EVALUATING MORPHOLOGY AND GEOGRAPHIC RANGE EXTENT OF GENETICALLY DELIMITED SPECIES OF MUSHROOMS

by

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Abstract

Species delimitation directly affects interpretation of evolution and biogeography. Following speciation, independently evolving lineages are expected to fix different characters that eventually distinguish them from their closest relatives. However, rates of fixation vary. I delimited species in the mushroom genus *Russula* based on the fungal nuclear internal transcribed spacer 2 (ITS2) DNA barcode region. I sampled 713 ITS2 sequences of American Pacific Northwest specimens collected by Seattle architect Benjamin Woo (1923-2008). I compared the morphology within and among DNA-delimited species, according to morphological character state data that Woo had recorded for each of specimen. To Woo's data, I added spore measurements for 23 species. The characters in *Russula* varied within and overlapped between my delimited species. My multivariate analysis showed that the centroids of morphological characters usually differed significantly between pairs of genetically defined species, indicating evolutionary divergence at the level of morphology. However, because of the variation among and within conspecific collections, morphological characters only correctly predicted the identity of ~50% of the individual specimens. Of the delimited species, nine had been collected ten or more times each and were, based on morphology and sequence analysis, undescribed and restricted to North America. I describe the nine as new species, reporting their character variation. I used data from public databases to ask how frequently geographical ranges are intercontinental as opposed to intracontinental among mushroom-forming species. I calculated the 'range extent' (maximum geographical distance) recorded for 2324 species world-wide and 341 species from the Pacific Northwest, representing 12 genera. The ranges of most species extended only to ~2000 km (shorter distances than a continent). By permutation of the data, I showed that this pattern vanished if geographical coordinates were randomized with respect to species suggesting the pattern I found in the data was not due to random sampling. More sampling would be needed to resolve whether the pattern arose from sampling bias or a high frequency of regional endemism. However, because it reflects a common pattern seen in the best sampled fungi and in narrower studies of genera and families, I hypothesize that regional endemism is the general pattern in well-studied genera and more generally fungal biogeography.

Lay summary

Species delimitation in fungi is important when untangling pathogen impact on humans and crops, characterizing ecological relationships, describing and quantifying diversity, or making conservation decisions. I analyzed DNA-barcode sequences to better understand within-species variation and geographic distributions, studying over 700 collections of *Russula* mushrooms from Seattle architect Benjamin Woo (1923-2008). I showed that the notorious difficulty of identifications in this genus stems from tremendous within-species morphological variation. Taking the variation into account, I described nine new *Russula* species with identification keys now available to the public. To investigate the extent of geographical ranges of mushroom-forming fungi, I retrieved collection localities for 2324 fungal species in 12 genera from a public fungal database. While the ranges of mushroom species were mostly under 2000 km, host trees of the mushrooms had even smaller geographical ranges, suggesting that host preferences do not restrict mushrooms to single host tree species.

Preface

Chapter 2 has been published: Bazzicalupo, A.L., Buyck, B., Saar, I., Vauras, J., Carmean, D., & Berbee, M.L. 2017. Troubles with mycorrhizal mushroom identification where morphological differentiation lags behind barcode sequence divergence. *Taxon* 66:791-810. I was responsible for most DNA extractions, all DNA analyses, all microscopic investigation and analyses, and wrote most of the manuscript. Buyck shared his experience with microscopic methods for working with Russulas and his knowledge of type specimens. Saar and Vauras contributed collections and ITS sequencing of the European mushrooms, Carmean helped me design the database. Berbee helped design the study and provided feedback on all analyses and on drafts of the manuscript.

Chapter 3 has been published: Hyde, K.D., Norphanphoun, C., Abreu, V.P., Bazzicalupo, A., [...] 2017. Fungal diversity notes 603–708: taxonomic and phylogenetic notes on genera and species. Fungal Diversity 87:1-235. 10.1007/s13225-017-0391-3. In this publication ~100 species of fungi were described by many authors – a 'compilation publication'. I contributed 9 species of *Russula* with B. Buyck, D. Miller and M. Berbee. I collected most of the data used in the summary and wrote most of the manuscript and descriptions, took the micrograph pictures and assembled all the figure plates. Miller collected data on field morphology. Berbee, Buyck, and Miller contributed to the manuscript.

I performed all the wet lab-work and analyses outlined in chapter 4. The data from the UNITE database (https://unite.ut.ee/index.php, a database dedicated to fungal ITS sequences for systematic classification of fungi) were made available by Kessy Abarenkov from University of Tartu, Estonia. I designed the permutation tests with J. Whitton. M. Berbee contributed to the manuscript.

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Chapter One: Introduction

Summary

During and after speciation, daughter species will accumulate differences independently of each other. In this chapter I will review the ways differences are thought to accumulate, and how we humans detect them. I hypothesize that in mushroom-forming fungi, these differences between sister taxa are not in the structures that we usually use for identification, and I review the evidence from the species level to higher taxonomic ranks that support this idea. In fungi, I expect the consequences of selection, drift and speciation on form and function to be similar to other sexually reproducing eukaryotes, but these differences may occur in microscopic mycelial structures in nascent species. Expectations for ecologically divergent speciation processes in fungi have been experimentally tested in *Saccharomyces* and *Neurospora*. Evidence for ecological specialization in environments of sister taxa is reported in studies of mycorrhizal fungi such as *Suillus*. At higher taxonomic ranks, another process that has hindered the identification and successful classification of fungi has been convergent evolution through selection for similar forms in different lineages. It emerges that there is a decoupling of identity and morphological traits, and I propose that this is a consequence of the microscopic scale and number of characters that selection or drift can change.

Speciation and the importance of species in fungi

In evolution and ecology, the knowledge of species and their diversity is the basic literacy required to be able to ask questions and test hypotheses, and it informs our practical choices for management action (Bickford et al., 2007).

Among fungi, as in other eukaryotes, species concepts are based on expectations of the products of speciation. Dobzhansky (1937, 1940) described biological speciation as a stage in an evolutionary process where breeding between two ‘arrays’ is no longer possible. From there, Ernst Mayr (1940, 1942) formalized the definition of the biological species concept as "interbreeding or potentially interbreeding groups of individuals that do not interbreed with other such groups." In organisms where breeding cannot be tested,

other species concepts are used to circumscribe species, and are based on observable differences that accumulate during and after speciation (e.g. ecology, morphology, phylogeny, or genetic clustering) (De Queiroz, 2007). Simpson (1951) suggested that there are two major forms of evidence used in delimitation of species, one is non-arbitrary and one is arbitrary. Non-arbitrary delimitation is reached by using characters that are discontinuous between species, in the case of the biological species concept, it would be discontinuous breeding, or in the morphological species concept, a set of morphological characters. A delimitation is arbitrary if the underlying distribution of characters is continuous. In a speciation event, a community of individuals that had been exchanging genes, homogenizing their genomes over generations, becomes divided. After subdivision, lineages become reproductively isolated and their genomes diverge independently of one another. Soon after this happens, De Queiroz (2007) predicts that characters may not fixed in either lineage, and the delimitation may be 'arbitrary' in Simpson's terms.

Understanding species has practical implications for conservation, where knowledge of species is used in prioritization of species protection efforts. On the 'Species at Risk Public Registry' in the 'Species Index' webpage (http://www.registrelep-sararegistry.gc.ca/sar/index/default_e.cfm) on the Government of Canada website (Accessed March 27th, 2018), a list of all species found to be endangered in Canada includes 22 lichens, but no other species of fungi. In the IUCN Red List of Threatened Species there are 57 species of fungi (http://www.iucnredlist.org/search). The number of species of fungi has been debated over the past two decades with estimates spanning about an order of magnitude (Blackwell, 2011). Fungal species have been estimated with several methods to be from 611,000 (Mora et al., 2011) to 1.5 million (Hawksworth, 2012) to 5 million (O'Brien et al., 2005). However, all the estimates are far higher than ~100,000 species described (Kirk et al., 2008). Likely, a lack of knowledge of the species of fungi and not a lack of environmental threats is responsible for their absence from endangered species lists.

In the fungi, the importance of species and reproductive isolation is clear when human lives are at risk. For example, the severity of fungal infections caused by the human pathogenic isolates of *Cryptococcus* depends in part on the degree of reproductive

isolation among the species involved. *Cryptococcus neoformans* and *C. gattii* form a species complex where seven 'serotypes' (a serotype is a serologically distinguishable strain of an organism) have now been recognized as species based on multilocus phylogenetic analyses and pathogenicity data (Hagen et al., 2015; Lin and Heitman, 2006). Cryptococcosis infections have been more common and harder to treat in Africa than in the rest of the world since the spread of AIDS. The nearly equal proportion of opposite mating types may indicate a history of within species sexual recombination contributing to the increased diversity and possible higher virulence of *Cryptococcus* species in some parts of Africa (Litvintseva et al., 2003). In France, studies of *Cryptococcus neoformans* showed one species to be asexual while another showed evidence of sexual reproduction. Hybrids between the two species have been found in Africa and in France. In France the severity of the infection was equal for all hybrids and all patients survived. For reasons unknown but possibly related to factors explained above, none of the eight hybrid-infected Ugandan individuals survived (Desnos-Ollivier et al., 2015).

Evolutionary processes at population and species levels also influence the pathogenicity and virulence of fungi parasitizing crops (McDonald and Linde, 2002a). The discovery that the plant pathogenic genus *Microbotryum* comprised not one, but several phylogenetically distinct taxa (Refrégier et al., 2008) enabled testing for co-evolution or co-speciation with its host genus *Silene* and distinguishing species based on their host (De Vienne et al., 2009; Denchev et al., 2009; Le Gac et al., 2007a). *Magnaporthe oryzae* and *Mycosphaerella graminicola* crop pathogens of rice and wheat speciated from ancestral populations infecting the wild ancestors of the crops, and then diverged synchronously with the domestication of their hosts (Couch et al., 2005; Stukenbrock et al., 2007). Understanding how traits that increase virulence are maintained or how they arise contributes to managing pathogens of crops (McDonald and Linde, 2002a). Characteristics that help plant pathogens overcome plant resistance include large populations that allow the persistence of variation, sexual reproduction, and outcrossing with frequent gene flow allowing spread of infectious variants (McDonald and Linde, 2002a). To slow the build-up of fungal strains that can overcome crop resistance, crop rotation can be used to remove standing variation in the pathogens,

subjecting the parasitic fungus to frequent population bottlenecks (McDonald and Linde, 2002b). Quarantine regulations prevent spread of pathogens across countries as in Australia's quarantine status against *Puccinia* species attacking wheat or guava (Langrell et al., 2008; Wellings et al., 1987). Legislation for quarantine regulations can be subject to status review, and uncertainty about the pathogen's ability to spread can cause controversy as in the case of deciding whether a quarantine period is required or not for species of *Tilletia* (Sansford et al., 2008).

Consequences of selection, drift and speciation on form and function

I hypothesize that speciation among mushroom-forming fungi often involves selection on the belowground or somatic part of life history and that cryptic species are common because drift is more important than selection on change in morphology of aboveground fruiting bodies. In a speciation event, a community of individuals that had been exchanging genes, homogenizing their genomes over generations, becomes divided. After subdivision, lineages become reproductively isolated and their genomes diverge independently of one another (De Queiroz, 2007).

In large populations, selection can fix characters more quickly than drift can (Crow and Kimura, 1970). Divergent selection for growth in different environments may increase the speed of evolution of distinctive characters between species. One result of selection in divergent environments can be an adaptive radiation as in the Hawaiian Silverswords (Robichaux et al., 1990), which are several closely-related species that have recently diversified to show striking variation in forms: shrubs, trees, cushion plants, lianas, and rosettes (Baldwin and Sanderson, 1998; Carr, 1985). Sometimes the character selected upon can be involved in reproduction where visual cues are used for mate recognition, making it easy for visual animals such as humans to pick out those differences. Reproductive isolation can be detected in different pollination syndromes of *Mimulus* sister taxa, where mostly geographical separation, but also visual cues for pollinators will tend to prevent the two species from homogenizing their genomes (Bradshaw Jr et al., 1995; Ramsey et al., 2003). Visual traits in animals and plants involved in sexual selection are easily detected by humans. These traits can be studied and have linked sexual selection to increased lineage diversification in jumping spiders

(Masta and Maddison, 2002) and sticklebacks (Boughman, 2001). To avoid inferior hybrid offspring, differences in mate-choice traits between sympatric species may be accentuated. In *Hyla* frogs, where mate choice happens through song, the song trait difference is stronger in sympatric populations. Hybrid males of two species singing hybrid songs were shown to be chosen less frequently by females from populations where the two species are sympatric (Höbel et al., 2003). The *Hyla* frog example suggests that sensory cues other than visual cues can be used by humans to distinguish reproductively isolated groups. Another example is birdsong: a trait involved in reproductive isolation and mate recognition, and it has been shown to be useful in distinguishing species of birds (Freeman and Montgomery, 2017).

In the above examples, speciation involves divergence of characters that humans can readily perceive, but this need not always be the case. Bickford et al. (2007) defined 'cryptic species' as different species that were or are classified as one due to morphological characters that are at least superficially indistinguishable. With this definition, species are especially prone to being considered as 'cryptic' if their characters are difficult to measure. Not only neutral processes, but even divergent selection in these species may act on characters that we are poorly equipped to measure or to detect. Marine species using pheromones to find mates have more cryptic species than other marine species that use visual cues (Bickford et al., 2007; Knowlton, 2000). Terrestrial taxa that use pheromones show similar problems with morphology. Species of *Bembidion* beetles revealed by molecular data initially believed to be morphologically indistinguishable were found to consistently have different sperm sizes or different chromosome numbers (Maddison, 2008). As fungi use pheromones to find compatible mates (Fraser et al., 2007), they too could show some degree of cryptic morphologies after speciation.

Kohn (2005) and Giraud et al. (2008) have thoroughly reviewed speciation in fungi. For example, two of the first experimental speciation studies in eukaryotes were done in yeast and *Neurospora* (Dettman et al., 2008; Dettman et al., 2007). In both studies, the fungi were evolved in different environments (high salt and low temperature) and showed hybrid inferiority in the opposite environment and Bateson-Dobzhansky-Muller incompatibilities. Evolution of reproductive isolation has also been shown in species of *Microbotryum* (De Vienne et al., 2009; Le Gac et al., 2007b). Despite experimental and

observational evidence that reproductive barriers had developed, species of *Neurospora* (Dettman et al., 2003), *Saccharomyces* (Kurtzman and Fell, 2006) and *Microbotryum* (Denchev et al., 2009; Le Gac et al., 2007a) were indistinguishable from their close relatives based solely on morphological traits.

Among mushroom-forming fungi, speciation may begin, for example, when an ancestral mycorrhizal species splits into daughter species adapted to different host tree taxa. While at first, the two, nascent species may be generalists on both hosts, adaptation in the biochemical signals that are involved in the root-tip and fungal interaction may lead to divergent specialization. Their hybrids could be inferior for mycorrhizal association compared to the parents. While this scenario has not been tested, some data suggest it may happen. I have found in my second chapter, two pairs of sister species specializing on different hosts (Bazzicalupo et al., 2017), while most Russulas are thought to be generalists at least to the level of taxa within angiosperms or Pinaceae (Looney et al., 2016). *Suillus subarueus* is nested in a clade of *Suillus* that are specialized on Pinaceae hosts. Descending from a specialist ancestor, this species has acquired the ability to also associate with *Quercus* (angiosperm), an association experimentally shown to fail in its sister taxa (Lofgren et al., 2018; Nguyen et al., 2016). The differences between these taxa should be found in the biochemical signals and receptors that allow ecological specialization. Mate recognition in a mushroom-forming fungus takes place between belowground haploid hyphae. As in the example of the beetles and the marine invertebrates, fungi use pheromones for mate recognition and they mostly live and interact with the world in the dark, underground, inside plant tissues, forming small colonies on plants, animals, or your neglected yoghurt, so maybe it is to be expected that fungi would have more cryptic species. Most of the selection for local adaptation and reproductive isolation likely happens in microscopic mycelial structures (Figure 1.1).

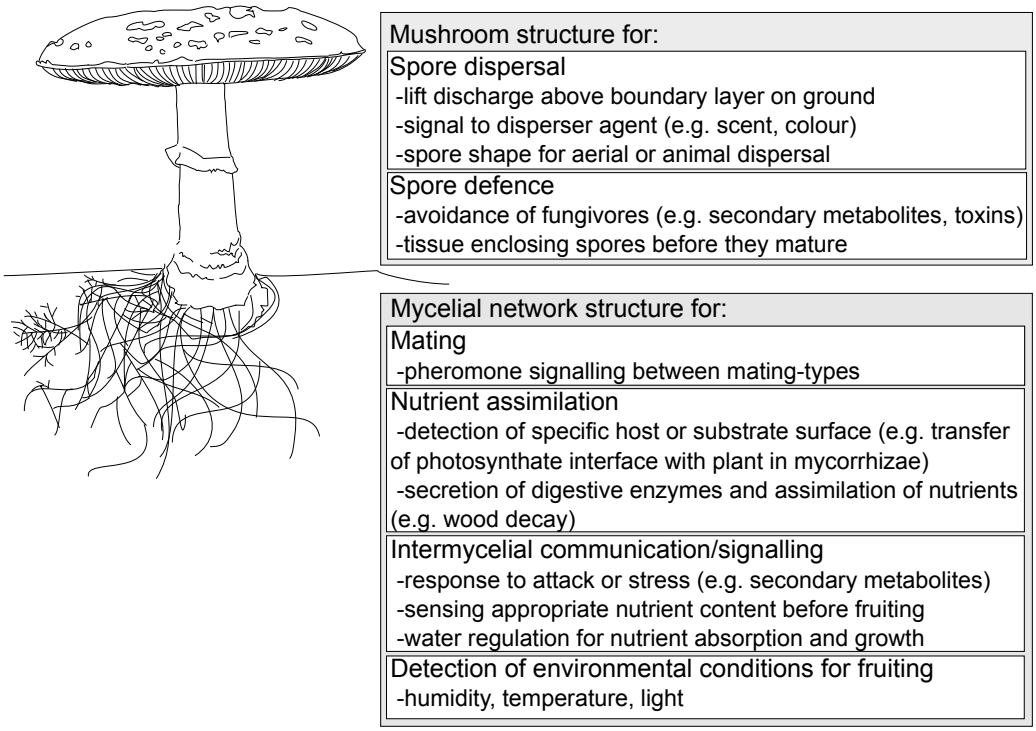

Figure 1.1 Mushroom structures and function.

Possible fungal structures under selection for different aspects of their function. Many processes under selection for diverging environments and mate recognition are in microscopic mycelial structures and not in the fruiting body we use for identification.

Among mushroom-forming fungi, delimitation of species has typically been based on morphological characters of fruiting bodies, but the mushroom has no role in mate recognition and no direct role in interactions with a host (Fig. 1.1). This may mean that neutral processes play a large role in character evolution in mushrooms. The accumulation of species-level morphological differences through drift may be slow, especially when the number of traits is small and populations are large. Paraphrasing Taylor et al. (2006): a two-celled organism will have twice as many characters as a unicellular organism where different character states may become fixed in a population. It follows that we should generally expect fewer character differences between a pair of sister taxa with fewer features than a pair of sister species with multiple cell types. A plethora of human-scale, detectable characters can distinguish sister species such as

chimps and humans. Fewer morphological characters are available to separate mushroom species.

Multivariate analyses have been applied to detect combinations of morphological characters that distinguish species and to assign new specimens to species based on their characters. To test if a group of specimens assigned to a species *a priori* is distinct in its characters from a second group, discriminant analyses have been common (Wiley, 1981). The null hypothesis of a Canonical Variates Analysis is that the distributions of morphological character states in the species are completely overlapping (Fig 1.2 A and C). If species are distinct, their distributions in morphospace should not overlap, and their distributions would be significantly different. Samples that fall into an intermediate zone between species show characters shared among species through either convergent evolution or ancestral polymorphisms (Fig 1.2 B). Based on the hypothetical morphological characters plotted, Figure 1.2a could be considered a non-arbitrary delimitation in Simpson's terms, while Figures 1.2b and c would be arbitrary delimitation.

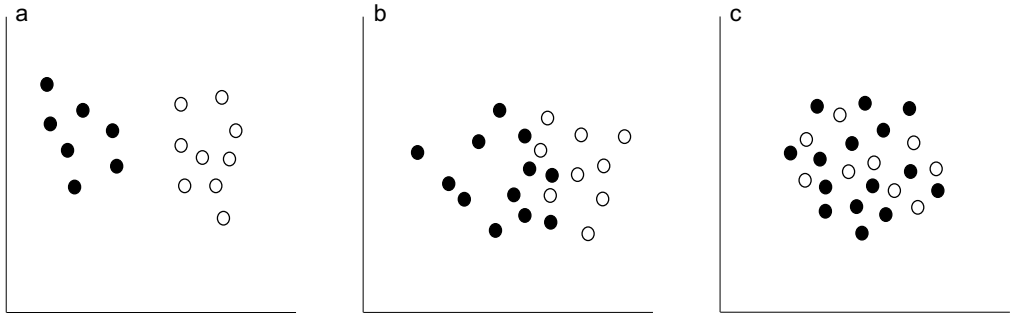

Figure 1.2 Morphological variation and overlap may lead to cryptic species.

Hypothetical samples of two species (black and white) plotted in morphospace. The identity of the samples is assigned a priori. *(a) Distinct species: the two species' morphologies do not overlap, and are far from the trait values of the other species. (b) The two species' morphologies somewhat overlap, but their centroids are still significantly different. (c) Cryptic species: the two species' morphologies completely overlap, and the centroids are not significantly different.*

If drift rather than selection dominates in the evolution of mushroom morphology, conspecific mushrooms may frequently inhabit overlapping clouds in morphospace. In such a scenario, an average mushroom specimen with average states for all species-level characters should be measurably different from an equally average specimen of another species. However, any mushroom in any species may not fall in the average part of the cloud, but at the margins. Overlapping morphologies are expected through convergence or ancestral polymorphism of characters. In this thesis, I show in Chapter 2 that specimens of the genus *Russula* assigned to species a priori based on DNA barcode delimitation show the pattern found in Fig 1.2 b (Bazzicalupo et al., 2017). Other examples of reported overlapping mushroom morphology are in genus *Hebeloma* (Eberhardt et al., 2016; Grilli et al., 2016), species of the *Russula clavipes* complex (Adamčík et al., 2016b), and *Cantharellus* (Dunham et al., 2003). Studies have compared morphological delimitation with molecular delimitation, as in the spiny lizards, and concluded systematics is sensitive to the delimitation tools used (Wiens and Penkrot, 2002).

Because much of fungal biology takes place belowground, we may expect niche partitioning or divergent selection to be more evident there. Molecular identification is beginning to connect fungal species with their ecological roles. Simard et al. (2012) has shown exchange of nutrients between trees through a mycorrhizal connection by carbon labelling, but more work is needed to reveal exactly which fungi are involved in this type of exchange (Selosse et al., 2006). Vertical soil partitioning in fungal species is thought to be important functionally as the proportion of organic N changes at different soil depths, and Mujic et al. (2016) showed that *Rhizopogon* competition in sister species results in niche partitioning and co-existence, despite an overlapping fundamental niche. Taylor et al. (2014) found that from 1001 OTUs (99% cutoff on ITS region) from Alaskan *Picea mariana* soil cores they could only match 33% to sequences stored in databases that were well annotated with a sample, and 67% could only be matched to another environmental sample. They also provided some evidence that closely-related taxa would often be consistently found in soil with different pH levels, giving some support to the idea of niche partitioning (divergent selection for ecological traits) in sister taxa.

Higher taxa and the contributions of molecular systematics to revealing convergence in mushroom forms

Evidence for a mismatch between morphology and identity extends to higher taxonomic ranks in Fungi where classification has changed extensively since the use of phylogenetics. Morphology was what first allowed taxonomic ranks to be distinguished in three multicellular eukaryotic lineages. The sexual system for plants by Linnaeus aligns well with the idea of reproductive isolation, as he observed and built his classification based on the shape and structure of flowers, which are reproductive organs. The classification of plants was eventually refined with molecular phylogenies (Bremer et al., 2009). In placental mammals, most groups were recognized based on morphological characters and consolidated with molecular phylogenies (Springer et al., 2004). Fungal morphology has brought insight in distinguishing phyla like Basidiomycota characterized by meiosis taking place in a specialized cell called a basidium, Ascomycota (where meiosis takes place in an ascus), and Chytridiomycota (where propagules retain an ancestral posterior flagellum that was lost in most fungal lineages (James et al., 2006)). Extensive convergences have historically made evolutionary reconstruction and classification of fungal lineages difficult. One example of a recent phylogenetic resolution representing higher taxonomic ranks is the circumscription of Mucoromycota and Zoopagomycota (Spatafora et al., 2016). Even the typical hyphal form was shown to have convergently evolved at least four times in the fungal tree (Dee et al., 2015).

Within the Basidiomycota and Ascomycota, classification has been in flux since DNA phylogenetics began to reveal extensive convergence among morphological characters. The earliest classification of mushroom-forming fungi in the Basidiomycota by Elias Fries was based on spore colour and fruiting body shape: whether a mushroom had gills or pores or teeth, or was truffle-like and underground, or club-shaped, or crust-like, or coral-like (Fries, 1838), and most current mushroom guides still use this grouping. However, by the 1970s it started to emerge that these characters were not predictive of fungal relatedness. Donk (1971) argued that the genus *Bonderzawia*, originally placed in the order Polyporales or 'shelf fungi', should be classified instead in the order Russulales

based on microscopic characters. As it turned out, Donk's classification still holds up based on molecular data (Binder et al., 2005). Classification of basidiomycete groups has been changing based on molecular data, and the different forms of the mushroom fruiting bodies have been shown to be convergently derived (Hibbett, 2007; Hibbett et al., 1997).

Sexual structures in Ascomycota also evolved multiple times, affecting the classification inside this phylum (Schmitt et al., 2009; Schoch et al., 2009). In Ascomycota, clonal reproduction is common, and the morphology and development of clonal propagules has been studied extensively. Clonal propagule morphology and development is successfully used for identification of common moulds, but has been shown to also be convergent (Kendrick, 1979). Fungi that were thought to only have an asexual lifestyle have been tied to their sexual morphology using DNA evidence (Taylor, 2011).

In this thesis, I use the genus *Russula* to address questions of morphological variation in DNA-delimited species. This was made possible by the collection of fresh specimen characters recorded by Benjamin Woo. No other collection that I know of has such consistent recording of mushroom macro-morphology. The multiple specimens per species also allowed me to observe variation within species.

Thesis objectives

In this thesis, my goals are to explore the consequences of re-formulating mushroom species identities based on taxonomy and biogeography. Specifically, I will:

(i) Evaluate morphological characters of mushrooms from an extensive collection of specimens of the many species of the genus *Russula* from the American Pacific Northwest.

(ii) Describe nine new species of *Russula*, including in my descriptions the morphological variation I found.

(iii) Evaluate the degree of geographical range extent of mushroom species delimited based on the fungal DNA barcode sequence (Schoch et al., 2012).

The data I used for my first and second aim come from specimens of the genus *Russula*. This genus is mycorrhizal, occurs all over the world, and is notoriously difficult

to identify to species. For the phylogenetic structure, I used a combination of my own collections of *Russula* from the Pacific Northwest and herbarium specimens from Europe. For name application, I used type specimens of species from Western North America. To test morphological characters and new species descriptions, I used 713 specimens collected by Seattle architect Benjamin Woo (1923-2008). For my third aim I used 2324 and 341 species of mushrooms circumscribed in the public database UNITE (Abarenkov et al., 2010a; Kõljalg et al., 2005).

In this thesis, I use operational taxonomic units based on the ITS fungal DNA barcode (Schoch et al., 2012) to test mushroom morphology and endemism and to describe new species. I acknowledge that a one-locus phylogeny is probably not a perfect species phylogeny (Maddison, 1997), as no single locus is perfect for delimitation of all species. Some of the species delimited based on a DNA barcode will likely fail other tests of species boundaries. In the well-studied plant pathogenic genus *Fusarium*, the ITS region was shown to have too little variation and failed to delimit haplotypes as accurately as other loci (O'Donnell et al., 2008) or had several variants of the region in the same species (O'Donnell, 1992). Nevertheless, by delimiting species based on a DNA barcode I was able to evaluate the characters we are using and assess how well they predict species (or at least barcode-specific species groupings). Large datasets of multiple loci and samples will help us get statistical support for the use of certain characters over others and understand which characters may be convergent, which may be variable, and how much they vary.

Chapter Two: Troubles with mycorrhizal mushroom identification where morphological differentiation lags behind barcode sequence divergence

Summary

Species of *Russula* (Russulaceae), a large, cosmopolitan, ectomycorrhizal fungal genus, are notoriously difficult to identify. To delimit species and to evaluate their morphology, I sequenced the ~400 bp ITS2 nuclear ribosomal DNA region from 713 Pacific Northwest *Russula* specimens from Benjamin Woo's exceptional collection. As a topological constraint for analysis of the ITS2, I sequenced and inferred a phylogeny from the ITS, LSU, RPB2 and EF1-α regions from 50 European and North American specimens of major clades in *Russula*. I delimited 72 candidate species from Woo's collection's ITS2 sequences using ABGD, GMYC, PTP, and mothur software. To guide application of names, I sequenced a ~200 bp of the ITS1 from 18 American type specimens. Of the 72 delimited species, 28 matched (99% along the sequence) a type or a currently barcoded European species. Among the remaining, 44 are previously unsequenced or undescribed species. I tested the congruence of morphology with delimitations for 23 species represented by 10 or more specimens each. No morphological character alone was consistently diagnostic across all specimens of any of the 23 candidate species. Ordination of combined field characters followed by pairwise multivariate analysis of variance showed that centroids were significantly different in 221 of 253 species pair comparisons. Ordination also showed that specimens from the same species were widely dispersed, morphologically overlapping with specimens from other species. This explains why only 48.5% of specimens were correctly assigned to their species in a canonical variates analysis of combined field and spore characters. Based on sequence comparisons, I contribute to correcting the broad and confusing misapplications of European names that have long obscured patterns of *Russula*'s geographical distribution and diversification. My evidence suggests that morphology in *Russula* diverges slowly and that phenotypic plasticity, convergence, or retention of ancestral polymorphisms blur the distinctions among recently derived species.

Introduction

Through the lens of molecular evolution, speciation followed by drift and selection can permit morphological divergence between lineages. Despite this, morphological divergence after speciation is far from inevitable, as is indicated by numerous examples of morphologically cryptic species in widely varying organisms including metazoans (Pfenninger and Schwenk, 2007), butterflies (Hebert et al., 2004), ancient clades of rotifers (Gómez et al., 2002), marine diatoms and other small marine eukaryotes (Amato et al., 2007; Šlapeta et al., 2006), algae and other plants (Dauphin et al., 2014; Hind et al., 2015) and fungi (Buyck et al., 2016; Crespo and Lumbsch, 2010; Dunham et al., 2003; Le Gac et al., 2007a). In mushroom-forming fungi, cryptic species may be too simple morphologically or too recent in origin to have fixed diagnostic morphological characters. In addition, in fungi, species-specific differences in ecology or biochemistry may evolve unobserved in underground growing mycelium rather than in mushrooms, the ephemeral, aboveground reproductive structures upon which fungal classification is based. For fungi, uncovering cryptic species is an important step towards improving understanding of their diversity, biogeography and speciation processes, niches and conservation needs (Bickford et al., 2007).

My study focuses on species-level variation in *Russula* Pers. (Russulaceae, Russulales) of the American Pacific Northwest. *Russula* is a large genus of common mushroom-forming obligate ectomycorrhizal symbionts of trees and shrubs from arctic and alpine regions (Gardes and Bruns, 1993; Kernaghan and Currah, 1998; Richardson, 1970) to the tropics (Buyck et al., 1996). In old-growth Douglas fir forest soils in Oregon, *Russula* was one of three dominant and most abundant mycorrhizal taxa on root tips and in bulk soil (Hesse, 2012). Despite *Russula*'s importance, identifying its species is difficult or sometimes impossible (Adamčík et al., 2016a; Kuo, 2009; Smith and Lebel, 2001). Species recognition is particularly difficult in areas such as North America west of the Rockies, where the species richness is high and European names are applied widely and often inappropriately to uncharacterized native species (Buyck et al., 2015).

Most North American and European work on *Russula* systematics date from the pre-molecular era (Bills and Miller Jr, 1984; Bon, 1988; Burlingham, 1913; Grund, 1965;

Romagnesi, 1967; Sarnari, 1998-2005; Shaffer, 1962, 1964, 1972, 1975; Singer, 1975). Buyck (1990) monographed species of this genus from central Africa. Roberts (2007) described with detailed drawings the species occurring in the coastal forests of Vancouver Island. Adamčík and Buyck (2010, 2011, 2012); Adamčík et al. (2013); Buyck and Adamčík (2011a, 2011b, 2013); Buyck et al. (2008a) re-described the micromorphology of many type specimens for species occurring in eastern North America. More recently, they extended their type studies to western North American species as well (Buyck et al., 2015). Over the past 10 years, molecular systematic studies have aimed to clarify the genus-level phylogeny of *Russula*, and its relationship with related genera: *Lactarius* Pers., *Multifurca* Buyck and V. Hofstetter and *Lactifluus* (Pers.) Roussel, by focusing on identified exemplar specimens. These studies have shown only moderate concordance between morphology and phylogeny (Buyck et al., 2008b; Eberhardt, 2002; Eberhardt and Verbeken, 2004; Miller and Buyck, 2002). Critical analyses of species delimitation and intraspecific variation have been few in number and restricted to *Russula*'s smaller clades (Adamčík et al., 2016a; Melera et al., 2017).

I was able to delimit candidate species and then analyze multiple collections of the same species to address the stability of morphology within clades thanks to the work of Benjamin Woo (1923-2008). Woo, an architect from the Seattle (WA) area, who is considered a foremost regional expert on *Russula*. He collected, photographed and documented 1076 *Russula* specimens with great care and consistency. Each collection was paired with a data sheet that detailed locality, habitat, macromorphological characters (e.g. cap colour or stipe staining), and staining in response to chemical spot tests, all in a consistent format (Fig. 2.1).

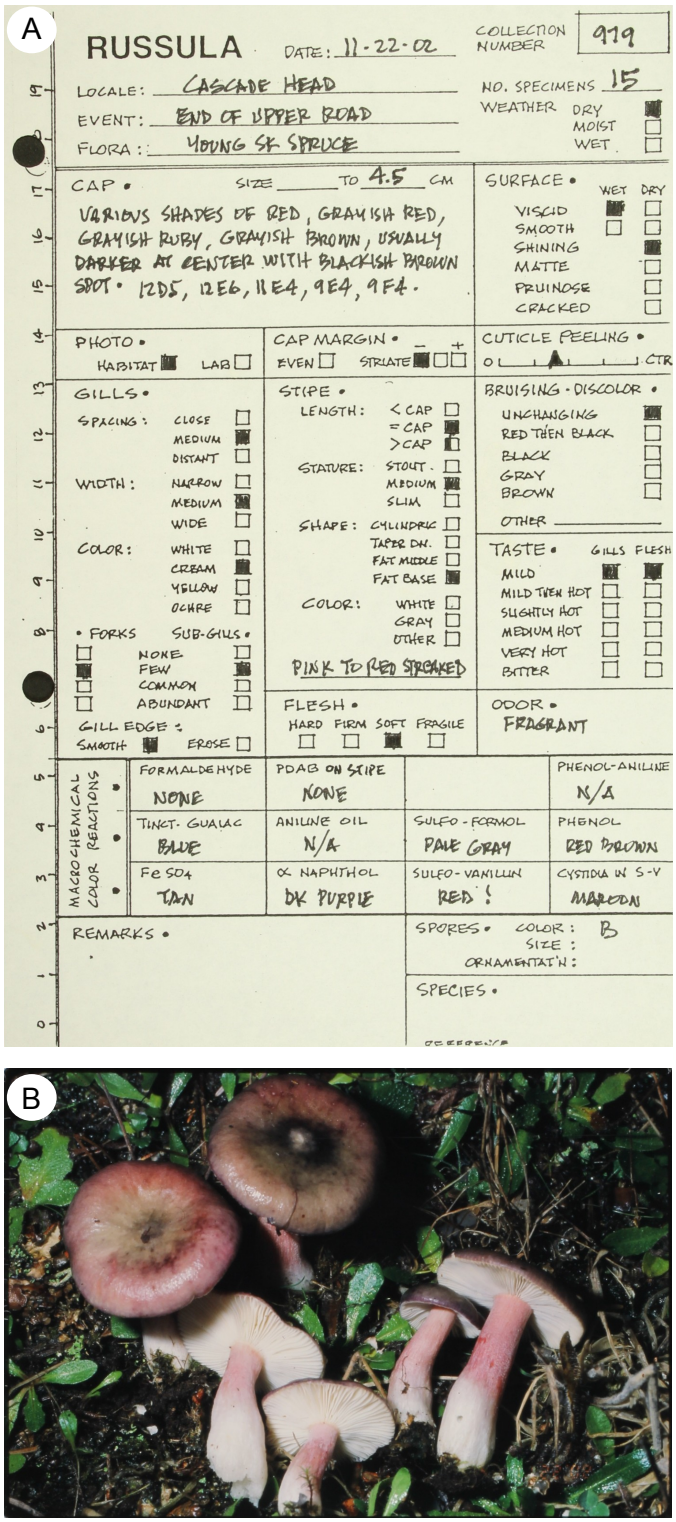

Figure 2.1 Specimen of Russula queletii *collected by Woo with collection sheet.*

Russula queletii *Fr. voucher BW 979.* ***A,*** *Woo's collection sheet showing data fields.* ***B,*** *Specimen photograph (By Benjamin Woo, with permission from the Burke Museum).*

Multiple years of fieldwork are necessary to capture the species richness of mushrooms due to their irregular or infrequent fruiting. Watling (1995) found that it took at least five years to observe fructifications of most of the macrofungal flora in a given area. Orton (1986) maintained that at least 10 years were needed. Straatsma and Krisai-Greilhuber (2003) found that after seven years, their species accumulation curve still failed to reach an asymptote. Straatsma et al. (2001) recorded new species every year including the last season over a ~20 year survey of fungal fruiting bodies in permanent plots. Woo collected over ~30 years, from 1974 to 2006. Unlike other more sporadic collectors, Woo may have captured a substantial fraction of the regional species richness.

To guide application of names to western taxa, I planned to borrow type specimens and sequence a part of the ITS region from them as well. Unfortunately, the quality of preservation of DNA in Woo's specimens did not match the depth of the collection. The nuclear ribosomal internal transcribed spacer (ITS) is the official fungal barcode (Schoch et al., 2012). Given the Woo specimens' fragmented total genomic DNA, the longest region amenable to sequencing across a wide range of sample quality was the ~400 bp long ITS2 region (Appendix 1.1). The problem of degraded total genomic DNA was even more severe for the types, and I was only able to sequence the short ~200 bp ITS1 region. To put the ITS sequences into a phylogenetic context, I sequenced and analyzed additional loci (ITS, LSU, RPB2 and EF1-α) from better-preserved recent collections to provide a topological constraint. I then used the constrained ITS2 phylogeny for species delimitation.

Previous studies have demonstrated success in applying species delimitation software to DNA sequence data sets, even for organisms with unknown diversity, sporadic availability of collections, and many undescribed species (Esselstyn et al., 2012; Leliaert et al., 2009; Pons et al., 2006). Studies on the fungal genera *Entoloma* P. Kumm. (Morgado et al., 2013) and *Xanthoparmelia* (Vain.) Hale (Leavitt et al., 2011), and on the species groups *Cladonia cariosa* (Ach.) Spreng. (Pino-Bodas et al., 2012) and *Amanita muscaria* (L.) Lam. (Geml et al., 2006) began with species molecular-based delimitation and went on to evaluate morphological, geographical, and ecological characters that can be important evolutionarily or for identification.

My working definition of 'species' is a group of specimens clustered based on explicit evolutionary expectations, as determined from the ITS2 region of the fungal barcode. I used four approaches to delimitation. One approach, Automatic Barcode Gap Discovery (ABGD) (Puillandre et al., 2012), uses aligned sequences as input and assumes that sequence divergence between species usually exceeds divergence within species. The transition from intraspecific to interspecific pairwise distances will then result in a detectable 'barcode gap'. The second approach uses a Generalized Mixed Yule Coalescent (GMYC) model (Fujisawa and Barraclough, 2013) and takes, as input, a single ultrametric tree of unique sequence types. It assumes that species are monophyletic, and then compares the likelihood of assigning branching events to speciation events or intraspecific coalescence taking time estimates into account. The third approach involves a Poisson tree processes (PTP) model (Zhang et al., 2013) and uses as input a maximum likelihood tree including all individuals to be classified. The PTP model does not require an ultrametric tree because it estimates speciation rates using numbers of substitutions rather than time. It assumes that branch lengths are generated by two non-overlapping processes. One process consists of speciation events where the average number of substitutions until the next species-level branching event follows an exponential distribution. The second process again uses an exponential distribution but to describe the probabilities of intraspecific branching and divergence. Both GMYC and PTP assume that probabilities of substitutions contributing to branch length between speciation events will be higher among species, while probabilities of intraspecific branching will be higher within species.

As a fourth method, I used the software mothur (Schloss et al., 2009) to cluster sequences using a 99% similarity cutoff point. In *Cortinarius*, Harrower et al. (2011) found that the 99% threshold correlated with phylogenetic, morphological, and ecological delimitations of species. However, an appropriate cutoff percentage may vary by clade (Nilsson et al., 2008). Hughes et al. (2009) suggested that within-species ITS variation could be estimated from the percentage of heterozygous positions in sampled mushrooms because mushroom tissue is dikaryotic and it carries equal proportions of the haploid genomes from both of its parents. Hughes et al. (2009) analyzed 100 mushrooms that represented various genera and that had heterozygous ITS regions. They found a

maximum 3.27% difference between haplotypes in a single mushroom. In almost 93% of mushrooms with heterozygous ITS sequences, the two constituent haplotypes were more than 99% identical. Following the logic of Hughes et al. (2009), I searched for shared polymorphic sites to provide evidence regarding the divergence of interbreeding parents to help calibrate expectations for within species variation.

To delimit species of *Russula* in the Pacific Northwest, my approach combined analysis of the ITS2 region from Benjamin Woo's collection, sequences from type specimens, and a framework of a genus-level multi-locus phylogeny from exemplar specimens. To assess evidence for morphological divergence across species and clades, I combined phylogenetic and multivariate statistical approaches to analyze spore and field characters from delimited species represented by 10 or more specimens. My work provides an example of how a deep, multi-year collection can contribute to uncovering cryptic species, improving species delimitation, and relating morphological evolution to a sequence-based phylogeny.

Materials and Methods

Sampling and database

For sequencing, I borrowed all 1076 of the *Russula* specimens of the Woo collection from the Burke Museum (Seattle, WA), along with scanned versions of Woo's detailed collection sheets and photographs (Fig. 2.1). The Woo collection spans ~30 years ca. from 1974 to 2006, and covers the Pacific Northwest concentrating mostly in Washington and Oregon, with a few samples from northern Idaho and northern California. Each collection sheet has 63 fields for chemical spot test results, cap colour, spore colour, locality etc. To compare the morphological and chemical characters scored by Woo with the DNA results, I designed a searchable, online database and populated it with the images and the data from all 63 fields with Dave Carmean (SFU). Michael Beug (Emeritus Professor, The Evergreen State College), was familiar with Woo's collection localities, and entered geographical coordinates for each site. The Benjamin Woo *Russula* Database is hosted on the SFU server using FileMaker® Pro 11 server software. The main database table contains 1191 records where each record represents a specimen

collected by Woo (these include non-*Russula* specimens). The online searchable version is available at: http://advance.science.sfu.ca/fungi/index.php?-link=Home.

For insight into the application of names, I borrowed type specimens of 18 species of *Russula* from western North America (Buyck et al., 2015). Of these, eight were described from Oregon and Washington (Table 2.1). In order to improve the support for the relationships between major *Russula* clades I sampled 50 recently collected, reliably by J. Vauras and I. Saar identified specimens from herbaria of the University of Tartu (Estonia), Uppsala University (Sweden), and the University of British Columbia (Canada) (Appendix 1.2). For this, I selected species based on previous *Russula* phylogenies to represent, as evenly as possible, the diversity of clades of *Russula* in temperate regions (Buyck et al., 2008b; Miller and Buyck, 2002).

Table 2.1 Pacific Northwest Russula *type specimens*

Type specimens sequenced for the study and matching GenBank sequences used in analysis.

Type name (species epithet)	**Author**	**State**	**Herbarium Accession**	**Clade**	**GB type /GB ITS2**[1]	Year of Collection	Year of Publication
alcalinicola [2]	Burl.	WY	MICH-618776	28	KX812817 /DQ974759	1920	1924
atroviolacea	Burl.	CO	NY-333779		KX812818 /JX630968	1914	1915
avellaneiceps	Fatto	CO	NY-00253509		KX812819 /KF007951	1997	1999
bicolor	Burl.	VT	NY-00618785		KX812820 /AY656976	1911	1913
californiensis	Burl.	CA	MICH-12193		KX812821 /AY245542	1928	1936
cascadensis	Shaffer	OR	MICH-12194	22	KX812822 /KJ146726		1964
cerolens	Shaffer	OR	MICH-9611	19	KF245486 /KF245486	1935	1972

cochisei	Fatto	AZ	NY-00618830		KX812823 /KF810136	1994	2000
crassotunicata	Singer	WA	MICH-12200	2	KX812824 /DQ384580	1935	1938
grundii	Thiers	CA	HDT-51480		KX812825 /DQ974829	1988	1997
mendocinensis	Thiers	CA	HDT-53479		KX812826 /DQ367913	1990	1997
montana [3]	Shaffer	CO	MICH-12231	35	KX812827 /EU057106	1972	1975
mordax	Burl.	WA	NYGB-653969	67	KX812828 /AF335442	1927	1936
rosacea var *macropseudocystidiata*	Grund	WA	ACAD-12870	31	KX812829 /HQ604840	1962	1979
sierrensis	Thiers	CA	HDT-52894	69	KX812830 /JF834336	1989	1997
stuntzii	Grund	WA	ACAD-12868	25	KX812831 /AY281091	1962	1979
viridofusca	Grund	WA	ACAD-12867	70	KX812832 /KJ748434	1962	1979
zelleri	Burl.	OR	NY-761009	46	KX812833 /JF834326	1927	1936

[1]These are the GenBank accession numbers of the ITS1 of the type specimen. I report the GenBank accession for the corresponding ITS2, also shown in Appendix 1.1.

[2] ITS1 sequence of *R. alcalinicola* Burl. 1924 is identical to the ITS1 sequence *R. exalbicans*. If synonymous, *R. exalbicans* has priority as it was applied earliest at the species level (*Agaricus exalbicans* (Pers.) J. Otto 1816).

[3] ITS1 sequence of *R. montana* Shaffer 1975 is identical to the ITS1 sequence of *Russula griseascens* (Bon & Gaugué) Marti 1984. If synonymous, *R. montana* has priority.

DNA extraction, amplification and sequence assembly

Gill tissue from each sample was ground using a TissueLyser machine (Qiagen, Retsch MM301 Mixer Mill Pulverizer). Genomic DNA was extracted from the Woo samples following the DNeasy 96-well Protocol from Qiagen. After preliminary tests of amplification from the 10-40 year old specimens, I chose to amplify the ~400 bp ITS2 region rather than the shorter ITS1 or the complete ITS1/2 region (Gardes and Bruns, 1993; White et al., 1990). This represented a compromise between optimizing the consistency and information content of the results. All samples were extracted twice in two separate plates so that I could detect contamination, which can be a problem with older samples having degraded DNA. The British Columbia Cancer Research Centre amplified and sequenced the ITS2 regions using Sanger sequencing and primers ITS3 and ITS4 (White et al., 1990). Each sample should have had four chromatograms (two forward, two reverse) representing the two replicates of the extraction. For my analyses, I only included the chromatograms that had been confirmed by both replicates. This resulted in a ~70% success rate and left 713 sequences from vouchers from the Woo collection. I edited and automatically trimmed the Woo sample sequences with Sequencher 5.1 DNA software. I aligned all the raw sequences in MAFFT (Katoh and Standley, 2013) and generated a 'rapid bootstrap' maximum likelihood (ML) tree with 100 replicates in raxmlGUI (Silvestro and Michalak, 2012) to recover preliminary clades. For each clade of nearly identical sequences from multiple collections, I then re-examined all chromatograms using Sequencher 5.1, recording polymorphisms, and correcting sequencing errors. Mushroom tissues are mostly dikaryotic and as in diploids, both parental alleles occupy the same cell. Double peaks in the ITS region chromatograms could either indicate variation among the tandem repeats of the ITS, or positions that were heterozygous between the two different nuclei of the dikaryon. I interpreted overlapping peaks as likely heterozygous positions when the superimposed peaks were similar to one another in area but with roughly half the surface area of neighboring single peaks, especially when both alternative alleles occurred as homozygotes in the population (Hughes et al., 2009). I indicated the polymorphisms using International Union of Pure and Applied Chemistry (IUPAC) nucleotide codes (such as 'Y' for 'C' or 'T').

I extracted genomic DNA from the type specimens using Qiagen's DNeasy Plant Mini Kit. The DNA in type specimens dating from 1915 to 1997 (Table 2.1) was too degraded for successful amplification and sequencing of even the 400 bp ITS2 region (primers ITS3 and ITS4). However, I successfully amplified and sequenced the ITS1 region from 18 specimens using primers ITS1F and ITS2. To represent each type species in analyses, I used a complete ITS sequence retrieved from GenBank that matched the type's ITS1 region (Appendix 1.1, Table 2.1). To make sure that the types and GenBank matches to the types also corresponded to delimited Woo species, I sequenced ITS1 regions from selected Woo species exemplars (Appendix 1.1). Cycling conditions were: initial denaturation (5 min, 94 °C), followed by 35 cycles (94 °C, 10 s; 55 °C, 20 s; 72 °C for 30 s plus 4 additional seconds per cycle), and then a final extension at 72 °C for 7 min. The product was diluted 100 times and re-amplified using the same primers with an annealing temperature of 48°C. The University of BC Nucleic Acid Protein Service Unit sequenced the types, and I analyzed the chromatograms in Mesquite's Chromaseq (Maddison and Maddison, 2005).

DNAs from the 50 specimens used for the multi-locus constraint tree were amplified using ITS1F (Gardes and Bruns, 1993) and ITS4 (White et al., 1990) for the ITS; LR0R and LR5 (Vilgalys and Hester, 1990) for the LSU; RPB26F (Matheny, 2005) and RPB27cR (Liu et al., 1999) for RPB2; and EF1-α 1577f and 2218R (Rehner and Buckley, 2005) for tef-1. Sequencing was performed at the Innovation Centre at McGill University and Génome Québec. Appendix 1.2 gives herbarium and sequence accession numbers.

Phylogenetic analysis

I aligned data from each of the four loci (ITS, LSU, RPB2 and EF1-α) from the 50 more recent samples using MAFFT, with manual editing in Mesquite (3.1) (Maddison and Maddison, 2015). I excluded introns in the RPB2 and EF1-α from the analysis. I analyzed the four loci individually and then together following concatenation. As a model of substitution, jModelTest (Posada, 2008) selected GTR+GAMMA for each single gene. No contradicting topology with support above 70% bootstrap (BP) support was recovered from single gene phylogenies (Appendix 1.3).

PartitionFinder (Lanfear et al., 2012) applied to the concatenated alignment found different models of evolution for each gene and codon position. PartitionFinder selected GTR+GAMMA for ITS and 28S. TrNef+I was selected for the 5.8S. TrN+I, HKY+G, and TIMef+I+G were selected for first, second and third codon position in EF1-α. GTR+I+G, K81uf+I+G, and TIMef+I+G were selected for first, second and third codon position in the RPB2 gene. I inferred several maximum likelihood trees based on the concatenated alignment of the genes using different partitions. I computed a maximum likelihood phylogeny partitioned by gene and unpartitioned using RAxML BlackBox with default settings under a GTR+GAMMA model of evolution (Stamatakis et al., 2008). I also produced a maximum likelihood tree with Garli 2.01 (Bazinet et al., 2014; Zwickl, 2006) to account for different rates of evolution to account for different gene partitioning. All multi-locus phylogenies recovered from different analytical strategies showed the same topology (Appendix 1.4), which I used as a topological constraint.

For further comparison with the Woo ITS2 sequences, I added 66 GenBank sequences from Miller and Buyck (2002). I also added the ITS2 of the samples from the multi-locus phylogeny; the GenBank sequences that matched the types; and five sequences from UNITE, chosen because they were 100% identical to my samples (or the closest match in the case of *R. badia* Quél. UDB016002) (Abarenkov et al., 2010a). In RAxML BlackBox (Stamatakis et al., 2008) I again used a GTR+GAMMA model of substitution and constrained the topology of the ITS2 alignment with the multi-locus phylogeny shown in Appendix 1.4. I considered bootstrap values over 95% to indicate strong support for a branch. I show values over 60% on figures because, depending on substitution rates and modes, this level of support may indicate underlying phylogenetic signal and is worthy of further testing. As outgroups for both the multi-locus and ITS phylogenies, I used *Pseudoxenasma* and *Gloeopeniophorella*, two related genera that are not included in the monophyletic group formed by *Russula* and *Lactarius* (Miller et al., 2006).

Candidate species delimitation

I ran the ABGD (Puillandre et al., 2012) analysis, the first of four methods of species delimitation, on the web version of the software (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) with default settings, using as

input the aligned ITS2 regions of Woo samples and other representative sequences. In the GMYC (Fujisawa and Barraclough, 2013) analysis, the ML value of lambda (Moran estimator) was estimated as the number of splitting events divided by the branch length (Nee, 2001), and so the software could not handle polytomies or zero-length branches. I selected one representative of each sequence type by excluding identical sequences using raxmlGUI (Silvestro and Michalak, 2012). From the resulting alignment of 416 *Russula* sequences, I inferred an ML tree using ITS2 data and a GTR+GAMMA model, constrained by the multi-locus backbone topology. I transformed the ML tree into an ultrametric tree using r8s with the Langley and Fitch method with the Powell algorithm with multiple restarts (Sanderson, 2003). I then trimmed all of the terminal taxa that were forming polytomies. I carried out the analysis on the remaining 150 representative taxa in R (R Core Team, 2014) using the splits package (Ezard et al., 2009). I used the single threshold analysis as it reportedly performed better for species delimitation (Sanderson, 2003).

The Langley and Fitch method, which assumes a single rate of substitution, was appropriate for my dataset because rates of substitution, although not clocklike, were uncorrelated through the tree (Sanderson, 2003), and because the ITS2 offered too little data for reliable parameter estimates in a more complex clock model. Autocorrelation of rates across clades was rejected by tests that showed that the value of an optimality criterion (likelihood minus a smoothing penalty) was highest in the absence of smoothing under a penalized likelihood model with the truncated Newton algorithm. I explored the effect on the GMYC delimitation of the penalized likelihood method (no smoothing; allowing rate variation throughout the tree). Using a penalized likelihood model resulted in delimitation of fewer species (50 vs 81 with Langley and Fitch) due to lumping of species e.g., *Russula pallescens* with *R. crassotunicata* that could be separated by morphology, ecology, or sequences. I did not consider the penalized likelihood model further. For the Bayesian PTP (bPTP) (Zhang et al., 2013) analysis, I again used the ITS2 ML tree with the multi-locus backbone constraint as input, implementing the software through the web interface (http://species.h-its.org/ptp/). I ran the mothur analysis Version 1.38.1 with the 99% cutoff point that was consistent with observed within-specimen polymorphism.

As a working criterion for delimiting species, on a first pass, I grouped specimens based on agreement among at least three of the four delimitation methods. My approach was conservative in the sense of Carstens et al. (2013) in that I looked for support from multiple methods before subdividing a clade into smaller groups of species. When only two methods agreed, I avoided proposing separate species for single specimens and for example, I included BW_523 in *R. stuntzii* Grund.

The PTP's behaviour was occasionally inconsistent, sometimes splitting (e.g. Woo sp. 20) and sometimes lumping specimens (e.g. *R. viridofusca* Grund, *R. xerampelina* (Schaeff.) Fr., and Woo sp. 60) where the other methods did not. In the case of *R. firmula* Jul. Schäff., no two delimitations agreed, and so I delimited this species as the largest set of isolates put into a single species by any of the methods.

Light microscopy

I measured characters of 30 basidiospores from each of at least eight specimens from each of the 23 species represented by 10 or more collections. I mounted the spores in Melzer's reagent. To illustrate examples of other characters used in *Russula* systematics, I examined the cap cuticle and hymenium from dried mushroom samples in 10% KOH. Photographs were taken with differential interference contrast imaging at 1000 x magnification with a Leica DFC420 digital colour camera mounted on a Leica DMRB (Leitz) DIC microscope. Using ImageJ (Abràmoff et al., 2004; Schneider et al., 2012) and Photoshop CS5 (Adobe Systems Inc.), I processed images and measured the length and width (excluding ornamentation) of mature spores in profile view. Also in ImageJ, I calculated the basidiospore width to length ratio and the maximum height of ornamentation of a spore in profile view on the distal side from the suprahilar appendix.

Phylogenetic evaluation and coding of field characters

I mapped the character states for 36 coded macromorphological characters onto the 715 sequence (713 from Woo samples, two from outgroups) ITS2 tree. I calculated the Retention Index (RI) for each character through the tree to evaluate its consistency through the phylogeny using Mesquite. RI values indicate the amount of homoplasy in a tree and how measure how well synapomorphies explain a tree. To investigate the stability of characters within species, I graphed the percentage of times that each state of

the 36 characters occurred in each of the 23 species with 10 or more collections. Woo recorded the colour of the cap (or pileus) of each specimen using terms and codes from Methuen (Kornerup and Wanscher, 1978). I reported the range of colours recorded among collections of delimited species in pie charts that display the palette web safe colour in Excel (MS) that was closest to Methuen colour recorded for each specimen. Woo recorded the colour of the gills and spore print based on charts in Crawshay (1930). Based again on specimen records, I selected colours using the Color Wheel palette in Excel (MS) to show inter- and intraspecific variation using pie charts.

Multivariate analyses of morphological characters to test for character divergence among species

To test whether the combined categorical (non-numerical) characters differed between species, I performed a multiple correspondence analysis (MCA) with the FactoMineR package in R (Abdi and Valentin, 2007; Lê et al., 2008). As input I used 11 characters with RI values greater than or equal to 0.3, from the species represented by 10 or more specimens. I coded the coordinates of the first two axes of the MCA according to the species identity of each individual collection. This allowed me to test whether the difference in coordinates between species was significant using a pairwise multivariate analysis of variance (MANOVA).

For species with ITS sequence polymorphisms, I looked for linked variation in sequences, spore length, width, width/length ratio, or ornamentation height that might offer evidence for additional species nested within delimited species. I performed an ANOVA and least significant difference (LSD) test to detect significant differences among spore measurements of specimens of different sequence types within a delimited species. Compared to a Tukey test, the LSD test is more relaxed and more likely to alert us to small differences. All statistical analyses were performed in the R package ggplot2 (R Core Team, 2014; Wickham, 2009). My data did not lend themselves to formal analysis of linkage disequilibrium but I looked by eye for linked traits such as, for example, association of a sequence variant with statistically larger spores having smaller ornamentation.

To test for significant differences of individual spore characters among species, I performed a series of ANOVAs and subsequent Tukey tests. To test whether specimens

from the same species grouped together using a combination of the spore measurement characters, I performed principal components analyses (PCAs) based on average (standardized) log values of 30 spores for each specimen.

To test whether the combination of both the categorical field traits and continuous spore measurements could group specimens by species, I performed a factor analysis of mixed data (FAMD) implemented in FactoMineR (Husson et al., 2016). Using FAMD, I weighted the continuous (spore measurement) and categorical variables equally to balance their influence, and then performed a PCA on the standardized data (Audigier et al., 2016). I used only samples for which field and spore traits were complete to avoid potential bias arising from missing data. I represented the results in a dendrogram after performing a hierarchical agglomerative cluster analysis of the first five axes from the FAMD and represented it with a dendrogram in the Cluster package in R (Maechler et al., 2012). If morphology predicted the 23 species, samples from the same species would be expected to group in clusters.

Multivariate analyses of probability of correct species identification, based on recorded field and spore characters

I implemented a canonical variate analysis (CVA) in the Morpho package in R (Schlager, 2014) (i) to test support from morphological characters for the delimited species and (ii) to estimate the probability of assigning specimens to the correct species using the recorded morphological characters. This type of analysis is used in morphometrics, especially of fossil taxa to estimate how well the groups defined by the study are supported by morphology, (Schlager, 2014; Webster and Sheets, 2010). Assumptions include that the number of groups is defined and that every new specimen will fall into one of the pre-defined groups. Statistical testing uses a cross-validation procedure with replicated runs, each time with exclusion of a small, random set of samples. The proportion of times that the previously excluded samples are reassigned to a delimited species is reported as a percentage (Webster and Sheets, 2010). I performed this analysis on the first five dimensions of the MCA, the PCA, and the FAMD. If the morphology predicted species delimitation perfectly, then, in cross validation runs, specimens would be assigned to the correct species 100% of the time. On the other hand,

if the morphology of delimited species overlapped or converged, the assignment percentage could be split among several species.

Results

Delimitation supported 72 candidate species from 713 sequences

In total, the 713 specimen sequences from B. Woo's collection represented 72 candidate species following my delimitation criteria and multi-test working species concept (Fig. 2.2, Appendix 1.5). This represents a conservative consensus from results from individual methods, which yielded different numbers of species (Appendix 1.6). GMYC delimited 81 species from an input of 150 sequence types. Input for ABGD, mothur, and PTP included the 713 specimen sequences. ABGD gave 76 species. Mothur delimited 93 putative species given a 99% identity threshold. As expected, mothur's strict 1% within-species maximum divergence resulted in the exclusion of the occasional specimen or two that the more relaxed ABGD or GMYC included in a single species. I counted the polymorphic sites for each sample in the Woo collection. Of the 713 samples, 85% were homozygous while 15% were polymorphic at one or more sites in the ITS2 region. Supporting interpretation of polymorphic sites as heterozygosity, I recovered homozygous individuals of both 'parental' types in well-sampled populations (Appendix 1.7). Of the heterozygous samples, most (62) had only one heterozygous site, 0.19% of the aligned ITS2. All sequences detected as heterozygotes would correctly be placed in the same species by mothur using the 99% cutoff from aligned sites because the maximum number of heterozygous sites, found in three sequences, was five or 0.95% of the 525 site ITS2 alignment. PTP delimited 78 candidate species from the 713 samples. My sequence dataset is not complete. Specifically, it is missing most of the white Russulas (Section *Lactarioideae* Maire, except for *R. cascadensis* Shaffer (22), and Woo sp. 21) and blackening Russulas (Section *Compactae* Fr.) because I was unable to amplify and sequence DNA from these taxa. I am unaware of any other biases in the collecting or sequencing.

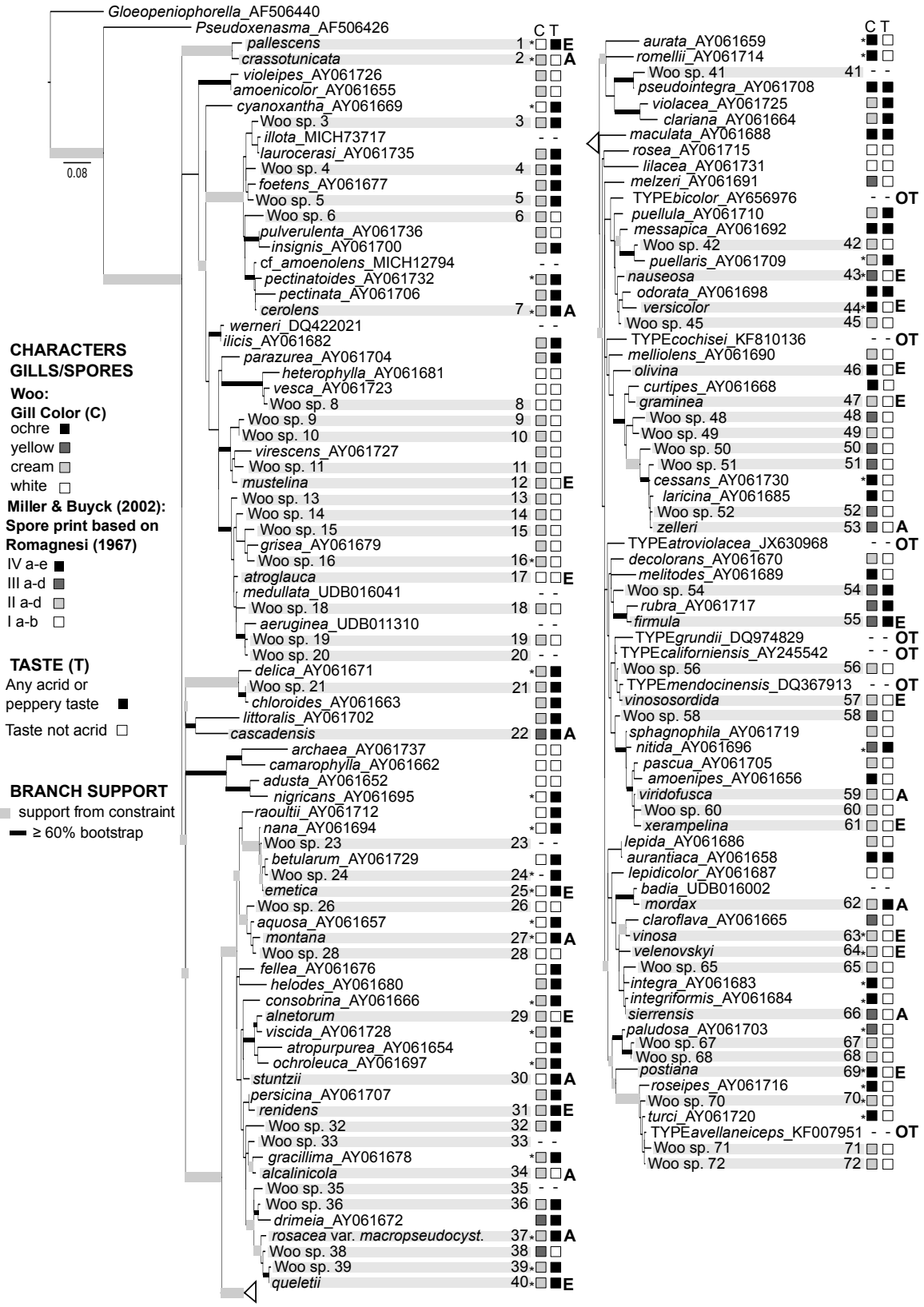

Figure 2.2 Woo samples in a phylogeny of Russula

Maximum likelihood phylogeny placing ITS2 barcode sequences of the Pacific Northwest Russula *species within a multilocus constraint tree. Each northwest species from the Woo collection is indicated by grey highlighting and a clade number. Specific epithets and accession numbers indicate sequences from GenBank or UNITE. Character states were coded based on the majority of the samples in that clade. Characters for taxa from Miller and Buyck (2002) were included for comparison. We collapsed the multiple, alternative taste character states from Miller and Buyck (2002) to binary 'mild' vs 'acrid' coding to be consistent with our findings. Symbols: 'E' sequence identical to a European reference specimen; 'A' sequence matched to a Pacific Northwest type specimen; 'OT' sequence known only from the type collection. '*' a taxon used in the constraint tree. For the complete phylogeny of ITS2 haplotypes see Appendix 1.5.*

In the Woo collection, I found 11 candidate species that matched only sequences from types of western North America (Appendix 1.1). Another 17 taxa only matched sequences from Europe. Sequences of two western North American types matched European sequences. These were the type of *R. alcalinicola* Burl. with a 100% match to European *R. exalbicans* (Pers.) Melzer & Zvára, and *R. montana* Shaffer, with a 100% match with *R. griseascens* (Bon & Gaugué) Marti (Table 2.1). The remaining 44 taxa could not be matched to any reliable source, and these may be either undescribed or unsequenced, described species. Of the species delimited, 20 were represented by a single sample, and 14 of those were part of the 44 potentially undescribed species. Without multiple samples to reveal within-species variation, my study lacked a basis for inferences about characters that would distinguish these clades.

