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The search for Australian relatives of the eucalypt-associated *Russula prolifica* (Russulales) from Madagascar reveals new Oceanian taxa in subsection *Auratinae*

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Abstract

The authors examine the possibility that *Russula prolifica*, a commercially important edible mushroom associated with exotic *Eucalyptus* on the Central Plateau of Madagascar, is a recent introduction from Australia. The new Australian species, described here as *R. discolor* sp. nov., closely resembles *R. prolifica* and associates with eucalypts in south-western Australia. While *R. prolifica* has previously been placed in subsect. *Virescentinae* of subgen. *Heterophyllidiae*, this Australian look-alike is not a member of *Virescentinae*, but belongs in the crown clade of *R.* subgen. *Russula*, more precisely in the *R. wielangtae* lineage of subsect. *Auratinae*. This paper further provides a detailed type study for *R. atroviridis* from New Zealand, another presumed member of the *R. wielangtae*-lineage. However, we show that the current concept of *R. atroviridis* does not conform to the type specimen which clearly belongs in subsect. *Tricholomopsidum* of subgen. *Russula*. The sequences previously deposited in GenBank under the name *R. atroviridis* represent a different and new species that is here described as *R. cooperiana* sp. nov. We distinguish two morphologically and genetically different varieties of this species: the type variety associates with Nothofagaceae, while the new var. *myrtacearum* associates with Myrtaceae. Other potential members of the *R. wielangtae* lineage are also discussed, including the Australian *R. wollumbina*. The wider circum-scription of subsect. *Auratinae* is here further supported and its various lineages discussed.

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Introduction

Introduction

Madagascar is not only a hotspot for biodiversity, it also presents a unique situation for eucalypt-associated ectomycorrhizal fungi. Indeed, as far as we are aware, there is no other place in the world where introduced eucalypts associate with so many different species of ectomycorrhizal Basidiomycota (Buyck 2008). Nearly all of these fungi appear to have made the host switch from endemic African or Malagasy trees to the Australian eucalypts that were introduced on the island less than two centuries ago (Buyck 2008). This host switch was most likely responsible for an impressive acceleration of the speciation process in the case of several endemic *Cantharellus* species (Buyck in Ariyawansa et al. 2015).

Plantations of *Eucalyptus robusta* Sm. that are regularly cut back constitute in Madagascar the favourite habitat for two edible and widely marketed *Russula* species, viz *R. madagassensis* Heim and *R. prolifica* Buyck. The latter species is harvested in impressive quantities to be sold in all major towns, along roadsides or in village markets on the Central Plateau (fig.1). First referred to as *R. pseudovesca* nom. prov. (Buyck 2002), a name pre-empted by Ying (1989), it was later published as *R. prolifica* Buyck sp. nov. (Buyck 2008) and has since been firmly placed in *Russula* subgenus *Heterophyllidiae* subsection *Virescentinae* using a multigene phylogenetic approach (Buyck et al. 2018).

Several facts suggest that R. prolifica was not common until long after the eucalypts were introduced in Madagascar more than two centuries ago. Our first argument lies in the fact that it is quite hard to imagine that R. pro*lifica* would have escaped the attention of Heim, Decary, Bouriquet and other French mycologists who collected mushrooms in Madagascar between 1930 and 1970, unless this species was absent or rare at that time. Second, Heim (1938a) mentions a general dislike among Malagasy people for species of Russula and Lactarius, something that is absolutely no longer the case today. On the contrary, Russula is at present the most commonly consumed and economically important mushroom genus in Madagascar, closely followed by Cantharellus (Buyck 2008). In Madagascar, russulas are presently for sale at almost any market, most frequently after having been stripped of their cap cuticle, a practice likewise not mentioned by Heim. This practice could find its origin in the fact that European expatriates in Madagascar prefer to buy white, clean mushrooms, reminiscent of the Agaricus bisporus (J. E. Lange) Pilát to which they were accustomed in Europe. Skinning the russulas not only removes dirt clinging to the cap cuticle, but also exposes the white flesh underneath producing thus the familiar mushroom type for expatriate Europeans. Finally, R. madagassensis also occurs under eucalypts side by side with *R. prolifica*, but it is much less abundant and is easily distinguished from the latter by the darker, more homogeneously reddish or vinaceous cap colour and much less firm texture. Notwithstanding its much lesser abundance, *R. madagassensis* was collected and described by Heim (1938 a,b), but not *R. prolifica*. We, therefore, assume that *R. prolifica* was much less common or perhaps even absent from Madagascar in the first half of the twentieth century.

There remains thus the possibility that *R. prolifica* was more recently imported into Madagascar, either by spore dispersal or, more likely, together with its Australian host as suggested by Cooper (2021). This hypothesis seems supported by the fact that we never observed a similar Russula species, neither in endemic Malagasy vegetation (typically dominated by the ectomycorrhizal families Phyllanthaceae, Asteropeiaceae and Sarcolaenaceae), nor on the African continent, during a period of more than 30 years of mushroom collecting in these parts of the world. One impediment to answer this question of origin is that Australian russulas are very poorly known. Indeed, Lebel & Tonkin (2007) studied principally sequestrate species that were previously placed in different genera, while the impressive Agaricales monograph by Grgurinovic (1997) mentions very few Russula species, none of which correspond morphologically to the new species that will be introduced hereafter. The only potential Australian candidate of a Russula that appears extremely similar in the field to R. prolifica was a species (see fig. 2) presented under the name 'R. aff cyanoxantha' in Bougher and Syme (1998) but also referred to there as 'Multicoloured Russula' and 'R. multicolor' [nom. inval.]. The present paper will address this issue in detail.

Methods

Morphological study

Fresh fruiting bodies were photographed in the field and in further detail immediately after collection, descriptive notes were made before drying and spore prints were taken. The color codes given in the description of *R. discolor* are from Kornerup & Wanscher (1967). A total of 13 collections corresponding to the Australian 'R. multicolor nom. ad int.' were borrowed from PERTH for microscopic study and sequencing. Micromorphological characters were studied using a Nikon Eclipse E400 microscope under oil-immersion lens at a magnification of 1000×. All drawings of microscopical structures were made with a 'camera lucida' using a Nikon Y-IDT drawing attachment at a projection scale of 2400×. Contents of hymenial cystidia and pileocystidia in the illustrations are indicated schematically, with the exception of a few elements where contents are indicated as observed in Congo red preparations from dried material. Spores were observed in Melzer's reagent and statistics for measurements based on 20 spores with mean values indicated in bold, accompanied by values minus/ plus standard deviation with outliers placed in parentheses. All other microscopic observations were made in ammoniacal Congo red, after a short aqueous KOH pretreatment near boiling temperature to improve tissue dissociation through gelatinous matrix dissolution. All tissues were also examined for the presence of orthoor metachromatic contents or incrustations in Cresyl blue as explained in Buyck (1989).

