

1 ***Russula* (Basidiomycota, Russulales, Russulaceae) subsect. *Roseinae* “down under”**

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14 Abstract. The present contribution presents species of *Russula* subsect. *Roseinae* Sarnari from
15 the southern hemisphere. *Russula incrustata* Buyck, sp. nov. and *R. koniamboensis* Buyck, sp.
16 nov. are described from New Caledonia, *R. purpureotincta* R. F. R. McNabb from New
17 Zealand is redescribed in detail and two secotioid species, *R. albobrunnea* T. Lebel from
18 Australia and *R. kermesina* (R. F. R. McNabb) T. Lebel from New Zealand are shown to be
19 the first known secotioid taxa in *Roseinae*. The systematic placement and importance of these
20 southern taxa is discussed.

21 Key words: Australia, biogeography, New Caledonia, New Zealand, *Nothofagus*, phylogeny,
22 *Russula* crown clade

23

24 **Introduction**

25

26 Extant species of *Russula* subsect. *Roseinae* have been suggested to have diverged about three
27 to one million years ago (Looney et al., 2020) and are part of the most diverse, major lineage
28 of the genus in the northern hemisphere, viz. the Crown clade of subgenus *Russula* (Buyck et
29 al. 2018). Species in subsect. *Roseinae* are morphologically characterized by the pink, red or
30 whitish to yellowish pileus, the white to pale cream spore print, the predominantly mild taste,
31 the context and lamellae turning eosin red with sulfovanillin and the lack of typical

32 gloeocystidia in pileipellis and context. In addition, the involved species possess “primordial
33 hyphae”, i.e. hyphal terminations with acid-resistant incrustations (see Romagnesi 1967: 58-
34 59) which are generally emerging above the other hyphal extremities. All *Roseinae* also have
35 inflated cells in the lower pileipellis composing a more or less well-developed
36 pseudoparenchymatic layer from which the hyphal terminations originate.

37 In Europe, *Russula* subsect. *Roseinae* is represented by merely two widely accepted
38 species, *R. velutipes* Velen. (syn.: *R. rosea* Quél.) and *R. minutula* Velen., but the subsection
39 is much more diverse in other regions of the northern hemisphere. Indeed, recent taxonomic
40 studies on *Roseinae* report seven described North American species and an additional six
41 species that are still not formally described (Looney et al., 2020; Manz et al., 2021): *R.*
42 *cordata* Looney, *R. rheubarbarina* Looney, *R. rubellipes* Fatto, *R. peckii* Singer, *R.*
43 *pseudopeckii* Fatto and one undescribed relative, *R. cardinalis* Looney, *R. albida* Peck (syn.:
44 *R. purpureomaculata* Shaffer) with two undescribed American relatives, one undescribed *R.*
45 *minutula* aff, as well as two undescribed species in the ‘*magnarosea*’-lineage). In addition,
46 four more species of *Roseinae* were described from the Central American montane forests in
47 Western Panama (Manz et al., 2021: *R. cornicolor* Manz & F. Hampe, *R. zephyrovelutipes*
48 Manz & F. Hampe, *R. oreomunneae* Manz, F. Hampe & Corrales and *R. cynorhodon* Manz &
49 F. Hampe), now bringing the total of putative American species in *Roseinae* (s. l.) to 17 taxa.
50 These same authors (Manz et al. 2021) confirmed or suggested the position of the following
51 thirteen Asian phylogenetic species in *Roseinae*: *R. dhakuriana* K. Das, J. R. Sharma & S. L.
52 Mill., *R. hakkae* G. J. Li, H. A. Wen & R. L. Zhao, *R. kewzingensis* K. Das, D. Chakr. &
53 Buyck with one undescribed relative, *R. guangxiensis* G. J. Li, H. A. Wen & R. L. Zhao with
54 one undescribed sister species, two undescribed Asian relatives to *R. velutipes*, two more
55 undescribed Asian species close to *R. pseudopeckii*, two undescribed Asian relatives to *R.*
56 *peckii*, as well as one sister species to *R. cynorhodon*.

57 *Russula rimosa* Murrill, again a North American species, is only known from the type
58 collection and was classified in subsect. *Roseinae* based on its morphology (Adamčík &
59 Buyck, 2012), but so far, any attempt of sequencing the DNA of this species has been
60 unsuccessful. Sequence data are also needed for the Indian *R. sharmae* K. Das, Atri & Buyck,
61 another potential member of subsect. *Roseinae* based on its microscopic features, but it is
62 unusual in producing an almost yellowish spore print (Das et al., 2013). This brings the total
63 of potential northern hemisphere *Roseinae* to 30 recognized species or about five times more
64 than hardly a few years ago !

65

66 Looney et al. (2020) suggested a northern hemisphere distribution for *Roseinae* and inferred
67 an Appalachian origin for the subsection in North America, followed by in situ diversification
68 of these species in the Appalachian Mountains roughly since the mid-Miocene. Whereas
69 published sequence data had already suggested the existence of several potential *Roseinae* in
70 Oceania or Australasia (Buyck et al., 2018; Lebel & Tonkin, 2007; Cooper & Leonard, 2014;
71 Cooper, 2021), none of these southern species has ever been discussed in the literature. The
72 aim of this study is to identify the actual taxonomic status and phylogenetic position of these
73 southern hemisphere relatives.

74

75

76 **Materials and Methods**

77

78 *Morphological study*

79 Fresh fruiting bodies were photographed in the field and in the lab, descriptive notes and
80 spore prints were taken and tissue samples transferred in CTAB solution for subsequent
81 sequencing.

82 Micromorphological characters were studied using a Nikon Eclipse E400 microscope under
83 oil-immersion lens at a magnification of x1000. All drawings of microscopical structures were
84 made with a ‘camera lucida’ using a Nikon Y-IDT drawing attachment at a projection scale of
85 x2400. Contents of hymenial cystidia and pileocystidia in the figures are indicated
86 schematically, with the exception of a few elements where contents are indicated as observed
87 in Congo red preparations from dried material. Spores were observed in Melzer’s reagent. All
88 other microscopic observations were made in ammoniacal Congo red, after a short aqueous
89 KOH pre-treatment near boiling temperature to improve tissue dissociation through gelatinous
90 matrix dissolution. All tissues were also examined for the presence of ortho- or
91 metachromatic contents or incrustations in Cresyl blue as explained in Buyck (1989).

92

93 *DNA extraction, PCR and sequencing*

94 Fungal genomic DNA was isolated from fresh material stored in cetyl-trimethyl-
95 ammonium bromide buffer (CTAB 1x). Five loci were tentatively amplified: 900–1400 base
96 pairs of the ribosomal nuclear large subunit (nuLSU) using primers LROR and LR7; 600

97 base pairs of the ribosomal mitochondrial small subunit (mitSSU) with primers MS1 and MS2
98 (White et al. 1990); 1300 base pairs of the largest subunit of the RNA polymerase II (RPB1)
99 with primers RPB1-AF (Stiller & Hall, 1997) and RPB1-CR (Matheny et al., 2009); 700 base
100 pairs of the second largest subunit of the RNA polymerase II (RPB2) using primers RPB2-6F
101 and fRPB2-7cR (Liu & Hall, 2004) and 900 base pairs of the translation elongation factor 1-
102 alpha (TEF1) using primers EF1-F and EF1-R (Morehouse et al., 2003). Amplifications were
103 performed under the conditions and with the reagents of the Taq PCR core kit (QIAGEN,
104 Inc., Valencia, California, USA). Sequencing used the amplification primers, reagents and
105 conditions of the BigDye Terminator v3.1 Cycle sequencing Kit and an automated capillary
106 sequencer ABI 3700 DNA analyzer (Perkin Elmer, Applied Biosystems, Foster City, CA,
107 USA). Most sequences for these various loci were obtained for *R. incrustata* and have already
108 been deposited in GenBank (as *R. roseinae* sp. *VH-2016n strain 735/BB 09.172*) and
109 published in the context of a *Russula* world phylogeny (Buyck et al. 2018). The newly
110 published sequences have all been deposited in GenBank (www.ncbi.nlm.nih.gov), viz. ITS
111 (OM397456-OM397459) and *tef1* (OM365994-OM365996, OM370807) for both new
112 species from New Caledonia, eight ITS sequences for *R. purpureotincta* R.F.R.McNabb
113 (OR348209, OR348217, OR348277-OR348281), including one from the holotype, and a
114 single ITS sequence for *R. kermesina* (R.F.R.McNabb) T.Lebel (OR348284).

115

116 *Phylogenetic analysis*

117 Separate phylogenetic analyses based on ITS and *tef1* performed with Maximum Likelihood
118 method were chosen in function of available sequence data for the southern species. The
119 sampling of northern hemisphere taxa is based on Manz et al. (2021). Each dataset was
120 automatically aligned by MAFFT v 7.427 (Kato & Standley, 2013), then manually adjusted
121 and trimmed with BioEdit v7.0.9 (Hall, 1999). The final ITS alignment consisted of 70
122 sequences and comprised 750 bp; the *tef1* alignment was 921 bp long (excluding introns) and
123 comprised 41 sequences, *Russula emeticicolor* (Jul.Schäff.) Singer and *R. lilacea* QuéL.
124 belonging to subsect *Lilaceinae* (Melzer & Zvára) Jul.Schäff. were chosen as the outgroup. A
125 rapid bootstrapping (BS) algorithm of 1000 replicates was executed in RAxML 7.2.6
126 (Stamatakis, 2006), followed by a heuristic ML search for the best tree using the
127 GTRGAMMA model. All parameters in RAxML analysis were kept at default. Bootstrap
128 value (BS) exceeding or equal to 70% was considered to represent significant support.

