Russulas of Southern Vancouver Island Coastal Forests

Volume 1

by

Christine Roberts

B.Sc. University of Lancaster, 1991

M.S. Oregon State University, 1994

A Dissertation Submitted in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in the Department of Biology

Your file Votre reference
ISBN: 978-0-494-47323-8
Our file Notre reference
ISBN: 978-0-494-47323-8

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Abstract

The *Russula* flora of Vancouver Island is diverse, colourful, abundant, ecologically important, but poorly documented, with the literature spread in many diverse journals and books from across North America and Europe in various languages. Keys and field guides to local species emphasise macroscopic and spore characters but distinctive structures in the epicutis are not described. As Russulas are prone to environmentally affected colour variation and a number of species have a similar appearance, correct identification may require microscopic examination and a suite of chemicals, a barrier to many people. The existence of synonyms and conflicting concepts for several species adds to the frustration in identification.

Presented here are detailed illustrated descriptions of locally collected species, with discussions on nomenclatural and taxonomic issues where these cause confusion, some of these confirm past records, and some are new records or new species. Three aids to identification are examined: 1. A simplified chromatography method is described that identifies Russulas to subgenus and in some cases section and subsection level, enabling differentiation between some lookalike species without recourse to microscopy.

2. A method often used to match ectomycorrhizae with nearby basidiomata by comparing their restriction fragment length polymorphisms (RFLPs) of amplified ITS rDNA, can also be compared with virtual RFLP's from sequence data downloaded from NCBI and EMBI to aid identification. The restriction enzymes Hinfl, Alul and Sau3 A, resolved identities to subgeneric and section level, rarely to species. 3. Using published sequence data and Bayesian analysis, a phylogeny was sought with better resolution in the upper clades than had been found with other analysis methods. Various characters from published descriptions and from Vancouver Island collections were then examined for correlation with branching order or clade in this phylogeny, with basidia width, spore colour, pileocystidia shape and spore shape having highest correlation.

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Acknowledgments

I thank my husband Bob Trotta for his patience and support while I worked on this dissertion. I also thank my off-campus supervisor Dr. Bryce Kendrick for financial support and much useful advice. My other committee members have provided advice, assistance and laboratory facilities as needed. I am grateful to Dr. William Hintz for his help with the DNA analyses, and to Dr. Tony Trofymow for the use of office, laboratory and library facilities for a while at the Pacific Forestry Centre. I thank Dr. Shannon Berch, David Dunn and Helga Mehl for the use of laboratory space and equipment at the Ministry of Forests Research Branch.

I am indebted to the members of the South Vancouver Island Mycological Society and the members of the Pacific Northwest Key Council for collecting Russulas, and epecially to both Ian Gibson for his development of the Matchmaker program, which has been very useful, and to Ben Woo, who wrote the original PNWKC *Russula* key, collected the literature for hundreds *of Russula* species, and shared this with me.

Chapter 1

Introduction

The genus *Russula* is a member of the *Russulaceae* within the order *Russulales* (Kirk *et al.* 2001). All *Russulales* have amyloid ornamentation on the spores evident as a blue-black reaction to iodine, usually applied as Melzer's reagent. They also have gloeocystidia in the hymenium (spore-bearing tissue) and the epicutis, vascular hyphae with contents that change colour with sulphuric benzaldehydes such as sulphovanillin (SV), and the hyphae lacking clamp connections. The *Russulaceae* consists of the agaric genera *Russula* and *Lactarius* and the closely related sequestrate genera of *Cystangium, Elasmomyces, Gymnomyces, Macowanites, Martellia* (close to *Russula),* and *Archangeliella* and *Zelleromyces* (close to *Lactarius).* These sequestrate taxa are not truly separate genera; rather they are an adaptation to a different spore dissemination strategy than that of agarics (Miller *et al.* 2001). The *Russulaceae* have heteromerous trama; as well as typical cylindrical hyphae they also contain sphaerocytes in varying degrees, which as the name suggests are approximately spherical cells that give the fungi a brittle, crumbly texture. *Russula* differs from *Lactarius* in its lack of fluid latex, and some *Lactarius* species have strongly coloured flesh whereas in *Russula* the colour is restricted to the pileal and stipe cutis. Both genera have species that change colour when damaged. In temperate regions these two genera are morphologically and, in some studies, phylogenetically distinct (Simono *et al.* 2004), but with tropical species this distinction between genera is blurred (Buyck 1995). In Eberhardt (2002), Hibbett *et al.* (2005), Miller *et al.* (2001) and Moncalvo *et al.* (2002), Lactarius is a sister clade to *Russula,* with the *Lactaroides* (for example *Russula delica, R. brevipes* and *R. chloroides)* basal to both clades.

Russulas are conspicuous, fleshy, often brightly coloured species, and may be among the most abundant epigeous fungi in Pacific Northwest (PNW) forests in the fall, playing a significant role in nutrient cycling. Smith *et al.* (2002) found that of the epigeous fungi, the biomass of Russulas was greater than that of other ectomycorrhizal genera, comprising 23% in an old-growth forest, 10% in a 40-50 year old stand and 7% in a young forest. In the PNW Russulas are more abundant and diverse in old and mature forests (Durall *et al.* 1999, Roberts *et al.* 2004, Smith *et al.* 2002), although Norvell and Exeter (2004) found no significant difference in *Russula* diversity between young (26 year old), mature and late stage (55 and 150 year old) stands, much depended on the characteristics of the site. Some Russulas may also be useful indicator species of site characteristics (O'Dell *et al.* 1999, Roberts *et al.* 2004).

Russulas are all ectomycorrhizal with trees and shrubs, as far as is known, and exhibit a range of host specificity (Trappe 1962, Molina *et al.* 1992). Epigeous Russulas have been recorded as ectomycorrhizal with members of the *Salicaceae, Betulaceae, Pinaceae, Fagaceae, Rosaceae* and *Polygonaceae* (Molina *et al* 1992). Observations made while collecting data for this dissertation and other studies have shown that while some *Russula* species are cosmopolitan and capable of forming associations with one or more hosts in a range of habitats, others were more constrained in either host or habitat or both (Roberts *et al.* 2004). Ectomycorrhizal fungi have been shown to be important in uptake of nutrients and their translocation to the host plant, increasing the viability of seedling trees over those without fungal mutualists (Trappe and Strand 1969). Simard *et al.* (1997) showed that nearby trees of different families linked by a common ectomycorrhizal system could obtain carbohydrates from one another via this link when one of the pair was under shade, an important means of survival of young trees in a mature forest until they reach the canopy.

Some *Russula* species are commonly found in wood of varying stages of decay. Tedersoo *et al.* (2003) found three *Russula* species colonizing roots in coarse woody debris, and in this study three *Russula* species were always collected from decaying wood. While it is not known if any *Russula* species can decompose wood, Leake *et al.* (2001) found that ectomycorrhizal fungi translocated to the host plant labelled nutrients from wood colonised by wood-decomposing saprotrophic fungi.

Russulas provide a seasonal food source for slugs, squirrels and deer (Cazares and Trappe 1994, Maser *et al.* 1978, Maser *et al.* 1985, Buller 1958, Keller and Snell 2002). In the Pacific Northwest, *Ariolimax columbianus*, the banana slug, preferentially consumed species of *Russula* when they were available; in a short study, 55% of all Russulas seen had slug damage averaging over 27% loss of tissue (Roberts 1997, unpublished data). Humans also collect several species of *Russula* for food, but in the

Pacific Northwest only *Russula brevipes* parasitized with *Hypomyces laclifluorum*, known as the lobster mushroom, is collected commercially.

All told, the ecological roles of Russulas are complex and may involve net movement of nutrients from soil and wood to host trees, between trees, and from host tree to various animals, probably in quite significant amounts, thus helping retain nutrients within the forest biomass.

Apart from a general appreciation of their usefulness and aesthetic value, relatively few Russulas are well known locally, and many remain unidentified. Researchers conducting diversity studies or examining reactions of mycoflora after any forest treatment need to know which and how many species are on their plots. Identification difficulties arise with Russulas because of diagnostic characters which are subjective or variable, such as taste and odour, pigments which wash out in rain, and natural colour variation within a species, as well as the paucity of adequate literature pertinent to this area. What is needed are good descriptions of local species, and different approaches to reaching an identification for people with a range of experience, equipment and needs.

In the following chapters, a simplified chromatography method and a modification of a commonly used DNA comparison method is described and their respective taxonomic values discussed. A phylogenetic tree derived using Bayesian analysis of previously published sequence data was used as a basis to examine morphological characters for evolutionary trends and clade specificity within the species used in the phylogeny, and within the Vancouver Island Russulas, for which more data could be collected. Habitat preferences were examined for species specificity and for clade-related patterns for use in the keys. Two types of key, a polychotomous and a synoptic key to the Vancouver Island species described, incorporated the most useful of these various methods and findings. A synoptic key allows more species to be added without major revision of the key, but the polychotomous key is more helpful for those unfamiliar with Russulas, as it teaches what to look for and why. Russulas used in the experimental processes were collected from Vancouver Island over an 8 year period, as mentioned below, and described in Appendix 3.

The environment of the Southern Vancouver Island coastal forests

Vancouver Island originated as a volcanic chain in the Pacific ocean, and was scraped off the Insular plate, a fragment of the Farallon plate, along with seafloor sediments when this was subducted under the North American plate 115 to 57 million years ago (Townsend and Figge, 2004). This episode also gave rise to the coastal mountains on the mainland. Vancouver Island's origins can be seen in outcrops of basalt, limestone, sandstone and other sedimetary rocks and intrusions. Most of the present-day vegetation has colonised Vancouver Island over the last 12,000 years or so since the retreat of the ice after the last glaciation, with Douglas-fir, Sitka spruce, western hemlock and western red cedar being present in varying abundances since that time, and garry oaks arriving 8,000 years ago (Hebda 1996). During the last glaciation, the Brooks peninsula on the north west coast was not covered in ice and many species found a refugium there, becoming a source for repopulation once the ice retreated (Hebda and Haggarty 1997, Rosenberg *et al.* 2003). Humans and many species of animals migrated along the coast and from mainland to islands from pre-glacial times to the present day, bringing fungal spores or mycelia deliberately or inadvertently on plant roots, as food, in the gut or in soil. Vancouver Island *Russula* populations may therefore originate from ancient populations with a different evolutionary path to their mainland ancestors, or they may have arrived recently from almost anywhere on the Earth with human immigrants bringing in favourite plants, or even in air currents if the spores remained viable long enough. We would therefore expect to find some *Russulas* that are indistinguishable from their counterparts in mainland North America, Europe and Asia, some with varying degrees of local adaptation, and some that have become indigenous, distinct species.

Most of Vancouver Island is within the coastal western hemlock (CWH) biogeoclimatic zone, except for high elevation sites and a narrow band of coastal Douglas-fir moist maritime zone (CDFmm), along the southern and southeastern coastline below 150m elevation.

The CDFmm zone is in the rain shadow of the Olympic mountains in Washington, and also covers the Gulf Islands up to and including part of Texada Island, and a strip along the mainland coast from the Puget Sound up to around Powell River.

On Vancouver island it extends from Comox south to the southern tip of the island around East Sooke Park.

The Victoria International airport weather station on the Saanich peninsula has reported mean annual temperatures range from 9.2°C to 10.5°C, with mean monthly temperatures of 3.4°C (January) 16.2°C (July and August). Although the coldest measurement made was -21.1°C, freezing temperatures do not usually last many days, and soils beneath the forest canopy rarely freeze (Brown and Hebda, 2002, Nuszdorfer *et al.* 1991).

Mean annual precipitation in the CDFmm zone varies from 647mm to 1263mm with around 5% as snow from November to April, the most rainfall is in December with a mean of 151.6mm, and the lowest rainfall in July with a mean of 17.6mm (Brown and Hebda, 2002, Nuszdorfer *et al.* 1991, Pojar and Meidinger, 1991).

Several types of forest communities occur in the CDFmm zone on Vancouver Island. *Pseudotsuga menziesii* (Douglas-fir) is commonly associated with an understory of *Gaultheria shallon* (salal) and *Mahonia nervosa* (dull Oregon-grape). Depending on the site, *Thuja plicata* (western red cedar), *Abies grandis* (grand fir), *Arbutus menziesii* (arbutus), *Alnus rubra* (red alder) and *Acer macrophyllum* (big-leaf maple) *Cornus nuttallii* (western flowering dogwood) and in some sites *Tsuga heterophylla* (western hemlock) are common tree associates. Rocky outcrops, shallow soils and open, well drained sites also support *Quercus garryana* (Garry oak), which occasionally forms almost pure stands (Nuszdorfer *et al.* 1991,). There are also a few rare *Picea sitchensis* (Sitka spruce) and occasional *Pinus contorta* (shore pine) in the area, the latter occurring on dry sites and in rare bogs. All the aforementioned trees and shrubs are hosts for ectomycorrhizal or ericoid-mycorrhizal fungi except for western red cedar and big-leaf maple. Similarly mycorrhizal common understory shrubs include *Vaccinium parvifolium* (red huckleberry), *Rosa gymnocarpa* (baldhip rose), *Rubus ursinus* (trailing blackberry) and *Symphoricarpos mollis* (trailing snowberry). The trees and plants mentioned above are just the more prevalent species; complete lists of plant associations for each site order found in the CDFmm region, together with details of soil characteristics, can be found in Nuszdorfer *et al.* (1991).

The Southern Coastal Western Hemlock very wet hypermaritime biogeoclimatic subzone (CWHvhl) extends along the west coast of Vancouver Island and inland to about 10km (Nuzdorfer *et* aZ.1991, Pojar *et* a/.1991). Mean annual precipitation at Bamfield, approximately 120km up the west coast from East Sooke Park, is 2876mm, ranging from from 61.5mm (July) to 412.7mm (November), and mean monthly temperatures ranging from 4.4°C (January) to 14.5°C (August) with an annual mean of 9.1°C, (Brown and Hebda, 2002).

The dominant tree in this zone on Vancouver Island is of course western hemlock, and, depending on the site, associated tree species may include western red cedar, Sitka spruce, shore pine, red alder, big-leaf maple and occasional *Abies amabilis* (amabilis fir). Extensive logging has reduced the once abundant amabilis fir to an occasional tree, mostly in remaining old-growth stands. *Chamaecyparis nootkatensis,(yellow* cedar), is much less common than on mainland sites in this zone; it is occasionally found in bogs, but this habitat more commonly has shore pine and western red cedar with western hemlocks only at the dryer margins. Along the coastline, Sitka spruce becomes dominant, occasionally in almost pure stands that form kmmmholz along the forest-beach margin (Harcombe 1974, Pojar *et al,* 1991, Roberts *et al.* 2004). Sitka spruce and amabilis fir also support ectomycorrhizal fungi. Ericaceous plants are particularly well represented in this area, and shrubs include *Ledum groenlandicum* (Labrador tea), salal, red and evergreen huckleberry *(Vaccinium ovalifolium), Menziesia ferruginea, Rubus spectabilis* (salmonberry) and other small *Rubus* species, *Rosa nutkana* and, on well-drained forest edges such as sand dunes, extensive mats of *Arctostaphylos uva-ursi* (kinnikinnik). (Harcombe 1974, Pojar *et al.* 1991, Roberts *et al.* 2004).

Towards the southern part of the Vancouver Island coast, Douglas-fir also becomes an increasing component of the CWH forests as the precipitation approaches that of the CDF zone.

The areas from which the Russulas were collected for this study are principally coastal, from Clayoquot Sound and Nanaimo southwards, concentrating more on undisturbed rather than regenerating forest. A few collections were made at Cape Scott, the Cowichan Lake area, Cathedral grove and Saturna Island. Collections were given an individual code composed of the initials of the collector, year, month, date and order for the day. Collectors contributing material included Oluna Ceska, John Dennis, Ian Gibson, Pam Janszen, Bryce Kendrick, Paul Kroeger, Renata Outerbridge, Bob Trotta, and anonymous contributors to the South Vancouver Island Mycological Society shows and forays.

Most collections were photographed or illustrated, a spore print taken, chemical tests made on stipe and cap tissue, and dimensions, odour, colour, texture, bruising reactions and taste noted while the material was fresh. Specimens were sliced in half longitudinally and the caps removed from the stipes of larger basidiomata for drying, which was accomplished in a home food dehydrator. Dried material was later examined microscopically and further measurements, drawings and photographs of hymenial and cuticular characters made. Further material was used in the chromatography and RFLP analyses as described in Chapters 2 and 3. Collections were sealed in ziplock bags and stored in polythene sleeves with their data sheet pending accession to the herbarium at the University of British Columbia.

Taxonomy of the genus *Russula*

Persoon first proposed the genus *Russula* in 1796 in his *Observationes Mycologicae,* while studying fungi in Northern France, basing his new taxon on the fleshy fruit bodies, depressed cap and equal gills. He reduced it to the rank of tribe in the genus *Agaricus* in 1801. Fries similarly regarded *Russula* as a tribe of *Agaricus* in the *Systerna Mycologicum* (1821), but later raised it to generic rank in the *Systerna Orbis Vegetabilis,* in 1825. Gray (1821) meanwhile had also recognized *Russula* Pers. as a genus, in his *Natural Arrangement of British Plants.*

Russula is a large genus with well defined groups of species that share characters, so the advantage of splitting it into more manageable subgenera and lower taxa was soon recognized.

Fries (1836-1838) divided the genus into five groups; *Compactae, Heterophyllae, Furcatae, Rigidae* and *Fragiles* based on macroscopic characters such as the presence or absence of lamellulae, gill forking, marginal striations and fragility. The first two of these groups are still recognised today, the *Compactae* being sufficiently distinct from other *Russula* that this subgenus has undergone minimal modification over the years. Quelet (1888) felt it to be a link with the sister genus *Lactarius,* calling his version of this group *Portentosae.* In 1907, Bataille split the *Compactae* into two subtaxa: *Lactaroideae,* (later *Plorantinae)* which do not blacken, and *Nigricantes* which do. With minor modification and occasional nomenclatural adjustments, this sytem was followed by Maire (1910), Melzer and Zvara (1927); (who renamed the subgenus *Lactairoides* and the non-blackening subtaxa *Delicinae,)* Singer (1932-1935a, 1935b), Schaeffer (1935), Konrad and Josserand, (1934), Heim (1937-1938), Romagnesi (1967,1985), Bon (1986, 1988) and Sarnari (1998). Singer added the subsection *Elephantinae* to house *R. elephantina* (= *R. mustelina),* but did not retain it in his 1986 classification. This species apparently posed some difficulty in placement as it has been moved several times. Heim (1938) added section *Archaeinae,* which is still recognised and Bon (1986) amended the *Nigricantes,* reverting to the name *Compactae* Fries.

Russulas in the remaining subgenera or sections, grouped under the *Eurussulae* (Melzer and Zvara 1927) or the *Genuinae* (Konrad and Josserand 1934), have had relatively stable lower taxa once determined (e.g. sections, subsections, and series), but the interrelationships of these groups were not so easily understood. Differing opinions on the phylogenetic importance of certain characters led to variation between classification systems.

Quelet (1888) formed two major subgeneric groups based on spore colour: *Leucosporae,* containing the *Portentosae, Ingratae, Sapidae* and *Piperinae;* and *Xanthosporae,* containing *Versicolores, Insidiosae* and *Tenellae. Ingratae* is still recognised, allbeit with a narrower concept, *{Portentosae* approximates Fries' *Compactae* and *Sapidae* the *Heterophyllae).* Massee (1893) recognized the taxonomic usefulness of taste of the pileal flesh as a base for infrageneric groupings. These important characters of taste and spore colour were subsequently adopted by most mycologists and are still two of the most useful macroscopic taxonomic characters today.

Arnould and Goris (1907) found sulphovanillin useful for examining spore walls, and Maire (1910) made an important contribution in which the microscopic morphology and histochemical reactions were described for their taxonomic value. In particular he discovered pileocystidia and other cuficular hyphal structures. Maire (1910) reduced the scope of the *Ingratae* to more or less its current circumscription, and *Heterophyllae* to the white-spored species currently in this group. He introduced subgenus *Polychromae* containing the *Decolorantes, Integrae* and *Urentes,* and subgenus *Alutaceae,* with a wider range than in more recent classifications. The latter has been removed from this subgenus but the former two remain as lower taxa. Kauffman (1918) retained the *Compactae* and *Fragiles* of Fries but divided the rest of the genus into *Rigidae* and *Subrigidae,* based on the macroscopic appearance of the cuticle and lamellae.

Melzer (1921-1924) described the preparation and use of an iodine solution, now known as Melzer's Reagent, which stains the ornamentation on the spores of the *Russulales,* making it easy to see under the microscope. Melzer and Zvara based their 1927 classification on taste, spore colour, gill shape and texture, reactions to bruising, FeS04 and sulphovanillin, and macroscopic and microscopic characters of the pileal cutis and spores. They divided the subgenus *Eurussulae* (all taxa other than the *Compactae)* which was further divided into *Ingratae* and *Gratae.* Melzer and Zvara's concept of the *Ingratae* included the *Emeticinae, Rubrinae* and *Sanguininae* as well as the *Foetentinae* and *Felleinae.* Within the *Gratae* they separated out the *Indolentes* (= *R. cyanoxantha* group) and *Viridantes* (= *R. xerampelina* group), from the *Rutilantes,* based on differing FeS04 reactions. They did not follow Maire's separation of the *Heterophyllae* at subgeneric level, instead this group appears as *Lividinae* within the white-spored *Immutatae* (non-blackening species) of *Rutilantes,* along with the *Lilaceinae,* that lacked pileocystidia, and *Lepidinae.* This latter had previously been included in the *Heterophyllae* because of its firm texture and velvety cap, which gave it a superficial resemblance to *R. virescens,* Melzer later discovered the use of SV for staining the incrustations on primordial hyphae (Melzer 1934).

Crawshay (1930) published a book including spore print and gill colours, and detailed illustrations of the spores of 92 European Russulas, showing beautifully the taxonomic usefulness of their ornamentation as seen in Melzer's reagent. In the preface to Crawshay's book, Bataille credits the following mycologists (other than those mentioned above) with contributions towards *Russula* taxonomy: Gillet and Cooke each began to give spore measurements in their descriptions of Russulas in the period 1877 to 1895; Bresadola and Patouillard each gave measurements of basidia and hymenial cystidia in *Russula* descriptions made between 1881 and 1892; Ricken in 1915 and Rea in 1922 followed suit. Unfortunately these measurements have not been consistently given in descriptions since.

Singer (1932, 1935a, 1935b) had studied American and tropical *Russulas* prior to making his classification, and had sections rather than subgenera as the highest taxon level, hi addition to refining (and in some cases renaming) some of the taxa of Maire and of Melzer and Zvara, he separated out subsections *Chamaeleontina, Subcompactinae* (~ *Griseinae* Schaeffer) and *Puellarinae* within his section *Constantes;* subsection *Melliolentinae* within section *Decolorantes;* and subsection *Olivaceinae* within section *Rigidae.* The three sections do not directly correlate with sections or subgenera of other authors, and Romagnesi (1967) commented on the rather heterogeneous nature of Singer's sections, which relied more on macroscopic characters than microscopic ones. In his 1986 classification, Singer had narrowed the concepts of sections *Compactae, Decolorantes* and *Rigidae,* but the latter remained a rather mixed group, united only by a velutinous cutis; those with a viscid, gelatinous cutis were placed in section *Russula.* (The term *Rigidae* has since been used for *R. cyanoxantha* and related species by Hongo (1960), and for the *Lepidinae* by Bon (1988), and its use should be discontinued in favour of more definitive terms.)

By contrast, Schaeffer (1935) essentially refined Melzer and Zvara's classification, breaking the *Integrinae* into two: die *Paludosinae* with filamentous pileocystidia, and the *Russulinae* Shroeter, without. Other contemporary classifications were made by Konrad and Josserand (1934), Lange (1940) and by Heim (1938) who introduced section *Fistulosinae* in Group *Ingratae,* and Group *Pelliculariae*, both originally of "exotic" species (from Madagascar). Bills and Miller (1984) regard *R. ballouii* Peck, from the Southern Appalachians to be in *Fistulosinae.*

The value of macrochemical reactions in identification and systematics was investigated by Bataille (1948), who published the results of putting KOH, NH4OH, guaiac, phenol, SO4OH, SV and aniline on gills, trama or stipe of 106 *Russula* species and varieties. Grund (1965) similarly investigated the reactions of Washington state Russulas.

This first half-century of *Russula* classification saw a gradual refinement of the lower, and some higher taxa into comparatively homogeneous groups, and the use of "rest groups", taxa housing those species that did not fit elsewhere. Since then, there has been a greater effort to understand the phylogeny, culminating in recent years in the study of DNA sequence relationships.

Perhaps the most comprehensive monograph on European and North African Russulas is that of Romagnesi (1967,1985), who documented 467 species and varieties, the majority with a detailed description and illustrations of microscopic features. His 1967 classification was based on macroscopic, chemical, ecological and microscopic characters of hymenium and pileal cutis, and has since undergone relatively minor modifications by other authors. His understanding of phylogenetic relationships is borne out by the similarities between his groupings and those determined by DNA analyses such as those of Miller *et al.* (2001) and Eberhardt (2002). Romagnesi originally split the genus between subgenera *Compactae* Fries and *Russula,* which was subdivided into eight supersections; these he raised to subgenera in 1987. Romagnesi united the mild tasting species with incrusted primordial hyphae into his subgenus *Incrustatula,* but left the *Lepidinae* and *Rubrinae,* also with epicutal incrustations, in subgenus *Russula,* along with the pale-spored, peppery species. Following the studies *of Russula* pigments by Gluchoff (1969), Romagnesi (1985) transferred three species out of the *Ingratae: R. ochroleuca* was put with the *Incrustatae,* and *R. consobrina* and *R. fellea* were placed into the *Piperinae.* He considered these latter two species to be a bridge between the *Ingratae* and the *Piperinae.* Bon split section *Plorantes* of subgenus *Compacta* into *Delicinae* and *Pallidosporinae,* a division subsequently supported by DNA analyses (Miller and Buyck 2002) but unfortunately not followed by Sarnari (1998), who wrote the most recent monograph on the European Russulas.

In volume 1 of his monograph, Sarnari covers his classification of genus *Russula,* and illustrated descriptions of 138 species in the subgenera *Compactae, Heterophyllidia, Amoenula, Ingratula* and *Russula* section *Russula.* This is the system followed in this dissertation. Sarnari felt that *Russula amoena* and related species different enough from the parent group, the *Heterophyllidia,* to warrant their own subgenus, the *Amoenula.* This group is characterised by a velutinous cap, coloured stipe, lack of SV reactive structures and other microscopic features. He also moved subsection *Olivaceinae* Singer into subgenus *Incrustatula,* and out of Romagnesi's *Polychromidia,* as he recognised the presence of (non-incrusted) primordial hyphae in the epicutis. Species in the *Olivaceinae* are unusual in having a bright purple reaction with phenol solution and an epicutis lacking cystidia.

Miller and Buyck (2002) and Eberhardt (2002), each generated a phylogenetic tree of European Russulas based on the internal transcribed spacer of the ribosomal DNA, which has sufficient variation at the infrageneric level. These phylogenies supported many of Romagnesi's and Sarnari's groupings, although not necessarily at the taxonomic levels accorded to them in these traditional nomenclatures. Some clades did not relate to any previous grouping and require further investigation; these are discussed in chapter 3 of this dissertation. A limited number of morphological characters were mapped onto phylogenetic trees in these studies, but more useful characters remain to be considered.

The issue of whether a particular group of species is defined as a subgenus, section, subsection and so on may soon be irrelevant as a clade-based nomenclature, termed the phylocode, has been proposed as a more stable alternative to hierarchical divisions (Cantino and de Queiroz 2006).

A summary and comparison of the classifications of Romagnesi (1987), Bon (1988) and Sarnari (1998) follows at the end of this chapter; the sections and subsections within each subgenus have been rearranged from the order in which they were published to better align them with one another. A description of each taxon is given in the original literature, and in some cases in the keys and species descriptions in Appendix 3 of this dissertation.

North American knowledge of Russulas

In America, Murrill described several new species of Russulas from Florida between 1938 to 1945, but unfortunately did not stain the spores with iodine and consequently did not describe in detail the ornamentation. Peck published the New York Species of *Russula* in 1907 and Burlingham (1915) compiled the known North American species, publishing them in her paper *Russula* which appeared in North American Flora; she also published several more papers on die genus, some of which related to Russulas of the Pacific Northwest. Beardslee (1918) published a monograph of the Russulas of North Carolina, and Kauffman (1918) included Russulas of the north midwest in his book *The Agaricaceae of Michigan.* Singer (1957) published descriptions and redescriptions of many species. Unfortunately many of these early descriptions lack details of some of the characters considered necessary in today's taxonomy. Hesler (1960) addressed some deficiencies by publishing descriptions and illustrations of the epicutis microscopic structures and spores of 159 North American Russulas, originally published by Singer, Murrill, Kauffman, Burlingham, Peck, Beardslee and others. In 1961, Hesler published a further 34 descriptions of Julius Schaeffer's collections from Europe. Several more of these early descriptions have been redescribed in various publications and dates by Shaffer, Singer, Bills and Fatto.

Recent useful contributions to the identification of North American Russulas include keys and descriptions by Kibby and Fatto (1990), and Fatto (1998, 1999, 2000, 2002), these deal mainly with eastern and northern species. Kibby and Fatto (2005) also have an on-line synoptic key to Russulas that includes both European and North American species.

Shaffer published descriptions of 14 North American species and forms of Section *Compactae* subsection *Compactae* in 1962, 10 species of subsection *Lactaroideae* in 1964, 18 of various sections in 1970,10 of section *Ingratae* subsection *Foetentinae* in 1972, and 8 of subsection *Emeticinae* in 1975. All Shaffers' descriptions were based on North American collections of new and known species, some of which occur in the Pacific Northwest (the taxonomic groupings here mentioned are as he published them). Other eastern species descriptions have been published by Bills and Miller (1984). On the west coast, Harriet Peters (1962) documented Russulas of the San Francisco area in her Masters thesis and Grund (1965) those of Washington State. In Grund's doctoral thesis a number of species were given provisional names as they were assumed to be previously undescribed, just five of these were subsequently validly published, four by Grund (1979) and one by Thiers, who also published one of Peters' discoveries (Thiers 1997a). Thiers (1997b) published a monograph with succinct descriptions of 102 Russulas of Northern California, and Woo (1989) published the PNW Key Council keys to PNW Russulas that had been documented as locally present in various publications.

The amount of detail and quality of illustration in descriptions ranges from a relatively brief summary with a sketch of the spores as in Thiers (1997) to a detailed treatise with ample drawings of hymenial and cuticular structures, supplemented with SEM photographs of spores, as in Bills and Miller (1984), Buyck and Ovrebo (2002) and Kong *et al.* (2002).

Woo compiled the literature for approximately 325 species of *Russula* reported or described from North America by numerous authors and mycological societies, and which he made available in 1997 to members of the Pacific Northwest Key Council interested in Russulas. This compilation has been of great assistance in the identification of several Vancouver Island collections described in the appendix of this dissertation, since the searching through abundant, sometimes hard to obtain literature was much reduced. One of the main problems facing a researcher is that of simply knowing of the existence of a description. This has historically led to a species being described more than once, under different names, and these are only slowly being weeded out as herbarium type collections are re-examined. An example is *Russula atropurpurea* (Krom.) Britz., which Sarnari (1998) estimates to have 9 synonyms and to have been described as different varieties and forms of these synonyms at least 23 times. Local species can and do sometimes vary in a given character from the specifications given in the original descriptions, and such variations should be explicit in descriptions. In some cases this variation from the type is large enough and sufficiently consistent to warrant a new variant name, or to be considered a new species. Currently, for collections that do not match existing North American descriptions, European and Asian publications may provide useful information if the language is not a barrier. In addition to those texts mentioned above, collections of useful descriptions and/or keys include those of Arora (1986), Blum (1963), Bon (1987,1988), Buczacki (1992), Courtecuisse and Duhem (1995), Einhellinger (1987), Moser (1983), Phillips (1991), and Rayner (1977).

The current level of knowledge of Russulas in western Canada

Redhead (1997) estimated that only 1% of B.C. macrofungi had been systematically studied, and only 20 *Russula* species had been documented for B.C. Of the 150 or more species in the genus originally estimated to be in the Pacific Northwest

(PNW) by Grand (1965), 78 (agarics) have been recorded (Woo, 1989, Gibson *et al.* 2006), with 37 reported from Vancouver Island and the Gulf Islands, mostly from observations made during forays by members of the South Vancouver Island Mycological Society, Vancouver Mycological Society and the PNW Key Council (Gibson 1998). Some of these records were unconfirmed, and they are also incomplete. Roberts *et al.* (2004) recorded 34 species of *Russula* in the forests around Clayoquot Sound between 1997 and 2001. Some of these species and those found elsewhere on Vancouver Island were difficult to identify, since one or more of their characters did not match available published descriptions.

Questions addressed by this dissertation

1. Are there are more species of *Russula* to be found on Vancouver Island than are currently recorded from local foray data and from published accounts of Pacific Northwest collections, and what are the characteristics and habitats of local forms of recorded and newly observed species?

2. While traditional identifications rest on morphological characters, can the RFLPs of an amplified piece of DNA, specifically the ITS region of the rDNA gene be usefully compared with virtual restrictions of published sequence data to confirm an identification, and can paper chromatography of cuticle pigments be employed in the identification of taxonomic groups?

3. How does the suite of morphological characters used in traditional classification within the genus *Russula* relate to the clade structure within a phylogenetic tree based on DNA sequences, and can a more efficient identification key be based on such characters sorted by the taxonomic level at which each is useful?

Summary of three recent classification systems.

S ome taxa that approximate one another but with dissimilar names are linked with shading, *i* Section, Ss ** Subsections.

Romagnesi 1987

<u>Subgenus *Comoacta* (Fr.) Bon.</u>
Type *R. nigricans.*
S.: *Nigricantinae* Bat,
Plorantinae Bat.
Archaeinae Heim: Romagn.

<u>Subgenus *Insratula* Romagn.</u>
typeiJLfeetens
S.: *Foetentinae* Mlz. & Zv.
Groups: *R,foetens*
R. pectinata
S,: *Felleinae* Mlz, & Zv.

<u>Subgenus *Meterophvllidia* Romagn.</u>
Type:!?, ^rfe^fl
S.; *Virescentinae* Sing.
Indolentinae Mlz. & Zv.
Heterophyllinae Maire
Griseinae Sehaef.
llicinae Romagn.
Amoeninae Sing.

Bon 1988

<u>Subgenus *Campacta (Fr.)* Bon.</u>

S.: *Compactae* Fries
Plorantes (Bat.) Sing.
Ss..: *Delicinae* Bat.
Pallidosporinae Bon

<u>Subgenus*Russula*</u>
S,: *Ingratae* Qu^let
Ss,,: *Foetentinae* Mlz. & Zv.
Pectinaiae Bon

S.: *Heterophyllae* Fries
Ss..: *Virescentinae* Sing.
Indolentinae Mlz. & Zv.
Heterophyllinae (Fries) Sehaef.
Griseinae Sehaef. ex Bon
Amoeninae Sing.

Sarnari 1998

Sub<u>genus *CompuctaVPtX* Bon</u>

S.: *Compactae* Fries
Lactarioides (Bat.) Konr. & Joss.
Archaeinae Heim ex Buyck & Sarnari

<u>Subgenus /iffrata/a Romagn.</u>
SL: *Ingratae* (Quel.) Maire
Ss,.; *Foetentinae* (Mlz & Zv) Sing.
Farinipedes Sing.
S.: *Felleinae* (Mlz. & Zv) Sarnari
Subvelatae Sing.
Fistulosae Heim

<u>Subgenus Heterophvllidia Romagn.</u>
S.: *Griseoflaccidae* Sarnari ad int.
Virescentinae (Sing.) Sarnari
Heterophyllae Fries
Ss,.: *Cyanoocanthinae* Sing.
Heterophyllae (Fr.) Schaeff.
Griseinae Schaeff.
llicinae (Romagn.) Buyck
Subgenus *Amoenula* Sarnari.
Type: *R. amoena*

Romagnesi 1987 (continued)

<u>Subgenus *Russula (Pers.)* Fries amend.</u>
Rotnagn. Type:!?, *emetica*

1, Piperinae-rubroflavae:
S..' *Lepidinae* Mlz. & Zv.
Rubrinae Sing. ss. str,
Citrinae (Romagn.) Bon
Emeticinae Mlz. & Zv. ss. str.
Persicinae Romagn.
2, Piperinae-variecolores:
S.: *Atropurpurinae* Romagn.
Violacinae Romagn.
Exalbicantinae Sing.
Sardoninae Sing.

<u>Subgenus *Insidiosula*</u> <u>Romagn.</u>
Type: *R. veternosa*
S..' *Urentinae* Maire ss. str.
Maculatinae Konr. & Joss.

<u>Subgenus *Coccinula* Romagn</u>.
S.; *Decolorantinae* Maire ss. str.
Paludosinae Schaef.
Laetinae Romagn.

Bon 1988 (continued)

<u>**Subgenus *Russula*** (continued)</u>
S.: *Rigidae* Fries
Ss.: *Lepidinae* Mlz. & Zv<
Rubrinae Mlz. & Zv. amend,
Romagn.
S.: *Russula (=Fragiles* Fries)
Ss.: *Felleinae* Mlz. & Zv.
Citrinae (Romagn.) Bon
Emeticinae Mlz. & Zv.
Atropurpurinae Romagn.

S.: *Violaceae* Romagn.

S.: *Firmae* Fries
Ss.: *Exalbicantinae* Sing.
Sanguininae Mlz. & Zv.

S.: *Insidiosae* Romagn.
Ss.: *Cupreinae* Bon
Urentinae Maire

S.: *Russulinae* (Schroet.) Sing.
Ss,: *Integrinae* Maire
Paludosinae Schaef.
Laetinae Romagn.

Sarnari 1998 (continued)

<u>Subgenus *Russula* Romagn. amend.</u>
Type: *R.emedca.*
S.: *Russula* (Romagn.) Sarnari
Ss.: *Consobrinae* Sarnari,
Rubrinae (Mlz. & Zv) Sing.
Russula (Romagn.) Sarnari
Sardoninae (Sing.) Sarnari
Urentes Maire
Violaceinae (Romagn.) Sarnari

S.: *Viscidinae* (Sarnari) Sarnari
Messapicae Sarnari
Paraincrustatae Sarnari
Ss.: *Integrae* (Maire) Sarnari
Lepidinae (Mlz. & Zv) Sing.

Romagnesi 1987 (continued)	**Bon 1988** (continued)	**Sarnari 1998** (continued)
Subgenus *Polvchromidia* Romagn.	*Polychromae* Maire	S.: *Polychromae* (Maire) Sarnari
Type: *R, Integra.*	*Decolorantinae* Maire	Ss.: *Auratinae* Bon,
S..' *Melliolentinae* Sing,	*Melliolentinae* Sing,	*Melliolentinae* (Sing.) Sarnari,
Iniegroidinae Romagn.	*Laeticolorinae* Bon	*Integriforminae* (Bon) Sarnari.
Olivaceinae Sing. ss. str.	*Integriforminae* Bon	*Paraintegrinae* Sarnari,
Integrinae Maire ss. str.		*Xerampelinae* Sing.
Viridantinae Mlz. & Zv.	*Viridantes* Mlz. & Zv.	
	Alutaceae Maire	
	Olivaceinae Sing.	
	Auratinae Bon	
Subgenus *Tenellula* Romagn.		
Type: *R. puellaris.*	*Tenellae* Quelet. amend. Romagn.	S.: *Tenellae* Quel
S.: *Sphagnophilinae* Sing.	*Sphagnophilinae* Sing.	Ss.: *Betulinae* (Romagn.) Sarnari
Rhodellinae Romagn.	*Rhodellinae* Romagn.	*Rhodellinae* (Romagn.) Bon
Laricinae Romagn.	*Laricinae* (Romagn.) Bon	*Laricinae* (Romagn.) Bon
Puellarinae Sing,	*Puellarinae* Sing.	*Puellarinae* Sing.
	Odoratinae Bon	
Subgenus *Incrustatula* Romagn.		**Subgenus *Incrustatula* Romagn. emend**
Type: *R. lilacea*	*Lilaceae* (Mlz. & Zv.) Konr. & Joss.	S.: *Lilaceinae* (Mlz. & Zv) Konr. & Joss
1. Incrustatae-leucosporae	*Roseinae* Sing.,	Ss.: *Roseinae* Sing, ex Sarnari
S.." *Roseinae* Sing.,	*Lilaceinae* Mlz. & Zv.	*Lilaceinae* (Mlz. & Zv) Schaff.,
Lilaceinae Mlz. & Zv. ss. str.	*Amethystinae* (Romagn.) Bon	
2.Incrustatae-xanthosporae	*Chamaeleontinae* Sing.	S.: *Amethystinae* (Romagn.) Sarnari
S.: *Amethystinae* Romagn.	*Iniegroidinae* (Romagn.) Bon	Ss.: *Amethystinae* (Romagn.) Bon,
Chamaeleontinae (Sing.) amend. Romagn.	*Qchroleucinae* (Romagn.) Bon	*Chamaeleontinae* Sing,
		Integroidinae Romagn. in Bon,
Ochroleiicime Romagn.		*Olivaceinae* Sing.

Chapter 2

Chromatographic analyses of cuticle pigments

Introduction

The colours of *Russulas* are amongst the most confusing of characters in the genus, since the incident light and weather affect the development of pigments and their relative longevity in the cutis. Many species are very similarly coloured, but at the same time the colour can vary dramatically between basidiomata of the same species.

The analysis of cuticle pigments by paper chromatography was examined for its potential in differentiating between taxa, and for finding natural groupings among species which could be compared with other data used for taxonomic divisions within the genus, such as those based on classical morphology and those arising from phylogenies based on DNA analyses. The initial experiments with cuticle pigments suggested that a simple procedure using widely available materials worked reasonably well, and as such could be of use to ecologists, forestry workers and amateur mycologists involved in diversity studies to aid identification when only a temporary field laboratory is available.

Comparison of naturally occurring pigments with watercolour pigments.

In the preparation of the watercolour illustrations for this thesis it became apparent that for most of the variably coloured Russulas three and occasionally four pigments provided the most accurate rendition of the colours of the cap cuticles; a primary red, yellow, a greenish blue and an ochre yellow. The behaviour of these watercolour pigments in mixtures and dilutions was similar to the behaviour of the *Russula* pigments in situ. Carmine red (St. Petersburg artists' watercolour), is close to the magenta ink found in ink jet printers, but gives a brighter red in concentrated form such as that of iR. *sanguinea* and *R. americana* var. *modicaspora* nom. prov., as well as a more purple pink in dilution as in *R. fragilis.* The blue pigments found in Russulas are of a greenish blue, duplicated in paint by phthalocyanine blue (DaVinci artists' watercolour, although most quality brands will be similar, since this pigment is a standardized manufactured product); this blue is darker and greyer than the cyan ink used for ink-jet printers. In Russulas the blue pigment was rarely, if ever, found alone, and both the natural and the watercolour blue pigments produced the range of blues, greys, violets and purples when in combination with the red, greens when combined with yellow and browns and black when combined with yellow and red. Although seven yellow pigments have been found in Russulas, discussed below, usually only two or three of these were found in any one species. The yellows were either a bright clear lemon-yellow or a browner shade of yellow. For most illustrations quinacridone gold (Daniel Smith artists' watercolour) duplicated both the deeper and lighter yellows and those in brown, green and mixed colour Russulas. Arylide yellow (DaVinci), similar to yellow ink-jet printer ink, was used when a clear citrus yellow was required, such as for *R. gilva*.

Russulas in the subgenera *Compactae* and *Ingratae* were better illustrated with a different set of pigments: ivory black and the earth colours raw and burnt umber, and raw and burnt sienna (either Winsor and Newton or Maimeri artists' watercolours).

In mixing the above colours and diluting them an appreciation was gained for the variety of hues possible with two and three pigment mixes, so that published descriptions of species listing colours ranging from green to grey to pink to brown became easier to visualize and understand. With experience, duplicating the colours of a fresh collection in watercolour pigment yielded information on whether one, two or three pigment groups (i.e., whether just reds, or reds and yellows, etc.) were likely present, and their approximate relative amounts. While such information could be confirmed only by chromatographic analysis of the actual cuticle extract, in practice this quick paint analysis sometimes facilitated the identification process. Analysis of colours by image manipulation programs on a computer of photographs of mushrooms was less reliable because the variation in light and shade on such three-dimensional objects affects local colour, although it is possible to reduce this source of variation by flattening a section of the mushroom cap onto the bed of a scanner and scanning in the image.

In the field, *Russula* pigments varied in their response to weather and light conditions. In some species, light is necessary for the development of the red pigment, as in *R. fragilis* (personal observation) and *R. xerampelina* (O. Ceska, personal communication). The red pigments also were more water soluble in the living mushroom than the blue and yellow - at least in the subgenera *Russula* and *Incrustatula* (sensu

Romagnesi 1987 and Sarnari 1998). The yellow colouration was sometimes observed within the dermatocystidia, where it appeared to be held more tightly than the red vacuolar pigments, and in age a dull yellowish hue appeared in many species as tissues degenerated and oxidized; a change not due to pigmentation. A general tendency was for an enrichment and darkening of pigmentation as the immature basidioma matured and emerged above the litter, followed by weathering, in which rain washed out the colours, diluting them toward a grey to yellowish-grey hue, or yellow to white in species lacking blue pigment. In dry weather colours sometimes changed toward brownish hues as decomposition set in. In very dry weather, the colours were often preserved as the mushroom dried before decomposition affected it. With this understanding of the behaviour of *Russula* pigments and of paint pigments, it was easier (but not infallible) to avoid making either mixed species collections or to split collections unnecessarily in the field, and also to recognize a particular species in the field in part by the weathering/aging pattern of its pigments.

Pigments from the cuticles of Russulas have been separated chromatographically and analyzed spectroscopically by Gluchoff, (1969, 1975) and Iten *et al.* (1984). In these studies, the chemical identity of the pigments was sought and isolation of each fraction was brought about by chromatographic separation on paper, thin layer silica gel, and columns of silica gel, using a variety of solvents including water, ethanol, acetic acid, pyridine and phenol. Identification of the reds was through spectrographic analysis and comparison with known compounds. Gluchoff (1969) was interested both in the chemical identity and taxonomic potential of the pigments and isolated three red pigments which she termed Russularhodines (Rrl, Rrll and Rra); three blue pigments termed Russulacyanines (Rcl, Re II and Re *d'Heterophyllae);* and seven yellow pigments termed Russulaxanthines (Rx I to VI and Rx d' *Heterophyllae).* No green pigments were detected. Gluchoff isolated cuticle pigments from 37 species of *Russula* spread among eight supersections of subgenus *Russula* sensu Romagnesi (1967). These analyses showed, with some notable exceptions, that the *Ingratae* and members of the *Heterophyllidia* Romagnesi lacked the suite of pigments found in the other groups. The exceptions, namely *R. ochroleuca* and!?, *consobrina* in *Ingratae* andi?. *amoena* and *R.*

violeipes in *Heterophyllae*, have recently been reclassified by Sarnari (1998) following Gluchoff s findings: *R. ochroleuca* into the subgenus *Incrustatula, R. consobrina* into subgenus *Russula* section *Russula* and the last two into a new subgenus, *Amoenula* (Sarnari 1998, Miller and Buyck 2002). Gluchoff (1969) also concluded that the supersection *Piperinae* sensu Romagnesi formed a natural group having two red and two yellow pigments in common, and within this group, all members of the section *Sardoninae* also had a blue pigment in common. This pattern was not exclusive of species in other groups.

Russularhodine I is a strongly polar hexose or pentose sugar-based nitrogenous heterosidic molecule found to be very soluble in water and 5% acetic acid; Rr II is soluble in 50% acetic acid or 50% pyridine, less soluble in water. A third red, Rr a, migrates along with the other two reds on paper in 5% acetic acid and superimposes on them. These pigments all fluoresced orange in UV light (360nM) (Gluchoff, 1969).

Russulacyanine I and II can be separated in a bidimensional paper chromatograph by running first with 40% acetic acid and secondly in the perpendicular direction with a butanol/pyridine/water mixture. Neither of these pigments fluoresce in UV light. The russulacyanine specific to the *Heterophyllae* sensu Romagnesi fluoresces orangish in UV light Gluchoff (1969).

Of the russulaxanthines, Gluchoff (1969) found Rx I and V to be water soluble; whileRx V and Rr I were found to be interconvertible; Iten *et al.* (1984) confirmed that three of the yellow compounds (lumazines) were derived by the hydrolysis of a red pigment. Rx VI was isolated from *R. vesca* and a further yellow from *R. cyanoxantha,* both in the *Heterophyllae.* Gluchoff did not discuss the other yellow pigments except to give the colour in daylight and UV light. Gluchoff also found a colourless pigment that fluoresced violet in UV light which she termed 'I.V.'. A table of the visible and UV fluorescence properties is reproduced from Gluchoff (1969) in the appendix. Also reproduced is her table of the pigments found in each of 37 *Russula* species using Whatman nol paper with 40% acetic acid and silica gel with 4:1 phenohwater. Iten *et al.* (1984) analyzed colourless and yellow pigments, which they termed Russupteridines, from *Russula* collections, and found several ribitol compounds, some of which are intermediates between two others. One of the yellows was riboflavin, a common pigment in *Russula* cuticles. Iten *et al.* (1984) commented on the difficulty of obtaining separations, stating that 1kg of Sephadex gel (in a column) was required to separate lOmg of previously purified fraction. Their methods also used single species of *Russula* in 200 to 300kg amounts. The chromatography methods worked out below are designed to make use of the limited amount of material that is normally available in a collection, which can sometimes consist of only one basidioma, and a very simple procedure that nevertheless gives useful taxonornic information.

Materials and Methods

Extraction of Pigments

The pigments in *Russula* are found within the cutis, which in many species will separate readily from the underlying tissues at least at die cap margin in fresh specimens. The pigments in most species are vacuolar. In the Ingratae the pigment is in the hyphal walls, and in *R. ochroleuca* (and perhaps other encrusted groups) a yellow pigment encrusts the outside of the hyphae (Gluchoff 1969, Romagnesi 1967). Slivers of cuticle totalling approximately 1cm^2 and cleaned of underlying tramal tissues from either fresh or dried collections were placed in a small boiling tube or 1.5ml Eppendorf tube and covered with distilled water. The amount of tissue used depended upon the amount available, retaining sufficient undamaged material for deposition in an herbarium. In practice this meant using between 1 and 4cm^2 of cuticle tissue and about 0.5 to 1.5ml of water. For single small samples a quick method was to microwave the contents of the boiling tube at maximum power for 10 seconds if 0.5ml water was used. For several or larger samples the mixture was heated in a heating block at 60-65°C if in an Eppendorf or on a hotplate set on low to medium (use a thermometer in a beaker of water to determine setting) if in a glass flat-bottomed tube, until the pigments were seen to be released into the water at about 60°C. This happened quite abruptly, and after 2 to 5 minutes in hot water very little colour remained in the tissue, so this was removed. The pigment solution was then reduced to 50-100ui to sufficiently concentrate the colour, by leaving it on the hotplate or placing it in an oven at 50-60°C and checking frequently. Completely dried extracts formed a crystalline deposit which will keep in a closed container away from light for several weeks without deterioration. Gluchoff (1975) analysed the pigments often collections of *R. norvegica,* some of which were fresh and others of various ages from a herbarium and found the pigment profiles sufficiently similar to assume chemical stability in dry storage. Dried extracts could be rehydrated with about lOOul water depending on colour density and consistency of the deposit. With some species, particularly members of the *Ingratae,* the concentrate was somewhat gelatinous and needed to be diluted more than that of other groups. This gelatinous substance sometimes rendered part of the pigment spot immobile on the chromatograph baseline.

Chromatography

Whatman No. 1 paper was cut into strips approximately 8mm wide by 30cm long and marked with a horizontal pencil line 5cm from one end to denote the starting point, and labelled at the other end. The use of strips rather than a sheet limited the horizontal spread of the pigments, a necessary precaution when minute amounts of pigment require maximum concentration if they are to be seen. An occasional disadvantage was that a colour band was pushed to the side of the strip rather than progressing upwards, necessitating a repeat. The extract was spotted onto the line. It was necessary to allow the spot to dry and repeatedly apply it to concentrate the pigments further on many samples, particularly pale species.

The strips were suspended with the lower 2.5cm in *2%* acetic acid solvent in a closed glass jar at room temperature, and the solvent front allowed to progress 21-24cm up from the baseline, which took approximately 3 hours. The chromatographs were removed from the solvent, the solvent line marked, and the strips allowed to dry. Pigment bands visible in daylight were noted, then those fluorescing in blue light emitted from a blue light-emitting diode (LED) flashlight at a wavelength centered on 470nm, and finally those fluorescing in ultra-violet light (UV), at a wavelength of 365nm, (as in a portable counterfeit currency detector light.) The blue LED and the UV observations were made in a darkroom. It was necessary to shine the blue LED onto the chromatograph for 20-30 seconds to impart sufficient energy to allow fluorescence, then move it slightly aside to check for a reaction. Red and orange LED lights were also tried but these have a longer wavelength and consequently lower energy, so they were not expected to incite fluorescence, and did not do so.

Results

There was little difference in the number and colours of bands formed when the running solvent was distilled water or 5% acetic acid, although the acetic acid generally gave slightly cleaner separation of yellow bands. When 5% ammonia was used, bands tended to smear more, except for the magenta bands for which separation into two fractions was slightly improved. The solvent ultimately chosen for further work was 2% acetic acid, which gavesufficiently clean band separation in the yellows and still allowed the reds to move off the blue pigments.

UV light caused several pigment bands to fluoresce, including some not visible in daylight. No fluorescence was seen with red, green or orange LEDs, although blue pigments appeared dark with red or orange, and non-fluorescing brownish or orange pigments appeared dark under blue light. No bands were seen under blue LED light that were not visible under either daylight or UV light, although the yellow pigment appearing about 4/10 of the way up the chromatograph, which was sometimes too pale to see in daylight, could still be detected with the blue LED. The pigment bands and the effects on them of UV and blue light are summarized below in the pattern diagrams.

Pigment bands fell into five basic patterns. Pattern 1 (fig. 1) and its variations were associated mostly with subgenera *Russula* and *lncrustatula* and is shown below. In general this pattern showed in daylight a blue-green to blue-grey at the baseline, two red pigments, not well separated, a bright yellow, a pale orange-brown spot and a diffuse pale brownish-orange up to the solvent front.

The red pigments did not usually separate, but appeared as one elongated band which was orange-magenta at the lower end and a pure magenta at the upper end.

The bright yellow band at 4/10 of the way up varied in intensity with different species (as did the other pigments), and in some species had a poorly differentiated band directly below it which fluoresced a green-blue in UV light. It was not clear if this was simply the yellow pigment that had smeared or another fraction, since the yellow shone greenish-yellow in UV light when fairly intense in the visible spectrum, becoming bluer at weaker intensities. This yellow fluoresced orange with blue light from an LED, being visible in this and UV light when too weak to be seen in daylight. Other yellow pigments included a pale orange-brown that migrated half way to the solvent front, usually in a well defined spot, which showed as a dark spot or band in UV light just above the yellow and below the blue-green fluorescing band when the latter was present. A third yellow which appeared in visible light as a pale brownish-orange migrated in a diffuse band up to the solvent front, where it was most concentrated. In UV light a band of blue-green was visible just above half-way in some species, and a band of bright violet at the 2/3 to 3/4 mark; above this band to the solvent front the pigment had a low light orange fluorescence, becoming bluer when very weak, and in some cases with fluorescence only in the lower half. These dull yellows did not fluoresce in blue LED light (470nm).

Variations in **Pattern 1** (fig. 1) involved the balance of various pigments, such that some were much reduced compared to others. In the *Xerampelinae* the red pigments fluoresced in UV light but not under blue light from an LED. Gluchoff (1975) found that in many groups of Russulas, including the *Xerampelinae,* there was a large proportion of Rr. I, with or without one or both of the other two reds. The *Xerampelinae* she tested lacked a second red or had only minor amounts of one, so the lack of fluorescence in blue light can be taken as indicative of the presence of Rr. I alone. Other Russulas tested by Gluchoff showed one or both of Rr. II and Rr. a in addition to Rr. I, and unfortunately the first two could not be separated by these simple methods. Collections of *Russula aeruginea* (or *R. stenotricha)* of subgenus *Heterophyllidia*, showed a pattern intermediate between pattern 1 and pattern 3, where other members of this subgenus were placed.

Pattern 2 (fig. 2), e.g., *R. occidentalis*, was similar to pattern 1 but with fewer bands. Pattern 2 had a blue baseline pigment which appeared somewhat brownish to purple-grey, was not fluorescent in UV light, but orange-pink in blue light. Above it and incompletely separated was an orange-pink spot which fluoresced that colour in UV light. As these two pigments partially overlaid one another, the fluorescence or lack thereof may have been due to the optical mixing. About 1/4 of the way up was a yellow-brown spot which fluoresced light blue in UV and orange-pink in blue light. At half-way was a pale yellowish band fluorescing yellow-green to green-blue, similar to that of pattern 1. From there to the solvent front was a diffuse pale orange-brown area which contained a strongly fluorescent violet band directly above the yellow, and above that a dimly bluish to pale orange fluorescence in UV light, in one case with a non-fluorescent area above this.

Pattern 3 (fig. 3), e.g., *R. medullata* and *R. brunneola,* was much simplified, with a greyish or brownish spot on the baseline which fluoresced orangeish in UV but not in blue light, and which seemed to be equivalent to Gluchoff s (1969) Russulacyanine specific to the *Heterophyllae* sensu RomagnesL Above that at about 1⁄3 to 1⁄2 way was a yellow-brown spot which fluoresced bright blue in UV but with a non-fluorescent spot in the lower half. Neither of these fluoresced in blue light.

Continuing up to the solvent front was a diffuse very pale dull yellow which was undifferentiated into bands in UV light but fluoresced blue to violet-blue.

Pattern 4 (fig. 4), e.g., *R. crassotunicata,* was simpler still with a brownish blue-grey spot at the baseline which fluoresced orange in both UV and blue light, and about 1⁄3 of the way up to the solvent front was a brownish yellow which fluoresced light orange in UV light.

Pattern 5 (fig. 5), e.g., *R. adusta* and *R. cerolens,* had a brownish blue-grey which fluoresced orange in UV light at the baseline, 1⁄4 of the way up was a colourless band which fluoresced orange in UV light; above that lay a pale yellow band to 1⁄2 way up; which fluoresced yellowish white in UV light, and above that to the solvent front was a brownish yellow area which fluoresced orange in UV light. There was no fluorescence with the blue LED.

Table 1 *Russula* species with pattern 1 pigmentation.

Pattern 1	Blue-grey	w 5* 2 » + <+	3 J» fS era m 3 O <+	G < a	cT D +	S 3. CD o 3 3 << 3 eg •<CTQ 3 n>	e. CO O n> •	< 0 v: P 3	ffq	< 3 CIQ g .
R. aeruginea LB	Tr	Tr	Tr	Tr	+				+	
R. aeruginea LB	+	Tr	Tr	+	+	+			+	
R. aeruginea Kok	+	+(led-)		+	Tr	+				Tr
R. viscida	++	++(led-)		+		Tr		+	+	
R. raoultii	Tr	Tr				+?		Tr	+	
R. fragilis	+				+	+		+	+	
R. fragilis	++				+	+		+	tr	
R. laccata	+		i+		+	+		+	+	
R. silvicola	Tr				+	+		+	+	
R. silvicola	Tr				++			+	+	
R. sanguinaria	+				Tr	Tr		Tr	+	
R. sanguinaria	+	++			+	+		+	+	
R. lutea	Tr	+	+	Tr	++	++	Tr			
*R. lepidiformis**	Tr	++	+	+	+ led-	Tr	+	+		Tr
R. murrillii	++	+(led-)	+(led-)		++		Tr	+	+	
R. veternosa	+	+	+	Tr	+	+		+	+	
R. veternosa	+	+	+	Tr	+	+	+	+	+	
R. veternosa	+	++	+	Tr		+	+	+	+	
R. veternosa	+	+	+	+		+		+	+	
R. olivaceae	++	+	+	+		+	+	+	+	
R. xerampelina	++	h (led-)	++ (led-)	Tr		+	+	+	0	Tr
R. xerampelina	++	h (led-)	i- (led-)	Tr		+	+	+	0	Tr
R. semirubra	+	h (led-)	K (led-)	0		+	0	+	0	
R. cf. *pruinosa*	++	+ (led-)	+ (led-)	+				+		+

Pattern 1										
/?. *eleodes*		+ (led-)	+ (led-)							+
R. isabelliniceps	1+	+ (led-)	+ (led-)							+
R. isabelliniceps		+(led-)	+(led-)						Tr	Tr
R. abietina			+ (UV&led-)						tr	
R. cessans						+			Tr	
R. aureofulva	+		[Tr			+	Tr			
R. nauseosa		+ (led-)	!+ (led-)			Tr				
R. aeruginoides		+	ITr	iTr	iTr				Tr	

Abbreviations and symbols: LB =Longbeach area, near Tofino, Pacific Rim National Park; Kok=Koksilah river valley, north side; * the yellow not visible in daylight, or LED, only UV.

Table *2 Russula* species with pattern 2 pigmentation.

Pattern 2	Brownish -blue-grey, LED-orange	Magenta	Yellow-brown UVblue					
R. occidentalis	+	+	+		+		+	+
R. occidentalis	+	+	+		+	+	+	
R. occidentalis	+	+	+		Tr	+	+	
R. occidentalis	+	+	+		Tr	+	+	

Table 3 *Russula* species **with pattern 3 pigmentation.**

Pattern 1 raiiernj	**C 3?** ^ g SK* O i CM ^ »	^ ^ ^ ^ d - B O p. ^ ^ 2 B	^ *< < ^ di 0 * 0 £f 2 S	**C J** ^^ iO O o (M y ° « Si O
/?. *brunneola*	+	++	++	++
i?. *brunneola*	+	?	+	+
i?. *medullata*	+	+	+	+
R. parazurea	+	+	+ (UV yellowish)	+
i?. *sublevispora*	+	Tr	++	+

Table 4 *Russula* species **with pattern 4** pigmentation.

Pattern 4	W d r m O ange	d < o	own p- fcd ^ ^ V!	UVOr d er2/3	Brown f How,
/?. *crassotunicata*	+		++		
i?. *crenulata*	+		++		

Table 5 *Russula* species with pattern 5 pigmentation.

Pattern 5	Grey, UV & blue LED-ve	Colourless, UV orange, blue LED-ve	Faint yellow, UV yellowish white	Brownish yellow, UVOr	Grey-brown, UVnil
R. adusta	Tr	+	+	++	
R. adusta	Tr	+	+	++	
R. dissimulans	+			++	
R. dissimulans	+			++	
R. ef. *pectinata*	+			++	
R. cerolens	Tr		+	+	+
R. fragrantissima	+		+	Tr	

Explanation of symbols in Tables 1 to 5; + = present, ++ = present and relatively abundant, Tr = trace amounts, ? = difficult to tell if there was a trace amount because of masking from other pigment bands.

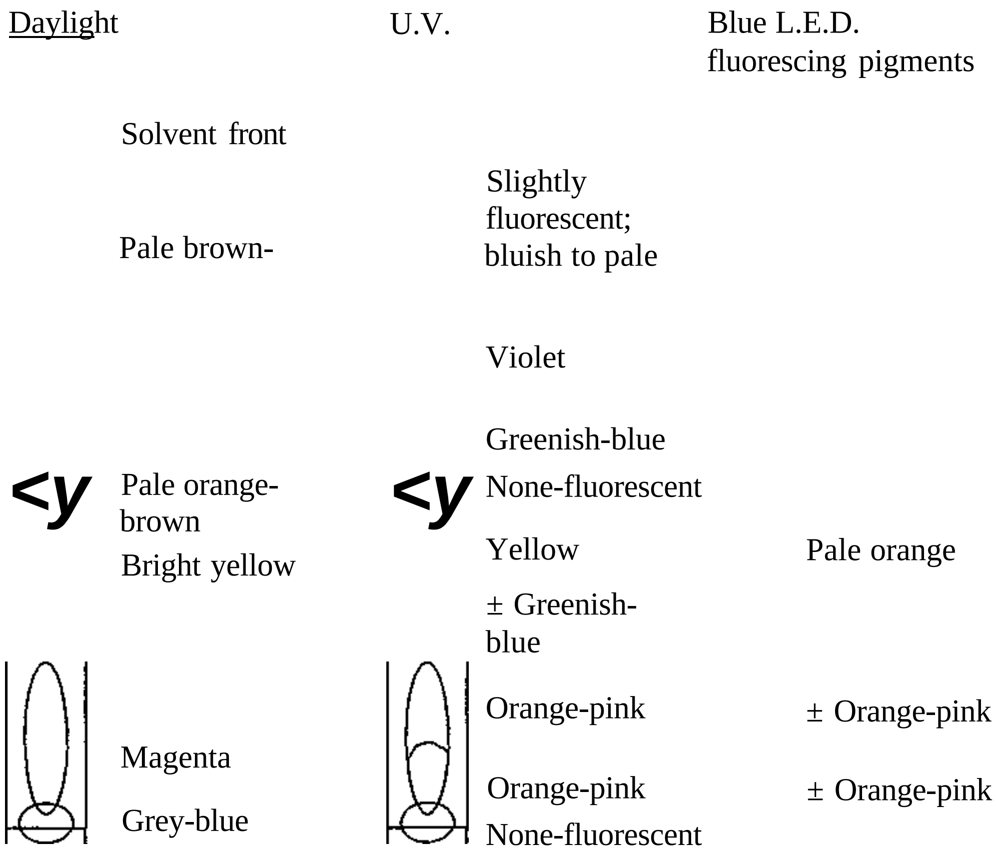


Figure 1 Pattern 1 e.g. *R. veternosa.*

Daylight	U.V.	Blue L.E.D. fluorescing pigments
Solvent front		
Pale brown-orange	Slightly fluorescent; bluish to pale orange	
	Violet	
Pale yellowish	Yellow-green to greenish-blue	
Yellow-brown	Light blue	(Orange-pink)
+ Orange-pink Brownish to purple-grey	Orange-pink Non-fluorescent	Orange-pink

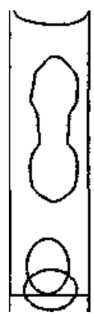


Figure 2 Pattern 2, e.g. *R. occidentalis.*

Daylight		U.V.		Blue L.E.D. None fluorescing
	Solvent front			
	Very pale dull yellow		Blue to violet-blue, not differentiated into bands	
A		A		
	Yellow-brown		Bright blue	
V		in	Non-fluorescent	
Oi	Pink-brown or greyish	o	Orangeish	

Figure 3 Pattern 3 e.g. *R. medullata*.

Daylight U.V. Blue L.E.D.

Solvent front

Brownish yellow Orange

O Brownish blue-grey O Orangeish Orange

Figure 4 Pattern 4 e.g. *R. crassotunicata.*

Daylight		U.V.		Blue L.E.D. No fluorescence
	Solvent front			
	Brownish yellow		Orange	
	Pale yellow		Yellowish-white	
	Colourless		Orange	
-©•	Brownish blue- grey	O	Orangeish	

Figure 5 Pattern 5 e.g. *R. adusta*

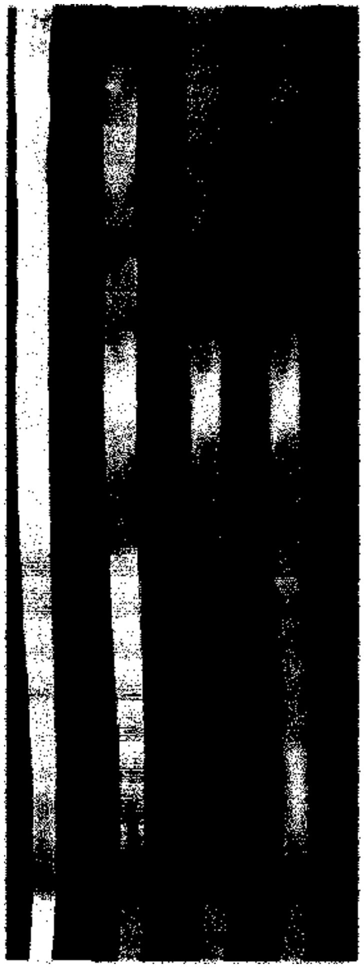


Figure 6 Paper chromatograph under three light conditions, on left, *Russula xerampelina* pigments under natural light, second from left, in UV light, third from left, under a blue LED light, and right, the effect of a blue LED light on a chromatograph with the red pigment fluorescing, for example *Russula silvicola.* The chromatographs shown were done with a high concentration of pigment, extracted from about half of a large basidioma, with the colour digitally saturated with photographic manipulation software (Arcsoft Photostudio 3.0), in order to show the colour bands, which are normally much paler. The chromatographs shown in UV and blue LED light were difficult to photograph because of the low light levels and the colours that the eye detects were not apparent on the photograph. In order to show what the eye sees, colours were digitally superimposed where appropriate on the chromatograph.

Conclusions and Discussion

In searching for effective extraction and chromatography solvents, acetone, chloroform, ethanol, ethanol-water mixtures, 5% ammonia solution and 5% acetic acid solution were tried, but gave results inferior to water. Both the blue and the red pigments are altered by strong acids and alkalis while in the cutis, and even 5% ammonia solution can in some species effect a colour change in the tissues, so their use in extraction had potential problems.

The extraction method above contrasts with that of Gluchoff (1969) who used ethanol:water in a 1:1 ratio followed by multiple aqueous pyridine extractions at room temperature. The method adopted in the present study was simple and used easily obtainable non-toxic materials, and since the isolation and chemical analysis of individual pigments was not the intent, it proved adequate for defining overall patterns associated with taxonomic groupings. It is possible that not all of the pigments were extracted with hot water, and that some additional cell proteins were included since the heat may break cells. However, similarities with the pigment patterns obtained by Gluchoff (1969, 1975) suggest that this hot water extraction was quite effective.

The use of narrow waveband light such as can be emitted by LED lights shows promise as a highly portable spectrum examination system. The blue light at 470nM had less available energy than a UV light to excite the electrons to a higher orbit in fluorescent materials, so fluorescence tended to be lower, a different colour, or affected fewer pigments. Different molecules fluoresce under different and precise spectra and if the molecule and the wavelength required to excite it are known, in theory a light of that specific wavelength should be able to detect its presence or absence in a chromatograph.

Direct comparisons with the pigments detected by Gluchoff (1969, 1975) and Iten *et al.* (1984) could not be done without subjecting the cuticles to the same procedures they used, which required considerably more material than the few mm^2 of cutis used here. However, some similarities exist: specifically, the red which did not fluoresce under blue LED light appeared to be analogous to Rr I, and those that did fluoresce in both UV and blue light were similar to Rr II and Rr a, which do not easily separate anyway and were identified spectroscopically by Gluchoff (1975). Most of the species tested in 1975 by Gluchoff had only one of two blue pigments; Re I for the majority, and

Re II for *R. nauseosa, R. heterochroa* and *R. maculata,* plus the blue specific to the *Heterophyllae* sensu Romagnesi, which fluoresced in UV light, and which was evident in the Vancouver Island species in this group. In view of the fluorescence with a blue LED of the blue pigment in four of the collections of/?, *occidentalis* further investigation with narrow waveband blue light could well find specific patterns of fluorescence in these pigments. Of the seven yellow pigments Gluchoff (1969) isolated, some of which Iten *et al.* (1984) suggested may be intermediate products or decomposition products of the other yellow pigments, one that seemed to be in most species of both Vancouver Island and Gluchoff s (1969,1975) collections, and the first yellow to be eluted in water was the pale orangish colour that ran up to the solvent line, probably analogous to Rx I, and which matched her description of a dull yellow fluorescence in UV.

The bright clear yellow that ran about 1/3 to 1/2 of the distance to the solvent front was most likely analogous to Rx V, which was found in varying quantities in all but the *Ingratae* and *Heterophyllae* in Gluchoff (1969, 1975), and in the same groups here, and also in *R. aeruginea.* The identity of the other yellows was not clear.

Interestingly, *R. aeruginea* in section *Heterophyllae* had a pigmentation pattern intermediate between that of subgenus *Russula* and that of the other members of subsection *Griseinae,* This, together with the reduction in the cutis of multiple septae in the epicutis hyphae and few inflated epicutal hyphal cells, and the relatively large spore ornamentation for this subgenus, suggests that local *R. aeruginea* could be be a link between the two subgenera.

Russula adusta, a member of subgenus *Compactae* (Fries) Bon, Section *Compactae* Fries, showed traces of the yellow pigment in the same position on the chromatograph as the yellow in those of subgenus *Russula.* There were also traces of pigment that were not visible in daylight but showed slightly orange in UV light in the same position as the UV+ magenta in red and purple Russulas. These pigments were not seen on the chromatograph of *R. nigricans,* a species in the same section as *R. adusta* and with a similar blackening of damaged flesh. The pigment patterns point to a closer relationship between *R. adusta* and subgenus *Russula* than is found in other sections of subgenus *Compactae.* The *Foetentinae* shared the same basic pattern as these members of the *Compactae.* Miller and Buyck (2002) in their phylogenetic analysis of die genus

Russula found that the clade with *R. adusta* and *R. nigricans* did not fall within the other *Compactae* examined but branched off after the *Heterophyllae* and *Foetentinae* clade, and was basal to the subgenus *Russula* sensu Romagnesi (1967), a position supported by this pigment analysis, (at least for *R. adusta).*

Members of the *Xerampelinae {Viridantinae* sensu Romagnesi 1967) including *R. xerampelina* var *xerampelina, R.* cf. *pruinosa, R. eleodes, R. isabelliniceps* and *R. semirubra* all shared a similar pattern and with the exception of a *R.* cf. *abietina* were the only ones so far tested that had a strong red pigment that did not fluoresce in blue LED light at 470nM. Their close relationship was confirmed by the similarities in the RFLP patterns of the ITS rDNA region, both within Vancouver Island collections and collections deposited in GenBank from Europe and North America.

The reduced pattern of the four *R. occidentalis* may be an artifact of the spotting technique on the chromatography paper, the extracts for most of these collections were very pale and needed repeated application with air drying in between applications to obtain sufficient deptii of colour to be visible when separated out. This may also have had the effect of concentrating the viscid substance generated by the cuticular hyphae, which appears to interfere with the ability of pigments to elute up the paper. *Russula occidentalis* is in the subsection *Integroidinae* (Romagn.) Bon and was unfortunately the only member of this group to be collected in this study, so comparisons cannot be made to ascertain which of the patterns is correct. Gluchoff (1969) examined the pigments of/?. *claroflava,* a yellow species also in the same subsection and clade as *R. occidentalis,* finding no red and blue pigments, but four yellow pigments and die colourless pigment that fluoresces violet in UV light.

Russula crassotunicatae and *R. crenulata* had a pattern not yet seen in another species, supporting the separation of these species into subsection *Crassotunicatae* Singer within the *Ingratae* (Quelet) Maire.

With the exception of *R. aeruginea,* which had a pigment pattern closer to pattern 1, species in subgenus *Heterophyllidia* Romagnesi had pattern 3 which lacked strong reds and clear yellows, even though greens and pinks appeared in the whole cutis. Evidently these pigments were not extracted by hot water, or they did not retain their original colours in the processing. Either way, the pattern formed seems to be indicative of members of this subgenus.

The pigment pattern analyses using the simple method outlined above on the whole supported the newer taxonomic divisions of Sarnari (1998) but also raised new questions regarding anomalous species. As an identification tool, it quickly resolved whether a species was in the *Compactae-Foetentinae* group, the *Heterophyllidia,* or the other subgenera of *Russula* and *Incrustatula* sensu Sarnari 1998, although these latter two are not differentiated by this tool. Even though tiiese groups are all fairly well defined in terms of microscopic morphological characters, it is not uncommon to encounter a collection in which one or more characters are ambiguous, particularly if the collection is very young, weathered, or at one extreme of the character diversity, and especially if it is one which the researcher has not encountered before. The *Xerampelinae* have in common red pigments lacking fluorescence under the blue LED light, a characteristic also found in some of the *Incrustatula,* some of which form a basal clade and some a sister clade to the former (Chapter 2). Any tool that can eliminate a range of possibilities increases the chance of an identification being made, and whilst this method of pigment analysis has its limitations, it also presents the potential for further developments and adaptations to make it more specific.

Chapter 3

DNA analyses Part 1: Evaluation of the RFLP of the ITS region of Russulas as a means of differentiating among them, and confirming their identity by comparison with published sequence data.

Introduction

In making an identification of a species of *Russula* the usual method of matching morphological characters of the species in hand with published descriptions does not always result in a satisfactory conclusion. Part of the problem is that descriptions vary in the amount and clarity of the information given, and in the accessibility and language of the journals in which they are published. Viewing holotypes is not always practical, especially when a number of species are being questioned, therefore, other methods of either confirming an identification or placing an unknown *Russula* species into a taxonomic unit within the genus would be helpful.

In the Pacific Northwest, in addition to purely local species, there are many species of Russula that are also found in the temperate zones of Europe and Asia. Increasingly, DNA sequences of parts of the genome of these species are being made publicly available in databases such as GenBank at NCBI and the European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI).

DNA sequences are an additional source of useful characters in systematics with different parts of the genome varying to different degrees. In general, more conserved regions are useful for elucidating phylogenetic relationships at higher taxonomic levels, while more variable regions are useful at lower taxonomic levels. Published sequences are, therefore, another set of characters available for comparison with an unidentified species.

Sequencing genes is becoming faster and more cost effective, but is still difficult for many researchers, particularly those who do not have ready access to sequencing facilities. Comparing restriction fragment length polymorphisms (RFLP) of an amplified gene or spacer DNA is one alternative with less precision than sequencing, but with the power to differentiate between taxa at the subgeneric level (Kernaghan *et al.* 1997, Suga *et al.* 2000). The results can also be compared with published RFLP data and with sequence data available through GenBank. Such comparisons to confirm or discover the identity of a basidioma would also be useful in ectomycorrhizal studies, since the below-ground fungal population often does not conveniently produce fruiting bodies with which to compare DNA (Gardes and Bruns 1996, Durall *et al.* 1999).

The use of rDNA genes and spacer regions for RFLP comparisons

An appropriate region of the genome to use for RFLP comparison needs to have sufficient variation to distinguish between species but little intraspecific variation. Phylogenetic studies have focused on the mitochondrial ribosomal DNA genes (rDNA) (Peter *et al.* 2001) or on nuclear rDNA as these sequences have both conserved and variable regions which can be selectively amplified (Gardes & Bruns 1993, Vilgalys and Hester 1990, White *et al.* 1990).

The ribosomal large sub-unit (LSU or 28S), a very conserved region of DNA, has been preferred for taxonomic analyses above the genus level (Drehmel *et al.* 1999, Eberhardt 2002, Henkel *et al.* 2000, Miller *et al.* 2001, Montcalvo *et al.* 2000, 2002). The small subunit (SSU or 18S) has similarly been used to establish phylogenetic relationships among the fungi (Bruns *et al.* 1992).

The internal transcribed spacer (ITS) of the nuclear ribosomal DNA (rDNA) gene is amongst the most widely sequenced DNA regions in fungi (Vilgalys 2001), as it is often used to determine intrageneric phylogenies (Kretzer *et al.* 1996, Aanen *et al.* 2000, H0iland and Hoist-Jensen 2000, Miller *et al.* 2001, Miller and Buyck 2002, Eberhardt 2002) and between isolates of a species or species complex (Gomes *et al.* 2000, Methven *et al.* 2000, Zervakis *et al.20Q4).* Gottlieb and Lichtwardt (2001), however, found it too variable to infer phylogenies in the *Harpellales.*

The internal transcribed spacer region (figs.7 and 8) is a variable region about 600-1000 base pairs long; in *Russula* it is usually between 800 and 920nt, containing two non-coding spacers between the highly conserved 18S and 28S subunits, and flanking the conserved 5.8S subunit of the ribosomal RNA gene in the nuclear genome (Gardes and Bruns 1993, 1996). The whole 18S-1TS1-5.8S-ITS2-28S complex occurs in multiple repeating units each separated by two intergenic spacers, IGS1 and 2, which flank the 5S subunit (fig. 7).

In *Schizophyllum commune,* the variation between repeating ITS units was found to be very low but variation was seen between IGS repeats (James *et al.* 2001). The non-coding region may be much more variable than the subunits of the gene, which has been a problem in its use for inferring phylogenies in some studies (Gottlieb and Lichtwardt 2001). In other cases, the variation between species has been found to be low, less than 1% in some groups of *Hebeloma* (Aanen *et al.* 2000). In fungi, variation among both the ITS and IGS regions can occur within a species (James *et al.* 2001, Pantou *et al.* 2003), and even between repeats within an individual (James *et al.* 2001, Gomes *et al.* 2002), but this is usually less than that between species. The large sub-unit (LSU or 28S), has been used for higher levels of taxonomic analyses than the ITS region (Henkel *et al.* 2000, Miller *et al.* 2001, Montcalvo *et al* 2000,2002, Eberhardt 2002). But the ITS region was used for infrageneric phylogenetic analyses of *Russula* as it has an appropriate level of variability for this purpose (Miller *et al.* 2002, Eberhardt 2002).

Primers

Gardes and Bruns (1993) developed the basidiomycete-specific primer pair ITS1-F/ITS4-B (table 6), which reduces the possibility of amplifying contaminant moulds, often a problem with field-collected specimens of fungi. *Russula* basidiomata are frequently infested with insect larvae, sometimes even in the primordial stage, and should not be assumed to be sterile inside the trama. Growing pure cultures of *Russula* from spores or even from basidiomata is still unreliable and difficult, and growth extremely slow (Ali and Jackson 1989, Hintikka and Niemi 1999), so fresh or dried basidiomata were used in this study. Primer pair ITS1-F/ITS4-B yields an amplification product about 14nt longer at the 5' end than the forward primers ITS5 and SR6R, and about 137nt longer at the 3' end than JTS4 (fig. 8), this latter being commonly used in published sequences.

Dunham *et al.* (2003) used the primer pair ITS IF and ITS4 (rather than ITS4-B) to amplify DNA from *Cantharellus* species after testing both reverse primers. Of 152 ITS1 sequences of *Russula* species held in the GenBank and EMBI databases, 38 commenced within the JTS1 primer site and 87 within the ITS5 site, the remainder commenced downstream of these sites. Of 150 *Russula* sequences containing the complete ITS2 region, 13 include or go beyond the ITS4-B recognition site, 90 terminate within the ITS4 recognition site and one terminated at the LR1 site. A more recent set of sequences placed in Genbank and listed in appendix 2, begin within US5, 20nt downstream from the 5' end of the ITS1-F site, and terminate beyond the ITS4-B site. Four *Russula* sequences covered the entire ITS1-F to ITS4-B region, three of these were from B.C., Canada (Durall *et al.* unpublished). The sequence string between the forward primers ITS1-F, ITS5 and ITS1 is within the conserved 18S subunit, and that between ITS4 and ITS4-B is within the conserved 28S subunit: both these regions show little variation among *Russula* species. Some commonly used ITS primers are shown in table 1, below, and their recognition sites are demonstrated in an example sequence of *Russula xerampelina* in fig. 8.

Table 6 Primers developed for the ITS region and shown in Figure 8

Primer	**Target sequence 5' to 3'**	**Developed by**
ITSl	TCCGTAGGTGAACCTGCGG	White *et al.* 1990
ITS5	GGAAGTAAAAGTCGTAACAAGG	White ef *al* 1990
ITS1-F	CTTGGTCATTTAGAGGAAGTAA	Gardes & Bruns, 1993
SR6R	AAGWAAAAGTCGTAACAAGG	Vilgalys lab*
Reverse primer	**Target sequence 3'to 5'-(same strand)**	
LR1	AGGAAAAGAAACCAACC	Vilgalys and Hester, 1990
ITS4	GCATATCAATAAGCGGAGGA	White ef *al* 1990
ITS4-B	CTGGACCGTGTACAAGTCTCCTG	Gardes & Bruns, 1993

*Unpublished data available on <http://www.botany.duke.edu/fungi/mycolab>

Restriction enzymes used in ITS-RFLP studies

Farmer and Sylvia (1998) tested seven restriction enzymes for their ability to differentiate among 69 species in 17 genera of ectomycorrhizal fungi, using the ITS1-F/TTS4 fragment. They found that almost all isolates had a restriction site for AM, Cfol, Hinfl and TaqI, but needed more than one enzyme to distinguish them, since members within a genus often shared restriction fragments. The ITS1-F/ITS4-B fragment, cut with the enzymes AM and Hinfl, has been successfully used to identify the fungal partners of ectomycorrhizae by matching the RFLP patterns with those of various species whose basidiomata were collected nearby (Gardes and Bruns 1996), and for separating two species of *Lactarius* (Kraigher *et al.* 1995). Goodman *et al* (1996) routinely include the Hinfl, AM and Rsa I RFLP patterns of the ITSl/NL6Bmun fragment, which includes slightly more of the large subunit gene than ITS4-B, for their descriptions of ectomycorrhizae. The restriction enzymes Hinfl, Alul and Ndell or its isoschizomer Mbol, have been used on the amplified ITS region in the identification of ectomycorrhizae (Smith and McKay personal communication, Sakakibara *et al.* 2002). Ndell, which recognises the GATC motif, has nine isoschizomers including Dpnll and Sau3AI. Kernaghan *et al.* (1997) used Hinfl, AM and Rsal and Hhal in their study of Russulaceous ectomycorrhizae and concluded that RFLP of the ITS region using these enzymes gave an "appropriate level of resolution for the taxa studied". They also noted that Rsal did not always cut the sequence. This was the experience of Suga *et al.* (2000) using Rsal in addition to three other enzymes to fragment *Fusarium solani* rDNA ITS region. The restriction sites for Hinfl, Alul and Sau3AI in a sequence of *Russula xerampelina,* together with the primer recognition sites listed above it, are given in fig. 8. All of these enzymes recognise a 4-base pair sequence motif hence cut DNA more frequently than enzymes that recognise 6 or more base-pair targets.

Extraction protocols

Extraction protocols vary depending on the source organism and the purpose for which the extracted DNA will be used. Different organisms vary in the amounts and type of contaminants, such as proteins and polysaccharides, which are extracted with the DNA: sometimes these contaminants interfere with amplification and have to be removed. In other cases simple dilution of the template solution solves problems. Extraction of DNA involves the physical or chemical breaking open of cell walls to release the cell contents. This is accomplished by freeze-drying, nitrogen freezing and crushing, freeze-thaw cycles or grinding tissue with or without sand. The breaking of cell membranes to release the nuclear material is usually accomplished with a detergent such as sodium dodecyl sulphate (SDS) or cetyl trimethyl ammonium bromide (CTAB), the latter also inhibiting the action of DNAase. Finally the released DNA needs to be separated from the extraction chemicals and cell debris.

Many extraction methods use phenol and chloroform to remove proteins from the extraction mixture (Moller *et al* 1992, Gardes and Bruns 1993, Bergemann and Miller 2002), and most protocols use several Eppendorf tubes for each sample as the DNA and cell debris are separated. Efforts have been made to reduce the use of hazardous chemicals and the number of transfers to new tubes, a particular consideration for automated systems. Aljanabi and Martinez (1997) used salt instead of chloroform to precipitate out proteins and carbohydrates from a variety of organisms including fungi. They obtained DNA of a quality and quantity suitable for multiple PCR reactions. Norvell (2000) extracted sufficient DNA from fresh spores of *Phaeocollybia* species by freeze-thaw cycling in TE buffer, which was then used directly as the template solution to amplify the ITS region without further modification. This method worked only on very fresh spores (Norvell, pers. communication). Similar techniques are applied to plant tissue using commercially prepared lysis buffer and a short heat-cool series in a thermocycler before being used directly as a PCR template (Burr *et al.* 2001). Shimono *et al.* (2004) used an SDS-based extraction buffer containing proteinase K for their phylogenetic study of members of the *Russulaceae*. After incubation with homogenised tissues and separation of the cell debris by centrifugation, the extraction buffer was directly used as template DNA. The many protocols and variations reported in the literature suggest that a certain degree of experimentation is required to extract DNA from a given organism adequate for the intended purpose, and that some steps may prove to be optional.

The objectives were: 1) To develop a method for isolating genomic DNA from fresh and air-dried *Russula* basidiomata, and for the amplification and digestion of the internal transcribed spacer (ITS) region, and 2) To evaluate the usefulness of the ITS region and its restriction fragment length polymorphisms (RFLPs) as a means of differentiating among species of *Russula*, as a tool for grouping similar species, and as a means of confirming identifications through comparison with published sequence data.

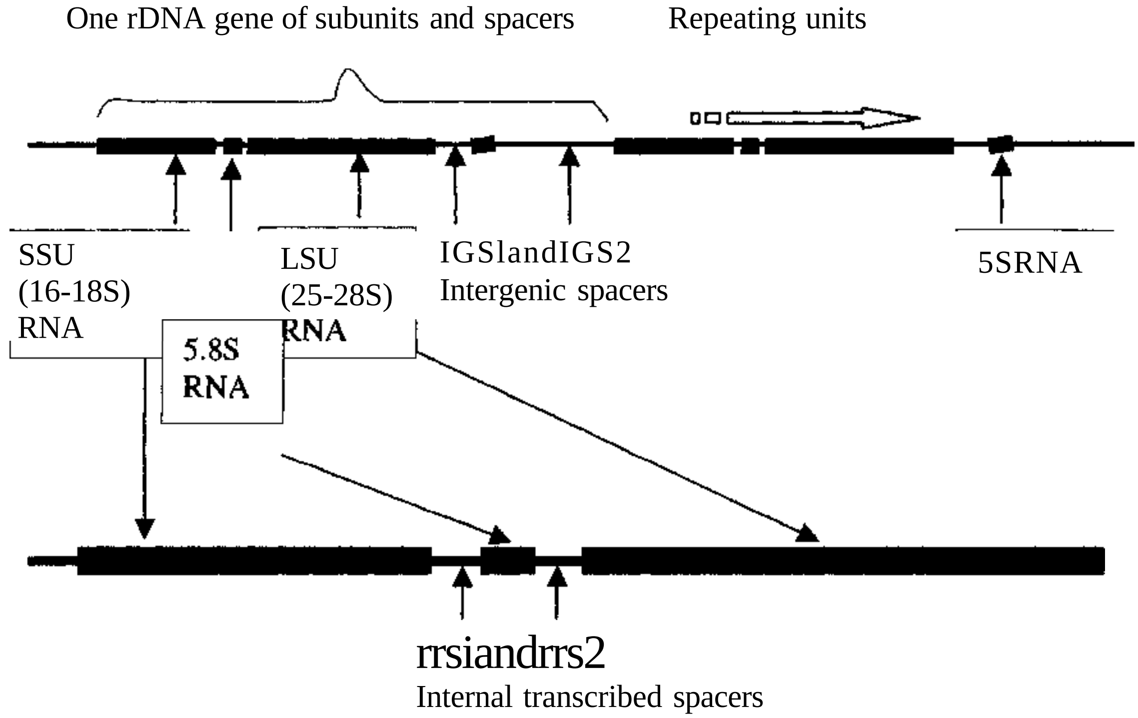


Figure 7 The rDNA coding subunits, internal transcribed spacers and intergenic spacers. Diagrammatical representation of repeating units of the ribosome genes, genes are separated by two intergenic spacers: IGS1 and IGS2, one either side of the 5S coding unit. The remaining three coding units are separated from one another by transcribed spacers: ITS1 lies between the small (18S) and the 5.8S subunits, and 1TS2 lies between the 5.8S and the large (28S) subunits.

rrsi-F

CTTGGTCAATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATCGTACAACG

1TS5, SR6R USl

GAGGTGTGAGGGCTGTCGCTGACCTTTAGAGGTCGTGCACGCCCGAGCACTTTCATACAATCCATCTCACCCCTTTGTGC

ATCACCGCGTGGGTCCCCTTTGCGGGAGGGCCCGCGTTTTCACATAAAACTCGATACAGTGTAGAATGTTCTTTTGCGAT

CACACGCAATCAATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATACGT

AATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCCCCTTGGCATTCCGAGGGGCACACCCG

TTTGAGTGTCGTGAAATCCTCAAAAACCCTTTTCTTGTCAGGAAAGGGATTTTTGGACTTGGAGGTTCAATGCTCGCCT^

CACTTITGAAAGTGAGCTCCTCTCAAATGAATTAGTGGGGTCCGCTTTGCTGATCCTTAACGTGATAAGATGCTTCTACG

TTTTGGACCCGGCTCTGTCCCTTGGATGCCTGCTTCTAACTGTCCCACGGACAATGATGGTGCTTCGGTCACTGCCATCTA

CATTGGCGGGAGGCTGGACCCACAAAATGAAAACCTTGACCTCAAATCGGGTGAGACTACCCGCTGAACTTAAGC

ITS4

ATATCAATAAGCGGAGGAAAAGAAACT^AACAAGGATTCCCCTAGTAACTGCGAGTGAAGCGGGAAAAGCTCAAATTT

LR1

AAAATCTGATGGTCTTTGGCCGTCCGAGTTGTAATTTAGAGAAGCGTCTTCCGCGCTGGACCGTGC*ACAAGTCTCCTG

ITS4-B

Figure 8 An example of the US 1 and 2 sequence with the forward primers ITS1-F, ITS5, SR6R and ITS1 and reverse primers ITS4, LR1 and ITS4-B under or overlined, differences between the primer nucleotides and the sequence nucleotides are marked with an asterisk. In bold shaded text are the restriction sites of Hinfl (GANTC), AM (AGCT) and Sau3al (GATC). The sequence is from *Russula xerampelina* var. *xerampelina*, GenBank accession number DQ367916.

Base differs from that of the primer.

Materials and Methods

Russula species were collected from coastal forests of southern Vancouver Island over the course of five fruiting seasons. A fruiting season generally runs from mid to late summer through fall and winter, with some Russulas withstanding light frosts but not fruiting at temperatures below freezing. Species were identified using the following references: Romagnesi (1967, 1985), Sarnari (1998), Gibson (2000), Thiers (1997), Woo (1989), Phillips (1991), Hesler (1960, 1961), Grand (1965), and Shaffer (1964,1970, 1972). As collections were destined eventually for an herbarium tihey were dried in a warm-air food drier; and for many Russulas this was done before a sample was taken for DNA analysis. Other collections made in the weeks before the DNA analyses were carried out had a sample removed and stored frozen in a 600ul aliquot of CTAB buffer or were extracted and the precipitated DNA stored dry and frozen at -20°C.

Selection of restriction enzymes

An initial selection of ITS sequences from 17 species of *Russula* and one each of a *Martellia, Lactarius* and *Rozites* were trimmed to a common start and end point. The location of all occurrences of the recognition site of each of 6 enzymes was found and the fragment sizes for each enzyme gave a virtual RFLP pattern for each sequence, which was examined for uniqueness among those of other sequences. The first enzyme, HinfT, which recognizes the motif GANTC, was found in all *Russulales* sequences at a double site within the 5.8 S conserved region, each cut site being 8 base pairs apart, effectively separating ITSl from ITS2. Some sequences also contained additional sites in the ITS2 region. However, this enzyme alone was insufficient to differentiate among all sequences.

The second enzyme Alul, which recognizes the motif AGCT, was found to have up to three recognition sites in ITS2, one of which consistently appeared around 87 base pairs before the downstream end of the ITS4-B primer site. Some sequences also contained a recognition site for Alul in ITSl. Whilst this enzyme in addition to Hinfl provided much more resolution among sequences, there remained a few that were not differentiated from one another. Three more enzymes, Rsal (GTAC), Mael (CTAG), and Haell (GGCC) were found to have recognition sites in some but not all sequences.

The enzyme Sau3AI, which recognizes the GATC motif, cut all sequences from 1 to 6 times, and provided good further resolution between test sequences. One site around 60nt downstream of the ITS1-F primer site was found in most sequences. The three enzymes finally selected for the study were Hinfl, AM and Sau3AI, which gave three levels of resolution.

DNA extraction procedure

Fresh and dried *Russula* tissues were extracted, amplified and digested using an adaptation of the basic protocols of Karen (1999), after also testing the methods of Aljanabi and Martinez (1997) which use sodium dodecyl sulphate (SDS) as the lysis agent and a saturated solution (6M) of NaCl to precipitate the polysaccharides and proteins.

In a trial using two collections of *Russula; R. brevipes* and *R. xerampelina,* comparisons between the SDS and CTAB extraction buffers, the salt and the chloroform method of precipitating cell debris, and the ethanol with the isopropanol precipitation of DNA were assessed. Test extractions were diluted to 1:10,1:100 and 1:1000, amplified and electrophoresed on 1.5% agarose gel. The results indicated that the CTAB buffer was more reliable than the SDS, that the salt precipitation worked about as well as the chloroform regardless of buffer, and that ethanol at 2.5 volumes was more successful than isopropanol at precipitating amplifiable DNA without salt also precipitating out (5/12 test extractions for isopropanol, 20/24 for ethanol). The salt precipitated cell debris did not form a solid pellet when centrifuged and was easily disturbed while removing the supernatant, requiring further cleaning of the supernatant with chloroform in several cases. Due to this and occasional problems with salt contamination of the DNA the salt precipitation method was not used for the main analyses.

A volume of about 3-5mm^3 of fresh tissue or its equivalent of dried tissue was taken from the youngest and cleanest specimen of a *Russula* collection and placed in a 2ml Eppendorf tube. As the moisture content varied in fresh material, weighing was not helpful. The gills of a basidioma have a greater concentration of genetic material per unit volume of tissue than the cap or stipe trama, since the latter contains many sphaerocytes. Gill tissue was therefore expected to release more DNA per mm^3 than stem or trama tissues and so was preferred. With mature, infested or dried specimens, judgment must be made in terms of the tissues and volume selected for extraction. The pattern of decay caused by contaminants such as moulds and insect larvae varies between individual basidiomata, but almost every *Russula* collected contained arthropod larvae. Specimens that had been dried for more than two years, as in herbaria, needed either more tissue or longer processing or both. None of the collections used for DNA analysis were freeze dried since this destroys their microscopic structures and renders them useless for herbarium specimens.

To the tissue in the Eppendorf tube, only 2 to 3 drops of 2% CTAB extraction buffer were added and the tissue ground by hand using a clean blue disposable pestle or a pestle made of 1/4-inch Teflon rod shaped with a pencil sharpener to fit the Eppendorf tube. A pounding, twirling and squashing motion homogenized the tissues most effectively. Fresh tissue could be ground immediately, but dried tissue was better left for up to an hour at room temperature (or overnight in a refrigerator at 4°C) to soften in no more than 1/2 ml of CTAB extraction buffer before grinding. Once a smooth paste was formed, more CTAB buffer was added to the tube contents to bring them to approximately 600pl and any remaining visible lumps carefully macerated with the pestle. Acid-washed sand was used as a grinding agent only as a last resort on somewhat rubbery samples, since it prevented adequate crushing of the tissues. Proteinase-K added to the CTAB solution (at 400pg/ml final concentration) was used when extracting from dried collections, particularly those over two years old, as the DNA becomes bound to the nuclear proteins more strongly over storage time and proteinase helps release it. In all cases amplifiable DNA was extracted without the use of p-mercapto-ethanol.

Each sample was heated to 60-65°C in a heating block or oven and maintained at that temperature for a minimum of one hour for macerates of fresh tissues, and up to overnight for macerates of dried tissues. Each tube was shaken manually at intervals. Removal of cell debris from the buffer solution was accomplished by filtering, since this gave a cleaner supernatant than centrifugation. Filters were made using cotton wool tightly packed into 1ml blue automatic pipette tips to a depth of about 10mm: the flat end of a clean disposable pestle worked well for this. The cotton wool was first soaked for an hour in two changes of 3% hydrogen peroxide, rinsed in three changes of sterile distilled water and oven dried. The filters were autoclaved prior to use. The filtrate was gathered into a new 2ml Eppendorf tube and the cotton filter rinsed through with sufficient CTAB buffer to bring the volume up to 500ul. DNA was then precipitated out with 2.5 volumes (1.25ml) of ice-cold ethanol or 1.5 volumes of ice-cold isopropanol. Ethanol precipitation was the preferred method since it brought down less salt than isopropanol (Aljanabi, personal communication). If DNA was seen forming as a jelly-like or cottony substance the tubes were immediately centrifuged, otherwise they were kept at -20°C for an hour to overnight until DNA was visible. Kar6n (1999) recommended a 30 minute spin but usually 5-10 minutes at 13,000 rpm brought down a visible pellet. The supernatant was then carefully drained off so as to retain the pellet, which was then washed with 3 changes of 70% ethanol, each of about 0.3-0.5ml, depending on the size of the pellet, and taking care not to dislodge it. Any pellets that became loose were spun at 13,000 rpm for 30 seconds to 1 minute to redeposit them. Tubes were placed upside-down onto paper towels to drain then left on their sides (to prevent contaminants from falling in) for an hour or two to air-dry. At this point the dried DNA could be stored at -20°C for several weeks.

In cases where the extraction solution was dark, cloudy, or the sample had failed to amplify on a previous attempt, the DNA was cleaned of proteins and lipids after filtering with a wash of 600ul of 24:1 chloroform: isoamyl alcohol, added under a fume hood and mixed well by hand. Cell debris collected and remained in the lower, discarded layer after centrifuging for 5 minutes at 13,000 rpm. The clear Eppendorf tubes sometimes burst in the centrifuge when used with chloroform at or above 10,000 rpm, but could be safely spun for 4 minutes at 7,000 rpm. The upper phase was removed to a clean tube and precipitated with ethanol as above. When this method was used on second attempts to extract amplifiable DNA after an earlier failure, it yielded an improvement in only two cases. With many *Russula* samples it did not make much difference whether a chloroform wash was used or not, because they amplified without this extra step.

Amplification protocol

For amplification, the DNA pellet was resuspended in lOOul of sterile double distilled water, which was found to give more consistent amplification success than TE buffer. When only a very small pellet of DNA was recovered, 50ul sdd H2O was used and for large or dark-coloured pellets, 200ul were used for resuspension. Tubes were flicked with a finger to mix and left about an hour at room temperature or overnight at 4°C to dissolve the DNA. A dilution of 1:100 or occasionally 1:50 of the above solution as the DNA template in an amount of lOul per 20ul PCR mix was usually successful.

For amplification a master mix of PCR reagents was prepared and portioned into labelled PCR tubes in lOul aliquots, followed by the template. All reagents and samples were kept on ice while mixing and until placed in the thermocycler. In each thermocycler run a blank containing the PCR mix and water instead of template DNA was included.

PCR mixes:-

For a 20ul reaction at 1.5mM MgCIj, the following reagents were added in order listed:

3.2ul ultra-pure water
2ul lOx Mg free buffer
2ul dNTP stock 2mM
lul Primer ITSl-F50uM
lul Primer ITS4-B 50uM
0.6ul 50mM $MgCl_2$ soln.
0.2ul Taq DNA polymerase
lOul DNA template

For buffers supplied with Mg already in, the above recipe was followed except that 3.8ul of water was added instead of water and $MgCl_2$ soln.

For a 3mM MgCk PCR reaction mix the recipe was as above but used:
2.6ul water
1.2ul50mMMgCl$_2$

Henegariu *et al.* (1997) examined the effects of different concentrations of MgCl2 on amplification and found an optimum at 3mM which resulted in more amplification product than when the more usual 1.5mM concentration was used. A 3mM MgQb concentration was tried on three collections, one of *R. mustelina* which had previously amplified only weakly, one of *R. crassotunicata* which had previously failed, and one of *R. aeruginea* which had amplified normally. The results, shown in figure 9, of a comparison of 1.5mM in the first of each pair of lanes, and 3mM in the second, show an increase in the amount of amplified DNA for *R. mustelina,* lanes 1-4, and no improvement or difference in the *R. crassotunicata* lanes 5 and 6, or *R. aeruginea,* lanes 7 and 8. This information was found after completing the bulk of the DNA work, but a final run of 16 previously failed samples resulted in 7 of them amplifying.

M I

Figure 9 Comparison of two concentrations of MgCk used in the PCR reaction. Lanes are in pairs starting from top right with the first of each pair using *MgCh* at 1.5mM and the second at 3mM. In lanes 1 and 2 a fresh extraction of *Russula mustelina* and in lanes 3 and 4, an older extraction of the same specimen which had amplified only weakly in a previous run. Lanes 5 and 6 contain a fresh extraction of *R. crassotunicata* which had previously failed, and lanes 7 and 8, *R. aemginea* which had successfully amplified in a previous run. Lane 9 is a lOObp ladder, brighter bands are at 1000 and 500bp. The last two lanes are blanks. Lane 6 shows very little terminal "cloud", suggesting the gel well leaked. The higher MgCk concentration resulted in an increase in the amplification product of both *R. mustelina* extractions but about the same for those of *R. aemginea*. An additional PCR product of approximately 250bp is apparent in lanes 7 and 8, the origin of which is unknown.

Amplification conditions:-

The PCR mixture was topped with a drop of mineral oil to prevent evaporation, spun briefly (about 15 seconds) to deposit the reagents at the base of the tube and placed in the thermocycler.

For ITS1-F and ITS4-B primers, the following thermocycler conditions were developed by Gardes and Bruns (1993.) and followed in this study:

Denature at 94°C for 1 minute, then cycle through the following denaturing, amplification and annealing stages :-

95°C for 35 seconds
55°Cfor55 " 13 cycles
72°Cfor45 "

95°C for 35 sec
55°C for 55 sec x22
72°C for 120 sec

95°C for 35 sec
55°C for 55 sec x8
72°Cforl80sec

72°C for 10 minutes

holdat4°C

Following this, the oil was removed by freezing the tubes, and while the aqueous phase was still frozen the oil was drawn off with a sliver of clean paper towel.

The amplification product was visualized on a 1% agarose gel using 3 pi of the PCR solution in a 12pl gel-well, the remainder of the volume consisting of 5 pi sterile distilled water and 4pl of a 0.2X solution of running dye. The running dye was diluted with 30% glycerol solution from a 6 X dye mix containing 0.25% of bromophenol blue and 0.25% xylene cyanol FF in a 30% solution of glycerol in water. This lower dye concentration was still visible when electrophoresed into its component blue and violet dyes, yet was not so dark that it masked any underlying DNA bands. A lOObp DNA ladder and a blank was run on each gel.

Gels were electrophoresed in TAE running buffer at 100V and 400 amps for about 1 hour 20 minutes or until the violet dye band was about half-way along the gel. Gels were stained with ethidium bromide, destained in water then viewed and a digital photograph taken under UV light.

Digestions

Aliquots of the PCR product were added to the restriction enzymes in 20ul reaction volumes as per the recipes below.

Digestion Recipes:

Hinfl (10,000 units/ml or Alul (8,000 units/ml)- per 20ul reaction:

0.2ul Hinfl (or Alul)
2ul lOx buffer
13.8ul sdd water
4ul PCR product

Sau3AI(4000 units/ml):

0.5ul Sau3AI
2ul lOx buffer
0.2ul lOOx BSA
13.3(j.l sdd water
4ul PCR product

Most reactions required 4ul PCR product in 20ul reaction, however, when the amplification produced a relatively abundant amount of DNA as observed by a very bright band under UV light on the stained gel, aliquots of l-2ul were digested and the reaction volume adjusted to 20ul with sterile double-distilled (sdd) water. Digestion mixtures were incubated overnight at 37°C. Aliquots of lOul of digestion product mixed with 2ul 0.2X running dye were loaded onto 1% agarose gels and electrophoresed as for die PCR product above but for approximately lhour 45 minutes or until the violet dye (which runs at about the same rate as the 500bp band) had travelled about 1/2 the length of the gel. Aliquots of the amplified blank were digested as for the other samples and run on each gel, a lOObp ladder was also included in each gel. Gels were stained and photographed as above.

Determining the size of DNA fragments

A subset of gel photographs were manually analysed using an interpolation method which determined the size of a band by comparing the distance it had travelled with that of the next smaller and next larger bands within the ladder according to the following equation:

$$X=B^{(1-Z)} * S^{z}$$

Where X was the size of the unknown DNA, B was the size of the larger flanking band, S the size of the smaller flanking band and Z the ratio of the distance the unknown band travelled past the larger band over the distance travelled by the smaller band past the larger. This equation assumed a straight line relationship between the log of the fragment size and the distance of band migration, but only between adjacent bands. The interpolation equation was found to be within 12bp and more usually within 3bp when estimating for example the 800bp ladder DNA from the 1000 and 900 bands, compared with an accuracy which varied up to 50bp for a best fit line method. This method was particularly useful on gels where the migration rate of DNA was uneven as indicated by the fuzzy terminal band of primer and incomplete amplification products, seen in fig. 9. This terminal band was a useful marker of the wave front of migrating bands and was retained in the digestions by using the entire PCR product. The band covered digestion products in the range of 50 to 150bp, however, there was sufficient variability in the larger digestion products to differentiate most species. McKay and Smith (personal communication) did not use any band below lOObp in their analyses of ectomycorrhizae using the same suite of restriction sites as in the present analysis.

Two software programs were used in reading the bulk of the gels: Lablmage version 2.7.1. (1999-2004 Kapelan GmbH) and TotalLab version 2003.03 (Nonlinear Dynamics Limited). The Lablmage analyses were interpolated using the bicubic option, which gave results very close to the manual interpolation method (usually less than lObp difference), and was simple to use provided the gel had run evenly across its width. This program did not have the option for corrections due to "smiling", a problem that occurred to a lesser or greater extent in about two thirds of the gels and appeared to be due to the expansion and consequent slight distortion of the gel as the electrophoresis bath became slightly warmer during the run. This was dealt with in two ways. The first involved manipulation of the image in a graphics program (Arcsoft Photostudio 3) to skew and stretch or shrink selected areas of the image to obtain a straight line-up of the terminal bands, prior to measuring band sizes with Lablmage. The second involved measuring the unadjusted image with the TotalLab program which had a means of manually adjusting the reading frame to the shape of the distortion. Whilst TotalLab gave results consistent with the manual interpolation and the best fit to the lOObp ladder bands using the cubic spline interpolation option on undistorted gels, the results for the distorted gels could vary as much as 50bp between reading methods. The problem with distorted gels was a lack of clarity concerning the point where one section of the gel began to migrate faster than another, so measurements were therefore an estimate of the DNA size. The worst affected samples were re-run on new gels, otherwise an average of readings was used.

The fragment sizes were entered into a spreadsheet from which the charts in figures 12 and 13 were created. Comparison with the virtual RFLPs from sequence data described below and shown in figures 10 and 11 was initially visual, since this gave an immediate sense of both the variation in RFLP patterns across species groups and the similarities or otherwise between query and known species. Following this the calculated fragment sizes were compared with those derived from sequence data.

Creation of RFLP data from published sequences

Sequence data of the ITS-rDNA of *Russula* species were selected from the GenBank and EMBL-EBI databases, imported into the BioEdit software (version 7; Hall 1999) and trimmed to the ITS1-F to ITS4-B sites when longer. The BioEdit restriction site recognition function was used to find cut sites. The number of nucleotides between cut sites was calculated to give the fragment lengths, and were entered into a spreadsheet (Excel 2000, Microsoft Corporation) (figs 10, 11). The sequences chosen included representatives of the species found on Vancouver Island where available, otherwise species within the same taxonomic groups were selected. Not all sequences extended to both sites, in which case the end fragments were incomplete and not comparable with real RFLP data. It is therefore possible that there was an underestimation of the number of cut sites for any enzyme in these shorter sequences. Where available, other sequences of the same species, covering a further part of the sequence, were aligned alongside the first to supply additional fragment data. While it is possible to splice two such sequences to get an estimate of all fragment sizes, there are two potential pitfalls to this: the length of the added piece from the donor sequence may differ from that of the recipient, and there may be site substitutions at a cut site erroneously included. The data must therefore be cautiously or conservatively interpreted.

Sequences beginning between the 3' end of the ITS 1-F site and the 3' end of the ITS 1 site are indicated on the charts (figs. 10 and 11) by a grey fragment size marker rather than a black one, to indicate that these are shorter by 20 to 57bp than the equivalent fragment from the V.I. RFLPs. A dot above such markers indicates the estimated fragment size were these missing nucleotides included. (Where the dot is above a black line there are two fragments of the same size at this position). This whole 57bp section is covered by various primer sites (fig. 8) and is set within a conserved region, so such estimates are likely correct within l-2bp.

Many sequences ended at or just before the "gagga" motif within the 1TS4 primer site, which is approximately 140bp short of the ITS4-B primer site and within the 28S subunit. End fragments from such short sequences were not used for comparison, as these are incomplete compared to the RFLPs of V.I. collections, and can vary, particularly in the presence or absence of a Hinfl cut site around lObp downstream of the

ITS4 primer site. This unfortunately means that the fragment patterns for these shorted sequences are incomplete, and it is harder to see species- or group-specific patterns. Shorter sequences which may only cover either ITS1 or ITS2 completely are so indicated in figures 10 and 11. Those sequences beginning from 1TS1-F to ITS1 and ending at ITS4-B -full length sequences or nearly so, are labelled in bold type. Sequence data shorter than 600bp in total is not shown to avoid confusion with fragment data. A table of Genbank accession numbers, authors and place of origin of sequences used in these analyses are given in Appendix 2. Also in Appendix 2 is a table of the collection numbers of V.I. species used in the RFLP analyses, further particulars of which are given in the descriptions of each species in Appendix 3.

Results and Discussion

The species were grouped into clades arranged from basal to upper levels as shown in figures 10 to 13, according to their relationship to the Russulas used in the phylogenetic analysis.

The calculated size of DNA bands varied between reading methods by 0 to 50bp, the latter problem arising when the gels ran unevenly. Repeat amplifications also varied slightly, as in the two versions *ofR. mustelina* in fig. 9 and 12, which were extracted and amplified from the same basidioma hence experiments were repeated until consistent results were obtained. There were also variations between collections of a species in the size of the restricted fragments both in the GenBank and Vancouver Island species; for example the Genbank *R. nigricans* showed two patterns in the Hinfl fragments due to a site substitution at a recognition site, with the 366bp fragment of the second being equivalent to two fragments of 284 and 78bp of the first (fig. 10,78nt fragment not shown). Several Vancouver Island (V.I.) collections also showed variation in Sau3AI fragment patterns; for example, *R. fragilis, R. silvicola* and *R. sanguinea,* in fig. 12. In other cases there was very little variation between collections of the same species. In fig. 13, the *R. xerampelina - eleodes - isabelliniceps* complex, there is little differentiation between these closely related species. With these factors in mind, when considering the pattern of a particular species, reference also needed to be made to its phylogenetic neighbours.

The PCR product

The ITS region in general averaged around 860bp for the V.I. Russulas with a range of 778-932. The GenBank full-length sequences ranged from 828-870bp with little distinction in size range between taxonomic groups. (Note that figures 10 and 11 full-length sequences are denoted by bold type for the species name, other sequence lengths are shorter than the V.I. species' PCR product, and sequence lengths below 600bp are not shown). The notable exception to these figures was *Russula olivacea,* which had a PCR product of 1141bp in the V.I. collection, comparable to sequence lengths of 1002nt and 979nt (ending circa 140nt before the ITS4-B site) from the GenBank collections of European specimens. The insertion of 250bp of novel sequence in the ITS1 region had not been observed to-date in any other *Russula.* Its PCR product alone can quickly identify this one species.

One V.I. collection of *Russula brevipes* and the Genbank *R. pallidospora* had a slightly smaller ITS than others in the *Lactaroides.* The former specimen was collected on the mainland for use as a standard, but did not amplify well enough for the restriction fragment patterns to be visible on the gels. (A collection *ofR. xerampelina* was subsequently used as a standard). The two V.I. *R. brevipes,* both var. *acrior,* had ITS regions of 885bp: this variety was much more common locally than *R. brevipes* var. *brevipes.*

Hinfl

The Hinfl restrictions showed four main patterns that were more or less indicative of a taxonomic group but with some overlap into neighbouring clades. The Genbank sequences showed that the main cut site divides ITS1 from ITS2, with one or two more cut sites in ITS2 being fairly common. Extra cut sites in ITS1 were more rare, and occurred in *R. pallidospora* and *R. aquosa* (latter not used in figures).

Pattern 1 showed two fragments of similar size, less than 40bp apart, in the region of 350-400, which appeared on the gels as a single, sometimes heavier band. This pattern occurred in the GenBank *Russula adusta, R. nigricans* 2 and *R. albonigra,* of the *Compactae* (fig. 10), also in the V.I. *R. albonigra,* all the V.I. *Ingratae (R. cerolens* to *R. farinipes)* and/?, *sublevispora* of the *Heterophyllae* (fig. 12). The Genbank

Heterophyllidia with this pattern included *R. cyanoxantha, R. heterophylla, R. parazurea* and *R. aeruginea.* The V.I. *R. aeruginea* and most others in this group had patterns closer to those of the Genbank *R. virescens* and R. *vesca,* in which the fragment in ITS2 had an extra cut site around 275bp downstream of the double Hinfl site in the 5.8S subunit.

Whilst pattern 1 seemed to be a good indicator of basal clades, the V.I. *R. stuntzii,* a *R. silvicola, R. queletii, R. Integra* and *R. abietina* and the Genbank *R. raoultii* (the first one), *R. fragilis R. betularum, R. persicina, R. cavipes* and *R. sanguinea* also had bands within 40bp of each other, making them difficult to place accurately within a taxonomic framework using Hinfl alone (figs. 10, 11, 12 and 13).

Pattern 2 showed two bands, one of which lay in the 350-405 range, the other between 220-270, with at least 120bp difference. The GenBank *R. chloroides, R. pectinatoides, R. farinipes, R. grisea* showed this pattern, as did the two V.L *R. brevipes,* the V.I. *R. aeruginea, R. brunneola,* and several species of subsection *Russula.* An unusually high number of cut sites occurred in the Genbank *R. pallidospora,* resulting in the largest fragment of 23 lbp with five other fragments ranging from 95 to 148bp, no other species showed this pattern.

Pattern 3 was found in most of the remaining members of the subgenera *Russula* and *Incrustatula,* and consisted of two bands between 40 and lOObp apart, but most within 60-70bp, with some variability in the sizes of die band pair. The larger band was generally in the 380-425 range but as low as 364bp in a V.L *R. queletii.* The smaller band generally ranged between 325 and 364 but was as low as 303 in a V.I. *R. isabelliniceps.*

A number of species had double band patterns somewhat intermediate between those of pattern 3 and pattern 2, notably *R. mustelina* and *R. virescens* in the GenBank set and most of the V.I. *Heterophyllae* and the *R. raoultii-fragilis-silvicola* group. The V.I. *R. raoultii* collections had one smaller fragment of around 275bp as opposed to that of die Genbank collection of North American origin, which contained an extra cut site dividing that fragment in two.

There were some species-specific patterns such as that of*R. olivaceae,* which showed bands in the regions of 230-240 and 180bp in the Genbank and V.I. collections, representing 2 fragments of similar size. A band around 140bp also appeared in both Genbank *collections.*

In figures 10 and *11, R. ochroleuca*, *R. violacea* and *R.* cf. *maculata* have bands at around 350, 230 and 150, and, except for die smaller band, in the V.I. *R. brunneoviolaceae,* one *R. puellaris* and *R. cessans,* indicating that this fragment pattern is associated in general with the upper clades but is no more specific than that, hi summary, the *Ingratula-Compacta* group, the *Lactaroides* and the subgenus *Russula* each had patterns shared by many members of the group, but these were not completely exclusive to the group and could occur in closely related groups.

Alul

The AM restriction patterns closely echoed the Hinfl patterns in terms of group specificity. By far the most common pattern was of a band around 500nt + 25 for the GenBank sequences, more variable for the V.L sequences, ranging from 456 to 569bp, with a band around 210bp smaller, in the range of 274-294bp for the GenBank species and between 250-333bp in the V.L species. These two bands occurred in the majority of the subgenera *Russula* and *Incrustatae,* which included the V.L species from *R. stuntzii* onwards in fig. 12 through all of fig. 13 and the Genbank *R. ochroleuca* on fig. 10 onwards through fig. 11. Amongst die Genbank *Ingratae* and the *Heterophyllidia* of both datasets a smaller fragment of around 220-265bp was more common. A Genbank and the V.L *Russula olivacea* each had three fragments differing slightly in size from other species in die upper clades; at 420-451, 363-379 and 242-269bp, the two larger bands also occurred in *R. lepida* (fig. 11). A fragment in the mid 300's also occurred in one V.L *R. americana* var. *modicaspora, R. occidentalis* and as an extra fragment in one *R. abietina. Russula turci* in the GenBank sequences had a double band around 300 and one at 190bp, the former appears to be equivalent to the band in the low 300's occurring in the closely related V.L *R. murrillii* (fig. 13).

The subsection *Ingratae* was also divided into two patterns, corresponding to the *R. cerolens-granulata* group and the *R. fragrantissima-farinipes* group (fig. 12). The former group were first segregated from the latter in the classification system of Romagnesi (1987), which was followed by Bon (1988) and Sarnari (1998) albeit at slightly different taxonomic levels. They belong to the series *Pectinata* (ad int. Sarnari 1998). The V.L *Pectinata* and the Genbank *R. amoenolens,* closely related to *R. cerolens,* had an extra AM cut site in ITS1, giving three fragments over lOObp long. However the Genbank *R. pectinatoides,* also in the *Pectinata,* lacked this cut site and showed a pattern like that of *R. foetens.* Two V.I. *R. fragrantissima* and the *R. farinipes* were in agreement with the GenBank *R. laurocerasci, R. farinipes* and *R. pallescens.* The V.I. *R. fragrantissima* collections were intermediate morphologically between that and *R. laurocerasi,* in common with several North American collections examined by Shaffer (1972).

A distinctive AM pattern was seen among the Genbank *Compactae* and *Lactaroides,* in which in addition to the fragment around 500bp, one or two small fragments around 127 and 155bp occurred instead of the circa 250bp band seen in other species. *Russula albonigra,* one *R. nigricans, R.* aff. *delica,* both *R. brevipes* and *R. chloroides* all showed these smaller fragments. Among the V.I. set one *R. dissimulans* (a close relative of *R. nigricans)* and two *R. brevipes* showed only the larger band, the smaller double band was not distinguishable on the gels but as no mid-range band was visible, this would suggest a similar pattern to the aforementioned Genbank species. A V.I. *R. dissimulans* and the Genbank *R. nigricans 4* had fragments of 705 and 640 respectively, larger than in all other species examined except the Genbank *R. parazurea,* and due to the lack of one cut site in ITS2. Neither the other V.I. *R. dissimulans* nor the *R. albonigra* amplified strongly enough to give a visible digestion product, a problem also encountered in several attempts to amplify *R. adusta.* An incomplete digestion is a possible reason for the faint extra fragment of 367bp occurring in the V.I. *R. cascadensis.*

Sau3AI and overall patterns

The Sau3AI patterns gave far more resolution between species within a group and showed some group-specific patterns, but as with the other enzymes, group-related patterns were not unique to a group. One noticeable pattern was of widely different fragment sizes as in the Genbank *R. fragilis* to *R. emetica* group (fig. 10) with fragments around 600 and 215bp. This pattern was also evident in three V.I. *R. silvicola, R. laccata* and *R. crenulata* and surprisingly, in the Genbank *R. decolorans* (fig. 11) and in the V.I. *R. cessans andR.* cf. *cessans,* unlike any of the GenBank *Tenellae* (fig. 12). The V.I. *R. albonigra* and one *R. dissimulans* had fragments of 632 and 647 respectively but no smaller bands were resolved on the gels. A similar pattern with fragments of around 550 and 235bp was seen in the GenBank *R. nigricans* and *R. aeruginea* (fig. 10), one V.I. *R. dissimulans, R. medullata* and *R. sublevispora* (fig. 12). The latter also had an extra fragment of 346bp. One of the V.I. *R. dissimulans* showed the same restriction fragment patterns for all enzymes as the GenBank collection of *R. nigricans 4,* -a closely related European species, the other did not, although these distinctive species were unlikely to have been misidentified. The one fully successful *R. dissimulans* amplification and restriction was from a freshly collected specimen, whereas the others in the *Compactae,* including several unsuccessfully amplified *R. adusta,* had been collected and dried 1-2 years prior to extraction.

Of the *Lactaroides,* the two V.I. *R. brevipes* (fig. 12) a Sau3AI fragments read as 226 for one and 245bp for the second, may represent two or three fragments of 200, 247 and 257bp, as in the Genbank sequences of this species. *Russula* aff. *delica* and/?. *chloroides,* both European species, had similar patterns to those of *R. brevipes* (fig. 10).

Russula cascadensis showed a pattern dissimilar to others in this group and more like that of the European *R. pallidospora.*

Sau3AI gave further differentiation between *R. cerolens, R.* cf. *pectinata* and *R.* cf. *pectinatoides,* in the V.I. *Ingratae,* but showed *R. granulata* with the same overall pattern as that of *R. cerolens.* The three V.I. collections of *R. cerolens* differed slightly in the Sau3AI fragments, and the first two, both collected from the same spot in Royal Roads University but two years apart, also differed in Alul fragment patterns (fig. 12). Shaffer (1972) considered *R. cerolens* the western equivalent *ofR. amoenolens,* which displays different patterns in the two Genbank sequences. The European *R. amoenolens* has two fragments analogous to those of the Genbank *R. pectinata,* but contains an extra cut site not found in the latter in the fragment downstream of these (not shown as they are incomplete). The North American sequence *{amoenolens 2)* is unusual in having a Sau3Al cut site due to a missing base just beyond the ITS4 site, where many other species have a Hinfl cut site, the second V.I. *R. cerolens* shows similarities in the larger fragment sizes. Wickaninnish collections in this group were slightly darker but otherwise morphologically identical to the Royal Roads collections of *R. cerolens,* but had a pattern more like that of *R. pectinata,* a species which had been previously reported from Crescent City in Northern California by Singer but subsequently determined as *R. cerolens* by Shaffer (1972). The collection was originally keyed out to *R. amoenolens* from which it differed only in having more reticulations on the spores, and unfortunately the RFLPs have not unambiguously clarified the identity. Series *Pectinata* consists of species with very slight differences not agreed upon by all authors, and in which Romagnesi (1987) and Shaffer (1972) consider *R. amoenolens* to be *R. sororia* in the sense of Schaeffer (1952) and *R. pectinata* in the sense of Singer (1957). This raises the question of whether differences between sequence data of a given species is due to natural variation or taxonomic confusion between species.

The V.I. *R.* cf. *pectinatoides,* which had an extra fragment of 586bp, which, when added to the other fragments was more than the amplification product, indicating either infragenic variation or incomplete digestion (fig. 12). The V.I. collections of *R. fragrantissima* show variation in fragment size, as do the two Genbank *R. laurocerasi,* due to a cut site in the second sequence that is lacking in the first. The V. I. collections identified as *R. fragrantissima* have several characters intermediate between that species and *R. laurocerasi,* and the variability of Sau3Al restriction fragments in each species does not clarify matters further. The GenBank *R. pallescens* and V.I. *R. farinipes* had similar RFLPs for all enzymes, the similarities and differences between these two species are discussed in the notes following the description in appendix 3.

Amongst the *Heterophyllae* two V.I. species: *R. mustelina* and *R. aeruginea,* had equivalents in the GenBank set (figs. 10 and 12); the former was a confirmed identification, but showed slight fragment size variation between the two extractions of the same basidioma. *Russula aeruginea* had fragment sizes closer to those of the GenBank *R. grisea.* Interestingly, the collections of/?, *aeruginea* from the Clayoquot area appeared to be intermediate between *R. aeruginea,* placed in subsection *Ilicinae* in Sarnari (1998) and *R. stenotricha,* placed in subsection *Griseinae* along with *R. grisea.* Although the morphology did not support the identification of this collection as *R. grisea,* the RFLPs suggested it belongs in *Griseinae* and is more likely *R. stenotricha. Russula medullata,* again in subsection *Griseinae,* had a Sau3AI and Alul pattern like the GenBank *R. aeruginea* but a Hinfl pattern like *R. grisea.* Of the two collections of *R. brunneola* (fig. 12), one had an extra AM cut site, and because of the variation in the

Sau3AI fragments this species could not be assigned to a subsection. Shaffer (1970) commented on the variability of this species, which he had had difficulty in identifying. *Russula* cf. *basifurcata* had a similar RFLP pattern to that of *R. brunneola* and *R. mustelina,* these three species also have the same cuticular micromorphology. The two V.I. *R. crassotunicata* differ in the Sau3AI pattern, the first collection matches those of the Genbank *R. crassotunicata* but differs slightly in one Alul and both Hinfl fragments. The second V.I. collection, labelled *R.* cf. *crassotunicata,* failed to show any Alul digestion products. This collection was morphologically intermediate between *R. crassotunicata* and *R. compacta.* The latter had no full ITS1-ITS2 sequence available through GenBank at the time of writing.

In the subgenus *Russula* subsection *Russula,* the V.I. *R. stuntzii, R- laccata* (in the *R. fragilis* complex), *R. betularum* and three of the *R. silvicola* showed a similar Sau3Al pattern to those of the GenBank/?. aff. *fragilis, R. betularum* and/?, *emetica,* but could not be further differentiated with these three enzymes. The V.I. *R. crenulata* also showed this pattern rather than that of the GenBank *R. ochroleuca,* a superficially similar species, as did the Genbank *R. decolorans* in a different taxonomic group. The three V.I. *R. raoultii* showed slight fragment length variations between them for all three enzymes, and the third collection had the smaller Alul fragment cut down to 124bp compared with the first two collections. The GenBank *R. raoultii* showed two closely sized fragments of 223 and 250bp, more or less analogous to the smaller of the two V.I. fragments of this species. The V.I. collections show a fragment of 326-346bp, which appears to be the end portion of the ITS2 section, only 195bp of this fragment in the Genbank collection were available in the sequence, so comparisons cannot be made. *Russula raoultii* can be difficult to identify with certainty since there are a number of closely related white species separated mainly by spore size, and some of the red species in this group have white forms.

The *R. fragilis* in the Pacific Northwest is very variable in taste and in microscopic but not macroscopic or habitat characters. Collections were made which bore a range of characteristics associated with *R. atropurpurea* such as pileocystidial shape, weakly peppery taste and pure white spore print, through to typical *R. fragilis* characters. The Genbank *R. fragilis* had two closely sized smaller fragments of 213 and 245bp, and a larger one of 338bp. This compared with the first three V.I. collections of this species, which had a smaller fragments ranging from 317 to 260bp, it was difficult to clearly see two separate bands on a gel when they were this close in size. The comparative larger fragments of five of the V.I. collections ranged from 305 to 354bp.

The remaining group on figs. 10 and 12 show the *Sardoninae*. Two V.I. *R.* cf. *fuscorubroid.es*, two *R. queletii* and the *R. cavipes* showed similar Sau3Al fragment patterns, the remaining two *R.* cf. *fuscorubroides* and one *R. queletii* shared similar restriction fragment patterns. In the Genbank *Sardoninae:- R. persicina* to *R. cavipes,* there were fragments in three size ranges of 185-215, 245-266 and 307-338bp, with variation between species and no common pattern. The double band seen in *R. queletii* was not detected as separate fragments in the V.I. collections, however three of them had fragments in the 253-270bp range, close in size to those of the Genbank collection.

In figure 13, the two collections *ofR. americana* var. *modicaspora* nom. prov. , the first from Mount Douglas Park in Victoria, the second from the Clayoquot rainforest, had uncorrelated patterns and may be different species. Whilst neither had RFLPs that completely matched other species in this group, the Mount Douglas collection had most similarity to the GenBank *R. violacea* RFLP pattern. The first V.I. *R. sanguinaria* (from Saturna Island) showed a similar pattern to that of the last V.I. *R. queletii,* although all fragments were slightly smaller, the second had one fragment of similar size at 277bp to one of the Genbank *R. sanguinea* (fig. 11). Unfortunately of the several Genbank sequences *ofR. sanguinea* and *R. rosacea,* most were too short for direct comparison with V.I. RFLPs, and were also quite variable. *Russula rosacea* and *R. sanguinaria* (as *R. sanguinea)* were considered synonymous by Romagnesi (1967), Sarnari (1998) and Thiers (1997b), but there is clearly much variation amongst populations of this species complex.

The majority of the remaining species in figure 13 had a Sau3AI fragment averaging 335bp and a smaller one around 220bp, the latter was also common in the Genbank set as it was within ITS1, which was complete in more sequences man was ITS2, in which the larger fragment occurred. A third fragment was more variable, in some cases being intermediate between the former two fragments, and in others, smaller than the 220 fragment and between 140 and 200bp in the V.I. set, and down to 106bp in the Genbank set (below the level of accurate resolution in the gels used for V.I. collections). In the V. I. set, the smaller two bands could not always be differentiated, and when a single band appeared it may have represented two closely sized fragments.

The next two groups included species in subgenus *Incrustatae* and sections *Paraincrustatae* and *Polychromae* of subgenus *Russula,* which were grouped together on the basis of the phylogenetic analysis (Part 2 of this chapter). *Russula albida* and *R. murrillii* had no GenBank equivalent. *Russula lepida* - a red-capped species, is closely related to the white *R. albida,* and there was a similarity of the Sau3Al overall pattern, but the fragment sizes were all 40-60bp larger in the V.I. *R. albida,* and the Alul patterns differed. The Genbank *Russula olivacea* showed three small Sau3AI fragments in the 115 - 167bp range and one in the 333 - 336bp range. The V. I. *R. olivacea* showed the larger fragment but the smaller ones were not differentiable, showing as a fragment of 195bp. *Russula Integra* showed a broad Hinfl band on the gels between about 360 and 380bp which was close to the size of the Hinfl fragment in the GenBank material, conversely, the Sau3AI digest revealed only a single band corresponding to the GenBank material's double bands at 225 and 257bp.

The V.I. *R. veternosa* collections showed some fragment size variability, mostly in the Alul fragments, but the overall pattern was consistent between collections (fig. 13). The two fragments of the GenBank *R. veternosa* were similar to those of the V.I. collections but were not distinct from those of the GenBank *R. firmula* or *R. amethystina* in the previous group (fig. 11). This is consistent with the morphology, which shows a close relationship of ft *veternosa* to the *Incrustatae.* The V.I. *R. Integra* differed from *R.* cf. *Integra* in fragment sizes for all three enzymes, the latter bearing a closer resemblance to the GenBank *R. velenovskyi,* which differs mainly in cap colour. The Genbank *R. Integra* showed a double Sau3AI fragment of 225 and 257bp where only one of 260bp was detected in the V.I. *R.* cf. *Integra* collections, the first of these also showed a double HinfT band of 360 and 380bp, the smaller of which was comparable to a GenBank fragment.

The GenBank *R. vinosa* and *R. occidentalis* had identical Sau3AI restriction fragments for the first part of the sequence, but the smaller fragment of the V.I. *R. occidentalis* was larger at 251bp than the largest ITS1 fragment in the GenBank set. The

GenBank and V.Ii?. *occidentalis* had a similar sized fragment in the ITS2 region (figs. 11 and 13). *Russula occidentalis* and *R. vinosa* have blackening flesh and a unique reaction to phenol, so are unlikely to be confused with other species.

Interestingly, the position of/?, *occidentalis* among species with incrusted pileal hyphae prompted a re-examination of the cutis with acid fuchsin rather than 5% KOH, whereupon incrustations were found. This was not mentioned in Singers' 1957 description, nor in Grand (1965) or Thiers (1997), but Thiers recognised it as a member of the *Coccineae* Romagn. which contains species with incrustations.

Russula sphagnophila and *R. brunneoviolacea* are related to the *Xerampelinae* according to the analysis in Part II of this chapter, yet they show different overall RFLPs from this group in both the GenBank and V.I. collections, except for two Sau3AI fragments in the V.I. *R.* cf. *brunneoviolacea* (figs. 11 and 13). The V.I. collection of *R. sphagnophila* has fragments that differ by up to 40bp from those of the GenBank collection, yet they have approximately the same relationships between fragment sizes. The two collections of V.I. *R. abietina* have somewhat varied RFLPs and were all from different locations. This species has an ambiguous original description and no GenBank representatives, the RFLP pattern shows similarities with both *R. sphagnophila* and *R. nauseosa,* it is placed among the *Sphagnophilae* because its cuticular pigments are like others in that group (see the notes under this species' description in appendix 3).

The V.I. collections *ofR. xerampelina* and its close relatives *R. elaeodes* and *R. isabelliniceps* show similar patterns for all enzymes, and these match those of the GenBank collections of/?, *xerampelina* (figs. 13 and 11). Another member of this group, *R. semirubra* differed only in the lack of a Sau3AI fragment around 270nt, presumably due to an extra cut site in this piece.

An immature, mild tasting *Russula* with undeveloped spores and flesh that stained blue-green in FeSC>4, was identified as *R. xerampelina* from its RFLP patterns, which were identical with those of a mature collection of this species.

The last group of V.I. species were of the *Tenellae.* The GenBank group also included *R.* cf. *maculata* in the *Urentes,* a species whose taxonomic position was not unambiguously resolved by the phylogenetic analysis in part II of this chapter, but which shows a Sau3AI pattern like those of *R. occidentalis* fig. 11). *Russula aureofulva,* a new species with mild taste and large spores which had some morphological characteristics of both the *Tenellae* and the *Urentes,* and showed most RFLP similarity with the GenBank *R. cuprea,* an acrid species of the *Urentes* with spore morphology and cap colours like those of/?, *aureofulva.* The V.I. *R. cessans* and *R.* cf. *cessans* had a Sau3AI fragment around 600nt and a Hinfl fragment of 249bp that were not cut (fig. 13), in contrast to that of the GenBank *R. cessans* (fig. 11). The *Macowanites* sp. could not be unambiguously identified from its RFLP pattern, the largest Sau3AI fragment appears to be incompletely digested as the fragment sizes add up to a greater length than that of the amplification product. The remaining Sau3AI and Hinfl fragments place this species close to *R. aureofulva.* With the exception of the smaller Hinfl fragments at 160bp, and some variation around the Sau3AI 215bp fragment, the second and third V.I. *R. puellaris* RFLPs are close to those of the GenBank collection. The GenBank sequence was not long enough to assess whether the circa 300bp Sau3AI fragment was present, but the lack of a cut site in the remaining nucleotides and a similar lack in a 28S sequence (AF325315, not shown), suggests this fragment is present. The first V.I. *R. puellaris* (CR021016-11) failed the Sau3AI digestion, and shows more similarity in Hinfl fragments to those of the V.I. and Genbank *R. nauseosa,* and these show different Sau3AI patterns. *Russula puellaris* and *R. nauseosa* can be hard to differentiate as they are closely related and share many characters, differing principally in the degree and extent to which they bruise yellow. The remaining V.I. species labelled with a collection number CR001007-04, an acrid species with incrustations, showed similarity to one of the V.I. *R. abietina,* a mild species lacking incrustations that is in the *Tenellae;* otherwise it had a very generalised pattern that could have placed it in several groups.

Conclusions

The three enzymes functioned overall in a nested division of Russulas, with Hinfl making divisions at roughly the subgeneric level, Alul making further divisions in some but not all groups and Sau3AI subdivided these and in some cases provided divisions not made by AM. Occasionally, as in the *Russula nitida-xerampelina* complex, Hinfl provided a greater resolution than Alul. The three enzymes were not always sufficient to resolve all the identities, however collating information derived from all three enzymes would be expected to give the best resolution of identities. In the higher clades particularly, between-species variation was low and there may not be an enzyme that would differentiate them. In such cases, resolution was limited to group level, but this was useful for confirming relationships between known and query taxa. To increase resolution, more enzymes could be employed, but the time and materials required for each additional restriction have to be weighed against the cost of sequencing, which returns far more information. Infrageneric variation occurred in several clades, some due to restriction-site substitutions; such variation may be explained by geographic separation, and sometimes by uncertainty in identification. In the collections of *R. veternosa,* small variations reflected population differences with the first collection from Breitenbush in the Oregon Cascade mountains, and the second and third from two southern Vancouver Island Douglas-fir forests.

The use of published sequence data to confirm an identification by comparing virtual RFLP patterns with those obtained by amplification and restriction using the enzymes Hinfl, Alul and Sau3AI was most appropriate when full-length sequences were available. Species with several analogous or partially overlapping sequences published were less useful. The gathering of published sequences and the process predicting enzyme recognition sites enabled an overview of the variability in RFLP in a given species or clade, and the origin of that variability. The use of sequences from different collections of a species, as mentioned above, brings a realisation that substitutions of a single base can result in changes in the fragment pattern, which makes identification using RFLP matching sometimes uncertain. Since these enzymes sample such a small portion of the overall sequence any difference can be interpreted as a large degree of divergence. Fragment sizes that changed due to a single site substitution tended to affect Sau3AI more than Alul or Hinfl because several of the recognition sites for Sau3AI were in most variable regions of the target DNAs. With query collections in which the sequence information was not available, adding the smaller fragments to see if they resulted in a missing larger fragment was one way of assessing whether a single extra cut site was a likely cause of the difference. Infrageneric variation in RFLPs was also seen between the pairs of GenBank *R. nigricans* and *R. laurocerasi* in fig. 10, and among the V.I. *R. fragilis* and *R. silvicola.*

Unfortunately, the resolution of bands below 150nt by agarose gel electrophoresis was a problem as incomplete amplification products and small restriction fragments produced a diffuse band. The elimination of the terminal band by using the DNA in the band from the PCR assessment gel brings in more steps and consequently more risks of contamination and sample loss. For many species, this step would not bring further resolution, as it was seen from the GenBank sequences that some of the smaller bands below 120nt were similar among species and originated in the conserved region.

In some species the fragment size distribution was remarkably stable, as in the GenBank and V.I. *R. xerampelina* complex, indicating little genetic variation even between geographically separated populations.

There were collections that had been identified as one particular species until the RFLP pattern showed a non-match either with other V.I. collections or the GenBank representative of that species or group. In these cases the characters of the V.I. collection were reviewed and some characters re-examined, and either the original identification confirmed or other possibilities considered. One such collection was assumed to be *R.* cf. *sanguinaria* but the RFLP pattern suggested otherwise, and on re-examination, this time using acid-fuchsin on the cap cuticle, incrustations were found which placed it as *R.cf. rubra.* This was a validation of this approach.

Vilgalys (2003) commented on the frustrating problem of misidentified sequences in the GenBank and EMBL-EBI databanks and Bridge *et al.* (2003) found up to 20% of published ITS sequences of *Helotiales, Amanita* and *Phoma* had been misidentified. Eberhardt (2002) found a GenBank sequence identified as *R. mairei* that was more likely to have been *R. compacta,* and in the phylogenetic analysis below, two sequences of/?. *amethystina* fell into different clades, one of which (AY061653) consistently appeared on the same branch as *R. firmula.* As some of the collections of Vancouver Island Russulas were difficult to identify either because of variation from the published descriptions or due to the occasional conflicting or confusing description, the same difficulties must exist for other authors, whose concept of a species may also vary. Therefore, when the RFLPs of a collection in hand failed to match those of a published species, or indicated a different species, some judgement was required to assess both the accuracy of the published data (by comparing it with that of sibling species), and the accuracy of the identification of the local collection. That said, the methods outlined above for treatment of published sequence data provided a useful framework into which query species could be placed, and in many cases verified. The method also found three misidentifications in the Vancouver Island collections that were subsequently confirmed as members of a different group. There were some disagreements between GenBank and V.I. RFLPs for a given species, but the local collections agreed with the published descriptions for these species (e.g. *R. cessans).*

Uncovering the reasons for such differences would require sequencing of local collections and comparisons with the GenBank sequences. The use of RFLP comparisons, as described here for identification of the *Russula* partner of ectomycorrhizal root tips, shows that in most cases the level of identification possible is within that of subgenus, sometimes down to subsection, and in rare cases to species level. A matching pattern does not necessarily return the correct identification, nor does a mismatch necessarily indicate a wrong identification. The degree of uncertainty should decrease as the sequence databases grow and anomalies are weeded out or re-examined.

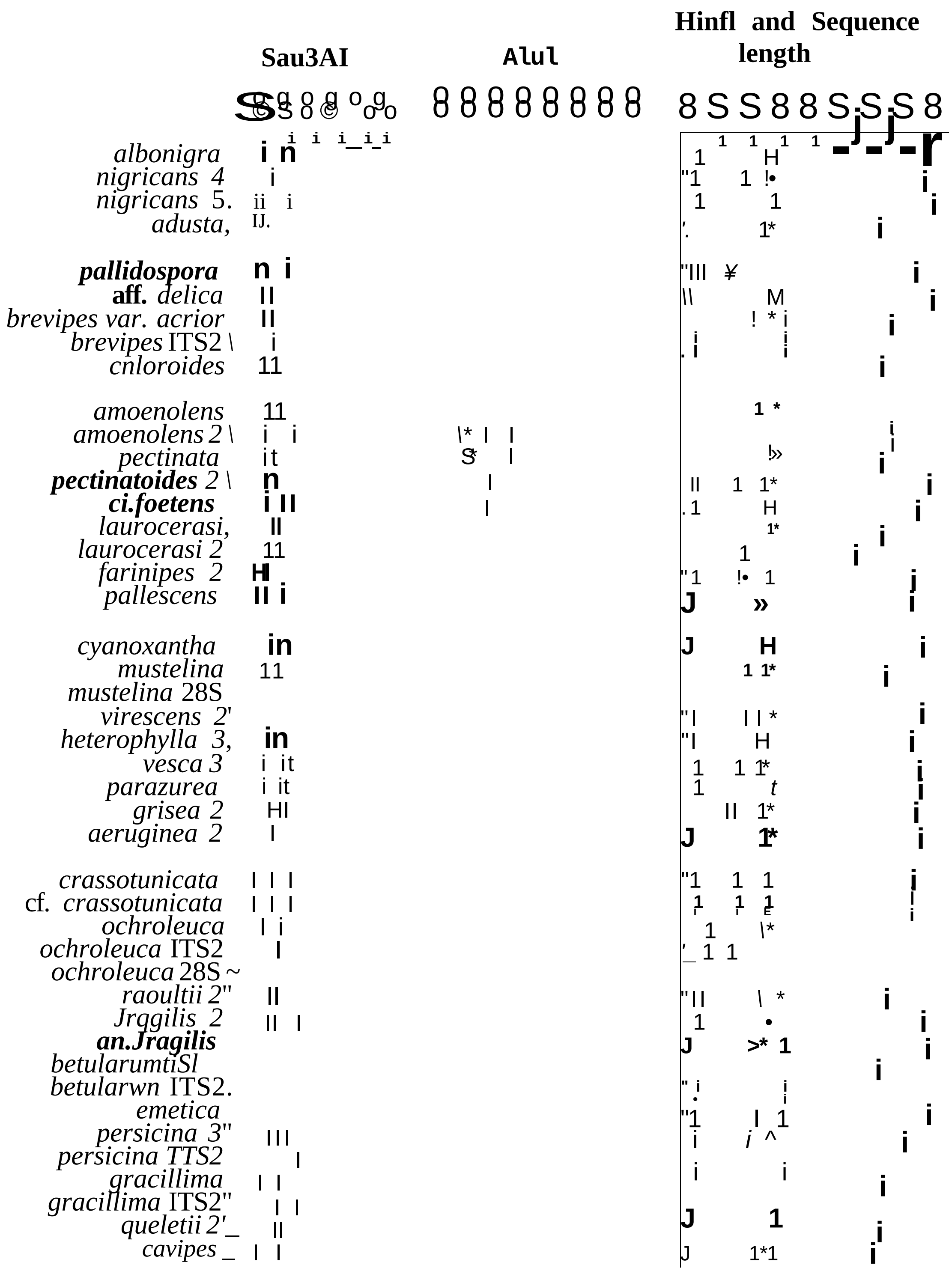


Figure 10 GenBank *Russula* ITS-rDNA virtual RFLP's of the *Compacta, Ingratula, Heterophyllidia, Russula* (subsections *Russula* and *Sardoninae)*. Black bands are complete fragments, grey bands are fragments beginning between the 5' end of the ITS 1-F primer site and the 3' end of the 1TS1 primer site, with a dot indicating the estimated size were they to extend to the ITS 1-F site. Only sequences in bold are full length, running between ITS 1-F (or rrSl)totheendofITS4-B.

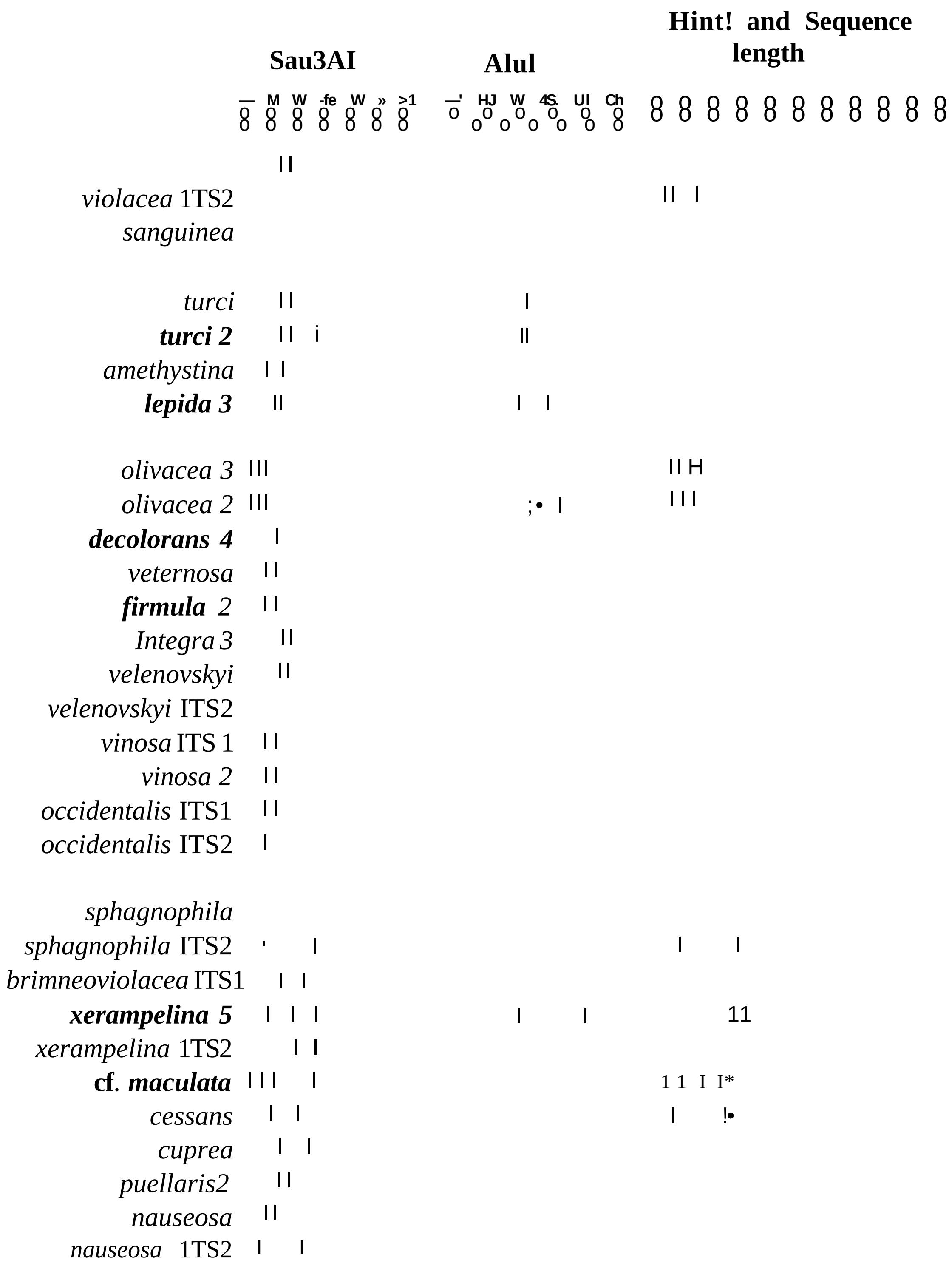
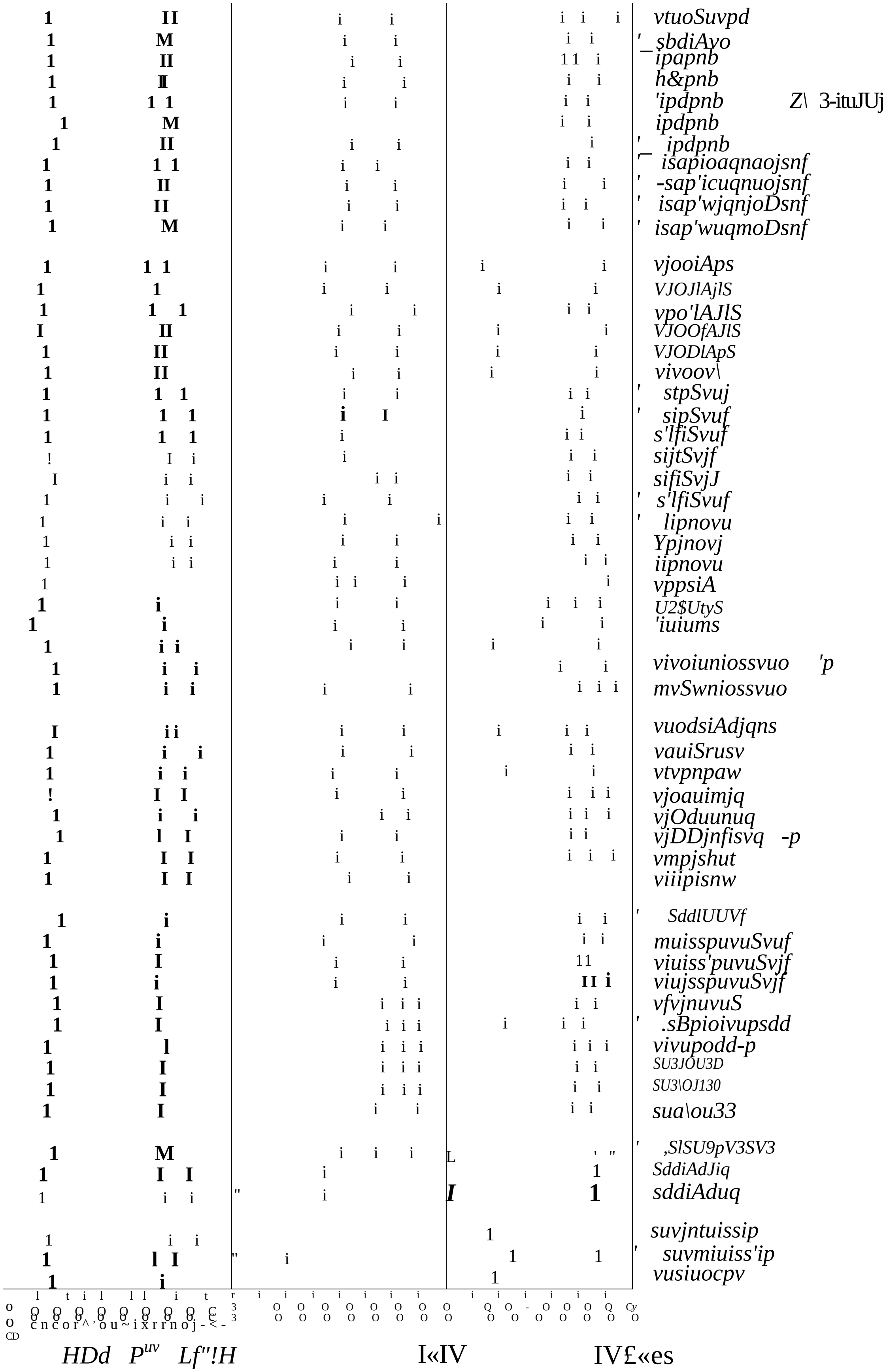


Figure 11 GenBank *Russuia* ITS-rDNA virtual RFLP's of subgenus *Russula* sections *Paraincrustatae, Polychromae* and *Tenellae; Russula* subsections *Violaceinae* and *Urentes,* and subgenus *Incrustatulae.* Black and grey bands, dots and bold type as in fig. 10.

Figure 12 Overleaf, Vancouver Island Russula ITS-rDNA RFLPs: subgenera *Compacta, Ingratula*, *Heterophyllidia, Russula* (subsections *Russula* and *Sardoninae),*

Figure 13 page 80, Vancouver Island Russula ITS-rDNA RFLPs: Subgenus *Russula* sections *Paraincrustatae*, *Polychromae*, *Tenellae* and *Russula* subsections *Urentes, Violaceinae* and *Rubrinae,* and subgenus *Incrustatula*.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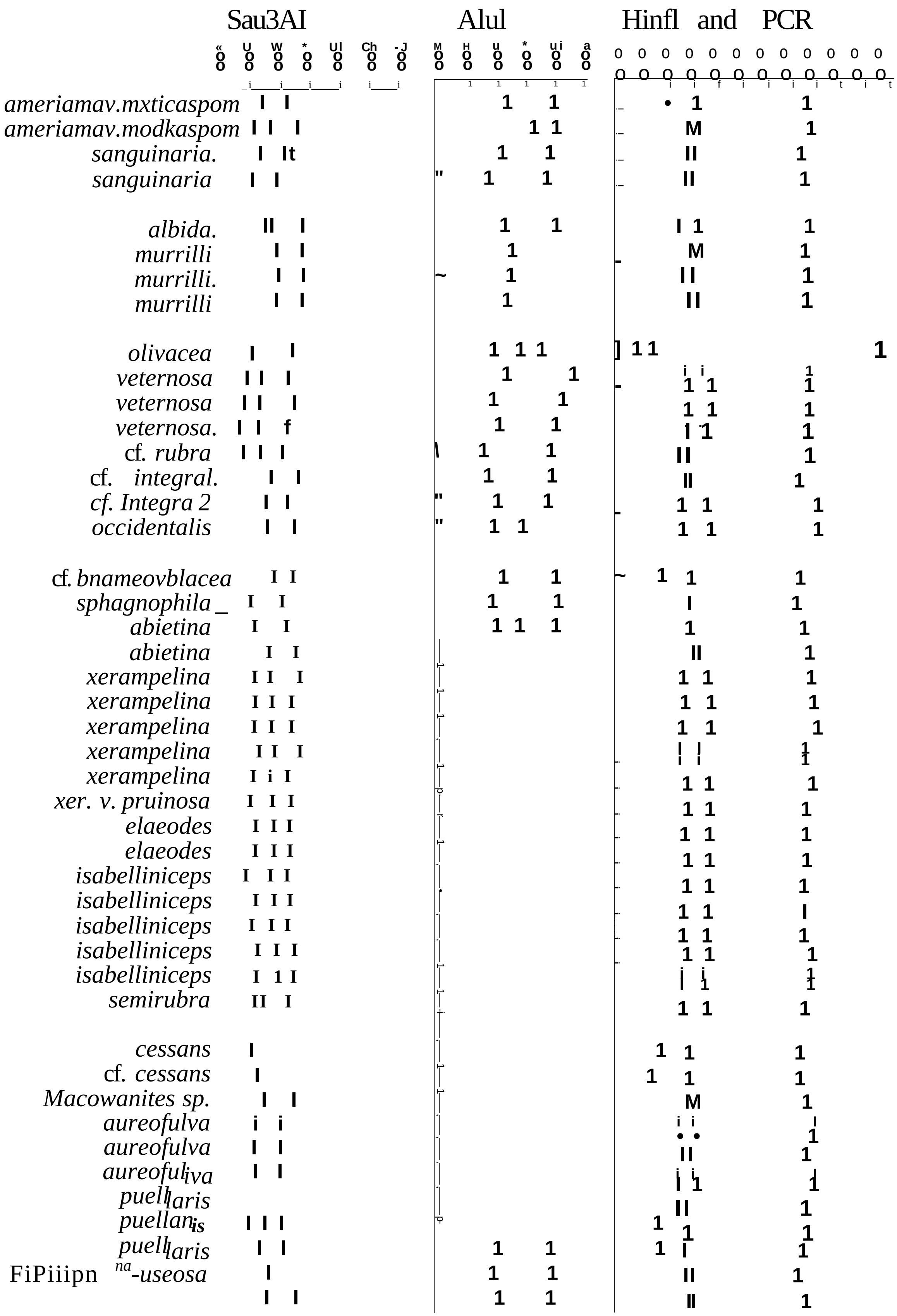
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DNA Analyses Part 2: Generating a phylogenetic tree from previously published sequence data as a basis for establishing the relative taxonomic importance of morphological characters.

Introduction

Classification within the genus *Russula* has in recent years increasingly recognised the importance of microscopic characters such as specialised hyphal structures in the epicutis and the ornamentation of the spores. The work of Romagnesi (1967, 1985) set a standard in classification of Russulas that has formed the basis of subsequent monographs. In more recent works, Romagnesi (1987) upgraded the supersections to subgenera. Bon (1988) returned to the more traditional two subgenera of *Compactae* and *Russula* but otherwise closely followed Romagnesi's classification. Sarnari (1998) maintained six subgenera, but combined most of Romagnesi's subgenera of *Tenellae, Polychromidia, Coccinula and Insidiosula* into *Russula* based on which characters he felt were more important taxonomically.

One of the problems with such a diverse genus is that although many small groups of species may be defined easily by a set of distinctive characteristics, the relationship between groups has not always been clear. Added to this problem are a few species that seem difficult to place with confidence since they possess characters in common with several groups, and these in particular can be confusing to identify. Attempts to clarify the infrageneric relationships of *Russula* by phylogenetic analysis of DNA sequences have been made by Eberardt (2002), Henkel *et al.* (2000) Miller *et al.* (2001), Miller and Buyck (2002), and Shimono *et al.* (2004). The upper clades of these phylogenies in particular show little structure analogous to the classifications of Sarnari (1998) and Romagnesi (1985). The reasons for this may be because little actual evolution has taken place in this piece of DNA within these upper clades, or because the analysis methods used are inadequate for the degree of variation present in the ITS region. Some of the morphological characters were mapped onto these phylogenies to assess their taxonomic correspondence. Eberhardt (2002) mapped taste, spore colour, cap colour, discolouration of flesh, four classes of epicutal hyphae and cystidia, SV reaction, host, and two classes of mycorrhizal type onto a tree of 26 species but found that only the mycorrhizal type corresponded to sequence data. Shimono *et al.* (2004) mapped spore colour and amyloidity of the suprahilar patch onto the LSU-DNA neighbour-joining tree of 95 *Russulaceae* (including 50 *Russula* species) and found spore colour to be related to the degree of apomorphy, and amyloidity to be useful at higher taxonomic levels within the genus. Miller and Buyck (2002), mapped spore colour, taste and presence of incrustations onto their most parsimonious tree, finding general trends with some exceptions from base to top of the tree, and correspondence to clades quite good for the last two features. Morphological and molecular data have also been integrated in estimating phylogenies (Lutzoni and Vilgalys 1995).

DNA sequences hold considerably more evolutionary information than morphological characters, but differences at variable regions of the sequence are of less importance functionally and phylogenetically than those in conserved regions, so the topography of the final tree depends to some extent on the weightings applied to different substitutions in different parts of the genome. Some published analyses have removed parts of the sequence that aligned ambiguously, for example Miller and Buyck (2002), H0iland and Hoist-Jensen (2000), Peinter *et al.* (2001). Although these residues are viewed as 'noise' when the number of sequences is small, as new sequences are added to an analysis, more of this'noise' becomes 'signal'. When many sequences are aligned, fewer of these 'unique to one sequence' sites occur, as pairs and groups with similarities in these sites are more likely. Thus, the more (related) sequences that are used in an alignment, the more sites are phylogenetically useful, and the more nuances of relationships between taxa are uncovered. Miadlikowska *et al.* (2003) formulated a coding system for hypervariable regions and re-integrated them into the dataset, since these regions contained phylogenetically useful information. Phylogenies generated by different authors for the *Russulaceae* and the genus *Russula* using either the LSU or the ITS regions have shown only partial agreement in their topology according to the analysis methods used. Neighbour-joining analyses are distance-based methods of inferring phylogenies, in which a match or mismatch at a site between pairs of sequences is scored. The method is quick but involves very little qualitative assessment of the data. Maximum parsimony analysis is a character-based method that finds the tree topology requiring the fewest changes to explain the data, but there may be several different, equally parsimonious trees. Maximum likelihood again is character based and looks among all possible trees for one that maximises the probability of observing the data by comparing one tree against the next closely related tree and moving on to the better one. The search suffers the risk of returning a suboptimal tree if that tree is "surrounded" by lesser trees, effectively blocking further progress. Eberhardt (2002) used maximum likelihood for her analysis of 62 *Russula* and *Lactarius* LSU-DNA sequences, but as this method requires long computing times it is used less often than the other two methods.

As the recognition of useful taxonomic characters rests upon a clear, unambiguous phylogeny, consideration was given to analytical methods other than those mentioned above, which purport to give a more realistic model of the evolutionary tree. One maximum likelihood-based method is Bayesian analysis, which has the power of maximum-likelihood but reduces the pitfall of getting stuck at a suboptimal tree.

Bayesian theory is named after its originator, the Reverend David Bayes (1702-1761), an English minister and mathematician. His theorem is summed up by von Baeyer (2003) as the new probability (the posterior probability) that something is true is based on the prior probability multiplied by a factor, greater or less than one, which is derived from new information. In a sense, the algorithm "learns" from experience. In Bayesian analyses of phylogenetic trees, any given proposed tree begins with an estimated probability that it is true based on the information to-date. One or more of the nodes are perturbed by a given amount (a tuning parameter) and the new tree examined for likelihood. If it is an improvement (i.e., fewer evolutionary steps are required), the factor to be multiplied with the prior probability, the likelihood, will be >1 and the new tree accepted together with the new probability, the posterior probability. This process may continue until the probability reaches a steady state beyond which no further improvements occur, termed convergence. This chain of successive tree searches with the updating of probability as more information is discovered is referred to as the Markov chain Monte Carlo (MCMC) (Huelsenbeck *et al.* 2001). Archibald *et al.* (2003) and von Baeyer (2003) give a non-technical explanation of Bayesian analysis methods, Huelsenbeck and Ronquist, (2001), Huelsenbeck *et al.* (2002) and Larget and Simon (1999) give a fuller, technical explanation of Bayesian methods and the MCMC chain as it pertains to phylogenetic inferences.

One of the main advantages of Bayesian phylogenetic inference methods is that they are computationally more efficient than standard maximum likelihood with bootstrapping methods, such that sets of more than 100 sequences can be analysed in several hours rather than days or weeks. When the number of cycles is sufficient to reach convergence, all trees sampled after the convergence point (when the log-log-likelihood values reach a plateau) can be used towards a consensus tree. Bootstrapping is not necessary, since the tree sampling from along the converged log-log-likelihood plateau performs a similar statistical function; in the consensus tree the branch-node scores indicate the number of trees in which the species leafwards of the node occurred, and posterior probabilities can be assigned to these figures. The difference between bootstrapping and tree-sampling is that in the former, a subset of the alignment data is sampled for each bootstrap cycle, whereas the MCMC chain always uses the full data, making it more sensitive to errors in the alignment since they would be incorporated into every cycle (Huelsenbeck *et al.* 2002).

Two currently available programs that run Bayesian analyses are MrBayes (Huelsenbeck and Ronquist, 2001), which can run several (usually four) MCMC chains simultaneously, and BAMBE (Simon and Larget, 2000), which runs one Metropolis-Hastings MCMC chain but which samples from a wider area. Hall (2001) gives a brief but useful summary of the MrBayes program with explanations on the output files and on determining parameters and the length of the run; information also relevant to the BAMBE program. Both programs can be run on an ordinary PC, but longer runs take several hours and stability can be a problem. The Pasteur Institute currently runs a BAMBE server on the Internet which accepts aligned sequence files, runs a Bayesian analysis according to user-defined parameters and returns the results within a few days.

A Bayesian phylogenetic analysis of previously published sequences was decided upon in an attempt to obtain a clearly defined clade structure of mainly temperate zone Russulas that may subsequently be used as a framework to place local species related to those in the analysis. Morphological characters to be mapped onto the tree included those represented in some form in all species and which were expected to form a gradient from basal to upper clades. Other characters were examined for clade specificity.

Methods

Sequence selection and treatment

A search of the Genbank database returned 365 entries for the ITS region of *Russula* species and the EMBL-EBI database returned 213 entries. These two organisations collaborate with one another so many of the entries are identical, however, the two searches together returned a few sequences not found by searching only one site. Some of these sequences were downloaded and opened in a sequence editing program (BioEdit, Hall 1999). Sequences were trimmed at the 5' end to a common starting motif (C ATT AT), and at the 3' end to a common ending motif (CATATCAAT). Shorter sequences which were missing data from either end were supplemented by data from 18 S or 28 S sequences of the same species when available. Sequences without either ITS1 or ITS2 were not used in the final analysis.

An initial alignment was conducted with ClustalW (Thompson *et al.* 1994), running on the EMBL-EBI site, using a gap open penalty of 10, a gap extension penalty of 0.05 and equal weighting for all nucleotides. The alignment was returned to the BioEdit program where duplicate sequences were removed and the two sequences of *Russula olivacea* had the large insertion of around 250bp in ITS 1 trimmed, leaving a short remnant of 4nt. Treatment of long insertions that do not appear in the rest of the sequences being aligned have been excluded in the cases of *Boletus edulis* by Shimono *et al.* (2004) and of *R. olivaceae* by Eberhardt (2002). Miller (2002) coded the insertion as a fifth base.

The remaining sequences were re-aligned with ClustalX (Thompson *et al.* 1997) using a gap opening penalty of 25 and a gap extension penalty of 0.05 for pairwise and multiple alignment parameters. A low gap extension penalty was chosen because in the variable regions insertions were often of repeated units such as multiples of A, T, or CT. These short repeats may be due to separate single events at a site, but were more likely a single insertion of multiple residues at a single site. A higher gap opening penalty reduced spurious gaps, but penalties higher than 25 for the whole sequence tended to result in misalignment. Poorly aligned sections were realigned using a lower gap opening penalty for those residues only. The alignment was transferred to BioEdit and fine-tuned by hand. One part of the alignment in ITS2 was very difficult to align satisfactorily because several different homologous sequence patterns were seen in that region. An initial neighbour-joining analysis with 1000 bootstraps for several variations of this alignment were compared, including one of the hypervariable section only, and it was found to give a clade structure similar to that derived from the whole sequence. As gap-rich regions can hold useful information (Miadlikowska *et al.* 2003), this part of the sequence was retained but not recoded.

The dataset still contained two or more non-identical sequences for nine of the species, and in the comparison of the aforementioned neighbour-joining trees, those consistently paired on a terminal branch had only one representative retained, preference being given to the one with fewest ambiguous characters and those derived from basidiomata rather than ectomycorrhizae. The two *R. olivacea,* two *R. xerampelina* and two *R. vesca* were retained to check constancy throughout the analyses. Two *R. amethystina* were retained, as these fell into quite different clades and were only 88.8% similar. *R. pallidispora* and *R. littoralis* were identical to one another, as were *R. cremoricolor* (AJ277910) and *R. raoultii* 2 (AY061712) and the first of each pair was retained. There remained 108 *Russula* sequences and a *Gymnomyces gilkeyii* which were re-aligned and fine-tuned as previously described, but with a gap-extension penalty of 5. The two outgroup sequences of *Albatrellus flettii* and *Gloeocystidiellum aculeatum.* were added to the alignment.

The final alignment had 870 sites, of which 647 were unique. Sites 1 to 6,286 to 445 (the 5.8S subunit) and 824 to 870 (the start of the 28S subunit) were highly conserved. Sequence similarities ranged from 71 % to 98% between pairs of Russulas and from 47 to 74% for pairs with an outgroup.

Bayesian analysis

The aligned sequences were categorised so that the parameter values would develop independently during analysis for differently conserved parts of the sequences. The three categories were; one for ITS1, the second for all conserved sites in the 5.8 S subunit and the beginning of the 28S subunit, and a third for ITS2. The alignment was next subjected to a number of short pre-analysis runs in the BAMBE program following the procedures outlined in the program documentation (Simon and Larget, 2000). The aim of these preliminary runs was to determine the starting and tuning parameters and an appropriate number of cycles and the burn-in. The burn-in cycles are perturbed by a global algorithm, in which a change can affect the topography of the whole tree. These initial burn-in trees were discarded from further analyses since they were at a coarse stage of resolution, the main-run trees begin at the last burn-in topology with perturbation confined to a local algorithm which only affects the topography of one branch-pair at a time, thus refining the resolution of the trees until they converge within a narrow range of likelihoods.

Huelsenbeck *et al.* (2002) comment that Bayesian analysis methods are sensitive to chosen prior distributions unless the MCMC chain is run long enough. To see if this was a problem, two sets of 600,000 cycles with a 3,000 cycle burn-in were run, each from a different seed number and starting the first using the BAMBE program default tuning parameters and the second using the calculated tuning parameters and using the final kappa and theta values from the first run as initial values for the second. These returned log-log likelihoods of -17602 and -17594 respectively with a plateau reached about half way through the cycles, indicating that prior conditions were not biasing the outcome. The overall tree topologies, assessed visually, were very similar. It should be mentioned that the likelihood plateau does not reach total stasis, but rather the values generally move up and down within a range of about 25-40 points, so a stable, trendless range is considered a plateau (Simon and Larget, 2000).

Hall (2001) recommends a run of about five times the number required to reach convergence, which called for a run of 1,500,000 cycles with local tuning with a burn-in of 300,000 with global tuning, and starting with the following empirically determined parameters: initial kappa = 3.8855, 5.1502, 3.7229; initial theta = 1.2423, 0.0429, 1.3414; global tune = 0.01, kappa tune = 0.16, theta tune = 3000, pi tune = 6000, local tune = 0.19. The HKY85 with molecular clock likelihood model was used (Hasegawa *et al.* 1985), with parameter updating every cycle, a tune interval of 200 cycles, and tree sampling every 200. This run was well into the plateau by cycle 300,000, but the first 1,500 trees sampled up to this point were discarded out of a total of 7,500 trees sampled from the full run. The final log-log likelihood was -17578.6, with the plateau range stabilizing about 20 points higher than that of the short runs. The last 6,000 trees were summarized using Consense in the PHYLIP package version 3.6 (Felsenstein 1989, 2004) after minor reformatting of the topology file, and using the extended majority rule method to generate the consensus tree. Unfortunately the BAMBE program's own summarize function could not deal with files of 111 taxa, and branch lengths were stored only for the initial and final trees and not with each tree in the topology file. As the branch lengths on the consensus tree do not relate to evolutionary distance, this and the final tree should be considered in concert. Trees were visualized in Tree View (Page, 1996).

Re-alignment and analysis of upper clades

A subset consisting of 54 Russulas forming the upper clades of the majority rule tree, which showed a few lower scoring branches, were realigned along with *Albatrellus flettii* as previously described. The alignment was then stripped of all fully conserved sites, leaving only variable sites, the intention being to exaggerate the differences to help resolve weakly resolved clade structures derived from the full data. The final alignment was of 530 sites of which 481 were unique. The analysis procedure on this alignment was the same as that for the full dataset, with the exception that all sites were treated as one category. In four short preliminary runs of 600,000 the final log-log-likelihoods ranged only between -7929 and -7943, and that of the 1,500,000 cycle run was -7938.8. The empirically determined initial parameters for the 1,500,000 cycle run were: initial kappa = 4.2070, initial theta=l, global tune = 0.01, kappa tune = 0.25, theta tune = 2000, pi tune = 4000 and local tune = 0.1900, burn-in, sampling, and consensus analysis as for the full set.

The sequence details and parameters used in the Bayesian analysis for the *Russula* phylogenetic analyses are detailed in appendix 2.

Mapping characters

Characters and character states, mostly of microscopic characters, were tabulated in clade order (Table 6). This table does not include the major macroscopic group - defining suites of morphological characters by which the lower clades in particular are differentiated. These are mentioned in the text where necessary but the reader is referred to monographs of *Russula* for full circumscriptions of taxonomic groups. The categorization of pileocystidial shape, tips, and septation is shown in figure 23 in Appendix 3. Pileocystidia are commonly included in species descriptions, but have not previously been categorised in a way suitable for determining the taxonomic position of a species.

Most of the character information was found in Bon (1988), Romagnesi (1985), Sarnari (1998) and Shaffer (1964,1970, 1972). Not all the information was available, so some fields have been left blank. In the pileocystidia columns, for clarity, zeros have been filled in only for those species with no pileocystidia, blanks in other rows do mean missing data if there is at least one category filled in. The epicutis terminal hyphae column contains information on features noted in the literature, and which is of varying taxonomic value: for instance the vacuolar pigment mentioned for *Russula emetica* is not unique, and most species have this to some degree, but it was very noticeable to the original author.

Results

Trees

The final tree of the Bayesian analysis of 111 *Russula* and outgroup taxa is shown in two halves in figures 14 and 15 and in radial format in figure 19, both of which express the branch length in terms of substitutions per site. This tree is one of more than 1,200,000 probable trees created after the algorithm converged, each of which has some variation in branching order and length. The radial format of the final tree (fig. 19) very clearly shows the separation between clades, and when this figure was adapted for a lecture by colour coding the branches according to taste of species, and adding the spore colours, die clade specificity of these characters was striking. Unfortunately the cap colours (not shown here) were rarely clade-specific. The clades' relationship to the classification of Sarnari (1998) has been indicated on the radial tree in place of the species names, and shows good correlation with taxonomic divisions in all but the lower left clades. The extended majority rule consensus tree is shown in two halves in figures 16 and 17 and includes scores for the frequency among the trees in which the species to the right of the node appeared, expressed as a percentage. Ten main clades have also been indicated, with 7,8,9 and 10 corresponding to the clades of mixed taxonomy at the lower left of the radial tree.

The consensus tree of the minimised sequences of the 54 upper clade Russulas and an *Albatrellus fletti* as outgroup is shown in figure 18, and also bears node scores.

The full tree showed high support, 70-100%, for most of the major clades and terminal clades. Scores as low as 40% were found within clade 10 and in the branching between clades 8, 9 and 10, probably due to the inclusion of/?, *maculata,* whose taxonomic position differed in several preliminary analyses. The minimalised alignment tree had the same suite of species in most of the terminal clades but these were grouped differently, with very low scores for these groupings. The *R. persicina* to *R. lepida* clade now included *R. maculata* which again lowered all the branch scores. *Russula olivacea* appeared basal to the clade containing *R. xerampelina,* with 99% of trees having this configuration. All told, this minimalizing method did not clarify relationships, since the tree topologies evidently were more variable.

Clade and character relationships

Spores: The amyloid reaction of the suprahilar patch is a major clade-specific trait, dividing the genus into two clade groups: 1, 3 and 4 (inamyloid or very weakly amyloid), from 2 and 5 to 10, in which this patch is strongly amyloid. The size of spores was considered (not shown in Table 6) but was found to be more or less species-specific, with the exception that clades 8d, 9 and 10a contained many species with comparatively large spores of 10 microns or more in length.

Spore colour: In general there was a gradient with palest colours in the lower clades and darkest in the upper clades. Spore colour was sub-clade specific for clade 5, dividing the lower two sub-clades from the upper one. In clades 5c to 10 spore colour was a within-clade, species-specific character.

Spore ornamentation: In general wart height increased from lower to upper clades but with numerous exceptions. The degree of reticulation divided clade 4 and 5 into subclades, while in other clades this character was species-specific.

Pileocystidia: Overall, there was a gradient from bottom to top of the phylogram with more capitate-strangulate cystidia (type 2) at the base of the tree and fewer occurrences towards the top. Septation and long, cylindrical cystidia with obtuse tips (type 5) increased towards the top of the phylogram. Most species had two or three cystidial types but the co-occurrence of types 2 and 5 were most frequent in clade 5.

Type 1, tapering and not or barely SV+, was clade-specific for clade 3b. The same shape but without the same SV reactions occurred in *R. melitodes* but this species also had other pileocystidial shapes.

Type 2, with capitate to strangulated tips, was loosely major-clade specific, occurring most often in clades 1 to 5 but also in clade 9. The occurrence of this type alone was species-specific within several clades.

Types 3 and 4, clavate with an obtuse tip, were not clade-specific, but a preponderance of type 3 in a cutis is more species-specific.

Type 5, long, cylindrical, with obtuse tips, was mainly clade-specific to clades 5 b,c and d and clade 10, this last clade all with multi-septate versions, a form more species-specific in clade 5. Occasional species-specific occurrences of non-septate to multi-septate type 5 pileocystidia were in other clades.

Type 6, with diverticulae, was clade-specific for clades lb and 10.

Type 7, incrusted pileocystidia, was clade-specific for clades 7 and 8 but did not define a subclade. Within these clades, and for *R. insignis* in clade 3, this character was species-specific.

Incrustations in the epicutis, usually but not exclusively associated with fucshin positive primordial hyphae, is clade specific, occurring in clades 7 and 8, and in *R. camarophylla* of clade 1, *R. pulverolenta* in clade 3 and *R. ochroleuca* of clade 5. The incrustations of*R. pulverolenta* are not analogous to those of clades 7 and 8, being pigmented and non-soluble in KOH.

Sulphovanillin reaction of pileocystidia: The basal clades 1 to 4 c generally had a null to weak reaction of the pileocystidia to sulphovanillin, appearing light grey or colourless, in some cases with small darker granules in the cytoplasm. Clade 4d of the *Heterophyllae* had weakly to positively reacting species. Clades 5 and 10 consisted of species with a positive SV reaction in which the pileocystidial contents stain purple to black. Clade 7 consisted of SV- negative species, but the tissues in general often stain deep pink or red in this reagent, a reaction not seen in the lower SV-negative clades. Clade 8 and 9 were mixed, with some negative or weakly staining species and some, particularly the peppery species in 8c, with a positive reaction. A positive SV reaction was frequently linked to a peppery taste, but these two characters could also be independent of one another.

Epicutal hyphae: The epicutal hyphae in most species in the upper clades tend to be interwoven, thin-walled, generative hyphae embedded in a gelatinous matrix, with tips often similar in shape to those of the pileocystidia, but smaller. Ampullate hyphal tips occurred in clades 8, 9 and 10: these have an inflated subapical cell and a tapered to capilliform apical cell, and sometimes this shape is formed by the terminal cell alone. Chains of inflated cells, or hyphae inflated between frequent septa so that they appear articulated like the leg of a crab, occurred mainly in the basal clades 1 and 4.

Macroscopic characters

Bruising: The tendency of the flesh to turn grey to black, sometimes with a red interphase, was clade-specific for clade lb and species-specific within clade 8. Weaker greying reactions occurred in clade 5 and were species-specific.

Yellow bruising was common in clades 5e, 9 and 10 and was species-specific within these clades: after the yellow stage the braised tissue turns brown in clade 9 species and in several others. Yellowing species occurred sporadically in clade 8.

Clades 2, 3 and 4 were composed of species bruising brown (a more reddish brown in clade 3). This character was also found sporadically in clades 6-10 but rarely in clade5: it is species-specific for these clades.

The waterlogged effect, in which the tissues of the whole stipe appear greyish or yellow-grey as if waterlogged in age was common in clade 5a and b and (from personal observation,) in 10b.

Taste: While peppery and mild species were found throughout the genus, some clades were specific for one character state. Clade 5 contained entirely peppery species, clades 4, 7 and 9 normally mild species, and clade 8 predominantly mild species, in which case pepperiness was species-specific. Other clades were mixed for this character.

Odours: This property is subjective, since people's sensitivity to different aromatic components varies, and the age and condition of the basidioma affects the odours given off. Unfortunately the chemical compounds responsible for the odours are known in only a few cases. However, there were both clade-specific and phylogenetic gradient properties noticed in *Russula* odours. Fruity or coconut odours, sometimes mixed with pelargonium, were common in the upper clades from 5 to 10 and also in clade 2, with the most frequent notations of this odour occurring in clade 5. In fact, 75-80% of peppery species had odours noted in the literature, compared with 62-70% of mild species. The odour of shellfish (trimethylamine) was specific for the subclade containing *R. xerampelina.* Honey or gingerbread odours were found in clade 8 and once in clade 10, and the basal clades 1 and 4 contained cheese or wine-barrel odours. Clade 3, the *Ingratae*, has long been defined in part by strong complex odours and these include benzaldehyde (almond), spermatic or bleach-like, rubbery and Jerusalem artichoke smells, which are rarely found outside of this group and then much weaker. In the upper clades (5 - 10), stewed apple, coconut or pelargonium odours appear to be plesiomorphic and honey, iodoform and menthol a more advanced character.

Conclusions and Discussion

Most useful characters

The characters mapped onto the clade structure represent only a few of the many that are recorded and used in systematics, and some groups, particularly the basal clades, are sufficiently distinctive to be segregated on macroscopic characters alone. These distinguishing characters have not been included in Table 6, but the main ones are, in brief: Clades 1, 2 and 3 lack bright coloured pigments, 1 and 2 have hard but brittle flesh, and fairly regularly spaced free subgills between the gills, clade 1 bruises grey to black, some also with a red phase first. Clade 3 members have a glutinous cap cuticle with brown pigments associated with the cell walls, strongly striate to tuberculate cap margins, regular lenticular cavities in the stipe and usually strong complex odours encompassing spermatic, rubbery, benzaldehyde and other nuances. Clade 4 contains the *Heterophyllae* which are the only taxa in this lower half of the tree to possess red, yellow and blue pigments in their cuticle, non-cavitate stipe and usually firm but elastic flesh.

Members of this group can be confused with the upper clades without microscopic examination or chromatographic pigment analysis.

The upper clades have fewer clade-specific macroscopic characters which unambiguously place them in a group, and as can be seen, some clades incorporated species from several taxa. That the structure of the last three clades showed much less segregation and consistent branching order than other clades indicates a more recent separation between groups. This is particularly apparent in figure 13.

Those characters that determine the position of a species relative to the phylogeny presented here were either clade-specific, species specific, or distributed along a gradient. Obviously the specific characters can be used to reach an identification, at least of a clade. The gradient characters including spore colour, pileocystidial shape and septation and, to a lesser degree, the braising reaction and odours, can be used to estimate the position and probable clade of a species that is less easy to identify and place in a traditional classification.

Ranking these characters in order of taxonomic usefulness is difficult because they tend to co-vary. However, following the major division with the amyloidity of the suprahilar patch, the pileocystidia and epicutal hyphal types showed a more consistent relationship to overall clade order than spore colour or taste. Spore ornamentation was generally the least clade-order related character of those considered.

