fusiform, often slightly flexuose, apically acute or obtuse, often with appendage 1–4(–5) µm long; contents strongly heteromorphous, refringent, reacting faintly (greying) in sulfovanillin; near the lamellae edges abundant, usually smaller and slender, (32–)40.7–*53.7*–66.7(–96) × (6–)6.2–*7.6*–9.0(–13) µm, fusiform, clavate or subcylindrical, apically often obtuse, without or with an appendage 3–5.3(–6); contents similar. Lamellae edges fertile; **marginal cells** not distinctive. **Pileipellis** orthochromatic in Cresyl Blue, not sharply delimited from the underlying context, 75–200 µm deep, vertically almost homogeneous, composed of irregularly oriented, non-gelatinized hyphae that become denser and more horizontally oriented towards the context. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin very flexuous, thin-walled, terminal cells (14–) 16.9–*28.7*–40.4(–56) × 3.6–*4.9*–6.2(–9) µm, cylindrical or clavate, apically obtuse; subterminal cells very irregular, sometimes branched. Hyphal terminations near the pileus centre, terminal cells (12–) 16.8–*24.4*–31.6(–39) × (3–)3.3–*4.5*–5.6(–7) µm, usually cylindrical, apically obtuse; subterminal cells very irregular, flexuous and often covered with glutinous hyaline coating. **Pileocystidia** near the pileus margin 1–to–3 celled, (26–)38.2–*71.6*–105(–158) × 4–*5.1*–6.2(–8) µm, narrowly clavate to subcylindrical, often flexuous, apically obtuse or acute, often with appendage, contents refringent, yellowish, no reaction in sulfovanillin. Pileocystidia near the pileus centre often shorter and less broad, (32–)33.9–*59.3*–84.8(–106) × (3–)3.2–*4.3*–5.4(–7) µm, usually clavate and with similar contents.

Habitat: Species collected on sandy loam soil.

Additional material studied: EUROPE**,** Belgium**,** Limburg, Vliermaalroot (Kortessem), Jongenbos, 50°52'45.5"N 5°26'21.1"E, R. Boeykens (RB 15 07 28 03); ibid., Limburg,

Diepenbeek/Kortessem, Netelbroekstraat, Nietelbroek (Netelbroek), 50°53'02.4"N 5°22'40.5"E, 23 Aug 2014, R. De Lange (RDL-19-23-08-2014).



Russula boeykensii; a) basidia and cystidia of the lamellae sides; b) basidia and cystidia of the lamellae edges; c) & f) spores; d) pileocystidia and hairs of the pileipellis centre; e) pileocystidia and hairs of the pileipellis centre. The scale is 10µm.

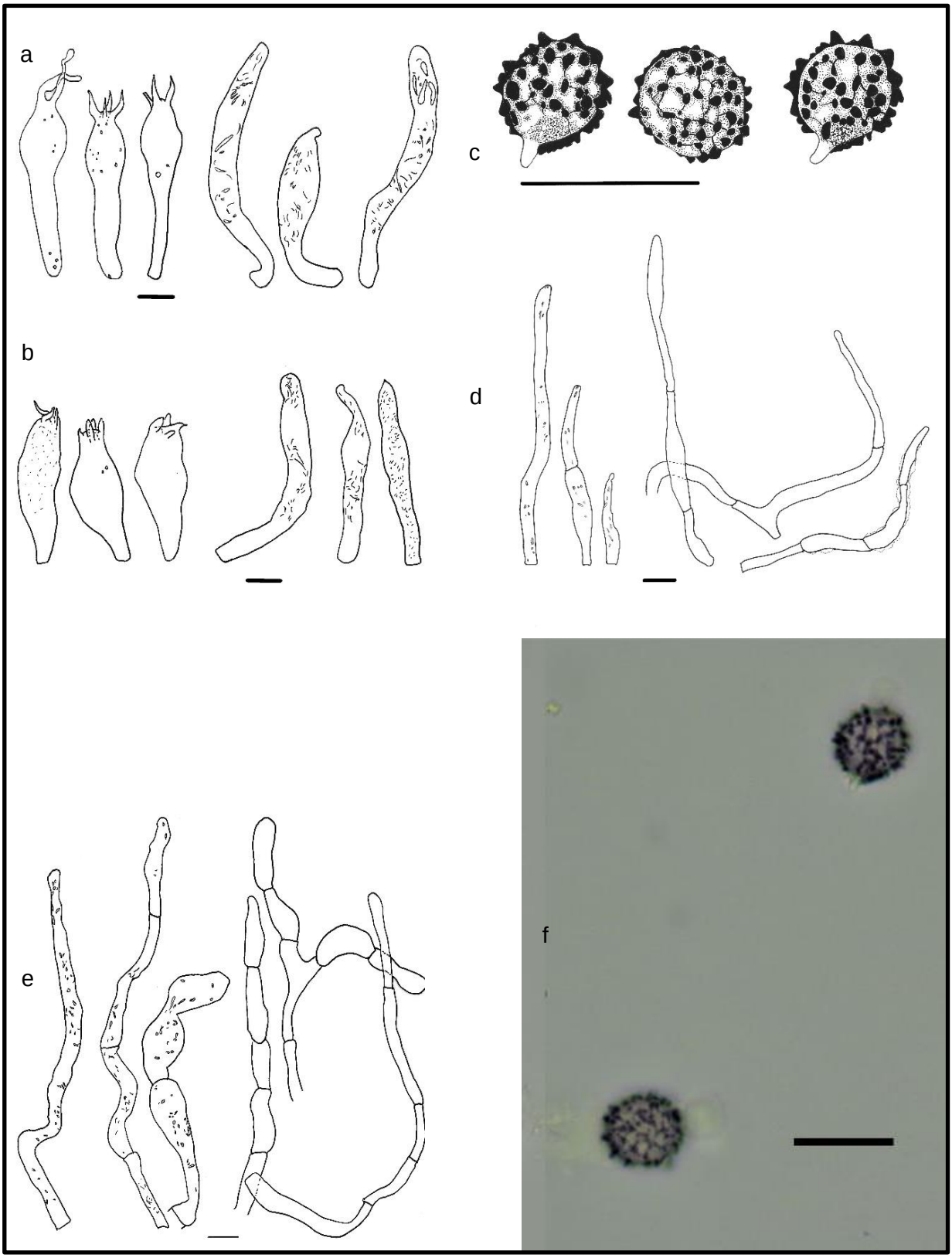
5.1.4. *Russula hampei*

Russula hampei Beel & De Lange **nom. prov.**
Holotype: **Central America**, Panama, Paso Ancho, Parque Nacional Volcan Baru, Chiriqui, 8°48'56.0"N 82°34'45.9"W, 16 June 2018, Felix Hampe & C. Manz, (FH 18-070).
Etymology: '*hampei*' refers to the collector of this species: Felix Hampe.

Pileus when mature, infundibuliform with a deep depression in the centre; white to pale yellowish white (2A2), yellow white (3A2, 4A2), light yellow (4A5) and dark yellow (4A8) with light yellow spots. **Lamellae** pale yellowish white (2A2) to yellow-white (3A2, 4A2); lamellulae general, of different lengths; furcation's absent or rare. **Stipe** white to pale yellowish white (2A2), yellow white (3A2, 4A2), light yellow (4A5) and dark yellow (4A8) with light yellow spots. **Context** not observed. **Spore print** not observed.

Spores (6.5–)7.1–*7.6*–8.2(–8.9) × (5.7–)6.0–*6.5*–7.1(–7.7) µm, subglobose to broadly ellipsoid, Q = (1.05–)1.13–*1.17*–1.22(–1.26), n= 40; ornamentation normal to high, moderately distant to very dense [4–13 in a 3 µm diam. circle] amyloid warts, 1.0–2.0 µm high, locally subreticulate, sometimes fused in chains (0–6 fusions in a 3 µm diam. circle), connected with occasional to abundant line connections [0–6(–9) line connections in a 3 µm diam. circle] suprahilar spot irregular, faintly amyloid to amyloid. **Basidia** (38–) 45.1–*49.5*–53.8 (–58) × (9–) 11.0–*12.1*–13.2 (–14) µm, narrowly clavate to clavate, 2–4–spored; basidiola first cylindrical, then clavate, ca. 4–11 µm wide; near the lamellae edges usually smaller (34–) 35.4–*40.8*–46.1 (–47) × (11–) 11.2–*12.7*–14.3 µm. **Hymenial cystidia** numerous to abundant, 2100–4200/mm², (50–) 56.8–*64.5*–73.2 (–75) × (6–) 6.9–*8.5*–10.0 (–12) µm, narrowly cylindrical to narrowly clavate or narrowly fusiform, often slightly flexuose, apically acute or obtuse, mostly without appendage 1–*3.5*(–7) µm long; contents strongly heteromorphous, refringent, reacting strongly (blackening) in sulfovanillin, weakly metachromatic walls in Cresyl Blue; near the lamellae edges more abundant, usually smaller and slender, (35–)41.4–*51.0*–60.6(–69) × (6–)6.3–*7.4*–8.5(–10) µm, fusiform, clavate or subcylindrical, apically often obtuse, (mostly) without appendages; contents similar. Lamellae edges fertile; **marginal cells** not distinctive. **Pileipellis** slightly metachromatic in Cresyl Blue, not sharply delimited from the underlying context, 210–300 µm deep, vertically almost homogeneous, composed of irregularly oriented, non-gelatinized hyphae that become denser and more horizontally oriented towards the context. Acid-resistant incrustations absent. Hyphal terminations near the pileus margin very flexuous, thin-walled, terminal cells (12–) 13.4–*26.0*–38.5(–71) × (3–)4.8–*6.6*–8.4(–10) µm, cylindrical or clavate, apically obtuse; subterminal cells very irregular, sometimes branched. Hyphal terminations near the pileus centre usually less broad, terminal cells (15–) 16.0–*26.2*–36.3(–50) × (3–)3.7–*4.8*–5.9(–7) µm, usually cylindrical, apically obtuse; subterminal cells very irregular, flexuous and often covered with glutinous hyaline coating, sometimes branched. Pileocystidia near the pileus margin 1–to–4 celled, (23–)33.3–*63.3*–93.2(–124) × (3–)3.4–*5.9*–8.4(–12) µm, narrowly clavate to subcylindrical, often flexuous, apically obtuse or acute, rarely with appendage, contents refringent, yellowish, no reaction in sulfovanillin. Pileocystidia near the pileus 1–to –2 celled centre often shorter and less broad, (18–)28.1–*48.9*–69.6(–90) × (3–)3.6–*4.4*–5.1(–6) µm, usually clavate and with similar contents.

Additional material studied: Central America, Panama, Paso Ancho, Parque Nacional Volcan Baru, Chiriqui, 8°48'56.0"N 82°34'45.9"W, 16 June 2018, Felix Hampe & C. Manz, (FH 18-072).



Russula hampei; a) basidia and cystidia of the lamellae sides; b) basidia and cystidia of the lamellae edges; c) & f) spores; d) pileocystidia and hairs of the pileipellis centre; e) pileocystidia and hairs of the pileipellis centre. The scale is 10µm.

5.2. Molecular work

The PCR success from the root tip samples are relatively low. From all the root tip samples, the ITS1 PCR-amplification was only successful for 45.6%. For ITS2 and the plant ITS this number is even lower, 24.9% and 11.4% respectively. The percentage of samples for which both ITS1 and ITS2 amplification was successful was just little lower than the success rate of ITS2, namely 21.4%. However, the success rate for all three amplification processes is very low, for merely 4.3% of all root tip samples all three amplification processes were successful.

Only 24.4 % and 11.3% of respectively the ITS1 and ITS2 Illumina reads from the root tips matched with OTU's assigned to the subgenus *Compactae* sensu lato (all compactoïd *Russula*).

In only 1 of the 4 newly described species all three primer PCR-amplification was successful. This was for *R. zebrihyphis*, the plant OUT's assigned to the root tip samples of *R. zebrihyphis* are *Salix caprea*, *Quercus dentata* and *Quercus petrea*.

The phylogenetic tree in figure 2 was compiled using ITS sequences of *R.* subg. *Brevipedum* species available in the Herbarium Universitatis Gandavensis and supplemented with other ITS sequences from *R.* subg. *Brevipedum* from GenBank.
Bootstrap values over 75 are seen as well-supported, values between 50 and 75 are seen as moderately supported and values lower than 50 are seen as little supported.
The first split within tis phylogenetic tree has moderate support, the next splits are well-supported except for the large group in which all the new described species of this work are placed. Support ranges from well-supported to barely supported.

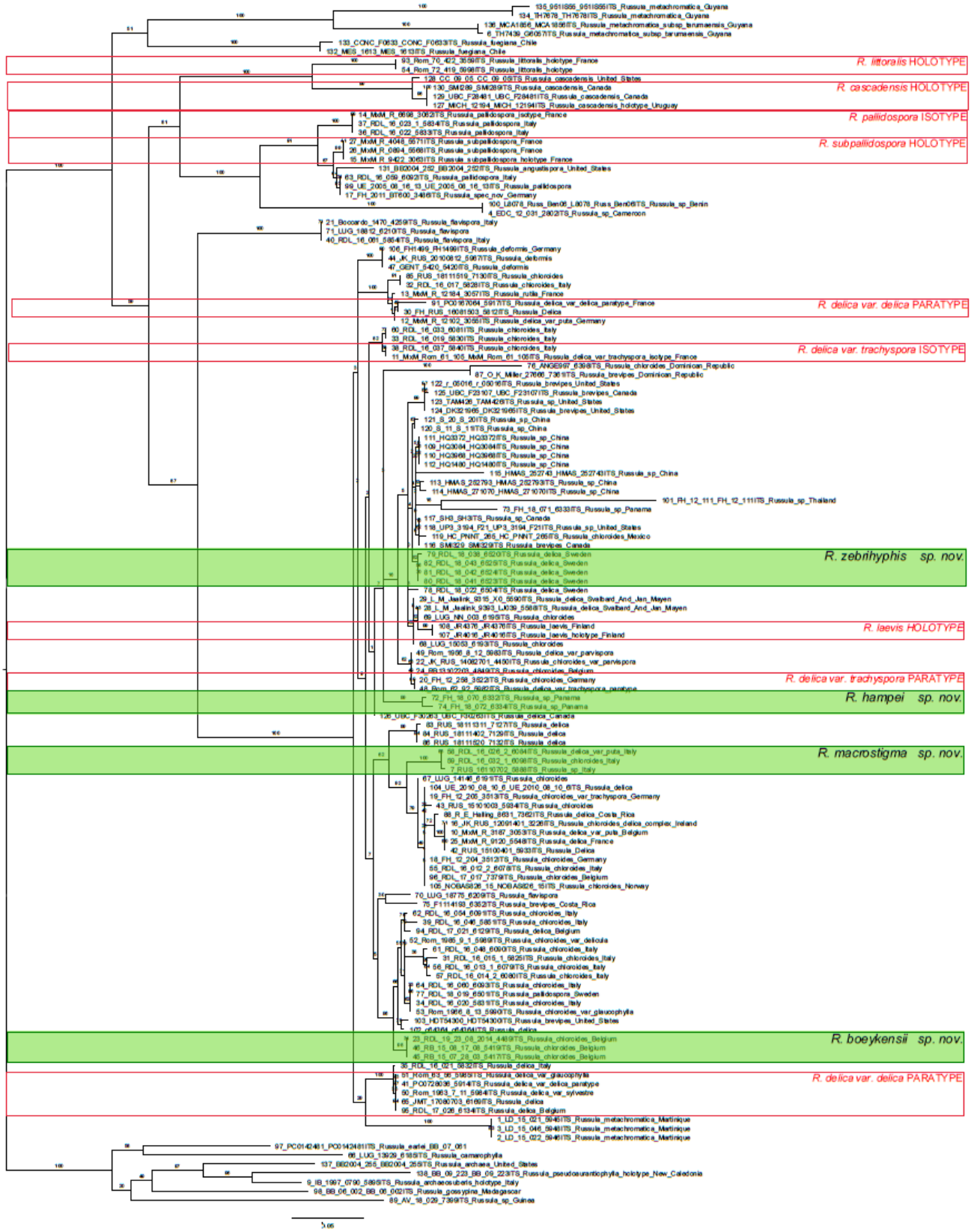


Figure 2. Phylogenetic tree o*f Russula* subgenus *Brevipedum*

6. Discussion

6.1. Comparison

A comparison of the newly described *R.* subg. *Brevipedum* species with the previously described species will be made in this section. The newly described species which are collected within Europe will be compared in depth with the previously described species of Europe and more briefly compared to those previously described in North America. Only the differences between species are described.

The conscious choice was made to compare the newly described species with all species descriptions from this subgenus from Romagnesi, Sarnari and Shaffer. Because of this a lot of the comparisons with European species are sectioned in Romagnesi, Sarnari and Shaffer, these sections refer to the comparison with the description found respectively in Romagnesi (1967), Sarnari (1998) and Shaffer (1964).

6.1.1. *R. macrostigma*

European species

R. macrostigma VS *R. delica*

1. Romagnesi
   Macroscopic: *R. delica* var. *puta* has a longer and less broad stipe (35–65 × 13–17 mm) compared to *R. macrostigma* (25–26 x 25–29 mm). *R. delica* and *R. delica* var. *trachyspora* has a complex odour of fruit and fish (one can dominate, var. *puta* only has very faint odour) while *R. macrostigma* has a fruity odour. *R. delica* and *R. delica* var. *trachyspora* has a slow, faint pink-orange reaction to $FeSO_4$, *R. delica* var. *puta* shows a pink-red after fifteen minutes, while *R. macrostigma* has a brown-orange reaction. Guaiac has a positive reaction, but not always immediate with *R. delica,* while *R. macrostigma* shows an immediate and strong positive reaction.
   Microscopic: *R. delica* spores are slightly larger (8–10–11.5 × 6.5–8.7 µm) compared to *R. macrostigma* spores ((7.1–)8.3–9.1–9.8(–10.8) × (5.3–)6.7–7.2–7.8 (–8.4) µm). Spore ornamentation of *R. delica* is slightly larger (0.5–0.7–1.0 µm, var. *trachyspora* 1.0–1.5 µm) than those of *R. macrostigma* (0.2–0.5 µm). The hymenial cystidia of *R. delica* are longer (65–150 µm, var. *trachyspora* 78–135 µm, var. *puta* 100–120 µm) compared to these of *R. macrostigma* ((43–)56.5–72.4–88.3 (–110) µm) and they have a strong reaction to sulfovanillin while these of *R. macrostigma* only show a faint reaction.
2. Sarnari
   Macroscopic: *R. delica* var. *puta* has a slender stipe (15–20 mm) compared to *R. macrostigma* (25–26 x 25–29 mm). *R. delica* var. *delica* has a longer stipe (25–48 mm) compared to *R. macrostigma* (25–26 x 25–29 mm). *R. delica* var. *delica* has a strong and unpleasant odour, like peach or salt with fruity components when young and a peaty flavour in the lamellae, while *R. macrostigma* has a fruity odour and has a mild and later slight acrid taste. *R. delica* var. *delica* reacts pale pink with $FeSO_4$, *R. macrostigma* has a brown-orange reaction.

Microscopic: *R. delica* var. *delica* has larger spores (8.5–11.2 × 7–9 µm) which are subglobose with higher ornamentation (0.8–1.0 µm, var. *puta* 1–1.2 µm) compared to the broadly ellipsoid to ellipsoid spores from *R. macrostigma* ((7.1–)8.3–9.1–9.8(–10.8) × (5.3–) 6.7–7.2–7.8 (–8.4) µm and ornamentation height 0.2–0.5 µm high). The hymenial cystidia of *R. delica* var. *delica* are longer and thicker (78–150 × 9–13 µm) compared to those from *R. macrostigma* ((43–) 56.5–72.4–88.3 (–110) × (4–)6.1–7.7–9.3 (–11) µm).

