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Russula hixsonii Murrill, a rare and intriguing southern species of uncertain systematic position, rediscovered in Georgia, USA

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Abstract – *R. hixsonii* Murrill, only known from its type locality in Florida, USA, is described and illustrated from a recent collection in southern Georgia and compared with the type specimen. Its very unusual combination of morphological features and eventual affinities are discussed.

***Cresyl blue* / Florida / *metachromatic color reaction* / morphology / North America / *Russula* sect. *Metachromaticae* / taxonomy**

INTRODUCTION

Trying to identify russulas in the United States is still a very disappointing endeavour due to the absence of modern and detailed morphological descriptions for most described species. Especially the lack of detailed illustrations for the microscopic features for the nearly 400 type specimens makes identification virtually impossible. In the past decennia, some progress towards this end was made thanks to the publications of, for example, Shaffer (1962, 1964, 1970, 1972, 1975, 1990), and more recently by a series of type studies of American species in some selected subsections *Xerampelinae*, *Roseinae*, *Heterophyllinae* (e.g.: Adamčík & Buyck 2011a, b; Buyck & Adamčík 2011) and well-illustrated and detailed descriptions of new taxa (e.g.: Adamčík *et al.* 2011, Adamčík & Buyck 2010, Buyck *& al.* 2008). For most taxa, these modern type studies have considerably altered the often widely accepted, previous species concepts.

In this contribution, the authors report on a very recent collection of an enigmatic southern *Russula*, which was only known from the type locality near Gainesville, Florida.

MATERIALS AND METHODS

Micromorphological characters were observed using Olympus CX-41 and Nikon Eclipse E400 microscopes using oil-immersion lenses at a magnification of 1000x. All drawings of microscopical structures, with the exception of spores, were

made with a 'camera lucida' using a Nikon Y-IDT drawing attachment at a projection scale of 2400x. Contents of hymenial cystidia and pileocystidia are indicated schematically in the illustrations, with the exception of a single or a few elements where contents are indicated as observed in Congo red preparations from dried material. Spores were observed on the gills in Melzer's reagent. All other microscopic observations were made in ammoniacal Congo red, after a short treatment in warm, aqueous KOH to dissolve the gelatinous matrix and improve tissue dissociation. All tissues were also examined in an alcoholic Cresyl blue solution to verify presence of ortho- or metachromatic reactions as explained in Buyck (1989). Trama and cystidia were examined for reactions of cell contents in a sulfovanillin solution. The presence of acidoresistant incrustations (such as described for primordial hyphae) was observed in distilled water after an initial coloration in karbolfuchsin followed then by a brief staining (a few seconds) in a 10% solution of HCl (cf. Romagnesi 1967).

Spores were scanned with an Olympus Artcam camera and measured using Quick Micro Photo (version 2.1) software. Enlarged scanned pictures of spores were used for measuring with an accuracy of 0.1 µm and for making line drawings. Q gives length/width ratio of the spores. Measurements exclude ornamentation. Statistics for measurements of microscopical characters are based on 30 measurements and given as a mean value (underlined) plus/minus standard deviation ; values in parentheses give measured minimum or maximum values. An estimate for spore ornamentation density in descriptions is given following Adamčík & Marhold (2000).