Anomalous clade members

Some species had characters seemingly anomalous in relation to those in the rest of their clade. *Russula ochroleuca* fell amongst subsection *Russula* with *R. atropurpurea* and *R. viscida* where its position was well supported, appearing in 86% of trees, and in agreement with the findings of Eberhardt (2002) and Miller and Buyck (2002). This suggests that the incrustations (of yellow pigment) are of the same origin and type as is found in the *Ingratae* in species like *R. pulverulenta.* This species was placed in *Felleinae* in Bon (1987), then in subsection *Ochroleucinae* amongst other incrusted subsections in Bon (1988).

hi this analysis *Russula viscida* was not in the same group as *Russula melliolens* in the *Melliontinae* of Romagnesi (1967). Sarnari (1998) created Section *Viscidinae* in subgenus *Russula* to house this species. The inclusion of *R. viscida* in the clade with *R. stuntzii* was only supported in 50% of trees and morphological characters such as dermatocystidia with capitate ends as well as the long, cylindrical, multi-septate *"Tenellae"* type pileocystidia do not clarify its position. Moreover, the position of ft *melliolens* amongst the *Tenellae* and *Urentinae* only occurred in 40% of trees, so the taxonomy of neither species was resolved.

In clade 8, *R. veternosa, R. firmula* and *R. californiensis* are peppery, non-incrusted species amid mild, incrusted species. This clade was well supported in both the minimised sequence analysis and the full analysis. *Russula californiensis* has the greying flesh and colouration of/?, *decolorans* in this clade. *Russula firmula* and *R. veternosa* are in separate series within subsection *Urentes,* as is *R. maculata* (Sarnari 1998). This sub-clade should be investigated further, perhaps with more collections being sequenced and compared, before making any changes to the classification.

Russula messapica fell amongst the *Tenellae,* validating Sarnari's (1998) placement of this species near the *Tenellae,* but erecting a new section; the *Messapicae.* This veiled species was considered close to the *Ingratae* in Courtecuisse and Duhem (1995). *Russula Solaris,* also in this clade, has been previously placed close to *R. raoultii* (Bon, 1988, Sarnari, 1998, Romagnesi, 1967), but its strong yellow bruising, diverticulate pileocystidia and yellow spores are in keeping with the *Tenellae* of clade 10.

General taxonomic observations and recommendations based on these analyses

The basal clades reflect the existing classification, beginning anticlockwise from the outgroup, subgenus *Compactae,* and its subdivision into *Archeinae, Compactae* and *Lactaroides* (fig. 19). There was 95% consensus of this clade structure, which is in contrast to that of Miller and Buyck (2002) who found the *Lactarioides* to be basal to the *Heterophyllidia* and *Ingratae,* and, in agreement with Shimono *et al.* (2004) the *Compactae* to be basal to the remaining upper *Russula* clades. The amyloid patch on the spores would support this position. Clearly the relative positions of these groups was sensitive to the analysis methods and choice of other taxa.

The clade of subgenus *Ingratae* was subdivided into the groups corresponding to series *Foetens* and *Pectinata,* suggesting these groups could be raised to section level.

The inclusion of/?, *cyanoxantha* as basal to this clade rather than *Heterophyllidia* in agreement with Miller and Buyck (2002) is indicative of the evolutionary roots of the *Ingratae.* Its precise position may not be quite accurate in this analysis, in Shimono *et al.* (2004) the LSU showed a clade of *R. cyanoxantha* and *R. cutefracta* as basal to the *Ingratae, Heterophylla* and *Amoenula,* as did Eberhardt (2002).

Russula fellea allied with the *Russula* clade in this analysis and in that of Miller and Buyck (2002), and Eberhardt (2002) for both US and LSU rDNA, and Shimono (2004), a position recognised in Bon (1988) and supported by the morphological character of an amyloid suprahilar patch on the spores.

Clade 4 contained the *Heterophyllae* and showed the white-spored species branching off before the yellow-spored species, with a rough correspondence to the subsections.

In the upper clades, the first branch included the white and mostly lighter yellow spored peppery species, which contains the type for subgenus *Russula: R. emetica.* This well-defined clade included section *Russula* subsections *Consobrinae, Russula,* and *Sardoninae* but excluded *Violaceinae, Urentes* and *Rubrinae. la* Miller and Buyck's (2002) analysis, this clade was also seen, and included similar sub-clades but with some differences in the branching order. As this clade is so clear, the subgenus *Russula* should be redefined to exclude groups not in this clade in order to make it monophyletic.

A small clade of three species, *R. rosacea, R. violacea* and *R. aurata,* occurred in 98% of trees in the full analysis and 72% in the minimalised analysis. This found a parallel in the analysis of large subunit rDNA by Shimono *et al.* (2004), who found a small separate clade with *R. rosacea, R. aurea* (synonymous with *R. aurata)* and two of three *R. flavida,* all collections from Japan. Their collections of/?, *violacea* and *R. sanguinea,* which is considered a synonym of/?, *rosacea,* fell into the clade with the remaining members of subgenus *Russula.* These species do not seem to have morphological characters in common, so the clumping could be due to long branch attraction rather man a real relationship.

In recent classifications, the incrusted species are divided into two subgenera based on the presence or absence of pileocystidia. In agreement with Miller and Buyck (2002), this Bayesian analyses showed two mixed clades of incrusted species from both subgenera, indicating that in this group at least, the presence of pileocystidia was secondary to the possession of incrustations and/or primordial hyphae. The subgenus *Incrustatula* needs to be expanded to accommodate section *Paraincrustatae* Sarnari and incrusted species and groups from section *Polychromae* (Maire) Sarnari, presently in subgenus *Russula.* The Pacific northwestern species *R. occidentalis* was included in clade 8, which also held other grey-bruising species such as *R. decolorans.* An examination of cap cuticles of dried local collections, after staining with fuchsin and mounting in 5% HC1 showed that they possessed incrusted hyphae, a character not mentioned in any of the descriptions. Thiers (1997) placed it in subsection *Decolorantinae* of section *Coccineae,* subgenus *Russula,* (following Romagnesi, 1967), a section which does contain other incrusted species.

The *Xerampelinae* which make up part of clade 9 have some plesiomorphic characters, the pileocystidia, normally very sparse, are generally aseptate, often with capitate ends, and SV negative. Type 5 pileocystidia are not found. The green reaction with FeS04 is also seen in *R. cyanoxantha* and close relatives. In Miller and Buyck's (2002) analysis this clade branched off earlier, becoming basal to the incrusted species and all clades above, corresponding to clades 7, 8 and 10 in the present analysis.

The grouping of *Russula olivacea* in the clade with *R. xerampelina* in the minimised sequence tree, as in Romagnesi's 1967 classification, and its grouping (in only 63% of trees), in the neighbouring clade with *R. caerulea,* basal to several species with incrusted pileal hyphae as in Sarnari's 1998 classification based on the presence of primordial hyphae, shows that either classification could be right.

Clade 10 brought together yellow-spored, often yellow bruising species with long multi-septate pileocystidia, also in some species diverticulate pileocystidia, which in this group had taxonomic precedence over taste.

Clades 7 to 10 did not fall conveniently into two distinct clades of incrusted and non-incrusted species and so any reclassification of these groups has to be either phylogenetically correct but counter-intuitive, or imperfect but usable. Perhaps the better alternative would be to continue investigations into these terminal clades in pursuit of confirmation or correction, then erect taxa to house them. Henkel *et al.* (2000) and Buyck (1995) comment on the need to make a more fully integrated phylogeny for the

Russulas by including the many diverse species found in the tropics, which have been relatively little studied compared with temperate zone Russulas. These may shed more light on the relative importance of various characters and clarify some of the clade structures.

Character variability - hypotheses on developmental processes.

Characters found in basal clades may reappear in upper clades and the genetic mechanisms governing the formation of a given character and its evolution are not fully understood. Continuous characters, such as spore colour, pepperiness and density of each pigment in the cuticle tend to be of a similar nature within a clade, but the exceptions give a clue as to how these characters develop. One hypothesis is of a system whereby a set of genes code for the default or plesiomorphic character, perhaps an aseptate form of pileocystidia with strangulated tip, with other genes modifying the growth and form of these as they develop. In any cap cuticle it is possible to find several cystidial types, although one usually predominates, indicating that several different versions of the modifying genes may exist in one thallus. Spore colours in the sister genus *Lactarius* range from white to light orange, indicating that the ability to produce some yellow pigment in the spore wall is a common ancestral quality in *Russulaceae*. With continuous characters the basic genes may code for continuous production of spore pigments until switched off by an environmental or genetic trigger, a state which happens much sooner in basal clades. In this case a non-functional gene would give rise to white spores as in *R. azurea, R. lilacea, R. rosea* and *R. lepidicolor* in an otherwise yellow-spored group. An alternative explanation would involve simply multiple functional copies of the spore pigment gene in darker spored species, in which case white-spored species are harder to explain. These two hypotheses are not mutually exclusive.

The sesquiterpenes responsible for pepperiness and carried in the laticifers can be detected in the immature stages of some mild species, and in the gills but not the trama of some variably peppery species. The inclusion in some groups of peppery and mild species prompted the hypothesis that the default condition is one of continuous production of the peppery compounds until the genes responsible are inhibited at some developmental stage by a controller gene or genes. Mild species occur when this critical stage is reached early or if the genes for the production of sesquiterpenes (and/or laticifers) are not functional. Pepperiness occurs frequently in *Lactarius* and so is probably plesiomorphic: completely mild species occur mostly in upper clades.

A general hypothesis regarding genetic control of cuticle pigments would have to assume separate control and production genes for each pigment, probably with similar mechanisms as those hypothesised above for spore and sesquiterpene production. Cap colours vary between species because the production of one or more colours is considerably inhibited in relation to the others. Further discussion of pigments is in the chapter on Chromatography.

Table 7 Taxonomically important characters of Russulas used in the phylogenetic analyses, organised by clade (following 7 pages). Not all the information is available or remarkable, as is reflected by the absence of information in a given category. Epicutal terminal hyphae are (correctly or not) assumed to be undifferentiated filamentous hyphae unless noted otherwise.

Abbreviations: Col = colour of spores in Romagnesi's 1967 code. Am. Ptch=amyloid suprahilar patch. Wart ht. = height of warts as follows: 1= small, 0.1 - 0.4 um; 2 =medium, 0.4 - 0.9 um; 3 =large, 0.7- < 2 um; Retic = amount of reticulation: A = warts isolated, B = warts connected 2 - 3, C = warts connected by a partial reticulum, D = complete reticulum, E = warts catenate forming ridges, both according to Woo's and Bon's keys, see Figure 30 in Appendix 3. S V = reaction to sulphovanillin where grey to black to purple is a positive reaction, colourless or pink is a null reaction. Inc. = fucshin positive acid resistant incrustations. Prim hyphae = primordial hyphae, also staining pink in fucshin. Picy = pileocystidia. Taste codes: p = peppery, pm = mature basidiomata usually peppery but mild forms occur, m = mild, mp = mature basidiomata are usually mild but immature stages or gills may be peppery, b = bitter. Bruising codes: y = yellow, ybr = yellow then brown, br = brown, rg - red then grey to black, g = grey or black, wl = the stipe becomes greyish or yellow-grey as if waterlogged but is not a true bruising reaction.

Oade	Species	Spares				Pfleocysitidia			Epkutalhyphae			Macroscopic characters		
		CoL	Am. ptdi	Wart ht	Retie.	shape 1 2 3 4 5 6 7	# Sepia	**SV**	Inc	Prim. hyphae	Terminal hyphae	Taste	Bruising	Odours
la	*Rarchaea*	la	0						0	0		m	tar	
la	*R camarvphylki*	k	0	1	A	2	0	OH-	0	0	ckvate end cells	m	ybr	none or cheese rind
lb	*Rdensifblia*	k	0	1	B-C	2 6	0	0	0	0	inflated-atticulated	P	bk	weak, fruity
lb	*Radusta*	k	0	1	RC	2 5 6	0	+	0	0		mp	bk	weak, wine battels
lb	*R nigricans*	la	0	1	**BC**	4	0	0	0	0		P	bk	
2	*RpaOidospom*	Id	1	1-2	A-D	2	0	0,+	0	0		m£>	br	fhily
2	*Rchbrokks*	lb	1	1	R€	2	0		0	0		P	br	fruity
2	*Rbrevipes*	kb	1	23	BC				0	0		m-p	tar	
2	*Rdelica*	Ha	1	1-3	C-E	2	0	+	0	0		mp	br	fruity
3a	*RcyanoxaMia*	k	0	1	A	2	0	+	0	0		mp	br	
3b	*Rpectmatokks*	Id	0	2	A-B	1 2	0	0	0	0		P	ibr	rubber
3b	*R amoenolens*	Db	0	2	A-B	1	0	0	0	0		P	itar	Jerusalem artichokes
3b	*Rpectinata*	Dbd	0	2	B	1 2	0	0	0	0		P	ibr	burnt flesh, nauseous
3c	*Rpulverulenta*	He	0	23	B-C	0 0 0 0 0 0 0	0	0	+	0	warty yellow walls	mp	ybr	

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(lade	Species	Spores				PfleocystkHa ——shape —			Epfcutal hyphae			Macroscopic characters		
		CoL	Am. ptdi	Wart ht	Retic.	1 2 3 4 5 6 7	# Septa	SV	Inc.	ftim. hyphae	Terminal hyphae	Taste	Bruising	Odours
5a	*Ratropurpwm*	la	1	2	D	2 4	0	+	0	0		pm	ybr,wlg	apple
5a	*Rochwleuca*	lab	1	23	D	2	0	0	+	+	jellow walls	P	br	
5a	*Rviscida*	lib	1	1	D£	2 3 5	0-m	+	0		yellow picy& latirifas	mp		fruity
5a	*Rstuntzji*	lb	1	23	B	2 4	0-1	+	0			P	wl	weak, fruity
5b	*RfeUea*	lab	1	2,3	C	2 4	0-2		0		pdgmaitonhyphal walls	P		apple, pelaigonium
5b	*Raquosa*	lb	1	2	C	2 3	0	+	0			P	wl	weak, fruity
5b	*RraouMi*	k	1	2	D	3 4	0	+	0			P	wl	coconut
5b	*R cmnoriwbr*	k	1	1	B	2	0	+	0			P		
5b	*R mcdrei*	k	1	U	C	2 4 5	0-3+	+	0			P		
5b	*Rfiugilis*	lb	1	2	D	2 3 4	0-2	+	0			P	wl	amy! acetate, coconut
5b	*RbeUdanon*	kb	]	2	D	3 4 5	0 4	+	0			P	wl	fruity
5b	*Rnana*	k	1	1	D	2 4 5	0-1		0			P	wl	fruity
5b	*Rbicobr*	k	]	u	A-B	2		+	0			P	wl	weak, fruity
5b	*Remetica*	kb	1	23	D	2 4 5	3+	+	0		red vacuolar pigment	P	wl	weak, fruity

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Oade	Species	Spores				Pfleocystidia			Epicutalhjphae			Macroscopic characters		
		CoL	Am. pteh	Wart ht	Retic	shape—— 1 2 3 4 5 6 7	# Septa	SV	Inc.	Mm. hyphae	Terminal hyphae	Taste	Bruising	Odours
7a	*Rrvseipes*	**IVab**	**]L**	**2**	**BC**	0 2?3?0 0 0 7?	0	0	+	+				
7a	*Rturci*	**IVa**	**]L**	**2**	**DE**	0 0 0 0 0 0 0		0	+	+	pigmented between sub and epicutis,infL cells	m		iodcfcam at stipe base
7a	*Rpctktdosa*	lab	**][**	**23**	**C**	4 7	0-2	**(+)**	+			m		weak,fiuity
7a	*Rpostktna*								+					
7a	*R risigallina*	**IV**	**1**						+					
7b	*R rosea*	**lb**	**]L**	**1**	**BC**	0 0 0 0 0 0 0		red ttama	+	+	inflated cells subcutis	m		none
7b	*Rawea*	**k**	**]**			3	0-1	(+)	+	+	clavate end cells	m		none
7b	*Rlttacea*	**kb**	**1[**	**2,3**	**A**	0 0 0 0 0 0			+	+		m		weakly fruity
7b	*R lejMcohr*	**lb**	**1[**	**1**	**CE**	4	0	red tama	(+)	+		m	**br**	
7b	*Rawarniaca*	**IVc**	***1*L**	**3**	**A**	3 4 5 7	0-2	0	+		capillifiam hyphae	mp		fiuii/apple
7b	*Rlepida*	**Da**	**]L**	**1**	**ED**	4 5 7	0	0	+	+		b, menthol		cedar wood, menthol
8a	*Rcaendea*	**M>**	**]L**	**2**	**CD**	0 0 0 0 0 0 0	0	red ttama	+		infl cells, ampullate	m,b	gfor	
8a	*Rolivacea*	**IVc**	**1[**	**3**	**A**	0 0 0 0 0 0 0	0	0	0	+		m	ybr	weakly fruity
8b	*RmeUtodes*	**M>**	**]L**	**3**	**AB**	1 3 4	0-2	(+)	+	+		m	ybr	weak fruit, honey
8b	*R decolorans*	**Hdfii**	**]L**	**23**	**B**	4	0-2	+	0		picy numerous	m	**g**	weak honey, cheese

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Oade	Species	Spores				Pfleocystidia			Epicutalhyphae			Macroscopic characters		
		CoL	Am, ptdi	Wart ht	Retie.	shape 1 2 3 4 5 6 7	# Septa	SV	Inc.	Prim. hyphae	Terminal hyphae	Taste	Bruising	Odours
10a	*RmeUiokns*	Dab	**1**	**1**	**D**	5	**m**	+	0			**m**	ybr	honey, gingerbread
10a	*Rcuprea*	lVe	**1**	**3+**	**A**	4 5 6	**m**	+	0		ampuHate, divoliculate	**P**		weak fruity
10a	*Raduherina*	IVe	**1**	**3+**	**A**	2 4 6	**0-2**	+	0			**P**		stewed apples
10a	*Rcurtipes*	IVa	**1**	**2**	**CE**	3 4 5	**0-m**		0		ampullate,hair	**m**	ybr	weak fruity
10a	*R cessans*	rvbc	**1**	**2**	**CD**	0 4 5 6	**m**	+	0			**m**	**wl**	
10a	*Rlaricina*	lVcd	**1**	**2,3**	**BC**	0 4 5 6	##	+	0			**mp**	**wl**	
10b	*R mekeri*	IVb	**1**	**2 3**	**A**	3 4 5	**m**	**+?**	0			**m**		fruity
10b	*Rpuelktla*	nbc	**1**	**2**	**B**	5	**m**	+	0			**P**	y	
10b	*Rpuellaris*	He	**1**	**2,3**	**AB**	3 4	**0-3**	+	0			**P**	y	
10b	*R solans*	***m***	**1**	**3**	**B**	3 4 5 6	**0-m**		0	globules	of 5dlow pigment	**P**		vinegar
10b	*Rmessapica*	IVc				5	**m**		+		mciustredin KOH	**m**		quince
10b	*Rnauseosa*	IVb	**1**	**3**	**AB**	3 4	**0-2**	+	0			**P**		
10b	*Rocbrata*	IVc	**1**	**2 3**	**C**	4 5	**m**	+	0			**mp**	y	fruity, pelargonia
10b	*R versicolor*	mb	**1**	**1**	**BC**	4 5	**2-3**	+	0			**P**	y	

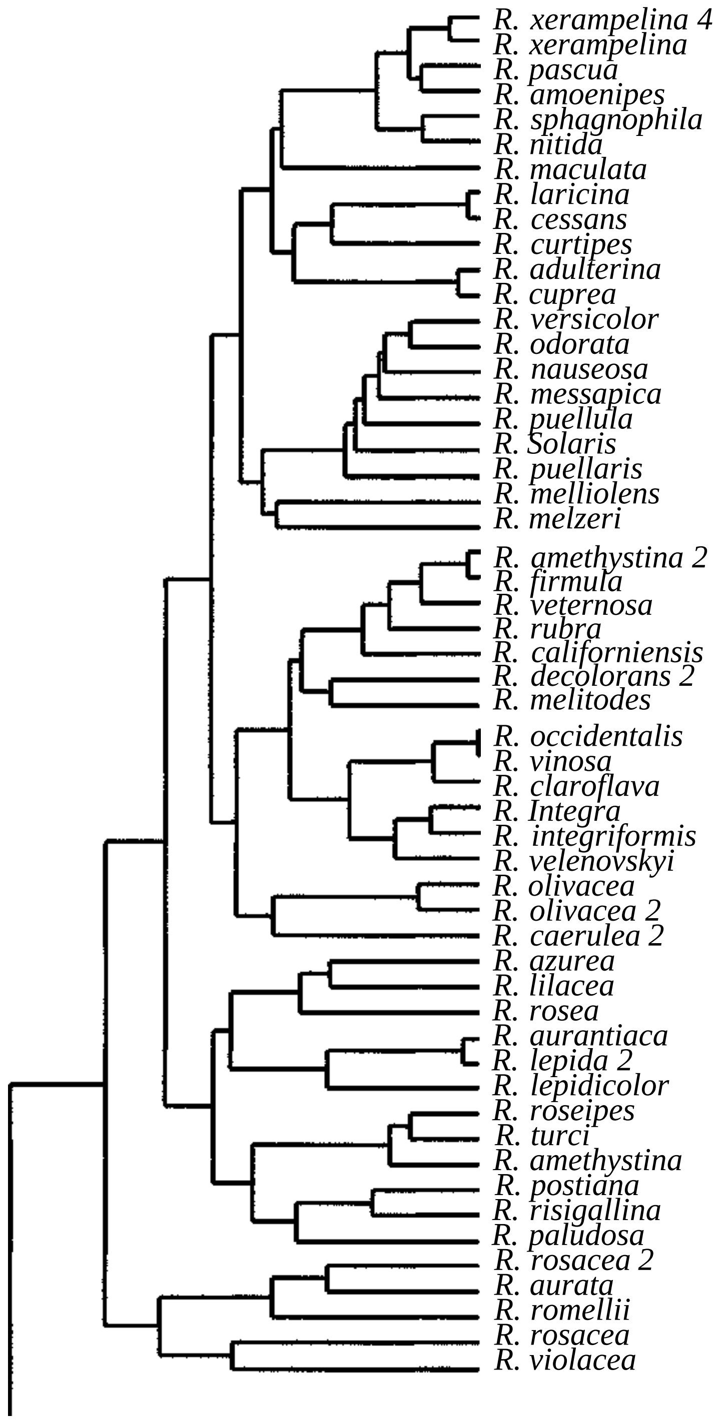

Figure 14 Bayesian maximum likelyhood tree of 111 taxa with molecular clock analysed using the HKY85 model (Haegawa, Kishino, and Yano, 1985), using Albatrellus flettii and Gloeocystidiellum aculeatum as outgroups. The tree is the final tree of 1500000 trees, with a log-log likelyhood of -17578.557772. Above are the upper clades and the figure is continued down to the lower clades in figure 15 .

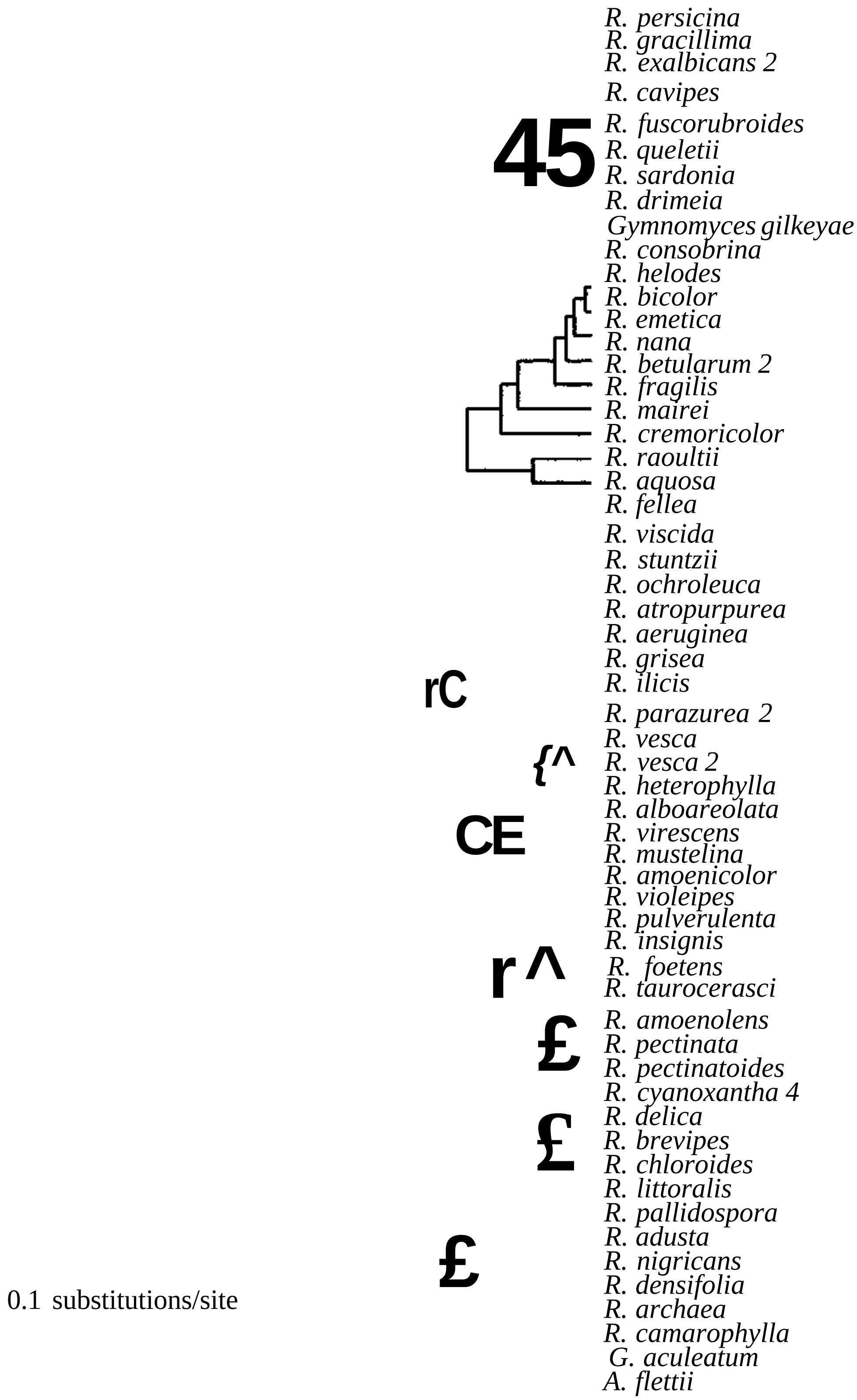

Figure 15 The continuation from figure14, the lower clades of the final Bayesian maximum likelyhood tree.

Figures 16 and 17 following pages. Extended majority rule consensus tree of the Bayesian maximum likelyhood with molecular clock analysis as outlined above. The numbers to the above left of the nodes indicate the percentage of times the group consisting of the species to the right of that node occurred among the trees, out of 6000 trees sampled at the rate of one in 200 from the 1200000 trees created after convergence. The terminal nodes are not marked except where the values were below 98%, heavy lines indicate values of 98-100%. Clades are marked and numbered, minor clades within each clade are given in Table 6.

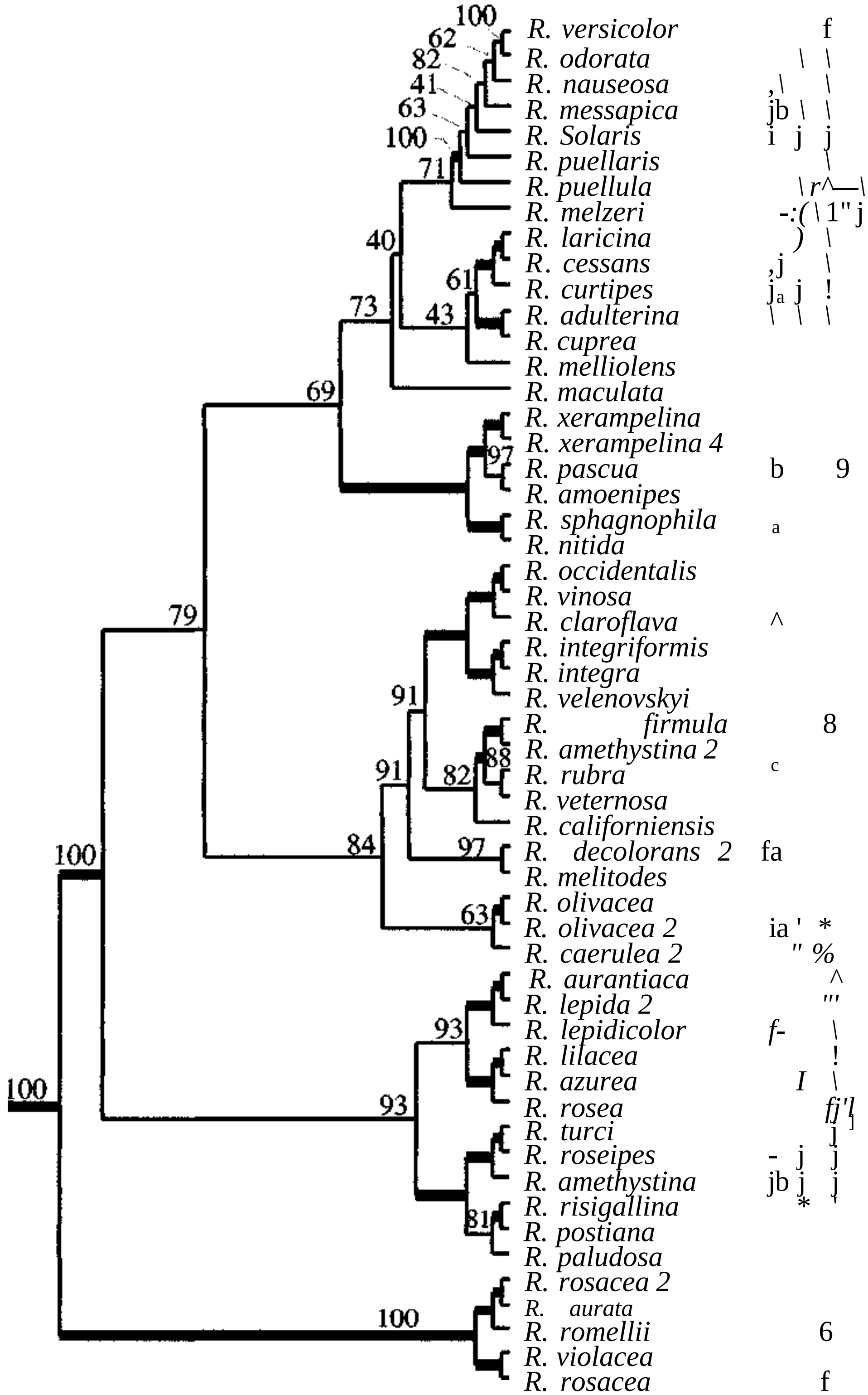

Figure 16 Extended majority rule tree, upper clades (numbered 6-10) of tree, the lower of which is continued downwards in figurel7.

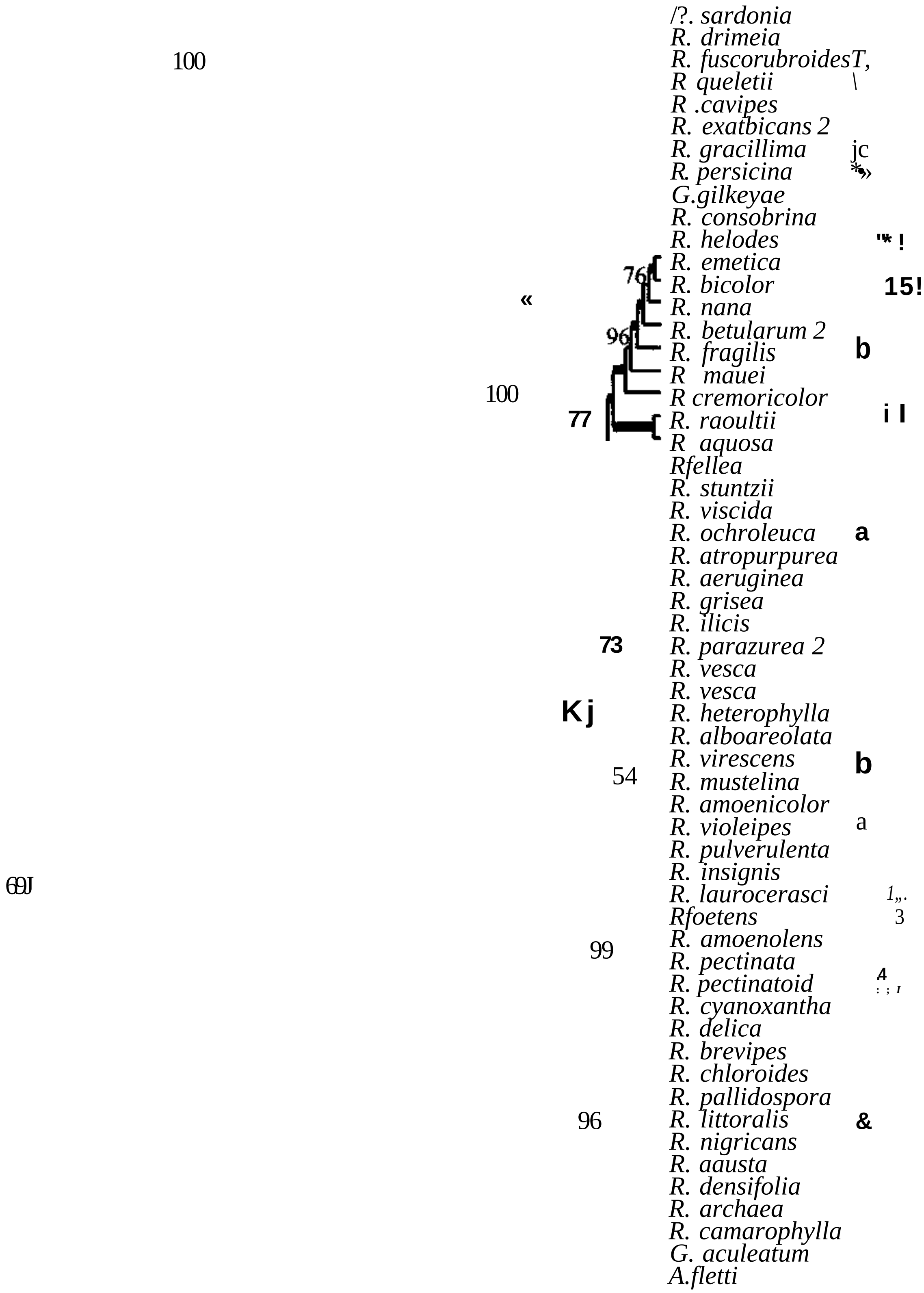

Figure 17 Lower clades (1-5) of extended majority rule tree, continued from figure 16. Full explanation of figure is given overleaf.

The numbers to the above left of the nodes indicate the percentage of times the group consisting of the species to the right of that fork occurred among the trees, out of 6000 trees.
The terminal nodes are not marked except where the values are below 98%,
heavy lines indicate values of 98-100%

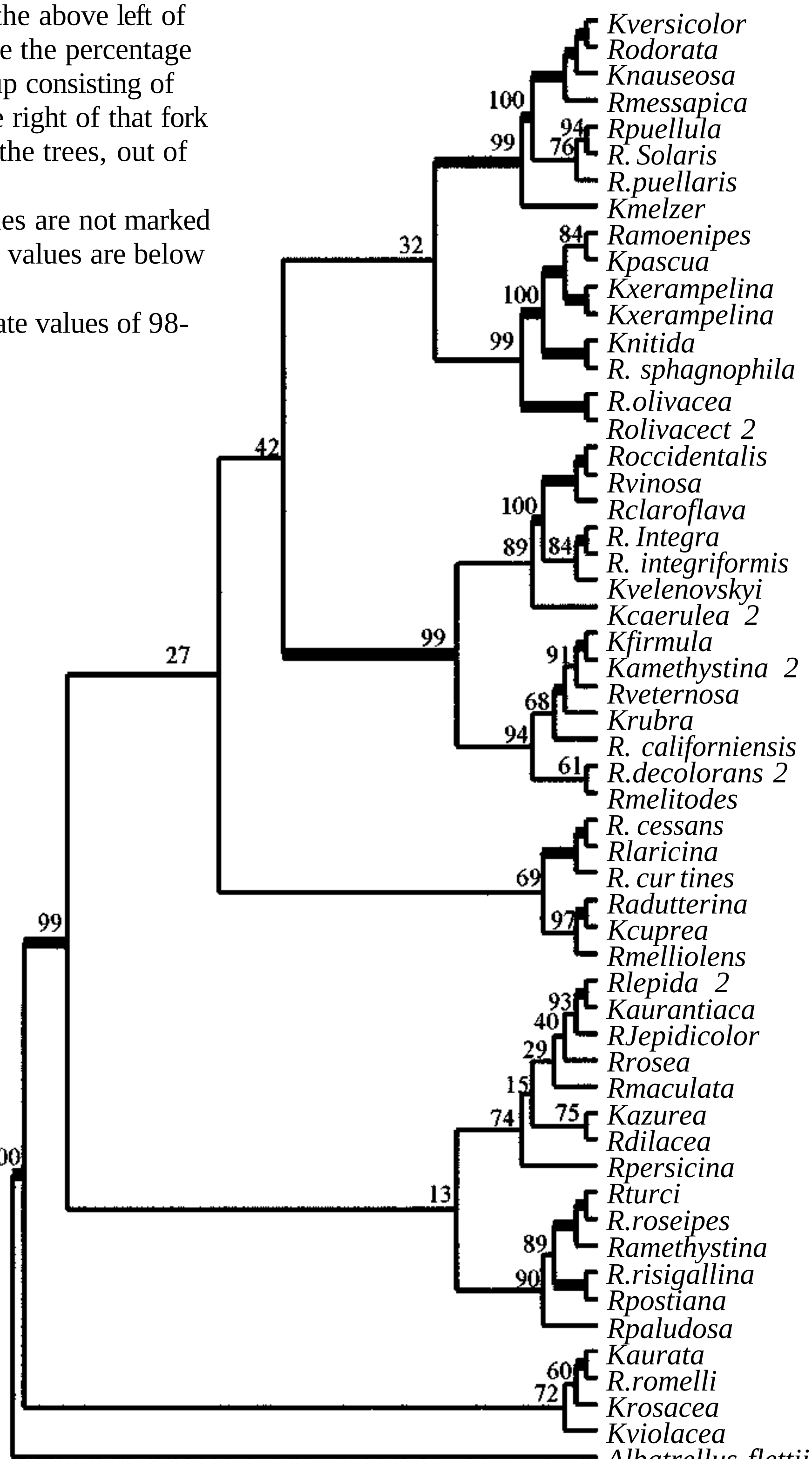

Figure 18 Extended majority rule consensus tree of 54 Russulas from the upper clades of the previous analysis plus an outgroup, re-analysed using sequence minimisation.

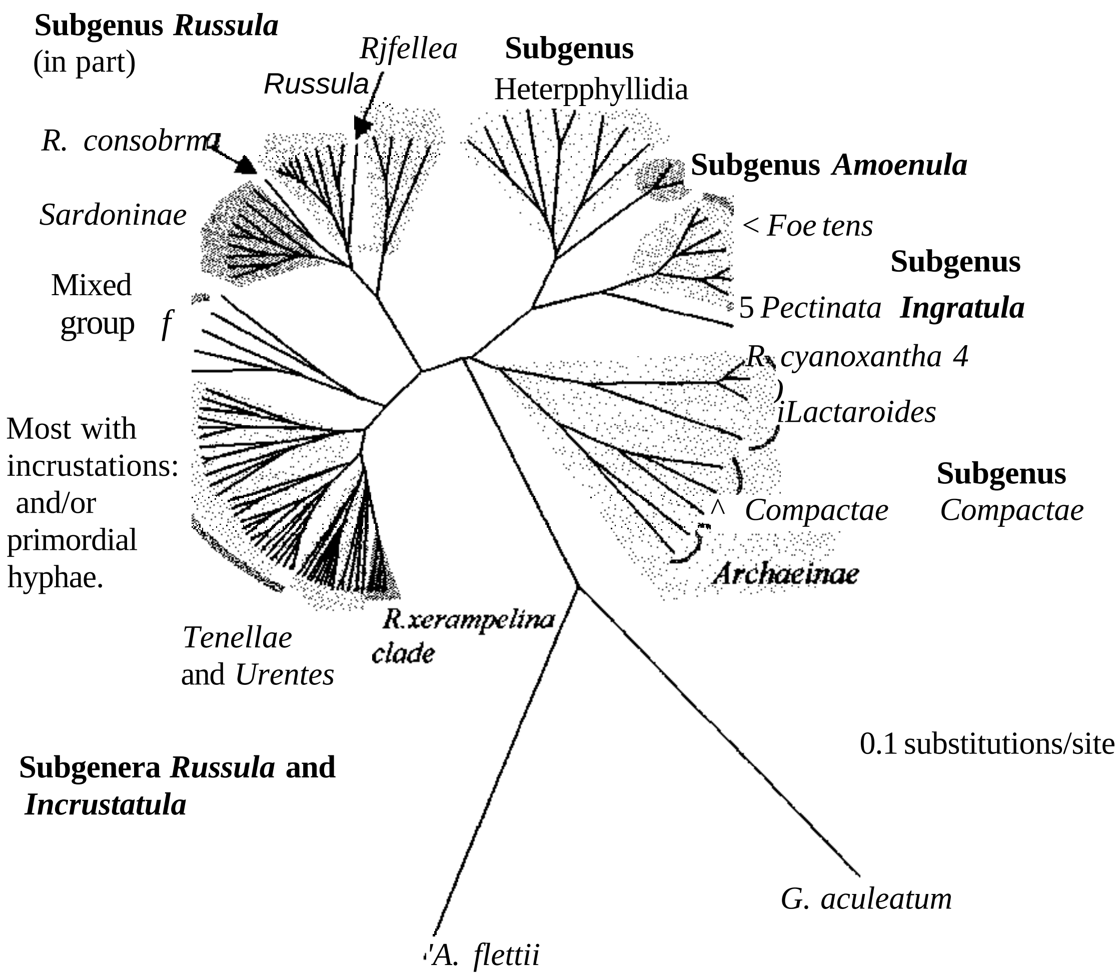

Figure 19 Unrooted Bayesian maximum likelyhood tree of 108 species of *Russula* and a *Gymnomyces gilkeyae. Albatrellus flettii* and *Gloeocystidiellum aculeatum* are the outgroup and are shown here with their branches truncated. This is the same tree as in figures 10 and 11 but this format shows more clearly the division within the genus of the subgenera *Russula* and *Incrustatula* on the left and the subgenera *Compactae, Ingratula, Amoenula* and *Heterophyllidia* on the right. The tree also shows that the subsections *Consobrinae, Sardoninae* and *Russula* which are currently in section *Russula* of subgenus *Russula* form a monophyletic group. The clades on the lower left include two groups in which most species have incrustations and/or primordial hyphae in the cap epicutis, and the terminal group of strongly coloured spores with no incrustations or primordial hyphae.

Chapter 4

Examination of character trends in Vancouver Island collections

Introduction

Russula taxonomists have considered characters such as relatively short, broad basidia as indicative of higher clades such as the *Temllae,* clade 10. However many such character variations have not been examined for phylogenetically ordered gradations. In the previous chapter the characters and character states of each species were gleaned from the literature of many different authors, whose methods of measurement, degree of detail, and perceptions of what may be taxonomically useful, varied. The 229 collections of Russulas from Vancouver Island offered the opportunity to examine first-hand the various characters, to measure them, to investigate other characters that have not to date been systematically examined, and to verify or question the clade related gradient findings from chapter 3 part II.

Methods

Estimating clade positions of Vancouver Island species

From a consideration of the phylogenetic analysis in chapter 3 part 2, the Vancouver Island Russulas were assigned an estimated clade position based on their relationships to the species used in the analysis. Morphological characters, RFLPs of rDNA analyses (chapter 3 part 1), chromatographic analyses of cuticle pigments (chapter 2) and reference to monographs on Russulas, particularly those of Bon (1988), Romagnesi (1967, 1985) and Sarnari (1998), were used in determining relationships between species. Estimated clade positions are shown in the table below and in the descriptions (Appendix 3). Sequence data for two local species, *Russula crassotunicata* and *Russula farinipes* has become available since the completion of most of this dissertation. A Bayesian maximum likelyhood analysis using a smaller set of sequences suggests these species branch off below those of the other *Russula* clades, closer to the *Archea,* than to the *Ingratae* to which they have been traditionally allied. They were placed in the lowest clade for the analyses of characters using the MacClade program described below.

Evaluation and examination of characters of Vancouver Island collections

The following characters which could be expressed numerically were plotted against the clade position of each of 56 species to see if any trends were apparent: Spore print colour, recoded from 1 to 10 following the colour codes of Romagnesi (1967), where l=Ia and 10=Ive. Spore shape, as the mean length to width ratio and its range (maximum L:W - minimum L:W); and maximum spore length of normal, mature spores, (not including the occasional oversized spores that normally occur in most spore prints, unless these comprise about 10% or more of the spores.) Maximum height of the spore ornamentation and degree of reticulation between warts, this latter based on WooV types (Appendix 3, figure 30), recoded numerically with type A=0, B=l, C=2, D=3 and E=4. These numbers actually relate closely to the number of connections the warts have to one another, with the exception of type E, in which the ornamentation is in the form of chains or ridges. Basidia dimensions, including minimum and maximum length and width of mature basidia.

While this provided an overall view of phylogenetically useful gradients, it was inappropriate to derive any significance from fitted trendlines. Characters showing trends were regressed against one another to see how they were correlated, and these regressions were examined for significance.

To examine trends further, character data were traced onto a phylogenetic tree using the MacClade program (version 4.03, Maddison and Maddison 1992), which calculates ancestral states for the branches. As few of the Vancouver Island species had sequences available with which to build a phylogenetic tree to use in the MacClade analyses, a hypothetical treewas created, organised as for the Bayesian majority rule tree (figs. 16 and 17), and based on the estimated clade positions of each of 56 Vancouver Island species as described in the previous paragraph.

Characters examined in the MacClade program were treated as continuous in the case of basidia and spore dimensions, or categorical in the case of degree of spore reticulation. Spore colour, for convenience, is commonly treated in descriptions as categorical. It is actually a continuous character but has occasional occurrences of white-spored species appearing within clades of yellow-spored species (compare *Russula lilacea* with *R. aurantaica*, table 7: clade 7), suggesting that pigment loss takes fewer evolutionary steps than pigment gain. Using MacClade, the spore colour evolution was traced as a continuous character, although various asymmetrical step-matrices were also tried and compared. Continuous characters were also entered as categorical into the MacClade database in order to examine tree statistics, as the program does not include continuous characters for these statistics.

Pileocystidia of four shapes and incrusted primordial hyphae were either present or absent in a given species. Unfortunately a pure binary scoring for pileocystidia shapes would be incorrect since several species commonly have more than one shape, and the relative proportions of these can vary by collection and by age. As a compromise, a score of 1 was recorded when one pileocystidia shape only was found, 0.5 for each of two shapes found and 0.33 for each of three shapes found; no species had four shapes present. These figures and the original binary score were put through the following principal component analysis to see if the results differed. The pileocystidia binary figures were also among the characters used in the calculation of tree statistics in the MacClade program.

To examine how well a clade could be defined by a set of character measurements, a principal component analysis was done using the following microscopic characters: mean length to width ratio of spores, maximum spore length, spore ornamentation height, pileocystidia shape and presence of incrustations in the epicutis. This analysis of 54 Vancouver Island species used the Biplot 1.1 add-in software for Excel (Smith and Lipkovich 1999-2002). Data columns were centred and standardized for the singular value decomposition transformation, and 2 components (axis) were extracted, the transformed data being plotted using a row scaling adjustment factor of 1.228, automatically calculated by the Biplot software.

Results

Trends

Several clade related trends were found in Vancouver Island collections. Positive trends from lower to upper clades included spore colour (lighter to darker), the height of spore ornamentation, maximum spore length, and basidia width, (both minimum and maximum width). The basidia length showed very little trend, save for a slight reduction in clade 5. Decreasing trends were seen in mean spore L:W, spore L:W range (amount of variation), and the degree of reticulation on the spore walls. These characters were traced onto the phylogenetic tree in MacClade, which calculates and displays on the branches the ancestral states, all but the last two are shown in figs. 20-24. All characters showed scatter around the trendline, displayed in the variety of character states within a clade on the branch ends of the hypothesised evolutionary trees shown in figs. 20-24.

The Vancouver Island collections had no white-spored species above clade 6, and none with spores darker than shade 6 (Romagnesi Ilia) below clade 7 (fig. 20). There are localized trends within this overall picture, including a steep increase in spore colour from clade 5 to 7, confirming the usefulness of this character on several taxonomic levels. In the phylogenetic analysis in chapter 3 part II, clade 7 contains 3 white-spored species, but otherwise has the same overall light to dark trend, suggesting a loss of functionality of one or more pigment genes. With this in mind the reconstructed ancestral character states for clade 5, also with mixed pigmented and colourless spores, could be darker than is indicated in fig. 20.

The spore length to width ratio shows a reduction from lower to upper clades (fig. 21, top). The variability of the spore shape (not shown) closely follows the clade-related pattern of the L:W ratio, with the exception of clades lb and 2 (the *Compactae* and *Lactaroides),* with variability like that of clades 6 upwards. Essentially spores are mostly ellipsoidal but quite variable in shape in the lower clades and mostly subglobose and less variable in the upper clades. Spore maximum length is generally higher in clades 7 to 10 than in lower clades (fig. 21 bottom). Two species with large spores, *R. olivacea* and *R. aureqfulva,* in clades 8 and 10 respectively, may be biasing the calculated ancestral state, although other members of these taxonomic groups not included in this analysis have larger than average spores.

The spore ornamentation height shows some variation in the states at the branch tips, but this translates as a gradual trend in the ancestral states from lower to upper clades, as shown by the shade gradations between deeper nodes (fig. 22, top). The degree of reticulation between spore warts tends to be polymorphic for most species. Spores from several collections of a species, or often from a single piece of gill, may have quite a range of reticulation, although one category may predominate. (In the descriptions in Appendix 3 these ranges are given). This does not translate easily to the analysis mode in MacClade, in that polymorphisms are not calculated into the ancestral states. The resulting traced tree gives the illusion of much better defined states than is the reality. The chart (fig. 22, bottom) gives one a more realistic idea and shows a slight tendency for reticulated spores to be more frequent in the lower clades. This overall trend is perhaps less useful than the smaller trends such as the highly reticulate spores in clades 1-2 and 5a-b, and the frequency of none- to partially-reticulate spores in clades 3, 4 and 7-10. The occurrence of type E ornamentation -lines of fused warts forming ridges, is scattered throughout clades 1-8 and is peculiar to a species rather than showing a trend.

Basidial width at maturity showed a strong positive trend from lower to upper clades when plotted against species in clade order, with a slightly better line fit for the maximum width at which developed spores are seen. This trend is apparent in the reconstructed ancestral states traced onto the phylogenetic tree, which show mostly narrow basidia in clades 1-4, mid-sized ones in clades 5-7, and larger ones in clades 8-10 (fig. 23 top). Basidial length however, shows very little trend save that those in clades 5 and 6 are generally shorter than lower and higher clades.

The statistics for the tree used in figures 20-23 calculated by the MacClade program were: Treelength 208; minimum possible treelength 54; maximum possible treelength 257; consistency index 0.26; retention index 0.24; rescaled consistency index 0.06. These figures were derived from 12 unordered characters in category format with equal weight: Mean spore L:W, spore L:W range, maximum spore length, spore colour, spore reticulations, spores with ridge formations, basidia minimum width, four pileocystidia types, and presence of incrustations (none, traces or present).

Correlations between characters

There is little correlation between the basidia width and length (table 8), presumably because basidial length is a function of the age of a basidioma, and of the size and proliferation of the basidial support cells. Those basidia arising from lower in the hymenium naturally have a longer base than those arising nearer the surface, since they must all bear their spores at the surface. This suggests basidia can elongate from the base once the apex is differentiated into sterigmata and spores.

The basidial width and spore ornamentation height show a positive significant correlation of 0.61 (a = 0.05, P< 0.0001; table 8 and fig. 24). Spore length to width ratio also shows a significant negative correlation of 0.33 with basidial width (a = 0.05, P= 0.001, table 8 and fig. 25), and basidial width is up to 53% significantly positively correlated with maximum spore length and 56% with spore colour.

Correlations between characters regressed against one another are summarized in the following table.

Table 8 Regression analyses results

	vs	Correlation	R^2	P (a = 0.05)
Basidia minimum width	Basidia minimum length	0.18	0.032	0.18
Basidia maximum width	Basidia maximum length	0.09	0.008	0.53
Basidia minimum width	Spore ornamentation height	0.61	0.38	<0.001
Basidia minimum width	Spore maximun length	0.53	0.28	<0.001
Basidia minimum width	Mean L:W spores	-0.33	0.11	0.013
Basidia minimum width	Spore colour	0.56	0.31	O.001
Mean L:W spores	Spore ornamentation height	0.43	0.18	0.001

In the principal component analysis (fig. 26) clades 1 and 4 cluster mainly in the upper left quadrant influenced by having strangulate tips to the pileocystidia, ellipsoidal spores, and (negatively) by having smaller than average spore ornamentation and narrower basidia. Clade 3 clusters mostly in the lower left quadrant, where the pileocystidia are tapered and the spore L:W ratio has more influence than other spore or basidial dimensions. Two exceptions are *Russula crassotunicata, R. farinipes,* which cluster with others in clade 1; supporting the finding based on sequence information published since these analyses were completed, that these species branch off below those of other *Russula* clades. Most of clade 5 cluster along the y axis, having close to average dimensions in spores and basidia, but some having strangulate pileocystidia tips (above the x axis) and others with obtuse pileocystidia tips (below the x axis). The three clade 6 species are above the x axis where they are split between strangulate and diverticulate pileocystidia. Clades 7-10 are in or close to the lower right quadrant, influenced mainly by the obtuse pileocystidia tips, incrustations and larger spore and basidium dimensions. The fact that those species do not cluster with other clade members shows that although there are general trends which are quite reliable indicators of clade position, there are also frequent exceptions in one or more characters.

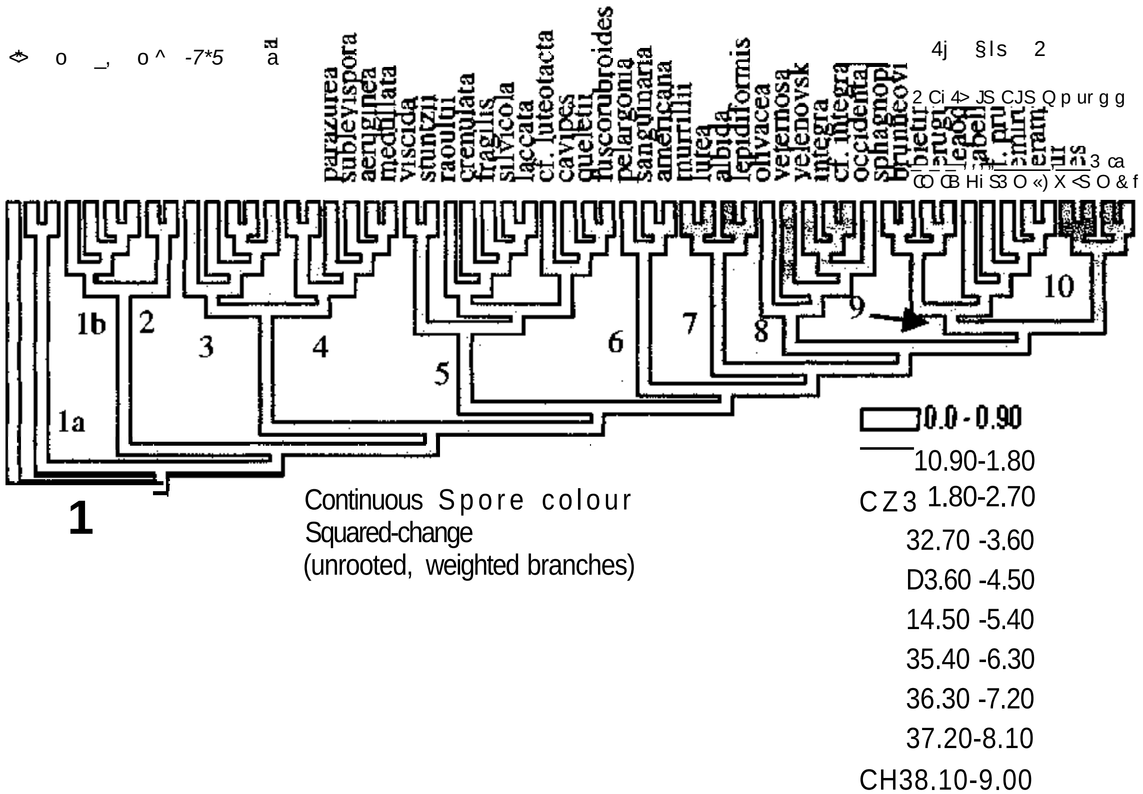

Figure 20 Spore colour of Vancouver Island collections with hypothesised ancestral states traced onto a phylogenetic tree. Colours are those of Romagnesi, coded 0 for white spores to 9 for deep ochraceous yellow spores, the colour scale is approximate. Character treated as continuous, clades are numbered adjacent to the supporting branch.

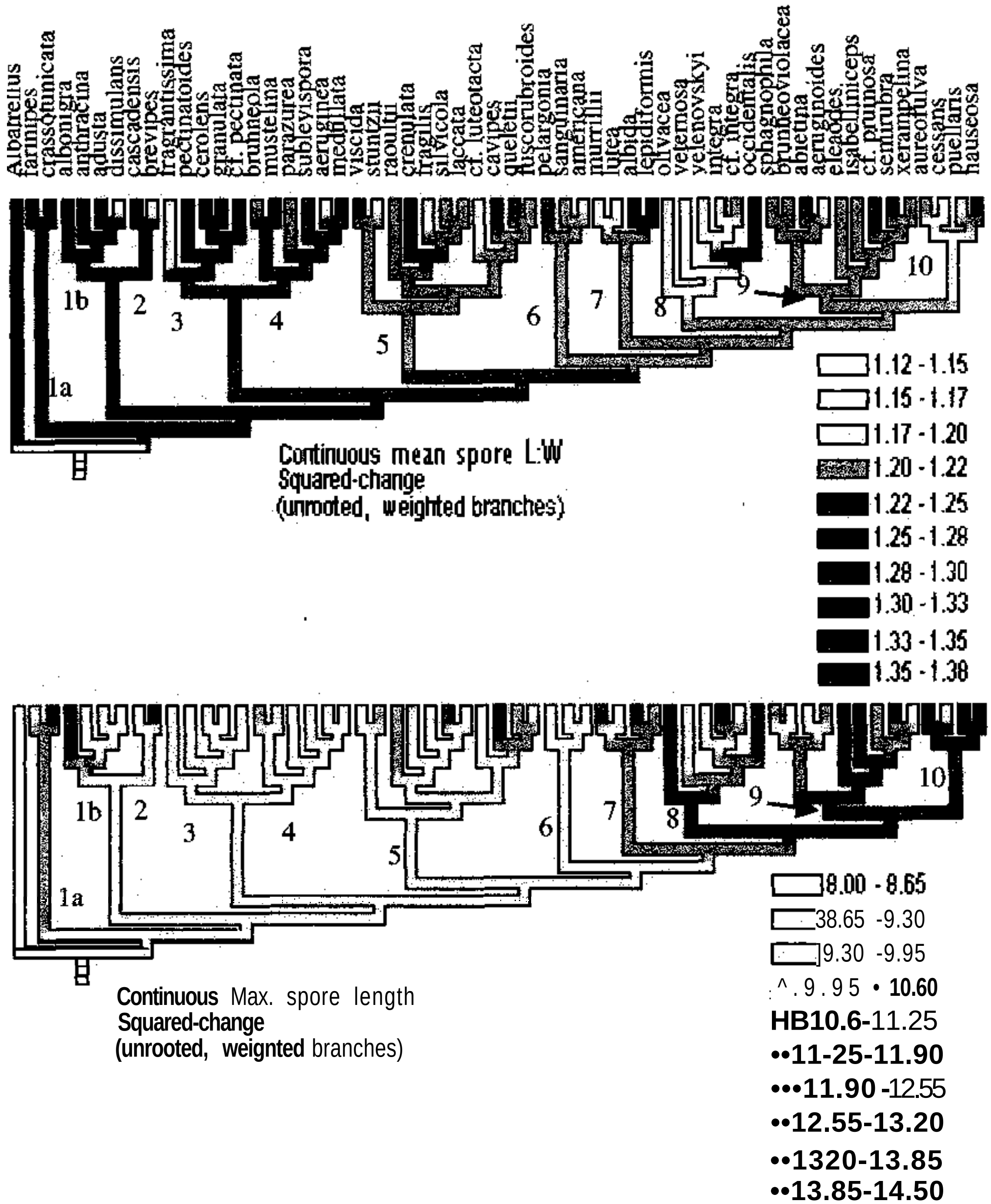

Figure 21 Spore shape and size with calculated ancestral states traced onto a hypothetical phylogenetic tree. Top: mean L:W ratios of spores showing a trend towards more globose spores in the upper clades; bottom, maximum length of (normal sized) spores, showing an increase in clade 7 and above. Albatrellus flettii, the outgroup on the left, has spores under 4.8um long. Clades are numbered adjacent to the supporting branch. Both characters are treated as continuous, dimensions are in urn.

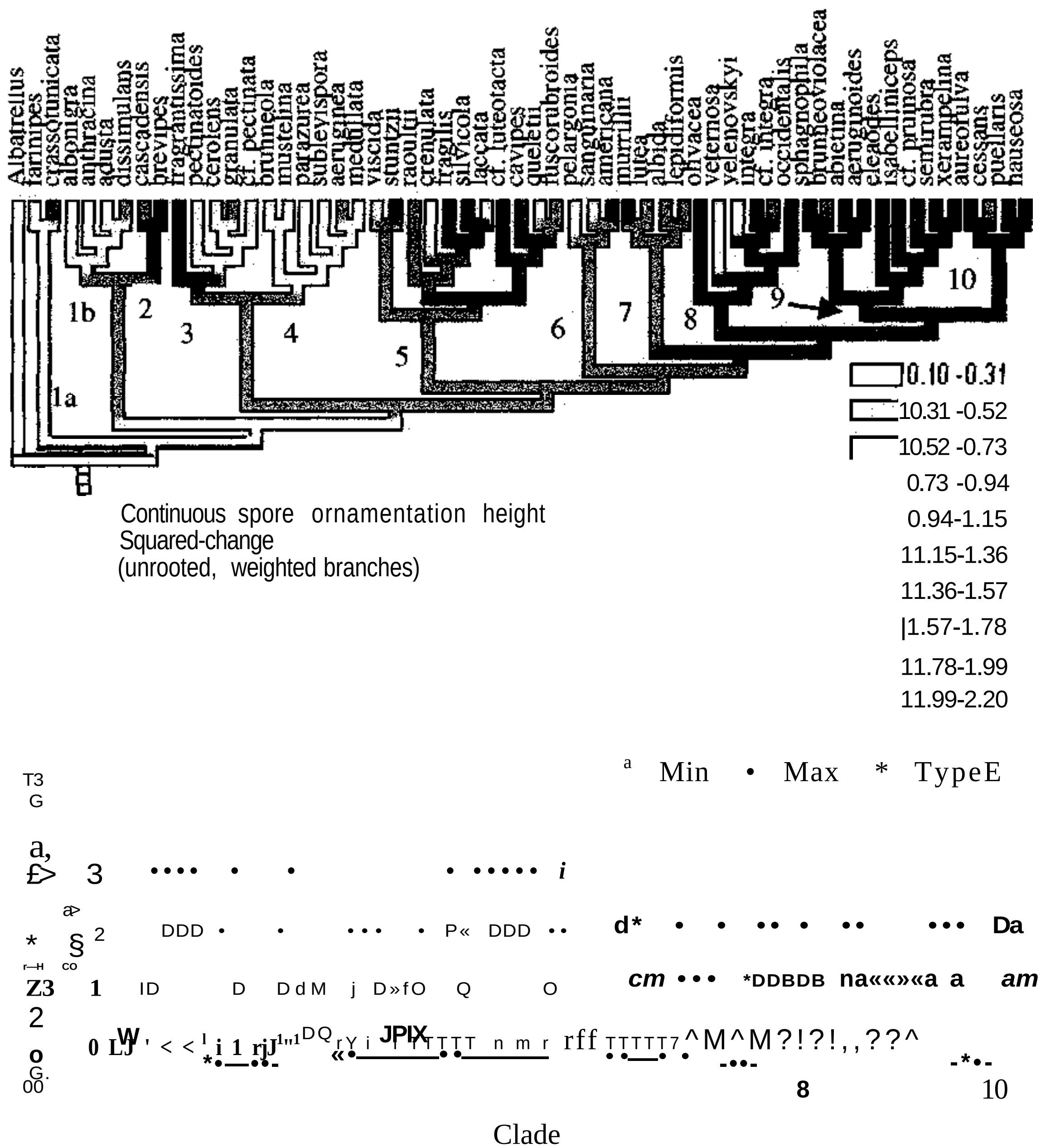

Figure 22 Spore ornamentation and its hypothesised ancestral states: Top, maximum height of the spore ornamentation in μm traced onto phylogenetic tree, showing an overall increase towards higher clades; bottom, amount of reticulation between warts on the spore wall. Most Russulas have a range of ornamentation, i.e. they are polymorphic and this is better portrayed by plotting the range against the species. The numerical divisions on the y axis approximate the number of connections between one wart and its neighbours, with the exception of 4, which stands for type E of the Woo spore ornamentation coding. This coding system also translates on this figure as 0=type A, 1=type B, 2=type C and 3=type D. Species are in the same order for both diagrams.

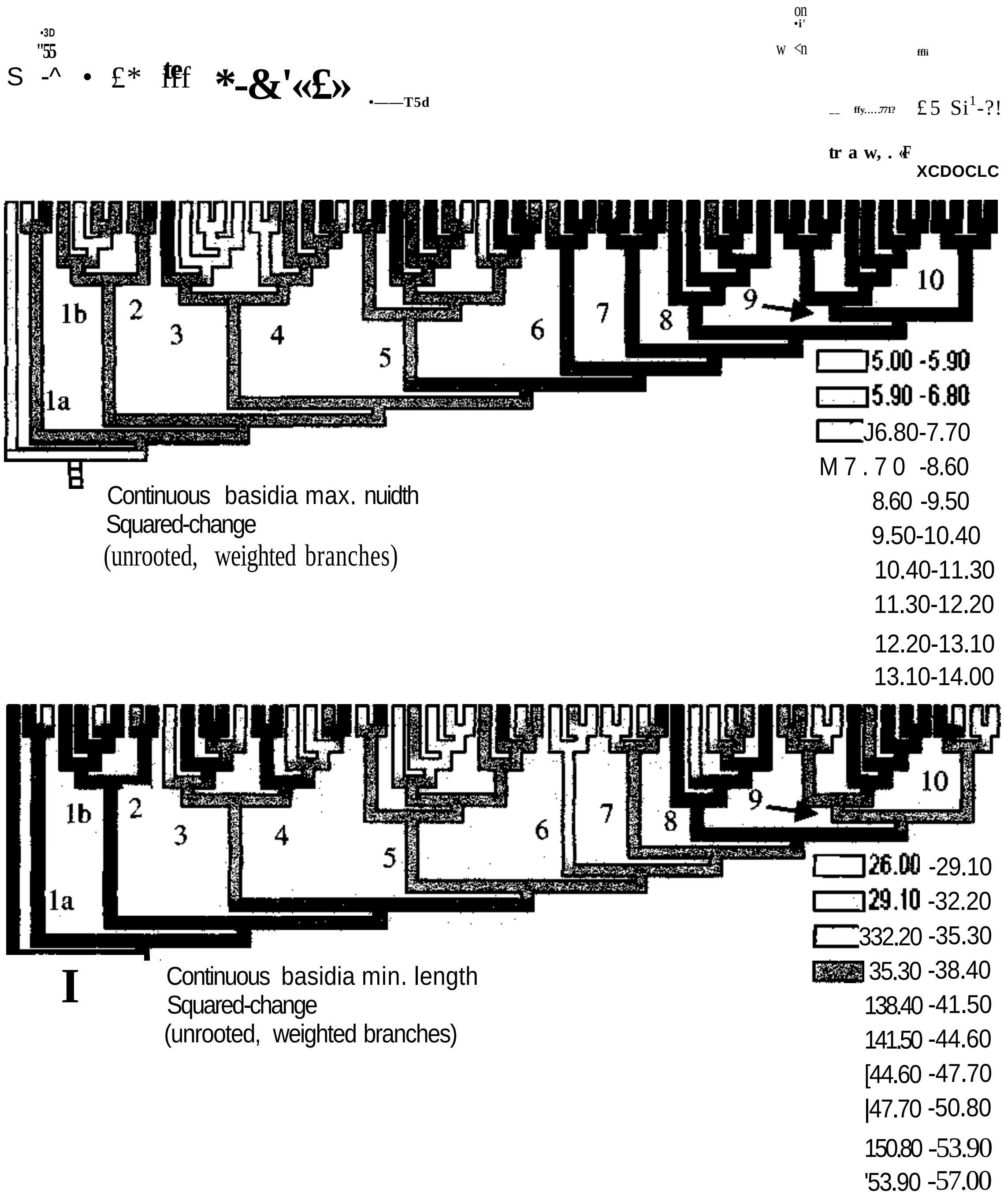

Figure 23 Dimensions of basidia with hypothesised ancestral states traced onto phylogenetic tree: Top, maximum width of basidia showing broader forms in clade 7 and above; bottom, minimum length of mature basidia showing a decrease in clades 5-7 but no overall trend. Scale is in um.

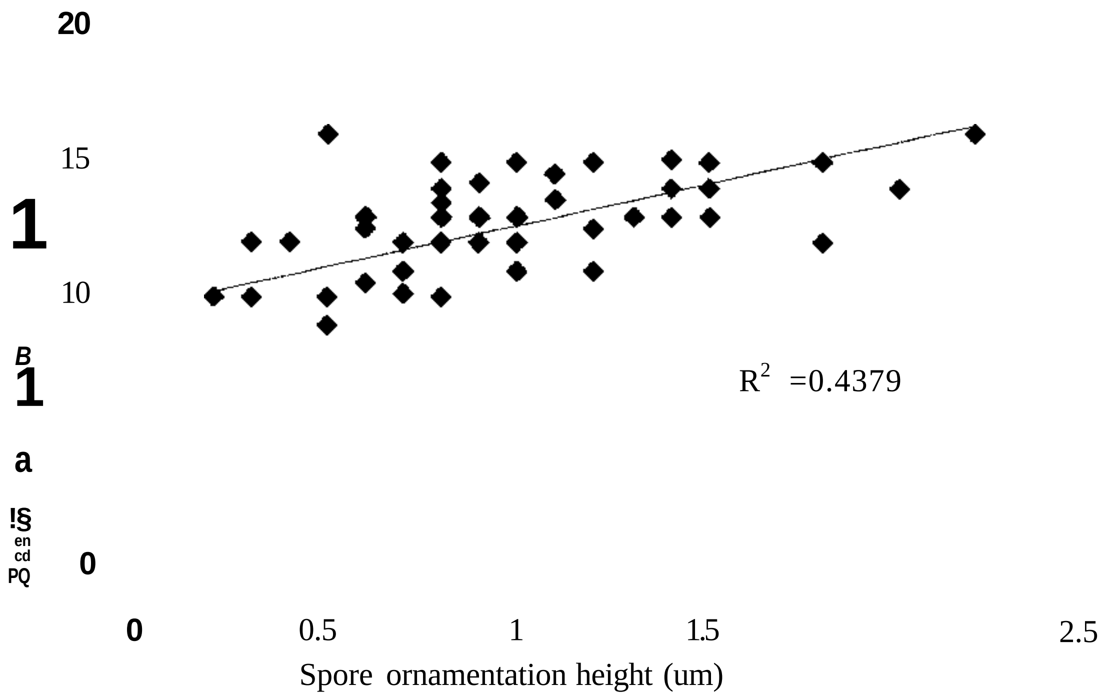

Figure 24 Basidia maximum width regressed against spore ornamentation height: they are 66% correlated (a = 0.05, P= 0.3"[7]).

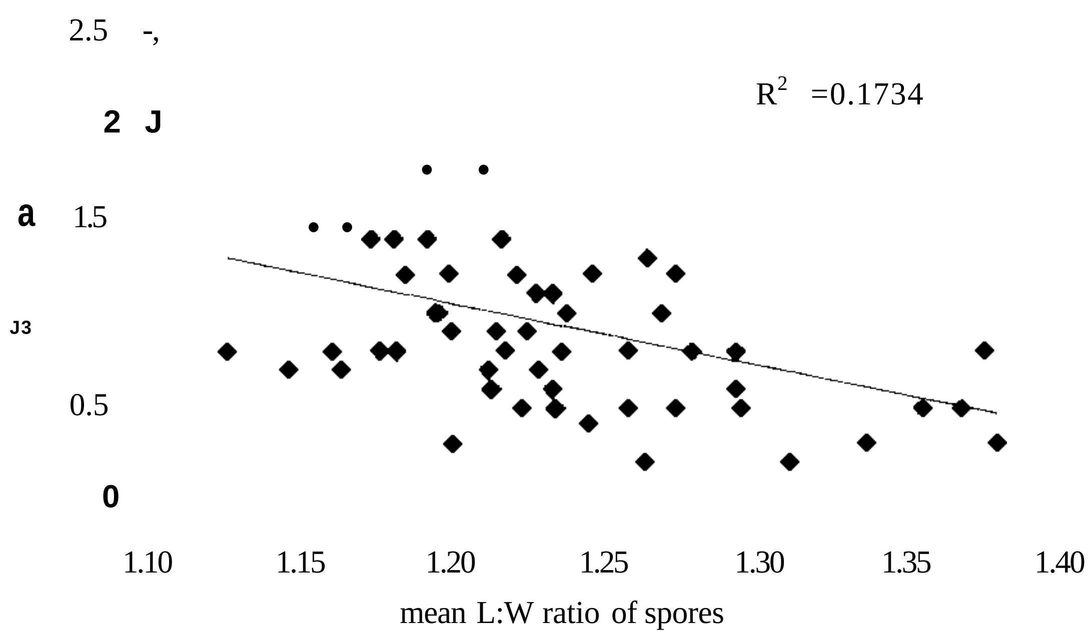

Figure 25 Spore wart height regressed against the mean length to width ratio of spores: they are 43% correlated (a = 0.05, P= 0.001).

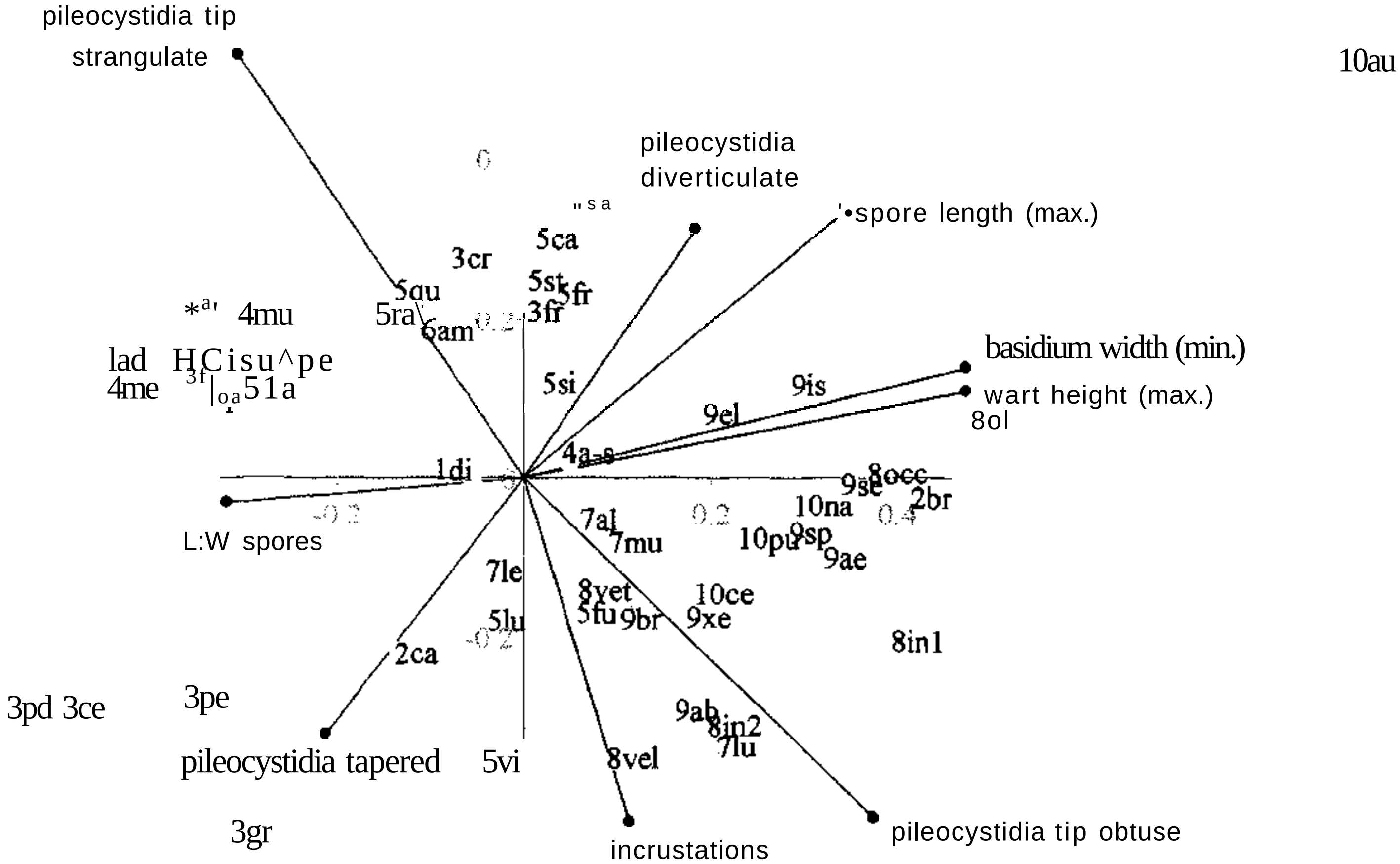

Figure 26 Principal component analysis of 54 *Russula* species from Vancouver Island, (each represented by a code). The species are arranged in space according to the influence of the following components: wart height, basidium width, spore length, length to width ratio of spores, shapes of pileocystidia - strangulate tips, tapered, obtuse tips, with diverticulae. Eigenvalues and key to codes on the following two

Summary of values computed in the principle component analysis in fig. 26

Singular and eigenvalues for the SVD (U LAMBDA V)

Singular values	Eigen values	Cumulative % of Eigenvalues	Eigen values	Cumulative % of Eigenvalues
1.7367	3.01613	0.33513	3.01613	0.33513
1.27927	1.63652	0.51696	1.63652	0.51696
Sum of eigenvalues	9		9	

Table 9 Key to codes representing species used in the principal component analysis in figure 26. Codes consist of the clade number followed by 2-3 digits of the species epithet.

Code	Species	Clade	Code	Species	Clade
lad	*R. adusta*		5qu	*R queletii*	
lad	*R. anthracina*	lb	5fu	*R* .cf. *fuscorubriodes*	5e
lal	*R. albonigra*		5ca	*R. cavipes*	
ldi	*R. dissimulans*		6pe	*R. pelargonia*	
2br	*R. brevipes*		6sa	*R. sanguined*	**6**
2ca	*R. cascadensis*	**2b**	6am	*R. americana* var *modicaspora*	
3ce	*R. cerolens*		71u	*R. lutea*	7a
3pe	*R. pectinatoides*	3b	7mu	*R. murrillii*	
3pd	*R. di.pectinata*		7al	*R. albida*	7b
3gr	*R. granidata*		71e	*R. lepidiformis*	
3fr	*R. fragrantissima*	3c (or la)	8ol	*R. olivacea*	**8**
3pa	*R. pallescens*		8vet	*R. veternosa*	8c
3cr	*R. crassotunicata*	3d	8vel	*R. velenovskyi*	
4mu	*R. mustelina*	4b	8inl	*R.* cf. *integra* 1	**8d**
4br	*R. brunneola*		8in2	*R.* cf. *integra 2*	
4pa	*R. parazurea*		8occ	*R. occidentalis*	
4me	*R. medulatta*	4d	9sp	*R. sphagnophUa*	9a
4su	*R.* cf. *sublevispora*		9br	*R. brunneoviolacea*	
4a-s	*R. aeruginea*		9ab	*R. abietina*	

Code	Species	Clade
5vi	*R. viscida*	5a
5 st	*R. stuntzii*	
5ra	*R. raoultii*	5b
5cr	*R. crenulata*	
5fr	*R. fragilis*	
51a	*R. laccata*	
5 si	*R. silvicola*	
51u	*R. luteotacta*	5d

Code	Species	Clade
9ae	*R. aeruginoides* nom prov	
9xe	*R. xerampelina*	**9b**
9se	*R. semirubra*	
9is	*R. isabelliniceps*	
9el	*R. elaeodes*	
lOau	*R. aureofulva*	10a
lOce	*R. cessans*	
lOpu	*R. puellaris*	10b
lOna	*R. nauseosa*	

Conclusions

The best indicators of phylogenetic position amongst the characters assessed above are, in order: basidium width, spore colour, spore ornamentation height, spore L:W, spore length and the maximum amount of reticulation on the spores. Basidium length is a poor predictor of phylogenetic position. These numerically expressible characters are in addition to the qualitative characters such as odours, taste, gill arrangement and amyloidity of the spore suprahilar patch, that are described in the keys to Vancouver Island Russulas that follow in Appendix 3, and to those previously discussed. Another microscopic character that appears to be linked to the phylogenetic branching order is average pileocystidia septation, which tends to increase in upper clades.

One character that is linked to clade, but not forming an overall trend, is the texture of the basidioma. Some are firm and elastic, others are firm and brittle, while others are quite soft and fragile at an equivalent growth stage, however this does not translate to easily measurable characters of the trama such as the relative amounts of sphaerocytes to generative hyphae. One may possibly measure the amount of force needed to break a certain thickness of flesh, but even this would have to take into account weather conditions, age of the basidioma and the amount of fungivorous arthropod infestation.

One of the issues brought up by the phylogenetic analyses in chapter 3 part II was the apparent mismatch between clade 8 and the current taxonomic system, where species with incrustations in the cap cutis were aligned with those without, and the fact that clades 7 and 8 have primordial hyphae but clades 9 and 10 are considered to lack this trait. By examining first-hand all the collections from these groups, I found hyphae in the epicutis of some of the *Tenellae* (clade 10) that show similarities to primordial hyphae, but are not encrusted (see *R. puellaris* and *R. nauseosa),* and occasional traces of incrustations in the epicutis of *R. veternosa*. This finding supports the phylogenetic analyses and indicates that the upper clades 7-10 could legitimately be placed together within subgenus *Incrustatae.*

The results of the character analyses of Vancouver Island collections supports, clarifies, and adds to those findings made from the literature in chapter 3 part II.

Taken together, microscopic characters alone can be used to narrow down an identification to within two or three clades, as shown by the principal component analysis. The spore colour narrows this down further and in theory at least, the additional use of macroscopic and habitat characters would narrow the possibilities down to one or two species.

Chapter 5

Summary

Russula species found in Vancouver Island coastal forests

The first question addressed in this thesis was "Are there are more species of *Russula* to be found on Vancouver Island than are currently recorded from local foray data and from published accounts of Pacific Northwest collections, and what are the characteristics and habitats of local forms of recorded and newly observed species?"

Sixty-one species and varieties of *Russula* have been described below (Appendix 3), 60 of which are represented by 229 collections from Vancouver Island. Thirty-one of these species are among the 78 (non-sequestrate) *Russula* species previously reported from the Pacific Northwest (Woo, 1988, Gibson and Gibson, 2004), confirming their occurrence in this region. There are two new species: *Russula auruginoides* nom. prov. and *R. aureofulva* nom. prov.; two new varieties: *R. americana* var. *modicaspora* nom. prov., and *R. fragilis* var. *mitis* nom. prov. and a proposed raising to species status of *Russula xerampelina* var. *isabelliniceps,* renamed *Russula isabelliniceps.* New records for the Pacific Northwest include 11 species: *Russula albida, R. anthracina* var. *insipida, R.c€ luteotacta, R. ctpruinosa, R.* c£ *subvelispora, R.ct fuscorubroides, R. laccata, R. lepidiformis, R. medullata, R. nauseosa* and *R. velenovskyi* and one variety: *R. queletii* cf. var. *flavovirens.* Some of these names derive from European descriptions and in some cases these lacked sufficient detail to make a confirmed identification; in others the Vancouver Island collections varied slightly from the European species. These issues are discussed in the notes section of each of the descriptions in Appendix 3.

The descriptions of Vancouver Island material contain new information and illustrations that will enable other taxonomists of the genus to judge the veracity of the identifications and compare them with their own collections more critically. It serves as a partial monograph to local species, building on those of Grund (1965) and Woo (1998). In addition, a further 48 species are included in the key, since these have been reported from the Pacific Northwest in a variety of publications and foray lists (Gibson 2003, Woo 1998,). Some of these have not been confirmed, a few could be erroneous, but their inclusion in the key widens the scope of possible identifications of a given collection. Several Vancouver Island collections have not been satisfactorily identified to date, and are not included in these descriptions. Such problems are common here where studies of the local fungal flora have barely begun.

Habitat specificity

Of the 60 Vancouver Island species 42 were found in the coastal Douglas-fir moist maritime biogeoclimatic zone (CDFmm), of which 26 were only found in that zone, 3 in stands which also included pines or pine-madrone, 3 in garry oak stands, and one with willows bordering a lake. Not counting the latter 7 species, there were 6 species found only in old-growth Douglas-fir forest, 13 found only in regeneration forest, and 10 species found in both ages of forest.

A total of 34 species were found in the coastal western hemlock very wet hypermaritime zone (CWHvhl), of which 18 were found only in that zone. The CWHvhl zone has four categories in this thesis, two are age classes of western hemlock forest, a third, the spruce fringe, is a strip of forest along the seafront that is particularly rich in Sitka spruce, many of the trees are stunted and small, but the sites have not generally been logged. The seafront dunes are somewhat similar but with more shore pine, salal and kinnickkinnick and a loose, sandy substrate. The western hemlock old growth, regeneration forest, spruce fringe and dunes had, respectively, 26, 19, 17 and 8 *Russula* species, of which 3,1,1 and 2 were found only in that one habitat. Just 5 species were found in all CWHvhl habitats.

Present in both zones were 16 species, which most likely have western hemlock as a mycorrhizal host and/or red alder or ericaceous plants, since these too are in both zones. These species have a tolerance of a relatively wide moisture range. Ubiquitous in all habitats except garry-oak stands were *Russula brevipes, R. brevipes* var. *acrior* and *R. fragilis.* These species have a wide distribution throughout North America and may have a range of host trees, although locally *R. fragilis* has consistently been observed with western hemlock in its vicinity, sometimes merely an understory seedling.

Although many species are occasionally found on rotten wood, simply because it is a major part of the forest floor, especially in old growth forest, 5 species are regularly if not always on rotten wood: *Russula stuntzii, R. raoultii, R fragilis, R. fragilis* var. *mitis* and *R. silvicola. Russula stuntzii* is found on wood in an advanced stage of decay, the others can colonize wood that retains some integrity. *R. aeruginoides* was found just once, on wood, which may or may not be its required substrate. The table below summarizes the main habitats for each species.

The habitat is not useful in defining any clades, at least not with the resolution the 61 Vancouver Island species and those used in the phylogenetic analyses can provide. Of interest are the fact that 5 of the 6 species on woody debris were in clades 5 a and b, all representatives of clade lwere found in regeneration forests, not old-growth, and those of clade 3 were all found less than 2km, and in most cases less than 1km from the sea shore.

Table 9 Summary of biogeoclimatic zones and habitats of Vancouver Island Russulas

	Zone	**CDFmm**				**CWHvhl zone**				**Specific habitats**
Clade	Species	DF1	DF2	Garry oak	Other hosts	WH1	WH2	**SF**	**D**	
la	*R. farinipes*					WH1				
la	*R. crassotunicata*					WH1		**SF**	**D**	
lb	*R. adusta*		DF2							
lb	*R. anthracina* var. *insipida*		DF2							
lb	*R. albonigra*		DF2							
lb	*R. dissimulans*		DF2				WH2			
2b	*R. brevipes*	DF1	DF2			WH1	WH2	**SF**	**D**	
2b	*R. brevipes* var. *acrior*	DF1	DF2			WH1	WH2	**SF**	**D**	
2b	*R. cascadensis*		DF2			WH1				
3b	*R. cerolens*		DF2							
3b	*R. pectinatoides*	DF1	DF2							
3b	*R. cf.pectinata*								**D**	
3b	*R. granulata*	DF1								
3c	*R. fragrantissima*					WH1	WH2	**SF**	**D**	
4a	*R. smithii**					WH1				
4b	*R mustelina*		DF2							
4b	*R. brunneola*					WH1	WH2	**SF**		
4d	*R. parazurea*		DF2							
4d	*R. medullata*								**D**	
4d	*R.* cf. *subvelispora*		DF2							
4d	***R. aeruginea***		DF2			WH1	WH2	**SF**	**D**	
5a	*R. viscida*				Madrone -pine					
5a	*R. stuntzii*	DF1	DF2			WH1				On woody debris
5b	*R raoultii*	DF1	DF2			WH1	WH2			On woody debris
5b	*R. crenulata*			Oaks						
5b	*R. fragilis*	DF1	DF2			WH1	WH2	**SF**	**D**	On woody

	Zone	**CDFmm**				**CWHvhl zone**				**Specific habitats**
Clade	Species	DF1	DF2	Garry oak	Other hosts	WH1	WH2	**SF**	**D**	
										debris
5b	*R. fragilis* var. *mitis*					WH1	WH2	**SF**		On woody debris
5b	*R. laccata*				Willow					
5b	*R. silvicola*		DF2			WH1		**SF**		On woody debris
5d	*R. luteotacta*						WH2			Alders
5e	*R. queletii*					WH1		**SF**		
5e	*R. queletii* cf. var. *flavovirens*							**SF**		
5e	*R. fuscorubroid.es*					WH1	WH2			
5e	*R. cavipes*	DF1								
6	*R. pelargonia*		DF2							
6	*R. sanguinea*				Pine		WH2			sometimes in bogs
6	*R. americana* var. *modicaspora*	DF1				WH1	WH2			wet seeps
7a	*R. lutea*			Oaks						
7a	*R. murrillii*	DF1	DF2			WH1	WH2			
7b	*R. albida*	DF1								
7b	*R. lepidiformis*			Oak?						
8a	*R. olivacea*	DF1								
8c	*R. veternosa*		DF2							
8d	*R. velenovskyi*				Madrone /pine					
8d	*R. integra* 1					WH1		**SF**		
8d	*R. integra* 2		DF2							
8d	*R. occidentalis*	DF1	DF2			WH1	WH2			
9a	*R. sphagnophila*					WH1		**SF**		
9a	*R brunneoviolacea*		DF2							
9a	*R. abietina*	DF1	DF2							
9a	*R. aeruginoides*					WH1				On woody debris

	Zone	**CDFmm**				**CWHvhl zone**				Specific habitats
Clade	Species	DF1	DF2	Garry oak	Other hosts	WH1	WH2	SF	D	
9b	*R. xerampelina*	DF1	DF2				WH2 ?			
9b	*R. semirubra*		DF2			WH1				
9b	*R. isabelliniceps*					WH1	WH2	SF		
9b	*R. ctpruinosa*	DF1								
9b	*R. elaeodes*	DF1				WH1	WH2	SF		
10a	*R. aureofulva*	DF1								
10a	*R. cessans*		DF2							
10b	*R. puellaris*					WH1		SF		
10b	*R. nauseosa*						WH2	SF		

Abbreviations used in the table above: DF1= old growth Douglas-fir forest and mixed-age stands with veteran trees, DF2. = regeneration Douglas-fir forest, WHl=old growth western hemlock forest, WH2 regeneration western hemlock forest, SF = spruce fringe, D =dunes, **Russula smithii* was not yet found on Vancouver Island.

Evaluation of the various tools of identification

The second question addressed in this thesis asks "While traditional identifications rest on morphological characters, can the RFLPs of an amplified piece of DNA, specifically the ITS region of the rDNA gene be usefully compared with virtual restrictions of published sequence data to confirm an identification, and can paper chromatography of cuticle pigments be employed in the identification of taxonomic groups?"

Both the RFLP's of the ITS region of rDNA and the chromatography methods were able to place collections into major clades, but identifying individual species requires the macroscopic and in most cases the microscopic investigation of morphology. What is useful about all these methods is that they support one another in the majority of cases, providing confirmation of the identification. Where there is ambiguity in morphological characters, such as in immature basidiomata or difficulty matching all characters to a given description, then the RFLP data or the chromatography data or both supply additional information towards a resolution. Examples from this study include an immature basidioma, in which the RFLP analysis showed a pattern closer to that of *R. xerampelina* rather than its close relative *R. isabelliniceps*.

The chromatographs showed that the new species *R. aeruginoides*, was closer to the *Sphagnophilae* in clade 9 than it was to the *Laricinae* in clade 10, because its magenta pigments were more like those of the *Xerampelinae* of clade 9. Not all the collections yielded sufficient pigment to run a chromatograph. Pale, faded specimens do not make good candidates for this analysis and it is difficult to get a clean separation of pigments in those with a very viscid cutis. Attempts to precipitate the viscid substance with alcohols failed because the pigment co-precipitates. In conclusion, neither the RFLP patterns of ITS rDNA nor the chromatographs can substitute for an examination of morphological characters; their most useful role is in pre-screening of multiple collections and support or clarification of other data.

Evaluation of morphological characters in relation to phylogenetic relationships and clade structure

The final question this thesis addresses is: "How does the suite of morphological characters used in traditional classification within the genus *Russula* relate to the clade structure within a phylogenetic tree based on DNA sequences, and can a more efficient identification key be based on those characters sorted by the taxonomic level at which each is useful?"

Morphological characters and clade structure

There are both continuous and discontinuous characters following the branching order of the phylogenetic tree. There is a general increase in spore colour, the height of spore ornamentation, and basidium width from basal to upper clades, and a decrease in the length to width ratio of spores and also in the amount of variation in this character. As long as it is borne in mind that there are variations about this trend, a set of these characters will quickly give an approximate position along the phylogenetic tree. The charts of these characters in chapter 4 are useful for this. Continuous characters are more difficult to use in a key, which necessarily breaks species down into groups, but are very useful in placing new species, especially when these have reduced epicutis characters as in *R. olivacea* group. Discontinuous characters are the ones most useful in keys, particularly for those seeking to familiarize themselves with the genus.

The keys given in Appendix 3 for Vancouver Island Russulas and those reported from the Pacific Northwest take two approaches; the polychotomous key assumes a relative unfamiliarity with the genus and divides groups of species by more obvious and initially macroscopic characters. These follow phylogenetic order in the major clade divisions and those of the finer divisions that can be unambiguously segregated by the relative novice. Other species are keyed out as groups of superficially similar species. The synoptic keys divide species up by each character individually, beginning with macroscopic characters and working down to microscopic ones. This type of key lends itself to computerized searches which tend to be more useful for those with more familiarity with the genus, since they can concentrate only on relevant characters and thereby shortcut through higher clade divisions. The synoptic keys are not sorted into a taxonomically based order in which they should be consulted; while this would be easy to do, it would make finding a given character unwieldy, and would not be relevant anyway were these keys on computer and accessed by search terms.

Microscopically the primary division is based on the amyloid reaction of the suprahilar patch on the spores, splitting clades 1, 3 and 4 from 5-10, with clade 2. The keys to the Vancouver Island Russulas (Appendix 3) use several macroscopic characters in the earlier queries which further divide the two primary clades. The type of character that defines one higher level clade differs from that which defines another. In the polychotomous keys clades 1 and 2 are defined by the gill arrangement, and distinguished from one another by bruising reactions. Clade 3 is defined by a number of characters including the appearance of the cap and the odour. Clade 4 is defined mostly by microscopic characters (e.g. spores, epicutis) but also a particular stature and feel, which is easier to demonstrate by touch than it is to put into words or photographs. This clade also differs in cutis pigments from similarly coloured species in higher clades. Clades 5 - 10 can be defined macroscopically by cutis pigments, and separated by a combination of spore colour, taste, bruising reactions, pileocystidia and/or primordial hyphae and the tendency or otherwise of the cap margin to become striate. Clade 9b is defined by its reaction with FeS04 and cuticular structure). Cap colour is useful but plays a much less important role in *Russula* taxonomy than it does in many other agaric genera, yet it is often the first thing people look for in a key.

Taxonomic observations and recommendations

Amongst the microscopic characters the epicutis structures and their chemical reactions have been used taxonomically for at least 40 years to group species, and remain among the primary tools for this. Primordial hyphae, which were considered to be confined to mild tasting species in clades 7 and 8, also occurred in peppery species, and in clades 9 and 10 stuctures appearing similar to non-incrusted primordial hyphae were occasionally seen. The colour, shape and ornamentation height of spores, and particularly the width of mature basidia show continua from basal to upper clades that allow a fairly accurate placement of query species within the phylogeny. However the basidium length is less important than has been previously assumed, save that clades 5 and 6 have generally the shortest basidia.

Within clades the basidial maximum length may be important, but this requires further examination with a larger sample size. Spore ornamentation, particularly the degree of reticulation, and again, spore colour are useful in conjunction with macroscopic characters.

In view of the fact that a relatively few species *of Russula,* taken mostly from the northern temperate zones of Europe and North America were phylogenetically analyzed, it would not be prudent to make major taxonomic revisions until a wider sampling of Russulas has been similarly analyzed, both through DNA and morphological studies. Having said that, all the data in this study point to narrowing the scope of subgenus *Russula* sensu Samari (1998) to include only section *Russula* subsections *Consobrinae* Sarnari, *Russula* (Romagn.) Sarnari, and *Sardoninae* (Singer) Sarnari.

The subgenus *Incrustatae* (Romagn.) Sarnari might then be expanded (and perhaps renamed) to house those species that have incrusted or none-incrusted primordial hyphae and broad basidia. Since clades 7-10 do not show high level phylogenetic divisions, this would entail their inclusion in the expanded subgenus. Ostensibly subgenus *Incrustatae* amended would include sections *Messapica* Sarnari, *Paraincrustatae* Sarnari, *Polychromae* (Maire) Sarnari (except for subsection *Auratinae* Bon), section *Tenellae,* and subsections *Rubrinae* (Metzer and Zvara) Singer and *(/rentes* Maire; all currently in subgenus *Russula.* Those groups represented by species forming clade 6 should undergo further study to verify and understand their position.

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Appendix 1

Table 11 Excerpt 1 from Crluchoff 1969; Etude chimiotaxinomique des pigments des Russules: Proprietes spectrales et chromatographiques des Pigments de Russules

The first part of Table II dealing with the visible and UV colours:

COMPOSES	COULEUR		
	visible	UV	+NH4OH
Russularhodines			
Rrl	rose-orange	orange	rose-violace (vis.)
Rr.II	rose-violace	orange	
Rr.a	rose-orange	orange	
Rr. *d'Heterophyllae*	rose-maUVe	orange	
Russulacvanines			
Rc.I	bleu-vert	nen	nen
Rc.II	bleu	rien	rien
Re. *d'Heterophyllae*	bleu-violet	orange?	
Russulaxanthines			
Rx.I ("aurataxanthine")	jaune-or	ocre	
Rx.II	jaune	jaune-citrin	jaune+ pale
Rx.III	jaune-citrin	jaune-vert	bleu-vert (UV)
Rx.IV	jaune-citrin	jaune-vert	bleu-vert (UV)
Rx.V	jaune-citrin	jaune-vert	J.+pale(vis.)verdatre(UV)
Rx.VI	jaune	jaune-vert	
Rx. *d'Heterophyllae*	jaune	jaune-or	

Table 12 Excerpt 2 from Gluchoff 1969; Etude chimiotaxinomique des pigments des Russules.

	Rrl	RrH	Rra	RcI	RcJI	RxJ	RxJI	]EUDI	RxJV	Rx.V	Rx.VI	VI
PPHUNAE												
Rkpida	**4+**	**(+)**	**(+)**	?		+	?	-	-	(+)UV	-	
Emetidme												
Remeticafgp.)		(+)UV	?	-		(+)	+UV	-	-	+		
RMcdrd		**(+)**	**(+)**	-			++UV	-	-	(+)	-	
Persicinae												
Rluteofacta	**++**	**(+)**	**(+)**	-		+UV		-	-	++UV	-	
Attvputpurinae												
RAttxjpurpurea	**(+)**	**-H-**	**?**		+	+UV	(+)UV	-	-	(+pv	**-**	
Rfcctgilis	+	++UV		(+)	-	(+)UV	(+)UV	-	-	(+)	-	
Sanfomnae												
Rscaiguinea	+	(+)UV	(+)	(+)	-	+UV	(+)UV	-	-	(+)	-	
Rscrdam	+	+	(+)	+	(+)	(+)UV	(+)UV	-	-	(+)	-	
Rcavipes	+	(+)UV		+			(+)UV	-	-	+		
Rquektii	++	?	(+)	+	(+)	?	(+)UV	-	-	**-H-**		
Rbadia	-++	+	(+)	+	(+)	(+)uv	(+XJV			++		
NCRUSMEAE												
Raseinae												
Rrosea		?	(+)UV	-	(+)	?	+UV			++	- i	
RmimMda				-			+UV	-	-	++UV	-	**+**
Rpseudcmtegra		?	(+)UV	-		?	(+)UV	-	-	(+)UV	-	**+**
Rfflacea	**+**			+	?		(+)UV	-	-	+		
Amdhystmcte												
Rturci		(+)UV	(+)UV	+	(+)	+UV	(+)UV	-	-	++UV	-	
Chamekontinae												

	Rrl	RrJI	Rra	RcI	RcJI	Rxl	RxJI	RxJU	RxIV	RxV	RxVI	VI
R chanaekontina	(+)UV	(+)				(+)		++	+	-KJV	-	+
R vitellim						(+)		+	?	(+)UV	-	+
TENEUAE												
Sphcgjtxjphihe												
Rbrwmoviolacea	+++	(+)UV	(+)	+	+		(+)UV			(+)		
POLYCHROMAE												
Meffiokntinae												
Rmeltiokns	(+)	(+)			(+)		(+)uv			+		
Viridantinae												
Retythrcpoda	+++	(+)UV	(+)	+	(+)		(+)UV			(+)		
Integradnae												
Rdcovflam	-	(+)	-			(+)		++	+	+uv		+
Integrinae												
Rirtfegra	+	(+)UV		+	(+)	(+)UV	(+)UV			(+)		
COGONAE												
Decdorcaitmae												
Rdecobnms	(+)	(+)UV		(+)		(+)	(+)					
PoUomae												
Rpaludosa	4+	(+)		(+)	?	?	(+)UV			+		
Laetmae												
Rcmata	+	(+)UV	?		(+)	-H-	+					
JMDI0S4E												
Urentime												
Raduherim	-H-	(+XJV		+		(+)UV	(+)UV			+		
MGRAT4E												
Felkinae												
Rfellea	-											
Rcomdrrim		(+)W										
Rochrokuca	–	-						4++	+	4UV		

	Rrl	RrH	Rra	RoIRcJI	Rxl	RxH	RxDI	RcIV	RxV	RxVI	VI
<u>*Foetentinae*</u>											
R pectinate/ides											
Rkoffvcems											
<u>*HETEROPHYUAE*</u>											
<u>*Virescenlmae*</u>											
Ramoem	(+)			+		(+)UV			(+)UV		
Rviokipes		(+)UV		(+)		-HJV			?		
<u>*Indolentincie*</u>											
R cyanaocailha											(+)
Grisemae											
										+	
<u>*HeterqjhMnce*</u>											
Rvesca										+	

Q =50(D.O.)mlg[1]	RrJ	RrH	Rr.a	Re.I	Rail	RxJ	RxH	RxHI	RxJV	RxV
Remetica 72-65	●					D			a	
Remetica 72-8	D					D			a	
Rmma 65-68	_j	•	a			D			•	
Rmma 2-08-72						D			0	
Rmma 13-08-71	[Z3	a	a			0			D	
Rmaculata 69-253	a	D			•	Q				
ft *heterochroa* 67-110	0	D			D	•				
R heterochroa 71-9	a	CD			a					
Rncmseosa 73-335	a	o			a	i	o			
Rpurpwofusca *12-60*			a	a		D				]
R saliceticokt 72-31			a	D		a				
ft *saliceticola* 72-13			D	D					a	
it *saliceticola* 72-72			a	D		a				
ft *saliceticola* 66-135	n		Q	●		•				
ft *saliceticola* 72-111			0	*CD*		0				
ft *saliceticokt* 6646			0	D		0				
Rnarvegica f *norvegica* 72-134			o	●					D	
	RrJ	Rr.H	Rr.a	Re. I	Re. II	RxJ	Rx.II	Rx.111	Rx.IV	Rx.V

Russules alpines: contenu pigmentaire de la cuticule pileique.

Rr.I, II, a: russularhodines I, II, a.

Rc.I, II: russulacyanines I, II.

Rx.I, II, III, IV, V: russulaxanthines I, II, III, IV, V.

Figure 27 Pigment analysis of cuticles of alpine Russulas, reproduced from Gluchoff 1975.

• = 5 0 p £) i r i g J	RrJ	Rr.H	Rr.a	Rcl	Rc.II	Rxl	Rx.II	Rx.ni	Rx.IV	RxV
ft *norvegica* f. *norvegica* 71-10			0	D		D				D
ft *norvegica* f. *norvegica* 22-08-72			0	●		a				D
R. norvegica 72-177	CD		a	●		0				D
R. norvegica 66-151	●		D	D		a				
ft *norvegica* 14-08-72			a	a		D				0
ft *norvegica* 14-08-72 v ~~			a	a		a				D
ft *norvegica* 69-305 —	"●		o	●		a				●
ft *norvegica* f. *rubromarginata* 22-08-72			•	a		O				a
f. *rubromarginata* 64-105			0	●		a				D
f. *rubromarginata* 71-19	●		a	a		a				
ft *amoenipes* 72-122			a	D			D			a
ft *chamiteae* 73-332				a	o	0				D
ft *chamiteae* 71-90	a			a		•				0
ft *chamiteae* 69-123	a			0		•				D
ft. *chamiteae* 71-104	a			0		a				a
ft *pascua* 69-126										D
ft. *pseadocampestris* 72-15	a			a		0	|R«.H'!|			0
ft *claroflava* **'"' 72-15										
	RrJ	Rr.H	Rr.a	Re.I	Re.H	Rx.1	RxH	Rx.ni	Rx.IV	Rx.V

Fto. 2.

Figure 28 Pigment analysis of cuticles of alpine *Russulas*, reproduced from Gluchoff 1975.

Appendix 2

Table 13 Sequences downloaded from GenBank and EMBL-EBI and adapted for phylogenetic and RFLP analyses.

FP = final phylogenetic analyses, P = used in earlier phylogenetic analyses but omitted from the final tree, R = RFLP analyses. The remainder of the sequences not used for these analyses were used in initial alignments and as supplemental data.

Species	Accession number	Author	Origin	Analyses	
Russula adulterina	AY061651	Miller, S.L. and Buyck, B.	Europe	FP	
R. adusta	AY061652	Miller, S.L. and Buyck, B.	Europe	FP	R
R. aeruginea	AF418612	Eberhardt,U.	Europe	FP	
R. aeruginea 2	DQ421999	Eberhardt,U.	Europe		R
R. alboareolata	AF345247	Teaumroong, N., Manassila, M. Boonkerd, N. and Rodtong, S.	Thailand	FP	
R albonigra	DQ422029	Eberhardt,U.	Europe		R
R. amethystina	AF418640	Eberhardt, U.	Europe	FP	R
R. amethystina 2	AY061653	Miller, S.L. and Buyck, B.	Europe	FP	
R. amoenicolor	AY061655	Miller, S.L. and Buyck, B.	Europe	FP	
R. amoenipes	AY061656	Miller, S.L. and Buyck, B.	Europe	FP	
R amoenolens	AF418615	Eberhardt, U.	Europe	FP	R
R. amoenolens 2	DQ822824	Peay, K.G., Bruns, T.D., Kennedy, P.G., Bergemann, S.E. and Garbelotto, M.	U.S.A. (CA)		R
R. aquosa	AY061657	Miller, S.L. and Buyck, B.	Europe	FP	
R. archaea	AY061737	Miller, S.L. and Buyck, B.	Africa	FP	
R. atropurpurea	AF418618	Eberhardt, U.	Europe	FP	
R. atropurpurea 2	AY061654	Miller, S.L. and Buyck, B.	U.S.A.	P	
R. aurantiaca	AY061658	Miller, S.L. and Buyck, B.	Europe	FP	
R. aurantiaca 2	AF506427	Larsson,E. andLarsson, K.-H.	Sweden	P	
R. aurata	AY061659	Miller, S.L. and Buyck, B.	Europe	FP	
R. azurea	AY061660	Miller, S.L. and Buyck, B.	Europe	FP	

Species	Accession number	Author	Origin	Analyses	
R. betularum	AY061729	Miller, S.L. and Buyck, B.	Europe	P	R
R. betularum ITS2	AJ534937	Tedersoo, L., Hallenberg, N., Larsson, K.H. and Koljalg, U.	Europe (Estonia)	FP	R
R. bicolor	AY750161	Cline, E.	U.S.A. (WA)	FP	
R. brevipes	AF349714	Bidartondo, M.I. and Bruns, T.D.	U.S.A.	FP	
R. brevipes ITS 2	DQ367912	Durall, D.M., Gamiet, S., Simard, S.W., Kudraa, L. and Sakakibara, S.M.	Canada (B.C.)		R
R. brevipes var. *acrior*	EF411133	Morris, M.H., Smith, M.E., Rizzo, DM., Rejmanek, M. and Bledsoe, C.S.	U.S.A. (CA)		R
R. brunneoviolacea ITS1	AMI 13956	Kjoller, R.	Europe (Denmark)		R
R. caerulea 1	AF418633	Eberhardt, U.	Europe	FP	
R. caerulea 2	AY061661	Miller, S.L. and Buyck, B	Europe	P	
R. californiensis•	AY245542	Davis, R.M. and Wolfe,C.R.	U.S.A. (CA)	FP	
R. camarophylla	AY061662	Miller, S.L. and Buyck, B.	Europe	FP	
R. cavipes	AF418623	Eberhardt, U.	Europe	FP	R
R. cessans	AY061730	Miller, S.L. and Buyck, B.	U.S.A.	FP	R
R. chloroides	AY061663	Miller, S.L. and Buyck, B	Europe	FP	R
R. chloroides 2	AF418604	Eberhardt, U.	Europe	P	
R. claroflava	AY061665	Miller, S.L. and Buyck, B.	Europe	FP	
R. consobrina	AY061666	Miller, S.L. and Buyck, B.	Europe	FP	
R. crassotunicata	EU057119	Wright, S.H.A., Carruthers, E., Lim, S. and Berbee, ML.	Canada (B.C.)		R
R.cl crassotunicata	DQ384580	Berbee, M.L., Wright, S.H.A, Sihota, N.J.J, and Lim, S.	Canada (B.C.)		R
R. cremoricolor 1	AJ277910	Redecker, D., Szaro, T.M., Bowman, R. and Bruns, T.D	U.S.A. (CA)	FP	
R. cremoricolor 2	AJ277911	Redecker, D., Szaro, T.M., Bowman, R. and Bruns, T.D.	U.S.A. (CA)	P	
R. cuprea	AY061667	Miller, S.L. and Buyck, B.	Europe	FP	

Species	Accession number	Author	Origin	An
R. curtipes	AY061668	Miller, S.L. and Buyck, B.	Europe	FP
R. cyanoxantha	AF418608	Eberhardt, U	Europe	P
R. cyanoxantha 2	AY061669	Miller, S.L. and Buyck, B.	Europe	P
R. cyanoxantha 3	AF291361	Weiss, M. and Oberwinkler, F.	Germany	P
R. cyanoxantha 4	AF345251	Teaumroong, N., Manassila, M., Boonkerd, N. and Rodtong, S.	Thailand	FP
R. cyanoxantha 5	DQ422033	Eberhardt, U.	Europe	
R. decolorans	AY061670	Miller, S.L. and Buyck, B.	Europe	P
R. decolorans 2	AF418637	Eberhardt, U.	Europe	FP
R. decolorans 3	AY194601	Fransson, P.	Europe (Sweden)	P
R. decolorans 4	DQ367913	Durall, D.M., Gamiet, S., Simard, S.W., Kudrna, L. and Sakakibara, S.M.	Canada (B.C.)	
R. delica 1	AF418605	Eberhardt, U.	Europe	FP
R delica 2	AF096987	Marin, M., Ibarra, M., Garcia,L. and Ferrer, S.	Europe (Spain)	P
R. delica 3	AF345250	Teaumroong, N., Manassila, M., Boonkerd, N. and Rodtong, S.	Thailand	P
R. delica 4	AY061671	Miller, S.L. and Buyck, B.	Europe	P
R, aff. *delica*	DQ422005	Eberhardt, U.	Europe	
R. densifolia	AF418606	Eberhardt, U.	Europe	FP
R. drimeia	AY061672	Miller, S.L. and Buyck, B.	Europe	FP
R. emetica	DQ421997	Eberhardt, U.	Europe	
R. emetica 1	AY061673	Miller, S.L. and Buyck, B.	Europe	FP
R. emetica 2	AF418619	Eberhardt, U.	Europe	P
R. cf. emetica 3	AY228350	Sheldrake, M., Berbee, ML., Inderbitzin, P. and Fischer, A.L.	Canada (B.C.)	P
R. cf. emetica 4	AY228360	Tso, A, Berbee, ML., Inderbitzin, P. and Fischer, A.L.	Canada (B.C.)	P
R. exalbicans	AY293156	Binder, M., Hibbett, D.S., Larsson, K.-H., Larsson,E., Langer, E. and Langer, G.	U.S.A.	P

Species	Accession number	Author	Origin	Analyses	
R. exalbicans 2	AY061674	Miller, S.L. and Buyck, B.	Europe	FP	
R. farinipes	AY061675	Miller, S.L. and Buyck, B.	Europe	FP	
R. farinipes 2	DQ421983	Eberhardt, U.	Europe		R
R. fellea	AF418616	Eberhardt, U.	Europe	FP	
R.fellea2	AY061676	Miller, S.L. and Buyck, B.	Europe	P	
R. firmula	AF418631	Eberhardt, U.	Europe	FP	
R. firmula 2	DQ422017	Eberhardt, U.	Europe	R	
R. foetens	AY061677	Miller, S.L. and Buyck, B	Europe	FP	
R. foetens 2	AF418613	Eberhardt, U.	Europe	P	
R. foetens 3	AF230895	Calonge, F.D. and Martin, M.P.	Europe (Spain)	P	
R.* cf. *foetens	DQ422023	Eberhardt, U.	Europe		R
R. fragilis	AF230897	Calonge, F.D. and Martin, M.P.	Europe (Spain)	FP	
R. fragilis 2	DQ367914	Durall, D.M., Gamiet, S., Simard, S.W., Kudrna, L. and Sakakibara, S.M.	B.C., Canada		R
R. aff. *fragilis*	AF335443	Berbee, M.L., Inderbitzin, P. and Zhang, G	Canada (B.C.)	P	R
R fuscorubroides	AF418624	Eberhardt, U.	Europe	FP	
R. gracillima	AY061678	Miller, S.L. and Buyck, B.	Europe	FP	R
R. gracillima 2	DQ422004	Eberhardt,U.	Europe		R
R. grisea	AY061679	Miller, S.L. and Buyck, B.	Europe	FP	
R. grisea 2	DQ422030	Eberhardt, U.	Europe		R
R. helodes	AY061680	Miller, S.L. and Buyck, B.	Europe	P	
R. heterophylla	AF418609	Eberhardt, U	Europe	FP	
R. heterophylla 7	AY061681	Miller, S.L. and Buyck, B.	Europe	P	
R. heterophylla 3	DQ422006	Eberhardt, U.	Europe		R
R. ilicis	AY061682	Miller, S.L. and Buyck, B.	Europe	FP	
R. insignis	AY061700	Miller, S.L. and Buyck, B.	Europe	FP	
R. integra	AF230896	Martin, M.P. and Calonge, F.D.	Europe	FP	
R. integra 2	AF418636	Eberhardt, U.	Europe	P	

Species	Accession number	Author	Origin	Analyses	
R. integra 3	AY061683	Miller, S.L. and Buyck, B.	Europe	P	R
R. integriformis	AY061684	Miller, S.L. and Buyck, B.	Europe	FP	
R. laricina	AY061685	Miller, S.L. and Buyck, B.	Europe	FP	
R. laurocerasi 1	AY061735	Miller, S.L. and Buyck, B.	U.S.A.	FP	R
R. laurocerasi 2	AF418614	Eberhardt, U.	Europe	P	R
R. lepida	AF418641	Eberhardt, U.	Europe	P	
R. lepida 2	AY061686	Miller, S.L. and Buyck, B.	Europe	FP	
R. lepida 3	DQ422013	Eberhardt, U.	Europe		R
R. lepidicolor	AY061687	Miller, S.L. and Buyck, B.	Europe	FP	
R. lilacea	AY061731	Miller, S.L. and Buyck, B.	U.S.A.	FP	
R. littoralis	AY061702	Miller, S.L. and Buyck, B.	Europe	FP	
R. maculata	AY061688	Miller, S.L. and Buyck, B.	Europe	FP	
R cf. maculata	DQ422015	Eberhardt, U.	Europe		R
R. mairei 1	AF418620	Eberhardt, U.	Europe	FP	
R. mairei 2	AF230899	Calonge, F.D. and Martin, M.P.	Europe (Spain)	P	
R. melitodes	AY061689	Miller, S.L. and Buyck, B.	Europe	FP	
R. melliolens	AY061690	Miller, S.L. and Buyck, B.	U.S.A.	FP	
R. melzeri	AY061691	Miller, S.L. and Buyck, B.	Europe	FP	
R. messapica	AY061692	Miller, S.L. and Buyck, B.	Europe	FP	
R. mustelina	AY061693	Miller, S.L. and Buyck, B.	Europe	FP	R
R. mustelina 28S	AY606967	Eberhardt, U. and Verbeken, A.	Europe		R
R. nana	AY061694	Miller, S.L. and Buyck, B.	Europe	FP	
R. nauseosa	AY061733	Miller, S.L. and Buyck, B.	U.S.A.	FP	R
R. nauseosa ITS2	AF506462	Larsson, E. and Larsson, K.-H	Europe (Sweden)		R
R. nigricans	AF418607	Eberhardt, U	Europe	FP	
R. nigricans 2	AY061695	Miller, S.L. and Buyck, B.	Europe	P	
R. nigricans 3 (partial ITS 1)	AY228357	Gendron, R, Berbee, MX., Inderbitzin, P. and Fischer, A.L.	Canada (B.C.)	P	
R. nigricans 4	DQ422010	Eberhardt, U.	Europe		R

Species	Accession number	Author	Origin	Analyses	
R. nigricans 5	DQ367915	Durall, DM., Gamiet, S., Simard, S.W., Kudraa, L. and Sakakibara, S.M.	B.C., Canada		R
R. nitida	AY061696	Miller, S.L. and Buyck, B.	Europe	FP	
R. occidentalis ITSI and partial ITS2	AY534206	Horton, T.R, Molina, R. and Hood, K	U.S.A. (OR)	P	R
R. occidentalis ITS2	AY228349	Karst, J., Berbee, ML., Inderbitzin, P. and Fischer, A.L.	Canada (B.C.)	FP	
R. ochroleuca	AY061697	Miller, S.L. and Buyck, B	Europe	FP	R
R. ochroleuca 2	AF418617	Eberhardt, U	Europe	P	
R. ochroleuca 28S	AF325313	Eberhardt, U.	Europe		R
R, ochroleuca ITS2	AY254880	Smit, E., Veenman, C. and Baar, J.	Europe (Holland)	P	R
R. odorata	AY061698	Miller, S.L. and Buyck, B.	Europe	FP	
R. olivacea 1	AF418635	Eberhardt, U.	Europe	FP	
R. olivacea 2	AF418634	Eberhardt, U.	Europe	FP	R
R. olivacea 3	AY061699	Miller, S.L. and Buyck, B.	Europe	P	R
R. pallescens	DQ421987	Eberhardt, U.	Europe		R
R. pallidospora	AY061701	Miller, S.L. and Buyck, B.	Europe	FP	
R. pallidospora	DQ422032	Eberhardt, U.	Europe		R
R. paludosa	AY061703	Miller, S.L. and Buyck, B.	Europe	FP	
R. parazurea	DQ422007	Eberhardt, U.	Europe		R
R. parazurea 2	AY061704	Miller, S.L. and Buyck, B.	Europe	FP	
R, parazurea 3	AF418611	Eberhardt, U.	Europe	P	
R. pascua	AY061705	Miller, S.L. and Buyck, B.	Europe	FP	
R. pectinata	AY061706	Miller, S.L. and Buyck, B.	Europe	FP	R
R. pectinatoides	AY061732	Miller, S.L. and Buyck, B.	U.S.A.	FP	
R. pectinatoides 2	DQ422026	Eberhardt, U.	Europe		R
R. persicina	AF506463	Larsson, E. and Larsson, K.-H.	Sweden	P	
R. persicina 2	AY061707	Miller, S.L. and Buyck, B.	Europe	FP	
R. persicina 3	DQ422019	Eberhardt, U.	Europe		R

Species	Accession number	Author	Origin	Analyses	
R. persicina ITS2	AF506463	Larsson, E. and Larsson, K.-H.	Europe		R
R. postiana	AF230898	Calonge, F.D. and Martin, M.P.	Europe (Spain)	FP	
R. pseudointegra	AY061708	Miller, S.L. and Buyck, B.	Europe	FP	
R. puellaris	AF418628	Eberhardt, U.	Europe	FP	
R. puellaris 2	AY061709	Miller, S.L. and Buyck, B.	Europe	P	R
R piielhda	AY061710	Miller, S.L. and Buyck, B.	Europe	FP	
R. pulverulenta	AY061736	Miller, S.L. and Buyck, B.	U.S.A.	FP	
R. queletii	AF418625	Eberhardt, U	Europe	FP	
R. queletii 2	AY061711	Miller, S.L. and Buyck, B.	Europe	P	
R. raoultii	AF418621	Eberhardt, U	Europe	FP	
R. raoultii 2	AY061712	Miller, S.L. and Buyck, B.	U.S.A.	P	R
R. risigallina	AY061713	Miller, S.L. and Buyck, B.	Europe	FP	
R. romellii	AY061714	Miller, S.L. and Buyck, B.	Europe	FP	
R rosacea	AF096978	Marin, M., Ibarra, M., Garcia, L. and Ferrer, S.	Europe (Spain)	P	
R rosacea 2	AF345249	Teaumroong, N., Manassila, M., Boonkerd, N. and Rodtong, S.	Thailand	FP	
R. rosea	AY061715	Miller, S.L. and Buyck, B.	Europe	FP	
R. roseipes	AY061716	Miller, S.L. and Buyck, B.	Europe	FP	
R. rubra	AY061717	Miller, S.L. and Buyck, B.	Europe	FP	
R. sanguinea	AY061718	Miller, S.L. and Buyck, B.	Europe	P	R
R. sardonia	AF418626	Eberhardt, U.	Europe	FP	
R. Solaris	AF418627	Eberhardt, U.	Europe	FP	
R. sphagnophila	AY061719	Miller, S.L. and Buyck, B.	Europe	FP	R
R. sphagnophila ITS2	AF506464	Larsson,E. and Larsson, K.-H.	Europe (Sweden)	P	R
R. stuntzii	AY281091	Davis, R.M.	U.S.A. (CA)	FP	
R turd	AY061720	Miller, S.L. and Buyck, B.	Europe	FP	R
R. turci 2	EF530935	Denis, M.W., Carruthers, E.K., Wright, S.H. A. and Berbee, M.L.	Canada (B.C.)		R

Species	Accession number	Author	Origin	An	
R. velenovskyi	AY061721	Miller, S.L. and Buyck, B.	Europe	FP	
R. velenovskyi	AY061721	Miller, S.L. and Buyck, B.	Europe		R
R velenovskyi ITS2	AJ966748	Tedersoo, L.; Suvi, T.; Larsson, E.; Koljalg, U.	Europe (Estonia)		R
R. versicolor	AY061722	Miller, S.L. and Buyck, B.	Europe	FP	
R. vesca	AF418610	Eberhardt, U.	Europe	FP	
R. vesca 2	AY061723	Miller, S.L. and Buyck, B.	Europe	P	
R. vesca 3	DQ422018	Eberhardt, U.	Europe		R
R. veternosa	AF418630	Eberhardt, U.	Europe	FP	R
R. vinosa	AF418638	Eberhardt, U	Europe (Estonia)	FP	
R. vinosa 2	AY061724	Miller, S.L. and Buyck, B.	Europe	P	R
R. vinosa ITS 1	AJ534938	Tedersoo L., Hallenberg N., Larsson K.H., Koljalg U.	Europe (Estonia)		R
R. violacea	AY061725	Miller, S.L. and Buyck, B.	Europe	FP	R
R. violacea ITS2	AF506465	Larsson, E. and Larsson, K.-H.	Europe (Sweden) ITS2 only	P	R
R. violeipes	AY061726	Miller, S.L. and Buyck, B.	Europe	FP	
R. virescens	AY061727	Miller, S.L. and Buyck, B.	Europe	FP	
R. virescens 2	DQ422014	Eberhardt, U.	Europe		R
R. viscida	AY061728	Miller, S.L. and Buyck, B.	Europe	FP	
R. xerampelina I	AF418632	Eberhardt, U.	Europe	FP	
R. xerampelina 2	AY061734	Miller, S.L. and Buyck, B.	Europe	P	
R. xerampelina 28S	AY534210	Horton, T.R., Molina, R. and Hood, K.	U.S.A. (OR)	P	
R xerampelina 4	AF540385	Davis, R.M.	U.S.A. (CA)	FP	
R. xerampelina 5	DQ367916	Durall, D.M., Gamiet, S., Simard, S.W., Kudrna, L. and Sakakibara, S.M.	Canada, (B.C.)		R
R. xerampelina ITS2	AY228344	Fischer, A., Berbee, ML., Inderbitzin, P. and Fischer, A.L.	Canada, (B.C.)	P	R

Species	Accession number	Author	Origin	Analyses
Albatrellus flettii	AY061738	Miller, S.L. and Buyck, B.	U.S.A.	FP
Gloeocystidiellum aculeatum	AY061739	Miller, S.L. and Buyck, B.	China	FP
Gymnomyces gilkeyae	AY239346	Whitbeck, K.L., Castellano, M.A. and Spatafora, J.W.	U.S.A.?	FP
Martelliapila	AF230894	Calonge,F.D. and Martin, M.P.	Europe (Spain)	R

Table 14 *Russula* collections used for RFLP analyses in chapter 2 part I, in the order in which they appear in figures 12 and 13. Full descriptions of the species together with information on the origin and habitat of each collection appear in appendix 3, (except *R* cf. *basifurcata* and *Macowanites* sp.).

Taxonomic group	Species	Collection number
Sg. *Compacta* S. *Compactae*	*R albonigra*	BKO10904-01
	R. dissimulans	CR010814-04
	R. dissimulans	SVIMS021027-RN
Sg. *Compacta* S. *Lactaroides*	*R. brevipes*	CR001105-br/a
	R brevipes	CR001001-05
	R. cascadensis	CR001007-cas
Sg. *Ingratula* S. *Ingratae* *Ss.Foetentinae* s. *Pectinata*	*R cerolens*	CR001121-01
	R cerolens	CR021219-01
	R cerolens	CR001007-01
	R. cf. *pectinata*	CR021016-14
	R. pectinatoides	CR001002-02
	R. gi'anulata	CR001108-01
Sg. *Ingratula* S. *Ingratae* *Ss.Foetentinae* s. *Foetens*	*R. fragrantissima*	CR030927-01
	R. fragrantissima	CR030924-01
	R. fragrantissima	CR010814-05
Sg. *Ingratula* S. *Ingratae* *Ss.Farinipedes*	*R. farinipes*	SVIMS021020-02
Sg. *Heterophyllidia* S. *Heterophyllae* Ss. *Heterophyllae*	*R. mustelina*	OC020717-01
	R. mustelina	OC020717-01 (repeat)
	R cf. *basifurcata*	CR001011-57
	R. brunneola	PJ010919-04 W
	R. brunneola	CR030924-02 PR
Sg. *Heterophyllidia* S. *Heterophyllae* Ss. *Griseinae*	*R. medullata*	CR021016-21
	R. aeruginea	CR021016-22
	R cf. *sublevispora*	CR010909-03

Sg. *Ingratula* Ss. *Crassotunicatinae*	*R. crassotunicata*	CR011112-LB.RF.
	R. crassotunicata	CR030924-06
Sg. *Russula* S. *Russula* Ss. *Russula* s. *Atropurpurea*	*R. crenulata*	CR030304-01
	R. stuntzii	CR001024-01
	R. stuntzii	BT021101-01
	R. viscida	CR981114-06
	R. raoultii	CR021118-02
	R. raoultii	CR001127-04
	R. raoultii	CROO1108-05
	R fragilis	CR980825-01
	R. fragilis	CR981014-02
	R. fragilis	CR031026-01
	R. fragilis	CR031130-01
	R. fragilis	CR030924-03
	R. fragilis	CR021015-09
	R. laccata	OC030526-01
	R. silvicola	CR021016-10
	R. silvicola	CR021016-10 repeat
	R. silvicola	CR981202-05
	R. silvicola	CR001011-53a
	R. silvicola	CR981014-01
Sg. *Russula* S. *Russula* Ss. *Sardoninae* s. *Sardonia*	*R fuscorubroides*	CR981013-07
	R. fuscorubroides	CR000919-02
	R. fuscorubroides	CR001012-23
	R fuscorubroides	CR021015-21b
	R queletii	CR981029-0 lb
	R. queletii	CR010919-05
	R. queletii	CR010919-05 repeat
	R. queletii	CR020927-04
	R. queletii cf. var. *flavovirans*	CR981029-02
	R. cavipes	CR021201-01

Sg. *Russula* S. *Russula* Ss. *Violaceinae*	*R. pelargonia*	CR981003-02
Sg. *Russula* S. *Russula* Ss. *Sardoninae* s. *Sanguinea*	*R. americana* var. *modicaspora*	CR981118-01
	R. americana var. *modicaspora*	CR001012-21
	R. sanguinaria	PJ981124-03
	R. sanguinaria	CR030110-01
Sg. *Russula* S. *Paraincrustatae* Ss. *Lepidinae*	*R. albida*	CRO10516-01
Sg. *Incrustatula* S. *Amethystinae* Ss. *Amethystinae*	*R. murrillii*	CROO1104-02
	R. murrillii	CR021015-13
	R. murrillii	CR011111-02
Sg. *Incrustatula* S. *Amethystinae* Ss. *Olivaceinae*	*R. olivacea*	CR021019-01
Sg. *Russula* S. *Russula* Ss. *Urentes*	*R. veternosa*	CR001029-Breit
	R. veternosa	CROO 1108-03
	R. veternosa	CRO 10909-01
Sg. *Russula* S. *Russula* Ss. *Rubrinae*	*R.* cf. *rubra*	CR001202-04
Sg. *Russula* S. *Paraincrustatae* Ss. *Integrae*	*R.* cf. *Integra*	CR021027-04
	R. cf. *Integra*	CR021028-01
Sg. *Incrustatula* S. *Amethystinae* Ss. *Integroidinae*	*R. occidentalis*	JJ021020-03
Sg. *Russula* S. *Tenellae* Ss. *Sphagnophilae*	*R.* cf. *brunneoviolacea*	CR981114-01
	R. sphagnophila	CR011030-03
	R. abietina	CR001104-01
	R. abietina	DGO10902-01

Sg. *Russula* S. *Polychromae* Ss. *Xerampelinae*	*R. xerampelina*	CR001028 Breit
	R. xerampelina	CROO1001-02
	R. xerampelina	CROO1127-01
	R. xerampelina	CROO 1202-02
	R. xerampelina	CR010814-03
	R. xer.ct pruinosa	PJO10922-02
	R. elaeodes	PJ010919-PJ
	R. elaeodes	CR001011-55
	R. isabelliniceps	CR000920-04
	R. isabelliniceps	PK021112-01
	R. isabelliniceps	JD021027-02
	R. isabelliniceps	CR030924-08
	R. isabelliniceps	CR030924-05
	R. semirubra	CR001007-02
Sg. *Russula* S. *Tenellae* Ss. *Laricinae*	*R cessans*	CR981120-01
	R. cf. *cessans*	CRO11020-03
	R. Macowanites sp.	CR030924-
	R. aureofulva	CROO1108-06
	R. aureofulva	CR021009-01
	R. aureofulva	CR031101-01
Sg. *Tenellae* S. *Tenellae* Ss. *Puellarinae*	*R. puellaris*	CR021016-11
	R. puellaris	BK0U030-BKrf
	R. puellaris	CR020927-03
	R. nauseosa	CR010814-01
	001007-04	CROO 1007-04

Output files of the Bam be program running from the Pasteur Institute server

Summary of sequence data of all 111 sequences in the alignment and the initial parameters selected.

Output generated by BAMBE version 2.03 Beta

Data Summary

111 number of taxa

870 total number of sites

647 total number of unique sites

Number of constant sites

Category	A	G	C	T	Total
1	2	3	3	4	12
2	47	35	34	42	158
3	8	8	6	9	31
Total	57	46	43	55	201

Overall	Count	Proportion
A	16870	0.1747
G	16843	0.1744
C	18802	0.1947
T	20552	0.2128
-	23488	0.2432
R	3	0.0000
M	0	0.0000
S	1	0.0000
V	0	0.0000
W	2	0.0000
K	3	0.0000
D	0	0.0000
Y	6	0.0001

H	0	0.0000
B	0	0.0000

Category		A	G	C	T		other
1	Pi	0.2145	0.2245	0.2835	0.2775		
	Prop	0.1495	0.1565	0.1976	0.1934	0.3030	0.0001
2	Pi	0.2813	0.2283	0.2506	0.2397		
	Prop	0.2797	0.2270	0.2491	0.2383	0.0058	0.0001
3	Pi	0.2028	0.2370	0.2423	0.3179		
	Prop	0.1362	0.1592	0.1627	0.2135	0.3282	0.0002

Taxon	A	G	C	T	-	other
1	0.1701	0.1770	0.2080	0.2103	0.2345	0.0000
2	0.1701	0.1759	0.2034	0.2046	0.2460	0.0000
3	0.1736	0.1805	0.2080	0.2080	0.2299	0.0000
4	0.1701	0.1747	0.1977	0.2069	0.2506	0.0000
5	0.1701	0.1747	0.1977	0.2069	0.2506	0.0000
6	0.1759	0.1690	0.1874	0.2207	0.2471	0.0000
7	0.1736	0.1690	0.1943	0.2161	0.2471	0.0000
8	0.1724	0.1713	0.1839	0.2264	0.2460	0.0000
9	0.1690	0.1690	0.1770	0.2333	0.2517	0.0000
10	0.1759	0.1667	0.1839	0.2241	0.2494	0.0000
11	0.1747	0.1724	0.1862	0.2172	0.2494	0.0000
12	0.1770	0.1655	0.1851	0.2253	0.2471	0.0000
13	0.1609	0.1563	0.1782	0.2138	0.2908	0.0000
14	0.1632	0.1575	0.1770	0.1966	0.3046	0.0011
15	0.1667	0.1690	0.1920	0.2184	0.2540	0.0000
16	0.1667	0.1690	0.1920	0.2184	0.2540	0.0000
17	0.1563	0.1759	0.1908	0.2011	0.2759	0.0000
18	0.1644	0.1713	0.2011	0.2161	0.2471	0.0000
19	0.1598	0.1690	0.1908	0.2092	0.2713	0.0000

20	0.1644	0.1632	0.1874	0.2218	0.2632	0.0000
21	0.1678	0.1701	0.1920	0.2103	0.2598	0.0000
22	0.1644	0.1713	0.2115	0.2092	0.2437	0.0000
23	0.1724	0.1540	0.1989	0.2000	0.2747	0.0000
24	0.1770	0.1621	0.1989	0.2184	0.2437	0.0000
25	0.1632	0.1678	0.1862	0.1920	0.2908	0.0000
26	0.1770	0.1736	0.1897	0.2207	0.2391	0.0000
27	0.1816	0.1770	0.1885	0.2241	0.2287	0.0000
28	0.1816	0.1816	0.1954	0.2218	0.2195	0.0000
29	0.1897	0.1793	0.1931	0.2264	0.2115	0.0000
30	0.1839	0.1908	0.2000	0.2195	0.2057	0.0000
31	0.1632	0.1667	0.2023	0.1897	0.2782	0.0000
32	0.1621	0.1644	0.2011	0.1862	0.2862	0.0000
33	0.1759	0.1782	0.1943	0.1989	0.2529	0.0000
34	0.1713	0.1759	0.1931	0.2069	0.2529	0.0000
35	0.1770	0.1793	0.1954	0.2195	0.2287	0.0000
36	0.1667	0.1782	0.1977	0.2000	0.2575	0.0000
37	0.1793	0.1713	0.2069	0.2057	0.2368	0.0000
38	0.1828	0.1770	0.1966	0.2172	0.2264	0.0000
39	0.1747	0.1701	0.2000	0.2046	0.2506	0.0000
40	0.1770	0.1713	0.2023	0.2092	0.2391	0.0011
41	0.1839	0.1805	0.2023	0.2184	0.2149	0.0000
42	0.1701	0.1701	0.1977	0.2057	0.2563	0.0000
43	0.1770	0.1770	0.2046	0.2057	0.2356	0.0000
44	0.1897	0.1759	0.1989	0.2230	0.2126	0.0000
45	0.1828	0.1805	0.1989	0.2069	0.2310	0.0000
46	0.1816	0.1770	0.2034	0.2069	0.2310	0.0000
47	0.1828	0.1793	0.1966	0.2126	0.2287	0.0000
48	0.1839	0.1793	0.1943	0.2195	0.2230	0.0000
49	0.1805	0.1805	0.2011	0.2080	0.2299	0.0000
50	0.1770	0.1724	0.1897	0.2138	0.2471	0.0000

51	0.1828	0.1816	0.1943	0.2230	0.2184	0.0000
52	0.1897	0.1805	0.1931	0.2207	0.2161	0.0000
53	0.1828	0.1816	0.1897	0.2172	0.2276	0.0011
54	0.1851	0.1736	0.1874	0.2299	0.2241	0.0000
55	0.1851	0.1724	0.1885	0.2299	0.2241	0.0000
56	0.1839	0.1713	0.1966	0.2253	0.2230	-0.0000
57	0.1862	0.1690	0.1862	0.2299	0.2287	0.0000
58	0.1966	0.1586	0.1862	0.2218	0.2368	0.0000
59	0.1862	0.1690	0.1920	0.2161	0.2368	0.0000
60	0.1782	0.1724	0.1954	0.2011	0.2529	0.0000
61	0.1874	0.1713	0.1759	0.2195	0.2460	0.0000
62	0.1897	0.1793	0.1851	0.2230	0.2230	0.0000
63	0.1851	0.1724	0.1782	0.2310	0.2333	0.0000
64	0.1966	0.1632	0.1678	0.2333	0.2391	0.0000
65	0.1770	0.1816	0.1966	0.2149	0.2299	0.0000
66	0.1874	0.1736	0.1954	0.2115	0.2322	0.0000
67	0.1851	0.1736	0.1862	0.2218	0.2333	0.0000
68	0.1816	0.1747	0.1920	0.2126	0.2333	0.0057
69	0.1828	0.1805	0.1943	0.2069	0.2356	0.0000
70	0.1770	0.1816	0.1885	0.2149	0.2356	0.0023
71	0.1874	0.1793	0.1989	0.2172	0.2172	0.0000
72	0.1908	0.1793	0.2115	0.2138	0.2034	0.0011
73	0.1851	0.1724	0.1954	0.2046	0.2425	0.0000
74	0.1793	0.1724	0.1931	0.2207	0.2345	0.0000
75	0.1851	0.1678	0.1897	0.2046	0.2529	0.0000
76	0.1644	0.1621	0.1943	0.1966	0.2828	0.0000
77	0.2000	0.1805	0.1862	0.2310	0.2023	0.0000
78	0.1862	0.1736	0.1897	0.2310	0.2195	0.0000
79	0.1747	0.1655	0.1885	0.2092	0.2621	0.0000
80	0.1655	0.1782	0.1977	0.2046	0.2540	0.0000
81	0.1713	0.1724	0.1874	0.2103	0.2586	0.0000

82	0.1678	0.1747	0.1989	0.2115	0.2471	0.0000
83	0.1506	0.1816	0.1931	0.2126	0.2621	0.0000
84	0.1667	0.1862	0.2046	0.2230	0.2195	0.0000
85	0.1678	0.1678	0.1989	0.1966	0.2690	0.0000
86	0.1667	0.1770	0.1897	0.2080	0.2586	0.0000
87	0.1736	0.1770	0.1908	0.2103	0.2483	0.0000
88	0.1736	0.1736	0.1943	0.2092	0.2494	0.0000
89	0.1759	0.1713	0.1931	0.2092	0.2506	0.0000
90	0.1678	0.1839	0.1966	0.2195	0.2322	0.0000
91	0.1678	0.1839	0.1977	0.2184	0.2322	0.0000
92	0.1644	0.1851	0.2023	0.2126	0.2356	0.0000
93	0.1667	0.1828	0.2011	0.2149	0.2345	0.0000
94	0.1713	0.1782	0.1954	0.2287	0.2264	0.0000
95	0.1736	0.1816	0.1977	0.2126	0.2345	0.0000
96	0.1713	0.1713	0.1874	0.2207	0.2471	0.0023
97	0.1632	0.1793	0.1966	0.2126	0.2483	0.0000
98	0.1632	0.1759	0.1908	0.2057	0.2644	0.0000
99	0.1701	0.1793	0.1920	0.2207	0.2379	0.0000
100	0.1609	0.1828	0.1931	0.2069	0.2563	0.0000
101	0.1736	0.1862	0.2011	0.2241	0.2138	0.0011
102	0.1609	0.1851	0.2046	0.2057	0.2437	0.0000
103	0.1678	0.1644	0.1931	0.2046	0.2701	0.0000
104	0.1747	0.1701	0.2000	0.2000	0.2552	0.0000
105	0.1644	0.1851	0.2241	0.1782	0.2483	0.0000
106	0.1644	0.1897	0.2207	0.1839	0.2414	0.0000
107	0.1678	0.1713	0.1989	0.2034	0.2586	0.0000
108	0.1678	0.1701	0.2057	0.2023	0.2540	0.0000
109	0.1690	0.1713	0.1931	0.2126	0.2529	0.0011
110	0.1701	0.1724	0.2023	0.2184	0.2368	0.0000
111	0.1598	0.1874	0.2000	0.1908	0.2621	0.0000

Run Settings:

seed=24922567

burn=300000

burn-algorithm=global

main-algorithm=local

cycles-1500000

sample-interval=200

parameter-update-interval= 1

update-kappa=true

update-theta=trae

update-pi=true

update-ttp=true

update-gamma==true

tune-interval=200

window-interval=200

molecular-clock=true

likelihood-model=HKYS5

category-list=l^A285,2^A160,3^A378,2*

single-kappa-false

initial-kappa=3.8855,5.1502,3.7229

initial-theta=l .2423,0.0429,1.3414

estimate-pi^true

data-file=allfri5. aln

outgroup=l

global-tune=0.0100

kappa-tune=0.1600

theta-tune=3000.0000

pi-tune=6000.0000

local-tune=0.1900

use-beta=false

max-initial-tree-neight=0.1000

file-root^results

initial-tree-type=random

newick-format=true

Input Taxa names: (in alignment order)

1 delica	31 laricina	61 lilacea	93 betularum2
2 chloroides	32 cessans	62 aurantiaca	94 fragilis
3 brevipes	33 curtipes	63 lepida2	95 mairei
4 Uttoralis	34 versicolor	64 lepidicolor	96 cremoricolor
5 pallidospora	35 solans	65 rosea	97 raoultii
6 pulverulenta	36 nauseosa	66 roseipes	98 aquosa
7 insignis	37 messapica	67 turci	99 ochroleuca
8 foetens	38 puellula	68 amethystina	100 viscida
9 laurocerasci	39 odorata	69 postiana	101 stuntzii
10 amoenolens	40 puellaris	70 risigallina	102 atropurpurea
11 pectinata	41 melzeri	73 paludosa	103 cavipes
12 pectinatoid	42 adulterina	74 caerulea2	104 gracillima
13 4cyanoxantha	43 cuprea	75 rosacea	105 archaea
14 heterophylla	44 maculata	76 violacea	106 camarophylla
15 vesca	45 sphagnophila	77 rosacea 2	107 adusta
16 vesca 2	46 nitida	78 aurata	110 G. aculeatum
17 aeruginea	47 xerampelina4	79 romellii	111 A. flettii
18 ilicis	48 xerampelina	80 persicina	
19 parazurea2	49 pascua	81 G. gilkeyae	
20 alboareolata	50 amoenipes	82 consobrina	
21 virescens	51 amethystina2	83 helodes	
22 mustelina	52 firmula	84 fellea	
23 amoenicolor	53 californiensis	85 exalbicans2	
24 violeipes	54 occidentalis	86 fuscorubroides	
25 grisea	55 vinosa	87 queletii	
26 melliolens	56 claroflava	88 sardonia	
27 decolorans2	57 integra	89 drimeia	
28 melitodes	58 integriformis	90 bicolor	
29 veternosa	59 velenovskyi	91 emetica	
30 rubra	60 azurea	92 nana	

Summary of sequence data and the initial parameters selected of the top clade of 55 sequences which have been stripped of all identical positions in the alignment.

Output generated by BAMBE version 2.03 Beta

Data Summary

55	number of taxa
530	total number of sites
481	total number of unique sites

Number of constant sites

Category	A	G	C	T	Total
1	1	0	2	0	3
Total	1	0	2	0	3

Overall	Count	Proportion
A	4794	0.1645
G	4691	0.1609
C	6261	0.2148
T	6497	0.2229
-	6897	0.2366
R	2	0.0001
M	0	0.0000
S	1	0.0000
V	0	0.0000
W	1	0.0000
K	1	0.0000
D	0	0.0000
Y	5	0.0002
H	0	0.0000
B	0	0.0000

Category A G C other

1 Pi 0.2155 0.2109 0.2815 0.2921

Prop 0.1645 0.1609 0.2148 0.2229 0.2366 0.0003

Taxon	A	G	C	T	-	other
1	0.1679	0.1698	0.2226	0.2113	0.2283	0.0000
2	0.1660	0.1642	0.2302	0.2113	0.2283	0.0000
3	0.1585	0.1566	0.2075	0.2226	0.2547	0.0000
4	0.1642	0.1698	0.2264	0.2132	0.2264	0.0000
5	0.1755	0.1679	0.2226	0.2283	0.2057	0.0000
6	0.1811	0.1679	0.2434	0.2226	0.1830	0.0019
7	0.1679	0.1717	0.2151	0.2377	0.2075	0.0000
8	0.1792	0.1698	0.2132	0.2340	0.2038	0.0000
9	0.1792	0.1679	0.2132	0.2434	0.1962	0.0000
10	0.1698	0.1868	0.2245	0.2321	0.1868	0.0000
11	0.1679	0.1717	0.2075	0.2283	0.2226	0.0019
12	0.1660	0.1642	0.2057	0.2396	0.2245	0.0000
13	0.1660	0.1717	0.2170	0.2358	0.2094	0.0000
14	0.1717	0.1585	0.2038	0.2491	0.2170	0.0000
15	0.1717	0.1566	0.2057	0.2491	0.2170	0.0000
16	0.1698	0.1547	0.2189	0.2415	0.2151	0.0000
17	0.1736	0.1509	0.2019	0.2491	0.2245	0.0000
18	0.1887	0.1358	0.2019	0.2358	0.2377	0.0000
19	0.1736	0.1509	0.2113	0.2264	0.2377	0.0000
20	0.1623	0.1566	0.2132	0.2340	0.2340	0.0000
21	0.1717	0.1566	0.2170	0.2075	0.2472	0.0000
22	0.1491	0.1623	0.2132	0.2113	0.2642	0.0000
23	0.1547	0.1528	0.2245	0.2075	0.2604	0.0000
24	0.1623	0.1547	0.2358	0.2094	0.2377	0.0000
25	0.1415	0.1660	0.2208	0.2000	0.2717	0.0000
26	0.1585	0.1679	0.2170	0.2321	0.2245	0.0000
27	0.1679	0.1642	0.2189	0.2283	0.2208	0.0000

28	0.1585	0.1547	0.2283	0.2151	0.2415	0.0019
29	0.1698	0.1698	0.2283	0.2302	0.2019	0.0000
30	0.1755	0.1585	0.2170	0.2189	0.2302	0.0000
31	0.1717	0.1585	0.2019	0.2358	0.2321	0.0000
32	0.1660	0.1604	0.2113	0.2208	0.2321	0.0094
33	0.1566	0.1660	0.2151	0.1981	0.2642	0.0000
34	0.1340	0.1434	0.2264	0.1774	0.3189	0.0000
35	0.1358	0.1472	0.2283	0.1830	0.3057	0.0000
36	0.1679	0.1698	0.2151	0.2113	0.2358	0.0000
37	0.1585	0.1717	0.2057	0.2245	0.2358	0.0038
38	0.1792	0.1679	0.2019	0.2358	0.2151	0.0000
39	0.1717	0.1566	0.1906	0.2491	0.2321	0.0000
40	0.1585	0.1642	0.2321	0.2094	0.2358	0.0000
41	0.1472	0.1528	0.2208	0.2094	0.2698	0.0000
42	0.1792	0.1623	0.2226	0.2377	0.1981	0.0000
43	0.1906	0.1415	0.1717	0.2547	0.2415	0.0000
44	0.1604	0.1566	0.2170	0.2019	0.2642	0.0000
45	0.1755	0.1547	0.1849	0.2321	0.2528	0.0000
46	0.1585	0.1717	0.2189	0.2245	0.2264	0.0000
47	0.1396	0.1660	0.2208	0.2075	0.2660	0.0000
48	0.1377	0.1396	0.2151	0.1943	0.3132	0.0000
49	0.1736	0.1585	0.2075	0.2509	0.2094	0.0000
50	0.1547	0.1453	0.2057	0.2151	0.2792	0.0000
51	0.1717	0.1491	0.2075	0.2075	0.2642	0.0000
52	0.1585	0.1585	0.2075	0.2340	0.2415	0.0000
53	0.1679	0.1679	0.2189	0.2208	0.2245	0.0000
54	0.1698	0.1679	0.2151	0.2321	0.2151	0.0000
55	0.1302	0.1811	0.2245	0.1849	0.2792	0.0000

Run settings:

seed=98452531
burn=30000
burn-algorithm=global
main-algorithm=local
cycles= 1500000
sample-interval=200
parameter-update-interval= 1
update-kappa=true
update-theta=true
update-pi=true
update-ttp=true
update-gamma=true
tune-intervaKJOO
window-interval^OO
molecular-clock=true
likelihood-model=HKY85
category-list=l*

single-kappa=false
initial-kappa=4.2070
initial-theta=1.0000
estimate-pi^true
data-file=allfii3 topcladestrip. aln
outgroup=l
global-tune=0.0100
kappa-tune=0.2500
theta-tune=2000.0000
pi-tune=4000.0000
local-tune=0.1900
use-beta=false
max-initial-tree-height=0.1000
file-root=results
initial-tree-type=random
newick-format=true

Input Taxa names: (in alignment order)

1 sphagnophila
2 nitida
3 amoenipes
4 pascua
5 olivacea
6 olivacea 2
7 amethystina2
8 firmula
9 veternosa
10 rubra
11 californiensis
12 decolorans2
13 melitodes
14 occidental
15 vinosa
16 claroflava
17 integra
18 integriformis
19 velenovsky
20 caerulea 2
21 paludosa
22 versicolor
23 odorata
24 messapica
25 nauseosa
26 Solaris
27 puellula
28 puellaris
29 melzeri
30 roseipes
31 turci
32 amethystina
33 curtipes
34 cessans
35 laricina
36 postiana
37 risigallina
38 aurantiaca
39 lepida2
40 cuprea
41 adulterina
42 maculata
43 lepidicolor
44 azurea
45 lilacea
46 rosea
47 persicina
48 violacea
49 aurata
50 romellii
51 rosacea
52 melliolens
53 xerampelina4
54 xerampelina
55 Albatrellus

Russulas of Southern Vancouver Island Coastal Forests

Volume 2

by

Christine Roberts

B.Sc. University of Lancaster, 1991

M.S. Oregon State University, 1994

A Dissertation Submitted in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in the Department of Biology

Appendix 3

Keys and Descriptions of Russulas found in southern Vancouver Island coastal forests

Diagnostic characters

The figures below show three of the more important diagnostic characters of *Russula.* The spore colour is referred to in the following descriptions with a Romagnesi coded colour. Descriptions found in other literature may refer to Crawshay or to their own colour codes. The ones that are most likely to be encountered are shown in their approximate chromatic and tonal position relative to the swatches of Romagnesi's 1967 colour chart.

The spore ornamentation chart drawn by Ben Woo for the Pacific Northwest Key Council is ordered by the amount and type of reticulation from A to E, and the height of the warts from 1 -small, to 3 -large. Woo adapted and simplified a similar system designed by Patterson (1979). A Woo type consists of letter and number coordinates that can be easily referred to on the chart (figure 23) and used as a shorthand descriptor. Bon (1988) uses virtually identical categories.

The chart of epicutal structures shows common pileocystidia shapes. Type 1 is almost exclusively found in the *Pectinatae* such as *Russula pectinata.* Types 2, 3 and 4 are more common in the lower clades (1-6), types 5 and 6 are more common in clades 7-10 and the incrusted pileocystidia together with the incrusted primordial hyphae are found in clades 7 and 8. In any sliver of epicutis more than one shape may be found, but one or occasionally two will predominate, and these are associated with taxonomic position.

The macroscopic and microscopic reactions to a number of chemicals are noted in the descriptions. However, some are more useful than others. In addition, microscopic examination is facilitated by a further set of reagents. A summary of these follows.

Macroscopic use:

FeS(>4 (Ferrous sulphate); very useful - rub a crystal on the fresh stipe tissue, reaction ranges from salmon pink to blue-green, differentiates some taxonomic groups, e.g. the *Xerampelinae.*

KOH (potassium hydroxide) - a 3-5% aqueous solution; used on fresh stipe tissue may accelerate natural bruising reactions, turns flesh pink to red in *Russula cavipes,* on cap cutis it usually bleaches out the blue pigment.

NH4OH (ammonia) - a 3% aqueous solution; useful in the *Sardoninae,* turns stipe tissue of *Russula sardonia* and *R. cavipes* pink to red.

Phenol - 2% in water; toxic and of limited use - place a drop on fresh stipe tissue, it turns brownish purple in almost every species except *Russula olivacea* and *R. alutacea,* in which it becomes black-currant purple.

Gum guaiac - 1:5 w/v dissolved in 70% ethanol; of limited use - a drop on fresh stipe tissue turns quickly blue-green in most groups, weaker, slower and more grey in *Russula fragiles* and some close relatives.

Guaiacol - preprepared tincture; not often tested so taxonomic usefulness not fully assessed - a drop on stipe tissue usually turns pink.

SV (sulphovanillin) - one drop of 50% sulphuric acid to a forceps-pinch of vanillin crystals, gently warmed to dissolve the crystals, hazardous but useful - applied to slivers of fresh or dried cutis and gill tissue, reactions range from bright red, deep pink, purple to blue-black or sometimes merely a loss of colour to a pale grey or brown.

Microscopic use:

Extra wet water - to about 25ml water add 3 drops glycerol and 1-2 drops Kodak photoflow or similar wetting agent, for initial examination and measurement of cutis, gelatinous material and pigment are left intact, at least for several minutes.

KOH or NH4OH - as above, for general viewing of tissues, removes the gelatinous substance from cutis tissues and usually also the pigment and most incrustations.

Congo red - 1:100 w:v in 3% ammonia, use KOH or ammonia to rinse stain off tissue and as mounting fluid - stains cell walls and septa, most useful for viewing cutis hyphae and thin gill sections or squash mounts, gives more contrast for photography.

SV - as for macroscopic use, stains contents of vascular hyphae and cystidia, the colour and intensity of the reaction is specific for several taxonomic groups.

Acid fuchsin - about lg dissolved in 20ml 5% acetic acid, applied to sliver of cutis and allowed to soak 5-15 minutes. If it dries on the tissues the staining is improved, rinsed off in 2% hydrochloric acid for 1 minute then mounted in water. This is useful in species with encrusted hyphae, but the incrustations are also visible in S V and in water mounts with methylene blue or cotton blue (soak tissues in 2% hydrochloric acid first, then rinse well in water, to leave only acid resistant incrustations.)

Melzer's reagent: 0.5gm iodine, 1.5gm potassium iodide, 20ml water and 20ml chloral hydrate - essential for seeing spore ornamentation.

At minimum, FeSC>4, SV, Melzer's reagent, water and 3% ammonia would suffice for a macroscopic and microscopic examination of most *Russula* species, adequate for making an identification with reasonable confidence. The microscopic staining of gill and cuticular cystidia in SV can be observed with a dissecting scope with a x25 objective, or even a very good hand lens with a magnification of at least x15, under which tiny dark dots on the gills or threads within the gelatinous matrix of the epicutis can be observed. The acid fuchsin reaction can be similarly observed.

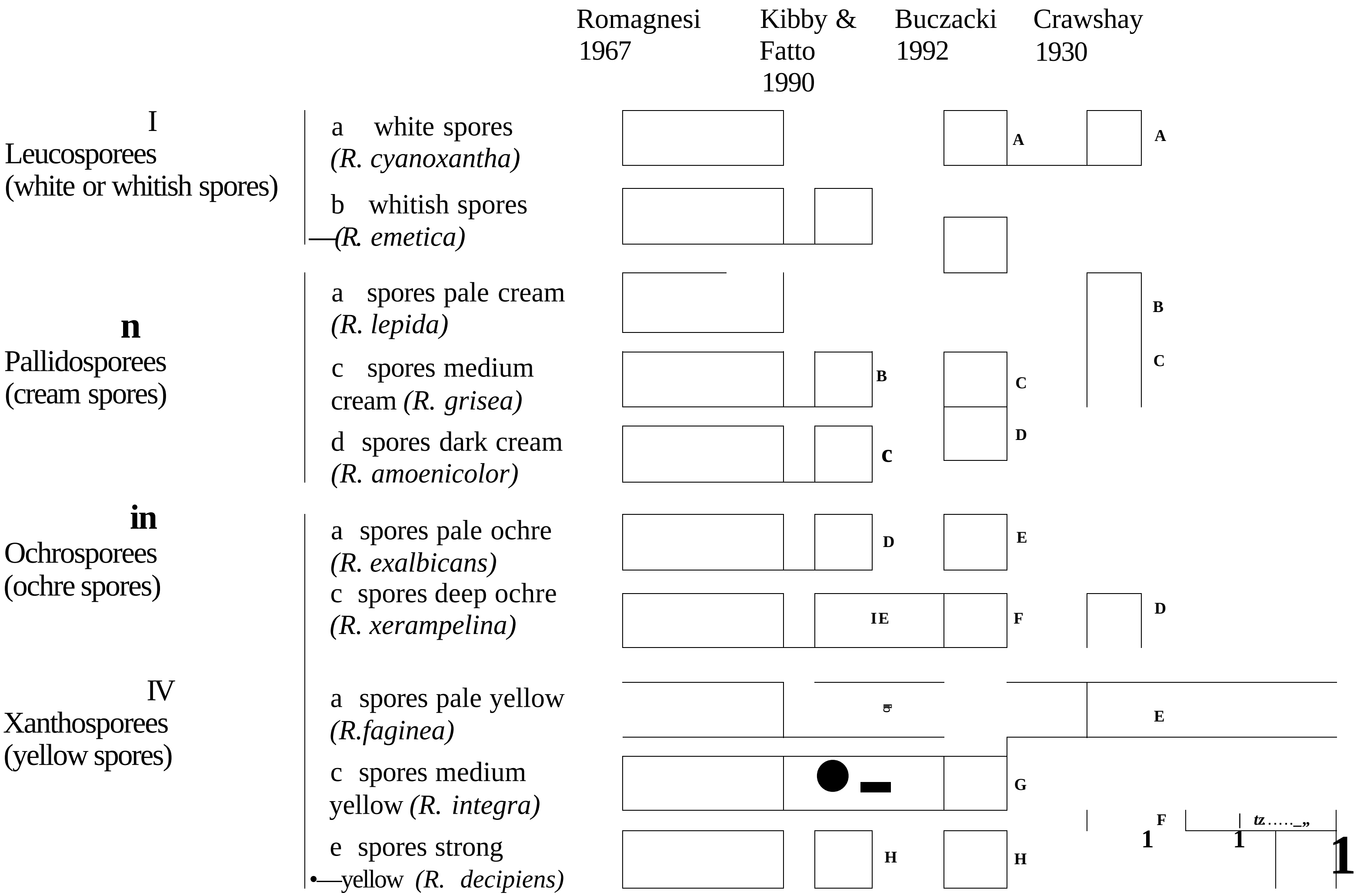


Figure 29 Spore print colour and code comparison between four authors. On a colour monitor the red-green-blue measurements for the Romagnesi swatches are as follows: la 255,255,255; lb 254,255,241; Ha 253,252,217; He 253,248,198; lid 253,239,182; Hla 252,233,178; fflc 250,225,159; IVa 251,225,159; IVc 251,216,137; IVe 242,186,103. (On this scale 0,0,0 is black).

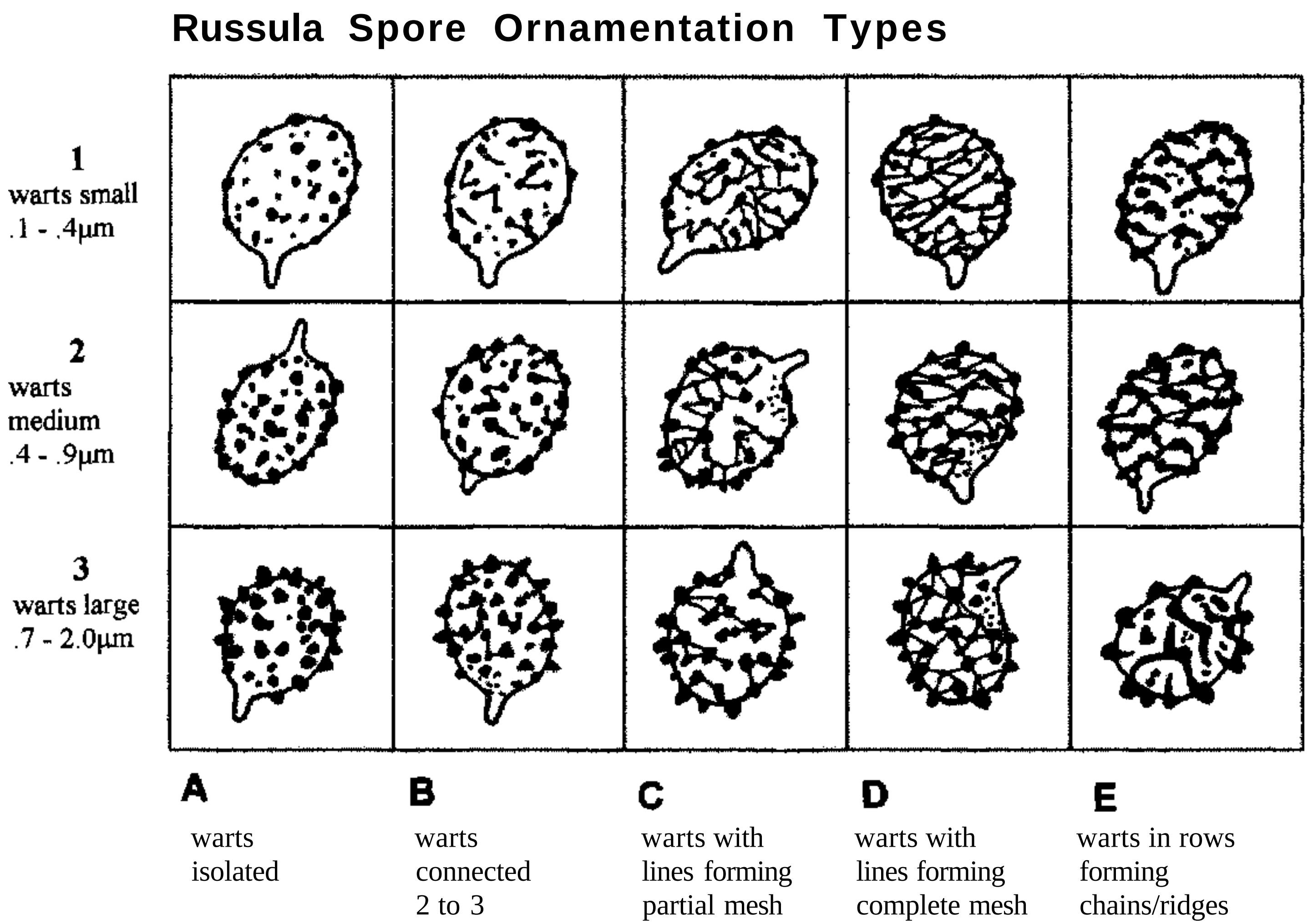


Figure 30 *Russula* spore ornamentation chart of Woo, drawn for the Pacific Northwest Key Council *Russula* Keys (Woo, 1989), with size and reticulation categories A to D being close to that of Bon, (1988), but with the addition of types E, in which the warts are catenate.

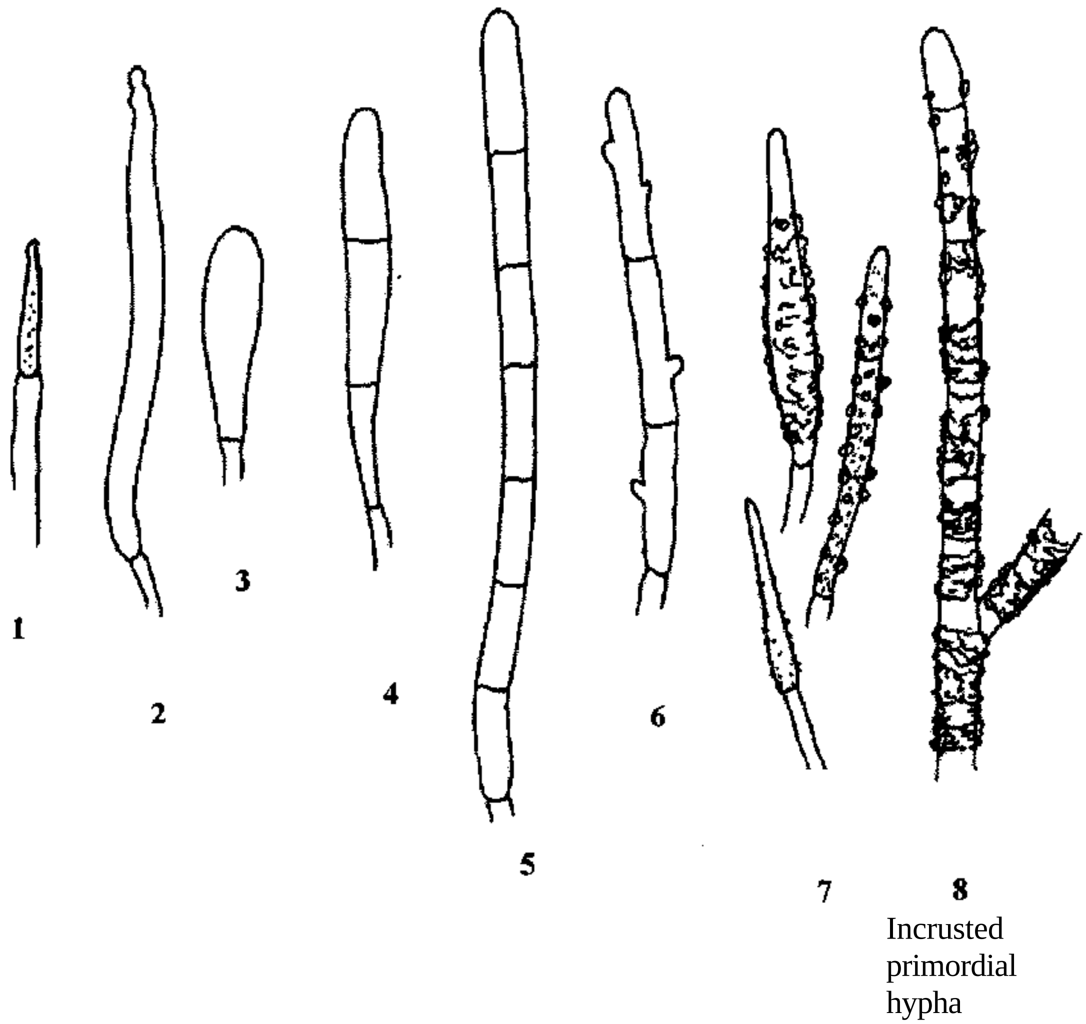


Figure 31 Specialized structures of the epicutis, 1-7 are pileocystidia: 1, aciculate or pointed; 2, ± cylindrical with capitate or strangulate terminus; 3, shortly clavate or broad with obtuse end; 4, narrowly clavate with obtuse tip; 5, long, cylindrical with obtuse tip; 6, diverticulate; 7, various shapes -incrusted. Septation has here been considered separately but type 2 is occasionally up to 2 septate, rarely 3; types 4, 5 and 6 are usually septate, types 4 and 6 often with 0-2 septa, and type 5 with 2-5 or more. Types 2 and 3 have 0-2 septa in a few cases but these are more commonly aseptate. Incrusted primordial hyphae are normally regularly septate, branched and do not stain in sulphovanillin.

Keys *to* Russulas of Vancouver Island and the Pacific Northwest

Species in bold type are described in the following pages, others have been reported from the Pacific Northwest in various literature and in foray lists, and are included in this key for more complete coverage. Species keyed out but not described below may be looked up in Matchmaker (Gibson and Gibson 2003), or in Woo (1989) or the original publication. The keys are based on a traditional dichotomous key, except where more than two distinct characters can separate species or groups of species. Cap colour, spore colour, taste and some cases reaction to FeSC»4 are the initial differential characters, since those are the first to be observed. Finer divisions are made with bruising reactions, habitat and microscopic characters. Sometimes more information is given than is strictly necessary to segregate groups so that the key is useable by people with a wide range of experience and access to microscopes and laboratory facilities.

Polychotomous keys to major groups:

1. Gills with frequent subgills that are more or less regularly distributed in 2-3 tiers, cap white to cream, shallowly funnel-shaped, margins smooth, cutis not peelable or viscid, may bruise brownish in age but not bruising red, grey or black.......**Key 2: *Lactaroides***

2. Gills as above, cap white, cream or shades of brown, margins smooth, cutis peelable only at the margins, dry or viscid, flesh bruising grey to black, sometimes with a pink to red interphase, spores with inamyloid suprahilar patch....................**Key** 1: *Compactae*

3. Cap some shade of light to dark brown, lacking red, green, purple or clear yellow pigments, always with a deeply grooved (tuberculate) margin, even when young, surface viscid and sticky when moist, flesh usually browning, stipe with 4 or more lenticular cavities, odours usually strong, taste peppery to acrid with a soapy aftertaste, spores with an inamyloid suprahilar patch. Chromatograph of hot water extract of cutis having only browns and dull yellows, and lacking bands of blue, magenta or bright yellow...**Key 3: *lngratula***

Cap colour various but not bright red or yellow, often pruinose, stipe usually solid, not developing a series of 3 or more cavities (but may be hollowed out by insect larvae), flesh usually firm and less brittle than other Russulas, usually mild or only slightly peppery, gills mostly all the same length or sometimes forked, acute to adnate at the cap margin (not obtuse), subgills infrequent and irregularly distributed, spores with an inamyloid suprahilar patch, often also with low ornamentation, basidia 6-12um wide, cutis pigment poorly extracted by hot water and distinct magenta bands not separated by paper chromatography..Key 4: *Heterophyllidia*

Key

Key 1: *Compactae* **-Clade lb**

1.1 a) Flesh turning distinctly red before greying or blackening, taste usually peppery... ....1.2

b) Flesh graying or blackening directly or with at most a dingy pink interphase, taste mild 1.4

1.2 a) Gills thick and distant, only 4-5 per cm at mid-radius 1.3

b) Gills thinner, more closely spaced .... *Russula densifolia* (Seer.) Gillet (see notes under *R. adusta).*

1.3 a) Spores with ornamentation up to 0.3 microns, European .... *R. nigricans* Fries (see notes under *R. dissimulans).*

b) Spores with ornamentation up to 0.7 microns, North American .... ***Russula*** *dissimulans* **Shaffer**

1.4 a) Cap cutis pure white when undamaged, flesh blackening strongly within a minute of bruising, no pink interphase ___ *Russula* ***albonigra*** **(Krombh.) Fries**

b) Cap cream to dingy yellow-brown when undamaged, bruising reaction slower and weaker, blackening only in age or after several hours 1.6

1.5 a) Cap smooth, viscid when wet, stipe smooth, hyphae of cap epicutis with yellow-brown pigment globules in 5% KOH or water mounts .... *Russula adusta* Fries

b) Cap dry, felty, stipe with tiny floccules, hyphae of cap epicutis with dark brown pigment globules in 5% KOH or water mounts .... *Russula anthracina* var. *insipida* ***JHom&gn.***

Key 2: *Lactaroides* —Clade 2b

1 a) Taste mild to only slightly peppery...2.2

b) Taste strongly peppery to acrid...2.3

2 a) Gills thick and distant, spore ornamentation generally under 1um high, not confirmed for North America.......................................*Russula delica* Fries

b) Gills thin and narrow, close to crowded, common in the Pacific Northwest..........
....***Russula brevipes*** **var.** ***brevipes*** **Peck**

3 a) Cap diameter medium to large, usually 10cm or more, spores 8-11.3 x 7.8-9.4um, gills with blue-green tints near the stipe..........***Russula brevipes*** **var.** ***acrior*** **Shaffer**

b) Cap smaller, lacking blue-green tints on gills, spores 6.2-8.5 x 4.5-6um...............
....***Russula cascadensis*** **Shaffer**

Key 3 *Ingratae* -Clade 3

3.1 a) Cap cream, margin smooth when young, cutis up to 1mm thick, rubbery and easy to peel almost completely, odour mild or of coconut. ***Russula crassotunicata* Singer**

b) Cutis not as above, cap margins striate to tuberculate when young..................3.2

3.2 a) Strong odour of marzipan, maraschino cherries (benzaldehyde) or almond essence, sometimes with a foetid component, pileocystidia ± cylindrical.............3.3

b) Odour not as above..3.5

3.3 a) Spore ornamentation of ridges forming a partial to complete reticulum__3.4

b) Spore ornamentation of mostly isolated warts, odour with a more pronounced foetid component..*Russula foetens Fries*

3.4 a) Spore ornamentation under 1.8 um high, habitat with Sitka spruce.......................
....***Russula fragrantissima Rom&gn.***

b) Spore ornamentation up to 2.6um high.....................*Russula laurocerasi* Melzer (see notes under *R. fragrantissima)*

3.5 a) Spore print white, Romagnesi 1a-1b, cap generally below 7cm diameter, stipe with more than 10 lenticular cavities, pileocystidia cylindrical and voluminous, SV +, odour subtle, ± fruity,..3.6

b) Spore print cream to pale yellow, odour strong, not fruity, stipe usually with fewer cavities, pileocystidia small, inconspicuous and lanceolate with contents showing only a few weakly staining granules in SV.......................................3.7

3.6 a) Under conifers..***Russulapallescens* Karsten**

b) Under broadleaved trees..***Russulafarinipes* Romell.**

3.7 a) Cap cutis with minute granular appearance, at least over central area, the granules usually red-brown, microscopically they are composed of clumps of upright smooth-walled articulated hyphae and pileocystidia, habitat with Douglas fir ....***Russula granulata*** **Peck.**

b) Cap cutis smooth, fibrillose or becoming areolate but these patches not granular in appearance..3.8

3.8 a) On beach dunes at the edge of Sitka spruce, pine and western hemlock forest, odour with spermatic, Jerusalem artichokes or fishy-almond components, usually dark greyish yellow-brown..3.9

b) In the coastal Douglas-fir zone...3.10

3.9 a) Spores with a partial reticulum..................................***Russula cf.pectinata*** **Fries**

b) Spores with mostly isolated warts...........................*Russula amoenolens* Romagn. (see notes under *R. cerolens* and *R.* cf. *pectinata).*

3.10 a) Cap 5.3-9cm diameter, pale to mid grey-brown, odour like bleach but also with a fresh ozone or cottonwood component, spores mid to deep cream, Romagnesi IIc-d, ellipsoidal, meanL:W around 1.33, articulated hyphae common in epicutis............. ....***Russula cerolens*** **Shaffer**

b) Cap under 5 cm diameter, pale dull yellow-brown, articulated hyphae rare in epicutis, spores pale cream, Romagnesi Ib-IIa, spores broadly ellipsoidal, mean L:W around 0.26...***Russula pectinatoides*** **Peck**

c) Not quite matching key choices from 3.7 on.......................*Russula sororia* group (see notes under *R. cerolens*

Key 4 *Heterophyllidia*

4.1 a) Spore print white, Romagnesi la to lb..4.2
b) Spore print cream to yellow, Romagnesi Ha and darker.................................4.6

4.2 a) Cap dark brown centrally with brown, olive or green margins and with subtle radial wrinkles..4.3
b) Brighter, paler or with more pink..4.4

4.3 a) Under Douglas firs, stipe with rosy flush, cap margins green, gills tinged yellow-green, no pileocystidia...***Russula smithii* Singer**
b) Under Sitka spruce or western hemlock, stipe usually with a pale pink-grey to green-grey pruina, gills cream and bruising brown, pileocystidia present but may be sparse..***Russula brunneola* Burlingham**

4.4 a) Flesh salmon pink to greyish pink in FeSC>4, epicutis hyphae with short, often inflated cells..4.5
b) Flesh slowly grey to greenish in FeS04, gills pliable, cap with a mixture of green, purple and violet, epicutis hyphae narrow and filamentous..........................
....*Russula cyanoxantha* Fries

4.5 a) Cap green, at the margins the cutis breaking up into angular patches, cap cutis mostly cellular with sphaerocytes in the epicutis and no pileocystidia, spores very pale cream, Romagnesi Ib-IIa, with deciduous trees..
....*Russula virescens* (Shaeffer) Fries
b) Cap light green to brownish green, spores under 7um long, epicutis with short cylindrical pileocystidia and occasional thick-walled hair-like cells over the centre..
....*Russula heterophylla* Fries
c) Cap light pinkish brown, light purple with buff to dull yellow or cream, epicutis as in previous but spores over 7um long.................................*Russula vesca* Fries

6 a) Cap various dull colours overlain with a pale greyish pruina, drying matte.... 4.10

b) Cap colours as above or paler, not pruinose and drying subglossy……………… 4.9

c) Cap blue-green, grass-green to yellow-green, not pruinose………………………… 4.8

d) Cap reddish to purple, stipe pink to purple……………………………………………… 4.7

7 a) Cap velvety to suede-like, usually under 7cm diameter, mostly dark reddish to purple with a pale grey bloom, sometimes with paler or browner areas, margins strongly striate, pleurocystidia voluminous, 10-20um wide and contents not refractive, vascular hyphae absent (Bills and Miller 1984), spores reticulate………… ……………………………………………………………………………… *Russula mariae* Peck

b) Cap velvety to suede-like, larger (~11cm) brownish-yellow centrally with purple to violet, smooth margin, pleurocystidia under 8um wide, mostly embedded and contents refractive, vascular hyphae and pileocystidia present, spores not reticulate **...*Russula* cf. *sublevispora* (Romagn.) Kuhn. Romagn.**

c) Cap large, up to 22cm diameter, dark purple, spores reticulate………………………… ……………………………………………………………………………… *Russula maxima* Burl.

(This species is placed in the *Heterophyllae* subsection *Griseinae* by Thiers (1997), but Singer (1942) placed it in the *Integrinae* (roughly equivalent to *Integrae* in Sarnari 1998)

8 a) Cutis breaking up into angular patches near the cap margins, spores very pale cream, Romagnesi Ib-IIa, with deciduous trees...*Russula virescens* (Schaeffer) Fries

b) Cutis not breaking up, can be peeled to at least half-radius, sometimes completely, spores light cream, Romagnesi Ila-c.........***R aeruginea* Lindbl.** ex **Fr.**

9 a) Dingy white, tinged with pink or yellow, spores yellow, Crawshay E, or about Romagnesi Illc-IVa, stipe unchanging, taste with bitter component……………………… ……………………………………………………………………………… *Russula basifurcata* Peck

b) Cap yellow-brown, pink-brown to light chocolate brown, spores cream, Romagnesi Ila-c, taste mild..***Russula mustelina* Fries**

c) Cap light to mid grey with tinges of blue, green and pink, spores deep cream, Romagnesi lid to Ilia, stipe bruising yellowish to brown, taste mild to slightly peppery, with shore pine and Sitka spruce.................***Russula medullata* Romagn.**

d) As for previous but spores paler and habitat with beech... *Russula grisea* (Seer.) Fr. (see notes under *R. medullata)*

4.10 a) Cap grey-green to greenish brown, very pruinose, convex to pulvinate at maturity, epicutis sometimes with pseudoparenchymatous layer, with a variety of tree hosts but especially oak..*Russula modesta* Peck

b) Cap pinkish brown to grey-brown to blue-grey with pale grey pruina, becoming funnel-shaped with margins uplifted before fully mature, epicutis with some inflated cells but not forming a pseudoparenchymatous layer...***Russula parazurea* Schaeffer**

c) Cap ochre in centre, margins purple, cutis margins suede-like..***Russula* cf. *sublevispora* (Romagn.) Kuhn. Romagn.**

Key 5 Other *Russula* subgenera

Russulas with various colours including bright red and yellow, with an amyloid suprahilar patch on the spores, and with easily hot-water extractable pigments that are separable by paper chromatography into blue, magenta and yellow bands, (at least in coloured species). Cap is assumed to be viscid when wet unless described otherwise.