3. Shaffer
   Microscopic: Spore ornamentation of *R. delica* is higher (0.4–1.0 µm) compared to *R. macrostigma* (0.2–0.5 µm). Suprahilar spot weakly amyloid for *R. delica*, while suprahilar spot is large and strongly amyloid for *R. macrostigma*.

R. macrostigma VS. *R. chloroides*

1. Romagnesi
   Macroscopic: *R. chloroides* can have a smaller pileus (var. *chloroides* 45–130 mm, var. *parvispora* 45–100 mm) and longer stipe (var. *chloroides* (15–)30–50(–90) mm, var. *parvispora* 25–40 mm) compared to *R. macrostigma* (49–81 mm pileus diameter and 10–25 mm stipe length). *R. chloroides* var. *chloroides* $FeSO_4$ dirty red reaction, *R. chloroides* var. *parvispora* pink-orange reaction, *R. macrostigma* brown-orange.
   Microscopic: *R. chloroides* var. *parvispora* has smaller spores (6.5–8.0 × 6.0–6.7 µm) compared to the spores of *R. macrostigma* ((7.1–)8.3–9.1–9.8(–10.8) × (5.3–)6.7–7.2–7.8(–8.4) µm). *R. chloroides* var. *chloroides* has higher spore ornamentation (1–1.5 µm) compared to *R. macrostigma* (0.2–0.5 µm high). *R. chloroides* var. *parvispora* hymenial cystidia colouring black in SV.
2. Sarnari
   Macroscopic: *R. chloroides* can have a smaller pileus (var. *chloroides* 65–150 mm) compared to *R. macrostigma* (49–81 mm). $FeSO_4$ *R. chloroides* var. *trachyspora* pinkish reaction, *R. chloroides* var. *chloroides* slow (sometimes faint) pink-orange reaction. Guaiac *R. chloroides* var. *chloroides* slow, green reaction.
   Microscopic: *R. chloroides* spores are slightly bigger (var. *chloroides* 8–11.2 × 7.2–8.8 µm, var. *trachyspora* 9.5–11.4 × 8–10.5 µm), higher spore ornamentation (1.3–1.6 µm) compared to *R. macrostigma* ((7.1–)8.3–9.1–9.8(–10.8) × (5.3–)6.7–7.2–7.8(–8.4) µm, 0.2–0.5 µm high). *R. chloroides* var. *parvispora* has smaller spores (6.4–8.0 × 6.0–6.7 µm) compared to the spores of *R. macrostigma* ((7.1–)8.3–9.1–9.8(–10.8) × (5.3–)6.7–7.2–7.8(–8.4) µm).

R. macrostigma VS. *R. pseudodelica*

1. Romagnesi
   Macroscopic: *R. pseudodelica* has an acrid taste, while *R. macrostigma*'s taste starts of mild taste and afterwards just slightly acrid. The spore print of *R. pseudodelica* is darker (pale, ochraceous cream) compared to *R. macrostigma* (Ib–IIa).

Microscopic: The spore ornamentation of *R. pseudodelica* reaches up to 1.25 µm which is higher compared to these of *R. macrostigma* (0.2–0.5 µm). The suprahilar spot is faintly amyloid in *R. pseudodelica* while being strongly amyloid in *R. macrostigma*.

2. Shaffer
   Macroscopic: *R. pseudodelica* has a pungent taste, while *R. macrostigma*'s taste starts of mild taste and afterwards just slightly acrid. The spore print of *R. pseudodelica* is darker (custard-ochraceous) compared to *R. macrostigma* (Ib–IIa).
   Microscopic: The spores of *R. pseudodelica* are less broad (6.9–9.3 × 6.3–7.0 µm) compared to the spores of *R. macrostigma* ((7.1–)8.3–9.1–9.8(–10.8) × (5.3–)6.7–7.2–7.8(–8.4) µm). The spore ornamentation of *R. pseudodelica* is higher (0.5–1.3 µm) compared to these of *R. macrostigma* (0.2–0.5 µm). The suprahilar spot of *R. pseudodelica* is ornamented like the remainder of the spore but the ornamentation is lower and often weakly amyloid, while the suprahilar spot of *R. macrostigma* is amyloid but not ornamented. The hyphae in the pileipellis are less broad in *R. pseudodelica* (hyaline hyphae 1.0–2.6 µm broad and oleiferous hyphae 2.0–5.3 µm broad).

<u>*R. macrostigma* VS. *R. pallidospora*</u>

1. Romagnesi
   Macroscopic: *R. pallidospora* has a longer stipe (40–45 mm) compared to *R. macrostigma* (pileus diameter 49–80 mm, stipe length 10–25 mm). *R. pallidospora* has a complex smell, with a fruity component (*R. macrostigma* fruity smell) and it has a mild (slightly refreshing) taste, later bitter (*R. macrostigma* mild taste, later slightly acrid). *R. pallidospora* reacts pinkish with $FeSO_4$, while *R. macrostigma* reacts brown-orange. *R. pallidospora* has a darker spore print (IId) compared to *R. macrostigma* (Ib–IIa).

   Microscopic: The spores of *R. pallidospora* are smaller (especially more slender; 7.2–9.0 × 5.9–6.8 µm) compared to *R. macrostigma* ((7.1–)8.3–9.1–9.8(–10.8) × (5.3–)6.7–7.2–7.8(–8.4) µm). The basidia of *R. pallidospora* are slender (8–11 µm) compared to those of *R. macrostigma* ((5–) 8.9–11.4–13.9 (–19) µm).
2. Sarnari
   Macroscopic: *R. pallidospora* can have a bigger pileus (60–150 mm) and longer stipe (25–50 mm) compared to *R. macrostigma* (pileus diameter 77–127 mm, stipe length 25–29 mm). *R. pallidospora* reacts pale pink-orange with $FeSO_4$, while *R. macrostigma* reacts brown-orange. *R. pallidospora* reacts slow and dirty blue with Guaiac, while *R. macrostigma* has an immediate strong reaction. *R. pallidospora* has a darker spore print (IIc) compared to *R. macrostigma* (Ib–IIa).
   Microscopic: The spores of *R. pallidospora* are smaller (especially more slender; 7.5–8.75 × 6.0–7.0 µm) compared to *R. macrostigma* ((7.1–)8.3–9.1–9.8(–10.8) × (5.3–)6.7–7.2–7.8(–8.4) µm). The basidia of *R. pallidospora* are slender (9–11 µm) compared to those of *R. macrostigma* ((5–) 8.9–11.4–13.9 (–19) µm). The hymenial cystidia of *R. pallidospora* are longer (65–165 µm) compared to those of *R. macrostigma* ((43–) 56.5–72.4–88.3 (–110) µm).

R. macrostigma VS. *R. flavispora*

1. Romagnesi
   Macroscopic: *R. flavispora* has a remarkable odour with fruity notes, the taste is bitter in the stipe and acrid in the lamellae while *R. macrostigma* has a fruity odour and mild, later acrid taste. $FeSO_4$ reacts an intense dirty pink for *R. flavispora* and brown-orange for *R. macrostigma*. Spore print (light golden yellow IVb) and lamellae (ochre)darker than spore print *R. macrostigma* (Ib–IIa).
   Microscopic: *R. flavispora* has smaller spores (7.5–8 × 6.2–6.7 µm) compared to these of *R. macrostigma* ((7.1–)8.3–9.1–9.8(–10.8) × (5.3–)6.7–7.2–7.8(–8.4) µm). The spore ornamentation of *R. flavispora* is larger (up to 0.75 µm, *R. macrostigma* 0.2–0.5 µm) the warts are more isolated and the suprahilar spot is vague and very faintly amyloid (*R. macrostigma* has a distinct suprahilar spot which is strongly amyloid). The hymenial cystidia of *R. flavispora* are longer (72–120 µm) than these of *R. macrostigma* ((43–) 56.5–72.4–88.3 (–110) µm). *R. flavispora* pileipellis has elements which have a greying reaction to sulfovanillin while the pileipellis elements of *R. macrostigma* show no reaction.
2. Sarnari
   Macroscopic: *R. flavispora* has a complex odour (stronger when cut) with notes of fish, boiled herbs and fruit, it has an acrid and bitter while *R. macrostigma* has a fruity odour and mild, later acrid taste. $FeSO_4$ reacts pink-orange for *R. flavispora* and brown-orange for *R. macrostigma*. Spore print (bright yellow IVb) and lamellae (ochre) darker than spore print *R. macrostigma* (Ib–IIa).
   Microscopic: *R. flavispora* has smaller spores (6.4–8.6 × 5.5–6.8 µm) compared to these of *R. macrostigma* ((7.1–)8.3–9.1–9.8(–10.8) × (5.3–)6.7–7.2–7.8(–8.4) µm). The warts of *R. flavispora* are more isolated and the suprahilar spot is vague and very faintly amyloid (*R. macrostigma* has a distinct suprahilar spot which is strongly amyloid). The hymenial cystidia of *R. flavispora* are longer (70–140 µm) than these of *R. macrostigma* ((43–) 56.5–72.4–88.3 (–110) µm).

R. macrostigma VS. *R. littoralis*

1. Romagnesi
   Macroscopic: *R. littoralis* has an odour similar to those of some *Lactarius* species and a mild taste in the stipe, very bitter in the lamellae (*R. macrostigma* mild, later acrid). *R. littoralis* reacts pink with $FeSO_4$, *R. macrostigma* has a brown-orange reaction. The spore print of *R. littoralis* is darker (creme IIc) compared to *R. macrostigma*.
   Microscopic: The spores of *R. littoralis* are less broad (5.5–6.5(–6.7) µm) compared to the spores of *R. macrostigma* ((5.3–)6.7–7.2–7.8(–8.4) µm). *R. littoralis* does not have a clearly differentiated suprahilar spot in contrast to *R. macrostigma*. The basidia of *R. littoralis* are small and slender (46–56 × 8.5–10 µm) compared to those of *R. macrostigma*((38–) 45.2–54.3–63.4 (–80) × (5–) 8.9–11.4–13.9 (–19) µm). The hymenial cystidia of *R. littoralis* show a strong blackening with sulfovanillin, while those of *R. macrostigma* merely have a faint greying reaction.

R. macrostigma VS. *R. laevis*

Macroscopic: *R. laevis* has a smaller, shiny pileus (40–75 mm) compared to the matt pileus of *R. macrostigma* (77–127 mm). The spore print of *R. laevis* is slightly darker (IIb–d) compared to *R. macrostigma* (Ib–IIa).
Microscopic: *R. laevis* has slightly larger spores ((9.2–)9.5–10–10.5(–11.3) × (7.6–)8–8.5–8.9(–9.6) µm) and lower Q ((1.14–)1.16–1.18–1.21(–1.26)) compared to *R. macrostigma* ((7.1–)8.3–9.1–9.8(–10.8) × (5.3–)6.7–7.2–7.8(–8.4) µm, Q = (1.15–)1.19–1.25–1.31(–1.43)). The ornamentation of *R. laevis* (0.8–1.1(–1.3) µm) is higher compared to *R. macrostigma* (0.2–0.5 µm). The suprahilar spot of *R. laevis* is small, partly amyloid to amyloid, while these of *R. macrostigma* is large and distinctly amyloid. The hymenial cystidia of *R. laevis* (moderately numerous, 850–900/mm²) are considerably less dense and have a stronger reaction in SV (dark grey-brown) compared to *R. macrostigma* (abundant 30000–50000/mm², light grey in SV). The hyphal termination of *R. laevis* are longer, both at the margin ((20–)36.5–53.6–71.5(–116) × (4–)5–6–7(–9) µm) and the centre of the pileus ((20–)23.5–31.9–40.5(–56) × (3.5–)4–5–6(–7) µm, compared to *R. macrostigma* (pileus margin (12–) 20–28–35.9(–42) × 3–5.2–7.4(–11) µm, pileus centre (10–)16.1–27.2–38.3(–60) × (2–)2.92–3.91–4.91(–7) µm). *R. laevis* has pileocystidia which are slightly metachromatic and are always 1–celled while these of *R. macrostigma* are orthochromatic and can consist of up to 5 cells.

North American species

R. macrostigma can be ruled out not to be one of the American species based upon a few clear differences.

R. brevipes has spores with a wider width, resulting in an average lower Q value (Q= (1.09–)1.11–1.16–1.21(–1.29)) compared to *R. macrostigma* (Q= (1.15–)1.19–1.25–1.31(–1.43)). The spore ornamentation of *R. brevipes* is higher (0.7–1.7 µm) than these of *R. macrostigma* (0.2–0.5 µm). The hymenial cystidia of *R. brevipes* have weakly metachromatic walls while *R. macrostigma* is orthochromatic. Of the 2 variations described by Shaffer (1964) *R. brevipes var. acrior* has higher spore ornamentation (0.7–1.7 µm), and the suprahilar spot is weakly amyloid. *R. brevipes var. megaspore has* larger spores (9.3–14.1 × 8.0–12.0 µm).

R. inopina has smaller spores ((6.5–)6.9–7.2–7.5(–7.8) × (5–)5.2–5.5–5.7(–6) µm) and higher spore ornamentation (0.4–0.7 µm) compared to *R. macrostigma* ((7.07–)8.31–9.05–9.78(–10.84) × (5.32–)6.71–7.24–7.77(–8.42) µm, 0.2–0.5 µm high). The suprahilar spot is small and inamyloid to partly amyloid for *R. inopina*, compared to fully amyloid for *R. macrostigma*. The basidia of *R. inopina* are slender (8–9,5 µm) compared to these of *R. macrostigma* ((5–)8.89–11.4–13.91(–19) µm). The hymenial cystidia of *R. inopina* do not have appendices while these of *R. macrostigma* often do.

R. romagnesiana has smaller spores ((5.8–)6–6.3–6.7(–7.3) × (5.1–)5.2–5.4–5.7(–6.1) µm), a lower Q–value ((1.09–)1.12–1.16–1.2(–1.25)) and a smaller suprahilar spot compared to *R. macrostigma* (((7.07–)8.31–9.05–9.78(–10.84) × (5.32–)6.71–7.24–7.77(–8.42) µm, Q=(1.15–

)1.19–1.25–1.31(–1.43)). The hymenial cystidia of *R. romagnesiana* have weakly metachromatic walls while *R. macrostigma* is orthochromatic. *R. romagnesiana* has shorter and less broad basidia (40–44–48 × 8.5–9.7–11 µm) compared to *R. macrostigma* ((38–) 45.2–54.3–63.4 (–80) × (5–) 8.9–11.4–13.9 (–19) µm).

R. vesicatoria has smaller spores ((7–)7.3–7.6–7.9(–8.2) × (5.8–)6–6.3–6.7(–7) µm), a lower Q value ((1.12–)1.15–1.19–1.23(–1.31)) and a smaller suprahilar spot which is not amyloid compared to *R. macrostigma* ((7.07–)8.31–9.05–9.78(–10.84) × (5.32–)6.71–7.24–7.77(–8.42) µm, Q=(1.15–)1.19–1.25–1.31(–1.43)) with a large, strongly amyloid suprahilar spot. *R. vesicatoria* is distinctly metachromatic in the subhymenium, while *R. macrostigma* is orthochromatic. *R. vesicatoria* has moderately numerous hymenial cystidia, which are insensitive to sulfovanillin, the hymenial cystidia of *R. macrostigma* are numerous to abundant and have a weak reaction to sulfovanillin. *R. vesicatoria* has shorter and less broad basidia (41–46–50 × 8–9.5–10.5 µm) compared to *R. macrostigma* ((38–) 45.2–54.3–63.4 (–80) × (5–) 8.9–11.4–13.9 (–19) µm).

R. fuegiana spores (6.8–8.5 × 5.3–7.3 µm) are smaller compared to *R. macrostigma* ((7.07–)8.31–9.05–9.78(–10.84) × (5.32–)6.71–7.24–7.77(–8.42) µm). Ornamentation height similar, but rarely forming a partial to broken reticulum while *R. macrostigma* is often subreticulate. Suprahilar spot of *R. fuegiana* is finely and faintly ornamented while these of *R. macrostigma* are large and amyloid. The hymenial cystidia of *R. fuegiana* do not have appendices while these of *R. macrostigma* often do.

R. cascadensis is easily discernibly different by its intense acrid taste. *R. cascadensis* spores (6.7–8.2 × 4.8–6.7 µm) are smaller compared to *R. macrostigma* ((7.07–)8.31–9.05–9.78(–10.84) × (5.32–)6.71–7.24–7.77(–8.42) µm). Suprahilar spot of *R. cascadensis* has weakly amyloid to almost no ornamentation while these of *R. macrostigma* are large and amyloid. *R. cascadensis* has shorter and less broad basidia (40–52 × 8–10.6 µm) compared to *R. macrostigma* ((38–) 45.2–54.3–63.4 (–80) × (5–) 8.9–11.4–13.9 (–19) µm). The hymenial cystidia of *R. cascadensis* do not have appendices while these of *R. macrostigma* often do.

R. delicula both spores (8.1–10.6 × 7–9.4 µm) and spore ornamentation (0.5–1.6 µm) are big compared to *R. macrostigma* ((7.07–)8.31–9.05–9.78(–10.84) × (5.32–)6.71–7.24–7.77(–8.42) µm and ornamentation height 0.2–0.5 µm). Suprahilar spot of *R. delicula* is weakly amyloid, while these of *R. macrostigma* is strongly amyloid.

Intermediate conclusion: *R. macrostigma*

R. macrostigma is most similar to *R. delica* as described in Shaffer (1964), *R. delica var. trachyspora* and *R. delica var puta*. *R. macrostigma* differs from *R. delica* by lower spore ornamentation and a distinct amyloid suprahilar spot. *R. macrostigma* differs from *R. delica var. trachyspora* by lower spore ornamentation and shorter hymenial cystidia, a less strong reaction to sulfovanillin and an odour that lacks a fish component. *R. macrostigma* differs from *R. delica var. puta* by a broader stipe, lower spore ornamentation and shorter hymenial cystidia which react less to sulfovanillin.

R. macrostigma is can be distinguished mostly by a light spore print, a large and distinctly amyloid suprahilar spot, quite large spores and broad basidia.

6.1.2. *R. zebrihyphis*

European species

R. zebrihyphis VS *R. delica*

1. Romagnesi
   Macroscopic: *R. delica* and *R. delica* var. *trachyspora* has a slow, faint pink-orange reaction to $FeSO_4$, *R. delica* var. *puta* shows a pink-red after fifteen minutes, while *R. zebrihyphis* has no reaction. Guaiac has a positive reaction, but not always immediate with *R. delica,* while *R. zebrihyphis* shows an immediate and strong positive reaction.
   Microscopic: Spore ornamentation of *R. delica* is smaller (0.5–0.7–1.0 µm, var. *puta* up to 0.85 µm) than those of *R. zebrihyphis* (0.9–1.6 µm). The hymenial cystidia of *R. delica* are longer and slender (65–150 × (6.5–)7.2–11.5(–13.5) µm, var. *trachyspora* 78–135 × 6–11.5 µm, var. *puta* 100–120 × 6.5–10 µm) compared to these of *R. zebrihyphis* ((45–) 50.9–65.9–80.8 (–108) × (7–) 7.9–9.1–10.1 (–11) µm).
2. Sarnari
   Macroscopic: *R. delica* var. *delica* has a strong and unpleasant odour, like peach or salt with fruity components when young and a peaty flavour in the lamellae, while *R. zebrihyphis* has a fruity, flowery, pectinatoides odour (fishy when old) and has a mild taste, sometimes a very light sharp tinge. *R. delica* var. *delica* reacts pale pink with $FeSO_4$, *R. zebrihyphis* has no reaction.
   Microscopic: *R. delica* has smaller spore ornamentation (var. *delica* 0.8–1.0 µm, var. *puta* 1.0–1.2 µm) compared to *R. zebrihyphis* (0.9–1.6 µm). The hymenial cystidia of *R. delica* var. *delica* are longer and thicker (78–150 × 9–13 µm) compared to those from *R. zebrihyphis* ((45–) 50.9–65.9–80.8 (–108) × (7–) 7.9–9.1–10.1 (–11) µm).
3. Shaffer
   Microscopic: *R. delica* has more slender spores (8.2–10.8 × 6.9–8.1 µm) and lower spore ornamentation (0.4–1.0 µm) compared to *R. zebrihyphis* 2 ((7.7–)8.5–9.4–10.2(–11.6) × (6.6–) 7.2–7.9–8.6(–10.2) µm, ornamentation 0.9–1.6 µm). Suprahilar spot weakly amyloid for *R. delica*, while suprahilar spot is large and strongly amyloid for *R. zebrihyphis*.