Phylogeny

Seven identical ITS sequences were newly produced for R. discolor sp. nov. and deposited in GenBank (OR441036-OR441042). As a first approach towards identifying close taxa, we used a sequence similarity search for one of these new sequences in GenBank (Megablast option) retaining environmental sequences. The distance tree (not shown) proposed by GenBank for these blast results obviously pointed towards subsection Auratinae sensu Gosh et al. (2023), a subsection belonging to the crown clade of subgenus Russula. Based on this result, as well as on previous multigene analyses, (Adamčík et al. 2019, Khatua et al. 2017, Gosh et al. 2023). We then selected 40 sequences representative of all most similar sequences in GenBank, adding two sequences for Russula romellii as outgroup based on Buyck et al. (2018). Phylogenetic analysis of these 42 sequences was performed in MAFFT V.7 (Katoh et al. 2019) using multiple sequence alignment and the neighbour-joining (NJ) algorithm for all gap-free sites (449) in an alignment totalling 817 base pairs. Subsequently, we ran an analysis for a subsample of 18 ITS sequences representative of all named taxa in the /wielangtae lineage using sequence EU598170 for R. flavida as outgroup. (Fig. 3b). Environmental sequences were discarded from this analysis as these do not impact nomenclatural decisions concerning new taxa. Sequences were aligned by eye in MacClade v. 4.08a (Maddison and Maddison, 2003). The final alignment included 644 characters after exclusion of ambiguously aligned regions. Three independent runs for the most likely tree were performed in PhyML v. 3.0 (Guindon and Gascuel 2003), with the evolutionary model = GTR (Abadi et al. 2019) and other parameters estimated during the search. Branch support was considered significant when maximum likelihood bootstrap values (MLbs) were \geq 70% (Alfaro et al. 2003).

Results

Phylogeny

The NJ tree (Fig. 3a) for subsection *Auratinae* sensu Gosh et al. (2023) inferred from 42 sequences (including two outgroup sequences for *R. romellii*) retrieved significant support for four principal lineages in this subsection, some of which had already been identified in previous multigene analyses (Adamcik et al. 2019, Ghosh et al. 2023): viz. the /aurea (100%), /flavida (99%), /pseudo-flavida (87%) and /wielangtae (100%) lineages (red cir-

cles in Fig. 3a). The relationships between these individual lineages is, however, not resolved here. *Russula alatoreticula* placed in *Auratinae* by Khatua et al. (2017) may not be a member of the subsection (see general discussion for more explanations) and takes here an isolated position on a long branch. This NJ analysis indicated very clearly that sequences of the here newly described taxa (*R. discolor, R. cooperiana* and its var. *myrtacearum*) all nest in the /wielangtae lineage with full support with two unidentified environmental sequences (JN969386 and AB854693), obtained from Dipterocarpaceae ectomycorrhiza collected in Thailand, placed within the same lineage and highly supported to be sister to the rest of the taxa in this lineage (MLbs = 100%).

Figure 3b shows the most likely tree (-In = 1287.94105) obtained in the ML analysis for the 18 sequences that are representative of all named taxa in the /wielangtae lineage. The /wielangtae-lineage was originally created for two Oceanian species, a misinterpreted R. atroviridis (see taxonomy section below) and the more recently described R. wielangtae (Adamčík et al. 2019). The species of this species complex are all within a 96% similarity range for their ITS sequences when performing BLASTn. The most likely tree obtained from this new analysis places all agaricoid species of the in-group in a strongly supported clade obtaining 97% bootstrap support in the Maximum Likelyhood analysis (MLbs=97%), and well-separated from the sequestrate Russula [Cystangium] theodoroui. In this agaricoid clade, R. discolor sits on a very short branch with limited, yet significant support (MLbs=71%). It is, however, well separated from the other taxa in this lineage as these are all highly supported: R. wielangtae (MLbs = 98%) and a fully supported subclade (MLbs = 100%) for the newly described R. cooperiana and its var. myrtacearum (MLbs=96%). See the taxonomic part below for comments on R. atroviridis and R. wollumbing.

Discussion

General discussion

As the result of this study, we can conclude that it is not *R. atroviridis* that belongs in the /wielangtae lineage as mentioned in several recent publications (Adamčík et al. 2019, Vidal et al. 2019, Cooper 2021, Gosh et al. 2023), but the here newly described *R. cooperiana. Russula atroviridis* is unrelated and almost certainly a synomyn of *R. roseostipitata*, a species that belongs in subsect. *Tricholomopsidum*.

We have also shown here that the newly described *R. cooperiana* exhibits an impressive colour variability in the field depending on the associated host tree family. This confirms the unreliability of general colouration of pileus and stipe for the identification of most *Russula* species in New Zealand as already reported by Cooper (2021).



Figure 1. a. Freshly collected *Russula prolifica* near the capital of Antananarivo. b. Fresh fruiting bodies for sale in the capital being stripped of their pileipellis. c. Women and children returning from nearby *Eucalyptus* stands with their morning harvest. Photo credits: B. Buyck.



Figure 2. Field habit of Australian *Russula discolor* (a. PERTH 08162794, b. PERTH 06670334, c. R. Robinson 989WA, d. R. Robinson 841WA; photo credits R. Robinson) and Malagasy *Russula prolifica*.(e-f, photo credits B. Buyck).



Figure 3a. MAFFT NJ phylogeny inferred from an alignment of 42 ITS sequences representing all taxa that have been related to subsection *Auratinae* sensu Gosh et al. (2023). The analysis is based on all gap-free sites (449) of a 815 base pairs alignment. The four well-supported clades (indicated by a red circle) have all been confirmed to be part of this subsection in multigene phylogenies (Adamcik et al. 2019, Gosh et al. 2023). *Russula alatoreticula* from India may not be part of this subsection (see general discussion for more detailed comments).