5.1 a) Cap white, cream or yellow, lacking other colours..5.2
b) Cap with colours other than above..5.8

5.2 a) Taste mild to slightly peppery or bitter, spores some shade of yellow__5.3
b) Taste acrid, spores white..5.6

5.3 a) Cap yellow, flesh greying to blackening, habitat often in swampy areas, epicutis with incrusted hyphae..*Russula claroflava* Grove
b) Cap cream to yellow, flesh unchanging..5.4

5.4 a) Cap cream with dull yellow centre, spore print light yellow, Romagnesi Ilia, epicutis with incrusted primordial hyphae...............................***Russula albida* Peck**
b) Cap bright clear yellow, generally below 8cm diameter, pileocystidia none or doubtful, not staining in SV..5.5
c) Cap pale yellow, up to 10cm diameter, epicutis with frequent pileocystidia staining grey in S V and taste sometimes slightly peppery and/or bitter...*Russula flaviceps* P'eck

5.5 a) Spore print dark yellow, Romagnesi IVe, epicutis with incrusted primordial hyphae, pileocystidia none or doubtful, odour of apricot, with oaks..***Russula lutea* (Huds.: Fr.) Gray**
b) Cap as previous or more buff coloured, no special odour, spores paler, Romagnesi IVa, epicutis as previous, with hardwoods.............*Russula gilva* Zvara

c) As above but with conifers, spores Romagnesi IVd........*R. chamaeleontina* Fries

Cap white, taste acrid, spores white

6 a) Cutis up to 1mm thick, rubbery and easy to peel almost completely, taste acrid, stipe grey-green in FeS04..***Russula crassotunicata* Singer**

b) Cutis thinner, less than 500um thick even in the cap centre, peelable no more than 2/3 the radius, stipe pinkish in FeS04..5.7

7 a) Cap cream with pale yellow centre, developing slightly greyish yellow tinges as the flesh beneath the cutis turns dingy with a waterlogged appearance, fragile, spores strongly reticulate, pileocystidia clavate with 0-1-septa, habitat with conifers on or near rotten wood..***Russula raoultii* Quel.**

b) Cap pale cream with dull yellow centre, firm, pileocystidia cylindical to clavate with up to 4 septa, spores finely reticulate, in oak-Douglas fir forest.........................
...*Russula crenulata* Burl.

c) Deep cream, sometimes as dark as yellow-brown on the disc, spores not reticulate, with conifers in the Pacific Northwest (Arora 1986)..................................
....*Russula cremoricolor* Earle

8 a) Flesh of stipe blue-green with FeSC«4, flesh bruising yellow then brown, odour often of shellfish, taste mild when mature______5.9

b) Flesh of stipe brownish with FeS04, (possibly green) and bright blackcurrant-juice purple with 2% phenol, flesh not bruising as above but stipe may bruise brown without a yellow phase, taste mild, hard-fleshed, cap surface dry, like kid leather, epicutis lacking pileocystidia and incrusted hyphae..5.12

c) Flesh of stipe not blue-green with FeSC«4, but may be salmon pink, pinkish grey brownish or greyish, odour and taste variable..5.13

Cap various colours, flesh blue-green with FeS04

5.9 a) Cap primary red to deep purple, lacking greenish or grey hues........................5.10

b) Cap brown to dark brown with olive, greyish or ochraceous tints...................5.11

c) Cap paler, light tones of olive to brownish pink or brown, with a brownish bloom, spores Romagnesi Ilc-d or IIIc, 8.2-12um x 7-10um, epicutis with numerous long, free, hair-like hyphal ends, habitat western hemlock-Sitka spruce stands.................. **..*Russula isabelliniceps* Grund**

5.10 a) Cap deep purple sometimes with ochraceous patches, spores Romagnesi Illb-c, 6.2-10 x 5-8 pm, mostly in Douglas fir forest. ***Russula xerampelina* (Schaeff.) Fries**

b) Cap primary red to bright magenta on the margins, almost black or brownish centrally, paler in age, spores Romagnesi Ilia, 8- 12pm x 6.5-9pm........................... ....***Russula semirubra* Singer**

5.11 a) Olive, brown and purple with a dense pruina in lighter and greyer hues than the base colours, as if the cap were mouldy, epicutis with numerous erect hair-like hyphae around 2pm wide and up to 120pm long ***Russula* cf. *pruinosa* Vel.**

b) Cap very dark brown centrally, finely velvety, olive towards the margins, which may also have a subtle vinaceous tint, margins smooth.. **...*Russula* *elaeodes* (Bres.:Romagn.) Bon**

c) Cap as b) but bald and margins striate-tuberculate *Russula viridofusca* Grund (see notes under *R. elaeodes)*

5.12 a) Cap a mixture of olive green and purple, becoming dull brown tinted in patches with these colours, no shellfish odour, spore print deep yellow, Romagnesi IVc, spores with mostly isolated warts up to 2|im high. ***Russula olivacea* (Schaeff.) Fries**

b) Cap with more red to purple than above, otherwise similar, spores with warts generally under 0.5 pm high, connected with a partial to complete reticulum............ ....*Russula alutacea* Fries

5.13 a) Cap bright red to blood red, sometimes fading, in some cases with orange, yellow or cream areas, lacking purple, violet, grey or green..5.14

b) Cap lacking bright to blood red..5.19

Flesh not blue-green in FeS04:

Cap red, with or without orange and yellow

5.14 a) Taste mild or slightly bitter, spores white to pale cream Romagnesi Ia-IIa......5.15

b) Taste mild or slightly bitter, spores yellow to deep yellow, Romagnesi Illb-IVd 5.16

c) Taste distinctly peppery to acrid, spores white, Romagnesi la-lb, stipe white .5.17

d) Taste distinctly peppery to acrid, spores yellow, Romagnesi Ha or darker, stipe pink..5.18

5.15 a) Cap deep velvety red, (but may be pink with yellow margin, Kibby and Fatto 1990) not or barely viscid when wet, cutis not peelable, spores pale cream, Romagnesi Ib-IIa, epicutis with incrusted pileocystidia.. ....*Russula lepidiformis* **Murrill**

b) Cap pink to deep red to brownish red, spores pure white, Romagnesi la, cutis peelable 1/2 to completely, small species only 3cm diameter or less, incrusted primordial hyphae and inflated cells in epicutis, with oaks...................................... ....*Russula praetenuis* Murrill

5.16 a) Buttons orange to red, more yellow when young and in age, flesh greying to blackening, spores light yellow, Romagnesi Illb-c, epicutis with pileocystidia, with conifers..*Russula decolorans* Fries

b) Cap yellow mottled with orange and red, spores deep yellow, Romagnesi IVd, epicutis lacking pileocystidia but with incrusted primordial hyphae, in deciduous or coniferous forest...*Russula chamaeleontina* Fries

5.17 a) Usually on decaying wood, unchanging, cap red ± yellow patches, fading dramatically to almost white, spore warts 0.8-1.4pm high, reticulate, epicutis with abundant 0-3 -septate pileocystidia______***Rusmla silvicola*** **Shaffer**

b) In soil or rotten wood, cap red mottled with yellow or yellow centrally with an orange-pink to copper red margin, spores with mostly isolated warts under lpm high, pileocystidia infrequent, inconspicuous............................*Russula bicolor* Burl

c) In sphagnum, cap red, occasionally with orange patches, spores reticulate, warts up to 1.7um high, epicutis with frequent multiseptate pileocystidia...*Russula emetica* Fr. ex S.F. Gray

5.18 a) Under pines, cap red ± cream areas near the margin, barely viscid, drying matte, margin smooth, stipe bruising yellow, flesh firm, spores 6.6-8.9 x 5.2-7.5pm...........**...*Russula sanguinaria* (Schumach.) Rauschert**

(=*R. rosacea* (Pers.ex Seer.) S.F.Gray)

b) Similar to a) but under *Abies* and *Tsuga*, more fragile and spores larger, 8.5-11.5 x 7-10.8um...*Russula americana* Singer

c) Similar to b) but with viscid cap drying glossy, striate margin, stipe not bruising yellow, habitat in wet seeps under *Tsuga* and spores 7-9.5 x 6-7.5pm.......................**..*Russula americana* var. *modicaspora* nom. prov.**

5.19 a) Cap pale pink, purplish pink or light terracotta with a cream to yellow centre, or mottled with these colours, taste peppery to acrid...5.21

b) As for a) but taste mild..5.23

c) Cap predominantly of various green, olive, grey, yellow-brown, grey- brown or green-brown hues, mostly lacking pink, red or purple, reaction with FeSC>4 often weak or greyish...5.24

d) Cap predominantly of vinaceous, red-brown, purple, violet or purple-brown hues, sometimes with green, black or yellow-brown areas, usually in the centre, in some cases fading dramatically to pale, dingy versions of these colours__5.33

Cap pinkish with cream to yellow centre

5.20 a) Spores white, stipe white but bruising bright yellow... ***Russula* cf. *luteotacta* Rea**

b) Spores white, stipe white, not bruising..5.17

c) Spores yellow...5.22

5.21 a) Stipe sometimes with a pink flush, bruising yellow-brown at the base, spores ochraceous yellow, Romagnesi IVb, Woo A2, or in local collections, B2-C2, mushroom with subtle odour of honey or gingerbread, in Douglas fir forest............. ***Russula veternosa* Fries**

b) Stipe white + yellow stains at the base, cap deep brownish pink on the margin with brown centre when young, fading to a pinkish margin with dull yellow centre, spores reticulate, estimated Romagnesi IIIc........................*Russula inconstans* Burl.

5.22 a) Small species mostly under 6cm cap diameter, spores deep yellow, Romagnesi IVd, epicutis lacking pileocystidia but with incrusted primordial hyphae.................. ....*Russula chamaeleontina* Fries

b) Cap diameter up to 10cm, spores deep yellowish cream, Romagnesi Ilia, epicutis with incrusted pileocystidia..........................***Russula** velenovskyi* **Melzer & Zvara**

Cap brown, olive, green, yellow-brown or grey, lacking purple or bright colours

5.23 a) Taste mild..5.24

b) Taste peppery to acrid..5.28

5.24 a) Flesh firm and robust, cap margin remaining smooth into late maturity..........5.25

b) Flesh softer and more fragile, cap margin becoming striate at maturity...........5.27

5.25 a) Flesh where damaged turning reddish then grey to black, cap colours very variable, PDAB (paradimethylaminobenzaldehyde) - vivid magenta on stipe (Woo, 1989), epicutis lacking pileocystidia but with incrusted primoridal hyphae..............

....***Russula occidentatis*** **Singer**

b) Flesh not bruising as above..5.26

5.26 a) Cap not or barely viscid, breaking concentrically into small areoli especially near the margin, not peelable in mature basidiomata, flesh bright blackcurrent-juice purple in 2% phenol, epicutis lacking pileocystidia and incrustations, spore print deep yellow, Romagnesi IVc, spores with mostly isolated warts up to 2um high ....***Russula olivacea*** **(Schaeff.) Fries**

b) Flesh reaction with 2% phenol normal, (brownish-purple), cap viscid when wet, peelable 1 /2-2/3 the radius, spore print variable -Romagnesi IIIc-IVc, epicutis with pileocystidia that barely react with SV, and primordial hyphae with contents pink in acid fuchsin but incrustations sporadic...................................***Russula Integra*** **Fries**

5.27 a) Cap bright golden brown, more red-brown centrally with an olivaceous margin, becoming a rich brownish-yellow, spores orange-yellow, Romagnesi IVd, up to 14 x 11.5um with isolated warts up to 2.2um high..........***Russula aureofulva*** **nom prov**

b) Cap light grass green, spores light orange-yellow, Romagnesi IIIc, up to 10 x 8.3um with warts up to 2um high........................***Russula aeruginoides*** **nom. prov.**

c) Small species under 3 cm wide, cap colour variable but often brown centrally with a grey margin, spore print Crawshay E, sometimes listed as peppery, under birches, possibly synonymous with *R versicolor*..........................*Russula blackfordae* Peck

5.28 a) Cap dark gray brown or olive brown, flesh bruising reddish then greying, spores cream, Romagnesi Ila-c..*Russula consobrina* Fries

b) Spore print darker, flesh not bruising as above..5.29

5.29 a) Cap brown and yellow or olive-grey...5.30

b)Cap a clearer, brighter green to olive-green..5.31

5.30 a) Cap dark brown with yellow margin, spores crawshay D-E, habitat deciduous woods-possibly Eastern N. A. only____*Russula disparilis* Burl.

b) Cap light to deep olive-grey, spores yellow ochre, Romagnesi IVd, spores with warts up to 0.4umhigh, reticulate..*Russula murina* Burl

5.31 a) Cap yellow-green, green or olive green, slight purple tints at the margin are possible but not recorded from North American material, stipe white, bruising pale yellow to yellow-brown, spores deep ochraceous yellow, about Romagnesi IVd, sometimes slightly paler, spore warts 0.7-1.5um high, isolated..*Russula wens* Romell apud Schaeff.

b) Cap as above but stipe with pink flush and staining bright yellow at the base spores paler -light orange, Romagnesi IIIc, spore warts mostly under 0.7um, + isolated................................***Russula queletii* cf. *var.flavovirens* (Bomm.-Rouss.)**

Cap purple with or without other colours

5.32 a) Taste peppery to acrid, spores white, Romagnesi Ia-b....................................5.33

b) Taste peppery to acrid, spores cream to yellow, Romagnesi Ila-IVe...............5.35

c) Taste mild, spores white, Romagnesi Ia-b..5.45

d) Taste mild, spores cream to yellow, Romagnesi Ila-IVe................................5.46

5.33 a) Cap a more or less uniform pale pink-grey to pale purple-grey, margin smooth, only slightly striate in age, spores white to pale buff, Romagnesi Ia-IIa, growing on very decayed wood...***Russula** stuntui* **Grund**

b) Cap with two or more colours...5.34

5.34 a) Cap purple to violet with a darker, black or greenish centre, buttons often green only, in age fading to pale purple at the margin and pale greyish to light brown centrally, margin soon striate, soft and fragile at maturity, stipe not browning, odour of stewed apples or coconut, usually on or near woody debris, with conifers, spores reticulate, subglobose, ornamentation^ 0.8um ... ***Russula fragilis* (Pers.: Fr.) Fries**

b) As a) but more robust, sometimes with more brown centrally or with dull yellow patches, on ground in deciduous or coniferous forest, stipe browning strongly at base, spores narrowly ellipsoidal................................*Russula krombholzii* Shaffer (=R. atropurpurea (Krombh.)Britzelm.)

c) As for a) but a more intense deep purple, under willows, spore ornamentation < 0.8um high...**Russula laccata Huijsman**

Cap purple ± other colours, taste acrid, spores cream to yellow

5.35 a) Stipe inner flesh turning pink to red in ISIH4OH or KOH (test fresh not dried material)..5.36

b) Stipe white, not reddening in alkaline solutions..5.37

c) Stipe with a partial to complete pink, reddish, purple or violet flush, at least in some basidiomata of any collection, not reddening in alkaline solutions_5.41

5.36 a) Stipe pink to violet, gills yellow when young, firm, on sandy soil with pines, spores light yellow, Romagnesi Ilia, reticulate......................*Russula sardonia* Fries (= *R. drimea* Cooke)

b) Stipe white or with just a flush of pink, gills cream when young, spores pale cream, Romagnesi Ha or slightly paler, with Douglas fir and grand fir......................
....**Russula cavipes Britzelmayr**

5.37 a) Cap a more or less unifora pale greyish purple, spores white to pale buff, Romagnesi lb-IIa but pinker, stipe not yellowing, on decayed wood that has cubical brown-rot...**Russula stuntm Grund**

b) Purplish hues usually mixed with other colours and stipe at least yellowing in age or when damaged...5.38

5.38 a) Small fragile species, cap generally under 5cm diameter, aspect like *R. fragilis* (key entry 5.3 lb) habitat birch or other deciduous woods.................................5.39

b) Medium to large species generally over 5 cm up to 13 cm cap diameter, habitat with Douglas fir 5.40

5.39 a) Under birch, slightly peppery to acrid, spores yellow, Romagnesi Ild-IIIb, stipe and flesh bruising yellow, spores up to 9pm long, partially reticulate, odour faintly fruity .... *Russula versicolor* Schaeff.

b) Cap violet with a green centre, stipe yellowing to browning, spore print cream, Romagnesi Ila-b, spores 9-1 lpm long, warts pointed, isolated, odour of pelargonium and menthol, in moist deciduous woods .... *Russula violacea* Quelet

c) Cap, stipe and spore print as b) above, weakly yellowing to browning, spores up to 8.5pm long, with complete reticulum, habitat moist coniferous or mixed woods.... .... *Russula olivaceoviolascens* Gillet

5.40 a) Cap vinaceous to violet, darker in the centre, up to 10.3cm diameter, margin striate-tuberculate, spore print light yellow, Romagnesi Illb approximately, spores up to 9pm long, ornamentation Woo C2 and E2, mostly with Douglas fir but occasionally with pine .... *Russula placita* Burl.

b) Cap redder than above, vinaceous to light purple with a brown to yellowish centre, up to 13 cm diameter, margin pruinose when young, striate in age, stipe staining yellow at the base, spores very similar to above but slightly darker en mass -an estimated Romagnesi IIIc .... *Russula inconstans* Burl.

5.41 a) Habitat in Douglas fir-western hemlock forest 5.42

b) Habitat with coastal Sitka spruce 5.44

c) Habitat with pines on calcareous or basaltic soils, fleshy species similar to *R. queletii* (key entry 5.44a) but more robust with a relatively short pink to violet stipe, gills cream rather than yellow when young, odour of apples spores light yellow, Romagnesi Ild-IIIa, and more reticulate, nodulose or diverticulate pileocystidia .... *Russula torulosa* Bres.

5.42 a) Cap a slightly brownish to greyish purple, stipe flushed pink, bruising yellow-brown at base, odour of geraniums or stewed fruit, spores cream, Romagnesi Ha or slightly lighter (but darker than lb), Woo types CI-2 to Dl-2, pileocystidia sometimes diverticulate..***Russula pelargonia* Niolle**

b) Odour not distinct, spores darker..5.43

5.43 a) Macroscopically like *R. jragilis* (key entry 5.31b), sometimes larger, but stipe white ± pink flush and spores light yellow, Romagnesi Ilc-IIIa, ornamentation of isolated warts up to lurn high with little or no reticulum, locally on ground under Douglas fir, but also with birch..*Russula gracilis* Burl.

b) Cap larger, up to 13cm diameter, deep brownish red, pinkish brown, darker centrally, margin smooth, stipe flushed reddish near the base, spores deep yellow, Romagnesi Illd-IVa, spores up to 1 lum long (Thiers 1997), warts isolated or joined in heavy ridges, partially reticulate..*Russula mordax* Burl.

5.44 a) Cap 3-11 cm diameter, a mixture of green and purple to violet, sometimes fading to pale greyish pink, firm when young, soon becoming soft and fragile, stipe with a partial to complete pink to violet pruina and staining bright yellow at the base, roughly equal in length to cap diameter. Within the stipe cortex at the very base is a thin layer of bright yellow tissue (more distinct in young basidiomata). Odour of stewed apples, gills cream when young, spore print varies from cream to light yellow Romagnesi IIc-IIIc, spores with mostly isolated warts, pileocystidia occasionally diverticulate...***Russula queletii* Fries**

b) As above but with more vinaceous and less green hues, stipe only partially coloured, sometimes white, the base yellow-brown rather than bright yellow, spores light cream, Romagnesi Ila-c, microscopic characters similar to those of *R. queletii*, habitat under Sitka spruce and western hemlock but within the rain forest rather than along the coastal fringe..***Russula* cf. *fuscorubroides* Bon**

Cap purple ± other colours, taste mild, spores white

5.45 a) Like *Rfragilis* key entry 5.31b, in all macroscopic and microscopic characters but taste completely mild............................***Russula fragilis*** **var.** ***mitis*** **nom. prov.**

b) Cap pinkish to vinaceous, sometimes more blue-grey or yellow-green, pruinose, peelable 1/4 the radius, stipe with pink flush, habitat deciduous woods, mild or occasionally slightly peppery, epicutis lacking pileocystidia but with incrusted primordial hyphae..*Russula lilacea* Quelet

c) Cap brownish to greyish violet, centre darker, more brown or black, pruinose, peelable 3/4 the radius, (like *R. murrillii,* key entry 5.57a, but with white spores) epicutis lacking pileocystidia but with incrusted primordial hyphae, habitat conifers especially spruce..*Russula azurea* Bresad.

5.46 a) Spores cream to light yellow, Romagnesi Ila-IId..5.47

b) Spores deep yellow Ilia and darker..5.52

Cap purple ± other colours, taste mild, spores cream to light yellow

5.47 a) Robust species, cap colours mixed, dark to pale tones of green, purple and yellow-brown, flesh bruising grey to deep grey-brown to black usually with a reddish interphase...***Russula occidentalis*** **Singer**

b) Not bruising as above but may bruise brown or yellow..................................5.48

5.48 a) Small to medium fragile species generally under 5cm (-6.5cm) across, with striate margins, pileocystidia usually 2-5 septate and constricted at the septa, SV+, sometimes weakly so, incrustations in epicutis none or rare..............................5.49

b) Medium to large robust species generally over 5cm across, margins smooth.. 5.51

5.49 a) Whole mushroom slowly staining dull yellow where bruised or handled, pileocystidia septate, epicutal hyphae sometimes filled with a deep yellow material. Habitat under Sitka spruce, alder and birch..........................***Russula puellaris*** **Fries**

b) Whole mushroom not strongly yellowing, but may yellow at stipe base........5.50

5.50 a) Habitat in wet coastal Sitka spruce-western hemlock forest, not always in sphagnum moss, cap dark to light purple tinged with brown or olive, spores up to 10.2um, partially reticulate, warts 0.5-1. lum high..***Russula sphagnophila* Kauffman**

b) Habitat with oaks and apparently in one case, western hemlock, cap purple to violet tinged with brown, brownish in the centre, spores under 9um long, wart height and reticulation variable, subcutis or hypodermis sometimes with sphaerocytes amongst the interwoven hyphae../?«ssw/a ***brunneoviolacea* Crawshay**

5.51 a) Cap large, up to 22cm diameter, dark purple, almost black on disc, stipe flushed with pink, spores partially reticulate, warts 0.5-1.5um high, pileocystidia few..*Russula maxima* Burl.

b) Cap up to 12um, brownish vinaceous, sometimes with yellow brown spots or centre, stipe usually white, rarely with a touch of pink, sometimes peppery in the gills or when young, spores reticulate, warts under 0.5u,m high, pileocystidia abundant, deeper ones with incrustations...........................***Russula viscida* Kudrna**

Cap purple ± other colours, taste mild, spores deep yellow

5.52 a) Remarkably firm fleshed species, not yellowing nor browning much, cap margins smooth except in age..5.53

b) Flesh texture normal to fragile, stipe sometimes yellowing or browning at the base...5.56

5.53 a) Cap surface the texture of kid leather, not or barely viscid when wet, spores deep orange-yellow, Romagnesi IVb-d, flesh of stipe turning bright blackcurrent-juice purple in 2% phenol, epicutis lacking pileocystidia and incrusted hyphae...........5.54

b) Flesh not brittle except in age, with smooth, viscid cap surface, cap brown with vinaceous or green areas, gills close and almost paper-thin, stipe white, not bruising but may brown slightly at the base, cutis peelable to at least 1/2 the cap radius, epicutiswith pileocystidia and sparsely incrusted hyphae................................5.55

5.54 a) Cap of drab olive, brown and/or purple, cutis not peelable when mature, breaking into small concentric areoli near the margin, stipe pink, spores with isolated warts 0.5-1.4um high, DNA of ITS-F to ITS4-B is around 1141bp, (around 240-300bp longer than other Russulas).......................................***Russula*** *olivacea* **(Schaeff.) Fries**

b) Cap vinaceous to purple, sometimes brownish, marbled with or olive or dull yellow or these colours in the centre, cutis peelable to at least 1/2 the radius, stipe white + a pink flush, spores reticulate, warts 0.2-0.5 microns high . *Russula alutacea* Fries

5.55 a) Cap purplish brown with deep green centre, stipe white, unchanging, spores deep orangish-cream, Romagnesi IIIc, quite densely waited, Woo types 3 A-3B, habitat Sitka spruce, huckleberry and salal..............***Russula* cf. *Integra* Fries (1st variety)**

b) Cap some shade of brown marbled with vinaceous and olive areas, stipe weakly browning at the base, spores deep orange-yellow, Romagnesi IVc, Woo types (A2) -B2-C2 (-E2), habitat Dougles fir-western hemlock forest.. ....***Russula* cf. *Integra* Fries (2nd variety)**

5.56 a) Cap generally a mixture of mid to light olive green and brownish-yellow, with pinkish purple towards the margins when mature, colours quite variable when very young, even within one collection, stipe not yellowing or browning at the base, spores deep warm cream to pale orange, Romagnesi Illa-c, Woo types A2-3, B2-3, warts 0.6-1.2um stipe unchanging to slightly greying...........***Russula abietina* Peck**

b) Cap predominantly purple to violet, brown, olive and green colours, when present, are only over the disc..5.57

5.57 a) Cap an opaque, chalky purple to violet, sometimes with black, brown or olive tints in the centre, often a darker zone around the disc which looks rather like a smear of charcoal, cap viscid, drying matte, stipe white, base sometimes browning or yellowing and with an iodoform odour, epicutis with abundant heavily incrusted primordial hyphae, pileocystidia absent to rare, subcutis cellular........................5.58

b) Cap of similar colours to above or a more reddish or brownish purple, but with a more translucent appearance, epicutis lacking incrustations but with frequent multiseptate pileocystidia greying in SV..5.59

5.58 a) Habitat with Douglas fir, stipe base occasionally spores orange-yellow, Romagnesi IVa-c, with a partial, broken reticulum, stipe base usually not yellowing but occasionally has an iodoform odour................................***Russula murrillii* Burl.**

b) As above but stipe more often yellowing at the base, spores with a complete reticulum and habitat with spruce and pine..............................*Russula turd* Bresad.

5.59 a) Small species, cap 2- 5.4cm diameter, mid to deep violet with a black to deep green centre, fading to greyed versions of these colours, margin striate, stipe discolouring light yellow-brown where bruised, taste slightly peppery in gills, spores light dull orange to ochre, Romagnesi Illb-c, with mostly isolated warts. Habitat Sitka spruce-western hemlock forest..........***Russula nauseosa* (Pers.) Fries**

b) Size ranging larger, stipe unchanging to slightly greying, cap colours a more reddish or brownish purple, spores darker..5.60

5.60 a) Cap up to 8.5cm diameter, reddish purple with a very dark centre which sometimes fades to yellow-brown, colours usually radially streaked, margin smooth until old, spores deep ochre yellow, Romagnesi IVd to IVe, reticulate, habitat with Douglas fir..***Russula cessans* Pearson**

b) Cap usually up to 9cm diameter, sometimes to 15 cm, vinaceous to purple, usually with a brown centre, spores pale ochre, Crawshay D-F, habitat with Sitka spruce...*Russula zelleri* Burl.

Synoptic keys

Each number codes for the species or variant as listed below this key, in approximate clade order, numbers in bold type are described in the following pages. Species may appear in more than one character category when it is variable for that character, for example, *R. brevipes* (coded 8) may be mild or slightly peppery.

Predominant cap colours	**Taste peppery**	**Taste mild**
White to cream to pale yellowish	2,6,7,8,9,10,22,41, 42,43	**1,3,4,5,6,7,8,31,75**
Bright yellow		**67, 68, 69, 87,** 88
Bright red, ± orange, yellow or cream areas	**47,** 48, 49, **64, 65, 66**	**76,79**
Pink to light terracotta with yellowish areas	51, **52,** 80, **81**	**27, 69, 74, 82, 86, 96**
Deep red to vinaceous	**38, 39, 46, 53, 55, 56, 57, 58, 59, 62, 63, 80, 108**	**24, 38, 76, 77, 84, 94, 95,**103, **104, 105,** 108
Purple, violet, blue, ± green, black or brown areas	**32,39,40,44,51,53,56, 57, 58, 61, 62, 63,** 100, **106,** 107, 108	**23, 24, 32, 33, 35, 45, 70, 71, 72, 73, 78, 85, 86, 90, 91, 92, 94, 97,** 103, **104, 105,**106, 108
Green	**23, 25, 26, 28, 36, 37, 61, 78, 83, 86,**	**44, 54, 93**
Browns and greys	**2, 3, 6, 11,12,13, 14, 15, 16, 17,18,19,20,21,32, 38,40,60,89,** 108	**1, 3, 4, 5, 6, 12,16, 25, 29, 30, 32,** 33, **34, 35,** 37, **38,** 77, **78, 83, 84, 90, 91, 92, 96, 97, 98, 99, 102, 105,**108
Other	**34** - pale, tints of pink, green and grey **40** - pale purplish grey 89 - yellow margin, dark brown disc	**102** - golden brown and olive green 108 - grey with yellow margin

Taste sometimes also with bitter component:

3,**10,15,** 17, 18, **22,** 31, 40, 64, 65, 66, 75, 76, 87, **103.**

Cap margins	
Striate to tuberculate at or before early maturity	67,**11,12,13,** 14, **15,** 16, 17, 18, 19, **20, 21,** 68, 80, 82, 90, 99, 100, 105,106,107, 108
Smooth when young, margin usually somewhat striate in maturity	36, 37, 41,42, 44, 45, 46, 47, 48, 49, 51, **52,** 53, 54, **55,** 56, 60, 61, 62, 63, 65, 66, 69, 70, 72, 74, 77, 81, 87, 88, 89, 91, 93, 96, 101, **102,103,** 104
Smooth, not or barely becoming striate in age	1, 2, 3, 4, 5, 6, 7, 8, 9,**10, 22,** 23, 24, **25,** 26, 27, 28, 29, **30,** 31, **32,** 33, 34, **35,** 38, 39, 40, 43, 50, 57, 58, 59, 64, 71, 73, 75, 76, 78, 79, 83, 84, 85, 86, 92, 94, 95, 97,98

Cap peelable - proportion of radius	
0 - 1/4	2, 3, 4, 5, 7, 8, 9,**10,11,** 14, 16, 18, 19, **20, 21,** 25, 26, 31, 35, 38, 42, 49, **55,** 57, 58, 64, 65, 76, 93
>1/4 - 3/4	1, 6,**13,**15, 17, **22,** 23, 24, 27, 28, 29,**30,32,** 33, **36,** 37, 39, 41, 40, 43, 47, 48, 50, **53,** 54, 56, 59, 60, 61, 62, **63,** 66, 69, 70, 71, 72, 75, 77, 78, 79, 80, 81, 82, 83, 84, 86, 88, 89, 90, 91, 92, 94, 96, 97, 98, 100, 101, **102,** 104, **105,106,** 108
3/4-completely	**22,** 36, 47, 67, 68, 73, 74, 107, 108

Pattern type of chromatograph of extracted pigments	
Pattern 1	**40, 44,** 45, **46,** 47, 53, 55, 56, **64, 66,** 67, 76, 78, 81, 82, 83, 84, 93,**102,103** *24, 39, 48, 49, 51, 57, 58, **63,** 65, 68, 69, 77, 79, 101
Pattern 1 -*Xerampelinae* type with magenta bands not fluorescing in blue LED light.	38, 71, 91, 94, 95, 96, 97, 98, 99, **106** *70, 72, 73
Pattern 1, other variations	**36** -very little magenta, not fluorescing in LED **41** -very little magenta or yellow 92 -the upper magenta band not fluorescing in LED or UV *50 -reduced magenta and blue *88 -no magenta bands
Pattern 3	**29, 30, 32, 34, 35** *23, 26, 27, 28, 31, 33, 37
Pattern 4	**22,43,** *42
Pattern 5	**1, 3, 4, 5,11,12,13,15,18, 20, 21** *2, 6, 14, 16, 17, 19

* These sets of species have not been tested here but are assumed to have their particular pattern because of their clade position and, in some cases, they have been assessed by Gluchoff (1969, 1975)

Gills and subgills	
Gills with frequent, more or less regularly distributed subgills sometimes forming 2-3 tiers (cap margin also inrolled)	1,2,3,4,5,6,7,8,9, **10**
Gills all the same length or with few, irregularly distributed subgills and/or forked gills	11-108 inclusive

Stipe surface colour	
Stipe white to cream	1, 2, 3, 4, 5, 6, 7, 8, 9,10,11,12,13, 14, 15, 16, 17, 18,19, 20, 21, 22, 23, 26, 27, 28, 29, 31, 32, 34, 37, 36, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 60, 61, 62, 67, 68, 69, 70, 71, 73, 75, 76, 79, 80, 83, 84, 86, 87, 88, 89, 91, 92, 93, 98, 99, 100, 101,103, 104, 105,106, 107, 108
Stipe usually or occasionally flushed with pink to violet	24, 25,30, 33, 35, 37, 51, 52, 53, 54, 55, 56, 57, 58, 59, 63, 64, 65, 66, 72, 74, 77, 78, 81, 82, 85, 90, 94, 95, 96, 97,102, (23, 27, 39, 44, 45, 69, 70, 84)
Stipe usually or occasionally flushed with grey-green	30, 32, 35, 36

Species in parenthesis normally have a white stipe but coloured flushes have occasionally been reported.

Bruising reactions of the flesh	
Trama bruising pink or red, then grey to black	2, 3, 5, 6, 86 (50 -red to grey, not blackening)
Trama bruising grey to black - no distinct reddish phase	1, 4, 79, 88 (87 -slight or inconsistent reaction)
Trama bruising ochre yellow, ± browning later	10, 20, 21, 22, 35, 57, 61, 62, 64, 81, 90, 94, 95, 96, 97, 98, 99, 105, 107, 108 (24, 25, 28, 29, 30, 69, 91,106, -slightly yellow-brown only)
Trama bruising grey-brown to red-brown	11,12,13, 14, 15, 16, 17, 18, 19, 26, 27
Stipe base bruising bright yellow, (may turn brownish later)	52, 53, 54, 57, 64 (55, 56, 58, 60, 62, 63, 65, 66, 70, 80, 90, 91,105, 106,107 -dull yellow only)
Stipe base bruising brown	1, 2, 3, 4, 5, 6, 7, 8, 9,10,11,12,13, 14, 15, 16, 17, 18,19, 20, 21, 22, 23, 29, 30, 31, 32, 33, 34,35, 36, 37, 38, 39, 42, 43, 55, 56, 58, 60, 75, 76, 77, 78, 86, 93, 94, 95, 96, 97, 98, 99, 101, 108 (67, 71, 81, 84 -browning only slightly)
Aging a buff-grey or yellowish grey as if waterlogged	39, 40, 41, 44, 45, 46, 47, 48, 51, 52, 53, 54, 55, 56, 58, 59, 60, 62, 63, 65, 66,102,103, 104, 105,106, 108

Reactions to chemicals:		
FeS04 on stipe cortex:	Blue-green	94, 95, 96, 97, 98, 99
	Grey-green or grey	1, 2, 3, 4, 5, 6, 12,15, 22, 23, 37, 86, 90
	Reaction none or very weak	52, 63, 71, 84, 102
	Salmon pink to brownish pink (normal)	All remaining species
NH4OH and KOH	Red on stipe cortex and gills	56,57
	No reaction or accelerating normal bruising reactions on stipe cortex	All remaining species
2% phenol on stipe surface	Blackcurrent purple	77,78
	Weak to pinkish	5
	Brownish purple (normal)	All remaining species
Sulphovanillin	Cutis staining bright pink to red (this reaction is not often recorded for species not described in this thesis)	35, 75, 95
	Gills staining as above	24, 25, 30, 35, 36, 75, 92, 93, 95,101
	Other reactions	36, 95 cap trama deep pink 52 red at stipe base 86 primordial hyphae pink 93 pileocystidia pink
	Pileocystidia not or barely staining, (or no pileocystidia)	1, 2, 3, 4, 5, 6, 7, 10,11,12, 13, 14,15, 16, 19, 23, 24, 25, 26, 27, 28, 29, 30, 34, 35, 67, 68, 69, 70, 71, 72, 73, 74, 75, 77, 78, 82, 83, 84, 85, 86, 87, 88, 91, (92 see above), 93,94, 95, 96, 97, 98, 101, 104
	Normal reaction, vascular hyphae and cystidia stain grey, purple or black, cutis and gills stain purple, brown or grey:	8,9,17,18,20,21,22,30,31, 32, 33, 36-66 inclusive, 76, 79, 81, 99, 100, 102,103, 105,106, 107, 108

Spore colour - Romagnesi codes, figure 29	
White la-lb	1, 2, 3, 4, 5, 6, 8, 9,12,13, 20, 21, 22, 23, 25, 26, 27, 28, 39, 42, 43, 46, 49, 52, 73, 74, 30, 41, 44, 45, 47, 48, 72, 76
Cream Ila-IId	7, 10,11,12,13, 14, 15, 16, 17, 18,19,24, 28, 29, 31, 32, 33, 34, 35, 36, 37, 38, 40, 50, 51, 53, 55, 56, 58, 61, 62, 63, 76, 79, 85, 86, 90, 91, 96, 98, *99*, 105
Yellow Illa-IIIc	31, 34, 53, 54, 57, 58, 59, 64, 65, 66, 75, 80, 82, 83, 88, 79, 89, 90, 92, 93, 94, 95, 96,100, 104, 106,107, 108
Orange-yellow IVa-IVe	59, 60, 67, 68, 69, 70, 71, 77, 78, 81, 84, 87, 89, 97, 101, 102,103, 104, 106

Spore ornamentation using Woo's codes, figure 30					
1	20, 21, 23, 28, 30, 34, 35,42, 49	1, 2, 4, 6, 11,12,16, 20,21,27,30,31,32, 33, 34, 35,36,42, 49, 60, 71, 82, 89	1,2,3,4,5,6,7,11, 12, 16, 29, 30, 32, 33, 36, 37, 38, 43, 62,63,71, 80,82	1,3,4,5, 11,38,41, 43, 46, 58, 62,63	7,33, 36,82
2	13, 14, 15, 17,20,21, 22, 25, 30, 34, 49, 75, J*?} JHt^ ^+J^ 61, 67, 78, 84, 99, 101, 92, 104	8, 9,11,12,13, 14, 15,17, 20,21, 22, 25, 26, 30, 34, 36, 49, 53, 54,55,51,52,58,59, 64, 65, 66, 67, 68, 69, 73, 71, 74, 75, 78, 81, 82, 84, 88, 89, 90, 91, 96, 97, 99, 92, 104, 107, 108	5, 7, 8, 9,10,11,12, 13, 28, 33, 36, 37, 43, 44, 45, 47, 56, 57, 58, 59, 62, 63, 64, 65, 67, 68, 71, 74, 76, 77, 81, 82, 84, 87, 88, 79, 90, 91, 94, 96, 97,100, 103, 107	5,8,9,24, 28, 36, 39, 41, 43, 44, 45, 46, 47, 48, 50, 57, 62, 63, 70, 77, 85, 103	7,8,9, 18, 24, 36, 41, 70, 84, 100, 105
3	25, 55, 61, 72, 78, 83, 86, 79, 89, 93, 95, 96, 98, 99, 101, 102, 92, 105	8, 9, 25, 40, 52, 55, 66, 69, 78, 83, 86,, 90, 93, 94, 95, 96, 98, 99, 92,105,106	7, 8, 9, 40, 44, 45, 47, 52, 56, 79, 90, 94, 98,105	8,9,24, 44, 45, 47, 48	7,18, 19,56
	A	B	C	D	E

Spores: suprahilar patch	
Most spores with an inamyloid to weakly amyloid area or a poorly defined area	1, 2, 3, 4, 5, 6, **10,11,12,13,** 14, 15,16, 17, 18, 19, **20, 21, 22,** 23, 26, 27, 28, 29, **30,** 31, **32,** 33, **34,** 35, 36,37
Most spores with a well defined, distinctly amyloid area	7, 38, 39, **40, 41,** 42, **44,** 45, **46,** 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, **64,** 65, **66,** 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101,**102,**103104 105 106 107 108
Spores variable or difficult to assign to one of the above two categories	7, 8, 9,**10,** 24, 25

Spore length to width ratio -(shape)	
Subglobose, 1.10-1.19	5, 8, 9, 16, 18, 36, 40, 44, 45, 52, 66, 67, 78, 81, 82, 83, 93, 103, 105
Broadly ellipsoidal, 1.20- 1.29	**1,12,15, 20, 21, 22, 25, 29, 30, 32, 35, 38, 41, 43, 46, 47, 53, 54, 55, 56, 63, 64, 71, 75, 76, 84, 90, 91, 94, 95, 96, 97, 98,102, 92,106**
Ellipsoidal, 1.30-1.39	**3,4,10,11,13, 14,34,86**

Cutis, characters other than the normal ixotrichodermis	
Epicutal hyphae with incrustations:	67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 79, 82, 83, 84, 85, 86, 87, 88
With mere traces of incrustation:	38, 47, 52, 92
Not known if incrustations present:	80
Cutis predominantly of spherical and inflated cells	28
Epicutis with multiseptate hyphae looking like chains of short, sometimes inflated cells (excluding cystidia)	11,12, 13, 14, 15, 24, 25, 26, 29, 30, 31, 32, 33, 34, 35, 37, 78 22 also has thickened walls
Epicutis with (a few) thick walled long, tapered, hair cells	26,27
Subcutis cellular	70, 71, 72, 74 91 ± cellular clusters in an otherwise interwoven subcutis

Pileocystidia types, figure 31 (types not mutually exclusive)	
1 -small, tapered	**11,12,**13, 14, **15,** 16, **22,** 97
2 -long, cylindrical, tip strangulate-capitate, usually 0-2 septate	1, 2, 3, 7, 17, **18,** 19, **20, 21, 22,** 23, 29, **30,** 31, **32,** 33, 34, 35, **36, 38,** 39, 40, 41, 42, 43, 44, 45, 48, 49, **52, 53,** 54, **55, 56,** 57, 58, 60, 62, **63,** 64, 65, **66, 81,** 94, 98,**102** , 104 95, 96, 97, 37 -few or rare, not consistently present
3 -clavate, tip obtuse, 0-1 septate	**18,** 19, 26, 27, 36, 41, 44, 46, 47, 61, 62, **63, 83,105**
4 -cylindro-clavate, tip obtuse, usually 0-2 septate, occasionally 3	6, 26, 27, 29, **30,** 33, 34,**35,** 37, **38,** 40, 41,42, 43, 44, 45, 46, 47, 48, 50, 51, **52,** 55, 58, 59, 60, 61, 62, **66,** 79, **81, 82, 83,** 84, 87, 89, 90, 91, 92, 93, 98, 99, 100, 101, **102,** 104, **105,106,** 107, 108 95 -few to rare 85 -assumed present
5 -long, cylindrical, tip obtuse, mostly >2 septate	**38,** 47, 48, 50, 51, **52, 53,** 54, **55,** 59, 60, 61, 64, 65, **82,** 84, 87, 92, 94, **102,103,105,** 107, 108 67 -poorly differentiated, uncertain
6 -diverticulate, generally 0-3 septate	2, **3, 46, 55, 56,** 58, **63, 64,** 65, **66,** 101, **103**
7 incrusted pileocystidia	**76,82** **75** -pileocystidia poorly differentiated 83, 84 -weakly and variably incrusted
8 incrusted primordial hyphae	**67,** 68, 69, 70, **71,** 72, 73, 74, 75, **76, 86,** 88, **106** **83,** 84, 92 -structures appearing like primordial hyphae but lacking obvious incrusting material
Pseudocystidia also present in epicutis	17, **18,** 19, **20, 21,** 29, 31, **36,** 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 50, 51, **52, 53,** 54, **55, 56,** 57, 58, 59, 60, 61, 62, **63,** 64, 65, **66,** 69, **76,** 89, 91, 92, 93, 98, 101, **103, 106** 8, 9,**10,11,**15,**12,13,**14, 16, 67 -rare to occasional
Pileocystidia and pseudocystidia lacking or doubtful.	78, 4, 5, 8, 9, **10,** 24, 25, 28, 68, 69, 70, 71, 72, 73, 74, 77, 86,88
No data available for these characters	80

Habitat and tree associations. Most species are found in habitats with a mixture of trees, so the host tree may be any or all of those in the habitat, therefore these habitat categories are not mutually exclusive.	
Under Douglas firs	1, 3, 4, 8, 9,**10,11,12,**15, 24, 29, 31, 32,35, **36,** 38, 41, 44, 51, 56,59, 60, **63,** 71, 75, 78, 80, **81,** 82, 84, 91, 92, 94, 95, 97, 100, **103**
Under western hemlocks	41, 44, 45, 47, 65, **66,** 71, 75, 78, **86,** 91, 92, 93, 94, 95, 99, **102,**13,**18,** 52, 55, 96, 98,**106**
Under Sitka spruce	**18,** 22, **30,** 47, 53, 54, 70, 83, 90, 96, 98, 101, 104, **105, 106,**13, 55, 8, 9,1 50 -unspecified spruce species
Under alders	**1,10, 52, 84**
Under oaks	7, 16, 24, 28, 50, 74, 87, **76, 43, 67**
Under true firs (Abies)	**41, 55, 56,** 65
Under pines	**13,34,**48, 57, 58, 60, **64,38, 82,10,**14, 70, 87, 100,
Under birches	28, 49, 88, 107, 108
Under madrone	**38,82**
Under willows	**46**
In boggy places or wet seeps ± sphagnum	**48, 62, 66, 88, 90**
Usually on rotten wood	**40, 41, 44, 45, 47, 93**
Coniferous forest*	**3, 4, 6, 17, 21, 32, 42, 62, 69, 73, 79, 95**
Deciduous forest*	**6, 14, 16, 17, 20, 24, 26, 27, 28, 32, 61, 68, 72, 74, 89**
Mixed forest*	**2, 19, 23, 33, 37, 39, 49, 77, 85**

* These categories include species for which the available data is non-specific and species with a wide range of host or habitat. The species listed here are in addition to those given in more specific habitats above.

Table 15 Species code numbers used in the synoptic key

Key number	species	Clade	Polychotomous key entry
1	***/£. adusta***	lb	1.6
2	*R. densifolia*	lb	1.4
3	*R anthracina* var. *insipida*	lb	1.6
4	***R albonigra***	lb	1.5
5	***R dissimulans***	lb	1.3
6	*R nigricans*	lb	1.3
7	*R delica*	2b	2.2
8	***R brevipes***	2b	2.2
9	***R brevipes* var. *acrior***	2b	2.3
10	***R cascadensis***	2b	2.3
11	***R cerolens***	3b	3.10
12	***R pectinatoides***	3b	3.10
13	***R cf.pectinata***	3b	3.9
14	*R. amoenolens*	3b	3.9
15	***R granulata***	3b	3.7
16	*R. sororia* group	3b	3.10
17	*R. foetens*	3c	3.3
18	***R fragrantissima***	3c	3.4
19	*R laurocerasi*	3c	3.4
20	***R farinipes***	3b	3.6
21	***R pallescens***	3b	3.6
22	***R crassotunicata****	3d	3.1,5.6
23	*R. cyanoxantha*	3a	4.4
24	*R mariae*	4a	4.7
25	***R smithii***	4a	4.3
26	*R. heterophylla*	4c	4.5
27	*R. vesca*	4c	4.5
28	*R virescens*	4b	4.5,4.8
29	***R mustelina***	4b	4.9

fragilis
fragilis var. mil
laccata
silvicola
emetica
bicolor
consobrina
gracilis*
cf. luteotacta
queletii
queletii cf. var.
fuscorubroides
cavipes
sardonia
torulosa
mordax*

5.33, 5.37

Key number	species	Clade	Polychotomous key entry
60	*R. murina**	5e	5.30
61	*R. violacea*	6	5.39
62	*R. olivaceoviolascens**	6	5.39
63	***R, pelargonia***	6	5.42
64	***R. sanguinaria***	6	5.18
65	*R. americana*	6	5.18
66	***R. americana var modicaspora***	6	5.18
67	***R. lutea***	7a	5.5
68	*R. gilva*	7a	5.5
69	*R. chamaeleontina*	7a	5.5,5.16,5.22
70	*R. turci*	7a	5.58
71	***R murrillii***	7a	5.58
72	*R. lilacea*	7b	5.45
73	*R. azurea*	7b	5.45
74	*R. praetenuis**	7b	5.15
75	***R. albida***	7b	5.4
76	***R lepidiformis***	7b	5.15
77	*R. alutacea*	8a	5.12
78	***R olivacea***	8a	5.12, 5.22, 5.54
80 79	*R. decolorans*	8b	5.16
8180	*R. inconstans**	8c	5.21, 5.40
82 81	***R veternosa***	8c	5.21
83 82	***R. velenovskyi***	8d	5.22
84 83	***R. Integra 1***	8d	5.26, 5.55
85 84	***R Integra 2***	8d	5.26, 5.55
86 85	*R. maxima**	8d	4.7, 5.51
87 86	***R. occidentals***	8d	5.25, 5.47
88 87	*R. flaviceps**	8d	5.4
88 88	*R. claroflava*	8d	5.3
89	*R. disparilis**	9a	5.30

Key number•	species	Clade	Polychotomous key entry
90	***R sphagnophila***	9a	5.50
91	***R brunneoviolacea***	9a	5.50
92	***R abietina***	9a	5.56
93	***R aeruginoides***	9a	5.27
94	***R xerampelina***	9b	5.10
95	***R semirubra***	9b	5.10
96	***R isabelttniceps***	9b	5.9
97	***R cf. pruinosa***	9b	5.11
98	***R elaeodes***	9b	5.11
99	*R. viridofusca*	9b	5.11
100	*R. placita* *	10a	5.40
101	*R. urens*	10a	5.31
102	***R aureofulva***	10a	5.27
103	***R. cessans***	10a	5.60
104	***R. zelleri****	10a?	5.60
105	***R puellaris***	10b	5.49
106	***R nauseosa***	10b	5.59
107	***R. versicolor***	10b	5.39
108	***R blackfordae***	10b	5.27

* Approximate clade position is a best estimate based on available descriptions, since herbarium or fresh specimens were not examined.

Other species clade positions that were not verified by DNA analyses in this thesis or in referenced literature are based on morphological similarities to species whose clade position has been verified.

Descriptions of Vancouver Island Russulas

Clade lb

Subgenus *Compactae* (Fries) Bon

Section *Compactae* Fries (= *Nigricantinae* in Romagnesi 1967)

***Russulu adusta* (Pers.) Fr.**

Epicrisis Systematis Mycologici 350. 1838

Cap 5.5-10cm diameter, convex to pulvinate when young, becoming more or less plane or more often developing a broad, shallow central depression, the margins usually remaining somewhat downcurved and inrolled even in age, not striate, cutis peelable less than 1/4 or up to 1/3 of the radius. Colour a pale slightly greyish cream, developing dull yellow to yellowish brown to dark greyish brown streaks and mottles, the margin usually not discolouring as much. Centrally, the colour may be lighter or darker than the rest of the cap. Eventually the whole cutis becomes grey-brown, generally not blackening until in a state of decay. Surface viscid when wet, drying matte, glabrous. Flesh white, firm, where cut changing to grey or greyish pink, slowly darkening to grey-brown and sometimes blackish, the reaction is slow compared to others in this subgenus.

Gills warm pale cream, bruising or aging pale dingy brown, sometimes with a pinkish-grey-brown phase, eventually nearly black, close, with more or less regular subgills interspersed, no forking seen, falciform, adnate to adnexed and sometimes ascending at the stipe, acute at the cap margin, about 1/3 to 1/2 the depth of the cap trama at half-radius.

Stipe 2.7-4.8 x 1.8-2.3cm, (up to 4cm in larger basidiomata that were seen but not collected), about equal in length to that of the cap diameter, cylindrical to clavate, sometimes also broadening at the apex and base, white, pubescent near the apex, bruising slowly pinkish brown or directly brown to almost black. The stipe rind is around 3 mm thick and encloses a firm bread-textured solid trama that may be hollowed out by insect larvae in age, and which bruises like the cap trama, not much darker than the stipe cap cutis in dried specimens.

Texture firm, not particularly brittle at first but becoming so in age.

Taste mild, like bread, sometimes with a faint fleeting pepperiness in the gills of immature basidiomata.

Odour not distinctive, slightly of bread.

Spore colour white, Romagnesi la.

Spores 6.5-9.5 (-10) x 5-7.5um, L.W1.03 -1.56, mean 1.26 (n=30), globose, subglobose, broadly ellipsoidal to ellipsoidal. **Ornamentation** of blunt to pointed, often triangular warts 0. l-0.2um high generally, but occasionally up to 0.4um, rarely isolated, mostly connected by fine to heavy lines forming a broken to complete reticulum, **Woo types** Cl-Dl. **Suprahilar patch** inamyloid, a smooth elliptical area about 3 urn long, sometimes with tiny warts and lines. **Hiliferous appendix** 1.2-1.9u,m long, 1.2-1.8um wide near the base. **Basidia** mostly 4-spored, some 2-spored, more rarely 1 and 3-spored, 30-52 x 8-10um, clavate when immature, narrowly clavate to almost cylindrical but slightly bulbous in the upper 1/3 when mature. Sterigmata 5-7um long, around 1.2um wide near the base. **Pleurocystidia** frequent to abundant, 32-84 x 5-7.5um, originating in the outer subhymenium, protruding hardly at all, at most to about 13um, cylindrical to fusoid, sometimes irregularly constricted, tips rounded, acute, capitate, contents refractive, yellowish in KOH, grey to black in SV. **Cheilocystidia** similar to pleurocystidia in shape but barely reacting with SV. Subhymenium 20-3Oum thick, pseudoparenchymatous. **Gill trama** of sphaerocytes, vascular hyphae rare.

Cutis 90-280um thick, quite elastic, in some sections appearing to have a lower pale layer but on microscopic examination this is actually an unblackened layer of the cap trama overlying the darkened trama. **Subcutis** interwoven, embedded in a weak gelatinous matrix that is inconspicuous in 5% KOH, of septate, branched hyphae 2.5-4um wide, partially filled with an amorphous to slightly refractive yellow-brown pigment. **Epicutis** not distinct from the subcutis, interwoven with erect free hyphal ends, 2-5 um wide but with frequent short inflated sections up to lOum wide, and often with the terminal or subterminal cell inflated into an ampullate shape. Near the cap margin, some of the hyphal ends appear cystidioid in shape, and a few contain yellowish (in SV) refractive contents while others have amorphous, pale grey contents. Since the hyphal ends are also varied in form, some with a long tapering end cell, many others with a short, slightly inflated end cell, and some containing pigment, it is difficult to differentiate between cystidia and hyphal ends. In SV the light yellow-brown vacuolar pigment in the epicuticular hyphae darkens to a grey-brown. Pileocystidia rare, mainly at cap margin, 14-117 x 3-8um cylindrical, mostly non-septate, capitate, occasionally with bi-lobed tips like a double capitum, with yellowish refractive contents not reacting in SV or with a few black granulations. **Hypodermis** of small slightly flattened sphaerocytes appearing greyish in KOH and water mounts.

Trama of discrete clusters of sphaerocytes bound by a hyphal mesh, often with brown pigment, and occasional vascular hyphae.

Chemical reactions: FeSC>4 - greenish-grey; KOH - no reaction on cap or stipe; NH4OH - no reaction; Phenol - deep red-brown; SV - dark grey-brown on the gills, light pink-brown on the cuticle, hymenial cystidia grey to black, vascular hyphae and pileocystidia not or barely reacting.

Habitat and tree associations: On the forest floor in moist, deep duff under regeneration Douglas fir forest with western hemlock and red alder, with or without veteran trees or wild cherry, with undergrowth including sword fern, huckleberry and salal.

Collections: CRO10902-02, along the High Ridge trail near an old growth Douglas fir and western red cedar amongst salal, Francis King Park N48.4837^0, W123.4505^0 (CDF zone). CRO 10909-04, in a swale containing red alder, cherry and Douglas fir, by the trail to Kemp Lake N48.3784^0 W123.7904^0 (western very dry maritime CWH).

Notes. The identification of these collections as *Russula adusta* rather than *R. densifolia* was made on the basis of a mild taste, the lack of a distinct red phase in the bruising reaction, which was at most a pinkish-brown, their similarities to a European collection of *R. adusta* and the difference between these collections and that *of & Russula densifolia* from Oregon. Although the spores are very close in size and description between the two species, the Vancouver Island collections had spores with a slightly lower ornamentation than that of the Oregon *R. densifolia,* in keeping with the observations of Shaffer (1962), Samari, (1998) and Romagnesi (1967). The spores however still have slightly coarser and higher ornamentation than in those authors' descriptions, and are more like the drawings and descriptions of spores of *R. adusta* by Thiers (1997) of California material. The European collection of it *adusta* had a weak but definite odour of wine corks and a very slow bruising reaction, the flesh only becoming dingy grey a day after cutting. Pacific Northwest collections of *R. adusta* do not seem to have this wine cork odour, but they taste and sometimes smell a bit like bread, another fermentation product of yeast. There are some anomalies with the Vancouver Island collections - *R. adusta* should have a brownish pink reaction with FeS04 rather than the greenish grey that is typical for *R. densifolia {sensu* Shaffer 1962, but not Singer, 1957). Both Shaffer (1962) and Thiers (1997) comment on the occurrence of collections that appear to intergrade between *R. densifolia* and *R. adusta,* in taste, spores and bruising reactions, so the differentiation between these species can be difficult.

Unfortunately, even with fresh material no amplifiable rDNA could be extracted from these collections.

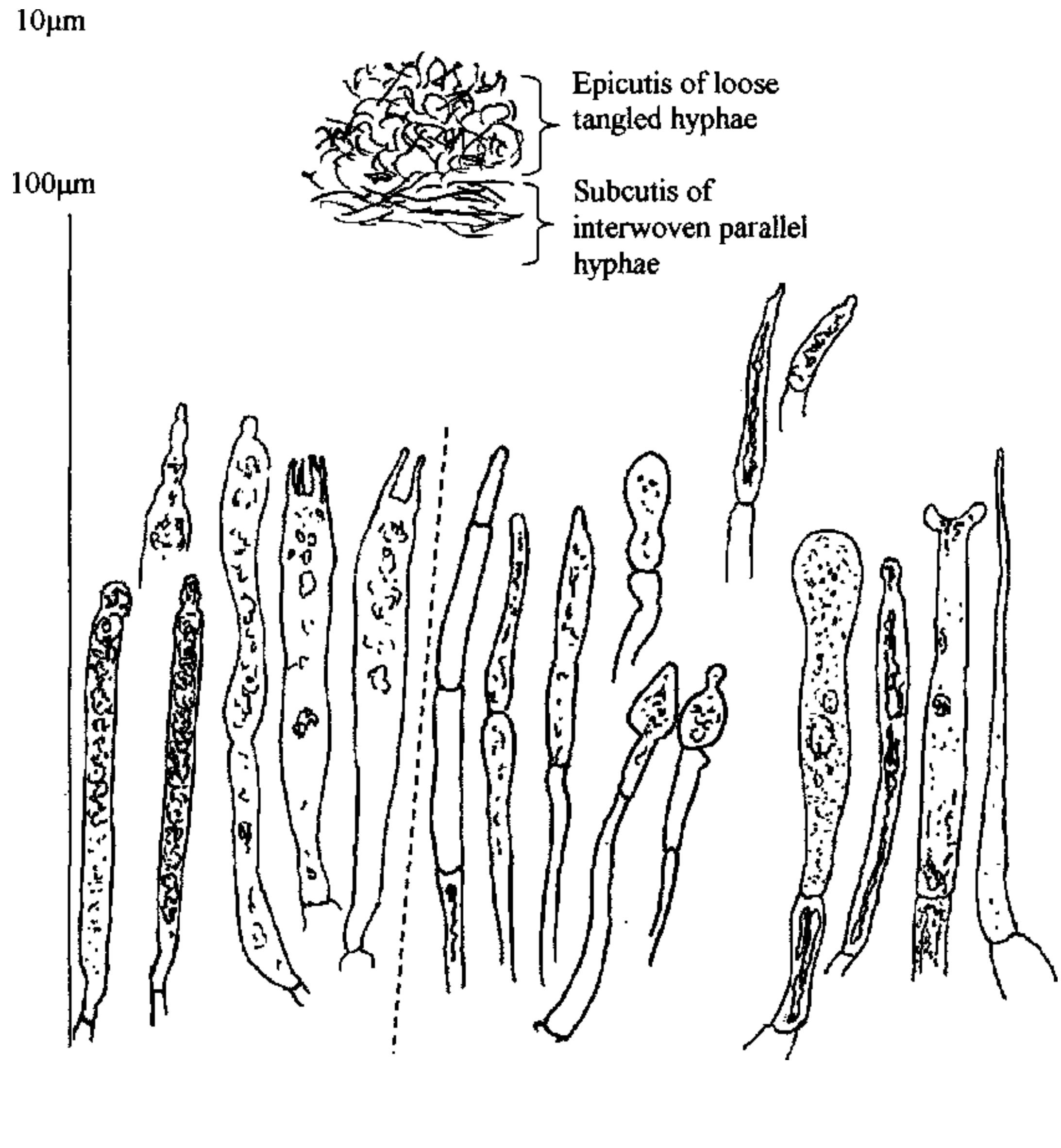


Figure 32 Microscopic characters of *Russula adusta*. Top, spores with 10 um scale bar; middle, diagram of section through cutis; bottom left, hymenial cystidia and basidia; bottom right, hyphal ends and pileocystidia from the epicutis, the six drawings on the far right are from the cap margin; lower scale bar is 100 um.

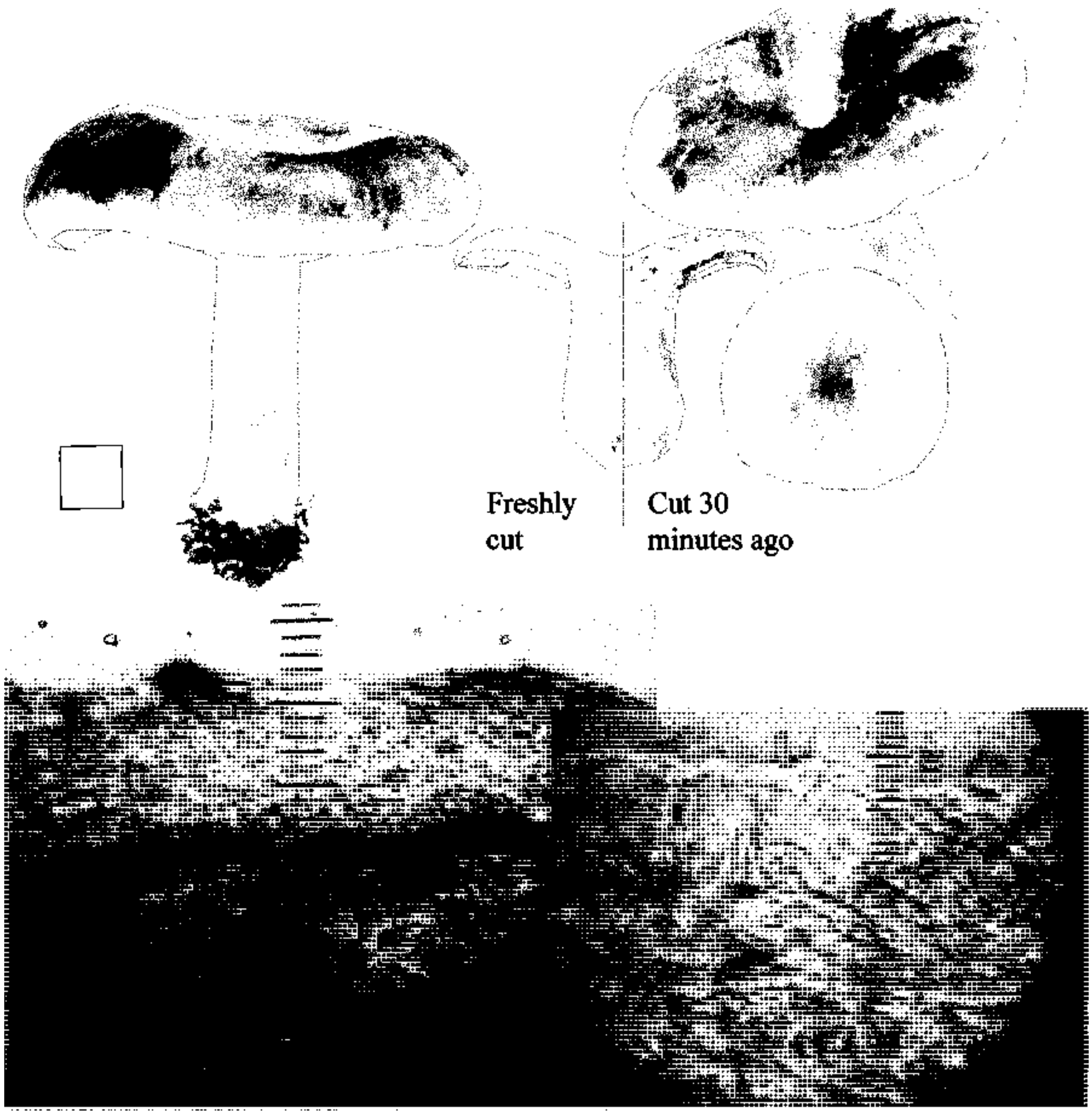


Figure 33 Macroscopic and cutis characters of *Russula adusta:* Top, illustration showing profile and cap surface of young and mature basidiomata, and a longitudinal section showing the bruising reaction of the flesh when freshly cut (left side of section) and after 30 minutes (right side); the square is 1 cm^2 and shows spore colour. Below left, section through cap cutis showing the light yellow-brown pigment in the cuticular hyphae, the hypodermis is the grey layer betweeN40 and 42 on the scale, in which 10 divisions are 100 um. Bottom right, section through the epicutis showing the viscid layer above the free hyphal ends (arrowed), 10 divisions on the scale are 25 um.

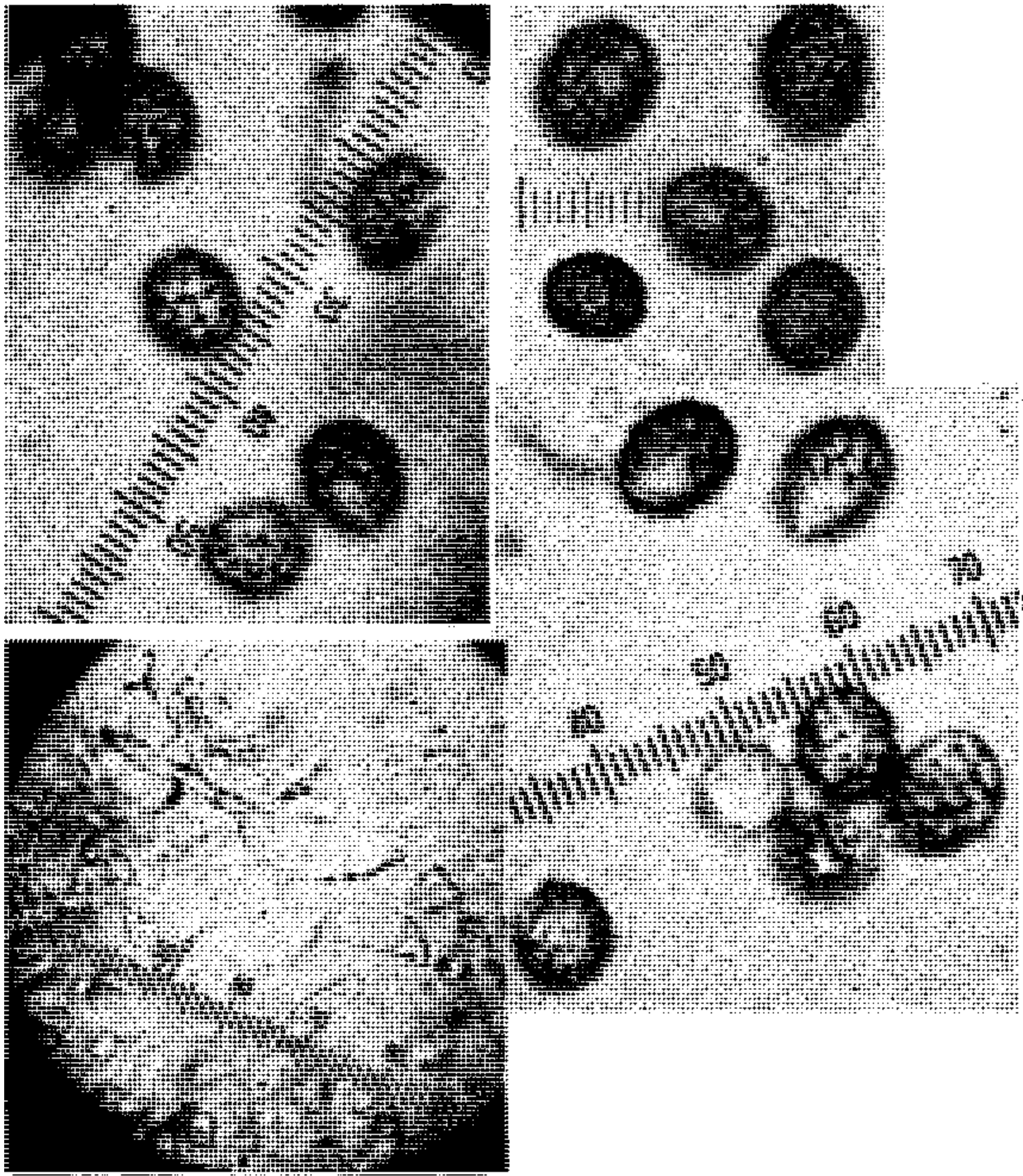


Figure 34 Spores and epicutis *ofRussula adusta:* Top left, spores from a Vancouver Island collection; top right, for comparison, spores from a Bulgarian collection of *R adusta;* bottom right, spores from an Oregon collection of *R densifolia*, scale is in 1 urn divisions on the latter three photographs. Bottom left, surface view of the epicutis of the cap margin showing hyphal ends and pileocystidia of varying shapes, 10 scale divisions are 25um.

***Russula anthracina* var. *insipida* Romagnesi** nom. inval, art. 37.1 ICBN

Bulletin Mensuel de la Societe Linneenne de Lyon 31: 173. 1962 (basionym)

Les Russules d'Europe et d'Afrique du Nord. 1967 (var. *insipida)*

Cap 3.9-8.5cm diameter, plane with a central depression, becoming shallowly infiindibuliform, dirty cream with dull brown to pinkish grey-brown mottling, becoming darker with handling and in age until dark grey to olive brown on the margin and almost black in the centre. General appearance almost identical to that *of Russula adusta* (see fig. 33 under that species description). Surface dry, somewhat felty to tangled-fibrillose especially over the disc, margins smooth, inrolled, cutis barely separable at the margin only, flesh creamy white, turning grey then black with very little to no reddish phase. In the dried basidiomata, the trama is black but the cutis on the cap and stipe are dark brown.

Gills pale cream with a slightly orange cast when seen edge-on, bruising and aging through grey to almost black, the white spores giving an ashy appearance. The gills are arched, 1/2 to 2/3 the depth of the cap trama at half radius, acute at the cap margin, only slightly decurrent, sometimes adnexed and ascending at the stipe, close to crowded, frequent lamellulae and some forking and anastomosing near the stipe in the immature basidioma, none in the older ones. Under a hand lens, the gill margins are minutely fringed due to protruding cheilocystidia.

Stipe 1.5-3.5 x 1.5-2.8cm, short and stubby, practically equal in both dimensions, length less than half the cap diameter, tapering slightly downwards. Surface white, minutely floccose, longitudinally rugulose, bruising grey-brown with the floccules darker, trama solid, becoming crumbly in age, cream at first, soon discolouring greyish and finally soot-black.

Texture hard but brittle.

Taste mild, in the older basidiomata slightly bitter.

Odour not distinctive.

Spore colour white, Romagnesi la.

Spores 7.6-9.6 x 5.8-7.5um, L:W 1.17-1.45 with amean of 1.31 (n-30), ellipsoidal to broadly ellipsoidal, some toward pyriform (the narrower end near the hiliferous appendix) occasionally slightly allantoid. **Ornamentation** of low, small, rounded to pointed warts 0.l-0.2u,m high, occasionally up to 0.4um, often in rows partially around the spore, mostly connected by fine lines, rarely heavy lines, sometimes with small isolated warts especially near the suprahilar patch, otherwise forming an almost complete reticulum, **Woo types** Cl-Dl. **Suprahilar patch** an elliptical inamyloid and unornamented patch about 3 urn long. **Hiliferous appendix** 1.3-1.5 urn long, 1.2-1.4um wide at the base. **Basidia** 47-65 x 6.5-7 urn, most 4 -spored but 2 -spored ones frequent, narrowly clavate and almost cylindrical, the spent basidia filled with a brownish pigment. Sterigmata 4-8um long and 1-1.3um wide near the base. **Pleurocystidia** 48-120 x 6-lOum, rare broad ones to 13pm wide, with yellow-brown refractive contents, staining grey in SV but variably so, some without refractive contents, originating at various levels within the subhymenium, protruding 10-40 urn. The basic shapes are more or less cylindrical to narrowly fusoid, often with constrictions, with diverse tips, many simply with a subacute to rounded end, others capitate or with a short to long appendage with a series of strangulations along their length. **Cheilocystidia** numerous, like pleurocystidia but barely reacting to SV. **Subhymenium** 25-35 um, interwoven but with frequent short cells and so appearing intermediate between pseudoparenchymatous and interwoven. **Gill trama** of small irregular sphaerocytes and very occasional vascular hyphae.

Cutis 120-250um thick, of two layers. **Subcutis** about 3/4 to 2/3 the total depth, of light brownish to hyaline, slightly gelatinized, more or less horizontal hyphae 1.5-4um wide, a few of which have dark contents like the epicutal hyphae. **Epicutis** interwoven with few free hyphal ends, of more or less repent hyphae varying in width along their length between about 2.5 and 7um, with occasional segments down to lum and up to lOum wide, containing large globules of amorphous dark brown pigment, sometimes this fills a hyphal cell completely, such that the epicutis resembles a tangle of transparent intestinal tracts. Some epicutal hyphae have colourless contents but are otherwise of similarly varying width. Hyphal ends undifferentiated to tapered in the centre of the cap, towards the margins a few hyphal ends may be inflated to short, broad clavate cells, around 10pm wide, few terminate in pileocystidia. **Pileocystidia** 53-145 x 5-10um, uncommon, found mainly on the cap margin, more or less fusiform with a capitate tip or cylindrical with a lobed tip, contents yellowish and refractive, incompletely filling the cell, not reacting in SV or with a very few dark granules. **Hypodermis** of dark, somewhat flattened tramal sphaerocytes and interwoven tramal hyphae that are continuous with those of the subcutis.

Stipe cortex containing frequent vascular hyphae 7-11pm wide, some terminating at the surface in pseudocaulocystidia with obtuse to slightly capitate ends, not reacting with S V or at most a few dark granules. The minute floccules visible through a hand lens consist of tufts of tangled free hyphal ends most of which contain dark brown necropigment.

Trama of discrete clusters of sphaerocytes bound by a hyphal mesh, with occasional vascular hyphae up to 7.5pm wide and sometimes with an inflated terminus up to 10pm, not reacting with SV.

Chemical reactions: FeSC>4 - greyish; KOH andNFLiOH - accelerating the blackening on all tissues; phenol - dark purple-brown; S V -on gills a deep red-brown, on cap cutis and stipe cortex magenta then purple-brown, not reacting with the contents of pileocystidia or vascular hyphae, or at best a few small dark granulations, staining pleurocystidia greyish, but this is difficult to assess since the contents are often greyish to begin with.

Habitat and tree associations: Both collections were made in moist areas with deep duff and a mixture of conifers and deciduous trees. Its host trees are probably Douglas fir and/or western hemlock, but may equally be alder and cottonwood. (Its European counterpart occurs with oaks on calcareous soils.)

Collections: CROO1007-03 an immature specimen and so not included in the spore measurements above, from a moist low-lying area close to the Cowichan River, under western hemlock, Douglas fir, red alder and cottonwood, also with many big-leaf maples, N48.756267^0, W123.8254^0 (eastern very dry maritime CWH subzone). CR021019-01 in a mixed age stand with old-growth trees, under western hemlock, Douglas fir and red alder, with big-leaf maples and western red cedars, Royal Roads University woodlands, N48.434833^0, W123.478417^0 (CDF zone).

Notes: Romagnesi recognised *Russula anthracina* as differing from *Russula albonigra* (Krombh.) Fries in spore ornamentation, the darker vacuolar pigment, the taste and the presence of pileocystidia, and within this species three varieties were described. *Russula anthracina* as a species distinct from *R. albonigra* has been supported in subsequent texts such as Bon (1988, 2002) and Sarnari (1998). In the latter monograph the validly published *Russula atramentosa* Sarnari was considered close to *R. anthracina* var. *insipida*, which name was invalid, however the Vancouver Island collections are closer to Romagnesi's description of this species than that Sarnari's *R. atramentosa*.

These collections were at first assumed to be *R. adusta* as they are similar macroscopically, but that species does not have the little floccules on the stipe, blackens less, is viscid, has a less fibrous-felty cap surface, lacks the distinctive dark brown globules in the epicutal hyphae and has more globose spores with stronger ornamentation. It also differed from the collection of *R. albonigra* below in the macroscopic appearance of the cutis, the pigment in the cuticular hyphae, the slower bruising reaction, the reaction of pleurocystidia with SV, the shape of the basidia and the flocculate stipe surface. *Russula dissimulans* or *Russula nigricans* have a much thinner cutis but a similar epicutis, but these species stain red before turning black, have more distant, thicker gills, and rounder, more strongly ornamented spores.

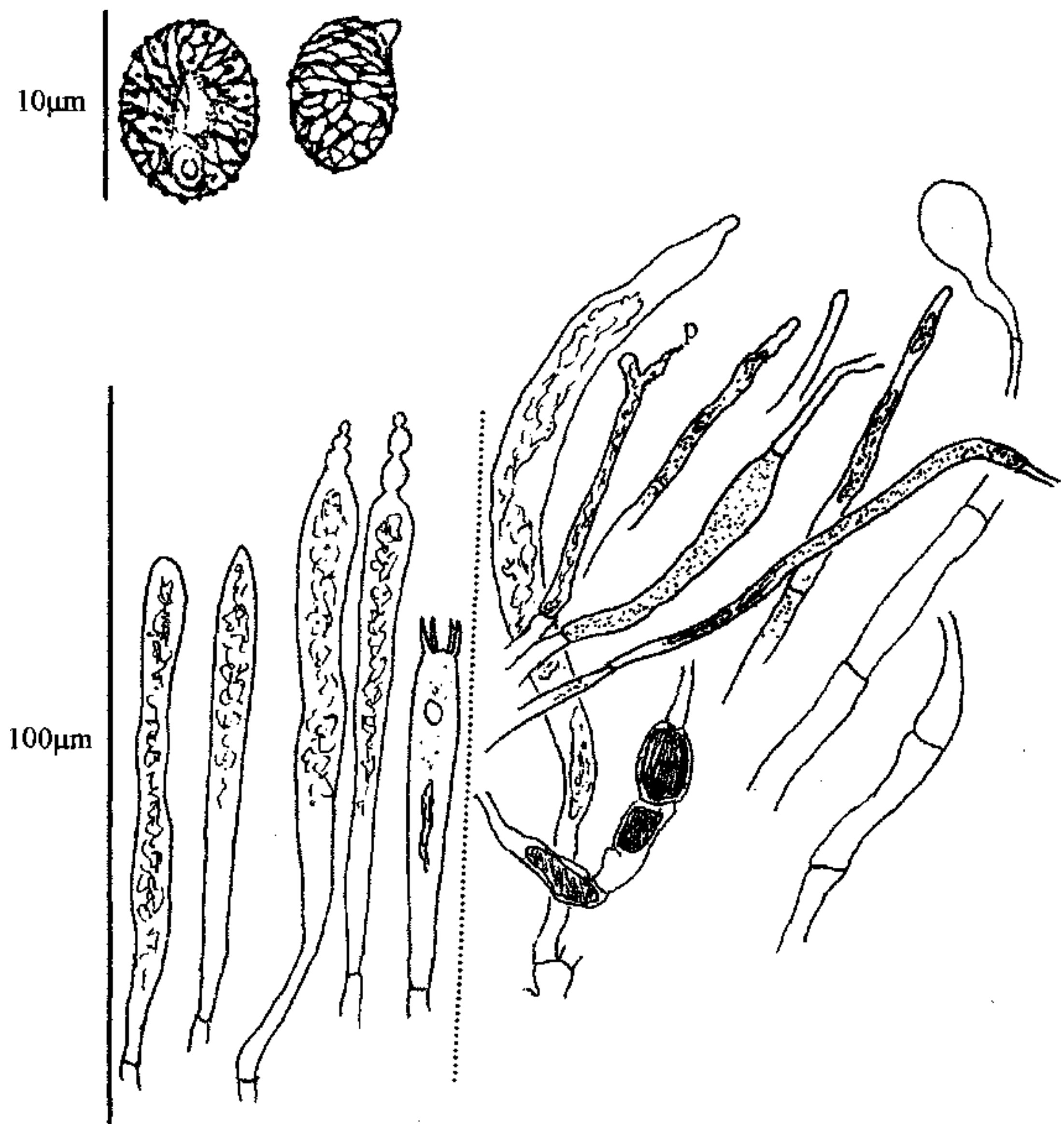


Figure 35 Microscopic characters *ofRussula anthracina* var. *insipida:* Top. Spores with 10 um scale bar; bottom left, hymenial cystidia and basidium; bottom right, hyphae and hyphal ends from the epicutis, the large capitate and small lobed pileocystidia are marked "p", note that some of the broader and ampullate shapes occur along the epicutal hyphae, rarely as the terminal cells. The lower scale bar is lOOum.

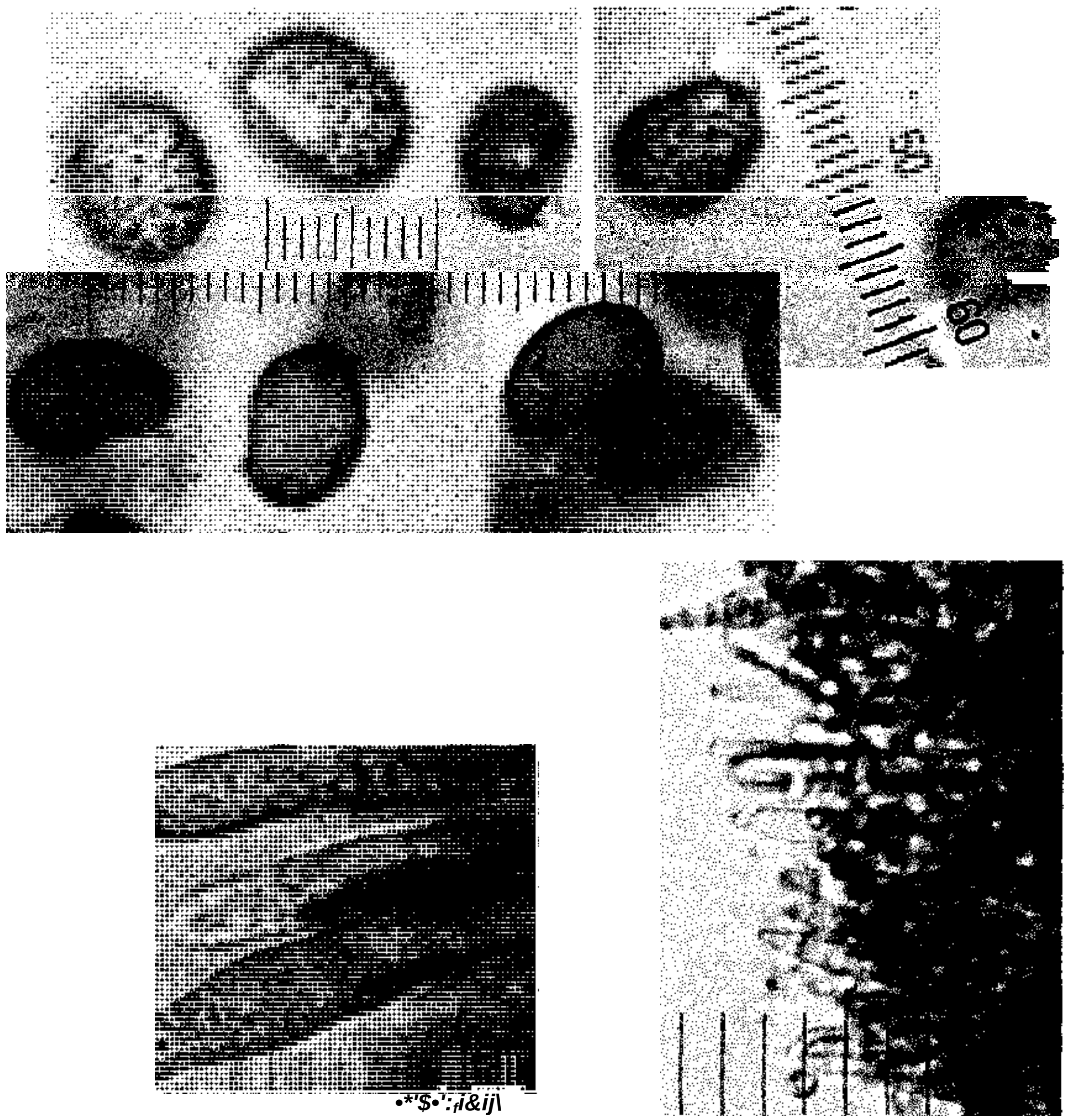


Figure 36 Hymenium of *Russula anthracina* var. *insipida:* Top, spores showing the surface ornamentation and below, profiles showing the variation in shapes, the scales are all in 1 um divisions; bottom left, a spent basidium which shows an accumulation of brownish pigment inside, a maturing one, and two cystidia, the scale is in lum divisions; below right, versiform cheilocystidia on the gill edge, with a scale in lOum divisions.

Figure 37 Cutis of *Russula anthracina* var. *insipida:* Top left, section through the cutis showing the mostly hyaline subcutis and the epicutis of tangled hyphae containing dark pigment, 10 divisions on the scale are lOOum; below left surface view of epicutal hyphae showing discontinuous pigmented contents, 10 scale divisions are 25um; top right, close up view of epicutal hyphal ends containing pigment globules, scale is in 1um divisions; lower right, pileocystidia from near the cap margin, (tip unfortunately missing), scale is in 2.5um divisions.

***Russula albonigra* (Krombh.) Fries**

Naturgetreue Abbildungen und Beschreibungen der Schwamme 9: 27. 1845
(*Agaricus alboniger* Krombholtz)
Hymenomycetes Europea: 440. 1874

Cap 6.2cm diameter, shallowly infundibuliform in this early mature collection, margin smooth, inrolled, colour pure white, subglossy, moist to slightly viscid, not bruising as quickly as the underlying flesh and remaining white in places even when the trama beneath is quite black, but eventually becoming grey-brown and finally almost black. Cutis peelable 1/4 of the cap radius. Cap trama firm and white at first, becoming directly black where damaged within about one minute, in the dried collection under a 25x lens the flesh is seen to have black spots amid a dark brownish flesh.

Gills white, becoming grey-brown and finally blackening, close to crowded with frequent lamellulae, not in regular tiers, adnexed at stipe, at this stage not decurrent, subacute at cap margin, broadening only slightly towards the margin or at mid-radius, about 1/3 to half the trama depth approximately 2-3 mm at half-radius.

Stipe 5 x 2cm more less cylindrical or slightly clavate, surface white, trama solid, with one horizontal cavity starting to develop in this collection, bruising a little brownish then quickly black both on the cortex and the stipe trama.

Taste mild.

Odour not distinctive.

Spore colour slightly creamy white, Romagnesi lb.

Spores 8.2-11 x 5.8-7.8um, L:W1.27-1.64, mean 1.38 (n=30), narrowly to broadly ellipsoidal, ornamentation of small blunt to conical warts, 0. l-0.3um high, isolated or connected by fine lines, forming a partial or sometimes almost complete reticulum, **Woo types Bl, CI** or **Dl. Suprahilar patch** a smooth inamyloid area. **Hiliferous appendix**

relatively small, 1.2-1.4 x l.l-1.2um. **Basidia** 4-spored, 40-52 x 8-12um, narrowly clavate or slightly bulbous in the upper 1/3, in general a little broader than in other species in the *Compactae*. Sterigmata 4-7um long by 1.2-1.7um wide near the base. **Pleurocystidia** frequent but sometimes hard to see, 45-100 x 5-1 lum, originating in the subhymenium, many not protruding beyond the basidioles, others protrude 20-25um more often towards the gill margin, cylindrical to slightly fusoid, tips obtuse, capitate, or with an appendix of a series of strangulations, contents refractive, light greyish in KOH, not reacting with SV, sometimes appearing partly empty. **Cheilocystidia** similar to pileocystidia except more of them protrude up to 25um. Subhymenium 15-35um, pseudoparenchymatous. **Gill trama** of sphaerocytes, vascular hyphae rare.

Cutis 50-160um thick in the dried basidioma, up to 200um at half-radius in fresh specimens, in some areas, mostly towards the margin, the cutis is thinner, not differentiated into distinct layers, of interwoven more or less parallel hyphae with a few free ends, 2-3 um wide, with occasional sections up to 15um wide, containing discontinuous light brownish pigment. Towards the centre of the cap the cutis is in two layers; with a subcutis of parallel interwoven hyphae and frequent to occasional vascular hyphae 6-7um wide with yellowish refractive contents not reacting in SV. **Epicutis** of mostly upright hyphae, the majority undifferentiated, and pileocystidia, in places with a thin layer of gluten visible on the upper layers. The subapical portions of many hyphae are narrow and helical, and hyphae at the surface frequently contain light to mid grey-brown globules of pigment. **Pileocystidia** that can be easily differentiated from the normal hyphal ends not seen, some of the hyphal ends terminate in ampullate or short-clavate cells that look similar to cystidia but the contents are rarely different from those of cuticular hyphae. Rarely a vascular hypha ascends through the cutis to terminate at the epicutis but does not form cystidioid shapes. **Hypodermis** compactly interwoven and including small flattened sphaerocytes, continuous with the tramal tissues below.

Trama of large clusters of sphaerocytes bound by a hyphal mesh. Some of the clusters are densely black, this appears to be in part due to hypha containing dark pigment, and in part to an intercellular matrix of this dark pigment. Vascular hyphae with yellowish refractive contents rare.

Chemical reactions: FeSCM -greenish-grey; phenol -purple-brown; SV -when fresh no colour at all on cutis or gills, not reacting with any hyphal type nor changing the colour of the tissues on the dried tissue.

Habitat and tree associations: in old-growth coniferous forest with Douglas fir and western red cedar, probably also with western hemlock in the understory.

Collections: BKO10904-01, from the Surveyers trail area, John Dean Park, Saanich Peninsula, approximately N48.613°, W123.445^0 (CDF zone).

		RFLP:		
Collection	ITSl-FtoITS4-B	Hinfl	Alul	Sau3A
BK010904-01	848	380	unsuccessful	632

Notes: Perhaps the most striking character of this species is the pure white cap in contrast to the rapid and dramatic blackening of the flesh, the two characters from which it takes its name. A similar species is *Russula atrata* Shaffer, which differs only in its thicker cutis, generally 200-450um thick, with an interwoven subcutis and a trichodermis above. The Vancouver Island *R. albonigra* in fact has this type of cutis in the centre of the cap, and the thinner, simple cutis towards the margins. *Russula atrata* tends to be associated more with oaks and *R. albonigra* with conifers, although both may be found in mixed forest.

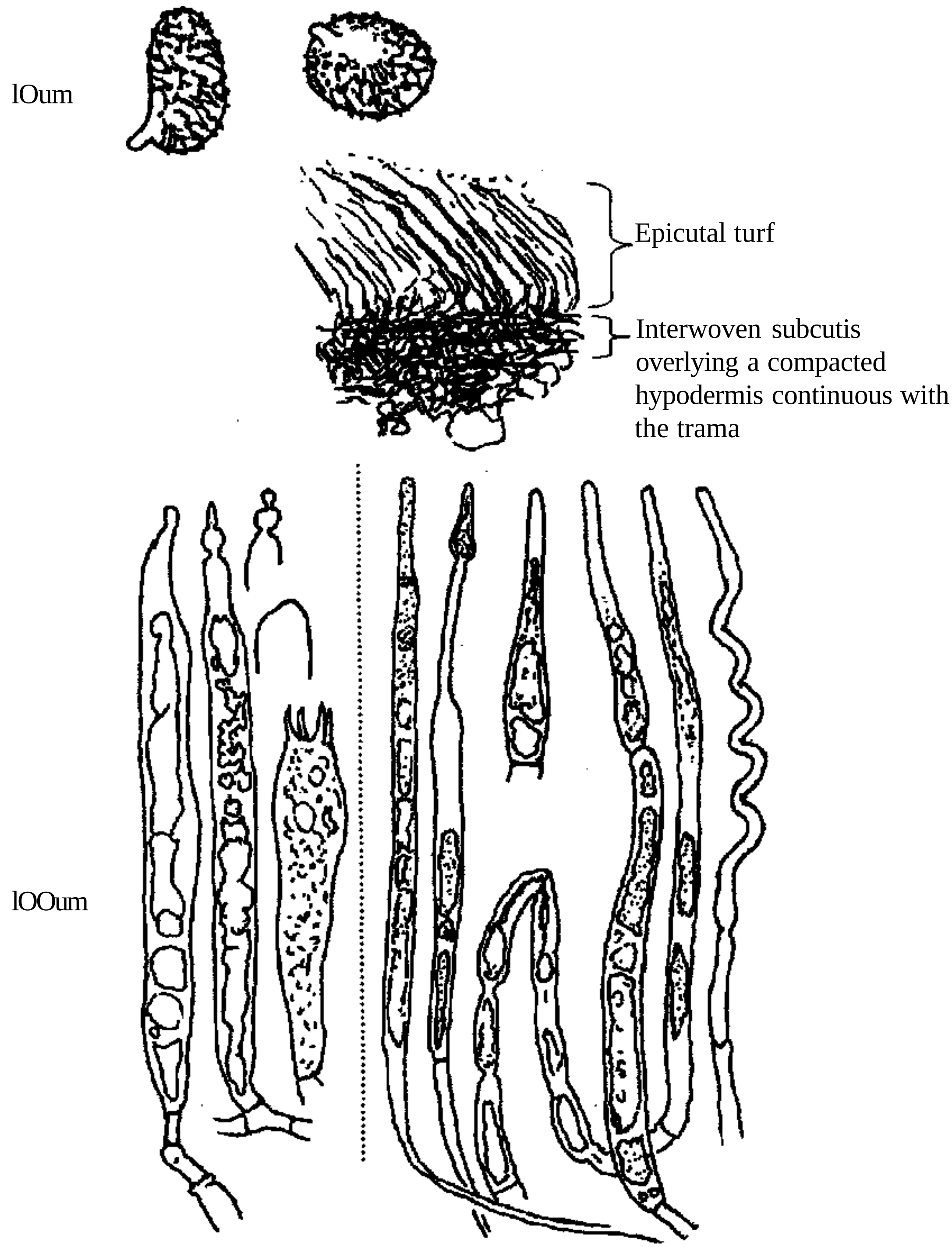


Figure 38 Microscopic characters of *Russula albonigra:* Top, spores with lOum scale bar; middle, diagram of the cutis structure; bottom left, hymenial cystidia and basidium; bottom right, hyphal ends from the epicutis, the lower scale bar is lOOum.

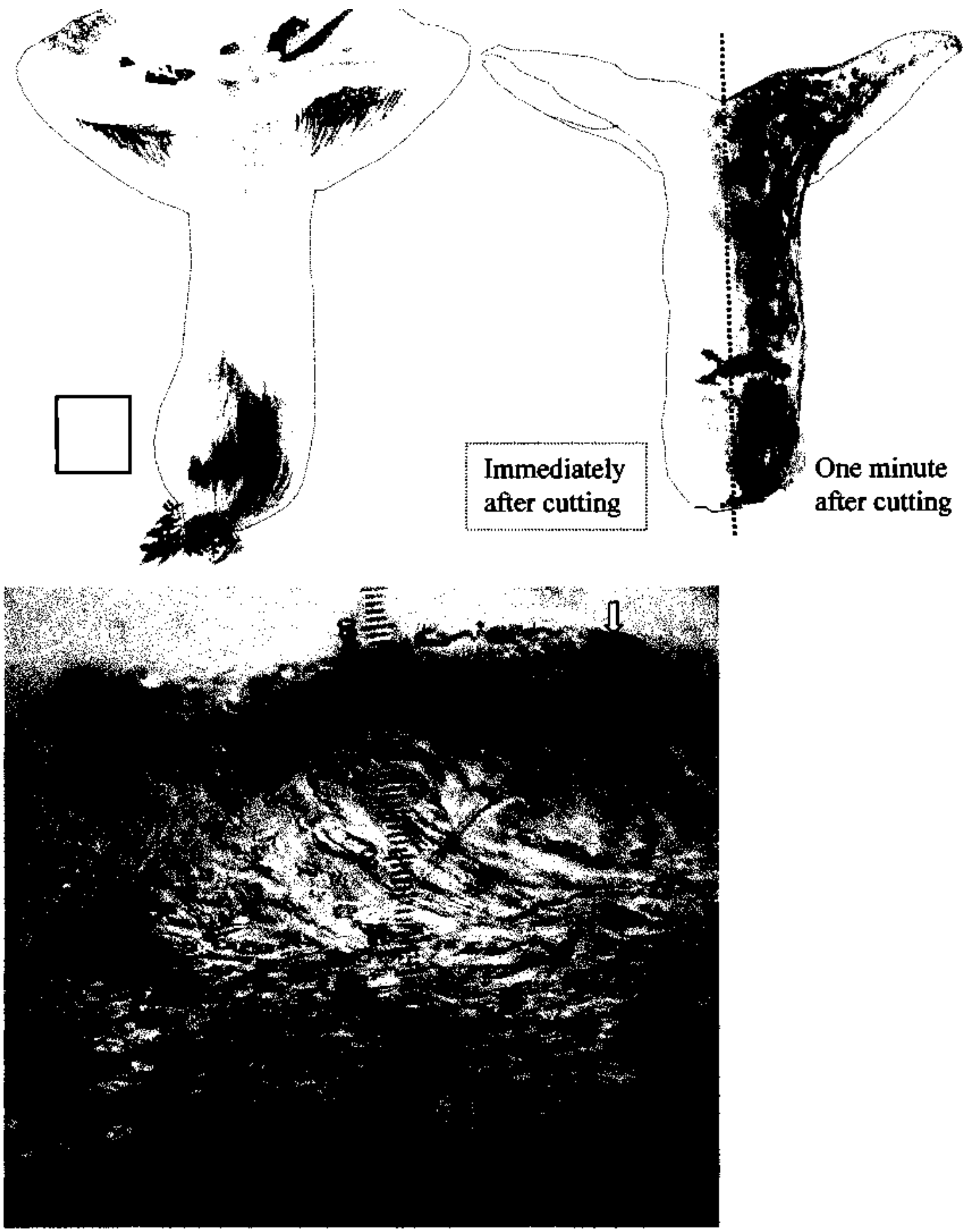


Figure 39 Macroscopic and cutis characters of *Russula albonigra:* Top, illustration of profile and longitudinal section, the left half of the which shows the flesh immediately after cutting and the right half after one minute, the square is 1cm^2 and shows spore colour; bottom, section through the cutis near the cap centre showing the upright epicutal hyphae above a subcutis of interwoven parallel hyphae, at the surface a hypha with vacuolar pigment globules is arrowed, 10 scale divisions are 25um.

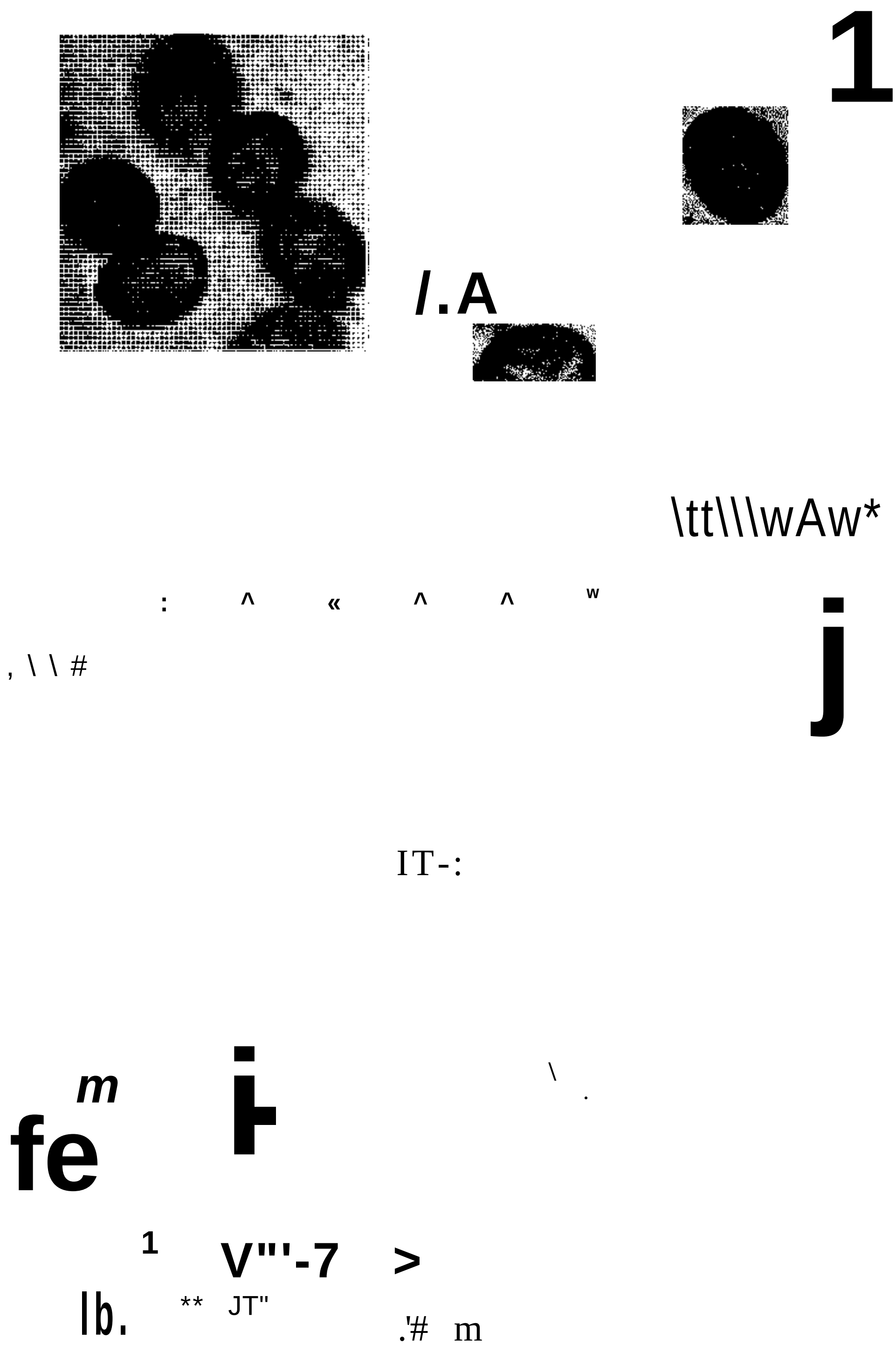


Figure 40 Hymenium of *Russula albonigra:* Top, spores with lum division scale; bottom, two basidia with basidioles, both are 4 -spored but two of the sterigmata are out of the focal plane in both pictures, the scale is in lum divisions.

***Russula dissimulans* Shaffer**

Brittonia, 14: 267-270, 1962

Cap 6-16cm diameter, convex to plane, with or without a central depression when young, becoming pulvinate or uneven, eventually shallowly infundibuliform, margin smooth, inrolled. Cap colour a dingy creamy white when young, soon becoming mottled with grey-brown, often retaining a few whitish spots, eventually completely dark brown and blackening in age, dry, not viscid even when wet, matte, minutely areolate, cutis peelable 0-1/4 the cap radius. Cap trama firm and creamy white when fresh and young, where damaged rapidly becoming a bright terracotta red, which dulls to a grey-brown and finally becomes almost black.

Gills pale cream, bruising red to reddish grey, then grey-brown to black, subdistant to distant and up to 1.5 mm thick, 2-3 tiers of lamellulae, adnexed and slightly ascending to adnate at stipe, only slightly decurrent in age, acute at cap margin, ventricose, 8-15 mm deep at half-radius, approximately equal to 1.5 times the depth of the trama at half-radius. The gill margins may be minutely fringed and sometimes darker due to the presence of cheilocystidia containing the reactive pigment. In wet weather the gill margins seep droplets of clear liquid, this may also be due to condensation.

Stipe 4-8.5 x 1.5-4cm, solid, clavate when young, becoming more or less cylindrical, surface white to pale cream, turning grey-brown, reacting like the cap trama when damaged, the trama bruising more strongly and rapidly than the cortex, becoming black.

Texture firm, brittle, the gills are exceptionally brittle.

Taste mild.

Odour not distinctive to faintly phenolic.

Spore colour white Romagnesi la.

Spores 7.3-9.1 x 6-8.lp.rn, occasionally spores measuring as little as 6.5 x 5.6 or up to 12.2 x 10 are found, normal spore L:W =1.05-1.36, mean 1.18 (n=63), globose, subglobose or broadly ellipsoidal, ornamentation of blunt, rounded to pyramidal warts 0.3-0.8um high, sometimes 2-3-catenate, forming short ridges, or connected by fine to heavy lines forming a partial to complete reticulum. The average size of warts varies among collections, some having warts mainly at the smaller end of the scale, others towards the larger end, but in any spore print there are several of each extreme, **Woo types** CI, C2, Dl and D2. **Suprahilar patch** inamyloid, smooth. **Hiliferous appendix** 1.5-1.8 um long and between 1-1.4 um wide near the base. **Basidia** 4-spored, or often 2-spored, 45-60 x 8-12pm, but most under lOum wide, narrowly clavate, slightly bulbous in the upper 1/3. Sterigmata 5-8um long and 1- 1.2um wide near the base. **Pleurocystidia** frequent to abundant but sometimes hard to see, 38-74 x 4-5 um, originating in the subhymenium, most are embedded in the hymenium, a few protrude up to about 12um, cylindrical, tips obtuse to rounded, contents refractive, yellowish in KOH, weakly greying in SV, sometimes only partly filled with refractive material. **Cheilocystidia** protruding 20-40um, some with light brown amorphous pigment, others hyaline, most lacking refractive contents (appearing empty), 35-60 x 6-10um, sometimes 1-2-septate, the terminal cell gradually tapering to a subacute or obtuse end. Subhymenium 25-50um thick, pseudoparenchymatous. **Gill trama** of sphaerocytes with occasional to frequent vascular hyphae containing dark brown pigment.

Cutis 30-100um thick, of one to two layers, in some areas of the cap the subcutis is absent or extremely thin, and only the epicutis is present directly overlying the tramal tissues. **Subcutis** about 1/2 to 3/4 the total depth of the cutis when present, a layer of interwoven, non-gelatinised, hyaline hyphae 1-7.5 urn wide. **Epicutis** of repent, interwoven hyphae 2.5-5um wide, with few to no erect hyphal ends, sometimes with inflated terminal or subterminal cells up to 10|j,m wide, most with globules of brown pigment giving them the appearance of transparent intestines. **Hypodermis,** none. **Pileocystidia** not seen.

Trama of sphaerocytes in fairly large clusters, for example, 4-5 deep by about 18 cells across, bound by a hyphal mesh, with few vascular hyphae visible.

Chemical reactions: FeSC>4- greyish; KOH - no reaction on cap or stipe; NaOFL,- no reaction on cap or stipe; phenol - pinkish; SV - brown on cutis but not reacting with any cuticular cells, weakly grey on hymenial cystidia.

Habitat and tree associations: on the forest floor, generally in mineral soil, in one case on a rocky knoll with shallow soil, often along trails, in mature regeneration forest; under western hemlock, or Douglas fir, or Sitka spruce, or red alder, or western red cedar; with understory sword fern.

Collections: CR000919-10, by the trail between cabins and dormitory on a rocky knoll, with western hemlock and western red cedar, Bamfield Marine Science Centre, N48.8315^{0}, W125.1345^{0} (southern very wet hypermaritime CWH subzone). CR010814-04, in a mixed age stand of western hemlock and Sitka spruce, Fairy Lakes trail, N48.585283^{0}, W124.35925^{0}. CR010909-02 amongst young regeneration, western hemlock and Douglas fir, with red alder and wild cherry, Kemp Lake area, N48.375283°, W123.78037°. 021020-SVIMS, a collection brought from either the Blueberry flats or Sooke Potholes area and deposited at the SVIMS mushroom show at Swan Lake, Victoria, the presence of western hemlock is indicated by needle litter adhering to the stipe bases. 021027-RN, collected in the Cowichan Lake area during a SVIMS foray, western hemlock and possibly Douglas fir presumed to be in the habitat (latter four in eastern very dry maritime CWH subzone)

		RFLP:		
Collection	ITSl-Fto ITS4-B	Hinfl	AM	Sau3A
CR010814-04	871	400, 326	705	565,230
021027-RN	857	349,244		647

Notes Shaffer (1962) separated *Russula dissimulans,* a North American species, from its close European relative *Russula nigricans,* which name has often been given to collections of the former. He comments that Singer (1958) considered the North American representatives of the *R. nigricans* complex to be a subspecies as they differed from the European species. The difference rests on the slightly thinner and closer gills of *R. dissimulans* and the larger spores with warts up to 0.7um rather than a maximum of 0.3 um for *R. nigricans.* A perusal of the many photographs of *R. nigricans* on European websites on the Internet shows that the gill spacing varies and that *R. dissimulans* does not differ from some of these collections. Sarnari (1998) mentions a number of 4-5 lamellae per cm near the edge of an 18cm diameter basidioma, and 2-3 mid-radius. In the 16cm diameter Vancouver Island basidioma, there are 5-6 lamellae and lamellulae per cm at the border and 4, sometimes 5, at mid-radius. Shaffer gives the spore size for *R. nigricans* as 6.3-7.9 x 5.3-6.8um and that of *R. dissimulans* as 7.7-10.8 x 6.5-9um, whereas Sarnari (1998) gives 7.2-9.2 x 6-7.5um for the former and the measurements given by Romagnesi (1967) fall in between. Clearly, if there is a difference between the European *R. nigricans* and the North American *R. dissimulans,* the characters of gill spacing and spore size are insufficient to separate the species.

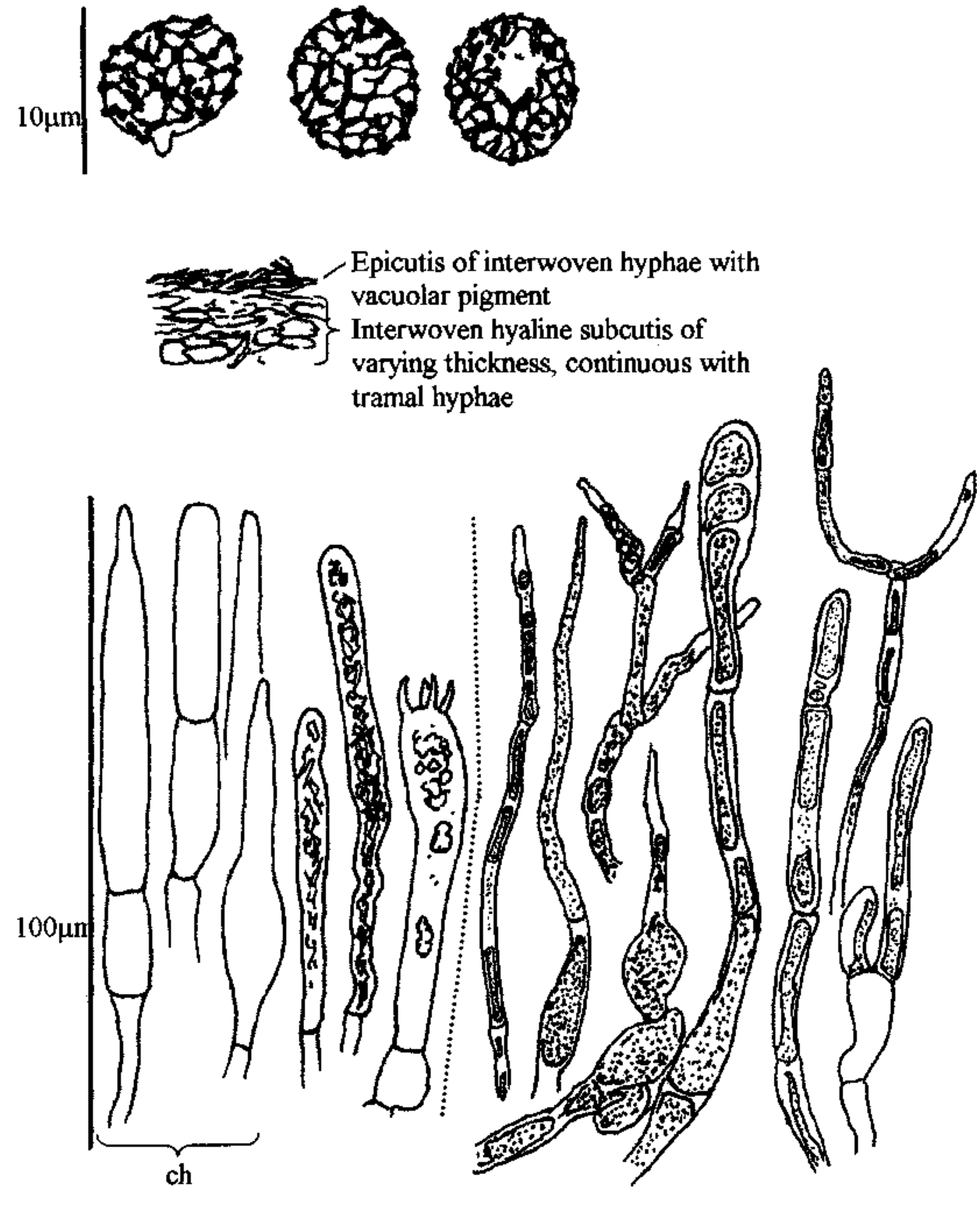


Figure 41 Microscopic characters *ofRussula dissimulans:* Top, spores with lOum scale bar; middle, diagram of the cutis structure; bottom left, cheilocystidia (ch), pleurocystidia and basidium; bottom right, hyphal ends from the epicutis, the lower scale bar is lOOum.

Figure 42 Macroscopic characters of *Russula dissimulans:* Top, view of the top and underside showing the initial red bruising stage and the exuded or condensed droplets on the gills, photograph by Bryce Kendrick; bottom, young basidioma and mature 16cm diameter one, where the stipe has turned dull brown and the broken gills still show some reddish staining but are starting to turn grey-brown.

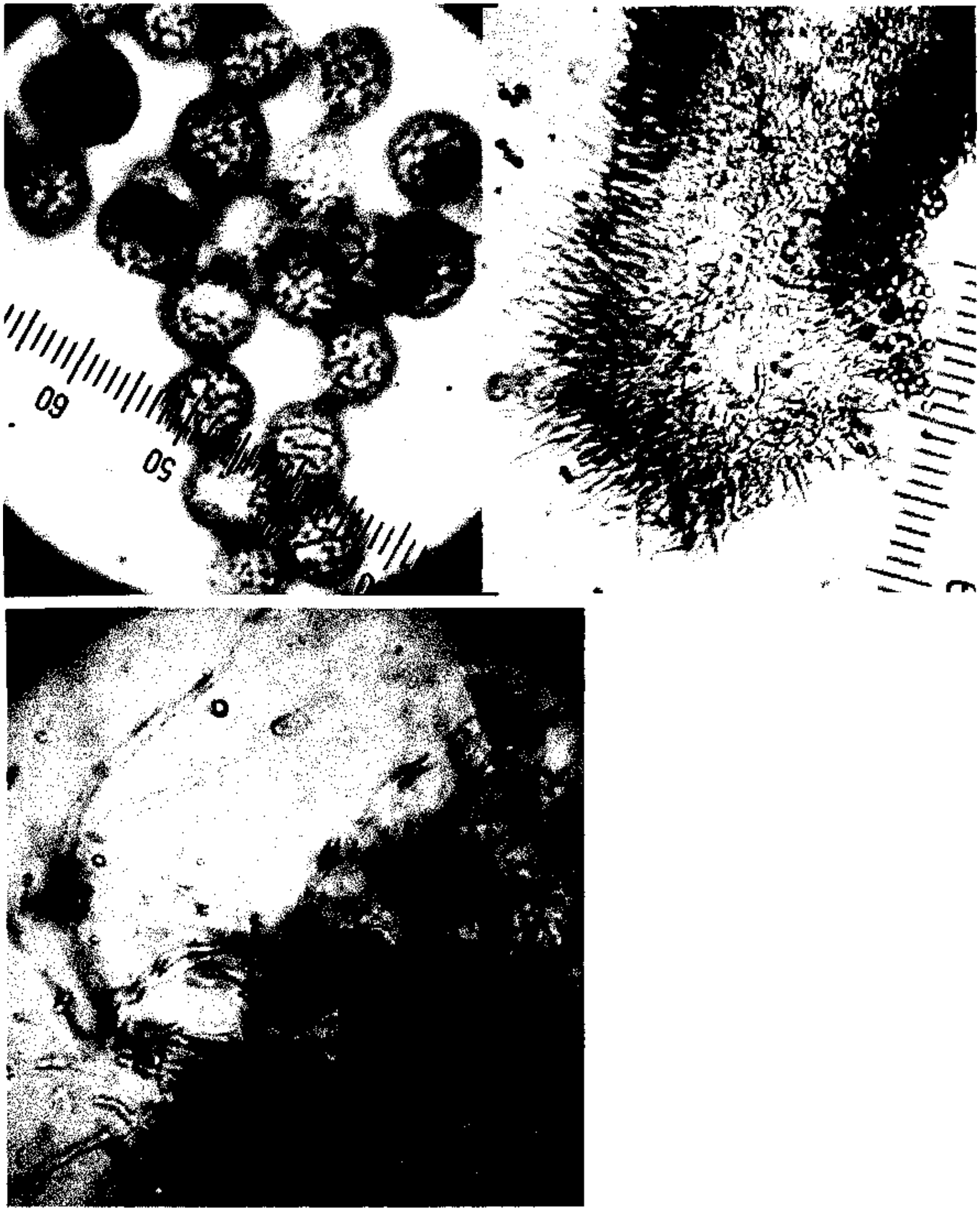


Figure 43 Hymenium and cutis of *Russula dissimulans:* Top left, spores with ljam division scale; top right, section through the gill at the margin showing dense numbers of cheilocystidia, 10 scale divisions arelOOum; bottom, hyphal ends of the epicutis showing brown pigment globules within, scale is in lum divisions.

Clade 2b

Subgenus *Compactae* (Fries) Bon

Section *Lactaroides* (Bataille) Konrad and Josserand

***Russula brevipes* var. *brevipes* Peck**

New York State Museum Annual Report 43: 20. 1890

***Russula brevipes* var. *acrior* Shaffer**

Mycologia 56: 223-225, 1964

Cap 3.7-over 20cm diameter, most often in the range of 10-15cm but in productive years (such as fall, 2004), basidiomata as large as 30cm diameter occur. The shape is typically broadly infundibuliform even when immature, regularly lifting a disc of duff on its top (fig. 45), margins becoming increasingly uplifted in age until the cap assumes a broad V-shaped cross section. The cap margins are inrolled and remain so until post-maturity, not becoming striate, cuticle not peelable. The cutis is a chalky white, dry, matte, soft and felty at the margins when young, developing light brown stains where damaged or where the margins dry out in warm weather: beneath the cuticle, the flesh is creamy-white, in var. *acrior* it may have a slight blue-green tinge when young, unchanging when cut but eventually turning dirty cream to brownish in age and around insect larval tunnels.

Gills close to crowded, usually with two tiers of subgills that are often relatively regularly distributed, (although this varies among individuals); occasionally some forking occurs also, sometimes water-like droplets are exuded in moist weather. The colour is a very pale cream, developing light brown spots and stains where damaged. In var. *acrior* the young gills often have a pale blue-green tint, gradually becoming cream but usually retaining a blue-green zone at the junction with the stipe into maturity, eventually this fades in age. The gills are arched, at least in the outer half of the cap radius, in some basidiomata, mostly when young, they may be sinuate or bellied towards the stipe as in figure 45 below, but as the cap expands they become arched throughout, adnate to decurrent at the stipe, narrow and acute at the cap margin, with a depth of about 1/4-1/2 the of the cap trama at half radius, brittle.

Stipe 3-8 x 0.9-4cm, relatively short, the length usually less than the cap diameter, most often cylindrical, occasionally tapering downward, firm, stuffed with a firm trama; unchanging when cut, but eventually hollow and brownish inside the rind. Surface a chalky white when young, becoming dingier and brownish with age; not or only slightly longitudinally rugulose, staining brown around the base and where damaged. In var. *acrior* the stipe has a blue-green band at the apex which fades in age, becoming indistinguishable from that of var. *brevipes*.

Texture hard but brittle, becoming crumbly in age.

Taste mild or with a slight to distinct latent pepperiness in var. *brevipes*, slowly peppery to acrid in var. *acrior* .

Odour not distinctive or mushroomy, becoming unpleasant or fishy in age.

Spore colour white to pale cream, Romagnesi la or lb, the latter being encountered less frequently locally than pure white, among the Vancouver Island collections all have white spores except one, a mild-tasting var. *brevipes* from Koksilah.

Spores 8-11.3 x 7.8-9.4um, L:W 1.03-1.29, with a mean of 1.17 (n = 46), subglobose to broadly ellipsoidal, ornamentation somewhat variable, of low blunt to high pointed warts 0.7-2.0um high, isolated or more commonly with light to heavy connectives forming a partial reticulum, occasionally the reticulum is complete, catenate warts are common, sometimes forming a low ridge. **Woo types** 3B, C and D and occasionally 2 B,C and D or between 2D and 2E. **Suprahilar patch** a weakly amyloid area of irregular outline, the borders of which are slightly more amyloid and raised than the centre. **Hiliferous appendix** 1.7-2.5um long, and 1.2-1.9um wide at base. **Basidia** 4-spored, 57-80 x 9-14um, narrowly clavate to clavate, sometimes with a relatively long, narrow base.

Sterigmata 7-10um long by 0.7um wide at the base. **Pleurocystidia** 75-125 x 7.5-*\0\im*, protruding up to 22um beyond the basidioles, originating in the subhymenium or outer trama, cylindrical, fusoid or narrowly clavate, tips obtuse or capitate, contents yellowish, refractive in KOH, dark grey in SV. **Cheilocystidia** similar to pleurocystidia in shape and distribution but often shorter, to about 80um. Subhymenium 15-35um thick, of interwoven hyphae, gill trama of sphaerocytes with common SV+vascular hyphae.

Cutis 50-3 OOum thick, not gelatinous, consisting of loosely interwoven, sometimes tortuous, septate, branched hyphae 3-6um wide, the upper 20-50um may be more compactly interwoven and of repent light brown hyphae over some parts of the cap, in other parts hyaline semi-repent to ascendant hyphae form a deep loosely interwoven cottony layer which gives the cap its chalky or felted appearance. Single or bundles of 2 to about 6 slender hyaline hyphae frequently emanate above the interwoven layers. Occasional branched vascular hyphae 4-6um wide with yellowish slightly refractive contents not reacting in S V permeate the cutis, sometimes ending in a non-differentiated terminus at the surface. Some of the surface hyphae appear in water and 5% KOH mounts to have occasional small droplets adhering to their surface, although such hyphae do not have clamp connections: it is possible they are foreign in origin as other, more distinctly foreign hyphae as well as soil particles become entrapped in the epicutis. No other descriptions consulted mention such droplets. **Hypodermis** none, the cuticular hyphae are a continuation of the tramal hyphae. **Pileocystidia** not seen, although some hyphae have slightly refractive contents in their terminal cells.

Trama of discrete clusters of sphaerocytes bound by a hyphal mesh, vascular hyphae rare in some basidiomata, common in others.

Chemical reactions: FeSO.4 - pinkish; KOH - no reaction to light yellowish brown on cap and stipe; NH4OH - no reaction; guaiac - blue-green; phenol - brownish purple; S V - purplish grey on the gills, blue-grey on the cuticle, cystidia and vascular hyphae grey to black.

Habitat and tree associations: With Douglas fir, western hemlock and possibly Sitka spruce as host trees, *R. brevipes* is found in old growth and mature regeneration forests, over about 40 years in general, being most numerous in forests about 70-120 years old where during its fruiting period it can be the most abundant epigeous mushroom present.

Collections: CR001202-br *R. brevipes* var. *brevipes,* Rocky Point in mature regeneration Douglas fir with large madrone and cedar N48.331617°, W123.5549^{0}; CR001105-01 *R. brevipes* var. *acrior,* Discovery Island along trail from campground to lighthouse, under regeneration Douglas fir, N48.4257^{0}, W123.2325^{0}; CR021102-br/ait *brevipes* var. *acrior,* Royal Roads near the small swamp with old-growth and understory Douglas fir and western hemlock with western red cedar and Oregon grape, N48.434467°, W123. 478867° (all in CDF zone). CR021015-01a#. *brevipes* var. *brevipes,* Rainforest trail east side of the road, Long beach area, P.R.N.P. in old growth western hemlock and Sitka spruce with western red cedar, N49.049^{0}, W125.699° (southern very wet hypermaritime CWH). CR980827-01 *R. brevipes* var. *acrior,* in forest next to the junction of the road to Bamfield with branch 167 in mature regeneration western hemlock, Sitka spruce and Douglas fir N48.8875°, W124.9155° (submontaine very wet hypermaritime CWH subzone). CR981114-03 and CR001001-05 *R. brevipes* var. *acrior,* Koksilah ridge, on southeast facing slope in regeneration Douglas fir with madrone, salal and Oregon grape, N48.6559^{0}, W123.7292 ° (eastern very dry maritime CWH subzone). CR010909-br/a*R. brevipes* var. *acrior,* Kemp Lake area, under regeneration Douglas fir with red alder and salal, N48.378°, W123.7873° (western very dry maritime CWH subzone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR001105-br/a	885	378,265	56!	226
CR001001-05	885	378,265	565	245
CR010909-br/a	816			

Notes. *Russula brevipes* is locally probably the most common species *of Russula* encountered. It is found throughout the coastal forests and in the coastal mountains along the mainland of the Pacific Northwest. It may be confused with white species of *Lactarius,* locally *Lactarius controversus* has a very similar colour and stature, but the flesh exudes a white latex and the gills are a little pinker and more crowded. *Russula cascadensis* appears almost identical but smaller, has an acrid taste and lacks any blue-green tints, and it has smaller, narrower spores. The spore size is the only really reliable means of distinguishing *R. cascadensis* from *R. brevipes* var. *acrior* as the latter varies greatly in size; this factor appears to be very dependent on local conditions. In Europe, two species are considered close to the two varieties of North American *Russula brevipes: R delica* Fr. (similar to var. *brevipes)* and *R chloroides* (Krom.) Bres. (similar to var. *acrior),* and their rDNA sequences show them to be closely related but not identical. When Shaffer described *Russula brevipes* var. *acrior* he also wrote a comprehensive description with illustrations of *R. brevipes* var. *brevipes* in the same publication (pages 220-223), after examining collections from mainland North America including Washington and Oregon. These descriptions provide further useful information and details on this species.

On Vancouver Island, *Russula brevipes,* (presumably both varieties) is frequently parasitized by *Hypomyces lactifluorum,* turning it into what is commonly known as a lobster mushroom, collected locally for food.

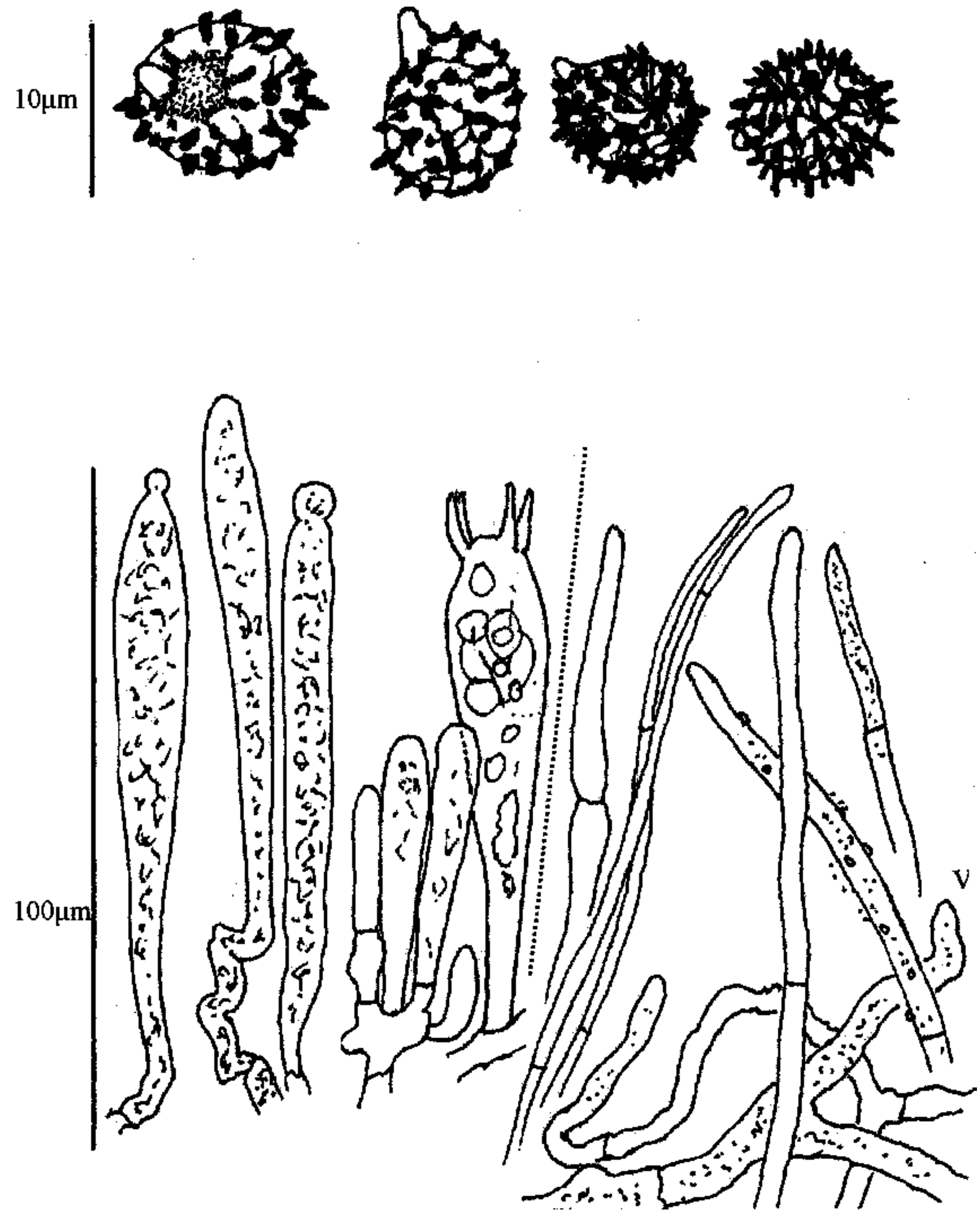
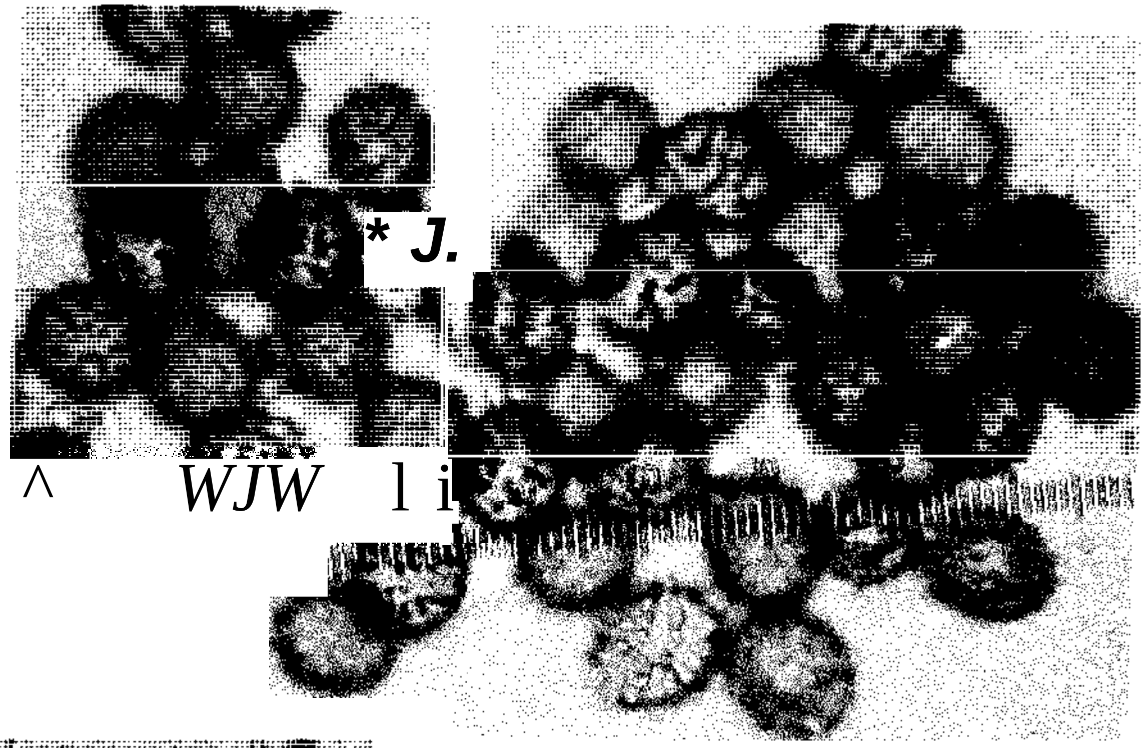


Figure 44 Microscopic characters of *Russula brevipes:* Top, spores, scale bar is lOum; bottom left, pleurocystidia, basidioles on basal cell and basidia; bottom right, hyphae from the cutis including long narrow hyphae, one with droplets on the surface, and a vascular hyphae (V), lower scale bar is lOOum.

Figure 45 Macroscopic characters of *Russula brevipes:* Top, illustration of a small basidioma of var. *acrior* showing the blue-green tints typical in the young to early mature stage and the light brown bruising where dry or damaged, the square is 1 cm^2 and shows spore colour. Bottom, typical appearance in habitat with a wad of duff being lifted by the expanding caps and the incorporation into the cutis surface of soil and debris.



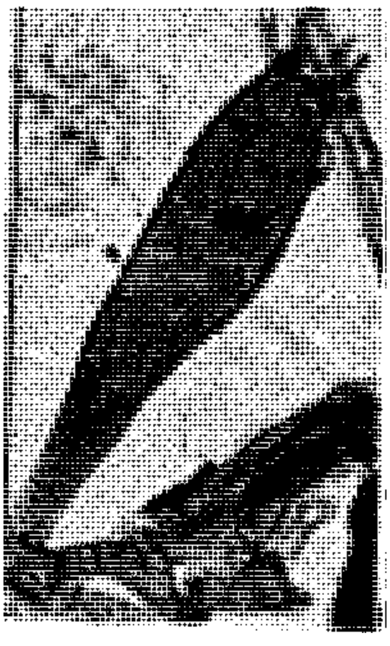


Figure 46 Hymenium and cutis *oiRussula brevipes* var. *acrior:* Top, spores, one division on the scale is lum; bottom left, basidia stained with Congo red, scale bar is 50um; bottom right, hyphal ends from the epicutis, scale bar is lOOum.

Russula cascadensis **Shaffer**

Mycologia 56: 212. 1964

Cap 4-4.7cm diameter, creamy white but developing light red-brown marks, dry, matte, minutely felty, centrally depressed, becoming broadly infundibuliform, margins smooth, inrolled and remaining so throughout maturity, peelable less than 1/4 the radius. Soil particles and debris are embedded in the cap cutis. Cap trama about 4-5 mm deep at half-radius, not bruising when cut but developing light brownish discolourations around insect larval channels, eventually the whole cap aging this colour.

Gills pale cream without any traces of blue-green, close to crowded with frequent subgills, thin, narrow, about 3mm deep at the deepest part, just beyond half-radius, adnate to slightly decurrent at the stipe, acute at the margin, arched, not particularly brittle, spotting brownish where damaged and in age.

Stipe 2.1-2.7cm x 1.1-1.6cm, short, about half the cap diameter in length, clavate, white, very firm, not bruising when cut but browning lightly around insect damage and round the base, solid.

Texture firm, but no more brittle than the average *Russula.*

Taste more or less bitter at first, then acrid.

Odour mild, mushroomy, not distinctive.

Spore colour pale buff, Romagnesi Ila-b.

Spores 6.2-8.5 x 4.5-6um, L:W 1.19-1.46 with a mean of 1.37 (n=30), most are ellipsoidal, but oblong or tear-drop shaped spores are common, ornamentation of low rounded, sometimes heavy warts, mostly 0.2-0.4jxm, occasional warts reach to about 0.8um high, warts often in rows, with thin to heavy connectives forming a partial reticulum, some isolated warts occur, Woo type C2. **Suprahilar patch** variable, on some spores an unornamented inamyloid area, on others a small lightly amyloid patch, on many an area of smaller but similar ornamentation to the rest of the spore, with tiny warts sometimes forming rows radiation from the hiliferous appendix. **Hiliferous appendix** 1.5-2um long, 1.2pm wide near the base. **Basidia** 4 -spored, 37-52 x 8-10pm, narrowly clavate, some almost cylindrical. Sterigmata 4-5 pm long and 1.2-2pm wide at the base, some appearing relatively short and fat, others normally proportioned. **Pleurocystidia** abundant but sparse near the gill margins, 50-75 x 6-10pm protruding less than 20pm, originating in the subhymenium or outer trama, fiisoid or irregularly cylindrical, tips rounded, capitate, or with a short to long (sometimes half the length of the cystidia) narrowed extension. Contents refractive in KOH, brownish grey in SV, occasional ones with non-refractive contents. **Cheilocystidia** sparse, similar to pleurocystidia. **Subhymenium** 15-40pm thick, pseudoparenchymatous but often including interweaving hyphae, gill trama of sphaerocytes and vascular hyphae with yellowish contents staining weakly grey in SV.

Cutis 130-220um thick at half-radius, of similar and locally variable thickness elsewhere. **Subcutis** tightly interwoven with no gelatinous matrix, of hyaline hyphae 1.5-4pm wide, but becoming brownish towards the trama and continuous with the hyphal network of the trama, and containing ascendant vascular hyphae 2.5-5um wide that mostly terminate below the surface in an undifferentiated or capitate end. There appears a somewhat distinct irregular lower boundary where clusters of sphaerocytes abut the cutis. **Epicutis** not uniformly distinct from the subcutis, of compacted repent hyphae in parts, elsewhere of semi-erect unbranched non to rarely septate hyphal ends, usually in tufts and emerging up to 150pm beyond the surface, sometimes small clusters of hyphae adhere together along their length forming a loose rope-like structure. **Pileocystidia** none seen, very rarely a vascular hypha forms a capitate pseudocystidium at the surface.

Trama of small discrete clusters of sphaerocytes bound by a dense hyphal mesh with frequent vascular hyphae.

Chemical reactions: $FeSO_4$ - light brownish pink; KOH and NHUOH - no reaction; phenol - pinkish brown; S V - grey-brown on the cuticle and gills, cystidia and vascular hyphae brownish grey, sometimes very weak.

Habitat and tree associations: In shrubby river valleys with red alder and western hemlock and/or Douglas fir.

Collections: CR981013-03, Cape Scott park near the beginning of the San Joseph bay trail, with western hemlock, Sitka spruce, red alder, huckleberry and salal, N 50.773°, W 128.403° (southern very wet hypermaritime CWH subzone). CR001007-cas, Koksilah River area, near the road at the Park boundary, with Douglas fir and red alder, N 48.6531°, W 123.7325°; CR001001-06 Cowichan River trail under Douglas fir, western hemlock and red alder with big-leaf maple and western red cedar, N 48.762°, W 123.7796° (eastern very dry maritime CWH subzone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR001007-cas	840	369,346	500, 367,230	272,213, 172

Notes. *Russula cascadensis* is named after the Cascade mountains of the Pacific Northwest, where the holotype originated, although it ranges as far as Michigan (Shaffer 1964). It looks like a miniature *Russula brevipes* and can really only be distinguished from peppery forms of that species by microscopic examination of the spores, (see notes under *R. brevipes).* The spore print of *R. cascadensis* is darker than that of most *R. brevipes,* which locally at least, is most often white. The taste of local collections of *R. cascadensis* includes an initial slight bitter component, and vascular hyphae are common in the cutis, characters not typical for this species according to Shaffer's description. Another similar species, *Russula vesicatoria* Burl, has a bitter then acrid taste and also common vascular hyphae in the cutis, and otherwise differs from *R. cascadensis* only in its slightly larger basidiomata, up to 11cm diameter, stronger odour (like *Lactarius camphoratus)* and slightly larger spores (6.8-9.3 x 5.6-7.3um). Burlingham collected *Russula vesicatoria* from Florida and New York State but to my knowledge it has not been reported from the Pacific Northwest. The cap diameters of these Vancouver Island collections are at the low end of the scale, Shaffer described them as between 4 and 9cm, and it may be that larger ones have simply been overlooked and assumed to be *R. brevipes*.

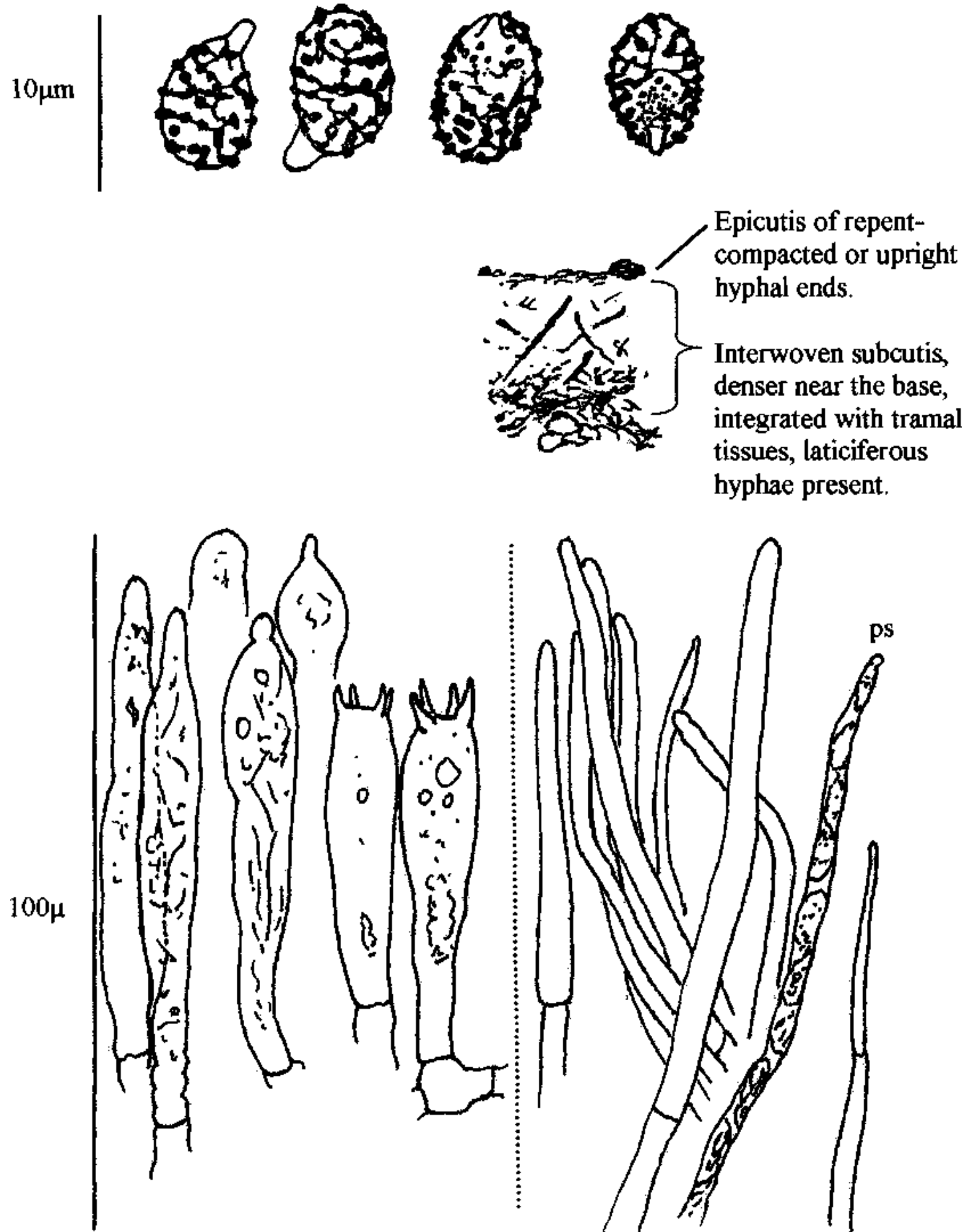


Figure 47 Microscopic characters of *Russula cascadensis:* Top, spores with lOum scale bar; middle, diagram of section through cutis; bottom left, hymeniaJ cystidia, the most common two shapes on the left, and basidia; bottom right, epicutal hyphal ends and a laticiferous hyphal end (pseudocystidia, labelled ps) that remains embedded within the cutis, lower scale bar is lOOum.

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Figure 48 Macroscopic characters *oiRussula cascadensis* showing profile and longitudinal section, note the Sequent subgills, the square is 1cm^2 and shows the spore colour.

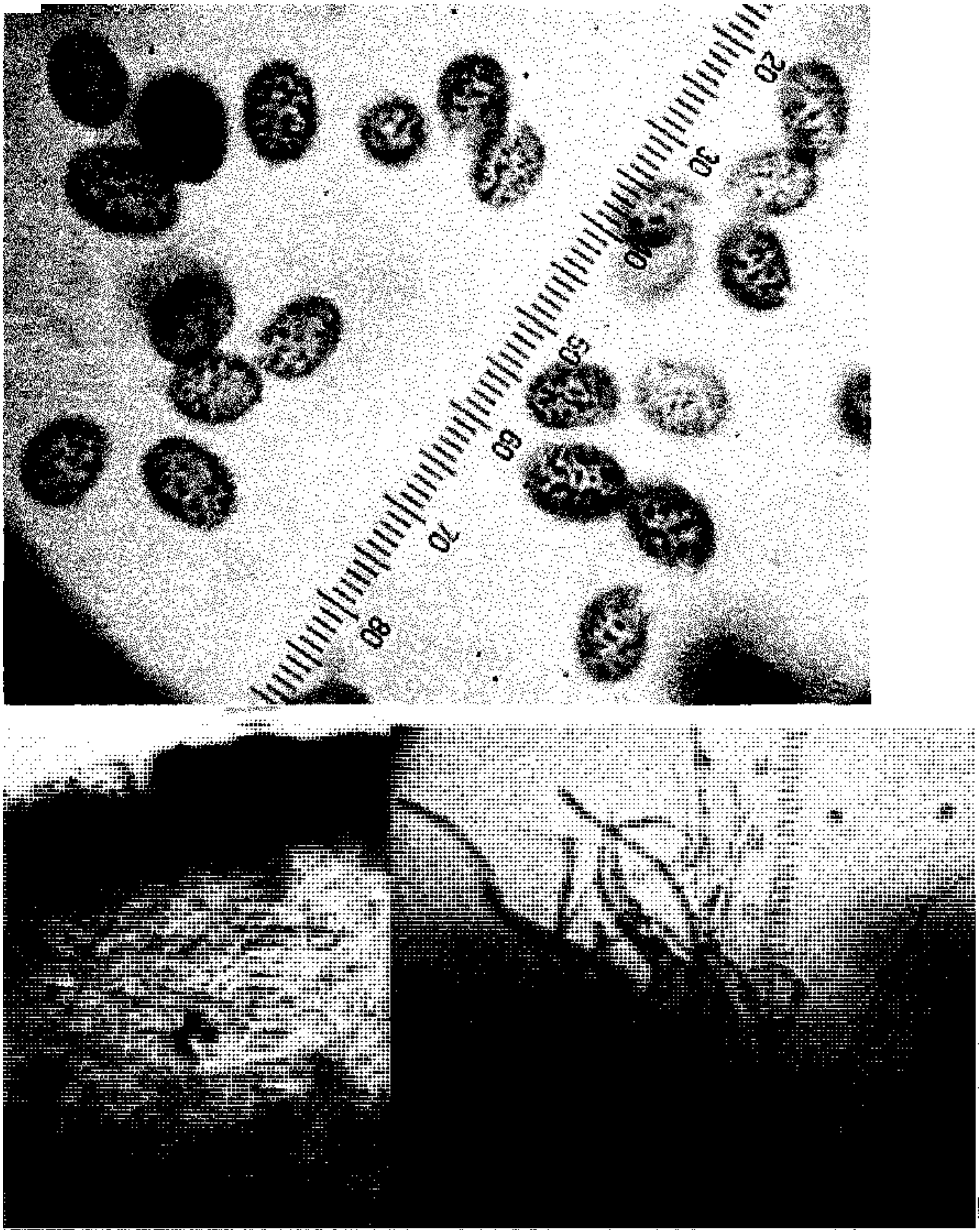

Figure 49 Spores and cutis *ofRussula cascadensis:* Top, spores, one scale division is lum; bottom left, section through the cutis showing a few ascendant vascular hyphae, one of which is arrowed, 10 scale divisions are lOOum, and the trama/cutis interface is at the 63 mark in this section. Bottom right, a tuft of upright epicuticular hyphal ends, 10 scale divisions are 25 urn.

Clade 3

Subgenus *Ingratula* Romagnesi

Section *Ingratae* (Quel.) Maire

Subgenus *Ingratula* consists of *Russula* species with brown, yellow-brown, grey-brown or red-brown cuticle pigmentation with no traces of red, purple or green. Typically the cuticle is thicker, sticky and often more gelatinous than in other groups *of Russula,* and the margins of the cap are deeply striate-tuberculate even when immature due to the thinness of the trama towards the cap margin. Section *Ingratae* contains numerous species with the strongest odours found in *Russula,* such as bleach (chlorine), spermatic, fish, almonds (benzaldehyde), maraschino cherries, rubber, Jerusalem artichokes and fruit, often with mixtures of these odours. The taste is often acrid with a soapy or oily element, but some species are mild. The stipe is generally creamy white with a tendency to bruise brown, and the trama has a series of four or more lenticular cavities from an early stage, a feature rare in other *Russula* groups, that generally have fewer, irregularly shaped cavities. In older basidiomata the stipe becomes completely hollow. The spores are cream, rarely white, never deep yellow. Microscopically, the spores have little to no amyloid ornamentation on the suprahilar patch, and often incompletely amyloid warts. The pileocystidia react only very weakly to sulphovanillin, yet the vascular hyphae in the subcutis and the hymenial cystidia generally react strongly.

Macroscopically this subgenus can be differentiated from similarly coloured Russulas in subgenera *Russula* and *Incrustatae* by a chromatograph of the cuticle extract (Chapter 2). In the *Ingratula* the chromatograph shows no reds or blues, just a greyish zone at the baseline and a yellow-brown zone behind the solvent line, which may split into three yellowish zones: a lighter yellow that is pale yellow in UV light, a darker yellow which is orangeish in UV light, and at the solvent line a UV-negative greyish-brown.

Oade3b

Subsection *Foetentinae* (Melzer & Zvara) Singer

Series *Pectinata* Sarnari ad int. *(Pectinatae* **Bon)**

In addition to the main features of the *Ingratae*, the *Pectinata* have in common spores with normal *Russula* ornamentation of conical or hemispherical warts and no wing-like ridges. Spore shapes are a useful identifying character at the species level, with globose to subglobose spores less common than elongate-ellipsoidal spores. The *Pectinata* all have very small inconspicuous lanceolate pileocystidia, not reacting or barely greying in SV, little differentiated from cuticle hyphal end cells. In some texts, for example Thiers (1997), species in this group are considered to lack pileocystidia. The subcutis contains numerous vascular hyphae with granular contents greying strongly in SV. The odours in this group are usually strong with a spermatic or bleach component, sometimes fishy, rubbery or of Jerusalem artichokes.

The literature in this group is rife with synonyms and homonyms, and the microscopic differences between species are often very slight. Odour and colour play a large role in species differentiation, the former being subjective, the latter somewhat variable. In the species described below, it was difficult to determine an unambiguous identification and in future these collections may be re-identified as a different taxon, perhaps a local variant. As several collections had characters intermediate between species and other collections assumed to be of the same species could be variable, it is probable that closely related species in this group hybridize. This may have given rise to the confusion in the literature mentioned below in the notes accompanying species descriptions. With this in mind, the descriptions below should not be regarded as definitive for the species named.

***Russula cerolens* Shaffer**

Mycologia 64: 1036. 1972

Cap 5.3-9cm, pulvinate when young, remaining centrally depressed with the margins eventually unevenly flared, grey-brown to dull yellowish brown, darker in the centre, becoming pallid in age, no reddish brown areas. Margin striate-tuberculate even when young, up to 2cm or around 1/3 of the radius, peelable only at the margin, surface sticky, glutinous when wet, drying matte, occasionally with patches breaking into minute areoli. Trama creamy white, unchanging.

Gills cream, subdistant, adnexed, ventricose, acute at margin, around twice the depth of the cap trama at half radius, narrowing towards either end, occasional subgills, no forking or only very close to the stipe, not discolouring.

Stipe 4-11 **x** 1.4-2.8cm, clavate or fusoid, with 5-6 lenticular cavities, becoming hollow in age. Stipe rind around 5 mm thick, trama bready-textured, dirty white and faintly greying where cut, stipe surface white, smooth, turning pale grey-brown where handled, base of stipe bruising strongly red-brown in some specimens, patchy or paler in others, not turning red with KOH.

Texture firm, not very brittle to brittle, gills pliable.

Taste unpleasant, slowly peppery to acrid, especially in the gills, and with an oily, soapy component.

Odour strong, at first somewhat like bleach but with fresh, pleasant overtones, of ozone or cottonwoods in spring, eventually smelling strongly spermatic or of bleach, even after drying. Shaffer (1972) describes the odour as waxy, Thiers (1997) as oily, unpleasant.

Spore colour light cream-yellow, Romagnesi lie to d.

Spores 6-8.5 (-10) x 4.8-6.8um, narrowly or broadly ellipsoidal, sometimes pip-shaped, L:W 1.07-1.73, average 1.33 (n=76). **Ornamentation** of low rounded warts up to about

0.5um, occasionally to 0.8um, isolated, 2-3 catenate or with fine to thick connectives forming a partial to almost complete reticulum, sometimes with several longitudinally oriented rows of isolated or joined warts. **Woo types Bl-2,** Cl-2 and **Dl. Suprahilar patch** inamyloid or barely amyloid, in Melzers' reagent appearing as a well defined very pale grey area with little or no ornamentation, on some spores there is a small darker patch within this area at the base of the hiliferous appendix, which is 1.5 to 2um long. **Basidia** 40-50 x 6.5-9um, columnar to narrowly clavate, sterigmata up to 8um long and slender, often under lum wide. **Pleurocystidia** abundant and densely distributed, 57-100 (140) x 6-10um, most are around 8 urn wide, more or less cylindrical, narrowly clavate or fusoid, ends mostly capitate or with a series of constrictions ending in a terminal button, some merely acute, arising from the inner subhymenium or the trama, sometimes embedded in the hymenium, sometimes protruding up to 40um, contents refractive in KOH, black or with black globules in SV. Shaffer (1972) comments on seeing some with inflated apices up to 17um wide in some basidiomes. **Cheilocystidia** frequent, 50-70 x 5- 8um, protruding 15-20um, ends rounded or more frequently ampullate or capitate contents refractive or not, staining purple to black in SV. **Subhymenium** about 40-50um thick, pseudoparenchymatous. **Gill trama** of sphaerocytes and frequent vascular hyphae.

Cutis around 280um thick at half radius, ranging from lOOum at the margin to up to 750|xm at the cap centre in mature basidiomata; an ixodermis with patches of trichoixodermis. **Subcutis** of radially oriented interwoven repent hyphae and numerous vascular hyphae which sometimes continue into the epidermis, contents yellowish in KOH, dark grey and granular in SV. **Epicutis** interwoven in surface view with occasional free hyphal ends, also with clusters of vertical multiseptate hyphal ends, around 3-5um wide, appearing articulated or sometimes like a string of beads, the terminal cell tapering to a point, 25-40um long, and often with refractive or granular contents. Cuticular hyphae 2-7.5u,m wide, most at the narrow end of the range with occasional broader ones. **Pileocystidia** infrequent, clustered, mostly tapered and sometimes with a tiny capitum, 25-50 x 3-5um, differentiated only by the contents being more refractive and sometimes slightly greyer in SV than similarly shaped hyphal ends. **Pseudocystidia** not differentiated, just the occasional terminus of a laticifer, mostly in the subcutis and rarely emerging into the epicutis. **Hypodermis** a brown layer of small flattened cells.

Trama of clusters of sphaerocytes bound by a hyphal mesh.

Chemical reactions: FeSC>4 - pinkish brown; KOH - no reaction on cuticle, yellow brown on stipe; NH4OH - slightly brownish; guaiac - rapidly deep blue-green; guaiacol - red; phenol - chocolate brown; SV - cutis purple in sulphovanillin, gills magenta at first, rapidly turning dark grey.

Habitat and tree associations: trooping in shrubby woodland edges with Douglas fir and Garry oaks; shrub and ground cover may include ocean spray, broom, grass, salal and blackberry, September to December.

Collections: CROO1007-01, alongside a trail under Douglas fir, western hemlock and red alder with big-leaf maple, western red cedar, sword fern and shrubs. Cowichan River trail, N48. 756267°, W123.8254° (eastern very dry maritime CWH subzone). CR021219-01 and CR040923-01 atN48.4364°, W123.4806^0 and CR040919-01 at N48.4361^0, W123.4821^0, all from Royal Roads University woodland near the roadside in grass under Douglas fir with garry oak, broom and trailing blackberry *(Rubus ursinus).* BKO10904-02, Thomson Cabin trail in John Dean Park on the Saanich Peninsula, N48.613°, W123.443^0. CR040927-01 atN48.453350°, W123.491067° from the lower part of the garden situated in a small ravine between Wentwich Road and Rainville, under Douglas fir, garry oak, ocean spray *{Holodiscus discolor)* and trailing blackberry. CR001121-01 at N48.4599^0, W123.3129° at the side of a chip trail on the south side of the University of Victoria in mixed woodland of Douglas fir, madrone,garry oak, ocean spray, blackberry and alder. This last collection was smaller, darker in colour and with fewer inflated-articulated hyphae in the epicutis, and a less acrid taste, (last 5 collections from CDF zone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR001007-01	856	375	340, 260, 200	310,235
CR001121-01	870	382	365, 205	327, 254
CR021219-01	857	376	340, 255, 195	320, 226, 190

Notes *Russula cerolens* is closely related to *R. amoenolens* Romagn. and is separated only by the reticulation of the spores (Shaffer 1972). Romagnesi (1967) considered the latter species synonymous with *R. sororia* described by Schaeffer, renaming it *R. amoenolens* after recognising it as a different species to *R. sororia* (Fries) Romell ss. Boud. Sarnari (1998) separates these two species on the basis of size, odour, habitat and reaction with guaiac; *R. amoenolens* is smaller, smells of unripe Camembert cheese as well as spermatic (Jerusalem artichokes in Bon 1987), has a strong, rapid reaction with guaiac and grows with oak and shoreline pines; *R. sororia* has a weak guaiac reaction and a similar odour to *R. cerolens* but grows with broadleaved trees, Moser (1978) gives *R. sororia* as having the odour of Jerusalem artichokes). *Russula cerolens* is not a European species so was not considered by either Romagnesi or Sarnari. However, it appears to be very similar to *R. sororia* but for the increased reticulation on the spores of the former and preference for coastal conifers or mixed conifer-hardwood. *Russula sororia* differs from both *R. amoenolens andR. cerolens* in its subglobose rather than ellipsoidal spores, smaller warts which are only up to 0.4um, and flesh, especially the stipe, which discolours strongly red-brown on handling and in age, and has only a weak reaction with guaiac. This species has not been recorded from the Pacific Northwest.

The Vancouver Island collections of *R. cerolens* were made at the edge of the Douglas fir forest, in an area with broom, oak, often with ocean-spray, and all with blackberry, which may indicate more than one mycorrhizal partner. Shaffer (1972) gives the habitat for *R. cerolens* as under spruce and pine, sometimes on dunes, and made his description based on material collected in Oregon and northern California by Alexander Smith, mostly from coastal regions but also from Blue River, which is in the foothills of the western Cascade mountains. The collections identified as *R. amoenolens* in North

America came from Illinois, Massachusetts and Michigan, which suggests that this mushroom is an eastern species while *R. cerolens* is western. Thiers (1997) gives both for California, finding *R. cerolens* under conifers and hardwood-conifer forests in coastal counties of northern California, and *R. amoenolens* under pines, especially in coastal regions. He comments that the separating factor of spore ornamentation is not consistent and questions the distinctness of the two species. Collection CR001121-01, appeared to have characters intermediate between *R cerolens* and *R pectinatoides*. A comparison of spore sizes (fig. 59) shows most similarity with *R. cerolens*, the ornamentation being the same.

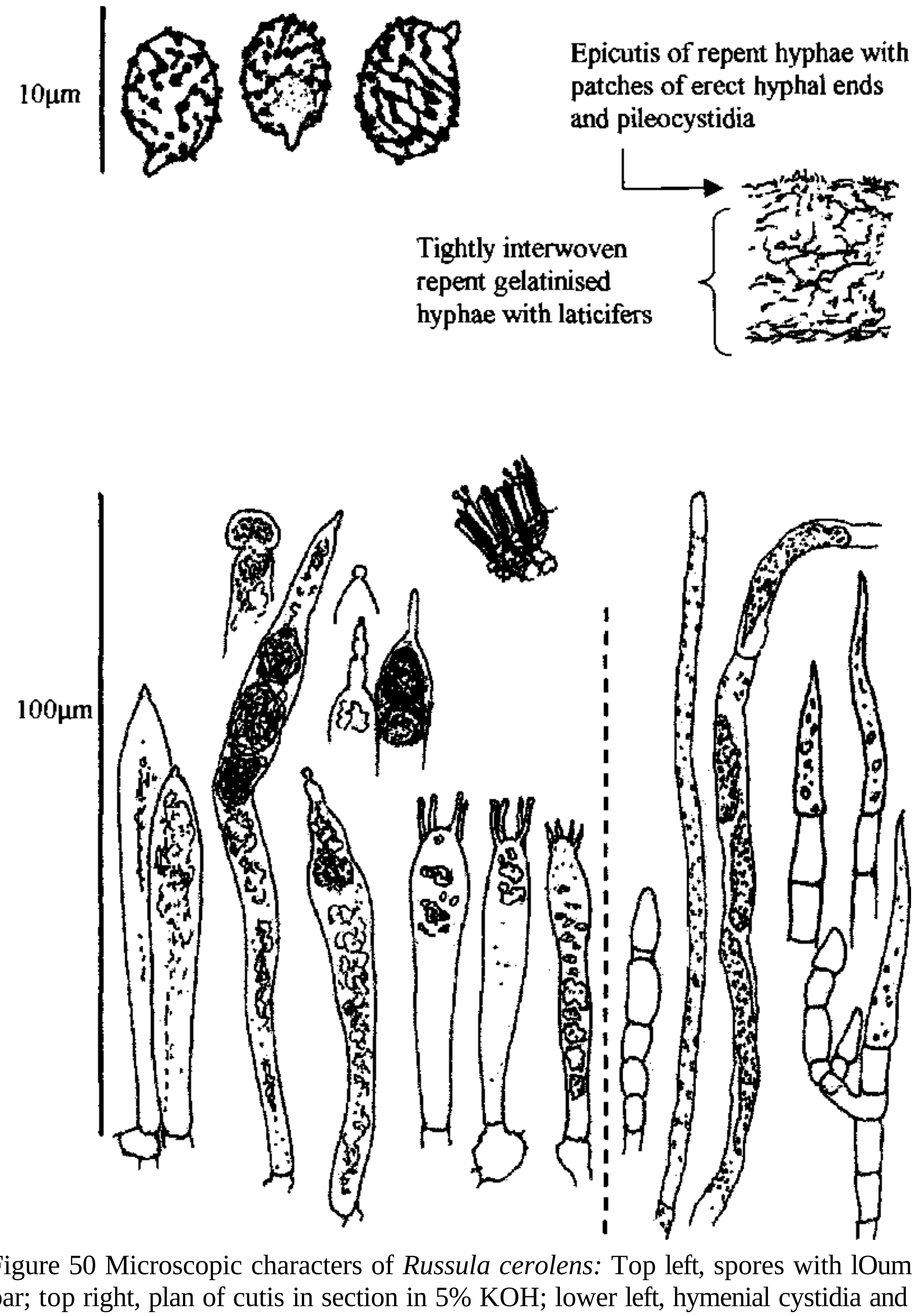

Figure 50 Microscopic characters of *Russula cerolens:* Top left, spores with lOum scale bar; top right, plan of cutis in section in 5% KOH; lower left, hymenial cystidia and basidia, the two on the left are cheilocystidia, those in the centre are pleurocystidia with different forms of apices, basidia on the right, and above these a plan of the hymenium; lower right, epicutal hyphal ends, a laticifer (centre) and pileocystidia, lower scale bar is lOOum.

Figure 51 Macroscopic characters of *Russula cerolens:,* Immature and mature basidiomata in situ and in profile.

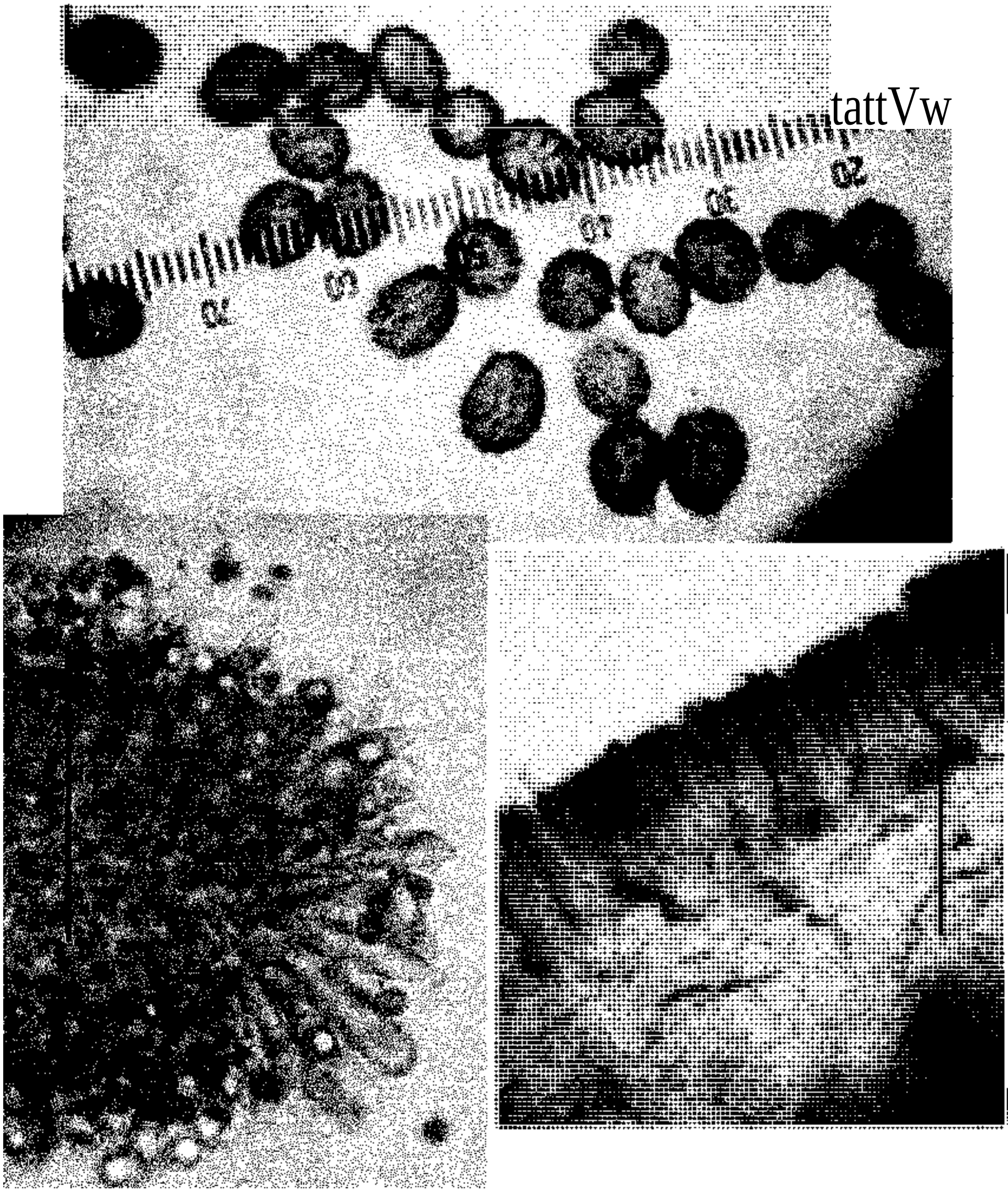

Figure 52 Hymenium of *Russula cerolens:* Top. spores with lum division scale; bottom left, section through the gill edge showing several cheilocystidia, some with refractive contents and some without, scale bar is lOOum: bottom right, section through hymenium showing dark pleurocystidia with origins mostly in the lower subhymenium, and either embedded or with protruding tips, scale bar is lOOum.

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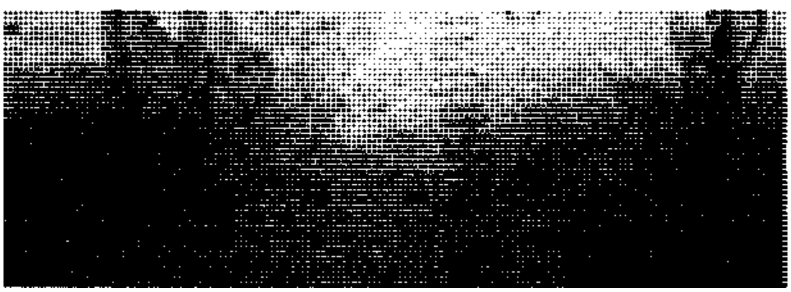

Figure 53 Epicutis of *Russula cerolens.* Top, surface view of an epicutal trichodermal patch of showing the numerous hyphal ends of chains of short cells; bottom, hyphal ends and pileocysidia in the epicutis, 10 scale divisions are 25um for both photographs.

***Russula pectinatoides* Peck**

Bulletin of the New York State Museum 116 : 43 1907

Cap 3.1-3.9cm, at first rounded with a central depression and incurved margin, expanding to plane or centrally depressed, margins long incurved, striate-tuberculate with the cutis stretched and splitting over the striations, which extend approximately 1/3 of the radius. Cutis a pallid yellow-brown, to yellow brown or dark grey-brown to dark greyish yellow-brown, becoming paler in age, usually darkest in the centre, may appear fibrillose, sticky to viscid, drying matte, sometimes with a whitish bloom when young, peelable 1/3-1/2 the radius, thick and elastic near margin, flesh immediately beneath grey-brown. Cap trama pale cream, about 0.5cm deep at half-radius when mature, unchanging when damaged.

Gills pale warm cream, close to crowded, lamellulae not seen, forking at stipe and out to half-radius, free to very narrow to notched at stipe, falciform, acute at cap margin, about 1/2 to 3/4 as deep as the cap trama at half radius

Stipe 2.9-3.5cm x 1.0-1.3 m, narrowly clavate, white, pruinose when young, sometimes rugulose to ridged, stuffed with a bready-textured trama that has 4 to 5 lenticular cavities, eventually becoming hollow, bruising lightly grey-brown round insect holes and at stipe base but otherwise unchanging. Stipe length approximately equal to cap diameter.

Texture firm, becoming brittle in age.

Taste mild at first and slightly sweet, finally peppery to acrid and leaving a soapy, oily aftertaste.

Odour fishy with rubbery or almond and rancid components.

Spore colour pale cream, Romagnesi Ib-IIa

Spores 5.8-8.2 x 5-6.2um, tear-drop-shaped or ellipsoidal, occasionally subglobose, L.W 1.08-1.53, average 1.26, warts generally small, most up to 0.5um high, occasionally to

0.7um high, rounded or bluntly conical, isolated or more usually with fine or occasionally heavy connections between two to several warts, sometimes 2-3 warts catenate or in longitudinal chains, spores with a partial reticulum quite common. Spore ornamentation and shape quite varied within one basidioma **Woo types Bl-2,** Cl-2. **Suprahilar patch** inamyloid or barely amyloid, in Melzers' reagent appearing as a very pale grey area with little or no ornamentation, on some spores there is a small darker patch within this area at the base of the hiliferous appendix, which is 1-1.5um long. **Basidia** 50-58 x 7.5-9um, very narrowly clavate, almost cylindrical with a slight broadening near the top, 4-spored, sterigmata 3-5um long. **Cheilocystidia** and pleurocystidia, abundant and densely distributed, 55-90 x 5-lOum, arising from the gill trama, embedded in the hymenium or protruding only about 15um, contorted-cylindrical, apices rounded or with a tiny point or capitum, with yellow refractive contents, dark purple-grey to black in S V, cheilocystidia orange in SV. **Subhymenium** 20-3Oum thick, pseudoparenchymatous. **Gill trama** of sphaerocytes and vascular hyphae.

Cutis 120-300 (-500)um thick. **Subcutis** of radially aligned, tightly packed interwoven repent hyphae with numerous vascular hyphae containing yellow droplets, staining grey and granular in SV. **Epicutis** of repent to patchily upright hyphal ends, 2-4um wide, mostly filamentous with either an undifferentiated hyphal terminus or of cystidioid tapering, ampulliform or fusiform-capitate end cells supported on one or two short, sometimes branched cells up to 5um wide. Very few articulated hyphae. **Pileocystidia,** 11-45 x 4.5-5um at the widest, hard to find and see, tapering as for hyphal end cells but with slightly granular contents, not staining in SV. **Pseudocystidia** rare, just an occasional undifferentiated terminus of a vascular hyphae, usually within the subcutis but occasionally reaching the surface. **Hypodermis** none.

Trama of discrete clusters of sphaerocytes enmeshed in hyphae.

Chemical reactions: FeS04- grey-brown; KOH -no change on cap cutis, yellowish on stipe; NHUOH - no reaction; guaiac - strongly blue-green; phenol - brownish purple; SV - colourless to brownish on cutis, purple-grey on gills.

Habitat and tree associations: Mature and regeneration Douglas fir, with or without western hemlock, along the edges of trails or in more open areas with shrubs.

Collections: CR001002-02, with regeneration Douglas fir in lawn by the ringroad at the University of Victoria, N48.4605^0, W123.3111°. CR001024-02, with Douglas fir, western hemlock, big-leaf maple and western red cedar, by the trail through Mystic Vale, University of Victoria N48.4594^0, W123.3093^0 (both CDF zone).

Collection	ITSl-Fto ITS4-B	RFLP.	Hinfl	Alul	Sau3A
CR001002-02	826	395		322,260,200	586,355,285

Notes: In this thesis, collections identified as *R. pectinatoides* differed from collections identified as *R. cerolens* in their smaller stature, milder taste and stipe with only four to five cavities, whereas *R cerolens* generally has five or six. Microscopically *R. cerolens* has many more of the chains of short inflated cells in its epicutis than does the local *R. pectinatoides*, which also has paler, rounder, spores with a shorter hiliferous appendix, but both species have spores with a partial reticulum. Collections of local *R. pectinatoides* have the spore ornamentation of *R. pectinatoides* (Peck), but the spores are paler and the extreme base of the stipe lacks the strong reddish bruising. Peck's description states the taste is mild; Romagnesi (1967) who describes *R. pectinatoides* sensu Singer, says it is fleetingly hot and sometimes bitter, Shaffer (1972) describes it as moderately acrid in the gills. The pale colour, small size and broadly ellipsoid to subglobose spores distinguish this species from other local *Pectinata.*

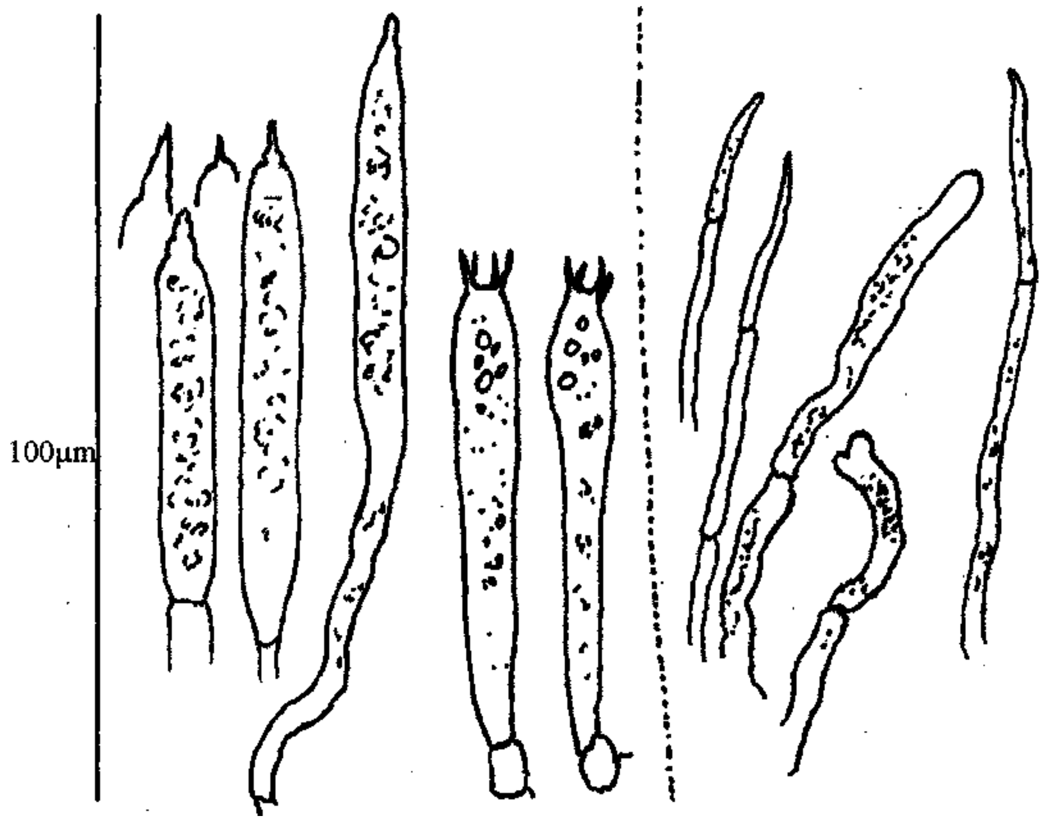

Figure 54 Microscopic characters *ofRussulapectinatoides:* Top, spores with lO^m scale bar; lower left, hymerdal cystidia and basidia; lower right, pileocystidia, laticifers and hyphal ends in epicutis, lower scale bar islOOmn.

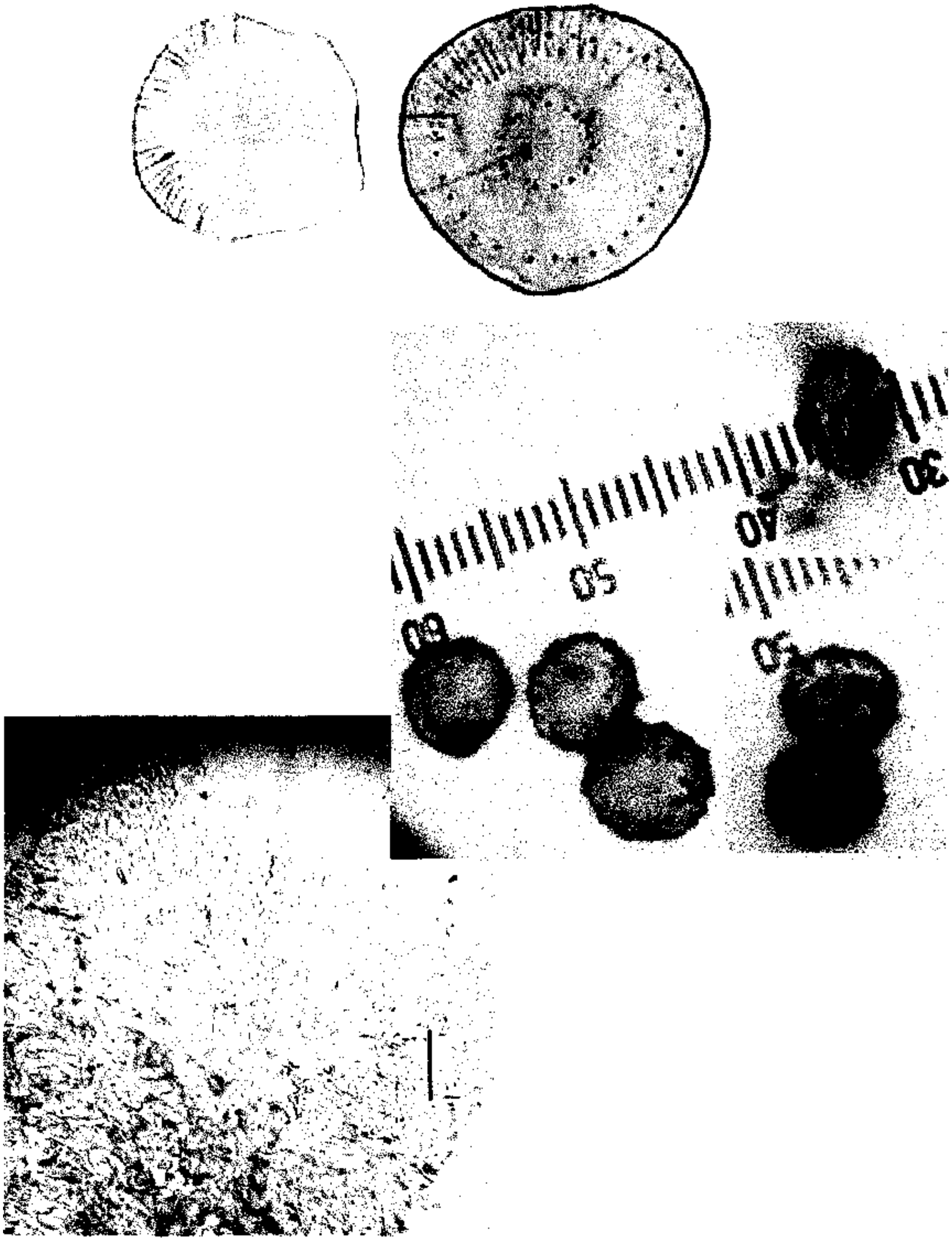

Figure 55 Cutis and spores of *Russulapectinatoides:* Top, field sketch of cap surface showing colours; middle right, spores with lum division scale; bottom left, section of cutis from *R. pectinatoides* showing a gradual merging of the interwoven subcutis with the tramal tissue, scale bar is lOOum.

Russula cf. *pectinata* **(Bull.) Fries**

Epicrisis Systematis Mycologici: 358. 1838

Cap 2.9-11.1cm, convex to pulvinate when young, becoming plane or more usually centrally depressed with the margins acute, sometimes lobed; grey-brown or dark yellow-brown when young, the mature caps paler, dull yellowish brown to dull brown, darker in the centre, developing reddish brown areas and spots particularly in the centre. Margin striate-tuberculate even when young, between 1/4-1/3 or occasionally to 1/2 of the radius, some peelable only 1/4, others up to 3/4 the radius. Surface sticky-viscid, drying matte in the centre, otherwise subshining with a silky appearance. When fresh slightly radially fibrillose, and occasionally with scurfy patches toward the centre, breaking into minute areoli. Cap trama creamy white, unchanging, tinged with cap colour directly beneath the cuticle.

Gills pale warm cream, sometimes with pinkish tints when viewed edge-on, subdistant to close, narrowly adnexed to almost free, falcate or arched, acute at margin, deepest at the mid-point, shallow at first becoming slightly deeper than the cap flesh at mid-radius, usually developing red-brown spots in age.

Stipe 2.4-6.9 x 0.7-2.4cm, more or less cylindrical or broadening at the base, stuffed, developing 4-5 lenticular cavities and becoming hollow in age. Stipe surface white, smooth to slightly rugulose, turning pale grey-brown where handled, base of stipe speckled with red-brown spots and stains, flesh unchanging where cut but browning around insect larval damage.

Texture firm, becoming more fragile in age, gills neither particularly brittle nor pliable, normal for *Russula*.

Taste initially bitter in three collections, then slowly peppery to acrid, especially in the gills, sometimes with a soapy aftertaste.

Odour spermatic to bleach with almond and fishy components, or in one case also hints of Jerusalem artichokes.

Spore colour pale cream, Romagnesi lb to Ha.

Spores 6.8-9 (-10) x 4.8-7um, mean 7.6 x 5.6, narrowly or broadly ellipsoidal, L:W 1.14-1.7, mean 1.36 (n=66). **Ornamentation** of low rounded warts up to about 0.5um, 2-3 catenate or with fine connectives forming a partial, broken reticulum, and often also with isolated warts, occasional spores with mostly isolated warts. **Woo types** A2, B2-C2. **Suprahilar patch** inamyloid or barely amyloid, non-ornamented or sometimes with a speckling of tiny warts, often bordered by normal warts. **Hiliferous appendix** 1.5 to 2um long. **Basidia** 35-50 x 5-lOum, columnar to narrowly clavate, sterigmata up to 6um long and slender, often under lum wide. **Cheilocystidia** infrequent to frequent, 50-90 x 9-lOum, non-staining or staining purple in SV, otherwise very similar to pleurocystidia. **Pleurocystidia** abundant and densely distributed, 33-100 x 7-13um, cylindrical, fusoid to clavate but often contorted and pinched at intervals, embedded in the hymenium or only protruding about 15 urn, arising from the inner subhymenium or the trama, contents refractive in KOH but occasionally not, yellowish near base or throughout, usually dark purple to grey in SV. **Subhymenium** about 25-30um thick, pseudoparenchymatous. **Gill trama** of sphaerocytes and frequent vascular hyphae.

Cutis 200-440um thick at half-radius, in some caps distinctly in two layers. **Subcutis** about 200pjn thick, of tightly interwoven hyphae around 2um wide, light pink in SV, and with numerous vascular hyphae containing yellow oily droplets which stain weakly and appear granular or bubbled in SV and which often extend into the epicutis. **Epicutis** around 240um thick or about half the depth of the cutis, an uneven trichodermis with patches of more or less upright hyphal tips, stretching at the margins to a thin broken layer with more repent hyphae. Cuticular hyphae *2-8\im* wide, most at the narrow end of the range with occasional broader ones mostly in the epicutis and often terminating in an articulate hyphal tip of chains of short, slightly inflated cells, sometimes with a tapered apical cell. **Pileocystidia** infrequent, clustered, mostly tapered and sometimes with a tiny capitum, most 12-35 x 3-5u,m at the base, more rarely long ones up to 1 lOum can be found, generally near the margins, and these may be cylindrical-capitate or tapered. Pileocystidia differentiated only by the contents being more refractive and sometimes slightly greyer and more granular in SV than similarly shaped hyphal ends. **Pseudocystidia** not differentiated, just the occasional terminus of a laticifer which sometimes emerge into the epicutis particularly near the cap margin.

Trama of clusters of sphaerocytes bound by a hyphal mesh.

Chemical reactions: FeS04 -greyish salmon to brownish pink; KOH -no reaction or slightly darkening the cuticle, yellow brown on stipe; NH4OH -no reaction; guaiac -strongly blue-green; phenol -pinkish brown; SV -cutis purplish pink, gills dull magenta at first, rapidly turning deep purple.

Habitat and tree associations: In troops at the edge of forested areas in the dunes and the spruce fringe along the shore in the western hemlock very wet maritime subzone, with western hemlock, shore pine, Sitka spruce, near shrubby areas of salal, kinnikinnik and blackberry.

Collections: CR001114-28, PJ010919-20, CR011031-06 from the Wickanninish dunes area to the north end, at the side of the trail close to the parking lot at the edge of the forested area, all approximately N49.02167^0, W125.67467^0. PJ010919-10, CR 021016-14 and CR021016-04, from the spruce fringe area alongside the southern part of the Wickanninish boardwalk, N49.015733^0, W125.67335^0 (both in the southern very wet hypermaritime CWH subzone).

		RFLP:		
Collection	ITSl-FtoITS4-B	Hinfl	Alul	Sau3A
CR021016-14	865	365	340,258, 190	320,257, 193

Notes: A.H. Smith made a collection he identified as *Russula pectinata* from Crescent City, a coastal town in Northern California, and which was subsequently studied by Singer (1957). The collections from Wickanninish match the description of *R. pectinata* given by Romagnesi (1967) except that the local collection has larger spores, up to 9 x 7um instead of up to 7.7 x 5.7um. The spore size does match that of the description of *R. pectinata* by Singer (1957) for mostly North American material. The colour of local collections is darker than that of Romagnesi's description, but he mentions the texture of the cuticle which sometimes breaks up into areolae, and the presence (in his illustrations) of longer, more cylindrical pileocystidia such as those very occasionally seen in Wickanninish collections. Romagnesi states that the taste is acrid, not bitter, *R praetervisa* (= *R. pectinatoides* f. *amarescens* Romagn.(nom. inval.)) is very similar to the Wickanninish collections in taste as well as most morphological characters and is a Mediterranean species of coniferous-hardwood forests on sandy soils. Singer (1957) gives the habitat for *R. pectinata* as under conifers and *Fagales* in damp depressions while Romagnesi gives it as under broadleaved trees in grassy openings on clay soils. The Wickanninish soils are sandy but close enough to the ocean to be kept moist by the fog-belt and rain. *Russula cerolens* is very similar and these collections were at first thought to be a darker variant of that species, since it is common further south, but the RFLP patterns of *R.* cf. *pectinata* were most similar to those derived from *R. pectinata* sequence data published through GenBank. However, very few of the *Pectinata* had been sequenced at the time of writing and there may be other species with similar RFLP patterns. In consequence, and pending further information, the Wickanninish collections can only be regarded as a species close to *R. pectinata*.

Russula amoenolens Romagn. is very similar, with an odour and appearance that matches the above collections, the spores have warts that are mostly isolated and the habitat is with coastal pines and oak. Certainly the above collections cannot be ruled out from being this species.