R. zebrihyphis VS. *R. chloroides*

1. Romagnesi
   Macroscopic: *R. chloroides* has a mild taste in the stipe, but acrid and unpleasant in the lamellae, while *R. zebrihyphis* has a mild taste (sometimes very faint sharp tinge). *R. chloroides* can have a longer stipe (var. *chloroides (*15–)30–50(–90) mm) compared to *R. zebrihyphis* (25–55 mm). *R. chloroides* var. *chloroides* $FeSO_4$ dirty red reaction, *R. chloroides* var. *parvispora* pink-orange reaction, *R. zebrihyphis* no reaction.
   Microscopic: *R. chloroides* var. *parvispora* has smaller spores (6.5–8.0 × 6.0–6.7 µm) compared to the spores of *R. zebrihyphis* ((7.7–)8.5–9.4–10.2(–11.6) × (6.6–)7.2–7.9–8.6(–10.2) µm).
2. Sarnari

Macroscopic: *R. chloroides* has an acrid flavour while *R. zebrihyphis* has a mild taste, with sometimes a slight sharp tinge. $FeSO_4$ *R. chloroides* var. *trachyspora* pinkish reaction, *R. chloroides* var. *chloroides* slow (sometimes faint) pink-orange reaction, *R. zebrihyphis* no reaction. Guaiac *R. chloroides* var. *chloroides* slow, green reaction; *R. zebrihyphis* strong and immediate reaction.
Microscopic: *R. chloroides* var. *parvispora* has smaller spores (6.4–8.0 × 6.0–6.7 µm) compared to the spores of species 2 ((7.7–)8.5–9.4–10.2(–11.6) × (6.6–)7.2–7.9–8.6(–10.2) µm). The basidia of *R. chloroides* var. *parvispora* are slender (8–11 µm) compared to those of *R. zebrihyphis*(37–) 43.9–52.4–60.8 (–69) × (10–) 11.1–13.4–15.9 (–19) µm). The hymenial cystidia of *R. chloroides* exceed 100 µm while these of *R. zebrihyphis* are mostly shorter ((45–) 50.9–65.9–80.8 (–108) × (7–) 7.9–9.1–10.1 (–11) µm).

R. zebrihyphis VS. *R. pseudodelica*

1. Romagnesi
   Macroscopic: *R. pseudodelica* has an acrid taste, while *R. zebrihyphis*'s taste starts of mild taste and sometimes has a very slight sharp tinge. The spore print of *R. pseudodelica* is darker (pale, ochraceous cream) compared to *R. zebrihyphis* (Ib–IIa).
   Microscopic: *R. pseudodelica* has smaller spores (8–9.25 × 6.2–6.7 µm) and spore ornamentation (up to 1.25 µm) compared to *R. zebrihyphis* ((7.7–)8.5–9.4–10.2(–11.6) × (6.6–)7.2–7.9–8.6(–10.2) µm). The suprahilar spot is faintly amyloid in *R. pseudodelica* while being strongly amyloid in *R. zebrihyphis*. The basidia (7–8 µm) and hymenial cystidia (7–9 µm) of *R. pseudodelica* are slender compared to these of *R. zebrihyphis* ((basidia 37–) 43.9–52.4–60.8 (–69) × (10–) 11.1–13.4–15.9 (–19) µm, cystidia (45–) 50.9–65.9–80.8 (–108) × (7–) 7.9–9.1–10.1 (–11) µm).
2. Shaffer
   Macroscopic: *R. pseudodelica* has a pungent taste, while *R. zebrihyphis*'s taste starts of mild taste and sometimes has a very slight sharp tinge. The spore print of *R. pseudodelica* is darker (custard-ochraceous) compared to *R. zebrihyphis* (Ib–IIa).
   Microscopic: The spores of *R. pseudodelica* are smaller (6.9–9.3 × 6.3–7.0 µm) compared to the spores of *R. zebrihyphis* ((7.7–)8.5–9.4–10.2(–11.6) × (6.6–)7.2–7.9–8.6(–10.2) µm). The suprahilar spot of *R. pseudodelica* is ornamented like the remainder of the spore but the ornamentation is lower and often weakly amyloid, while the suprahilar spot of *R. zebrihyphis* is amyloid but not ornamented. The hyphae in the pileipellis are less broad in *R. pseudodelica* (hyaline hyphae 1.0–2.6 µm broad and oleiferous hyphae 2.0–5.3 µm broad) compared to *R. zebrihyphis* ((7–) 7.9–9.1–10.1 (–11) µm).

R. zebrihyphis VS. *R. pallidospora*

1. Romagnesi
   Macroscopic: *R. pallidospora* has a complex odour, with a fruity component (*R. zebrihyphis* fruity, flowery, pectinatoides odour) and it has a mild (slightly refreshing) taste, later bitter (*R. zebrihyphis* mild taste, sometimes very slight sharp tinge). *R. pallidospora* reacts pinkish with $FeSO_4$ reacts slowly dirty blue with Guaiac, while *R. zebrihyphis* reacts brown-orange

with $FeSO_4$ and has a strong, immediate reaction with Guaiac. The spore print of *R. pallidospora* is darker (IId) compared to these of *R. zebrihyphis* (Ia–IIb).
Microscopic: The spores of *R. pallidospora* are smaller (especially more slender; 7.5–8.75 × 6.0–7.0 µm) compared to *R. zebrihyphis* ((7.7–)8.5–9.4–10.2(–11.6) × (6.6–)7.2–7.9–8.6(–10.2) µm). The basidia of *R. pallidospora* are slender (52–60 × 9–11 µm) compared to those of *R. zebrihyphis* ((5–) 8.9–11.4–13.9 (–19) µm).

2. Sarnari
Macroscopic: *R. pallidospora* has a smaller pileus (60–130 mm) compared to *R. zebrihyphis* (85–160 mm). *R. pallidospora* reacts pale pink-orange with $FeSO_4$, while *R. zebrihyphis* has no reaction. *R. pallidospora* reacts slow and dirty blue with Guaiac, while *R. zebrihyphis* has an immediate strong reaction. The spore print of *R. pallidospora* is darker (IIc) compared to these of *R. zebrihyphis* (Ib–IIa).
Microscopic: The spores of *R. pallidospora* are smaller (especially less broad; 7.2–9.0 × 5.9–6.8 µm) compared to *R. zebrihyphis* ((7.7–)8.5–9.4–10.2(–11.6) × (6.6–)7.2–7.9–8.6(–10.2) µm). The basidia of *R. pallidospora* are slender (48–62 × 8–11 µm) compared to those of *R. zebrihyphis* ((37–) 43.9–52.4–60.8 (–69) × (10–) 11.1–13.4–15.9 (–19) µm). The hymenial cystidia of *R. pallidospora* are become greyish in SV while those of *R. zebrihyphis* become black.

R. zebrihyphis VS. *R. flavispora*

1. Romagnesi
Macroscopic: *R. flavispora* has a bitter taste in the stipe and acrid in the lamellae while *R. zebrihyphis* has mild taste, with sometimes a very light sharp tinge. $FeSO_4$ reacts an intense dirty pink for *R. flavispora* and *R. zebrihyphis* has no reaction. Spore print (light golden yellow IVb) and lamellae (ochre) darker than spore print *R. zebrihyphis* (Ib–IIa).
Microscopic: *R. flavispora* has smaller spores (7.5–8 × 6.2–6.7 µm) and ornamentation (up to 0.75 µm) compared to these of *R. zebrihyphis* ((7.7–)8.5–9.4–10.2(–11.6) × (6.6–)7.2–7.9–8.6(–10.2) µm, ornamentation 0.9–1.6 µm). The warts are more isolated and the suprahilar spot is vague and very faintly amyloid (*R. zebrihyphis* has a distinct suprahilar spot which is strongly amyloid). The hymenial cystidia of *R. flavispora* are longer (72–120 µm) than these of *R. zebrihyphis* ((45–) 50.9–65.9–80.8 (–108) × (7–) 7.9–9.1–10.1 (–11) µm) and have a milder reaction in SV (no to light greying vs blackening). *R. flavispora* pileipellis has elements which has a greying reaction to sulfovanillin while the pileipellis elements of *R. zebrihyphis* show no reaction.

2. Sarnari
Macroscopic: *R. flavispora* has an complex odour (stronger when cut) with notes of fish, boiled herbs and fruit, it has an acrid and bitter while *R. zebrihyphis* has a fruity, flowery, pectinatoides odour and mild taste, with sometimes a very light sharp tinge. *R. flavispora* has a smaller pileus (50–105 mm) compared to *R. zebrihyphis* (85–160 mm). $FeSO_4$ reacts pink-orange for *R. flavispora* and *R. zebrihyphis* has no reaction. Spore print (bright yellow IVb) and lamellae (ochre) darker than spore print *R. zebrihyphis* (Ib–IIa).
Microscopic: *R. flavispora* has smaller spores (6.4–8.6 × 5.5–6.8 µm) compared to these of *R. zebrihyphis* ((7.7–)8.5–9.4–10.2(–11.6) × (6.6–)7.2–7.9–8.6(–10.2) µm). The warts of *R.*

flavispora are more isolated and the suprahilar spot is vague and very faintly amyloid (*R. zebrihyphis* has a distinct suprahilar spot which is strongly amyloid). The hymenial cystidia of *R. flavispora* are longer (70–140 µm) than these of *R. zebrihyphis* ((45–) 50.9–65.9–80.8 (–108) × (7–) 7.9–9.1–10.1 (–11) µm).

R. zebrihyphis VS. *R. littoralis*

1. Romagnesi
   Macroscopic: *R. littoralis* has a mild taste in the stipe, very bitter in the lamellae (*R. zebrihyphis* mild, with sometimes a very light sharp tinge). *R. littoralis* has a smaller pileus (60–85 mm) compared to *R. zebrihyphis* (85–160 mm). *R. littoralis* reacts pink with $FeSO_4$, *R. zebrihyphis* has a no reaction. The spore print of *R. littoralis* is darker (crème IIc) compared to *R. zebrihyphis* (Ib–IIa).
   Microscopic: The spores of *R. littoralis* are smaller (6.2–8(–10) × 5.5–6.5(–6.7) µm) compared to the spores of *R. zebrihyphis* ((7.7–)8.5–9.4–10.2(–11.6) × (6.6–)7.2–7.9–8.6(–10.2) µm). *R. littoralis* does not have a clearly differentiated suprahilar spot in contrast to *R. zebrihyphis*. The basidia of *R. littoralis* are slender (46–56 × 8.5–10 µm) compared to those of *R. zebrihyphis* ((37–) 43.9–52.4–60.8 (–69) × (10–) 11.1–13.4–15.9 (–19) µm). The hymenial cystidia of *R. littoralis* are slender (70–80 × 5–8.5 µm) compared to those of *R. zebrihyphis* ((45–) 50.9–65.9–80.8 (–108) × (7–) 7.9–9.1–10.1 (–11) µm).

R. zebrihyphis VS. *R. laevis*

1. Adamcik et al.
   Macroscopic: *R. laevis* has a smaller, shiny pileus compared to the bigger, matt pileus of *R. zebrihyphis*. *R. laevis* has a slender stipe (12–20 mm) compared to *R. zebrihyphis* (20–40 mm). *R. laevis* have darker spore print (IIb–d) compared to *R. zebrihyphis* (Ib–IIa).
   Microscopic: *R. laevis* have less fused warts (1–3 fusions in a 3 µm diam. circle) compared to *R. zebrihyphis* (0–9 fusions in a 3 µm diam. circle). The hymenial cystidia of *R. laevis* are less numerous (moderately numerous, ca. 850–900/mm²), longer and slender ((72–)79.5–86.8–94(–98) × (7–)7.5–8–8.5(–9.5) µm) compared to *R. zebrihyphis* (abundant, 5000–20000/mm², (45–) 50.9–65.9–80.8 (–108) × (7–) 7.9–9.1–10.1 (–11) µm). The pileipellis of *R. laevis* is less deep (110–130 µm) compared to these of *R. zebrihyphis* (150–540 µm). *R. laevis* have longer and less broad hyphae ends in the pileipellis ((20–)36.5–53.6–71.5(–116) × (4–)5–6–7(–9) µm) compared to *R. zebrihyphis* ((10–) 18.4–37.3–56.3(–118) × (2–)3.4–6.5–9.6(–13) µm). The pileocystidia of *R. laevis* are always 1–celled and slightly metachromatic while these of *R. zebrihyphis* are often more celled (up to 4–celled) orthochromatic. The pileocystidia of *R. laevis* are longer and slender ((40–)46.5–79.8–113(>200) × 4.5–5.9–7(–8) µm), frequently with appendages compared to these of *R. zebrihyphis* ((28–)45.5–91.4–137(–237) × (3–)3.8–7.8–11.7(–23) µm, rarely with appendage).

North American species

R. zebrihyphis can be ruled out not to be one of the American species based upon a few clear differences.

R. brevipes (var. *brevipes,* var. *acrior*) tastes mild and slowly becomes acrid and has an indistinct odour, *R. zebrihyphis* does not become acrid and has a fruity, flowery odour. *R. brevipes* (var. *brevipes*) has slightly slender spores ((8.5–)8.7–9.1–9.5(–9.9) × (7.2–)7.5–7.9–8.2(–8.6) µm) compared to *R. zebrihyphis* ((7.7–)8.5–9.4–10.2(–11.6) × (6.6–)7.2–7.9–8.6(–10.2) µm). . *R. brevipes var. megaspore* has larger spores (9.3–14.1 × 8.0–12.0 µm). The basidia of *R. brevipes* var. *brevipes* ((45–) 55.5–60.7–68 × 9.5–11.4–14 µm) are slender, var. *acrior* (49–74 × 8.0–14.3 µm) are slender and longer, var*. megaspora* (53–73 × 9.3–16.0 µm) are longer compared by these of *R. zebrihyphis* ((37–) 43.9–52.4–60.8 (–69) × (10–) 11.1–13.4–15.9 (–19) µm). The hymenial cystidia of *R. brevipes* have weakly metachromatic walls and react only weakly with sulfovanillin, while *R. zebrihyphis* is orthochromatic and the hymenial cystidia have a strong reaction with sulfovanillin. The suprahilar spot is of *R. brevipes* var. *acrior* is weakly amyloid

R. inopina has an indistinct odour while *R. zebrihyphis* has a fruity, flowery odour. *R. inopina* has smaller spores ((6.5–)6.9–7.2–7.5(–7.8) × (5–)5.2–5.5–5.7(–6) µm) and a higher Q ((1.22–)1.25–1.31–1.37(–1.44)) compared to *R. zebrihyphis* ((7.7–)8.5–9.4–10.2(–11.6) × (6.6–)7.2–7.9–8.6(–10.2) , Q = (1.1–)1.12–1.19–1.25(–1.33)). The suprahilar spot is small and inamyloid to partly amyloid for *R. inopina*, compared to fully amyloid for *R. zebrihyphis*. The basidia of *R. inopina* are slender (8–9,5 µm) compared to these of *R. zebrihyphis* ((10–) 11.1–13.4–15.9 (–19) µm). The hymenial cystidia of *R. inopina* do not have appendices while these of *R. zebrihyphis* often do. The pileipellis of *R. inopina* has weak reaction in SV, while *R. zebrihyphis* has no reaction to SV.

R. romagnesiana has an indistinct odour while *R. zebrihyphis* has a fruity, flowery odour. *R. romagnesiana* has smaller spores ((5.8–)6–6.3–6.7(–7.3) × (5.1–)5.2–5.4–5.7(–6.1) µm), smaller ornamentation (0.6–0.9 µm) and a smaller suprahilar spot compared to species2 ((7.7–)8.5–9.4–10.2(–11.6) × (6.6–)7.2–7.9–8.6(–10.2) µm, ornamentation 0.9–1.6 µm). The hymenial cystidia of *R. romagnesiana* have weakly metachromatic walls while *R. zebrihyphis* is orthochromatic. *R. romagnesiana* has shorter and less broad basidia (40–44–48 × 8.5–9.7–11 µm) compared to *R. zebrihyphis* ((37–) 43.9–52.4–60.8 (–69) × (10–) 11.1–13.4–15.9 (–19) µm).

R. vesicatoria has a strong, unpleasant odour and an astringent taste which becomes intensely and persistently acrid compared to *R. zebrihyphis* which has a fruity, flowery odour and a mild taste. *R. vesicatoria* has smaller spores ((7–)7.3–7.6–7.9(–8.2) × (5.8–)6–6.3–6.7(–7) µm), smaller ornamentation (0.4–0.7 µm) and a smaller suprahilar spot which is not amyloid compared to *R. zebrihyphis* ((7.7–)8.5–9.4–10.2(–11.6) × (6.6–)7.2–7.9–8.6(–10.2) µm, ornamentation 0.9–1.6 µm) with a large, strongly amyloid suprahilar spot. *R. vesicatoria* is distinctly metachromatic in the subhymenium, while *R. zebrihyphis* is orthochromatic. *R. vesicatoria* has moderately numerous hymenial cystidia, which are insensitive to sulfovanillin, the hymenial cystidia of *R. zebrihyphis* are abundant and have a strong reaction to sulfovanillin. *R. vesicatoria* has shorter and less broad basidia (41–46–50 × 8–9.5–10.5 µm) compared to *R. zebrihyphis* ((37–) 43.9–52.4–60.8 (–69) × (10–) 11.1–13.4–15.9 (–19) µm).

R. fuegiana has an odour like applesauce or *R. maculate* and an acrid taste with a bitter component while *R. zebrihyphis* has a fruity, flowery odour and mild taste. *R. fuegiana* spores (6.8–8.5 × 5.3–7.3 µm) and ornamentation (0.3–0.7 µ) are smaller compared to *R. zebrihyphis* ((7.7–)8.5–9.4–10.2(–11.6) × (6.6–)7.2–7.9–8.6(–10.2) µm, ornamentation 0.9–1.6 µm). Suprahilar spot of *R. fuegiana* is finely and faintly ornamented while these of *R. zebrihyphis* are large and amyloid. The hymenial cystidia of *R. fuegiana* do not have appendices while these of *R. zebrihyphis* often do.

R. cascadensis has an indistinct odour and intense acrid taste while *R. zebrihyphis* has a fruity, flowery odour and mild taste. *R. cascadensis* is easily discernibly different by its intense acrid taste. *R. cascadensis* spores (6.7–8.2 × 4.8–6.7 µm) and ornamentation (0.2–0.7 µm) are smaller compared to *R. zebrihyphis* ((7.7–)8.5–9.4–10.2(–11.6) × (6.6–)7.2–7.9–8.6(–10.2) µm, ornamentation 0.9–1.6 µm). Suprahilar spot of *R. cascadensis* has weakly amyloid to almost no ornamentation while these of *R. zebrihyphis* are large and amyloid. *R. cascadensis* has shorter and less broad basidia (40–52 × 8–10.6 µm) compared to *R. zebrihyphis* ((37–) 43.9–52.4–60.8 (–69) × (10–) 11.1–13.4–15.9 (–19) µm). The hymenial cystidia of *R. cascadensis* do not have appendices while these of *R. zebrihyphis* often do.

R. delicula spores (8.1–10.6 × 7–9.4 µm) are slightly bigger compared to *R. zebrihyphis* ((7.7–)8.5–9.4–10.2(–11.6) × (6.6–)7.2–7.9–8.6(–10.2) µm). Suprahilar spot of *R. delicula* is weakly amyloid, while these of *R. zebrihyphis* is strongly amyloid.