TAXONOMY

Russula hixsonii Murrill, Lloydia 6 (3): 212. 1943 [as *R. hixsoni*]. Figs. 1-12

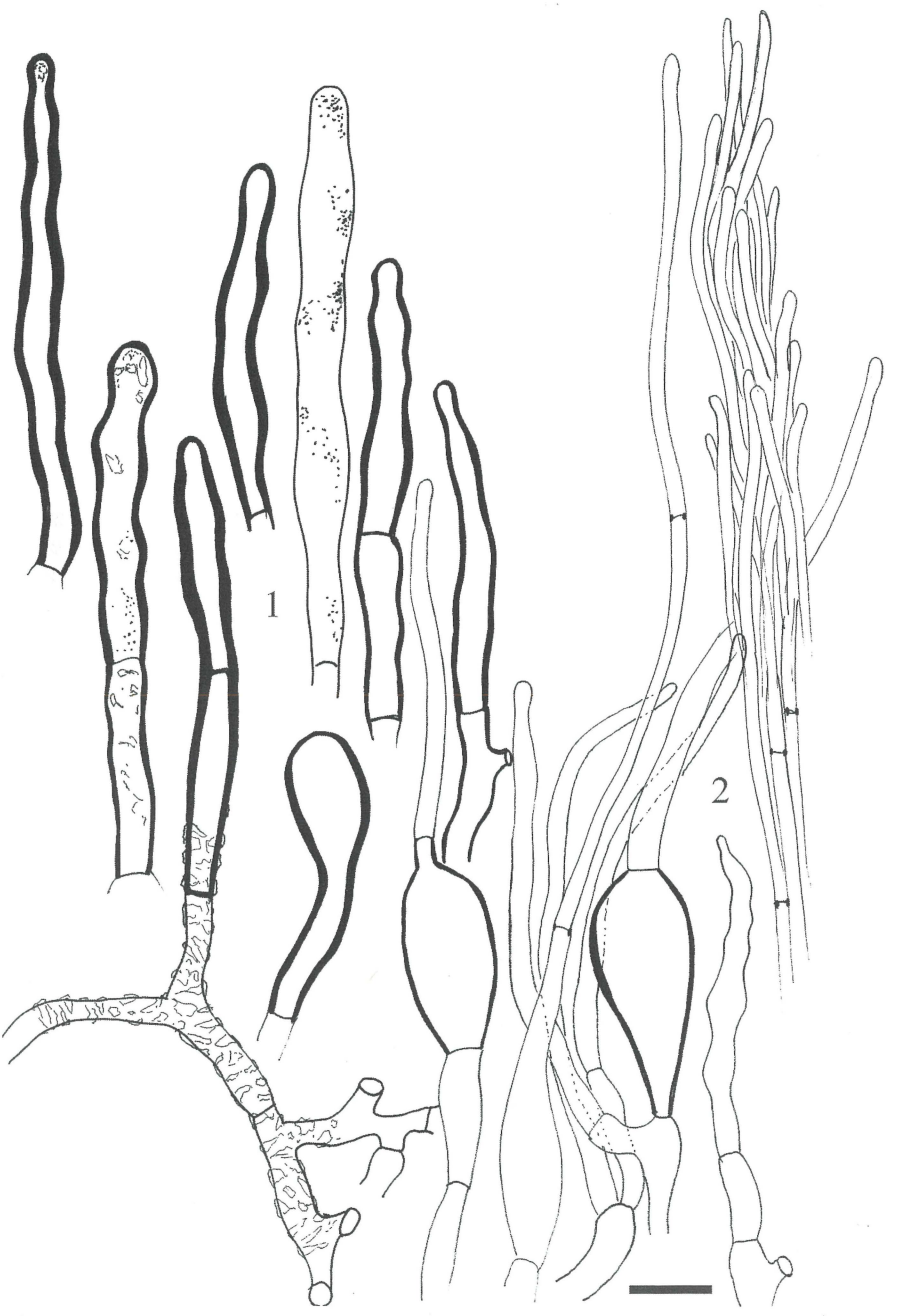
Original description: Pileo convexo-depresso, 15 cm. lato, roseo ad incarnato, grato; lamellis adnatis, latis, distantibus, pallidis ad fumosis; sporis ellipsoideis, echinulatis, stramineis, 10-12 × 8-10µ; stipite albo, 7 × 2-3 cm.

Pileus convex to depressed, gregarious, 15 cm. broad; surface dry, smooth, glabrous, roseous to incarnate, margin thick, even, entire, not peeling readily; context thick, white, unchanging, mild, with very agreeable odor on drying; lamellae adnate, many forked at base, broad, ventricose, distant, firm, entire, pallid, becoming avellaneous to fumosous on drying; spores broadly ellipsoid, prominently echinulate, 1-guttulate, stramineous, 10-12 × 8-10µ; stipe nearly equal, smooth, glabrous, white, unchanging, 7 × 2-3 cm.