129

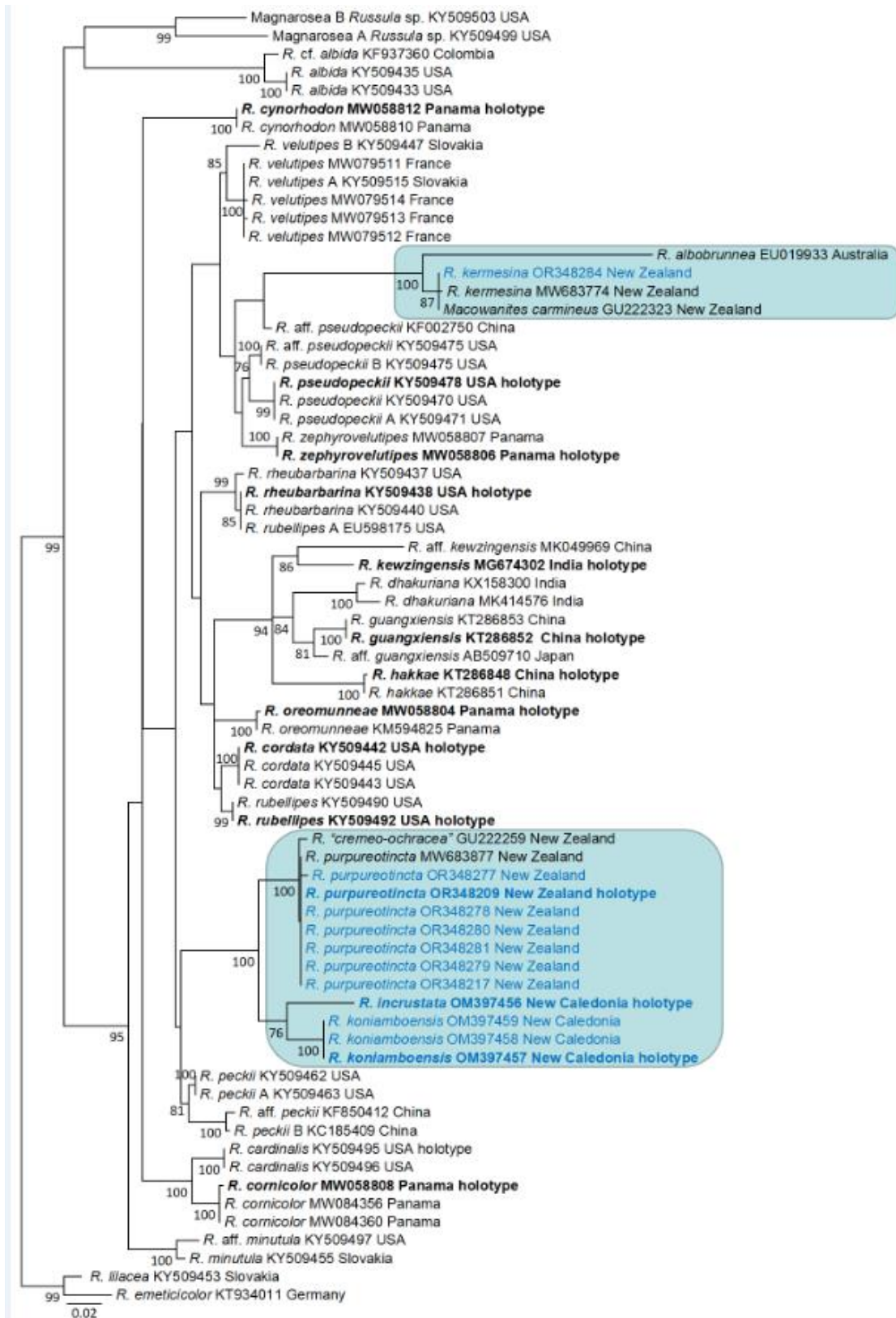
130 **Results**

131 *Phylogeny*

132 The ITS phylogeny (Fig. 1) comprises 68 sequences representative of taxa in *Roseinae* and
133 two sequences of *Lilaceinae* chosen as out-group. Southern *Roseinae* are represented by six
134 previously deposited sequences in GenBank and twelve newly generated sequences (see
135 above). In this phylogeny, the three New Caledonian specimens of *R. koniamboensis* are
136 placed sister with significant, although moderate support (MLbs=76%) to our second new
137 species from New Caledonia, *R. incrustata*. Both New Caledonian species are again placed
138 sister with full support (MLbs=100%) to a clade composed of all sequences of *R.*
139 *purpureotincta* from New Zealand (one was previously deposited under the wrong name of *R.*
140 *cremeoohracea* in GenBank). It is for the very first time that we also place two secotioid
141 species in subsect. *Roseinae*: *R. kermesina* from New Zealand and *R. albobrunnea* from
142 Australia. For both, only ITS sequence data are available and our analysis reveals that these
143 two secotioid taxa are monophyletic with full support (MLbs=100%). We obtained no
144 significant support to place this clade in relation to any other particular clade, but our
145 phylogeny suggests close relationship with a clade composed of *R. pseudopeckii*, *R.*
146 *zephyrovelutipes* and related taxa.

147 The *tefl* phylogeny (Fig. 2) comprises 41 sequences and places only the New Caledonian
148 species as no *tefl* sequences are available for the other southern species. Whereas the exact
149 relationships of both New Caledonian species with the other species in the subsection
150 remained unresolved in the ITS phylogeny (fig. 1), the *tefl* phylogeny offers now strong
151 support (MLbs=97%) to put both New Caledonian sister to the /cardinalis lineage as delimited
152 in Manz et al. (2021). The latter lineage comprises merely two species, viz. *R. cornicolor*
153 associated with *Oreomunnea* Oerst. (fam. Juglandaceae) in Western Panama and *R. cardinalis*
154 associated with *Quercus* L. in the Appalachian Smoky Mts of Tennessee, USA.

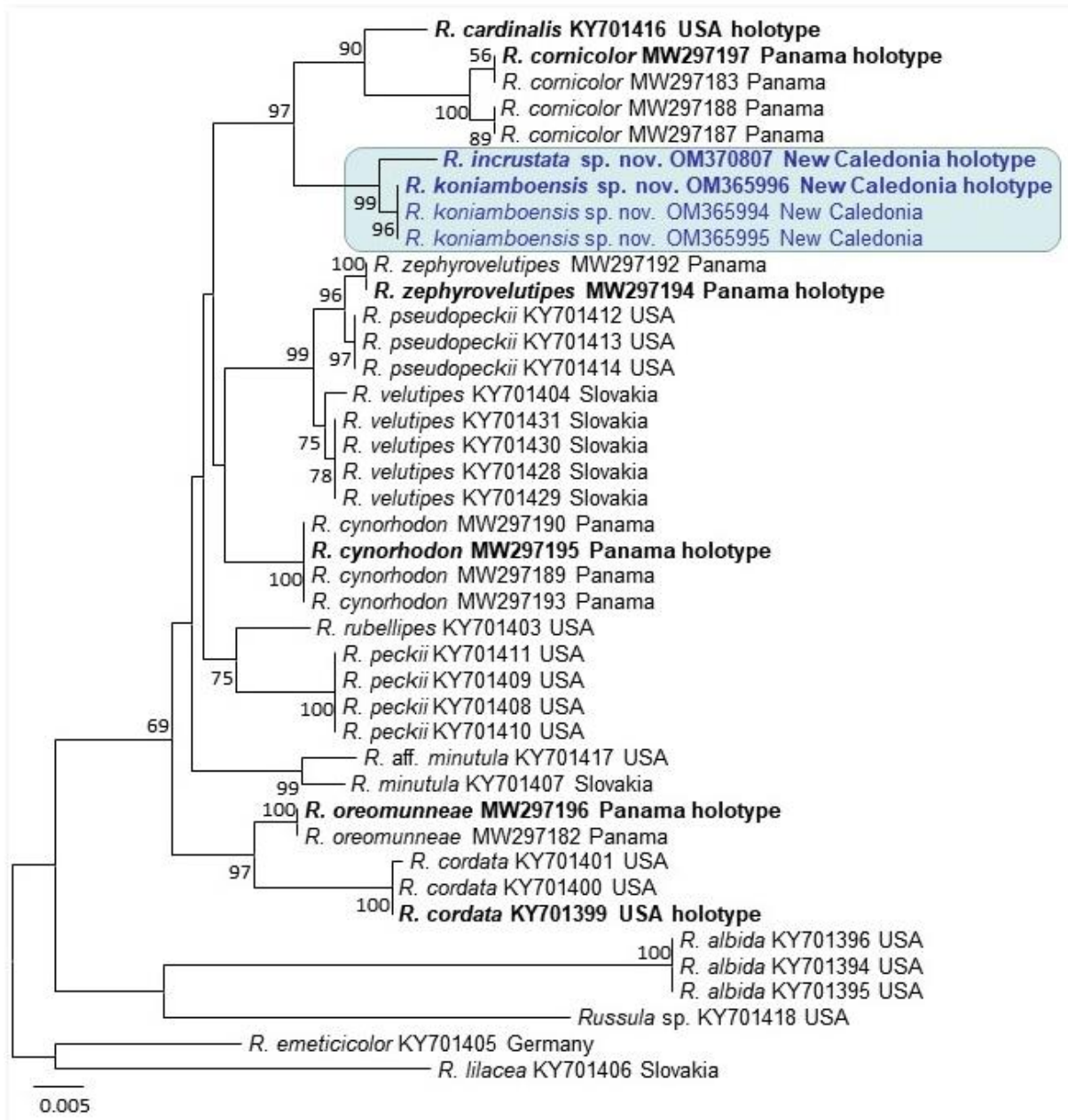
155 Considering the topologies obtained in our ITS and *tefl* analyses and those obtained in the
156 abovementioned papers with multigene analyses (see introduction), it seems likely that
157 southern hemisphere *Roseinae* occur in at least two different lineages within the subsection:
158 the /cardinalis-lineage for the agaricoid species from New Caledonia and New Zealand, and a
159 mixed American-Asian lineage for both secotioid taxa.



160

161 Fig. 1. — ITS phylogeny of *Russula* subsect *Roseinae*. Southern hemisphere species are

162 indicated by coloured rectangles. Type specimens are indicated in bold.



163

164 Fig. 2. — The *tef1* phylogeny of *Russula* subsect. *Roseinae* placing both New Caledonian
165 species (blue rectangle). Type specimens are indicated in bold.

166

167 Taxonomy

168 In the below paragraphs we will provide descriptions for the two new New Caledonian
169 species, as well as a description for the here newly reported collections for the related *R.*
170 *purpureotincta* from New Zealand.



171

172 Fig. 3. — *Russula incrustata* Buyck, sp. nov. (holotype): Fresh basidiomata. a, in situ; b,
173 detail of the pileus surface; c, view of pileus and stipe surface. Photo credit: B. Buyck.

174



175

176 Fig. 4. — *Russula koniamboensis* Buyck, sp. nov. Fresh basidiomata. a-c, holotype; d-e,

177 730/BB09.170. Photo credits: B. Buyck

178

179 **1. *Russula incrustata* Buyck, *sp. nov.***

180 **Figs.: 3, 5-8, 9d**

181 Differs from *R. koniamboensis* Buyck, *sp. nov.* in the vividly colored pileus, pinkish stipe, the
182 somewhat smaller spore size and its occurrence under endemic *Arillastrum gummiferum*.

183 Holotype. — NEW CALEDONIA: Les Bois du Sud, near blockhut, S22.171713- E166.760103, ca.
184 300 m alt., on ultramafic soil in monodominant rain forest of endemic *Arillastrum gummiferum*
185 (Brongn. & Gris) Pancher ex Baill. [fam. Myrtaceae], 27 March 2009, 735/BB 09.172 (PC0142414).

186 Mycobank. — xxxx

187 Genbank. — OM370807 (ITS), KU237588 (LSU), KU237436 (mitSSU), KU237873 (*rpb2*),
188 KU238015 (*tef1*), KU237728 (*rpb1*)

189 Etymology. — the epithet refers to the distinct incrustations present on the cell walls of the lower
190 pseudoparenchyma of the pileipellis and other parts of the context.

191

192 *Pileus*

193 Medium-sized, 64 mm diam., plane or very gently and widely depressed in the center,
194 inconspicuously striate near margin; surface peeling 2/3 radius, dull, not hygrophanous, felty-
195 velutinous, never viscid, not fragmenting in areolae or squamae but slightly concentrically
196 pruinose, colour range from pale brown, orange, pink, lilac, purple or vinaceous, not paler in
197 the center with age.

198 *Stipe*

199 51 x 11-12 mm, central, cylindrical; surface smooth, slightly longitudinally wrinkled, not
200 pruinose, entirely pinkish but white at the extreme base, firm, context soft spongy without
201 cavities, basal mycelium absent.

202 *Lamellae*

203 Equal in length, adnate, without anastomoses or forks, brittle, 8 mm high, off-white.

204 *Context*

205 Brittle, white, colour unchanging on injury or with age, about 6 mm thick in pileus above gill
206 attachment to stipe, with FeSO₄ hardly changing color, merely faintly grey inside stipe and

207 weakly pinkish on stipe surface, insensitive to Guaiac reaction negative. Taste and odor not
208 distinctive.

209 *Spore print*

210 White.

211

212 *Spores*

213 (6.46)6.89–**7.27**–7.65(7.92) x (5.42)5.67–**5.92**–6.17(6.46) μm , broadly ellipsoid, Q =
214 (1.10)1.17–**1.23**–1.29(1.36); ornamentation subreticulate, composed of large, prominent,
215 moderately distant, conical and strongly amyloid spines, up to 1.5 μm high, connected by
216 frequent fine lines into an incomplete network; suprahilar spot well–developed, varying from
217 strongly amyloid to verruculose, grayish and poorly amyloid.