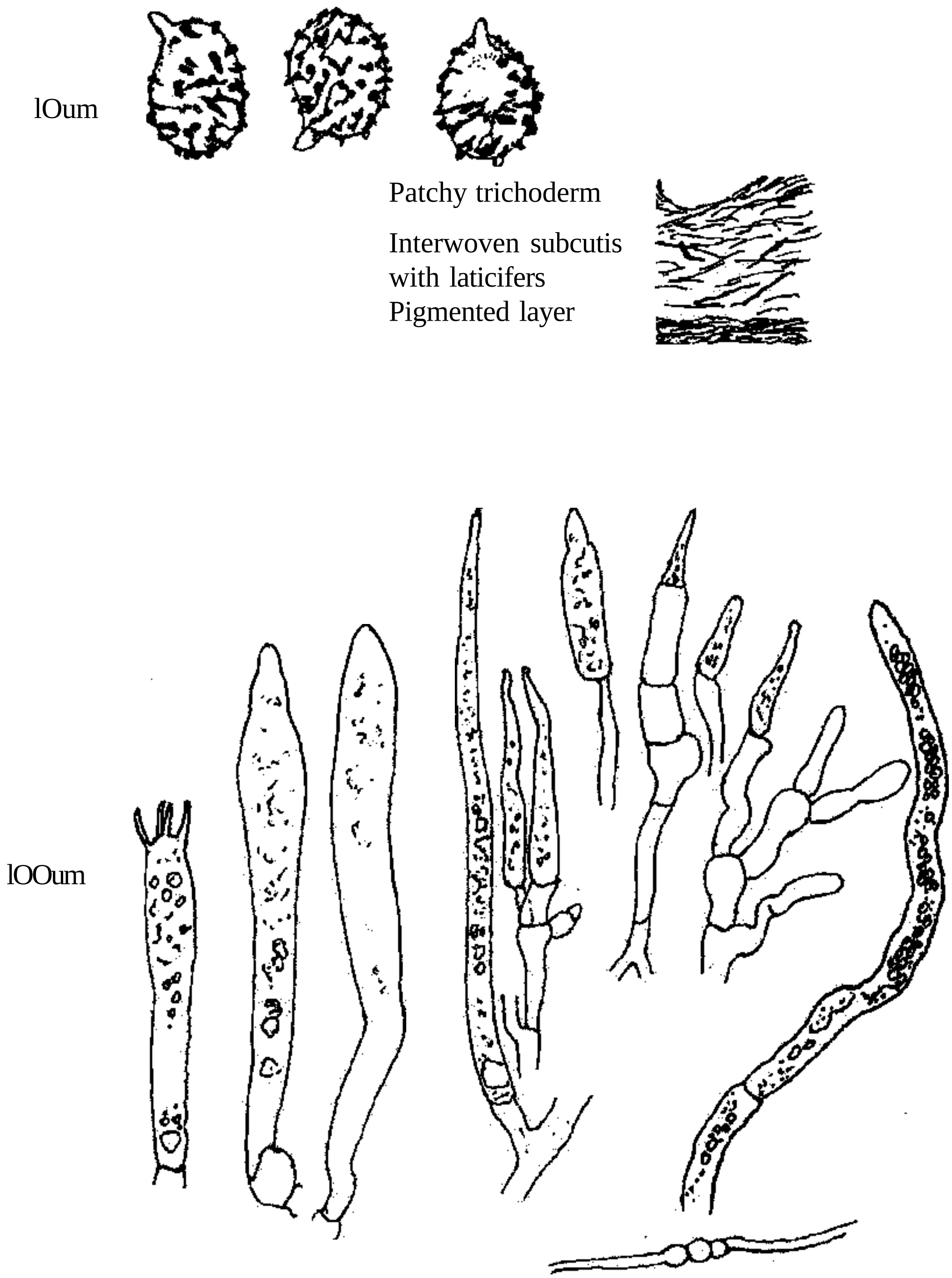

Figure 56 Microscopic characters *of Russula* cf. *pectinata:* Top, spores with lOum scale bar; lower left, basidia and hymenial cystidia; lower right, pileocystidia, hyphal ends in epicutis and laticifer, bottom, occasionally occurring bulbous hyphae near septum, lower scale bar islOOum.

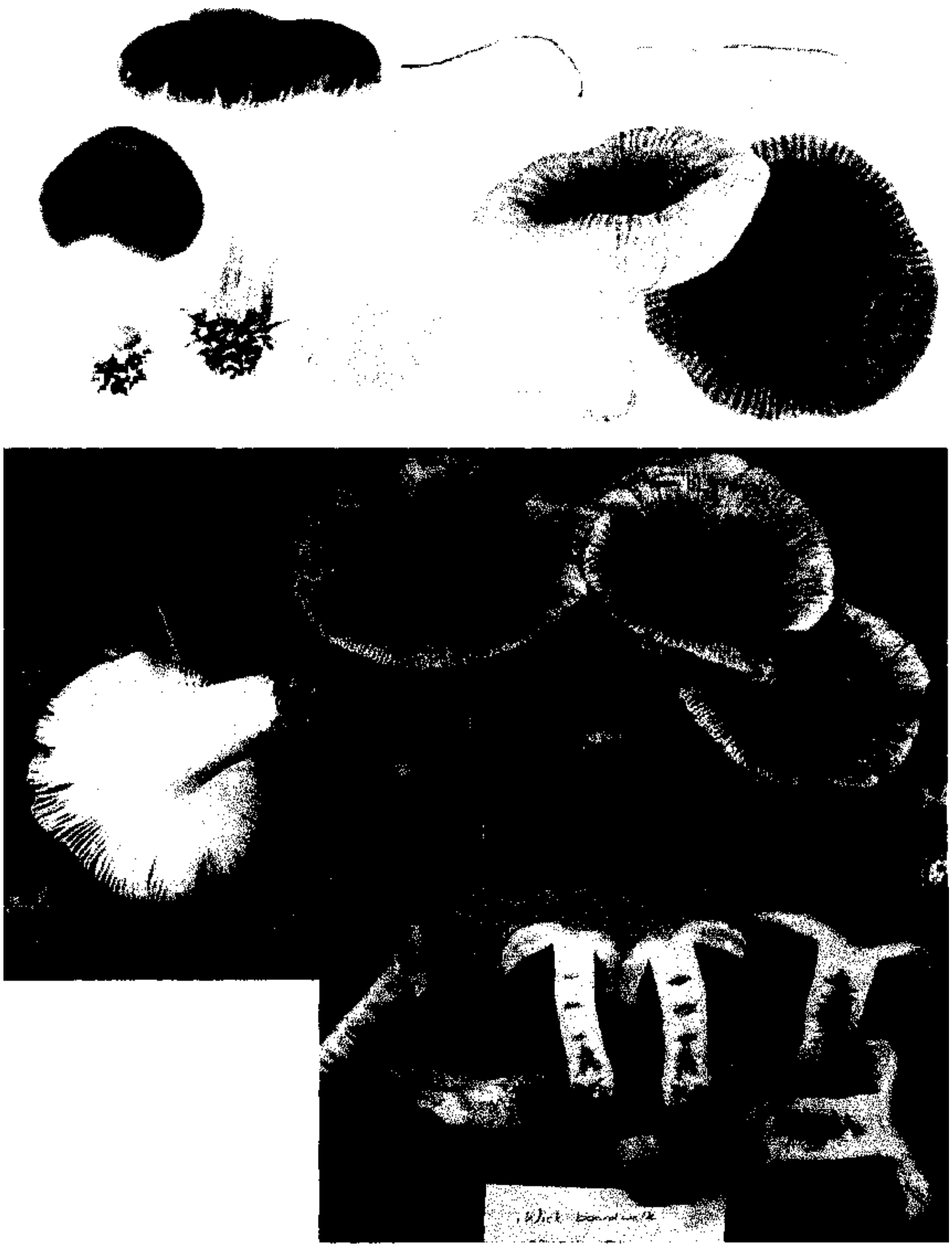

Figure 57 Macroscopic characters of *Russula* cf. *pectinata:* Top, illustration showing the cap and gill colour of immature, mature, longitudinal section and gills, coloured square is $1cm^2$ and shows spore colour; middle, *R.* cf. *pectinata* in situ (photograph by A. Ceska); bottom, specimens showing typical lenticular stipe cavities, red-brown tints and bruising on the cap and stipe base and where damaged inside by insect larvae.

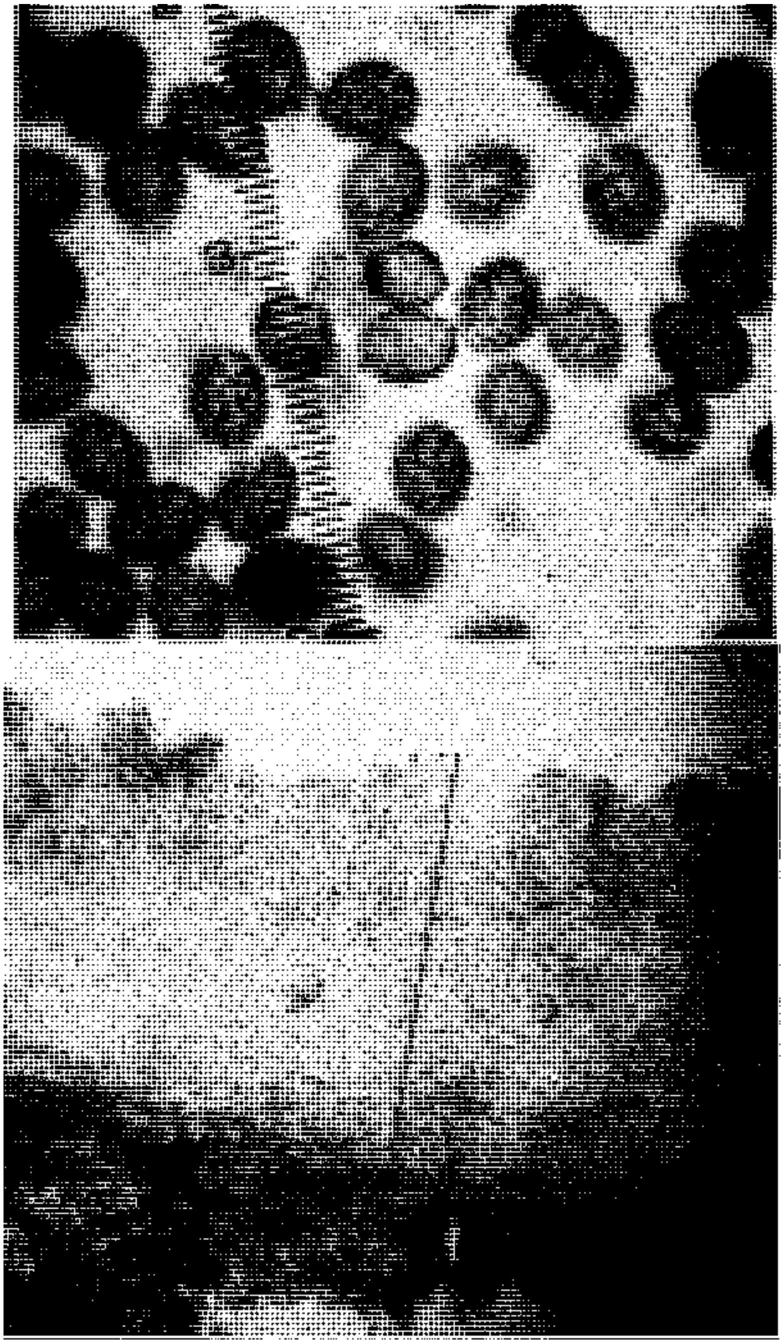

Figure 58 Spores and cutis of *Russula* cf. *pectinata:* Top, spores with lum division scale; bottom, section through cutis showing uneven epidermal layer, tightly interwoven subcutis and more pigmented hypodermis at the junction with cap trama, scale bar is 500um.

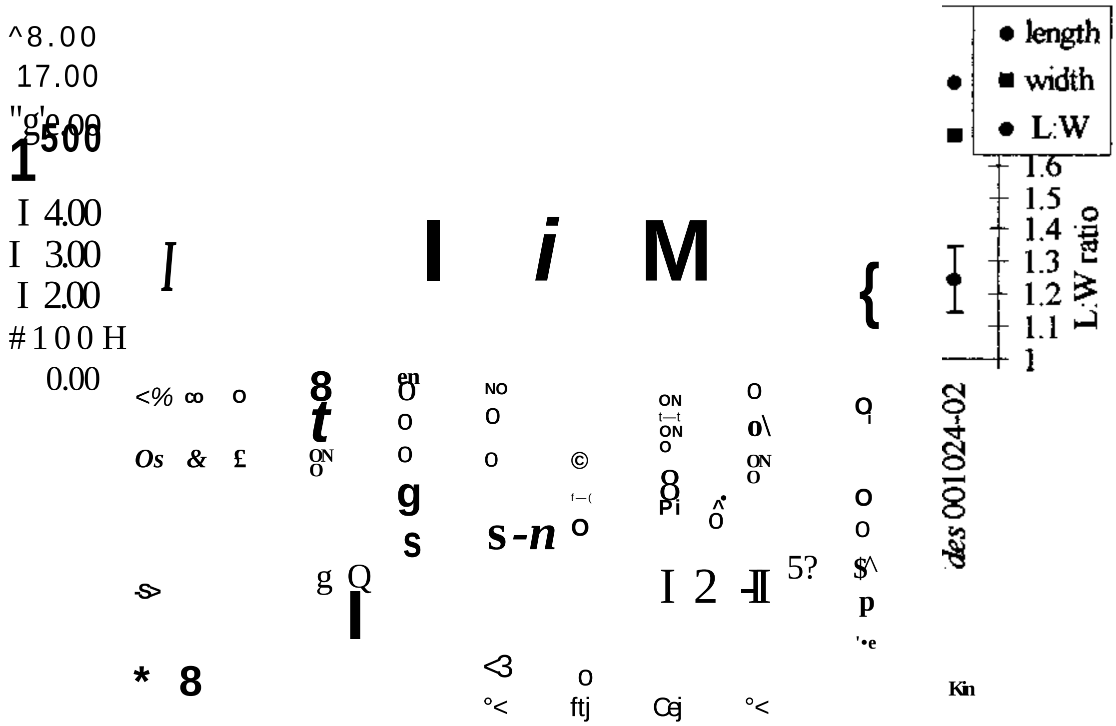

Figure 59 Comparison of spore dimensions and L:W ratio of *Russula cerolens, R* cf. *pectinata* and *R pectinatoides*. Error bars on ratio are one standard deviation and the difference between *R. cerolens* and *R* cf *pectinata* are not significant at a = 0.5, but *R pectinatoides* differs significantly from the other two (p = 0.002).

Table 16 Morphological characters of closely related species in the *Pectinata* obtained from the literature as mentioned.

***Russula* species**	***R.sororia*** sensu Romagnesi 1967	***R amoenolens*** sensu Romagnesi 1967	***R cerolens*** sensu Shaffer 1972	***R pectinatoides*** sensu Shaffer 1972	***R pectinata*** sensu Singer 1957
Cap diameter (cm)	5-10 (12) robust, rare	3.5-6, firm	4-8, firm Shaffer, 5-10 Thiers	2.5-8 fragile	4-7, firm at first, soon fragile
Stipe length (cm)	3.5-5 (7)	3-4.5	3-5	2.5-5.1	2-4.3
Stipe width (cm)	1-2.5	0.9-1.5	1-2	0.5-2.0	0.6-1.4
Bruising	rusty inside stipe	yellowing in stipe, grey-brown	rusty at base of stipe only	brownish at base of stipe	grey-brown at stipe base, not reddish brown
Odour	spermatic, like amoenolens but weaker, fruity	Jerusalem artichokes, Camembert, spermatic	bleach-ozone	spermatic-waxy with fruity, fishy or rubber component	smoked herring, spermatic, camembert or pleasant, of Jerusalem artichokes when young
Taste	slowly acrid	slowly strongly acrid, oily	slowly acrid & soapy	spermatic, waxy, mild to faintly acrid	acrid, sometimes slowly
Guaiac reaction	feeble	strong and rapid	strong and rapid	strong and rapid	not known
Spore colour	Ilb-c	lib	Ilc-d	Ilc-d	lib
Spore size (microns)	subglobose 6-8.2x5.7-7.2	ellipsoidal 7-8.5 (9) x 5-6.7	ellipsoidal 6-8.7x4.8-6.5	varied, broadly elliptic-elongate 5.4-8.4x4.6-7.3 up to 10.9 x 7.7 in some basidiomata	subglobose 6-10x6-8.5, most 7.3-9 x 6-8

***Russula* species**	***R.sororia*** sensu Romagnesi 1967	***R amoenolens*** sensu Romagnesi 1967	***R cerolens*** sensu Shaffer 1972	***R pectinatoides*** sensu Shaffer 1972	***R pectinata*** sensu Singer 1957
L.W			1.15-1.45		
Spore ornamentation (urn)	up to 0.4 Woo 1B-1C mostly isolated or lines joining pairs	up to 0.75 warts mostly isolated, partial reticulum rare, Woo2B	up to 0.8 partially reticulate Woo 2C-2D	up to 1.0(1.4) Woo 2A-2B(3A), isolated (Singer 1958), forming an incomplete reticulum (Shaffer 1972)	0.6-1.0 mostly isolated or lines joining pairs
Basidia (urn)	40-65x7.5-10	38-55x6.5-10	38-67 x 5-11.3	34-51x6.2-11.3	35-40 x 8-10
Hymenial cystidia ((am)	63-110(225) x 7-10, SV++	60-115x6.5-9.5pm, sometimes of two types, SV++ sometimes with yellow oily inclusions in base	37-97x5.3-10.2p.rn, occasionally with inlated tips to 17p.m. SV++, embedded or projecting to 40pm.	sometimes two types; 40-79x5.7-10pm greyish-yellow SV+, embedded, and 39-90 x 7.2-13.6pm, constricted apices, almost SV-, hyaline, projecting to 36pm	40-78 x 7-10 versiform, often appendiculate SV+
Pileocystidia	tapered	tapered, black in SV, yellow globules	tapered, SV- or weak S V+	subfusiform or tapered, sometimes terminal button, sparse SV+ granules	

***Russula* species**	***R .sororia*** sensu Romagnesi 1967	***R amoenolens*** sensu Romagnesi 1967	***R cerolens*** sensu Shaffer 1972	***R pectinatoides*** sensu Shaffer 1972	***R pectinata*** sensu Singer 1957
Epicutis hyphae	ramified, articulate, some thin, some wide	ramified, ± articulate, some thin, some wide	articulate, inflated cells to 5.7 or lOum, common.	articulate, ± inflated or subfusiform, some with granular contents, ends ± capitate	filamentous, with oleiferous hyphae
Habitat	broadleaved, (conifers?) clay, chalk and sandy soils	littoral pines, deciduous oak woods in warm, dry areas, mediterranean zone.	Under pine and spruce, sometimes on dunes, weedy edges of Douglas fir, oak, blackberry, salal.	open areas in mixed woods, sometimes on rotten wood	Under conifers and *Fagales* in damp depressions
Peelable	Not noted	1/4 to 3/4	less than 1/4	1/3-2/3	Not noted
Other			5% NH_3OH no effect	15%NH_3OH strong red-brown	

***Russula granulata* Peck (sensu Shaffer)**

New York State Museum Annual Report 53: 843. 1901

Mycologia 64: 1019. 1972

Cap 4.7-8.6cm, dome-shaped when young with incurved margin, becoming centrally depressed at maturity but retaining the incurved margin, sometimes lobed, striate to slightly tuberculate, but finer and not as lumpy as others in the *Ingratae*, from 1/5 to 1/4 the radius. Colour dark yellow-brown to dull brown with reddish-brown speckles over the centre when young and with a whitish bloom, becoming a paler dull yellow brown at maturity with red-brown streaks and mottles from the centre outwards almost to the striations, feeling rough to the touch and visually with a minutely granular texture. The granules look loose but are in fact part of the cutis. Buttons not viscid, mature caps viscid when wet, drying matte, becoming areolate, peelable 1/4-3/4 the radius with the trama immediately beneath tinted pale brownish.

Trama pale greyish cream, not discolouring when cut.

Gills pale cream, bruising and spotting rust-brown, close to crowded, narrowly adnexed to almost free, adnate at the margin, arched, about 1.5 times the depth of the cap trama at mid-radius, occasional lamellulae and some forking throughout the radius.

Stipe 5.0-7.6 x 1.4-2.2cm, clavate, firm, stuffed but developing 4-5 lenticular to irregular cavities. Stipe surface dingy white, pruinose at the apex, smooth to slightly rugulose below, with red-brown stains at the base, unchanging where cut.

Texture firm and not particularly brittle.

Taste mild at first, slowly becoming increasingly peppery with a soapy component.

Odour strong, spermatic with coconut or maraschino and almond components but less sweet and more soapy. Shaffer mentions a cocoa component to the odour which was not apparent in these collections.

Spore colour pinkish cream Romagnesi IIc-d in tone but a little pinker.

Spores 5-8 um x 4.2-6um, ellipsoidal to broadly ellipsoidal, L:W 1.1-1.6, warts 0.5-0.8um, bluntly conical, isolated, in rows roughly longitudinally oriented or 2-3 joined by fine lines, rarely forming a broken reticulum. **Woo types 2A, 2B. Suprahilar patch** inamyloid, or a very pale greyish area in Melzer's reagent, with a scattering of very tiny warts. **Hiliferous appendix** 1.5-2um long, around 1.2um wide at base. **Basidia** 40-58 x 7.5-10um, clavate or narrowly clavate, 4-spored. Sterigmata 4-6um long and up to 2um wide at the base. **Cheilocystidia** and **Pleurocystidia** 50-110 x 7.5-10um, protruding 10-22pm beyond basidioles, arising from within the subhymenium or occasionally the trama, narrowly clavate or fusoid, shape fairly smooth and even rather than contorted as in other *Pectinata,* tips mostly rounded, occasionally with a small button, contents refractive, yellow in 5% KOH or water, purple in SV. **Subhymenium** about 20-3 5 pm thick, pseudoparenchymatous, gill trama of sphaerocytes of varying sizes but very few vascular hyphae.

Cutis 250-550um thick, consisting of an uneven light brownish epicutis 40-70um thick but missing in patches and a thick subcutis of interwoven pale yellow hyphae which become slightly darker towards the bottom and merge gradually into the trama so that there is no strongly defined division between the two tissues. **Subcutis** interwoven, becoming looser towards the top and embedded in a gelatinous matrix and with many yellow, refractive vascular hyphae 3-8um wide that rarely react with SV. Cuticular hyphae l-4um wide, of uneven diameter with occasional short inflated sections giving some of them a knobbly appearence. **Epicutis** of loosely interwoven hyphae terminating in more or less upright, chains of short, moderately inflated cells giving them an articulated appearance, the apical cell may be rounded and undifferentiated or a cystidium. The septa between these articulated cells appear yellow and thickened at the edges. Hyphal ends tend to be clumped to form the "granules" seen macroscopically, and are bound with a brownish gelatinous matrix through which some of the end cells protrude, while others are confined by it. **Pileocystidia** frequent but clumped into the granules, of more or less capitate lanceolate to tapered cells, 10-7 5nm by 3-6 wide at their base, with light brown refractive contents not staining in SV.

Trama of clusters of sphaerocytes bound by a hyphal mesh with occasional SV+ vascular hyphae.

Chemical reactions: FeSC>4 - greyish on stipe surface, pinkish on trama; KOH - darkening the cuticle, slightly yellowish on stipe, on dried material the cutis becomes bright red-brown; guaiac - blue-green; phenol -purplish brown; SV - no effect on cutis, purple on gills. Shaffer notes that 30% KOH turns the flesh strongly red-brown, but this was not tested on local material.

Habitat and tree associations: In a stand of 50-70-year-old Douglas fir with understory holly, Canadian dogwood, snowberry, Pacific blackberry and grass, in early November.

Collections: CR001108-01 and CR001108-02, in a stand of mature Douglas fir, Royal Roads University, to the west of the main entrance, N48.439150^0, W123.478183^0; and N48.437767^0, W123. 479517° respectively (CDF zone).

	ITSl-Fto	RFLP:		
Collection	ITS4-B	Hinfl	Alul	Sau3A
CR001108-01	827	390	340,260,200	310,235

Notes The Vancouver Island collections agree very well with the descriptions in Shaffer (1972), from which further information may be derived. *Russula granulata,* according to Shaffer (1972), Singer (1957) and Bills (1984), is rather cosmopolitan in its habitat, having been recorded from mountainous and lowland areas under a variety of hardwood and coniferous trees. The collections described here are the only ones made from Vancouver Island to-date and the first records for British Columbia. Another collection made from a similar habitat at Rocky Point in December of the same year (2000), initially thought to be this species, was found to be closer to *R. pectinatoides*

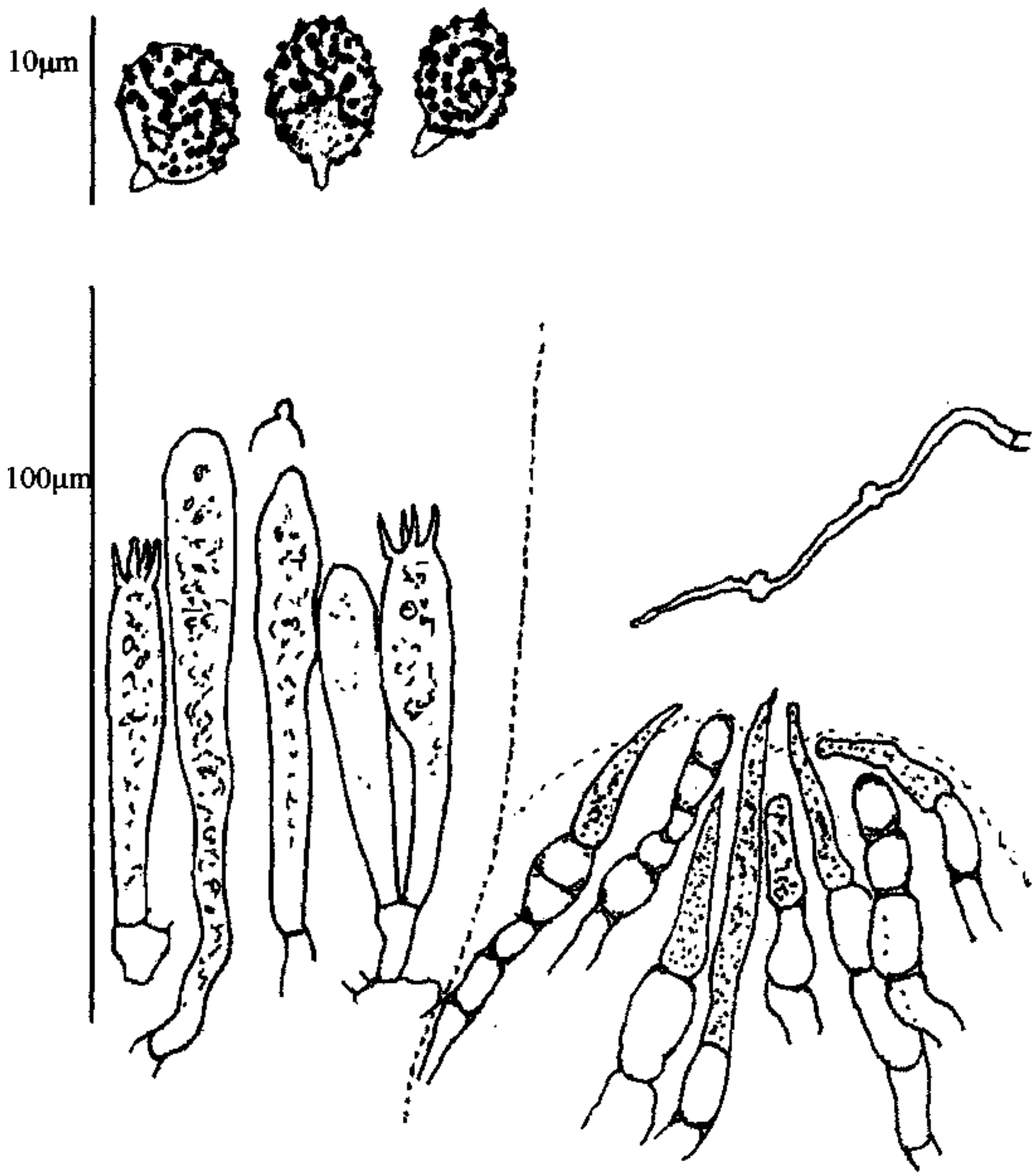

Figure 60 Microscopic characters *ofRussuIagramilata:* Top, spores with lOum scale bar; lower left, basidia, hymenial cystidia and basidia with basidiole; lower right, pileocystidia and hyphal ends in epicutal granule, faint dotted line over them represents the gelatinous matrix. Above is a common form of articular hyphae with uneven diameter and bulbous sections, lower scale bar is lOOum.

Figure 61 Macroscopic characters of *Russula granulata:* Illustration of immature, mature and longitudinal section, coloured square is 1cm^2 and shows spore print colour.

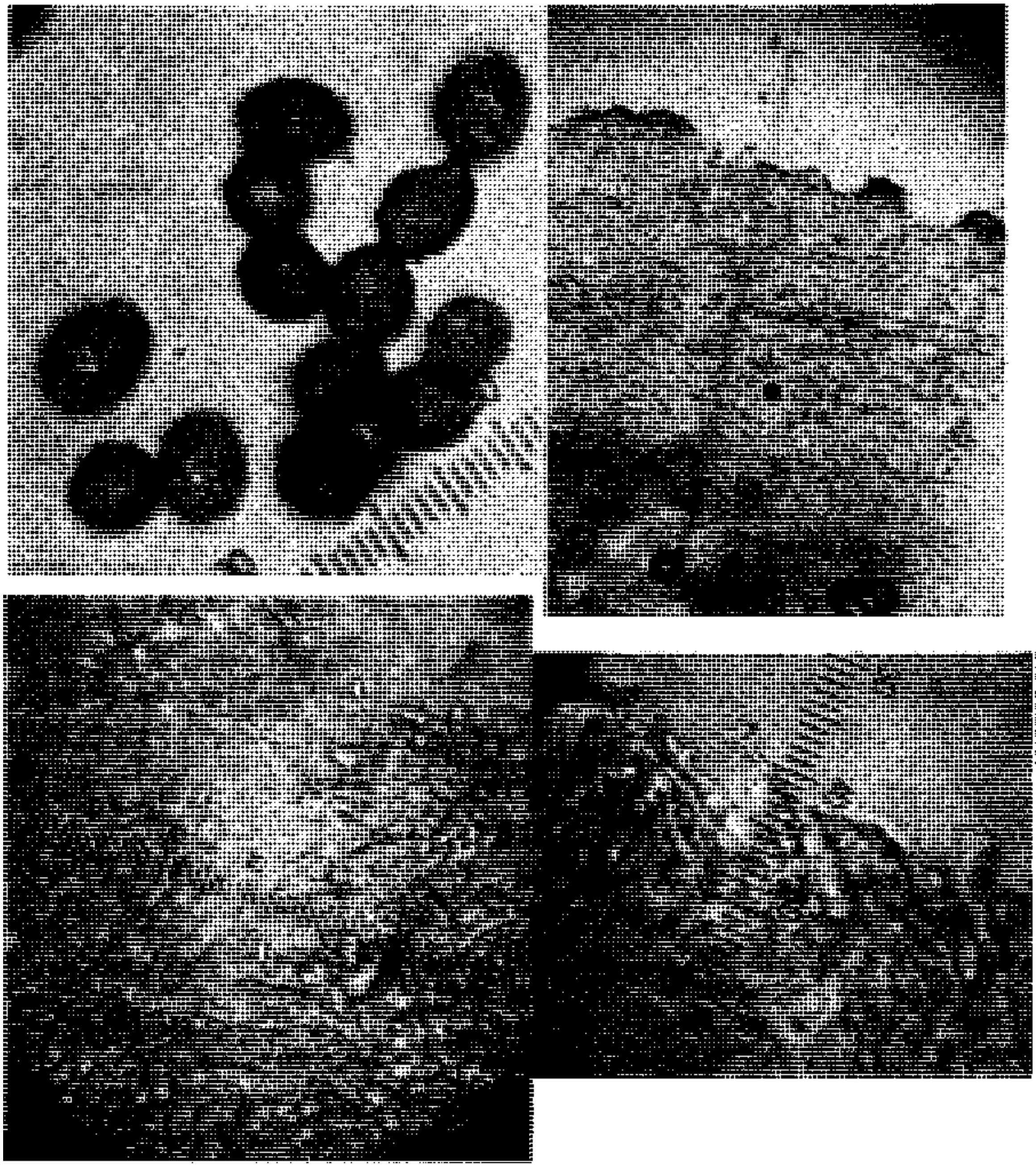

Figure 62 Hymenium and cutis *ofRussulagranulata:* Top left, spores with lum division scale; top right, section through cutis showing the relatively deep subcutis and the uneven epicutis, the raised bumps give the granular appearence to the surface, 10 scale divisions are lOOum; bottom left, surface view of the epicutis showing many lanceolate-capitate pileocystidia (two are arrowed) and articulated inflated hyphal ends; bottom right, section through one of the epicutal "granules" showing the same structures clustered, the gelatinous coating which normally binds the clump together has been dissolved by the 5% KOH used in the mount, 10 scale divisions are 25um for both lower photographs.

Clade 3c

Series *Foetens* Sarnari ad int. (Foetintinae ss. Str. Bon)

This group shares the strong odours, simple, yellow-brown cuticular pigments, browning flesh of the stipe and cream spore print found in the sister taxa of Series *Pectinata*. Series *Foetens* differs in having generally larger basidiomata, pigment mainly membranal (rather than vacuolar), cylindrical to clavate pileocystidia of normal size rather than tiny lanceolate ones, and larger spore ornamentation which can form wing-like ridges.

***Russulafragrantissima* Romagn. (sensu Shaffer)**

Russules d''Europe et d'Afrique du Nord: 350. 1967

Mycologia 64: 1044. 1972

Cap 6-16cm, spherical when young, becoming plane with a depressed centre, margin acute, not or rarely upturned, striate-tuberculate about 1/3 to occasionally almost 1/2 the radius. Colour pale ochraceous yellow to clear yellow-brown, sometimes with light reddish-brown marks or radial streaks especially in the centre, the centre usually a little darker than the margins, colours often darkening more with age. Surface radially moderately rugulose, viscid when wet and very sticky, especially when young, and leaving a glutinous residue on the fingers, drying subshining, sometimes becoming areolate toward margin, peelable only about 1/4 the radius with the trama immediately beneath tinted the cap colour.

Trama creamy white, unchanging when cut but browning where insect damaged and in the stipe.

Gills pale cream, bruising and spotting rust-brown, subdistant to close, narrowly adnexed to almost free, occasional lamellulae and forking, mostly towards the margin but sometimes also near the stipe, slightly decurrent at stipe and acute to narrowly obtuse at the margin. Shape somewhat ventricose (see fig 64) with the deepest point about 1/3 in from the margin, deeper than cap trama even when very young, becoming up to twice the depth at mid-radius. Both Shaffer and Romagnesi mention that the gills are often beaded with moisture, and this was also observed in these local collections.

Stipe 4-11.4 x 1.2-4.9cm, generally shorter than cap diameter at maturity, ventricose, stuffed, developing 8-9 lenticular cavities, becoming hollow in age, and staining strongly brown inside, surface cream, smooth to slightly rugulose, sometimes with broad grooves, turning yellow-brown to reddish-brown where handled and at the base. All collections of young buttons had a light blue-green layer of tissue inside the stipe rind at the base of the stipe and extending 1/4-1/3 up the stipe, a character not mentioned in other descriptions.

Texture firm and not particularly brittle but becoming so in age.

Taste slightly bitter and becoming peppery to acrid even in the stipe.

Odour strong, of maraschino cherries or cheap almond essence, developing an oily, rancid or stale component as it ages.

Spore colour pale cream Romagnesi Ha.

Spores 6.5-9 x 5.8-7.5um, subglobose to broadly ellipsoidal, L.W 1-1.35, average 1.17. **Ornamentation** of warts up to 1.4um, a few to 1.8um, none as high as 2um, bluntly conical to peg-like, some isolated, most joined with a heavy partial to complete reticulum made up of narrow wavy ridges up to the height of the warts and unevenly amyloid, **Woo types** 2-3E. **Suprahilar patch** inamyloid to very weakly amyloid, a poorly defined pale area with tiny warts, or often with similar but lower ornamentation to the rest of the spore. **Hiliferous appendix** 1.5 to 2um long, around 1.2um wide at base. **Basidia** 30-54 x 10-15um, clavate with a tapering base, 4-spored, similar in shape to those found in subgenus *Russula* rather than the narrow cylindrical shape associated with the *Pectinata*. Sterigmata 5-7um long and around 1.5 urn wide at the base. **Cheilocystidia** around 60-80 x 7-12um, abundant in young caps but lost in older caps, gill margins almost sterile, more or less cylindrical to fusoid and tapering downwards, arising in the subhymenium, ends obtuse or mucronate or with a small button, yellow in 5% KOH, colourless to brownish pink in SV. **Pleurocystidia** abundant, fairly densely distributed, 50-100 x 6-

8um, cylindrical to narrowly clavate arising within or from the base of the subhymenium, protruding about 10-18um beyond basidioles contents amorphous, yellow in 5% KOH and slowly grey to deep purple-brown in SV. **Subhymenium** about 20-35um thick, pseudoparenchymatous, gill trama of sphaerocytes and frequent vascular hyphae.

Cutis 200-250um thick at half-radius, in young but mature caps 250um at the margin to 600um over the disc with many vascular hyphae staining a granular dark brown in S V. In three layers: **Subcutis** 80-100um thick (of a 250um thick cutis section), of hyaline, interwoven radially aligned hyphae 1.5-4um wide, embedded in a gelatinous matrix and many yellowish vascular hyphae with refractive contents staining dark brown in SV. **Epicutis** approximately 50um thick, of more or less repent hyphae and free hyphal tips with many pseudocystidia 3-6um wide and often over lOOum long, originating in the subcutis and generally being the terminal cells of vascular hyphae, with tips obtuse to strangulated or capitate, the latter the most common type. On the epicutal surface lie many long, narrow, curved, yellowish hyphae of uneven diameter but on average about 1.5urn wide. **Pileocystidia** uncommon, mostly in young caps, 38-100 x 5-8um, narrowly clavate to cylindrical, with refractive contents staining brown in SV. In mature caps the pseudocystidia are so dense as to make it hard to pick out the pileocystidia. **Hypodermis** of densely packed radially aligned brown hyphae and flattened cells, with many vascular hyphae

Trama quite dense, of clusters of sphaerocytes bound by a hyphal mesh and frequent vascular hyphae.

Chemical reactions: FeS04 - greyish salmon to brownish pink; KOH - no reaction or slightly darkening the cuticle, orange-brown on stipe; NH4OH - no reaction; guaiac - strongly blue-green; phenol - pinkish brown; SV - cutis and gills dark brown.

Habitat and tree associations: common under Sitka spruce in forested glades on beach dunes, in the spruce fringe behind the dunes and under old-growth Sitka spruce-western hemlock forest alongside an estuary, and inland/upstream from this area in lowland western hemlock forest with occasional Sitka spruce. Season late August to early November.

Collections: CR 980825-03 and CR010814-05 from under a large Sitka spruce in estuarine forest at Port Renfrew, collections made three years apart, N48 5767°, W124. 3933°. CR030924-01 from a stand of old-growth Sitka spruce, western hemlock and western red cedar at the corner of Island Road in estuarine forest at Port Renfrew, N 48.5660°, W 124.3990°. CR030927-01, from regeneration western hemlock forest in the Lizard Lake area upstream from the Port Renfrew estuary N 48.603833°, W 126.205833°. CR001011-56 in an open stand of Sitka spruce with salal and red alder, within the spruce fringe alongside the boardwalk at Wickanninish dunes; CR011031-RF and PJ010919-03 under closed canopy forest in the same area, and 021016-WD from a forested pocket within Wickanninish dunes under Sitka spruce, shore pine and western hemlock, Pacific Rim National Park, N 49.016317°, W125.673400^{0}. (All collections from the southern very wet hypermaritime CWH subzone).

Collection	ITS1-F to ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR010814-05	870	400	569,220	285,211
CR030924-01	845	395	522,260	305,260
CR030927-01	845	405	522,250	285,260,180

Notes The Vancouver Island collections agree on most characters, particularly the spores, with Shaffer's 1972 description of North American material, to which the reader should refer for additional information. Local collections peel only at the margin as in Romagnesi's description, rather than up to 3/4 as in Shaffer's, the vascular hyphae and pseudocystidia do stain in SV, and the flesh is peppery, characters associated with Shaffer's 1972 description of North American *Russula laurocerasi.* Shaffer also notes that he made collections that had some characters of both *R. fragrantissima* and *R. laurocerasi.*

Russulafoetens (Pers. ex Fr.) Fries has mostly isolated spore warts and a more foetid odour, but is otherwise similar to *R. fragrantissima* and *R. laurocerasci*, and these latter species have probably been mis-identified as *R. foetens* in some reports.

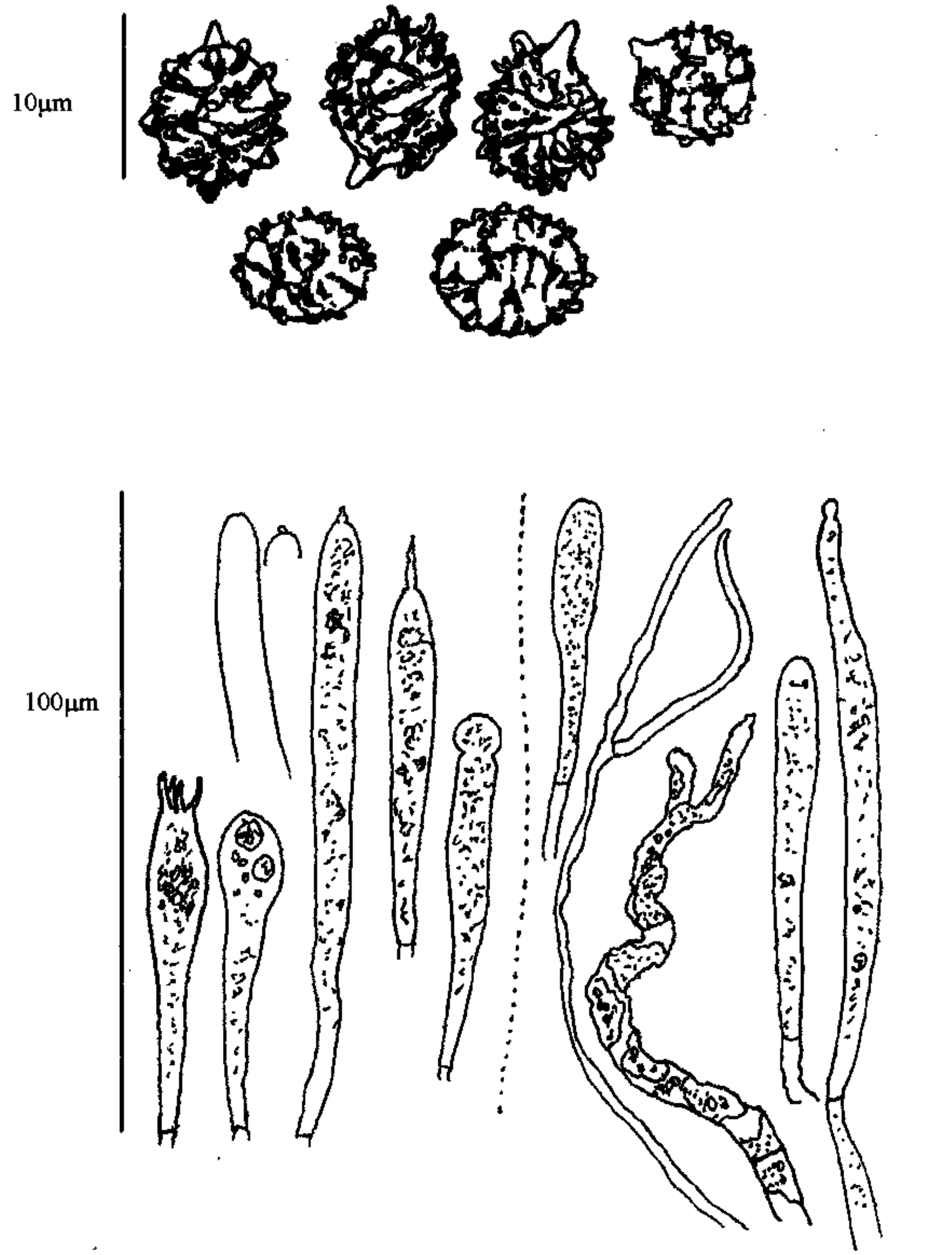

Figure 63 Microscopic characters *ofRussula fragrantissima:* Top, spores with lOum scale bar; lower left, basidia, basidiole and hymenial cystidia; lower right, pileocystidia, hyphal ends, vascular hypha and pseudocystidia in epicutis, lower scale bar is 100pm.

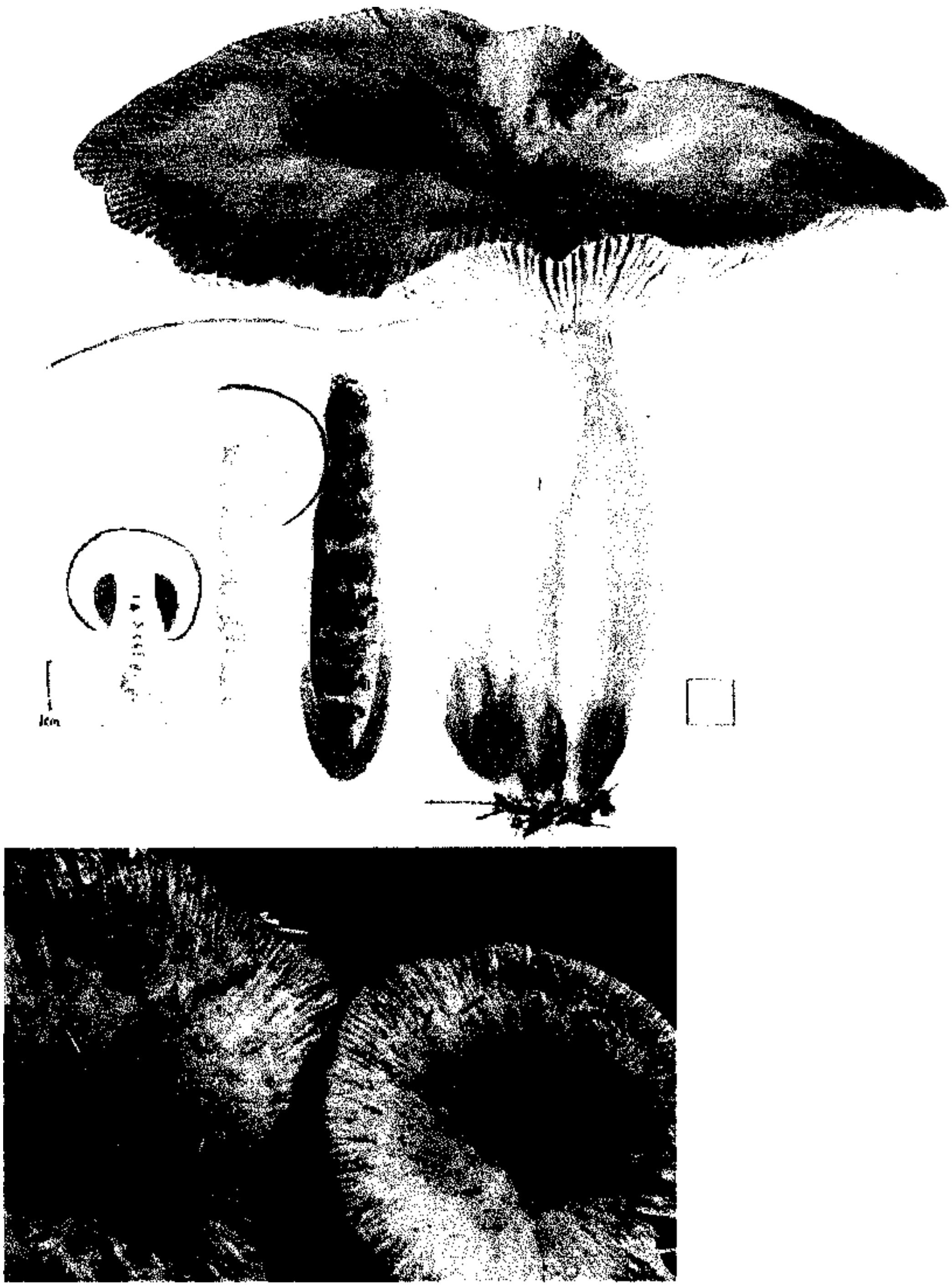

Figure 64 Macroscopic characters of *Russulafragrantissima:* Top, illustration of sectioned immature stages in which the cavitation and discolouration of the stipe is evident, note the pale blue-green layer of tissue in the extreme base of the stipe in the buttons, also a mature basidioma in profile, the square is 1cm^2 and shows spore colour; bottom, surface of cap showing the ochraceous yellow colour and the ruberculate margins typical of the *Ingratae*.

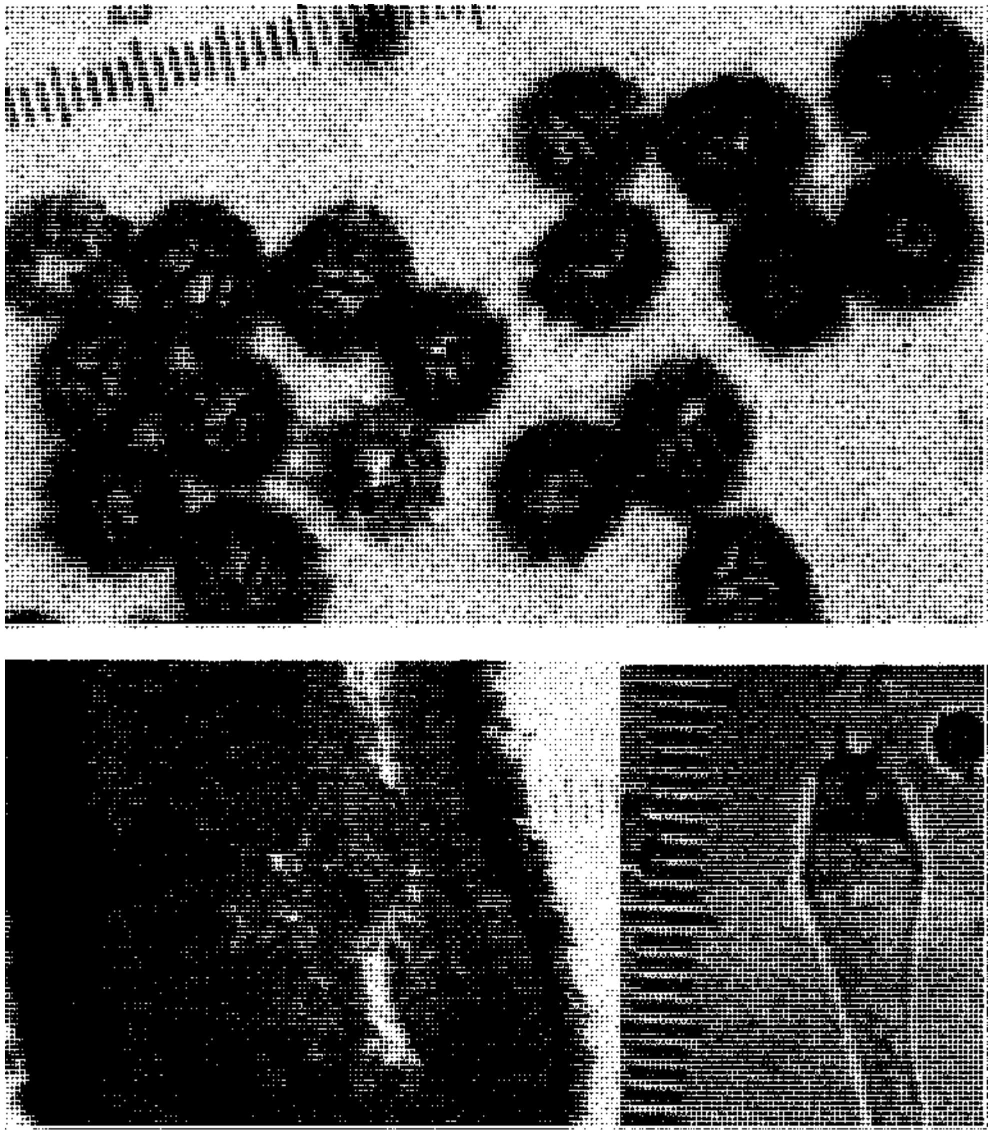

Figure 65 Hymenium *otRussulafragrantissima:* Top, spores with lum division scale; bottom left, section of gill mounted in 5% KOH, white bar shows extent of subhymenium, 10 scale divisions are 25um; bottom right, basidia with adjacent immature spore, scale as left.

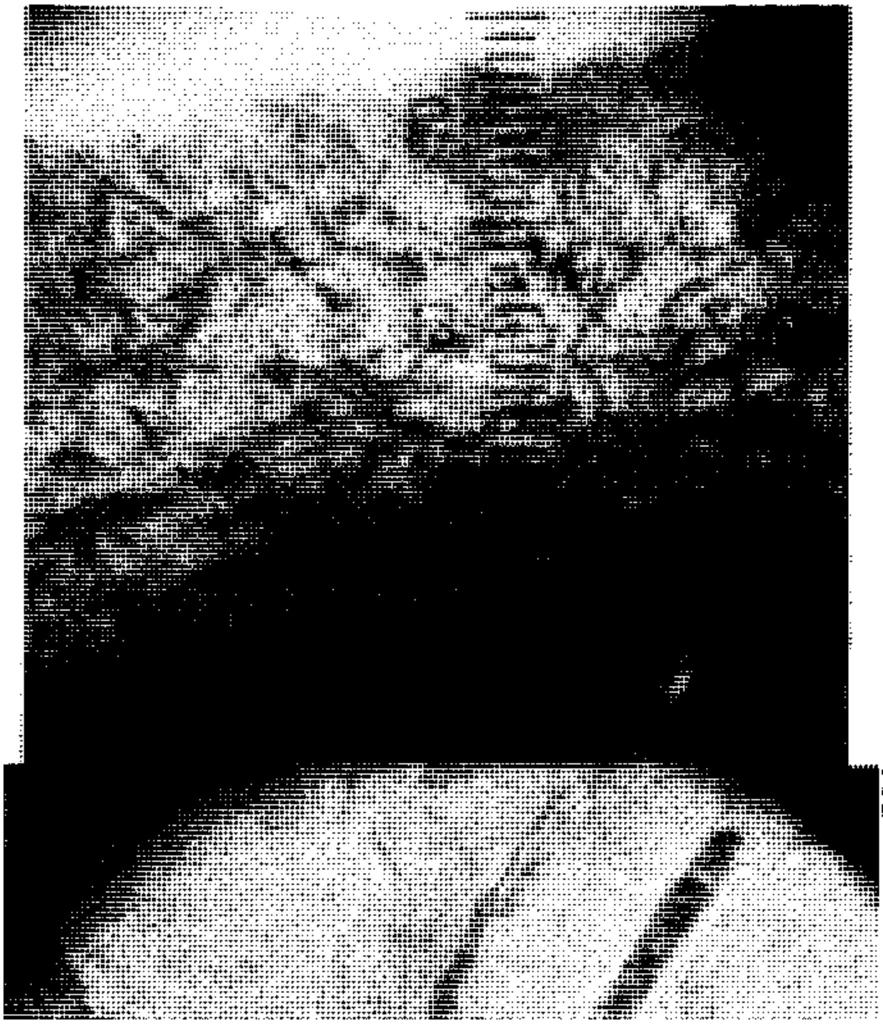

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Figure 66 Cutis of *Russulafragrantissima:* Top, section through entire cutis, 10 scale divisions are lOOum; bottom, surface view of epicutis with pileocystidia in SV, 10 scale divisions are 25um.

Subsection *Farinipedes* Singer

Russulas in this subsection share many of the general characteristics of the *Ingratae*, namely the simple yellow-brown articular pigmentation, the inamyloid to weakly amyloid suprahilar patch on the spores, and the lenticular cavities in the stipe trama. The *Farinipedes* differ in the white spore print, voluminous pileocystidia, and fruity rather than bleach, spermatic or benzaldehyde odours. The characters of this group have similarities with both subgenus *Ingratula* and subgenus *Russula*. A phylogenetic analysis using sequence data published after this dissertation was completed shows *Russula farinipes, R. pallescens* and *R. crassotunicata* as a clade basal to the other *Russula* clades. The rationale for including them here in their traditional classification order is because their morphology is closer to that of the clade 3 and clade 4 species, and is where one might more logically look for them.

***Russula farinipes* Romell**

In Britzelmayr: Hymenomyceten aus Siidbayern 9:239. 1893

***Russula pallescens* Karsten**

Kritisk Ofversigt Finlands Basidsvampar: 463. 1889

Cap 2.4-7.Ocm, immature caps assumed to be convex, expanding to broadly dish-shaped with downcurved margins, eventually margins upcurved to form a deep bowl shape, all the while retaining a central flattened umbo. Margins tuberculate to almost 1/2 the radius, more or less radially rugulose over the rest of the cap. Colour cream to pale ochraceous yellow, fairly uniform in some caps, darker in the centre in others, with damaged areas light yellow-brown. Surface sticky to viscid when wet, drying matte, not peelable at all.

Trama pale yellowish cream, unchanging to becoming dull yellow where cut, very thin, 1.5 -3 mm at half-radius and thinning to a few cells thick outwards, virtually non-existent at the margin.

Gills whitish to pale cream with slight brownish spotting in age, distant, intervenose, acute at cap margin, adnate with a decurrent tooth at stipe, margin entire, more or less straight-edged or ventricose and deepest at half-radius, 3-7 mm deep depending on the size of the pileus, 4 to 5 times the depth of the cap trama at that point. Lamellulae very few, one or two per cap, not forked or only near margin.

Stipe 3.8-9.0 x 0.5-1. lcm, equal to or longer than the cap diameter, cylindrical but slightly widening at the apex, cream, becoming brownish-yellow on handling and at the base, otherwise unchanging where cut, pruinose at apex, longitudinally slightly rugulose, stuffed, with 12 to 16 small lenticular cavities which remain distinct into maturity but eventually converge, the stipe becoming hollow.

Texture quite tough and firm to average *Russula* texture, not particularly fragile, gills pliable.

Taste acrid.

Odour a complex but subtle mixture of fruity, perfume and fishy components.

Spore colour Romagnesi between la and lb.

Spores 7-9 x 5.5-7um, bean shaped to broadly ellipsoidal, warts hemispherical to bluntly conical, most up to 0.5pm but a few to 0.8pm, many small ones dotted between the larger ones but this character varies from spore to spore; warts isolated or 2-3 or more joined by fine lines, not forming a reticulum, **Woo types** Al-2, Bl-2. **Suprahilar patch** a pale, inamyloid to weakly amyloid well-defined area bordered with warts, often speckled with several minute warts radiating out from the hiliferous appendix. **Hiliferous appendix** 1.5-2um long, about 1.2pm wide near the base. **Basidia** 45-52 x 7-10pm, narrowly clavate to clavate, 4-spored but 2-spored ones common. Sterigmata 7-10pm long, slender, 1-1.5pm wide at the base. **Cheilocystidia** variable in abundance, some parts of the gill with very few, others with similar density to that of the pleurocystidia on the gill face. **Pleurocystidia** abundant, 55-100 x 7-10pm, with yellow refractive contents, staining strongly blue-black in SV, fusoid, ends acute, capitate, or with a short series of narrowing strangulations, arising in the inner regions of the subhymenium or the trama.

Subhymenium 20-3 Oum thick, pseudoparenchymatous. **Gill trama** of sphaerocytes of quite varying sizes, with frequent vascular hyphae.

Cutis 120-150um thick over the disc, thinning to 60um or less at the margin. **Subcutis** comprising about 1/2 to 2/3 the depth of the cutis, of repent, somewhat compressed hyaline to pale yellow hyphae l-3um wide, embedded in a gelatinous matrix, and becoming darker in the lower approximately 50um, forming a sometimes distinct, sometimes gradual boundary with the trama. The subcutis contains abundant vascular hyphae 4-9um wide, tortuous, with yellowish refractive contents. **Epicutis** 40-60um thick, of more or less upright, loosely interwoven brownish hyphal ends and pileocystidia embedded in the gelatin, the uppermost surface is almost entirely of pileocystidia. Epicutal hyphae 1.5-5um, sometimes nodulose, this being somewhat variable between basidiomata, but with undifferentiated terminal cells, inflated-articulate termini not seen. **Pileocystidia** abundant, voluminous, 55-150 x 5-9um, unicellular, with pale yellow refractive contents that stain weakly to strongly grey in S V, tips acute, capitate or with a strangulated appendix, accompanied by occasional pseudocystidia, the terminals of laticiferous hyphae and with slightly stronger yellow highly refractive contents that stain purple-black in SV.

Trama of clustered sphaerocytes with hyphal mesh and frequent vascular hyphae.

Chemical reactions: $FeS0_4$ - pale brownish; KOH - orange-brown on cap cutis, no reaction on stipe; NH4OH - bright ochre-yellow on cap, no reaction on stipe; guaiac - weakly blue-green; phenol - purplish-brown; SV - almost no effect on cutis, purple on gills.

Habitat and tree associations: The collection from Carmanah was found under old growth Sitka spruce, western hemlock and western red cedar, the collection assumed to be from the Sooke area was reported as from a second-growth western hemlock coastal forest, with blueberry.

Collections: CR020927-02 from Carmanah grove between the Fallen Giant and Heaven grove, N48.65700^0, W124. 697167° (southern very wet hypermaritime CWH subzone). SVUVIS021020-02, brought in to the South Vancouver Island Mycological Society annual show 2002, location unrecorded but probably near the Sooke area.

Collection	ITS1-F to ITS4-B	RFLP:Hinfl	Alul	Sau3A
(as R farinipes)	809	364	493,252	300,200

Notes This mushroom is commonly regarded as *Russulafarinipes* and may be that species, but in European literature it is reported as under broadleaved trees, particularly *Fagales,* whereas the very similar *Russula pallescens* is its equivalent from coniferous forests. Sarnari (1998) reports the latter as a rare northern species (in Europe). Morphological differences are that *R pallescens* has a flattened umbo, slightly larger spores (7.2-9 x .4-7.4) with low warts and some ridges as opposed to those of *R farinipes* with spores 6.4-8 x 5.8-6.7 with small, isolated, pointed warts. The cuticular hyphae of *R pallescens* have oddly-shaped, sometimes inflated-articulate ends giving it a jigsaw-puzzle appearance in surface view through a microscope (Sarnari 1998) whereas those of it *farinipes* are normal hyphae. In the Vancouver Island material, the epicutal hyphae were generally of the normal type, although the occasional oddly shaped, inflated element could be found. The spores of local material are closer to those of *R pallescens* and the habitat is coniferous forest, although broadleaved trees such as red alder and many shrubs are common in forest openings and cannot be excluded as the host. The flattened umbo was quite apparent in the collection from the Sooke area, less so in the Carmanah collection. The mushroom is quite rare, with very few records from the Pacific Northwest. Grund (1962) collected this species once from coniferous forest in the White River Valley, Washington State. His description shows the spores to have some reticulation and to be of the size and ornamentation fori?, *pallescens* rather than *R farinipes*. Grund identified this species as *R farinipes* because *R pallescens* was unlikely to have been known to him, its description not being in the literature available to him. Local collections are closer to *R pallescens,* although the above description appears under both names, partly because *R. farinipes* is locally known, correctly or not, and partly because it is possible that both species do co-exist.

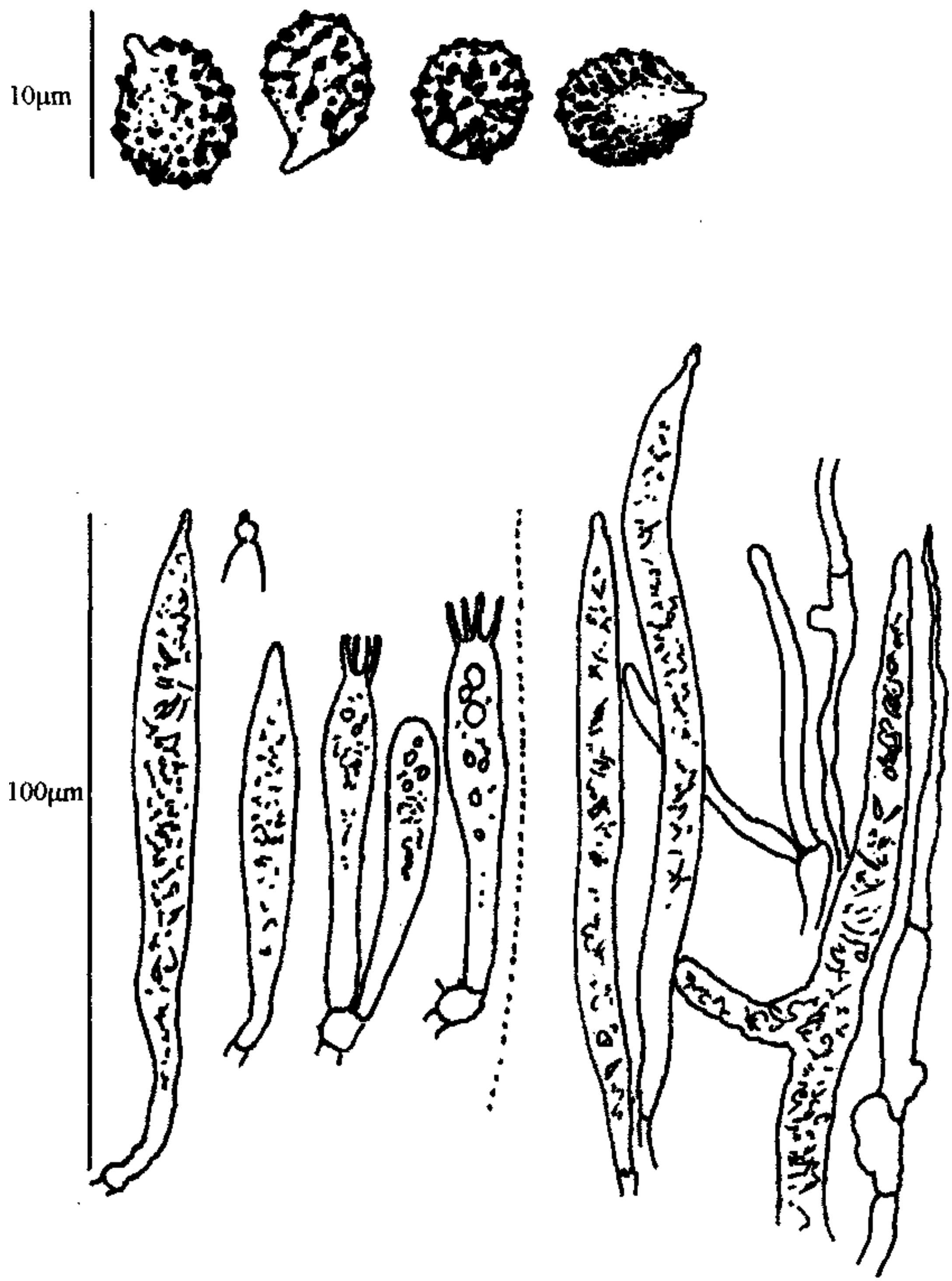


Figure 67 Microscopic characters of *Russulafarinipes;* top, spores, bar is lOum; bottom left, hymenial cystidia, basidia and basidiole; bottom right, pileocystidia, epicutal hyphae and terminus of laticifer, bar is lOOum.

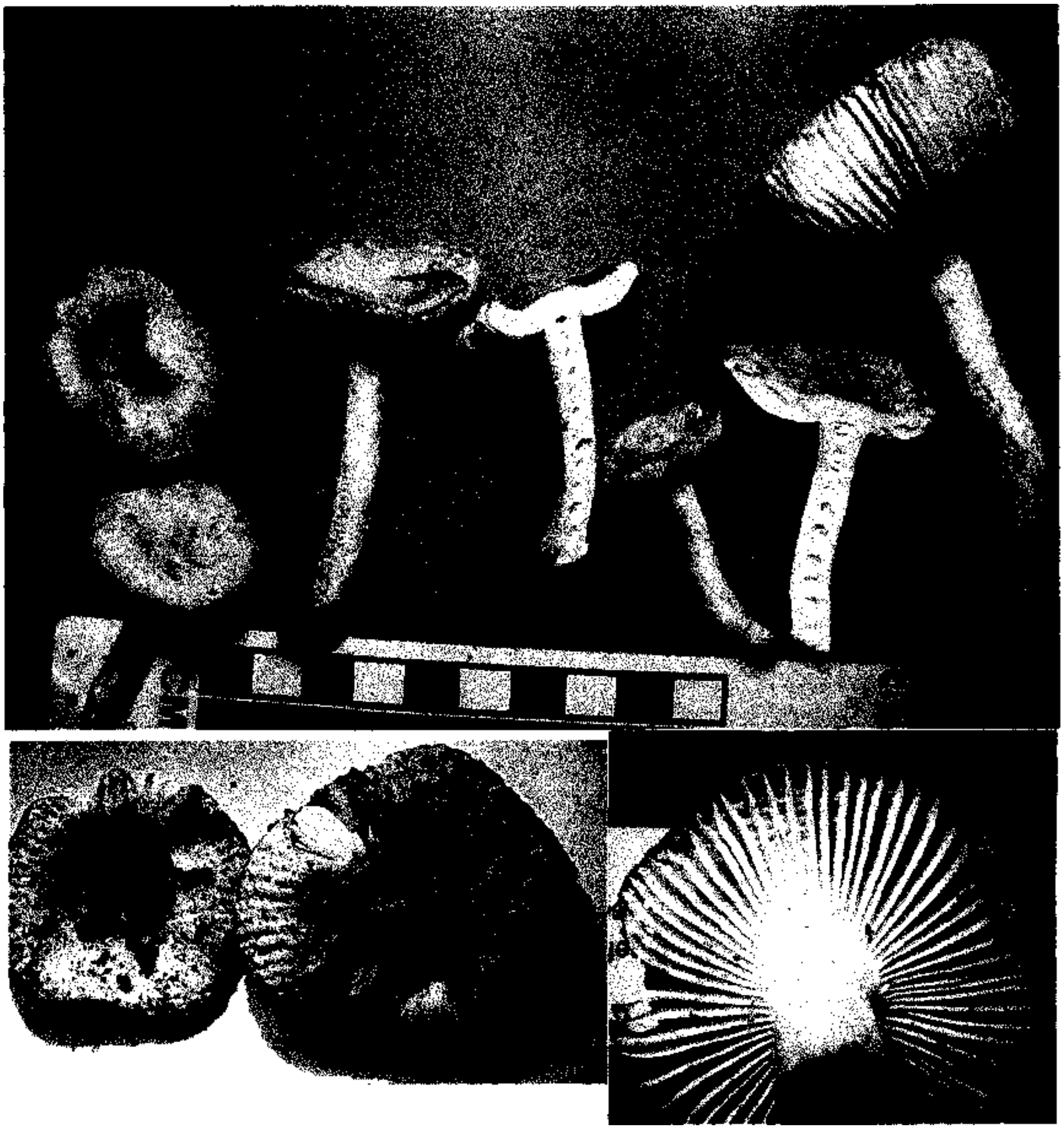


Figure 68 Macroscopic characters of *Russulafarinipes:* Top, collection of mature basidiomata with one cut longitudinally to show lenticular cavities and the flattened umbo, lower left, cap of collection from Carmanah grove showing tuberculate margins and light browning where damaged by slugs, lower right, view of gills showing the distant spacing and mterveining.

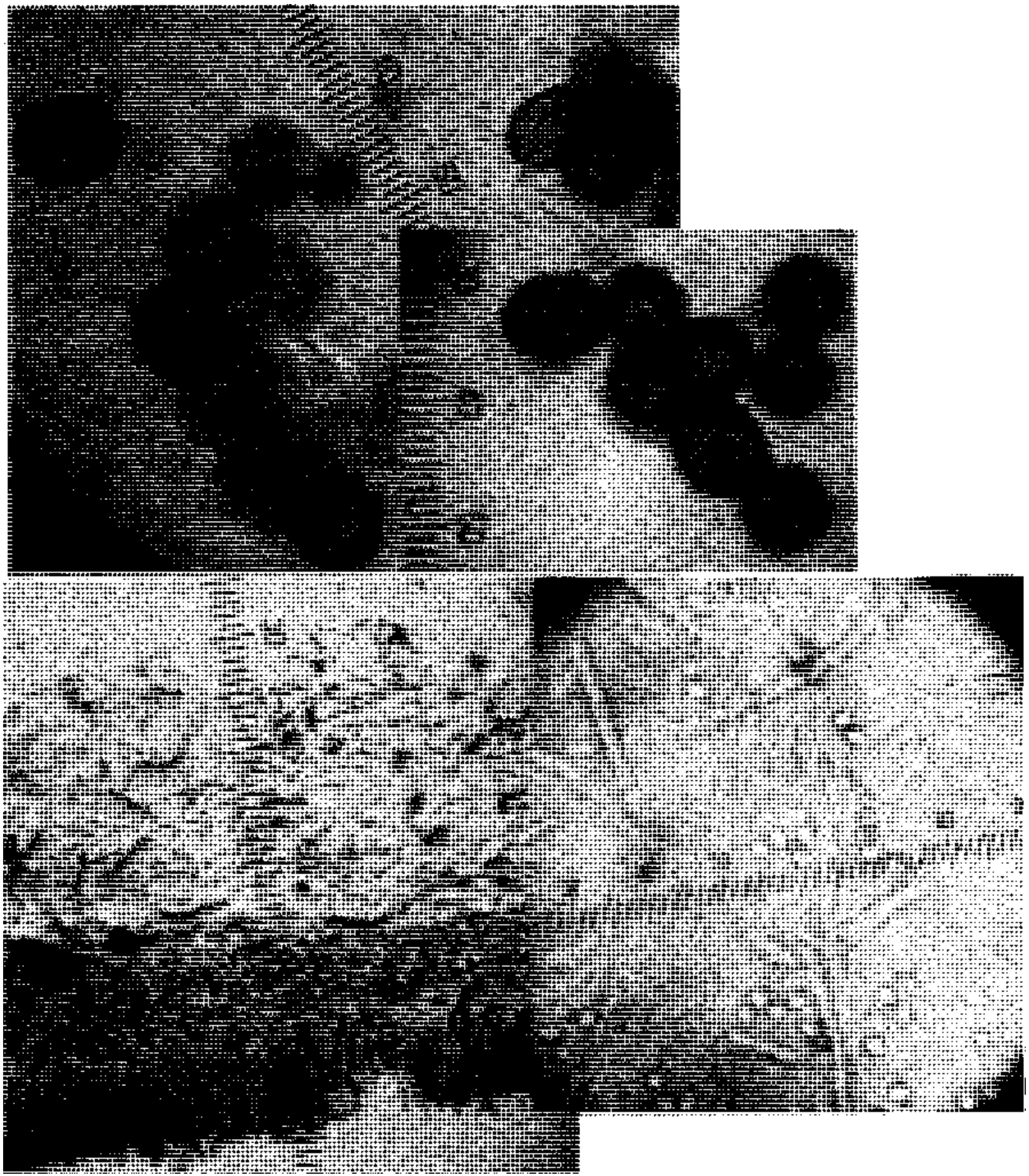


Figure 69 Hymenium and cutis of *Russulafarinipes:* Top, spores with lum division scale; bottom left, section through cutis and trama just beyond the half-radius point, showing some pileocystidia near the surface and many laticifers which appear dark in this view, below the cutis is the trama of sphaerocytes about 60um thick at this point, and immediately below that is the subhymenium and hymenium, with the cystidia appearing dark, 10 scale divisions are lOOum; bottom right, a surface view of the cutis showing the pileocystidia, some spores are also visible, ten divisions on the scale are 25 um. Both mounts were in 5% KOH.

Subsection *Crassotumcatinae* Singer

Russula crassotunicata Singer

Bulletin de la Societe Mycologique de France 54:132. 1938

Cap 3.2-8cm, cream to pale ochre yellow, fairly uniform or slightly more ochraceous over the disc, developing yellow-brown stains where damaged and in age (fig. 71). Immature basidiomata tend to be pulvinate with inrolled margins, this shape is maintained into maturity with the margins becoming incurved rather than inrolled, in age the centre is depressed but the margins mostly remain downcurved to some extent. Margins smooth, not striate until well aged. Stature usually fairly squat and thick-fleshed. This species is notable for its up to 1mm thick, translucent, rubbery cap cutis which peels 1/2 to completely, viscid when wet but more often with a matte, smooth appearence rather like frosted glass, a little pruinose when young or centrally, sometimes areolate in dry weather and at maturity, drying matte. Flesh white, bruising brown, white under the cuticle.

Gills whitish to pale cream, developing dark brown stains in age and where damaged, arched to plane, narrowing at stipe and cap margin, about equal in depth to that of the cap trama at half radius (about 5mm on an average sized cap) adnate to slightly decurrent at the stipe, acute at the margin, edges entire, close to subdistant, frequent lamellulae not regularly distributed.

Stipe 1.9-7.5 x 0.7-2.6cm, cylindrical or clavate, firm, longitudinally rugulose, white but developing dull yellow bruising where handled and cut, particularly the rind, eventually browning, solid when young, developing usually three lenticular cavitities which become irregular and eventually extend to create a hollow stipe in age. The cavitation is intermediate between that of other subgeneus *Ingratae* where generally more than three regularly spaced cavities develop and that of subgenus *Russula* in which usually three rather irregular cavities develop.

Texture firm, slightly elastic and not very brittle.

Taste peppery, the intensity varies between collections.

Odour not distinctive or more often of coconut, sometimes with fruity, yeasty or rubbery components, becoming fishy in age.

Spore colour white, Romagnesi la.

Spores 8.3-11.5 x 6.3-lOum, L:W 1.1-1.56 with a mean of 1.27 (n = 49), subgloboseto broadly ellipsoidal. **Ornamentation** of rounded to bluntly conical or triangular warts, some quite heavy, many tiny ones in between, 0.2 -lum, isolated or less commonly two to three joined, no reticulum, **Woo type** A2, occasionally B2. **Suprahilar patch** inamyloid, unornamented or with a few tiny dots, surrounded by small warts, hiliferous appendix quite large, 1.8-3um long, l.l-1.3um wide at base, in one case 2um. **Basidia** 4 spored, 35-55 x 9-12um, clavate but relatively slender. Sterigmata 5-9um long and 1.7-2.2um wide at the base. **Pleurocystidia** 45-125 x 9-12um protruding 10-35um, originating in the subhymenium, mostly fiisoid, some unevenly cylindrical, tips tapered, often the taper begins in the upper 2/3, sometimes at a slight shoulder, and ends with small appendage, contents yellowish and refractive in KOH, grey-black in SV,. **Cheilocystidia** 75-110x10-13 numerous, protruding around 25-50um and forming a fringe along the gill margins, more or less fiisoid with long tapered tips sometimes ending in a small appendage or button. Both types of hymenial cystidia and the vascular hypahe stain dark purple in SV. **Subhymenium** 25-40 um thick, interwoven but with a few parenchyma shaped cells, gill trama of remarkably even-sized sphaerocytes with occasional vascular hyphae.

Cutis 300-600um thick at half-radius, an ixodermis consisting of a thick subcutis and a relatively thin epicutis which becomes broken up at maturity or dry weather. **Subcutis** of tightly to loosely interwoven gelatinized pale yellow-brown hyphae 2-5 *im* wide, occasional inflated septate hyphae up to 13um wide, often with thickened walls up to 0.8urn in parts, and with a tapering terminal cell, laticiferous hyphae 4 -7um wide with yellowish refractive contents frequent in the basal layers, black in SV. **Epicutis** 60-lOOum thick, of hyphal ends embedded in a fairly tenaceous brownish matrix that adheres the hyphal ends in clumps, sometimes incrusting them, and which renders it very difficult to see them individually. By dampening the cutis of a dried basidioma and scraping the surface with sharp forceps some of the epicutis can be loosened and spread thinly on a slide with 5% KOH for viewing. The inflated-thick-walled hyphal ends make up a large part of the epicutis, the rest being of bluntly terminating vascular hyphae, refractive tapering pileocystidia and the tapering termini of ordinary hyphae. One collection in particular (CR03 0924-06) is somewhat unusual in that the cuticle is thinner than most of the other collections of *R. crassotunicata* at 300-350 and has an overgrowth of narrower mostly thin-walled hyphae 1-2.5 wide forming a pubescent layer over the centre of the cap. **Pileocystidia** 18-75 x 5-9u.m, aseptate, with yellowish refractive SV negative contents, tapering, cylindrical with strangulate ends or short-cylindrical with rounded ends, infrequent and sometimes deeply embedded. **Hypodermis** a few layers of brownish, flattened cells.

Trama of individual and clusters of sphaerocytes bound by a thickish hyphal mesh with numerous vascular hyphae.

Chemical reactions: FeSC>4 -greyish to grey green; KOH -no reaction to slightly yellowish on cap surface, no reaction on stipe; NH3OH -no reaction; Phenol -slowly brownish purple; SV -brownish purple on the gills, grey-brown on the cuticle, cystidia and vascular hyphae purple to dark grey; acid-fuchsin stains vascular hyphae pink and the epicutal compactions blue-grey.

Habitat and tree associations: In coastal forests and coastal mountains with western hemlock, from August through November. In the Clayoquot area it was recorded from forested pockets in the dunes, the spruce fringe, old growth and second growth (circa 50 year old) rain forest. The substrate may be the forest floor or often on accumulations of woody debris in advanced decay stages. It appears to require a moist climate. I have not found it in the coastal Douglas fir zone.

Collections: CR000831-02 and CR030924-06, fruiting in moss over a deep litter of very decayed coarse woody debris in an area with many old stumps under regeneration western hemlock and Sitka spruce, China Beach park, N 48.4373°, W 124.0926°. CR000919-01, in sheltered coastal stand of regeneration western hemlock with red alder, Cape Beale trail, Bamfield, N 48.826517°, W 125.145400°. CR00101 l-07a, near boardwalk with salal under western hemlock in old growth Sitka-spruce-western hemlock-western red cedar sheltered coastal stand on Meares Island, Clayoquot Sound, N 49.1510°, W 125.8600°. CR001012-15, on steep slope with much decayed coarse woody debris under western hemlock and shore pine with salal and huckleberry understory, southwest corner of Kennedy Lake Park just off Grice Bay main logging road, N 49.016883°, W 125.581533°. CR011112-LB.RF. on a decayed log in an old-growth Sitka spruce-western hemlock-western red cedar stand with abundant and diverse understory shrubs along the Central Beach trail at P.R.N.P. south of Torino N 49.053°, W 125.7155°. (All above collections from the southern very wet hypermaritime CWH subzone) One inland collection; CR980818-01, from Elk River Valley, Strathcona Park, amongst western hemlock, Douglas fir and western red cedar, N49.8190^{0}, W 125.8810° (windward moist maritime MH subzone).

Collection	ITS1-F to ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR011112-LB.RF	827	365,257	565,234	300,226,159
CR030924-06	832	374,244	unsuccessful	375,200

Notes: *Russula crassotunicata* can be distinguished from other white-spored, peppery, whitish capped Russulas by its thick peelable elastic cutis, locally often 1mm thick or more when fresh, brown bruising and firm pulvinate cap with margins that rarely become uplifted. A similar species, *Russula compacta* has reticulate spores and a cutis that is thinner and peels only up to 1/2 the radius. *Russula crassotunicata* has been found throughout the Pacific northwestern coastal and coastal mountain forests (Shaffer 1970, Singer 1957, Thiers 1998), and the Vancouver Islands collections closely match these other descriptions of this species, but extend slightly the range of spore widths (from 7.0-9.3 um in Shaffer 1970). Singer mentions that the distribution of/?, *crassotunicata* is predominantly along the west coast, but extends through the boreal forests as far as the Great Lakes region. While Singers' 1957 redescription is thorough, Shaffers' (1970) adds further detail and excellent drawings of the spores, hymenial cystidia and cutis hyphal ends and should be referred to for more information. The epicutis has been described by Shaffer as lacking a gelatinous matrix but being compacted into yellowish brown masses, or by Singer as incrusted with a brownish (in KOH) substance. Whatever the substance, it occasionally leaves an acid and alkali resistant residue on the hyphal ends that appears as an incrustation where the compaction is broken apart.

This species bridges subgenus *Ingratula* of clade 3 and subgenus *Russula* of clade 5. It has the strong browning reaction, lenticular cavities in the stipe, tapered pileocystidia and inamyloid suprahilar patch on the spores of the *Ingratula*, with the separable cutis, coconut odour, incrustations staining in acid fuchsin, smooth cap margins and just three cavities in the stipe, all of which are more characteristic of subgenus *Russula*. Singer placed it in subsection *Crassotunicatinae* within what is now the subgenus *Ingratula*, and it would be appropriate to raise it to the level of section.

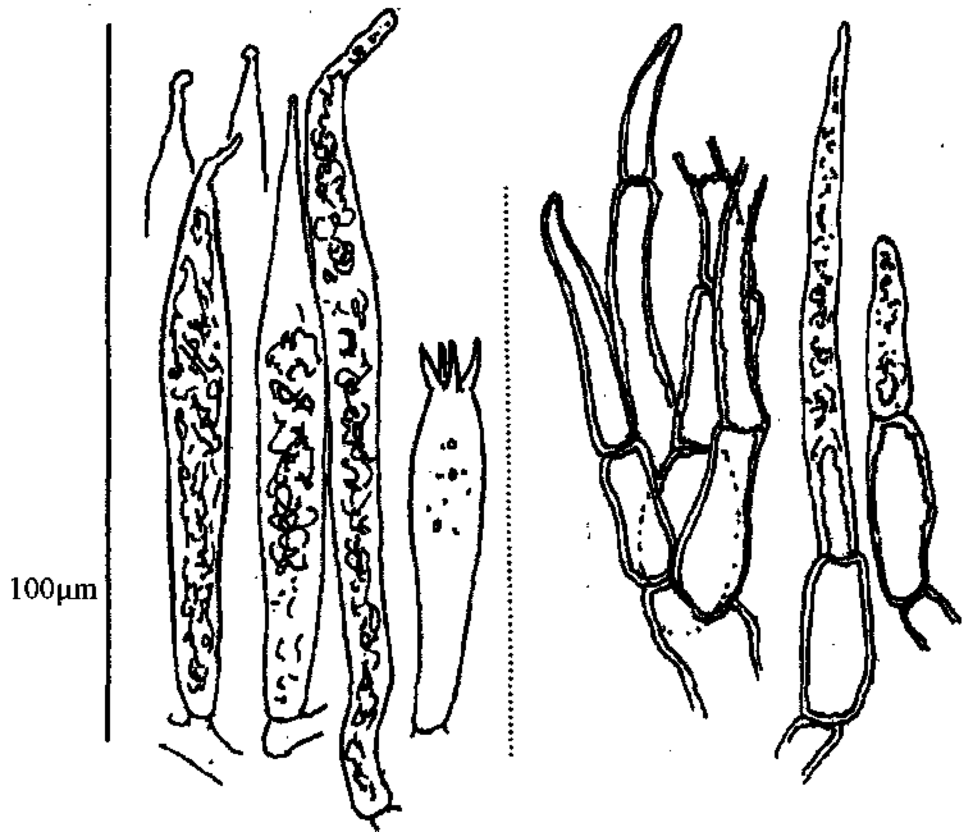


Figure 70 Microscopic characters *oiRussula crassotunicata:* Top, spores with 10 um scale bar; bottom left, hymenial cystidia and basidia; bottom right, inflated thick-walled hyphal ends and pileocystidia from epicutis.

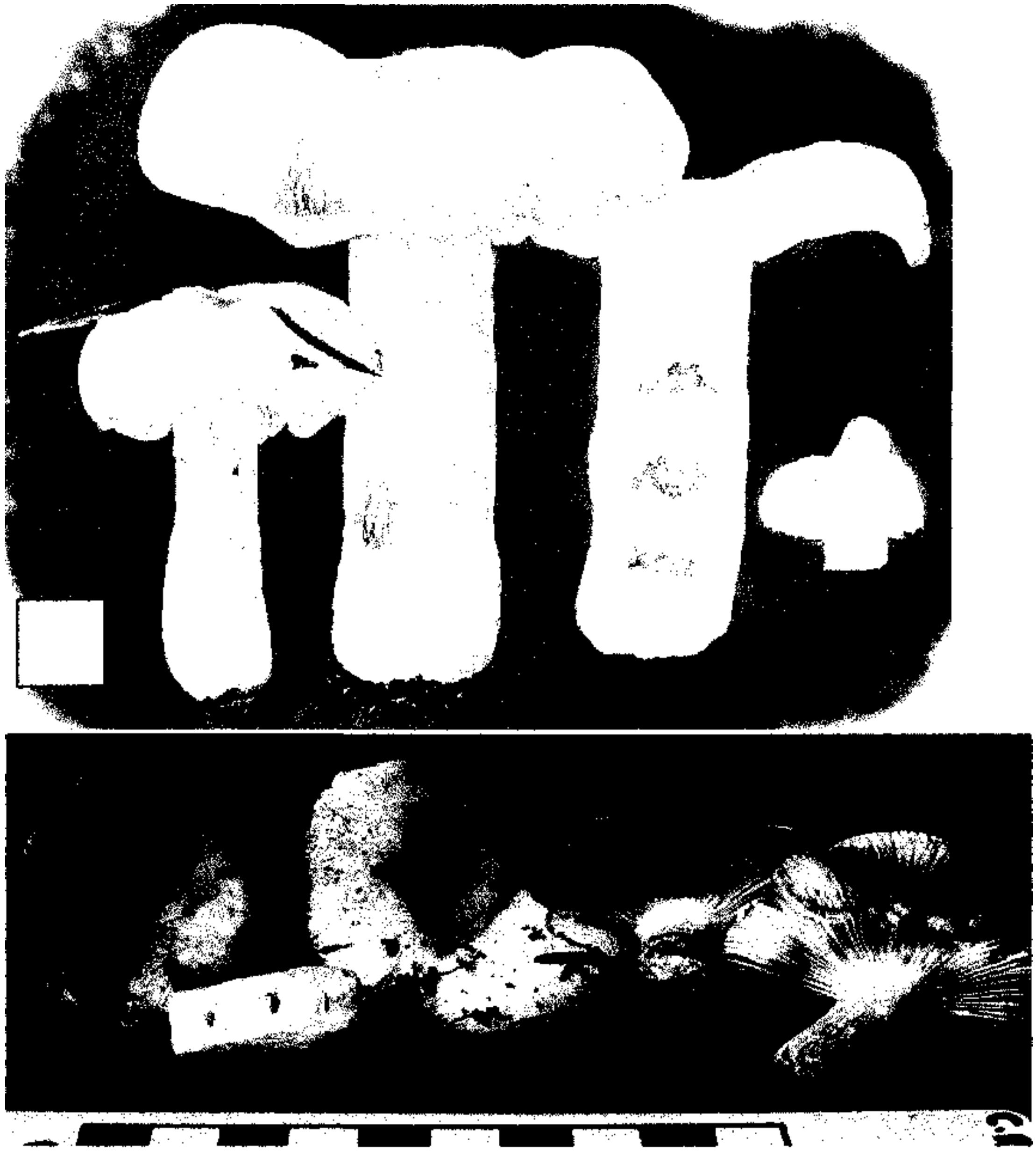


Figure 71 Macroscopic characters of *Russula crassotunicata:* Top, illustration showing profiles of irnmature, mature and a section through basidiomata, the square is 1cm^2 and shows spore colour; bottom, a collection from the P.R.N.P. near Torino, of mature basidiomata showing a considerable amount of bruising on the gills, cap and stipe, the three lenticular cavities within the cut stipe trama, and the initial yellowing that occurs where cut. The black and white scale in the foreground is in 1cm divisions.

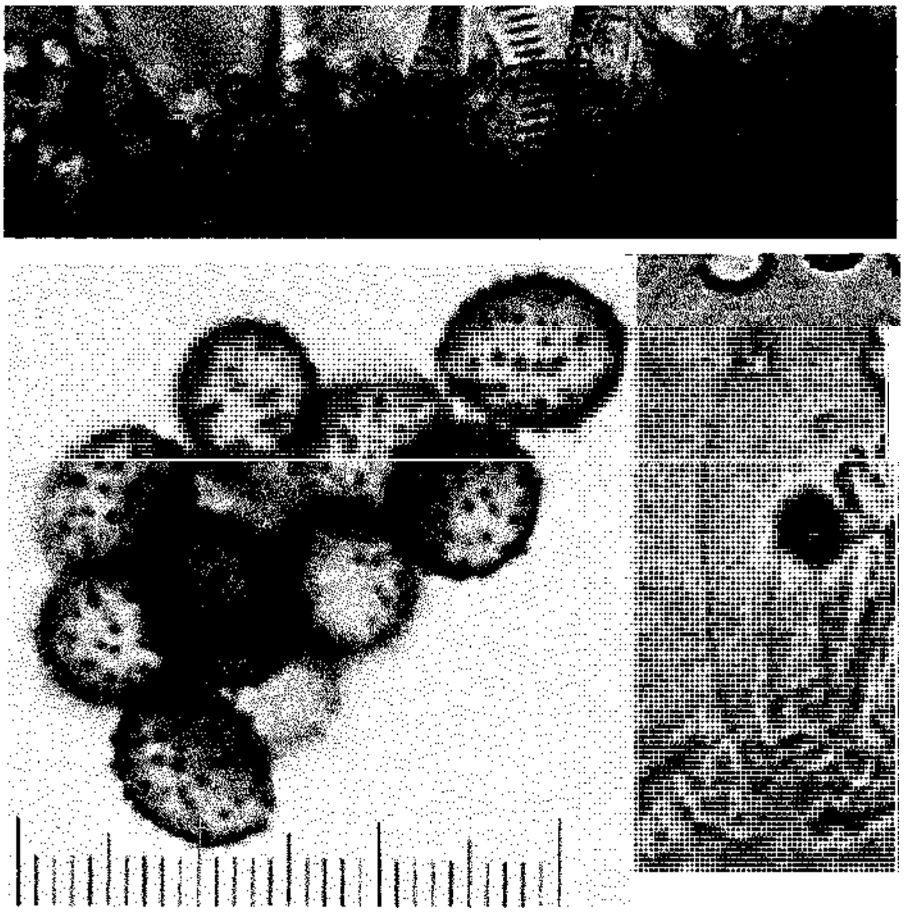


Figure 72 Hymenium of *Russula crassotunicata:* Top; gill margins showing cheilocystidia and basidia; below left, spores with 1 um division scale, composite photograph of two depths of focus; right, basidia and basidioles, 10 divisions on scale are 25 um.

Figure 73 Cutis of *Russula crassotunicata:* Top left, section through cutis showing the thick interwoven subcutis and the epicutal hyphae embedded in a dark matrix, 10 scale divisions are 100pm; top right, pileocystidia; lower two photographs show thick-walled inflated hyphal ends found in the upper subcutis and epicutis, note that two of the hyphal ends in the bottom left photograph appear incrusted with a yellowish substance that adheres the epicutal hyphal ends in clumps, 10 scale divisions are 25 um for the latter three photographs.

Clade 4a

Subgenus *Amoenula* Sarnari

Russula smithii Singer

Bulletin de la Societe Mycologique de France 54:140. 1938

Not yet found on Vancouver Island, but potentially it could exist here since its host tree is Douglas fir. Description excerpted and interpreted from Singer's original Latin description and Grund's 1965 translation of it, supplemented by photomicrographic illustrations and additional information from examination of the holotype. Examined: one basidioma, small section of cutis from about half radius, one piece of gill tissue, small piece of cutis of one of the broken off pieces, which was from near the cap margin.

Cap 8-15cm diameter, convex at first, later depressed, dark red-brown to purple-brown to very dark brown centrally, pea green to Lincoln green (a deep clear leaf green without a blue or yellow bias). Singer described the centre as reticulate-venose, it is not entirely clear if this referred to texture or pigment, but on examining the holotype the cutis over the disc was wrinkled radially, with the wrinkles interweaving to form a reticulation, and Singer noted that this is under the gluten. Surface viscid becoming sub-viscid and sub-smooth, pruinose when dry (the dried specimen appears minutely velutinous) margin smooth, not peelable easily. **Trama** white becoming pale yellowish, greenish-brown under the cutis at the margin. Singer noted that the dried pilei were irregularly applanate with a central depression, blackish brown centrally merging through olivaceous to green at the margin. (Even after 45 years the greenish tint remains apparent, though neither bright nor strong and a little brownish.)

Gills cream becoming tinged yellow-green, close, narrowly adnexed at stipe, rounded at cap margin, strongly anastomosing, subochraceous in dried basidiomata and only about 4mm deep.

Stipe 6-8 x 2-4.5cm, firm, hard, cylindrical or broadening slightly at the base, solid, surface pruinose-fibrillose at first, white but with a rose-pink flush at the apex and over most of the surface.

Texture firm to hard, not brittle. In the herbarium specimen, very hard.

Taste mild.

Odour not recorded.

Spore colour white, presumably Romagnesi la or close.

Spores 8-11.5 x 7.5-10um, subglobose to short-ellipsoidal, ornamentation of rounded to peg-like warts, some incompletely amyloid, mostly isolated, occasionally two or three joined with lines, not forming a reticulum, small dots interspersed, warts 6-1.2pm but mostly 0.9-1.2pm, **Woo types** 2A, 3 A, 2B and 3B. (Singer described the spores as type VI, VIII, seldom IV, and rarely Illb in his own key). **Suprahilar patch** lightly amyloid, irregular in outline and bordered by small warts. **Subhymenium** well developed, 40-60um thick, interwoven, gill trama of sphaerocytes. **Basidia** 2- or 4-spored, clavate, 37-55 x 10-15p.m. Ripe basidia and cystidia protruding well beyond basidioles, to 1/3-1/2 their length. **Pleurocystidia** and cheilocystidia numerous, thin-walled, empty or with refractive contents towards the tip in 3% KOH, 62-85um long, width very variable, 5-17um (Singer's measurements were 5.8-13.0pm). Shapes range from irregular to cylindrical to clavate to fusoid, often with constricted sections. Tips are rounded, broadly acute, elongated or capitate. Singer noted that the contents were granular towards the tip and that occasional ones were encrusted. Cystidioid hairs from the gill edge are an unusual character in *Russula.* They are not frequent, but are distinctive, and sometimes have refractive contents in the terminal cell. They are narrowly clavate, 4-8-septate, sometimes constricted at the septa, sometimes with a side appendage or outgrowth, and around 70-90um long and up to 11pm wide. Singer described the gill margins as heteromorphic, with cylindrical to acute-fusoid, frequently septate, cystidioid hairs 37-68 x 8-9um, and with variously shaped septate hyphae with mostly acute tips, 3-4u.m (-8um) wide and sometimes bifurcate or laterally appendiculate.

Cutis interwoven, with no clear division between the subcutis and the tramal tissues, approximately 300-350um thick at half-radius. **Epicutis** not really distinct from the subcutis, of free hyphal ends emanating from an interwoven layer, not forming a regular turf, just irregular free articulated hairs, constricted at the septa, upright to semi-repent, 90-110 x 2.5-9um, the terminal cell frequently acuminate (narrowing to a point), basal inflated cells not seen. No refractive contents in any elements, no vascular hyphae seen, no granular cytoplasmic pigments, and no acid-resistant incrustations seen.

Trama of discrete clusters of sphaerocytes bound by a hyphal mesh.

Chemical reactions: KOH - transitorily pale reddish-brown on cap surface; SV - on gills, red with no darkening at all of the hymenial cystidia beyond the overall red, on cutis brown, no pileocystidia seen, no elements staining positively (grey, black or blue).

Habitat and tree associations: In the Olympic mountains under *Pseudotsuga menziesii* (Douglas fir). (In Singer's description it is listed as under *Pseudotsuga taxifolia,* an older name for *Pseudotsuga menziesii.)*

Collection examined: Smith A. H. 2466, holotype, collected 14th September 1935, from Boulder Creek, Olympic Mountains, Olympic National Forest, Clallam County, Washington State.

Notes: *Russula smithii* is known only from the single type collection and is therefore very rare. *Russula smithii* bears some similarities to *R. brunneola,* namely, the spore colour, the cap colour and texture and the close taxonomic position. The green tints on some collections of *R. brunneola* were initially misleading in making an identification since that colour is not mentioned in the descriptions examined (and discussed under that species' description), and they were considered to potentially be *R. smithii.* Once the holotype of *R. smithii* was examined, it differed in the spore ornamentation, which consists of isolated warts and a lightly amyloid suprahilar patch (unusual in this taxonomic group), the presence of septate cystidioid hairs on the gill margin and in its lack of pileocystidia. I have personally not examined other members of subgenus *Amoenula* so can not adequately judge the taxonomic position Singer applied to *R. smithii*, however, the spores have a size, shape, ornamentation and amyloid suprahilar patch very like those of clades 8-10, and the relatively broad basidia are more in keeping with those clades. The septate cystidiod hairs of the gill margin are also seen in clade 9 (see *R brunneoviolacea),* as are the hymenial cystidia in having refractive contents mostly at the tips. The firm trama is also found in several species in clade 8, for example *R, Integra,* and the epicutal hyphae of/?, *smithii* show similarities in septation and shape as those in clade 8, some species of which have few to no pileocystidia.

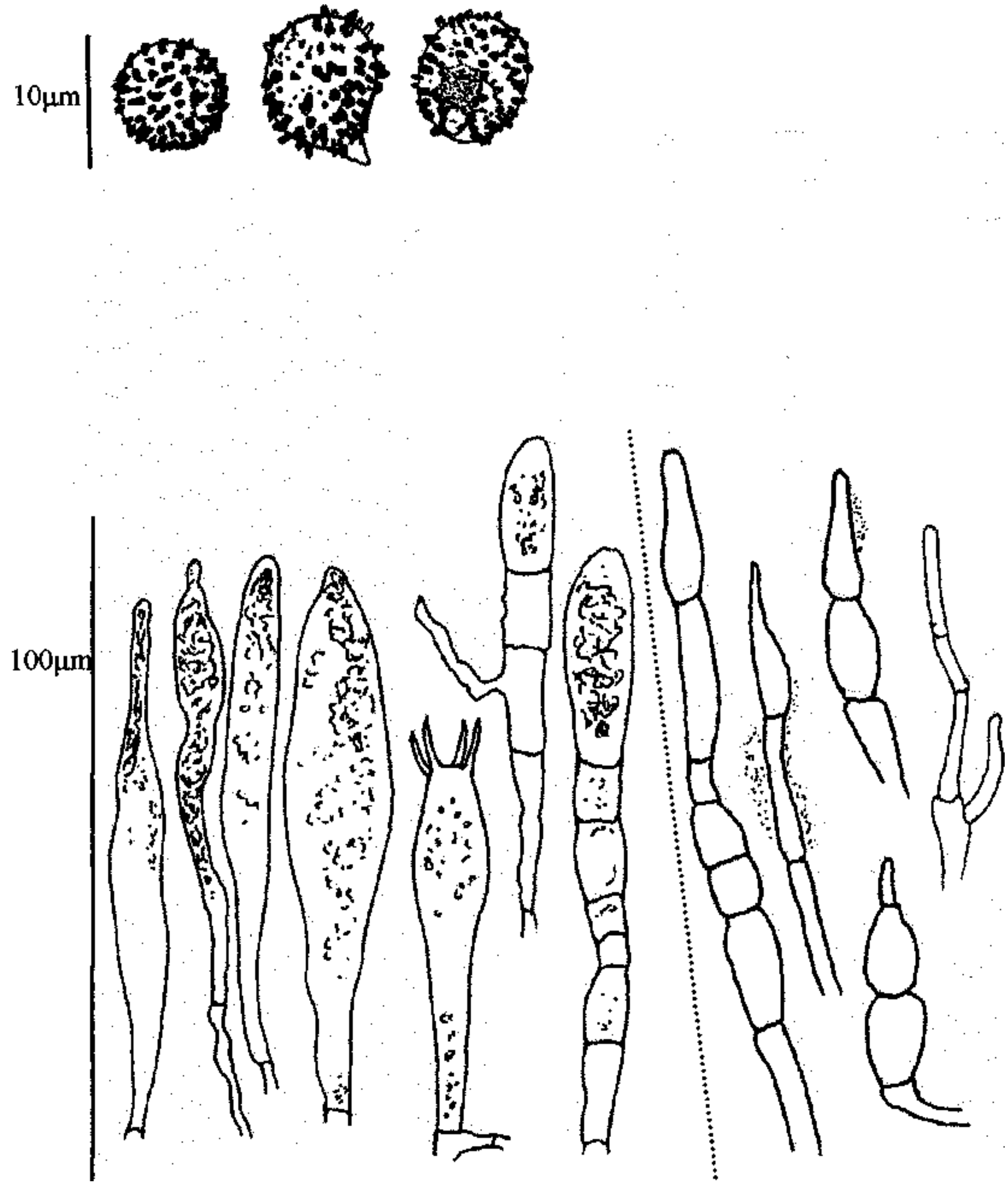


Figure 74 Microscopic characters *ofRussula smithii* holotype 2466: Top, spores with lOum scale bar, bottom left, hymenial cystidia, basidia, and two septate cystidioid hairs from the gill margin, marked 'c h'; bottom right, hyphal ends from the epicutis, lower scale bar is lOOum.

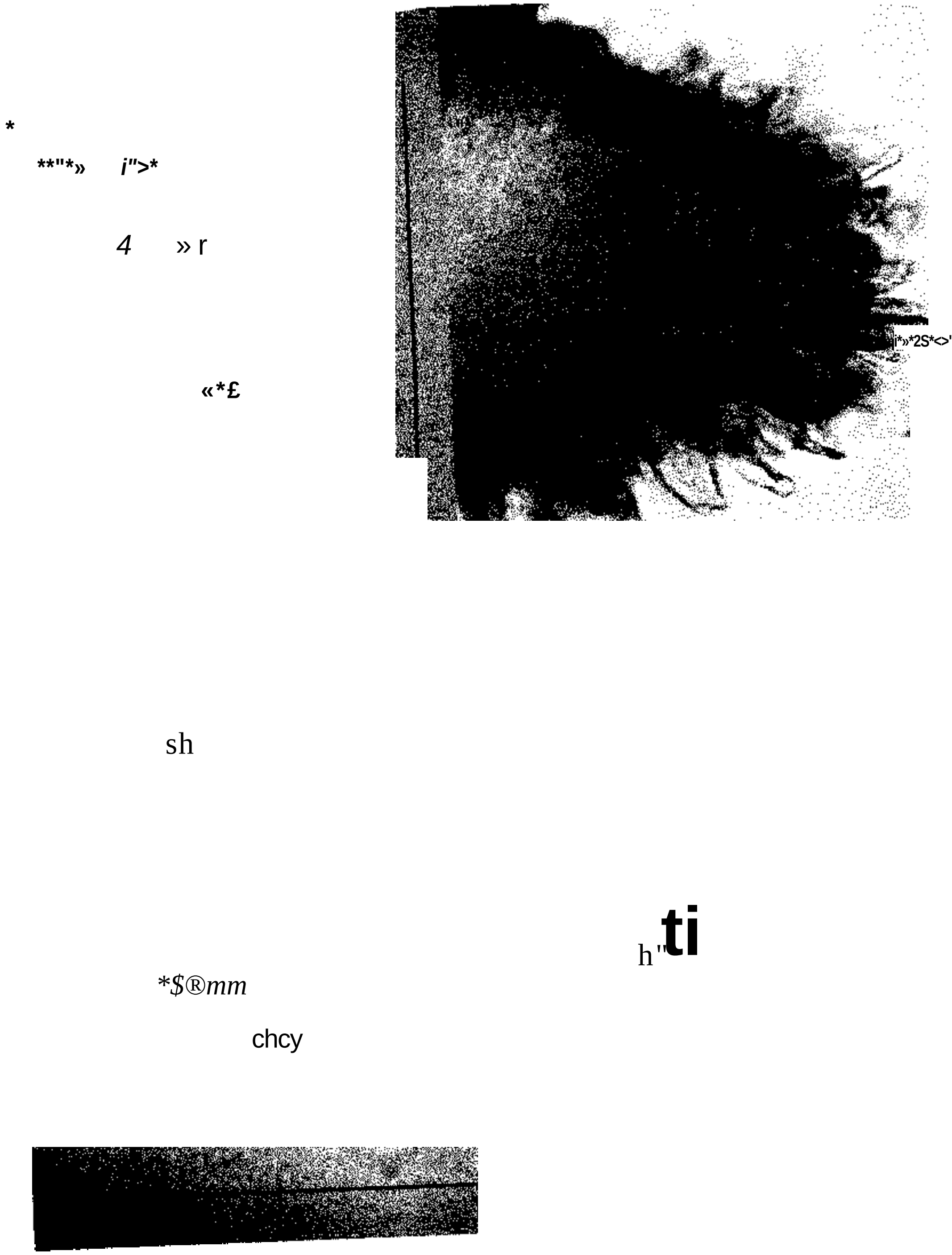


Figure 75 Hymenium of *Russula smithii:* Top left, section through gill showing pleurocystidia protruding above the basidioles; top right, section through gill margin showing cheilocystidia, cystidioid hairs are not obvious except in crush mounts as in the lower photograph, this shows a septate acute-tipped hyphae (s h), a cheilocystidia (chcy) with its base to the right, a laterally appendiculate cystidioid hair (c h) with its base to the top, and a basidium (b). All scale bars are 10 µm, the gill margin and crush mount were stained in Congo red.

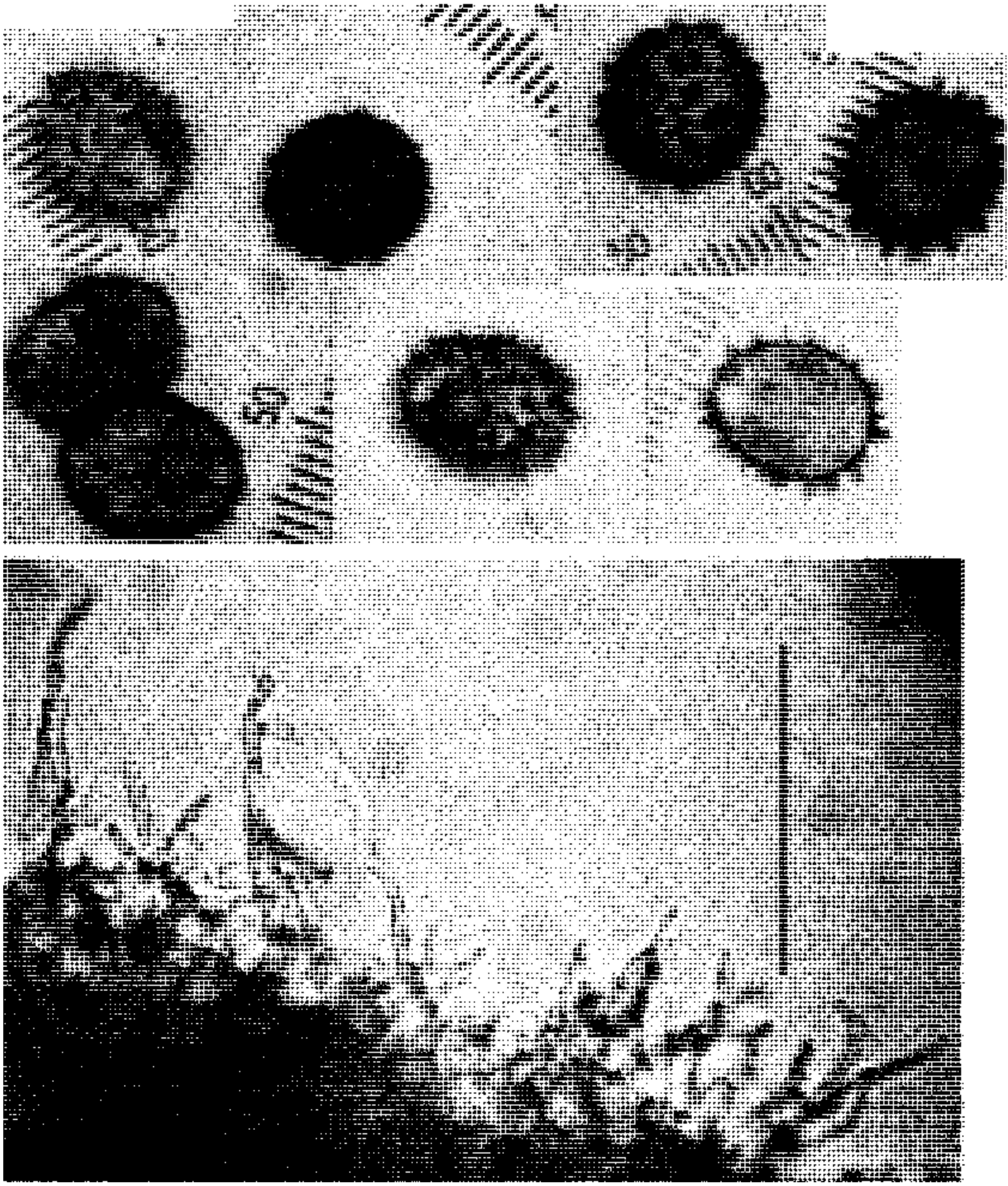


Figure 76 Spores and epicutis of*Russula smithii:* Top: spores the middle two of the top row are composites of two depths of focus, the middle and right of the bottom row are the same spore in surface and equatorial view, note the lightly amyloid suprahilar patch and the variation in wart height between individual spores, scales are in 1um divisions; bottom, epicutal hyphal ends *ofR smithii,* scale bar is lOOum.

Clade 4b

Subgenus *Heterophyllidia* Romagn. emend. Sarnari

Section *Heterophyllae* Fries

Subsection *Heterophyllae* (Fries) Schaeffer

***Russula mustelina* Fries**

Epicrisis Systematis Mycologici: 3 51. 1838

Cap 9.8cm diameter, at maturity, plano-convex with a slight depression over the disc, mid to light yellowish-brown, slightly darker in the centre, slightly more pinkish-brown at the margin, glabrous, with a fine darker appressed, radially fibrillose texture, margins smooth, viscid drying almost matte. Flesh white, tinted pale vinaceous under the cuticle, unchanging, very firm, peelable to 1/3 the radius.

Gills deep cream with a pale orange cast when seen edge-on, subdistant, with occasional lamellulae, forking near the stipe and near the cap margin, and most gills anastomosed with their neighbour at the outer end. Gills are adnexed-notched at the stipe, falcate, the broader part closer to the stipe than the margin, and narrowly rounded at the cap margin with the cap cutis reaching about half-way around the end, leaving the lower part of the gill ends visible (fig. 78).

Stipe 6.1 x 1.4cm, ventricose, pale cream, white at the apex, firm, solid or at least stuffed with a firm trama, longitudinally finely rugulose, pruinose at the apex, unchanging to slightly yellowing where cut, becoming pale yellowish brown where handled and at the base.

Texture very firm and not brittle except for the gills.

Taste mild.

Odour not distinctive, but with a strong, unpleasant rotting meat smell after refrigeration for two days.

Spore colour light cream, Romagnesi Ila-b.

Spores 7.2-9.8 x 5-7.8um, L.W 1.06-1.5 with a mean of 1.29, ornamentation of low blunt crests or rows of roughly hemispherical warts 0.2-0.5um high, joined by fine connectives forming a partial reticulum, sometimes incompletely amyloid, occasional isolated warts, Woo type 1C. **Suprahilar** patch inamyloid, sometimes with fine warts, hiliferous appendix short, around l-1.5um long, 1.2um wide at base. Basidia 4-spored, 50-62 x 8-10um, narrowly clavate or widest in the upper 1/3 and then also with a long, narrow base, generally slender. Sterigmata 3-5um long and 1.5um wide at the base. **Pleurocystidia** 62-130 x 8-12um protruding up to 40um, originating in the subhymenium, contents refractive in KOH, grey in SV, but sometimes only in the upper half, overall mostly fusoid or clavate, sometimes contorted at the base, sometimes broadening gradually from the base to a shoulder, many with a capitum of varying size up to the full width of the cystidia, mucronate or with a long appendage with a series of constrictions, sometimes with the appendage emerging from a capitum giving it the shape of a minaret. **Cheilocystidia** very sparse, protruding around 15-20um, similar to pleurocystidia but the irregularly distributed refractive material only light purplish grey in SV. **Subhymenium** 70-100um thick, interwoven with occasional subspherical cells, dissolving into an amorphous material in 5% KOH, possibly gelatinizing, gill trama relatively narrow, of sphaerocytes with occasional refractive vascular hyphae.

Cutis 230-320um thick at half-radius, 400um on disc. **Subcutis** of interwoven hyphae 2.5-5um wide, brownish in the more compressed lower layers, hyaline centrally, and light brown towards the epicutis, infrequent vascular hyphae 3-7.5um wide, unstaining in SV. **Epicutis** not well differentiated from the subcutis, an interwoven layer of light brownish free hyphal ends that are repent or, more often, with the free part curving back down towards the surface, or upright and clustered, the hyphae mostly 2-4 um wide, with light greenish to brownish cytoplasm, ends undifferentiated or more or less tapered. Very few inflated or articulate elements in this collection. **Pseudocystidia** occasional, originating as vascular hyphae and rising to just below the epicutis or through it, 2-5 p.m wide. **Pileocystidia** frequent, 27-60 x 3-5um, aseptate, mostly cylindrical or slightly tapering to an obtuse or capitate end, less often fusoid or narrowly clavate, often recurved like the hyphal ends, contents slightly refractive, not staining in SV. **Hypodermis** not distinct from the subcutis, merely the basal layers of the interwoven subcutis that are more compressed and lie directly on normal tramal tissues.

Trama of discrete clusters of sphaerocytes bound by a dense hyphal mesh and vascular hyphae, which are mostly in the trama beneath the cutis.

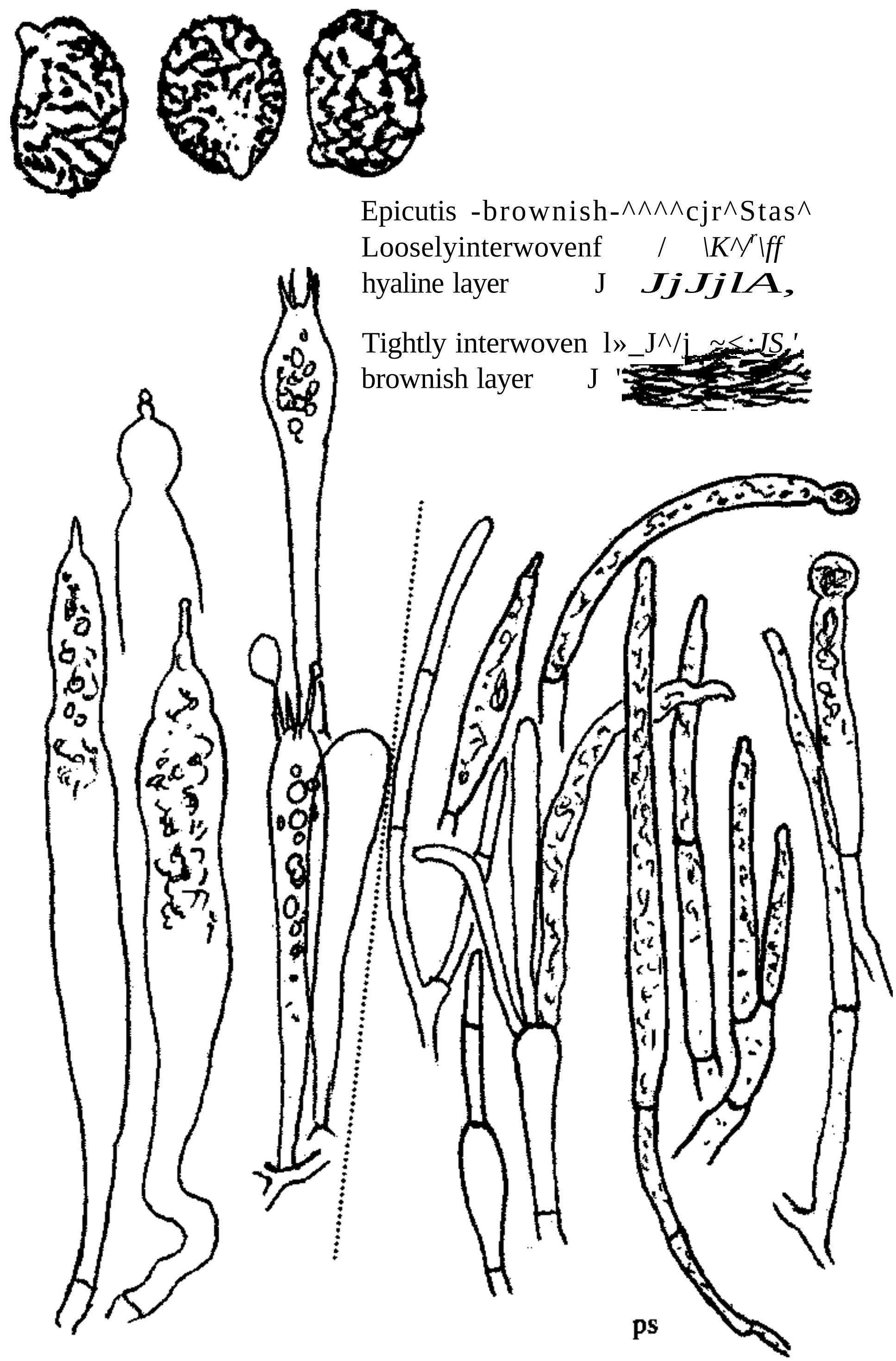
Chemical reactions: FeS04 - brownish salmon pink; KOH - no reaction on cap, slightly yellowish on stipe; phenol - dark brown; SV - pinkish on the gills and cap trama when fresh, brownish on dried tissue, brownish pink on the cutis, cystidia and vascular hyphae colourless to pale pinkish grey.

Habitat and tree associations: In moss *(Eurhynchium oreganum)* on the ground in dry regeneration Douglas fir forest with understory salal *{Gaultheria shallori),* dwarf rose *{Rosa gymnocarpa),* ocean spray *{Holodiscus discolor)* and orange honeysuckle *(Lonicera hispiduld).*

Collections: OC 020717-01, of one basidioma, from dry Douglas fir forest, Highlands area, north of Matson Lake, Victoria, approximately N 48.4896°, W 123.5137° (CDF zone).

	ITSl-Fto	RFLP:		
Collection	ITS4-B	Hinfl	Alul	Sau3A
OC020717-01	863	358, 272	471,236	320,260, 180
OC020717-01 (repeat)	867	364,260	515,265	340,260,170

Notes: This collection of*Russula mustelina* constitutes a first record of this species for Vancouver Island. It matches the description given by Romagnesi (1967) and Sarnari (1998) for European material very closely, the hymenial cystidia range from about 20um shorter but are otherwise as described. Grand (1965) collected and described a *Russula* he identified as *R. mustelina* that was a chocolate brown colour centrally, had smaller basidia, pileocystidia not differentiated, hymenial cystidia with obtuse tips and smaller less well defined spore ornamentation. From his description I surmise that his collection was probably an immature basidioma rather than a local variation lOum



lOOum

Figure 77. Microscopic characters of *Russula mustelina:* Top, spores with lOum scale bar; middle, diagram of section through cutis; bottom left, hymenial cystidia, basidia and basidiole; bottom right, hyphal ends, pileocystidia and a pseudocystidia (ps); bottom scale bar is lOOum.

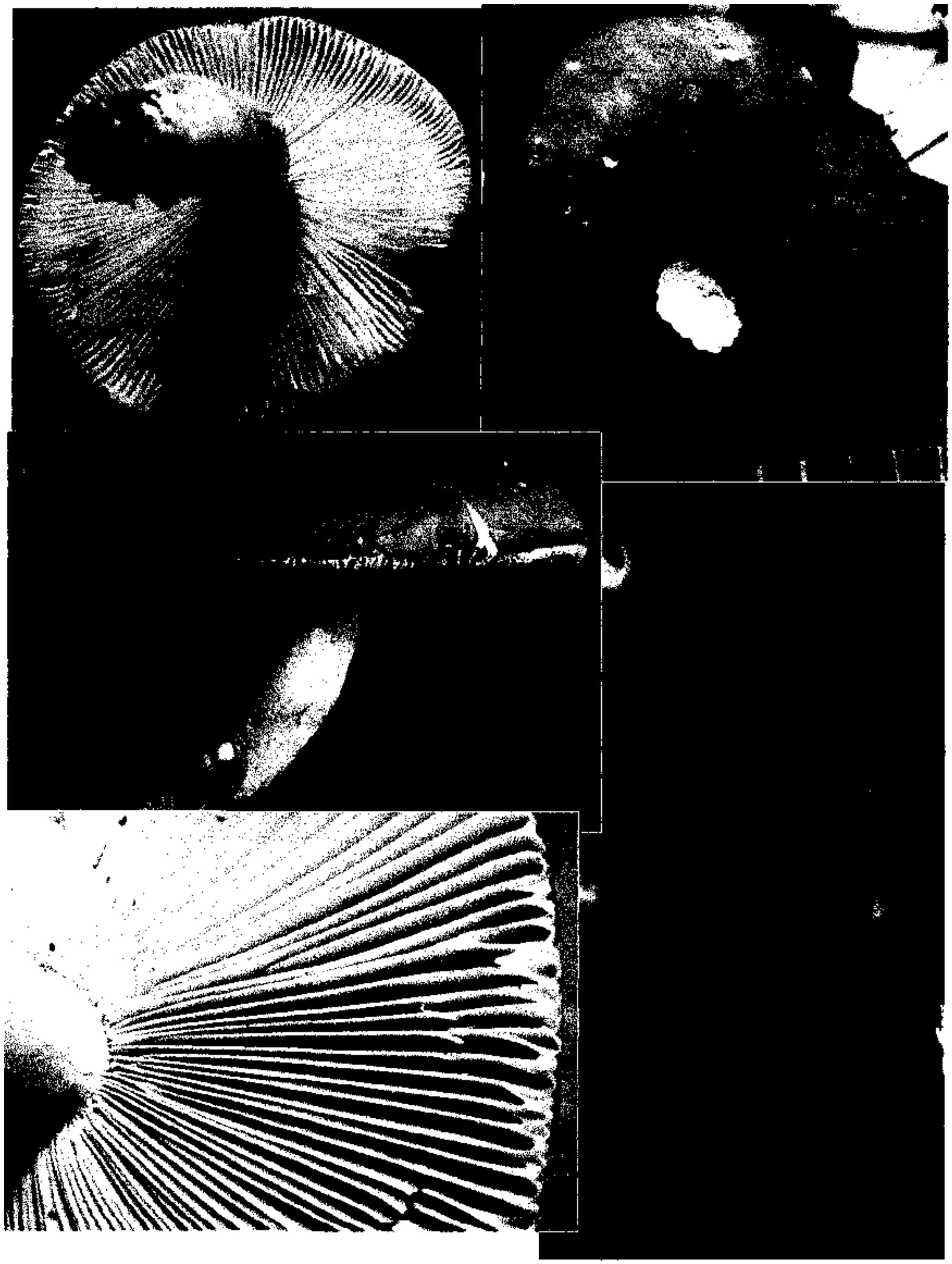


Figure 78. Macroscopic characters *ofRussula mustelina:* Top and middle, gills, cap surface and profile of one basidioma; below left, detail of the gills in which a few lamellulae are visible and the anastamoses at the cap margin; below right, close up of the cap cutis showing the minute darker fibrillose-like surface texture. All photographs are of a 9.8cm diameter basidioma.

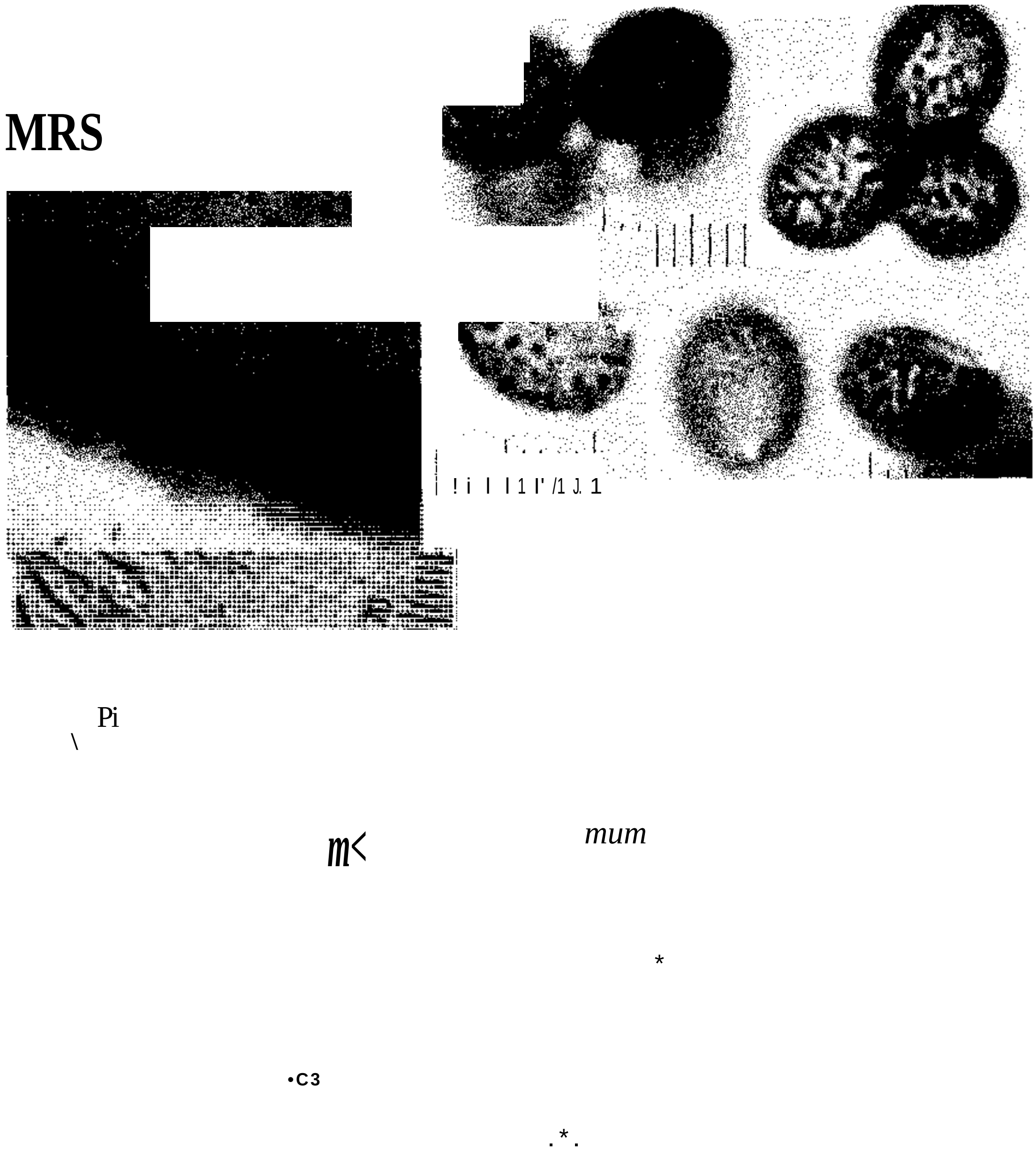


Figure 79 Hymenium of *Russula mustelina:* Top left, face of gills showing the sparse hymenial cystidia (dark spots, stained with SV), scale bar is 100um; top right, spores, scale is in 1um division and applies to all spores shown; bottom, crush mount of gill tissue in which two pleurocystidia (pi), several basidia and basidioles can be seen, ten scale divisions are 25um.

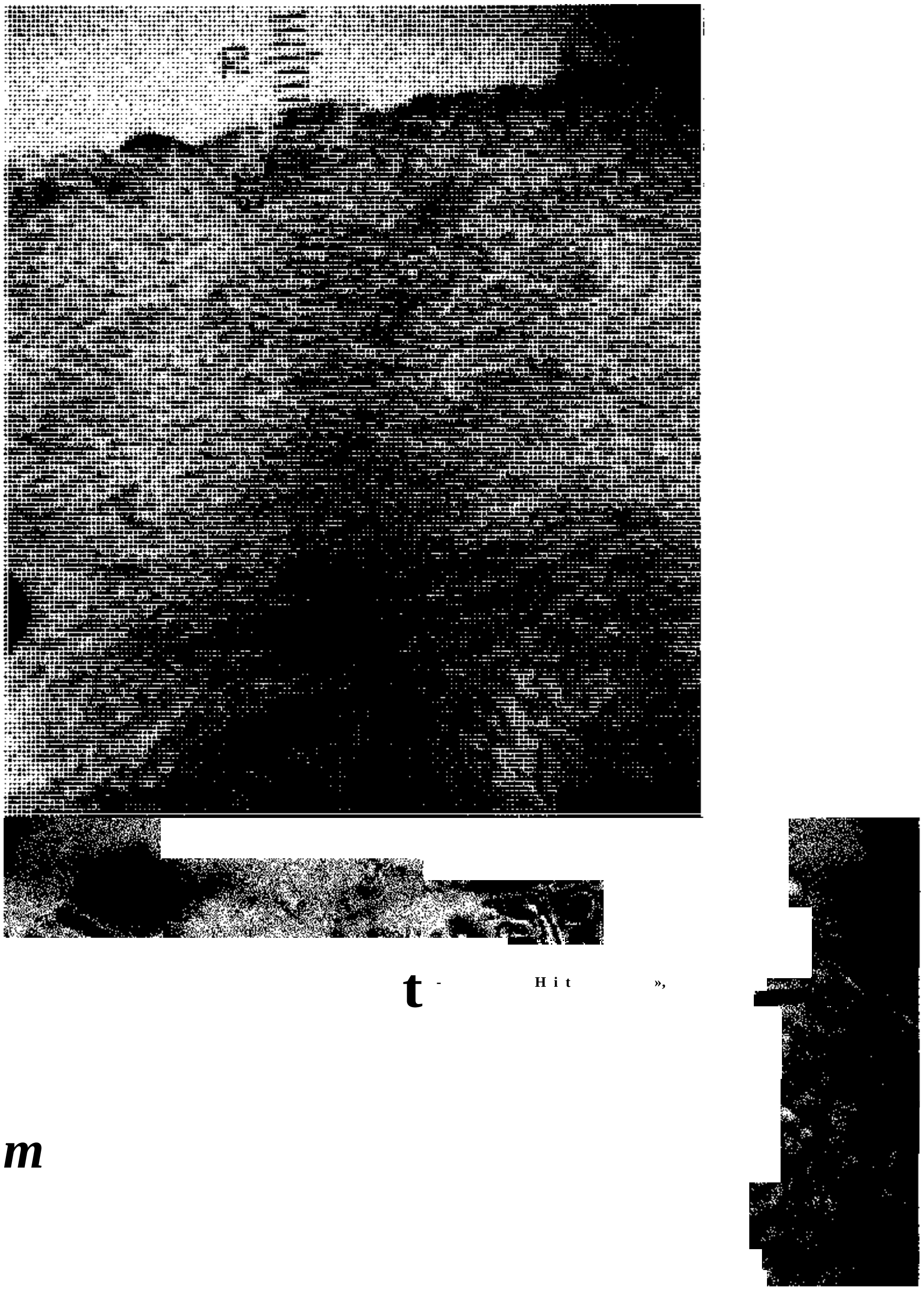


Figure 80 Cutis *otRussula mustelina:* Top, section through the cutis near the disc, the subcutis- trama border is at about the level of the 24 mark on the scale, 10 scale divisions arelOOum; bottom, the epicutis showing a tuft of upright hyphal ends beneath the viscid pellicle, the surface of which is marked with an arrow, the divisions on the scale are 25um.

***Russula brunneola* Burlingham**

North American Flora 9:233. 1915

Cap 5.2-15cm diameter, convex when young, becoming plane to shallowly cyathiform when fully expanded, margin smooth, becoming a little striate in age, sometimes with a narrow free margin. Colour variable but some shade of brown, when very young a light to dark yellow-brown to red-brown, the pigmentation becoming darker as it matures, becoming a velvety vinaceous brown to dark greenish-brown to almost black centrally and deep yellow-brown to olive-brown to olive at the margin; some basidiomata lack any greenish tints. As the cap ages, the colours lighten again, the surface becomes glabrous and the olive tints may be more apparent; over the disc the cap may remain darker or develop paler reddish brown areas. Overall, the redder tints tend to be centrally located while the greenish tints are toward the margin. Surface viscid, drying matte or with a low sheen, minutely radially rugulose, usually with a velvety bloom which is lost in age, the rugulosity becoming more apparent then and seeming to be a reliable character in this variable species. Cuticle peelable 1⁄4 to 1⁄3 the radius, flesh greenish or brownish under cuticle near disc, unchanging when cut but sometimes yellowish to pale brownish around insect larval channels.

Gills pale cream, browning slightly to strongly where damaged, close to subdistant, pliable at first but soon brittle, forking mostly near the stipe, sometimes with a few lamellulae, adnexed to adnate at stipe, arched and equal at first and subacute at cap margin, becoming more or less ventricose and rounded at the cap margin. Gill depth is approximately equal to double that of the cap trama at half radius, for instance in a mature cap of 12.5cm the gills are 10mm.

Stipe 3.6-9.0cm x 1.1-3.8cm wide, most are more or less cylindrical with a slight broadening near the base, surface longitudinally slightly rugulose, whitish, or more commonly with a grey, violet-grey, brownish-grey or, in those with green caps, grey-green to green-grey bloom, sometimes with a pink flush at the base or apex, unchanging where cut but the surface browning slightly where handled and at the base, solid at first, then rind around 3-4mm thick and inner stuffed with a firm bread textured trama eventually developing generally 3 irregular cavities.

Texture Moderately firm and not brittle but becoming so in age, thick-fleshed.

Taste mild, sometimes nutty.

Odour not distinctive.

Spore colour pale cream, lighter than Romagnesi lb, or on the green forms, pure white, Romagnesi la.

Spores 6.0-10um (-Hum) x 5.25-8um, L:W = 1.0-1.45, mean 1.22 (n = 90), variable in shape, size and ornamentation, with no distinct range related to population, yet the average L:W ratio is remarkably constant (fig. 81). Shape subglobose, bean-shaped, broadly ellipsoidal, ellipsoidal, or elongate. **Ornamention** of warts mostly 0.1-0.3um high, most under 0.5u,m high but occasional ones to 8um high, hemispherical to bluntly conical or low short ridges, isolated, 2-3 catenate, or with fine connectives between two or three warts but sometimes forming a partial reticulum. **Woo types** A-Cl, **A2,** or **B2.** **Suprahilar patch** inamyloid, a relatively large area free of warts or with only minute punctae, occasional spores with a lightly amyloid area. **Hiliferous appendix** 1-1.8um long by 1-1.8 um wide at the base, often quite short and stubby. **Basidia** 4-spored, rarely 2-spored, approximately 42-95um long by 6-10um wide, almost cylindrical to narrowly clavate. **Cheilocystidia** abundant, similar to pleurocystidia but less reactive to SV. **Pleurocystidia,** normally to sparsely distributed, 68-92 (-115) x 8-12um (-14)um wide, protruding 20-3 Oum beyond basidioles or sometimes embedded up to the tip, the lower 1/3 not staining, the upper staining red to purple to grey in sulphovanillin but unevenly as if partly devoid of contents, (younger caps with more grey staining, older more red or pink). The shape very variable, more or less cylindrical to fusoid to clavate with the terminus expanded-obtuse, narrowed-obtuse, pointed or with strangulate-elongate or capitate appendages, originating within the subhymenium or trama, occasionally almost from the middle of the gill. **Subhymenium** about 20-40um, interwoven of hyphae of variable width, often with pseudoparenchymatous cells, the cells being 5-10um diameter. **Gill trama** of sphaerocytes, some of which are quite large, reaching 42um diameter, with a few hyphae interspersed. Microscope slides of gill tissue from some dried basidiomata contain many oil droplets, and when slicing the tissues, an oily substance is exuded with pressure from the razor blade.

Cutis 150-400 thick at half-radius, 220-500um thick over the disc, (young caps with a thicker cutis than aged ones), the upper layers with a greenish granular pigment which dissolves in KOH, and a red amorphous cytoplasmic pigment, also some of the enlarged surface cells have a brownish cytoplasm. **Subcutis** of repent, interwoven hyphae 2 -3 urn wide, embedded in a gelatinous matrix, the lower part, approximately half the depth, more gelatinous and very densely interwoven, the upper half looser, vascular hyphae rare. No distinct hypodermium, the basal layers of the subcutis may incorporate openings or sphaerocytes 10-15um deep by 10 -30um across so the transition to the tramal tissues is not abrupt. **Epicutis** of free hyphal ends and pileocystidia, sometimes clumped, in places a turf 60-150um deep, particularly over the disc and in young basidiomata, in others reduced to a few free repent hyphal ends. Many hyphae have long tapering terminal cells 4um at the base and narrowing to 2um wide and 20-50um long. Some hyphal ends are articulated with short chains of cells inflated up to lOum wide or with a series of short clavate, lemon-shaped, tarsi-shaped or bulbous cells, or with the subterminal cells inflated and the terminal cell tapered, thus forming an ampullate structure. Sometimes the terminal few cells have darker contents. In areas where there are many inflated-clavate structures the epicutis is almost hymeniform. **Pileocystidia** variable in density but generally sparse, sometimes grouped, with refractive contents that stain grey to black in SV or sometimes unstaining, 15-80um by 2.5-8 (-lO)urn wide, but mostly 4-5um wide, cylindrical or more or less clavate or tapered, tips rounded, mucronate or capitate, mostly non-septate, some with one septum. Stipe surface with sparse caulocystidia similar to the shorter pileocystidia.

Trama mainly of sphaerocytes with hyphae interspersed, and rare vascular hyphae with refractive contents sometimes staining magenta in SV.

Chemical reactions: $FeSO_4$- pinkish-brown to salmon; KOH and NH4OH •* no reaction to browner or yellowish green on cap, yellowish on stipe; guaiac - quickly blue-green; phenol - not much reaction to purplish-brown; SV - macroscopically, cutis stains pink, gills stain pink to deep red, microscopically, pileocystidia stain grey to blackish, pleurocystidia purple and cheilocystidia stain pale to deep pink to purple.

Habitat and tree associations: In small groups on the forest floor among regeneration or old growth stands of Sitka spruce and western hemlock, or in pure stands of either species; both tree species appear to be hosts.

Collections: CR980825-02, CR030824-02 and CR030824-02, under western hemlock, Sitka spruce, western red cedar, big-leaf maple, and red alder near the estuary at Port Renfrew, N 48.577167°, W 124.393333°. PJ 010919-04, in the spruce fringe inland of the beach under Sitka spruce, western hemlock and shore pine Wickaninnish, N 49.02167°, W 125.67467°. CR001011-53 and CR001011-54, Wickaninnish boardwalk under krummholtz of Sitka spruce only, N 49.017°, W 125.6740°. PJ 010919-20, Wickaninnish boardwalk in the spruce fringe inland of the beach under Sitka spruce, western hemlock and shore pine, N 49.02167°, W 125.67467°. CR021016-09, Sitka spruce krummholz, Wickaninnish boardwalk, N 49.017°, W 125.6740°. CR021016-16b, Sitka spruce krummholz along the Wickaninnish dunes, N 49.0215°, W 125.6745°. CR03 0927-03, regeneration western hemlock with occasional Sitka spruce, near Lizard Lake, Port Renfrew, N 48.6037°, W 124.2268°. (All collections from southern very wet hypermaritime CWH subzone).

	ITSl-Fto	RFLP:		
Collection	ITS4-B	Hinfl	Alul	Sau3A
PJ010919-04	829	395,249	344,243	334,274, 190
CR030924-02	849	390,290	515,260	340,247,186

Notes: The greener form of *R. brunneola* was first thought to be a different species, possibly *R. smithii,* compared to the more identifiable tawny brown form. However the two were found together at Port Renfrew and seemed to intergrade. In particular a young tawny-brown specimen was collected which developed the dark vinaceous-olive velvety colouring and bloom after a day in the refrigerator. The greener form consistently has a pure white spore print whereas those with no green have an off-white print. Both have the greyish bloom on the stipe, though this varies in hue and can be close to white sometimes, but so far the green hues occur only when there is noticeable green pigment in the cap. The variation in stipe colouration is due to the balance of green and pink pigments, these colours are complimentary and appear grey where overlapping. Most of the green forms were found only under Sitka spruce, however, one specimen was found in a pure western hemlock stand near Lake Cowichan (not vouchered because of poor condition). Burlingham (1915) described the stipe colour as slate-violet, Shaffer (1970) and Thiers (1998) described it as yellowish white, purplish white or light reddish-brown, even though some of the collections examined were from the west coast including the Olympic Peninsula, Washington State. I collected *R. brunneola* from Priest Lake, Idaho, which had a yellowish white stipe, so it seems the greenish-grey stipe colour may be a local variation. Ironically, perhaps the strongest defining character of this species is its variability, yet it cannot be broken up taxonomically since much variation exists within a collection and often within a basidioma. A superficially similar species with which it may be confused is *R. eleaodes*, however that species is in the *Xerampelinae* and has yellow spores, a green reaction with $FeSO_4$ and other characteristics of that group. *R. mustelina* is another pale-spored, mild, brownish species in subsection *Heterophyllae* but this species is rarely pruinose or velvety, is more robust, with a generally paler brown cap and slightly darker spores (Romagnesi lib).

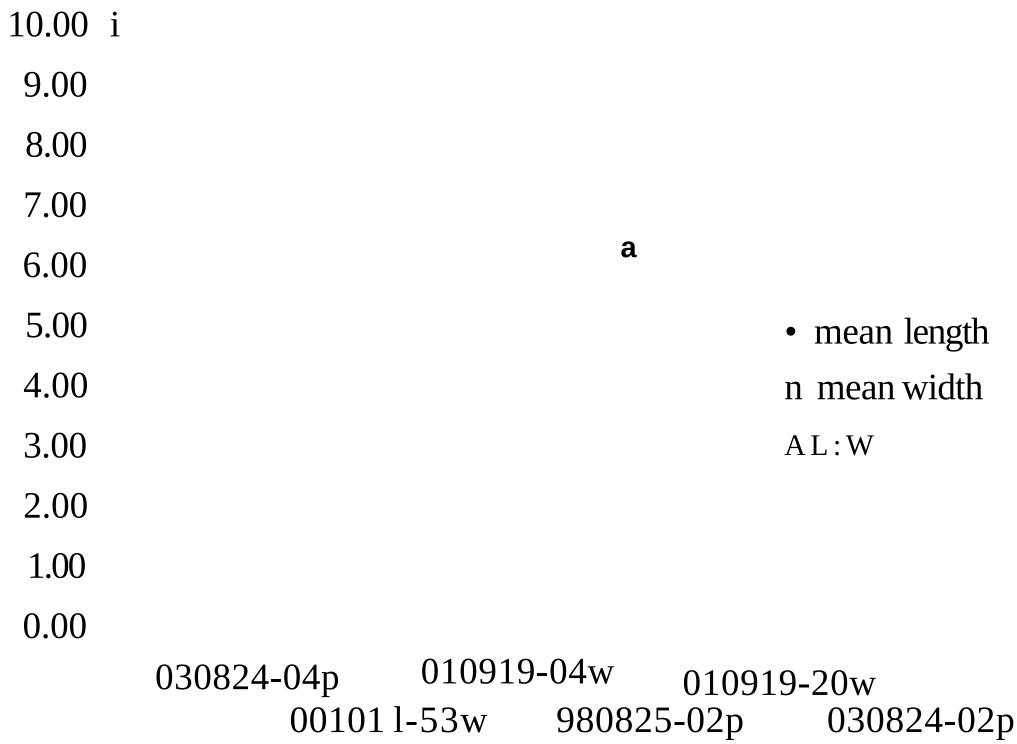


Figure 81 Chart of means (n = 15 for each) of spore length, width and L:W ratio for six collections of *R. brurmeola*, the collection number of each is followed by a letter denoting the collection area, 'p' for Port Renfrew, V for Wickaninnish.

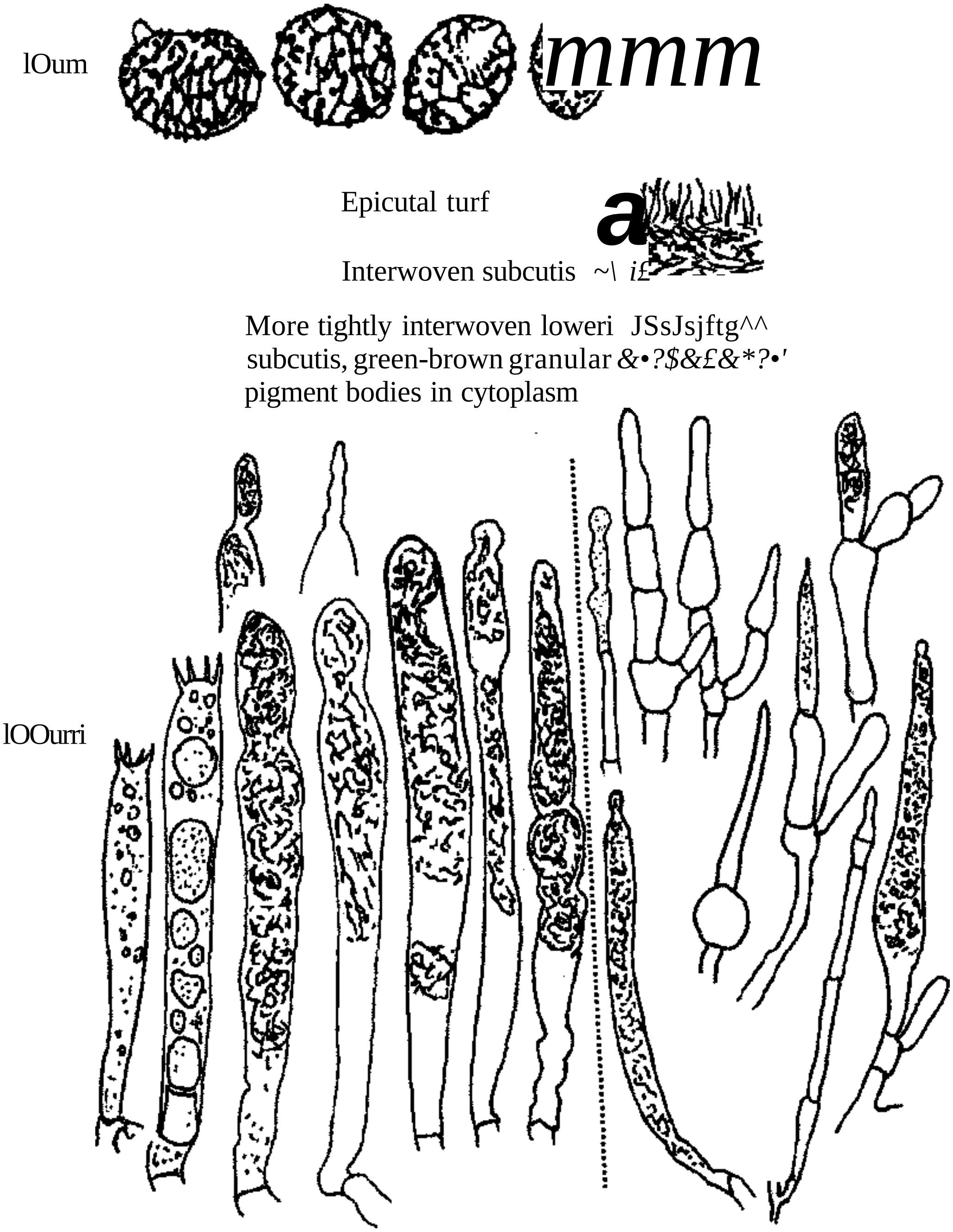


Figure 82. Microscopic characters of *Russula brunneolcr*. Top, spores with lOum scale bar; middle, diagram of section through cutis; bottom left, hymenial cystidia and basidia; bottom right, pileocystidia and hyphal ends from the epicutis, scale bar is lOOum.

Figure 83

Figure 83 Overleaf, macroscopic characters of*Russula brurmeola:* top, collection from the Wickaninnish area, with one basidioma having an unusually short stipe, photograph by Pam Janzsen; middle left, cap surface of an olive-green tinted collection and middle right, detail of the greenish-grey pruina on the stipe surface; bottom, a collection from Port Renfrew showing the matte, finely velvety texture of the young caps, (the minute speckles are insect eggs) and pinkish-grey tints on the stipe.

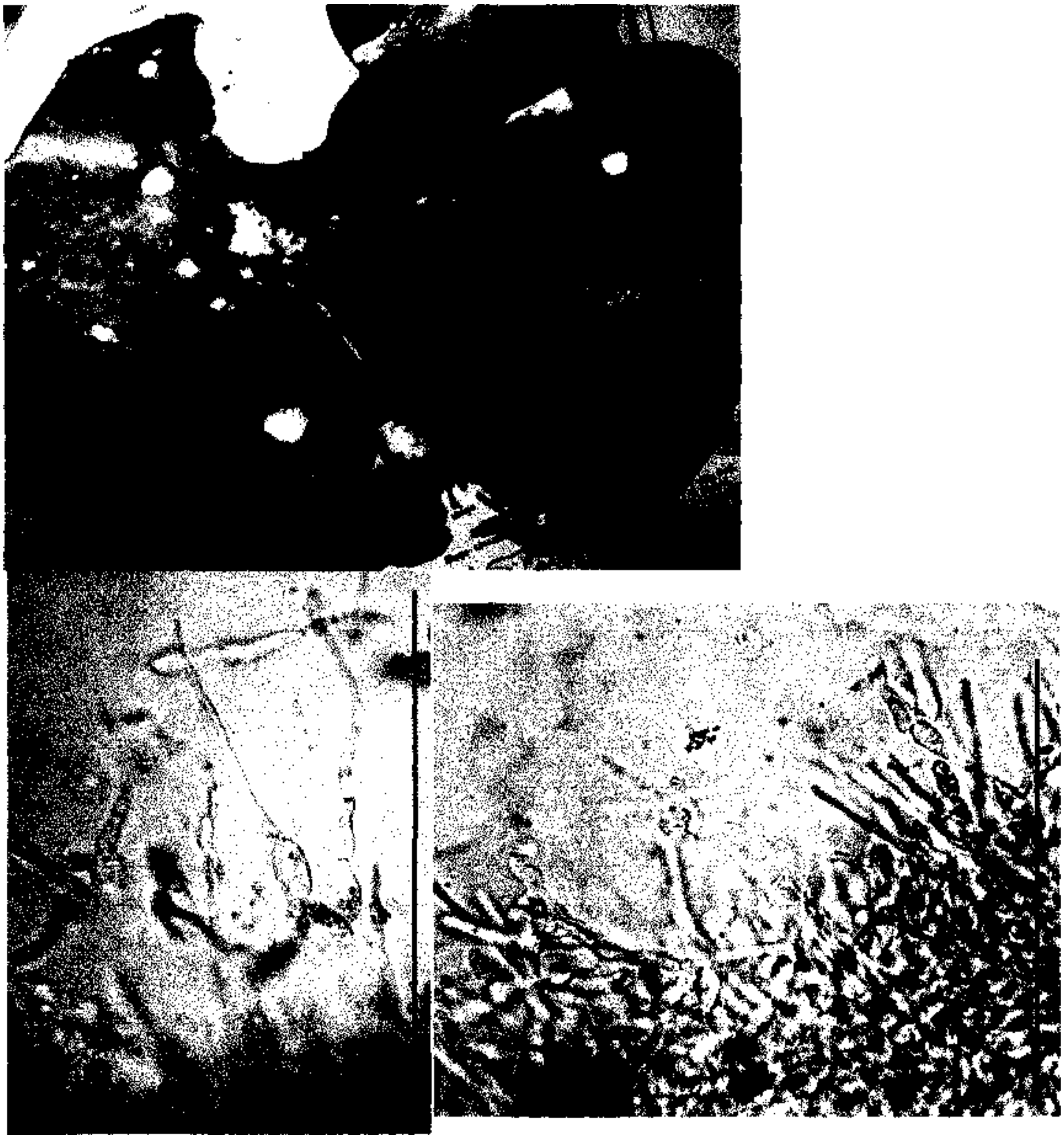


Figure 84 Cutis *ofRussula brunneola:* Top, surface view of a mature basidioma showing the typical radial rugulosiry; bottom, two views of hyphal ends in the epicutis, on the left, two ampullate structures can be seen, on the right, many narrow or tapered hyphal ends and some articulated hyphae with variform cells. The scale bars for both lower photographs are lOOum.

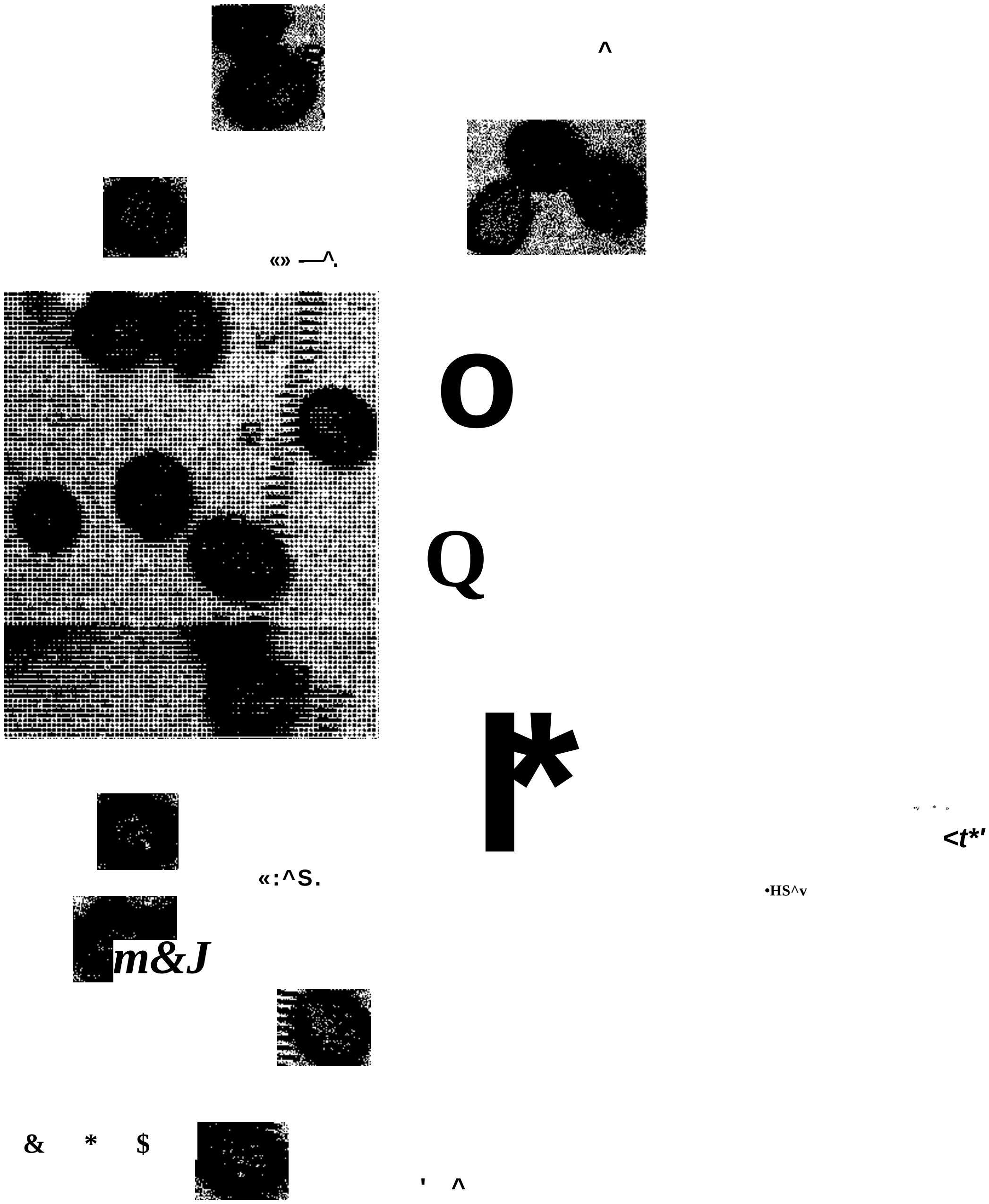


Figure 85 Spores *ofRussula brurmeola:* the upper and lower left-hand photographs are of the same spores focussed at the equatorial and surface plane respectively, note that most have an inamyloid suprahilar patch but one at lower left has an amyloid patch, such anomalous spores do occur occasionally in many *Russuh* species. On the right at the same scale are spores from another collection that are smaller with finer ornamentation. All scales are in 1 Um divisions.

Clade 4d

Subsection *Griseinae* Schaeffer and Subsection *flicinae* (Romagnesi) Buyck

Both these subsections combine in Clade 4d

***Russula parazurea* Schaeffer**

Zeitschrift fur Pilzkunde: 105. 1931

Cap 5.3cm diameter, depressed in centre becoming uplifted towards the margins but not inverting at the edge. Colour, red brown to pink brown or grey brown toward the margin, darker brown to almost black in the centre, all overlain with a white pruina giving the whole a dusty, greyish appearance. Margin smooth, pulling away slightly from the edge of the flesh and exposing the end of the gills, peelable to 1/2 the radius. This collection was made in dry weather and the surface was dry and matte, but became viscid when wetted.

Gills light pinkish cream, not bruising brown except at the cap margin, close, occasional sub-gills, infrequent forking near stipe, narrowly adnexed, narrowly obtuse at the margin, ventricose, varying between 1/2 to 1 1/2 times the depth of the trama at half radius, edges entire.

Stipe 4.5 x 2 cm at mid-point, tapering downward, white, slightly rugulose, solid to stuffed with a firm trama, unchanging.

Texture firm, not brittle, gills pliable.

Taste mild with a very faint peppery aftertaste.

Odour not distinctive.

Spore colour pinkish cream, Romagnesi lib but a little pinker.

Spores (6-) 7-8.5 (-10)|jmby 5-7.3um, L:W from 1.04-1.33, subglobose, broadly ellipsoidal, or oblong, warts tiny, 0.1-0.3 um high, occasional ones up to 0.5um, isolated or more commonly joined by fine to heavy lines in a partial reticulum, **Woo types IB** and **1C. Suprahilar patch** a small unornamented inamyloid to pale grey area, hiliferous appendix 1.2-1.6um by about 1.4um at the base. **Basidia** 35-53 x 8-10um, narrowly clavate, tapering very gradually from the widest point downward, some more bulbous in the upper 1/3, most 4-spored, some 2-spored. Sterigmata 5-7um long. **Cheilocystidia** and pleurocystidia abundant, strongly staining black in SV, 65-100 x 7-1 lum, arising from the inner layer of the subhymenium, narrowly fusoid to more less cylindrical, ends rounded, pointed or more often with a small capitum, contents refractive. **Subhymenium** 40-50um thick, of tightly interwoven hyphae. **Gill trama** of sphaerocytes with occasional vascular hyphae.

Cutis up to 600um thick, 40-70um thick at cap margin, an ixotrichodermis. **Subcutis** basal layers of compacted and parallel hyphae, upper region of subcutis is more loosely interwoven, of hyphae 2-4um wide. **Epicutis** of repent to upright hyphae 2-7um wide, generally wider than subcutal hyphae, some hyphal ends of short chains of cells 2-4 times as long as the hyphal width, sometimes slightly inflated, sometimes with constrictions at the septa and swellings either side giving a tibiiform cell shape. Terminal cells lanceolate, tapered or cylindrical-sausage shaped. **Pileocystidia,** abundant, staining red or black in SV, 30-120 x 5-10um, more or less cylindrical or narrowly fusoid, ends pointed to tapering into a narrow extension or more often with a small capitum, aseptate. **Pseudocystidia** not seen.

Trama of sphaerocytes in discrete, medium to large clusters bound with hyphal mesh.

Chemical reactions: FeSCV brownish-pink; KOH - reddish orange on cap, no reaction on stipe; NH_3OH - no reaction; guaiac- slowly blue-green; phenol - brownish purple, SV - purple on gills and cutis, very pale pinkish on cap trama.

Habitat and tree associations: On a south-facing limestone slope with Douglas fir.

Collections: TT040904-02, Koksilah ridge, northeast of Burnt Bridge park, exact location not recorded (eastern very dry maritime CWH subzone).

Notes: This collection of one mushroom agrees with the description for *R. parazurea* m Romagnesi (1967) in most characters. The description in Thiers (1997) has a spore size for the California material as 6-10.5 x 5-6um, whereas in Romagnesi the spores are only up to 8.5urn long and up to 6.5urn wide. In the Koksilah collection described above, the spores are closer to the California material in size, although most are under lOum long, the ornamentation is not as reticulated as either the European or California descriptions and the wart size is closer to that in Romagnesi's description. Thiers (1997) describes the pileocystidia as poorly differentiated, whereas the Koksilah collection matches those illustrated in Romagnesi (1967) which are clearly differentiated.

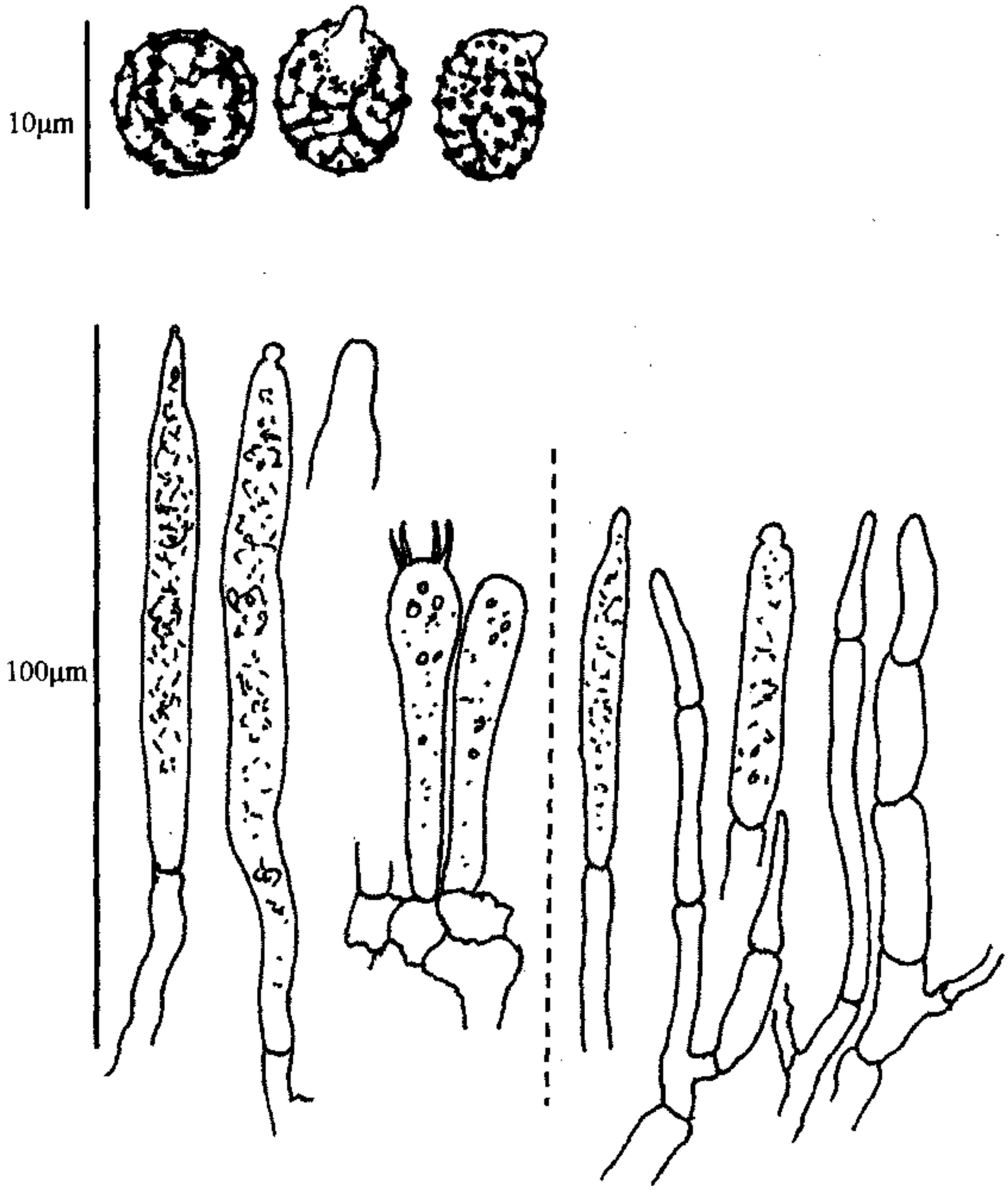
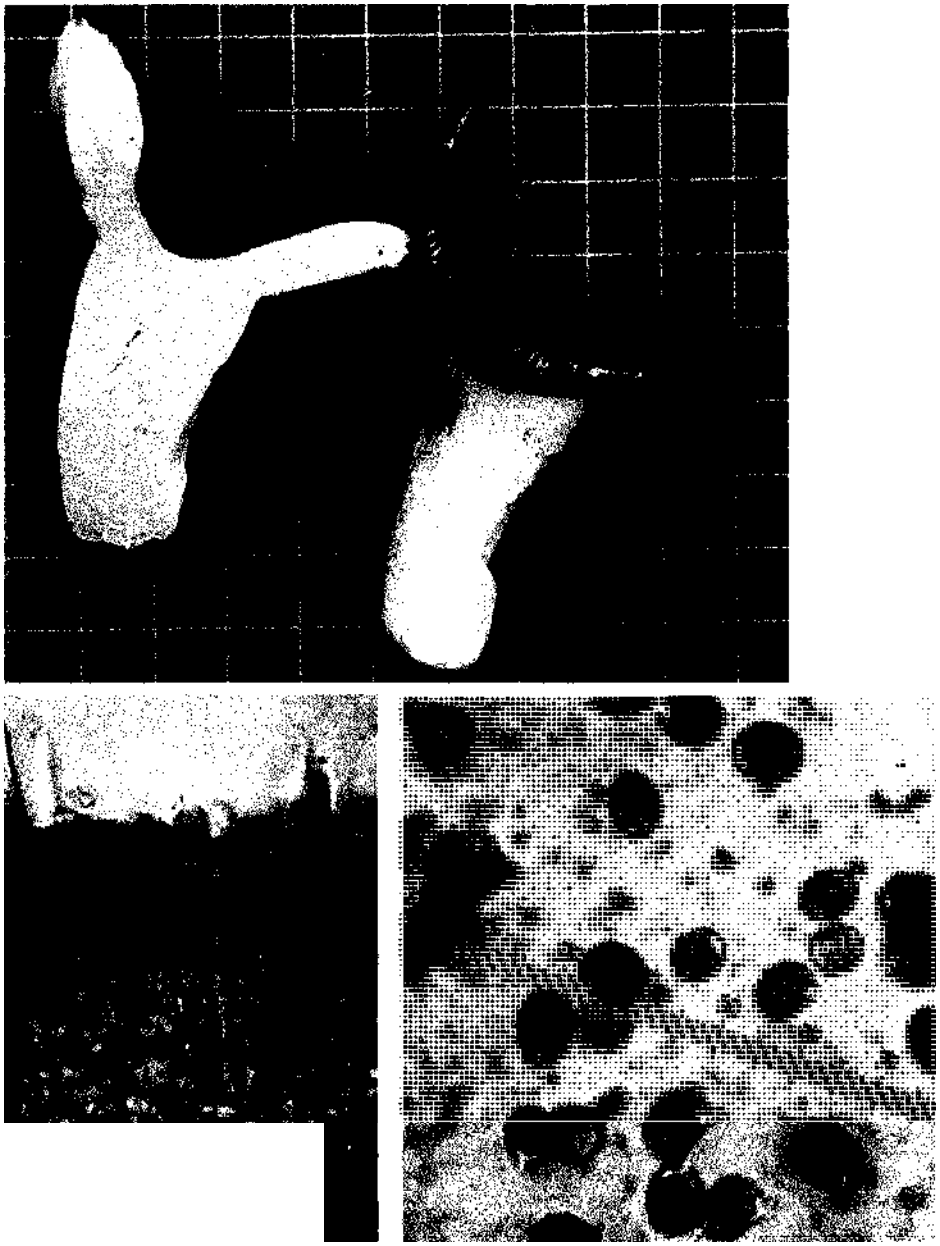


Figure 86. Microscopic characters *ofRussulaparazurea;* top, spores with 10|xm scale bar; lower left, hymenial cystidia and basidia, lower right, pileocystidia and epicutal hyphae, lower scale bar is 100pm.



JK**v„ M,

Figure 87. Macroscopic and hymenial structures of *Russulaparazurea:* Top, basidiorna with $1cm^2$ grid as background, note the bloom on the cap surface and the upturned margin in this early-stage mature specimen; bottom left, hymenium showing several pleurocystidia, including the base of one in the foreground that originates in the inner layers of the subhymenium, bar is lOOum; bottom right, spores with lum division scale (right), show typical low ornamentation and inamyloid plage of the subgenus *Heterophyllidia.*

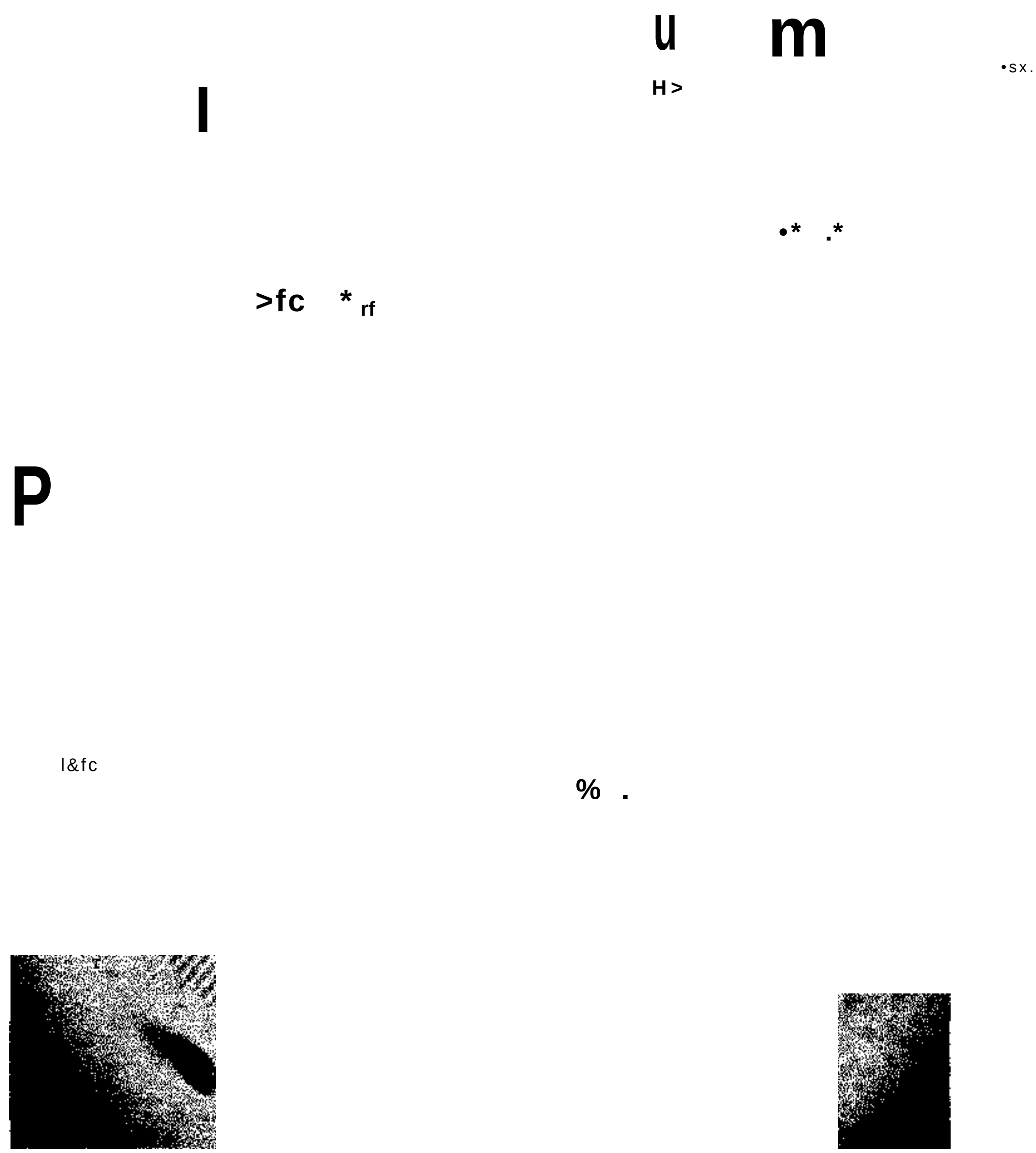


Figure 88 Epiutis of *Russulaparazurea*, at top is a section through epicutis showing pileocystidia (arrowed) and the hyphal ends which are mostly hair-like with no inflated basal cells; beneath is a surface view of the cutis in SV showing darkly stained pileocystidia. Ten divisions on the scale at lower left are 25 um, both photographs are the same scale.

***Russula medullata* Romagn.**

Documents Mycologiques 106:53 1997

Cap 6.5-9.5 cm diameter, plane when young, becoming depressed in the centre when fully expanded, but retaining a broadly rounded margin which remains smooth. Colour a mottled light to mid blue-grey to green-grey with patches of greyed pink, dull green, cream and light brown, in general, the margins more blue-grey and the disc lighter and more cream or light brown. The colours seemed to darken and develop more after collection and refrigeration, which may be natural aging or a bruising reaction. Surface viscid, drying subglossy and minutely radially rugulose. Cuticle peelable 1/3-1/2 the radius, cap trama whitish to pale greyish directly beneath. Cap flesh thicker, ca. 8 mm, at half radius than on disc or margin, unchanging where cut.

Gills subdistant to moderately close, frequent unevenly distributed subgills, forking and anastomoses near stipe and towards margin, pliable when young and fresh, adnate at the stipe and margin, gill margins entire. Colour cream, becoming deep cream.

Stipe 5.2-7.2 x 2.0-3.1cm, cylindrical or widening near the base, white, staining rusty brown near the base, stuffed with a firm, bread textured trama which eventually develops irregular cavities, (just two large ones in two of the basidiomata) and presumably eventually becomes hollow.

Texture firm, not brittle.

Taste mild but with a very faint hint of pepperiness.

Odour mild, not distinctive.

Spore colour deep cream, Romagnesi lid to Ilia.

Spores variable in shape, bean-shaped, ellipsoidal, subglobose or frequently elongate, 6.5-9 (-11) x 5-8um, most are around 8 x 6um, L:W - 1.08 tol.6. **Ornamentation** of warts mostly 0.1 -0.3 um high, with a maximum about 0.5 urn, rounded to bluntly conical, isolated or very occasionally with fine connectives between two or three warts. **Woo types** A1, B1, A2, or B2. **Suprahilar patch** inamyloid, a relatively large area free of warts or with only minute punctae. **Basidia** 4-spored, rarely 2-spored, approximately 45-52um long by 7.5-10um wide, narrowly clavate, sterigmata around 5um long and up to 2um across near the base. **Cheilocystidia** abundant, 60-130 x 10-12um. **Pleurocystidia,** average to sparsely distributed, staining red or grey in sulphovanillin, (younger caps with more grey, older more red), 80-115 x 10-14u,m wide, protruding 20-30um beyond basidioles, fusoid to clavate with most commonly acute tips or sometimes with elongate and capitate tips. **Subhymenium** about 20-40um thick, of small cells 5-10um diameter, gill trama of sphaerocytes with a few hyphae.

Cutis 200-400um thick, a gelatinised ixodermis. **Subcutis** with the upper half more loosely interwoven than the lower half. **Epicutis** not well differentiated from the subcutis, of repent hyphal ends and pileocystidia. Hyphal ends of chains of inflated, lemon-shaped or short cylindrical or irregularly shaped cells, sometimes with darker cell contents than in the generative hypha, and many with a lance-shaped terminal cell which tapers to a point. The majority of these hyphal end cells are 4-5 u,m wide, some reach 7-8um. **Pileocystidia** sparse, around 30-80 x 4-10um wide, most at the 4-5um end of the range, cylindrical to clavate, tips rounded, mucronate or minutely capitate, mostly non-septate, some one septate.

Trama mainly of sphaerocytes, with binding hyphae interspersed.

Chemical reactions: $FeS0_4$ - buff; KOH - no reaction on cutis or stipe; SV - microscopically, gills stain red or purple, cutis stains magenta then brown, pileocystidia stain lightly grey, becoming non-staining in older basidiomata, pleurocystidia and cheilocystidia stain red or grey, with more grey ones in the younger caps, more red in the older.

Habitat and *tree* associations: Around a small 2m tall *Pinus contorta* among kinnikinnick in the open coastal dunes beyond the margin of a pocket forest (a small area of forest in a dune swale.)

Collections CR021016-21 from the dunes at Wickaninnish, Pacific Rim National Park, N 49.0220°, W 125.6750 (southern very wet hypermaritime CWH subzone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A	
_ _ _ _ _ _ _ _ _ _		390,290	532,285	582,246	"~

Notes: The published collections of species within subsection *Griseinae* Schaef. from the Pacific Northwest include *R. parazurea* J. Schaeff, *R. grisea* Persoon ex Fries and *R. basifurcata* Peck, all of which are similar to *R. medullata* J. Schaeff. The Wickaninnish collection, however, matches well the original description for *R. medullata* in Romagnesi (1967) and that of Sarnari (1998), and differs from both *R. grisea* and *R. parazurea* in spore and cuticle characters. Descriptions of the latter two species in the American literature differ slightly from those in the European literature and may represent local subspecies. *R. parazurea* differs from *R. medullata* in its paler spore print and reticulated spores. *R. grisea* has spores only slightly paler than this collection of *R. medullata,* whose spores are at the paler end of the described range, *R. grisea* also differs from *R. medullata* in the spore ornamentation-the warts are up to 1.25um high and form chains and connections more frequently, *R. grisea* has a strong salmon reaction with FeSC*4 and its habitat is with broadleaved trees especially *Fagus,* on calcarous soils. Romagnesi noted that *R. medullata* is not rare and has been found with many broadleaved trees and with pines and firs. This collection of *R. medullata* shares several characters with *R. basifurcata;* the spores, in particular the occasional occurrence of large spores (up to 14.2um in *R. basifurcata)* the hymenial cystidia protruding up to 30um beyond the basidioles, and the size and shape of the pileocystidia. Differences include the paler cap of *R. basifurcata,* and its thinner, non gelatinised cuticle (60um as opposed to 250-300um) which separates only at the margin, the abundance of pileocystidia, plentiful in *R. basifurcata,* sparse in *R. medullata,* and the unchanging flesh of *R. basifurcata* as opposed to the browning, especially at the base of the stipe, in *R. medullata.* Grund (1965) described a collection of one specimen of *R. basifurcata* from Whidbey Island, Washington, U.S.A., which differs from the Wickaninnish collection of *R. medullata.*

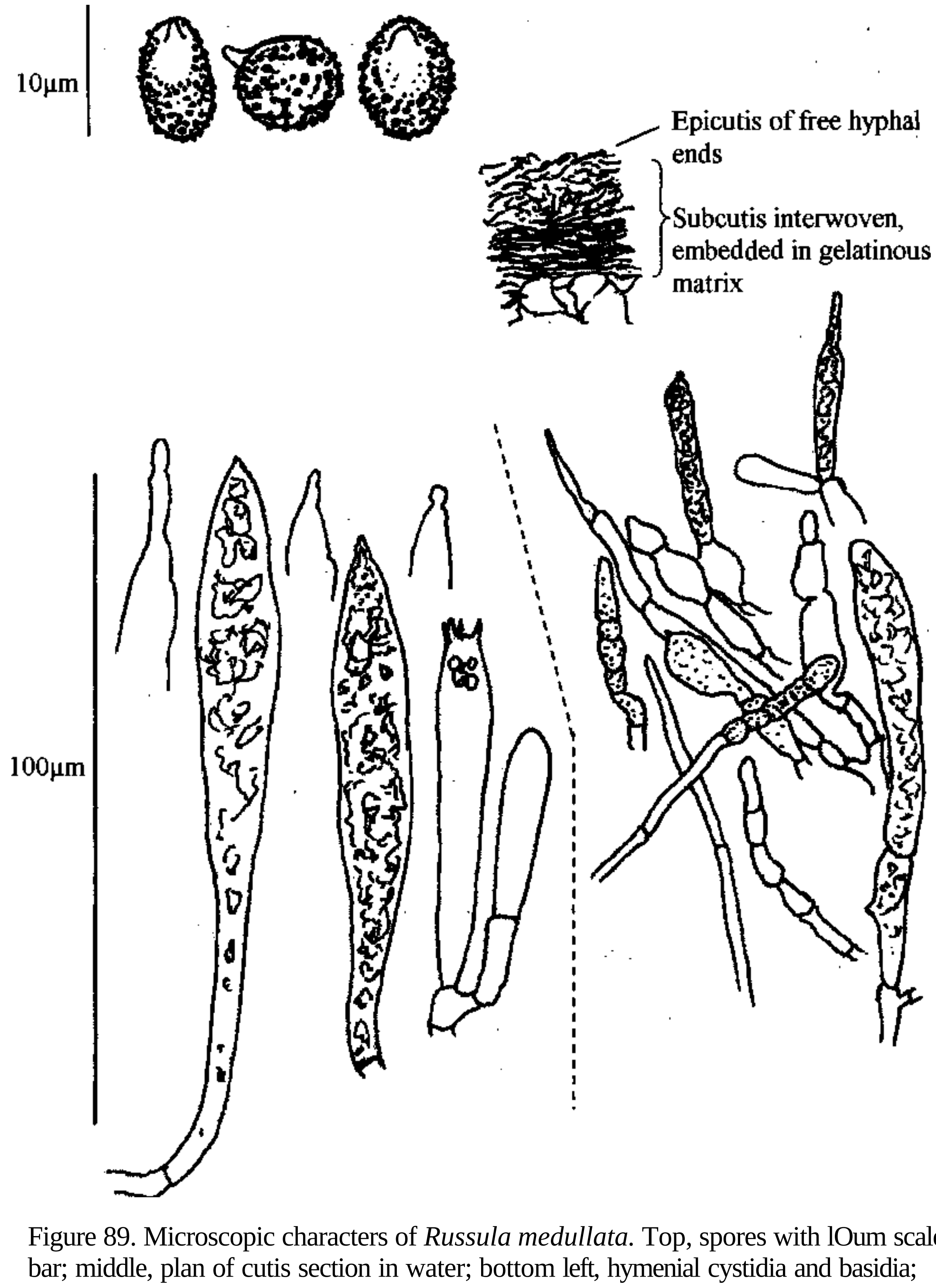


Figure 89. Microscopic characters of *Russula medullata*. Top, spores with lOum scale bar; middle, plan of cutis section in water; bottom left, hymenial cystidia and basidia; lower right, pileocystidia and hyphal ends in epicutis, lower scale bar is lOOum.