The almost exclusively Oceanian /wielangtae lineage composes, together with the tropical /pseudoflavida lineage and the northern hemisphere /flavida and /aurea lineages, subsect. *Auratinae* (Gosh et al. 2023). Below, we will shortly discuss these different lineages.

It is also of interest that the /wielangtae lineage contains some sequestrate species, but apparently much less than the number of sequestrate species associated with the above-mentioned subsection *Tricholomopsidum* where *R. atroviridis/roseostipitata* belongs (Trierveiler-Pereira et al. 2015). *Russula [Cystangium] theodoroui* is most likely the closest relative to our new *R. discolor.* It is a hypogeous to semi-epigeous *Russula* known to occurr in *Eucalyptus* woodland in Queensland (Lebel 2003). Apart from its much smaller size, both the overall colour and microscopical features of pileipellis and hymenium are extremely similar.

Our ITS phylogeny for the /wielangtae lineage (Fig. 3a) retrieved very high support to separate both varieties

of *R. cooperiana*, but only moderate, yet significant, support to recognize *R. discolor*, which is mainly confirmed as independant species by the very high support obtained for the other taxa in this small lineage. There are few apomorphic characters to support *R. discolor*, a possible indication for a long term stable environment for this species.

Our NJ ITS phylogeny for subsection *Auratinae* (Fig. 3a) places available sequences that are representative of all described and undescribed taxa that have been related to this subsection.

Russula aurea, the type species, is the only European species in the entire subsection. The /aurea lineage is still an exclusively northern hemisphere lineage. It comprises further the extremely close *R. aurantioflava* from Pakistan (which might well be a later synonym) and a second, still unpublished Pakistani species (for a morphological description, see Adamčík et al. 2019). We here confirm for the first time the placement of the



Figure 3b. ITS phylogeny of the *Russula wielangtae* lineage placing the new taxa that are here introduced. The newly sequenced specimens are in blue. Sequences from holotypes of the taxa as indicated on the right are in bold. Bootstrap values are indicated above branches that are significantly supported.

American *R. xantho* (see Buyck 2005) in this lineage thanks to several ITS sequences of Canadian collections that were very recently deposited in GenBank.

The /flavida lineage, equally an exclusively northern hemisphere lineage, comprises essentially the American *R. flavida* complex and harbours several still undescribed close taxa (probably subspecies or varieties), including one undescribed species from Asia.

The very recently published Indian look-alike of *R. flavida*, *R. pseudoflavida* (see Gosh et al. 2023), has a surprisingly divergent ITS sequence for a species that is morphologically so highly similar. However, we can confirm the conclusion of Ghosh et al. (2023) that there is high support to group *R. pseudoflavida* with an undescribed tropical Australian species (*Russula* sp. 3) collected in the Northern territory near Darwin. Together, they constitute a separate lineage.

Russula alatoreticula K. Acharya, S. Khatua, A.K. Dutta & S. Paloi, placed in subsect. *Auratinae* by its authors (Khatua et al. 2017) is certainly an odd-ball and may not belong here at all. It was described on the basis of a very limited phylogenetic sampling and an incomplete description. It is a pinkish red species with white lamellae, producing a white spore print and has dis-

tinctly winged (sub)globose spores. It further forms very narrow pyriform to subulate hyphal terminations in the pileipellis; pileocystidia are not mentioned, but caulocystidia are said to be present. Both the white spore print colour, the presence of caulocystidia and apparently also the type of pileipellis are arguing against its inclusion in this subsection.

The Indian *R. pseudoflavida* and *R. alatoreticula* are species associated with dipterocarp trees and it is interesting to note that the basal taxon that is here for the first time firmly placed in the /wielangtae lineage is represented by two environmental (ectomycorrhizal) sequences that were also obtained from dipterocarp forest (but in Thailand), extending thereby considerably the host range of this lineage which includes now various Myrtaceae, Nothofagaceae and Dipterocarpaceae.

All in all, there are now at least eight confirmed different taxa in the /wielangtae lineage when accounting also for environmental sequences. The latter sequences extend the distribution of this lineage in Australia, as it is now also present in the northern part where *Russula* sp. 8 (KP012851) was collected in Berry Springs Nature Park, near Darwin, Northern Territory, while the sequence (MF503292) of a (most likely sequestrate) *Russula* was obtained from scats of the endangered northern bet-

tong (*Bettongia tropica* Wakefield), a marsupial which is a dietary fungal specialist living in the *Eucalyptus* dominated woodlands bordering the tropical rain forest in Northern Queensland (Abel et al. 2006). The sequence of *R. wollumbina* is most likely just a specimen of *R. wielangtae* (originally described from Tasmania) as there are merely two reliable base pair differences.

Russula romellii R. Maire, chosen here as our out-group (fig. 3a-b) is a European species traditionally placed in either subsection *Integrinae* (Romagnesi 1967) or *Integriforminae* (Sarnari 2005). Buyck et al. (2018), in their world phylogeny for *Russula*, demonstrated that the real affinities of *R. romellii* are elsewhere as already suggested earlier (Buyck 2005). Indeed, it forms with *Auratinae* a well-supported clade (MLbs=99%, BPP=1) that constitutes the sister-lineage (MLbs=82) to the tropical African *R. cf sesenagula* (Buyck et al. 2018). All of the above-mentioned species are then again placed sister to another, entirely tropical, African clade and, together, all these species constitute one of the most ancient lineages in the *Russula* crown clade.

Taxonomy

Russula discolor Buyck & Bougher, sp. nov. Index Fungorum: IF900892

Holotype: Western Australia, Walpole, property 1683, Dingo Flat Rd, 34°57′0.000″S, 116°50′0.000″ E, in litter under *Allocasuarina decussata, Eucalyptus marginata, E. jacksonii* and *E. guilfoylei*, 5 June 1995, N.L. Bougher, K. Syme & M.E. Alosi E5412 (PERTH 07551681). *GenBank:* OR441039 (ITS).

= *R. multicolor* Bougher & Syme, Fungi Southern Australia 146 (1998), nom. inval.

Diagnosis: *Russula discolor* is characterised by its occurrence in the eucalypt forests of southwestern Australia, its rapidly discolouring reddish pileus becoming mottled with red, maroon, cream, yellow and grey or greenish grey in variable proportions, by its unchanging white stipe surface and context, the mild taste and the yellow to cream, equal gills producing an egg yellow spore print.