218 *Basidia*

219 30–40(–58) \times 11–15 μm , narrowly to broadly clavate, 4–spored with stout sterigmata;
220 basidiola clavate.

221 *Subhymenium*

222 pseudoparenchymatic.

223 *Hymenial gloeocystidia*

224 On lamella sides mostly 50–74 \times 8–10 μm , clavate to fusiform, frequently mucronate to
225 appendiculate at apex, up to 10 μm long, rarely obtuse rounded, originating in subhymenium
226 and longer as basidia, walls up to 2(–2.5) μm thick; contents mainly restricted to some
227 refringent inclusions at apex, not reacting to sulfovanillin, rarely with up to 5 secondary septa;
228 cystidia near the lamellae edges smaller, up to 30 μm long.

229 *Marginal cells*

230 Occupying most of the lamellar edges, sitting on 1–2 basal cells, mostly 13–29 \times (3–)5–9 μm ,
231 very variable in shape, several reminding of the terminal cells in the suprapellis (but smaller),
232 but usually with 1–4 diverticulate pointed to obtuse–rounded outgrowths.

233 *Pileipellis*

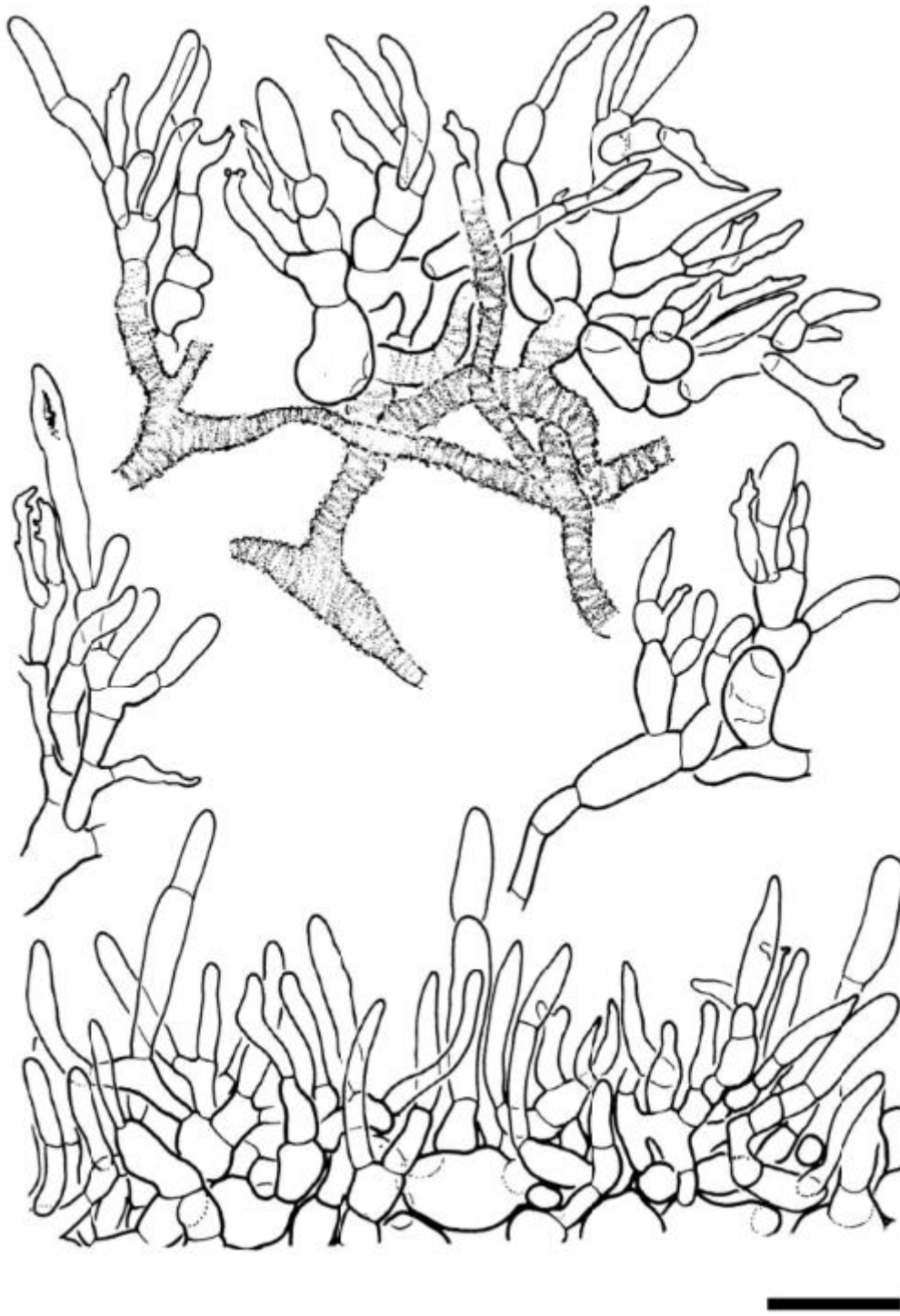
234 Two-layered, not distinctly delimited from the underlying context; subpellis composed of a
235 40–60 µm thick, loose pseudoparenchyma composed of intertwined and strongly ramifying,
236 ascending to erect, irregularly shaped cells, in the lower part with very distinct orthochromatic
237 zebroid incrustations, larger inflated cells at the base up to 12(–15) µm wide, giving rise to
238 smooth-walled 2–4(–5) narrower cells at the pileus surface; subterminal cells frequently
239 branched, thin-walled, barrel-shaped or subcylindrical; terminal cells mostly longer in
240 comparison, (8–)16–23(–39) × 2–4(6) µm, often undulated in outline, typically attenuating
241 towards a minutely capitate apex or with one subapical lateral diverticulum. Primordial
242 hyphae difficult to distinguish in shape from other extremities, recognizable at the terminal
243 cell measuring 15–31 × 3–5 µm and filled with refringent, granular-heteromorphous contents
244 in their very upper part. Cystidioid hyphae absent from subpellis and context. Oleiferous
245 hyphae or hyphal fragments abundant in subpellis.

246 *Clamp connections*

247 absent.

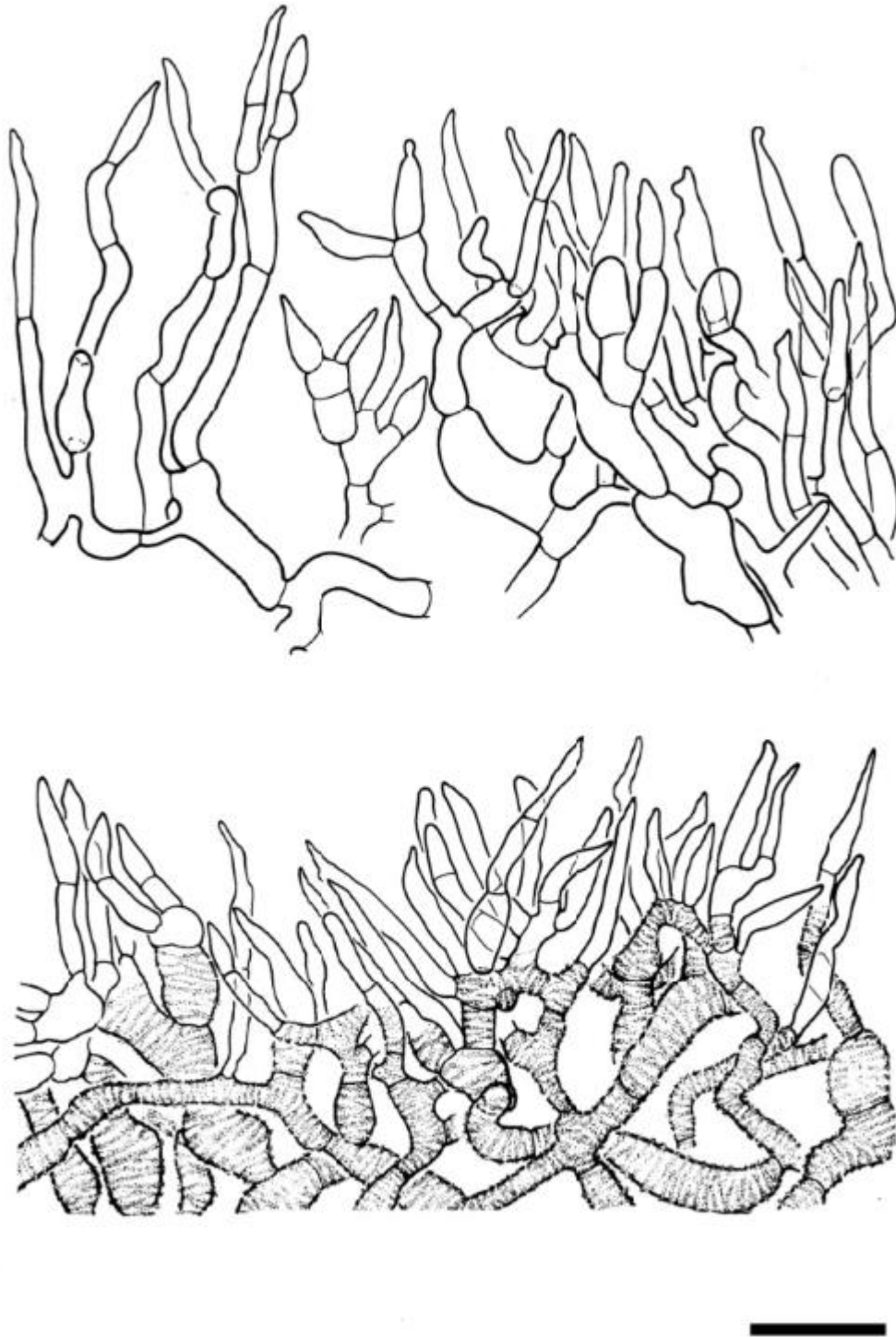
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249 *Notes:* *Russula incrustata* is a beautiful and colourful species that we found only once. There are no
250 environmental sequences available for it, which could indicate that this taxon is rather rare. Perhaps its
251 ectomycorrhizal association with the rare and endemic *Arrilastrum gummiferum* is the explanation. In
252 the multigene phylogenetic analysis of worldwide *Russula* (Buyck et al., 2018), this species was
253 already clearly placed in *Roseinae* as “*R.roseinae* sp. ined”.



255 Fig. 5. — *Russula incrustata* Buyck, sp. nov. (735/BB09.172, holotype): Details of the
256 pileipellis. - Scale bar = 20 μm . Drawings B. Duhem.