I found few shared nucleotide polymorphisms between candidate species. For each species and each aligned site that had additive double peaks indicative of within-species polymorphisms (Appendix 1.6), I analyzed all of the sequence variants shared among specimens. I then looked for shared polymorphisms between closely related species (Appendix 1.7). No polymorphisms were shared between Woo sp. 28 and the closely related *R. montana* (sp. 27) (Appendix 1.7). While closely related Woo sp. 39 and *Russula queletii* Fr. (sp. 40) have between them four sites with double peaks, none of their polymorphisms were shared (Appendix 1.8). For example, specimens of Woo sp. 39 have a double peak, 'G/T', or a 'G', or a 'T' at site 72, where all *R. queletii* specimens had a

'G'. Mothur split *R. queletii* into three groups but delimiting these as separate species was not justified by sequence patterns (Appendix 1.8), which were consistent with homozygous and heterozygous alleles in a single interbreeding population.

Field characters and chemical spot tests showed low levels of congruence with the phylogeny

Although low bootstrap support at the deeper nodes indicated remaining uncertainty about relationships, I estimated the distribution of character states recorded by Woo using the phylogeny constrained by the multi-locus tree. The multi-locus phylogeny (Appendix 1.4) clearly improved the support for some clades compared with support from ITS sequences alone. Given the phylogeny, none of the 36 characters recorded by Woo were perfectly consistent throughout clades. RI values ranged from 0-1, with 1 showing the fewest state changes, and 0 showing most state changes. The characters with the highest RI values were 0.69/0.68, taste of flesh/gills; 0.49, colour change in sulfovanillin; 0.46, colour change in sulfoformol; 0.45, gill colour; 0.45, cap margin striation; 0.42, stipe colour flush/stain; 0.4, bruising; and 0.37, fragrance (Appendix 1.9). Some characters scored by Woo had to be reinterpreted before patterns were apparent. For example, the different degrees of 'hot' tastes were highly variable among conspecific specimens (Appendix 1.10 C, D) yet taste of the gills and flesh were the characters with the highest RI values as long as subcategories for the degrees of 'hot' or 'peppery' were lumped together as 'hot'. I mapped taste and gill colour onto the phylogenetic tree using characters from Woo and data from Miller and Buyck (2002) (Fig. 2.2). Woo recorded spore print colour for some specimens but he recorded gill colour (which comes from spores) for almost all specimens. The coding that Woo applied to gills and the coding that Miller and Buyck (2002) applied to spore prints had comparable character states and were thus easy to combine to indicate general colour patterns across clades in Fig. 2.2. Based on my analysis of variation within the 23 delimited species represented by 10 or more samples, I re-interpreted tastes as hot or mild from the original three categories used by Miller and Buyck (2002).

I also analyzed character-state variability within the 23 species with 10 or more specimens (Fig. 2.3, Appendices 1.10-1.18). In contrast to patterns among nucleotide polymorphisms, all macromorphological or chemical characters that varied among

species also varied within species, and shared polymorphisms were the rule. Even characters with the highest RI values were not constant within candidate species. I considered a character as 'potentially useful' as long as one state was recorded among 50% or more of collections of one or more species, while being rare or absent in any other species (Appendix 1.18). Woo grouped characters by the mushroom part involved (e.g. cap or stipe); staining or colour changes; colour changes in response to chemical spot tests; and spore print colour (Appendices 1.10-1.17). In each category, at least one character, usually a character with a relatively high RI value, has the potential to contribute to identification.

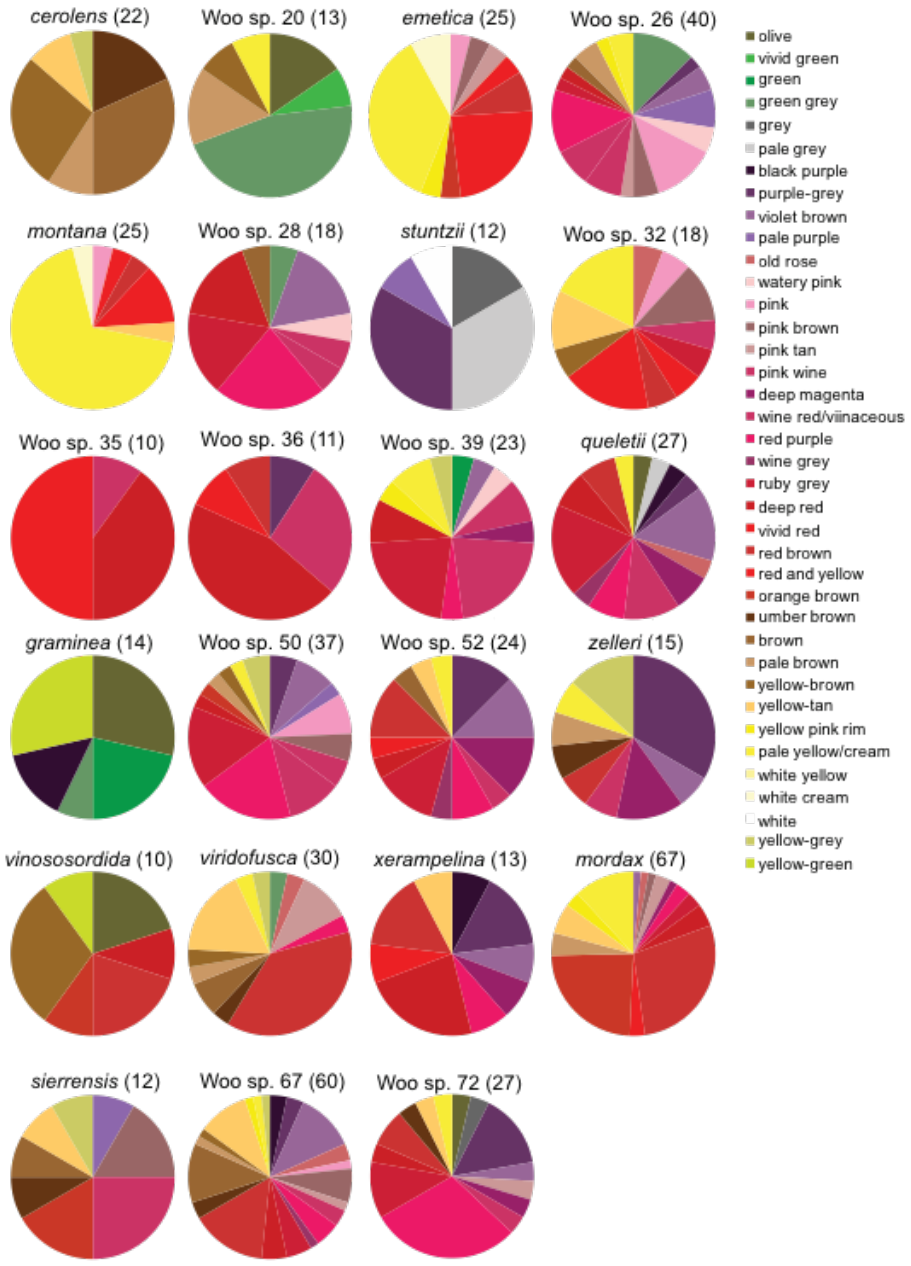

Figure 2.3 Russula *species cap colours*

Variation in cap colours among conspecific collections of *Russula*. Each pie chart represents one species with 10 or more specimens. Width of a coloured section is proportional to the fraction of specimens that shared the same predominant cap colour. The number of specimens is in parentheses and follows the specific epithet or species code. Colours approximate the Methuen chart colours (Kornerup and Wanscher, 1978) recorded in collection notes for each specimen.

Chemical characters consisted of records of colour changes in fungal tissue in response to drops of various chemicals (Appendices 1.18-1.19, and 1.14-1.15). The $FeSO_4$ test is probably the most widely used chemical test for identification, producing a distinctive blue or green reaction on flesh of *R. xerampelina* and *R. viridofusca*, versus an orange and pink reaction for other species (RI 0.35, Appendix 1.15 E). As described on Woo's data sheets, reactions to spot tests with sulfovanillin were inconsistent within candidate species. To code this character, I grouped black and purple colour reactions as 'dark' vs. gray or pink as 'pale,' thereby increasing within-species consistency (Appendix 1.14 D). The relatively high RI of response to sulfoformol reflected in part the small number of alternative states for this character (Appendix 1.14 C). Gill colour and spore print colour, which Woo had coded by matching with colour tiles from Crawshay (1930), distinguished some candidate species groups, but still varied within-species (Appendices 1.16-1.17). When cap margins with any degree of striation were coded simply as 'striate', ignoring variation in extent of striation, the character appeared to be more consistent within species than when different degrees of striation were considered as separate states (Appendix 1.11 A). Stipe colour was recorded by Woo as all 'white' except for *R. queletii* that was scored as 'other', with an RI value of 0.16 (Appendix 1.13 A). A brown reaction to bruising separated *R. xerampelina* and *R. viridofusca* from the other 21 candidate species, which were recorded by Woo as 'unchanging' (Appendix 1.10 A). Fragrance can be distinct in several candidate species, for example: *R. cerolens* Shaffer has a gassy/spermatic scent; *R. xerampelina* and *R. viridofusca* have a crab/fish scent; and Woo sp. 36, Woo sp. 39, and *R. queletii* have a fruity and pelargonium scent.

Lamellulae ('subgills' in Woo's records) are 'short' gills that do not join the stipe. These may be more common in Woo sp. 36 and Woo sp. 35 than in other species, although the original descriptions of character states suggested that specimens from the same collection varied (i.e., difference between: 'common abundant' and 'few common,' Appendix 1.12 D). The blackening or white *Russula* candidate species (21-22 clades, Fig. 2.2) definitely have abundant lamellulae but were not well enough represented to include in my analyses.

Some characters did not vary enough among species to be useful. The surface of the cap was consistently scored as viscid when wet and variably 'matte', 'shining' or

'smooth' when dry (Appendix 1.11 B, C). How much of the cap cuticle could be peeled off (Appendix 1.18 and Appendix 1.11 D) varied such that no state was recorded in more than 30% of conspecific specimens. Some characters might have been useful if they had a quantitative reference to a scale of the measurement: for example, width and spacing of gills would require a scale for categories such as 'wide' or 'medium' (Appendix 1.12 B). Length of stipe was always 'more than cap.' Again, this character would distinguish taxa that were not included in my data set. All gill edges were coded as 'smooth' (Appendix 1.12 A, 1.13 D).

Cap colours of conspecific specimens varied widely in most candidate species. Due to the large number of colours recorded within species and the lack of concordance between phylogeny and colour range (Fig. 2.3), Mesquite crashed rather than calculating an RI value for this character. While a few species showed a narrow range of colours, for example, green tones in Woo sp. 10 and *R. graminea* Ruots., H.-G. Unger & Vauras, grey/purple in *R. stuntzii* Grund, and mostly brown in *R. cerolens*, others showed confusing mixtures of reds, yellows and purples (Fig. 2.3).

Association with host helped to distinguish *R. queletii*, growing with *Picea* or *Pinus*, from closely related Woo sp. 39, recorded with *Pseudotsuga* and *Tsuga* (Appendix 1.18 A). Similarly, *R. zelleri* was usually with *Pinus* or *Picea,* while closely related Woo sp. 52 was usually associated with *Pseudotsuga* and *Tsuga* (Appendix 1.18 B). Patterns in host specificity were otherwise hard to detect because specimens were often recorded as growing near 'mixed conifers' or in an area with several unrelated host trees.

Multivariate analyses of morphological characters provided evidence for significant differences between centroids of pairs of delimited species

Results from MCA followed by MANOVA showed significant differences at $P < 0.05$ between the centroids (~multidimensional averages) for 221 out of the 253 possible pairwise comparisons for 23 species (Table 2.2). Three characters contributed the most to the overall separation of candidate species in the MCA and distinguished *R. xerampelina* and *R. viridofusca* from all other taxa: (i) the green reaction to $FeSO_4$, (ii) the browning reaction with age or bruising, and (iii) the fishy fragrance (Appendix 1.20 A). Other variables that distinguished species were the taste of the flesh and the gills and the reaction in sulfovanillin. A dark sulfovanillin reaction and the hot taste often co-occurred

among the same specimens and candidate species. Only the most closely related species were not significantly different. *Russula viridofusca* and *R. xerampelina* differed from all other taxa yet they were not distinct from one another. The peppery tasting *R. montana* and *R. emetica* were not distinct from one another and overlapped even with *R. cerolens*. In spite of significant differences between centroids of most candidate species in pairwise comparisons, the dispersion of the conspecific specimens was wide and specimens from different species overlapped in multivariate space (Appendix 1.20 A).

As with field characters, spore length, width, width/length ratio, and maximum height of ornamentation showed significant differences between candidate species, but the spread of the data was largely overlapping (Appendix 1.21). Tukey test results (not shown) generally indicated that species were significantly different when their SE bars (illustrated, Appendix 1.21) did not overlap. The PCA of the combined spore characters also showed that samples from the same species grouped together but with extensive overlap with other species (Appendix 1.20 B). The two best separated candidate species were *R. cerolens*, with smaller than average spores, and *R. viridofusca,* with larger spores and taller than average ornamentation.

I saw no evidence of further, even narrower, candidate species nested within my delimited candidate species. Within delimited candidate species, ANOVA and LSD tests did not reveal congruence between sequence polymorphisms (Appendix 1.22, Appendix 1.7) and statistically different spore lengths, widths and ornamentation heights (Appendix 1.22).

As expected, a FAMD using a combination of field and spore traits performed better in separating candidate species and grouping samples into candidate species compared with the MCA based solely on field characters, or PCA based solely on spore measurements (Appendix 1.20 C). However, most species did not appear monophyletic in morphological analysis on the FAMD axes (Appendix 1.23). Even the distinctive species *R. cerolens* and *R. viridofusca* appeared polyphyletic (Appendix 1.23).

Multivariate analyses show up to ~48.5% average probability of correct identification of candidate species using field or spore characters

In the CVA with cross validation based on the MCA of field data, the overall probability of correct assignment of a specimen to a candidate species was 31%. For the

spore data from the PCA, the overall probability of correct identification was 21%. Combining the data types in FAMD resulted in the highest probability of accurate classification at 48.5%. Through cross-validation, FAMD with CVA also provided species-by-species estimates of the probability of correct identification (Table 2.3). *Russula cerolens* (the sole representative of clade 'Ingratula I', Appendix 1.4) was the only candidate species with a 100% probability of correct classification. The probability of correct identification was over 50% for an additional 12 candidate species and over 80% for three of these (Woo sp. 38, *R. viridofusca* and *R. mordax*). For *R. viridofusca*, the combination of odour, bruising reaction, and relatively large spores with strikingly tall ornamentation (Fig. 2.4, Appendix 1.21) were often diagnostic. Even when the probability of correctly identifying a specimen to its species was high, the probability that members of other candidate species would be incorrectly assigned to the same species was also high. As an example, specimens of *R. mordax* had an 87.5% probability of being correctly identified, yet specimens of *R. montana*, Woo spp. 35, 39, 32, and *R. queletii* would sometimes be incorrectly identified as *R. mordax* (Table 2.3). Table 3 shows several groups of candidate species likely to be confused with each other (e.g. *R. montana*, *R. emetica*, *R. stuntzii*, Woo sp. 28, 26, 36). Even though *R. montana* has, on average, slightly smaller spores and shorter ornamentation compared with *R. emetica* (Fig. 2.5, Appendix 1.21), the CVA shows that *R. montana* specimens were assigned to *R. emetica* and similar species rather than to their own species. Overall, the significant morphological differences between candidate species pairs (Table 2.2) contrasted strikingly with the low probability of specimen assignment to the correct candidate species (Table 2.3).

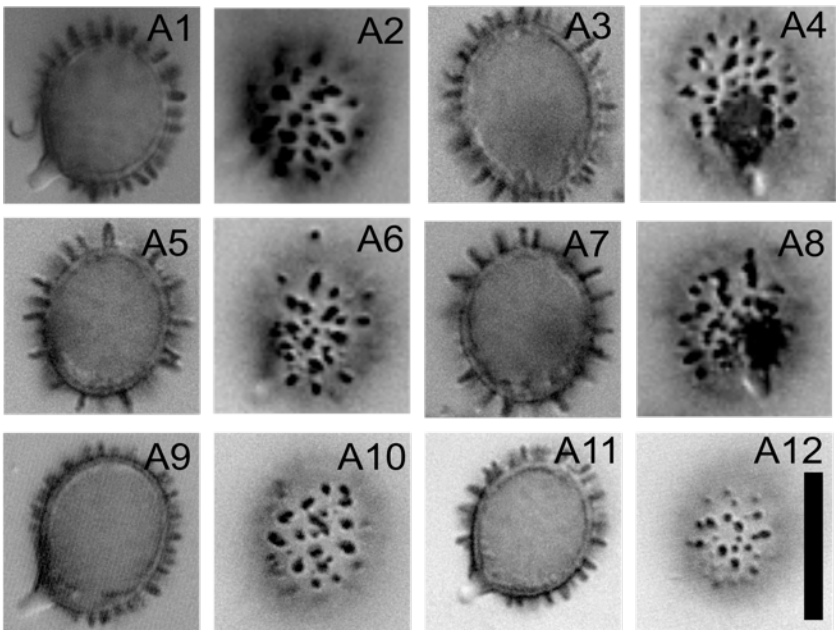

Figure 2.4 Spores of R. viridofusca

An example of similar spores in two Russula species. All scale bars 10 μm. Spores in median optical section and surface view in Melzer's reagent showing the iodine reaction ofthe ornamentation (BW followed by numerals designate Ben Woo samples): A, Russula montana (clade 27): A1 & A2, BW 725; A3 & A4, BW 753; A5 & A6, BW 821; A7 & A8, BW 912; A9 & A10, BW 883; B, Russula emetica (clade 25): B1 & B2, BW 484; B3–B6, BW 520; B7–B10, BW 513.

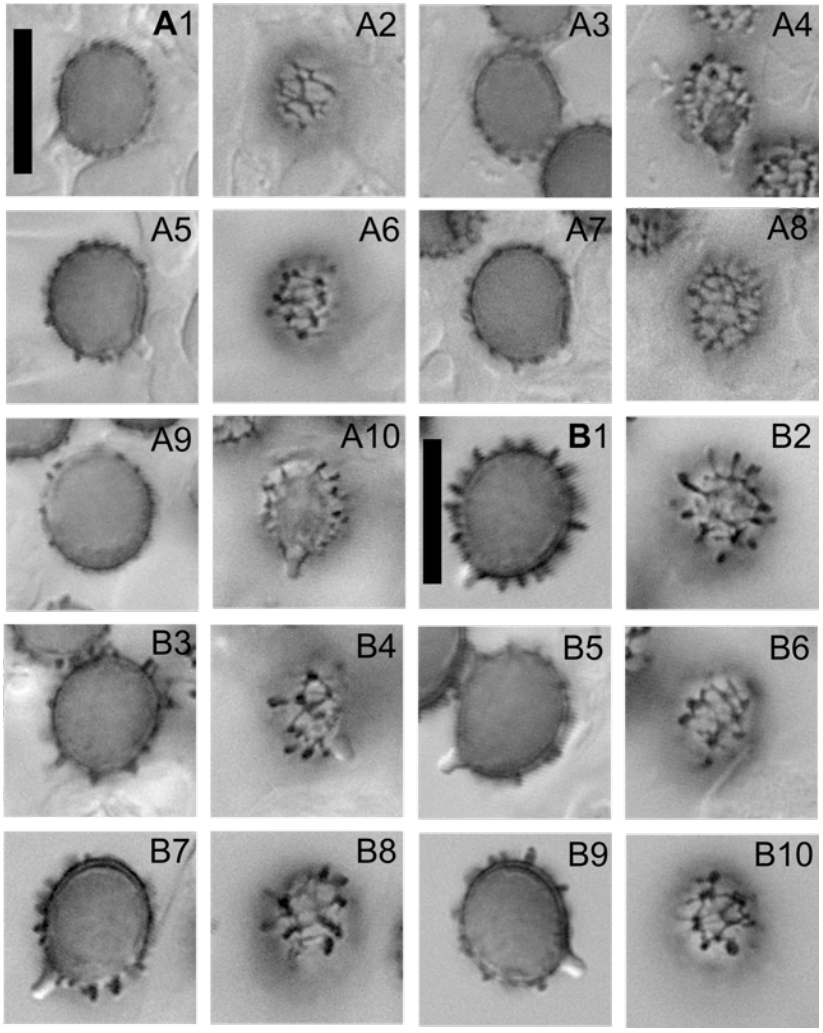

Figure 2.5 Spores of Russula montana *and* R. emetica

Similar microscopic features in two closely related Russula *species. BW followed by numerals designate Ben Woo samples. All scale bars 10 µm.* ***A-E,*** Russula montana *(clade 27):* ***A,*** *Spores in median optical section and surface view in Melzer's reagent (**A1-A2**, BW725; **A3-A4**, BW753; **A5-A6**, BW821; **A7-A8**, BW912; **A9-A10**, BW883).*

Table 2.2 Pairwise difference of species based on morphology

*Morphological divergence between most species pairs was evident in the significant differences of the centroids from multiple correspondence analysis of characters recorded by Woo. Significant difference is indicated by: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '-' 1*

Species (clade)	-	7	25	26	27	28	30	32	35	36	39	40	47	50	52	53	57	59	61	62	66	67	72
cerolens (7)		***	***	***	***	***	***	***	**	**	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Woo sp. 20			***	***	***	***	***	***	***	***	***	***	**	***	***	***	.	***	***	***	***	***	**
emetica (25)				***	-	**	-	-	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Woo sp. 26					***	-	***	*	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
montana (27)						*	-	.	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Woo sp. 28							.	.	***	***	***	***	***	***	***	***	***	***	***	**	***	***	***
stuntzii (30)								-	***	***	*	**	***	***	***	***	***	***	***	***	***	***	***
Woo sp. 32									**	*	-	.	***	***	***	***	***	***	***	*	***	***	***
Woo sp. 35										.	*	*	***	***	***	***	***	***	***	***	***	***	***
Woo sp. 36											-	-	***	***	***	***	***	***	***	***	***	***	***
Woo sp. 39												-	***	***	***	***	***	***	***	***	***	***	***
queletii (40)													***	***	***	***	***	***	***	**	***	***	***
gramin. (47)														-	-	-	-	***	***	***	-	-	-
Woo sp. 50															.	*	*	***	***	***	*	.	***
Woo sp. 52																-	-	***	***	***	-	*	**
zelleri (53)																	**	***	***	***	-	**	**
vinosos. (57)																		***	***	***	-	**	-
viridof. (59)																			-	***	***	***	***
xeram. (61)																				***	***	***	***
mordax (62)																					***	***	***
sierren. (66)																						**	-
Woo sp. 67																							***
Woo sp. 72																							

Table 2.3 CVA identification

Estimated probability of correct identification vs. misidentification based on a canonical variates analysis of field and spore characters. Each cell gives the probability that a specimen from a species on the left will be classified into the species along the top; the diagonal gives the probability of correct identification.

Species (clade)	N/A[1]	7	20	25	26	27	28	30	32	35	36	39	40	47	50	52	53	57	59	61	62	66	67	72
cerolens (7)		100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Woo sp. 20		-	14.3	-	-	-	-	-	28.6	-	-	-	-	-	-	-	-	-	-	14.3	-	-	-	42.8
emetica (25)		-	-	42.8	-	-	-	42.8	-	-	-	-	14.4	-	-	-	-	-	-	-	-	-	-	14.3
Woo sp. 26		-	-	-	50	-	25	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
mont. (27)		-	-	12.5	-	-	-	12.5	-	12.5	25	-	25	-	-	-	-	-	-	-	12.5	-	-	-
Woo sp. 28		-	-	-	28.6	-	42.8	14.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14.3	-
stuntzii (30)		-	-	-	16.7	16.7	-	66.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Woo sp. 32	16.6	-	16.6	-	-	-	-	-	-	-	16.7	16.7	16.7	-	-	-	-	-	-	-	16.7	-	-	-
Woo sp. 35		-	-	-	-	-	-	-	-	83.3	-	-	16.7	-	-	-	-	-	-	-	16.7	-	-	-
Woo sp. 36		-	-	-	-	12.5	-	12.5	-	12.5	37.5	12.5	12.5	-	-	-	-	-	-	-	-	-	-	-
Woo sp. 39	8.3	-	-	-	-	-	-		-	-	8.3	58.4	16.7	-	-	-	-	-	-	-	8.3	-	-	-
queletii (40)		-	-	-	-	16.7	8.3	-	-	8.3	25	-	16.7	8.3	-	-	-	-	-	-	16.7	-	-	-
gramin. (47)		-	-	-	-	-	14.3	-	-	-	-	-	-	14.3	-	-	-	-	-	-	-	-	57.1	14.3
Woo sp. 50		-	-	-	25	-	-	-	-	-	-	-	-	-	50	25	-	-	-	-	-	-	-	-
Woo sp. 52		-	-	-	-	-	7.1	-	-	-	-	-	-	-	28.6	35.8	-	-	-	-	-	21.4	-	7.1
zelleri (53)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	75	-	-	-	-	12.5	12.5	-
vinosos. (57)	16.7	-	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	33.3	-	-	-	-	-	-
viridof. (59)	14.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	85.7	-	-	-	-	-
xeram. (61)	28.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14.3	57.1	-	-	-	-
mordax (62)		-	-	-	-	-	-	-	-	12.5	-	-	-	-	-	-	-	-	-	-	87.5	-	-	-
sierren. (66)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	33.3	-	-	-	-	66.7	-	-
Woo sp. 67		-	-	-	-	-	-	-	-	-	-	-	-	28.6	-	14.3	-	-	-	-	-	-	57.1	-
Woo sp. 72	14.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14.3	-	71.4

[1] These specimens were not predicted to fall into any of the species groups.

Discussion

Delimited species are a starting point for critical species descriptions

Methods available for species delimitation have increased in recent years to take advantage of predictions based on explicit evolutionary assumptions and the wider availability of multi-locus data (Carstens et al., 2013). I hypothesize that the species I delimited represent 'unique evolutionary lineages' but acknowledge that not all of my candidate species will prove to be species by other evolutionary or biological criteria and some may require future subdivision into even narrower units. Because methods of delimitation rely on comparisons of within versus among species variation, 10 or more samples may be needed to produce robust species hypotheses (Carstens et al., 2013). Of my candidate species, the 23 with more than 10 collections each are correspondingly more likely than the others to be 'evolutionarily distinct lineages' in the sense of De Queiroz (2007). By presenting delimited candidate species, specimens with their herbarium accession numbers, sequences and morphological notes, I have made future description or synonymizing of species much easier.

Convincing corroborating evidence for distinctly evolving species tends to accumulate over time and so I looked for evidence of divergence in habitat, morphology, and sequence patterns that would support delimitation (De Queiroz, 2007; Taylor et al., 2000). One of the advantages of using species delimitation software is the detachment from arbitrary fixed cut-off points such as the 97% that is commonly used in barcoding studies (Ryberg et al., 2008). My delimited species were narrow, ranging from 98-99.5% identity, in some cases clustering closely with identified species (shown in Fig. 2.2). Overall morphological divergence between most pairs of delimited species were statistically significant based on the MANOVA following MCA of the morphological characters, uggesting that I was not systematically subdividing too narrowly. Evidence of different host preferences supported distinctions between the closely related *R. queletii* and Woo sp. 39, and between *R. zelleri* and Woo sp. 52. This is broadly consistent with the suggestion that host switching was a driver of diversification in *Russula* (Looney et al., 2016). Fewer than 1% of positions in the ITS2 alignment appeared heterozygous,

suggesting that interbreeding might be limited between haploid individuals differing by more than 1%. Polymorphisms were not shared between species; retention of ancestral polymorphisms or genetic exchange that would suggest overly narrow species boundaries were not evident.

Gene flow is expected among sympatric populations. Gene flow between populations of generalist wood-decay fungi occurs over vast distances (James and Vilgalys, 2001). Geml et al. (2006) found that three non-interbreeding, closely related lineages of the ectomycorrhizal *Amanita muscaria* s.l. had dispersed widely, resulting in circumboreal distributions. The area covered by Woo's collections is much smaller than the ones reported in Geml et al. (2006) and James and Vilgalys (2001), so it is at least possible that all or most of the *Russula* species are sympatric. The lack of evidence for gene flow between *Russula* candidate species suggests that many of them are indeed separate, non-interbreeding lineages.

My delimited species may still be too broad, encompassing narrower species. However, in *Russula*, none of the characters available to us--ITS2 region sequences, macromorphology, spore characters or host associations--have the power to further subdivide species. I saw no evidence in the form of linkage among alternative character states that would have supported narrower, nested species. A more critical approach would involve testing for linkage disequilibrium across multiple unlinked genetic loci. This would require new collections because of the low DNA quality in the Woo specimens. My public database of specimen information should help guide researchers to the localities where they can collect target species for further study.

In *Russula*, within-species variation and among-species overlap in morphological characters led to ~50% incorrect identification in resampling tests

In contrast to the rarity of shared interspecific ITS polymorphisms, shared interspecific morphological variation was the rule in *Russula*. By comparing morphology-based identifications and DNA barcode based identification Adamčík et al. (2016a) found that closely related species pairs such as *R. pascua* (F.H. Møller & Jul. Schäff.) Kühner and *R. clavipes* Velen. did not have distinguishing morphologies. In my study, the low RI of characters, wide dispersion of conspecific specimens in ordinations, and low probabilities of correct species assignment in CVA analysis all document a loose

connection between morphological character states and species boundaries. Average morphology differed among species, but few specimens were average, and this fact limited the probability of successful identification based on my recorded field and microscopic characters. If field and spore characters do not reliably distinguish among 23 candidate species, identification will be even more difficult upon factoring in the additional 49 candidate species that are represented by nine or fewer specimens.

My results showcase where morphological identifications would usually succeed. CVA did correctly assign *R. cerolens* specimens to their candidate species 100% of the time. As *R. cerolens* was the only representative of the distinctive Ingratula I clade, this illustrates that distinguishing among species will be easier where fewer species are expected, for example, in narrower geographical areas or specialized habitats. The CVA analysis included many but not all characters that could separate *Russula* species. Adding additional species-specific characters would be expected to improve assignment accuracy. *Russula graminea* specimens often have a green cap and large spores with tall ornamentation, and as a result most of them are readily identifiable to their candidate species. However, the CVA did not include cap colour due to high within-species variation that made character-state coding essentially impossible, and so *R. graminea* had a low probability of correct assignment to species. In some species, microscopic characters of the non-reproductive cells of the hymenium or cap cuticle may have diverged enough to allow species identification.

In spite of variation within and overlap between candidate species, spore colour, gill colour and taste generally help refer specimens to wider clades. In Hygrophoraceae, when lineages could be defined by characters, it was by several traits in combination (Lodge et al., 2014). In *Russula*, spore colour and taste have long been used to identify groups (Blum and Heim, 1962; Crawshay, 1930; Lange, 1935-1940; Massee, 1902; Romagnesi, 1967; Thiers, 1997). Miller and Buyck (2002) showed phylogenetically that spore colour was an important character. I similarly found limited within-species variation in spore and gill colour, even though spores tend to darken with maturity and can vary with the age of the mushroom. As Miller and Buyck (2002) also showed phylogenetically, taste is a useful character for identification across the genus. I found taste to be predictive especially if coded simply as 'mild' *vs* 'hot,' ignoring the nuances

of pepperiness recorded by Woo. The sequence-based phylogeny allowed us to identify convergent evolution that was not obvious to earlier authors. For example, the peppery taste and creamy spore colour of *R. mordax* Burl. incorrectly predicted its affinity with cream-spored and peppery Russulas in clades 29-40, while I recovered its phylogenetic position among mild-tasting members of clades 62-66 (Fig. 2.2). The peppery taste and creamy spores of *R. veternosa* Fr. (not included in my phylogeny but close to *R. mordax* based on a BLAST search) may be similarly misleading. Although Romagnesi relied on taste and colour to key the species, he used microscopic and macroscopic characters to classify it in Sect. *Maculatinae*, distant from the other spicy, light-spored taxa (Romagnesi, 1967).

Malagòn et al. (2014) pointed out that the compounds responsible for pungent or peppery *Russula* tastes might be useful in distinguishing among *Russula* species. Compounds responsible for the hot taste have been characterized as sesquiterpenoid unsaturated dialdehydes in *Lactarius* and *Russula* species (Clericuzio et al., 1998; Clericuzio and Sterner, 1997; Hanson, 2008; Malagòn et al., 2014; Vidari and Vita-Finzi, 1995; Wang et al., 2006). Rapid enzymatic conversions turn a mild compound found in intact fruiting bodies to pungent tasting compounds once the mushrooms are injured. In *Lactarius*, these compounds had an anti-feeding effect on the insect pest *Tribolium* (Daniewski et al., 1993; Daniewski et al., 1995). As in earlier studies, I found that taxa with a pungent taste tended to exhibit a dark (purple or black) reaction to sulfovanillin (Favre-Bonvin and Bernillon, 1982) (Appendix 1.10 C-D, and 1.14 D).

Cap colours of Russulas are strikingly variable within species and distantly related species often showed similar colours (Adamčík et al., 2016a; Roberts, 2007; Romagnesi, 1967; Sarnari, 1998-2005). Colours vary in other fungal species as well. Closely related strains of *Amanita muscaria* can have red or yellow caps (Geml et al., 2006). Cap colour varies within species of *Cantharellus* of the Pacific Northwest (Buyck et al., 2016; Dunham et al., 2003). However, the range of colour within many *Russula* species is unusual. Several researchers have separated and characterized *Russula* pigments (Eugster et al., 1970; Fröde et al., 1995; Gill and Steglich, 1987; Iten et al., 1984; Watson, 1966). Russulas may also have pigments that are usually colourless but fluoresce yellow, red, or blue-violet in UV light. Eugster et al. (1970) extracted and

characterized the pigment from 6 – 200 kg of fungal tissue of each of several species. The red compounds were glycosides of dimeric pteridine ribosides (Eugster et al., 1970; Gill and Steglich, 1987). Orange-yellow colours in Asian and Eastern North American fruiting bodies of *R. flavida* Frost were from lipophilic pigments, related to riboflavin and pteridine metabolism. The structures of the blue-violet pigments are still unknown (Gill and Steglich, 1987). Many of the pigments are water-soluble and so the fruiting bodies show a different appearance before and after rain (Eugster et al., 1970; Gill and Steglich, 1987; Roberts, 2007; Watson, 1966). The difficulties in identifying *Russula* species have been a confounding factor that complicates the interpretation of previous pigment studies. I hope that future chemical and genomic analyses of specimens identified by matches to DNA barcodes will help uncover more consistent overall patterns of colour and its evolution.

While spore sizes and ornamentation, characters of the cells of the hymenium and cap cuticle are usually reported in systematic studies of *Russula* (Adamčík et al., 2016a; Adamčík and Marhold, 2000; Buyck and Mitchell, 2003; Romagnesi, 1967; Sarnari, 1998-2005; Shaffer, 1962, 1964, 1972; Vauras et al., 2013), as Miller and Buyck (2002) pointed out, they are not usually evaluated statistically or morphometrically. Spore characters, unlike hymenial or cuticular characters do lend themselves to statistical analysis. My results conformed to expectation; within candidate species dispersion in size and ornamentation of spores was considerable yet using these characters improved the odds of correct candidate species assignment from 31% to 48.5% in the CVA.

Of the recorded morphological variation, some may be due to developmental stage at the time of sampling, to phenotypic plasticity, to convergence, or to miscoding of characters. Retention of ancestral polymorphisms may be occurring so that ancestral variation persists through speciation events. Mushrooms are ephemeral and their only function is production and release of spores. They do not attract a pollinator or choose a mate, functions that result in well-documented selection for species-specific morphological divergence in flowers of plants (Caruso, 2000; Delph et al., 2004; Galen, 1989; Johnston, 1991) or genitalia of animals (Arnqvist, 1998; Hosken and Stockley, 2004; Hotzy et al., 2012). Lodge et al. (2014) attributed a lack of synapomorphies across Hygrophoraceae (Basidiomycota) to a preponderance of traits used in traditional

mushroom classification that were non-adaptive and therefore not under strong selection. Similarly, whatever its cause, variation within and across candidate species may reflect weak selection on aspects of mushroom morphology in *Russula*.

Analysis does not support widespread application of European names to Pacific Northwest taxa

I applied names to candidate species that matched a type specimen or a sequence from a reliably identified European species. While type specimens from Oregon and Washington matched sequences from the Woo specimens, most of the types from California as well as the types from Vermont and Arizona did not find matches. The only North American *Russula* candidate species found by Woo that was described in California rather than the Pacific Northwest was *Russula sierrensis* Thiers. *Russula bicolor* Burl. Is noteworthy as it may be rare or absent in the Pacific Northwest even though its name is used frequently (21 records UBC herbarium; 9 records OSU herbarium). Burlingham (1913) described the type as a *Russula* with a coppery red with yellow or ochre intermixed cap, collected under yellow birch in Newfane, VT (U.S.A.). Murrill may have initiated use of the name in the Pacific Northwest when he applied it to 'specimen 807', collected in Oregon, as cited by Burlingham (1913). The sequence of the type is near the *Puellarinae* group, Woo clades 42-45 (Fig. 2.2). However, the three 'red and yellow' specimens that Woo named *R. bicolor* (BW545, BW694 and BW513) were instead *R. montana* or *R. emetica* (Schaeff.) Pers. of the emeticoid clade (23-28 in Fig. 2.2). Other names that are commonly applied in the Pacific Northwest (D. Miller, pers. comm., (Gibson et al., 2010)) and represented by sequences in the UNITE database (notably: *R. olivacea* Pers., *R. aeruginea* Lindblad ex Fr., *R. pelargonia* Niolle, *R. cuprea* J.E. Lange, *R. brunneoviolacea* Crawshay, *R. lilacea* Quél., *R. amoenolens* Romagn.) did not match samples in the Woo collection.

Most but not all of the type specimens from western North America gave reliable sequences. A commonly used name without corresponding type sequence is *R. smithii* Singer. My failure to sequence the ITS1 region from *R. occidentalis* may have been due to DNA damage related to the oxidizing, blackening reaction in the specimens. *Russula modesta* Peck was shown morphologically to have been incorrectly applied (Adamčík et

al., 2013). *Russula atroglauca* Einhell. is a candidate species newly recorded for the Pacific Northwest collected only once by Woo in Alaska.

My results call into question the widespread application of European names to Pacific Northwest *Russula* specimens in herbaria or ecological studies. The number of candidate species in the Pacific Northwest found by this study (72) is comparable to the 89 species, mostly European, that Woo presented in his key to *Russula* (Woo, 1989). Tellingly, although he wrote the key, Woo only identified 10% of his *Russula* collections to species, perhaps due to a well-founded concern that Pacific Northwest specimens did not match described, mostly European, species (Appendix 1.24). Through comparisons with my new type sequences and sequences from carefully identified reference specimens, I was only able to apply names to 39% of 72 candidate species and only 17 of the 72 taxa I delimited matched currently barcoded European taxa. Of the 44 candidate species that could not be named, some are probably described species that have never previously been sequenced while others are likely as yet undescribed. In the study of *Russula* species from Alaskan spruce forests, Geml et al. (2010) applied names to 22 (52.4%) of the 42 species detected. The relatively high proportion of named Alaskan specimens could reflect a broader species concept or perhaps a circumboreal *Russula* community of higher latitudes known from northern Europe.

In contrast to the high proportion of candidate species that I could not identify by their sequences, 775 out of 812 *Russula* in the UBC herbarium database and 1494 out of the 1566 *Russula* in the OSU herbarium were determined to species. It seems likely that many of these specimens were assigned names of European species based on morphological characters that are not altogether reliable for specimen identification. Harrower (2011) barcoded species of *Cortinarius* for British Columbia, and the sorting of specimens and identifications through barcoding made it easier to describe new species like *Cortinarius parkeri* Ammirati, Seidl, and Ceska (Ammirati et al., 2012). I hope that new species description will be rendered a simpler task thanks to available reference sequence and the availability of the specimens. Comparison of carefully delimited species from Eastern North America, Europe and Asia will offer insights into *Russula* biology and seems likely to reveal species ranges that are restricted by climate, hosts or geographical barriers.

Conclusion

My study is the first sequence-based survey of Russulas of the Pacific Northwest and the first broad exploration of within species morphological variation for the genus. It showcases the contribution of Benjamin Woo's long-term collection towards revealing the distribution of character states within and among species. The resulting improvements in application of names will inform identifications of *Russula* in herbaria, ecological and metagenomic studies. Improved species delimitations will lead to further studies and better resolution of species' evolution and biogeographical origins.

Chapter Three: Nine new species of *Russula* from the Pacific Northwest

Summary

In this chapter I make detailed taxonomic descriptions of nine species of *Russula* new to science. I describe *Russula benwooi*, *R. hypofragilis*, *R. obscurozelleri*, *R. parapallens*, *R. phoencea*, *R. pseudopelargonia*, *R. pseudotsugarum*, *R. rhodocephala*, and *R. salishensis*. These species are described based on the collections made by Benjamin Woo (studied in Ch. 2), and are commonly found and somewhat distinctive species of *Russula* of the Pacific Northwest.

Introduction

The phylogenetic neighbourhood of *Russula* is diverse in form and ecology. The genus *Russula* belongs to the order *Russulales* Kreisel ex P.M. Kirk et al. and family *Russulaceae* Lotsy. Apart from the corticoid (crust-like) genera *Boidinia*, *Gloeopeniophorella,* and *Pseudoxenasma* (Larsson, 2007), this family harbors four predominantly agaricoid (mushroom-forming) genera, i.e. *Lactifluus*, *Lactarius*, *Multifurca* and *Russula* (Buyck et al., 2008b; Buyck et al., 2010), some of which may also contain secotioid to hypogeous (truffle-like) or pleurotoid (mushroom-like with an eccentric connection between cap and stem) species.

Russulaceae is one of the most common mushroom families in the Pacific temperate rainforest ecoregion (Hesse, 2012). *Russula* species are important as ectomycorrhizal partners of the Pacific region's dominant forest trees, yet they remain poorly characterized taxonomically and ecologically (Buyck et al., 2015). The nine new *Russula* species that I describe here emerged from the work of the late *Russula* expert Benjamin Woo (1926-2008), whose collections encompassed much of the diversity in the region and were analyzed in Chapter 2 of this thesis (Bazzicalupo et al., 2017). Each new species is based on Woo's vouchers collected from 1974 to 2007. Here, I document within-species variation by summarizing macromorphological characters from databased versions of Woo's detailed specimen collection notes for 10 to 61 specimens per species.

I then add data on microscopic morphology based on examination of eight or more specimens per species.

Methods

Phylogenetic placement

I sampled previously published sequences to provide a phylogenetic context for the new species. For sequencing protocols and species delimitation, see Chapter 2. I included taxa in subgenus *Russula* with *R. adusta* as an outgroup. To represent each of Woo's species in the subgenus *Russula*, I added internal transcribed spacer 2 (ITS2) sequences from 64 OTUs from the phylogeny in Chapter 2 (Appendix 1.5). I included 118 other OTUs from GenBank and UNITE (Abarenkov et al., 2010a; Abarenkov et al., 2010b; Kõljalg et al., 2005) that were relevant to morphological comparisons and that appeared to be close to the new species based on BLAST searches. Sequences were aligned with MAFFT (Katoh and Standley, 2013) followed by manual editing in Mesquite v. 3.03 (Maddison and Maddison, 2015). The maximum likelihood tree was produced using the constraint topology from four loci (ITS, RPB2, LSU, and EF1- α) produced in Chapter 2, with RAxML-GUI, a GTR + G model of evolution, and 100 bootstrap replicates.

Specimens and characters

As type material, I selected from among Woo's specimens in the Burke Museum (Seattle). Woo's careful notes and photographs are available in an online database: http://advance.science.sfu.ca/fungi/index.php?-link=Home. Appendix 2.1 lists all specimens used in the descriptions and others considered conspecific in the Woo collection.

When comparing ITS sequences through BLAST searches, I recorded specimens that are similar in public databases up to >3% cutoff. Based on results from Chapter 2 and studies in other fungi (Garnica et al., 2016), I consider specimens that differ in ITS more than 3% to be different species.

Where conspecific specimens varied in their morphology, I present the percentages of recorded alternative character states. Woo recorded spore print colour using coding from (Crawshay, 1930): A, white, B-C pale cream to cream, D-E yellow, F-H ochre. Woo

recorded taste as "mild", "slightly hot", "hot", "acrid" etc. but I pooled all slightly or extremely spicy variants as "hot", which improved the consistency of this character within species based on my findings in Chapter 2. Measurements of microscopic characters are presented as minimum size (in parenthesis), mean minus one standard error, mean (in bold), mean plus one standard error, and maximum size (in parenthesis). Spores were measured without spines, ornamentation was measured separately. Microscopy protocols are outlined in Chapter 2. Maps of species distributions were produced from analysis of Woo's specimens' metadata using the dismo R package (Hijmans and Elith, 2015; Hijmans et al., 2012). Additional data on localities extracted from records from GenBank and UNITE sequences identical to ITS sequences of my new species are listed in the 'Habitat and Distribution' sections that follow.

Results

The maximum likelihood tree shows the phylogenetic placement of the specimens used in the new species descriptions and taxa closest to them (Fig 3.1).

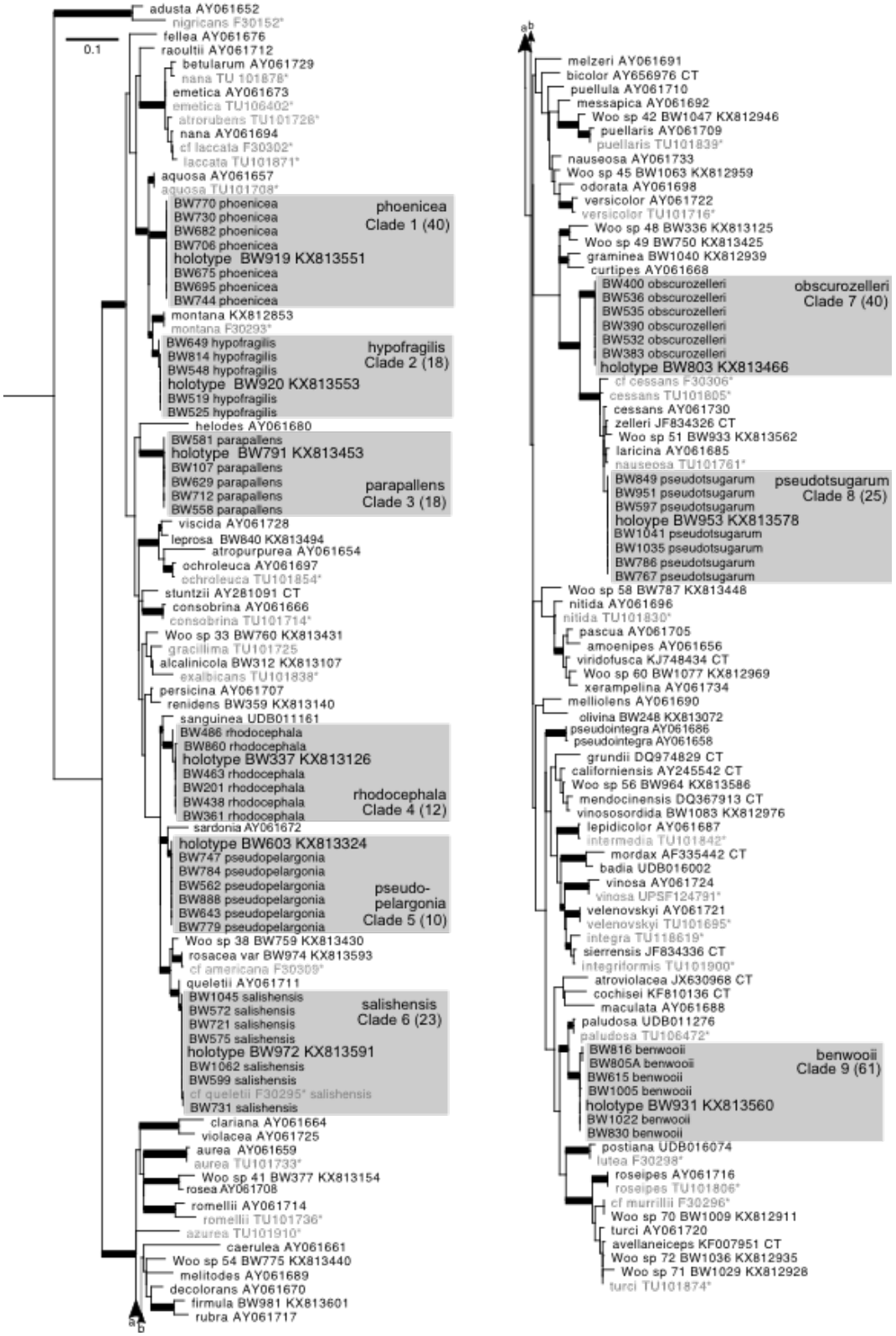

Figure 3.1 Placement of new Russula *species in the genus phylogeny.*

Maximum likelihood phylogeny of nine new species of Russula. *Specimen codes and morphological descriptions are available through <http://advance.science.sfu.ca/fungi/index.php?-link=Home>. Bootstrap support 70% or more is indicated by thickened black branches. The grey shading of taxa indicates the samples of the new species described. Holotypes are designated along with their collection numbers and GB accessions. Clade numbers are assigned to new species and the total number of Woo's collections of each new species is in parentheses. 'CT' next to taxon name indicates the sample was confirmed with a type specimen sequence; an asterisk '*' indicates a sample from the backbone constraint tree. GenBank sequences R. aurea, R. leprosa, R. sardonia, R. rosea, and R. pseudointegra were re-named in this figure based on recent sequencing of the species by Bart Buyck.*

Taxonomy

***Russula benwooii* Bazzicalupo, D. Miller & Buyck., *sp. nov*.**

Index fungorum nr: IF553815; *FacesofFungi nr*: FoF 03648; Fig. 3.2

Etymology: Named for Benjamin Woo, whose collections were the basis of this study.

Holotype: BW931 (WTU, sub nr. F-038559)

Pileus firm and fleshy, (4-)8-11(-16) cm diam., convex, then becoming gently depressed in the centre, but never deeply funnel-shaped, sometimes radially fissuring from the margin inwards, with mostly even margin or - in less fleshy specimens - also weakly striate; surface viscid when wet, matte to shiny when dry, variably peeling, extremely variable in colour and with the centre either paler or darker than the rest of the cap, mostly coming in tones of brown to brownish tan, mixed or not with shades of olive, reddish-pink, wine red or purple, but also sometimes with dominance of single colours, for example, entirely wine red, olive-green or intense purple. *Lamellae* adnate, normally spaced (ca. 1 L/mm) or sometimes wider, pale cream to yellow (of Woo specimens, ~85% recorded as 'cream'; ~15% as 'yellow'), occasionally bifurcating near the cap margin, lamellulae of variable length often present. *Stipe* mostly distinctly widening toward the base, shorter than the cap diam., white or partly to entirely tinged with purple or red, sometimes staining brown. *Odour* none. *Taste* mild, sometimes slightly spicy in gills (of Woo specimens, ~20% recorded as having hot gills). *Spore print* cream (of Woo specimens, ~60% Crawshay B-C, ~40% Crawshay D-E).

Spores broadly ellipsoid, (6.9-) 9.16-**9.22**-9.28 (-12) × (5-) 7.09-**7.14**-7.18 (-8.6) μm, Q=(1-) 1.28-**1.29**-1.3 (-1.5), ornamentation subreticulate, composed of strongly amyloid, sometimes slightly curved, conical warts, (0.4-) 1.1-**1.12**-1.14 (-1.9) μm high, with some interconnections; suprahilar spot present as a strongly amyloid patch. *Basidia* (44-) 51-**56**-61 (-65) × (10.5-) 11.5-**12.5**-13.5 (-14.5) μm, 4-spored, clavate. *Lamellar trama*

mainly composed of sphaerocytes, mixed with some cystidioid hyphae. *Hymenial cystidia* broadly clavate, obtuse-rounded at the top, 60-75(-85) μm long and 10-12 μm wide, similar near the gill edge, most often weakly SV+ (reaction in sulfovanillin: ~40% colouring grey, ~25% no reaction, others turning red, violet, and purple). *Pileipellis* not sharply delimited from the underlying context of filamentous hyphae and sphaerocytes; suprapellis composed of densely packed narrow hyphae, 2-4 μm wide, with terminal cylindrical cells with narrowing, sometimes capitate tips. Pileocystidial cells ~30-35 μm long and up to 7 μm in width, with obtuse tips and refringent contents . Acidoresistant incrustations absent. *Clamp connections* absent in all parts. $FeSO_4$: none to tan.
Habitat and distribution: associated with *Tsuga heterophylla* (as evidenced by deposited ectomycorrhizal sequences for western hemlock), possibly also with *Pseudotsuga menziesii* as the trees frequently co-occur, and potentially also other conifers that were occasionally present: *Picea sitchensis*, *Pinus contorta*, *Abies*, and *Larix* (Larch). Only known from the Pacific Northwest (USA: Oregon, Washington, Idaho; Canada: British Columbia).
Material examined: U.S.A., Oregon, Clackamas County, Wildcat Mountain, 04 Sep 1999, B. Woo BW805A, F-038724 (WTU), GenBank ITS2: KX813469; ibidem, Lincoln City, East Devils Lake Park, 123.997778°W, 44.966667°N, 8 m alt., 26 Oct 2001, B. Woo BW931, F-038559 (WTU, **holotype !**), GenBank ITS2: KX813560; Washington State, Asahel Curtis Loop Trail, 121.474722°W, 47.390833°N, 650 m alt., 30 Sep 1999, B. Woo BW830, F-039368 (WTU), GenBank ITS2: KX813487; ibidem, Millersylvania State Park, 122.9083°W, 46.91°N, 67 m alt., 20 Oct 2004, B. Woo BW1005, F-038884 (WTU), GenBank ITS2: KX812908; ibidem, Sloan Creek Camp, 121.287778°W, 48.0575°N, 630 m alt., 19 Sep 1999, B. Woo BW816, F-039305, (WTU), GenBank ITS2: KX813478.
Notes: Russula benwooii corresponded to clade 9 in the phylogeny (Fig. 3.1), to Woo sp. 67 in Chapter 2) and UNITE SH DOI: https://plutof.ut.ee/#/datacite/10.15156/BIO/SH299776.07FU**.** An 'SH' in the UNITE database stands for 'Species Hypothesis', and it represents a group of sequences clustered at a certain cut-off. In this study I used the 99% cutoff. It appeared as a well-supported sister to European *R. paludosa*, but its phylogenetic position was otherwise unresolved. This mild-tasting, firm *Russula* could be easily confused with equally variable species in

subsections *Xerampelinae* (particularly *R. xerampelina*) and *Integrinae* (particularly *R. integra*), all species that are known as strict conifer associates. Woo's database records showed that *R. benwooii* had been confused in the past with *R. xerampelina* (particularly when specimens had a browning or tinted stipe). *Russula benwooii* can usually be distinguished from *R. xerampelina* by its absence of a green $FeSO_4$ reaction and lack of a fishy smell. *Russula benwoii* was confused with *R. vinosa* (known as '*R. occidentalis*' in the Pacific Northwest), but as shown in Fig. 3.1, the two are phylogenetically distinct. I concluded that *R. maxima* Burl., another taxon described from the Pacific Northwest, was not synonymous with our species. The type of *R. maxima* was not sequenced but a comparison of morphological descriptions showed that the spore ornamentation was much shorter than *R. benwooi*, below 0.5 (-1) μm high in Burlingham's species (Buyck et al., 2015; Hesler, 1961).

The closest species in the phylogeny (Fig. 3.1) and in the UNITE database was *R. paludosa* (SH299756.07FU), with a 95% match. Samples with identical ITS sequences to *R. benwooi* have only been reported from the Pacific Northwest to date [Canada: Campbell River, BC (KP403057, EU597055, DQ367916), BC (FJ152488, JF899571, KP889681)].

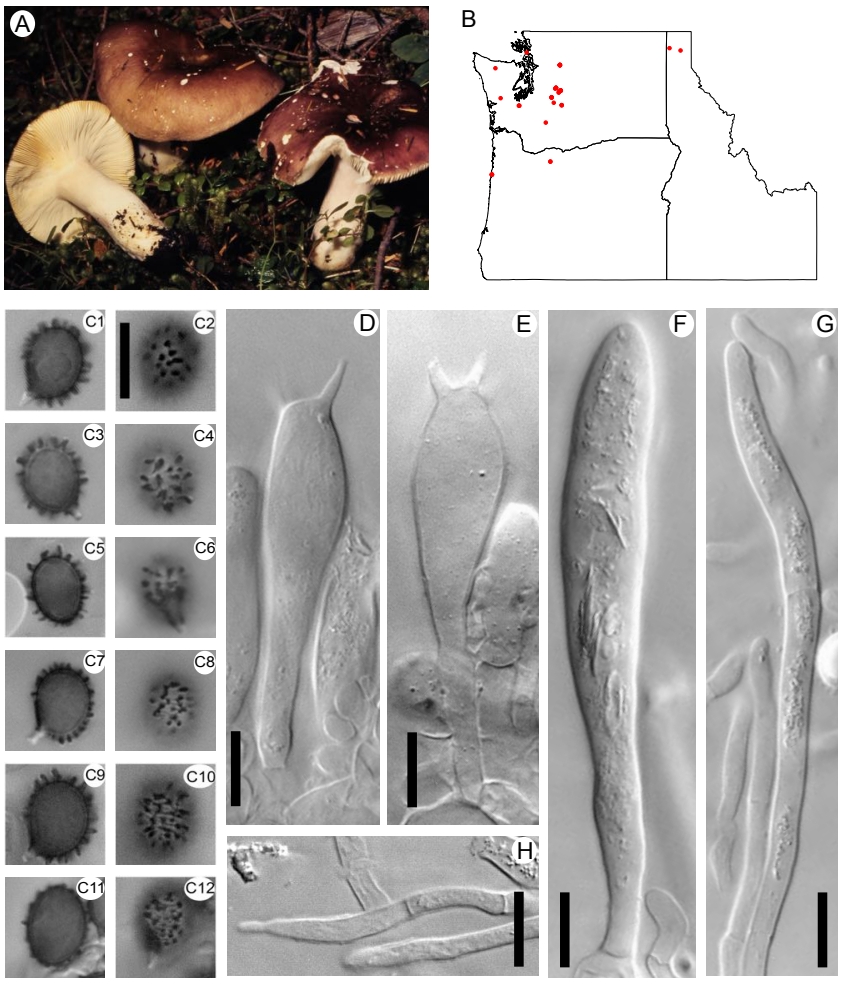

Figure 3.2 Russula benwooi *morphology and map of specimen locations*.

Morphology and specimen distribution of Clade 9 *Russula benwooii* (Woo sp. 67). BW followed by numerals designate Ben Woo samples. **A,** Photograph of fresh specimen (BW805A). **B,** Distribution of specimens of the Woo collections in Pacific Northwest States. **C-H** Micromorphology, all 1000x magnification. All scale bars 10 μm. **C,** Spores in median optical section and surface view in Melzer's reagent (**C**1-**C**2, BW931; **C**3-**C**4, BW1005; **C**5-**C**6, BW1034; **C**7-**C**8, BW931; **C**9-**C**10, BW931; **C**11-**C**12, BW816); **D-E,** Basidia (BW1034, BW931); **F,** Hymenial cystidium (BW816); **G-H,** Cap cuticle terminal cells with refringent contents (BW931, BW830).

***Russula hypofragilis* Bazzicalupo, D. Miller & Buyck., *sp. nov*.**

Index fungorum nr: IF553816; *FacesofFungi nr*: FoF 03649; Fig. 3.3

Etymology: refers to the resemblance to taxon *R. fragilis*

Holotype: BW920 (WTU, sub nr. F-038403)

Pileus 4-11.5 cm diam., convex, then becoming deeply concave and with striate margin; surface smooth, purple, purplish red, vinaceous to grayish olive. *Lamellae* adnate,

normally spaced (ca 1L/mm), equal or with some rare lamellulae and none to few bifurcations, white to cream coloured (Woo specimens, ~90% recorded for 'white'; ~10% for 'cream'). *Stipe* less or equal to cap, cylindrical, mostly firm and solid. *Context* white, unchanging with age or on injury, turning tan to grey-pink with $FeSO_4$. *Odour* none. *Taste* mild (mostly) to slightly hot (of Woo specimens, 'hot' for flesh in ~33%; for gills, ~50%). *Spore print* very pale (of Woo specimens, ~90% Crawshay A, ~10% Crawshay B).

Spores ellipsoid, (7.1-) 8.41-**8.45**-8.49 (-10.2) × (5.5-) 6.71-**6.73**-6.67 (-8.1) μm, Q=(1.1-) 1.25-**1.26**-1.3 (-1.5); ornamentation subreticulate, composed of amyloid, conical warts, (0.2-) 0.92-**0.94**-0.95 (-1.6) μm high, sometimes slightly curved at the tips, interconnected by web-like patterns or thin lines between warts; suprahilar spot present as a patch, but not highly amyloid. *Basidia* (33.5-) 39-**44**-49 (-60.5) × (8-) 9.5-**11**-12 (-13) μm, 4-spored, stout and clavate with swollen top; basidiola similar. *Lamellar trama* mainly composed of sphaerocytes, intermixed with cystidioid hyphae. *Hymenial cystidia* 80-95 × 8-10 μm, broadly clavate to fusiform, thin-walled, SV+ and turning dark purple in sulfovanillin. *Pileipellis* not sharply delimited from the underlying context of filamentous hyphae and sphaerocytes; suprapellis composed of loosely arranged, branching hyphal terminations, with cylindrical terminal cells. Pileocystidia at pileus surface measuring (20-) 21.5-**22**-23.5 (-30.5) × (2.5-) 5-**6.5**-7.5 (-8.5) μm, occasionally constricted at the tips; contents refringent, continuing as cystidioid hyphae with refractory contents in subpellis and trama. Acidoresistant incrustations absent. *Clamp connections* absent in all parts.

Habitat and distribution: consistently reported with *Abies*, sometimes mixed with *Pinus contorta*, *Picea sitchensis*, *Pseudotsuga menziesii*, and *Tsuga heterophylla*. Only known from the Pacific Northwest (USA: Washington; Canada: British Columbia).

Examined material: U.S.A., Washington, Lake Kachess, Road 4934, 121.25°W, 47.366944°N, 710-800 m alt., 13 Oct 1996, B. Woo BW649, F-038961 (WTU), GenBank ITS2: KX813353; ibidem, Sloan Creek, 121.287778°W, 48.0575°N, 630 m alt., 04 Oct 1992, B. Woo BW519, F-039439 (WTU), GenBank ITS2: KX813256; B. Woo BW525, F-039441 (WTU), GenBank ITS2: KX813260; ibidem, 03 Oct 1993, B. Woo BW548, F-038825 (WTU), GenBank ITS2: KX813283; ibidem, 19 Sep 1999, B. Woo BW814, F-

038713 (WTU), GenBank ITS2: KX813476; ibidem, 23 Sep 2001, B. Woo BW920, F-038403 (WTU, **holotype !**), GenBank ITS2: KX813553.

Notes: Russula hypofragilis corresponds to Clade 2 in Fig. 3.1, to Woo sp. 28 in Chapter 2 and to UNITE SH DOI: https://plutof.ut.ee/#/datacite/10.15156/BIO/SH297355.07FU. *R. hypofragilis* was placed in the phylogeny (Fig. 3.1) as sister without support to *R. montana*, in a well-supported clade together with *R. aquosa* and *R. phoenicea*. As is evident from the above description, this new species could be mistaken for *R. phoenicea*, given macromorphology and the size and form of its microscopic features. The European *R. aquosa*, as yet unknown from the Pacific Northwest, is also similar morphologically and would be difficult to distinguish without sequencing. *R. hypofragilis* was always recorded in the presence of *Abies,* while its look-alike, *R. phoenicea*, was usually with *Pseudotsuga*. *R. hypofragilis* is common and may have been recorded as *R. atropurpurea* in species lists in the Pacific Northwest.

Geographically, samples with identical ITS2 sequences to *R. hypofragilis* have only been recorded from the Pacific Northwest to date [Canada, British Columbia; GenBank nrs HQ604847, KP889642), Campbell River, Vancouver Island, BC (GenBank nrs EU597058, KP406576)]. As of March 2017, other sequences with 1% difference have been recorded from Tennessee (GenBank nrs HQ022216, KF359620, KF359619), and a 3% difference hits collections attributed to *R. montana* in Europe and in the United States and Canada (see Fig 3.1).