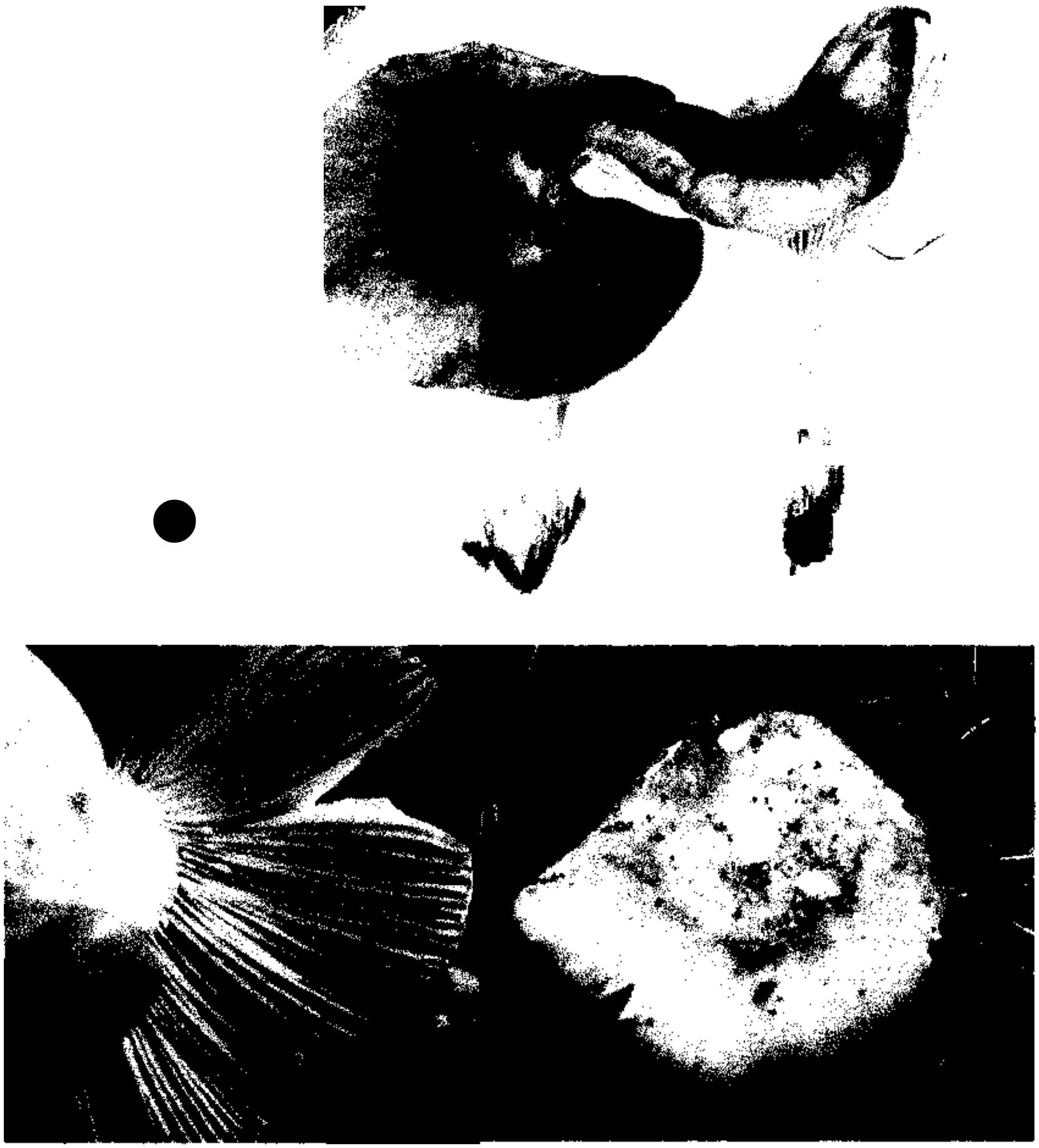


Figure 90. Macroscopic characters of *Russula medullata:* Top, illustration of profiles and longitudinal section, small square is 1cm^2 and shows spore print colour; bottom, gills and a basidioma in its habitat. Note that the colours become more pronounced after collection and refrigeration.

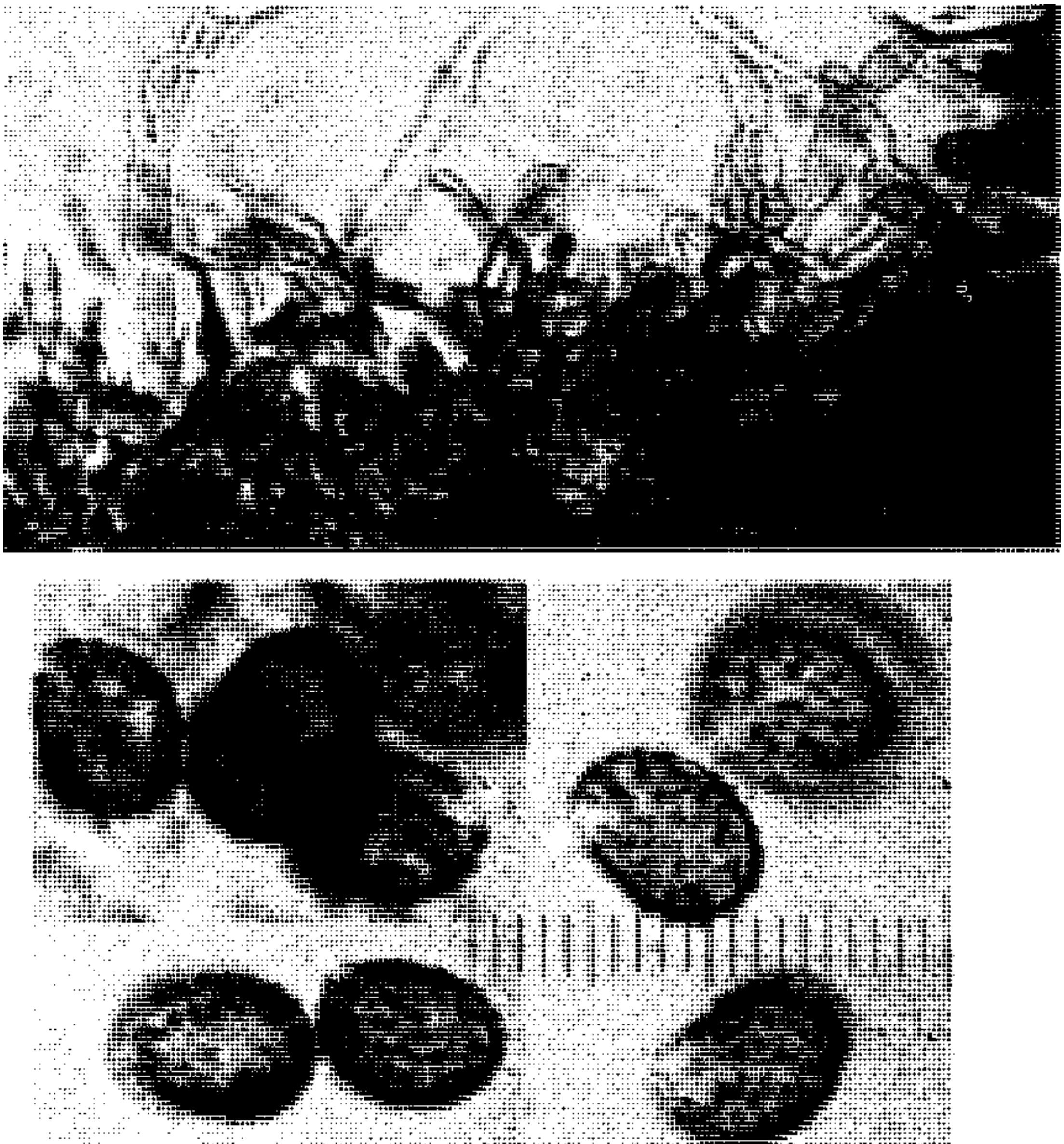


Figure 91 Epicutis and spores of *Russula medullata:* Top, articulated hyphal ends from the epicutis; bottom, spores, composite photograph of two depths of focus, both scales are in lum divisions.

Russula **cf.** ***mblevispora*** **(Romagn.) Romagn.**

Les Russules d'Europe et d'Afrique du Nord: 299. 1967

(-Russula ferreri var. *sublevispora* Romagn.

Bulletin Mensuel de la Societe Linneenne de Lyon 9: 94. 1940)

Cap 11cm diameter, more or less plane but with about half of the margin slightly uplifted, margin smooth, cuticle peelable less than 1/4 the cap radius. Colours are a yellow brown (ochre) over the central area, mottled with brownish grey and purple, the purple increasing towards the margin and becoming more violet, overlain with a light greyish bloom. Cap surface matte and velvety when dry, towards the margins the surface texture becomes suede-like with minute granulations. Cap flesh white, 5-10mm thick at half radius, unchanging to slightly browning when cut.

Gills pale cream, occasionally developing red-brown spots, subdistant, some forking near the stipe, approximately equal to or up to 25% broader than the depth of the cap trama at half radius, adnate to notched at stipe, acute at cap margin, brittle.

Stipe 8 x 2.2cm, broadest at the apex and tapered downwards, mostly white with a faint flush of very pale pink, appearing almost pearlescent, surface longitudinally rugulose, stuffed, solid, developing yellow-brown stains where damaged and particularly around the base.

Texture firm, not brittle, the stature fairly robust.

Taste mild with a brief, fleeting hint of pepperiness.

Odour mild, woody, nutty, and in the base of the stipe where cut, faintly spermatic.

Spore colour cream, Romagnesi lie.

Spores 7.25-9 (-10) x 5.75-8.3um, L:W 1.11-1.5, mean 1.24 (n = 30), subgloboseto broadly ellipsoidal. **Ornamentation** of fairly densely distributed small, blunt, isolated warts under 0.4um high, strongly staining in Melzer's reagent, occasional fine connectives between 2 or 3 warts, and occasionally few catenate or joined warts. **Suprahilar patch** a pale smoothish oval area very faintly amyloid. **Woo types Al** to **Bl. Basidia** 4-spored, 30-52 x 8-12um (most 8 to lOum), narrowly clavate to slightly bulbous in the upper 1/3, often with one or two basidioles arising from the same basal cell. Sterigmata to about 5urn long and 1.5-1.8urn wide at the base. **Pleurocystidia** and **cheilocystidia** sparsely distributed, some patches of the gill with none. Both range from 35-70 by 7.5-8um, and arise from deep in the subhymenium. Many pleurocystidia remain embedded in the hymenium completely or with just the tip protruding. Shapes are more or less cylindrical to narrowly clavate but often with constricted sections, tips tapered, mucronate or capitate, contents refractive, weakly staining pink or grey in sulphovanillin. **Subhymenium** around 15-25um thick, pseudoparenchymatous. **Gill trama** of sphaerocytes with rare vascular hyphae.

Cutis 170um thick near the cap margin to 400um over the disc, an ixotrichoderm. **Subcutis** of densely interwoven light brownish hyphae 3-5um wide and containing pigment granules that appear dark grey in water mounts. Occasional vascular hyphae 4-6um wide occur and some terminate in **pseudocystidia** in the epicutis. **Epicutis** of variable thickness, in patches just a few free upright hyphal ends, in others clumps of hyphal ends 3-5um wide and cystidia, these clumps mostly in the outer half of the radius and being visible to the naked eye as a suede-like texture or little granules. **Pileocystidia** 18-62 x 5um, fairly regularly dispersed over the surface, some longer and cylindrical with elongated capitate-strangulate tips, others narrowly clavate, around 25 um long, with rounded or mucronate tips, aseptate, filled with yellowish refractive contents that stain weakly grey or pink in SV. **Hypodermis** not distinct, although there is a fairly abrupt differentiation between the cutis and tramal tissue; the cuticular and tramal hyphae interconnect in small areas between clusters of tramal sphaerocytes.

Trama of larger (up to 80um) sphaerocytes in loose small clusters interleaved with denser, small irregular cells all bound with a hyphal mesh, with occasional vascular hyphae reacting weakly with SV.

Chemical reactions: $FeSO_4$ - greyish to pinkish; KOH - orange on cap surface, no reaction on stipe; NH4OH - no reaction; phenol - brownish pinkish purple; S V - stains gills and cutis pink, vascular hyphae and cystidia pink to grey.

Habitat and tree associations: Found in deep duff on the forest floor on a SE facing well drained slope with little to no undergrowth amid a mixed age stand of 30-40-year-old Douglas fir, western red cedar and occasional western hemlock saplings.

Collections: CRO10909-03 from regeneration Douglas fir forest in the Kemp Lake area, Sooke, near a trail from the houses along Kemp Lake Road towards the lake, N 48.22.517°, W 123.46.822° (western very dry maritime CWH subzone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR010909-03	~832	358,321	500,259	610,346,270

Notes. This *Russula* keyed out to *R. sublevispora* in Romagnesi (1985) and matches the description very well except for the habitat and having slightly stronger spore ornamentation. In Romagnesi this is described as in grassy or muddy lanes through broadleaved woods. The Vancouver Island collection was found under mainly Douglas fir, but a number *of Russula* species recorded from Europe under broadleaved trees also occur with Douglas fir and other local coniferous hosts. Additionally, red alder is also a common tree in these sites and may have been present but overlooked, so too deciduous shrubs. The macroscopic and microscopic morphology of this *Russula* places it in the *Heterophyllae* yet the DNA restriction fragments are dissimilar to those of the others in this group, in particular the Hinfl fragments. These show more similarity to members of subgenus *Russula* section *Russula* subsections *Russula* and *Sardoninae.* The cutis pigments show similarities to those of *R. aeruginea/stenotricha* with a small amount of water extractable blue-grey and magenta pigment but the much stronger yellow pigment was more like that of *R. parazurea*, i.e., a brownish yellow strongly fluorescing yellowish in UV light.

Romagnesi first described *R sublevispora* as a variety of *Kferreri* Singer, but on checking Singer's original description it was apparent that that species differs in having reticulate spores but is otherwise similar. Both are closely related to *R. grisea*.

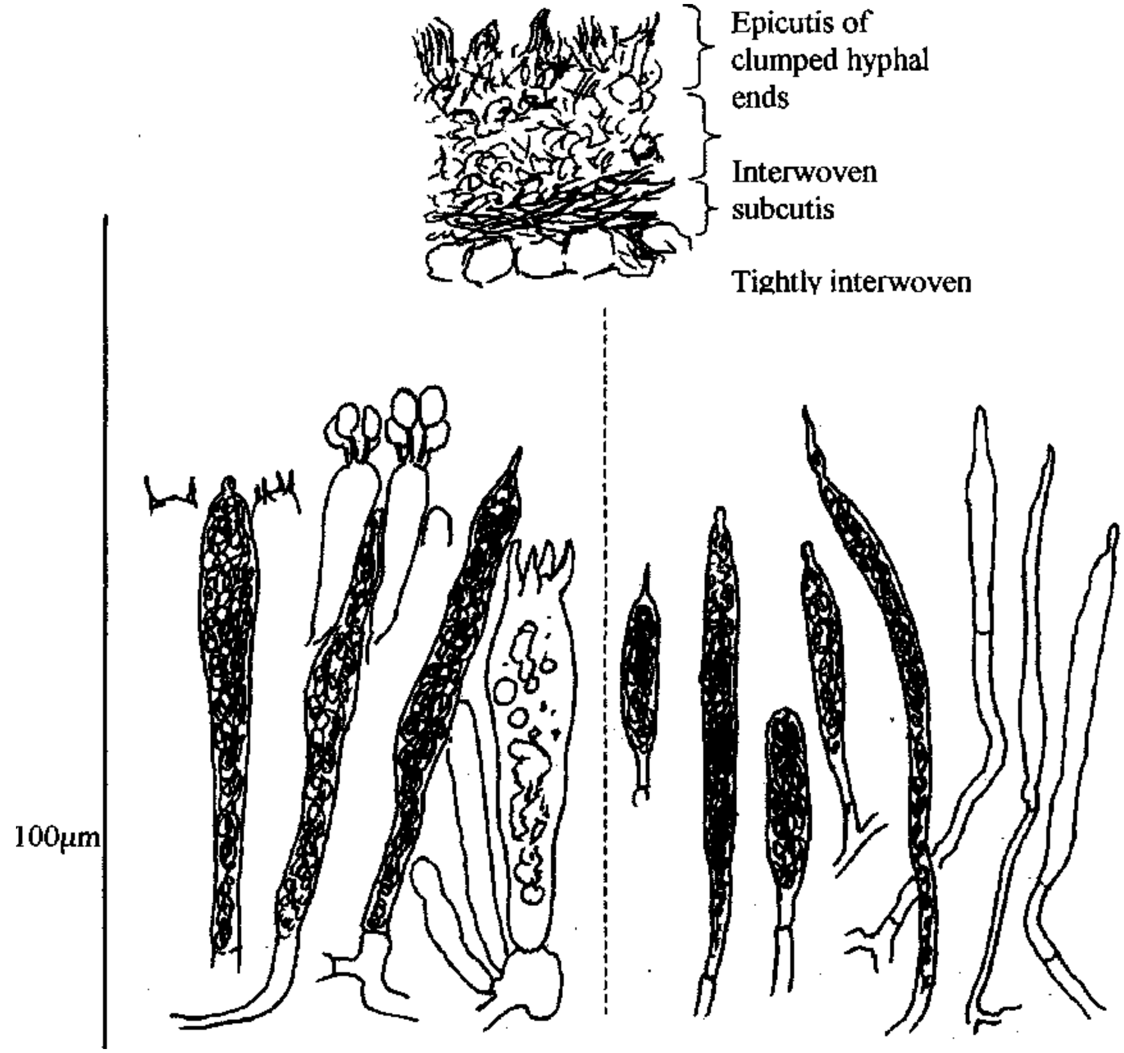


Figure 92 Microscopic characters *ofRussula* cf. *sublevispora:* Top. Spores with lOum scale bar; middle, diagram of section through cutis; bottom left, hymenial cystidia (in SV) and basidia; bottom right, pileocystidia (in SV) and epcutal hyphal ends. Lower scale bar is lOOum.

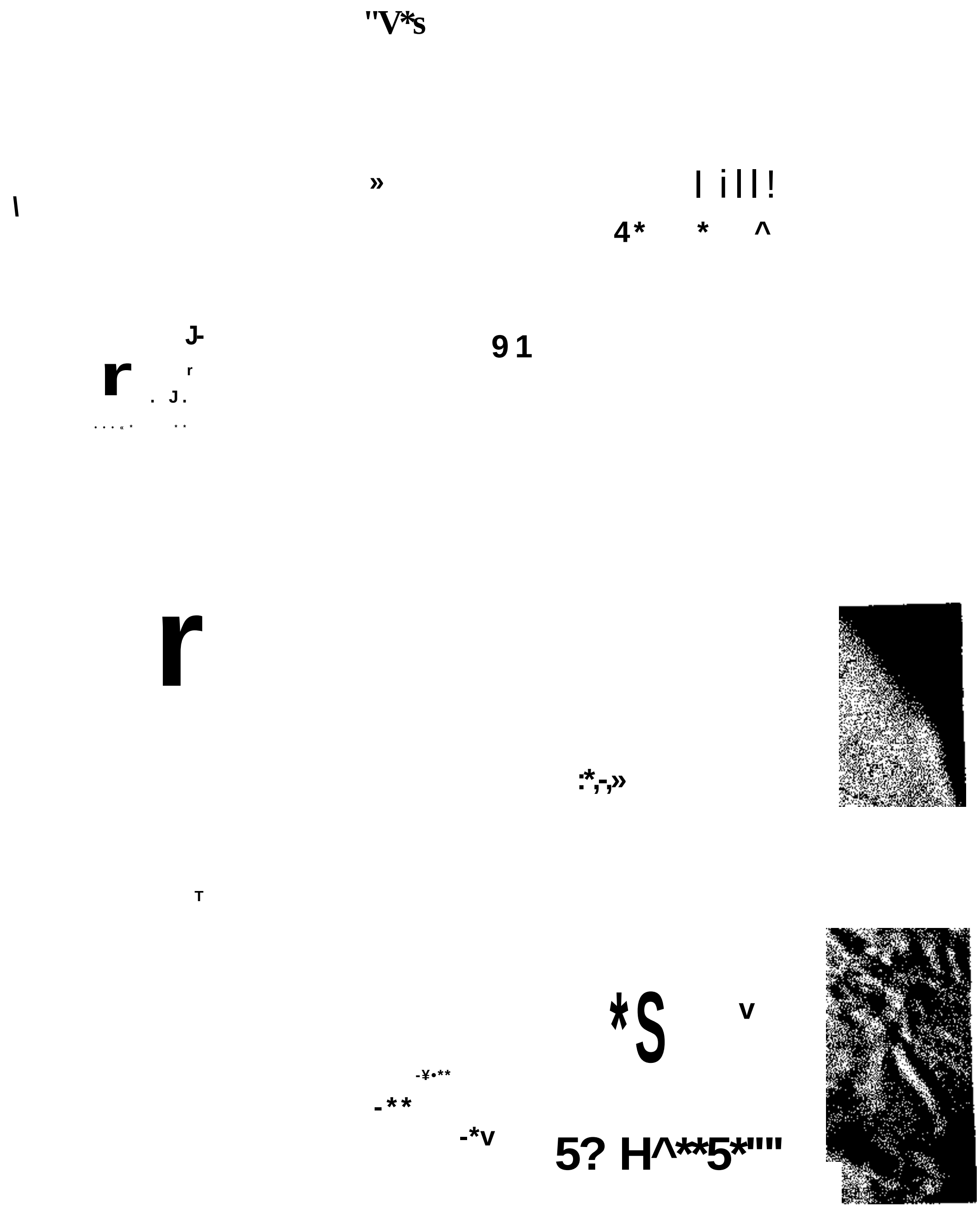


Figure 93 Epicutis and hymenium *oiRussula* cf. *sublevispora:* Top, spores with lum division scale; middle left, basidia with lum division scale; middle right, surface view of cutis in which the dark pigment granules show up inside the hyphae, scale bar is Sum; bottom, cluster of hyphal ends from epicutis, 10 divisions on the scale are 25 um.

Russula aemginea **Lindbl. ex Fr.**

Monographia Hymenomycetum Sueciae 2: 198. 1863

Russula **cf.** ***stenotricha*** **Romagn.**

Les Russules d'Europe et d'Afrique du Nord.: 290, 1967

These collections have been described together because it is not clear whether they represent one variable species, as has been assumed in several previous descriptions, or two species with many similarities.

Cap 3.6-10.5cm diameter, almost spherical with a flattened top, in some cases with a small central depression when young, and becoming shallowly centrally depressed with the edges flared unevenly. Colour grass green with tints of olive, yellowish green or, in the Koksilah collection, deep green, like a greyed viridian, sometimes minutely radially streaky, the colours usually strong but may fade somewhat in age, but never really pale, radially finely rugulose, viscid drying subglossy to matte. Margin smooth at first, becoming striate at maturity. Cuticle peelable at least half and sometimes completely, sometimes pulling away from the cap margin to expose the ends of the gills, flesh grey under cutis.

Gills close to subdistant, occasional anastomoses, occasional separate subgills, arched, becoming ventricose, depth increasing with age from about half to approximately equal that of the trama at half-radius, adnate at margin and adnate to almost sinuate at stipe, thin, pale cream, not or only slightly bruising brownish, brittle.

Stipe 3.0-8.5 x 1.2-2.5cm, more or less cylindrical, surface white or, in one button, with a green flush, sometimes with brown bruising at the base, stuffed with firm to bread textured trama, uncavitate, unchanging.

Texture ranges from quite firm to soft and pliable.

Taste mild or more often, slightly peppery especially in the gills.

Odour not distinctive.

Spore colour pale cream, Romagnesi Ha. The Koksilah collection lie.

Spores subglobose to broadly ellipsoidal, mostly with little size variation within one collection with the exception of the occasional large spore. Three collections have spores of 5.5-8 (-9.5) x 5-7um, L.W = 1 to 1.38, one collection from Lizard Lake near Port Renfrew has spores of 6.75-9 by 6.5-8um. The Koksilah collection has variably shaped spores taken from the spore deposit of one mushroom, the majority are oblong but many are subglobose, 6-9.5 x 5-7.5um, L:W averages 1.31 and ranges from 1.19 to 1.5. **Ornamention:** Woo types Bl, B2, CI, C2, D2; the Lizard Lake collection E1-E2, the warts mostly 0.2-0.8um, rarely up to lum, rounded, isolated or more usually in chains forming a ridge, with some broad or fine connective lines, forming a partial reticulum. The reticulum on the Koksilah collection spores tends to be finer than that on the coastal collections. **Suprahilar patch** inamyloid or barely amyloid, appearing as a small unornamented region below the hilar appendage. **Basidia** mostly 4-spored, approximately 37-40um x 10-12um wide, clavate to bulbous in upper third. **Cheilocystidia** and pleurocystidia, abundant, cheilocystidia brownish purple in SV, protruding 20 to 30um beyond basidioles, pleurocystidia purple to black in S V, some pink-staining ones in younger basidiomata, originating in the subhymenium, protruding about 10 to 25 um, both cystidia types 55-85 x 8-12um, fusoid or cylindrical, often of uneven diameter, the tips rounded, broadly pointed or shortly mucronate. **Subhymenium** well differentiated, about 35um thick, of small irregular cells, gill trama of sphaerocytes.

Cutis 150-230um thick at half-radius, up to 350um over the disc, an ixotrichoderm containing occasional vascular hyphae. **Subcutis** of repent interwoven hyphae gradually becoming more loosely interwoven toward the surface, the **epicutis** forming a loosely interwoven turf of hair-like hyphal ends, 1.5-4um wide, with blunt or narrowed tips, and without the frequent septa or inflated cells common within the subgenus. Cuticular hyphae and cystidia with dark pigment granules in the cytoplasm. **Pileocystidia,**

abundant, 20-65 x 7-10um, the predominant shape is clavate, relatively short and broad, mostly non-septate, occasionally with one septum, the Lizard Lake collection with some two-septate pileocystidia, and these 35-75um long by 5-8um wide. The Koksilah collection has pileocystidia 30-80 x 4-7um, cylindrical, most with a small capitum and aseptate, strongly staining in SV but with some non-staining ones too. **Pseudocystidia** occasional, 3.5-6.5um wide, more or less cylindrical with rounded ends or with a small capitum, refractive in KOH and greyish in SV.

Chemical reactions: FeSC»4 - weak, slowly light brownish pink; KOH - ochre on cap, yellowish on stipe; NH4OH - no reaction; phenol - pale brownish pink; SV - cutis brownish or dull pink, gills and cap trama stain bright magenta.

Habitat and tree associations: The coastal type with clavate pileocystidia and more yellow-green cap colours in coastal stands of western hemlock with or without sitka spruce, usually solitary, uncommon. The more typical *R. aeruginea* type with cylindrical-capitate pileocystidia from Koksilah Ridge was under regeneration Douglas fir, also uncommon.

Collections: CR000919-03 (2 specimens,) Bamfield area; P.K.27/V/00 Clayoquot Island (2 specimens), CRO10920-3 8b (1 specimen) CR021016-22 (1 specimen) Long Beach area, Pacific Rim National Park; CR03 0927-02 (1 specimen) Lizard Lake, Port Renfrew (all from southern very wet hypermaritime CWH subzone). TT040904-01 (3 specimens) from Koksilah Ridge (eastern very dry maritime CWH subzone).

	ITS1-F to	RFLP:		
Collection	ITS4-B	HinFl	Alul	Sau3A
CR021016-22	855	376,229	493,229	334,250

Notes Romagnesi (1967) recognized *Russula stenotricha* as a separate species from *R. aeruginea*, basing his conclusion on the consistent characteristic of the pileocystidia, which are clavate and broad, generally 5-9 (-12)um broad and 25-50um long, and lack a terminal appendage in *R. stenotricha*, whereas in *R. aeruginea* the pileocystidia are much longer, only 4.5-7um wide and mostly capitate. Otherwise the two species are very similar. Romagnesi considered *R. stenotricha* to be closely related to *R. aeruginea*, whereas Sarnari (1998) considered it to be more closely related to *R. pseudoaeruginea*. However, this latter species differs in its epicutal hyphae of articulated, often inflated cells. In European literature *R. stenotricha* is reported as being found under broadleaved trees on chalky-clay soils but *R. aeruginea* is found under spruce and pine. The Vancouver Island collections all have the short, clavate pileocystidia typical of *R stenotricha* and no articulated inflated epicutis hyphae, the spore print is slightly paler than is described for either *R. aeruginea* or *R. stenotricha*, and the habitat always includes western hemlock. *R. aeruginea* is included in Thiers' Agaricales of California (1997), Grund (1965) described a collection of *R. aeruginea* as var. *mutabilis* nom. prov., from Washington State, which had larger warts on the spores than the above collections and a variety of pileocystidia shapes that echoes the Lizard Lake collection. When compared with the Genbank sequence for *R. aeruginea*, a Vancouver Island collection had a slightly longer ITS region, and differing sizes for each of the three enzymes. The GenBank sequence could have a base-pair substitution at a Sau3 A site, leaving the 544 nucleotide fragment intact. The 274 nucleotide fragment of the PRNP collection may be masking a second fragment only slightly smaller in size.

Shaffer (1970) made several collections of *R. auruginea* from Michigan and commented on the variation in spore ornamenation and in the pileocystidia, which can vary between specimens in the same collection or even within the same pileus. One explanation for the variability in North American material compared with the two distinct forms found in Europe is that the North American *R. aeruginea* is a remnant ancestral species to the two European species *R. stenotricha* and *R. aeruginea*. The two types found on Vancouver Island in two different habitats may well represent two species rather than one variable one, so hopefully future collections will resolve this question.

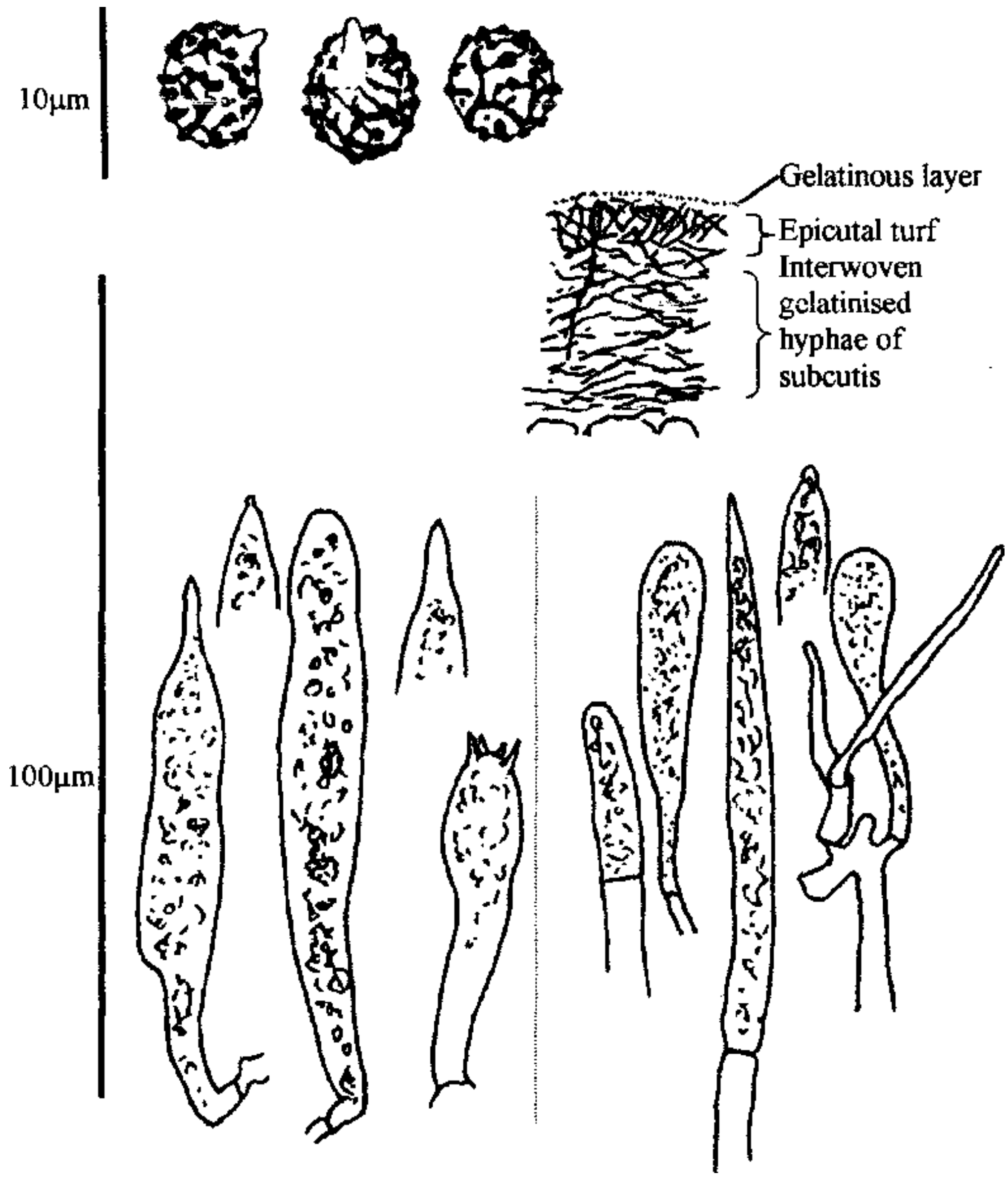


Figure 94 Microscopic characters *ofRussula aeruginea:* Top, spores with lOum scale bar; middle, plan of cutis section in 5% KOH; bottom left, hymenial cystidia and basidia; lower right, pileocystidia and hyphal ends in epicutis, lower scale bar is lOOum. The clavate pileocystidia are typical *ofRussula* cf. *stenotricha*, and the cylindrical ones with strangulate tips are those of typical *Russula aeruginea*.

Figure 95 Macroscopic characters of *Russula* aeruginea: Top and inset, *Russula* cf. *stenotricha* basidiomata from the Long-beach area of the PRNP, shown alongside a 1 cm scale; bottom, the more typical form of *Russula aeruginea* from Koksilah ridge, the spore print is visible on the white paper and the mushrooms are on a 1cm grid mat.

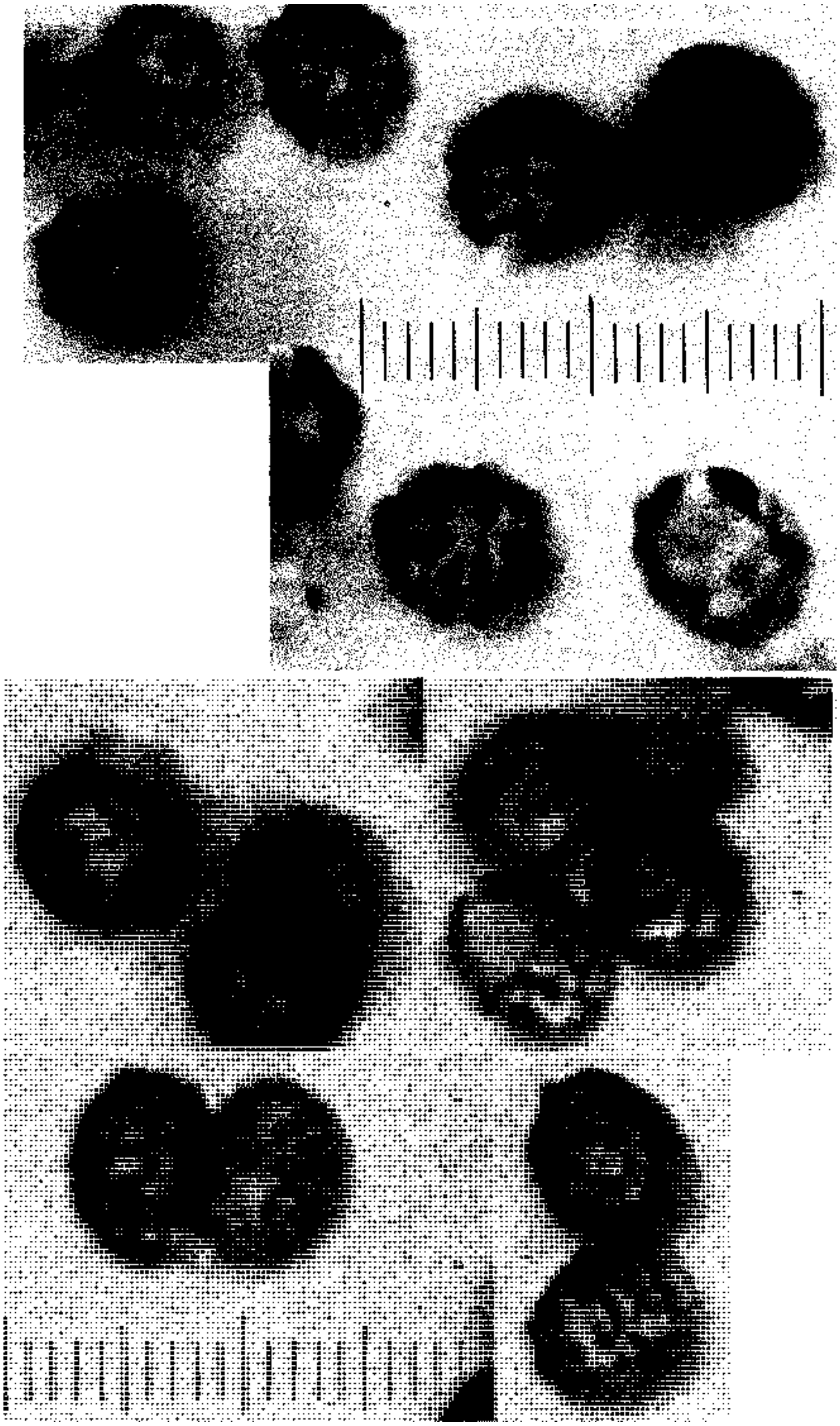


Figure 96 Spores of *Russula aeruginea:* Top, those of *Russula* cf. *stenotricha* from the Long-beach area of the PRNP, bottom, those of the more typical form *of Russula aeruginea* from Koksilah ridge. In both sets the scale is in 1um divisions.

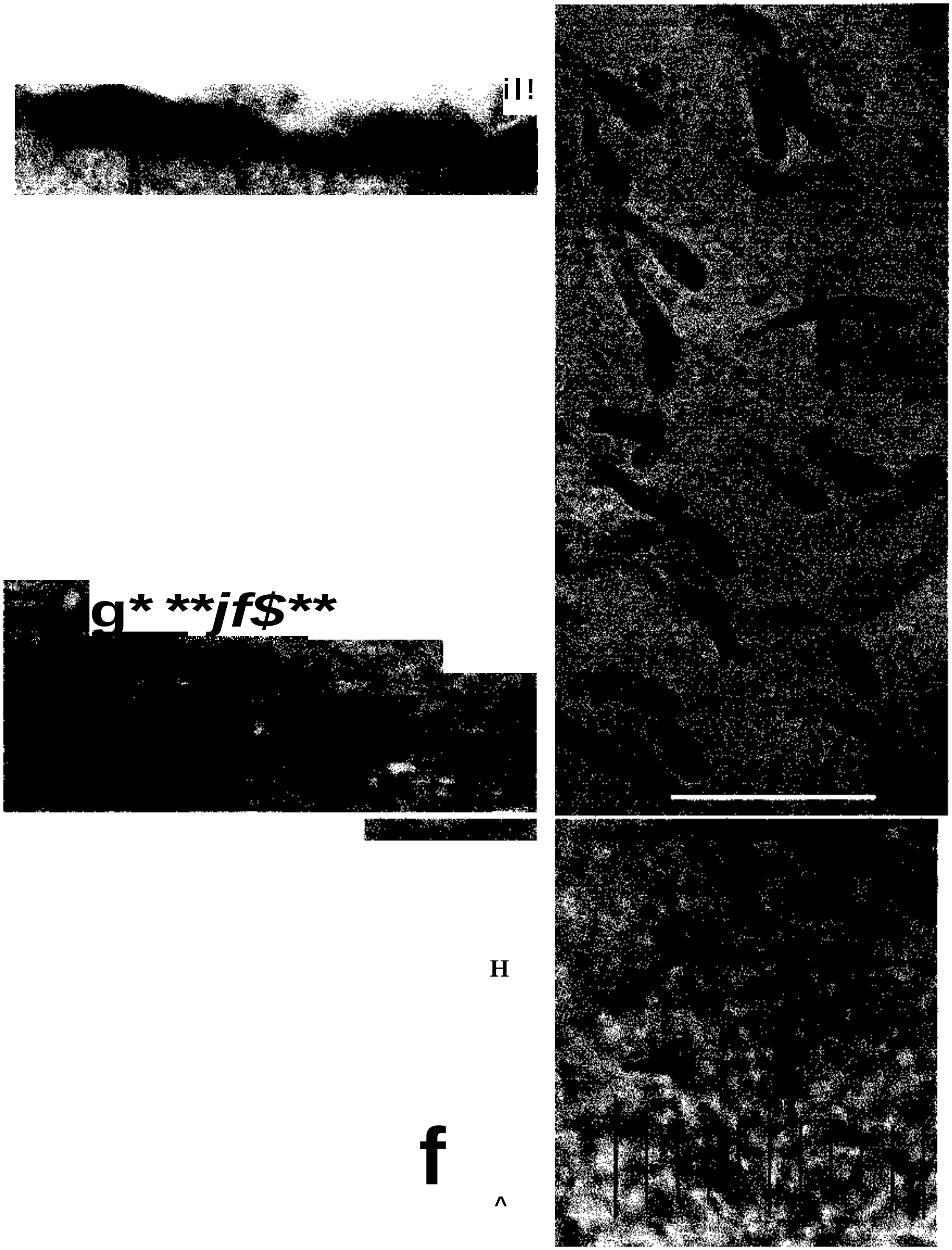


Figure 97 Cutis of /?. *aeruginea:* Top left, section through cutis with scale bar of lOOum, top right, surface view of epicutis with scale bar of 50um, both of *Russula* cf *stenotricha)* from the Long-beach area of the PRNP; bottom, surface view of epicutis of *R. aeruginea* from Koksilah ridge with lOum division scale.

Clade 5a

Subgenus *Russula* Romagn.

Includes subsections *Russula, Consobrinae* and *Sardoninae* of Section *Russula* sensu Sarnari (1998).

***Russula viscida* Kudrna**

Mycologia5:56. 1928

Cap 5.3-7.5cm, convex at first, becoming depressed in the centre and sometimes uplifted towards the margins, but with the margin remaining curved around the top outer edge of the gills, margins smooth. Colour brownish vinaceous, mottled with pallid, brown and dull brownish-yellow areas, viscid (but not exceptionally so), drying matte, minutely concentrically areolate near the margin. Cutis peelable less than 1/4 the radius. Cap trama pinkish under cutis, otherwise light cream, at least twice the depth of the gills at half-radius, unchanging when cut.

Gills arched, adnate, appearing slightly decurrent, fairly shallow, close to crowded, deep cream to ochre yellow, bruising yellow-brown, brittle, with some forking especially near the stipe.

Stipe 4.5-4.8 x 1.8-2.5cm, short and stocky, widest at the apex, very firm, stuffed, not cavitate in this collection, the rind about 1/4 the thickness of the stipe diameter. White, becoming yellow-brown where scraped and at the base, somewhat rugulose.

Texture firm, not brittle

Taste mild.

Odour mild, slightly fruity or nutty.

Spore colour not verified but estimated as cream, Romagnesi (1967) states it as about lib.

Spores 6.2-9.8 (-11.2) x 5.2-8.5 (-10)um, L:W 1.03-1.5, mean 1.27, most broadly ellipsoidal but some narrowly ellipsoidal, globose or bean shaped and occasionally large spores found, the overall impression is of fairly variable shape and size. **Ornamentation** of warts under 0.5um high, bluntly conical to crestate, joined in chains or by fine to heavy lines forming a nearly complete to complete reticulum, **Woo types CI** and **Dl. Suprahilar patch** an irregular, strongly amyloid patch with warts bordering it sometimes giving it a raised appearance, and in some spores the patch forms an irregular collar around the base of the hiliferous appendix. **Hiliferous appendix** relatively large at 0.8-2.2um long and about 1.4um at the base, distinctly 2-tiered. Bastdia 4- and 2-spored, very occasionally 1-spored, 35-52 x 9-15um at the widest point, most are at the smaller end of the range, between 9-12um, the rather more voluminous type (fig. 99) are rather rare. Sterigmata 7-8um long and about 0.8um wide at the base. **Cheilocystidia** and pleurocystidia, 52-85 x 8 -12um, sparse, weakly reacting to SV in dried material, light reddish brown to grey, refractive and yellowish in 5% KOH, projecting about 20um beyond the basidioles, most fusoid, some narrowly clavate or irregularly cylindrical, sometimes with a contorted base originating from the subhymenium. Tips acute or with a short appendage. Subhymeniunt about 30um thick, of jigsaw-puzzle-like pseudoparenchymatous cells, gill trama of irregular sphaerocytes and vascular hyphae

Cutis 150-300um thick at half-radius, subcutis about 100-150um thick. **Subcutis** of interwoven hyphae 2.5-4um wide, occasional ones to 5um, basal layers with vascular hyphae 4-8um wide, many of which stain grey in both SV and acid-fuchsin, others have brownish-yellow contents in SV and in water mounts. Some of these vascular hyphae terminate in pseudocystidia at or below the epicutis. The basal layers of the subcutis are interwoven with the upper layers of the trama so the division between the two tissues is not abrupt. Epicutis an upright to repent turf of yellowish brown hyphal ends, 3-5um wide, some of which in acid mounts appear loosely incrusted with small granular or globular particles. Romagnesi (1962) and Bon (1988) mention dark to yellow-brown pigment inclusions in the hyphal ends, these may not be immediately apparent save that the uppermost layer of the epicutis is somewhat browner; higher magnification reveals these end cells contain 2-3 greyish-yellow globules (a necropigment), almost the width of the hypha. The terminal cells may be clavate, undifferentiated or more commonly tapered, and subapical cells occasionally have diverticulae. **Pileocystidia** abundant, 50-112 x (3-) 6-7.5um, cylindrical to narrowly clavate, 1-3 (- 6)-septate, end cells mostly cylindrical or a little inflated at the base or tip or both, some fusoid, tips obtuse, pointed or capitate. Most stain dark grey in SV, but some appear brownish-yellow, or often with the lower cell yellowish and the terminal cells black, occasionally vice-versa. Some of these have acid-resistant incrustations, especially those more deeply embedded in the epicutis.

Trama of discrete small clusters of sphaerocytes interspersed between thickish bands of interwoven hyphae, the latter being more abundant and denser than in most Russulas.

Chemical reactions: FeS04 - salmon-pink, KOH - bright red on cap cutis, no reaction on mid-stipe but red-brown at the very base of the stipe, guaiac - blue-green, NH4OH - no reaction, phenol - brownish, SV - pinkish-brown on gills and cutis.

Habitat and tree **associations:** This collection was with madrone *(Arbutus menziesii),* with nearby Douglas fir and salal, spruce was absent.

Collections: CR981114-06, Koksilah ridge eastward of Burnt Bridge park, on a roughly south-facing limestone bluff, in young and mature regeneration Douglas fir with pine and madrone, N 48.6558°, W 123.7357° (eastern very dry maritime CWH subzone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR981114-06	874"	*	515,448,254	189*

* This collection amplified only on the second attempt, and then poorly; in the Hinfl and Sau3 AI digestions the larger DNA bands were not visible, a problem when there was insufficient DNA.

Notes: This species has the characteristics of the *Melliontinae* sensu Romagnesi (1967), in which it was placed by him and by Bon (1988). It also keys out to section *Paraincrustatae* subsection *Lepidinae* sensu Sarnari (1998) except for the positive reaction of the above species with SV. Sarnari created a new section, the *Viscidinae* (Sarnari) Sarnari in 1991 for this and related species. It is an unusual species in that the phylogenetic analyses described in Chapter 2 and in Miller and Buyck (2002) placed *Russula viscida* (based on sequence data from a European collection) in a clade with *R. ochroleuca* and *R. atropurpurea* basal to the peppery, pale-spored clade of Russulas which includes *R. fragilis* and *R. queletii,* rather than the higher clades with the *Lepidinae* or *Melliontinae*. It shares with *R. ochroleuca* similar reactions with SV and acid fuchsin, both having scattered occasional incrustations on cuticular hyphae, and is described as sometimes having a slight pepperiness to the gills when young, and both have reticulate spores. Like *R. ochroleuca*, *R. viscida* has cylindrical septate pileocystidia, a character usually associated with higher clades such as the *Tenellae*, which is why both species have traditionally been placed within the upper clades even though the pale spore print is more commonly found in the basal clades. The chromatograph of the Vancouver Island *R. viscida* has a pattern closer to that of *Xerampelinae* of clade 9.

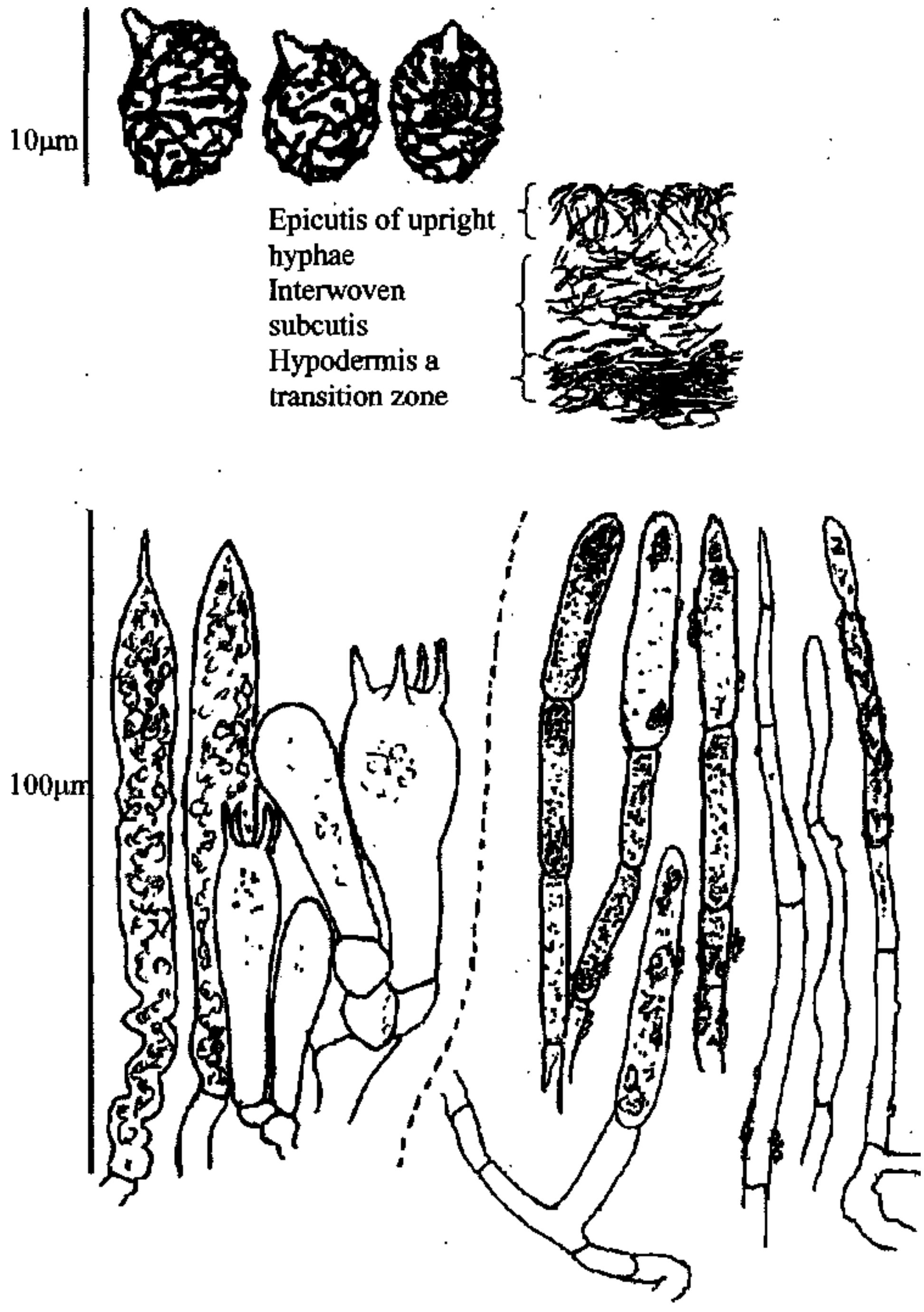


Figure 98 Microscopic characters of *Russula viscida;* top, spores with lOum scale bar; middle, diagram of section through cutis; bottom left, hymenial cystidia and a normal and a large basidium with basidioles; bottom right, pileocystidia and epicutal hyphal ends.

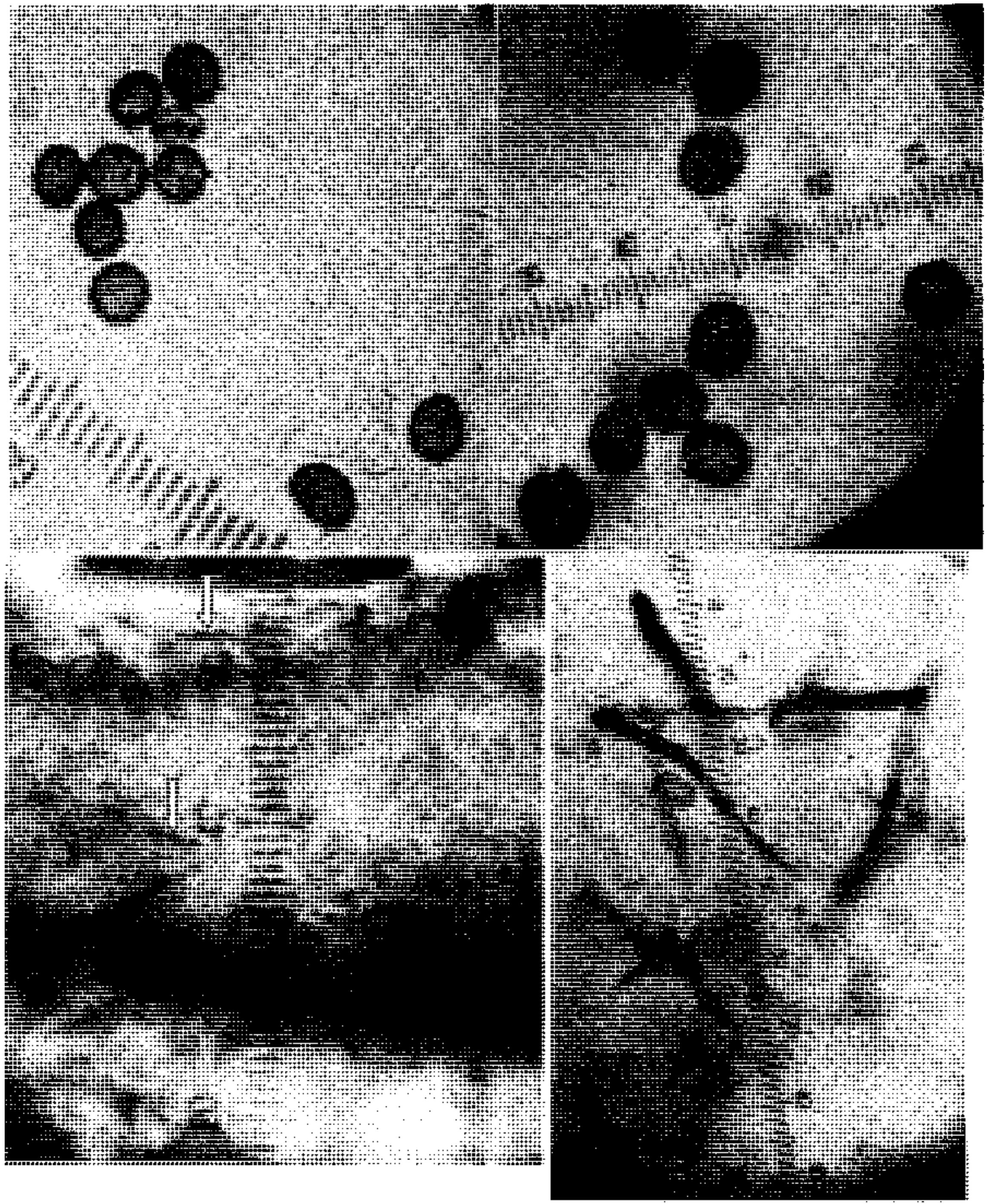


Figure 99 Hymenium and cutis of *Russula viscida:* Top left, basidium and basidioles, 10 scale divisions are 25um; top right, spores with 1um division scale; bottom right, section through cutis of *R. viscida* in S V, in which pileocystidia at the surface and laticifers in the subcutis (arrowed) are darkly stained, 10 scale divisions are lOOum; bottom right, surface view showing several pileocystidia, some of which have cells that do not stain black in SV. Ten divisions of the scale are 25um.

***Russula stuntzii* Grund**

Mycotaxon 9:97 1979

Cap 4.0-10.5cm, hemispherical to convex when young, becoming plane and eventually centrally depressed, sometimes retaining a low umbo, but long retaining a rounded margin, margin mostly smooth developing striations only in age. Colour a rather uniform pale slightly purplish grey, sometimes with a more violet cast, other times more towards pink, perceived colour varies with the light source (fig. 101), usually slightly darker or browner in the centre and occasionally almost white towards the margin, never with yellow or green hues, not discolouring much at maturity, viscid, drying subglossy, peelable 1/4 to 1/2 the radius. Flesh white, unchanging, white to slightly grey under the cuticle. The colour of dried basidiomata is light olive-brown.

Gills white to pale cream, unchanging when bruised, subdistant to close, occasional lamellulae, forking common, mostly near the stipe, adnate to adnexed, obtuse to subacute at the margin, about 5mm deep at half-radius on an averaged size mature basidioma of 6.5cm diameter, this depth being about 1.3-1.5 the depth of the cap trama. Gills pliable when young occasionally slightly greasy feeling as in the *Heterophyllidia,* margins entire.

Stipe 4.9-11.2 x 1.1-2.9cm, more or less cylindrical or widening slightly at the base or apex, white, when young a white pruina may overlay the pale cream surface, not braising but turning buff-grey as if waterlogged in age, stuffed with a bread-textured trama that develops irregularly shaped and distributed cavities, rind firm, pale greyish, around 2mm thick.

Texture fairly firm and pliable when young, becoming fragile in age.

Taste sometimes slightly bitter at first, then peppery to acrid, sometimes the acridity is immediate.

Odour not distinctive to faintly fruity, sometimes also with a faint cedar component.

Spore **colour** pale buff, Romagnesi Ha but less yellow and more pink.

Spores 6.8-10 (-11) x 6.2-8.9nm, L:W 1-1.43 with a mean of 1.17 (n = 50), globose, subglobose or broadly ellipsoidal. **Ornamentation** of conical warts, occasionally heavy, 0.8-1.4um or sometimes up to 2um high, isolated or with mostly fine connectives not forming a reticulum or at most a partial one, Woo type B3 -C3. **Suprahilar patch** amyloid, more or less rounded but with irregular margins that are often more strongly amyloid or raised slightly, and sometimes with a lower but otherwise similar ornamentation to that of the rest of the spore. **Hiliferous appendix** relatively large, 2-2.8um long by 1.2-1.7um wide at the base. **Basidia** mostly 4-spored, occasionally 2-spored, 47-60 x 9-13 urn, clavate and slightly bulbous in the upper 1/3 to 1/2. Sterigmata 5-8um long and 1.5-2.5um wide at the base. Pleurocystidia abundant, 52-105 x 7-1 lum, protruding 15-25um, originating in the subhymenium or the outer gill trama, blue-black in SV (but reacting only weakly in one collection), contents refractive in KOH, fusoid to narrowly clavate, occasionally cylindrical, bases variable in length and shape, tips acute or mucronate, less often capitate. Cheilocystidia frequent, sometimes forming a microscopic fringe, protruding 15-50um, similar to pleurocystidia in shape, blue-black in SV, sometimes with yellow granulations. **Subhymenium** 20-45 um thick, pseudoparenchymatous, sometimes also with a narrow layer of interwoven hyphae directly beneath the hymenium, gill trama of sphaerocytes with frequent vascular hyphae.

Cutis 170-250um thick at half radius, an ixotrichoderm of three layers. Ilypodermis of repent, tightly interwoven hyphae and numerous vascular hyphae, all greyish purple in SV and brownish in KOH. Strbcuiis of hyaline more loosely interwoven gelatinized hyphae 2-4um wide, through which penetrate frequent upright vascular hyphae, about 4um wide but sometimes broadening tolOum, and extending into the epicutis, dark grey in SV. **Epicutis** a turf of upright hyphal ends 1.5-5 urn wide, some of which terminate in a small cystidioid cell with refractive contents, others undifferentiated, many with a capitate terminus, interspersed with abundant long cystidia lying along the epicutal surface for part of their length. Pileoeystidia unevenly distributed, in small tufts of 6-7 or single, 60-230 x 5-10um, but most in the mid-range width, 0-1-septate, cylindrical, tips mostly capitate, some obtuse, SV+. Intermediates between the small cystidioid hyphal ends (from 10 x 3 urn) to the larger-sized cystidia can sometimes be found.

Pseudocystidia abundant, much more frequent than pileocystidia, generally regularly septate, sometimes with inflated sections up to 13um or allantoid (sausage-shaped) cells, tips obtuse or capitate in roughly equal proportions, contents refractive, staining dark grey in SV.

Trama of irregularly sized and shaped clusters of sphaerocytes bound by a hyphal mesh with frequent vascular hyphae.

Chemical reactions: FeS04 - light greyish pink; KOH - no reaction to slightly pinker or browner on cap surface, no reaction to yellowish on stipe; NH4OH - no reaction; guaiac - blue-green; phenol - brownish purple; SV - reddish purple on the gills, magenta then greyish purple on cuticle, cystidia and vascular hyphae grey to black.

Habitat and tree associations: in small groups of two or three but in one case trooping, on conifer logs that have brown cubical rot and become friable, with western hemlock, other trees within the habitats may include red alder, Douglas-fir or Sitka spruce. The primary host tree is probably western hemlock as that was consistently present in *R, stuntzii* habitats. October to February, generally ceasing fruiting after heavy frosts but in one case fruiting two days after a 20cm snowfall.

Collections: CR981029-RF8, on large cedar log of decay class 4, on a small section of more advanced decay, near western hemlock, Sitka spruce and western red cedar, Rain Forest trail east side of highway, P.R.N.P. south of Tofino, N49.446778^0, Wl 25.531694° (southern very wet hypermaritime CWH subzone). CR001024-01 on partially buried decayed log near old growth Douglas fir, western hemlock, red alder and big-leaf maple, Mystic vale, University of Victoria grounds N4834598^0, W123.3071^0. CR001127-03 on very decayed moss-covered logs, possibly hemlock and woody debris in mature regeneration forest with some old growth Douglas fir, also western hemlock (mature and understory saplings), western red cedar and oregon grape, Royal Roads University woodlands, N48.434467^0, W123.478867^0. OC010218-01 from a buried decayed Douglas fir log amongst old growth and mature regeneration Douglas fir and western hemlock, Royal Roads University woodlands, exact location not recorded. BT021101-01 on decayed Douglas fir log and woody debris with old growth western hemlock, western red cedar and salal, Royal Roads University woodland, N48.433417^{0}, W123.477417^{0} (last 4 collections from CDF zone).

Collection	ITS1-F to ITS4-B	RFLP: Hinfl	Alul	Sau3A
CROO1024-01	932~	375	526,262	445,214
BT021101-01	892	400	517,286	421,316,221

Notes: *Russula stuntzii* has a distinctive colour that can be distinguished from *R. fragilis* by its distinctive nearly uniform colour with no traces of greens or dark, clear purples or violets, it tends to be firmer, larger, and usually has a straight stipe, whereas that of J¹?. *fragilis* is often curved and the spore colour differs. *Russula stuntzii* has no clear yellow in the cap unlike *R. raoultii* and pale forms of *R. bicolor* and *R. silvicola,* both of which usually grow on wood. Grund's description of *Russula stuntzii* states that the spore print is white, and suggests that collections with pale salmon coloured spores could be *R. albiduliformis* Murr. or *R. pantoleuca* Singer but those species are mild, the former has SV negative cuticular cystidia and is milk-white, the latter a rare eastern species of oak woodlands with pale brownish or greenish tints in the mostly white cap. Thiers (1997) described *R. stuntzii* spores as white to pale yellow and the cap colour as ranging from white to greyish with purple tones. The Vancouver Island material all had pale buff coloured spores and purple or pinkish-grey caps, even old faded basidiomata retained some purplish-grey tints. The spores also extend the upper size limit by lum on the length and 1.4um on the width, and the ornamentation ranges higher than the lum limit for Californian and Washington material, all other characters agree with Thiers and Grund's descriptions. Grund first described *R. stuntzii* in his thesis in 1965 as *R. pallidolivida* nom prov. after finding it twice over a 3-year period. He considered it rare in Washington. On Vancouver Island the five collections were made in old-growth forests with ample decayed woody debris, a habitat rare in itself. Thiers (1997) noted that it is the only *Russula* species in California that often grows on well-rotted conifer logs, and that it is found in coastal and montane forests throughout the state.

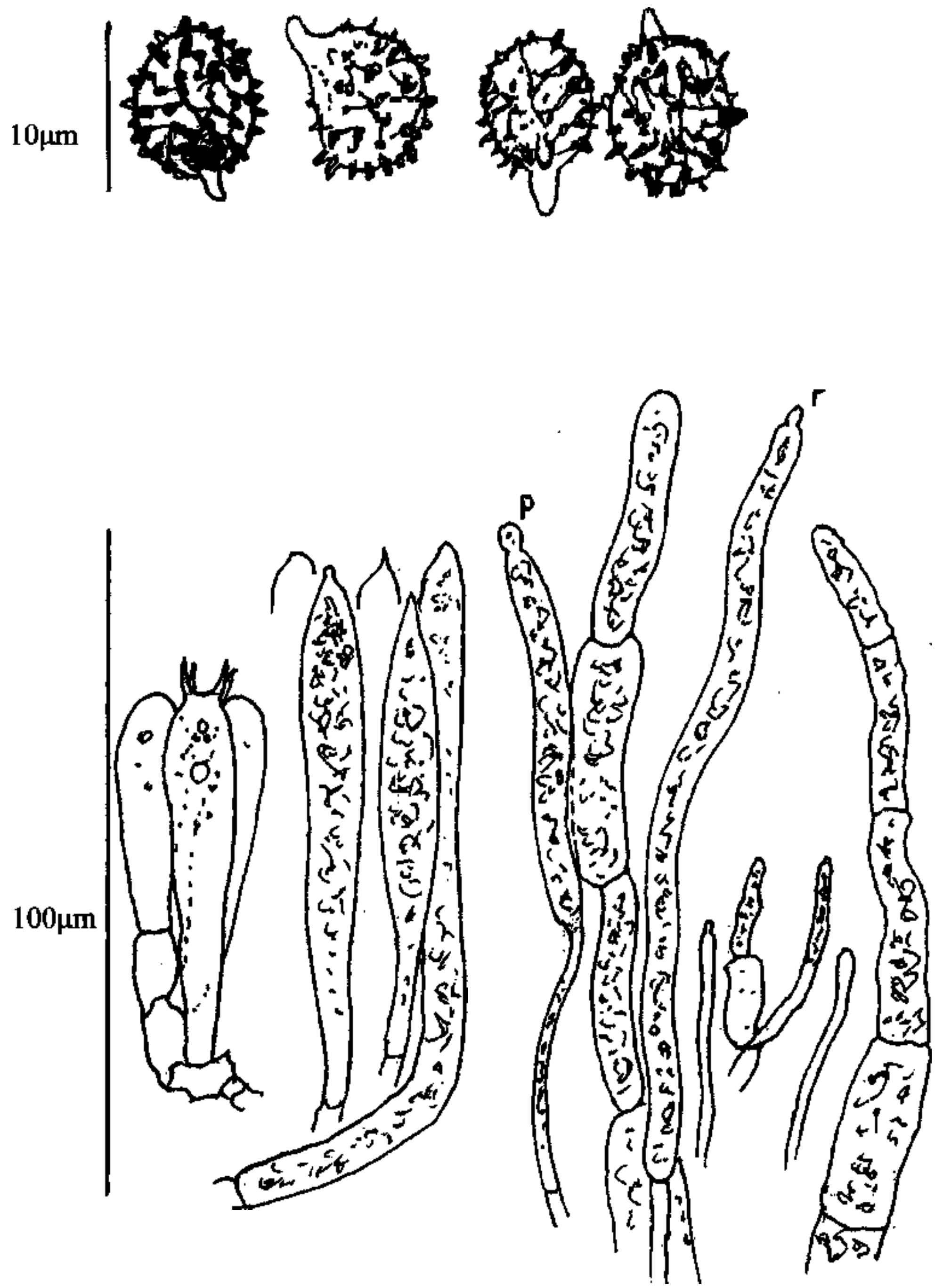


Figure 100 Microscopic characters *ofRussula stuntzii:* Top, spores with lOum scale bar; botton left, basidium and basidioles and hymenial cystidia; bottom right, pileocystidia (marked 'p'), pseudocystidia and hyphal ends from the epicutis, scale bar is lOOum.

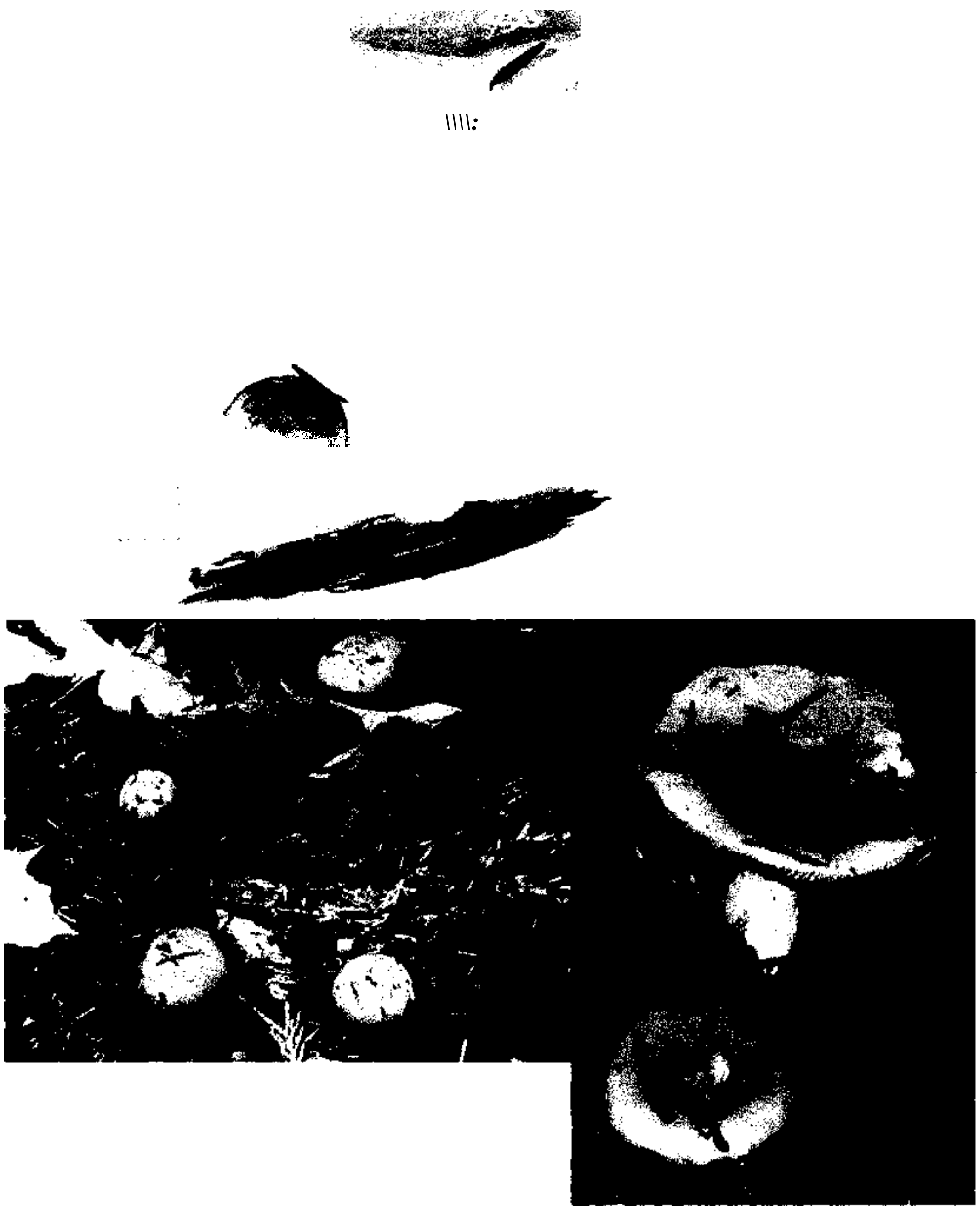


Figure 101 Macroscopic characters of *Russula stuntzii:* Top, illustration showing profile of immature and mature basidiomata and a longitudinal section, the $1cm^2$ square shows spore colour; bottom, in situ on rotten logs, on the left are buttons photographed in daylight, on the right, an immature and mature specimen photographed with an electronic flash, note that the colours appear pinker with the flash. (Photograph on the right by Bryce Kendrick).

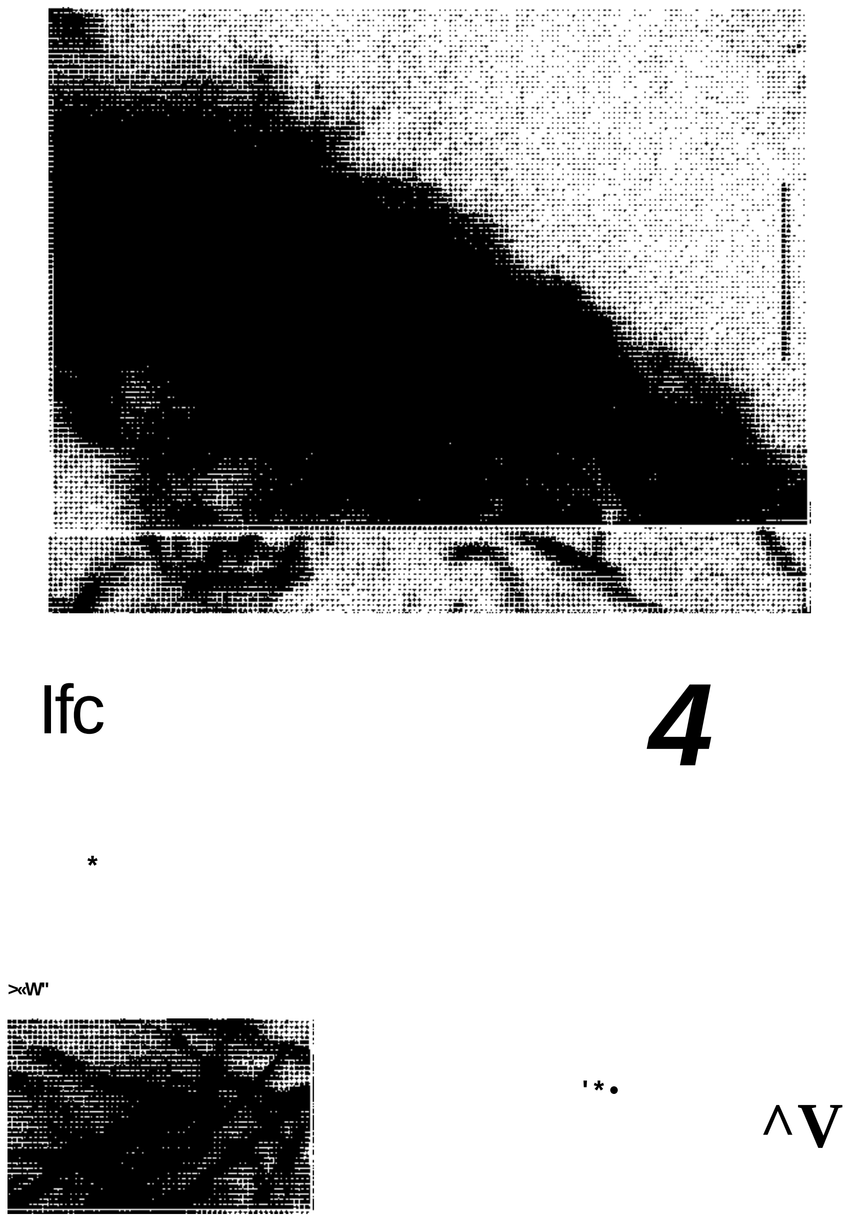


Figure 102 Cutis of *Russula stuntzii*, top, section through the cutis in SV showing the many dark pseudocystidia arising at or below the basal layer of the subcutis and emerging at or near the epicutis, bottom, surface view of pileocystidia and pseudocystidia. Scale bars are lOOum.

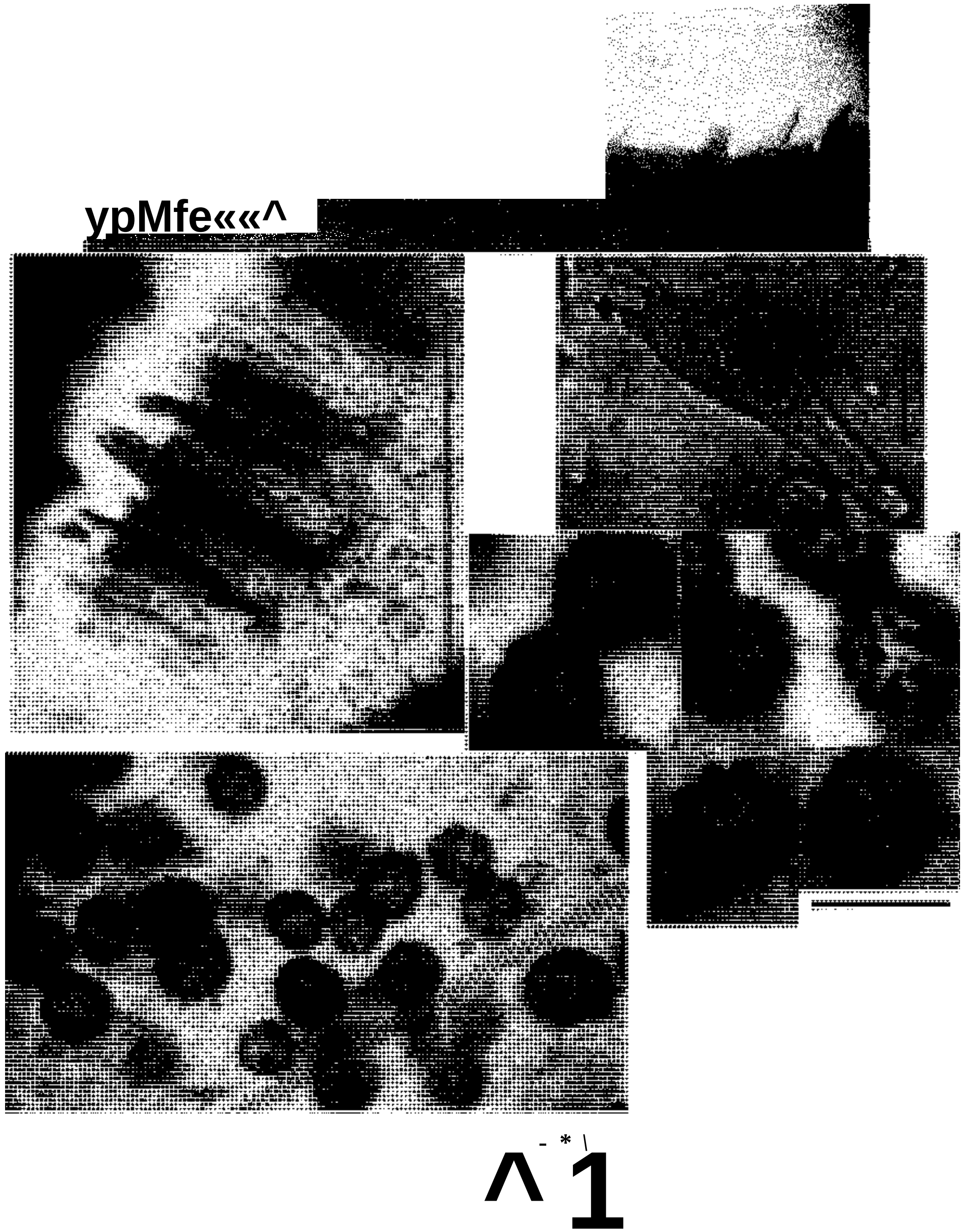


Figure 103 Hymenium of *R. stuntzii*. Top, gill margin with cheilocystidia stained with congo red, ten scale divisions are lOOum; middle left, section of gill showing pleurocystidia in SV, scale bar is lOOum, middle right, a basidium (2-spored) with lOum scale bar; below basidium, details of spore ornamentation, composites of two depths of focus, scale bar is lOum; bottom, spores in Melzer's reagent, 1 scale division is lum .

Clade 5b

Section *Russula* Bon

Series *Atropurpurea* Romagn.

***Russula raoultii* Quel.**

Compte Rendu de l'Association Francaise pour l'Avancement des Sciences 14:449. 1886

Cap 2.5-6. Ocm, convex with a small flattened umbo when young, becoming shallowly depressed at maturity and sometimes retaining a vestige of the flattened umbo. Margins smooth, becoming striate about 1/5 the radius from the margin in age. Colour pale yellow to cream over the disc, paler towards the margins which are whitish, sometimes a very faint pinkish tinge is discernible in the outer 2-3 mm, but more often a greyish to greyish yellow occurs as the trama beneath the cutis discolours in age. This greying is more of a waterlogging rather than a bruising effect. Surface viscid drying matte to glossy depending on age. Cutis peelable 1/4 to 1/3, rarely to 1/2, flesh beneath white, unchanging where bruised but acquiring a yellowish-grey waterlogged appearance in age.

Gills white to very pale cream, occasionally with a slightly pinkish cast when young, close to crowded, sometimes subdistant, lamellulae occasional to frequent, absent in some basidiomata, lamellae generally with some forking. Shape arched to fusoid, equal to about 1.3 the depth of the cap trama at half-radius, acute at margin and stipe, very narrowly adnexed to free, margins entire and smooth on most collections, and it appears from microscopic examination that cuticular-like gelatinizing hyphae continue along the gill margins in many basidiomata. Gills not bruising, pliable when young, brittle in age.

Stipe 2.0-9.0 x 0.8-1.8cm, ranging from shorter to longer than cap diameter but most are longer, white, smooth, stuffed with soft bread-textured trama, becoming hollow with age, unchanging when bruised but like the rest of the trama developing a greyish waterlogged appearance in age.

Texture soft and fragile.

Taste quickly very acrid.

Odour fruity and sometimes also of coconut, occasionally faintly reminiscent of the *Ingratae* (spermatic-rubbery).

Spore colour white, Romagnesi la to paler than lb.

Spores of two size ranges: collection CR021118-02 is within the range expected for *Russula raoultii;* 6-8 x 5-6.8um, L:W 1-1.42 with a mean of,1.19 (n = 15); all other collections are 6.8-10 x 5.5-8um, averaging 8.3 x 6.8 (n = 95) with a L:W of 1-1.45, mean 1.21. Spores are subglobose to broadly bean-shaped to ellipsoidal. **Ornamentation** (for all collections) is of low short ridges or angular warts with blunt to pointed tips, 0.2 - 0.8um, rarely, the occasional wart may reach 1.0-1.2um. Warts rarely isolated, most catenate or with fine to heavy connectives forming a complete reticulum or nearly so, **Woo types** Dl, D2 -E2. **Suprahilar patch** amyloid, sometimes also with small warts. **Hiliferous appendix** around 1.8um long, lum wide at base. **Basidia** mostly 4-spored but 2-spored ones common, 35-45 x 9-12um, clavate to bulbous in the upper 1/3, sterigmata relatively large, 5 -lOum long and 2um wide at the base. **Pleurocystidia** 32-75 x 6-1 lum, occasionally to lOOum long or to 15um wide, protruding around 20-25 urn, originating in the subhymenium, brownish purple in SV, contents yellowish and refractive in KOH, fusoid, tips obtuse or mucronate, the appendage short, pointed or button-like, those with an apical exudate quite common. **Cheilocystidia** numerous, absent in occasional sections, reddish or black in SV, making up most of the gill margin which is otherwise nearly sterile, protruding around 30um, similar to pleurocystidia in contents and shape, however many of them appear to leak their contents or exude them from the tip, eventually the protruding part becoming repent and agglutinated along the gill margin. This phenomenon occurs more in some basidiomata than in others. **Subhymenium** 20-25 urn thick, intermediate between interwoven and pseudoparenchymatous, i.e., an interwoven layer with many inflated cells. **Gill trama** of quite large sphaerocytes, up to 50um across, with occasional SV+ vascular hyphae.

Cutis 100-200um thick, occasionally to 300um on the disc but more often towards the thinner end of the range, an ixotrichoderm. **Subcutis** about half the depth of the cutis, of tightly interwoven hyaline gelatinised hyphae l-3um wide, with vascular hyphae in the basal layers, many of which rise into the epicutis and terminate in clavate pseudocystidia. **Epicutis** a turf of upright undifferentiated hyphal ends 1-3 urn wide, also some with non-refractive cystidiform end cells up to 9um wide but mostly narrower, embedded in a gelatinous matrix. Vascular hyphae frequent, dark grey in SV. **Pileocystidia** numerous, 60-100 x 6-9 (-12.5)um, 0-1-septate, clavate, ends mostly rounded, some capitate-strangulate, contents yellow, refractive, and unstaining to red to strongly blackening in SV, the reaction is strongest on fresh material and seems to decline after drying. **Pseudocystidia** frequent and similar in shape and SV reaction to pileocystidia. **Hypodermis** a distinct layer of compressed yellow-brown cells.

Trama of loose clusters of sphaerocytes bound by a hyphal mesh and frequent SV+ vascular hyphae.

Chemical reactions: FeSCU- light greyish pink; KOH - no reaction on cap surface, none to slightly yellowish on stipe; NH4OH - no reaction; guaiac - rapidly blue-green; guaiacol - light purple; Phenol - slowly pink then brownish purple; S V - grey on the gills, deep pinkish on the cuticle.

Habitat and tree associations: On or near very decayed coniferous wood (class 5) in old-growth and mixed age stands of Douglas fir or Sitka spruce-western hemlock-western red cedar forest, often with the understory shrubs salal and huckleberry.

Collections: CR981013-06 from Cape Scott, near the San Joseph trail, in a mixed age stand of western hemlock with huckleberry understorey, N 50.768°, W 128.342°. CR 010920-10 from the PRNP Rain Forest trail (eastern side of the highway) on a decay class 4 spruce log with salal and huckleberry under western hemlock and Sitka spruce, N

49. 446778° W 125. 531694°. CR001012-15, from the SW corner of Kennedy Lake Park, offGrice Bay main logging road in the Ucluelet area, under western hemlock with salal and huckleberry, N 49.016883°, W 125.58153° (all above from southern very wet hypermaritime CWH subzone). CR001108-05 and CR001127-04, both from Royal Roads University woodland amongst woody debris near an old-growth Douglas fir N 48.4358°, W 123.47893°. BT021101-02 and CR 021118-02 from Royal Roads University woodland on decayed logs in mature regeneration forest with western hemlock, western red cedar and salal, N 48.433250°, W123.4780^0 and N 48. 434167°, W 123. 4765° respectively (last 2 collections from CDF zone).

Collection	ITS1-F to ITS4-B	RFLP: HinfI	AluI	Sau3A
CR021118-02 Royal Roads	868	339, 272	527, 286	281, 200
CR001127-04 Royal Roads	873	344,270	495,286	326,227
CR001108-05 Royal Roads	884	380,279	485,124	346,254

Notes: This species differs from the following species; *Russula crenulata,* in its smaller, more fragile stature, its shorter more clavate 0-1-septate pileocystidia, and the coarser warts and reticulum on the spores. This latter character is difficult to visualize without a side-by-side comparison; the above collections have spores very like those *of Russula fragilis* but with lower warts, and *R. crenulata* has spores that resemble those of some of the *Heteropnyllae* except for the amyloid ornamentation on the suprahilar patch. Shaffer (1975) considered American forms of *R. raoultii* as close to *R. silvicola* Shaffer, a red-capped species inhabiting woody debris, and in which all the characters agree except for cap colour. The characters are also very similar to those of *R. fragilis,* a species with many named colour forms, *R. fragilis* f. *nivea* Cooke is a white form and f *raoultii* Quelet is pale yellow. However, there do not appear to be intermediates, as might be suggested by the subspecific taxon, at least locally, between the above *Russula* and *R. fragilis.* The Vancouver Island *R. raoultii* agrees with the descriptions in Romagnesi (1967) and Shaffer, (1975) for eastern North American collections with the exception of the larger spores. Romagnesi mentions the hymenial cystidia as often having a voluminous tip, which may or may not be the same as the exudate described above;

neither he nor Shaffer mention a gelatinous gill margin. Shaffer examined one collection growing in sphagnum from Michigan that had spores up to lOum long and 9.0um wide but was otherwise the same as other collections of *R. raoultii*. Vancouver Island collections represent a form or subspecies of *R. raoultii* with a larger range of spore sizes up to 10 x 8um.

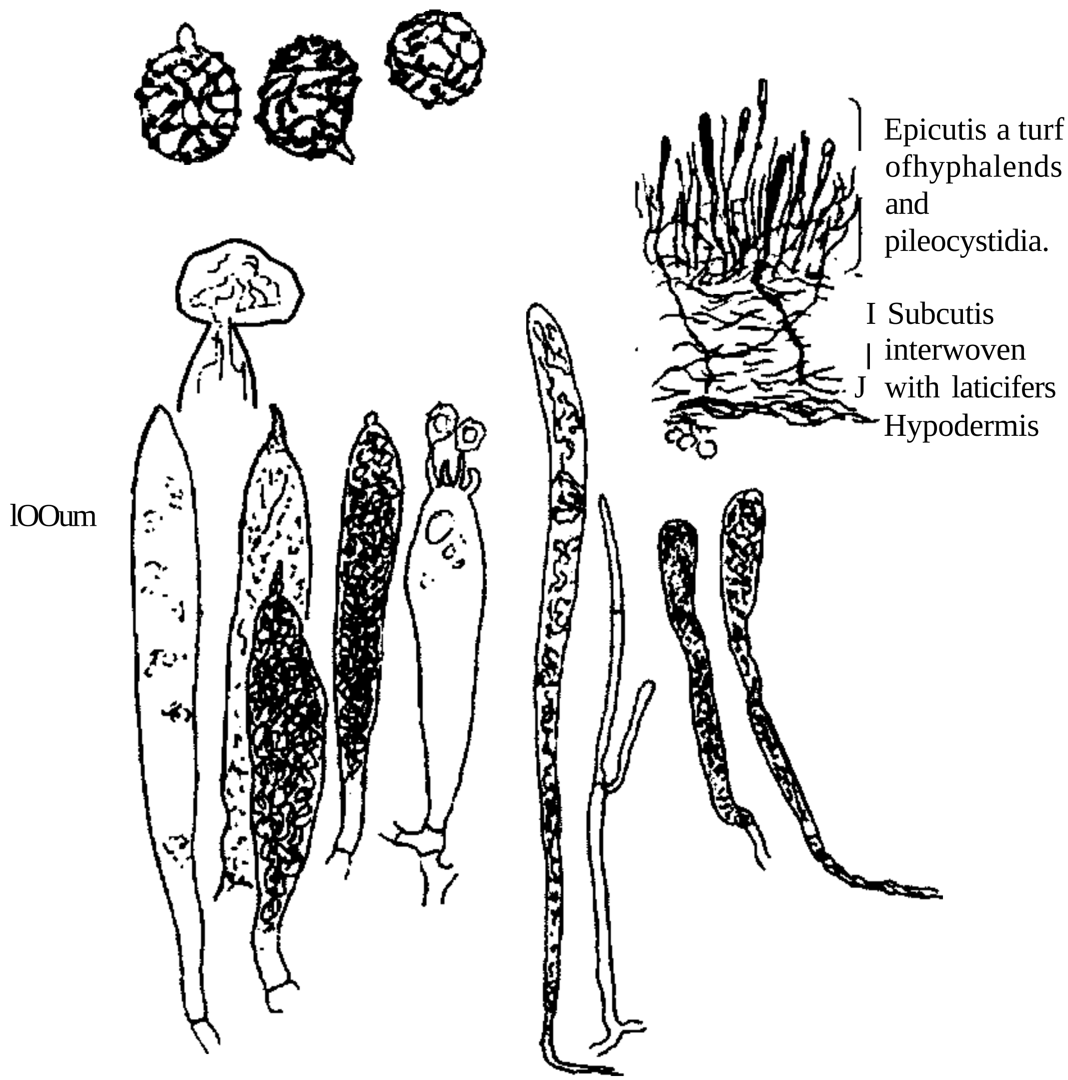


Figure 104 Microscopic characters *ofRussula raoultii.* Top, spores with 10 um scale bar; upper right, section of cutis; bottom left, hymenial cystidia in KOH and in SV (the darker two) and a basidium; bottom right, pileocystidia, epicutal hyphal end and pseudocystidia, scale bar is 100 um.

Figure 105 Macroscopic characters of *Russula raoultii:* Top, immature and mature, in profile, top view and section, square is 1cm^2 and shows spore colour; bottom, a collection from the rain forest near Long Beach in the Pacific Rim National Park, which was growing amongst mosses and woody litter as seen from the stipe bases.

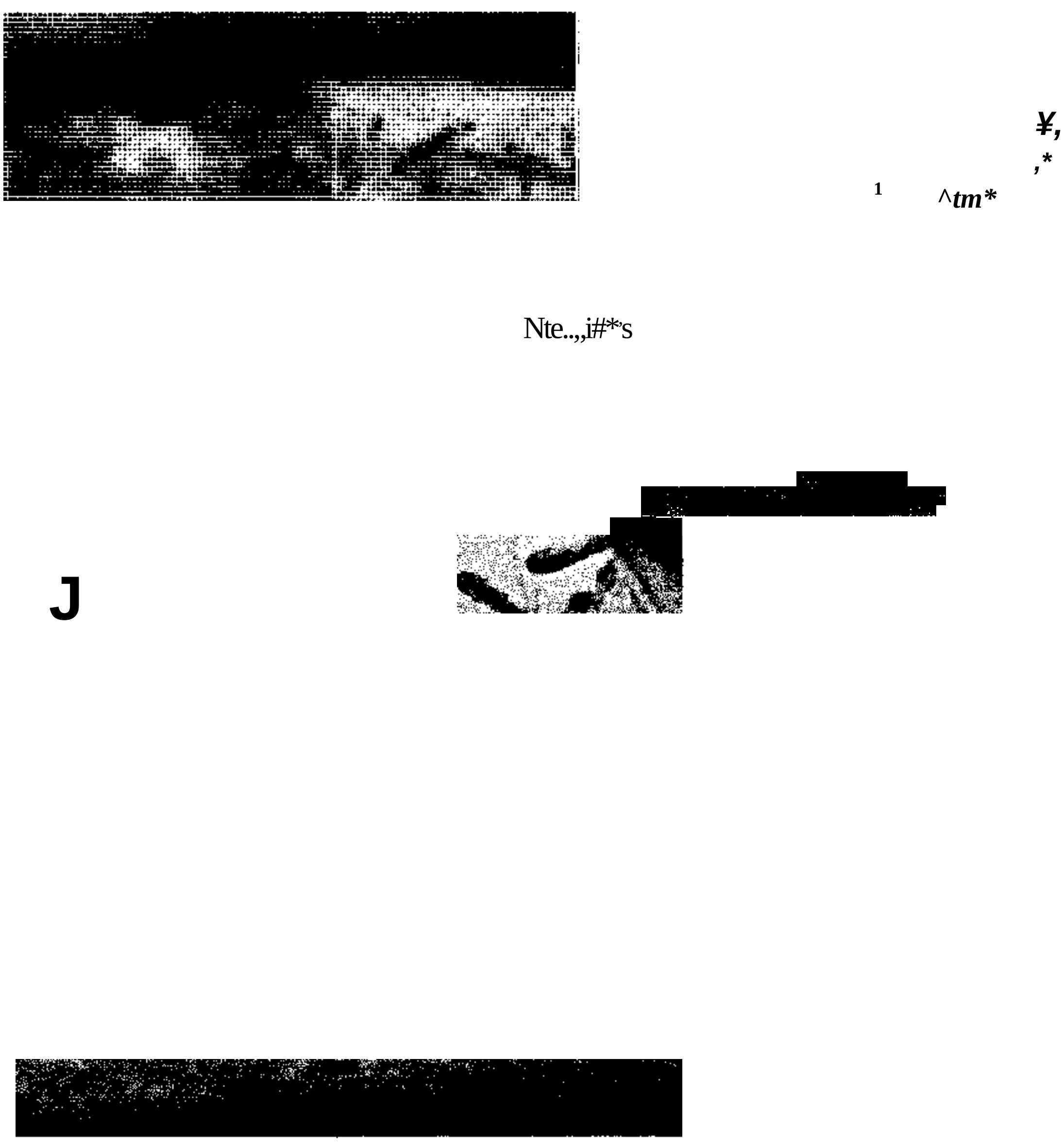


Figure 106 Cutis of *Russula raoultii:* Top left, section through cutis showing darker hypodermium; middle, surface view showing numerous pileocystidia and pseudocystidia scale bars are both lOOum; bottom, section through epicutis with many pileocystidia, 10 divisions on scale are 25 um.

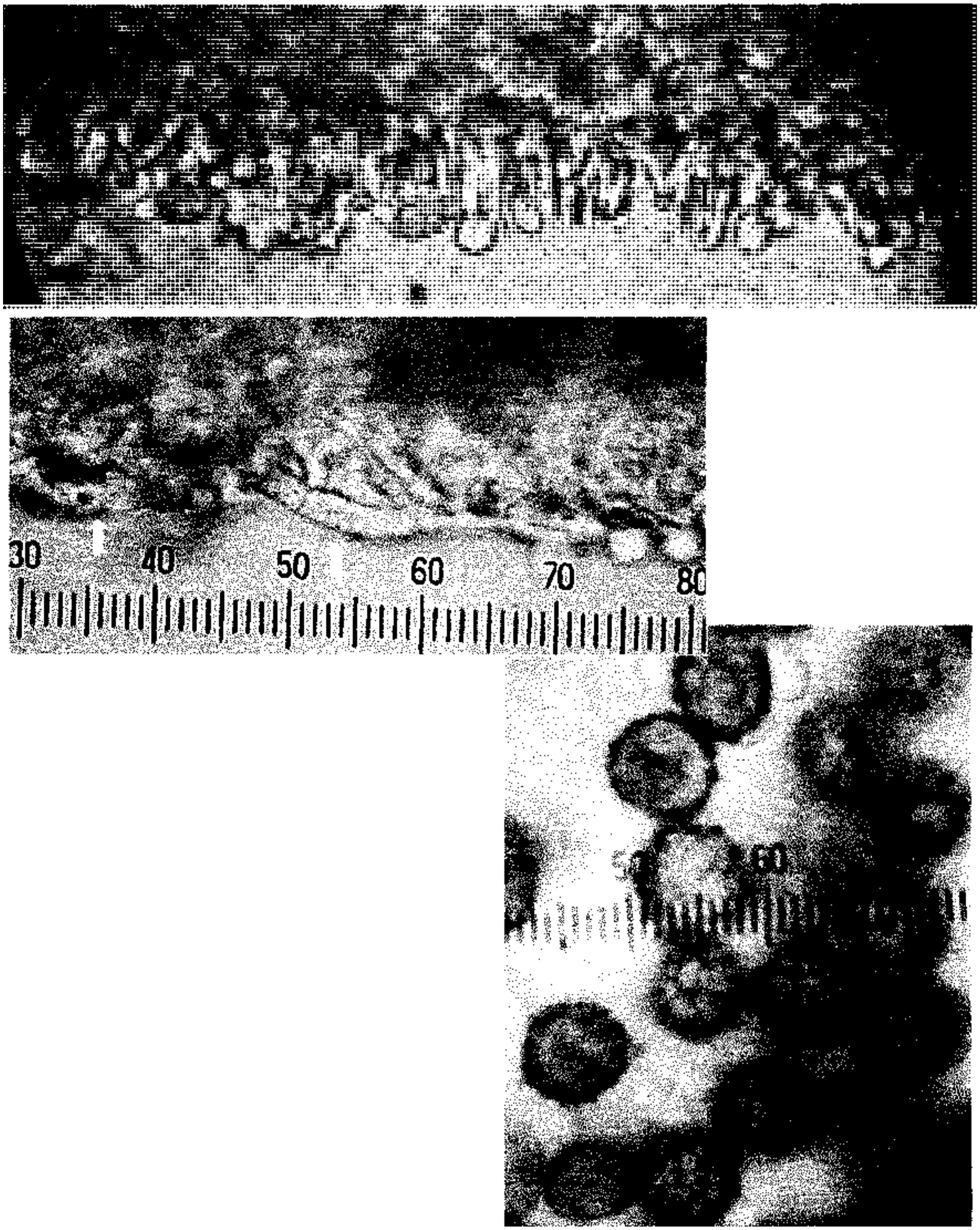


Figure 107 Hymenium of *Russula raoultii:* Top, gill margins of a young basidioma, the cheilocystidia are erect but many have an exudate appearing as a refractive gelatinous blob at the tip, as if a remarkable number of tips have broken off and released their contents (scale bar is 25um); middle, in maturity the cheilocystidia (arrowed) become adhered along their length to the gill edge by this gelatinous substance, 10 scale divisions are 25um; bottom, spores with lum scale.

***Russula crenulata* Burl.**

Mycologia 5:310. 1911

Cap 4.9-8.9cm diameter, convex when young, becoming shallowly and broadly centrally depressed, unevenly uplifted towards the margins, but with the margin remaining downcurved, forming an adnate junction with the gills, margins smooth, not inrolled, cream with slight hints of pinkish tones, darker and more ochraceous in the centre, paler to almost white at the margins, viscid when moist, drying matte, peelable to 1/4 (Mill Hill collections) to 2/3 (Mesachie Lake collection) the radius. Cap trama white, firm, approximately equal in depth to that of the gills at half radius, about 7 mm, and maintaining more or less this thickness outwards to about 3/4 the radius, where it tapers to the margin, unchanging where cut or damaged.

Gills white to pale cream with warm, very pale orange cast when seen edge on, almost free to distinctly sinuate to notched at the stipe, even to slightly ventricose, approximately equal in depth to cap trama at half-radius, adnate at margin, pliable, margins entire and not always obviously crenellate to minutely unevenly fringed, subdistant to close with occasional short gills and a small amount of forking towards the margin, unchanging in colour where bruised.

Stipe 5-7.8 x 1.4-2. lcm, white, somewhat rugulose, unchanging when cut, slightly yellowing and with some light rusty stains at the base where bruised, stuffed with a firm, bread-textured trama, not cavitate and long remaining so, but developing a cavity in age, rind approximately 3-4 mm thick in the larger specimen and about 1/5 to 1/4 the diameter of the stipe in other specimens.

Texture firm but fairly brittle.

Taste initially mild, then on chewing, slightly to strongly peppery.

Odour mild, mushroomy, and with a slight coconut to rubbery smell.

Spore colour white, Romagnesi la.

Spores 7.8-9.5 (-10) x 6.0-7.5um, L:W 1.18-1.45, average 1.29, narrowly to broadly ellipsoidal. **Ornamentation** of warts 0.4-0.6um high, mostly low, small, rounded to bluntly conical, some catenate, some joined by fine to heavy ridges and lines forming an almost complete reticulum, and in the larger interstices of the mesh, the spore wall often bears light greyish amyloid areas. **Woo types C 1-2, D 1-2. Suprahilar patch** variable in shape, size, and amyloid reaction, on some spores one or two small irregular lightly amyloid patches occur on an otherwise unornamented area, in others a smaller version of the general spore ornamentation continues over an inamyloid area, making it difficult to detect any form of patch, and on others there is a combination of amyloid patches and small warts. **Hiliferous appendix** 1.4-1.8um long and around 1.2um at the base. **Basidia** 37-50 x 8-13um, narrowly clavate, becoming broader towards the upper 1/3 at maturity, mostly 4-spored. Sterigmata 3-7um long and about lum wide at the base. **Pleurocystidia** 50-120 x 7-10um, arising at various levels within the subhymenium, protruding around 20-25 um (not including the appendage), beyond the basidioles, fusoid, tips acute, capitate or mucronate with the appendage generally short and slender, contents refractive and yellowish in 5% KOH, most blackening in SV, some merely stain dark pink. **Cheiiocystidia** sparse, to frequent and clustered, similar in shape to pleurocystidia or sometimes rather broader, to 15um, with contents less refractive and less reactive in SV than pleurocystidia, protruding up to 50um. **Subhymenium** about 35-40um thick, pseudoparenchymatous.

Cutis 150-250um thick, the subcutis about 80- lOOum thick, of hyaline interwoven repent, more or less parallel hyphae 1.5-2um wide, embedded in a gelatinous matrix, and overlaying a more yellowish layer containing many vascular hyphae, some of which ascend to the epicutis as pseudocystidia. This lower layer grades into a sometimes poorly differentiated hypodermis. **Epicutis** a turf of slender erect hyphal ends about 2-2.5um wide and numerous pileocystidia with little gelatinous matrix. **Pileocystidia** abundant, 50-120 x 7-12um, cylindrical to clavate or often with bulbous ends, shorter ones aseptate, most with up to 4 septa, also numerous cylindrical, regularly septate **pseudocystidia**

emanating from the subcutis, most staining black in S V, but some showing much weaker reactions.

Trama of discrete and rather distantly separated clusters of sphaerocytes surrounded by hyphae with frequent vascular hyphae.

Chemical reactions: FeS04- pale brownish pink; KOH - no reaction on cap or stipe; NH4OH - no reaction; guaiac - brownish at first, becoming blue-green after several minutes, phenol - slowly grey-purple to brownish; S V - initially magenta on cap trama and gills, then brownish purple.

Habitat and tree associations: On Mill Hill in Garry oak woodland on rocky east-facing slope with mature oak trees, some broom and other small shrubs, no Douglas fir in the immediate area, but many nearby. At Mesachie Lake the habitat is a mixture of conifers and deciduous trees including Douglas fir and oaks.

Collections: CR030304-01 found on Mill Hill, near the top to the east side, N 48.457°, W 123.478917°; OC 040931-01, from the same habitat, about 100m NW of the former collection (CDF zone). JD021027-03, from the grounds of the Mesachie Lake Forest Research Centre, exact location unknown, brought in during a S VIMS foray (western very dry maritime CWH subzone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul		Sau3A
CR030304-01	865	380,321	466,260	~	632,227

Notes: These collections are not as acrid, have lower spore ornamentation, and are firmer fleshed than is described for *R. crenulata.* The identification rests on the spore size and reticulations, habitat with oak and the distinctive septate pileocystidia, the gill margins are not crenulate in the Mill Hill collections but they are in the Mesachie Lake collection. Thiers (1998) comments that the crenulate margins may not be a reliable character. This species has since been found in the same general area (Mill Hill park just off the summit), in the fall of 2004 (O. Ceska, pers. comm.) It bears macroscopic and spore similarities to *R. ochroleuca* but that species lacks pileocystidia. Other similar white-spored, none bruising species include *R. raoultii,* which has reticulate spores but they are smaller, and the pileocystidia are mostly aseptate; and *R. cremoricolor,* which has non-reticulate spores but a similar wart height to these Vancouver Island collections. Both are acrid. Grund (1965) made four collections from coniferous forest that he identified as *R crenulata,* that have little to no reticulation on the spores, he did not mention any crenellations on the gill margins nor septation of the pileocystidia, and they appear to be different from the Mill Hill collection. He also made one collection of what he provisionally named *Russula pallidostraminea* from Friday Harbour Laboratories on San Juan Island, that has similar trama characteristics and spore size with the low, reticulate ornamentation found in the above collections. A distinguishing feature of Grund's collection was the strong orange reaction with guaiacol, and brown with aniline, unfortunately neither test was administered to the Vancouver Island collections while fresh. There are several species of cream coloured, white-spored, peppery Russulas on Vancouver Island, and they may recognisably differ. However, matching them to a description is not always easy. A table summarizing the differences between peppery, white-spored yellowish-white Russulas of similar size-range and all with close to subdistant lamellae follows, and it can be seen that the above species has several character matches with all four species. The information was taken from Thiers (1997), Romagnesi (1967), (Sarnari 1998) Shaffer (1975) and supplemented from the original literature sourced from Woo (1997).

The weakly amyloid suprahilar patch on the spores and the solid stipe suggests that *Russula crenulata* has a close relationship with the *Heterophyllae* of clade 4, but has cuticular characteristics closer to the above species of clade 5.

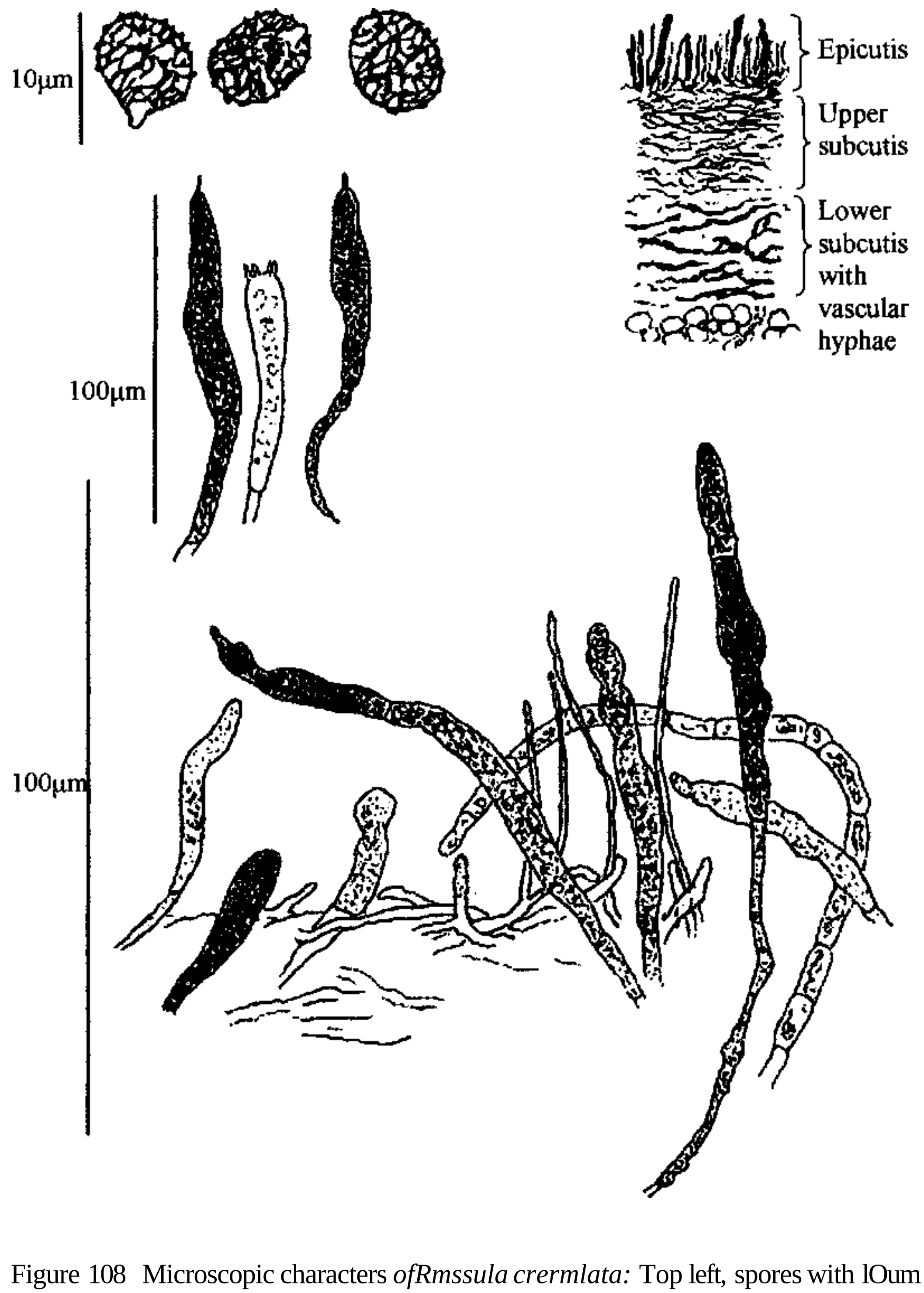


Figure 108 Microscopic characters *ofRmssula crermlata:* Top left, spores with lOum scale bar; top right, diagram of section through cutis showing epicutal turf, interwoven subcutis and more compact lower subcutis with laticifers; middle, hymenial cystidia and basidia with lOOum scale bar; bottom, articular hyphae and pileocystidia as seen in SV, scale bar is lOOum.

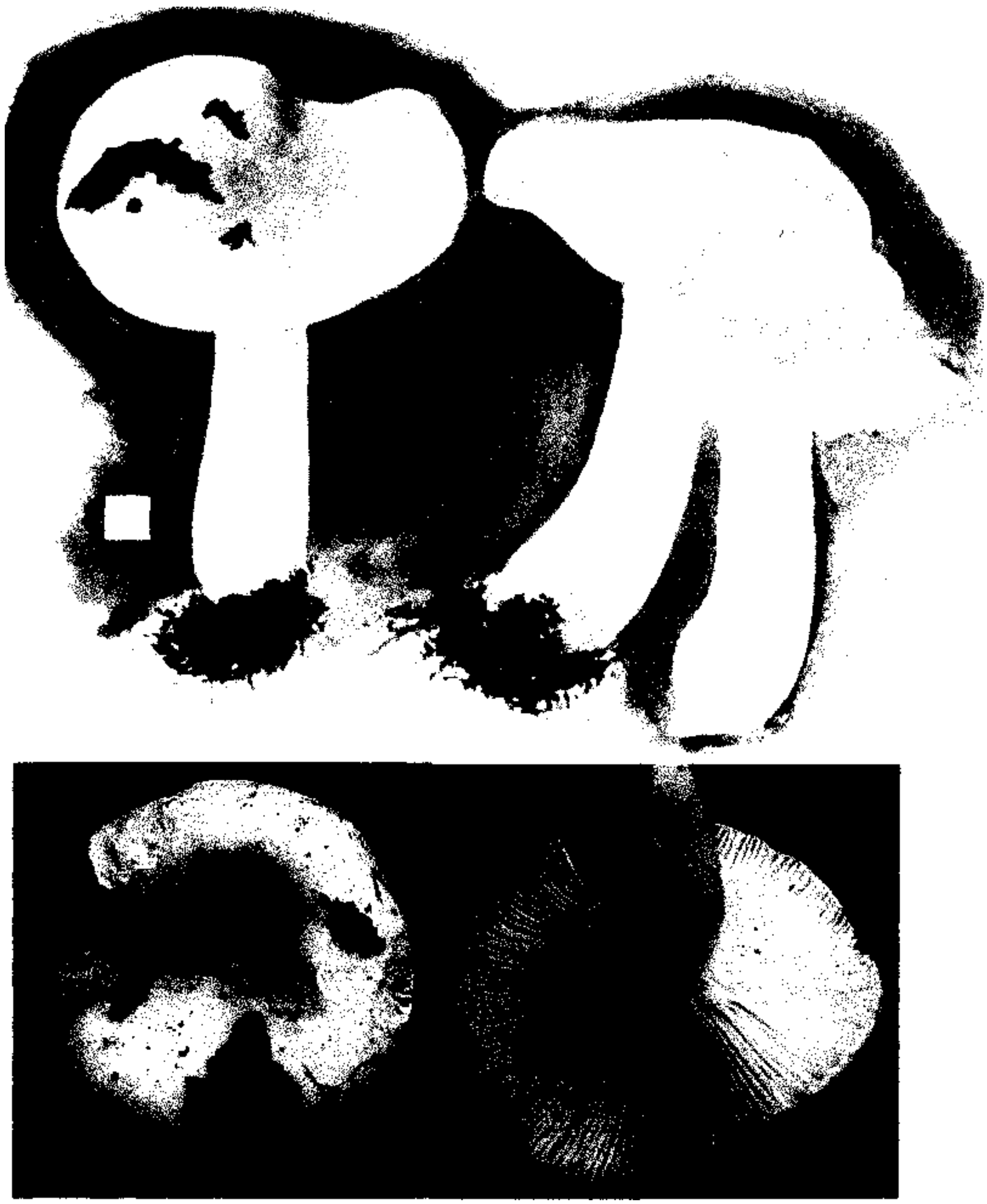


Figure 109 Macroscopic characters of *Russula crenulata;* top, illustration showing general stature in profile and section, the 1cm^2 square shows scale and spore print colour; bottom left, pileus and bottom right, gills of the same mushroom.

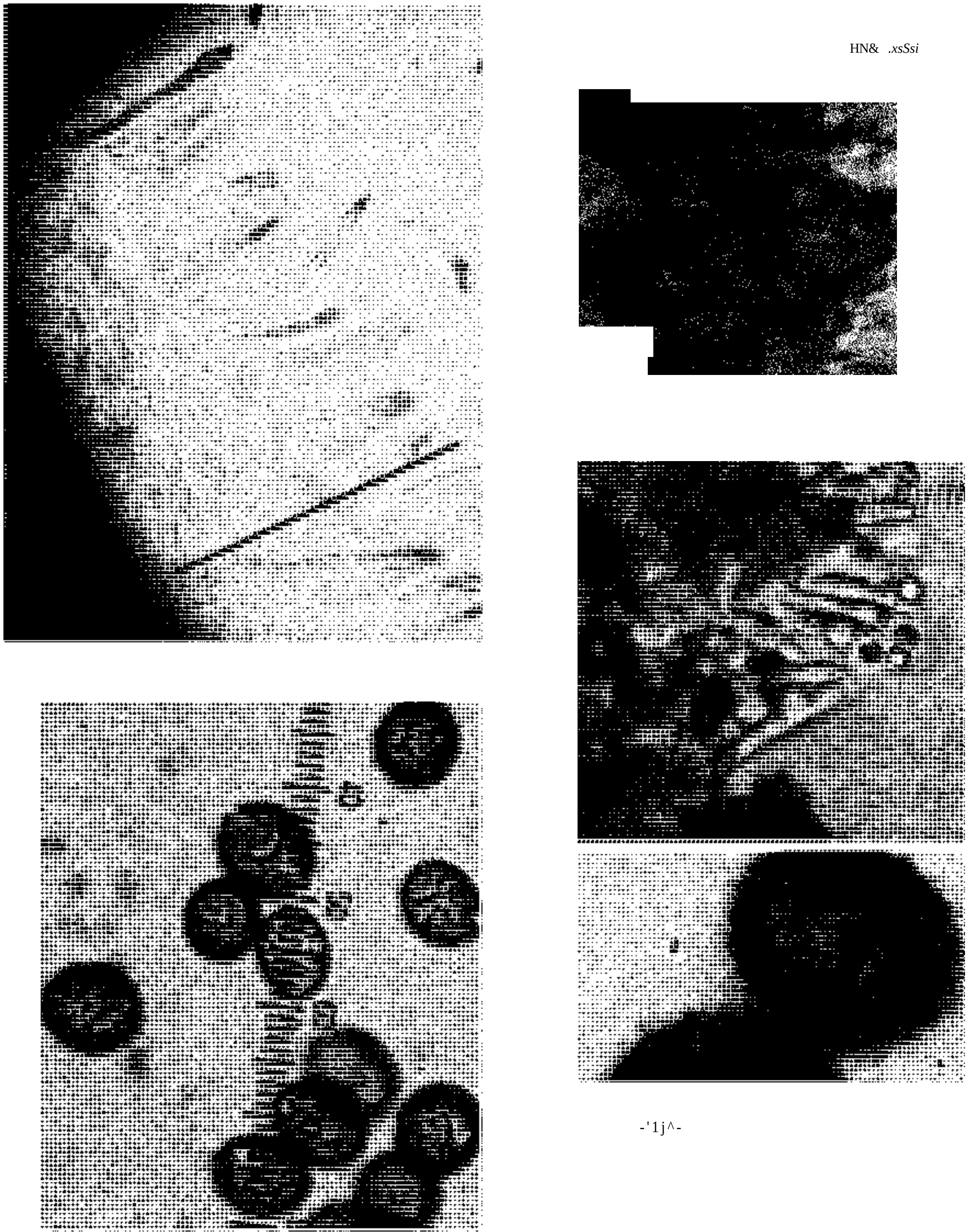


Figure 110 Epicutis and hymenium of *Russula crenulata:* Top left, section through epicutis in SV showing several pileocystidia (scale bar is lOOum.); top right, cheilocystidia clusters which give the crenellated effect, 10 divisions on scale are 25 um; bottom left, spores (one division on scale is lum); bottom right, ventral view of two spores showing the patchy amyloid reaction of the suprahilar patch, scale bar is 5um.

Table 17 Comparison of differential characters of four cream-capped peppery species of *Russula*, the information was derived from Burlingham (1913), Thiers (1997), Bon (1986) and Romagnesi (1967,1985).

	R. cremoricolor	***R crenulata***	***R ochroleuca***	***R. raoultii***
Spore size	7.6-9.5 x 5.7-6.7 (8.4)um	8.2-10.4x6.5-7.6um	8-10.5x6.5-8.2um	5.7-7.9 x 5.4-6.8um
Spores: reticulations	Some ridges only, not reticulate	Partial reticulations	Partial to complete	Complete
Wart height	0.2-0.5um	0.8-1.2um	0.8-1.0um(to 2.0um, Singer)	0.2-0.8um
Suprahilar patch	Small warts	Not specified (patchily amyloid in V.I. collections)	amyloid	Uneven, low, diffuse ornamentation
Lamellar insertion at stipe	Subsinuous-adnexed	Adnate-adnexed	Adnate-notched	Adnate-adnexed
Peelability of cutis	1/3-1/2	1/2 or more	To 1/4	To 1/4
Pileocystidia & septation	Numerous, septation not indicated	Numerous, 1-4 septate	No epicutal cystidia, subcutal ones aseptate	Frequent, 0-1 septate
Unusual epicutal hyphae	None mentioned	None mentioned	Fuchsin + incrustations	Long, slender hyphal ends
Habitat	Conifers & hardwoods	Oak-pine forest	conifers	Woody debris, conifers & deciduous
Stipe bruising	None	None	Ochraceaous-brownish, ± greying	None
Cap margin	Striate	Striate in age	Smooth	Smooth
Other notes	Interwoven subhymenium.	Gill margins crenellate	Mild to peppery	

***Russulafragihs* (Pers.: Fr.) Fries**

Stirpum Agri Femsionensis Index: 57. 1825.

Epicrisis Systematis Mycologici: 359. 1838

Cap 2.2-9.4cm diameter; convex when young, becoming plane to shallowly depressed, with the margins often remaining downcurved, margin striate up to about 1/3 the radius. When first emergent often quite dark, either green or grey, becoming more pinkish-purple as it matures and in the presence of light. The centre of the cap may be almost black in early maturity grading into an olive green to a pink to purple margin. The colours fade in age and are washed out by rain, with the purple more soluble than the green, with the end result of a pale cap with greenish-grey, to brownish grey-green disc and tinges of purple to greyed pink towards the margin, sometimes becoming almost entirely pallid with merely a trace of pigment. Cutis often just sticky more than viscid, drying matte to subglossy, cutis peelable 1/3 to 3/4, or sometimes almost completely, the tissue quite elastic, trama white, often tinged purple or greenish-grey under the cutis, soft and fragile unchanging when cut.

Gills white to pale cream, unchanging when bruised, pliable, subdistant to close, with occasional to frequent lamellulae, shallowly adnexed to almost free at the stipe, subacute to acute at cap margin, sometimes with the cap cuticle curving around the end of the gills, ventricose to broadening towards the margin, equal to twice the depth of the trama at half-radius or sometimes 4-5 times the depth, 3-9 mm deep. Gill edges entire, neither eroded nor serrated in most basidiomata, more rarely with clustered to scattered protruding cheilocystidia forming an irregular fringe on parts of the gill margin.

Stipe 2.5-6.8 x 0.5-1.8cm, in general roughly equal in length to the cap diameter, more or less cylindrical to clavate, white, rarely flushed with pink, smooth, stuffed with a bready-textured trama, developing usually three irregular cavities, eventually becoming hollow, fragile, unchanging when cut or bruised, aging pale greyish or pale yellowish-grey as if waterlogged, and when handled the flesh may collapse and turn slightly yellowish.

Texture sometimes fairly firm when young, becoming soft and fragile in age.

Taste immediately peppery to acrid, occasionally slightly bitter at first, then peppery.

Odour not distinctive to faintly fruity, of stewed apples or plums, sometimes of coconut.

Spore colour pure white in many collections, Romagnesi la, less commonly pale creamy-white, Romagnesi lb.

Spores 7-9.5 x 5.5-8.3um, L:W 1-1.46, mean (n=64) 1.19, globose, subglobose or broadly ellipsoidal. **Ornamentation** of conical or peg-like, fine to heavy warts, 0.5-1.8um, occasionally isolated, mostly connected by fine to heavy lines, 2-3 catenate sometimes forming short ridges, forming a partial to complete reticulum, **Woo type** C2, or C3, or D2, or D3. Some basidiomata with spore ornamentation mostly below l.Oum, others with mostly higher ornamentation, commonly the wart height is in the range of 0.8-1.5um. **Suprahilar patch** an irregular lightly amyloid patch bordered by, or sometimes including, small warts. **Hiliferous appendix** 1.6-2.1 x 0.9-1.2um. **Basidia** most 4-spored, occasionally 2-spored, 26-47 x 8.7-12um, clavate, bulbous in the upper 1/3, relatively short and broad. Sterigmata 5-10um long, 1.7-2.1 urn wide near the base. **Pleurocystidia** frequent to abundant, 37-90 x 7-13, originating in the subhymenium, protruding 5-25, fusoid to clavate, tips subacute, rostrate, mucronate, or with a short irregularly shaped appendage, contents refractive, yellowish in KOH, dark pink, purple to black in SV, entire hymenium initially bright magenta in SV. **Cheiiocystidia** similar to pleurocystidia but protruding up to 30u.m and reacting less strongly with SV. **Subhymenium** 20-30u.m, pseudoparenchymatous. **Gill trama** of sphaerocytes with occasional to rare vascular hyphae.

Cutis 80-250u.m thick, generally about 150-180um thick at half-radius, an ixotrichoderm. **Subcutis** about 1/2-2/3 the depth of the cutis, of hyaline gelatinised hyphae, 2.5-3.5um wide, and including many vascular hyphae 2-7jim wide, grey in SV, giving rise to frequent, narrowly clavate to cylindrical pseudocystidia with obtuse to capitate tips. The lower layer of the subcutis stains greyish magenta in SV. **Epicutis** a turf of more or less upright undifferentiated hyphal ends, l-3u,m wide, embedded in a viscid matrix,with numerous pileocystidia and frequent pseudocystidia. **Pileocystidia** abundant, 20-137 x 3-

8um, clavate or cylindrical, sometimes in tufts of 2-3 arising from a common hypha, occasionally Y-shaped, tips obtuse or capitate, 0-2 -septate, contents greyish to purple in SV Miniature versions of the pileocystidia are frequently found, around 2um wide, and usually in small tufts, (fig, 114). **Pseudocystidia** frequent, of similar width and shape as pileocystidia, arising from the subcutis, S V+. **Hypodermis** of small flattened sphaerocytes and compactly interwoven hyphae.

Trama of discrete clusters of sphaerocytes bound by a hyphal mesh, with frequent yellowish refractive vascular hyphae 3-1 Oum wide, grey to black in SV.

Chemical reactions: FeS04 -light greyish pink; KOH -salmon on cap cutis, no reaction on stipe; NH4OH -no reaction on cap or stipe; guaiac -slowly and weakly becoming grey-green, although in a few basidiomata, the reaction is faster; guiaicol -pinkish orange; phenol -slowly pinkish; SV -turning cutis and gills bright magenta at first, becoming more purple as the cystidia darken to purple of grey.

Habitat and tree associations: Widespread and common on or near coarse woody debris and on logs penetrated by tree roots, in association with *Tsuga heterophylla* in mature second growth and old-growth coastal forests, August to November. Found throughout Vancouver Island in coastal forests where western hemlock occurs. The white-spored form mostly occurs in the coastal western hemlock zone and the cream-spored form in the coastal Douglas fir zone.

Collections: CR980825-01 in a stand of mixed-age Sitka spruce, western hemlock and Douglas fir, Port Renfrew N 48.555667°, W 124.39233°. CR 980825-DF25 and CR 980916-01, from a single-species plantation of Douglas fir, Port Renfrew area, N 48.5997°, W 124.3325°. CR 980916-WC23 adjacent to a single-species plantation of western red cedar with understory western hemlock saplings N 48.5959°, W 124.3341°. CR 981013-05 in a mature stand of western hemlock, western red cedar and understory salal, near San Joseph trail, Cape Scott, N 50.776°, W 128.3975°. CR 000919-06 on decayed log under western hemlock, western red cedar and understory salal and salmonberry, Cape Beale trail, Bamfield, N 48.820083° W 125.151533°. CR 001012-22 in a pocket forest in coastal dunes under western hemlock and Sitka spruce with kinnikinnik ground cover, Wickanninish, N 49.022°, W 125.674°. CR 010920rfb, on decayed wood under western hemlock, red alder and amabilis fir, Rainforest trail (west of road), Pacific Rim N. P., N 49.04°, W 125.68°. CR 021015-09 and 021015-11 on a large (1.5 m diameter) fallen cedar log at decay stage 4, with western hemlock, western red cedar, salal and huckleberry growing on top, Rainforest trail (east of road), Pacific Rim N. P. N 49.446778°, W 125.531694°. CR030924-03, on decayed wood under regeneration Sitka spruce and western hemlock, along trail to Mystic beach, China Beach park, N 48.4368°, W124.0922^0. (All above from southern very wet hypermaritime CWH subzone). CR 981014-02 on well decayed wood in an old-growth western hemlock and western red cedar stand north of Sayward N 50.3255°, W 126.0905° (submontaine very wet hypermaritime CWH subzone). CR 011013 -02, near woody debris under regeneration western hemlock and Douglas fir, Mesachie Lake area, N 48.815°, W 124.1358°; CR031026-01, on decayed logs in regeneration western hemlock forest west of Youbou, N 48.8960°, W 124.2765° (western very dry maritime CWH subzone). PJ 981128-ER187 in Douglas fir-western hemlock-western red cedar mature forest, Saturna Island Ecological Reserve, N 48.786725°, W 123.16782° JD031130-01 Metchosin wilderness park, Victoria N 48.3675°, W 123.5776°. CR040930-01 on rotten wood in an area with western hemlock and occasional Douglas fir, Blueberry flats, near Sooke N 48.396°, W 123.9664°. CR041114-02 Royal Roads University woodlands in a mixed-age stand of Douglas fir, western red cedar and western hemlock. (Latter 3 collections from CDF zone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR980825-01	870	369, 224	569,313	305, 234
CR981014-02	827	369, 267	364, 289	345, 260
CR021015-09	874	383, 294	491, 284	341, 270
CR030924-03	872	364, 258	500, 327	295
CR031026-01	852	356, 258	487	335, 242
CR031130-01	868	367, 255	500	354, 291

Notes: *Russula jragilis* can be a very variable species in colour, taste, size, and pileocystidia shape. Its growth on or near wood, with western hemlock (sometimes only small saplings), generally purple and dull green colours, white to creamy-white spores and mostly peppery taste are reliable characters. It is not always acrid and fragile, although never robust or tough

In addition to the Vancouver Island collections of this species a collection from Whidby Island, north of Seattle, U.S.A., was examined and found to correspond more closely to the European concept *of Russula Jragilis,* with a pale cream spore print, (Romagnesi lb), rather than the pure white of many Vancouver Island collections. The Whidby Island collection also had a mixture of pileocystidia types, many were the clavate, septate ones considered diagnostic for *R. jragilis,* and others were the elongate, 0-1 -septate ones associated with descriptions of *R. atropurpurea.* A further collection from the Olympic peninsula had the pure white spore print (Romagnesi la) of most Vancouver Island collections, the typical slow, weak guaiac reaction of *R. jragilis* and clavate, 0-1 -septate pileocystidia. In both the above collections the spores were similar to those of the Vancouver Island collections. These collections from three areas of the west coast of North America appear to almost bridge the gap between *R. jragilis* and *R. atropurpurea.* Other similar closely related species include *Russula aquosa,* which grows in sphagnum, *R. violescens,* under conifers in marshy areas, *R. laccata,* a dark red-purple species that grows with willows, and *R. alnetorum,* that is associated with alder. In Europe, one distinguishing feature of *R. jragilis* is its odour of amyl acetate (like pears), which is rarely apparent in the Pacific Northwest forms, which also differ in their generally higher spore ornamentation and infrequently serrated gill margins. Neither the amyl acetate odour nor the fringed or crenellated margins stressed in many descriptions and keys are reliable characters in local collections.

The name *R. atropurpurea* has been used by Peck for a species in the *R. xerampelina* group, without regard for its previous usage in 1845 by Krombholz. Shaffer (1970) therefore considered it necessary to rename the original *R. atropurpurea,* and he chose the honorific epithet *krombholzii.* The current name has reverted to *Russula atropurpurea*

(Krombh.) Britzelm. (Index Fungorum 2004), but both synonyms should be checked when looking for descriptions. Sarnari lists i?. *atropurpurea* (Singer) Crawshay, 1930, andi?. *atropurpurea* var. *atropurpurea* (Sing.) Singer, 1932, as synonyms for *Russula fragilis*, so the difficulty in distinguishing between these species is not without precedent. Sarnari also lists 27 synonyms for *Russula atropurpurea*, clearly this is a variable species if collections have been thought a different taxa from the pre-existing published descriptions. *Russula fragilis* differs from *R. atropurpurea* in its weak reaction with guaiac, its subglobose rather than ellipsoidal spores, and its generally smaller, more fragile stature (sensu Bon 1988 and Shaffer 1970).

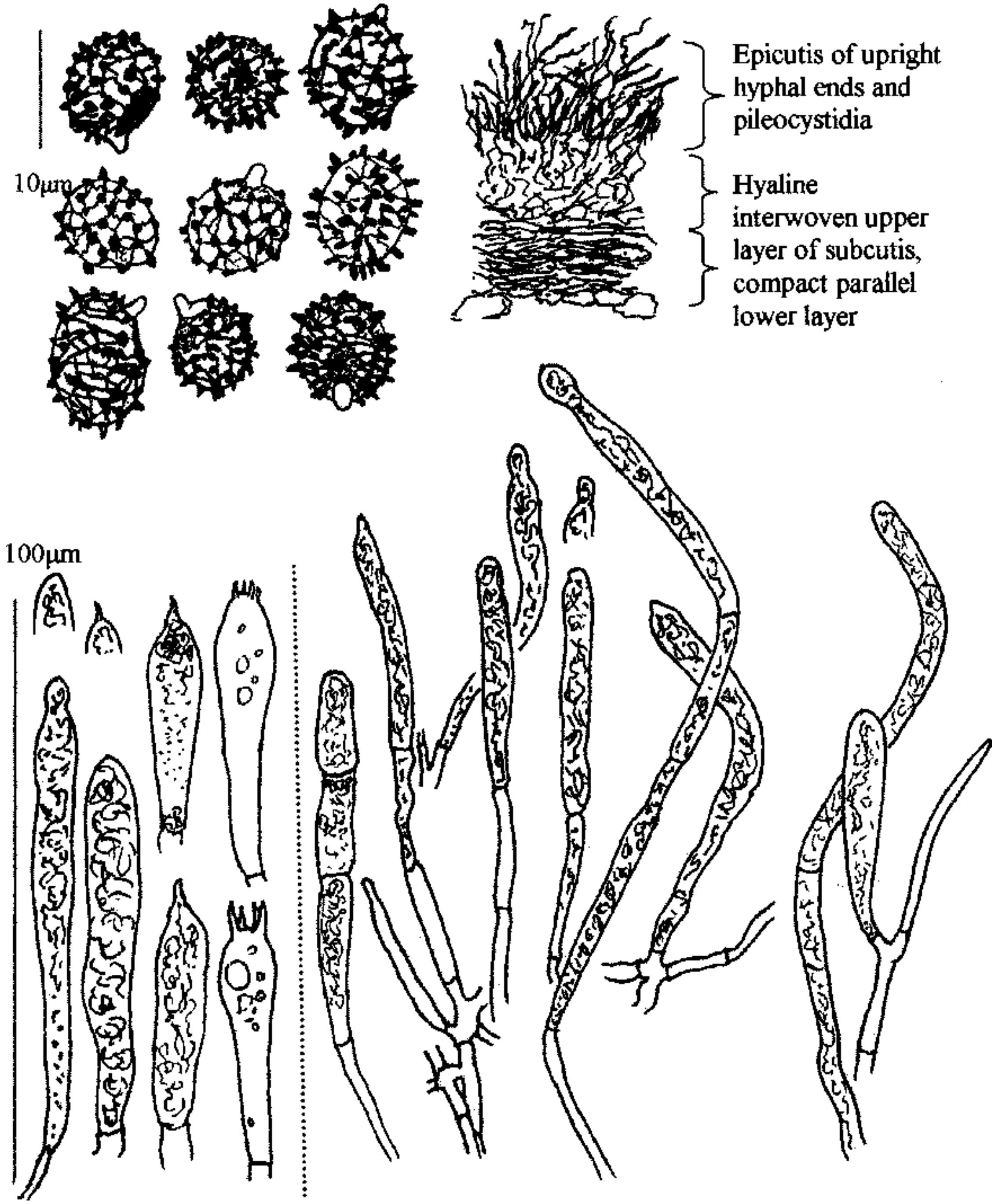


Figure 111 Microscopic characters *ofRussulafragilis:* Top left, spores with 10 um scale bar; top right, diagram of section through cutis; bottom left, hymenial cystidia and basidia; bottom right, pileocystidia and pseudocystidia from the epicutis; lower scale bar is 100 um.

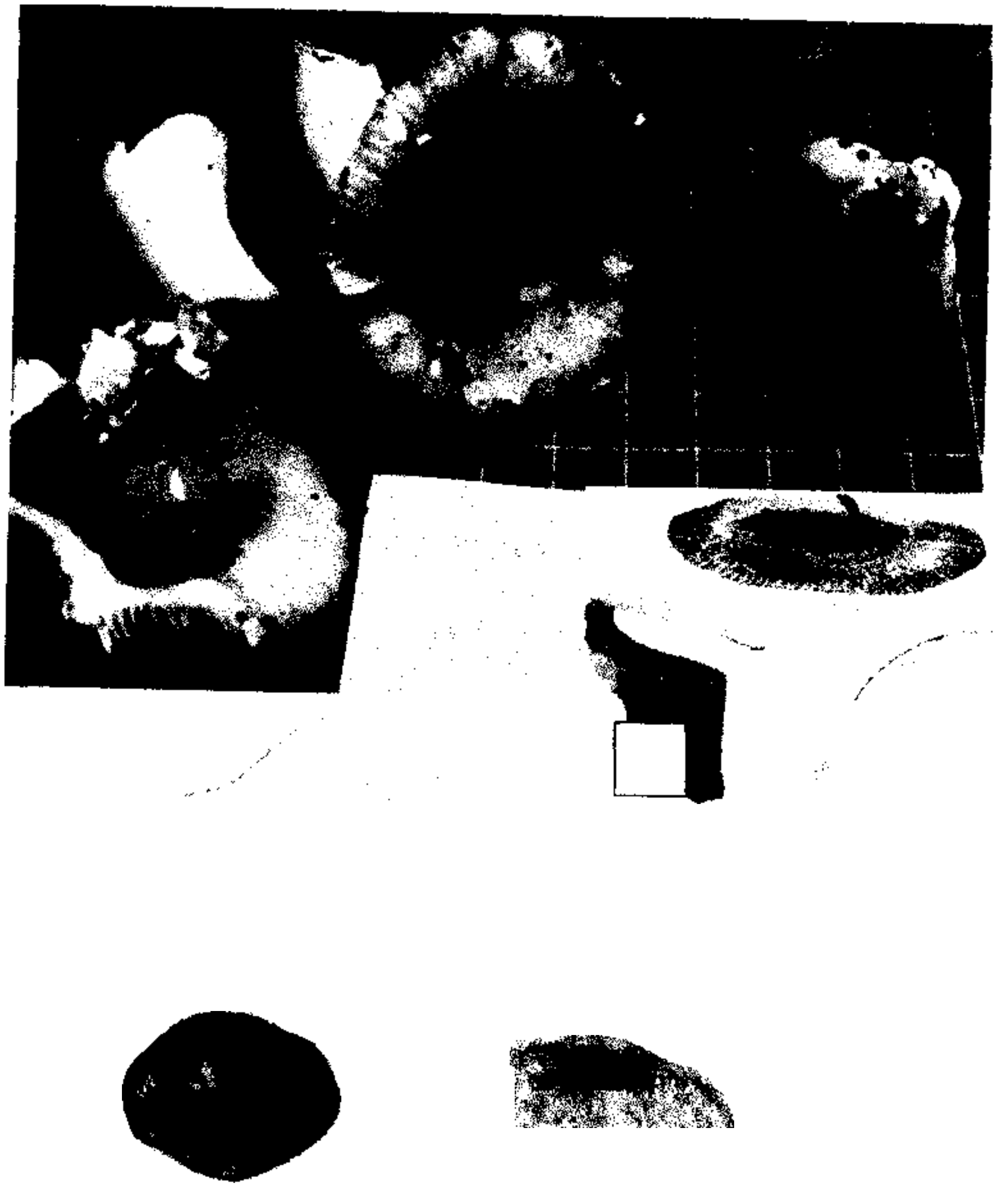


Figure 112 Macroscopic characters of *Russulafragilis:* Top, photograph of the cap surface of three basidiomata set on a 1cm^2 grid; below, illustrations of profiles, longitutudinal sections and a view of the gills, the squares show the spore colour and are 1cm^2.

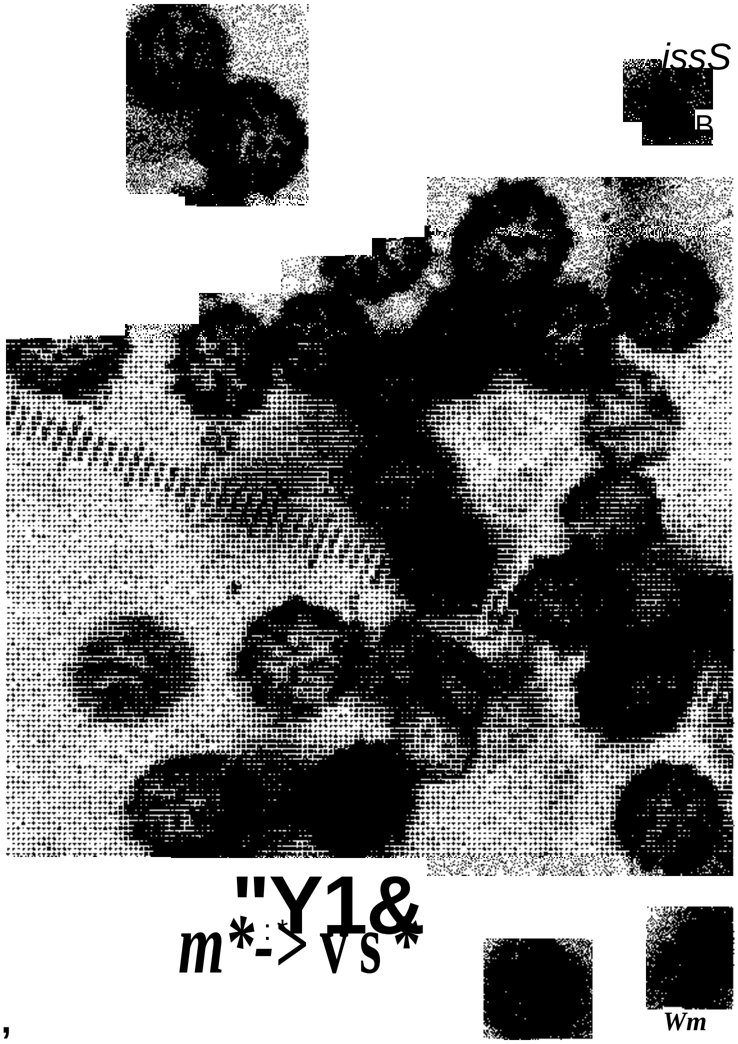


Figure 113 Hymenium *of Russula fragilis:* Left, spores with 1um division scale; right, basidia and basidiole, scale bar is 50um.

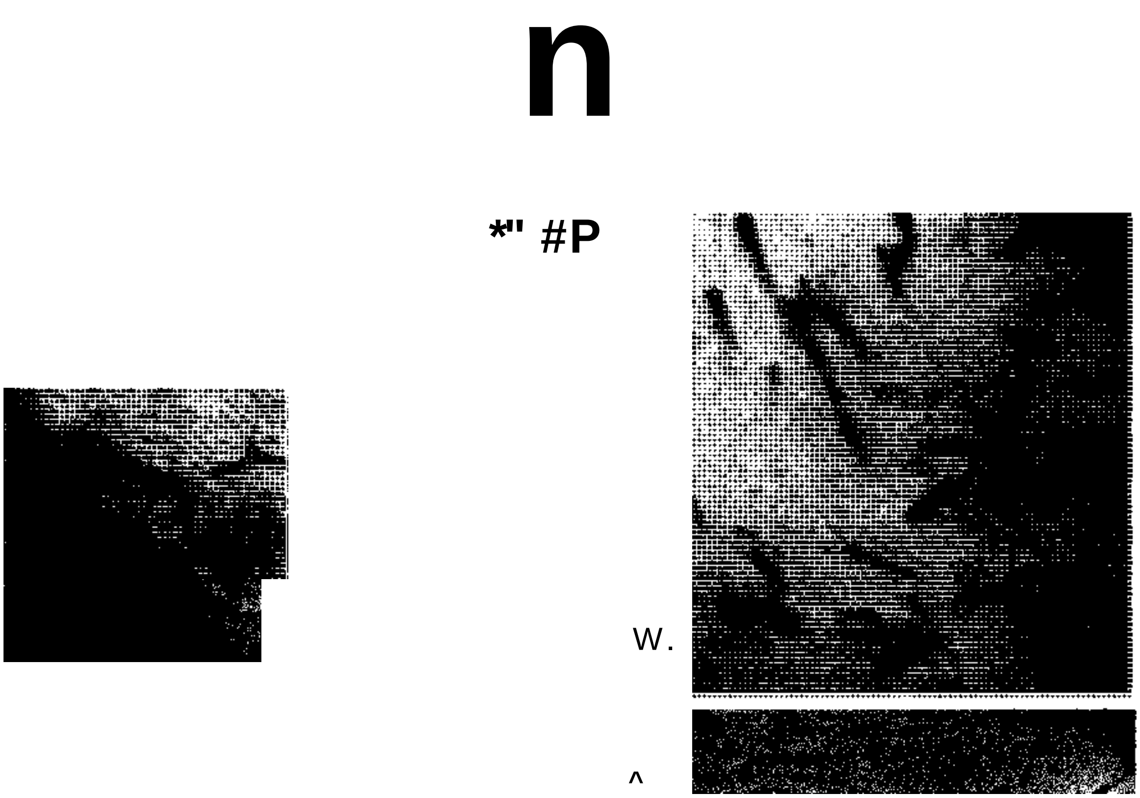


Figure 114 Cutis of *Russula fragilis:* Top left, section through cutis in SV, with the compact lower layers of the subhymenium to the lower left between 76 and 82 on the scale, 10 scale divisions are 25 urn; upper right, surface view of the cutis in SV of a basidioma with clavate, 0-2 -septate pileocystidia; below right, surface view of the cutis in KOH of a basidioma with cylindrical capitate, mostly aseptate pileocystidia, both scale bars are 100 um; lower left, view of a tuft of miniature pileocystidia, arising from 3 hyphae, scale bar is lOum

***Russulafragihs* var. *mihs* nom. prov.**

Three collections of what appear to be *Russula fragilis* but are mild-tasting were found. Macroscopically and microscopically they are virtually identical and fall within the range of characters for *R. fragilis,* and this description can be referred to for information on these characters. All have pileocystidia that are narrowly clavate to cylindrical, very commonly capitate, and 0-1 septate, and the subcutis in water mounts tends to be pink. They have no more than a faint latent pepperiness and a nondescript to slightly fruity-peppery or coconut odour, two collections were on rotten logs under western hemlock, the third in a stand of red alder between the rain-forest boardwalk and the road, with much woody debris on the ground, and surrounded by old-growth Sitka spruce and western hemlock.

Two species of *stkps fragilis* with a mild taste and purplish cap that seem closest to these Vancouver Island collections have been described in the European literature: *Russula aquosa* Leclaire (Bull. Soc. Myc. France 48:303, 1932); and *Russula alnetorum* Romagnesi (Bull. Soc. Linn. Ly. 25: 183. *Russula aquosa* is found in moist habitats with or without sphagnum, and in moist coniferous forests, there is no mention of its occurring on rotten wood. Its spores are smaller by about 1um in each dimension, with warts around 0.6-0.7um high, and it has slightly longer hymenial cystidia than the Vancouver Island collections but is otherwise very similar. *Russula alnetorum* is associated with alders in Europe, and its spores are more narrowly ellipsoidal than those of *R. fragilis*, and have a rather elongated suprahilar patch, the hymenial cystidia also tend to be longer on average (ie. 80-85^im). Neither of these species has been recorded in North America to my knowledge, however, similar habitats and related host trees occur here.

In summary, the three Vancouver Island collections do not match exactly the descriptions for *R. fragilis* (in taste), *R. aquosa* or *R. alnetorum,* yet are not so different to discount them from being local varieties. Although alders were not recorded in the vicinity of two of the collections, they are very common in both areas and may have been overlooked.

The growth on rotten wood is typical of *R. fragilis* and until more collections are made that suggest otherwise these mild forms are considered a variant of *R. fragilis.*

Collections. CR 981014-03 on very decayed log, under old-growth western hemlock, with western red cedar and huckleberry, in roadside forest about 40K north of Sayward, N50.3255^0, W126.0905^0 (submontaine very wet hypermaritime CWH subzone); **Spores** 8-9 x 6-7.8, mean L:W 1.23 (n=10), warts to 1.5um high. CR000920-03 growing from broken branch on a decaying log, under old growth western hemlock with huckleberry, near the Pachina River close to Pachina Bay, N48.805083^0, W125.1122° (southern very wet hypermaritime CWH subzone); **Spores** 7.9-9 x 6.7-7.6, mean L:W 1.17 (n=10), warts to 1. lum high. CR011030-19 on the ground under a stand of alders adjacent to the Tofino road, with young western red cedar, amidst old growth Sitka spruce and western hemlock, Rainforest trail east side, PRNP, N49.446782^0, W125.5317^0; **Spores** 8-9.6 x 6.2-7.7, mean L:W 1.26 (n=10), warts to 1.4um high (southern very wet hypermaritime CWH subzone).

Figure 115 Characters of*Russulafragilis* var. m///y: Top, illustration of profile, top and longitudinal section with 1cm^2 box showing spore colour; middle, spores with lum division scale, bottom left, surface view of cutis showing pileocystidia in water mount, round objects are spores stuck to the surface, scale is in lOum divisions; bottom right, clavate round-tipped pileocystidia with 40um scale bar.

***Russula laccata* Huijsman**

Fungus, 25:40, pl.8, f. I-II. 1955

Cap 2.9-4.7cm diameter, convex when young, becoming plane or with a slightly depressed centre when fully expanded, cap margin smooth, becoming striate in age, purple to violet with black on the disc when mature, spotted with pink, grey-pink and yellowish patches on immature basidioma, viscid when wet, drying subglossy. Cuticle peelable 1/3 to 1/2 the radius, trama beneath tinged purple, unchanging when damaged.

Gills white, becoming pale cream, pliable, moderately close, with occasional subgills, no forking seen, arched, adnate at the stipe, subacute at the cap margin, margins entire, not serrated.

Stipe 3.0-4.2 x 1.3 to 2.0cm, ventricose or widening near the base, white, one with a pale pinkish brown bruising at the base as if waterlogged, otherwise unchanging when bruised, stuffed with a bready textured trama, becoming hollow in age.

Texture somewhat fragile.

Taste acrid.

Odour nutty with a rubbery component.

Spore colour very pale cream, Romagnesi lb.

Spores 7.0-8.5 x 5.2-6.5um, L:W = 1.21 tol.5. mean 1.34 (n=30), ellipsoidal to bean-shaped. **Ornamention** of warts mostly under 0.6um (-0.8um) high, rounded to bluntly conical, catenate or connected by a complete or almost complete reticulum, **Woo types** 1D-2D. **Suprahilar patch** amyloid, lilac-grey in Melzers' reagent, irregular in outline. **Basidia** 4-spored, approximately 34-50 x 7.5 to 12.5um, cylindrical to clavate, in general more slender than those of *R. fragilis.* Sterigmata 4-7um long, around 1.2um wide near the base. **Cheilocystidia** and pleurocystidia, densely distributed, staining purple-black to black in sulphovanillin, 42-80 x 7-10um, or occasionally to 12um wide, protruding about

12um beyond the hymenium, fiisoid to clavate with acute, mucronate or shortly tapering tips. **Subhymenium** 20-3 5 um thick, pseudoparenchymatous, gill trama of sphaerocytes and frequent vascular hyphae.

Cutis 80 to lOOum thick at half radius, an ixotrichoderm with numerous erect hyphal ends, pileocystidia, and pseudocystidia. **Subcutis** of hyaline densely interwoven hyphae mostly about 3um wide, and frequent vascular hyphae that give rise to pseudocystidia. **Hypodermis** of small, tightly packed sphaerocytes. **Epicutis** of erect hyphal ends 2 - 6um wide, terminating in a tapered or narrowly clavate end cell, sometimes with refractive contents like small cystidia. **Pileocystidia** 20-28um by 6-8um wide, cylindrical to clavate, sometimes forked, tips rounded or with a short, blunt point, less often capitate, 0 -2 -septate, mostly dark grey in S V but occasionally partially or completely none reacting. **Pseudocystidia** around 83 um long in the epicutis, narrowly clavate, septate, both staining and none-staining cystidia are apparent in SV.

Trama of sphaerocytes loosely arranged in clusters with a sparse hyphal network and frequent vascular hyphae interspersed.

Chemical reactions: FeS04 - light brownish pink; NH4 OH - no reaction on cap or stipe; KOH - turns cuticle more orange, stipe no reaction; phenol - slowly brownish purple; guaiac - slowly, weakly blue-green; SV - gills and cap trama stain deep violet, pileocystidia unreacting or grey, pleurocystidia and cheilocystidia black.

Habitat and tree associations: Along the shore of Langford Lake, Victoria, close to the water, with willows, Douglas fir and western hemlock nearby. Found in late May.

Collections: OC030526-01 from under willows and Douglas fir, at the western shore of Langford Lake, Victoria (CDF zone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A
OC030526-01	865	414,374	456,279	641,238

Notes: This collection is very similar to *R. fragilis* (Persoon:Fr.) Fries except the spores are smaller and the pileocystidia are 0-1 septate, *R. fragilis* pileocystidia usually have more septa. Sarnari (1998) gives *R. fragilis* var.*alpestris* Boudier, *R. norvegica* Reid (nom. inval.) and *R. norvegica vdx.rubromarginata* Kiihner as synonyms, indicating the affinity with the *R. fragilis* group. This collection has the slow reaction with guaiac and the pale cream rather than pure white spores typical of *stivpsfragilis,* and the small spore size with low warts and reticulations of *R. laccata.* The local collection had a nutty-rubbery smell rather than the fruity-pear smell indicated in the type and were not as shiny when dry, but the habitat close to water with *Salix,* the microscopic and other macroscopic characters match the description given in Sarnari, 1998. The RFLP's of the rDNA were more like those of *R. betularum,* another species of moist sites, than those of *R. fragilis.*

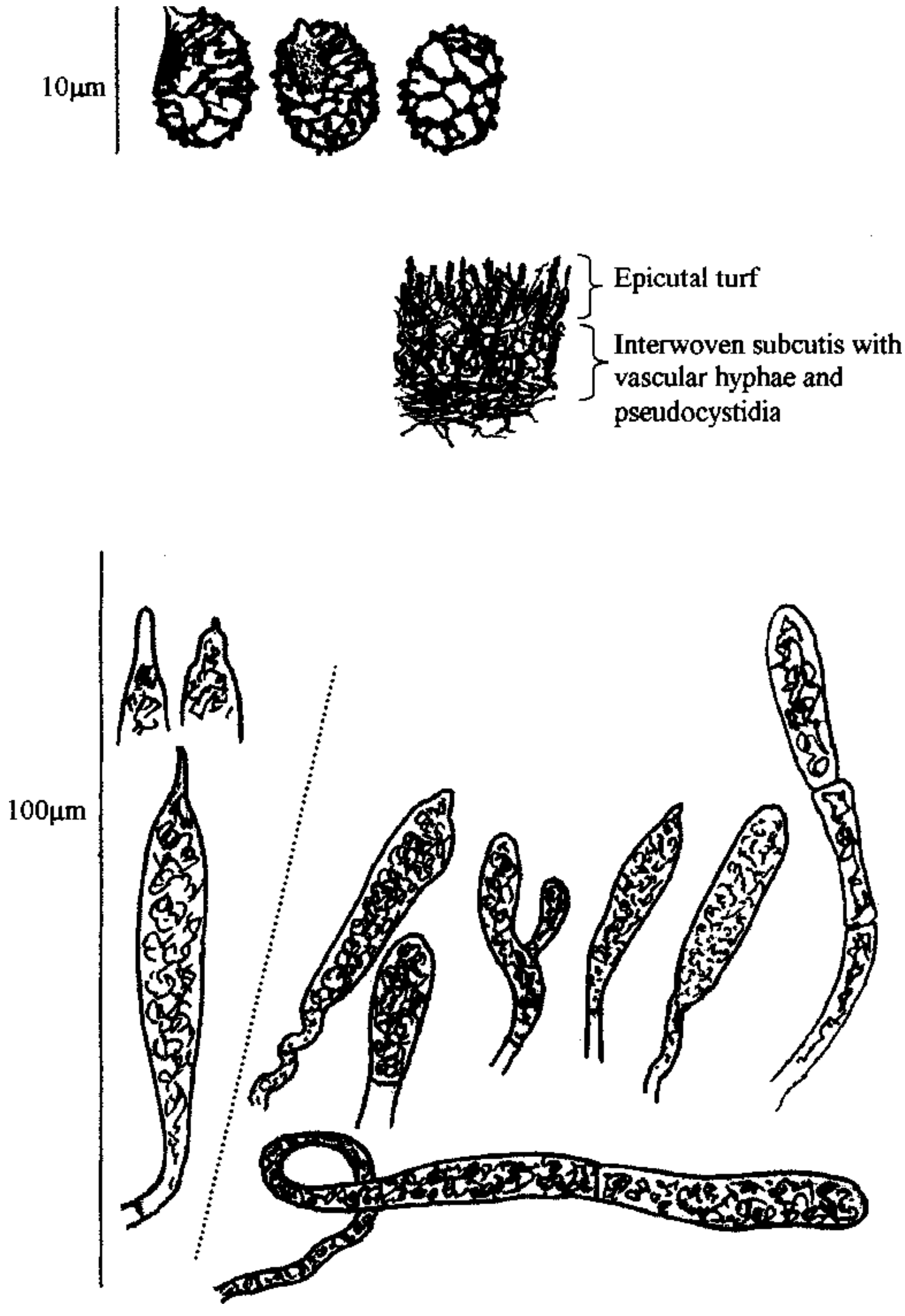


Figure 116 Microscopic characters of *Russula laccata:* Top, spores with 10 pm scale bar; bottom left, hymenial cystidia; bottom right, pileocystidia with pseudocystidia below, lower scale bar is 100 |im.

Figure 117 Cutis of *Russula laccata:* Top, colour notes of cap surface of mature and young basidiomata; lower left, section through cutis with 100 urn scale bar positioned just above the hypodermis, arrow point is at level of gluten; lower right, surface view of cutis with pileocystidia stained in SV, scale bar is 100 urn

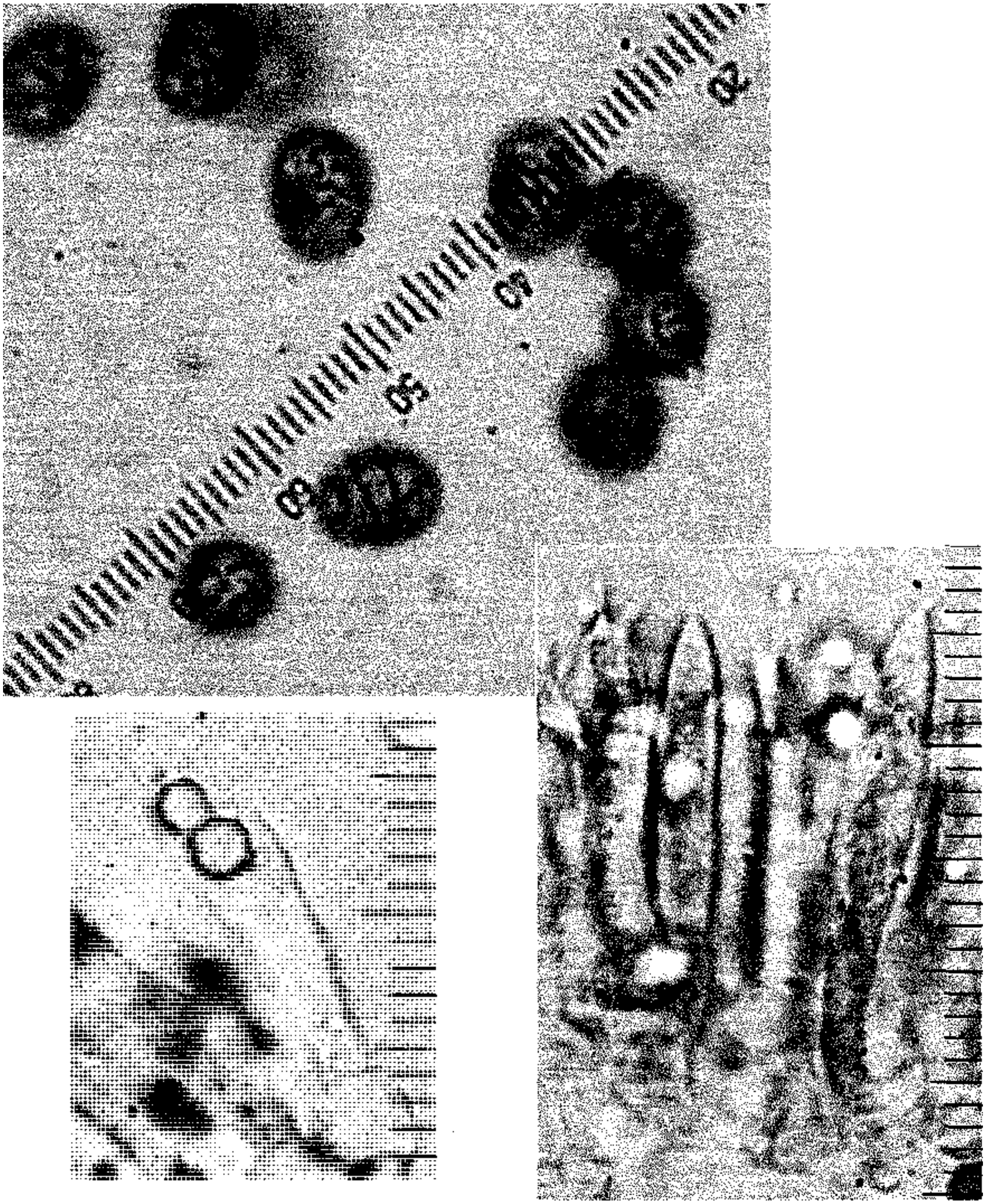


Figure 118 Hymenium of *Russula laccata:* Top, spores with lum division scale, below, basidia and below right, section through hymenium showing basidia, basidioles and two pleurocystidia and the subhymanial cells they originate from, 10 divisions are 25 urn for both lower scales.

***Russula silvicola* Shaffer**

Nova Hedwigia 51:229-234. 1975

Cap 1.9-7.5cm diameter, convex to pulvinate when young, sometimes with a small flattened umbo, becoming plane to shallowly depressed, eventually shallowly infundibuliform, sometimes with a low umbo and often retaining rounded margins, margin smooth, striate in age. When very young pale yellow, developing pink to red blotches and becoming completely scarlet red or retaining some cream to light yellow orange areas, fading in age, sometimes to almost white in rainy weather. Two collections were cream with light pink blotches only (fig. 120). Surface sticky to viscid, drying matte, cutis peelable 1/3 to almost completely, cap trama white, occasionally pink under the cutis, unchanging or becoming pale pinkish.

Gills white to pale cream, unchanging where bruised, subdistant to close, frequently forked near stipe or margin, with occasional lamellulae, narrowly adnexed to almost free, or adnate to slightly decurrent at stipe, subacute to obtuse at cap margin, ventricose with the broadest part towards the margin, depth 2-5 mm at half-radius, generally equal to 1.5 times the depth of the trama, margins generally minutely fringed, sometimes crenellate.

Stipe 2.7-8.3 x 0.6-2cm, in general longer than cap diameter, more or less cylindrical but widening at the base and sometimes the apex, surface white, smooth, longitudinally finely rugulose, stuffed with a bready-textured trama, becoming hollow in age, unchanging to pale pinkish where damaged, becoming a light yellow-grey in age as if waterlogged.

Texture relatively firm and pliable when young, soon becoming soft and fragile.

Taste slightly bitter, slowly to quickly acrid.

Odour slightly fruity, of stewed rhubarb, with a rubbery component or sometimes a sort of waxy, coconut smell reminiscent of gorse flowers.

Spore colour white to slightly creamy white, Romagnesi Ia-Ib.

Spores 7.2-11.2 x 6.5-8.8um, L:W 1.03-1.36, mean (n=40) 1.21, mostly broadly ellipsoidal, occasionally subglobose, ornamentation of conical, peg-like or rounded, mostly heavy warts, 0.8-1.4um high, isolated or connected by fine to heavy lines, some 2-3 catanate forming short ridges, forming an often relatively wide partial reticulum, less often a complete reticulum, **Woo types C2, C3, D2, D3. Suprahilar patch** amyloid, often only partially so, a light greyish irregular area in Melzers* reagent, often with a lower but similar ornamention to that of the rest of the spore. **Hiliferous appendix** 1.2-2um long, around 1.5um wide near the base. **Basidia** mostly 4-spored, rarely 3 and 2-spored, 32-48 x 8-14um, clavate, bulbous in the upper 1/3, relatively short and broad. Sterigmata, 3-8um long, 2-2.5um wide near the base. **Pleurocystidia** regularly and densely distributed, 38-80 x 6-13um, originating in the subhymenium or the outer trama, protruding 12-20um, fusoid, narrowly clavate to clavate, tips rounded, subacute, acute, mucronate, or with a short appendix or tiny button, contents refractive, yellowish in KOH, pink to reddish-brown to dark grey in S V. **Cheilocystidia** often very densely clustered, usually the only cell type discernible on the gill margins, up to 90um long and 3.5-12um wide, similar in shape to pleurocystidia but protruding up to 40um, in some basidiomata the tips are very varied in shape and may be subacute, mucronate, capitate, or with a long, roughly cylindrical appendix 2-3 urn wide, with walls that appear lumpy or pitted. Contents lightly to strongly refractive, variably staining in SV. **Subhymenium** 15-35um, pseudoparenchymatous, gill trama of sphaerocytes, with many vascular hyphae.

Cutis 80-120um thick, an ixotrichodermis with a pink pigment in the lower layers which dissolves slowly in water. **Subcutis** about 2/3-1/2 the cutis thickness, of parallel, interwoven, hyaline gelatinised hyphae 1.0-3.0um wide, with many vascular hyphae 2.5-10pm wide, dark grey in SV, that sometimes give rise to pseudocystidia in the epicutis. **Epicutis** of erect free hyphal ends 1.0-3.0jxm wide, undifferentiated or with slightly inflated terminal cells up to 6um wide, with abundant pileocystidia, embedded in a gelatinous matrix. **Pileocystidia** 20-120 x 3-1 lum, most are clavate with slightly inflated rounded tips, but cylindrical ones are common, tips obtuse, 0-3 -septate, variously reacting with SV with some remaining yellowish, others dark grey, many having a gradation along the length with the terminal cells darkest. The walls appear incrusted slightly in places in S V. **Pseudocystidia** occasional, reacting similarly to pileocystidia in SV and of similarly shaped ends. **Hypodermis** of small flattened sphaerocytes and compactly interwoven hyphae with vascular hyphae.

Trama of discrete clusters of sphaerocytes bound by a hyphal mesh, with frequent vascular hyphae, 4-7um wide, light grey in SV, grey to black in SV.

Chemical reactions: FeSC>4- light greyish pink; KOH - yellow on cap cutis, orange on cap cutis, no reaction on stipe; NaOHt - no reaction on cap or stipe; phenol - brownish; guaiac - brownish initially, slowly turning grey-green to black; S V - brownish red on cutis and gills, cystidia reaction varied, pinkish to grey, weakest on the gill margins.

Habitat and tree associations: gregarious, on well decayed logs, occasionally the forest floor, in one case on the bark of dead and living trees, in old growth and mature western hemlock or Sitka spruce, often with huckleberry and salal. Douglas fir and western red cedar may be present also,.

Collections: CR981013-09a and -09a on well decayed log with mixed age Sitka spruce, western hemlock, red alder and western red cedar, near the beach, San Joseph trail, Cape Scott, N 50.768°, W128.342°. CR021113-02 on decaying large log under regeneration Sitka spruce and western hemlock, Gold Mine trail, near Uclulet, N 49.011°, W125.627°. CR001011-53a,N 49.015733°, W125.67335°; CR021016-10,N 49.0155°, W125.6745^0; CR021016-12 and CR010615-14, N 49.0215°, W125.6735°, all on decayed logs in areas with little undergrowth under Sitka spruce and western hemlock krummholz, Wickaninnish, PRNP. (All above from southern very wet hypermaritime CWH subzone). CR 981014-01 on log and bark of tree in old-growth western hemlock, north of Sayward, N 50.3255°, W126.0905^0, CR010930-01, on small log under western hemlock near eastbound one-way road between Bamfield and Cowichan Lake, N 48.875°, W124.5965° (both from submontaine very wet hypermaritime CWH subzone). CR981202-05 on log under regeneration western hemlock and Douglas fir, Thetis Lake park, Victoria, N 48.4715°, W123.4744° (CDF zone). JD021027-01 on forest floor under western hemlock and possibly Douglas fir, Cowichan lake area, exact location unrecorded (western very dry maritime CWH subzone).

Collection	ITSl-FtoITS4-B	RFLP. Hinfl	Alul	Sau3A
CR981014-01	870	424, 365	565, 295	672, 208
CR981202-05	884	409, 298	464, 221	343, 265
CR001011-53a	898	407	569, 322	609, 243
CR021016-10	874	410, 374	521, 284	616, 236
CR021016-10 (repeat)	892	382, 347	315,275	614, 200

Notes. These collections were at first thought mistakenly to be *Russula bicolor* Burl, or *R. simillima* Peck, however they differ from the former in the abundant pileocystidia, the higher ornamentation and stronger reticulation formation on the spores, and growth on wood. Thiers (1997) comments that *R. bicolor* inhabits coastal Sitka spruce forests but is rare. *R. simillima* is described as yellowish with salmon pink (in Thiers 1997) with the pink often at the margin, a description fitting some local collections of *R. silvicola,* in addition the spores are very similar in size and ornamentation. They differ in the cutis, which in some basidiomata of *R. simillima* has inflated cells, in the pileocystidia which are mostly aseptate, and in the yellowish stipe and slightly browner cap colours. Thiers also notes that *R. silvicola* is similar to *R. paxilloides* Earle, but that species does not become bright red, has infrequent pileocystidia and grows with oaks. The Vancouver Island collections of *R. silvicola* match well the original description by Shaffer, which is clear and well illustrated with drawings of the microscopic characters. It is closely related to *R. fragilis* and has probably had that name applied to collections in the PNW, particularly as there have been many colour forms of that species recognised including red, some of which have been published as separate species. In contrast with local collections of *R. fragilis, Russula silvicola* shows a remarkable consistency in the shape of the pileocystidia from one collection to another, even those from different habitats and areas. The local collections commonly have a mixture of yellow and pink colours in the cap, completely red ones are less common and seem to occur before or early in maturity, subsequently fading. Shaffer commented on the variation in cap colour and also in spore size, yet these differences were uncorrelated with other characters.

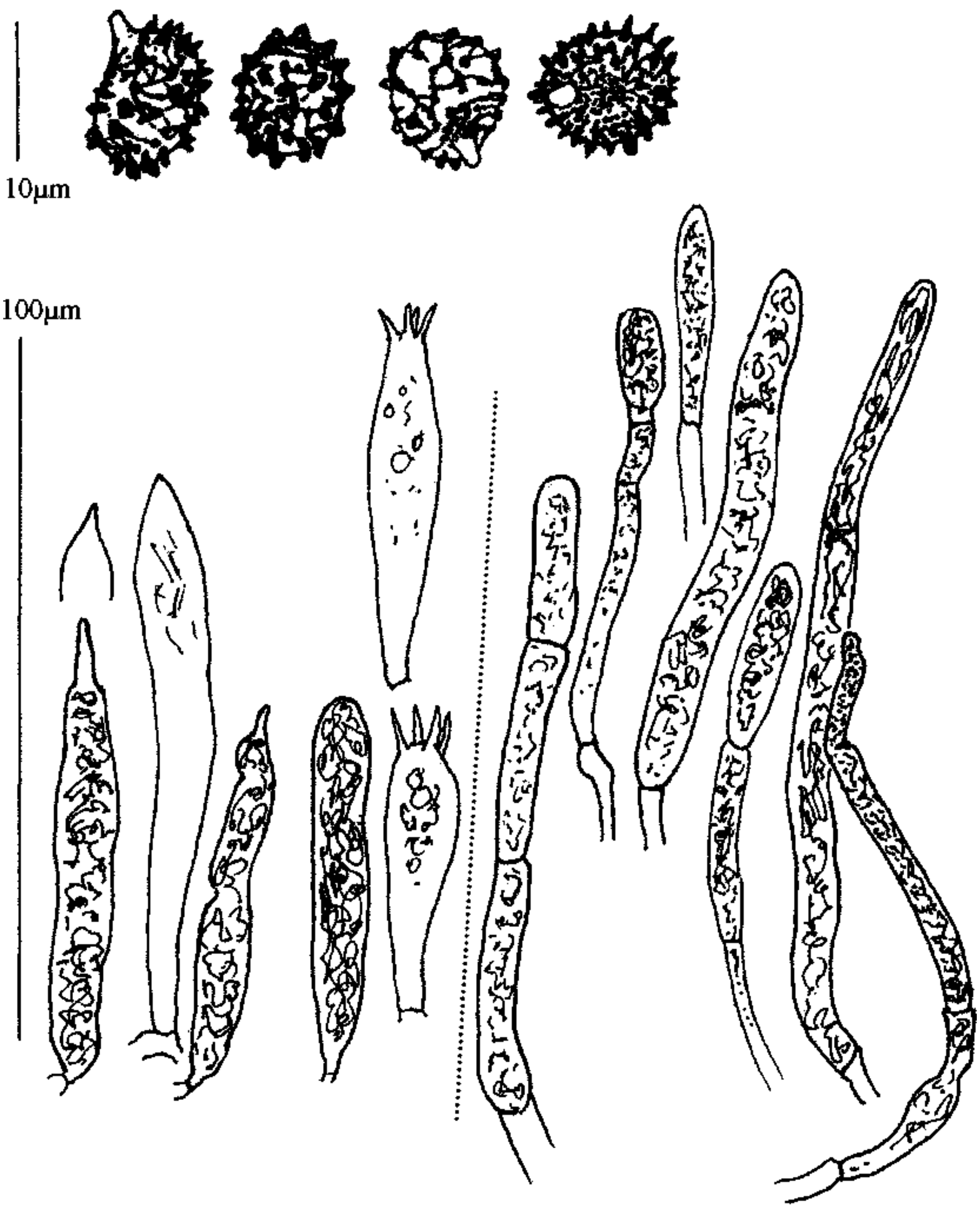


Figure 119 Microscopic characters *otRussula silvicola:* Top. Spores with lOum scale bar; bottom left, hymenial cystidia, the larger one is typical of the cheilocystidia, and two basidia; bottom right, pileocystidia from the epicutis, the most common types are the clavate, septate ones as first, second and fifth from the left, bottom scale bar is lOOum.

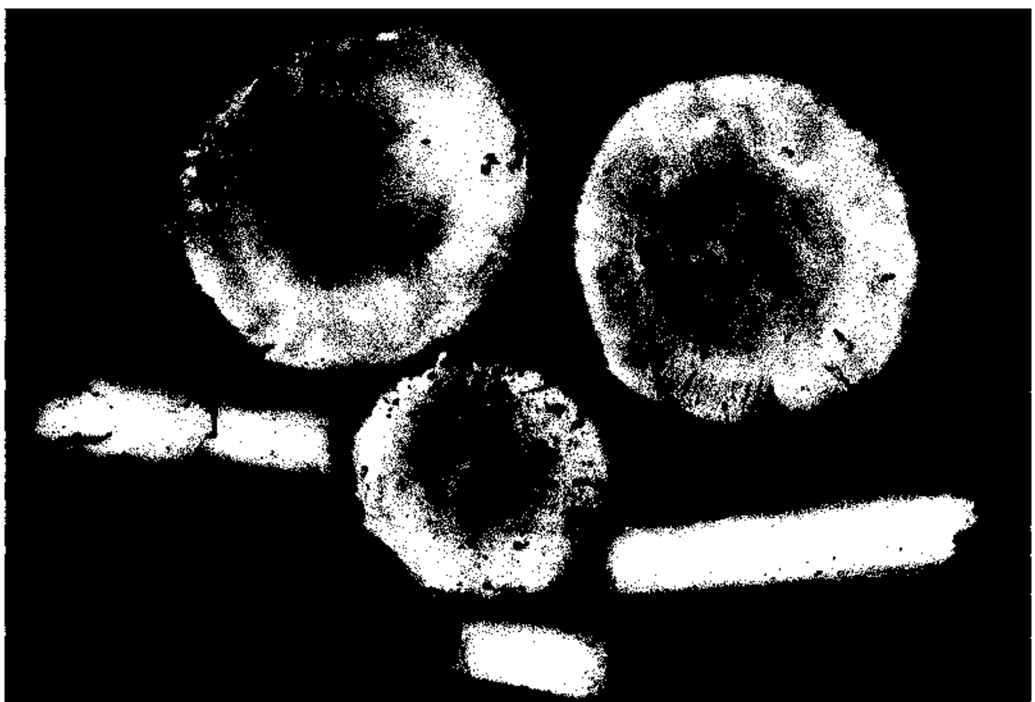


Figure 120 Macroscopic characters *oiRussula silvicola:* Top, illustration showing profiles and top of young to mature basidiomata, and a longitudinal section, the square is $1cm^2$ and shows spore print colour; bottom, a collection from the Cowichan Lake area that is exceptionally pale, photographed on a scanner for more accurate colour rendition.

Figure 121 Hymenium of *Russula silvicola;* Top left, cheilocystidia in situ stained with SV, scale bar is 50um; lower right, close-up of hymenial cystidia tips, scale is in 1um divisions; lower left, spores with 1um division scale.

Figure 122 Cutis of *Russula silvicola.* Top, section through cutis in water mount, 10 divisions on scale are 25 um; bottom, surface view of cutis showing pileocystidia stained in SV, one scale division is lOum.

Clade 5d

Subsection *Sardoninae* (Singer) Sarnari

Series *Persicina* Romagn. emend. Sarnari

***Russula* cf. *luteotacta* Rea**

British Basidiomycetes: 469. 1922

Cap 2.7-6.Ocm diameter, a mixture of light cream and pink, in one collection almost entirely cream with only touches of rose pink, on the other a delicate light pink with a light brown tint over the disc, slightly viscid, drying matte. Pulvinate when young, soon becoming shallowly depressed with a curved, striate margin. Cutis peelable 1/3-2/3 the radius, flesh white, unchanging where cut.

Gills white, unchanging where damaged, subdistant to close, few to frequent lamellulae, forking not seen, falcate, broadest closer to the stipe, adnexed at the stipe, subacute to obtuse at the cap margin, about twice the depth of the trama at half-radius, up to about 6mm, becoming crisped in the dried basidiomata, but whether this character was not recorded when fresh.

Stipe 3.1-7.3cm x 0.9-1.9cm, cylindrical, longer than the cap diameter, stuffed, becoming hollow, white, staining bright yellow at the base, yellow-brown above the base, the upper half or so unchanging when cut, in the dried material the stipes are a strong red-brown. The stipe base stains red in SV.

Texture about average for a *Russula,* not particularly fragile, not noticably firm, but rather thin-fleshed.

Taste acrid, sometimes after a few seconds of chewing.

Odour not distinctive to slightly fruity, of orange.

Spore colour white, Romagnesi la.

Spores 7-8.5 (-9) x (5.5) 6.1-7.5um, L:W 0.96-1.32 with a mean of 1.18 (n=30), obovoid. **Ornamentation** of quite heavy warts, 0.6 -1.2 high, most often under lum high, isolated or joined in rows to form ridges, with a few fine lines, not forming a reticulum, **Woo types B2, B3, C3.** **Suprahilar patch** a roundish to elongate (up to 4um long) lightly amyloid patch bordered by strongly amyloid warts and low ridges. **Hiliferous appendix** around 1.3-1.5 long by 1-1.2pm wide near the base. **Basidia** 4 - spored, 36-45 x 7-1 lum, clavate, sometimes broadly so. Sterigmata 5-8um long and 1.3-2pm wide near the base. **Pleurocystidia** abundant, 40-85 x 6-14um, protruding 15-30pm, originating in the subhymenium, clavate to fusoid, tips obtuse when young, acute to shortly tapering, sometimes with a small terminal button when mature, contents yellowish refractive in KOH, black in S V. **Cheilocystidia** sparse to frequent, similar to pleurocystidia but protruding to 40pm. **Subhymenium** 15-25pm thick, interwoven, **gill trama** of sphaerocytes with uncommon narrow vascular hyphae around 2pm wide.

Cutis 80-120pm thick at half-radius, in two layers, **Subcutis** about half the depth of the cutis, of loosely interwoven of hyphae 2-3 pm wide, the basal layers more compact and repent, with a pinkish hue in patches, and with vascular hyphae 5-7um wide, and embedded in a gelatinous matrix. **Hypodermis** -none. **Epicutis** a tangled turf of mostly unbranched hyphal ends l-3um wide, some tapering, others with slightly refractive contents like small cystidia, sometimes contorted or strangulate, in a viscid matrix. **Pileocystidia** 70-250 x 5-8um, most are in the 70-125um range, narrowly clavate to cylindrical, mostly with obtuse tips but some with a small capitum, arising from the lower subcutis. Many are aseptate, otherwise 1-3 septate. **Pseudocystidia** remaining embedded in the subcutis, merely the undifferentiated termini of vascular hyphae.

Trama of discrete clusters of sphaerocytes bound by a hyphal mesh and vascular hyphae.

Chemical reactions: FeSCU - no reaction; KOH - no reaction to pale orange on cap surface, no reaction to pale yellow on stipe; NH4OH - no reaction; phenol - pale brownish pink; S V - greyish on the gills and cuticle, cystidia and vascular hyphae grey to black with some red, red at the base of the stipe.

Habitat and tree associations: on the forest floor under red alder with salmonberry, huckleberry and salal, in openings of regeneration western hemlock and Sitka spruce coastal forest.

Collections: BT000919-07 and CR000919-08, near the Cape Beale trail on the western side of the Bamfield inlet, N48.822117°, W125.150583° and N48.824183^0, W125.150583° respectively (southern very wet hypermaritime CWH subzone).

Notes: These collections are closer to the *R. queletii* group than to the *R. fragilis* group, as they share similarities in the spores, pileocystidia and the yellow flesh within the base of the stipe. The former group has cream to yellow spores, not white, and usually purple and greenish tints in the cap. The European *R. luteotacta* has firmer flesh, barely peels, has more distant gills and more of the flesh stains yellow, albeit slowly, and it is found under broadleaved trees, particularly *Carpinus* (Hornbeam) in moist copses often near ponds (Bon, 1988, Romagnesi 1967). Romagnesi (1967) described *R. luteotacta* form *immuable* that lacked the yellow bruising reaction. The spores, epicutis, colour and pileocystidia of the Vancouver Island collections match the descriptions for the European *R. luteotacta* and the habitat amongst alders in moist coastal forest is similar. Romagnesi placed *R. luteotacta* in subsection *Persicinae* of section *Piperinae,* which places it in clade 5d.