Intermediate conclusion: *R. zebrihyphis*

R. zebrihyphis is most similar to *R. littoralis*, *R. delica var. puta/trachyspora*. *R. zebrihyphis* differs from *R. delica var. puta* by larger spore ornamentation (and shorter and thicker hymenial cystidia, pink-red reaction to $FeSO_4$). *R. zebrihyphis* differs from *R. delica var. trachyspora* by shorter and broader hymenial cystidia (odour of *R. zebrihyphis* is mainly flowery, only fishy when old, while *R. delica var. trachyspora* has a complex odour of fruit and fish).

R. zebrihyphis can be distinguished mostly by light spore print, a strong reaction to sulfovanillin in the hymenium, quite large spores, broad basidia and the presence of zebroid incrustations on the hyphae of the pileipellis.

6.1.3. *Russula boeykensii*

European species

Russula boeykensii VS *R. delica*

1. Romagnesi
   Macroscopic: *R. delica* has a complex odour of fruit and fish compared to the fruity odour of *Russula boeykensii*. *R. delica* var. *puta* has a bright pink-red reaction to $FeSO_4$ while *Russula boeykensii* shows an orange reaction. The reaction of *R. delica* to Guaiac is not always immediate while these of *Russula boeykensii* is immediate and strong.
   Microscopic: *R. delica* has larger spores (8–10–11.5 × 6.5–8.7 µm) and smaller spore ornamentation (0.5–0.7–1.0 µm, var. *puta* up to 0.85 µm, var. *trachyspora* 1–1.5µm) compared to *Russula boeykensii* ((7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm, ornamentation 0.6–2.1 µm). The hymenial cystidia of *R. delica* are longer and slender (65–150 × (6.5–)7.2–11.5(–13.5) µm, var. *puta* 100–120 × 6.5–10 µm, var. *trachyspora* 78–135 × 6–11.5 µm) and react strong on SV compared to these of *Russula boeykensii* ((57–) 62.2–71.9–81.5 (–94) × (7–) 7.2–8.2–9.2 (–10) µm) which have a faint reaction in SV.
2. Sarnari
   Macroscopic: *R. delica* has a strong and unpleasant odour with peachy and salty components compared to the fruity odour of *Russula boeykensii*. *R. delica* var. *delica* has a peaty taste in the lamellae while *Russula boeykensii* has an acrid taste. *R. delica* var. *delica* has a pale pink reaction to $FeSO_4$ while *Russula boeykensii* shows an orange/pink reaction.
   Microscopic: *R. delica* has larger spores and smaller spore ornamentation (var. *delica* 0.8–1.0 µm, var. *puta* 1.0–1.2 µm) compared to *Russula boeykensii* ((7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm, ornamentation 0.6–2.1 µm). The hymenial cystidia of *R. delica* var. *delica* are longer and thicker (78–150 × 9–13 µm) compared to those from *Russula boeykensii* ((57–) 62.2–71.9–81.5 (–94) × (7–) 7.2–8.2–9.2 (–10) µm).
3. Shaffer
   Microscopic: *R. delica* has larger spores (8.2–10.8 × 6.9–8.1 µm) and lower spore ornamentation (0.4–1.0 µm) compared to *Russula boeykensii* ((7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm, ornamentation 0.6–2.1 µm).

Russula boeykensii VS. *R. chloroides*

1. Romagnesi
   Macroscopic: *R. chloroides* has an unpleasant odour, with fruity and brackish notes while *Russula boeykensii* has a fruity odour.
   Microscopic: *R. chloroides* var. *parvispora* has smaller spores (6.5–8.0 × 6.0–6.7 µm), var. *chloroides* (7–10–11 × 6–8.7 µm) has larger spores and both have smaller spore ornamentation (var. *chloroides* up to 1.5 µm and var. *parvispora* up to 0.75 µm) compared to the spores of *Russula boeykensii* ((7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm, ornamentation 0.6–2.1 µm). The hymenial cystidia of *R. chloroides* are longer (var.

chloroides 50–130 µm and even longer, var. *parvispora* (57–)65–115 µm) compared to *Russula boeykensii* ((57–) 62.2–71.9–81.5 (–94) × (7–) 7.2–8.2–9.2 (–10) µm).

2. Sarnari

   Microscopic: *R. chloroides* var. *parvispora* has smaller spores (6.4–8.0 × 6.0–6.7 µm), var. *chloroides* (8–11.2 × 7.2–8.8 µm) and var. *trachyspora* (8–10.4 × 6.6–8.2 µm) have larger spores and the spore ornamentation is smaller (var. *chloroides* 1.3–1.6 µm, var. *trachyspora* up to 1.5 µm, var. *parvispora* up to 1 µm) compared to the spores of *Russula boeykensii* ((7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm, ornamentation 0.6–2.1 µm). The basidia of *R. chloroides* var. *parvispora* are slender (8–11 µm) compared to those of *Russula boeykensii* (40–) 52.5–58.9–65.2 (–72) × (9–) 10.7–12.1–13.5 (–14) µm). The hymenial cystidia of *R. chloroides* exceed 100 µm while these of *Russula boeykensii* are shorter ((57–) 62.2–71.9–81.5 (–94) × (7–) 7.2–8.2–9.2 (–10) µm).

Russula boeykensii VS. *R. pseudodelica*

1. Romagnesi

   Macroscopic: The spore print of *R. pseudodelica* is darker (pale ochraceous cream) compared to *Russula boeykensii* (Ib–IIb).

   Microscopic: *R. pseudodelica* has slightly larger spores (8–9.25 × 6.2–6.7 µm) and smaller spore ornamentation (up to 1.25 µm) compared to *Russula boeykensii* ((7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm, ornamentation 0.6–2.1 µm). The basidia (7–8 µm) and hymenial cystidia (7–9 µm) of *R. pseudodelica* are slender compared to these of *Russula boeykensii* ((basidia (40–) 52.5–58.9–65.2 (–72) × (9–) 10.7–12.1–13.5 (–14) µm, cystidia (57–) 62.2–71.9–81.5 (–94) × (7–) 7.2–8.2–9.2 (–10) µm). The hymenial cystidia of *R. pseudodelica* react stronger with SV (blackening) compared to the faint (greying) reaction of *Russula boeykensii*.

2. Shaffer

   Macroscopic: The spore print of *R. pseudodelica* is darker (custard–ochraceous) compared to *Russula boeykensii* (Ib–IIb).

   Microscopic: *R. pseudodelica* has spores (6.9–9.3 × 6.3–7.0 µm) and ornamentation (0.5–1.3 µm) that are smaller compared to the spores of *Russula boeykensii* ((7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm, ornamentation 0.6–2.1 µm). The hyphae in the pileipellis are less broad in *R. pseudodelica* (hyaline hyphae 1.0–2.6 µm broad and oleiferous hyphae 2.0–5.3 µm broad) compared to *Russula boeykensii* (3.6–4.9–6.2(–9) µm).

Russula boeykensii VS. *R. pallidospora*

1. Romagnesi

   Macroscopic: *R. pallidospora* has a complex odour, with fruity components and a mild taste, later acrid compared to *Russula boeykensii* with a fruity odour and an acrid taste. The spore print of *R. pallidospora* is darker (IId) compared to these of *Russula boeykensii* (Ib–IIb).

   Microscopic: The basidia of *R. pallidospora* are slender (52–60 × 9–11 µm) compared to those of *Russula boeykensii* ((9–) 10.7–12.1–13.5 (–14) µm). The hymenial cystidia of *R.*

pallidospora (65–160 µm) can be longer than these of *Russula boeykensii* ((57–) 62.2–71.9–81.5 (–94) µm).

2. Sarnari
   Macroscopic: The spore print of *R. pallidospora* is darker (IIc) compared to these of *Russula boeykensii* (Ib–IIb).
   Microscopic: The basidia of *R. pallidospora* are slender (48–62 × 8–11 µm) compared to those of *Russula boeykensii* ((40–) 52.5–58.9–65.2 (–72) × (9–) 10.7–12.1–13.5 (–14) µm.

Russula boeykensii VS. *R. flavispora*

1. Romagnesi
   Macroscopic: *R. flavispora* has a remarkable odour, with fruity components and a bitter taste in the stipe and acrid taste in the lamellae compared to *Russula boeykensii* with a fruity odour and an acrid taste. *R. flavispora* has an intense dirty pink reaction to $FeSO_4$ while *Russula boeykensii* has an orange/pink reaction. Spore print (light golden yellow IVb) and lamellae (ochre) darker than spore print of *Russula boeykensii* (Ib–IIb).
   Microscopic: *R. flavispora* has slender spores (7.5–8 × 6.2–6.7 µm) and smaller ornamentation (up to 0.75 µm) compared to these of *Russula boeykensii* ((7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm, ornamentation 0.6–2.1 µm). The warts are more isolated. The hymenial cystidia of *R. flavispora* are longer (72–120 µm) than these of *Russula boeykensii* ((57–) 62.2–71.9–81.5 (–94) × (7–) 7.2–8.2–9.2 (–10) µm). *R. flavispora* pileipellis has elements which has a greying reaction to sulfovanillin while the pileipellis elements of *Russula boeykensii* show no reaction.
2. Sarnari
   Macroscopic: *R. flavispora* has a remarkable odour, with fruity components and a bitter taste in the stipe and acrid taste in the lamellae compared to *Russula boeykensii* with a fruity odour and an acrid taste. The spore print (bright yellow IVb) and lamellae (ochre) of *R. flavispora* are darker than spore print and lamellae of *Russula boeykensii* (Ib–IIb).
   Microscopic: The warts of *R. flavispora* are more isolated compared to *Russula boeykensii*. The hymenial cystidia of *R. flavispora* are longer (70–140 µm) than these of *Russula boeykensii* ((57–) 62.2–71.9–81.5 (–94) × (7–) 7.2–8.2–9.2 (–10) µm).

Russula boeykensii VS. *R. littoralis*

1. Romagnesi
   Macroscopic: *R. littoralis* has an odour like some Lactaria and a mild taste in the stipe and very bitter in the lamellae compared to *Russula boeykensii* with a fruity odour and an acrid taste. The spore print of *R. littoralis* is darker (crème IIc) compared to *Russula boeykensii* (Ib–IIb).
   Microscopic: The spores of *R. littoralis* (6.2–8(–10) × 5.5–6.5(–6.7) µm) are slender compared to the spores of *Russula boeykensii* ((7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm). The basidia of *R. littoralis* are smaller and slender (46–56 × 8.5–10 µm) compared to those of *Russula boeykensii* ((40–) 52.5–58.9–65.2 (–72) × (9–) 10.7–12.1–13.5 (–14) µm). The hymenial cystidia of *R. littoralis* are slender (70–80 × 5–8.5 µm) and

have a stronger reaction in SV (blackening) compared to those of *Russula boeykensii* ((57–) 62.2–71.9–81.5 (–94) × (7–) 7.2–8.2–9.2 (–10) µm, greying in SV).

Russula boeykensii VS. *R. laevis*

1. Adamcik et al.
   Macroscopic: *R. laevis* has an indistinct odour and a slowly acrid taste compared to the fruity odour and acrid taste of *Russula boeykensii*. *R. laevis* has a darker spore print (IIb–d) compared to *Russula boeykensii* (Ib–IIb).
   Microscopic: *R. laevis* has larger spores ((9.2–)9.5–10–10.5(–11.3) × (7.6–)8–8.5–8.9(–9.6) µm) with less fused warts (1–3 fusions in a 3 µm diam. circle) compared to *Russula boeykensii* (7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm, (0–6 fusions in a 3 µm diam. circle). The hymenial cystidia of *R. laevis* are less numerous (moderately numerous, ca. 850–900/mm²) compared to *Russula boeykensii* (abundant, 12000–220000/mm²). *R. laevis* has longer hyphal terminations ((20–)36.5–53.6–71.5(–116) × (4–)5–6–7(–9) µm) compared to *Russula boeykensii* ((14–) 16.9–28.7–40.4(–56) × 3.6–4.9–6.2(–9) µm). The pileocystidia of *R. laevis* are always 1–celled and slightly metachromatic while these of *Russula boeykensii* are often more celled (up to 3–celled) and orthochromatic. The pileocystidia of *R. laevis* are longer ((40–)46.5–79.8–113(>200) × 4.5–5.9–7(–8) µm) compared to these of *Russula boeykensii* ((26–)38.2–71.6–105(–158) × 4–5.1–6.2(–8) µm).

North American species

Russula boeykensii can be ruled out not to be one of the American species based upon a few clear differences.

R. brevipes have larger spores (var. *brevipes* (8.5–)8.7–9.1–9.5(–9.9) × (7.2–)7.5–7.9–8.2(–8.6) µm, *var. megaspora* 9.3–14.1 × 8.0–12.0 µm, var. *acrior* 8–10.6 × 6.7–8.6(–9.6) µm) compared to *Russula boeykensii* ((7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm). The basidia of *R. brevipes* are slightly longer (var. *brevipes* (45–) 55.5–60.7–68 × 9.5–11.4–14 µm) or slender (var. *acrior* 49–74 × 8.0–14.3 µm, var*. megaspora* 53–73 × 9.3–16.0 µm) compared to these of *Russula boeykensii* ((40–) 52.5–58.9–65.2 (–72) × (9–) 10.7–12.1–13.5 (–14) µm). The hymenial cystidia of *R. brevipes* have weakly metachromatic walls while *Russula boeykensii* has orthochromatic cystidia.

R. inopina has smaller spores ((6.5–)6.9–7.2–7.5(–7.8) × (5–)5.2–5.5–5.7(–6) µm), lower spore ornamentation (0.4–0.7 µm) and a higher Q ((1.22–)1.25–1.31–1.37(–1.44)) compared to *Russula boeykensii* ((7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm, ornamentation 0.6–2.1 µm, Q = (1.14–)1.16–1.2–1.24(–1.29)). The basidia of *R. inopina* are slender (8–9,5 µm) compared to these of *Russula boeykensii* ((9–) 10.7–12.1–13.5 (–14) µm). The hymenial cystidia of *R. inopina* do not have appendices while these of *Russula boeykensii* often do.

R. romagnesiana has smaller spores ((5.8–)6–6.3–6.7(–7.3) × (5.1–)5.2–5.4–5.7(–6.1) µm) and smaller ornamentation (0.6–0.9 µm) compared to *Russula boeykensii* ((7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm, ornamentation 0.6–2.1 µm). The hymenial cystidia of *R.*

romagnesiana have weakly metachromatic walls while *Russula boeykensii* is orthochromatic. *R. romagnesiana* has shorter and less broad basidia (40–44–48 × 8.5–9.7–11 µm) compared to *Russula boeykensii* ((40–) 52.5–58.9–65.2 (–72) × (9–) 10.7–12.1–13.5 (–14) µm).

R. vesicatoria has smaller spores ((7–)7.3–7.6–7.9(–8.2) × (5.8–)6–6.3–6.7(–7) µm), smaller ornamentation (0.4–0.7 µm) and an inamyloid suprahilar spot compared to *Russula boeykensii* ((7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm, ornamentation 0.6–2.1 µm) with faintly amyloid to amyloid suprahilar spot. *R. vesicatoria* is distinctly metachromatic in the subhymenium, while *Russula boeykensii* is orthochromatic. *R. vesicatoria* has moderately numerous hymenial cystidia, which are insensitive to sulfovanillin, the hymenial cystidia of *Russula boeykensii* are abundant and have a faint reaction to sulfovanillin. *R. vesicatoria* has shorter and less broad basidia (41–46–50 × 8–9.5–10.5 µm) compared to *Russula boeykensii* ((40–) 52.5–58.9–65.2 (–72) × (9–) 10.7–12.1–13.5 (–14) µm).

R. fuegiana spores (6.8–8.5 × 5.3–7.3 µm) and ornamentation (0.3–0.7 µ) are smaller compared to *Russula boeykensii* ((7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm, ornamentation 0.6–2.1 µm). *R. fuegiana* have slender and shorter basidia (45–57 × 6.7–10.6 µm) compared to *Russula boeykensii* ((40–) 52.5–58.9–65.2 (–72) × (9–) 10.7–12.1–13.5 (–14) µm). The hymenial cystidia of *R. fuegiana* do not have appendices while these of *Russula boeykensii* often do.

R. cascadensis spores (6.7–8.2 × 4.8–6.7 µm) and ornamentation (0.2–0.7 µm) are smaller compared to *Russula boeykensii* ((7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm, ornamentation 0.6–2.1 µm). *R. cascadensis* has shorter and less broad basidia (40–52 × 8–10.6 µm) compared to *Russula boeykensii* ((40–) 52.5–58.9–65.2 (–72) × (9–) 10.7–12.1–13.5 (–14) µm). The hymenial cystidia of *R. cascadensis* do not have appendices while these of *Russula boeykensii* often do.

R. delicula spores (8.1–10.6 × 7–9.4 µm) are larger and ornamentation (0.5–1.6 µm) is smaller compared to *Russula boeykensii* ((7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm, ornamentation 0.6–2.1 µm). The Pileus cuticle of *R. delicula* is thinner (up to 130 µm thick) compared to *Russula boeykensii* (up to 200 µm thick).

Intermediate conclusion: *Russula boeykensii*

Russula boeykensii is most similar to *R. pallidospora* and *R. delicula*. *Russula boeykensii* can be differentiated from *R*. *pallidospora* by a different taste (acrid in *Russula boeykensii* and bitter in *R. pallidospora*) a lighter spore print, broader basidia and shorter hymenial cystidia. *Russula boeykensii* differs from *R. delicula* with a different taste (acrid in *Russula boeykensii* and mild in *R.* delicula) smaller spores and spore ornamentation and a thinner pileipellis.

Russula boeykensii can be distinguished mostly by a light spore print, broad basidia and a large spore ornamentation.

6.1.4. *Russula hampei*

Russula hampei was a species found in Panama, therefore a detailed comparison of this species with the described species of North and South America will be made here.

American species

Russula hampei VS *R. brevipes*

Microscopic: The spores of *R. brevipes* are larger ((8.5–)8.7–9.1–9.5(–9.9) × (7.2–)7.5–7.9–8.2(–8.6) µm) and the spore ornamentation is smaller (0.8–1.3 µm) compared to *Russula hampei* ((6.5–)7.1–7.6–8.2(–8.9) × (5.7–)6.0–6.5–7.1(–7.7) µm, ornamentation 1.0–2.0 µm). The basidia of *R. brevipes* are slightly longer ((45–)55.5–60.7–68 × 9.5–11.4–14 µm) and always 4–spored compared to those *Russula hampei* ((38–) 45.1–49.5–53.8 (–58) × (9–) 11.0–12.1–13.2 (–14) µm) which are often 2–or 3–spored. The hymenial cystidia of *R. brevipes* are less numerous (1500–2500/mm²) and react less strong in sulfovanillin compared to these of *Russula hampei* (2100–4200/mm²). The pileipellis of *R. brevipes* is less deep (ca. 100 µm) and the hyphal terminations are less broad at the margin ((15–)24.5–30.8–43 × 4.5–5.9–7.5 µm) but broader at the centre (14–22.3–28.5(–38) × 5–7.3–10 µm) compared to *Russula hampei* (pileipellis depth 210–300 µm, hyphal termination at the margin(12–) 13.4–26.0–38.5(–71) × (3–)4.8–6.6–8.4(–10) µm and at the centre (15–) 16.0–26.2–36.3(–50) × (3–)3.7–4.8–5.9(–7) µm). The pileocystidia of *R. brevipes* are mostly non-septate (occasionally 2–3 celled), weakly greying in sulfovanillin and the size is less variable compared to those *Russula hampei* which often are septate (1–to 4–celled) and don't react in sulfovanillin.