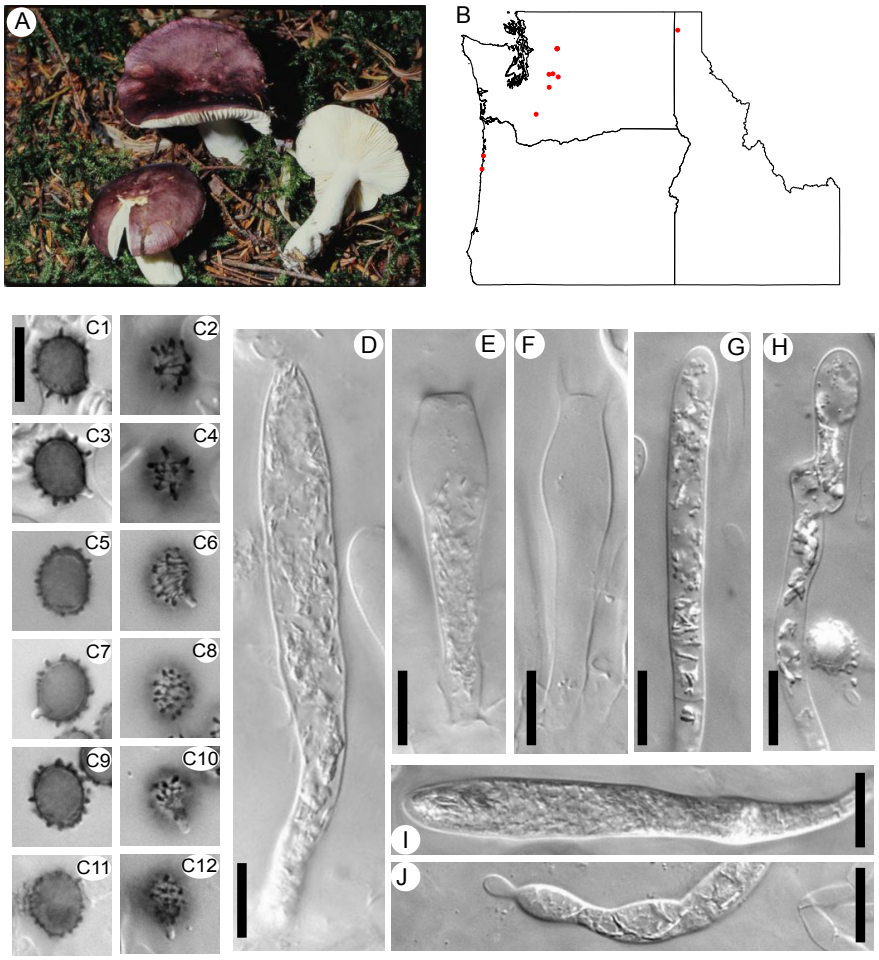

Figure 3.3 Russula hypofragilis *morphology and map of specimen locations.*

Morphology and specimen distribution of Clade 2 Russula hypofragilis *(Woo sp. 28). BW followed by numerals designate Ben Woo samples.* ***A,*** *photograph of fresh specimen (BW920).* ***B,*** *Distribution of specimens of the Woo collections in Pacific Northwest States.* ***C-J*** *Micromorphology, all 1000x magnification. All scale bars 10 µm.* ***C,*** *Spores in median optical section and surface view in Melzer's reagent (****C****1-****C****2, BW519;* ***C****3-****C****4, BW519;* ***C****5-****C****6, BW548;* ***C****7-****C****8, BW814;* ***C****9-****C****10, BW649;* ***C****11-****C****12, BW649);* ***D,*** *Hymenial cystidium (BW814);* ***E-F,*** *Basidia (BW814, BW814);* ***G-J,*** *Cap cuticle terminal cells with refringent contents (BW548, BW525, BW519, BW519).*

***Russula obscurozelleri* Bazzicalupo, D. Miller & Buyck., *sp. nov*.**

Index fungorum nr: IF553817; *FacesofFungi nr*: FoF 03650; Fig. 3.4

Etymology: refers to its similarity to *R. zelleri*

Holotype: B. Woo BW803, (WTU, sub nr. F-038663)

Pileus 3-7(-12) cm diam., fragile, young convex with inrolled margin, later plano-convex and often irregularly wavy-lobed, shallowly depressed but not becoming funnel-shaped in

age, slightly striate at the margin; surface strongly viscid when wet, shining to dull when dry, peeling half way, variable in colour but nearly always, at least when young, with a very dark, sometimes near blackish centre, elsewhere dark purplish red to brownish red, dark pinkish red to brownish violet, later discolouring irregularly and developing paler yellowish tan to vinaceous pink spots. *Lamellae* adnate to almost subfree, equal or nearly so, rarely with occasional bifurcations, mostly normally spaced (ca. 1L/mm) or somewhat more distant, with transversal anastomoses between gills, cream (of Woo specimens, ~25% recorded as white to pale cream) with maturity becoming distinctly yellowish (~75% of Woo specimens recorded as yellow to ochre); edge even, concolourous. *Stipe* mostly distinctly shorter than the cap diam., typically widening toward the base, chalk white, sometimes with rusty stains, fragile. *Context* white, unchanging, sometimes with pinkish tones underneath the cap cuticle, reacting pale tan with $FeSO_4$. *Odour* none. *Taste* mild in gills and flesh. *Spore print* cream to dark cream (of Woo specimens, ~15% Crawshay A-C, ~85% Crawshay D-F).

Spores broadly ellipsoid, (5.9-) 7.66-**7.7**-7.75 (-10.7) × (5.3-) 6.54-**6.58**-6.61 (-7.9) μm, Q= 1.1-**1.17**-1.18 (-1.4), ornamentation subreticulate, crested, with amyloid web-like interconnections and pointy warts at line intersections, up to (0.2-) 0.64-**0.65**-0.66 (-1.1) μm high; suprahilar spot present as a moderately amyloid patch. *Basidia* (38-) 40-**44.5**-49 (-61) × (9-) 10-**11**-12 (-13) μm, 4-spored, clavate; basidiola similar. *Lamellar trama* mainly composed of sphaerocytes, intermixed with cystidioid hyphae. *Hymenial cystidia* 70-85 × 8-10 μm, slightly clavate, sometimes capitate, reacting weakly to sulfovanillin (~80% grey and ~20% dark purple reaction). *Pileipellis* not sharply delimited from the underlying context of filamentous hyphae and sphaerocytes; suprapellis composed of loosely arranged hyphae. Pileocystidia in pileus terminal, measuring (27-) 31-**40**-48.5 (-53.5) × (4-) 5-**6**-7.5 (-9) μm, obtuse-rounded at the tips; contents refringent. Acidoresistant incrustations absent. *Clamp connections* absent in all parts.

Habitat and distribution: recorded with *Pseudotsuga menziesii*, *Tsuga heterophylla*, and *Pinus contorta*. The species seems to have a wide northern distribution in Canada (not shown here) including Newfoundland and Pacific Northwest (USA: California, Idaho, Oregon, Washington; Canada: British Columbia, Newfoundland).

Examined material: U.S.A., Oregon, Gerlinger Tree farm, 123.5°W, 44.90083°N, 215-

600 m alt., 07 Nov 1992, B. Woo BW536, F-039388 (WTU), GenBank ITS2: KX813272; 08 Nov 1992, B. Woo BW535, F-039400 (WTU), GenBank ITS2: KX813271; Roseburg, Weandell Simpson, 123.35°W, 43.25083°N, 140 m, 19 Nov 1983, B. Woo BW383, F-039004 (WTU), GenBank ITS2: KX813159; Washington: Millersylvania State Park, 122.9083°W, 46.91°N, 67 m, 04 Nov 1984, B. Woo BW400, F-038523 (WTU), GenBank ITS2: KX813173; Olympia, Priest Point Park, 122.8961°W, 47.06972°N, 30 m, 24 Oct 1992, B. Woo BW532, F-039444 (WTU), GenBank ITS2: KX813268; Olympia, Tolmie State Park, 122.7762°W, 47.120694°N, 2 m, 18 Nov 1998, B. Woo BW803, F-038663 (WTU, **holotype !**), GenBank ITS2: KX813466; Union Powerline - pole 93 S, 123.066667°W, 47.250833°N, 60 m, 21 Oct 1984, B. Woo BW390, F-038949 (WTU), GenBank ITS2: KX813164.

Notes: Russula obscurozelleri corresponds to Clade 7 in the phylogeny (Fig. 3.1), to Woo sp. 50 in Chapter 2 and to UNITE SH DOI: https://plutof.ut.ee/#/datacite/10.15156/BIO/SH270408.07FU

R. obscurozelleri was placed with support as the sister to a clade including *R. cessans*, *R. nauseosa*, *R. laricina*, *R. pseudotsugarum* (clade 8, Fig. 3.1) and *R. zelleri*. *R. obscurozelleri* could easily be confused (both in micro- and macroscopic characters) with *R. zelleri* and *R. pseudotsugarum* in the Pacific Northwest region (Bazzicalupo et al., 2017), and it also resembles the European *R. laricina, R. nauseosa*, and *R. cessans* (Romagnesi, 1967; Sarnari, 1998-2005). *Russula cessans* and *R. laricina* have not been recorded from the Pacific Northwest. The taxon '*R. nauseosa*' in Fig. 3.1 has no matches to ITS sequences of species found in the Pacific Northwest. Host might distinguish *R. obscurozelleri* from *R. zelleri*. *Russula zelleri* was most often reported with *Picea sitchensis* or *Picea engelmannii* (Engelmann spruce), although sometimes in mixtures with *Abies lasiocarpa* (alpine fir) or *Pinus contorta*. *R. zelleri* caps were more likely to be brownish-greenish-yellowish. When the caps of *R. zelleri* were darker, they were more pinkish-brown to vinaceous or even purplish. When darker, *R. zelleri* caps had a mottled centre with paler yellowish-tan spots. Standard errors of the means of spore lengths across individual collections of *R. zelleri*, *R. pseudotsugarum*, and *R. obscurozelleri* overlapped and so spore measurements cannot be used for identification of individual collections. However, the average spore length for *R. zelleri* was 8.9 μm, while the

average spore length for *R. pseudotsugarum* was 8.1 μm, and for *R. obscurozelleri*, 7.7 μm.

Identical sequences were from Canada: Capilano Regional Park, Vancouver, BC (KX579784), Morne Trail, NL (KX579804); and West coast U.S.A.: California (EU248590, GQ221634) Washington (KJ748443, KJ748441, KJ748445). This taxon has also been collected in Newfoundland (KX579804 (Bazzicalupo et al., 2016), which could indicate a northern distribution in Canada. Outside of the identical sequences listed above, the only other similar sequences in GenBank (as of March 2017) are more than 3% different.

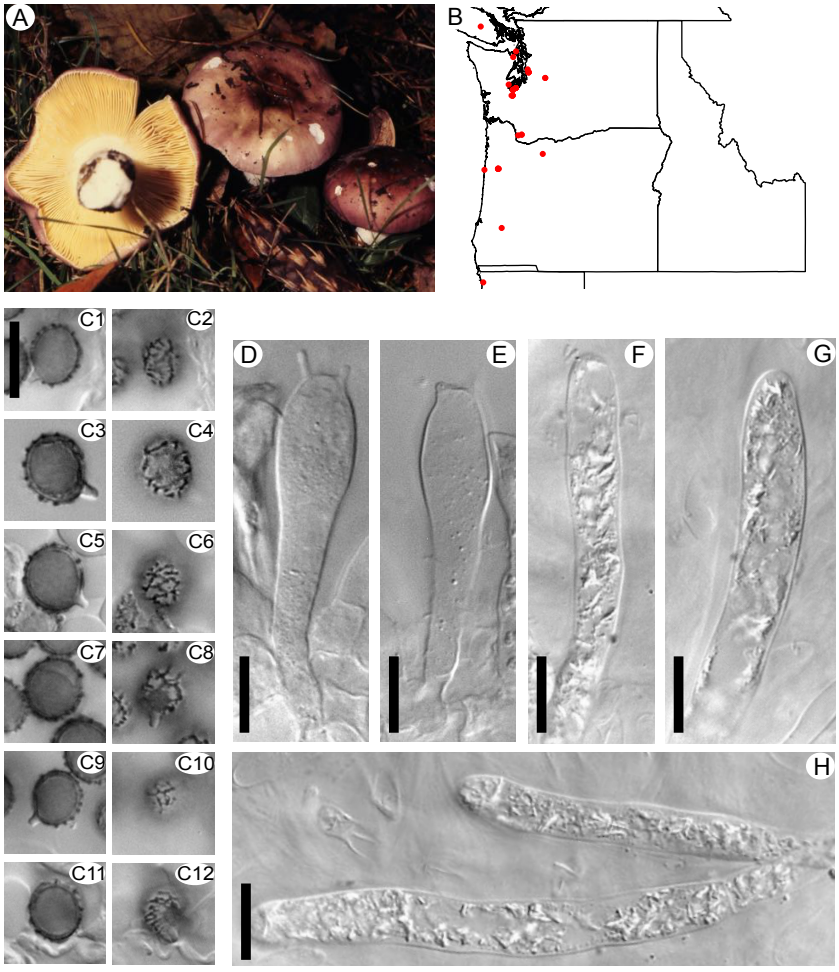

Figure 3.4 Russula obscurozelleri *morphology and map of specimen locations.*

Morphology and specimen distribution of Clade 7 Russula obscurozelleri *(Woo sp. 50). BW followed by numerals designate Ben Woo samples.* ***A,*** *photograph of fresh specimen (BW803).* ***B,*** *Distribution of specimens of the Woo*

*collections in Pacific Northwest States and Province. **C-H,** Micromorphology, all 1000x magnification. All scale bars 10 µm. **C,** Spores in median optical section and surface view in Melzer's reagent (**C**1-**C**2, BW383; **C**3-**C**4, BW400; **C**5-**C**6, BW535; **C**7-**C**8, BW536; **C**9-**C**10, BW535; **C**11-**C**12, BW535); **D-E,** Basidia (BW532, BW390); **F-H,** Cap cuticle terminal cells with refringent contents (BW536, BW536, BW536).*

***Russula parapallens* Bazzicalupo, D. Miller & Buyck., *sp. nov*.**

Index fungorum nr: IF553818; *FacesofFungi nr*: FoF 03651; Fig. 3.5

Etymology: refers to the sometimes pale colour of the cap

Holotype: BW791 (WTU)

Pileus 2-10 cm diam., plano-convex, not becoming infundibuliform with age, shortly striate at margin; surface smooth, patchy mixture of paler and darker shades of pinkish, tan, brownish wine red. *Lamellae* adnate, equal or with few lamellulae, bifurcations few or absent, normally spaced (ca 1 L/mm) and narrow in width, white to pale cream (of Woo specimens, ~50% recorded as 'white', ~50% as 'cream'); edge even, concolourous. Stipe mostly longer than the cap diam., slender, cylindrical, firm, white or sometimes with a pinkish blush. *Context* white, unchanging with age or on injury, reacting tan to $FeSO_4$. *Odour* fruity. *Taste* variable (noted as hot or mild in both gills and flesh). *Spore print* very pale (of Woo specimens, 90% Crawshay A, ~10% Crawshay B-C).

Spores broadly ellipsoid, (6.1-) 7.32-**7.35**-7.39 (-8.8) × (4.9-) 6.12-**6.15**-6.18 (-7.7) µm, Q=1.19-**1.2**-1.25 (-1.4); ornamentation reticulate, composed of interconnected amyloid, conical warts, (0.2-) 0.67-**0.68**-0.69 (-1.3) µm high; suprahilar spot present as a strongly amyloid patch. *Basidia* (28.5-) 36.5-**41**-45.5 (-49) × (8-) 9-**10**-11 (-13) µm, 4-spored, stout and slightly clavate. *Lamellar trama* mainly composed of sphaerocytes, intermixed with cystidioid hyphae. *Hymenial cystidia* 45-60(-65) × 6-7 µm, broadly clavate to fusiform, thin-walled, SV+, turning dark purple in sulfovanillin: (~25% specimens had a grey reaction). *Pileipellis* not sharply delimited from the underlying context of filamentous hyphae and sphaerocytes; suprapellis composed of loosely arranged hyphae with cylindrical terminal cells having obtuse tips. Pileocystidia so long that their length was hard to determine (i.e. the first septum proximal to their tip was often difficult to find), up to 9 µm thick; contents refringent. Acidoresistant incrustations absent. *Clamp connections* absent in all parts.

Habitat and distribution: host association unknown, but recorded with species including *Picea sitchensis*, *Pseudotsuga menziesii*, *Tsuga heterophylla*, and *Pinus contorta*. Only known from the Pacific Northwest (USA: Alaska, Oregon, Washington; Canada: British Columbia).

Examined material: U.S.A., Oregon, Lincoln City, East Devils Lake Park, 123.997778°W, 44.966667°N, 8 m alt., 13 Nov 1998, B. Woo BW791, F-038394 (WTU, **holotype !**), GenBank ITS2: KX813453; Washington, Denny Creek Camp, 121.4425°W, 47.412778°N, 680 m alt., 07 Oct 1997, B. Woo BW712, F-039290 (WTU), GenBank ITS2: KX813398; ibidem, La Push, Mora Campground, 124.606944°W, 47.918056°N, 10 m alt., 28 Oct 1994, B. Woo BW581, F-038864 (WTU), GenBank ITS2: KX813309; ibidem, Sloan Creek Camp, 121.287778°W, 48.0575°N, 630 m alt., 08 Sep 1996, B. Woo BW629, F-039202 (WTU), GenBank ITS2: KX813343; ibidem, Sol Duc Campground, 123.857778°W, 47.966944°N, 510 m alt., 11 Oct 1993, B. Woo BW558, F-038823 (WTU), GenBank ITS2: KX813292; ibidem, Spirit Lake, 03 Oct 1976, B. Woo BW107, F-039383 (WTU), GenBank ITS2: KX812964.

Notes: *Russula parapallens* corresponds to Clade 3 in the phylogeny (Fig. 3.1) and to Woo sp. 32 in Chapter 2. The phylogenetic placement of *R. parapallens* within subgenus *Russula* was unsupported and its closest relatives were unresolved. Although without support, the closest distinct species in the phylogeny (Fig. 3.1), *R. helodes*, is considered rare in Europe and characteristic of sphagnum peat bogs with birch and spruce (Sarnari, 1998-2005). The closest SH found in UNITE was *R. luteotacta* with a 94% match (UNITE SH DOI: https://plutof.ut.ee/#/datacite/10.15156/BIO/SH284902.07FU). *R. luteotacta* is ecologically very different, as it associates mostly with *Quercus*, *Carpinus*, and *Castanea* (Sarnari, 1998-2005). Other species that appeared genetically closely related when looking at BLAST results included species of subsection *Citrinae* sensu Romagnesi (but not *R. solaris* Ferdinandsen & Winge), as well as some of the paler acrid-tasting west coast Russulas such as *R. cremoricolor*.

As of March 2017, in UNITE and in PlutoF, no SH matched the ITS2 of this taxon. However, identical sequences were recorded from Canada (March 2017): Port Renfrew, Vancouver Island BC (UDB031531), Capilano Regional Park, Vancouver BC

(KX579783); USA: Delta Junction, Alaska (EU222979), Bonanza Creek, Alaska (KF617596).

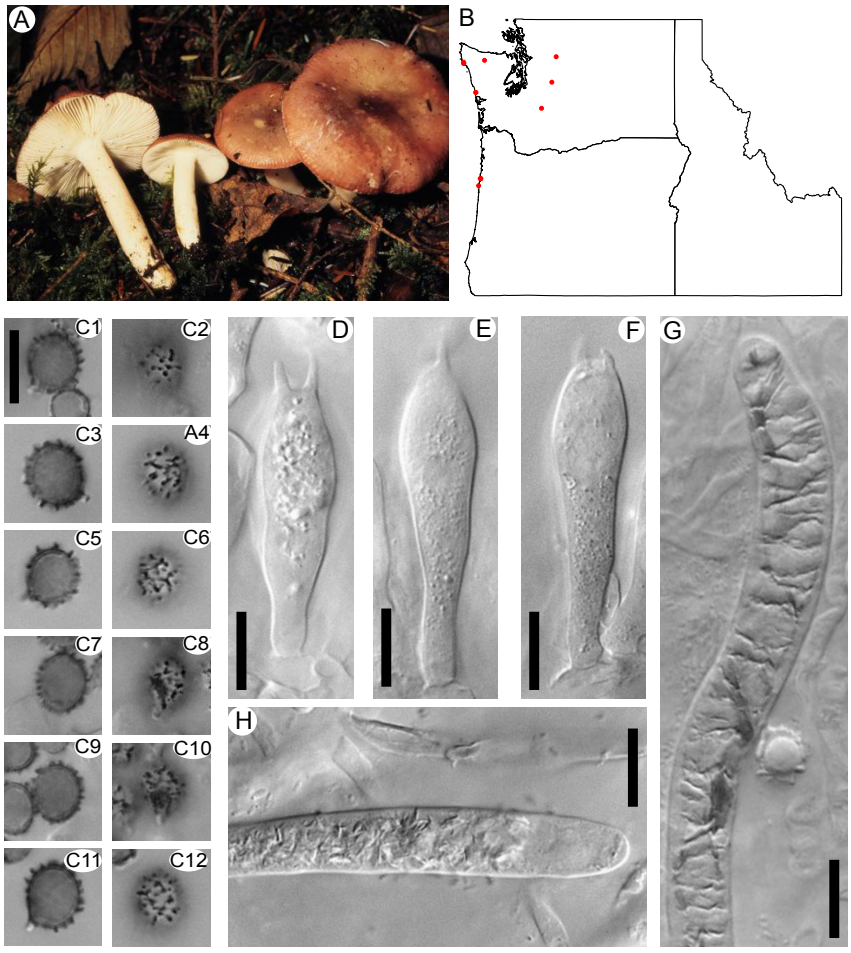

Figure 3.5 Russula parapallens morphology and map of specimen locations.

Morphology and specimen distribution of Clade 3 Russula parapallens *(Woo sp. 32). BW followed by numerals designate Ben Woo samples.* ***A,*** *photograph of fresh specimen (BW791).* ***B,*** *Distribution of specimens of the Woo collections in Pacific Northwest States.* ***C-H,*** *Micromorphology, all 1000x magnification. All scale bars 10 µm.* ***C,*** *Spores in median optical section and surface view in Melzer's reagent (**C**1-**C**2, BW107; **C**3-**C**4, BW107; **C**5-**C**6, BW107; **C**7-**C**8, BW712; **C**9-**C**10, BW712; **C**11-**C**12, BW558);* ***D-F,*** *Basidia (BW581, BW629, BW629);* ***G-H,*** *Cap cuticle terminal cells with refringent contents (BW558, BW712).*

Russula phoenicea* Bazzicalupo, D. Miller & Buyck., *sp. nov.

Index Fungorum nr: IF553819; *FacesofFungi nr*: FoF 03652; Fig. 3.6

Etymology: phoenix-like, a reference to its pleasing colouration

Holotype: Ben Woo 919 (WTU, sub nr. F-038577)

Pileus 3.5-12 cm diam., slightly depressed in the centre and with weakly striate margin; surface smooth, tinged with pale shades of pink, greyish pink, vinaceous or green, often with some patches of paler discolouration. *Lamellae* (L) adnate, equal or with few lamellulae and few to no bifurcations, normally spaced (ca 1 L/mm), white to pale cream (of Woo specimens, 'white' recorded for ~85%; 'cream' for ~15%); gill edge concolourous, even. *Stipe* mostly equal to cap diameter, cylindrical, with wrinkled surface, translucent and fragile; interior mostly soft. *Context* unchanging or turning pale pink with $FeSO_4$. *Odour* none. *Taste* mild to faintly acrid in gills and flesh (of Woo specimens; flesh at least somewhat hot in ~15%; gills somewhat hot in ~27%). *Spore deposit* very pale (of Woo specimens, Crawshay A ~75%; B-C ~25%).

Spores broadly ellipsoid, (6.8-) 8.09-**8.1**-8.16 (-9.4) × (5.3-) 6.52-**6.5**-6.59 (-8) μm, Q=(1-) 1.15-**1.24**-1.25 (-1.5); ornamentation subreticulate, composed of amyloid, conical warts, (0.3-) 0.89-**0.9**-0.92 (-1.6) μm high and sometimes slightly curved and the tips, with low and thin interconnections; suprahilar spot not highly amyloid although present as a patch. *Basidia* (33.5-) 40.5-**45**-49.5 (-55) × (8.5-) 10-**11**-12 (-13) μm, 4-spored, stout and clavate with swollen top; basidiola also stout and clavate. *Lamellar trama* mainly composed of sphaerocytes; cystidioid hyphae present. *Hymenial cystidia* 80-95 × 8-10 μm, broadly clavate to fusiform, mostly clavate near the gill edge, obtuse-rounded at the top, SV+ and turning dark purple in sulfovanillin (although ~20% of Woo specimens showed a grey reaction). *Pileipellis* not sharply delimited from the underlying context of filamentous hyphae and sphaerocytes; suprapellis very similar between cap centre and margin, composed of loosely arranged, branching hyphal terminations, with cylindrical terminal cells. Pileocystidia at the cap surface (20-) 48-**55**-60 (-62) × (3.5-) 5-**6**-7 (-8) μm, sometimes slightly constricted at the tips; contents refringent, SV+, continuing as cystidioid hyphae with refractory contents in subpellis and trama. Acidoresistant incrustations absent. *Clamp connections* absent in all parts.

Habitat and distribution: *Pseudotsuga menziesii* (Douglas fir) was consistently recorded near collections, sometimes mixed with *Pinus contorta* (also known as lodgepole pine, shore pine or twisted pine), *Picea sitchensis* (Sitka spruce), *Tsuga heterophylla* (western

hemlock) and *Abies* (true firs). Only known from the Pacific Northwest (USA: Oregon, Washington; Canada: British Columbia).

Examined material: CANADA, British Columbia, Golden Ears Provincial Park, in camp loop "Kalmia", 122.543056°W, 49.245278°N, 100 m alt., 04 Oct 1997, B. Woo BW706, F-039284 (WTU), GenBank ITS2: KX813394; U.S.A., Oregon State, East Devils Lake Park, Lincoln City, 123.997778°W, 44.966667°N, 8 m alt., 10 Nov 1996, B. Woo BW675, F-039222 (WTU), GenBank ITS2: KX813372; ibidem, South Beach State Park, Newport, 124.060556°W, 44.602222°N, 15 m alt., 10 Nov 1996, B. Woo BW682, F-038547 (WTU), GenBank ITS2: KX813376; Washington State, 3rd Beach Trail, La Push, 124.165°W, 47.888889°N, 6 m alt., 30 Oct 1998, B. Woo BW770, F-038905 (WTU), GenBank ITS2: KX813437; ibidem, Old Fort Townsend State Park, 122.790556°W, 48.074444°N, 60 m alt., 27 Oct 1997, B. Woo BW744, F-038937 (WTU), GenBank ITS2: KX813421; ibidem, Sloan Creek, 121.254°W, 48.0575°N, 630 m alt., 28 Sep 1997, B. Woo BW695, F-038577 (WTU), GenBank ITS2: KX813388; ibidem, 23 Sep 2001, B. Woo BW919, F-038477 (WTU, **holotype !**), GenBank ITS2: KX813551; ibidem, Talapus Lake Trail, 121.585°W, 47.401°N, 805 m alt, 17 Oct 1997, B. Woo BW730, F-038215 (WTU), GenBank ITS2: KX813410.

Notes: R. phoenicea corresponds to Clade 1 in the phylogeny (Fig. 3.1), to Woo sp. 26 in Chapter 2 and to UNITE SH DOI: https://plutof.ut.ee/#/datacite/10.15156/BIO/SH297356.07FU

In the Pacific Northwest, *R. phoenicea* could most easily be confused with *R. hypofragilis*. (described below) but the two species are not sister taxa (Fig. 3.1). Host association may differentiate the two. Both were found in mixed conifer stands, however, *R. hypofragilis* was consistently reported in the presence of *Abies,* while *R. phoenicea* was consistently recorded with *Pseudotsuga*. Morphological characters overlapped although gills in *R. hypofragilis* were more frequently recorded as being at least somewhat hot, and specimens of *R. hypofragilis* were less likely to have olive or green tones compared with *R. phoenicea*. Cap colour in both species ranged from shades of purples, greens and pink grey, and sizes of their microscopic features overlapped. The red to yellow cap colours so typical of *R. montana* Shaffer (=*griseascens* (Bon & Gaugué) Marti, see Chapter 2, *R. emetica* and other species in the clade were less common than

purplish or greenish tones in both *R. phoenicea* and *R. hypofragilis*. While flesh and gill tastes were hot in >90% of specimens of *R. montana* and in 100% of specimens of *R. emetica*, flesh and gill taste of *R. phoenicea* was usually mild. The macro- and micro-morphology of *R. phoenicea* overlapped with descriptions from Sarnari (1998-2005) for *R. fragilis* Fr., and *R. aquosa* LeClair, however these latter species are at this time unknown from the Pacific Northwest based on UNITE SH. Identical (100% match) ITS2 sequences to *R. phoenicea* have only been found in the Pacific Northwest [Canada: British Columbia (EU057098, FJ152483, KP889552, KP889829); Sooke Reservoir, Victoria, BC (UDB024994); Capilano Regional Park, Vancouver, BC (KC581327); Campbell River, BC (KP406551)]. The only other sequence available as of March 2017 with a 1%-3% difference was KF835445 from India.

Related west coast species (see Buyck et al. 2015) include *R. crenulata* Burl., which was described from Oregon (Burlingham, 1913) but differs in its very pale, white to yellowish cap colour, distinctly crenulate gill edges and very acrid taste; *R. cremoricolor* Earle, which is similarly coloured and equally hot but is an oak-associated species described from California (Earle, 1902); and *R. stuntzii* Grund, a whitish and very acrid species that is phylogenetically closer to *R. consobrina* and *R. helodes* (see Chapter 2). The more reddish *R. subveternosa* Sing., an acrid species with darker and differently ornamentated spores described from *Populus* stands in Wyoming (Singer, 1939) probably belongs to a different clade given the difference in cap-colour, host tree, and colour of spores.

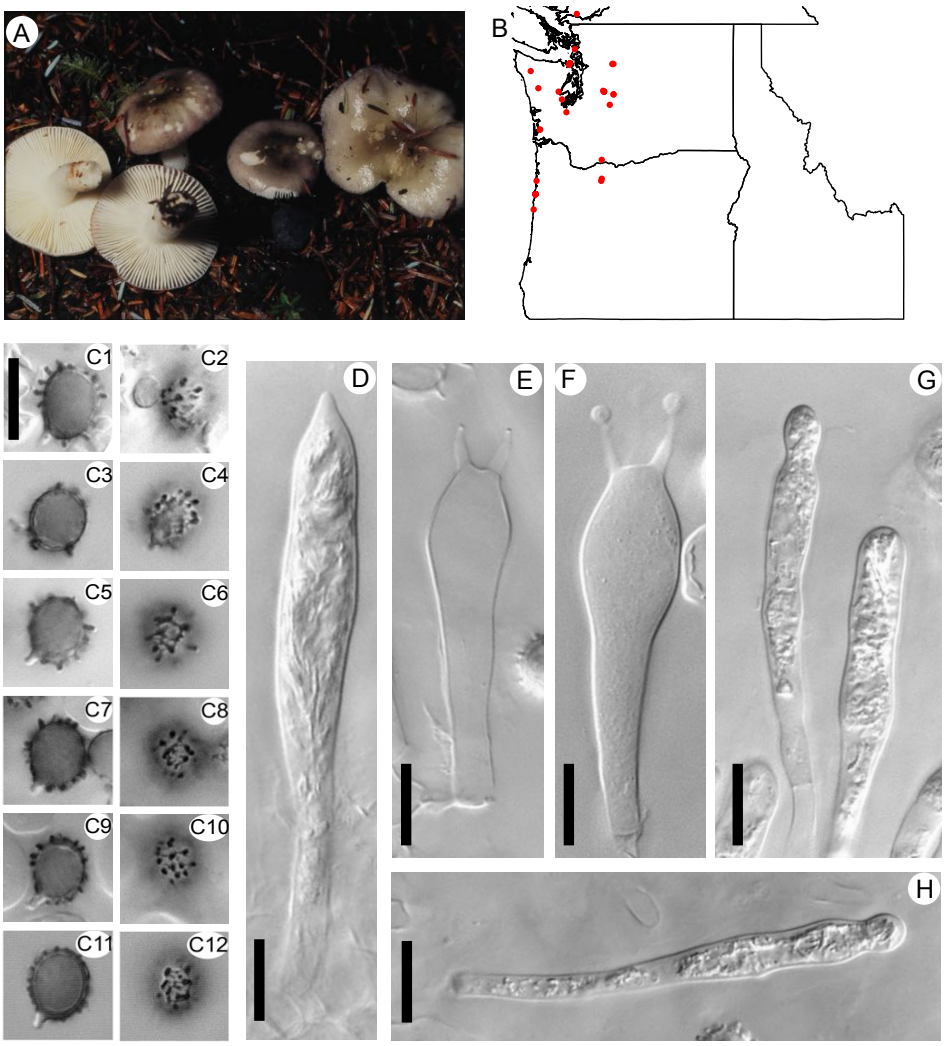

Figure 3.6 Russula phoenicea morphology and map of specimen locations.

Morphology and specimen distribution of Clade 1 Russula phoenicea *(Woo sp. 26). BW followed by numerals designate Ben Woo samples.* ***A,*** *Photograph of fresh specimen (BW770).* ***B,*** *Distribution of specimens of the Woo collections in Pacific Northwest States and Provinces.* ***C-H,*** *Micromorphology, all 1000x magnification. All scale bars 10 µm.* ***C,*** *Spores in median optical section and surface view in Melzer's reagent (**C**1-**C**2, BW675; **C**3-**C**4, BW682; **C**5-**C**6, BW695; **C**7-**C**8, BW744; **C**9-**C**10, BW744; **C**11-**C**12, BW730);* ***D,*** *Hymenial cystidium (BW695);* ***E-F,*** *Basidia (BW706, BW744);* ***G-H,*** *Cap cuticle terminal cells with refringent contents (BW695, BW682).*

***Russula pseudopelargonia* Bazzicalupo, D. Miller & Buyck., *sp. nov*.**

Index fungorum nr: IF553820; *FacesofFungi nr*: FoF 03653; Fig. 3.7

Etymology: from its prior confusion with *R. pelargonia*

Holotype: BW603 (WTU, sub nr F-038653)

Pileus up to 7.5(10.5) cm in diam., plano-convex to gently depressed in the centre, often uneven, with irregularly- wavy, striate margin; surface deep red to vinaceous red or brownish red, usually darker in the centre, sometimes pinkish toward margin, often irregularly mottled with yellow splotches or discolouring toward the margin with age, when young often with the extreme margin white, peeling up to mid-radius, strongly viscid to glutinous when wet. *Lamellae* adnate, cream-coloured (of Woo specimens, ~75 recorded as 'cream', ~25% as 'white'), normally spaced (ca 1 L/mm), equal or with a few, sometimes very short lamellulae, and occasional bifurcations; gill edge concolourous, even. *Stipe* most often shorter than the cap diam., subcylindrical or more frequently widening toward the base, white but often with a pinkish flush in the lower half, not bruising or with some yellowish brown stains at the base. *Context* white, unchanging, turning grey-pink to tan with $FeSO_4$. *Odour* fruity or more frequently, clearly reminiscent of *Pelargonium* (geranium). *Taste* medium to very hot in gills and flesh. *Spore print* cream (of Woo specimens, ~25% Crawshay A; ~75% Crawshay B-C). *Spores* broadly ellipsoid, (5.9-) 7.8-**7.85**-7.9 (-9.8) × (4.8-) 6.27-**6.31**-6.35 (-7.9) μm, Q=(1.1-) 1.24-**1.25**-1.27 (-1.4), ornamented with amyloid, relatively high, conical warts, (0.2-) 0.66-**0.68**-0.69 (-1.4) μm, with rare interconnections; suprahilar spot present as a strongly amyloid patch. *Basidia* (33-) 38-**43.5**-49 (-53.5) × (7-) 9-**10.5**-12 (-13) μm, 4-spored, stout and clavate with slightly swollen top. *Lamellar trama* composed mainly of sphaerocytes, intermixed with cystidioid hyphae. *Hymenial cystidia* 60-70 × 7-8 μm, clavate, SV+ (of specimens, ~50% grey/~50% dark purple). *Pileipellis* not sharply delimited from the underlying context of filamentous hyphae and sphaerocytes. Suprapellis composed of loosely arranged, branching and slender hyphal terminations. Pileocystidia sometimes so long that it is hard to determine their length (i.e. find the first septum from their tip); when measurable with terminal cells ~35-40 μm long, up to 7.5 μm in width and with obtuse tips; contents refringent. Cystidioid hyphae containing refractory contents also very abundant in subpellis and trama. Acidoresistant incrustations absent. *Clamp connections* absent in all parts.

Habitat and distribution: consistently associated with *Pseudotsuga menziesii* or *Tsuga heterophylla*, often intermixed with other trees; only known from Washington west of the Cascades and southern BC.

Examined material: U.S.A., Washington, Denny Creek Camp, 121.441667°W, 47.412778°N, 680 m alt., 24 Oct 1993, B. Woo BW562, F-038819 (WTU), GenBank ITS2: KX813295; 11 Oct 1996, B. Woo BW643, F-039024 (WTU), GenBank ITS2: KX813349; 13 Sep 1995, B. Woo BW603, F-038653 (WTU, **holotype !**), GenBank ITS2: KX813324; ibidem, Olympia, Priest Point Park, 122.8961°W, 47.06972°N, 30 m alt., 31 Oct 1997, B. Woo BW747, F-038935 (WTU), GenBank ITS2: KX813422; 02 Nov 1998, B. Woo BW779, F-038903 (WTU), GenBank ITS2: KX813442; B. Woo BW784, F-038906 (WTU), GenBank ITS2: KX813446; 03 Nov 2000, B. Woo BW888, F-039282 (WTU), GenBank ITS2: KX813529.

Notes: *Russula pseudopelargonia* corresponds to Clade 5 in the phylogeny (Fig. 3.1), to Woo sp. 36 in Chapter 2 and to UNITE SH DOI: https://plutof.ut.ee/#/datacite/10.15156/BIO/SH297365.07FU . In the phylogeny (Fig. 3.1) *R. pseudopelargonia* was placed as a well-supported sister to the European *R. sardonia*, the type species of subsection *Sardoninae*. Aside from the fruity odour and very acrid taste of *R. sardonia* (Marxmüller et al., 2014), the species lacks strong morphological similarity to *R. pseudopelargonia* and as a strict associate of *Pinus*, it contrasts in host preference.

In the Pacific Northwest, the *Pelargonium* scent of the carpophore possibly led to previous records of this species as '*R. pelargonia* Niolle'. *Russula pelargonia* lies phylogenetically in the unrelated subsect. *Violaceinae* (Miller and Buyck, 2002). *R. pseudopelargonia* could also be confused with many colour forms of *R. salishensis* but the more consistently hot taste of its flesh would sometimes help to distinguish it.

Distribution of ITS sequences identical to *R. pseudopelargonia*, including environmental samples, confirmed its restricted distribution to the Pacific Northwest [Canada: Port Renfrew, BC (UDB031534), BC (HQ604842)]. Except for these identical sequences, no other sequences in public databases were 97% or more identical to *R. pseudopelargonia* (as of March 2017).

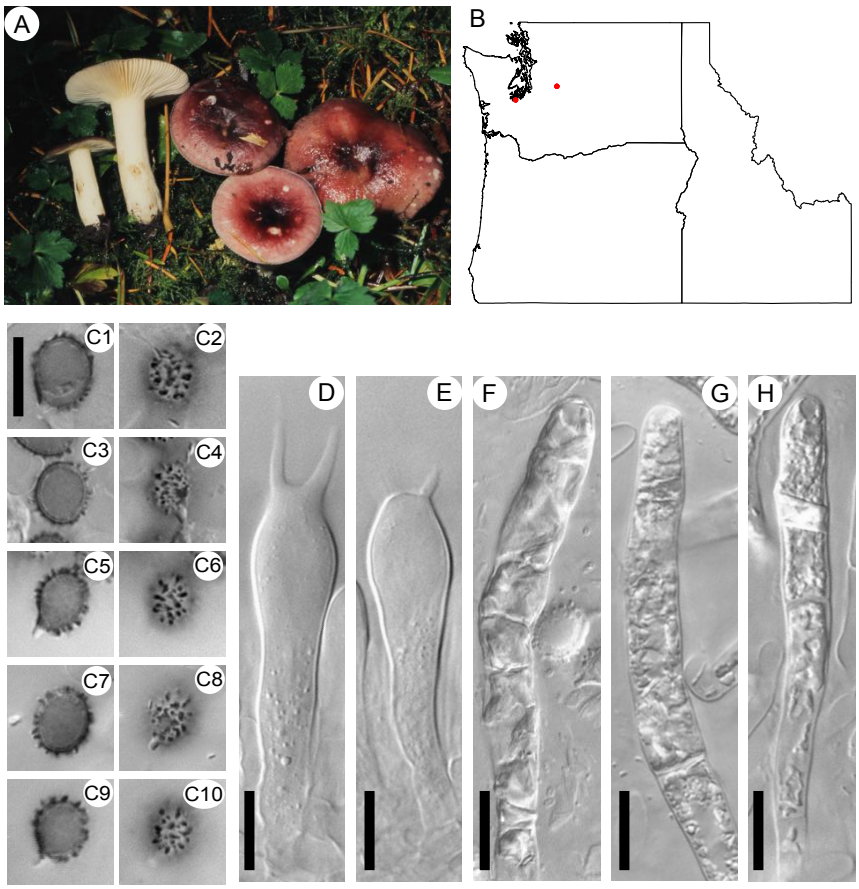

Figure 3.7 Russula pseuopelargonia *morphology and map of specimen locations.*

Morphology and specimen distribution of Clade 5 Russula pseudopelargonia *(Woo sp. 36). BW followed by numerals designate Ben Woo samples.* ***A,*** *Photograph of fresh specimen (BW784).* ***B,*** *Distribution of specimens of the Woo collections in Pacific Northwest States.* ***C-H,*** *Micromorphology, all 1000x magnification. All scale bars 10 µm.* ***C,*** *Spores in median optical section and surface view in Melzer's reagent (**C**1-**C**2, BW562; **C**3-**C**4, BW562; **C**5-**C**6, BW603; **C**7-**C**8, BW888; **C**9-**C**10, BW888);* ***D-E,*** *Basidia (BW562, BW562);* ***F-H,*** *Cap cuticle terminal cells with refringent contents (BW779, BW747, BW643).*

***Russula pseudotsugarum* Bazzicalupo, D. Miller & Buyck., *sp. nov*.**

Index fungorum nr: IF553821; *FacesofFungi nr*: FoF 03654; Fig. 3.8

Etymology: refers to its presumed association with *Pseudotsuga*

Holotype: Woo BW953 (WTU, sub nr. F-038562)

Pileus 3-8(-16) cm diam., convex, becoming gently depressed in the centre, never deeply funnel-shaped, with shortly striate margin; very variable in colour, greenish, flesh-coloured to pinkish red, vinaceous to ruby red, reddish purple, violet brown to brown,

even uniformly dark purple, mostly with distinctly darker centre. *Lamellae* adnate, equal or with an occasional lamellula or bifurcation, normally spaced (ca. 1 L/mm), becoming distinctly yellowish; gill edge even, concolourous. *Stipe* cylindrical or more often widening downward, variable in length, and both distinctly longer or shorter than the cap diam., slender to distinctly inflated, often also bent in its lower part, white. *Context* fragile, white, unchanging, insensitive to $FeSO_4$ (~20% of Woo specimens recorded as tan or yellow). *Odour* none. *Taste* mild in gills and flesh. *Spore print* cream to yellow (~25% Crawshay D-E, ~75% Crawshay F-G).

Spores broadly ellipsoid, (6.25-) 8.07-**8.13**-8.18 (-10.6) × (4.7-) 3.31-**6.35**-6.39 (-8.1) μm, Q=(1.2-) 1.27-**1.28**-1.29 (-1.6); ornamentation (sub)reticulate, with amyloid warts, (0.2-) 0.59-**0.6**-0.61 (-1) μm high, fused in short crests or with thin interconnections; suprahilar spot present as a distinct amyloid patch. *Basidia* (23-) 32-**38**-43.5(-56) × (9-) 10-**11**-12 (-13.5) μm, 4-spored, stout and slightly clavate; basidiola similar. *Lamellar trama* composed mainly of sphaerocytes, intermixed with cystidioid hyphae. *Hymenial cystidia* 60-65 × 9-11 μm, clavate to fusiform, thin-walled, weakly SV+ and pale grey in sulfovanillin (~20% of Woo specimens recorded as maroon or pink). *Marginal cells* not differentiated. *Pileipellis* not sharply delimited from the underlying context of filamentous hyphae and sphaerocytes; suprapellis composed of loosely arranged hyphae with cylindrical terminal cells having obtuse tips. Pileocystidia at pileus surface measuring (23.5-) 27-**31**-35.5 (-41.5) × (4-) 5-**6**-7.5 (-9) μm, septate, with short terminal cells, often somewhat clavate or inflated, obtuse-rounded at the tip; contents refringent. Acidoresistant incrustations absent. *Clamp connections* absent in all parts.

Habitat and distribution: although *Pseudotsuga menziesii* was present at each collection locality, other conifers such as *Tsuga heterophylla*, *Picea sitchensis,* and *Pinus contorta* were usually present as well.

Examined material: U.S.A., Washington, Chimacum County Park, 48˚0'53" N; -122˚46'39" W, 40 m alt., 11 Nov 2001, B. Woo BW951, F-038563 (WTU), GenBank ITS2: KX813576; ibidem, Greenwater Road 70, 1176 Trailhead, 121.619167˚W, 47.140278˚N, 600 m alt., 10 Sep 1995, B. Woo BW597, F-038602 (WTU), GenBank ITS2: KX81331809; ibidem, Oct 2005, B. Woo BW1041, F-038641 (WTU), GenBank ITS2: KX812940; Old Fort Townsend Jefferson County, 122.790556˚W, 48.074˚N, 60 m

alt., 29 Oct 1998, B. Woo BW767, F-038908 (WTU), GenBank ITS2: KX813433; ibidem, 11 Nov 2001, B. Woo BW953, F-038562 (WTU, **holotype !**), GenBank ITS2: KX813578; ibidem, Olympia, Priest Point Park, 122.8961°W, 47.06972°N, 30 m alt., 02 Nov 1998, B. Woo BW786, F-038887 (WTU), GenBank ITS2: KX813447; ibidem, Talapus Lake Trailhead FS road 9030, 121.585°W, 47.401°N, 805 m alt., 05 Oct 2005, B. Woo BW1035, F-038628 (WTU), GenBank ITS2: KX812934; ibidem, Olympia, Tolmie State Park, 122.7761°W, 47.120556°N, 2 m alt., 11 Nov 1999, B. Woo BW849, F-039125 (WTU), GenBank ITS2: KX813499.

Notes: Russula pseudotsugarum corresponded to Clade 8 in the phylogeny (Fig. 3.1), to Woo sp. 52 in Chapter 2 and to UNITE SH DOI: **https://plutof.ut.ee/#/datacite/10.15156/BIO/SH315582.07FU**

All 25 samples of *Russula pseudotsugarum* formed a monophyletic group nested within a well-supported clade that included *R. cessans* Pearson, *R. zelleri* Burl., Woo sp. 51, *R. laricina* Velenovsky and *R. nauseosa*. The European *R. olivina,* Ruotsalainen & Vauras, well characterized by its two-spored basidia and particular spore ornamentation, considered as a close relative to these species in Sarnari (1998-2005), was only distantly related in the phylogeny (Fig. 3.1). *R. pseudotsugarum* is difficult to separate morphologically from *R. zelleri* and *R. obscurozelleri*, but as its name suggests, it has been found consistently with *Pseudotsuga*, while *R. zelleri* is found with *Picea*. *R. pseudotsugarum* has probably been identified as *R. lilacea* in the Pacific Northwest based on the Grund (1965) key, but *R. lilacea* may not occur in the region. *R. pseudotsugarum* could easily be confused with *R. obscurozelleri* (Clade 7); see notes under that species.

Geographically, samples with sequences identical to *R. pseudotsugarum* ranged from the Pacific Northwest to Mexico: Canada: Sooke Reservoir, Vancouver Island, BC (UDB031541); Bella Coola, BC (HQ650754); Pemberton, BC (JN652960); Campbell River, BC (KP403052, KP403055); BC (KM402893, KP406550, KP406553, KT272154, KT272155); Mexico: Tlaxcala (KP781012) USA: Mt St Helen, Washington (UDB012199); HJ Andrews Experimental Forest, Cascade Range, Oregon (EU526011); California (JF834345, JF834494).

Sequences1.5% different from *R. pseudotsugarum* were recorded from western and eastern North America and Europe, likely representing *R. zelleri*, *R. cessans*, and *R. laricina*: https://plutof.ut.ee/#/datacite/10.15156%2FBIO%2FSH177309.07FU.

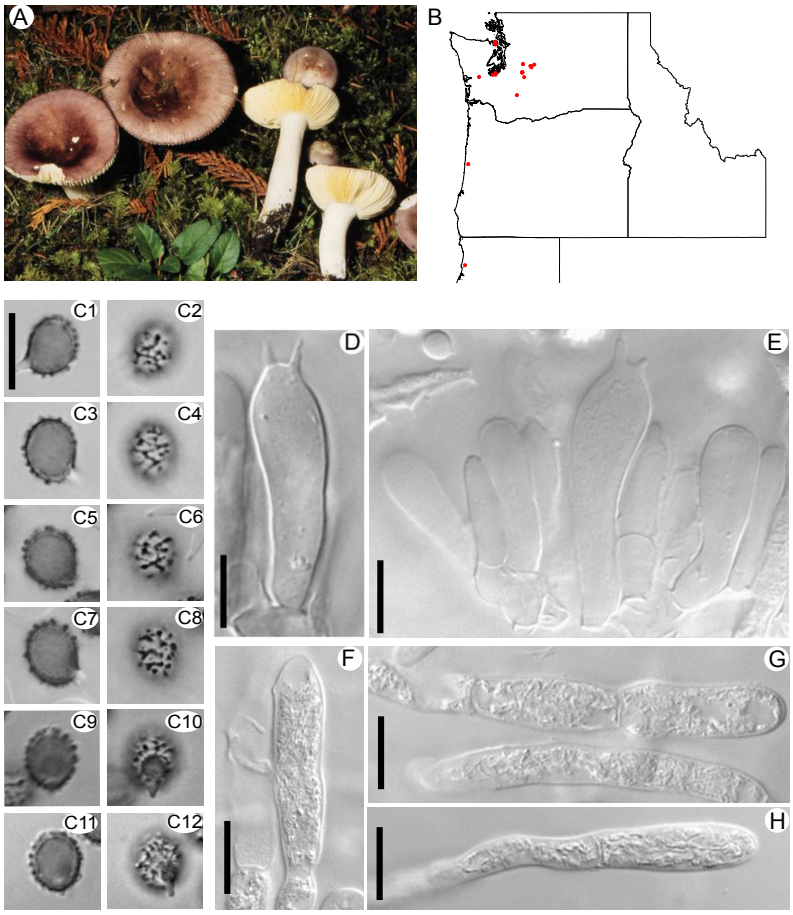

Figure 3.8 Russula pseudotsugarum *morphology and map of specimen locations.*

Morphology and specimen distribution of Clade 8 Russula pseudotsugarum *(Woo sp. 52). BW followed by numerals designate Ben Woo samples.* ***A,*** *Photograph of fresh specimen (BW953).* ***B,*** *Distribution of specimens of the Woo collections in Pacific Northwest States.* ***C-H,*** *Micromorphology, all 1000x magnification. All scale bars 10 µm.* ***C,*** *Spores in median optical section and surface view in Melzer's reagent (**C**1-**C**2, BW767; **C**3-**C**4, BW767; **C**5-**C**6, BW786; **C**7-**C**8, BW786; **C**9-**C**10, BW953; **C**11-**C**12, BW953);* ***D-E,*** *Basidia (BW951, BW1041);* ***F-H,*** *Cap cuticle terminal cells with refringent contents (BW849, BW1035, BW1035).*

***Russula rhodocephala* Bazzicalupo, D. Miller & Buyck., *sp. nov*.**

Index fungorum nr: IF553822; *FacesofFungi nr*: FoF 03655; Fig. 3.9

Etymology: refers to the red colour of the cap

Holotype: BW337 (WTU, sub nr. F-039507)

Pileus 2-12 cm diam., very fleshy and firm, convex to shallowly depressed or irregularly wavy with downward margin without striations; surface bright scarlet, deep crimson red to brownish reddish orange. *Lamellae* adnate to slightly decurrent, spacing normal (ca. 1 L/mm) to slightly wider, mostly equal although lamellulae can be common and bifurcations occasionally present, cream coloured. *Stipe* shorter than the cap diam. in mature fruiting bodies, robust and cylindrical, flushed with pink or red. *Context* white, unchanging with age or on injury, the lower stipe may bruise yellow, turning pink with $FeSO_4$. *Odour* none or weakly pleasant. *Taste* very hot in gills and flesh, (of Woo specimens, 100% recorded as 'hot' in both gills and flesh, 50% of flesh recorded as some degree of 'acrid'). *Spore print* yellowish, (~40% of Woo specimens recorded as Crawshay B-C, ~60% D-E).

Spores broadly ellipsoid, (6.2-) 7.83-**7.87**-7.92 (-10) × (4.8-) 6.28-**6.32**-6.35 (-7.5) μm, Q=(1-) 1.24-**1.25**-1.26 (-1.5), ornamented with mostly isolated, amyloid, conical warts, (0.2-) 0.68-**0.69**-0.71 (-1.4) μm high, with rare connections; suprahilar spot a strongly amyloid patch. *Basidia* (36.5-) 41-**45**-49 (-55) × (9-) 10-**11**-12 (-14) μm, 4-spored. *Lamellar trama* mainly composed of sphaerocytes, intermixed with cystidioid hyphae. *Hymenial cystidia* 60-65(-70) × 7-8 μm, broadly clavate, obtuse-rounded at the tip, SV+ and dark purple in sulfovanillin. *Pileipellis* not sharply delimited from the underlying context of filamentous hyphae and sphaerocytes; suprapellis composed of loosely arranged, branching hyphal terminations, with cylindrical terminal cells. Pileocystidia sometimes so long that it was hard to determine their length, when measurable up to ~40 μm long and up to 8 μm in width and with obtuse tips; contents refringent, also abundantly continuing as cystidioid hyphae with refractory contents in subpellis and trama. Acidoresistant incrustations absent. *Clamp connections* absent in all parts.

Habitat and distribution: *Pinus contorta*. Known from USA: California, Idaho, Oregon, Washington; Canada: British Columbia.

Examined material: U.S.A., Idaho, Priest Lake, 116.916667°W, 48.565556°N, 765-900 m alt., 29 Sep 1978, B. Woo BW201, F-038413 (WTU), GenBank ITS2: KX813043; ibidem, Priest Lake, North Lake Road, 116.816667°W, 48.748889°N, 770 m alt., 24 Sep 1983, B. Woo BW361, F-038604 (WTU), GenBank ITS2: KX813143; Oregon, Lincoln

City, East Devils Lake Park, 124.01194°W, 44.97°N, 6 m alt., 15 Nov 1986, B. Woo BW438, F-038632 (WTU), GenBank ITS2: KX813199; ibidem, 123.997778°W, 44.97°N, 6 m alt., 15 Nov 1999, B. Woo BW860, F-039103 (WTU), GenBank ITS2: KX813506; ibidem, Astoria, Fort Stevens, 123.96861°W, 46.185278°N, 15 m alt., 11 Nov 1989, B. Woo BW486, F-038655 (WTU), GenBank ITS2: KX813231; Washington, Fort Canby State Park, 124.063889°W, 46.285833°N, 6 m alt., 12 Nov 1988, B. Woo BW463, F-038363 (WTU), GenBank ITS2: KX813218; ibidem, Shelton power line, 123.066667°W, 47.250833°N, 60 m alt., 24 Oct 1982, B. Woo BW337, F-039507 (WTU, **holotype !),** GenBank ITS2: KX813126.

Notes: Russula rhodocephala corresponds to Clade 4 in the phylogeny (Fig. 3.1), to Woo sp. 35 in Chapter 2 and to UNITE SH DOI https://plutof.ut.ee/#/datacite/10.15156%2FBIO%2FSH218433.07FU (100% match ITS2).

Until now, *R. rhodocephala* has been referred to as *R. sanguinea* Fr.. Both *R. rhodocephala* and *R. sanguinea* are associated with *Pinus* (Bills and Miller Jr, 1984). Even though the European *R. sanguinea* appeared as the sister to this species with significant support (Fig. 3.1), it differed from *R. rhodocephala* by more than 3% in the ITS2 region. PlutoF maps showed that their distributions differed. The only records of sequences matching *R. rhodocephala* at 99.5% identity cutoff were from the American west: [Canada: Rocky Point, Victoria, BC (UDB031015), U.S.A.: California (GU180315), corresponding to UNITE SH DOI: https://plutof.ut.ee/#/datacite/10.15156/BIO/SH297359.07FU]. Relaxing the identity cutoff to 99%, samples with a wider geographical distribution across the United States and Mexico were included: https://plutof.ut.ee/#/datacite/10.15156/BIO/SH130463.07FU. Further relaxing the cutoff to 97% cutoff included sequences found from Korea, China and Japan (March 2017): https://plutof.ut.ee/#/datacite/10.15156%2FBIO%2FSH030433.07FU. Reflecting their sequence differences, European *Russula sanguinea* corresponded to UNITE SH218425 represented by sample *R. sanguinea* UDB011161, while N. American *R. rhodocephala* (accessioned into GenBank as "*R. sanguinea*") corresponded to UNITE SH218433.

The spore print colour described should be verified due to the surprisingly wide range recorded by Woo. However, a similar wide variation in spore print colour is given on Mushroomexpert.com for the eastern U.S. taxon identified as *R. sanguinea* ("creamy to yellowish or orange-yellow") (Kuo, 2009).

The species could be confused with *R. americana* Singer, which appears a somewhat less robust taxon associating with *Tsuga* and perhaps also *Abies*, and has larger spores, 8.5-11.5 x 7-l0.8 (Singer 1939). *R. americana* also matches the description of *R. rosacea* var. *macropseudocystidiata* Grund. Detailed descriptions can be found in Roberts (2007) who distinguished *R. americana* from *R. rhodocephala* (under the name '*R. sanguinaria*') by its taller and more slender habit and by its association with western hemlock (and possibly *Abies*) rather than pines. Unlike *R. americana, R. rhodocephala* is generally found in wet areas, often shows yellow staining on the lower stipe, and has a more evenly coloured and shiny cap with the epicutis an ixotrichodermis. Most other red-capped, acrid species in the area produce whitish gills and spore prints, while the otherwise very similar *R. californiensis* Burl. grows with pine and oak in California, has a pale yellowish spore print, a more distinctly greying stipe, and especially, a distinctly more reticulate spore ornamentation (Burlingham, 1936).

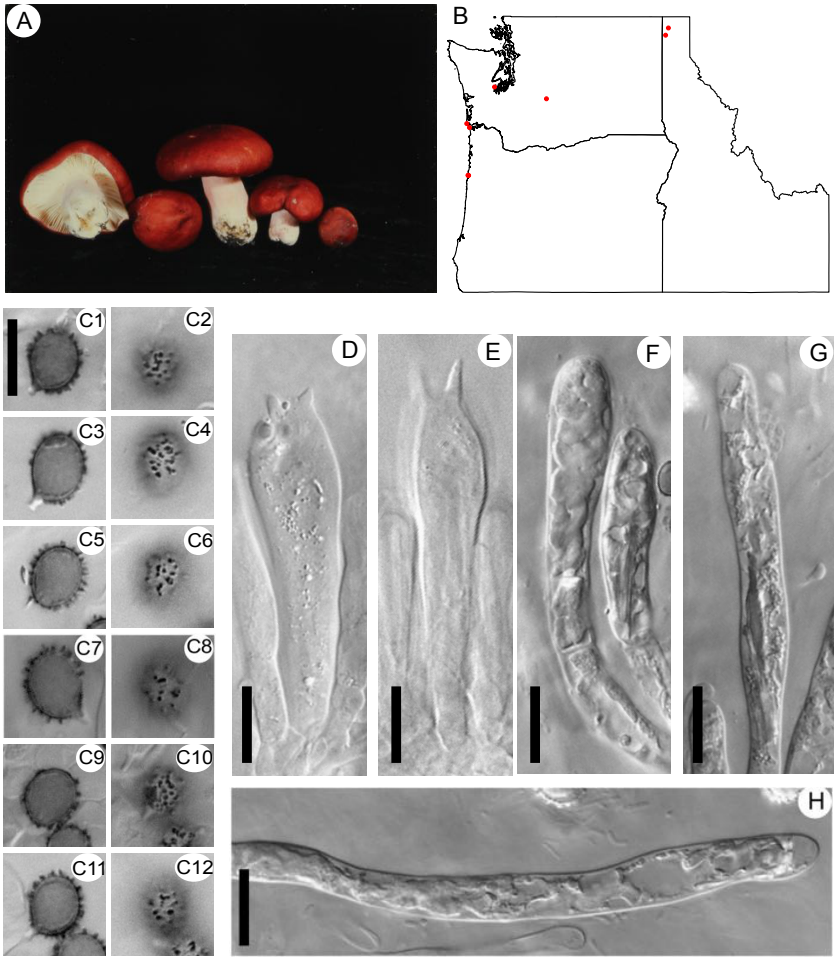

Figure 3.9 Russula rhodocephala *morphology and map of specimen locations.*

Morphology and specimen distribution of Clade 4 Russula rhodocephala *(Woo sp. 35). BW followed by numerals designate Ben Woo samples.* ***A,*** *Photograph of fresh specimen (BW337).* ***B,*** *Distribution of specimens of the Woo collections in Pacific Northwest States.* ***C-H,*** *Micromorphology, all 1000x magnification. All scale bars 10 µm.* ***C,*** *Spores in median optical section and surface view in Melzer's reagent (**C**1-**C**2, BW438; **C**3-**C**4, BW438; **C**5-**A**6, BW337; **C**7-**C**8, BW860; **C**9-**C**10, BW337; **C**11-**C**12, BW463);* ***D-E,*** *Basidia (BW361, BW463);* ***F-H,*** *Cap cuticle terminal cells with refringent contents (BW337, BW201, BW201).*

***Russula salishensis* Bazzicalupo, D. Miller & Buyck., *sp. nov*.**

Index fungorum nr: IF553823; *FacesofFungi nr*: FoF 03656; Fig. 3.10

Etymology: refers to the Salish Sea

Holotype: BW972 (WTU, sub nr. F-038984)

Pileus 3-8(9.5) cm diam., plano-convex, becoming gently depressed in the centre, with the margin slightly striate; surface viscid when wet, pale to deep pinkish red, wine red,

more rarely purplish red, usually darker in the centre, occasionally with yellow to brown splotches or producing forms that are much paler, yellowish or pinkish to flesh coloured, or toward the margin tinted with pale greenish grey. *Lamellae* adnate, equal, normally spaced (ca. 1 L/mm), cream to yellow coloured (of Woo specimens, ~60% recorded as 'cream'; ~40% as 'yellow'). *Stipe* rather slender to robust, length roughly equal to cap diam., cylindrical or broadening downward, white, often also with a faint pink flush, or sometimes with the very base spotted with rusty yellow. *Odour* fruity or sometimes reminiscent of *Pelargonium*. *Context* white, unchanging, insensitive or turning pale buff with $FeSO_4$. *Taste* slightly hot to mild in flesh, but usually very hot in gills. *Spore print* pale cream (of Woo specimens, ~25% Crawshay B, ~35% Crawshay C-D, ~40% Crawshay E).

Spores broadly ellipsoid, (5.7-) 7.67-**7.72**-7.76 (-10.5) × (4.8-) 6.14-**6.18**-6.22 (-9.1) µm, Q=(1.15-**1.25**-1.26 (-1.6), ornamentation subreticulate, composed of amyloid, conical warts up to (0.2-) 0.39-**0.4**-0.41 (-0.9) µm high, locally confluent in short crests or with thin interconnections, suprahilar spot present as a not highly amyloid patch. *Basidia* (36.5-) 41.5-**46.5**-52 (-59.5) × (7.5-) 8.5-**9.5**-10.5 (-12) µm, 4-spored, stout and clavate with swollen top; basidiola also stout and clavate. *Lamellar trama* mainly composed of sphaerocytes, intermixed with cystidioid hyphae. *Hymenial cystidia* broadly clavate, measuring 70-85(-90) × 9-12 µm, sometimes capitate, contents SV+ (dark purple). *Pileipellis* not sharply delimited from the underlying context of filamentous hyphae and sphaerocytes; suprapellis composed of loosely arranged hyphae with cylindrical terminal cells with obtuse tips. Pileocystidia so long that their length was difficult to determine, up to 7.5 µm thick; contents refringent, SV+ (dark purple). Acidoresistant incrustations absent. *Clamp connections* absent in all parts.

Habitat and distribution: probably associates with both *Pseudotsuga menziesii* and *Tsuga heterophylla,* and is mostly found in forests where both occur; as yet known only from the Pacific Northwest (Oregon, Washington east and west of the Cascades, British Columbia), up to 1200 m alt.

Examined material: U.S.A., Washington, Dol Duc Road, 123.866667°W, 48.00083°N, 400-600 m alt., 15 Oct 2006, B. Woo BW1062, F-038415 (WTU), GenBank ITS2: KX812958; ibidem, Gifford Pinchot Rd 24, 121.666667°W, 46.03361°N, 925-1200 m

alt., 15 Oct 1994, B. Woo BW572, F-039045 (WTU), GenBank ITS2: KX813303; ibidem, Greenwater Road 7030, 121.619167°W, 47.140278°N, 600 m alt., 10 Sep 1995, B. Woo BW599, F-038592 (WTU), GenBank ITS2: KX813320; ibidem, Greenwater Road 70, End Pavement, 121.442°W, 47.103056°N, 1270 m alt., 09 Oct 2005, B. Woo BW1045, F-038666 (WTU), GenBank ITS2: KX812944; ibidem, Lake Kachess, Road 4832, 121.31361°W, 47.3172°N, 820 m alt., 12 Oct 1997, B. Woo BW721, F-038194 (WTU), GenBank ITS2: KX813404; ibidem, Sloan Creek Camp, 121.287778°W, 48.0575°N, 630 m alt., 23 Oct 1994, B. Woo BW575, F-039027 (WTU), GenBank ITS2: KX813306; ibidem, Sloan Creek Horsecamp, 121.287778°W, 48.0575°N, 630 m alt., 22 Oct 2002, B. Woo BW972, F-038984 (WTU, **holotype !**), GenBank ITS2: KX813591; ibidem, Talapus Lake Trail, 121.585°W, 47.401°N, 805 m alt., 17 Oct 1997, B. Woo BW731, F-038220 (WTU), GenBank ITS2: KX813411.

Notes: Russula salishensis corresponds to Clade 6 in the phylogeny (Fig. 3.1) and to Woo sp. 39 in Chapter 2. As of March 2017 *R. salishensis* did not have a unique SH in UNITE probably because of the close similarity of its ITS2 sequence to *R. queletii*.

Russula salishensis appeared as a strongly supported clade closely related to *R. queletii,* a species originally described from Europe and principally associated with *Picea* on calcareous soils, but also reported, rarely, from other European conifers. The European *R. queletii* also occurs in the Pacific Northwest, consistently with *Picea* (*P. sitchensis*). A detailed description of *R. queletii* specimens from the Pacific Northwest can be found in Roberts (2007). This species does not produce the intense red to pink coloured stipes so typical of *R. queletii*. Although both *Russula queletii* and *R. salishensis* had white-stipe forms occasionally with a yellowish-rusty spotted stipe base, *R. queletii* more frequently had a pink flush to its stipe compared with *R. salishensis*. Finally, the two species differed significantly in spore ornamentation, *R. queletii* having spores with isolated spines.

The colour forms of *R. salishensis* that had more red than purple could be easily confused with *R. pseudopelargonia*. In the past it is likely that this species was mistaken for and recorded as *R. pelargonia*.