Peck described *R. luteobasis* from North America (Torrey Botanical Club Bulletin 31:179, 1904) as a rosy colour that became paler with a yellowish centre and a yellow base of the stipe, but this species is mild, has yellow spores and has incrusted primordial hyphae in the epicutis. *Russula alborosea* Reumaux, a species decribed from France, is macroscopically similar to the above Vancouver Island collections and is found under *Fagus* and oaks in calcareous clay soils, the spores are larger at 7-10 x 6-8 urn, and the pileocystidia are shorter, up to 90um. This species is placed in the *Emeticinae.*

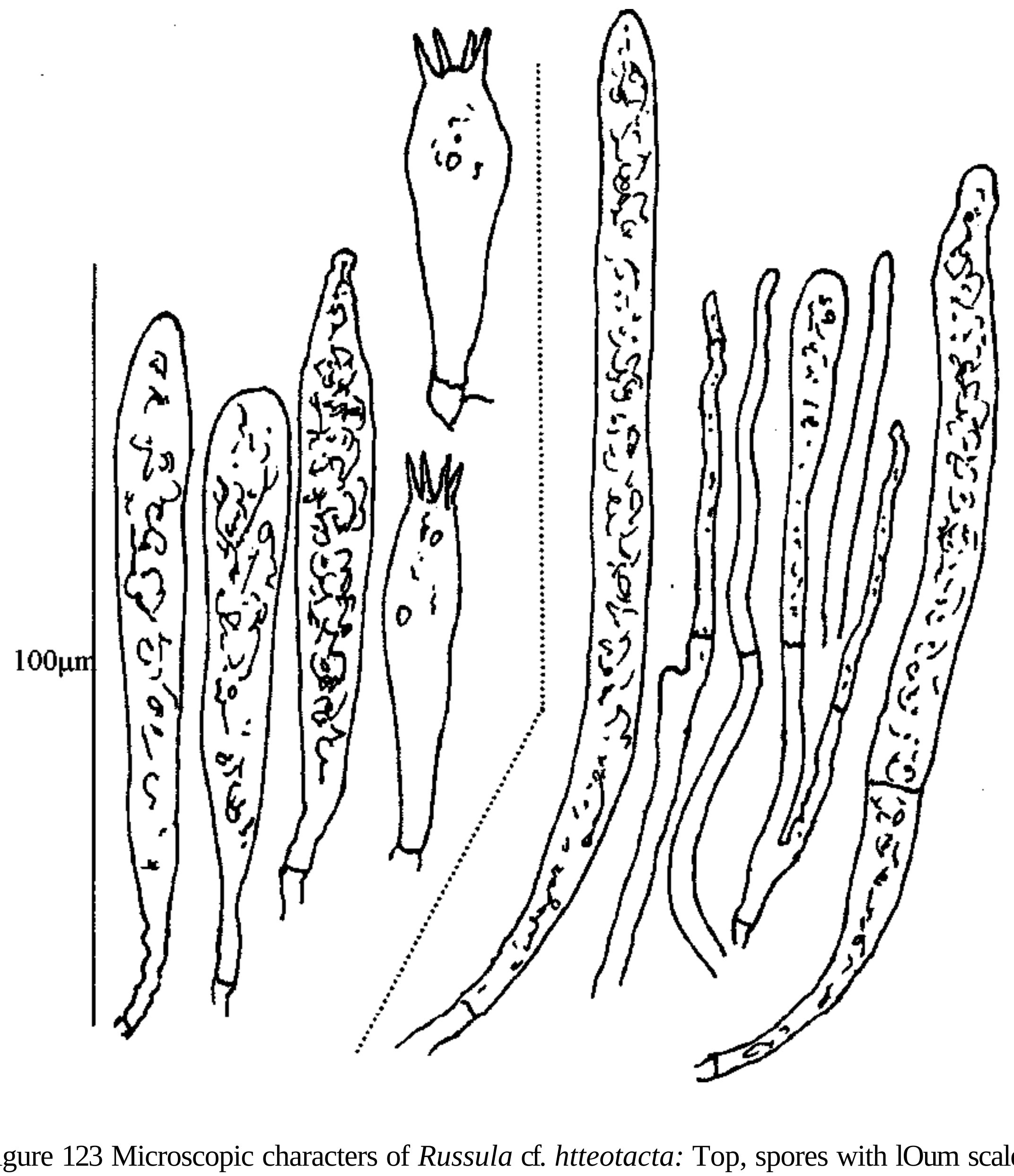


Figure 123 Microscopic characters of *Russula* cf. *htteotacta:* Top, spores with lOum scale bar; bottom left, hymenial cystidia and basidia; bottom right, pileocystidia and epicutal hyphal ends, lower scale bar is lOOum.

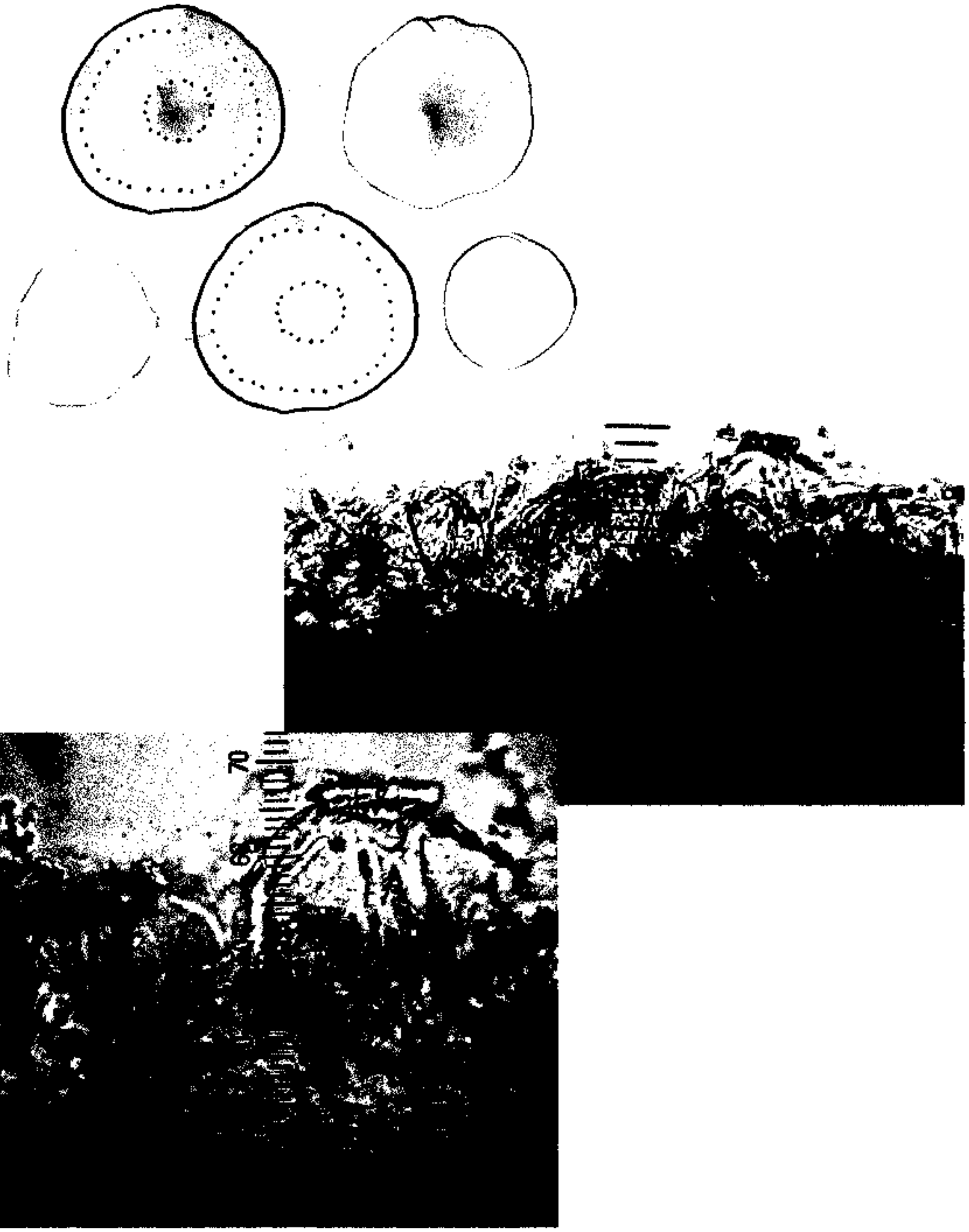


Figure 124 Cutis of*Russula* cf. *luteotacta:* Top, field colour notes of the caps of two collections; middle, section through cutis in water of a young basidioma at about 1/2 radius, 10 scale divisions are lOOum, and on this section there is almost no trama, the subcutis-subhymenium boundary is about at the 40 mark; bottom, a section of cutis at higher magnification showing a pileocystidia, hyphal ends and a vascular hypha at lower right, 10 scale divisions are 25um.

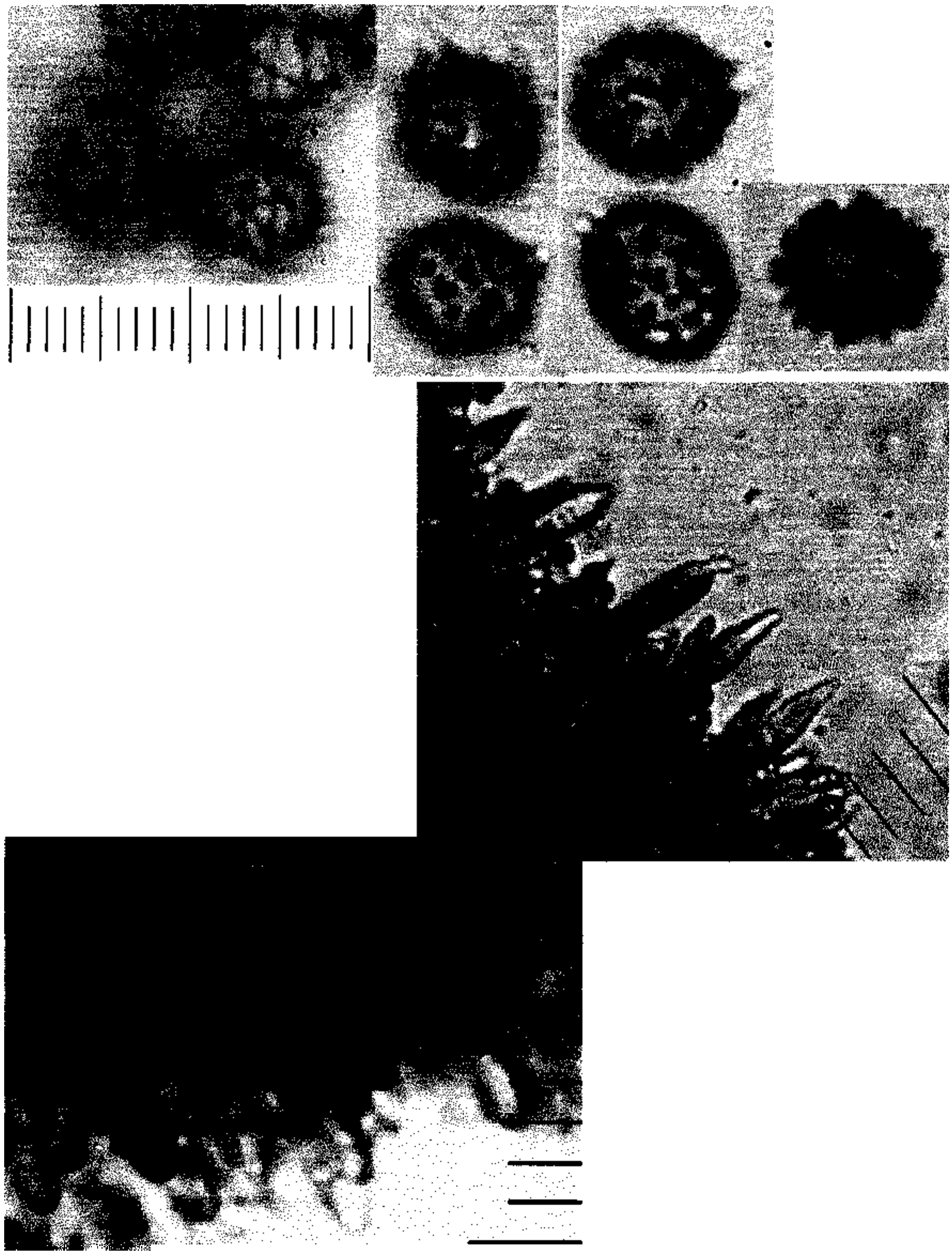


Figure 124 Hymenium *oiRussula* cf. *luteotacta:* Top, spores with lum division scale; bottom left, pleurocystidia stained in SV of a young basidioma; middle right, cheilocystidia of a mature basidioma stained in Congo red, scale is in lOum divisions for both photographs.

Clade 5e

Series *Sardonia* Sarnari

Russula queletii Fries

Memoires de la Societe d'emulation de Montbeliard 2nd series, 5: 185 1872

Cap 2.7-11cm diameter, convex when young, becoming plane with a central depression, margins smooth, striate only in age, rounded and curving over the gill ends until fully expanded. Colours range from deep red-purple to purple to violet, to a grey or brownish violet or purple, with a blackish to olive to brownish centre, very dark in young basidiomata, fading markedly to greyish or brownish pink, grey, grey-green or a mixture of these colours, sometimes remaining darker in the centre, otherwise the centre fading to brownish or dull yellowish. Although there appears to be variability in colour, the principal pigments are magenta and blue-green, usually with the greenish hues centrally and the magenta throughout or more towards the margin. Surface viscid to sticky when wet, drying subglossy to matte, sometimes minutely velvety to bumpy over the disc, usually with a pale pruina in younger basidiomata. **Cutis** peelable 1/2 to 2/3, flesh beneath tinted pink or dull violet, cap flesh creamy white, unchanging where damaged.

Gills pale cream to cream, (lacking lemon yellow tints), close to subdistant, sometimes becoming distant in age, occasional lamellulae and forking. Adnate or sometimes slightly decurrent in age, generally narrowest at stipe, broadening slightly outwards or of more or less of equal depth in the outer 2/3, obtuse at cap margin, depth roughly equal to that of cap trama at half-radius, brittle and fairly soft.

Stipe 2.4-9 x 0.7- 2.1cm, on average approximately equal in length to cap diameter, stuffed with a bread- textured trama which develops several small irregular cavities when young, becoming hollow. **Stipe** surface usually partially or completely deep pink to violet, occasionally white, pruinose, unchanging when cut or browning slightly, greying as if waterlogged in age. The base of the stipe stains bright yellow and when cut shows a thin layer of yellow tissue just under the stipe cutis at the tip (fig 127).

Texture firm and quite hard at first, becoming soft and fragile by late maturity.

Taste mostly peppery rather than acrid, occasionally more intense.

Odour mild and slightly fruity, of stewed apple or sometimes like jam, the strength of the odour is weather dependent and reduced by cold or dry conditions.

Spore colour pale to deep cream, somewhat variable between collections, generally slightly darker than that of European material; Romagnesi IIc-IIIc.

Spores 6.9-10.5 x 6-8um with means of 8.5 x 7um, L:W 1.07-1.39 with a mean of 1.23, (n=94), warts 0.2-0.7um on most spores, occasionally to l.Oum. **Warts** bluntly conical, sometimes crestate, often quite heavy, in some spores mostly isolated, in others also with low connections between 2-4 warts forming irregular lines and crests, rare spores with almost all the warts in connected groups, not forming a reticulum or sometimes a broken one. **Woo types** A2-B2, rarely to C2. **Suprahilar patch** an amyloid, commonly elongate area around 3urn long, bordered by low broad warts. **Hiliferous appendix** 1.6-1.9um long, 1-1.5um wide near the base. **Basidia** 4 spored, 32-50 x 9 -12um, clavate to slightly fusoid, not particularly bulbous. Sterigmata 6-8 um long and about 0.7um wide at the base, slender. **Pleurocystidia** 45-92 x 9-15um protruding 20-30um, originating in the subhymenium, fusoid, tips acute, mucronate, small-capitate, or sometimes with a slender extension, contents yellowish and refractive in KOH, deep purple to black in SV. **Cheilocystidia** sparse to numerous but clustered, around 50-60 x 6-8 um, protruding about lOurn or less, roughly but irregularly cylindrical with tips similar to pleurocystidia, reddish to purple in SV. **Subhymenium** 25-45 um thick, interwoven, sometimes incorporating a few cells, **gill trama** of hyphae and sphaerocytes with abundant vascular hyphae 5-8um wide in the thicker, upper part of the gill, narrowing to nothing towards the gill margins.

Cutis 60-100um thick at half-radius, up to 400um thick on disc, an ixotrichoderm with a turf of hyphal ends and numerous pileocystidia and pseudocystidia. **Subcutis** comprising about half the depth of the cutis, of tightly interwoven repent hyphae 2-4um wide with pinkish contents, and a network of numerous vascular hyphae, staining red or grey in SV. **Epicutis** more loosely interwoven, of semi-upright hyaline hyphal ends 4-6um wide, pileocystidia, and abundant pseudocystidia whose terminal 50-100ujm or more lie on the surface and give the cutis its minutely bumpy texture, all embedded in a gelatinous matrix. The hyphal ends often have refractive contents in the terminal cell. **Pileocystidia** 32 -70 x 3-8um, 0-1 septate, cylindrical, occasionally bifurcate or diverticulate, tips usually obtuse, sometimes capitate, with refractive and often banded contents, weakly to strongly staining in SV. **Pseudocystidia** arising from the lower subhymenium or hypodermis, septate, with terminal cells similar in shape and contents to pileocystidia. It is quite common in pseudocystidia and sometimes pileocystidia to have a terminal cell 25-30um long, while below that septa occur at greater distances apart. **Hypodermis** of flattened cells and vascular hyphae.

Trama of discrete clusters of sphaerocytes generally under 40um diameter, bound by a hyphal mesh and numerous vascular hyphae.

Chemical reactions: $FeS0_4$ - pale pinkish; KOH - reddish orange on cap cutis, yellowish to salmon pink on the coloured stipes, no reaction on the white part and inner trama; NEUOH - no reaction; phenol - very slowly brownish (after 4-5 minutes); SV - initially magenta on the gills turning brownish -grey, purple on the cuticle, cystidia and vascular hyphae grey to black.

Habitat and tree associations: Under coastal Sitka spruce just above the highest tides, in sandy soil with seashell fragments.

Collections: CR981013-04, under western hemlock, western red cedar and salal Cape Scott and San Joseph trail junction, N50.7725^0, W128.414^0,. CR981013-04a and -04b, along the shoreline forest of Sitka spruce, western hemlock and salal, San Joseph trail, Cape Scott, N50.773^0, W128.403^0. CR981029-01 and -01b, at base of Sitka spruce, Combers Beach Spruce trail, Long Beach area, PRNP, N49.045^0, W125.70633^0. CR001011-43, CR001114-02, CR001114-05, PJ 010919-05 and CR021016-01 under Sitka spruce and western hemlock shoreline forest along the trail to Florencia bay, Wickaninnish, PRNP, in the vicinity of N49.0125^0, W125.6755^0. CR011030-02, under small Sitka spruce, Long beach picnic area, PRNP, N49.0445^0, W125.702^0. CR020927-04 under old growth western hemlock and Sitka spruce with western red cedar, alongside Carmanah river, Carmanah grove N48.67°, W124.68667^0. (All collections from southern very wet hypermaritime CWH subzone).

	ITSl-Fto	RFLP:		
Collection	ITS4-B	Hinfl	Alul	Sau3A
CR981029-01b	832	388, 349	460, 280	253
PJO10919-05	849	408, 352	486, 292	359, 270
PJ010919-05 (repeat)	854	388, 371	489, 259	346, 227
CR020927-04	857	388,354	460, 274	371,315,231

Notes: The amount of reticulation on the spores of the above collections is sometimes greater than is given in descriptions for *R. queletii* by Bon (1988), Romagnesi (1967) Sarnari (1998), or Thiers (1997), and the spore prints are a shade or two darker. It is not clear whether the above collections of *R. queletii* and those described below as *R.* cf. *fuscorubroides* are actually two species or a single variable species. The average spore size differs by almost 0.5um in length and 0.3um in width with *R. queletii* having the larger dimensions ($p< 0.001$, $a = 0.05$ for each dimension). None of the above collections had a red reaction with either KOH or NH_4OH so they are neither *R. sardonia* nor *R. cavipes*. *Russula sardonia* Fries has fairly narrow pileocystidia, under 6um wide, lemon yellow tinted gills and a pink reaction with ammonia (Sarnari 1998). *Russula torulosa* Bresad. is very similar to *R. queletii* but larger and more robust, with a shorter stipe. It has reticulated spores and occurs on sandy soils with pines, rarely spruce (Bon 1988, Sarnari 1998).

Other purple capped, cream-spored, peppery Russulas include *Russulaplacita* Burl., with more reticulated spores and occurring under Douglas fir. *Russula gracilis* Burl., a small fragile species with a purple, violet and green cap colours that fade and often a pinkish stipe, the spores are pale yellow: Romagnesi Ila-b, with low ornamentation 0.2-0.4 um high of isolated warts, and Thiers (1997) reports it from coastal forests in California.

Russula mordax Burl, has more brownish tints in the cap, is quite large, up to 13cm across the cap, and occurs under Douglas firs (Burlingham 1936). This latter is closer in appearance to the *R* cf. *fuscorubroides* described below.

Russula pelargonia Niolle has a purple to greyish purple cap sometimes with olive tints and a purple to lavender stipe and is distinguished by its odour of geraniums (pelargonium), the spores are reticulated and 6-8.6 x 5-8um, slightly smaller than any of the collections from the Clayoquot area, Romagnesi (1967) reports the habitat as under *Populus* and other broadleaved trees and Woo (1989) describes Pacific northwest collections as in Douglas fir-westem hemlock forest. *Russula queletii* and *R. fuscorubroides* may also have pelargonia-like odour components sensu Bon (1988).

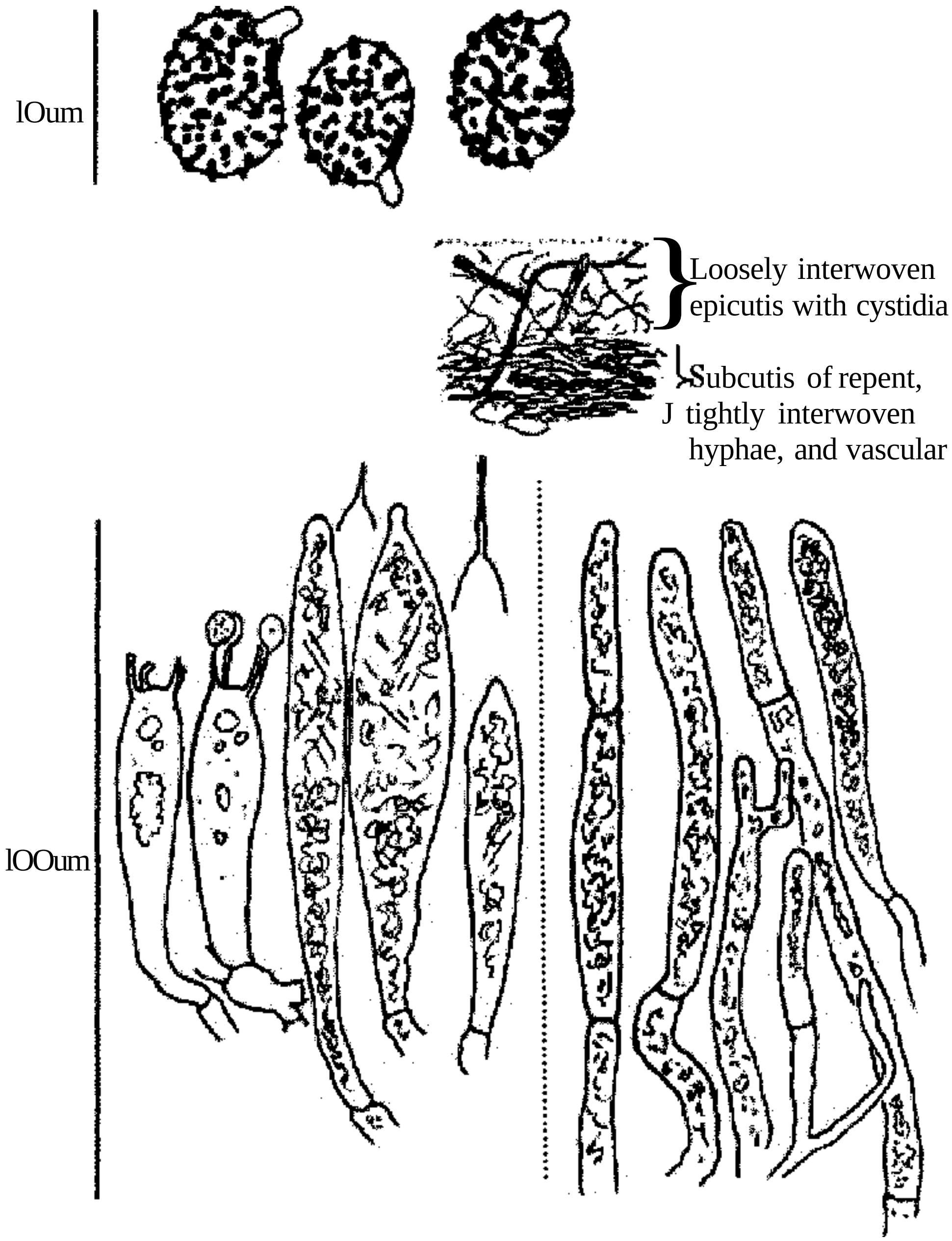


Figure 126 Microscopic characters of *Russula queletii*: Top, spores with lOum scale bar; middle, diagram of section through cutis; bottom left, basidia and hymenial cystidia; bottom right, pseudocystidia, pileocystidia and hyphal ends from the epicutis, lower scale bar is lOOum.

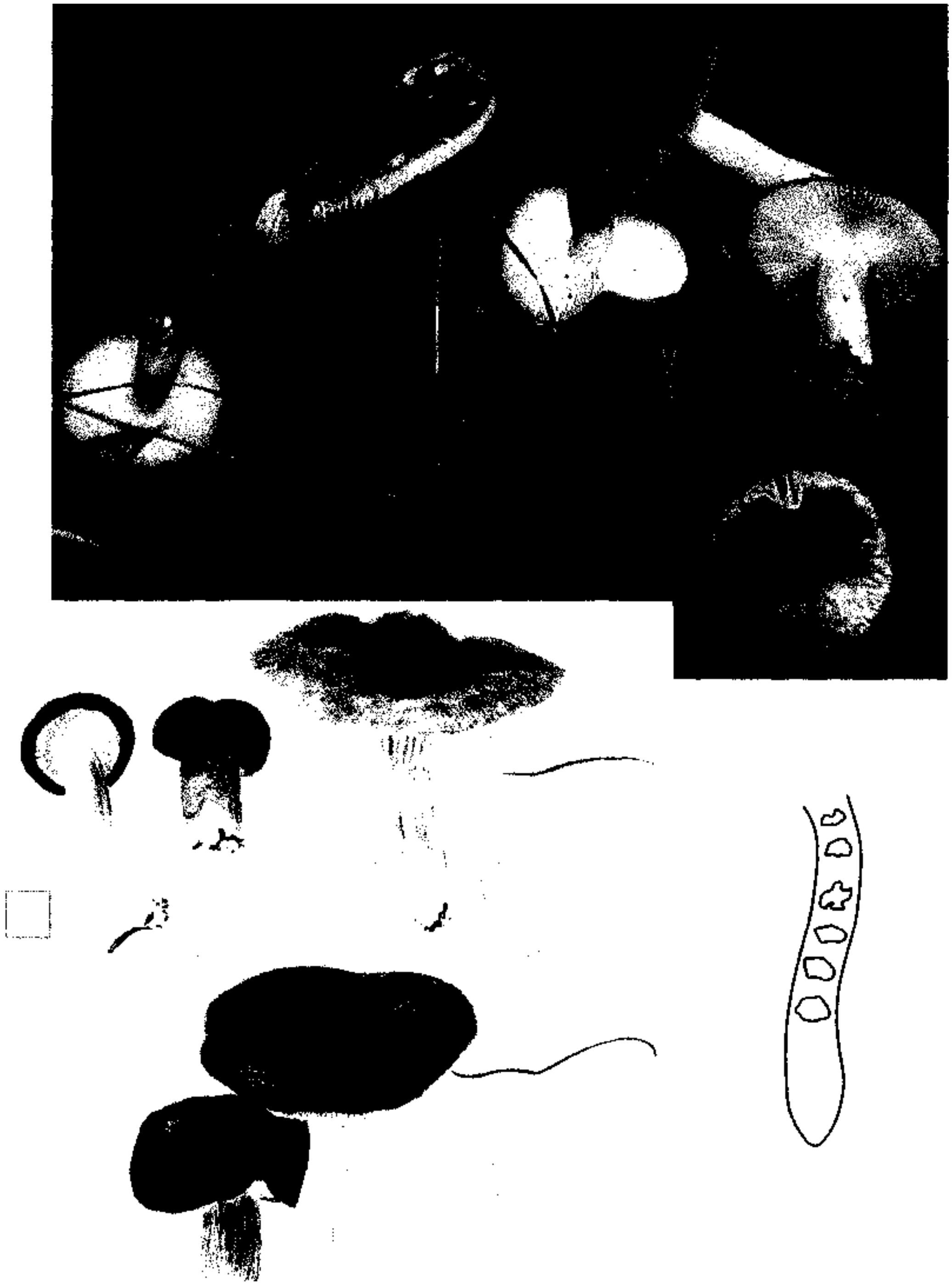


Figure 127 Macroscopic characters of *Russula queletii:* Top, several basidiomata in habitat (under shorline Sitka spruce), photograph by A. Ceska, inset, a faded specimen; below, illustrations of two collections showing the yellow tissue at the stipe base, the squares are 1cm^2 and show spore colours: below left, diagram of cavities in a young stipe.

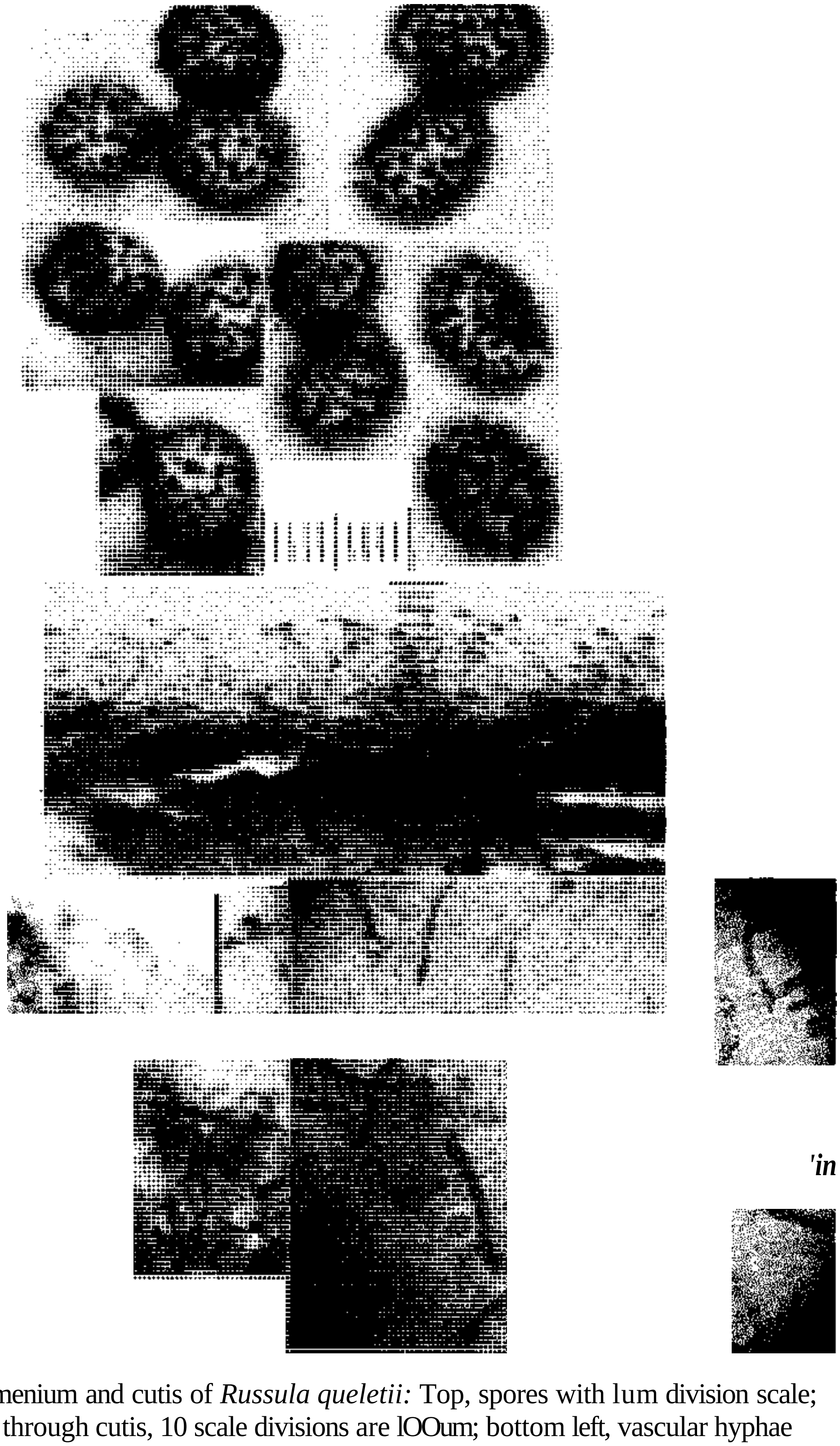


Figure 128 Hymenium and cutis of *Russula queletii:* Top, spores with lum division scale; middle, section through cutis, 10 scale divisions are lOOum; bottom left, vascular hyphae in the hypodermis, scale bar is lOOum; bottom right, pseudocystidia and pileocystidia in the epicutis.

***Russula queletii* cf. *xnr.flavovirens* Bommer & Rousseau**

Flora mycologique de Beige Suppl. 1: 58. 1887

One collection *of Russula queletii* had an almost entirely green cap (fig. 129) with mere traces of purple at the margins. These basidiomata had pink flushes on the stipe and the characteristic bright yellow bruising at its base, and were microscopically almost identical to the purple-capped *R. queletii* found in the same habitat, but differed in the following characters:

Cap green throughout but darkest in the centre, mottled with yellow-green, becoming greyer towards the margins with purplish tints at the very margin, peelable less than 1/4. **Odour** mild but slightly sour. Spore print light orange, Romagnesi IIIc. **Spores** 7-9 x 6-7.9u,m, L:W 0.96-1.33, mean 1.18 (n=30) warts up to 0.7um, occasionally to lum, mostly isolated, sometimes 2-3 joined with usually heavy lines or ridges, Woo A2-B2, rarely B3. The spores tend to have a slightly broader shape on average than those of the purple *R. queletii* but have the same ornamentation.

Collections: CR981029-02, in Sitka spruce krummholz at the top of the beach, Combers beach trail, Long Beach area, PRNP, N49.0435^0, W125.7035^0 (southern very wet hypermaritime CWH subzone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR981029-02	800 ~~	364,325	unsuccessful	368,269

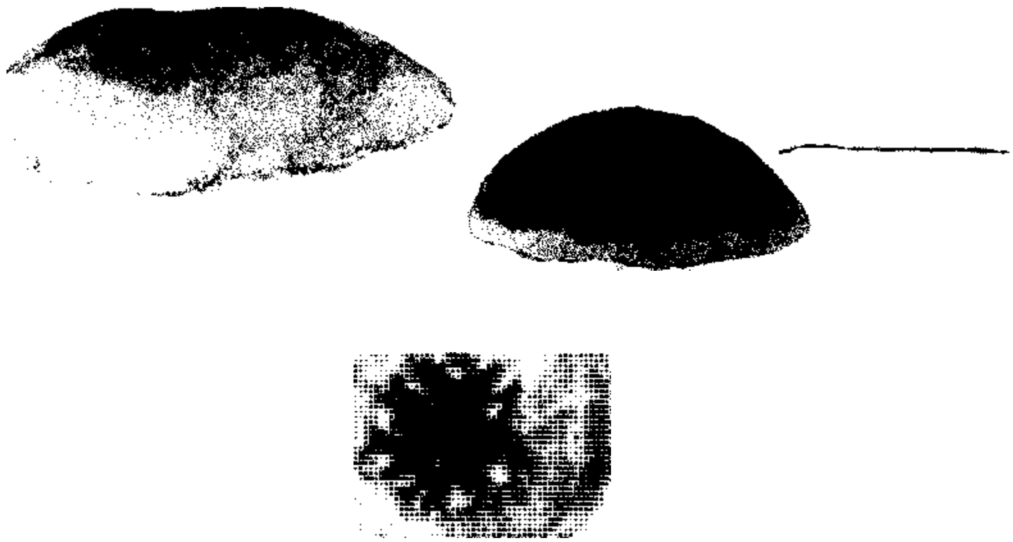


Figure 129 Macroscopic characters of *Russula queletii* cf. var. *flavovirens,* the square shows spore print colour and is 1cm^2.

***Russula* cf. *fuscorubroides* Bon**

Bulletin de la Societe Mycologique de France 91 (4). 1975

Cap 4.6-11.3cm diameter, mid to dark purplish red, black or nearly so over the disc when young, then sometimes with brownish or olivaceous tints, not fading much, peelable less than 1/4 the radius, viscid when wet, drying subglossy and minutely bumpy when veiwed with a handlens, more so near the centre. Hemispherical when young, becoming plane with a central depression, margins eventually unevenly uplifted. Margins smooth when young becoming striate to 1/4 the radius when fully mature. Flesh creamy white or pinkish under the cuticle, unchanging when cut.

Gills deep cream when young, becoming pale cream at maturity, unchanging to bruising brownish where damaged, subdistant to distant in larger basidiomata, adnate at stipe, subacute at margins, ventricose, approximately equal to depth of trama at half-radius in early maturity, almost twice the depth in age, forking and presence of lamellulae varying from none on some basidiomata to many on others.

Stipe 3-8.8cm x 1.1 -3.2cm, more or less cylindrical or broadening at the base, white or with a flush of pink at the base or less commonly over most of the surface, pruinose when young, surface longitudinally rugulose, unchanging when cut but yellowish brown stains developing near the base. In age the flesh becomes slightly greyish and soft.

Texture firm when young and early maturity, becoming quite fragile in age.

Taste immediately acrid.

Odour faint, slightly fruity, of apples or plums.

Spore colour light cream, Romagnesi Ila-c.

Spores 7-9.4 (-10) x 5.8-7.6 (-8)um, with means of 8.1 x 6.7um, L:W 1.02-1.34 with a mean of 1.2 (n=65), broadly ellipsoidal. **Ornamentation** of fairly heavy conical to pyramidal to crestate warts mostly 0.4-0.9um high, occasionally to 1.2um high, in general isolated, sometimes in rows or clustered, 2-3 catenate or with a low, often broad connection that appears weakly amyloid, not forming a reticulum, smaller warts sometimes scattered between the larger ones. **Woo types** A2-3 to **B2 -3. Suprahilar patch** an irregular amyloid area bordered by small or normal sized warts, hiliferous appendix 1.4-2.1um long, around 1.2pm wide at base. **Basidia** 4 spored, 37-53 x 8 - 12pm, clavate to bulbous in the upper 1/3. Sterigmata up to 10pm long and lpm wide at the base. **Pleurocystidia** 60 -140 x 8-12pm, occasional broad ones to 21pm wide, protruding 20-40pm, originating in the subhymenium or outer trama, narrowly fusoid or almost cylindrical, tips mostly tapered, acute or occasionally with a tiny capitum, contents refractive, yellowish in KOH, dark grey to black in SV, tips acute, sometimes with a tapering extension. **Cheilocystidia** patchily distributed, generally shorter up to 60pm, and more irregularly shaped than pleurocystidia, protruding around 25pm, sometimes agglutinated. **Subhymenium** 20-50pm thick, pseudoparenchymatous, gill trama of fairly spherical sphaerocytes 20-40pm diameter, with occasional vascular hyphae 5-8pm wide.

Cutis 90 -120pm thick, an ixotrichodermis. **Subcutis** of tightly interwoven repent hyphae with pinkish-brown contents 2- 4pm wide, laticiferous hyphae frequent, staining red or grey in S V, and terminating in the epicutis as pseudocystidia, all embedded in a gelatinous matrix. **Epicutis** of repent to semi-upright hyphal ends 3-5pm wide, undiffentiated or with irregularly shaped, slightly inflated, strangulated or nodulose terminal and subterminal cells. **Pileocystidia** 26-100 x 4-8pm, common, sometimes distributed in clusters, cylindrical or narrowly clavate, sometimes diverticulate, occasionally forked or branched, ends obtuse or capitate, 0-3 septate but the majority aseptate or with one septum separating a shorter terminal cell, contents yellowish and refractive, sometimes banded. In the case of banded contents, there are frequently narrow breaks in the refractive material that can appear like septa, this is less apparent if the cutis is mounted in Melzers' reagent. Sometimes the hyphal wall forms a small asymmetrical shoulder just below a septum. **Pseudocystidia** numerous, septate, arising in the lower subcutis, contents, width and terminus similar to those of pileocystidia. **Hypodermis** of flattened sphaerocytes and repent hyphae.

Trama of small to large discrete clusters of sphaerocytes bound and permeated with a comparatively dense hyphal mesh.

Chemical reactions: FeSC>4 - pale pinkish; KOH - brownish orange on cap surface, no reaction on stipe; NH4OH -no reaction; guaiac - blue-green; guaiacol - pinkish; phenol, - brownish purple; SV - grey on the gills, purple on the cuticle, cystidia and vascular hyphae grey to black.

Habitat and tree associations: In coastal western hemlock forest but inland of the Sitka spruce dominated coastal fringe, associated with western hemlock and possibly also amabilis fir, and often alongside trails where there are young trees.

Collections: CR981013-07, on a very decayed stump under mixed-age western hemlock and Sitka spruce, San Joseph trail, Cape Scott, approximately N50.773°, W128.403^0. CR000919-02 alongside the Cape Beale trail, Bamfield, under young western hemlock, N48.82658^0, W125.1458^0. CR001012-22 and -23, along the roadside of Grice Bay mainline logging road near Kennedy Lake Park, under red alder, western hemlock and amabilis fir, N49.016883^0, W125.58153°. OC010920-RFB and CR021015-21b, in young stand of western hemlock-amabilis fir at the edge of the seaward side rainforest trail, PRNP, N49.05^0, W125.7095^0. (All above from southern very wet hypermaritime CWH subzone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR981013-07	848	388,329	500,333	343,213
CR000919-02	865	412,369	474,285	364,275
CR001012-23	865	395,365	482,295	361,208
CR021015-21b	874	385,329	500,361	349,269

Notes: This species was first identified as *Russula queletii* with which it morphologically very similar, the slight differences in colour and habitat from collections also identified as *R. queletii* from the coastal spruce fringe suggest either a variant or a different species. These collections of i?.cf. *fuscorubroides* have on average a redder cap than local *R.*

queletii, and only a yellow-brown rather than bright yellow staining at the stipe base. *Russulafuscorubroides* is perhaps the closest identification, a species preferring acidic soils but usually associated with *Picea,* often in habitats with *Vaccinium* also present. A rough test with ph strips dipped into the moisture squeezed from a handful of rainforest soil showed that they are generally acid, around pH 4-5, whereas the spruce fringe along the upper shoreline is pH 5-6. The above collections have slightly paler spores than the lid for *R. fuscorubroides* given in Bon (1988).

Russula torulosa is a very similar species that may have quite large basidiomata on a par with the above collections, it has in general more reticulations and fewer isolated warts on the spores than the above collections and is associated mainly with pines on neutral to calcareous sandy soils, rarely with spruce on acid soils. *Russula fuscorubra,* another associate of spruce on calcareous soils has darker and more reticulate spores and broader pileocystidia (8-12um wide). *Russulapunicea* Thiers, is an evenly coloured dark red species found under coastal Sitka spruce in California, it has yellow spores, a white stipe lacking any coloured flushes and spores with similar but slightly lower ornamentation (0.3-0.7um high) to the above collections. Thiers (1997) reports that *R. punicea* is known from only one collection so the full range of variation within this species may not have been observed. Other acrid tasting dark red to purple species that may be found in coastal forests include *Russula mordax* Burl.a reddish brown species, rare in California (Thiers 1997) and considered by Singer to be synonymous with *Russula badia* Quelet. It has clavate to subclavate pileocystidia, spores 8.5-1 lum long with isolated spines 0.4-lum high and heavy ridges (Thiers 1997), and a spore print of Romagnesi Illd-IVa (Romagnesi 1967 fori?, *badia).*

10p,m

lOOum

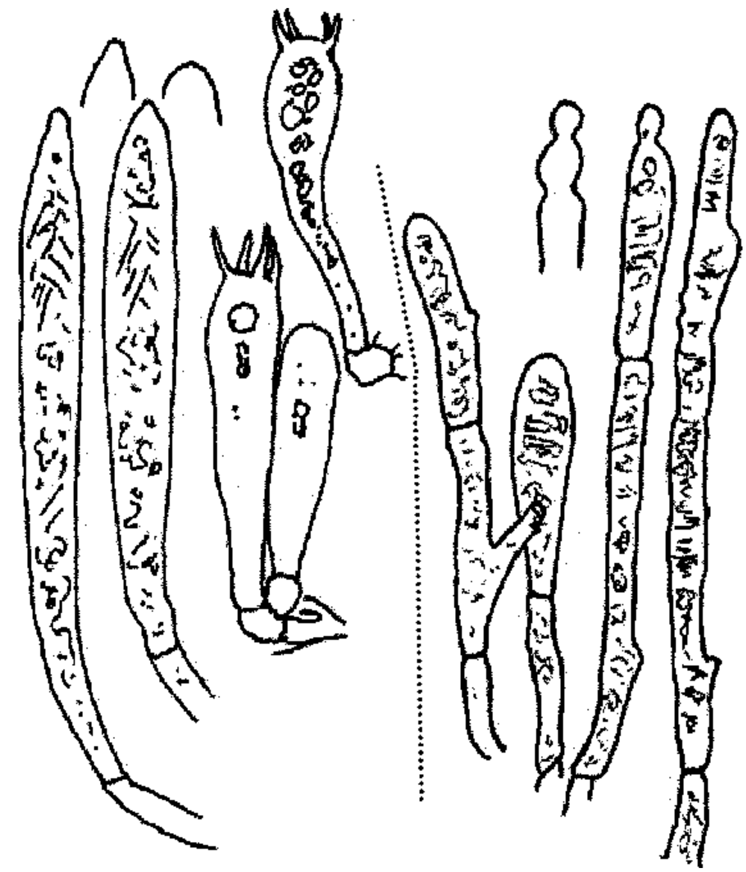


Figure 130 Microscopic characters of *Russula ctfuscorubroides*: Top spores with lOum scale bar; bottom left, cheilocystidia (ch), pleurocystidia and basidia; bottom right, pileocystidia, lower scale bar is lOOum.

Figure 131 Macroscopic characters *ofRussula* *zi.fuscorubroid.es:* Top, illustrations of a collection from Cape Scott (left) and Bamfield (right), showing profiles of mature and immature basidiomata and a longitudinal section, the squares are $1cm^2$ and show spore print colour; bottom, photograph of mature basidiomata in situ alongside an unpaved road beneath red alder, western hemlock and amabilis fir.

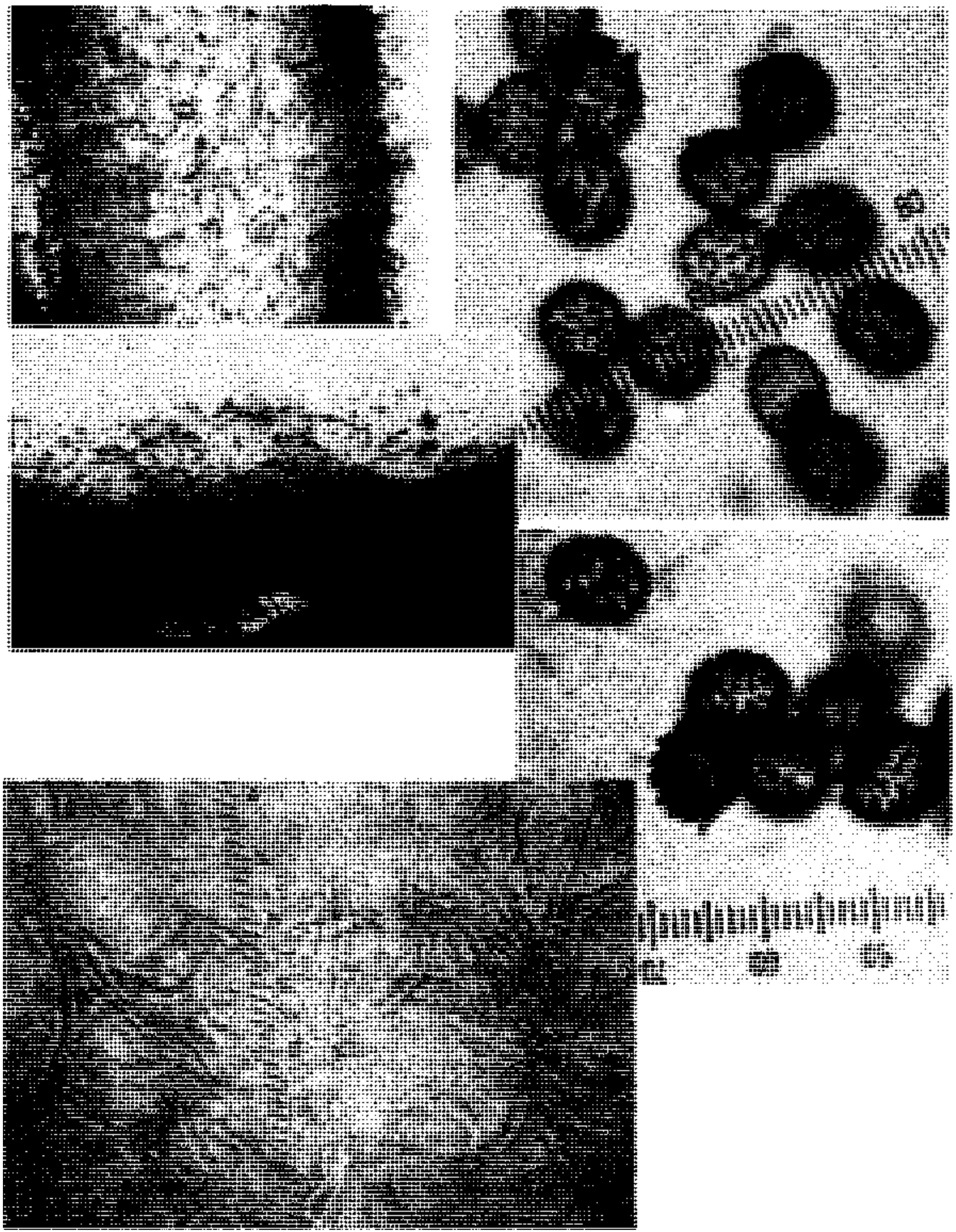


Figure 132 Hymenium and cutis *otRussula ctfuscorubroides:* Top left, section through a giU which shows the lamellar trama of sphaerocytes, 10 scale divisions are 25 um; right, two photographs of spores with lum division scale; middle left, section through cutis, 10 scale divisions are lOOum; bottom, surface view of cutis in Melzers' reagent showing pileocystidia and pseudocystidia, 10 scale divisions are 25 um.

***Russula** cavipes* **Britzelmayr**

Hymenomyceten aus Sudbayern 9:17, 1893.

Cap 3.3-10.lcm diameter, convex at first, soon becoming plane with a central depression, at maturity the margins often lobed, wavy and unevenly uplifted, margin smooth, striate in age. Colour deep vinaceous, black over the disc or occasionally yellow-brown, fading quite dramatically to a purplish grey-brown, often with pale olive to yellowish brown patches, eventually becoming pale pinkish-brown, viscid when wet, or drying drying matte to slightly velvety over the disc. Cutis peelable 1/4 to 1/3, cap trama cream, tinted greyish under the cutis, becoming greyish in age but otherwise unchanging where damaged.

Gills pale to deep cream, becoming yellowish, unchanging where bruised, subdistant to close, lamellulae occasional to frequent, and forking common, adnate at stipe, acute at cap margin, ventricose, approximately equal in depth to cap trama at half-radius.

Stipe 3.1-8cm x 0.8-2cm, in general roughly equal in length to cap diameter, cylindrical or more often somewhat clavate, white or with a slight pinkish flush, unchanging or bruising yellow-brown at the base, becoming yellowish to greyish as if waterlogged in age. Surface pruinose, longitudinally finely rugulose, cortex stuffed with a bready-textured trama which is solid at first but develops irregular cavities and eventually becomes hollow.

Texture firm and not brittle when young, becoming soft and fragile in maturity.

Taste slightly bitter at first, then peppery to acrid and of apple at the same time.

Odour slightly fruity, of stewed apples.

Spore colour pale cream; Romagnesi Ha or slightly paler.

Spores 7.8-11.5 x 6.2-8.6um, L:W 1.03-1.37, mean (n=30) 1.23, broadly ellipsoidal to obovate, occasionally slightly pyriform. **Ornamentation** of bluntly conical to crestate.

sometimes incompletely amyloid, often heavy warts, 0.5-1.1um high, these often in rows connected by fine to heavy lines, catenate, or forming short ridges, forming a partial reticulum or zebroid pattern, interspersed occasionally with small isolated warts, **Woo types** C2 to C3, and E2 to E3. **Suprahilar patch** an irregular often eccentric amyloid patch bordered by warts, and sometimes containing one or two. **HHiferous appendix** 1.6-2.6 x 1.3-1.5um. **Basidia** most 4-spored, some 2-spored, 42-62 x 10-13.6um, clavate to bulbous in the upper 1/3. Sterigmata 6-10um long, 1.5-2.3um wide near the base. **Pleurocystidia** frequent in young basidiomata, sparse in old ones, (as if the basidia multiply as the gills enlarge but not the cystidia), 62-120 x 7-13.6um, originating in the subhymenium, protruding 15-30um, narrowly clavate or fusoid, tips acute, subacute or with a small capitatum or short appendix, contents yellowish and refractive in KOH, dark grey to black in S V. **Cheilocystidia** abundant, becoming sparser in age, generally a little shorter than pleurocystidia, around 60um, otherwise similar in shape and contents. **Subhymenium** 30-50um thick, pseudoparenchymatous. **Gill trama** of sphaerocytes, or with occasional vascular hyphae.

Cutis 90-170um thick, an ixotrichoderm of two layers, with a pink pigment mostly in the epicutis and which appears to be dissolved in the gelatinous matrix. **Subcutis** brownish (in water mounts), about 1/3 the depth of the cutis, of parallel interwoven hyphae 2 -5um wide, and many vascular hyphae 5-8 um wide, which frequently terminate in the epicutis or at the surface in pseudocystidia. **Epicutis** of loosely tangled semi-upright hyaline hyphae 1.5-3.5um wide which may be tortuous and strangulated subapically, with slightly clavate or capitate ends up to 5um wide, sometimes with refractive contents, also abundant pileo- and pseudocystidia, embedded in a pink stained gelatinous matrix. **Pileocystidia** frequent to abundant, 40-100 x 3-9um, most around 6um wide, cylindrical to clavate, 0-2 -septate, tips obtuse or capitate, in age forked tips are quite common, contents refractive, light grey to black in S V, the reaction stronger in mature and aged basidiomata. **Pseudocystidia** abundant, sometimes more frequent than pileocystidia, arising from the subcutis, most around 6um wide, septate, shape and contents similar to pileocystidia. **Hypodermis** compactly interwoven and including small flattened sphaerocytes and numerous vascular hyphae, the basal layers of the subcutis are interwoven with the upper layers of the trama near the cap centre, so the division between the two tissues is not abrupt.

Trama of discrete clusters of sphaerocytes bound by a hyphal mesh and frequent vascular hyphae, 5-7um wide.

Chemical reactions. FeSCu salmon pink; KOH bright red to red-brown on cap cutis, pink to red on stipe trama; NaOFL* - pink to red on stipe trama; phenol - brownish; guaiac - blue-green; S V- red to magenta initially, quickly turning grey-brown on gill, brown on cutis, grey to black on all cystidia and vascular hyphae.

Habitat and tree associations: on ground under mature Douglas fir, grand fir and western red cedar in moist streamside soil.

Collections: CR001102-01, CR001102-03, CR011015-01, CR021201-01, all from the same area in moist bottomlands by the river Millstream, under mature Douglas fir and western red cedar with understory western hemlock and an old-growth grand fir *(Abies grandis)* about 20m away, N48.452433^0, W123.48382^0 (CDF zone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR 021201-01	857	391,354	491,295	349,260

Notes: The Vancouver Island collections *oiRussula cavipes* are distinguished from similarly coloured species such as *R. gracilis, R. pelargonia, R. queletii, R. torulosa* and *R. fuscorubriod.es* by the pink to red reaction of the flesh of mature basidiomata with alkaline solutions particularly ammonia (household ammonia). The only other species with this reaction as far as is known is *Russula sardonia,* which prefers sandy soils with pine, has lemon yellow tints in the gills and flesh, a coloured stipe, lower spore ornamentation (up to 0.5um) and slightly narrower cuticular cystidia. Occasionally, immature individuals of *R. queletii* also show this red reaction in dry weather. The above collections of *R. cavipes* match descriptions given for European material (Romagnesi 1967, Samari 1998), with the exception of a blue-green rather than null or weak guaiac reaction, and the pileocystidia which reach a maximum of 9um, rather than the 6-1 lum of European material.

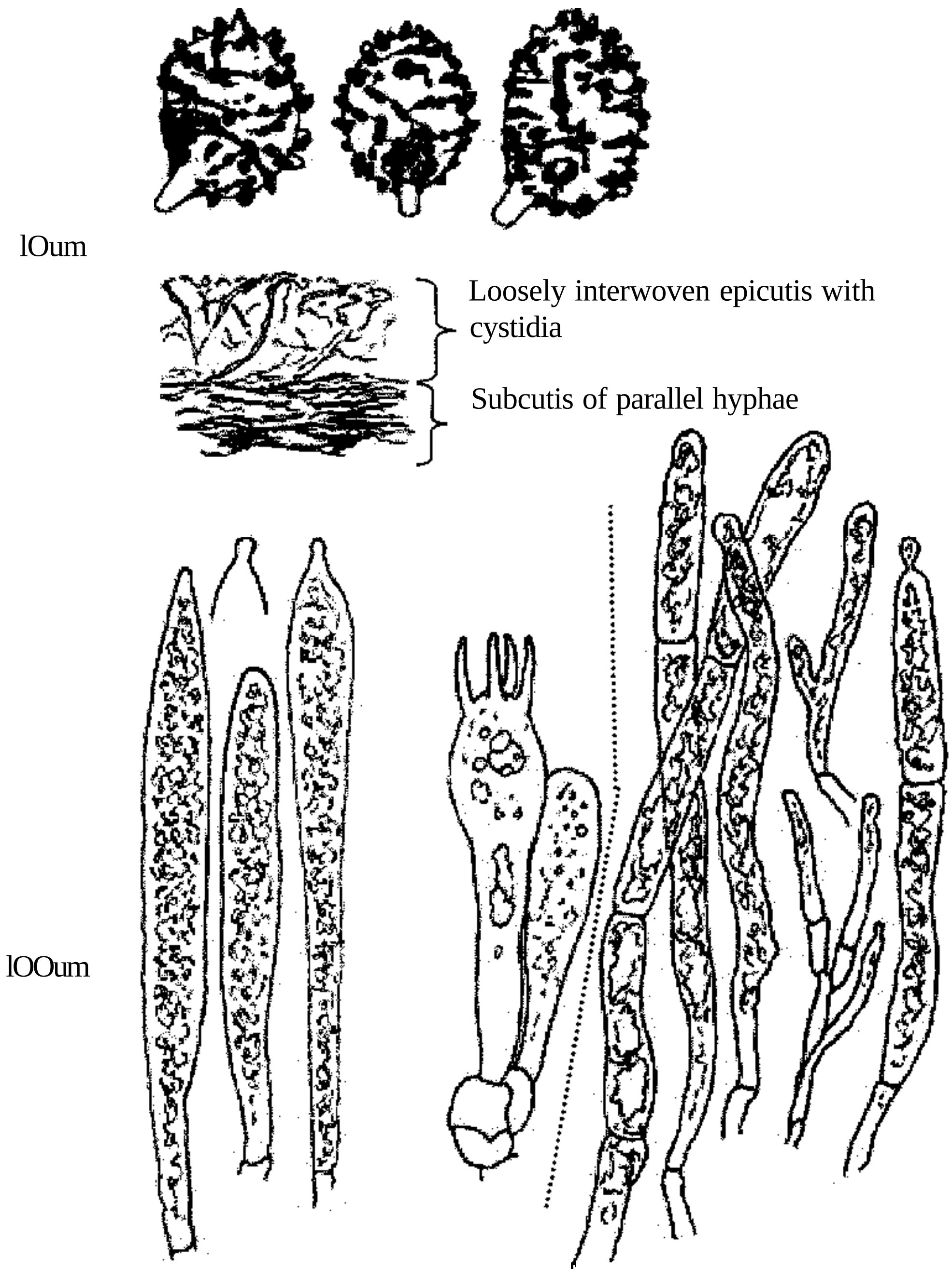


Figure 133 Microscopic characters *ofRussula cavipes*: Top, spores with lOum scale bar; middle, diagram of section through cutis; bottom left, hymenial cystidia and basidium; bottom right, pseudocystidia, pileocystidia and hyphal ends from the epicutis, lower scale bar is lOOum.

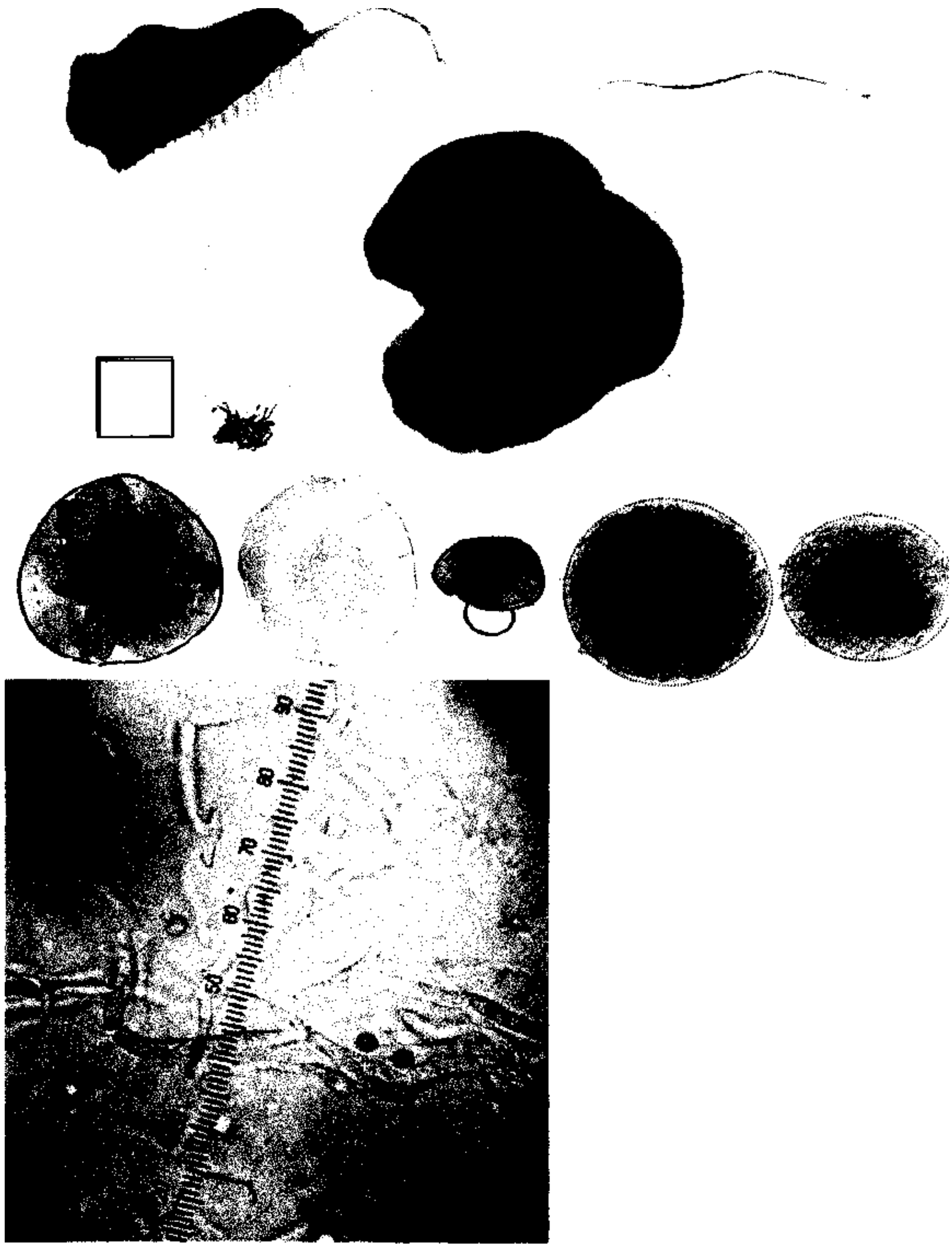


Figure 134 Macroscopic and cuticular characters of *Russula cavipes:* Top, illustration of early mature basidiomata in profile and longitudinal section, with $1cm^2$ square showing spore colour; middle, a row of cap colour notations from field data sheets of various stages of maturity; bottom, surface view of the epicutis in NH4OH showing pileocystidia, ten scale divisions are 25um.

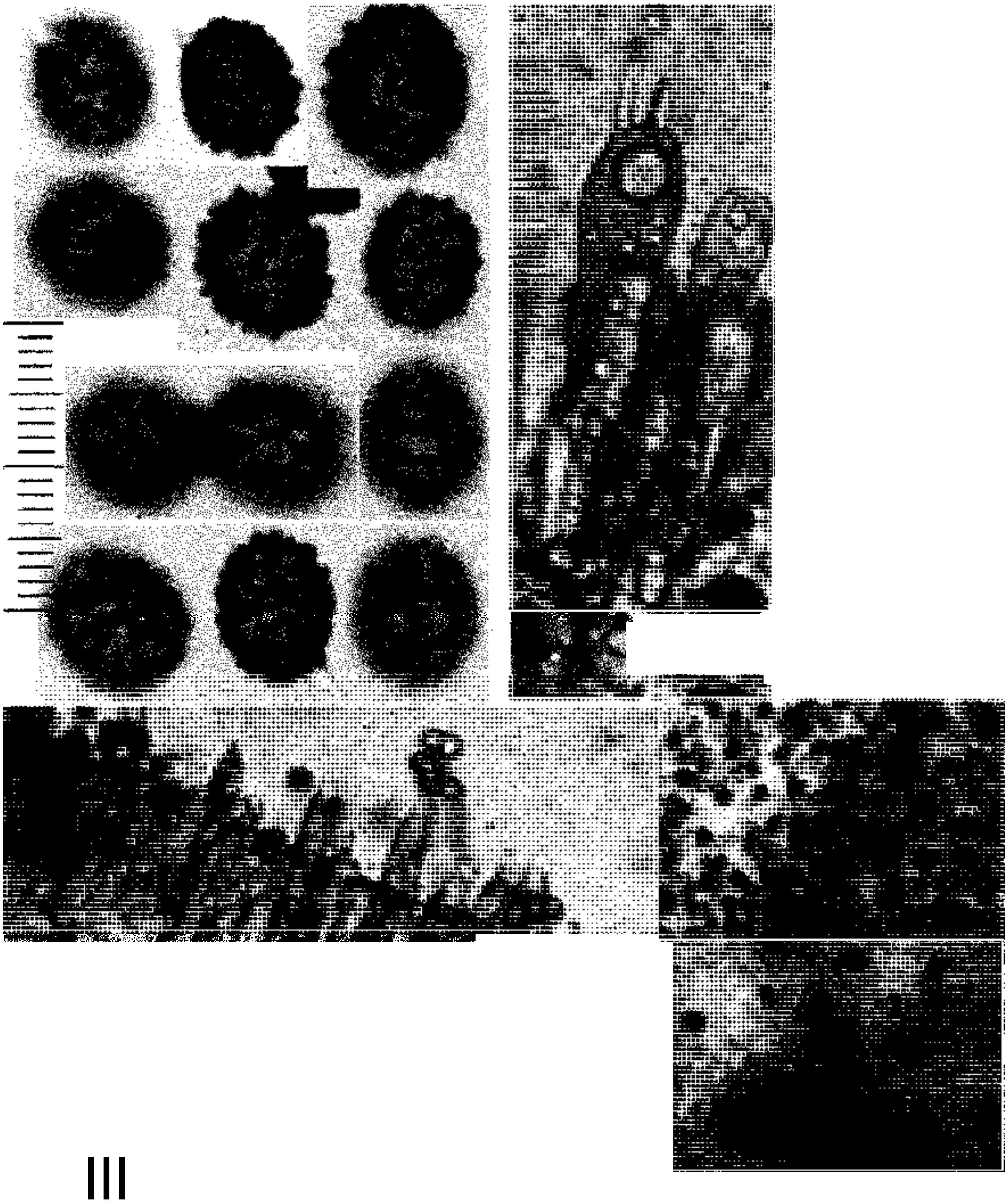


Figure 135 Hymenium *ofRussula cavipes:* Top left, spores and top right, basidia, both with lum division scales; bottom left, section through hymenium showing pleurocystidia, scale is in lOum divisions; bottom right, paired photograph of surface of gill stained in SV showing a denser distribution of cystidia (dark spots) in a young basidioma (upper) than in an old one (lower), scale is in lOum divisions.

Table 18 Comparison of principle differential characters of the purple capped *Sardoninae*. Information derived from Bon (1988), Romagnesi, (1967) and Samari (1998).

Species	**Spores (urn)**	**Spore type, colour**	**Pileocystidia tip -shape, width, septation**	**Habitat: soil type, host**	NH_4	**Other**
R torulosa	8-9 x 6-8	BC2-D1 Ild-IIIa	Strangulate-nodulose, 4-8.5um, ± aseptate	Sandy, calcareous or basaltic *Pinus (Picea)*	- ve	Firm, stipe short, coloured.
R. fuscorubra	7.7-9 x 6.5-7.5	C2 Illb-c	Obtuse, 8-12um, 0-1 septate	Calcareous *Picea*	- ve	Firm, stipe coloured
R. fuscorubriodes	8-9.5 x 6.5-8	B2 (B3,C2) lid	Obtuse 6-8 (10)um 0-3 septate	Acid *Picea* ± *Vaccinium*	- ve	Firm, stipe coloured
R. queletii	7.3 -10 x 6.7-9	A2-3 Ilc-d	± Capitate 5-8 or 12-14um 0-1 septate	Calcareous *Picea*	- ve	Fragile, stipe coloured
R. sardonia	7-9 x 5.8-7.6	C2-D2 Ilia	± Capitate 4-6um, 0-3 septate	Sandy *Pinus*	pink	Firm, stipe coloured, gills yellow
R. cavipes	7.2-9.5 x 6.5-8	C2-3 Ha	Capitate or obtuse 6-llu.m ± aseptate	Moist *Abies, (Picea)*	pink	Fragile, stipe white ± pink flush
R. violacea *(Violaceinae)*	7.5-8.2 x6.2-7.2	A2-B2 Ha-b	Obtuse ± clavate, 5-12um, 0-3 septate	*Populus, Betula*, conifers	- ve	Small, fragile, stipe white, menthol odour

Observations in brackets are uncommon. Spore types refer to Woo codes, spore colours to those of Romagnesi (1967).

Clade 6

Subsection *Violaceinae* **(Romagn.) Sarnari**

***Russula pelargonia* Niolle**

Annals Mycologici. 39:66. 1941

Cap 4-4.8cm diameter, almost hemispherical when young, becoming more or less plane with a depressed centre and rounded, smooth margins that become striate in age. Colour is slightly greyish purple to pinkish-brown, approaching dull violet at the margins or with tints of brown, dark brownish red centrally. Viscid, drying matte to subglossy, peelable to about 1/3, flesh white, unchanging, to pinkish under the cuticle.

Gills pale cream, unchanging where bruised, subdistant, subgills and forking rare, adnate or sometimes slightly decurrent at stipe, obtuse and broadest at cap margin, about equal in depth to the cap trama at half-radius, pliable.

Stipe 5.4-7.5cm x 1. 1-2.0cm, generally longer than cap diameter, white with a flush of pink, longitudinally rugulose, unchanging where damaged but developing yellow-brown stains at the base, and becoming greyish as if waterlogged in age, stuffed with a bread-textured trama that develops several irregularly shaped cavities along its length, eventually becoming hollow.

Texture slightly firmer than average.

Taste slowly peppery, not very intense.

Odour of cooked apple or plums or *of Pelargonium* (geraniums), according to individual (human) sensitivity.

Spore colour pale cream, Romagnesi Ha or slightly lighter (but darker than lb).

Spores 6.6-8.8 x 5.7-7. lum, L:W 1.08-1.4 with a mean of 1.23 (n=30), ellipsoidal, occasionally subglobose. **Ornamentation** of low conical warts generally below 0.6um high, connected by fine to heavy lines, forming a partial reticulum, the heavy connections often linking rows of warts in latitudinal bands. **Woo types** Cl-2 to Dl-2. **Suprahilar patch** weakly to patchily amyloid, irregular, sometimes bordered by warts, on some spores scarcely different from the rest of the spore surface. **Hiliferous appendix** 1.2-1.4pm long, 0.9-1. lpm wide at base. **Basidia** 4-spored, rarely 2-spored, 32-43 x 8.4-10.5pm, clavate to almost fusoid, tapering gradually from the widest part. Sterigmata 5- 7pm long and 1.3-1.9pm wide at the base. **Pleurocystidia** frequent, 7-12 x 50-100pm originating in the subhymenium or outer trama, protruding 20-3 0pm, fusoid, occasionally cylindrical, tips subacute or frequently bottle-shaped to capitate, sometimes with a short, broad, wavy extension, contents yellowish and refractive in KOH, purple in SV and often banded or crumpled in appearance. **Cheilocystidia** numerous, protruding around 25-40pm, often more cylindrical than fusoid, ends obtuse or subacute. **Subhymenium** 20-3 0pm thick, interwoven. **Gill trama** scarcely differentiated from subhymenium, of compact cells and hyphae with vascular hyphae.

Cutis 150-180pm thick on disc, an ixotrichoderm of two layers. **Subcutis** of tightly interwoven parallel pinkish-brown hyphae 2-5 pm wide, with numerous vascular hyphae that give rise to pseudocystidia, which terminate at any level between the subcutis and the epicutis. **Epicutis** around 2/3 the thickness of the cutis, of tangled, semi-upright hyaline hyphae 2-3 pm wide with undifferentiated tips, or often with the end cell or two with refractive contents, all embedded in a gelatinous matrix. The whole epicutis is permeated with pseudocystidia and the upper 1/3 with pileocystidia that often lie along the surface. **Pileocystidia** abundant, originating in the epicutis, 25-150 x 3-6pm, 0-3-septate, cylindrical to clavate, the majority capitate, sometimes diverticulate or with irregularly shaped short outgrowths, contents refractive and greying in SV. **Pseudocystidia** frequent, regularly septate, sometimes about every 30-50um, others more distant, with contents and termini similar to those of pileocystidia. **Hypodermis** interwoven and incorporating flattened sphaerocytes and a network of vascular hyphae, interwoven with the subcutis.

Trama of discrete clusters of sphaerocytes bound by a hyphal mesh and numerous vascular hyphae.

Chemical reactions: $FeSO_4$- barely reacting; KOH - orange-pink on cap surface, no reaction on stipe; NH4OH - no reaction; phenol -brownish; SV - reddish brown on the gills and cutis, cystidia and vascular hyphae grey or purple, not always strongly staining.

Habitat and tree associations: Under Douglas fir on a limestone slope.

Collections: CR981114-02 with Douglas fir and salal on limestone bluffs north of Koksilah river, N48.6539^0, W123.7302^0 (eastern very dry maritime CWH subzone).

Collection	ITS1-F to ITS4-B	RFLP: Hinfl	AluI	Sau3A
CR981114-02	865	382,329	510,305	368,294, 154

Notes: This Vancouver Island collection of *R. pelargonia* is comparable with collections from Metaline Falls area, Washington State, with the exception that the latter reach up to 8cm diameter (Woo 1997).

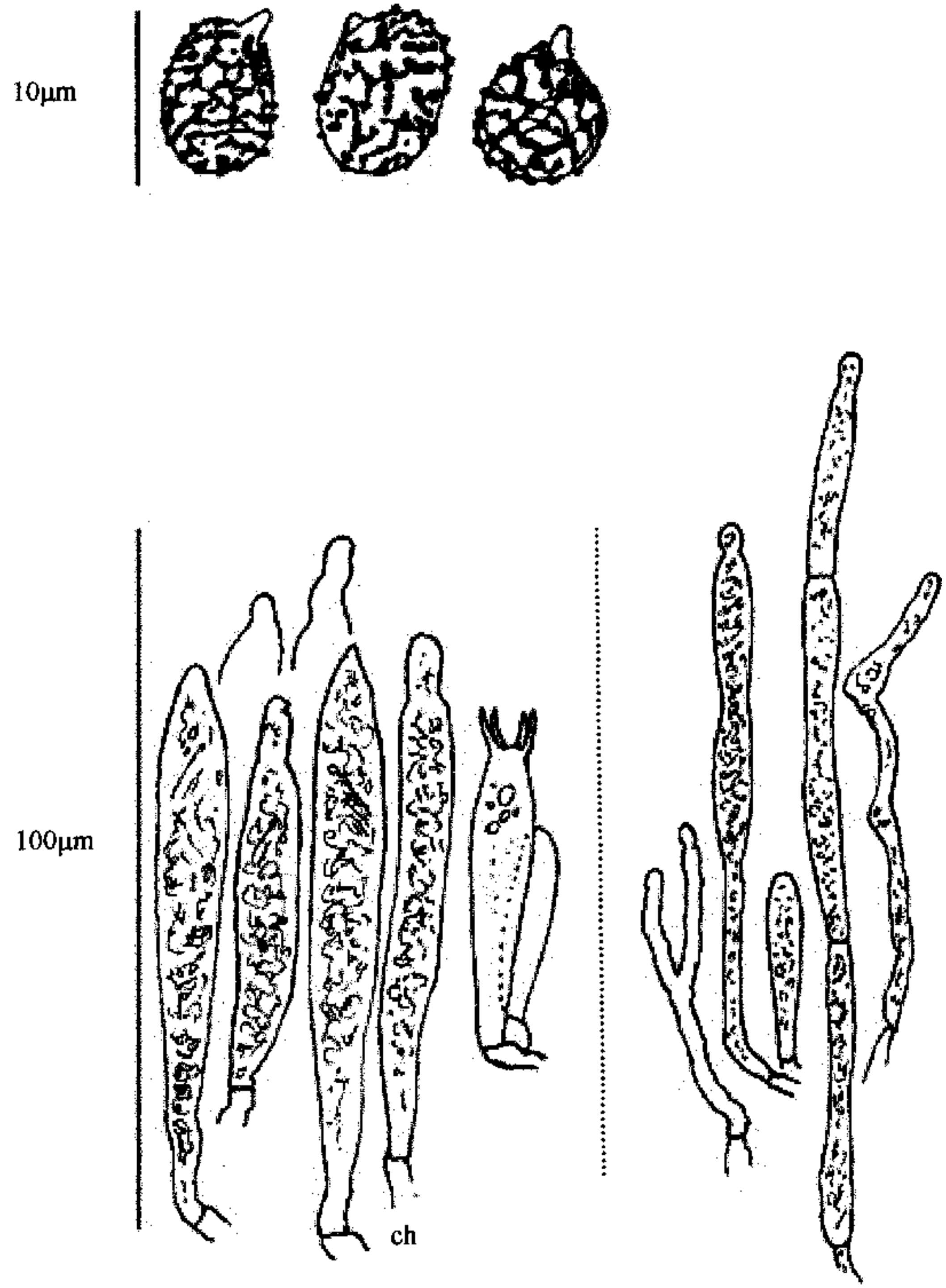
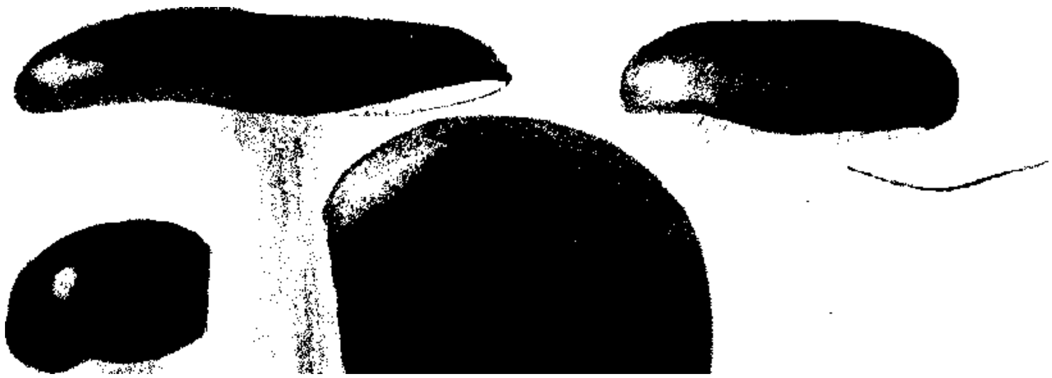


Figure 136 Microscopic characters of *Russulapelargonia:* Top, spores with lOum scale bar; bottom left, three pleurocystidia, a cheilocystidia (ch) and a basidium; bottom right, hyphal ends, pileocystidia and a pseudocystidium from the epicutis, lower scale bar is lOOum.



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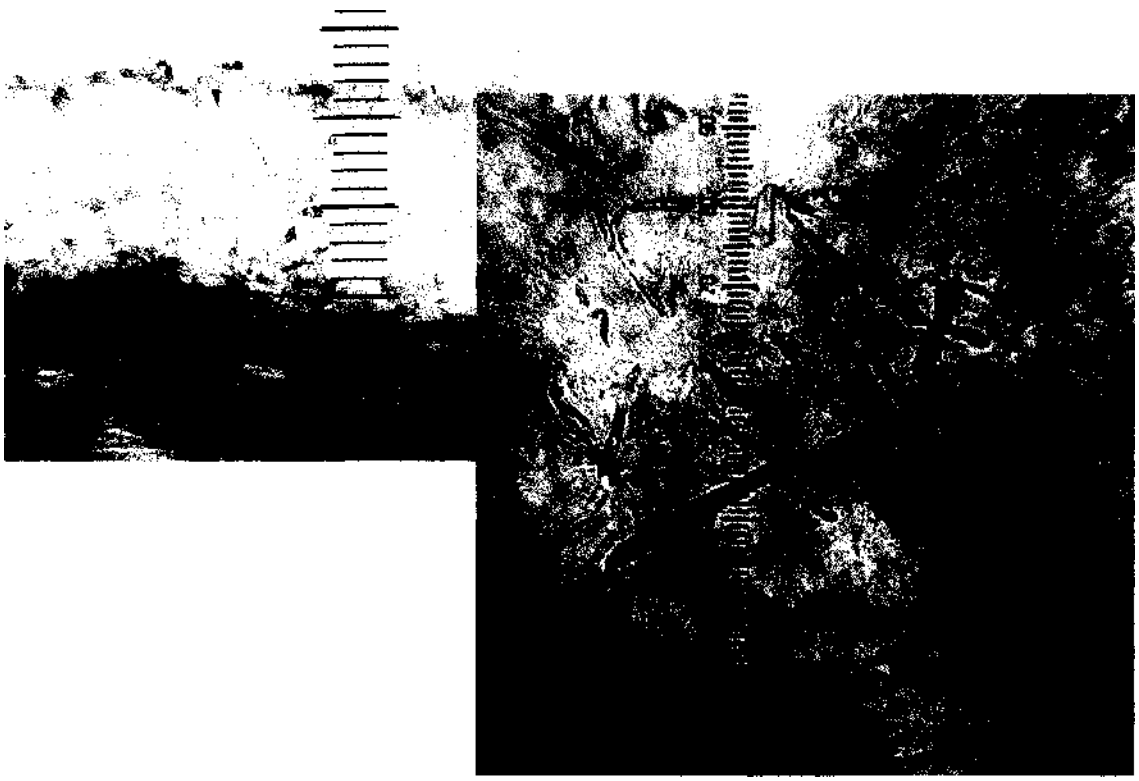

Figure 137 Macroscopic and cuticuiar characters of *Russulapelargonia:* Top, illustrations of young and mature basidiomata, the three on the left are of a collection fromWashington State, tihie other, including the longitudinal section, is from Vancouver Island, the square is $1cm^2$ and shows spore print colour; bottom left, section through cutis, 10 scale divisions are lOOum; bottom right, pileocystidia on the surface of the cutis, stained with SV, 10 scale divisions are 25um.

Figure 138 Hymenium *ofRussulapelargonia:* Top left, fragment of the hymenium with basidia and basidioles, scale bar is lOum; top right, spores, the leftmost two photographs are of the same group of spores at two depths of focus, the scale is in lum divisions; bottom left, cheilocystidia on the gill margin, scale is in lOum divisions; bottom right, a pleurocystidia stained in SV which shows the banded cell contents, scale bar is lOum.

Subsection *Sardomnae* Singer emend. Sarnari

Series *Sanguinea* Sarnari

***Russula sanguinaria* (Schumach.) Rauschert**

Ceskoslovenska vedecka spolecnost pro mykologii 43(4): 204. 1989

Agaricus sanguinarius Schumach. (Basionym)

=*Russula rosacea* (Pers.ex Seer.) S.F. Gray em.Fries

-*Russula sanguinea* (Bull.) Fries

Description of Vancouver Island and Saturna Island collections:

Cap 2.7-12.2cm diameter, convex when young, usually with a small central indentation, becoming plane to shallowly centrally depressed but long retaining a downturned (but not inrolled), smooth margin, often irregular in diameter to almost lobed and with some parts of the cap upturned while others are flat or downturned. Surface bright carmine red, more intense over the disc, sometimes with paler or white to cream areas especially near the margin, slightly viscid, soon drying matte, margin smooth until old or dry. Cutis not peelable or only at the very margin, flesh beneath pink, otherwise white, unchanging to becoming slightly yellow or greyish when damaged.

Gills white at first, becoming a warm deep cream at maturity, bruising yellow, close to subdistant, with occasional lamellulae and occasional to frequent forking throughout the radius, adnate to narrowly decurrent at stipe, arched, shallow, half to equal the depth of the trama at mid-radius, subacute to obtuse at cap margin with the edge of the cap curving down as far as the gill lower edge in parts.

Stipe 2.3 to 7.4cm by 0.8 to 2.4cm, ± cylindrical or broadening slightly towards the base, solid, with firm unchanging or lightly greying trama, surface white or more often flushed partly or entirely with pink, paler than the cap, pruinose, bruising bright yellow which turns to dull brownish yellow.

Texture Quite firm.

Taste slightly bitter then peppery to acrid.

Odour varied, in some collections not distinctive, in others fruity, of apples or plums and vanilla.

Spore colour between a deep cream and pale orange, Romagnesi Ilia.

Spores broadly ellipsoidal to obovoid, sometimes broadly tear-drop shaped, 6.6-8.5 (-8.9) x (5.2-)5.7-7.5um, L:W = 1.03-1.41, mean 1.21 (n=40). **Ornamentation** of warts mostly 0.4-0.7um high, convex to bluntly conical, isolated or in short chains with a few heavy connectives, not forming a reticulum, Woo B2, occasionally C2. **Suprahilar patch** amyloid, in Melzer's reagent, a small light grey irregular patch bordered by small warts. **Basidia** 4-spored, approximately 36-44pm long by 10-1 lum wide, clavate to slightly bulbous in upper third, often relatively slender, sterigmata 4-lOu.m long, around 2um wide near the base. **Pleurocystidia** abundant and sometimes densely distributed, 63-100um by 6.3-14um wide, arising from the subhymenium or occasionally the trama, protruding about 18 to 30um, cylindrical to fusoid with subacute to acute apices, occasionally mucronate or with a small capitum, contents sometimes banded, in KOH filled with yellow globular contents, almost black in SV. **Cheilocystidia** frequent, often forming a fringe along the gill edge, 33-70um by 5-7.5p.rn wide, protruding up to 42um beyond basidioles, similar in shape to pleurocystidia. **Subhymenium** about 15-20um thick, interwoven to pseudoparenchymatous, hyphae around 3 urn in diameter, gill trama of sphaerocytes with many vascular hyphae.

Cutis 80-250um thick, a gelatinised ixodermis of two layers. **Subcutis** of repent, interwoven hyphae l-3.5p.rn wide, appearing red in SV and water, the colour quickly dispersing into water mounts, and with many vascular hyphae that are dark grey in S V. **Epicutis** of repent, interwoven, hyaline hyphae with a few free undifferentiated ends and many pileocystidia and pseudocystidia. **Pileocystidia** frequent but sometimes patchily distributed, 57-120umby 5-10um wide, cylindrical, sometimes diverticulate, tips obtuse or occasionally capitate, mostly non-septate, with strikingly banded contents weakly to strongly staining in SV. **Pseudocystidia** abundant, ranging from 4-11pm wide but most

5-7um wide, sometimes branched near terminus, ends blunt, tapering or with a small capitum, strongly refractive in KOH and with banded or bubbled contents in SV.

Trama of sphaerocytes in loosely defined clusters with many SV+ vascular hyphae.

Chemical reactions: FeSCU - salmon pink; KOH - turns both cap and coloured parts of stipe orange, NH4OH - no reaction on cap, slightly grey on stipe flesh; Guaiac - quickly blue-green; phenol - immediately bright pink, becoming brown; SV - gills dark purple-grey, cutis pink-grey.

Habitat and tree associations: In three collections, associated with *Pinus contorta* in either a dry or boggy area, with or without western hemlock and Douglas fir, the Saturna Island collection from East Point park with Douglas fir, *Pinus* not seen.

Collections: OC 981031-GL1, under shore pine, western hemlock and western red cedar, near Glintz Lake, Sooke area, exact location not recorded, this collection is a much deeper red (dark blood red) than the others but is otherwise the same (western very dry maritime CWH subzone). PJ 981124-03 along the side of the road under mature *Pinus contorta*, Douglas fir ,western red cedar and western hemlock, ClifFside Road, Saturna Island, N 48.780706°, W 123.05717°. PJ981130-3a from scrubby regrowth with Douglas fir near the lighthouse, other conifers across the road, East Point Park, Saturna Island, N 48.780712°, W 123.04574°. CR011111-01 on ground beneath a stand of *Pinus contorta* (shore pine) adjacent to the parking lot, Royal Roads University, N 48. 436667°, W 123. 474583°. OC031010-01 on ground near shore pine Rithet's Bog, Victoria, N 48.4895°, W 123.3815°. (Last 4 collections from CDF zone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A
PJ981124-03	839	404,364	482,300	337,254,225
CR030110-01	853	392,354	471,263	277,196

Notes: *Russula sanguinaria* (as *R. sanguinea* or *R. rosacea)* has been fully described from both Europe and North America (Singer 1957, Hesler 1961, Bills and Miller 1984, Romagnesi 1967, Sarnari 1998). Grund (1979) distinguished a variety, *R rosaceae* var *macropseudocystidia,* from Washington State in which the hymenial cystidia were in the same size range as this Vancouver Island collection, but it differs from this collection in its polished cutis, which may be environmentally affected. Bills and Miller's description fits well with the Vancouver Island material, including the larger sized pleurocystidia (up to 160um long), except local collections have slightly larger spores and shorter basidia. Singer (1938) described a similar species: *Russula americana* from the Olympic peninsula, that has larger spores, (9-11 x 8.2-9.5urn) than *Russula sanguinaria,* and a habitat with *Abies* and *Tsuga* species rather than *Pinus* (but see notes under the description of Vancouver Island collections of *R. americana* var. *modicaspora* nom prov.). One of the Saturna Island collections had no mention of shore pine in the habitat description, but it does not differ from the other three collections in spore size or other characters, and is not likely to be *Russula americana.* Vancouver Island collections also agree well with Romagnesi's (1967) description of European material. *Russula sanguinaria* is a very distinctive mushroom with its vivid red dry cap, pink to red stipe with yellow staining at the base, firm flesh and habitat usually with pines.

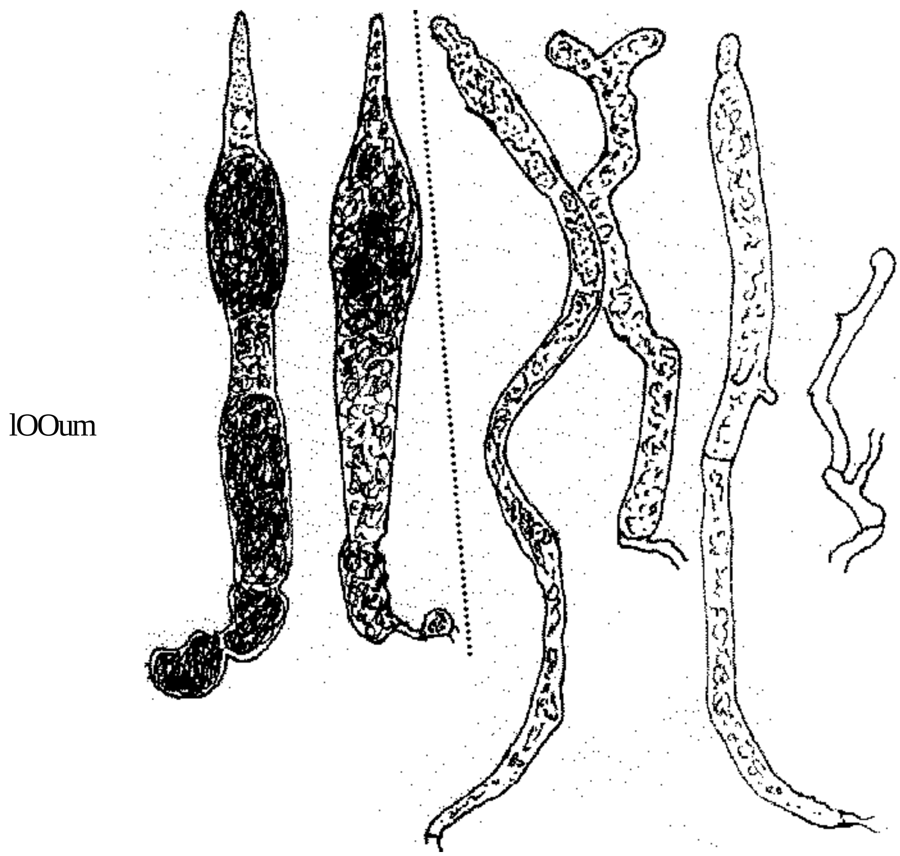

Figure 139 Microscopic characters *ofRussula sanguinaria:* Top, spores with lOum scale bar; bottom left, hymenial cystidia (in SV); bottom right, pileocystidia and epicutal hyphal ends, lower scale bar is lOOum.

Figure 140 Macroscopic characters of *Russula sanguinaria*: Top, in situ under shore pine; below, collection made under pine in Rithet's Bog, Victoria, the grid is in $1cm^2$ squares, inset square shows spore print colour. Note the yellow bruising reaction on the stipes of several basidiomata.

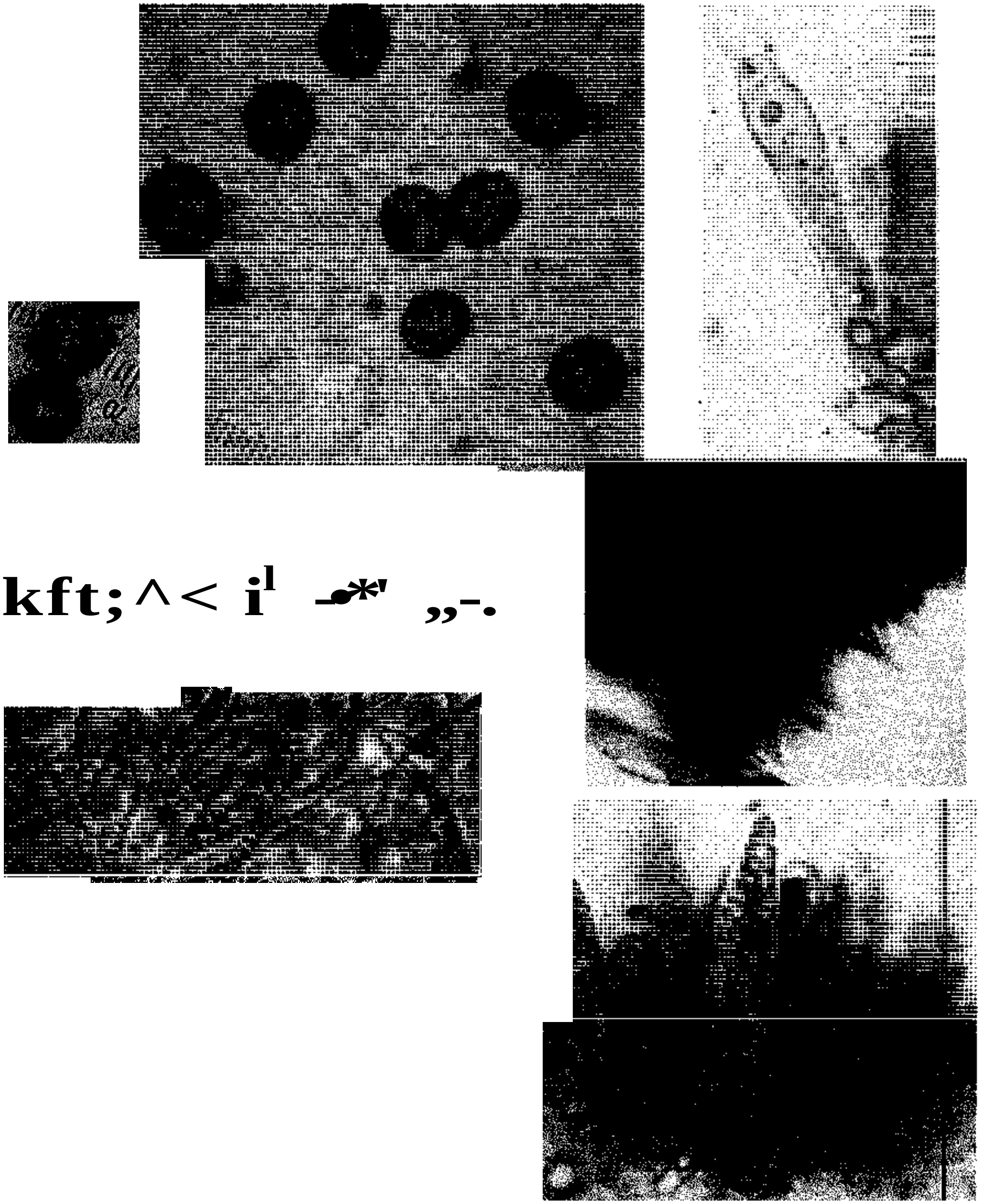

Figure 141 Hymenium and cutis of *Russula sanguinaria:* Top left, spores with lum division scale; top right, basidia with lum division scale; middle right, edge of gill showing cheilocytidia in SV with lOOum scale bar; bottom right, pleurocystidia in KOH showing the refractive globular contents, basidioles are also visible, scale bar is 90um; bottom left, surface view of cutis in KOH showing the banded contents of pileocystidia, scale bar is lOOum.

Russula amencana var. _modicaspora_ nom. prov.

Russula americana (Singer) Singer (Basionym)
Bulletin de la Societe Mycologique de France 55: 264. 1939.

Cap 1.8cm to 5.6cm diameter, convex with a flattish umbo when young, becoming plane to shallowly depressed but retaining a small umbo, margins remaining somewhat curved until quite mature, eventually uplifted, smooth at first but developing striations at maturity. Surface bright scarlet red, a little darker over the disc when young and sometimes fading centrally to a pinkish yellow, but usually a fairly even red, viscid, drying glossy. Cutis peelable from less than 1/4 up to 1/2, flesh beneath pink, trama white, unchanging when damaged.

Gills white at first, becoming a pale cream to deep cream at maturity, sometimes developing brownish bruising, generally subdistant, sometimes close, rarely forked or with lamellulae, adnate to narrowly adnexed at the stipe, subacute to rounded at the margin, ventricose to broadening outwards, at least equal in depth to the trama at half-radius, in mature basidiomata more than twice the depth. In mature basidiomata the gills appear quite broad when the cap margins are uplifted.

Stipe 3 to 8.5cm by 0.7 to 2.5cm, usually longer than cap diameter, fusoid to clavate, stuffed with a bread-textured trama that soon develops a series of 6-8 cavities that are more irregular in shape than lenticular, eventually becoming hollow. Stipe surface flushed with pink to red, paler than the cap, usually only the base white, slightly pruinose at the apex, somewhat rugulose, bruising brown at the base but unchanging to lightly greying in the rind when cut, developing a greyish waterlogged appearance in age.

Texture about average for *Russula*, not as hard and firm as *R. sanguinaria,* softer, but more pliable than fragile.

Taste often bitter at first then peppery to acrid, gills more acrid.

Odour not distinctive or slightly fruity, sometimes with flowery or nutty components, in several older basidiomata faintly like dental disinfectant (thymol), in one collection rather like ladybird beetles *(Coccinellidae).*

Spore colour variable in shade between collections, light to mid buff rather than deep cream or yellow, Kibby and Fatto (1990) shade C to E, Romagnesi Ilia to Illb or occasionally as light as lid but with less yellow and more pink.

Spores 7-9.5 x 6-7.5um, L:W = 1.03 tol.38, mean 1.19 (n=61), subglobose to broadly ellipsoidal. **Ornamentation** of mostly isolated conical to peg-like warts 0.2 -1.0u,m high, occasionally to 1.2um, occasionally 2-3 warts catenate and forming a short ridge, occasionally with fine lines joining 2-3 warts, not forming a reticulum, Woo B2-B3. **Suprahilar patch** an irregular amyloid patch, sometimes relatively small, bordered by small warts. **Basidia** 4-spored, approximately 28-48um long by 9-11 urn wide, clavate to slightly bulbous in upper third. Sterigmata slender, 5-9um long, around l-1.5|xm wide near the base. **Pleurocystidia** frequent, 37-100 x 5-14um, occasionally to 18um wide, arising from the subhymenium, protruding about 18-30u.m, fusoid, mostly with acute apices, occasionally mucronate or with a small capitum, or an apical exudate, filled with yellow refractive contents, sometimes banded or with bubbles, black in SV. **Cheilocystidia** frequent to sparse, sometimes forming a fringe along the gill edge, other times embedded, 50-87 x 8-12.5um, protruding up to 35um beyond basidioles, similar in shape to pleurocystidia. **Subhymenium** about 12-25um thick, in parts appearing interwoven, in others pseudoparenchymatous, in parts with an interwoven layer below a cellular layer, gill trama of fairly spherical sphaerocytes about 20-40um diameter, interspersed with many vascular hyphae.

Cutis 120-220um thick, a gelatinised ixotrichodermis of two layers. **Subcutis** 40-1 lOum thick, of tightly interwoven parallel hyphae appearing brownish red in water, with many vascular hyphae 5-8u,m wide with yellowish refractive contents, dark grey in SV. **Epicutis** up to 120um thick, of tangled, fairly densely packed upright to semi-repent hyphal ends 2 -5um wide, and pileocystidia and pseudocystidia, embedded in a gelatinous matrix, the whole of which is pink in water mounts from the release of red pigment, the origin of which is hard to determine. **Pileocystidia** patchily distributed, more frequent on younger basidiomata, 48-130um by 5-8um wide, commonly narrowly clavate, sometimes cylindrical, occasionally diverticulate, tips obtuse or capitate, 0-3-septate, contents refractive, weakly to strongly staining in S V, some banded to bubbled but in general these are fewer or less striking than those *ofR.* ***sanguinaria.*** **Pseudocystidia** abundant, ranging from 4-1 lum wide but most about 5-7um wide, ends obtuse or with a small capitum, strongly refractive in KOH and with banded or bubbled contents in SV. **Hypodermis** of small flattened cells beneath the subcutis.

Trama of sphaerocytes scattered to clustered within a hyphal mesh, and common SV+ vascular hyphae.

Chemical reactions: FeS04- no change to pinkish; KOH - turns both cap and coloured parts of stipe orange, stipe flesh unreacting or yellowish to slightly greenish; phenol - brownish, sometimes weak; SV - gills dark purple-grey, cutis dark purple, vascular hyphae and cystidia mostly reacting strongly, purple to black, rarely reddish.

Habitat and tree associations: In wettish areas such as seeps, sphagnum or skunk cabbage sometimes in the habitat, otherwise just wet forest duff, with western hemlock, western red cedar often present, other trees in the vicinity may include red alder, Sitka spruce, Amabilis fir and big-leaf maple. October and November.

Collections: CR981029-br and CR021015-14, from the same site in a small patch of sphagnum with understory western hemlock, under old-growth Sitka spruce and western red cedar, at the northern part of east-side rainforest trail loop, near Tofino, N 49.0490°, W 125.6975°; CR001012-20/21, under western hemlock, red alder and Amabilis fir, by the Grice bay mainline Logging road, near Uclulet, N 49.016883°, W 125.58153° (southern very wet hypermaritime CWH subzone). CR981118-01, in a seep between clumps of sword fern in a mixed age stand of western hemlock and Douglas fir, on the east slope of Mount Douglas, Victoria; CR001102-02 from bottomlands near river, amidst skunk cabbage, western hemlock, big-leaf maple and western red cedar, Mill Hill park, approximately 30' from N 48.452433°, W 123.48382° (CDF zone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Mil	Sau3A
CR981118-01	856	382,278	493,320	311,230
CR001012-21	872	417,366	500,413	346,254,200

Notes: Singer's description *ofRussula americana* is consistent with these Vancouver Island collections with the following two main exceptions: The spores of local collections are consistently smaller, those of *R. americana* are given as 9-11 x 8.2-9.5um; and the gills of *R. americana* are described as frequently forked and becoming subdecurrent in fully mature basidiomata, both these characters can be quite variable but were not observed in any of the local collections, other notations of gill shape and colour agree very well. The spores of these Vancouver Island collections were nevertheless larger by about 0.5-1.Oum in the longer dimension than the spores of local collections of *R. sanguinaria.* Some texts describe *R. americana* as identical to *R. sanguinaria (=R. sanguined)* in all but spore size, however, this is not strictly true. Singer's description mentions the greater fragility of *R. americana,* and its different habitat, which is under *Abies* and *Tsuga.* Singer's collections were made in Oregon, Washington and California, usually in river valleys, but he does not mention anything about his collections growing in damp or wet areas. Side by side, *R. sanguinaria* and *R. americana* var. *modicaspora* are easily distinguishable, the latter is taller and more slender, grows with western hemlock (and possibly *Abies)* rather than pines, is generally in wet areas, does not stain yellow on the stipe, and has a more evenly coloured and shiny cap with the epicutis an ixotrichodermis.

Russula renidens Ruotsalainen, Sarnari and Vauras, described in Sarnari (1998), is a red-capped, red-stiped species that matches the above collections extremely well in general stature and in spore colour and size, the spore ornamentation is of smaller warts to 0.5urn on average but is otherwise similar, and cuticular characters differ. It may include darker reds and yellow hues in the cap and is found in similarly wet patches and streamsides, sometimes in sphagnum, but under birch.

Other local peppery red-capped species include *Russula silvicola,* which has white spores and a white stipe, *R* cf. *luteotacta,* which stains yellow at the base of the stipe, has whitish spores, different pileocystidia and is much paler, *R. bicolor,* with white spores and more yellow on the cap, *R. rhodopoda* which is mild to only slightly peppery and can have a darker red cap and reticulate spores, *R. queletii* and *R. ctfuscorubroid.es*, which have more purple and often greenish tints in the cap and are never such a brilliant red as *R. americana*. *Russula paludosa*, found in Northern California in boggy areas tends to be broader capped, is only weakly peppery, has more yellow in the cap and the spores are reticulate, and *R. californiensis* is paler but otherwise somewhat like *R. sanguinaria*, however, the stipe is white with just flushes of pink, and the spores have very small warts up to 0.5um high and are reticulate.

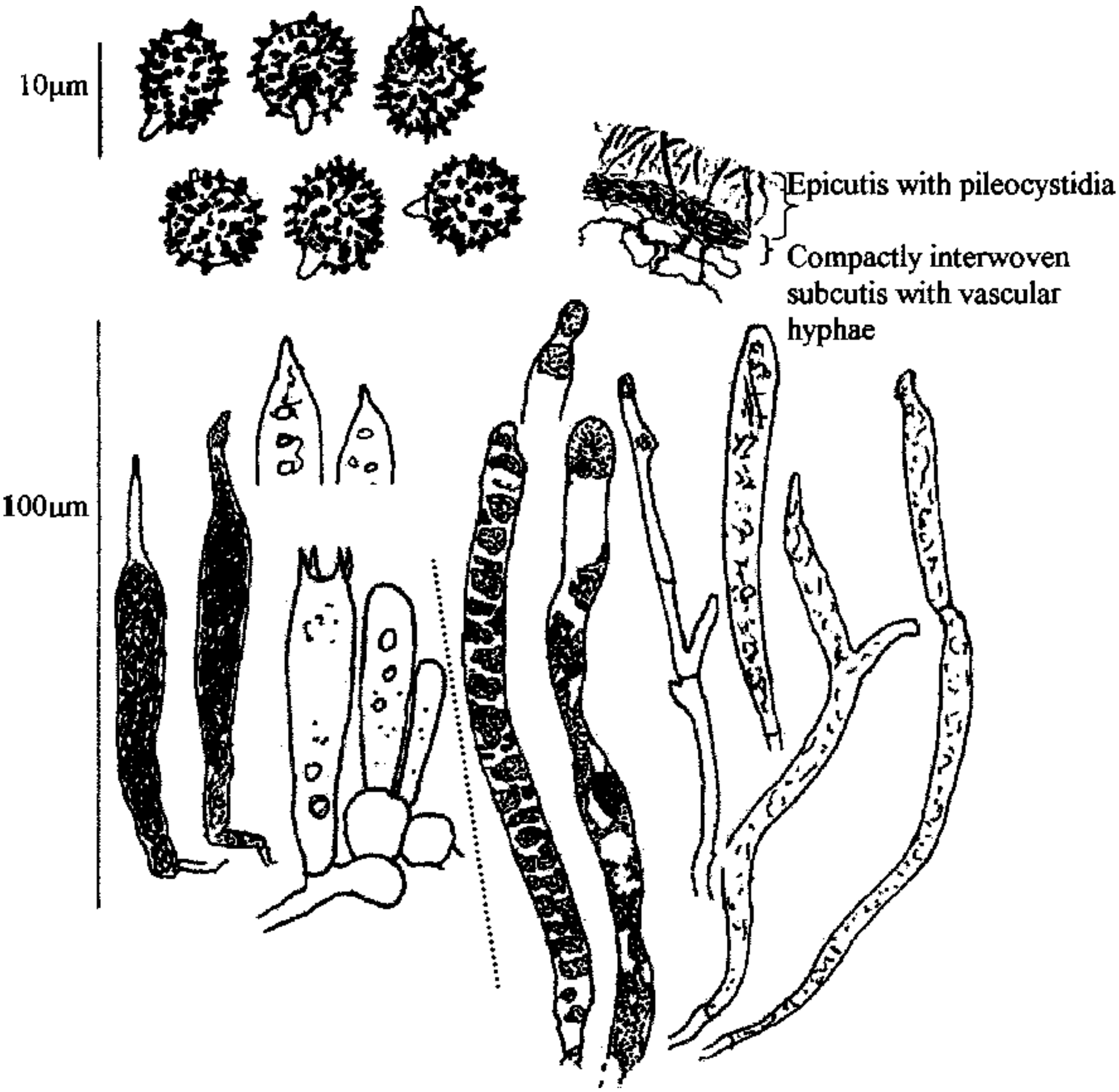

Figure 142 Microscopic characters of *Russula americana var. modicaspora:* Top, spores with lOum scale bar; middle, diagram of section through cutis; bottom left, hymenial cystidia (in SV) and in KOH, (tips only shown), and basidium with basidioles; bottom right, pileocystidia and hyphal ends as they appear in S V and, far right, in KOH, lower scale bar is lOOum.

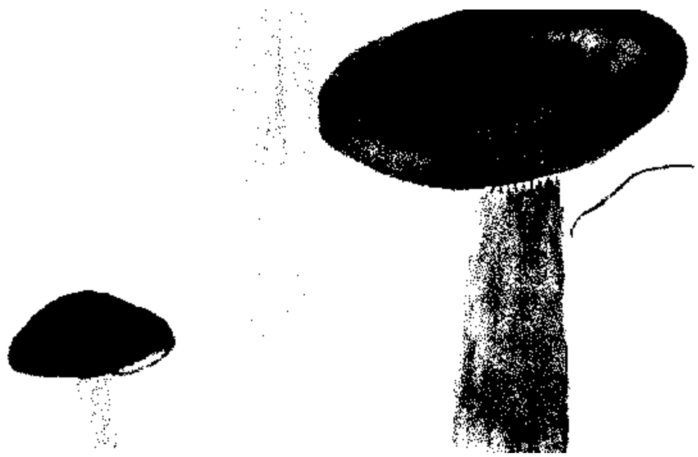

Figure 143 Macroscopic characters of *Russula americana var. modicaspora:* Illustrations of profiles and longitudinal sections of two collections, the upper one with immature ans well as mature basidiomata, the small squares are 1cm^2 and show the spore colour.

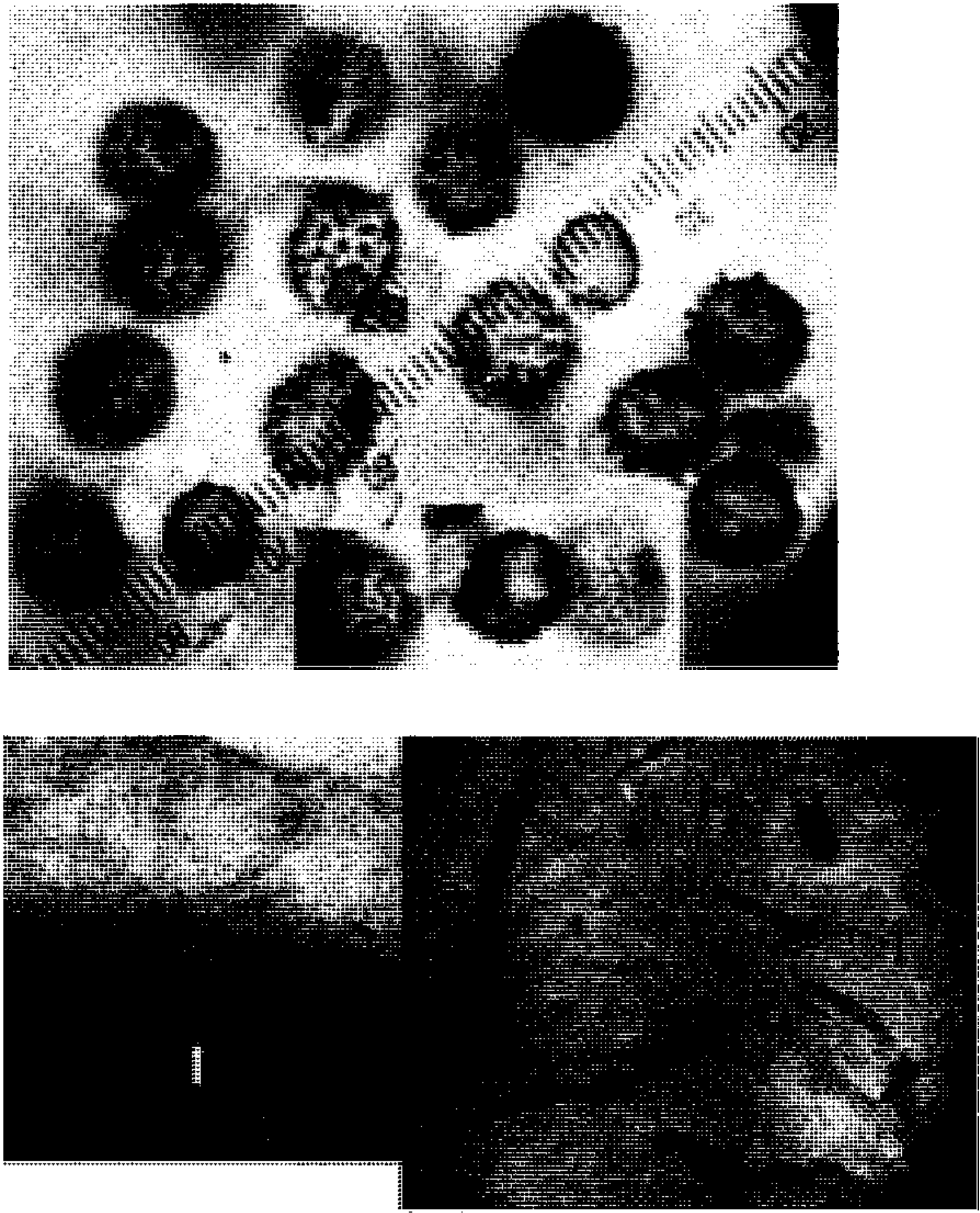

Figure 144 Hymenium and cutis of *Russula americana vat. modicaspora:* Top, composite photograph of spores with lum division scale; bottom left, section through cutis showing the lighter epicutis above a darker subcutis, the base of the subcutis is arrowed; bottom right, surface view of cutis in SV showing the darkened pileocystidia, 10 scale divisions are 25 um.

Clade 7a

Subgenus *Incrustatula* Romagnesi

Section *Amethystinae* Romagnesi

Subsection *Chamaeleontinae* Singer

Beih. Bot. Centralbl. 49 (2): 236, 1932

Russula lutea (Huds.: Fr.) Grays

Nat. Arr. Brit. PL: 618. 1821

Cap 4-7cm diameter, plane, soon becoming uplifted at the margin, finally centrally depressed with a low central umbo, margin striate to tuberculate, viscid drying matte except at the margin where it is subglossy over the striations, Colour a bright egg-yolk yellow, sometimes with orange patches toward the centre, becoming pale yellow at the margins. One specimen greying in patches on the cap surface. Cuticle peelable almost completely. Cap trama whitish, pale yellow under cuticle, unchanging when bruised.

Gills adnexed, ventricose, depth to 1cm at mid-radius (where cap trama is about 2mm deep) obtuse to somewhat rounded at the cap margin, occasional forking toward the stipe, subdistant to moderately close, no subgills. Light orange at maturity, unchanging where damaged.

Stipe 5-9 x 1.1-1.5cm, cylindrical to fusoid, white, surface slightly longitudinally rugulose, stuffed with soft bread-textured trama, becoming hollowed in age, rind about 1.5mm thick, flesh unchanging when bruised but browning slightly in age at the base.

Texture average to soft and fragile.

Taste mild.

Odour like a chanterelle, weakly of apricot.

Spore colour brownish yellow-orange, Romagnesi IVe. While a spore print is being obtained, the mushroom leaves rusty stains on the paper where moisture has seeped out from it.

Spores 7-8.2umx6-7.2um, L:W 1.13-1.23, withameanof 1.17, (n=30), subgloboseto broadly ellipsoidal. **Ornamentation** of bluntly conical warts, mostly 0.5 to 0.8um high, occasionally to lum, isolated or often short to long catenate, frequently forming one or more chains running around the circumference of the spore, or zig-zags roughly parallel to the suprahilar patch margin. Occasional spores are zebroid, reticulum partial to none. **Woo types** 2A-2B, occasionally to 2C. The spores are strongly yellow when mounted in water and viewed under the microscope, and it can be seen that the warts on the spores are filled with yellow pigment. Broken, empty spores remain yellow indicating a cell wall pigment. **Suprahilar patch** amyloid, an irregular patch bordered by and containing small warts. **Basidia** mostly 4-spored, but 2-spored ones quite frequent and 1-spored basidia occasional, 33-43um x 1 l-13um, broadly clavate to bulbous in upper third. Sterigmata up to 8um long. Many basidia close to the gill margins have alongside and originating from the same basal cell one or two roughly clavate structures without refractive contents and with one or two septa. These structures appear to function as packing or spacers between basidia. These are more apparent in some mounts than others and should not be confused with basidioles which have refractive or oil drop contents even while quite immature, and also often originate from the same cell as more mature basidia. **Pleurocystidia** and **cheilocystidia,** sparsely and unevenly distributed, 47-80 x 7 -Hum, not or scarcely protruding beyond basidioles, arising from deep within the subhymenium or the central trama, cylindrical to narrowly clavate, tips acute, often with a small sharp projection, very occasionally with a long narrow projection especially in the older specimen, contents refractive in KOH, staining dark red to grey in SV. **Subhymenium** 20-30um thick, of small irregularly shaped cells 5-8|i.m in diameter, **gill trama** of sphaerocytes with occasional hyphae, vascular hyphae rare.

Cutis 60-150um thick, an ixotrichodermis, the glutinous matrix of which forms a layer 5-lOum thick over the epicutis, and is visible in sections because of the debris trapped at its surface. **Subcutis** of repent, interwoven hyphae 1.5-3 urn wide, the lower portion of which has more yellow pigment than the upper, with occasional vascular hyphae in subcutis, weakly greying in SV in fresh material, unreacting in dried material. **Epicutis** a turf about 25u,m deep, of erect hyphae 2-4u,m wide, with yellowish cell walls and containing bright yellow pigment globules, terminating in a clavate, bottle shaped, capitate or cylindrical terminal cell 3-8jxm wide and 7-22um long, often with a similarly enlarged cell beneath. Amongst these are numerous primordial hyphae and rare cystidium-like hyphal ends. **Primordial hyphae** 3-8um wide are common in the epicutis of younger basidiomata, becoming sparser with age, their incrustation appears as an uneven, refractive, gelatinous layer over parts of the surface: it takes up acid fuchsin less strongly than do the cell contents, does not darken in S V, and is sometimes hard to discern. **Pileocystidia** rare, possibly part of the primordial hyphal system rather than true cystidia, 15-42um long by around 3um wide, cylindrical with rounded ends, with yellowish refractive contents that are often banded or bubbled, not staining in SV. **Pseudocystidia** rare, 3-6um wide, most with rounded ends, contents as for pileocystidia, not staining in SV. Both cystidial forms more frequent on the younger specimen. **Hypodermis** none.

Trama of aggregations of sphaerocytes, binding hyphae and occasional vascular hyphae staining purple in SV.

Chemical reactions: FeS04 - light pinkish, KOH and NH3OH - no reaction on cap or stem, guaiac - at first brownish pink, very slowly turning blue-grey; phenol - brownish-purple, guaiacol -purplish red, SV - at first red, gradually turning purple-grey.

Habitat and tree associations: in fairly dry conditions under *Quercus garryana*, in May. Rare.

Collections: OC040528-01 found under Garry oaks with some madrone in Beacon Hill Park, Victoria. N48.416^0, W123.363^0 This is the first collection in 7 years of a yellow *Russula* under oaks (CDF zone).

Notes: This Vancouver Island collection is identified as *Russula lutea* sensu Phillips (1981) and Romagnesi (1967), who considered this species a variety of *R. chamaeleontina.* Phillips mentions the apricot odour. The spores in these two descriptions are mostly with isolated warts whereas those of this V.I. collection have more catenations.

This collection was initially thought to be *R. flaviceps* because of its habitat with oaks, and the similarities with Grand's (1965) description of that species. *Russula flaviceps* differs from *Russula lutea* in its slightly paler, larger spores with higher ornamentation, thicker cutis (sensu Grand), bluing reaction of the pileocystidia in SV and sometimes peppery to bitter taste. Another mild yellow species, also reported by Grand is *Russula gilva,* which has incrasted primordial hyphae in the cutis and is also in subsection *Chamaeleontinae.* It differs from *R. lutea* in its less vivid cap colours and lighter spore colour.

In mounts of cutis from a mature specimen stained with fuchsin or methylene blue the incrustations are difficult to see, due to the presence of bacterial colonies and other debris in the surface gluten that also take up the stain. In water mounts the debris is less visible and the incrustations appear as yellowish droplets, many of which seem to be shed into the mounting fluid when fresh material is observed.

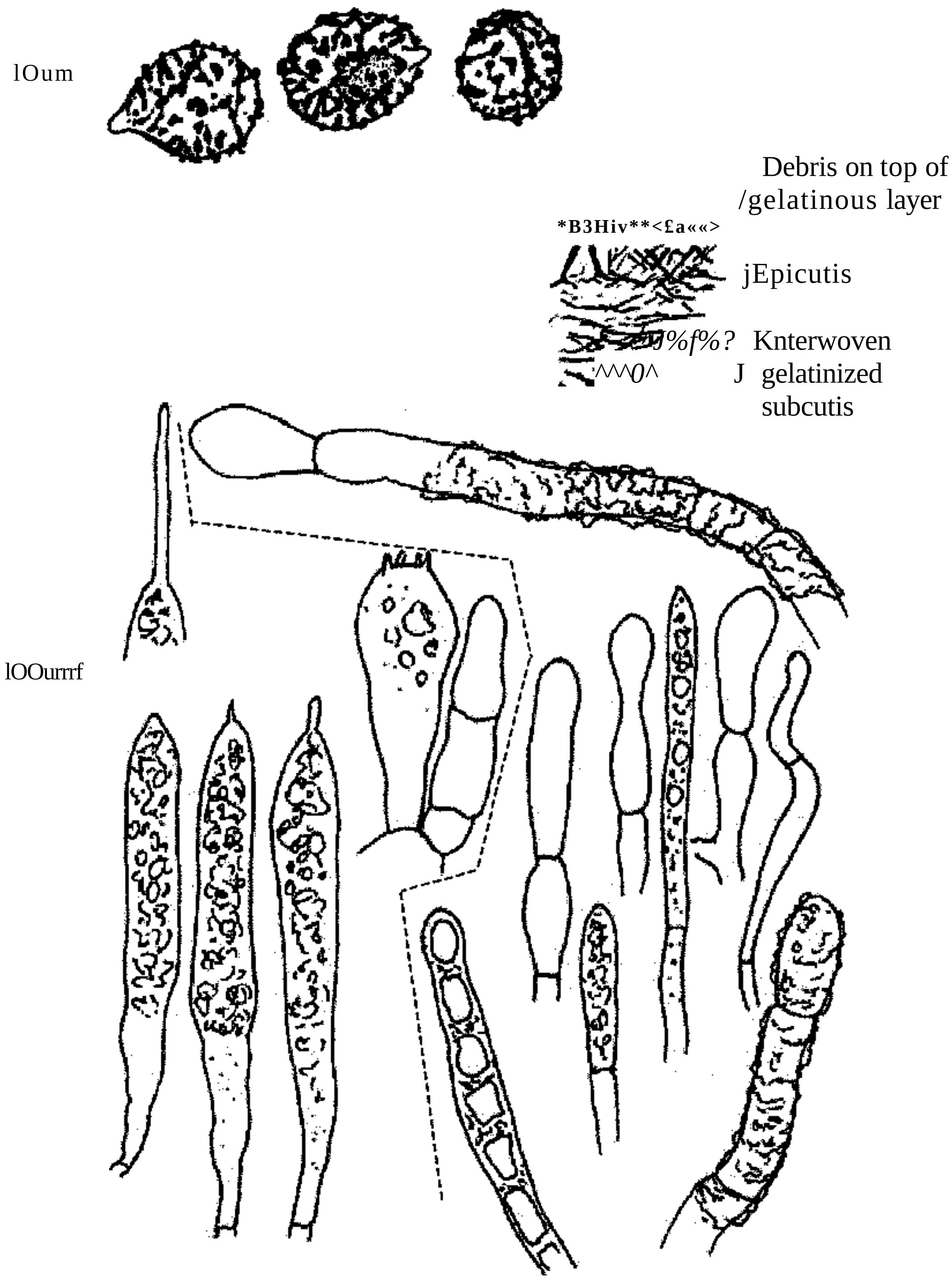

Figure 145 Microscopic characters *of Russula lutea:* Top, spores with lOum scale bar; middle, diagram of section through cutis; bottom left, hymenial cystidia and basidium with packing cells; bottom, to right of dotted line, hyphal ends, incrusted primordial hyphae and cystidioid cells, lower scale bar is lOOum.

Figure 146 Macroscopic characters of *Russula lutea:* a collection from under oaks, background grid is in $1cm^2$, inset square shows spore colour, the soil at the collection site has stained the stipe at left greyish, this is not a bruising reaction.

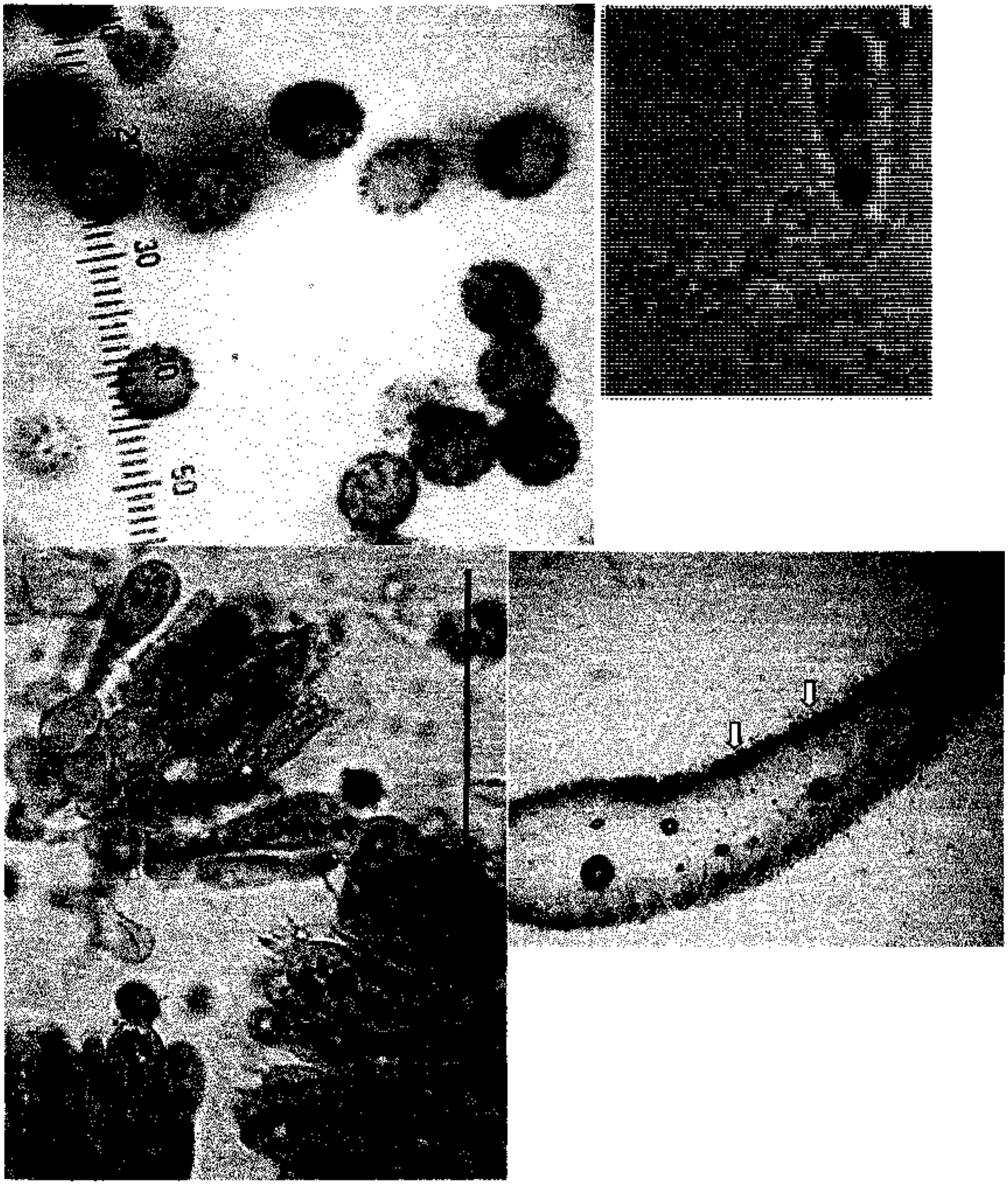

Figure 147 Hymenium *ofRussula lutea:* Top left, spores with lum division scale; top right, basidioles with packing cells to their right (arrowed); bottom left, fragments of the hymenium showing basidioles and a segment of subhymenium at upper left and mature basidia at lower right, scale bar is 50um; bottom right, section through gill stained in SV illustrating the sparse nature of the hymenial cystidia which are stained dark, arrows point to two of them.

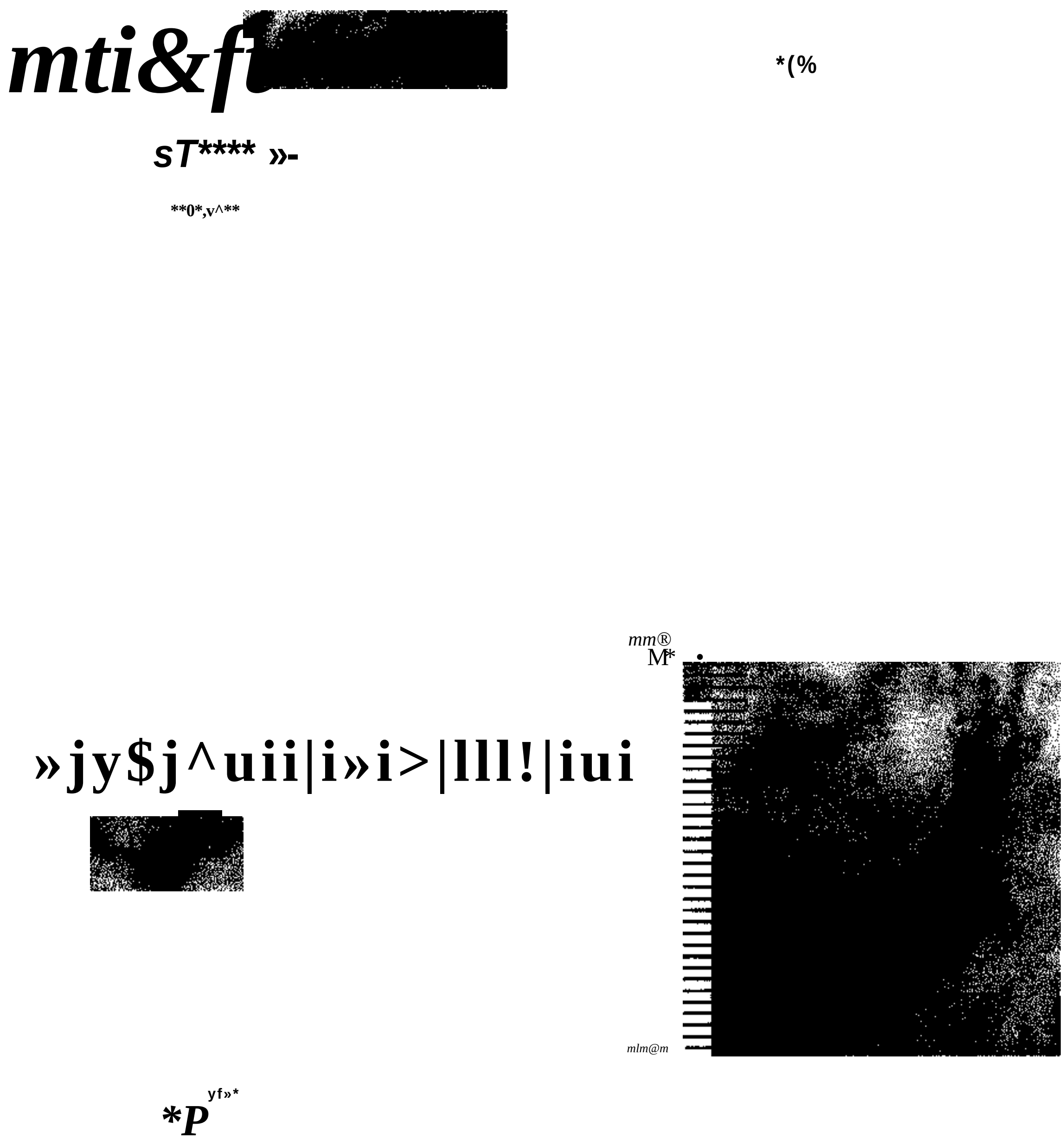

Figure 148 Cutis of *Russula lutea:* Top right, section through cutis showing the dark layer of debris lying on the glutinous matrix, primordial and generative hyphae forming the epicutal turf and the subcutis which rests directly on the trama with no defined hypodermis, scale bar is lOOum; top middle, a cystidioid hyphae with banded contents: top right, two primordial hyphae stained in acid fuchsin in which the incrustations show as darker areas along the walls, the top part of the left hyphae is naked and has banded refractive contents; bottom, detail of a primordial hyphae in SV in which the incrustation shows as a sheath of varying thickness, the scales for the latter three photographs are in lum divisions.

Subsection *Amethystinae* (Romagn.) Bon

Russula munillii Burlingham

Mycologia 5:310. 1913

Cap 3.7-10.3cm diameter, pulvinate when young, sometimes with a flattish umbo within the central depression, expanding to shallowly centrally depressed, usually with rounded margins, but those growing in clusters becoming rather irregularly uplifted, margins smooth, slight striations developing in age and dry conditions. Colour a greyish dark pink to a greyish violet, often dull brownish or with olive tints centrally, becoming more brownish-grey in age, the colours opaque and chalky, usually with a ring of minute blackish floccules around the central depression but not always within it, on occasion the blue pigment is much reduced and the colour resembles that *of Russula veternosa*, i.e. dull yellowish on the disc and pinkish towards the margins, buttons may be these colours initially. The appearance of the cap surface is as if coloured with chalk pastels and often lightly rubbed around the depression with charcoal, and is quite distinctive. The cutis is viscid when wet, drying matte with a greyish bloom, and minutely concentrically areolate throughout, this character being more pronounced in the cap centre. Peelable 1/2 to 3/4 or sometimes completely, flesh white, tinged pink under the cutis, unchanging.

Gills cream at first, becoming a deep cream to light orange-yellow, unchanging where damaged, ventricose, narrowly adnexed to free at stipe, acute to subacute or sometimes obtuse at cap margin, up to twice the depth of the cap trama at 1/2-radius, subdistant, subgills occasional, a small degree of forking common, texture average to slightly pliable.

Stipe 5.2-10.1 x 1.1-2.6cm, chalky white, pruinose, particularly near the base, longitudinally slightly rugulose, unchanging where damaged but sometimes browning slightly at the very base, in dried material the stipe retains its white surface. Shape generally cylindrical to clavate but when growing in clusters may taper downwards or be irregular in shape, stuffed with a soft bread-textured trama that develops 1-3 cavities, becoming hollow in age. Microscopically, incrusted primordial hyphae may be found in the surface of the base of the stipe in young basidiomata.

Texture quite firm.

Taste mild and nutty.

Odour not distinctive usually, one collection with a slight fruity-flowery smell, sometimes with an iodoform smell at the stipe base or when deteriorating in an enclosed space.

Spore colour orange-yellow, Romagnesi IVa-c.

Spores 7.3-10.8 x 6-8.5 (-9)pm, L:W 1.06-1.6 with a mean of 1.24 (n=50). **Ornamentation** 0.2-0.8pm high, of hemispherical, isolated warts, elongated, blunt, low crest-like warts and warts joined with short to long, branched, generally heavy lines, some spores with a partial reticulum, less commonly with mostly isolated warts. **Woo types** Bl-2, Cl-2, rarely Al-2. **Suprahilar patch** amyloid, roundish, around 3pm diameter, warts not generally forming a border. **Hiliferous appendix** up to 2.0pm long, 1.6pm wide at base. **Basidia** mostly 4 -spored but 2 -spored ones common, 32-48 x 9.5-13.Sum, clavate and bulbous in the upper 1/3, embedded amid many basidioles. Sterigmata fairly short, 3-5pm long and 1.3-1.5pm wide at the base. **Pleurocystidia** very sparse, (they are difficult to find on the gill as they are not strongly differentiated from the submature basidia), 47-70 x 5-1 lpm, protruding up to 15 pm, most embedded except for the tip, originating in the subhymenium, fusoid, cylindrical or with constrictions toward the apex, sometimes appearing thicker walled than in other Russulas, tips obtuse, mammillate, or with a short strangulate appendage; with refractive contents in the apex, not reacting in SV. **Cheilocystidia** sparse, only the tips protruding, mostly under 8pm wide and usually with more constrictions than pleurocystidia, contents similar. **Subhymenium** 25-30pm thick, pseudoparenchymatous, gill trama of sphaerocytes, vascular hyphae not seen.

Cutis 50-150jim thick, up to 175um thick over disc, consisting of a more or less colourless subcutis and a pinkish epicutis embedded in a gelatinous matrix that may extend 20u.m or more beyond the epicutis in water mounts. **Subcutis** cellular, of roundish to flattened sphaerocytes that are about 12 -20 *(-45)[im* across in the mid subcutis, becoming smaller near the boundaries between the epicutis and hypodermis, lacking vascular hyphae. **Epicutis** an ixotrichodermis, of erect, branched, somewhat tortuous and nodulose hyphae 2-4 um wide, sometimes incrusted, containing many pink granular pigment bodies up to 2.5urn across, hyphal tips rounded, sometimes capitate, often with a small yellowish refractive body in the tip that slowly turns bluish in SV. **Primordial hyphae** 5-8um wide, project from the epicutal surface and often lie repent on it. They are multi-septate, incrusted, the contents staining pink in acid fuchsin. They may be branched in their lower portions but the terminal 3-4 cells, around 60-1 lOum total length, are unbranched and narrowly clavate, and often include a small yellowish refractive body, bluing slightly in SV, at the terminus. The incrustations appear as a roughened, closely fitting sleeve with small globular extrusions, greying slightly in SV, not very visible in water mounts, as droplets in aqueous methylene blue mounts, disappearing in KOH, and breaking into horizontal bands or squarish blocks in acid fuchsin mounts. The areoli seen macroscopically are clumps of hyphal ends and primordial hyphae protruding around 10-15urn above the general epicutis level. **Pileocystidia** and pseudocystidia not seen, though the primordial hyphae appear somewhat like cystidia. **Hypodermis** a narrow layer of compact tramal tissue and distinctly more parallel interwoven hyphae than in the subcutis.

Trama of large discrete clusters of sphaerocytes bound by a hyphal mesh, vascular hyphae apparently absent, although short sections of some hyphae may have refractive contents.

Chemical reactions: FeS(>4 - unreacting to slightly yellowish or pale brownish; KOH - pinkish to brownish orange on cap surface, no reaction to slightly yellowish on stipe; NH4OH - no reaction; guaiac - blue-green; guaiacol - faintly pinkish; phenol - slowly brownish purple (after more than 5 minutes); SV - initially pink on the gills and cuticle, becoming brownish, slowly staining some refractive bodies and incrustations bluish.

Habitat and tree associations: Gregarious, sometimes clustered, rarely singly, under Douglas fir or western hemlock, usually under canopy openings and well lit patches of forest floor, often in patches of broom or huckleberry, October to November.

Collections: CR981029-01 and CR 021015-13, under roadside red alder with old-growth western hemlock with western red cedar and huckleberry, just inside the beginning of the Rainforest trail (east side), P.R.N.P., near Tofino N 49.036°, W 125.667°; CR001115-07 under old-growth western hemlock and Sitka spruce with western red cedar, huckleberry and salal, Clayoquot Island, N 49.1542°, W 125.9191° (southern very wet hypermaritime CWH subzone). CR 021027-03, at the forest edge by the road, under regeneration Douglas fir, Mesachie Lake Forest Research Station, N 48.826113°, W124.13635° (western very dry maritime CWH subzone). CR001104-02 under regeneration Douglas fir and western hemlock with big-leaf maple, Pacific blackberry, salal, ocean spray *(Holodiscus discolor)* and sword fern, Gowen Todd peninsula trail, N 48.5187°, W 123.52935; CR011111-02, CR011111-03, CR 041114-01 and CR 041011-01, at the forest edge under mature and sapling Douglas fir with broom, Royal Roads N 48.4375°, W 123.4744° (CDF zone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR001104-02	849	428,374	337	365,276
CR011111-02	864	420,369	320	363,280
CR021015-13	866	404,346	342	495,370,285

Notes: Burlingham (1913) commented on the beauty *ofRussula murrillii* with its "violet cap and pure-white stem", a type she collected from Corvallis, Oregon. It is indeed an attractive and clean-looking mushroom, with its contrast between the violet cap, white stipe and egg-yolk yellow gills. The particular colour and texture of the cap cutis, described above, is quite distinctive and apparent even in old, faded basidiomata. It can be quite common in the coastal Douglas fir forests. Its range and different tree hosts could suggest more than one species but there do not appear to be any macroscopic or microscopic differences between the coastal Douglas fir populations and those from the coastal western hemlock zone.

Singer (1942) considered *Russula murrillii* to be synonymous with *Russula punctata* Krombholz *(-Russula amethystina* Quelet). However by 1957, after examining more collections, he conceded that *Russula murrillii* was an autonomous Pacific Northwestern species. It is very similar to *R. amethystina* sensu Romagnesi (1967), which grows with *Picea* and *Abies,* more rarely with *Pinus.* The spore print of *R, murrillii* is described in Singer (1947 and 1957) as Crawshay D-E, which is paler than that of most of the Vancouver Island collections which are generally Crawshay E or slightly darker, a shade or two deeper than Romagnesi's description of *R, amethystina* (spore print Illd-IVa). The spore size *of Russula murrillii* ranges larger than those of *R. amethystina* (7-9 x 5.5-8) but have the same ornamentation. Romagnesi's drawing of the primordial hyphae shows a thicker, rougher layer of incrustations than is normal fori?, *murrillii,* but other characters including the occasional iodoform odour, the weak reaction with guaiacol, and the macroscopic appearance are similar.

Russula turd is quite similar but has more consistently reticulate spores, stipe occasionally with pink tints and yellowing at the base, cap colour more vinaceous or dark red, a thicker subcutis (around 150um), thicker incrustations on the primordial hyphae and softer flesh, but the same spore colour as that of *R. murrillii. Russula turci* grows with *Pinus* and *Picea.*

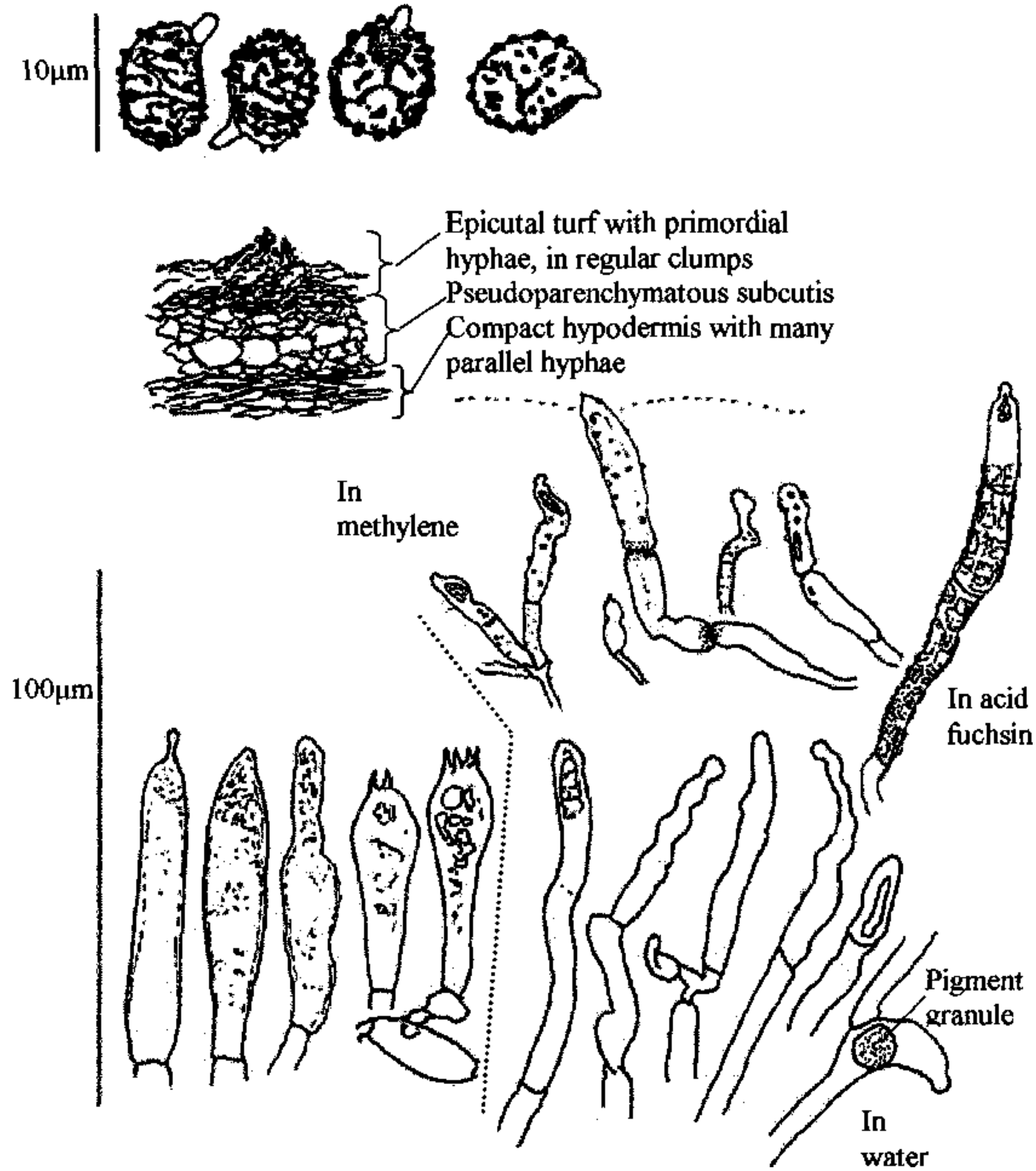

Figure 149 Microscopic characters *ofRussula murrillti:* Top, spores with 10pm scale bar; middle, diagram of section through cutis; bottom left, hymenial cystidia, a two -spored and a 4-spored basidium; bottom right, hyphal ends and primordial hyphae from epicutis in water, above which are these structures in aqueous methylene blue and to the right of them is a primordial hyphae in acid fiichsin; lower scale bar is lOOum.

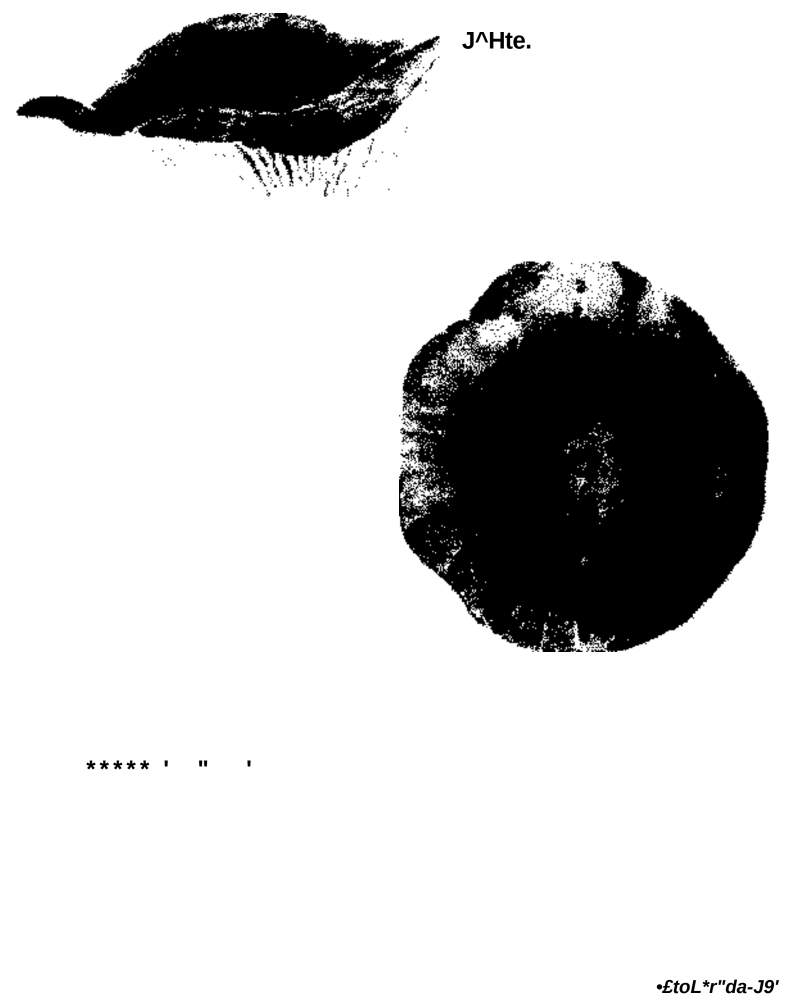

Figure 150 Macroscopic characters of *R. murrillii;* Top, illustration of profile, cap and longitudinal section of mature basidiomata, small square is $1cm^2$ and shows spore colour; bottom left, top of cap showing unchanging white flesh and yellow gills where slug-damaged; bottom right, close-up of same cap cutis showing minute areolae.

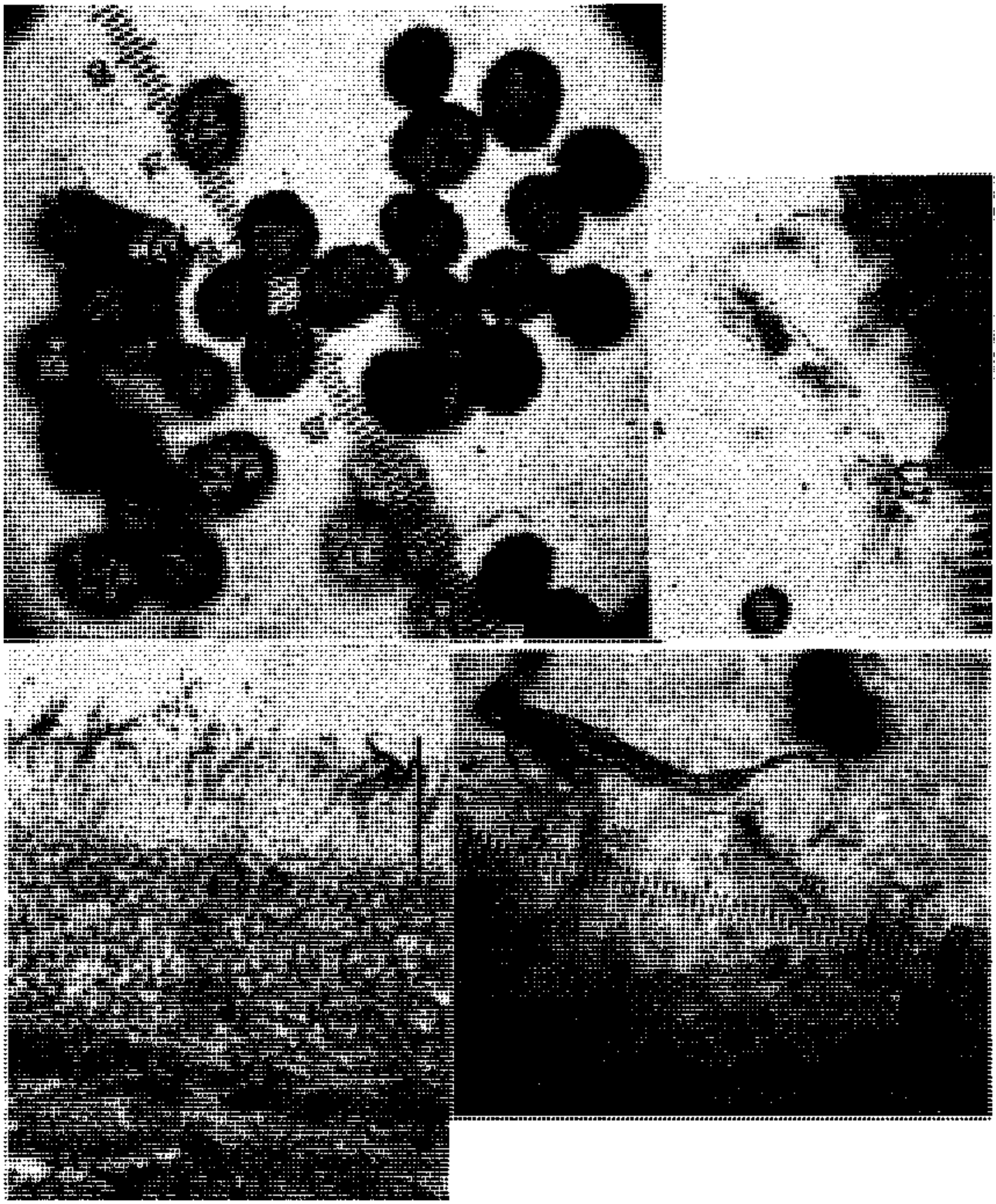

Figure 151 Spores and cutis of *Russula murrillii:* Top left, spores with 1 um division scale; bottom left, section through cutis showing an ixotrichodermal epicutis with darker primordial hyphae, above a pseudoparenchymatous subcutis and a darker, denser, hypodermis, stained in acid fuchsin, scale bar is lOOum; top right, a primordial hypha in SV showing a darkened refractive body within and a darkened, cracked sheath-like incrustation with small globular exudates; bottom right, detail of the epicutis stained in acid fuchsin, showing three primordial hyphae with dark-stained incrustations; 10 scale divisions are 25um for both right-hand photographs.

Clade 7b

Section *Paraincrustatae* Sarnari

Subsection *Lepidinae* (Melzer and Zvara) Singer

***Russula albida* Peck**

Bulletin of the New York State Museum 1:10. 1888

Cap 3-6cm diameter, plano-convex when young with an incurved margin, becoming shallowly depressed with irregularly uplifted margins that remain smooth, pale cream initially, eventually a pale clear yellow with an ochre yellow centre, yet the impression is of a cream coloured cap rather than a yellow one. Not very viscid when wet, drying matte or with a slight sheen, peelable to 1/2 the radius, flesh white, unchanging.

Gills pale cream when young, deep cream to pale orange-yellow at maturity, not bruising, adnate to adnexed, ventricose, about 1.5 times the depth of the trama at half-radius when mature but very shallow at first, subacute at margin, close to crowded, no forking or subgills seen, brittle.

Stipe 2.2-5.8 x 0.9-1.7cm, white, smooth, slightly pruinose especially near the base when young, cylindrical to slightly clavate, stuffed with a firm bread-textured trama that develops 3 or 4 cavities, unchanging or with slight yellow-brown bruising at the very base.

Texture somewhat firmer and more brittle than an average *Russula* but not as firm as the *Compactae.*

Taste mild and nutty, lacking any bitter taste.

Odour pleasant, nutty and fruity, of brazil nut or floral, depending on one's interpretation.

Spore colour pale orange-yellow. Romagnesi IIia.

Spores 9-11(12) x7-9pm,L:W 1.13-1.54 with a mean of 1.29, (n=30), subgloboseto broadly ellipsoidal, the largest spores more ellipsoidal to obovoid. **Ornamentation** of pointed, narrow conical to peg-like warts 0.2-. 0.8 pm or occasionally to lpm high, generally the full height range on each spore, many warts isolated but short catenations and fine to heavy lines linking several warts occur on most spores, not forming a reticulum. **Woo types** A2-B2. **Suprahilar patch** amyloid, an irregularly shaped area with or without warts at its border. **Hiliferous appendix** relatively short for the spore size, around 1.4-2.2pm long, 1.4-1.7pm wide at base. **Basidia** 4-spored, occasionally 2-spored, 30-55 x 10-13 pm, with the older ones having a longer base than the shorter developing ones, clavate, the longer ones sometimes more bulbous in the upper 1/3, the hymenium expands outwards by one to two cells with each generation of basidia. Sterigmata up to 7.5pm long and 2.2pm wide at the base. **Pleurocystidia** sparse, 60-75 x 5-10pm, embedded or protrudingup to 20pm, originating in the subhymenium, cylindrical, fiisoid or occasionally contorted, tips acute, mucronate, or with a short narrow appendage, contents refractive in KOH, pinkish brown and darker than basidia in SV. **Cheilocystidia** occasional, similar to pleurocystidia. **Subhymenium** 25-50pm thick, pseudoparenchymatous, sometimes not well defined, separated from the gill trama by a one or two layers of hyphae. **Gill trama** of voluminous sphaerocytes 25-60pm across, no vascular hyphae seen.

Cutis 120-250um thick, an ixodermis composed of a thick subcutis and a barely differentiated epicutis 20-50pm thick. **Subcutis** of tightly interwoven semi-upright hyphae 2-4um wide, slightly yellowish towards the subcutis base, more or less hyaline above, embedded in a gelatinous matrix, vascular hyphae rare. **Epicutis** of tangled hyphal ends which are towards the thinner end of the width range in general, ends obtuse, sometimes slightly capitate, accompanied by numerous septate, occasionally branched, incrusted **primordial hyphae,** mostly 4-5 pm wide but ranging from 2-6pm wide, and with up to about 100pm lying on the epicutal surface, contents slightly refractive and sometimes yellowish banded to globular, tips usually subacute. The hyphae and incrustations stain pink in acid fuchsin but are harder to see than in SV, which stains the whole epicutis pink initially, with the primordial hyphae and incrustations a deeper pink, and some of their contents light grey, often but not always in the terminal cells. The incrustations appear as an incomplete and patchy granular or darkly dotted sheath, some hyphae are almost naked and the ends of many are naked. **Pileocystidia** rare and uncertain, some of the primordial hyphae have refractive contents, lightly greying in SV, in the terminal 1-3 cells, which are sometimes narrowly clavate and occasionally arise from a narrower hyphae, are patchily incrusted and otherwise similar to the rest of the primordial hyphae, but which do look somewhat cystidioid. These structures are 25-75um long and 4-6um wide in general. **Hypodermis** none.

Trama of discrete well defined clusters of sphaerocytes bound by a hyphal mesh, vascular hyphae not seen.

Chemical reactions: FeSC>4 - very weakly pinkish grey; KOH - no reaction on cap, slightly yellowish on stipe; phenol - pinkish-brown, becoming almost black after 10 minutes; SV - pinkish on the gills, bright pink on the cuticle, both turning brown after 10-15 minutes, pinkish brown on the stipe trama, cystidia and vascular hyphae not reacting to slightly brownish.

Habitat and tree associations: In dry mineral soil under Douglas fir and western hemlock.

Collections: CRO10516-01, under mature regeneration Douglas fir with understory western hemlock and ivy ground cover, in May during fairly dry weather, Tillicum Park, N 48.45750°, W 123.385450° (CDF zone). One other collection, 981114-04, from Koksilah river area on a limestone slope with Douglas fir and salal, passed on to me by forayers, has similar spores to the above collection but with a size range extended down to 7um, and a pink reaction of the cutis with SV, but the poor condition of the cutis, which had been grazed by slugs, prevented a positive identification. The description above does not include data from this collection.

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR010516-01	866	390,330	500,309	375,271,242

Notes: Singer (1947) and Thiers (1997) both placed *Russula albida* in the *Lepidinae*, which has both incrusted primordial hyphae and SV -ve pileocystidia that may also have incrustations. Neither author specifically mentions incrusted hyphae although Singer refers to the presence of primordial hyphae in the cutis; both authors note a lack of pileocystidia. The spore size given by Singer is 8.8-10.8 x 7.8-9.2um, which is consistent with the size range of most of the spores in the Vancouver Island collection, though large spores occasionally up to 12um long but of average width are common. This collection lacked the slight bitter taste that is reported for *Russula albida,* although this character is recorded as mild to bitterish, indicating variation. Singer also mentions that the hymenial cystidia are numerous, which was not the case in the V.I. collection, he also notes the pink to red reaction of the flesh with SV, which occurred in the V.I. collection with the cutis and gills but less so with the stipe trama.

Other white to cream or light yellow Russulas include *Russula brevipes* which is sometimes quite mild, but much larger, more robust, with white spores and a non-separable cutis. *Russula raoultii, R. crenulata, R. cremoricolor* and *R. cascadensis* are all acrid tasting and have white spores. *Russula basifurcata* is a yellowish or dingy white, mild-tasting, yellow-spored species that may sometimes stain red in SV, but the cap cutis is thinner and barely peels, the gills are frequently forked, and microscopically it lacks incrusted hyphae and has an inamyloid suprahilar patch and much smaller ornamentation on the spores. *Russula lutea* and *R flaviceps* are more yellow and have darker spores which are also smaller, and the latter species bruises greyish. Pale yellow forms of *Russula chamaeleontina* have a similar spore size and ornamentation, incrusted primordial hyphae and are mild-tasting, they differ only in having a darker spore print.

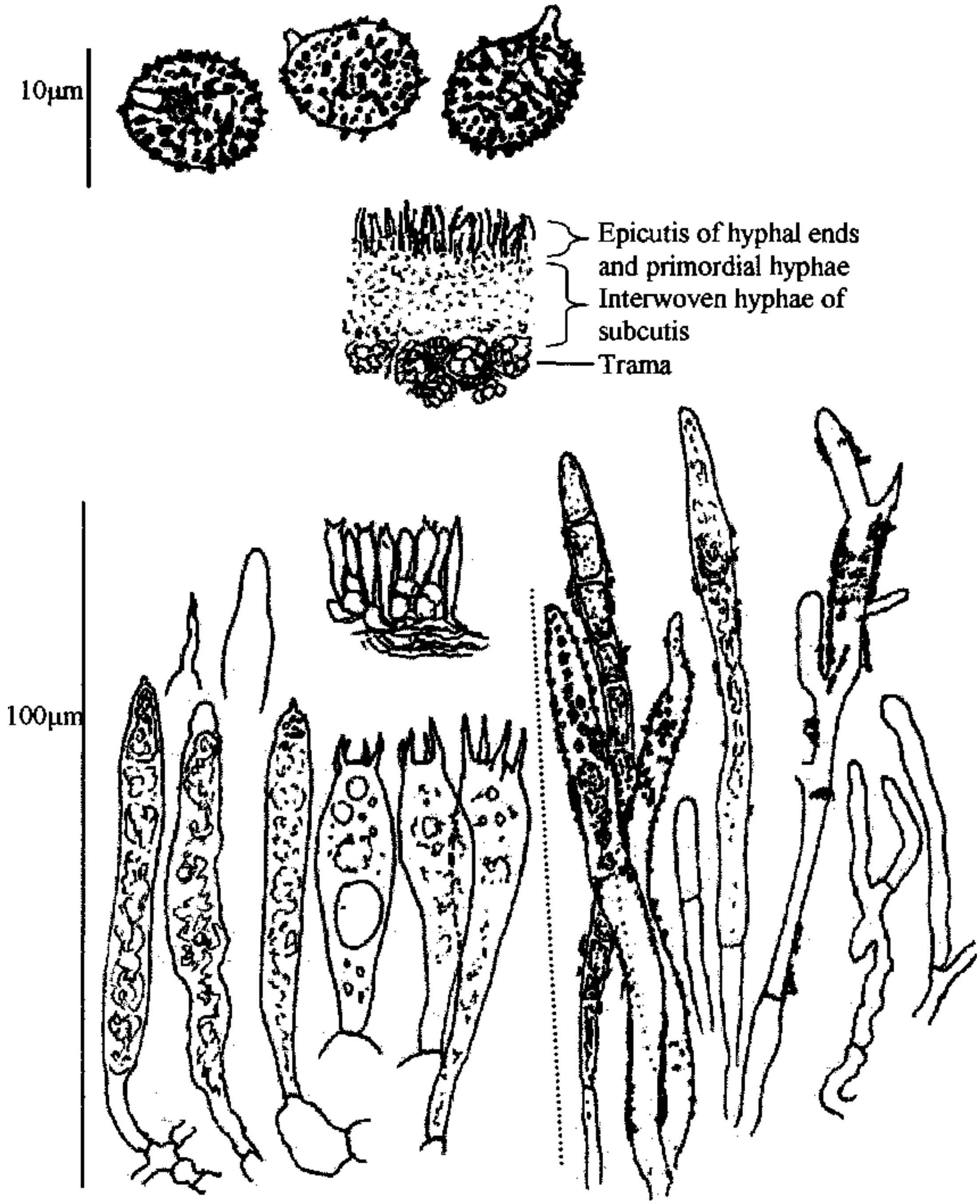

Figure 152 Microscopic characters *oiRussulaalbida*. Top, spores with lOum scale bar; middle, diagram of cutis in SV, which dissolves the gelatinous matrix allowing the epicutis to expand upwards; bottom left, hymenial cystidia and basidiomata with, above them a diagram of the structure of the hymenium showing the raising of newer basidiomata above the origin of the older ones by means of an inflated basal cell; lower right, incrusted primordial hyphae and epicutal hyphae, lower scale bar is lOOum.

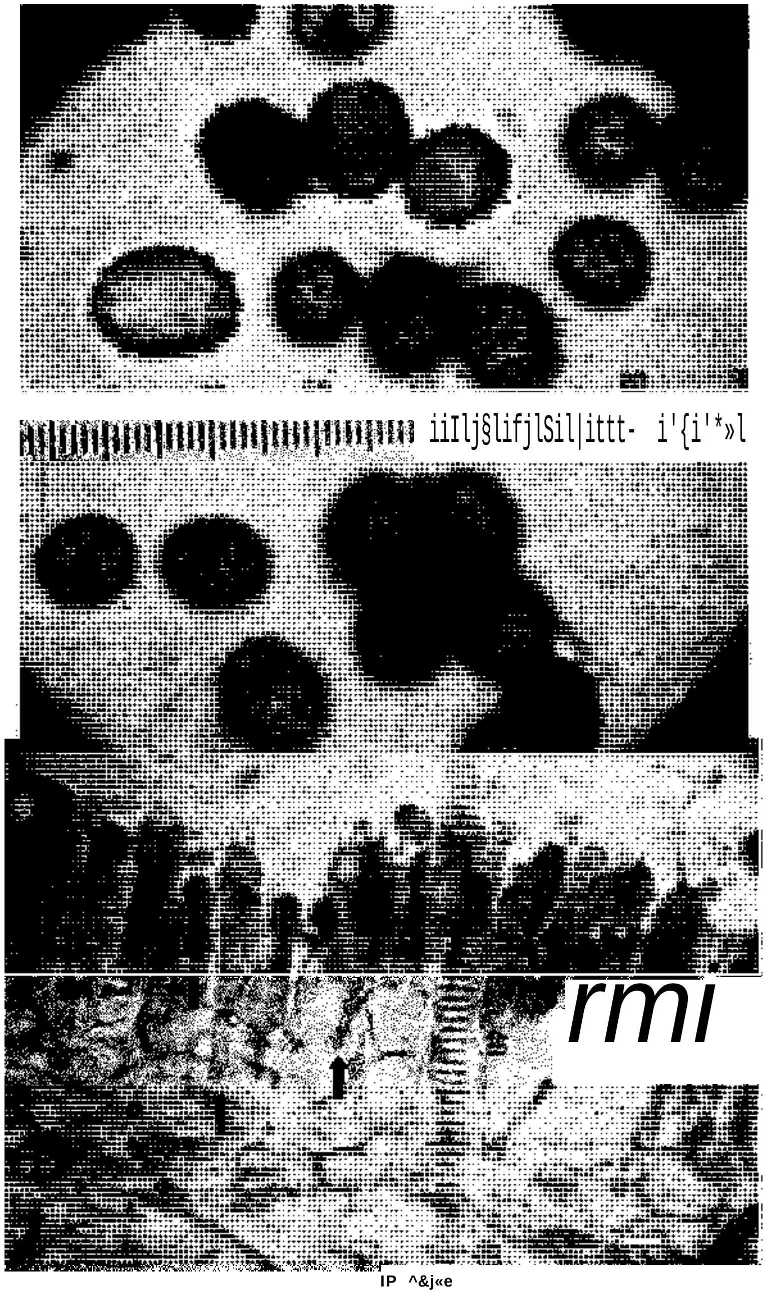

Figure 153 Hymenium of *Russula albida.* Top, spores with 1um division scale; bottom, section through gill stained in phloxine, the white arrow points to the narrow band of hyphae at the base of the subhymenium, black arrows point to the bases of basidia at different levels, the uppermost one is just starting to develop, note also the large sphaerocytes of the gill trama, 10 scale divisions are 25um.

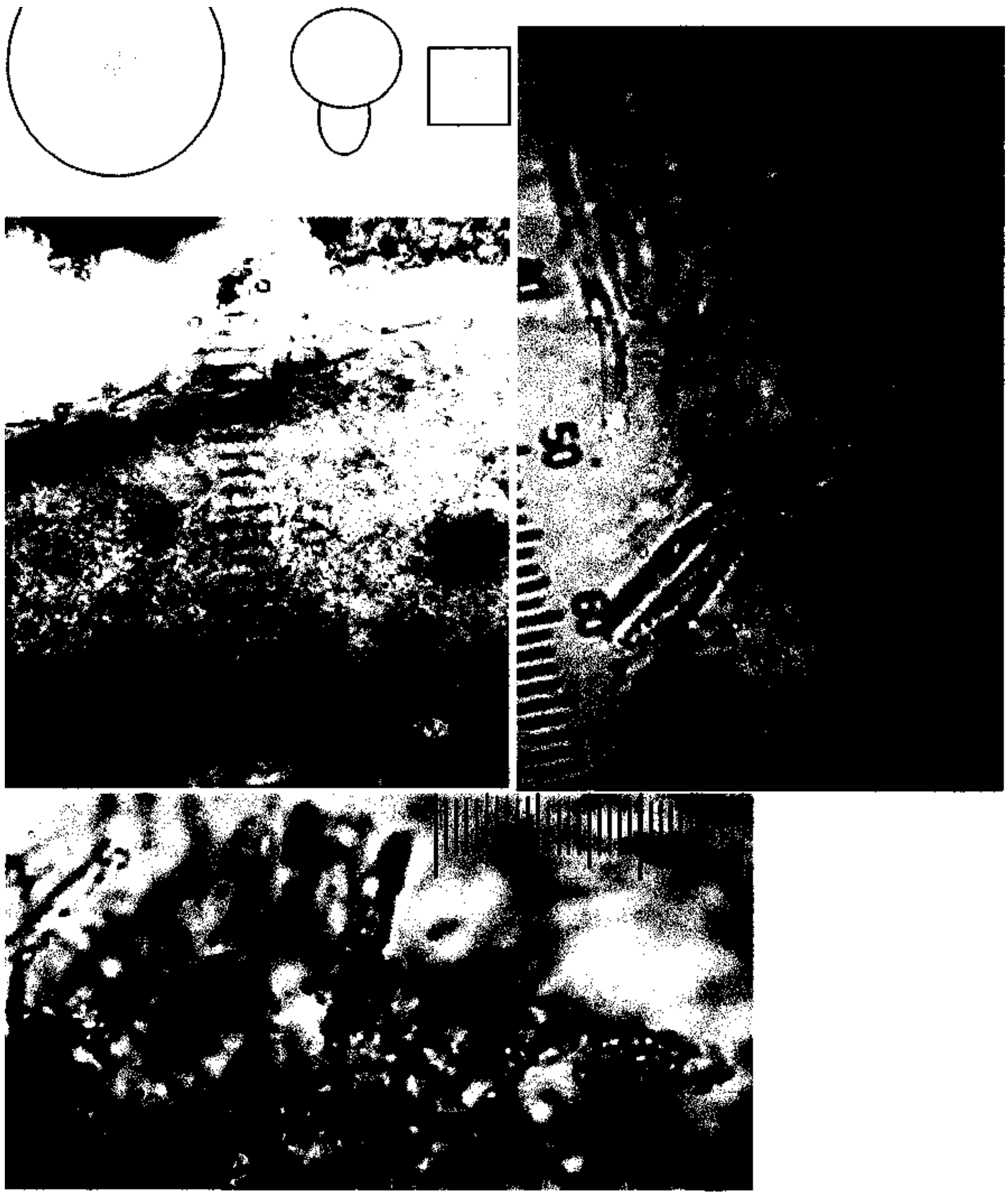

Figure 154 Cutis of *Russula albida:* Top left, field sketch of cap colours; middle left, section through cutis mounted in water, 10 scale divisions are 100um; top right, articular hyphae in SV showing some greying hyphal contents in some cells, 10 scale divisions are 25um; bottom, close up of incrusted primordial hyphae seen in SV, scale is in 1um divisions.

Russula lepidiformis **Murrill**

Mycologia 30:363 1938

Cap 5.5-8cm diameter, pulvinate when young, becoming shallowly depressed, margins curved down over gill edges, margins smooth. Colour an intense deep red, more brownish red in the centre, not fading or changing much, young and mature basidiomata about the same colour, not or barely viscid when wet, quickly drying matte and finely velvety. Cutis not peelable, flesh beneath tinted pink, cap trama white, unchanging.

Gills creamy white, close to subdistant, subgills occasional, forking not seen, narrowly adnexed at the stipe, ventricose, broadest at about 3/4 the radius, up to twice the depth of the cap trama at half-radius, at cap margin the gill is rounded up to where it becomes free of the cap cutis, where there is a near 90° angle with the gill margin.

Stipe 4-6 x 1.3-2cm, white, smooth to finely longitudinally rugulose, sometimes pruinose especially near the apex, firm, solid to firmly stuffed, not cavitate, more or less cylindrical, unchanging where cut but browning a little where handled. Microscopically the vascular hyphae in the stipe cortex do not stain in S V.

Texture firm, like a crisp apple, but not particularly brittle.

Taste bitter, but not strongly so, otherwise mild to faintly peppery.

Odour not distinctive.

Spore colour pale cream, Romagnesi Ib-IIa.

Spores 8.0-10.2 x 6.8-8.6um, L:W 1.15 -1.4 with a mean of 1.25, (n=30), mostly ellipsoidal to ovoid, rarely subglobose. **Ornamentation** of small conical warts 0.4-0.8um high, very occasionally up to lum high, isolated or 2-3-catenate or joined by very fine lines forming a partial reticulum, with most spores having fairly crowded warts. At a magnification of 400x, the spores appear as if with mostly isolated warts or wart clusters, but the reticulum is apparent at lOOOx magnification. **Woo type 2C.**

Suprahilar patch lightly amyloid, irregular in shape and often with a nearly continuous strongly amyloid border containing warts. **Hiliferous appendix** around 1.8-2.2um long, L2-l.5u.rn wide at base. **Basidia** 4 -spored, occasionally 2 -spored, 48-60 x 11-1 Sum, broadly clavate, light grey-brown in 5% KOH, successive basidia developing one cell up from the basal cell of the older basidia. Sterigmata rather fat, up to 7um long and 1.9-2.5um wide at the base. **Pleurocystidia** frequent, 70-95 x 7-10um, most protruding less than 20um, originating in the subhymenium, more or less fusoid, contents yellowish, refractive in KOH, grey to brownish purple in SV, tips subacute to acute, occasionally with a tiny button. **Cheilocystidia** frequent, 55-70um protruding up to 20um, tips acute, obtuse, with a small button or strangulate appendage, barely reacting with S V, occasionally lacking refractive contents, otherwise similar to pleurocystidia. Approximately 200-300um of the gill margin stains yellow-brown in SV in a clearly delineated band. **Subhymenium** 20-400um thick, well differentiated, pseudoparenchymatous. **Gill trama** of sphaerocytes with occasional vascular hyphae.

Cutis 150-260um thick, an ixotrichodermis when young, losing any surface viscid material when mature to become a trichodermis. **Subcutis** about 1/2-2/3 the thickness of the cutis, of hyphae 1.5 -3.5 urn wide, pink in water mounts and containing occasional to frequent small globular bodies of dark pink pigment. The lower 30-50um of the subcutis is a brownish pink and contains frequent vascular hyphae 3-7um wide, with occasional ones ascending into the epicutis, forming pseudocystidia about 5um wide. These are often incrusted, terminate below the surface, with tips obtuse, undifferentiated or with a clavate end cell that broadens up to 8um wide, staining grey in SV and temporarily pink in acid fuchsin. **Epicutis** of erect, tangled, hyaline to pinkish hyphae 2-5 um wide, many of which are partially incrusted, endings capitate, nodulose, contorted or occasionally with series of short cells occasionally up to 7um wide, sometimes the terminal few cells with brownish contents. Accompanying the epicutal hyphae are clusters of pileocystidia and primordial hyphae, mostly lying along the surface, all of which are incrusted with a broken brownish material, often angular as if crystalline, with much of this material loose between the epicutal hyphae and appearing as debris, except that it is within the interweavings of the epicutis. All these elements form a brown crust-like layer that cracks apart into areoli in mature basidiomata. **Pileocystidia** 60-110 x 5-8um, 0-2 -septate, cylindrical, incrusted, capitate, occasionally with a crown of incrustations, with yellow-brown amorphous to refractive contents that stain temporarily red in acid fuchsin and grey in SV. **Primordial hyphae** 3-4um wide through the subcutis where they arise, broadening up to 7.5 urn wide at the surface, sometimes branched, incrusted more than subcutis hyphae, with colourless to yellowish contents more refractive than epicutal hyphae but less so than pseudocystidia and vascular hyphae, sometimes with a cystidioid terminus, staining pink in acid fuchsin, the incrustations staining brownish grey and the contents unreacting to light grey in SV. **Hypodermis** not strongly differentiated, but separable from the lowermost layers of the subcutis in parts, merely a slightly more compact layer of tramal tissue.

Trama of discrete large clusters, 130-250um across, of sphaerocytes, bound by a hyphal mesh, with occasional incrusted hyphae and frequent vascular hyphae 4-6um wide that stain grey-brown in SV.

Chemical reactions: FeSC>4- light salmon pink; KOH - brownish orange on cap surface, no reaction on stipe; NH4OH - no reaction; guaiac - blue-green; guaiacol - pinkish; phenol - brownish purple; SV - purple-brown on the gills but unstaining on the gill margins, brownish on the cuticle and trama, cystidia and vascular hyphae non-reacting to greyish.

Habitat and tree associations: not recorded.

Collections: SVEVIS 041030, a collection brought in to the October 2004 South Vancouver Island Mycological Society annual show at Swan Lake Nature House. Site of collection and habitat details not known.

Notes: This collection keyed out as *Russula lepida* Fries in Bon (1988) and Romagnesi (1967) with the exception of the positive reaction of the cystidia with SV and larger spores; and its North American equivalent: *R. lepidiformis* Peck in Kibby and Fatto (1990). The cap colour of this collection is more intense and darker than is common for both those species, but not outside of the range. Singer (1957) expressed some doubt as to whether *R. lepida* and *R. lepidiformis* were separate species; the European *R. lepida* has no SV positive elements according to Romagnesi, but Singer queries this based on his own observations of *R. lepida*. The Vancouver Island collection agrees reasonably well with Murrill's original rather brief description and with Singer's more detailed description made from the type and supplementary collections. *Russula lepidiformis* has larger spores than *R. lepida:* 7.5-11.7 x 7-10.2p.m. Minor differences between the Vancouver Island collection and Singer's description of *R. lepidiformis* include the slightly shorter basidia, 33-42um, and lack of reaction of hymenial cystidia with SV. Murrill found the type in Florida under turkey oak, a species not present on Vancouver Island, so it is hoped that more collections of this rather beautiful *Russula* will be found and a habitat ascertained.

The Kibby and Fatto (1990) key also led to *R. subvelutina* Peck, a dark red, mild species which does have SV positive cystidia, albeit scarce. This species has darker, smaller spores, and at the time of writing I have not acquired a full description with which to judge any similarities with the Vancouver Island collection. *Russula rubra* Fries is another red to deep red species with similarly hard flesh and incrusted hyphae in the cap cutis, with cap and hymenial cystidia staining dark grey in SV, but an intensely acrid taste. *Russula rhodopoda* Svara in Melzer and Zvara, is a mild tasting red species reported from the Pacific northwest (Thiers 1997), but has a glossy cap, yellow spores, and a cutis lacking incrustations but with many well differentiated pileocystidia.

If the microscope slide of the cutis is illuminated with a white LED bulb, the yellow-brown pileocystidia show up better and are easier to differentiate from the primordial hyphae than when a tungsten bulb is used.

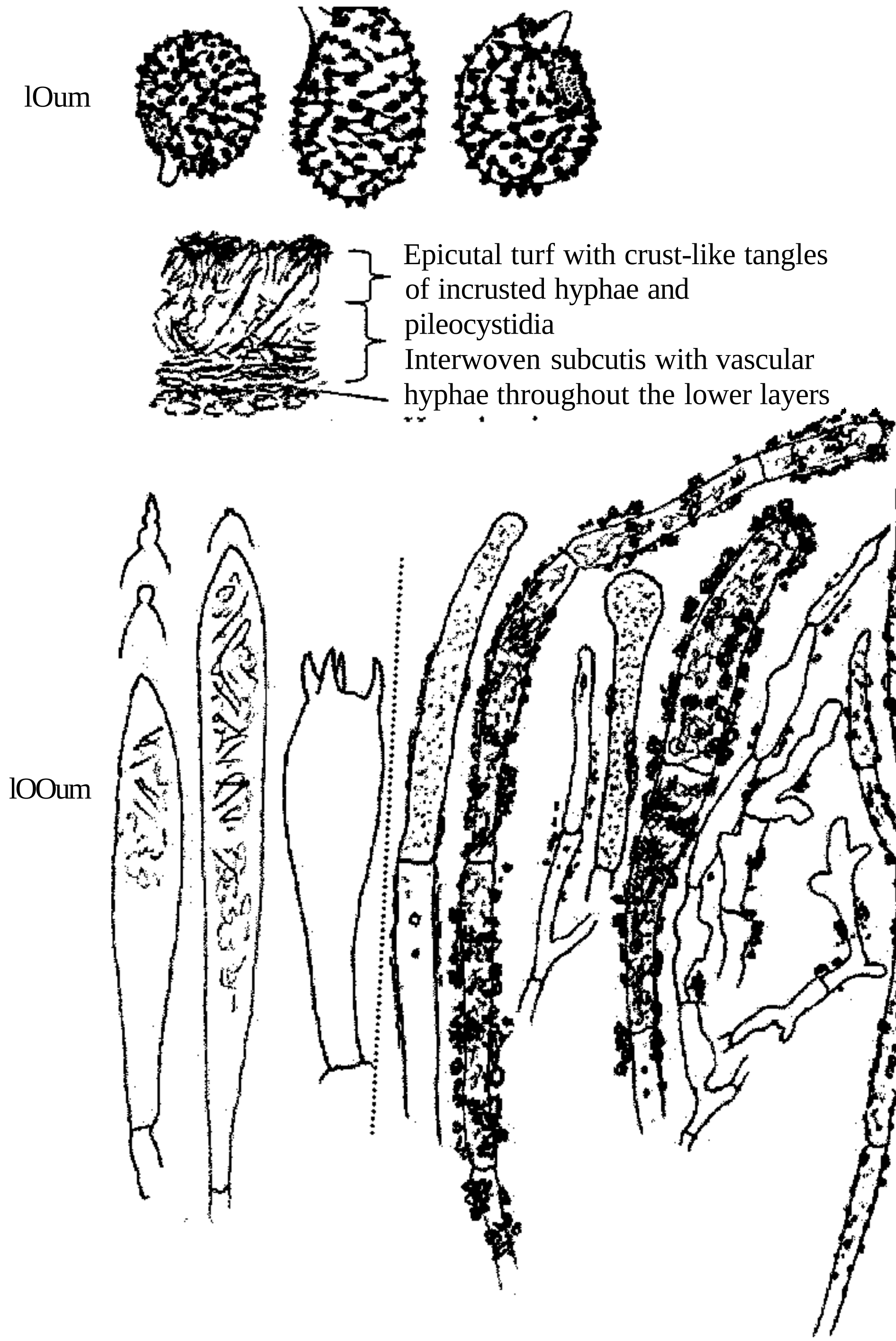

Figure 155 Microscopic characters of *Russula lepicHformis:* Top, spores with lOum scale bar; middle, diagram of section through cutis; bottom left, cheilocystidia, pleurocystidia and basidium; bottom right, pileocystidia, primordial hyphae and hyphal ends from the epicutis, lower scale bar is lOOum.