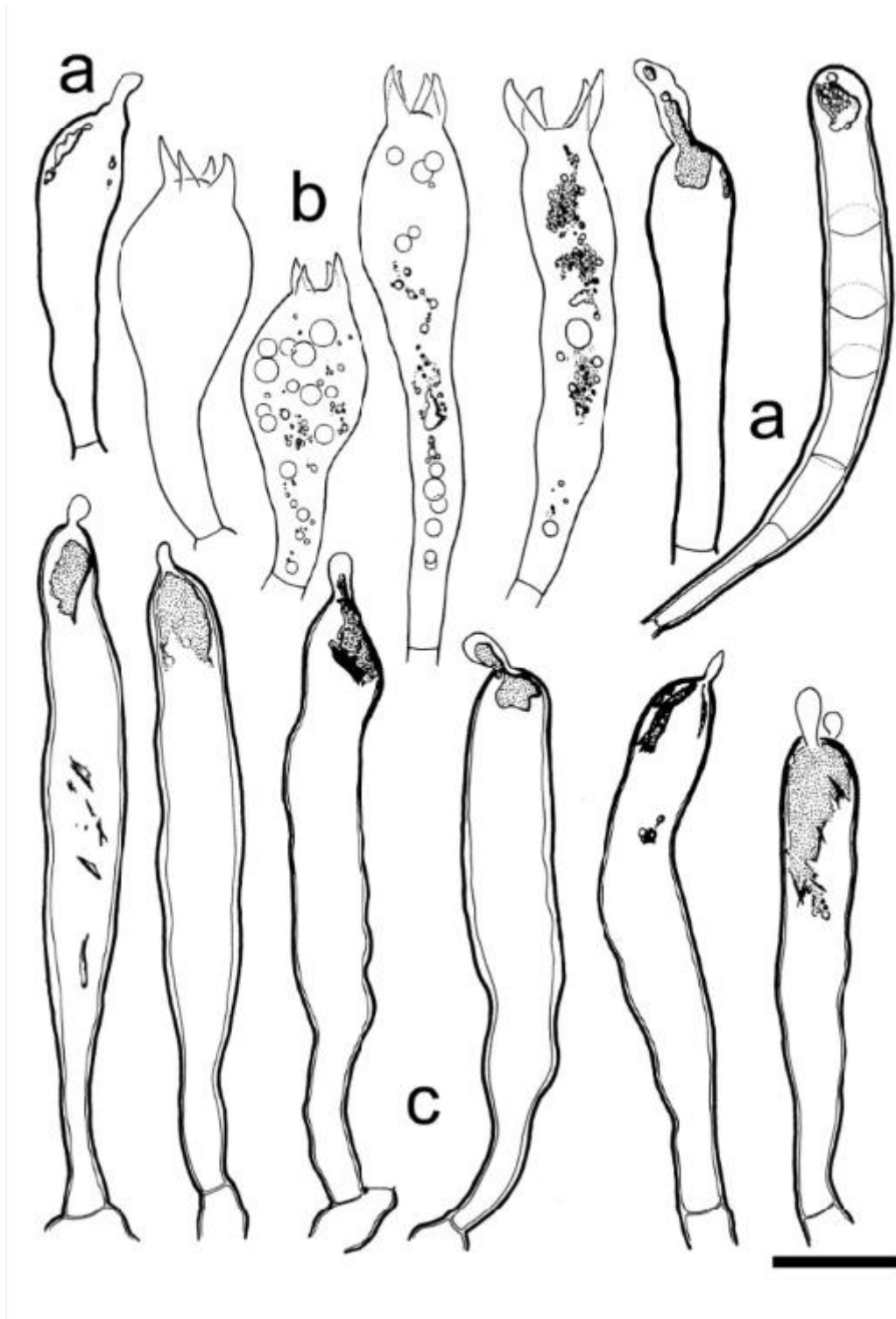
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259 Fig. 6. — *Russula incrustata* Buyck, sp. nov. (735/BB09.172, holotype): Hyphal terminations
260 of the pileipellis, continued. - Scale bar = 20 μ m. Drawings B. Duhem.

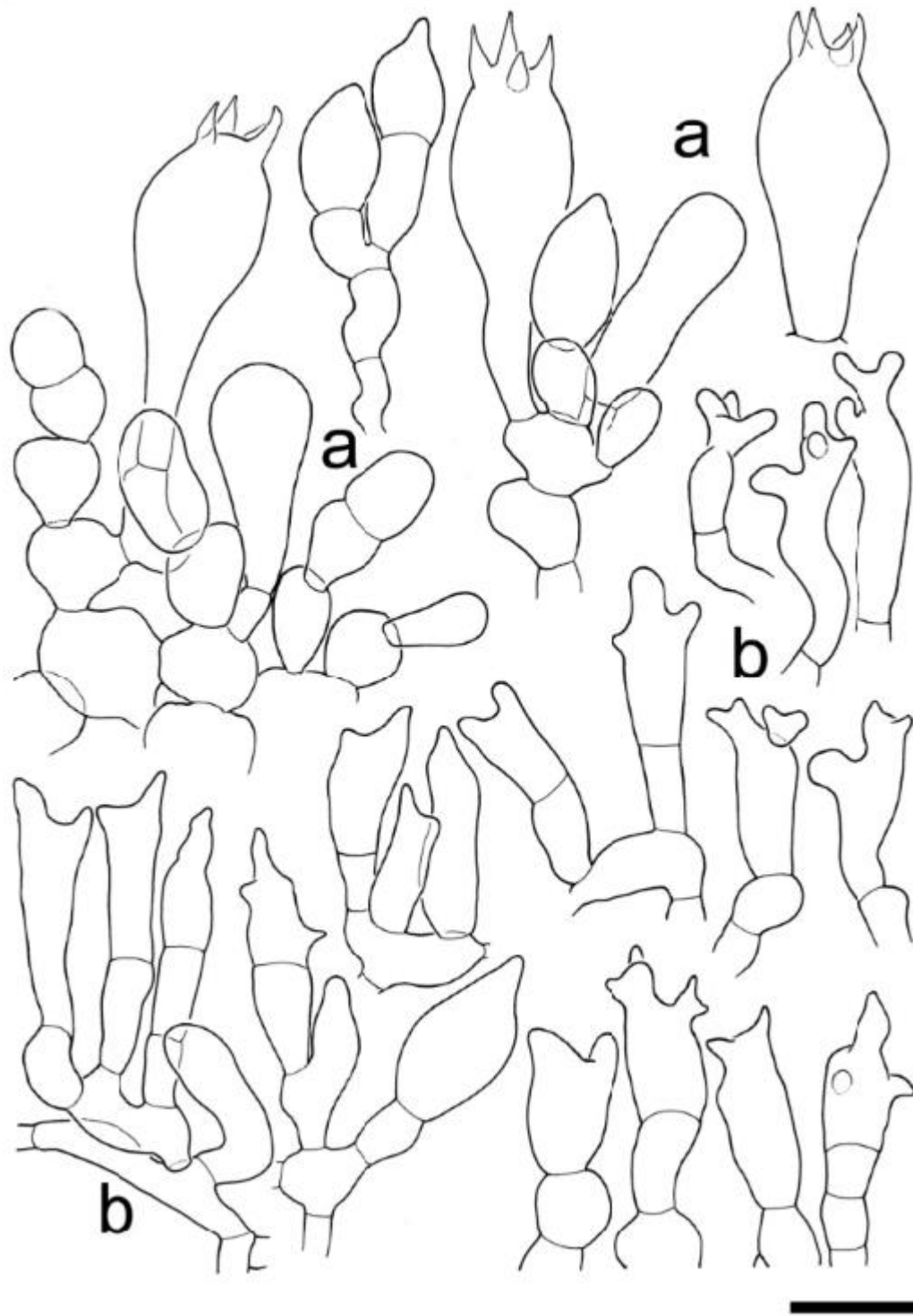
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286 Fig. 7. — *Russula incrustata* Buyck, sp. nov. (735/BB09.172, holotype): Details of the
287 hymenium: a, cheilogloeocystidia, b, basidia; c, gloeocystidium with secondary septa; d,
288 pleurogloeocystidia. - Scale bar = 10 μ m. Drawings B. Duhem.

289

290

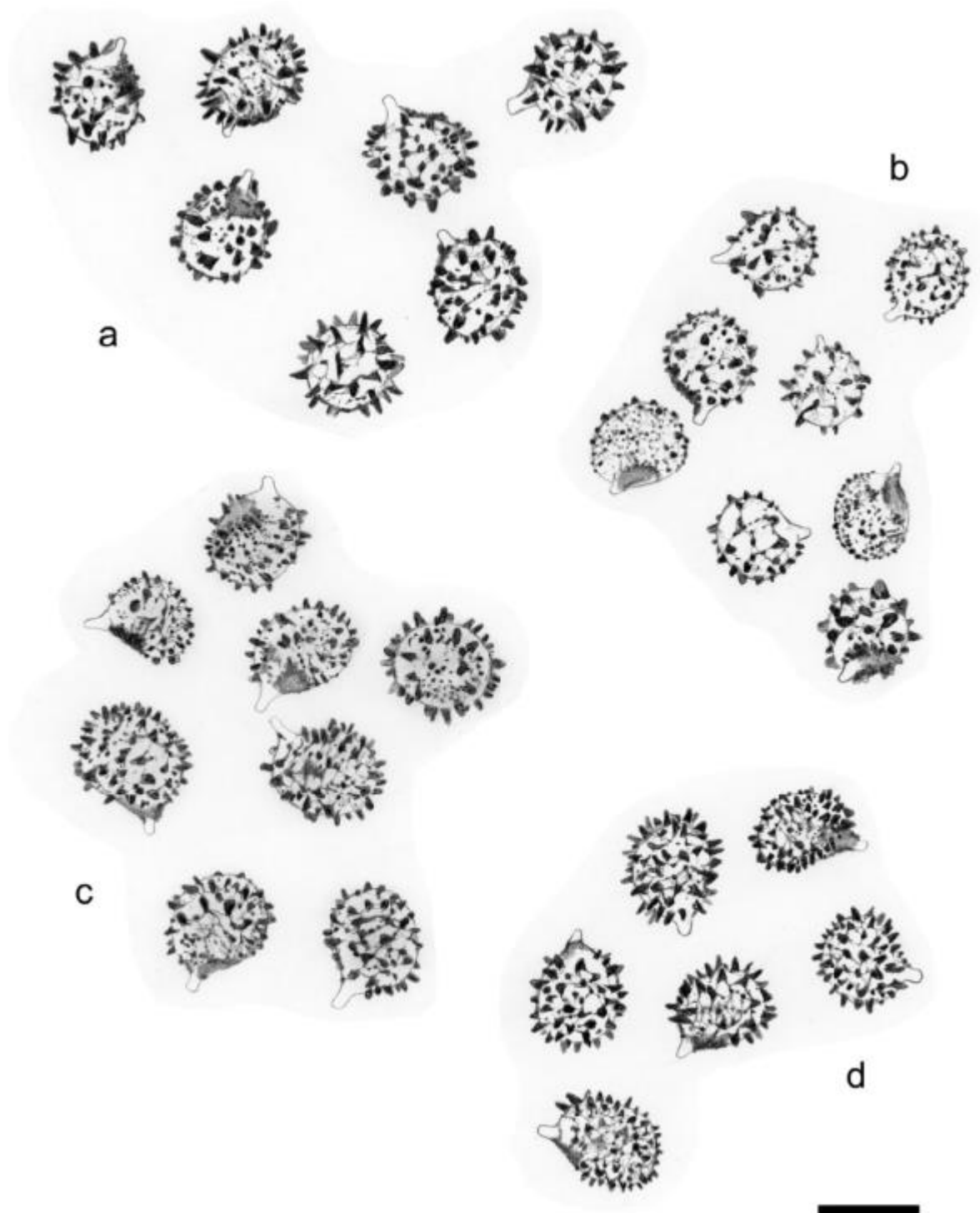


291

292 Fig. 8. — *Russula incrustata* Buyck, sp. nov. (735/BB09.172, holotype). Details of the
293 hymenium near the gill edge: a, basidiola and basidia; b, marginal cells. - Scale bar = 10 μ m.

294 Drawings B. Duhem.

295



296

297 Fig. 9. — Spore clouds. a-c. *Russula koniamboensis* Buyck, sp. nov. (a: 730/BB09.11, b: 742/
298 BB09.346, c: 722/ BB09.022, holotype). d, *Russula incrustata* Buyck, sp. nov. (holotype).