Russula salishensis shares its host, *Pseudotsuga* with *R. phoenicea*. *Russula phoenicea* could be distinguished because it lacked pinkish shades on its stipe and rusty-yellow

tones on the stipe base. In addition, *Russula phoenicea* usually had paler caps, milder gill taste, paler spore prints and gills, as well spores with taller, more strongly reticulate, interconnected but not crested ornamentation. *Russula hypofragilis* was another look-alike but was consistently associated with *Abies* in the PNW.

Identical sequences were reported only from the Pacific Northwest [Canada: Campbell River, Vancouver Island, BC (KP406552); BC (EF218807, UDB031028); Sooke Reservoir, BC (UDB031005, UDB031003); U.S.A.: Oregon (HM488501, FJ440932)]. Although *R. salishensis* could perhaps form partnerships with the *Pseudotsuga* species of eastern Eurasia, it has yet to appear among sequence records from that region.

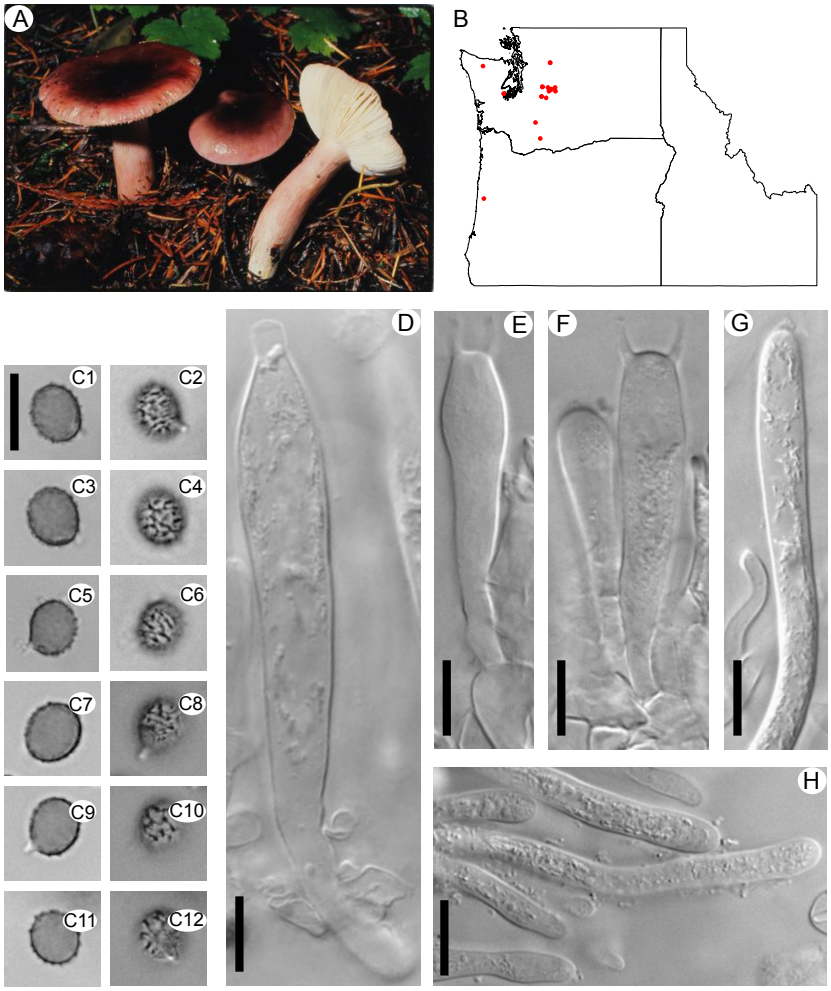

Figure 3.10 Russula salishensis *morphology and map of specimen locations.*

Morphology and specimen distribution of Clade 6 Russula salishensis *(Woo sp. 39). BW followed by numerals designate Ben Woo samples.* ***A,*** *photograph of fresh specimen (BW575).* ***B,*** *Distribution of specimens of the Woo*

collections in Pacific Northwest States. ***C-H,*** *Micromorphology, all 1000x magnification. All scale bars 10 µm.* ***C,*** *Spores in median optical section and surface view in Melzer's reagent (****C****1-****C****2, BW575;* ***C****3-****C****4, BW599;* ***C****5-****C****6, BW721;* ***C****7-****C****8, BW1045;* ***C****9-****C****10, BW1062;* ***C****11-****C****12, BW1062);* ***D,*** *Hymenial cystidium (BW731);* ***E-F,*** *Basidia (BW1045, BW972);* ***G-H,*** *Cap cuticle terminal cells with refringent contents (BW1045, BW713).*

In conclusion, I have described nine new species of *Russula* from the Pacific Northwest. These descriptions include the character variation found in multiple specimens to more reflect the description of a species.

Chapter Four: Large-scale geographic range extents of mushrooms based on available ITS and georeference metadata

Summary

Geographical range extents have important implications for understanding species habitat requirements and patterns of species richness. Here, I analyze the distribution of range extents of two datasets of 2324 and 341 genetically defined species of mushrooms in Agaricomycetes which represent 7.4% of species in this class in the publicly available UNITE fungal database.

In the first analysis, I combined 2912 nuclear ribosomal internal transcribed spacer (ITS) sequences from recent herbarium samples collected in the American Pacific Northwest with records from UNITE, and clustered them in 341 OTUs using 99% sequence identity as a proxy for species boundaries. In the second analysis, I used 15,373 sequences from 12 mushroom genera clustered into 2324 OTUs and 1240 sequences of woody trees clustered in 178 OTUs, all based on 99% identity. I calculated the maximum within-species geographical distance for each OTU. This maximum distance, the 'range extent', served as an estimate of the known geographical distribution of the OTU. I compared the range extents of 2324 OTUs with permuted data within each genus to estimate significance of the distribution of range estimates compared to a random distribution of range extent estimates, and to control for biased sampling. In a third analysis, I compared the range extents of the host tree and mushroom species.

Observed range extents of mushrooms were significantly lower than extents estimated from permuted data. Some of the few taxa with low genetic divergence and high geographic distance between samples may have been transported by humans beyond their natural ranges. Most mushroom species had larger range extents than most species of host trees. If, as this result suggests, most mushroom species are not restricted to single tree species, current estimates based on plant:fungus ratios exaggerate global mushroom diversity, as also predicted by Hawksworth (2001).

Introduction

Fungi play essential roles in terrestrial ecosystems by decaying wood, associating with plant hosts as pathogens, as endophytes (not causing disease), and forming mycorrhizal partnerships. Increased CO_2 and temperatures change rates of wood decay (Allison and Treseder, 2008), increase the susceptibility of plants to fungal infections (Chen et al., 2007), and expand (Pringle et al., 2009) or potentially contract (Ellis et al., 2007) fungal geographical ranges. Elucidating current spatial distributional ranges is an important first step for predicting how key ecosystem services may shift with increasingly changing environments (Peay et al., 2010). Fungal species have long been recognized as difficult to delimit because of their challenging morphology or cryptic nature (Petersen and Hughes, 1999; Taylor et al., 2000); if species are poorly delimited, so are their geographical ranges. The morphology of fungal species is sometimes too simple or too poorly understood for us to distinguish closely related taxa, and as a consequence, in some parts of the world, names applied to mushrooms are frequently incorrect for native species (Bazzicalupo et al., 2017; Harrower et al., 2011; Nilsson et al., 2006; Richard et al., 2015).

Discovery of new taxa and DNA barcoding efforts around the world are challenging our understanding of fungal biogeography (Truong et al., 2017). Even without the use of DNA barcoding, studies of intercontinental biogeographical patterns have flagged the issues associated with morphological identifications (Petersen and Hughes, 2007; Wu and Mueller, 1997). Using DNA markers and phylogenetic inference, genetic diversity estimates from increasing numbers of studies have been revealing multiple species where only a single species had been recognized previously. After dense sampling unveiled several independent lineages comprising what were previously thought to be single species in *Flammulina*, *Amanita*, and *Cantharellus*, biogeographical history could then be re-investigated (Anderson et al., 1980; Dunham et al., 2003; Geml et al., 2006; Geml et al., 2008; Hughes et al., 1999). These studies consistently found little evidence for pan-continental distributions. Similarly, studies from restricted geographical areas have shown fewer species to be shared across continents than had previously been assumed in genera such as *Cortinarius*, *Russula* (see Chapter 2), *Morchella*, *Laccaria*, and *Hebeloma* (Bazzicalupo et al., 2017; Geml, 2011; Geml et al.,

2012; Grilli et al., 2016; Harrower et al., 2011; Meiser et al., 2014; Mueller et al., 2001; Richard et al., 2015; Tedersoo et al., 2014; Wilson et al., 2017). In contrast, invasive species, often associated with humans, show very low genetic diversity and geographic distributions that span over continents (Schwartz et al., 2006; Vellinga et al., 2009). Among them, the deadly *Amanita phalloides* is the most notorious example (Pringle et al., 2009; Wolfe et al., 2010).

In this study, I used genetic data and geographical metadata of mushroom specimens in publicly available databases representing a broad sampling of geographical distributions of mushroom species. Available data included operational Taxonomic Units (OTUs) defined using a cutoff of 99% identical fungal nuclear ribosomal internal transcribed spacer region (ITS) sequences in the UNITE database, which served as a proxy for clades of individuals that are at approximately the level of species (Abarenkov et al., 2010a; Kõljalg et al., 2005; Nilsson et al., 2008). As a genetic marker, the ITS fungal DNA barcode (Schoch et al., 2012) is widely used in fungal taxonomy, systematics, and biogeography studies (Nilsson et al., 2011; Tedersoo, 2017; Tedersoo et al., 2014). The UNITE database takes all publicly available fungal ITS data, clustering them into OTUs (='Species Hypotheses'). Pre-calculated clusters can then be selected from among the ~89,000 UNITE OTUs at the 99% identity level (available < https://unite.ut.ee/index.php> 07 December, 2017). As an estimate of species geographical range extent, I selected the maximum from among the linear distances between collection localities of pairs of individuals of the same OTU (Gaston, 1996). Having access to a large number and a wide sampling of fungal OTUs with geographical metadata provided an opportunity to test the statistical support for general patterns of geographical distributions in mushroom-forming fungi.

In these analyses, I used ITS data from two fungal and one set of tree samples. In the first analysis, I asked how frequently the range extents of mushroom OTUs found in the American Pacific Northwest extended across continents and oceans. I took advantage of recent herbarium specimens from UBC Beaty Museum and University of Washington Burke Museum (WTU) collections and of recent studies done on Pacific Northwest mushrooms. As a second analysis, I evaluated the range extents of OTUs defined in UNITE by 99% cutoff representing worldwide collections of 12 genera of mushrooms.

For comparison with observed data in this second analysis, I modelled the null distribution of OTUs in the absence of geographical structure by performing permutations. For the third analysis, as a basis for comparison with the mushrooms, I downloaded ITS sequences and metadata from GenBank and calculated the range extents for species from seven genera of host trees. Because trees are associated with mushrooms as mycorrhizal and wood-decay hosts, this comparison seemed potentially biologically relevant.

I aimed to address three main questions: (i) To what extent are mushrooms of the American Pacific Northwest endemic to the western half of the continent? (ii) What are the average range extents of species for 12 mushroom-forming fungal genera? (iii) Do the range extents of host tree species predict the range extents of mushroom forming fungi?

Methods

Analysis 1: Pacific Northwest mushrooms ITS sequences

To test the proportion of species of mushrooms found in the Pacific Northwest that are known from outside western N. America, I analyzed a combination of new and pre-existing sequences of herbarium samples. New sequences included a sample of 960 UBC Beaty Museum mushroom specimens, representing 289 species from 75 genera. DNA extraction was performed as in Chapter 2 (Bazzicalupo et al., 2017). Amplification and sequencing was performed by the Canada Genome Cancer Centre in Vancouver BC. For the amplification I used primers ITS1F and ITS4 (White et al., 1990). I processed the sequences using BLAST searches of GenBank, and used the UNITE (Abarenkov et al., 2010a) databases to apply names to the specimens. After manual editing and cleaning of the sequences, 604 ITS sequences were used in the subsequent analyses, a ~63% sequencing success rate. I deposited the sequences in GenBank (MF954608-MF955211). Pre-existing data included 988 sequences of *Cortinarius* (AF335446, AY228343, AY228359, DQ384589, DQ384593, DQ481670-DQ481864, EF530931, EF530945, EU057080, EU057087, EU057089-EU057091, EU057093, EU057094-EU057097, EU057109, EU057110, EU057122, EU057124, EU486445, EU486455, EU486459, EU821651-EU821697, FJ039534-FJ039578, FJ039581-FJ039635, FJ039637-FJ039685, FJ039692-FJ039710, FJ152499-FJ152503, FJ152506-FJ152513, FJ152515-FJ152517,

FJ157001-FJ157062, FJ157064-FJ157127, FJ157129-FJ157132, FJ157134-FJ157147, FJ627024, FJ717495-FJ717521, FJ717523-FJ717563, FJ717565-FJ717574, FJ717576-FJ717580, FJ717582-FJ717605, GQ159762-GQ159789, GQ159791-GQ159918, GQ159920-GQ159921, HM068559-HM068562, HM240522-HM240523, HQ604641-HQ604739, JN976979-JN976990, KC581329-KC581330, KC581333, KC581349, KJ019014-KJ019015, KJ146703-KJ146705, KP454013, KP454023); 607 *Inocybe* sequences (HQ604068-HQ604640, HQ604751, HQ604776-HQ604787, HQ604794, HQ604803-HQ604822); and 713 *Russula* sequences (KX812903–KX813614, MF457916, from Chapter 2). To analyze the geographical distribution of the OTUs including BC collections, I used the UNITE database of fungal ITS sequences. From this, I recovered all 13,211 sequence records from 522 OTUs that matched our 2912 sequences at a 99% similarity cut-off. I excluded the 181 OTUs composed only of one sequence, as their range extent would inevitably be '0', and used the remaining 341 OTUs.

Analysis 2: ITS sequences from 12 mushroom genera

I sampled UNITE data for 12 well-studied genera of mushroom-forming agaric fungi: *Amanita*, *Agaricus*, *Cortinarius*, *Galerina*, *Hebeloma*, *Hydnum*, *Hygrocybe*, *Hygrophorus*, *Inocybe*, *Lepiota*, *Pholiota*, and *Russula+Lactarius*. For these genera, I had 17,509 sequences, representing 4096 OTUs at the 99% similarity cut-off (Garnica et al., 2016). I removed 1772 singleton OTUs and analyzed the remaining 15,373 ITS sequences representing 2324 OTUs. Although the correspondence between ITS sequence similarity and species delimitation probably varies by clade and may be inconsistent even among closely related species, I began with OTUs defined by a cutoff of 99% similarity following (Garnica et al., 2016). I also analyzed geographical distributions of OTUs at 98% and 97% cutoffs. I also downloaded country of provenance information for specimen sequences from UNITE.

Analysis 3: Host tree taxa

For biologically relevant comparisons to the estimates of range extents of mushrooms, I used ITS sequences for seven genera of long-lived, woody trees (*Abies*, *Betula*, *Fagus*, *Pinus*, *Quercus*, *Salix*, and *Tsuga*). I downloaded 1240 ITS sequences with country information from GenBank. I aligned the genera individually with mafft

(Katoh and Standley, 2013) and manually edited the alignments in Mesquite v.3.01 (Maddison and Maddison, 2015). By using the software mothur (Schloss et al., 2009), I delimited 178 clusters at 99% similarity. I analyzed the tree clusters like the mushroom clusters as described in the following sections except we did not run permutation tests on the tree data.

Range extent of OTUs

I estimated the range extent in each OTU as the maximum distance from all the pairwise geographic distances among collections following Gaston (1996). I extracted the maximum distance between all possible pairs of specimen geographical coordinates within a UNITE-defined OTU (Fig 4.1, Appendix 3.1). Detailed geographic coordinates were only present for 10% of the samples in the UNITE data. For a more complete dataset, I assigned each sequence record the coordinates of its country's centroid coordinate from https://developers.google.com/public-data/docs/canonical/countries_csv. Range extent was calculated by using the Vincenty equation implemented in the GeoPy package v.1.10.0 in Python 2.7 to find the shortest distance between geographical coordinates, assuming the Earth is a sphere bulging in the middle. The Vincenty calculation was used for all four datasets: Pacific Northwest mushrooms, mushroom genera, mushroom genera permutation tests, and host trees.

Sampling of mushroom ITS barcodes is far from uniform across the world. I plotted the number of samples against their range extent to explore the relationship between number of collections of an OTU and range extent. I expected a relationship for two reasons. For OTUs with narrow geographic ranges, the probability of being represented by multiple barcoded collections was likely lower than for OTUs with wide geographical ranges. Secondly, the probability of capturing the entire OTU range extent likely increased with increased sampling. I tested the effects of removing OTUs sampled four or fewer times or 30 or more times on estimates of range extent. Although limited by the lack of a world-wide random sampling of mushrooms, this study draws on what may be the most extensive fungal barcode dataset to date. The sampling was wide; even countries like the United States and Estonia that have the highest sampling densities each contributed ~1% or less of the overall dataset of the 12 genera (Appendix 3.2).

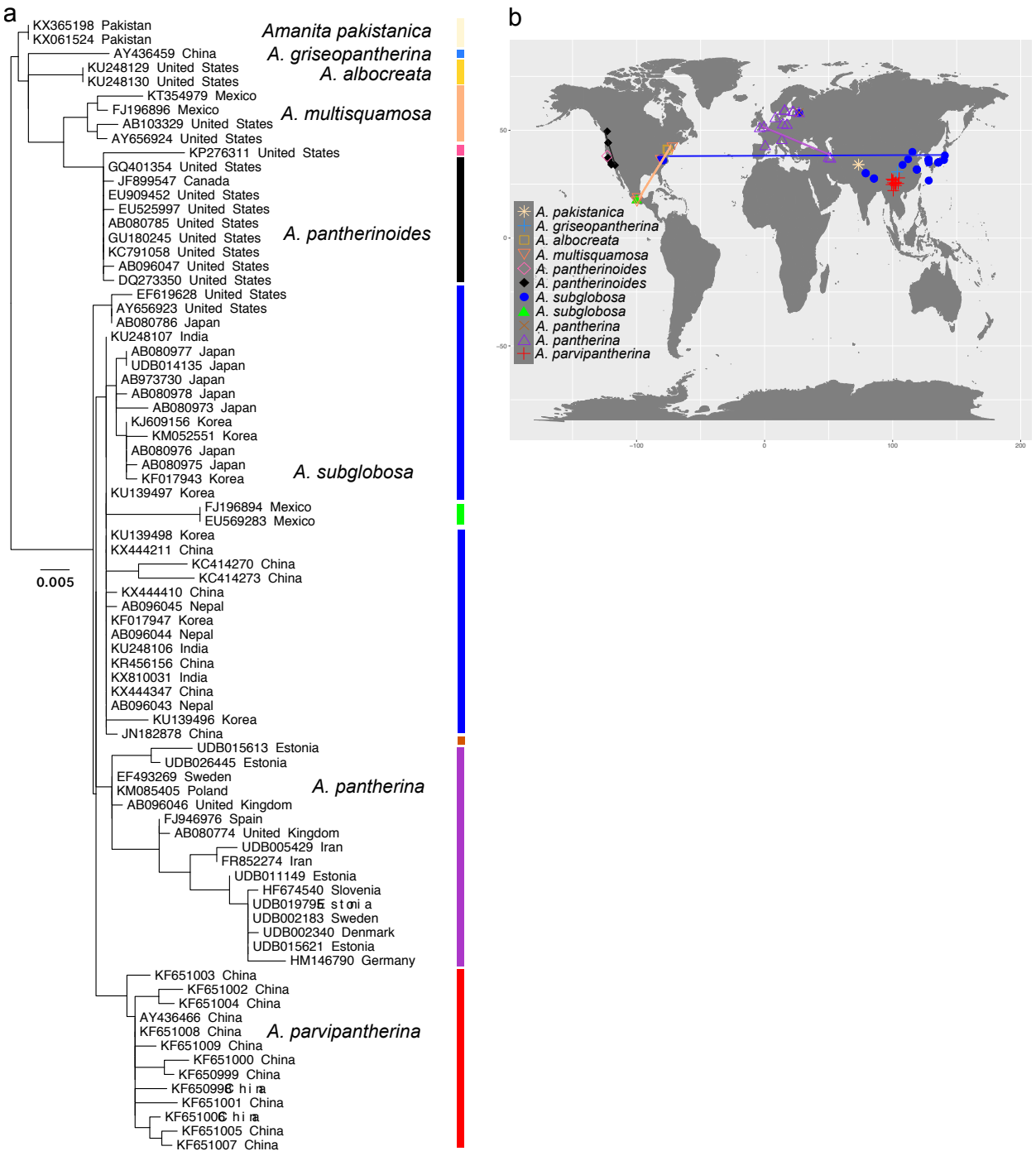

Figure 4.1Panther Amanitas range extents.

(a) Phylogeny and matching OTUs of samples for the 'Panther' Amanita *species complex and (b) the geographic distribution of the samples colour-coded based on their Species Hypothesis. Lines represent the range extent, the maximum recorded distance within an OTU. See Figure S1 to see how the range extent of the clusters changes with more inclusive clusters in the 'Panther'* Amanita.

To explore the effects of lack of geographical precision in the metadata, I compared the range extent within OTUs based on country centroids vs geographical coordinates for the Panther Amanitas dataset from Fig 4.1. Panther amanitas are well-sampled and distinctive mushrooms, making them a good example for my analysis. I plotted the range extent of the 'Panther' *Amanita* OTUs in Appendix 3.3 for comparison. For this group of species, I found no significant difference between centroids and geographical coordinates on range size estimates based on a Kolmogorov-Smirnov test (data not shown). Although we found no difference between the country centroid and precise data, using country centroid data systematically increases the frequency of 'zero' maximum distances for larger countries such as the United States, Canada, China, and the Russian Federation. The Russian Federation represents ~0.3% of the total data.

Mushroom species travel at different rates and by different modes (Golan and Pringle, 2017). Species that migrated unusually quickly, possibly due to transport by humans would be expected to show a high range extent and low genetic diversity. To identify OTUs that may have been dispersed by humans, I plotted each range extent against the average genetic distance within each OTU. I calculated genetic distances for each group of sequences in an OTU using the PyCogent package v.1.5.3 (Knight et al., 2007) with the GTR model of evolution. I extracted the average genetic distance from each genetic distance matrix.

Permutation tests of observed data and random geographical data set up a null expectation for range extents

To test whether the observed range extents were significantly different from a null expectation of range extents if the OTUs showed no geographical structure, I first re-sampled the geographical coordinates of the observed data of 12 mushroom genera OTUs, and secondly, I randomly assigned geographical coordinates of country centroids to the 12 mushroom genera OTUs. I sampled with replacement so that observations of samples were independent of one another (Appendix 3.4). Code to carry out the permutations was written using the python 2.7 package NumPy v.1.14.0 (van der Walt et al., 2011).

To re-sample the geographical coordinates, in each permutation, each sequence record in a genus was assigned random coordinates from the set of observed coordinates within the genus (Appendix 3.4). In each of the 100 replicates, frequency of representation of each country was approximately proportional to its observed frequency in sequence records in the genus. Evidence of geographical structure remaining after the first permutation test could stem from bias in global geographical sampling and it is likely, for example, that coordinates from 'N. America' and 'Europe' were heavily represented.

Secondly, I re-sampled from a pool of country centroids to strip the dataset of structure that may have resulted from the frequency distribution of the real geographical coordinates (Appendix 3.4). I re-assigned each OTU to the centroid of a country randomly drawn from among all of the world's countries. I again used 100 replicates and then calculated the maximum within-OTU range extent.

I then compared the range extent of 2324 mushroom OTUs from the real data with the range extents simulated from the two permutation tests, as well as with the range extents of the 178 tree clusters. I show the results as quantiles of the frequency distributions. To test the probability that real range extent distributions differed from permutated distributions, I applied Wilcoxon rank sum tests. All figures were produced in R (R Core Team, 2014), with the ggplot2 (Wickham, 2009) and ggridges (https://CRAN.R-project.org/package=ggridges) packages.

Results

Regional endemics predominated among American Pacific Northwest and worldwide mushrooms

The OTUs barcoded in the Pacific Northwest were generally not represented by sequences from anywhere else in the world, consistent with restricted geographical distributions of mushroom-forming fungi. Of the 341 Pacific Northwest OTUs, the known range extents of 189 were from 0-2,000 km (median = 0 km). This was so even when OTUs were re-defined to be more inclusive by changing the cutoff from 99% ITS sequence identity to 98% or 97% (Fig 4.2, Appendix 3.5). Across OTUs from all 12 genera, 1696 out of 2324 OTUs showed range extents below 4000 km, and 1277 OTUs

showed range extents below 2000 km (Fig 4.3). Considering the world-wide set of 2324 OTUs from the 12 mushroom genera, making the OTUs within the 12 genera more inclusive had little effect on the range extents (Appendix 3.6). Among the 12 genera, overall median range extents values were for 99% identity: 1431 km, 98% identity: 1831 km, and 97% identity: 2073 km (Fig. 4.3a, Appendix 3.6, 3.7). I found no difference in geographical range extent patterns between the mycorrhizal and saprotrophic genera. Appendices 3.8-3.19 in Supporting Information show the same pattern across genera while comparing real data and permutations at three sequence similarity cut-offs. Ranges of OTUs of *Amanita*, which are comparatively well documented show the same pattern (Appendix 3.9) as OTUs in genera that are species rich and difficult to identify, such as *Inocybe, Cortinarius* and *Russula* (Appendices 3.10, 3.14, 3.19). For comparison, the range extents of the 178 OTUs of seven genera of trees showed a much lower range extent median value (0 km) than the mushroom genera (1431 km) (Fig 4.3d, Appendix 3.7).

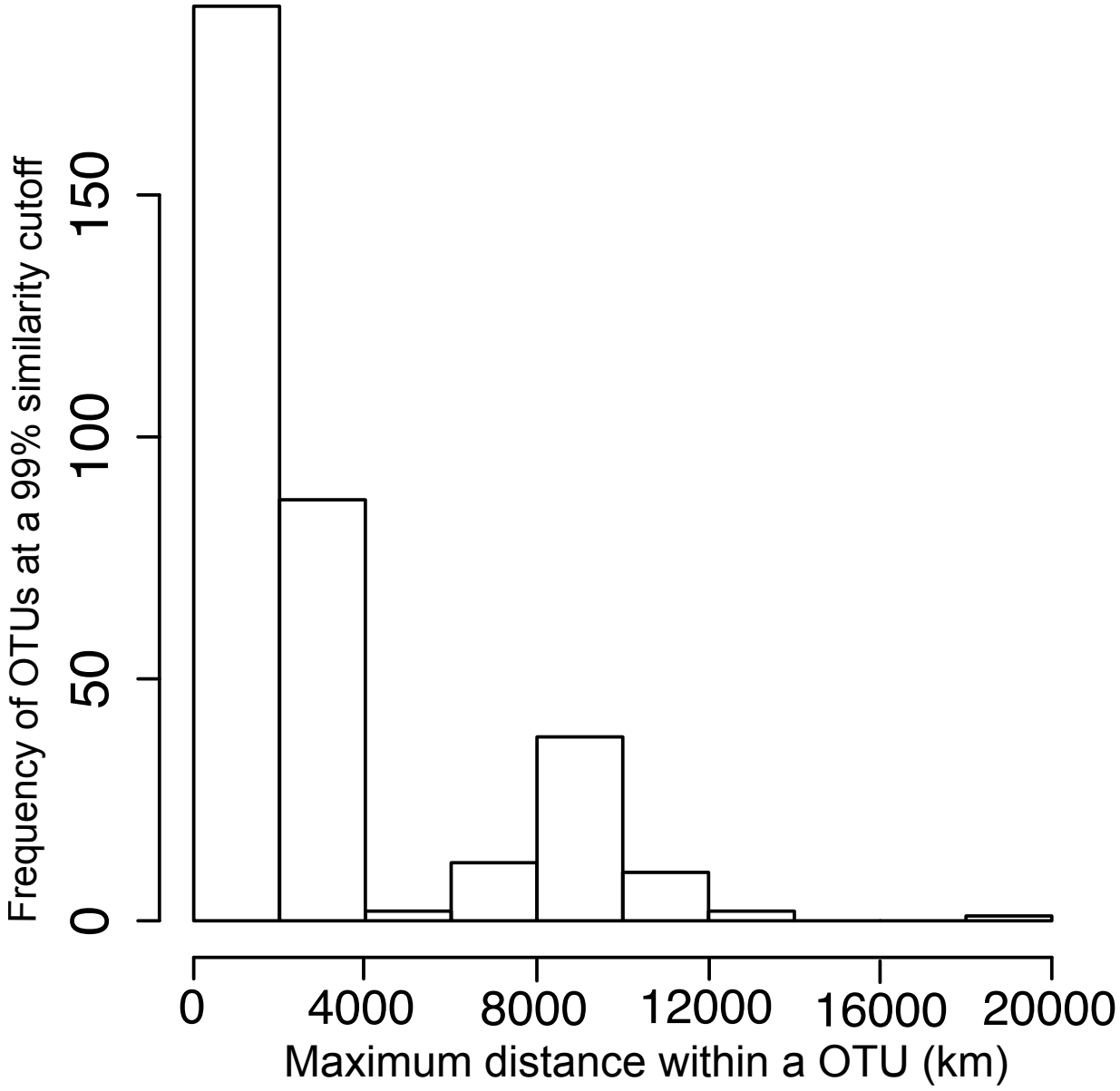

Figure 4.2 Pacific Northwest mushrooms range extent frequency.

Regional endemics predominated among mushroom-forming OTUs from the American Pacific Northwest. ***Frequency of range extent (km) of the 341 ITS-based OTUs collected from the N. American Pacific Northwest (99% similarity). For reference, Baltimore (U.S. East Coast) is ~4500 km from San Francisco (U.S. West Coast); New York, U.S. is ~5800 km from Paris, France; and Vancouver, Canada is ~7500 km from Tokyo, Japan; 20,000 km is approximately half the circumference of the earth.***

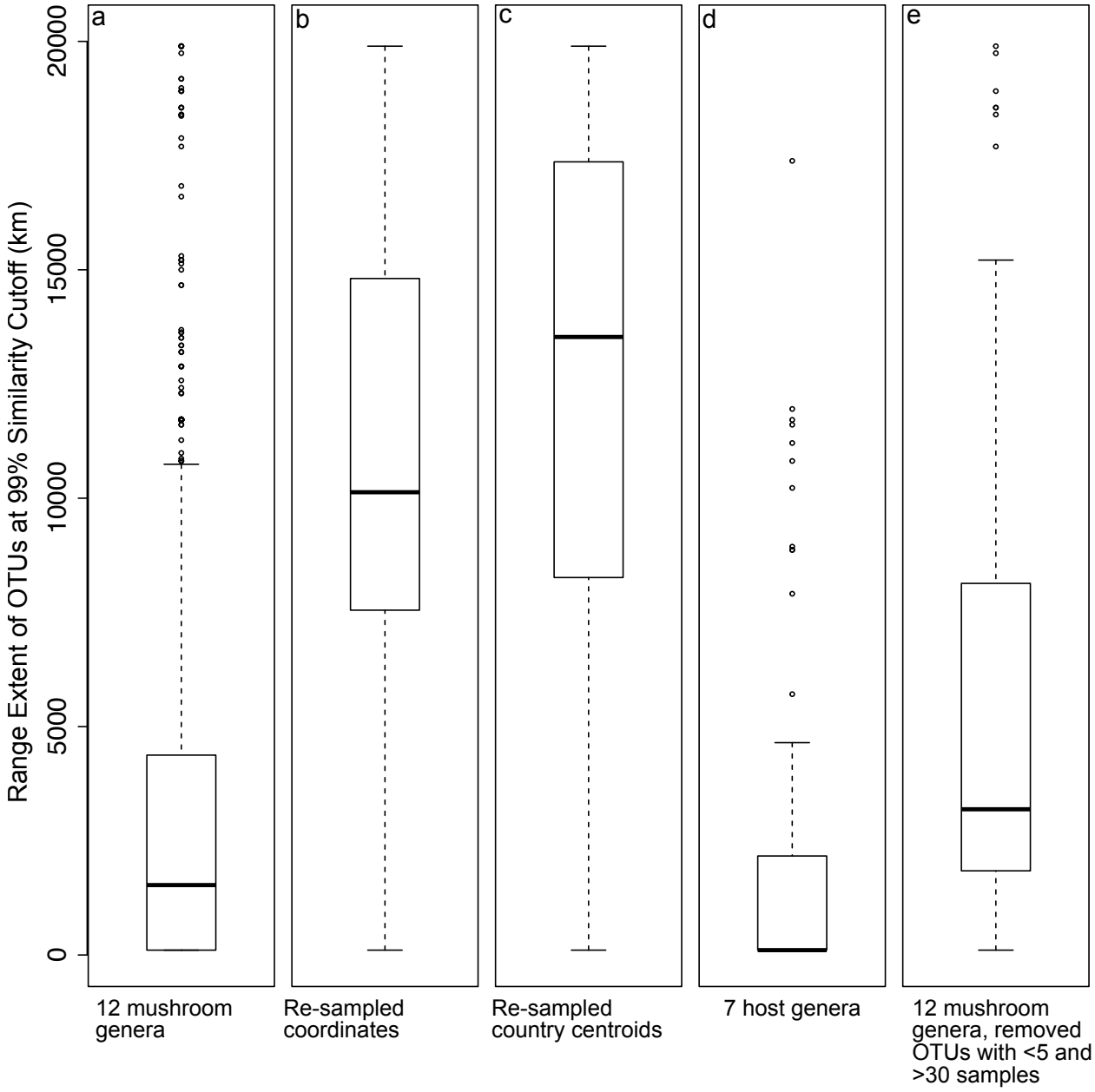

Figure 4.3 Mushroom and tree OTUs range extent boxplots.

Observed range extents of mushroom-forming OTUs differed significantly from randomized range extents. Boxplots show distributions of range extents (km) from worldwide collections of 2324 OTUs (99% similarity) from 12 genera. Observed data (a), the two simulations re-sampled coordinates (b) and re-sampled country centroids (c), host tree genera (d), and the observed data excluding OTUs composed of more than 30 samples and less than 5 samples (e). The solid black line is the median value. The box indicates the interquartile range. Whiskers are outside 1.5 times the interquartile range above the upper quartile and below the lower quartile.

Among the OTUs, a few did have broad range extents (Fig 4.4). Ten OTUs stood out as potentially transported by humans (circled in red in Fig 4.4). They were characterized by high range extent (>15,000 km) and a relatively low average genetic distance in the ITS (≤0.005).

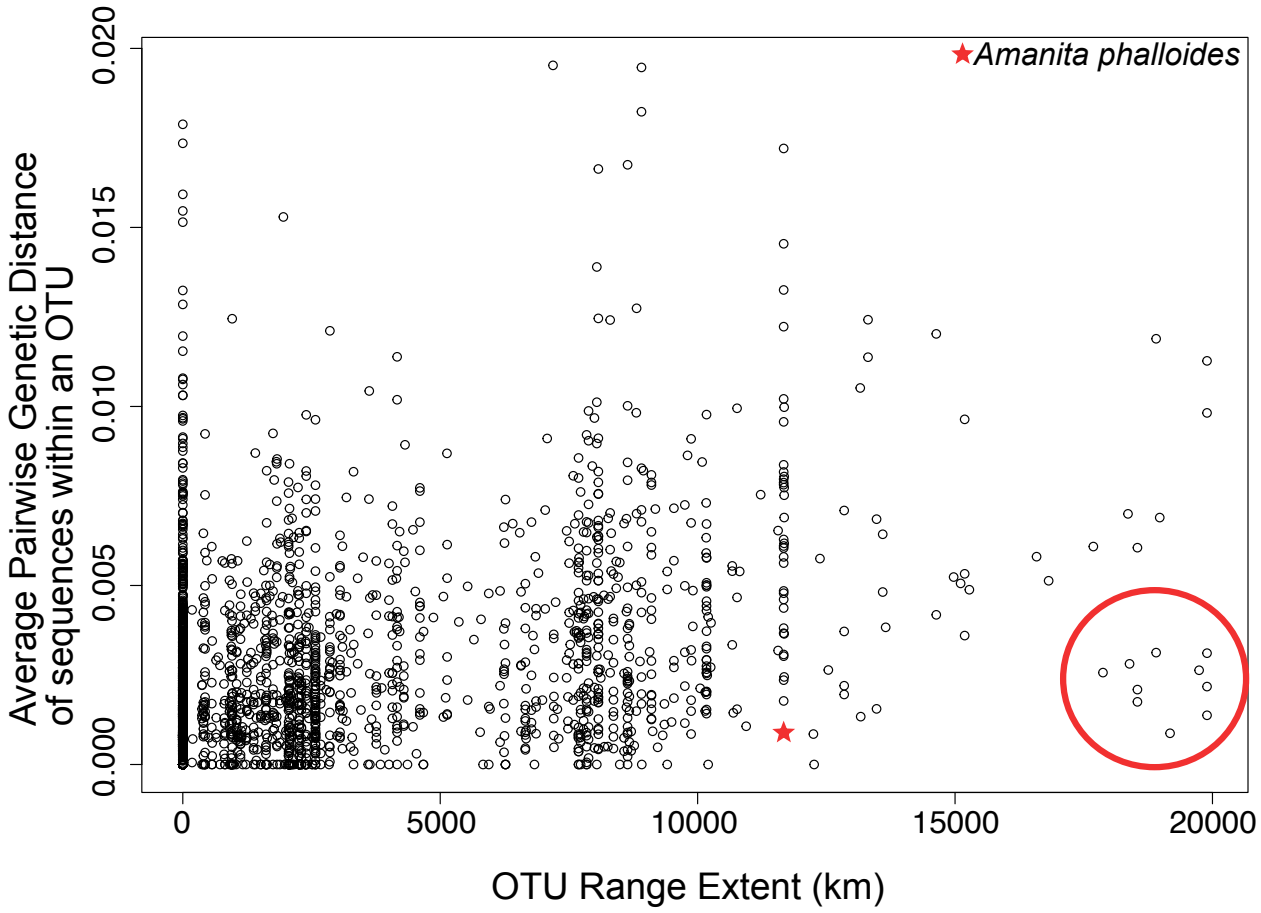

Figure 4.4 Range extent against ITS pairwise distance of OTUs.

Range extent plotted against average genetic distance for mushroom OTUs. Circled in red are 10 OTUs that have a large range extent (>15,000 km) and a relatively low average genetic distance in the ITS (≤0.005). The red star is the Amanita phalloides *OTU.*

Observed range extents differed significantly from randomized range extents

After geographical coordinates were randomized with respect to samples in each of the 12 genera, fewer OTUs had maximum range extents from 0-4000 km but many

more had extents between ~5,000-10,000 km (Fig 4.3b, Appendix 3.7). A non-parametric Wilcoxon rank sum test showed that the real data (Fig 4.3a) were significantly different from the permutated data (Fig. 4.3b, Appendix 3.20). Using the re-sampled country centroids permutation test to remove the effect of geographical bias in sampling effort resulted in significantly higher range extents (Fig. 4.3c) compared to the real data (Fig 4.3a). Compared with the re-sampled coordinates permutation test, fewer OTU pairs had range extents between 5,000 and 10,000 km in the re-sampled country centroid comparison (Appendix 3.7).

Frequency distributions of geographical distances from the two permutation tests were consistent even when OTUs were defined by more inclusive 98% or 97% identity cutoffs (Appendix 3.20, 3.21, 3.22). The real data were again significantly different than either permutation test. Each of the 100 permutation replicates in each test gave similar results (Appendix 3.20, 3.21).

Mushroom range extent vs. number of samples in OTUs

I used the 2324 OTUs from the 12 genera dataset to explore the relationship between number of collections and range extent. Consistent with expectations, the number of collections per OTU was positively correlated with the maximum range extent (Appendix 3.23) in the OTU and slopes were significant in all analyses described below ($p \leq 0.0001$). Depending on which OTUs were included, the R^2 values suggested that number of collections per OTU could explain up to 30% of the variation in range extent. Including all OTUs gave an adjusted R^2=0.24 (Appendix 3.23). The slope became less steep after excluding all OTUs with more than 50 sequences each (1% of the whole data) adjusted R^2=0.30, or all OTUs with more than 30 sequences (5% of the data) adjusted R^2=0.27. To test if the positive slope was due to the many sparsely collected species as well as the abundantly collected ones, I excluded all OTUs represented by fewer than five collections or more than 30 collections. With the remaining 844 OTUs, the adjusted R^2 was lower (0.16, Appendix 3.24a) but the slope was still positive and significant. Even after excluding 1480 OTUs with fewer than five or more than 30 collections, a frequency histogram of 844 OTUs still showed that most OTUs had restricted range extents

(Appendix 3.24b) and the Wilcoxon rank sum test showed that the distribution was still different from the permutated distributions (Fig 4.3).

Although I fit a line to the relationship between number of samples and range extent, the highest density of plotted points formed two clouds along the y-axis (Appendix 3.23, 3.24a). The 0-2000 km cloud was consistent with the many OTUs with ranges within Europe or within N. America. The cloud centred around 8000-10,000 km was consistent with OTUs known from both Europe and N. America. Intermediate ranges would have been limited by a combination of oceans and patterns of world-wide sampling.

Discussion

Mushroom vs. tree species range extents

Knowing what controls fungal distributions is fundamental to understanding fungal ecology and assessing global fungal diversity (Peay et al., 2010). The median range extent for the mushroom genera was 1431 km compared to the median value of 0 km for the host trees. Permutation tests showed that the known range extents of mushrooms were neither random nor global. However, similar analyses showed that most host tree species have even smaller geographical ranges (median = 0). These numbers mean that most species of mushroom forming fungi are not restricted to single species of trees. Hawksworth (2001) used observed ratios of fungi per host plant to estimate global numbers of undescribed fungi to be "at least 1.5, but probably as many as 3 million", while acknowledging that the degree of specificity of fungi to host is critical for the estimates. Here, I only considered a subset of mushroom-forming fungi, not the entire range of kinds of fungi that may have different levels of host specificity. Tedersoo et al. (2014) found from their global analysis, that fungal diversity is currently overestimated by 1.5-2.5 times, supporting this study and also suggesting that considerations based on ratio of fungi to tree species would overestimate the number of undescribed mushroom-forming fungi. As climatic change response efforts depend also on identities of both plant and fungal species (Van der Putten et al., 2010), the more limited than expected specificity to host species shown in this thesis should be considered in predictions of fungal ecosystem function and response to changing climate.

Limitation and expansion in fungal range extents

Previous broad studies of fungal communities found few shared OTUs across the world (Meiser et al., 2014; Tedersoo et al., 2014). Similarly, more narrowly focused systematic studies have generally shown a surprising number of cryptic species with restricted geographical distributions (Geml et al., 2012; Geml et al., 2008; Hughes et al., 1999; James and Vilgalys, 2001; Wilson et al., 2017), and this thesis Chapter 2. Known mushroom dispersal extent measurements are comparable to these findings as summarized in Appendix 3.25 (Vincenot and Selosse, 2017).

While in this analysis the contribution of dispersal limitation is unknown, dispersal ability is thought to be important in the distribution of fungi (Golan and Pringle, 2017). Dispersal limitation was shown to be involved in structuring communities of mycorrhizal fungi (Peay et al., 2012). Sampling *Russula brevipes*, Bergemann et al. (2006) found no geographical structure to three populations separated by several kilometres. Most evidence for successful long-distance dispersal among fungi comes from plant pathogens. Outside the mushroom-forming fungi, spores of *Cladosporium*, *Alternaria*, and rust urediniospores were roughly equally common in air sampled from over the North America continent and the Pacific Ocean (Holzapfel, 1978). Plant pathogens recorded as having dispersed more than 500 km included sugarcane rust, wheat stem rust, and coffee leaf rust, which were probably aerially dispersed across continents, and potato late blight and wheat yellow rust, which were transported in infected plant material. Relatively few data are available documenting dispersal on the scale of thousands of kilometers but pathogens (fungal and other) have been reported to travel from Australia to New Zealand, about a 2000 km distance (Close et al., 1978). White pine blister rust urediniospores survive and are able to germinate after air transport of 500 km in jet streams (Dalton et al., 2010). Among fungi, dramatic range expansions resulted when uniformly susceptible host populations were available to feed the white pine blister rust and the chestnut blight fungi introduced by humans (Brown and Hovmøller, 2002).

The range expansions of plant pathogens show that the ability to establish, persist and spread contribute to determining range extent. As for other multicellular eukaryotes, barriers to establishment or persistence may contribute to restricting the biogeographical

zones of mushroom OTUs much as they do for other land plants and animals (Cox, 2001). Gaston (1996) showed that a high frequency of species expected to have a restricted range extent. Ecological determinants that would limit establishment such as habitat/host availability and, environmental tolerances are a few of the many interacting processes that would result in a signal of limited range size of species (Gaston, 1996). Similar factors likely affect ranges in some mushroom-forming fungi and environmental conditions and were used to predict distributions of mushrooms in Norway (Wollan et al., 2008).

Among fungi, the dichotomy between saprotrophic and mycorrhizal taxa is a fundamental difference in mode of nutrition. The distribution of a fungus has been shown to be linked to the availability of its host in mycorrhizal taxa associated with alder trees (Põlme et al., 2013). Although I found the range extents among mushrooms and trees to be comparable, I detected no difference between the range extents of mycorrhizal and saprotrophic mushroom genera. This was unexpected, given that Sato et al. (2012) modelled host, environment, and probability of detection to predict that ranges of mycorrhizal taxa would be limited by host availability and therefore more restricted than ranges of saprotrophs. These results raise the possibility that empirical data might not fit the Sato et al. (2012) model. However, it is also possible that the model of Sato et al. (2012) is correct and the difference between ranges of mycorrhizal vs saprotrophic taxa would be evident with more precise geographical sampling than we were able achieve using estimates from country centroids.

Considering crop pathogens, human dispersal of hosts may have been important in the distribution of the mushroom-forming OTUs with range extents approaching the maximum of 20,000 km or half way around the world. Of ten OTUs that had high range extents (>15,000 km) and a relatively low average genetic distance for ITS (≤0.005), two showed some geographical structure at a lower cutoff: *Lactarius deliciosus* (SH132494.07FU) found in Europe and China; and *Cortinarius* sp. (SH094290.07FU) always found under *Alnus* in Europe, South America, East Asia, Western North America. These species may have dispersed prior to human intervention. Humans have however transported fungi when exporting plants, and some of the widespread OTUs have previously been flagged as possible introductions of widely dispersed mycorrhizal fungi

(Vellinga et al., 2009). Two OTUs were known as widely distributed, introduced species: *Amanita muscaria* (SH082297.07FU) (Geml et al., 2006) and the cultivated edible, *Agaricus bisporus* (SH077010.07FU). Six of the OTUs had a European or European/North American distribution with one to two samples collected in New Zealand under *Pinus radiata* and *Pinus contorta*, tree species introduced from western North America. The six were: *Inocybe sidonia* (SH079481.07FU), *Hebeloma sacchariolens* (SH127585.07FU), *Cortinarius saniosus* (SH094389.07FU), *Inocybe pseudorubens* (SH083303.07FU), *Russula amoenolens* (SH133387.07FU), *Hebeloma hiemale* (SH127571.07FU). The co-invasion of the pine with its mycorrhizal fungi has been thoroughly documented in New Zealand and suggested as a form of invasion meltdown, where co-invasive species will more quickly impact native ecosystems through their interaction (Dickie et al., 2010; Simberloff and Von Holle, 1999).

In this study, I am unable to distinguish mushroom OTUs that were introduced by humans from indigenous OTUs, and I cannot estimate how many of the wider distributions of OTUs were because of accidental introductions. Such accidental introductions may inflate fungal range extents relative to tree ranges. Pyšek et al. (2017) estimated that in temperate regions about 25% of plants are invasive due to human intervention. From the quantile breakdown of the mushroom range extents (Appendix 3.22), the upper 25% are ~4500 km ranges and higher. This suggests that if I were able discern and remove the mushrooms collected outside their natural range, I might recover range extents more closely resembling their host trees. In two examples, I found many collections in Europe under native trees, and at a ~5,000 km distance I found one specimen collected under a non-native tree. Of 30 samples found in total of European *Russula sardonia* (SH091396.07FU), one (EU557320) was collected under pine in Argentina. Of the 13 samples of the European *Amanita excelsa* (SH133634.07FU), one (EF031124) was collected under *Pinus patula* in South Africa. This pattern supports the finding in the fungal pathogen *Sclerotinia*, where mitochondrial DNA haplotypes were shown to have narrow distributions in wild populations and wide geographical ranges when associated with canola crops (Kohli and Kohn, 1996).

Are regionally endemic species rarely collected? Are widely collected species rarely regionally endemic?

I found that range extent was positively correlated with the number of samples for an OTU, and various factors likely contributed to this trend. First, under-sampling almost certainly played a role, and some OTUs currently known only as narrow endemics undoubtedly have larger range extents that could be detected by more sampling. Lack of sampling is especially likely in genera such as *Cortinarius*, *Inocybe* and *Galerina*, which have small, drab mushrooms that are difficult to identify. Excluding the OTUs represented by four or fewer collections did increase the average range extent, although median extent was still far lower than permutated samples. While range extents of many OTUs will expand with better sampling, I predict an even larger increase in the number of new species that will be discovered to have well-characterized, restricted ranges.

Secondly, species with narrow geographical ranges may have an inherently lower probability of being sampled, compared with species with broad distributions. In many groups of organisms including in birds where sampling density is good, range size is correlated with abundance (Gaston et al., 2000). If a similar relationship holds in mushroom-forming fungi, species with narrow ranges might also be uncommon within their ranges while species with wide ranges might be abundant across their ranges. Even if fungal species with narrow ranges are just as abundant locally as species with wide ranges, the distribution of researchers looking for fungi is sparse and multiple records of the same species are much more likely for wide ranging fungi that overlap the territories of several mycologists.

If sampling intensity were the main factor in restricted range distributions, then obvious and well-sampled taxa should have broader ranges (Gaston et al., 2000). Large, colourful, edible or poisonous mushroom species that are more easily placed in their taxonomic neighborhood with field characters often have extensive specimen collections. Species of the genus *Amanita* are among the best sampled across the world because on top of being large and flashy, humans take an interest in their other characteristics like the culinary appeal of Cesar's Amanita (*A. cesarea*), the toxicity of the Panther Amanita (*A. pantherina*), and the iconic, story-book appearance of the Fly Agaric (*A. muscaria*). These morphologically defined groups have been shown to be composed of multiple

species, which have restricted range extents. Their collections are dense enough and their morphology so distinctive, that I do not expect them to show world-wide distributions, even with increased, systematic sampling.

Improved understanding of geographical distributions of species still requires improved taxon sampling, better species delimitation, and precise and accurate geographical coordinates for samples. Taxon sampling is particularly important. Systematic sampling and records of absences as well as presence would improve the accuracy of estimates of species overlap across their ranges. Absence data will be difficult to obtain by sampling mushrooms because their mycelia persist unseen underground, often for many years, before fruiting. This analysis of 2324 OTUs from 12 genera did include metagenomic data from environmental samples, which can better detect hidden fungal mycelia. However, meaningful estimates of presence/absence will still require extensive sampling over time and space.

Conclusion

This analysis suggests that mushroom-forming agaric fungi have wider geographical ranges than host tree species. It is possible that mushroom specimens that have been moved and recorded outside of their natural range are resulting in an overestimate of range extents. In order to better address fungal geographic ranges and consequences to diversity, it is important to be able to identify mushrooms that are outside of their natural range, although it might be a difficult task as historical records of fungi are sparse and humans have become more efficient and effective as vectors of introduced species, or as native habitats degrade.

Chapter Five: Conclusions

Delimitation of species has important implications for understanding the biology of fungi, including for the management of fungal pathogens and conservation of fungal diversity. The correct circumscription of a species can lead to improved precision in estimates of geographical distributions and habitat requirements. Recent systematic studies of mushroom-forming fungi have tended to reveal that historical species concepts were too broad. For example, Geml et al. (2006) showed cryptic speciation among three Fly Agaric species that had all been lumped in *Amanita muscaria*. The geographical range extents of many species including *A. muscaria* have been exaggerated due to the application of overly broad species concepts. The segregate species in *A. muscaria* have restricted geographical ranges but pooling them all, their distribution appeared global. Biologically relevant species delimitations are a prerequisite for establishing not only species-specific geographical ranges but also for more accurately characterizing symbiotic interactions and habitat requirements.

Strengths and significance

While previous research has repeatedly revealed problems with fungal species delimitations, my approach has been broad, allowing me to generalize about patterns of diversity within and across genera. I systematically barcoded ~700 *Russula* collections and 604 collections of fungi representing 12 additional large genera of mushrooms by sequencing their internal transcribed spacer DNA regions. I added ~18,000 barcode sequences to my analyses, and then used automatic species delimitation to develop narrower, genetically based species concepts for the species-rich fungal genera.

For analysis of species in *Russula*, I was able to analyze morphology in parallel to phylogeny. This was possible because I had access to the Woo herbarium collection with its unusually detailed notes on morphology of freshly collected specimens. Studies of mushroom systematics draw on observations of fresh specimens where possible, but mushrooms fruit irregularly, and a single mycelium (=fungal individual) often fruits only once every several years. This means that systematic studies typically draw heavily on

herbarium specimens collected over decades, which better capture diversity. However, as dried specimens, mushrooms lose many of the features, such as colour, size and odour, considered important in identification. Critical analysis of within vs among species variation of these diagnostic characters has therefore been difficult. I was able to circumvent this problem by creating an electronic version Woo's morphological notes. This database lent itself to formal analysis. Based on the DNA barcode species delimitation in *Russula* and the characters recorded for them, I described nine of the more common Russulas in the American Pacific Northwest. I found that the morphology of mushroom species in the genus *Russula* lags behind barcode sequence divergence. *Russula* species have been notoriously difficult to identify, and my multivariate analysis provided statistical support for the overlap of character states among even distantly related species. I hypothesize that mushroom morphology may be subject to lower levels of diversifying selection compared to reproductive organs such as flowers, and this may account for the high numbers of cryptic species being discovered among fungi.

My *Russula* database also included Woo's notes on the host trees that co-occurred with collections. Analysis of patterns of host-fungus associations revealed differences in host preference between pairs of closely related *Russula* sister taxa. Woo noted his collection localities, but not their GPS coordinates. Michael Beug (Evergreen State) kindly supplied coordinates, drawing on his familiarity with Woo's favourite collecting sites.

Analyzing broad patterns of geographical occurrences of species delimited by barcode sequences representing 12 large fungal genera led me to hypothesize that the range extents of the majority of fungal species are restricted by oceans and mountain ranges. Even though fungal spores may disperse over vast distances, the probability of successful range expansion may be limited by factors including competition and availability of host and habitat. I analyzed the distribution of range extents of genetically defined species of mushrooms from the publicly available UNITE fungal database. I found half mushroom species to be continental endemics with range extents below 2000 km. Some of the taxa with low genetic divergence and high geographic distance between samples may have been transported by humans beyond their natural ranges.

Limitations

Although sampling and species delimitation are strengths of this thesis, my work was nonetheless limited by available sampling and the species delimitations.

Sampling in fungi is made problematic by the nature of the organisms. They are most of their lives invisible underground and perform most of their metabolism and mating in that state, while we sample only fruiting bodies that are available about three days per year or less (Ceska and Ceska, 2013). Straatsma et al. (2001), Watling (1995), Orton (1986) observed that when doing a mushroom species survey of a site, it will take several years to reach a plateau in a species accumulation curve. These characteristics make thorough sampling very challenging. Long-term sampling and herbarium collections alleviate that burden, even though the DNA quality decays with time and collections are biased. In plants, broad sampling is geographically patchy, and biased by season, with over-representation of certain taxa, and most collections are contributed by a small number of botanists (Daru et al., 2018). Mushrooms are likely be subject to similar biased sampling.

Species delimitations in this study are based on a single locus, and that can be a great limitation. One locus may not be able to reconstruct species relationships due to incomplete lineage sorting, and the support from independent loci should help circumscribe species with more support.

The geographical metadata used in Chapter 4 was limited to country centroids, and much of the resolution at the geographical scale below continents was inaccessible. Precise coordinates of a samples would have been more informative at a local scale and the distribution of range extents at the lower end of the distribution (0-2000 km).

Applications

The Woo collection analyzed in this thesis illustrates well the contribution of non-academic collections (Hill, 2017). The importance in mycology of collections done by citizen scientists and non-professional experts has been recognized in the context of the biodiversity crisis and the need to describe the natural world (Kuo, 2007). The establishment of the North American Mycoflora Project was a recent step towards

recognizing and facilitating the work mushroom clubs perform to make inventories and descriptions of mushrooms (2017).

Russula species delimitation and characters recorded in this study have been useful to summarize for a mushroom identification online application aimed at nature enthusiasts (Berbee et al., 2018) and the *Russula* section from pictoral identification key of Pacific Northwest mushrooms of the Puget Sound Mycological Society (Miller, 2017). The online App pages describing common *Russula* species (choice edibles or not) have been written as of April 2018, but have yet to be posted online. GenBank sequences with accurate names are referenced in the App, and provide a reference for a better-curated public database. Future ecological studies in the Pacific Northwest may find the sequences by using GenBank to identify their mushrooms by using the BLAST tool. Our delimitation will make their identifications more consistent and probably less painful.

Future directions

These projects set up the scene for several possible avenues to continue research on mycorrhizal fungi, on *Russula* systematics, and on the factors influencing the geographical distribution of a mushroom. After identifying two pairs of *Russula* species with different host preferences, I extracted DNA from eight mushrooms and sequenced their genomes. I hope to analyze these in the near future. One of the members of each pair is associated with Sitka spruce and the other with Douglas fir. The sequences will contribute additional information on *Russula* genomes. So far the sampling has been on representative of major clades of *Russula* (Looney et al., 2018) as opposed to taxa that are closely related. I plan to compare the putative secreted proteins across species pairs to find candidate genes with possible roles in host adaptation. Fungi secrete proteins to signal, to interact with their host plants, and to digest nutrients from their surrounding environment. Experimental confirmation of mycorrhizal adaptation to a specific host would be difficult, but secreted proteins that differ between hosts would be candidates for factors involved in host adaptation. Evidence of positive selection might also point to genes with roles in adaptation to hosts.

Even with complete genomes of fungi adapted to different hosts, identifying proteins involved in host specificity has been difficult in other fungi and will likely be

challenging in *Russula* as well. From a broad sampling of mycorrhizal and saprotrophic Fungi, Kohler et al. (2015) found a rapid turnover of genes involved in symbiosis. Badouin et al. (2017) looked for genes for host specialization in sister species of *Microbotryum* parasitizing two different hosts. Which genes were under positive selection varied by species. These results suggest that symbiosis can be modulated in many ways and it has been difficult to recognize repeated patterns in its initiation. Repeated evolution of species associating with a host may provide a test to see whether association to the same host may have applied similar selective pressures on similar genes in independent lineages.

Future sampling of *Russula* for further analyses with newer specimens will be much easier due to my published work, and thanks to the GPS coordinates for Woo's localities that are available through my public online database (Bazzicalupo and Carmean, 2017). One of the main problems in extracting good enough DNA for the amplification of multiple loci was the age of the specimens. One future avenue of research could be to re-collect samples for a multilocus analysis from localities where they are known to occur. It is also now possible to predict additional localities for species based on similar host and environmental variables. Other species of *Russula* still remain undescribed, and locality and appearance information may help with collecting new samples to describe them.

I would also like to test if the predicted distribution of the fungus through environmental modeling software such as MaxEnt (Phillips et al., 2004), can be better predicted by using host information in combination with environmental factors. This has been shown to be the case in woodpeckers that track *Quercus* species producing the acorns they feed on (Freeman and Mason, 2015). It is possible that mycorrhizal fungi do the same, and knowing their host may help with predicting their geographical range.

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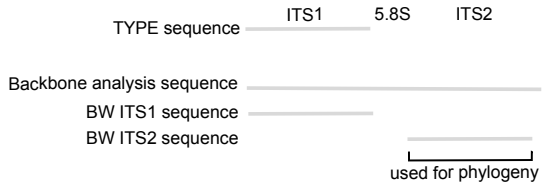
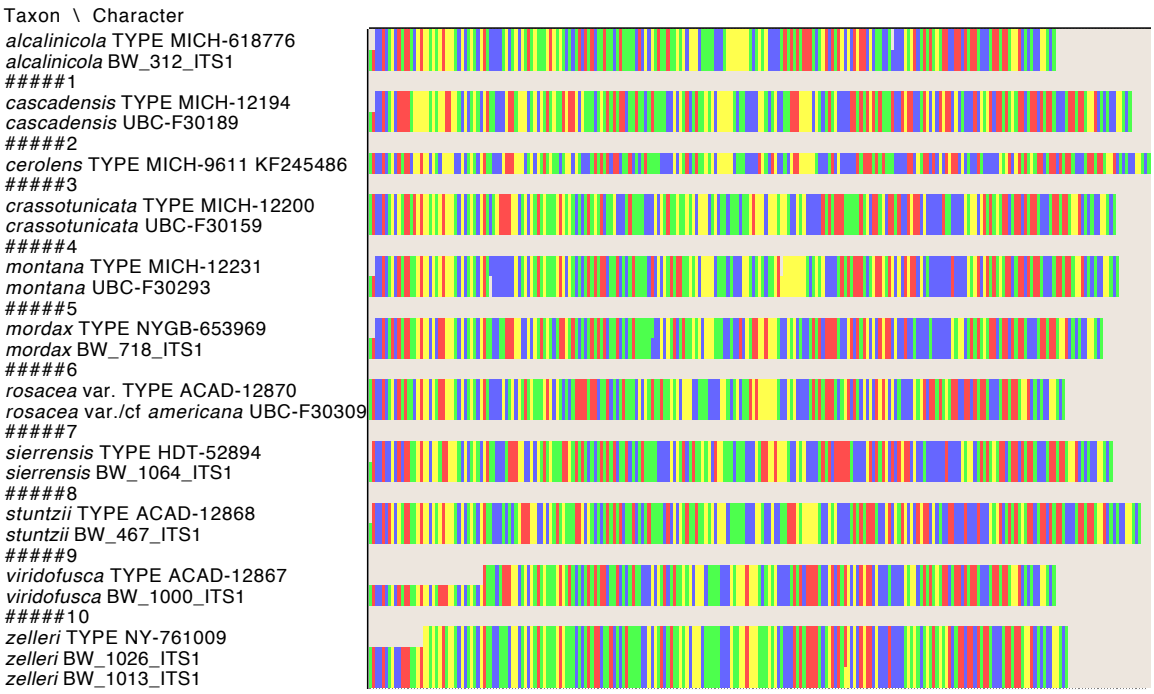
Appendix 1

Appendix 1.1 Cartoon of ITS and ITS1 sequence of type specimens

S1A



S1B



A. Cartoon map showing relative positions of the sequenced ITS1 fragment of a type; a complete ITS sequence from a 'backbone analysis' and our constraint tree; an ITS1 sequence from an exemplar Ben Woo (=BW) collection; and one of the 713 ITS2 sequence fragments determined for the BW collections. I sequenced the ITS1 region from 18 N. American type specimens. Because the DNA of the types was partially degraded, we were unable to sequence longer regions, not even the ITS2 upon which our species delimitation was based. We found and used full-length ITS1-ITS2 sequences in GenBank that were identical to the ITS1 region of each type to represent the type in the phylogeny and in species delimitation. **B.** Exemplar ITS1 sequences from the Woo collection were more than 99% identical to the 11 corresponding conspecific types. Taxon names are given as specific epithet followed by voucher specimen

identifier. Identical colours indicate the same nucleotide in these bird's eye views from Mesquite 3.1 (A=red, T=blue, C=green, G=yellow).

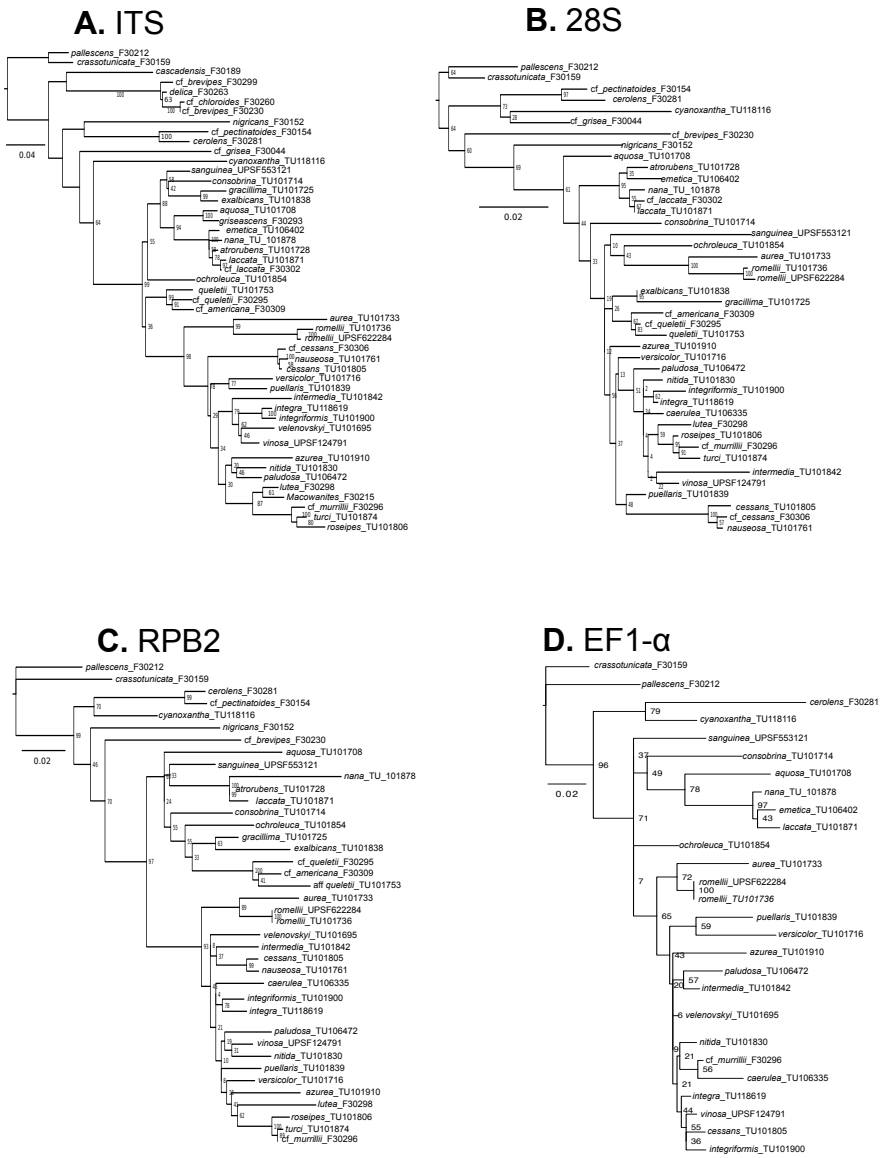
Appendix 1.2 Specimens for multilocus phylogeny

Samples and their herbarium accession numbers used for the multi-locus phylogeny.