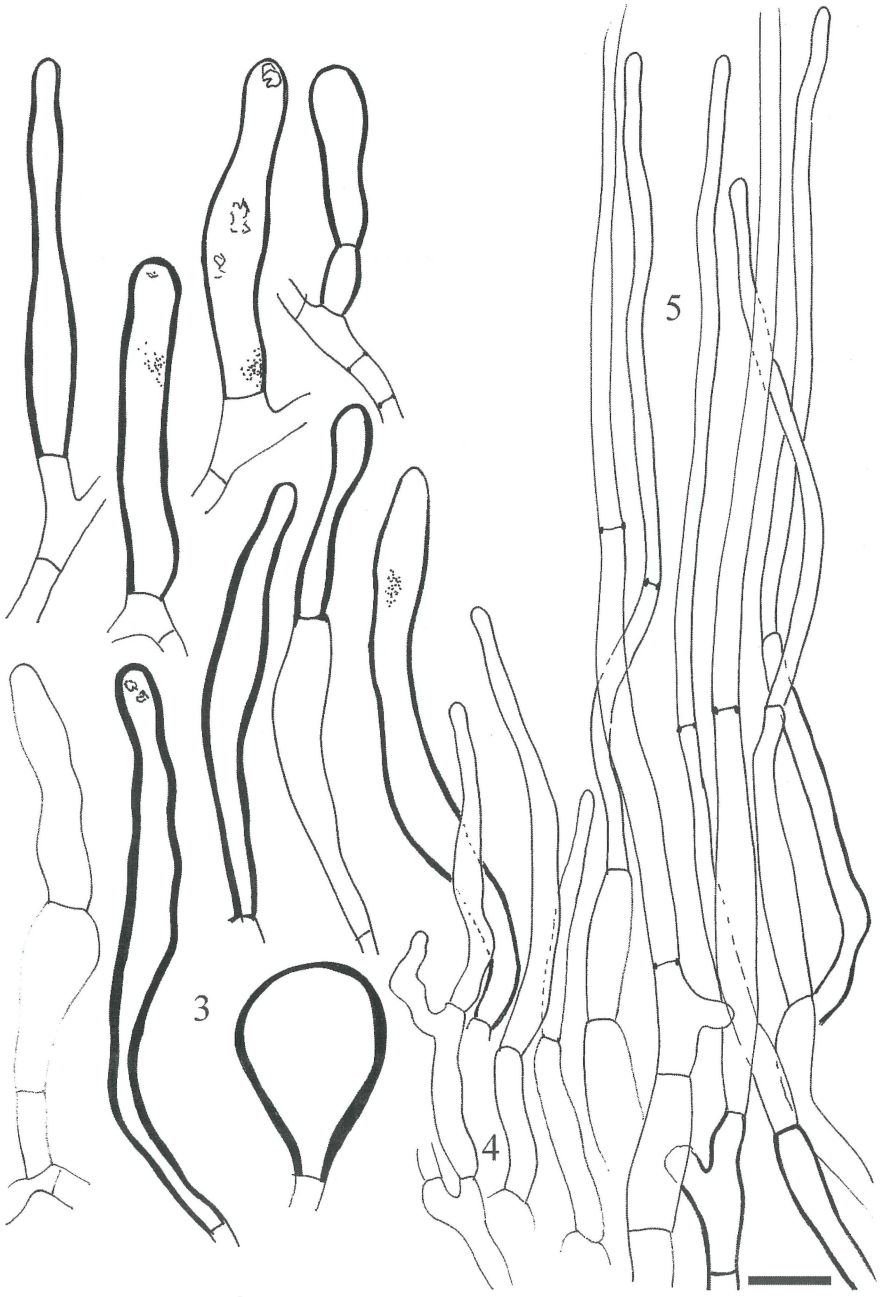
Type collected by Prof. Homer Hixson on a moist shaded slope near sweet gums west of Newnan's Lake, Alachua Co., Fla., Oct. 31, 1942 (*F 19081*). One of the largest and most beautiful species of the genus. While drying and for some time afterwards it smells like freshly baked cake.

Holotypus: [USA], Florida, Gainesville, Alachua Co., west of Newman's Lake, moist shaded slope near sweet gums, 10-31-42, leg. prof. Homer Hixson (FLAS F19081).