Etymology: in reference to its variegated colours and to the fact that different fruiting bodies may be of different dominant colouration

Pileus 40-85(120) mm broad, convex with incurved margin, soon flat-convex with plane margin, then expanding to flat or with a shallow central depression; margin thin, smooth or very shortly translucent-striate; surface when young mainly red (8D-E8, 9-10C7, 10E5, 10-11D8), soon discolouring and becoming mottled with various colours - e.g. pale pastel yellow (2A2), cream (3-4A4), yellow (2-5A4), pink (10D7, 11C6), orange (4A6), tan brown (7C3), maroon (11F7), violet brown (11E4), grey (2B3-3B2-3) or greyish green (5C3); mature caps may vary from being heavily mottled with a mixture of these

colours to remaining mainly red or, on the contrary, ending up completely discoloured and mainly cream, but usually retaining some red traces at the center; sticky when young in damp conditions, readily drying to become smooth but dull, often with a distinct white bloom or scurfy aspect (particularly under hand lens), concentrically wrinkled if excessively drying out (airdried specimens show this feature). Lamellae adnate or subdecurrent, becoming narrowly adnexed when mature, equal in length or with occasional shorter lamellulae, up to 9 mm high, normally disposed (meaning ca. 1L/mm), fragile, some anastomoses present, cream (2A3-3A2) at first, then deeper yellow-cream (4A4-4A5), unchanging when bruised; lamella edge smooth, entire, becoming more yellow than sides with age. Stipe 35-50(-60) x 10-25 mm, cylindric or sometimes slightly bulbous at the base, white, smooth yet finely and longitudinally rugulose, not bruising in age or when injured; inside solid then stuffed with soft tissue, sometimes developing 1-2 cavities. Veil absent. Context white, unchanging. Basal mycelium white, sparse. Odour not distinctive. Taste mild. Spore deposit butter to egg yellow (4A4-5).

Spores subglobose to broadly ellipsoid, (7.5-)7.9-**8.20**-8.5(-8.7) x (6.5-)6.7-**7.05**-7.4(-7.9) μm, Q (1.06-)1.11-**1.16**-1.22(-1.29) [E5412 Holotype] (7.1–)7.7–**8.21**–8.7(–9.2) x (6.2–)6.5–**6.88**–7.3(–7.7) μm, Q = (1.06-)1.12-1.28(-1.38) [E5000]; ornamentation up to 1 µm high, subreticulate to reticulate, heavily crested with crests composed of linearly aligned, obtuse to conical warts in a ramifying pattern that is usually transversely oriented to the spore axis, with rare and short linear connections or isolated warts; suprahilar spot moderately amyloid, becoming greyish but not black in Melzer's reagent, not elevated, often just verruculose or only partly amyloid; apiculus well-developed. **Basidia** 29–42(–52) x 9–11(–12) µm, mostly short, hardly clavate, often somewhat narrowed in the middle, hyaline, sterigmata up to 6(-9) µm long, 4-spored. Marginal cells not observed; the gill edge being mainly composed of cheilogloeocystidia. Hymenial gloeocystidia abundant on sides of lamellae, 55-85 x 8-11(-15) µm, arising from the lamellar trama, hardly emergent above the basidia, thin-walled, slender, fusiform to cylindrical, with rounded or mucronate apex, with yellowish or greyish granular contents; those near or on the lamallar edges very abundant and slightly smaller, 40–70 x 5–10 µm, with rounded or tapering apex, mainly hyaline, originating in the hymenium, subhymenium or trama. Lamellar trama predominantly composed of sphaerocytes up to 50 µm broad, mixed with few hyphae 2-5 µm broad and oleiferous hyphal fragments. Subhymenium pseudoparenchymatic, composed of tightly packed, irregular cells up to 12 µm broad. Pileipellis hyaline in KOH, pinkish in water, orthochromatic in Cresyl blue, two-layered and composed of a strongly developed pseudoparenchymatic suprapellis covering a subpellis composed of gelatinised hyphae 2–4 µm broad.



Fig. 4. *Russula discolor* sp. nov. Fresh basidiomata. a. PERTH 07554699. b. PERTH 07551681 (holotype). c. PERTH 07710259. Photo credits: N. Bougher.

Suprapellis arranged more or less in a palisade from pileus center to pileus margin, composed of long, multi-

celled (up to 10 cells or more outside the pileus center), strongly ramifying, diverticulate, thin-walled hyphal end-



Figure 5. *Russula discolor* sp. nov. (holotype). Hyphal extremities of the pileipellis near the pileus margin (a) and in the centre (b). Scale bar = $10 \mu m$. Drawings B. Buyck.



Figure 6. *Russula discolor* sp. nov. (holotype). Hyphal extremities of the stipitipellis from upper (a) and lower part of the stipe (b). Scale bar = $10 \mu m$. Drawings B. Buyck.



Figure 7. *Russula discolor* sp. nov. (holotype). Elements of the hymenium: a. Spores as observed in Melzer's reagent. b. Basidia. c. Basidiola. d. Gloeocystidia on sides of lamellae with contents as observed in Congo red in two cells. Scale bar = 10 µm, but only 5 µm for spores. Drawings B. Buyck.

ings that are composed of often strongly and irregularly inflated cells that are globose, barrel-shaped, clavate, ellipsoid or variously shaped, 3–25 μ m diam., many with lateral branches or outgrowths; the terminal cell often also very irregular, particularly in the pileus center, but frequently narrowing upward, often (very) short, mostly 12–36 μ m long and less than 5 μ m wide, some up to 10 μ m wide, bottle-shaped. Primordial hyphae, pileogloeocystidia and cystidioid hyphae absent. **Stipitipellis** less developed; hyphal endings more slender, with terminal cells often short, narrow or irregularly sinuate, toward the stipe base with more irregular, nodulose-sinuate terminal cells as in pileipellis. Caulocystidia not observed. **Clamp connections** absent in all tissues.

Habitat: on soil amongst litter in Karri (*Eucalyptus diversicolor*) and Tingle (*E. jacksonii* & *E. guilfoylei*) forests, and Jarrah (*E. marginata*) - Marri (*Corymbia calophylla*) forests in south-west Australia. Usually in small groups, can be locally abundant.