Name	Herbarium	Specimen voucher	ITS	LSU	RPB2	EF1
aquosa	Tartu	TU-101708	UDB011290	KX812873	KX813654	KX813620
atrorubens	Tartu	TU-101728	UDB011308	KX812877	KX813658	
aurea	Tartu	TU-101733	UDB011363	KX812878	KX813659	KX813623
azurea	Tartu	TU-101910	UDB016046	KX812894	KX813675	KX813635
caerulea	Tartu	TU-106335	UDB011211	KX812895	KX813676	
cascadensis	UBC	F30189	KX812838			
cerolens	UBC	F30282	KX812844	KX812865	KX813648	KX813617
cessans	Tartu	TU-101805	UDB015971	KX812883	KX813664	KX813626
cf. brevipes	UBC	F30230	KX812841	KX812863	KX813647	
cf. brevipes	UBC	F30299	KX812848	KX812869		
cf. cessans	UBC	F30306	KX812850	KX812871		
cf. delica	UBC	F30260	KX812852			
cf. grisea	UBC	F30044	KX812834	KX812858		
cf. laccata	UBC	F30302	KX812849	KX812870		
cf. murrillii	UBC	F30296	KX812846	KX812867	KX813650	KX813618
cf. pectinatoides	UBC	F30281	KX812843			
cf. queletii	UBC	F30295	KX812845	KX812866	KX813649	
cf. samguinaria	UBC	F30309	KX812851	KX812872	KX813652	
consobrina	Tartu	TU-101714	UDB011295	KX812874	KX813655	KX813621
crassotunicata	UBC	F30159	KX812837	KX812861	KX813645	KX813615
cyanoxantha	Tartu	TU-118116	UDB011230	KX812898	KX813678	KX813638
delica	UBC	F30263	KX812842	KX812864		
emetica	Tartu	TU-106402	UDB011171	KX812896		KX813636
exalbicans	Tartu	TU-101838	UDB015994	KX812886	KX813667	
gracillima	Tartu	TU-101725	UDB011361	KX812876	KX813657	
montana	UBC	F30293	KX812853			
integra	Tartu	TU-118619	UDB018021	KX812899	KX813679	KX813639
integriformis	Tartu	TU-101900	UDB016042	KX812893	KX813674	KX813634
intermedia	Tartu	TU-101842	UDB015997	KX812888	KX813669	KX813630

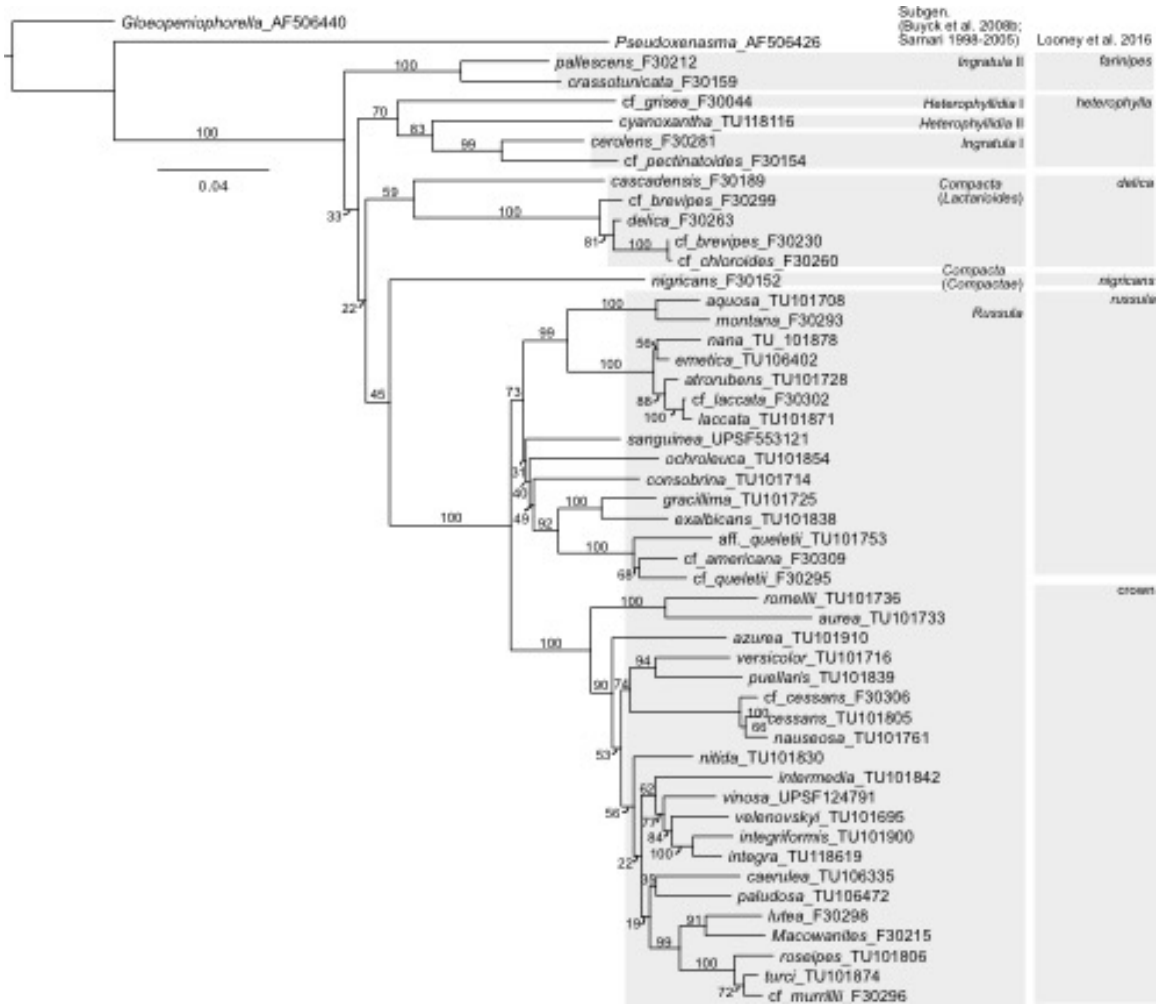
laccata	Tartu	TU-101871	UDB016024	KX812890	KX813671	KX813632
lutea	UBC	F30298	KX812847	KX812868	KX813651	
Macowanites	UBC	F30215	KX812840			
nana	Tartu	TU-101878	UDB016029	KX812892	KX813673	KX813633
nauseosa	Tartu	TU-101761	UDB011329	KX812882	KX813663	
nigricans	UBC	F30152	KX812835	KX812859	KX813643	
nitida	Tartu	TU-101830	UDB015987	KX812885	KX813666	KX813628
ochroleuca	Tartu	TU-101854	UDB016009	KX812889	KX813670	KX813631
pallescens	UBC	F30212	KX812839	KX812862	KX813646	KX813616
paludosa	Tartu	TU-106472	UDB011179	KX812897	KX813677	KX813637
puellaris	Tartu	TU-101839	UDB015995	KX812887	KX813668	KX813629
Aff. queletii	Tartu	TU-101753	UDB011324	KX812880	KX813661	
romellii	Tartu	TU-101736	UDB011365	KX812879	KX813660	KX813624
roseipes	Tartu	TU-101806	UDB015972	KX812884	KX813665	KX813627
sanguinea	Uppsala	UPS-F-553121	KX812856	KX812901	KX813681	KX813641
sp. 3	UBC	F30154	KX812836	KX812860	KX813644	
sp. 6	Uppsala	UPS-F-622284	KX812855	KX812902	KX813682	KX813642
turci	Tartu	TU-101874	UDB016082	KX812891	KX813672	
velenovskyi	Tartu	TU-101695	UDB011282		KX813653	KX813619
versicolor	Tartu	TU-101716	UDB011297	KX812875	KX813656	KX813622
vinosa	Uppsala	UPS-F-124791	KX812857	KX812900	KX813680	KX813640

Appendix 1.3 Single-gene phylogenies (ITS, LSU, RPB2, and EF1-α)



Maximum likelihood trees obtained from alignment of single genes (indicated at the top of each tree) **A**, ITS; **B**, 28S (LSU); **C**, RPB2; **D**, EF1- α . Branches with 70% and above bootstrap support are congruent among individual gene trees.

Appendix 1.4 Multi-locus phylogeny



Maximum likelihood phylogeny of *Russula* from concatenated ITS, LSU, RPB2 and EF1-α sequences that served as a constraint for the phylogeny of the ITS2 sequences from the Pacific Northwest Woo material. Numerals at nodes are bootstrap values. Subgeneric groups from Buyck et al. (2008b) (with names from Sarnari (1998)) and Looney et al. (2016) (with names from their study) are highlighted. Ingratula II takes a different position in our study, in Looney et al., and in Buyck et al. but always without bootstrap support. We found *R. cyanoxantha* to have an 83% BP support as sister of Ingratula I. Although branching order differed for these taxa in the other two studies, support was below 70%. Branching order here and in Looney et al.'s Table S2 is identical for 'crown' taxa *R. versicolor*, *R. romellii*, *R. integra* and *R. nitida*. In Looney et al.'s 'russula' *R. emetica*, *R. aquosa*, *R. ochroleuca*, *R. gracillima*, *R. queletii* are in positions fully congruent with those illustrated here but our *R. sanguinea* is sister to the other 'russula' rather than sister to *R. queletii* as in Looney et al. (2016). These details show that branching order across multilocus studies differs in details but overall is congruent.

Appendix 1.5 Phylogeny of *Russula* Woo samples

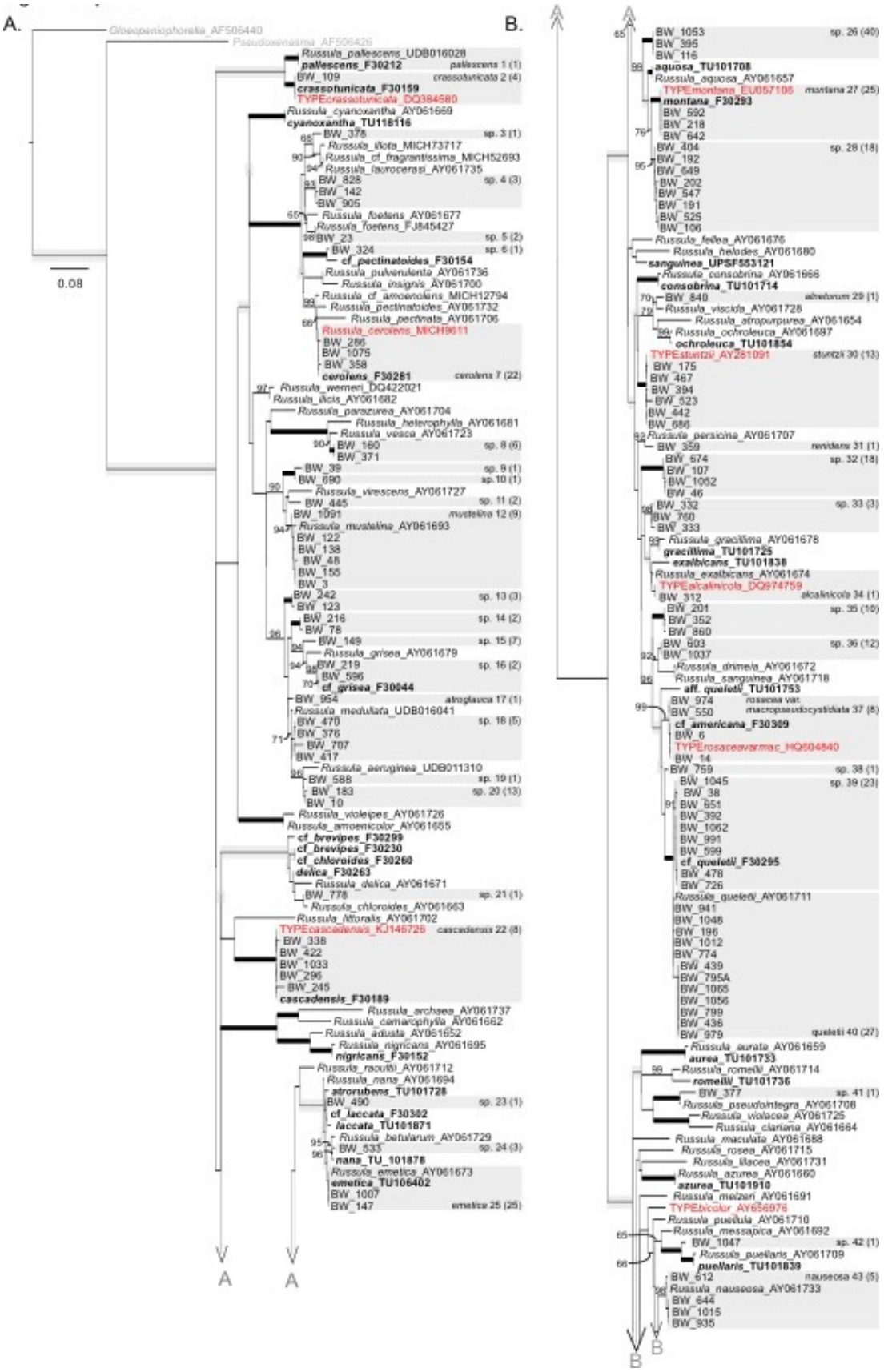
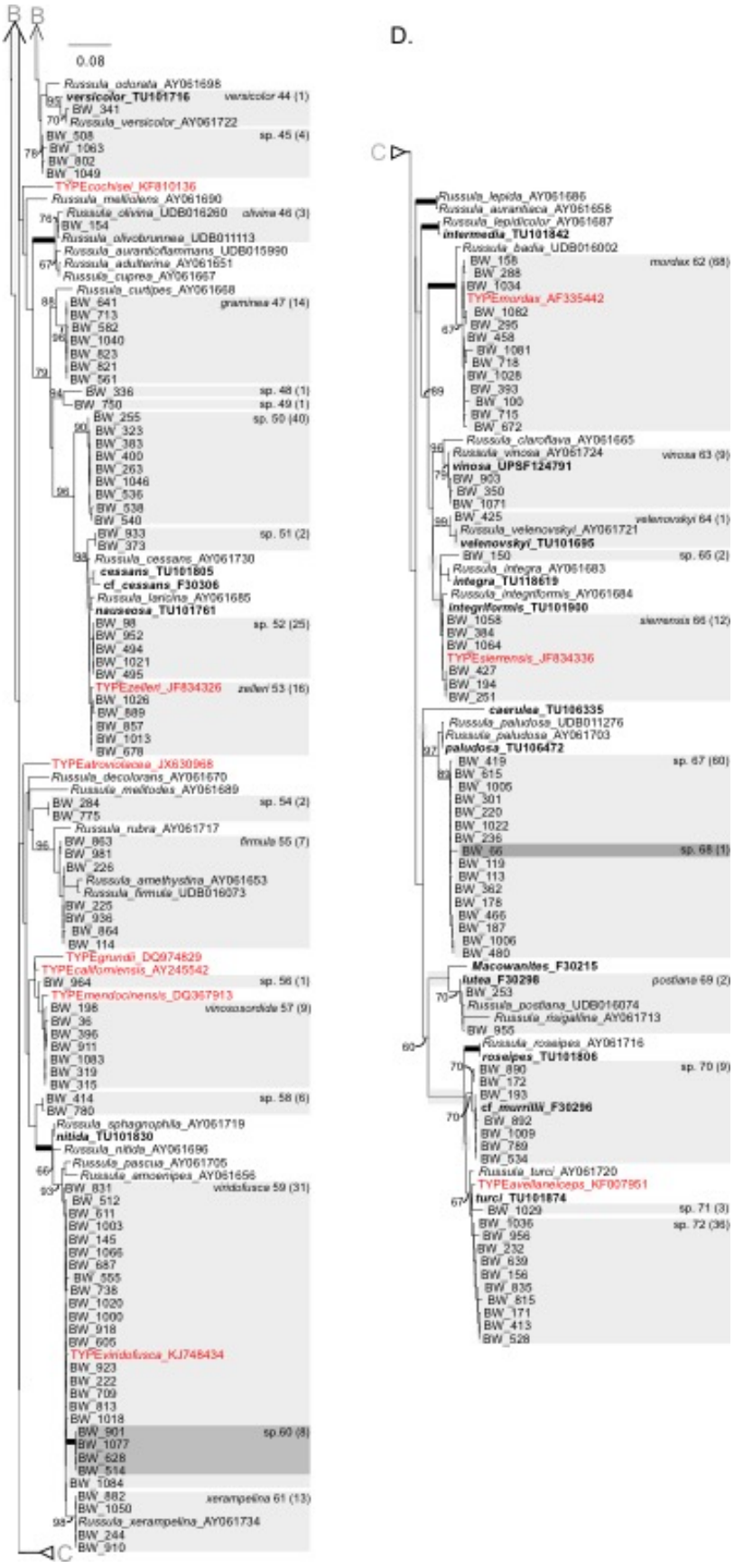

A.
B.
Gloeopeniophorella_AF506440
Pseudoxenasma_AF506426
0.08
Russula_pallescens_UDB016028
pallescens_F30212
pallescens 1 (1)
BW_109
crassotunicata 2 (4)
crassotunicata_F30159
TYPEcrassotunicata_DQ384580
Russula_cyanoxantha_AY061669
cyanoxantha_TU118116
BW_378
sp. 3 (1)
Russula_illota_MICH73717
Russula_cf_fragrantissima_MICH52693
Russula_laurocerasi_AY061735
BW_828
BW_142
BW_905
sp. 4 (3)
Russula_foetens_AY061677
Russula_foetens_FJ845427
BW_23
sp. 5 (2)
BW_324
sp. 6 (1)
cf_pectinatoides_F30154
Russula_pulverulenta_AY061736
Russula_insignis_AY061700
Russula_cf_amoenolens_MICH12794
Russula_pectinatoides_AY061732
Russula_pectinata_AY061706
Russula_cerolens_MICH9611
BW_286
BW_1075
BW_358
cerolens_F30281
cerolens 7 (22)
Russula_werneri_DQ422021
Russula_illota_AY061682
Russula_parazurea_AY061704
Russula_heterophylla_AY061681
Russula_vesca_AY061723
BW_160
BW_371
sp. 8 (6)
BW_39
sp. 9 (1)
BW_690
sp.10 (1)
Russula_virescens_AY061727
BW_445
sp. 11 (2)
BW_1091
mustelina 12 (9)
Russula_mustelina_AY061693
BW_122
BW_138
BW_48
BW_155
BW_3
BW_242
sp. 13 (3)
BW_123
BW_216
sp. 14 (2)
BW_78
BW_149
sp. 15 (7)
Russula_grisea_AY061679
BW_219
BW_596
sp. 16 (2)
cf_grisea_F30044
BW_954
atroglauca 17 (1)
Russula_medullata_UDB016041
BW_470
BW_376
sp. 18 (5)
BW_707
BW_417
Russula_aeruginea_UDB011310
BW_588
sp. 19 (1)
BW_183
BW_10
sp. 20 (13)
Russula_violeipes_AY061726
Russula_amoenicolor_AY061655
cf_brevipes_F30299
cf_brevipes_F30230
cf_chloroides_F30260
delica_F30263
Russula_delica_AY061671
BW_778
sp. 21 (1)
Russula_chloroides_AY061663
Russula_litoralis_AY061702
TYPEcascadensis_KJ146726
cascadensis 22 (8)
BW_338
BW_422
BW_1033
BW_296
BW_245
cascadensis_F30189
Russula_archaea_AY061737
Russula_camarophylla_AY061662
Russula_adusta_AY061652
Russula_nigricans_AY061695
nigricans_F30152
Russula_raoultii_AY061712
Russula_nana_AY061694
atrorubens_TU101728
BW_490
sp. 23 (1)
cf_laccata_F30302
laccata_TU101871
Russula_betularum_AY061729
BW_533
sp. 24 (3)
nana_TU_101878
Russula_emetica_AY061673
emetica_TU106402
BW_1007
BW_147
emetica 25 (25)
A
A
BW_1053
sp. 26 (40)
BW_395
BW_116
aguosa_TU101708
Russula_aguosa_AY061657
TYPEmontana_EU057105
montana 27 (25)
montana_F30293
BW_592
BW_218
BW_642
BW_404
sp. 28 (18)
BW_192
BW_649
BW_202
BW_547
BW_191
BW_525
BW_106
Russula_fellea_AY061676
Russula_helodes_AY061680
sanguinea_UPSF553121
Russula_consobrina_AY061666
consobrina_TU101714
BW_840
alnetorum 29 (1)
Russula_viscida_AY061728
Russula_atropurpurea_AY061654
Russula_ochroleuca_AY061697
ochroleuca_TU101854
TYPEstuntzii_AY281091
stuntzii 30 (13)
BW_175
BW_467
BW_394
BW_523
BW_642
BW_686
Russula_persicina_AY061707
BW_359
rendens 31 (1)
BW_674
sp. 32 (18)
BW_107
BW_1052
BW_46
BW_332
sp. 33 (3)
BW_760
BW_333
Russula_gracillima_AY061678
gracillima_TU101725
exalbicans_TU101838
Russula_exalbicans_AY061674
TYPEalcalinicola_DQ974759
BW_312
alcalinicola 34 (1)
BW_201
sp. 35 (10)
BW_352
BW_860
BW_603
sp. 36 (12)
BW_1037
Russula_drimeia_AY061672
Russula_sanguinea_AY061718
aff._queletii_TU101753
BW_974
rosacea var. macropseudocystidiata 37 (8)
BW_550
cf_americana_F30309
BW_6
TYPErosaceavarmac_HQ504840
BW_14
BW_759
sp. 38 (1)
BW_1045
sp. 39 (23)
BW_38
BW_651
BW_392
BW_1062
BW_991
BW_599
cf_queletii_F30295
BW_478
BW_726
Russula_queletii_AY061711
BW_941
BW_1048
BW_196
BW_1012
BW_774
BW_439
BW_795A
BW_1065
BW_1056
BW_799
BW_436
BW_979
queletii 40 (27)
Russula_aurata_AY061659
aurea_TU101733
Russula_romellii_AY061714
romellii_TU101736
BW_377
sp. 41 (1)
Russula_pseudointegra_AY061706
Russula_violacea_AY061725
Russula_clariana_AY061664
Russula_maculata_AY061688
Russula_rosea_AY061715
Russula_lilacea_AY061731
Russula_azurea_AY061660
azurea_TU101910
Russula_melzeri_AY061691
TYPEbicolor_AY656976
Russula_puellula_AY061710
Russula_messapica_AY061692
BW_1047
sp. 42 (1)
Russula_puellaris_AY061709
puellaris_TU101839
BW_612
nauseosa 43 (5)
Russula_nauseosa_AY061733
BW_644
BW_1015
BW_935
B
B

Figure S4 part 2



Likelihood phylogeny showing all unique sequences from the Pacific Northwest Woo collection. Shaded light-grey boxes indicate a delimited species; dark grey boxes indicate delimited species that are nested within another species. Taxa from the constraint tree are in bold. Types are indicated in red. Each delimited species is indicated by a clade number. The total number of specimens in the species is in parentheses. Branches from the constraint topology are shaded grey, bootstrap values above 60% are indicated at branches and 100% support is indicated by thickened black branches.

Appendix 1.6 Woo *Russula* specimens ITS2 grouped in species by 4 software

Clade number (first column), species delimitations[1], and the list of samples of the Woo collection. Shaded specimen codes represent collections excluded from the species concept by one or two of the delimitation methods.

	Species Delimitation species delimitation/ closest DOI UNITE SH#[2]	**#**	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
		# samples	ABGD (part 4)	mothur 99% cutoff	GMYC	PTP	Double Peaks (heterozygous dikaryons) and variable sites represented by 2 or more samples within a species[3]	Gill Colour[4]	Taste Gills
		BW taxa included>	713	713	150	713			
		Input file >	Alignment	Alignment	Ultrametric tree	ML tree			
		# BW sp. Del. >	76	93	81	78			
1	**pallescens† 10.15156/BIO/SH250224.07FU**	**1**	BW_571	BW_571	BW_571	BW_571		white	mild then hot
2	**crassotunicata* 10.15156/BIO/SH250225.07FU**	**4**	BW_109, BW_40, BW_446, BW_62, DQ384580	BW_109, BW_40, BW_446, BW_62, DQ384580	BW_109, DQ384580	BW_109, BW_40, BW_446, BW_62, DQ384580		cream (2)	mild (2), very hot (2)
3	**Woo sp. 3**	**1**	BW_378	BW_378	BW_378	BW_378		cream	medium hot

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
	10.15156/BIO/SH270547.07FU								
4	**Woo sp. 4 10.15156/BIO/SH301439.07FU**	**3**	BW_142, BW_828, BW_905	BW_142, BW_828, BW_905	BW_142, BW_828	BW_142, BW_828, BW_905		cream (2), ochre (1)	very hot (2), mild (1)
5	**Woo sp. 5 10.15156/BIO/SH301513.07FU**	**2**	BW_23, BW_75	BW_23, BW_75	BW_23	BW_23, BW_75		cream (1), yellow (1)	medium hot (2)
6	**Woo sp. 6 10.15156/BIO/SH272114.07FU**	**1**	BW_324	BW_324	BW_324	BW_324		cream	mild
7	**cerolens* 10.15156/BIO/SH301399.07FU**	**22**	BW_1075, BW_139, BW_161, BW_168, BW_240, BW_261, BW_286, BW_358, BW_382, BW_453, BW_460, BW_49, BW_507, BW_614, BW_63, BW_663, BW_788, BW_796, BW_797, BW_865, BW_949, BW_982, KF245486	BW_1075, BW_139, BW_161, BW_168, BW_240, BW_261, BW_286, BW_358, BW_382, BW_453, BW_460, BW_49, BW_507, BW_614, BW_63, BW_663, BW_788, BW_796, BW_797, BW_865, BW_949, BW_982, KF245486	BW_1075, BW_286, BW_358	BW_1075, BW_139, BW_161, BW_168, BW_240, BW_261, BW_286, BW_358, BW_382, BW_453, BW_460, BW_49, BW_507, BW_614, BW_63, BW_663, BW_788, BW_796, BW_797, BW_865, BW_949, BW_982, KF245486		cream (16), which cream (3), cream yellow (1), white (1)	very hot (10), medium hot (8), slightly acrid (1), slightly hot (1), very hot bitter (1)
8	**Woo sp. 8 10.15156/BIO/SH230394.07FU**	**6**	BW_160, BW_163, BW_353, BW_367,	BW_160, BW_163, BW_353, BW_367,	BW_160, BW_371	BW_160, BW_163, BW_353, BW_367,		white (4), cream (1)	mild (6)

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
			BW_371, BW_904	BW_371, BW_904		BW_371, BW_904			
9	**Woo sp. 9 10.15156/BIO/SH233869.07FU**	**1**	BW_39	BW_39	BW_39	BW_39, BW_690		cream (2)	mild (2)
10	**Woo sp. 10** 10.15156/BIO/SH233869.07FU	**1**	BW_690	BW_690	BW_690				
11	**Woo sp. 11 10.15156/BIO/SH233867.07FU**	**2**	BW_445, BW_839	BW_445, BW_839	BW_445	BW_445, BW_839		cream (1), white (1)	mild (2)
12	**mustelina† 10.15156/BIO/SH233858.07FU**	**9**	BW_1091, BW_122, BW_126, BW_138, BW_155, BW_3, BW_430, BW_48, BW_563	BW_1091, BW_122, BW_126, BW_430, BW_138, BW_563 BW_155, BW_3, BW_48	BW_1091, BW_138, BW_155, BW_3	BW_1091, BW_122, BW_126, BW_138, BW_155, BW_3, BW_430, BW_48, BW_563		cream (6), yellow (1)	mild (8)
13	**Woo sp. 13 10.15156/BIO/SH233857.07FU**	**3**	BW_123, BW_242, BW_913	BW_123, BW_242, BW_913	BW_123, BW_242	BW_123, BW_242, BW_913		cream (2), white cream (1)	mild (3)
14	**Woo sp. 14 10.15156/BIO/SH233873.07FU**	**2**	BW_216, BW_78	BW_216, BW_78	BW_216, BW_78	BW_216, BW_78		cream (1)	mild bitter (1)
15	**Woo sp. 15 10.15156/BIO/SH233853.07FU**	**7**	BW_149, BW_179, BW_227, BW_440, BW_473, BW_591, BW_702	BW_149, BW_179, BW_227, BW_440, BW_473, BW_591, BW_702	BW_149	BW_149, BW_179, BW_227, BW_440, BW_473, BW_591, BW_702		cream (5), white (2)	mild (7)
16	**Woo sp. 16 SH559820.07FU**	**2**	BW_219, BW_596	BW_219, BW_596	BW_219, BW_596	BW_219, BW_596		cream (2)	mild (2)
17	**atroglauca†**	**1**	BW_954	BW_954	BW_954	BW_954		white cream	mild

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
	10.15156/BIO/SH324598.07FU								
18	**Woo sp. 18** SH564580.07FU	**5**	BW_376, BW_391, BW_417, BW_470, BW_707	BW_376, BW_391, BW_417, BW_470, BW_707	BW_376, BW_417 BW_470 BW_707	BW_376, BW_391, BW_417, BW_470, BW_707		cream (4), cream yellow (1)	mild (5)
19	**Woo sp. 19** **10.15156/BIO/SH233863.07FU**	**1**	BW_588	BW_588	BW_588	BW_588		-	-
20	**Woo sp. 20** **10.15156/BIO/SH233912.07FU**	**13**	BW_10, BW_183, BW_243, BW_307, BW_330, BW_505, BW_51, BW_585, BW_734, BW_74, BW_77, BW_79, BW_995	BW_10, BW_183, BW_243, BW_307, BW_330, BW_505, BW_51, BW_585, BW_734, BW_74, BW_77, BW_79, BW_995	BW_10, BW_183	BW_10 BW_183 BW_243 BW_307 BW_330 BW_505 BW_51 BW_585 BW_734 BW_74 BW_77 BW_79 BW_995		cream (10), white cream (1), cream yellow (1)	mild (10), slightly acrid (2), mild lightly hot (1)

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
21	**Woo sp. 21 10.15156/BIO/SH300881.07FU**	**1**	BW_778	BW_778	BW_778	BW_778		cream	slightly hot
22	**cascadensis* 10.15156/BIO/SH245028.07FU**	**8**	BW_1033, BW_296, BW_338, BW_422, BW_434, BW_610, BW_834, KJ1467262	BW_1033, BW_245, BW_296, BW_338, BW_422, BW_434, BW_610, BW_834, KJ1467262	BW_1033, BW_422, BW_338	BW_1033, BW_245, BW_296, BW_338, BW_422, BW_434, BW_610, BW_834, KJ1467262		cream (4), white cream (1), white (1), yellow (2)	very hot (6), slightly hot (1), slightly acrid (1)
22			BW_245		BW_245				
23	**Woo sp. 23 10.15156/BIO/SH297335.07FU**	**1**	BW_490	BW_490	BW_490			-	-
24	**Woo sp. 24 10.15156/BIO/SH297394.07FU**	**3**	BW_533, BW_544, BW_948	BW_533, BW_544, BW_948	BW_533				very hot (2), medium hot (1)
25	**emetica† 10.15156/BIO/SH297334.07FU**	**25**	BW_1007, BW_1014, BW_105, BW_108, BW_128, BW_131, BW_147, BW_237, BW_429, BW_461, BW_484, BW_504, BW_513, BW_520, BW_541, BW_556, BW_601, BW_647,	BW_1007, BW_1014, BW_105, BW_108, BW_128, BW_131, BW_147, BW_237, BW_429, BW_461, BW_484, BW_504, BW_513, BW_520, BW_541, BW_556, BW_601, BW_647,	BW_1007	BW_1007, BW_1014, BW_105, BW_108, BW_128, BW_131, BW_147, BW_237, BW_429, BW_461, BW_484, BW_490, BW_504, BW_513, BW_520, BW_541, BW_533, BW_544,		white (24), white cream (1)	very hot (14), medium hot (7), slightly hot (3)

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
			BW_758, BW_768, BW_851, BW_908, BW_922, BW_959, BW_963	BW_758, BW_768, BW_851, BW_908, BW_922, BW_959, BW_963		BW_556, BW_601, BW_647, BW_758, BW_768, BW_851, BW_908, BW_922, BW_948, BW_959, BW_963			
26	**Woo sp. 26 10.15156/BIO/SH297356.07FU**	**40**	BW_1053, BW_1069, BW_116, BW_129, BW_241, BW_270, BW_290, BW_297, BW_299, BW_300, BW_311, BW_366, BW_395, BW_476, BW_479, BW_620, BW_621, BW_622, BW_645, BW_673, BW_675, BW_676, BW_682, BW_695, BW_706,	BW_1053, BW_1069, BW_116, BW_129, BW_241, BW_270, BW_290, BW_297, BW_299, BW_300, BW_311, BW_366, BW_395, BW_476, BW_479, BW_620, BW_621, BW_622, BW_645, BW_673, BW_675, BW_676, BW_682, BW_695, BW_706,	BW_1053	BW_1053, BW_1069, BW_116, BW_129, BW_241, BW_270, BW_290, BW_297, BW_299, BW_300, BW_311, BW_366, BW_395, BW_476, BW_479, BW_620, BW_621, BW_622, BW_645, BW_673, BW_675, BW_676, BW_682, BW_695, BW_706,	pos 330: **2r**, 38g; g shared with Woo sp. 28 and montana (27)	white (33), cream (4), white cream (1)	mild (29), slightly hot (6), slightly acrid (4), mild slightly hot (1)

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
			BW_730, BW_732, BW_744, BW_749, BW_752, BW_770, BW_790, BW_793, BW_801, BW_872, BW_875, BW_919, BW_932, BW_971, BW_980	BW_730, BW_732, BW_744, BW_749, BW_752, BW_770, BW_790, BW_793, BW_801, BW_872, BW_875, BW_919, BW_932, BW_971, BW_980		BW_730, BW_732, BW_744, BW_749, BW_752, BW_770, BW_790, BW_793, BW_801, BW_872, BW_875, BW_919, BW_932, BW_971, BW_980			
27	**griseascens†/montana* 10.15156/BIO/SH297351.07FU**	**25**	BW_218, BW_2182, BW_247, BW_310, BW_314, BW_322, BW_334, BW_443, BW_492, BW_524, BW_545, BW_592, BW_593, BW_642, BW_664, BW_694, BW_697, BW_711, BW_725, BW_753, BW_812,	BW_218, BW_2182, BW_247, BW_310, BW_314, BW_322, BW_334, BW_443, BW_492, BW_524, BW_545, BW_592, BW_593, BW_642, BW_664, BW_694, BW_697, BW_711, BW_725, BW_753, BW_812,	BW_218, BW_642	BW_218, BW_2182, BW_247, BW_310, BW_314, BW_322, BW_334, BW_443, BW_492, BW_524, BW_545, BW_592, BW_593, BW_642, BW_664, BW_694, BW_697, BW_711, BW_725, BW_753, BW_812,	pos 127: **1y**, 24t; t shared with Woo sp. 28, Woo sp. 26 - pos 291: **1r**, 24a; a shared with Woo sp. 28, Woo sp. 26	white (24), cream (1)	very hot (15), medium hot (5), slightly hot (2), mild (1), mild slightly hot (1), mild very hot (1)

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
			BW_829, BW_883, BW_912, BW_969, EU057106	BW_829, BW_883, BW_912, BW_969, EU057106		BW_829, BW_883, BW_912, BW_969, EU057106			
28	**Woo sp. 28 10.15156/BIO/SH297355.07FU**	**18**	BW_106, BW_191, BW_192, BW_202, BW_368, BW_404, BW_428, BW_503, BW_519, BW_525, BW_547, BW_548, BW_559, BW_649, BW_814, BW_873, BW_920, BW_993	BW_106, BW_191, BW_192, BW_202, BW_368, BW_404, BW_428, BW_503, BW_519, BW_525, BW_547, BW_548, BW_559, BW_649, BW_814, BW_873, BW_920, BW_993	BW_106, BW_202, BW_547, BW_649	BW_106, BW_191, BW_192, BW_202, BW_368, BW_404, BW_428, BW_503, BW_519, BW_525, BW_547, BW_548, BW_559, BW_649, BW_814, BW_873, BW_920, BW_993	pos 413: **5y**, 7t, 5c; C shared with montana (27) and Woo sp. 26	white (16), cream (1), yellow (1)	mild (8), medium hot (3), slightly acrid (3), mild slightly hot (3), slightly hot (1)
29	**alnetorum† 10.15156/BIO/SH359435.07FU**	**1**	BW_840	BW_840	BW_840	BW_840		cream	mild

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
30	**stuntzii*** **10.15156/BIO/SH284903.07FU**	**13**	AY281091, BW_175, BW_348, BW_394, BW_442, BW_467, BW_491, BW_546, BW_570, BW_665, BW_684, BW_686, BW_757 BW_523	AY281091, BW_175, BW_348, BW_442, BW_467, BW_491, BW_546, BW_570, BW_665, BW_684, BW_686, BW_757 BW_523	AY28109, BW_394, BW_523	AY281091, BW_175, BW_348, BW_394, BW_442, BW_467, BW_491, BW_523, BW_546, BW_570, BW_665, BW_684, BW_686, BW_757	pos 238: **1y**, 6c, 4t	white (10), cream (1)	slightly hot (4), medium hot (3), slightly acrid (2), mild (2), mild slightly acrid (1) very hot (1)
31	**renidens†** **10.15156/BIO/SH244456.07FU**	**1**	BW_359	BW_359	BW_359	BW_359		cream	medium hot
32	**Woo sp. 32** **10.15156/BIO/SH284902.07FU**	**18**	BW_1052, BW_107, BW_134, BW_223, BW_441, BW_46, BW_542, BW_558, BW_581, BW_626, BW_629, BW_674, BW_685, BW_712, BW_769, BW_771, BW_791, BW_792	BW_1052, BW_107, BW_134, BW_223, BW_441, BW_542, BW_558, BW_581, BW_626, BW_629, BW_674, BW_685, BW_712, BW_769, BW_771, BW_791, BW_792 BW_46	BW_1052, BW_107, BW_46, BW_674	BW_1052, BW_107, BW_134, BW_223, BW_441, BW_46, BW_542, BW_558, BW_581, BW_626, BW_629, BW_674, BW_685, BW_712, BW_769, BW_771, BW_791, BW_792		white (7), cream (5), white cream (2), yellow (1)	mild (7), slightly hot (4), medium hot (2), slightly acrid (2), very hot (1)

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
33	**Woo sp. 33 10.15156/BIO/SH244462.07FU**	**3**	BW_332, BW_333, BW_760	BW_332, BW_760 BW_333	BW_332, BW_333, BW_760	BW_332, BW_333, BW_760		cream (1), yellow (1)	mild (1)
34	**exalbicans†/ alcalinicola* 10.15156/BIO/SH244463.07FU**	**1**	BW_312, DQ974759	BW_312, DQ974759	BW_312, DQ974759	BW_312, DQ974759		cream	mild
35	**Woo sp. 35 10.15156/BIO/SH297359.07FU**	**10**	BW_201, BW_337, BW_352, BW_361, BW_438, BW_463, BW_486, BW_500, BW_860, BW_92	BW_201, BW_337, BW_352, BW_361, BW_438, BW_463, BW_486, BW_500, BW_860, BW_92	BW_201, BW_352	BW_201, BW_337, BW_352, BW_361, BW_438, BW_463, BW_486, BW_500, BW_860, BW_92		cream (8), white (1), yellow (1)	very hot (5), medium hot (4), slightly hot (1)
36	**Woo sp. 36 10.15156/BIO/SH297365.07FU**	**12**	BW_1037, BW_180, BW_562, BW_566, BW_603, BW_643, BW_740, BW_747, BW_779, BW_784, BW_888, BW_9	BW_1037, BW_180, BW_562, BW_566, BW_603, BW_643, BW_740, BW_747, BW_779, BW_784, BW_888, BW_9	BW_1037, BW_603	BW_1037, BW_180, BW_562, BW_566, BW_603, BW_643, BW_740, BW_747, BW_779, BW_784, BW_888, BW_9		cream (11)	very hot (5), medium hot (4), slightly acrid (10)

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
37	**rosacea var. macropseudocystidiata* 10.15156/BIO/SH244475.07FU**	**8**	BW_14, BW_249, BW_475, BW_550, BW_6, BW_974, BW_990	BW_14, BW_249, BW_475, BW_550, BW_974, BW_990 BW_6	BW_14, BW_550, BW_974		pos 156: **1y**, 6c, 1t; c shared with Woo sp. 38, Woo sp. 39 and queletii (40) - pos 374: **1k**, 7g; g shared with Woo sp. 38, Woo sp. 39 and queletii (40)	cream (4), white (1), white cream (1), yellow (1)	mild (3), medium hot (1), mild then hot (1), mild slightly hot (1), very hot (1), slightly acrid (1)
38	**Woo sp. 38 10.15156/BIO/SH244485.07FU**	**1**	BW_759	BW_759	BW_759			yellow	mild
39	**Woo sp. 39** 10.15156/BIO/SH244458.07FU	**23**	BW_1045, BW_1062, BW_176, BW_273, BW_335, BW_38, BW_392, BW_478, BW_529, BW_53, BW_543, BW_572, BW_575, BW_598, BW_599, BW_637, BW_651, BW_721, BW_726, BW_731,	BW_1045, BW_1062, BW_176, BW_273, BW_335, BW_38, BW_392, BW_478, BW_529, BW_53, BW_543, BW_572, BW_575, BW_598, BW_599, BW_637, BW_651, BW_721, BW_726, BW_731,	BW_1045		pos 191: **15k**, 4g, 4t; g shared with queletii (40) and rosacea var. mac. (39) - pos 374: **1r**, 22g;g shared with queletii (40) and rosacea var. mac. (37) - pos 413: **1y**, 22c; c shared with queletii (40) and rosacea var. mac. (37)	cream (12), yellow (9), white (1), white cream (1)	slightly hot (7), mild (6), medium hot (4), very hot (2), mild slightly hot (1), mild slightly acrid (1), slightly acrid (1)

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
			BW_733, BW_972, BW_991	BW_733, BW_972, BW_991					
40	**queletii**† 10.15156/BIO/SH244458.07FU	**27**	BW_1012, BW_1016, BW_1048, BW_1056, BW_1065, BW_124, BW_136, BW_196, BW_432, BW_435, BW_436, BW_439, BW_462, BW_502, BW_509, BW_774, BW_795, BW_795A, BW_798, BW_799, BW_800, BW_847, BW_854, BW_867, BW_941, BW_944, BW_979	BW_1012, BW_1016, BW_1056, BW_1065, BW_124, BW_136, BW_432, BW_439, BW_502, BW_509, BW_774, BW_795, BW_795A, BW_798, BW_944	BW_1012	BW_14, BW_249, BW_475, BW_550, BW_6, BW_974, BW_990 BW_759 BW_1012, BW_1016, BW_1048, BW_1056, BW_1065, BW_124, BW_136, BW_196, BW_432, BW_435, BW_436, BW_439, BW_462, BW_502, BW_509, BW_774, BW_795, BW_795A, BW_798, BW_799, BW_800, BW_847, BW_854, BW_867,	pos 155: **1y**, 26t; t shared with Woo sp. 39 - pos 335: **14y**, 8t, 4c; t shared with Woo sp. 39 - pos 339: **14r**, 7g, 4a; g shared with Woo sp. 39 - pos 344: **14y**, 8t, 4c; t shared with Woo sp. 39 - pos 373: **4y**, 1t, 22c; c shared with Woo sp. 39 - pos 465: **1m**, 26c; c shared with Woo sp. 39 - pos 466: **3m**, 6a, 17c; c shared with Woo sp. 39	cream (21), yellow (4), cream yellow (1)	mild (9), medium hot (9), slightly hot (6), slightly acrid (2)

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
						BW_941, BW_944, BW_979 -- BW_1045, BW_1062, BW_176, BW_273, BW_335, BW_38, BW_392, BW_478, BW_529, BW_53, BW_543, BW_572, BW_575, BW_598, BW_599, BW_637, BW_651, BW_721, BW_726, BW_731, BW_733, BW_972, BW_991			
				BW_436, BW_799, BW_800, BW_979 BW_1048, BW_196, BW_435, BW_462, BW_847, BW_854,					

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
				BW_867, BW_941					
41	**Woo sp. 41 10.15156/BIO/SH231372.07FU**	**1**	BW_377	BW_377	BW_377	BW_377		-	-
42	**Woo sp. 42 10.15156/BIO/SH246690.07FU**	**1**	BW_1047	BW_1047	BW_1047	BW_1047		cream	mild
43	**nauseosa†/puellaris† 10.15156/BIO/SH246703.07FU**	**5**	BW_1015, BW_612, BW_644, BW_861, BW_935	BW_1015, BW_612, BW_644, BW_861, BW_935	BW_1015, BW_612, BW_644, BW_935	BW_1015, BW_612, BW_644, BW_861, BW_935		yellow (2), cream yellow (1), cream (1)	mild (4), slightly hot (1)
44	**versicolor† 10.15156/BIO/SH299757.07FU**	**1**	BW_341	BW_341	BW_341	BW_341		ochre	mild
45	**Woo sp. 45 10.15156/BIO/SH246656.07FU**	**4**	BW_1049, BW_1063, BW_508, BW_802	BW_1049, BW_508, BW_802 BW_1063	BW_1049, BW_1063, BW_508	BW_1049, BW_1063, BW_802 BW_508		cream (3)	mild (4)
46	**olivina† 10.15156/BIO/SH246688.07FU**	**3**	BW_154, BW_248, BW_27	BW_154, BW_248, BW_27	BW_154	BW_154, BW_248, BW_27		ochre (2), yellow (1)	mild (2)
47	**graminea† 10.15156/BIO/SH228737.07FU**	**14**	BW_1040, BW_518, BW_561, BW_582, BW_641, BW_713, BW_821, BW_823, BW_885, BW_927, BW_945, BW_946,	BW_1040, BW_518, BW_561, BW_582, BW_641, BW_713, BW_821, BW_823, BW_885, BW_927, BW_945, BW_946,	BW_1040	BW_1040, BW_518, BW_561, BW_582, BW_641, BW_713, BW_821, BW_823, BW_885, BW_927, BW_945, BW_946,	pos 310: **3r**, 11a; a shared with Woo sp. 48, Woo sp. 49	cream (8), yellow (4), ochre (2)	mild (12), slightly hot (2)

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
			BW_998, BW_999	BW_998, BW_999		BW_998, BW_999			
48	**Woo sp. 48 10.15156/BIO/SH228756.07FU**	**1**	BW_336	BW_336	BW_336	BW_336		yellow	mild
49	**Woo sp. 49 10.15156/BIO/SH228734.07FU**	**1**	BW_750	BW_750	BW_750	BW_750		cream	mild
50	**Woo sp. 50 10.15156/BIO/SH270408.07FU**	**40**	BW_1046, BW_1057, BW_1085, BW_1086, BW_1087, BW_1088, BW_1089, BW_1090, BW_130, BW_169, BW_170, BW_255, BW_263, BW_323, BW_325, BW_342, BW_343, BW_349, BW_357, BW_379, BW_383, BW_390, BW_398, BW_400, BW_451, BW_452,	BW_1046, BW_1057, BW_1085, BW_1086, BW_1087, BW_1088, BW_1089, BW_1090, BW_130, BW_169, BW_170, BW_255, BW_263, BW_323, BW_325, BW_342, BW_343, BW_349, BW_357, BW_379, BW_383, BW_390, BW_398, BW_400, BW_451, BW_452,	BW_1046, BW_255, BW_323, BW_536	BW_1046, BW_1057, BW_1085, BW_1086, BW_1087, BW_1088, BW_1089, BW_1090, BW_130, BW_169, BW_170, BW_255, BW_263, BW_323, BW_325, BW_342, BW_343, BW_349, BW_357, BW_379, BW_383, BW_390, BW_398, BW_400, BW_451, BW_452,	pos 149: **1y**, 2t, 37c - pos 344 **1y**, 39c; c shared with Woo sp. 37 (53), Woo sp. 38 (54) - pos 367: **7y**, 2t, 31c; c shared with Woo sp. 37 (53), Woo sp. 38 (54)	yellow (17), cream (13), ochre (2), cream yellow (2), white cream (1), white (1)	mild (34), mild slightly acrid (1), slightly hot (1), very hot (1)

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
			BW_532, BW_535, BW_536, BW_538, BW_539, BW_540, BW_658, BW_659, BW_662, BW_729, BW_803, BW_842, BW_852, BW_994	BW_532, BW_535, BW_536, BW_538, BW_539, BW_540, BW_658, BW_659, BW_662, BW_729, BW_803, BW_842, BW_852, BW_994		BW_532, BW_535, BW_536, BW_538, BW_539, BW_540, BW_658, BW_659, BW_662, BW_729, BW_803, BW_842, BW_852, BW_994			
51	**Woo sp. 51 10.15156/BIO/SH228768.07FU**	**2**	BW_373, BW_933	BW_373, BW_933	BW_373		pos 154: **1y**, 24t; t shared with Woo sp. 52	yellow (2)	mild (1), slightly acrid (1)
52	**Woo sp. 52 10.15156/BIO/SH228736.07FU**	**25**	BW_1021, BW_1035, BW_1041, BW_1042, BW_1043, BW_177, BW_276, BW_305, BW_369, BW_494, BW_495, BW_526, BW_527, BW_597, BW_762, BW_767, BW_786, BW_849,	BW_1021, BW_1035, BW_1041, BW_1042, BW_1043, BW_177, BW_276, BW_305, BW_369, BW_494, BW_495, BW_526, BW_527, BW_597, BW_762, BW_767, BW_786, BW_849,	BW_1021		pos 150: **1y**, 24c; c shared with Woo sp. 50, Woo sp. 51	yellow (14), cream (5), cream yellow (2), yellow ochre (2), ochre (1)	mild (22), slightly hot (1)

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
			BW_850, BW_868, BW_951, BW_952, BW_953, BW_98, BW_985	BW_850, BW_868, BW_951, BW_952, BW_953, BW_98, BW_985					
53	**zelleri*** **10.15156/BIO/SH228735.07FU**	**16**	BW_1013, BW_1026, BW_1027, BW_482, BW_501, BW_678, BW_855, BW_857, BW_862, BW_889, BW_896, BW_897, BW_937, BW_942, BW_943, BW_978, JF834326	BW_1013, BW_1026, BW_1027, BW_482, BW_501, BW_678, BW_855, BW_857, BW_862, BW_896, BW_897, BW_937, BW_942, BW_943, BW_978, JF834326	BW_1013, BW_889	BW_1013, BW_1026, BW_1027, BW_482, BW_501, BW_678, BW_855, BW_857, BW_862, BW_889, BW_896, BW_897, BW_937, BW_942, BW_943, BW_978, JF834326 BW_1021, BW_1035, BW_1041, BW_1042, BW_1043, BW_177, BW_276, BW_305, BW_369, BW_494, BW_495, BW_526,	pos 139: 3c, 13t; t shared with Woo sp. 51, Woo sp. 52 - pos 155: **2m**, 14c; c shared with Woo sp. 51, Woo sp. 52 - pos 191: 1a, 15g; g shared with Woo sp. 51, Woo sp. 52 - pos 338: **1k**, 15g; g shared with Woo sp. 51, Woo sp. 52	yellow (8), ochre (3), cream (2), cream yellow (2)	mild (15)

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
						BW_527, BW_597, BW_762, BW_767, BW_786, BW_849, BW_850, BW_868, BW_951, BW_952, BW_953, BW_98, BW_985 BW_373, BW_933			
				BW_889					
54	**Woo sp. 54 10.15156/BIO/SH227482.07FU**	**2**	BW_284, BW_775	BW_284, BW_775	BW_284, BW_775	BW_284, BW_775	pos 296: **1y**, 1t	yellow (2)	medium hot (1), mild (1)
55	**firmula† 10.15156/BIO/SH252013.07FU**	**7**	BW_114, BW_225, BW_226, BW_863, BW_864, BW_936, BW_981	BW_114, BW_225, BW_863, BW_864, BW_936, BW_981	BW_114	BW_114	pos 141: **1m**, 6a; a shared with Woo sp. 54 - pos 180: **1k**, 6t - pos 193: **5w**, 1a, 1t - pos 249: **4y**, 2t, 1c - pos 481: **4r**, 3a - pos 482: **2r**, 3a, 2g - pos 483: **2m**, 2a	yellow (5), cream (2)	medium hot (2), slightly hot (2), mild (1), very hot (1)
						BW_225			
				BW_226		BW_226			
						BW_863			
					BW_981	BW_864, BW_981			

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
					BW_936	BW_936			
56	**Woo sp. 56 10.15156/BIO/SH299755.07FU**	**1**	BW_964	BW_964	BW_964			cream	mild
57	**vinososordida† 10.15156/BIO/SH299755.07FU**	**9**	BW_1083, BW_198, BW_315, BW_319, BW_34, BW_36, BW_396, BW_87, BW_911	BW_1083, BW_198, BW_315, BW_319, BW_34, BW_36, BW_396, BW_87, BW_911	BW_1083, BW_36 BW_396	BW_1083, BW_198, BW_315, BW_319, BW_34, BW_36, BW_396, BW_87, BW_911, BW_964		cream (4), yellow (3), cream yellow (2)	mild (8), mild slightly acrid (1), slightly acrid (1)
58	**Woo sp. 58 SH568905.07FU**	**6**	BW_414, BW_426, BW_627, BW_692, BW_693, BW_780, BW_787	BW_414, BW_426, BW_627, BW_692, BW_693, BW_780, BW_787	BW_414, BW_780	BW_414, BW_426, BW_627, BW_692, BW_693, BW_780, BW_787		yellow (2), white (1), cream (1), ochre (1)	mild (5)
59	**viridofusca* 10.15156/BIO/SH251973.07FU**	**31**	BW_1000, BW_1001, BW_1003, BW_1010, BW_1017, BW_1018, BW_1019, BW_1020, BW_1030, BW_1031, BW_1066, BW_1084, BW_145, BW_222, BW_512,	BW_1000, BW_1001, BW_1003, BW_1010, BW_1017, BW_1019, BW_1020, BW_1030, BW_1031, BW_1066, BW_1084, BW_145, BW_222, BW_512, BW_555,	BW_1000, BW_1018, BW_1084, BW_605, BW_831, BW_923, KJ748434	BW_1000, BW_1001, BW_1003, BW_1010, BW_1017, BW_1018, BW_1019, BW_1020, BW_1030, BW_1031, BW_1066, BW_1084, BW_145, BW_222, BW_512,	pos 141: **1y**, 30 t; t shared with xerampelina (61) - pos 149: 1c, 30a; a shared with xerampelina - pos 150: **1m**, 30c; c shared with xerampelina - pos 269: 1c, 30t; t shared with	cream (23), white (3), ochre (2), white cream (1)	mild (26), slightly hot (1), slightly acrid (1)

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
			BW_555, BW_557, BW_605, BW_607, BW_611, BW_687, BW_709, BW_738, BW_811, BW_813, BW_831, BW_918, BW_923, BW_929, BW_962, BW_975, KJ748434	BW_557, BW_605, BW_607, BW_611, BW_687, BW_709, BW_811, BW_813, BW_831, BW_918, BW_923, BW_929, BW_962, BW_975, KJ748434		BW_555, BW_557, BW_605, BW_607, BW_611, BW_687, BW_709, BW_738, BW_811, BW_813, BW_831, BW_918, BW_923, BW_929, BW_962, BW_975, KJ748434, BW_1077, BW_514, BW_628, BW_698, BW_901, BW_909, BW_938, BW_1050, BW_244, BW_272, BW_389, BW_55, BW_617, BW_652, BW_841, BW_879, BW_882, BW_887, BW_916,	xerampelina - pos 447: **1k**, 30g; g shared with xerampelina		

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
				BW_1018 BW_738		BW_950, BW_910			
60	**Woo sp. 60** **10.15156/BIO/SH251968.07FU**	**8**	BW_1077, BW_514, BW_628, BW_698, BW_901, BW_909, BW_938	BW_1077, BW_514, BW_628, BW_698, BW_901, BW_909, BW_938	BW_1077		pos 181: **1m**, 7c; c shared with xerampelina (61) - pos 193: **1r**, 7g	cream (5), cream yellow (1), yellow (1)	mild (7)
61	**xerampelina†** **10.15156/BIO/SH251969.07FU**	**13**	BW_1050, BW_244, BW_272, BW_389, BW_55, BW_617, BW_652, BW_841, BW_879, BW_882, BW_887, BW_916, BW_950, BW_910	BW_1050, BW_244, BW_272, BW_389, BW_55, BW_617, BW_652, BW_841, BW_879, BW_882, BW_887, BW_916, BW_950, BW_910	BW_1050			cream (6), yellow (5), ochre (1)	mild (11), very hot (1)

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
62	**mordax*** **10.15156/BIO/SH227461.07FU**	**68**	AF335442, BW_100, BW_1028, BW_1032, BW_1034, BW_1038, BW_1060, BW_1078, BW_1081, BW_1082, BW_148, BW_158, BW_162, BW_166, BW_167, BW_188, BW_230, BW_246, BW_254, BW_258, BW_259, BW_271, BW_275, BW_288, BW_295, BW_345, BW_365, BW_387, BW_393, BW_405, BW_424, BW_45, BW_450, BW_457, BW_458, BW_517,	AF335442, BW_1028, BW_1032, BW_1034, BW_1038, BW_1060, BW_1078, BW_1082, BW_148, BW_158, BW_162, BW_166, BW_167, BW_188, BW_230, BW_246, BW_254, BW_258, BW_259, BW_271, BW_275, BW_288, BW_295, BW_345, BW_365, BW_387, BW_393, BW_405, BW_424, BW_45, BW_450, BW_457, BW_458, BW_517, BW_549, BW_569,	AF335442, BW_100, BW_1028, BW_1081, BW_1082, BW_158, BW_288, BW_295, BW_393, BW_672, BW_718	AF335442, BW_100, BW_1028, BW_1032, BW_1034, BW_1038, BW_1060, BW_1078, BW_1081, BW_1082, BW_148, BW_158, BW_162, BW_166, BW_167, BW_188, BW_230, BW_246, BW_254, BW_258, BW_259, BW_271, BW_275, BW_288, BW_295, BW_345, BW_365, BW_387, BW_393, BW_405, BW_424, BW_45, BW_450, BW_457, BW_458, BW_517,	pos 141: 2g, 64a - pos 154: 1c, 65t - pos 373: 37t, 29c	cream (41), yellow (17), cream yellow (4), white cream (1), ochre (1)	very hot (26), medium hot (20), mild (8), slightly hot (6), slightly acrid (2), mild then hot (1), mild slightly acrid (1), bitter (1), slightly hot medium hot (1)

Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
		BW_549, BW_569, BW_573, BW_574, BW_609, BW_654, BW_656, BW_657, BW_671, BW_672, BW_691, BW_701, BW_705, BW_714, BW_715, BW_718, BW_720, BW_723, BW_736, BW_741, BW_818, BW_826, BW_878, BW_88, BW_884, BW_891, BW_924, BW_925, BW_960, BW_966, BW_968, BW_977, BW_997	BW_573, BW_574, BW_609, BW_654, BW_656, BW_657, BW_671, BW_672, BW_691, BW_701, BW_705, BW_714, BW_715, BW_720, BW_723, BW_736, BW_741, BW_818, BW_826, BW_878, BW_88, BW_884, BW_891, BW_924, BW_925, BW_960, BW_966, BW_968, BW_977, BW_997 BW_100 BW_1081 BW_718		BW_549, BW_569, BW_573, BW_574, BW_609, BW_654, BW_656, BW_657, BW_671, BW_672, BW_691, BW_701, BW_705, BW_714, BW_715, BW_718, BW_720, BW_723, BW_736, BW_741, BW_818, BW_826, BW_878, BW_88, BW_884, BW_891, BW_924, BW_925, BW_960, BW_966, BW_968, BW_977, BW_997			

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
63	**vinosa† 10.15156/BIO/SH227454.07FU**	**9**	BW_1071, BW_1072, BW_1080, BW_234, BW_302, BW_350, BW_822, BW_894, BW_903	BW_1071, BW_1072, BW_1080, BW_234, BW_302, BW_350, BW_822, BW_894, BW_903	BW_1071, BW_350, BW_903	BW_1071, BW_1072, BW_1080, BW_234, BW_302, BW_350, BW_822, BW_894, BW_903		cream (7)	mild (8)
64	**velenovskyi† 10.15156/BIO/SH251965.07FU**	**1**	BW_425	BW_425	BW_425	BW_425		cream	mild
65	**Woo sp. 65 10.15156/BIO/SH299800.07FU**	**2**	BW_150, BW_819	BW_150, BW_819	BW_150	BW_150, BW_819		cream (1), yellow (1)	mild (2)
66	**sierrensis* 10.15156/BIO/SH299855.07FU**	**12**	BW_1058, BW_1064, BW_194, BW_251, BW_384, BW_427, BW_531, BW_57, BW_653, BW_782, BW_917, BW_983, JF834336	BW_1058, BW_1064, BW_194, BW_251, BW_384, BW_427, BW_531, BW_57, BW_653, BW_782, BW_917, BW_983, JF834336	BW_1058, BW_427	BW_1058, BW_1064, BW_194, BW_251, BW_384, BW_427, BW_531, BW_57, BW_653, BW_782, BW_917, BW_983, JF834336		yellow (6), ochre (4), cream (2)	mild (12)

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
67	**Woo sp. 67** **10.15156/BIO/SH299776.07FU**	**60**	BW_1005, BW_1006, BW_1022, BW_1023, BW_1039, BW_1044, BW_1076, BW_1079, BW_113, BW_118, BW_119, BW_178, BW_187, BW_189, BW_220, BW_228, BW_231, BW_233, BW_236, BW_25, BW_269, BW_301, BW_316, BW_317, BW_360, BW_362, BW_408, BW_409, BW_410, BW_411, BW_412, BW_418, BW_419, BW_420, BW_466, BW_480,	BW_1005, BW_1006, BW_1022, BW_1023, BW_1039, BW_1044, BW_1076, BW_1079, BW_118, BW_178, BW_187, BW_189, BW_220, BW_228, BW_231, BW_233, BW_236, BW_25, BW_269, BW_301, BW_316, BW_317, BW_360, BW_362, BW_408, BW_409, BW_410, BW_411, BW_412, BW_418, BW_420, BW_466, BW_480, BW_497, BW_498, BW_499,	BW_1005, BW_187, BW_362, BW_466	BW_1005, BW_1006, BW_1022, BW_1023, BW_1039, BW_1044, BW_1076, BW_1079, BW_113, BW_118, BW_119, BW_178, BW_187, BW_189, BW_220, BW_228, BW_231, BW_233, BW_236, BW_25, BW_269, BW_301, BW_316, BW_317, BW_360, BW_362, BW_408, BW_409, BW_410, BW_411, BW_412, BW_418, BW_419, BW_420, BW_466, BW_480,	pos 127: **1y**, 59t - pos 165: **6y**, 9t, 45c; t shared with Woo sp. 68 - pos 269: **2y**, 5c, 53t; t shared with Woo sp. 68 - pos 296: **3y**, 12c, 45t; t shared with Woo sp. 68	cream (44), yellow (8), white (4), white cream (2)	mild (48), slihgtly acrid (5), slightly hot (4), mild bitter (1), mild slightly hot (1)

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
			BW_497, BW_498, BW_499, BW_553, BW_554, BW_565, BW_578, BW_600, BW_615, BW_618, BW_623, BW_677, BW_689, BW_742, BW_804, BW_805, BW_805A, BW_806, BW_808, BW_816, BW_830, BW_836, BW_838, BW_931	BW_553, BW_554, BW_565, BW_578, BW_600, BW_615, BW_618, BW_623, BW_677, BW_689, BW_742, BW_804, BW_805, BW_805A, BW_806, BW_808, BW_816, BW_830, BW_836, BW_838, BW_931 BW_113 BW_178 BW_480		BW_497, BW_498, BW_499, BW_553, BW_554, BW_565, BW_578, BW_600, BW_615, BW_618, BW_623, BW_66, BW_677, BW_689, BW_742, BW_804, BW_805, BW_805A, BW_806, BW_808, BW_816, BW_830, BW_836, BW_838, BW_931			
68	**Woo sp. 68 10.15156/BIO/SH299776.07FU**	**1**	BW_66	BW_66	BW_66		pos 127: 1k	cream	mild
69	**postiana† 10.15156/BIO/SH242653.07FU**	**2**	BW_253, BW_955	BW_253, BW_955	BW_253 BW_955	BW_253, BW_955	pos 335: **1y**, 1t - pos 339: 1r,1g - pos 344: **1y**, 1t	ochre (1)	mild (1)

	Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
70	**Woo sp. 70 10.15156/BIO/SH299775.07FU**	**9**	BW_1009, BW_172, BW_193, BW_197, BW_534, BW_683, BW_789, BW_890, BW_892	BW_1009, BW_172, BW_193, BW_197, BW_534, BW_683, BW_789, BW_890, BW_892	BW_1009, BW_172, BW_193, BW_890		pos 156: **3w**, 5t	yellow (3), cream (6), ochre (1)	mild (9)
71	**Woo sp. 71 SH299839.07FU**	**3**	BW_1029, BW_973, BW_976	BW_1029, BW_973, BW_976	BW_1029			cream (3)	mild (3)
72	**Woo sp. 72 10.15156/BIO/SH299768.07FU**	**36**	BW_1036, BW_156, BW_159, BW_171, BW_229, BW_232, BW_318, BW_320, BW_327, BW_363, BW_413, BW_489, BW_528, BW_530, BW_639, BW_650, BW_724, BW_80, BW_81, BW_815, BW_835, BW_844, BW_876, BW_880,	BW_1036, BW_156, BW_159, BW_171, BW_229, BW_232, BW_318, BW_320, BW_327, BW_363, BW_413, BW_489, BW_528, BW_530, BW_639, BW_650, BW_724, BW_80, BW_81, BW_835, BW_844, BW_876, BW_880, BW_881,	BW_1036, BW_156, BW_171, BW_232, BW_413, BW_528, BW_639, BW_815, BW_835	BW_1029,BW_973,BW_976, BW_1036, BW_156, BW_159, BW_171, BW_229, BW_232, BW_318, BW_320, BW_327, BW_363, BW_413, BW_489, BW_528, BW_530, BW_639, BW_650, BW_724, BW_80, BW_81, BW_815, BW_835,	pos 413: 27c, 9t - pos 447: 26a, 10g	cream (16), yellow (9)	mild (34)

Species Delimitation	#	ABGD	mothur	GMYC	PTP	polymorphic	Gill Col.	Taste
		BW_881, BW_956, BW_992, KF007951	BW_992, KF007951 BW_815 BW_956		BW_844, BW_876, BW_880, BW_881, BW_956, BW_992 BW_1009, BW_193, BW_197, BW_534, BW_577, BW_683, BW_789, BW_892 BW_193 BW_172, BW_890			

[1]Each cell with an isolate code represents a delimited species. When one or more methods disagreed with the delimitation, specimens that they excluded are highlighted in light gray.

[2]† indicates sequences identical to *Russula* sequences in Europe; * symbolises sequences matched to a type specimen described in the Pacific Northwest. In the case of *R. queletii* and Woo sp. 39, one Species Hypothesis encompassed more than one of our candidate species. Bolded species hypothesis codes represent a 99% or better match. A few instances did not have a DOI, so the SH code is reported.