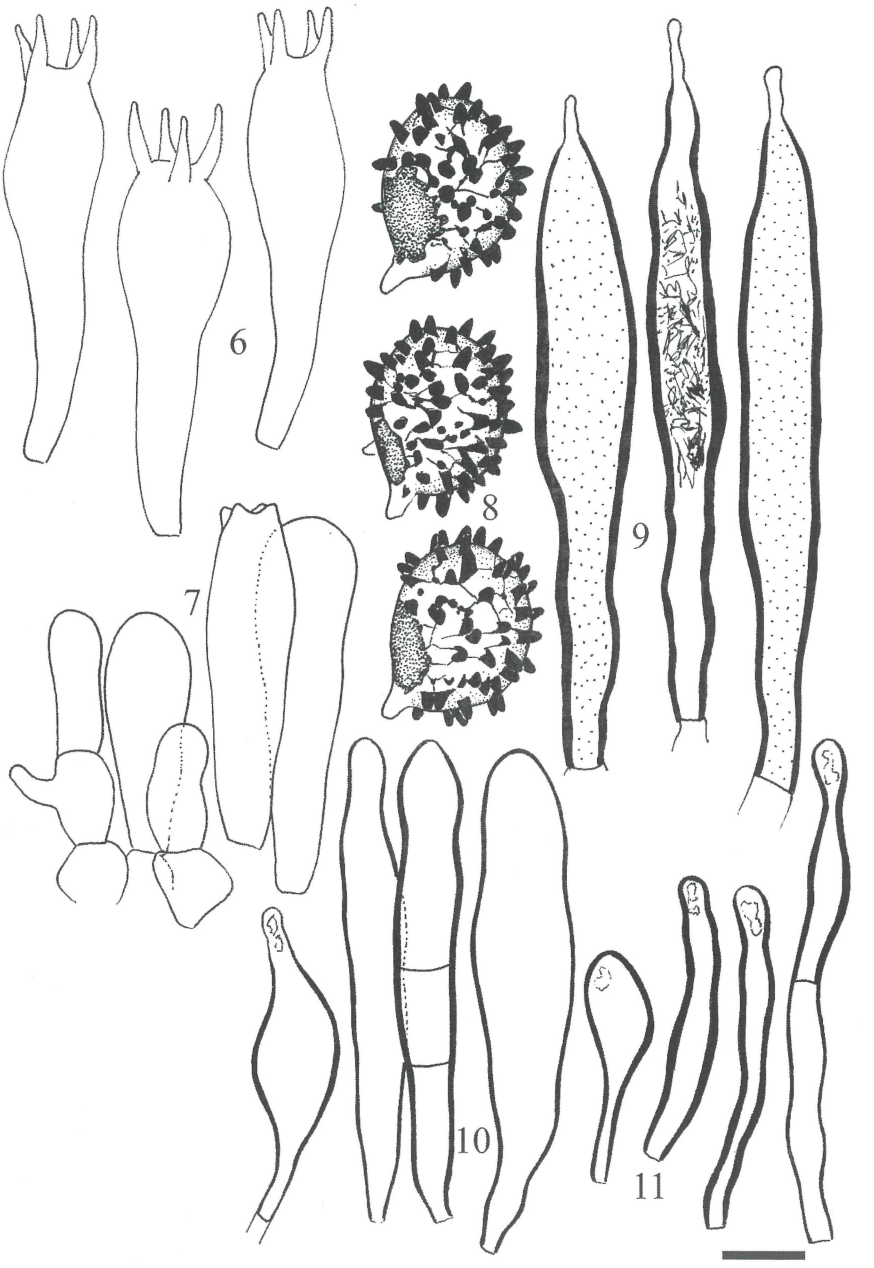
Cap very large, up to 20 cm diam., with an incurved margin when young, becoming uplifted to nearly plane in age with a depressed center and sometimes very shortly pectinate margin; buttons and young specimens white, mottled with pinks and yellows, then turning an intense rose-pink as they mature, with the center fading to whitish mottled with brown; the dry surface first dull, smooth but



Figs. 1-2. *Russula hixsonii* (PC 0084824). **1.** Metachromatic, mostly distinctly thick-walled terminal elements of the cap surface (here interpreted as pileocystidia); some elements with contents as seen in Congo red; one element in lower left corner with incrustations as seen in Cresyl blue and Congo red. **2.** Tips of typical, long hyphal extremities near the cap margin clinging together in tufts. Scale bar equals 10 µm.