Additional material examined:

AUSTRALIA. Western Australia, Walpole-Nornalup National Park: Hilltop Road, to Giant Tingle Tree track, in litter under Eucalyptus diversicolor, E. jacksonii and Allocasuarina decussata, 35°1'0.000" S, 116°40'0.000" E, 12 June 1988, leg. R.N. Hilton E4068 (PERTH 07550308); ibid., on track between Gully Rd and Giant Tingle Tree, 34°59'0.000"S, 116°47'0.000"E, 05 June 1992, N.L. Bougher & K. Syme E4743 (PERTH 07554648); ibid., Knoll Drive walk, Walpole, in litter under Corymbia calophylla, Eucalyptus diversicolor, E. marginata, Agonis flexuosa and Allocasuarina decussata, 34°59'0.000"S, 116°43'0.000"E, 06 June 1992, Leg. N.L. Bougher & K. Syme E4746 (PERTH 08792275); ibid., same habitat, 06 June 1992, leg. K. Syme & N.L. Bougher E4748 (PERTH 07554699); ibid., under Eucalyptus diversicolor and E. calophylla, 11 June 1993, N.L. Bougher & K. Syme E5000 (PERTH 07652631). Western Australia, Hull Rd, off North Walpole Rd, near Walpole, under mixed 30 year old rough & smooth barked Eucalyptus species, Agonis parviceps and Acacia pentadenia, 34°53'0.000" S, 116°41'50.000" E, 04 June 1992, K. Syme & N.L. Bougher E4654 (PERTH 07710259). Western Australia, Mount Shadforth Reserve, Denmark area, 34°58'0.000" S, 117°16'0.000" E, under Eucalyptus diversicolor, 23 April 1995, leg. K. Syme KS786/95/E5615 (PERTH 07676336, "Morphological details of this collection are described and illustrated in Bougher & Syme 1998 as Russula aff. cyanoxantha"). Western Australia, Amphion Forest Block: Murray River Rd, Dwellingup, 32°47′0.000″ S, 116°11′0.000″ E, under unburnt Eucalyptus marginata & Corymbia calophylla, 11 June 1998, leg. N.L. Bougher & S.Q. Bolsenbroek E5943 (PERTH 07605056) & E5949 (PERTH 07605102); ibid., same habitat, 14 June 1998, S.Q. Bolsenbroek E5961 (PERTH 07605226); ibid, under E. marginata, 18 July 1996, N.L. Bougher s.n. (PERTH 07711980). Western Australia, Alcoa Mine, Nettleton Rd, 32°34'0.000" S, 116°4'0.000" E,

under *Eucalyptus marginata* mixed forest, 14 June 2000, leg. N.L. Bougher s.n. (PERTH 07676336).

Notes:

The description of the new *R. discolor* depicts a species with a variable pileus colour. In general terms, it could be described as an overall reddish species that discolours easily and rapidly, while developing different tinges. Also the colour of the lamellae changes distinctly between young and mature fruiting bodies. Notwithstanding this important colour variation, the species is considered in Australia to be 'easily recognizable in the field' (Richardson 2011, Bougher & Syme 1998). This was confirmed by our sequencing results because all of the received collections (see studied material) produced identical ITS sequences. It seems to be widespread in southwestern Australia where it is exclusively associated with various Eucalyptus species that compose the Karri (Eucalyptus diversicolor), Tingle (E. jacksonii & E. guilfoylei) and Jarrah (E. marginata) - Marri (Corymbia calophylla) forest stands. We can also confirm here that the voucher that served for the illustration and description in Bougher & Syme (1998) of R. aff. cyanoxantha / R. multicolor [as listed on p. 380 of that work] was successfully sequenced and represents indeed R. discolor.

Russula wielangtae, described from Tasmania and equally a eucalypt associate, is definitely close (ITS similarity between 97-98%) to the here newly described R. discolor and shares with it a very similar spore ornamentation, a similar composition of the pileipellis and even a similar overall colour, although less variable. The presence of 'pileocystidia' was considered 'doubtful' in the original description of *R. wielangtae* and the legend of fig. 74 in Adamčík et al. (2019), mentioning twice 'pileocystidia', probably needs correction as none of the hyphal terminations in the pileipellis of these Oceanian species has the typical contents of the generally accepted definition of gloeocystidia in the genus. Russula wielangtae might also occur on the Australian continent as suggested by the collection MEL 2238232 (see Fig. 3a,b). The ITS sequence for this collection was deposited in Gen-Bank associated with the name R. wollumbing Grgur. and still represents the single deposited ITS sequence under that name (Lebel & Tonkin 2007). Unfortunately this sequence seems of unsufficient quality, lacking more than 70 base pairs at the start of ITS1 and having several ambiguous base pairs further down the sequence. The specimen was identified and collected by J. E. Tonkin in eucalypt woodland in southeastern Australia near Mt Richmond (https://biocache.ala.org.au/ occurrences/

9063ba82-91d0-4c74-9e1f-329ed608b9db). Tonkin's identification was questioned by Adamčík et al. (2019) who assumed that it rather represents their newly described *R. wielangtae* (we agree on the basis of the ITS sequence similarity) as the spores of the type specimen of *R. wollumbina* were described and illustrated as having much more isolated warts and scarcely any ridges

(Grgurinovic 1997), a feature that was confirmed for the type in Adamčík et al. (2019).

There are many more collections reported as R. wollumbina the 'Atlas of Living in Australia' (https://www.ala.org.au), but none of these have been sequenced. The fact that the type of R. wollumbina is based on a collection by Cleland (1934) originally identified as *R. xerampelina*, a species often reported from Australia in the past (e.g.: Dickinson & Lucas 1979, Gill & Ashton 1971, Shepherd & Totterdell 1988, Sinnott 1976) seems to be responsible for a misleading shortcut. Indeed, certain websites on Australian mushrooms automatically seem to assume that all mentions of R. xerampelina in Australia correspond now to R. wollumbi*na*, which is absolutely not the case. An interesting and recent find that might correspond to R. wollumbing is a description by P. Leonard for a specimen identified as such and uploaded on https://qldfungi.org.au/wp-content/uploads/2022/02/Russula-wollumbina.pdf. This specimen indeed shows some overall resemblance to close relatives of R. xerampelina, the earlier interpretation of R. wollumbina. Leonard's description mentions pale gills that produce a whitish or very pale cream spore print (IIa following the Romagnesi 1967 scale), not the ochre or yellow spore print observed for R. wielangtae or R. discolor, respectively, and stresses the often strongly tapering stipe, two characters that match the original interpretation of Cleland (1934, as 'R. xerampelina'). However, the spores of Leonard's collection are described as having strongly amyloid ridges, which seems in contradiction with the spore description given by Grgurinovic (1997) for the type specimen of R. wollumbina. Leonard's interpretation of R. wollumbina suggests a very different species compared to the here newly described *R. discolor* which has more variegated colours, much darker spore print and a cylindrical to slightly bulbous stipe. The stipe of *R. discolor* also lacks the pinkish tints observed on nearly all of the other species that are discussed here. A correct interpretation of R. wollumbina remains therefore problematic for the moment.