Russula hampei VS *R. inopina*

Microscopic: *R. inopina* has slightly smaller spores (6.5–)6.9–7.2–7.5(–7.8) × (5–)5.2–5.5–5.7(–6) µm) with a higher Q-value ((1.22–)1.25–1.31–1.37(–1.44)) and lower spore ornamentation (0.4–0.7 µm) compared to *Russula hampei* ((6.5–)7.1–7.6–8.2(–8.9) × (5.7–)6.0–6.5–7.1(–7.7) µm, Q = (1.05–)1.13–1.17–1.22(–1.26), spore ornamentation 1.0–2.0 µm). The suprahilar spot is inamyloid to partly amyloid in *R. inopina* while these of *Russula hampei* are amyloid. The 4–spored basidia of *R. inopina* are longer and less broad ((52–)57–63.5–70(–80) × 8–9.5 µm) compared to the 2–to 4–spored basidia of *Russula hampei* (38–) 45.1–49.5–53.8 (–58) × (9–) 11.0–12.1–13.2 (–14) µm. The hymenial cystidia of *R. inopina* are longer (71–83.3–93(–110) × 7–8.6–10 µm) compared to those of *Russula hampei* ((50–) 56.8–64.5–73.2 (–75) × (6–) 6.9–8.5–10.0 (–12) µm). The pileocystidia of *R. inopina* react weakly greying in sulfovanillin and are less broad (3.5–5–7(–8) µm) compared to *Russula hampei* ((3–)–5.1–8.4–(12) µm), which show no reaction in sulfovanillin.

Russula hampei VS R. romagnesiana

Microscopic: *R. romagnesiana* has smaller spores ((5.8–)6–6.3–6.7(–7.3) × (5.1–)5.2–5.4–5.7(–6.1) µm) and spore ornamentation (0.6–0.9 µm) compared to *Russula hampei* ((6.5–)7.1–7.6–8.2(–8.9) × (5.7–)6.0–6.5–7.1(–7.7) µm, ornamentation 1.0–2.0 µm). The 4–spored basidia of *R. romagnesiana* are smaller and less broad (40–44–48 × 8.5–9.7–11 µm) compared to *Russula*

hampei which has 2–to 4–spored basidia ((38–) 45.1–49.5–53.8 (–58) × (9–) 11.0–12.1–13.2 (–14) µm). *R. romagnesiana* have longer and less broad hymenial cystidia ((47–)60.5–75.6–91(–103) × 7–7.3–8 µm) that react less to sulfovanillin (weak) compared to *Russula hampei* ((50–) 56.8–64.5–73.2 (–75) × (6–) 6.9–8.5–10.0 (–12) µm, blackening in sulfovanillin). The pileipellis of *R. romagnesiana* is less deep (100 µm) and has distinct zebroid wall incrustations compared to these of *Russula hampei* (210–300 µm) without zebroid wall incrustations. *R. romagnesiana* has less broad pileipellis hyphae (3.5–4.5–6 µm) compared to *Russula hampei* ((3–)4.8–6.6–8.4(–10) µm). The pileocystidia of *R. romagnesiana* are mostly 1–celled while these of *Russula hampei* are often more celled.

Russula hampei VS R. vesicatoria

R. vesicatoria has slightly smaller spores ((7–)7.3–7.6–7.9(–8.2) × (5.8–)6–6.3–6.7(–7) µm), smaller spore ornamentation (0.4–0.7 µm) and slightly higher Q-value (Q=(1.12–)1.15–1.19–1.23(–1.31)) compared to *Russula hampei* ((6.5–)7.1–7.6–8.2(–8.9) × (5.7–)6.0–6.5–7.1(–7.7) µm, ornamentation 1.0–2.0 µm, Q=(1.05–)1.13–1.17–1.22(–1.26)). The suprahilar spot of *R. vesicatoria* is inamyloid, while this of *Russula hampei* is amyloid. The 4–spored basidia of *R. vesicatoria* are smaller and less broad (41–46–50 × 8–9.5–10.5 µm) compared to these of *Russula hampei* ((38–) 45.1–49.5–53.8 (–58) × (9–) 11.0–12.1–13.2 (–14) µm), which are 2–to 4–spored. *R. vesicatoria* has less numerous (ca. 1000–1200/mm²), longer and less broad hymenial cystidia, which are insensitive to sulfovanillin and Cresyl blue (orthochromatic), compared to *Russula hampei* (density 2100–4200/mm², (50–) 56.8–64.5–73.2 (–75) × (6–) 6.9–8.5–10.0 (–12) µm, strong reaction to sulfovanillin and slightly metachromatic). The pileipellis of *R. vesicatoria* are orthochromatic and less deep (ca. 150–250 µm) compared to *Russula hampei* (metachromatic and 210–300 µm deep). *R. vesicatoria* has longer and more slender hyphal terminations ((29–)37–52–67(–91) × 2–3 µm) compared to *Russula hampei* (cells (12–) 13.4–26.0–38.5(–71) × (3–)4.8–6.6–8.4(–10) µm). The pileocystidia of *R. vesicatoria* are always 1–celled while these of *Russula hampei* are often more celled.

Russula hampei VS *R. fuegiana*

R. fuegiana has similar spore sizes but smaller spore ornamentation (0.3–0.7 µm) and less conspicuous suprahilar spot (finely and faintly ornamented, occasionally devoid of ornamentation) compared to *Russula hampei* (spore ornamentation 1.0–2.0 µm, suprahilar spot distinctly amyloid). The basidia of *R. fuegiana* are less broad (45–57 × 6.7–10.6 µm) and 4–spored compared to *Russula hampei* which have 2–to 4–spored basidia ((38–) 45.1–49.5–53.8 (–58) × (9–) 11.0–12.1–13.2 (–14) µm). The hymenial cystidia of *R. fuegiana* are longer and less broad (53–90 x 6.7–8.6 µm) compared to *Russula hampei* ((50–) 56.8–64.5–73.2 (–75) × (6–) 6.9–8.5–10.0 (–12) µm). *R. fuegiana* pileipellis is less deep (120–173 µm) and the hyphae are less broad (1.0–5.3(–4.3) µm) compared to *Russula hampei* (pileipellis depth 210–300 µm, hyphae width (3–)4.8–6.6–8.4(–10) µm).

Russula hampei VS R. cascadensis

R. cascadensis spores are less broad (6.7–8.2 × 4.8–6.7 µm), have smaller ornamentation (0.2–0.7 µm) and a weaker amyloid suprahilar spot (weakly amyloid) compared to *Russula hampei* ((6.5–)7.1–7.6–8.2(–8.9) × (5.7–)6.0–6.5–7.1(–7.7) µm, ornamentation 1.0–2.0 µm, suprahilar spot amyloid). The basidia of *R. cascadensis* are less broad (40–52 × 8.0–10.6 µm) and 4–spored compared to *Russula hampei* ((38–) 45.1–49.5–53.8 (–58) × (9–) 11.0–12.1–13.2 (–14) µm) which are 2–to 4–spored. The hymenial cystidia of *R. cascadensis* are less broad (47–86 × 5.3–8.0 µm) compared to *Russula hampei* ((50–) 56.8–64.5–73.2 (–75) × (6–) 6.9–8.5–10.0 (–12) µm). The pileipellis hyphae are less broad (1.3–5.3 µm) compared to *Russula hampei* ((3–)4.8–6.6–8.4(–10) µm).

Russula hampei VS *R. delicula*

R. delicula has larger spores (8.1–10.6 × 7.0– 9.4 µm), slightly smaller ornamentation (0.5–1.6 µm) and a less amyloid suprahilar spot (Weakly amyloid) compared to *Russula hampei* ((6.5–)7.1–7.6–8.2(–8.9) × (5.7–)6.0–6.5–7.1(–7.7) µm, ornamentation 1.0–2.0 µm, suprahilar spot amyloid). The basidia of *R. delicula* are longer, less broad and 4–spored (47–64 × 8.0–13.3 µm) compared to *Russula hampei* ((38–) 45.1–49.5–53.8 (–58) × (9–) 11.0–12.1–13.2 (–14) µm, 2–to 4–spored). The hymenial cystidia of *R. delicula* often are appendiculate while these of *Russula hampei* rarely are appendiculate. The pileipellis of *R. delicula* are less deep (up to 130µm) and have less broad hyphae (1.0–5.3 µm) compared to *Russula hampei* (pileipellis depth 210–300 µm, hyphae width (3–) 4.8–6.6–8.4(–10) µm).

Russula hampei VS. *R. idroboi*

R. idroboi has larger spores (8.8–11 × 8.2–9 µm) and smaller ornamentation (0.6–1 µm) compared to *Russula hampei* ((6.5–)7.1–7.6–8.2(–8.9) × (5.7–)6.0–6.5–7.1(–7.7) µm, ornamentation 1.0–2.0 µm). The basidia of *R. idroboi* are always 4-spored while these of *Russula hampei* are often 2- or 3-spored. *R. idroboi* has hymenial cystidia with banded contents, these contents are absent in *Russula hampei*.

Russula hampei VS R. littoralis

The spores of *R. littoralis* are less broad (5.5–6.5– (6.7) µm) and have lower ornamentation (low) compared to *Russula hampei* ((5.7–)6.0–6.5–7.1(–7.7) µm, ornamentation normal to high 1–2 µm). *R. littoralis* has less broad 4–spored basidia (8.5–10 µm) compared to *Russula hampei* with 2–to 4-spored basidia ((9–) 11.0–12.1–13.2 (–14) µm). The hymenial cystidia of *R. littoralis* are longer and less broad (70–80 × 5–8.5 µm) compared to *Russula hampei* ((50–) 56.8–64.5–73.2 (–75) × (6–) 6.9–8.5–10.0 (–12) µm). The pileocystidia of *R. littoralis* are less broad (1.5–4 µm) compared to *Russula hampei* ((3–)3.4–5.9–8.4(–12) µm).

Russula hampei VS *R. aucarum*

R. aucarum Singer (Singer, 1975) is the type species from the section *Delicoarchaeae* Singer (Singer, Araujo, & Ivory, 1983). There has been debate about the distinction between section *Delicoarchaeae* and subgenus *Brevipedum* (the former subsection *Lactarioideae*) on the one hand and section *Delicoarchaeae* and section *Metachromaticae* Singer (Pegler & Singer, 1980) on the other hand (Buyck & Ovrebo, 2002; Barbosa, 2016).
The basidia of *R. aucarum* are similar in size compared to these of *Russula hampei* however they always are 4-spored while these of *Russula hampei* are often 2- or 3-spored. The hymenial cystidia of *R. aucarum* are less dense (600–900/mm²), smaller and slender (44–63 × 7–8 µm) and less reactive to sulfovanillin (scarcely reacting) compared to *Russula hampei* (density 2100 –4200/mm², (50–) 56.8–64.5–73.2 (–75) × (6–) 6.9–8.5–10.0 (–12) µm, strongly reacting in sulfovanillin). The hyphae (2–4 µm) and pileocystidia (2–5 µm) of the pileipellis of *R. aucarum* are slender compared to *Russula hampei* (hyphae width (3–)4.8–6.6–8.4(–10) µm, pileocystidia width (3–)3.4–5.9–8.4(–12) µm). In the description of *R. aucarum* is nothing mentioned about the reaction in Cresyl Blue despite the mentioning of this in the other described species (Buyck & Ovrebo, 2002).

Russula hampei VS. *R. herrerae*

Russula hampei has no veil residue and is thus different from *R. herrerae.*

Russula hampei VS. R. metachromatica

Since *Russula hampei* is metachromatic a comparison with *R. metachromatica* Singer (Singer, 1952) is also made. This species belongs to the section *Metachromaticae* which is characterised by hymenial cystidia with thick metachromatic walls in Cresyl Blue.

R. metachromatica has larger spores ((9–)11–12.3 × (8–)10–11 µm) and smaller spore ornamentation ((1–)1.2–1.4 µm) compared to *Russula hampei* ((6.5–) 7.1–7.6–8.2(–8.9) × (5.7–)6.0–6.5–7.1(–7.7) µm, ornamentation 1.0–2.0 µm). The basidia of *R. metachromatica* colour blue in Cresyl Blue, while the basidia of *Russula hampei* do not react to Cresyl Blue. The hymenial cystidia of *R. metachromatica* broader (48–96 × 9.5–15.5 µm) and have thicker walls which are strongly metachromatic compared to *Russula hampei* ((50–) 56.8–64.5–73.2 (–75) × (6–) 6.9–8.5–10.0 (–12) µm) with thin walls which are slightly metachromatic.

European species

Russula hampei is slightly metachromatic in Cresyl Blue both in the lamellae and the pileipellis, this character distinguishes this species from the European species which are all orthochromatic (except for *R. laevis*). Therefore, no comparison with these species will be made, except for a very short comparison with *R. laevis*. *R. laevis* has larger spores, a higher Q-value and longer hymenial cystidia compared to *Russula hampei*, based on these we can assume they are different species.

Intermediate conclusion: *Russula hampei*

Russula hampei is most similar to *R. aucarum* despite the lack of information about Cresyl Blue reaction. *Russula hampei* differs from *R. aucarum* by basidia that can have less than 4 sterigmata, higher hymenial cystidia density, which have a strong reaction to sulfovanillin and the hyphae and pileocystidia from the pileipellis are broader.

Russula hampei is mostly characterised by basidia which are 2-to 4-spored, a strong reaction to sulfovanillin in the hymenium and slightly metachromatic (lamellae stronger reaction compared to pileipellis) reaction to Cresyl Blue.

6.1.5. Comparison of the 4 newly described species

	R. macrostigma	*R. zebrihyphis*	*Russula boeykensii*	*Russula hampei*
Pileus	77–127 mm diam.	85–160 mm diam.		
Lamellae	6–9L + 3–5l/cm, furcation's absent or rare	5–8L + 1–2l/cm, furcation's absent or rare		
Stipe	25–26 x 25–29 mm	20–40 x 25–55 mm		
Taste and odour	Taste mild, afterwards slightly acrid Odour fruity	Taste mild, sometimes very light sharp tinge Odour fruity, flowery, like *R. pectinatoides* (fishy when old).	Taste acrid Odour fruity	
Spore print	White to pale cream (Ib–IIa)	White to pale cream (Ib–IIa)	Whitish to pale cream (Ia–IIb)	
FeSO4	Brown-orange	No reaction	Orange or pink, afterwards grey	
Guaiac	Strong and fast reaction	Strong and fast reaction	Strong and fast reaction	
KOH	Stipe and lamellae yellowish, context yellow to slightly reddening	No reaction		
Spore size	(7.1–)8.3–9.1–9.8(–10.8) × (5.3–)6.7–7.2–7.8(–8.4) µm	(7.7–)8.5–9.4–10.2(–11.6) × (6.6–)7.2–7.9–8.6(–10.2) µm	(7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm	(6.5–)7.1–7.6–8.2(–8.9) × (5.7–)6.0–6.5–7.1(–7.7) µm
Spore shape	Broadly ellipsoid to ellipsoid, Q = (1.15–)1.19–1.25–1.31(–1.43)	Subglobose to broadly ellipsoid, Q = (1.1–)1.12–1.19–1.25(–1.33)	Subglobose to broadly ellipsoid, Q = (1.14–)1.16–1.2–1.24(–1.29)	Subglobose to broadly ellipsoid, Q = (1.05–)1.13–1.17–1.22(–1.26)
Spore ornamentation	Small sized (0.2–0.5 µm high), moderately distant to dense [3–8 in a 3 µm diam. circle] amyloid warts, , locally subreticulate, sometimes fused in chains (3–5 fusions in a 3 µm diam. circle), connected with occasional line connections [0–3(–7) line connections in a 3 µm diam. circle]	Normal to high (0.9–1.6 µm), moderately distant to very dense [4–12 in a 3 µm diam. circle] amyloid warts, locally subreticulate, sometimes fused in chains (0–9 fusions in a 3 µm diam. circle), connected with occasional to frequent line connections [0–2(–6) line connections in a 3 µm diam. circle]	Normal to high (0.6–2.1 µm), distant to very dense [2–12 in a 3 µm diam. circle] amyloid warts, locally subreticulate, sometimes fused in chains (0–6 fusions in a 3 µm diam. circle), connected with occasional to frequent line connections [0–2(–6) line connections in a 3 µm diam. circle]	Normal to high (0.1–0.2), moderately distant to very dense [4–13 in a 3 µm diam. circle] amyloid warts, locally subreticulate, sometimes fused in chains (0–6 fusions in a 3 µm diam. circle), connected with occasional to abundant line connections [0–6(–9) line connections in a 3 µm diam. circle]
Suprahilar spot	Large, amyloid	Large, amyloid	Irregular, faintly amyloid to amyloid	Irregular, amyloid
Basidia	(38–) 45.2–54.3–63.4 (–80) × (5–) 8.9–11.4–13.9 (–19) µm, 4-spored	(37–) 43.9–52.4–60.8 (–69) × (10–) 11.1–13.4–15.9 (–19) µm, 4-spored	(40–) 52.5–58.9–65.2 (–72) × (9–) 10.7–12.1–13.5 (–14) µm, 4-spored	(38–) 45.1–49.5–53.8 (–58) × (9–) 11.0–12.1–13.2 (–14) µm, 2-to 4-spored
Hymenial cystidia	Numerous to abundant, ca. 38000/mm², (43–) 56.5–72.4–88.3 (–110) × (4–) 6.1–7.7–9.3 (–11) µm, with or without appendage 2–6.63(–10) µm long. React weakly (greyish) in sulfovanillin	Abundant, 5000–20000/mm², (45–) 50.9–65.9–80.8 (–108) × (7–) 7.9–9.1–10.1 (–11) µm, mostly with appendage 1–4.1(–9) µm long. React strong (blackening) in sulfovanillin	Moderately numerous to numerous, 1200–2200/mm², (57–) 62.2–71.9–81.5 (–94) × (7–) 7.2–8.2–9.2 (–10) µm, often with appendage 1–4(–5) µm long. React weakly (greyish) in sulfovanillin	Numerous to abundant, 2100–4200/mm², (50–) 56.8–64.5–73.2 (–75) × (6–) 6.9–8.5–10.0 (–12) µm, mostly without appendage 1–3.5(–7) µm long React strong (blackening) in sulfovanillin, slightly metachromatic
Pileipellis	Orthochromatic, terminal hyphae cells (12–) 20.04–28–35.96(–42) × 3–5.22–7.43(–11) µm 140–250 µm deep	Orthochromatic, terminal hyphae cells (10–) 18.4–37.3–56.3(–118) × (2–)3.4–6.5–9.6(–13) µm 150–540 µm deep	Orthochromatic, terminal hyphae cells (14–) 16.9–28.7–40.4(–56) × 3.6–4.9–6.2(–9) µm 75–200 µm deep	Slightly metachromatic, terminal hyphae cells (12–) 13.4–26.0–38.5(–71) × (3–)4.8–6.6–8.4(–10) µm 210–300 µm deep
Pileocystidia near pileus margin	1-to-5 celled, (14–)34.8–58.5– 82.3(–131) × (2–)3.45–5.19–6.93(–10) µm	1-to-4 celled, (28–)45.5–91.4–137(–237) × (3–)3.8–7.8–11.7(–23) µm	1–to–3 celled, (26–)38.2–71.6–105(–158) × 4–5.1–6.2(–8) µm, often with appendage	1-to-4 celled, (23–)33.3–63.3–93.2(–124) × (3–)3.4–5.9–8.4(–12) µm, rarely with appendage
Pileocystidia near pileus centre	(20–)39.7–65.9–92.1(135) × (3–)2.97–5.16–7.34(–11) µm	(27–)30.7–47–63.3(–84) × (3–)3.5–5.6–7.7(–11) µm	(32–)33.9–59.3–84.8(–106) × (3–)3.2–4.3–5.4(–7) µm	1- to 2-celled, (18–)28.1–48.9–69.6(–90) × (3–)3.6–4.4–5.1(–6) µm

6.2. Species description

Not all possible characters are described within this manuscript, characters such as sphaerocytes and surface of the stipe are not described. The characters that are described are deemed the most diagnostic (Adamčík *et al.*, 2019).