Figs. 2-5. *Russula hixsonii* (holotype). **3.** Metachromatic, mostly distinctly thick-walled terminal elements of the cap surface (here interpreted as pileocystidia) with contents indicated in some elements as seen in Congo red. **4.** Thin-walled hyphal extremities in the cap center. **5.** Thin-walled hyphal extremities (some truncated) closer towards cap margin. Scale bar equals 10 µm.



Figs. 6-11. *Russula hixsonii* (6-7, 9-11 PC 0084824; 8 holotype). **6.** Basidia. **7.** Basidiola. **8.** Basidiospores in Melzer's reagent. **9.** Pleurocystidia with contents indicated in one element as seen in Congo red. **10-11.** Metachromatic elements of variable size on the gill edge. Scale bar equals 10 μm, but only 5 μm for spores.

not glabrous, towards the margin even strongly pruinose-tomentose, finally cracking into quilt-like patterns in the center with age; peeling about 1/3 to center of cap. **Gills** adnate to subfree, obtuse-rounded at the cap margin and up to 1 cm broad, narrowing towards the stipe, moderately spaced (ca 1/mm at the cap margin), predominantly equal (some dispersed lamellulae present), often forked especially near the stipe, white to off-white, changing to grayish black with age and on handling. **Stipe** 10-12 cm long, up to 3.5 cm thick, white, solid. **Context** ca 1 cm thick above the gill attachment, firm but not hard, white, strongly graying with age, without any rapid color changes on injury. **Taste** entirely mild. **Odor** not distinctive. **Spore print** "golden yellow" [IIIa (pale ochre) in Romagnesi's chart (Romagnesi 1967), scale 9 in Dagron's chart (Anonym 1993)].

Spores large, shortly ellipsoid, (9.2-)9.4-9.9-10.3(-10.9) × (7.6-)7.9-8.2-8.5(-9) µm, Q = (1.14-)1.16-1.2-1.25(-1.28); ornamentation subreticulate, composed of conical, amyloid, 1-1.4 µm high spines [(2-)3-5 in a 3 µm circle on spore surface] which are connected by numerous, thin line connections [2-3(-4) in the circle] or occasionally fused [(0-)1-2 fusions in the circle]; suprahilar spot strongly amyloid and large. **Basidia** mostly 45-56 × 12-15 µm, 4-spored, distinctly inflated in their upper part; basidiola first ellipsoid or cylindrical, then clavate. **Subhymenium** thick and densely pseudoparenchymatic, with deeply embedded bases of hymenial cystidia. **Lamellar trama** composed of sphaerocytes and generative hyphae, forming a rather compact tissue. **Hymenial cystidia** on gill sides widely dispersed, ca. 300-350/mm^2, numerous to abundant near the gill edge, fusiform or clavate and mostly mucronate-appendiculate (the appendix up to 20 µm long), with mostly distinctly thickened walls (0.5-1 µm thick) that are metachromatic in Cresyl blue (strongest at the gill edge), measuring mostly 87-110 × 8-12 µm, without any or with very few and mostly apical, heteromorphous to granular contents, not graying in sulfovanillin. **Marginal cells** resembling either terminal elements of the cap or hymenial cystidia, but smaller and never forming filiform projections, mostly obtuse, with or without some inconspicuous heteromorphous inclusions, some with one or more secondary septa, many thick-walled and then metachromatic. **Pileipellis** ochrochromatic in Cresyl blue except for the thick-walled elements, not sharply delimited from the underlying context, hardly gelatinized, vaguely divided in a 50-100(-200) µm deep suprapellis and 150-250 µm deep subpellis; the latter a dense, not gelatinous layer of intertwined, narrow, 4-6 µm wide hyphae. In subpellis and trama with numerous refringent, oleipherous fragments that are nodulose and constricted at septa. Suprapellis forming an extremely dense tissue of strongly ramifying, ascending elements in the cap center, giving it an almost subhymenium-like aspect, terminating at the cap surface in a trichodermal layer of mostly subcylindrical but undulate or sinuous cells rarely exceeding 80 µm in length. Outside the cap center the apical cells quickly grow longer towards the cap margin, protruding often far beyond the other surface elements and reaching sometimes 200-300 µm in length, forming very narrow, 2-3 µm diam., hypha-like projections with distant septa that cling together in tufts at their tips or over most of their length. These hyphal projections originate frequently from variably inflated, sometimes ampullaceous or even subglobose subapical cells having thin- to slightly thickened walls. Distinct irregular to zebroid incrustations are often present in the lower portion of the trichodermal layer and continue on some elements also deeper in the suprapellis, in subpellis and even on some hyphae in the trama. Pileocystidia ill-differentiated, one- or two-celled, mostly 36-74 × 5-10 µm, some with secondary septa in their basal part, arising in between the other terminal elements or sometimes originating from the very base of the suprapellis, absent from subpellis or underlying trama, mostly subcylindrical or fusiform,