The only other species that has been placed in the /wielangtae lineage in recently published phylogenies was identified as *R. atroviridis* Buyck from New Zealand (Adamčík et al. 2019, Cooper 2021). It differs already from both other Oceanian species in this lineage because it associates not with eucalypts, but principally with *Nothofagus*. Our phylogeny (Fig. 3b) suggests that there are possibly two different, but very close phylogenetic entities. Although considerable effort has been made to obtain sequences for most of the *Russula* species of New Zealand (Cooper & Leonard 2014, Cooper 2021), there remains an urgent need for better and more detailed descriptions, particularly for the type specimens. We, therefore, provide below our own microscopic examination of the isotype of *R. atroviridis*.

As discussed below, the results of this re-examination raised more questions.

Russula atroviridis Buyck, Bull. Jard. Bot. Nat. Belg. 60(1-2): 202 (1990) MB127270

Holotype: New Zealand: Prov. Mid-Canterbury, Craigieburn Range, Kowai Bush, on soil under *Nothofagus solandri*, 5 Febr. 1969, leg. E. Horak (PDD 30655 holotype, ZT69.305 isotype)

= R. atrovirens R.F.R. McNabb New Zealand journal of botany 11: 703, *nom. illeg.*, non Beeli (1928)

Spores broadly ellipsoid, (7.9-)8.0-8.45-8.9(-9.4) x (5.8-)6.5-6.98-7.5(-7.7) μm, Ο (1.08–)1.14–**1.21**–1.29(–1.36), sparsely to typically very densely ornamented with strongly amyloid, cylindrical to conical or smaller, obtuse-rounded warts; the warts isolated or more often grouped with two or more in dense clusters or aligned into short crests that never encircle the spore, low and short linear interconnections are sometimes present but never to such a degree that the spore could be considered subrecticulate; suprahilar spot large and mostly distinct, often even strongly amyloid, but sometimes also just greying in Melzer's reagent or simply verruculose. Basidia strongly clavate, not pedicellate, 35-40(-45) x 11-14 µm, 4-spored; sterigmata robust, ca 8 x 2 µm. Hymenial gloeocystidia on sides of lamellae clavate to almost fusiformous, 40–60 x 10–14 µm, mucronate or more frequently appendiculate; the appendix measuring up to 25 µm long, not thick-walled, filled with abundant, refringent, granular-crystalline contents. Marginal cells not observed; the lamella edge has basidia and cheilocystidia similar to pleurocystidia, but (much) smaller, sometimes as small as 20 x 7 µm. *Pileipellis* two-layered, a gelified subpellis of intertwined hyphae is present beneath the pseudoparenchyma at the surface; the pseudoparenchyma composed of hyphal terminations that start with basal, strongly inflated, globose to ellipsoid cells, sometimes up to 25 µm diam, from which arise short chains of 3-6 gradually smaller cells, rarely ramified, that are mostly barrel-shaped to almost cylindrical with the terminal cell typically short and slightly narrowing, utriform to even clavate, measuring 8–20 x 4-8 µm; these terminations mixed with much longer, more slender ones that are composed of 4 to 8 subcylindrical cells, forming 'hairs' that extend up to more than 100 µm and are strongly emergent, the uppermost 1-2 cells banded with refringent contents (pigments ?) particularly near the septa; the terminal cell mostly narrowing, up to 40 µm long, sometimes subcapitate (and reminiscent of mucronate pileocystidia); toward the pileus margin parts of the suprapellis has seceded leaving the narrow, thin-walled hyphae of the subpellis exposed; these hyphae individually distanced from each other by gelification and intermixed with oleiferous fragments that are more strongly undulate-tortuous. Typical



Figure 8. *Russula atroviridis* (isotype). a. Hyphal terminations of the pileipellis constituting the pseudoparenchymatic layer. b. Emerging 'hairs' from the pseudoparenchyma containing refringent bands that are clearly visible in Congo red mounts. Scale bar = 10 µm. Drawings B. Buyck



Figure 9. *Russula atroviridis* (isotype). Elements of the hymenium. a. Spores as observed in Melzer's reagent. b. Basidia. c. Basidiola. d. Gloeocystidia on sides of lamellae with indication of contents as observed in Congo red in two cells. e. Gloeocystidia on edge of lamellae. Scale bar = 10μ m, but only 5 μ m for spores. Drawings B. Buyck.

pileocystidia and cystidioid hyphae not observed. *Clamp connections* absent.

Notes: The original description of *R. atroviridis* pictures a mild, medium-sized (up to 65 mm diam.) species with a multicoloured pileus, predominantly tinged with dull greyish magenta, greyish green, greenish-yellow and dark brown, a pinkish stipe, 40 x 15 mm, and pale coloured lamellae (McNabb 1973: as *R. atrovirens*). It was originally collected under *Nothofagus solandri*, but is also listed with several other *Nothofagus*.

Our examination of the isotype of *Russula atroviridis* revealed a pileipellis that could be described as a pseudoparenchyma. It differs from a typical 'virescens'-structure in the presence of dispersed, septate, cylindrical, emerging 'hairs' (fig. 8b). These 'hairs' are easily located in microscopic preparations as their terminal and sometimes subterminal cell are filled with distinct, mostly banded or agglutinated, refringent contents. Also our observations on the spores of the isotype (fig. 9a) correspond very well to McNabb's description which mentions a 'densely verrucose' ornamentation.