Figure 156 Spores *ofRussula lepidiformis* in surface view (left) and equatorial view (right), scale is in 1 urn divisions.

Figure 157 Cutis *oiRussula lepidiformis:* Top left, section through cutis mounted in water, scale bar is lOOum; top and middle right, two views of pileocystidia in SV; bottom, surface view of cutis mounted in water of a younger basidiomata showing the yellow-brown pileocystidia and the paler and slightly broader primordial hyphae at top and bottom of picture, the incrusting material is less obvious in this mount, 10 scale divisions are 25 um for all three pileocystidia photographs.

Clade 8a

Section *Amethystinae* Romagn. emend Sarnari

Subsection *Olivaceinae* Singer

Russula olivacea (SchaefF.) Fries
Epicrisis Systematis Mycologici: 356. 1838

Cap (4.5 -immature) 8.4-11.4cm diameter, convex or pulvinate when young, expanding to irregularly plane with a central shallow depression or in one collection illustrated below, convex with a flattish centre and rim, the shape of an inverted soup bowl, the cap margins smooth and downcurved over the gill ends but not actually inrolled. The colours are mostly drab, with olive green and purple hues that mix to form a dull brown, dry, not or barely viscid when wet, pruinose when young, later finely felted like kid leather, the surface breaking up into concentric areoli especially towards the margin. Cutis peelable up to 1/2 when young, later barely at all, flesh beneath with a dull green or greyish purple tint, otherwise creamy white, unchanging.

Gills cream at first, becoming deep egg-yolk yellow at maturity, unchanging where damaged, close, little forking, subgills common and often reaching almost to the stipe, relatively thin, slightly decurrent to adnate at the stipe, subacute at cap margin, of even depth at first, becoming ventricose, from around 1/3 to equal the depth of the cap trama at half-radius, the gill edges tinted with the cap colour along the outer 2-5mm.

Stipe 3.2-6.9 x 1.8-2.4cm, slightly more clavate than cylindrical, firm, generally with a partial to complete flush of pink, otherwise creamy white, pruinose, fairly smooth to slightly rugulose, browning at the base, solid to stuffed with a firm trama that does not form cavities, although it may be hollowed out by insects in age, unchanging to faintly greyish-brown where damaged.

Texture hard, robust, not brittle except in the gills.

Taste mild, nutty, slightly sweet.

Odour not distinctive to fruity or like meat.

Spore colour deep ochre-yellow, Romagnesi IVc.

Spores 8.6-13.6 x 7-11.8um, L.W 1.04-1.39 with a mean of 1.19 (n=30), varying considerably in size within one spore print or piece of gill, subglobose to ellipsoidal to obovoid, large spores may be pyriform, globose or narrowly obovoid instead of the more normal shape. **Ornamentation** of warts 0.5-1.4um high, on most spores below lum, conical, pointed, or often shortly crestate or arc-shaped, mostly isolated, sometimes in rows, sometimes linked to close neighbours with low, fine lines, not forming a reticulum, often incompletely amyloid. **Spores** may have mostly well-spaced heavy warts, or commonly a mixture of heavy warts with fine warts scattered among them. **Woo types** 2A-3A-2B-3B. **Suprahilar patch** a lightly amyloid irregularly shaped area with warts within and at its borders. Hiliferous appendix around 1.9-4.8um long, 1.5-3.0um wide near the base. **Basidia** 4- and 2-spored, 45-77 x 11-13 um, the older and spent basidia with long narrow bases. Sterigmata 7-12um long and 1.6-3 um wide at the base, those of 2-spored basidia generally larger than those of 4-spored ones. **Pleurocystidia** frequent, 90-150 x 8-12um, protruding up to 55um, less so toward the gill margin, originating in the subhymenium or outer trama, cylindrical to narrowly fusoid, contents yellowish and refractive in KOH, often apparently empty except for the tip, tips acute, more often mucronate to tapering with a long filamentous strangulate appendage, red to a slightly more brownish red than the basidia in SV. **Cheilocystidia** none near the cap margin, instead the gill edge has a gelatinous substance and contorted, stunted basidioles, over the remainder of the gill margins are numerous cheilocystidia, the tips protruding only up to 30um, narrow and contorted, sometimes capitate and sometimes tapering 37-57 (-75) x 5-7um, very occasionally septate, and rarely branched, often lacking refractive contents partially or entirely. **Subhymenium** 40- 70um thick, sometimes merging gradually into the gill trama, pseudoparenchymatous, expanding outward by one to two cells with successive developing basidia. **Gill trama** of sphaerocytes and occasional vascular hyphae.

Cutis 90-150 um thick, a weakly gelatinised trichodermis. **Subcutis** a greyish pink zone up to half the cutis depth, consisting of the tangled bases of the epicutal hyphae and hyphae 3-6 um wide, continuous with those of the trama, with no distinct demarcation line. However, in freshly prepared sections in water the air trapped in the more porous trama distinguishes it from the denser subcutis. The subcutis is thinner and even less differentiated from the trama toward the margins. Vascular hyphae not seen. **Epicutis** of erect, hyaline to pinkish hyphae 5-7um wide, most with obtuse undifferentiated tips, others with small capitate or tapered tips, and fairly frequent inflated cells up to lOum wide, one to three of which often form a fiisoid base to a narrower tip making an ampulliform structure, sometimes these cells are articulated or shaped like tarsals. On a few of the inflated sections the cell walls appear minutely roughened. Some of the terminal cells have refractive contents or globules, which become slightly browner than the others in S V, but lack any typical greying reaction. Some hyphae lying along the surface have greenish brown contents. **Primordial hyphae** not seen, no one structure stains differentially in acid fuchsin, instead, the whole epidermis, especially the deeper tissues, retains a slight pink hue. **Pileocystidia** - none that are unambiguously obvious. Some of the hyphae have slightly refractive contents and these sometimes terminate in cystidia-shaped cells, but lack any reaction with SV. **Hypodermis** none.

Trama of discrete clusters of sphaerocytes bound by a hyphal mesh, vascular hyphae not seen.

Chemical reactions: $FeS0_4$ - brown; KOH - reddish on cap surface, no reaction on stipe; NH4OH - no reaction; phenol - bright blackcurrant-juice purple; S V - bright red on the gills, brown on the cuticle (hyphae pink under the microscope), hymenial cystidia weakly greying or browning.

Habitat and tree associations: In old-growth stands of Douglas fir, western hemlock and western red cedar. The species is uncommon locally.

Collections: CR001010-01, beside a trail under old-growth Douglas fir, western hemlock and western red cedar, Cathedral Grove, near Cameron lake, N49.291161°, W124.662468° (eastern very dry maritime CWH subzone). CR021019-01, on ground in a mixed-age stand with old-growth western hemlock, Douglas fir and western red cedar, Royal Roads University woodlands, N48.433667°, W123.477333° (CDF zone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR021019-01	1141	229, 180	451,379,269	337, 195

Notes: The similarities between *Russula olivacea* and others in clade 8 are the generally firm texture, solid stipe, and the epicutal hyphae which are upright, crowded, with the majority of an even width, around 4-5 um, bringing to mind the tentacles of certain tropical sea anemones associated with clown-fish or of a twist-pile carpet.

Russula olivacea is superficially similar to several large, mild, yellow-spored Russulas with mixtures of purple and olive in the cap. *Russula xerampelina* and other species in that group have a fishy odour, stain blue-green with FeSC«4, brownish purple with 2% phenol, bruise yellow then brown, and have microscopic differences in the cutis and spores. *Russula Integra* has a similar robust texture and colours to *R. olivacea* but differs in its reaction to phenol, the texture of the cap cutis and microscopically to acid fuchsin and S V on the cutis. *Russula occidentalis* has a more viscid cutis, a pink to grey to black bruising reaction, and incrusted hyphae in the cutis. Two other members of the *Olivaceinae: Russula alutacea* and *R. vinosobrunnea,* occur on the west coast, (Thiers 1997, Woo 1988), both of which have the blackcurrant colour reaction with phenol, the former has a reddish-purple cap and reticulate spores and the latter, not recorded north of California, is a smaller species generally with slightly smaller spores (up to lOum long) with lower, more catenate ornamentation. It is not yet known if these species also carry the approximately 250 bp insertion in the ITS region.

The occasionally septate cheilocystidia on these Vancouver Island collections are unusual, they have also been found on the gill margins of *R. smithii,* a green and brown, white-spored species in the subgenus *Amoenula.*

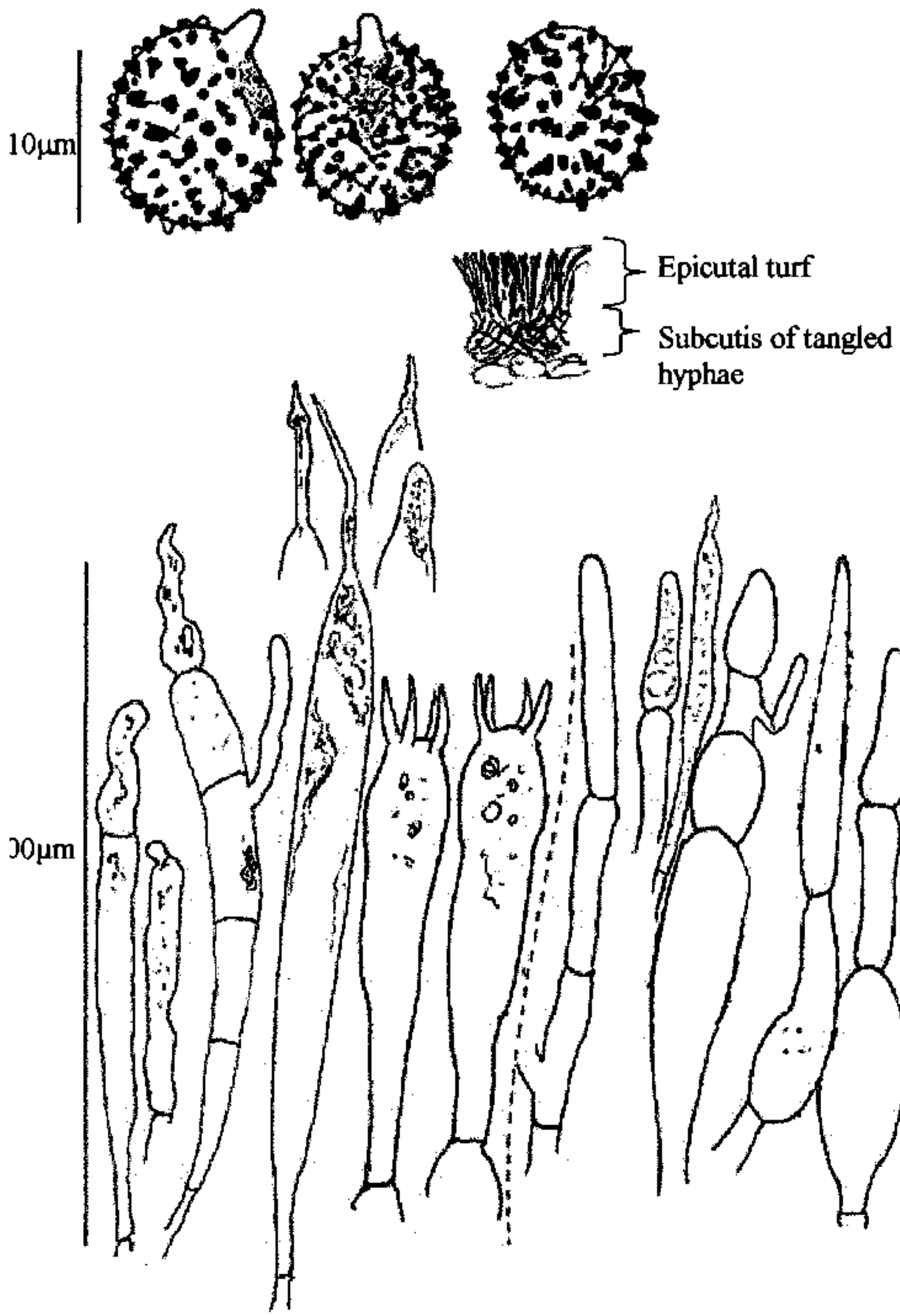

Figure 158 Microscopic characters of *Russula olivacea:* Top, spores with 10 um scale bar; middle, diagram of section through cutis; bottom left, three cheilocystidia, a pleurocystidia and various tip shapes and two basidia; bottom right, hyphal ends from the epicutis, the most numerous type is the left-hand one adjacent to the dotted line, very occasionally elements with minutely roughened hyphal walls as is shown in two of the inflated hyphae, lower scale bar is 100 um.

Figure 159 Macroscopic characters of *Russula olivacea:* Top, two basidiomata measuring 11.4cm and 8.4cm diameter, the larger is mature; middle left, illustration of immature and young-mature basidiomata in profile and in longitudinal section, square shows spore colour and is 1cm^2; bottom right, the underside of the larger basidioma.

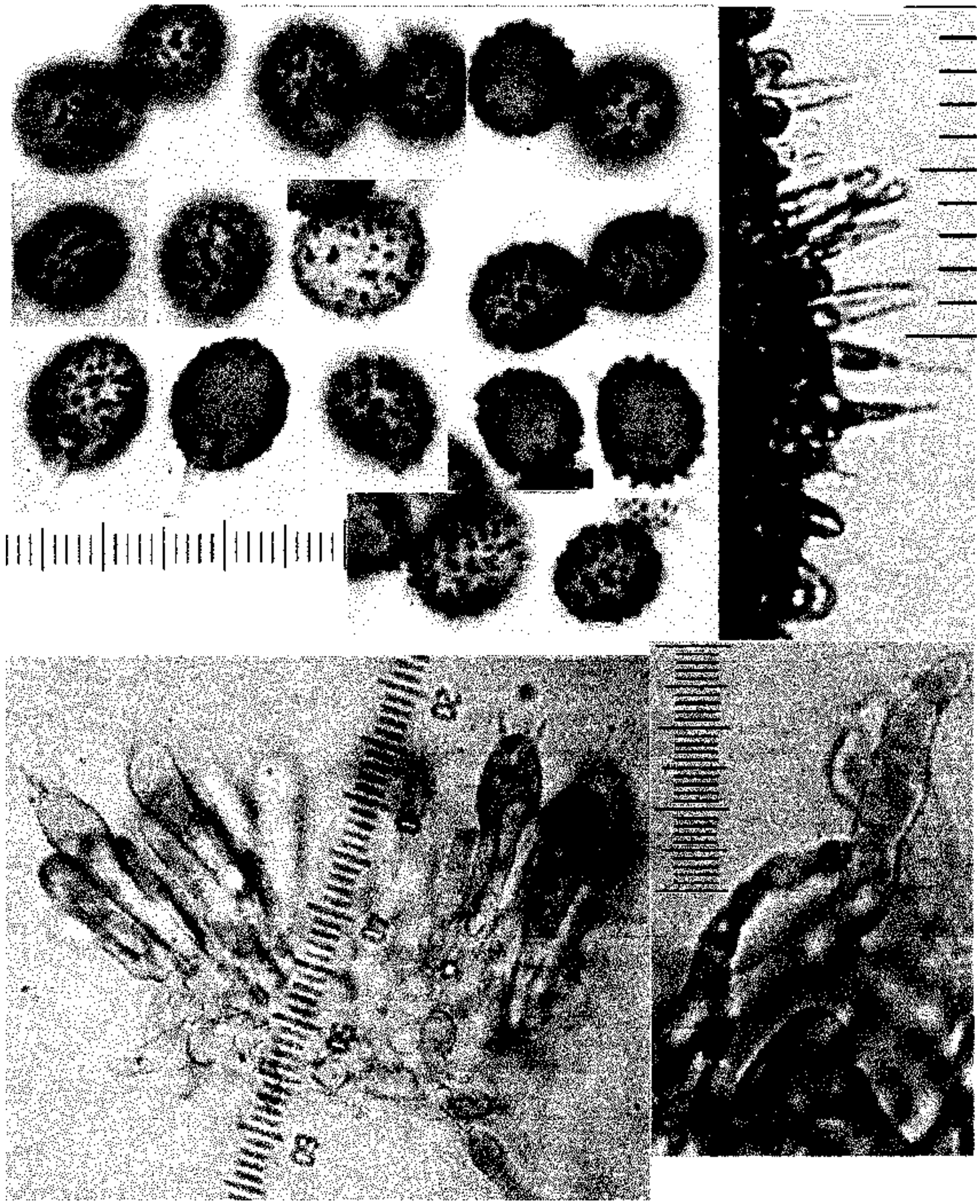

Figure 160 Hymenium of *Russula olivacea:* Top left, spores with lum division scale; top right, pleurocystidia with a variety of tip shapes protruding from hymenium, scale is in lOum divisions; bottom right, septate cheilocystidia with lum division scale; bottom left, basidia and basidioles with subhymenium, 10 scale divisions are 25um.

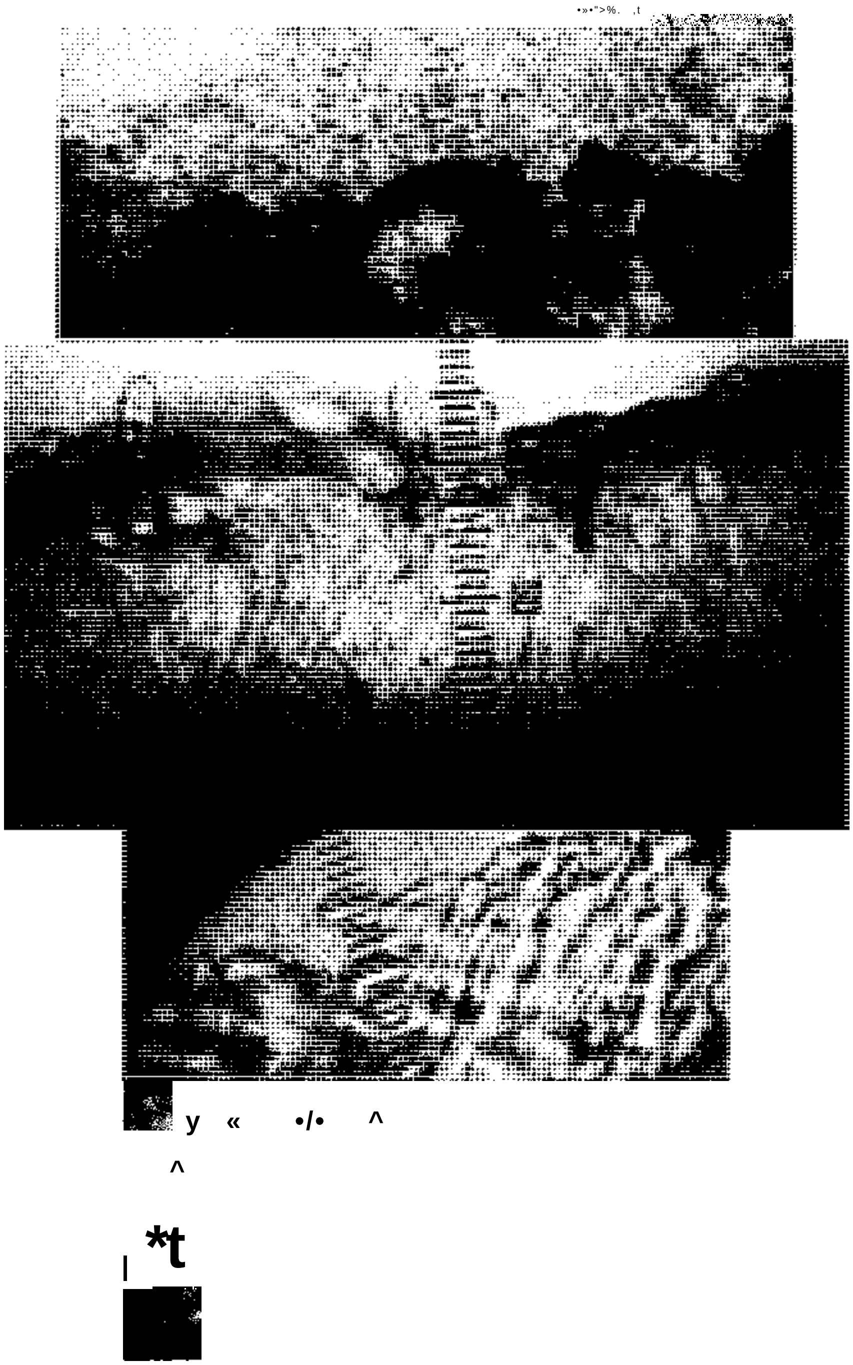

Figure 161 Cutis of *Russula olivacea:* Top, section through cutis with tramai tissues below, in which the trapped air appears as dark patches, 10 scale divisions are lOOum; middle and bottom, two views of the epicutal turf in water mounts, from near the cap centre and near the margin respectively, 10 scale divisions are 25um.

Clade 8c

Subsection *Urentes* Maire

Series *Veternosa* **Sarnari**

Russula veternosa Fries

Epicrisis Systematis Mycologici: 357. 1838

Cap 3.9-8cm diameter, convex with a small central depression at first, expanding to shallowly and broadly centrally depressed or almost infundibuliform, margin smooth, striate in age, long remaining downcurved over the end of the gills (not inrolled), colour light to mid terracotta colour (brownish-pink), usually with a cream to light brownish-yellow centre, this sometimes with olive tints, occasionally this colour over all but for the slightly pinker margin, colours generally paler when young, moist to slightly viscid, viscid when wet, drying matte, cutis peelable 1/4 to 1/3, cap trama firm, creamy white, pink under the cutis, unchanging where damaged or at most becoming very pale grey-brown.

Gills deep yellowish cream, becoming light orange-yellow at maturity, unchanging where bruised, close to crowded, with occasional lamellulae and forking near stipe, adnexed to ascendant to almost free at stipe, subacute to obtuse at cap margin, of equal depth along most of their length but narrowing towards the stipe, 5-7 mm deep at half-radius, approximately twice the depth of the trama, texture pliable to slightly brittle.

Stipe 2.2-8cm, x l-2.7cm, length in general roughly equal or slightly longer than cap diameter, more or less cylindrical to slightly clavate, sometimes widening at the apex, surface white, often with a pinkish flush near the base, longitudinally rugulose, solid, stuffed with a bread-textured trama that does not usually develop cavities, unchanging when cut or bruising weakly light grey-brown.

Texture firm, not brittle, but becoming softer in age.

Taste peppery to acrid, sometimes with a slightly bitter component at first.

Odour slightly fruity and faintly of ginger biscuits.

Spore colour ochraceous yellow, Romagnesi IVb.

Spores 6.5-8.8 x 5.7-7.5um, L:W 1-1.28, mean (n = 60) 1.14, broadly ellipsoidal, ellipsoidal to obovoid. **Ornamentation** of conical to peg-like or occasionally crestate warts 0.4-0.7 urn, rarely to 1 .Oum, 2-3-catenate or connected by fine to heavy lines often forming horizontal or vertical bands, with occasional isolated warts, sometimes forming a broken reticulum, Woo type B2-C2. **Suprahilar patch** amyloid, a more or less round patch bordered by warts and lines. **Hiliferous appendix** 1.7-1.9um long, 1.1-1.3um wide near the base. **Basidia** mostly 4-spored, some 2-spored, 34-52 x 10-12um, clavate. Sterigmata up to 8 urn long, 1-1.5 jam wide near the base. **Pleurocystidia** frequent, regularly distributed, 35-80 x 7.5-10um, rarely to 12um wide, originating in the subhymenium, embedded or only protruding up to 20um, fusoid to clavate, tips obtuse, subacute, with a small button or short appendix, or occasionally inflated, contents refractive, yellowish in KOH, dark grey to purple in SV. **Cheilocystidia** numerous, protruding up to 30um in some cases, or sometimes adhering to the gill margin, contents and shape similar to pleurocystidia but usually more slender, 6-8um wide. **Subhymenium** 20-40um, a dense and interlocked pseudoparenchymatous layer. **Gill trama** of sphaerocytes with occasional vascular hyphae.

Cutis 120-200um thick at half-radius, about 2/3 the depth consisting of a pinkish subcutis, and an epicutis with many pileocystidia embedded in a conspicuous glutinous layer. **Subcutis** of compactly interwoven parallel or tangled narrow hyphae mostly under 2um wide, with rare to occasional vascular hyphae that may ascend and terminate within the epicutis, embedded in a gelatinous matrix. **Epicutis** a trichodermis of erect free hyphal ends mostly under 2um wide, undifferentiated, with a small capitum, or quite commonly ending in a small cystidium, embedded in a viscid matrix. **Pileocystidia** abundant, 40-100 x 4-8um, occasionally up to 180um in length, cylindro-clavate to clavate, tips obtuse, with a small capitatum or a narrowed but not constricted tip, generally 3-4 septate with septa spaced about 10-3 5 urn apart, somewhat articulated, rarely aseptate and these usually short and small, contents greyish to purple in SV. Rare pileocystidia appear to have an exudate or irregular mucilaginous sheath which may stain grey in SV but not or only fleetingly in acid fucshin. **Pseudocystidia** none or rare. **Hypodermis** a layer of compacted tramal tissue up to twice the cutis depth.

Trama of discrete clusters of sphaerocytes within a denser hyphal network, with occasional vascular hyphae, purple in SV.

Chemical reactions: FeS04- light greyish pink; KOH - orange to yellowish on cap cutis, faintly yellow on stipe; NaOFL;. no reaction on cap or stipe; phenol - purple-brown; SV - greyish-purple on gills and cutis, deep purple to black on vascular hyphae and cystidia.

Habitat and tree associations: gregarious under Douglas fir with or without western hemlock or western red cedar and understory huckleberry, commonly in regeneration stands and in well drained mineral soil rather than duff.

Collections: CRO10902-03 in a mixed-age stand with veteran trees of Douglas fir with western red cedar, huckleberry and sword fern, near the ridge-top in well-drained soil, near the High Ridge trail, Francis King Park, Victoria, N 48.4833°, W 123.4502°. CR001108-03 in regeneration Douglas fir with western red cedar on south-facing slope, Royal Roads N 48.436°, W 123.474°. NP981107-01 with young Douglas fir, western hemlock and western red cedar, on private land, Hutchenson road, Mill Bay. (All above collections from CDF zone). CR 010909-01, in young regeneration Douglas fir with western red cedar, Kemp Lake trail N 48.375283°, W 123.78037° (western very dry maritime CWH subzone).

	ITSl-Fto	RFLP:		
Collection	ITS4-B	Hinfl	Alul	Sau3A
CR001108-03	865	432, 359	526, 271	380, 220, 171
CRO 10909-01	860	423, 364	500, 291	323, 220, 155
CR001029-Breit (Oregon collection)		430,334	385,298	340,251

Notes: These collections *01 Russula veternosa* could equally have been identified as the closely related *Russula maculata,* as the spore ornamentation is closer to that of the latter species than the former. *Russula veternosa* should have spores with isolated pointed warts up to lum rather than with connections as in the Vancouver Island collections. The spore size, however, is consistent with *R. veternosa* sensu Romagnesi (1967), as are the multiseptate pileocystidia, the smaller hymenial cystidia, the faint ginger smell, the more muted cap colours and the slightly grey-brown rather than yellowish bruising reaction. *Russula maculata* tends to have a brighter red and yellowish mottled and spotted cap, spore sizes 8.2-10 x 7-8.7um, hymenial cystidia from 80 - 115um long, and many aseptate pileocystidia, otherwise 1-2-septate. The identification of these Vancouver Island collections as *Russula veternosa* called for a judgment as to whether the cuticular and macroscopic characters bore more taxonomic weight than the spore ornamentation: others may disagree, and it may be that this is a species distinct from either of those discussed here.

Figure 162 Microscopic characters *ofRussula veternosa:* Top left, spores with lOum scale bar; top right, diagram of pileocystidia and micro-cystidia; middle, diagram of section through cutis; bottom left, hymenial cystidia; bottom right, pileocystidia, hyphal ends and micro-cystidia from the epicutis, lower scale bar is lOOum.

•Mfet

Figure 163 Macroscopic characters ofRussula vetemosa: Top. Illustration of immature and mature basidiomata with longitudinal section, the square shows spore print colour and is lcm^2; bottom, collection of *R vetemosa* in habitat showing the typical terracotta and dull cream-yellow cap colours.

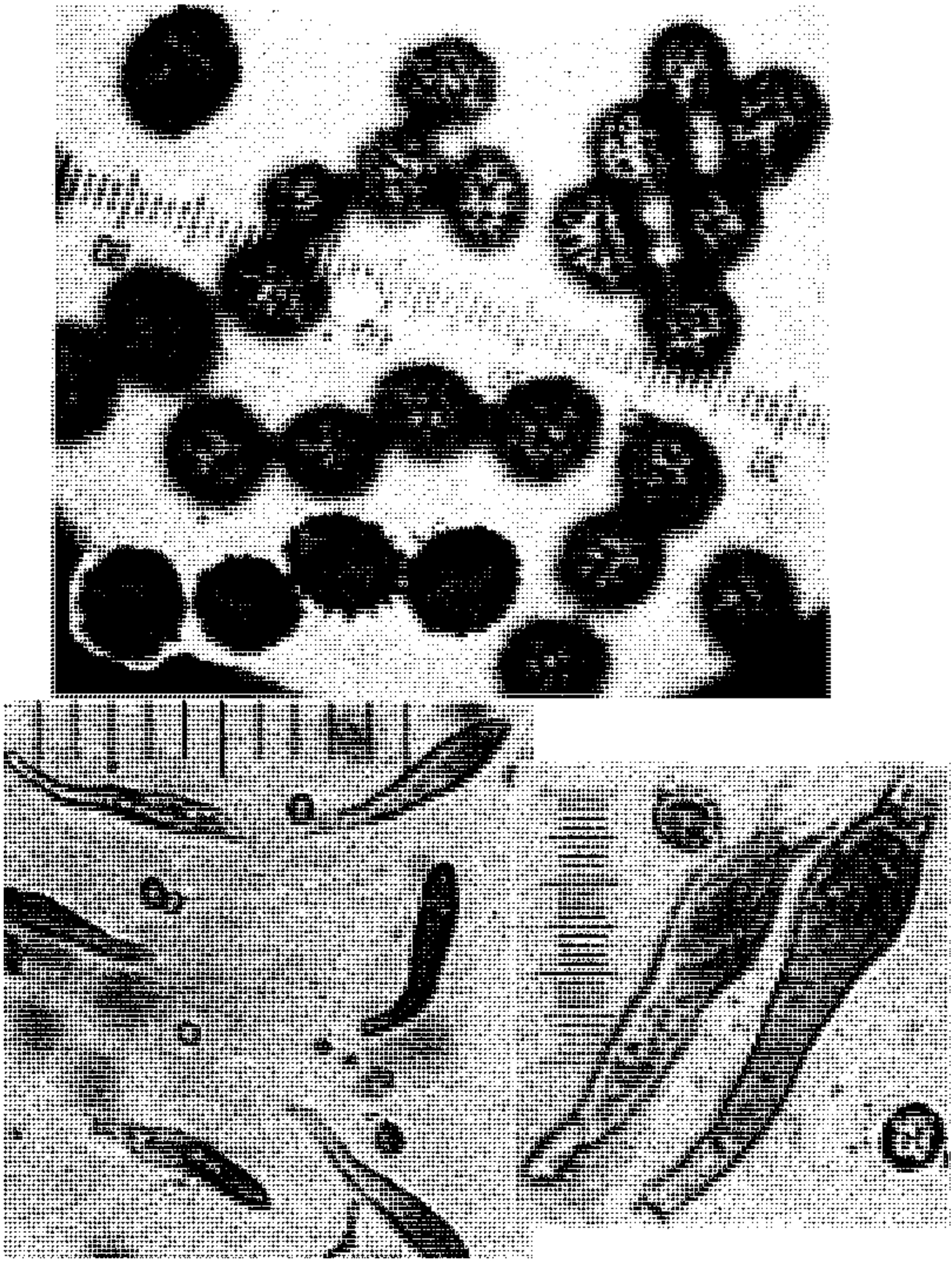

Figure 164 Hymenium of *Russula vetemosa*. Top, surface view of spores with inset equatorial view of four of the spores below the scale, scale is in lum divisions; bottom left, hymenial cysitidia in a crush mount, scale is in lOum divisions; bottom right, two basidia, the left a four-spored and the right a two-spored one, scale is in lum divisions.

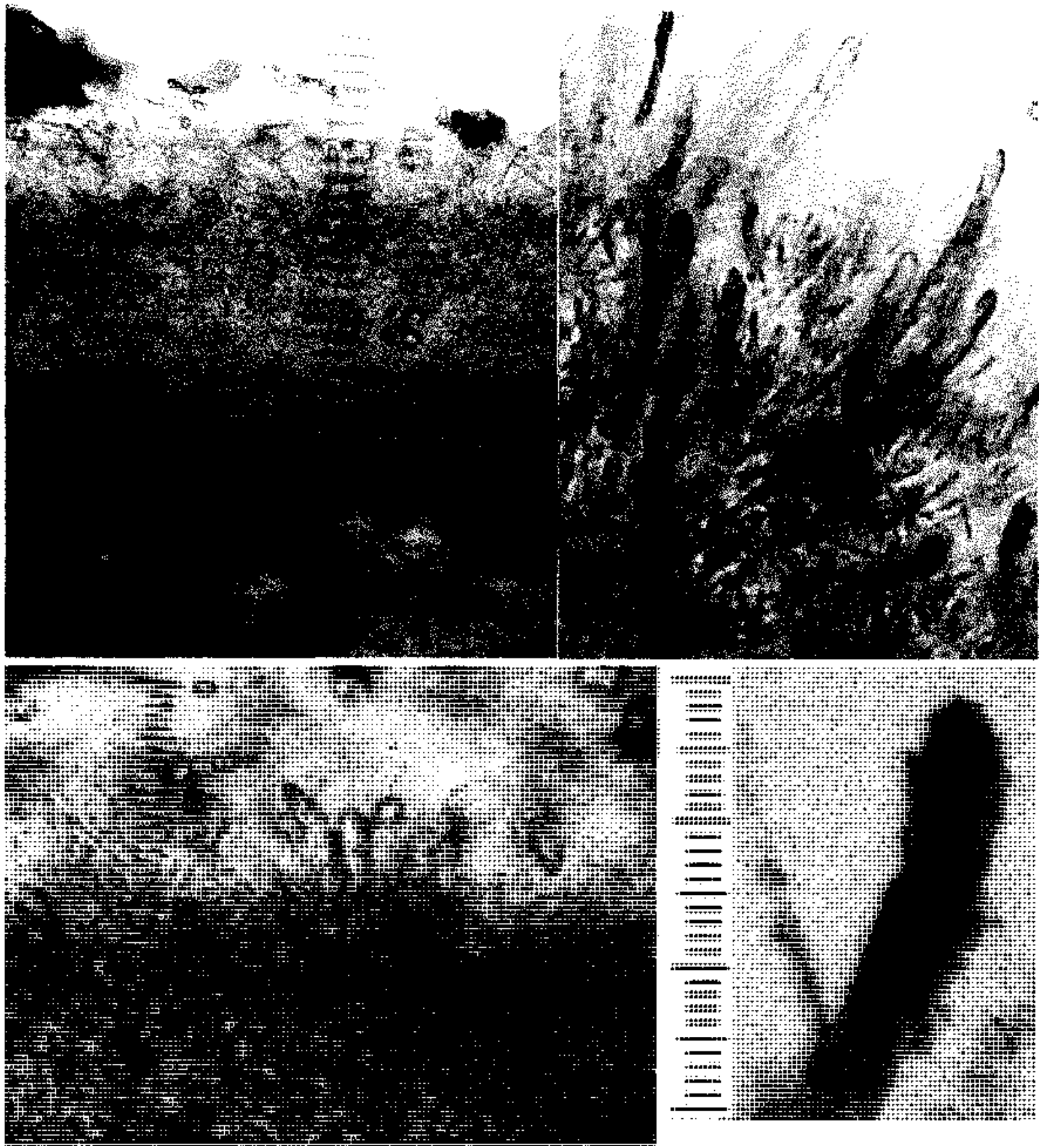

Figure 165 Cutis *ofRussula vetemosa.* Top left, section through cutis, the epicutis is between the 40 and 43 mark, the subcutis to about the 53 mark and the hypodermis the 50-80um compact layer below this, scale is in lOum divisions; top right, oblique view of epicutis in SV showing pileocystidia amidst erect hyphal ends some of which are also darkened by SV, scale is approximately the same as in the bottom photograph; bottom left, section of cutis in NH4OH showing pileocystidia, 10 scale divisions are 25 um; bottom right, rare pileocystidia end with exudate stained with SV, scale is in lum divisions.

Clade 8d

Section *Paraincrustatae* Sarnari

Subsection *Integrate* (Maire) Sarnari

Russula velenovskyi **Melzer & Zvara**

Archiv pro Pfirodovedecky Vyzkum Cech 17: 92. 1927

Cap 10cm diameter, this mature collection of one basidioma is more or less shallowly infundibuliform, a bright brownish red, (light terracotta), darker at the margin, dull yellow to dark cream centrally, viscid when wet, drying matte, marginal 5mm striate to tuberculate, peelable to 1/2 the radius, flesh beneath firm and white.

Gills deep cream, brittle, adnate to adnexed, broadening outwards, about 5mm deep - shallower than cap flesh at half-radius, obtuse at cap margin, close, not noticeably forked, with the cutis colour extending 2-3mm along the outer gill edges.

Stipe 9.4 x 2.8cm, flared at the apex and to a lesser extent at the base, stuffed with a firm bread-textured trama, not cavitate, surface white with rose-pink flush on the lower half, unchanging where damaged.

Texture firm, not brittle.

Taste mild with a faint peppery aftertaste.

Odour mild, not distinctive.

Spore colour deep yellowish cream, Romagnesi Ilia.

Spores 6.7-8.8 x 5.7-7.6um, L:W 1.07 -1.25 with a mean of 1.16, (n=32), subglobose to broadly ellipsoidal. **Ornamentation** of peg-shaped to crestate warts up to 0.7um high but most often up to 0.5pm high, sometimes incompletely amyloid. Some spores have ornamentation of mostly isolated warts with just a few fine connectives linking 2-3 warts or 2-3 catenate, other spores have zebroid ornamentation in which most of the warts are linked by crests, forming a broken reticulum, the majority of spores are in between these extremes with some crests, some fine-line linkages and some isolated warts. **Woo types** B1-2, CI-2 to E1. **Suprahilar patch** an irregular amyloid area often bordered by warts, hiliferous appendix often relatively long and slender, 1.3-2. lum long, l.l-1.5um wide near the base. **Basidia** mostly 4 spored, 35-50 x 8-10. lum, clavate. Sterigmata up to 8um long and 1.5um wide at the base. **Pleurocystidia** somewhat sparse, on average 50-80um apart, 50-110 x 7-1 lum, protruding 10-35um, originating in the subhymenium, more or less fusoid, tips obtuse, acute, capitate or with a wavy to strangulated appendix, contents refractive in KOH, purple to black in SV. **Cheilocystidia** absent towards the cap margins, otherwise patchily distributed, similar to pleurocystidia but reacting less strongly with SV. **Subhymenium** 30-45um thick, pseudoparenchymatous. **GUI trama** of large sphaerocytes 30-60um wide, with rare vascular hyphae.

Cutis 100-170um thick, in cross sections in NH4OH appearing as a light brownish lower layer, overlain by a thick pale pink layer then a thin slightly greyish layer directly beneath the thin, pink epicutis. Gelatinous matrix not evident. **Subcutis** comprising the aforementioned brownish, hyaline and greyish layers, of tightly interwoven, mostly parallel hyphae, 1.5-4um wide, the greyish layer appears so in water and NH4OH mounts because of air trapped within interstices of a more openly woven layer, in KOH this is not seen. Vascular hyphae rare. **Epicutis** of more or less repent, compactly interwoven hyphae somewhat more tortuous and more interlocked than those in the subcutis, ends undifferentiated or sometimes with slightly refractive contents, accompanied by pileocystidia that lay along the surface and appear adhered to it. **Pileocystidia** frequent, originating in the upper subcutis or within the epicutis, 25-100 (-130) x 2.5-7um wide, the shorter ones aseptate, longer ones 2-3 septate, cylindrical to narrowly clavate, most often the terminal cell is clavate, ends obtuse or slightly capitate, contents refractive, banded, staining light grey in SV, pink in acid fucshin with acid-resistant exudates that appear as small surface granules in patches, often towards the base of the cystidia. **Pseudocystidia** not seen. **Hypodermis** a layer of more compact tramal tissue with flattened sphaerocytes.

Trama of discrete clusters of sphaerocytes bound by a hyphal mesh and occasional vascular hyphae.

Chemical reactions: FeSC>4 - salmon pink; KOH - bright red on cap surface, slightly yellowish on stipe; phenol- purple-brown; SV - brownish red on the gills, purplish on the cuticle, cystidia and vascular hyphae grey to black.

Habitat and tree associations: on limestone south-facing slope with madrone, Douglas fir and shore pine with salal understory.

Collections. CR981114-05, from the north valley slope along the Koksilah river on the forest floor in a mixed-age stand of Douglas fir, madrone and a few pines, N 48.6550°, W 123.7303° (eastern very dry maritime CWH subzone).

Notes: *Russula velenovskyi* is indistinguishable in macroscopic appearance from *Russula veternosa,* and has a similar spore size and ornamentation. It differs in its mild taste, paler spore print, no odour of gingerbread or honey, the habitat in Douglas fir-madrone-pine forest and the rather easy to overlook incrustations that stain pink in acid fucshin. It occurs in Northeastern North America (Kibby and Fatto 1990), but has not previously been recorded from the Pacific Northwest. The spores of this collection are slightly larger than is given in Romagnesi (1967) for European material, 6.5-8.5 x 5.5-6.5um) but close to those for Northeastern North America sensu Kibby and Fatto (1990) (6.5-9 x 5.5-7.5urn), they also have more connectives between the warts than is given in either of those descriptions. *Russula paludosa,* a similarly coloured mild species also recorded from California, has larger spores with larger warts and a cellular subcutis sensu Thiers (1997).

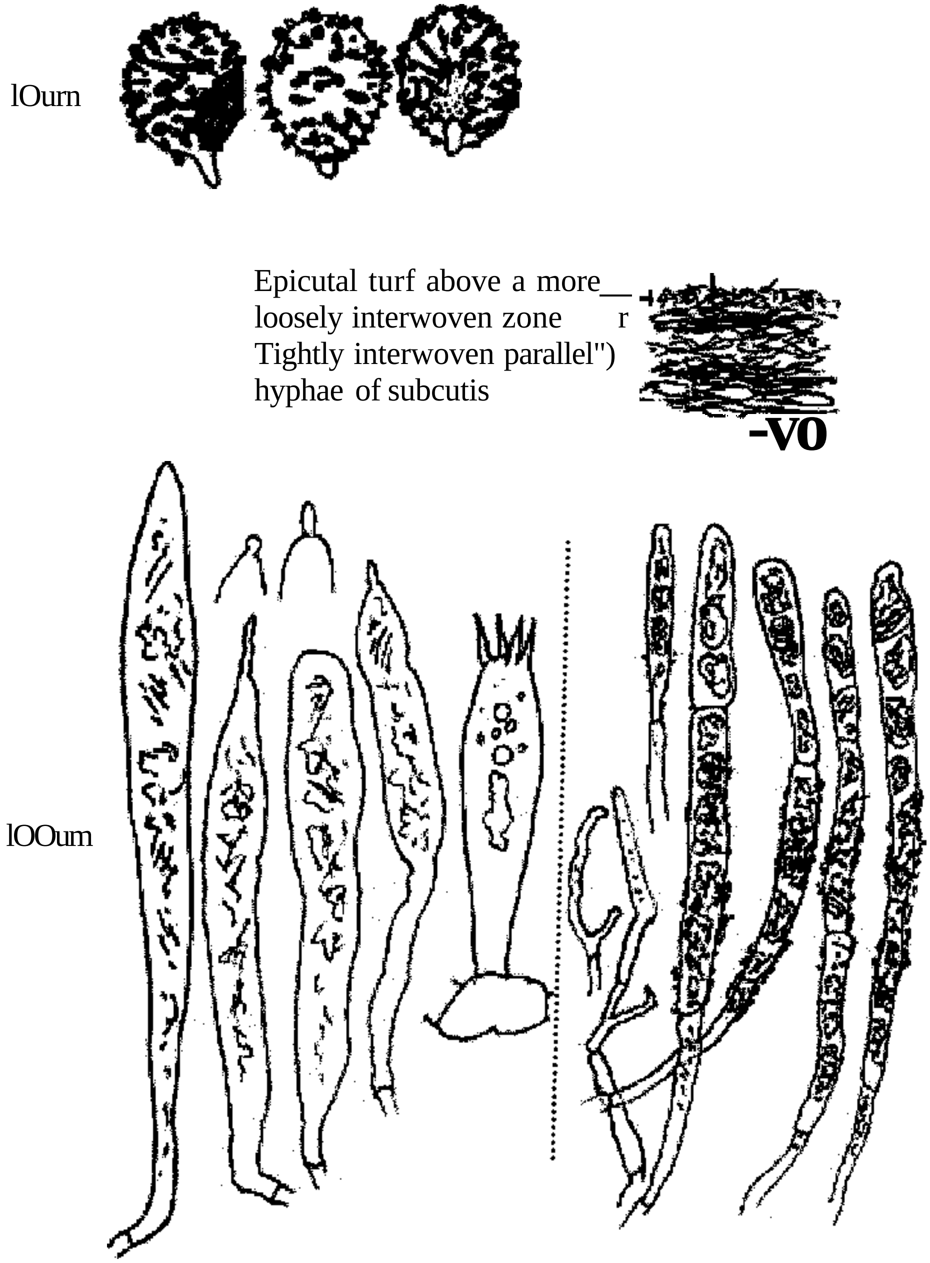

Figure 166 Microscopic characters of *Russula velenovskyi*: Top, spores with lOum scale bar; middle, diagram of section through cutis; bottom left, hymenial cystidia and basidium; bottom right, hyphal ends and pileocystidia from the epicutis, lower scale bar is lOOum.

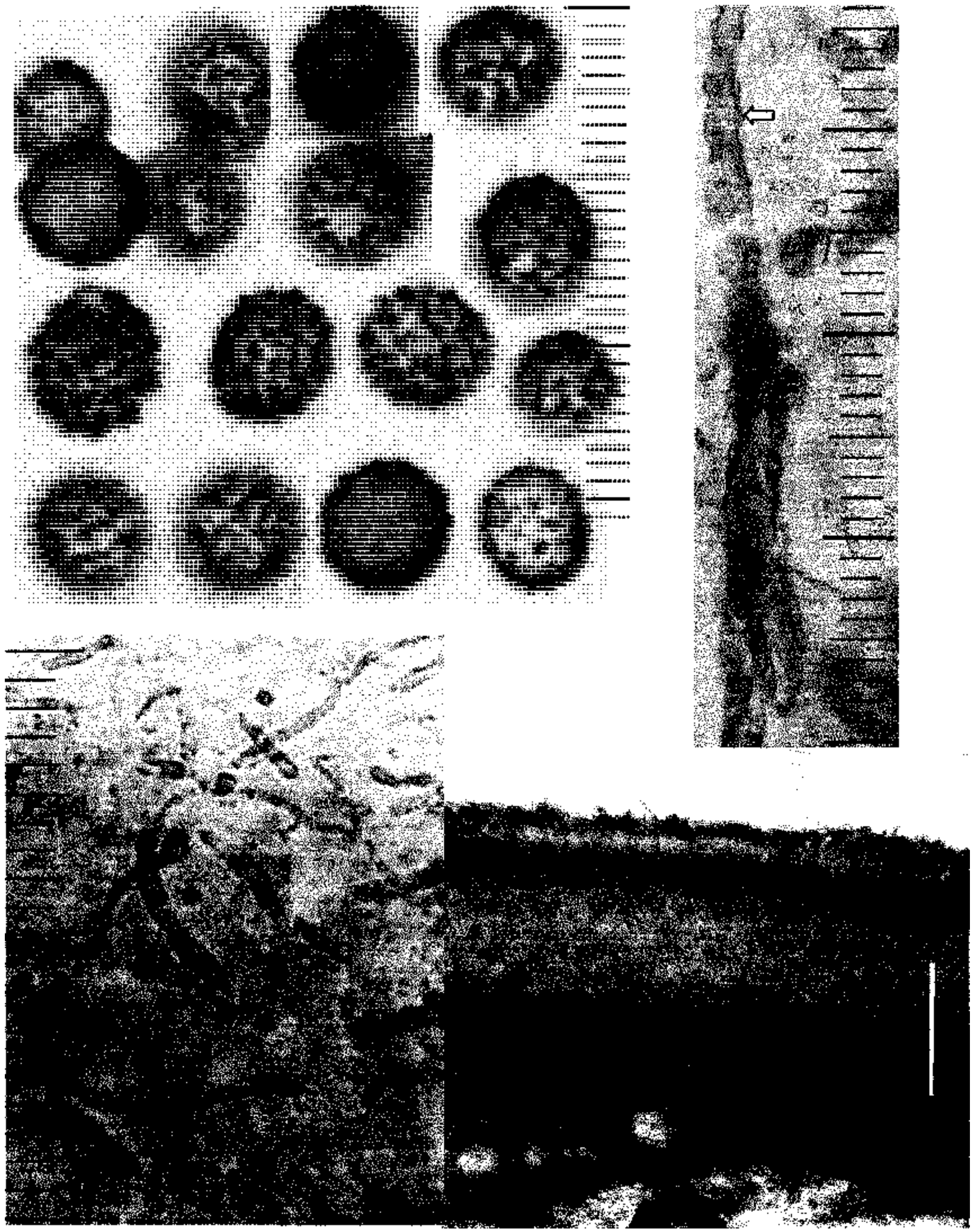

Figure 167 Hymenium and cutis of *Russula velenovshyi:* Top left, spores withlum division scale; top right, a pileocystidium stained with acid facshin, both the contents and the exudate are stained, arrow points to an irregular, broken sheath of exuded material, scale is in 2.5 um divisions; bottom left, surface view of pileocystidia stained in SV, 1 scale division is lOum; bottom right, section through cutis mounted in NH4OH, note the darker band below the epicutis which is due to trapped air bubbles, scale bar is lOOum.

***Russula* cf. *integra* Fries (1st variety)**

Epicrisis Systematis Mycologici: 360. 1838

Cap 5.1cm diameter, one mature basidioma only, shallowly centrally depressed with broadly downcurved smooth margins that extend over the rounded ends of the gills, deep green centrally and dull brown with subtle vinaceous tints at the margins, like bronze, with a darker band at the shoulder where the pigments overlap, viscid when wet, drying subglossy. Cutis peelable up to 2/3 the radius, flesh white, light grey-brown under the cutis.

Gills cream, close, occasional subgills, forking not seen, adnexed and slightly ascendant at the stipe, broadening a little towards the margins, rounded at the cap margin, about equal the depth of the cap trama at half-radius, thinner than in most Russulas, not much thicker than paper.

Stipe 3.7 x 1.4cm, more or less cylindrical to clavate, white, longitudinally markedly rugulose, stuffed with a bread-textured trama with no cavities at this stage, rind firm, 2.5-3mm thick, flesh unchanging where damaged.

Texture fairly firm.

Taste mild and nutty.

Odour not distinctive.

Spore colour deep orangish-cream, Romagnesi IIIc but with a pinker cast.

Spores 8.9-11.4 x 7.4-9.9um, L:W 1.05-1.45 with a mean of 1.15, (n=30), subglobose to broadly ellipsoidal. **Ornamentation** of large, conical, pointed, peg-like or short-crestate warts 0.5-1.5um high, mostly isolated or clustered in connected groups of 2-4, rather like the markings of a leopard, fairly densely distributed, connecting lines occasional, on many spores the wall between the warts is also lightly amyloid making the whole spore quite dark compared to other species. **Woo types** 3A-3B. **Suprahilar patch** an irregularly shaped amyloid patch, the borders with warts and darker in general. **Hiliferous appendix** 1.9-2.9um long, 1.3-1.9pm wide near the base. **Basidia** 4 and 2 - spored, 37-55 x 10 -13pm, clavate, broadest in the upper 1/3 but generally with a gradual taper downwards. Sterigmata 4- 7.3pm long and 1.4 -1 9pm wide at the base. **Pleurocystidia** 65-110 x 8-12p.m, the majority are 10pm wide, protruding up to 50pm, originating in the subhymenium or outer trama, fusoid to narrowly clavate, tips obtuse to subacute, occasionally with a small button, contents yellowish, refractive in KOH, grey in SV. Cheilocystidia abundant, taking up most of the gill margin, 9-13 urn protruding 20-40um beyond the gill margin, partially filled with yellowish refractive contents, mostly towards the tip, that barely if at all stain in SV. Tips mostly obtuse to subacute. **Subhymenium** 12-25 pm thick, mixed, of hyphae of variable diameter with short inflated irregularly shaped cells frequent. **Gill trama** of sphaerocytes with occasional vascular hyphae.

Cutis 160-pm thick at half-radius, an ixotrichoderm consisting of a thick subcutis and a poorly differentiated epicutis. **Subcutis** of tightly interwoven branched hyphae 1 5-3pm wide, that are repent in the lower layers, gradually becoming more upright towards the epicutis, which is a continuation of the process. In the lower layers are vascular hyphae with yellowish refractive contents, 3-8pm wide, not reacting with SV, which sometimes ascend through the subcutis to give rise to clavate pseudocystidia at the surface, and with contents that stain pink in acid fuchsin. **Epicutis** of more or less erect hyphal ends 2-5 pm wide, with undifferentiated obtuse tips or sometimes slightly capitate or clavate at the end. **Pileocystidia** 25-100 x 5-10pm, occasionally longer, numerous, lying along the surface, clavate or cylindrical with yellowish refractive contents, not reacting with SV but with contents staining pink in acid fuchsin, only rarely can one be found with a small amount of incrustation. These structures bear more resemblance to cystidia than to primordial hyphae as they arise from narrower hyphae lacking refractive contents and not staining in acid fuchsin, although they are usually the terminus of such hyphae. The clavate refractive part has 0-3 septa. **Primordial hyphae** 3-6pm wide, with weakly refractive contents, occasionally with a few specks of incrusting material, infrequently septate, behind the cystidioid terminus there may be a 100pm or more between septa.

Hypodermis a yellowish band 10-25 urn thick of interwoven parallel hyphae, interwoven with the trama.

Trama of discrete clusters of sphaerocytes bound by a dense hyphal mesh, with occasional vascular hyphae around 5um wide, not reacting with SV.

Chemical reactions: FeSC>4 - pale salmon-pink; KOH - brownish orange on cap surface, no reaction on stipe; phenol - brownish purple; SV - greyish on the gills, pale grey on the cutis, cystidia and vascular hyphae not reacting.

Habitat and tree associations: On the bark of a living old growth Sitka spruce, about 1.5m up from the ground, and with huckleberry also sprouting from crevices in the bark. October.

Collections: CR981013-01, from a Sitka spruce in the St. Joseph River campground at Cape Scott Park, N 50.761075° W 128.363231° (southern very wet hypermaritime CWH subzone).

Notes: The bronze and greenish colours together with the large spores with long pointed warts and the cuticular elements staining with acid fuchsin place this species in the *Russula integra* group, however, the spore print is slightly paler and the degree of incrustation is small, and it does not exactly match any of the several varieties *of Russula integra* in Bon (1988) and Romagnesi (1967). Several of these aforesaid varieties are found with spruce, but although this Vancouver Island collection was found on spruce, it would seem unlikely that the roots of the tree were available to the mycelium were it confined to the crevices in the bark, and more likely that its host is one of the *Vaccinium* species such as huckleberry or salal that grow in soil pockets in the bark.

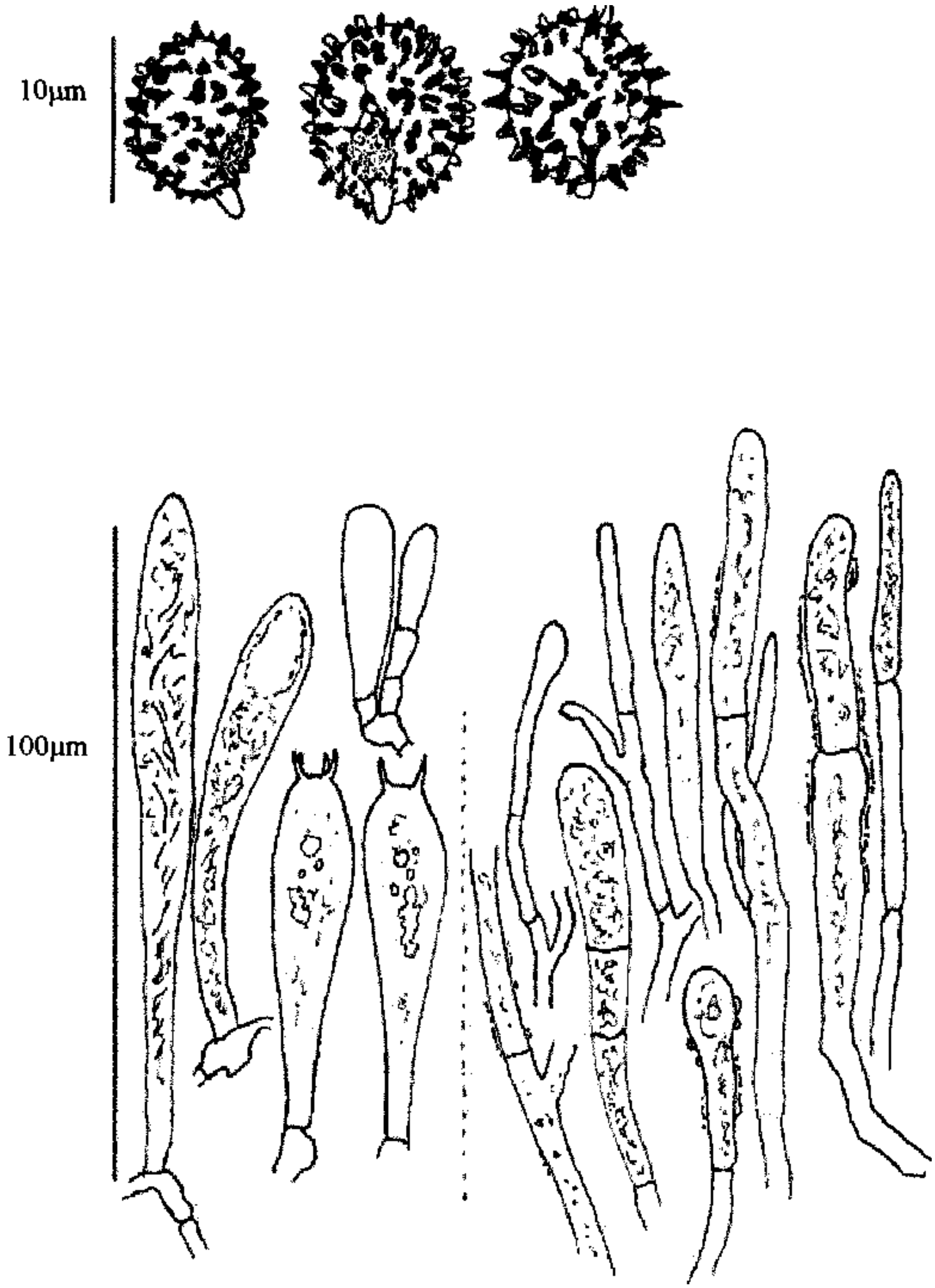

Figure 168 Microscopic characters of *Russula* cf. *integra* 1st var.: Top, spores with lOum scale bar; bottom left, hymenial cystidia, two basidia and basidioles; bottom right, hyphal ends, primordial hyphae and cystidioid terminal cells from the epicutis, lower scale bar is lOOum.

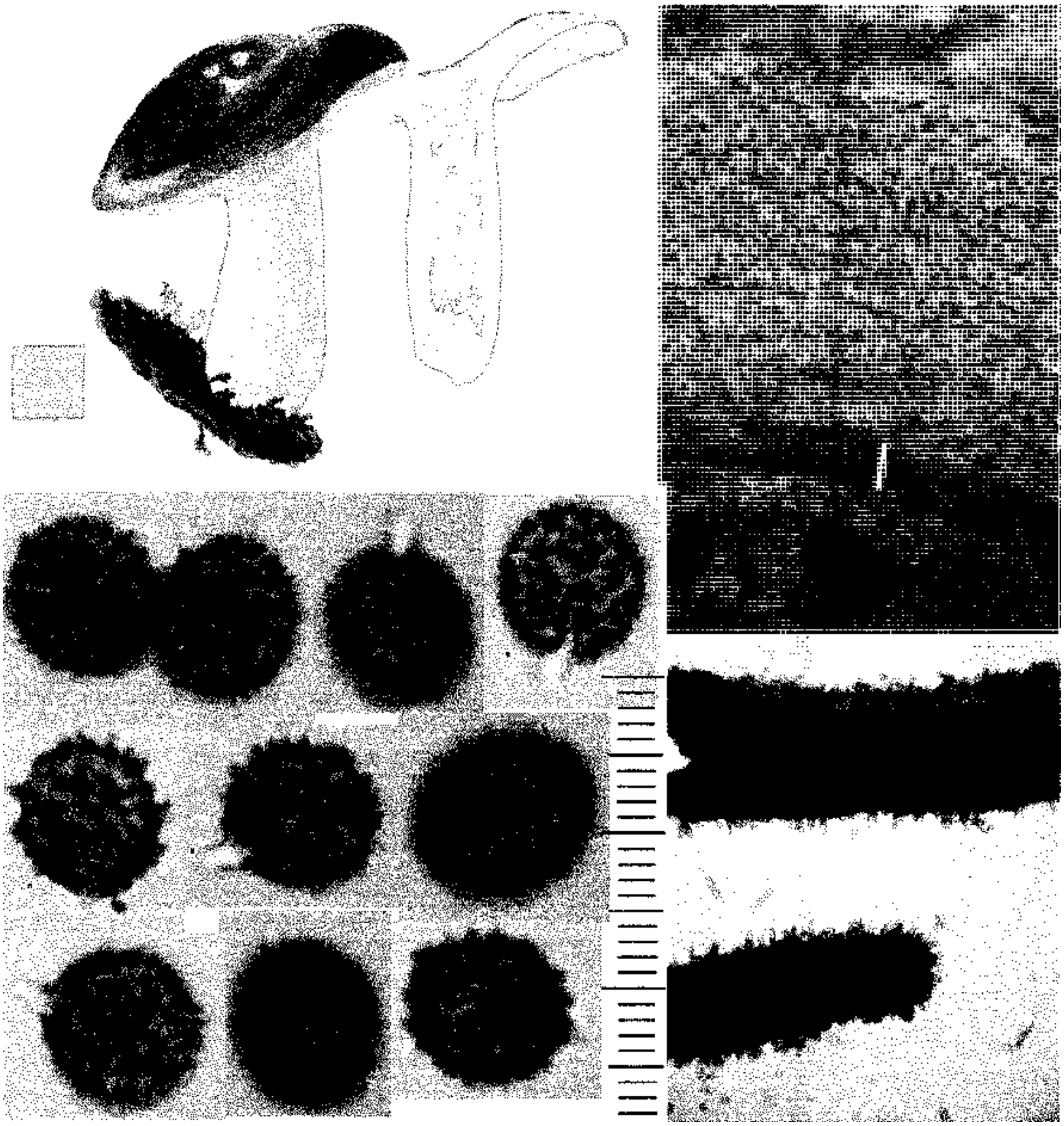

Figure 169 *Russula.* cf. *Integra* 1st var.: Top left, illustration of profile and longitudinal section as found on Sitka spruce bark, small square is $1cm^2$ and shows spore colour; bottom left, spores with 1 um division scale; top right, section through cutis in water, the hypodermis is indicated with the 25um long white bar, 10 scale divisions are 25um; bottom right, sections through gill showing the protruding pleurocystidia and cheilocystidia, scale is in lOum divisions.

***Russula* cf. *integra* Fries (2nd variety with reticulate spores)**

Epicrisis Systematis Mycologici: 360. 1838

Cap 4.9-12cm diameter, convex when young, becoming shallowly depressed at maturity with margins remaining curved down over the gill ends, sometimes becoming lobed, smooth, not or barely developing marginal striations in age. Colour overall brownish, sometimes greyish-brown when young, at maturity more yellow-brown centrally and more vinaceous-brown towards the margins, with or without blackish zones between, sometimes dull purple at the margin, sometimes slightly olivaceous tints marbled through, generally bronze-hues. The Saturna Island collection has more of the vinaceous to purple hues mixed with dull brown. Cutis viscid when wet, drying almost matte, peelable up to 1/2 the radius, flesh beneath light purple, cap trama pale cream, unchanging where damaged.

Gills cream when young but quite soon becoming deep yellow, subdistant, with occasional subgills but no forking, narrow and ascending to free at the stipe, broadening outwards, rounded at the cap margin, up to 1.5 times the depth of the cap trama at half-radius, thinner than in most Russulas, not much thicker than paper, not bruising.

Stipe 4.2-11 x 1.8-2.1cm, cylindrical or slightly ventricose, shorter or longer than the cap diameter (the longest stipe not belonging to the widest cap), longitudinally finely rugulose. Surface and trama very pale yellow, browning weakly at the base, elsewhere unchanging to slightly buff where damaged, solid to firmly stuffed, not cavitate.

Texture very firm and somewhat elastic, robust and not breaking easily, becoming more fragile in age.

Taste mild and nutty.

Odour mild, slightly of rubber.

Spore colour a deep strong yellow bordering on orange, Romagnesi IVc.

Spores 8-9.2 (-10.3) x 6.5-8pm, L:W 1.09-1.34 with a mean of, 1.20 (n=30), subglobose to broadly ellipsoidal, often slightly pyriform. **Ornamentation** of blunt or occasionally conical or crestate warts 0.3 -0.9pm high with most spores having warts in the mid size range. On some spores the warts are mostly isolated with just a few heavy lines connecting groups of 2-3 warts, other spores are partially zebroid with scattered isolated warts and a well developed partial reticulum, most have a combination of both elements with no strong reticulum. **Woo types** B2-C2, occasionally A2 or E2. **Suprahilar patch** an irregular amyloid patch, often rectangular and up to 4pm long, generally with warts at its borders. **Hiliferous appendix** relatively large, 1.6-2.6 x 1.3-1.6p.m. **Basidia** 4 -spored, occasionally 2 -spored, 35 -57 x 9-13pm, clavate to slightly bulbous in the upper 1/3. As the hymenium grows outwards by a cell or two for each successive basidia developed the older basidia have longer narrower bases than the newer ones. Sterigmata relatively slender, up to 9pm long and 1.2 -1.3 um wide at the base. **Pleurocystidia** 57-100 x 7.5 -11pm protruding up to 20pm, originating in the subhymenium, fiisoid, tips acute or usually with a terminal button, filamentous or allantoid appendage, contents yellowish, refractive in KOH, sometimes only in the upper half, unreacting to greyish to dark in SV, with all reaction types occurring on one small piece of gill. The pleurocystidia are patchily distributed, they appear infrequent because many are embedded in the hymenium and do not react with S V, and so are hard to see, some patches of gill seem to be devoid of them, in others they are spaced about 50-100pm apart. **Cheilocystidia** 48-90 x 7.5-9pm numerous, protruding 20-40pm, cylindrical to fusoid, tips subacute or more often with a filamentous appendage or small button, not or barely reacting with SV. **Subhymenium** 30-50um thick, pseudoparenchymatous. **Gill trama** of sphaerocytes uo to 28pm across, with occasional vascular hyphae.

Cutis 170-200pm thick, of two layers plus a hypodermis. **Subcutis** about 3/4 or more of the depth of the cutis, of tightly interwoven and tangled semi-upright hyphae 2.5-5pm wide, appearing pink in water mounts and containing occasional pinkish globules of pigment, occasional vascular hyphae in the lower layers, staining weakly grey in S V. **Epicutis** of upright, septate, branched hyphae, pileocystidia and probably primordial hyphae that lie along the surface. Epicutal hyphae in general very slightly wider than in the subcutis, 4-6u.ni with rare inflated cells up to 12um wide, the terminal cells may be short, sometimes contorted, ends obtuse or often with a longer tapering terminal cell. **Pileocystidia** numerous, cylindrical to narrowly clavate, 4-7 x 55-120um or sometimes longer, rarely aseptate, mostly 3-4 septate, the end cell generally the broadest, tips obtuse, contents refractive, weakly greying and often with a granular appearance in S V, rarely with small inconspicuous dots of incrusting material. **Primordial hyphae** not clearly differentiated from pileocystidia which may form as a terminus, contents slightly refractive and fleetingly staining pink in acid fuchsin, weakly greying in SV, mostly without incrusting material but very rarely one may be found with a sheath of incrustation or more often just a few dots over a short section. **Primordial hyphae** differ from pileocystidia in being more regularly septate, about every 25-50um, branched, and more or less cylindrical except for slightly clavate end cells. **Hypodermis** a layer of repent parallel hyphae about 12-25um thick, absent in places, interwoven with the trama and the subcutis hyphae.

Trama of scattered and irregular clusters of sphaerocytes bound by a loosely interwoven hyphal mesh that in places forms bundles up to 50pm across. Vascular hyphae occasional, with amorphous contents that stain weakly grey in S V.

Chemical reactions: FeSC>4 - very little reaction, slightly greyish; KOH - orange-brown on cap surface, no reaction on stipe; NH4OH, - no reaction; guaiac - blue-green; phenol - slowly brownish purple; SV - deep greyish pink on cutis, gills and stipe trama.

Habitat and tree associations: In a moist north-facing slope with mature regeneration Douglas fir and western hemlock, the presence of red alder not noted at the time but likely to be close by, October.

Collections: PJ981213-01, under mature regeneration Douglas fir with understory salal and pacific blackberry, by a driveway, Saturna Island, N48.799110°, W123.181273° (CDF zone). CR021027-04 from alongside the lakeside trail under mature regeneration Douglas fir and western hemlock with salal, Mesachie Lake Forest Research Centre, approximately N48.815^{0}, W124.1358°; CR021028-01 on completely rotten log under mature regeneration Douglas fir and western hemlock, Youbou lookout, N48.86433^{0}, W124.25927^{0} (eastern very dry maritime CWH subzone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR021027-04	832	360,380	489,254	355,260
CR021028-01	900	430,334	385,298	340,251

Notes: These collections are undoubtedly close to *Russula integra,* they have the robust flesh that, when young, will withstand being dropped onto a hard surface from chest height without breaking, although this is not a recommended taxonomic method. They differ however in the spores, which in *R. integra* are larger, up to 1 lum long and 9u.m broad, with isolated spines up to 1.75um long. Romagnesi (1967) mentions the variability in the amount of incrustation seen on the pileocystidia and primordial hyphae in the epicutis, which can be absent in some collections, and points out that primordial hyphae are usually rare. In Romagnesi's monograph (1967) he recognises a number of varieties of *R. integra,* some of which, in Bon, (1988) are raised to species status. Among these, *R. integra* var. *areas,* found with spruce, is macroscopically similar to those of the Vancouver Island collection, and has the same spore size and ornamentation, however it is described as fleshy but not robust. The epicutis of *Russula carpini* Heinemann-Girard is most like that of the Vancouver Island collection, but that species bruises yellow, has spores with isolated spines and is found under *Carpinus* (hornbeam). Arora (1986) treats *R. integra* as found in California as a species complex, Thiers (1997) reports it from two counties in California but his description states that the epicutis lacks pileocystidia. The collection described above is the second of two local varieties or species in the *Integrinae* that almost but not quite matches published descriptions for species in this group.

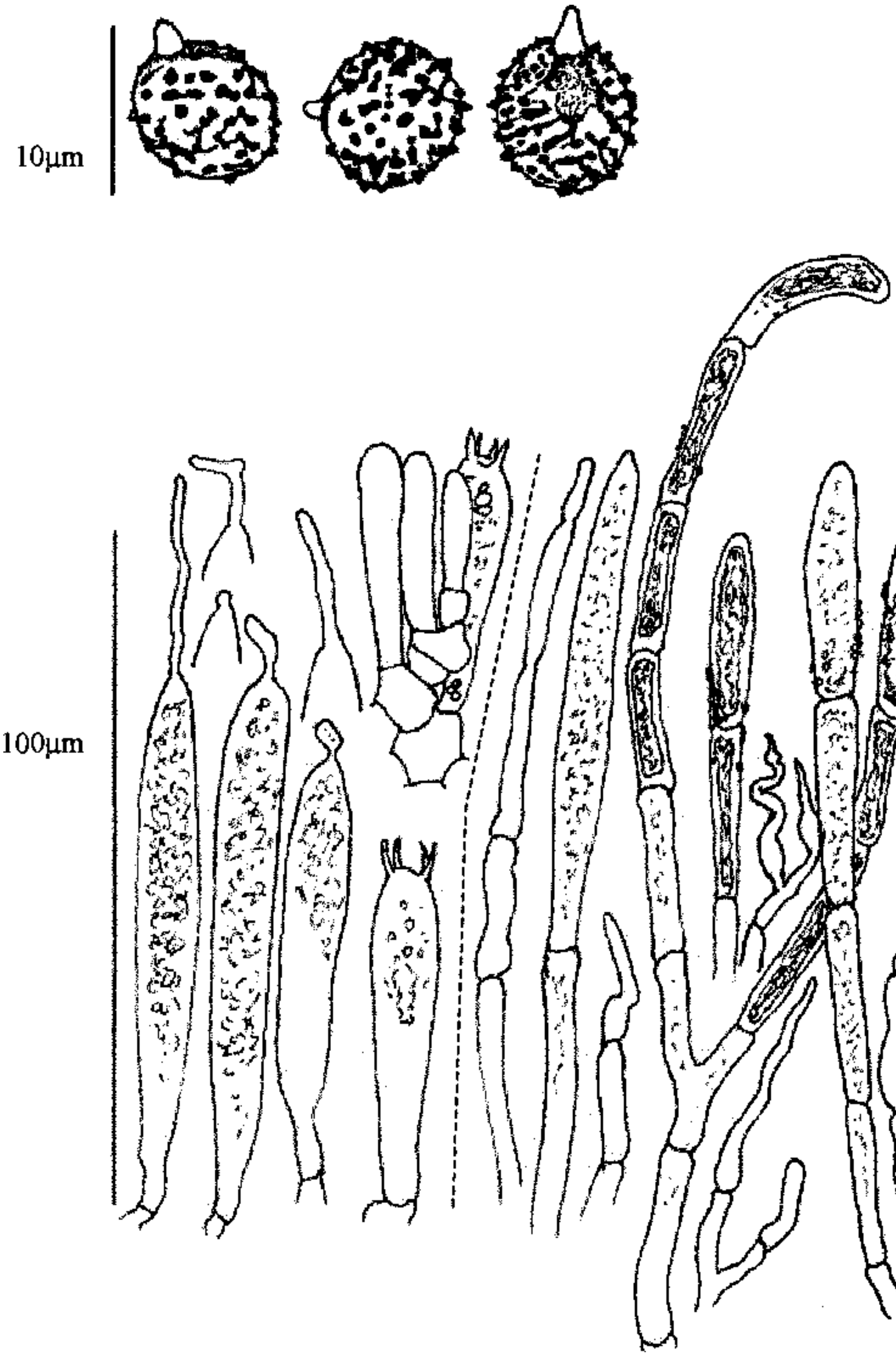

Figure 170 Microscopic characters of *Russula* cf. *Integra* 2nd var.: Top, spores with lOum scale bar; bottom left, hymenial cystidia, a basidium and above it a basidium with basidioles showing the build-up of cellular subymenium; bottom right, hyphal ends, primordial hyphae and pileocystidia from the epicutis, the two marked S V are shown as they appear in S V, lower scale bar is lOOum.

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Figure 171 Macroscopic characters of *Russula* cf. *integra* 2nd var. photographed on a scanner, showing basidiomata at various stages of maturity, small square is $1cm^2$ and shows spore colour.

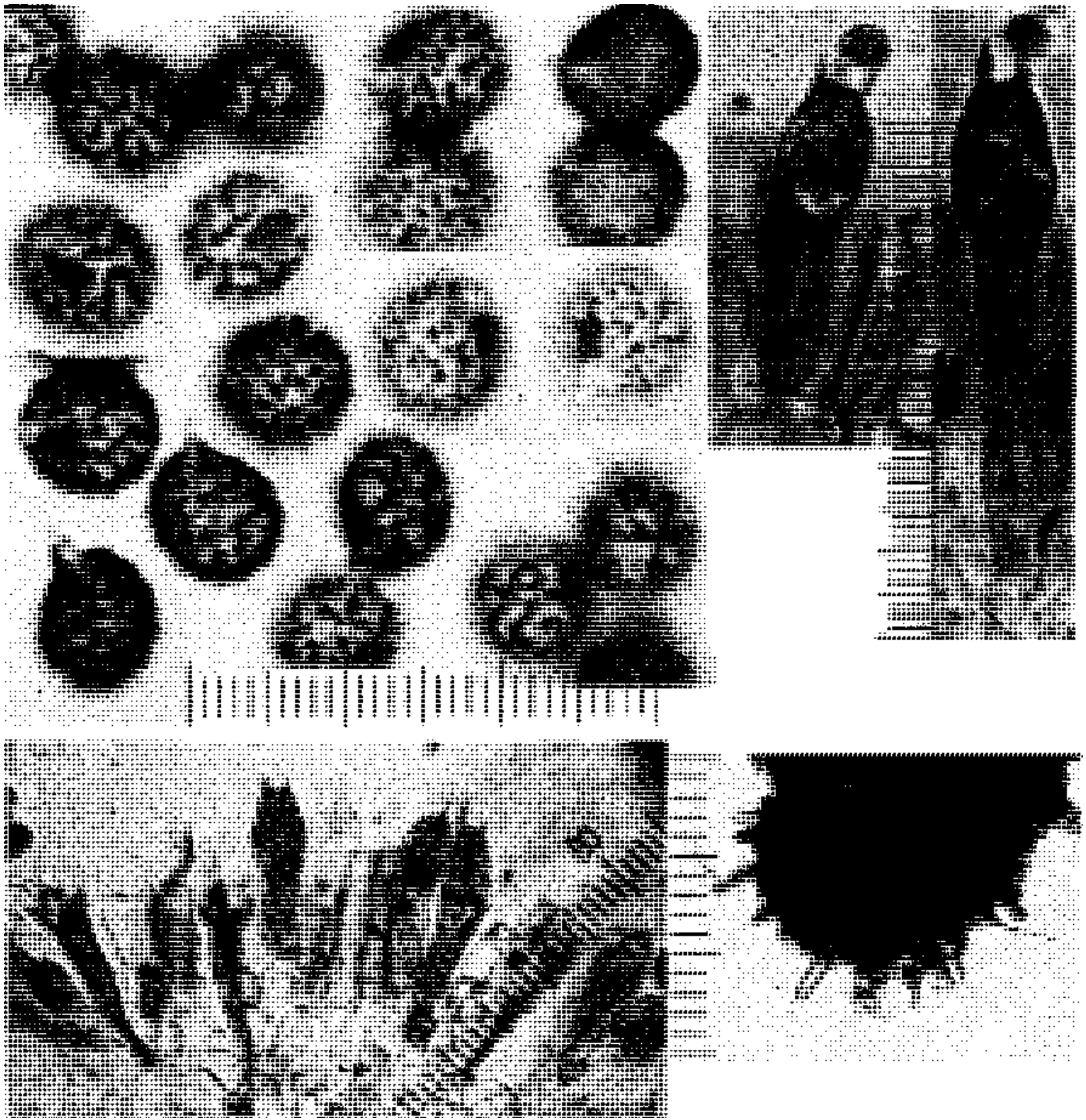


Figure 172 Hymenium of *Russula* cf *integra* 2nd var.: Top left, spores with 1 um division scale; top right, variation in basidia length between new (left) and old (right), shown with lum division scale; bottom left, small section of gill tissue stained in phloxine showing the structure of the subhymenium and hymenium with spent basidia (left) emanating from deeper levels than new basidia (centre), scale is in 2.5pm divisions; bottom right, cheilocystidia on gill section, scale is in lOum divisions..

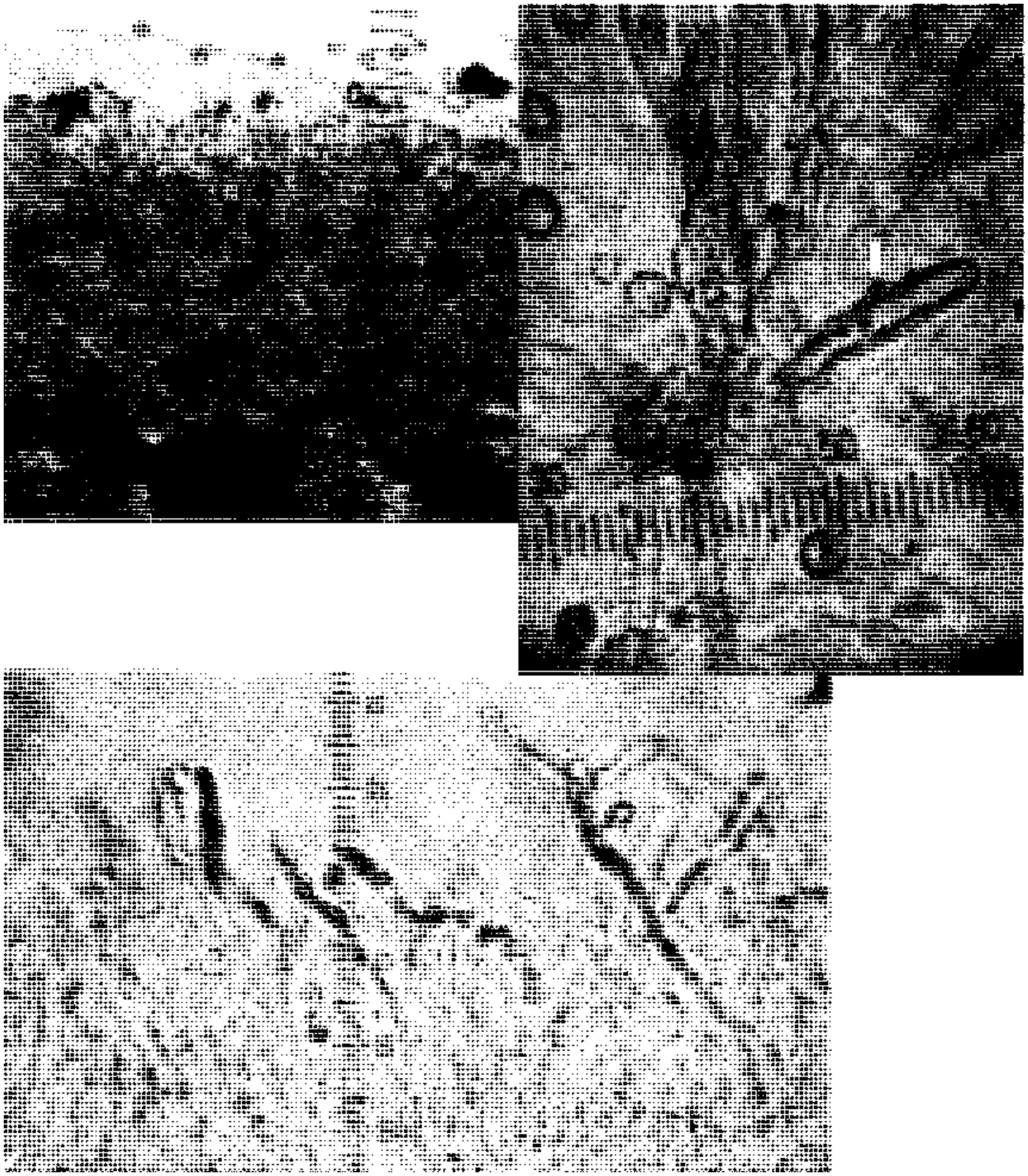


Figure 173 Cutis of *Russula* cf. *Integra* 2nd var.: Top left, section through cutis with lOum division scale, the viscid surface appears 10 - 30um above the epicutis in this water mount, the hypodermis is around the 48 - 50 scale marks; top right, surface view of epicutis showing pileocystidia and (or) primordial hyphae that are mostly naked, as can be seen from the smooth walls, but occasional small dots of incrusting material such as that arrowed can be seen, scale for this and the bottom photograph is in 2.5um divisions; bottom, epicutis stained with SV.

Section *Amethystinae* Romag. emend. Sarnari

Subsection *Integroidinae* Romagn. in Bon

***Russula occidentalis* Singer**

Sydowiall: 155. 1957

Cap 5.5 -13cm diameter, convex, closr to hemispherical when young, expanding through more or less plane to shallowly depressed with unevenly uplifted margins, the outer 2-4 mm of which curve down over the edge of the gills giving a slightly inrolled appearance, although not truly inrolled, remaining smooth. The colour is very variable but includes pinkish purple and olive hues, often also yellow-brown, sometimes red-brown, these colours may be marbled throughout or with the olive and brown in the centre and the pinks and purples toward the margin. Some basidiomata are strongly coloured, almost black in the centre, others are quite pale, almost buff, some lack pink and purple hues and are green to brown only. Cutis slightly viscid when wet, drying matte and slightly pruinose or minutely concentrically areolate towards the margin, centre subglossy, peelable to 3/4 the radius, flesh beneath greyish pink, cap trama creamy white, discolouring faintly dirty pink, finally dark grey, although this may take many hours in some cases, particularly in dry weather.

Gills light cream to cream, edges bruising grey to black, brittle, close to almost crowded, occasional subgills, generally some forking throughout the radius, adnexed to ascending at the stipe, broadening outwards, acute to obtuse and rounded at cap margin, both may be seen in one basidiomata (fig. 176), around 1.5-1.7 times the depth of the cap trama at half-radius, up to 12mm deep.

Stipe 3 -11.2cm x 1.5-4.2cm, roughly equal in length to cap diameter on average, white to creamy white, developing dingy pink stains where handled or damaged, becoming dark grey to brownish-grey, eventually black, often also with some browning near the base, longitudinally rugulose, pruinose when young, clavate, cylindrical, or broadest at the apex, solid, not developing cavities. In dry weather the bruising reaction is paler and slower and the flesh may not fully blacken.

Texture firm and robust.

Taste mild, sometimes nutty.

Odour not distinctive, in one collection slightly cheesy.

Spore colour warm cream, Romagnesi Ilc-d.

Spores 7.9-11.2 x 6.7-8.9um, L:W 1.11-1.46 with a mean of 1.30, (n=30). The wide range of spore sizes is due to the basidia having sometimes 2 and sometimes 4 spores, which translates to a dichotomy in size within one spore print, although the larger range is more common, there is some variation in relative proportions between basidiomata. **Spores** are ellipsoidal to obovate, rarely subglobose. **Ornamentation** is of mostly conical, pointed, isolated warts mostly around lum high but ranging between 0.4-1.7um high, occasionally a few warts may be linked by fine to heavy lines with catenations rare. Woo type 3A, occasionally 3B. **Suprahilar patch** amyloid, irregularly shaped, with warts at its border. **Hiliferous appendix** around 2.lum long, 1.7-1.8 um wide near the base. **Basidia** 4 and 2 -spored, 52-62 x 14 -17.7p.rn, voluminous, often abruptly bulbous in the upper 1/3. Like others in this clade the hymenium grows a cell or two for successively developing basidia, however because the cells are relatively small there is not such a wide range of basidia lengths. Sterigmata relatively stubby, up to 8u,m long and 2.0-2.8um wide at the base, those of two-spored basidia being broader than those of 4 -spored. **Pleurocystidia** frequent and evenly distributed, 70-125 x 9-13um protruding 15-40um, appearing elliptical in end view (when gill surface is examined), originating in the lower subhymenium, almost cylindrical to fusoid to narrowly clavate, tips subacute, contents partially yellowish and refractive in KOH, sometimes very weakly or rarely appearing empty, unreacting or with brownish-purple upper half in SV. **Cheilocystidia** 60-90 x 9-13, numerous, protruding up to 30um, fusoid or clavate, ends subacute to obtuse or sometimes with the terminal 25-30um narrower, around 6-8um, aseptate, contents partially refractive, usually at the tip, unreactmg to brownish in S V. **Subhymenium** 20 -30pm thick, of interwoven hyphae and small cells. **Gill trama** of sphaerocytes generally under 25 um, with a central core of more or less parallel hyphae, no vascular hyphae seen.

Cutis 80-160um thick, an ixotrichodermis of which about 2/3 the thickness is subcutis, with a well differentiated epicutis, lacking pileocystidia. **Subcutis** of tightly interwoven repent to semi-upright hyphae 1.5-3 urn wide, tinted pinkish or greenish en masse, and a few hyphae contain greyish or greenish vacuolar pigment globules, vascular hyphae not seen. **Epicutis** of erect hyphal ends 2-4um wide, undifferentiated or frequently with a tapering terminal cell up to 50um long, and numerous incrusted, septate primordial hyphae 4 -6um wide, rarely with inflated cells up to 8u,m wide, the incrustations varying from a thin broken sheath to fine or heavy droplets up to 2pm diameter, visible in water mounts and bright pink in SV, but neither the hyphal contents nor the incrustations staining much in acid fuchsin, although the latter remain visible. **Pileocystidia** absent. **Hypodermis** a band around 25 pm thick of repent, parallel light yellowish brown hyphae interwoven with the subcutis and trama.

Trama of discrete well separated clusters of sphaerocytes interspersed between bundles of hyphae, vascular hyphae rare, not staining in SV.

Chemical reactions: FeS04- weakly salmon pink or greyish green on young material; KOH - yellow-brown to orange on cap surface, no reaction to brownish on stipe; phenol, -dull reddish purple; SV - deep pink initially, then browning on the gills, trama and cuticle, hymenial cystidia brown or grey, vascular hyphae not reacting, primordial hyphae and incrustations bright strong pink; PDAB (paradimethylaminobenzaldehyde) - vivid magenta on stipe (Woo, 1989), not tested on Vancouver Island collections.

Habitat and tree associations: under western hemlock in natural and regeneration stands with or without either Douglas fir or Sitka Spruce.

Collections: CR980821-03, from old growth Douglas fir with understory western hemlock at about 300m elevation on Phillips Ridge trail, Strathcona Park, N 49.5900°, W 125.583° (windward moist maritime MH). PJ990731, from a mixed-age stand of western hemlock with veteran Douglas fir and western red cedar, Saturna Island crown land section 4, N 48.776474°, W 123.295362° (CDF zone). OC011013-01, from a stand of young (< 50 years) western hemlock, near Mesachie Lake Forest Research station, approximately N 48.8257°, W 124.1348° (western very dry maritime CWH subzone). JJ021020-03, from regeneration western hemlock and Sitka spruce, China Beach area, approximately N 48.4362°, W 124.0888° (southern very wet hypermaritime CWH subzone).

Collection	ITS1-F to ITS4-B	RFLP: Hinfl	Alul	Sau3A
JJ021020-03	900	430,334	385,298	340,251

Notes: *Russula occidentalis* is superficially similar to *R. olivacea, R. xerampelina, R. isabelliniceps andR. integra* none of which have the pink to grey to black colour changes of the flesh. *Russula decolorans, R. claroflava,* and *R. pacifica* also bruise grey, these have, respectively, a red cap, a yellow cap and a purple cap, the latter also is the only *Russula* species that shows no reaction to phenol, it also has S V positive pileocystidia and a thicker cutis. Other greying and blackening Russulas are of subgenus *Compactae* and lack any of the red, yellow and blue pigments in the cutis, and have spores with an inamyloid suprahilar patch, these species include *R. adusta, R. albonigra, R anthracina, R. dissimulans* and *R. nigricans.*

The incrustations on the primordial hyphae have apparently been overlooked by other authors in their descriptions *of Russula occidentalis.* Singer (1957) in his original description states "epicutis of pileus formed by hyphae which are equal, smooth, "empty", clampless, l-3.5um thick, filamentous, and making up a trichoderm which becomes depressed at an early age. Thiers (1997) notes that the California material has poorly differentiated pileocystidia, which may or may not be the same as the primordial hyphae in Vancouver Island collections. Singer had published *R. occidentalis* as a subspecies *01R. vinosa* in 1940, which species does possess heavily incrusted primordial hyphae and is very similar to the Vancouver Island collections described above. The incrustations dissolve in 3% ammonia and 5% KOH and if the cutis had previously always been examined in these reagents as is a common practice, this would explain the ommission in other descriptions, however it is also common practice to examine the cutis in SV to determine the presence of pileocystidia, in which case the bright pink incrustations would be hard to miss were they present. This would indicate a great amount of variation in the presence or absence of primordial hyphae and pileocystidia in the cutis. In *Russula* phylogenetic analyses *R. occidentalis* and *R. vinosa* fall in the same terminal clade as *R. integra* and *R. claroflava*, both of which have incrustations that redden in S V.

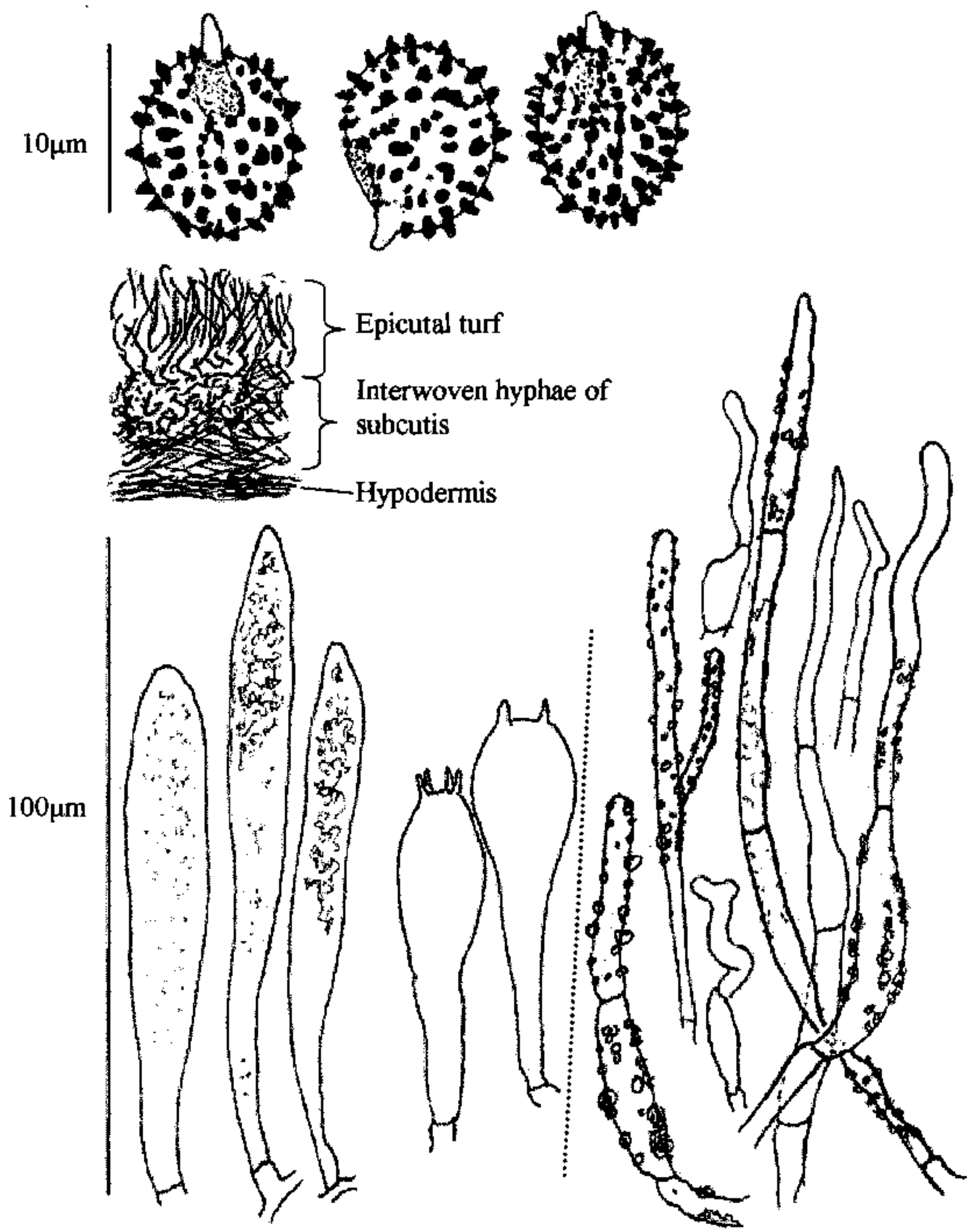


Figure 174 Microscopic characters *ofRussukt occidentatis:* Top, spores with lOum scale bar; middle, diagram of section through cutis; bottom left, cheilocystidia, two pleurocystidia, a four and a two-spored basidium; bottom right, primordial hyphae and hyphal ends from the epicutis, lower scale bar is lOOum.

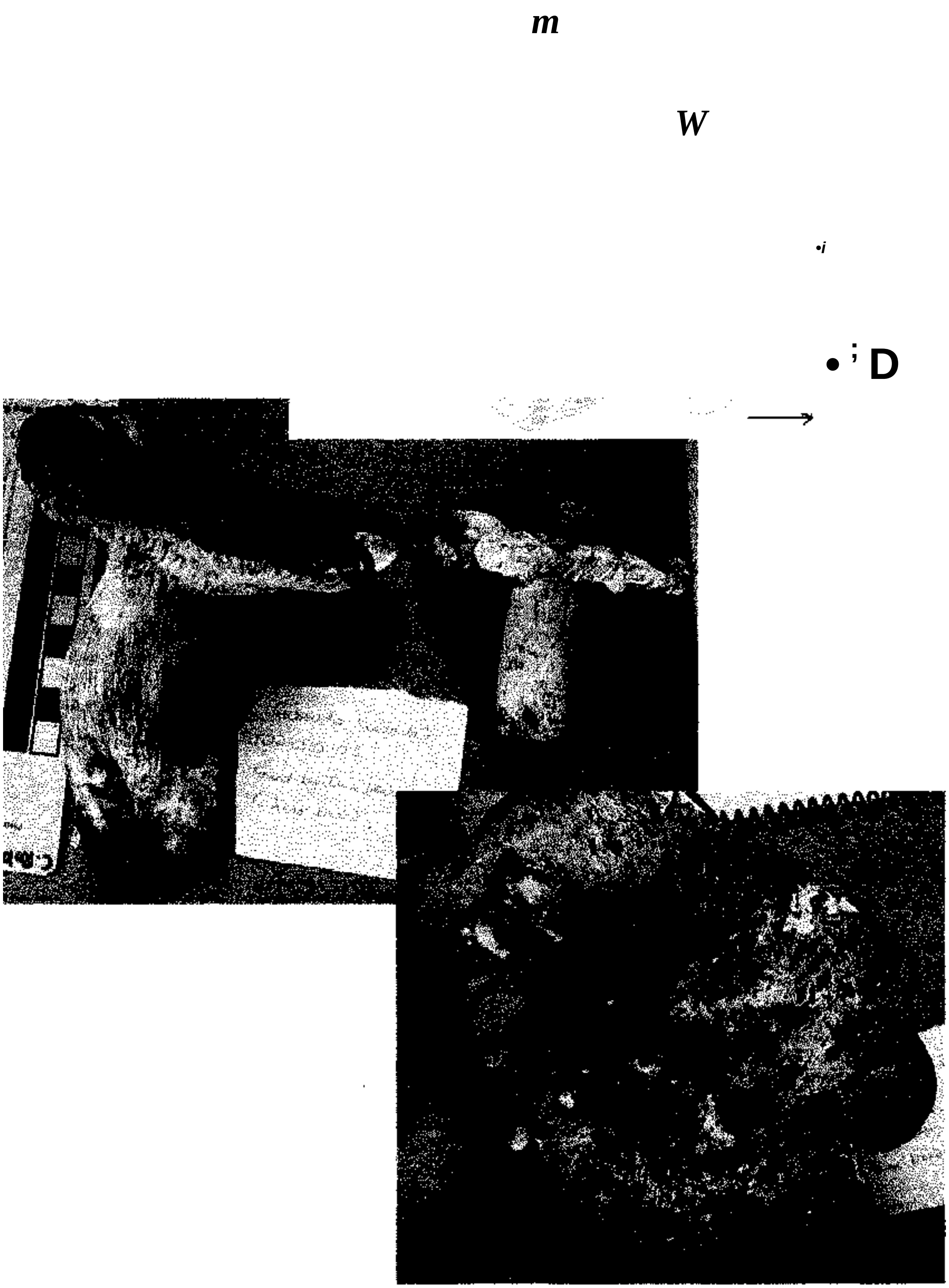


Figure 175 Macroscopic characters *ofRussula occidentalis:* Top, illustration of profile and longitudinal section showing progressive bruising reaction, small square is $1cm^2$ and shows spore colour; middle, collection with 1cm division scale, note the bruising on the stipe; bottom, view of the cap surface of the larger basidioma.

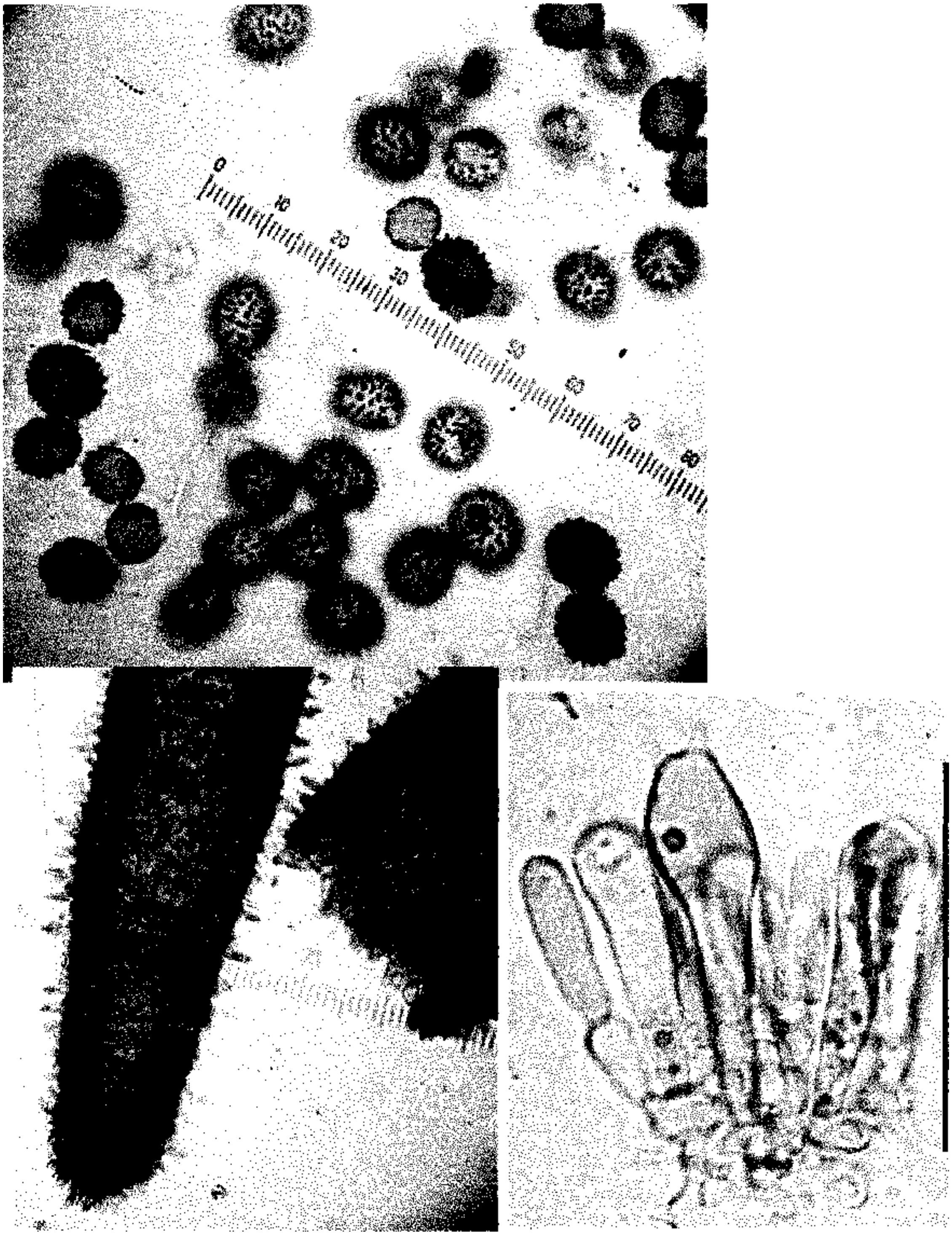


Figure 176 Hymenium *ofRussula occidentalis:* Top, spores with lum division scale; bottom left, section through gill showing the protruding pleurocystidia and compact gill trama, 10 scale divisions are lOOum; bottom right, fragment of hymenium showing basidium and basidioles supported on stepped basal cells, scale bar is 50um.

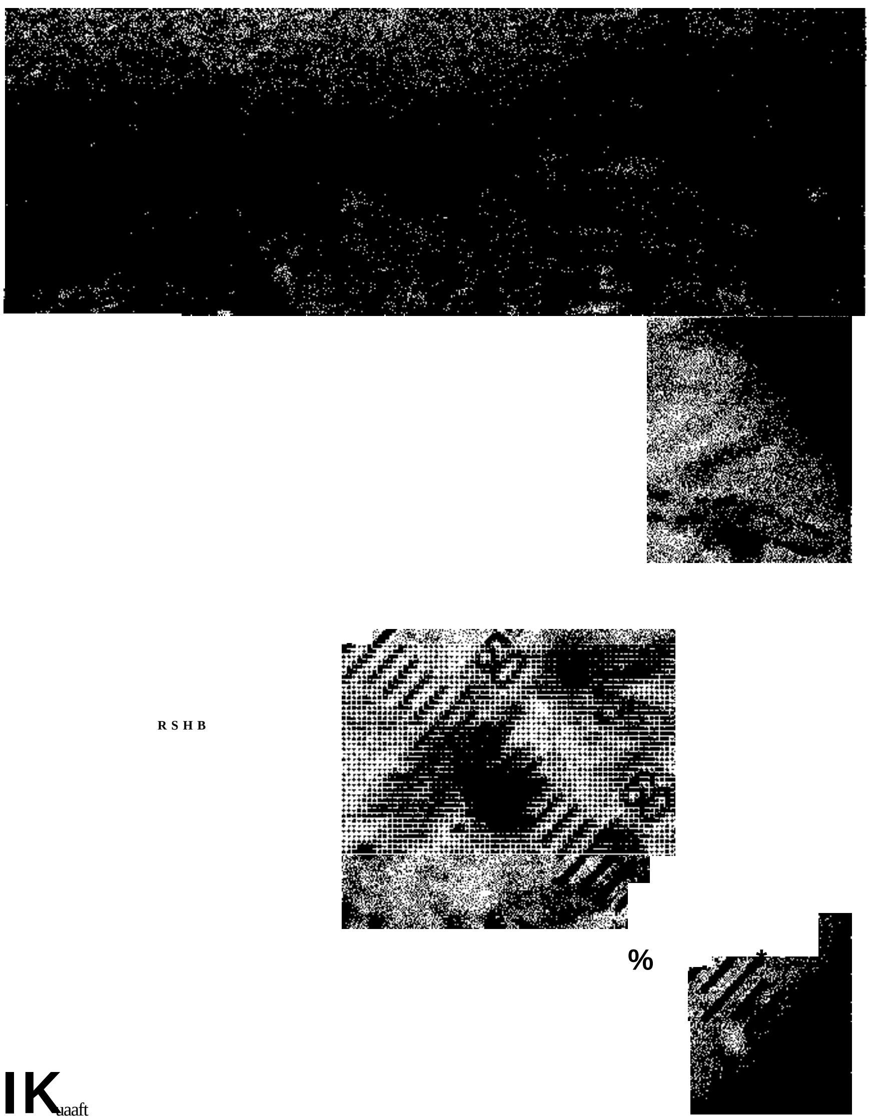


Figure 177 Cutis of *Russula occidentalis:* top, section through cutis in which the epicutis shows a clear differentiation from the subcutis at about the 55 mark on the scale, of which, 10 divisions are lOOum; bottom, surface view of epicutis in SV showing naked epicutal hyphae in the background and incrusted primordial hyphae in the foreground, 10 scale divisions are 25um.

Clade 9a

Section *Tenellae* Quelet

Subsection *Sphagnophilae* Singer

***Russula sphagnophila* Kauffman**

Michigan Academy of Science Report 11: 86. 1909

Cap 1.7-6.5cm diameter, convex when young, becoming plano-convex with a shallow central depression, margins remaining curved over the gill ends, sometimes almost inrolled in places, smooth when young, soon becoming striate, finally tuberculate. Colour very dark vinaceous purple to brownish purple to brownish violet centrally, lighter purple towards the margin, fading to brownish pink to greyish olive on the margin and olive to yellow-brown or grey-brown over the disc, with a duller brown or grey zone between. Cutis slightly viscid when wet, drying matte, peelable 1/2-3/4, flesh beneath tinted pink, otherwise creamy white, not or barely yellowing, fairly thin, barely 2mm thick at half-radius on a 6cm diameter cap.

Gills white at first, becoming pale cream, subdistant, subgills and forking uncommon, rounded to sinuate at stipe, ventricose or broadest close to the margin, 2-4 times the depth of the trama at half-radius, up to 3 mm deep, rounded at the cap margin up to an acute junction with the cutis, pliable, not bruising.

Stipe (1.6-) 4.3-5.0 x (0.4-)l-1.3cm, longer than cap diameter, cylindrical but usually broadening slightly at the base, longitudinally rugulose, soft and compressible, stuffed, becoming cavitate and finally hollow, white, staining dull yellow especially near the base.

Texture fairly soft, but not exceptionally fragile.

Taste mild or with a slightly peppery aftertaste.

Odour slight, bread- or yeast-like in one collection, slightly fruity in the other.

Spore colour deep cream to pale orange-yellow, Romagnesi lid -Ilia.

Spores 8.2-10.2 x 6.3-8.2um, L:W 1.11-1.36 with a mean of 1.23 (n=50), narrowly to broadly ellipsoidal. **Ornamentation** of blunt conical warts 0.5-1.lum high, often a mixture of heavy warts with finer ones interspersed, mostly isolated or 2-4-catenate, or warts joined by a heavy lines, on some spores catenations will run almost the length of the spore, fine lines occasional, these plus catenations forming a partial reticulum. **Woo types** B2-3 to C2-3. **Suprahilar patch** a roundish amyloid area bordered by warts, occasionally including them. **Hiliferous appendix** 1.3-1.6 urn long, 0.8-1.2um wide near the base. **Basidia** 4-spored, uncommonly 2-spored, 37-50 x 12-14.5um, strongly clavate to bulbous in the upper half. Sterigmata up to 8u,m long and 2. lum wide near the base. Pleurocystidia frequent, 43-68 x 7-12.5um, elliptical in end view, protruding less than 20um, originating in the subhymenium, fiisoid to cylindrical or slightly clavate, tips obtuse or sometimes with a small button, contents refractive, pale yellow in KOH, weakly greying in SV. **Cheilocystidia** frequent, 35-40 x 8-10um, protruding around 20um, otherwise similar to pleurocystidia. **Subhymenium** 20-3 5 urn thick, pseudoparenchymatous, of relatively large cells up to 13 urn wide, with younger basidia raised one to two cells above the mature ones, **gill trama** of sphaerocytes with occasional vascular hyphae.

Cutis 100-1 lOum thick, an ixotrichodermis of two distinct layers. **Subcutis** aroundl/2 the depth of the cutis, compactly interwoven, hyphae 1.5-2.5um wide, repent and more or less parallel, occasional vascular hyphae weakly greying in SV. **Epicutis** of erect hyaline to pinkish hyphae 1.5-4um wide, and abundant pileocystidia. Hyphal ends undifferentiated, occasionally obtuse, or most often with a tapering terminal cell that sometimes ends in a small button. **Pileocystidia** abundant, 28-80 x 6-10 (-13)um the majority around 40-60u,m long, clavate to cylindroclavate, 0-5-septate, only the shorter ones aseptate, most 2-3-septate, sometimes constricted at the septa, contents pale yellow, refractive, often only partially filled, greying in SV, sometimes only weakly.
Hypodermis a narrow layer of compacted tramal tissues.

Trama of loosely defined clusters of sphaerocytes bound by a hyphal mesh and occasional vascular hyphae.

Chemical reactions: FeS04 - pale grey to pinkish grey; KOH - salmon pink to orange on cap surface, no reaction on stipe; acid fuchsin - no reaction on cuticular hyphae and cystidia; phenol - pale pink to pale brown; SV - pink initially on the gills and cutis, then brown, cystidia and vascular hyphae weakly greying, sometimes not reacting.

Habitat and tree associations: In very wet maritime forests, with Sitka spruce, not necessarily with sphagnum.

Collections: CR001011-38 and -39 on and beside the root pad of a fallen old-growth Sitka spruce, Meares Island boardwalk, N49.1560°, W125.8695^0. CR001011-42, in grass and moss (not sphagnum) under mixed age with old-growth Sitka spruce and red alder, alongside the south part of the boardwalk fromWickanninish bay parking lot, PRNP, N49.012617^0, W125.674650^0. CR011030-03, in grass under young Sitka spruce, Long beach picnic area, PRNP, N49.070061^0, W125.7545^0. CR020927-01, on duff near base of western hemlock in a valley bottom with old-growth Sitka spruce and western red cedar, Carmanah grove, N48.6585^0, W124.6895^0 (all from southern very wet hypermaritime CWH subzone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR011030-03	816	374	511,267	305, 191

Notes: The spores of *R. sphagnophila* should have more reticulations than is apparent in the above collections, whose spores are intermediate in size and ornamentation between those of *R. brunneoviolacea* and *R. nauseosa.* The collections differ from the latter in having paler spores and from the former in being softer-fleshed with a stronger and earlier development of striations on the cap margin. *Russula brunneoviolacea* prefers a drier forest habitat wherein Douglas fir is found, and *R. sphagnophila* a moister habitat with Sitka spruce, but where these habitats overlap the difference between the two species becomes difficult to discern. *Russula puellaris* is very similar to *R. sphagnophila* in colour and stature, but the entire basidioma yellows, and some of the pileocystidia are filled with a deep yellow refractive material.

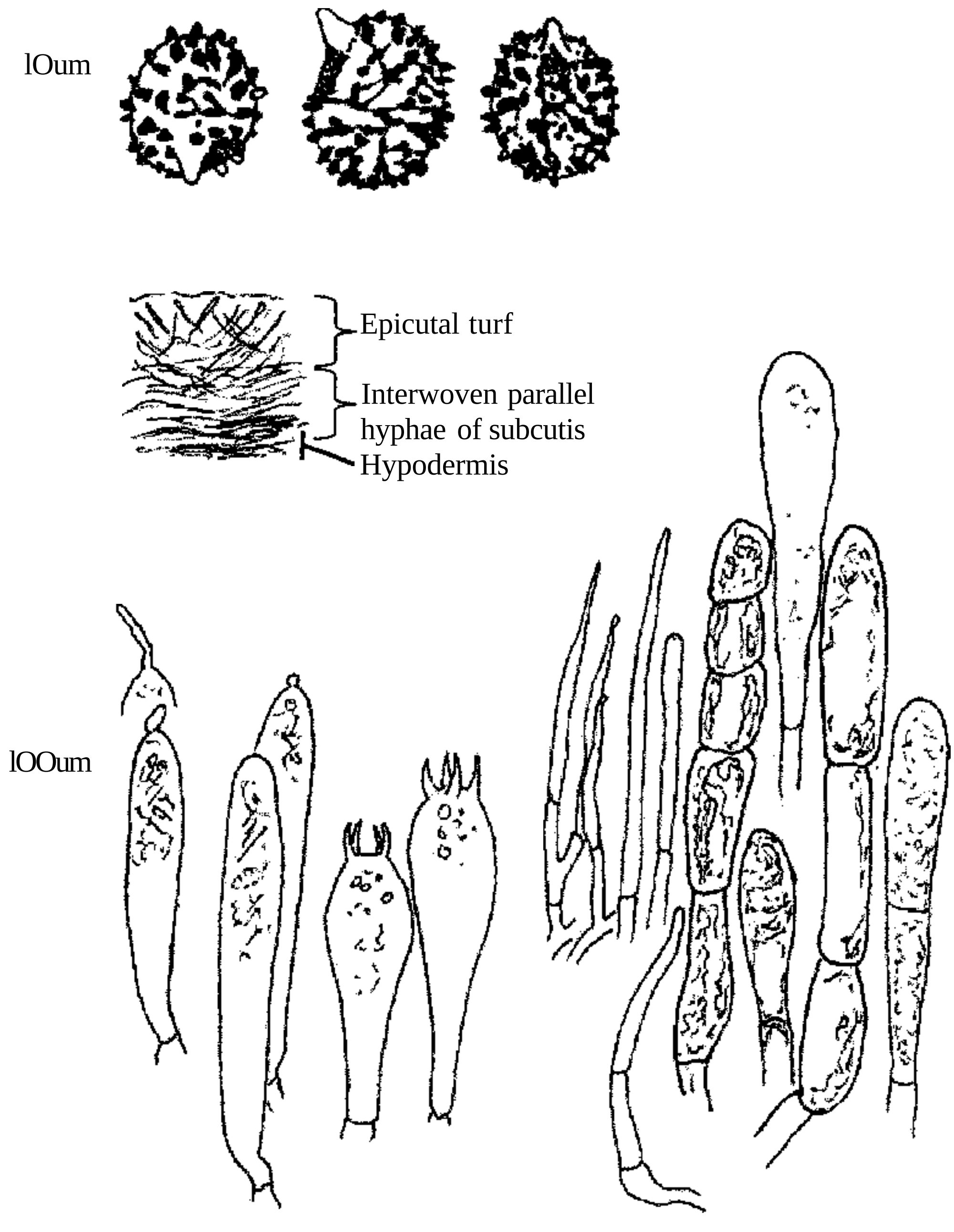
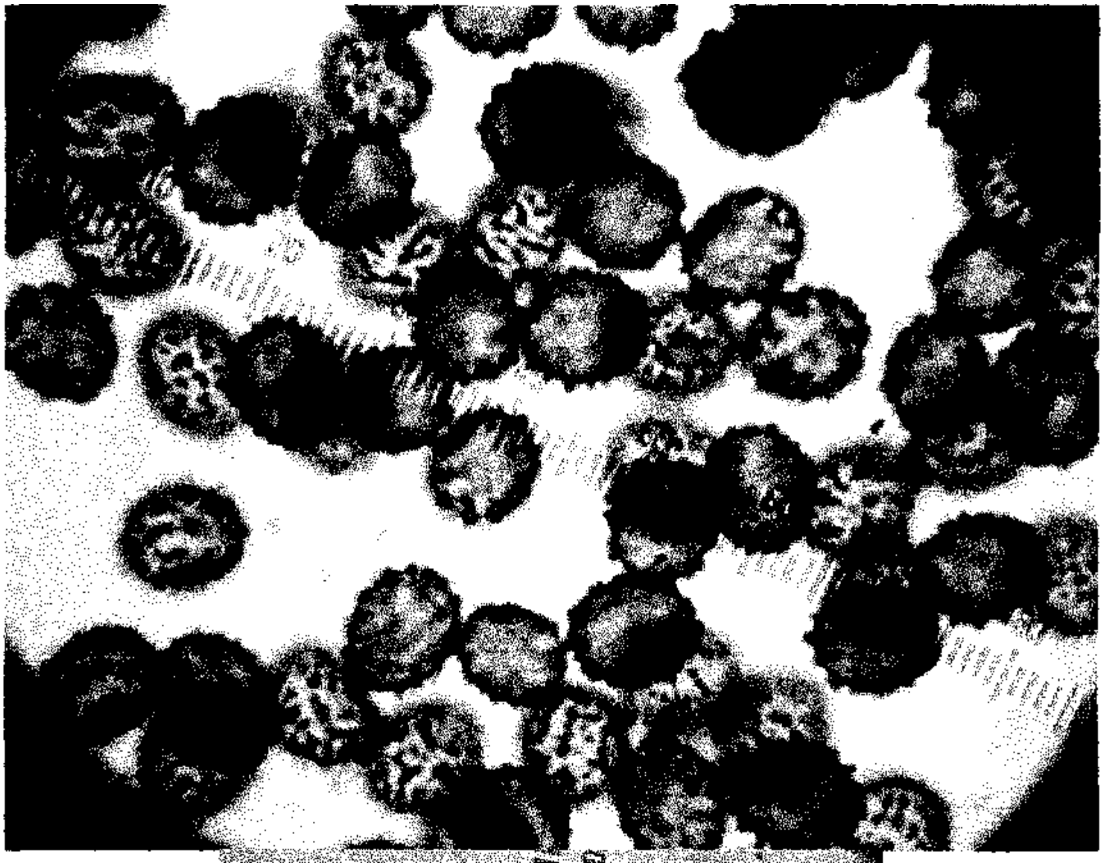


Figure 178 Microscopic characters *otRussula sphagnophila:* Top, spores with 10 um scale bar; middle, diagram of section through cutis; bottom left, cheilocystidia and two pleurocystidia, two basidia; bottom right, hyphal ends and pileocystidia from the epicutis, lower scale bar is 100 um.

Figure 179 Macroscopic characters of *Russula sphagnophila:* Upper (top) and underside (bottom) of a rather small but fully mature basidioma from Carmanah Grove, the cap is 1.7cm across on the longer dimension.



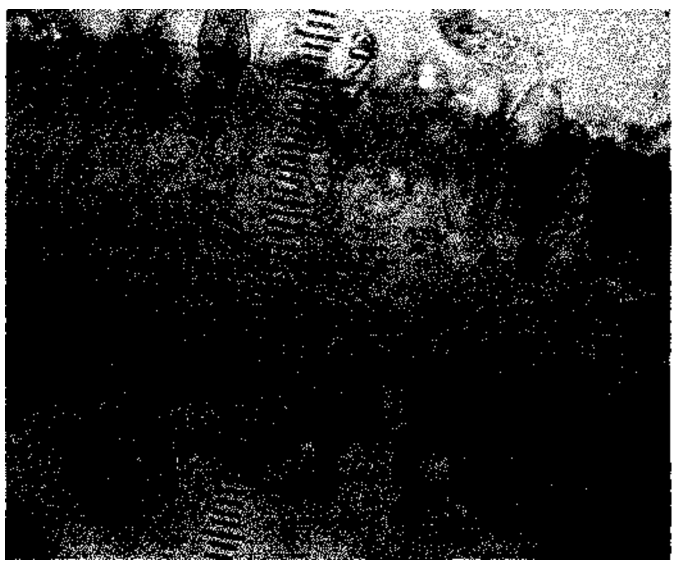


Figure 180 Hymenium *of Russula sphagnophila:* Top, spores with lum division scale; bottom, section through gill near the margin showing several basidia and basidioles, 10 scale divisions are 25 um.

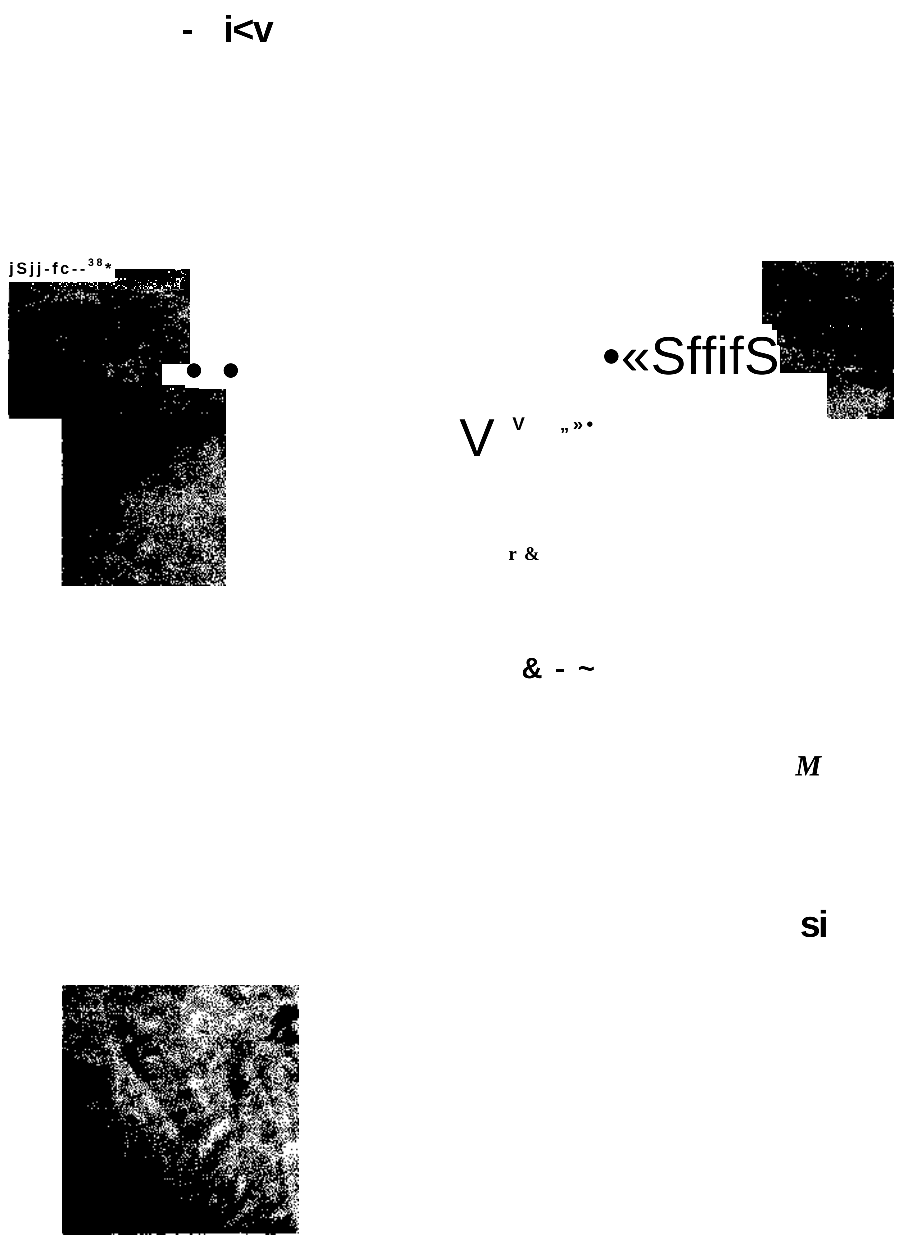


Figure 181 Cutis of *Russula sphagnophila:* Top, section through cutis in NH4OH, with hyphal ends but no pileocystidia in view, 10 scale divisions are 25 urn for this and the bottom photograph; bottom, surface view of cutis with several septate pileocystidia.

Russula brunneoviolacea **Crawshay**

Spore Ornamentation of the Russulas. p90. 1930

Cap 3.3-3.9cm diameter, almost spherical with a flattened disc area when young, becoming plane with a shallow central depression and downcurved, margins smooth when young, becoming striate in age. Colour a more or less even dark vinaceous purple when young, becoming brownish violet to brownish purple, usually more brown to almost black centrally with clearer hues towards the margin. Viscid when wet, soon drying subglossy with a matte centre. Cutis peelable 2/3-3/4 the radius, trama beneath tinted pink, otherwise white, unchanging.

Gills deep cream to pale orange at maturity, subdistant, subgills and forking uncommon, adnexed at the stipe, broadening outwards, obtuse to rounded at cap margin, 2-4 times the depth of the cap trama at half-radius.

Stipe 3.5-6.0 x 0.9-1.4cm, usually longer than the cap diameter, cylindrical to narrowly clavate, flexuous, white, glabrous, longitudinally slightly rugulose, stuffed with a soft bread-textured trama which may develop three or more small cavities that merge to form a hollow stipe, unchanging or with a faintly buff to yellowish bruising especially at the base.

Texture fairly soft and fragile.

Taste mild, nutty or sweet.

Odour not distinctive to slightly nutty or fruity, sometimes with a faint phenolic, iodine or disinfectant smell at the very base of the stipe on young basidiomata.

Spore colour Romagnesi Ilb-c.

Spores 7.4-8.8 (-9.8) x 6.1-7.1 (-8.6)um, L.W 1.14-1.31, with a mean of 1.22 (n=30), subglobose to broadly ellipsoidal. **Ornamentation** of warts 0.5-0.9um high, mostly hemispherical, sometimes conical, occasionally crestate, often heavy, mostly joined in rows by thin to thick lines which often run more or less vertically but can be in any direction, generally forming a very broken reticulum, occasionally an almost complete one, or sometimes with a lot of isolated warts or clusters of warts with little reticulum development. **Woo types** B2-C2, rarely D2. **Suprahilar patch** strongly amyloid, irregularly shaped, often with warts within and on its borders. **Hiliferous appendix** around 1.3 um long and 0.8um wide near the base. **Basidia** 4-spored, occasionally 2-spored, 37-52 xl0-14.2um, short and broad, clavate and bulbous in the upper 1/3, often abruptly so. Sterigmata relatively short, 5.6-6.2x1.6-2.2um. **Pleurocystidia** common, normally distributed (i.e., neither numerous nor sparse), 55-75 x 7.5-14.6um, protruding up to 30um, sometimes embedded, originating in the subhymenium or outer trama, cylindrical to fusoid, tips acute to subacute or sometimes rather blunt and rounded, contents refractive, weakly greying in SV. **Cheilocystidia** variable in frequency and distribution, 37-55 x 8-13um, embedded or protruding 10-15um, fusoid to clavate, often crumpled, sometimes with one septum, tips acute, rounded and somewhat irregular, or often with a tiny button, contents refractive, although often only at the tip, brown or grey in SV. **Subhymenium** 10-25um thick, pseudoparenchymatous, sometimes with longer, narrower cells but not interwoven hyphae, **gill trama** of sphaerocytes, vascular hyphae uncommon.

Cutis 110-130um thick, an ixotrichodermis. **Subcutis** about 2/3 the cutis depth, of tightly interwoven semi-upright hyaline hyphae 2-6um wide, the lower layers repent and parallel, with a few vascular hyphae weakly staining grey in S V, occasionally giving rise to pseudocystidia that terminate at the surface alongside the pileocystidia. Occasionally a small area has a few sphaerocytes and inflated hyphae in the interwoven zone. **Epicutis** of upright hyphae 2-5 um wide, generally septate every 10-25um, tips undifferentiated, clavate or sometimes capitate and generally at or near the upper width range (4-5um). Pileocystidia abundant, 19-70 x 5-10 (-13)um, the majority under 50um long, clavate, 1-5-septate, most with 2 septa, tips obtuse, very occasionally capitate, contents refractive, yellowish in KOH, light to dark grey in S V, sometimes only partially staining. **Pseudocystidia** similar in width and septation to pileocystidia but emanating from the lower subcutis and hence, longer. **Hypodermis** a layer of parallel hyphae interwoven with the subcutis and less so with the upper trama. In places, below the interwoven subcutis and above the normal trama, there is a layer up to 150um thick of loosely interwoven more or less parallel hyphae with inflated segments, air spaces and sphaerocytes up to about 20um across.

Trama mostly of sphaerocytes in loosely defined clusters and as a layer above the hymenium, bound by a hyphal mesh and occasional vascular hyphae.

Chemical reactions: FeSC>4 - pale salmon pink; KOH - deep red, turning orange- brown on cap surface, slightly pale green on stipe; NH4OH - no reaction; guaiac - blue-green; guaiacol - a strong deep pink; phenol -brownish purple; SV - pink at first, then brownish on the gills and cutis, cystidia and vascular hyphae grey.

Habitat and tree associations: Gregarious on the forest floor in mature regeneration western hemlock stand or Douglas fir-madrone-garry oak woodland.

Collections: OC011013-04, Mesachie Lake Forest Research Centre, amongst western hemlocks, exact location not recorded but in the vicinity of N48.8257^0, W124.1348^0 (western very dry maritime CWH subzone). Collection CR981114-01, from a limestone outcrop under young Douglas fir with madrone, occasional small oaks and salal, differs from the above description in the following characters: the spores are slightly smaller, 6.5-8.9 x 5.8-6.9um, and although the mean L:W is similar at 1.21, the range is larger at 1.06-1.41, the spore colour could not be accurately ascertained but appears slightly darker at lid or Ilia, and there was no reaction with FeSC>4. Other characters including the spore ornamentation are comparable with the Mesachie Lake collection. The location of the latter collection site is in the Koksilah river valley, north side, near N48.6559^0, W123.7294° (eastern very dry maritime CWH subzone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR981114-01	832	369, 263	500, 305	343, 271

Notes: The North American collections of *Russula brunmoviolacae* differ in spore ornamentation from those in Europe as described by Romagnesi (1967), that have long, mostly isolated spines, but drawings by Crawshay (1930) show spores with ornamentation much like the North American and Vancouver Island collections. The basidia of the Vancouver Island material are larger than the dimensions (31-42 x 7.9-10.2um) given by Shaffer (1970) for Michigan material, but the spores are very close to his variant 1. The subcutis of Vancouver Island collections varied in the amount and position of inflated cells, sometimes within the subcutis, sometimes within the hypodermis, and sometimes lacking. Romagnesi (1967) described such a layer beneath the cuticle and distinguished it from the interwoven layer beneath the epicutis. Shaffer also observed this character in some but not all of the basidiomata in his collections, noting that he did not think this significant due to the inherent variability of this character. *Russula nauseosa*, described and illustrated below, is similar in many microscopic characters and, with the exception of spore colour, virtually indistinguishable macroscopically from *Russula brunneoviolacea*, the larger spore size differentiates it microscopically. *Russula puellaris* is also quite similar, but bruises yellow all over, and usually is red-brown to purple rather than violet, becoming quite brown in age. Two collections with macroscopic characters virtually identical to *R. brunneoviolacea* and with spores of comparable size and ornamentation but darker (Romagnesi Ilia and IVa), and with slightly longer pileocystidia, were made from the coastal Douglas fir zone. These are close to *R. laricina*. *Russula sphagnophila* differs from *R. brunneoviolacea* in its more striate to tuberculate cap margin, its association with wetter forests that sometimes include sphagnum, larger spores and softer flesh. *Russula brunneoviolacea* is normally associated with deciduous trees, especially oaks, of which there are a few in the vicinity of the Mesachie Lake Forest Research Centre, as well as red alder and some willows, so the association with western hemlock may or may not be correct.

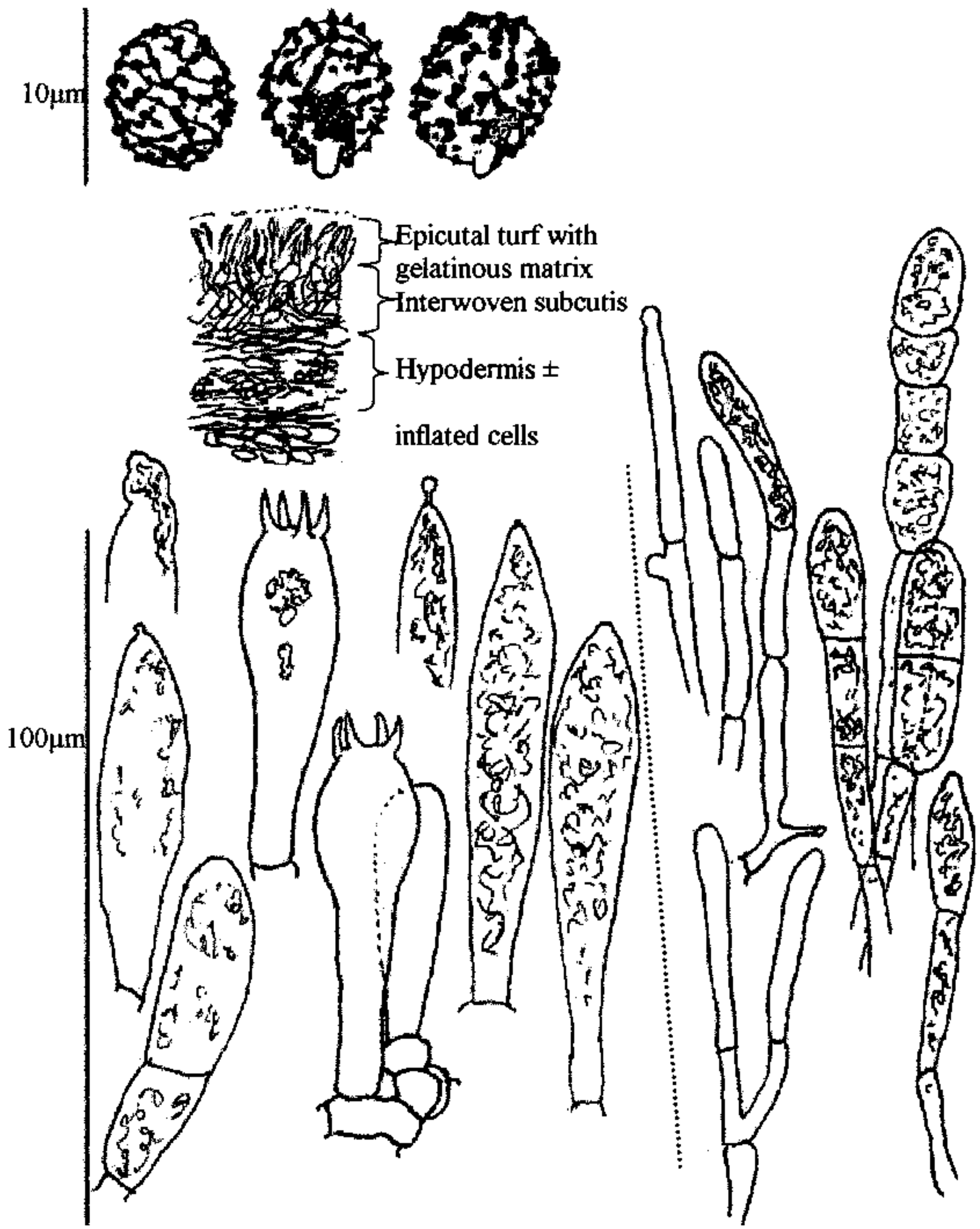


Figure 182 Microscopic characters of *Russula brurmeoviolaea:* Top, spores with lOum scale bar; middle, diagram of section through cutis; bottom left, cheilocystidia cystidia, basidia and pleurocystidia; bottom right, hyphal ends and pileocystidia from the epicutis, lower scale bar is lOOum.

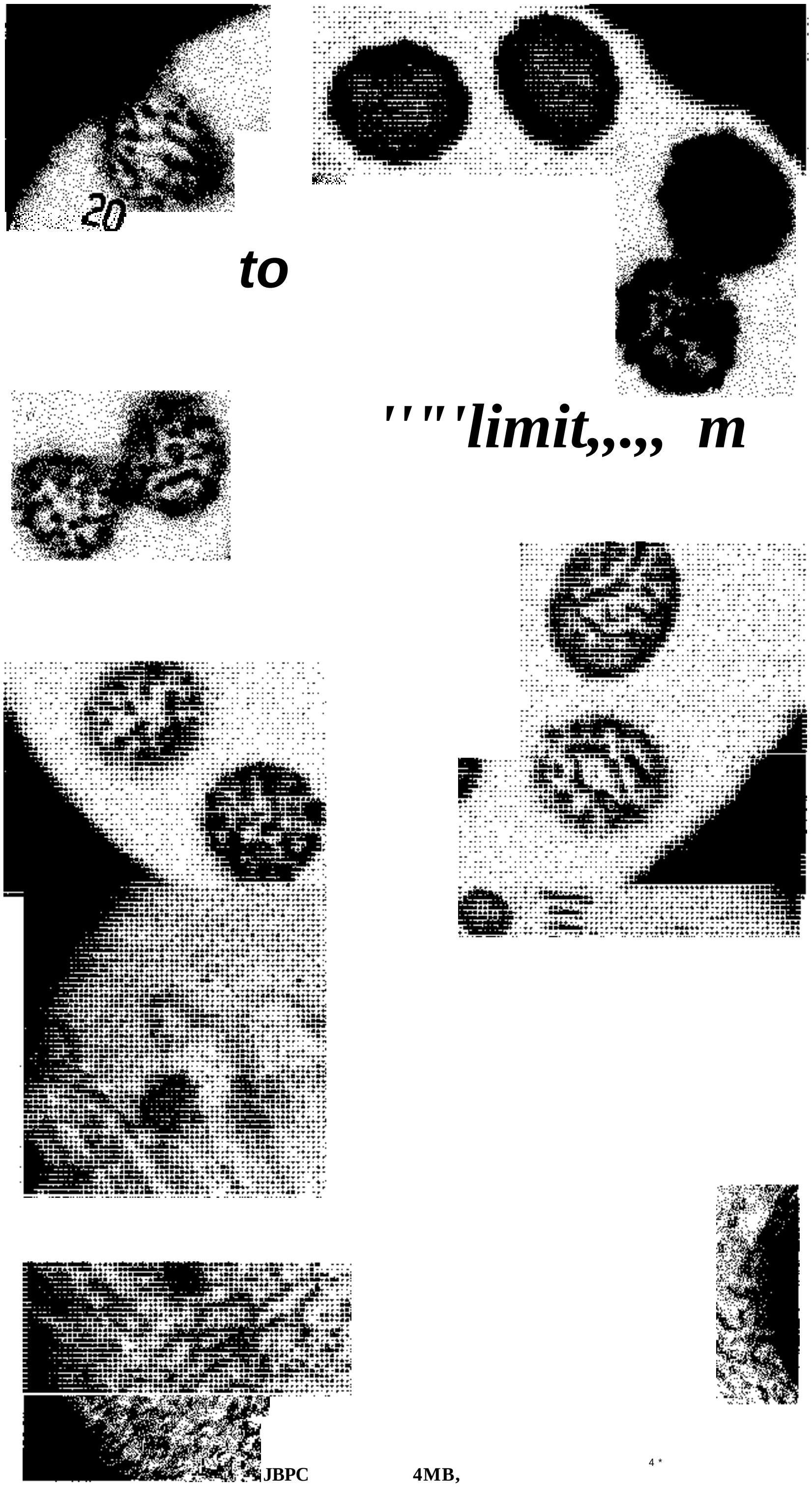


Figure 183 Hymenium of *Russula brunneoviolacea:* Top, spores with lum division scale; bottom, section through hymenium including subhymenium, mounted in Melzer's reagent, 10 scale divisions are 25um.

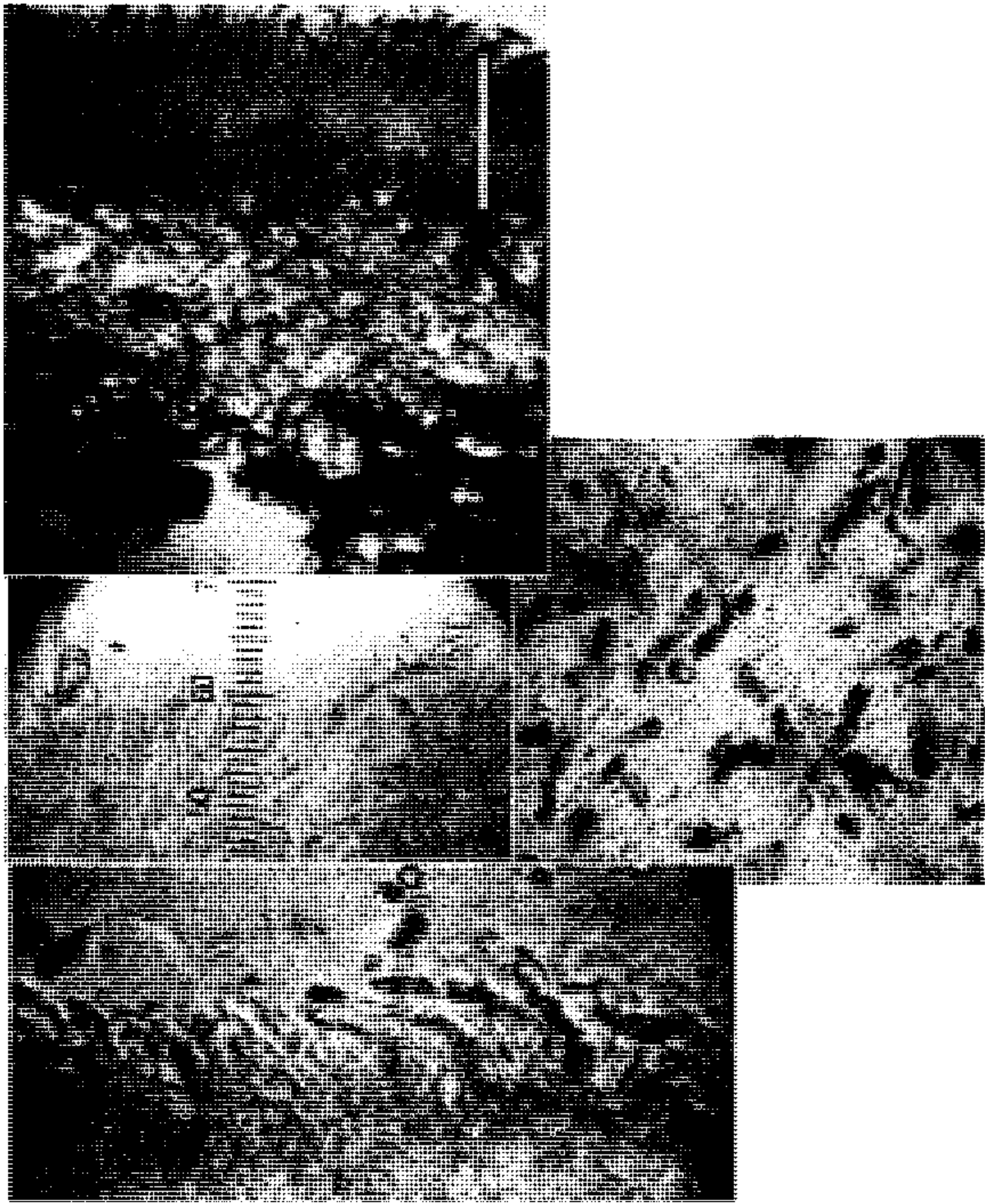


Figure 184 Cutis *ofRussula brunneoviolacea.* Top: section through cutis and underlying tissues, the tramal tissue is darkly stained at the bottom of the photograph, the area between this and the denser subcutis is the hypodermis filled with inflated hyphae, air spaces and sphaerocytes, in this patch of cutis it is wide, in other areas it lacks inflated elements and consists only of parallel hyphae, scale bar is lOOum; middle left, epicutal hyphae and pileocystidia stained in Congo red; middle right, surface view of cutis stained in SV showing darker pileocystidia; bottom, sectional view of pileocystidia stained in SV at epicutal surface, 10 scale divisions are 25 um for the last three scales.

***Russula abietina* Peck**

Annual Report of the New York State Museum 54:180. 1901

Cap 3.3-9cm diameter, almost spherical when young, becoming convex with a small central depression, eventually almost plane but shallowly depressed, the margin curved over the gill ends, eventually uplifted, smooth, the outer 5mm or so striate in age. Colour, generally a mixture of olive green and brownish-yellow centrally and pinkish purple towards the margins, but with a wide range in colour balance and tone. Young caps may be deep brownish purple, almost black, yellowish pink, yellow-brown or olive, mature caps may be zoned or mottled with brownish red, bronze, grey-brown, or pink and green, fading to pale dull green with traces of pink at the margin, generally, dull green is rarely absent. Cutis viscid when wet, drying glossy to subglossy, peelable 1/4 to 1/2 radius, flesh white, pinkish just under the cutis, unchanging, up to 5mm thick at half-radius.

Gills cream at first, becoming a light orange-yellow at maturity, adnexed to ascending, sometimes with a tiny decurrent tooth, to free, close to subdistant, sometimes distant, usually with subgills and forking throughout the radius, ventricose, around twice the depth of the cap trama at half-radius when mature, obtuse to rounded at the cap margin, unchanging when bruised.

Stipe 2.6-7.5 x 1.4-2.5cm, generally longer than the cap diameter, cylindrical to slightly clavate, white, longitudinally slightly rugulose, stuffed with a fairly firm bread-textured trama that becomes cavitate and finally hollow in age, unchanging to slightly greying beneath the rind when cut. Sometimes a wad of soil-encrusted mycelium remains attached to the stipe base on collection.

Texture about average, firmer when young, quite soft and fragile in age.

Taste mild, sometimes nutty, with a faint peppery aftertaste.

Odour not distinctive to slightly nutty.

Spore colour a deep warm cream to pale orange, Romagnesi IIIa-c.

Spores 7.5-9.5 x 6-8um, L:W 1.1-1.38 with a mean of 1.24 (n=66), subglobose to broadly ellipsoidal for the majority of spores (from 4-spored basidia), also large spores ranging from around 10.3 x 8um up to 12.1 x 9um and sometimes as narrow as 6.9um, obovoid to narrowly ellipsoidal, from 2-spored basidia, these are more common in some basidiomata than others. **Ornamentation** of conical, peg-like to short-crestate warts 0.6-1.2um (-1.5) um high, that are mostly isolated, sometimes 2-4 clustered or occasionally catenate, with few to no fine lines and no reticulum. **Woo types** A2-3, B2-3, rarely C2-3. **Suprahilar patch** an irregular roughly elliptical to rectangular amyloid patch with warts on its borders. **Hiliferous appendix** around 1.5-1.9 um long, 1-1.2um wide near the base. **Basidia** the majority 4-spored, occasionally 2- and rarely 1-spored, 34-53 x 9-12.5ujn, clavate to slightly bulbous in the upper 1/3, generally rather smoothly tapering downward. Sterigmata 5-7um long and 1.4-1.9um wide at the base. **Pleurocystidia** common to sometimes sparse, irregularly distributed, 52-85 x 8-12um, occasionally up to 1 lOum long, protruding 10-30u,m, originating at all levels within the subhymenium, narrowly clavate or fiisoid, tips acute, rostrate, or sometimes with a narrow meandering appendage up to 20um long, contents pale yellowish and refractive in KOH, unreacting or purple in SV, whole patches of gill may have only unstaining ones. **Cheilocystidia** abundant to rare, protruding to 20um, 50-55 x 5-10um wide, fusoid, tips blunt and irregularly shaped, mucronate, tapered or capitate, partially filled with refractive material, not reacting in SV. **Subhymenium** 20-30um thick, pseudoparenchymatous, expanding by one cell for each successive basidium formed. **Gill trama** of sphaerocytes with occasional vascular hyphae 5-6um wide.

Cutis from 70um thick near the cap margin, to 200um thick over the disc, an ixotrichodermis of three layers, an epicutal turf, a interwoven layer of subcutis and a repent layer of subcutis. **Subcutis** of tightly interwoven and tangled pinkish hyphae around 2um wide, toward the base of the subcutis are bundles of repent hyphae which are interwoven with the upper trama, vascular hyphae rare. **Epicutis** around 40-50um thick, of erect hyphae and abundant pileocystidia, upright erect hyaline hyphae 1.6-2.6 wide, tips undifferentiated or more often with a long tapered end cell up to 50um long, sometimes tortuous and/or knobbly, accompanied by pileocystidia and pseudocystidia in variable densities. **Pileocystidia** 25-90 x 3-6um, cylindrical, sometimes with a clavate or broader cylindrical terminal cell 6-7um wide and then with a spathulate outline, tips obtuse, some pileocystidia short-clavate 25-55 x 5-8um, those under 50um are 0-1-septate, longer ones are 2-3-septate, or with a septum approximately every 20-30um, contents partially refractive, pale yellow, unstaining to weakly greying in SV. There is usually a mixture of long-cylindrical, short-clavate and cylindrical cystidia with clavate ends, but the predominance of one or the other type varies between basidiomata and between the cap centre and margin. **Pseudocystidia** common toward the cap margins, infrequent over the disc, it is possible that some of these structures are actually primordial hyphae, since the contents are only weakly refractive, partially greying in SV. **Primordial hyphae** probably present, in young basidiomata especially, some of the cystidioid surface hyphae stain temporarily in acid fuchsia, although this is mostly the cell contents, in some SV mounts and in aqueous methylene blue, patches of cystidioid hyphae have minutely roughened walls, usually adjacent to a septum, but fuchsin does not differentially stain these areas. **Hypodermis** not differentiated from trama.

Trama of discrete clusters of sphaerocytes bound by a hyphal mesh with very occasional vascular hyphae around 4um wide, not reacting in SV.

Chemical reactions: FeSC>4 - greyish pink to salmon pink; KOH - brownish orange to reddish on cap surface, unreacting to yellowish on stipe; NH4OH - no reaction; guaiac - blue-green; phenol - slowly brownish purple, taking up to 10 minutes to react; SV - deep brownish pink on gills and cutis, cystidia and vascular hyphae weakly to strongly grey or purple.

Habitat and tree associations: On well drained soils on east or west-facing slopes under a broad age range of Douglas fir and western hemlock, with understorey of sword fern and huckleberry. Grand fir may be present in the vicinity as it is common in these forest stands, but it was not looked for at the time of collection.

Collections: CR001104-01 along a trail downslope from the parking lot, on duff under young regeneration western hemlock and Douglas fir, Gowland Tod Provincial Park N48.527°,W123.529°. DGO10902-01, amidst sword fern and huckleberry under mixed age Douglas fir, western hemlock and western red cedar, just off the High Ridge trail, Francis King Park, N48.4813^0, W123.449317^0. CR021009-02, on duff with understory and old-growth western hemlock and Douglas fir with western red cedar, Royal Roads N48.434100^0, W123. 478350° (all from the CDF zone).

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR001104-01	848	363	500,358,285	322,205
DG010902-01	874	419,390	491,290	357,255

Notes: Although the type of *Russula abietina* Peck is considered by Singer (1947) to be a mixture of two other species: *R. sphagnophila* and *R. blackfordae.* The above collections differ from these latter species macroscopically in stature, cap colour and spore colour. The Vancouver Island collections match the concept of *R. abietina* sensu Grand (1965) and Thiers (1997), except for ranging slightly larger than the upper cap diameter of 7.5cm given by Thiers. Whether or not Peck's type of *R. abietina* is invalid, collections answering the general description and concept of this species turn up regularly in the Pacific Northwest, so clarification of the taxonomy of this species would be welcome. This species shows the same pigment pattern as members of the *Xerampelinae* and is therefore placed in the closely related *Sphagnophilae,* with which it shares most other characters.

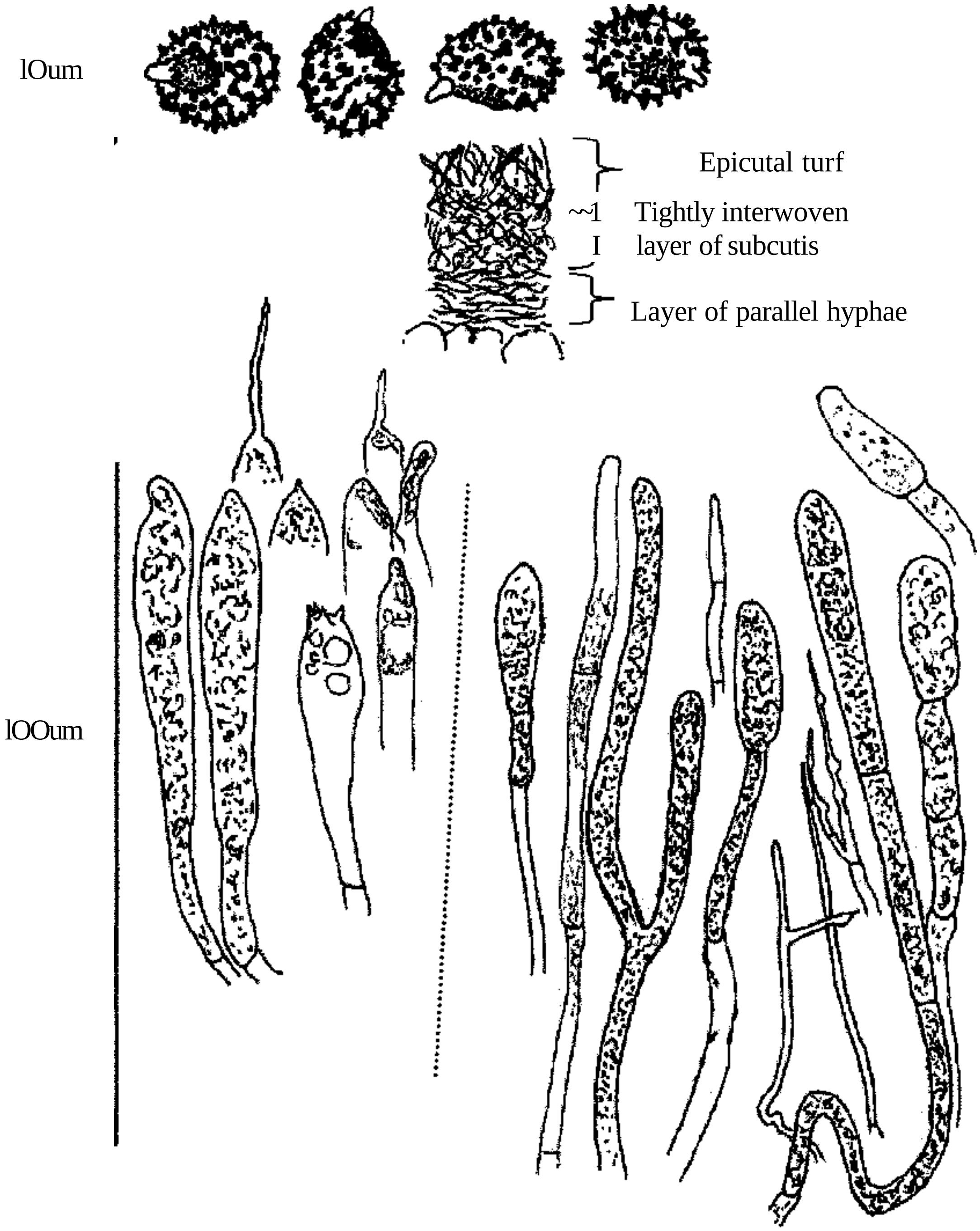


Figure 185 Microscopic characters *ofRussula abietina:* Top, spores with 10um scale bar; middle, diagram of section through cutis; bottom left, pleurocystidia, a basidium and to its right, cheilocystidia; bottom right, pileocystidia, pseudocystidia and hyphal ends from the epicutis, lower scale bar is lOOum.

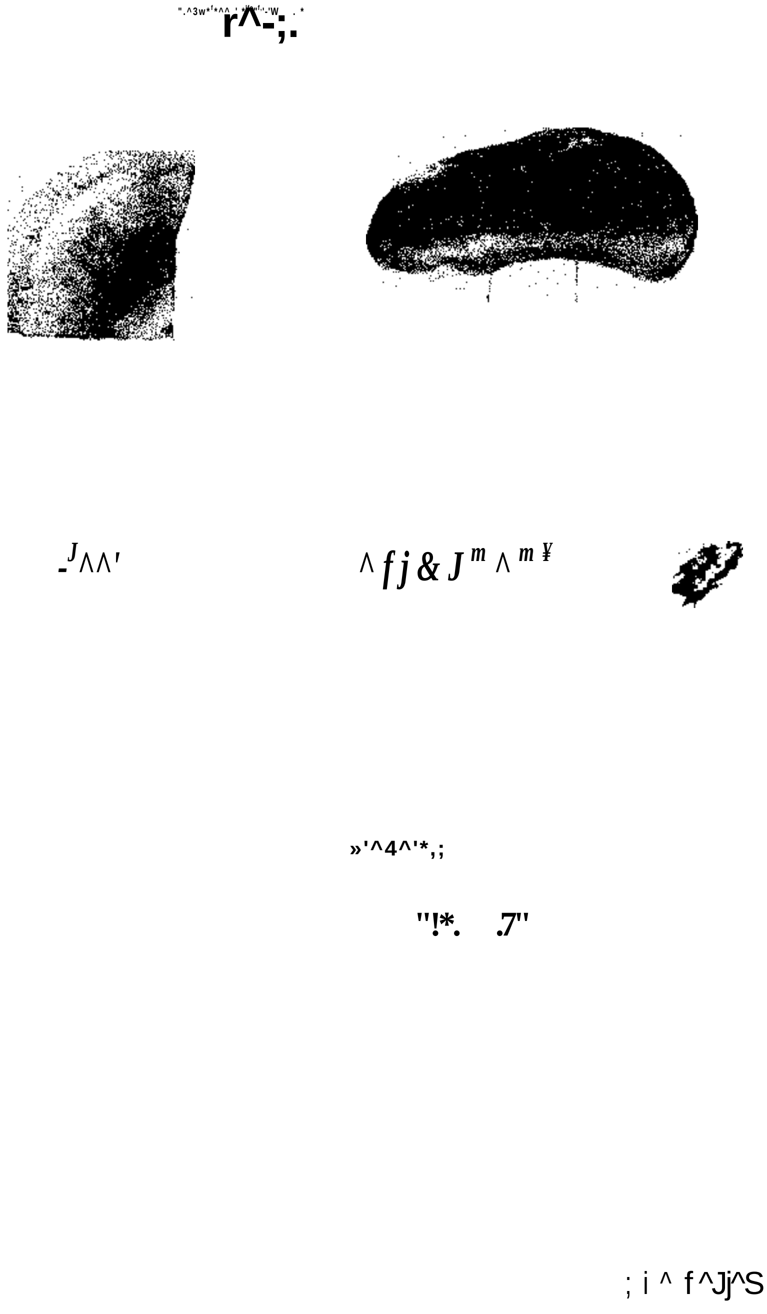


Figure 186 Macroscopic characters of *Russula abietina:* Top, illustration of profiles, top views and a longitudinal section of immature to mature basidiomata showing the colour range, the square is $1cm^2$ and shows spore colour; bottom, mature basidiomata in habitat.

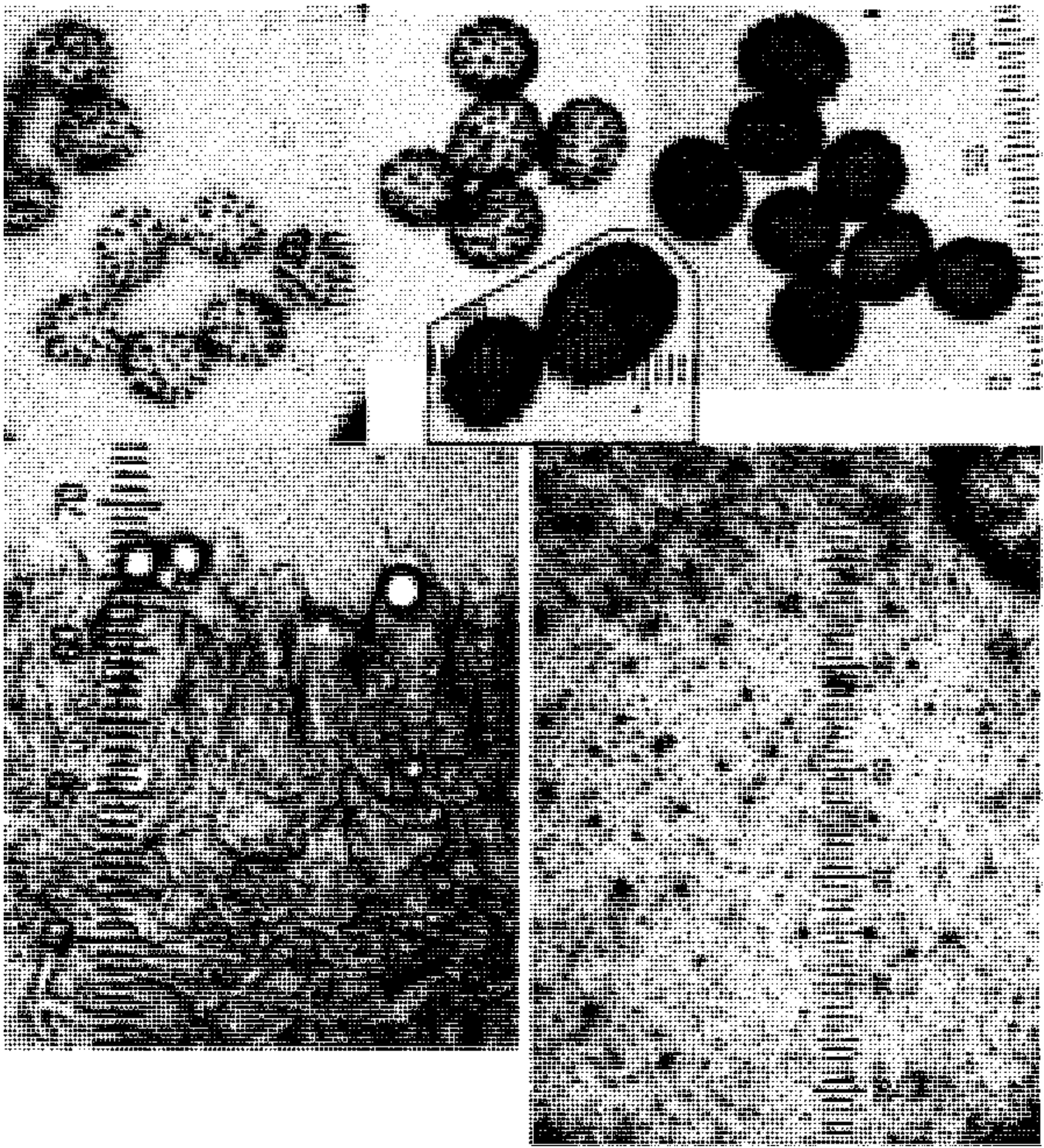


Figure 187 Hymenium *ofRussula abietina:* Top, spores in surface and equatorial view with (centre) a composite of two focal depths and inset, a spore from a 4-spored basidia and a larger one from a 2-spored basidia, scales are all in lum divisions; bottom left, section of hymenium showing immature, mature and spent basidia upon a pseudoparenchymatous subhymenium and part of the gill trama below the 40 mark, 10 scale divisions are 25um; bottom right, surface view of gill stained in SV showing the patchy distribution and reactivity to SV of pleurocystidia (dark spots), scale is in lOum divisions.

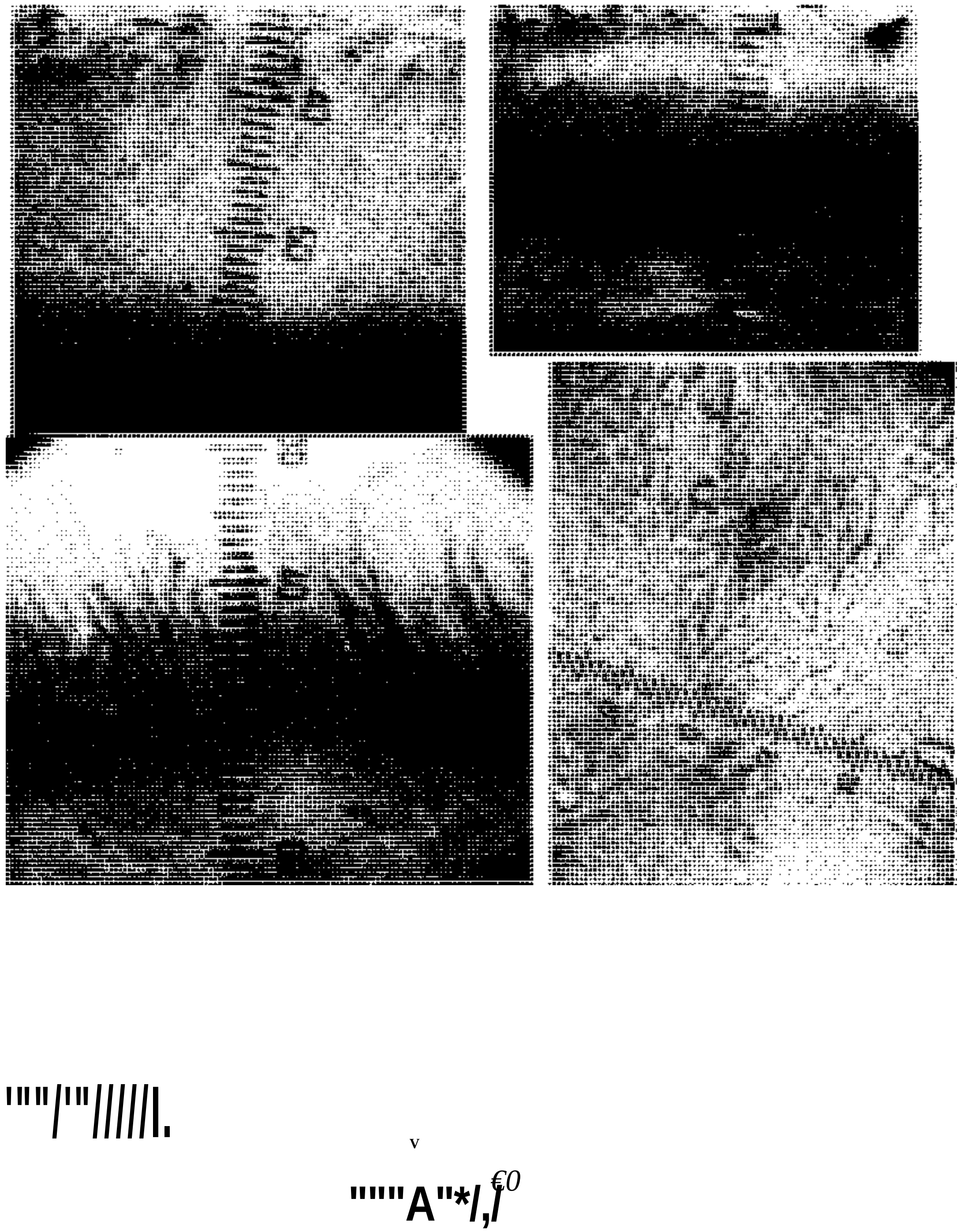


Figure 188 Cutis of *Russula abietina.* Top, section through cutis near the cap centre (left) and the margin (right), 10 scale divisions are lOOum; middle left, hyphal ends from the epicutis, stained in Congo red, 10 scale divisions are 25um; middle right, surface view of cutis in NH4OH, showing a cluster of pileocystidia, scale as left; bottom, pseudocystidia (or primordial hyphae) in SV, scale as above.

***Russula aeruginoides* nom. prov.**

Cap 10cm diameter, almost plane but with a broad, shallow central depression. Colour a clear light olive green, with a darker green concentric band around the central depression, margin striate on this mature specimen. Cutis viscid, drying subglossy, peelable 1/4 the radius, cap trama white, dull yellow under cuticle, unchanging, around 5-6mm deep at half-radius.

Gills deep yellowish cream with rusty spotting on the eroded margins, subdistant to close, some forking towards cap margin, narrowly adnexed and ascending to almost free at stipe, broadening outwards, approximately twice the depth of the cap trama at mid-radius, rounded at cap margin, brittle.

Stipe 5.5 x 2.4cm, slightly more clavate than cylindrical, creamy white, bruising a red-brown at the base, stipe rind and cap trama immediately above the gills slightly greyish, stipe stuffed with a bread-textured trama.

Texture of average *Russula* texture.

Taste mild and nutty.

Odour not distinctive.

Spore colour light orange-yellow, Romagnesi IIIc but slightly more pink.

Spores 7.1-10 x 6.1-8.3um, L:W 1.03-1.36 with a mean of 1.16, (n=30), subglobose to broadly ellipsoidal. **Ornamention** of pointed, peg-like or occasionally short-crestate, heavy, incompletely amyloid warts 1-1.5pm high, occasionally up to 2pm high, mostly isolated, sometimes with fine lines joining 2-3 warts or short catenations, not forming a reticulum. Fine, low dots are sometimes interspersed between the larger warts, especially towards the suprahilar patch. **Woo types** A3-B3. **Suprahilar patch** strongly amyloid, with small to medium sized warts on its border. **Hiliferous appendix** relatively small, 1.4-1.8pm long, 1-1.2pm wide near the base. **Basidia** most 4-spored, some 2-spored, 35-

52 x 10-14um, the majority of mature basidia are close to 37-40um long, clavate to slightly bulbous in the upper 1/2. Sterigmata about 6-10um long and 2-2.6um wide near the base. **Pleurocystidia** frequent, 60-95 x 9-13 (-15) urn, protruding 10-30um, arising from the subhymenium, fusoid, tips obtuse, acute, mucronate or most commonly with a small terminal button or allantoid appendage, contents refractive, colourless to pale yellow in KOH, red-brown or grey in S V. **Cheilocystidia** locally abundant but irregularly distributed, similar to pleurocystidia but fewer react to SV, occasional ones lack refractive contents. **Subhymenium** 12-25 um thick, pseudoparenchymatous, **gill trama** of sphaerocytes of irregular outline, with occasional vascular hyphae.

Cutis 80-150um thick, an ixotrichodermis, the turf layer becoming repent towards the margin, pigment granular, and darker in the epicutis and lower subcutis. **Subcutis** of tangled hyphae 2-4um wide, becoming more loosely interwoven toward the top and more repent and yellowish-green toward the base, embedded in a gelatinous matrix, vascular hyphae uncommon. **Epicutis** consisting of upright hyphae, 2.5-3.5um wide, containing a vacuolar and granular green pigment, hyphal ends obtuse, not differentiated, with frequent pileocystidia, embedded in a sometimes conspicuous gelatinous matrix. No incrusted hyphae and nothing staining in acid fuchsin. **Pileocystidia** 40-87 x 4-10pm, 1-3-septate, the majority being 2-septate, rarely aseptate, often constricted at the septa, clavate or with a clavate to broadly cylindrical terminal cell on a narrower base, tips obtuse, contents yellow-green refractive, staining pink in SV. **Pseudocystidia** occasional, arising from the subcutis, slightly refractive. **Hypodermis** a narrow layer up to 20pm thick, of parallel hyphae interwoven with the tramal and subcutis hyphae.

Trama of discrete clusters of sphaerocytes bound by a hyphal mesh, vascular hyphae uncommon.

Chemical reactions: FeS04 - faint salmon pink; KOH - tan to orange on the cap surface, no reaction on stipe; phenol - slowly brown; S V - red-brown on cap cutis, red on gills, pileocystidia pink, hymenial cystidia mostly stain brownish red, some grey, some non-staining.

Habitat and tree associations: On rotten log, amongst western hemlock, huckleberry and salal, in moist, level, mixed-age stand with some old growth close to an estuary.

Collections: CR981013-08 near estuary along San Joseph trail, Cape Scott, approximately N50.768°, W128.342^0 (southern very wet hypermaritime CWH subzone).

Notes: The spores are heavily ornamented and have an amyloid suprahilar patch, indicating that this species is not *R. aeruginea,* nor does it have other characteristics of the *Heterophyllidia.* This collection has the microscopic characters and outward appearance of *R. wens,* but it is not peppery and the cystidia are rarely positive in SV (pepperiness and SV positive staining are often correlated). *Russula olivacea,* which can be greenish, tends to also have purple hues in the cap, pink on the stipe, and a dry, concentrically areolate cutis with a smooth cap margin, and lacks pileocystidia. The granular pigment is similar to that found in *Russula murrillii* and *R. puellaris,* save that it is green rather than pink. This collection fits best in the *Sphagnophilae* and is closest to *Russula nitida,* a slightly smaller species (up to 7cm cap diameter), which occasionally fades to a greenish colour from brownish purple, and grows in moist, acidic soils, though rarely with spruce or other conifers. Unfortunately, only one collection of one mature basidiomata has been found, so it would seem to be very rare.

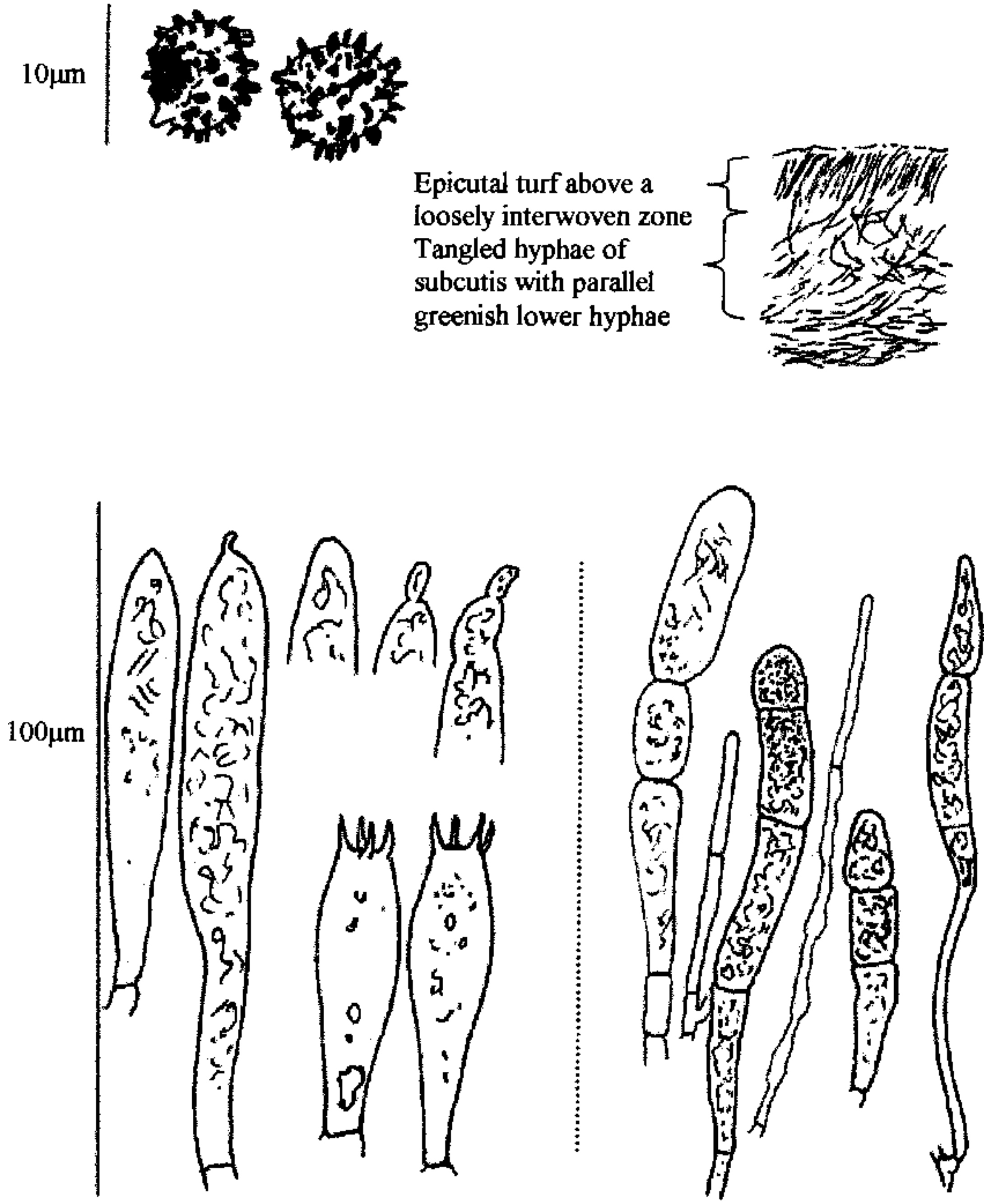


Figure 189 Microscopic characters of *Russula aeruginoides:* Top, spores with lOum scale bar; middle, diagram of section through cutis; bottom left, hymenial cystidia and basidia; bottom right, pileocystidia, hyphal ends, lower scale bar is lOOum.

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Figure 190 Macroscopic and articular pigmentation characters of *Russula aeruginoides:* Top, profile and longitudinal section of a mature basidioma, square is $1 cm^2$ and shows spore colour; bottom, two colour photomicrographs of the surface of the epicutis showing the green vacuolar pigment which is more apparent in the left picture, and the granular inclusions, more apparent at a lower focal point in the right picture, scale for both photographs is in 1pm divisions.

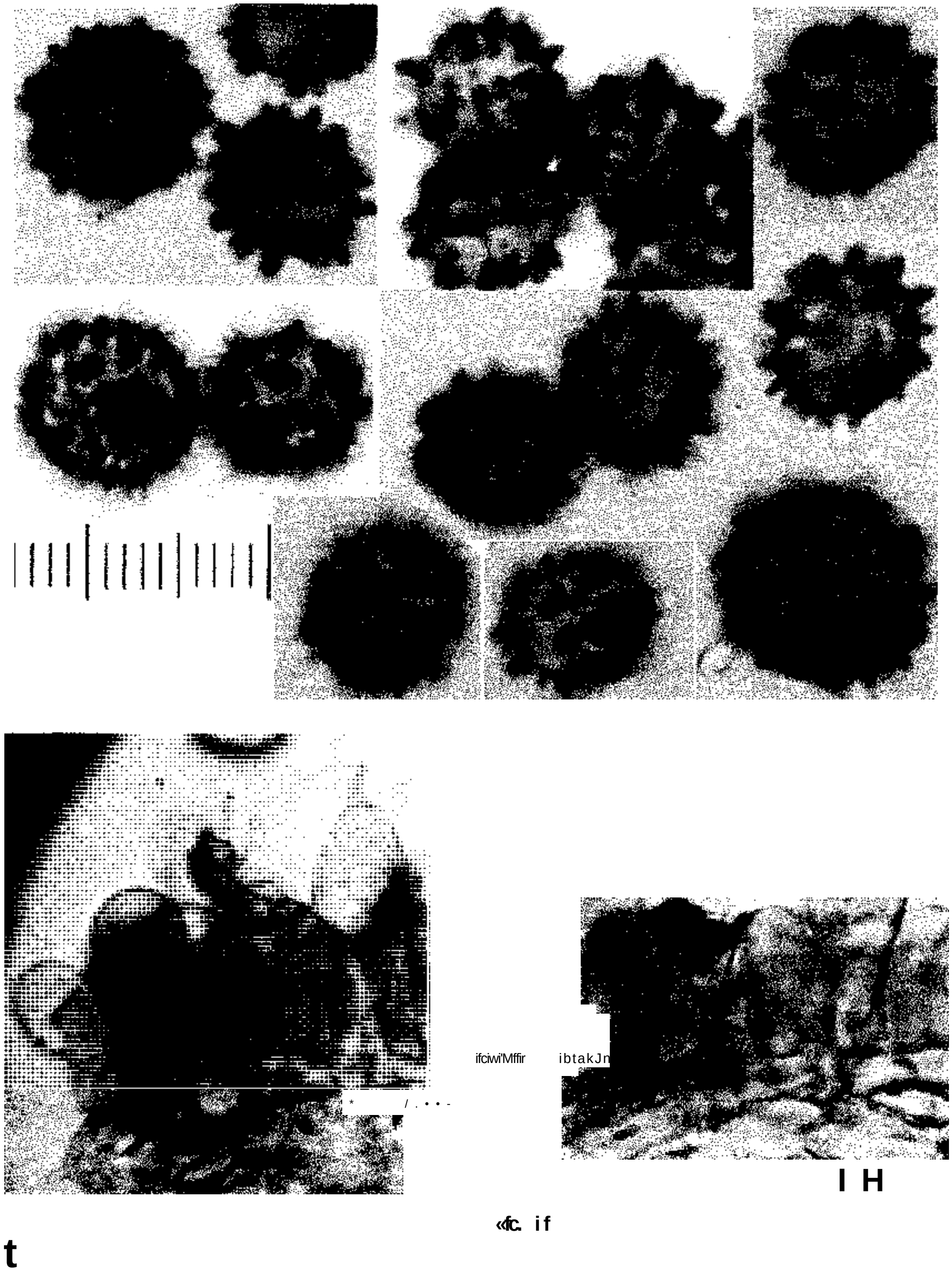

Figure 191 Characters of the hymenium of *Russula aeruginoides:* Top, spores with lum division scale; bottom, section of hymenium showing basidia, basidioles and subhymenium, 10 scale divisions are 25 um.

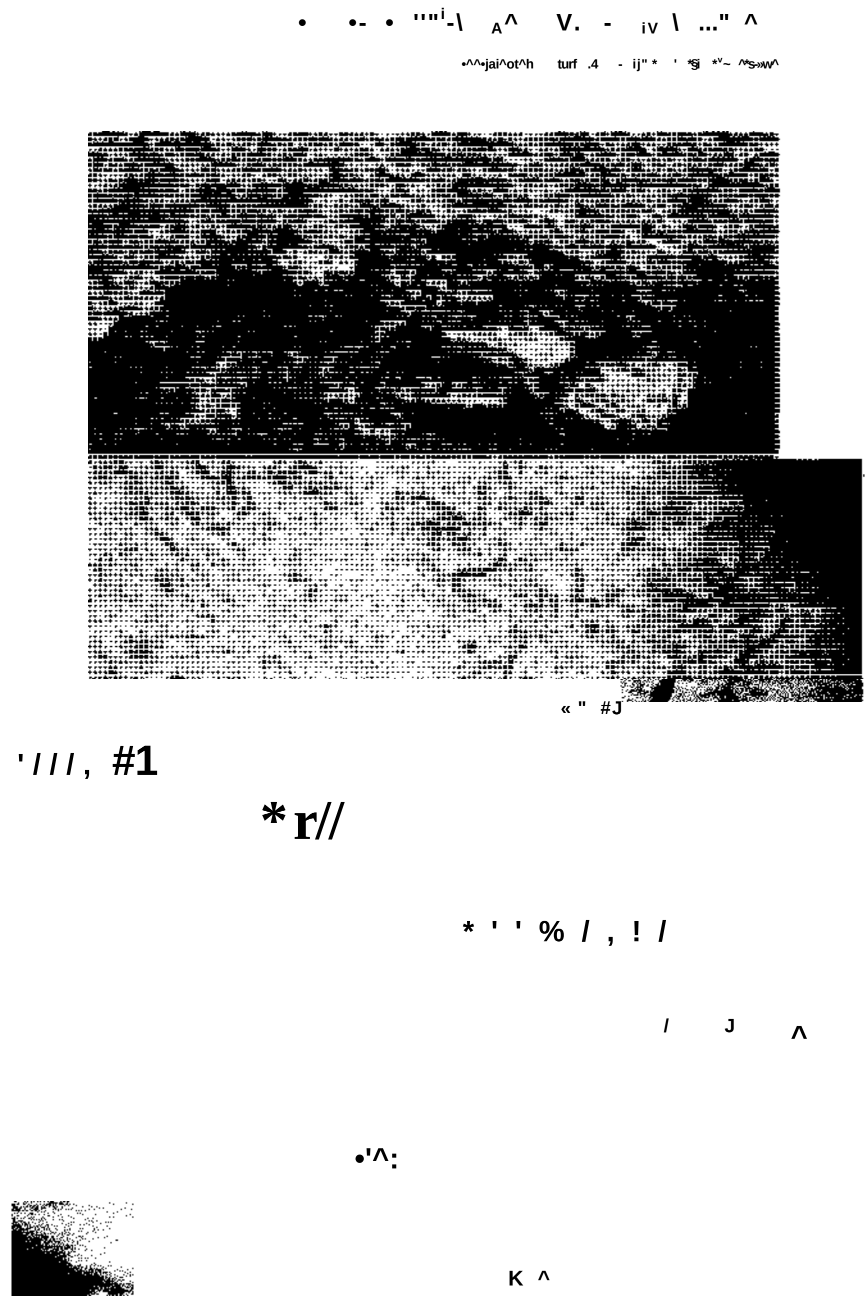

Figure 192 Cutis of *Russula aeruginoides:* Top, section through cutis, 10 scale divisions are lOOum; bottom, surface view of cutis in 3% NH4OH showing epicutal hyphal ends and broad, septate pileocystidia with refractive contents, 10 scale divisions are 25 um.