299 Scale bar = 10 μ m.

300

301

302 **2. *Russula koniamboensis* Buyck, *sp. nov.***

303 **Figs. 4, 9a-c, 10-14**

304 Differs from *R. incrustata* in the pale yellowish pileus, white stipe, the somewhat larger spore size and
305 its occurrence under *Nothofagus*; from *R. purpureotincta* principally in the pileus colour and its
306 geographic distribution.

307 *Holotype*. — **New Caledonia**. Northern Prov., Massif du Koniambo, near Voh, in the nickel mine
308 exploitation site called ‘Niko’, S 21° 00’22” – E 164°49’51”, at 724 m alt., on ultramafic soil under
309 *Nothofagus balansae*, 17 March 2009, leg. B. Buyck, 722/BB 09.022 (PC0142407).

310 Mycobank. —

311 *Additional examined material*. — **New Caledonia**. Northern Prov., Massif du Koniambo, near Voh,
312 734 m alt., in the “île nickel” mine exploitation site, S 21° 00’42” – E 164°49’50”, on ultramafic soil
313 under *Nothofagus balansae*, 19 March 2009, leg. B. Buyck 730/BB 09.117 (PC0714855); Massif du
314 Koniambo, near the Trazy entry of the nickel mine exploitation site, on ultramafic soil under
315 *Nothofagus codonandra*, 9 April 2009, leg. B. Buyck, 742/BB 09.346 (PC0714856).

316 Etymology. — named after the type locality.

317 *Pileus*

318 25-30 mm diam., convex, slightly depressed in the center, near the margin striate over 1/3-1/4
319 of the radius, surface dull, smooth to finely or even distinctly farinaceous in the center and
320 more or less concentrically deposited, peeling 2/3 of the radius, pale yellowish in the center,
321 cream towards margin.

322 *Lamellae*

323 Equal in length, adnate, distant and ca. 1-2 L/mm at pileus margin, off-white to cream color,
324 obtusely rounded at the pileus margin; entire edge concolorous.

325 *Stipe*

326 30-34 x 6-8 mm, central, cylindrical to slightly obclavate, glabrous, smooth to finely
327 longitudinally striate, white to ivory, pale greyish towards base, brittle, spongy inside, lacunes
328 absent.

329 *Context*

330 Very thin toward the margin, white, distinctly greying in age. Taste and odor not distinctive.

331 *Spore print*

332 white.

333

334 *Spores*

335 (6.67)7.51–8.01–8.51(8.75) x (6.25)6.52–6.83–7.15(7.29) μm , Q = (1.06)1.10–1.17–

336 1.24(1.31), subglobose to broadly ellipsoid; ornamentation subreticulate, composed of large,

337 prominent, moderately distant, conical to hemispherical and strongly amyloid spines, up to

338 1(–1.5) μm long, connected by dispersed to frequent fine lines into a (very) incomplete

339 network; suprahilar spot well–developed, varying from strongly amyloid to verruculose and

340 grayish to poorly amyloid.

341 *Basidia*

342 30–42 \times 12–17 μm , clavate, of variable shape, fusiformous to distinctly clavate, with (2–)4

343 stout sterigmata; basidiola clavate.

344 *Subhymenium*

345 pseudoparenchymatic.

346 *Hymenial gloeocystidia*

347 68–94 \times 7–12 μm on lamellar sides, smaller near the lamella edges, up to 45 μm long,

348 narrowly clavate to fusiform or subcylindrical, frequently mucronate to appendiculate at the

349 apex, up to 14 μm long, originating in subhymenium and protruding beyond the basidia, thin–

350 walled with walls up to 1 μm thick; contents in Congo Red mainly restricted to dispersed

351 refringent inclusions of variable size that do not react to sulfovanillin.

352 *Marginal cells*

353 15–26(–34) \times 4–6(–9) μm , sitting on 1–2 short, subterminal cells, small, occupying most of

354 the lamellar edges, extremely variable in shape, similar to the smaller terminal cells in the

355 suprapellis in having 1–4 diverticulate, obtuse lobes or outgrowths.

356 *Pileipellis*

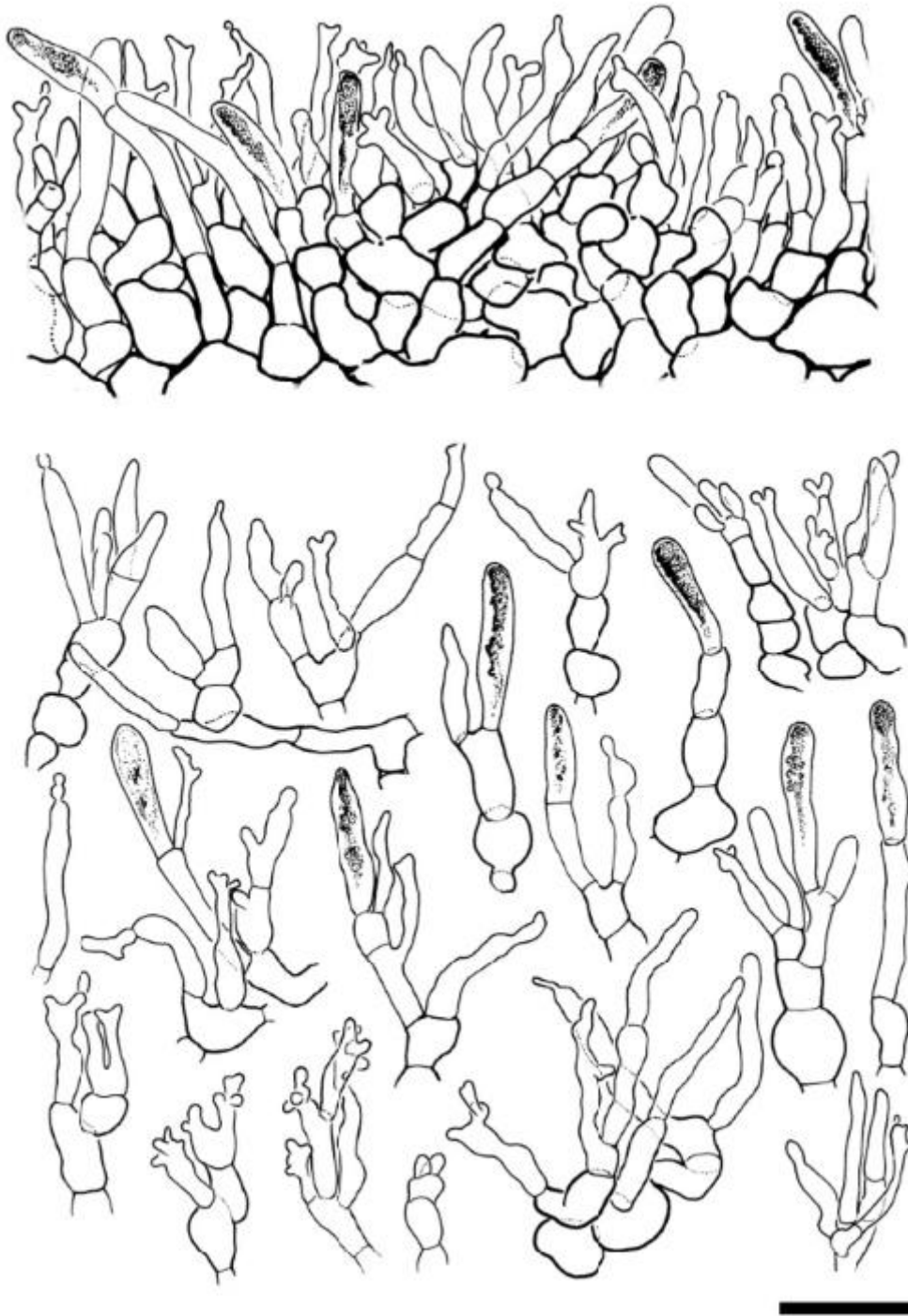
357 40–60 μm thick, two–layered, not well delimited from the underlying context, in the lower

358 part composed of hyphae with or without distinct orthochromatic incrustations, forming a

359 pseudoparenchyma of intertwined and strongly ramifying, ascending to erect, irregularly
360 shaped, thin-walled cells; basal cells sometimes up to 20(–25) μm wide, near the surface
361 giving rise to short chains composed of 2–4(–5), narrower cells that are slightly inflated and
362 barrel-shaped, ellipsoid to subcylindrical, up to 10 μm wide; terminal cells of variable size
363 and either longer or shorter than subterminal cells, (8–)16–23(–34) \times 2–4(–6) μm , often very
364 irregular or undulate in outline, slightly attenuating toward the frequently capitate,
365 appendiculate diverticula at apex. Primordial hyphae recognizable mostly by their somewhat
366 more regular outline, but especially by the refringent granular–heteromorphic contents of the
367 terminal cell that mostly measures 15–25 \times 4–5 μm , narrowly clavate to subcylindrical in
368 outline, obtuse rounded at the apex, diverticula or appendages absent, thin-walled. Cystidioid
369 hyphae absent in subpellis and context. Oleiferous hyphae rare.

370 *Clamp connections*

371 absent.

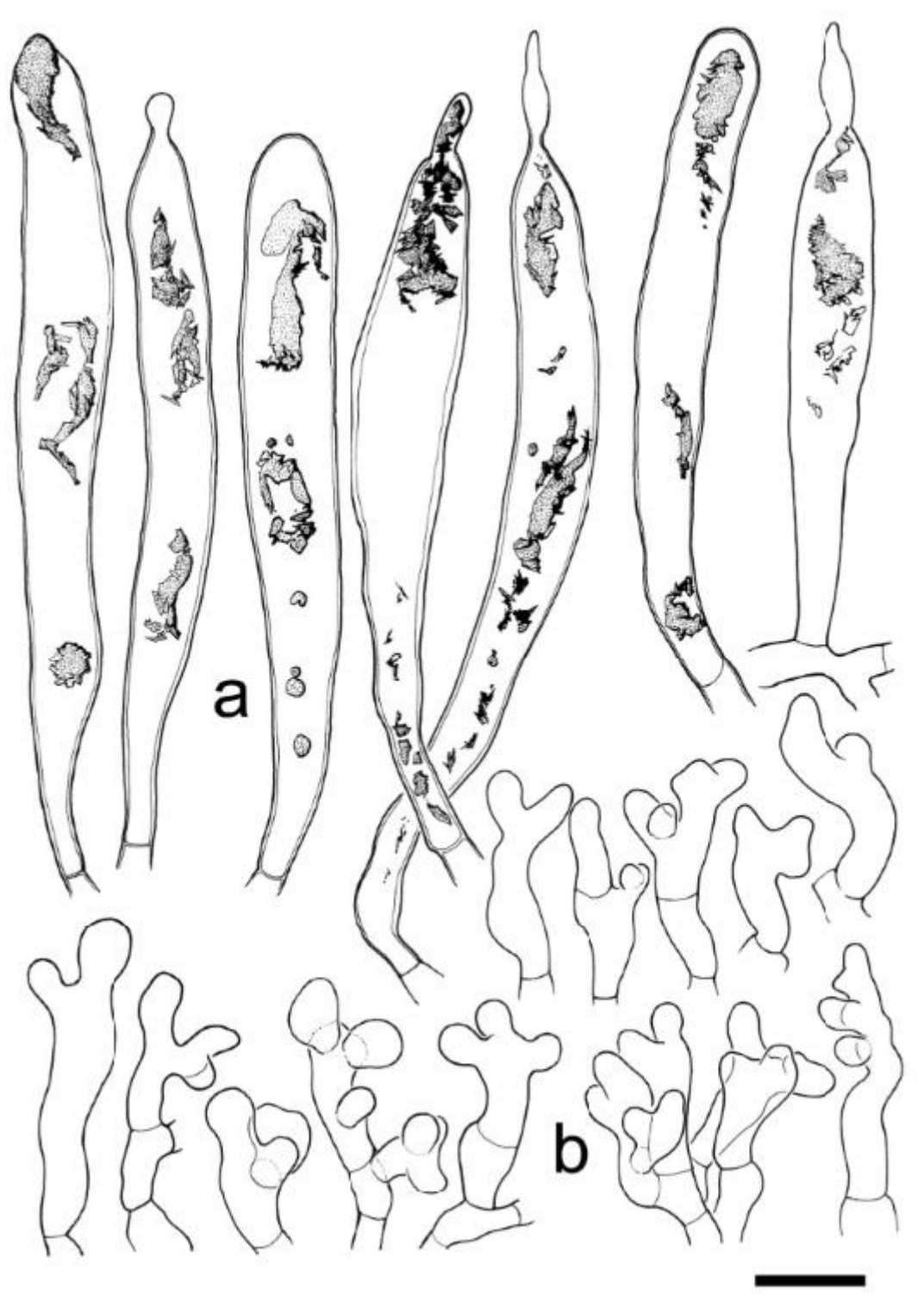


396 Fig. 10. — *Russula koniamboensis* Buyck, sp. nov. (722/BB09.022, holotype). Details of the
397 pileipellis. Note that all capitate-mucronate endings are optically empty; these are not
398 pileocystidia, notwithstanding they are morphologically very similar to the pileocystidia of
399 species in subg. *Heterophyllidiae*, while all terminal cells of primordial hyphae are obtuse-
400 rounded at their apex and possess refringent contents reminiscent of typical pileocystidia.
401 Scale bar = 20 μ m. Drawings B. Duhem.