While neither the type nor any of the original material of R. atroviridis has been sequenced, all of the recently published ITS and LSU sequences deposited under that name place McNabb's species in the /wielangtae-lineage (Adamčík et al. 2019, Ghosh et al. 2023) and the most disconcerting fact resulting from our microscopic examination of the type collection, is that the pileipellis of R. atroviridis is very different from the only two other known species in the /wielangtae-lineage. Indeed, the type has exactly the same pileipellis structure as found in Oceanian species that are part of the unrelated subsection Tricholomopsidum Buyck & V. Hofst. (Buyck in Rossi et al. 2020). The latter subsection is by far the most common and diverse Russula lineage in New Zealand and harbours several species with very similar overall colouration and equally associated with Nothofagus trees.

When looking at images of the microscopic features of these sequenced "*R. atroviridis*" collections (available on https://scd.landcareresearch.co.nz/), it is impossible not to notice a major discrepancy been these specimens and the type specimen. These sequenced collections all possess strongly ornamented subreticulate spores, which is in complete disagreement both with McNabb's original description (1973: 703-704) and our own observations on the type. It is therefore obvious that the modern interpretation of *R. atroviridis* in New Zealand does not conform to the type.

The complexity of the situation does not end here, however, because a comparison between the type collection of *R. atroviridis* and the other species described by McNabb (1973) reveals near-identical features for *R. roseostipitata* McNabb. The similarities are such that we suspect that McNabb's holotype of *R. atroviridis* is a specimen of *R. roseostipitata*. Whether or not *R. atro*-

viridis becomes indeed a synonym of R. roseostipitata will need a more in-depth study of this species complex as there appear to exist other closely related but still undescribed taxa based on deposited sequences in GenBank. This possible synonymy is not without interest in the context of our original quest as R. roseostipitata was suggested by McNabb to be closely related to Virescentinge (exactly the subsection where the Malagasy R. prolifica is placed). However, McNabb ignored the importance of the strongly amyloid suprahilar spot on the spores of his species, which excludes such affinity. Available sequences for R. roseostipitata place it in subsect. Tricholomopsidum and this placement conforms to our observations on the R. atroviridis type specimen. It is also interesting that the two earliest deposited sequences for R. atroviridis (GU222285 and MW296245) are identical to the more recently deposited sequences for R. roseostipitata.

The recent misinterpretation of *"R. atroviridis"*, however, corresponds to the best of our knowledge to a still undescribed species. This misinterpreted *"R. atroviridis"*, which is part of the /wielangtae lineage, does indeed possess the typical features of this lineage and is described below as *R. cooperiana* sp. nov.

Russula cooperiana Buyck & E. Horak, sp. nov.

Index Fungorum: IF 900893

Holotype: North Canterbury, Glentui Bush, 2449076E 5778573N (WGS84 -43.197777 172.250253), in beech broadleaved forest under *Nothofagus [Fuscospora] solandri*, 2 March 2009, leg. J.A. Cooper JAC10864 (PDD95332). *GenBank*: MW683758 (ITS).

= R. αtroviridis sensu Adamčík et al. (2019), Ghosh et al. (2023) and Cooper (2021)

= *R. roseostipitata* sensu Bohorquez et al. (2021)

Diagnosis: differs from other known species in the /wielangtae lineage by the combination of its overall color and geographic distribution in New Zealand.

Etymology: dedicated to Jerry Cooper, who documented this species online (as *R. atroviridis*) with many illustrations.

Russula cooperiana Buyck & E. Horak var. *cooperiana*

Pileus up to 90 mm diam., when young convex, sometimes more or less plane in the center, then becoming slightly depressed; margin smooth or very shortly striate; surface viscous when humid, soon dry, dull, olive- to moss-green and minutely and densely warty-punctuate from much darker dots (pointillist aspect), sometimes discolouring and becoming more brownish-yellowish in the center, peeling to mid-radius and then revealing



Figure 10. *Russula cooperiana* var. *cooperiana*. Fresh basidiomata. a-d, Views of pileus, gills and stipe (PDD95409). e-f. view of the contrasting colour of the subcuticle layer (PDD95409). g. macrochemical reactions (Top left: guaiac. Bottom left: iron sulfate. Right: KOH on cap). h. specimen Horak (ZT68.60). Photo credits: J.A. Cooper, except h from E. Horak.



Figure 11. *Russula cooperiana* var. *cooperiana* (ZT 68.60). Hyphal terminations of the pileipellis near margin (a) and in center (b). Scale bar = 10 µm. Drawings B. Buyck.



Figure 12. *Russula cooperiana* var. *cooperiana* (ZT 68.60). Elements of the hymenium. a. Spores observed in Melzer's reagent. b. Basidia c. Basidiola. d. Gloeocystidia on sides of lamellae with indication of contents as observed in Congo red in two cells. e. Detail of lamellar edge. Drawings B. Buyck. Scale bar = 10 µm, but only 5 µm for spores. Drawings B. Buyck.

a pinkish-purple context underneath. Lamellae shortly adnate, equal or with rare, rather short lamellulae (up to mid-radius), moderately spaced (7-14/cm at pileus margin), quite thick at the pileus attachement and with concentric anastomoses between gills, young cream coloured, turning butter yellow in age, obtuse-rounded near the pileus margin; lamellar edges even, concolorous. Stipe always shorter than the pileus diameter, mostly 22–39 x 15–18 mm, cylindrical, or slightly widening downward, minutely striate lengthwise, white but always partially with a pinkish red blush, with age yellowing in the lower half, toward the base rounded, finely felted to tomentose; the stipe context spongy and brittle, without cavities. **Context** whitish, unchanging, just underneath the green pileipellis a thin part of the context is pinkish orange, mauve to purplish violet, then turning yellowish. Macrochemical reactions: FeSO4 salmon; guaiac strong, bluish; KOH on pileus surface pale orange to orange red, NHOH on pileus surface nil. Taste mild. Smell indistinct. Spore print yellowish (F).