[3]Polymorphic positions are specified by 'pos' followed by a site number from the alignment. Following a colon, the numeral is the number of specimens with each variant and the letter is the nucleotide observed at the polymorphic site. Only states present in 2 or more conspecific samples are included. The IUPAC codes indicate double peaks. Sequence variants shared with closely related species are specified.

[4]Character states mapped on the phylogeny (Fig. 2) were not invariant within species and the numbers in parentheses are the number of specimens with each alternative character state.

Appendix 1.7 Polymorphisms shared between species

Polymorphisms[1] shared between species. 'AP'= Alignment Position

***R. montana* (sp. 27) and Woo sp. 28**

Specimen	Species	AP[3]		L[2]	W	O	R
Specimen	Species	149	413				
BW_218	*montana*	g	c				
BW_218	*montana*	g	c				
BW_247	*montana*	g	c				
BW_310	*montana*	g	c				
BW_314	*montana*	g	c				
BW_322	*montana*	g	c				
BW_334	*montana*	g	c				
BW_443	*montana*	g	c				
BW_492	*montana*	g	c				
BW_524	*montana*	g	c				
BW_545	*montana*	g	c				
BW_592	*montana*	g	c				
BW_593	*montana*	g	c				
BW_642	*montana*	g	c				
BW_664	*montana*	g	c				
BW_694	*montana*	g	c				
BW_697	*montana*	g	c				
BW_711	*montana*	g	c				
BW_725	*montana*	g	c				
BW_753	*montana*	g	c				
BW_812	*montana*	g	c				
BW_829	*montana*	g	c				
BW_883	*montana*	g	c				
BW_912	*montana*	g	c				
BW_969	*montana*	g	c				
BW_106	Woo sp. 28	a	Y				
BW_191	Woo sp. 28	a	Y				
BW_192	Woo sp. 28	a	c				
BW_202	Woo sp. 28	a	t				
BW_368	Woo sp. 28	a	c				
BW_404	Woo sp. 28	a	t				
BW_428	Woo sp. 28	a	t				

Specimen	Species	AP[1]		L[2]	W	O	R
BW_503	Woo sp. 28	a	T/c				
BW_519	Woo sp. 28	a	t	BCD	B	AB	A
BW_525	Woo sp. 28	a	c	B	C	A	A
BW_547	Woo sp. 28	a	Y	A	B	AB	A
BW_548	Woo sp. 28	a	c	BC	C	A	A
BW_559	Woo sp. 28	a	c	A	A	A	A
BW_649	Woo sp. 28	a	Y	B	C	B	A
Specimen	Species	AP					
		149	413	L	W	O	R
BW_814	Woo sp. 28	a	t	D	D	C	A
BW_873	Woo sp. 28	a	Y	CD	C	AB	A
BW_920	Woo sp. 28	a	t				

***R. queletii* (sp. 40) & Woo sp. 39**

Specimen	Species	141	191	335	339	344	L	W	O	R
BW_1045	Woo sp. 39	a	g	t	g	t	BC	EF	A	AB
BW_1062	Woo sp. 39	a	K	t	g	t	BC	AB	A	AB
BW_176	Woo sp. 39	a	g	t	g	t				
BW_273	Woo sp. 39	a	K	t	g	t				
BW_335	Woo sp. 39	a	K	t	g	t				
BW_38	Woo sp. 39	a	g	t	g	t				
BW_392	Woo sp. 39	a	K	t	g	t				
BW_478	Woo sp. 39	a	t	t	g	t				
BW_529	Woo sp. 39	a	g	t	g	t				
BW_53	Woo sp. 39	a	K	t	g	t				
BW_543	Woo sp. 39	a	K	t	g	t				
BW_572	Woo sp. 39	a	K	t	g	t	EF	G	CD	AB
BW_575	Woo sp. 39	a	G/t	t	g	t	DEF	CDEF	BCD	AB
BW_598	Woo sp. 39	a	t	t	g	t	BCD	EFG	CD	B
BW_599	Woo sp. 39	a	K	t	g	t	B	ABC	BC	AB
BW_637	Woo sp. 39	a	K	t	g	t	A	A	BC	B
BW_651	Woo sp. 39	a	K	t	g	t	CDEF	DEF	CD	AB
BW_721	Woo sp. 39	a	K	t	g	t	B	ABC	CD	AB
BW_726	Woo sp. 39	a	t	t	g	t	F	BCDE	BCD	A
BW_731	Woo sp. 39	a	T/g	t	g	t	BCD	FG	D	B

Specimen	Species	141	191	335	339	344	L	W	O	R
BW_733	Woo sp. 39	a	K	t	g	t	CDE	DEF	ABC	AB
BW_972	Woo sp. 39	a	K	t	g	t	BC	ABCD	AB	AB
BW_991	Woo sp. 39	a	K	t	g	t				
BW_1012	*queletii*	c	g	Y	R	Y	EFG	DEF	A	AB
BW_1016	*queletii*	c	g	T/c	A/g	T/c	CDE	CD	A	AB
BW_1048	*queletii*	c	g	Y	R	Y	EF	DEFG	A	AB
BW_1056	*queletii*	c	g	C/t	A/g	C/t	BC	DE	A	AB
BW_1065	*queletii*	c	g	Y	A/g	C/t	FG	H	AB	AB
BW_124	*queletii*	c	g	Y	R	C/t				
BW_136	*queletii*	c	g	t	g	t				
BW_196	*queletii*	c	g	Y	R	Y				
Specimen	Species	141	191	335	339	344	L	W	O	R
BW_432	*queletii*	c	g	t	g	t				
BW_435	*queletii*	c	g	c	a	c				
BW_436	*queletii*	c	g	Y	R	Y				
BW_439	*queletii*	c	g	t	g	t				
BW_462	*queletii*	c	g	Y	R	Y				
BW_502	*queletii*	c	g	Y	R	Y				
BW_509	*queletii*	c	g	Y	R	Y				
BW_774	*queletii*	c	g	Y	R	Y	BC	BC	A	AB
BW_795	*queletii*	c	g	T/c	G/a	T/c	DEF	EFGH	A	AB
BW_795A	*queletii*	c	g	Y	R	Y	B	A	A	A
BW_798	*queletii*	c	g	c	a	c	FG	GH	AB	AB
BW_799	*queletii*	c	g	c	a	c	FG	EFGH	A	AB
BW_800	*queletii*	c	g	t	g	t	G	FGH	AB	AB
BW_847	*queletii*	c	g	t	g	t				
BW_854	*queletii*	c	g	t	g	t				
BW_867	*queletii*	c	g	t	g	t	EFG	FGH	B	AB
BW_941	*queletii*	c	g	Y	R	Y	A	B	A	B
BW_944	*queletii*	c	g	c	a	c	CD	BCD	A	AB

R. mordax

Specimen	Species	AP	L	W	O	R

Specimen	Species	141	373
BW_100	*mordax*	a	t
BW_1028	*mordax*	a	t

Specimen	Species	AP		L	W	O	R
BW_1032	*mordax*	a	y	AB	AB	C	A
BW_1034	*mordax*	a	c	D	E	BC	A
BW_1038	*mordax*	a	c	A	CDE	ABC	A
BW_1060	*mordax*	a	T/c				
BW_1078	*mordax*	a	t	ABC	AB	ABC	A
BW_1081	*mordax*	a	y				
BW_1082	*mordax*	a	t				
BW_148	*mordax*	a	c				
BW_158	*mordax*	g	c				
BW_162	*mordax*	a	c				
BW_166	*mordax*	a	c				
BW_167	*mordax*	a	T/c				
BW_188	*mordax*	a	c				
BW_230	*mordax*	a	t				
BW_246	*mordax*	a	y				
BW_254	*mordax*	a	y				
BW_258	*mordax*	a	t				
BW_259	*mordax*	a	t				
Specimen	Species	141	373	L	W	O	R
BW_271	*mordax*	a	c				
BW_275	*mordax*	a	T/c				
BW_288	*mordax*	g	y				
BW_295	*mordax*	a	y				
BW_345	*mordax*	a	t				
BW_365	*mordax*	a	T/c				
BW_387	*mordax*	a	c				
BW_393	*mordax*	a	t				
BW_405	*mordax*	a	c				
BW_424	*mordax*	a	y				
BW_45	*mordax*	a	c				
BW_450	*mordax*	a	y				
BW_457	*mordax*	a	c				
BW_458	*mordax*	a	T/c				
BW_517	*mordax*	a	T/c				
BW_549	*mordax*	a	t				
BW_569	*mordax*	a	y				
BW_573	*mordax*	a	c				

Specimen	Species	AP		L	W	O	R
BW_574	*mordax*	a	T/c				
BW_609	*mordax*	a	y				
BW_654	*mordax*	a	c				
BW_656	*mordax*	a	c				
BW_657	*mordax*	a	t				
BW_671	*mordax*	a	T/c				
BW_672	*mordax*	a	T/c				
BW_691	*mordax*	a	c				
BW_701	*mordax*	a	c				
BW_705	*mordax*	a	T/c				
BW_714	*mordax*	a	y				
BW_715	*mordax*	a	y				
BW_718	*mordax*	a	t				
BW_720	*mordax*	a	y				
BW_723	*mordax*	a	t				
BW_736	*mordax*	a	c				
BW_741	*mordax*	a	c				
BW_818	*mordax*	a	T/c				
BW_826	*mordax*	a	t				
BW_878	*mordax*	a	T/c				
BW_88	*mordax*	a	y				
BW_884	*mordax*	a	c				
BW_891	*mordax*	a	c	ABC	BCD	ABC	A
BW_924	*mordax*	a	t				
BW_925	*mordax*	a	y				
Specimen	Species	141	373				
BW_960	*mordax*	a	T/c				
BW_966	*mordax*	a	c	CD	ABC	A	A
BW_968	*mordax*	a	T/c	CD	DE	AB	A
BW_977	*mordax*	a	t	BC	A	A	A

R. Woo sp. 72

Specimen	Species	413	447	L	W	O	R
		AP					
BW_1036	Woo sp. 72	c	g	D	CD	A	A
BW_156	Woo sp. 72	c	a				

Specimen	Species	413	447	L	W	O	R
BW_159	Woo sp. 72	c	g				
BW_171	Woo sp. 72	t	a				
BW_229	Woo sp. 72	c	g				
BW_232	Woo sp. 72	c	g				
BW_318	Woo sp. 72	c	a				
BW_320	Woo sp. 72	c	g				
BW_327	Woo sp. 72	c	g				
BW_363	Woo sp. 72	c	a				
BW_413	Woo sp. 72	t	a				
BW_489	Woo sp. 72	c	g				
BW_528	Woo sp. 72	t	a				
BW_530	Woo sp. 72	c	a				
BW_639	Woo sp. 72	c	g				
BW_650	Woo sp. 72	C/t	a				
BW_724	Woo sp. 72	y	a				
BW_80	Woo sp. 72	c	r				
BW_81	Woo sp. 72	c	a				
BW_815	Woo sp. 72	t	a				
BW_835	Woo sp. 72	y	a	CD	CD	A	A
BW_844	Woo sp. 72	y	r	BC	BCD	A	A
BW_876	Woo sp. 72	T/c	a	B	ABC	A	A
BW_880	Woo sp. 72	c	a	BC	CD	A	A
BW_881	Woo sp. 72	t	a	AB	AB	A	A
BW_956	Woo sp. 72	t	g	A	A	A	A
BW_992	Woo sp. 72	t	r	CD	D	A	A

R. Woo sp. 70

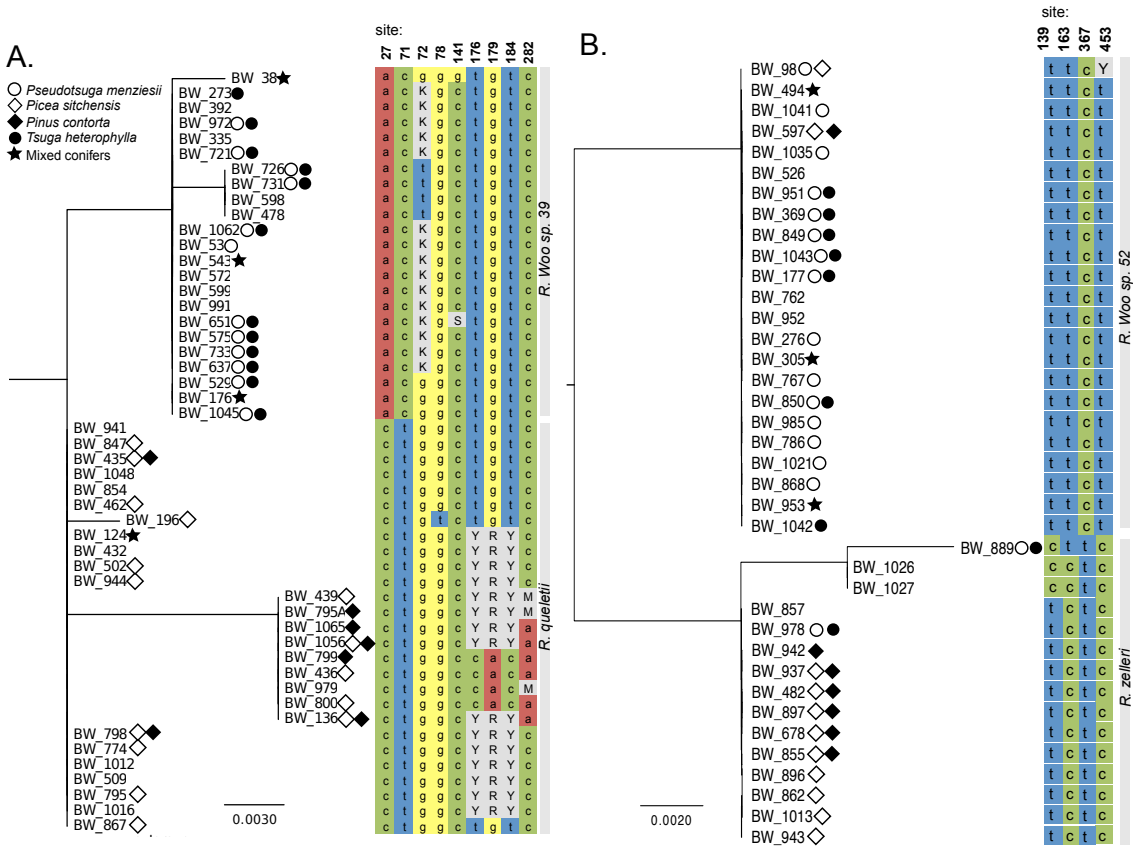
Specimen		156	279	347	413
BW_1009	Woo sp. 70	t	c	c	c
BW_197	Woo sp. 70	t	c	c	c
BW_534	Woo sp. 70	W	c	c	c
BW_892	Woo sp. 70	t	c	c	c
BW_683	Woo sp. 70	t	c	c	c
BW_789	Woo sp. 70	t	c	c	c
BW_172	Woo sp. 70	W	t	t	t
BW_890	Woo sp. 70	W	t	t	t

[1] Closely related species pairs *R. queletii* and Woo sp. 39; Woo sp. 26 and *R. montana;* and *R. mordax* and Woo sp. 70 and Woo sp. 72 shared no double peaks. The capital letter indicates which one of the two peaks was the larger.

[2] 'AP' = Alignment position

[3] 'L' = length, 'W' = width, 'R' = ratio, 'O' = ornamentation. Groups based on the LSD test show that they significantly different groups do not correspond to different polymorphisms (an in-depth example of *R.* Woo sp. 52 is shown in Fig. S16).

Appendix 1.8 Species pairs associated with distinct hosts



Distinctions among hosts, sequenced variable sites, and the lack of shared sequence polymorphisms support recognition of some closely related taxa as different species. Phylogeny of samples of **A,** *R. queletii* and Woo sp. 39, and **B**, *R. zelleri* and Woo sp. 52 with corresponding variable sites and polymorphisms. Polymorphisms are indicated by IUPAC ambiguity codes. The legend and symbols give putative hosts recorded by Woo.

Appendix 1.9 Macromorphological character Retention Index

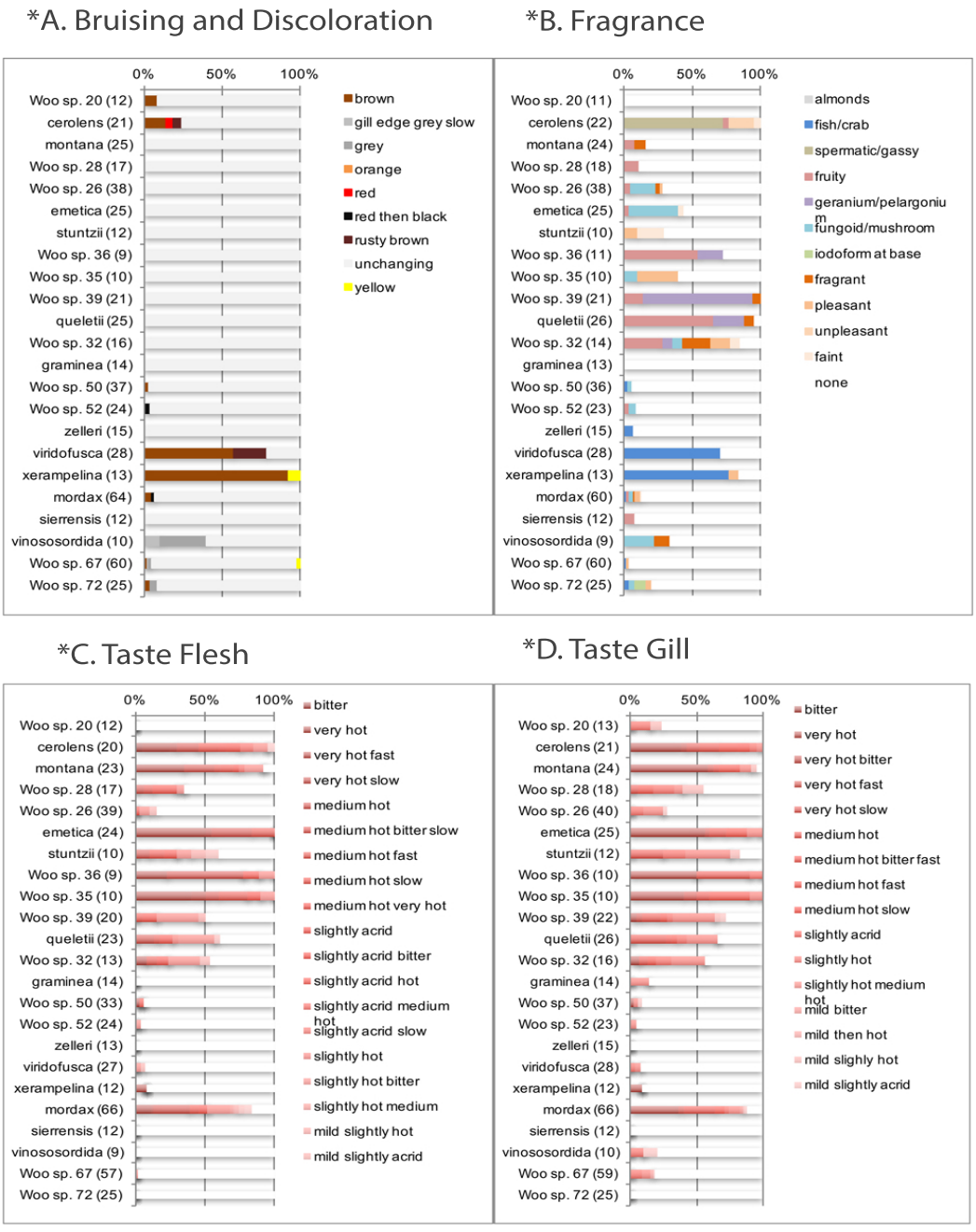
RI values for 36 macromorphological characters and chemical spot tests calculated in Mesquite (3.1). Characters state definitions follow Woo's records.

Character	RI
Taste Flesh	0.69
Taste Gills	0.68
Sulfovanillin	0.49
Pileus Margin	0.45
Gill Colour	0.45
Sulfoformol	0.45
Stipe Colour – flush/stain	0.42
Bruising	0.40
Fragrance	0.37
Stipe Length	0.36
$FeSO_4$	0.35
Spore Print	0.29
Formaldehyde	0.29
SV cystidia	0.27
Pileus Surface Dry	0.26
Gill Width	0.25
Stipe Stature	0.24
Pileus Cuticle Peeling	0.24
PDAB on stipe	0.22
Aniline Oil	0.22
Gill Spacing	0.20
Guaiacol	0.20
Gill Forks	0.20
Gill Subgills	0.18
Stipe Colour	0.16
Stipe Flesh	0.16
Stipe Shape	0.16
Phenol	0.12
α-naphthol	0.12
Pileus Surface Wet	0.11
Pyrogallol	0.10
Stipe Texture	0.10
Tinct Guaiac	0.09

Character	RI
Phenol Aniline	0.09
Gill Edge	0.00
Most common pileus colour	(N/A)

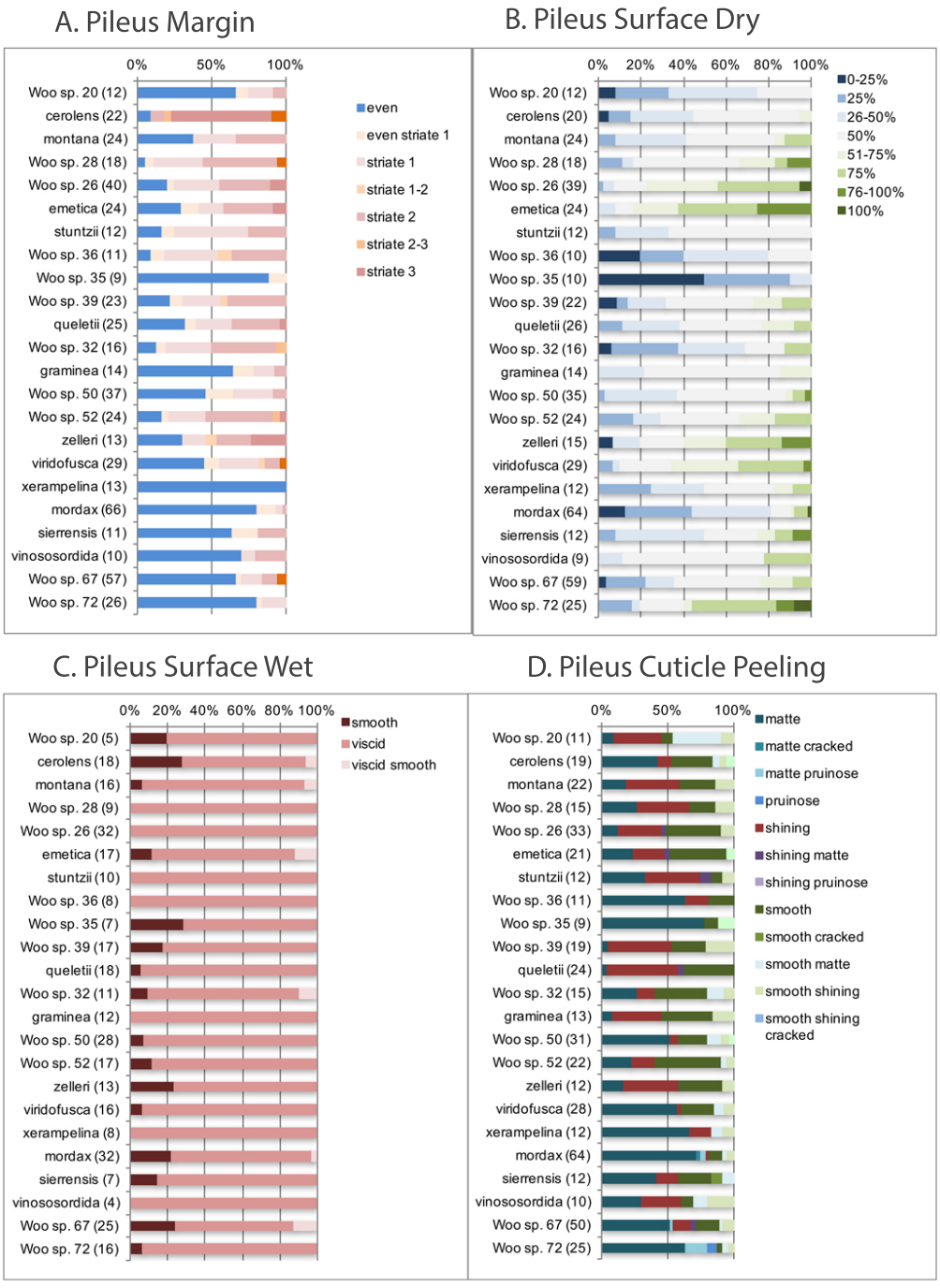
Appendices 1.10-1.17
Bar graphs showing the percentages of specimens scored for each character state, for each species with at least 10 specimens. Asterisks (*) around the title name indicate characters that are potentially useful for identification. The number of specimens scored for each character, given in parentheses, may be considered in interpreting reliability. For example, stipe texture was wrinkled in 100% of *R. zelleri* but it was only recorded for two samples so it may not be consistent within the species. We coded apparently contradictory character states from the Woo collection sheets such as 'few common' or 'pruinose matte' as separate, distinct character states but these likely represent variation among different fruiting bodies in a collection.

Appendix 1.10 Bruising, fragrance, and taste



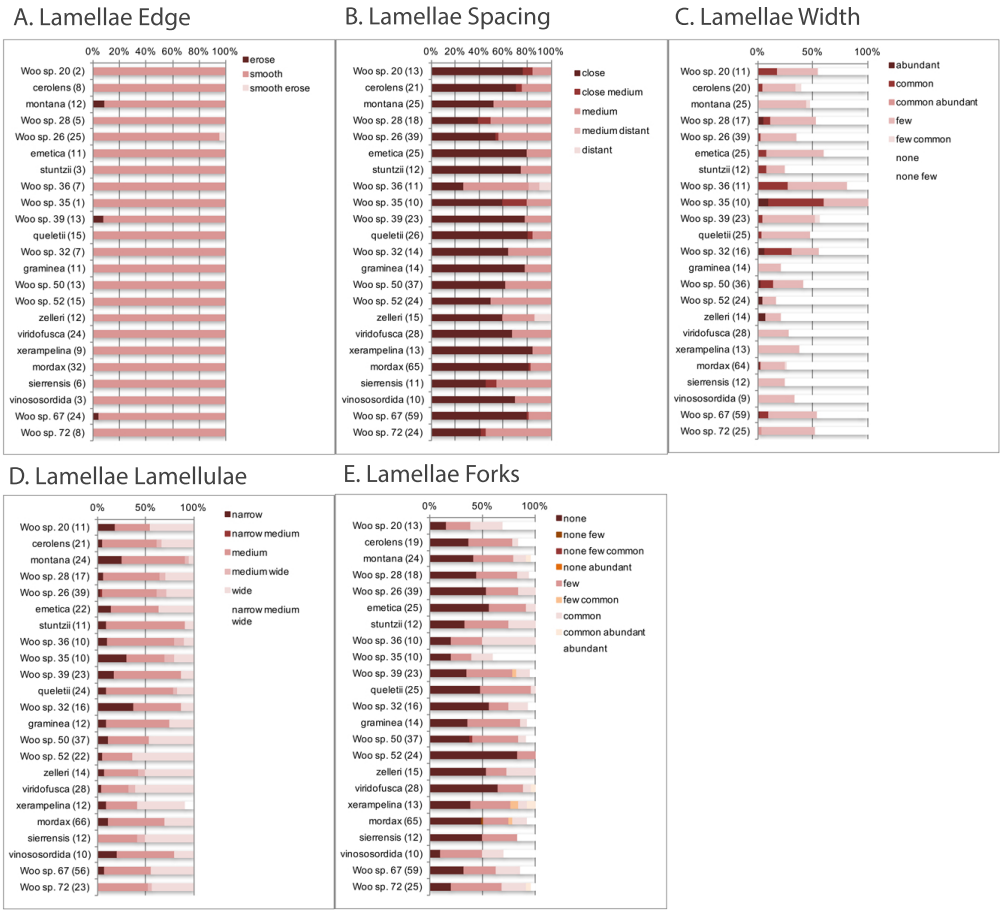
Bruising, fragrance and taste characters

Appendix 1.11 Pileus characters



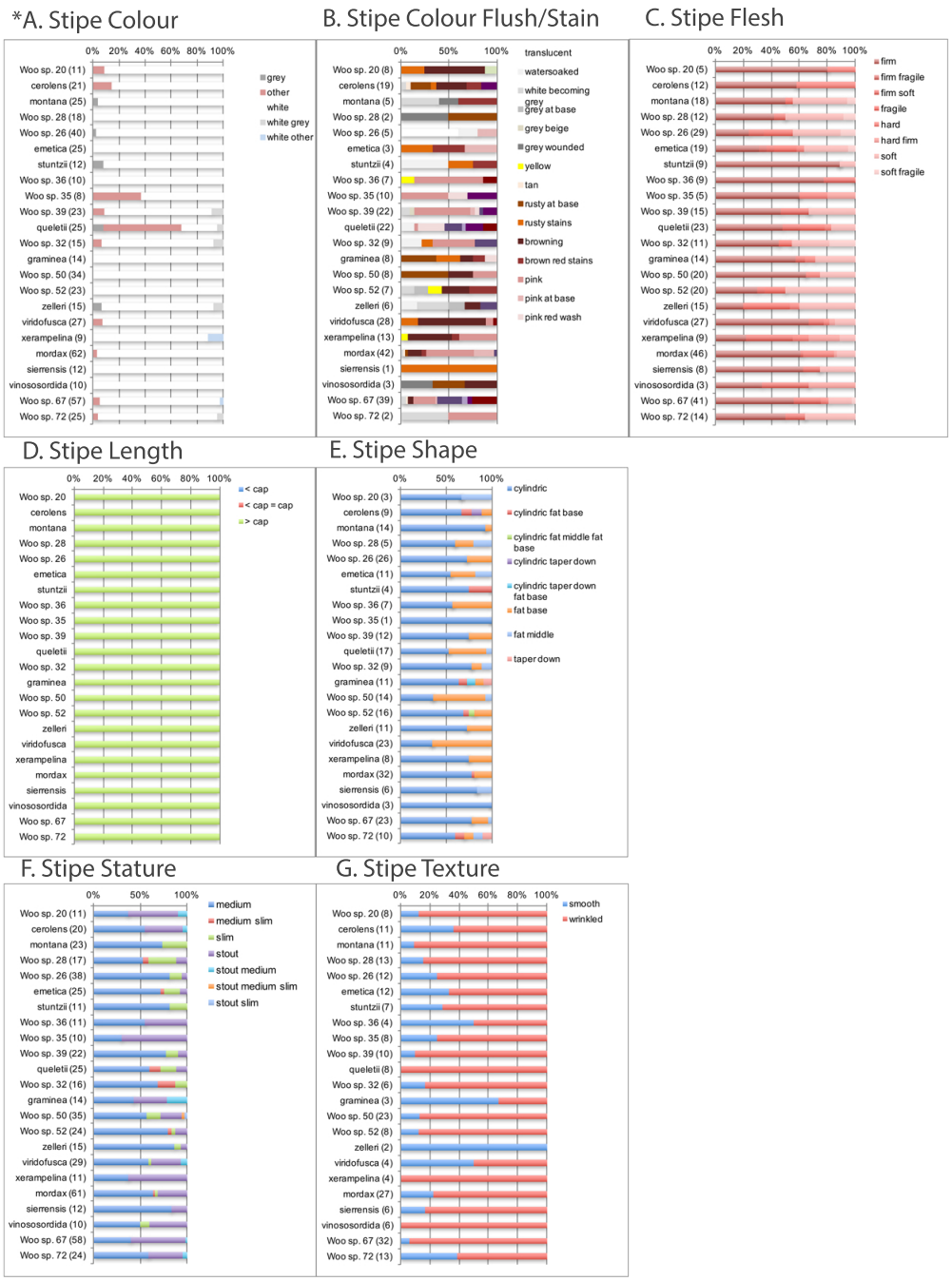
A. 'Pileus margin' refers to whether the edge of the cap is smooth ('even'), or has perpendicular lines radiating at the edge ('striate'); **B.** Pileus surface dry – appearance of the cap in dry weather; **C.** Pileus surface wet – documenting that almost all specimens are 'viscid' when wet; **D.** Pileus cuticle peeling – the cuticle could be peeled off from about half of the cap in most species, with considerable variation from specimen to specimen.

Appendix 1.12 Lamellae characters



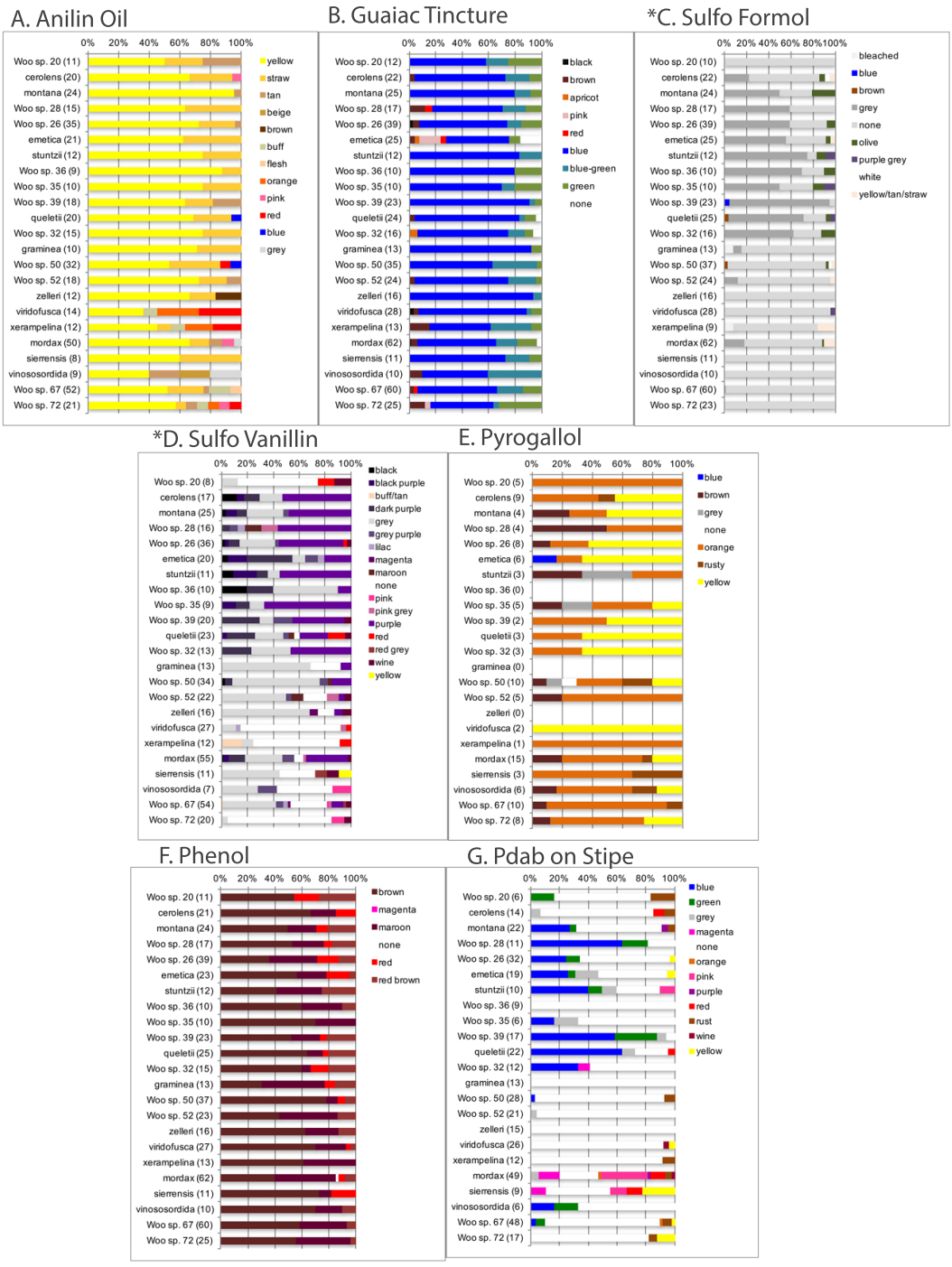
Lamellae characters.

Appendix 1.13 Stipe characters



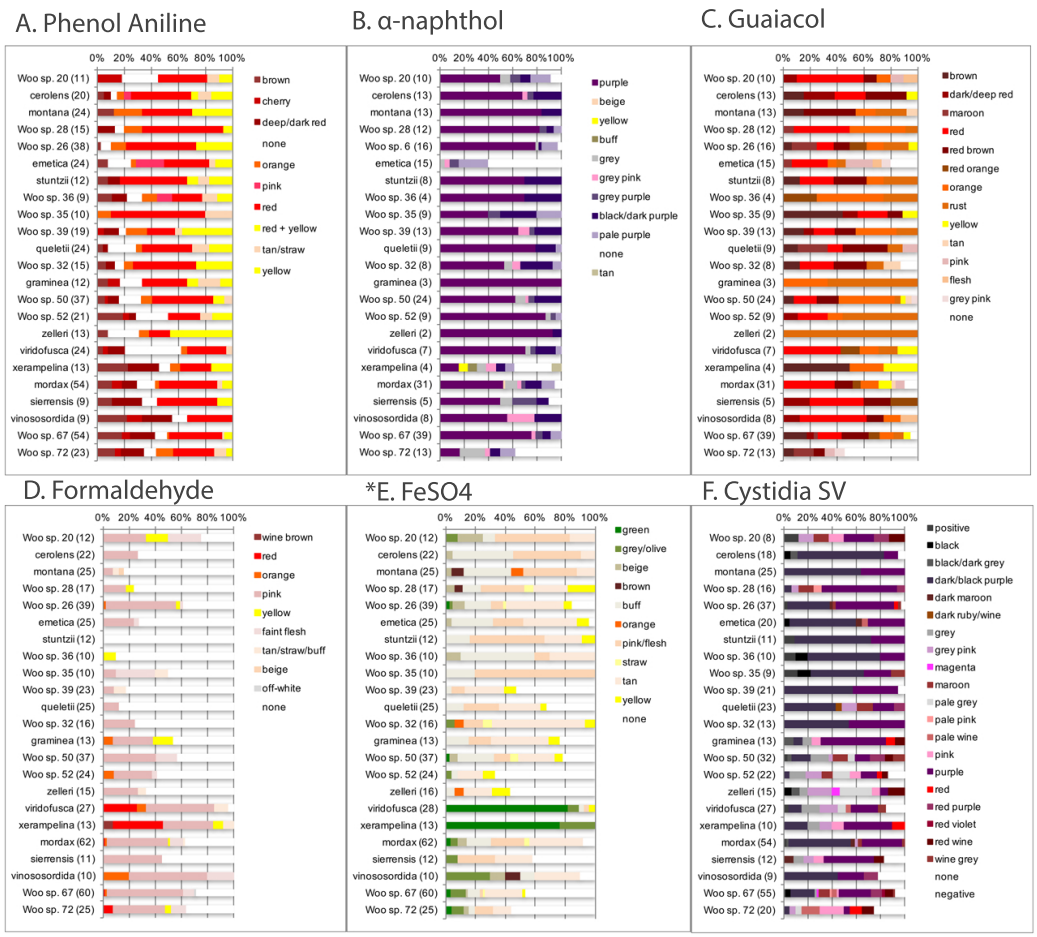
Stipe characters.

Appendix 1.14 Chemical spot tests 1



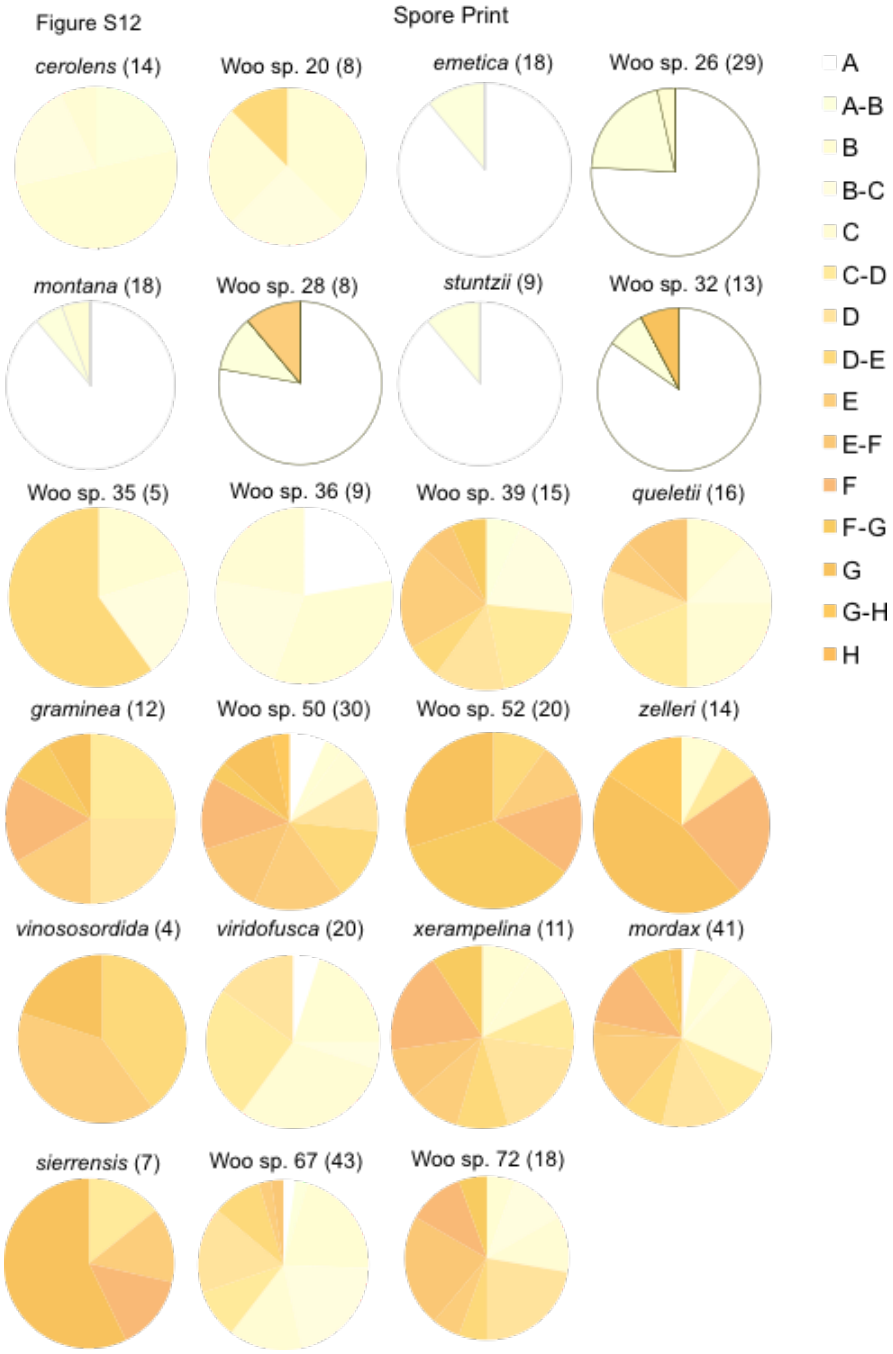
Chemical characters (1); colour changes in the mushroom flesh in response to a spot of the chemical. See Table S6 for chemical names and formulas.

Appendix 1.15 Chemical spot tests 2



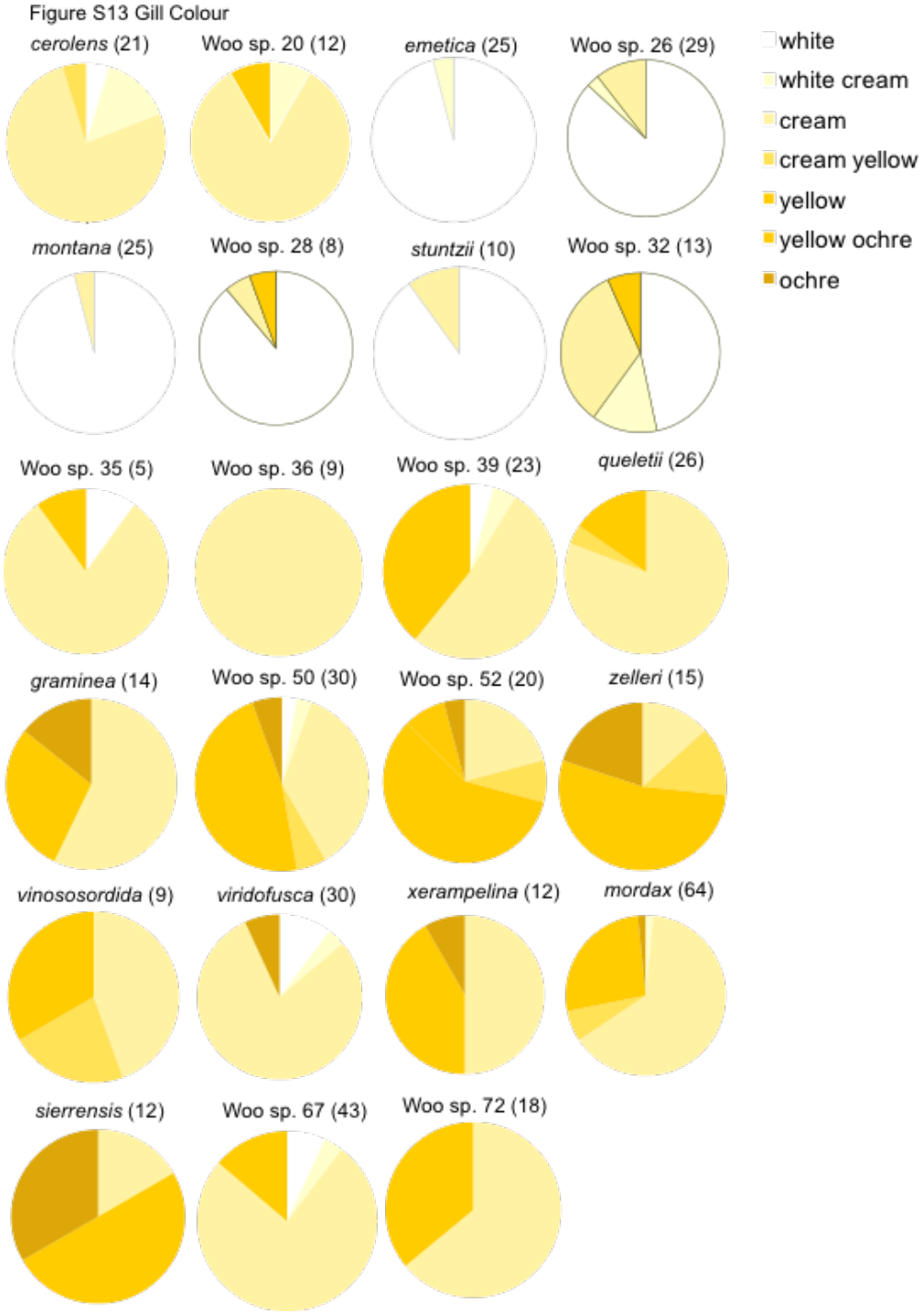
Chemical characters (2); colour changes in the mushroom flesh in response to a spot of the chemical. See Table S6 for chemical names and formulas.

Appendix 1.16 Spore print



Variation in spore print colours among conspecific collections of *Russula*. Each pie chart represents one species with 10 or more specimens. Width of a coloured section is proportional to the fraction of specimens that shared the same spore print colour. The number of specimens is in parentheses and follows the specific epithet or species code. Intensity of colour in the figure approximates the Crawshay (1930) code in the collection record for each specimen. For more accurate colour representation, see the original field notes and Crawshay's colour chips.

Appendix 1.17 Gill colour



Variation in gill colours among conspecific collections of *Russula*. Each pie chart represents one species with 10 or more specimens. Width of a coloured section is proportional to the fraction of specimens that shared the same gill colour. Intensity of colour in the figure approximates the Crawshay (1930) code in the collection record for each specimen. For more accurate colour representation, see the original field notes and Crawshay's colour chips.

Appendix 1.18 Characters states

Character states scored as being present in the majority of specimens in species represented by 10 or more specimens.

Supp. Fig.	Character	RI	Woo sp. 20	cerolens	montana	Woo sp. 28	Woo sp. 26	emetica	stuntzii
1.10 A	**Bruising**	0.40	unchanging	unchanging	unchanging	unchanging	unchanging	unchanging	unchanging
1.10 B	**Fragrance**	0.37	none	spermatic/gassy	none	none	none	none	none
1.10 C	**Taste - Flesh**	0.69	mild	hot	hot	mild/hot	mild	hot	mild/hot
1.10 D	**Taste - Gills**	0.68	mild	hot	hot	mild	mild	hot	hot
1.11 A	**Pileus - Margin**	0.23	even	striate	striate	striate	striate	striate	striate
1.11 B	**Pileus - Surface dry**	0.26							
1.11 C	**Pileus - Surface wet**	0.11	viscid	viscid	viscid	viscid	viscid	viscid	viscid
1.11 D	**Pileus - cuticle peeling**	0.24	0-25%	0-75%	25-75%	25-100%	25-100%	25-100%	25-50%
1.12 A	**Gill - edge**	0.00							
1.12 B	**Gill - Spacing**	0.20	close	close	close/madium	close/madium	close/madium	close	close
1.12 C	**Gill - Width**	0.25							
1.12 D	**Gill - Forks**	0.20							
1.12 E	**Gill - Sublamellae**	0.18	none	none	none	none	none	none	none
1.13 A	**Stipe - Colour**	0.16	white	white	white	white	white	white	white
1.13 B	**Stipe - Colour Flush/Stain**	0.42							
1.13 C	**Stipe - Flesh**	0.16							
1.13 D	**Stipe - Length**	0.36							
1.13 E	**Stipe - Shape**	0.16	cylindric	cylindric	cylindric	cylindric	cylindric	cylindric	cylindric
1.13 F	**Stipe - Stature**	0.24	medium	medium	medium	medium	medium	medium	medium
1.13 G	**Stipe - Texture**	0.10							
1.14 A	**Aniline Oil**	0.22	yellow	yellow	yellow	yellow	yellow	none	yellow
1.14 B	**Tinct Guaiac**	0.09							
1.14 C	**Sulfoformol**	0.45	none	none	grey	grey	grey	grey	grey

Appendix	Character	RI	Woo sp. 20	cerolens	montana	Woo sp. 28	Woo sp. 26	emetica	stuntzii
1.14 D	**Sulfovanillin**	0.49	none	black purple	black purple	black purple	black purple	black purple	black purple
1.14 E	**pyrogallol**	0.10	orange			orange	yellow	yellow	
1.14 F	**phenol**	0.12							
1.14 G	**pdab on stipe**	0.23	none	none	none	blue	none	none	none
1.15 A	**phenol aniline**	0.09							
1.15 B	**α-naphthol**	0.12	buff	buff	buff	buff	buff	none	buff
1.15 C	**guaiacol**	0.20							
1.15 D	**formaldehyde**	0.29	none	none	none	none	pink/red	none	none
1.15 E	**$FeSO_4$**	0.35	orange and none	orange and none	orange and none	orange and none	orange and none	orange and none	orange and none
1.15 F	**cystidia in SV**	0.27							
Fig. 2.3	**approx cap colour**	**N/A**	green shades	brown shades	yellow (red)	red-violet-brown	red-violet-brown	yellow red	pale purple grey
Fig. 1.16	**approx spore colour**	0.29	pale	pale	white	white	white	white	white
Fig. 1.17	**approx gill colour**	0.45	cream	cream	white	white	white	white	white

Character	Woo sp. 36	Woo sp. 35	Woo sp. 39	queletii	Woo sp. 32	graminea	Woo sp. 50	Woo sp. 52
Bruising	unchanging	unchanging	unchanging	unchanging	unchanging	unchanging	unchanging	unchanging
Fragrance	fruity/pelargonium	none	fruity/pelargonium	fruity/pelargonium	none	none	none	none
Taste - Flesh	hot	hot	mild/hot	mild/hot	mild/hot	mild	mild	mild
Taste - Gills	hot	hot	hot	hot	hot	mild	mild	mild
Pileus - Margin	striate	even	striate	striate	striate	even	striate	striate
Pileus - Surface dry	matte	matte						
Pileus - Surface wet	viscid	viscid	viscid	viscid	viscid	viscid	viscid	viscid
Pileus - cuticle peeling	0-50%	0-50%	0-75%	25-75%	0-75%	25-75%	25-100%	25-75%
Gill - edge								
Gill - Spacing		close	close	close	close	close	close	close/medium
Gill - Width								
Gill - Forks								
Gill - Sublamellae	few + common	few + common	none	none	none	none	none	none
Stipe - Colour	white	white	white	other	white	white	white	white
Stipe - Colour Flush/Stain								
Stipe - Flesh								
Stipe - Length								
Stipe - Shape	cylindric	cylindric	cylindric	cylindric	cylindric	cylindric	cylindric	cylindric
Stipe - Stature	medium	stout	medium	medium	medium	medium	medium	medium
Stipe - Texture								
Aniline Oil	yellow	yellow	yellow	yellow	yellow	yellow	yellow	yellow
Tinct Guaiac								
Sulfoformol	grey	grey	grey	grey	grey	none	none	none

Character	Woo sp. 36	Woo sp. 35	Woo sp. 39	queletii	Woo sp. 32	graminea	Woo sp. 50	Woo sp. 52
Sulfovanillin	grey/black purple	black purple	black purple	black purple	grey	grey	grey	grey
pyrogallol				yellow	yellow			orange
phenol								
pdab on stipe	none	none	blue	blue	none	none	none	none
phenol aniline								
α-naphthol	buff	buff	buff	buff	buff	buff	buff	buff
guaiacol						none		none
formaldehyde	none	none	none	none	none	none	none	none
$FeSO_4$	orange and none	orange and none	orange and none	orange and none	orange and none	orange and none	orange and none	orange and none
cystidia in SV								
approx cap colour	red violet	red (violet)	red-violet-yellow	red-violet-brown	red-violet-brown-yellow	green shades	violet-brown	red-violet-brown
approx spore colour	pale	pale-dark	pale-dark	pale-dark	white	dark	dark	dark
approx gill colour	cream	cream	cream	cream	white	cream	yellow	yellow

Character	zelleri	viridofusca	xerampelina	mordax	sierrensis	vinososordida	Woo sp. 67	Woo sp. 72
Bruising	unchanging	brown/rusty brown	brown/rusty brown	unchanging	unchanging	unchanging	unchanging	unchanging
Fragrance	none	crab/fish	crab/fish	none	none	none	none	none
Taste - Flesh	mild	mild	mild	hot	mild	mild	mild	mild
Taste - Gills	mild	mild	mild	hot	mild	mild	mild	mild
Pileus - Margin	striate	striate	even	even	even	even	even	even
Pileus - Surface dry			matte	matte				matte
Pileus - Surface wet	viscid	viscid	viscid	viscid	viscid	viscid	viscid	viscid
Pileus - cuticle peeling	0-100%	25-100%	25-75%	0-75%	25-100%	25-75%	0-75%	25-100%
Gill - edge								
Gill - Spacing	close	close	close	close	close/medium	close	close	close/medium
Gill - Width								
Gill - Forks								
Gill - Sublamellae	none	none	none	none	none	none	none	none
Stipe - Colour	white	white	white	white	white	white	white	white
Stipe - Colour Flush/Stain								
Stipe - Flesh								
Stipe - Length								
Stipe - Shape	cylindric	fat base	cylindric	cylindric	cylindric	cylindric	cylindric	cylindric
Stipe - Stature	medium	medium	stout	medium	medium	medium	stout	medium
Stipe - Texture								

Character	zelleri	viridofusca	xerampelina	mordax	sierrensis	vinososordida	Woo sp. 67	Woo sp. 72
Aniline Oil	yellow	yellow	yellow	yellow	yellow	yellow	yellow	yellow
Tinct Guaiac								
Sulfoformol	none	none	none	none	none	none	none	none
Sulfovanillin	grey	none	none	grey/black purple	grey/none	grey/none	grey/none	none
pyrogallol		yellow	orange	orange	orange		orange	orange
phenol								
pdab on stipe	none	none	none	none	none	none	none	none
phenol aniline								
α-naphthol	buff	buff	buff	buff	buff	buff	buff	buff
guaiacol	none							none
formaldehyde	none	pink/red	pink/red	pink/red	pink/red	pink/red	pink/red	pink/red
$FeSO_4$	orange and none	green or olive	green or olive	orange and none	orange and none	orange and none	orange and none	orange and none
cystidia in SV								
approx cap colour	violet-brown	red-brown	red-violet-brown	yellow-red-brown	red-violet-brown	red-green-brown	red-violet-brown	red-violet-brown
approx spore colour	dark	pale-dark	pale-dark	pale-dark	dark	dark	pale-dark	pale-dark
approx gill colour	yellow	cream	cream	cream	yellow	cream	cream	cream

[1] Cells are shaded grey when no character state was found in the majority of samples. Figs S6-S13 give the proportion of each character state for each character and each of these species.

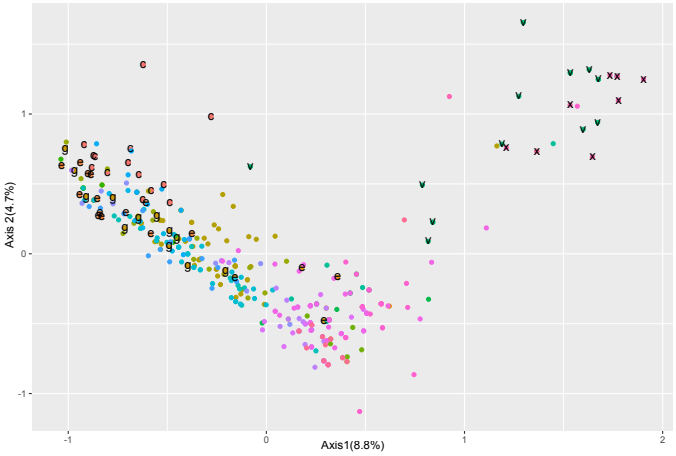
Appendix 1.19 Chemicals used in spot tests

Common names of chemicals and their formulas used in spot tests by Woo.

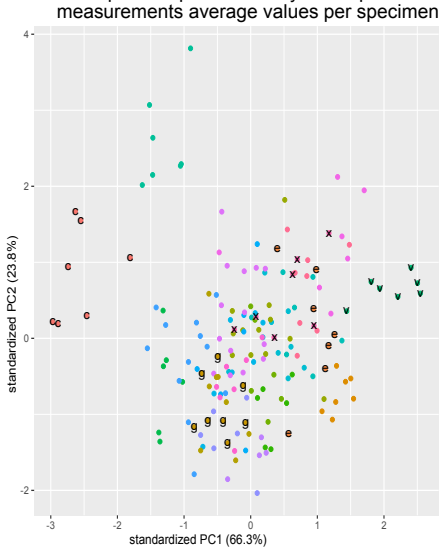
	Chemical Formula	Background including other names
Aniline Oil	C_6H_5 - NH_2	phenylamine, analine, aminobenzene, aminophene
Tinct Guaiac	not available	solvent made from Guaiac Gum/resin
Sulfoformol	H_2SO_4 and CH_2O	mixture of sulfuric acid and formol (or formaldehyde, aldehyde)
Sulfovanillin	H_2SO_4 and $C_8H_8O_3$	mixture of sulfuric acid and vanillin (or vanillic aldehyde, methoxy 3 - hydroxy 4 benzaldehyde)
pyrogallol	$C_6H_6O_3$	1,2,3-benzenetriol
phenol	C_6H_6O	
pdab on stipe	$C_9H_{11}NO$	4-(dimethylamino)benzaldehyde
phenol aniline	C_6H_6O and H_2SO_4 and C_6H_5 - NH_2	mixture of phenol, sulfuric acid and aniline
α-naphthol	$C_{10}H_7$-OH	naphthalen-1-ol
guaiacol	$C_7H_8O_2$	2-methoxyphenol
formaldehyde	CH_20	or formol
Ferrous sulfate	$FeSO_4$	iron(II)sulfate

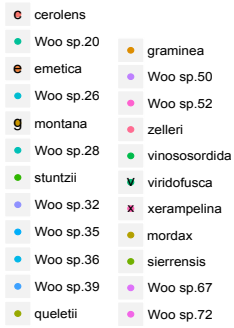
Appendix 1.20 Multivariate analyses of morphological characters

A. Multiple Correspondence Analysis of field characters

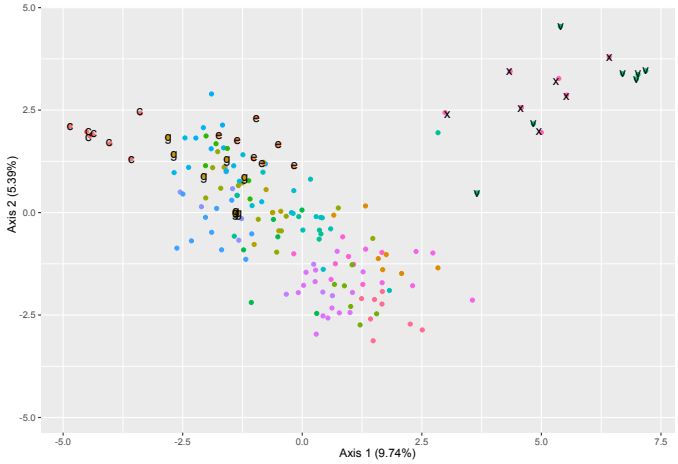


B. Principal Components Analysis of spore measurements average values per specimen



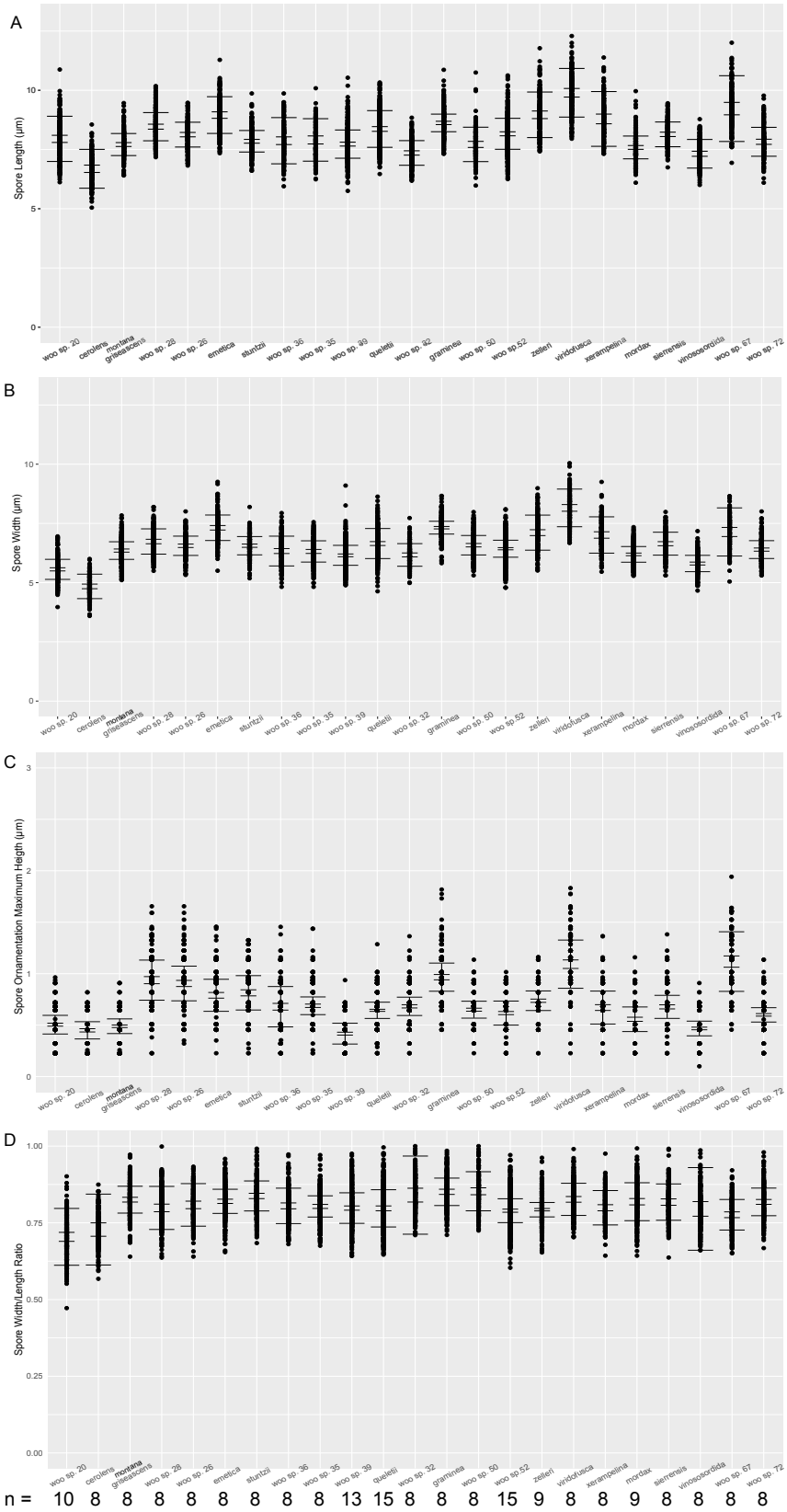


C. Factor Analysis of Mixed Data of spore measurements and field characters



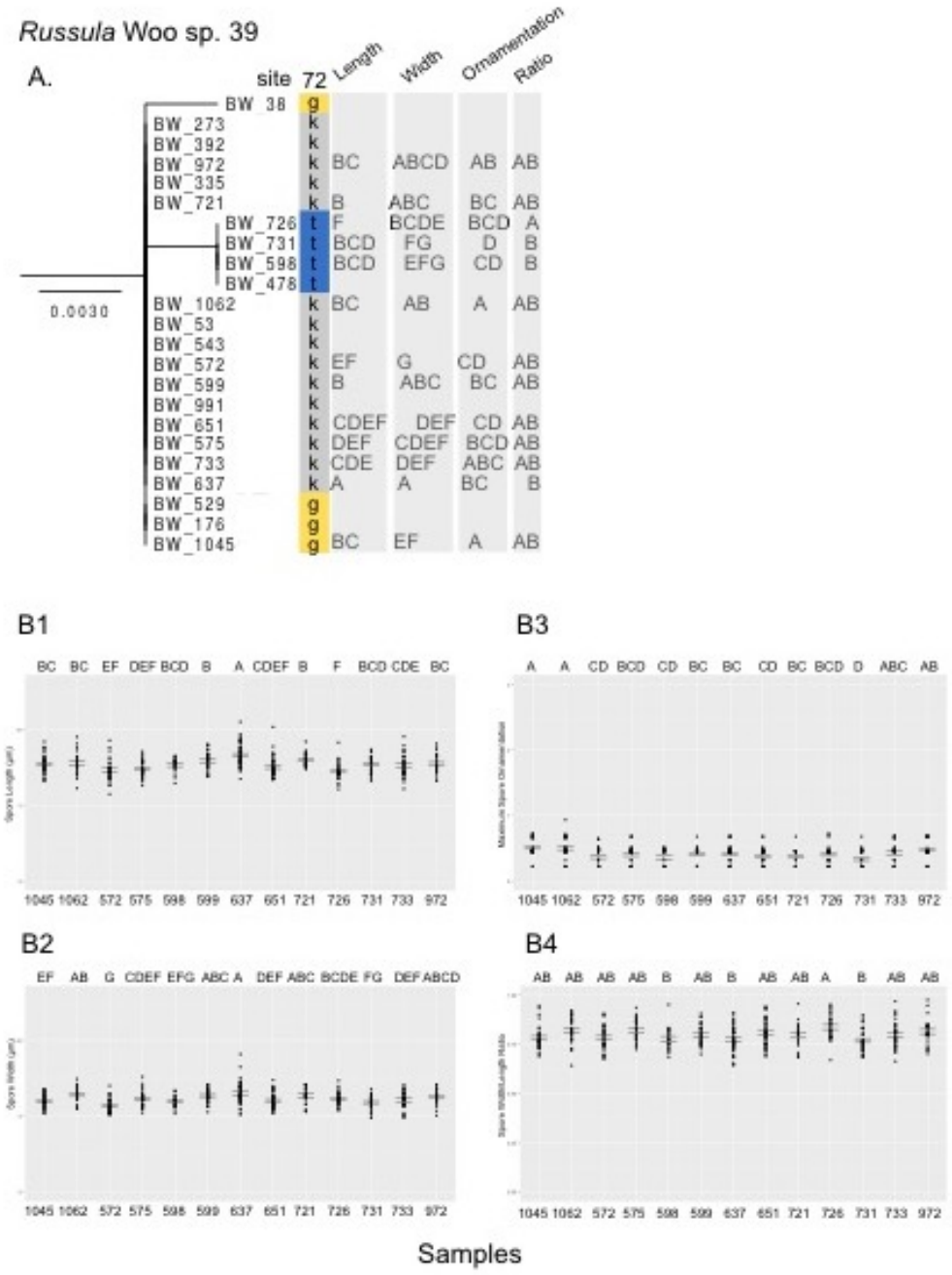
Multivariate analyses of morphological characters. Dots correspond to individual specimens and their colours correspond to their species. Letters designate examples of species that are relatively distinctive: *R. cerolens* (c), *R. viridofusca* (v), *R. xerampelina* (x), or difficult to separate: *R. montana* (g), and *R. emetica* (e).
A. The multiple correspondence analysis of the 23 species with 10 or more specimens, based on categorical morphological variables having retention indices above 0.3. Within morphospace, conspecific collections are widely dispersed and collections of different species overlap even when species' centroids are significantly different. The centroids of *R. xerampelina* and *R. viridofusca* are significantly different from all other species but not from one another (reported in Table S7). Species *R. emetica* and *R. montana* do not differ significantly from one another.
B. Principal components analyses of spore measurements, performed on the log of the average measurement for each specimen. The average measurements contribute to distinguishing some species, for example *R. cerolens* because of the smaller, narrower spores and *R. viridofusca* with large spores.
C. Factor analysis of mixed data of the combined categorical morphological variables and the spore measurements distinguish *R. cerolens*, *R. viridofusca* and *R. xerampelina* from the rest. Most of the 23 species are still overlapping even though their species samples group within the larger swarm, as is evident from *R. montana* and *R. emetica*.