Fig. 12. *Russula hixsonii in situ* (PC 0084824). Photo: Arleen Bessette.

mostly undulate-flexuous, sometimes more inflated in their upper part and then clavate, pyriform, ampullaceous to even subglobose, obtuse-rounded at the tip, more rarely distinctly capitate; their walls distinctly thickened (0.5-1 µm) and

metachromatic in Cresyl blue, bearing in the lower half often incrustations that react clearly acidoresistant (as in primordial hyphae), optically empty or with very few, refringent, minutely granular or heteromorphous, SV-negative inclusions in their apical part. **Clamp connections** absent in all parts.

Examined material. – UNITED STATES. **Florida**. Alachua Co. Gainesville, west of Newman's Lake, on moist shaded slope near sweet gums (*Liquidambar*), 31 Oct. 1942, leg. prof. Homer Hixson (FLAS F19081-holotype). **Georgia**. Camden Co., Crooked River State Park, Saint Marys, scattered or in groups under Live Oak (*Quercus virginiana*) and Longleaf Pine (*Pinus palustris*), 20 Oct. 2011, leg. Arleen Bessette (PC 0084824)

Commentary. – Our description is entirely based on the Georgia collection but resembles the type collection (Figs. 2-5, 8) in nearly all respects (spores of type somewhat longer: (9.8-)10-10.5-11(-11.7) × (7.6-)7.8-8.2-8.6(-9) µm, Q = (1.16-)1.23-1.28-1.33(-1.39)). The type of *R. hixsonii* was already studied by Hesler (1960) and also by Singer (1958), who published an updated description that was based on his own collection from the type locality. Singer (l.c.) also added more precision concerning the habitat at the type locality: "under *Quercus* with some pines, *Serrenao*, *Liquidambar*. Fruiting from May until October". Both at the type locality and at the Georgia locality, *R. hixsonii* seems to fruit abundantly. Yet, it is undoubtedly a very rare species and the senior author has never found it, notwithstanding ten years of collecting *Russula* in the southeastern states, including near Gainesville, nor do we know of any report that this species was found by other mycologists. The large size and impressive intensity of the pink cap color make *R. hixsonii* one of the most beautiful species in the genus (an appreciation that was mentioned explicitly in the original description, as well as in the notes on the Georgia collection). For this reason alone, a wider occurrence of such a species in the southeast would somehow have been reported. We think, therefore, that this *Russula* possibly requires specific soil or habitat conditions.

Singer's description (1958) presents some important variation with respect to the original description and the Georgia collection: (1) the stipe surface can also, partly to almost entirely, be of the same color as the cap; (2) the stipe may become eventually hollow with age, and (3) the taste is mild at first, but then becomes astringent to subacrid or even burning in the throat. On the other hand, one very obvious feature of the Georgia specimens is the strongly graying context. This may also be a quite variable feature as Murrill (1943) noted only a smoky color for drying gills and both he and Singer noted a white, unchanging context.