Even though macro-morphological descriptions are important for species descriptions and delimitation, parts of the description are lacking for *Russula hampei* this due to lack of or missing of the notes taken by the collectors. The dried specimens of *Russula hampei* were accompanied by 2 photographs without reference scale, which made measuring on the photographs impossible. *Russula boeykensii* was not accompanied by any photographs or size measurements.

The spores that have been observed were extracted from the lamellae due to lack of spore prints. The handling needed to extract the spores from the lamellae can damage ornamentation, make the ornamentation less regular, seem more connected and more numerous (Adamčík *et al.*, 2019).

R. sect. Metachromaticae and *R.* sect. *Delicoarchaeae* have both been proposed to be synonyms to *R.* subg. *Brevipedum* (Buyck & Ovrebo, 2002; Barbosa, 2016). The tropical species within *R.* subg. *Brevipedum* are probably more ancient compared to the temperate species (Buyck *et al.*, 2018). Further molecular research is still needed to fully understand the relationship and placement of the different species within the phylogeny of *R.* subg. *Brevipedum*. And if *R.* sect. *Metachromaticae* and *R.* sect. *Delicoarchaeae* should be absorbed within *R.* subg. *Brevipedum*.

6.3. Molecular analysis

6.3.1. Host of *R. zebrihyphis*

The plant OTU's assigned to the root tip samples of *R. zebrihyphis* are *Salix caprea*, *Quercus dentata* and *Quercus petrea*. Since the used marker, ITS1, is not the most optimal plant primer we only consider the genus and not the specific species. The host of *R. zebrihyphis* is most probably a *Salix* or *Quercus* species. This is based upon DNA extracted and amplified from root tips. Despite these genera not being listed as being in close proximity it is still possible since the distance between host plant and occurring fruitbodies can be quite substantial.

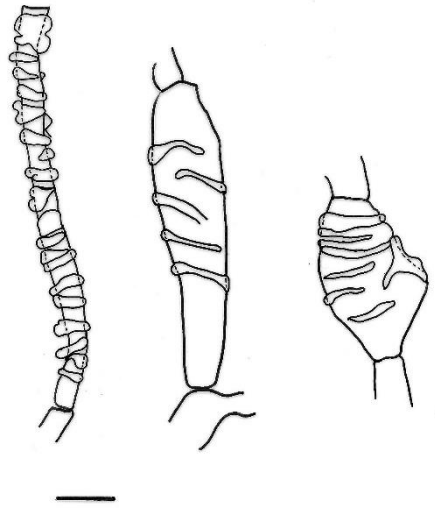
6.3.2. Molecular likeness

Only a few holotypes, isotypes and paratypes can be found within the phylogenetic tree (figure 2). Despite the limited number of 'types' present within this tree, there are 2 paratypes for the same species, namely *R. delica* var. *delica,* and an isotype and paratype for another species, namely *R. delica* var. *trachyspora*. These types for both species are not placed next to each other or even close together. Some descriptions appear to be based upon collections of specimens which do not belong to the same species, probably caused by the high likeliness between species within this subgenus. This is one more indication of the importance and the necessity of a thorough revision of all described species and the correct assignment of type species.
This tree is used to determine to which species the newly described species are closest related. For this only the holotypes, paratypes and isotypes are considered since we cannot assume that the assigned names are correct when working with a group with so many cryptic species. The references below to the closest related species is thus based upon the presence of type species in the phylogentic tree. The true closest related species is often not yet described. *R. zebrihyphis* is

closest related to *R. laevis*, both these species are found in Scandinavia. For the other species it is less clear; *R. macrostigma* and *Russula boeykensii* are quite closely related; *Russula hampei* is quite closely related to the isotype of *R. delica* var. *trachyspora*.

6.4. Zebroid incrustations

The zebroid incrustations that were observed appear to be banded incrustations on the hyphae of the pileipellis, both on slender and inflated cystidia segments, but never on 2 or more consecutive segments. The incrustations appear to be like the stripes of zebras, meaning that it is not a closed connective network but separate irregular bands. These incrustations were mostly observed in *R. zebrihyphis* where they are fairly abundant, it can also be observed in the other species but less frequent.



6.5. American species

A feature that seems to be present in some of the American species of *R.* subg. *Brevipedum*, and is absent in all European species (except R. laevis) of this subgenus, is metachromatic reaction to Cresyl Blue. Can this be a way to differentiate American and European species or is this due to lack of use of this reagent in, the often older, description of European species? A revision of the European species could also provide an answer this question since the widespread use of Cresyl Blue is only documented from 1989 onwards (Buyk, 1989).

6.6. Improvements

6.6.1. Uniform description

As mentioned in the introduction, species descriptions are often not complete, and this makes the process of comparing different species challenging. This is especially true for the species within R. subg. Brevipedum since at first glance they all look alike, with their whitish cap, stipe and lamellae. A revision of the described species within *R.* subg. *Brevipedum*, mainly the European species, could be a great improvement to have more uniform descriptions, clarification of the confusion around the different description of *R. delica* and could contribute to the discovery of new pseudo-cryptic species. Older descriptions especially lack the reaction of some reagents, density of hymenial cystidia and the differences between the lamellae sides and lamellae edge on the one hand and pileipellis edge and pileipellis centre on the other hand.

A comprehensive description of freshly collected specimens should be strived for since elements like colour, odour and taste change during the drying process. Using templates (Adamčík *et al.*, 2019) for all microscopical characters will help unify the species description and this will make comparing species easier.

6.6.2. Cresyl Blue

In the article of (Adamčík *et al.*, 2019) Cresyl Blue is referred to as a reagent to detect metachromatic incrustation in the pileipellis, despite previous use of it to detect metachromatic incrustations or cystidia walls in hymenium. In *Russula hampei* the metachromatic reaction is barely visible in the pileipellis and could therefore be overlooked but the reaction in the hymenium is stronger, while still less compared to *R. metachromatica.* Cresyl Blue should be used both in pileus and hymenium.

6.6.3. Molecular work

Only a limited number of markers have been used for this study, more markers could give an even clearer view and increase the bootstrap support. Analysis of root tip samples should always be combined with fruitbody samples to enhance the success rate.

7. Conclusion

Russula subgenus *Brevipedum* is a group which demands further investigation to discover all the species and clear up the relationship of this subgenus and *R.* sect. *Metachromaticae* and *R.* sect. *Delicoarchaeae*. All the species within this subgenus are characterised by a whitish cap colour and the presence of lamellulae. It is a challenging group that consist of a few described species and a bunch of pseudo-cryptic species. Only 7 species of this subgenus have been described within Europe despite DNA analysis predictions of 31 European species.

Within this work 3 new European species and 1 American species are described and compared to the previous described species. Differences were found, this indicates that these are pseudo-cryptic species instead of cryptic species since they only have molecular differences.

R. macrostigma is can be distinguished mostly by a light spore print, a large and distinctly amyloid suprahilar spot, quite large spores and broad basidia. It is closely related to *Russula boeykensii* and is most similar to *R. delica* as described in Shaffer (1964), *R. delica* var*. trachyspora* and *R. delica* var *puta*. *R. zebrihyphis* can be distinguished by light spore print, large spores, a strong reaction to sulfovanillin in the hymenium, broad basidia and the presence of zebroid incrustations on the hyphae of the pileipellis. It is closely related to another Scandinavian species; *R. laevis* and is most similar to *R. littoralis*, *R. delica var. puta* and *R. delica var. trachyspora*. *Russula boeykensii* can be distinguished by a light spore print, broad basidia and large spore ornamentation. As said above it is closely related to *R. macrostigma* and is most similar to *R. pallidospora* and *R. delicula. Russula hampei* most characteristic features are the 2- to 4-spored basidia, the strong reaction in sulfovanillin in the hymenium and the slightly metachromatic in Cresyl Blue both in the hymenium and pileipellis. It is most closely related to *R. delica* var. *trachyspora* and is most similar to *R. aucarum*.

The PCR success rate from the root tip samples was very low this could be due to the few markers that were used. Only for *R. zebrihyphis* the amplification of the fungal markers ITS1, ITS2 and the plant marker ITS1 was successful. The host of *R. zebrihyphis* are most likely *Salix* and *Quercus*.

There are efforts to increase the uniformity of *Russula* descriptions, this will also make comparison species easier. Slavomir et al. (2019) have made templates for the microscopic description of species. These could introduce a consensus about what features should be measured and which reagent should be used and how.

There is a lack of types species within *Russula* subgenus *Brevipedum* and the few that are present showcase discrepancy. Type species are necessary to delimit all species within this genus and should provide correct information.
We can conclude that this subgenus still demands research; a revision of the described species and the correct assignment of type species needs to be made, on top of the description and delimitation of new species.

8. Comparisons in table form

8.1. *R. macrostigma*

8.1.1. *R. macrostigma* VS. European species (Romagnesi, Sarnari, Shaffer)

	R. macrostigma	*R. delica*	*R. delica* var. *puta*	*R. delica* var. *trachyspora*	*R. delica* var. *delica*	*R. delica* var. *puta*	*R. delica*
Odour and taste	Odour fruity, taste mild and afterwards acrid	complex odour of fruit and fish	very faint odour	complex odour of fruit and fish	strong and un-pleasant odour, peachy and salty (fruity when young), peaty flavour in the lamellae		
stipe	25–26 × 25–29 mm		longer and less broad (35–65 × 13–17 mm)		longer (25–48 mm)	less broad (15–20 mm)	
$FeSO_4$	Brown-orange	faint pink-orange			pale pink		
Guaiac	Immediate strong positive reaction	positive reaction but not always immediate					
spore print	Pale cream Ib–IIa						
spore size, shape, Q-value	8.31–9.05–9.78 × 6.71–7.24–7.77 µm, broadly ellipsoid to ellipsoid, Q = 1.19–1.25–1.31	larger (8–10–11.5 × 6.5–8.7 µm)			larger (8.5–11.2 × 7–9 µm), subglobose		
spore ornamentation	Small; 0.2–0.5 µm	larger (0.5–0.7–1.0 µm)		higher (1.0–1.5 µm)	higher (0.8–1.0 µm)	higher (1.0 –1.2 µm)	higher (0.4 – 1.0 µm)
suprahilar spot	Large and strongly amyloid						weakly amyloid
hymenial cystidia	56.5–72.4–88.3 × 6.1–7.7–9.3 µm, SV faint reaction	longer (65–150µm), SV strong reaction	longer (100–120µm), SV strong reaction	longer (78–135µm), SV strong reaction	longer and thicker (78–150 × 9–13 µm)		

	R. macrostigma	*R. chloroides*	*R. pseudodelica*	*R. pallidospora*	*R. flavispora*	*R. littoralis*	*R. laevis*
Odour and taste	Odour fruity, taste mild and afterwards acrid		Acrid-pungent taste	Complex smell, with a fruity component, mild (slightly refreshing) taste, later bitter	Remarkable odour with fruity notes, taste is bitter in the stipe and acrid in the lamellae	Odour similar to Lactarius, mild taste in stipe, very bitter in the lamellae	
Pileus	77–127 mm	Bigger (var. *chloroides* 45–150 mm), smaller (var. *parvispora* 45–100 mm)		Bigger (60–150 mm)			Smaller (40–75 mm), Shiny
Stipe	25–26 × 25–29 mm	Longer (var. *chloroides (*15–)30–50(–90) mm, var. *parvispora* 25–40 mm)		Longer (25–50 mm)			
$FeSO_4$	Brown-orange	dirty red, var. *chloroides* & var. *parvispora* pink-orange, var. *trachyspora* pinkish		Pinkish/ pale pink-orange	Intense dirty pink/ pink-orange	Pink	
Guaiac	Immediate strong positive reaction	var. *chloroides* slow, green		Slow, dirty blue			
Spore print	Pale cream Ib–IIa		Darker (ochraceous custard)	Darker (IIc–d)	Darker (bright yellow IVb)	Darker (crème IIc)	Darker (IIb–d)
Spore size, shape, Q-value	8.3–9.1–9.8 × 6.7–7.3–7.8 µm, broadly ellipsoid to ellipsoid, Q = 1.19–1.25–1.31	Smaller var. *parvispora* (6.4–8.0 × 6.0–6.7 µm), bigger var. *chloroides* 8–11.2 × 7.2–8.8 µm, var. *trachyspora* 9.5–11.4 × 8–10.5 µm	Less broad (6.9–9.3 × 6.3–7.0 µm)	Smaller (7.2–9.0 × 5.9–7 µm)	Smaller (6.4–8.6 × 5.5–6.8 µm)	Slender (5.5–6.5(–6.7) µm)	Larger (9.5–10–10.5 × 8–8.5–8.9 µm), lower Q (1.16–1.18–1.21)
Spore ornamentation	Small; 0.2–0.5 µm, subreticulate	Higher (1–1.6 µm)	Higher (0.5–1.3 µm)		Higher (up to 0.75 µm), warts more isolated		Higher (0.8–1.1(–1.3) µm)
Suprahilar spot	Large and strongly amyloid		Ornamented but lower, often weakly amyloid		Vague, very faintly amyloid	Not clearly differentiated	Small and irregular, partly amyloid to amyloid
Basidia	(45.2–54.3–63.4 × 8.9–11.4–13.9 µm)		Slender (8–11 µm)			Smaller, slender (46–56 × 8.5–10 µm)	
Hymenial cystidia	56.5–72.4–88.3 × 6.1–7.7–9.3 µm, SV faint reaction, orthochromatic, numerous-abundant (38000/mm²)	var. *parvispora* colouring black in SV	Longer (65–165 µm)		Longer (70–140 µm)	Blackening in SV	Less dense (moderately numerous, 850–900/mm²), dark grey-brown in SV
Pileipellis			Slender (hyaline hyphae 1.0–2.6 µm broad and oleiferous hyphae 2.0–5.3 µm broad).		Greying in SV		Hyphal terminations longer (36.5–53.6–71.5 × 5–6–7 µm), pileocystidia slightly metachromatic, always 1–celled

8.1.2. *R. macrostigma* VS. North American species

	R. macrostigma	*R. brevipes*	*R. inopina*	*R. romagnesiana*	*R. vesicatoria*	*R. fuegiana*	*R. cascadensis*	*R. delicula*
Odour and taste	Odour fruity, taste mild and afterwards acrid						Intense acrid taste	
Spore size, shape, Q-value	8.31–9.05–9.78 × 6.71–7.24–7.77 µm, broadly ellipsoid to ellipsoid, Q = 1.19–1.25–1.31	Broader; lower Q (1.11–1.16–1.21) Var. *megaspore* larger spores (9.3–14.1 × 8.0–12.0 µm)	Smaller (6–6.3–6.7× 5.2–5.4–5.7µm), lower Q (1.12–1.16–1.2)	Smaller (6–6.3–6.7 × 5.2–5.4–5.7 µm), lower Q (1.12–1.16–1.2)	Smaller (7.3–7.6–7.9 × 6–6.3–6.7 µm), lower Q (1.15–1.19–1.23)	Smaller (6.8–8.5 × 5.3–7.3 µm)	Smaller (6.7–8.2 × 4.8–6.7 µm)	Bigger (8.1–10.6 × 7–9.4 µm)
Spore ornamentation	Small; 0.2–0.5 µm, subreticulate	Higher (0.7–1.7 µm) also for var. *acrior*				Rarely forming a partial to broken reticulum		Higher (0.5–1.6 µm)
Suprahilar spot	Large and strongly amyloid	Var. *acrior* weakly amyloid	Small, inamyloid – partly amyloid	Small	Small, inamyloid	Finely and faintly ornamented	Weakly amyloid – no ornamentation	Weakly amyloid
Basidia	(45.2–54.3–63.4 × 8.9–11.4–13.9 µm)		Slender (8–9,5 µm)	Shorter, slender (40–44–48 × 8.5–9.7–11 µm)	Shorter, slender (41–46–50 × 8–9.5–10.5 µm)		Shorter, slender (40–52 × 8–10.6 µm)	
Hymenial cystidia	56.5–72.4–88.3 × 6.1–7.7–9.3 µm, often with appendices, SV faint reaction, orthochromatic, numerous-abundant (38000/mm²)	Weakly metachromatic walls	No appendices	Weakly metachromatic walls	Metachromatic, insensitive to SV, moderately numerous hymenial cystidia (1000–1200/mm²)	No appendices	No appendices	

8.2. *R. zebrihyphis*

8.2.1. *R. zebrihyphis* VS European species (Romagnesi, Sarnari, Shaffer)

	R. zebrihyphis	*R. delica*	*R. delica* var. *puta*	*R. delica var. trachyspora*	*R. delica* var. *delica*	*R. delica* var. *puta*	*R. delica*
Odour and taste	Mild taste (sometimes slight sharp tinge), odour fruity, flowery, pectinatoides (fishy when old)	complex odour of fruit and fish	very faint odour	complex odour of fruit and fish	strong and un-pleasant odour, peachy and salty (fruity when young), peaty flavour in the lamellae		
$FeSO_4$	No reaction	faint pink-orange	pink-red		pale pink		
Guaiac	Immediate strong positive reaction	positive reaction but not always immediate					
spore size, shape, Q-value	(7.7–)8.5–9.4–10.2(–11.6) × (6.6–)7.2–7.9–8.6(–10.2) µm, Q= (1.1–)1.12–1.19–1.25(–1.33)						Slender (8.2–10.8 × 6.9–8.1 µm)
spore ornamentation	0.9–1.6 µm, subreticulate	Smaller (0.5–0.7–1.0 µm	Smaller (up to 0.85 µm)		Smaller (0.8–1.0 µm)	Smaller (1.0 –1.2 µm)	Smaller (0.4 – 1.0 µm)
Suprahilar spot	Large and strongly amyloid						Weakly amyloid
Basidia	(37–) 43.9–52.4–60.8 (–69) × (10–) 11.1–13.4–15.9 (–19) µm						
hymenial cystidia	56.5–72.4–88.3 × 6.1–7.7–9.3 µm, often with appendices, SV strong reaction, orthochromatic	Longer and slender (65–150 × (6.5–)7.2–11.5(–13.5) µm	Longer and slender (100–120 × 6.5–10 µm)	Longer and slender (78–135 × 6–11.5 µm)	longer and thicker (78–150 × 9–13 µm)		