402

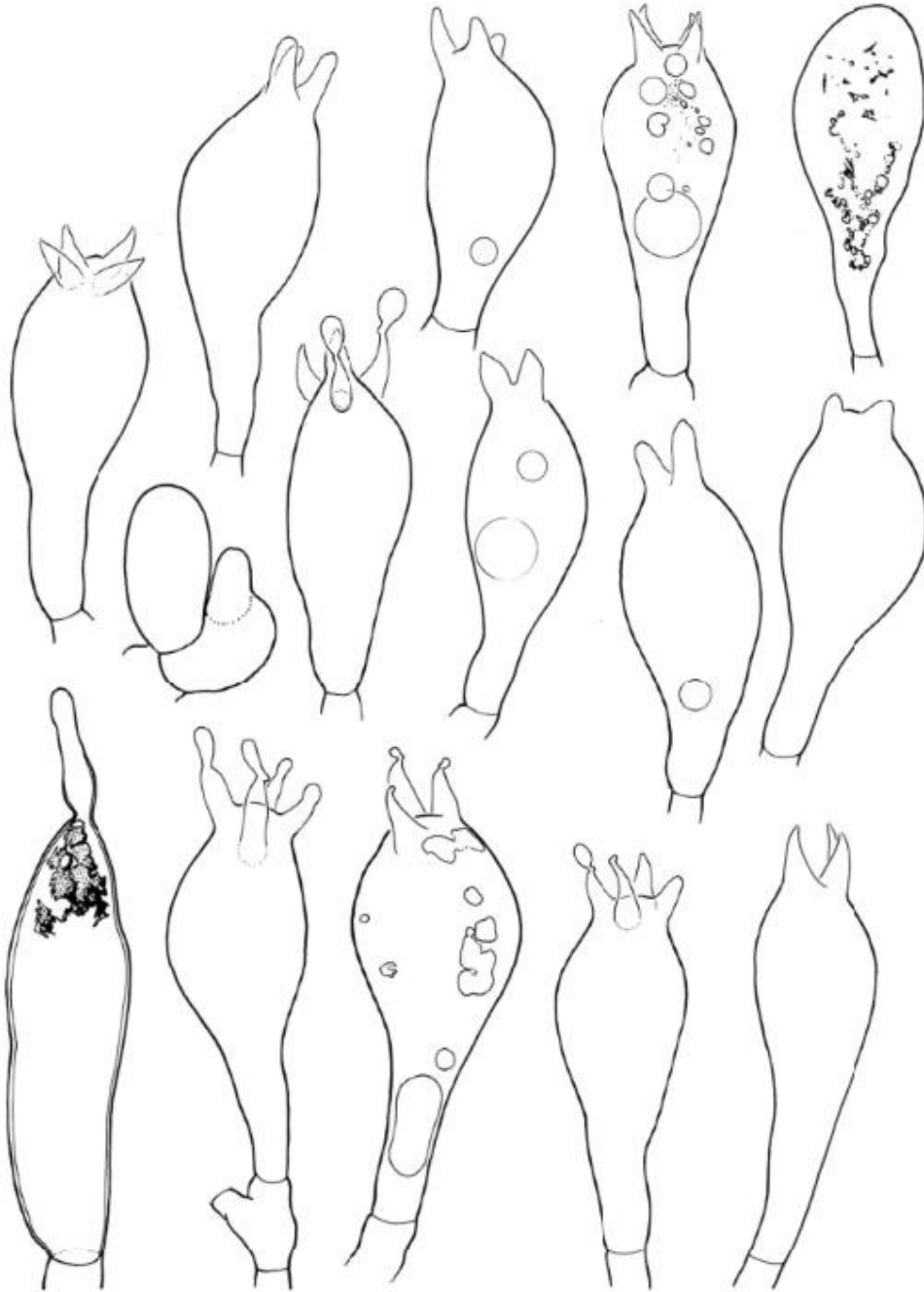
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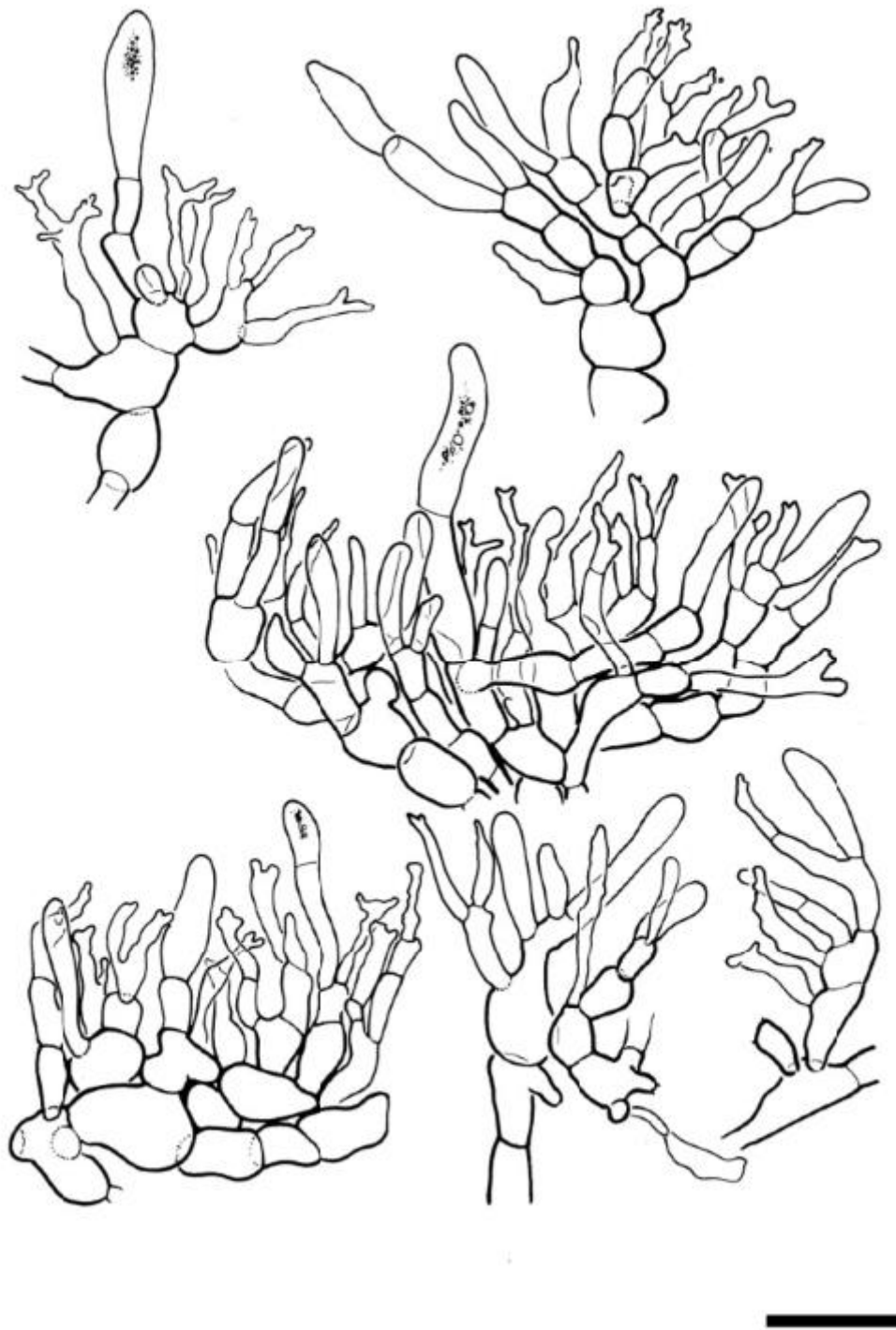
406 Fig. 11. — *Russula koniamboensis* Buyck, sp. nov. (722/BB09.022, holotype). Elements of
407 the hymenium; top row: pleurogloeocystidia with indication of contents; bottom, marginal
408 cells. Scale bar = 10 μ m. Drawings B. Duhem.



409

410 Fig. 12. — *Russula koniamboensis* Buyck, sp. nov. (722/BB09.022, holotype). Elements of
411 the hymenium; cheilogleocystidium; basidia and basidiola. - Scale bar = 10 μ m. Drawings B.
412 Duhem.