DISCUSSION

Singer changed his opinion on the systematic placement of this species several times: from subsections *Lilaceinae* Melzer & Zvára (Singer 1951) or *Virescentinae* Singer (Singer 1958) to finally *Lepidinae* (Melzer & Zvára) Singer (Singer 1975, 1986). However, in our opinion, this species does not fit in any of these nor other well-known *Russula* groups that occur also in Europe. In the field, it does indeed remind one of *Lepidinae* or *Virescentinae* because of the firmness and size of the fruit bodies, the pale gills and the cracking of the cap center with age. This very same habit separates it from the much smaller, more fragile

Lilaceinae. The pale ochre spore print (IIIa in Romagnesi's chart, 9 in code Dagron) is really a most unexpected feature considering the very pale color of the gills, even at maturity. According to Singer the spore print color is even deeper yellow when still fresh, but then changes to pale ochre with desiccation. Although spore print color has lost much of its taxonomic value in recent classifications of the genus, it would make a placement in any of the above-mentioned pale-spored groups unsatisfactory, especially in consideration also of the other microscopic differences. We agree with Singer (1958) that the aspect of the pileipellis with ill-differentiated dermatocystidia vaguely reminds of *Virescentinae* (or even the acystidiate *Amoeninae*) and *Lepidinae*, whereas general color and distinct acido-resistant incrustations on pileal elements surely are responsible for suggesting a placement in *Lilaceinae*, a group of exclusively (off-)white-spored, mostly (very) small species. The absence of an intense, red macrochemical reaction to sulfovanilline (Singer 1958) excludes affinities with the closely related subsection *Roseinae* Singer ex Sarnari. The metachromatic reaction to Cresyl blue of the thick-walled elements is here noted for the first time. It is a strong argument against any of the previously cited subsections, while the strong amyloidity of the suprahilar spot on the spores – another important taxonomic feature – exclude *Virescentinae* or other groups in subgenus *Heterophyllidia* Romagn. (including subsection *Cyanoxanthinae* Singer which possesses similar, strong (and in this case metachromatic) incrustations and metachromatic cell walls of terminal elements in the cap in Cresyl blue – see Buyck 1989, Buyck & Ovrebo 2002).

The morphological characters of *R. hixsonii* are indeed quite extraordinary. It is, for example, not possible to ascertain whether we are dealing with 'primordial hyphae' or with 'true dermatocystidia' at the cap and stipe surface. If choosing for the former, then the strongly graying to almost blackening context and gills might suggest a place close to the equally graying-blackening *R. vinosa* Lindbl. or *R. claroflava* Grove (both lacking dermatocystidia but with distinct primordial hyphae) for example. However, the fact that the wall incrustations in *R. hixsonii* are most evident in the lower portion of the suprapellis (and thus not typically present on the longest and surpassing elements of the suprapellis as with normal primordial hyphae) and even on certain hyphae in the deeper tissues, suggests that we are not dealing with typical 'primordial hyphae' as described for subgenus *Incrustatula* Romagn. Furthermore, none of the known *Incrustatae* show any metachromatic reaction in Cresyl blue (Buyck 1989).

On the other hand, the absence of any reaction to sulfovanilline and the very poor to absent, almost pigment-like contents of the thick-walled cap elements make it difficult to decide whether true dermatocystidia are absent or present. It was finally the comparison with the hymenial cystidia, variably thick-walled and with often poor contents that argued in favor of accepting similar cells as true dermatocystidia in the surface tissues. We unfortunately did not examine very young specimens, but Singer (1958) mentions that contents are more distinct in young specimens and then tend to disappear with age. Furthermore, no known acystidiate group in *Russula* would fit for *R. hixsonii*.

Finally, when looking for possible affinities with tropical taxa, the metachromatic reaction to cresyl blue for thick-walled cystidia has been described for the tropical American *R. metachromatica* Singer, a species possible very close to *R. delicoarchaea* Singer (see Buyck & Ovrebo 2002). Moreover, characters of spores (type of ornamentation, decurrent large amyloid spot) and basidia (subterminal strongest inflation and large, robust sterigmata) are nearly identical for these three species. On the other hand, the obtuse-rounded gills and vivid coloration of the cap (and apparently sometimes of the stipe as well) would argue

against close affinities to sections *Metachromaticae* Singer or *Delicoarchaeae* Singer. Spore print color of the latter two species-groups is unknown, but likely to be very pale.

In conclusion, the systematic position of *R. hixsonii* remains problematic and seems impossible to solve without molecular data. From a morphological point of view, Murrill's species remains unique and may well represent a distinct lineage within the genus.

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