Appendix 1.21 Spore measurements of 23 species



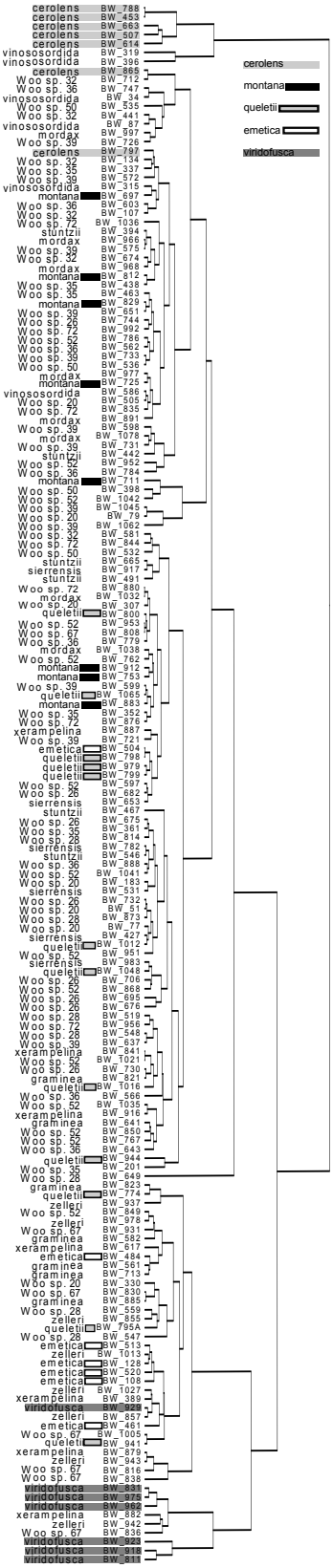
Data points for each species show the spread of spore measurements. Each point represents the average measurement of 30 spores from one collection. Spore length (**A**), width (**B**), maximum height of ornamentation (**C**), and the width/length ratio (**D**), for 23 species of *Russula*. The narrower horizontal lines show the mean plus or minus one standard error of the mean for each species. The wider horizontal lines show the mean plus or minus one standard deviation from the mean. 'N' indicates the number of collections used in spore measurements for each species.

Appendix 1.22 Spore measurements and base-pair variation



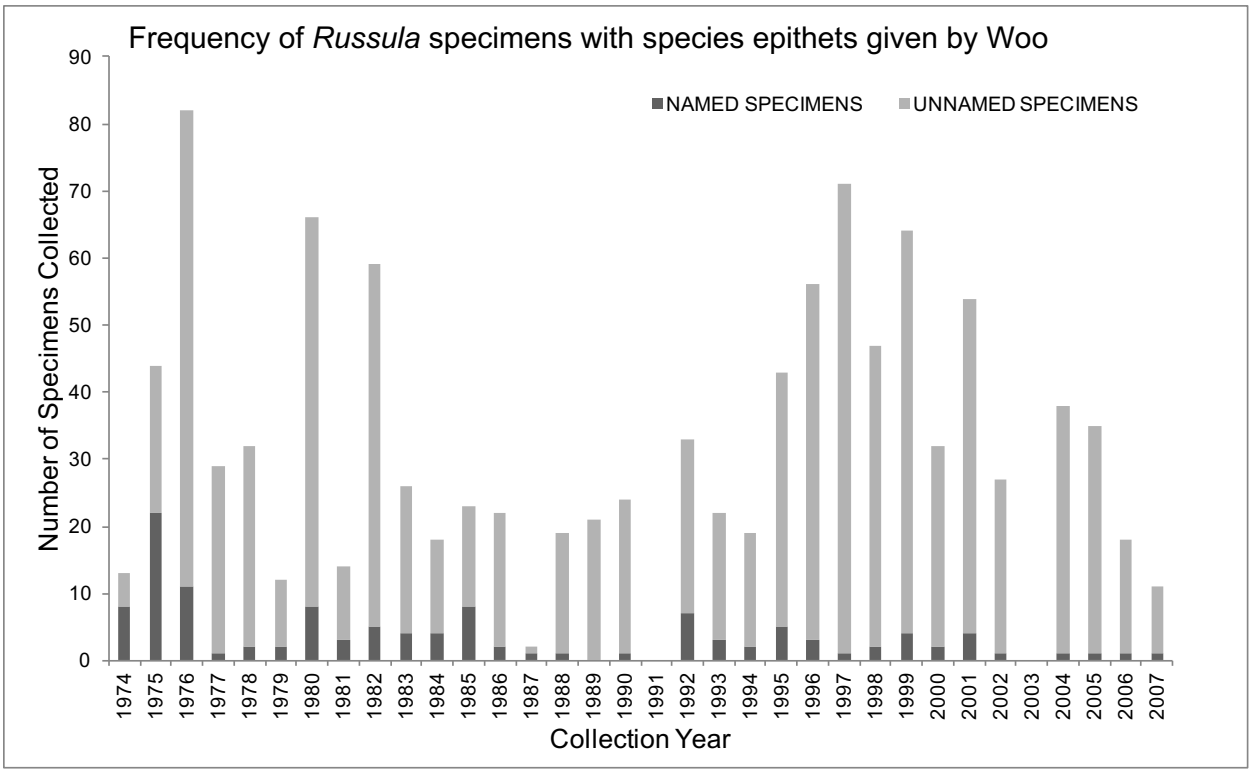
Sequence genotypes below the species level was not correlated with detectable differences in spore measurements. **A.** Sequence variation at site 72 of the alignment in samples in Woo sp. 39 was not correlated with significant differences in spore sizes or ornamentation height based on Tukey tests. Different letters in the column to the right designate significant differences in average sizes. For similar examples in other species, see Table S3. **B.** shows the data points for individual spores from collections of Woo sp. 39. **B1.** Spore length; **B2.** Spore width; **B3.** Maximum height of ornamentation; **B4.** Spore width/length ratio. Numbers at the bottom of each graph are the Ben Woo collection numbers. Different letters at the top of the column indicate statistically different measurements. The horizontal bars show the standard error of the mean.

Appendix 1.23 Agglomerative nesting cluster analysis



Conspecific samples do not usually form clades in a dendrogram based on agglomerative nesting cluster analysis of the sample distances calculated from the first 5 dimensions of the factor analysis of mixed, combined field and microscopic characters. We highlighted samples from 5 exemplar taxa. Of the species, *R. cerolens* and *R. viridofusca* have the most samples clustered into conspecific clades. *R. queletii*, *R. montana* and *R. emetica* show a more common pattern and are interspersed with other specimens from other species.

Appendix 1.24 Named and unnamed specimens in Woo collection



Bar graph showing the number of specimens that Woo collected and either identified or left unidentified in each of 34 years. In his fourth year of collecting, the proportion that Woo identified to species dropped and did not increase over subsequent years, suggesting that Woo recognized that the Pacific Northwest species could not be identified using the available European keys.

Appendix 2

Appendix 2.1 New species specimens

Specimens of new species from the Woo collection. Bolded rows indicate specimens used in new species descriptions. Asterisks (*) indicate holotype specimens.

sp. nov.	Species code	Coll. Num.	WTU Accession	GB ITS2	Date	Country	Pr
benwooii	Woo sp. 67	BW_1005	WTU-F-038884	KX812908	20-Oct-2004	U.S.A.	WA
benwooii	Woo sp. 67	BW_1006	WTU-F-038893	KX812909	20-Oct-2004	U.S.A.	WA
benwooii	Woo sp. 67	BW_1022	WTU-F-038417	KX812923	11-Sep-2005	U.S.A.	WA
benwooii	Woo sp. 67	BW_1023	WTU-F-039180	KX812924	11-Sep-2005	U.S.A.	WA
benwooii	Woo sp. 67	BW_1039	WTU-F-038634	KX812938	5-Oct-2005	U.S.A.	WA
benwooii	Woo sp. 67	BW_1044	WTU-F-038644	KX812943	9-Oct-2005	U.S.A.	WA
benwooii	Woo sp. 67	BW_1076	WTU-F-038408	KX812968	2-Sep-2007	U.S.A.	WA
benwooii	Woo sp. 67	BW_1079	WTU-F-038367	KX812971	2-Sep-2007	U.S.A.	WA
benwooii	Woo sp. 67	BW_113	WTU-F-039508	KX812986	3-Oct-1976	U.S.A.	WA
benwooii	Woo sp. 67	BW_118	WTU-F-039514	KX812989	3-Oct-1976	U.S.A.	WA
benwooii	Woo sp. 67	BW_119	WTU-F-039469	KX812990	3-Oct-1976	U.S.A.	WA
benwooii	Woo sp. 67	BW_178	WTU-F-039013	KX813029	22-Oct-1977	U.S.A.	WA
benwooii	Woo sp. 67	BW_187	WTU-F-038853	KX813033	17-Sep-1978	U.S.A.	WA
benwooii	Woo sp. 67	BW_189	WTU-F-038802	KX813035	24-Sep-1978	U.S.A.	WA
benwooii	Woo sp. 67	BW_220	WTU-F-038490	KX813048	27-Oct-1979	U.S.A.	WA
benwooii	Woo sp. 67	BW_228	WTU-F-039259	KX813054	20-Sep-1980	U.S.A.	WA
benwooii	Woo sp. 67	BW_231	WTU-F-039247	KX813058	20-Sep-1980	U.S.A.	WA
benwooii	Woo sp. 67	BW_233	WTU-F-039253	KX813060	27-Sep-1980	U.S.A.	WA
benwooii	Woo sp. 67	BW_236	WTU-F-039233	KX813062	27-Sep-1980	U.S.A.	WA

sp. nov.	Species code	Coll. Num.	WTU Accession	GB ITS2	Date	Country	Pr
benwooii	Woo sp. 67	BW_25	WTU-F-038400	KX813074	21-Sep-1975	U.S.A.	WA
benwooii	Woo sp. 67	BW_269	WTU-F-038491	KX813083	2-Nov-1980	U.S.A.	WA
benwooii	Woo sp. 67	BW_301	WTU-F-038834	KX813101	3-Oct-1982	U.S.A.	WA
benwooii	Woo sp. 67	BW_316	WTU-F-039006	KX813110	8-Oct-1982	U.S.A.	oR
benwooii	Woo sp. 67	BW_317	WTU-F-039055	KX813111	8-Oct-1982	U.S.A.	OR
benwooii	Woo sp. 67	BW_360	WTU-F-038603	KX813142	24-Sep-1983	U.S.A.	ID
benwooii	Woo sp. 67	BW_362	WTU-F-038605	KX813144	24-Sep-1983	U.S.A.	ID
benwooii	Woo sp. 67	BW_408	WTU-F-038337	KX813176	28-Sep-1985	U.S.A.	WA
benwooii	Woo sp. 67	BW_409	WTU-F-038388	KX813177	28-Sep-1985	U.S.A.	WA
benwooii	Woo sp. 67	BW_410	WTU-F-038435	KX813178	28-Sep-1985	U.S.A.	WA
benwooii	Woo sp. 67	BW_411	WTU-F-038347	KX813179	28-Sep-1985	U.S.A.	WA
benwooii	Woo sp. 67	BW_412	WTU-F-038381	KX813180	4-Oct-1985	U.S.A.	WA
benwooii	Woo sp. 67	BW_418	WTU-F-038350	KX813184	12-Oct-1985	U.S.A.	WA
benwooii	Woo sp. 67	BW_419	WTU-F-038444	KX813185	12-Oct-1985	U.S.A.	WA
benwooii	Woo sp. 67	BW_420	WTU-F-038387	KX813186	12-Oct-1985	U.S.A.	WA
benwooii	Woo sp. 67	BW_466	WTU-F-038431	KX813219	1-Oct-1989	U.S.A.	WA
benwooii	Woo sp. 67	BW_480	WTU-F-038621	KX813228	28-Oct-1989	U.S.A.	WA
benwooii	Woo sp. 67	BW_497	WTU-F-038185	KX813238	4-Nov-1990	U.S.A.	OR
benwooii	Woo sp. 67	BW_498	WTU-F-038204	KX813239	4-Nov-1990	U.S.A.	OR
benwooii	Woo sp. 67	BW_499	WTU-F-038199	KX813240	4-Nov-1990	U.S.A.	OR
benwooii	Woo sp. 67	BW_553	WTU-F-038854	KX813287	3-Oct-1993	U.S.A.	WA
benwooii	Woo sp. 67	BW_554	WTU-F-038817	KX813288	3-Oct-1993	U.S.A.	WA

sp. nov.	Species code	Coll. Num.	WTU Accession	GB ITS2	Date	Country	Pr
benwooii	Woo sp. 67	BW_565	WTU-F-039033	KX813297	23-Oct-1993	U.S.A.	WA
benwooii	Woo sp. 67	BW_578	WTU-F-038867	KX813308	27-Oct-1994	U.S.A.	WA
benwooii	Woo sp. 67	BW_600	WTU-F-038606	KX813322	10-Sep-1995	U.S.A.	WA
benwooii	Woo sp. 67	BW_615	WTU-F-039168	KX813332	30-Oct-1995	U.S.A.	WA
benwooii	Woo sp. 67	BW_618	WTU-F-038385	KX813334	4-Nov-1995	U.S.A.	WA
benwooii	Woo sp. 67	BW_623	WTU-F-039165	KX813339	13-Nov-1995	U.S.A.	OR
benwooii	Woo sp. 67	BW_677	WTU-F-038553	KX813374	10-Nov-1996	U.S.A.	OR
benwooii	Woo sp. 67	BW_689	WTU-F-038584	KX813382	14-Sep-1997	U.S.A.	WA
benwooii	Woo sp. 67	BW_742	WTU-F-038894	KX813420	22-Oct-1997	U.S.A.	WA
benwooii	Woo sp. 67	BW_804	WTU-F-038660	KX813467	4-Sep-1999	U.S.A.	OR
benwooii	Woo sp. 67	BW_805	WTU-F-038725	KX813468	4-Sep-1999	U.S.A.	OR
benwooii	Woo sp. 67	BW_805A	WTU-F-038724	KX813469	4-Sep-1999	U.S.A.	OR
benwooii	Woo sp. 67	BW_806	WTU-F-038661	KX813470	4-Sep-1999	U.S.A.	OR
benwooii	Woo sp. 67	BW_808	WTU-F-038723	KX813471	4-Sep-1999	U.S.A.	OR
benwooii	Woo sp. 67	BW_816	WTU-F-039305	KX813478	19-Sep-1999	U.S.A.	WA
benwooii	Woo sp. 67	BW_830	WTU-F-039368	KX813487	30-Sep-1999	U.S.A.	WA
benwooii	Woo sp. 67	BW_836	WTU-F-039373	KX813491		U.S.A.	WA
benwooii	Woo sp. 67	BW_838	WTU-F-039149	KX813492	15-Oct-1999	U.S.A.	WA
benwooii	Woo sp. 67	BW_931*	WTU-F-038559	KX813560	26-Oct-2001	U.S.A.	OR
hypofragilis	Woo sp. 28	BW_106	WTU-F-039396	KX812956	3-Oct-1976	U.S.A.	WA
hypofragilis	Woo sp. 28	BW_191	WTU-F-039074	KX813036	24-Sep-1978	U.S.A.	WA

sp. nov.	Species code	Coll. Num.	WTU Accession	GB ITS2	Date	Country	Pr
hypofragilis	Woo sp. 28	BW_192	WTU-F-039000	KX813037	24-Sep-1978	U.S.A.	WA
hypofragilis	Woo sp. 28	BW_202	WTU-F-039214	KX813044	30-Sep-1978	U.S.A.	ID
hypofragilis	Woo sp. 28	BW_368	WTU-F-039028	KX813149	12-Nov-1983	U.S.A.	OR
hypofragilis	Woo sp. 28	BW_404	WTU-F-038531	KX813174	22-Sep-1985	U.S.A.	WA
hypofragilis	Woo sp. 28	BW_428	WTU-F-038596	KX813192	11-Oct-1986	U.S.A.	WA
hypofragilis	Woo sp. 28	BW_503	WTU-F-038200	KX813244	4-Nov-1990	U.S.A.	OR
hypofragilis	Woo sp. 28	BW_519	WTU-F-039439	KX813256	4-Oct-1992	U.S.A.	WA
hypofragilis	Woo sp. 28	BW_525	WTU-F-039441	KX813260	4-Oct-1992	U.S.A.	WA
hypofragilis	Woo sp. 28	BW_547	WTU-F-038821	KX813282	3-Oct-1993	U.S.A.	WA
hypofragilis	Woo sp. 28	BW_548	WTU-F-038825	KX813283	3-Oct-1993	U.S.A.	WA
hypofragilis	Woo sp. 28	BW_559	WTU-F-039501	KX813293	24-Oct-1993	U.S.A.	WA
hypofragilis	Woo sp. 28	BW_649	WTU-F-038961	KX813353	13-Oct-1996	U.S.A.	WA
hypofragilis	Woo sp. 28	BW_814	WTU-F-038713	KX813476	19-Sep-1999	U.S.A.	WA
hypofragilis	Woo sp. 28	BW_873	WTU-F-038948	KX813516	28-Oct-2000	U.S.A.	WA
hypofragilis	Woo sp. 28	BW_920*	WTU-F-038403	KX813553	23-Sep-2001	U.S.A.	WA
hypofragilis	Woo sp. 28	BW_993	WTU-F-039413	KX813609	30-Sep-2004	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_1046	WTU-F-038642	KX812945	20-Oct-2005	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_1057	WTU-F-038382	KX812954	7-Nov-2005	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_1085	WTU-F-039185	KX812978	22-Oct-2007	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_1086	WTU-F-039162	KX812979	22-Oct-2007	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_1087	WTU-F-039232	KX812980	22-Oct-2007	U.S.A.	WA

sp. nov.	Species code	Coll. Num.	WTU Accession	GB ITS2	Date	Country	Pr
obscurozelleri	Woo sp. 50	BW_1088	WTU-F-039231	KX812981		U.S.A.	WA?
obscurozelleri	Woo sp. 50	BW_1089	WTU-F-039206	KX812982		U.S.A.	WA?
obscurozelleri	Woo sp. 50	BW_1090	WTU-F-038505	KX812984		U.S.A.	WA?
obscurozelleri	Woo sp. 50	BW_130	WTU-F-039118	KX812997	23-Oct-1976	U.S.A.	OR
obscurozelleri	Woo sp. 50	BW_169	WTU-F-039117	KX813022	1-Oct-1977	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_170	WTU-F-039158	KX813023	1-Oct-1977	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_255	WTU-F-038433	KX813078	18-Oct-1980	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_263	WTU-F-038470	KX813082	19-Oct-1980	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_323	WTU-F-038914	KX813116	10-Oct-1982	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_325	WTU-F-038910	KX813118	13-Oct-1982	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_342	WTU-F-038469	KX813130	29-Oct-1982	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_343	WTU-F-038687	KX813131	30-Oct-1982	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_349	WTU-F-038712	KX813134	30-Oct-1982	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_357	WTU-F-038598	KX813138	7-Jan-1982	U.S.A.	OR
obscurozelleri	Woo sp. 50	BW_379	WTU-F-038805	KX813156	18-Nov-1983	U.S.A.	CA
obscurozelleri	Woo sp. 50	BW_383	WTU-F-039004	KX813159	19-Nov-1983	U.S.A.	OR
obscurozelleri	Woo sp. 50	BW_390	WTU-F-038949	KX813164	21-Oct-1984	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_398	WTU-F-038565	KX813171	4-Nov-1984	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_400	WTU-F-038523	KX813173	4-Nov-1984	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_451	WTU-F-039215	KX813209	4-Nov-1988	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_452	WTU-F-039216	KX813210	4-Nov-1988	U.S.A.	WA

sp. nov.	Species code	Coll. Num.	WTU Accession	GB ITS2	Date	Country	Pr
obscurozelleri	Woo sp. 50	BW_532	WTU-F-039444	KX813268	24-Oct-1992	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_535	WTU-F-039400	KX813271	8-Nov-1992	U.S.A.	OR
obscurozelleri	Woo sp. 50	BW_536	WTU-F-039388	KX813272	7-Nov-1992	U.S.A.	OR
obscurozelleri	Woo sp. 50	BW_538	WTU-F-039438	KX813273	7-Nov-1992	U.S.A.	OR
obscurozelleri	Woo sp. 50	BW_539	WTU-F-038484	KX813274	7-Nov-1992	U.S.A.	OR
obscurozelleri	Woo sp. 50	BW_540	WTU-F-039394	KX813275	7-Nov-1992	U.S.A.	OR
obscurozelleri	Woo sp. 50	BW_658	WTU-F-039212	KX813361	16-Oct-1996	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_659	WTU-F-039191	KX813362	16-Oct-1996	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_662	WTU-F-039193	KX813364	21-Oct-1996	U.S.A.	wa
obscurozelleri	Woo sp. 50	BW_729	WTU-F-038217	KX813409	17-Oct-1997	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_803*	WTU-F-038663	KX813466	18-Nov-1998	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_842	WTU-F-039094	KX813496	30-Oct-1999	Canada	BC
obscurozelleri	Woo sp. 50	BW_852	WTU-F-039112	KX813502	14-Nov-1999	U.S.A.	WA
obscurozelleri	Woo sp. 50	BW_994	WTU-F-039257	KX813610	30-Sep-2004	U.S.A.	WA
parapallens	Woo sp. 32	BW_1052	WTU-F-038673	KX812951	6-Nov-2005	U.S.A.	OR
parapallens	Woo sp. 32	BW_107	WTU-F-039383	KX812964	3-Oct-1976	U.S.A.	WA
parapallens	Woo sp. 32	BW_134	WTU-F-039147	KX812999	26-Oct-1976	U.S.A.	Wa
parapallens	Woo sp. 32	BW_223	WTU-F-038449	KX813050	14-Sep-1980	U.S.A.	WA
parapallens	Woo sp. 32	BW_441	WTU-F-038665	KX813202	16-Nov-1986	U.S.A.	OR
parapallens	Woo sp. 32	BW_46	WTU-F-039362	KX813214	28-Sep-1975	U.S.A.	WA

sp. nov.	Species code	Coll. Num.	WTU Accession	GB ITS2	Date	Country	Pr
parapallens	Woo sp. 32	BW_542	WTU-F-039440	KX813277	13-Nov-1992	U.S.A.	OR
parapallens	Woo sp. 32	BW_558	WTU-F-038823	KX813292	11-Oct-1993	U.S.A.	WA
parapallens	Woo sp. 32	BW_581	WTU-F-038864	KX813309	28-Oct-1994	U.S.A.	WA
parapallens	Woo sp. 32	BW_626	WTU-F-039219	KX813340	13-Nov-1995	U.S.A.	OR
parapallens	Woo sp. 32	BW_629	WTU-F-039202	KX813343	8-Sep-1996	U.S.A.	WA
parapallens	Woo sp. 32	BW_674	WTU-F-038341	KX813371	10-Nov-1996	U.S.A.	OR
parapallens	Woo sp. 32	BW_685	WTU-F-038582	KX813379	16-Nov-1996	U.S.A.	WA
parapallens	Woo sp. 32	BW_712	WTU-F-039290	KX813398	7-Oct-1997	U.S.A.	WA
parapallens	Woo sp. 32	BW_769	WTU-F-038904	KX813435	30-Oct-1998	U.S.A.	WA
parapallens	Woo sp. 32	BW_771	WTU-F-038877	KX813438	30-Oct-1998	U.S.A.	Wa
parapallens	Woo sp. 32	BW_791*	WTU-F-038394	KX813453	13-Nov-1998	U.S.A.	OR
parapallens	Woo sp. 32	BW_792	WTU-F-038436	KX813454	13-Nov-1998	U.S.A.	OR
phoenicea	Woo sp. 26	BW_1053	WTU-F-038630	KX812952	6-Nov-2005	U.S.A.	OR
phoenicea	Woo sp. 26	BW_1069	WTU-F-038476	KX812963	4-Nov-2006	U.S.A.	WA
phoenicea	Woo sp. 26	BW_116	WTU-F-039481	KX812988	3-Oct-1976	U.S.A.	WA
phoenicea	Woo sp. 26	BW_129	WTU-F-039334	KX812996	23-Oct-1976	U.S.A.	WA
phoenicea	Woo sp. 26	BW_241	WTU-F-039240	KX813065	4-Oct-1980	U.S.A.	WA
phoenicea	Woo sp. 26	BW_270	WTU-F-038511	KX813085	2-Nov-1980	U.S.A.	WA
phoenicea	Woo sp. 26	BW_290	WTU-F-039061	KX813094	21-Oct-1981	U.S.A.	WA

sp. nov.	Species code	Coll. Num.	WTU Accession	GB ITS2	Date	Country	Pr
phoenicea	Woo sp. 26	BW_297	WTU-F-038847	KX813097	7-Nov-1981	U.S.A.	WA
phoenicea	Woo sp. 26	BW_299	WTU-F-039052	KX813098	22-Nov-1981	U.S.A.	WA
phoenicea	Woo sp. 26	BW_300	WTU-F-038861	KX813100	22-Nov-1981	U.S.A.	WA
phoenicea	Woo sp. 26	BW_311	WTU-F-039054	KX813106	8-Oct-1982	U.S.A.	OR
phoenicea	Woo sp. 26	BW_366	WTU-F-038985	KX813147	29-Oct-1983	U.S.A.	WA
phoenicea	Woo sp. 26	BW_395	WTU-F-038552	KX813169	28-Oct-1984	U.S.A.	WA
phoenicea	Woo sp. 26	BW_476	WTU-F-038704	KX813224	8-Oct-1989	U.S.A.	WA
phoenicea	Woo sp. 26	BW_479	WTU-F-038623	KX813226	28-Oct-1989	U.S.A.	WA
phoenicea	Woo sp. 26	BW_620	WTU-F-039174	KX813336	13-Nov-1995	U.S.A.	OR
phoenicea	Woo sp. 26	BW_621	WTU-F-038333	KX813337	13-Nov-1995	U.S.A.	OR
phoenicea	Woo sp. 26	BW_622	WTU-F-039170	KX813338	13-Nov-1995	U.S.A.	OR
phoenicea	Woo sp. 26	BW_645	WTU-F-039075	KX813351	11-Oct-1996	U.S.A.	WA
phoenicea	Woo sp. 26	BW_673	WTU-F-039229	KX813370	28-Oct-1996	U.S.A.	WA
phoenicea	Woo sp. 26	BW_675	WTU-F-039222	KX813372	10-Nov-1996	U.S.A.	OR
phoenicea	Woo sp. 26	BW_676	WTU-F-039172	KX813373	10-Nov-1996	U.S.A.	OR
phoenicea	Woo sp. 26	BW_682	WTU-F-038547	KX813376	10-Nov-1996	U.S.A.	OR
phoenicea	Woo sp. 26	BW_695	WTU-F-038577	KX813388	28-Sep-1997	U.S.A.	WA
phoenicea	Woo sp. 26	BW_706	WTU-F-039284	KX813394	4-Oct-1997	Canada	BC

sp. nov.	Species code	Coll. Num.	WTU Accession	GB ITS2	Date	Country	Pr
phoenicea	Woo sp. 26	BW_730	WTU-F-038215	KX813410	17-Oct-1997	U.S.A.	WA
phoenicea	Woo sp. 26	BW_732	WTU-F-038219	KX813412	17-Oct-1997	U.S.A.	WA
phoenicea	Woo sp. 26	BW_744	WTU-F-038937	KX813421	27-Oct-1997	U.S.A.	WA
phoenicea	Woo sp. 26	BW_749	WTU-F-038934	KX813423	31-Oct-1997	U.S.A.	WA
phoenicea	Woo sp. 26	BW_752	WTU-F-038921	KX813426	10-Nov-1997	U.S.A.	WA
phoenicea	Woo sp. 26	BW_770	WTU-F-038905	KX813437	30-Oct-1998	U.S.A.	WA
phoenicea	Woo sp. 26	BW_790	WTU-F-038487	KX813452	13-Nov-1998	U.S.A.	OR
phoenicea	Woo sp. 26	BW_793	WTU-F-038390	KX813455	13-Nov-1998	U.S.A.	OR
phoenicea	Woo sp. 26	BW_801	WTU-F-038726	KX813464	14-Nov-1998	U.S.A.	OR
phoenicea	Woo sp. 26	BW_872	WTU-F-039020	KX813515	28-Oct-2000	U.S.A.	WA
phoenicea	Woo sp. 26	BW_875	WTU-F-038982	KX813517	28-Oct-2000	U.S.A.	WA
phoenicea	Woo sp. 26	BW_919*	WTU-F-038477	KX813551	23-Sep-2001	U.S.A.	WA
phoenicea	Woo sp. 26	BW_932	WTU-F-038555	KX813561	26-Oct-2001	U.S.A.	OR
phoenicea	Woo sp. 26	BW_971	WTU-F-038831	KX813590	22-Oct-2002	U.S.A.	WA
phoenicea	Woo sp. 26	BW_980	WTU-F-038845	KX813600	23-Nov-2002	U.S.A.	OR
pseudopelargonia	Woo sp. 36	BW_1037	WTU-F-038631	KX812936	5-Oct-2005	U.S.A.	WA
pseudopelargonia	Woo sp. 36	BW_180	WTU-F-039070	KX813031	17-Sep-1978	U.S.A.	WA
pseudopelargonia	Woo sp. 36	BW_562	WTU-F-038819	KX813295	24-Oct-1993	U.S.A.	WA
pseudopelargonia	Woo sp. 36	BW_566	WTU-F-039034	KX813298	23-Oct-1993	U.S.A.	WA

sp. nov.	Species code	Coll. Num.	WTU Accession	GB ITS2	Date	Country	Pr
pseudopelargonia	Woo sp. 36	BW_603*	WTU-F-038653	KX813324	13-Sep-1995	U.S.A.	WA
pseudopelargonia	Woo sp. 36	BW_643	WTU-F-039024	KX813349	11-Oct-1996	U.S.A.	WA
pseudopelargonia	Woo sp. 36	BW_740	WTU-F-038212	KX813418	22-Oct-1997	U.S.A.	WA
pseudopelargonia	Woo sp. 36	BW_747	WTU-F-038935	KX813422	31-Oct-1997	U.S.A.	WA
pseudopelargonia	Woo sp. 36	BW_779	WTU-F-038903	KX813442	2-Nov-1998	U.S.A.	WA
pseudopelargonia	Woo sp. 36	BW_784	WTU-F-038906	KX813446	2-Nov-1998	U.S.A.	WA
pseudopelargonia	Woo sp. 36	BW_888	WTU-F-039282	KX813529	3-Nov-2000	U.S.A.	WA
pseudopelargonia	Woo sp. 36	BW_9	WTU-F-038410	KX813537	20-Oct-1974	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_1021	WTU-F-039205	KX812922	24-Oct-2004	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_1035	WTU-F-038628	KX812934	5-Oct-2005	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_1041	WTU-F-038641	KX812940	9-Oct-2005	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_1042	WTU-F-038636	KX812941	9-Oct-2005	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_1043	WTU-F-038633	KX812942	9-Oct-2005	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_177	WTU-F-039022	KX813028	22-Oct-1977	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_276	WTU-F-038987	KX813090	8-Nov-1980	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_305	WTU-F-039008	KX813103	4-Oct-1982	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_369	WTU-F-038990	KX813150	12-Nov-1983	U.S.A.	OR
pseudotsugarum	Woo sp. 52	BW_494	WTU-F-038624	KX813236	28-Oct-1990	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_495	WTU-F-038668	KX813237	28-Oct-1990	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_526	WTU-F-039429	KX813261	11-Oct-1992	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_527	WTU-F-039430	KX813262	11-Oct-1992	U.S.A.	WA

sp. nov.	Species code	Coll. Num.	WTU Accession	GB ITS2	Date	Country	Pr
pseudotsugarum	Woo sp. 52	BW_597	WTU-F-038602	KX813318	10-Sep-1995	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_762	WTU-F-038907	KX813432	16-Oct-1998	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_767	WTU-F-038908	KX813433	29-Oct-1998	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_786	WTU-F-038887	KX813447	2-Nov-1998	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_849	WTU-F-039125	KX813499	11-Nov-1999	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_850	WTU-F-039140	KX813500	11-Nov-1999	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_868	WTU-F-038965	KX813513	28-Nov-1999	U.S.A.	CA
pseudotsugarum	Woo sp. 52	BW_951	WTU-F-038563	KX813576	11-Nov-2001	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_952	WTU-F-038538	KX813577	11-Nov-2001	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_953*	WTU-F-038562	KX813578	11-Nov-2001	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_98	WTU-F-039398	KX813599	25-Sep-1976	U.S.A.	WA
pseudotsugarum	Woo sp. 52	BW_985	WTU-F-039402	KX813604	5-Sep-2004	U.S.A.	WA
rhodocephala	Woo sp. 35	BW_201	WTU-F-038413	KX813043	29-Sep-1978	U.S.A.	ID
rhodocephala	Woo sp. 35	BW_337*	WTU-F-039507	KX813126	24-Oct-1982	U.S.A.	WA
rhodocephala	Woo sp. 35	BW_352	WTU-F-038711	KX813136	6-Nov-1982	U.S.A.	OR
rhodocephala	Woo sp. 35	BW_361	WTU-F-038604	KX813143	24-Sep-1983	U.S.A.	ID
rhodocephala	Woo sp. 35	BW_438	WTU-F-038632	KX813199	15-Nov-1986	U.S.A.	OR
rhodocephala	Woo sp. 35	BW_463	WTU-F-038363	KX813218	12-Nov-1988	U.S.A.	WA

sp. nov.	Species code	Coll. Num.	WTU Accession	GB ITS2	Date	Country	Pr
rhodocephala	Woo sp. 35	BW_486	WTU-F-038655	KX813231	11-Nov-1989	U.S.A.	OR
rhodocephala	Woo sp. 35	BW_500	WTU-F-038209	KX813241	4-Nov-1990	U.S.A.	OR
rhodocephala	Woo sp. 35	BW_860	WTU-F-039103	KX813506	15-Nov-1999	U.S.A.	OR
rhodocephala	Woo sp. 35	BW_92	WTU-F-038950	KX813552	26-Sep-1976	U.S.A.	WA
salishensis	Woo sp. 39	BW_1045	WTU-F-038666	KX812944	9-Oct-2005	U.S.A.	WA
salishensis	Woo sp. 39	BW_1062	WTU-F-038415	KX812958	15-Oct-2006	U.S.A.	WA
salishensis	Woo sp. 39	BW_176	WTU-F-039012	KX813027	22-Oct-1977	U.S.A.	WA
salishensis	Woo sp. 39	BW_273	WTU-F-038396	KX813088	8-Nov-1980	U.S.A.	WA
salishensis	Woo sp. 39	BW_335	WTU-F-038312	KX813124	24-Oct-1982	U.S.A.	WA
salishensis	Woo sp. 39	BW_38	WTU-F-039345	KX813157	28-Sep-1975	U.S.A.	WA
salishensis	Woo sp. 39	BW_392	WTU-F-038809	KX813166	21-Oct-1984	U.S.A.	WA
salishensis	Woo sp. 39	BW_478	WTU-F-038622	KX813225	28-Oct-1989	U.S.A.	WA
salishensis	Woo sp. 39	BW_529	WTU-F-039389	KX813264	11-Oct-1992	U.S.A.	WA
salishensis	Woo sp. 39	BW_53	WTU-F-039364	KX813265	11-Oct-1975	U.S.A.	WA
salishensis	Woo sp. 39	BW_543	WTU-F-038824	KX813278	15-Nov-1992	U.S.A.	OR
salishensis	Woo sp. 39	BW_572	WTU-F-039045	KX813303	15-Oct-1994	U.S.A.	WA
salishensis	Woo sp. 39	BW_575	WTU-F-039027	KX813306	23-Oct-1994	U.S.A.	WA
salishensis	Woo sp. 39	BW_598	WTU-F-038617	KX813319	10-Sep-1995	U.S.A.	WA
salishensis	Woo sp. 39	BW_599	WTU-F-038592	KX813320	10-Sep-1995	U.S.A.	WA
salishensis	Woo sp. 39	BW_637	WTU-F-039190	KX813345	6-Oct-1996	U.S.A.	WA

sp. nov.	Species code	Coll. Num.	WTU Accession	GB ITS2	Date	Country	Pr
salishensis	Woo sp. 39	BW_651	WTU-F-039065	KX813355	13-Oct-1996	U.S.A.	WA
salishensis	Woo sp. 39	BW_721	WTU-F-038194	KX813404	12-Oct-1997	U.S.A.	WA
salishensis	Woo sp. 39	BW_726	WTU-F-038223	KX813408	12-Oct-1997	U.S.A.	WA
salishensis	Woo sp. 39	BW_731	WTU-F-038220	KX813411	17-Oct-1997	U.S.A.	WA
salishensis	Woo sp. 39	BW_733	WTU-F-038187	KX813413	17-Oct-1997	U.S.A.	WA
salishensis	Woo sp. 39	BW_972*	WTU-F-038984	KX813591	22-Oct-2002	U.S.A.	WA
salishensis	Woo sp. 39	BW_991	WTU-F-039434	KX813607	30-Sep-2004	U.S.A.	WA

Appendix 3

Appendix 3.1 Samples of panther Amanitas

Panther Amanitas samples used in Figure 4.1. UNITE and PlutoF structure of 'Species Hypothesis' and clusters of sequences. An example of Amanita patherina from version 7 of the database. Percentage represents the amount of dissimilarity between the sequences within that cluster. 'SH' stands for 'species hypothesis' and it is followed by its UNITE code.

GenBank Accession	Species	**0%**	**1%**	**2%**	**3%**	Country	Latitude	Longitude
HM189721	OUTGROUP cf *gemmata*	outgroup	outgroup	outgroup	outgroup	Germany	14.1	52.5
KX365198	*Amanita pakistanica*	SH661484	SH645386	SH635771	SH003266	Pakistan	73.372968	34.074314
KP276311	*Amanita sp.*	SH617299	SH550713	SH038017	SH003266	United States	-122.8495	38.0586
GQ401354	*Amanita pantherinoides*	SH320408	SH082304	SH038017	SH003266	United States	-121.797052	36.392727
EU909452	*Amanita pantherinoides*	SH320408	SH082304	SH038017	SH003266	United States	-119.758022	34.019129
AB080785	*Amanita pantherinoides*	SH320408	SH082304	SH038017	SH003266	United States	-119.724079	34.490117
GU180245	*Amanita pantherinoides*	SH320408	SH082304	SH038017	SH003266	United States	-121.8	36.4
KC791058	*Amanita pantherinoides*	SH320408	SH082304	SH038017	SH003266	United States	-116.7778	33.8083
JF899547	*Amanita pantherinoides*	SH320473	SH082304	SH038017	SH003266	Canada	-122.9	49.5
EU525997	*Amanita pantherinoides*	SH320505	SH082304	SH038017	SH003266	United States	-122.22	44.23
AB096047	*Amanita pantherinoides*	SH320507	SH082304	SH038017	SH003266	United States	-122.308389	37.271054
DQ273350	*Amanita pantherinoides*	SH320508	SH082304	SH038017	SH003266	United States	-122.22	44.23
KU248130	*Amanita albocreata*	SH581001	SH536382	SH038017	SH003266	United States	-75.293048	40.784313
KU248129	*Amanita albocreata*	SH594228	SH536382	SH038017	SH003266	United States	-76.274	41.3036
AY436459	*Amanita griseopantherina*	SH320589	SH082343	SH038050	SH003266	China	103.6	30.7
KT354979	*Amanita multisquamosa*	SH622277	SH082312	SH038025	SH003266	Mexico	-99.3	19.2
AY656924	*Amanita multisquamosa*	SH320459	SH082312	SH038025	SH003266	United States	-81.2	36.9

GenBank Accession	Species	**0%**	**1%**	**2%**	**3%**	Country	Latitude	Longitude
FJ196896	*Amanita multisquamosa*	SH320468	SH082312	SH038025	SH003266	Mexico	-99.7	17.7
AB103329	*Amanita multisquamosa*	SH320504	SH082312	SH038025	SH003266	United States	-72.1	42.7
KX061524	*Amanita pakistanica*	SH661484	SH645386	SH635771	SH003266	Pakistan	73.377078	34.07699
KM052551	*Amanita cf. subglobosa*	SH593247	SH082299	SH038015	SH003264	Korea	128.01	36.33
UDB026445	*Amanita cf. subglobosa*	SH607355	SH082299	SH038015	SH003264	Estonia	27.067004	58.062183
KU139496	*Amanita cf. subglobosa*	SH585028	SH082299	SH038015	SH003264	Korea	128.01	36.33
KU248106	*Amanita cf. subglobosa*	SH615441	SH082299	SH038015	SH003264	India	79.027976	30.04648
KU139497	*Amanita cf. subglobosa*	SH615852	SH082299	SH038015	SH003264	Korea	128.01	36.33
KX444410	*Amanita cf. subglobosa*	SH662421	SH082299	SH038015	SH003264	China	115.43	39.97
AB080976	*Amanita cf. subglobosa*	SH320412	SH082299	SH038015	SH003264	Japan	135.67	35.05
KF017943	*Amanita cf. subglobosa*	SH320412	SH082299	SH038015	SH003264	Korea	128.01	36.33
KJ609156	*Amanita cf. subglobosa*	SH320412	SH082299	SH038015	SH003264	Korea	128.19	35.06
AB096044	*Amanita cf. subglobosa*	SH320422	SH082299	SH038015	SH003264	Nepal	85.405508	27.570948
KR456156	*Amanita cf. subglobosa*	SH320422	SH082299	SH038015	SH003264	China	118.9	31.8
KU139498	*Amanita cf. subglobosa*	SH320422	SH082299	SH038015	SH003264	Korea	128.01	36.33
KU248107	*Amanita cf. subglobosa*	SH320422	SH082299	SH038015	SH003264	India	79.027976	30.04648
KX444347	*Amanita cf. subglobosa*	SH320422	SH082299	SH038015	SH003264	China	112.087404	36.593023
KX444211	*Amanita cf. subglobosa*	SH320422	SH082299	SH038015	SH003264	China	107.771472	33.948608
KX810031	*Amanita cf. subglobosa*	SH320422	SH082299	SH038015	SH003264	India	78.867533	30.170283
AB080977	*Amanita cf. subglobosa*	SH320429	SH082299	SH038015	SH003264	Japan	135.67	35.05
UDB014135	*Amanita cf. subglobosa*	SH320429	SH082299	SH038015	SH003264	Japan	140.102	36.234
KC414273	*Amanita cf. subglobosa*	SH320432	SH082299	SH038015	SH003264	China	118.9	31.8

GenBank Accession	Species	**0%**	**1%**	**2%**	**3%**	Country	Latitude	Longitude
KC414270	*Amanita cf. subglobosa*	SH320432	SH082299	SH038015	SH003264	China	118.9	31.8
AB973730	*Amanita cf. subglobosa*	SH320491	SH082299	SH038015	SH003264	Japan	128.182697	26.663944
AB096045	*Amanita cf. subglobosa*	SH320521	SH082299	SH038015	SH003264	Nepal	85.405508	27.570948
AB096043	*Amanita cf. subglobosa*	SH320522	SH082299	SH038015	SH003264	Nepal	85.514759	27.724199
KF017947	*Amanita cf. subglobosa*	SH320525	SH082299	SH038015	SH003264	Korea	128.01	36.33
AB080975	*Amanita cf. subglobosa*	SH320528	SH082299	SH038015	SH003264	Japan	135.891406	35.224822
JN182878	*Amanita cf. subglobosa*	SH320587	SH082299	SH038015	SH003264	China	118.9	31.8
UDB000213	*Amanita pantherina*	SH320411	SH082301	SH038015	SH003264	Sweden	15.64	58.9
UDB015621	*Amanita pantherina*	SH320411	SH082301	SH038015	SH003264	Estonia	26.934	57.7445
FR852274	*Amanita pantherina*	SH320442	SH082301	SH038015	SH003264	Iran, Islamic Republ	50.009	36.997
HM146790	*Amanita pantherina*	SH320447	SH082301	SH038015	SH003264	Germany	14.1	52.5
UDB011149	*Amanita pantherina*	SH320458	SH082301	SH038015	SH003264	Estonia	22.439	58.237
HF674540	*Amanita pantherina*	SH320469	SH082301	SH038015	SH003264	Slovenia	13.49	45.27
UDB005429	*Amanita pantherina*	SH320478	SH082301	SH038015	SH003264	Iran, Islamic Republ	51.48	36.62
UDB019795	*Amanita pantherina*	SH320511	SH082301	SH038015	SH003264	Estonia	22.077	58.309
UDB002340	*Amanita pantherina*	SH320515	SH082301	SH038015	SH003264	Denmark	8.427	55.415
AY436466	*Amanita parvipantherina*	SH320503	SH082303	SH038015	SH003264	China	100.26	26.78
KF651009	*Amanita parvipantherina*	SH320529	SH082303	SH038015	SH003264	China	102.608	25.906
KF651008	*Amanita parvipantherina*	SH320530	SH082303	SH038015	SH003264	China	102.362	25.548
KF651007	*Amanita parvipantherina*	SH320531	SH082303	SH038015	SH003264	China	105.127	27.928
KF651006	*Amanita parvipantherina*	SH320532	SH082303	SH038015	SH003264	China	102.583	24.326

GenBank Accession	Species	**0%**	**1%**	**2%**	**3%**	Country	Latitude	Longitude
KF651005	*Amanita parvipantherina*	SH320533	SH082303	SH038015	SH003264	China	104.35	25.31
AB080786	*Amanita sp.*	SH320509	SH082299	SH038015	SH003264	Japan	135.67	35.05
AY656923	*Amanita sp.*	SH320590	SH082299	SH038015	SH003264	United States	-80.905	36.989
AB096046	*Amanita pantherina*	SH320433	SH082301	SH038015	SH003264	United Kingdom	-0.396	51.217
KM085405	*Amanita pantherina*	SH320433	SH082301	SH038015	SH003264	Poland	17.4	52.3
AB080774	*Amanita pantherina*	SH320519	SH082301	SH038015	SH003264	United Kingdom	-3.8	50.8
FJ946976	*Amanita pantherina*	SH320586	SH082301	SH038015	SH003264	Spain	0.5	42.4
UDB015613	*Amanita pantherina*	SH320463	SH082329	SH038015	SH003264	Estonia	26.941	58.254
EF619628	*Amanita sp.*	SH320494	SH082299	SH038015	SH003264	United States	-79.12	36.04
AB080978	*Amanita sp.*	SH320523	SH082299	SH038015	SH003264	Japan	135.67	35.05
KF651004	*Amanita parvipantherina*	SH320534	SH082303	SH038015	SH003264	China	101.85	25.29
KF651003	*Amanita parvipantherina*	SH320535	SH082303	SH038015	SH003264	China	101.450515	25.078876
KF651002	*Amanita parvipantherina*	SH320536	SH082303	SH038015	SH003264	China	100.162845	25.638227
KF651001	*Amanita parvipantherina*	SH320537	SH082303	SH038015	SH003264	China	100.505558	22.037622
KF651000	*Amanita parvipantherina*	SH320538	SH082303	SH038015	SH003264	China	99.198	27.341
KF650999	*Amanita parvipantherina*	SH320539	SH082303	SH038015	SH003264	China	99.961	26.863
KF650998	*Amanita parvipantherina*	SH320540	SH082303	SH038015	SH003264	China	99.147	24.805
AB080973	*Amanita sp.*	SH320541	SH082299	SH038015	SH003264	Japan	140.761	38.3
EF493269	*Amanita pantherina*	SH320593	SH082301	SH038015	SH003264	Sweden	15.64	58.9
FJ196894	*Amanita sp.*	SH320483	SH179046	SH038015	SH003264	Mexico	-99.84	17.57
EU569283	*Amanita sp.*	SH320484	SH179046	SH038015	SH003264	Mexico	-99.84	17.57

Appendix 3.2 Sampling frequency by country

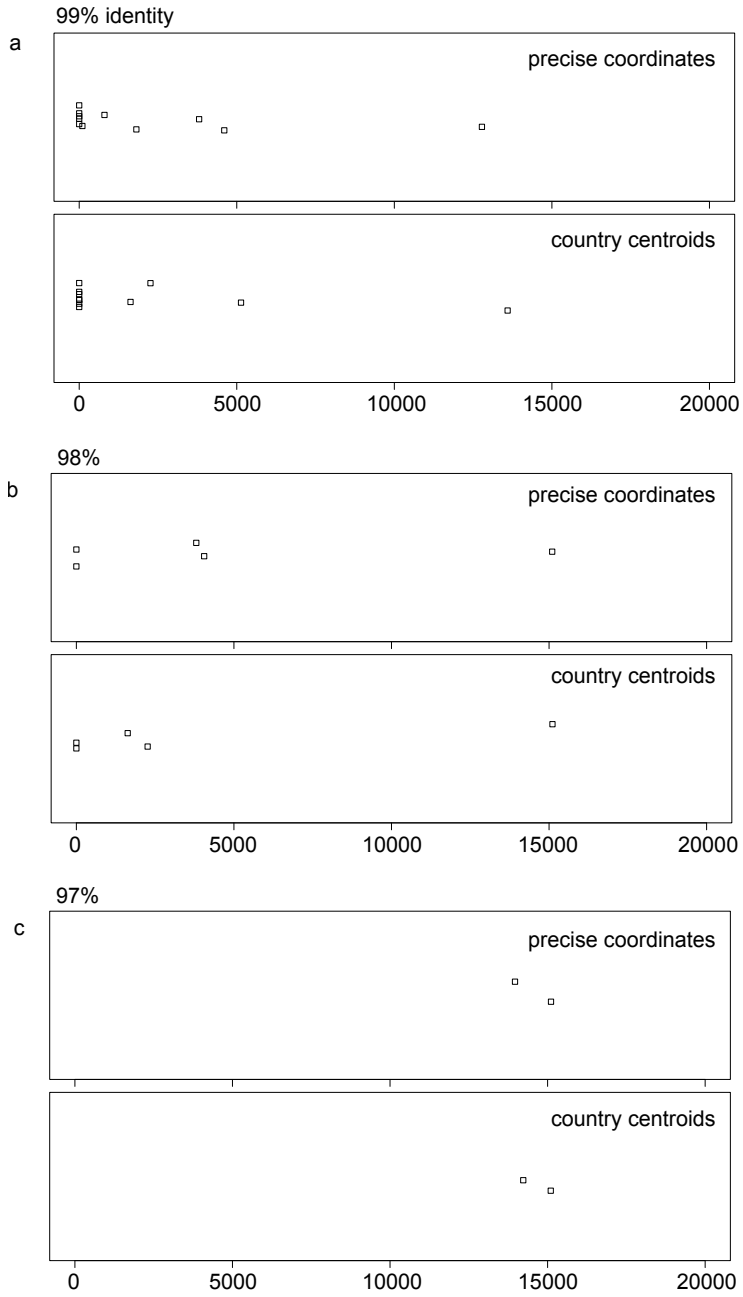
Country sampling frequency.

times sampled	country
3610	United States
1876	Estonia
1075	Sweden
1074	China
995	Canada
922	Finland
776	Germany
772	Italy
771	France
635	Australia
580	Japan
549	Norway
339	United Kingdom
314	Thailand
285	New Zealand
250	Denmark
248	Spain
242	Iran
212	Austria
210	Mexico
202	Svalbard and Jan Mayen
200	Argentina
169	Madagascar
169	Zambia
138	Cameroon
131	Hungary
123	Papua New Guinea
113	Poland
105	Belgium
100	South Korea
93	India
92	Czech Republic
82	Netherlands

times sampled	country
80	Guyana
73	Benin
73	Switzerland
68	Slovenia
66	Gabon
66	Russia
60	Portugal
55	Malaysia
53	Greenland
52	Slovakia
52	Ecuador
43	Costa Rica
42	Chile
39	New Caledonia
38	Vietnam
36	Pakistan
34	Latvia
26	Togo
21	Israel
17	Guinea
16	Iceland
15	Lithuania
15	Romania
14	Zimbabwe
14	Colombia
13	Turkey
10	Sri Lanka
10	Nepal
10	Puerto Rico
9	Seychelles
9	Panama
8	South Africa
8	Laos
8	Croatia
8	Montenegro
7	Bulgaria
7	Ireland

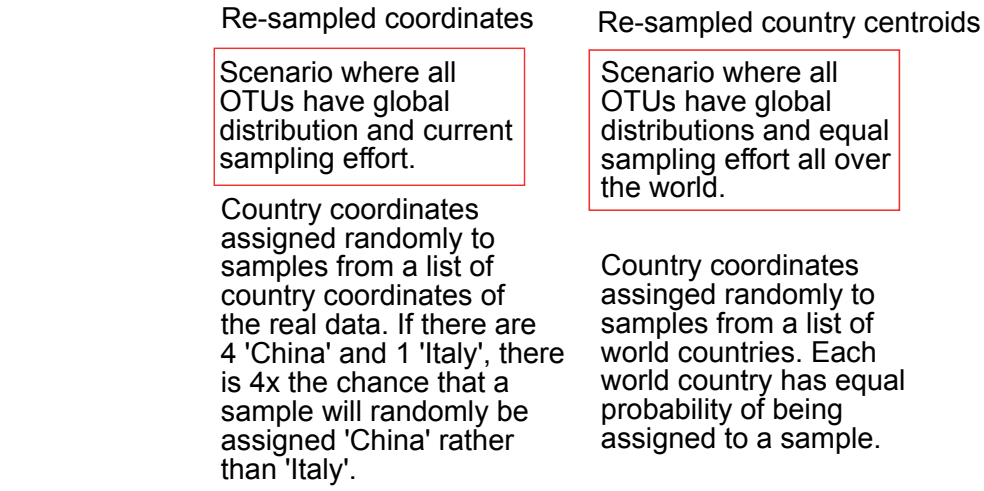
times sampled	country
6	Burundi
6	Ethiopia
6	Belize
5	Senegal
5	Netherlands Antilles
5	Brazil
5	Venezuela
4	São Tomé and Príncipe
4	Andorra
4	Luxembourg
4	Serbia
3	Martinique
3	Cyprus
2	Burkina Faso
2	Morocco
2	Indonesia
2	Greece
2	Macedonia [FYROM]
2	Ukraine
2	Dominican Republic
1	Malawi
1	Tanzania
1	Georgia
1	Cambodia
1	Northern Mariana Islands
1	Cuba

Appendix 3.3 Country centroids vs. precise coordinates range extents



Comparison of *Amanita pantherina* maximum distance measurements between precise coordinates and country centroids. This graph shows that using country centroids to estimate range extent at this scale will not result in differences that are orders of magnitude off.

Appendix 3.4 Permutation design



Mock data-set and permutations

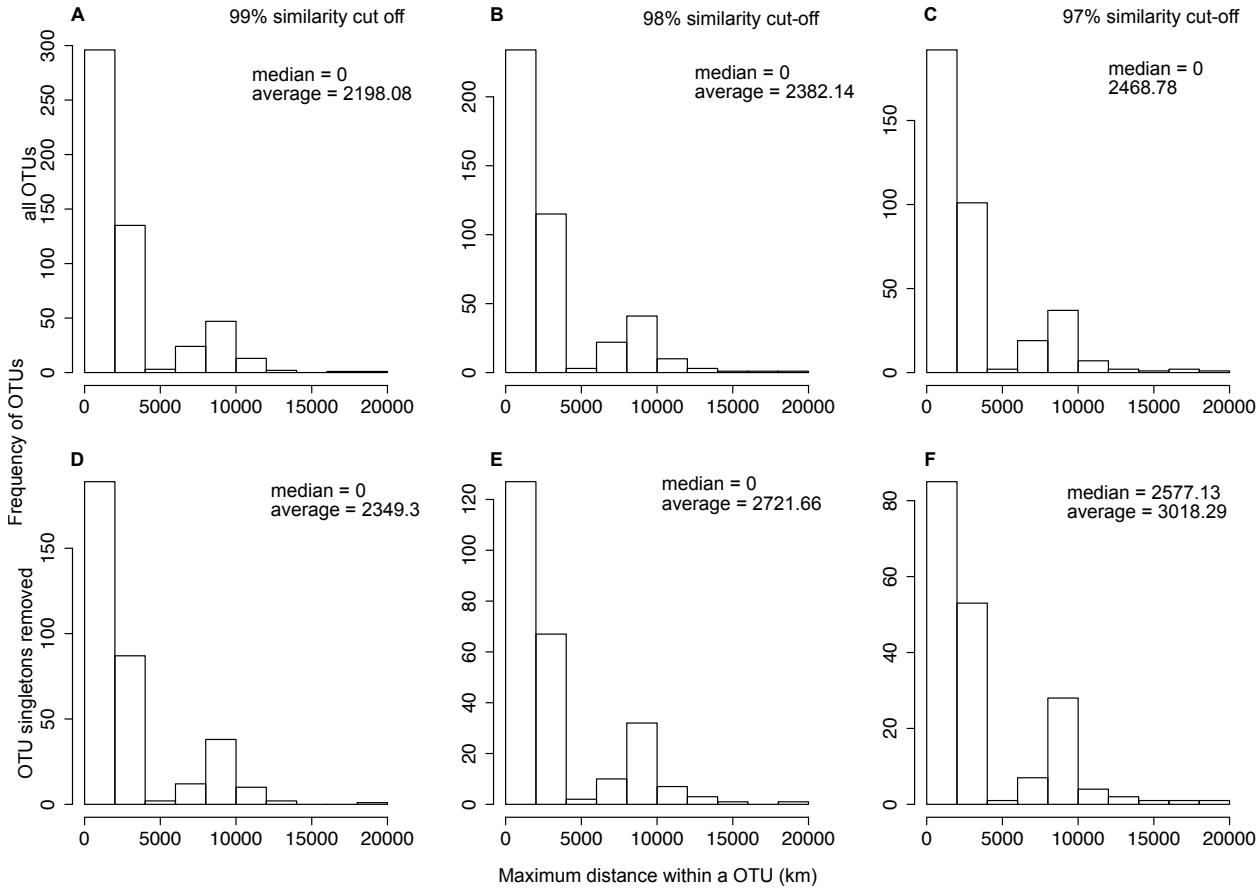
OTUs	Sample	Country real data	re-sampled coordinates	re-sampled country centroids
	[redacted]	Canada	China	Iran
	[redacted]	U.S.	U.S.	Italy
	[redacted]	U.S.	China	U.S.
	[redacted]	Korea	China	New Zealand
	[redacted]	China	U.S.	Estonia
	[redacted]	China	Korea	South Afirca
	[redacted]	China	Canada	Panama
	[redacted]	China	China	Senegal
	[redacted]	Japan	U.S.	Greenland

Countries in the analysis are replaced by their geographical coordinate centroid.

Range extent (maximum distance between all pairs of coordinates/countries) is calculated for each OTU for the real data and the two permutations tests. We compared the frequency of the range extents.

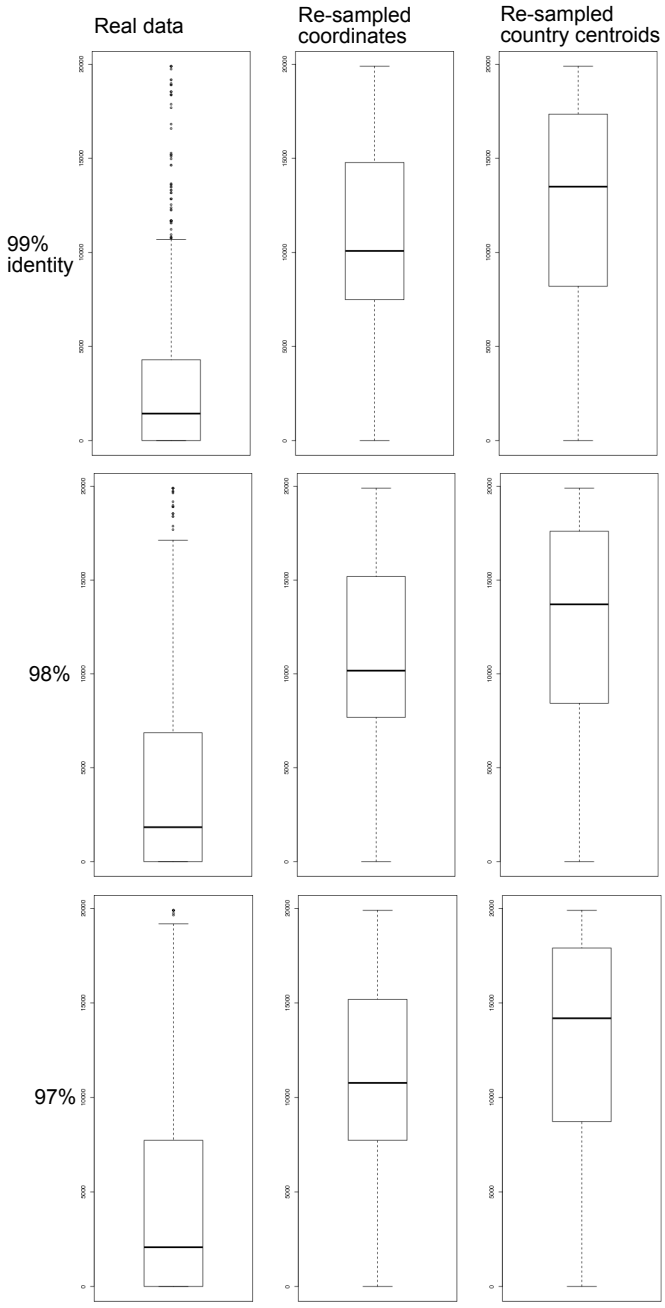
Top boxes describe the two permutation tests. The table below shows a mock example of a data-set where two OTUs are composed of several samples. One OTU is from Asia and the other is found in North America. In the first permutation test the same countries are re-sampled and randomly assigned to the samples. In the second permutation, the countries are sampled from a list of all world countries.

Appendix 3.5 Range extent frequency of species in the Pacific Northwest



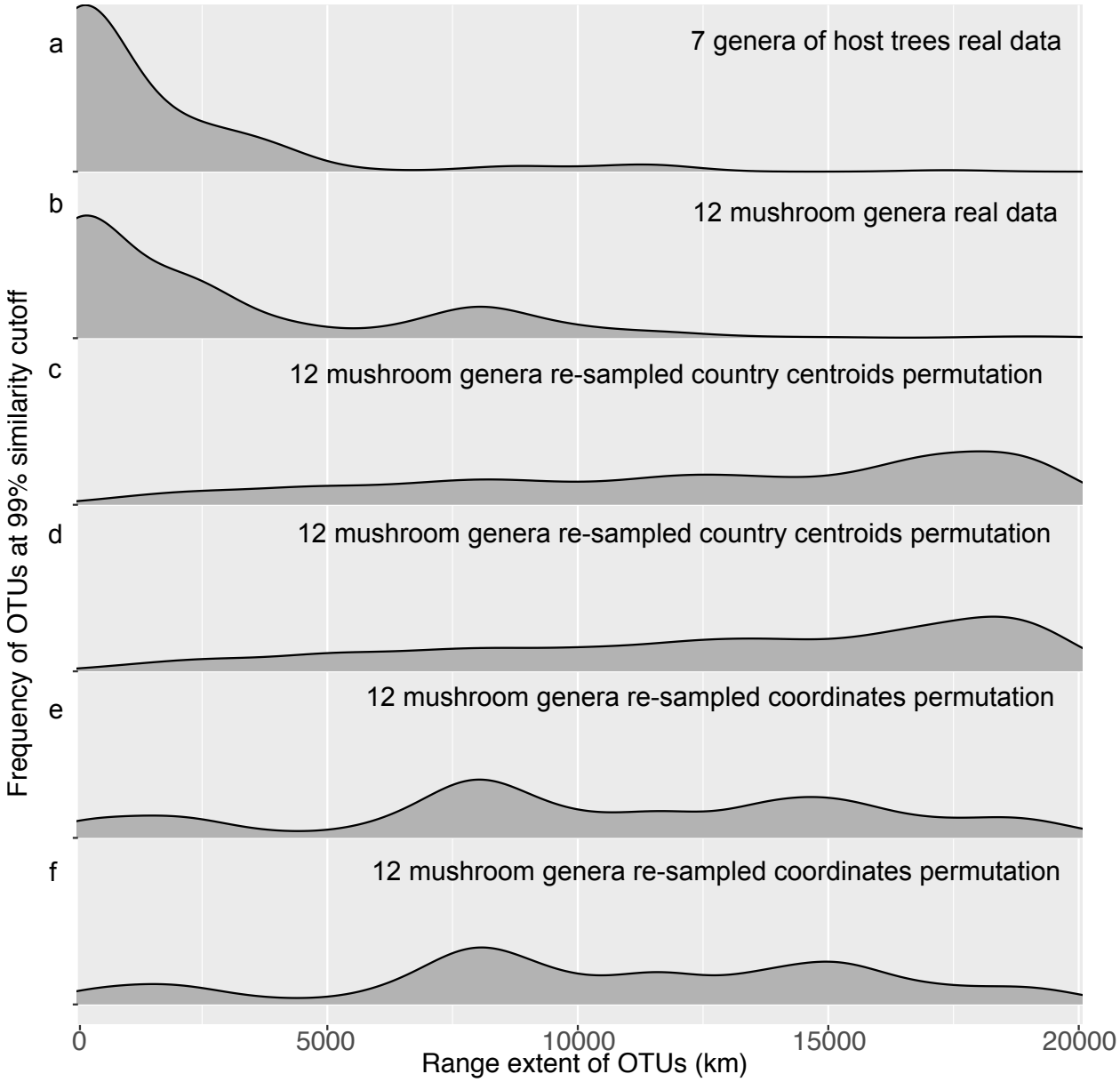
Range extent (km) between all pairs sequences from the Pacific Northwest within a cluster is mostly 0-2000 km, regardless of sequence dissimilarity (1-3 % differences). Note different scale bars in all histograms. A, B, and C include clusters that have at all cutoff levels a maximum distance of '0'. D, E, and F compared with the row above have a scale bar that is roughly half, but show the same pattern despite the removal of the 'singleton' clusters.