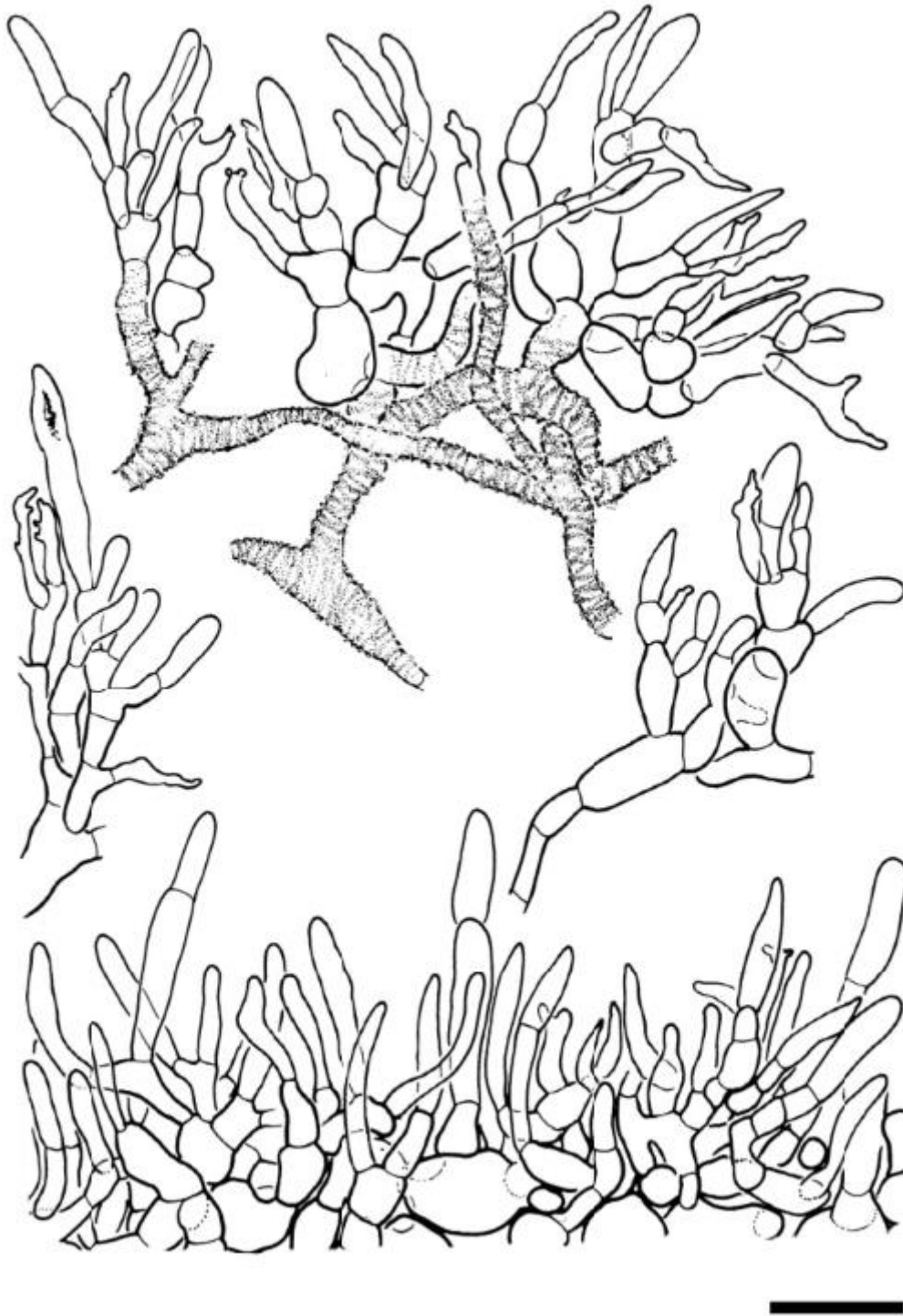
413



414

415 Fig. 13. — *Russula koniamboensis* Buyck, sp. nov. (722/BB09.022, holotype). Fragments of
416 the suprapellis. - Scale bar = 20 μ m. Drawings B. Duhem.

417



418

419 Fig. 14. — *Russula koniamboensis* Buyck, sp. nov. (730/BB09.117): Details of the
420 suprapellis. - Scale bar = 20 μ m. Drawings B. Duhem.

421

422

423 *Notes:* The above description is based on the holotype collection. However, the two other collections
424 of this species (having identical ITS sequences) clearly show that its microscopic features are quite
425 variable among different collections. For the pileipellis this variation concerns the size of the
426 sphaerocytes in the lower suprapellis which are sometimes up to 35 μm diam., giving rise to 8-celled
427 chains (as in *BB09.346*); observed variations apply also to the presence/absence of distinct
428 orthochromatic zebroid incrustations on the cell walls in the lower pileipellis (present in *BB09.117*)
429 and finally also to the form of the terminal cells on the hyphae. The latter can be either more regular in
430 outline and similar to those of *R. incrustata* (as in *BB09.346* and *BB09.117*) or strongly diverticulate
431 as in the type collection. On the lamella edge, cheilocystidia can be abundant and well-developed (as
432 in *BB09.346*) or just dispersed among the other elements. The spores in *BB09.346* are near-identical in
433 size and ornamentation to the holotype [(8.13)8.20-**8.63**-9.05(9.38) x (6.25)6.47-**6.86**-7.26(7.50) μm ,
434 $Q = (1.11)1.17$ -**1.26**-1.35(1.40)], while spores in *BB09.117* produced several aberrant sizes and
435 ornamentations indicating an unfavourable development. These spores were, therefore, not used for
436 measurements.

437

438

439 3. *Russula purpureotincta* R.F.R. McNabb, *New Zealand Journal of Botany* 11: 711.
440 1973.

441 Figs. 15-18

442 *Examined material.* — **New Zealand.** Prov. Nelson, Springs Junction, Upper Grey, among *Sphagnum*
443 under *Nothofagus [Fuscospora] solandri* var. *cliffortioides* and *Nothofagus [Lophozonia] menziesii*,
444 25 Febr. 1968, *E. Horak* (ZT 68-086); Prov. Nelson, W of Tophonse Saddle, at the border of a swampy
445 locality between moss (not *Sphagnum*) under *Nothofagus [Fuscospora] solandri* var. *cliffortioides* and
446 *Nothofagus [Lophozonia] menziesii*, 3 March 1968, *E. Horak* (ZT 68-105).

447

448 *Pileus*

449 Up to 75 mm diam., at first convex, becoming slightly depressed in the center with age, near
450 the margin smooth to shortly striate; surface dull, when humid weakly viscid, at first reddish
451 lilac to purplish, but rapidly discoloring, particularly between the center and the very margin,
452 and then becoming beige-brownish-pinkish to flesh-colored, pileipellis separable up to mid-
453 radius.

454 *Stipe*

455 36-80 x 10-19 mm, cylindrical or slightly narrowing towards apex, entirely white, smooth or
456 finely striate lengthwise.

457 *Lamellae*

458 Equal in length or with rare lamellulae, 4-6 mm wide, normally spaced (ca. 1L/mm at the
459 pileus margin), white, becoming cream with age, not anastomosing in the dorsal interspaces,
460 obtusely rounded at the pileus margin; gill edge smooth, concolorous.

461 *Context*

462 brittle, spongy, white, unchanging on exposure. Odour not distinctive. Taste mild.

463 *Spore print*

464 white.

465

466 *Spores*

467 (8.13–)8.32–~~8.74–8.93~~–9.43(–10.21) x (6.67–)6.99–~~7.28–7.29~~–7.57(–7.92) μm , Q = (1.11–
468)1.14–~~1.20–1.23~~–1.28(–1.34), large, subglobose to broadly ellipsoid, densely ornamented
469 with strongly to partly amyloid spines or cylindrical warts, these up to 2 μm high in many
470 spores and frequently curved, often in pairs, with few smaller granular warts and rare
471 interconnecting fine lines; suprahilar spot distinctly amyloid.

472 *Basidia*

473 40–48 x 12–15 μm , clavate, 4–spored; sterigmata stout, mostly 7–8 x 2–3 μm .

474 *Hymenial gloeocystidia*

475 Mostly 70–90 x 12–15 μm , abundant, on lamellar sides clavate to fusiform, thin– to slightly
476 thick–walled; near lamellar edges distinctly thick–walled (up to 2–3 μm thick in middle
477 portion) and smaller, mostly 40–60 x 7–12 μm , rarely with an additional septum in the upper
478 part.

479 *Marginal cells*

480 Not differentiated.

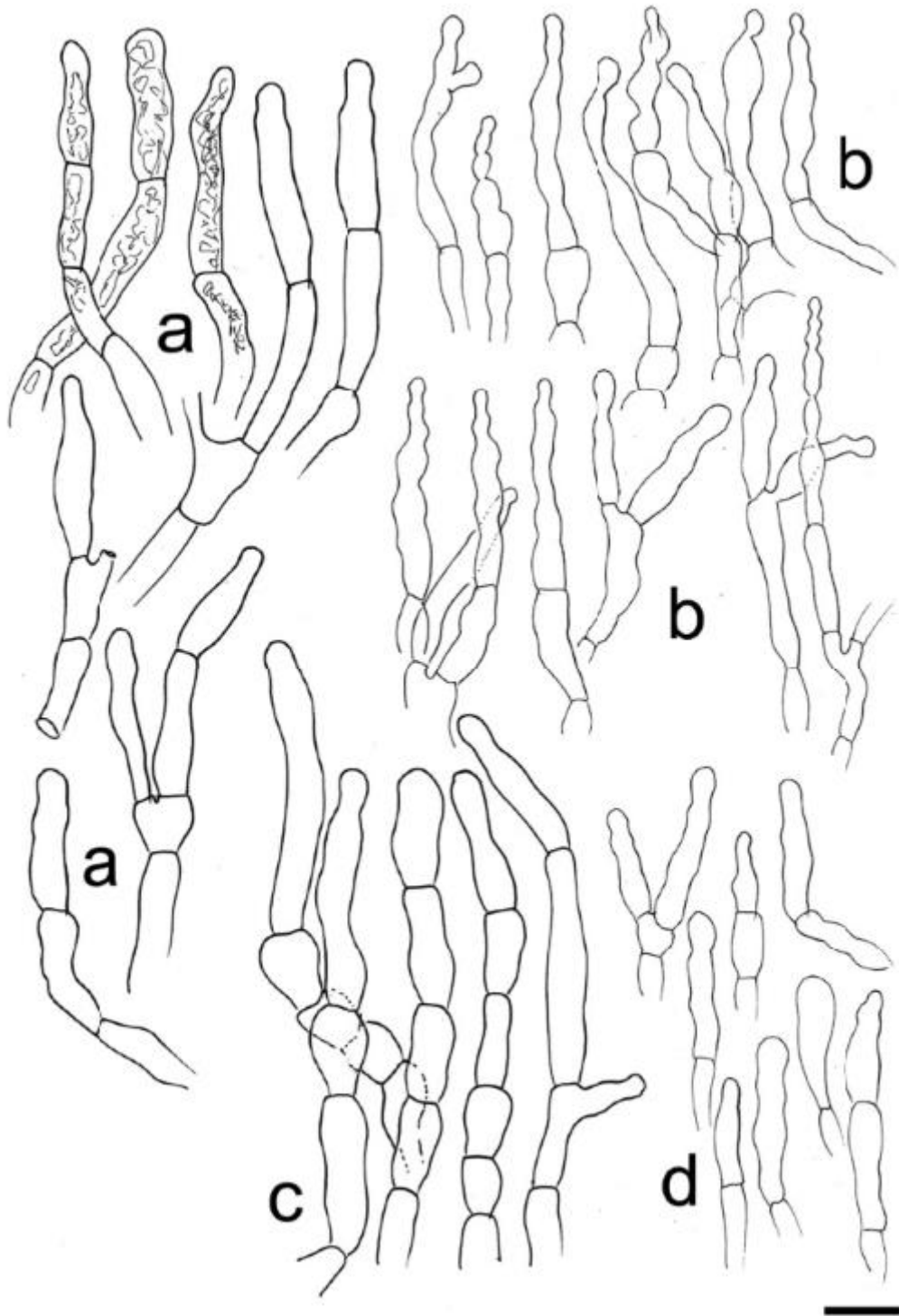
481 *Pileipellis*

482 Two-layered: the suprapellis forming a trichoderm of densely packed, narrow and thin-
483 walled hyphal extremities composed of 3–6 short cells and originating from a
484 pseudoparenchymatic layer underneath; the latter is composed of much more inflated,
485 sausage-like, ellipsoid or spherical cells up to 30 μm diam., without zebroid incrustations on
486 the cell walls; suprapellis composed of hyphal extremities with terminal cells in the pileus
487 center short to very short, rarely exceeding 20 x 3–5 μm , either narrowing in the upper part or
488 clavate, often slightly undulate in outline, toward the pileus margin more irregular in outline
489 and very frequently globose to moniliform at apex. Typical pileocystidia absent. Primordial
490 hyphae emerging from the surface of the suprapellis, slightly more voluminous than the other
491 hyphal extremities and composed of 3–6, thin-walled cells, usually more strongly septate in
492 the pileus center; their cells shortly cylindrical to barrel-shaped, and mostly also recognized
493 by distinctly refringent, granular contents, up to 8 μm diam., the terminal cell frequently up to
494 35 μm long, more regular in outline compared to the other sometimes subcapitate hyphal
495 extremities. Cystidioid and oleiferous hyphae lacking.