	R. zebrihyphis	*R. chloroides*	*R. pseudodelica*	*R. pallidospora*	*R. flavispora*	*R. littoralis*	*R. laevis*
Odour and taste	Mild taste (sometimes slight sharp tinge), odour fruity, flowery, pectinatoides (fishy when old)	Mild in stipe, acrid and unpleasant in lamellae	Acrid-pungent taste	Complex smell, with a fruity component, mild (slightly refreshing) taste, later bitter	Remarkable odour with fruity notes, taste is bitter in the stipe and acrid in the lamellae	Mild taste in stipe, very bitter in the lamellae	
Pileus	85–160 mm			Smaller (60–130 mm)		Smaller (60–85 mm)	Smaller (40–75 mm), Shiny
Stipe	25–26 × 25–29 mm, matt	Longer (var. *chloroides (*15–)30–50(–90) mm, var. *parvispora* 25–40 mm)					Slender (12–20 mm)
$FeSO_4$	No reaction	dirty red, var. *chloroides* slow (faint) pink-orange, var. *parvispora* pink-orange, var. *trachyspora* pinkish		Pinkish/ pale pink-orange	Intense dirty pink/ pink-orange	Pink	
Guaiac	Immediate strong positive reaction	var. *chloroides* slow, green		Slow, dirty blue			
Spore print	Pale cream Ib–IIa		Darker (ochraceous custard, ochraceous cream)	Darker (IIc–d)	Darker (bright yellow IVb)	Darker (crème IIc)	Darker (IIb–d)
Spore size, shape, Q-value	8.31–9.05–9.78 × 6.71–7.24–7.77 µm, broadly ellipsoid to ellipsoid, Q = 1.19–1.25–1.31	Smaller var. *parvispora* (6.4–8.0 × 6.0–6.7 µm)	Smaller (6.9–9.3 × 6.3–7.0 µm)	Smaller (7.2–9.0 × 5.9–7 µm)	Smaller (6.4–8.6 × 5.5–6.8 µm)	Smaller (6.2–8(–10) × 5.5–6.5(–6.7) µm)	
Spore ornamentation	Small; 0.2–0.5 µm, subreticulate, 0–9 fusions in a 3 µm diam. circle	Higher (1–1.6 µm)	Smaller (up to 1.25 µm)		Smaller (up to 0.75 µm), warts more isolated		Less fused (1–3 fusions in a 3 µm diam. circle)
Suprahilar spot	Large and strongly amyloid		Ornamented but lower, often weakly amyloid		Vague, very faintly amyloid	Not clearly differentiated	Small and irregular, partly amyloid to amyloid
Basidia	(45.2–54.3–63.4 × 8.9–11.4–13.9 µm)	var. *parvispora* slender (8–11 µm)	Slender (8–11 µm)	Slender (48–62 × 9–11 µm)		Slender (46–56 × 8.5–10 µm)	
Hymenial cystidia	56.5–72.4–88.3 × 6.1–7.7–9.3 µm, often with appendices, SV strong reaction, orthochromatic, numerous-abundant (38000/mm²)	>100 µm	Slender (7–9 µm)	Greyish (weak reaction) in SV	Longer (70–140 µm), greying (weak reaction) in SV	Slender (70–80 × 5–8.5 µm)	Less dense (moderately numerous, 850–900/mm²), dark grey-brown in SV
Pileipellis	No reaction in SV Pileocystidia 1–4 celled, ((28–)45.5–		Slender (hyaline hyphae 1.0–2.6 µm broad and oleiferous		Greying in SV		Hyphal terminations longer (36.5–53.6–71.5 × 5–6–7 µm),

	91.4–137(–237) × (3–)3.8–7.8–11.7(–23) µm, rarely with appendage, orthochromatic		hyphae 2.0–5.3 µm broad).				Pileocystidia slightly metachromatic, always 1–celled, longer and slender ((40–)46.5–79.8–113(>200) × 4.5–5.9–7(–8) µm), frequently with appendages

8.2.2. *R. zebrihyphis* VS North-American species

	R. zebrihyphis	*R. brevipes*	*R. inopina*	*R. romagnesiana*	*R. vesicatoria*	*R. fuegiana*	*R. cascadensis*	*R. delicula*
Odour and taste	Odour fruity, flowery Mild taste	Odour indistinct Taste mild, slowly becoming acrid	Odour indistinct Taste mild	Odour indistinct Taste indistinct	Odour strong and pleasant Taste astringent to bitter taste, which becomes extremely and persistently acrid	Odour slightly like applesauce or *R. maculata* Taste non-burning acrid, with a bitter component	No or indistinct odour Taste intense acrid	Odour faintly fruity Taste mild
Spore size, shape, Q-value	(7.7–)8.5–9.4–10.2(–11.6) × (6.6–)7.2–7.9–8.6(–10.2) µm, Q= (1.1–)1.12–1.19–1.25(–1.33)	Slender (var. brevipes: (8.5–)8.7–9.1–9.5(–9.9) × (7.2–)7.5–7.9–8.2(–8.6) µm); larger (var. megaspora: (9.3–14.1 × 8.0–12.0 µm)	Smaller (6–6.3–6.7× 5.2–5.4–5.7µm), greater Q= (1.22–)1.25–1.31–1.37(–1.44)	Smaller (6–6.3–6.7 × 5.2–5.4–5.7 µm),	Smaller (7.3–7.6–7.9 × 6–6.3–6.7 µm)	Smaller (6.8–8.5 × 5.3–7.3 µm)	Smaller (6.7–8.2 × 4.8–6.7 µm)	Slightly bigger (8.1–10.6 × 7–9.4 µm)
Spore ornamentation	0.9–1.6 µm, subreticulate			Smaller (0.6–0.9 µm)	Smaller (0.4–0.7 µm)	Smaller (0.3–0.7 µm), Rarely forming a partial to broken reticulum	Smaller (0.2–0.7 µm)	
Suprahilar spot	Large and strongly amyloid	Var. *acrior* weakly amyloid	Small, inamyloid – partly amyloid	Small	Small, inamyloid	Finely and faintly ornamented	Weakly amyloid – no ornamentation	Weakly amyloid
Basidia	(37–) 43.9–52.4–60.8 (–69) × (10–) 11.1–13.4–15.9 (–19) µm	Slender (var. *brevipes* (45–) 55.5–60.7–68 × 9.5–11.4–14 µm), slender and longer (var. *acrior* 49–74 × 8.0–14.3 µm), longer (var. *megaspora* 53–73 × 9.3–16.0 µm)	Slender (8–9,5 µm)	Shorter, slender (40–44–48 × 8.5–9.7–11 µm)	Shorter, slender (41–46–50 × 8–9.5–10.5 µm)		Shorter, slender (40–52 × 8–10.6 µm)	
Hymenial cystidia	56.5–72.4–88.3 × 6.1–7.7–9.3 µm, often with appendices, SV strong reaction, orthochromatic, abundant (3800/mm²)	Weakly metachromatic walls, weak reaction with SV	No appendices, weak reaction with SV	Metachromatic	Metachromatic, insensitive to SV, moderately numerous hymenial cystidia (1000–1200/mm²)	No appendices	No appendices	

8.3. *Russula boeykensii*

8.3.1. *Russula boeykensii* VS European species (Romagnesi, Sarnari, Shaffer)

	Russula boeykensii	*R. delica*	*R. delica* var. *puta*	*R. delica* var. *trachyspora*	*R. delica* var. *delica*	*R. delica* var. *puta*	*R. delica*
Odour and taste	Odour fruity Taste acrid	Complex odour of fruit and fish Taste mild in stipe, acrid in lamellae (faint to strong)	Complex odour of fruit and fish Taste mild	Complex odour of fruit and fish Taste mild to slowly acrid in stipe, acrid in lamellae	Strong and un-pleasant odour, peachy and salty (fruity when young), Taste peaty in the lamellae	Brackish odour Taste faint acrid in stipe, more acrid in lamellae	/
$FeSO_4$	Orange/pink, afterwards grey	Faint pink-orange	First very faint, later bright pink-red	Pink-orange	Pale pink	Mediocre intensity	/
Guaiac	Immediate strong positive reaction	positive reaction but not always immediate	/	/			/
Spore size	(7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm	Larger (8–10–11.5 × 6.5–8.7 µm)			Larger (8.5–11.2 ×7–9 µm)	Larger (8–9.6 × 6.7–8 µm)	Larger (8.2–10.8 × 6.9–8.1 µm)
Spore ornamentation	0.6–2.1 µm	Smaller (0.5–0.7–1.0 µm)	Smaller (up to 0.85 µm)	Smaller (1–1.5 µm)	Smaller (0.8–1.0 µm)	Smaller (1.0–1.2 µm)	Smaller (0.4–1.0 µm)
Basidia	(40–) 52.5–58.9–65.2 (–72) × (9–) 10.7–12.1–13.5 (–14) µm						
Hymenial cystidia	(57–) 62.2–71.9–81.5 (–94) × (7–) 7.2–8.2–9.2 (–10) µm, faint reaction (greying) in SV	Longer and slender (65–150 × (6.5–)7.2–11.5(–13.5) µm), stronger reaction (blackening) in SV	Longer and slender (100–120 × 6.5–10 µm), stronger reaction (blackening) in SV	Longer and slender (78–135 × 6–11.5 µm), stronger reaction (blackening) in SV			

	Russula boeykensii	R. *chloroides*	R. *pseudodelica*	R. *pallidospora*	R. *flavispora*	R. *littoralis*	R. *laevis*
Odour and taste	Odour fruity Taste acrid	Odour unpleasant, with fruity and brackish notes Taste mild in stipe, acrid and unpleasant in lamellae	Taste acrid-pungent	Complex odour, with a fruity component Taste mild (slightly refreshing), later acrid	Remarkable odour with fruity notes, Taste bitter in stipe and acrid in lamellae	Odour like some Lactaria Taste mild in stipe, very bitter in lamellae	Odour indistinct Taste slowly acrid
$FeSO_4$	Orange/pink, afterwards grey	Dirty red, var. *chloroides;* var. *trachyspora* pinkish	/	Pinkish/ pale pink-orange	Intense dirty pink/ pink-orange	Pink	/
Guaiac	Immediate strong positive reaction	var. *chloroides* slow, green	/	Slow, dirty blue			/
Spore print	Ia–IIb		Darker (pale ochraceous crème/custard creme)	Darker (IIc–d)	Darker (IVb light golden yellow)	Darker (IIc, crème)	Darker (IIb–d)
Spore size	(7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm	Smaller (var. *parvispora* 6.4–8 × 6–6.7 µm) Larger (var. *chloroides* 7–10–11.2 × 6–8.8 µm, var. *trachyspora* 8–10.4 × 6.6–8.2 µm)	Slightly larger (8–9.25 × 6.2–6.7 µm) to slightly smaller (6.9–9.3 × 6.3–7.0 µm)		Slender (7.5–8 × 6.2–6.7 µm)	Slender (6.2–8(–10) × 5.5–6.5(–6.7) µm)	Larger ((9.2–)9.5–10–10.5(–11.3) × (7.6–)8–8.5–8.9(–9.6) µm)
Spore ornamentation	0.6–2.1 µm, 0–6 fusions in a 3 µm diam. circle	Smaller (var. *chloroides* up to 1.6 µm and var. *parvispora* up to 1 µm, var. *trachyspora* up to 1.5 µm)	Smaller (up to 1.3 µm)		Smaller (up to 0.75 µm), more isolated		Less fused warts (1–3 fusions in a 3 µm diam. circle)
Basidia	(40–) 52.5–58.9–65.2 (–72) × (9–) 10.7–12.1–13.5 (–14) µm	Slender var. *parvispora* (8–11 µm)	Slender (7–8 µm)	Slender (48–62 × 8–11 µm)		Smaller and slender (46–56 × 8.5–10 µm)	
Hymenial cystidia	(57–) 62.2–71.9–81.5 (–94) × (7–) 7.2–8.2–9.2 (–10) µm, abundant, 12000–220000/mm², faint reaction (greying) in SV	Longer (var. chloroides 50–130 µm and even longer, var. *parvispora* (57–)65–115 µm)	Slender (7–9 µm), (hyaline hyphae 1.0–2.6 µm broad and oleiferous hyphae 2.0–5.3 µm broad) Stronger reaction with SV (blackening)	Longer (65–160 µm)	Longer (70–140 µm)	Slender (70–80 × 5–8.5 µm) Stronger reaction in SV (blackening)	Less numerous (moderately numerous, ca. 850–900/mm²)
Pileipellis	No reaction in SV Hyphal terminations (14–) 16.9–28.7–40.4(–56) × 3.6–4.9–6.2(–9) µm				Greying in SV		Longer hyphal terminations ((20–)36.5–53.6–71.5(–116) × (4–)5–6–7(–9) µm)
Pileocystidia	(26–)38.2–71.6–105(–158) × 4–5.1–6.2(–8) µm						Longer ((40–)46.5–79.8–113(>200) × 4.5–5.9–7(–8) µm)

8.3.2. *Russula boeykensii* VS North American species

	Russula boeykensii	*R. brevipes*	*R. inopina*	*R. romagnesiana*	*R. vesicatoria*	*R. fuegiana*	*R. cascadensis*	*R. delicula*
Odour and taste	Odour fruity Taste acrid	Odour indistinct Taste mild, slowly becoming acrid	Odour indistinct Taste mild	Odour indistinct Taste indistinct	Odour strong and pleasant Taste astringent to bitter taste, which becomes extremely and persistently acrid	Odour slightly like applesauce or *R. maculate* Taste non-burning acrid, with a bitter component	No or indistinct odour Taste intense acrid	Odour faintly fruity Taste mild
$FeSO_4$	Orange/pink, afterwards grey	Positive (+)	Positive (+)	/	/	/	/	/
Spore size	(7.2–)7.7–8.2–8.6(–9.0) × (6.0–)6.4–6.8–7.2(–7.5) µm Q = (1.14–)1.16–1.2–1.24(–1.29)	Larger (var. *brevipes* (8.5–)8.7–9.1–9.5(–9.9) × (7.2–)7.5–7.9–8.2(–8.6) µm, *var. megaspora* 9.3–14.1 × 8.0–12.0 µm, var. *acrior* 8–10.6 × 6.7–8.6(–9.6) µm)	Smaller ((6.5–)6.9–7.2–7.5(–7.8) × (5–)5.2–5.5–5.7(–6) µm) higher Q ((1.22–)1.25–1.31–1.37(–1.44))	Smaller ((5.8–)6–6.3–6.7(–7.3) × (5.1–)5.2–5.4–5.7(–6.1) µm)	Smaller ((7–)7.3–7.6–7.9(–8.2) × (5.8–)6–6.3–6.7(–7) µm)	Smaller (6.8–8.5 × 5.3–7.3 µm)	Larger (8.1–10.6 × 7–9.4 µm)	Larger (8.1–10.6 × 7–9.4 µm)
Spore ornamentation	0.6–2.1 µm, 0–6 fusions in a 3 µm diam. Circle Suprahilar spot faintly amyloid to amyloid		Smaller (0.4–0.7 µm)	Smaller (0.6–0.9 µm)	Smaller (0.4–0.7 µm) Suprahilar spot inamyloid	Smaller (0.3–0.7 µ)	Smaller (0.5–1.6 µm)	Smaller (0.5–1.6 µm)
Basidia	(40–) 52.5–58.9–65.2 (–72) × (9–) 10.7–12.1–13.5 (–14) µm	Slightly longer (var. *brevipes* (45–) 55.5–60.7–68 × 9.5–11.4–14 µm) Slender (var. *acrior* 49–74 × 8.0–14.3 µm, var. *megaspora* 53–73 × 9.3–16.0 µm)	Slender (8–9,5 µm)	Shorter and slender (40–44–48 × 8.5–9.7–11 µm)	Shorter and slender (41–46–50 × 8–9.5–10.5 µm)	Shorter and slender (45–57 × 6.7–10.6 µm)	Shorter and slender (40–52 × 8–10.6 µm)	No reaction to SV
Hymenial cystidia	(57–) 62.2–71.9–81.5 (–94) × (7–) 7.2–8.2–9.2 (–10) µm, often with appendices, abundant (12000–220000/mm²), faint reaction (greying) in SV, orthochromatic	Weakly metachromatic walls	No appendices	Weakly metachromatic walls	Less numerous (moderately numerous) Insensitive to SV Distinctly metachromatic	No appendices	No appendices	
Pileipellis	No reaction in SV Hyphal terminations (14–)16.9–28.7–							Thinner cuticle (up to 130 µm)

	40.4 (–56) × 3.6–4.9–6.2(–9) µm; Cuticle ≤ 200 µm thick							

8.4. *Russula hampei*

8.4.1. *Russula hampei* VS. American species

	Russula hampei	*R. brevipes*	*R. inopina*	*R. romagnesiana*	*R. vesicatoria*	*R. fuegiana*
Spore size	(6.5–)7.1–7.6–8.2(–8.9) × (5.7–)6.0–6.5–7.1(–7.7) µm	Larger ((8.5–)8.7–9.1–9.5(–9.9) × (7.2–)7.5–7.9–8.2(–8.6) µm)	slightly smaller spores (6.5–)6.9–7.2–7.5(–7.8) × (5–)5.2–5.5–5.7(–6) µm)	Smaller spores ((5.8–)6–6.3–6.7(–7.3) × (5.1–)5.2–5.4–5.7(–6.1) µm)	Slightly smaller spores ((7–)7.3–7.6–7.9(–8.2) × (5.8–)6–6.3–6.7(–7) µm)	
Spore shape	Subglobose to broadly ellipsoid, Q = (1.05–)1.13–1.17–1.22(–1.26)		Higher Q-value ((1.22–)1.25–1.31–1.37(–1.44)		Slightly higher Q-value (Q= (1.12–)1.15–1.19–1.23(–1.31))	
Spore ornamentation	Normal to high (1.0-2.0 µm)	Smaller (0.8–1.3 µm)	Lower spore ornamentation (0.4–0.7 µm)	Smaller (0.6–0.9 µm)	Smaller (0.4–0.7 µm)	Smaller (0.3–0.7 µm)
Suprahilar spot	Irregular, amyloid		Inamyloid to partly amyloid		Inamyloid	Less conspicuous (finely and faintly ornamented, occasionally devoid of ornamentation)
Basidia	(38–) 45.1–49.5–53.8 (–58) × (9–) 11.0–12.1–13.2 (–14) µm, 2-to 4-spored	Slightly longer ((45–)55.5–60.7–68 × 9.5–11.4–14 µm) and always 4–spored	Longer and less broad ((52–)57–63.5–70(–80) × 8–9.5 µm) 4-spored	Smaller and less broad (40–44–48 × 8.5–9.7–11 µm), 4-spored	Smaller and less broad (41–46–50 × 8–9.5–10.5 µm), 4-spored	Less broad (45–57 × 6.7–10.6 µm), 4-spored
Hymenial cystidia	Numerous to abundant, 2100–4200/mm², (50–) 56.8–64.5–73.2 (–75) × (6–) 6.9–8.5–10.0 (–12) µm, mostly without appendage 1–3.5(–7) µm long React strong (blackening) in sulfovanillin, slightly metachromatic	less numerous (1500–2500/mm²) and react less strong to sulfovanillin	Longer (71–83.3–93(–110) × 7–8.6–10 µm)	Longer and less broad hymenial cystidia ((47–)60.5–75.6–91(–103) × 7–7.3–8 µm), react less to sulfovanillin (weak)	Less numerous (ca. 1000–1200/mm²), longer and less broad hymenial cystidia, insensitive to sulfovanillin and orthochromatic	Longer and less broad (53–90 x 6.7–8.6 µm)
Pileipellis	Slightly metachromatic, terminal hyphae cells (12–) 13.4–26.0–38.5(–71) × (3–)4.8–6.6–8.4(–10) µm 210–300 µm deep	less broad at the margin ((15–)24.5–30.8–43 × 4.5–5.9–7.5 µm) but broader at the centre (14–22.3–28.5(–38) × 5–7.3–10 µm) Less deep (ca. 100 µm)		Less broad hyphae (3.5–4.5–6 µm, distinct zebroid wall incrustations Less deep (100 µm)	Longer and more slender hyphal terminations ((29–)37–52–67(–91) × 2–3 µm) Orthochromatic and less deep (ca. 150–250 µm)	Hyphae are less broad (1.0–5.3(–4.3) µm) Less deep (120–173 µm)
Pileocystidia	1-to-4 celled, (23–)33.3–63.3–93.2(–124) × (3–)3.4–5.9–8.4(–12) µm, rarely with appendage No reaction in sulfovanillin	Mostly non-septate (occasionally 2–3 celled), weakly greying in sulfovanillin and the size is less variable	Less broad (3.5–5–7(–8) µm), weakly greying in sulfovanillin	Mostly 1–celled	Always 1-celled	