Appendix 3.6 3 clustering distances show similar pattern



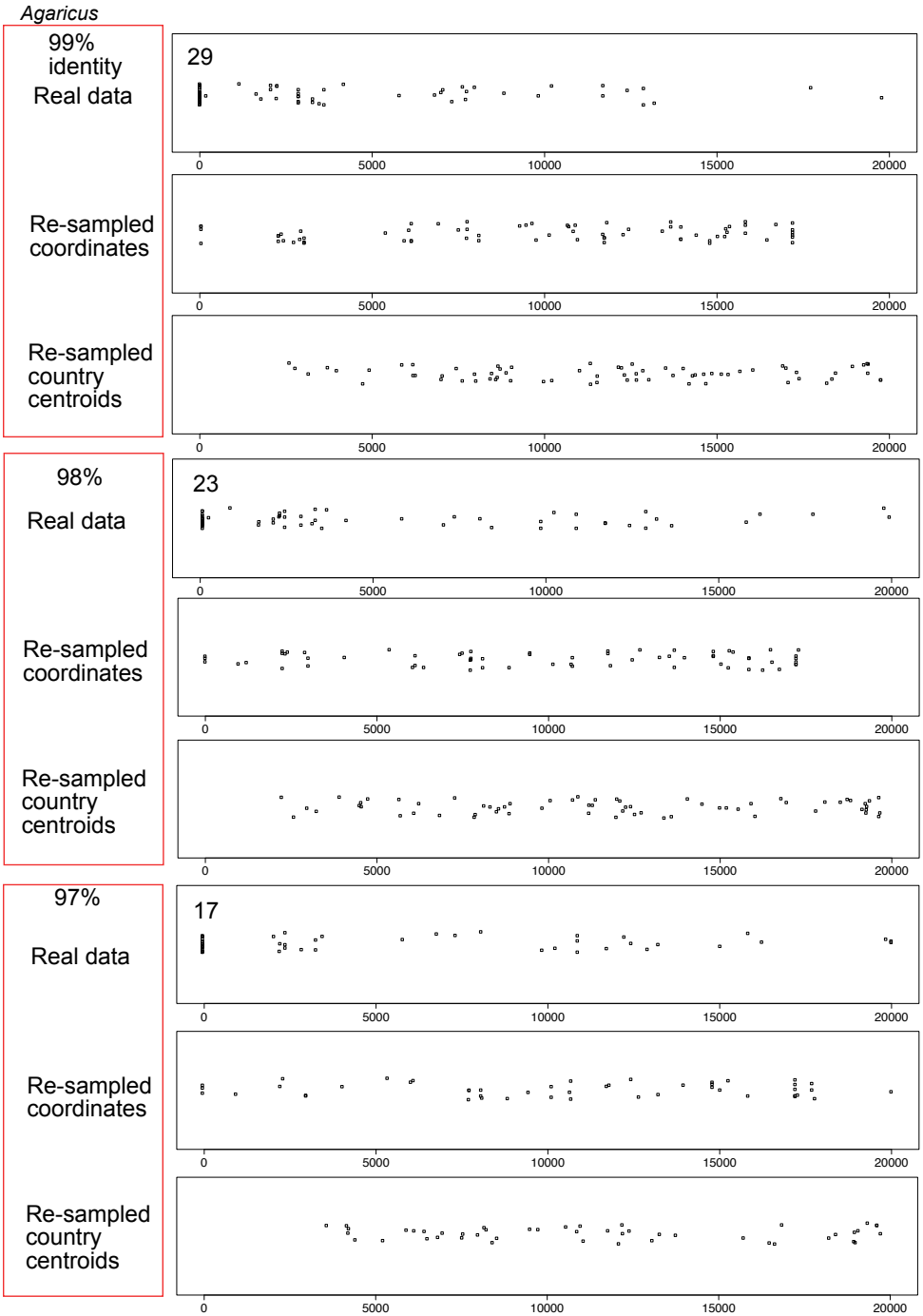
Boxplots compare the real data with the re-sampled coordinates and re-sampled country centroids permutation tests at three sequence clustering distances.

Appendix 3.7 Mushroom, tree, and permutations OTU frequencies



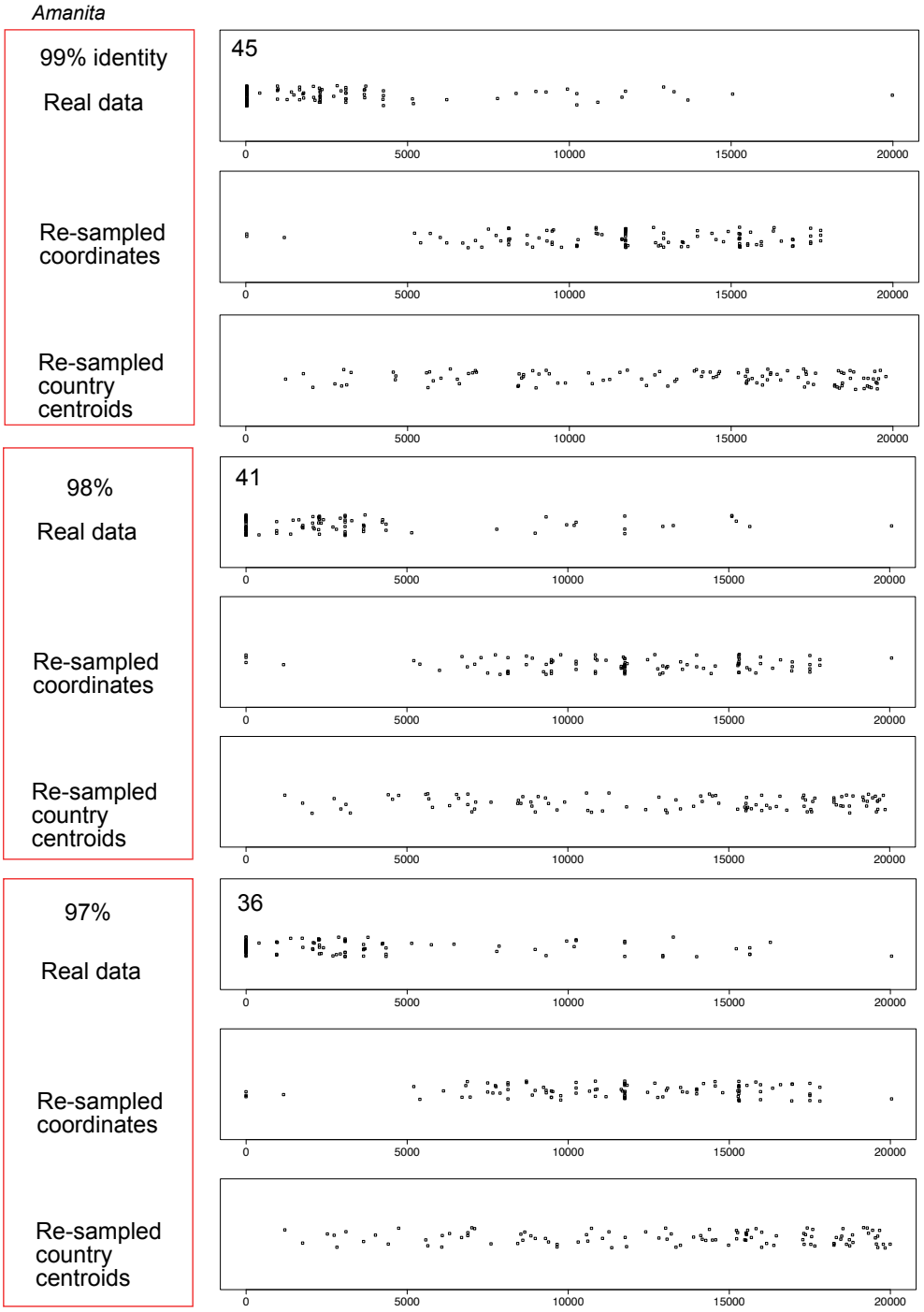
Frequency curves of ITS sequence Species Hypotheses (99% similarity) against their range extent (km). For host trees (a), the mushroom genera real data (b), the two simulations (c, d) and (e, f).

Appendix 3.8 *Agaricus* range extents



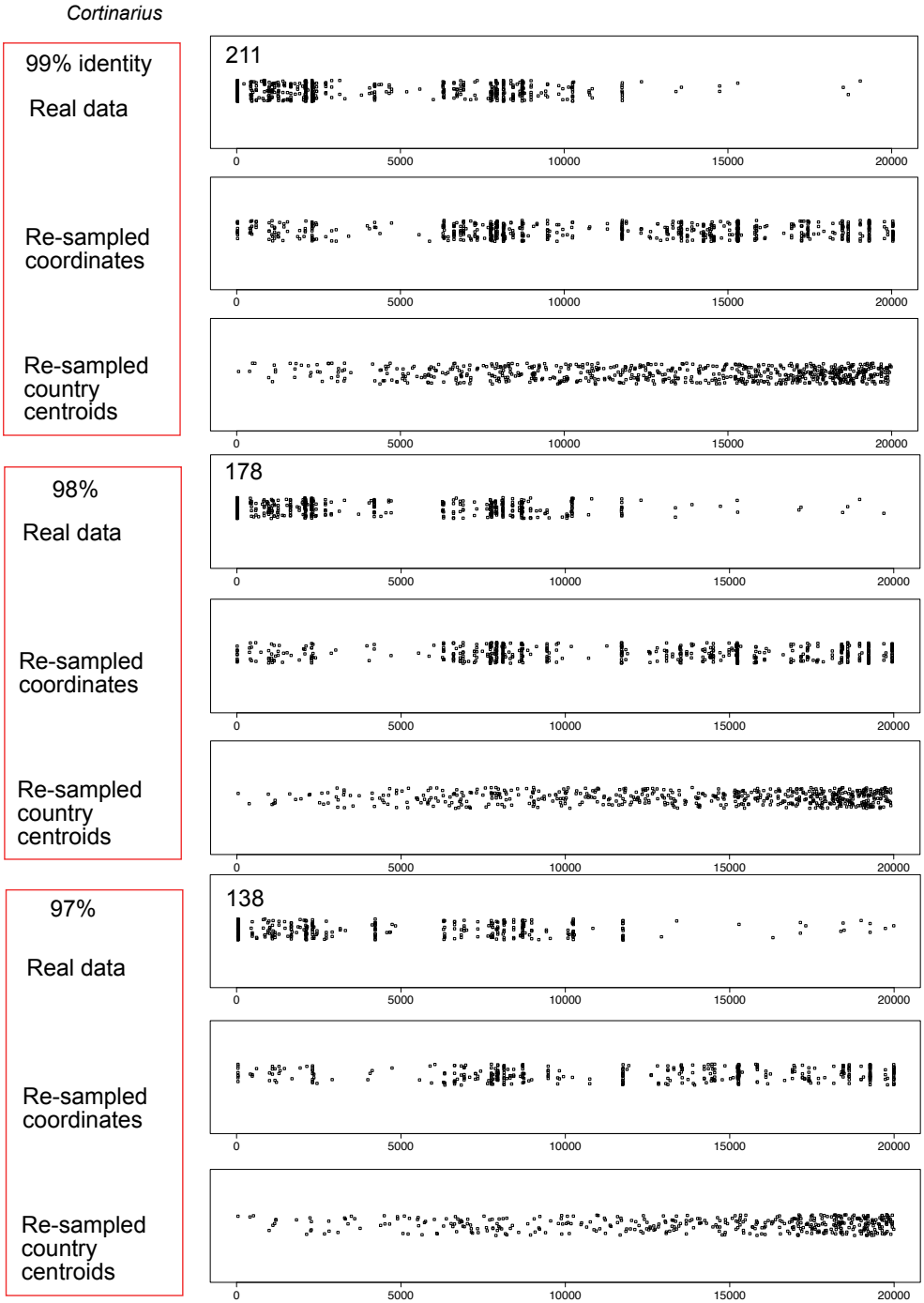
Agaricus Sequence clusters plotted along their maximum distance at 3 levels of percentage sequence similarity. For each cut-off, the first box 'real data' shows the real range extents, the second shows the re-sampled coordinates permutation, and the third is re-sampled country centroids permutation. In the 'real data' box a number indicates OTUs with '0' maximum distance .

Appendix 3.9 *Amanita* range extents



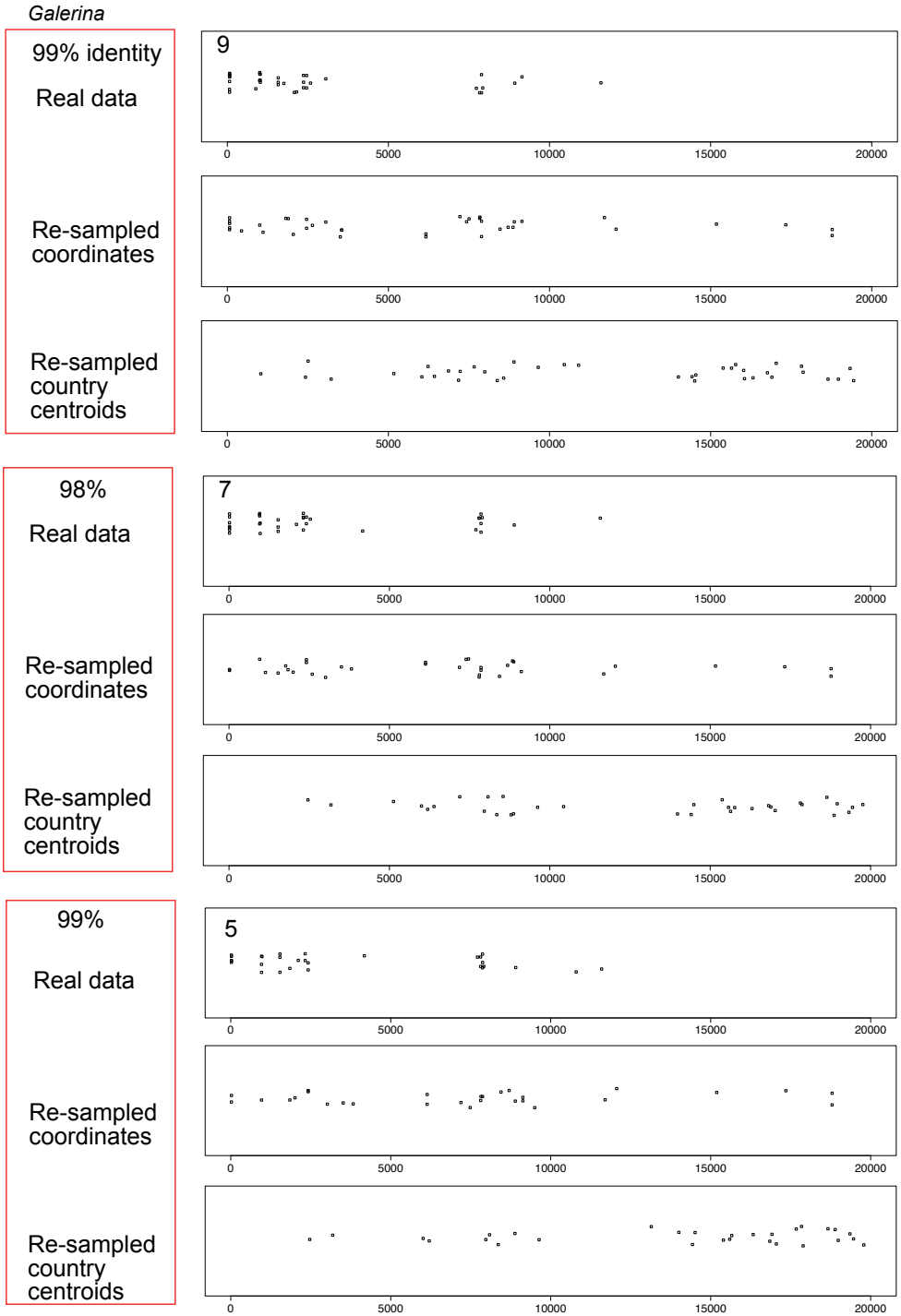
Amanita Sequence clusters plotted along their maximum distance at 3 levels of percentage sequence similarity. For each cut-off, the first box 'real data' shows the real range extents, the second shows the re-sampled coordinates permutation, and the third is re-sampled country centroids permutation. In the 'real data' box a number indicates OTUs with '0' maximum distance .

Appendix 3.10 *Cortinarius* range extents



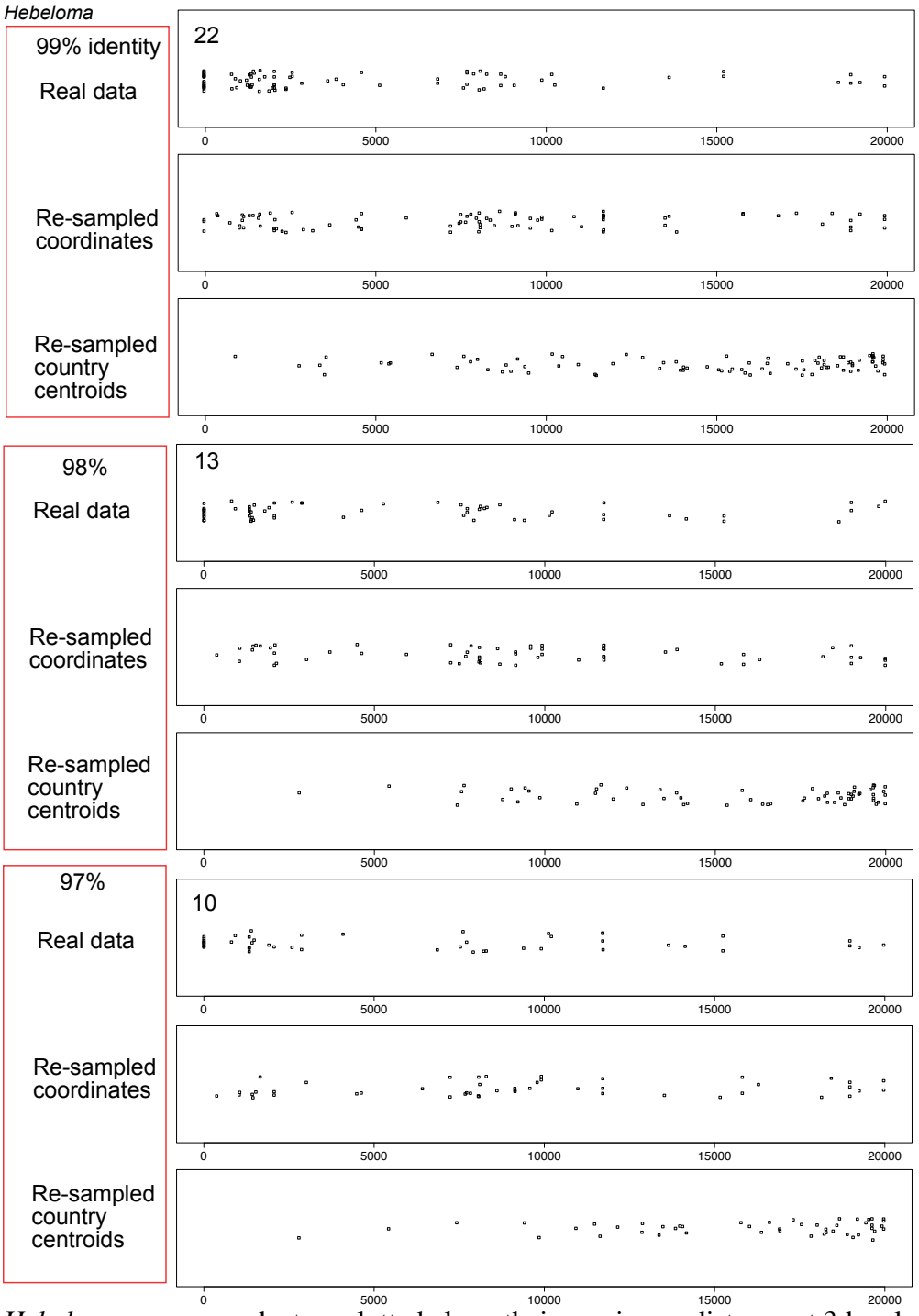
Cortinarius Sequence clusters plotted along their maximum distance at 3 levels of percentage sequence similarity. For each cut-off, the first box 'real data' shows the real range extents, the second shows the re-sampled coordinates permutation, and the third is re-sampled country centroids permutation. In the 'real data' box a number indicates OTUs with '0' maximum distance .

Appendix 3.11 *Galerina* range extents



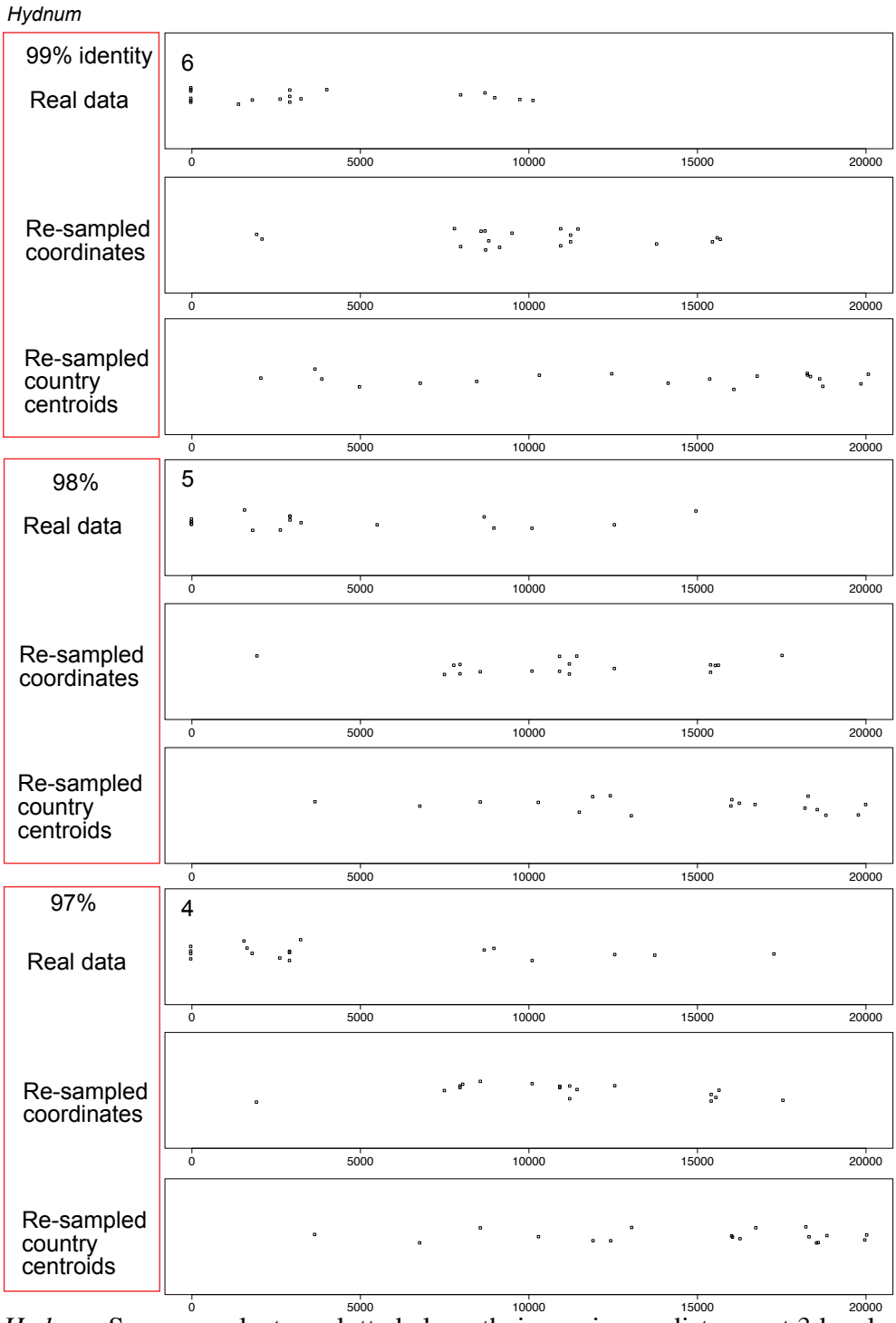
Galerina sequence clusters plotted along their maximum distance at 3 levels of percentage sequence similarity. For each cut-off, the first box 'real data' shows the real range extents, the second shows the re-sampled coordinates permutation, and the third is re-sampled country centroids permutation. In the 'real data' box a number indicates OTUs with '0' maximum distance .

Appendix 3.12 *Hebeloma* range extents



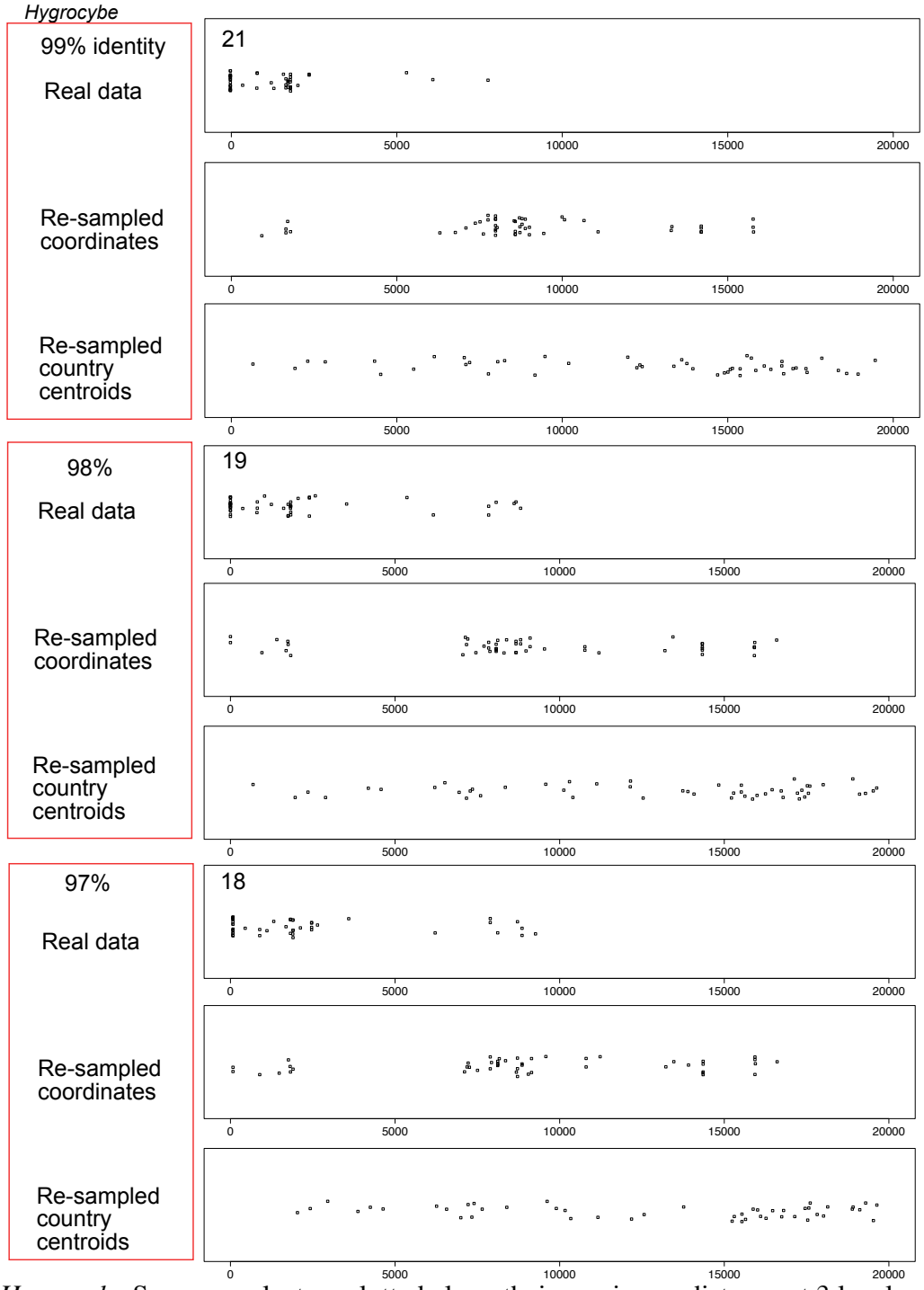
Hebeloma sequence clusters plotted along their maximum distance at 3 levels of percentage sequence similarity. For each cut-off, the first box 'real data' shows the real range extents, the second shows the re-sampled coordinates permutation, and the third is re-sampled country centroids permutation. In the 'real data' box a number indicates OTUs with '0' maximum distance.

Appendix 3.13 *Hydnum* range extents



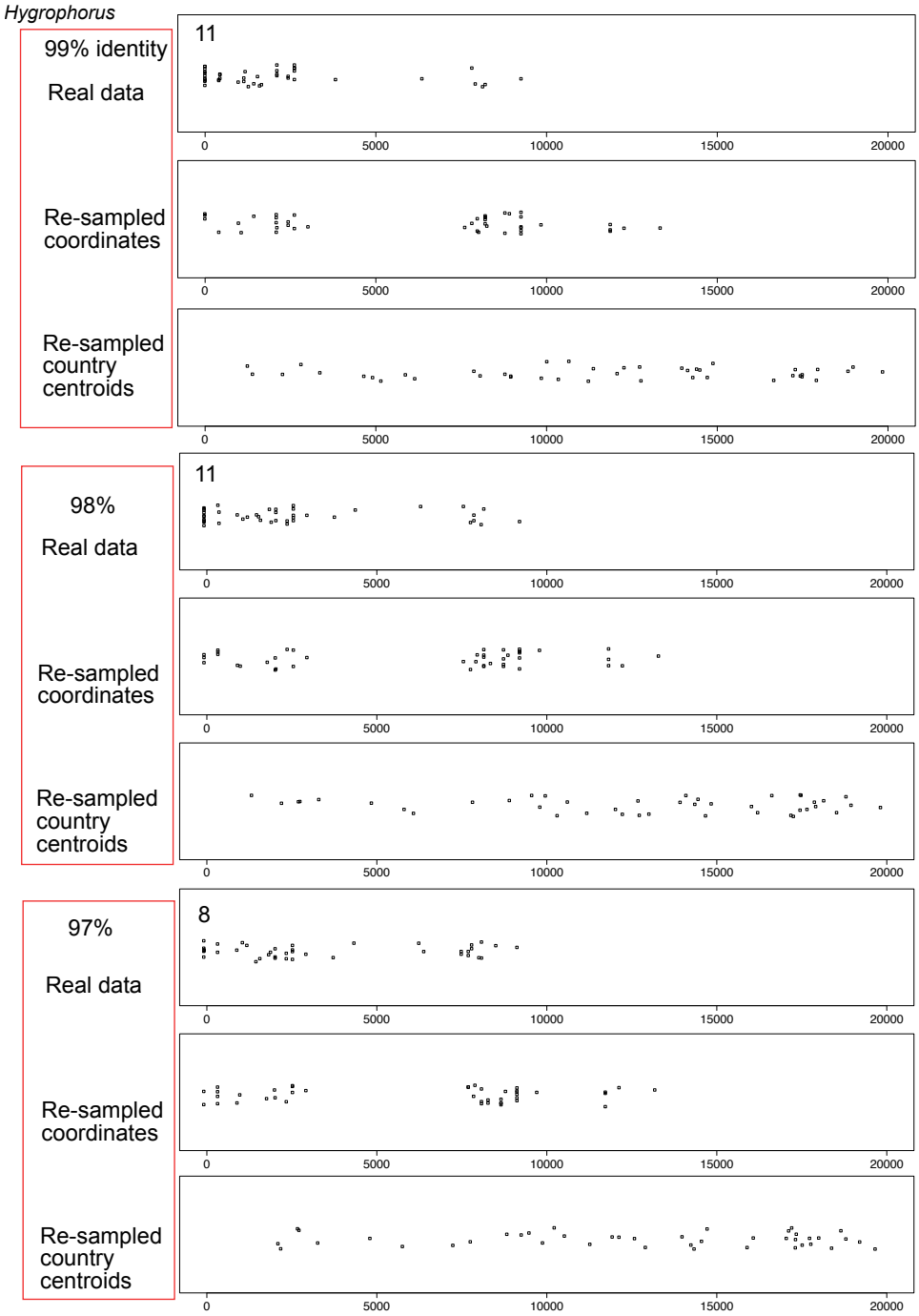
Hydnum Sequence clusters plotted along their maximum distance at 3 levels of percentage sequence similarity. For each cut-off, the first box 'real data' shows the real range extents, the second shows the re-sampled coordinates permutation, and the third is re-sampled country centroids permutation. In the 'real data' box a number indicates OTUs with '0' maximum distance.

Appendix 3.14 *Hygrocybe* range extents



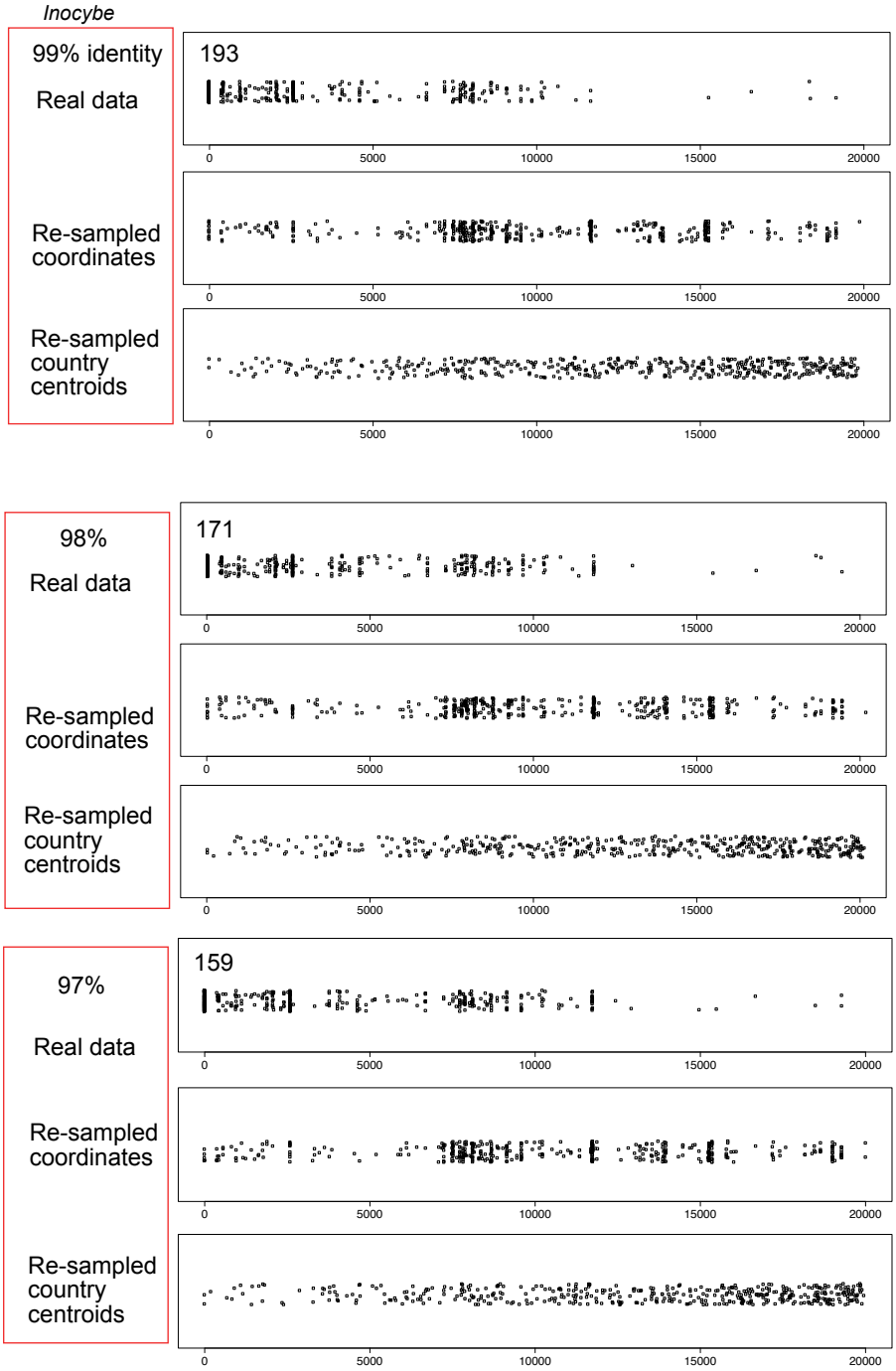
Hygrocybe Sequence clusters plotted along their maximum distance at 3 levels of percentage sequence similarity. For each cut-off, the first box 'real data' shows the real range extents, the second shows the re-sampled coordinates permutation, and the third is re-sampled country centroids permutation. In the 'real data' box a number indicates OTUs with '0' maximum distance.

Appendix 3.15 *Hygrophorus* range extents



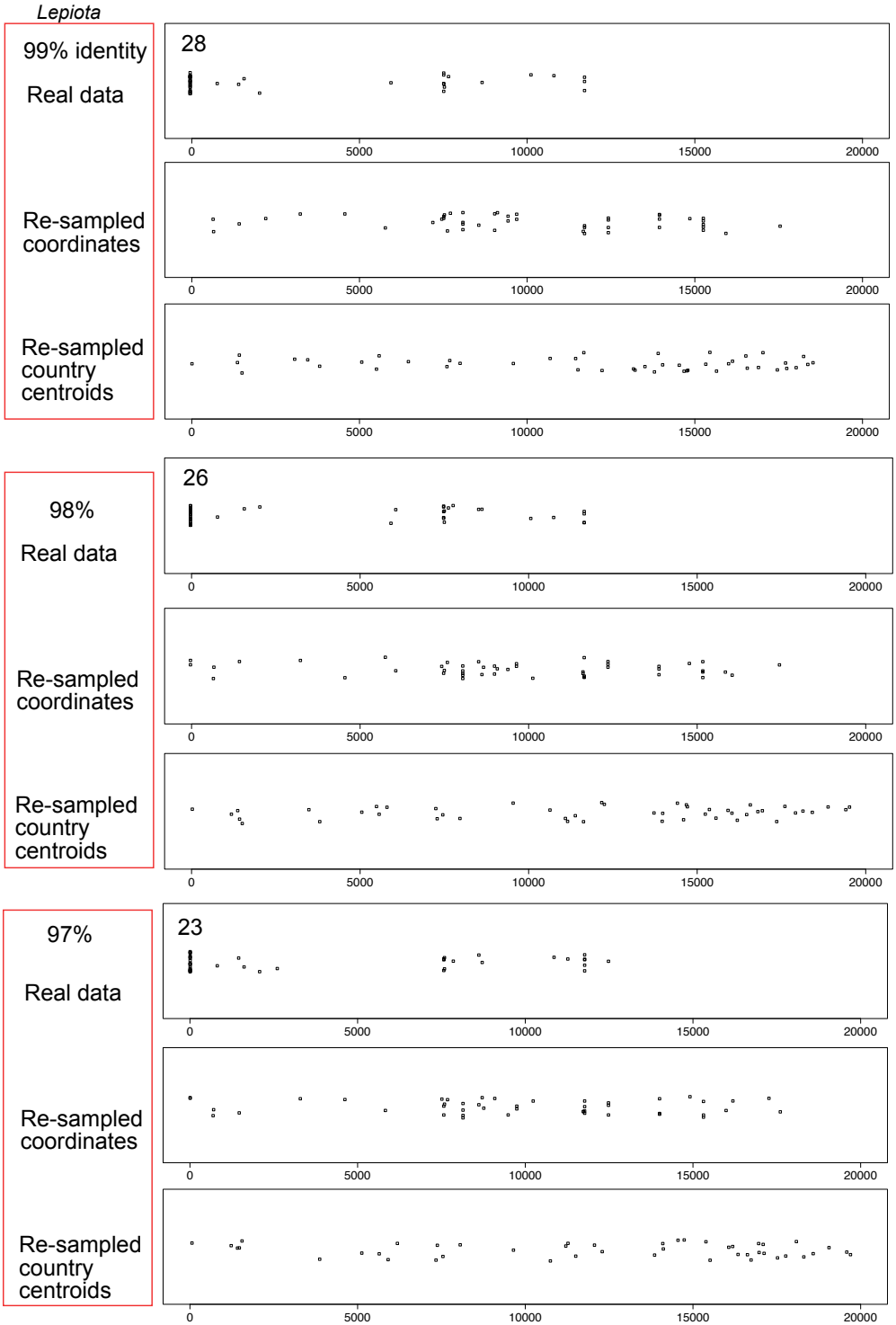
Hygrophorus Sequence clusters plotted along their maximum distance at 3 levels of percentage sequence similarity. For each cut-off, the first box 'real data' shows the real range extents, the second shows the re-sampled coordinates permutation, and the third is re-sampled country centroids permutation. In the 'real data' box a number indicates OTUs with '0' maximum distance.

Appendix 3.16 *Inocybe* range extents



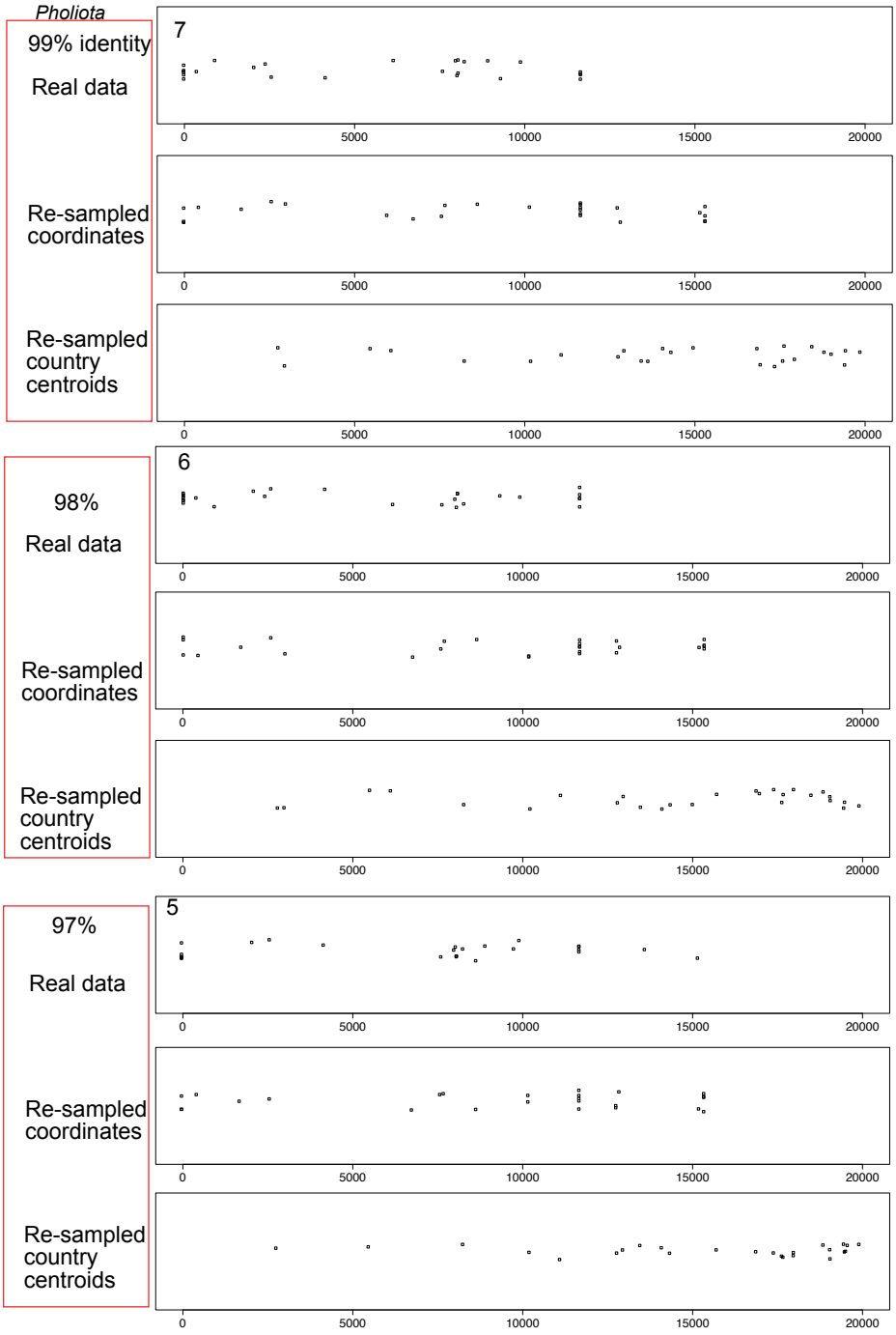
Inocybe Sequence clusters plotted along their maximum distance at 3 levels of percentage sequence similarity. For each cut-off, the first box 'real data' shows the real range extents, the second shows the re-sampled coordinates permutation, and the third is re-sampled country centroids permutation. In the 'real data' box a number indicates OTUs with '0' maximum distance.

Appendix 3.17 *Lepiota* range extents



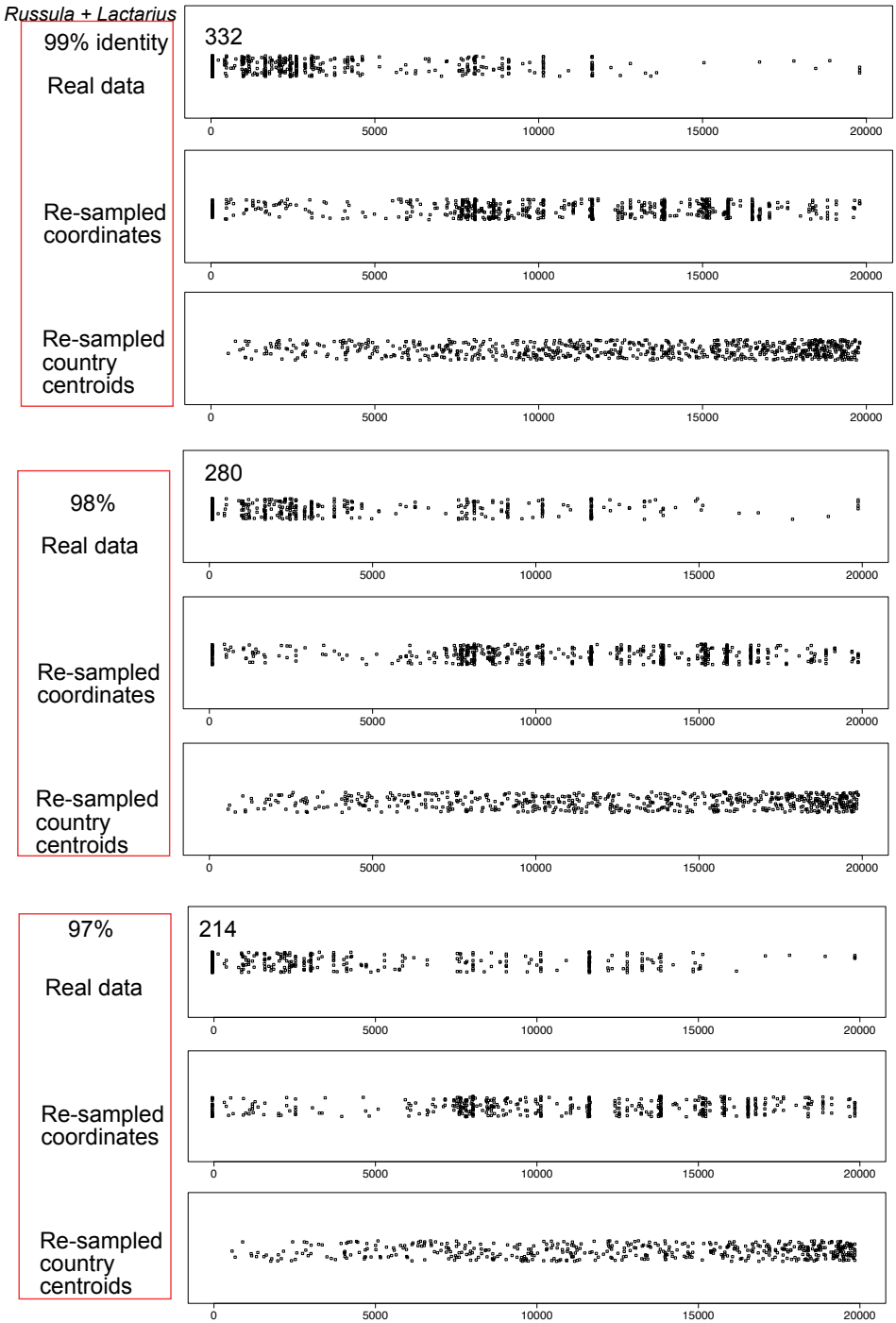
Lepiota Sequence clusters plotted along their maximum distance at 3 levels of percentage sequence similarity. For each cut-off, the first box 'real data' shows the real range extents, the second shows the re-sampled coordinates permutation, and the third is re-sampled country centroids permutation. In the 'real data' box a number indicates OTUs with '0' maximum distance.

Appendix 3.18 *Pholiota* range extents



Pholiota Sequence clusters plotted along their maximum distance at 3 levels of percentage sequence similarity. For each cut-off, the first box 'real data' shows the real range extents, the second shows the re-sampled coordinates permutation, and the third is re-sampled country centroids permutation. In the 'real data' box a number indicates OTUs with '0' maximum distance.

Appendix 3.19 *Russula* and *Lactarius* range extents



Russula and *Lactarius* Sequence clusters plotted along their maximum distance at 3 levels of percentage sequence similarity. For each cut-off, the first box 'real data' shows the real range extents, the second shows the re-sampled coordinates permutation, and the third is re-sampled country centroids permutation. In the 'real data' box a number indicates OTUs with '0' maximum distance.

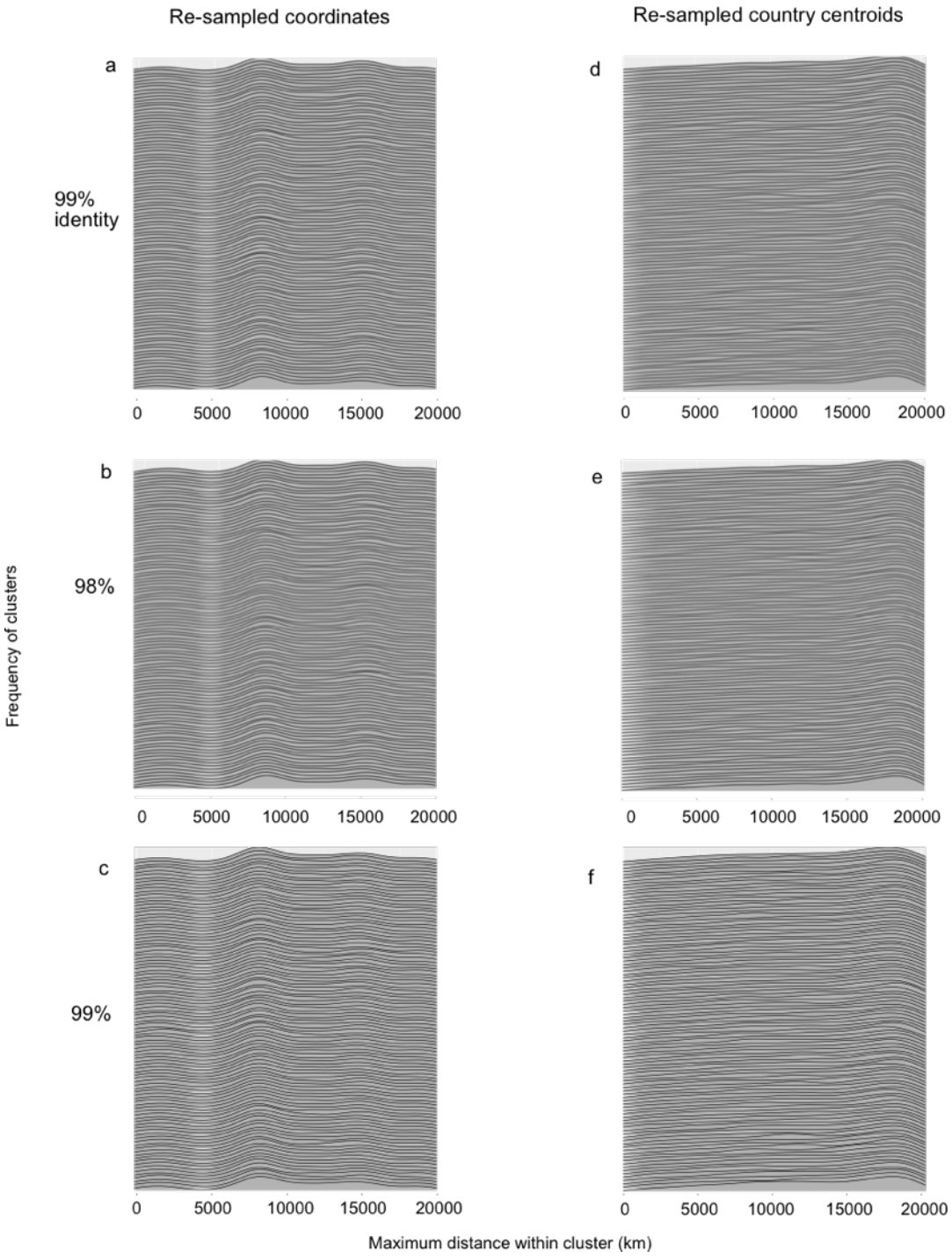
Appendix 3.20 Wilcoxon test

Results of the Wilcoxon test.

99% identity	re-sampled coordinates replicate 68	re-sampled coordinates replicate 69	re-sampled country centroids replicate 5	re-sampled country centroids replicate 54	real data excluding OTUs with more than 30 samples and less than 5 samples
real data	W = 689950, p-value < 2.2e-16	W = 767760, p-value < 2.2e-16	W = 415090, p-value < 2.2e-16	W = 454520, p-value < 2.2e-16	W = 664030, p-value < 2.2e-16
re-sampled coordinates replicate 68		W = 2776000, p-value = 0.09883	W = 2015600, p-value < 2.2e-16	W = 2083900, p-value < 2.2e-16	
re-sampled coordinates replicate 69			W = 3431700, p-value < 2.2e-16	W = 3364700, p-value < 2.2e-16	
re-sampled country centroids replicate 5				W = 2638700, p-value = 0.1767	
98%	re-sampled coordinates replicate 68	re-sampled coordinates replicate 69	re-sampled country centroids replicate 5	re-sampled country centroids replicate 54	
real data	W = 612320, p-value < 2.2e-16	W = 671890, p-value < 2.2e-16	W = 372540, p-value < 2.2e-16	W = 425580, p-value < 2.2e-16	
re-sampled coordinates replicate 68		W = 2165500, p-value = 0.1904	W = 1551000, p-value < 2.2e-16	W = 1641200, p-value < 2.2e-16	
re-sampled coordinates replicate 69			W = 2706800, p-value < 2.2e-16	W = 2618000, p-value < 2.2e-16	
re-sampled country centroids replicate 5				W = 2039600, p-value = 0.04585	

97%	re-sampled coordinates replicate 68	re-sampled coordinates replicate 69	re-sampled country centroids replicate 5	re-sampled country centroids replicate 54	
real data	W = 482500, p-value < 2.2e-16	W = 538620, p-value < 2.2e-16	W = 309990, p-value < 2.2e-16	W = 352990, p-value < 2.2e-16	
re-sampled coordinates replicate 68		W = 1586700, p-value = 0.07254	W = 1121500, p-value < 2.2e-16	W = 1192000, p-value < 2.2e-16	
re-sampled coordinates replicate 69			W = 1981000, p-value < 2.2e-16	W = 1913000, p-value < 2.2e-16	
re-sampled country centroids replicate 5				W = 1473100, p-value = 0.04513	

Appendix 3.21 Permutation replicates



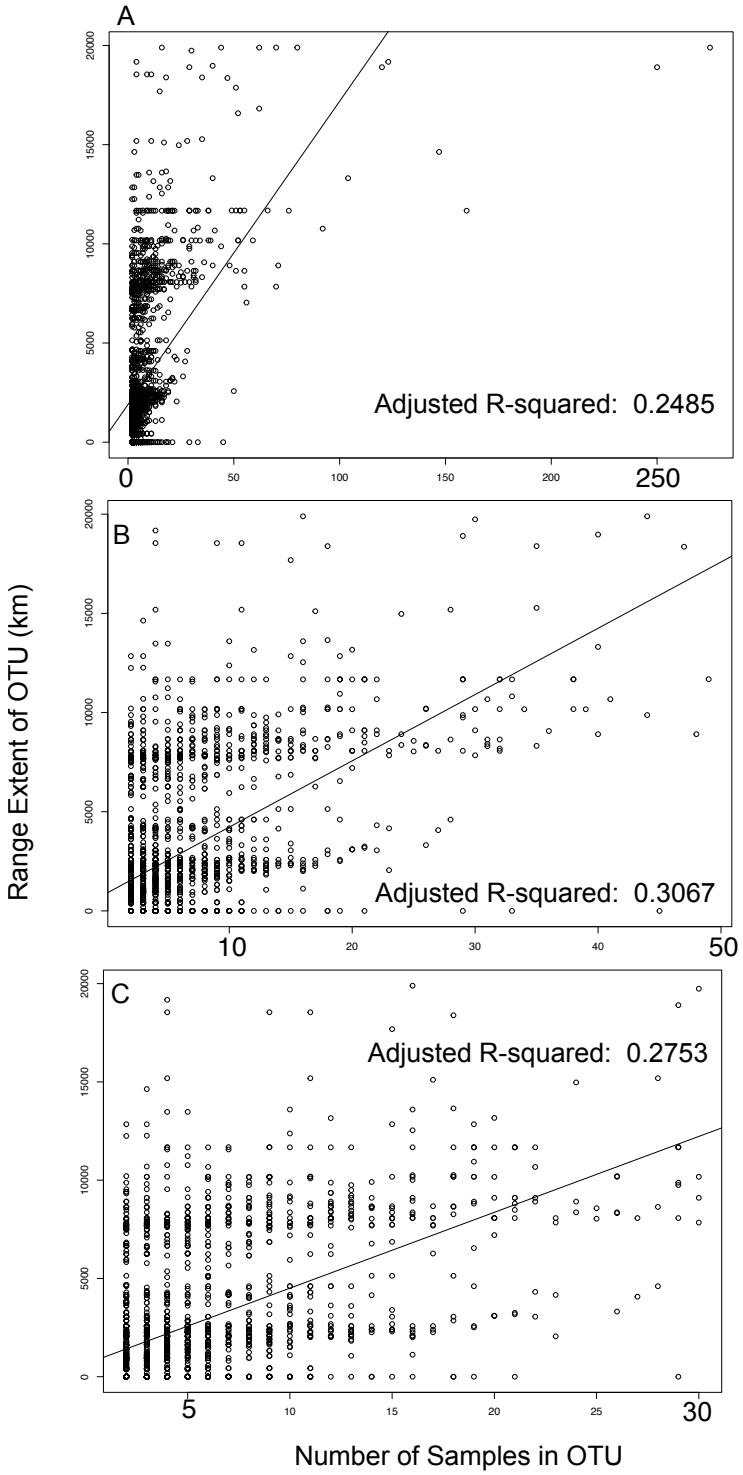
Each panel shows 100 frequency curves for the two permutation tests, where random assignments to the OTUs. a, b, and c are all 100 frequency curves for re-sampled coordinates. d, e, and f are all 100 frequency curves for re-sampled country centroids.

Appendix 3.22 Quantiles

Results from comparing quantiles of the different permutations.

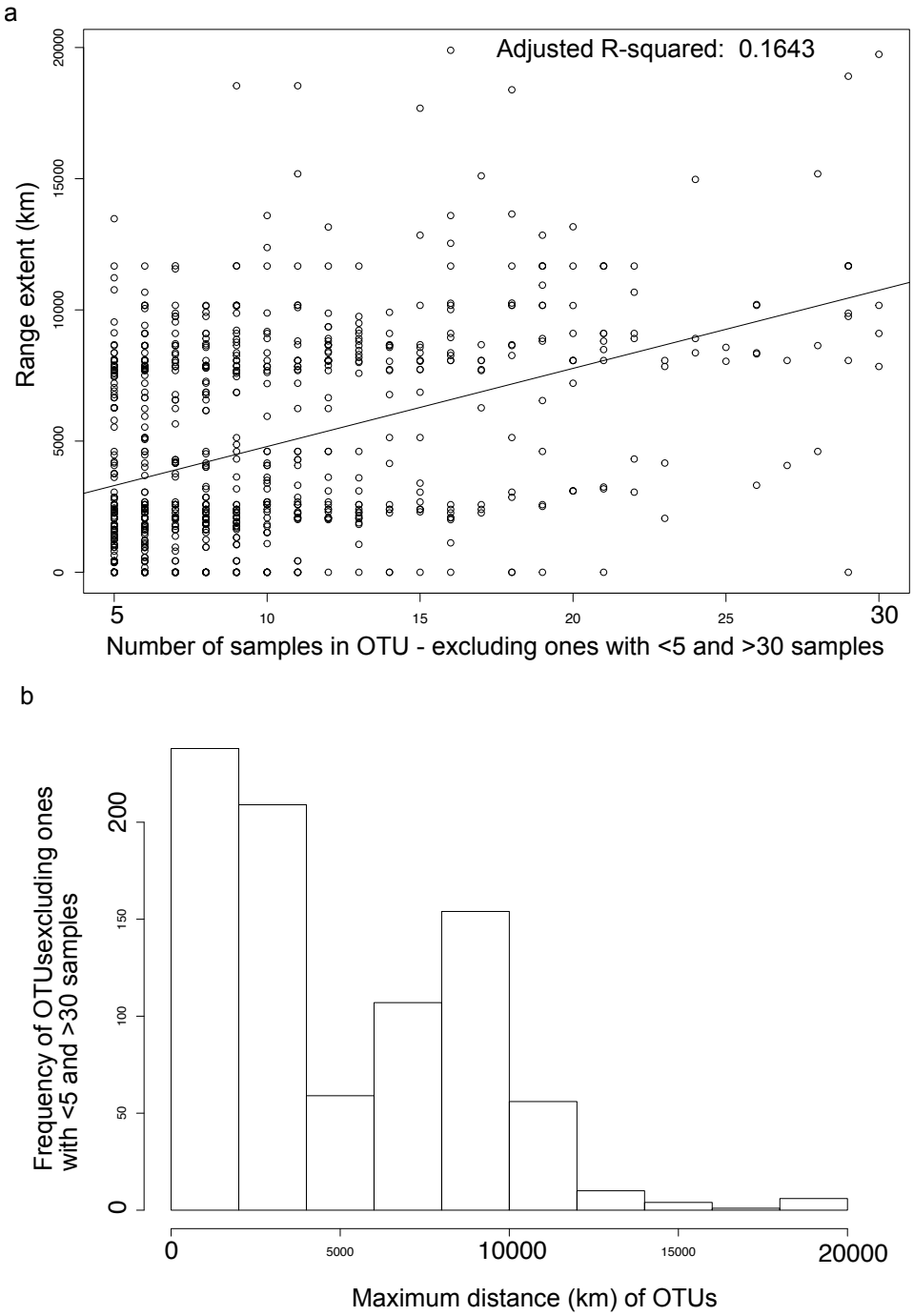
Quantiles	0	0.25	0.5	0.75	1
99% real data	0	0	1447.966	4441.671	19894.268
resampled coordinates	0	7473.015	9991.251	14752.991	19894.268
resampled country centroids	0	8231.093	13475.862	17335.748	19894.268
98% real data	0	0	1849.296	6958.43	19894.268
resampled coordinates	0	7633.859	10274.374	15165.4	19894.268
resampled country centroids	0	8422.479	13764.987	17589.523	19894.268
97% real data	0	0	2078.007	7758.415	19894.268
resampled coordinates	0	7738.812	10728.239	15184.894	19894.268
resampled country centroids	0	8745.201	14177.659	17904.139	19894.268

Appendix 3.23 Number of samples per OTU vs. range extent



Number of samples within a OTUs against the range extent of OTUs. A. All samples show a strong positive trend mostly driven by ~30 OTUs with more than 50 samples, about 1% of the whole dataset. Once those are removed the trend decreases. B and C show the line becomes less steep as the OTUs with more than 50 or more than 30 samples are removed.

Appendix 3.24 Number of samples (excluding <5,>30) per OTU vs. range extent



Number of samples vs. range extent of OTUs. (a) We excluded all OTUs with less than 5 samples and more than 30. (b) We plotted a histogram of the frequency and see the same pattern as the complete data set.

Appendix 3.25 Dispersal extent from other studies

Vincenot and Selosse summary of dispersal extent of mushroom dispersal extent.

Species	Distance (km)
Cenococcum geophilium	10-100
Rhizopogon vinicolor	~100
Tuber malanosporum	100-500
Tuber aestivum	~500
Pisolithus microcarpus	~500
Tuber magnatum	~500
Rhizopogon roseolus	~1000
Pisolithus tinctorius	100-3000
Tricholoma matsutake	100-3000
Laccaria sp. A	100
Suillus brevipes	100
Tricholoma scalpturatum	1000
Russula brevipes	1000
Tricholoma populinum	1000-5000
Laccaria amethystina	500-10000
Amanita phalloides	1000-5000
Suillus luteus	100-500
Suillus spraguei	~2000
Russula virescens	~7000