Clade 9b

Subsection *Xerampelinae* Singer

Beih. Bot. Centralbl. 49(2): 240. 1932

Subsection *Xerampelinae* Singer is in section *Polychromae* R. Maire subgenus *Russula,* in the classification system of Saraari (1998), and replaces the name *Viridantinae* Melzer-Zvara which has been in use since 1927 for this group. Taxonomically this is a well defined group whose member species are relatively easy to recognize macroscopically from the combination of mild taste, odour of trimethylamine (shellfish), deep cream to yellow spores, flesh which turns ochre yellow and eventually brownish where damaged, and a grey-green to blue-green reaction with $FeSO_4$. Most stain red in aniline (Grand, 1965, Romagnesi 1967). Microscopically, they have few pileocystidia, which, together with the hymenial cystidia, stain weakly if at all in SV, and spores with an ornamentation of conical or bluntly conical warts, mostly 0.8 to 1.2um high, isolated or catenate with very little reticulum development.

There are several varieties of this species, named according to the colour and texture of the cap cuticle. A few of these varieties such as *Russula elaeodes* (Bresad.: Romagn.) Bon, *R. grundii* Thiers and *R. semirubra* have been raised to the status of species. One variety, *R. xerampelina* var *isabellinaceps* published as nom prov. by Grand (1965) in his Ph.D. thesis, has consistent morphological differences from the sister species and does not appear to intergrade with any of them, and warrants being raised to species level. The restriction fragment lengths of the ITS region of the rDNA gene of *R. elaeodes, R. isabelliniceps, R. semirubra* and *R. xerampelina* var. *xerampelina* all show very similar patterns, and these in turn closely match the RFLP patterns of the sequenced ITS regions of four isolates of *R. xerampelina* whose sequences were downloaded from Genbank.

Russula erythropoda and *R. xerampelina* var. *erythropoda* are considered synonyms of *R. xerampelina* by many authors including Romagnesi (1967), Bon (1987), Buczacki

(1992), Courtecuisse and Duhem (1995) and Adamclk, who designated a neotype of the species (2002).

One of the collections, *R. ctpruinosa* was initially thought to be a member of subgenus *Amoenula* Samari, because of the lack of pileocystidia and cheilocystidia in several of the mounts, and because of the elongated terminal cells of the epicutal hyphae. However, the RFLP pattern of the ITS region matched that of other *Xerampelinae* and did not match that derived from a DNA sequence of *R. amoenicolor* from GenBank. Also, the chromatograph of cuticle pigments showed a red which did not fluoresce in blue LED light, in common with that found in other *Xerampelinae*.

***Russula xerampelina* (Schaeff.) Fries**

Epicrisis Systematis Mycologici: 356. 1838

Cap 5.2-12.5cm diameter, convex at first becoming centrally depressed at maturity, margin smooth, rounded with the cutis often extending down and under the edge of the cap trama so that it looks somewhat inrolled, and the pigment extends along the outer gill margin a short distance to up to halfway. Colour ochraceous-olive when very young, becoming deep purple with a darker purple to black centre, occasionally with mottled areas of yellow ochre or brownish-olive, sometimes this colour in the cap centre. Surface viscid and usually with a slight bloom, drying matte. Cutis peels 1/2 to 2/3 of the radius. Cap trama cream, purplish-pink under the cutis.

Gills close to subdistant, sometimes quite coarse, adnexed to sinuate at stipe, acute at margin, arched, narrowest at stipe and broadening outwards, approximately equal in depth to that of the cap trama at half-radius, the margins parallel to the cap surface, margins entire. Colour cream when young, becoming warm yellow to light orange-yellow at maturity and developing brown stains where damaged and on the gill margins. Anastomoses frequent, especially near the stipe but often distributed throughout the radius, (fig. 195) free sub-gills rare.

Stipe 5.0-8.2 x 1.1-3.1cm, cylindrical to clavate, with a firm rind 2-5mm thick, stuffed with a bread-textured trama that develops irregular cavities and turns pinkish-brown on cutting and around insect damage. Surface longitudinally finely rugulose, pruinose, cream to light yellow, flushed partly or entirely with pink, bruising ochre-yellow when scratched, eventually browning.

Texture quite firm and somewhat brittle, gills brittle.

Taste mild, sweetish or nutty, sometimes slightly peppery, especially when young.

Odour not distinctive or mushroomy when young, sometimes with a rubbery component, gradually developing a crab or shrimp smell as it ages, eventually smelling strongly fishy. In dry weather, the mushroom may become too dry to detect much odour.

Spore colour light orange-yellow, Romagnesi Illb-c.

Spores 6.2-8.5 x 5-7.5um for the Strathcona collection, 6.8-10 (-12) x 6-8um (-10pm) for the other lowland collections, overall mean 7.56 x 6.34um; L:W ratio 0.94-1.45, mean 1.2 (n=30), ellipsoidal, many a curved teardrop shape with the suprahilar patch area flattened or slightly concave in side view. **Ornamentation** of sharply or bluntly conical warts, 0.8-1.2um (-1.5pm) high, isolated or catenate, a few joined by lines but not forming a reticulum, or occasionally a partial reticulum seen. **Woo types B3** to **C2** to **C3. Suprahilar patch** amyloid, a distinct grey area in Melzer's reagent, with uneven edges and with occasional low warts at the margin. **Basidia** 40-55 x 10-15um, clavate, mostly 4 -spored but 2 -spored ones common, giving rise to the larger end of the size range of spores. Sterigmata 5-8um long. **Cheilocystidia** rare, not staining in SV or staining pinkish to red, as does the gill margin. **Pleurocystidia,** scattered to evenly distributed, staining reddish or weakly grey in SV, protruding only 10-25um beyond basidioles, 67-120 x 9-15 p.m, but most 10-11 urn wide, many elliptical in end view, clavate, sometimes pinched or crumpled, ends narrowing, blunt, developing one to a series of 2 to 3 capita in diminishing sizes (ampullaceous) in aging sporocarps, (fig. 194.) **Subhyinenium** about 50-70um thick, parenchymatous, gill trama of sphaerocytes without vascular hyphae.

Cutis 60-140um thick at half radius, up to 250pm thick on disc, of tightly interwoven and felted hyphae within a gelatinous matrix. **Subcutis** about half the depth of the cutis but not well differentiated, the **epicutis** merely of slightly more upright felted hyphae with few free hyphal ends visible. In water mounts of cutis sections, the red-purple colour is stronger in a layer just above the trama and in the epicutis, with a paler band between, the colour visibly leaching out into the mount. **Pileocystidia,** unevenly distributed with some areas of the cutis apparently devoid of them, other parts with small clusters of them, 30-70um x 5-7.5pm, 0-1-septate, cylindrical to narrowly clavate with rounded ends, many with a pinched or narrowed end, contents refractive but not as strongly so as on most other Russulas. **Pseudocystidia,** none seen. Cuticular hyphae 2.5-4um wide, with hyphal ends at the cap surface with blunt cylindrical, tapering or lance-shaped end cells up to 5 um wide, occasionally forming low clusters protruding about 20um beyond the surrounding surface.

Trama of bundles of hyphae enclosing clusters of sphaerocytes, lacking SV-positive laticiferous hyphae.

Chemical reactions: FeS(>4 - blue-green; KOH - red to orange red on cap surface (slightly browner on young, still yellowish caps), yellowish on stipe; MHUOH - no reaction; phenol - brownish purple; S V - on gills brownish-purple to purple-grey, on cutis pink-grey.

Habitat and tree associations: Found from low-lying coastal regions to at least 300m, in well drained and often dry soils from August to December among Douglas fir and hemlocks and mixed coniferous forest containing one or both of these trees. *Russula xerampelina* generally occurs singly or in small groups of 2 or 3, but may be more abundant in good years such as 2004.

Collections: CR010814-03, Fairy Lake near Port Renfrew, N48.5840^0, W124.0035^0 (southern very wet hypermaritime CWH subzone), two immature sporocarps with yellow ochre to olive colours and a finely areolate cuticle texture, found with mature western hemlock and Sitka spruce. This latter collection has the texture and habitat associated with *R. isabelliniceps* and may be that species, but the DNA analysis, in particular the RFLP pattern for the enzyme AluI showed a closer relationship to *R. xerampelina*. CR001001-02 Koksilah Ridge N48.6558^0, W123.7357^0 on a rocky knoll with a small Douglas fir, Oregon grape and huckleberry (eastern very dry maritime CWH subzone). CR980821-05 Strathcona Park, Phillips Ridge trail at about 300m, N49.59°, W125.583^0 amongst Douglas fir, hemlock sp. and Abies sp. (windward moist maritime MH subzone). CR980807-03 Rocky Point N48.3162^0, W123.5818^0 amongst old growth Douglas fir. CR001202-02 Rocky Point N48.3313^0, W123.5551^0, amongst second growth Douglas fir, with some western hemlock and madrone. CR001127-01 Royal

Roads N48.4369^0, W123.4793^0 in a mixed-age stand of Douglas fir. Collections from Saturaa Island: PJ981122 from under Douglas fir on a roughly south facing slope on a lawn, N48.799114^0, W123.178658°; PJ981018-ER17 from Saturna Island Ecological Reserve, amongst mature Douglas fir with understory western hemlock, N48.774588°, W123.155597°. (The latter 5 collections from the CDF zone).

Two collections were made from the forests around the Breitenbush area in the Oregon Cascade mountains for comparison with the Vancouver Island material. These showed very slight differences in morphology in that the terminal hyphae in the cutis are more upright, patches of cutis have broader hyphae at 4.5 to 8urn (within a couple of millimetres from areas of the narrower type), and the cutis is generally 200 to 250um thick. Other characters are no different, and the DNA is very similar to the Rocky Point collection in particular.

		RFLPs:		
Collections	ITSl-FtoITS4-B	Hinfl	Alul	Sau3A
CR001001-02	885	427,343	531,295	341,266,205
CR001127-01	900	427, 336	530, 282	341, 266, 205
CR001202-02	852	416, 340	506, 295	367, 270, 218
CR010814-03	880	419,353	511,278	326,261,200
R. xerampelina (Breitenbush, OR)	874	416, 340	506, 295	367, 252, 205

Notes: These collections agree very well with the descriptions of Northern California *Russula xerampelina* sensu Thiers (1997), Washington collections (Grund, 1964), and the European description sensu Romagnesi (1967), and with the description and illustrations for var. *xerampelina* in particular of AdamcTk (2002). The spores of the Vancouver Island collections span a greater range than those of the neotype of Adamclk (2002) which range from 8.2-10.6um x 6.3-8.2um. Romagnesi (1967) gives the spore size range from 7.7-11.5um x 7-8.5um. The ITS rDNA region shows some variation between individuals but the general pattern of all the RFLP's is remarkably consistent within the species, other members of *the XerampeHnae* group and to those derived from sequences ofi?. *xerampelina* from GenBank.

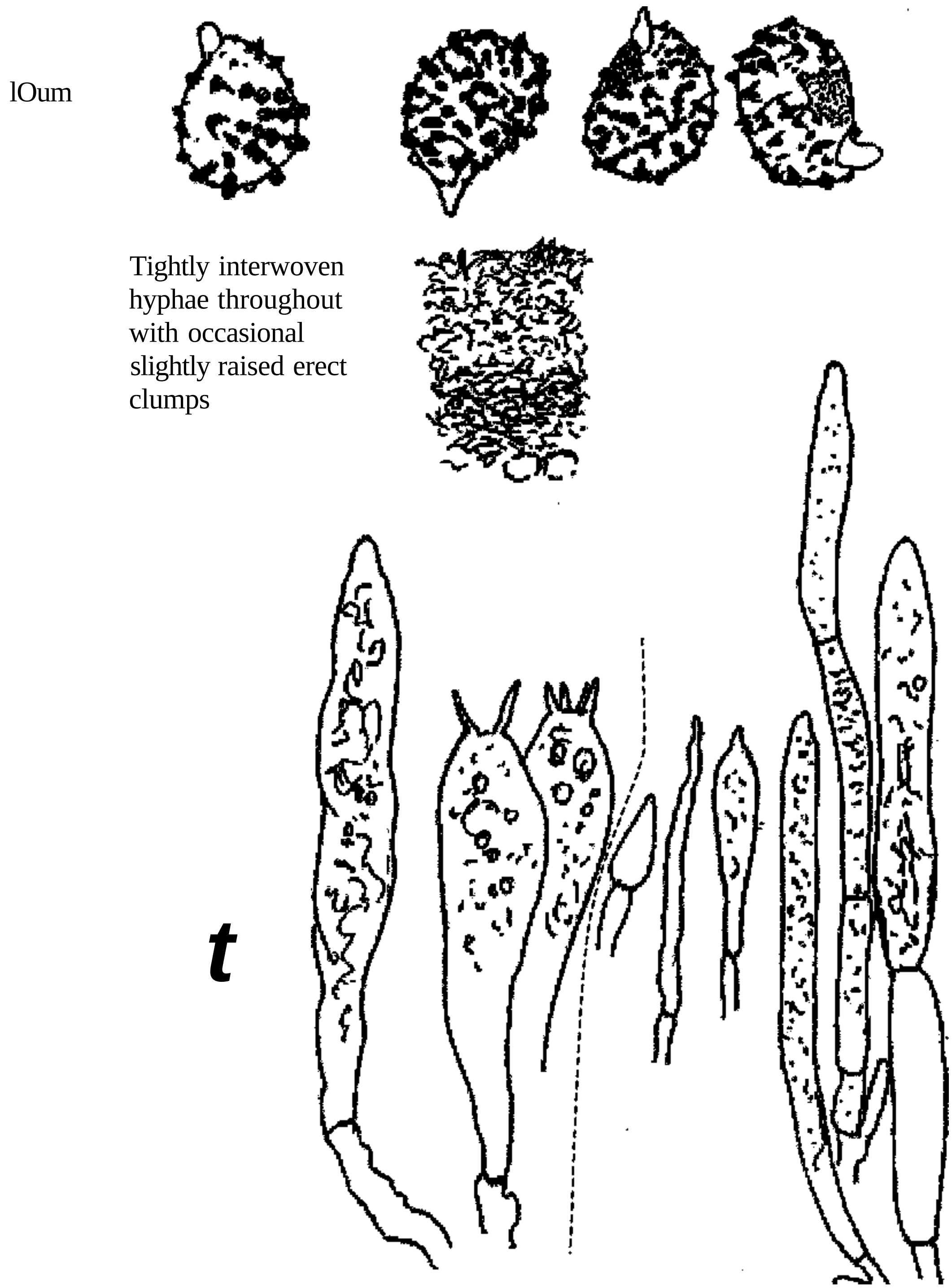

Figure 193 Microscopic characters of *Russula xerampelina:* Top, spores withlOum scale bar; middle, plan of cutis in section in 5% KOH; lower left, basidia and hymenial cystidia; lower right, pileocystidia and hyphal ends in cutis, lower scale bar is lOOum.

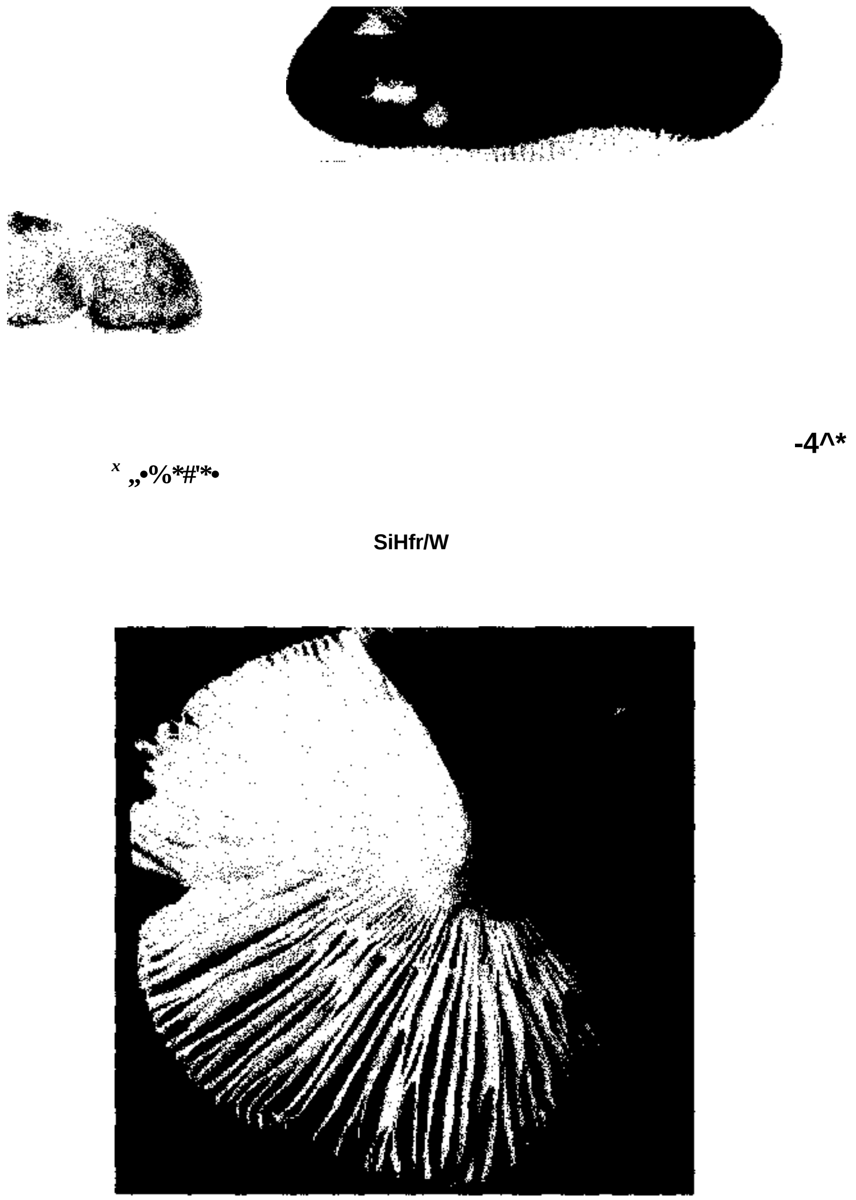

Figure 194 Macroscopic characters of *Russula xerampelina:* Top, illustration of three phases in development from yellowish when young to purple in maturity, profiles, longitudinal sections and gills, square is 1cm^2 and shows spore deposit colour; bottom, *R xerampelina* gills showing anastomoses common in this species, note also the almost completely pink stipe.

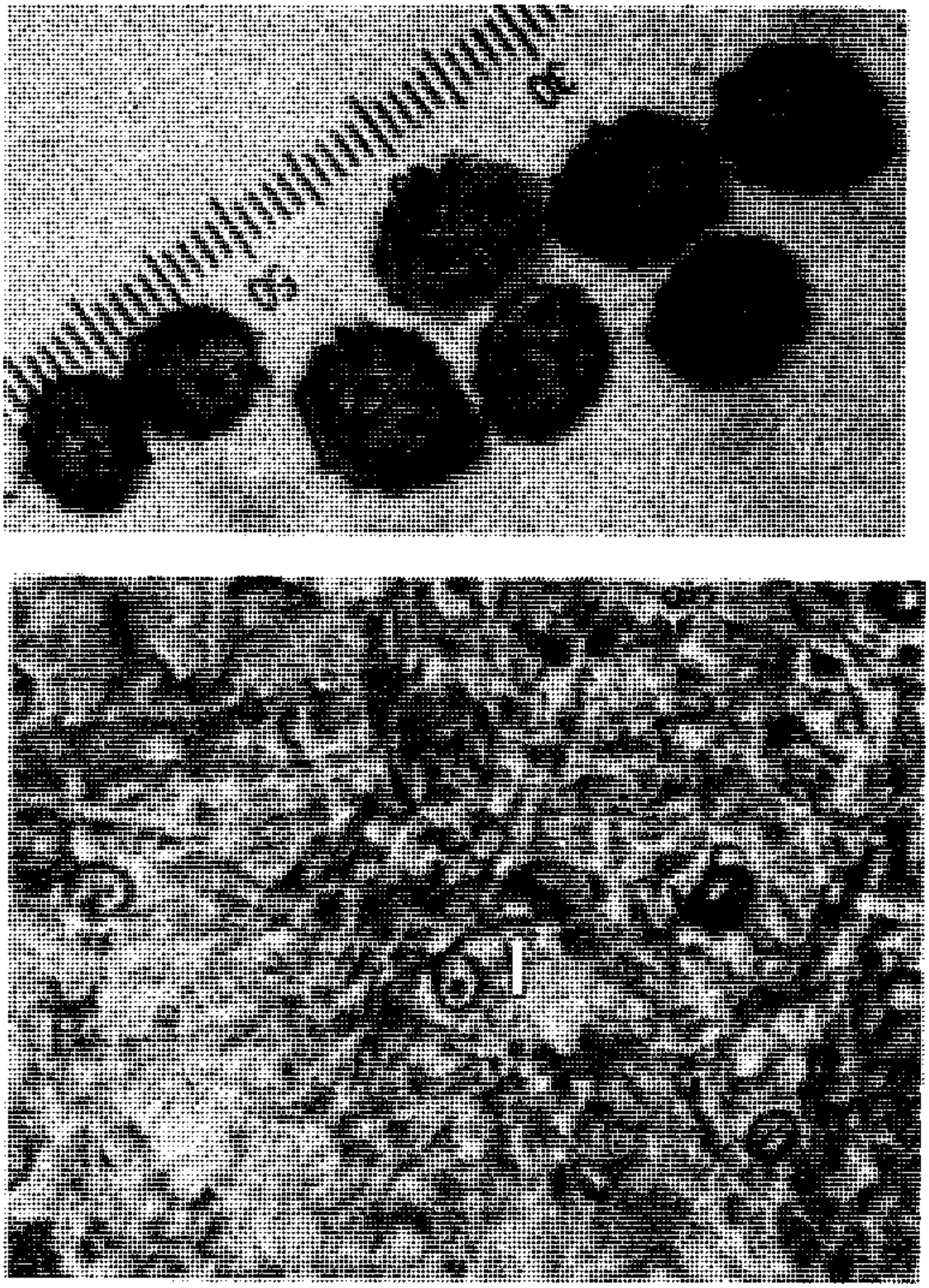

Figure 195 Hymenium and cutis of *Russula xerampelina:* Top, spores with lum division scale (composite photograph of two depths of focus); bottom, surface view of cap cutis, arrow points out a pileocystidia, scale bar is lOum (rounded objects are spores.)

***Russula semiruhra* Singer**

Sydowia 11:218. 1957

Cap 3.7-16cm diameter, convex at first becoming plane to shallowly centrally depressed at maturity, margin smooth, rounded with cutis colour extending l-2mm along the outer gill margin. Colour a strong primary red to deep carmine tending to black or darker red at the centre, in older caps the red fading to a pinkish-red with a deep pinkish-red to red-brick colour in the centre, and with a whitish bloom. Surface viscid, drying matte. Cutis peels 1/3 to 1/2 of the radius. Cap flesh cream, deep rose-pink under the cutis.

GUIs Subdistant to moderately close, sometimes quite coarse, adnexed to sinuate to free at stipe, rounded at margin, ventricose, slightly broader than the depth of the cap trama at half-radius, up to 2cm on larger caps, gill margins entire, staining brown in age and where damaged. Colour cream when young, becoming deep cream to pale orange-yellow at maturity. Some anastomoses near the stipe, free sub-gills rare.

Stipe 1.3-12.4 x 1.0-2.8cm, cylindrical to clavate, with a firm pale yellow rind 2 to 5mm thick, and an interior stuffed with a bread-textured trama that develops irregular cavities and turns an orange-brown to pink-brown on cutting and around insect damage. Surface longitudinally finely rugulose, pruinose, cream to light yellow, flushed partly or entirely with pink, bruising ochre-yellow when scratched, eventually browning.

Texture quite firm, not brittle when young, becoming so in age, gills brittle.

Taste mild, sweetish or nutty, sometimes slightly peppery.

Odour fishy, or of crab or shrimp, becoming stronger in age.

Spore colour deep cream or pale orange-yellow, Romagnesi Ilia

Spores 8-12um x 6.5-9um, L:W ratiol. 14-1.43, mean (N=30) 1.27, broadly ellipsoidal to subglobose. **Ornamentation** of sharply or bluntly conical warts, 0.5-1.5 (-2.0pm) high, isolated or catenate, a few joined by lines but not forming a reticulum, or occasionally a partial reticulum seen. **Woo types** B2, B3, C2 or C3. In two collections the ornamentation is similar to that of *R. xerampelina* with warts mostly 0.8-1 um high but ranging from 0.5-1.5pm. The Combers Beach trail collection has spores with more pointed, narrow warts up to 2pm high, with fewer connectives between them, of Woo type 3A or 3B. **Suprahilar patch** amyloid, a distinct grey area in Melzer's with uneven edges with a few low warts at the margin, on some spores this grey-stained area encloses the lower part of the hyliferous appendix like a collar. **Basidia** 40-70um x 10-15pm, most in the 50-60um x 12-14pm range, clavate, 4-spored or occasionally 2-spored, many with a sterile companion cell arising from the same basal cell. Sterigmata 5-8um long. **Cheilocystidia** rare, not staining in SV or staining pinkish to red, as does the gill margin. **Pleurocystidia,** scattered to evenly distributed, staining dark red to purple in SV, sometimes banded or incompletely staining, not protruding or only protruding 10 to 20um beyond basidioles, originating in the subhymenium, 55-120pm x 8-l5um, but most arel0-12um wide, many elliptical in end view, cylindrical to narrowly clavate or fusoid, ends blunt, with an obtuse point, a short tapering terminus which may be constricted at intervals, or with a small capitum. **Subhymenium** 25-50um thick, parenchymatous, **gill trama** of sphaerocytes without vascular hyphae.

Cutis 160-250pm thick. **Subcutis** approximately 1/2 to 2/3 of the cutis thickness and consisting of tightly interwoven hyphae within a gelatinous matrix. **Epicutis** of more or less erect hyphae forming a turf in which hyphae are clumped into fascicles, only slightly gelatinised. In water mounts of cutis sections, the subcutis is pinkish and the epicutis more brownish-pink. Cuticular hyphae arel.7-5um wide, most at the narrower end of the range, except in the epicutis, hyphal ends septate and sometimes broadening around the septum, end cells cylindrical, tapering or ampulliform. On the outer 1/4 of the cap diameter, the cutis thins to 100pm and the epicutis becomes interwoven and indistinct from the subcutis. **Pileocystidia,** rare and gathered into small patches on the cutis, absent elsewhere, cylindrical to narrowly clavate with obtuse ends, 0-1 septate, 30-100um long x 5-10pm wide, contents refractive but not as strongly so as on most other *Russulas,* light grey in SV. **Pseudocystidia** not seen.

Trama of bundles of hyphae enclosing clusters of sphaerocytes, sometimes appearing layered, laticiferous hyphae rare or none.

Chemical reactions: FeSO* - blue-green; KOH - clear red on cap surface, yellow-brown on stipe; phenol - brownish purple; SV - on gills deep red, on the cutis pink-grey.

Habitat and tree associations: Found in coastal forests, generally in moister sites that *R. xerampelina,* and with mature Douglas fir, western hemlock, madrone, red alder or Sitka spruce as potential hosts. Red alder may have been present in the mixed forest at Koksilah but was not noted at the time. *R. semirubra* was found singly in the following collections.

Collections: CROO1007-02 from the forest floor in a mixed age stand with mature trees including Douglas fir, western hemlock, red alder, western red cedar, cottonwood and big-leaf maple, Cowichan River trail N48.7563^0, W123.8254^0; CR001001-04 on south facing slope under madrone and Douglas fir, Koksilah Ridge, N48.656^0, W123.736^0 (eastern very dry maritime CWH subzone). CRO10920-3 5 in old growth rain forest in more open area under red alder and Sitka spruce, Combers Beach trail, Pacific Rim National Park N49.045^0, W125.70633^0 (southern very wet hypermaritime CWH subzone).

Collections	ITSl-FtoITS4-B	RFLPs: Hinfl	Alul	Sau3A
CR001007-02	852	416,340	482,291	332,226,207

Notes: This mushroom differs from *R. xerampelina* in its strongly magenta-red colouration, larger cap size, up to 16cm wide in these collections, paler spore print that is deep cream to pale yellow-orange, Romagnesi Ilia as opposed to Illb-c fori?. *xerampelina,* larger spores with fewer connectives between the warts, and turf-like epicutis distinct from the subcutis rather than the more homogeneous cutis of *R. xerampelina.* The RFLPs of the ITS region of rDNA gene of *R. semirubra* show a similar pattern to that of *R. xerampelina* except for the fragments from the Sau3 A digestion, of which one at 226 is 37bp smaller than the average size of that fragment in *R. xerampelina.*

Thiers' (1997) description of the Californian material has the cutis at only 65-70um thick. However, the cuticle thicknesses of many species described by Thiers is consistently thinner than those of the same species on Vancouver Island. This discrepancy is either due to climatic differences affecting the cuticle, or to a difference in the method of measuring. The Californian material is also described as having hymenial cystidia of 36-70um x 9-16um, shorter and perhaps a little broader than in the Vancouver Island collections, but otherwise similar in shape. Variation in length of cystidia can be due to the age of the collection, since the terminus of hymenial cystidia frequently elongates in age. Grund (1965) was most likely describing *R. semirubra* in his thesis when he described briefly the variety of/?, *xerampelina* var. *rubromarginata* nom. prov.

The collections from Cowichan and Koksilah differ slightly in spore ornamentation and habitat from the Pacific Rim N. P. collection, which consisted of one large sporocarp. It may be a different species or merely a local variation, a question that was not settled by the DNA analysis since the Pacific Rim N. P. collection failed to amplify.

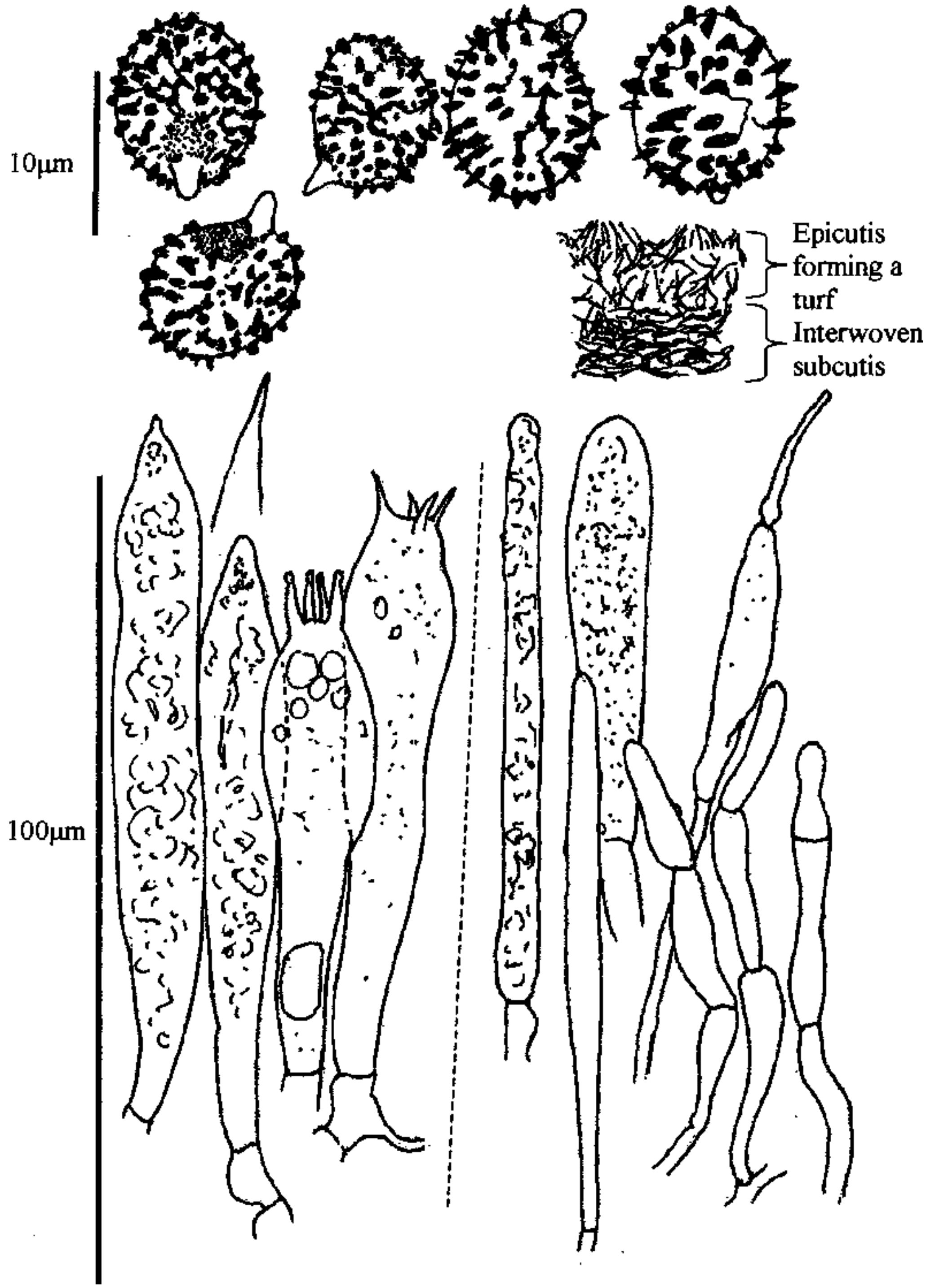

Figure 196 Microscopic characters of *R. semirubra:* Top, spores, the three on the right from Cowichan and Koksilah collections, the two on the left from Combers Beach trail, P.R.N.P., scale bar is lOum; middle right, plan of cutis in section in 5% KOH; bottom left, basidia and hymenial cystidia; lower right, pileocystidia and hyphal ends in cutis, lower scale bar is lOOum.

Figure 197 Macroscopic characters of *Russula semirubra*, top, (Koksilah collection) in side view, top view and longitudinal section, square is 1cm^2 and shows spore colour en masse. Beneath is the Combers Beach Trail, P.R.N.P. collection of *R semirubra* with a 16cm wide cap and less pink on the stipe.

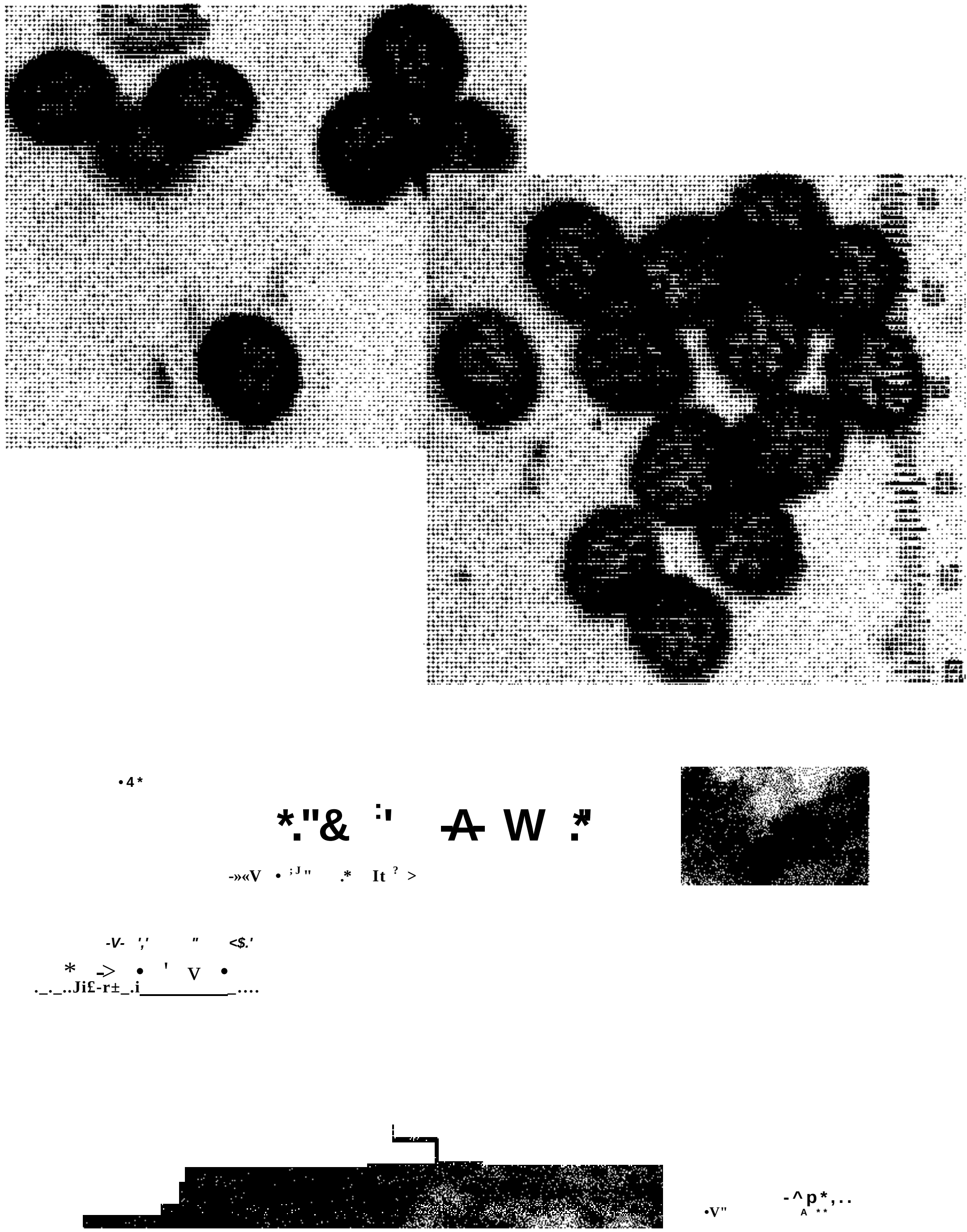

Figure 198 Spores and cutis of *Russula semirubra:* Top left, spores of Koksilah collection; right, spores of the P.R.N.P. collection, at the same scale of lum divisions; bottom, section through cutis showing upright turf of hyphal ends clustered into loose fascicles above an interwoven subcutis, scale bar is 250um.

***Russula isabelliniceps* nom. prov**

(=*R. xerampelina* var. *isabelliniceps* Grund nom. nud.)

A Survey of the genus *Russula* in Washington State. PhD Thesis, Washington State University 1965

Cap 7.4-16.8cm diameter, pulvinate at first, soon developing the distinctive deep, broad central depression with the broadly rounded margin, central depression often cyathiform, margin smooth but may develop striations in age or in dry conditions. The margin usually does not become uplifted in age. Often the caps are remarkably even and round. Colour ranges from a mid to light brownish olive or ochraceous olive, light brown, tending towards a brownish pink at the margin or sometimes completely this colour, the colour and texture often reminiscent of suntanned Caucasian skin. Commonly the olive and ochre hues are in the centre and the pinker hues towards the margin. Surface viscid and with a brown bloom which is more easily seen in tangential view, drying matte. The cutis breaks into more or less concentric bands of minute areolae formed by the clumping of the epicutal hyphal ends. Cutis peels 1/4 to 3/4 of the radius. Cap trama cream, purplish-pink under the cutis, browning where damaged.

Gills moderately close to subdistant, sometimes distant, ventricose, adnate to adnexed at stipe, rounded at margin, with the cap margin curving downwards over the ends, approximately equal in depth to that of the cap trama at half-radius, margins entire. Colour cream when young, becoming deep cream to ochre-cream to light orange-yellow at maturity, developing reddish-brown stains where damaged. Anastomoses occasional, mainly near the stipe, free sub-gills rare.

Stipe 5.0-8.3 xl.6-3.5cm, clavate, often strongly so, stocky, with a firm rind around 5mm thick, stuffed with a firm bread-textured trama which turns pinkish-brown on cutting and around insect damage, but which remains solid and without cavities well into maturity. Surface longitudinally finely rugulose, pruinose, white, occasionally lightly flushed with pink, bruising ochre-yellow where damaged, eventually browning.

Texture quite firm and somewhat brittle, gills brittle.

Taste mild, sweetish or nutty.

Odour mild and slightly fishy, eventually smelling strongly fishy, of trimethylamine.

Spore colour warm deep cream to light orange-yellow, Romagnesi Ilc-d or IIIc.

Spores 8.2-12um (-15)um x (6.5-) 7-10um (-1 l)um, mean 10.3 x 8.6um, L:W ratio 1.04-1.38, mean 1.2 (n=30), ellipsoidal. **Ornamentation** of sharply or bluntly conical warts, 0.8-1.8um high, occasionally to 2 um high, isolated or 2-3 catenate, a few joined by lines but not forming a reticulum, or occasionally a partial reticulum. Spores vary between individual basidioma in that some have isolated pointed warts and others have blunter warts with more catenations and connections (very similar to those of *R. xerampelina).* **Woo types** A3 or B2-3, occasionally C2 to C3. **Suprahilar patch** amyloid, a distinct grey area in Melzer's reagent with uneven edges and low warts at the margin, hiliferous appendix up to 3um long. **Basidia** 38-62 x 1 l-15um, clavate, sometimes broadly clavate, 4-spored occasionally 2-spored. Sterigmata7-10umlong. **Cheiiocystidia** sparse, not staining in S V or staining pinkish, otherwise similar to pleurocystidia. **Pleurocystidia,** unevenly or evenly distributed, sometimes sparse, arising in the subhymenium, staining red or weakly grey in SV, protruding only 10-25um beyond basidioles, 55-103 x 9-13um, but most 10-1 lum wide, ellipsoidal in end view, cylindrical to narrowly clavate to fusoid, ends mostly acute, elongating in older caps to a tapering or lanceolate rather than mucronate extension, this sometimes with a series of constrictions. **Subhymenium** parenchymatous, about 3 0-100pm thick, gill trama of sphaerocytes with rare vascular hyphae staining light grey in S V.

Cutis 150-300um thick at half radius, up to 500um on disc, an ixotrichoderm. As a result of the irregular surface described below the cutis thickness can vary between 300 and 500fim in a horizontal distance of 200um. **Subcutis** of mostly repent hyphae in the basal layers becoming gradually more upright and less tightly packed toward the epicutis. **Epicutis** about 1/5 to 1/4 the depth of the cutis and consists of the hyphal ends bunched into broad fascicles which form the areolae in macroscopic view. The cutis is gelatinised and in some sections continues above the epicutis as a clear pellicle-like layer. In water mounts of cutis sections, the subcutis is pale and the epicutis brownish.

Pileocystidia, very rare, several 2-3 mm^2 pieces of cutis may be examined without finding any, then only 2 or 3 together, embedded and interwoven with cuticular hyphae rather than freely emergent, contents refractive but not as much as in most Russulas, not staining in SV. Mostly cylindrical, none septate, occasionally 1-septate, ends more or less capitate, rounded or tapered, 35-76 x 4-10um, most 4-7um wide. **Pseudocystidia,** none seen. Cuticular hyphae 3-5um wide, with the terminal cells frequently shortest, 20-25 um long, curved-cylindrical or contorted or papillate at the end, often the last 2 or 3 cells forming an ampullate shape with the basal cell inflated up to 12um. Also common are long narrow hair-like extensions 150um long and 2 to 2.5um wide, consisting of about 3 cells. Some hyphae, including sections of these hair-like extensions, have a more or less spiral internal thickening of the cell wall which appears at first sight as small internal incrustations which can be seen to be linked on focussing up and down at high power (lOOOx). Laticiferous hyphae very rare, bright pink in SV, (two seen out of multiple sections of cutis and underlying trama from 4 collections).

Trama of bundles of hyphae enclosing lenticular clusters of sphaerocytes.

Chemical reactions: FeSC«4 - blue-green; guaiac - rapidly blue-green; KOH - no change to yellow-brown on cap surface, yellowish on stipe; NH4OH - no reaction; phenol - slowly brownish purple; S V - gills, pinkish-brown to purple-grey, cutis magenta to pink-grey, cap trama magenta.

Habitat and tree associations: These collections were made in coastal forests, often in association with rotted coarse woody debris penetrated by tree roots, always with western hemlock and usually with Sitka spruce.

Collections: CR981013-11 on a rotten stump with western hemlock and salal, San Joseph trail near the estuary, Cape Scott, N50.768^0, W128.342^0. CR021015rfa in old growth forest beneath fallen logs and in a stump hollow, with western hemlock, amabilis fir and Sitka spruce along the rainforest trail A, Longbeach area, Pacific Rim National Park, N49.0490^0, W125.6975^0. PK 021112-01, from regeneration western hemlock and Sitka spruce Goldbeach campground, Pacific Rim National Park, N49.0565^0, W125.7220^0.

CR03 0924-05 and CR03 0924-08 from Port Renfrew area in mature Sitka spruce and western hemlock, N48.5660^0, W124.3990^0 (southern very wet hypermaritime CWH subzone). OC011013-01 under western hemlock and Douglas fir, Mesachie Lake, N48.8257, W124.1348 (western very dry maritime CWH subzone). JD021027-02 in coarse woody debris with western hemlock, Cowichan Lake area towards Bamfield, precise location not recorded.

Collections	ITS1-F to ITS4-BRFLPs:	Hinfl	Alul	Sau3A
CR021015-rfa	849	416,340	476,286	337,280,207
JD021027-02	845	416,340	481,296	327,271, 196
CR030924-05	876	431,350	482,285	349,284,211
CR030924-08	879	419,353	500,300	352,285,214

Notes: *Russula isabelliniceps* is a large, fleshy mushroom that often looks as if it has been turned on a lathe because of its broad vase-like shape, even diameter and concentric markings. The cutis is quite skin-like or sometimes chamois-like, the gills and spore print are a little paler than in *R. xerampelina* and the stipe is white or with only a small amount of pink colouration. The mushroom most likely to be confused with *R. isabelliniceps* is *R. olivacea*, which is also a large, fleshy, mild tasting, yellow spored mushroom with similar colours and with a cutis devoid of pileocystidia and which breaks into concentrically arranged areolae. In Phillips (1991) the reaction of *R. olivacea* with FeSCv is given as green but in the Vancouver Island collection of this species an orange-pink reaction occurred in agreement with Romagnesi (1967), Blum (1962), Bataille (1948) and Buczacki (1992). It is possible that Phillips' collection labelled *R. olivacea* is actually *R. isabelliniceps*. The green reaction with FeSCu of *R. isabelliniceps* and brownish purple reaction with phenol effectively distinguishes it from *R. olivacea*. Other differences include the length of the ITS region which is around 270bp longer *inR. olivacea*, its complete lack of pileocystidia, the more complete pink colouration of the stipe, the drier and more strongly areolate cap surface and darker colouration, which can become deep vinaceous, the non-separable cuticle, the harder flesh, more lobed and uneven shape, darker spore print and bright blackcurrent purple reaction of the flesh to phenol in *R. olivacea*. *Russula isabelliniceps*, at least in the above collections, always remains a light to mid tone, never becoming dark.

The characters distinguishing *R. isabelliniceps* from *R. xerampelina* are the lighter pigmentation of the cap and the lack of strong purples or dark reds, the brownish bloom, larger spores and the habitat.

One of the interesting characters photographed below and noticed in some other members of the *Xerampelinae* but not previously mentioned in the texts consulted is the presence in the cutis of hyphae with spiral internal wall thickenings, not seen in other *Russula* groups.

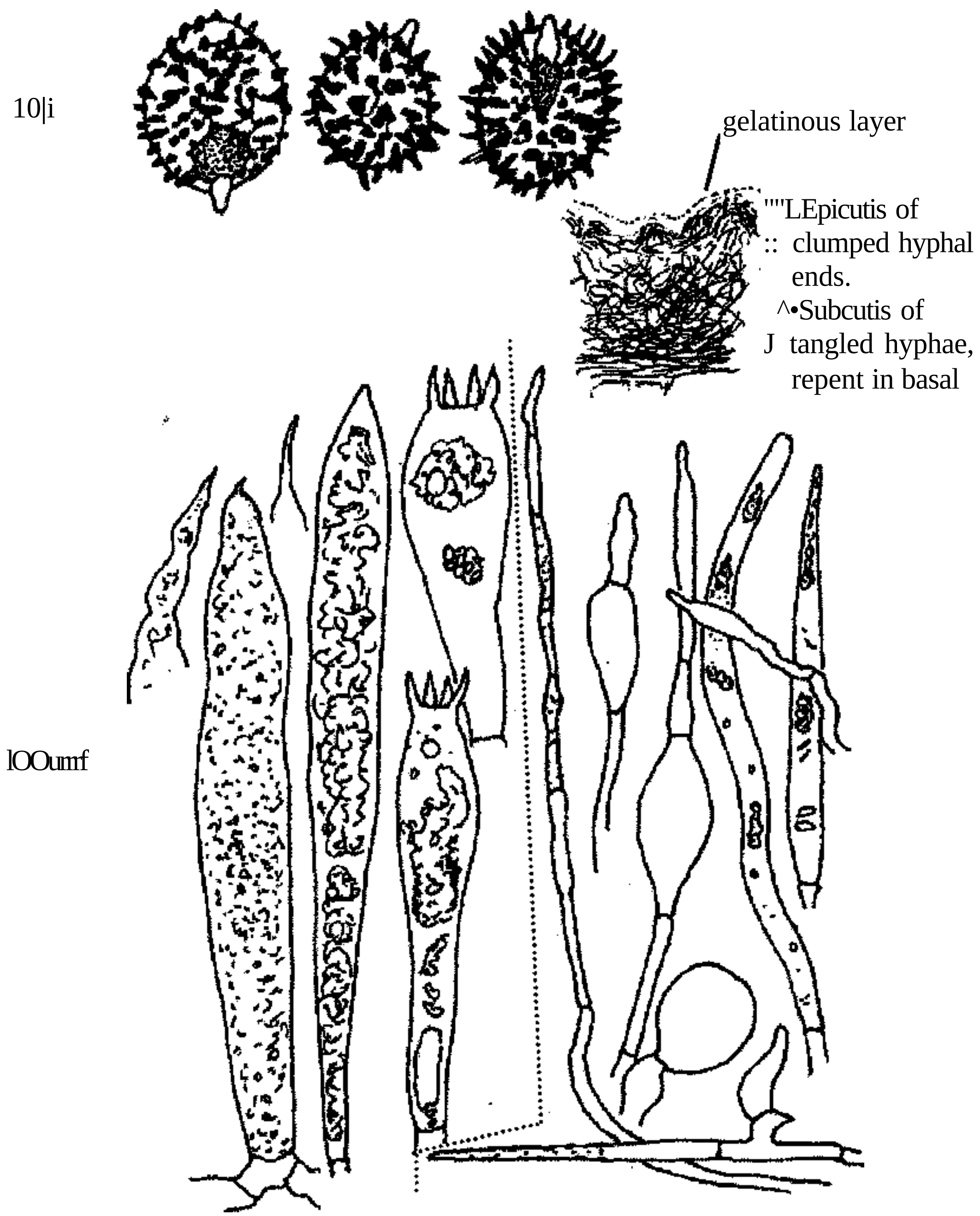

Figure 199 Microscopic characters of *Russula isabelliniceps;* Top, spores with 10um scale bar; middle, diagram of cutis in section in 5% KOH; bottom left, hymenial cystidia and basidia; bottom right, pileocystidia and hyphal ends in cutis, lower scale bar is 100um.

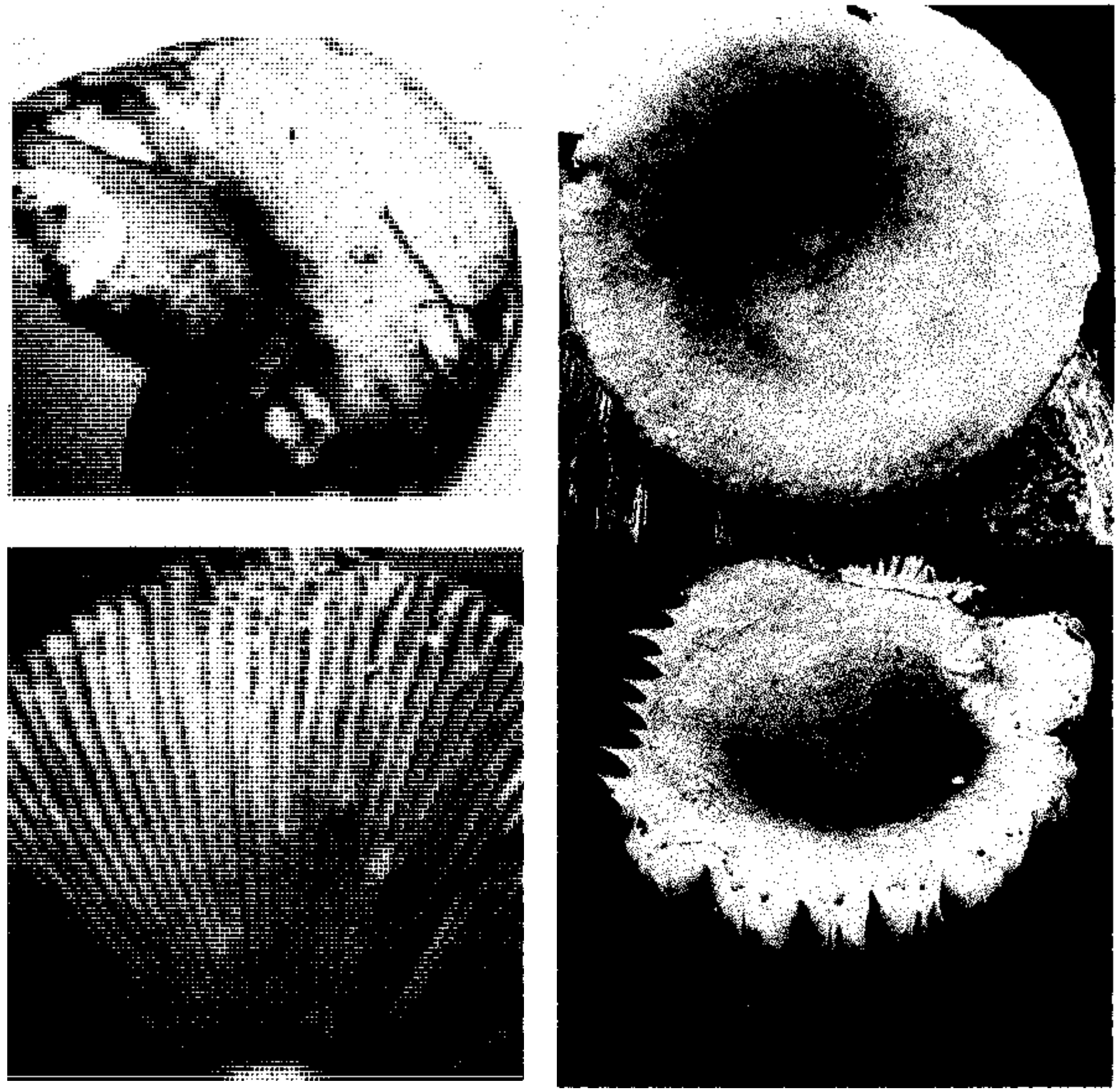

Figure 200 Macroscopic characters of *Russula isabelliniceps:* Top left, the olive form showing the browning of damaged flesh typical of the *Xerampelinae;* top right the brownish pink form showing the typical even shape with the cyathiform depression and broad margin; lower right, the brownish form showing the concentric floccules and brown bloom on the surface, and the ventricose gills, (the splitting at the margin is an artifact of weather conditions); bottom left, gills showing some forking near stipe. The basidiocarps in this figure are between 12 to 16cm in diameter.

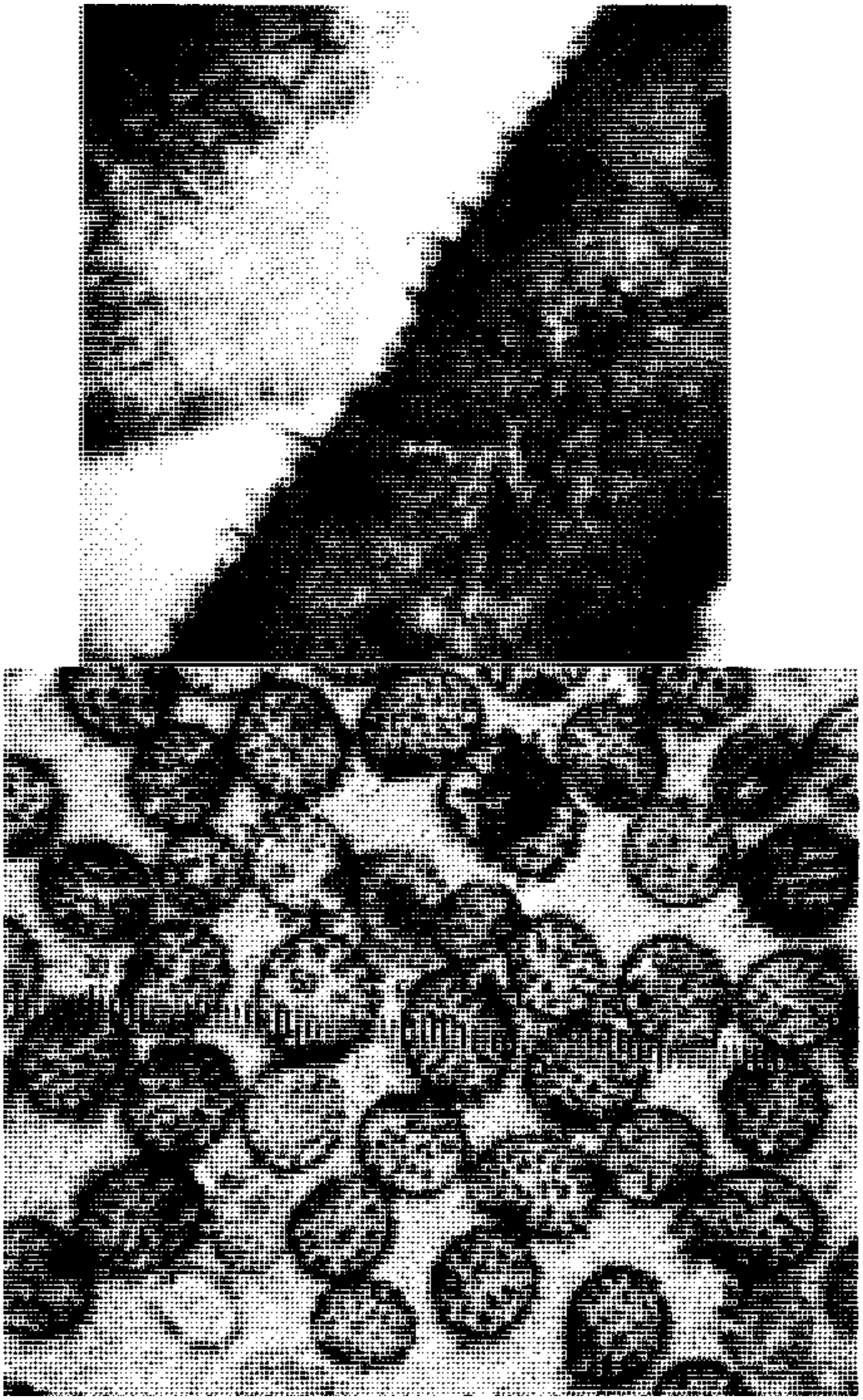

Figure 201 Hymenium of *Russula isabelliniceps:* Top, gill sections of an older (left) and young but sporulating basidioma (right), note the increase in thickness of the subhymenium from approximately 50um in the young gill to lOOum in the older gill, and the pleurocystidia which protrude more in the young gill of this specimen, (scale bar positioned over the subhymenium is 50um); Bottom, spores with lum division scale.

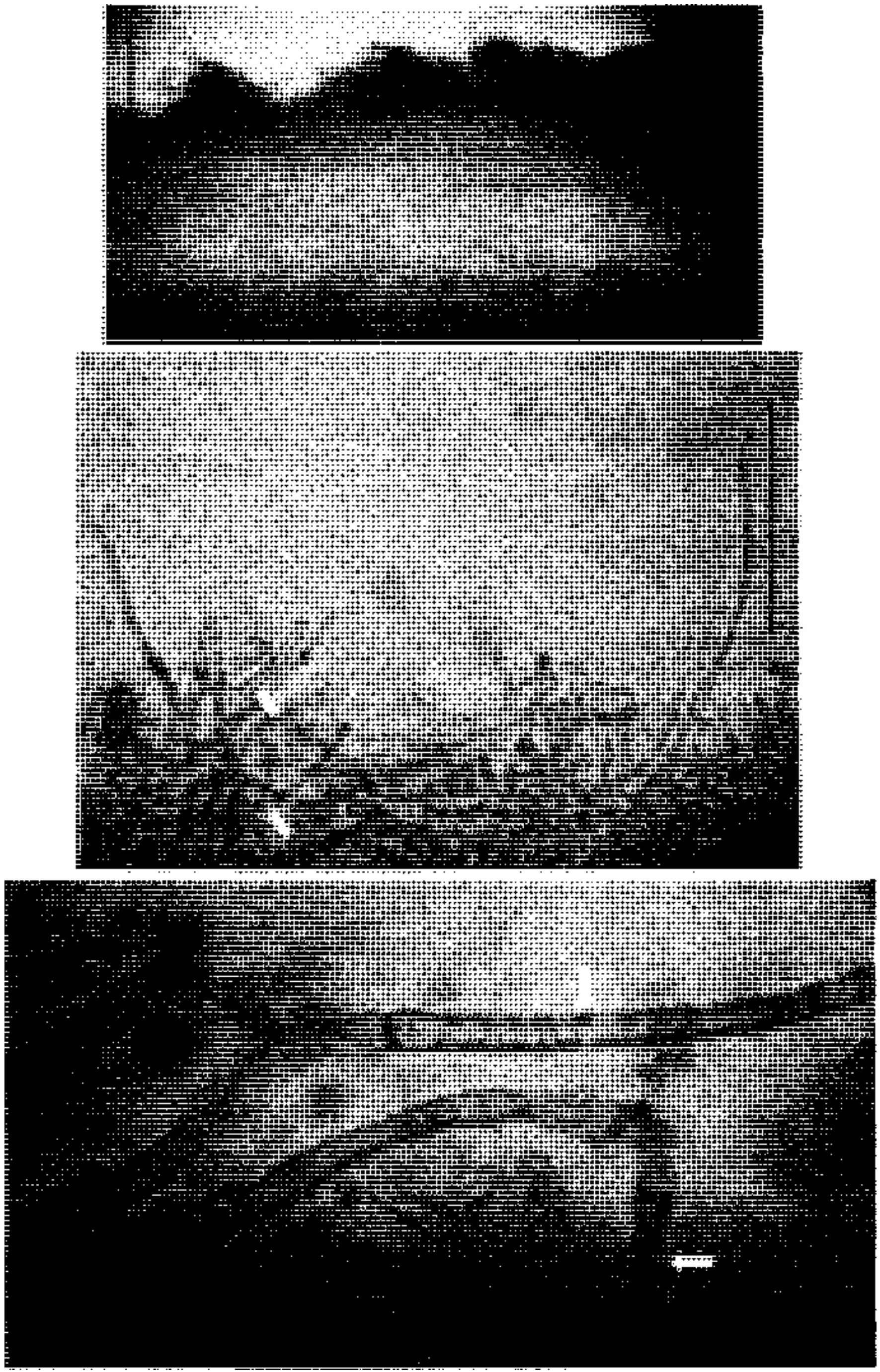

Figure 202 Cutis of *Russula isabelliniceps*. Top, section through cutis showing the clumped hyphal ends of the epicutis which form the floccules on the cap surface, scale bar is 100am; middle, epicutal hyphal ends showing the long hair-like form on the right, and the ampullate forms left, arrowed, scale bar is 50um; bottom, epicutal hyphal cells of 2-3um in diameter, which have internal spirally arranged wall thickenings (arrowed).

***Russula* cf. *pruinosa* VelenovskL**

Ceskehouby 1:154. 1920

Cap 1 l-12cm diameter, strongly lobed and deeply rounded at the margin, but not inrolled. The colour is a mixture of greyish violet and purple in the centre, tending to a deep grey-brown olive towards the margin, and marked with yellow-brown to greenish brown spots like watermarks, particularly at the margin. The whole surface is matte and dry with a dense pruina which gives it the look of having mould growth on the surface. The pruina is a mixture of greyish violet, yellowish brown and pale grey, with small more or less discrete patches of individual colours. The cutis peels about 1/3, and the flesh directly beneath is greenish grey. Cap trama cream, turning pinkish brown on cutting.

Gills Deep cream to light yellow ochre, browning where larvae have damaged them, otherwise not browning much, moderate to close, free at stipe, broadening outward in wedge-shape, equal to or more than the depth of the cap trama at half-radius, around 12mm at broadest point. At the cap margin the gills are rounded and enclosed almost to the lower margin by the cap cutis. Gill margins entire.

Stipe 5.4-5.7 x 2.2cm, cylindrical, creamy white with or without a partial delicate pink flush, surface smooth, with a normal degree of pruina. Rind firm, 2-3 mm thick, pale yellow on cutting, stuffed with a bread-textured trama which turns pinkish-brown in age and on cutting.

Texture very firm, not brittle except in the gills.

Taste mild

Odour mild, mushroomy.

Spore colour an orange-yellow, Romagnesi. IVa-b

Spores 8.8-10 x 6.8-8.2um, mean 9.38 x 7.61 um (n=30), L:W 1.13-1.29, mean 1.2, ellipsoidal, warts 0.5-1.0um high, catenate sometimes forming chains, occasionally isolated, most joined with a partial reticulum of fine lines. Woo type 2B to 2C. **Suprahilar patch** an amyloid area appearing greyish, of uneven shape and with small warts and thickenings around the edge. **Basidia** 45-55 x 12-15um at the widest part, clavate, but with a relatively long narrow base. Sterigmata 7-10um long. **Cheilocystidia** and pleurocystidia sparse, breaking more easily than in other Russulas so that in a crush mount it is hard to find whole ones. **Cheilocystidia** around 75-80 x 8-12um, with acute or elongated tips, lightly refractive contents which do not stain in SV. **Pleurocystidia** 60-108 x 8-10um, laterally flattened or elliptical in end view, more or less cylindrical but of uneven diameter, with rounded or capitate tips, filled with refractive contents, staining red in SV. **Subhymenium** distinct, parenchymatous, 40-50um thick gill trama of sphaerocytes with no vascular hyphae seen.

Cutisl20-150um thick. **Subcutis** has a basal layer of interwoven hyphae lying parallel to the cap surface, above which is a tangled somewhat gelatinised mass of hyphae 2.5-3.5um wide in general, with enlarged elements up to 8um wide common. **Epicutis** of upright, non-interwoven hyphae up to 120um in length. Small patches are devoid of this turf, especially near the cap margin. The aerial epicutal hyphae are rarely septate beyond the surface, are 40-120um long x 1.5-2um wide, cylindrical or tapering, sometimes stuck together into narrow fascicles of 2-6 hyphal ends of varying length, not gelatinised. Very few hyphae seen with the internal spiral wall thickenings noted in other members of the *Xerampelinae*, and those with more randomly arranged thickenings. **Pileocystidia,** very rare, contents barely refractive, not free of the surface, 17-25 x 4-7.5p.rn, ends tapered or papillate, difficult to determine whether they are true cystidia or not, not staining in SV. **Pseudocystidia** not seen.

Trama of sphaerocytes in small globular clusters, sometimes not well defined, bounded by bundles of hyphae or sometimes a loose hyphal network.

Chemical reactions: FeSC>4 - strongly blue-green; KOH - red-brown on cap, yellow-brown on stipe; NH4OH - vinaceous on cap; phenol - brownish purple; SV - brownish-dull violet on cutis and brownish on gills.

Habitat and tree associations: In remnant old growth forest close to the highway, in mineral soil with Douglas fir and western hemlock, at about 1000ft altitude.

Collections: CRO10922-02, in mineral soil under old growth Douglas fir and western hemlock, within 12m of the highway, Cathedral Grove (MacMillan Provincial Park) N49.2885^0, W124.6705^0 (western very dry maritime CWH subzone).

Collection	ITSl-Fto ITS4-B	RFLPs: Hinfl	Alul	Sau3A
CR010922-02	859	423,358	494,295	338,271, 191

Notes: The heavily pruinose or velvety surface together with the colour mixture of olive and purple and the large, lobed cap which brings to mind *R. amoena* (or *R. amoenicolor).* It matches the rather brief description of *R. pruinosa* in Blum (1962) apart from the spore ornamentation which is up to 3um in height and of isolated spines. Romagnesi (1967) also briefly describes *R. pruinosa* as being a robust species, very firm, with a large cap which is matte and pruinose, violet, seldom partly olive, with a white stipe, broad gills 10-12mm deep, under firs in mountainous regions of the Central Pyrenees. Without a more complete description of the holotype it is difficult to ascertain if this species from Vancouver Island is the presumably rare *R pruinosa.* Neither Blum nor Romagnesi actually saw the fresh mushroom. In Sarnari (1998) the photograph of *R. amoenicolor* matches this Vancouver Island species remarkably well, and some of the microscopic characters of the cutis are similar. However, it is not *R. amoenicolor* because unlike this species the spores have a strongly amyloid suprahilar patch, refractive contents in the pleurocystidia, a strong green reaction with FeSC«4, and a darker spore print than *R. amoenicolor* or *R. amoena,* and the RFLP's of the ITS region are like those of the other *Xerampelinae* and unlike that of *R. amoenolens* derived from sequence data from GenBank.

Other similar velutinous species in the *Xerampelinae includeRussula duportii* Phil, that is generally smaller, to 6cm, mostly green to olivaceous and with isolated warts on the spores, a paler spore print and growth under deciduous trees. *Russula cretata* Romagn. *ad* *int*. olivaceous on the centre with violaceous margin, with paler spores (IIc-IIIa) but similar ornamentation and size to the above collection, under deciduous trees on limestone-clay soils. *Russula amoenoides* Romagn. with colours similar to the above collection or paler, spores paler, lid - Illb and with isolated warts and long, cylindrical pileocystidia, under deciduous trees in clay or sandy soils. These species all vary from the above collection on small details which may or may not be taxonomically relevant. The descriptions are all based on one or two collections that would indicate they are all quite rare.

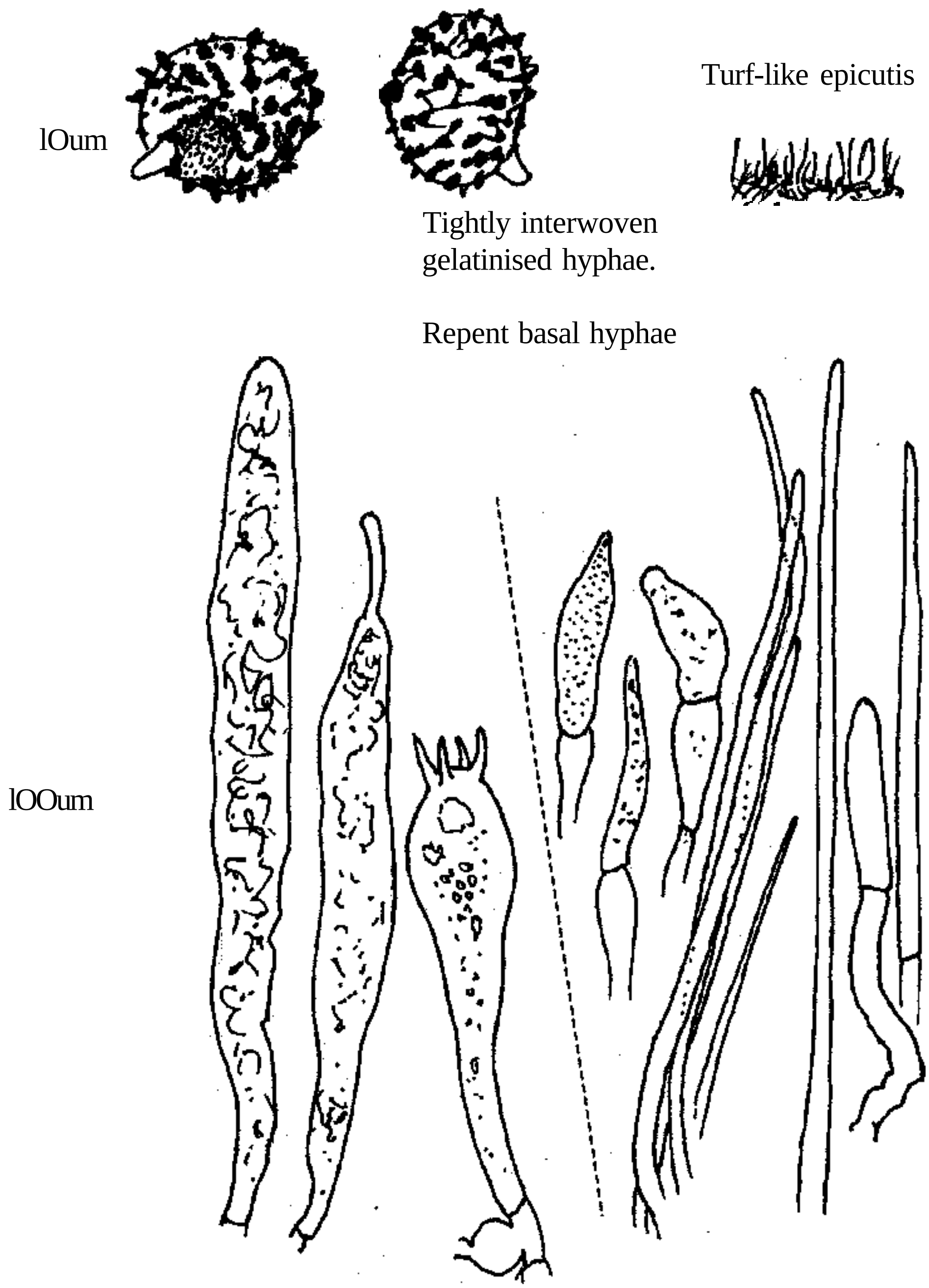

Figure 203 Microscopic characters *ofRussula* cf. *pruinosa:* Top, spores with lOum scale bar; upper right, plan of cutis in section in 5% KOH; bottom left,from left, pleurocystidia, cheilocystidia and basidia; bottom right pileocystidia and hyphal ends in cutis, scale bar is lOOum.

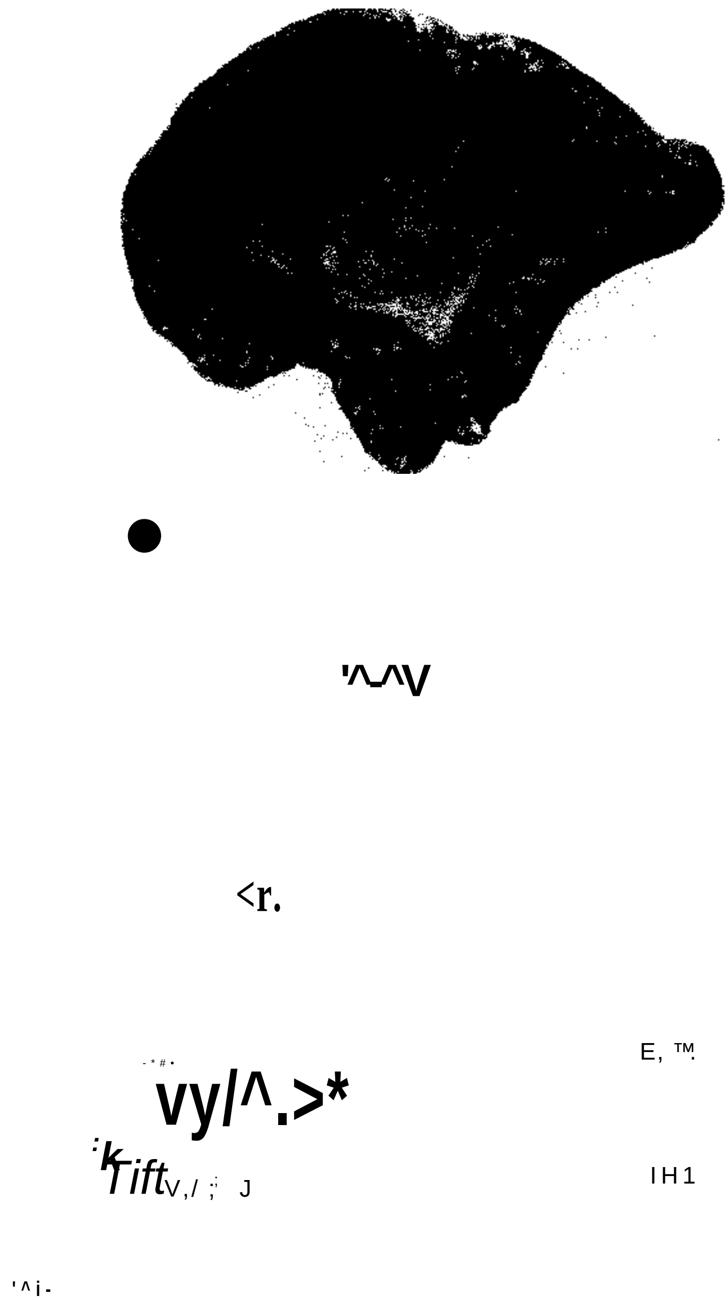

Figure 204 Macroscopic characters of *Russula* cf *pruinosa:* Top, profile of mature basidioma and a longitudinal section, coloured square is $1cm^2$ and represents spore deposit colour; bottom, gills and cap surface. (Photograph by Bryce Kendrick.)

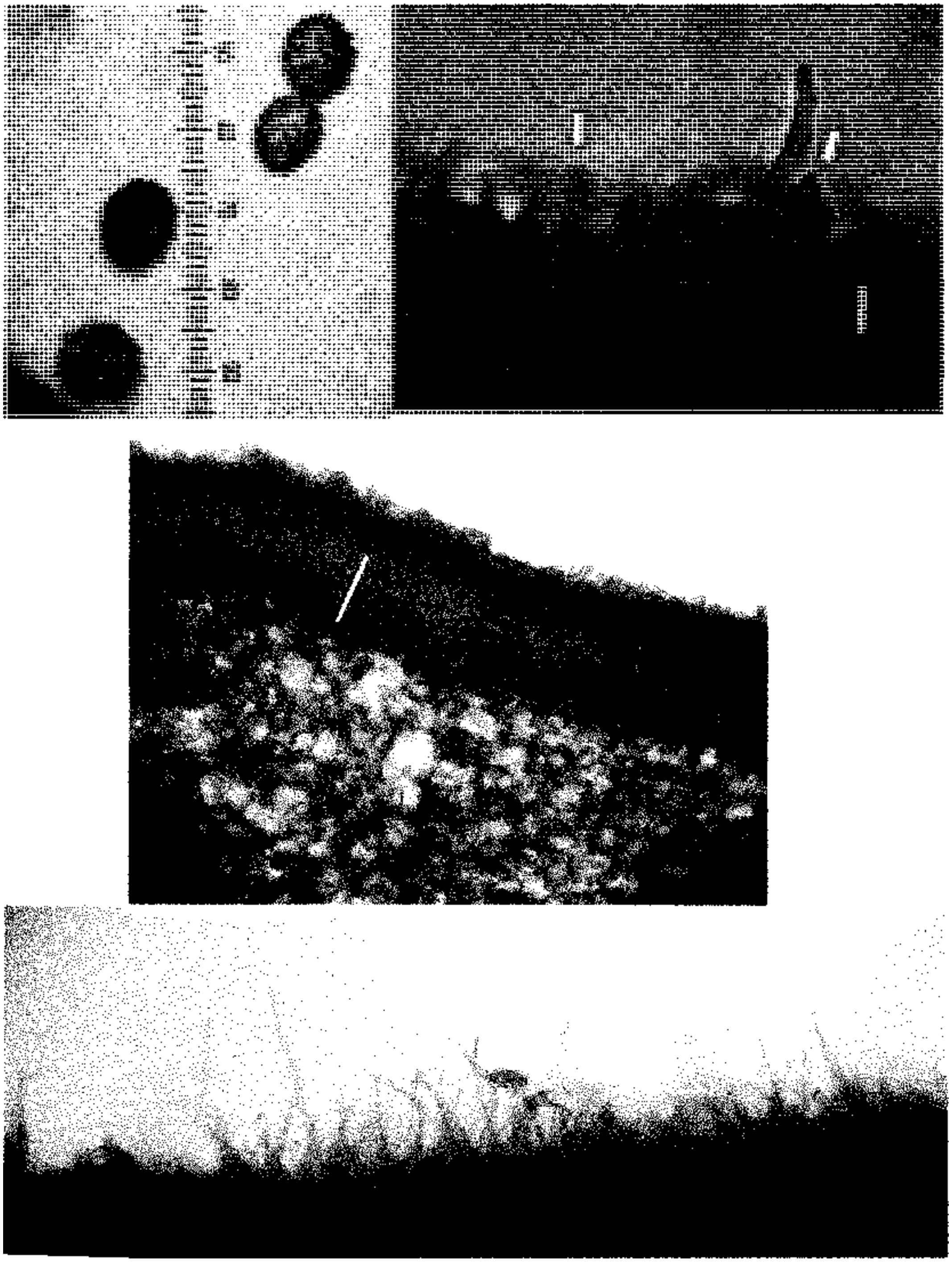

Figure 205 Hymenium and epicutis *oiRussula* cf. *pruinosa*. Top left, spores with lum division scale; top right, gill margin showing cheilocystidia, two protrude about lOum (arrowed), the longer one is less common, scale bar is lOum; middle, gill section showing the pseudoparenchymatous subhymenium marked by the 50um scale bar, and the gill trama of sphaerocytes beneath; bottom, hyphal ends of the epicutis, the longest of which extends 120um above the general surface.

***Russula elaeodes* (Bres.:Romagn.) Bon**

Iconographia mycologica 9:470 1929

Cap 6.0-12cm diameter, squarish in side view when young, becoming plane to centrally depressed, trama deeper than gills at half radius, fleshy and stocky, colour dark brown to almost black in the centre becoming a yellow-brown outwards and greyer or more olive towards the margin, sometimes with very dark vinaceous tints, may be mottled with mixtures of these colours or with minute concentric aereolations. Edge of the cap even or lobed, margin smooth, and maintaining curvature round gill ends as in other *Xerampelinae*, cutis peelable 1/4 to 3/4 of the radius. Flesh dull pink to grey-purple beneath the cutis. Texture dry or only slightly viscid, finely velvety with a whitish bloom. Cap trama light cream becoming dark pinkish cream on cutting.

Gills pale cream becoming pale yellow to a very light orange at maturity, unchanging or slightly bruising reddish brown, close to subdistant, subgills infrequent, occasional forking near the stipe, brittle, adnexed to almost free, broadly rounded at cap margin.

Stipe 2.5-8.3 x 1.3-3.6cm, clavate or fusiform, white with or without a pink flush, bruising ochre yellow, eventually brown, finely rugulose, stuffed with a fairly firm solid stuffing, occasionally with one or two cavities, bruising pinkish-brown.

Texture fairly firm, not particularly brittle but becoming so in age.

Taste mild.

Odour mild, nutty, or with a slight to strong fishy odour.

Spore colour deep-cream to pale yellow, Romagnesi lie

Spores 9-11.3 (-12.2) x 7.5-10.9, L:W from 1.01 to 1.41, mean 1.22 (n=30), subglose. **Ornamentation** of warts 0.8 -1.2um (-1.5)um, conical to peg-like, sometimes wing-like, mostly isolated, some short chains of two or three, occasional fine lines between warts, not forming a reticulum or occasionally forming a partial reticulum, **Woo types 3 A, 3B,**

occasionally to 3C. **Suprahilar patch** a distinct amyloid patch with irregular edges and small warts bordering it, hiliferous appendix 2-3 urn. **Basidia** 40-70 x 12-15um, the upper 1/3 bulbous, below which the cell is more or less cylindrical or very gradually tapering downwards, 4 or sometimes 2-spored, and some apparently with a single enlarged sterigmata, presumably one-spored though none with a developing spore seen, these could be rather short, broad cystidia but the contents are similar to that of normal basidia. Sterigmata 5-10um long. **Cheilocystidia** and pleurocystidia regularly distributed and of average density compared to most Russulas but much denser than other *Xerampelinae*, elliptical to flattened in end view, fusiform to clavate, sometimes broad only in the upper half, the lower tapering sometimes quite abruptly to a narrow base. Originating in the subhymenium, measuring 55-90 x 9-14um. protruding 15-30um beyond basidioles, most with an obtuse point, but many also with a small, sometimes constricted extension, or a capitum, and some mucronate. Contents of pleurocystidia staining reddish in SV, cheilocystidia stain weakly red if at all. **Subhymenium** about 20um thick, of jigsaw-puzzle-shaped pseudoparenchymatous cells, very convoluted, **gill trama** of sphaerocytes, often of irregular shape, with occasional vascular hyphae.

Cutis 120-250um thick, an ixotrichodermis, pink in water mounts with cystidia with yellowish refractive contents. **Subcutis** of tightly interwoven hyphae with very occasional vascular hyphae terminating in pseudocystidia in the epicutis. **Epicutis** around 40um deep, a layer of more or less erect, tangled hyphae with pileocystidia and free hyphal ends that are occasionally clumped into fascicles over small areas. Hyphal ends are a mixture of shapes, cylindrical, ampullate or tapering, mostly formed of 2-3 cells with occasional inflated cells up to 13um wide, septate, with individual cells in the order of 12-30um long, occasionally shorter giving the hypha a jointed appearance. **Pileocystidia,** frequent, not staining in SV, more or less cylindrical to narrowly clavate or fusiform, ends rounded or with a small capitum, 28 to 70um by 5 to 8um, 0 to 3 septate. **Pseudocystidia,** occasional, cylindrical, about 5 to 7um wide, with septae about every 20 to 50um in the terminal portion, with refractive contents not staining in S V. Cuticular hyphae 2-4um wide, most being 3um wide.

Trama of large clusters of sphaerocytes bound by hyphae.

Chemical reactions: FeS04 - blue-green to greenish grey; KOH - bright red to reddish orange on the cap, light yellow-brown on the stipe; NH3OH - no reaction; phenol - light brownish purple; SV - reddish brown on gills and cutis.

Habitat and tree associations: Coastal Sitka spruce zone, often in krummholz where there is little light and undergrowth, on sandy soils, also in old-growth coastal Douglas fir with western hemlock, again with little undergrowth. Sitka spruce and western hemlock are both possible hosts.

Collections: CR001011-55, Wickanninish south beach trail along krummholz, in a stand of Sitka spruce with no undergrowth inland of the beach N49.015733^{0} W125.67335^{0}. CR021016-06, location and habitat as for previous collection. CR000831-04, near Juan de Fuca trail at China Beach park, N48.4370^{0}, W124.0924^{0}, on and near very decomposed coarse woody debris by a small boggy patch under regeneration Sitka spruce and western hemlocks up to 20cm diameter. PJ010919 Wickanninish, in a forested part of the rear dunes, with Sitka spruce, western hemlock, shore pine and a salal understory N49.02167^{0}, W125.67467^{0}. (All above from the southern very wet hypermaritime CWH subzone). CRO10922-01, Cathedral Grove, N49.2885^{0}, W124.6705^{0}, under old-growth Douglas fir and western hemlock with very little undergrowth (western very dry maritime CWH subzone).

Collection	ITSl-FtoITS4-B length:	RFLP: Hinfl:	Alul:	Sau3A:
PJ 010919	859	423,346	494,291	336,276,209
CR001011-55	859	423,358	494,291	336,276,209

Notes: *Russula elaeodes* differs from the other members of the *Xerampelinae* described earlier in this paper in the increased frequency of articular and hymenial cystidia. The epicutis in these collections also lacks the long narrow hyphal ends with internal spiral thickenings as seen in *R. isabelliniceps* and *R. semirubra*. *Russula elaeodes* has characters typical of'the *Xerampelinae* such as the trimethylamine odour in age, the spore ornamentation, the green reaction with $FeSO_4$ and the negative reaction of the cystidia with SV.

The Vancouver Island collections differ from those described from California by Thiers (1997) in the spore size, which is 9-11.3 x 7.5-10.9um, larger than the 7-9um x 6- 7.5um in Thiers' description, which matches that of Romagnesi (1967). Thiers also notes the cutis as being only up to 100um thick, the warts on the spores as up to 1um high, and the pileocystidia as being poorly differentiated, although the remainder of his description matches the above collections very closely.

Russula viridqfusca Grund is similar in cap and spore colour with spores measuring (9)10-13 x 8-10um, slightly larger than the above collections. Grund (1979) states that the cap margin is striate to tuberculate and lacks the typical fishy odour typical of this subsection, and notes that it differs from *R. eleodes* on this latter character and on the spore size. Grund found *R. viridofusca* only twice in a 3-year period, and rated it rare.

The species most likely to be initially confused with *R. eleodes* is *R. brunneola,* which occurs in the same habitats and has a similarly coloured and textured cap, but lacks the green reaction with $FeSO_4$ and has a white spore print.

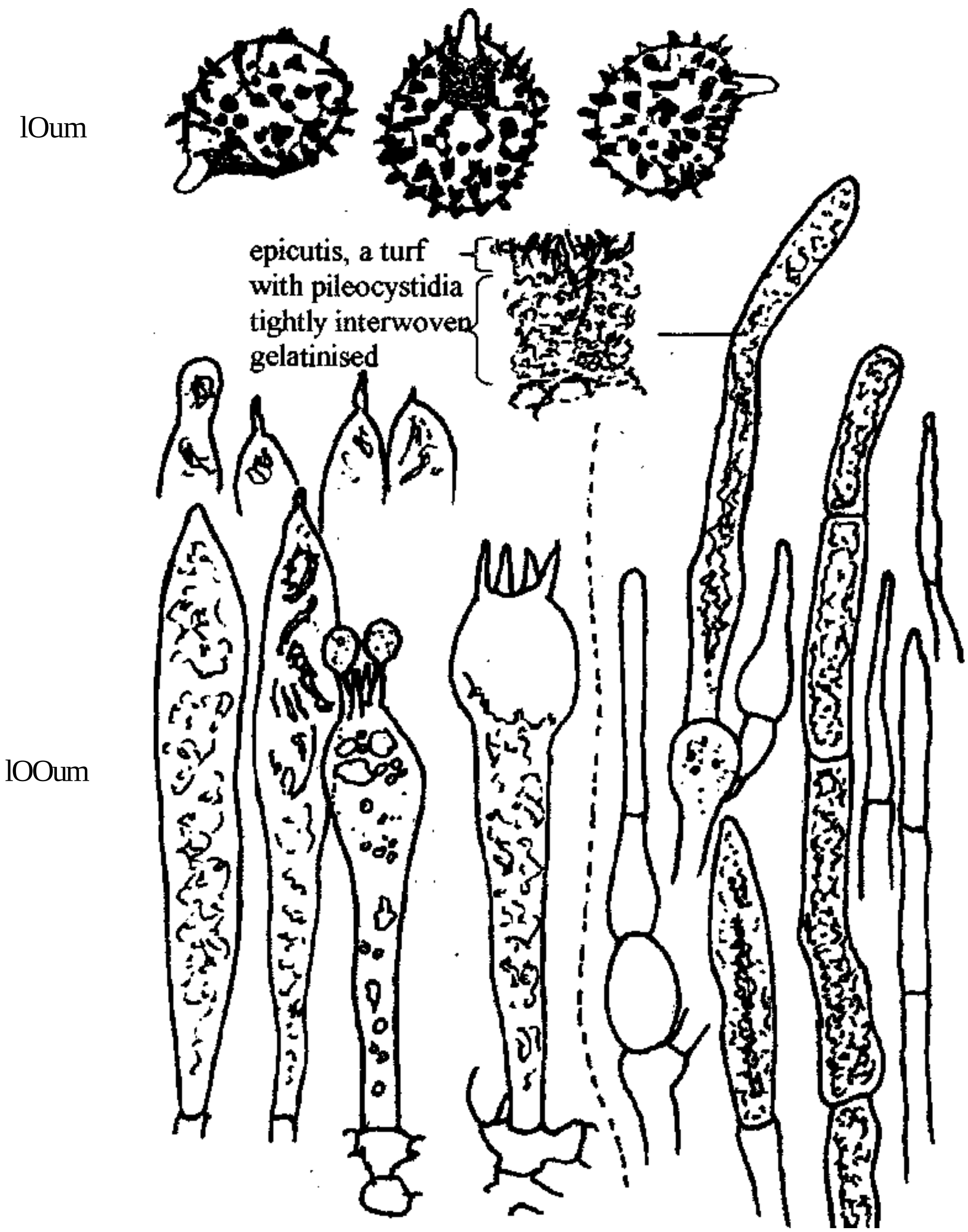

Figure 206 Microscopic characters *ofRussula elaeodes:* Top, spores with lOum scale bar; middle, plan of cutis in section in *5%* KOH; bottom left, hymenial cystidia and basidia; bottom right, pileocystidia and hyphal ends in cutis, lower scale bar is lOOum.

Figure 207 Macroscopic characters of *Russula elaeodes:* Illustrations of immature and mature basidiomata and longitudinal sections of two collections, at top centre, the cap surface shows white flesh where nibbled by slugs and pinkish where the cutis has been peeled off. The squares are $1cm^2$ and show the spore colour.

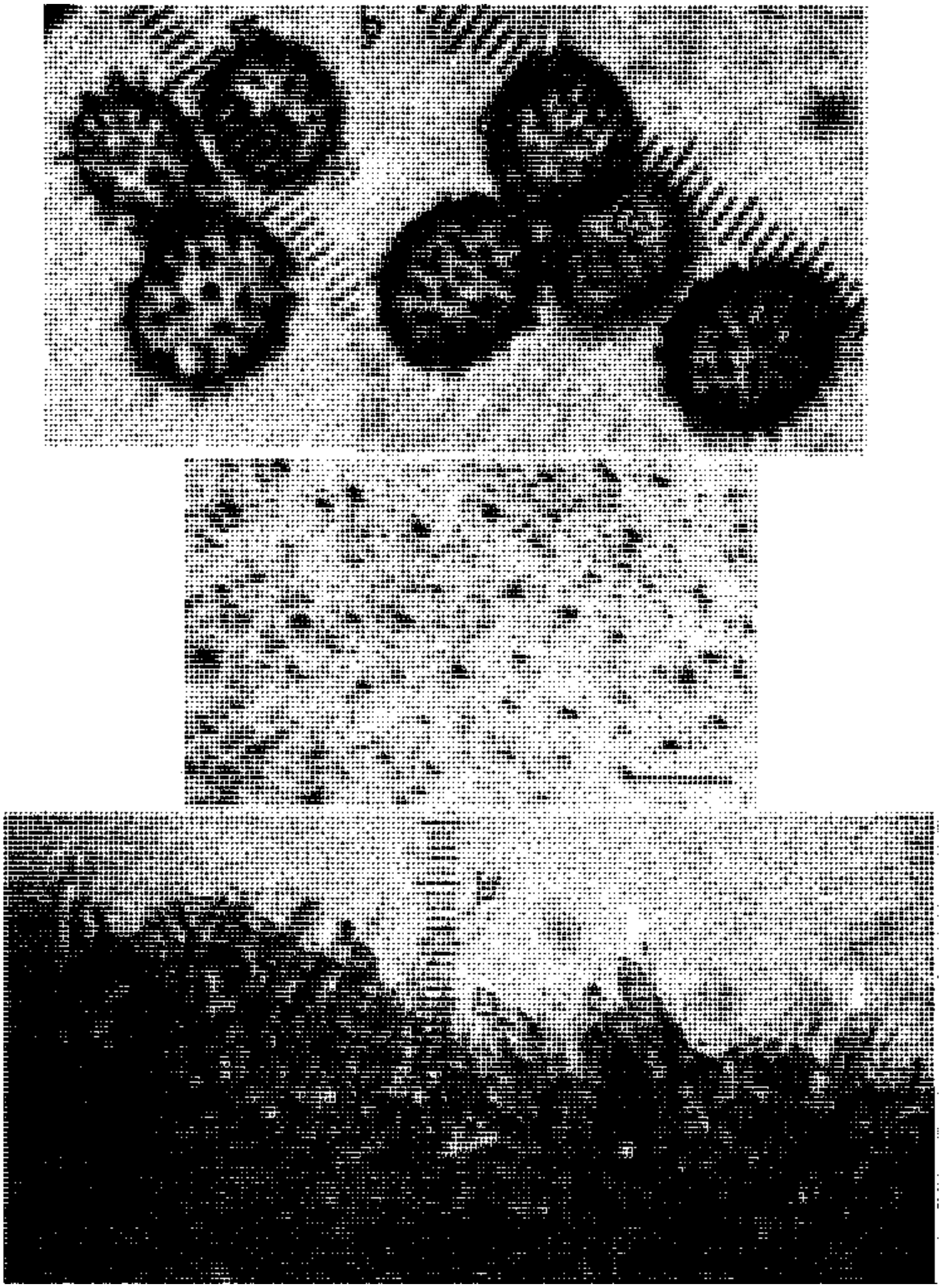

Figure 208 Hymenium and cutis *Russula elaeodes.* Top, spores with lum division scale; middle, face view of gill stained with SV showing the pleurocystidia as elliptical in end view (dark spots), and their fairly even distribution compared with other *Xerampelinae,* scale bar is lOOum; bottom, section through epicutis stained in Congo red showing the turf-like hyphal ends and pileocystidia (arrowed), 10 scale divisions are 25 um.

Clade 10a

Subsection *Laricinae* (Romagn.) Bon, emend. Sarnari

***Russula aureofulva* nom prov.**

Cap 3.5-6.8cm diameter convex with a slightly flattened umbo when young, becoming plane with a depressed centre when fully expanded, cap margin rounded, smooth, becoming striate in age, bright golden brown, more orange-brown to red-brown in the centre, slightly to strongly olivaceous towards the margin and when immature, viscid when wet, drying subshining, glabrous. Young caps are more olivaceous but with red-brown disc (figs. 211 and 212). At all stages there is a definite underlying dark yellow component to the cap colours. Cutis peelable to half the radius.

Gills subdistant to moderately close, with rare to occasional anastomoses and subgills, broad and rounded at pileus margin, adnexed to sinuate and almost free at the stipe, about twice the depth of the cap trama at half radius, pale cream at first, becoming deep cream at maturity, pliable at first, becoming brittle in age.

Stipe 3.7-8.6cm x 1.2-2.3cm, cylindrical or widening near the base, white, some with a pink flush at the base or apex, unchanging when bruised, stuffed with a bready textured trama, becoming unevenly cavitate to hollow in age, surface longitudinally finely rugulose.

Texture average for *Russula,* neither very firm nor particularly fragile at maturity.

Taste mild, nutty.

Odour not distinctive.

Spore colour orange-yellow to ochre, Romagnesi IVd.

Spores subglobose, 9.5-14 x 8-11.5nm, mean 12.2 x 10.2um (n = 45 inclusive of both collections), L:W = 0.97-1.34, mean 1.2. **Ornamention** of warts mostly 0.5-1.5um, but some up to 2.2um high, isolated, incompletely amyloid, no reticulations or chains of warts. Woo type A3. **Suprahilar patch** amyloid, irregular in outline with small warts at the margin. **Basidia** mostly two-spored, approximately 50-70um long by 14-16um wide, clavate or more often with the distal 1/4-1/3 bulbous, occasional basidia are 3 spored, 4-spored basidia are rare, sterigmata number can be best seen by focussing up and down at 400x on the face of a piece of gill which has been rinsed clear of most of the spores. Sterigmata relatively wide, around 7um long and around 2um near the base. **Cheilocystidia** occasional, not numerous, like pleurocystidia. **Pleurocystidia** patchily distributed, 70-100 x 8-9um at widest point, protruding around 25um beyond the basidia and basidioles, fusoid to clavate with elongated tips, many with the tips cylindrical or tapering, some forming one or two progressively smaller bulb shaped swellings between constrictions, contents refractive in 5% KOH and in S V. **Subhymenium** of small sphaerocytes and **gill trama** of sphaerocytes, laticiferous hyphae not seen.

Cutis 140-200um thick at half radius, (in SV or water,) an ixodermis not differentiated into layers, of tightly interwoven hyphae approximately 2-3 um wide, not incrusted, embedded in a gelatinous matrix, with very few free hyphal ends above the surface. In 5% KOH, the gelatinous matrix is dissolved, and the epicutis shows more free hyphal ends around 3um wide which form an interwoven somewhat repent turf. Some hyphal ends have refractive contents. **Pileocystidia** cylindrical to narrowly clavate, occasionally with tiny diverticulae, tips mostly obtuse, a few with a narrower extension, none to several septate, with septa spaced about 25-35um apart, most are 45-80um long but range from 25-125um long and up to 7um wide, occasional ones to lOum wide, contents refractive in KOH, staining patchily and weakly in SV. Caulocystidia few, similar to hymenial cystidia.

Trama mostly of sphaerocytes, evenly distributed throughout the trama, with generative hyphae interspersed, occasional vascular hyphae under the cutis, barely staining with S V.

Chemical reactions: 2% phenol - brownish purple; FeSC«4 - no change to slightly darker cream to faint pinkish; 5% N_3OH - no reaction; 5% KOH - turns cuticle more orange, stipe no reaction; guaiac - strongly blue-green; SV - macroscopically, gills stain deep pink, cuticle none staining, microscopically, pileocystidia none staining or with occasional ones grey or with small aggregations of grey stained ones, pleurocystidia pink, occasional ones grey-purple, cheilocystidia not staining.

Habitat and tree associations: Trooping under mixed age stands of western hemlocks with red alder, Douglas fir or western red cedars nearby. All collections were found amongst small understory hemlocks underneath large mature (around 0.8 to lm diameter) hemlocks. Western hemlock is the most likely mycorrhizal partner since it is the tree consistently present.

Collections: DAVFP 28769 -holotype, (11 specimens), DAVFP 28771 (9 specimens), at GPS reference N48° 26.090' W 123° 28.705' and DAVFP 28768 (1 specimen), DAVFP 28770 (6 specimens) at N 48° 26.045' W 123° 28.700', all from Royal Roads University woodlands, Victoria (CDF zone). This species has not to date been found elsewhere. Collections are deposited in the herbarium of the Pacific Forestry Centre, Burneside Road, Victoria, B.C. Canada.

Collection	ITSl-Fto ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR001108-06	868	400, 349	477, 259	295, 208
CR021009-01	884	392, 334	477, 279	295, 212
CR031101-01	879	396,339	472,279	295,212

Notes: The brown colour of *Russula aureofulva* is suggestive of the *Ingratae* but the pigments are blue-green, yellow and magenta as in subsection *Russula*, there is no strong odour, the margin is not tuberculate, the cuticle is not so heavily gelatinised as is usual in the *Ingratae* and the stipe does not have the regular lenticular cavities that are found in subgenus *Ingratula*. The spores with their incomplete amyloid staining are reminiscent of *R. laurocerasci* and *R. fragrantissima*, but the warts do not form chains or ridges as in those species and the suprahilar patch is amyloid. The weak FeSCu and S V reactions, deep yellow, large spores and long, multiseptate pileocystidia *of Russula aereofulva* are typical for the *Tenellae.*

All collections of *Russula aureofulva* were growing in deep shade, in which the magenta pigments fail to develop in some other *Russula* species. Nearby in sunlight were two light brick-red and olive Russulas, collection number CR021009-02, which were initially thought to be the same as the shade-grown *R. aureofulva* collections, however, on closer examination, they were found to be *R. abietina,* differing in spore colour, size and ornamentation. The possibility that purple hues might be within the normal colour range of this species were it exposed to more light has been taken into account in the search for its identity. *Russula aureofulva* shares most morphological similarities with the *Tenellae* sensu Romagnesi (1967).

Similar species include *R. aurata* (Blum) which has more red in the cap, flesh bruising yellowish, and smaller spores with some reticulations. *R. aurantiaca* (J, Schaef) Romagn. in subsection *Laetinae* (Romagnesi 1967), has a brick orange cap, pink flush on the stipe, a mild taste, a similar spore colour and ornamentation to *R. aureofulva,* but the spores of/?, *aurantiaca* are smaller, and some of the basal cuticular hyphae are incrusted with small acid-resistant granules. *Russula globispora* (Blum) has similar large spores with large isolated warts and deep yellow spore print to *R. aureofulva,* but differs in its lighter, more red-brown cap and acrid taste. *Russula adulterina* Fries has large dark yellow spores with large isolated warts and yellow to olive brown to purple brown cap colours, but it is peppery and has a fruity, pelargonium odour. The most similar species is *Russula straminea* Malencon, as described in Romagnesi (1967), which has similar colouring, odour, taste, spore colour, size and ornamentation, pileoocystidia and lamellae. This species differs slightly from *R. aureofulva* in its larger size, grey reaction with FeSC>4, browning of the flesh and habitat with oaks. The above species are European.

Russula olivacea (Schaeff.) Fr. has many similarities including the spore colour and ornamentation, but it is much more robust, has no pileocystidia and the stipe flesh has a distinctive blackcurrent colour with 2% phenol. Grand (1965) reported from Washington State four brownish-capped, mild tasting species with yellow spores having large ornamentation, all with 2 and 4-spored basidia: *Russula disparilis* Burl., which differs from *R. aureofulva* in having a paler spore print and smaller spores, larger basidiomata and no pileocystidia; *Russula sphagnophila* Kauffin. also has paler spores and is described as very fragile in Romagnesi (1967), and *R. alachuana* Murr. is larger, with paler spores but otherwise similar microscopically apart from the cuticle which is thicker and composed of two distinct layers, with constricted terminal hyphal cells in the epicutis (Hesler 1960). The latter two species have more purple in the cap. *Russula viridqfusca* Grund is superficially very similar to *R. aureofulva* and occurs under western hemlock, but is in the subsection *Viridantinae* Meltzer-Zvara and has the greenish reaction with FeS04and browning of bruised flesh typical of that subsection, and the spores have smaller warts up to 1.1 um high.

The combination of a brassy to golden cap, mild taste, deep orange-yellow spores which are almost twice the dimensions of those of many other Russulas, spores with isolated warts, non-incrusted cuticular hyphae and habitat with large western hemlock with understory hemlocks are the distinctive characteristics of *Russula aureofulva*.

All collections were damaged by slugs and millipedes. When collection CR001108-06 was made, approximately 30 stipes were scattered around bearing small rodent toothmarks, consistent with squirrel damage, indicating that these animals had harvested the caps. When collection CR021009-01 was made there were many remnants of stipes in the ground, the remainder having apparently been eaten by slugs.

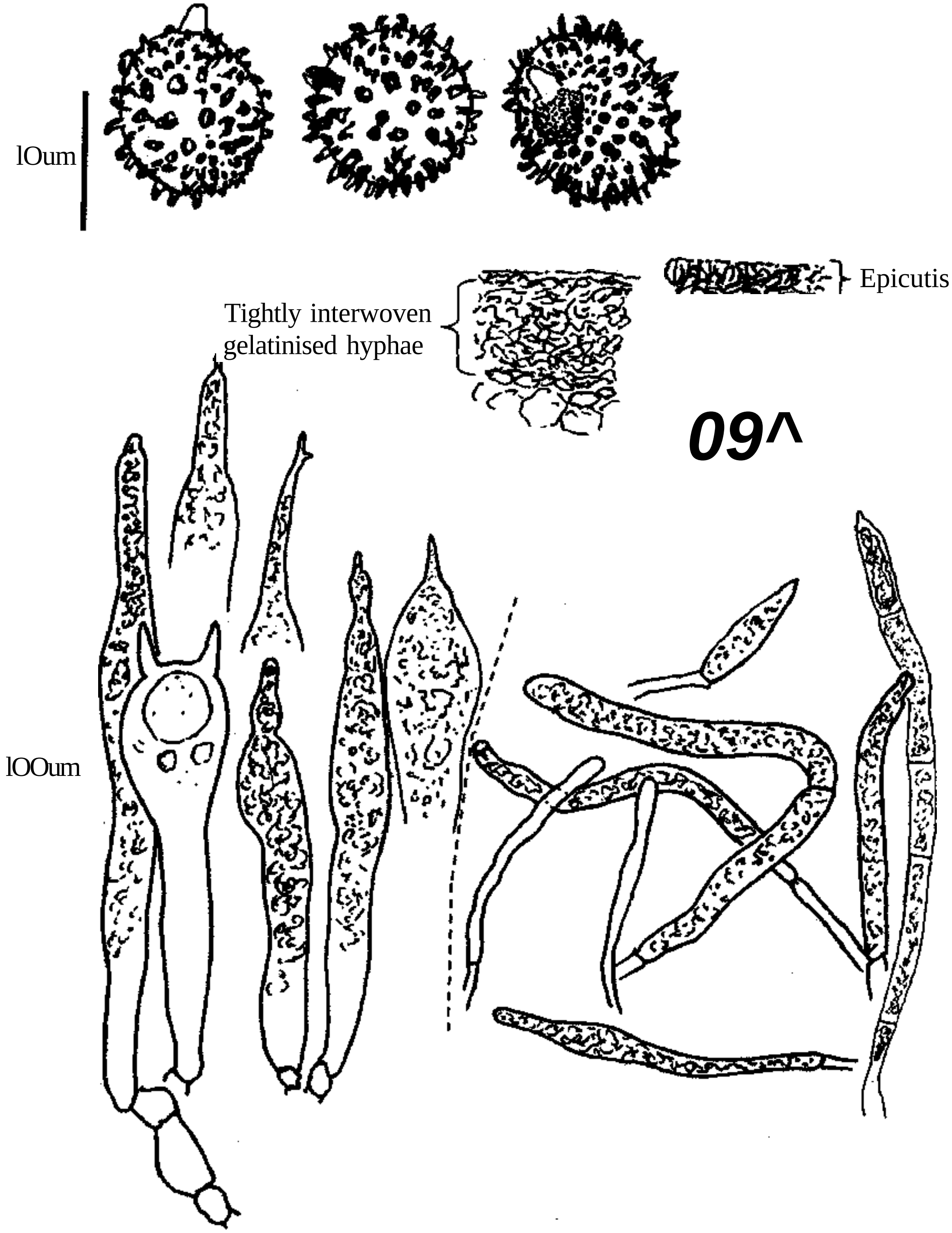

Figure 209 Microscopic characters *otRussula aureofulva*. Top, spores, with lOum scale bar; middle, plan of cutis in section in SV (left) and in 5% KOH (right); bottom left, basidia and hymenial cystidia; bottom right, pileocystidia and hyphal ends in cutis, lower scale bar is lOOum.

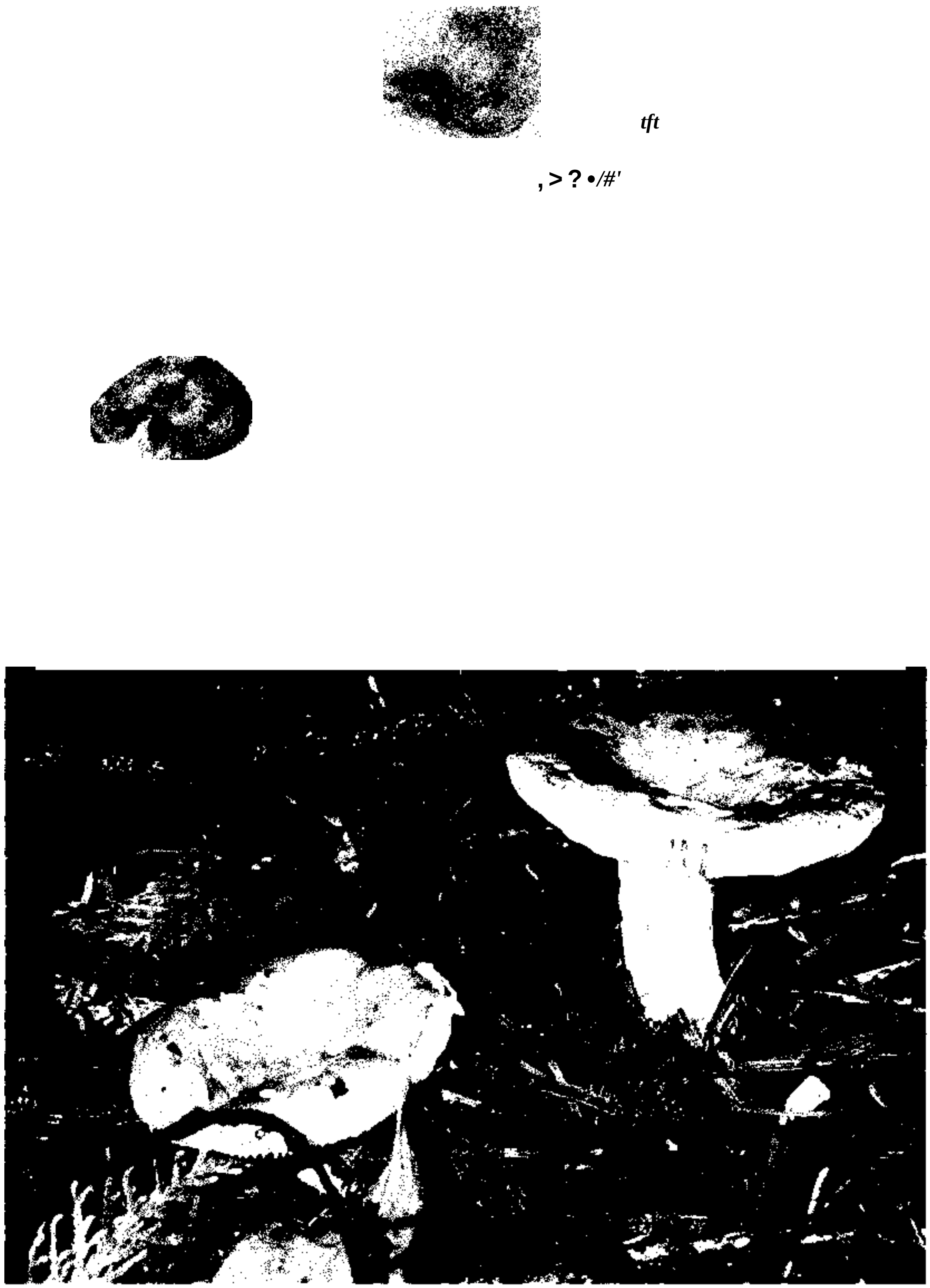

Figure 210 Macroscopic characters of *Russula aureofulva:* Top, lectotype of immature, mature and longitudinal section of basidiomata, coloured square is 1cm and represents spore deposit colour; bottom, mature *R. aureofulva.* Note the broadly rounded gills at the cap margin.

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Figure 211 Immature basidiomata of *Russula aureofulva* with 1cm scale (background) and colour scale. The larger two stipes show a pink flush over the parts of surface.

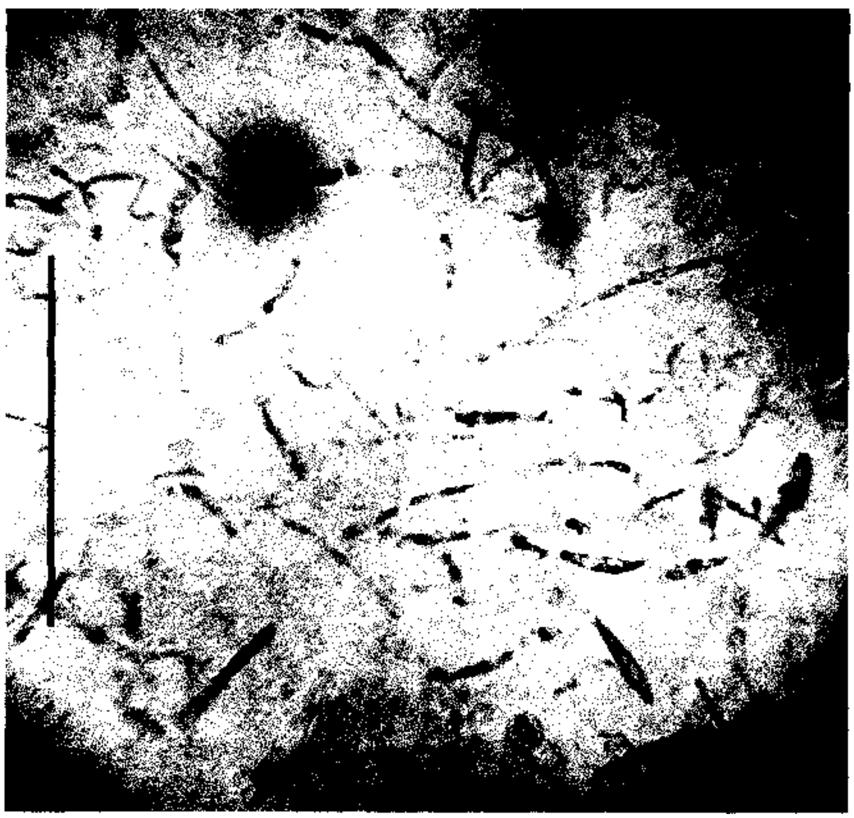

Figure 212 Cutis *of Russula aureofulva* with pileocystidia of varying shapes partially stained grey with SV, scale bar is lOOum.

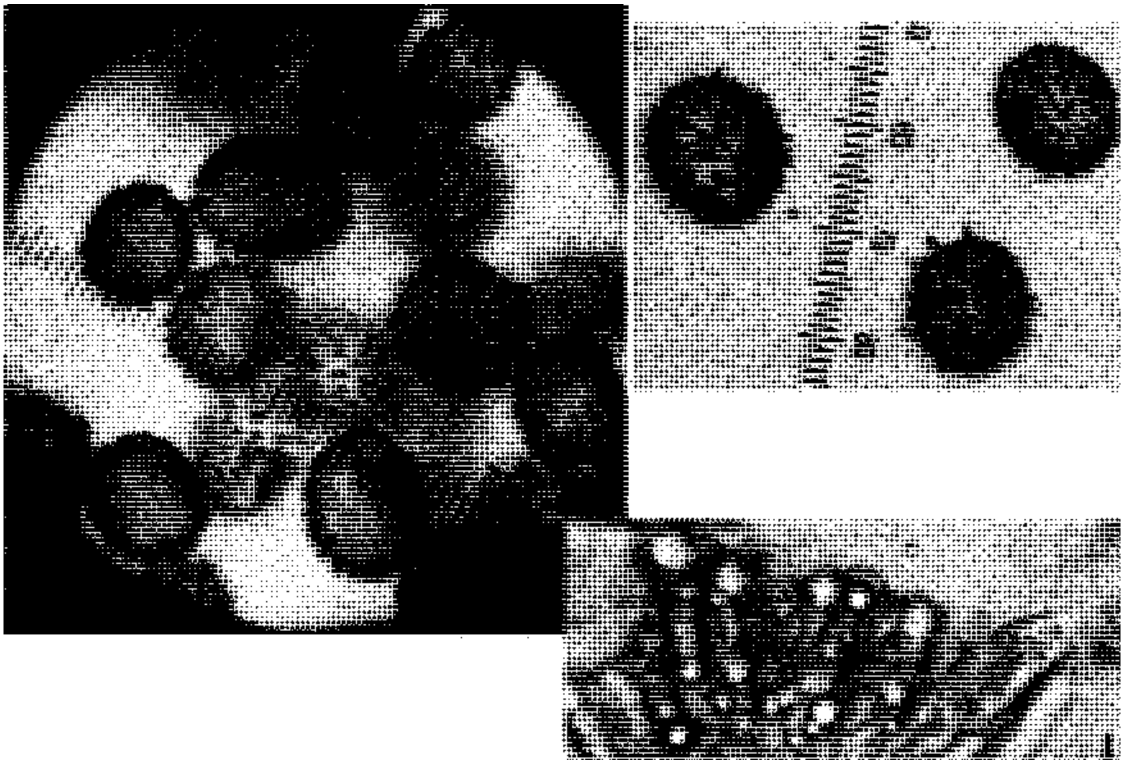

Figure 213 Hymenium *c&Russula aureofulva:* Top left, spores with lum division scale; top right, composite photograph of spores at surface and equatorial depths of field, scale as before; bottom, squash mount of hymenium showing basidioles and pleurocystidia, scale bar is lOOum.

***Russula** cessans* **Pearson**

The Naturalist: 101. 1950

Cap 3.3-8.5cm diameter, convex when young, becoming plane or with a barely depressed centre, often with a central low umbo, margin smooth, becoming striate in age, peelable 1/3 to 1/2 the radius. Colour a clear purplish red, usually darker in the centre, sometimes almost black, sometimes with light brownish to olive areas towards the centre especially in older basidiomata, the darker central colours usually in radiating streaks, viscid drying subglossy. The cuticle appears as a thin translucent rather than opaque coloured layer over the white flesh. Flesh white, unchanging, white to pink under the cuticle, thinning quite steeply off the disc.

Gills white when young, turning cream then finally orange-yellow, narrowly adnexed, acute at the cap margin, broadest about mid-radius, usually deeper than cap trama at mid-radius by 1.5 to 2 times, close to moderately close, with few to no subgills and anastomoses.

Stipe 3.2-10.2 x l.l-2.4cm, pure white, with no flushes of colour, more or less cylindrical but often somewhat flexuous, longitudinally rugulose, smooth, unchanging. Solid and firm at first, becoming stuffed with a firm bread-textured trama, gradually unevenly cavitate and faintly greying in age. The stipe is generally longer than the diameter of the cap in these collections, giving the mushroom a tall, slender stature.

Texture about that of average Russulas or slightly firmer, but becoming softer and more brittle in age.

Taste Mild, sometimes a slightly soapy aftertaste.

Odour not distinctive.

Spore colour deep ochre yellow, Romagnesi IVd to IVe.

Spores 6.5-8.8 (-9.5) x 6-8um, globose to subglobose to broadly ellipsoidal, L:W from 0.93 to 1.23 with a mean of 1.12 (n=30). **Ornamentation** of rounded warts 0.2-0.8um high, in chains or with heavy connectives forming a partial to complete reticulum, more mesh-like than zebroid. Woo type C2 to D2. **Suprahilar patch** amyloid, irregular in outline and with the border heavily amyloid and waited. **Hiliferous appendix** 1.1-2um long, 0.9um wide near the base. **Basidia** mostly 4-spored, 33-50 x 10-14um, of two basic shapes: The shorter ones arise from the upper cells of the subhymenium and are cylindrical-clavate, the base being relatively wide and the taper very gradual, the longer ones arise from cells lower down and have a bulbous upper part with a steep or abrupt taper to a narrow base. The hymenium expands by one or two cells for successively developing basidia, but the older ones do not collapse until after the next level reaches maturity, apparently elongating to compensate for the growth of the hymenium. Sterigmata 5-8um long. **Cheilocystidia** and pleurocystidia, grey in SV, cheilocystidia few, protruding only around lOum, pleurocystidia regularly distributed, dark grey to black in SV, with refractive contents in KOH, 55-87 x 7.5-10um, clavate to fusiform, acute, rounded or mucronate, arising within the subhymenium. **Subhymenium** 20-50um thick, of small sphaerocytes, gill trama of sphaerocytes with occasional vascular hyphae.

Cutis 200um thick on disc, thinning to 20um near margin, an ixotrichoderm with a turf of hyphal ends and numerous pileocystidia and pseudocystidia with refractive and often banded contents, unstaining to strongly staining dark grey in SV. **Subcutis** of interwoven hyphae with pink contents around 2.5um wide, quite loosely interwoven in the upper half, laticiferous hyphae frequent, staining red or grey in SV. **Epicutis** of semi-upright to repent hyphal ends 2-4um wide, with undifferentiated, obtuse tips embedded in a conspicuous gelatinous matrix. Often the epicutis is not well differentiated from the subcutis. **Pileocystidia** 25-200 x 4-8um, (up to 277urn long in Singer's 1957 description) occasional ones inflated tolOjjjn wide. Both cystidia types are septate about every 15 to 35um although some pileocystidia are aseptate, ends rounded, occasionally capitate or clavate, greying in SV. Small diverticulae are sometimes present, not much more than a small bump in the hyphal wall. **Hypodermis** not differentiated from the cap trama.

Trama of sphaerocytes in clusters bound by a hyphal mesh.

Chemical reactions: FeSC»4 - pale pinkish; KOH - brownish orange on cap surface, no reaction on stipe; NH4OH - no reaction; guaiac - brownish-green; guaiacol - light purple; phenol - very slowly brownish purple; SV - grey on the gills, purple on the cuticle, cystidia and vascular hyphae grey to black. Singer (1957) determined that formalin gave a negative reaction on the stipe.

Habitat and tree associations: Under Douglas fir in grass, September to November.

Collections: CR981120-01 at N 48.4603, W123.3128 from University of Victoria campus by the side of the ring-road just north of the Cunningham parking lot entrance, under Douglas firs. CR040918-01 at N 48.43915, W 123. 478183, Royal Roads University campus, in the stand of mature Douglas fir between Sooke Road and the sports field. (Both from the CDF zone).

Collection	ITS1-F to ITS4-B	RFLP: Hinfl	Alul	Sau3A
CR981120-01	832	364,249	497,289	600

Notes: The Vancouver Island collections match very closely the descriptions of *R. cessans* in Romagnesi (1967) for Europe, and those of Singer (1957) and Thiers (1997) for North America in all morphological characters. Singer examined collections of *R. cessans* from Austria, Sweden, Germany, England and Michigan, North America. The habitat in these descriptions is given as conifers, especially pine. In the Royal Roads collection they were growing in a stand of mature Douglas fir, and under Douglas fir and madrone on the University of Victoria campus.

Russula cessans grows in proximity to, and in similar habitats to *R. murrillii*, which is also purple with a white stipe, dark yellow spore print and mild taste. The latter is usually shorter in stature, and has an opaque, matte, chalky appearance to the cuticle that has abundant incrusted primordial hyphae. *R. cessans* may also fruit near *R. xerampelina*, also of similar colours and taste, however, this latter species is usually larger, often has pink on the stipe, has a paler spore sprint, usually a shellfish odour, and bruises dull yellow then brown. *Russula abietina,* in the *Sphagnophilae* has overlapping habitat, but more green in the cap, a paler spore print, and non-reticulate spores.

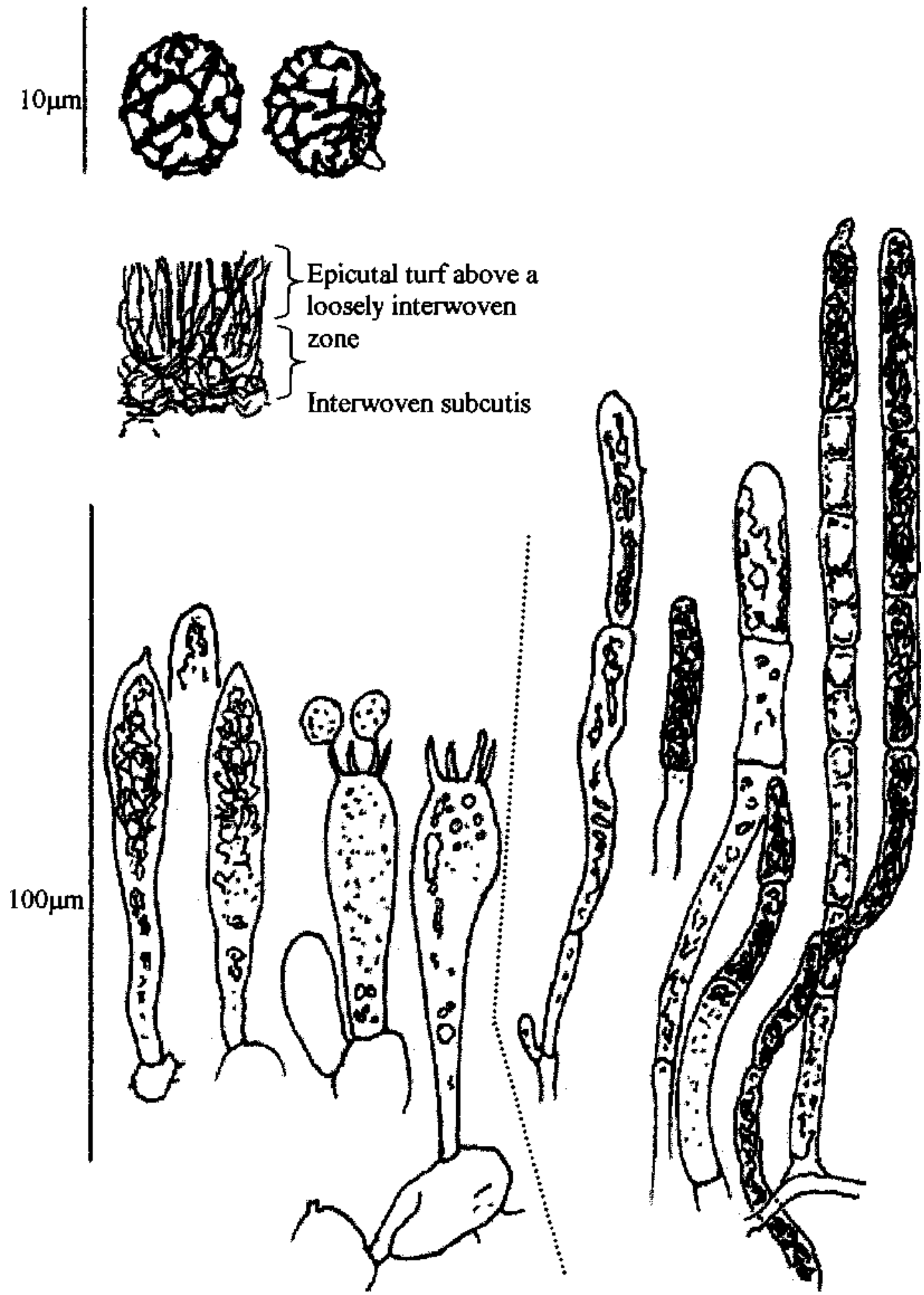

Figure 214 Microscopic characters of *Russula cessans:* Top left, spores with 10 urn scale bar; top right, plan of cutis in section in 5% KOH; bottom left, hymenial cystidia and basidia; bottom right, pileocystidia and pseudocystidia in cutis, lower scale bar islOOum.

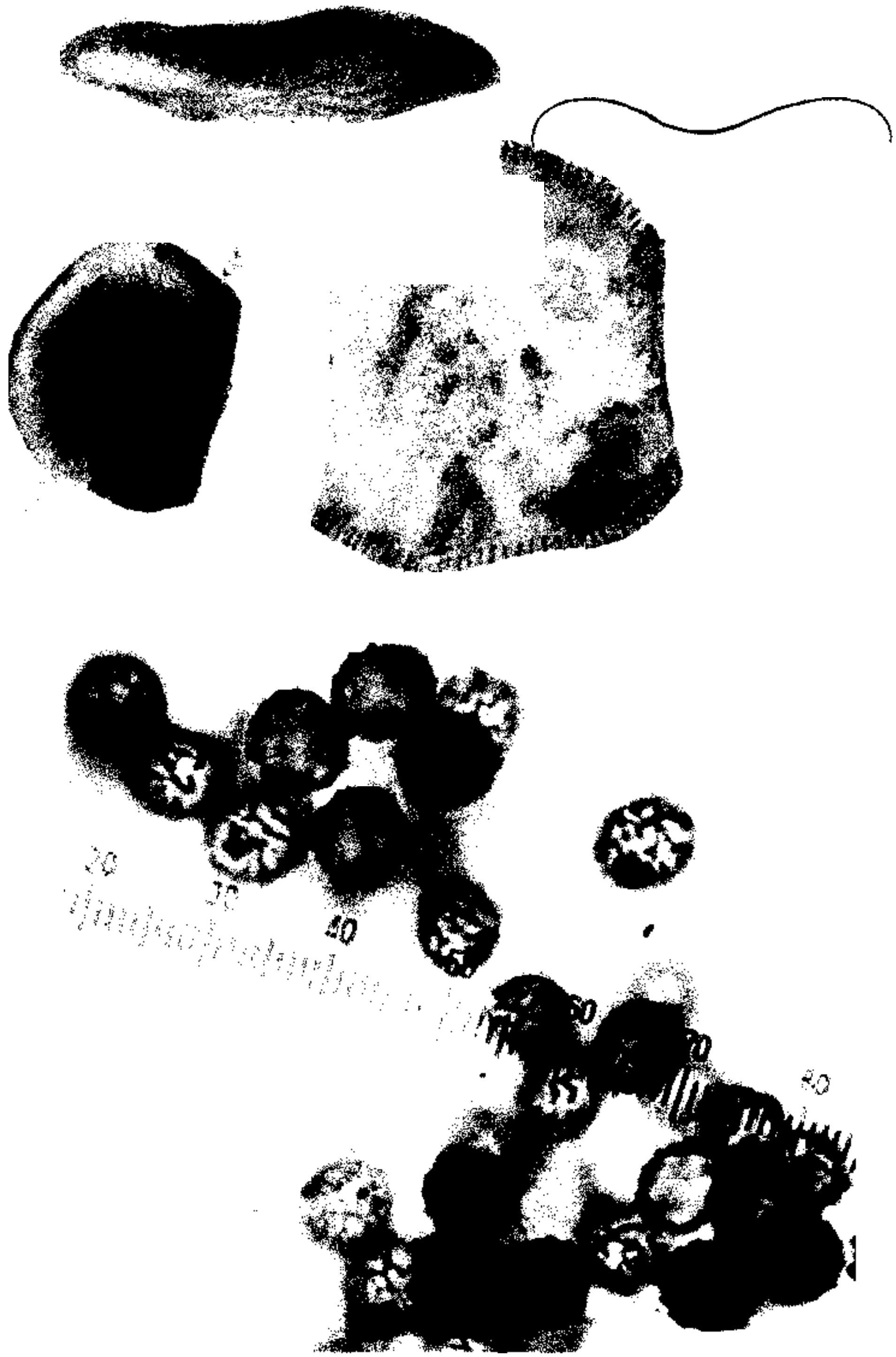

Figure 215 Macroscopic characters and spores of *Russida* cessans: Top, profile, immature and mature top view and a longitudinal section, square is 1cm^2 and shows spore print colour; bottom, spores with 1um division scale.

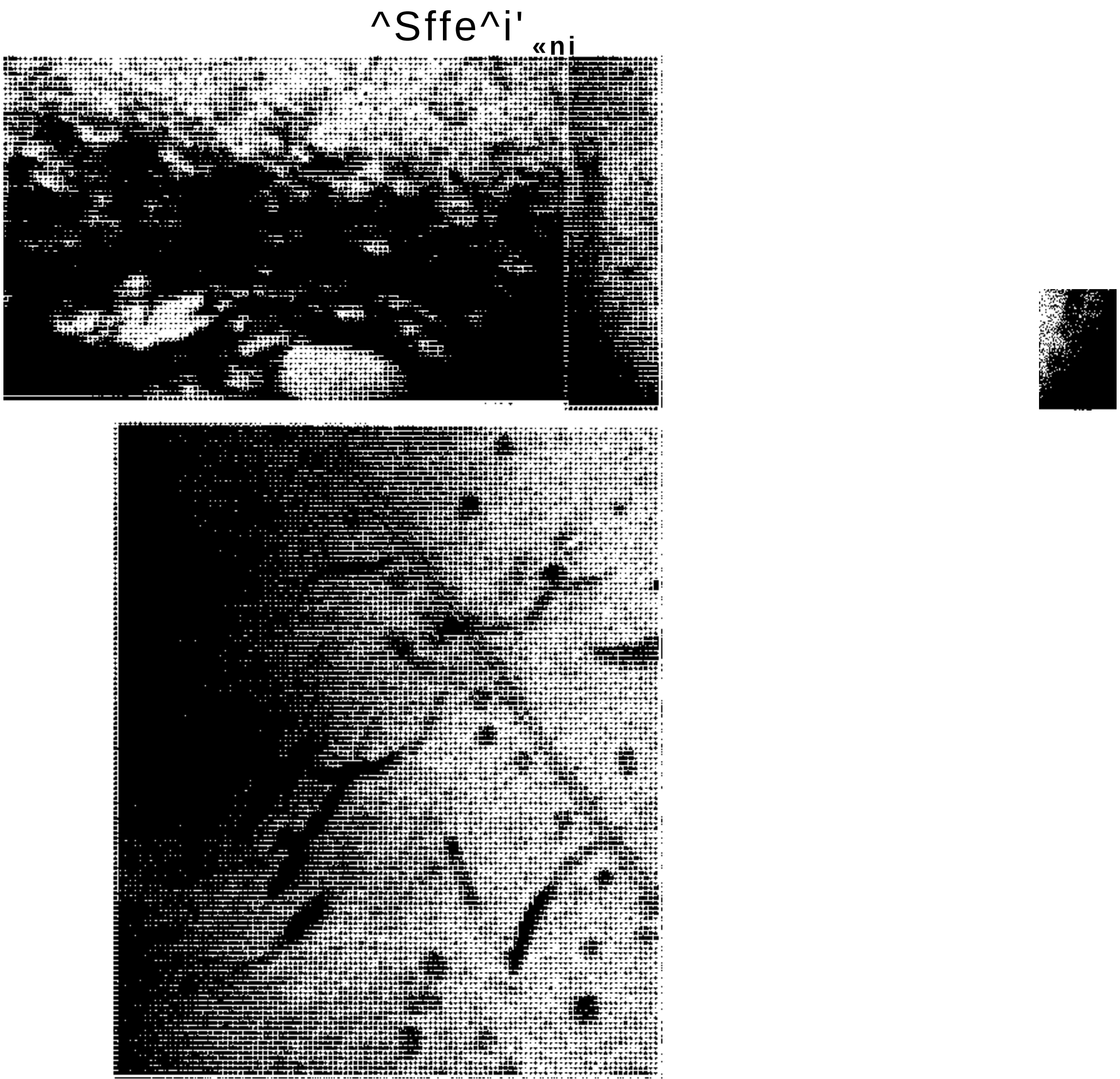

Figure 216 Cutis *ofRussula cessans:* Top left, section of cutis mounted in 3% NH_4OH, 10 scale divisions are lOOum; top right, detail of the epicutis showing pileocystidia with refractive and banded contents, 10 scale divisions are 25um; bottom, surface view of cutis stained in SV showing pileocystidia and pseudocystidia, 10 scale divisions are 25 urn.

Clade 10b

Subsection *Puellarinae* Singer

Russula puellaris **Fries**

Epicrisis Systematis Mycologici: 383. 1838

Cap 3.7-6.5cm diameter, convex when young, becoming plane with a shallow central depression, margins soon striate, becoming tuberculate, to about 1/3 the cap radius. Colour a dark vinaceous purple, almost black centrally, lighter purple to vinaceous to brownish pink towards the margin, becoming browner as the flesh beneath yellows and shows through the translucent cutis, which also fades in age. Cutis slightly viscid when wet, soon drying matte or with a slight sheen and minutely punctate, peelable 1/2-3/4 the radius, trama beneath tinted pink at first, otherwise creamy white, bruising dull yellow, quite thin, around 2mm at half-radius on a 6.5cm wide cap.

Gills pale to deep cream, bruising dull yellow sometimes also with slightly brown edges, subdistant, occasionally close, subgills and forking uncommon, adnate or sometimes with a tiny decurrent tooth at the stipe, strongly ventricose, up to 10mm or about 5 times the thickness of the trama at half-radius, broadly acute at the cap margin, usually more pliable than brittle.

Stipe 3.0-6.7 x 0.9-1.8 cm, usually longer than the cap diameter, cylindrical to narrowly clavate, longitudinally slightly rugulose, stuffed with a soft bread-textured trama which may develop 3-4 small cavities that merge to form a hollow stipe, white, entirely discolouring dull yellow in age or after handling, and also having a waterlogged look, although this may take several hours to develop.

Texture slightly softer than the average *Russula*.

Taste mild.

Odour slightly fruity, sometimes none.

Spore colour light buff-cream, Romagnesi Ila-c.

Spores 8.3-10.9 x 6.7-9.2um, L:W 1.02-1.21, with a mean of 1.09 (n=20) in the collection from the Rainforest trail, PRNP, and 1.06-1.31, with a mean of 1.18 (n=50) for the remaining collections; globose to subglobose to broadly ellipsoidal. **Ornamentation** of warts 0.5-1.2um high, mostly in the 0.7-1 .Oum range, conical to bluntly conical or occasionally peg-like, 2-several-catenate, or joined by thin to moderate lines forming a partial reticulum on some spores, or a very broken one on others, occasional spores with mostly isolated warts. Both the spore shape and degree of reticulation vary among collections, although none are completely reticulate. **Woo types** B3 to C3, often with partial E2 type ornamentation, sometimes A3. **Suprahilar patch** strongly amyloid, irregularly shaped, often with warts within and on its borders, sometimes relatively small, 2um or less across. **Hiliferous appendix** 1.4-2.4um long, 0.9-1.5um wide near the base. **Basidia** 4-spored, occasionally 2-spored, 32-45 x 10-13um, short and broad, often almost columnar pre-maturity, becoming clavate and slightly bulbous in the upper half. A few of the basidia are filled with a deep yellow material, most commonly older and spent ones. Sterigmata 5-7.5 x 1.6-2.4um. **Pleurocystidia** frequent to sparse, up to 80um apart near the gill origin to around 3 Oum apart near the gill edge, 50-80 x 7-12um, protruding up to 25 um, sometimes embedded, originating in the subhymenium, often quite shallowly, cylindrical to clavate, most tips capitate, otherwise acute to subacute or sometimes rather blunt and rounded, contents refractive, yellow-brown, unreacting to purple in SV, the strongest staining occurs towards the gill origin. **Cheilocystidia** common, 28 45- x 5.5-8um, embedded or protruding 10- 15 pm, fusoid to roughly cylindrical but occasionally with slight constrictions, often crumpled, rarely with 1-2 septa and then "empty" (fig. 218), tips capitate, mucronate or with a short irregularly shaped extension, contents refractive, pink in S V. The gill edges sometimes have a thin gelatinous coating in parts which adheres the protruding portion of cystidia to the gill edge, and forms a lumpy coating on the cells, this may be in response to some environmental damage since the affected gill edges are also strongly yellowed. **Subhymenium** 20-40um thick, pseudoparenchymatous, expanding by one cell for each subsequently developing basidium, of relatively large, roughly ellipsoidal cells up to 20 x lOum, **gill trama** of sphaerocytes up to 40um across, vascular hyphae occasional.

Cutis 70-160um thick, an ixotrichodermis but with a sometimes inconspicuous gelatinous matrix. **Subcutis** about 2/3 the cutis depth but merging gradually into the epicutis without a clear demarcation, of tightly interwoven semi-upright hyphae 2-6um wide, containing a water soluble pink vacuolar pigment that gives them a granular appearance en masse, the lower layers repent and parallel, merging into the hypodermis. Vascular hyphae uncommon, with yellow refractive contents, barely staining in SV. **Epicutis** of upright hyphae 3-5um wide, generally septate every 10-25um, tips undifferentiated or most often with a short tapered cell, occasionally bearing a tiny terminal button. Clumps of hyphal tips can be found in which the contents are deep yellow, accompanied by yellow pileocystidia. **Pileocystidia** abundant, of two types: small, clavate ones 25-38 x 4-7um, 1-2-septate, mostly at the surface, with deep yellow contents (a necropigment) in NH4OH, sometimes banded, becoming brownish yellow in SV, and longer, broader, 1-4-septate, cylindro-clavate ones with grey to black granular contents in SV, emanating from the subcutis. These latter measure 5 0-130x6-Hum with the terminal 1-3 cells forming a clavate end to a cylindrical base. Occasionally the two types are found within one pileocystidium with the greyish ones forming the terminal cells or vice-versa. The yellow hyphal ends and pileocystidia are variable in their occurrence, increasing in older and more discoloured basidiomata. Very rarely, small particles of a loose incrustation may be found over short sections of the cystidia or their supporting hyphae. **Hypodermis** a layer of parallel hyphae with frequent septa appearing in places as a compressed pseudoparenchymatous layer with normally septate hyphae permeating it.

Trama mostly of sphaerocytes in loosely defined clusters and scattered within a hyphal network, vascular hyphae common, up to 9um wide, contents either weakly greying and granular or strongly yellow in SV.

Chemical reactions: FeS04 - usually weak, pinkish to brownish-grey to grey; KOH - yellowish to orange-brown on cap surface, unreacting to yellowish on stipe; NH4OH - no reaction to dull yellow on stipe; phenol -brownish purple, sometimes weak or slow; S V -purplish-pink on the gills, brownish-purple on the cutis, cystidia and vascular hyphae brownish pink to dark grey.

Habitat and tree associations: Singly or in small groups on sandy soils under Sitka spruce, in warm weather May through October.

Collections: PK000527-SchRp on the forest floor towards the lower (seaward) part of the trail, under old-growth Sitka spruce, western hemlock and salal, approximately N49.0665^0, W125.7885^0. CR010613-27, on duff along the trail inland of the house and campground under regeneration Sitka spruce, western hemlock, western red cedar, salal and grasses, N49.172822°, W125.968405^0. BK/OC011030-rfa on duff beneath old-growth Sitka spruce, western hemlock and western red cedar, along the Rainforest trail east of the highway, PRNP around N49.4468°, W125.5317°. CR021016-11, on duff under Sitka spruce krummholz at the beach edge of Wickaninnish bay, PRNP, N49.01262^0, W125.67470^0. CR020927-03, in old growth valley bottom forest, on very decayed wood and soil mixture at the base of a western hemlock, with Sitka spruce, western red cedar and huckleberry, Carmanah grove, N48.6660^0, W 124.6840°. (All collections from the southern very wet hypermaritime CWH subzone)

	ITSl-Fto	RFLP:		
Collection	ITS4-B	Hinfl	Alul	Sau3A
BK/OC011030-rfa	866	380,241	511,291	300,245, 193
CR020927-03	849	358,250	490,291	305,227
CR021016-11	869	386,330	473	300,250,190

Notes: *Russulapuellaris* is distinctive in the tendency of the flesh of all tissues to age and bruise ochre yellow. Other macroscopically similar species such as *R. brimneoviolacea, R. blackfordae* and *R. sphagnophila* may show some yellowing at the base of the stipe, but this does not extend to the cutis, cap trama or gills. Some of the collections described here were not strongly yellowed but still showed occasional intensely yellowed cells in the cap trama and cutis when viewed under the microscope in several mounting media. This seems to be the main difference between *R. puellaris* and

R. sphagnophila, which have very similar spore colour, size and ornamentation and similar habitat.

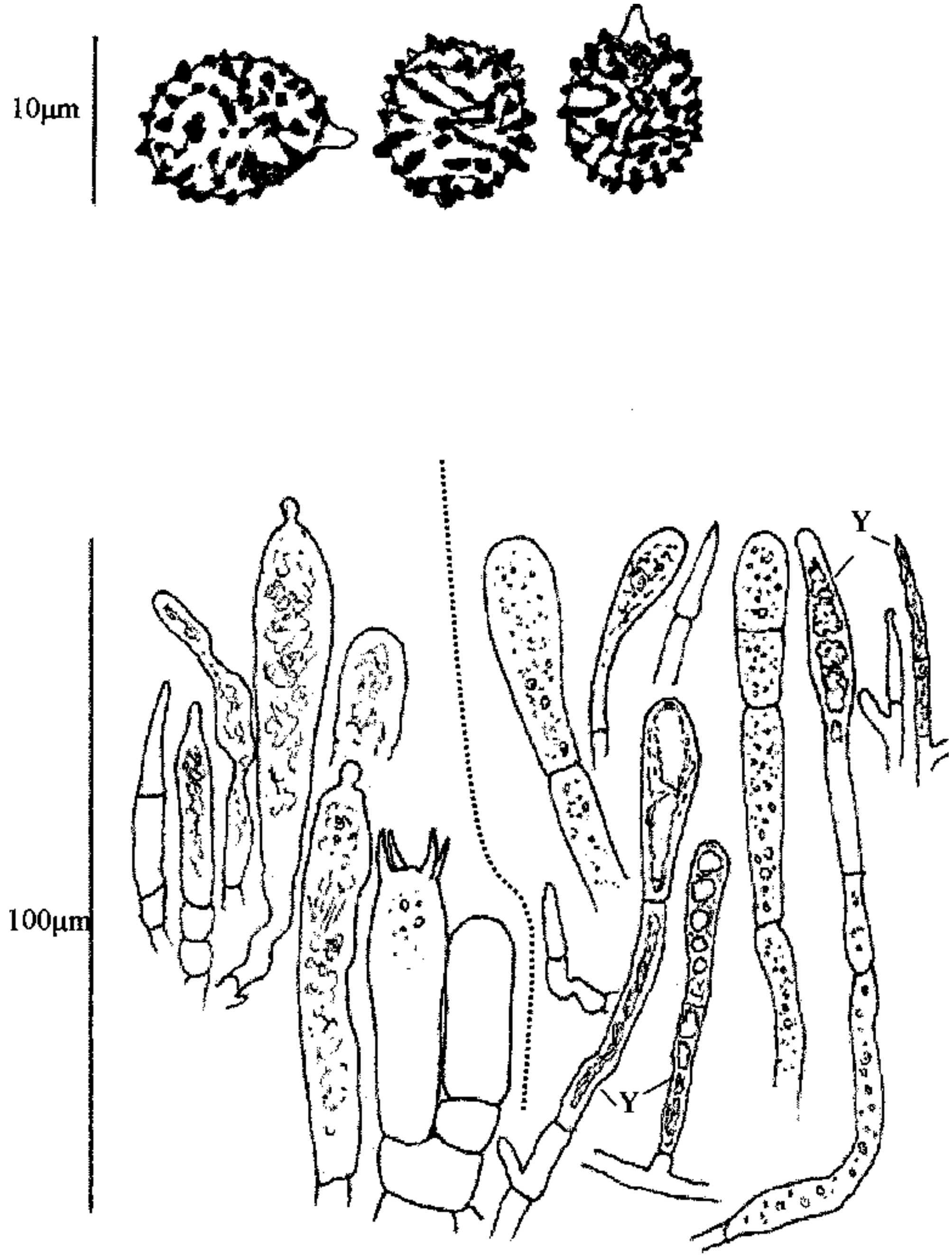

Figure 217 Microscopic characters of *Russula puellaris:* Top, spores with 10 um scale bar; bottom left, three cheilocystidia, pleurocystidia and basidium with basidiole; bottom right, hyphal ends and pileocystidia from the epicutis, those filled with deep yellow contents are marked Y, lower scale bar is 100 um.

Figure 218 Macroscopic characters of *Russulapuellaris:* Top, fresh basidiomata that have not yet yellowed (photograph on left by A. Ceska, that on right showing 1cm scale); below, top and underside of a similarly sized and coloured basidioma several hours after collection, in which the yellowing reaction is well advanced and has changed the purple colour of the cap to brown (photographs by B. Kendrick); small square is $1cm^2$ and shows spore colour.

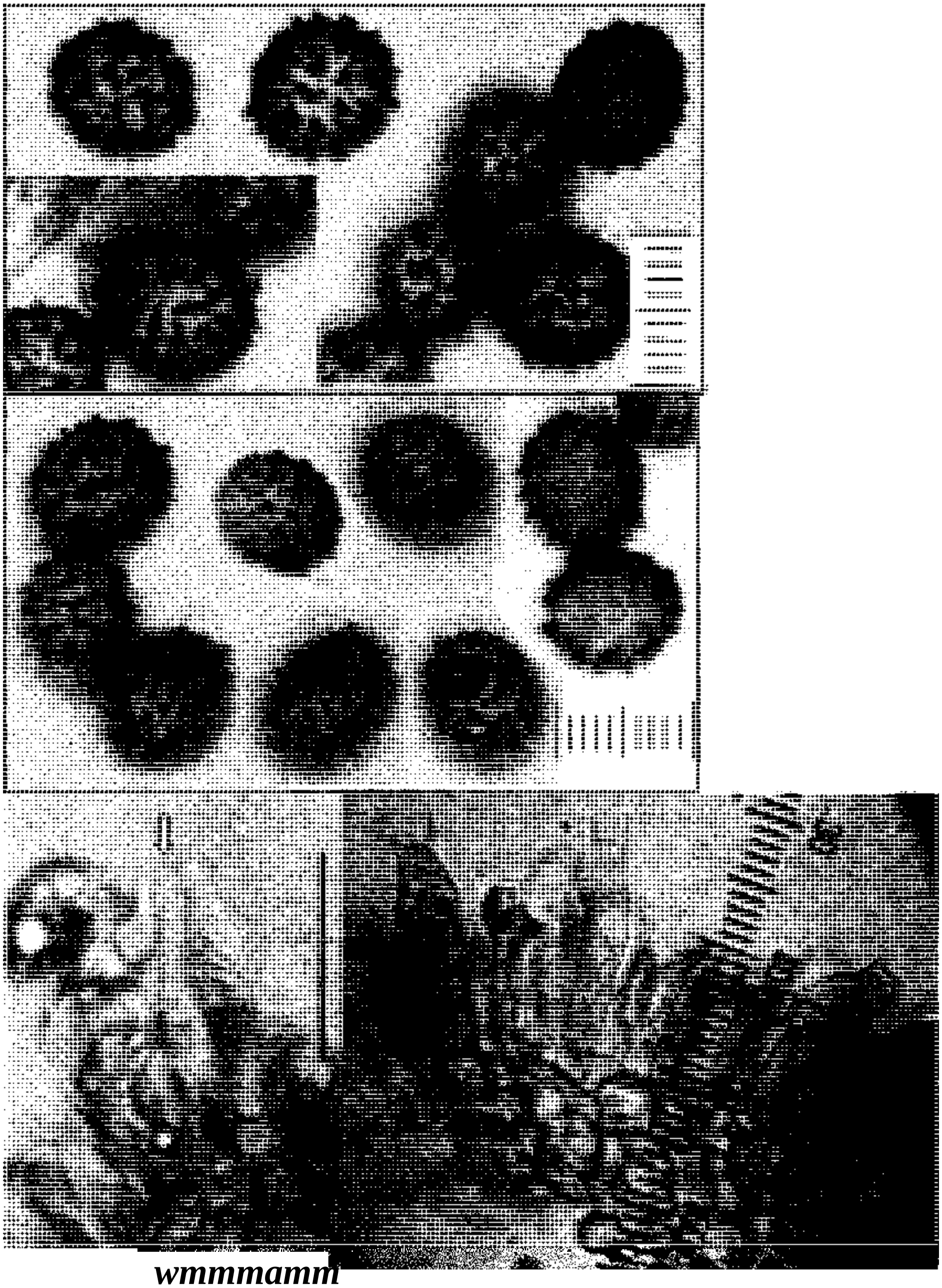

Figure 219 Hymenium of *Russulapuellaris:* Top, spores of the Wickaninnish collection; middle, spores of the Schooner beach collection, both with lum division scale; bottom left, 2-septate "empty" cheilocystidia (arrowed) with a non-septate one to its right, developing directly from the subhymenium, scale bar is 2um; bottom right, fragment of hymenium showing mature and developing basidia upon the pseudoparenchymatous subhymenium.

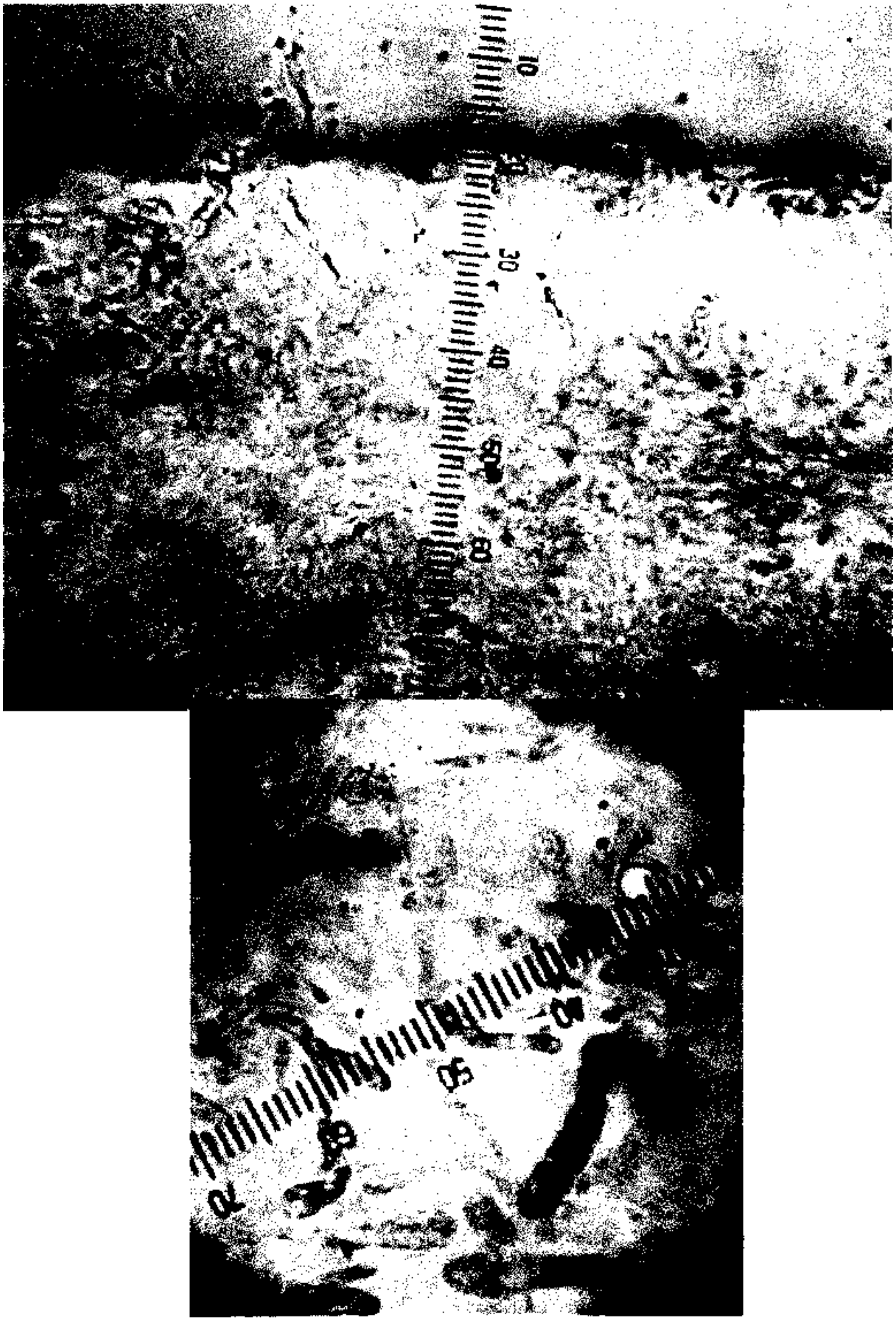

Figure 220 Cutis of *Russula puellaris:* Top, section through cutis in which a cluster of hyphal tips and pileocystidia with yellow contents can be seen to the left of the scale, and normal pileocystidia to the right, the section is mounted in 3% NH4OH, 10 scale divisions are 25um; bottom, surface view of epicutis stained in SV showing normal pileocystidia, stained grey, and smaller yellow ones which often have banded contents, scale as above.

Subsection *Laricinae* **(Romagnesi) Bon**

Russula nauseosa **(Pers.) Fr.**

Epicrisis Systematis Mycologici: 363. 1838.

Cap 2- 5.4cm diameter, hemispherical when young, sometimes with a slight umbo, becoming convex or plano-convex usually with a small central depression, margins striate even from quite young, in age almost tuberculate. Colour when young almost black with a purple margin, becoming vinaceous to violet, generally darker and browner centrally, sometimes with an olive tint over the disc, sometimes deep grey-brown, fading in age to greyish. Viscid when wet, drying matte or with a dull sheen and minutely punctate. Cutis peelable 3/4 the radius, flesh beneath white to cream, unchanging to discolouring a very pale yellowish brown, trama steeply reducing in thickness to 1mm or less by half-radius.

Gills white becoming cream and eventually pale orange cream, slightly bruising light yellow brown, subdistant to close, sub gills not seen, occasional forking toward the cap margin, adnexed at stipe broadening outwards, obtuse to rounded at the cap margin, 2-4 times the depth of the trama (3 to 6 mm) at half radius, brittle.

Stipe 2.7-5.7 x 0.6-1.3cm, longer than or often twice the cap diameter, white, usually narrowly clavate, stuffed with a soft, bread-textured trama that develops three or more cavities, eventually becoming hollow, discolouring very pale tan to light ochre where bruised and damaged.

Texture firm but quite fragile, later soft and fragile.

Taste mild, at first the gills very slightly peppery.

Odour not distinctive to fruity, one basidioma from the Long Beach picnic site had an apricot, chanterelle-like odour.

Spore colour light dull orange to ochre, Romagnesi Illb-c.

Spores 8.3-11.4 (-13) x 6.7-9.2um, L:W 1.13 to 1.4 with a mean of 1.26 [n - 48], ellipsoidal to broadly ellipsoidal, occasionally obovoid. **Ornamentation** of fine to heavy, blunt to pointed conical warts 0.5 to 1.3um high, mostly isolated, or 3-5-catenate, occasionally more, sometimes connected in small clusters of 2-4, occasionally linked by fine lines, not forming a reticulum, on some spores the warts are quite crowded. Woo type B3. **Suprahilar patch** amyloid, irregularly shaped, warts at its borders and sometimes within. **Hiliferous appendix** 1.5-2.2um long by 1.2-1.5um wide near the base. **Basidia** 2- and 4-spored, both types equally common, 35-50 x 1 l-13um, short and broad, clavate. Sterigmata relatively short, up to 7x 2um. **Fleurocystidia** numerous, regularly distributed, 48-80 x 8-12ujm, protruding 10-25 microns, sometimes embedded, narrowly clavate to fusoid, tips acute, occasionally obtuse or more commonly with a tiny capitatum, contents refractive, blue-black in SV. **Cheilocystidia** frequent to none or sometimes hard to see, 45-55 x 7-10um, embedded or protruding 15-20um, broadly fusoid to clavate, tips capitate, mucronate, occasionally obtuse, not or only weakly staining in SV. **Subhymenium** 20-30um thick, pseudoparenchymatous, merging gradually into trama near the origin of the gill. **Gill trama** of sphaerocytes, generally up to 30pm diam., vascular hyphae uncommon.

Cutis 80-160um thick, an ixotrichodermis in two distinctive layers embedded in a gelatinous matrix. **Subcutis** 1/3 to 1/2 the depth of the cutis, tightly interwoven of repent, parallel hyphae containing globular or amorphous pink pigment that rapidly leaches into the mounting fluid, 2.5-5 um wide, occasionally with inflated sections, vascular hyphae common, some of which ascend to the epicutal surface and terminate in pseudocystidia, grey in SV. **Epicutis** of upright to semi-upright hyphae with pinkish contents, hyphal ends 2.5-4um wide, undifferentiated, accompanied by multiple pileocystidia. **Pileocystidia** abundant, 30-110 x 5-10um, occasionally up to 12 um wide, most in the middle of this range, clavate to narrowly clavate, occasionally almost cylindrical, arising in the lower epicutis, 1-3-septate, rarely aseptate, quite often constricted at the septa, frequently with a shorter terminal cell about 25-30um long, tips obtuse to rounded, contents yellowish and refractive in KOH, turning gray to black in SV and pink in acid fuchsin. Sometimes darker pink patches occur with the latter reagent but they do not appear to be outside of the hyphal wall. Very rarely one can find a cystidium with small patches of incrustation below the terminal cell, apparent in fuchsin but not SV mounts. **Pseudocystidia** common, similar in shape and septation to pileocystidia, staining pink in acid fuchsin, generally not incrusted although occasional segments of wall stain more strongly, possibly a very tightly adhering material. **Primordial hyphae:** occasionally long, regularly septate broad hyphae around 7-8um wide and partially filled with refractive contents may be found that are more like primordial hyphae in appearance than cystidia, but they are not noticeably incrusted, stain weakly grey in S V and patchily pink in acid fuchsin. **Hypodermis** not differentiated from the subcutis or trama.

Trama mostly of sphaerocytes in well defined clusters bound by a hyphal mesh that is more substantial than is generally found in other small Russulas, vascular hyphae occasional.

Chemical reactions: FeSC«4 - no change to a light pinkish brown; KOH - red or orange on cap no reaction on stipe; NH4OH - no reaction; phenol - brownish purple; SV - initially violet on the gills and cap trama, cystidia and vascular hyphae grey to black.

Habitat and tree associations: On the forest floor beneath western hemlock and Sitka spruce, with understory salmonberry and mosses. Sitka spruce is the most likely tree symbiont.

Collections CRO10814-01, in a mature regeneration stand of Sitka spruce and western hemlock along the Fairy Lakes trail near Port Renfrew, N48.583150^0, W124.355217^0; CR011030-01, in grass under young Sitka spruce with mature Sitka spruce and western hemlock nearby, Long Beach picnic area, PRNP, N49.070061^0, W125.754495^0 (southern very wet hypermaritime CWH subzone).

Collection	ITSl-Fto ITS4-B	RFLP: Mnfl	Alul	Sau3A
CR010814-01	832	400,364	495,274	440,257

Notes: The spore print colour is paler than is given for *Russula nauseosa* in Romagnesi (1967), more like that of *R. nitida*, which also has spores similar in size and ornamentation to the above collection. However, this latter species is associated with birch, of which there is none in the vicinity of the collection area, in fact this tree symbiont is very rare on Vancouver Island. The flesh of *R. nauseosa* does not bruise yellow like that of*R. puellaris,* although it may become weakly yellow-brown especially at the stipe base, and does not have the conspicuously incrusted primordial hyphae of *R. turci* and *R. murrillii,* two other violet to purple, mild, yellow-spored species. *Russula brunneoviolacea* is also macroscopically similar, but that species has paler, smaller spores and flesh that yellows more than that of *R. nauseosa,* and it is found in a different habitat. It is not clear why the epithet *nauseosa* was given to this species, since neither the taste nor the odour is suggestive of vomit.

Russula nauseosa has been placed in the *Laricinae* by Bon (1988) and Romagnesi (1967, 1985), yet in the DNA analysis in Chapter 2 it groups with the *Puellarinae* and, if one follows Bon (1988), with the *Odoratinae,* a group he segregated by its odour. In Europe it fruits in late spring to early summer, on Vancouver Island along the coast, spring-like weather may persist in some years for much of the year, so fruiting seasons for some species are extended, the above collections were made in mid-August and late October.

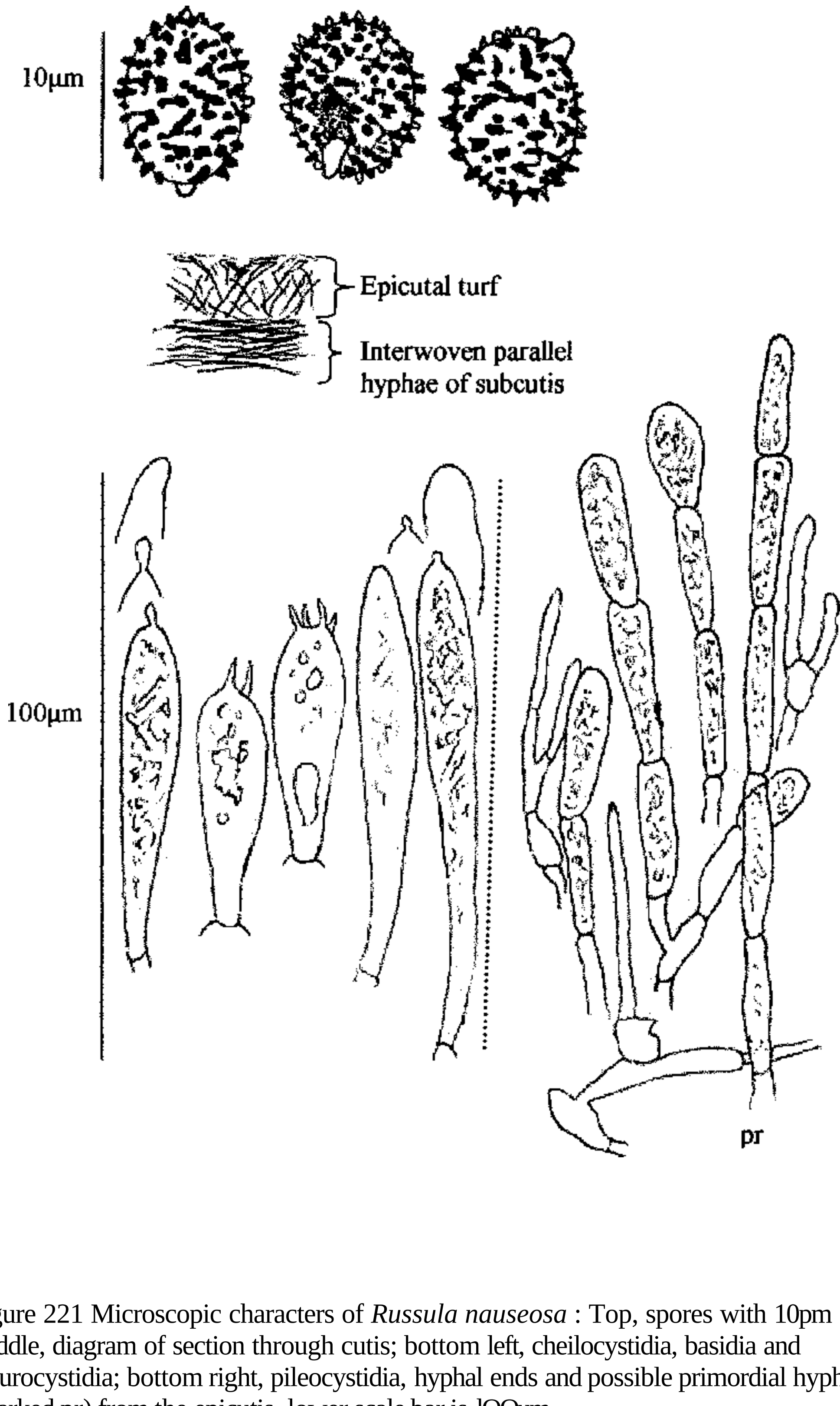

Figure 221 Microscopic characters of *Russula nauseosa* : Top, spores with 10pm scale bar; middle, diagram of section through cutis; bottom left, cheilocystidia, basidia and pleurocystidia; bottom right, pileocystidia, hyphal ends and possible primordial hyphae (marked pr) from the epicutis, lower scale bar is lOOum.

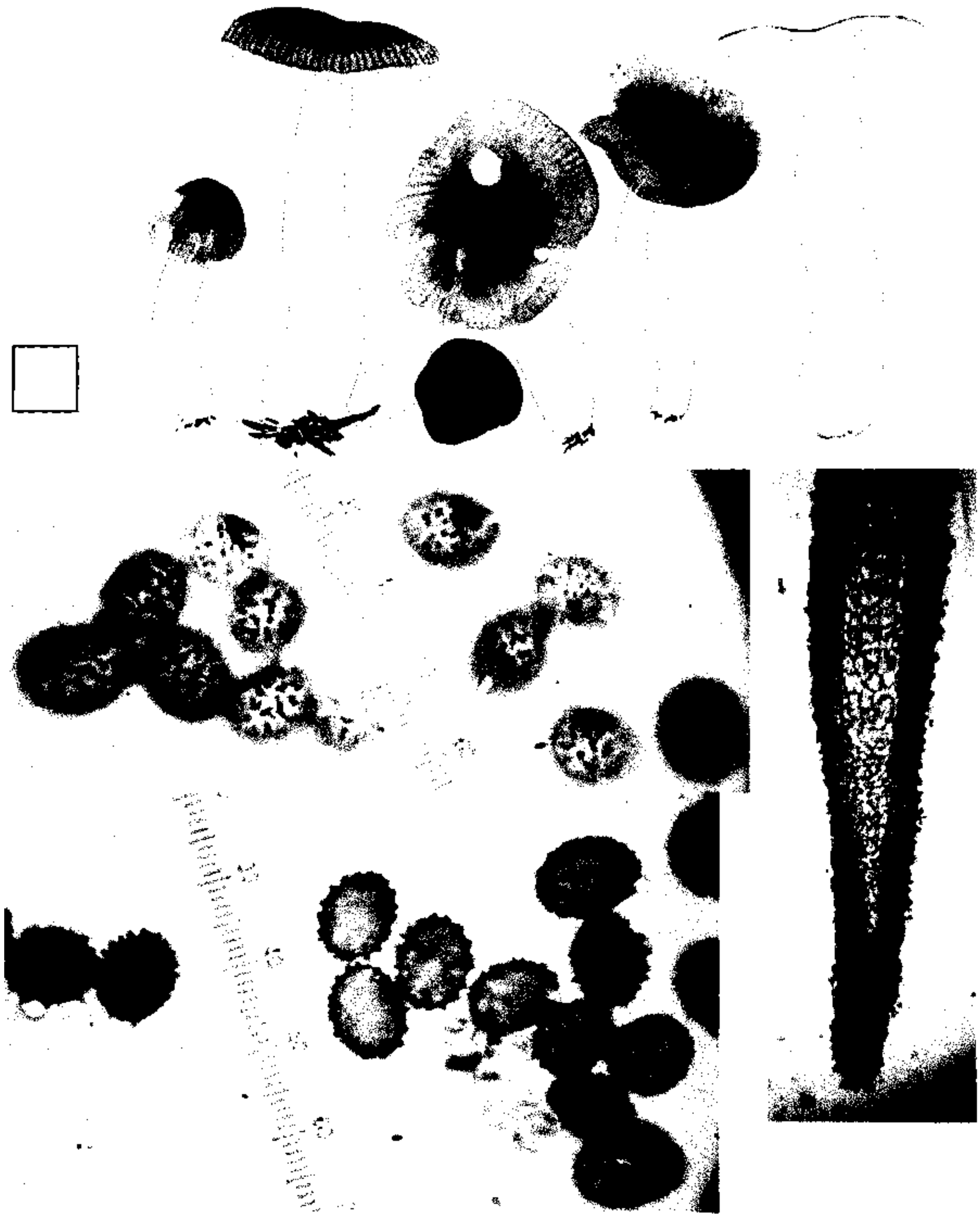

Figure 222 Macroscopic and hymenial characters of *Russule nauseosa:* Top, illustration showing profiles and top of several basidiomata of varying maturity, with a longitudinal section and spore colour in the $1cm^2$ square; bottom left, spores in surface and equatorial view with lum division scales; bottom right, section through gill showing gill trama of spherocytes and differentiated subhymenium which becomes less strongly differentiated towards the origin, 10 scale divisions are lOOum.

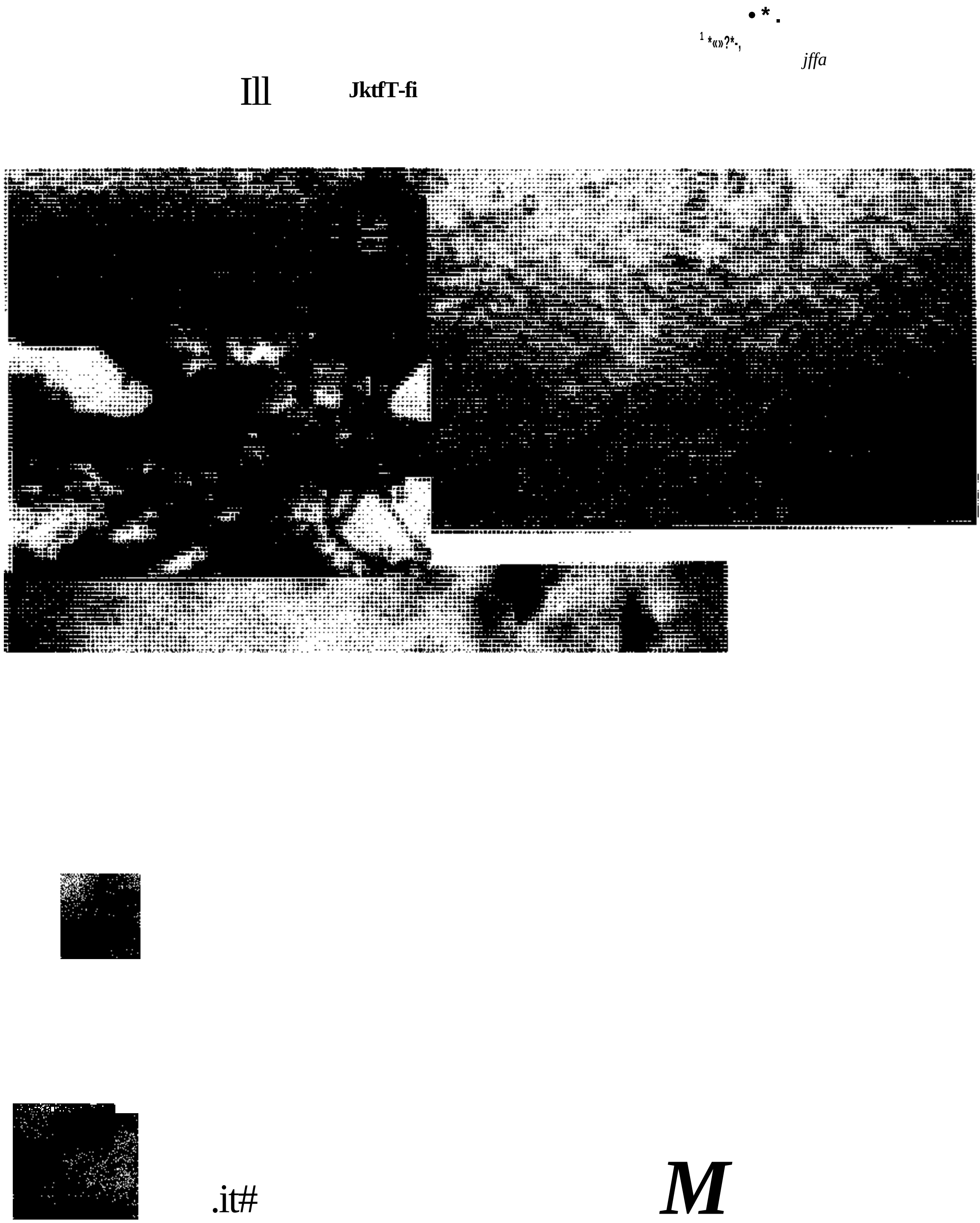

Figure 223 Cutis of *Russula nauseosa:* Top left, section through cutis and part of trama stained in Congo red, the cutis-trama boundary is about the 35 mark on the scale, 10 scale divisions are lOOum; top right, closer view of the epicutal hyphae in which a few slightly inflated sections can be seen, as can the tip of a pileocystidia, upper right, 10 scale divisions are 25 um; bottom, surface view of cutis stained in acid fiichsin, several clavate pileocystidia can be seen and also a primordial hyphae (arrowed), 10 scale divisions are 25pm.

Table 19 Comparison of key characters of small, mild-tasting, purple to violet, cream and yellow-spored Russulas. All *bntR murrillii* are in section *Tenellae* Quelet. Information from local collections.

<table>
<tr><th>Species</th><th>Spore size um</th><th>Spore colour</th><th>Spore ornamentation</th><th>Yellowing</th><th>Habitat</th></tr>
<tr><td>R. brunneoviolacea</td><td>7.4-8.8 x 6.1-7.1</td><td rowspan="3">Cream

Ila-d</td><td rowspan="2">Partial reticulum</td><td rowspan="2">Base of stipe only</td><td>Oaks, Douglas fir, western hemlock</td></tr>
<tr><td>R. sphagnophila</td><td>8.2-10.2 x 6.3-8.2</td><td rowspan="3"></td></tr>
<tr><td>R. puellaris</td><td>6.5-10 x 5.5-8</td><td rowspan="2">iliiiiiSsiiiiii</td><td>lUKUtKSi</td></tr>
<tr><td>R. nauseosa</td><td>8.3-11.4 x 6.7-9.2</td><td rowspan="3">Deep yellow

Ulc-fVc</td><td>Base of stipe only</td></tr>
<tr><td>R. cessans (larger basidiomata -up to 9cm)</td><td>6.5-8.8 x6-8</td><td rowspan="2">Partial reticulum</td><td rowspan="2">None</td><td rowspan="2">Douglas fir and western hemlock</td></tr>
<tr><td>R. murrillii (appears chalky, has incrusted hyphae)</td><td>7.3-10.8 x 6-8.5</td></tr>
</table>

There are a number of small, purple to violet, mild, non-yellowing, gregarious Russulas in Vancouver Island coastal forests within the *Tenellae* that can be extremely frustrating to identify. Since they are closely related there is scant difference between cuticular characters, so obtaining a good spore print with which the colour can be accurately assessed is crucial. Unfortunately, one may come across collections that differ only in one character such as spore colour, ornamentation or size, or that differ in habitat or slight macroscopic characters, but have virtually identical microscopic characteristics. In the European literature there are many more species described in this general group than in North America, and it is quite likely that some local species are undescribed and that others have more variation in certain characters than has been so far recorded.

Glossary

Allantoid -sausage shaped.

Basidioma, pi. basidiomata -the structure supporting multiple basidia; the mushroom.

Basidium, pi. basidia -a specialized cell in which conjuction of two nuclei occurs followed by meiotic division. The basidia support and give rise to the spores, most commonly 4 in *Russula.*

Cheilocystidia - (plural) cystidia or pseudocystidia found on the margin of the gills, see also pseudocystidia.

Cystidia - (plural) specialized structures, containing material that differs from that of ordinary generative hyphae, in *Russula* this is usually refractive, turns red to blue-black with sulphobenzaldehydes (e.g., sulphovanillin) and contains sesquiterpenes that give the mushroom a peppery taste. The term cystidia is usually prefixed with a modifier that gives type or position, eg. pileocystidia =on the pileus. True cystidia arise from generative hyphae.

Context -the trama or inner flesh, often used in reference to the stipe in which the softer inner tissue is the context.

Cortex -of the stipe, the firm layer or rind of the outer surface.

Diverticulae - small projections or short branches from the main hypha, usually not separated from it by a septum; diverticulate -having such projections.

Epicutis -the outer layer of the cutis, usually referring to that of the pileus.

Falcate -sickle-shaped, like a tapered, flat blade in an arc shape.

Free -of gills, not joined to the stipe but terminating under the cap tissue at or distant to its junction with the stipe.

Hair cells -long, tapering, terminal hyphal cells of the epicutis, with thicker than normal cell walls which may be pigmented yellow-brown, sometimes colourless, found in *Russula vesca* and *R. heterophylla.*

Hiliferous appendix -the unornamented, inamyloid, bluntly conical appendage toward the base on the ventral side of the spore, which is the remnant of the attachment to the sterigma on the basidium. Synonyms: hilar appendix, hilum, apicus.

Hypodermis - a layer of tissue beneath the subcutis, often a compacted form of the tramal tissues. In some literature the subcutis is termed the hypodermis.

Lamellae -gills, singular= lamella.

Lamellulae -subgills, those not reaching the stipe. Singular: lamellula.

Laticifer, laticiferous hyphae - (plural) hyphae containing latex or a latex-like substance, usually appearing refractive, called gloeoplera in the most recent terminology. See vascular hyphae.

Mucronate -with an abrupt beak-like extension, often used in relation to cystidia.

Oleiferous hyphae -hyphae containing an homogeneous, oily appearing substance not reacting in SV. Sometimes only a section of a hypha has such contents, occurring occasionally in the trama of Russulas. See vascular hyphae.

Pileocystidia -sometimes termed (plural) dermatocystidia, specialized termini to epicutal generative hyphae of the pileus, containing refractive contents like those of vascular hyphae and which often react differentially in S V. Note that some authors call the terminal epicutal hyphal cells dermatocystidia if they are differentiated by shape but not contents, and call those differentiated by contents pseudocystidia.

Pileus -cap, including gills.

Pleurocystidia - (plural) cystidia or pseudocystidia found on the surface of the gills in the hymenium, sometimes called macrocystidia or gloeocystidia, see pseudocystidia.

Primordial hypha -found in the epicutis and base of the stipe of some *Russula* species, originating from the primordium, a hypha (singular) that is usually broader than generative epicutal hyphae, is normally septate and branched, has slightly refractive contents and often incrusting material that stains pink in acid fuchsia, but has very little reaction to SV.

Pseudocystidia - (plural) cystidia-like cells that terminate vascular hyphae (laticifers) at a surface such as the pileus epicutis or the stipe outer cortex. In sections through the pileal cutis, pseudocystidia are those emanating from the hypodermis, trama or lower subcutis. Technically, the majority of hymenial cystidia are pseudocystidia, also termed gloeocystidia in *Russulales.*

Sphaerocytes -short sections of hyphae that are inflated so as to become spherical or nearly so, found singly or more usually in clusters in the pileal, stipe and gill trama. Sometimes erroneously called sphaerocysts.

Sterigma, pi. sterigmata -tubular projections from the basidium that support the developing spores.

Stipe - a non-vascular stem.

Subcutis -usually referring to that of the pileus, the layer of tissue beneath the epicutis and usually interwoven with it, and above the hypodermis or tramal tissues.

Subgills -gills that do not extend inwards as far as the stipe, or at least as far as the longest gills when these are free. Synonym: lamellulae.

Suprahilar patch -a small, variously shaped area just above the hiliferous appendix on the ventral side of the spore. This area may be colourless in Melzer's reagent, or be a diffuse, low amyloid area (grey in Melzer's reagent), with or without a reduced version of the ornamentaion found over the rest of the spore wall.

Vascular hyphae -general term referring to hyphae carrying refractive or oily-appearing material, usually of larger diameter than generative hyphae, and often reacting to stains such as SV. In this thesis the terms "laticifers" or "laticiferous hyphae", and "oleiferous hyphae" have been replaced by the term "vascular hyphae" with a description of the contents, since the former types are not always easy to differentiate and are occasionally used interchangeably by some authors.