	Russula hampei	*R. cascadensis*	*R. delicula*	*R. idroboi*	*R. littoralis*	*R. aucarum*	*R. metachromatica*
Spore size	(6.5–)7.1–7.6–8.2(–8.9) × (5.7–)6.0–6.5–7.1(–7.7) µm	Less broad (6.7–8.2 × 4.8–6.7 µm)	Larger (8.1–10.6 × 7.0–9.4 µm)	Larger (8.8–11 × 8.2–9 µm)	Less broad (5.5–6.5–(6.7) µm)		Larger (9–)11–12.3 × (8–)10–11 µm
Spore shape	Subglobose to broadly ellipsoid, Q = (1.05–)1.13–1.17–1.22(–1.26)						
Spore ornamentation	Normal to high (1.0–2.0 µm)	Smaller ornamentation (0.2–0.7 µm)	Slightly smaller ornamentation (0.5–1.6 µm)	Smaller (0.6–1 µm)	Smaller (low)		Smaller ((1–)1.2–1.4 µm)
Suprahilar spot	Irregular, amyloid	Weakly amyloid	Weakly amyloid				
Basidia	(38–) 45.1–49.5–53.8 (–58) × (9–) 11.0–12.1–13.2 (–14) µm, 2-to 4-spored	Less broad (40–52 × 8.0–10.6 µm) 4–spored	Longer, less broad (47–64 × 8.0–13.3 µm) 4-spored	4-spored	Less (8.5–10 µm), 4-spored	4–spored	
Hymenial cystidia	Numerous to abundant, 2100–4200/mm², (50–)56.8–64.5–73.2 (–75) × (6–) 6.9–8.5–10.0 (–12) µm, mostly without appendage 1–3.5(–7) µm long React strong (blackening) in sulfovanillin, slightly metachromatic	Less broad (47–86 × 5.3–8.0 µm)	Often appendiculate	Banded contents	Longer and less broad (70–80 × 5–8.5 µm)	Less dense (600–900/mm²), smaller and less broad (44–63 × 7–8 µm) Less reactive to sulfovanillin (scarcely reacting)	Broader (48–96 × 9.5–15.5 µm), thicker walls which are strongly metachromatic
Pileipellis	Slightly metachromatic, terminal hyphae cells (12–) 13.4–26.0–38.5(–71) × (3–)4.8–6.6–8.4(–10) µm 210–300 µm deep	Less broad (1.3–5.3 µm)	Less broad hyphae (1.0–5.3 µm) Less deep (up to 130µm)			Less broad (2–4µm)	
Pileocystidia	1-to-4 celled, (23–)33.3–63.3–93.2(–124) × (3–)3.4–5.9–8.4(–12) µm, rarely with appendage No reaction in sulfovanillin				Less broad (1.5–4 µm)	Less broad (2–5 µm)	

9. Glossary

Carbolfuchsin	Reagent used to detect the primordial hyphae
Congo red	Reagent used to observe the microscopic elements in fungi, it improves contrast
Cresyl Blue	Reagent to detect metachromatic incrustations or walls. A pink reaction indicates metachromatic, the negative reaction is called orthochromatic.
Dermatocystidia	Cystidia of the pileipellis
Ectomycorrhiza	The symbiotic relationship between a fungi and plants, whereby the fungi forms a mycorrhizal network around the roots of the plants
Epicutis	The top layer of the pileipellis
Hymenium	The fertile part of fungi in which asci or basidia are produced, in this case the lamellae
Marginal veil	A temporary structure that connects the cap edge and stipe, it disintegrates while the fruitbody matures and residue can be found at the cap edge or at the top of the stipe
Melzer	Reagent used to observe spores and spore ornamentation, black reaction indicates amyloidy, no reaction is called amyloid
Pileipellis	The skin of the cap
Sphaerocytes	Rounded infertile cells, that form the trama and cause the brittleness of *Russula*'s, *Lactarius, Multifurca* and *Lactifluus*
Sterigmata	The spike-like extensions to basidia that bears the spores
Sulfovanilin	Reagent used to observe cystidia content
Suprahilar spot	Spot on the spore immediately above the apiculus, it can be amyloid, inamyloid, anything in between, smooth or ornamented
Trama	The flesh of fungi
Biological Species Concept	Species are defined by the potential of interbreeding of populations
Morphological Species Concept	Species are defined by distinctive morphological characters (macroscopic, microscopic, production of secondary metabolites, presence of pigments etc.
Phylogenetic Species Concept	Species are defined as the smallest group of populations with a common lineage that share a combination of defining traits
Lamellulae	Lamellae that don't extend from to cap edge to the stipe (stalk)
Holotype	The specimen that is designated as the type of a species by the original author at the time the species name and description was published
Isotype	A duplicate specimen of the holotype
Paratype	A specimen not formally designated as a type but cited along with the type collection in the original description of a taxon

10. Summary

Russula is a genus of ectomycorrhizal fungi (ECM) which belongs to one of the dominant ECM genera in many ecosystems worldwide. It holds a great diversity in cap colour and other macro- and micromorphological features. This genus has been studied elaborately, nevertheless there is still a large unknown diversity. Unequal sampling and the existence of species complexes contribute to this unknown diversity. Most *Russula* research has been done within Europe, this resulted in undersampling and restricted knowledge of species from other parts of the world.

Species complexes consist of species with a high morphological likeliness and these can be cryptic or pseudo-cryptic species. Pseudo-cryptic species have morphological differences while these are absent in cryptic species, where only molecular differences can be found.
Another issue is the inconsistency of species descriptions; most descriptions of *Russulales* are incomplete and author specific. This makes comparing species difficult to even impossible. The use of reagent is inconsistent, and when describing microscopical elements of the hymenium or pileipellis the location is often not mentioned despite evidence of several species in which the shape and measurements of these elements depends on location. Nowadays efforts are made to make descriptions more consistent with the use of templates and organisation of microscopy workshops.
The aim of this master's thesis was to describe and delimit some new species within *Russula* subgenus *Brevipedum*. This is framed within a larger project of Ruben De Lange on the former *R.* subg. *Compactae*. This subgenus has been divided into 5 subgenera: *R.* subg*. Compactae, R.* subg*. Glutinosae, R.* subg*. Archaeae, R.* subg*. Malodorae* and *R.* subg*. Brevipedum*. A preliminary analysis, based upon ITS (internal transcribed spacer) sequences, estimates the existence of 31 European species, this is in sharp contrast with the 7 described species at this moment. This could be explained by the presence of pseudo-cryptic species within this subgenus. *R.* subg. *Brevipedum* is a cosmopolitan subgenus with not only species in Europe, but also in America, Asia and Africa. The species within this subgenus are characterised by a whitish cap, a white to yellow spore print and the presence of lamellulae. The relation between *R.* subg. *Brevipedum* and *R.* sect. *Metachromaticae* on the one hand and *R.* subg. *Brevipedum* and *R.* sect. *Delicoarchaeae* on the other hand is still unclear. *R.* sect. *Delicoarchaeae* has been suggested as the tropical synonym of *R.* subg. *Brevipedum*, others suggest *R.* sect. *Delicoarchaeae* being placed within *R.* sect. *Metachromaticae*.

The specimens used for this work are collected in Europe and Central America, by different collectors. At least 2 dried specimens were available per species and all available specimens were used. A description of macro- and micromorphological features was compiled for each species using the template made by Adamčík et al. to contribute to the uniformisation in species descriptions. Not all possible characters were described, only those which are deemed the most diagnostic. The microscopic observations and measurements were done on dried specimes in the recommended reagentia. Drawings of the elements, except of the spores, were made using a microscope with a drawing tube. For the spore drawings stacking images were compiled. Molecular analysis were done, both on DNA extracted from fruitbodies and DNA extracted from root tips. The fungal DNA was used to compile a phylogentic tree, which was used to study the relationships between the *Russula* subg. *Brevipedum* species. The references below to the closest related species is based upon the presence of type species in the phylogentic tree. The true closest related species is often not yet described. The purpose of the root tip samples was to determine the host species. Therefore the fungal markers were ITS1 and ITS2 were used, and the used plant marker was ITS1.
The four newly described species within this work seem to be scattered in the phylogentic tree of *R.* subg. *Brevipedum*. The only species for which the PCR amlification for all primers of the root tip samples was successful, is *R. zebrihyphis*. From the analysis from these sequences we can conclude that the hosts of *R. zebrihyphis* are of the genera *Salix* and *Quercus*. Since ITS1 is not the best plant marker we only consider the genus level.

R. macrostigma was collected in Italy and is closest related to *Russula boeykensii*. *R. macrostigma* is most similar to *R. delica* as described by Shaffer, *R. delica* var. *trachyspora* and *R. delica* var. *puta*. *R. macrostigma* differs from *R. delica* by lower spore ornamentation and a distinct amyloid suprahilar spot. *R. macrostigma* differs from *R. delica* var. *trachyspora* by lower spore ornamentation and shorter hymenial cytidia, a less strong reaction to sulfovanilin and an odour that lacks a fish component. *R. macrostigma* differs from *R. delica* var. *puta* by a broader stipe, lower spore ornamentation and shorter hymenial cystidia which react less to sulfovanilin. The most charasteristic features of *R. macrostigma* are a light spore print, a large and distinctly amyloid suprahilar spot, quite large spores and broad basidia.

R. zebrihyphis was collected in Sweden and is closest related to *R. laevis*, which is another species found in Scandinavia. *R. zebrihyphis* is most similar to *R. delica* var. *puta* and *R. delica* var. *trachyspora*. *R. zebrihyphis* differs from *R. delica* var. *puta* by larger spore ornamentation (and shorter and thicker hymenial cystidia, pink-red reaction to FeSO4). *R. zebrihyphis* differs from *R. delica* var. *trachyspora* by shorter and broader hymenial cystidia (odour of *R. zebrihyphis* is mainly flowery, only fishy when old, while *R. delica* var. *trachyspora* has a complex odour of fruit and fish). The most charasteristic features of *R. zebrihyphis* is a light spore print, a strong reaction to sulfovanilin in the hymenium, quite large spores, broad basidia and zebroid incrustations on the hyphae of the pileipellis.

Russula boeykensii was collected in Belgium and is closest related to *R. macrostigma*. *Russula boeykensii* is most similar to *R. pallidospora* and *R. delicula*. *Russula boeykensii* can be differentiated from *R. pallidospora* by a different taste (acrid in *Russula boeykensii* and bitter in *R. pallidospora*), a lighter spore print, broader basidia and shorter hymenial cystidia. *Russula boeykensii* differs from *R. delicula* with a different taste (acrid in *Russula boeykensii* and mild in *R. delicula*), smaller spores and spore ornamentation and a thinner pileipellis. The most characteristic features of *Russula boeykensii* are a light spore print, broad basidia and a large spore ornamentation.

Russula hampei was collected in Panama and is closest related to the isotype of *R. delica var. trachyspora*. *Russula hampei* is most similar to *R. aucarum* despite the lack of information about Cresyl Blue reaction. *Russula hampei* differs from *R. aucarum* by basidia that can have less than 4 sterigmata, higher hymenial cystia density, which have a strong reaction to sulfovanilin and the hyphae and pileocystidia from the pileipellis are broader. The most characteristic features of *Russula hampei* are 2- to 4-spored basidia, a strong reaction to sulfovanilin in the hymenium and slighty metachromatic (lamellae stronger reaction compared to pileipellis) reaction to Cresyl Blue.

We can conclude that this subgenus still demands research; a revision of the described species and the correct assignment of type species needs to be made, on top of the description and delimitation of new species.

11. Samenvatting

Russula is een genus van ectomycorrhiza fungi (ECM) dat behoort tot één van de dominantste ECM-genera aanwezig in verschillende ecosystemen over de hele wereld. Dit genus wordt gekenmerkt door een grote diversiteit in kleur van de hoedhuid, en in andere macro- en micromorfologische kenmerken. Hoewel het een uitvoerig onderzocht genus is, is er nog steeds sprake van een grote onbekende diversiteit. Dit valt te wijten aan ongelijke bemonstering en aan de aanwezigheid van soortcomplexen. Het grootste deel van onderzoek op *Russula* heeft plaatsgevonden binnen Europa, wat heeft geleid tot een onder bemonstering en een tekort aan kennis in de andere werelddelen. Soortcomplexen bestaan uit soorten met een grote morfologische gelijkenis; deze soorten kunnen cryptisch of pseudo-cryptisch zijn. Pseudo-cryptische soorten hebben morfologische verschillen terwijl deze afwezig zijn in cryptische soorten, die enkel moleculair verschillen.
Een bijkomend probleem is inconsistentie in soortbeschrijvingen; de meeste beschrijvingen van *Russulales* zijn onvolledig en auteur-specifiek. Dit zorgt ervoor dat soorten vergelijken moeilijk tot zelfs onmogelijk wordt. Het gebruik van reagentia is inconsistent, en desondanks dat er in verschillende soorten *Russula* de locatie van de microscopische elementen een effect heeft op vorm en afmetingen ervan, wordt deze vaak niet vermeld. Tegenwoordig worden er inspanningen gedaan om de beschrijvingen consistenter te maken door het ontwikkelen van sjablonen en het organiseren van workshops voor microscopie.

Het doel van deze master thesis is om enkele nieuwe soorten binnen *Russula* subg. *Brevipedum* te beschrijven en af te bakenen van de andere soorten. Dit onderzoek is gekaderd binnen een groter project van Ruben De Lange op het voormalige *Russula* subg. *Compactae*. Dit subgenus is intussen opgedeeld in 5 subgenera: *R.* subg*. Compactae, R.* subg*. Glutinosae, R.* subg*. Archaeae, R.* subg*. Malodorae* en *R.* subg*. Brevipedum*. In een voorlopige analyse, gebaseerd op ITS (internal transcribed spacer) sequenties, schat men dat er 31 Europese soorten zijn. Dit is veel meer dan de 7 soorten die tot nu toe zijn beschreven. Dit kan verklaard worden door de aanwezigheid van soortcomplexen. *R.* subg*. Brevipedum* is een kosmopolitisch subgenus met niet enkel Europese soorten, maar ook Amerikaanse, Afrikaanse en Aziatische soorten. De soorten binnen dit subgenus zijn gekenmerkt door een wittige hoed, een witte tot gele sporenafdruk en de aanwezigheid van tussenlamellen. De relatie tussen *R.* subg. *Brevipedum* en *R.* sect. *Metachromaticae* aan de ene kant en *R.* subg. *Brevipedum* en *R.* sect. *Delicoarchaeae* aan de andere kant is nog onduidelijk. Van *R.* sect. *Delicoarchaeae* en *R.* sect. *Metachromaticae* wordt gesuggereerd dat beiden synoniemen kunnen zijn voor *R.* subg. *Brevipedum*; anderen suggereren dat *R.* sect. *Delicoarchaeae* ondergebracht zou moeten worden in *R.* sect. *Metachromaticae*.

De specimens gebruikt in dit werk zijn door verschillende wetenschappers ingezameld in Europa en Centraal-Amerika. Per soort waren er minstens 2 specimen beschikbaar en alle beschikbare specimens zijn gebruikt. Bij het opstellen van de macro- en micromorfologische beschrijving is gebruik gemaakt van de sjablonen opgesteld door Adamčík et al., wat bijdraagt aan de uniformisering van de soortbeschrijvingen. De microscopische observaties en metingen zijn gebeurd op gedroogd materiaal in de voorgeschreven reagentia. De tekeningen van de elementen, behalve deze van de sporen, zijn gerealiseerd door gebruik van een microscoop met tekentubulus. Voor de sporen werden stacking foto's samengesteld en op basis daarvan tekeningen vervaardigd. Moleculaire analyses zijn uitgevoerd zowel op DNA ge-extraheerd uit de vruchtlichamen en op

DNA geextraheerd uit worteltop stalen. De analyses van de fungi DNA werd gebruikt om een fylogenetische boom op te stellen en zo verwantschappen te kunen onderzoeken. De verwijzing hieronder naar de dichtst gerelateerde soort is gebaseerd op de type soorten aanwezig in de fylogenetische boom. De effectief dichtst gerelateerde soort is vaak nog niet beschreven. De worteltop stalen werden gebruikt om te kunnen achterhalen wat de gastheer is. Hiervoor werden de fungi merkers ITS1 en ITS2 gebruikt, alsook de plant merker ITS1.

De 4 nieuw beschreven soorten die in dit werk behandeld worden, lijken verspreid te zitten in de fylogenetische boom van *R.* subg. *Brevipedum*. Enkel bij *R. zebrihyphis* waren de PCR-amplificaties van de worteltop stalen voor alle merkers succesvol. Uit de analyse van deze sequenties kunnen we concluderen dat de gastheren van *R. zebrihyphis* behoren tot de genera *Salix* en *Quercus*. Aangezien ITS1 niet de beste plant merker is, kijken we enkel maar op genus level.

R. macrostigma werd ingezameld in Italië en is het dichtst gerelateerd aan *Russula boeykensii*. *R. macrostigma* lijkt meest op *R. delica* zoals beschreven door Shaffer, *R. delica* var. *trachyspora* en *R. delica* var. *puta*. *R. macrostigma* onderscheidt zich van *R. delica* door een lagere ornamentatie van de sporen en een opvallend amyloide suprahilaire vlek. *R. macrostigma* onderscheidt zich van *R. delica* var. *trachyspora* door een lagere ornamentatie van de sporen en kortere cystidia in het hymenium, een minder sterke reactie op sulfovaniline en de afwezigheid van visgeur. *R. macrostigma* onderscheid zich van *R. delica* var. *puta* door een bredere stipe, lagere ornamentatie van de sporen en kortere cystidia in het hymenium die minder reageren op sulfovaniline. De meest opvallende kenmerken van *R. macrostigma* zijn een zeer lichte sporenafdruk, een grote, opvallende amyloide suprahilar vlek, relatief grote sporen en brede basidia.

R. zebrihyphis werd ingezameld in Zweden en is dicht gerelateerd aan *R. laevis*, een andere scandinavische soort. *R. zebrihyphis* lijkt het meest op R. delica var. puta en R. delica var. trachyspora. *R. zebrihyphis* onderscheidt zich van R. delica var. puta door een grotere ornamentatie van de sporen, kortere en dikkere cystidia in het hymenium en een roze-rode reactie op FeSO4. *R. zebrihyphis* onderscheidt zich van R. delica var. trachyspora door kortere en dikkere cystidia van het hymenium (de geur van *R. zebrihyphis* is voornamelijk floraal en heeft enkel een visgeur wanneer hij oud is, terwijl R. delica var. trachyspora een complexe geur van fuit en vis heeft). De meest kenmerkende eigenschappen van *R. zebrihyphis* zijn een zeer lichte sporenafdruk, een sterke reactie in sulfovaniline in het hymenium, relatief grote sporen, brede basidia en zebroide incrustaties op de hyphen van de pileipellis.

Russula boeykensii werd ingezameld in België en is het dichtst gerelateerd aan *R. macrostigma*. *Russula boeykensii* heeft de grootste gelijkenis met R. pallidospora en R. delicula. *Russula boeykensii* onderscheidt zich van R. pallidospora door een verschillende smaak (pikant in *Russula boeykensii* en bitter in R. pallidospora), een lichtere sporenafdruk, bredere basidia en kortere cystidia in het hymenium. *Russula boeykensii* onderscheidt zich van R. delicula door een verschillende smaak (pikant in *Russula boeykensii* en mild in R. delicula), kleinere sporen en ornamentatie van de sporen en een dunnere pileipellis. De meest kenmerkende eigenschappen

van *Russula boeykensii* zijn een zeer lichte sporenafdruk, brede basidia en grote ornamentatie van de sporen.

Russula hampei werd verzameld in Panama en is het dichtst gerelateerd aan het isotype van *R. delica var. trachyspora*. *Russula hampei* toont de grootste gelijkenis met R. aucarum, ondanks er in de beschrijving hiervan geen informatie over de reactie in Cresyl Blue staat. *Russula hampei* verschilt van R. aucarum door de basidia die minder dan 4 sterigmata kan dragen, een grotere densiteit aan cystidia in het hymenium, die een sterke reactie in sulfovanilin vertonen en de hyphen en pileocystia van de pileipellis zijn breder. De meest kenmerkende eigenschappen zijn de 2- tot 4-sporen dragende basidia, een sterke reactie in sulfovaniline in het hymenium en licht metachromatische reactie in Cresyl Blue (reactie sterker in de lammelen i.v.m. de pileipellis).

We kunnen de concluderen dat er in dit subgenus nog veel onderzoek nodig is; een grondige revisie van de beschreven soorten en de correcte toewijzing van type soorten moet gemaakt worden, bovenop de beschrijving en afbakeningen van nieuwe soorten.

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