Spores shortly ellipsoid, (7.9–)8.0–**8.20**–8.4(–8.5) x (6.2-)6.4-6.69-7.0(-7.3) μm, 0 (1.14-)1.18-1.23-1.28(-1.32), subreticulate, when young with a network of low crests without strongly developed wrats, the latter developing only later with crests becoming then chains of well-developed cilindrical to conical warts or even spines, straight or curved, here and there still leaving low lines, some individual, low and isolated warts present but rare; suprahilar spot distinctly amyloid, often somewhat descending on the apiculus. Basidia (35-)40-50(-60) x (13-)14-16 µm, rapidly strongly clavate, rarely pedicellate-clavate, 4-spored. Hymenial cystidia (70–)100–120(–140) x (11–)13–18 μm, dispersed, emergent up to 40 μm, thin-walled, clavulate to fusiform and usually with mucronate or appendiculate tip, filled with poorly differentiated contents that are mostly more or less granular to minutely crystalline and concentrated in the upper part. Marginal cells very difficult to observe, resisting to rehydration, agglutinated in dense tufts along the entire edge rendering it probably sterile, at least some resembling the lageniform terminal cells of the pileus because of the similar capitate apex, mixed with cheilocystidia. Lamellar trama predominantly composed of sphaerocytes, mixed with hyphae and oleiferous hyphae. Pileipellis orthochromatic in Cresyl blue, two-layered, composed of a strongly developed trichodermal suprapellis covering a subpellis composed of gelatinised hyphae, 2–4 µm broad. Suprapellis arranged more or less in a palisade from pileus center to pileus margin, composed of long, multi-celled, sparsely ramifying and thin-walled hyphal endings; terminations near the pileus margin mostly composed of 4 to 8 irregularly inflated cells that are barrel-shaped, lageniform, ellipsoid or subcylindrical, (4–)6–12(–15) µm diam., few with lateral branches or outgrowths; the terminal cell often quite small, mostly attenuating upward to conical, (10-)16-34(-51) long, of similar diameter, some minutely mucronate to subcapitate and reminiscent of pileocystidia but optically empty. Hyphal extremities in the pileus center composed of less cells, these mostly cylindrical and narrow, 4–8 μ m diam.; terminal cells very variable and irregular in outline, frequently repeatedly constricted, undulate to moniliformous; quite many optically empty terminal cells reminiscent of pileocystidia because of their mucronate to even appendiculate tip, and these mostly 12–36 µm long and less than 5 µm wide; some rare endcells inflated up to 10 µm diam. and then ampullaceous to almost globose. Primordial hyphae and pileocystidia with differentiated contents absent. Cystidioid hyphae absent. Oleiferous hyphae frequent. **Clamp connections** absent from all tissues.

Other examined material: **New Zealand**. Oxford, Cooper's Creek, 2435494E 5772008N, (WGS84 -43.255649 172.082262), in broadleaved forest under *Nothofagus [Fuscospora] solandri* var. *cliffortioides*, 10 April 2009, leg. J.A. Cooper JAC10953 (PDD95409); Nelson, Abel Tasman N.P., Rameka Track, N26 2499000 6031200 (WGS84 -40.925682 172.869573), under *N. fusca*, 19 Jan 2003, leg. P. Leonard PL12103 (PDD77744); Nelson, Lake Rotoiti, under *N. fusca* and *N. menziesii*, 4 February 1968, leg. E. Horak (ZT68.60)

Differs morphologically from the new var. *myrtacearum* in the predominantly greenish pileus, even in young specimens, and in the pinkish flush on the stipe which also takes yellowish tinges with age. It further differs ecologically in its host association with *Nothofagus* species instead of with Myrtaceae and in ITS sequence data.

Russula cooperiana var. *myrtacearum* Buyck & J.A. Cooper, var. nov.

Index Fungorum: IF900894

Holotype: New Zealand. Auckland, Waitakere, Huia, Big Muddy Creek, 2653880E 6469325N (WGS84 -36.966752 174.611713), in *Leptospermum* and Podocarp mixed forest, 22 April 2006, leg. C. Shirley CS R33 in Cooper JAC13218 (PDD92357). *GenBank:* MW683828 (ITS), MW683665 (LSU).

Diagnosis: differs from the type variety in the often predominantly pinkish, magenta to dark purplish pileus color, but particularly in the always entirely white stipe and in its association with Myrtaceae instead of Nothofagaceae, as well as in four consistent base pair changes in ITS sequences.

Etymology: refers to Myrtaceae, the family of host trees it is associated with.

Other examined material: Auckland, Waitakere Ranges, near start of Parau Track and Farley Track, 2650122E-6466446N (WGS84 -36.993321 174.570115), under *Kunzea ericoides* (A.Rich.) Joy Thomps. (Myrtaceae), 29 Jan 2014, leg. P.R. Johnston, J.M. Ryder, O.K.



Figure 13. *Russula cooperiana var. myrtacearum* var. nov. Field habit (a. PDD104176, b-c. PDD92357, d. E. Horak ZT939. Note that the green colours clearly visible in ZT939 were not present at the time of collecting, but developed only afterwards as explicitly mentioned in the field notes. Photo credits J.Cooper (a), C.Shirley (b-c), E. Horak (d).



Figure 14. *Russula cooperiana var. myrtacearum* var. nov. a-c. Spores as observed with scanning electron microscope (a. PDD104176, b-c. PDD92357, holotype). photo credits J.A. Cooper.

Sigglekow, B.C. Robson, in JAC13171 (PDD104176); Little Barrier Island, Thumb track, in *Leptospermum* forest, 13 Feb 1981, E. Horak (ZT 939).

Notes: Russula cooperiana is characterized by the strongly subreticulate to almost reticulate, interconnected to crested spore ornamentation. The spores are not 'winged', but the long connectives or ridges between the well-developed warts or spines can in some cases almost encircle the spores and are often horizontally oriented (the orientation of the longest ridges is more or less at right angles with the spore's vertical axis).

The type variety associates with *Nothofagus* and develops pinkish tinges on the stipe. It has a predominantly greenish pileus surface sitting on a pinkish-orange coloured context layer just underneath the surface. The 'pointillist' aspect of the pileus surface may not always be very apparent, giving the pileus surface thus a more homogeneous, smoother aspect.

In the field, the here newly described variety *myrtacearum* differs essentially in the always white stipe and in its association with Myrtaceae. The overall colour of this variety is also different, being predominantly pinkish, greyish magenta or purplish, but greenish tints may appear with age. Microscopically speaking, both varieties are completely similar. As already mentioned in the phylogenetic results, there is a 0.5% genetic difference between the ITS sequences of both varieties as the result from four consistent base pair differences.

Disclosures

There are no conflicts of interest.

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