496 *Clamp connections*

497 absent.

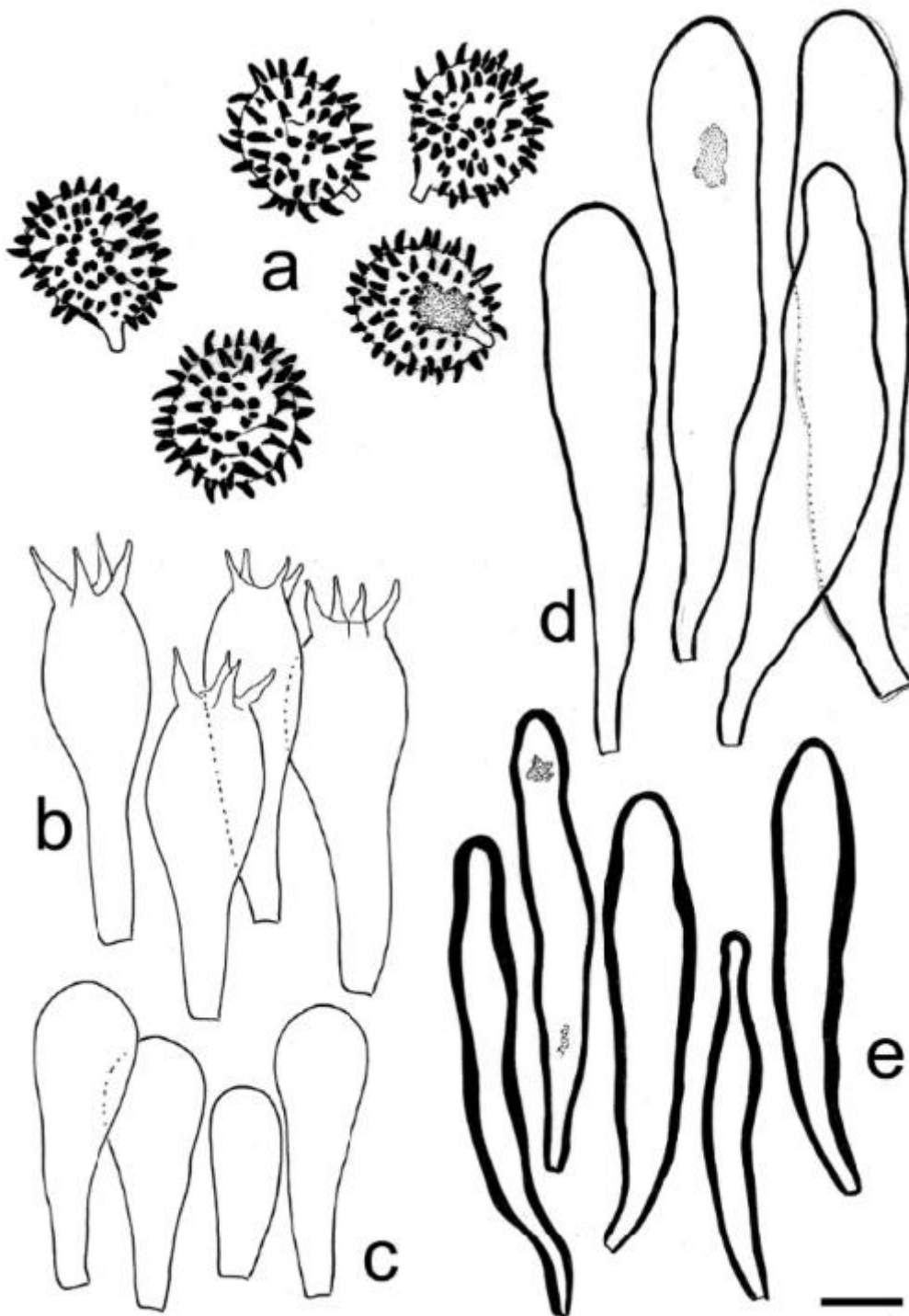
498



499

500 Fig. 15. — *Russula purpureotincta* (ZT 68-105): a-b. Elements of the pileipellis near pileus
501 margin: a, primordial hyphae with indication of contents in few cells; b, terminal cells of the
502 other hyphal extremities; c-d. Elements of the pileipellis in the pileus center: c, primordial
503 hyphae without indication of contents; d, terminal cells of other hyphal extremities. Scale bar
504 = 10 μ m (Drawings B. Buyck)

505



506

507 Fig. 16. — *Russula purpureotincta*. Elements of the hymenium (all from ZT 68-105, except b
508 from ZT 68-086). a-b, basidiospores; c, basidia; d, basidiola; e, pleurocystidia; f,
509 cheilocystidia. Scale bar = 10 μ m. Drawings B. Buyck.

510



511

512 Fig. 17. — *Russula purpureotincta*. Fresh basidiomata (ZT 68-105). Photo E. Horak.

513



514

515 Fig. 18. — *Russula purpureotincta*. Fresh basidiomata (ZT 68-068). Watercolour by E. Horak.

516

517 *Notes:* The description is based on two collections gathered by E. Horak one year before the species
518 was described by R.F.R. McNabb (1973) as *R. purpureotincta*. Our description is highly similar to the
519 original description, taking into account that the spore size given by McNabb (9-12 x 8.5-10.5 μm)
520 includes the spore ornamentation. In addition, McNabb was probably not aware of the concept of
521 ‘primordial hyphae’.

522 This endemic species of New Zealand seems to be widely distributed and is not rare as many records
523 are reported online (e.g. on <https://scd.landcareresearch.co.nz>). The online available field images,
524 illustrations of microscopic features and SEM pictures of the spores for these collections (including for
525 the type collection) clearly confirm our own observations on Horak’s specimens.

526 *Russula purpureotincta* is represented in our ITS phylogeny by nine sequences, including one newly
527 produced sequence from the type specimen and one [GU222259 for PDD77740] that corresponds to a
528 very pale collection initially identified as *Russula cremeo-ochracea* (and still deposited as such in
529 GenBank). McNabb’s species resembles our *R. incrustata* because of the faint greyish magenta,

530 greyish purple to reddish grey tints present in the pileus, but it lacks the warmer orange-red tints of our
531 New Caledonian species which, in addition, has a coloured stipe. As evident from online published
532 pictures for *R. purpureotincta*, the stipe can frequently be obclavate and can vary considerably in
533 length between different specimens.

534 *Russula purpureotincta* is very variable in overall colour, but typically discolours very rapidly leaving
535 a pale sordid whitish-isabelline pileus with only faint greenish-pinkish hues remaining in the center
536 and at the very margin of the pileus. Such discoloured specimens are more reminiscent of equally
537 discoloured forms of several (but acrid !) species in the *Russula* core clade, rather than of other species
538 of subsect. *Roseinae*.

539 Microscopic differences between *Russula purpureotincta* and both above-mentioned New Caledonian
540 species are very subtle. Spore ornamentation in McNabb's species seems less reticulate, its
541 cheilocystidia more strongly thick-walled, and the contents in hymenial cystidia more abundant.

542

543 **Discussion**

544 Subsect. *Roseinae* is sister to subsect. *Lilaceinae* in all recent multigene phylogenies. Both
545 subsections have always been considered – even in the traditional morphotaxonomic
546 classifications (Romagnesi, 1967; Sarnari, 2003) – either as one single or as two extremely
547 close species groups. In older morphology-based classifications, both subsections were part of
548 subgen. *Incrustatula* Romagnesi (1967) together with other subsections having more coloured
549 spore prints. In the first multigene phylogeny that was more or less representative of world-
550 wide *Russula* species, Buyck et al. (2018) demonstrated the artificial concept of subgen.
551 *Incrustatula* with dark-spored subsect. *Amethystinae* (Romagn.) Bon and *Chamaeleontinae*
552 Singer of *Russula* subgen. *Incrustatula* being unrelated to the pale-spored taxa in subsect.
553 *Roseinae* and *Lilaceinae*. This was later confirmed by the same authors in Rossi et al. (2020)
554 when providing significant, although moderate support to group the northern hemisphere,
555 dark-spored subsections *Amethystinae* and *Chamaeleontinae*, together with the Oceanian
556 subsection *Tricholomopsisidum* Buyck & V. Hofst. and the Asian subsect. *Castanopsisidum*
557 Buyck & X. H. Wang and not with the *Roseinae* – *Lilaceinae* clade.

558 Secotioid or hypogeous *Roseinae* have not yet been reported from the northern
559 hemisphere. We here place two secotioid southern species in the subsection: *R.*
560 [*Macowanites*] *kermesina* from New Zealand and *R. albobrunnea* T. Lebel from Australia.
561 Our ITS phylogeny suggests that these southern secotioid taxa are related to different lineages

562 of northern hemisphere *Roseinae* compared to the agaricoid *Roseinae* from New Caledonia
563 and New Zealand (Fig. 1).

564 The southern *Roseinae* associate rarely with host trees from family Myrtaceae (so far
565 only *R. incrustata*) and seem to prefer *Nothofagus* as a host (*R. purpureotincta*, *R. kermesina*,
566 *R. koniamboensis*, *R. albobrunnea*). It is not clear yet whether these hosts have been more
567 recently invaded by these fungi or not but, so far, no *Roseinae* have been reported from
568 *Nothofagus* forests in South America.

569 Arguing that the traditional interpretation of *Roseinae* should be considered at the
570 section level, Looney et al. (2022) described subsect. *Albidinae* Looney, Manz & Adamčik,
571 for merely two species (*Russula albida* and one still undescribed species). The same authors
572 also suggested that a separate subsection was needed for the American /magnarosea clade,
573 another tiny subclade based so far on two undescribed collections. In our opinion, proposals
574 to elevate such extremely small entities within terminal clades to higher ranks should be
575 postponed until more worldwide samplings for the various *Russula* clades become available.

576 Buyck et al. (2018) noticed that many of the southern hemisphere *Russula* have a
577 pseudoparenchymatic pileipellis structure. This observation was based on the fact that all of
578 species in the hyperdiverse subsect. *Tricholomopsidum* have a pseudoparenchyma in the
579 lower suprapellis. The latter feature also applies to all *Roseinae* that are discussed here. It is
580 yet unclear whether such a pileipellis structure represents an advantage for the involved
581 species.

582 **Acknowledgements**

583 B. Buyck obtained funds for field work in New Caledonia both by ANR (French National
584 Agency for Research), BIONEOCAL ANR-07-BDIV-006 (PI P. Grandcolas, CNRS-MNHN)
585 and ULTRABIO ANR-07-BDIV-010 (PI M. Ducouso, CIRAD). Sequencing of the
586 collections was financed through a 2014 project submitted to the ATM « Emergences » (Dir.
587 S. Peigné) of the National Natural History Museum in Paris. The first author is also thankful
588 to the late B. Duhem for the microscopic drawings of the New Caledonian species. Mr. Denis
589 Poignonec of Koniambo Nickel SAS is thanked for access to the Koniambo Massif mining
590 area. Mr. David Paulaud of the “Direction de l’Environnement” for Southern Prov. is thanked
591 for the collecting permits in New Caledonia. E. Horak is grateful for logistic support offered
592 by the New Zealand Forest Research Institute (Rangiora) and the Herbarium PDD in

593 Auckland. Song acknowledges funding from the Research Initiation Project of Shaanxi
594 University of Technology (SLGRCQD2214) and the Science and Technology Department of
595 Shaanxi Province project (2022JQ-199).

596

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659 **Figure legends**

660

661